



**Genética Molecular**  
**RIB0102 Ano 2020**

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**Grupo B**

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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 ( $T_{ann}$ );

Resposta:

☐ **Primer 1**

- [CG] = 50%
- $T_m = 2*(A+T) + 4*(C+G) = 60^\circ\text{C}$
- $T_{ann} = T_m - 4^\circ\text{C} = 56^\circ\text{C}$

☐ **Primer 2**

- [CG] = 50%
- $T_m = 2*(A+T) + 4*(C+G) = 60^\circ\text{C}$
- $T_{ann} = T_m - 4^\circ\text{C} = 56^\circ\text{C}$

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

Resposta: 327pb

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

Resposta: Temperatura do PCR =  $56^\circ\text{C}$

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

Resposta: Foi trocado um nucleotídeo A (adenina) em GAC por um G (guanina), logo foi uma substituição de transição, visto que foram trocadas bases púricas.

## Gene F9



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1 aaagacaagctacaggctggagacaatatatttaatccacctatctatgaaaggactcat
61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaaaatcagactaact
121 ggaccactcatacattgctgatggaaatgtaaagtggtacagccatttttggtaaacatca
181 ttgctcgctgacgaaatagcgccggtccgactgaggaaccgtgccccacggttaaacgt
241 agcgactcggccgatctcgattcggaaagacggtcccttcccgtttccgacccaaatcgg
301 ccgccggtggcagaagcccacgaaatcagaggtgaaatttaataatgaccactgccattc
361 tcttcacttgtcccgaagaggccattggaaatagtccaaagaccattgagggagatggac
421 attatttcccagaagtaaacacagctcagcttgtactttggtacaactaatccaccttac
1      M Q R V N M I M A E S P G L
481 cactttcacaatctgctagcATGCAGCGCGTGAACATGATCATGGCAGAAATCACCAGGCC
15    I T I C L L G Y L L S A E C T V
541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGgtttgtttcctt
601 ttttaaaaatacattgagtatgcttgccttttagatatagaaaatatctgatgctgtcttct
661 tcactaaattttgattacatgatttgacagcaatattgaagagtctaacagccagcagcgc
// 721 aggttggttaagtactggttctttgtagctaggttttcttcttcttcatTTTTAAAACTA
// 6541 actaaaagtaaaattgaattttaattcctaaatctccatgtgtatacagtactgtgggaa
6601 catcacagattttggctccatgccctaaagagaaaattggctttcagattatttggattaa
6661 aaacaaagacttttctaagagatgtaaaaattttcatgatgttttcttttttgctaaaact
31      F L D H E N A N K I L
6721 aaagaattattcttttacatttcagTTTTCTTGATCATGAAAAAGCCCAACAAAAATCTG
42    N R P K R Y N S G K L E E F V Q G N L E
6781 AATCGGCCAAAGAGGTATAAATTCAGGTAAAATGGGAAGTTTGTTCAGGGAACCTTGAG
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 GAAAGAACAgtgagtatttccacataataacccttcagatgcagagcatagaatagaaaat
6961 ctttaaaaagacacttctcttttaaaattttaagcatccatataatattatgtatgtaa
7021 atggtataaaaagataggaaatcaataccaaaacactttagatattaccgttaatttgtct
85      T E F W K Q Y V D
7081 tcttttattctttatagACTGAATTTTGGGAAGCAGTATGTTGgtaagcaattcattttat
7141 cctctagctaatatatagaaacatatgagaattatgtgggtttttctctgcataaataga
7201 taatatattaaactttgtcaaaaggactcagaaaagatcagccaaccctctaaccatata
// 7261 tgaatggtgatatactacagggttatgccagtgtgggaactatcgctggtaaataagttt
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10621 atctttggtttgggtggcttttagaaaactcaggaagacaggagcatcatatgcctataggc
10681 agctggcttccaggtcagtagttttgctctgaccctaaaatcagactcccatccaatga
10741 gtatctacaggggaggaccgggcattctaagcagtttacgtgcccaattcaatttcttaac
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 GGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTTGAAGGAAAGAACTGTGA
130    L D
10921 ATTAGgtaagtaactatTTTTTgaatactcatggttcaaagtttccctctgaaacaagtt
10981 gaaactggaaaatgcaatattgggtgatcataatTTTTTcttaaaaacatacctttgatgc
11041 ttataaacatttcattttagtagtatgattttcaggatatgagttcaagaagctacattaa
// 11101 aatcaataacaatatttggtaactaatattaagtaataatgatgttccactcacttatta
// 17881 tatagtgtaccatcatttttatgcattattgagaagtttattttacccttctttccact
17941 cttatttcaaggctccaaaatttctctccccaacgtatattggggggcaacatgaatgcc
18001 ccaatgtatatttgaccacatacatgagtcagtagttccatgtacttttttagaaatgcatg
132      V T C N I K N
18061 ttaaagtatgctgttactgtctatTTTTgcttcttttagATGTAACATGTAACATTAAGAA
139    G R C E Q F C K N S A D N K V V C S C T
18121 TGGCAGATGCGAGCAGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGTCTCCTGTAC

