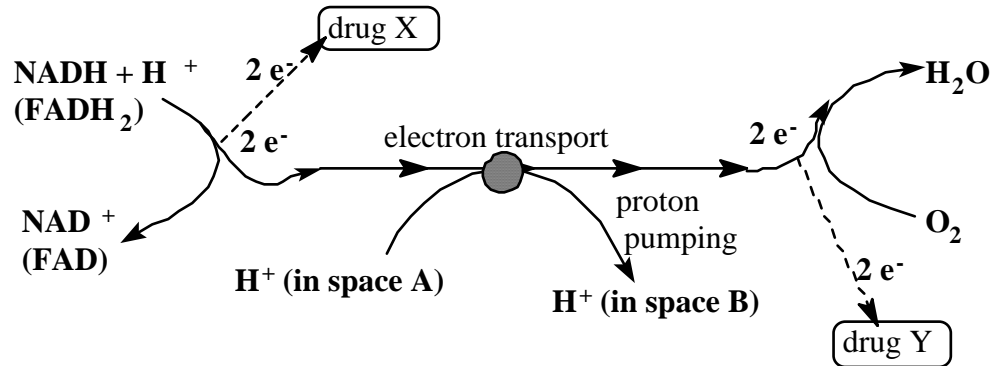


7.014 Quiz III Handout

****This will be a closed book exam****

Question 1

Shown below is the portion of the diagram from page 9 that shows the electron transport pathway. In this pathway, electrons are transferred from NADH to O_2 ; the energy of this reaction is coupled to pumping of H^+ .



Under normal circumstances, cells carrying out respiration:

- consume O_2
- produce CO_2
- produce 36 ATP per glucose consumed

Two drugs, drug X and drug Y, can pick up electrons from particular intermediates in this pathway. This is shown above.

a) You treat cells carrying out respiration with a saturating dose of drug X, so that all the electrons which would normally continue along the pathway are captured by drug X. Under these conditions:

- Will the cells continue to consume O_2 ?
 - Will the cells continue to produce CO_2 ?
 - Will the rate of ATP synthesis increase or decrease or stay the same?
- Explain your reasoning.

b) You treat cells carrying out respiration with a saturating dose of drug Y, so that all the electrons which would normally continue along the pathway are captured by drug Y. Under these conditions:

- Will the cells continue to consume O_2 ?
 - Will the cells continue to produce CO_2 ?
 - Will the rate of ATP synthesis increase or decrease or stay the same?
- Explain your reasoning.

Question 2

Agrobacterium tumefaciens is a bacterium that infects a wide range of broad-leaved plants. Most of the *Agrobacterium* genes are found on its large chromosome, but some of the genes that allow this bacterium to infect plant cells are found on the bacterial T_i plasmid. During infection, the bacterium transfers the T_i plasmid DNA to the plant and the plasmid DNA is integrated into the genome of the plant. This new DNA encodes plant hormones that stimulate cell division of the infected cells. This excessive cell division produces a tumor.

The T_i plasmid also carries the genes responsible for opine production. Opines are modified amino acids that only plant cells infected with *Agrobacteria* produce. These opines can be used by *Agrobacteria* as the SOLE carbon and energy source, and are not used by other organisms.

a) Circle the correct answer...

Agrobacterium is an autotroph or a heterotroph.

b) For the T_i plasmid DNA to have an effect within the plant cell, an appropriate promoter must be included for each gene.

i) What is a promoter?

ii) Describe the potential problem(s) of trying to express genes from a eukaryote in a prokaryote.

iii) Would the genes carried on the *Agrobacterium* T_i plasmid have a prokaryotic or eukaryotic promoter? Why?

iv) What is the likely origin of the T_i plasmid DNA sequences?

The major staple food for hundreds of millions of people is rice. However, rice lacks carotenoids that are converted into beta-carotene, a precursor of vitamin A. Millions of people suffer from vitamin A deficiencies. Rice has most of the biochemical pathway for producing beta-carotene, but is lacking two enzymes. Using *Agrobacterium tumefaciens* and modified T_i plasmids, scientists have created rice plants that have the needed enzymes and can make beta-carotene. These plants produce yellowish colored rice grains, called Golden Rice, which is high in beta-carotene.

