Required Watching: Chromosome 11 Flyover
http://www.dnalc.org/ddnalc/resources/chr11.html

Reading Assignments in Text

PCR, cDNA & Dideoxysequencing
- Chapter 10 Section 10.1: pgs 341-348 especially PCR and cDNA. Look carefully at Figure 10-1 (How to amplify a gene of interest)
- Chapter 10 Section 10.4 on Determining the Base Sequence of a DNA segment

DNA Fingerprinting & microsatellite markers
- Pgs 138 & 139 on Single nucleotide polymorphisms & Simple sequence length polymorphisms
- Also Figure 4-16: Phenotypic and Molecular markers mapped on human chromosome 1

Hardy-Weinberg and allele frequencies: Box 18-1 (Calculation of allele frequencies) on pg 645 & 644-647

Required Reading and Problem Assignments in 9th edition of text

Additional Problems

If a problem number is underlined, a detailed answer will be available.

Problem 1
A PCR reaction is set up with the following primers. (Note: primers are typically longer than those shown here.)
5’ GAAGTTCTC  3’
5’ TGTCTCTAA   3’

Draw a cartoon of the PCR product, which is about 200 base pairs long. Indicate the sequence of both strands at the ends of the product. Be sure to label the 5’ and 3’ ends. Assume that you are looking at a typical product late in the cycling process.

Problem 2
Choose the pair of primers that would amplify the sequence shown below. (Note: primers are typically longer than those shown here.)

5' GTCATTATCTCTAA.........................................CCTTGGGT TACGTACG 3'
3' CAGTAATAGAGT T..........................................GGAACCCAATGCATGC 5'

primer     # 1   5'  CAGTAAT  3'                 #2   5'  GTCATTA  3'
            #3   5'  GCATGCA  3'                 #4   5'  CGTACGT  3'
            #5   5'  TACGTAG  3'                 #6   5'  TAATGAC  3'
**Problem 3**  Here is a portion of the human β globin gene:

```
5’AGATTAGTCCAGGCAGAAACAGTTAGATGTCCCCAGTTAACCTCCTATTT
GACACCACTGATTACCACCCATGATAGTCACACTTTGGGTTGTAAGTGACCTTT
TATTATTTGTATTTTTTGAAGTGATTAAGGGCTCTTAGTTTTTTATCTCTTTGTTT
CCCAAAACCTAATAAGTAACACTAATGCACACAGACGACAATGTGATTATTTAT
TCTATTTT AGACATAATTATTAGCATGCATGACAAATAAATAGAAAAACAA
CAACAAATGAATGCATATATATGTATATGTATGT GTGTATATATACACATATA
TATATATATATTTTCTTTCTTT 3’
```

da. You are planning to set up a PCR reaction to amplify the 154 bp region between the `<`’s. The PCR product should include all of (but not extend beyond) the designated region. List the first five bases of each primer. Your primer sequences must read in the 5’ to 3’ direction.

Primer A:
Primer B:
b. The template DNA for your PCR is total human genomic DNA. What is the size of the human genome in base pairs?
c. What biochemical “limitation” of DNA polymerase ensures that, among the vast sea of sequences in genomic DNA, only the β globin sequences will be amplified? **one sentence**

**Problem 4**  A friend of yours is doing a series of PCR reactions and comes to you for advice. She has purchased, three sets of primers hoping that one set would amplify the template sequence shown below. She wasn’t exactly sure how to design the primers, so she tried three different strategies (as shown below for the 3 primer pairs). She is using DNA polymerase from *Thermus aquaticus*, which does not contain a 3’--→5’ exonuclease activity.

