



Genética Molecular RIB0102 Ano 2020

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1. Identifique a posição da mutação **G254D** no gene F9 da coagulação e desenhe um par de *primers* para amplificar a região que se encontra a mutação para validá-la por sequenciamento numa população de 100 hemofílicos. Para tanto, responda as seguintes questões:

(Obs: o tamanho do produto da amplificação deve ser de aproximadamente 350pb)

- a) Para **cada primer**, informe a porcentagem de [CG], a temperatura de melting (T_m), e a temperatura de anelamento ($T_{ann.}$);
R: **Primer 1**(**gctcacattccagaaacat**): 8 CG=40% CG.
 $T_m=(7 \times 2)+(5 \times 2)+(2 \times 4)+(6 \times 4)=44^\circ\text{C}$
 $T_{ann.}=44-4=40^\circ\text{C}$
Primer 2(**ttgggctaaaggcagaaggg**): 11 CG=55% CG.
 $T_m=(3 \times 2)+(6 \times 2)+(9 \times 4)+(2 \times 4)=62^\circ\text{C}$
 $T_{ann.}=62-4=58^\circ\text{C}$
- b) Qual o tamanho exato do produto de PCR?;
R: 376 pares de bases.
- c) Considerando as três temperaturas que são definidas para a reação de PCR, qual seria a temperatura de melting do PCR em questão?;
R: $(40+58)/2=49^\circ\text{C}$
- d) Informe o tipo de substituição (transição ou transversão) e os nucleotídeos trocados.
R: Transversão, trocou uma Guanina por Citosina.

Gene F9



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1 aaagacaagctacaggctggagacaatatatttaatccacctatctatgaaaggactcat
61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaaatcagactaact
121 ggaccactcatacattgctgatggaaatgtaaagtggtacagccatcttggtaaacatca
181 ttgctcgctgacgaagatacggcggggtccgactgaggaaccgtgcgggccacggtaaacgt
241 agcgactcggccgatctcgattcgggaagacgcgtcccttcccgtttccgacccaaatcgg
301 ccgcggtggcagaagcccacgaaatcagaggtgaaatttaataatgaccactgcccattc
361 tcttcacttgcccaagaggccattggaaatagtccaaagaccattgaggggatggac
421 attatttcccagaagtaaaatcacagctcagcttgtactttggtacaactaatccaccttac
1 M Q R V N M I M A E S P G L
481 cactttcacaaatctgctagcATGCAGCGGTGAACATGATCATGGCAGAATCACCAGGCC
15 I T I C L L G Y L L S A E C T V
541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGTGAATGTACAGgtttgtttcctt
601 ttttaaaatacattgagtatgcttgccttttagatatagaaatatctgatgctgtcttct
661 tcactaaattttgattacatgatttgacagcaatattgaagagtctaacagccagcacgc
