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SARAH BRAGA RODRIGUES NUNES

BACTERIÓFAGOS RECOMBINANTES E miRNAs COMO ESTRATÉGIAS PARA O CONTROLE DE S*podoptera frugiperda*

PATOS DE MINAS – MG
FEVEREIRO DE 2019

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Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para obtenção do título de Mestre em Biotecnologia.

Orientadora: Profa. Dra. Thaise Gonçalves Araújo

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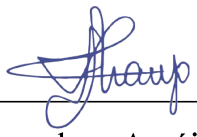

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RESUMO

A lagarta *Spodoptera frugiperda* é considerada uma das principais pragas agrícolas no Brasil, responsável por severos danos a culturas importantes. Inseticidas sintéticos e biopesticidas têm enfrentado limitações como o aumento no impacto ambiental e resistência dos insetos às toxinas recombinantes. A biotecnologia se mostra uma alternativa promissora ao permitir o desenvolvimento de estratégias efetoras para o combate da lagarta. O objetivo deste estudo foi gerar novas moléculas para o controle de *S. frugiperda* por meio da ferramenta biotecnológica de *Phage Display* (PhD), ao selecionar peptídeos ligantes a proteínas intestinais, e predizer microRNAs por meio de ferramentas de bioinformática. Utilizando a tecnologia de PhD, conseguimos selecionar sete clones cuja capacidade de se ligar a proteínas intestinais de *S. frugiperda* foi validada por ELISA e imuno-histoquímica. O clone SfF3, quando usado em combinação com a toxina de *Bacillus thuringiensis*, promoveu um aumento da mortalidade das lagartas neonatas. Além disso, demonstrou similaridade com as proteínas cassetes de ligação de ATP subfamília C2 e citocromo P450, as quais são importantes para a sobrevivência desta praga. Quanto aos miRNAs, foram preditos 350 precursores de microRNAs presentes no genoma de *S. frugiperda*, sendo 60 específicos para o gênero *Spodoptera*. Além disso, realizamos o refinamento de bibliotecas de RNA-seq disponíveis no banco de dados do NCBI na busca de possíveis moléculas bioinseticidas e 91 destas sequencias ainda não foram descritas na literatura, evidenciando as perspectivas de novas sequências enquanto possíveis alternativas para a regulação de transcritos e consequente alteração na biologia da lagarta. Nossos resultados validam essas tecnologias como uma abordagem agrobiotecnológica para o controle de pragas e identifica novas moléculas com atividade bioinseticidas, podendo ser utilizadas combinadas ou isoladamente.

Palavras-chave: Lagarta do cartucho, Phage Display, RNAi, peptídeos, bioinseticidas.

ABSTRACT

The caterpillar *Spodoptera frugiperda* is considered one of the main agricultural pests in Brazil, responsible for severe damage to important crops. Synthetic insecticides and biopesticides have faced limitations such as increased environmental impact and insect resistance to recombinant toxins. Biotechnology is a promising alternative that allow the development of effective strategies to caterpillar combat. The objective of this study was to generate new molecules for the control of *S. frugiperda* using the biotechnological tool of Phage Display (PhD), when selecting ligand peptides to intestinal proteins, and to predict microRNAs through bioinformatics tools. Using the technology of PhD, we were able to select seven clones whose ability to bind intestinal proteins of *S. frugiperda* was validated by ELISA and immunohistochemistry. Clone SfF3, when used in combination with *Bacillus thuringiensis* toxin, promoted an increase in mortality in newborn caterpillars. In addition, it demonstrated similarity to the ATP binding cassette subfamily C2 and cytochrome P450 proteins, which are important for the survival of this pest. As for the miRNAs, 350 precursors of microRNAs present in the genome of *S. frugiperda* were predicted, being 60 specifics for the genus *Spodoptera*. In addition, we performed the refinement of RNA-seq libraries available in the NCBI database in search of possible bioinsecticides molecules and 91 of these sequences have not yet been described in the literature, evidencing the perspectives of new sequences as possible alternatives for the regulation of transcripts and consequent alteration in the biology of the caterpillar. Our results validate these technologies as an agrobiotechnological approach to pest control and identify new molecules with bioinsecticide activity, which can be used in combination or in isolation.

Key words: Fall armyworm, Phage Display, iRNA, peptides, bioinsecticides.

LISTA DE ABREVIATURAS E SÍMBOLOS

AGO-1	Argonauta-1
ABCC2	cassetes de ligação de ATP subfamília C2
AMFE	Energia Livre Mínima Ajustada
Bt	*Bacillus thuringiensis*
C-	Controle Negativo
C+	Controle Positivo
DAB	3, 3-diaminobenzidina
Div	Diversidade de conjuntos
dsRNA	RNA dupla fita
Freq	Conjunto de Energia Livre Termal
GC content	Conteúdo de Guanina e Citosina
MFE	Energia Livre Mínima
MFEE	Energia Livre Mínima do Conjunto Termodinâmico
MFEI	Índice Mínimo de Energia Livre
miRNA	microRNA
mRNA	RNA mensageiro
NTC	Controle não alvo
PhD	*Phage Display*
RISC	Complexo silenciador induzido por RNA
RNAi	RNA de interferência
scL	Cistatina da soja L
scN	Cistatina da soja N
siRNA	pequeno RNA de interferência
ssDNA	DNA circular de cadeia simples
ssDNA	DNA circular de cadeia simples
WT	Fago selvagem

SUMÁRIO

INTRODUÇÃO **11**
OBJETIVOS 12
CAPÍTULO I **13**
1 *Spodoptera frugiperda* **14**
2 *PHAGE DISPLAY* E microRNAs PARA O CONTROLE DE PRAGAS **16**
2.1 *Phage Display* 16
2.2 microRNAs 20
REFERÊNCIAS **23**
CAPÍTULO II **29**
Abstract **31**
Introduction **31**
Materials and Methods **33**
Spodoptera frugiperda and protein extraction 33
Phage Display and biopanning 34
Phage-ELISA 34
Phage-clone binding 35
DNA extraction and *in silico* analysis 35
Immunohistochemistry 36
In vivo assay 37
Statistical analyzes 38
Results **38**
Discussion **50**
Acknowledgment **53**
References **54**
CAPÍTULO III **60**
Abstract **62**
1 Introduction **62**
2 Materials and Methods **64**
2.1 Prediction of mature miRNAs and their precursors (pre-miRNAs) 64
2.2 Identification of protostome-specific miRNAs 65

2.3 Sequencing analysis of the RNA-seq libraries 65

3 Results 65

4 Discussion 70

5 Conclusion 71

Acknowledgment 71

Declaration of Interest 72

Authors' contributions 72

References 72

Supplementary Tables 80

3 CONCLUSÃO 207

ANEXO A - Normas para revista Plos one 208

ANEXO B - Normas para revista Journal of Invertebrate Pathology 248

INTRODUÇÃO

A lagarta-do-cartucho (*Spodoptera frugiperda)*, da ordem Lepidoptera e família Noctuidae, é considerada uma das principais pragas no Brasil. Os danos provocados por este inseto podem reduzir a produtividade do milho em até 53% da produção média anual, com valor estimado de perdas de 6.187 milhões de dólares (Abrahams et al., 2017). Apesar das tentativas de combate químico, a severidade do ataque da lagarta-do-cartucho aumentou em várias regiões brasileiras, o que tem evidenciado a necessidade de novas estratégias para seu controle. A biotecnologia surge como uma alternativa ao oferecer métodos inovadores de interesse agronômico.

Dentre as ferramentas disponíveis, o *Phage Display* (PhD) vem sendo amplamente utilizado nas mais diferentes áreas, incluindo saúde e agropecuária. Esta tecnologia é uma plataforma de seleção molecular, em que peptídeos ou fragmentos de anticorpos podem ser bioengenheirados e selecionados contra alvos de interesse. No PhD, peptídeos aleatórios são expressos em fagos e uma biosseleção é realizada com a finalidade de se obter ligantes altamente específicos. Uma das principais vantagens inclui a seleção sem o conhecimento prévio do alvo, o que não compromete sua eficácia. Além disso, no PhD, há uma conexão entre o fenótipo (o peptídeo exibido no fago) e o seu genótipo (o DNA que o codifica), viabilizando a análise dos recombinantes e sua posterior seleção. A técnica é eficiente, robusta e específica, além de se mostrar promissora na obtenção de ligantes, que, por sua vez, podem ser conduzidos ao controle de pragas agrícolas através da seleção de moléculas com ação inseticida.

Em nível transcricional, os microRNAs (miRNAs) se destacam. Estas moléculas estão envolvidas na regulação pós-transcricional silenciando, por RNA de interferência (RNAi), genes específicos. Este mecanismo serve como a principal linha de defesa antiviral, tanto em plantas quanto em insetos (Gan *et al.*, 2008). Portanto, sua manipulação se traduz em perspectivas ao administrar, através da injeção ou do alimento do inseto, esses miRNAs. Sua principal vantagem é a especificidade ao gene-inseto-alvo, permitindo uma abordagem mais ecológica reduzindo os impactos ambientais na agricultura.

Nesse trabalho hipotetizou-se que peptídeos expressos na superfície de bacteriófagos filamentosos e miRNAs possam combater ou auxiliar no controle de *S. frugiperda.* Para isso, foram selecionados fagos recombinantes contra proteínas do intestino da lagarta, posteriormente validados em ensaios *in vivo*. Adicionalmente,

precursores de miRNAs foram preditos a partir do genoma de *S. frugiperda*, permitindo identificar possíveis moléculas com efeito inseticida. Oferecemos assim, ferramentas para uma dupla estratégia na contenção dessa praga que ainda compromete a produção e o desenvolvimento econômico do país. Novos caminhos são, portanto, abertos na busca de alternativas biotecnológicas e inovadoras voltadas para a agricultura.

OBJETIVOS

O objetivo primordial deste trabalho foi desenvolver novas moléculas para o controle de *S. frugiperda* utilizando as ferramentas biotecnológicas de *Phage Display* para selecionar peptídeos ligantes a proteínas intestinais, e ferramentas de bioinformática para identificar possíveis miRNAs com ação inseticida. Secundariamente, objetivamos estabelecer uma estratégia eficiente de biosselação e predizer para a obtenção de moléculas biologicamente ativas.

CAPÍTULO I

FUNDAMENTAÇÃO TEÓRICA

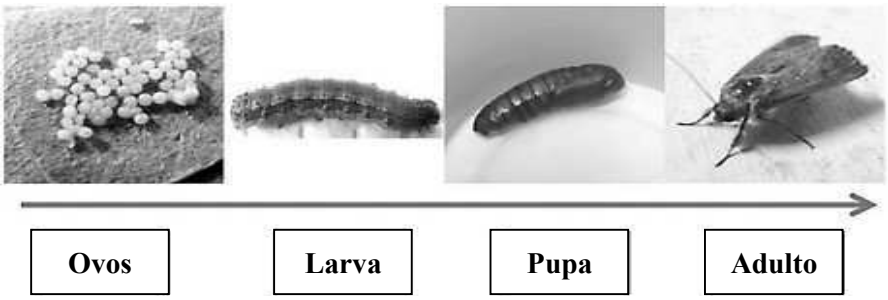
Normas ABNT

1 *Spodoptera frugiperda*

A *S. frugiperda* é considerada uma das principais pragas agrícolas no Brasil, e pode se alimentar de mais de 100 espécies de plantas de uma ampla gama de famílias (CABI, 2018). Este inseto ocorre naturalmente em regiões tropicais e subtropicais. Contudo, a alimentação diversificada e disponível durante todo o ano, além das condições climáticas favoráveis, agrava sua distribuição geográfica em solo brasileiro (Cruz, 1995; Cruz, 1999; Cruz *et al.*, 2009).

O ciclo de vida desta lagarta (Figura 1), sob condições laboratoriais controladas, dura aproximadamente 30 dias e inicia quando os ovos são colocados em massa na parte superior das folhas. Após 3 dias, a 25ºC, estes eclodem e originam as lagartas. Inicialmente, estas se alimentam da casca dos próprios ovos e depois raspam as folhas mais novas da planta, ocasionando o sintoma característico denominado “folhas raspadas". À medida que cresce, a larva se desloca para a região do cartucho, onde ocasiona severos danos. No interior dessa estrutura, o período larval varia de 12 a 30 dias. Quando completamente desenvolvida, a lagarta sai do cartucho e penetra no solo, onde se transforma em pupa, com aproximadamente 15 mm de comprimento. A fase de pupa dura, em média, de 10 a 12 dias. Já a mariposa mede cerca de 35 mm de envergadura e possui coloração das asas anteriores parda-escura e posteriores branca-acinzentada, com pontos claros na região central de cada asa. A longevidade do adulto é de aproximadamente 12 dias. Durante o estágio larval, as lagartas podem passar por até sete instares, dependendo da temperatura e do tipo de alimento. A larva recém-nascida tece um fio de seda que é usado como meio de dispersão e escape de inimigos naturais. No final dessa fase, chega a atingir 50 mm de comprimento (Cruz, 1995).

Figura 1 - Ciclo de vida da *S. frugiperda*. Inicialmente, os ovos são colocados em massa na parte superior das folhas, então eclodem e dão origem as lagartas. Quando completamente desenvolvida, a lagarta sai do cartucho e penetra no solo, onde se transforma em pupa e em seguida origina ao adulto.



Fonte: Adaptado de Schmidt-Duran *et al.* (2015)

As lagartas danificam o limbo foliar das folhas mais novas, produzindo injúrias de "raspagem". Já as lagartas maiores perfuram as folhas e se desenvolvem no cartucho do milho. Os danos biológicos provocados diminuem a área fotossintética da planta comprometendo sua produtividade. Apesar das lesões não serem devastadoras, abrem orifícios que facilitam a penetração de patógenos. O controle ineficaz desses indivíduos pode reduzir a produtividade do milho em até 53%, valor correspondente a US $ 6.187 milhões (Abrahams et al., 2017)

O principal controle desta praga é a utilização de inseticidas sintéticos da classe de carbamatos, organofosforados e piretróides. O uso destes produtos promove o surgimento de populações resistentes e gera impactos ao ambiente e inimigos naturais, além de possuírem um elevado custo econômico (Martinelli e Omoto, 2006). A utilização de biopesticidas, como as toxinas Cry produzidas por *B. thuringiensis* (Bt), também é uma importante forma de manejo, proporcionando bons resultados e reduzindo o impacto ambiental (Bobrowski *et al.*, 2003). Evidências sugerem que essas toxinas se ligam a proteínas da membrana do epitélio do intestino médio, levando à lise ou conduzindo à morte celular (Bravo *et al.*, 2007). Apesar das vantagens, uma das dificuldades do uso dos produtos Bt é a susceptibilidade reduzida das larvas do terceiro instar, comparada as mais novas (Ferro e Lyon, 1991; Navon, 2000). Além disso, o número de casos de resistência das plantas geneticamente modificadas para expressar a toxina Bt mais do que triplicou desde 2002 (Tabashnik e Carrière, 2017).

A severidade dos danos causados também aumentou em várias regiões brasileiras. A eliminação dos inimigos naturais, aparecimento de populações resistentes a inseticidas, além da maior disponibilidade de alimento, criam condições favoráveis para a sobrevivência do inseto (Cruz, 1999). Nesse contexto, novas estratégias de controle tornam-se necessárias, envolvendo tecnologias inovadoras que possam gerar moléculas efetoras no controle dessa praga.

2 *PHAGE DISPLAY* E microRNAs PARA O CONTROLE DE PRAGAS

A biotecnologia emerge como uma promissora alternativa ao oferecer ferramentas para o desenvolvimento de novos ligantes. O *Phage Display* (PhD) se destaca como uma plataforma de seleção molecular, em que peptídeos ou fragmentos de anticorpos podem ser bioengenheirados e selecionados contra alvos específicos. Os miRNAs, por sua vez, são moléculas de pequeno tamanho (20 a 23 nucleotídeos) envolvidas na regulação pós-transcricional. Estes pequenos RNAs permitem o silenciamento gênico mediado por RNA de interferência (RNAi) de alvos específicos e, portanto, com nítida interface econômica. Tratam-se, portanto, de duas técnicas que, isoladamente ou combinadas, podem introduzir novos elementos no controle de pragas, transpondo inúmeras barreiras químicas até então enfrentadas pela economia do país.

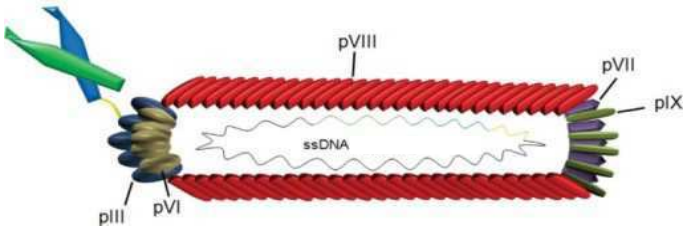
2.1 *PHAGE DISPLAY*

PhD é uma ferramenta molecular utilizada para apresentar, enriquecer e amadurecer a afinidade de vários peptídeos/proteínas a partir de uma biblioteca com mais de $2x10^9$ variantes expressa em vetores virais. A apresentação dos ligantes envolve a utilização de bacteriófagos, os quais são manipulados geneticamente para expressar uma molécula exógena fusionada a uma de suas proteínas, em geral de seu capsídeo (Smith, 1985; Scott e Smith, 1990).

Bacteriófagos filamentosos da família Inoviridae (M13, f1 e fd), parasitas de bactérias gram negativa que contêm o pílus F, são os vetores utilizados nesse processo. A partícula do fago M13, mostrada na Figura 2, é formada por um DNA circular de cadeia simples (ssDNA) cercado por proteínas de revestimento (pIII, pVI, pVII, pVIII e pIX),

formando o capsídeo viral. Existem aproximadamente 2700 cópias da pVIII e, em uma das extremidades do fago, 5 cópias da pIII e pVI, estas envolvidas na ligação à célula hospedeira e no término do processo de montagem da partícula viral, respectivamente. Na outra extremidade, são encontradas 5 cópias de pVII, responsável pelo início da montagem viral e pIX, essencial para a manutenção da estabilidade do fago (Phizicky e Fields, 1995; Webster, 2001).

Figura 2 - Estrutura da partícula de fago M13 exibindo uma proteína exógena fusionada à região N-terminal da proteína de revestimento pIII. As demais proteínas do capsídeo viral pIII, pVI, pVII, pVIII e pIX também são demonstradas, e em seu interior, o DNA fita simples circular.



Fonte: Adaptado de Kierny *et al.* (2012)

O bacteriófago necessita da maquinaria de replicação, transcrição e tradução da bactéria para sua reprodução. A infecção das células de *Escherichia coli* se inicia com a interação entre a proteína pIII e a parte superior do pilus F, um tubo proteico composto por subunidades de pilina, as quais são despolimerizadas retraindo o pilus e internalizando o fago. Após o DNA viral penetrar no citoplasma, é convertido em uma molécula replicativa dupla fita e o material genético do vírus é utilizado como molde para a transcrição e tradução de suas proteínas. Após a síntese da proteína pV, forma-se o complexo DNA-pV, utilizado na montagem do bacteriófago entre a parede e membrana celulares. O fago M13 não provoca a lise da célula hospedeira, mas induz um estado em que a bactéria origina e libera continuamente partículas virais, ocasionando uma queda na sua taxa de reprodução (Maneewannakul *et al.*, 1993; Tikunova e Morozova, 2009).

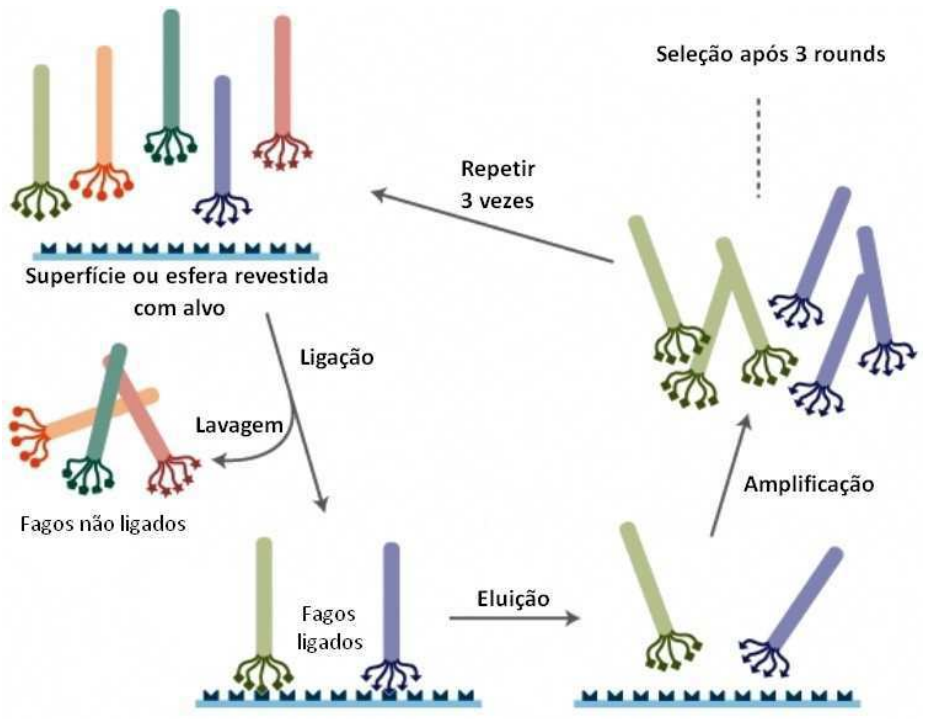
No PhD, o gene exógeno é fusionado, em geral, na região N-terminal das proteínas de revestimento pIII ou pVIII. A escolha da proteína que exibe a biblioteca determina a valência do processo. Utilizando a pIII, as 5 cópias podem expressar peptídeos curtos sem interferir na infectividade do fago. Quando se utiliza a pVIII, aproximadamente 10% destas podem ser alteradas, o que determina uma elevada valência (aproximadamente 200

cópias por vírus) em detrimento da afinidade durante o processo de seleção de recombinantes. Portanto, o sistema baseado na expressão em pIII permite a seleção de ligantes de alta afinidade. O processo de seleção para apresentar, enriquecer e amadurecer a afinidade é denominado de *biopanning* ou biosseleção (Gram *et al.*, 1992; Peltomaa *et al.*, 2016).

O *biopanning* consiste em ciclos ou *rounds* sequenciais de exibição, seleção e enriquecimento (Figura 3). No primeiro toda a biblioteca é exposta ao alvo e uma subpopulação de fagos específicos permanece ligada, enquanto o restante dos vírus é removido por lavagens. As partículas de fagos que ligaram ao alvo são eluídas, e em seguida, infectam bactérias para serem amplificadas em meios seletivos, permitindo aumentar o número de cópias de fagos específicos. A eluição dos ligantes pode ser realizada com pH extremo, clivagem de protease ou com o uso de competidores. O produto do primeiro *round* é utilizado nos ciclos seguintes permitindo enriquecer os recombinantes que se ligam especificamente ao alvo de interesse (Tikunova e Morozova, 2009; Kierny *et al.*, 2012). Para a seleção, o antígeno pode ser imobilizado em suporte sólido ou mantido em solução, utilizando-se partículas que se movem livremente, como esferas paramagnéticas (Kierny *et al.*, 2012).

Figura 3 - Representação do processo de seleção (*biopanning*). A biblioteca de fagos recombinantes é incubada com o antígeno alvo (imobilizado ou em solução). As partículas virais não ligantes são removidas por sucessivas lavagens; as específicas são eluídas (eluição ácida ou competitiva) e posteriormente amplificadas através da infecção

de bactérias. O produto do primeiro *round* é utilizado nos ciclos seguintes permitindo enriquecer os fagos com maior afinidade.



Fonte: Adaptado Biolabs (2017).

O PhD se destaca em relação a outras metodologias por permitir uma ligação direta entre o genótipo encapsidado e o fenótipo expresso em fusão, o que facilita a caracterização da proteína após o processo de seleção. Além disso, são construídas bibliotecas que variam entre 10^6 a 10^{11} variantes expressos, o que amplia a possibilidade de se obter ligantes de interesse (Nixon *et al.*, 2014). Não somente peptídeos, mas também anticorpos, ou até mesmo seus fragmentos, podem ser expressos e selecionados a partir de bacteriófagos recombinantes. A utilização de fragmentos de anticorpos é uma alternativa interessante, pois são menores e apresentam propriedades farmacocinéticas e farmacodinâmicas melhoradas, embora falte a função efetora (Chames *et al.*, 2009; Aghebati-Maleki *et al.*, 2016).

A não utilização de animais de laboratório, a relativa simplicidade dos protocolos e a possibilidade de rastrear uma grande quantidade de moléculas candidatas em um curto período de tempo torna o PhD vantajoso. Peptídeos e anticorpos são selecionados contra diferentes antígenos, incluindo substâncias tóxicas ou vírus altamente perigosos, os quais, por razões éticas, não podem ser utilizados na imunização (Koiwa *et al.*, 1998; Carmen e Jermutus, 2002; Nascimento *et al.*, 2009; Tikunova e Morozova, 2009).

A versatilidade dessa tecnologia é traduzida em diferentes estratégias e moléculas selecionadas com atividades defensivas. Koiwa *et al.* (1998) identificaram o papel inseticida de duas isoformas proteicas de cistatinas da soja, a cistatina da soja N (scN) e a cistatina da soja L (scL) por meio da técnica de PhD. Após o *biopanning*, a afinidade de ligação da scN foi superior a scL, sendo capaz de retardar o crescimento e desenvolvimento de *Callosobruchus maculatus*.

Também expressas na superfície viral, variantes de inibidores de tripsina foram selecionadas quanto à sua capacidade de ligação ao alvo. A Chy8 se mostrou altamente tóxica para as ninfas de *Acyrthosiphon pisum* e moderadamente tóxica para as ninfas de *Aphisgossypii* e *Myzus persicae*, além de cerca de oito vezes mais eficiente que a selvagem. Em outro estudo, variantes da toxina produzida pelo Bt foram exibidas e selecionadas contra o vetor *Aedes aegypti*. Duas moléculas, Cry1Aa13-A8 e Cry1Aa13-A12, foram capazes de induzir uma alta mortalidade (90%) nas populações larvais do mosquito. Outro mutante, Cry1Aa13-A10, não demonstrou efeito no estágio larval, mas induziu a mortalidade tardia das pupas, evidenciando a aplicabilidade do PhD enquanto estratégia para o controle de diferentes pragas (Domínguez-Flores *et al.* (2017).

Considerando o princípio dessa metodologia, o PhD se mostra como uma alternativa biotecnológica interessante no desenvolvimento de inibidores contra pragas agrícolas, gerando novas moléculas inseticidas ou tornando as existentes mais efetivas. Esta tecnologia permite selecionar mutantes contra proteínas ou moléculas receptoras intestinais de insetos, e estas podem mostrar uma nova atividade inseticida, ou podem melhorar a toxicidade contra um alvo específico, ou mesmo demonstrar ser ativa contra insetos resistentes (Bravo *et al.*, 2013).

2.2 microRNAS

Os microRNAs (miRNAs) e os pequenos RNAs de interferência (siRNAs) são moléculas utilizadas em ensaios para o silenciamento. Possuem propriedades físico-químicas e função semelhantes, mas mecanismos distintos (Ahmadzada *et al.*, 2018). Ambos são duplex de RNAs curtos formados por uma cadeia ativa (guia) e uma cadeia complementar inativa (passageiro). Os siRNAs são considerados RNAs exógenos e apresentam complementariedade total ao seu alvo, promovendo, assim, a clivagem do esqueleto fosfodiéster. Os miRNAs são endógenos e reprimem a tradução ao conduzir a um emparelhamento de bases imperfeito. Uma diferença importante no que concerne à

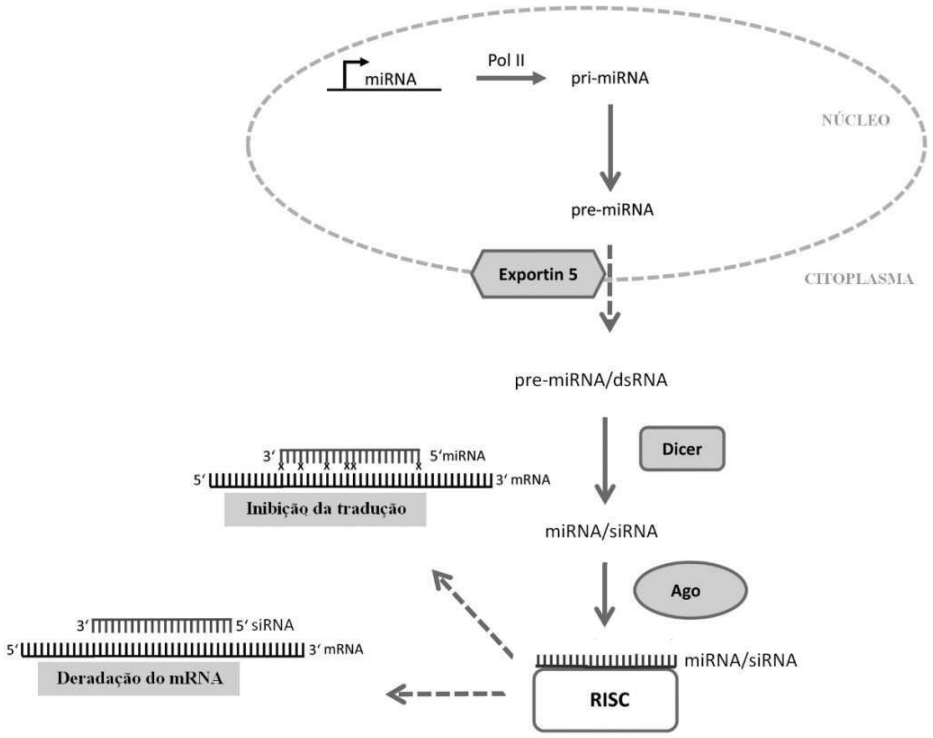
atividade dessas moléculas é a complementariedade do siRNA a uma sequência específica de um único mRNA, inibindo a expressão de um único gene. Já o miRNA pode ter múltiplos alvos e regular diferentes genes em decorrência de seu reconhecimento imperfeito (Lam *et al.*, 2015). Contudo, é possível identificar, por meio de análises *in silico*, miRNAs específicos a um determinado alvo. Os algoritmos utilizados permitem gerenciar a sensibilidade ou o nível de precisão das moléculas preditas (Akhtar *et al.*, 2015).

Para ambos, o silenciamento envolve o mecanismo de RNAi. Primeiramente, um longo RNA dupla fita (dsRNA) expresso ou introduzido na célula é digerido em pequenos RNAs de cadeia dupla pela enzima Dicer. Em seguida, as fitas são separadas e uma destas, conhecida como guia, é acoplada ao complexo RISC. Este é dirigido para a cadeia alvo de seu RNA mensageiro (mRNA) complementar. Após a ligação ocorre o bloqueio da tradução ou degradação do alvo (Winter *et al.*, 2009).

No caso dos miRNAs, estes são primeiramente transcritos em pri-miRNA no núcleo da célula pela RNA polimerase II ou III e possuem sequências complementares em tandem que permitem a dobradura da molécula, gerando um dsRNA (Siomi e Siomi, 2009). Os pri-miRNAs são processados a uma molécula precursora, o pré-miRNA (65 a 70 nucleotídeos). Estes precursores são transportados para o citoplasma pela Exportin 5, onde são clivados em dsRNAs curtos (miRNAs) pela Dicer. A cadeia com menor estabilidade de combinação de bases é carregada no complexo RISC por ligação direta à proteína Argonauta-1 (AGO-1). O complexo RISC/miRNA é guiado até o mRNA para que ocorra a regulação de sua expressão. O grau de complementaridade com o mRNA é crucial para determinar o mecanismo de interferência. Quando ocorre uma complementaridade parcial entre a sequência da extremidade 5' do miRNA (*seed sequence*) com a região do mRNA alvo, é desencadeada a inibição da tradução. O silenciamento por siRNA se inicia com a introdução de uma molécula de dsRNA artificial na célula. Esta é processada pela Dicer em siRNA e também carregada no complexo RISC. Nesse caso, a complementariedade total entre o mRNA e o siRNA conduz à degradação da molécula alvo. Ambos os mecanismos são descritos na Figura 4.

Figura 4 - Mecanismos de silenciamento de genes por miRNA e siRNA. O silenciamento através dos miRNAs inicia-se pela transcrição do pri-miRNA pela RNA polimerase II ou III gerando um dsRNA. Os pri-miRNAs originam ao pré-miRNA que é transportado para o citoplasma pela Exportin 5 e clivado em dsRNA curto (miRNA) pela

Dicer. Uma das cadeias é carregada no complexo RISC por ligação direta à proteína AGO-1 e este complexo é guiado até o mRNA para que ocorra a regulação de sua expressão. A complementaridade parcial entre a *seed sequence* com a região do mRNA alvo desencadeia a inibição da tradução. Já o silenciamento por siRNA se inicia com a introdução de uma molécula de dsRNA artificial na célula. Esta é processada pela Dicer em siRNA e também carregada no complexo RISC. Nesse caso, a complementariedade total entre o mRNA e o siRNA conduz à degradação da molécula alvo



Fonte: Adaptado de Wagner *et al.* (2015)

O RNAi é um mecanismo de regulação pós-transcricional, encontrado em plantas e animais, incluindo insetos (Burand e Hunter, 2013) e visa silenciar a expressão de genes endógenos ou de invasores, parasitas e patógenos, como vírus e elementos genéticos móveis, incluindo os transposons. Como é a principal linha de defesa em plantas, é considerado parte de seu sistema imune inato (Gordon e Waterhouse, 2007). Este mecanismo vem sendo extensivamente explorado para o desenvolvimento de métodos para controle de pragas e vetores de doenças. Várias pesquisas mostraram que as moléculas de dsRNAs administradas por injeção ou ingestão são capazes de penetrar nas células e ativar a via de silenciamento (Matranga e Zamore, 2007; Van Rij e Berezikov, 2009; Berezikov, 2011). Um exemplo é o estudo desenvolvido por Mogilicherla *et al.* (2018), em que foi realizada a administração oral de dsRNA em *Halyomorpha halys* com

a consequente morte do inseto. A administração oral do miR-2703 em ninfas de *Nilaparvata lugens* silenciou o gene A da síntese de quitina, também levando a fenótipos letais (Li *et al.*, 2017). Já o silenciamento dos genes alatostatina C e alototropina 2 de *S. frugiperda,* por RNAi, promoveu uma redução nas taxas de oviposição das fêmeas adultas (Griebler *et al.*, 2008). Em 2018, o grupo de pesquisa de Wang inibiu o gene da calmodulina, relacionada ao desenvolvimento de insetos, utilizando dsRNA em *N. lugens* com consequente alterações fenotípicas e a mortalidade dos indivíduos tratados (Wang *et al.*, 2018).

A escolha dos miRNAs para serem usados como estratégia inseticida exige minuciosas análises *in silico,* de forma a garantir sua especificidade ao inseto de interesse. miRNAs, ao contrário de siRNAs, são conservados (Grody *et al.*, 2009). Interessantemente, ao definir como alvo essas sequências conservadas, há uma maior chance de se promover a morte do inseto, pois regiões importantes para o funcionamento do organismo seriam silenciadas (Lai, 2005).

De fato, essas moléculas são potencialmente promissoras. A sequência usada pode ser cuidadosamente desenhada para o uso contra uma única praga, ou grupo de espécies relacionadas, sem afetar insetos não-alvo. Assim, a principal vantagem desta tecnologia é sua alta especificidade e explorar esse mecanismo se mostra interessante, especialmente no manejo de pragas agrícolas (Whyard *et al.*, 2009; Parsons *et al.*, 2018).

REFERÊNCIAS

ABRAHAMS, P., BATEMAN, M., BEALE, T., CLOTTEY, V., COCK, M., COLMENAREZ, Y., … WITT, A. (2017). Fall armyworm: impacts and implications for Africa. Evidence Note (2), September 2017. Wallingford, UK: CABI.Disponível em: < **DOI:** https://doi.org/10.1564/v28_oct_02>.

AGHEBATI-MALEKI, L. et al. Phage display as a promising approach for vaccine development. **Journal of Biomedical Science,** v. 23, n. 1, p. 66, September 29 2016. ISSN 1423-0127. Disponível em: < https://doi.org/10.1186/s12929-016-0285-9 >.

AHMADZADA, T.; REID, G.; MCKENZIE, D. R. Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. **Biophysical Reviews,** v. 10, n. 1, p. 69-86, February 01 2018. ISSN 1867-2469. Disponível em: < https://doi.org/10.1007/s12551-017-0392-1 >.

AKHTAR, M. M. et al. Bioinformatic tools for microRNA dissection. **Nucleic acids research,** v. 44, n. 1, p. 24-44, 2015. ISSN 0305-1048. Disponível em: < https://doi.org/10.1093/nar/gkv1221>.

BEREZIKOV, E. Evolution of microRNA diversity and regulation in animals. **Nature Reviews Genetics,** v. 12, n. 12, p. 846, 2011. ISSN 1471-0064. Disponível em: < https://doi.org/10.1038/nrg3079 >.

BIOLABS, N. E. Panning with a pentavalent peptide library displayed on pIII., Disponível em: < https://www.neb.com/faqs/0001/01/01/whatisphdphagedisplay >.

BOBROWSKI, V. L. et al. Genes de Bacillus thuringiensis: uma estratégia para conferir resistência a insetos em plantas. **Ciência rural. Santa Maria. v. 33, n. 5, p. 843-850**, 2003. ISSN 0103-8478. Disponível em: < http://www.scielo.br/pdf/%0D/cr/v33n5/17128.pdf >.

BRAVO, A.; GILL, S. S.; SOBERÓN, M. Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. **Toxicon,** v. 49, n. 4, p. 423-435, 2007/03/15/ 2007. ISSN 0041-0101. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0041010106004387 >.

BRAVO, A. et al. Evolution of Bacillus thuringiensis Cry toxins insecticidal activity. **Microbial Biotechnology,** v. 6, n. 1, p. 17-26, 2013. ISSN 1751-7915. Disponível em: < http://dx.doi.org/10.1111/j.1751-7915.2012.00342.x >.

BURAND, J. P.; HUNTER, W. B. RNAi: Future in insect management. **Journal of Invertebrate Pathology,** v. 112, p. S68-S74, 2013/03/01/ 2013. ISSN 0022-2011. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0022201112001814 >.

CABI (CAB International). Crop Protection Compendium, 2018. Disponível em: < https://www.cabi.org/cpc/>.

CARMEN, S.; JERMUTUS, L. Concepts in antibody phage display. **Briefings in Functional Genomics,** v. 1, n. 2, p. 189-203, 2002. ISSN 2041-2649. Disponível em: < http://dx.doi.org/10.1093/bfgp/1.2.189 >.

CHAMES, P. et al. Therapeutic antibodies: successes, limitations and hopes for the future. **Br J Pharmacol,** v. 157, n. 2, p. 220-33, May 2009. ISSN 1476-5381 (Electronic) 0007-1188 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19459844 >.

CRUZ, I. A lagarta-do-cartucho na cultura do milho. Sete Lagoas: **EMBRAPA-CNPMS,** 1995. 45 p. (EMBRAPA-CNPMS. Circular técnica, 21). Disponível em: < https://core.ac.uk/download/pdf/15437047.pdf >.

CRUZ, I. Manejo de Pragas da Cultura do Milho. In: REUNION LATIONOAMERICANA DEL MAIZ, 18., 1999, Sete Lagoas. Memorias... Sete Lagoas: **EMBRAPA-CNPMS**; Mexico:CIMMYT, 1999. p. 51-56.

CRUZ, I.; VIANA, P. A.; WAQUIL, J. M. Pragas: pragas da fase vegetativa e reprodutiva. In: CRUZ, J. C. (Ed.). Cultivo do milho. 5. ed. Sete Lagoas: **Embrapa Milho e Sorgo**, 2009.

DOMÍNGUEZ-FLORES, T. et al. Using phage display technology to obtain Crybodies active against non-target insects. **Scientific Reports,** v. 7, n. 1, p. 14922, 2017/11/02 2017. ISSN 2045-2322. Disponível em: < https://doi.org/10.1038/s41598-017-09384-x >.

FERRO, D. N.; LYON, S. M. Colorado potato beetle (Coleoptera: Chrysomelidae) larval mortality: operative effects of Bacillus thuringiensis subsp. san diego. **Journal of economic entomology,** v. 84, n. 3, p. 806-809, 1991. ISSN 1938-291X. Disponível em: < https://doi.org/10.1093/jee/84.3.806 >.

GAN, Q.-H.; CHI, X.-Y.; QIN, S. Roles of microRNA in plant defense and virus offense interaction. **Plant cell reports,** v. 27, n. 10, p. 1571-1579, 2008. ISSN 0721-7714. Disponível em: < https://doi.org/10.1007/s00299-008-0584-z >.

GORDON, K. H.; WATERHOUSE, P. M. RNAi for insect-proof plants. **Nature biotechnology,** v. 25, n. 11, p. 1231, 2007. ISSN 1546-1696. Disponível em: < https://doi.org/10.1038/nbt1107-1231 >.

GRAM, H. et al. In vitro selection and affinity maturation of antibodies from a naive combinatorial immunoglobulin library. **Proceedings of the National Academy of Sciences,** v. 89, n. 8, p. 3576-3580, April 15, 1992 1992. Disponível em: < http://www.pnas.org/content/89/8/3576.abstract >.

GRIEBLER, M. et al. RNA interference with the allatoregulating neuropeptide genes from the fall armyworm Spodoptera frugiperda and its effects on the JH titer in the hemolymph. **Journal of Insect Physiology,** v. 54, n. 6, p. 997-1007, 2008/06/01/ 2008. ISSN 0022-1910. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0022191008000887 >.

GRODY, W. W. et al. Molecular diagnostics: techniques and applications for the clinical laboratory. **Academic Press,** 2009. ISBN 0080919049.

KIERNY, M. R.; CUNNINGHAM, T. D.; KAY, B. K. Detection of biomarkers using recombinant antibodies coupled to nanostructured platforms. **Nano Reviews,** v. 3, p. 10.3402/nano.v3i0.17240, 2012. ISSN 2000-5121. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3404449/ >.

KOIWA, H. et al. Phage display selection can differentiate insecticidal activity of soybean cystatins. **The Plant Journal,** v. 14, n. 3, p. 371-379, 1998. ISSN 1365-313X. Disponível em: < http://dx.doi.org/10.1046/j.1365-313X.1998.00119.x >.