<table>
<thead>
<tr>
<th>Primer Pair #1</th>
<th>Primer Pair #2</th>
<th>Primer Pair #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ GTCCAGC 3’ &amp; 5’ CCTGAAC 3’</td>
<td>5’ GGACTTG 3’ &amp; 5’ GCTGGAC 3’</td>
<td>5’ GTCCAGG 3’ &amp; 5’ CAAGTCC 3’</td>
</tr>
</tbody>
</table>

Template  5’ ... GTCCAGCTAGGG............ATTCCGGACTTG........3’
3’.....CAGGTCTGATCTCC.............TAAGCCTGAAC........5’

a. None of the three primer pairs produced any products. Carefully examine each primer pair and explain why it didn’t work. Be sure to indicate whether both of the primers are at fault or if just one primer is the problem.
b. Your friend doesn’t want to buy new primers. She asks you whether she can salvage this experiment. What do you tell her to do?
c. For reasons known only to her, she ignores your advice and decides to redo the PCR reactions with the same primer pairs and use *E. coli* DNA polymerase instead. At the annealing step in each cycle she adds a dollop of fresh polymerase. She gets product with one set of primers. Which primer pair gave her a product and why?
d. Why did she add fresh *E. coli* DNA polymerase at each cycle?
Problem 5  Males exhibiting FMPP (familial precocious puberty) generally show signs of puberty by age 4. The gene that is mutated in this syndrome codes for the LH (lutenizing hormone) receptor protein. The wild-type receptor protein, when bound to lutenizing hormone, transmits a signal to the cell to increase testosterone production in gonadal cells. The mutant receptor protein sends this signal independent of the presence of lutenizing hormone. This is a very rare disease state.  SEE data on the next page.

For each term indicate whether it
A (applies) to the FMPP mutation  C is contradicted by the information given
N (not enough information)

<table>
<thead>
<tr>
<th>Transition</th>
<th>Autosomal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense</td>
<td>X-linked</td>
</tr>
<tr>
<td>DNA polymorphism</td>
<td>Dominant</td>
</tr>
<tr>
<td>Y linked</td>
<td>Incomplete penetrance</td>
</tr>
</tbody>
</table>

The bottom panel of the figure below shows PCR products generated using primers 1 and 2 and genomic DNA from various members of the pedigree. The PCR products were treated with Msp I before agarose gel electrophoresis

The molecular analysis for each person is shown directly below the pedigree symbol. For example, the first lane of the agarose gel gives the results of this molecular test for III 1, the second lane for III 2, etc.

The 53 bp piece was run off the bottom of the gel.

40 additional controls were examined with the same results as N1-N3.
N= normal
Problem 6  Cystic Fibrosis (CF) is one of the most common autosomal recessive diseases in the Caucasian population. CF results from mutations in the CFTR gene, which codes for a transmembrane chloride transporter. Genetic tests to determine individuals at risk are currently available. Here is a table lifted from the web site of the Michigan State University (MSU) DNA Diagnostic Program. using the MSU tests.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Carrier Risk</th>
<th>MSU Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jewish</td>
<td>1/24</td>
<td>94%</td>
</tr>
<tr>
<td>Non-Hispanic Caucasian</td>
<td>1/25</td>
<td>90%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1/46</td>
<td>74%</td>
</tr>
<tr>
<td>African American</td>
<td>1/65</td>
<td>66%</td>
</tr>
<tr>
<td>Asian American</td>
<td>1/94</td>
<td>55%</td>
</tr>
</tbody>
</table>

Methods:
Direct DNA analysis of the CFTR gene is performed using the Invader DNA assay.

Specimen Requirements:
5.7 ml of whole blood in an EDTA (purple top) or citrate tube.

http://www.phd.msu.edu/Divisions/HumanGenetics/TestMenu/tabid/91/ctl/LabDetails/mid/514/labTestGuid/fbcf9a8e-48fb-4f13-b89f-470167e11831/Default.aspx

a. Why is the detection rate not 100% for Caucasians or any other group? (NOTE: assume no mistakes are made in the genotyping). One or two sentence explanation.
b. The same “genotype detection tests” are administered to all individuals independent of ethnicity. Why is the detection rate different for different ethnic groups? One sentence.
Problem 7

