Bacterial Genetics
GMS6038
Final Exam
Fall 2006

Make up a code name for yourself and put it here. ___________________________

Write down the code with your name on a sheet for the class so I can grade the exam in a blinded manner.

You have 2 hours from when you arrived to complete the exam.

Please note - this is a closed book, closed note exam. All backpacks and notebooks must be against the wall, not at your table.

You may use the rest room one person at a time.

Any cheating will result in a 0 for the exam and failure of the course.

Shown below is pGULIG-8. Starting at the top, note the following genetic elements:

At the top is the origin of replication of the ColE1 plasmid. The promoter for RNA-II has been replaced by the lac promoter (for lacZYA).

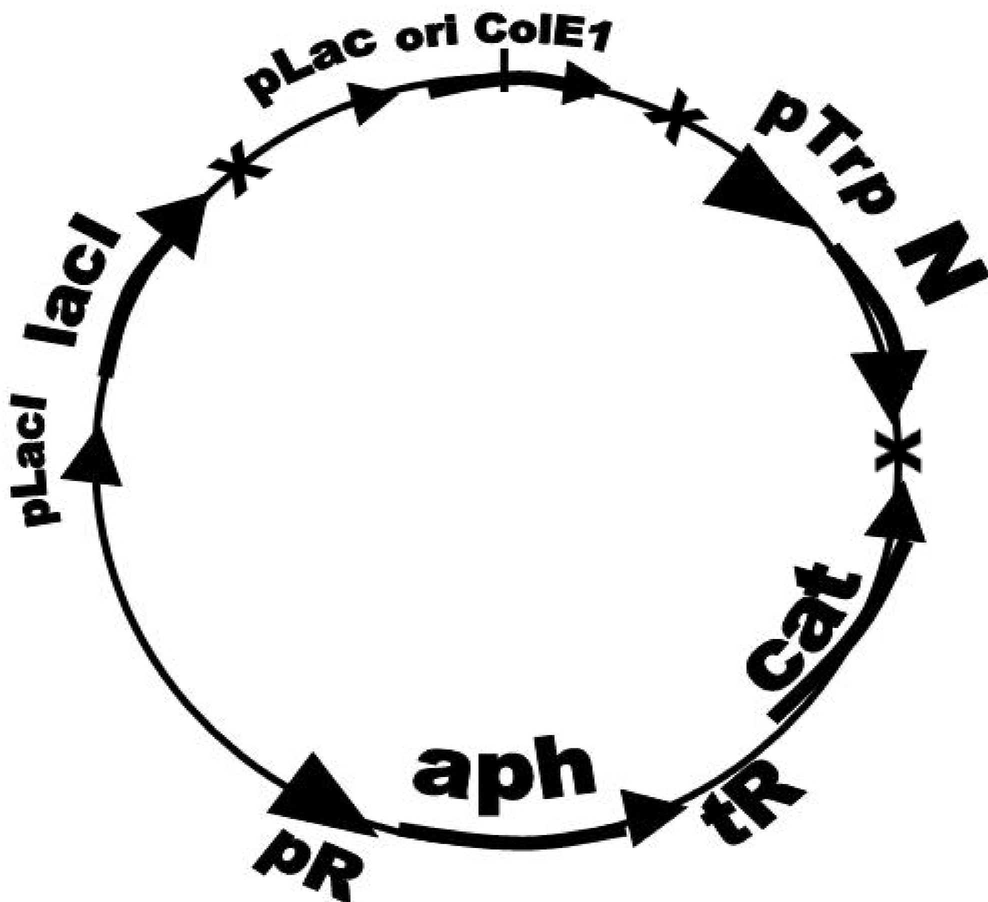
In the upper right is the Lambda phage N gene expressed from the trp promoter (does not include trpL).

In the bottom right is the Lambda phage pR promoter driving expression of the aph gene followed by the Lambda phage tR terminator followed by the cat gene. Note that the cat gene does not have its own promoter.

At the left is the lacI gene driven by its own promoter.

Assume that all genes have appropriate translation sequences. The x's on the map represent typical factor-independent terminators that will prevent transcriptional read through between these different elements.

Don't forget that the host E. coli K12 strain has the normal chromosomal genes, no F plasmid, and no Lambda phage or any other phages.



NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. Keep to what is being asked. Adding extra material hoping to include the right answer somewhere in the middle could count against you if your answer reveals a lack of understanding or includes incorrect information, even if unrelated to the original question.

Write your answers legibly on separate lined paper with your code on each page.

Questions 1 through 4 are based on pGULIG-8. Here is a clue to help work these questions. Start at the genetic element that is being asked about and then work your way through the rest of the plasmid looking for genes that are related to those. Don't try to take in the whole plasmid at once. Don't forget to consider genes contributed by the E. coli host.