LAI, E. C. miRNAs: whys and wherefores of miRNA-mediated regulation. **Current Biology,** v. 15, n. 12, p. R458-R460, 2005. ISSN 0960-9822. Disponível em: < https://doi.org/10.1016/j.cub.2005.06.015 >.

LAM, J. K. et al. siRNA versus miRNA as therapeutics for gene silencing. **Molecular Therapy-Nucleic Acids,** v. 4, 2015. ISSN 2162-2531. Disponível em: < https://doi.org/10.1038/mtna.2015.23 >.

LI, T. et al. MicroRNA and dsRNA targeting chitin synthase A reveal a great potential for pest management of the hemipteran insect Nilaparvata lugens. **Pest Management Science,** v. 73, n. 7, p. 1529-1537, 2017. ISSN 1526-4998. Disponível em: < http://dx.doi.org/10.1002/ps.4492 >.

MANEEWANNAKUL, K.; MANEEWANNAKUL, S.; IPPEN-IHLER, K. Synthesis of F pilin. **Journal of Bacteriology,** v. 175, n. 5, p. 1384-1391, 1993. ISSN 0021-9193 1098-5530. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC193225/ >.

MARTINELLI, S.; OMOTO, C. Resistencia de lepidoteros-praga a inseticidas na cultura do algodao no Brasil. **Revista Brasileira de Oleaginosas e Fibrosas,** v. 10, n. 3, 2006. Disponível em: < https://www.researchgate.net/profile/Celso_Omoto/publication/228489802_Resistencia_de_lepidoteros-praga_a_inseticidas_na_cultura_do_algodao_no_Brasil/links/0deec51878cf4e862b000000/Resistencia-de-lepidoteros-praga-a-inseticidas-na-cultura-do-algodao-no-Brasil.pdf >.

MATRANGA, C.; ZAMORE, P. D. Small silencing RNAs. **Current Biology,** v. 17, n. 18, p. R789-R793, 2007. ISSN 0960-9822. Disponível em: < https://doi.org/10.1038/nrg2504 >.

MOGILICHERLA, K.; HOWELL, J. L.; PALLI, S. R. Improving RNAi in the Brown Marmorated Stink Bug: Identification of target genes and reference genes for RT-qPCR. **Scientific Reports,** v. 8, n. 1, p. 3720, 2018/02/27 2018. ISSN 2045-2322. Disponível em: < https://doi.org/10.1038/s41598-018-22035-z >.

NASCIMENTO, R.; KERR, W. E.; FILHO, L. R. G. **Construção de uma biblioteca de anticorpos monoclonais apresentados em fagos para seleção e caracterização de scFv ligante a proteínas intestinais de Diatraea Saccharalis**. UBERLÂNDIA, MG, p.97. 2009. (Dissertação de Mestrado). Disponível em: < https://repositorio.ufu.br/handle/123456789/15817 >.

NAVON, A. Bacillus thuringiensis insecticides in crop protection—reality and prospects. **Crop protection,** v. 19, n. 8-10, p. 669-676, 2000. ISSN 0261-2194. Disponível em: < https://doi.org/10.1016/S0261-2194(00)00089-2 >.

NIXON, A. E.; SEXTON, D. J.; LADNER, R. C. Drugs derived from phage display. **mAbs,** v. 6, n. 1, p. 73-85, 2014/01/01 2014. ISSN 1942-0862. Disponível em: < http://dx.doi.org/10.4161/mabs.27240 >.

PARSONS, K. H. et al. Guanidinium-Functionalized Interpolyelectrolyte Complexes Enabling RNAi in Resistant Insect Pests. **Biomacromolecules**, 2018/02/15 2018. ISSN 1525-7797. Disponível em: < https://doi.org/10.1021/acs.biomac.7b01717 >.

PELTOMAA, R. et al. Application of bacteriophages in sensor development. **Analytical and Bioanalytical Chemistry,** v. 408, n. 7, p. 1805-1828, March 01 2016. ISSN 1618-2650. Disponível em: < https://doi.org/10.1007/s00216-015-9087-2 >.

PHIZICKY, E. M.; FIELDS, S. Protein-protein interactions: methods for detection and analysis. **Microbiological Reviews,** v. 59, n. 1, p. 94-123, 1995. ISSN 0146-0749. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC239356/ >.

SCHMIDT-DURAN, A. et al. Larval stage prediction model of Spodoptera frugiperdacollected in fig (Ficus carica) and discovery of Apantelessp. as its parasitoid. **Revista Tecnología en Marcha,** v. 28, n. 1, p. 47-58, 2015. ISSN 0379-3982. Disponível em: < http://www.scielo.sa.cr/scielo.php?script=sci_abstract&pid=S0379-39822015000100047&lng=en&nrm=iso >.

SCOTT, J. K.; SMITH, G. P. Searching for peptide ligands with an epitope library. **Science,** v. 249, n. 4967, p. 386-90, Jul 27 1990. ISSN 0036-8075 (Print) 0036-8075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1696028 >.

SIOMI, H.; SIOMI, M. C. On the road to reading the RNA-interference code. **Nature,** v. 457, n. 7228, p. 396, 2009. ISSN 1476-4687. Disponível em: < https://doi.org/10.1038/nature07754 >.

SMITH, G. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. **Science,** v. 228, n. 4705, p. 1315-1317, 1985. Disponível em: < https://doi.org/10.1126/science.4001944 >.

TABASHNIK, B. E.; CARRIÈRE, Y. Surge in insect resistance to transgenic crops and prospects for sustainability. **Nature biotechnology,** v. 35, n. 10, p. 926, 2017. ISSN 1546-1696. Disponível em: < https://doi.org/10.1038/nbt.3974 >.

TIKUNOVA, N. V.; MOROZOVA, V. V. Phage Display on the Base of Filamentous Bacteriophages: Application for Recombinant Antibodies Selection. **Acta Naturae,** v. 1, n. 3, p. 20-28, 2009. ISSN 2075-8251. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347532/ >.

VAN RIJ, R. P.; BEREZIKOV, E. Small RNAs and the control of transposons and viruses in Drosophila. **Trends in microbiology,** v. 17, n. 4, p. 163-171, 2009. ISSN 0966-842X. Disponível em: < https://www.sciencedirect.com/science/article/pii/S0966842X09000432 >.

WAGNER, A. E. et al. Food derived microRNAs. **Food & function,** v. 6, n. 3, p. 714-718, 2015. ISSN 2042-650X. DOI: 10.1039/C4FO01119H

WANG, W. et al. dsRNA targeting calmodulin reveal a potential target for pest management of Nilaparvata lugens. **Pest Management Science**, p. n/a-n/a, 2018. ISSN 1526-4998. Disponível em: < http://dx.doi.org/10.1002/ps.4865 >.

WEBSTER, R. Filamentous phage biology. **Phage Barbas CF, Burton DR, Scott JK and Silverman (eds) Phage Display: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor pp**, p. 1.1-1.37, 2001. Disponível em: < http://www.caister.com/cimb/v/v13/51.pdf >.

WHYARD, S.; SINGH, A. D.; WONG, S. Ingested double-stranded RNAs can act as species-specific insecticides. **Insect Biochemistry and Molecular Biology,** v. 39, n. 11, p. 824-832, 2009/11/01/ 2009. ISSN 0965-1748. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0965174809001374 >.

WINTER, J. et al. Many roads to maturity: microRNA biogenesis pathways and their regulation. **Nature cell biology,** v. 11, n. 3, p. 228, 2009. ISSN 1476-4679. Disponível em: < https://doi.org/10.1038/ncb0309-228 >.

CAPÍTULO II

INSECTICIDAL RECOMBINANT PHAGES AGAINST S*podoptera frugiperda*: NEW PERSPECTIVES FOR BRAZILIAN AGRICULTURE

Instructions according to **Plos one**

Title: Insecticidal recombinant phages against *Spodoptera frugiperda*: new perspectives for Brazilian agriculture

Short title: New recombinant phages to control *Spodoptera frugiperda*

Autorship

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Abstract

The *Spodoptera frugiperda* is an important pest that promotes large productivity losses of maize. Insecticidal proteins from *Bacillus thuringiensis* has promoted important advances for this pest control. However, the occurrence of resistant insects challenges this technology, and has become important to develop new effective products through innovative techniques in the selection of new binders. The aim of this study was to select recombinant phages expressing gut exogenous peptides binders with insecticidal activity against *S. frugiperda.* Using Phage Display technology, seven clones were successful selected and bound to intestinal proteins. SfF3 clone when combined with the *B. thuringiensis* toxin, has increased mortality of *S. frugiperda* neonates, and also demonstrated similarity to the truncated ABC transporter subfamily C2 and cytochrome P450, which are important proteins to this pest survival. Our innovative study validated the reliability of Phage Display technology as an agribiotechnological approache for pest control, expanding the options to identify new molecules with bioinsecticides activities.

Keywords: Fall armyworm, peptides, phage display, bioinsecticides

Introduction

The fall armyworm (*Spodoptera frugiperda*) is a polyphagous insect that feeds on more than 60 species of plants, being an important pest of corn, rice, sorghum and cotton in Americas [1]. This species can reduce maize production in up to 53% of the annual averaged production, with estimated loss of$6,187m [2].

The climatic conditions are determinant for the occurrence of lepidopteran pests, interfering in the development, behavior and feeding of the insects [3]. In Brazil, corn

cultivation in two seasons in the central-west region favors the occurrence of high infestations during all phases of plant growth [4]. In the southern, planting is carried out from August to January, thus, the first crops result in low infestations, but in the late months there is an increase in the pest population density [5]. Therefore, frequent applications of insecticides are necessary to reduce the insect population and avoid economic damages [6]. Bioinsecticidal molecules that lead to reduced use of chemical agents have been prominent in the area. In fact, the introduction of transgenic maize lines expressing insecticidal proteins from *B. thuringiensis* (Bt) is considered one of the most successful biotechnology achievements for the pest control. However, reports of insects resistance to transgenic crops arose in 2006 in TC1507 corn in Puerto Rico [6-8].

This event has also been reported in Brazil. Farias et al. [5] detected resistance of *S. frugiperda* to TC1507 corn in Brazil. Besides, low frequency of resistant alleles to *B. thuringiensis* has already been identified in fall armyworm population, demonstrating the potential evolution risk of resistance in Brazil [9]. Farias et al. [10] also demonstrated a significant reduction in susceptibility to the Cry1F toxin in *S. frugiperda* over the years 2010 to 2013, especially in regions with intensive maize production in Brazil. In this context, it becomes necessary to develop new effective products through innovative techniques in the selection of new binders.

Phage Display (PhD) is a high throughput profiling technology based on peptide libraries present on the surface of bacteriophage [11]. This technique has become a tool for finding high affinity bioactive peptides exploring the interaction sites between targets and ligands [12,13]. Therefore, through this technology, it is possible to trace and identify new peptides that bind to the midgut proteins of the caterpillar. Oligopeptides can therefore be identified through an affinity selection strategy, making this tool extremely suitable for the identification of new molecules that act as biopesticides [14].

Although there are no reports of recombinant phage peptides for *S. frugiperda*, different studies have been conducted with this methodology, with particularly promising results. Koiwa et al. [15] used the PhD technology to identify the insecticidal role of two protein isoforms of soybean cystatin, N (scN) and L (scL). After the biopanning, the binding affinity of scN was superior to scL, being able to retard the growth and development of *Callosobruchus maculatus*. The trypsin inhibitor variant Chy8 was selected by PhD by Ceci et al. [16] and was toxic to *Acyrthosiphon pisum*, *Aphisgossypii* and *Myzus persicae* nymphs. In another study, variants of the toxin produced by Bt were displayed on recombinant phages and selected against *Aedes aegypti*, and two molecules, Cry1Aa13-A8 and Cry1Aa13-A12, were able to induce a high mortality (90%) in the larval populations of this mosquito [17].

Therefore, PhD is an innovative agribiotechnological approache for pest control and was explored in this work. The aim was to select recombinant phages expressing gut exogenous peptides binders with insecticidal activity against genotypes of *S. frugiperda* adapted for Brazil.

Materials and Methods

Spodoptera frugiperda and protein extraction

Fifteen larvae of *S. frugiperda* (Lepidoptera: Noctuidae) from the Laboratório de Bio Controle Farroupilha/LALLEMAND (Patos de Minas, MG) were used. They were maintained at 25 ± 2°C, 70 ± 10% of relative humidity and 12 hours of photophase. Fall armyworm close to the last instar remained fasted for 24 hours. After this period, they were immobilized for 15 minutes at -4°C to be dissected in saline solution (NaCl 0.215 mM). The first and last segments were withdrawn and the intestine removed and stored at -80°C.

The intestines were microdissected and the extraction buffer (125 mM Tris-HCl pH 7.0, 100 mM NaCl, 0.5% Triton X-100, 0.5% Tween-20, e 0.1% Genapol C-100 or SDS 0.1%) was

added (125mg/mL). Then, the sample was homogenized on ice for 1 minute using a homogenizer, followed by 1 minute of rest. This step was repeated five times. After centrifugation at 10,000 x g for 15 minutes at 4°C, the supernatant was discarded, and 100mM phenylmethylsulfonyl fluoride, 1µL 100mM benzamidine and 1µL phosphatase inhibitor cocktail was added to the extracted proteins.

Phage Display and biopanning

The Ph.D.-C7C™ Peptide Library Kit (New England Biolabs) was used for phage selection. Wells of a 96-well high binding plate (Nunc, Denmark), one for each round, were coated with 10µg of midgut proteins in bicarbonate buffer (0.1M $NaHCO_3$, pH 8.6) overnight at 4ºC followed by blocking with $NaHCO_3$-BSA 5% (0.1M $NaHCO_3$, pH 8.6; BSA 5mg/mL) for 1h. Wells were washed six times with TBST (Tris-buffered saline containing 0.1% Tween 20), and proteins were incubated with phage library (10^{11}/100uL) diluted in TBST for 1h at 37ºC. The supernatant (with non-binding particles) was removed though 10 washes with TBST, and the remained bound phages were eluted in 50µL of 0.2 M glycine buffer, pH 2.0 for 10 min. Neutralization of acid pH was performed with 1 M Tris-base pH 9.0.

The phages were amplified using early-log *Escherichia coli* ER2738 as a host, purified by PEG-8000/NaCl precipitation and titered on LB/IPTG/Xgal plates as described previously [18]. The selection was repeated for two more times (a total of three rounds for enrichment) each one using the previous amplified eluted phages.

Phage-ELISA

For screening of selected clones, 96-well Maxisorp microtiter plates (Nunc, Denmark) were coated with 5µg of midgut protein in 100 mM NaHCO3, pH 8.6 and overnight at 4°C. A

separate set of wells without gut proteins was coated with blocking buffer TBS-BSA 5% (Tris-buffered saline, BSA 5%) and used as no target control (NTC). Plates was washed twice with TBS 1X and then blocked with TBS-BSA 5%, for 90 min at 37°C. After washing with TBS 1X, culture supernatant (50uL) from 54 amplified phage particles randomly picked from the second round (~10^{10} pfu/mL) were incubated for 1 h at 37°C. The wells were washed four times with TBS-T 0.1% followed by incubation the peroxidase-conjugated anti-M13 antibody (GE Healthhcare, Chicago, IL) diluted 1:5000 in blocking buffer at 37 ° C for 1 hour.

Plates were further washed four times in TBS-T 0.1%, revealed with OPD SigmaFastTM (Sigma-Aldrich) and read at 492 nm. The ELISA assay was performed in triplicate for each of de 54 clones. The same protocol was performed to evaluate the rate of recovered phage clones using the supernatant of *E. coli* infected with the pool of phages from each round.

Phage-clone binding

A new format-ELISA assay was conducted for the seven selected clones (SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8). The immunoassay was conducted as above, and the culture supernatant was replaced by 1x10^{9}, 1x10^{10} and 1x10^{11} phages/well. The wild type M13 phage vector (no peptide) was also purified and used as control. Phages were purified as previously published [18].

DNA extraction and *in silico* analysis

For DNA extraction and sequencing, isolated colonies from the seven purified phages that were submitted to the above ELISA were transferred to DeepWell plates. DNA extraction and sequencing were performed according to Ferreira et al. [19].

The amino acid sequences were deduced according to the nucleotide sequences using the tool available in ExPASy Bioformatics Resources Portal (http://web.expasy.org/translate/). Peptide sequences were also analyzed with SAROTUP program to identify redundant sequences. Similarity of the peptides to proteins was performed using BLASTp (http://www.ncbi.nlm.nih.gov/BLAST) against the proteins of *S. frugiperda*.

The peptides were modeled by the de novo method using the PEPFOLD3 - RPBS software (http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3). Clusters and standard parameters of the program were used (Label: PEPFOLD; Type of simulation: Short) without structural reference. As input, it was submitted the FASTA amino acid sequences of each peptide flanked by cysteines. The program ranks the five best hits based on the free energy and melting temperature and provides the structural odds chart according to the amino acid sequence (output). For this work, it was selected only the first best result of each peptide.

Immunohistochemistry

Caterpillars close to the last instar remained fasted for 24 hours. Subsequently, they were immobilized for 15 minutes at -4°C to be dissected in saline solution (NaCl 0.215 mM). The first and last segments were removed to extraction of the gut, which were fixed in modified Zamboni solution (10% formaldehyde, 15% picric acid in 0.1 μM sodium phosphate buffer) for 24 h at 4°C [20]. Then, they were washed in 10% neutral buffered formalin (1M of formaldehyde, 33mM of sodium phosphate monobasic, 46mM of dibasic sodium phosphate) at 4°C until complete removal of the picric acid. Dehydration of slides was carried out in baths of increasing concentrations of ethanol (70%, 85%, 100%) to include in paraffin.

For immunohistochemical staining, the sections were submitted to antigenic recovery with 10mM citrate buffer pH 6 in microwaves for 15 minutes after xylol and alcohol baths

147 (100%, 85%, 70%). Endogenous peroxidase was blocked with 3% H_2O_2 (vol. 10). Non-specific
148 sites were blocked with 10% BSA in PBS 1X for 40 min. 1 x 10^{11} phages/slide diluted in buffer
149 (Novocastra IHC Diluent) were incubated for 30 minutes at 37°C and then overnight at 4°C in
150 a humid chamber. After washing with PBS, the slides were incubated for 1h at 37°C with HRP-
151 labeled anti-M13 (1: 650) (GE Healthhcare, Chicago, IL). The slides were revealed with
152 NovoLink polymer (Leica Biosystems) according to the manufacturer's recommendations, 0.5
153 mg/mL 3, 3-diaminobenzidine (DAB) (Sigma-Aldrich), and counterstaining with Harris
154 Hematoxylin (Dinamica). Histological analysis was performed using a microscope (Aperio-
155 Leica Biosystems) and the images were captured (20x).
156

157 ## *In vivo* assay

158 The bioassays were conducted adapting the droplet feeding method [21,22]. Briefly, the
159 solution for the negative control (C-) was prepared using 1 g/L of blue food powder dye
160 (Iceberg), 25 mM sucrose and PBS 1X. This solution was used for the preparation of all other
161 treatment. For the positive control (C +), was used then insecticide Agree® at 1 x 10^6 CFU/µL.
162 For the treatment with the strain of *B. thuringiensis* GF 07 from Laboratório de Bio Controle
163 Farroupilha/LALLEMAND we used 3 x 10^3 CFU/µL to visualize the increment of mortality
164 promoted by the peptides. We used 1x10^{11} phages/µL for the seven selected clones (SfF3, SfC4,
165 SfD5, SfB6, SfH6, SfF7 and SfC8). Wild-type phage were used as a control to check whether
166 the exogenous non-peptide phage is capable of killing larvae. Twenty-five newborn hatchlings
167 were used for each of the treatments in trials with five replicates using completely randomized
168 design. The larvae were isolated and stored within 2mL tubes and 0.5µL of each treatment was
169 deposited for ingestion. After five hours, the larvae were transferred to vessels with artificial
170 diet without formaldehyde and incubated at room temperature. After 3 days, the insects were

171 transferred to a new container containing artificial diet with 25% formaldehyde. Mortality
172 assessments were performed after seven days.
173

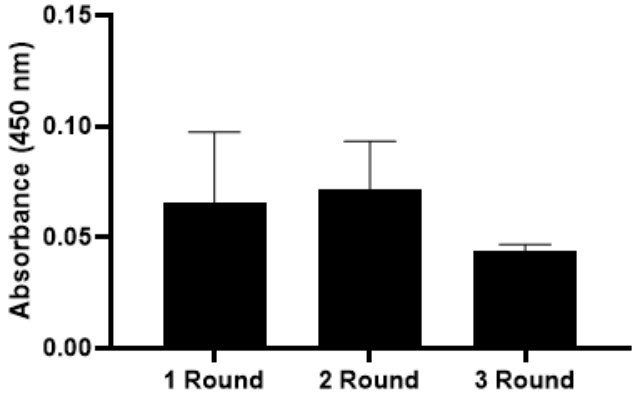
174 Statistical analyzes

175 Results were expressed by average ± standard deviation of the groups. The normality
176 analysis was performed using the D'Agostino & Pearson test. ANOVA test using Dunnett's
177 post-test for multiple comparisons was used to compare the results between groups. Statistical
178 analyses were carried out using GraphPad Prism software (version 6.0 for Windows).
179 Differences were considered significant when $p < 0.05$.
180 Comparisons for *in vivo* assay were carried out by software R using ANOVA test and
181 Scott Knot test which is a hierarchical clustering algorithm. Differences were considered
182 significant when $p < 0.01$.
183

184 Results

185 In order to identify phage displayed peptides that could bind to midgut proteins of *S.*
186 *frugiperda* a constrained 7-mer phage library was used for positive panning on immobilized
187 total proteins. The enrichment of recovered phage after each biopanning cycle was determined
188 by ELISA assay (Fig 1). Our data indicated a successful affinity selection of phage clones that
189 recognized the midgut proteins of caterpillar. However, in the last cycle, there was a decrease
190 in absorbance, characterizing a decrease in the enrichment process. Therefore, the phages
191 obtained in the second cycle were used in the subsequent analyses.
192

193 **Fig 1. Enrichment profile of the phage displayed library during the biopanning against**
194 **midgut proteins of *S. frugiperda*.** Plate was coated with 10µg of midgut proteins and ELISA
195 was conducted to evaluate the enrichment of phages in each of the three rounds. Absorbance of
196 coated wells was subtracted from uncoated wells.



197

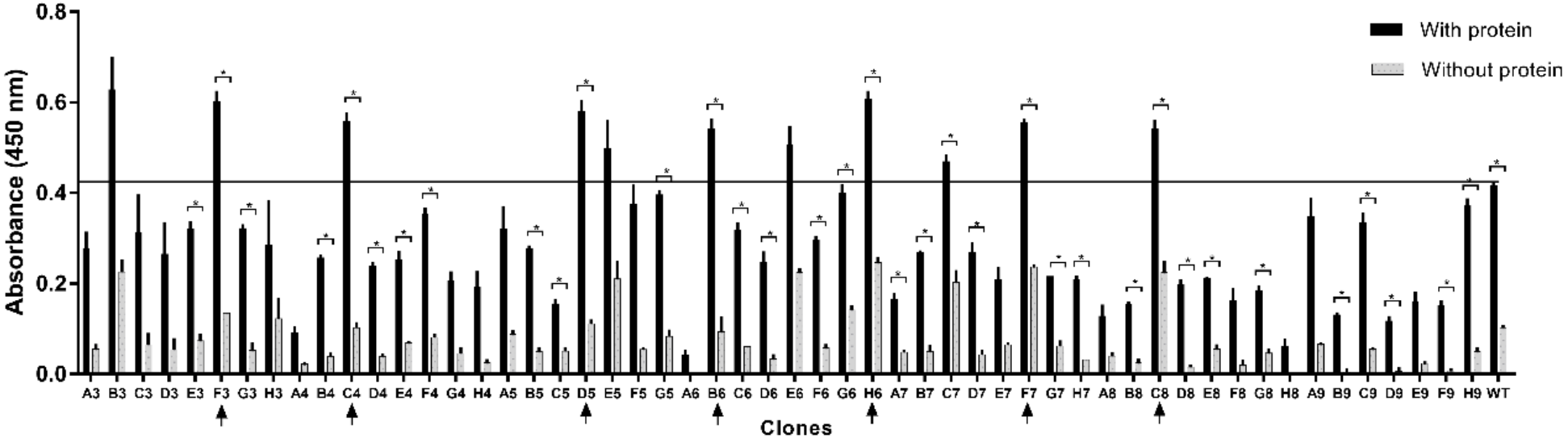
198 Fifty-four binders were randomly selected after the second cycle of biopanning. Fig 2
199 shows the reactivity of these clones against caterpillar proteins. The absorbance observed for
200 35 clones differed significantly from that obtained for uncoated wells ($p < 0.01$). Only the
201 reactivity of the seven clones SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8 differed from the
202 wild-type phage (displaying no peptide on its surface). Although SfC7 clone differed
203 significantly from uncoated wells, the absorbance was not significant different from wild-type.

204

205 **Fig 2. Phage-ELISA for the clones eluted after the second selection cycle.** The
206 threshold was based on the absorbance of the Wild-type phage (WT). Selected phages with
207 absorbance greater than WT and significant different from uncoated wells were selected. Of the
208 54 clones, 35 reacted significantly (*$p < 0.01$) to midgut proteins when compared to uncoated
209 wells. Of these, seven (SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8 – indicated by arrows)
210 showed higher absorbance than WT ($p<0.05$). Data are presented as mean ±SD D'Agostino &
211 Pearson test was accomplished to compare coated and uncoated wells. ANOVA test using

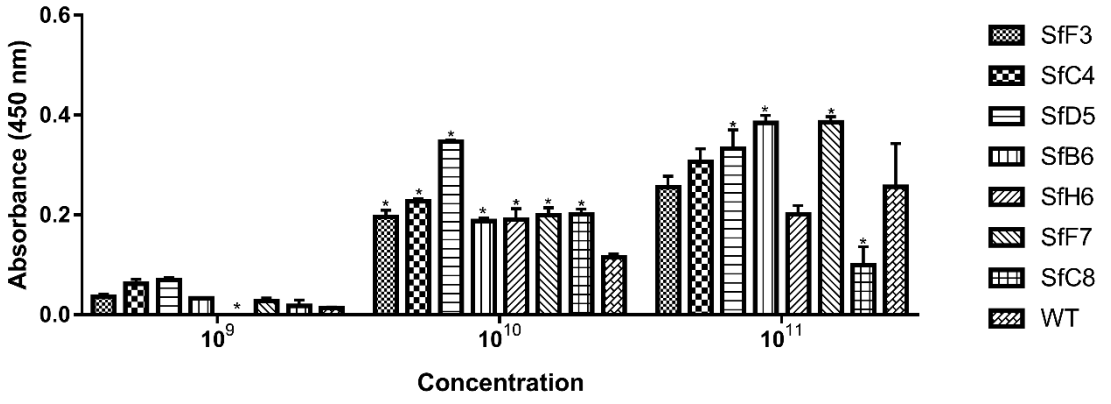
212 Dunnett's post-test for multiple comparisons was used to compare the results between the 54
213 clones and WT.

214


With protein
Without protein
Absorbance (450 nm)
0.0
0.2
0.4
0.6
0.8
A3 B3 C3 D3 E3 F3 G3 H3 A4 B4 C4 D4 E4 F4 G4 H4 A5 B5 C5 D5 E5 F5 G5 A6 B6 C6 D6 E6 F6 G6 H6 A7 B7 C7 D7 E7 F7 G7 H7 A8 B8 C8 D8 E8 F8 G8 H8 A9 B9 C9 D9 E9 F9 H9 WT
Clones

Clones highlighted in the previous assay were purified and a new format-ELISA was conducted to evaluate the differential reactivity of these promising phages to the midgut proteins of the cartridge caterpillar. Phage clones were using in different titration. As shown in Fig 3, the promising clones only when titrated at 10^{10} and 10^{11} differentially reacted to *S. frugiperda* gut proteins comparing to the wild-type. At 10^{10}, all seven phages differed from the wild-type phage. At 10^{11}, only the SfD5, SfB6 and SfF7 clones showed higher absorbance than the wild-type. Although three clones demonstrated better reactivity, the all seven were evaluated in the further experiments because the selection was performed using the midgut proteins in their non-native conformation (coated in 96-well Maxisorp microtiter plates), and immunohistochemistry and *in vivo* assays included native proteins. Moreover, the conformational library used in this work may allow the selection of molecules with different structures, that may interact with midgut proteins in their native conformation.

Fig 3. Reactivity of purified phage clones against gut proteins from *S. frugiperda*. Only seven clones SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8 were used based on previous screening assay. Three different phage titers were tested and statistical differences (through ANOVA test using Dunnett's post-test for multiple comparisons) between the selected clones and the wild-type M13 phage (WT) control were observed (*$p<0.05$).



233 The sequencing results allowed the characterization of the seven peptides. Alignment
234 analyses were performed using the peptide sequences and no consensus motifs were
235 determined. Result already expected since we use a conformational library. The potential
236 ligands to gut proteins from *S. frugiperda* were predicted through Blastp tool and Fig 4
237 represents the peptides sequences of the five best-ranked proteins. Using a conformational
238 library is allow selected peptides with different structure, thus were predicted the 3D structure
239 of each peptide predict by PEPFOLD3 – RPBS software.
240
241 **Fig 4. Exogenous peptides expressed by phage particles.** Peptides sequences of the seven
242 phage clones (SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8) and their 3D structures were
243 obtained. The potential ligands to midgut proteins of *S. frugiperda* were predicted through
244 BLASTp alignment and the five best-ranked proteins are represented. Conformational library

245 for biopanning was chosen to allow interaction between the 3D-structure of the peptides and
246 the midgut proteins.

A

Peptide SfF3 - NQSGSSV

Aligned proteins	Cover	Identity
cytochrome oxidase subunit I [Spodoptera frugiperda]	86%	88%
cytochrome oxidase subunit I [Spodoptera frugiperda]	86 %	88%
cytochrome P450 CYP321B1 [Spodoptera frugiperda]	100%	71%
truncated ABC transporter subfamily C2 [Spodoptera frugiperda]	57%	100%
fam1 R882 ABCC2 transporter [Spodoptera frugiperda]	57%	100%

B

Peptide SfC4 - SPYTRYF

Aligned proteins	Cover	Identity
PRO [Spodoptera frugiperda]	57%	100%
prophenoloxidase subunit 2 [Spodoptera frugiperda]	57%	100%
chitin synthase [Spodoptera frugiperda]	57%	75%
cytochrome P450 CYP324A6 [Spodoptera frugiperda]	28%	100%
Dicer-1-PA [Spodoptera frugiperda]	28%	100%

C

Peptide SfD5 - NNHGYWW

Aligned proteins	Cover	Identity
cytochrome P450 CYP6AN4 [Spodoptera frugiperda]	71%	80%
acid digestive lipase [Spodoptera frugiperda]	85%	44%
cytochrome c oxidase subunit 1 [Spodoptera frugiperda]	85%	50%
cytochrome c oxidase subunit I [Spodoptera frugiperda]	85%	50%
cytochrome c oxidase subunit 1 [Spodoptera frugiperda]	85%	50%

D

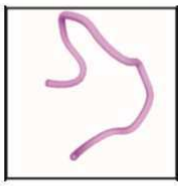
Peptide SfB6 - HPSAARF

Aligned proteins	Cover	Identity
RecName: Full=60S ribosomal protein L32	57%	100%
ribosomal protein L12 [Spodoptera frugiperda]	57%	100%
charged multivesicular body protein 2b [Spodoptera frugiperda]	71%	80%
charged multivesicular body protein 5 [Spodoptera frugiperda]	57%	100%
actin related protein 2/3 complex subunit 2 [Spodoptera frugiperda]	57%	100%

E

Peptide SfH6 - KSNISLP

Aligned proteins	Cover	Identity
RecName: Full=40S ribosomal protein S4	100%	71%
prophenoloxidase subunit 1 [Spodoptera frugiperda]	71%	80%
DSF 2 [Spodoptera frugiperda]	85%	88%
fam155 ABCC2 transporter [Spodoptera frugiperda]	57%	100%
ABC transporter subfamily C2 [Spodoptera frugiperda]	57%	100%



F

Peptide SfF7 - HWPAKHI

Aligned proteins	Cover	Identity
putative CAD trifunctional protein [Spodoptera frugiperda]	71%	80%
Dicer-1-PA [Spodoptera frugiperda]	57%	75%
Chain A, Mechanisms Of Crippling Actin-dependent Phagocytosis By Vapo	71%	60%
polyadenylate binding protein [Spodoptera frugiperda]	71%	80%
CBH1 [Spodoptera frugiperda]	71%	60%

G

Peptide SfC8 - PSAFHTG

Aligned proteins	Cover	Identity
RecName: Full=40S ribosomal protein S9	57%	100%
chymotrypsin-like serine protease precursor [Spodoptera frugiperda]	57%	100%
trypsin-like serine protease precursor [Spodoptera frugiperda]	57%	100%
scavenger receptor SR-C-like protein [Spodoptera frugiperda]	85%	38%
scavenger receptor SR-C-like protein [Spodoptera frugiperda]	85%	38%

247 Considering the protein that aligned with the selected peptides, all presented similarity
248 to *S. frugiperda*. A literature search was performed (Table 1) to identify possible molecules that
249 interact with each BLASTp-aligned protein. Only the SfF7 peptide did not present a target
250 described for the genus *Spodoptera*.

251 The results demonstrated that both, SfF3 and SfF5, presented similarity to cytochrome
252 oxidase. Interestingly, SfF3, SfC4 and SfD5 were similar to cytochrome P450, a detoxification
253 enzyme regulated by endogenous miRNAs [23]. SfF3 also aligned with ABCC2 transporter, a
254 receptor of Cry1A toxins [24], and SfC4 and SfF7 also targeted miRNAs, which are also
255 involved in resistance mechanisms [23].

256

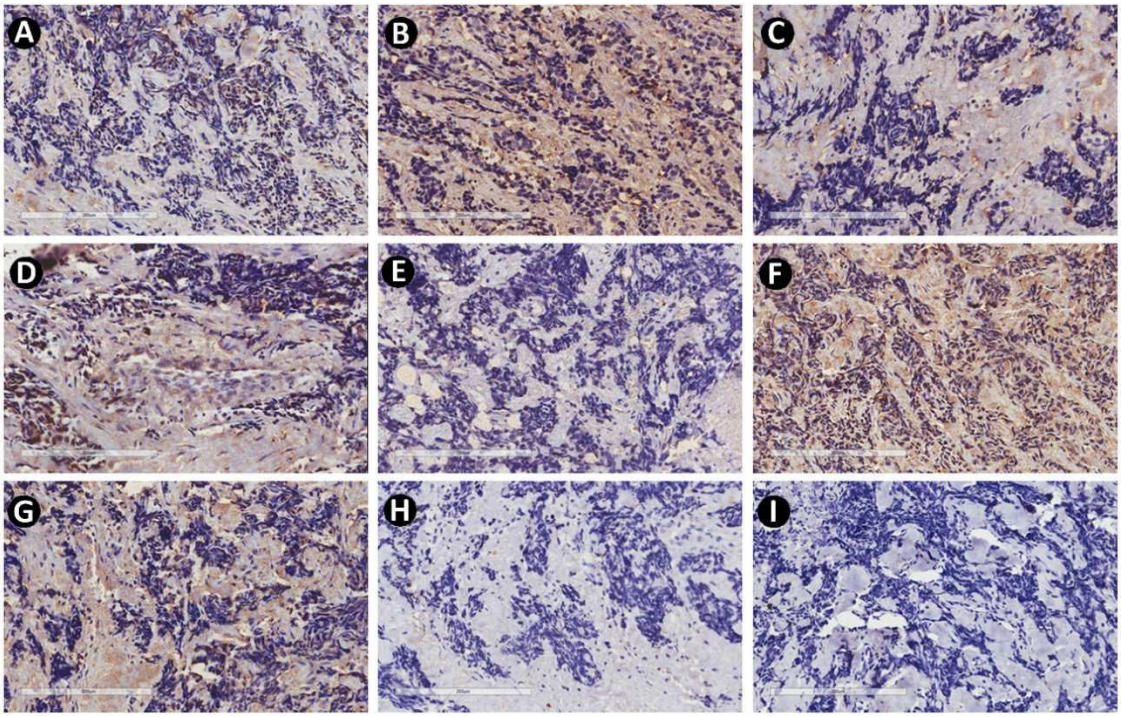
257 **Table 1. Similarities between the peptide sequences and *S. frugiperda* proteins registered**
258 **in the GenBank database.** The target in the same genus of this caterpillar was recorded, and
259 studies in different insects were reviewed.

Peptides	Potential ligands to gut proteins	Described targets	Organisms	References
SfF3	cytochrome oxidase subunit I [Spodoptera frugiperda]	Other subunits	*Omphisa fuscidentalis*	[25]
	cytochrome oxidase subunit I [Spodoptera frugiperda]			
	cytochrome P450 CYP321B1 [Spodoptera frugiperda]	Insecticides	*Spodoptera litura*	[26]
	truncated ABC transporter subfamily C2 [Spodoptera frugiperda]	Cry protein receptor	*Spodoptera frugiperda*	[27] and [28]
	fam1 R2R2 ABCC2 transporter [Spodoptera frugiperda]			
SfC4	PRO [Spodoptera frugiperda]	-	-	-
	prophenoloxidase subunit 2 [Spodoptera frugiperda]	Phenolic substances	*Bombyx mori*	[29]
	chitin synthase [Spodoptera frugiperda]	Sugar	Insect	[30]
	cytochrome P450 CY321A8 [Spodoptera frugiperda]	Insecticides	*Spodoptera litura*	[26]
	Dicer-1-PA [Spodoptera frugiperda]	miRNAs	*Drosophila*	[31]
SfD5	cytochrome P450 CYP6AN4 [Spodoptera frugiperda]	Insecticides	*Spodoptera litura*	[26]
	acid digestive lipase [Spodoptera frugiperda]	Lipids	*Drosophila melanogaster*	[32]
	cytochrome c oxidase subunit 1 [Spodoptera frugiperda]	Oxygen		
	cytochrome c oxidase subunit I [Spodoptera frugiperda]	Oxygen	*Omphisa fuscidentalis*	[25]
	cytochrome c oxidase subunit 1 [Spodoptera frugiperda]	Oxygen		
SfB6	RecName: Full=60S ribosomal protein L32	rRNA / Polypeptides	*Spodoptera frugiperda*	[33]
	ribosomal protein L12 [Spodoptera frugiperda]	rRNA / Polypeptides		
	charged multivesicular body protein 2b [Spodoptera frugiperda]	Polymers	*Homo sapiens*	[34]
	charged multivesicular body protein 5 [Spodoptera frugiperda]	Polymers		
	actin related protein 2/3 complex subunit 2 [Spodoptera frugiperda]	Actin binding proteins	*Drosophila*	[35]
SfH6	RecName: Full=40S ribosomal protein S4	rRNA / Polypeptides	*Spodoptera frugiperda*	[33]
	prophenoloxidase subunit 1 [Spodoptera frugiperda]	Phenolic substances	*Bombyx mori*	[29]
	ORF 7 [Spodoptera frugiperda]	-	-	-
	fam1 SS ABCC2 transporter [Spodoptera frugiperda]	Cry protein receptor	*Spodoptera frugiperda*	[27,28]
	ABC transporter subfamily C2 [Spodoptera frugiperda]			
SfF7	putative CAD trifunctional protein [Spodoptera frugiperda]	Nucleotides	*Drosophila melanogaster*	[36]
	Dicer-1-PA [Spodoptera frugiperda]	miRNAs	*Drosophila*	[31]
	Chain A, Mechanisms of Crippling Actin-dependent Phagocytosis By Yopo	-	-	-
	polyadenylate binding protein [Spodoptera frugiperda]	Nucleotides	*Drosophila*	[37]
	CRM1 [Spodoptera frugiperda]	rRNA / Protein	Mammal	[38]
SfC8	RecName: Full=40S ribosomal protein S8	rRNA / Polypeptides	*Spodoptera frugiperda*	[33]
	chymotrypsin-like serine protease precursor [Spodoptera frugiperda]	Proteins	*Anticarsia gemmatalis*	[39]
	trypsin-like serine protease precursor [Spodoptera frugiperda]			
	scavenger receptor SR-C-like protein [Spodoptera frugiperda]	Vip protein	*Spodoptera frugiperda*	[40]
	scavenger receptor SR-C-like protein [Spodoptera frugiperda]			

The targets are described in agreements with published data.

In order to map the binding of the phages in the midgut tissue of the caterpillar, an immunohistochemistry analysis was performed (Fig 5). The reactivity of the seven clones was observed preferentially in the cytoplasm (Fig 5A-G). As negative control, non-recombinant wild-type phage (Fig 5H) were used, showing poor marking to the gut of the caterpillar. Control of the reaction, in which the virus was suppressed, did not show immunostaining (Fig 5I).

Fig 5. Immunohistochemical analysis of the midgut tissue of *S. frugiperda* (20X) demonstrating the labeling of the selected peptides. A: SfF3; B: SfC4; C: SfD5; D: SfB6; E: SfH6; F: SfF7; G: C8; H: wild-phage and I: negative control without phage-clones. Counterstaining with Harris-Hematoxylin.