// 721 aggttggttaagtactggttctttgtagctaggttttcttcttcttcatTTTTAAACTA
// 6541 actaaaagtaaaattgaattttaattcctaaatctccatgtgtatacagtactgtgggaa
6601 catcacagattttggctccatgccctaaagagaaaattggctttcagattatttggattaa
6661 aaacaaagacttttctaagagatgtaaaattttcatgatgttttctttttgctaaaact
31 F L D H E N A N K I L
6721 aaagaattattcttttacatttcagTTTTCTTGATCATGAAAACGCCAACAAAATTCGTG
42 N R P K R Y N S G K L E E F V Q G N L E
6781 AATCGGCCAAAAGAGGTATAAATTCAGGTAAAATTGGAAGAGTTTGTTC AAGGGAACCTTGAG
62 R E C M E E K C S F E E A R E V F E N T
6841 AGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTTTGA AACACT
82 E R T
6901 GAAAGAACAgtgagtattttccacataataacccttcagatgcagagcatagaatagaaaat
6961 ctttaaaaagacacttctcttttaaaattttaaaagcatccatataatattatgtatgtaa
7021 atgttataaaaagataggaatcaataccaaaacacttttagatattaccgtaaatttgtct
85 T E F W K Q Y V D
7081 tcttttattctttatagACTGAATTTTGGGAAGCAGTATGTTGgtaagcaattcattttat
7141 cctctagctaatatatgaaacatatgagaattatgtgggttttttctctgcataaaataga
7201 taatatattaaactttgtcaaaaggactcagaaaagatcagccaaccctctaaccatata
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10681 agctggccttcaggtcagtagttttgctctgaccctaaaatcagactcccacccaatga
10741 gtatctacaggggaggaccgggcattctaagcagtttacgtgccaattcaatttctaac
94 G D Q C E S N P C L N G G S C K
10801 ctatctcaaagATGGAGATCAGTGTGAGTCCAATCCATGTTTAAATGGCGGCAGTTGCAA
110 D D I N S Y E C W C P F G F E G K N C E
10861 GGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTGAAGGAAAGAACTGTGA
130 L D
10921 ATTAGgtaagtaactatTTTTgaatactcatggttcaaagtttccctctgaaacaagtt
10981 gaaactggaaaatgcaatattggtgatcataatTTTTcttaaaaacatacctttgatgc
11041 ttataaacatttccattttagtgatagttttcaggatatgagttcaagaagctacattaa
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17941 cttatttcaaggctccaaaatttctctcccacgtatattgggggcaacatgaatgcc
18001 ccaatgtatatttgaccatacatgagtcagtagttccatgtacttttagaatgcatg
132 V T C N I K N
18061 ttaaatgatgctgttactgtctatTTTTgcttcttttagATGTAACATGTAACATTAAGAA
139 G R C E Q F C K N S A D N K V V C S C T
18121 TGGCAGATGCGAGCAGTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGTCTCTGTAC
159 E G Y R L A E N Q K S C E P A V
18181 TGAGGGATATCGACTTGCAGAAAACAGAACTCCTGTGAACCAGCAGgtcataatctgaa
18241 taagattttttaaagaaaatctgtatctgaaacttcagcattttaacaaacctacataat
18301 ttaattcctacttgaatctgcttcttttgaaatcatagaaaatcatcagtagcttgaat

