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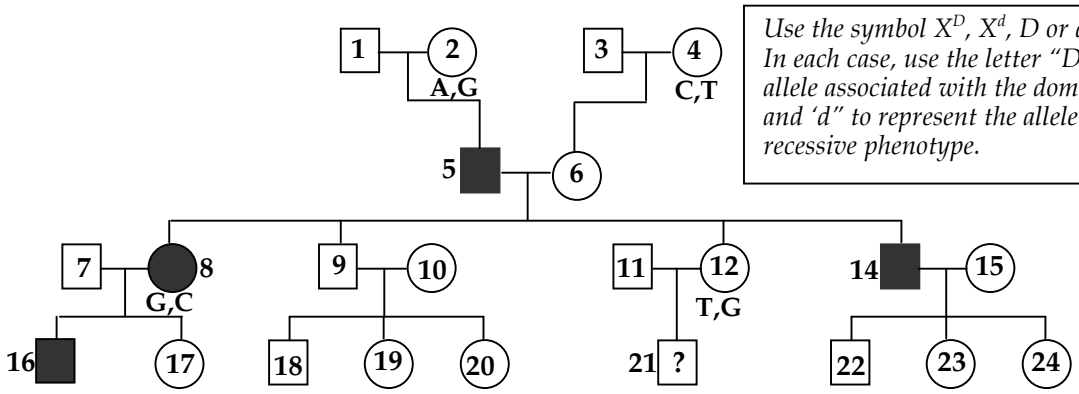
7.013 Problem Set 4- 2013

Question 1

The following human pedigree shows the inheritance of a specific disease. ***Please note:*** *The filled squares or circles represent the abnormal phenotype. The individuals marrying into the family do not have the disease associated allele. Assume that no other mutation arises within the pedigree. Also assume complete penetrance.*



Use the symbol X^D, X^d, D or d where appropriate. In each case, use the letter "D" to represent the allele associated with the dominant phenotype and 'd" to represent the allele associated with the recessive phenotype.

a) What is the **most likely** mode of inheritance of this disease?

b) Write **all possible genotypes** of the following individuals in the pedigree.

#6:	#19:

c) What is the probability of **Individual #21** being **affected**?

d) The disease shown by the pedigree above is caused by a mutation in Gene D that encodes Protein D. You identify a SNP that is **tightly linked** to Gene D and may be used **as a marker for the disease**. The alleles (*A, G, T, C*) of this SNP for some individuals are given in the pedigree above.

i. Identify the SNP(s) that is/are tightly linked with the mutant allele of Gene D.

ii. Write the SNP genotypes of the following individuals.

#5:	#14:

e) One can use the SNP microarrays to determine the SNP genotype of an individual. Briefly describe how the SNP microarrays work.

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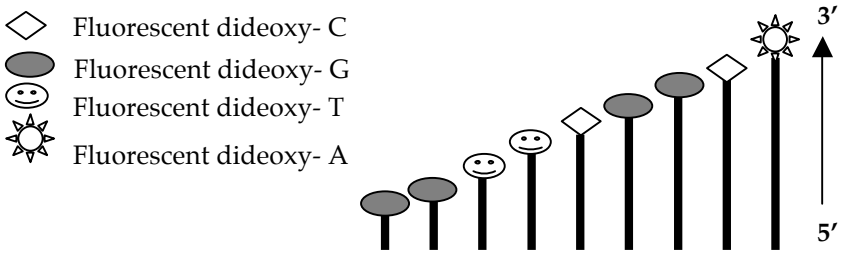
Question 1 continued

f) Although SNPs can be in the coding, non-coding or intergenic (regions between two genes) of the genes, you observe that this SNP is located within the noncoding region of Gene D. However, it still changes the sequence of the protein encoded by Gene D. Provide an **explanation** that supports this observation.

g) You purify the wild- type and disease- associated forms of Protein D and determine their amino acid sequence. The only difference you find is at the **6th position** shown in **bold** below.

Wild- type variant: N-gly^{5}-**trp^{6}**-ala^{7}-C
Mutant variant: N-gly^{5}-**ser^{6}**-ala^{7}-C

Shown below is a portion of the fluorescence dideoxy- sequencing gel, which gives the sequence of the **non- template/ mRNA like strand** of the DNA that corresponds to amino acids 5-7 of the **mutant form of Protein D**.



