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HST.161 Molecular Biology and Genetics in Modern Medicine
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HST161 Problem Set 3 Answer Key

1.

a) Early onset bilateral retinoblastoma suggests an inherited form of retinoblastoma. Also, lack of family history of retinoblastoma suggests that George's case is due to a *de novo* germline mutation in the RB1 gene. There is a 50% chance that he will pass this faulty copy of RB1 to his children, and there is a 90% penetrance rate, given a mutated form of RB1. 0.5 * 0.9 leads to an approximately 45% chance that each child of George will develop retinoblastoma.

b) Southern blotting is suitable for detecting large deletions, inversions, or mutations in the restriction enzyme sites used to cut genomic DNA in preparation for blotting. PCR is suitable for detecting point mutations, insertions, and deletions.

One of the reasons that we can be confident that a mutation is present in RB1 is because bilateral, early-onset retinoblastoma, almost always is associated with a mutated, non-functional RB1.

Other types of changes which could lead to functional changes undetectable by Southern blotting or PCR include mutations in the RB1 promoter, mutations in the intron, or changes in the epigenetic state of RB1 (hypermethylation, etc.).

c) It is known that a mutation in RB1 is more likely to be inherited from the father (presumably because *de novo* mutations are more likely to occur during spermatogenesis than oogenesis). Thus, assuming that George has inherited a faulty RB1 from either his father or mother, it is more likely that George inherited the faulty RB1 from his father. We can then attribute much higher risk of retinoblastoma to a child who has inherited Elton's copy of RB1 as compared to a child who has inherited Shania's copy of RB1.

d) Elton's genotype is 1,2. He contributed allele 1 to Elton and allele 2 to his brothers. It is not possible to determine, based on these four SNPs, whether George has passed Elton's or Shania's RB1 gene to his child, because George is homozygous at each of the four SNPs.

e) George is the only one with marker 8, which he inherited from Elton. It seems likely that this marker is associated with the defective RB1 copy. If the fetus has the 8 marker, it increases his risk of retinoblastoma. Of course, because of the limited size of this family, we cannot be 100% sure about linkage between marker 8 and RB1.

f) While the four SNPs are uninformative of disease linkage, the VNTR 505 locus shows that Alanis has not inherited allele 8, which is likely linked to disease. Barring a recombination event, she should not have familial retinoblastoma and is at a risk no greater than the general population of developing the sporadic form of the disease.

g) The four SNPs are uninformative of disease linkage as above, but here the VNTR 505 locus shows that Sid has not inherited allele 5, suggesting that he does not possess a copy

of defective RB1. However, there may be slightly increased risk of developing retinoblastoma due to fetal exchange of cells in utero.

h) The markers would be expected to be the same as in other cells previously genotyped, as long as the point mutation (Glutamine->STOP) is not found within one of these markers. i.e. 1,2; 1,2; 2,2; 1,1; 4,8

i)A non-disjunction which is responsible for the second hit will eliminate the second good copy of RB1. Jewel would be left with: 2,-; 1,-; 2,-; 1,-; 8,-

2.

a) If Southern blots are performed with DNA that has been obtained directly from surgically-removed tumors, the lanes corresponding to tumor would contain the same sets of bands as lanes corresponding to unaffected tissues. This is because the DNA from tumor cells, which is homozygous or hemizygous for markers of interest (as in the case of non-disjunction, mitotic recombination, gene conversion or deletion), would be contaminated with DNA from unaffected cells, which are often heterozygous for these markers. As a result the Southern blot for tumor DNA would appear the same as a Southern blot for DNA from unaffected cells.

b) The critical marker for demonstrating mitotic crossover in the tumor is p7F12. In constitutional cells, the markers p7F12, p9D11 and p1E8 are all heterozygous. p7F12 is found between the RB1 locus and the centromere, while the latter two markers are both located between RB1 and the telomere. In tumor cells, which have two mutant copies of the Rb gene, p9D11 and p1E8 both become homozygous for alleles found on the mutant chromosome, while p7F12 remains heterozygous. This pattern can only be explained by a mitotic recombination event that occurs between the mutant and normal chromosome at a point between the p7F12 marker and the RB1 locus.

c) If the tumor arose by non-disjunction, the normal copy of chromosome 13 would be lost with only the copy containing the mutant RB1 gene present in tumor cells. Therefore, the p7F12 marker would be hemizygous for allele 1, which is found on the mutant chromosome. If non-disjunction is followed by reduplication of the mutant chromosome, we would expect the p7F12 marker to be homozygous for allele 1.