PLNT2530 PLANT BIOTECHNOLOGY

# MID-TERM EXAMINATION

11:30 am to 12:20 pm Monday, March 14, 2022

Answer any combination of questions totaling to exactly 100 points. If you answer questions totaling more than 100 points, answers will be discarded at random until the total points equal 100. The questions total to 120 points. This exam is worth 20% of the course grade.

Hand in these question sheets along with your exam book. Question sheets will be shredded.

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Ways to write a readable and concise answer:
i. Just answer the question. Save time by specifically addressing what is asked. Don't give irrelevant background if it doesn't contribute to the question that was asked.
ii. Avoid stream of consciousness. Plan your answer by organizing your key points, and then write a concise, coherent answer. Make your point once, clearly, rather than repeating the same thing several times with no new information.
iii. Point form, diagrams, tables, bar graphs, figures are welcome. Often they get the point across more clearly than a long paragraph.
iv. Your writing must be legible. If I can't read it, I can't give you any credit.

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1. (10 points) Why is it that plants cells in tissue culture can regenerate into complete organ systems such as shoots or roots? In particular, in what way do plants lend themselves to regeneration, where animals do not?

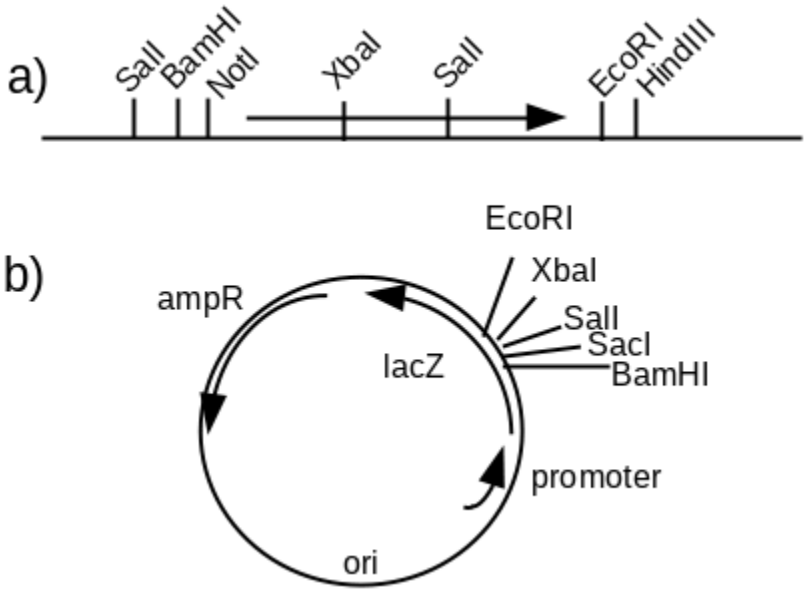
2. (10 points) When making a cDNA library with a Lambda phage vector, the protocol typically includes cutting with a restriction enzyme, followed by dephosphorylation of the vector using alkaline phosphatase. Alternatively, one might consider dephosphorylating the cDNA instead of the Lambda arms. What would be the problem with this alternative approach?

3. (5 points) The simplest way to construct an expression library would be to synthesize a double-stranded cDNA, make the ends blunt using DNA polymerase, and then ligate identical adaptors for a restriction enzyme to both ends. For example, if the adaptors had overhanging ends for a BamHI site, you could clone the cDNA into the BamHI site of a vector.

Suppose you screened the library using a hybridization probe, and found 24 positive clones. How many of them do you expect would express the protein?

Hint: This is NOT directional cloning.

4. (10 points) Based on the restriction maps for a gene (a) and a vector (b), choose a pair of restriction enzymes that could be used to cut the entire gene from a, to be cloned between any two compatible sites in the vector.




5. (10 points)

a) Imagine that you are testing a self-driving car on a street with 20 houses. In each trial, the computer chooses an address at random, and drives to that address. After 20 trials, would you expect to have visited all 20 houses? Explain your answer.

