PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

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

Answer any combination of questions totaling to <u>exactly</u> 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.

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.

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.

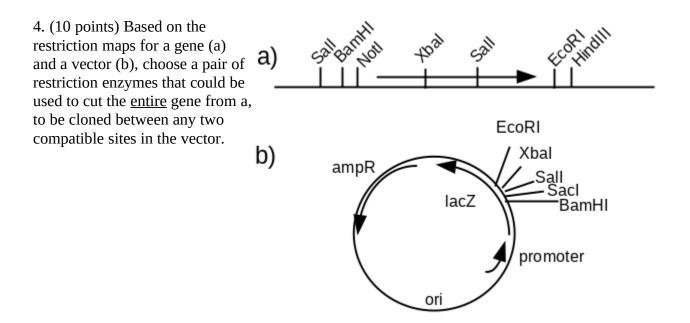
Suppose you screened the library using a hybridization probe, and found 24 positive clones. How many of them do you expect would express the protein?

Hint: This is NOT directional cloning.

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.



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 $2 \ge 10^9$ bp long. You have made a BAC library with an insert size of $2 \ge 10^5$ 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.

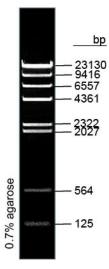
	GmPDIM		 NW_/ # + 1011 N# 010 F + 000 > + 000 -
GmPDIM 800	Gmf Gm Gmf <u>1</u> 600 <u>2</u> 400	PDIMIM (GmPDIM (3200 400	Gr C (Gm Gn Gn Gn PD IM 4 0 <u>4</u> 800 <u>5</u> 600

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
АраІ	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:

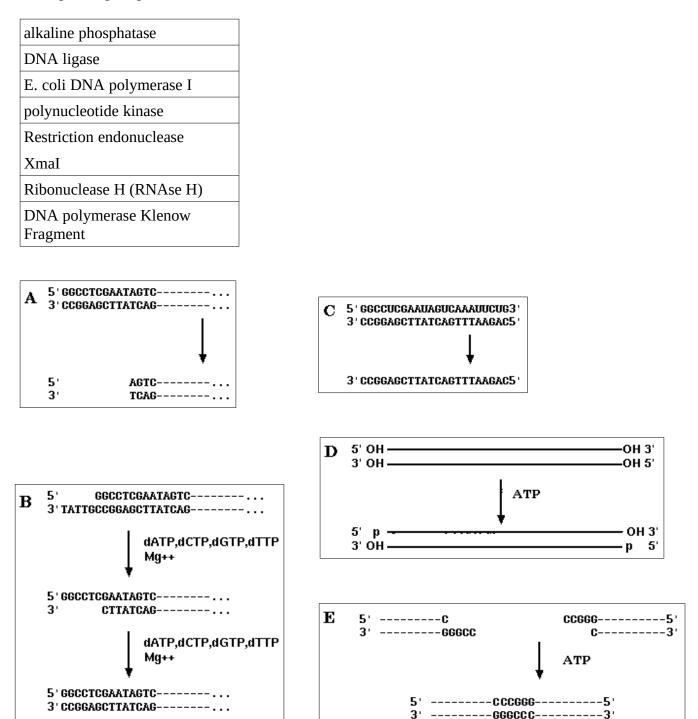
- 1. none
- 2. HaeII
- 3. NotI
- 4. AoxI
- 5. ApaI
- 6. BamHI
- 7. ApaI, AoxI
- 8. AoxI, NotI
- 9. ApaI, NotI

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?

Α							
🔄 웅 - Find:	✓ <		- Q, 🜆 📑	<u><u></u></u>	🔀 Tools -	🔹 Tracks 🗕 📩	Download 🗕 🎅 🤉 🗕
125,360 К1	25,380 K	125,400 K		125,420 k	· · · · · · · · · · · · · · · · · · ·	125,440 K	125,460 K
Gnomon Alignments Warning: No track data found in this range NG Alignments Warning: No track data found in this range Refseq Alignments							0 × 0 ×
Genes, NCBI Zea mays Annotati			0273397 NP_0011413	06.2		80283532 ┣┝╋┓ NP_001149904.	± © ×
RNA-seq exon coverage, aggreg	ate (filtered), I	NCBI Zea m	ays Annot	tation	Release 103	- log base 2	scaled 🗢 🛪
RNA-seq intron-spanning reads	, aggregate (fil	tered), NC	BI Zea ma	ays Ann	otation Rele	ease 103 - log	g base 2 sca∜eð
RNA-seq intron features, aggr	egate (filtered)	, NCB					¥ ⊕ ¥
B S NC 001666.2 → (C) (C) (C)				6			wnload • 🕾 🤊 •

	0 K 20	к 30 к	40 K 50	к	K. 70 K.	80 K 90	к 100 к	110 K	120 K	140,38
enes, NC	BI Zea ma	ys Annotation	n Release 1	03, 2020						± \$ 3
trnK	rp	oC2	psaA			rps12			Z	emaCr119
🚽 tRNA-Ly	ys NP_0430	17.1 🚬	E NP_04302	5.2			NP_043003.1		rRN8-2	23S ribosor
exon 1	P	etN	troM		psbB	ZemoCr1	14	ndhE		rpl2
psbZ	NP_	43014.1	tRNA-Me	t NP_	043049.1 📕	rRNA-23S ribos	omal 돈 📒	NP_043084.1	N	P_043110.1
P_043011.1	t	rnC	psaB	atpB		rpl2		ndhD		exon 1
psbC	tRI	NA-Cys	NP_043024.1	E NP_043	8032.1	NP_04306	6.1	NP_0430	87.1	ZemaCp11
043010.1		atpA	trnL	rpl3	3	exon 1	trnL	. trnN		NP_043112.
osbD	trnG	NP_043022.1	tRNA-Leu 🖁	NP_0430	345.1	ZemaCp064	tRNA-L	eu tRNA-Asn	1	trnI
Ø430 📕	H tRNA-Gly	atpF	exon 1	trnV	petD	NP_043065.	1 t	rnN n	dhA	tRNA-Ile
к	exon 1	NP_043021.1	troF	H tRNA-Val	NP_043054.1 H	trnI	t RI	NA-Ash 📙 NP.	043092.1	
Ø43	trnfM	exon 1	tRNA-Phe	exon 1	exon 1	tRNA-Ile	ccsA	ex	on 1	
I	tRNA-Met		ycf3		psbE	rpl16	NP_04308	6.1 📕	ndhB	
Ø43	rpoB		H NP_04		NP_043041.1	H NP_043061.1	ZemaCp077		NP_043102	
	VP_043015.1	3	exon 1		trnW	exon 1	NP_043078.1		-	1
-	trnS	atpH	tr		tRNA-Trp	rpoA	rps15		trni	

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.



Genetic code

				Secon	d base				
		U		С		А		G	
		UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	U	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	0	UUA	Leu	UCA	Ser	UAA	STOP	UGA	STOP
		UUG	Leu	UCG	Ser	UAG	STOP	UGG	Trp
		CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	С	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
First	C	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
base		CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
		AUU	lle	ACU	Thr	AAU	Asn	AGU	Ser
	А	AUC	lle	ACC	Thr	AAC	Asn	AGC	Ser
	~	AUA	lle	ACA	Thr	AAA	Lys	AGA	Arg
		AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
_		GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
Met	G	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
Wiet	•	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
		GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly
tRNA									
UAC									
5'AUG cod	on	— mRNA	`						

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	Mboll	GAAGA
6	4096	Hind III	AAGCTT
7	16384	Abe I	CCTCAGC
8	65536	Not I	GCGGCCGC

------ mRNA