7.012 Studying

12/15
10:30P

esp need to review stuff at start
- ~~Bad~~ bonding
- pedigrees

Oh they do drop lowest exam!
└ prob new psets

~~Skip~~ ~~basic~~
Skip basic stuff I know
└ for time purposes...

Organelles - components within a cell...

1. Observational Bio
2. Analytical Bio

Hydrogen

H

- ionic, negitive charge H^-

isotope

- same protons, diff neutrons

Carbon-12 = 6 protons + 6 neutrons

atomic # = protons

mass # = protons + neutrons

electrons much smaller $\left(\frac{1}{1736}\right)$ but same charge

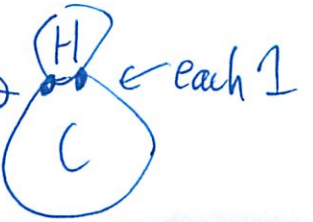
Carbon

4 **valance** electrons

- available to bond

Oxygen

6 valance electron

each contributes 1 → 

(3)

Nitrogen 5 epi valance electron

Phosphorus = 5

Sulfer = 6

(really need to remember!)

Bonds

Covalent

Sharing electrons

very strong

Ionic

Eletrostatic attraction

2 oppositly charged ions

Since # electrons ≠ # protons

Polar bonds

type of covalent bond b/w 2 atoms are shared unequally

so at end one has slight + or − charge

(4)

Electronegitivity

Symbol = Chi χ

tendency of atom or functional group

Hydrogen Bond

electongitive interaction of H and a electrongitive ion

is a polar bond

not as strong as covalent or ionic

VdW

"natural" attractive forces

very base

(5)

Solubility

likes to form H- bonds w/ water

ionization

Covalent bond being broken

Carboxyl

at neutral pH - gives up proton

Need to remember these groups!

Amino

likes to suck up protons

(+) charged

amine

(6)

—— line → flat

 wedge → toward observer

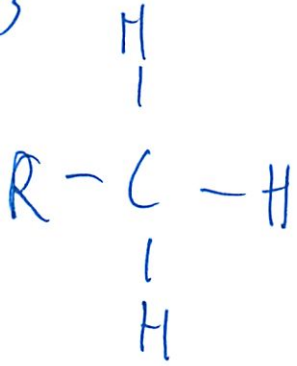
 dotted → away from observer

.... dotted → Hydrogen bond

Methyl

Can't ionize

CH_3



Condensation / dehydration

loss of water from molecules

hydrolosis → water added

(7)

Lipids

fats, waxes, steroids, vitamins etc

Cell membrane structure
important signaling molecules
bilayer

Carbohydrates

Carbon, hydrogen, oxygen
Sugars (saccarides)
-ose
Store energy
Structural component
backbone of RNA/DNA
play other key roles
in many foods

(8)

linear vs ring form

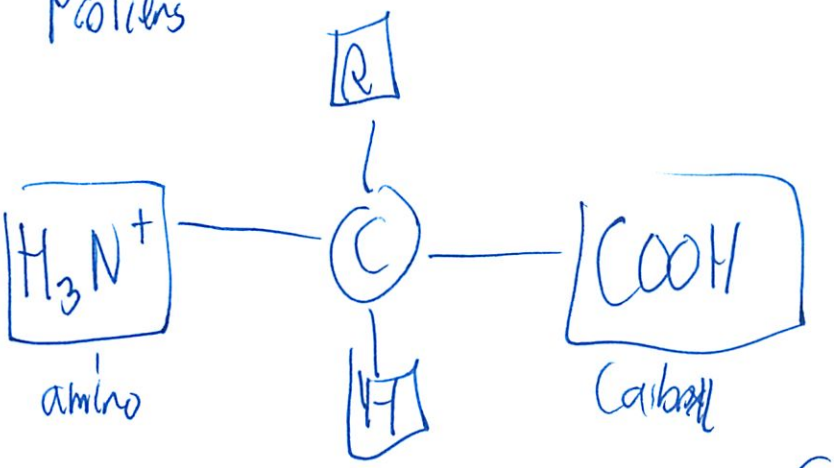
Can build long polymers

Starch makes fat, gives energy

Cellulose can't do much --- structure of cells

Glycogen stores energy

Proteins

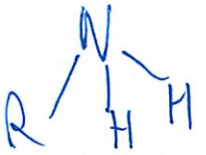


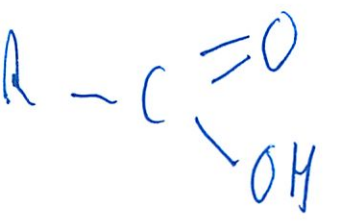
↑ C terminus

N terminus ——transfer——→ $-COOH$

$-NH_2$

↑ exits last





Carbonyl + hydroxyl
H^+ leaves

(9)

peptide bond

makes protiens

N → C

20 diff amino acids

---

primary structure - amino acids

Secondary - local segments 3D

Tertiary - helix or β sheets

Quadrary - β protiens stick together in certain way

Bond order

Covalent
ionic
H bonding
polar
Vandewals

(10)

yeah pretty sure H weaker than polar

H is a intermolecular force (weaker than a bond)

polar are covalent but diff negitivites (

↳ yeah shared unequally

(need to do a lot of bonding pratice!)

(review P-sets at some pt...)

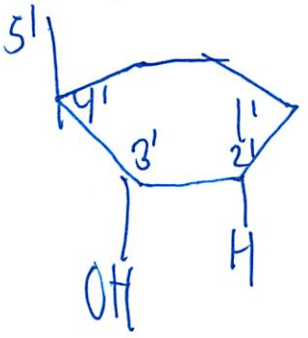
---

denature proteins

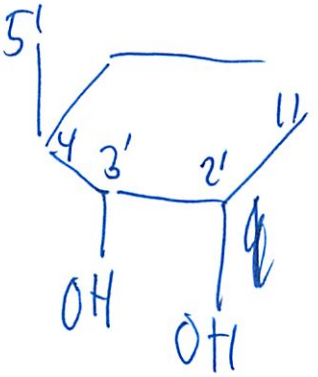
break down 2nd, 3rd structure

primary unchanged

DNA



RNA



↑ lost an oxygen (↑ so this more stable!)