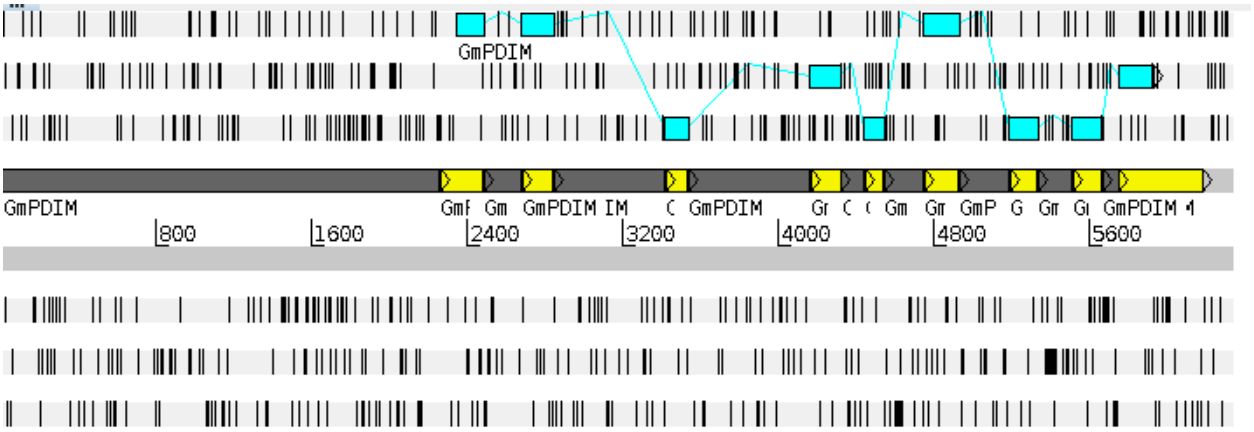
b) Now, let's apply this reasoning to genomic libraries. The maize haploid genome is $2 \times 10^9$ bp long. You have made a BAC library with an insert size of $2 \times 10^5$ bp. The library contains 10,000 clones. Would you expect that, somewhere in your library, every gene in the maize genome is represented? Explain your answer.

6. (10 points) Which do you think would yield more DNA: 100 mg of embryos, or 100 mg of tissue from fully expanded leaves?
Hint: Think about cell division and cell elongation as part of development.

7. (5 points) Media for co-cultivation of Agrobacterium with plant tissue needs Acetosyringone in order to induce Agrobacterium genes required for transformation. All other components are heat stabile, but Acetorsyringne would be destroyed by heat. How could you sterilize the acetosyringone so that it could be added to sterile media?

8. (10 points) The Td for a 100 bp DNA fragment is 72 °C at standard hybridization conditions. What would be the Td for a 500 bp fragment, given similar conditions? Show your work.

9. (15 points) The image below depicts a clone for a gene for disulfide isomerase. In each of the 3 reading frames on the forward strand (top) and 3 reading frames on the reverse strand (bottom), stop codons are shown as vertical tick marks. A map of the gene is shown between the two strands.

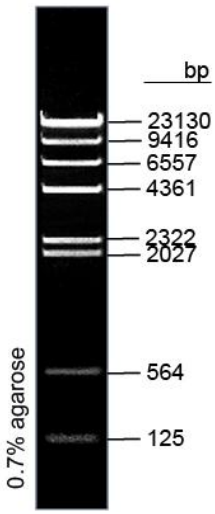



a) Which boxes are exons, and which are introns? What lets you distinguish between exons and introns in this diagram?

b) Is this from a cDNA library, or a genomic library?

c) The gene shown was cloned into the multiple cloning region of the pUC18 cloning vector. Many white colonies were screened, but the disulfide isomerase protein could not be detected in any of the clones. What is the most likely reason that the protein was not produced in any of the clones?

10. (5 points) The figure at right shows Lambda phage DNA digested with HindIII, separated by electrophoresis and stained with RedSafe. The length of each restriction fragment is indicated at right. Explain why some bands are brighter than others on the gel.



