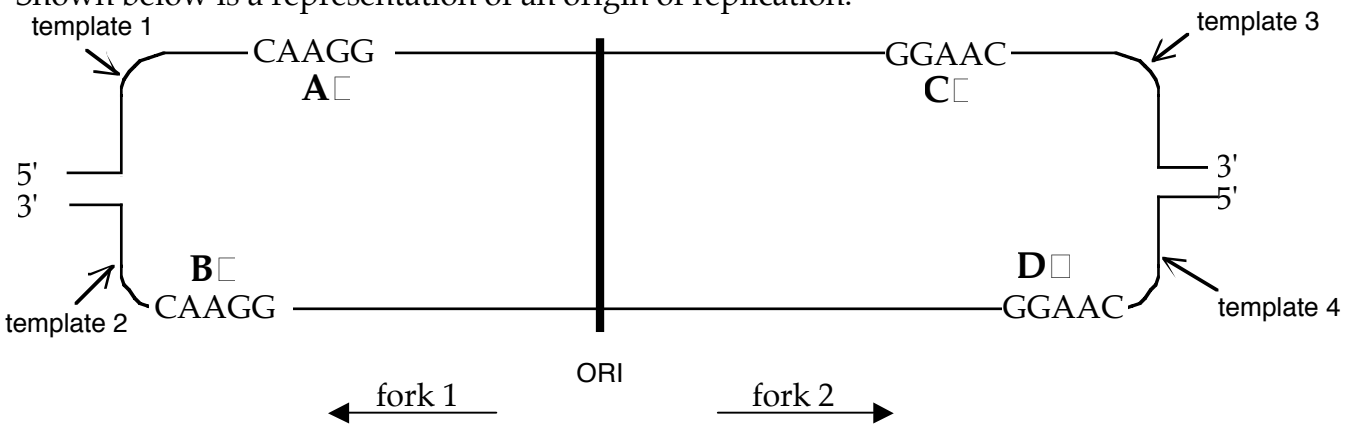
7.014 Quiz II Handout

This will be a closed book exam

Question 1

Shown below is a representation of an origin of replication.



a) For the following, use sites A and B with respect to fork 1 and sites C and D with respect to fork 2.

i) On which strand(s) will replication be continuous?

template 1 template 2 template 3 template 4

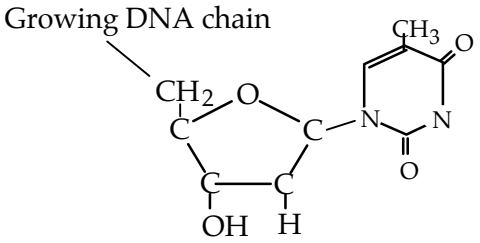
ii) To which site or sites (**A**, **B**, **C**, or **D)** can the primer 5'-GUUCC-3' bind to initiate replication?

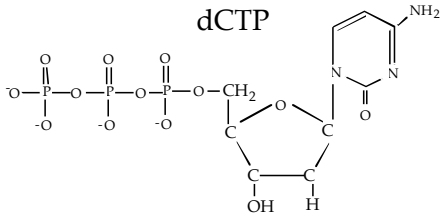
iii) When DNA ligase is inhibited, it differentially affects the synthesis from the leading and the lagging strands. Explain which strand (leading or lagging) is more affected by the lack of DNA ligase and why.

Question 1, continued

b) The next nucleotide to be added to a growing DNA strand is dCTP (shown).

- Circle the part of the growing DNA chain to which the next base is attached.
- Circle the part of the dCTP that is incorporated into the growing DNA chain.





c) DNA Replication involves many different enzymatic activities. Match each enzyme activity listed below with the function(s) that it has in the replication process. The first one is done for you.

