

Original	1	GGTACCGCTTGTCCTCTTGAGAAAGTCGCAATTTGTGCGGTGGAGGTTCGGCTTGTCCTCCT
Otimizada	1	GGTACCGCGTGTCCGCTGGAAAAAGCCACCTGTGTGGCGGTGGTTCTGCGGTGTCCACCG
Proteína		G T A C P L E K S H L C G G S A C P P
Original	61	GAGAAGATTCAATGTGTGCGGTGGAGGTTTCGGCTTGTCGACTGAGAAGAAGCATGTTTGC
Otimizada	61	GAAAAAATTCACTGTGTGTGGAGGCGGCAGCGCGTGTCCGACCGAAAGAAACATGTGTGC
Proteína		E K I H V C G G G S A C P T E K K H V C
Original	121	GGTGGAGGTTCCGATGCTTGGAAGATGCGGGTGTCTCAGATGTATGATGGTGGAGGTTTCG
Otimizada	121	GGCGCGGCTCCGATCGGTGGAAGATGCGCCTGTCTCAGATGTATGATGGTGGTGGAAAGC
Proteína		G G G S D A W K M R L S Q M Y D G G G S
Original	181	ATTGATCCGCTGATGTTTAAGTATTGGTCGAATATGGGTGGAGGTTTCGTCTAATAATGCG
Otimizada	181	ATTGACCCGCTGATGTTTAAATATTGGAGCAACAATGGGTGGCGGCTCGAGTAACAACGCC
Proteína		I D P L M F K Y W S N M G G S S N N A
Original	241	GATTATAAGCAGTCGCTGCTGGTGGAGGTTTCGTCTATTCCGACGTATACCTCCTGAT
Otimizada	241	GACTATAAACAGAGCCTGCTGCTGGCGGCGGCAGCAGCATCCCGACCTATACGCCGGAT
Proteína		D Y K Q S L L L G G G S I P T Y T P D
Original	301	AAGGTGACGTATGGTGGAGGTTTCGGTGAATTGGAATTCTTGGCATAAGACTAATCTGTCT
Otimizada	301	AAAGTCACCTACGTTGGGGATCTGTCAACTGGAATTCA TGGCATAAACCAATCTGTCA
Proteína		K V T Y G G G S V N W N S W H K T N L S
Original	361	GGTGGAGGTTCTGTGTGAGGAGCGGTATTATGCTTTTGTGCGACTGGTGGAGGTTTCG
Otimizada	361	GGTGGCGGAAGCTCTGTTCAGGAACGTTATTACGCGTTCTGGTCTACCGGAGGTGGTTCA
Proteína		G G G S S V Q E R Y Y A F W S T G G G S
Original	421	ATGGCGATTACTGATCATCCTGGTCTGCGATGGTGGTGGAGGTTTCGTCGGCCGC
Otimizada	421	ATGGCGATTACCGATCATCCGGGTTCTGCCATGGTGGCGGTGGA TCGTGGGCCCGC
Proteína		M A I T D H P G S A M V G G G S C G R

Figura 29 - Gene artificial A: alinhamento de DNA (região otimizada). Os códons alterados estão indicados com letras claras: resultados da tradução GENE A = 5'3' Frame 1

Original	1	GGTACCGCTTGTCCTCTTGAGAAAGTCGCATTTGTGCGGTGAGGTTGCGCTTGTCCTCTT
Otimizada	1	GGTACCGCTGCCCCGCTGGAAAAAGCCATCTGTGTGGCGGTGGCAGCGCCTGTCCGCTG
Proteína		G T A C P L E K S H L C G G S A C P L
Original	61	GAGAAAGTCGATTTGTGCGGTGAGGTTGCGCTTGTCCTCTTGAGAAAGTCGATTTGTGC
Otimizada	61	GAAAAATCCCATCTGTGCGGGCGGGCTCTGCTTGCCCACTGGAAAAAGTCATTTATGT
Proteína		E K S H L C G G S A C P L E K S H L C
Original	121	GGTGAGGTTGCGCTTGTCCTCTTGAGAAAGTCGCATTTGTGCGGTGAGGTTGCGCTTGT
Otimizada	121	GGGGCGGTTCTGCGTGGCCGTTAGAGAAATCCCATCTGTGCGGTGGTGGCTCGGCATGT
Proteína		G G G S A C P L E K S H L C G G S A C
Original	181	CCTCTTGAGAAAGTCGCATTTGTGCGGTGAGGTTGCGCTTGTCCTCTTGAGAAAGTCGCAT
Otimizada	181	CCTCTGGAAAAAAGTCACCTTTGCGGGCGGTGGTTCGGCTGCCCTTTGGAAAAATCACAT
Proteína		P L E K S H L C G G S A C P L E K S H
Original	241	TTGTGCGGTGAGGTTGCGCTTGTCCTCTTGAGAAAGTCGCATTTGTGCGGTGAGGTTTCG
Otimizada	241	CTGTGCGGAGGTGGCTCGGCGTGTGTCCTCTTGAAAAATCGACCTATGCGGTGGCGGTCT
Proteína		L C G G S A C P L E K S H L C G G S
Original	301	GCTTGTCCTCTTGAGAAAGTCGCATTTGTGCGGTGAGGTTGCGCTTGTCCTCTTGAGAAAG
Otimizada	301	GCTGTCCCCCTGGAAAAAAGCCATTTGTGCGGTGGCGGTGGCAGCGCATGTCCACTGGAGAAA
Proteína		A C P L E K S H L C G G S A C P L E K
Original	361	TCGCATTTGTGCGGTGAGGTTGCGCTTGTCCTCTTGAGAAAGTCGCATTTGTGCGGTGGA
Otimizada	361	TCTCACCTGTGCGGAGGCGGTAGCGCATGCCCGCTGGAGAAATCGCATCTGTGTGGCGGC
Proteína		S H L C G G S A C P L E K S H L C G G
Original	421	GGTTCGTGCGGCCGC
Otimizada	421	GGCTCGTGGGCCGC
Proteína		G S C G R

