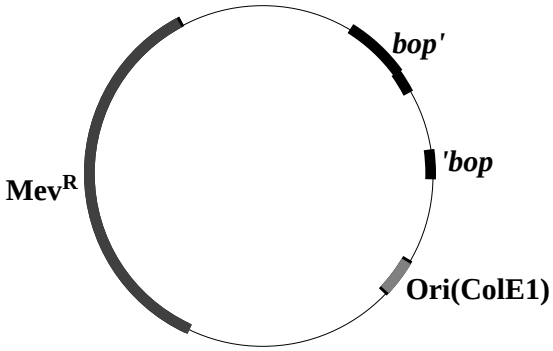
Exam 3 **Name _____**
Mcbio 316

Q1. (10) ColE1 plasmids replicate in enteric bacteria but cannot replicate in *Halobacterium salinarium*. The *bop* gene from *H. salinarium* was cloned into a ColE1 plasmid, and an insertion mutation constructed that disrupted the plasmid encoded *bop* gene (indicated by *bop'-'bop* in the figure below). This plasmid was then transformed into *H. salinarium* with selection for resistance to the antibiotic mevinolin (Mev^R).



a. The plasmid does not have any functional replication origin and, except for the *bop* gene, the plasmid lacks any homology with the *H. salinarium* chromosome. How do the Mev^R transformants arise? [Show a diagram and briefly describe your answer.]
ANSWER: The plasmid cannot replicate, so the Mev^R colonies must arise by integration of the plasmid into the chromosome by homologous recombination between the *bop* genes (see the figure on the following page).

b. When the resulting transformants were subsequently grown for many generations without mevinolin, some Mev^S colonies were obtained. About half of these Mev^S colonies were Bop^+ and half were Bop^-. How do the Mev^S Bop^- colonies arise? [Show a diagram and briefly describe your answer.]
ANSWER: Segregration of the chromosomal *bop* duplication will yield Mev^S colonies. Sometimes the recombination will occur on the same side of the *bop* disruption as the initial integration event and these will result in bop^+ colonies [crossover (i) in the figure below]. Sometimes the recombination will occur on the other side of the *bop* disruption relative to the initial integration event and these will result in bop^- colonies [crossover (ii) in the figure below].

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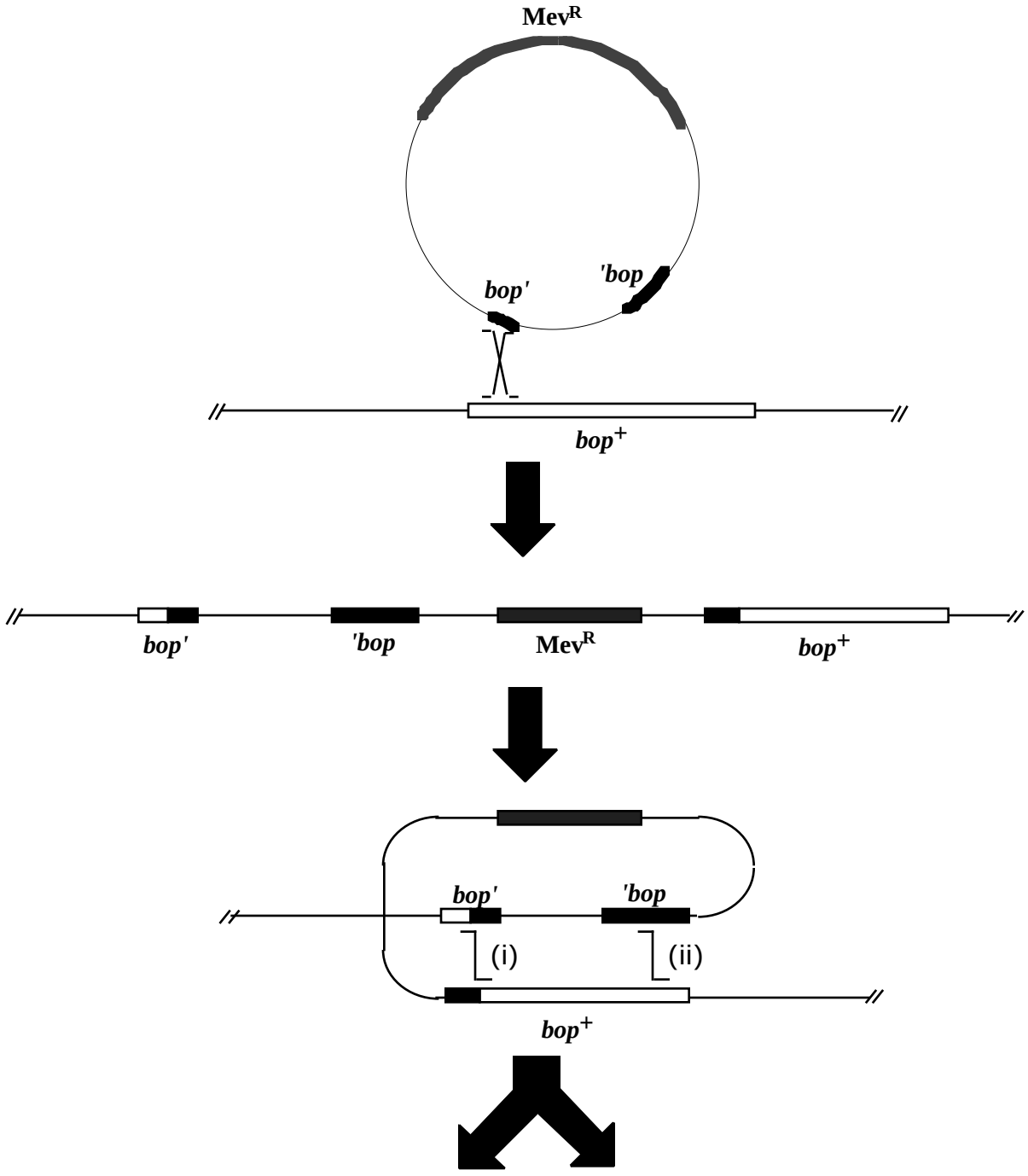