(11)

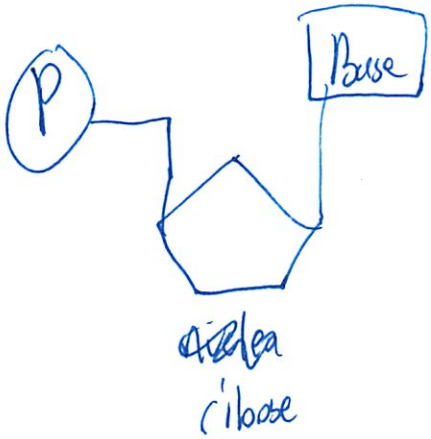
hydroxyl

Oxygen + hydrogen

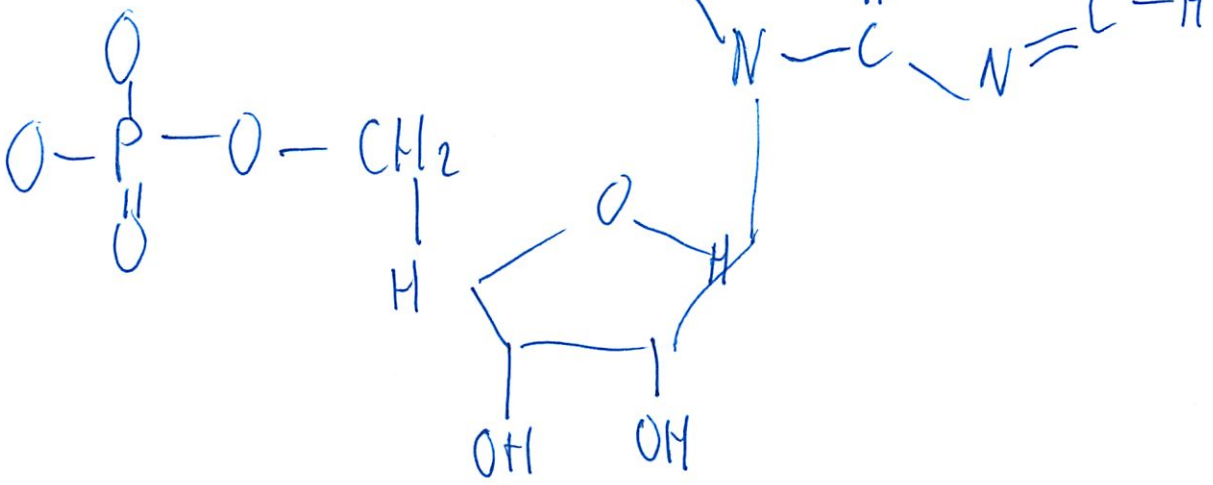
L w/ covalent bond

part of substrate of water molecule

Nucleotide



- either C, T, U, A, G



(1.2)

% of A = % of T A–T
% of C = % of G C–G

3' → 5'
5' → 3'

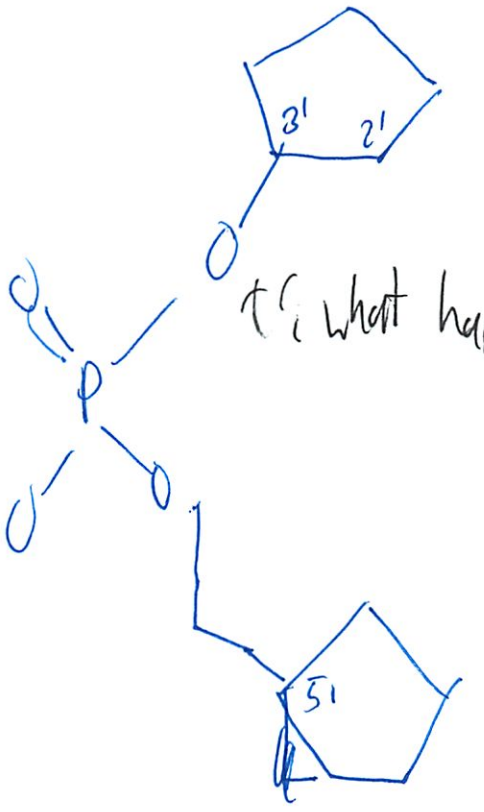
So stuff joins at 5' end
the phosphorus end

* Only appends to 3' end w/ OH

Phosphodiester bond — Strong covalent bond

2 carbohydrates over 2 ester bonds
–OH replaced by –O

(13)



↑ ; what happens to 2nd O ?
and H ?
; condense ; ;

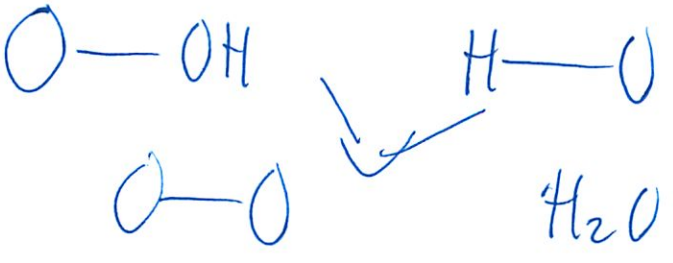
intercept w/ didi-oxycibose

We do get rid of a phosphate

usually 3 phosphates → high energy

"polymeration process"

Condensation



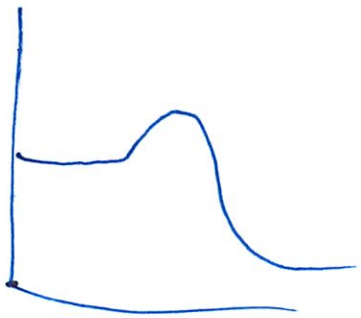
(19)

Hydrolosis → add water

Polymerization is w/ condensation

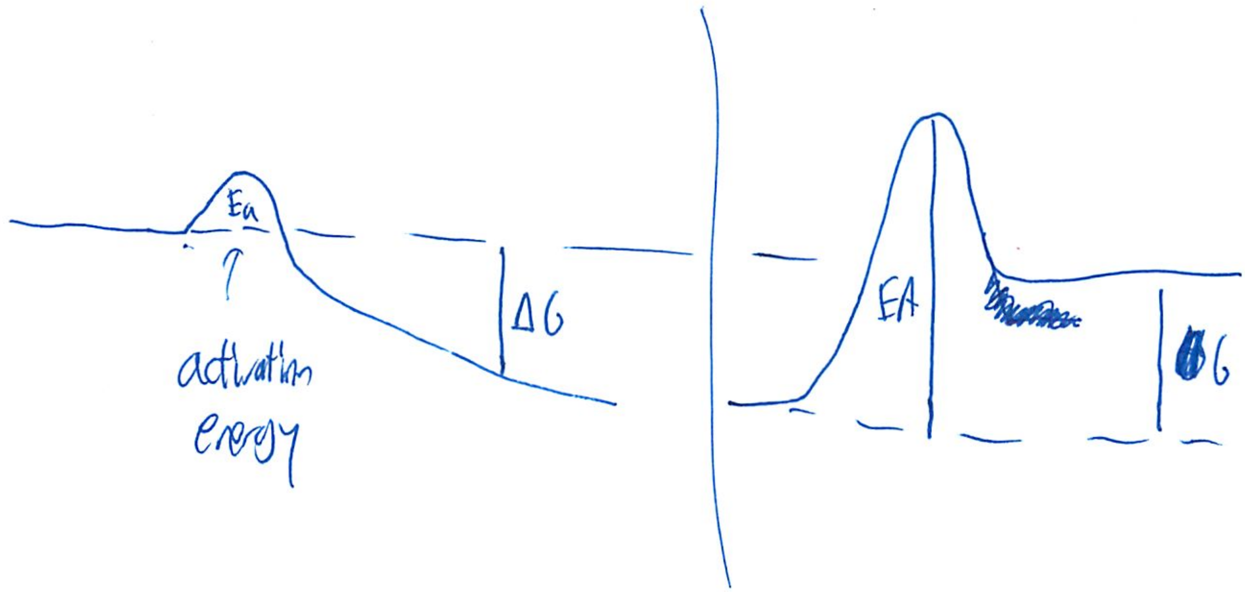
Activation Energy

Should do pratice problems
These are tricky!