Write the sequence of the double stranded DNA that corresponds to amino acids 5-7 of the **wild-type form of Protein D** and label its 5′ and 3′ ends. ***Note:*** *A codon chart is provided on the last page of the problem set.*

h) The following is the DNA sequence of the **wild type allele of Gene D** that you want to amplify using the polymerase chain reaction (PCR).

```
5′CTCGAGGTGAATATGAAAG----[ Gene D ]----CATTTGGCGCGTAATCGATA3′
3′GAGCTCCACTTATACTTTC----[ Gene D ]----GTAAACCGCGCATTAGCTAT5′
```

i. If you amplify a DNA sequence through PCR, what are the reaction components that you would **absolutely need**? Briefly **state the function** of each of these components.

ii. Give the sequence (10 bases long) of a **set of primers**, which you would use for the PCR reaction.

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Question 1 continued

iii. In the PCR reaction, you need a three- step reaction cycle, which results in a chain reaction that produces an exponentially growing population of identical DNA molecules. Each step of a reaction cycle is performed at a specific temperature i.e. 94ºC for Step 1, 60ºC for step 2 and 72ºC for Step 3. Briefly **explain** why the three steps are performed under different temperatures.

iv. When PCR amplification is used to provide a specific DNA fragment for cloning, the resulting clones are sequenced to make sure that inserts have the correct base sequence. What activity does Taq DNA polymerase enzyme lack that may explain the errors that occur during PCR amplification?

Question 2

You need a large amount of Protein D encoded by Gene D. Therefore you decide to engineer a mouse cell line that will secrete a large amount of Protein D, so that you can purify Protein D from the medium. ***Note: A cell line is a single type of cell, which continuously grows in culture***.

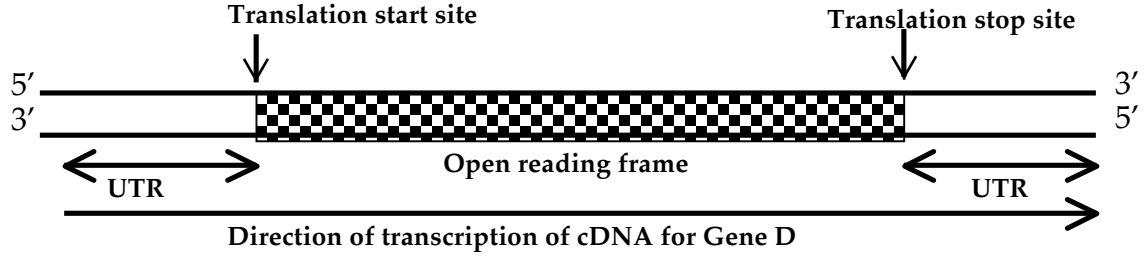
a) List **any four components**, of the host eukaryotic cell translation machinery, which are **absolutely** required for synthesis of proteins, and briefly (few words) indicate what each does.

b) List **two components**, of the host eukaryotic cell translation machinery, which are **absolutely** required for **export of Protein D from the cell?**

c) You isolate a cDNA for Gene D. However, when you transfect the normal cell line with a vector containing Gene D (*i.e. insert a vector containing Gene D into the cells*), you find that Protein D is produced in the cytoplasm but not secreted. You conclude that something is therefore wrong with the Gene D.

i. What modifications would you make to the cDNA for Gene D that would plausibly allow Protein D to be secreted?

ii. The following is a schematic of the cDNA for Gene D. **Circle** the region in Gene D, where you would make the above modification.



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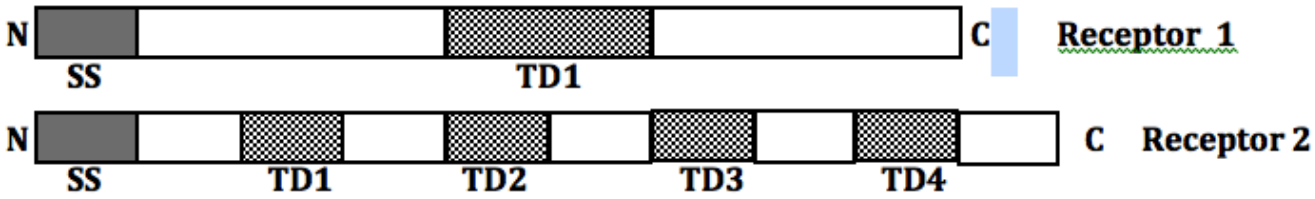
Question 2 continued

iii. Assuming your manipulation in part (i) is successful, would the modified/secreted version of Protein D be of the same size, larger than, smaller than its unmodified/cytoplasmic version? **Explain** your answer.

d) Interestingly, you find that this protein, when secreted, can bind to either of following cell membrane receptors (**Receptor 1 and Receptor 2**). The transmembrane domains (TD) and the signal sequence (SS) are shown in the schematic.