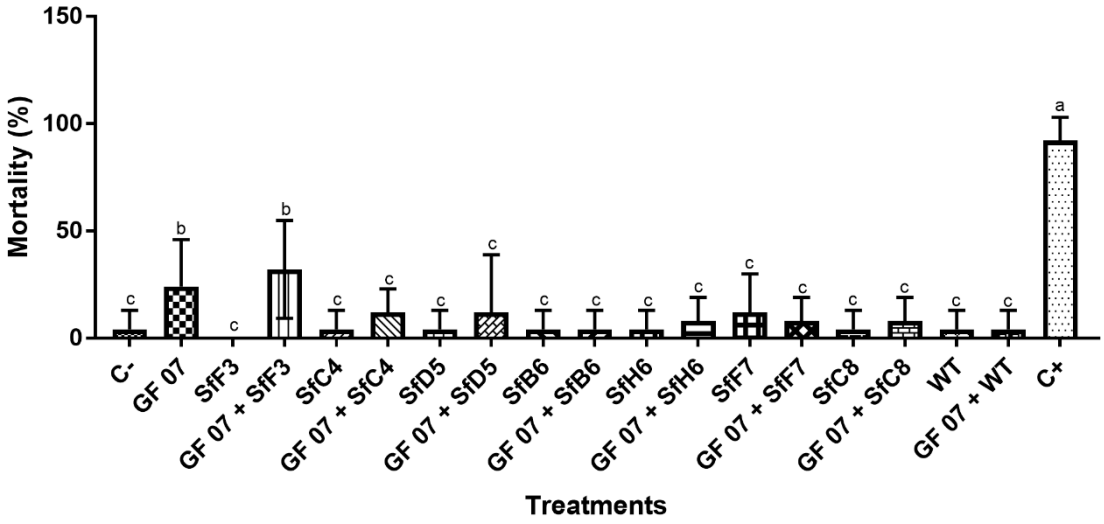


Finally, we carried out the *in vivo* assay to verify the mortality of the newborn fall armyworm fed with the selected seven phages (SfF3, SfC4, SfD5, SfB6, SfH6, SfF7 and SfC8) and the wild type. We also performed the combination of the strain GF 07 with the peptides aiming to observe a synergistic action between both. The results were compared

277 to the negative control (Fig 6). This consisted of solution without phage or Bt toxins,
278 demonstrating, as expected, low mortality (4%). We used, as a positive control, the
279 Agree® which contains *B. thuringiensis* aizawai transconjugated with *B. thuringiensis*
280 kurstaki. This product has already been tested and was efficient in controlling *S.*
281 *frugiperda* [41,42], *Plutella xylostella* [43], *Bonagota salubricola* [44] and *Duponchelia*
282 *fovealis* [45]. In our experiments the mortality of Agree® was 92%.

283 In this work, we also used *strain* of *B. thuringiensis* GF 07 combined with selected
284 peptides to increase the application spectrum of biopesticides. This combination is
285 interesting due to resistance reports from different pests to bacteria *B. thuringiensis* [46].
286 In addition, studies indicated that Bt toxins are toxic to caterpillars from 1st to 4th instar,
287 failing to act in the late stages, when damages are more severe [47,48].

Fig 6. *In vivo* assay using the droplet feeding methodology in *S. frugiperda* larvae. The fall armyworms were fed by the phages SfF3, SfC4, SfD5, SfB6, SfH6, SfF7, SfC8 and wild-type (WT). We also combined these phages with the *B. thuringiensis* strain GF 07 to observe the synergistic effect. As a positive control (C+) we used the bioinsecticide Agree®. All treatments were compared with the negative control (C-) using Scott-Knott test and statistical difference was considered when $p < 0.01$. Treatments followed by the same letter are not statistically different at the 0.01 level as determined by Scott-Knott test.



The analysis of variance using the Scott-Knott's test allowed treatments grouping and consists of partitioning the original set of treatments. This partitioning aims at a maximum differentiation between groups and is interrupted when the groups obtained are no longer significantly different [49]. This process is quite interesting when the number of treatments is large, such as the assay performed in this work.

The treatment with GF 07, GF 07+SfF3 and the positive control were statistically different from de negative control. The treatments lead to a low mortality, except for the positive control (92%). Strain GF 07 resulted in a significantly higher mortality (24%)

306 comparing to the negative control. Although no phage was capable of killing *S. frugiperda*
307 larvae alone, when associated to GF 07, the GF 07+SfF3 complex caused significantly
308 mortality (32%) compared to the negative control. Thus, by combining this peptide and
309 the GF 07 we achieved an 8% increase in mortality of *S. frugiperda* to the strain GF 07.
310

311 Discussion

312 This is the first study that aimed to use the PhD in searching a new strategy for
313 fall armyworm control. This pest control is economic interest and necessary, since the
314 transgenic plants no longer proves to be effective. For this purpose, the midgut proteins
315 of *S. frugiperda* were extracted for the selection of peptides fused to bacteriophages
316 capsids. The use of peptide libraries expressed on the surface of filamentous phage is a
317 robust *in vitro* selection tool and has been very successful in identifying ligands in
318 different systems [50]. Our strategy, therefore, is innovative when exploring a
319 biotechnological tool that allows selecting molecules with insecticidal activity.
320 During the biopanning process, after three cycles, there was a reduction in
321 enrichment. In fact, phages may evolve during the process due to their higher infectivity,
322 in detriment of their specificity. The biological bias during this selection functions as an
323 important driving force for the isolation of irrelevant peptides, leading some to be
324 enriched or removed during biopanning. Consequently, these peptides show a higher than
325 expected frequency within the library [51]. By performing fewer rounds of selection, the
326 bias can be reduced by decreasing the loss of relevant sequences [52]. Therefore, the
327 second round was selected for the isolation of phage clones, which were subsequently
328 selective against midgut proteins.
329 Based on the objectives of this work, the ELISA test was crucial for the validation
330 of the selection process, as well as for the identification of promising clones. This

technique demonstrated the affinity to the target and not only the numbers of viral particles. Ngubane et al. [53] used the same validation technique and achieved a successful enrichment, allowing the identification of specific peptides for mycobacteria. This immunoassay was also previously performed to demonstrate the reactive phage particles to the desired target by [54,55]. Considering our results, the process was successful, since we selected seven clones reactive to the target, compared to the wild-type, a filamentous virus that does not express external peptide fused to protein III. At the end of the selection process, using purified phage, we obtained seven promising clones capable of specifically recognizing midgut proteins of *S. frugiperda*. This reactivity was further confirmed by the immunohistochemistry assay, in which the phage clones recognized proteins in the cytoplasm of intestinal cells.

Throughout the bioinformatics analysis, no protein structural motif was identified during the alignment of the sequences of the seven clones. This result was expected because we used a conformational library for biopanning process. This library is particularly interesting because allows the selection of peptides with different conformations, which can interact with different midgut proteins.

In the *in vivo* assays, we used the combination of phages with the *B. thuringiensis*, a gram-positive bacterium that during sporulation produces lethal endotoxins for different larvae species. These toxins are synthesized in the form of inactive pro-toxins that are solubilized and proteolytically activated in the insect gut. After binding to its receptor, the toxin leads to swelling of the midgut cells and eventual lysis [7,8]. Therefore, the combination of the clone with these bacteria can promote a synergism leading to improved mortality.

With exception of SfF3 clone, no phages were able to promote *S. frugiperda* larvae death, even in combination with *B. thuringiensis* GF 07. Even not toxic, they may

be used as delivery vehicles in a new method design based on biotechnology approaches, once they are ligands of intestinal proteins. Besides, these six peptides may still contribute to fall armyworm control in more advanced instars. Some studies suggest that products based on Bt technology only act on larvae of up to 4th instar [47,48], making them advantageous the be tested as insecticides that act in the other phases

The SfF3 clone, although in isolation has not caused the death of *S. frugiperda* larvae, increased the mortality to the strain GF 07. We predicted similarity of the expressed peptide to the truncated ABC transporter subfamily C2 (ABCC2) by performing alignment through BLASTp tool. Recent studies have shown that mutations in ABCC2 protein confer resistance against Bt toxins in *Bombyx mori* [56], *Helicoverpa armigera* [57], *Spodoptera exigua* [58], *Plutella xylostella* [59] and *S. frugiperda* [27,60]. Researchers suggest that the proteins of the Cry1 family interact with ABCC2 to initiate the formation of pores in the intestinal membrane of the insect and when different mutations render the ABCC2 the protein becomes nonfunctional, leading to resistance [61,62]. ABCC2 sequences possess an aberrant splicing and its function is still unknown [28]. We suggest in this work that the peptide expressed by the SfF3 clone can interact with the Bt toxin and this small sequence of amino acids can reverse the resistant phenotypes, since this peptide showed interaction with the Cry protein receptor. However, experiments with different individuals are needed to test this notion.

Sf3F was also similar to cytochrome P450, which is an enzyme involved in the detoxification of chemical insecticides [63,64] and on pest resistance [65,66]. Recent studies have demonstrated that Bt toxin is capable of inducing the expression of cytochrome P450 in *Manduca sexta* larvae [67]. Furthermore, this enzyme is involved in the resistance of *Spodoptera exigua* to beta-cypermethrin [68]. Interestingly, some

researchers have also reported that microRNAs (miRNAs) can regulate the expression of this enzyme, suggesting new pathways associated to insects' resistance [69-71].

When exposing larvae of *Plutella xylostella* to the insecticide chlorantraniliprole, Etebari et al. [72] found that miR-2b-3p was differentially expressed. This miRNA was able to regulate the transcriptional levels of cytochrome P450, thus playing a role in metabolic resistance. Consequently, the synergistic effect observed when combining SfF3 and strain GF 07 might have occurred due to the modulation of cytochrome P450 through miRNA induction, which need be further evaluated

In summary, this is the first work that used the PhD technology to select clones for *S. frugiperda* control. Seven clones were successful selected and bound to intestinal proteins. Among then, the SfF3 when used in combination with the *B. thuringiensis* toxin, increased the mortality of *S. frugiperda* neonates. However, new trials with fall armyworm in the last instars should be performed, and resistant individuals should be included to evaluate the role of these peptides in reversing pesticide resistance. This study also validated the reliability of PhD technology as an agribiotechnological approaches for pest control.

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References

1. Sparks AN (1979) A review of the biology of the fall armyworm. Florida Entomologist: 82-87. https://www.jstor.org/stable/3494083
2. Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., … Witt, A. (2017). Fall armyworm: impacts and implications for Africa. Evidence Note (2), September 2017. Wallingford, UK: CABI. https://doi.org/10.1564/v28_oct_02
3. Bavaresco A, Garcia MS, Grützmacher AD, Foresti J, Ringenberg R (2002) Biology and thermal requirements of Spodoptera cosmioides (Walk.)(Lepidoptera: Noctuidae). Neotropical Entomology 31: 49-54. http://dx.doi.org/10.1590/S1519-566X2002000100007
4. Barros EM, Torres JB, Bueno AF (2010) Oviposition, development, and reproduction of Spodoptera frugiperda (JE Smith)(Lepidoptera: Noctuidae) fed on different hosts of economic importance. Neotropical entomology 39: 996-1001. http://dx.doi.org/10.1590/S1519-566X2010000600023
5. Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, et al. (2014) Field-evolved resistance to Cry1F maize by Spodoptera frugiperda (Lepidoptera: Noctuidae) in Brazil. Crop protection 64: 150-158. https://doi.org/10.1016/j.cropro.2014.06.019
6. Storer NP, Kubiszak ME, Ed King J, Thompson GD, Santos AC (2012) Status of resistance to Bt maize in Spodoptera frugiperda: Lessons from Puerto Rico. Journal of Invertebrate Pathology 110: 294-300. https://doi.org/10.1016/j.jip.2012.04.007
7. VAN RIE J, JANSENS S, HÖFTE H, DEGHEELE D, VAN MELLAERT H (1989) Specificity of Bacillus thuringiensisδ- endotoxins: Importance of specific receptors on the brush border membrane of the mid- gut of target insects. European Journal of Biochemistry 186: 239-247. https://doi.org/10.1111/j.1432-1033.1989.tb15201.x
8. Sacchi VF, Parenti P, Hanozet GM, Giordana B, Lüthy P, et al. (1986) Bacillus thuringiensis toxin inhibits K+- gradient- dependent amino acid transport across the brush border membrane of Pieris brassicae midgut cells. Febs Letters 204: 213-218. https://doi.org/10.1016/0014-5793(86)80814-6
9. Bernardi O, Bernardi D, Ribeiro RS, Okuma DM, Salmeron E, et al. (2015) Frequency of resistance to Vip3Aa20 toxin from Bacillus thuringiensis in Spodoptera frugiperda (Lepidoptera: Noctuidae) populations in Brazil. Crop Protection 76: 7-14. https://doi.org/10.1016/j.cropro.2015.06.006
10. Farias JR, Horikoshi RJ, Santos AC, Omoto C (2014) Geographical and temporal variability in susceptibility to Cry1F toxin from Bacillus thuringiensis in Spodoptera frugiperda (Lepidoptera: Noctuidae) populations in Brazil. Journal of economic entomology 107: 2182-2189. https://doi.org/10.1603/EC14190
11. Wu C-H, Liu IJ, Lu R-M, Wu H-C (2016) Advancement and applications of peptide phage display technology in biomedical science. Journal of Biomedical Science 23: 8. https://doi.org/10.1186/s12929-016-0223-x
12. Ladner RC, Sato AK, Gorzelany J, de Souza M (2004) Phage display-derived peptides as therapeutic alternatives to antibodies. Drug discovery today 9: 525-529. https://doi.org/10.1016/S1359-6446(04)03104-6

13. Yu M, Li X, Liang R, Yang J, Zhang Y, et al. (2018) A new ligand of CD105 screened out by phage display technology provides a reliable identification of recurrent or metastasizing pleomorphic adenoma from pleomorphic adenoma. International immunopharmacology 65: 37-43. https://doi.org/10.1016/j.intimp.2018.09.042
14. Yun S, Lee S, Park JP, Choo J, Lee EK (2019) Modification of phage display technique for improved screening of high-affinity binding peptides. Journal of Biotechnology 289: 88-92. https://doi.org/10.1016/j.jbiotec.2018.11.020
15. Koiwa H, Shade RE, Zhu-Salzman K, Subramanian L, Murdock LL, et al. (1998) Phage display selection can differentiate insecticidal activity of soybean cystatins. The Plant Journal 14: 371-379. https://doi.org/10.1046/j.1365-313X.1998.00119.x
16. Ceci LR, Volpicella M, Rahbé Y, Gallerani R, Beekwilder J, et al. (2003) Selection by phage display of a variant mustard trypsin inhibitor toxic against aphids. The Plant Journal 33: 557-566. https://doi.org/10.1046/j.1365-313X.2003.01645.x
17. Domínguez-Flores T, Romero-Bosquet MD, Gantiva-Díaz DM, Luque-Navas MJ, Berry C, et al. (2017) Using phage display technology to obtain Crybodies active against non-target insects. Scientific Reports 7: 14922. https://doi.org/10.1038/s41598-017-09384-x
18. Costa LE, Goulart LR, de Jesus Pereira NC, Lima MIS, Duarte MC, et al. (2014) Mimotope-based vaccines of Leishmania infantum antigens and their protective efficacy against visceral leishmaniasis. PLoS One 9: e110014. https://doi.org/10.1371/journal.pone.0110014
19. Ferreira BC, Lima-Ribeiro AMC, Coimbra NC, Goulart LR, Cardoso R, et al. (2012) Seleção de peptídeos específicos para anticorpos anti Leptospira interrogans.
20. Peres KC, Trinca V, Oliveira FP, Oliveira LJ, Bressan FF, et al. (2015) Caracterization of caveolin-1 and-2 proteins in cloned and transgenic placenta of cattle. Pesquisa Veterinária Brasileira 35: 477-485. http://dx.doi.org/10.1590/S0100-736X-2015000500016
21. Frankenhuyzen KV, Gringorten L, Dedes J, Gauthier D (1997) Susceptibility of different instars of the spruce budworm (Lepidoptera: Tortricidae) to Bacillus thuringiensis var. kurstaki estimated with a droplet-feeding method. Journal of Economic Entomology 90: 560-565. https://doi.org/10.1093/jee/90.2.560
22. Hughes P, Van Beek N, Wood H (1986) A modified droplet feeding method for rapid assay of Bacillus thuringiensis and baculoviruses in noctuid larvae. Journal of Invertebrate Pathology 48: 187-192. https://doi.org/10.1016/0022-2011(86)90122-9
23. Li C, Wong A, Wang S, Jia Q, Chuang W-P, et al. (2018) miRNA-Mediated Interactions in and between Plants and Insects. International journal of molecular sciences 19: 3239. https://doi.org/10.3390/ijms19103239
24. Ren X-L, Jiang W-L, Ma Y-J, Hu H-Y, Ma X-Y, et al. (2016) The Spodoptera exigua (Lepidoptera: Noctuidae) ABCC2 Mediates Cry1Ac Cytotoxicity and, in Conjunction with Cadherin, Contributes to Enhance Cry1Ca Toxicity in Sf9 Cells. Journal of economic entomology 109: 2281-2289. https://doi.org/10.1093/jee/tow193

25. Singtripop T, Saeangsakda M, Tatun N, Kaneko Y, Sakurai S (2007) Correlation of oxygen consumption, cytochrome c oxidase, and cytochrome c oxidase subunit I gene expression in the termination of larval diapause in the bamboo borer, Omphisa fuscidentalis. Journal of insect physiology 53: 933-939. https://doi.org/10.1016/j.jinsphys.2007.03.005
26. Wang RL, Zhu- Salzman K, Baerson SR, Xin XW, Li J, et al. (2017) Identification of a novel cytochrome P450 CYP321B1 gene from tobacco cutworm (Spodoptera litura) and RNA interference to evaluate its role in commonly used insecticides. Insect science 24: 235-247. https://doi.org/10.1111/1744-7917.12315
27. Tanaka S, Miyamoto K, Noda H, Jurat- Fuentes JL, Yoshizawa Y, et al. (2013) The ATP- binding cassette transporter subfamily C member 2 in B ombyx mori larvae is a functional receptor for C ry toxins from B acillus thuringiensis. The FEBS journal 280: 1782-1794. https://doi.org/10.1111/febs.12200
28. Flagel L, Lee YW, Wanjugi H, Swarup S, Brown A, et al. (2018) Mutational disruption of the ABCC2 gene in fall armyworm, Spodoptera frugiperda, confers resistance to the Cry1Fa and Cry1A. 105 insecticidal proteins. Scientific reports 8: 7255. https://doi.org/10.1038/s41598-018-25491-9
29. Asano T, Ashida M (2001) Cuticular pro-phenoloxidase of the silkworm, Bombyx mori purification and demonstration of its transport from hemolymph. Journal of Biological Chemistry 276: 11100-11112. http://www.jbc.org/content/276/14/11100.full
30. Merzendorfer H (2006) Insect chitin synthases: a review. Journal of Comparative Physiology B 176: 1-15. https://doi.org/10.1007/s00360-005-0005-3
31. Lee YS, Nakahara K, Pham JW, Kim K, He Z, et al. (2004) Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. cell 117: 69-81. https://doi.org/10.1016/S0092-8674(04)00261-2
32. Pistillo D, Manzi A, Tino A, Boyl PP, Graziani F, et al. (1998) The Drosophila melanogaster lipase homologs: a gene family with tissue and developmental specific expression1. Journal of molecular biology 276: 877-885. https://doi.org/10.1006/jmbi.1997.1536
33. Landais I, Ogliastro M, Mita K, Nohata J, López-Ferber M, et al. (2003) Annotation pattern of ESTs from Spodoptera frugiperda Sf 9 cells and analysis of the ribosomal protein genes reveal insect-specific features and unexpectedly low codon usage bias. Bioinformatics 19: 2343-2350. https://doi.org/10.1093/bioinformatics/btg324
34. Bodon G, Chassefeyre R, Pernet-Gallay K, Martinelli N, Effantin G, et al. (2011) Charged multivesicular body protein-2B (CHMP2B) of the endosomal sorting complex required for transport-III (ESCRT-III) polymerizes into helical structures deforming the plasma membrane. Journal of Biological Chemistry: jbc. M111. 283671. https://doi.org/10.1074/jbc.M111.283671
35. Hudson AM, Cooley L (2002) A subset of dynamic actin rearrangements in Drosophila requires the Arp2/3 complex. The Journal of cell biology 156: 677-687. https://doi.org/10.1083/jcb.200109065
36. Moulton JK, Wiegmann BM (2004) Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta). Molecular phylogenetics and evolution 31: 363-378. https://doi.org/10.1016/S1055-7903(03)00284-7

548 37. Blagden SP, Gatt MK, Archambault V, Lada K, Ichihara K, et al. (2009)
549 Drosophila Larp associates with poly (A)-binding protein and is required
550 for male fertility and syncytial embryo development. Developmental
551 biology 334: 186-197. https://doi.org/10.1016/j.ydbio.2009.07.016
552 38. Nguyen KT, Holloway MP, Altura RA (2012) The CRM1 nuclear export protein
553 in normal development and disease. International journal of biochemistry
554 and molecular biology 3: 137.
555 39. Xavier LP, Oliveira MA, Guedes RNC, Santos AV, De Simone SG (2005)
556 Trypsin-like activity of membrane-bound midgut proteases from
557 Anticarsia gemmatalis (Lepidoptera: Noctuidae). European Journal of
558 Entomology 102: 147. https://doi.org/10.14411/eje.2005.023
559 40. Jiang K, Hou X-y, Tan T-t, Cao Z-l, Mei S-q, et al. (2018) Scavenger receptor-C
560 acts as a receptor for Bacillus thuringiensis vegetative insecticidal protein
561 Vip3Aa and mediates the internalization of Vip3Aa via endocytosis. PLoS
562 pathogens 14: e1007347. https://doi.org/10.1371/journal.ppat.1007347
563 41. da Silva CA, Cruz I, Redoan ACM, da SILVA R, AMANCIO M, et al. Eficiência
564 de produtos registrados para a cultura do milho no controle de Spodoptera
565 frugiperda; 2014. In: CONGRESSO NACIONAL DE MILHO E SORGO,
566 30.; SIMPÓSIO SOBRE LEPDÓPTEROS ….
567 https://www.alice.cnptia.embrapa.br/bitstream/doc/992718/1/Eficienciapro
568 dutos.pdf
569 42. SOUZA C, Mendes SM, MARTINS LdO, Guimarães AF, RIBEIRO PdA, et al.
570 Compatibilidade de milho Bt e bioinseticidas à base de Bacillus
571 thuringiensis para o manejo da lagarta-do-cartucho do milho; 2016. In:
572 CONGRESSO NACIONAL DE MILHO E SORGO, 31., 2016, Bento
573 Gonçalves. Milho e ….
574 https://www.alice.cnptia.embrapa.br/bitstream/doc/1054385/1/Compatibili
575 dademilho.pdf
576 43. Moraes CPd, Foerster LA (2012) Toxicity and residual control of Plutella
577 xylostella L.(Lepidoptera: Plutellidae) with Bacillus thuringiensis Berliner
578 and insecticides. Ciência Rural 42: 1335-1340.
579 http://dx.doi.org/10.1590/S0103-84782012000800001
580 44. do Amaral RO, dos Santos RSS, Lucia R (2013) Sucetibilidade de Bonagota
581 salubricola (Meyrick)(Lepidoptera: Tortricidae) a Bacillus thuringiensis
582 var. aizawai+ kurstaki.
583 http://www.conhecer.org.br/enciclop/2013a/agrarias/suscetibilidade%20de
584 %20bonagota.pdf
585 45. Benicá PCNT, Ataide JO, Fragoso DFM, Pratissoli D (2017) CONTROLE DA
586 LAGARTA DO MORANGUEIRO PELO BIOINSETICIDA Bacillus
587 thuringiensis. SEAGRO: ANAIS DA SEMANA ACADÊMICA DO
588 CURSO DE AGRONOMIA DO CCAE/UFES 1.
589 http://www.publicacoes.ufes.br/SEAGRO/article/view/17450/12030
590 46. Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to Bt crops:
591 lessons from the first billion acres. Nature biotechnology 31: 510.
592 https://doi.org/10.1038/nbt.2597
593 47. Halcomb JL, Benedict JH, Cook B, Ring DR (1996) Survival and Growth of
594 Bollworm and Tobacco Budworm on Nontransgenic and Transgenic
595 Cotton Expressing a CryIA Insecticidal Protein (Lepidoptera: Noctuidae).
596 Environmental Entomology 25: 250-255.
597 https://doi.org/10.1093/ee/25.2.250

48. Nayar J, Knight J, Ali A, Carlson DB, O'Bryan PD (1999) Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of Bacillus thuringiensis serovar. israelensis against two Florida mosquito species. Journal of the American Mosquito Control Association 15: 32-42. http://europepmc.org/abstract/MED/10342266
49. Bhering LL, Cruz CD, Vasconcelos Ed, Ferreira A, Resende Junior Md (2008) Alternative methodology for Scott-Knott test. Embrapa Agroenergia-Artigo em periódico indexado (ALICE). http://www.sbmp.org.br/cbab/siscbab/uploads/bd6b9df0-1143-c8dc.pdf
50. Vaz ER, Fujimura PT, Araujo GR, da Silva CAT, Silva RL, et al. (2015) A Short Peptide That Mimics the Binding Domain of TGF-β1 Presents Potent Anti-Inflammatory Activity. PLOS ONE 10: e0136116. https://doi.org/10.1371/journal.pone.0136116
51. Zade HM, Keshavarz R, Shekarabi HSZ, Bakhshinejad B (2017) Biased selection of propagation-related TUPs from phage display peptide libraries. Amino Acids 49: 1293-1308. https://doi.org/10.1007/s00726-017-2452-z
52. Christiansen A, Kringelum JV, Hansen CS, Bøgh KL, Sullivan E, et al. (2015) High-throughput sequencing enhanced phage display enables the identification of patient-specific epitope motifs in serum. Scientific reports 5: 12913. https://doi.org/10.1038/srep12913
53. Ngubane NA, Gresh L, Ioerger TR, Sacchettini JC, Zhang YJ, et al. (2013) High-throughput sequencing enhanced phage display identifies peptides that bind mycobacteria. PloS one 8: e77844. https://doi.org/10.1371/journal.pone.0077844
54. Hou P, Zhao G, He C, Wang H, He H (2018) Biopanning of polypeptides binding to bovine ephemeral fever virus G 1 protein from phage display peptide library. BMC veterinary research 14: 3. https://doi.org/10.1186/s12917-017-1315-x
55. Wang J, Song J, Zhou S, Fu Y, Bailey J, et al. (2018) Screening and identification of RhD antigen mimic epitopes from a phage display random peptide library for the serodiagnosis of haemolytic disease of the foetus and newborn. Blood transfusion= Trasfusione del sangue: 1-7. https://doi.org/10.2450/2018.0176-17
56. Atsumi S, Miyamoto K, Yamamoto K, Narukawa J, Kawai S, et al. (2012) Single amino acid mutation in an ATP-binding cassette transporter gene causes resistance to Bt toxin Cry1Ab in the silkworm, Bombyx mori. https://doi.org/10.1073/pnas.1120698109 Proceedings of the National Academy of Sciences 109: E1591-E1598. https://doi.org/10.1073/pnas.1120698109
57. Xiao Y, Zhang T, Liu C, Heckel DG, Li X, et al. (2014) Mis-splicing of the ABCC2 gene linked with Bt toxin resistance in Helicoverpa armigera. Scientific Reports 4: 6184. https://doi.org/10.1038/srep06184
58. Park Y, González-Martínez RM, Navarro-Cerrillo G, Chakroun M, Kim Y, et al. (2014) ABCC transporters mediate insect resistance to multiple Bt toxins revealed by bulk segregant analysis. BMC Biology 12: 46. https://doi.org/10.1186/1741-7007-12-46
59. Baxter SW, Badenes-Pérez FR, Morrison A, Vogel H, Crickmore N, et al. (2011) Parallel Evolution of Bacillus thuringiensis Toxin Resistance in Lepidoptera. Genetics 189: 675-679. https://doi.org/10.1534/genetics.111.130971

60. Flagel L, Lee YW, Wanjugi H, Swarup S, Brown A, et al. (2018) Mutational disruption of the ABCC2 gene in fall armyworm, Spodoptera frugiperda, confers resistance to the Cry1Fa and Cry1A.105 insecticidal proteins. Scientific Reports 8: 7255. https://doi.org/10.1038/s41598-018-25491-9
61. Heckel DG (2012) Learning the ABCs of Bt: ABC transporters and insect resistance to Bacillus thuringiensis provide clues to a crucial step in toxin mode of action. Pesticide Biochemistry and Physiology 104: 103-110. https://doi.org/10.1016/j.pestbp.2012.05.007
62. Bravo A, Gill SS, Soberón M (2007) Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. Toxicon 49: 423-435. https://doi.org/10.1016/j.toxicon.2006.11.022
63. Li C, Wong AYP, Wang S, Jia Q, Chuang W-P, et al. (2018) miRNA-Mediated Interactions in and between Plants and Insects. International Journal of Molecular Sciences 19: 3239. https://doi.org/10.3390/ijms19103239
64. Feyereisen R (2012) 8 - Insect CYP Genes and P450 Enzymes. In: Gilbert LI, editor. Insect Molecular Biology and Biochemistry. San Diego: Academic Press. pp. 236-316. https://doi.org/10.1016/B978-0-12-384747-8.10008-X
65. Feyereisen R (2006) Evolution of insect P450. Biochemical Society Transactions 34: 1252-1255. DOI: 10.1042/BST0341252. http://www.biochemsoctrans.org/content/34/6/1252
66. Bergé J, Feyereisen R, Amichot M (1998) Cytochrome P450 monooxygenases and insecticide resistance in insects. Philosophical Transactions of the Royal Society of London B: Biological Sciences 353: 1701-1705. https://doi.org/10.1098/rstb.1998.0321
67. Van Munster M, Préfontaine G, Meunier L, Elias M, Mazza A, et al. (2007) Altered gene expression in Choristoneura fumiferana and Manduca sexta in response to sublethal intoxication by Bacillus thuringiensis Cry1Ab toxin. Insect Molecular Biology 16: 25-35. https://doi.org/10.1111/j.1365-2583.2006.00692.x
68. Wang X, Xiang X, Yu H, Liu S, Yin Y, et al. (2018) Monitoring and biochemical characterization of beta-cypermethrin resistance in Spodoptera exigua (Lepidoptera: Noctuidae) in Sichuan Province, China. Pesticide Biochemistry and Physiology 146: 71-79. https://doi.org/10.1016/j.pestbp.2018.02.008
69. Ma K-S, Li F, Liu Y, Liang P-Z, Chen X-W, et al. (2017) Identification of microRNAs and their response to the stress of plant allelochemicals in Aphis gossypii (Hemiptera: Aphididae). BMC Molecular Biology 18: 5. https://doi.org/10.1186/s12867-017-0080-5
70. Peng T, Pan Y, Gao X, Xi J, Zhang L, et al. (2016) Reduced abundance of the CYP6CY3-targeting let-7 and miR-100 miRNAs accounts for host adaptation of Myzus persicae nicotianae. Insect Biochemistry and Molecular Biology 75: 89-97. https://doi.org/10.1016/j.ibmb.2016.06.002
71. Tian M, Liu B, Hu H, Li X, Guo Q, et al. (2016) MiR-285 targets P450 (CYP6N23) to regulate pyrethroid resistance in Culex pipiens pallens. Parasitology research 115: 4511-4517. https://doi.org/10.1007/s00436-016-5238-4
72. Etebari K, Afrad MH, Tang B, Silva R, Furlong MJ, et al. (2018) Involvement of microRNA miR-2b-3p in regulation of metabolic resistance to insecticides in Plutella xylostella. Insect Molecular Biology 27: 478-491. https://doi.org/10.1111/imb.12387

CAPÍTULO III

IDENTIFICATION OF miRNAS IN *Spodoptera frugiperda*

Instructions according to **Journal of invertebrate pathology**

Title: Identification of miRNAs in *Spodoptera frugiperda*

Autorship

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ABSTRACT

Spodoptera frugiperda is one of the main agricultural pests causing severe productivity losses. A promising alternative to this pest control is miRNA mechanism, which regulates gene expression and protein translation. In the present study we aimed to identify and characterize, by *in silico* analysis, the miRNAs in the genome of *S. frugiperda*. Using robust software with optimized algorithm, we were able to predict 350 miRNAs precursors, and 60 miRNAs were specific for genus *Spodoptera*. Subsequently, we performed the refinement of 10 RNA-seq and we identified 91 miRNAs not yet described, demonstrating the possibilities of this sequences in describing new important regulatory mechanisms in crop protection. Our results expand the knowledge about the miRNAs in the genome of *S. frugiperda* and bring new possibilities to understand their roles in the biology of this important pest.

Keywords: Fall armyworm, miRNA, bioinformatics, bioinsecticide

1 INTRODUCTION

Fall armyworm (*Spodoptera frugiperda,* Lepidoptera: Noctuidae) is one of the main agricultural pests presents in the tropical and subtropical regions of the American continent (Bateman et al., 2018). The larvae are polyphagous but well adapted to feed on grasses; causing severe damage to economically important crops such as corn, rice, sorghum and sugarcane. In addition, losses are also accounted in other crops such as cabbage, beet, peanut, soybean, alfalfa, onion, cotton, grass, millet, tomato, potato and cotton (Luginbill, 1928). This insect can potentially feed on over 100 species of plants from a wide range of families (CABI, 2018). In Brazil, it is considered the most important corn pest, causing severe productivity losses (Cruz and Turpin, 1983; Sarmento et al., 2002). Recent studies show yield losses in a range of 21% to 53% of annual average maize production, estimated at $ 2,481 million - $ 6,187 million (Abrahams et al., 2017).

In corn, young larvae hide in the cartridge during the day, but leave at night to feed on developing leaves. Older larvae remain inside the cartridge, and reduce product quality by compromising plant reproductive structures (Cruz, 1995). This behavior makes the pest control difficult, especially when using chemical products that depend on direct contact with the larva, which is protected (Bateman et al., 2018). In addition, these

pesticides cause severe health and environment damages (FAO and WHO, 2016). In this context, alternative strategies of combat are particularly interesting.

The use of Cry toxins from *Bacillus thuringiensis* expressed in plants brought significant advances in *S. frugiperda* control. However, resistant insects have already emerged, highlighting the importance of new methods in agriculture field (Flagel et al., 2018). An increasingly exciting option is the use of interference RNA (RNAi) technology, in which microRNAs (miRNAs) or small interfering RNAs (siRNAs) trigger a reduction of transcripts that are complementary to their sequences (Kim et al., 2009; Swevers et al., 2018). They are prominent by their specificity (Bachman et al., 2013; Chen et al., 2018a) and disruption of miRNA genes may cause serious physiological consequences, including developmental defects in invertebrates (Bartel, 2018; Li et al., 2018).

Firstly, described in Caenorhabditis *elegans* (Lee et al., 1993) miRNAs compromise protein expression through inhibition of translation, degradation or decay of mRNA using Argonaute (Ago)/RNA-induced silencing complex (RISC) mechanism (Fu et al., 2017). Insects express different miRNAs during development and have evolved strategies to overcome plants defenses against their attack modulating small non-condign RNAs (Li et al., 2018). It is therefore reasonable to assume that changes in endogenous miRNAs of the insect may also affect its biological functions, and survival.

Bioinformatic tools for prediction of miRNAs have been widely and successfully used (Chen et al., 2018b; Yang et al., 2018). Considering *S. frugiperda*, its genome was recently sequenced (Nandakumar et al., 2017). However, a greater understanding and deeper annotation of its sequences, including the identification of miRNAs, is still incipient and essential as a strategy for this pest control.

The objective of this study was, therefore, to identify and characterize, by *in silico* analysis, the miRNAs present in the genome of *S. frugiperda*. The understanding of these sequences will certainly allow their later use as a tool to control the carcass caterpillar when being included in artificial diets or templates for genetically modified plants.

2 MATERIALS AND METHODS

2.1 PREDICTION OF MATURE miRNAS AND THEIR PRECURSORS (pre-miRNAS)

The most recent data and information on the genome of *S. frugiperda* were accessed and downloaded from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The search for probable miRNAs and their precursors was carried out using software developed in several stages based on conserved characteristics of these molecules (de Souza Gomes et al., 2011). First, sequences of the genome of *S. frugiperda* with potential hairpin formation or similar miRNA precursor structures were obtained. The BLASTn and EINVERTED programs (EMBOSS tool) were used. The undesirable sequences were removed, selecting only those corresponding to probable miRNAs. The filters for removal were based on conserved characteristics of miRNA precursors as well as characteristics of other known regions which have no potential for formation of miRNA precursors. The filters used were: GC content (guanine and cytosine), minimum free energy (MFE), homology with mature miRNAs, homology with protein coding regions, homology with repetitive regions and homology with non-coding RNAs.

We also used a miRNA classifier based on previous features of possible real miRNAs, pseudo-miRNAs and non-real miRNAs, as well as information extracted from machine learning technology "Random forest" (Jiang et al., 2007). For the search for non-conserved miRNAs, an extra step was added to the protocol to search for mature sequences of miRNAs in the probable secondary structures of their respective precursors (Gkirtzou et al., 2010). Prediction of the secondary structure of pre-miRNAs was performed using the RNAfold (Vienna RNA Package) with adjusted parameters (Zuker and Stiegler, 1981).

Additional analyses were performed with the objective of comparing the putative miRNAs with the set of miRNAs found in other organisms. These analyzes involved the study of some previously used parameters and other additional parameters such as: Adjusted Minimum Free Energy (AMFE), Minimum Free Energy Index (MFEI), GC content, Minimal Free Energy of the thermodynamic Ensemble (MFEE), Ensemble Diversity and frequency of the MFE structure in the ensemble.

2.2 IDENTIFICATION OF PROTOSTOME-SPECIFIC miRNAS

To identify the protostome-specific miRNAs, the previously identified precursor sequences were aligned using the BLASTn tool and the miRBase database (Altschul et al., 1990; Kozomara and Griffiths-Jones, 2013).

2.3 SEQUENCING ANALYSIS OF THE RNA-SEQ LIBRARIES

We used 17 sequencing (SRR6670043, SRR6670042, SRR6670041, SRR6670040, SRR1536060, SRR1536059, SRR1536058, SRR850249, SRR850248, SRR850247, SRR850246, SRR850245, SRR850244, SRR850243, SRR850242, SRR496499, SRR496500) and the quality of the RNA-seq libraries was evaluated using the FastQC software. The adapters were removed with Trimmomatic, discarding reads with a score below 20 and a length of less than 17 and greater than 30 nucleotides.

The filtered sequences were mapped and quantified using miRDeep. The miRDeep2 and Perl scripts were used in each sequence separately to generate the number of reads for each identified miRNA. The R program was used to merge the counts of the different sequencing.

3 RESULTS

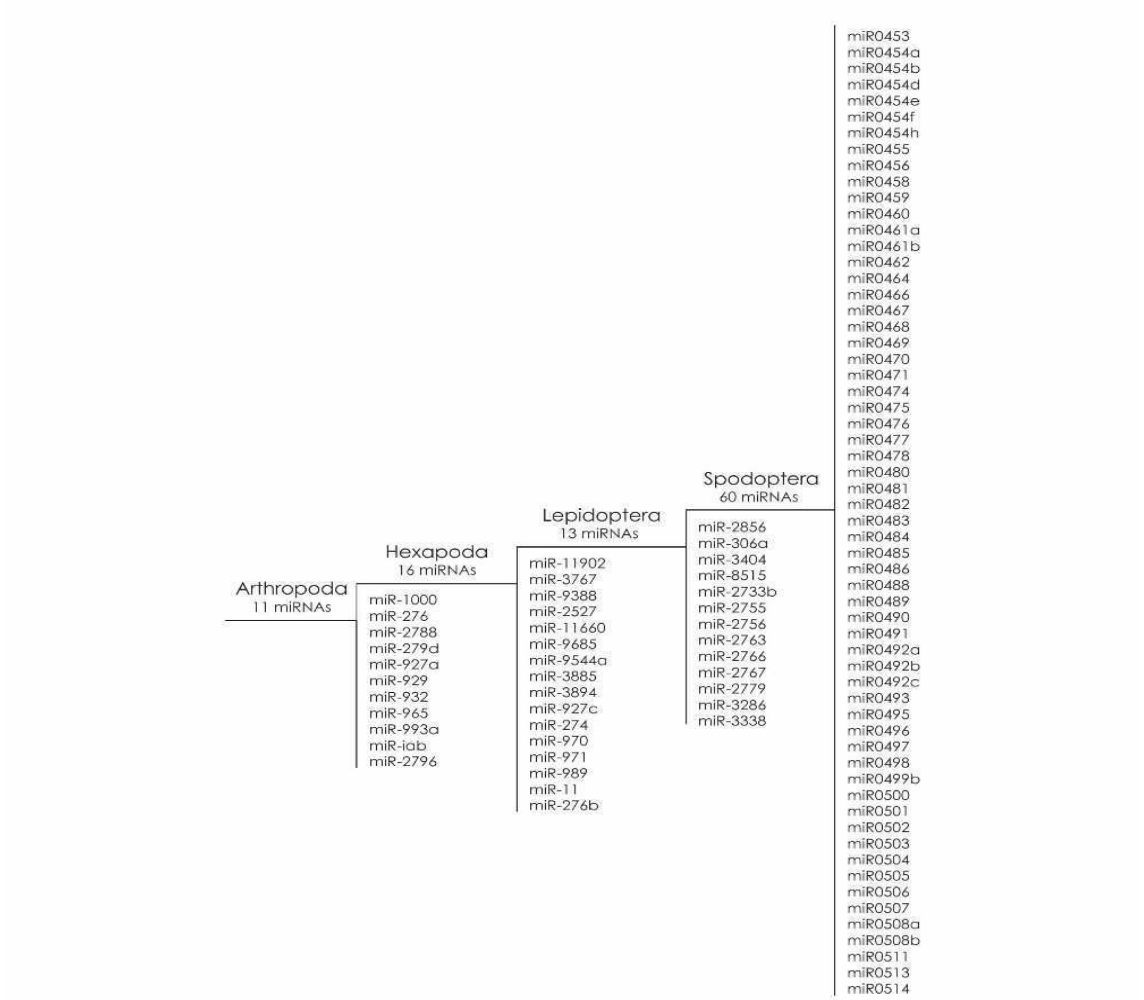
Once miRNAs regulation of gene expression and protein translation is a critical event in insect's physiology, we predicted the miRNAs and their precursors in the genome of *S. frugipeda*. We believe that these sequences may be biotechnologically exploited feeding the larvae with miRNA mimics added to the diet or even expressed by transgenic plants (Peng et al., 2016).

When applying an optimized algorithm, we identified 350 precursor miRNAs and their structural and thermodynamic characteristics are described in Table S1. The MFE of the pre-miRNAs ranged from -18.50 to -86.40 kcal.mol^{-1}, with a mean of -34.97 kcal.mol^{-1}; an average AMFE of -38.78 kcal.mol^{-1} and MFEI -0.87, similar to the values reported by other studies (Joshi, 2018; Nageshbabu et al., 2012; Sahoo et al., 2018; Sunkar and Jagadeeswaran, 2008; Zhang et al., 2006a; Zhang et al., 2006b; Zhang et al., 2008). The MFE of the precursor miRNAs is generally below -18 kcal.mol^{-1} and can be distinguished from other classes of RNAs by presenting values of MFEI less than 0.85 (Joshi, 2018; Nageshbabu et al., 2012; Zhang et al., 2006a; Zhang et al., 2006b). In addition, RNA sequences with an AMFE close to -46 kcal.mol^{-1} are considered likely to

be true miRNAs, compared to the less negative values typical of other classes of RNAs (Zhang et al., 2006a; Zhang et al., 2006b). Our results showed an average AMFE of -38.78 kcal.mol^{-1}, contributing to conclude that the predicted molecules are probable sequences of genuine miRNAs.