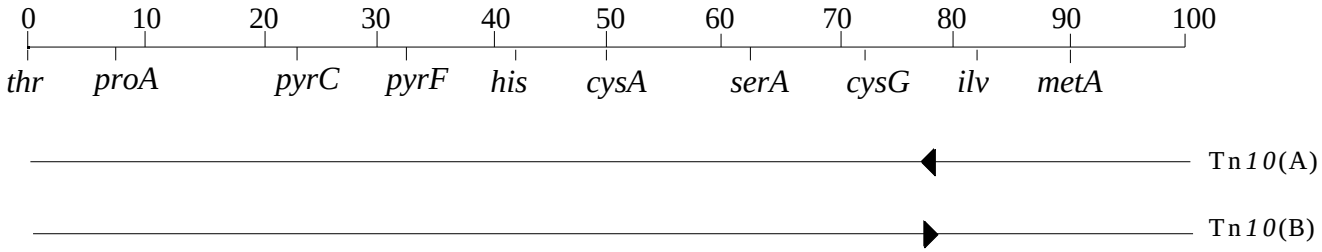
(i) Mev^S *bop*$^+$ (ii) Mev^S *bop*$^-$

Q2. (10) Two different F' (Ts) Tn*10* *lac*$^+$ plasmids were integrated into a chromosomal Tn*10* insertion located near a new mutation called *proZ* in *S. typhimurium*. The two F's were identical except for the orientation of the Tn*10* insertions on the plasmid: the Tn*10* insertions labeled (A) and (B) are in the same site but in opposite orientations on the F'. To determine the map position of the *proZ* gene, the Hfr's were mapped by mating with a collection of Str^R auxotrophic recipients. The results are shown in the table below.

Recipient	Map position (min)	Number of recombinants	
		Hfr Tn*10*(A)	Hfr Tn*10*(B)
thrA	0	++	−
proA	7	+	−
pyrC	23	+	+
pyrF	33	+	+
his	42	−	+
cysA	50	−	++
serA	63	−	++
cysG	73	−	+++
ilv	83	+++	−
metA	90	+++	−

