



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 às 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 ($T_{ann.}$);

Resposta:

Primer1: ccagcacctagaagccaata → [GC]=50%

Primer2: attgggctaaaggcagaagg → [GC]=50%

Temp de melting = $(A+T)2+(C+G)4$

T_m primer 1 ⇒ $(10)2+(10)4=20+40=60^\circ\text{C}$

T_m primer 2 ⇒ $(10)2+(10)4=20+40=60^\circ\text{C}$

Temp de anelamento = $T_m - 4^\circ\text{C}$

T_{ann} primer 1 ⇒ $60^\circ\text{C}-4^\circ\text{C} = 56^\circ\text{C}$

T_{ann} primer 2 ⇒ $60^\circ\text{C}-4^\circ\text{C} = 56^\circ\text{C}$

- b) Qual o tamanho exato do produto de PCR?;

Resposta: 345 pb

- c) Considerando as três temperaturas que são definidas para a reação de PCR, qual seria a temperatura de melting do PCR em questão?;

Resposta: T_m de melting do PCR: $[(60+60)/2]=60^\circ\text{C}$

- d) Informe o tipo de substituição (transição ou transversão) e os nucleotídeos trocados.

Resposta: A substituição é do tipo transição pois a substituição ocorre entre bases da mesma categoria (purina por purina). O nucleotídeo trocado foi GGT (Gly) para GAT (Asp).

Gene F9



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1 aaagacaagctacaggctggagacaatatatttaatccacctatctatgaaaggactcat
61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaaaaatcagactaact
121 ggaccactcatacattgctgatggaaatgtaaagtggtacagccattttggtaaacatca
181 ttqctcqtgacqaaqatacqqcqqgtccqactgaggaaccgtgcgqccacqgtaaacqg
241 aqcqactcqqccqatctcqattcqqaaqacqctcccttcccgtttccqaccaaatcqq
301 ccqccqgtgqcaqaaqcccacqaaatcagaggtgaaatttaataatgaccactgccattc
361 tcttcacttgtcccaagaggccattggaaatagtccaagaccattgagggagatggac
421 attatttcccagaagtaatacacgctcagcttgtactttggtacactaatccaccttac
1 M Q R V N M I M A E S P G L
481 cactttcacaaatctgctagcATGCAGCGCGTGAACATGATCATGGCAGAATCACCAGGCC
15 I T I C L L G Y L L S A E C T V
541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGTGAATGTACAGgtttgtttcctt
601 ttttaaaatacattgagtatgcttgccttttagatatagaaatatctgatgctgtcttct
661 tcactaaattttgattacatgatttgacagcaaatattgaagagtctaacagccagcagc
// 721 aggttggttaagtactggttcctttgttagctaggttttcttcttcttcatttttaaacta
// 6541 actaaaagtaaaattgaatttttaattcctaaatctccatgtgtatacagtagctgtggaa
6601 catcacagattttggctccatgcccctaaagagaaattggctttcagattatttggattaa
6661 aaacaaagacttttcttaagagatgtaaaattttcatgatgttttcttctttttgctaaaact
31 F L D H E N A N K I L
6721 aaagaattattcttttacatttcagTTTTTCTTTGATCATGAAAACGCCAACAAAATTTCTG
42 N R P K R Y N S G K L E E F V Q G N L E
6781 AATCGGCCAAAGAGGTATAAATTCAGGTAAAATFGAAGAGTTTGTTC AAGGGAACCTTGAG
62 R E C M E E K C S F E E A R E V F E N T
6841 AGAGAATGTATGGAAGAAAAGTGTAGTTTTGAAGAAGCACGAGAAGTTTTTGAAAACACT
82 E R T
6901 GAAAGAACAgtgagtatttccacataatacccttcagatgcagagcatagaatagaaaat
6961 ctttaaaaagacacttctctttaaattttaaagcatccatataatattatgtatgtaa
7021 atggtataaaaagataggaaatcaataccaaaacacttttagatattaccgttaatttgtct
85 T E F W K Q Y V D
7081 tcttttattctttatagACTGAATTTTGAAGCAGTATGTTGgtaagcaattcattttat
7141 cctctagctaatatataaacaatatagagaattatgtgggttttttctctgcataaaataga
7201 taatatattaactttgtcaaaaggactcagaaagatcagtccaaccctctaaccatata
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10621 atctttggttgggtggcttttagaaactcaggaagacaggagcatcatatgcctataggc
10681 agctggcttccaggtcagtagttttgctctgaccctaaaatcagactcccatcccaatga
10741 gtatctacagggggaggaccgggcattcctaagcagtttacgtgccaattcaatttcttaac
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 GGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTTGAAGGAAAGAAGTGTGA
130 L D
10921 ATTAGgtaagtaactattttttgaatactcatggttcaaagtttccctctgaacaagtt
10981 gaaactggaaaatgcaatattgggtgatcataatttttcttaaaaacatacctttgatgc
11041 ttataaacatttcatttggtagtgatagttttcaggatatgagttcaagaagctacattaa
// 11101 aatcaataacaatatttggtaactaatattaagtaataatgatgttccactcactatta
// 17881 tatagtgtaccatcatttttatgcattattgagaagtttattttaccttcttccact
17941 cttatttcaaggctccaaaatttctctcccaacgtatattgggggcaacatgaatgcc
18001 ccaatgtatatttgaccatacatgagtcagtagttccatgtacttttttagaaatgcatg
132 V T C N I K N
18061 ttaaagatgctgttactgtctatttttagATGTAACATGTAACATTAAGAA
139 G R C E Q F C K N S A D N K V V C S C T

