Section A: Genetic Engineering Overview

1. What is genetic engineering?

2. Put the steps of genetic engineering in order.
   _________ Recombinant product is isolated, purified and analyzed before marketing.
   _________ The DNA is identified and isolated from a cell.
   _________ Transformation occurs when the host cell incorporates the recombinant DNA in culture.
   _________ Restriction digestion occurs, a gel is performed and probes are used to isolate the gene.
   _________ Assays are used to confirm the inserted gene is expressed.
   _________ Recombinant DNA is pasted into a plasmid vector.
   _________ Recombinant cells are grown in larger cultures during the scale up process.

3. Using chymosin and rennin as an example, explain the rationale for the creation of the genetically engineered product, chymosin.

4. For more than 10 years, scientists have been genetically modifying species of fish. These GMOs have resulted in bigger fish that can better resist disease or environmental changes.
   Read the excerpt and study the table showing the fish species and target genes that have been added to them.

“The most popular gene used in aquatic species is growth hormone (GH). GH has been widely used in terrestrial species and as the gene sequence is highly conserved; the product is readily utilized across species boundaries. It may also be noted that, at least in some cases, enhanced growth is associated with more effective utilization of food.
Cold water temperatures are often a major problem in aquaculture in temperate climates when an unusually cold winter can severely damage both production and brood fish stocks of fish. Some marine teleosts (fish) have high levels of serum anti-freeze proteins (AFP) or glycoproteins (AFGP) which reduce the freezing temperature by preventing ice-crystal growth.”

<table>
<thead>
<tr>
<th>Species</th>
<th>Target gene</th>
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<tbody>
<tr>
<td>At. Salmon</td>
<td>GH</td>
</tr>
<tr>
<td>At. Salmon</td>
<td>AFP</td>
</tr>
<tr>
<td>Carp</td>
<td>GH</td>
</tr>
<tr>
<td>Tilapia</td>
<td>GH</td>
</tr>
<tr>
<td>Common carp</td>
<td>AFP GH</td>
</tr>
</tbody>
</table>

⇒ What traits does the GH gene confer to the GMO fish?

⇒ What traits does the AFP gene confer to the GMO fish?

⇒ List a potential pro and a potential con of engineering this gene into these fish.
Section B: Plasmids
1. What are plasmids?
__________________________________________________________________________
__________________________________________________________________________
2. What is ori?
__________________________________________________________________________
3. What are the 2 main uses of plasmids?
__________________________________________________________________________
__________________________________________________________________________
4. What are multiple cloning sites and what are they used for?
__________________________________________________________________________
__________________________________________________________________________
5. Read the article on website – “Plasmid History”. Give a brief background and scientific contribution to recombinant DNA technology for the following scientists.
Paul Berg –
Herbert W. Boyer –
Stanley N. Cohen –

Section C: Comparing Vectors (Plasmids)
An expression vector is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the protein that is encoded by the gene is produced by the ribosomal. The goal of an expression vector is the production of large amounts of messenger RNA and in extension, proteins. After expression of the gene product, the purification of the protein is required; but since the vector is introduced to a host cell, the protein of interest should be purified from the proteins of the host cell. Therefore, to make the purification process easy, the cloned gene should have a tag.

Cloning vectors involve the same process of introducing a new gene into a plasmid as expression vectors, but the plasmid is then added into bacteria for replication purposes. In general, DNA vectors that are used in many molecular-biology gene-cloning experiments need not result in the expression of a protein.
A shuttle vector is a vector that can propagate in two different host species; hence, inserted DNA can be tested or manipulated in two different cell types. The main advantage of these vectors is that they can be manipulated in E. coli and then used in a system which is more difficult or slower to use. Shuttle vectors can be used in both eukaryotes and prokaryotes. Shuttle vectors are frequently used to quickly make multiple copies of the gene in E. coli (amplification). They can also be used for in vitro experiments and modifications such as mutagenesis and PCR. One of the most common types of shuttle vectors is the yeast shuttle vector that contains components allowing for the replication and selection in both E. coli cells and yeast cells. Source: Boundless. “Shuttle Vectors and Expression Vectors.” Boundless Microbiology. Boundless, 21 Jul. 2015.

Compare the three types of plasmid vectors.
Section D: Isolating Genetic Information
1. What must occur to retrieve the DNA in a cell? ____________________________________________

2. What must be eliminated during the retrieval process? ______________________________________

3. What is the function of lysozyme in the buffer solution? _____________________________________

4. What is the function of detergent in the retrieval process? ____________________________

5. What can be added with the detergent and why is it needed? ______________________________

6. Why centrifuge the sample? ___________________________________________________________

7. What is in the supernatant? ____________________________________________________________

8. What is the function of alcohol in the retrieval process? _________________________________

9. What do scientists add to separate the plasmids from the bacterial chromosome? ________________

10. What is not needed when isolating DNA in animal cells? Explain. ____________________________

Section E: Probing DNA for Genes of Interest
1. What is a probe and what are probes used for? __________________________________________

2. How are restriction enzymes used to isolate genes? _______________________________________

3. What happens to restriction fragments on a gel electrophoresis? __________________________

4. How are probes used in gel electrophoresis to isolate genes of interest? ____________________

5. What is hybridization? ___________________________________________________________________

6. How can probes be visualized? __________________________________________________________

7. How can southern blotting and probes be used to isolate genes of interest? __________________

8. What does PCR use instead of probes to isolate genes? _____________________________________

9. How is PCR used in the gene isolation process? ___________________________________________

10. A technician has found a section of DNA responsible for an antifreeze phenotype in an ocean fish. She wants to make several copies of the gene for genetic engineering purposes. What technique(s) and instruments could be used to make multiple copies of the gene? ____________________________

