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Genética Molecular e de Populações
RIB0102 Ano 2019

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1. Identifique a posição da mutação ALA250GLY 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:

- Para cada primer, informe a percentagem de [CG], temperatura de melting (T_m), temperatura de anelamento (T_{an});
- Qual o tamanho exato do produto de PCR?
- Considerando as três temperaturas que são definidas para a reação de PCR, qual seria as temperaturas para o PCR em questão?
- 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;
- Tomando como base a resposta sobre a mutação, esta pode ser considerada patogênica? Por que?

a) $P_1 = \text{TGC AAAGCTCACATT.TCCAG}$
 $[\text{CG}] = 45\% / T_m = 2 \cdot (11) + 4(9) = 58^\circ\text{C} \quad T_{an} = 54^\circ\text{C}$

$P_2 = \text{CCTTCTGCCCTTTAGCCCAAT}$
 $[\text{CG}] = 50\% / T_m = 2 \cdot (10) + 4(10) = 60^\circ\text{C} \quad T_{an} = 56^\circ\text{C}$

b) 381 b

c) $T_{desm} = 95^\circ\text{C} \quad T_{an} = 55^\circ\text{C} \quad T_{ex} = 72^\circ\text{C}$

d) $\overset{\text{A}}{\text{GCA}} \rightarrow \overset{\text{E}}{\text{GAA}}$ mutação de transição
Alamina: tiny e hidrofóbico
Ácido glutâmico: não small e polar } não conservativa

e) Como esses aminoácidos possuem tamanho e polaridade diferentes, a mutação é provavelmente patogênica.

5'UTR

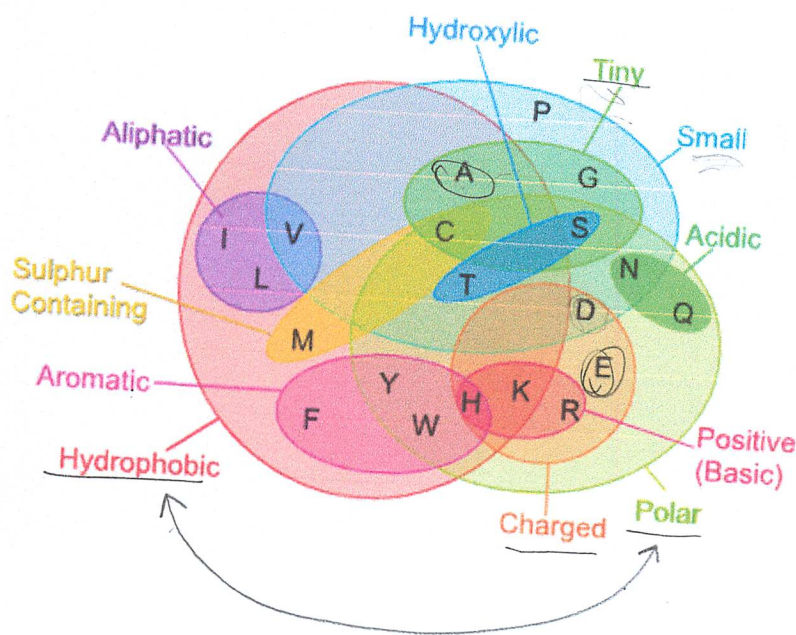
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1 M Q R V N M I M A E S P G L  
481 cactttcacaaatctgctagcATGCAGCGCVTGAACATGATCAGAGAATCACCAAGGCC  
15 I T I C L L G Y L L S A E C T V  
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6661 aaacaaagactttcttaagagatgtaaaaatttcatgatgttttcttttttgctaaaact  
31 F L D H E N A N K I L  
6721 aaagaattattctttttacatttcagTTTTCTTGATCATGAAAACGCCAACAAATTCTG  
42 N R P K R Y N S G K L E E F V Q G N L E  
6781 AATCGGCCAAGAGGTATAATTTCAGGTAAATGGAAAGATTGTTCAGGGAACTTCAG  
62 R E C M E E K C S F E E A R E E V F E N T  
6841 AGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTTTGAAACACT  
82 E R T  
6901 GAAAGAACAgtgagtattttccacataatacccttcagatgcagagcatagaatagaaaat  
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94 G D Q C E S N P C L N G G S C K  
10801 ctatctcaaagATGGAGATCAGTGTGAGTCCAATCCATGTTTAATGGCGGCAGTTGCAA  
110 D D I N S Y E C W C P F G F E G K N C E  
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130 L D  
10921 ATTAGgtaagtaactattttttgaatactcatggttcaaagtttccctctgaaacaagtt  
10981 gaaactggaaaatgcaatattgggtgatcataatttttcttaaaaacatacctttgatgc  
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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 TGGCAGATGCGAGCAGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGTCTCCTGTAC  
159 E G Y R L A E N Q K S C E P A V  
18181 TGAGGGATATCGACTTGCAGAAAACCGAAGTCCTGTGAACCAAGgtcataatctgaa  
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350 ALA/ASD
GLU

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182 S V S Q T S K L T R A E T V F P D V D Y
20821 TCTGTTTCACAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTCTGTATGTGGACTAT
202 V N S T E A E T I L D N I T Q S T Q S F
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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
30421 attttttctagatcaaatgtattatgcagtaagagtcttaattttgttttcacagGTTG
244 L N G K V D A F C G G S I V N E K W I V
30481 TTTTGAATGGTAAAGTTGATGCTTCTGTGGAGGCTCTATCGTTAATGAAAAATGGATTG
264 T A A H C V E T G V K I T V V A G
30541 TAACTGCTGCCCACTGTGTTGAAACTGGTGTAAATTACAGTTGTCGCAGgtaaataca
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31201 agaaaatctgtgtatgtgaaatactgtttgtgacttaaaatgaaattttatttttaatagG
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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 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
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401 E G G R D S C Q G D S G G P H V T E V E
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441 Y G I Y T K V S R Y V N W I K E K T K L
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461 T *
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300 + 29 + 52
= 381

	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		



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)