Enzyme Activity	Function(s)
Topoiosmerase	k
Primase (synthesizes primer)	
DNA polymerase to elongate new DNA strand	
Helicase to unwind DNA	
DNA polymerase to replace RNA with DNA	
Processivity factor	

Choose From:

a) 3′→ 5′ growth of new DNA strand

b) 5′→ 3′ growth of new DNA strand

c) 3′→ 5′ exonuclease

d) 5′→ 3′ exonuclease

e) Makes RNA primer complementary to the lagging strand

f) Makes RNA primer complementary to the leading strand

g) Makes peptide bonds

h) Separates the two DNA strands

i) Maintains DNA polymerase on template

j) Provides 3′ hydroxyl for initiation of DNA polymerization

k) Untangles super-coiled DNA

Question 2

Below is the partial sequence of the *sevenohwunforin* (*7014in*) gene, hypothesized to be mutant in students who take 7.013 Introductory Biology and in those students at the other school up the river. The promoter is underlined and transcription begins at and includes the bold G/C base pair.

```
5′ TGCCA TCCGA TTGGT GTTCC TTCCA TGAAG GATGC ACAAC GCAAA 3′
3′ ACGGT AGGCT AACCA CAAGG AAGGT ACTTC CTACG TGTTG CGTTT 5′

5′ TACAC GCTTA GCTGA CTATA AGGAC GAATC GCTAC AACGA TGCGA 3′
3′ ATGTG CGAAT CGACT GATAT TCCTG CTTAG CGATG TTGCT ACGCT 5′

5′ TGCCA TCCGA TTGGT GTTCC TTCCA TGAAG GATGC ACAAC GCAAA 3′
3′ ACGGT AGGCT AACCA CAAGG AAGGT ACTTC CTACG TGTTG CGTTT 5′
```

a) What are the first 12 nucleotides of the transcript encoded by the *7014in* gene? Label the 5′ and 3′ ends.

__′- | | | | | | | | | | | | -__′

b) On the DNA sequence above, **circle** the DNA bases that encode the first amino acid of the protein.

c) What are the first four amino acids encoded by the *7014in* transcript? Label the N- and C-terminus

____- | | | | | - ___

d) You want to create a system to translate a specific mRNA in a test tube. To an appropriate water and salt solution you add many copies of this mRNA and ATP (energy). What other key components must you add?

You succeed in translating the mRNA in your test tube. You repeat the experiment with two identical test tubes. You add limiting amounts of the antibiotic puromycin to test tube 2 only. Puromycin is a molecule that has structural similarities to the 3′ end of a charged tRNA. It can enter the ribosome and be incorporated into the growing protein. When puromycin is incorporated into the polypeptide, it stalls the ribosome and the polypeptide is released. You do not know if puromycin recognizes a specific codon or not.

e) What effect would puromycin have on transcription?

f) What effect would puromycin have on translation?

Question 2, continued

g) You examine the length of the polypeptide produced in both test tubes.

i) In test tube 1 (no puromycin) you get a polypeptide that is 100 amino acids long. At least how many bases was the mRNA that you added?

ii) Which of the following would you find in test tube 2 (has limiting amounts of puromycin) if puromycin does **NOT** recognize a specific codon.

Only a single type of polypeptide

Only 2 types of polypeptides that are each different lengths

Only 3 types of polypeptides that are each different lengths

Only 4 types of polypeptides that are each different lengths

Polypeptides of all sizes, i.e., dipeptides, tripeptides, … a polypeptide that is 100 amino acids long

iii) Which of the following would you find in test tube 2 (has limiting amounts of puromycin) if puromycin recognizes a specific codon that occurs three times in the mRNA.

Only a single type of polypeptide

Only 2 types of polypeptides that are each different lengths

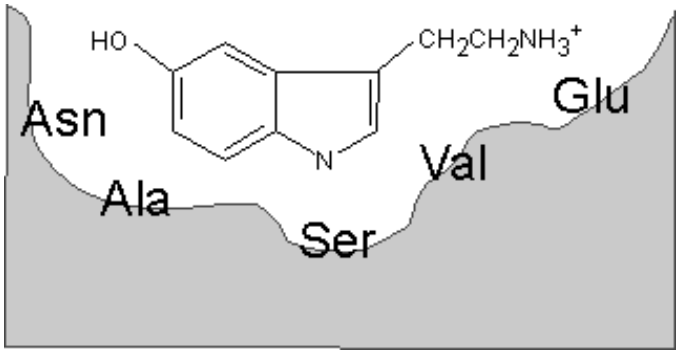
Only 3 types of polypeptides that are each different lengths

