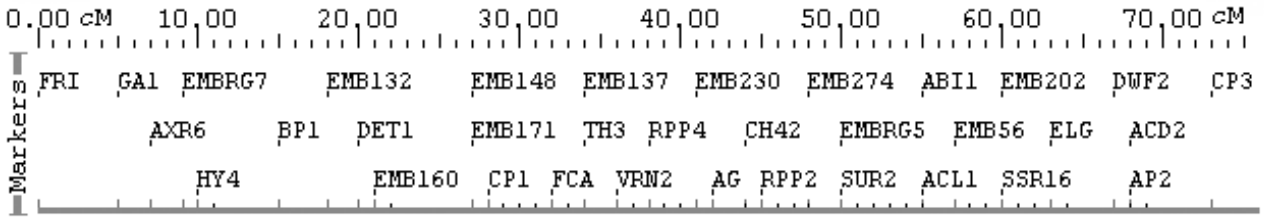
**Bio321 S'06 Young Exam #2. (150 pts)** **Name:**_____

**1. (18 pts)** The following is the classical map of chromosome 1 in *Arabidopsis thaliana*. Consider HY4 at 10 cM and CP1 at 28 cM.




**a. (2 pts)** what is the percent of observed recombination between these two genes?

_____

**b.** if you looked at 1000 progeny from the following cross ...

$$\frac{HY4\ \ CP1}{hy4\ \ cp1} \times \frac{hy4\ \ cp1}{hy4\ \ cp1}$$

(hy4 mutants have long hypocotyls, HY4 is wt)
(CP1 codes for a cystein protease, cp1 is the mutant)

**i. (2 pts)** how many recombinant progeny would you expect? _____

**ii. (2 pts)** what are the recombinant progeny genotypes?

_____ _____

**c. ( 2 pts)** if a plant were homozygous for a chromosomal deletion from the BP1 locus (15 cM) to the *DET1* locus (20 cM), what would the percentage of recombination be between HY4 and CP1?

_____

**d. (2 pts)** for a plant heterozygous for the deletion described in 1c., and with the recessive *emb132* (embryo lethal gene) on the wild-type chromosome, what would be the phenotype of the plant?

_____

**e. (2 pts)** if a plant, homozygous for an inversion in the region from the BP1 locus (15 cM) to the *DET1* locus (20 cM) (inclusive), self pollinates, what would the percentage of observed recombination be between HY4 and CP1 in the offspring?

_____

**f. (2 pts)** if the plant in 1e. were heterozygous at the BP1, EMB132 and EMB160 loci, would these alleles be able to recombine and produce viable crossover gametes?

Yes No

**g. (2 pts)** if the plant in 1f had one inverted and one normal chromosome, would the BP1, EMB132 and EMB160 genes be able to recombine and produce viable crossover gametes?

Yes No

**f. (2 pts)** for the plant in 1e., would the embryo specific wt genes EMB132 and EMB160 ever recombine and produce viable crossover gametes if the plant were crossed with a wt (chromosome) plant with emb132 and emb160 genotypes (mutant)?

Yes No

**2. ( 16 pts)** For the following questions, use the amino sequence, in tandem with the codon usage table, on the Reference Page (it's the last page on your exam, and can be detached as you won't be asked to hand it in). The 888 amino acid sequence codes for a complete glutamate receptor (neurotransmitter receptor) from *Loligo opalescents,* a myopsid squid. Single letter amino acid designations refer to specific codons (i.e. M = Met: AUG).

*Note: Use the topmost codon if the code is degenerate at that position.*

**a. (2 pts)** How many DNA base pairs code for the squid receptor protein? _____

**b. (8 pts)** Design 12 base <u>DNA</u> primers that you can use to PCR amplify the receptor gene from a cDNA library, starting at the serine (s) at position 11, through to the end of the sequence.

