Genética Molecular e de Populações

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Wilson A. Silva Jr

1. Identifique a posição da mutação C178X 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 (Tm), temperatura de anelamento (T.an.);
   b) Qual a tamanho exato do produto de PCR?;
   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?

Gene F9

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        1 aaagacaagctacaggctggagacaatatatttaatccacctatctatgaaaggactcat
       61 atctagaatatataaacaaccttaagaatctgacagtaaaaaaaaaaaatcagactaact
      121 ggaccactcatacattgctgatggaaatgtaaagtggtacagccattttggtaaacatca
      181 ttgctcgctgacgaagatacggcgggtccgactgaggaaccgtgcggccacggtaaacgt
      241 agcgactcggccgatctcgattcggaagacgcgtcccttcccgtttccgacccaaatcgg
      301 ccgcggtggcagaagcccacgaaatcagaggtgaaatttaataatgaccactgcccattc
      361 tcttcacttgtcccaagaggccattggaaatagtccaaagacccattgagggagatggac
      421 attatttcccagaagtaaatacagctcagcttgtactttggtacaactaatccaccttac
        1                     M  Q  R  V  N  M  I  M  A  E  S  P  G  L
      481 cactttcacaatctgctagcATGCAGCGCGTGAACATGATCATGGCAGAATCACCAGGCC
       15   I  T  I  C  L  L  G  Y  L  L  S  A  E  C  T  V
      541 TCATCACCATCTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGgtttgtttcctt
      601 ttttaaaatacattgagtatgcttgccttttagatatagaaatatctgatgctgtcttct
      661 tcactaaattttgattacatgatttgacagcaatattgaagagtctaacagccagcacgc
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     6661 aaacaaagactttcttaagagatgtaaaattttcatgatgttttcttttttgctaaaact
       31                            F  L  D  H  E  N  A  N  K  I  L
     6721 aaagaattattcttttacatttcagTTTTTCTTGATCATGAAAACGCCAACAAAATTCTG
       42 N  R  P  K  R  Y  N  S  G  K  L  E  E  F  V  Q  G  N  L  E
     6781 AATCGGCCAAAGAGGTATAATTCAGGTAAATTGGAAGAGTTTGTTCAAGGGAACCTTGAG
       62 R  E  C  M  E  E  K  C  S  F  E  E  A  R  E  V  F  E  N  T
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       82 E  R  T
     6901 GAAAGAACAgtgagtatttccacataatacccttcagatgcagagcatagaatagaaaat
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     7021 atgttataaaagataggaaatcaataccaaaacactttagatattaccgttaatttgtct
       85                  T  E  F  W  K  Q  Y  V  D
     7081 tcttttattctttatagACTGAATTTTGGAAGCAGTATGTTGgtaagcaattcattttat
     7141 cctctagctaatatatgaaacatatgagaattatgtgggtttttctctgcataaataga
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    10621 atctttgtttgggtggcttttagaaactcaggaagacaggagcatcatatgcctataggc
    10681 agctggcttccaggtcagtagttttgctctgaccctaaaatcagactcccatcccaatga
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       94              G  D  Q  C  E  S  N  P  C  L  N  G  G  S  C  K
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    18061 ttaaatgatgctgttactgtctattttgcttcttttagATGTAACATGTAACATTAAGAA
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	T			C			A			G		
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	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
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G	GTT	Val	V	GCT	Ala	A	GAT	Asp	D	GGT	Gly	G
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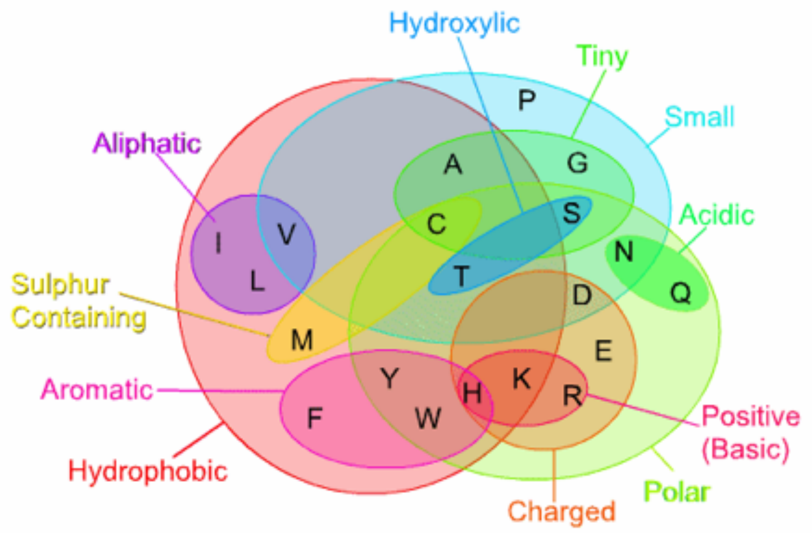

Amino Acids

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 metioneine (met)
F phenyalanine (phe)
P proline (pro)
S serine (ser)
T threonine (thr)
W trytophan (trp)
Y tyrosine (tyr)