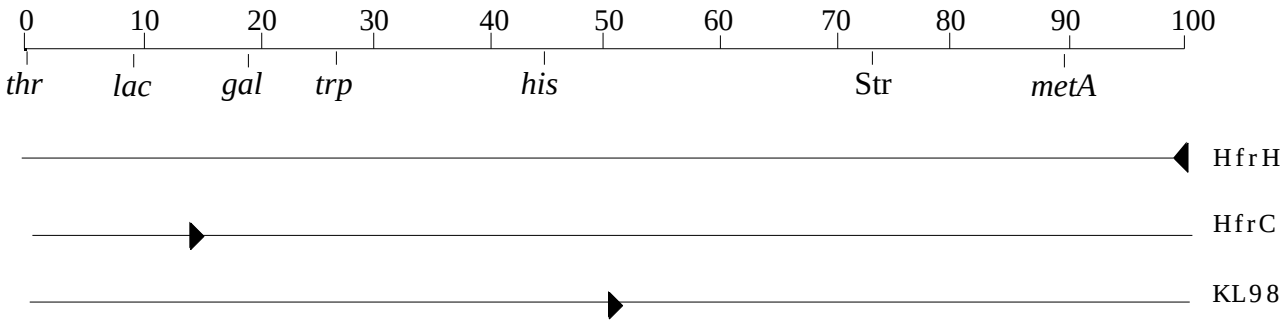
+++ indicates over 1000 colonies, ++ indicates 200 to 1000 colonies, + indicates 50 to 200 colonies, and - indicates less than 50 colonies.

a. Based on these results, draw a map showing the position of each Hfr insertion (relative to the chromosomal genes) with arrowheads showing the direction of transfer.
ANSWER:



b. Based on these results, where does the *proZ* gene map?
ANSWER: Between *cysG* at 73 min and *ilv* at 83 min.

Q3. (20) Several Hfrs from *E. coli* are shown in the figure below with the direction of transfer indicated relative to the map shown at the top of the figure. Each of the Hfr strains is *metA*$^-$, otherwise, the Hfr strains carry wild-type copies of the genes shown on the map (i.e., the genotype of the Hfr strains is *thr*$^+$, *lac*$^+$, *gal*$^+$, *trp*$^+$, *his*$^+$, *str*^{S}, *metA*$^-$). Each of these Hfr's was mated with a F$^-$ *gal*$^-$ *thr*$^-$ recipient.



a. What medium would you use to select for Gal$^+$ Thr$^+$ recombinants?
ANSWER: Minimal medium with galactose as a carbon source + tryptophan + histidine

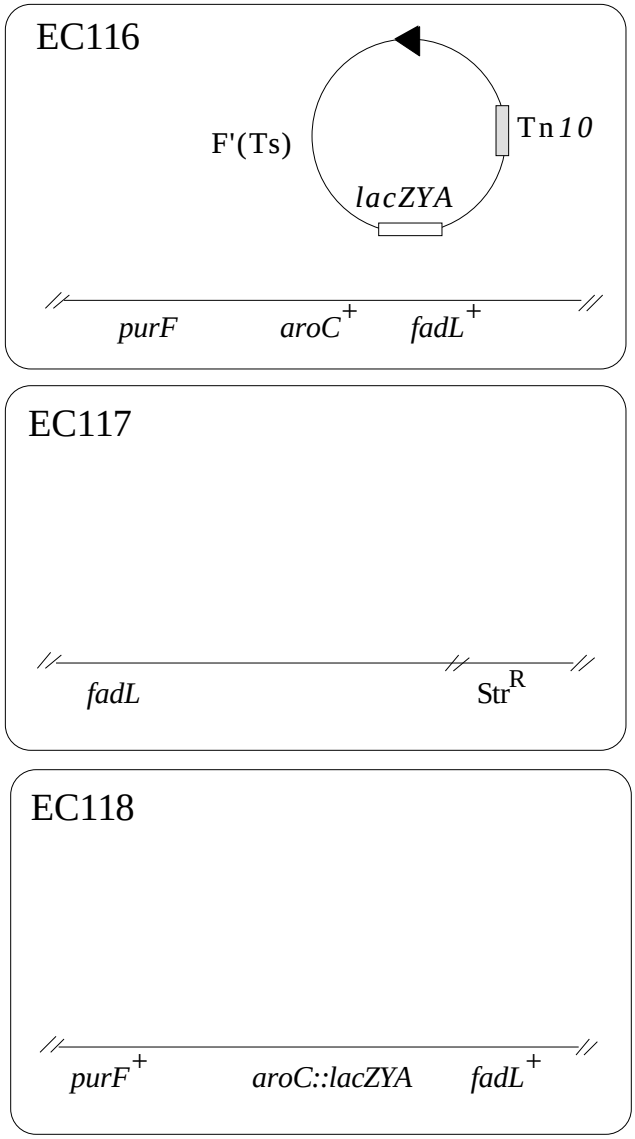
b. How would you counterselect against the donors?
ANSWER: Plate on minimal medium without methionine: the recipient strain will grow because it is Met$^+$ but the donor strain will not grow because it is *metA*$^-$.