Only 4 types of polypeptides that are each different lengths

Polypeptides of all sizes, i.e., dipeptides, tripeptides, … a polypeptide that is 100 amino acids long

Question 2, continued

The *7014in* gene encodes a protein (7014IN) that binds the neurotransmitter serotonin, as shown below. The five amino acids 7014IN involved in binding serotonin are shown.



To understand the difference between introductory biology students, you have determined the DNA sequence for the *7014in* gene in a group of 7.013 and 7.014 students. Below is the 7014IN protein and the DNA sequence that encodes it. The amino acids depicted in the picture above are <u>underlined</u>.

From a 7.014 student:

```
DNA         5′ ACC AAT GGA CCA GCA GGA AGC GGG GTA GCT GAG TAC 3′
            3′ TGG TTA CCT GGT CGT CCT TCG CCC CAT CGA CTC ATG 5′

Protein     N- Thr Asn Gly Pro Ala Gly Ser Gly Val Ala Glu Tyr –C
```

h) You find that 7.013 student 1 has the following DNA sequence for the 7014IN:

```
5′ ACC AAT GGA CCA GCA GGA TAG CGG GGT AGC TGA GTAC 3′
3′ TGG TTA CCT GGT CGT CCT ATC GCC CCA TCG ACT CATG 5′
```

i) Indicate (circle/underline) the site of the mutation on the sequence directly above.

ii) Does student 1 have an insertion, deletion, or substitution mutation?

iii) Would you expect this DNA sequence to encode a protein that binds serotonin? Why or why not? A chart of the amino acids is found on page 10.

i) You find that 7.013 student 2 has the following DNA sequence for the 7014IN:

```
5′ ACC AAT GGA CCA GCA GGA AGC GGG GTA GCT GAT TAC 3′
3′ TGG TTA CCT GGT CGT CCT TCG CCC CAT CGA CTA ATG 5′
```

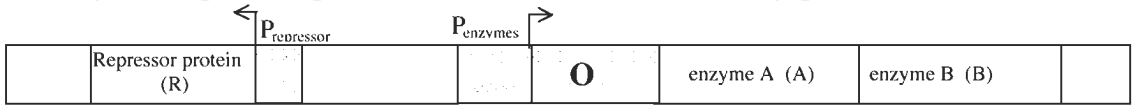
i) Indicate (circle/underline) the site of the mutation on the above sequence.

ii) Does student 2 have an insertion, deletion, or substitution mutation?

iii) Would you expect this DNA sequence to encode a protein that binds serotonin? Why or why not? A chart of the amino acids is found on page 10.

Question 3

Enzymes A and B are both required for the breakdown of maltose. The wild-type operon is regulated by the repressor protein (R), which is continuously produced.



You have three mutants (m1, m2 and m3), each one is the result of a loss-of-function mutation in a single component shown in the diagram. The mutants m1, m2 and m3 exhibit the following phenotypes when grown with or without maltose in the medium.

	with maltose		without maltose	
	Enzyme A activity	Enzyme B activity	Enzyme A activity	Enzyme B activity
wild-type (+)	high	high	low	low
m1	low	low	low	low
m2	high	high	high	high
m3	high	high	high	high

a) A mutation in which component could produce the phenotype seen in the m1 mutant? Why?

b) Name three different components that could produce the phenotype seen in m2 and m3 when mutated.

c) How could maltose act to induce transcription of this operon?

