PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm Wednesday, February 27, 2019

Answer any combination of questions totalling to exactly 100 points. If you answer questions totalling 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.

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. (15 points) In your exam booklet, re-write the table, using the terms below. Some terms may be used more than once. Some may not be used at all.

	plastid	mitochondrion	nucleus
genome size (bp)			
copies of genome per cell			
chromosome topology			
number of genes			
inheritance			

maternal paternal both parents

1 2 <100 > 100 50 - 200 10^5

$> 10^4$ 10^5 - 10^6 10^7 - 10^{12}

linear circular branched

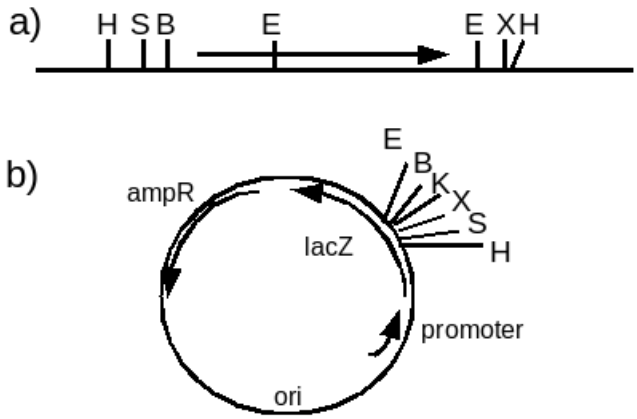
2. (15 points) A restriction map of a gene that you want to clone is shown in a). The direction of transcription of the gene is indicated by an arrow. Your goal is to use directional cloning to clone the transcribed sequence of the gene into the vector (b) in the sense orientation. In other words, your construct should have the insert in the correct orientation to produce the protein coded for by the gene, under the control of the lacZ promoter.

Describe a directional cloning strategy with the following infomation

- Which enzyme(s) would you use to cut the gene?
- Which enzyme(s) would you use to cut the vector?
- Draw a map of the final construct

There are several possible cloning strategies. Only describe one of those.

H = HindIII; S=SalI; B=BamHI; E=EcoRI; K=KpnI; X=XbaI



3. (10 points) Design a set of DNA hybridization probes for a protein that contains the following amino acid sequence:

Ile Met His Ser Trp Tyr

For reference, the standard Genetic Code is included on the last page of this exam.
In total, how many unique oligonucleotides would you have to make to account for all possible 18-mers that could encode this polypeptide?

4. (15 points) When growing cells in callus culture, there are three categories of components that must be included in the media: essential elements, organic supplements, and a carbon source. Briefly define what each of these does and give an example of each.

Explain why we need to provide these things to cells in culture, but not to mature plants growing in soil.

5. (10 points) In cell elongation, what is the mechanism by which plant cells can increase their total volume without having a substantial increase in cytoplasmic volume? Draw a simple diagram to illustrate your point.

6. (10 points) List two aspects of DNA structure that cause single-stranded DNA to spontaneously reanneal into double-stranded DNA.

7. (5 points) Which of the following would you expect to see in a cDNA library:

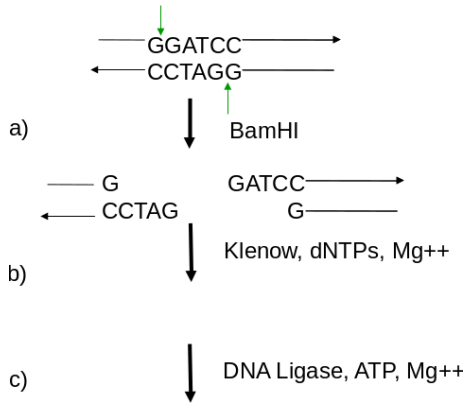
a) exon
b) intron
c) TATA box
d) promoter
e) 3'UTR

8. (10 points) Draw a simple diagram illustrating the posttranscriptional processing of pre-mRNA transcripts into mature mRNA. Also show the translation step, indicating the N-terminal and C-terminal ends of the polypeptide product. Show steps in the order in which they occur.

9. (5 points) PCR primers used to amplify a single gene from total genomic DNA are typically around 20 nucleotides long. Why can't these primers be, for example, 10 nucleotides long?
Hint: See the table of Restriction Site Frequencies on page 5.

10. (10 points) In expression libraries, each clone expresses the protein encoded by a single cDNA insert. Why is it not possible to make expression libraries in BAC vectors carrying large fragments (eg. 100 kb) of plant genomic DNA as inserts? Give at least 2 reasons.
Hint: Keep in mind that you are trying to express a plant gene in E. coli.

11. (15 points) In step a), the Bluescript vector was cut with BamHI, as illustrated in the accompanying figure. Redraw the figure, showing what the result would be after steps b and c.