c. Rank the three Hfr donors to indicate the relative frequency of Gal$^+$ Thr$^+$ exconjugants expected on each mating plate.
ANSWER: HfrH > KL98 >> HfrC

d. Given a new Hfr with an unknown location and a multiply mutant strain with the genotype *thr*, *lac*, *gal*$^-$, *trp*$^-$, *his*$^-$, *str*^{R}, *metA*$^-$, how would you determine the location of the Hfr? [Briefly describe how you would map the Hfr, including the medium you would use for any selections and how you would interpret the results.]
ANSWER: (1) Mate the new Hfr with the multiply mutant recipient selecting for repair of each recipient marker individually (i.e. select Thr$^+$ by growth on minimal medium with tryptophan, histidine, and methionine; select Lac$^+$ by growth on minimal medium with lactose as a carbon source and supplemented with threonine, tryptophan, histidine, and methionine; etc) and counterselecting for Str^R. (2) Count the number of recombinants obtained -- the closer the selected markers that are closer to the origin of transfer of the Hfr (in the correct direction) will be inherited most frequently. Note that both the donor and

recipient are *metA* so the Met$^+$ recombinants will probably be rare no matter where the Hfr is located.

Q4. (15) The *fadL* gene product is required for *Salmonella typhimurium* to grow on long chain fatty acids. How could you use the following three strains (EC116, EC117, and EC118) to construct an F'(Ts) *fadL*$^+$? [Clearly descibe each step required, including the selections used, and the genotype of each derivative. Hint: the *aroC* gene product is required for biosynthesis of aromatic amino acids and the *purF* gene product is required for biosynthesis of purines. Note that the F' in strain EC116 has no homology with the wild-type *S. typhimurium* chromosome.]



ANSWER: Construction of the desired F' would require several steps.

(1) Mate EC116 with EC118 at 30°C selecting Tet^R with counter-selection for Pur^+. This will yield EC118 / F'(Ts) Tn*10* lac^+. (This step should be done at 30°C because if you shift the cells to 42°C the mating efficiency will drop dramatically because replication of the F' is temperature sensitive.)

(2) Select for integration of F'(Ts) into the chromosome by demanding growth at 42°C on medium with tetracycline. (It is important to select for Tet^R because otherwise the F'(Ts) may simply segregrate from the cell at 42°C. Note that both the F' and the chromosome carry the lac^+ genes.) This will yield EC118 with an Hfr integrated within *aroC::lac* (and hence resulting in a duplication of the *lac* genes.)

(3) Grow the EC118 Hfr at 30°C to allow segregration of the F'.

(4) Mate the resulting population of cells (including EC118 Hfr, EC118 F' Tn*10* lac^+, and any EC118 F's arising due to abbarent excision of the integrated Hfr) with EC117 at 30°C, selecting for $FadL^+$ (growth on long chain fatty acids) with counter-selection for Str^R. Interrupt the mating after 20 to 30 min to prevent transfer of late markers (such as $fadL^+$) from the Hfr. This will yield EC117 with a F' (Ts) carrying $fadL^+$.

Q5. (5) Describe one good reason for determining the map location of a gene on the chromosome. [Your answer should include both why the map location is useful and how the map location is used to achieve the indicated goal.]

ANSWER: There are many possible answers to this question. A few examples are:

- **To use nearby markers as a selection for strain construction or localized mutagenesis.**
- **To construct defined chromosomal deletions or duplications of the gene and/or neighboring genes for use in complementation analysis or to select for dominant mutations.**

Q6 (10) Phage λ was grown on three different strains of *E. coli* and the resulting lysates were then titered on each of these three strains. (Strain P was obtained by transduction of strain K with phage P1.) The results are shown in the following table. Suggest an explanation for these results and briefly describe your logic.

