Hands-On TEN
Beta Thalassemia in Morocco

A) We are going to use a gene predictor to see the exons and introns of the beta globin gene.

- Obtain the sequence "beta_globin_sequence" from: www.cs.sjsu.edu/faculty/khuri/Rabat_2013/beta_globin_sequence.pdf
- Open a web browser and go to the GenScan server at: http://genes.mit.edu/GENSCAN.html
- Paste the sequence into the DNA field.
- Keep the default options and press "Run GENSCAN".
- Read the following explanation and answer the questions related to the table that GenScan generated.

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Gn.Ex : gene number, exon number (for reference)
Type  : Init = Initial exon (ATG to 5' splice site)
        Intr = Internal exon (3' splice site to 5' splice site)
        Term = Terminal exon (3' splice site to stop codon)
        Sngl = Single-exon gene (ATG to stop)
        Prom = Promoter (TATA box / initation site)
        PlyA = poly-A signal (consensus: AATAAA)
S     : DNA strand (+ = input strand; - = opposite strand)
Begin : beginning of exon or signal (numbered on input strand)
End   : end point of exon or signal (numbered on input strand)
Len   : length of exon or signal (bp)
Fr    : reading frame (a forward strand codon ending at x has frame x mod 3)
Ph    : net phase of exon (exon length modulo 3)
I/Ac  : initiation signal or 3' splice site score (tenth bit units)
Do/T  : 5' splice site or termination signal score (tenth bit units)
CodRg : coding region score (tenth bit units)
P     : probability of exon (sum over all parses containing exon)
Tscr  : exon score (depends on length, I/Ac, Do/T and CodRg scores)
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1. How many exons are predicted? _____ .
2. On which strand (+ or -) is the gene located? _____ .
3. Fill in the table:

Exon or Signal Number	Is it an Exon?	Exon or Signal Start Position	Exon or Signal End Position	Probability
1				
2				
3				
4				

Note that we can click on "here" to "view a PDF image of the predicted gene(s)" (assuming that Acrobat Reader is installed on the computers).

B) "Molecular Basis of β-Thalassemia in Morocco: Possible Origins of the Molecular Heterogeneity" by Imane Agouti, et al. was published in the "Genetic Testing" in 2008 (volume 12, number 4, pages 563 to 568).

Table 1 of the article gives the distribution of beta globin gene mutations and haplotypes that lead to β-thalassemia in Morocco. A closer look at the table reveals that the top eight mutations are responsible for around 76% of all beta thalassemias in Morocco.

Note that most of these mutations were already reported in 2003, in: "Spectrum of β–Thalassemia Mutations and HbF Levels in the Heterozygous Moroccan Population" by Lemsaddek et al., which was published in "American Journal of Hematology". See Table II on the next page of this Hands-On.

For each of the following mutations, locate the mutation on the beta globin sequence and explain what consequences on the protein it might have.

1) Codon 39 (C→T)

2) FSC-6 (-A)

3) IVS-I-110 (G>A) Hint: See the "Anatomy of an intron" on the next page.

TABLE 1. DISTRIBUTION OF β-GLOBIN GENE MUTATIONS AND HAPLOTYPES IN THE MOROCCAN POPULATION

Mutation	*Type*	*Number of chromosomes*	*Number of homo/hetero*	*Frequency (%)*	*Haplotypes*
Codon 39 (C → T)	β^0	42	16/10	26.58	I, II, Nd
FSC-8 (-AA)	β^0	22	7/8	13.91	IV, VI, VII
IVS-II-745 (C → G)	β^+	12	5/2	7.6	VII
−29 (A → G)	β^+	10	2/6	6.33	II, IX, 3 black, C
FSC-6 (-A)	β^0	9	2/5	5.7	III, IX
IVS-I-110 (G → A)	β^+	9	3/3	5.7	I, II
IVS-I-2 (T → C)	β^0	8	3/2	5.06	II, IX
IVS-I-1 (G → A)	β^0	8	2/4	5.06	V, IX
IVS-I-6 (T → C)	β^+	5	0/5	3.16	VI, VII
Codon 37 (G → A)	β^0	5	2/1	3.16	I
IVS-I-2 (T → G)	β^0	5	2/1	3.16	IX
IVS-II-1 (G → A)	β^0	4	0/4	2.5	III
−28 (A → C)	β^+	3	0/3	1.9	I
25pb Deletion	β^0	2	0/2	1.27	IX
FSC-5 (-CT)	β^0	2	1/0	1.27	III
IVS-II-726 (A → G)	?	2	1/0	1.27	IX
Codon 24 (T → A)	β^+	1	0/1	0.63	IX
−190 (G → A)	?	1	0/1	0.63	XI
HbS (codon 6 A → T)	β^S	4	0/4	2.5	Benin
Unidentified		4	0/4	2.5	–
Total		158	46/34	100.0	–