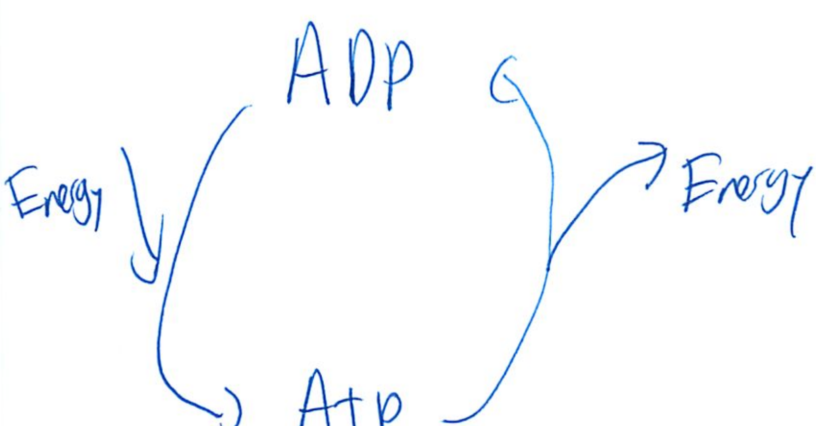
Decrease w/ enzymes
- They must be regenerated each tie

Enzymes can also break stuff down



Says nothing about kinetics (how Fast)

Ea lowered by enzyme
G same



endothermic = release energy
exothermic = takes energy

(16)

Went through that unit

I feel like I get it ok

The phosphorylation still kinda baffling me

But that is non-core...

Genetics

(This is tricky... need to be exacting!)

Mendall's Experiments

Peas

Rinkled vs Round

Certain ratios

Allele alt form R vs r

Genotype two alleles carried by indv

Homozygous two of the same RR

hetero Diff Rr

Pheno appearance

(17)

Law of Segregation (1st law)

random selection from parents | each

Law of Ind Assotment (2nd law)

Inheritance happens independently

Chromosomes two copies

| each one chromatid

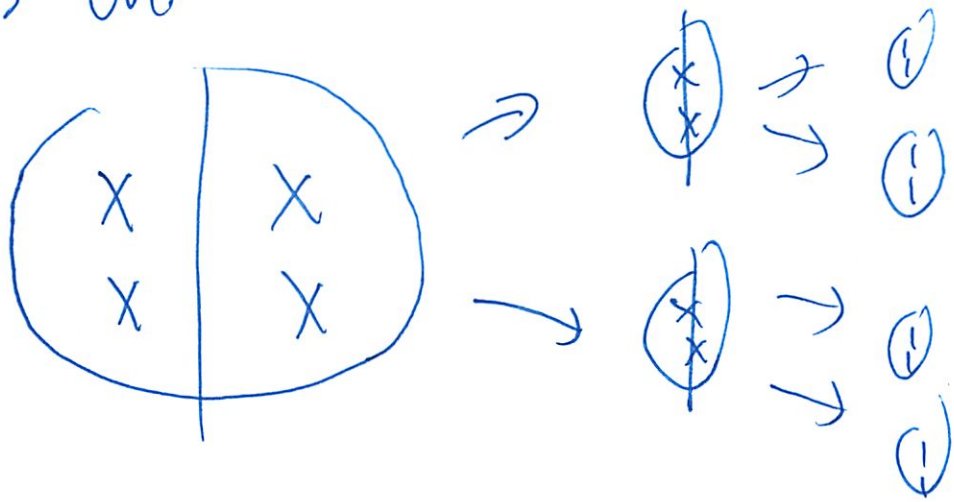
(need to do the mitosis/meiosis)

(18)

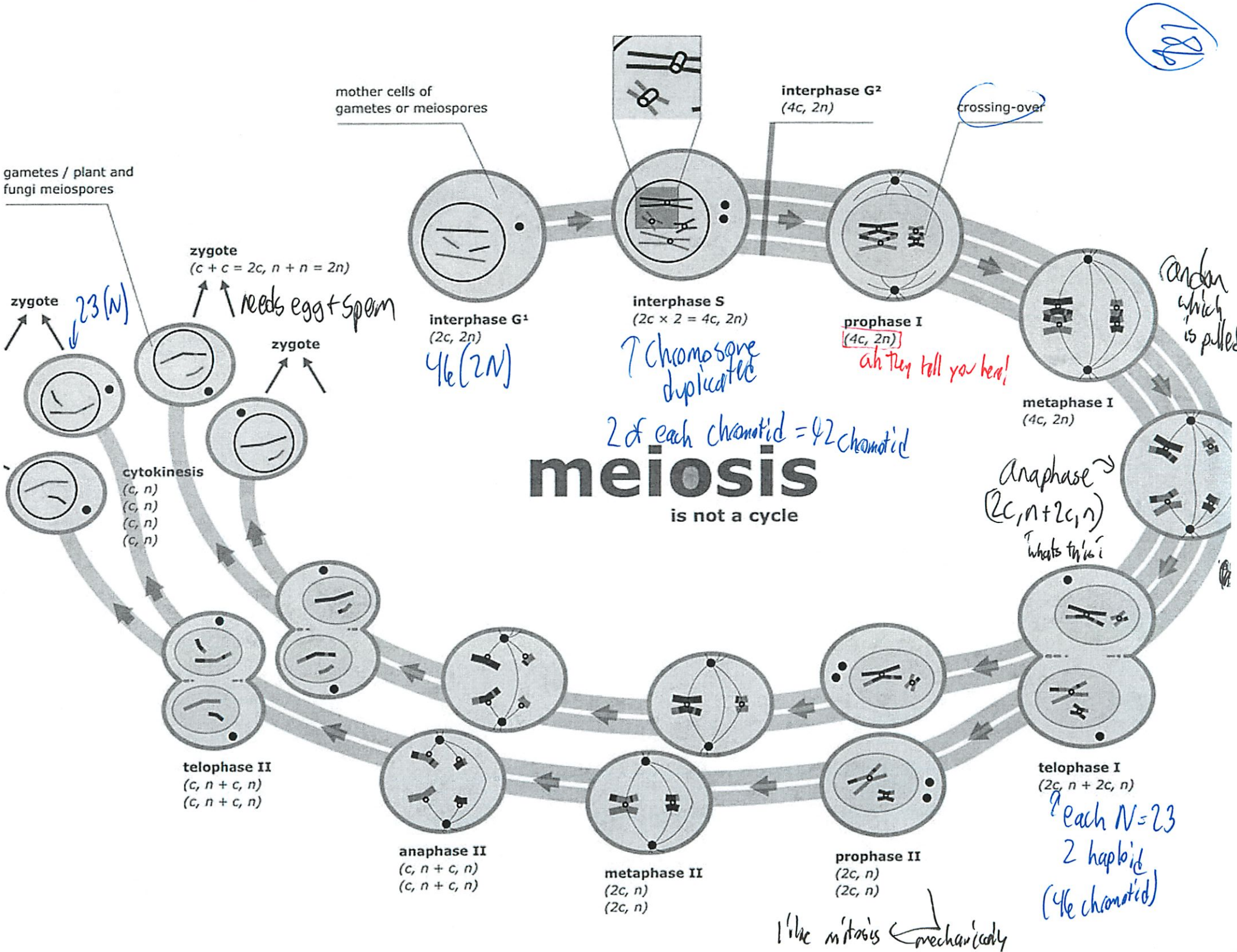
12/16
12:15p

Meiosis

Splits in 4 (sexual repo)
homologous chromosomes split up
Cross over



? sperm or eggcells (haploids)