A crucial consideration in RNAi technology is specificity for the target specie. No lethally is desired for unrelated insects such as fruit flies (*Drosophila* sp.) or pea aphids (*A. pisum*). To overcome this issue, we performed a phylogenetic distribution of the 350 molecules initially predicted between four different clades defining the protostome-specific sequences (Figure 1). We identified 100 specific molecules to the clade Arthropoda, 89 to *Hexapoda*, 73 to *Lepdoptera* and 60 specifics to genus *Spodoptera*. In this way, the miRNA present in the same genus of the carcass caterpillar were categorized, suggesting its possible application in the specific control of this pest.

Figure 1 Phylogenetic distribution of the miRNAs identified in the genome of *Spodoptera frugipeda*. Of the 100 molecules specific to Arthropoda, 89 were specific to Hexapoda, 73 to Lepdoptera and 60 to genus *Spodoptera*.



The next step was to analyze dataset of publicly available total RNAseq. Seventeen different sequencing of *S. frugiperda* were used (Table S2). Considering the strategy to pest control through the expression of miRNAs mimics, we selected 116 molecules based on the total of counts found in at least 10 sequencing. As such molecules are present in several different sequencing the probability of being real are greater. Of these 116 selected miRNAs, only 25 have already been studied and their biological effects have been described in the literature for other species (Table 1), including associated with mechanisms of resistance to insecticides. The remaining 91 sequences need to be explored and their role must be elucidated in *S. frugiperda.*

Table 1 miRNAs found in the genome of *S. frugiperda* with functions already described for other species. The prediction was performed through an algorithm developed by de Souza Gomes et al. (2011) using 17 RNA-seq libraries.

miRNA	Biological effect	Organism	References
miR-2765	Caste differentiation	*Reticulitermes speratus*	(Matsunami et al., 2018)
miR-285	Interacts with P450 to regulate resistance to pyrethroids	*Culex pipiens pallens*	(Tian et al., 2016)
miR-33	Gonadogenesis	*Drosophila*	(Yang et al., 2016a)
miR-279	Immune pathway regulator	*Drosophila melanogaster*	(Fullaondo and Lee, 2012)
miR-7	Immunity	*Plutella xylostella*	(Xu et al., 2017)
miR-932	Mechanism of resistance to insecticides	*Culex pipiens pallens*	(Liu et al., 2016)
miR-276	Synchronized hatching	*Locusta migratoria*	(He et al., 2016)
miR-34	Regulates innate immunity and ecdysone signaling	*Drosophila*	(Xiong et al., 2016)
miR-79	Regulates innate immunity	*Rhipicephalus haemaphysaloides*	(Wang et al., 2015)
miR-92	Inhibits premature differentiation of neuroblasts	*Drosophila*	(Yuva-Aydemir et al., 2015)
miR-9	Prevents apoptosis during wing development	*Drosophila*	(Bejarano et al., 2010)
miR-13	Apoptosis control	*Drosophila*	(Stark et al., 2003)
miR-1	Muscle development	*Drosophila*	(Zhu et al., 2017)
miR-308	Regulates immunity	*Plutella xylostella*	(Shakeel et al., 2018)
miR-745	Immunity	*Plutella xylostella*	(Xu et al., 2017)
miR-2	Immunity	*Plutella xylostella*	(Xu et al., 2017)
miR-278	Regulate resistance to pyrethroids	*Culex pipiens pallens*	(Lei et al., 2015)
miR-14	Regulation of autophagy	*Drosophila*	(Nelson et al., 2014)
miR-317	Embryogenesis	*Drosophila*	(Pushpavalli et al., 2014)
miR-10	Development	*Drosophila*	(Stark et al., 2007)
miR-71	Chitin metabolism	*Locusta migratoria*	(Yang et al., 2016b)
miR-277	Lipid metabolism	*Aedes aegypti*	(Ling et al., 2017)
miR-306	Regulates immunity	*Plutella xylostella*	(Shakeel et al., 2018)
miR-11	Apoptosis control	*Drosophila*	(Ge et al., 2012)
miR-263	Chitin metabolism	*Locusta migratoria*	(Yang et al., 2016b)

4 DISCUSSION

miRNAs are important non-coding regulators of physiological events in insects (Gordon and Waterhouse, 2007). Disrupt their expression thorough miRNA mimics may be an excited application in agriculture, controlling pest, decreasing pesticide resistance, and preventing non-target species death (Saini et al., 2018; Swevers et al., 2018). Actually, this is a promising technology that must be explored, especially considering the management of *S. frugiperda*.

S. frugiperda is one of the main agricultural pests and the climatic conditions are determinant for its occurrence (Bavaresco et al., 2002). This species can reduce maize production in up to 53% and frequent applications of insecticides are necessary to reduce the population avoiding economic damages (Abrahams et al., 2017). Despite the genome already described, the notation of its miRNAs is still restricted and describing them is necessary to overcome the challenges faced in plantations. In the present study, the prediction of miRNA precursors from *S. frugiperda* genome allowed the identification of 350 different molecules with determination of their thermodynamic and structural characteristics. The thermodynamic aspects are useful criteria to differentiate miRNAs from other types of RNAs (Zhang et al., 2006b), already the structural characteristics help in these sequences validation as interfering RNAs (de Sousa Cardoso et al., 2016). Considering the thermodynamic parameters obtained in this work we can affirm that the miRNAs identified *in silico* from the genome of *S. frugiperda* are genuine molecules. Thus, it is possible to use them as modulators of the expression of different genes and proteins.

For the design of the molecular interference strategy, special attention should be devoted to off-targets (Birmingham et al., 2006; Jackson et al., 2003; Ljepoja et al., 2018). Considering pest control, the miRNA must be species-specific (Lim et al., 2016) with minimal effect on beneficial insects to the crop. Through the phylogenetic distribution of the miRNAs it was possible to identify 60 molecules specific to genus *Spodoptera*.

Some miRNA molecules are naturally suppressed in healthy organisms. Exogenous administration and, consequently, increased expression may lead to physiological modifications ranging from growth retardation, making the organism more susceptible to control until its death. We initially identified 116 miRNA molecules present in 10 different RNA-seq of *S. frugiperda*. Of these, only 25 have already been studied in other organisms and have their biological effect described. The miR-276, for example, despite the small number of counts, is responsible for the synchrony of progeny hatching in locusts (He et al., 2016) and for the coordination of neural function in *Apis melifera* (Hori et al., 2011). On the other hand, miR-

932 targets a gene related to resistance to pyrethroids in *Culex pipiens pallens.* Liu et al. (2016) observed that overexpression of this miRNA renders more resistant mosquitoes while inhibition made them more sensitive to deltamethrin. This fact evidences the importance of a careful choice in the design of interfering sequences.

Another approach involving miRNAs may be adopted, including inhibitors of its expression with consequent insect combat. Saini et al. (2018) developed a miRNA capable of silencing the acetylcholinesterase gene of *Helicoverpa armigera*, an essential enzyme for the nervous system. By silencing this enzyme, the researchers observed a delay in larval growth and mortality, as well as the faulty emergence of adults, indicating that this miRNA can be used as an insecticidal molecule. In the present study, we identified 91 miRNAs that lack characterization and, therefore, open new ways in the exploration of these molecules in *S. frugiperda* control.

It is important to notice that, this agrobiotechnology approach for pest control can modify not only the target-gene expression, but also can modulate distinct physiological processes. Therefore, plant-insect interactions must be considered in analysis of the biological effects of the RNAi pathway.

5 CONCLUSION

The identification of miRNAs in *S. frugiperda* genome is an important tool to create alternatives for this pest control. We successfully applied an integrated computational analysis with the prediction of 350 miRNA precursors, being 60 specifics to the study genre. Subsequently, the refinement of 10 RNA-seq showed 91 miRNAs not yet described. Our results demonstrate the possibilities of the sequences described here, which are associated to an attractive regulatory mechanism as biotectenologically feasible in crop protection.

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DECLARATION OF INTEREST

We confirm that all authors fulfill all conditions required for authorship, and have read and approved the manuscript. We also confirm that there is no potential conflict of interest, as described in the Instruction for Authors.

AUTHORS' CONTRIBUTIONS

Experimental design and manuscript writing: TGA e SBRN

Optimization of in silico algorithms: MSG e LRA

In silico analysis: SBRN, TCSC e TCA

Project coordinator and senior author: TGA

REFERENCES

Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., … Witt, A. (2017). Fall armyworm: impacts and implications for Africa. Evidence Note (2), September 2017. Wallingford, UK: CABI.

Altschul, S. F., et al., 1990. Basic local alignment search tool. Journal of molecular biology. 215**,** 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bachman, P. M., et al., 2013. Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (Diabrotica virgifera virgifera LeConte). Transgenic research. 22**,** 1207-1222. https://doi.org/10.1007/s11248-013-9716-5

Bartel, D. P., 2018. Metazoan MicroRNAs. Cell. 173**,** 20-51. https://doi.org/10.1016/j.cell.2018.03.006

Bateman, M. L., et al., 2018. Assessment of potential biopesticide options for managing fall armyworm (Spodoptera frugiperda) in Africa. Journal of Applied Entomology. 142**,** 805-819. https://doi.org/10.1111/jen.12565

Bavaresco, A., et al., 2002. Biology and thermal requirements of Spodoptera cosmioides (Walk.)(Lepidoptera: Noctuidae). Neotropical Entomology. 31**,** 49-54. http://dx.doi.org/10.1590/S1519-566X2002000100007

Bejarano, F., et al., 2010. miR-9a prevents apoptosis during wing development by repressing Drosophila LIM-only. Developmental biology. 338**,** 63-73. https://doi.org/10.1016/j.ydbio.2009.11.025

Birmingham, A., et al., 2006. 3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets. Nature Methods. 3**,** 199. https://doi.org/10.1038/nmeth854

CABI (CAB International, 2018. Crop Protection Compendium. https://www.cabi.org/cpc/

Chen, J., et al., 2018a. Genome-Wide Screening and Functional Analysis Reveal That the Specific microRNA nlu-miR-173 Regulates Molting by Targeting Ftz-F1 in Nilaparvata lugens. Frontiers in Physiology. 9. https://doi.org/10.3389/fphys.2018.01854

Chen, X., et al., 2018b. DRMDA: deep representations- based miRNA–disease association prediction. Journal of cellular and molecular medicine. 22**,** 472-485. https://doi.org/10.1111/jcmm.13336

Cruz, I., 1995. A lagarta-do-cartucho na cultura do milho. Embrapa Milho e Sorgo-Circular Técnica (INFOTECA-E). https://core.ac.uk/download/pdf/15437047.pdf

Cruz, I., et al., 1999. Damage of Spodoptera frugiperda (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. International Journal of Pest Management. 45**,** 293-296. https://doi.org/10.1080/096708799227707

Cruz, I., Turpin, F., 1983. Yield impact of larval infestations of the fall armyworm (Lepidoptera: Noctuidae) to midwhorl growth stage of corn. Journal of Economic Entomology. 76**,** 1052-1054. https://doi.org/10.1093/jee/76.5.1052

de Sousa Cardoso, T., et al., 2016. Genome- wide identification and in silico characterisation of micro RNA s, their targets and processing pathway genes in Phaseolus vulgaris L. Plant Biology. 18**,** 206-219. https://doi.org/10.1111/plb.12377

de Souza Gomes, M., et al., 2011. Genome-wide identification of novel microRNAs and their target genes in the human parasite Schistosoma mansoni. Genomics. 98**,** 96-111.

FAO, F. a. A. O. o. t. U. N., WHO, W. H. O., International code of conduct on pesticide management: guidelines on highly hazardous pesticides. 2016. https://doi.org/10.1016/j.ygeno.2011.05.007

Flagel, L., et al., 2018. Mutational disruption of the ABCC2 gene in fall armyworm, Spodoptera frugiperda, confers resistance to the Cry1Fa and Cry1A.105 insecticidal proteins. Scientific Reports. 8**,** 7255. https://doi.org/10.1038/s41598-018-25491-9

Fu, X., et al., 2017. Association of microRNAs with Argonaute proteins in the malaria mosquito Anopheles gambiae after blood ingestion. Scientific Reports. 7**,** 6493. https://doi.org/10.1038/s41598-017-07013-1

Fullaondo, A., Lee, S. Y., 2012. Identification of putative miRNA involved in Drosophila melanogaster immune response. Developmental & Comparative Immunology. 36**,** 267-273. https://doi.org/10.1016/j.dci.2011.03.034

Ge, W., et al., 2012. Overlapping functions of microRNAs in control of apoptosis during Drosophila embryogenesis. Cell death and differentiation. 19**,** 839. https://doi.org/10.1038/cdd.2011.161

Gkirtzou, K., et al., 2010. MatureBayes: a probabilistic algorithm for identifying the mature miRNA within novel precursors. PloS one. 5**,** e11843. https://doi.org/10.1371/journal.pone.0011843

Gordon, K. H., Waterhouse, P. M., 2007. RNAi for insect-proof plants. Nature biotechnology. 25**,** 1231. https://doi.org/10.1038/nbt1107-1231

He, J., et al., 2016. MicroRNA-276 promotes egg-hatching synchrony by up-regulating brm in locusts. Proceedings of the National Academy of Sciences. 113**,** 584-589. https://doi.org/10.1073/pnas.1521098113

Hori, S., et al., 2011. Expression of two microRNAs, ame-mir-276 and -1000, in the adult honeybee (Apis mellifera) brain. Apidologie. 42**,** 89-102. https://doi.org/10.1051/apido/2010032

Jackson, A. L., et al., 2003. Expression profiling reveals off-target gene regulation by RNAi. Nature Biotechnology. 21**,** 635. https://doi.org/10.1038/nbt831

Jiang, P., et al., 2007. MiPred: classification of real and pseudo microRNA precursors using random forest prediction model with combined features. Nucleic acids research. 35**,** W339-W344. https://doi.org/10.1093/nar/gkm368

Joshi, H., 2018. In silico identification and target prediction of micrornas in Sesame (sesamum indicum L.) expressed sequence tags. Genetics and Molecular Research. 17. http://dx.doi.org/10.4238/gmr16039911

Kim, V. N., et al., 2009. Biogenesis of small RNAs in animals. Nature reviews Molecular cell biology. 10**,** 126. https://doi.org/10.1038/nrm2632

Kozomara, A., Griffiths-Jones, S., 2013. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic acids research. 42**,** D68-D73. https://doi.org/10.1093/nar/gkt1181

Lee, R. C., et al., 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. cell. 75**,** 843-854. https://doi.org/10.1016/0092-8674(93)90529-Y

Lei, Z., et al., 2015. MiR-278-3p regulates pyrethroid resistance in Culex pipiens pallens. Parasitology research. 114**,** 699-706. https://doi.org/10.1007/s00436-014-4236-7

Li, C., et al., 2018. miRNA-Mediated Interactions in and between Plants and Insects. International journal of molecular sciences. 19**,** 3239. https://doi.org/10.3390/ijms19103239

Lim, Z. X., et al., 2016. Diet-delivered RNAi in Helicoverpa armigera–progresses and challenges. Journal of insect physiology. 85**,** 86-93. https://doi.org/10.1016/j.jinsphys.2015.11.005

Ling, L., et al., 2017. MicroRNA-277 targets insulin-like peptides 7 and 8 to control lipid metabolism and reproduction in Aedes aegypti mosquitoes. Proceedings of the National Academy of Sciences. 114**,** E8017-E8024. https://doi.org/10.1073/pnas.1710970114

Liu, B., et al., 2016. MiR-932 regulates pyrethroid resistance in Culex pipiens pallens (Diptera: Culicidae). Journal of medical entomology. 53**,** 1205-1210. https://doi.org/10.1093/jme/tjw083

Ljepoja, B., et al., 2018. A proteomic analysis of an in vitro knock-out of miR-200c. Scientific Reports. 8**,** 6927. https://doi.org/10.1038/s41598-018-25240-y

Luginbill, P., 1928. The fall army worm. US Dept. of Agriculture.

Matsunami, M., et al., 2018. Caste- specific microRNA expression in termites: insights into soldier differentiation. Insect molecular biology. https://doi.org/10.1111/imb.12530

Nageshbabu, R., et al., 2012. Computational identification of conserved miRNAs and their potential targets in French bean (Phaseolus vulgaris). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 3**,** 562-568. DOI: https://doi.org/10.4238/2014.January.17.16

Nandakumar, S., et al., 2017. Whole-Genome Sequence of the Spodoptera frugiperda Sf9 Insect Cell Line. Genome announcements. 5**,** e00829-17. https://doi.org/10.1128/genomeA.00829-17

Nelson, C., et al., 2014. miR-14 regulates autophagy during developmental cell death by targeting ip3-kinase 2. Molecular cell. 56**,** 376-388. https://doi.org/10.1016/j.molcel.2014.09.011

Peng, T., et al., 2016. Reduced abundance of the CYP6CY3-targeting let-7 and miR-100 miRNAs accounts for host adaptation of Myzus persicae nicotianae. Insect biochemistry and molecular biology. 75**,** 89-97. https://doi.org/10.1016/j.ibmb.2016.06.002

Pushpavalli, S. N., et al., 2014. Argonaute-1 functions as a mitotic regulator by controlling Cyclin B during Drosophila early embryogenesis. The FASEB Journal. 28**,** 655-666. https://doi.org/10.1096/fj.13-231167

Sahoo, S., et al., 2018. Computational identification and characterization of conserved miRNAs and their putative target genes in Eclipta prostrata. Gene Reports. 11**,** 213-219. https://doi.org/10.1016/j.genrep.2018.03.020

Saini, R. P., et al., 2018. Silencing of HaAce1 gene by host-delivered artificial microRNA disrupts growth and development of Helicoverpa armigera. PloS one. 13**,** e0194150. https://doi.org/10.1371/journal.pone.0194150

Sarmento, R., et al., 2002. Revisão da biologia, ocorrência e controle de Spodoptera frugiperda (Lepidoptera, Noctuidae) em milho no Brasil. Bioscience Journal. 18.

Shakeel, M., et al., 2018. genome-Wide identification of Destruxin a-responsive immunity-related Micrornas in Diamondback Moth, Plutella xylostella. Frontiers in Immunology. 9**,** 185. https://doi.org/10.3389/fimmu.2018.00185

Stark, A., et al., 2003. Identification of Drosophila microRNA targets. PLoS biology. 1**,** e60. https://doi.org/10.1371/journal.pbio.0000060

Stark, A., et al., 2007. Systematic discovery and characterization of fly microRNAs using 12 Drosophila genomes. Genome research. 17**,** 000-000. http://www.genome.org/cgi/doi/10.1101/gr.6593807

Sunkar, R., Jagadeeswaran, G., 2008. In silico identification of conserved microRNAs in large number of diverse plant species. BMC plant biology. 8**,** 37. https://doi.org/10.1186/1471-2229-8-37

Swevers, L., et al., 2018. Defense Mechanisms against Viral Infection in Drosophila: RNAi and Non-RNAi. Viruses. 10**,** 230. https://doi.org/10.3390/v10050230

Tian, M., et al., 2016. MiR-285 targets P450 (CYP6N23) to regulate pyrethroid resistance in Culex pipiens pallens. Parasitology research. 115**,** 4511-4517. DOI: https://doi.org/10.1007/s00436-016-5238-4

Wang, F., et al., 2015. Lipopolysaccharide-induced differential expression of miRNAs in male and female Rhipicephalus haemaphysaloides ticks. PloS one. 10**,** e0139241. https://doi.org/10.1371/journal.pone.0139241

Xiong, X.-P., et al., 2016. miR-34 modulates innate immunity and ecdysone signaling in Drosophila. PLoS pathogens. 12**,** e1006034. https://doi.org/10.1371/journal.ppat.1006034

Xu, J., et al., 2017. Genome-Wide Profiling of Plutella xylostella Immunity-Related miRNAs after Isaria fumosorosea Infection. Frontiers in physiology. 8**,** 1054. https://doi.org/10.3389/fphys.2017.01054

Yang, H., et al., 2016a. MicroRNA-dependent roles of Drosha and Pasha in the Drosophila larval ovary morphogenesis. Developmental biology. 416**,** 312-323. https://doi.org/10.1016/j.ydbio.2016.06.026

Yang, M., et al., 2016b. miR-71 and miR-263 jointly regulate target genes chitin synthase and chitinase to control locust molting. PLoS genetics. 12**,** e1006257. https://doi.org/10.1371/journal.pgen.1006257

Yang, Y., et al., 2018. MiRGOFS: a GO-based functional similarity measurement for miRNAs, with applications to the prediction of miRNA subcellular localization and miRNA–disease association. Bioinformatics. 1**,** 10. https://doi.org/10.1093/bioinformatics/bty343

Yuva-Aydemir, Y., et al., 2015. Downregulation of the host gene jigr1 by miR-92 is essential for neuroblast self-renewal in Drosophila. PLoS genetics. 11**,** e1005264. https://doi.org/10.1371/journal.pgen.1005264

Zhang, B., et al., 2006a. Conservation and divergence of plant microRNA genes. The Plant Journal. 46**,** 243-259. https://doi.org/10.1111/j.1365-313X.2006.02697.x

Zhang, B., et al., 2006b. Evidence that miRNAs are different from other RNAs. Cellular and Molecular Life Sciences CMLS. 63**,** 246-254. https://doi.org/10.1007/s00018-005-5467-7

Zhang, B., et al., 2008. Identification of soybean microRNAs and their targets. Planta. 229**,** 161-182. https://doi.org/10.1007/s00425-008-0818-x

Zhu, J.-y., et al., 2017. The E3 ubiquitin ligase Nedd4/Nedd4L is directly regulated by microRNA 1. Development. 144**,** 866-875. DOI: 10.1242/dev.140368. https://doi.org/10.1242/dev.140368

Zuker, M., Stiegler, P., 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic acids research. 9**,** 133-148. https://doi.org/10.1093/nar/9.1.133

SUPPLEMENTARY TABLES

Table S1 Thermodynamic and structural characterization of *S. frugiperda* pre-miRNAs. The following parameters are described: size, G, A, C and U content, GC and AU content, UA and GC ratio, Minimum Free Energy (MFE), Minimal Free Energy of the thermodynamic Ensemble (MFEE), Free Minimum Thermal set (Freq), Ensemble Diversity (Div), Adjusted Minimum Free Energy (AMFE), Minimum Free Energy Index (MFEI).

Pre-miRNA	Sequence	Size	G	A	C	U	GC	AU	UA-ratio	GC-ratio	MFE	MFEE	Freq	Div	AMFE	MFEI
>sfr-miR-466f-1	CGUUUACAGGAAGUUUGUGCUCAUCUGUACACAGACAUACGUGUGUGUGCAUGUGAGCACGAAUACACCGUAACUA	77,00	23,38	27,27	20,78	27,27	44,16	54,55	1,00	1,13	-35,00	-32,30	0,23	3,19	-45,45	-1,03
>sfr-miR-274	CUUGUUAAGCGAAGUUGGUUUGUGACCGUCACUAACGGGCAGUACCUCUAUUUGCUCGUUUUGACGAUCGCAAAAUGAACUCGCUUGACUCU	93,00	22,58	21,51	22,58	32,26	45,16	53,76	1,50	1,00	-41,50	-41,50	0,06	6,09	-44,62	-0,99
>sfr-miR-970	UGUCCGCGAGAGCCUUGCGUGUGCUCUUAUUGGUAGUGUGGCUGAUAAUCUAUCAUAAGACACACGCGGCUCUCGCUGGCUC	83,00	27,71	16,87	25,30	28,92	53,01	45,78	1,71	1,10	-44,30	-43,40	0,19	4,31	-53,37	-1,01
>sfr-miR-281	UCUGUUAAUGAAGAGAGCUAUCCGUCGACAGUAUUGCCAGUAAACACUGUCAUGGAGUUGCUCUCUUUAUGAACGGU	78,00	23,08	25,64	19,23	30,77	42,31	56,41	1,20	1,20	-34,60	-34,60	0,30	2,44	-44,36	-1,05
>sfr-miR-10486	AGGAUUCAAUAAUUUCACUGUCCUGCAGGCG	100,00	22,00	23,00	19,00	35,00	41,00	58,00	1,52	1,16	-20,50	-13,10	0,03	23,94	-20,50	-0,50

	CCUUAUCGGCUCUGAUGACAAUUUUGUGAUUAUCUGAUGGCAUGCGGUCUAAUGCAUUUAUGAAUGUC														
>sfr-miR-10459-1	AAUGCUAUACGACAUGAUGCUAUGGAAGUAACUGAAAUACACUAGUCUUGCCGUGCCUAUGUCGGUUAGCUGU	74,00	22,97	27,03	18,92	29,73	41,89	56,76	1,10	1,21	-23,60	-23,60	0,51	2,11	-31,89	-0,76
>sfr-miR-10459-2	CCCACAGCAAAAUGCCAUAGACAUGAUGCUAUGGAAGUAACUGAAAUACACUAGUCUUGCCGUGCCUAUGUCGGUUAGCUGUCUG	86,00	22,09	27,91	23,26	25,58	45,35	53,49	0,92	0,95	-20,70	-20,70	0,30	13,93	-24,07	-0,53
>sfr-miR-10459-3	CCCACAGCAAAAUGCCAUACGACAUGAUGCUAUGGAAGUAACUGAAGUAUACUAGUCUUGCCGUGUCUAUGUCAGUUAGCUGUCUG	87,00	21,84	27,59	21,84	27,59	43,68	55,17	1,00	1,00	-20,40	-20,40	0,10	9,44	-23,45	-0,54
>sfr-miR-10459-4	GAAAUACACUAGUCUUGCCGUGCCUAUGUCGGUUAGCUGUCUGAUCUUCCAGACAGCGUAAGCUGCAGAGCUCAAUCGUCCCGCUAGUGAUGUA	95,00	24,21	22,11	25,26	27,37	49,47	49,47	1,24	0,96	-29,20	-25,80	0,07	14,34	-30,74	-0,62
>sfr-miR-10514	AGAAGCCAACCUCACCCCUAAACGGCCUGCCACAGGCCCGAAAUUGCGCGCUUGCCGAUGGCGGGUCGUCAAUUUCGUCUAGGGGUGAGGUUAGCUUCC	100,00	28,00	20,00	31,00	20,00	59,00	40,00	1,00	0,90	-60,10	-57,70	0,23	4,97	-60,10	-1,02

>sfr-miR-10468	CAGUACCUCCGCGUCG CGCGACUUCCCGUCGA AACGUGCAGUGCACUU GGCGAAGUUGCCUGU GACCGCGGACGGGUAG AC	82,00	31,71	17,07	31,71	18,29	63,41	35,37	1,07	1,00	-35,80	-35,80	0,10	6,90	-43,66	-0,69
>sfr-miR-307b	UGCCUCCUCCACGAUC GCUCACUCAAGGAGGU UGUGAUGGGCCGAGC AACUAGUGCACACAUC ACACCCAGGUUGAGUG AGUAACCGGGAAUGA GAUC	99,00	27,27	25,25	27,27	19,19	54,55	44,44	0,76	1,00	-36,90	-36,00	0,31	5,48	-37,27	-0,68
>sfr-miR-307	GAUCUCAUUCCCGGUU ACUCACUCAACCUGGG UGUGAUGUGUGCACU AGUUGCUCGGCCCAUC ACAACCUCCUUGAGUG AGCGAUCGUGGAGGA GGCA	99,00	27,27	19,19	27,27	25,25	54,55	44,44	1,32	1,00	-42,40	-40,40	0,03	7,66	-42,83	-0,79
>sfr-miR-2a-1	UUAAAUGUGGUUUGG CAUCAAAGUCGGCUUG UCAUAGGUCAUAACG UAGCUAUCACAGCCAG CUUUGAUGAGCAUGA CCUCAUUCCU	88,00	21,59	25,00	21,59	30,68	43,18	55,68	1,23	1,00	-29,60	-29,20	0,14	4,68	-33,64	-0,78
>sfr-miR-2b	AGGAAUGAGGUCAUG CUCAUCAAAGCUGGCU GUGAUAGCUACGUUA UGACCUAUGACAAGCC GACUUUGAUGCCAAAC CACAUUUAA	88,00	21,59	30,68	21,59	25,00	43,18	55,68	0,81	1,00	-27,00	-27,00	0,10	5,20	-30,68	-0,71
>sfr-miR-2a-2	CGCGAGUGCAGCGGCG CUCACAAAGUGGCUGU AAUGUGUGUCCCCGUA CAUAUCACAGCCAGCU	87,00	27,59	21,84	26,44	22,99	54,02	44,83	1,05	1,04	-43,90	-43,90	0,21	3,78	-50,46	-0,93

	UUGAUGAGCGCUGAG CAUCAUU														
>sfr-miR-2c	AAGCGCGUCAGGGGG UCAGCAAAGUGGUUG UGUCUUAUGAGAUUC AUGAACAUAUCACAGC CAGCUUUGUUGACUUC UUUGUCGCGUAU	90,00	26,67	22,22	18,89	31,11	45,56	53,33	1,40	1,41	-43,20	-43,20	0,06	7,97	-48,00	-1,05
>sfr-miR-11	UCUCGUGGCUAGCACU UUGGCUGUGACCUGU GUGUCGUAGUCACAUC ACAGUCAGAGUUCUA GCUACGCGG	72,00	27,78	16,67	25,00	29,17	52,78	45,83	1,75	1,11	-37,90	-37,90	0,11	5,38	-52,64	-1,00
>sfr-miR-10497	AAUACAACCACAAGCU GCCUUCACACCAAACG GUGUCUGGUCAGGUU CUUAUUCCCUGACCAG ACUCGUUUUGGUUGA GAGGCCGCUGUGGUUC GUUCG	100,00	24,00	20,00	27,00	28,00	51,00	48,00	1,40	0,89	-50,80	-46,40	0,02	7,85	-50,80	-1,00
>sfr-miR-10503-1	GGGCAUUGGAUCGUU CGAUUCCCUGCAACAU GGUUGAGUUGGACGG UGCUGGUGUACGUGU CAUGUUGGUGAACGA GCGCUGUCAACUGGGA	93,00	35,48	17,20	18,28	27,96	53,76	45,16	1,63	1,94	-30,80	-29,60	0,04	14,88	-33,12	-0,62
>sfr-miR-10503-2	GGGCAUUGGAUCGCUC GAUUCCCUGCAACAUG GUUGAGUUGGGCGGU GCUGGUGUACGUGUC AUGUUCGUGAACGGG CGCUGUCAACUGGGA	93,00	36,56	15,05	20,43	26,88	56,99	41,94	1,79	1,79	-32,70	-32,10	0,07	17,78	-35,16	-0,62
>sfr-miR-3192	GACGUACUUUUUUUU AAAUGGGACAACCCCA CCACAACCUCUGGGAG GUUGUAGCGGGUUUG	85,00	21,18	27,06	20,00	30,59	41,18	57,65	1,13	1,06	-23,40	-18,60	0,08	13,99	-27,53	-0,67

	UUAAUUUCAGAUAAC AUACUGA														
>sfr-miR-8515-1	UCCCUGACUGUUUCAU UCCAUUACCUACGCGA AAUCGUACGCGGCCGG UAAUGGAAUGAAACA AACAGAAC	72,00	19,44	30,56	26,39	22,22	45,83	52,78	0,73	0,74	-35,30	-35,30	0,92	0,20	-49,03	-1,07
>sfr-miR-8515-2	GCUCGCUUUCCCCUUC CUGACUGAUUCAUUCC AUUACCUACGCGAAUU CGUACGCGGCCGGUAA UGGAAUGAAUCAAAC AGUCGGGAUGGGAAC GCGAGC	101,00	24,75	22,77	27,72	23,76	52,48	46,53	1,04	0,89	-61,90	-61,90	0,30	2,61	-61,29	-1,17
>sfr-miR-10492b-1	ACAAAACGACGCAAGA GCGCGUUCGAUGCUCU GAUUGGUCGGUGAGA AUUAAUCAACCAAUCA GAGUAUCGAACGCGCU CUGGUUUCGUUUCGC	95,00	25,26	26,32	23,16	24,21	48,42	50,53	0,92	1,09	-59,90	-59,90	0,55	1,49	-63,05	-1,30
>sfr-miR-10492b-2	UUUAGCGCGUUCGGU GCUCUGAUUGGGCGGC UUGAAUAAAUCAACC AAUCAGAGUAUCGAA CGCGCUCUC	71,00	25,35	22,54	23,94	26,76	49,30	49,30	1,19	1,06	-42,20	-41,50	0,23	3,96	-59,44	-1,21
>sfr-miR-10492c-1	UAGAAACGAGAGCGC GUUCGGCGCUGUGAU UGGCCGGCUCUAAUGA ACCAACCAAUCACAAC GCUGAACGCGCUUCGU GUUGAC	85,00	27,06	24,71	27,06	20,00	54,12	44,71	0,81	1,00	-47,70	-47,70	0,23	5,28	-56,12	-1,04
>sfr-miR-10492c-2	UAAAGUAAUCAAAAC GAGAGCGCGUUCGGCG CUGUGAUUGGCCGGCU CGAAUAAACCAACCAA UCAGCGCGCCGAACGC	101,00	23,76	26,73	26,73	21,78	50,50	48,51	0,81	0,89	-66,80	-66,80	0,55	1,02	-66,14	-1,31

	GCUCUCGUUUCGAUUA CUUUA														
>sfr-miR-10492a-1	AGUAAUCGAAACGAG AGCGCGUUUGGCACUC UGAUUGGCCGGCUCGA AUUAACUAACCAAUCA GAGCGCCGAACGUGCU CUCGUUUCGAUUAUA	95,00	24,21	26,32	24,21	24,21	48,42	50,53	0,92	1,00	-63,70	-63,70	0,70	0,91	-67,05	-1,38
>sfr-miR-10492a-2	AGAGCGCGUUCGACGC UUUGAUUGGUCGGCU CGAAUAAACCAACCAA UCAGAGCGCCGAACGU GCGCA	69,00	27,54	26,09	27,54	17,39	55,07	43,48	0,67	1,00	-39,30	-39,10	0,94	0,24	-56,96	-1,03
>sfr-miR-10492c-3	GACGAUAUCGAAACG AAAAUGCGUUCGGCGC UGUGAUUGGCCGGCUC AAAUGAACUAUCCAA UUACAGCGCCGAAUGC ACUUUCGUUUCGAUA UCUUU	99,00	22,22	26,26	23,23	27,27	45,45	53,54	1,04	0,96	-63,10	-63,10	0,69	0,93	-63,74	-1,40
>sfr-miR-10492c-4	AACGAGUUCGAAAUG AAACCGCAUUCGGCGC UGCGAUUGGCCGGCUC CCAUGAAUCAACCAAU CAGAGCGCCGAACGUG CACUCGUUUCGAUCUC UUU	99,00	23,23	24,24	29,29	22,22	52,53	46,46	0,92	0,79	-44,30	-44,30	0,49	2,81	-44,75	-0,85
>sfr-miR-10492c-5	GAGAGUGUGUUCAGC GCUGUGAUUGGCCGG UUCGAAUAAACCAACC AAUCACAACGCUGAAC AACUAAG	70,00	24,29	31,43	22,86	20,00	47,14	51,43	0,64	1,06	-34,30	-34,30	0,57	1,34	-49,00	-1,04
>sfr-miR-10471-1	GGUAAACUAGAAUGC AUUCGGUACCGGUGCU CUGAUUGGUUAGUUC AUUGGAGCCGGCCAAU	97,00	26,80	19,59	22,68	29,90	49,48	49,48	1,53	1,18	-44,40	-44,40	0,24	6,23	-45,77	-0,93

	CAGAGCGCCGAUCGCC GUGUCAUUUCGAUUU CUU															
>sfr-miR-10492c-6	ACUAGAGCGCGUUCGG CGCUGUGAUUGGUUG GUAUAUUGGAGCCGG CCAAUCACAGCGCCGA ACGCACUCUCGA	75,00	30,67	20,00	26,67	21,33	57,33	41,33	1,07	1,15	-56,70	-56,70	0,83	0,35	-75,60	-1,32
>sfr-miR-10492a-3	UCUAAACGAGACCGCG UUCGGCGUUCUGAUU GGUUGGUUUAUUCGA ACUGGCCAAUCAGAGC GCCGAACGUGUAAUCG UUUCGA	85,00	27,06	21,18	22,35	28,24	49,41	49,41	1,33	1,21	-54,50	-54,50	0,34	2,29	-64,12	-1,30
>sfr-miR-10492a-4	UGAGAUAAAACGAGU UCGUCGCGUUCGGCGC UGUGAUUGUUGGGUU CAUUGAAGCCGGCCAA UCACAGCGCCGAACGU GUCUCGUCUCGUUUCG AUCUCG	101,00	28,71	17,82	24,75	27,72	53,47	45,54	1,56	1,16	-61,30	-61,30	0,21	2,90	-60,69	-1,14
>sfr-miR-10492b-3	UUUACGAGAGCGCGU UCGAUGCUCUGAUUG GUUGAUUUAUUUGAG CUGGCCAAUCAGAGUG CCGAACGCGCUCUCGU UUC	81,00	27,16	17,28	22,22	32,10	49,38	49,38	1,86	1,22	-50,00	-48,80	0,17	3,70	-61,73	-1,25
>sfr-miR-10492b-4	UGAAUUAUAACGAAA AAAGAGCGCGUUCGA UGCUCUGAUUGGUUG UUUUAUUCAAGCUGG CCAAUGAGAGCGCCGA ACGCAAUCUCGUUUCA AUAAUAAC	101,00	21,78	30,69	18,81	27,72	40,59	58,42	0,90	1,16	-38,70	-38,70	0,20	6,21	-38,32	-0,94
>sfr-miR-10492b-5	AACGAGAACACGUUCG AUGCUCUGAUUGGUU	76,00	26,32	21,05	23,68	27,63	50,00	48,68	1,31	1,11	-36,80	-36,80	0,19	5,53	-48,42	-0,97

	GUUCAUUCGCGCCGGU CAAUCAGAGCGCUGAA CGGAUUCUCGUU														
>sfr-miR-10492b-6	ACGUUGAAACGACACC GCGUUCGAUGCUCUGA UUGGUUAGUUCAUUG AAGUUGACCAAUCACA GUGACAAACACAGUU UCAUUUUCAAUAA	92,00	18,48	30,43	20,65	29,35	39,13	59,78	0,96	0,89	-23,50	-13,80	0,04	20,53	-25,54	-0,65
>sfr-miR-10862	AUCGAACCGUUACUGC AUUUCUGUCCUCUGAU UGGCUGCUCCGAGUUA AACGACCAAUCAGAGC GCUAGUAGCGGUAAC GUUUCAAA	88,00	21,59	25,00	25,00	27,27	46,59	52,27	1,09	0,86	-31,70	-31,30	0,17	5,40	-36,02	-0,77
>sfr-miR-10492a-5	AUGCGAUCAAAACAU UACGGCAUUUUGUCCU CUGAUUGGCUGAUUA ACUUGGAGCUGCCCAA UCAGAGUGCCGAAAG UCAUCAAAAUGUCGA UCGUGU	99,00	22,22	27,27	21,21	28,28	43,43	55,56	1,04	1,05	-29,40	-26,40	0,03	13,42	-29,70	-0,68
>sfr-miR-10492a-6	GCAACAGUUUUUGAG UUUAGCCCUCUGAUUG GUUGGUCGGCCAAUCA GAGCGCCGAACGUCAU AAACCUGUCGA	75,00	25,33	22,67	24,00	26,67	49,33	49,33	1,18	1,06	-30,40	-30,40	0,13	4,01	-40,53	-0,82
>sfr-miR-10492a-7	UUACCGCGUUUAGCUC UCUGAUUGGUUAGUU AGUCGGAACAGGCCAA UCAGAGCCCGAAGCUG CGGGCU	70,00	28,57	20,00	24,29	25,71	52,86	45,71	1,29	1,18	-28,60	-28,60	0,37	4,97	-40,86	-0,77
>sfr-miR-124c	AUGAGUCCUCUCUUGG CAUUCACCGCGUGCCU UAUGAUUGCUACAUA ACACAUAAAGGCAACG	90,00	25,56	26,67	23,33	23,33	48,89	50,00	0,88	1,10	-38,80	-34,90	0,02	6,83	-43,11	-0,88

	UAGUGAACGCGAGGA GGAGGACUGCU														
>sfr-miR-124	AGCAGUCCUCCUCCUC GCGUUCACUACGUUGC CUUUAUGUGUUAUGU AGCAAUCAUAAGGCAC GCGGUGAAUGCCAAG AGAGGACUCAU	90,00	23,33	23,33	25,56	26,67	48,89	50,00	1,14	0,91	-37,90	-37,90	0,02	7,27	-42,11	-0,86
>sfr-miR-184b	UAUUGUAUUGGACAU GCCCUUAUCAGUUCUC CGUCCAGUAGUAUGA AAGCACACGGGCAAGA AGAAUGACAAGGGCA CGGCACAAUACAUUC	93,00	22,58	31,18	22,58	22,58	45,16	53,76	0,72	1,00	-29,00	-25,60	0,03	9,61	-31,18	-0,69
>sfr-miR-9544a	GCAUCUUUUAUGGCG UAUCUAUUUGCACAG UGGUCUUGCAACAAU GCUUAGGCACGUCGUC GGCAAAUAAACGUCA AGACAGAACA	87,00	21,84	28,74	21,84	26,44	43,68	55,17	0,92	1,00	-21,80	-20,50	0,04	7,51	-25,06	-0,57
>sfr-miR-466o	AUAUACACCUGCAUUC AGGUACAUACAUGCAC ACAUACACCUGCAUUC AGGUACCUACUUGCAC AUGUACAC	73,00	12,33	31,51	30,14	24,66	42,47	56,16	0,78	0,41	-19,50	-19,50	0,54	1,33	-26,71	-0,63
>sfr-miR-10506	UGGAUUCGCGUCUUU UCCUGUUUCAGAUCUA CAUCUGGCAAUGGAG AACCCAUUGGACGAUG UAGUUGUGAAACAGG AAAAAACGCUAAUGA U	94,00	23,40	28,72	18,09	28,72	41,49	57,45	1,00	1,29	-44,10	-43,10	0,33	2,28	-46,91	-1,13
>sfr-miR-297a	UGUAUGUGUCUGCCCC CAUUGUAUGUAUGUG UGCAUGUCAUAAAGG AUUUACAUACAUACA	81,00	20,99	27,16	18,52	32,10	39,51	59,26	1,18	1,13	-25,90	-25,70	0,42	2,85	-31,98	-0,81