11. (10 points) Match statements a - e with one of the choices.

| Enzyme | Cutting site |
|---|---|
| BamHI | G^GATCC |
| ApaI | GGGCC^C |
| NotI | GC^GGCCGC |
| AoxI | ^GGCC |
| HaeII | RGCGC^Y |

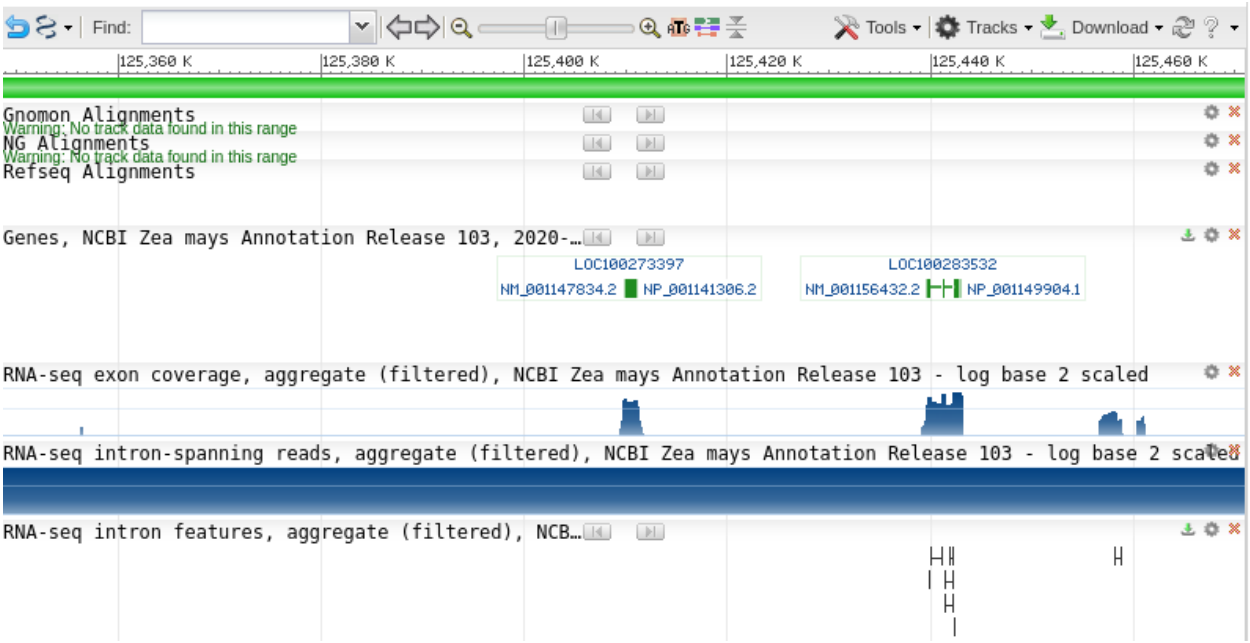
a) Digestion with this enzyme gives the largest fragments
b) Digestion with this enzyme gives the smallest fragments
c) Enzymes whose overhangs are compatible for ligation
d) Generates a 3' protruding end
e) Has an asymmetric recognition sequence

Choices:

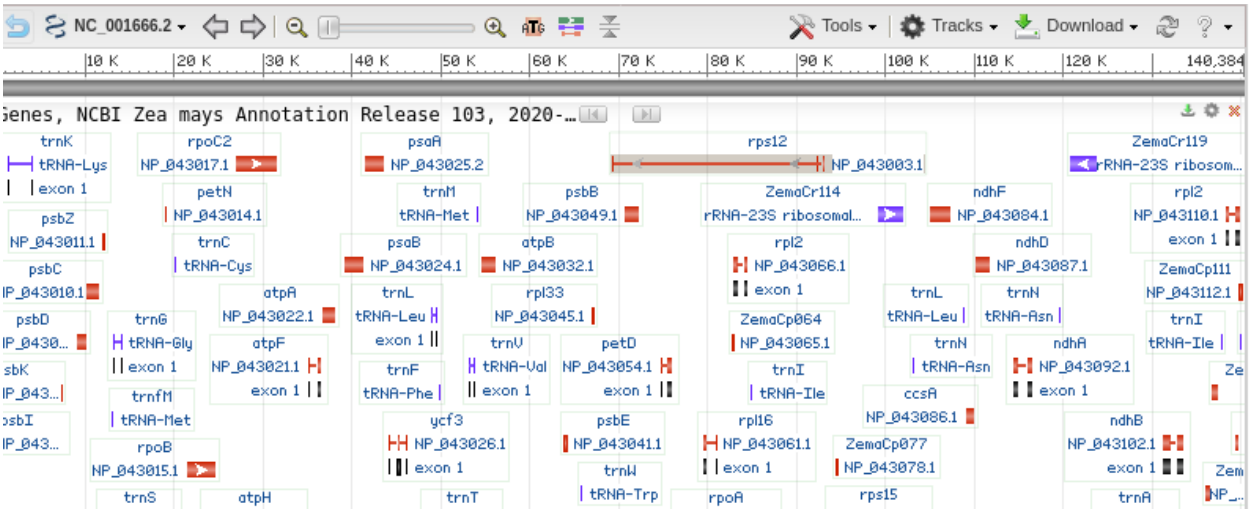
1. none
2. HaeII
3. NotI
4. AoxI
5. ApaI
6. BamHI
7. ApaI, AoxI
8. AoxI, NotI
9. ApaI, NotI