Draw the two receptors as they would be inserted into the Endoplasmic reticulum (ER) and plasma membrane, label their N and the C termini and include all the TD domains that are shown in the schematic above.

Receptor 1	Receptor 2
Cytosol ER membrane ER lumen	Cytosol ER membrane ER lumen

Receptor 1	Receptor 2
Extracellular Plasma membrane Cytosol	Extracellular Plasma membrane Cytosol

Question 3

You have identified an enzyme (E1) in a yeast strain (Strain 1) that catalyzes a step in a biochemical pathway, which results in the synthesis of the amino acid Arginine. This enzyme is encoded by Gene A. You isolate Gene A from the yeast cells, clone it into a plasmid vector and **amplify** it in the bacterial cells.

a) List the minimum features that a plasmid vector **must** have to allow the cloning and amplification of Gene A in bacterial cells.

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Question 3 continued

b) You decide to use the following plasmid vector to clone Gene A. The recognition sequence for each restriction enzyme is given below. An arrow represents the site at which the restriction enzyme cuts.

Nde I:

5′ CATATG
3′ GTATAC

Sal I:

5′ GTCGAC
3′ CAGCTG

EcoR I:

5′ GAATTC
3′ CTTAAG

BamHI:

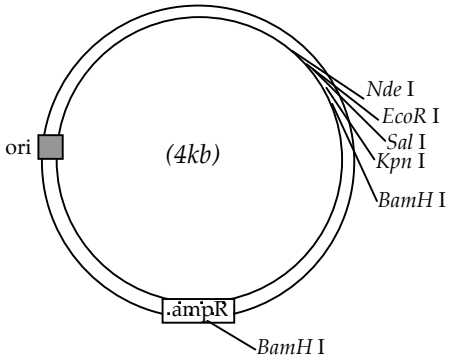
5′ GGATCC
3′ CCTAGG

Kpn I:

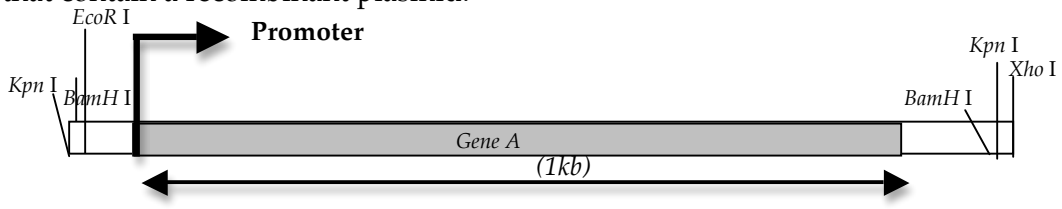
5′ GGTACC
3′ CCATGG

Xho I:

5′ CTCGAG
3′ GAGCTC



i. A schematic of Gene A is given below. You want to clone Gene A into the plasmid vector. Give three different strategies that you could use to clone Gene A into the vector, and obtain colonies that contain a recombinant plasmid.



Strategy	Restriction enzyme(s) used to cut….	
	Gene A	**Plasmid vector**
1		
2		
3		

ii. Which strategies (*Choose from Strategy 1, 2, 3*) would allow a **directional cloning**?

c) You then transform the bacterial cells with the ligation mix.

i. Briefly outline a procedure that would allow you to distinguish the **transformed** bacterial cells from the **untransformed** ones.

ii. Briefly outline how the DNA gel electrophoresis would allow you to distinguish between the bacterial cells transformed with the **self- ligated plasmid** from those transformed with the **recombinant plasmid**.

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Question 3 continued

d) You grow and amplify the recombinant plasmid in the bacterial cells. You then purify the recombinant plasmid from the bacterial cells and transform the yeast cells with it.

i. Why did you use the bacterial cells only for the amplification of the plasmid but **NOT** for the expression of Gene A?

ii. Give **an alteration** in your experiment that would have allowed you to both amplify and express Gene A in the bacterial cells.

iii. What media would you use to select the yeast cells that are expressing the enzyme encoded by Gene A?

e) You friend also does the same experiment to clone Gene A with the following flanking sequence. But he decides to select the bacterial cells transformed with the recombinant plasmid using colony hybridization.

```
5′-CCCGTACTTGAAATC.....[ Gene A ].....GGACTTACCATTGGG-3′
3′-GGGCATGAACTTTAG.....[ Gene A ].....CCTGAATGGTAACCC-5′
```

i. Give the sequence of the probe that he would use for colony hybridization and label the 5′ and the 3′ ends.

ii. Briefly describe the major steps of colony hybridization that would allow your friend to select the transformed bacterial colony.