	UACAUCGAGCAGCAAU GGU															
>sfr-miR-279d	AAUUUAACUUGAUGA UAUUUGUGGGCGAGU UUGCUUCUGGUGCAU GUUCAUUACUAAUCA UGACUAGAUCCAUACU CGUCUGCAGCCAUCAA CUUAAUCU	101,00	17,82	24,75	19,80	36,63	37,62	61,39	1,48	0,90	-34,10	-34,10	0,27	3,20	-33,76	-0,90
>sfr-miR-279	GGUCAAUUUCUUUCG AUGAGUGGAGGUUUA GUGCAUGUUUCUGUA CAUCAUGACUAGAUCC ACACUCAUCCAUGGAA GUUGCGA	85,00	23,53	23,53	18,82	32,94	42,35	56,47	1,40	1,25	-33,90	-33,90	0,18	2,37	-39,88	-0,94
>sfr-miR-10b	GAUUAACUGACACAA GUACCGACGUAUGGU AGGGCCAAGUACGUCU CUACAGUUCUAAUUG UCUAUCUACAUAAUG UACAUGUGUAGGUAC AC	94,00	20,21	29,79	20,21	28,72	40,43	58,51	0,96	1,00	-21,50	-20,60	0,04	20,59	-22,87	-0,57
>sfr-miR-10496	UCUUGGUCUUUGAUU GAAGGCACUGGUAUC UUGAGGGUACUACCU GUACCCUCUUGAUACU GCAGAUUGGCUCAAAC UCAGAUGAGCAGCA	92,00	23,91	22,83	21,74	30,43	45,65	53,26	1,33	1,10	-34,40	-31,10	0,09	10,76	-37,39	-0,82
>sfr-miR-10482	GAAUACGUAGUUCGG UAUCGGUGAAUGCCA ACGGAUUUCAUAGCU ACUAGCUAUACUCACA GAAAUCCUUUGAUAU UCACCGGUAUCGCGCU ACGUAACA	101,00	19,80	28,71	22,77	27,72	42,57	56,44	0,97	0,87	-48,60	-48,60	0,52	1,14	-48,12	-1,13

>sfr-miR-10488	UUUGUGCAAUCGAUGUUUUUUUCCAAUACUUUAGUUUUUAACCACCCUCUAGAAAACAACAUGAAUGCUGUCGGAAUAAACAUUGAUUAGGCACUCC	98,00	14,29	29,59	20,41	34,69	34,69	64,29	1,17	0,70	-20,50	-20,10	0,14	14,84	-20,92	-0,60
>sfr-miR-2335	CACAAUCAUACAUUGUCUCCCUUGUAUCUAUGUUCUGGCACACUAUGGGUUAAAUGUACCAUCUGGAUACAUAGAUAAUGAUGACUAAUUUAUUAUUUUA	101,00	13,86	29,70	17,82	37,62	31,68	67,33	1,27	0,78	-19,50	-18,00	0,09	18,21	-19,31	-0,61
>sfr-miR-71	GUGUGAAAGACAGGAGUAGUGAGAUGUCCUCGACAUCACAAAUUCUCACUACCUUGUCUUUCAUGC	67,00	20,90	26,87	22,39	28,36	43,28	55,22	1,06	0,93	-33,30	-33,30	0,47	2,73	-49,70	-1,15
>sfr-miR-9807	AACCAUUAACCUGUUGUCUGUCGGAACGCAGAUCUACGCGAGACACAAUGUGUUUUCUAUUGUGUCUUAUAUACCGACAGACAAGGGGUUAAAUGACU	99,00	21,21	28,28	20,20	29,29	41,41	57,58	1,04	1,05	-42,40	-42,40	0,15	3,73	-42,83	-1,03
>sfr-miR-10495-1	CUGCAUCAACCGGGGAUUGUCCUUUGUGUACUUCCAUAGUGCAUACAGGGAUAAUCGCCGGCUGAUGUCC	71,00	25,35	19,72	25,35	28,17	50,70	47,89	1,43	1,00	-40,00	-40,00	0,71	0,77	-56,34	-1,11
>sfr-miR-8485	GUGGUCCACACACACACACACACACACACACACGUUGUUUGUGCUUGUAUGUGUGUGGUACAUU	67,00	19,40	25,37	26,87	26,87	46,27	52,24	1,06	0,72	-27,60	-25,30	0,30	6,82	-41,19	-0,89

>sfr-miR-466f-2	UCUCACGCACUUUUAC ACACACGCAUACACAC ACACAUACACAAAGGA GGGUUGAAUUCUUAG UGUUGGUGUUUCAAA CAUUGCGGUGGUA	92,00	19,57	29,35	22,83	27,17	42,39	56,52	0,93	0,86	-22,50	-22,50	0,44	2,87	-24,46	-0,58
>sfr-miR-466f-3	CGAACUCACGCACUUU UACACACACGCAUACA CACACACAUACACAAG GGAGGGUUGAAUUCU UAGUGUUGGUGUUUC AAACAUUGCGGUGGU ACC	97,00	20,62	28,87	24,74	24,74	45,36	53,61	0,86	0,83	-22,70	-22,70	0,50	3,17	-23,40	-0,52
>sfr-miR-2733b-1	UUCGUCACUCAGUAGC GAUUGUCACACUCUCA GUAGACAGUGUGUCA UUGUGUCACUGGGUG UGUGAUAAUAGAUAC UGAUGACUAC	88,00	23,86	23,86	19,32	31,82	43,18	55,68	1,33	1,24	-40,60	-38,70	0,28	5,33	-46,14	-1,07
>sfr-miR-297b	UAUUAUGUGUGUGUG UGUGUGUCAUAUUUU UGUUGAAAUGGUGUA UGAUUGUAUUGUUGC UAUCCUAUUAUUCUU AUACAUACACACAUAC ACAUACAU	100,00	17,00	25,00	12,00	45,00	29,00	70,00	1,80	1,42	-30,90	-26,18	0,02	37,67	-30,90	-1,07
>sfr-miR-2733b-2	UGUGUGUCACUCAGU AGCCGUUGUCAUUCGC CCAGUACAUAGUGUG UCAUUGUGUCACUGG GUGUGUGAUAAUAGA UACUGUUGACUACCGU	93,00	25,81	19,35	19,35	34,41	45,16	53,76	1,78	1,33	-41,80	-40,30	0,35	3,76	-44,95	-1,00
>sfr-miR-12312	AUUAUUCAGUAUAGC AUACGCCAUACGAUUU UAAAUUUAAAUGACA AGUGAAUUAGUGGGU	88,00	20,45	30,68	11,36	36,36	31,82	67,05	1,19	1,80	-18,50	-11,70	0,05	25,16	-21,02	-0,66

	UGGAGCUGUGCUAUA UCUUUAAUGGU														
>sfr-miR-2489	UCGUAGUAGAUUGUU GUUUGUGAUGUUAAG GAAAUGUGUCAUUUU UUAUUGUAUGUUGUA UUUCGUUACUAUCGAC AAUCAAAUAUGUAAU ACAU	96,00	18,75	27,08	8,33	44,79	27,08	71,88	1,65	2,25	-19,40	-19,40	0,19	28,26	-20,21	-0,75
>sfr-miR-297	UGUAUGUAUAUUUAU AUGUAUGUAUGUAUG UGUGUGUGUAUGUAU GCAUAUAUGUAUGUA AGUAUGCAUCUA	73,00	21,92	26,03	4,11	46,58	26,03	72,60	1,79	5,33	-26,40	-26,40	0,22	2,51	-36,16	-1,39
>sfr-miR-574	CGAUUUAUUUAUGGA UCAUACAUAGACAUG UGUGUGUGUGUGUGU GUGUGUGUGAUGUGU ACAUAAUUAUGUAUU UACGAUAAAGUAUAA AGU	94,00	24,47	27,66	6,38	40,43	30,85	68,09	1,46	3,83	-21,50	-21,50	0,05	13,51	-22,87	-0,74
>sfr-miR-11660	UUCAACACAUUCGCGG GCUUUCGUCGCCAUUU UGUAAUUAAAACGUC ACUUUAUUAUUGACA UAUGUAAGAUGGCGA CGUGUCCUCGGAGUGU UUCC	98,00	20,41	22,45	21,43	34,69	41,84	57,14	1,55	0,95	-33,30	-33,00	0,10	6,25	-33,98	-0,81
>sfr-miR-1410	AAGGGAAUUUAGAUU CCGUUUAUAAUCACAA AGAGUCAUUAGUGAG UGAGACGGAGUCGUG AGUAAUUCCUAC	74,00	24,32	32,43	13,51	28,38	37,84	60,81	0,88	1,80	-24,00	-20,80	0,11	10,59	-32,43	-0,86
>sfr-miR-306a	UGACAGUCAGGUACU AGGUGACUCUGAGUG AGUGUGCCCGUGUCAG	70,00	34,29	18,57	20,00	25,71	54,29	44,29	1,38	1,71	-22,10	-20,90	0,39	6,69	-31,57	-0,58

	GUACUAGGUGACUCU GAGUGGCC														
>sfr-miR-466i-1	CCUACAGAGCACAGAU AAUCACAGGAAAUGU UUUCGGCUCAUGAAU AUUUAACGACAUUAG GAUGCAACGUAAUUCC UGUGUGUGUGUGUGU GUGUGUG	100,00	25,00	27,00	16,00	31,00	41,00	58,00	1,15	1,56	-27,20	-24,10	0,08	14,52	-27,20	-0,66
>sfr-miR-1b	CACGCCCGCGCAGCUC CAUACUUCUUUACAUU CCAUACGAUUAUAAGC ACUAUGGGAAGUAAG GAAGCACGGAACUCGC GCAAGGCUUU	90,00	21,11	27,78	27,78	22,22	48,89	50,00	0,80	0,76	-33,20	-33,20	0,67	1,87	-36,89	-0,75
>sfr-miR-1770	AUCGCAAGUUACUGA GGUGACGAGGGAAGU GAGGAGGCCUGCGGG GGAGGAGGGAGACCG GUCCAACCUCGUCACU AGGUAUACUCGCAGU	92,00	38,04	23,91	20,65	16,30	58,70	40,22	0,68	1,84	-34,90	-30,60	0,25	27,59	-37,93	-0,65
>sfr-miR-466f-4	GUAACACAUGCACAUG CGUGUAUACGUCGUA UGAUAGACGUGAAGA CGUGUGUGUGCAUGU GUGCA	67,00	29,85	25,37	16,42	26,87	46,27	52,24	1,06	1,82	-33,60	-33,60	0,24	4,36	-50,15	-1,08
>sfr-miR-1	AAAGCCUUGCGCGAGU UCCGUGCUUCCUUACU UCCCAUAGUGCUUAUA AUCGUAUGGAAUGUA AAGAAGUAUGGAGCU GCGCGGGCGUG	90,00	27,78	22,22	21,11	27,78	48,89	50,00	1,25	1,32	-42,70	-42,70	0,14	9,22	-47,44	-0,97
>sfr-miR-10235	CCCUGUAACGAAACGU CACGUGCAUCCUACUC CCUACACACCAUGUAA AUGUGUGGACGAGUU	93,00	21,51	27,96	25,81	23,66	47,31	51,61	0,85	0,83	-23,20	-23,20	0,10	10,52	-24,95	-0,53

	GACUGCGACAUGAGU AAUCGUGAGCAUUA															
>sfr-miR-92a	GGAGGCCCGUGUGGCU GGGCGGUGACUGGCGC UAUAUUCGGUUUGUG UGCGAUAUUGCACCAG UCCCGGCCUAUCCAAG CGGGCCCGU	89,00	35,96	12,36	26,97	23,60	62,92	35,96	1,91	1,33	-47,80	-47,70	0,08	5,88	-53,71	-0,85
>sfr-miR-10475	UGUGUGAAUUGAAGC GUCUCCGUGUGGUCGG CGAACACUGCAACAUG CGACAUGUCGGUACCA UACUCCACCACACGGA GAUGACUCUGAUUCAC UCA	99,00	24,24	24,24	27,27	23,23	51,52	47,47	0,96	0,89	-42,80	-42,20	0,07	9,00	-43,23	-0,84
>sfr-miR-989	GAAGUUCAGGGAUGA AAUGGCCAUUACCUCA CAGUCACGUGGUCAUG ACGAUACAACGUGUG AUGUGACGUAGUGGA AGUCUCGACCUGAACG GU	96,00	29,17	27,08	19,79	22,92	48,96	50,00	0,85	1,47	-37,80	-37,80	0,16	6,47	-39,38	-0,80
>sfr-miR-7398f	UCGAAGUGUAGAAGA GAGUGAUGCCGACGAC CACAUCGUCUGCAGCA CGCUGACUGUCGACAC UUAUU	69,00	26,09	26,09	24,64	21,74	50,72	47,83	0,83	1,06	-21,90	-21,30	0,18	7,11	-31,74	-0,63
>sfr-miR-4756	GCUGCUGAACAAGCGU GAAGCGUGAGUGAGG GAGGCGCUCACUCCUC GCCCUCACCGUCACCG UCCUCAGCGUUUUCAG CCGU	84,00	28,57	16,67	33,33	20,24	61,90	36,90	1,21	0,86	-30,80	-26,10	0,01	14,93	-36,67	-0,59
>sfr-miR-10508a-1	CGUCGCACGCGCCGCA UAGUACGCGCGAUUCC AGGCAGAGGGCGCGA	100,00	35,00	19,00	30,00	15,00	65,00	34,00	0,79	1,17	-34,10	-33,80	0,04	19,68	-34,10	-0,52

	UGGGCGCGAAACUCGA GUCGUCAGUAGCAGCG UGACAAUCGCCGCGUC GUGU														
>sfr-miR-10508b-1	UUUCAUGACCAGCUUU CUGCGACAAUCGUAUC GCGCGUAGCAGCGCUG CAAUCGCCGCGUCGUC GUGUGUCGCGGAAUG CUAAGUCCUGGUC	93,00	27,96	16,13	29,03	25,81	56,99	41,94	1,60	0,96	-36,70	-32,30	0,05	11,76	-39,46	-0,69
>sfr-miR-137b-1	UGGAGGUCACGCGUA UUCUUGGGAAUUAAC ACACAUUACGAGAUG UUAUUGCUUGAGAAU ACACGUAGCUGAC	74,00	24,32	28,38	17,57	28,38	41,89	56,76	1,00	1,38	-21,20	-21,10	0,06	3,84	-28,65	-0,68
>sfr-miR-283	GAAUCGGUUUCCCGGC UAAAUAUCAGCUGGU AAUUCUGGGGUGCUG UCGCCCAGACUACCAG UUGGUAUGAGGCCGG GAAUCGAACG	88,00	30,68	21,59	22,73	23,86	53,41	45,45	1,11	1,35	-50,70	-49,90	0,09	6,05	-57,61	-1,08
>sfr-miR-137b-2	GUCACGCGUAUUCUUG GGGAAUUAACACACA UUACGAGAUGUUAUU GCUUGAGAAUACACG UAGC	66,00	22,73	28,79	18,18	28,79	40,91	57,58	1,00	1,25	-18,80	-16,40	0,14	5,04	-28,48	-0,70
>sfr-miR-7086	UACAAUUACGAAACCG GUAUUCCUUGCGACAA AGCAAAGGAAGAGCA AGAGGAGAAAGGUUU CGUAGAUUACC	74,00	24,32	37,84	17,57	18,92	41,89	56,76	0,50	1,38	-21,10	-21,10	0,26	8,33	-28,51	-0,68
>sfr-miR-3529	UGAGCCGUACUUUGU AGGGAGAUUAGUGAU UUCUUGUUACCCAAAU CAAAACAACAAAAUCA	90,00	18,89	31,11	21,11	27,78	40,00	58,89	0,89	0,89	-33,90	-33,90	0,30	5,15	-37,67	-0,94

	CUAGUCUUCCAAACAA CGUGUGGCCUG														
>sfr-miR-7b	CAGGCCACACGUUGUU UGGAAGACUAGUGAU UUUGUUGUUUUGAUU UGGGUAACAAGAAAU CACUAAUCUCCCUACA AAGUACGGCUCA	90,00	21,11	27,78	18,89	31,11	40,00	58,89	1,12	1,12	-27,60	-27,60	0,03	12,33	-30,67	-0,77
>sfr-miR-34	CUUCUGUAAGGUAGU CCGCGUUGGCAGUGUG GUUAGCUGGUUGUUU AAAUUGCUACAACAGC CAGUAACGACACUGCC UCCGCGUGCUACCUAA AGCAA	100,00	25,00	23,00	24,00	27,00	49,00	50,00	1,17	1,04	-52,50	-52,50	0,12	4,04	-52,50	-1,07
>sfr-miR-33	AAUAACUAUUACGGG AGUGCAUUGUAGUUG CAUUGCACUGGUUUAC AUGAGUUGUGCAAUA UUACUACAAAGCAAA UCUCGCAUUAGUUGCA	93,00	20,43	30,11	16,13	32,26	36,56	62,37	1,07	1,27	-31,50	-30,60	0,24	3,50	-33,87	-0,93
>sfr-miR-10481	GUAAAACGGGGUGAA UAGAAUUCGAGGGGU UAAUAGAUACAGAAU AGGGGUGAAUAAAUG AUGGGAAAAUGUCCU UUGAAUACUAUUCACC CCGUUUCAC	101,00	25,74	34,65	12,87	25,74	38,61	60,40	0,74	2,00	-34,60	-34,04	0,03	8,70	-34,26	-0,89
>sfr-miR-509	GCAGAGUAGUUUCAA AAAGUGACUGAUUGA UACGUCUGUCAAUUCA GUUCUAUUUGCACUA UUCAAA	68,00	17,65	30,88	16,18	33,82	33,82	64,71	1,10	1,09	-20,10	-20,10	0,23	2,58	-29,56	-0,87
>sfr-miR-8327	UGAUUUAUUAAUACG AGUACAUUCCCUACCU AGUGAAGCACGUGUA	74,00	20,27	25,68	13,51	39,19	33,78	64,86	1,53	1,50	-21,20	-21,20	0,16	3,10	-28,65	-0,85

	GGUUUUGUAUUUCGU UUUGAUAAAGGU														
>sfr-miR-10483	UGAAGUGUGUACUUA CGUCACCUGUAAUUGU UCGUUCCCUUUUGUAA AUAAUUAAGGGGCCG AACAAUUCCAGACGAU GUUUGAACAGACUCG U	95,00	21,05	26,32	18,95	32,63	40,00	58,95	1,24	1,11	-34,90	-33,20	0,09	5,68	-36,74	-0,92
>sfr-miR-100	CGGUUAAGCGGAGAC AUUUCCACCCUUUGAA CAAGUUCUUGUUUAU GUUUGCUCAUUAAAC AAAUAAAAGUGGAAA UUCUGUCUUAAUCU	91,00	16,48	29,67	17,58	35,16	34,07	64,84	1,19	0,94	-23,60	-23,60	0,15	10,43	-25,93	-0,76
>sfr-miR-8456	CAAUUACAGUUUGCA ACAUUGUUUUAAUUA CUUACUUUGUUUUAU UUACUUUUUUAAACG AGUAGGUAUUGUUGG GAACCAGUGUUUCAA AUGAUAAGAU	101,00	15,84	28,71	10,89	43,56	26,73	72,28	1,52	1,45	-22,00	-22,00	0,05	10,15	-21,78	-0,81
>sfr-miR-11902	AUCAUAGCUUGAGCU AAUGACGCGUUAAAG UUCUAAACUUCAACGA AGCGUUGAAAAUAUG ACAAGUGAUAAGCGC AAGCGAUUCA	87,00	20,69	36,78	17,24	24,14	37,93	60,92	0,66	1,20	-20,20	-19,70	0,12	7,17	-23,22	-0,61
>sfr-miR-6399	CUCAGAGACAUUAAU GAUUACUUACACUAA UCUUUUGCAAUGAUG GUAUUAAUCGUAUCG UAUAUCGUAACAAUU AAGCAAUCAUUAAUU GUCUCUAAG	100,00	13,00	34,00	16,00	36,00	29,00	70,00	1,06	0,81	-23,00	-23,00	0,03	13,01	-23,00	-0,79

>sfr-miR-2767	GGAAAACUUUUACCG UCGACACAAGUAAAUC UCGUGCGGCUUGUGU UUAGAUUAAGCCGUU CGAGAUUUACUUAAG UCAAUGGUAAAUAUU UCUA	96,00	19,79	29,17	16,67	33,33	36,46	62,50	1,14	1,19	-43,80	-43,80	0,23	4,07	-45,63	-1,25
>sfr-miR-2218b	UGAAUAAAUCAAUGU GUCUUUAAACCAAAU UAUGAGUACGAUACA UUAGUGGUAUAUAAA UUUGUAGUUUGUAAG GAUAAAUUGAAGUAU GCC	94,00	18,09	37,23	8,51	35,11	26,60	72,34	0,94	2,13	-19,70	-19,70	0,24	7,92	-20,96	-0,79
>sfr-miR-10489-1	AUACCAAGUCGGUUA AUCUAUUUAGUGUAU GAUAUCAUGCCAAUA UUAAAAAUAUUUAAC GUUUUAAACAAAUAG AUCGACUUGGACA	89,00	13,48	38,20	13,48	33,71	26,97	71,91	0,88	1,00	-19,60	-18,70	0,04	13,09	-22,02	-0,82
>sfr-miR-10489-2	GUAGUGUGAUACAUA CUAAUAAUCAAAUCA AGCGAUGUCCAAGUCG AUCUAUUUGUUUAAA ACGUUAAAUAUUUUU AAUAUUGGCAUGAUA UCAUACACU	101,00	13,86	35,64	13,86	35,64	27,72	71,29	1,00	1,00	-18,90	-17,30	0,05	12,21	-18,71	-0,68
>sfr-miR-9518	UGGUCCAGAUCUAGA UUAUCAACCAUUAAU UAUUUUUAUUGAUAU UGUCUGCAAUAUCAU AAGAAUGUAUCAUUU GAGACAAUGAGUCUG GAAUA	96,00	15,63	33,33	12,50	37,50	28,13	70,83	1,13	1,25	-22,60	-20,70	0,06	8,64	-23,54	-0,84
>sfr-miR-10474-1	GAAUAACGCGGAAUU AGAUUGUGAUUUUUU	98,00	17,35	35,71	9,18	36,73	26,53	72,45	1,03	1,89	-25,60	-24,40	0,12	8,06	-26,12	-0,98

	UUAUGUUGAUAGAUU AACGUAUGAAGAGUA UACUAUAUAAAUAUC UCAAUCUAAUAUGGC GUUAAAC														
>sfr-miR-10262	UAAGUAGAAGAUGUG AUGUUAUGUUGGUAG CUUUUGGUAGGAUGA UGGUCUACCAACAUUA AUAACAAGUUCGACAC A	79,00	24,05	31,65	11,39	31,65	35,44	63,29	1,00	2,11	-20,10	-20,10	0,06	6,82	-25,44	-0,72
>sfr-miR-10504	UCAGACCUCUCGACCA GUCGUACAUAGAGACC UGAAGUAUUCACAGC UUCAAAUCUCUGUGU AUGAUUGGCCGCGGGC GUCCCA	85,00	22,35	23,53	28,24	24,71	50,59	48,24	1,05	0,79	-42,30	-42,30	0,90	0,38	-49,76	-0,98
>sfr-miR-10474-2	AUGAACGCGGAAUUA GAUUGUAUUUUUUUA UGUUGAUAGAUUAAC GUAUGAAGAGUAUAC UAUAUAAAUAUCUCA AUCUAAUAUGGCGUU AAA	94,00	17,02	36,17	8,51	37,23	25,53	73,40	1,03	2,00	-23,20	-22,00	0,23	6,42	-24,68	-0,97
>sfr-miR-10761	CGGGGAUAAAAGGCG UUACGAUUAUUCUUU UGAAUUUGUUCUUGU AUACUAACACGAAGA AUAAUAAAAGCAUUU CGUCACUGUUGUUCAU CCGUG	97,00	19,59	28,87	15,46	35,05	35,05	63,92	1,21	1,27	-19,20	-15,40	0,02	12,97	-19,79	-0,56
>sfr-miR-3924	UAAUAAGGUAUCGUU UUAGUAGUUAAGGAA UACAUAUAUUUUGGC ACUUCCCUCAAACUUA UAUAUGUAUAUGUGA	98,00	15,31	31,63	13,27	38,78	28,57	70,41	1,23	1,15	-19,20	-17,80	0,08	20,17	-19,59	-0,69

	CUUCAAAAGUUACGU GUACUU														
>sfr-miR-9051	GUGAUGUUUUAGAAU UUAGACUCUUGAUAG AAUAAGGACUUAUUU UGUAGCAGACUUUUU UUAAACAAGUGUCUA UGGACUCCAAAACAUU AU	94,00	17,02	31,91	11,70	38,30	28,72	70,21	1,20	1,45	-19,40	-19,40	0,03	12,42	-20,64	-0,72
>sfr-miR-10500-1	GACGCAUAUUCAGUCC AACAGUGACCGUCAUA GAGAUAAGGAAGCGU GUUCGGGCAUGUAUU CGUCGUAGGGGAGGU CACUUUGAACAAAAU UUGCAAA	100,00	27,00	30,00	18,00	24,00	45,00	54,00	0,80	1,50	-28,30	-28,30	0,28	4,38	-28,30	-0,63
>sfr-miR-10500-2	GUCCAAGAGUAACCGU CAUAGAGAUAAGGAA GUGUGUUCGGGCAUG UACUCGUCGUAGGGA AGGUUACUUUGAAC	76,00	30,26	27,63	15,79	25,00	46,05	52,63	0,90	1,92	-20,00	-20,00	0,20	4,83	-26,32	-0,57
>sfr-miR-10500-3	AAUUCUUCUCUGACGC AUAUUCAGCCCAAGAG UGACCGUCAUAGAGA UAAGGAAGUAUGUUC GGGCAUGUAUUCGUC GUAGGGAAGAUCA	91,00	25,27	28,57	18,68	26,37	43,96	54,95	0,92	1,35	-24,10	-24,10	0,07	10,71	-26,48	-0,60
>sfr-miR-279b-1	ACUCCUACUUGCUCCU AAUGGGUAUAUGUCU AGUGCCACAGUGAAU UAUUUCUGUGACUAG AUCUACACUCAUUGAG AGCUUUUAGGCCU	91,00	18,68	23,08	21,98	35,16	40,66	58,24	1,52	0,85	-32,20	-31,30	0,22	3,15	-35,38	-0,87
>sfr-miR-10461b-1	ACGGCUUGAAACUAG UCGAGUUCCUCGUUAU UUAUUAUGUUAUCGG	95,00	20,00	25,26	17,89	35,79	37,89	61,05	1,42	1,12	-44,80	-44,80	0,13	6,97	-47,16	-1,24

	CUUAGUCACGUUAUU GUAUGACGAGAAACU CGACUAGUUUCAAGCU AU														
>sfr-miR-279b-2	UGUAAGCAGCCUCCAC GGAAGAUAAGCGAUA UUCUAGUAUCAAGUU GUUUACGUAUCUUGA CUAGAUUAUCACUUA UCCUCUGAGGUCGCUU UCG	96,00	19,79	25,00	20,83	33,33	40,63	58,33	1,33	0,95	-34,10	-34,10	0,02	5,19	-35,52	-0,87
>sfr-miR-10847	AAUGAGAAUGAUUGA UUGCGUGUCCCGCUCG CACCUGACAGGAACAA UUUUACUACACACGAU CAAUCAUUCUCGAC	78,00	17,95	29,49	25,64	25,64	43,59	55,13	0,87	0,70	-26,40	-22,90	0,16	5,75	-33,85	-0,78
>sfr-miR-10480	GCAAGUUUCCGUGGCG UGUUUAGCCGUCACAG UGACAUUUUUAAACU ACAUAAUGUCACUUU GACAGCAAACCACAAC AUGGAAAACUAAC	92,00	17,39	32,61	22,83	26,09	40,22	58,70	0,80	0,76	-33,10	-33,10	0,05	6,62	-35,98	-0,89
>sfr-miR-2779-1	CCCAACCGAGCCAUCA UAAAAUGAUCCGGCUC GAAGGACCAUUUUAU GAGCGCUGCCGCGUUA AG	66,00	22,73	27,27	28,79	19,70	51,52	46,97	0,72	0,79	-20,00	-20,00	0,36	12,83	-30,30	-0,59
>sfr-miR-232b	GUCUCACAAUACCGAA CAAAGAGAAAAUGCA CCUUAACUUAGCAAUA UUUAAGGAUGUAUUU UGCUUGUUUGGAAAU GGAAUU	84,00	17,86	35,71	14,29	30,95	32,14	66,67	0,87	1,25	-20,50	-18,30	0,04	7,55	-24,40	-0,76
>sfr-miR-5394	AAUUUGUCGAUUUUC ACCAUCAGGAAAACUA CACUGUUACUUGGACA	93,00	17,20	37,63	17,20	26,88	34,41	64,52	0,71	1,00	-21,30	-21,30	0,41	3,62	-22,90	-0,67

	GAGCUAAAUUGUUAA AGCUAAACUGAUUAU GAAAAGAGACAACCG														
>sfr-miR-6948	UGGUGGAGUUCAUGC GGAAAUAAACUUGCA GCUGGCCGUGAGCGAU AAUUAGAUAAGUUCA GACAGGACUGCAAUU AAUUAACCUAUUGAA CAACCAGAU	101,00	23,76	33,66	16,83	24,75	40,59	58,42	0,74	1,41	-23,20	-23,20	0,10	8,32	-22,97	-0,57
>sfr-miR-83	CUCCUCAAGAUAGCAA UUACAAACUUAUUCA AAAGCAAUUCAUCCAA AACUGAAUUUAUGUG UGAAUGCGUUUGCAU CGUGACCUUGAGCGG	93,00	17,20	32,26	20,43	29,03	37,63	61,29	0,90	0,84	-19,40	-19,10	0,05	5,86	-20,86	-0,55
>sfr-miR-10469	GUGUGCAUGCUAGUA AAUAAACACAUUUGU CAUCAAACGGGAUCCU GUAAAAAACUGGACU GCCGUGUGAAAAGGC UGUAUGUAGGUAACU ACAGCAAAA	101,00	22,77	35,64	16,83	23,76	39,60	59,41	0,67	1,35	-23,80	-21,40	0,15	14,94	-23,56	-0,60
>sfr-miR-8468	CCGUAACUAUCUAAAU AUCCAAUAAUGUAUU AUGCAAUAAGUAAUU UGAACAAGGCAAACU GCUUUGUUGUAUUAU AAUAAUAGUUUUAGA UAGAUAUAA	101,00	12,87	39,60	10,89	35,64	23,76	75,25	0,90	1,18	-18,90	-14,20	0,01	23,52	-18,71	-0,79
>sfr-miR-2763	AAAUCUACAACGUUCU CCAAAGUAGUGAACG UAAUUUAUCGUGAGG UAAAUCAAUAGAUAU UAUGCUCAUUACUUU	95,00	16,84	34,74	13,68	33,68	30,53	68,42	0,97	1,23	-33,70	-33,70	0,17	6,67	-35,47	-1,16

	GGAUAUGUUGUACGAAUA														
>sfr-miR-2527	AUCCAAACACAUCAUCGCAUCCGUGUCAUUAACUCCUAAAGAAGUGAGUUCUUCAGCGCAGGCAUCAGCAUCUGAUGGAUGUAGGUGUGUGACU	95,00	22,11	27,37	23,16	26,32	45,26	53,68	0,96	0,95	-25,50	-25,50	0,14	16,62	-26,84	-0,59
>sfr-miR-95	UACGUAGUAACAGCAUUCAAUGGGUAUUUACGAGUGUGAAAUAGCCACAAAAUAUGCUAUUACUACAUU	70,00	17,14	35,71	15,71	30,00	32,86	65,71	0,84	1,09	-20,50	-20,50	0,13	6,65	-29,29	-0,89
>sfr-miR-10498	GCGAGUGUCUCGCGUCUUUGGUCAACGUUCAACACAGCAGUUCAGUUGCUGUUUUGGACAUUGGUGUAAGGCGCGGAUACUGUA	85,00	29,41	18,82	20,00	30,59	49,41	49,41	1,63	1,47	-38,30	-38,30	0,13	3,71	-45,06	-0,91
>sfr-miR-10460	UGAGCCAAUGUUCGUUAGUGAUGGAACUAUUCCCAAAGUUCAAUUACUUCCAUAGCAUUGGCAGA	66,00	19,70	28,79	19,70	30,30	39,39	59,09	1,05	1,00	-22,80	-22,80	0,47	1,35	-34,55	-0,88
>sfr-miR-1415	ACCCACUGACAUUUGCAGGCGCUGUAAUUCUGCUUCUGAAACCAUUCAGCAUAUUACAGGUGCCUGCAAAGCCAUUGUCC	81,00	18,52	24,69	28,40	27,16	46,91	51,85	1,10	0,65	-29,40	-29,40	0,06	7,21	-36,30	-0,77
>sfr-miR-2172	ACUCAUAUUUCUGUUUAUUGAUGCCUACAUGUAUAGUAUUACAACUAUGUGUUGUACAACAUAAUAUACCUAUCA	93,00	12,90	32,26	15,05	38,71	27,96	70,97	1,20	0,86	-19,10	-17,20	0,04	12,18	-20,54	-0,73

	AUGUAUAGGAUAUGUCA															
>sfr-miR-7360	CUUGCCAACAAUAUUGUAUCACAGAUUCAUCCUAGGUCAUGCUAUAAUACCUAGGAAGGAAAAAUAUGUACAUAUUUAUGGCCGU	86,00	16,28	33,72	18,60	30,23	34,88	63,95	0,90	0,88	-23,10	-23,10	0,14	6,02	-26,86	-0,77
>sfr-miR-7866	GAUCUGGGAUGAGACAUUGCAGUUUCUUGUUAAAUUAUGAUAAUUUUAAAGUAAGUAUGCAUAAUUUAAAUAAAUAUCAUUGUGAAUCGCAGGCA	96,00	18,75	35,42	9,38	35,42	28,13	70,83	1,00	2,00	-19,60	-19,30	0,06	9,57	-20,42	-0,73
>sfr-miR-8408	UUACGGGACGUCGGCCUGCACAAGUAUAUUGGUGAGUUAUGAGAUCUACUUCCCAUAAUUAUAUUAUACUAGCUAGCCCGGCAAACGUACCGCCU	96,00	20,83	26,04	23,96	28,13	44,79	54,17	1,08	0,87	-25,70	-23,40	0,04	16,15	-26,77	-0,60
>sfr-miR-308	GACGACGCUUCCGCGCGCAGUAUUAUACUUGUGAAUGUGUUCCUACUACAAUCACAGGAUAAUACUGCGAGUGGCGGCGUCCAC	85,00	24,71	23,53	25,88	24,71	50,59	48,24	1,05	0,95	-45,90	-45,90	0,51	1,87	-54,00	-1,07
>sfr-miR-3404	AGUAAGAGAUGGAAUCAUUUCUGCAAUAGAAUUACGAUAUAUUGCAGAACGUGGAGCUUCCAGACUCGUUCAG	74,00	22,97	32,43	16,22	27,03	39,19	59,46	0,83	1,42	-21,20	-21,20	0,56	2,46	-28,65	-0,73
>sfr-miR-12354	CCGCAUGUCUGUAAUAUCCAUUCGAGUACGGUACUACUUGUAGUUUC	97,00	18,56	25,77	16,49	38,14	35,05	63,92	1,48	1,13	-20,40	-20,20	0,02	10,39	-21,03	-0,60

	AUUUAAGUUAUUUGAGUGAUCUCCAUACUGAAUGAUUUCAAAGAUGUGU														
>sfr-miR-10453-1	GGUGCACCGUGCAAAUGUCAUUGAUACGCGUAUUCCAUUUUAUUUAGGAGCGUAAUUUCGCUACUAUAGUUUCAUGGACGAUUGGCCCAGUGCGUU	97,00	23,71	21,65	19,59	34,02	43,30	55,67	1,57	1,21	-21,40	-15,70	0,01	21,51	-22,06	-0,51
>sfr-miR-10453-2	GGUGCACCGCGUAAAUGUCAUUGAUACGCGUAUUCCAUUUUAUUUAGGAGCGUAAUUUCGCUUCUAUAGUUUCAUGGACGAUUGGCGCAGUGCGUU	97,00	24,74	20,62	18,56	35,05	43,30	55,67	1,70	1,33	-30,20	-29,80	0,07	6,48	-31,13	-0,72
>sfr-miR-3767	CUAAUCGAUACAAGCGAUGUAGAUAAUUGAGUAAAUAAACAUGUAGACACACAGUCUGCACGCUUUACACAUUAUUUACUAUCGACUGAUAUACUAUCAA	101,00	13,86	37,62	18,81	28,71	32,67	66,34	0,76	0,74	-22,40	-20,50	0,04	9,72	-22,18	-0,68
>sfr-miR-3618	UUUCUAGACCACAUCUGUCUACAUUAAUGAAUCAUAAUGUAUCAUUUCAUGCUAAUAGUAUGCGGUCUAGGCA	74,00	14,86	29,73	18,92	35,14	33,78	64,86	1,18	0,79	-20,30	-19,90	0,13	5,01	-27,43	-0,81
>sfr-miR-929	CCCAGCUCAUGGAUGUUAAAUUGACUCUAGUAGGGAGUCCGUAUGUACGCGCGACUCCCUAAUCGAGUCAGGUUGACUUCUUUGAGGCUAAC	93,00	24,73	22,58	23,66	27,96	48,39	50,54	1,24	1,05	-42,50	-42,50	0,17	3,33	-45,70	-0,94

>sfr-miR-10455	UGGUCUCGGAUGUCUUUCUAAAUCAAUCAAAUCUAUGAUGAGAUCUUGCUACAUAUCGACCUUCCUUGGGUAUAGUUAGACGUCCGAGAUGU	93,00	20,43	24,73	19,35	34,41	39,78	59,14	1,39	1,06	-28,70	-27,50	0,13	11,11	-30,86	-0,78
>sfr-miR-iab-4	CGCGUCGCCUUCUGUACGUAUACUGAAUGUAUCCUGAGUGCCUCAUUCUUUCCGGUAUACCUUCAGUAUACGUAACAGAAGAUGACACU	90,00	18,89	23,33	25,56	31,11	44,44	54,44	1,33	0,74	-38,30	-32,60	0,14	7,89	-42,56	-0,96
>sfr-miR-252a	GAAAAAACUAAGUCUUGUUCCUAAGUACUAGUGCCGCAGGAGUGUUACUAAUUUCUCCUGCUGCUUCAGUGCUUAUCAAUGAGUAUUUAGUGUUACA	98,00	19,39	26,53	18,37	34,69	37,76	61,22	1,31	1,06	-33,70	-33,70	0,28	3,43	-34,39	-0,91
>sfr-miR-3532	CUAAUUAUUUGUAGCACUAAUUAGCUGAUGUUGCACUGCAGCUGCUGAAAAACUUUCAGUGUAACAUACAAUGUAGUUGUACUGCGAAAUGAUAUG	97,00	19,59	30,93	15,46	32,99	35,05	63,92	1,07	1,27	-23,90	-23,00	0,04	10,33	-24,64	-0,70
>sfr-miR-2756	GUUGGGUUGGUAGCAUACCCUGUAGCUGCUAAGGGGCGCAGUUUAAUACCUCCCCUUUGCUGCUACAUUGUAUGCUACGGACCCUUG	88,00	26,14	17,05	25,00	30,68	51,14	47,73	1,80	1,05	-46,90	-45,90	0,27	2,82	-53,30	-1,04
>sfr-miR-10476	ACCGAAAGUUUACAUCGAGCGAUGGUUGGAAUCCAACUGUUGAUACA	89,00	21,35	26,97	17,98	32,58	39,33	59,55	1,21	1,19	-35,10	-35,10	0,35	4,02	-39,44	-1,00

	CAUUGGAUUUUAAGU UCCAUCGCUAGUUGUG GACUAUCUUA														
>sfr-miR-6844	AGCAAAUUAAUAGUU UUCUUUGUUUUUAAU UGGUUUUACGACAAG UUAUAUGCAAUUUGU CGUCUAUGAAAAACA UUCAAAACGUAUUUU UUGCU	96,00	13,54	30,21	11,46	43,75	25,00	73,96	1,45	1,18	-23,30	-23,30	0,12	5,95	-24,27	-0,97
>sfr-miR-9789	UUGAAAAAGGCAUUC UAAGUUUAGCUUCCCA UUCCAACUAGUUGGU AUUUAGAUUGAAGGC CAGUUAAACGACAAU GUUUUUUAUA	87,00	17,24	31,03	14,94	35,63	32,18	66,67	1,15	1,15	-19,00	-18,60	0,20	5,36	-21,84	-0,68
>sfr-miR-133	CGUUGUCCGCUUUAGC UGGUUGACUUCGGGU CAAAUUGUAAUUUGG AUAUCAUUUGGUCCCC UUCAACCAGCUGUAGU UGACAUCU	87,00	21,84	18,39	21,84	36,78	43,68	55,17	2,00	1,00	-33,70	-33,40	0,34	4,40	-38,74	-0,89
>sfr-miR-133c	AGAUGUCAACUACAGC UGGUUGAAGGGGACC AAAUGAUAUCCAAAU UACAAUUUGACCCGAA GUCAACCAGCUAAAGC GGACAACG	87,00	21,84	36,78	21,84	18,39	43,68	55,17	0,50	1,00	-29,80	-29,80	0,32	3,80	-34,25	-0,78
>sfr-miR-12357	CUGAAAUGAAAAUAA UUCGGAUAAUUUACU GGCACCCUAACUUCUG CCAGUUCAUCCGAACA AUUCAUUUUCC	74,00	12,16	31,08	24,32	31,08	36,49	62,16	1,00	0,50	-21,40	-21,40	0,28	3,69	-28,92	-0,79
>sfr-miR-10029	AGGUCUAUUAUCGUU UGGCAAAUUUGAAUA UUUGCUAAGAUCUGC	100,00	19,00	30,00	13,00	37,00	32,00	67,00	1,23	1,46	-20,60	-20,30	0,05	15,47	-20,60	-0,64

