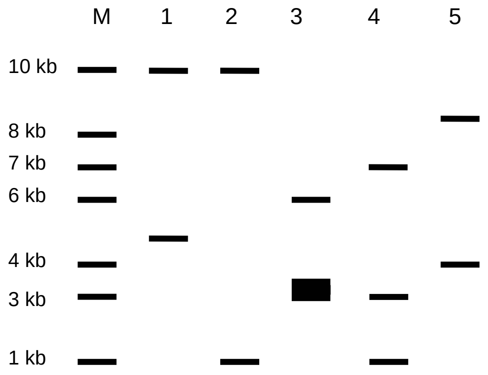
Dr. Chevrette's Questions (36 points)

16. (3 points) Which of the following statements is ***incorrect***.

a) The length of VNTRs varies between individuals and even in the same individual.
b) Because highly repetitive DNA sequences are present at more than 100,000 copies /genome, they will be seen easily on an ethidium bromide stained agarose gel upon digestion of human DNA.
c) Cesium chloride gradients can be used to isolate satellite DNA from eukaryotes.
d) The C-value paradox defining the lack of correlation between the size of the genome and the biological complexity of an organism can be explained largely by the presence of repetitive DNA.
e) Transposable elements, although present in the human genome, are rarely inserted into the exons of the genes.

17. (6 points) The following figure represents an ethidium bromide stained gel containing molecular weight markers ("M"), and fragments of DNA from 5 samples (1 to 5) that have been digested with restriction enzymes, and then separated according to their size.



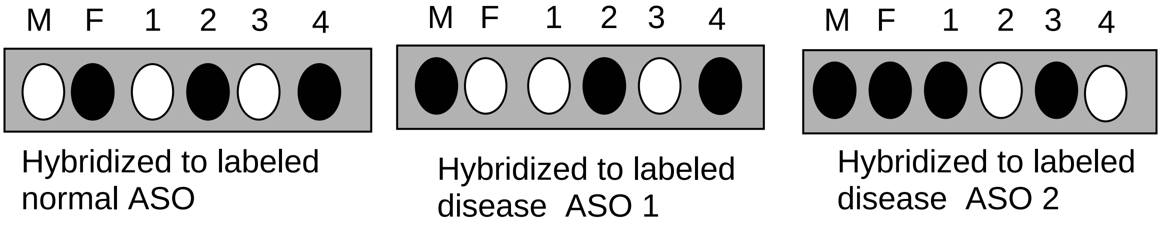
Based on this gel, which one of the following statements are ***true***.

a) This gel could represent the complete digestion of the same plasmid with 5 different restriction enzymes.
b) The 1 kb fragment seen in samples 2 and 4 will always be identical and will have the same DNA sequence.
c) If one was to use the 10 kb fragment of sample 2 as a probe in a Southern blot obtained from this gel, there will be a hybridization signal only in samples 1 and 2.
d) If one was to use the 7 kb fragment of sample 4 as a probe in a Southern blot obtained from this gel, there will always be a hybridization signal in all samples.
e) If two of the 5 samples are the same, sample 4 can be obtained by digesting sample 2 with an additional restriction enzyme.

18. (3 points) Which of the following statements is ***incorrect***.

a) Slipped mispairing during DNA replication is the main cause of microsatellite polymorphism.
b) A rapid analysis of RFLPs among individuals within a population can be performed by combining PCR amplification, restriction enzyme digestion and gel electrophoresis.
c) Combining PCR and gel electrophoresis is the method of choice to detect microsatellite variation between individuals.
d) PCR amplification of the VNTR sequence using primers complementary to the VNTR sequence has replaced the need of using Southern blot to detect the VNTRs.
e) In DNA fingerprinting, the restriction enzyme will digest the DNA on each side of a VNTR sequence.

19. (3 points) DNA obtained from different members of the same family (mother (M), father (F) and four children (1, 2, 3, and 4)) was tested for a disease using ASOs. Two different dominant disease alleles, identified by two different ASOs (ASO 1 and ASO 2), will cause the disease. The results of the analysis are illustrated on the following figure where hybridization with the ASOs is indicated by a black circle, and no hybridization is represented by a white circle.