mother cells of gametes or meiospores
interphase G¹ (2c, 2n)
interphase S (2c × 2 = 4c, 2n)
interphase G² (4c, 2n)
crossing-over
prophase I (4c, 2n)
metaphase I (4c, 2n)
anaphase I (2c, n + 2c, n)
telophase I (2c, n + 2c, n)
prophase II (2c, n) (2c, n)
metaphase II (2c, n) (2c, n)
anaphase II (c, n + c, n) (c, n + c, n)
telophase II (c, n + c, n) (c, n + c, n)
cytokinesis (c, n) (c, n) (c, n) (c, n)
zygote
zygote (c + c = 2c, n + n = 2n)
zygote
gametes / plant and fungi meiospores
meiosis
is not a cycle

19

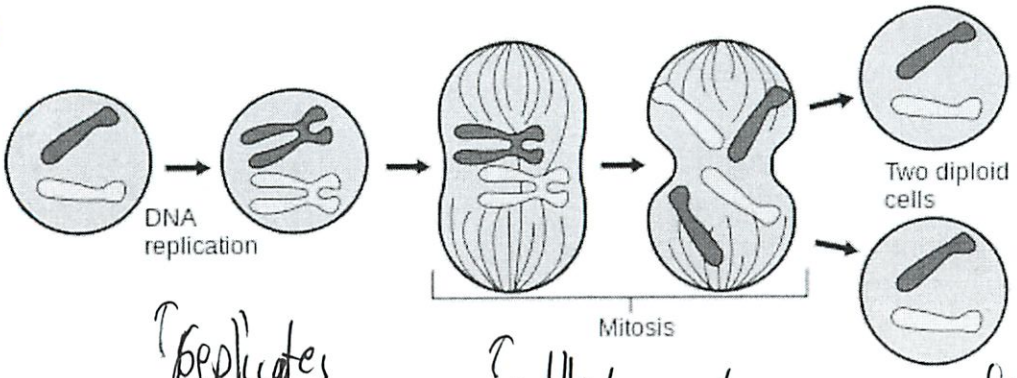
Mitosis

Splits in 2 (asexual reproduction)