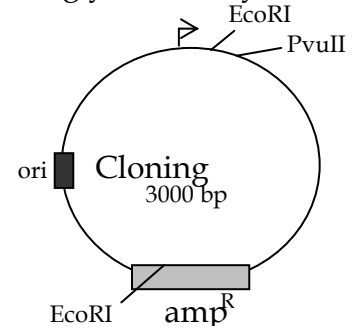
Question 2, continued

To create Golden Rice plants, two different T_i plasmids were made. In the first case, the T_i plasmid carried the *crt1* gene from the bacterium *E. uredovora*. The other T_i plasmid carried the daffodil gene *psy2*. You want to recreate the first of these modified T_i plasmids.

c) You are given *E. uredovora* genomic DNA and want to use this to make a library in *E. coli* cells. You purchase the following cloning vector for use in constructing your library. It encodes the gene for ampicillin resistance (amp^R).

PvuII: 5' -CAG | CTG-3'
3' -GTC | GAC-5'

EcoRI cuts: 5' -G | AATTC-3'
3' -CTTAA | G-5'



i) With what enzyme will you cut your cloning vector?

ii) With what enzyme will you cut your genomic DNA?

d) You successfully cut both your genomic DNA and your cloning vector. You mix them together and add DNA ligase.

i) In what other cellular process have we encountered DNA ligase?

ii) What does DNA ligase do?

e) You transform *E. coli* cells with your ligated DNA to make a library.

i) The *E. coli* cells that you use for transformation should have what phenotype prior to being transformed?

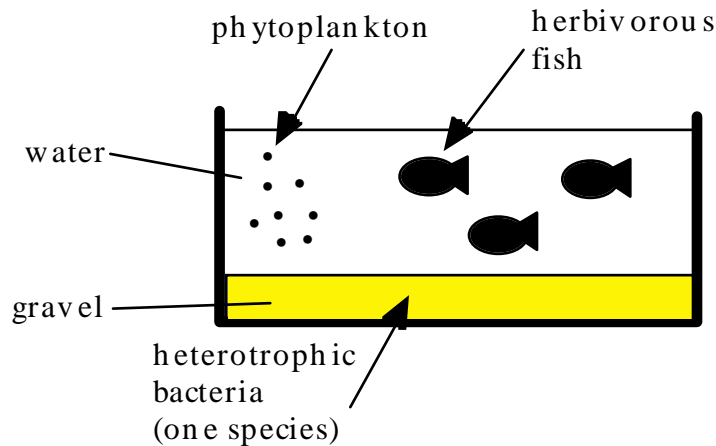
ii) Once transformed, how do you isolate the *E. coli* cells that have a plasmid away from the *E. coli* cells that don't have a plasmid?

f) You successfully create a wild type genomic DNA library from *E. uredovora*.

Assume that production of beta-carotene is essential for growth of *E. uredovora* cells on minimal media. How could you use your library to clone the *crt1* gene by complementation?

Question 3

Take the following simple ecosystem enclosed in a fish tank:



- the only energy input is light
- the fish eat only the phytoplankton
- humans harvest the fish
- nothing eats the bacteria
- the whole tank, including the sediments, is aerobic

a) Construct an energy flow model for this ecosystem.