5' - __ __ __ __ __ __ __ __ __ __ __ __ __ 3'  5' - __ __ __ __ __ __ __ __ __ __ __ __ __ 3'

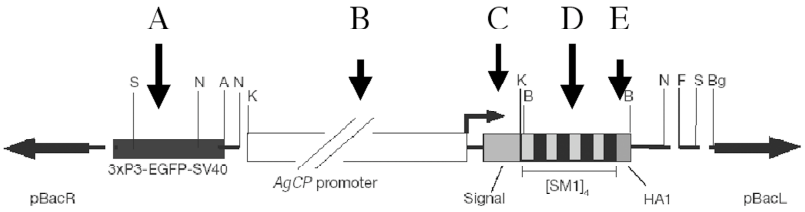
**c. (2 pts)** How long (in base pairs) is the PCR product from 2b? _____

**d. (4 pts)** You also have genomic DNA from *L. opalescents*. You use your PCR primers on the genomic template and get a PCR product that is nearly twice as long as the PCR product in 2c. I one word, why?

_____

**3. (8 pts)** The following figure is from the *Anopheles* paper...

**3a.** What is the function of the portion of vector labeled **Signal**?

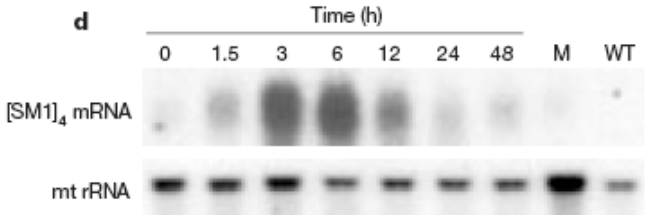



**3b.** What is the function of the portion of vector labeled **HA1,** and what experiment assayed it's function directly?

**4. (6 pts)** The following figure (d) is from the *Anopheles* paper...

**4a.** What type of blot is this?

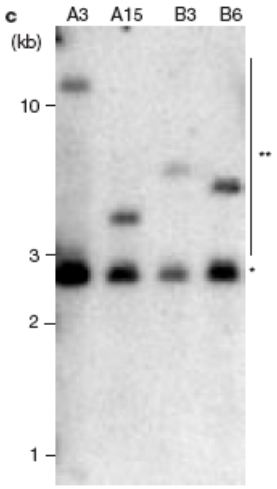
_____




**4b.** What portion of the vector depicted in **Question 3** <u>controls</u> the pattern of hybridization in the upper portion of figure d? Circle all correct answers…