	UCUUAUACCAGUUCGU AGUAGGCCAAAUAAC AUUUUUGAAAGGUUA AGAGAUUU														
>sfr-miR-4331	GUUAGUCAAAGAGUA UAGGCUGUGGCUGUG GUGUAGGUUAGUGUU AGUCUAUGGUCGUGU UGUGUGAAUGAUACG GCCUAAUGCUAUUUCA CUGAU	97,00	30,93	20,62	11,34	36,08	42,27	56,70	1,75	2,73	-28,40	-25,80	0,13	26,79	-29,28	-0,69
>sfr-miR-745	CAAAACUCUUGUCGGU UGCGGCUCAUCGUAUG GCAGUUUGCUGUGUA GUAUUGCCAGCUGCCU AGCGAAGGGCAACAA AUGACUGAGUUGAU	93,00	27,96	22,58	20,43	27,96	48,39	50,54	1,24	1,37	-37,90	-36,10	0,20	4,12	-40,75	-0,84
>sfr-miR-96	UUUCACUAAUCUUAU UCAUUGGUACACAUA GCUUGGCACUGGCGGA AACGGACUCAGCUAAG CUAUGUUUUUUAUAU GGAAAGAUACGUGCU A	94,00	20,21	27,66	18,09	32,98	38,30	60,64	1,19	1,12	-26,90	-25,80	0,17	4,91	-28,62	-0,75
>sfr-miR-10507-1	GGAGAACCGUCCAAGU UUAAUCUGUAGUGGU CUUUUGAGGAAAGAA CCACAGCAGUUUGAGC GGACUUGUUCAGA	76,00	27,63	27,63	17,11	26,32	44,74	53,95	0,95	1,62	-27,50	-27,50	0,23	4,28	-36,18	-0,81
>sfr-miR-375	GACAUGACAGCGAGAC AACCCGAGCGGAUUGA GCAAACUAUUUGUCU GUUAGCAAGUUUGUU CGCCCCGGCUCGAGUC GUCUGGCGUCAUACC	94,00	26,60	22,34	26,60	23,40	53,19	45,74	1,05	1,00	-39,30	-39,30	0,17	4,38	-41,81	-0,79

>sfr-miR-10507-2	GUCUCUUUUAAAGUCC AGACAAAAUCAAUUA AAGAUGUCGGAGAAC CGUGCAAGUUUAUUC UGUAGUGGUCUUUAG AGGAAAG	84,00	22,62	32,14	14,29	29,76	36,90	61,90	0,93	1,58	-20,00	-17,20	0,15	6,98	-23,81	-0,65
>sfr-let7f	GCUUACAUAAAUUGA AAUGGUUUUGGAGCU ACAGUAGGCUUUGGU UGUAGAGCUAUACAG UCUACUGUAGACCUAA AGACAAAAUCAAAAU AUUUAUUU	100,00	19,00	34,00	13,00	33,00	32,00	67,00	0,97	1,46	-28,80	-27,30	0,07	10,45	-28,80	-0,90
>sfr-miR-2796	AUUAAGUACAGUCAG UAGGGGUUUCUUUCG GCCUUCAGCUGUUGAU AGACUGUAGGCCGGCG GAAACUACUUGCCGAC UCUACUUCUA	89,00	24,72	21,35	22,47	30,34	47,19	51,69	1,42	1,10	-39,60	-39,60	0,28	2,14	-44,49	-0,94
>sfr-miR-10462-1	GUAAAAUUUAAGCAG UCGGGACCCAUUCUAG AAAGCAAGCCGUAUG UGCCCUCACAAAGUCU UUAUAUUUAGAAUGG GUCCCGACUGCUUAAA UUUUAC	100,00	19,00	30,00	21,00	29,00	40,00	59,00	0,97	0,90	-55,50	-54,50	0,36	2,81	-55,50	-1,39
>sfr-miR-10471-2	AGCUAAAAAAAGUAG ACCGUUCAGUACCUUU AGUACGAGUUUAUUU UACGUUUAACCGGUGC UCUGAUUGGUUAGUU UUUUUAUAC	87,00	18,39	26,44	14,94	39,08	33,33	65,52	1,48	1,23	-18,90	-15,20	0,02	12,03	-21,72	-0,65
>sfr-miR-10462-2	AGAAAAUUAAAGCAG UCGGGACCCAUUCAAG AAAGCCAGCCGUAUGU GCCCUUACUAAGUCUU	100,00	19,00	31,00	21,00	28,00	40,00	59,00	0,90	0,90	-42,50	-37,50	0,06	13,94	-42,50	-1,06

	CAUAUUUAGAAUGGG UCCCGACUGCUUAAAU UUUUA															
>sfr-miR-10462-3	GUCAUCGUAAAAUUU AAGCAAUCGGGACCCA UUCUAGAAAGCCAGCC AAAUAUGUACCCAUA UUUAGAAUGGGUCCC GACUGCUUAAAUUUU ACGAUGAC	101,00	17,82	32,67	21,78	26,73	39,60	59,41	0,82	0,82	-57,90	-57,60	0,22	4,60	-57,33	-1,45
>sfr-miR-10462-4	AAGUCAUCGUAAAAU UUAAGCAGUCGGGACC CAUUCUAGAAAGCCCU CACUAAGUCUAUAUU UAGAAUGGGUCCCGAC UGCUUAAAUAUUACG AUGACUU	101,00	17,82	31,68	20,79	28,71	38,61	60,40	0,91	0,86	-62,40	-62,10	0,14	3,64	-61,78	-1,60
>sfr-miR-10462-5	AGAAUACAAGAAAAA UUUAAGUAGUCGGGA CCCAUUCUAGAAAGAC AUAAUUCAUAAUAUU UAGAAUGGGUCCCGAC UGCUUAAAUUUUUCU UGUAUUCU	101,00	15,84	35,64	14,85	32,67	30,69	68,32	0,92	1,07	-63,20	-63,20	0,46	1,80	-62,57	-2,04
>sfr-miR-10462-6	GUAAAAUUUAAGCAG UCGGGACCCAUUCUAA AUAUAAAGACUGUGU GAGGACACAUACGGCU UGCUUUCUAGAAUGG GUCCCGACUGCUUAAA UUUUAC	100,00	21,00	30,00	19,00	29,00	40,00	59,00	0,97	1,11	-61,10	-61,10	0,71	1,06	-61,10	-1,53
>sfr-miR-3894	GCGGUUUAUUGGAAU UUGGUUUUUGACAAU CUUUGGAACUAUCUCA UUUUCAGAAUGACCA	90,00	17,78	33,33	15,56	32,22	33,33	65,56	0,97	1,14	-22,90	-17,20	0,08	13,23	-25,44	-0,76

	AAGAUAGCCAAAGCCA UUAAUAAACGAA														
>sfr-miR-5838	GAAAUUGUUAUUGGU CUUUGUGAGUAUUUU CGGUUUGAGUUUCCG AAGCAAAAAUACUUG CAUAUAAUAAUAAUA AUCGU	81,00	18,52	30,86	9,88	39,51	28,40	70,37	1,28	1,88	-22,40	-16,70	0,08	14,26	-27,65	-0,97
>sfr-miR-927a	GGGUUUGGUUUUAGA AUUCCUACGCUUUACC GUUGCCAAGCAGUGU UGGGCAAAGCGUUUG GAUUCUAAAACUAAA UAG	80,00	25,00	25,00	16,25	32,50	41,25	57,50	1,30	1,54	-32,50	-26,00	0,14	10,52	-40,63	-0,98
>sfr-miR-193	UAUGUCCACCACGGCC AGGGUCUUGGCGGUC UGGUCGGUGUAUGAU UCUCUUACUGGCCUGC UAAGUCCCAAGCCUUG GUGGACUUU	88,00	28,41	13,64	26,14	30,68	54,55	44,32	2,25	1,09	-45,60	-45,60	0,45	4,03	-51,82	-0,95
>sfr-miR-927c	CUAUUUAGUUUUAGA AUCCAAACGCUUUGCC CAACACUGCUUGGCAA CGGUAAAGCGUAGGA AUUCUAAAACCAAACC C	80,00	16,25	32,50	25,00	25,00	41,25	57,50	0,77	0,65	-26,20	-25,30	0,07	6,46	-32,75	-0,79
>sfr-miR-138	UAAAUUAGACAUGCG AUAGUUUCCGAUACA AAAUCAUUGCUAGCU GGUGUUGUGAAUGCU UUUUUAAUCAGGAAA UCGCGUGUUGUAAUG AU	93,00	21,51	29,03	12,90	35,48	34,41	64,52	1,22	1,67	-23,80	-22,60	0,04	11,07	-25,59	-0,74
>sfr-miR-1651	UCACAUAAGCUUAUU GGGUACUUAUAUGAA GGUGGUAAAACAGGC	99,00	20,20	33,33	18,18	27,27	38,38	60,61	0,82	1,11	-26,30	-25,40	0,10	5,85	-26,57	-0,69

	GCUAAGACUGAUAAA AUCCACUAUCACUAAG UACUGGAAUCGGCUU ACUGACU														
>sfr-miR-3885	CCAAGGCGGCGGCGGC GGUAAGUACUCAUUA UAUUGUACUCAAUAA GUACUCAUUAUUUAA GUAGCAGUAUGAAGA UUUACCGGGGCCCCGG CUCAA	98,00	24,49	27,55	21,43	25,51	45,92	53,06	0,93	1,14	-33,30	-30,50	0,09	10,14	-33,98	-0,74
>sfr-miR-9-1	GUCGUAGAUGGCACU UAACUCCGGUAACCUA GCUUUAUGAUGUCAG AAUACUCAUACAGCUA GAUAACCAAAGAUAA UUAACCGAUCUACUUU	94,00	15,96	32,98	21,28	28,72	37,23	61,70	0,87	0,75	-23,80	-23,80	0,20	5,48	-25,32	-0,68
>sfr-miR-1798	UGGCUGAAUCGAUUU UUAUGUGGUUUUCAG AAGUGUUUUUGUGUC UAGGAGCAGGUUUUA GUAUAUUAACCGUCA UAAAAAAAGAGGUUC AGGAC	96,00	25,00	27,08	10,42	36,46	35,42	63,54	1,35	2,40	-21,10	-19,80	0,05	17,32	-21,98	-0,62
>sfr-miR-9c	ACGCCGCGGCUCGUCU CUUUUGGUAUCCUAGC UGUAGGCGUGUCACGC AAACCCUAAAGUUAU GGUACCGAAGUUACG AGUCGCGGUAU	90,00	26,67	20,00	25,56	26,67	52,22	46,67	1,33	1,04	-45,10	-45,10	0,10	6,32	-50,11	-0,96
>sfr-miR-9-2	AAAGUAGAUCGGUUA AUUAUCUUUGGUUAU CUAGCUGUAUGAGUA UUCUGACAUCAUAAA GCUAGGUUACCGGAG	94,00	21,28	28,72	15,96	32,98	37,23	61,70	1,15	1,33	-37,00	-37,00	0,38	2,38	-39,36	-1,06

	UUAAGUGCCAUCUACGAC															
>sfr-miR-79	GACGCGCGGUUCGGCUCUUUGGCGAUUUAGCUCCGUGACGUGAAAAAAGCUCAUAAAGCUAGAUUACCAAAGCACCGUACCGUCGCACG	90,00	25,56	25,56	26,67	21,11	52,22	46,67	0,83	0,96	-38,90	-38,90	0,65	1,09	-43,22	-0,83
>sfr-miR-9b	CGUGCGACGGUACGGUGCUUUGGUAAUCUAGCUUUAUGAGCUUUUUUCACGUCACGGAGCUAAAUCGCCAAAGAGCCGAACCGCGCGUC	90,00	26,67	21,11	25,56	25,56	52,22	46,67	1,21	1,04	-37,60	-37,60	0,16	3,18	-41,78	-0,80
>sfr-miR-2755	UUAUAUGAGCUUGGUGACUCAAGGUGGCCUAGCAGCGUGUUUUCUCUUAAGGUUUGCACCCUGUCAGACCAUACUUGUUUCAACAUGUCUCUAAUC	97,00	20,62	20,62	22,68	35,05	43,30	55,67	1,70	0,91	-26,40	-25,30	0,01	5,12	-27,22	-0,63
>sfr-miR-513d	ACACACACAUUUGUAGUAGUUCACAACGAGGUGUCAUAACUUGUGUUACAACUUUAUUCGUUUCGCACCAUGUAUGUACAGUACACAUGUGUGGUG	97,00	19,59	26,80	19,59	32,99	39,18	59,79	1,23	1,00	-23,60	-20,40	0,06	22,81	-24,33	-0,62
>sfr-miR-7184	AGUCAUGUGAGCCUUCUGCUGGAGCAGGUCGGCUCGCGUGUCAUCGUGCUGGUGAGCUGAUCCAGUAGGGCUAAUGUAA	80,00	33,75	17,50	21,25	26,25	55,00	43,75	1,50	1,59	-33,60	-15,38	0,07	29,01	-42,00	-0,76

>sfr-miR-10590	CAAAGUCGCUAGCAACAGCUAGUAAUACAUAUUAUUUAAACUUGAACUAUUUAAUUUAAAGUCCUCAUGUGUUUACCUGGACAGUCUACUAGCGUCUCUC	101,00	13,86	30,69	20,79	33,66	34,65	64,36	1,10	0,67	-19,00	-14,90	0,07	12,89	-18,81	-0,54
>sfr-miR-10587	UCAGUUCGUUAGGUAACUUCUGAACCAAGUUCGGUCGAGUUUCUUUCGAACCUCGUAAUUUGGACCGGAACUCUCAGAAGUAAGCGCCUCAAUGAACAAA	101,00	20,79	27,72	22,77	27,72	43,56	55,45	1,00	0,91	-31,70	-30,30	0,06	12,51	-31,39	-0,72
>sfr-miR-81c	GCGACAUCUAUCGUGAGAUCAAAUUAGACGAAUGUACGUCUGAUGUUGUUUCAUGCAACAUAGUAUGUUGU	72,00	22,22	27,78	15,28	33,33	37,50	61,11	1,20	1,45	-25,50	-25,50	0,39	2,62	-35,42	-0,94
>sfr-miR-10-1	UAGUGCCCUACAUCUACCCUGUAGAUCCGAAUUUGUUUGAAGUGAGGCGACAAAUUCGGUUCUAGAGAGGUUUGUGUGGUGCAUGC	87,00	27,59	21,84	18,39	31,03	45,98	52,87	1,42	1,50	-37,00	-36,90	0,16	4,16	-42,53	-0,92
>sfr-miR-135a	AUUAUGGCUUUCAUCCUAUGUGGGCUCAUGUUUGAUGGGACAACGUCACACGUUCUUUACGAAUGAAUGACGCACAGAAUGAAACCAUUGA	92,00	21,74	28,26	19,57	29,35	41,30	57,61	1,04	1,11	-23,90	-23,90	0,21	10,81	-25,98	-0,63
>sfr-miR-993a-1	GCACAUUCGUGAUCUACCCUGUAGAUCCGGGCUUUUGUGACUUACACAUCAGAAGCUCGUUUC	86,00	23,26	22,09	24,42	29,07	47,67	51,16	1,32	0,95	-41,30	-41,30	0,15	5,44	-48,02	-1,01

	UACAGGUAGCUCACGA UUGGGA															
>sfr-miR-993a-2	AUUCGUGAUCUACCCU GUAGAUCCGGGCUUU UGUAGUUUAGUUCAC AUCAGAAGCUCGUCUC UACAGGUAUCUCACGG CU	81,00	20,99	19,75	24,69	33,33	45,68	53,09	1,69	0,85	-33,10	-33,10	0,08	3,83	-40,86	-0,89
>sfr-miR-2318	UAAAUAUGAAAUUAU UAAUACACGUGCGUA ACGAAUUUCGGAGUA AAUAAUGUCUCGCCUG CUCUAUAUCCGCCGUG UAUGAUGAAUUAUUC AUAUCAU	100,00	16,00	32,00	17,00	34,00	33,00	66,00	1,06	0,94	-20,00	-19,40	0,03	11,53	-20,00	-0,61
>sfr-miR-10484-1	CCUAAGAAAAGGAGG AUCCUCCGACCAGGCG AGGUGAUCAUGCCUG GCGGGCUUUCUGCCCU AUUUCUUUAA	73,00	26,03	21,92	26,03	24,66	52,05	46,58	1,13	1,00	-27,10	-17,90	0,07	12,75	-37,12	-0,71
>sfr-miR-276b	UACCCAAGAGCACGGU AUGAAGUUCCUACAG UGGUAGAAUACGUAG GAACUCUAUACCUCGC UAGUGGCAA	72,00	23,61	30,56	22,22	22,22	45,83	52,78	0,73	1,06	-29,50	-29,50	0,70	1,12	-40,97	-0,89
>sfr-miR-10484-2	GUAAGUAAAGUUCCC UAAGAAAAGCAGGAU CCUCCGACGAGGCGAG GUGAUCAUGUCUGGCC GGCUUUCUGCCCUGCC UUUCUGUUGGGAUUU UCUAUC	100,00	26,00	20,00	24,00	29,00	50,00	49,00	1,45	1,08	-30,10	-25,50	0,04	16,39	-30,10	-0,60
>sfr-miR-276	UUGCCACUAGCGAGGU AUAGAGUUCCUACGU AUUCUACCACUGUAGG	72,00	22,22	22,22	23,61	30,56	45,83	52,78	1,38	0,94	-31,00	-31,00	0,24	5,45	-43,06	-0,94

	AACUUCAUACCGUGCU CUUGGGUA														
>sfr-miR-10-2	UUAGCUCAUACGUGU AACCCUGUAGAUCCGA ACUUUAUCUAUUAAA CUUGAAUAACGUGCG GACCAACAGGAUUACA CUUGUGGGCACG	90,00	20,00	28,89	22,22	27,78	42,22	56,67	0,96	0,90	-32,10	-31,00	0,15	3,93	-35,67	-0,84
>sfr-miR-7675	CUCUAGGUAUCUACAA UCAAUGCAUGAAGUA GAAUGUAGAUGAAAU GCCACAAUUGUUUCAU UUGUUCGUUCUAUCA GCAUUAUAGCAAUAA GCUAAGA	100,00	17,00	34,00	16,00	32,00	33,00	66,00	0,94	1,06	-21,50	-21,50	0,05	7,95	-21,50	-0,65
>sfr-miR-277	UCCGGAGUUCGUGCCA GGAGUGCGUUUGCAA AGCACGCUACAAGUUU GCGUUAAGGCAAAGU UGACACUGUAAAUGC ACUAUCUGGUACGACA CCCCGGA	101,00	26,73	25,74	23,76	22,77	50,50	48,51	0,88	1,13	-48,30	-48,30	0,20	4,52	-47,82	-0,95
>sfr-miR-316	AAGCAUCAAGCAUAA GCAAGAAGAAGCAGA AGAGAGAAGCAACAG UUCUCCAUAAAUUUG UCUUUUUCCGCUUUGC UGCUGAUAAUUAUGA UC	94,00	19,15	35,11	18,09	26,60	37,23	61,70	0,76	1,06	-20,70	-18,10	0,06	11,69	-22,02	-0,59
>sfr-miR-4648	UAAGUGUUAGUGACG CCCAAUGUGGGACUGC AAAUGUUCGCGAGGCC CAGUUAGGGCGCCAAC ACCAU	69,00	28,99	24,64	24,64	20,29	53,62	44,93	0,82	1,18	-24,20	-23,30	0,17	5,58	-35,07	-0,65

>sfr-miR-3338	AGAAGAGUGUUUCAUAGCAAACACAGUAGUGUGCAAUGCUAAAGUCCACCAUGCAUGUACUUACUUUGUUUGUUCUGAAAUACUCUAAC	90,00	17,78	31,11	18,89	31,11	36,67	62,22	1,00	0,94	-35,90	-33,50	0,31	1,90	-39,89	-1,09
>sfr-miR-965	AUUGCUGUGUAACUGAACGGGAGAAGUUGUACCGCUAUAUGUUUUUAAUAAGGUCAUAAGCGUAUAGCUUUUCCCCUUGGUUAUGCAAGCCAG	94,00	23,40	25,53	17,02	32,98	40,43	58,51	1,29	1,38	-37,30	-37,30	0,07	7,41	-39,68	-0,98
>sfr-miR-87	AAGAAAGUACGGUACACUGAUGGGCCUGAAUUGUUGCUCGAACCUGUGUUUUGAUCAAGGUGAGCAAACUUUCAGGUGUGUUAGUGCCGACUUUUCA	98,00	26,53	24,49	17,35	30,61	43,88	55,10	1,25	1,53	-40,90	-40,90	0,06	8,43	-41,73	-0,95
>sfr-miR-9388	GAGGUUAACAUUGUAGUAACGGUGCAAGUACCUGUGUAUGUAUGUAUGUAUGUACAUACGUAGGUUCUCCACACGGCUCACAAGCUUACACAUA	95,00	22,11	28,42	18,95	29,47	41,05	57,89	1,04	1,17	-25,50	-24,50	0,03	11,75	-26,84	-0,65
>sfr-miR-10458	UGUGUGUGCUGCGCCGGGAUACGUGUUUACCGCAUGUGUGAUGUGUCUGCCUAAAACAUGUAUCCGGCUCAGCAACCCU	80,00	27,50	17,50	25,00	28,75	52,50	46,25	1,64	1,10	-31,20	-31,20	0,04	9,95	-39,00	-0,74
>sfr-miR-263a-1	AACUCGCACCAUGCACAGGCAAUGGCACUGGAAGAAUUCACGGGUU	100,00	26,00	23,00	24,00	26,00	50,00	49,00	1,13	1,08	-41,20	-41,20	0,12	7,61	-41,20	-0,82

	ACAUUUUAUUAGUCCC GUGGUCUCUUAGUGG CAUCACUGGUGCUGGG CGAUAC														
>sfr-miR-263b-1	AUCAGACCAUGUUGA GCCUUGGCACUGGGAG AAUUCACAGGAGUUC UGAAUAUACCGUGAA UUUCCUGAUGCCUUAG CUUAAUGCGGUCUUU U	94,00	23,40	23,40	20,21	31,91	43,62	55,32	1,36	1,16	-35,80	-35,80	0,07	5,02	-38,09	-0,87
>sfr-miR-263a-2	AACUCGCACCAUGCAC AGGCAAUGGCACUGG AAGAAUUCACGGGUU ACAUUUUAUUAGUCC GUGGUCUCUUAGUGG CAUCACUGGUGCUGGG CGAUAC	99,00	26,26	23,23	23,23	26,26	49,49	49,49	1,13	1,13	-39,40	-39,40	0,15	6,60	-39,80	-0,80
>sfr-miR-263b-3	AUCAGACCAUGUUGA GCCUUGGCACUGGGAG AAUUCACAGGAGUUC UGAAUAUACCCGUGA AUUUCCUGAUGCCUUA GCCUAAUGCGGUCUUU U	95,00	23,16	23,16	22,11	30,53	45,26	53,68	1,32	1,05	-32,70	-32,70	0,07	7,58	-34,42	-0,76
>sfr-miR-210	UGGUGAAAUGAAGCC CAAAGCAGCUGCUGGC CACUGCACAAGAUUAG UUAGAACUAUACUCU UGUGCGUGUGACAGC GGCUAUUGCGGGCUA UUCAUUG	100,00	27,00	25,00	21,00	26,00	48,00	51,00	1,04	1,29	-44,00	-43,40	0,10	10,02	-44,00	-0,92
>sfr-miR-3286	GCUUGAAGCCCAAAAC AACUAUUGGGCACUGC ACAGGACUAGUUACCC GAAUAUACUCUUGUG	97,00	22,68	25,77	22,68	27,84	45,36	53,61	1,08	1,00	-40,60	-40,40	0,07	7,05	-41,86	-0,92

	CGUGUUCUAAUAGUU GUUGUCGGAGCUUCAC AG															
>sfr-miR-317	GGGCUAUCGAACGACC GGGUGCCACGCUGUGC UCUCUCGGGAAUUAA UGCAGAGUGAACACA GCUGGUGGUAUCUCA GUUGUUCCCUAGUUC	93,00	29,03	19,35	24,73	25,81	53,76	45,16	1,33	1,17	-39,80	-39,70	0,28	4,30	-42,80	-0,80
>sfr-miR-210b	GACAAUGAAUAGCCCG CAAUAGCCGCUGUCAC ACGCACAAGAGUAUA GUUCUAACUAAUCUU GUGCAGUGGCCAGCAG CUGCUUUGGGCUUCAU UUCA	99,00	22,22	26,26	25,25	25,25	47,47	51,52	0,96	0,88	-41,50	-41,50	0,07	7,05	-41,92	-0,88
>sfr-miR-750	CUAGACCUCACGUCUG AGUUGGACAGGGGAU CUUGACAGUUUGAGC UAUGUGCUGCCAGAUC UAUCUUUCCAGCUCAC GCGUGAAGUCGCU	92,00	26,09	19,57	25,00	28,26	51,09	47,83	1,44	1,04	-39,90	-39,30	0,14	3,30	-43,37	-0,85
>sfr-miR-10467	GUAUCGAUUGCUCGGC UGUAUUGAUCGAUCA CUAUUCUCGUGAGAU GCACUCGAGCGAUGAA UAGAGCCGAGCGAUCG AAGC	83,00	27,71	24,10	21,69	25,30	49,40	49,40	1,05	1,28	-38,60	-35,00	0,08	8,73	-46,51	-0,94
>sfr-miR-932	GGAGGGUGUCGAGGC CUCAAUUCCGUAGUGC AUUGCAGUGCAUCCUG CCUCCUGCAAGCAGUG CGGAAGUGAGGCUGA GACACCCCAU	89,00	31,46	20,22	26,97	20,22	58,43	40,45	1,00	1,17	-55,20	-55,20	0,43	1,47	-62,02	-1,06
>sfr-miR-10505-1	AGCAUCGACUCGCCAG UUCAAAAGUAUAGGG	88,00	23,86	27,27	19,32	28,41	43,18	55,68	1,04	1,24	-21,50	-16,40	0,09	16,01	-24,43	-0,57

	UUAGAAACUUGGCUC UACACGGGAUCUGAU UACUUUUUGACAGUG CGAGCUAUAUG														
>sfr-miR-5700	CGGGUCGCAAGUAUA UAGAUAAUAAGGUGU ACAUAAUGCAUUAAA UUAUGGUUAUACAUG UAUAAUUUAUGUGCU UCUACUAA	84,00	17,86	34,52	10,71	35,71	28,57	70,24	1,03	1,67	-19,40	-18,00	0,18	10,66	-23,10	-0,81
>sfr-miR-6830	CGACCUCUCACUGUCG ACUUUUGUAGACUCU GGGCUUCUGCAAAGA UGAAAUGUCGCCAAG GAAGGAGGCUGCUAA AGGAGAGUGAAAGGG AC	94,00	29,79	27,66	20,21	21,28	50,00	48,94	0,77	1,47	-27,60	-27,60	0,16	8,90	-29,36	-0,59
>sfr-miR-16c	ACCGGUCACAAUUCCA GACUCCGUGCUACUGC UGAGAAAUUUUCGAA AAACCGAAAAAAACUC CAGUAUUACUUUGCU UGGAAUUGAACCCGA	94,00	17,02	32,98	24,47	24,47	41,49	57,45	0,74	0,70	-27,60	-27,00	0,39	3,05	-29,36	-0,71
>sfr-miR-10501-1	CAUCAAAAACCUAGAC CACUUCCAAUGGUCGU AUUGACUUGAAAUUU GGCAUGGAGGUAGGU CUUUGGGU	71,00	23,94	26,76	18,31	29,58	42,25	56,34	1,11	1,31	-22,90	-22,90	0,21	5,29	-32,25	-0,76
>sfr-miR-10501-2	ACUCAUCAAAAACCUA GACCACUUCCAAUGGU CGUAUUGACUUGAAA UUUGGCAUGGAGGUA GGUCUUUAUGUCA	76,00	19,74	28,95	19,74	30,26	39,47	59,21	1,05	1,00	-19,30	-19,30	0,29	7,94	-25,39	-0,64
>sfr-miR-10501-3	UCACUCAUCAAAAACC UAGACCACUUCCAAUG GUCGUAUUGACUUGA	82,00	21,95	28,05	19,51	29,27	41,46	57,32	1,04	1,13	-21,00	-19,30	0,14	10,57	-25,61	-0,62

	AAUUUGGCAUGGAGG UAGGUCUUUAGAUCA GGGU														
>sfr-miR-10501-4	CAUCAAAAACCUAGAC CACUUCCAAUGGUCGU AUUGACUUGAAAUUU GGCAUGGAGGUAGGU CUUUGGUC	71,00	22,54	26,76	19,72	29,58	42,25	56,34	1,11	1,14	-22,80	-22,80	0,40	3,59	-32,11	-0,76
>sfr-miR-10501-5	AAAACCUAGACCACUU CCAAUGGUCAUAUUG ACUUGAAAUUUGGCA UGGAGGUAGGUCUUU AGGUCAA	69,00	21,74	30,43	17,39	28,99	39,13	59,42	0,95	1,25	-24,10	-24,10	0,21	3,99	-34,93	-0,89
>sfr-miR-10456	GUUAAUUCGCAUGUA AACUUCAUAUGUGCG AUGCAGGAUAUUUAU GACGGCAUCCGUGGAU UUGCUCGUAGAUAGU CUAUGUGCGACUUCUG	93,00	24,73	22,58	17,20	34,41	41,94	56,99	1,52	1,44	-25,10	-21,40	0,05	20,95	-26,99	-0,64
>sfr-miR-10495-2	CUGCAUCAACCGGGGA UUGUCCUUUGUGUAC UUCCAUAGUGCAUACA GGGAUAAUCGCCGGCU GAUGUCU	71,00	25,35	19,72	23,94	29,58	49,30	49,30	1,50	1,06	-40,00	-40,00	0,71	0,77	-56,34	-1,14
>sfr-miR-10495-3	CACAAUAGUUGUGCA UCAACCGGGGAUUGUC CUUUGGUGUAUUUCC AUAGUGCAUACAGGG AUAAUCGCCGGUUGA UGUCCCAUUACAUUUA G	94,00	23,40	23,40	20,21	31,91	43,62	55,32	1,36	1,16	-41,70	-40,70	0,05	8,89	-44,36	-1,02
>sfr-miR-10495-4	CUUACAUCAGCCGGCG AUUGUCCUUUGGUGU ACUUCCAUAGUGCAUA CAGGGAUAAUCGCCGG CUGAUGUCCC	74,00	24,32	18,92	27,03	28,38	51,35	47,30	1,50	0,90	-48,70	-48,70	0,31	2,40	-65,81	-1,28

>sfr-miR-10495-5	CUGCAUCAACCGGGGA UUGUCCUUUGGUGUA CUUCCAUAGUGCAUAC AGGAUUAAUCGCCGAC UGAUGUCC	72,00	23,61	20,83	25,00	29,17	48,61	50,00	1,40	0,94	-30,20	-30,20	0,24	2,65	-41,94	-0,86
>sfr-miR-305	CAGCCGCCGCACGCCC AUUGUACUUCAUCAG GUGCUCUGGUGAUGA UGGUUCCAGGCGCUUG UUGGAGUACACUUACC GUGUCGGCGGUAG	92,00	30,43	15,22	27,17	26,09	57,61	41,30	1,71	1,12	-49,90	-49,90	0,53	1,83	-54,24	-0,94
>sfr-miR-1000	CUCGCGCGCCACGACC AUAUUGUCCUGUCACA GCAGUACGUGCACUGU UACUGCUGCGUCCGGA CAAACGGGCCGUGGCG UCGCACU	88,00	27,27	17,05	34,09	20,45	61,36	37,50	1,20	0,80	-44,70	-41,70	0,20	3,22	-50,80	-0,83
>sfr-miR-10454a-1	AGUCACCAGGGGCCUU AGUCUGCUAUUACAA UUGUGUCAGAAUGGU CGAUCAUUGAGCACAA UUGUAAUAUCAGACU AAGGCCCCAGGUGGAA	94,00	24,47	28,72	20,21	25,53	44,68	54,26	0,89	1,21	-56,70	-56,70	0,16	4,14	-60,32	-1,35
>sfr-miR-4819	UGAUUAUUUACUUUU AUUAUUACGUCCAUCC UGACGCGCUCGCGGCA CUCGCGUGUGAUUUA ACUUGGACAAAAUGG AUAUCAGUAGCAAUA AAAC	97,00	18,56	27,84	20,62	31,96	39,18	59,79	1,15	0,90	-21,70	-20,00	0,02	8,88	-22,37	-0,57
>sfr-miR-10454a-2	GAACAGGGGCCUUAG UCUGCUAUUACAAUU GUGUCAGAAUGAUCA GUGAUCGACCAUUCUG ACACAAUUGUAAUAG	96,00	22,92	29,17	20,83	26,04	43,75	55,21	0,89	1,10	-73,30	-73,30	0,82	1,09	-76,35	-1,75

	CAGACUAAGGCCCCUG GUA														
>sfr-miR-10454a-3	GGGGCCUUAUUCUGCU AUUACAAUUGUGUCA GAAUGGUCGAUCACU GAGAUGACGAUGAUC AAACAUUGUGACACA AUUGUAAUAGCAGAC UAAGGCCCC	101,00	22,77	29,70	19,80	26,73	42,57	56,44	0,90	1,15	-63,00	-63,00	0,72	0,88	-62,38	-1,47
>sfr-miR-10454a-4	GGCCUUAGUCUGCUAU UACAAUUGUGUCACA AUGGUCGAUUACUGA GCAGUGCAAGAGGGA UCGACCAUUCUGACAC AAUUGUAAUAGCAGA CUAAGGCC	101,00	23,76	28,71	20,79	25,74	44,55	54,46	0,90	1,14	-69,40	-69,40	0,28	2,57	-68,71	-1,54
>sfr-miR-10454a-5	GGGCCUUAGUCUGCUA UUACAAUUGUGUCAG AAUGGUCAAUCAAUC AACUGAUCAAUGAUC GACCAUUGUGACACAA UUGUAAUAGCAGACU AAGGCCC	100,00	20,00	31,00	21,00	27,00	41,00	58,00	0,87	0,95	-71,20	-71,20	0,78	0,79	-71,20	-1,74
>sfr-miR-10454a-6	CCUUAGUCUGCUAUUA CAAUUGUGUCGGAAU GGUCGGUCCGUCCCUC UCGCACUGCUCAGUGA UCGACUAUUCUGACAC AAUUGCAAUAGCAGA CUAAAG	101,00	20,79	23,76	25,74	28,71	46,53	52,48	1,21	0,81	-55,10	-54,00	0,11	5,63	-54,55	-1,17
>sfr-miR-10454a-7	UUUUGAUCGCCAUGG UAAAACAAUUGUGCC UUUGAACAGGGGCCU UAGUCUGCUAUUACA AUUGUGUCAGAAUGG UCGAUCACUG	86,00	23,26	24,42	18,60	32,56	41,86	56,98	1,33	1,25	-24,90	-24,90	0,23	5,21	-28,95	-0,69

>sfr-miR-10454a-8	UGUCCAGUCAUCUUGACUACUACACUGGGGCCUUAGUCUGCUAUUACAAUUGUGUCAGAAUGGUCGAUCACUGAGCAGUGCAAGAAGGACGGAGC	96,00	26,04	25,00	21,88	26,04	47,92	51,04	1,04	1,19	-25,20	-25,20	0,23	5,73	-26,25	-0,55
>sfr-miR-10454a-9	CAUAUUCUGAACUAGAAUAUUAUAUAGUGCCCUUCUAGGGCACAUUUAUCUGGGGCCUUAGUCUGCUAUUACAAUUGUGUCAGAAUGGU	90,00	20,00	25,56	17,78	35,56	37,78	61,11	1,39	1,13	-22,20	-12,00	0,04	25,91	-24,67	-0,65
>sfr-miR-10454a-10	AGUGACGAUAUGACACAAGUGUACAACUACUAACCACUGGCCUGUACUCCAGGGGCCUUAGUCUGCUAUUACAAUUGUGUAAAAAUGGUCGAU	94,00	21,28	29,79	21,28	26,60	42,55	56,38	0,89	1,00	-22,80	-17,70	0,09	13,59	-24,26	-0,57
>sfr-miR-10454a-11	GUUGACACUCCGGGGCCUUAGUCUGCUAUUACAAUUGUGUCACAAUGGUCGAUCACUGUAAUAGCAGACUAAGGCCCCUGAUGUCGCC	89,00	23,60	22,47	25,84	26,97	49,44	49,44	1,20	0,91	-51,40	-51,40	0,23	4,02	-57,75	-1,17
>sfr-miR-10454a-12	AGUCUGCUAUUACAAUUGUGUCACAAUGGUCGAUCACUGAACAGUGCAAGAGUGCAGUGCUCAGUGAUCGGCCAUUCUGACACAAUUGUAAUAGCAGACU	101,00	22,77	28,71	20,79	26,73	43,56	55,45	0,93	1,10	-73,40	-73,40	0,43	1,19	-72,67	-1,67
>sfr-miR-10454a-13	UCUGCUAUUACAAUUGUAUCAGAAUGGUCGAUCACUGAGCAGUGCGAGAGGGACGGAGCUA	100,00	23,00	32,00	20,00	24,00	43,00	56,00	0,75	1,15	-36,80	-31,10	0,03	15,36	-36,80	-0,86

	UACAACCUACAUAGCU CUGACACAAUUGUAA UAGCAGA															
>sfr-miR-10454h-1	CUGCUAUUACAAUUA UGUCAGAAUGAUCGA UCAUUGAGCAGUGCA AGAGGGACGGAGCUA UGUAGGUUGUAUAUU CUGACACAAUUGUAA UAGUAG	97,00	24,74	30,93	13,40	29,90	38,14	60,82	0,97	1,85	-37,50	-34,90	0,13	6,01	-38,66	-1,01
>sfr-miR-10454f	UGCUAUUACAAUUGU GUCAGAAUGGUCGAU CACUGAGCAGUGCAAG AGGGACGGAGCUAUG UAGGUUGUAUAGCUC CGUUCUGACACAAUUG UAAUAGCA	101,00	26,73	27,72	16,83	27,72	43,56	55,45	1,00	1,59	-48,80	-47,60	0,03	11,55	-48,32	-1,11
>sfr-miR-10454e-1	GCUAUUACAAUUGUG UCACAAUGGUCGAUCA CUGAGCAGUGCGAGA GGGACGGAGCUAUGU AGGUUGUAUAGCCAU UGUGACACAAUUGUA AUUGU	97,00	27,84	26,80	15,46	28,87	43,30	55,67	1,08	1,80	-43,90	-40,30	0,15	9,28	-45,26	-1,05
>sfr-miR-10454b	UAUUACAAUUGUGUC ACAAUGGUCGAUCACU GAGCAGUGCGAGAUG GACGGAGCUAUGUAA GUUGUAUUAUAUACG ACCAUUCUGACACAAU UGUAAUA	100,00	22,00	31,00	16,00	30,00	38,00	61,00	0,97	1,38	-37,00	-30,97	0,09	16,49	-37,00	-0,97
>sfr-miR-10454h-2	UAUUACAAUUGUGUC AGAAUAGUCGAUCGU UGAGCAAUGCAAGAG GGACGGAGCUAUGUA GGACUGCUCAAUGGUC	101,00	24,75	30,69	15,84	27,72	40,59	58,42	0,90	1,56	-59,00	-59,00	0,33	2,41	-58,42	-1,44

	GACUAUUCUGACACAA UUGUAAUA														
>sfr-miR-10454e-2	UACAAUUGUGUCACA AUGGUCGAUCACUGA GCAGUGCAAGAGGGA CGGAGCUAUGUUGGU UGUAUAGCUCCAUCCC AUGACCAUUGUGACAC GAUUGUA	100,00	26,00	26,00	20,00	27,00	46,00	53,00	1,04	1,30	-53,70	-53,70	0,16	2,90	-53,70	-1,17
>sfr-miR-10454h-3	UAAAAUUGUGUCAGA AUGGUCGAUCAUUGA GCAGUGCAAGAGGGA AGGAGCUAUACAACCU ACAUAGCUCAGUCCCU CGACCAUUCUGACACA AUUGUC	100,00	22,00	31,00	22,00	24,00	44,00	55,00	0,77	1,00	-48,20	-46,90	0,20	5,78	-48,20	-1,10
>sfr-miR-10454h-4	GUCAGAAUGAUCGAU CAUUGAGAAGUGCAA GAGGGACGGAGCUAU GUAGGUUGUAUAGCU UCUUCUCUCUUGCACU GCUCAAUGGUCGACUA UUCUGAC	100,00	26,00	24,00	19,00	30,00	45,00	54,00	1,25	1,37	-64,00	-64,00	0,29	1,99	-64,00	-1,42
>sfr-miR-10454h-5	UGUCAGAAUGGUCGA UCACUGAGCAGUGCGA GAGGGAUGGAGCUAU GUAGGUUGUAUAGCU CCAUCCCUCUCGCAAU GUCAAUGAUCGACCAU UCUGACA	101,00	26,73	24,75	21,78	25,74	48,51	50,50	1,04	1,23	-76,30	-76,30	0,12	3,29	-75,54	-1,56
>sfr-miR-10454h-6	UGUCAGAAUGGUCGA UCAUUGAGCAGUGCA AAGGGAAGGAGCCAU ACAACCUACAUAGCUU CGUCCCUCUUGCACUG	101,00	21,78	28,71	24,75	23,76	46,53	52,48	0,83	0,88	-64,80	-64,80	0,19	2,34	-64,16	-1,38