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// 18361 tagaccaattaatcttagattgcatcatatctttaaataataaactatgtaatcatctac
// 20581 caaatgttcttttcatgaaggatttgaaaactgtccatgaaaataacgcaatcaaccttt
20641 tagcttgagactctattcactgattagatttttttaataactgatgggcctgcttctcag
20701 aagtgacaaggatgggcctcaatctcaatcttttgtaatacatgttccatttgccaatgag
175 P F P C G R V
20761 aaatatacagggttactaatttttcttctatttttctagTGCCATTTCATGTGGAAGAGTT
182 S V S Q T S K L T R A E T V F P D V D Y
20821 TCTGTTTCACAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTTCTGATGTGGACTAT
202 V N S T E A E T I L D N I T Q S T Q S F
20881 GTAAATTTACTGAAGCTGAAACCATTTTGATAACATCACTCAAAGCACCAATCATT
222 N D F T R V V G G E D A K P G Q F P W Q
20941 AATGACTTCACTCGGGTTGTTGGTGGAGAAGATGCCAAACCAGGTCAATTCCCTTGCCAG
21001 gtactttatactgatgggtgtgtcaaaactggagctcagctggcaagacacaggccaggtg
// 21061 ggagactgaggctatcttacttagacagacctattgggatgtgagaagtatttaggcaagt
// 30241 gcttaagcttccctgtctctcattgtgtgttgccttcaatgcagttacataaatggcttt
30301 tttgtttatgcacaaaaaacactaattcatctgcaaa **gctcacatttccagaaacat** tcc
30361 atttctgccagcacctagaagccaatattttgcctattctgtaccagcacacatattt
242 V V
30421 attttttctagatcaaatgtattatgcagtaagagtcttaattttgttttcacagGTTG
244 L N G K V D A F C G G S I V N E K W I V
30481 TTTTGAATGGTAAAGTTGATGCATTCTGTG **GAG** GCTCTATCGTTAATGAAAAATGGATTG
264 T A A H C V E T G V K I T V V A G
30541 TAACTGCTGCCCCTGTGTTGAACTGGTGTAAAAATTACAGTTGTGCGAGgtaaataca
30601 cagaaagaataataatctgcagcaccactagctctttaatatgatttggtacacccatattt
30661 tactaagggtctaataaaattggtgttgaataaa **ttgggctaaaggcagaagg** tcataat
30721 ttcagaaccacgctgcaccgctcctccaagcatccatagttcttttgatatacccctatt
30781 atcactcatttcagtgagggtacaattagttcttgatgtagccatttccataaccagaaggc
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31021 atcaccaatgaaaggcttataacagcatgagtgaacagaaccatctctatgatagtctg
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31141 caatataagaatgagatctttaacattgccaattaggtcagtggtcccaagtagtcactt
281
31201 agaaaaatctgtgtatgtgaaatactgtttgtgacttaaaatgaaatttatttttaatagG
281 E H N I E E T E H T E Q K R N V I R I I
31261 TGAACATAATATTGAGGAGACAGAACATACAGAGCAAAAGCGAAATGTGATTGCAATTAT
301 P H H N Y N A A I N K Y N H D I A L L E
31321 TCCTCACCAACTACAATGCAGCTATTAATAAGTACAACCATGACATTGCCCTTCTGGA
321 L D E P L V L N S Y V T P I C I A D K E
31381 ACTGGACGAACCCCTTAGTGCTAAACAGCTACGTTACACCTATTTGCATTGCTGACAAGGA
341 Y T N I F L K F G S G Y V S G W G R V F
31441 ATACACGAACATCTTCCTCAAATTTGGATCTGGCTATGTAAGTGGCTGGGGAAGAGTCTT
361 H K G R S A L V L Q Y L R V P L V D R A
31501 CCACAAAGGGAGATCAGCTTTAGTTCTTCAGTACCTTAGAGTTCCACTTGTGACCGAGC
381 T C L R S T K F T I Y N N M F C A G F H
31561 CACATGTCTTCGATCTACAAAGTTCACCATCTATAACAACATGTTCTGTGCTGGCTTCCA
401 E G G R D S C Q G D S G G P H V T E V E
31621 TGAAGGAGGTAGAGATTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACTGAAGTGA
421 G T S F L T G I I S W G E E C A M K G K
31681 AGGGACCAGTTTCTTAACTGGAATTATTAGCTGGGGTGAAGAGTGTGCAATGAAAGGCCAA
441 Y G I Y T K V S R Y V N W I K E K T K L
31741 ATATGGAATATATACCAAGGTATCCCGGTATGTCAACTGGATTAAGGAAAAACAAAGCT
461 T *
31801 CACTTAAaccttggtttttgtggattccattgatgtgaatcagtcaccctgtatttgat
31861 gatgcatgggactactgacaaaatcactctgaccccgccaagctgctgcttctctctgcc
31921 ccaacctcaccagccaggcctcactcttgctagttccttttagttcttttagtcaata
31981 tttttgtcttcgcatataagtataaataaacatatttttaatttcttggtgggccc
32041 agtggctcacgcctataatcccagcacttctggaggccaagtgggcgatcacctgagg

	T	C	A	G
T	TTT Phe F	TCT Ser S	TAT Tyr Y	TGT Cys C
	TTC	TCC	TAC	TGC
	TTA Leu L	TCA	TAA STOP	TGA STOP
	TTG	TCG	TAG	TGG Trp W
C	CTT Leu L	CCT Pro P	CAT His H	CGT Arg R
	CTC	CCC	CAC	CGC
	CTA	CCA	CAA Gln Q	CGA
	CTG	CCG	CAG	CGG
A	ATT Ile I	ACT Thr T	AAT Asn N	AGT Ser S
	ATC	ACC	AAC	AGC
	ATA	ACA	AAA Lys K	AGA Arg R
	ATG Met M	ACG	AAG	AGG
G	GTT Val V	GCT Ala A	GAT Asp D	GGT Gly G
	GTC	GCC	GAC	GGC
	GTA	GCA	GAA Glu E	GGA
	GTG	GCG	GAG	GGG