There are many different slick molecular assays that can be used to directly test the genotype of a particular individual. One PCR-based approach is called ARMS, for amplification refractory mutation system. The most common mutation in the CFTR gene (mutated in cystic fibrosis) is the ΔF508 mutation shown below. In American populations of European descent, the ΔF508 mutation represents about 70% of the mutant copies of the gene. In an ARMS assay for this mutation, each template DNA is tested with two different sets of primers:
Primer combination a: F1 & R (with control primer pair that amplifies a non-polymorphic site elsewhere in the genome)
Primer combination b: F2 & R (with control primer pair that amplifies a non-polymorphic site elsewhere in the genome)

a. This test must be performed with a DNA polymerase that does not have 3’-5’ exonuclease function. Why is this important? Two sentences.
b. Controls are important for all methods of genotype detection. But, why is a positive control especially critical for an ARMS test?
c. Indicate the genotypes of each of the three individuals tested. No explanation necessary. Define allele symbols:
d. Cystic fibrosis is a very common disease in American populations of European descent. You want to use this ARMS test to set up a genetic screen to identify couples at risk for CF children. What fraction of at risk couples (both heterozygotes for a CF mutation) will be identified with this ARMS test? Show your work.
e. How would you reduce the number of false negatives (failure to identify at risk couples). Two sentences max.
**Problem 8**

**a.** Huntington disease (HD) is caused by a variable expressed but fully penetrant autosomal dominant mutation that causes late onset (post-reproductive) neurodegeneration. The HD gene was mapped to chromosome 4 in 1983 and cloned in 1993. The mutations that cause HD involve an expansion of a triplet repeat located in the coding region of the gene. Normal alleles of this gene have 27-35 CAG repeats. Alleles with >40 repeats confer a clear HD phenotype. Alleles with 36-39 repeats are in a twilight zone, with some individuals healthy and others not.

What molecular technique would you use to set up a direct detection of genotype for the HD gene? ______________________

Briefly explain/illustrate how the HD genotype would be assessed using this technique by creating a hypothetical set of data for
- two individuals who are homozygous for a normal allele
- two individuals who are heterozygous for a mutant allele
  - *Label your figure clearly*

**b.** As the number of repeated triplets increases, the age of onset in the patient decreases. Furthermore, because the unstable trinucleotide repeat can lengthen when passed from parent to child, the age of onset can decrease from one generation to the next.

Draw a diagram to show how an increase of *one repeat unit* can occur as a spontaneous mistake during DNA replication. Be sure to label the 5’ and 3’ ends of all DNA strands. Be sure to label the parental and daughter DNA strands.

**HINT:** *the initial mistake can occur in the replication of either parental strand so follow the replication of just one of the parentals*

**PARENTAL DNA**

```
------------CAGCAGCAGCAG--------------------------------
------------GTCGTCGTCGTC---------------------------------
```

### Solution

**Problem 8**

**a.** Huntington disease (HD) is caused by a variable expressed but fully penetrant autosomal dominant mutation that causes late onset (post-reproductive) neurodegeneration. The HD gene was mapped to chromosome 4 in 1983 and cloned in 1993. The mutations that cause HD involve an expansion of a triplet repeat located in the coding region of the gene. Normal alleles of this gene have 27-35 CAG repeats. Alleles with >40 repeats confer a clear HD phenotype. Alleles with 36-39 repeats are in a twilight zone, with some individuals healthy and others not.

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- two individuals who are homozygous for a normal allele
- two individuals who are heterozygous for a mutant allele
  - *Label your figure clearly*

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Draw a diagram to show how an increase of *one repeat unit* can occur as a spontaneous mistake during DNA replication. Be sure to label the 5’ and 3’ ends of all DNA strands. Be sure to label the parental and daughter DNA strands.