Question 3, continued

You construct the following diploids by inserting a second copy of the operon into each mutant. + indicates that the component is wild type, - indicates that the component is non-functional.

	with maltose		without maltose	
Strain	**Enzyme A activity**	**Enzyme B activity**	**Enzyme A activity**	**Enzyme B activity**
Wild type with $R^+ P_{enz}{}^+ O^+ A^+ B^+$	high	high	low	low
m1 with $R^+ P_{enz}{}^+ O^+ A^- B^-$	low	low	low	low
m1 with $R^+ P_{enz}{}^+ O^+ A^+ B^+$	high	high	low	low
m2 with $R^+ P_{enz}{}^+ O^+ A^- B^-$	high	high	high	high
m3 with $R^+ P_{enz}{}^+ O^+ A^- B^-$	high	high	low	low

d) Which one of the three mutants (m1, m2 or m3) has a mutation in the gene for the repressor protein? Briefly explain your reasoning.

e) You examine the number of mRNA molecules (transcripts) produced from the maltose operon(s) in each cell. Complete the table below.

	with maltose	without maltose
Cell	**Number of transcripts**	**Number of transcripts**
Wild type	1000	10
Wild type with $R^+ P^+ O^+ A^- B^-$	2000	20
$\underline{R^+ \mathbf{P^-} O^+ A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$		
$\underline{R^+ P^+ \mathbf{O^-} A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$		
$\underline{\mathbf{R^-} P^+ O^+ A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$		

Question 4

You have discovered a new strain of bacteria that form aesthetically pleasing snowflake shaped plaques when infected with bacteriophage. Further analyses show that these bacteria need to make a compound called Crystalin to form these pretty plaques. To examine the pathway involved in Crystalin synthesis, you undertake a mutant hunt. You isolated 7 mutants (M1- M7) that no longer form snowflake plaques and perform a complementation test as shown below.

	M1	M2	M3	M4	M5	M6	M7	Wild type
M1	-	+	-	+	+	+	+	+
M2		-	+	+	+	-	-	+
M3			-	+	+	+	+	+
M4				-	+	+	+	+
M5					-	+	+	+
M6						-	-	+
M7							-	+

a) Place the above mutants into complementation groups. What is the likely minimal number of genes in the Crystalin synthesis pathway?

You test the ability of each mutant to make Crystalin when given the intermediates in the pathway. + indicates that Crystalin is made, - indicates that Crystalin is not made.

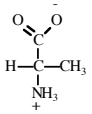
Intermediates	A	B	C	D
M1	+	+	+	-
M2	+	-	-	-
M3	+	+	+	-
M4	+	+	-	-
M5	-	-	-	-
M6	+	-	-	-
M7	+	-	-	-

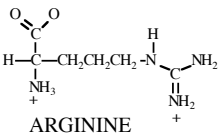
b) Complete the pathway by filling in each blank with an intermediate and by labeling each arrow with the mutants that cannot complete that step.

_____ → _____ → _____ → _____ → Crystalin

	U		C		A		G		
U	UUU	**phe (F)**	UCU	**ser (S)**	UAU	**tyr (Y)**	UGU	**cys (C)**	U
	UUC	**phe**	UCC	**ser**	UAC	**tyr**	UGC	**cys**	C
	UUA	**leu (L)**	UCA	**ser**	**UAA**	**STOP**	**UGA**	**STOP**	A
	UUG	**leu**	UCG	**ser**	**UAG**	**STOP**	UGG	**trp (W)**	G
C	CUU	**leu**	CCU	**pro (P)**	CAU	**his (H)**	CGU	**arg (R)**	U
	CUC	**leu**	CCC	**pro**	CAC	**his**	CGC	**arg**	C
	CUA	**leu**	CCA	**pro**	CAA	**gln (Q)**	CGA	**arg**	A
	CUG	**leu**	CCG	**pro**	CAG	**gln**	CGG	**arg**	G
A	AUU	**ile (I)**	ACU	**thr (T)**	AAU	**asn (N)**	AGU	**ser (S)**	U
	AUC	**ile**	ACC	**thr**	AAC	**asn**	AGC	**ser**	C
	AUA	**ile**	ACA	**thr**	AAA	**lys (K)**	AGA	**arg (R)**	A
	AUG	**met (M)**	ACG	**thr**	AAG	**lys**	AGG	**arg**	G
G	GUU	**val (V)**	GCU	**ala (A)**	GAU	**asp (D)**	GGU	**gly (G)**	U
	GUC	**val**	GCC	**ala**	GAC	**asp**	GGC	**gly**	C
	GUA	**val**	GCA	**ala**	GAA	**glu (E)**	GGA	**gly**	A
	GUG	**val**	GCG	**ala**	GAG	**glu**	GGG	**gly**	G