Figura 30 - Gene artificial B: alinhamento de DNA (região otimizada). Os codons alterados estão indicados com letras claras: Resultados da tradução GENE A = 5'3' Frame 1.

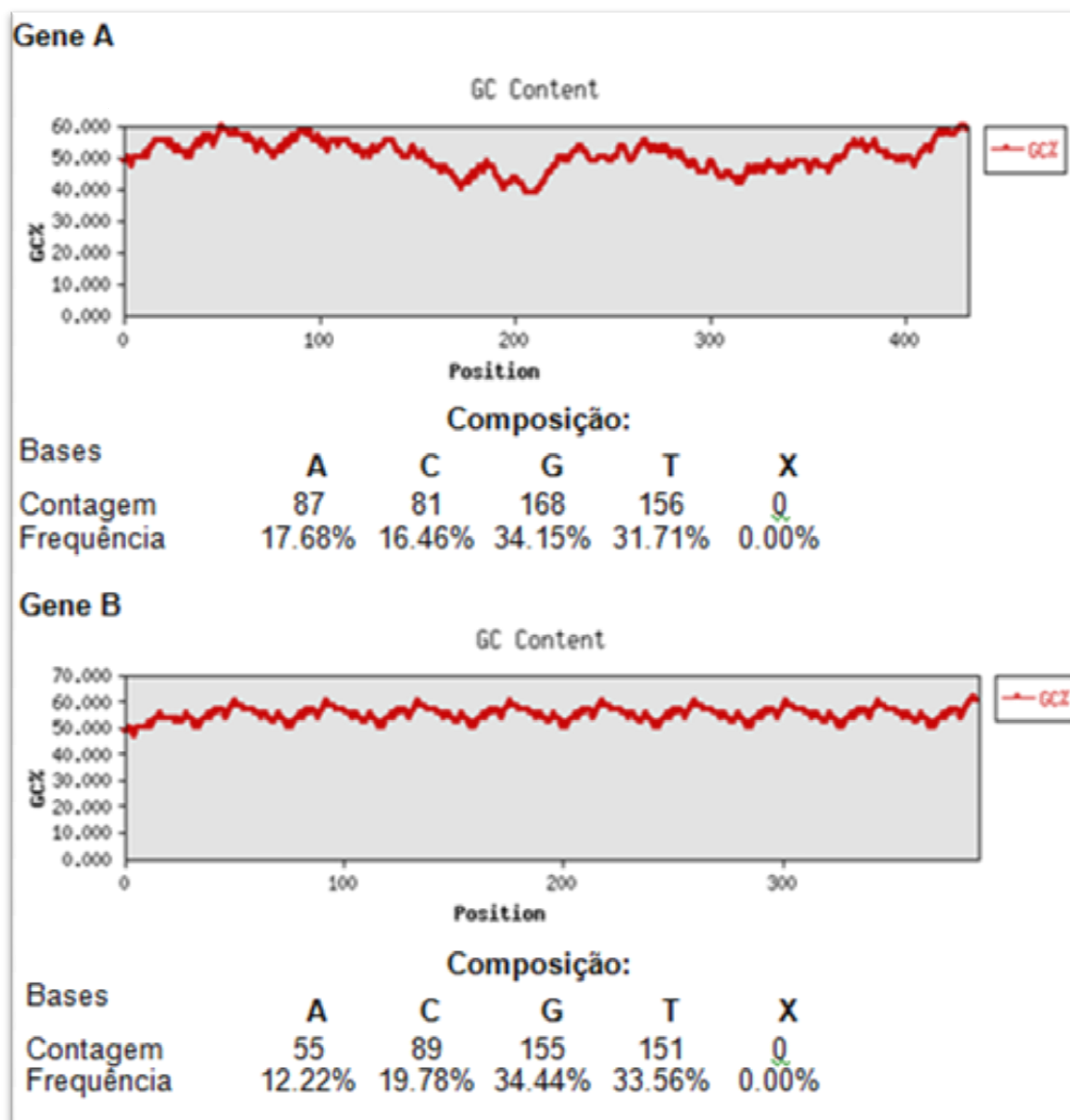


Figura 31: Análises de conteúdo GC (guanina e citosina) nas seqüências artificiais dos genes A e B. A- adenina, C-citosina, G-guanina e T-timina. A letra X representa qualquer letra distinta das citadas anteriormente.