12. (10 points) One of the figures below is from a region of a maize nuclear chromosome. The other is from the maize chloroplast genome. Both diagrams span around 140 Kb of DNA. State which is genomic, and which is from chroloplast. What observations support your choices?

**A**

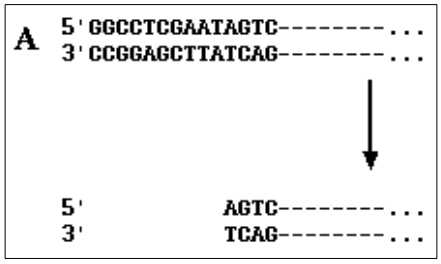



**B**

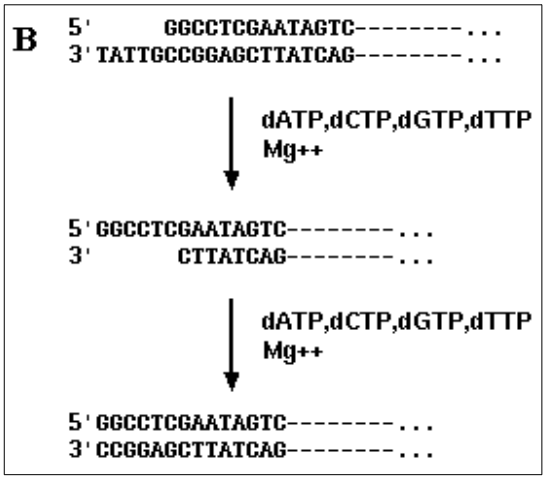


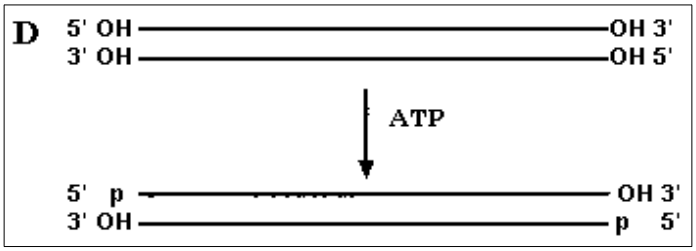
13. (10 points) For each of the diagrams A - E, match the name of the enzyme from the list below that BEST fits the reaction. Note that some enzymes in the list do not have a corresponding diagram.

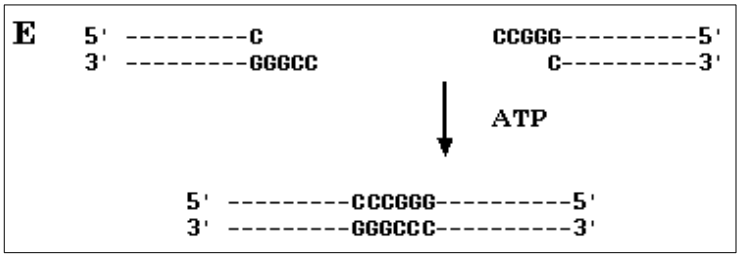
| |
|---|
| alkaline phosphatase |
| DNA ligase |
| E. coli DNA polymerase I |
| polynucleotide kinase |
| Restriction endonuclease<br>XmaI |
| Ribonuclease H (RNAse H) |
| DNA polymerase Klenow Fragment |