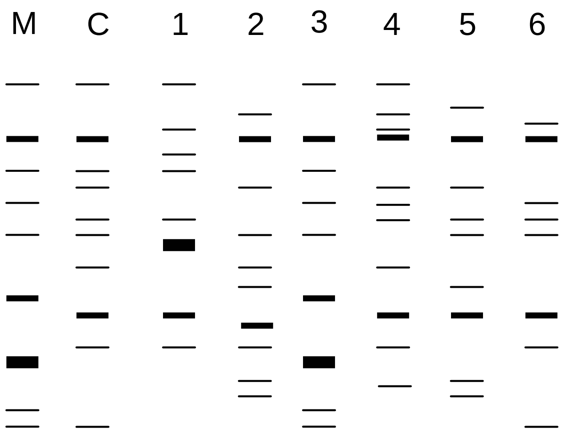
From these results, you can conclude:

a) Only the mother, children 1 and 3 will have the disease.
b) No member of this family will have the disease.
c) Only the mother, children 2 and 4 will never have the disease.
d) Every members of this family will eventually have the disease.
e) Only the parents, children 1 and 3 will have the disease.

20. (3 points) Which of the following statements is ***true***.

a) The presence of an origin of replication (ori) in a plasmid will allow its replication as an extrachromosomal entity in human cells.
b) In a plasmid containing an ampicillin resistance gene, cloning a DNA fragment into the sequence of the β-globin gene and plating the transformed bacteria on media containing ampicillin and X-Gal will allow selection for bacteria that have been transformed with plasmids that contain a foreign piece of DNA.
c) A cosmid vector is a plasmid containing the cos sites of a bacterial artificial chromosome (BAC) and can, upon ligation with DNA of appropriate size, be packaged into virus particles.
d) Genomic libraries can be obtained by generating genomic DNA fragments without the use of restriction endonucleases.
e) The number of clones to be screened to identify a particular gene in a genomic library depends only on the size of the genomic DNA that can be inserted in the cloning vector.

21. (3 points) The following figure represents a VNTR analysis of eight individuals including the mother (M), her child (C) and six other individuals (1, 2, 3, 4, 5 and 6). Which one of the following statements best describes these results:



a) Individual 3 is the real biological father of the child (C).
b) Based on this analysis, it is impossible to determine who (out of the 6 individuals labeled 1 to 6) is the real biological father of the child (C).
c) Individual 4 is the real biological father of the child (C).
d) Although the real father of child (C) can not be determined with certainty, it could be either individual 3 or 4.
e) None of these individuals could be the real biological father of this child (C).

22. (3 points) A cDNA has been cloned in the unique *Eco*RI site of the multiple cloning sites of a pUC24 plasmid (plasmid length of 2,4 kb). This multiple cloning site contains unique restriction sites for *Eco*RI, *Sal*I, *Mst*II, *Hind*III, and *Apa*I. Note that pUC24 itself has no restriction site for *Pst*I. After transformation of bacteria, plasmid DNA (containing the cDNA insert) has been digested with few restriction enzymes, and the size of the fragments resulting from an ethidium bromide stained agarose gel is illustrated below. Please note that the resolution of the gel will not allow you to differentiate fragments of the same length.

EcoRI	**PstI**	**HindIII**	**HindIII & PstI**	**HindIII & PstI & EcoRI**
	6.4 kb	6.4 kb		
			4.4 kb	
3.0 kb				
2.4 kb				2.4 kb
			2.0 kb	2.0 kb
1.O kb				1.O kb

Based on these results, digesting the pUC24 plasmid that contains the cDNA insert with *Pst*I and *Eco*RI will allow you to see the following fragments on an ethidium bromide-stained agarose gel:

a) One single fragment of 6.4 kb
b) Fragments of 2.4, 2.0 and 1.0 kb
c) Fragments of 3.0, 2.4 and 1.0 kb
d) Fragments of 6.4, 3.0, 2.4 and 1.0 kb
e) Fragments of 3.4 and 3.0 kb