1. What would you include or exclude from the growth medium to:

A. (5 points) cause the highest copy number of this plasmid? Explain.

+ IPTG or lactose, no glucose
LacI will repress the lac promoter, and IPTG/lactose is the inducer
The lac promoter is under catabolite repression, so adding glucose would prevent expression from the lac promoter, so leave it out.

It would have been ideal if you stated that initiation of replication is caused by transcription of RNA-II through the origin of replication.

B. (5 points) cause the lowest copy number of this plasmid (maybe even prevent its replication altogether)? Explain.

- IPTG, + glucose
don't want to transcribe lac promoter, so don't add inducer
adding glucose will cause catabolite repression

C. (2 points) If the lac promoter was exchanged with a trc or tac promoter, how would this affect your answers above? Explain.

Adding glucose would have no effect because these hybrid trp-lac promoters are insensitive to catabolite repression (trp is not under catabolite repression).

2. (4 points) What functions are encoded by the aph and cat genes?

aph – aminoglycoside phosphotransferase = kanamycin resistance
cat – chloramphenicol acetyl transferase = chloramphenicol resistance

3. A. (6 points) How would adding or omitting tryptophan in the growth medium of E. coli possessing this plasmid affect antibiotic resistance? Explain.

adding trp – the TrpR repressor protein would be encoded by the chromosome. trp bound to TrpR represses the trp promoter, so N gene and protein would not be made. N is required to read through the tR terminator, so only the aph gene would be expressed by the pR promoter. The bacteria would be kanamycin resistant.

Not adding trp – Without trp, TrpR will not repress the trp promoter, so N will be made and transcription will proceed through tR. The bacteria will be kanamycin and chloramphenicol resistant.

B. (2 points) If the trp sequences upstream of the N gene included the trpL gene, how would this affect your answer to part A? Explain.

trpL is where attenuation takes place if the ribosomes do not stall at the two trp codons because there is plenty of charged trp-tRNA. Since regulation of the trp promoter is already being done directly by TrpR, this likely wouldn't make a difference. It might enable tighter repression of transcription downstream of trpL if trp is provided. Without trp – no difference.

4. (4 points) If this plasmid was placed into an E. coli strain that was lysogenized by Lambda phage, how might this affect antibiotic resistance conferred by this plasmid?
Explain.

The Lambda lysogen will be expressing CI. CI will repress the pR promoter, so neither antibiotic resistance will be expressed.

The following questions are independent of pGULIG-8

5. A. (2 points) What is the definition of a facultative anaerobe?

a bacterium that can grow under both aerobic and anaerobic conditions. It tolerates a lack of oxygen.

B. (6 points) E. coli is a facultative anaerobe. How does this fact relate to the optimal growth conditions for enabling this bacterium to produce your favorite cloned gene? Explain. You only need to discuss optimal growth conditions related to being a facultative anaerobe, but if you discuss all aspects of optimal growth, you can get extra credit.

Even though E. coli can tolerate a lack of oxygen, it thrives better with oxygen because aerobic respiration enables more ATP generation per molecular of glucose provided. Put the culture in a flask and shake it well.

Extra credit – 37C because it is a mesophile. Induce at log phase, not stationary phage. Provide a rich growth medium with glucose, if possible.

6. (6 points) Name two unrelated (in different families) antibiotics that affect DNA synthesis. Very briefly explain how each interferes with DNA synthesis.

quinolones – nalidixic acid, ciprofloxacin – bind to DNA gyrase and inhibit supercoiling

metronidazole – metabolized by anaerobes and inhibits DNA synthesis

trimethoprim and sulfa drugs – inhibit folic acid metabolism which inhibits thymidine synthesis

(These are actually in different families structurally, but since they act in the same place, I hope that you didn't choose both of these as your examples and skip the examples immediately above.)

7. A. (6 points) Let's say that you want to convert your favorite cloning vector into one that can be moved by conjugation. What DNA sequences would you have to add to your vector and what DNA sequences/genetic elements would you need to add to your host donor E. coli strain? Briefly explain how this system will work for conjugation.

You need a mob site (origin of transfer) on your plasmid. This is cis active. This is the site where the tra functions act.

You need the transfer functions somewhere in the E. coli. These genes encode the pilus biosynthesis and the enzymes that copy the plasmid DNA into the recipient. These are trans active functions that don't have to be on the plasmid.

Your plasmid will be much smaller and more usable if you provide the tra genes elsewhere in the E. coli.

B. (6 points) To conjugate your plasmid from the donor to the recipient, you need a selectable genetic marker in the recipient strain so that you can plate the conjugation mixtures of the donor and recipient strains on this antibiotic to kill or inhibit the donor E. coli strain. So you need to create an antibiotic-resistant derivative of your recipient strain. Without using any plasmids or other genetic exchange, briefly explain how you will make your recipient strain antibiotic resistant. Explain at the genetic and functional level why your bacteria are now resistant.