**HINT:** *the initial mistake can occur in the replication of either parental strand so follow the replication of just one of the parentals*

**PARENTAL DNA**

```
------------CAGCAGCAGCAG--------------------------------
------------GTCGTCGTCGTC---------------------------------
```
Problem 9  Mental retardation (MR) affects 2-3% of the human population. Loss-of-function mutations in the AGTR2 gene affect brain development and cognitive function and can result in profound mental retardation.

Here is part of the wild-type sequence of an exon of this gene.

5’ …….TGG CCT ATT TTT TTT ATC ACC T…… 3’

trp pro ile phe phe ile thr

Here is the sequence of a mutant allele:

5’ …….T G G C C A T T T T T A T C A C C T…… 3’

Part a: Examine a genetic code table. Using proper “mutation jargon” describe the sequence data. [Three or four sentences using proper terminology at the DNA and protein level.] Be sure to distinguish between disease-causing mutations and neutral polymorphisms.

For members of the pedigree shown above, the region of the AGTR2 gene carrying the sequence shown on the previous page was amplified by PCR and the DNA products analyzed by gel electrophoresis. [This assay did not involve digestion by restriction enzymes]. On the left and right side of the gel, the arrows point to a 115 base pair band. The lower band is 114 base pairs. RM and RF = random normal male and female respectively. NOTE: do not draw any conclusions bases on band intensities.
**Part b**  This mutation is 100% penetrant. Given this information, is the mutant allele dominant or recessive?  [No explanation necessary.]

**Part c**  The pedigree data alone are consistent with either autosomal or X-linked inheritance.
- Does the genotyping data resolve this ambiguity? Assume the mates of all generation II females are normal and exhibit the RM pattern.
  - Circle **YES** or **NO**
- Defend your answer with one specific example.

**Part d**  What is the probability that III4 is heterozygous for this mutant allele?
No explanation required.

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**Problem 10**  The following info was taken from a web site on familial hypercholesterolemia. A fellow biology major wants you to explain what the jargon means (ALL OF IT) and how a geneticist would use the info.

Thoroughly define and explain all of this info to your friend. You may want provide additional labels on the figure, but be sure to summarize your response in a coherent paragraph. Be sure to decode as much as possible

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### Exon10 (G1413A) RFLP (MspI)

**First described by**: Warnich et al. (1992) Hum. Genet. 89:362  
**Method**:  
- primer Forward: 5'-CTCCTCCTGCCTCAGCACC-3'  
- primer Backward: 5'-ACGCACCCATGAACAGGATC-3'  
- recommended annealing temperature: 55°C  
- product size:  
**Variant**:  
- M1 -> A  
- M2 -> G  

**Frequency and population**:  
12 individuals  
- M1: 0.5  
- M2: 0.5  
**Heterozygosity**: not determined  
**Note**: This silent change is localised at codon 450 (Arg)
Problem 11  Mutations in the X-linked gene AGTR2 can result in mental retardation (MR). You are a reporter for the New York Times and are responsible for writing an article about mutations in the AGTR2 gene. Examine the table on the next page. Your charge is analyze the data presented in this table with respect to possibilities for genetic screening and prenatal diagnosis.

Address all points listed below.

- Be sure to use the proper terminology to briefly summarize the spectrum of mutations at the DNA and protein level (assume NYT readers know genetical terms.)
- Your readers will be very interested to hear about the potential for genetic tests. Examine the last column in the table (and the bottom rows) and describe the relevance of this information to the direct detection of sequence variation in the AGTR2 gene.
- Consider the usefulness of genotype analysis in a general genetic screen (where members of a population are screened at random to determine if they or their progeny are at risk for a specific genetic disease). Be sure to discuss how reliable such a screen would be and whether you would need additional information.
- Consider the usefulness of genotype analysis for prenatal diagnosis for a member of one of the families examined here.
- Ignore Patient 1 and the column labelled Domain.