λ grown on strain	EOP when plated on *E. coli* strain			**Interpretation**
	C	K	P	
E. coli C	1	$< 10^{-4}$	$< 10^{-4}$	$\mathbf{r^-m^-}$
E. coli K	1	1	$< 10^{-4}$	$\mathbf{r_K^+m_K^+}$
E. coli P	1	1	1	$\mathbf{r_K^+m_K^+\ r_P^+m_P^+}$

ANSWER:

***E. coli* C does not restrict or modify λ DNA grown on any of the three strains, thus it is probably r^-m^-.**

***E. coli* K does not restrict or modify λ DNA, grown on *E. coli* K because it would be modified by the $r_K^+m_K^+$ system. However, it will restrict the unmodified DNA from *E. coli* C.**

***E. coli* P does not restrict or modify λ DNA, grown on *E. coli* P because it would be modified by the $r_P^+m_P^+$ system. Furthermore, *E. coli* P is a derivative of *E. coli* K (obtained following infection with phage P1) and thus has the *E. coli* K restriction system, so it does not restrict λ grown in *E. coli* K (modified by the $r_K^+m_K^+$ system). However, it will restrict the unmodified DNA from *E. coli* C.**

The simplest explanation for these results is that *E. coli* P contains a phage P1 prophage (from the phage infection during transduction) and the P1 restriction system can cleave any incoming DNA not modified by $r_P^+m_P^+$.

Q7. (15) The *E. coli fnr* gene encodes a protein that regulates transcription of many genes required for anaerobic growth. It was proposed that Fnr protein becomes functional under anaerobic conditions due to the reduction of an iron-sulfur center in the protein. Such an iron-sulfur center could be formed by the coordination of 4 cysteine residues with iron. To determine if the cysteine residues of Fnr are essential for its function, each of the 5 cysteine codons of *fnr* was changed by site-directed mutagenesis using the *dut ung* method. The results are shown below.

Mutation	Fnr Activity
Cys 16 -> Ala	+
Cys 20 -> Ser	–
Cys 23 -> Gly	–
Cys 29 -> Gly	–
Cys 122 -> Ala	–

a. Do these results prove that any of the Cys residues are directly involved in the function of the Fnr protein? [Explain your answer.]

ANSWER: No. It is possible that the mutations at positions 20, 23, 29, and 122 result in loss of Fnr activity because they interfer with proper folding of the protein. In particular, the Cys -> Gly substitutions change both the size and polarity of the amino acid side group.

(This question does not explicitly state that there are no other mutations in the resulting protein, so it is also possible that in the process of site directed mutagenesis another mutation was produced at a different site in the Fnr protein and it is the second, unidentified mutation that results in loss of Fnr activity. When doing site directed mutagenesis, it is important to eliminate this possibility by either reversion analysis or sequencing the entire gene and associated regulatory regions.)

b. If you wanted to make "safe" amino acid substitutions at the Cys residues, what amino acid/codon substitutions would you have used and why?
ANSWER: Alanine which has a somewhat smaller side group but similar polarity, or Serine which has a similar size but is slightly more polar than Cysteine would be good bets. Note that although Proline is a small, nonpolar amino acid like Cysteine, Proline would not be a safe substitution because due to its ring structure it can dramatically affect the secondary structure of a polypeptide chain.

c. Draw a diagram showing each of the steps of the *dut ung* method of site-directed mutagenesis and briefly describe the rationale of each step.

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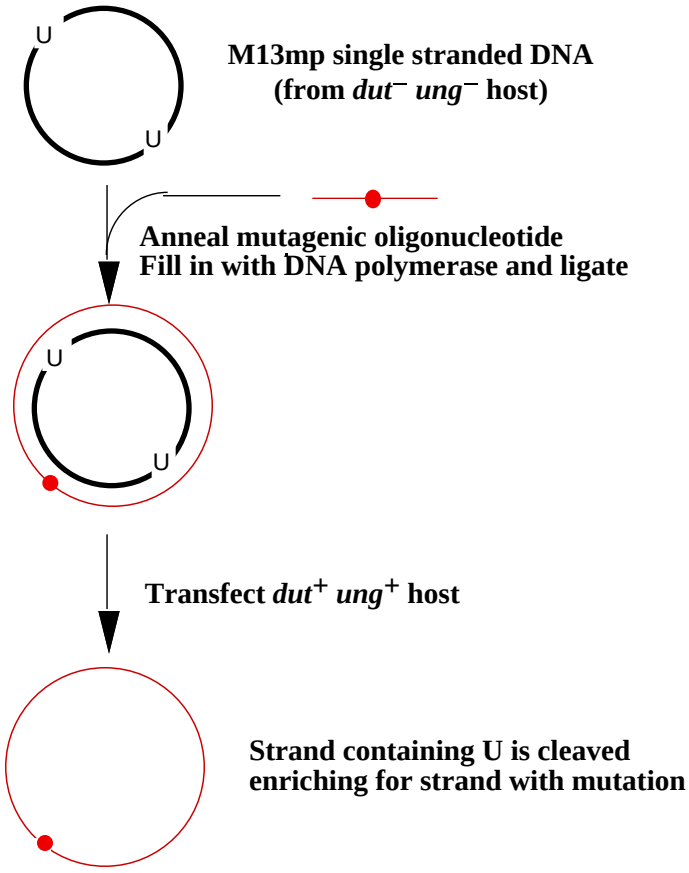