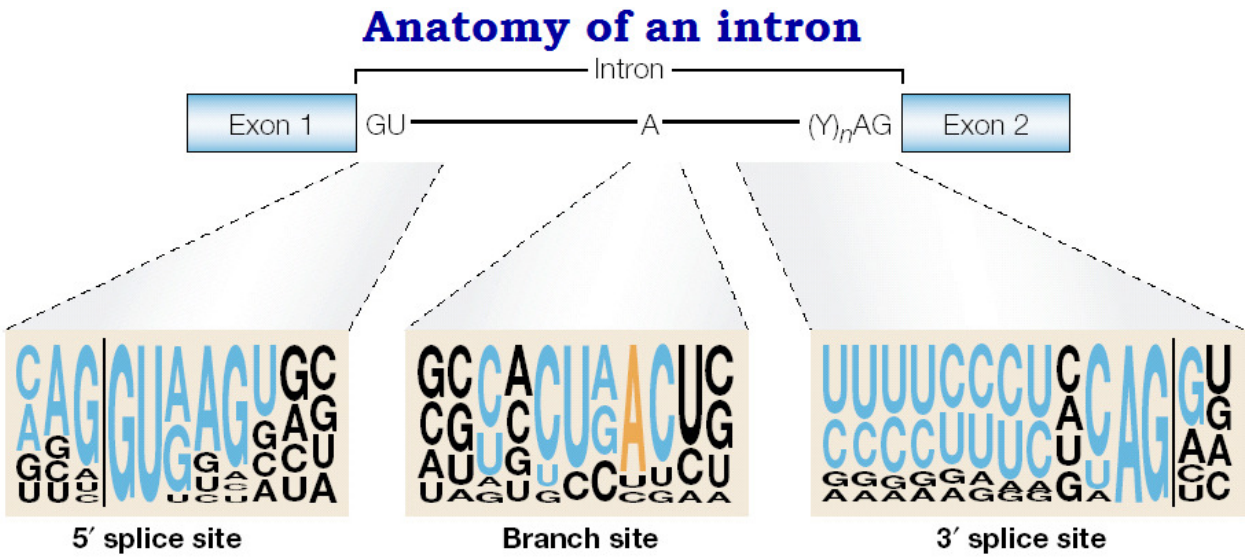

TABLE II. Frequency and Haplotype Distribution of β Globin Gene Mutations

Mutation	No. of chromosomes	Frequency	Haplotypes
$\beta^0$39 (C→T)	14	15.5	I, II
β^0FsCD8 (–AA)	14	15.5	IV, VI
β^+IVS1,nt6 (T→C)	13	14	VI, VII
β^0IVS1,nt1 (G→A)	12	13	V, IV, IX
β^0 fsCD6 (–A)	9	10	IX, III
β^+–29 (A→G)	6	7	3black, II, VI
β^0IVS1,nt2 (T→C)	3	3	II, IX
$\beta^0$37 (G→A)	2	2	VII, I
β^+IVS1,nt110 (G→A)	2	2	I
β^0IVS1,nt130 (G→A)	1	1	–
β^+–101 (C→T)	1	1	–
β^0IVS2,nt1 (G→A)	1	1	III
β^+–28 (A→G)	1	1	I
β^+IVS2,nt745 (C→G)	1	1	VII
β^++20 (C→T)	1	1	VII
β^+polyA (T→C)	2	2	1
$\beta^0$25bp del 3′IVS1	1	1	IX
Hb Newcastle	1	1	II
βS	3	3	Benin
nd[a]	3	3	3
Total	90	100	–