Section F: Transforming Cells
1. What is transformation? __________________________________________________________________

2. Do transformations occur naturally? Explain. ______________________________________________

3. What does it mean when a cell is “transformed”? __________________________________________
4. When scientists first tried transformations, the efficiency was low. What did they decide to try to increase transfer efficiency? __________________________________________________________________________________________

5. What is transduction? __________________________________________________________________________________________

6. What is transfection? __________________________________________________________________________________________

7. What must occur to plasmids so they can carry genes into a cell? __________________________________________________________________________________________

8. What is the function of restriction enzymes? __________________________________________________________________________________________

9. What are sticky ends? __________________________________________________________________________________________

10. Once the genes of interest are cut, what happens next? __________________________________________________________________________________________

11. What is the function of DNA ligase? __________________________________________________________________________________________

12. What explanation do scientist give for the presence of restriction enzymes in bacteria? __________________________________________________________________________________________

13. What does palindromic mean? __________________________________________________________________________________________

14. Why is it important to cut DNA into restriction fragments? __________________________________________________________________________________________

15. A plasmid has a gene of interest that you would like to transfer to another plasmid that is a better vector for transformation. How would you know which restriction enzymes to use to make the rDNA? __________________________________________________________________________________________

16. List and explain applications of Restriction Fragment Length Polymorphism (RFLP) analysis.
   a. __________________________________________________________________________________________
   b. __________________________________________________________________________________________
   c. __________________________________________________________________________________________


<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>DNA Recognition Site</th>
<th>Does the endonuclease produce sticky ends?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alu I</td>
<td>5’...AGCT...3’ &lt;br&gt;3’...TCGA...5’</td>
<td>How did you determine this?</td>
</tr>
<tr>
<td>EcoRI</td>
<td>5’...GAATTC...3’ &lt;br&gt;3’...CTTAAG...5’</td>
<td></td>
</tr>
<tr>
<td>Hind III</td>
<td>5’...AAGCTT...3’ &lt;br&gt;3’...CTCGA...5’</td>
<td></td>
</tr>
<tr>
<td>Pst I</td>
<td>5’...CTGCAG...3’ &lt;br&gt;3’...GACGT...5’</td>
<td></td>
</tr>
</tbody>
</table>
Section G: Performing a Transformation
1. How do you make host cells competent when doing a transformation? ________________________________
   ____________________________________________________________________________________
2. After adding the rDNA (plasmid), what do you do next? ______________________________________
   ____________________________________________________________________________________
3. Why do you do the heat shock/cold shock cycle? ___________________________________________
   ____________________________________________________________________________________
4. Why do you add a nutrient agar? __________________________________________________________
5. Why do you plate the cells? _____________________________________________________________
6. Explain how competency works. __________________________________________________________
7. What is electroporation? ________________________________________________________________
8. Explain how the heat shock/cold shock cycle works. _______________________________________ 
   ____________________________________________________________________________________
9. What is a recovery period? ______________________________________________________________
10. What is selection and why is this process essential? _________________________________________
    ____________________________________________________________________________________
11. What is transformation efficiency? _______________________________________________________ 
12. A technician is attempting to transform cells with a rather large plasmid and the transformation efficiency is very low. What might she do to increase the transformation efficiency? 
    ____________________________________________________________________________________
13. In a given transformation, only a relatively low number of cells are transformed. How can a technician tell if a transformation, such as transforming E.coli into amylase producers, actually occurs? 
    ____________________________________________________________________________________
14. Calculate the transformation efficiency using the formula in the notes. If bacteria are transformed with 1 μg of DNA and 76 CFU result from spreading 100 μl of the culture (from 500 μl final volume), what is the transformation efficiency (in CFU per μg of DNA)? 
    ____________________________________________________________________________________

Section H: Scale-Up Process
1. Why do biotechnology companies scale-up? ___________________________________________________
2. Explain the scale-up process. _____________________________________________________________
    ____________________________________________________________________________________
3. What must be measured during each scale-up? _____________________________________________ 
    ____________________________________________________________________________________
4. What requirements must be recorded and monitored through the scale-up process? 
   ____________________________________________________________________________________
5. What else are cultures monitored for? ____________________________________________________
6. What must workers do to ensure sterile conditions during the scale-up process? ____________________
7. What do the assays test for during the scale-up process? ____________________
8. What is R&D’s role during the manufacturing process? ____________________
9. What is QC’s role during the manufacturing process? ____________________
10. What is alcoholic fermentation? ____________________
11. What is lactic acid fermentation? ____________________
12. In reference to biotechnology, what is fermentation? ____________________
13. What is the job of fermentation departments? ____________________
14. Manufacturing teams want to keep cell cultures in exponential growth. What is exponential growth? ____________________
15. What is a seed colony? ____________________
16. How do scientist know a broth culture becomes concentrated with cells? ____________________
17. A cell culture is in a 2-L spinner flask. Overnight, the growth rate of cells slowed, and the culture is no longer in exponential growth. The density of cells in the culture is not high enough to “seed” another flask. What might the technician check? ____________________

Section I: Retrieving Plasmids after Transformation
1. What is the name of the procedure in which plasmids are extracted from cells? ____________________
2. Briefly explain the miniprep procedure. ____________________
3. How is plasmid DNA precipitated in the final steps of plasmid prep? ____________________
4. List 2 reasons for performing a prep. ____________________
5. Once plasmid is extracted from a cell, how can a technician know that it is the “correct” plasmid? ____________________
6. If a DNA sample gives a 260 nm reading of .8 au, what is the concentration? ____________
7. If a DNA sample gives a 260 nm reading of .8 au and a 280 nm reading of .5 au, what is its purity? ____________ Is this purity acceptable? ____________
8. What occurs after a sample is considered pure and concentration is determined? ____________________