STRUCTURES OF AMINO ACIDS


ALANINE
(ala)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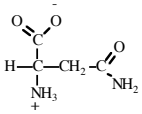
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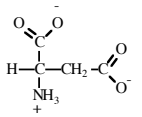
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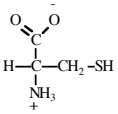
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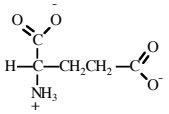
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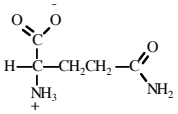
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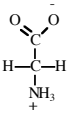
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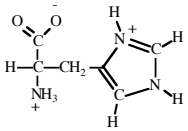
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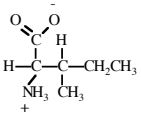
(his)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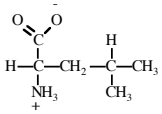
(ile)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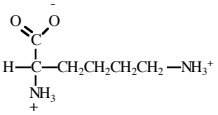
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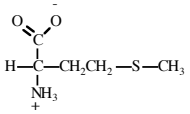
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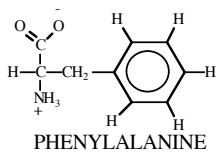
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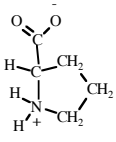
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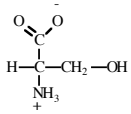
(pro)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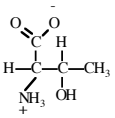
(ser)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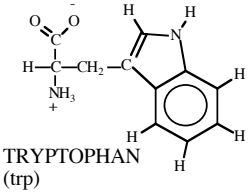
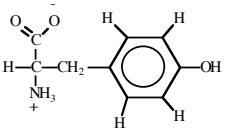
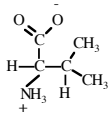
(thr)


TRYPTOPHAN
(trp)


TYROSINE
(tyr)


VALINE
(val)

Solutions:

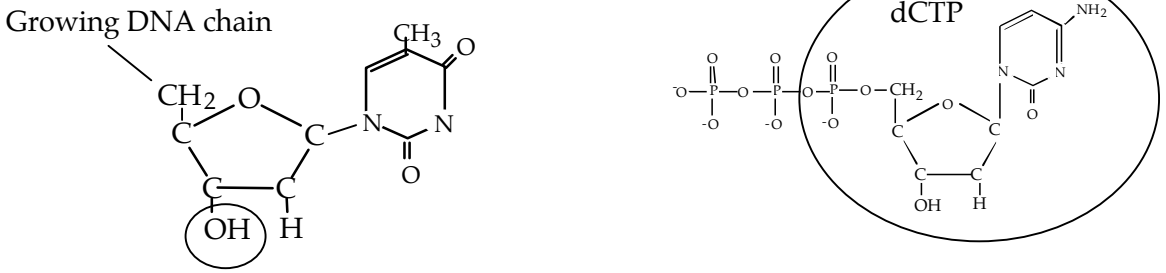
Question 1

a) i) template 1 template 2 template 3 template 4

ii) *B and C*

iii) *The lagging strand is more affected by the lack of DNA ligase. DNA replication on the lagging strand occurs in small stretches called Okasaki fragments. For replication of the lagging strand to be complete, a phosphodiester bond must be formed between the 3'OH on one Okasaki fragment and the 5' phosphate on the other. DNA ligase makes this bond.*

b)



c)

Enzyme Activity	Function(s)
Topoiosmerase	k
Primase (synthesizes primer)	*e, f, j*
DNA polymerase to elongate new DNA strand	*b, c*
Helicase to unwind DNA	*h*
DNA polymerase to replace RNA with DNA	*b, d*
Processivity factor	*i*

