



Genética Molecular e de Populações
RIB0102 Ano 2019

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1. Identifique a posição da mutação GLY106ALA no gene F9 da coagulação e desenhe um par de primers para amplificar a região da posição da mutação (tamanho do produto de amplificação de aproximadamente 350pb) para validá-la por sequenciamento numa população de 100 hemofílicos. Para tanto responda as seguintes questões:
- a) Para cada primer, informe a percentagem de [CG], temperatura de melting (T_m), temperatura de anelamento (T_{an});
 - b) Qual a tamanho exato do produto de PCR?; 350pb
 - c) Considerando as três temperaturas que são definidas para a reação de PCR, qual seria as temperaturas para o PCR em questão?;
 - d) Com relação a mutação descrita no enunciado, responda suas características em relação a substituição de nucleotídeos e aminoácidos. Use os quadros anexo para responder esta questão;
 - e) Tomando como base a resposta sobre a mutação, esta pode ser considerada patogênica? Por que?

LUIS GUSTAVO M. DOS REIS

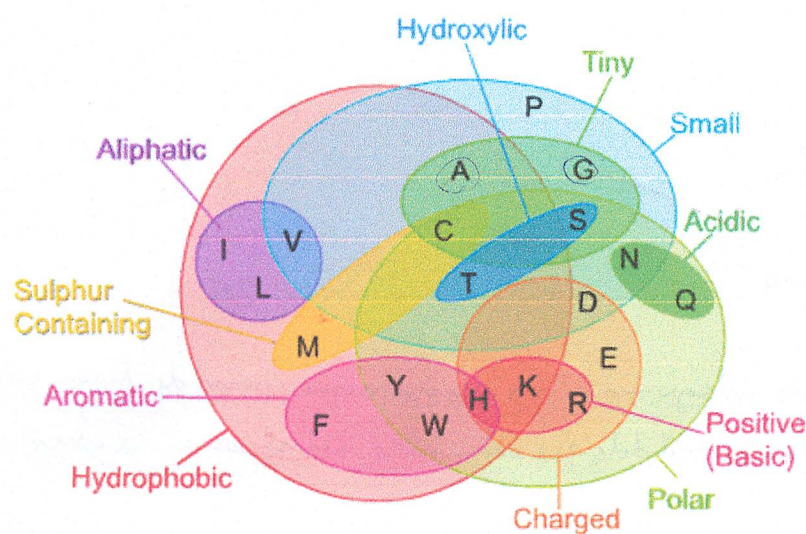
MIGUEL VIANNA DE SOUZA

GUSTAVO MANOEL BRANDÃO

1 aaagcaagctacaggtcgagacaaatatttataatccaccttctatgaaaggactcat
61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaatcagactaact
121 ggaccactcatacattgctgatggaatgtaaagtggtacagccatttttggtaaacatca
181 ttgctcgctgacgaagatacggcggggtccgactgaggaaccgtgcggccacggtaaacgt
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301 ccgcggtggcagaagcccacgaaatcagaggtgaaatttaataatgaccactgcccattc
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421 attatttcccagaagtaaatcacagctcagcttgtactttggtacaactaatccaccttac
1 M Q R V N M I M A E S P G L
481 cactttcacaaatctgctagcATGCAGCGCTGAACATGATCATGGCAGAATCACCAGGCC
15 I T I C L L G Y L L S A E C T V
541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGTGAATGTACAGgtttgtttcctt
601 ttttaaaatacattgagtatgcttgccttttagatatagaaatatctgatgctgtcttct
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6661 aaacaaagactttcttaagagatgtaaaatttcatgatgttttcttttttgctaaaact
31 F L D H E N A N K I L
6721 aaagaattattcttttacatttcagTTTTCTTGATCATGAAAACGCCAACAAAATTCTG
42 N R P K R Y N S G K L E E F V Q G N L E
6781 AATCGGCCAAAGAGGTATAAATCAGGTAAATTGGAAGAGTTTGTTCAGGGAACCTTGAG
62 R E C M E E K C S F E E A R E E V F E N T
6841 AGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTTTGAAAACACT
82 E R T
6901 GAAAGAACAgtagtattttccacataatacccttcagatgcagagcatagaatagaaaat
6961 ctttaaaaagacacttctctttaaatttttaagcatccatataatattatgtatgttaa
7021 atgttataaaaagataggaaatcaataccaaaacacttttagatattaccggttaatttgtct
85 T E F W K Q Y V D
7081 tcttttattctttatagACTGAATTTTGGAAACAGTATGTTGgtaagcaattcattttat
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10681 agctgcttccagggtcagtagtttgcctctgaccctaaaatcagactcccacccaatga
10741 gtatctacaggggaggaccgggcattctaagcagtttacgtgccaaattcaatttcttaac
94 G D Q C E S N P C L N G G S C K
10801 ctatctcaaaagATGGAGATCAGTGTGAGTCCAATCCATGTTTAAATGGCGGCAGTTGCAA
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 gaaactggaaaaatgcaatattggtgtatcataatttttcttaaaaaacatacctttgatgc
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18001 ccaatgtatatttgaccatacatgagtcagtagttccatgtactttttagaatgcatg
132 V T C N I K N
18061 ttaaatgatgctgttactgtctattttgcttcttttagATGTAACATGTAACATTAAGAA