Cellular reproduction

no crossing over

19b



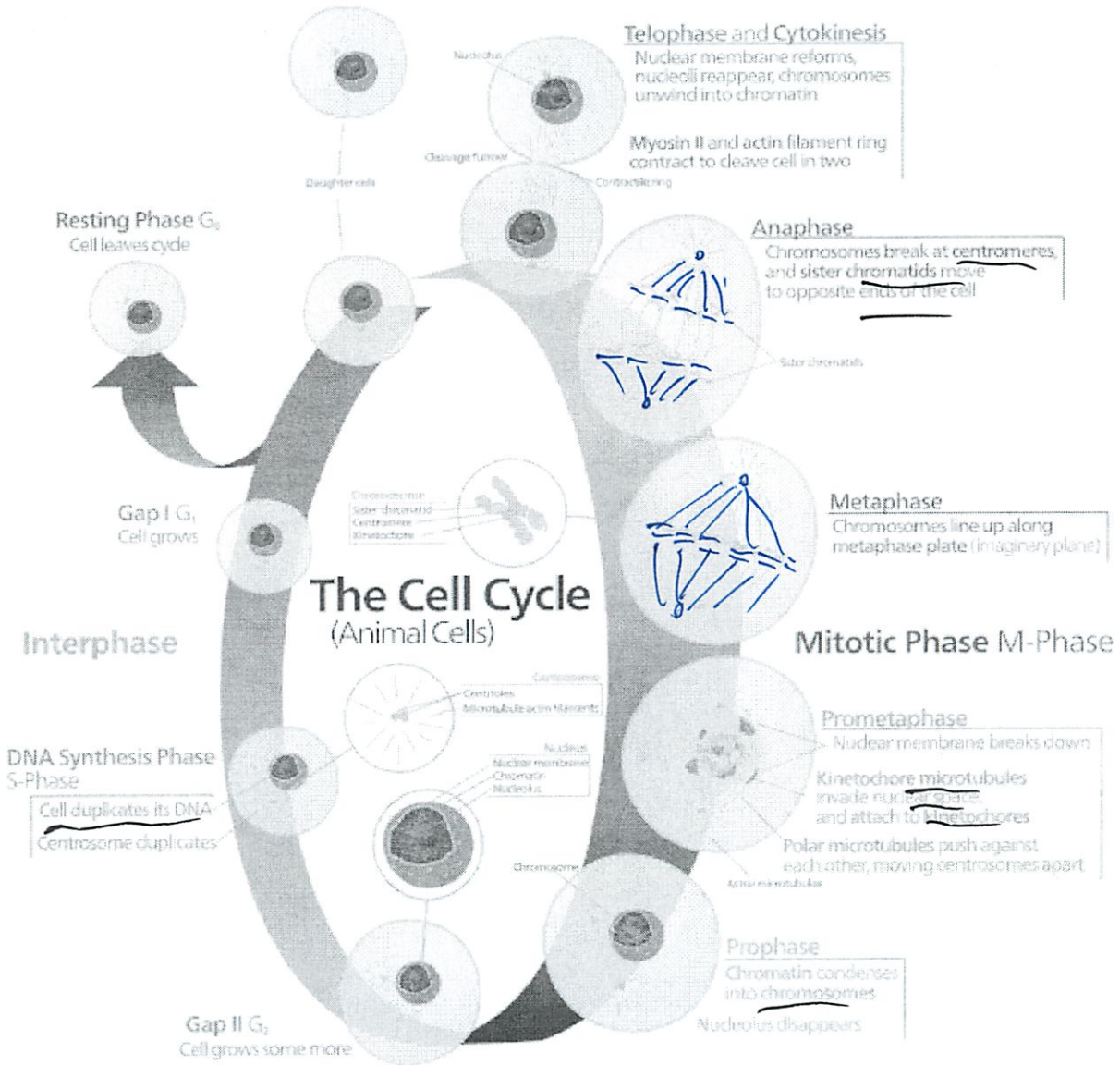
replicates

pulled apart

end w/ 2 identical cells

yes

19c

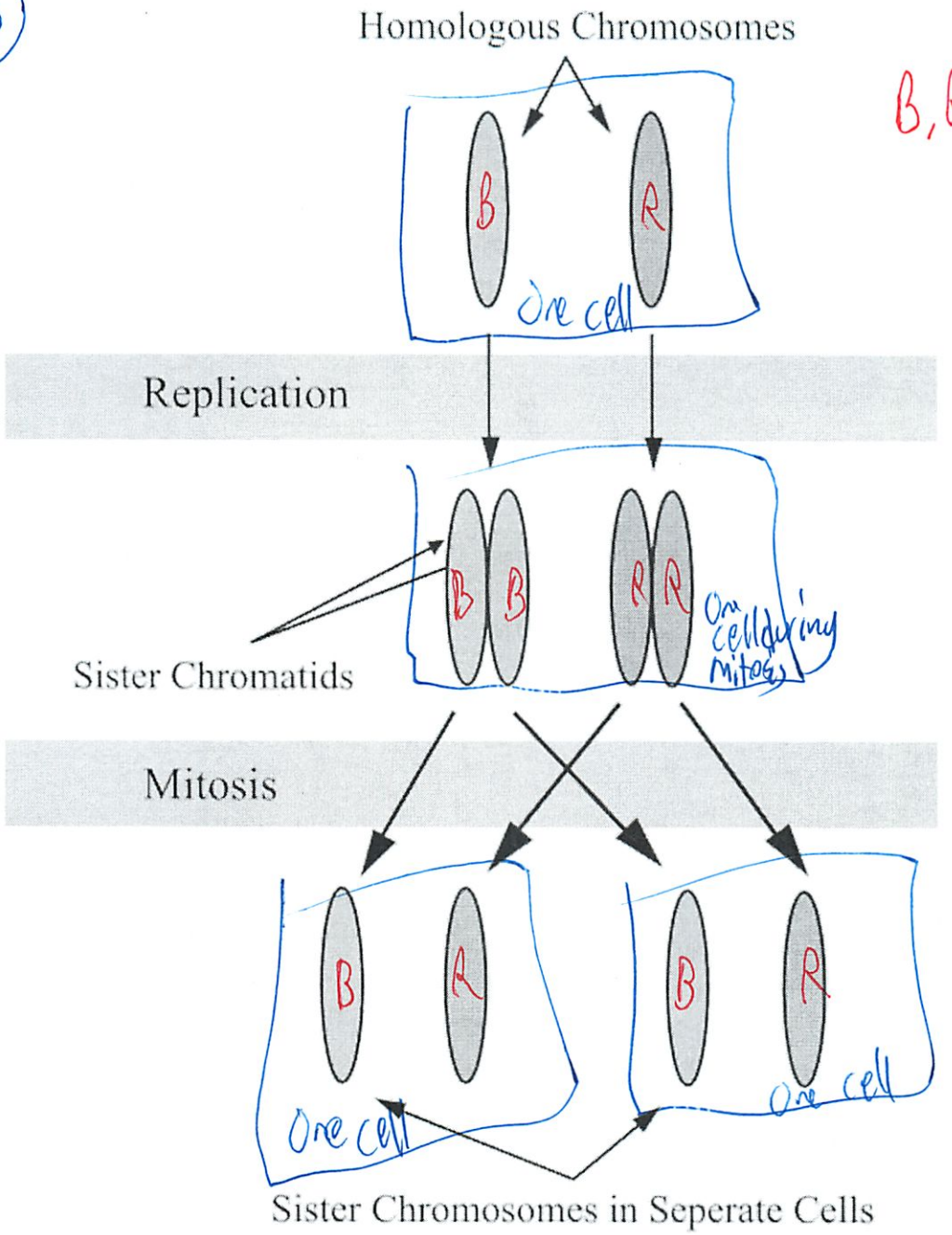


pulls apart

Sister chromatids - 2 identical copy of a chromatid

Chromosomes - 2 diff copies of chromatid (1 each parent)

19d


Homologous Chromosomes
B
R
One cell
Replication
B B
R R
One cell during mitosis
Sister Chromatids
Mitosis
B
R
One cell
B
R
One cell
Sister Chromosomes in Seperate Cells

B, R = cells

(20)

Enzymes

Can have anhibiters

Change Ea / Rate

not equilibium or ΔG

Kinase → add (P)

Phophotase → remove (P)

(really need to practice the genetics stuff!)

Same chromsome

dependent assothort

Unless crossing over ...

+ = wild tipe

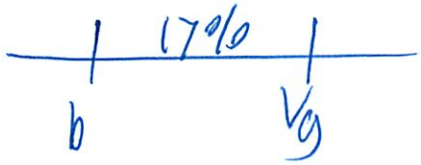
recombination rate $\frac{206 + 185}{465 + 444 + 206 + 185} = 17\%$

(206 + 185: due to recomb)

expected 1:1:0:0

(21)

Then can build a genetic map



but is it right or left?

(Should practice)!

At 50% uncorrelated

Sex Chromosomes

X-linked dom / recessive

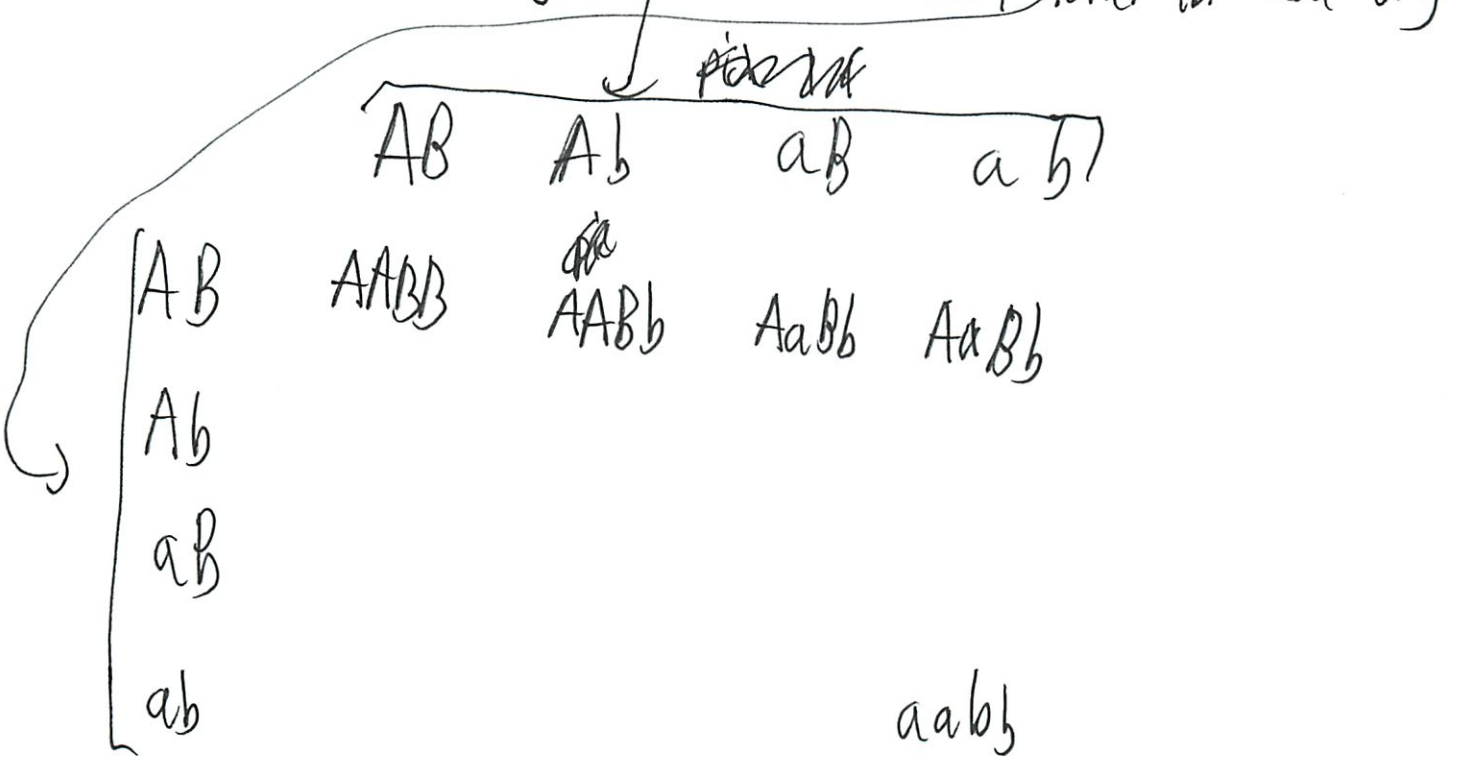
(22)