Question 4

Individuals who are homozygous for the mutation in Gene A (genotype = aa) suffer from a liver disease that shows an autosomal recessive mode of inheritance. You decide to do a UROP in a lab that is dedicated to using gene therapy to cure this hereditary disorder, in a mouse model.

a) Using **virus** as a vector that stably integrates into the genome of the host cell, you introduce a wild-type allele of Gene A in the liver cells of newborn mice whose genotype is "aa". If all cell types of the mouse have roughly the same genome why would you use only the liver cells to introduce the wild-type allele of Gene A?

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Question 4 continued

b) Based on what you have learnt in 7.013 lectures why is the **ex-vivo** gene therapy more successful than the **in- vivo** gene therapy? Give one reason.

c) Your supervisor suggests that you use embryonic cells instead of liver cells for ex-vivo gene therapy. **Explain** what may be the potential advantage of using embryonic cells compared to the liver cells.

d) You isolate embryonic cells from an affected mouse (genotype: aa) and infect them with a viral vector that has a wild- type allele of Gene A. You select the cells that have been infected with the virus containing the wild- type copy of Gene A and re-introduce them into the developing embryo of the affected mouse to obtain newborns. You trace the location and expression of Gene A in the newborn mice by adding a blue color dye that specifically binds to the protein encoded by Gene A. You obtain the following two sets of results. ***Note:*** *You may assume that the level of expression of the Gene A correlates with the intensity of the blue color in the cells. In wild-type mice, the dye stains only the liver cells.*

- **Set 1:** *When you add the dye you find that most of cells in the mouse, including the liver cells, turn blue.*
- **Set 2:** *Based on the results of Set 1, you modify your viral vector that contains Gene A, reinsert it into the embryonic cells and obtain newborns by following the same steps that were described above. When you add the dye you find that only the liver cells turn blue and the color is of the same intensity as in the liver cells of wild-type mice. In addition, these mice do not show the manifestations of the disease.*

How did you modify the viral vector so that the introduced Gene A was expressed only in the liver cells of newborns obtained in Set 2?

e) You isolate the cell from a developing embryo (at the blastula stage/ 8– cell stage) that is produced by the fusion of gametes from affected parents (genotype: aa). You infect these cells with a modified vector from Set 2 that has a wild type copy of Gene A. You then select the cells that have undergone homologous recombination and **now have a wild-type copy of Gene A**. You re-introduce them into the developing embryo (genotype: aa) to obtain newborns.

i. Give **all** the possible genotypes of the newborn obtained from this strategy? Briefly **explain** why you selected this genotype. ***Note:*** *Use the uppercase A to represent the allele responsible for the dominant phenotype and lowercase a to represent the allele responsible for the recessive phenotype.*

ii. You allow the newborn obtained from the strategy outlined above to mate with a wild- type female mouse (genotype: AA*).* Would you expect all the mice from this mating experiment to have a normal phenotype (*Yes*/ *No*)? **Explain** why you selected this option.

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Codon Chart

	U	**C**	**A**	**G**
U	UUU Phe (F) UUC " UUA Leu (L) UUG "	UCU Ser (S) UCC " UCA " UCG "	UAU Tyr (Y) UAC " UAA ***Stop*** UAG ***Stop***	UGU Cys (C) UGC " UGA ***Stop*** UGG Trp (W)
C	CUU Leu (L) CUC " CUA " CUG "	CCU Pro (P) CCC " CCA " CCG "	CAU His (H) CAC " CAA Gln (Q) CAG "	CGU Arg (R) CGC " CGA " CGG "
A	AUU Ile (I) AUC " AUA " **AUG** Met (M)	ACU Thr (T) ACC " ACA " ACG "	AAU Asn (N) AAC " AAA Lys (K) AAG "	AGU Ser (S) AGC " AGA Arg (R) AGG "
G	GUU Val (V) GUC " GUA " GUG "	GCU Ala (A) GCC " GCA " GCG "	GAU Asp (D) GAC " GAA Glu (E) GAG "	GGU Gly (G) GGC " GGA " GGG "

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