NOTE: G>A means that an A is substituted for G
## Supplemental Table 1. Sequence alterations detected in the AGTR2 gene

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Domain</th>
<th>Age</th>
<th>Phenotype</th>
<th>Restriction change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Patient DF</td>
<td>t(X;7)(q24;q22.3)</td>
<td>None</td>
<td>None</td>
<td>37 yr</td>
<td>Moderate MR (IQ44), borderline diabetes, kyphoscoliosis</td>
<td>None</td>
</tr>
<tr>
<td>2. CMS0890&lt;sup&gt;1&lt;/sup&gt;</td>
<td>395/402 Del T&lt;sup&gt;2&lt;/sup&gt;</td>
<td>FS at Phe&lt;sup&gt;133&lt;/sup&gt;, Term after 4 res.</td>
<td>TMD3</td>
<td>8 yr</td>
<td>Moderate MR, seizures, microcephaly, optic atrophy</td>
<td>None</td>
</tr>
<tr>
<td>CMS4481&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>6 yr</td>
<td>Moderate MR, seizures, optic atrophy, ACC</td>
<td></td>
</tr>
<tr>
<td>CMS5551</td>
<td>395/402 Del T</td>
<td>FS at Phe&lt;sup&gt;133&lt;/sup&gt;, Term. after 4 res.</td>
<td>TMD3</td>
<td>25 yr</td>
<td>Profound MR (IQ16), autistic behaviors</td>
<td></td>
</tr>
<tr>
<td>CMS3910&lt;sup&gt;3&lt;/sup&gt;</td>
<td>62 G&gt;T&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Gly21Val</td>
<td>ECD</td>
<td>50 yr</td>
<td>Profound MR (IQ21)</td>
<td>Drall (+)</td>
</tr>
<tr>
<td>CMS5103&lt;sup&gt;3&lt;/sup&gt;</td>
<td>62 G&gt;T</td>
<td>Gly21Val</td>
<td>ECD</td>
<td>63 yr</td>
<td>Profound MR (IQ18)</td>
<td></td>
</tr>
<tr>
<td>CMS5044</td>
<td>62 G&gt;T</td>
<td>Gly21Val</td>
<td>ECD</td>
<td>32 yr</td>
<td>Severe MR (IQ32), seizures, limited speech, autistic behaviors</td>
<td></td>
</tr>
<tr>
<td>4. CMS5778</td>
<td>971 G&gt;A&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Arg324Gln</td>
<td>ICD</td>
<td>32 yr</td>
<td>Moderate MR (IQ38), seizures, hypertension, diabetes</td>
<td>Hpall (-)</td>
</tr>
<tr>
<td>CMS4940</td>
<td>971 G&gt;A</td>
<td>Arg324Gln</td>
<td>ICD</td>
<td>3 yr</td>
<td>Developmental delay</td>
<td></td>
</tr>
<tr>
<td>CMS5124</td>
<td>971 G&gt;A</td>
<td>Arg324Gln</td>
<td>ICD</td>
<td>25 mo. Developmental delay, no recognizable speech</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. CMS4954</td>
<td>1009 A&gt;G&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Ileu337Val</td>
<td>ICD</td>
<td>73 yr</td>
<td>Profound MR (IQ8), seizure (once), limited speech, mild kyphosis</td>
<td>MaelII (+), Tsp5091(-)</td>
</tr>
</tbody>
</table>

### Polymorphic variants

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Domain</th>
<th>Age</th>
<th>Phenotype</th>
<th>Restriction change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>603 T/C</td>
<td>Pro201Pro</td>
<td>ECL2</td>
<td>-</td>
<td>-</td>
<td>Ddel (-)</td>
</tr>
<tr>
<td>2.</td>
<td>743 G/A</td>
<td>Arg248Lys</td>
<td>ICL3</td>
<td>-</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>3.</td>
<td>498 T/C</td>
<td>Leu166Leu</td>
<td>TMD4</td>
<td>-</td>
<td>-</td>
<td>Mnl1(+)</td>
</tr>
<tr>
<td>4.</td>
<td>1011 T/A</td>
<td>Ileu337Ileu</td>
<td>ICD</td>
<td>-</td>
<td>-</td>
<td>Tsp5091(-)</td>
</tr>
</tbody>
</table>

Restriction enzymes that distinguish alleles are as indicated, with (+) designating the creation and (-) the abolition of a restriction site caused by alterations. TMD, transmembrane domain; ECD, extracellular domain; ECL, extracellular loop; ICD, intracellular domain; ICL, intracellular loop; FS, frame-shift; ACC, anogenesis of.
Problem 12 Calculating a DNA profile frequency.