mono hybrid cross

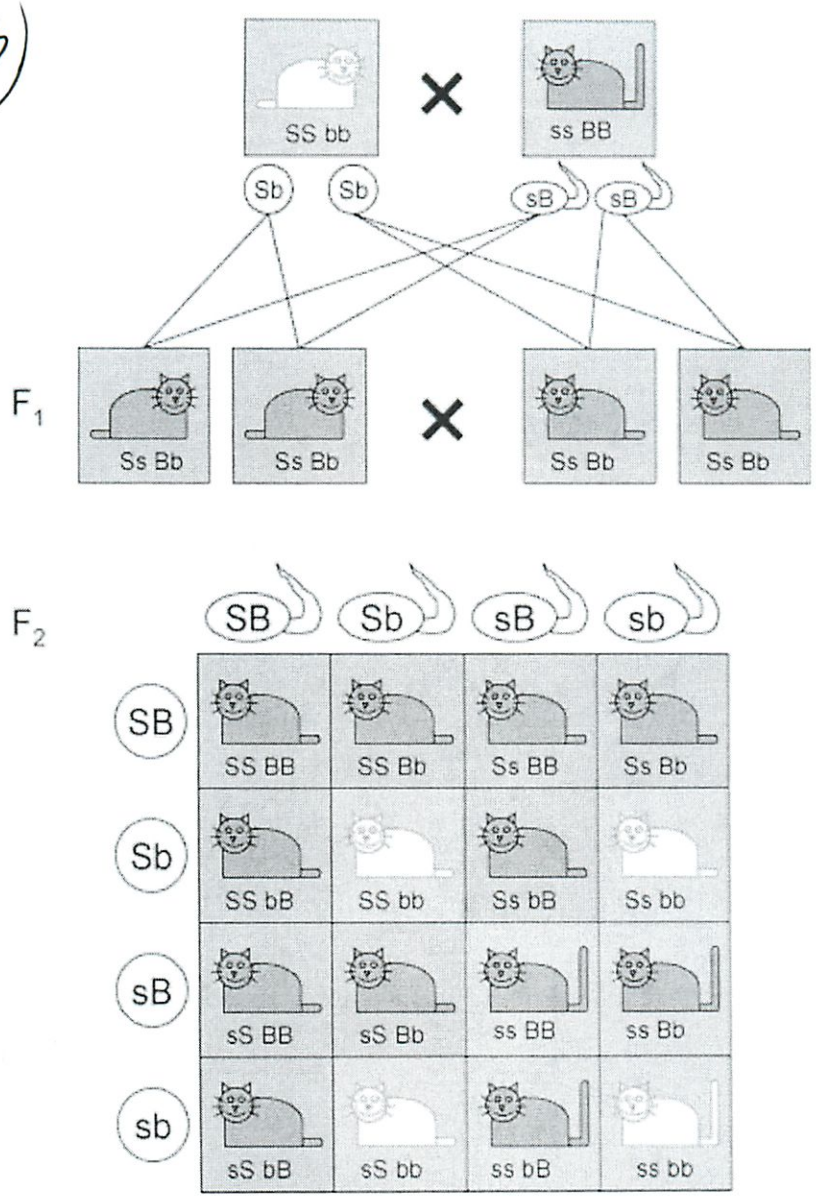
AABB vs aa bb

Dihybrid

AaBb vs Aa Bb



(23)



dihybrid cross

(24)

Charts/Pedigrees

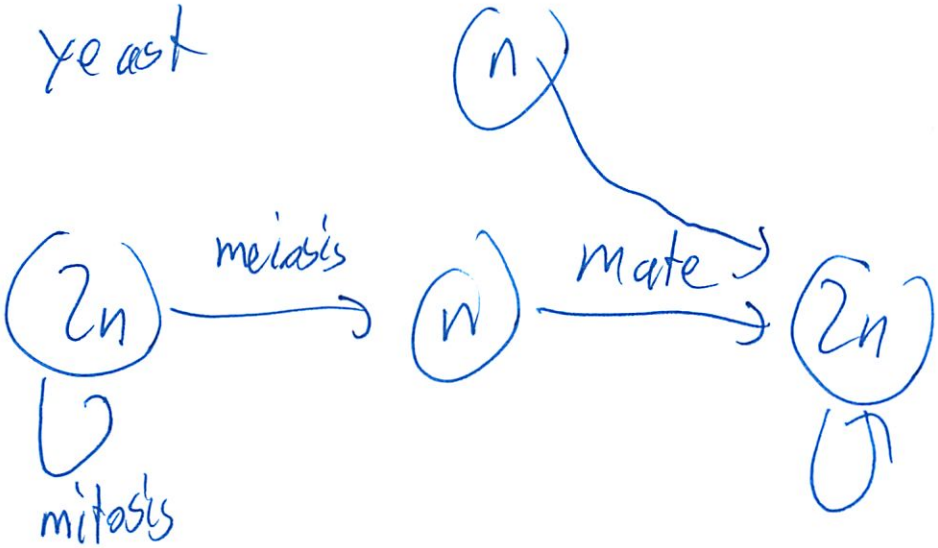
□ male

○ female

(really need to practice these!)

Urine based

Growing yeast



Growing in minimal media and rich medium
↳ study these qu!

~~Odd~~

auxotrophe needs some supplement in its medium

prototrope can grow in minimal medium

25

Mutant hunt

- ~~Gal~~ Screen
- Select

test for dominance

Cross it

See if it grows in minimal media

test cross

~~is ind dominant or recess~~

is ind homo or hetero for a dominant trait

RR w/ rr	Rr w/ rr
all dom phenotype	½ halt dom phenotype

26

backcross

hybrid (hetrozygas) w/ its parent
get a genotype close to parent

Complementation test

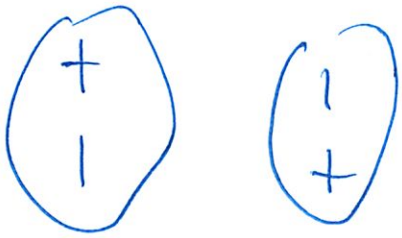
Complementation

relation b/w diff strains or org which both have homo, recessive mutations that produce same phenotype but don't reside on same homologous gene

(27)

Complementation test

test if in diff genes



if cross and get diploid

2 mutations complement each other

 will grow in minimal media

Otherwise won't grow

So complementation gene

ah since its ~~dominant~~ recessive → if both missing it

dominant

how many genes/proteins on bio pathway?
easiest to do on yeast

1. mutate haploids w/ chemicals + radiation
2. Fuse to getter
3. Look at phenotypes

If wild → genes complement
↳ diff genes
If mutation → in a complementation gp

Mutant screen

Plate on rich media → all grow
Replica plate to minimal media
See which colonies grow
Those that don't grow have some mutation
Then in minimal + tyr
└ have mutation in tyr production
(need to study these guys)

29

Non Mendelian

incomplete dominance red + white = pink

multiple alleles influence a trait

X-linked traits

Color blindness = X-linked recessive disease

hemophia = X-linked recessive

(must practice!)

