1. In humans almost all methylation is stripped from DNA in the fertilized egg and in spermiogenesis almost all histones are replaced by protamines that allow greater compaction. Yet we know that some methylation sites and histones are carried over;

   a) What is the general rule concerning DNA methylation as a factor in gene expression in animals?
      
      $5^\text{mC}$ in CG dinucleotides in the promoter (often in CG rich islands) lower the level of transcription.

   b) What are some mechanisms for creating, maintaining and eliminating $5^\text{mC}$?
      
      De-novo methylases (DNMTs) and maintenance methylases maintain the level of $^\text{mC}$ after DNA replication. In some cases methylation appears to be triggered by RNAi and levels of histone condensation. Demethylation occurs via action of C deaminase that creates a T and triggers mismatch repair. Also, adding an OH or other radical to the C triggers the TET response to replace the $5\text{MC}$ with a normal C. In addition, rapid DNA replication and 5AZA-C lead to deaminantion.

   c) What are some general rules concerning the role of histone alterations in affecting gene expression?
      
      Adding acetyl groups to exposed tails of histones loosens DNA compaction, removing reverses and lowers level of transcription. Adding methyl groups is even more compacting.

   d) What enzymes are involved in altering histones
      
      HATs and HDACs add and remove the acetyl, histone methyl transferases, kinases and phosphatases can add/remove phosphate and Swi/Snf type complexes with ATP can move histones along the DNA backbone.

   e) How do we know that not all of the DNA or marked histones are eliminated in the first cells of a human embryo?
      
      Basically, ‘imprinting’

2. A recent review included the statement that ‘at least some miRNAs feed into RNAi’. What do these terms mean and what is the direct implication?
   
   miRNAs are microRNAs made by dicer clipping of hairpin double strand regions in longer non-coding transcripts after introns have been spliced-out. RNAi is RNA interference in which short (siRNAs) are created from double stranded RNAs by dicer and carried by argonaut proteins into a RISC complex where a single stranded siRNA can anneal with a complementary segement of mRNA and prevent traslation or cleave the mRNA. Thhis implies that genes have evolved whose function is to stop expression of other genes as a mechanism of regulation.
3. a) Describe the steps, including enzymes and other tools that could be used to clone a gene from a plant or animal so that it would be expressed (at least so far as making the correct amino acid sequence) in E. coli.

One example—note the cDNA methods should be used
1. use oligo-dT beads or columns to extract mRNA from target tissue.
2. use oligo-dT as a primer to create first stand DNA copies of the mRNAs present using reverse transcriptase.
3. add RNAase H to create nicks in the RNA strand so that the RT or an added DNA polymerase can use replace the RNA created ds DNA copies (cDNA) of the messages.
4. add tails or restriction site linkers to the ends of the cDNAs
5. Open a pUC plasmid in the MCS site and cleave the added RE sites on the linkers with an restriction endonuclease.
6. mix the cDNAs and plasmids; allow to anneal and combine with DNA ligase
7. Transform into an E. coli lacking the lacZ gene function and grow on medium with X-gal
8. Select white colonies and test for the target gene based on expression, antibody interaction, sequencing, etc.

b) What might you try if the correct sequence is made but fails to function?
Exposing the cloned gene product to dog pancreas (or wheat germ) extract to allow post translational modifications to be made, or better, transform the clone into Pichia pastoris, a kind of yeast that makes modifications similar to higher eukaryotes ((standard yeast, Saccharomyces does not)

4. A diploid ant species has only two pairs of chromosomes. Assume a male is heterozygous for genes A and B, with A on the end of the long arm of chromosome 1 and B very near the centromere of chromosome 2.

a) Show labeled chromosomes in metaphase of a) mitosis and b) Meiosis I
b) Assuming one three strand double crossover occurs between A and its centromere and none for B, predict the types and frequency of gametes that would arise from your drawing of Meiosis.
25% each, AB, AB, aB, ab

5. What factors made yeast a good model organism for Hartwell’s work identifying genes that are involved in controlling the cell cycle. What are typical functions of many of the cdc genes?
1. It is a single celled eukaryote that can be grown as a haploid, making finding mutations relatively easy.
2. It divides by budding which can be tracked under a dissecting microscope, so that mutants that don't grow at the restrictive temperature can be identified.

