Biology 335-201 MIDTERM EXAM February 6, 2015

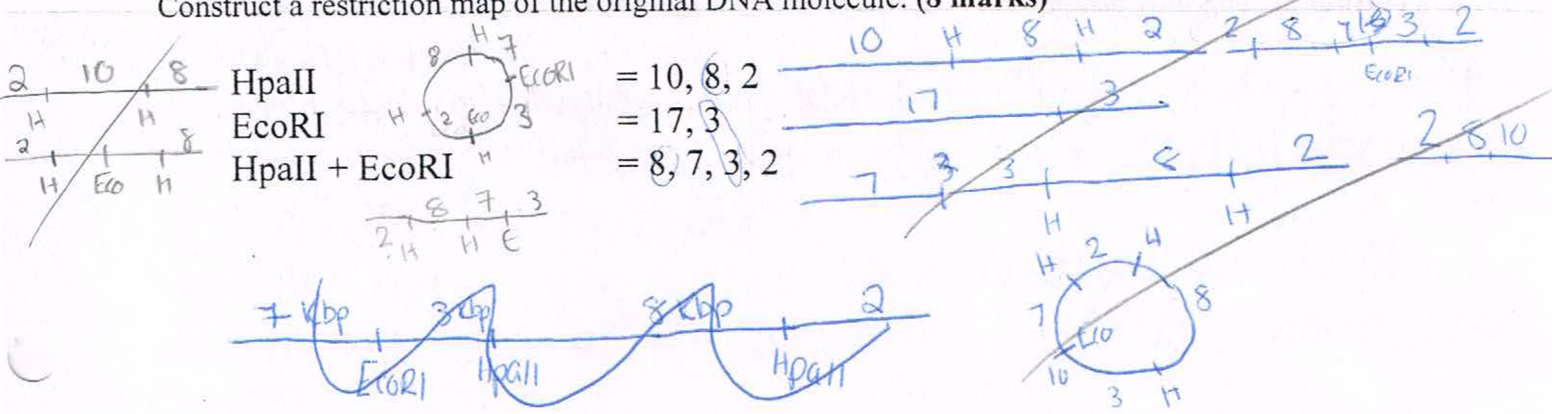
NAME _ Student No.

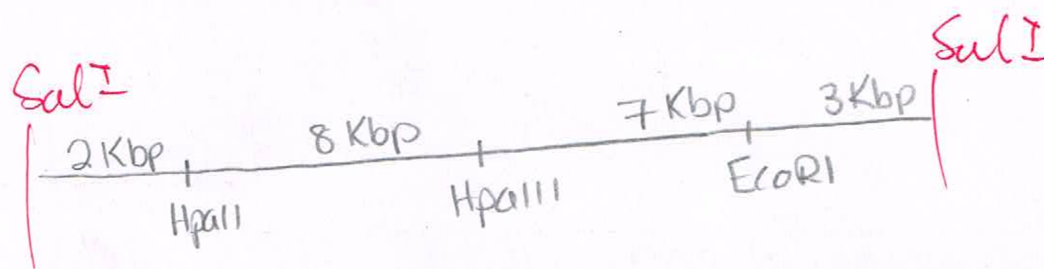
Q1	7/8
Q2	5
Q3a	1
Q3b	4
T/30	17

Instructions:
- **Answer all questions in the space provided**
- **Clearly show your logical steps for full marks**
- **Time = 50 minutes**

1. Restriction enzyme sites play an important role in characterizing clones as well as in gene mapping and diagnostics because they provide, among other things, heritable chromosomal reference points. The establishment of such sites, known as restriction mapping, is often accomplished by examining the DNA fragments formed by single and double digestions of DNA with restriction endonucleases. Below are the results of digests of a SalI derived cloned DNA insert giving the size of fragments produced in Kbp. Construct a restriction map of the original DNA molecule. **(8 marks)**

HpaII = 10, 8, 2
EcoRI = 17, 3
HpaII + EcoRI = 8, 7, 3, 2

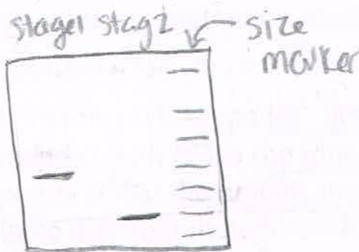




NAME J Student No.