The sequence of the region of the Bluescript vector that includes the BamHI site is shown below. This is the only BamHI site in the vector. We see that the BamHI site is within the protein coding region for the lacZ gene. Only the beginning of the protein coding region is shown.

```
lacZ gene ----------------->                                          KpnI                 XhoI
atg acc atg---> 802                 787                 772                 757                 742
ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA GGG AAC AAA AGC TGG GTA CCG GGC CCC CCC TCG
TAC TGG TAC TAA TGC GGT TCG AGC TTT AAT TGG GAG TGA TTT CCC TTG TTT TCG ACC CAT GGC CCG GGG GGG AGC
MET Thr MET Ile Thr Pro Ser Ser Lys Leu Thr Leu Thr Lys Gly Asn Lys Ser Trp Val Pro Gly Pro Pro Ser

  SalI        ClaI    HindIII EcoRV   EcoRI   PstI    SmaI      BamHI SpeI    XbaI      NotI
                727                 712                 697                 682                 667
AGG TCG ACG GTA TCG ATA AGC TTG ATA TCG AAT TCC TGC AGC CCG GGG GAT CCA CTA GTT CTA GAG CGG CCG CCA
TCC AGC TGC CAT AGC TAT TCG AAC TAT AGC TTA AGG ACG TCG GGC CCC CTA GGT GAT CAA GAT CTC GCC GGC GGT
Arg Ser Thr Val Ser Ile Ser Leu Ile Ser Asn Ser Cys Ser Pro Gly Asp Pro Leu Val Leu Glu Arg Pro Pro
SacII     SacI
               652                 637                 622                 607
CCG CGG TGG AGC TCC AAT TCG CCC TAT AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA C.......
GGC GCC ACC TCG AGG TTA AGC GGG ATA TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT G.......
Pro Arg Trp Ser Ser Asn Ser Pro Tyr Ser Glu Ser Tyr Tyr Asn Ser Leu Ala Val Val Leu Gln ........
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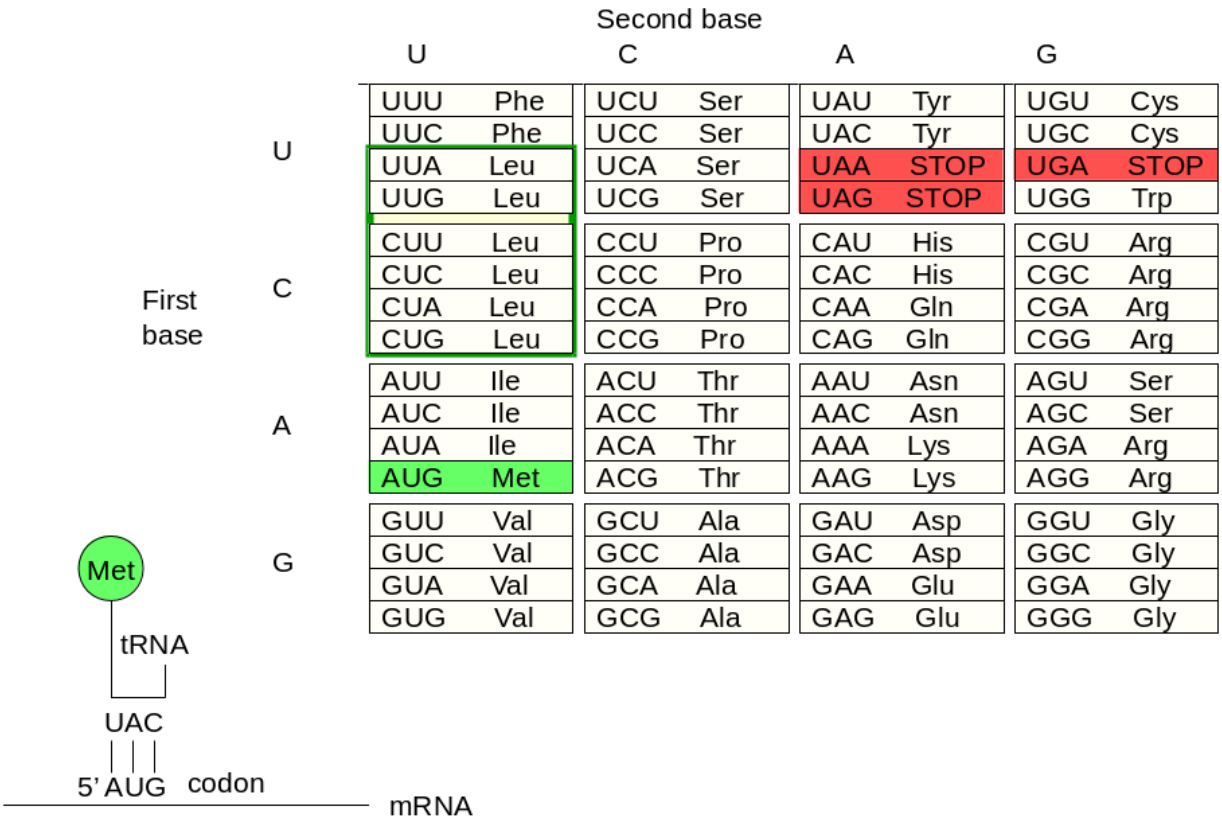
The Bluescript vector from step c was transformed into *E. coli*, and grown on media containing antibiotic and Xgal. The resultant colonies were all white.

Explain the result.

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