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Genética Molecular e de Populações RIB0102 Ano 2019

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file 210T#n

1. Identifique a posição da mutação 210 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?

RESPOSTAS

1-

a) Primer 1: percentagem de [CG] = 50%.

$$T_{M1} = (A+T)_2 + (C+G)_4$$

$$T_{M1} = (10)_2 + (10)_4$$

$$T_{M1} = 60^{\circ}\text{C}$$

Primer 2: percentagem de [CG] = 45%.

$$T_{M2} = (A+T)_2 + (C+G)_4$$

$$T_{M2} = (11)_2 + (9)_4$$

$$T_{M2} = 58^{\circ}\text{C}$$

OBS: FALTA A
 SEQUENCIA
 PRIMER 2

→ temperatura de melting

• Primer 1 = 60°C

• Primer 2 = 58°C

→ temperatura de anelamento

$$T_{AM} = \frac{(T_{M1} - 4) + (T_{M2} - 4)}{2} = \frac{(60 - 4) + (58 - 4)}{2} = \frac{56 + 54}{2} = 55^{\circ}\text{C}$$

b) 390 pares de bases

391

- c) → temperatura de denaturação = 95°C
→ temperatura de anelamento = 55°C
→ temperatura de extensão = 72°C

d) Transição não conservativa, pois há uma troca da base pirimidica, e troca de aminoácido (de alifático para hidrofílico)

e) Sim, porque houve uma mudança funcional das propriedades físico-químicas

F9

1 aaagacaagctacaggtcgtgagcaaatatatttaacccacctatcttatgaaaggactcat
61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaatacagactaact
121 ggaccactcatacattgctgatggaatgtaaaagtggtacagccatttttgtaaacaatca
181 ttgctcgcgtgacgaagatacggcggggtccgactgaggaaccgtgcgggccacggtaaacgt
241 agcgactcggccgatctcgattcggaaagacgcgtcccttcccgtttccgacccaaatcgg
301 ccgcggtggcagaagcccacgaaatcagaggtgaaatttaataatgaccactgcccattc
361 tcttcacttgtcccaagaggccattggaatatgtccaaagacccattgagggagatggac
421 attatttcccagaagtaataacagctcagcttgtacttttggtacaactaatccaccttac
1 M Q R V N M I M A E S P G L
481 cactttcacaaatctgctagcATG CAGCGCTGAACATGATCGGCAGAAATCACCAGGCC
15 I T I C L L G Y L L S A E C T V
541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGgttttgtttcctt
601 ttttaaaatacattgagtatgcttgcccttttagatatagaaatatctgatgctgtcttct
661 tcactaaattttgattacatgatttgacagcaatattgaagagtctaacagccagcacgc
// 721 aggttggttaagtactggttctttgttagctagggttttcttcttcttcatttttaaaacta
// 6541 actaaaagtaaaattgaatttttaattcctaaatctccatgtgtatacagctactgtgggaa
6601 catcacagattttggctccatgccctaaagagaaattggctttcagattatttgatttaa
6661 aaacaaagactttcttaagagatgtaaaattttcatgatgttttcttttttgctaaaact
31 F L D H E N A N K I L
6721 aaagaattattcttttacatttcagTTTTCTTGATCATGAAAACGCCAACAAAATCTG
42 N R P K R Y N S G K L E E F V Q G N L E
6781 AATCGGCCAAAGAGGTATAATTCAGGTAAATTGGAAGATTTGTTCAAGGGAACCTTGAG
62 R E C M E E K C S F E E A R E V F E N T
6841 AGAGAATGTATGGAAGAAAAGTGTAAGTTTTGAAGAAGCACGAGAAGTTTTTGAAAACACT
82 E R T
6901 GAAAGAACAgtgagtattttccacataataacccttcagatgcagagcatagaatagaaaat
6961 ctttaaaaagacacttctcttttaaaatttttaagcatccatatatatttatgtatgttaa
7021 atgttataaaaagataggaaatcaataccaaaacacttttagatattaccgttaatttgtct
85 T E F W K Q Y V D
7081 tcttttattctttatagACTGAATTTTGGAAGCAGTATGTTGgtaagcaattcattttat
7141 cctctagctaataatatgaaacatatgagaattatgtgggttttttctctgcataaaataga
7201 taatatattaaactttgtcaaaaaggactcagaaagatcagtcacaacctctaaccatat
// 7261 tgaatggtgatatactacaggggttatgccagtgtgggaactatcgctggtaaaataagttt
// 10561 tgcataaaactatgtacatgccttctcagggcacttttctaggacagtgtcagcctaagg
10621 atctttgtttgggtggttttagaaactcaggaagacaggagcatcatatgcctataggc
10681 agctgggttccaggctcagtagttttgctctgaccctaaaatcagactcccatcccaatga
10741 gtatctacaggggaggaccgggcatttctaagcagtttacgtgccaaattcaatttcttaac
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 GGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTGGAAGGAAAGAACTGTGA
130 L D
10921 ATTAGgtaagtaactattttttgaatactcatggttcaaagtttccctctgaaacaagtt
10981 gaaactggaaaaatgcaatattggtgtatcataatttttcttaaaaacataacctttgatgc
11041 ttataaacattttcatttgtagtgtatgattttcaggatattgagttcaagaagctacattaa
// 11101 aatcaataacaaatttttgtaactaataatagtaataatgatgttccactcatttatta
// 17881 tatagtgtaccatattttttagtcattattgagaagtttattttaccttttctttccact
17941 cttattttcaaggctccaaaatttctctccccaacgtatattgggggcaacatgaatgcc
18001 ccaatgtatatttgaccatacatgagtcagtagttccatgtactttttagaaatgcatg
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 TGGCAGATGCGAGCAGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTTGCTCCTGTAC
159 E G Y R L A E N Q K S C E P A V
18181 TGAGGGATATCGACTTGCAGAAAACCAGAAGTCCTGTGAACCGAGgtcataatctgaa
18241 taagatttttttaagaaaaatctgtatctgaaacttcagacttttaacaaacctacataat
18301 tttatttctacttgaatctgtcttcttctttgaaatcatagaaaatacagctagcttgaat
// 18361 taqaccaatttaattttctagattgcacatatttttaataataaaactatgtaaatcatctac