Question 2

a) *5' G A A U C G C U A C A A 3'*

Question 2, continued

b)

```
5′ TGCCA TCCGA TTGGT GTTCC TTCCA TGAAG GATGC ACAAC GCAAA 3′
3′ ACGGT AGGCT AACCA CAAGG AAGGT ACTTC CTACG TGTTG CGTTT 5′

5′ TACAC GCTTA GCTGA CTATA AGGAC GAATC GCTAC AACGA TGCGA 3′
3′ ATGTG CGAAT CGACT GATAT TCCTG CTTAG CGATG TTGCT ACGCT 5′

5′ TGCCA TCCGA TTGGT GTTCC TTCCA TGAAG GATGC ACAAC GCAAA 3′
3′ ACGGT AGGCT AACCA CAAGG AAGGT ACTTC CTACG TGTTG CGTTT 5′
```

c) *N- met-arg-cys-his -C*

d) *You would need a functional ribosome and all the tRNAs charged with the appropriate amino acid.*

e) *None.*

f) *Puromycin would halt translation and cause truncation of the proteins being produced.*

g) i) *The mRNA would be at least 303 nucleotides long. It is likely longer as the start codon is not usually the first three nucleotides at the 5' end*

ii) *Polypeptides of all sizes, i.e., dipeptides, tripeptides, … a polypeptide that is 100 amino acids long*

iii) *Only 4 types of polypeptides that are each different lengths*

h) You find that 7.013 student 1 has the following DNA sequence for the 7014IN:

i)

```
5′ ACC AAT GGA CCA GCA GGA TAG CGG GGT AGC TGA GTAC 3′
3′ TGG TTA CCT GGT CGT CCT ATC GCC CCA TCG ACT CATG 5′
```

ii) *insertion*

iii) *You would expect that the protein encoded by this sequence does NOT bind serotonin. This insertion changes the codon for Ser into a stop codon. The protein is truncated after the Gly. The three dimensional shape will be changed. A binding pocket no longer exists.*

i) You find that 7.013 student 2 has the following DNA sequence for the 7014IN:

i)

```
5′ ACC AAT GGA CCA GCA GGA AGC GGG GTA GCT GAT TAC 3′
3′ TGG TTA CCT GGT CGT CCT TCG CCC CAT CGA CTA ATG 5′
```

ii) *substitution*

iii) *Yes, this protein is altered only at one position, where we now have an Asp instead of Glu. These two amino acids are similar and can form the same type of interactions with seritonin.*

Question 3

a) *A mutation in the $P_{enzymes}$ would give you this phenotype. Without the $P_{enzymes}$, RNA polymerase can not bind, so no transcript is made and no proteins are produced.*

b) *Mutations in the gene that encodes the repressor protein, the operator region, or the P_R would give this phenotype.*

c) *By analogy to the lac operon, we expect that maltose would bind the repressor protein. This interaction changes the shape of the repressor such that it no longer binds to the operator region. This allows RNA polymerase to bind and begin transcription of the A and B genes.*

d) *m3 has a mutation in the gene for the repressor protein. An extra copy of the maltose operon can restore proper regulation only when the element mutated acts in trans. Proteins can act in trans as they are mobile. Operators and promoters act in cis because they are DNA elements and can only control expression of the genes to which they are attached.*

e)

	with maltose	**without maltose**
Cell	**Number of transcripts**	**Number of transcripts**
Wild type	1000	10
Wild type with $R^+ P^+ O^+ A^- B^-$	2000	20
$\underline{R^+ \mathbf{P^-} O^+ A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$	*1000*	*10*
$\underline{R^+ P^+ \mathbf{O^-} A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$	*2000*	*1010*
$\underline{\mathbf{R^-} P^+ O^+ A^- B^-}$ / $R^+ P^+ O^+ A^- B^-$	*2000*	*20*

Question 4

a) *group 1: m1, m3*
group 2: m2, m6, m7
group 3: m4
group 4: m5

There are at least 4 genes in the Crystalin synthesis pathway.

b)