139 G R C E Q F C K N S A D N K V V C S C T
18121 TGGCAGATGCGAGCAGTTTGTAAAAATAGTGTGATAACAAGGTGGTTTGTCTCCTGTAC
159 E G Y R L A E N Q K S C E P A V
18181 TGAGGGATATCGACTTGCAGAAAACCAGAAGTCTGTGAACCAGAGtcataatctgaa
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20701 aagtgacaaggatgggcctcaatctcaatttttgtaatacatgttccatttgccaatgag
175 P F P C G R V
20761 aaatatcaggttactaatttttcttctatttttctagTGCCATTTCCATGTGGAAGAGTT
182 S V S Q T S K L T R A E T V F P D V D Y
20821 TCTGTTTCACAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTCTGATGTGGACTAT
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
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242 V V
30421 attttttcttagatcaaatgtattatgcagtaagagtcttaattttgttttcacagGTTG
244 L N G K V D A F C G G S I V N E K W I V
30481 TTTTGAATGGTAAAGTTGATGCATTCTGTGGAGGCTCTATCGTTAATGAAAATGGATTG
264 T A A H C V E T G V K I T V V A G
30541 TAAGTCTGCTCCCACTGTGTTGAAACTGGTGTTAAATTACAGTTGTGCGAGgtaaataca
30601 cagaagaataataatctgcagcaccactagctctttaatatgattgggtacaccatattt
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281
31201 agaaaatctgtgtatgtgaaatactgtttgtgacttaaaatgaaattttatttttaatatgG
281 E H N I E E T E H T E Q K R N V I R I I
31261 TGAACATAATATTGAGGAGACAGAACATACAGAGCAAAGCGAAATGTGATTGCAATTAT
301 P H H N Y N A A I N K Y N H D I A L L E
31321 TCCTCACCACAACACTACAATGCAGCTATTAATAAGTACAACCATGACATTGCCCTTCTGGA
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 TGAAGGAGGTAGAGATTCAATGTCAAGGAGATAGTGGGGGACCCCATGTTACTGAAGTGGA
421 G T S F L T G I I S W G E E C A M K G K
31681 AGGGACCAGTTTCTTAAGTGAATATTAGCTGGGGTGAAGAGTGTGCAATGAAAGGCAA
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 CACTTAAaccttggctttttgtggattccattgatgtgaatcagtcaccctgtatttgat
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32041 agtggtcacgcctataatcccagcacttctggaggccaaggtgggcggatcacctgagg

	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	<u>Ala</u>	A	GAT	Asp	D	GGT	<u>Gly</u>	G
	GTC			<u>GCC</u> <i>666</i>			GAC			<u>GGC</u> <i>666</i>		
	GTA			GCA			GAA	Glu	E	GGA		
	GTG			GCG			GAG			GGG		



Amino Acids

- A alanine (ala)
- R arginine (arg)
- N asparagine (asn)
- D aspartic acid (asp)
- C cysteine (cys)
- Q glutamine (gln)
- E glutamic acid (glu)
- G glycine (gly)
- H histidine (his)
- I isoleucine (ile)
- L leucine (leu)
- K lysine (lys)
- M methionine (met)
- F phenylalanine (phe)
- P proline (pro)
- S serine (ser)
- T threonine (thr)
- W tryptophan (trp)
- Y tyrosine (tyr)

comparative / in comparative

106 → *proline para alanina*

Primer 1: GGCTTCCA, GGTCAGTAGT, TT

Primer 2: TTTC, AA, CTTG, TTTC, A, G, A, GGG

a) $IP_{P1} = \frac{10}{20} = 50\% [CG]$

$IP_{P2} = \frac{8}{20} = 40\% [CG]$

$T_m = 10 \cdot 2 + 10 \cdot 4 = 60^\circ C$

$T_m = 12 \cdot 2 + 8 \cdot 4 = 56^\circ C$

$T_{an} = 60 - 4 = 56^\circ C$

$T_{an} = 56 - 4 = 52^\circ C$

b) 300 pb

c) denaturação: $95^\circ C$

$T_{PCR} = \frac{56 + 52}{2} = 54^\circ C$

extensão: $72^\circ C$

d) ~~GGG~~

GGC $\rightarrow$ GCC

transcrição G por C

substituição glicina por alanina

e) Sim, esta pode ser considerada patogênica. Isso ocorre porque apesar de terem certas semelhanças, serem muito pequenos, há uma diferença dita que alanina é ~~hidrofóbica~~ e a glicina não é.