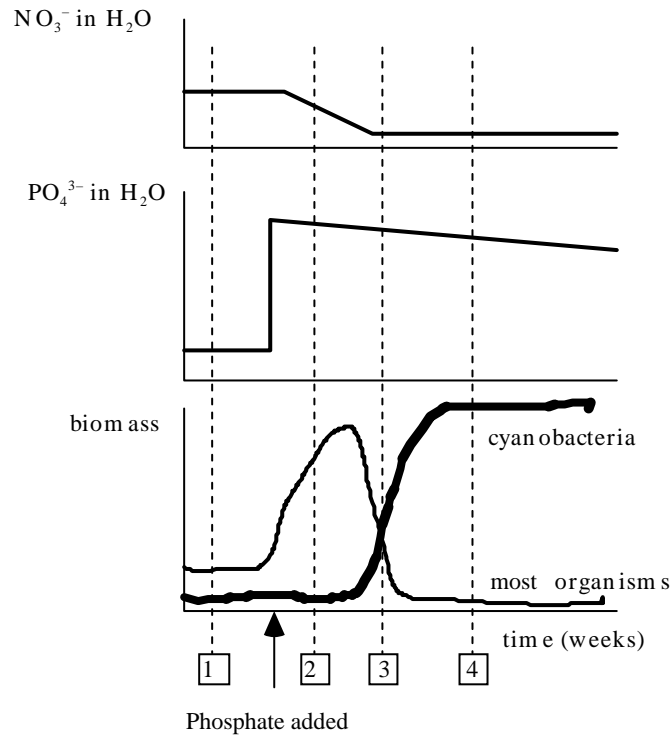
- i) Label all compartments and products
- ii) assign a different label to each of the energy flows
- iii) describe what each energy flow represents.

b) Suppose you added a worm to this ecosystem that only consumes waste produced by the fish. What would be the effect on the level of respiration by the heterotrophic bacteria (increase, decrease, stay the same) of adding this worm to the tank, if the worm's waste and detritus **was not** consumed by the detritivores. Explain your reasoning briefly.

c) Suppose you added a worm to this ecosystem that only consumes waste produced by the fish. What would be the effect on bacterial respiration (increase, decrease, stay the same) of adding this worm to the tank, if the worm's waste **was** consumed by the detritivores. Explain your reasoning briefly.

Question 4

Nitrogen (in the form of nitrate: NO_3^-), and phosphorus (in the form of phosphate: PO_4^{3-}), are often limiting nutrients in aquatic ecosystems. In the experiment below, you add phosphate fertilizer to a pond. You see a brief period of growth of most organisms followed by a large bloom of cyanobacteria. This is shown below:



a) Which of the above element(s) are limiting at each of the following times?

i) At time **(1)**? Explain your reasoning.

ii) At time **(4)**? Explain your reasoning.

b) Why is the $[\text{NO}_3^-]$ in the water decreasing at time (2)?

c) Why are the cyanobacteria at an advantage at time (4)?

Question 5

You are studying the genetics of the fruit fly. Body color (brown or black) is controlled by the *b* locus and wing size (long or short) is controlled by the *vg* locus. Use (+) for the alleles associated with the dominant characteristics, (*b* or *vg*) for the alleles associated with the recessive traits.

In cross 1, you mate two pure-breeding strains and obtain the following progeny:

cross 1: brown, short-winged male X black, long-winged female

F1: all brown, long-winged flies

a) Which phenotypes are dominant and which phenotypes are recessive?

i) The recessive trait for body color is _____.

ii) The recessive trait for wing size is _____.

b) In cross 2, you then mate many pairs of siblings from the F1 generation of cross 1:

cross 2: F1 sibling male X F1 sibling female

What genotypic and phenotypic ratios would you expect in the progeny (F2) of these crosses if independent assortment occurs?

The compiled progeny results from the cross 2 mating pairs are presented below:

<u>F2:</u>	<u>Phenotype</u>	<u># identified</u>
	brown, large-winged	512
	black, large-winged	244
	brown, short-winged	246
	black, short-winged	9

c) From this data, what might you conclude about these two loci?

d) What alleles are carried on each chromosome of each fly in cross 1?

e) You perform test crosses with F1 flies and homozygous recessive flies.

i) If the *b* and the *vg* genes are 100% linked, what phenotypes do you expect?

ii) The actual data from these crosses is shown below:

<u>Progeny:</u>	<u>Phenotype</u>	<u># identified</u>
	brown, large-winged	46
	black, large-winged	209
	brown, small-winged	213
	black, small-winged	49