A. Fill in the table.

For the ABC locus, the frequency of allele #2 is 0.1 and the frequency of allele #10 is 0.005. For the XYZ locus, the frequency for allele #3 is 0.08.

<table>
<thead>
<tr>
<th>Micro/minisatellite Locus</th>
<th>Genotype</th>
<th>Frequency of genotype (HW)</th>
<th>Combined frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>allele 2 / allele 10 (het for 2 &amp;10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XYZ</td>
<td>Homozygous for allele #3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Assume the calculation in A is extended until the combined frequency is \(1 \times 10^{-8}\). Very briefly indicate how this number would be used by a prosecuting attorney in an address to a jury.

C. Population data on micro/minisatellite loci is compiled and analyzed by the FBI. Obviously, the validity of the calculation shown above is dependent on the accuracy of the population database. For a number of years, the databases were small and somewhat biased (not representing all ethnic or racial groups) and the allele frequencies determined from them were suspect. For these reasons, the Ceiling Principle was adopted. The Ceiling Principle says that the allele frequency used for any given micro/minisatellite allele must be either:

1. the highest allele frequency found in any one of the three major population subgroups: Caucasian, Hispanic and Black (don’t know why Asians were not included here)
2. or 10%, if 10% is larger than the frequency in #1.

Does the Ceiling principle bias the calculation for or against a defendant? Briefly explain your answer. Your answer should address the ceiling principle and not what you know about the U.S. legal system.

Problem 13 Mini and microsatellite alleles are useful markers for DNA fingerprinting for all of the following reasons BUT,

a. They show codominance
b. They are highly polymorphic markers
c. They can be analyzed by simple PCR tests
d. Examination of a single locus can be used to establish the identity of a DNA sample, i.e. that two DNA samples are from the same individual
e. They show Mendelian patterns of inheritance
Problem 14 [From a letter to the editor of Nature 367: 696-693 1994]

Although, the capture and trading of great apes has been banned in 112 countries since 1973, it is estimated that about 1,000 chimpanzees are removed annually from Africa and smuggled into Europe, the U.S. and Japan. This illegal trade is often disguised by private (such as zoo or circus) owners by simulating births in captivity. Until recently, genetic identity tests to uncover these illegal activities have not been used because of the lack of availability of highly polymorphic markers and the difficulties of obtaining chimp blood samples. Recently, a study was reported in which DNA samples were extracted from freshly plucked chimp hair roots and used as templates for the Polymerase Chain Reaction. The primers used in these studies flank highly polymorphic sites in human DNA resulting from variable numbers of tandem nucleotide repeats. Several suspect chimp offspring and their supposed parents were tested to determine if the offspring were "legitimate" or were the "product of the illegal trading" and not the offspring of the putative parents. A sample of the data is shown below.

Examine this data carefully and choose the best conclusion:

a. None of the offspring are legitimate.
b. Offsprings B and C are not the products of these parents and were probably purchased on the illegal market. The data are consistent with offspring A being legitimate.
c. Offspring A and B are the products of the parents shown, but C is not and was therefore probably purchased on the illegal market.
d. Not enough data to draw any conclusions. Additional polymorphic sites should be examined.
e. No conclusion can be drawn since "human" primers were used.

Lane 1: father chimp
Lane 2: mother chimp
Lanes 3-5: putative offspring A, B, C.