**A  B  C  D  E**

**5. (8 pts)** This figure (c) is from the *Anopheles* paper...

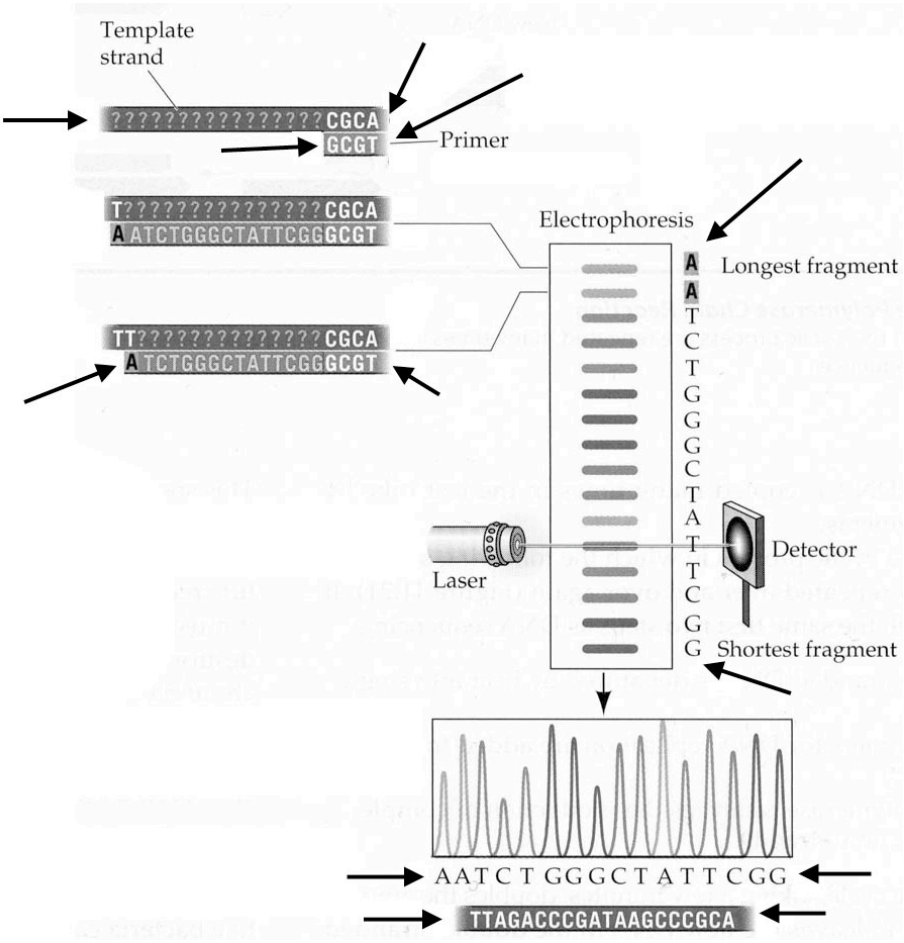



**5a.** What type of blot is this? _____

**5b.** What do the lanes (A3, A15, B3 and B6) represent?

**5c**. Why are the lanes different, and what is the significance of the differences?

**6. ( 12 pts)** Indicate 5' or 3' at each arrow location bellow…



**7. (4 pts)** You are given a suspension of bacteria and told that it contains 3 x $10^7$ viable cells per ml. How many 10-fold serial dilutions would you carry out so that 0.1 ml of the final dilution would contain approximately 200 viable cells?

_____

**8. (4 pts)** A suspension of bacteria was serially diluted through three dilutions of 100-fold each, and one dilution of 10-fold. From the final dilution, 0.5 ml was spread over nutrient agar in a Petri dish and incubated overnight. The next day, 131 colonies were visible. Estimate the number of viable bacteria per milliliter in the original undiluted suspension.

_____

**9. (18 pts)** Match the following with the best answer…

1. A process in which recipient cells acquire genes from free DNA molecules in the surrounding medium is called _____
2. A process in which DNA is transferred from a bacterial donor cell to a recipient cell by cell-to-cell contact is known as _____
3. A process in which DNA is transferred from a bacterial via a prophage step is termed _____
4. Phage DNA integrated into the chromosome is called a(n) _____
5. In a mating between Hfr and $F^-$ cells, the $F^-$ recipient _____
6. A phage that can undergo the lysogenic life cycle is said to be _____

A. Specialized transduction
B. Exconjugant
C. Temperate
D. Lysogen
E. Virulent
F. Generalized transduction
G. Transformation
H. Conjugation
I. Recombination
J. Remains $F^-$
K. Becomes $F^+$
L. Becomes Hfr
M. Becomes F′
N. Cannot establish lysogeny
O. Prophage
P. Lytic phage
Q. Specialized transducing phage
R. Insertion sequence
S. Transformant

**10. (16 pts)** In bacterial matings, prophage can be transferred from Hfr to F-. The prophage is automatically induced when it enters F- cells when there is no phage repressor, and the cell is then lysed. Several new Hfr strains of *E. coli* were independently isolated. All were wild type, except for Hfr 1 which was lysogenic for phage lambda. All Hfrs were then mated to a F- strain carrying mutations in the following genes: ara, gal, lys, pro, pyr, rha. The times of first appearance of individual Hfr genes (wild-type alleles) among the recombinants were as follows (in minutes):

| Hfr Marker | Hfr 1 | Hfr2 | Hfr3 |
|---|---|---|---|
| ara | 8 | 60 | 73 |
| gal | 24 | 44 | 89 |
| his | No recombinants | 21 | 12 |
| lys | No recombinants | 4 | 29 |
| pro | 20 | 48 | 85 |
| pyr | No recombinants | 40 | 93 |
| rha | No recombinants | 84 | 49 |

Draw a complete (circular) map of the *E. coli* chromosome, showing the distance (in terms of time) between each of the markers and the approximate location of the lambda prophage. Show the orientation and location of the F factor (i.e., the arrow). Assume a 100-minute map.

**11. ( 6 pts)** Cotransduction experiments were carried out to determine the order of the closely linked genes *tolC, metC,* and *ebg* in the chromosome of *E. coli*. P1 phage of the genotype $tolC^+$ $metC^+$ $ebg^+$ were used to transduce a recipient strain of genotype $tolC^-$ $metC^-$ $ebg^-$

The results are shown in the accompanying table.

What order of genes is consistent with these results?