Q8. (15) A plasmid clone of the *mucAB* genes is shown below. A linear representation of the *mucAB* genes are shown on the left side with the position of four complementary 20-mers (labeled a-d with the arrowhead at the 3' end of the oligonucleotides). There are no additional sites on this plasmid for any of the restriction enzymes shown in the cartoon.

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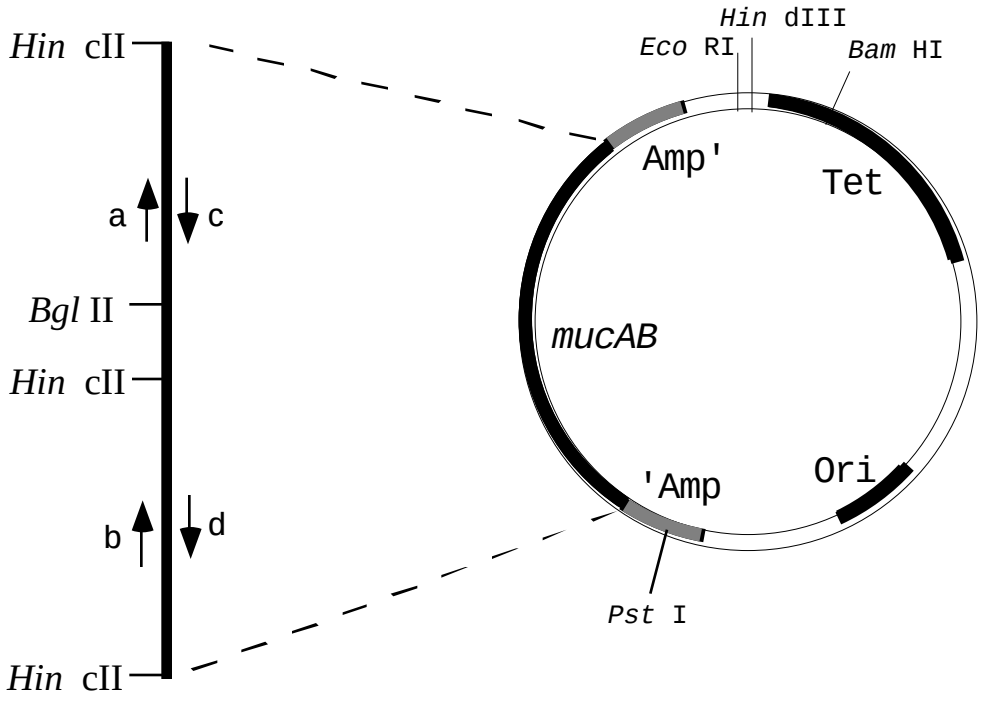

a. Using this plasmid and any of the four oligonucleotides shown, how could you use PCR to construct a deletion that removes the central portion of the *mucAB* genes. [Describe the primers you would use and how you would construct the new plasmid. You do not need to describe the details of PCR.]

ANSWER: Cut the plasmid with *Bgl* II. Use primers a and d for PCR. This will amplify the entire plasmid except the region between these two primers (that is, the region between the tips of the two arrowheads including the primer sequences). Ligate the resulting PCR fragment. (See the following diagram

b. How could you enrich for the desired deletion mutants?

After amplification digest the plasmids with *Bgl* II then transform into cells selecting for Tet^R. Any parental plasmids left over will be linearized and the linear DNA will be degraded by the RecBCD exonuclease upon entering the recipient. The desired deletion derivatives will not be digested with *Bgl* II and will thus yield Tet^R transformants.