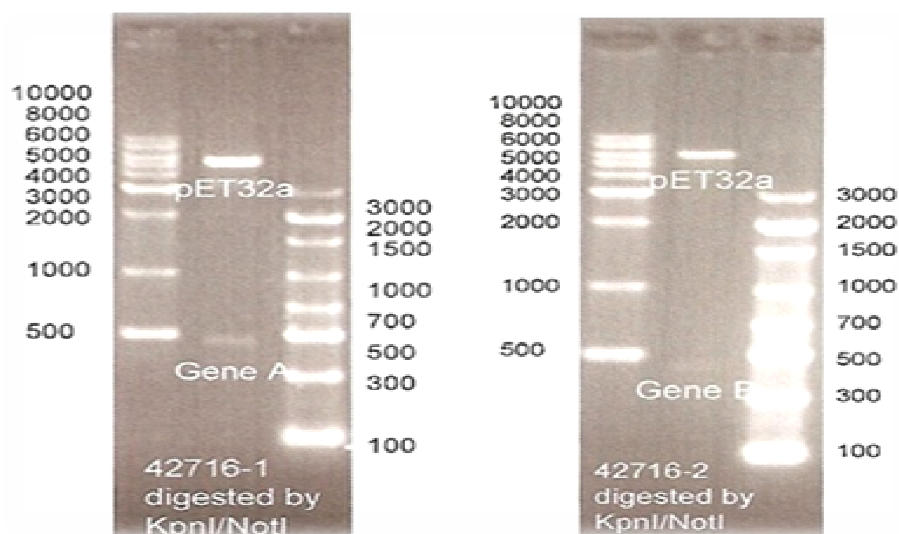


Figura 32 – Restrição enzimática do vetor pET32a contendo os genes sintéticos A e B com as enzimas *Kpn1* e *Not1* (representados por fragmentos de tamanho aproximado de 500pb). Marcador 100pb *DNA ladder* alto peso molecular (Colunas 1 e 4) Marcador 1kb *DNA ladder* baixo peso molecular (Colunas 3 e 6).

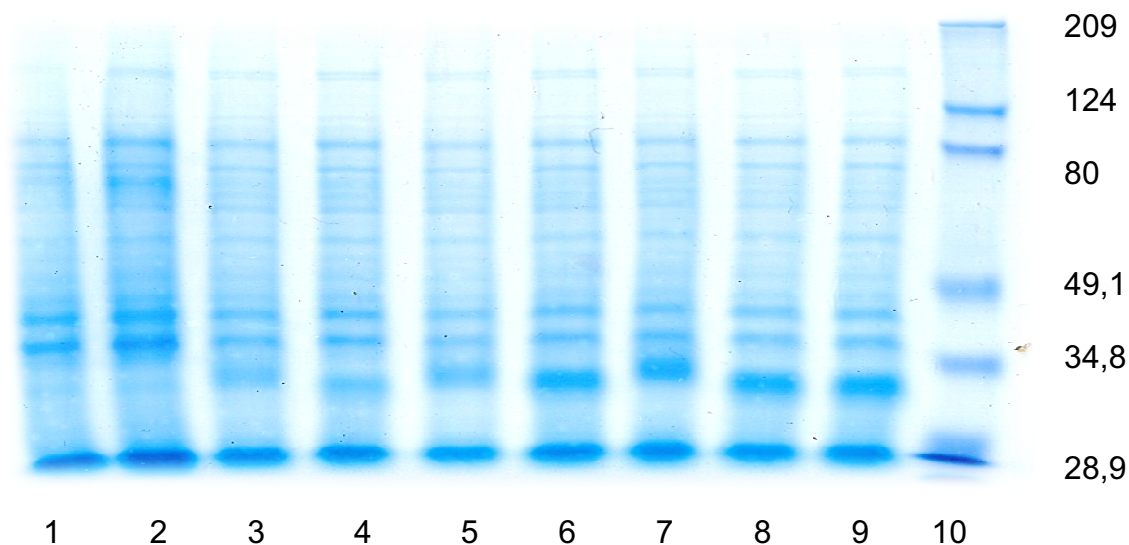


Figura 33 – Indução da expressão das proteínas PRA e PRB em extrato de *E. coli*. Amostras da cultura foram coletadas em diversos tempos, 0 (poço 1 e 2), 1 hora (poço 3 e 4), 2 horas (poço 5 e 6), 3 horas (poço 7 e 8), 5 horas (gene B, poço 9) e analisadas em gel SDS-PAGE 16%. O poço 10 representa o marcador de massa molecular em kDa (*Biorad Protein Ladder*).