Typical functions are cyclins and cyclin-dependent kinases that function at checkpoints during the cell cycle to monitor progression between phases.

6. Design simple pathways that would account for the following dihybrid F2 ratios in flower color. Assume the enzymes are coded by independent genes.

   a) 9 blue: 7 white: White → White → Blue
   b) 15 blue: 1 white: White → Blue ← White
   c) 9 lavender: 3 blue: 4 white: white → blue → lavender
   d) 9 lavender: 6 blue: 1 white: W → blue → lavender

Where each arrow is the enzyme produced by an independent gene
7. Strains of a haploid yeast were used to select 7 mutants (A-G) that would not grow at 37°C but would at 25°C. After crossing to a wild type strain of the opposite mating, the 7 recovered haploid strains were then paired to form diploids and tested for ability to grow at 37°C, with the following results.

(++ means good growth, + some growth, - no growth beyond background levels)

<table>
<thead>
<tr>
<th>α MT</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>++</td>
<td>+</td>
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<tr>
<td>C</td>
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<td>G</td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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</tbody>
</table>

a) How would the crosses to wild type segregate if each mutant strain carried a single gene defect? 1 mutant : 1 wild type

b) Why would it be necessary to recover each temperature sensitive mutation in each mating type?
Crosses to form a diploid must have one alpha and one a mating type allele

c) How many different genes were discovered and which mutations are in the same gene(s)?
likely 3 genes: A, D and F are alleles of one gene; C and E are alleles, B and G also appear to be in the same gene different from the others.

d) B and G were apparently somewhat ‘leaky’. Does this negate the results where they were included. Justify your answer.
No: although some growth occurs at 37, they each show strong growth with combined with each other and with the other mutants.

8. White face in cattle is a single gene trait where W_ is white face and ww is solid. A whiteface bull progeny from a Ww X Ww cross sires 4 white-faced calves when mated to 4 solid-face cows. What is the probability that he is: Ww? WW?

\[
P(Ww) = \frac{2/3 (1/2)^4}{2/3 (1/2)^4 + 1/3 (1)} = 1/9 \quad \text{so } P(WW) = 8/9
\]
9. Coffin-Lowry syndrome is a very rare human genetic disease. Most cases are new mutations. Full symptoms, seen almost entirely in males include mental retardation, large lips, and small stature due to poor bone growth. Two affected brothers differed in that one also had extreme spinal scoliosis. Very rarely, females are affected, but their symptoms are much less severe. Establish a hypothesis that could account for these observations. Be sure to include any terms that deal with complications of “Mendelian” inheritance.

Finding the severe symptoms entirely in males suggests a sex linked recessive is the cause; The trait shows pleiotrophy as multiple organs show phenotypic effects and variable expressivity as twin brothers showed a different level of one of the symptoms.

The lower level seen in rare females is easiest to explained by differential expression of X's based on Lyon's Law

10. The following legends describe kernel characteristics in maize.:

\[
\begin{align*}
C & \text{ red}, & W & \text{ normal}, & S & \text{ plump} \\
cc & \text{ white} & ww & \text{ waxy} & ss & \text{ shrunken}
\end{align*}
\]

A true-breeding plant with red, waxy shrunken seeds was crossed with a true-breeding white, normal, plump seeded plant. The F1 was then test-crossed to do 3 point mapping. Results of the test cross are shown below. In the center column, fill in the genotype of the gamete from heterozygous parent:

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Gamete from heterozygous parent</th>
<th>Genotypes</th>
<th>Gamete from heterozygous parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red, normal, shrunken</td>
<td>Cws</td>
<td>3967</td>
<td></td>
</tr>
<tr>
<td>White, waxy, plump</td>
<td>cwS</td>
<td>3868</td>
<td></td>
</tr>
<tr>
<td>Red, waxy, plump</td>
<td>CwS</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>White, normal, shrunken</td>
<td>cWs</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>Red, waxy, shrunken</td>
<td>Cws</td>
<td>919</td>
<td></td>
</tr>
<tr>
<td>White, normal, plump</td>
<td>cWS</td>
<td>894</td>
<td></td>
</tr>
<tr>
<td>Red, normal, plump</td>
<td>CWs</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>White, waxy, shrunken</td>
<td>cws</td>
<td>4</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>10000</td>
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</tr>
</tbody>
</table>

1) What is the order of the genes? C S W
2) Give genotypes for the two original parents, and the F1 and testcross plants. .

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>cS w</th>
<th>cS w</th>
<th>C s W</th>
<th>C s W</th>
<th>c s w</th>
</tr>
</thead>
<tbody>
<tr>
<td>C s W</td>
<td>c S w</td>
<td>c S w</td>
<td>C s W</td>
<td>C s W</td>
<td>c s w</td>
</tr>
</tbody>
</table>

3) Map the genes. 

C 3.5 S 18.3 W

4. Calculate interference and the coefficient of coincidence.

\[ \text{Cof C} = 15.6, \ I = 84.4 \]
11. Two abnormal sex chromosome karyotypes show clearly that sex determination in *Drosophila melanogaster* and *Homo sapiens* differ significantly, even though in both species XX = female and XY = male. What are the key abnormalities and what do they tell us about the keys to sex determination in each species. Include the names of the syndromes for the human examples.

XO is a Turner's female in humans but a male in Drosophila

XXY is a Klinefelter's male in humans but a fertile female in flies.

**TAKE HOME – OPEN BOOK and Web Use is OK**

As part of his Ph.D research, Jairo (now Dr.) Osario needed to identify F2 sorghum plants that were homozygous for resistance or susceptibility to head smut. Jairo crossed an inbred line that is highly susceptible to another that is resistant, grew the resulting F1 seeds to maturity, and allowed the plants to self-pollinate. He then grew 200 F2 plants to maturity, and again insured self pollination by bagging the heads before anthesis. F3 seeds from each of the heads were collected and used to test the resistance/susceptible genotype of each F2 plant by needle inoculation with spores of *S. reilianum*.

A) What F2 ratios (genotypic and phenotypic) would be expected among the 200 F2s if resistance results from (1) a single gene dominant trait, (2) a simple recessive (3) two unlinked dominant genes, either of which confers resistance.

1) (phenotypic ratios) 3 Res : 1 sus or 150: 50; 2) 1 Res : 3 Sus  3) 15 Res : 1 sus

B) How many F3 plants should be grown and tested for disease response for cases 1 & 2 to be above to be 95% certain that each F2 plant can be correctly classed as homozygous or heterozygous? What about case 3?

1 11 for 1 NS 2, 47 for case 3 since it would take that many in the event the plant was R1R1, R2R2. (Those that are R1R1 or R2Rc would all give only resistant progeny, but you would not know which ones these were without making test crosses.)

C) Now consider that in practice, on average, only 70% of the fully susceptible plants develop disease. (This occurs when the needle doesn’t hit the meristem, the fluid leaks out, or the environment is too dry.) Given this rate of ‘escapes’ what would be the expected ratios of ‘resistant’ and susceptible progeny for each of the models, and how many F3 plants should be tested to identify those that are heterozygous?

1) 165 to 35, 2) 105 Sus : 95 Res.  3) about 191 Res to 9 sus
grow 16, 16, and 67

D) Overall, 696 of 4,501 randomly selected F3 plants tested developed head smut. Describe a model for head smut response based on this and the prior information and test your model for goodness of fit.

None of these models fits: but if the detection rate is more like 60%, a single dominant R gene will give an acceptable Chi Square value