1. a) What is the basis for "blue/white" screening using pUC and many other cloning vectors?
   
   Expression of lacZ in the plasmid in a complementing host gives blue colonies on X-Gal medium; disruption of the lac Z by insertion of foreign DNA in the MCS (polylinker) gives white colonies.

   b) The blue/white system actually takes advantage of "complementation". Explain how and why this is the case.

   Only a part of the lac Z is in the plasmid; the remainder is in the host. Only if both parts are made, will functional beta-galactosidase be made.

2. Rank the following according to the size of insert they can carry (1 = smallest, 4 largest)

   2  Lambda gt11  1  pUC18  4  BAC  3  cosmid

3. The amino terminus of a highly purified protein has the amino acid sequence:
   
   arg•leu•his•pro•asp•met•trp•ser----

   a) Suggest a good 15 base primer that could be used to generate a nearly full-length cDNA clone for the gene. Indicate mixed bases where needed.

   skip arg and leu since each has 6 codons

   CAY-CCX-GAR-AUG-UGG  (Y = pyrimidine, R = purine X = any base)

   b) Assume mRNA from tissue induced to express this gene is available. Outline steps you could take (include enzymes used) to create a cDNA clone in a plasmid vector.
   (this is one of many possible approaches)

   1) reverse transcriptase using a oligo-dT as a primer to make the "first strand" DNA copy of the message starting from the poly a tail

   2) PCR amplify with TAQ polymerase using the primer from part A and oligo-dT
3) Verify correct size band by gel electrophoresis and clone into pre-T-tailed vector using TOPO-cloning (topoisomerase) or TA cloning (DNA ligase).

If a full length clone is needed, bases could be added to the second strand primer to add the arg and leu.

4. What is a nucleosome? Tell what it is made of.

A ball of histones (2 copies each of H2A, H2B, H3 and H4) wrapped by about two turns (180 bp) of DNA with one H1 histone as a linker.

5. Define and give examples of the following classes of DNA:

   a) moderately repeated sequences:

      present in around a thousand copies - rDNA

   b) highly repeated sequences

      100,000 to millions of copies; LINES, SINES ALU elements

6. Shown below are two Cot curves. Label the curves to show which came from humans and which came from E. coli. Also label the classes of DNA as defined by the curves.
7. What does the acronym FISH stand for? **Fluorescent in situ hybridization**

8. Show metaphase for a double heterozygote (Aa, Bb) in an organism where 2n = 4, and the genes are on different chromosomes. Include centrioles and spindles.

![Mitosis, Meiosis I, Meiosis II diagrams]

9. Why is endosperm tissue always triploid?

   *it results from the union of one sperm nucleus from the pollen with 2 polar nuclei in the egg*

10. Given the pigment pathway from hamsters:

    ![Pathway diagram from white to black](white E1 tan E2 black)

    A true breeding white female was crossed to a true breeding tan male. All the progeny were black.

    a) Give a legend that explains coat color inheritance involving these genes.

    b) Give the genotypes of the following individuals:

    **Female parent** ttBB **Male parent** TTbb **F1** Tt, Bb

    c) Predict the phenotypic ratio expected in the F2

    9 black: 3 tan: 4 white

    d) What is the name of the "Variation on Mendel's Theme" illustrated in this problem? **Epistasis**
11. What does the acronym RFLP stand for? Restriction Fragment Length Polymorphism

12. The following diagram shows an electrophoretogram of the PCR products amplified using a RAPD primer. The target was DNA extracted from 2 truebreeding pea cultivars and their F1. (S and F simply represent the slow and fast migrating forms seen on the gel.

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>S</td>
<td>F</td>
<td>F</td>
</tr>
</tbody>
</table>

a) If the bands seen in the 2 parents are amplified from alleles, what ratio will be predicted in the F2?

1 S : 2 F&S : 1 F

b) Now suppose the bands are from independent genes, with the "missing" bands accounted for by null alleles. What ratio would be predicted in the F2.

9 F&S : 3 F only : 3 S only : 1 no bands (ie heterozygotes for a null still show the band)

13. Differentiate between genetic heterogeneity and variable expressivity. Give an example of each.

Genetic heterogeneity refers to cases where multiple genes give the same phenotype; vision and hearing are good examples

Variable expressivity refers to differences in phenotype associated with a defect in one gene; brittle bones-blue sclera is an example.