You plate the bacteria on plates containing the antibiotics of choice – rifampicin, nalidixic acid, or streptomycin. Spontaneous resistant mutants will grow. The targets of the antibiotics will have been changed so they retain function but are not recognized by the antibiotic.

Here are the relevant genes – streptomycin – rpsL for ribosomal protein; nalidixic acid – gyrA for gyrase; rifampicin – rpoB for RNA polymerase

8. (6 points) Why is Lambda phage very good at specialized transduction but terrible at generalized transduction?

good at specialized transduction – it is temperate and integrates into the E. coli genome so it can accidentally pick up an adjacent gene (gal or bio) when it excises

bad at generalized transduction – it demands a cos site for packaging, so it cannot accidentally pick up pieces of host genome during lysis

9. A. (2 points) What is the mechanism of action of beta-lactamase?

It is an enzyme that cleaves beta-lactam antibiotics.

B. (4 points) Where (on what genetic element) would you normally expect the bla gene to be encoded? Why?

plasmid – Since this is a whole new enzyme (as opposed to the mutations described above) it has to be inherited in a preformed package from somewhere else.

C. (6 points) Beta-lactamase enzyme is normally found in the periplasm of E. coli. How does E. coli target beta-lactamase to the periplasm, both in terms of the critical elements of the protein itself and cellular components/functions that get the protein to the periplasm.

The protein has an N-terminal signal (leader) sequence that is recognized by the SecB protein (not the SRP since the SRP sends proteins into the inner membrane). SecB leads the protein to SecA and the General Export Pathway that pushes the protein into the periplasm. Leader peptidase will cleave off the leader sequence.

D. (2 points) If you specifically removed the portion of the bla gene that encodes the critical elements of the protein required for localization of the protein to the periplasm, do you believe that the protein would still enable resistance to beta-lactam antibiotics? Why or why not?

Without the leader sequence the beta-lactamase would end up in the cytoplasm. Since the beta-lactam antibiotics act in the periplasm where the penicillin binding proteins (e.g., transpeptidase) are located, the resistance mechanism will be useless.

10. (4 points) Why do bacteria have ribosome binding sites but eukaryotes do not?

Bacteria have polycistronic messages but eukaryotes do not. So ribosomes cannot simply scan for the first ATG start codon at the beginning of a message. They must be able to initiate translation internally in the messages.

11. (8 points total) Compare and contrast (a table or list is adequate) the type 2 and type 3 terminal secretion pathways in terms of:

A. final location of the secreted protein

2 – extracellular space 3 – injected into host cell

B. if the secreted protein utilizes the General Export Pathway

2 – Yes 3 – No

C. the cellular component that most resembles or depends on each pathway

2 – Type 4 pili (many secreted proteins use this) 3 – flagella

D. if the common laboratory E. coli strain K12 has the terminal pathway

2 – No 3 - No

12. (8 points) Using only a few words or possibly a diagram, describe the **single** most important/characteristic feature of each of the four bacterial cell wall types.

Gram-positive – thick peptidoglycan Gram-negative – outer membrane
Acid fast – waxes and lipids Wall-less – no cell wall or peptidoglycan

13. You clone your favorite gene into a common expression vector and electroporate it into E. coli. You choose a single colony grown on a suitable antibiotic to select for the plasmid and then grow a broth culture overnight. However, you failed to include the antibiotic in the overnight broth. The next day the culture is turbid because it grew to stationary phase. Realizing your mistake, you dilute the culture and plate it on plates with and without the antibiotic. You find that only 10% of the bacteria were capable of growing on the antibiotic-containing plates.

A. (4 points) Assuming that the antibiotic-sensitive bacteria are not contamination, what happened to allow these bacteria to appear?

As the bacteria replicated, the plasmid failed to either replicate or partition into each progeny cell. Once cured of the plasmid, the bacteria continued to grow without detriment. The bacteria did not exclude, spit out, exocytose, or degrade the plasmid.

B. (2 points) How might the gene that you cloned into the vector affect the appearance of the sensitive bacteria?

If your gene product taxed the energy or was otherwise toxic or detrimental to the host bacteria, the plasmid-free derivatives would grow faster and take over the culture.

14. Extra credit (up to 10 points) rpoH encodes the heat shock alternative sigma factor. When E. coli cells are subjected to a heat shock, the level of transcription of rpoH does not increase very much, but the levels of the RpoH sigma factor protein increase a lot. Explain why this happens. This answer could be longer than a couple of sentences!

DnaK is a heat shock protein that recognizes misfolded proteins in a shocked cell and targets the proteins for degradation. If there are no misfolded proteins (normal growth), DnaK binds to RpoH and causes it to be degraded. Under a heat shock condition, DnaK binds to the misfolded proteins and thereby lets RpoH become stable. Therefore, the levels of RpoH protein increase relative to the amount of mRNA and transcription. RpoH is responsible for transcribing dnaK.