Quiz | (review at some pt)

(did until on due to personal circumstances)

dominant = capital letter

(20)

Look at R grp if polar

⊕ ⇒ ionic, so polar

O, N most electroneg → so polar
ionic, H bond = polar

W H, O → H bonding possible
depending on other molecules

Phosphate grps ↓ are ⊖ so want ⊕ charge
for ionic

H–C not plr

OH polar

NH polar

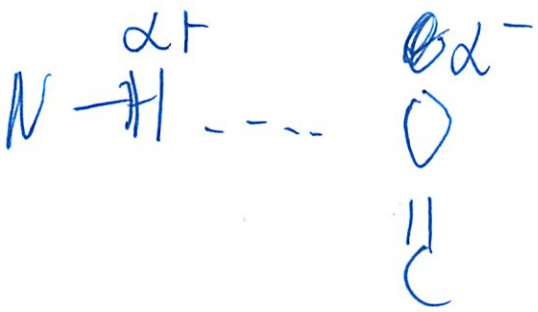
polar w/ non polar → VDW

ionic	polar ⊕	polar ⊖
hydrogen	polar	polar

Charge or uncharge

(31)

H must be electronegitive



So gave me chart on what is polar and what isn't

* first recognize amine and carboxly

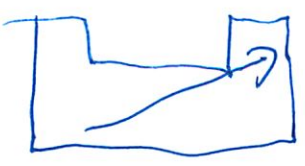
So if ⊕ → polar +

⊖ → polar −

just O → polar

6 valance electrons on O?

Oxy highly electronegivity



Since Oxy good at pulling electrons towards it
So $\delta-$

The higher the electronegativity the more ionic/polar the bond is

bonds that are part ionic + part covalent = polar bond

Carbon = Oxygen

Very prevalent
Covalent bond

electronegative

6 total valance electron

H bond

b/w polar H
and electro neg N, O, F

(33)

Still don't fully get
kinda get
Need pratice w/ at some pt...

OH also plar

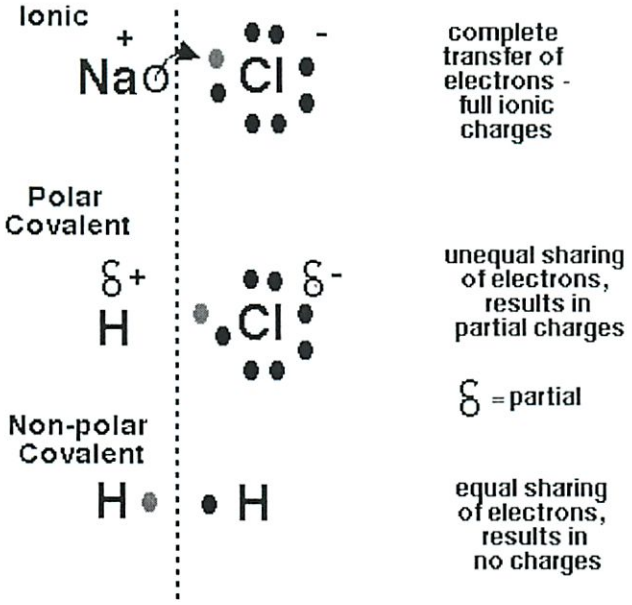
OH polar bond

polar uncharged

(Did I ever realize Covalent, polar, ionic
were actually somewhat related?)

(34)

Comparison of Bonding



35

Compare Ionic, Polar, and Non-polar Bonds

Definitions:

Ionic Bonding:

The formation of an **Ionic bond** is the result of the transfer of one or more electrons from a metal onto a non-metal.

Covalent Bonding:

Bonding between non-metals consists of two electrons shared between two atoms. In covalent bonding, the two electrons shared by the atoms are attracted to the nucleus of both atoms. Neither atom completely loses or gains electrons as in ionic bonding.

There are two types of covalent bonding:

1. **Non-polar bonding** with an equal sharing of electrons.

2. **Polar bonding** with an unequal sharing of electrons. The number of shared electrons depends on the number of electrons needed to complete the octet.

Comparison of Ionic, Polar and Non-Polar Bonding:

Whereas non-polar bonding involves the equal sharing of electrons between identical non-metal atoms, POLAR BONDING is the unequal sharing of electrons between two different non metal atoms. A proper understanding of polar bonding is gained by viewing the types of bonding on a continuum. Ionic bonding is on one extreme with a complete transfer of electrons forming charged ions. Non-polar covalent bonding with equal sharing of electrons is at the other extreme. Somewhere in the middle but favoring the covalent side is polar bonding with unequal sharing of electrons and partial but incomplete transfer of electrons.

Comparison of Lewis Diagrams of Ionic, Polar and Non-Polar Bonding:

The best way to show and represent the unequal sharing of electrons would be by comparison with NaCl and HCl, and H2 using Lewis diagrams.

The captions below correspond to the **graphic** on the right.

IONIC: Complete transfer of electrons, therefore Na becomes positive (lost e-) and Cl becomes negative (gained e-).

POLAR: Unequal sharing. Chlorine has a greater tendency to keep its own electron and also draw away hydrogen's electron. It is NOT completely successful. As a result only partial charges are established. Hydrogen becomes partially positive since it has lost control of its electron some of the time (H +). Chlorine becomes partially negative since it gains hydrogen's electron some of the time (Cl -).

In summary, a polar bond results when different atoms share electrons. One atom will attract the bonding electrons more strongly than the other atom and will acquire more than a half share of these

electrons. This leaves the other atom with less than a half share and makes the electron distribution unsymmetrical. On a time-average basis the electrons spending more time with one atom cause it to have a partial negative charge. The other atom deficient in electrons acquires a partial positive charge.

NON-POLAR: Equal Sharing. Neither atom can dominate the other, therefore the electrons are shared equally between them.