2. Using standard cloning techniques, you have obtained a genomic actin clone from chicken using an actin probe from quail. When you use your chicken genomic actin clone against total cellular RNA derived from a sample of skeletal muscle of chicken (Northern blot) you find that 2 different RNAs show strong hybridization to the chicken actin probe. After an extensive and exhaustive search against your chicken genomic library you are unable to obtain a second chicken genomic actin clone. The conclusion you reach is that the chicken genomic actin clone used as probe is hybridizing to two different sized mRNAs which represent differentially spliced variants of a single genomic actin gene. You clone the two cDNAs which represent the two mRNAs and after sequence comparisons with each other and with the genomic clone you determine they are in fact two splice variants of a single gene. Your next experiment is to quantitatively determine the relative amounts of the two mRNAs in samples obtained from skeletal muscle at different stages of development. Briefly outline in point form **two different** experimental procedure(s) you could perform in the lab to accomplish your goal. **(8 marks)**

Northern blot
- Isolate total cellular RNA from both stages of development + run through agarose gel + probe with radiolabelled chicken actin probe (3)
- also run a size marker so hybridized bands of mRNA can be compared to bands of known size.



RT-PCR:
- Make cDNA library for each developmental stage
- Oligo(dT) addition as 1st primer + reverse transcriptase to extend 1st sequence. (2)
- Perform PCR with 2nd primer to amplify target mRNAs + quantify the relative amounts in each developmental stage.

NAME Student No

3a. The following table gives the relative activities of the enzymes β-galactosidase and permease in cells with different genotypes at the lac operon in *E. coli*. The induced level of activity of each enzyme in wild-type cells is arbitrarily set at 1000 units, and all other enzyme levels are measured relative to this value. Based on the data given in the table for genotypes 1 through 4, fill in the levels of activity that would be expected for genotypes 5 and 6 in the spaces provided in parenthesis. **(8 marks)**

Genotype	β-galactosidase no inducer	β-galactosidase + inducer	Permease no inducer	Permease + inducer
1. $I^+ O^+ Z^+ Y^+$	0.5	1000	0.5	1000
2. $I^- O^+ Z^+ Y^+$ no represser	1000	1000	1000	1000
3. $I^+ O^c Z^+ Y^+$ represser falls off	50	1000	50	1000
4. $I^- O^+ Z^+ Y^- / F' I^- O^+ Z^+ Y^+$	2000	2000	1000	1000
5. $I^- O^c Z^- Y^+ / F' I^+ O^+ Z^+ Y^+$	(0.5)	(500)	(50)	(1000)
6. $I^+ O^c Z^+ Y^- / F' I^- O^c Z^+ Y^+$	(25)	(1000)	(25)	(500)

represser off : const.

1000 - Y

1

3b. You are studying mutations affecting the lactose operon. During a screen for mutants you find a lac⁻ strain and decide to run 2-D protein gels to compare the protein profiles between a wild-type lac⁺ strain and your newly acquired lac⁻ mutant when both are induced with IPTG (no glucose present). When you compare the two gels to each other you find that the mutant strain lacks three proteins relative to the wild-type strain. Propose three different mutations affecting the lactose operon that could explain your results. **(6 marks)**

2

- mutation in the represser gene I that causes it to bind to the operator with or without the presence of inducer (lacI^s)
- Frameshift mutation in first gene transcribed (Z gene) that alters the amino acid sequence of that gene and all genes following (Z, Y, A) so protein product degrades + no proteins in mutant detected.
- Promoter mutation - RNA polymerase unable to bind so protein product not produced in mutant.

2