Gene order: _____ _____ _____

| Selected Marker | Genotypes of unselected markers | Observed Percent |
|---|---|---|
| $tolC^+$ | $metC^+$ $ebg^+$ | 2 |
| | $metC^+$ $ebg^-$ | 12 |
| | $metC^-$ $ebg^+$ | 30 |
| | $metC^-$ $ebg^-$ | 56 |
| $metC^+$ | $tolC^+$ $ebg^+$ | 1 |
| | $tolC^-$ $ebg^+$ | 0 |
| | $tolC^+$ $ebg^-$ | 34 |
| | $tolC^-$ $ebg^-$ | 65 |

**12. (10 pts)** You need a pure culture of *E. coli* cells specifically auxitrophic for methionine and arginine. You also want the cells to be kanamycin resistant and able to use galactose as the sole carbon source. You already have the following *E. coli* strains;

| Strain | F' Genotype | Chromosomal Genotype |
|---|---|---|
| WWU1 | F' $gal^+$ $kan^r$ $str^r$ | $gal^-$ $arg^-$ $kan^s$ |
| WWU2 | F- | $gal^-$ $thi^-$ $leu^-$ $kan^s$ $str^s$ |
| WWU3 | F- | $gal^-$ $met^-$ $arg^-$ $kan^s$ |
| | | |

You have rich media broth for mating, but only two types of selection plates:

Media A MM + + glucose + thimine + leucine + kanamycin + streptomycin
Media B MM + glucose + methionine + arginine + kanamycin

Describe in detail how you would isolate the new strain. Include the strains used for mating, the media used for selection, and exactly which cells would be growing on the plate after selection. You will receive full credit only for a selection strategy that produces the desired pure strain. A flow chart may help you organize the experiment.

**13a. (5 pts)** In a general transduction experiment, T4 phage are grown on $leu^+$ $gal^+$ $str^R$ $tet^S$ *E. coli* and are used to transduce *E. coli* that are $leu^-$ $gal^-$ $str^S$ $tet^R$. What would you supplement minimal media (agar, salts) with to identify the $leu^+$ $gal^+$ $str^R$ $tet^R$ transductants that you plate on these media? gene symbols: leu (leucine synthesis), gal (galactose metabolism), str (streptomycin +/-). tet (tetracycline +/-).

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**13b. (5 pts)** How would you be able to identify the transductants described above.

colony plaques - or - bacteria colonies

**14. (14 pts)** Using a Punnett square or a forked line diagram, determine the theoretical risk of having a Down syndrome child if <u>one</u> parent is heterozygous for a 14q;21q Robersonian translocation. Show genotypes and phenotypes. Use the following designations:

| | |
|---|---|
| 14 | ...for wild-type Chromosome 14 |
| 21 | ...for wild-type Chromosome 21 |
| 14q;21q | ...Robersonian translocation for chromosome 14 and 21 |

For example; a translocation heterozygote would be 14, 21, 14q;21q, and viable. A wild-type would be 14, 14, 21, 21.

**Answer as a ratio:_____**

**- Extra Credit on the back of this page -**

## Extra Credit. (6 pts)

**Problem:** Although deletions were extremely important in Benzer's *rII* mapping experiments, they did not come labeled as "deletions" but had to be identified as deletions by experiments. Two principles were used in this identification: (1) deletions cannot undergo reverse mutation to wildtype, and (2) deletions do not map as "point mutations" in recombination experiments but fail to recombine with two or more mutations that do recombine with each other. This problem illustrates the second approach. The accompanying data are from some of Benzer's experiments showing the observed frequency of recombination between all combinations of 8 *rII* mutations. To avoid unnecessary complexity, the mutations have been renamed in sequential order, and the frequencies of recombination have been adjusted to make them perfectly additive. Identify which of the mutations are "point mutations" and which are deletions. Draw a genetic map showing the locations of the point mutations and the frequencies of recombination between adjacent mutations, and indicate the location and extent of each deletion.