	AUCAAUGAUCGACCAU UCUGACA														
>sfr-miR-10454h-7	UGUCAGAAUGGUCGA UCAUUGAGCAGUGCA AGAGGGAUAUAGCUA UACUACCUACAUAGCU GUCCCUCUCGCACUGC UCAAAGAUCGACCAUU CUGACA	100,00	21,00	28,00	25,00	25,00	46,00	53,00	0,89	0,84	-70,60	-70,60	0,18	2,87	-70,60	-1,53
>sfr-miR-10454e-3	UGUCACAAUGGUCGA UCACUGAGCAGUGCAA GAGGGAUGAAGCUAU ACAACCUACACAGCUC CGUCCCCUCGCACUGC UCAGUGAUCGACCAUU CUGACA	101,00	21,78	26,73	29,70	20,79	51,49	47,52	0,78	0,73	-67,10	-67,10	0,32	2,34	-66,44	-1,29
>sfr-miR-10454d	GUGUCAGAAUGGUCG AUCAUUGGGCAGUGC AAGAGGGAUUGAGCU AACUUACAUAGCUGCG UCCCUCUUGCACUGCU CAAUGAUCAACCAUUC UGACAC	100,00	24,00	25,00	24,00	26,00	48,00	51,00	1,04	1,00	-76,90	-76,90	0,82	0,46	-76,90	-1,60
>sfr-miR-10454e-4	UUGUGUCACAAUGGU CGAUCACUGAGCAGUG CAAGAGGGAUGGAGC UAUACAACCUACAUAG CUCCGUCACUGCUCAG UGAUCGACCAUUGUG ACACAA	100,00	24,00	28,00	24,00	23,00	48,00	51,00	0,82	1,00	-70,60	-70,60	0,26	1,95	-70,60	-1,47
>sfr-miR-7926	ACUCAGAAGCUCAGAU GAGUUGAUCAACUUC AAAGUCUCUAACAUUC CUUUAUUAUUAUCGU UUGAUUUUGAAAUCA	97,00	14,43	29,90	17,53	37,11	31,96	67,01	1,24	0,82	-20,40	-19,10	0,25	4,44	-21,03	-0,66

	UGUCAAAUGAUUUCU GCAG														
>sfr-miR-285	UAAGUCCACAAUUUG ACAACUGUAUUCGAG UGAGUGGAUAGAGUU GUGAUUCUCUAGCACC AUUCGAAUUCAGUGC UCGAAUUUGGACGAU	92,00	22,83	27,17	17,39	31,52	40,22	58,70	1,16	1,31	-38,80	-38,80	0,48	1,65	-42,17	-1,05
>sfr-miR-971	AAGAAGUCCGGUAUC UCGGCUCACUCUAAGU AUGAACGCCAAGCAUA UUGAAAAGAGUUUGG UGUUCUACCUUACAGU GAACUGAGUACUAGA CAUUCAU	101,00	20,79	30,69	19,80	27,72	40,59	58,42	0,90	1,05	-40,70	-40,70	0,30	3,49	-40,30	-0,99
>sfr-miR-7006	GUAGAAAAUUGAAGU CAAGAAGCUGGUAGA UGAUUUCAAGACCCAC AGGGUUUCUGACCUG GAUCCUGACUUCUCCU UUCGGU	84,00	23,81	26,19	20,24	28,57	44,05	54,76	1,09	1,18	-22,30	-20,80	0,16	6,09	-26,55	-0,60
>sfr-miR-10508a-2	AGCUAUUCUAGAGGC UCAUAUGCUGCAGUA GCAGCGCGACAAUCGG CGCGUCGUGAGUCGCG GAAUGCUCCUCAUGAA UAUGACCCUGAAAUA UGA	97,00	26,80	25,77	23,71	22,68	50,52	48,45	0,88	1,13	-30,50	-30,50	0,13	12,85	-31,44	-0,62
>sfr-miR-10508a-3	AGCCAUUCAAGAGGCU CAUAUGAUCAGUAGC AGCGCGACAAUCGCCG CGUCAUGUGUCGCGGA AUGCUGCUCAUGAAU AUGAGCCUCUAGCAUG ACU	98,00	25,51	25,51	25,51	22,45	51,02	47,96	0,88	1,00	-42,20	-37,60	0,04	21,05	-43,06	-0,84

>sfr-miR-10508b-2	UCAAGCCAUGCUAGAG GCUACUACGAGUAGCA GCGCGGCAAUCGCCGC GUCGUGUGUCGCGGA AUGCUGCUCAUGAAU AUGAGCCUCAAGCAUG GCUUGA	101,00	29,70	22,77	25,74	20,79	55,45	43,56	0,91	1,15	-56,60	-56,60	0,13	7,81	-56,04	-1,01
>sfr-miR-10508a-4	AGCCAUGCAAGAGGCU CAUAUUCAUGAGGAG CAUUUCGCGACACACG ACGCGGCGAUUGUCGC GAAAUGCUCCUCAUGA AUAUGAGCCUCUUGCA UGGCU	101,00	25,74	24,75	25,74	22,77	51,49	47,52	0,92	1,00	-86,40	-86,40	0,66	0,66	-85,54	-1,66
>sfr-miR-10508b-3	AAUUAUUCAUUCCAGC AGCAUUCCGCGACAAU CGCCGCGUCGUGUGUC GCGGAAUGCUGCUCAU GAAUAUGA	73,00	23,29	23,29	26,03	26,03	49,32	49,32	1,12	0,89	-40,20	-40,20	0,41	3,20	-55,07	-1,12
>sfr-miR-10461b-2	CUAGAAGCUCAUAUUC AUGAGCAGCAUUCCGC GACAAUCGCCGCGUCG UGUGUCGCGGAAUGC UACUGAUGAAACUAG UCGAGUUCCUC	90,00	24,44	23,33	26,67	24,44	51,11	47,78	1,05	0,92	-38,20	-38,10	0,05	7,99	-42,44	-0,83
>sfr-miR-10508b-4	UGUUUGUAUUAUAAG AAGCAGCGCGACCGGG GACAAUCACCGCGUCG UGUGUCGCGGAAUGC UGCUCAUGAAUAUAA GCC	81,00	28,40	24,69	22,22	23,46	50,62	48,15	0,95	1,28	-26,30	-10,15	0,03	22,26	-32,47	-0,64
>sfr-miR-10508a-5	ACAUCACACGAAUACG AAUAGUUCAUAUUCC GCGACAAUGUUGUCGC GGAAUGCUGCUCAUG	90,00	20,00	30,00	18,89	30,00	38,89	60,00	1,00	1,06	-25,60	-24,70	0,03	18,02	-28,44	-0,73

	AAUAUGAACUUUUAG CAUGUUUGAGAU														
>sfr-miR-10508a-6	UCAAGCCAUGCUAGAG GCUCACACACAUGCGU CGUGUGUCGCGAAAU GCUGCUCAUGAAUAU GAGCCUCUAGCAUGGC UAUU	83,00	24,10	24,10	25,30	25,30	49,40	49,40	1,05	0,95	-44,50	-44,50	0,60	8,12	-53,61	-1,09
>sfr-miR-10461a-1	GUUUGACGAGGAACU CGACUAGUGUCGUGU GUCGCGAAAUGCUGCU CAUGAAUAUAAGCCUC UAUCAUGGCUUGAAA CUAGUCGAGUUUCUCG UCAAAC	100,00	24,00	25,00	21,00	29,00	45,00	54,00	1,16	1,14	-52,00	-52,00	0,28	1,99	-52,00	-1,16
>sfr-miR-10461a-2	UUGACUAGGAACUCA ACUAGUUUAGUACUA GCGCGACUGUCGCCGC CUCUCUAGUAUGGCUU GAUAUGAAACUAGUC GAGUUUCUCGUCAA	92,00	21,74	23,91	22,83	30,43	44,57	54,35	1,27	0,95	-30,50	-24,57	0,02	14,96	-33,15	-0,74
>sfr-miR-10461b-3	CUGUUUGACGAAGACC UCGACUAGUUUCAAGC CGUACUAGAGACUUA UAAUUAUACUACUGA UCAUGAAUAUGAAAC UAGUCGAGUUCCUCGU CAAACAG	101,00	17,82	31,68	20,79	28,71	38,61	60,40	0,91	0,86	-38,70	-37,70	0,12	8,84	-38,32	-0,99
>sfr-miR-10461b-4	UUACUCACGUAACUGU UUGUCGAGGAAUGCU GCUCAUGUAUGAGUC UCUAACAUGGCUUGA AACUAGUCGAGUUCCU CGUCAAACAGUUACGU GAGUAA	100,00	22,00	26,00	20,00	31,00	42,00	57,00	1,19	1,10	-49,20	-49,00	0,06	12,85	-49,20	-1,17

>sfr-miR-10508a-7	CUACACUGUUACGUGU GUCGUGGAAUGCUGC UCAUGAAUAUGAGCC UCUAGCAUGGCUUGA AACUAGUCGAGUUUC UCGUCAAAACGUAACG UGAGU	98,00	24,49	24,49	20,41	29,59	44,90	54,08	1,21	1,20	-30,50	-28,70	0,10	7,95	-31,12	-0,69
>sfr-miR-10461b-5	ACUCACGUGACGUUUU GACGAGGAAUUCGAC UGCUCAUAAAUAUGA GCCUCUAGCAUGGCUU GAAACUAGUCGAGUU CCUCGUCAAACAGUUA CGUGAGU	101,00	22,77	26,73	21,78	27,72	44,55	54,46	1,04	1,05	-55,80	-55,80	0,17	10,49	-55,25	-1,24
>sfr-miR-10461b-6	AUUAUUAUUUCAACG GCUUACUCAUUGGCCU CUAGCAUGGCUUGAA ACUAGUCGAGUUCCUC GUCAAACAGUUACGU GAGUAAGCCGAUAAU AUAAUGAU	101,00	18,81	28,71	19,80	31,68	38,61	60,40	1,10	0,95	-30,80	-30,10	0,09	14,54	-30,50	-0,79
>sfr-miR-10461a-3	AAUUGUCCGACGAGG AACUUCAGUAGCAGCG CGACGGCGCUCAUAAA UAUGAGCCUUUAGUA UGGCUUGAAACUAGU CGAGUUUCUCGUCAAA CAGUU	99,00	24,24	27,27	21,21	26,26	45,45	53,54	0,96	1,14	-37,50	-36,30	0,20	5,51	-37,88	-0,83
>sfr-miR-92b	ACUGCGGUGGCCACGC UUAGGACGCGAUUCG GUGUAAACGUUGUGA CUUGUAUCAAAUUGC ACCAAUCCCGGCCUGC CUGUGGUCACCGCGCC	94,00	27,66	18,09	29,79	23,40	57,45	41,49	1,29	0,93	-41,50	-40,20	0,08	6,65	-44,15	-0,77
>sfr-miR-9685	GACGCCGGAGUGAAGC UGCUGCACUCGCCGAC	98,00	28,57	17,35	37,76	15,31	66,33	32,65	0,88	0,76	-33,10	-27,20	0,03	28,98	-33,78	-0,51

	CGGGUGCAUACAGUGC GUGUACCUCACCACGC CCAGCAUGCAGCUCGC CUUCAACGCCGUGCCG C														
>sfr-miR-10502-1	GAUAGAAAAUCCCAAC GAAGUUGGGCAGAAA GCCCGCCAGGCUGAUC ACCUCGCCUGGUCGGA GGAUCCUCCUUUUCUU UGGGAACUUUACUCUC	96,00	23,96	22,92	28,13	23,96	52,08	46,88	1,05	0,85	-34,90	-30,50	0,08	9,11	-36,35	-0,70
>sfr-miR-10502-2	AACAACAAAAGGGCA GAAAGCCCGCCAGGCU GAACACCUCGCCUGGU CGGAGGAUCCUCCUUU UCUUAGG	71,00	25,35	26,76	29,58	16,90	54,93	43,66	0,63	0,86	-24,90	-24,90	0,42	5,72	-35,07	-0,64
>sfr-miR-12237	GCGGGGCGCGGGGGG GCUGACCUGGUAGUA GACAGUGUUGGGGUG UCCUUGUCGGGGAUCC AGGUGACGACACCAGG GUUGCCCUCCUCGCAG ACCAGC	100,00	41,00	14,00	26,00	18,00	67,00	32,00	1,29	1,58	-44,60	-44,60	0,06	16,42	-44,60	-0,67
>sfr-miR-4705	UAAUUGUUGCUUAUA AGCGGUUGCGUGACCU GCUGGGUGAUUCGGU UACCGUCAAUCACUUG GUAAUGCAACCAAGU G	79,00	26,58	21,52	18,99	31,65	45,57	53,16	1,47	1,40	-25,30	-25,30	0,22	6,23	-32,03	-0,70
>sfr-miR-12389	CCGUCUGAAGUUAAG GUCGAGAAUCGCUGG GAAGGCGGCAGCACUA UGGUCCUCGAUGCGAU UACGUUGCGUAACUU GAGAAUU	85,00	30,59	23,53	20,00	24,71	50,59	48,24	1,05	1,53	-22,10	-15,70	0,05	19,20	-26,00	-0,51

>sfr-miR-2064	CCCGAGUCGACCGAAG CGAUACCACGGCCACG GUUAUAGGAAACCGG UGUGAACGGUUGCAU UGUGUUUCGCCGGGU GAGUAGGUUACGGCG ACCUCUUC	101,00	31,68	19,80	25,74	21,78	57,43	41,58	1,10	1,23	-33,50	-33,50	0,13	14,36	-33,17	-0,58
>sfr-miR-981	AAGAAUCCGCAUAUA AUUCGGGUUUCGGGA CGACGGAACCGUGAUU AAAUUGGUUCGUUGU CGACGAAACCUGCAAA AUGUGCGGUUGAA	91,00	27,47	28,57	17,58	25,27	45,05	53,85	0,88	1,56	-39,80	-39,80	0,10	5,12	-43,74	-0,97
>sfr-miR-11240-1	AGAACACAAAGAUUG CACGGUUGGUGCGUU AGCUGGGCAACCGGCU GCUGCGCAACGUGUAG CGGGUUCGAUUCCCGC ACGAAACUACUCUUUG UGUUAU	101,00	28,71	21,78	23,76	24,75	52,48	46,53	1,14	1,21	-45,90	-45,90	0,28	3,89	-45,45	-0,87
>sfr-miR-11240-2	UGCACGGUUGGUGCG GUGGCUGGGCAAACG GCUGCCACGUAGCGUG UAGCGGGUUCGAUUCC CGCACGGACCAACUGU UUG	82,00	36,59	14,63	25,61	21,95	62,20	36,59	1,50	1,43	-37,30	-37,30	0,41	4,94	-45,49	-0,73
>sfr-miR-11240-3	AGUUGCACGAAUAAA AUCGUCUGCACGGUUA GCGCAGUGAUUGGGU UCCGCGCAACGUGUAG CGGGUUCGAUUCCCGC ACAG	83,00	30,12	21,69	24,10	22,89	54,22	44,58	1,06	1,25	-31,70	-25,80	0,24	3,74	-38,19	-0,70
>sfr-miR-11240-4	AAUGAUUAUACAGUU GGUGCGUUGGCUGGA CAACCGGCUGGCGCGC AACGUGUAGCGGGUU	96,00	30,21	20,83	26,04	21,88	56,25	42,71	1,05	1,16	-43,90	-43,90	0,36	8,32	-45,73	-0,81

	CGAUUCCCGCACGGAG CAACCCUGUGUAAUCC AC															
>sfr-miR-2788	GCAAGUCGCCGUAGAG CUGGGGUUUCAAAGC GGCAUGUGCCACCUGC UAUGCAAUGCCCUUGG AAAUCCCAAACGUGCU GGCGACUGUC	90,00	28,89	21,11	27,78	21,11	56,67	42,22	1,00	1,04	-39,70	-39,50	0,10	6,42	-44,11	-0,78
>sfr-miR-10490-1	UUUUCUAUAAACAUC AGGGAACCCAAGACCU UUCCAACGAAUGCAAG ACCGUGGAAAUCGGU UCAUGCGUUCUGGAG UUAUAGAUAG	88,00	21,59	30,68	20,45	26,14	42,05	56,82	0,85	1,06	-22,50	-17,20	0,02	18,35	-25,57	-0,61
>sfr-miR-10490-2	UUUGCUAUAAACCUCA CGGAGCCCGAGACCUU UCCAACGAAUGCAAGA CCGUGGAAAUCGGUUC GUGCGUUCUGGAGUU AUAGCGUC	88,00	25,00	23,86	25,00	25,00	50,00	48,86	1,05	1,00	-29,80	-23,20	0,02	8,63	-33,86	-0,68
>sfr-miR-10490-3	CUGCUAUAAACCUCAC GGAGCCGAGACCUUUC CAACGAAUGCAAGACC GUGGAAAUCGGUCCG UGCGUUUUGUAGUUA	79,00	24,05	25,32	25,32	24,05	49,37	49,37	0,95	0,95	-28,60	-27,10	0,16	4,85	-36,20	-0,73
>sfr-miR-10511-1	CGACAGAUACUGUUCG ACAAACCAAUCGAUCG AUUGCCGGUCGAUGGC AUCGUUCAACGUCGAU CGAUGUUGAUCGACU GGUCUGGUA	89,00	25,84	23,60	23,60	25,84	49,44	49,44	1,10	1,10	-33,60	-33,60	0,23	8,59	-37,75	-0,76
>sfr-miR-10511-2	UCAUGCCAUCGAUCGA CAUUGAUCGAUUGGU GUGGUAGCUUUAAUU GAUCGGUUACCGGUCG	99,00	27,27	20,20	21,21	30,30	48,48	50,51	1,50	1,29	-50,60	-50,60	0,19	5,26	-51,11	-1,05

	AUUUACCAAUCGAUCGAUUGCCGGUCGAUGGCAGCG														
>sfr-miR-10464-1	GACUGCAUUUCAAUUGCAAUCCAACUGAACAUUGCAGUUCAAGGGCAGUUUCAAUGCAGUUGACACGACUGCAAUAGAACUGCAAGC	88,00	20,45	31,82	22,73	23,86	43,18	55,68	0,75	0,90	-29,80	-29,10	0,21	6,09	-33,86	-0,78
>sfr-miR-10464-2	UUGACACGACUGCAAUAGAACUGCAAAUCAAACGUGCGGUCCGAUUGUAGUUGAAUUGCAGUCGGUGUGCA	72,00	26,39	27,78	19,44	25,00	45,83	52,78	0,90	1,36	-29,30	-29,30	0,06	4,77	-40,69	-0,89
>sfr-miR-10464-3	AUGCAGUUGACACGACUGCAAUAGAACCGCAAACCAAACGUGUAGUCCGAUAGUAGUUCUAUUGCAAUCGUACCAGCUGCAU	83,00	20,48	31,33	24,10	22,89	44,58	54,22	0,73	0,85	-33,30	-33,30	0,33	2,42	-40,12	-0,90
>sfr-miR-10464-4	UGUGCAGUUCACAUAAUGCUAGGCUGCACGGUUGGCUUGCAGUACCAUUGCAGUCGUGUCAACUGCAUU	70,00	25,71	20,00	22,86	30,00	48,57	50,00	1,50	1,13	-24,60	-23,40	0,13	14,14	-35,14	-0,72
>sfr-miR-2856	GCCAUAGAAUAAUAAGUACAACUACGGCCUAAUAUUAAACGAAACUCCACAUUCGAGAACCGUAGUAGGCCCUAGGCUAUCUUAUCUUUUAUUUU	96,00	14,58	33,33	21,88	29,17	36,46	62,50	0,88	0,67	-18,60	-14,00	0,09	12,91	-19,38	-0,53
>sfr-miR-10466	UGCUAUAAUUAUCAGCACCGUCCAAAUGUCAAACACUGAUACUUUU	70,00	18,57	32,86	17,14	30,00	35,71	62,86	0,91	1,08	-23,40	-23,40	0,37	2,66	-33,43	-0,94

	GAGAGGUGCAGGUAA UUAUAAUG														
>sfr-miR-10505-2	UAGCUCGCACUGUCAA AAAGUAAUCAGAUCCC GUGUAGAGCCAAGUU UCUAACUCUAUACUUU UGAACUGGCGAGUCG	79,00	20,25	27,85	22,78	27,85	43,04	55,70	1,00	0,89	-19,90	-19,90	0,34	14,34	-25,19	-0,59
>sfr-miR-10513-1	UGAUAGGGGUGGGAC UUCUAACCCGUUAAGG CUGAGUACUACAUCUA AUUUUAUCGACAAAA GUCGGGGGUCGAAGG GGUCGAAUCCCCUCCC CCUAAAA	101,00	25,74	26,73	22,77	23,76	48,51	50,50	0,89	1,13	-30,70	-21,90	0,04	28,69	-30,40	-0,63
>sfr-miR-10513-2	CUCAUGCUCAGUAUCA AAGAACUUACGGUAU AGAGAGGUGCACAUA UACAAUGUGAUAGGU GUGGGACUUCUAACCC GUUAAGGCUGAGUAC UACAUCUA	101,00	22,77	30,69	18,81	26,73	41,58	57,43	0,87	1,21	-24,60	-20,80	0,02	24,11	-24,36	-0,59
>sfr-miR-10478	AGAGAGCCUUAAUGG CUCUGAUACCCGUGGG GAGGCGCUGGACUCGU CCAGCAAGUCCCACAC ACGGGUCCCAAGCUGG AAGGCGUCGGA	91,00	32,97	21,98	28,57	15,38	61,54	37,36	0,70	1,15	-42,40	-42,40	0,20	12,90	-46,59	-0,76
>sfr-miR-278	AAGAACAGUAUCAAG UGCUACGCCCGGACGA ACUUCCCAGCUCGGCC GACAUACACGGGUCGG UGGGAUCUUCGUCCGU UUGUAUCGCUUAUAC GUUUUU	101,00	24,75	20,79	26,73	26,73	51,49	47,52	1,29	0,93	-43,60	-43,40	0,08	4,47	-43,17	-0,84

>sfr-miR-7847	ACGAGCACUAUGCGUG CGUGGAGGACGAGGA GAAUAUGUCGGCGGC GCGCCACGCCUCGGCC UCCUCCUCCGCGUCGU ACUGCUGCA	88,00	32,95	15,91	32,95	17,05	65,91	32,95	1,07	1,00	-41,30	-41,30	0,19	13,13	-46,93	-0,71
>sfr-miR-2779-2	UUGAUCCGGCUCGAAG GACCAGAAUGUGCGG GUCGGAGGGAUCAGG CUGUGGAUCUAUUCCU CGACCCGCACAUUCUG GUCCUUCGAGCCGGAU UGU	98,00	31,63	17,35	25,51	24,49	57,14	41,84	1,41	1,24	-78,50	-78,00	0,13	7,26	-80,10	-1,40
>sfr-miR-10485	AAUCGCUGCGCGGGGG ACAGUCUACCCGGACA GGCCGACUACUGUAUG UCGCACUGGACGGGCA GGCCGCCCUCCUCACA AGCGUCA	88,00	30,68	19,32	34,09	14,77	64,77	34,09	0,76	0,90	-41,00	-38,60	0,32	5,94	-46,59	-0,72
>sfr-miR-10477-1	GUCGGCGACGGCGACC UCUCGCGCCCGGCACU GGUUCAGGCCAUGGU GCGGAGCGAGAGGAU GGGAUGCCGUCUCCUC C	80,00	37,50	12,50	32,50	16,25	70,00	28,75	1,30	1,15	-39,80	-39,80	0,08	10,18	-49,75	-0,71
>sfr-miR-10477-2	GUCGGCGACGGCAACC UCUCGCGCUCGGCACU GGUUCAGGCCAUGGU GCGGAGCGAGAGGGA CUGGGAUGCCGUCUCC UCC	82,00	36,59	13,41	31,71	17,07	68,29	30,49	1,27	1,15	-43,40	-42,30	0,19	4,66	-52,93	-0,77
>sfr-miR-10477-3	GCUGAGCACCGCCAAG UCCUCAGGGACCUCUC GCGCCCAGCACUGGUU CAGGCCAUGGUGCGGA	93,00	33,33	16,13	33,33	16,13	66,67	32,26	1,00	1,00	-42,30	-41,50	0,15	11,40	-45,48	-0,68

	GCGAGAGGGACUGGG AUGCCGUCUCCUC														
>sfr-miR-10477-4	CGCUAGGUACAUAACC UUGCGGAGCGAGAGG GACUGGGAUGCCGUCU CCUCCUUCUGCGAAGC AGUCAUGCUAGCUAA GG	81,00	30,86	20,99	25,93	20,99	56,79	41,98	1,00	1,19	-29,40	-25,50	0,10	14,58	-36,30	-0,64
>sfr-miR-7398p-1	UCUUGUGUACUCGCGC UCGCCGCCACCAGCUC CGUCAACUACUGCGGC GCCAAGAUGUGUGGA GGCAAGGAUACACAU AC	81,00	25,93	20,99	32,10	19,75	58,02	40,74	0,94	0,81	-25,20	-25,20	0,26	6,22	-31,11	-0,54
>sfr-miR-7398p-2	UCCUGUGUACUCGCGC UCGCCGCCACCAGCUC CGUCAACUACUGCGGC GCCAAGAUGUGUGGA GGCAAGGAUACACAU AC	81,00	25,93	20,99	33,33	18,52	59,26	39,51	0,88	0,78	-25,20	-25,20	0,25	7,09	-31,11	-0,52
>sfr-miR-10470-1	CCGUUUCUACUUCACC UUUUCGAGCCGGAGCC CCGGUAAACUUGCCAA CCUCAGUAUCUACUGG GCUCCAUCUGAAUACA GGUAAGCUUAGAAAU AU	98,00	18,37	24,49	28,57	27,55	46,94	52,04	1,13	0,64	-28,60	-28,60	0,04	10,35	-29,18	-0,62
>sfr-miR-10470-2	CUCCUGCUUGUCGAGC CGGAGCCCCGGUAAGC CCGCUAGGUAGUCCGC AGCUCCGGAUCACACA GCAGAAA	72,00	27,78	20,83	34,72	15,28	62,50	36,11	0,73	0,80	-32,70	-32,70	0,55	2,56	-45,42	-0,73
>sfr-miR-10470-3	GUGCCUAAUCCUGCUU UUCGAGCCGGAGCCCC GGUAAACCCGCUAGGU AGUCCGCAGCUCCGGA	83,00	26,51	24,10	30,12	18,07	56,63	42,17	0,75	0,88	-27,40	-19,30	0,08	13,91	-33,01	-0,58

	UCAGAACACAAUAGG GAA														
>sfr-miR-10470-4	GUUUCUACUCCUGCUU UUCGAGCCGGAGCCCC GGUAAGCGGUAGGUA GUUCGCAGCUCCGGAU AGCAGUUGUAGAGCU	79,00	30,38	16,46	25,32	26,58	55,70	43,04	1,62	1,20	-29,80	-23,20	0,05	17,37	-37,72	-0,68
>sfr-miR-10470-5	GCGUUCCUACUUCUAC UUUUCGAGCCGGAGCC CCGGUAACCCGUUAGG CAUUUCGCAGCUCCGG GUCAAUCGAAUGACA GUUAAAAUAGGAAUU U	96,00	22,92	22,92	26,04	27,08	48,96	50,00	1,18	0,88	-29,00	-25,10	0,05	10,93	-30,21	-0,62
>sfr-miR-10470-6	UCCUGCUUUUCGAGCC GGAGCCCCGGUAAACC CGCUAAGUAGUCCGCA GCUCCGAGCUUCAAAG GCAUAC	71,00	23,94	21,13	33,80	19,72	57,75	40,85	0,93	0,71	-28,10	-28,10	0,36	2,75	-39,58	-0,69
>sfr-miR-10493-1	UUCCCUCGUUCUGAUC CGAAGCUGCGGACUAC CUAGCGGGUUUACCGG GGCUCCGGGUCGGAAA GCAGGAGU	73,00	32,88	16,44	27,40	21,92	60,27	38,36	1,33	1,20	-35,20	-34,90	0,28	4,11	-48,22	-0,80
>sfr-miR-10493-2	GAUCCAUAUCUAGUU ACAAAUUUUCUGAUCC GAAGCUGCGGACUACC UAGCGGGUUAACCGG GGCUCCGGUUCGAAAA GCAGGAAUAGGUACG GGGU	98,00	28,57	25,51	21,43	23,47	50,00	48,98	0,92	1,33	-33,00	-31,80	0,11	7,07	-33,67	-0,67
>sfr-miR-10493-3	UUUAGAUGUAACUGC UAACUACCUACCGGGU UUACCGGGGCUCCGAC UCGCCAAGCAGGAGUA GGAACGGGGUGGUUU	99,00	29,29	23,23	21,21	25,25	50,51	48,48	1,09	1,38	-32,70	-26,40	0,13	12,30	-33,03	-0,65

	UUAGUCAGUAAGAGU CUGAC														
>sfr-miR-10493-4	UUACCGGGUGUCUGGC UCGAAAGGCAGGAGU AGAAACGGGGUGGUU UUUAGUCAGUAAGAA UCUGACACUCUCGCUC GCCUCGCCCGACGCCC GGAGA	99,00	31,31	21,21	25,25	21,21	56,57	42,42	1,00	1,24	-40,70	-34,80	0,14	12,89	-41,11	-0,73
>sfr-miR-10493-5	GGGUCUCCCGCUUGAG AAGCAAGAGUAGGAA CGGGGUGGUUUUUAG UCAGUAAGAGUCUGA CACUCCCUCUCGCUUC ACUCAAGGCGGGAGA AGU	96,00	31,25	22,92	21,88	22,92	53,13	45,83	1,00	1,43	-38,60	-37,30	0,05	13,84	-40,21	-0,76
>sfr-miR-1175	GACGCGUGAAGGAUC AAGUGGAGGUGUGAU CUCUUCACUUUUUAUG UUUAAAGUGAGAUUC AACUCCUCCAACUUAA UUCCGAAACGCUUU	92,00	20,65	26,09	19,57	32,61	40,22	58,70	1,25	1,06	-32,00	-32,00	0,03	8,62	-34,78	-0,86
>sfr-miR-10499b-1	CUCUGCAUGGGGCGGU ACAAGGGUUUGAAAC UCCAUACAUGUGUAU GAGAAUCUGCAUGGA GUUUCAAACUCUUGA AUUGCUAUAUGCAAU G	93,00	24,73	26,88	17,20	30,11	41,94	56,99	1,12	1,44	-44,50	-44,50	0,48	3,24	-47,85	-1,14
>sfr-miR-10499b-2	CUGCAUAGGGCAGUU AAGGGUUUGAAACUC CAUACAUGUAAAUGC GCUUCUGCAUGGAGU UUCAAACUCUUGAACU GCUCUGUGUAA	88,00	22,73	26,14	19,32	30,68	42,05	56,82	1,17	1,18	-47,90	-45,70	0,05	8,72	-54,43	-1,29

>sfr-miR-10499b-3	UAUCAAUGCAUGGGA CAGUGCAAGGGUUUA AAACUCCAUACAUGUA UAGCAUCUGCAUGGA GUUUCAAACUCUUGG AUUGCUCUGUGCAAU GUCA	96,00	21,88	28,13	18,75	30,21	40,63	58,33	1,07	1,17	-39,10	-39,10	0,08	6,99	-40,73	-1,00
>sfr-miR-2766	GAAAACGCCCGUCUCG UCCAUCCUUCGUCUCG ACUGGCGCUGCUUAAC GCUUCAGUCUUGUCGA AUGGUGGGUGAGAUU UGCGUUCUA	89,00	25,84	15,73	26,97	30,34	52,81	46,07	1,93	0,96	-38,40	-36,60	0,34	3,90	-43,15	-0,82
>sfr-miR-10060	AAGCAUGUAGUAAUA UGAAAUGUGUUAAUC CAUGCCAAGGAGCGUG UGGCGUUGCCGCAAAA UGCCAAGUGAUUAACC ACAAUCCAAUUUGCUC AUGCCC	101,00	21,78	30,69	21,78	24,75	43,56	55,45	0,81	1,00	-30,70	-30,70	0,18	5,20	-30,40	-0,70
>sfr-miR-10491-1	CAUCAAACUUAGUGCU GUGCUCACAUGUCAAC CAGGGGGUGACGCUCC AUGAUAGUGCCCUGA UUAACAUGUCAGUAC ACACUGAGCUUGUAC	94,00	22,34	25,53	25,53	25,53	47,87	51,06	1,00	0,88	-34,00	-32,50	0,20	6,54	-36,17	-0,76
>sfr-miR-10491-2	CUUCAAACUUGGUGCU GUGCUCACAUGUCAAC CAGGGGGUGACGCUCC AUGAUAGUGCCCUGA UUAACAUGUCAGUAC ACACUGAGCUUGUAC	94,00	23,40	23,40	25,53	26,60	48,94	50,00	1,14	0,92	-32,70	-31,20	0,20	6,49	-34,79	-0,71
>sfr-miR-466i	AGUGAAUGAGCCGGC AUGUCAUCGUGUGUG UGUGUGUGUGUGUGU GGCGCGCGCCACACGC	93,00	34,41	13,98	27,96	23,66	62,37	37,63	1,69	1,23	-49,20	-44,90	0,12	11,54	-52,90	-0,85

GCCAACGCAGACGCCA
CGUUGCCGCUCUUCUG
G

Table S2 Counts of miRNAs in 17 different sequencing of *S. frugiperda*. The penultimate column displays the sum of the counts of the 17 sequencing and the last one shows the number of sequencing in which the miRNA appeared. We selected 116 molecules that were identified in at least 10 sequencing, ensuring the choice of molecules with high chances of actually occurring. However, among these selected molecules, some are highly expressed in *S. frugiperda*.

miRNA	**Sequencing SRR6670043**	**Sequencing SRR6670042**	**Sequencing SRR6670041**	**Sequencing SRR6670040**	**Sequencing SRR1536060**	**Sequencing SRR1536059**	**Sequencing SRR1536058**	**Sequencing SRR850249**	**Sequencing SRR850248**	**Sequencing SRR850247**	**Sequencing SRR850246**	**Sequencing SRR850245**	**Sequencing SRR850244**	**Sequencing SRR850243**	**Sequencing SRR850242**	**Sequencing SRR496499**	**Sequencing SRR496500**	**Sum of the counts**	**N° of sequencing**
sfr-miR-10462-3-5p	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-10474-1-3p e sfr-miR-10474-2-3p	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1

b-10500-3-3p																			
sfr-miR b-10507-1-5p	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR b-8515-1-3p	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR b-8515-2-3p	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR b-10454a-6-3p	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR b-10508a-2-3p e sfr-miR	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1

b-10508a-4-3p																			
sfr-miR-b-5394-3p	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-b-10468-5p	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-b-10478-3p	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-b-6844-5p	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-2765-3p	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-b-10847-5p	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	1,00	1
sfr-miR-b-	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	1,00	1

2064-3p																			
sfr-miRb-2733b-1-3p e sfr-miRb-2733b-2-3p	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	1,00	1
sfr-miRb-297a-3p	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	1,00	1
sfr-miRb-10495-3-3p	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	1,00	1
sfr-miRb-10454h-2-3p e sfr-miRb-1045	NA	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	1

4h-4-3p																			
sfr-miRb-137b-2-5p	NA	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	1
sfr-miRb-10454h-7-3p	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	1
sfr-miRb-10475-5p	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	2,00	1
sfr-miR-10509-5p	1	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-10455-5p	1	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-10481-5p	1	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-	1	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	2,00	2

9807-5p																		
sfr-miRb-10462-5-5p	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	2,00	2
sfr-miRb-971-3p	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-10492c-4-5p	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-iab-4-3p	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-10476-3p	NA	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-10484-2-5p	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	2,00	2

sfr-miRb-10506-5p	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	2,00	2
sfr-miRb-10478-5p	NA	NA	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	2,00	2
sfr-miRb-297-5p	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	2,00	2
sfr-miRb-8485-5p	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	1	NA	NA	2,00	2
sfr-miRb-10454d-3p	NA	2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3,00	2
sfr-miRb-10474-1-5p	NA	2	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3,00	2
sfr-miRb-1047	NA	2	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3,00	2

4-2-5p																			
sfr-miRb-3192-5p	NA	NA	NA	NA	2	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	3,00	2
sfr-miRb-10503-2-3p	NA	NA	NA	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	NA	1	3,00	2
sfr-miRb-10454h-2-5p	NA	NA	3	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5,00	2
sfr-miRb-10459-3-3p	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	6,00	2
sfr-miRb-10475-3p	NA	3	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7,00	2
sfr-miRb-10498-3p	NA	NA	4	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,00	2

sfr-miR-10463-5p	NA	NA	NA	NA	NA	NA	NA	NA	1	1	NA	NA	NA	1	NA	NA	NA	3,00	3
sfr-miRb-10454h-1-5p	1	NA	2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4,00	3
sfr-miRb-10454h-4-5p	1	NA	2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4,00	3
sfr-miRb-9388-5p	1	NA	NA	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	1	NA	NA	4,00	3
sfr-miRb-33-3p	NA	NA	2	1	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	4,00	3
sfr-miRb-10484-2-3p	NA	NA	NA	NA	NA	2	NA	NA	NA	1	1	NA	NA	NA	NA	NA	NA	4,00	3
sfr-miRb-1049	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	1	NA	NA	NA	3	NA	NA	5,00	3

2a-4-3p																		
sfr-miRb-2788-5p	NA	1	4	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6,00	3
sfr-miRb-279b-2-5p	NA	2	2	3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7,00	3
sfr-miR-10487-5p	NA	2	5	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,00	3
sfr-miRb-252a-3p	NA	3	4	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,00	3
sfr-miRb-8468-5p	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	2	5	NA	NA	NA	NA	8,00	3
sfr-miRb-10492c-5-5p	2	NA	1	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,00	3
sfr-miRb-	NA	3	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	9,00	3

10466-5p																			
sfr-miRb-2756-3p	NA	2	5	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,00	3
sfr-miRb-193-5p	NA	4	6	3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13,00	3
sfr-miRb-10497-3p	NA	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11	13,00	3
sfr-miRb-970-5p	NA	4	3	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	14,00	3
sfr-miRb-10466-3p	NA	3	11	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18,00	3
sfr-miR-10494-5p	NA	1	16	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	22,00	3
sfr-miRb-1046	NA	NA	1	1	1	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	4,00	4

1b-3-5p																			
sfr-miRb-10492b-1-3p e sfr-miRb-10492b-2-3p	NA	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	1	NA	1	NA	NA	4,00	4
sfr-miRb-10500-2-5p	NA	NA	NA	1	NA	1	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	4,00	4
sfr-miRb-10490-3-3p	NA	NA	NA	NA	1	1	1	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	4,00	4
sfr-miRb-10492a-6-5p	NA	NA	NA	1	NA	NA	NA	1	2	NA	NA	NA	NA	NA	1	NA	NA	5,00	4
sfr-miRb-1047	NA	NA	NA	1	1	3	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	6,00	4

7-1-5p																		
sfr-miRb-10492c-1-3p e sfr-miRb-10492c-5-3p	NA	NA	NA	1	3	1	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	6,00	4
sfr-miRb-10453-1-3p e sfr-miRb-10453-2-3p	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	2	NA	NA	2	1	NA	NA	7,00	4
sfr-miRb-10459-1-3p; sfr-miRb-1045	NA	NA	NA	2	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	4	8,00	4

9-2-3p e sfr-miR b-1045 9-4-3p																			
sfr-miR b-2796 -5p	2	4	2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,00	4
sfr-miR-13a-5p	NA	2	4	1	NA	NA	NA	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	9,00	4
sfr-miR b-3286 -5p	1	5	3	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11,00	4
sfr-miR b-34-3p	2	NA	3	5	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	11,00	4
sfr-miR b-2755 -5p	1	2	6	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13,00	4
sfr-miR b-	1	NA	6	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	14,00	4

7b-3p																			
sfr-miR-971b-5p	4	9	12	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35,00	4
sfr-miR-3286-3p	1	9	18	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	36,00	4
sfr-miR-10506-3p	3	5	11	25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44,00	4
sfr-miR-929-3p	4	7	29	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50,00	4
sfr-miR-10499a-1-5p; sfr-miR-10499a-2-5p; sfr-miR-10499a-3-	8	17	6	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	54,00	4

5p e sfr-miR-10499a-5-5p																			
sfr-miR-10499a-4-5p	8	17	6	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	54,00	4
sfr-miRb-10499a-3-5p	8	17	6	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	54,00	4
sfr-miRb-277-5p	3	14	30	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	57,00	4
sfr-miRb-10485-3p	5	27	42	9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	83,00	4
sfr-miRb-71-5p	14	24	42	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	99,00	4
sfr-miRb-	5	49	55	21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	130,00	4

124-3p																			
sfr-miRb-137b-1-3p e sfr-miRb-137b-2-3p	39	55	87	55	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	236,00	4
sfr-miRb-10480-3p	34	46	121	94	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	295,00	4
sfr-miRb-1-5p	14	122	143	43	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	322,00	4
sfr-miRb-10498-5p	16	66	216	83	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	381,00	4
sfr-miRb-2788-3p	17	116	241	49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	423,00	4
sfr-miRb-	25	117	226	123	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	491,00	4

263a-1-3p e sfr-miR b-263a-2-3p																			
sfr-miR b-2a-2-5p	148	139	222	246	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	755,00	4
sfr-miR b-9-2-3p	131	346	359	170	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1006,00	4
sfr-miR-1047 2-5p	583	680	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	1265,00	4
sfr-miR b-274-3p	576	1005	1497	1083	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4161,00	4
sfr-miR b-281-3p	1849	3539	10009	4984	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	20381,00	4
sfr-miR b-9-2-5p	21765	38198	73314	44153	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	177430,00	4

sfr-miR b-10492a-7-5p	NA	NA	NA	NA	NA	1	NA	1	2	NA	NA	NA	NA	1	1	NA	NA	6,00	5
sfr-miR b-285-5p	2	NA	2	NA	1	NA	NA	NA	NA	1	NA	NA	NA	1	NA	NA	NA	7,00	5
sfr-miR b-10503-2-5p	NA	NA	1	NA	NA	1	2	1	NA	NA	3	NA	NA	NA	NA	NA	NA	8,00	5
sfr-miR b-10454b-5p	4	NA	1	3	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	1	NA	NA	10,00	5
sfr-miR b-2856-3p	NA	NA	NA	1	5	1	2	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	10,00	5
sfr-miR b-10497-5p	1	3	15	5	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	26,00	5
sfr-miR b-	3	12	14	3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	33,00	5

283-3p																			
sfr-miRb-210-3p	2	12	23	1	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	39,00	5
sfr-miR-10487-3p	6	19	103	22	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	152,00	5
sfr-miRb-3338-3p	14	64	112	19	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	210,00	5
sfr-miRb-10480-5p	27	37	75	72	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	212,00	5
sfr-miR-13b-5p	37	40	69	76	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	224,00	5
sfr-miRb-2796-3p	25	67	100	55	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	248,00	5
sfr-miRb-263b-1-5p e	19	146	55	40	44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	304,00	5

sfr-miRb-263b-3-5p																			
sfr-miRb-193-3p	24	138	332	95	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	590,00	5
sfr-miRb-993a-2-3p	91	248	646	289	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1275,00	5
sfr-miRb-750-5p	659	2421	3991	1771	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8844,00	5
sfr-miRb-316-5p	1187	2912	9260	2951	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	16311,00	5
sfr-miRb-10495-4-5p	NA	2	1	NA	2	3	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	10,00	6
sfr-miR-	1	NA	5	NA	2	2	NA	NA	NA	NA	1	NA	NA	1	NA	NA	NA	12,00	6