Isoleucina



Threonina

// 20581 caaatgttcttttcatgaaggatttgaaaactgtccatgaaaataacgcaatcaaccttt
20641 tagcttgagactctattcactgattagatttttttaataactgatgggctgcttctcag
20701 aagtgacaaggatgggcttcaatctcaatttttgtaatacatgttccatttgccaatgag
175 P F P C G R V
20761 aaatatcaggttactaatttttcttctatttttctagTGCCATTTCATGTGGAAGAGTT
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
20881 GTAAATTCTACTGAAGCTGAAACCTTTTGGATAACATCACTCAAAGCACCCAATCATT
222 N D F T R V V G G E D A K P G Q F P W Q
20941 AATGACTTCACTCGGGTTGTTGGTGGAGAAGATGCCAAACCAGGTCAATTCCCTTGGCAG
21001 gtactttatactgatgggtgtgtgcaaaactggagctcagctggcaagacacaggccaggtg
// 21061 ggagactgaggctatttttadtagacagacctattgggagtgagaaagtatttaggcaagt
// 30241 gcttaagcttccctgtctctcattgtgtgttgctttcaatgcagttacataaatggcttt
30301 tttgtttatgcacaaaaaacactaattcatctgcaaagctcacatttccagaaacattcc
30361 atttctgccagcacctagaagccaatattttgcctattcctgtaaccagcacacatattt
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 TTTTGAATGGTAAAGTTGATGCATTCTGTGGAGGCTCTATCGTTAATGAAAAATGGATTG
264 T A A H C V E T G G V K I T V V A G
30541 TAACTGCTGCCACTGTGTGAAACTGGTGTAAAATTACAGTTGTGCGAGgtaaatata
30601 cagaagaataataatctgcagcaccactagctctttaatatgattgggtacaccatattt
30661 tactaaggctctaataaaaattgttgaataaaattgggctaaggcagaaggtcataat
30721 ttcagaaccacagctgcacccgtctccaagcatccatagttcttttgatatacccctatt
30781 atcactcatttccagtgaggtacaattagttcttgatgtagccatttccataaccagaaggc
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31141 caatataagaatgagatctttaacattgccattaggtcagtggtcccaagtagtcactt
281
31201 agaaaatctgtgtatgtgaaataactgtttgtgacttaaaatgaaattttatttttaatagG
281 E H N I E E T E H T E Q K R N V I R I I
31261 TGAACATAATATTGAGGAGACAGAACATACAGAGCAAAAGCGAAATGTGATTCTGAATTAT
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
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 TGAAGGAGGTAGAGATTTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACTGAAAGTGA
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 CACTTAAaccttggcttttttgtggattccattgatgtgaatcagtcaccctgtatttgat
31861 gatgcatgggactactgacaaaatcactctgaccccgccaagctgctgccttctcctgcc
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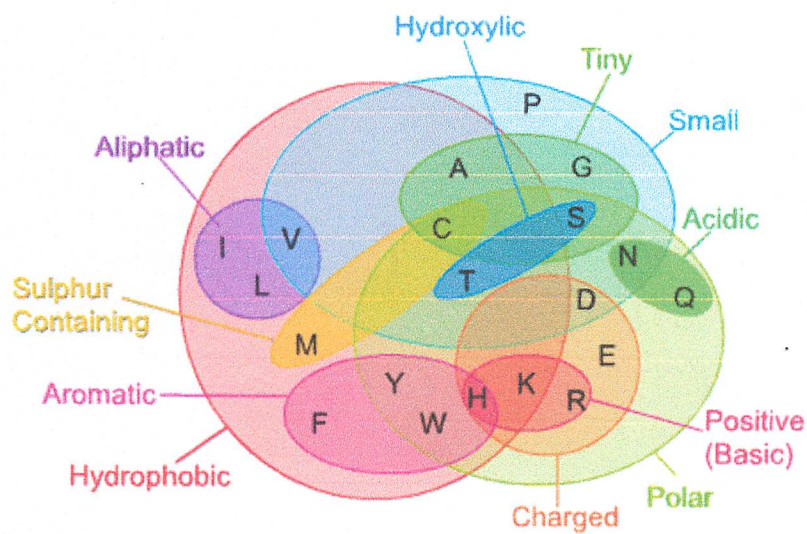
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91

P2
40

	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		

ATT



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)