| | *r1* | *r2* | *r3* | *r4* | *r5* | *r6* | *r7* | *r7* |
|---|---|---|---|---|---|---|---|---|
| *r1* | 0 | 0 | 0.6 | 1.0 | 1.1 | 0 | 1.3 | 2.5 |
| *r2* | | 0 | 0 | 0.1 | 0.2 | 0 | 0.4 | 1.6 |
| *r3* | | | 0 | 1.6 | 0.5 | 0 | 0.7 | 1.9 |
| *r4* | | | | 0 | 2.1 | 0.5 | 2.3 | 3.5 |
| *r5* | | | | | 0 | 0 | 0.2 | 1.4 |
| *r6* | | | | | | 0 | 0 | 0.9 |
| *r7* | | | | | | | 0 | 0 |
| *r8* | | | | | | | | 0 |

# Reference Page

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  1 mapaiglppa stfpqlcfvi lalsgsaiaa kgstkrisig svfdveseki qtafrfavdr
 61 fntiensaql klnplreeid dtdsfslgna lcsimskgvf avfgkanssm latvksysdt
121 fqipylttsm amnttdqspy mlflrpinir aivdliehlg wrvvhyiyis neglmrvqql
181 fqvmgksdlq mtlnvkrasd vnssyvilke lhhtnpeldi havldmsipm aselmnllse
241 dprvhnrrfh fllvepgiqe ldfakiglyg ynvsgfqlvd fnnmtvrlfl sdwtkidpae
301 wpgagvktit yeaalavdav slftramknl snlglfeslf vrarsganss ktcaaerlnv
361 wnkgkhvlka mketefdglt grvafddrgh rkeftldvld igitrgavki gywtprdglt
421 mlkrmvrpin apssenrtri vttiqtppyi mkkpkpidgh plignd kyeg ycvdlarkva
481 hevgfdyvfq mvkdgaygsk landswngmv gelirleadm aiapltisav rervidfskp
541 fmslgisimi kkpadqkahv fsfldplsye iwmcilfafi gvsvvlflvs rfspsgwhve
601 desnitndft isnslwfslg afmqqgcdfs prsisgrivg svwwfftlii issytanlaa
661 fltvermstp iesaedlakq teieygtlrs gtteaffkts kvavyermwa ymtsktpsvf
721 tdkiqdgitr vrdsngkyaf lvesstndyi nnrlpcdtmk vgsnldskgf giatpagsdl
781 gdkltlavlk lredgeldkl qkfwwvgkgq ctpqdkntdg gqsaltlsnv agifyiligg
841 lilaiivava eflykskvds kkskytytgp sqsmgfdtvp egnthtqv
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## The standard genetic code

| First position (5' end) | Second position | | | | Third position (3' end) |
|---|---|---|---|---|---|
| | U | C | A | G | |
| U | UUU Phe } F<br>UUC Phe }<br>UUA Leu } L<br>UUG Leu } | UCU Ser }<br>UCC Ser } S<br>UCA Ser }<br>UCG Ser } | UAU Tyr } Y<br>UAC Tyr }<br>**UAA Stop**<br>**UAG Stop** | UGU Cys } C<br>UGC Cys }<br>**UGA Stop**<br>UGG Trp W | U<br>C<br>A<br>G |
| C | CUU Leu }<br>CUC Leu } L<br>CUA Leu }<br>CUG Leu } | CCU Pro }<br>CCC Pro } P<br>CCA Pro }<br>CCG Pro } | CAU His } H<br>CAC His }<br>CAA Gln } Q<br>CAG Gln } | CGU Arg }<br>CGC Arg }<br>CGA Arg } R<br>CGG Arg } | U<br>C<br>A<br>G |
| A | AUU Ile }<br>AUC Ile } I<br>AUA Ile }<br>**AUG Met** M | ACU Thr }<br>ACC Thr } T<br>ACA Thr }<br>ACG Thr } | AAU Asn } N<br>AAC Asn }<br>AAA Lys } K<br>AAG Lys } | AGU Ser } S<br>AGC Ser }<br>AGA Arg } R<br>AGG Arg } | U<br>C<br>A<br>G |
| G | GUU Val }<br>GUC Val } V<br>GUA Val }<br>GUG Val } | GCU Ala }<br>GCC Ala } A<br>GCA Ala }<br>GCG Ala } | GAU Asp } D<br>GAC Asp }<br>GAA Glu } E<br>GAG Glu } | GGU Gly }<br>GGC Gly }<br>GGA Gly } G<br>GGG Gly } | U<br>C<br>A<br>G |