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159 E G Y R L A E N Q K S C E P A V  
18181 TGAGGGATATCGACTTGCAGAAAACAGAAAGTCTGTGAACCAGCAGgtcataatctgaa  
18241 taagatTTTTTaaagaaaatctgtatctgaaacttcagcattTTTaaacaaacctacataat  
18301 tTTaattcctacttgaatctgcttcctTTTgaaatcatagaaaatatcagtagcttgaat  
// 18361 tagaccaattaatTTTctagattgcatcatatTTTaaatataaaactatgtaatcatctac  
// 20581 caaatgttctTTTcatgaaggatttgaaaactgtccatgaaaataacgcaatcaaccttt  
20641 tagcttgagactctattcactgattagatTTTTTaaatactgatgggctgcttctcag  
20701 aagtgacaaggatgggctcaatctcaatTTTTgtatacatgttccatttgccaatgag  
175 P F P C G R V  
20761 aaatcaggttactaattTTTTcttctatTTTTctagTGCCATTTCCATGTGGAAGAGTT  
182 S V S Q T S K L T R A E T V F P D V D Y  
20821 TCTGTTTCACAAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTTCTGATGTGGACTAT  
202 V N S T E A E T I L D N I T Q S T Q S F  
20881 GTAAATTCTACTGAAGCTGAAACCATTTTGATAACATCACTCAAAGCACCCAATCATT  
222 N D F T R V V G G E D A K P G Q F P W Q  
20941 AATGACTTCACTCGGGTTGTTGGTGGAGAAGATGCCAAACCAGGTCAATTCCCTTGGCAG  
21001 gtactttatactgatgggtgtgtcaaaactggagctcagctggcaagacacaggccaggtg  
// 21061 ggagactgaggctatTTTactagacagacctattgggatgtgagaagtatttaggcaagt  
// 30241 gcttaagcttccctgtctctcattgtgtgttgcTTTcaatgcagttacataaatggcttt  
30301 tttgtttatgcacccaaaaactaattcatctgcaaagctcacatttccagaacattcc  
30361 atTTctgccagcacctagaagccaatatttTgcctattcctgtaaccagc acacatattt  
242 V V  
30421 atTTTTTctagatcaaatgtattatgcagtaagagctttaatTTTgtTTTcacagGTTG  
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 TAACTGCTGCCCCTGTGTTGAAACTGGTGTAAAAATTACAGTTGTTCGCAGgtaaataca  
30601 cagaaagaataataatctgcagcaccactagctctttaaatatgattggtacacatattt  
30661 tactaagggtctaataaaattgTTgttgaataaattgggctaaaggcagaagggtcat aat  
30721 ttcagaaccacgctcgcaccgctcctccaagcatccatagttctTTTgatataccctatt  
30781 atcactcatttcagtgagggtacaattagttcttgatgtagccatttccataaccagaaggc  
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30961 aggggtgaggatacttgaaatttggaaaatctaggataattcatgactagtggattcatt  
31021 atcaccaatgaaaggcttataacagcatgagtgaacagaacctctctatgatagtctctg  
31081 aatggctTTTTTggtctgaaaaatatgcattggctctcattacatttaacccaaaattatca  
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 ACTGGACGAACCCTTAGTGCTAAACAGCTACGTTACACCTATTTGCATTGCTGACAAGGA  
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 AGGGACCAGTTTCTTAACTGGAATTATTAGCTGGGGTGAAGAGTGTGCAATGAAAGGCAA  
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 agtggctcacgcctataatcccagcacttctggaggccaaggtgggaggatcacctgagg

	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		