<table>
<thead>
<tr>
<th>bp</th>
<th>Lane:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>148</td>
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<td>140</td>
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<td>120</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Aliquots of 20 ng of genomic DNA, extracted from hair samples, were used as templates in PCR reactions using MBP1 primer pairs. Amplified products were run on a polyacrylamide gel and silver-stained. The primers flank a region of variable numbers of tandem tetranucleotide repeats. bp = base pairs.
Problem 15  The SCN9A gene codes for a subunit of a sodium ion channel found in neurons that transmit pain signals. Answer this question using the information provided on the next two pages.

For each statement, indicate

True, False or N (not enough information to decide).

• Consider a statement false if any part of it is false. If there are two statements, only indicate True if both statements are True.
• 1 pt if no explanation required. 3 pts if explanation required.
• If you choose N and an explanation is required be sure to indicate what additional info you need.

1. The normal function of this sodium ion channel is to dampen (decrease) the sensation of pain.
   Circle: T F N  One sentence explanation/defense of your answer

2. In Panel 1, both transitions and transversions are seen in this disease state.
   Circle: T F N

3. Examine Panel 2. From the pattern of bands seen here in the wildtype individuals, it is likely that the researchers used two separate pairs of PCR primers to analyze the genotype of each individual
   Circle: T F N

4. Examine Panel 2. The mutation in this family results in a restriction enzyme recognition site which is not found in the sequence of the wild-type allele.
   Circle: T F N  One sentence explanation/defense of your answer

5. Examine the pedigrees only in Panel 1, 2 & 3. Based on the pedigree data only, the affected male shown in Panel 3, is likely to exhibit no pain phenotype.
   Circle: T F

6. Examine all of the data in Panel 3. The affected male is likely to show the dominant, gain-of-function phenotype. Even though neither parent exhibits the trait, we cannot conclude that the mutation is incompletely penetrant.
   Circle: T F N  One sentence explanation/defense of your answer
Panel 1  *NO PAIN: complete inability to sense pain*
Each of the three families shown below has a different mutation in the SCN9A gene.
Family 1:  G2691A resulting in Trp897STOP
Family 2: Deletion of a single T in the coding region of the gene
Family 3:  C1376G resulting in Ser459STOP
Panel 2: **Too much pain:** Intermittent burning pain with redness and heat in the extremities

T → A in the SCN9A gene resulting in a leucine→ histidine missense mutation.

For each individual, the results of PCR amplification and restriction digestion are shown under the appropriate symbol in the pedigree.

**Panel 3** No info on phenotype of affected individual

For each individual, the results of PCR amplification and restriction digestion are shown under the appropriate symbol in the pedigree. The restriction enzyme used recognizes the sequence ACTGG.
Problem 16
You are working in a forensics lab on a paternity case. You have done PCR reactions to test each individual involved, mother child and two potential fathers. You have tested 4 diagnostic loci A B C and D.

<table>
<thead>
<tr>
<th>Mother</th>
<th>Child</th>
<th>Man 1</th>
<th>Man 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
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<td>C</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
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</tr>
</tbody>
</table>

a. Which man do you suggest should be paying child support?
b. Which loci are informative for your decision?

Problem 17 From W13 quiz 4
Examine the information below. The pedigree below represents a family segregating a form of blindness called Leber’s congenital amaurosis. The mutation causing this syndrome is a T → G transversion.

Normal allele: 5’ TAGCATCA 3’
Mutant allele: 5’ TAGCAGCA 3’

The data below the pedigree represent PCR products treated with the restriction enzyme Fnu4 and analyzed by agarose gel electrophoresis.
a. Examine each panel carefully. Which set of data (Panel A or Panel B) are consistent with the pedigree and with the information given above? Circle: Panel A OR Panel B

b. Defend your answer in a couple of explicit sentences.

c. What is the probability that individual IV2 is heterozygous for the mutant allele? [NOTE: you do not have to get part a correct in order to answer this question correctly.]
No explanation required.