23. (3 points) For subcloning purposes, and from the gel above, your supervisor asks you to isolate all fragments derived only from the cDNA that are flanked (ended) by both *EcoRI* and *Pst*I sites (thus the cDNA fragment(s) of interest will have a *Pst*I site at one end and a *EcoRI* site at the other end). If the fragments are present in more than one sample, you need only to isolate it once. If the fragment is not pure, you still need to isolate it. Thus to obtain such fragment(s), you will cut out of the gel:

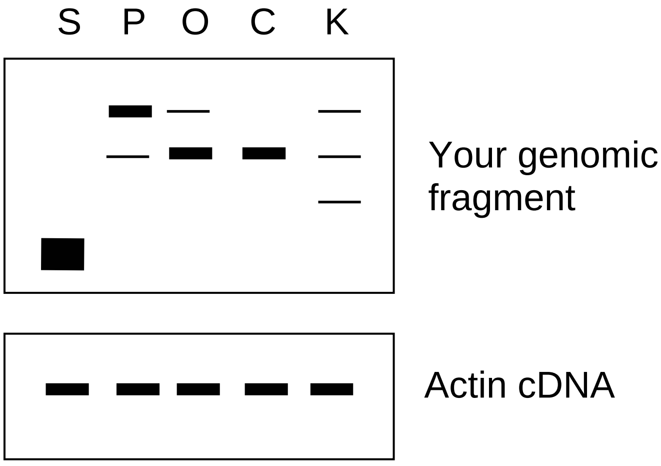
a) The three fragments of 3.0, 2.4 and 1.0 kb
b) The fragments of 2.0 and 1.0 kb from the triple digestions (*HindIII*, *PstI* and *EcoRI*).
c) The two fragments of 4.4 and 2.0 kb
d) Only the fragment of 1.0 kb from the triple digestions (*HindIII*, *PstI* and *EcoRI*).
e) There is no fragment that will have a *Pst*I site at one end and an *EcoRI* site at the other end.

24. (3 points) After finding a new species of squirrel in the deep forest of Lac-St-Jean, scientists decided to analyze its karyotype. Such analysis performed on the white blood cells of a female revealed 16 chromosomes (you can assume that the mode of sex determination in these squirrels is equivalent to that in humans). Based on this data, the minimum number of contigs that a male from this animal species will have upon full sequence analysis of its genome is:

a) 16
b) 32
c) 17
d) 9
e) 8

25. (6 points)You have just cloned a DNA fragment that you have isolated from the genomic library generated from the DNA of this squirrel. You know that this DNA fragment contains at least three large exons, so you perform a Northern blot analysis, using as a probe either your genomic fragment or an actin cDNA. You obtain the illustrated results from the RNA isolated from five different tissues :

S: spleen
P: prostate
O: ovary
C: colon
K: kidney



Please read the following possible conclusions from these results:
1) The three bands seen in the RNA from the kidney (K) correspond to the three exons present in your genomic fragment.
2) The lower band seen in the spleen (S) is indicative of degraded (and thus shorter) RNA.
3) The restriction enzymes that you may have used to generate the analyzed samples have only partially digested your samples, thus explaining the sizes of the different fragments.
4) The RNA from your gene is alternatively spliced in the different tissues analyzed.
5) Although not expressed at high level in kidney (K), this tissue contains all alternative spliced RNA forms of this gene.

Based on the previous statements, please select the correct reponse:
a) Only statement 3) is false, all others are true.
b) Statements 2), 4) and 5) are true, while statements 1) and 3) are false.
c) All statements are true.
d) Statements 1), 2) and 4) are true, while statements 3) and 5) are false.
e) Only statement 4) is true, all others are false.

Answers:16 b, 17 e, 18 d, 19 d, 20 d, 21 c, 22 b, 23 b, 24 d, 25 e.