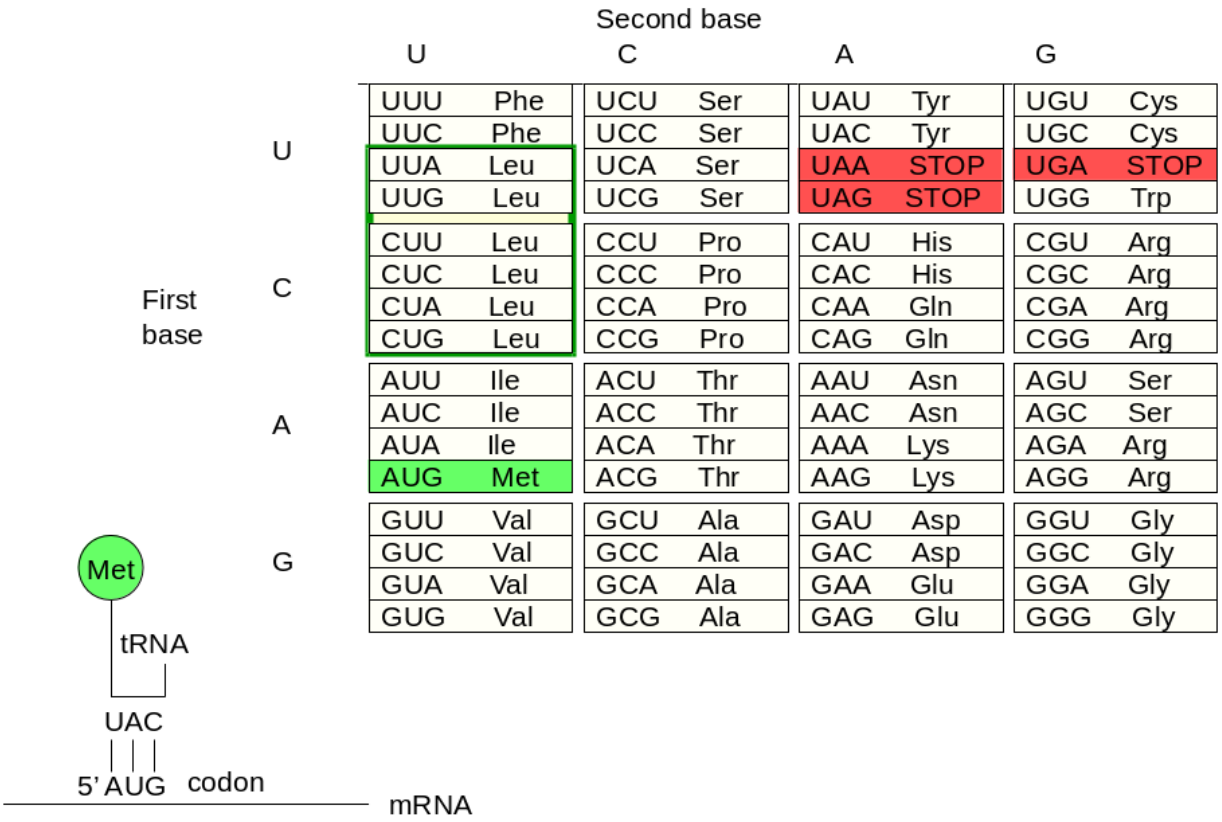




# Genetic code




## Frequencies of Restriction Sites (or other oligonucleotides)

| length n | frequency: occurs every $4^n$ | example | sequence |
|---|---|---|---|
| 1 | 4 | Single nucleotide | G |
| 2 | 16 | Di-nucleotide | GT |
| 3 | 64 | Codon | ATG |
| 4 | 256 | Taq I | TCGA |
| 5 | 1024 | MboII | GAAGA |
| 6 | 4096 | Hind III | AAGCTT |
| 7 | 16384 | Abe I | CCTCAGC |
| 8 | 65536 | Not I | GCGGCCGC |