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18121 TGGCAGATGCGAGCAGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGCTCCTGTAC
159 E G Y R L A E N Q K S C E P A V
18181 TGAGGGATATCGACTTGCAGAAAACAGAACTCCTGTGAACCAGCAGgtcataatctgaa
18241 taagattttttaagaaaatctgtatctgaaacttcagcatttttaacaaacctacataat
18301 ttttaattcctacttgaatctgcttccttttgaaatcatagaaaatatcagtagcttgaat
// 18361 tagaccaattaatcttagattgcatcatatcttaataataaaactatgtaatcatctac
// 20581 caaatgttcttttcatgaaggatttgaaaactgtccatgaaaataacgcaatcaaccttt
20641 tagcttgagactctattcactgattagatttttttaataactgatgggctgcttctcag
20701 aagtgacaaggatgggctcaatctcaatcttttgtaatacatgttccatttgccaatgag
175 P F P C G R V
20761 aaatatcaggttactaatttttcttctatcttttctagTGCCATTTCCATGTGGAAGAGTT
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 GTAAATTCTACTGAAGCTGAAACCATTTTGGATAACATCACTCAAAGCACCCAATCATT
222 N D F T R V V G G E D A K P G Q F P W Q
20941 AATGACTTCACTCGGGTGTGGTGGAGAAGATGCCAAACCAGGTCAATTCCCTTGGCAG
21001 gtactttatactgatgggtgtgtcaaaactggagctcagctggcaagacacaggccaggtg
// 21061 ggagactgaggctatcttactagacagacctattgggatgtgagaagtatttaggcaagt
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30361 atttctgccagcacctagaagccaataatcttgcctattcctgtaaccagcacacatattt
242 V V
30421 attttttctagatcaaatgtattatgcagtaagagctttaatcttttctacagGTTG
244 L N G K V D A F C G G S I V N E K W I V
30481 TTTTGAATGGTAAAGTTGATGCATTCTGTGGAGGCTCTATCGTTAATGAAAAATGGATTG
264 T A A H C V E T G V K I T V V A G
30541 TAACTGCTGCCCCTGTGTTGAAACTGGTGTAAAAATACAGTTGTGCGCAGgtaaataca
30601 cagaaagaataataatctgcagcaccactagctctttaatagattggtacaccatattt
30661 tactaagggtctaataaaattggtgttgaataaatgggctaaaggcagaagggtcataat
30721 ttcagaaccacgctcgaccgctcctccaagcatccatagttcttttgatatacccctatt
30781 atcactcatttcagtgaggtacaattagttcttgatgtagccatttccataaccagaaggc
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31081 aatggctttttggtctgaaaaatagcattggctctcattacatttaacaaaattatca
31141 caatataagaatgagatctttaacattgccaattaggtcagtggtcccaagtagtcactt
281
31201 agaaaatctgtgtatgtgaaatactgtttgtgacttaaaatgaaatttatttttaatagG
281 E H N I E E T E H T E Q K R N V I R I I
31261 TGAACATAATATTGAGGAGACAGAACATACAGAGCAAAGCGAAATGTGATTCGAATTAT
301 P H H N Y N A A I N K Y N H D I A L L E
31321 TCCTCACCACTACAATGCAGCTATTAATAAGTACAACCATGACATTGCCCTTCTGGA
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 CACATGTCTTCGATCTACAAAGTTCAACATCTATAACAACATGTTCTGTGCTGGCTTCCA
401 E G G R D S C Q G D S G G P H V T E V E
31621 TGAAGGAGGTAGAGATTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACTGAAAGTGA
421 G T S F L T G I I S W G E E C A M K G K
31681 AGGGACCAGTTTCTTAACTGGAATTTATAGCTGGGGTGAAGAGTGTGCAATGAAAGGCAA
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 CACTTAAaccttggctttttgtggattccattgatgtgaatcagtcaccctgtatattgat
31861 gatgcatgggactactgacaaaatcactctgaccccgccaagctgctgccttctcctgcc
31921 ccaacctcacccccagccaggcctcactcttgctagttcctttagttcttttagtcaata
31981 tatttttgtcttcgcatataagtataaataaacatatttttaaatttcttggctgggccc
32041 agtggctcacgcctataatcccagcacttctggaggccaaggtgggcggatcacctgagg

	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		