10510-3p																		
sfr-miRb-10455-3p	NA	6	1	NA	4	1	NA	1	NA	NA	1	NA	NA	NA	NA	NA	NA	14,00	6
sfr-miRb-10464-1-5p e sfr-miRb-10464-2-5p	NA	4	NA	1	4	11	1	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	22,00	6
sfr-miRb-10499b-1-5p	6	6	12	14	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	40,00	6
sfr-miRb-10499b-2-5p	6	6	12	14	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	40,00	6
sfr-miR-10494-3p	NA	1	17	7	13	3	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43,00	6

sfr-miR b-932-3p	NA	NA	1	NA	2	NA	NA	NA	1	NA	NA	NA	1	NA	NA	2	36	43,00	6
sfr-miR b-308-5p	9	6	18	8	1	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	44,00	6
sfr-miR b-375-3p	6	16	33	14	2	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	72,00	6
sfr-miR b-1050 4-5p	11	24	33	19	NA	NA	NA	NA	NA	1	2	NA	NA	NA	NA	NA	NA	90,00	6
sfr-miR-1051 0-5p	NA	49	11	4	44	9	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	129,00	6
sfr-miR b-1000 -5p	25	45	86	37	NA	1	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	195,00	6
sfr-miR b-2c-5p	44	105	142	111	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	2	405,00	6
sfr-miR b-	62	92	225	101	3	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	484,00	6

iab-4-5p																		
sfr-miRb-305-3p	69	275	560	212	1	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	1118,00	6
sfr-miRb-252a-5p	114	268	553	240	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	1177,00	6
sfr-miRb-1175-3p	553	117	1048	992	2	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2716,00	6
sfr-miRb-375-5p	45	2007	898	173	1	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	3125,00	6
sfr-miRb-1175-5p	1175	2441	5396	2683	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	11698,00	6
sfr-miRb-10461b-5-5p	1	2	NA	1	1	NA	NA	2	NA	1	NA	NA	NA	1	NA	NA	NA	9,00	7
sfr-miRb-	1	NA	NA	3	1	NA	NA	1	2	NA	1	NA	NA	NA	1	NA	NA	10,00	7

10471-1-5p e sfr-miR b-10471-2-5p																			
sfr-miR b-10492c-6-3p	NA	1	NA	2	1	NA	2	1	NA	NA	4	NA	NA	NA	3	NA	NA	14,00	7
sfr-miR b-10461a-1-3p; sfr-miR b-10461a-2-3p e sfr-miR b-10461a-3-3p	9	3	NA	1	NA	NA	NA	1	NA	1	1	NA	NA	1	NA	NA	NA	17,00	7
sfr-miR b-	1	1	NA	2	NA	NA	NA	NA	5	NA	2	NA	NA	2	6	NA	NA	19,00	7

11240-1-5p; sfr-miRb-11240-2-5p; sfr-miRb-11240-3-5p e sfr-miRb-11240-4-5p																			
sfr-miRb-10476-5p	2	4	7	5	5	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	30,00	7
sfr-miRb-10490-1-5p; sfr-miRb-1049	2	NA	NA	1	15	9	2	NA	NA	NA	NA	NA	NA	1	2	NA	NA	32,00	7

0-2-5p e sfr-miR b-10490-3-5p																		
sfr-miR b-10469-3p	NA	1	11	10	14	8	4	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	49,00	7
sfr-miR b-2767-5p	29	27	41	27	NA	NA	1	NA	NA	NA	NA	1	2	NA	NA	NA	NA	128,00	7
sfr-miR b-317-5p	8	24	89	30	NA	NA	NA	NA	NA	1	NA	NA	NA	1	1	NA	NA	154,00	7
sfr-miR b-929-5p	17	32	66	38	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	1	2	157,00	7
sfr-miR b-10458-5p	NA	11	7	1	212	48	53	NA	NA	NA	NA	NA	NA	NA	NA	NA	13	345,00	7
sfr-miR b-	67	350	375	158	25	14	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	997,00	7

263b-3-3p																		
sfr-miR-6094-5p	74	1039	849	206	7	2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2178,00	7
sfr-miRb-927a-5p	289	741	744	540	28	12	9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2363,00	7
sfr-miRb-133-3p	284	2045	5645	1187	NA	1	1	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	9164,00	7
sfr-miRb-133-5p	284	2045	5645	1187	NA	1	1	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	9164,00	7
sfr-miRb-750-3p	5356	22979	33723	12984	21	15	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	75082,00	7
sfr-miRb-274-5p	9856	29946	51900	22394	NA	2	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	114100,00	7
sfr-miRb-1050	1	NA	1	4	NA	1	2	NA	NA	1	NA	NA	NA	1	1	NA	NA	12,00	8

8a-1-5p																		
sfr-miRb-10454a-13-5p	1	4	2	1	3	2	NA	NA	NA	NA	NA	NA	NA	1	3	NA	NA	17,00	8
sfr-miRb-10470-6-3p	NA	NA	NA	4	4	NA	NA	2	3	3	2	NA	NA	2	1	NA	NA	21,00	8
sfr-miRb-965-5p	1	4	7	7	2	NA	1	NA	NA	NA	NA	NA	1	NA	1	NA	NA	24,00	8
sfr-miR-10509-3p	1	6	1	2	12	3	6	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	32,00	8
sfr-miRb-10464-4-3p	2	26	4	1	1	NA	2	NA	NA	NA	NA	NA	NA	1	NA	NA	1	38,00	8
sfr-miRb-466i-1-5pe sfr-	NA	3	NA	3	NA	NA	NA	8	4	5	NA	NA	NA	8	4	NA	3	38,00	8

miR b-466i-5p																			
sfr-miR b-574-5p	NA	3	NA	3	NA	NA	NA	8	4	5	NA	NA	NA	8	5	NA	3	39,00	8
sfr-miR b-10507-1-3p	7	9	14	12	NA	4	1	NA	1	1	NA	NA	NA	NA	NA	NA	NA	49,00	8
sfr-miR b-10486-5p	NA	20	1	4	3	12	2	NA	NA	NA	NA	NA	NA	1	NA	NA	7	50,00	8
sfr-miR b-10488-3p	3	9	21	5	52	13	7	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	111,00	8
sfr-miR b-965-3p	9	13	64	28	NA	1	NA	2	1	NA	NA	NA	NA	NA	NA	NA	1	119,00	8
sfr-miR b-981-3p	23	26	114	49	1	4	2	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	220,00	8

sfr-miRb-927a-3p	117	224	536	299	9	9	2	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	1197,00	8
sfr-miR-13a-3p	139	343	461	335	38	15	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1342,00	8
sfr-miR-10472-3p	442	1398	1567	502	3	3	4	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	3921,00	8
sfr-miRb-305-5p	727	1721	3698	1627	2	NA	NA	NA	NA	1	NA	2	5	NA	NA	NA	NA	7783,00	8
sfr-miRb-281-5p	28161	288483	730454	112824	3	8	NA	NA	NA	NA	NA	NA	NA	NA	2	NA	2	115993 7,00	8
sfr-miRb-10492b-1-5p	2	1	1	1	NA	1	NA	1	NA	1	1	NA	NA	1	NA	NA	NA	10,00	9
sfr-miRb-10492b-3-5p; sfr-	2	4	1	1	NA	2	NA	1	NA	2	1	NA	NA	1	NA	NA	NA	15,00	9

miR b-1049 2b-4-5p; sfr-miR b-1049 2b-5-5p e sfr-miR b-1049 2b-6-5p																		
sfr-miR b-307-5p	1	1	6	NA	7	NA	NA	1	1	1	NA	NA	NA	NA	1	NA	4	23,00	9
sfr-miR b-1047 0-2-3p e sfr-miR b-1047 0-3-3p	NA	NA	NA	4	4	1	NA	3	3	3	3	NA	NA	2	1	NA	NA	24,00	9
sfr-miR	3	13	3	1	NA	1	NA	NA	1	NA	1	NA	NA	3	1	NA	NA	27,00	9

b-1050-1-3p																			
sfr-miR b-1050-8a-3-3p; sfr-miR b-1050-8a-5-3p; sfr-miR b-1050-8a-6-3p e sfr-miR b-1050-8a-7-3p	7	10	1	4	1	2	NA	NA	3	NA	NA	NA	NA	1	3	NA	NA	32,00	9
sfr-miR b-1050-7-2-5p	6	5	8	4	4	NA	NA	1	NA	NA	2	NA	NA	2	NA	NA	1	33,00	9
sfr-miR	8	NA	1	16	NA	1	NA	3	NA	3	2	NA	NA	2	2	NA	NA	38,00	9

b-1049 0-2-3p sfr-miR b-1047 0-1-5p; sfr-miR b-1047 0-2-5p; sfr-miR b-1047 0-3-5p; sfr-miR b-1047 0-4-5p; sfr-miR b-1047 0-5-5p e sfr-miR	NA	1	NA	1	20	11	4	3	NA	1	NA	NA	NA	NA	1	NA	1	43,00	9

b-10470-6-5p																		
sfr-miR-10465-1-5p e sfr-miR-10465-2-5p	4	8	16	4	3	NA	NA	NA	1	1	NA	NA	NA	3	6	NA	NA	46,00	9
sfr-miR-10454c-5p	10	35	2	4	3	1	NA	NA	NA	NA	2	NA	NA	1	1	NA	NA	59,00	9
sfr-miRb-10489-2-3p	6	13	7	6	NA	1	NA	NA	1	NA	3	NA	NA	14	25	NA	NA	76,00	9
sfr-miRb-10503-1-5p	NA	NA	NA	NA	76	83	17	10	19	10	9	NA	NA	4	10	NA	NA	238,00	9
sfr-miRb-11-5p	32	102	175	64	5	1	NA	NA	NA	1	2	NA	NA	NA	NA	NA	1	383,00	9

sfr-miRb-989-3p	NA	1	1	NA	242	97	30	NA	NA	NA	1	1	NA	1	NA	NA	21	395,00	9
sfr-miRb-2a-1-5p	113	153	259	230	5	4	1	1	NA	NA	NA	NA	NA	NA	NA	NA	1	767,00	9
sfr-miRb-10485-5p	124	408	812	357	3	2	NA	NA	1	NA	NA	NA	NA	2	NA	NA	2	1711,00	9
sfr-miRb-10-2-5p	98	1677	542	233	332	187	129	NA	1	1	NA	NA	NA	NA	NA	NA	NA	3200,00	9
sfr-miR-2765-5p	4	NA	NA	1	2	4	3	1	1	NA	1	NA	NA	1	4	NA	NA	22,00	10
sfr-miRb-10503-1-3p	NA	NA	NA	NA	12	4	1	3	2	1	2	NA	NA	1	NA	1	1	28,00	10
sfr-miRb-10454e-4-3p	1	1	3	NA	12	5	4	NA	1	NA	NA	NA	NA	1	1	NA	1	30,00	10

sfr-miR b-10495-1-3p; sfr-miR b-10495-2-3p e sfr-miR b-10495-4-3p	1	NA	NA	NA	5	6	2	1	4	3	2	NA	NA	2	4	NA	NA	30,00	10
sfr-miR b-10456-3p	12	4	2	18	4	NA	NA	NA	1	3	2	NA	NA	7	5	NA	NA	58,00	10
sfr-miR b-10492a-1-5p	20	2	15	20	2	1	NA	2	3	1	2	NA	NA	NA	NA	NA	NA	68,00	10
sfr-miR b-33-5p	2	14	18	11	NA	NA	NA	13	7	NA	NA	2	1	1	NA	NA	1	70,00	10
sfr-miR	8	22	35	20	NA	NA	NA	1	NA	2	1	2	1	1	NA	NA	NA	93,00	10

b-279b-1-5p																			
sfr-miRb-10495-1-5p; sfr-miRb-10495-2-5p; sfr-miRb-10495-3-5p e sfr-miRb-10495-5-5p	NA	2	1	NA	82	38	24	2	2	1	1	NA	NA	NA	1	NA	NA	154,00	10
sfr-miRb-79-5p	91	273	425	215	4	1	NA	NA	NA	NA	1	2	1	NA	NA	NA	2	1015,00	10
sfr-miRb-	2	1	1	1	3	2	1	1	3	NA	1	NA	NA	NA	NA	NA	4	20,00	11

10459-1-5p e sfr-miRb-10459-3-5p																		
sfr-miRb-10492c-2-3p	3	4	8	3	NA	1	NA	1	1	2	4	NA	NA	2	2	NA	NA	31,00	11
sfr-miRb-285-3p	3	9	9	1	5	5	1	1	1	NA	1	NA	NA	NA	NA	NA	1	37,00	11
sfr-miRb-10477-2-3p; sfr-miRb-10477-3-3p e sfr-miRb-1047	2	NA	NA	4	12	11	NA	6	12	2	2	NA	1	1	1	NA	NA	54,00	11

7-4-3p																			
sfr-miRb-10492b-2-5p	10	6	8	19	2	1	1	1	3	NA	NA	NA	NA	2	3	NA	NA	56,00	11
sfr-miRb-10454e-1-5p; sfr-miRb-10454e-2-5p; sfr-miRb-10454e-3-5p e sfr-miRb-10454e-4-5p	NA	15	10	10	1	3	NA	4	NA	1	1	NA	NA	7	5	NA	1	58,00	11
sfr-miRb-1045	7	19	7	7	5	4	NA	1	NA	1	3	NA	NA	4	4	NA	NA	62,00	11

4a-10-5p																			
sfr-miRb-10508b-1-3p; sfr-miRb-10508b-2-3p; sfr-miRb-10508b-3-3pe sfr-miRb-10508b-4-3p	10	5	4	23	NA	2	NA	2	9	3	2	NA	NA	3	3	NA	NA	66,00	11
sfr-miRb-10454a-3-5p	3	14	12	9	12	4	NA	1	NA	2	2	NA	NA	4	5	NA	NA	68,00	11
sfr-miRb-	9	14	3	13	17	18	4	1	NA	3	1	NA	NA	3	NA	NA	NA	86,00	11

10501-1-3p; sfr-miRb-10501-2-3p; sfr-miRb-10501-3-3p; sfr-miRb-10501-4-3p e sfr-miRb-10501-5-3p																			
sfr-miRb-10482-3p	2	10	12	5	NA	1	NA	NA	NA	4	2	18	31	10	6	NA	NA	101,00	11
sfr-miRb-1045	6	38	13	11	12	7	NA	1	NA	2	3	NA	NA	4	6	NA	NA	103,00	11

4a-12-5p sfr-miRb-1045 4a-1-5p; sfr-miRb-1045 4a-11-5p; sfr-miRb-1045	12	38	17	15	12	7	NA	1	NA	2	3	NA	NA	4	7	NA	NA	118,00	11
4a-2-5p; sfr-miRb-1045 4a-4-5p; sfr-miRb-1045 4a-5-5p; sfr-miR																			

b-1045 4a-6-5p; sfr-miR b-1045 4a-7-5p; sfr-miR b-1045 4a-8-5p e sfr-miR b-1045 4a-9-5p																			
sfr-miR-279a-5p	13	26	43	28	NA	NA	NA	1	NA	2	1	2	1	1	NA	NA	2	120,00	11
sfr-miR b-1049 6-3p	15	80	26	9	13	15	NA	1	2	1	2	NA	NA	NA	5	NA	NA	169,00	11
sfr-miR b-1050 0-1-	8	66	38	36	6	7	1	NA	2	2	1	NA	NA	NA	4	NA	NA	171,00	11

5p e sfr-miR b-1050 0-3-5p																		
sfr-miR b-276-5p	30	84	124	68	4	21	3	NA	1	NA	NA	1	NA	NA	NA	1	6	343,00	11
sfr-miR b-1048 3-2-3p	56	223	423	211	10	4	10	NA	1	3	2	NA	NA	NA	2	NA	NA	945,00	11
sfr-miR b-1050 4-3p	122	167	376	254	NA	1	NA	NA	2	2	NA	9	11	NA	5	NA	26	975,00	11
sfr-miR b-1050 5-2-5p	216	1117	8	2	30	20	5	NA	3	NA	2	NA	NA	1	1	NA	NA	1405,0 0	11
sfr-miR b-9c-3p	260	482	723	465	8	4	1	1	NA	2	1	NA	NA	NA	1	NA	NA	1948,0 0	11
sfr-miR b-	2040	62664	85	15	44	27	10	NA	1	2	2	NA	NA	1	NA	NA	NA	64891, 00	11

10505-1-3p																			
sfr-miR-6094-3p	31753	170354	436089	100569	96	48	40	NA	1	1	NA	NA	NA	2	1	NA	NA	738954,00	11
sfr-miR-10454c-3p	1	2	NA	2	6	4	1	2	3	NA	3	NA	1	2	3	NA	NA	30,00	12
sfr-miRb-10500-2-3p	1	3	9	8	3	1	NA	2	1	1	2	NA	NA	1	5	NA	NA	37,00	12
sfr-miRb-10493-3-3p; sfr-miRb-10493-4-3p e sfr-miRb-10493-5-3p	8	1	1	5	5	5	1	6	10	NA	3	NA	NA	1	1	NA	NA	47,00	12

sfr-miRb-10489-1-5p	8	9	1	2	6	3	2	3	NA	2	2	NA	NA	6	9	NA	NA	53,00	12
sfr-miRb-10492c-6-5p	3	14	17	14	4	NA	3	2	3	5	2	NA	NA	1	2	NA	NA	70,00	12
sfr-miRb-10493-1-5p e sfr-miRb-10493-2-5p	3	1	1	NA	21	23	6	3	6	2	6	NA	NA	5	3	NA	NA	80,00	12
sfr-miRb-10454d-5p	6	24	12	28	2	4	NA	2	3	2	NA	NA	NA	2	1	NA	1	87,00	12
sfr-miRb-10454h-3-5p;	7	25	12	29	4	5	NA	2	4	2	NA	NA	NA	6	1	NA	1	98,00	12

sfr-miRb-10454h-6-5p e sfr-miRb-10454h-7-5p																			
sfr-miRb-10513-1-5p e sfr-miRb-10513 2-2-5p	4	9	34	10	15	7	1	1	4	8	NA	NA	NA	6	7	NA	NA	106,00	12
sfr-miRb-10454f-5p	16	27	14	41	9	5	NA	1	2	4	6	NA	NA	6	10	NA	NA	141,00	12
sfr-miRb-79-3p	102	251	644	257	NA	3	NA	1	3	4	NA	NA	1	NA	1	4	199	1470,00	12

sfr-miRb-10460-5p	551	224	711	906	9	23	3	13	11	1	1	NA	NA	NA	1	NA	NA	2454,00	12
sfr-miRb-283-5p	1624	3023	5934	3659	4	13	3	1	NA	NA	1	NA	1	NA	1	NA	1	14265,00	12
sfr-miR-14-5p e sfr-miR-14-5p	1186	4857	6334	4570	79	6	2	2	2	6	3	NA	NA	NA	NA	NA	14	17061,00	12
sfr-miRb-263a-1-5p e sfr-miRb-263a-2-5p	39471	631314	180500	80681	31	4	5	NA	NA	1	NA	3	2	NA	NA	-	1	932013,00	12
sfr-miRb-10454h-5-3p	1	7	13	11	1	7	1	2	1	1	1	NA	NA	3	2	NA	NA	51,00	13

e sfr-miR b-1045 4h-6-3p																			
sfr-miR b-1046 2-1-3p; sfr-miR b-1046 2-2-3p; sfr-miR b-1046 2-3-3p; sfr-miR b-1046 2-4-3p; sfr-miR b-1046 2-5-3p e	1	3	3	4	5	5	2	4	4	12	3	NA	NA	6	8	NA	NA	60,00	13

sfr-miRb-10462-6-3p																			
sfr-miRb-10501-1-5p; sfr-miRb-10501-2-5p; sfr-miRb-10501-3-5p e sfr-miRb-10501-4-5p	6	19	3	7	7	9	2	1	3	8	3	NA	NA	11	8	NA	NA	87,00	13
sfr-miRb-10492a-1-3p;	5	11	17	6	9	13	2	4	2	5	5	NA	NA	5	6	NA	NA	90,00	13

sfr-miR b-1049 2a-2-3p; sfr-miR b-1049 2a-3-3p e sfr-miR b-1049 2a-6-3p																		
sfr-miR b-1049 2c-4-3p	5	12	20	7	7	14	2	4	1	7	5	NA	NA	6	7	NA	NA	97,00	13
sfr-miR b-1049 2a-3-5p	5	17	22	24	8	2	5	7	4	5	2	NA	NA	1	2	NA	NA	104,00	13
sfr-miR b-1050 2-1-5p e	3	10	7	9	36	6	4	6	6	7	5	NA	NA	NA	3	NA	2	104,00	13

sfr-miRb-10502-2-5p																		
sfr-miRb-10462-2-5p	2	27	30	11	36	15	11	3	4	6	2	NA	NA	5	11	NA	NA	163,00	13
sfr-miRb-10461b-1-5p	38	42	27	44	4	9	1	3	1	1	1	NA	NA	6	3	NA	NA	180,00	13
sfr-miRb-10461b-1-3p; sfr-miRb-10461b-2-3p; sfr-miRb-10461b-3-3p;	29	31	20	32	17	9	4	NA	8	4	9	NA	1	14	16	NA	NA	194,00	13

sfr-miRb-1046 1b-4-3p; sfr-miRb-1046 1b-5-3p e sfr-miRb-1046 1b-6-3p																			
sfr-miRb-307-3p	17	52	94	32	29	58	12	5	6	1	NA	NA	NA	NA	1	15	177	499,00	13
sfr-miR-1046 5-1-3p	49	63	204	142	38	44	11	2	4	25	16	NA	NA	17	39	NA	NA	654,00	13
sfr-miRb-34-5p	113	62	448	297	1	2	NA	4	5	3	3	NA	2	NA	NA	1	3	944,00	13
sfr-miR-	197	401	791	597	19	26	6	2	6	NA	1	NA	2	NA	NA	4	21	2073,00	13

13b-3p																			
sfr-miRb-1-3p	311	414	1173	594	6	1	3	NA	1	3	6	NA	NA	NA	2	1	1	2516,00	13
sfr-miRb-10458-3p	878	4353	9481	2382	67	58	16	2	9	2	NA	NA	NA	NA	4	2	8	17262,00	13
sfr-miRb-10492c-1-5p; sfr-miRb-10492c-2-5p e sfr-miRb-10492c-3-5p	19	9	18	24	10	3	3	1	6	1	1	NA	NA	2	2	NA	1	100,00	14
sfr-miRb-10454f-3p	8	25	15	19	17	10	2	2	4	1	5	NA	1	6	7	NA	NA	122,00	14

sfr-miRb-10454b-3p	8	28	15	15	17	12	2	2	4	3	5	NA	1	6	8	NA	NA	126,00	14
sfr-miRb-932-5p	13	19	49	32	10	3	1	3	7	3	1	NA	NA	1	1	NA	9	152,00	14
sfr-miRb-10499b-1-3p e sfr-miRb-10499b-2-3p	18	26	45	37	10	31	2	1	3	1	1	NA	1	2	3	NA	NA	181,00	14
sfr-miRb-10454a-13-3p	18	81	49	35	19	39	4	1	3	3	4	NA	1	7	13	NA	NA	277,00	14
sfr-miRb-10454e-3-3p	7	86	36	37	23	31	6	7	4	14	10	1	NA	20	19	NA	NA	301,00	14

sfr-miR b-1045 4a-12-3p; sfr-miR b-1045 4a-2-3p; sfr-miR b-1045 4a-3-3p; sfr-miR b-1045 4a-4-3p e sfr-miR b-1045 4a-5-3p	28	86	56	53	23	39	4	2	3	4	5	NA	1	8	13	NA	NA	325,00	14
sfr-miR b-1045	10	74	50	47	20	28	7	7	9	10	7	NA	NA	33	23	NA	2	327,00	14

4h-5-5p																			
sfr-miRb-10462-1-5p; sfr-miRb-10462-4-5p e sfr-miRb-10462-6-5p	25	185	68	32	144	53	55	11	10	8	3	NA	NA	7	15	NA	2	618,00	14
sfr-miRb-10484-1-3p	1	NA	4	NA	64	113	14	189	225	145	130	6	5	262	257	NA	1	1416,00	14
sfr-miRb-87-3p	175	534	1308	539	16	29	4	1	1	NA	NA	1	1	1	NA	2	100	2712,00	14
sfr-miR-10465-2-3p	471	1485	3293	1414	28	26	5	3	2	18	13	NA	1	12	21	NA	NA	6792,00	14

sfr-miR b-1049 1-1-3p e sfr-miR b-1049 1-2-3p	2303	6063	17630	7835	803	991	190	212	269	35	24	NA	NA	36	38	NA	27	36456,00	14
sfr-miR b-277-3p	2728	7054	21953	7118	4	4	NA	3	4	5	NA	1	2	1	1	NA	1	38879,00	14
sfr-miR b-7b-5p	22	7	18	14	25	20	5	3	5	5	3	NA	5	2	1	NA	4	139,00	15
sfr-miR-1049 9a-1-3p; sfr-miR-1049 9a-2-3p; sfr-miR-1049 9a-3-	93	144	221	177	9	30	2	1	3	1	1	NA	1	2	3	1	NA	689,00	15

3p; sfr-miR-10499a-4-3p e sfr-miR-10499a-5-3p																		
sfr-miRb-10499b-3-3p	93	144	221	177	9	30	2	1	3	1	1	NA	1	2	3	1	NA	689,00	15
sfr-miRb-10491-1-5p e sfr-miRb-10491-2-5p	9	45	99	45	331	376	91	13	10	22	19	NA	NA	53	59	8	3	1183,00	15
sfr-miRb-2a-2-3p	291	1817	1962	774	1198	567	257	5	5	NA	1	1	1	NA	1	14	109	7003,00	15
sfr-miR	435	2283	2975	1137	1210	575	258	5	5	NA	1	1	1	NA	1	14	109	9010,00	15

b-2a-1-3p																		
sfr-miRb-993a-1-3p	925	3072	7863	2596	21	14	4	11	8	8	5	NA	NA	7	5	1	16	14556,00	15
sfr-miRb-71-3p	1631	8367	17816	5612	67	67	7	4	4	3	2	3	1	NA	1	NA	1	33586,00	15
sfr-miRb-278-5p	1502	28188	24263	6210	115	16	17	1	NA	4	5	5	5	1	2	1	NA	60335,00	15
sfr-miRb-2755-3p	6097	18247	30346	12625	189	259	63	4	6	20	15	5	5	4	7	NA	NA	67892,00	15
sfr-miRb-92a-3p	44	376	379	166	61	78	14	28	49	50	41	11	17	24	49	NA	6	1393,00	16
sfr-miRb-745-5p	429	1204	2747	1122	127	28	18	3	5	1	2	1	NA	2	1	2	153	5845,00	16
sfr-miRb-	1026	1394	1866	1903	46	80	15	39	44	31	18	5	2	21	38	NA	15	6543,00	16

993a-1-5p e sfr-miR b-993a-2-5p																			
sfr-miR b-2766-5p	414	3279	4051	1006	160	89	37	2	2	12	8	14	19	2	5	NA	73	9173,00	16
sfr-miR b-279-3p	683	2799	5463	2223	201	222	45	6	15	4	6	6	7	2	3	NA	26	11711,00	16
sfr-miR b-278-3p	611	2619	7329	1918	3	3	1	2	7	5	6	7	15	7	6	NA	3	12542,00	16
sfr-miR b-92b-3p	492	7037	5996	1822	122	290	40	251	278	823	629	165	211	304	470	NA	7	18937,00	16
sfr-miR b-970-3p	834	4851	15827	2816	237	106	30	8	4	8	1	1	3	NA	2	1	20	24749,00	16
sfr-miR	3655	4734	12667	9493	52	207	15	11	26	59	25	73	72	37	40	NA	5	31171,00	16

b-1048 2-5p																			
sfr-miR b-2c-3p	3193	17588	37303	12698	571	556	148	17	25	5	1	8	2	1	2	NA	11	72129,00	16
sfr-miR b-276-3p	12892	29455	85190	37084	266	201	48	15	19	5	3	3	4	8	5	NA	4	165202,00	16
sfr-miR b-2763-3p	87	468	630	93	31	13	2	12	26	11	11	4	5	8	15	1	120	1537,00	17
sfr-miR b-2756-5p	251	801	1708	689	117	135	33	1	11	5	3	5	8	7	2	3	158	3937,00	17
sfr-miR b-308-3p	206	927	1317	502	164	202	48	145	209	11	9	39	59	43	40	24	413	4358,00	17
sfr-miR b-745-3p	146	808	1410	420	10	75	8	31	30	7	3	9	5	4	6	7	1604	4583,00	17
sfr-miR b-	465	1920	5914	1784	143	480	27	152	180	201	181	1	4	175	212	25	267	12131,00	17

10483-5p																			
sfr-miRb-317-3p	2592	5572	11413	7650	321	396	50	44	69	11	5	3	5	7	8	2	9	28157,00	17
sfr-miRb-10-1-3p	3385	4869	12579	7687	46	40	16	14	16	1	7	15	6	3	8	3	6	28701,00	17
sfr-miR-279a-3p	3953	6782	12172	7813	53	83	13	13	21	6	11	9	11	2	11	5	13	30971,00	17
sfr-miRb-279b-1-3p	3953	6782	12172	7813	53	83	13	13	21	6	11	9	11	2	11	5	13	30971,00	17
sfr-miRb-279d-3p	2735	7941	14419	6601	48	98	10	55	73	78	54	97	107	112	148	47	295	32918,00	17
sfr-miRb-279b-2-3p	2983	7557	19176	7387	9	8	1	2	10	2	1	3	4	2	5	1	4	37155,00	17
sfr-miRb-	9	95	110	27	49	36	2	269	342	4177	3347	14741	18797	3220	2847	3	8	48079,00	17

2779-1-5p e sfr-miR b-2779-2-5p																			
sfr-miR b-306a-5p	5942	12557	28513	13258	31	82	14	55	60	25	17	18	30	21	30	1	13	60667,00	17
sfr-miR b-11-3p	2950	18339	27372	6405	10530	3972	1869	8	18	18	16	8	2	4	7	1	8	71527,00	17
sfr-miR b-2766-3p	2401	10376	17317	4355	22829	20676	4161	1780	2095	282	250	140	177	306	337	146	1453	89081,00	17
sfr-miR b-10-1-5p	303767	4111083	1346432	513258	2070	637	587	50	27	24	21	39	53	14	26	1	5	6278094,00	17

3 CONCLUSÃO

A biotecnologia oferece alternativas inovadoras que podem ser empregadas no controle de pragas de interesse agronômico. Utilizando o PhD, selecionamos sete clones capazes de se ligar a proteínas intestinais de *S. frugiperda*. O clone SfF3, quando usado em combinação com o *B. thuringiensis* GF 07, promoveu um aumento da mortalidade em lagartas neonatas. Além disso, demonstrou similaridade com as proteínas da cassetes de ligação de ATP subfamília C2 e citocromo P450, as quais são importantes para a sobrevivência desta praga. Já para entender como os miRNAs podem ser usados como uma ferramenta no controle da praga, foram preditos 350 precursores de miRNA no genoma de *S. frugiperda*, sendo 60 específicos para o gênero *Spodoptera*. Além disso, o refinamento de bibliotecas de RNA-seq na busca de possíveis moléculas bioinseticidas forneceu 91 sequências ainda não descritas, evidenciando novas alternativas na modulação de transcritos para o controle biológico do inseto. Nossos resultados validam essas tecnologias como uma abordagem agrobiotecnológica para o controle de pragas e identifica novas moléculas com possível atividades bioinseticidas.

ANEXO A - Normas para revista Plos one

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This is the article title

John Doe[1¶], Antonie Data[1¶], Johannes van Stats[1,#a], Marie Testperson[2*], David Ribosome Jr.3,5 , Gregory H.T. McBio 4,#b , Angela Reviewerson [1,2&], Marina Measure[1&], on behalf of The Bunny Genome Sequencing Consortium[^]

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[&]These authors also contributed equally to this work.

^Membership of the Bunny Genome Sequencing Consortium is provided in the Acknowledgments.

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Abstract

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Materials and methods

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$$p^2 + 2pq + q^2 = 1 \quad (1)$$

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Genotyping

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Whole genome RFLP analysis

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Table 1. This is the Table 1 Title.

	Chemical W	Chemical X	Chemical Y	Chemical Z
Chemical 1	Reaction 1W	Reaction 1X	Reaction 1Y	Reaction 1Z
Chemical 2	Reaction 2W	Reaction 2X	Reaction 2Y	Reaction 2Z
Chemical 3	Reaction 3W[a]	Reaction 3X	Reaction 3Y[b]	Reaction 3Z
Chemical 4	Reaction 4W	Reaction 4X	Reaction 4Y	Reaction 4Z
Chemical 5	Reaction 5W	Reaction 5X	Reaction 5Y	Reaction 5Z

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References

- Doe J, Data A, van Stats J, Testperson M, Ribosome D Jr, McBio GHT, et al. This is the article title. PLoS ONE. 2017;12(12):e00000. doi: 10.1371/journal.pone.0000000
- Doe J, Data A, van Stats J, Testperson M, Ribosome D Jr, McBio GHT, et al. Bunny dynamics in cartoon landscapes. PLoS ONE. Forthcoming 2017.

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Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

Summarize the study's contribution to the scientific literature

Relate the study to previously published work

Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)

Describe any prior interactions with PLOS regarding the submitted manuscript

Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)

List any opposed reviewers

IMPORTANT: Do not include requests to reduce or waive publication fees in the cover letter. This information will be entered separately in the online submission system.

Read about publication fee assistance.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

Describe the main objective(s) of the study

Explain how the study was done, including any model organisms used, without methodological detail

Summarize the most important results and their significance

Not exceed 300 words

Abstracts should not include:

Citations

Abbreviations, if possible

Introduction

The introduction should:

Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study

Define the problem addressed and why it is important

Include a brief review of the key literature

Note any relevant controversies or disagreements in the field

Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established,

authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information. We recommend depositing **laboratory protocols** at protocols.io. Read detailed instructions for depositing and sharing your laboratory protocols.

Human or animal subjects and/or tissue or field sampling

Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. For details, consult the reporting guidelines for specific study types.

Data

PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

Large data sets, including raw data, may be deposited in an appropriate public repository. See our list of recommended repositories.

For smaller data sets and certain data types, authors may provide their data within supporting information files accompanying the manuscript. Authors should take care to maximize the accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

Cell lines

Methods sections describing research using cell lines must state the origin of the cell lines used. See the reporting guidelines for cell line research.

Laboratory Protocols

To enhance the reproducibility of your results, we recommend and encourage you to deposit laboratory protocols in protocols.io, where protocols can be assigned their own persistent digital object identifiers (DOIs).

To include a link to a protocol in your article:

Describe your step-by-step protocol on protocols.io

Select **Get DOI** to issue your protocol a persistent digital object identifier (DOI)

Include the DOI link in the Methods section of your manuscript using the following format provided by protocols.io: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]

At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time by selecting **Publish** on the protocols.io site. Any referenced protocol(s) will automatically be made public when your article is published.

New taxon names

Methods sections of manuscripts adding new zoological, botanical, or fungal taxon names to the literature must follow the guidelines for new taxon names.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensible efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

Published or accepted manuscripts

Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.

Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts or author summaries.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	**Format**
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. *Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.*
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.

Source	Format
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available from: arXiv:1403.3301v1. Cited 17 March 2014.
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html Cited 17 March 2014.
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting Information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 10 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Figures and Tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").

A concise, descriptive title

The caption may also include a legend as needed.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current

repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

Deposit data in the integrated repository of choice.

Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.

Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a "Striking Image" that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

Specific grant numbers

Initials of authors who received each award

Full names of commercial companies that funded the study or authors

Initials of authors who received salary or other funding from commercial companies

URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

Study design

Data collection and analysis

Decision to publish

Preparation of the manuscript

If they had no role in the research, include this sentence: “The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.”

If the study was unfunded, include this sentence as the Financial Disclosure statement: “The author(s) received no specific funding for this work."

Competing Interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into “parts.” Each

submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Preprints

PLOS encourages authors to post preprints as a way to accelerate the dissemination of research and supports authors who wish to share their work early and receive feedback before formal peer review. Deposition of manuscripts with preprint servers does not impact consideration of the manuscript at any PLOS journal.

Authors posting on bioRxiv may concurrently submit directly to PLOS journals through bioRxiv's direct transfer to journal service.

Authors submitting manuscripts in the life sciences to *PLOS ONE* may opt-in to post their work on bioRxiv during the *PLOS ONE* initial submission process.

Guidelines for Specific Study Types

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the

Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in theInternational Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

The name of the approving institutional review board or equivalent committee(s). If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed

Whether informed consent was written or oral. If informed consent was oral, it must be stated in the manuscript:

Why written consent could not be obtained

That the Institutional Review Board (IRB) approved use of oral consent

How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

Explicitly describe their methods of categorizing human populations

Define categories in as much detail as the study protocol allows

Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency

Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms

of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

Register all related clinical trials and confirm they have done so in the Methods section

Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

Provide the registry name and number in the methods section of the manuscript

Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.

Include the CONSORT flow diagram as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

The full name of the relevant ethics committee that approved the work, and the associated permit number(s).

Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.

Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, The use of non-human primates in research, including:

Information about housing, feeding, and environmental enrichment.

Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance,

assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. Please see the NC3Rs guidelines for more information. Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-Neves, I. Anna S. Olsson (2012) How "Humane" Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.

The duration of the experiment.

The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.

How frequently animal health and behavior were monitored.

All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.

Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why

Whether the land accessed is privately owned or protected

Whether any protected species were sampled

Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- Sharing of data and materials. Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under PLOS ONE's data availability criterion.
- Ethics. PLOS ONE will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

Checklist: PDF or Word document

Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

State this in your cover letter

Select "Research Article" as your article type when submitting

Include the PRISMA flow diagram as Fig 1 (required where applicable)

Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in Systematic Reviews of Genetic Association Studies by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be

collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.

If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

Details of institutional review board or ethics committee approval; AND

For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

A reference to the published article that first described the cell line; AND/OR

The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Manuscripts reporting results from blots (including Western blots) and electrophoretic gels should follow these guidelines:

In accordance with our policy on image manipulation, the image should not be adjusted in any way that could affect the scientific information displayed, e.g. by modifying the background or contrast.

All blots and gels that support results reported in the manuscript should be provided.

Original uncropped and unadjusted blots and gels, including molecular size markers, should be provided in either the figures or the supplementary files.

Lanes should not be overcropped around the bands; the image should show most or all of the blot or gel. Any non-specific bands should be shown and an explanation of their nature should be given.

The image should include all relevant controls, and controls should be run on the same blot or gel as the samples.

A figure panel should not include composite images of bands originating from different blots or gels. If the figure shows non-adjacent bands from the same blot or gel, this should be clearly denoted by vertical black lines and the figure legend should provide details of how the figure was made.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.

The commercial supplier or source laboratory.

The catalogue or clone number and, if known, the batch number.

The antigen(s) used to raise the antibody.

For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

The final antibody concentration or dilution.

A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such asAntibodypedia or CiteAb.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility

The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation

Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability

If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified

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Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called "Nomenclatural Acts":

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All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore, the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording:

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording (this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum):

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from

the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

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Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

ANEXO B - Normas para revista Journal of Invertebrate Pathology

Introduction

The *Journal of Invertebrate Pathology* publishes articles on all aspects of original research concerned with the causation and manifestation (including immunologic responses) of infectious and noninfectious diseases of invertebrates, the suppression of such diseases in beneficial species, and the use of these pathogens in controlling undesirable species such as agricultural pests and vectors of pathogens transmissible to other organisms. In addition, this journal publishes the results of biochemical, physiological, morphological, genetic, and ecological studies related to the etiologic agents of diseases of invertebrates. The journal is particularly dedicated to the publication of contributions of a basic and fundamental nature, although it will accept suitable articles pertaining to the applications of invertebrate pathology. The editor-in-chief and members of the Editorial Board will examine contributions from any qualified worker in any country of the world.

Types of paper

The *Journal of Invertebrate Pathology* publishes the following types of articles:

Regular Articles. Manuscripts for Regular Articles are full-length papers the reports the results of a large and well-defined study. There is no page limit, but this type of article is usually in the range of eight published pages.

Short Communications. Manuscripts for Short Communications should be 1500 or fewer words and contain not more than two illustrations or two tables, or one of each. Manuscripts should contain an abstract of not more than 100 words. References should be kept to a minimum and should be styled according to the guidelines in the section on References.

Minireviews. Manuscripts for Minireviews typically range from four to six published pages and provide a succinct review of important and recent developments in any field of invertebrate pathology.

Forum Articles. Manuscripts for Forum Articles typically range from one to four published pages and focus on a topical issue in invertebrate pathology. It is the intent of Forum

Articles to stimulate discussion of controversial or unresolved issues relevant to all aspects of invertebrate pathology.

Important Note: *When you reach the submission page, you will see a drop down box with the label "Select Issue Type'. Please click on the drop down box and select "Regular issue" unless you are invited by a editor for a special issue.*

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One author has been designated as the corresponding author with contact details:

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All necessary files have been uploaded:

Manuscript:

Include keywords

All figures (include relevant captions)

All tables (including titles, description, footnotes)

Ensure all figure and table citations in the text match the files provided

Indicate clearly if color should be used for any figures in print *Graphical Abstracts / Highlights files* (where applicable) *Supplemental files* (where applicable)

Further considerations

Manuscript has been 'spell checked' and 'grammar checked'

All references mentioned in the Reference List are cited in the text, and vice versa

Permission has been obtained for use of copyrighted material from other sources (including the Internet)

A competing interests statement is provided, even if the authors have no competing interests to declare

Journal policies detailed in this guide have been reviewed

Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center. You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

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Ensure all figure and table citations in the text match the files provided

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All references mentioned in the Reference List are cited in the text, and vice versa

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Relevant declarations of interest have been made

Journal policies detailed in this guide have been reviewed

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Before you begin

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Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

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Preparation

Peer review

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of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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