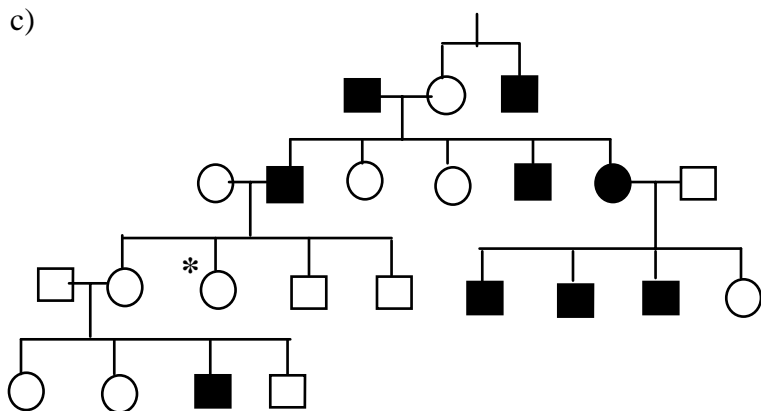
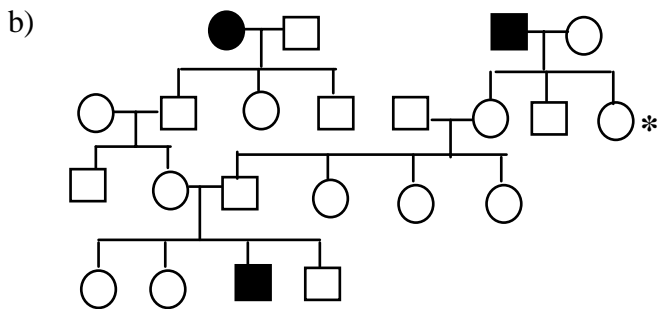
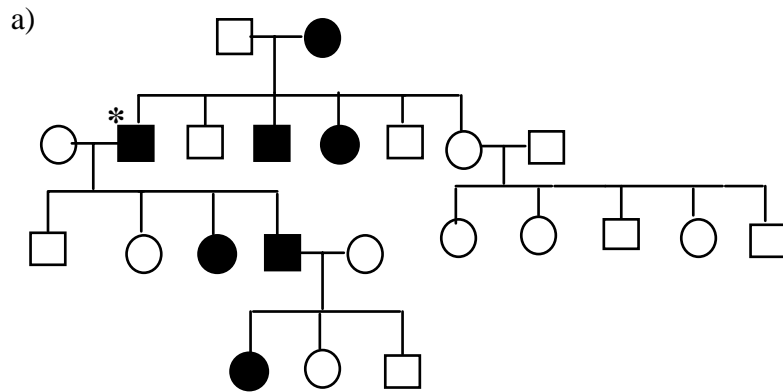
Calculate the recombination frequency between the body color and wing size loci:
Question 6

For the following human pedigrees, a-c, determine:

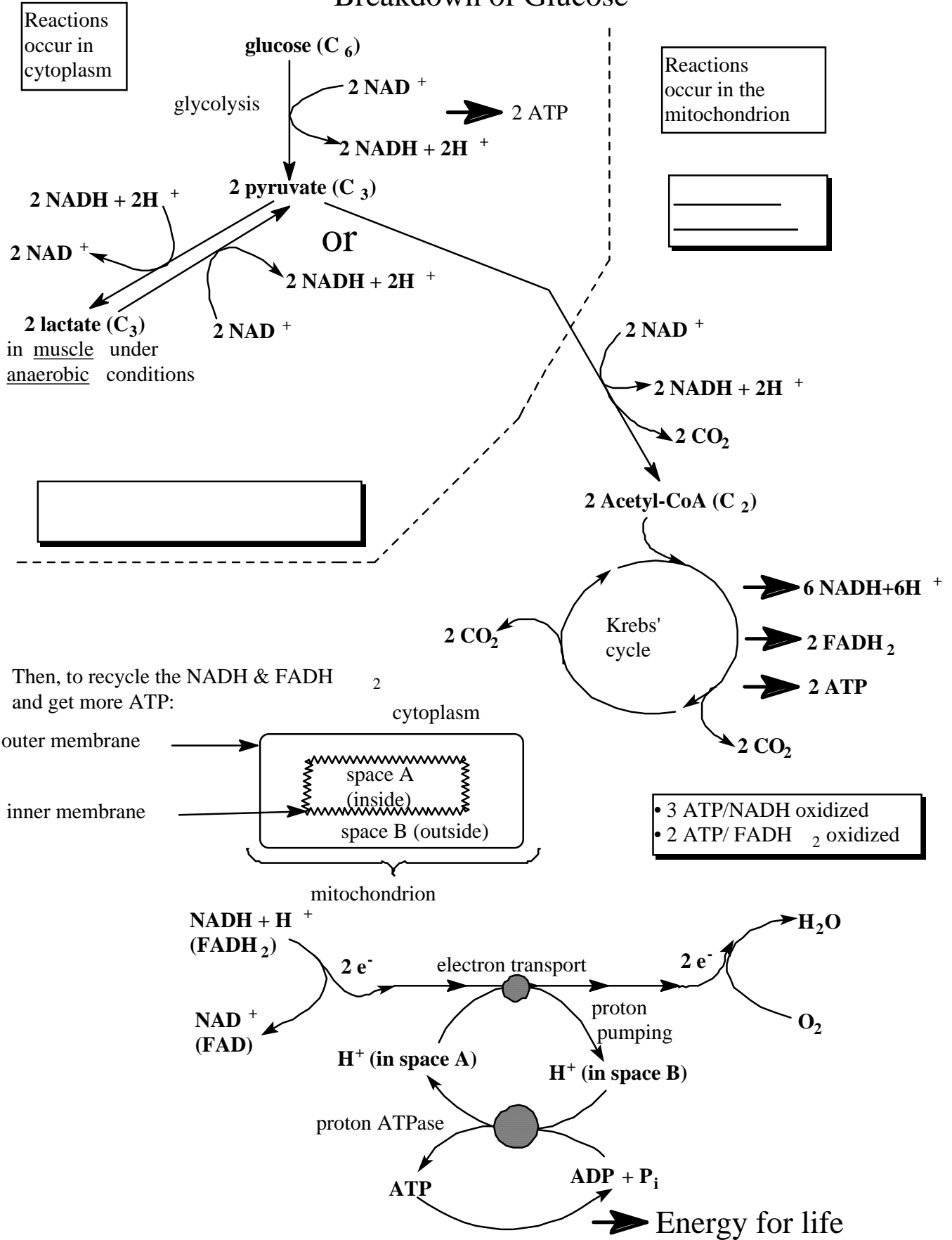
- i) the most likely mode of inheritance of the following traits (dominant or recessive **and** autosomal or sex-linked),
- ii) the probable genotype of the individual marked with an asterisk (*).

Assume that individuals that marry into the family do not have the defective allele. Explain your answer. Be sure to clearly define your symbols.

KEY: □ normal male ○ normal female
 ■ affected male ● affected female



Breakdown of Glucose



Solutions:

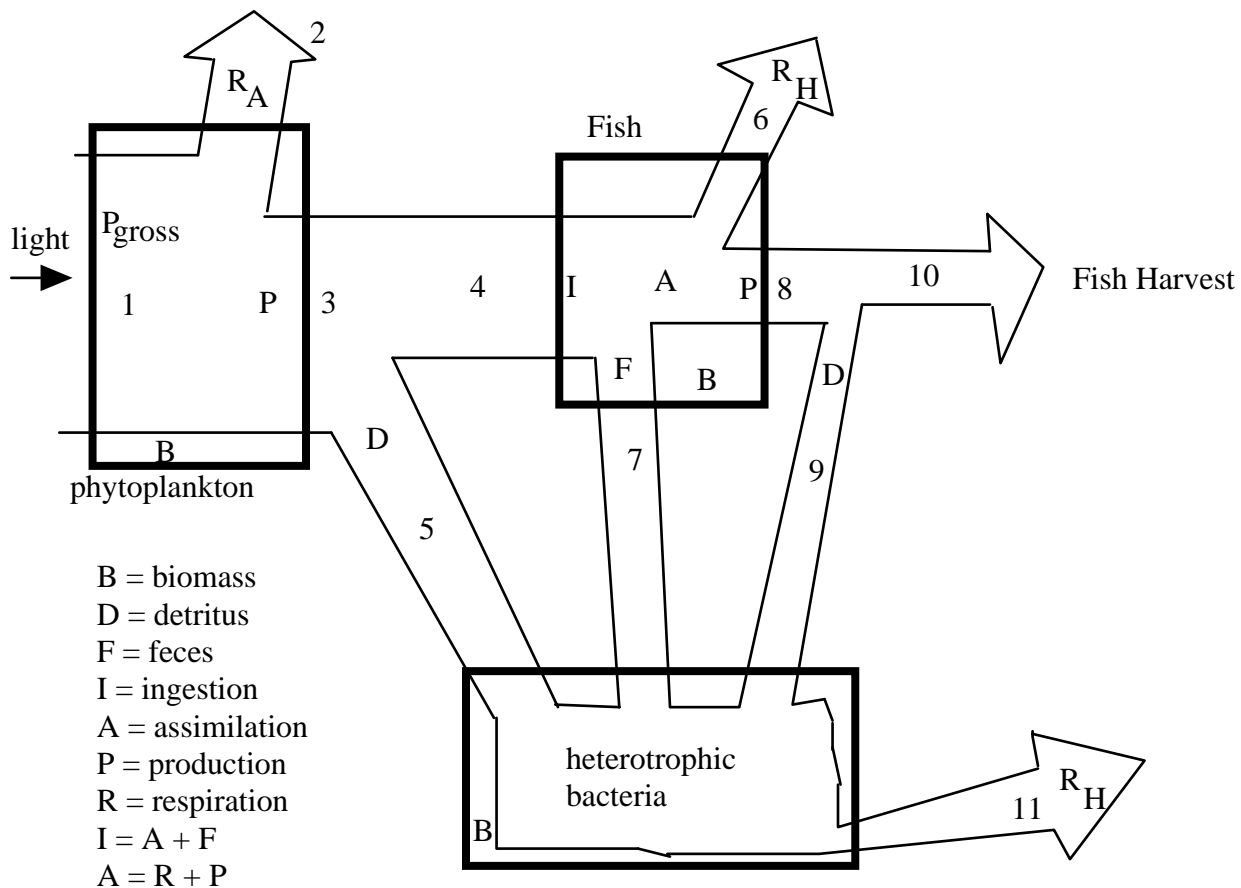
Question 1

- a) won't consume O₂; will produce CO₂; ATP will decrease because no H⁺ pumped
- b) won't consume O₂; will produce CO₂; ATP will stay the same because H⁺ still pumped.

Question 2

- a) *Agrobacterium* is an autotroph or a heterotroph.
- b)
 - i) A region of double-stranded DNA where RNA polymerase binds and begins transcription.
 - ii) 1) The DNA sequence that defines a promoter is specific to that organism. A eukaryotic promoter will not be recognized by prokaryotic RNA polymerase, so the eukaryotic gene is not transcribed.
2) Eukaryotic genes often have introns. An intron is a region of DNA that does not encode protein and is spliced out of the transcript prior to translation. Prokaryotic organisms do not have introns and can not remove them from the transcript so the protein produced would not be correct.
 - iii) The genes on the T_i plasmid are expressed in plant cells so they must have eukaryotic promoters.
 - iv) The T_i DNA sequences encode plant hormones, so they likely came originally from a plant.
- c)
 - i) PvuII
 - ii) PvuII or any other restriction enzyme that leaves blunt ends.
- d)
 - i) DNA Replication of lagging strand
 - ii) Forms a phosphodiester bond between the 3' OH and the 5' phosphate of two DNA fragments.
- e)
 - i) The *E. coli* cells prior to transformation must be sensitive to ampicillin.
 - ii) The *E. coli* cells that are transformed can be grown on media containing ampicillin. Only cells that carry a plasmid will survive.
- f) You would have or create *E. uredoovora* cells that are missing the *crt1* gene (*crt1*- cells). These cells will not grow on minimal medium. These *crt1*- cells can then be transformed with the plasmids from the library, and transformed cells would be grown on minimal media. Only cells with the wild type *crt1* gene will grow.