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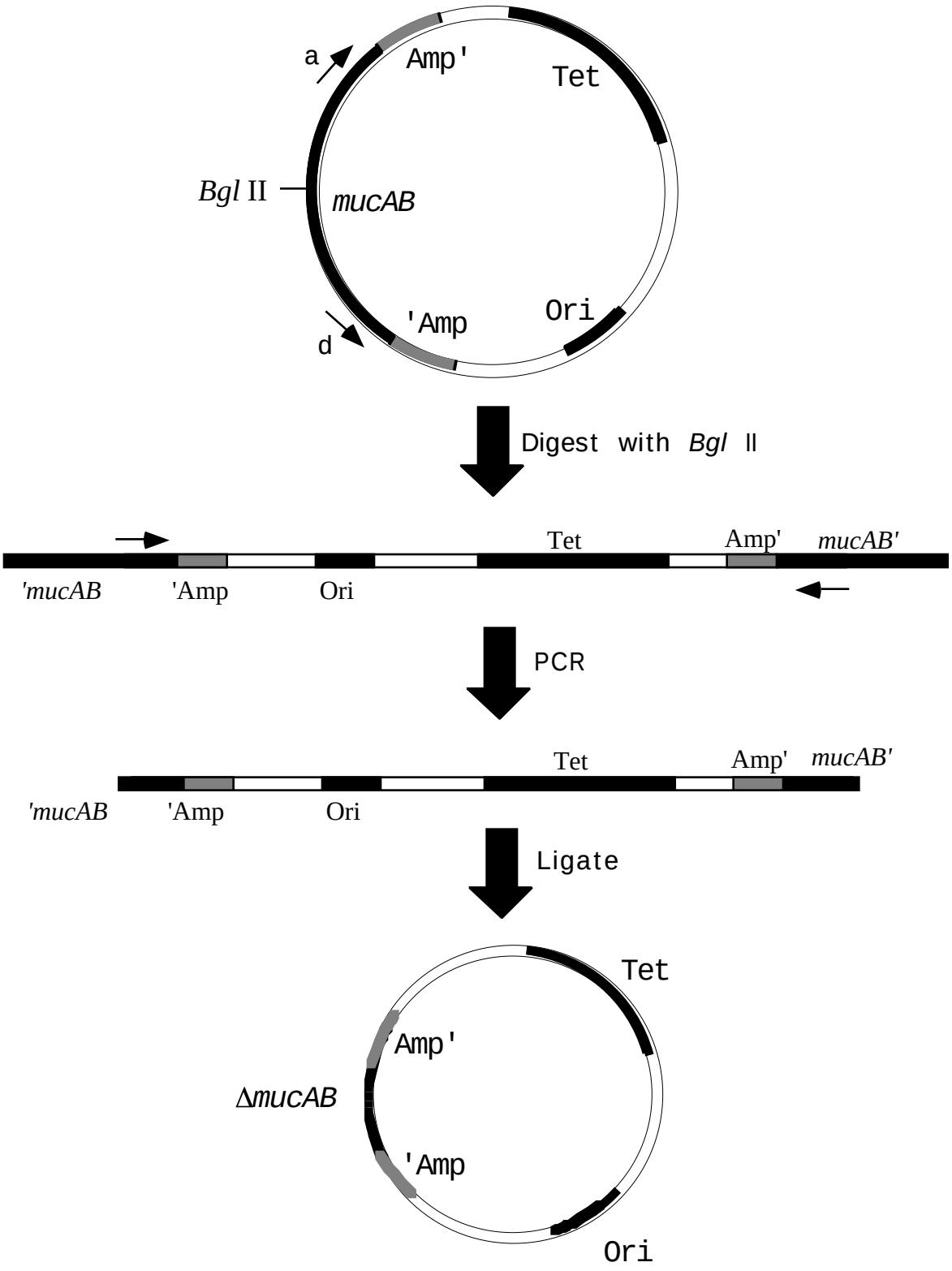
a
Amp'
Tet
Bgl II
mucAB
'Amp
Ori
d
Digest with Bgl II
Tet
Amp'
mucAB'
'mucAB
'Amp
Ori
PCR
Tet
Amp'
mucAB'
'mucAB
'Amp
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Ligate
Tet
Amp'
ΔmucAB
'Amp
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