14. Given the pedigree:

a) How is the trait inherited? simple autosomal recessive

b) What is the probability II-2 is heterozygous? 1

c) What is the probability that II-3 is homozygous? 1/3
15. Given the human pedigree:

Assuming a single gene is responsible, and that no problem such as lack of penetrance is involved,

a) Which of the following modes of inheritance could account for the trait: (Check correct answers)

- (a) sex-linked recessive
- (b) sex linked dominant
- (c) autosomal recessive
- (d) autosomal dominant
- (e) sex-influenced trait
- (f) sex-limited trait

b) Would any of these be eliminated if couple 2 has an affected son?  
(List letters of those eliminated) ____________ b, d, e

c) Would any of possibilities in part a be eliminated if couple 3 has a normal daughter?  
(List letters of those eliminated) ____________ b, e

16. Rapid feathering is a dominant sex-linked trait that has been used as a simple way to "sex" chicks. Breeders have developed near-isogenic lines in breeds such as white leghorns that have been developed for egg production.

a) Show the genotypes of males and females that could be used as breeding stock so that feathering patterns can be used in sexing.

- $X^R W$ female
- $X^r Z^Z$ rooster gives all rapid males and all slow females

b) How would breeding stock be produced for the next generation?

- maintain rapid and slow homozygous NIL flocks, or mate F1s and select fast females and slow males.
17. Inca-hairless dogs are heterozygous for a zygotic lethal gene.
   a) If an Inca-hairless female mated with an Inca-hairless male has 6 pups, what is the probability that 4 will be hairless and 2 normal? (show formula)

   \[ \frac{6!}{4!2!} \left( \frac{2}{3} \right)^4 \left( \frac{1}{3} \right)^2 \]

   b) What is the probability at least one of the pups will be normal?

   \[ 1 - \left( \frac{2}{3} \right)^6 \]

18. How are X inactivation and genomic imprinting alike? How do they differ?

   Both use DNA methylation to silence specific DNA sequences; X inactivation occurs early in development of females and randomly inactivates any X >1 in somatic cells; the same X remains inactive in daughter cells

   In imprinting, selective regions of specific chromosomes are silenced during gametogenesis, with different regions silenced in males and females.

19. Genes A and B have been reported to be 20 map units apart. Your cross of a doubly heterozygous female to a recessive male gives 17 A B; 33 a b; 32 a B; 18 a b.

   a) Give the genotypes of the 2 parents in the testcross.

   female A b/a B male a b/a b

   b) What is your estimate of the map distance between the A and B loci?

   35 map units (cM)

   c) Does your map differ significantly from the previously reported map?

   Expected 10 AB: 40 Ab: 40 aB: 10 ab; Chi squared = 14.12, so with 3 df, the results do differ significantly from expected under hypothesis.

20. In maize: C/_ colored Sh/_ full kernal Wx/_ starchy
     c/c colorless sh/sh shrunken wx/wx waxy
A mapping cross with 1,000 progeny tested gave the following results:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>colored, full, waxy</td>
<td>392</td>
</tr>
<tr>
<td>colored, shrunken, starchy</td>
<td>11</td>
</tr>
<tr>
<td>colored, full, starchy</td>
<td>97</td>
</tr>
<tr>
<td>colored, shrunken, waxy</td>
<td>1</td>
</tr>
<tr>
<td>colorless, shrunken, starchy</td>
<td>386</td>
</tr>
<tr>
<td>colorless, full, waxy</td>
<td>12</td>
</tr>
<tr>
<td>colorless, shrunken, waxy</td>
<td>101</td>
</tr>
</tbody>
</table>

**Total** 1,000

Map the genes, calculate the value of interference and the coefficient of coincidence.

map: $C \quad 2.4 \quad Sh \quad 19.9 \quad Wx$

Cross: $C - Sh - wx/ c - wx - Wx$ by $c - sh - wx/ c - sh - wx$

Interference = 0.209; Coefficient of Coincidence = 0.791

21. Draw 3 asci of a *Neurospora crassa* cross segregating for a white spore mutation that show different second division segregation patterns.

bbwwbbww; bbwwwwbb, wwbbwwbb etc.