Question 3



- 1 = GPP: gross CO₂ fixation
- 2 = phytoplankton respiration
- 3 = NPP: net primary production by phytoplankton
- 4 = phytoplankton eaten by fish
- 5 = phytoplankton detritus (phytoplankton that die before being eaten)
- 6 = fish respiration
- 7 = fish waste (feces)
- 8 = net production by fish
- 9 = fish that die without being harvested
- 10 = fish harvest
- 11 = respiration by detritivores (bacteria)

b) Decrease. Now two organisms compete for fish waste (7), so the detritivores necessarily consume a smaller portion of (7) than without a competitor.

c) Decrease, but decrease not as large as in (e). The two organisms still compete for fish detritus (7), now however the worm returns some part (necessarily less than 100%) of the portion of (7) it consumes as worm detritus.

Question 4

a) Which of the above element(s) are limiting at each of the following times?

i) At time **(1)**? Explain your reasoning.

Phosphate. We see that simply adding PO_4^{3-} is sufficient to cause growth of most organisms. This indicates that PO_4^{3-} was limiting.

ii) At time **(4)**? Explain your reasoning.

Nitrogen. We see from the graph that PO_4^{3-} levels remain high, but nitrogen levels have decreased, and the biomass of organisms is no longer increasing.

b) Why is the $[\text{NO}_3^-]$ in the water decreasing at time (2)?

As the biomass of organisms increases, so does the need for nitrogen, which is being used to form biomolecules, such as proteins, DNA, etc.

c) Why are the cyanobacteria at an advantage at time **(4)**?

Cyanobacteria can fix atmospheric N_2 into organic N and thus are not limited by the decreasing nitrogen (NO_3^-) levels.

Question 5

a) i) The recessive trait for body color is black.

ii) The recessive trait for wing size is short-winged .

b) You would expect ratios of:

- 9 brown, large-winged
- 3 black, large-winged
- 3 brown, short-winged
- 1 black, short-winged

c) The genes are not segregating independently because you do not obtain a 9:3:3:1 ratio. The genes for body surface and wing size may be linked on the chromosome.

d)

	chromosomes
	+ vg

brown, short-winged male	+ vg

	b +

black, long-winged female	b +

Question 5, continued

- e) i) 1/2 of the flies would be brown, short-winged
1/2 of the flies would be black, long-winged

ii) frequency = #recombinants/total # of progeny

The two recombinant classes are wild type and the double mutant black small-winged.

Therefore, the recombination frequency is:

$$(46 + 49)/(46 + 209 + 213 + 49) = 95/517 = 0.184$$

Multiply this by 100 to obtain a percent value of 18.4% recombination frequency.

Question 6

a) Autosomal dominant, because affected individuals can be of either sex, occur in all generations, and always have an affected parent. If d = normal allele and D = affected rare trait allele, then $*$ = Dd

b) Autosomal recessive; the disease is not present in every generation, intermarriage allows the trait to become evident. If D = normal allele and d = affected rare trait allele, then $*$ = Dd

c) X chromosome-linked (sex-linked) recessive; carrier females pass allele to one-half of their sons, affected males pass allele to all their daughters, all sons of affected females are affected.

If X^D = X chromosome with normal allele and X^d = X chromosome with disease allele, then $*$ = $X^D X^d$