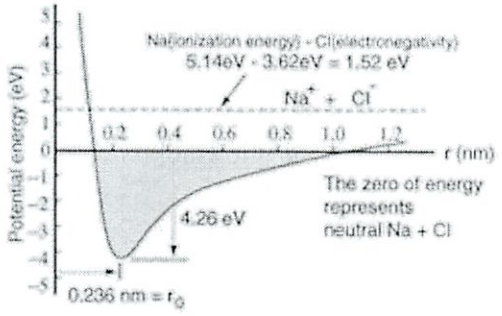
Chemical Bonding

Chemical compounds are formed by the joining of two or more atoms. A stable compound occurs when the total energy of the combination has lower energy than the separated atoms. The bound state implies a net attractive force between the atoms ... a chemical bond. The two extreme cases of chemical bonds are:

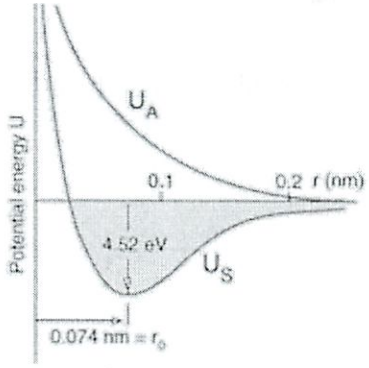
Covalent bond: bond in which one or more pairs of electrons are shared by two atoms.

Ionic bond: bond in which one or more electrons from one atom are removed and attached to another atom, resulting in positive and negative ions which attract each other.

Other types of bonds include metallic bonds and hydrogen bonding. The attractive forces between molecules in a liquid can be characterized as van der Waals bonds.



Sodium chloride
Ionic



Hydrogen molecule
Covalent

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Bond concepts

Bond data

Chemical concepts

HyperPhysics***** Quantum Physics ***** Chemistry

R Nave

Go Back

Covalent Bonds

Covalent chemical bonds involve the sharing of a pair of valence electrons by two atoms, in contrast to the transfer of electrons in ionic bonds. Such bonds lead to stable molecules if they share electrons in such a way as to create a noble gas configuration for each atom.

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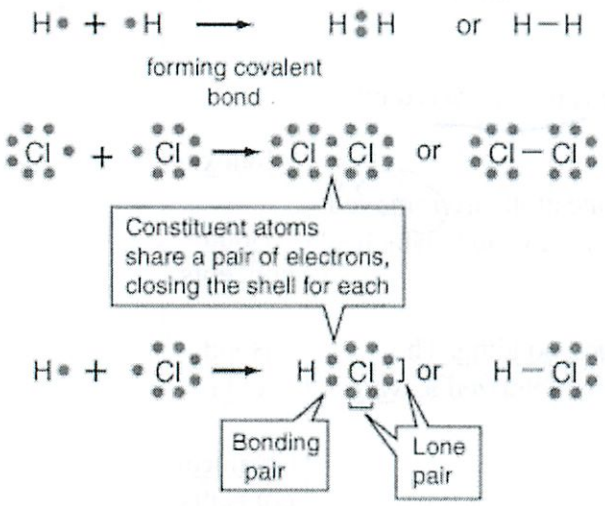
Bond concepts

Chemical concepts

Hydrogen gas forms the simplest covalent bond in the diatomic hydrogen molecule. The halogens such as chlorine also exist as diatomic gases by forming covalent bonds. The nitrogen and oxygen which makes up the bulk of the atmosphere also exhibits covalent bonding in forming diatomic molecules.



Share evenly

Covalent bonding can be visualized with the aid of Lewis diagrams.

Comparison of ionic and covalent materials.

Polar Covalent Bonds

Covalent bonds in which the sharing of the electron pair is unequal, with the electrons spending more time around the more nonmetallic atom, are called polar covalent bonds. In such a bond there is a charge separation with one atom being slightly more positive and the other more negative, i.e., the bond will produce a dipole moment. The ability of an atom to attract electrons in the presense of another atom is a measurable property called electronegativity.

δ δ

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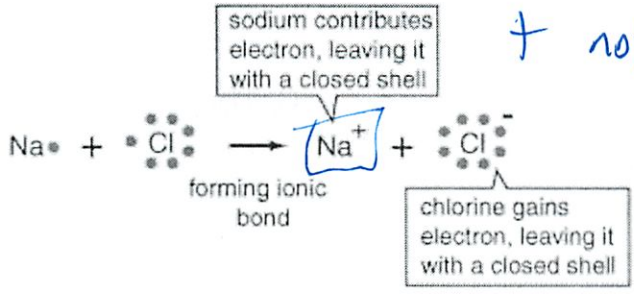
Bond concepts

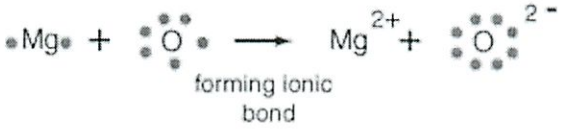
HyperPhysics***** Chemistry R Nave Go Back

Ionic Bonds

In chemical bonds, atoms can either transfer or share their valence electrons. In the extreme case where one or more atoms lose electrons and other atoms gain them in order to produce a noble gas electron configuration, the bond is called an ionic bond.

Typical of ionic bonds are those in the alkali halides such as sodium chloride, NaCl.





Ionic bonding can be visualized with the aid of Lewis diagrams.

Comparison of ionic and covalent materials.

Energy contributions to ionic bonds

Table of ionic diatomic bonds

Index

Bond concepts

HyperPhysics***** Chemistry R Nave Go Back

Metallic Bonds

The properties of metals suggest that their atoms possess strong bonds, yet the ease of conduction of heat and electricity suggest that electrons can move freely in all directions in a metal. The general observations give rise to a picture of "positive ions in a sea of electrons" to describe metallic bonding.

Index

Bond concepts

HyperPhysics***** Chemistry

R Nave

Go Back

Metal Properties

The general properties of metals include malleability and ductility and most are strong and durable. They are good conductors of heat and electricity. Their strength indicates that the atoms are difficult to separate, but malleability and ductility suggest that the atoms are relatively easy to move in various directions. The electrical conductivity suggests that it is easy to move electrons in any direction in these materials. The thermal conductivity also involves the motion of electrons. All of these properties suggest the nature of the metallic bonds between atoms.

Index

Bond concepts

HyperPhysics***** Chemistry R Nave | Go Back

Hydrogen Bonding

Hydrogen bonding differs from other uses of the word "bond" since it is a force of attraction between a hydrogen atom in one molecule and a small atom of high electronegativity in another molecule. That is, it is an intermolecular force, not an intramolecular force as in the common use of the word bond.

When hydrogen atoms are joined in a polar covalent bondwith a small atom of high electronegativity such as O, F or N, the partial positive charge on the hydrogen is highly concentrated because of its small size. If the hydrogen is close to another oxygen, fluorine or nitrogen in another molecule, then there is a force of attraction termed a dipole-dipole interaction. This attraction or "hydrogen bond" can have about 5% to 10% of the strength of a covalent bond.

Hydrogen bonding has a very important effect on the properties of water and ice. Hydrogen bonding is also very important in proteins and nucleic acids and therefore in life processes. The "unzipping" of DNA is a breaking of hydrogen bonds which help hold the two strands of the double helix together.

Index

Bond concepts

Go 1 w/ polar another w/ H-bonding!

HyperPhysics***** Chemistry R Nave | Go Back

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Unit 2

The "transforming principle"

injects in a mouse

dead smooth virulent still causes mouse to die

A G T C

Grows at 3' end!

Bacteria viruses

Hershy-Chase

radio labbe viruses

Saw it was in DNA

"transforming principle

Double Helix

Semi-conservative replication

Grow w/ heavy nitrogen

See which piece grows ...

Central Dogma

free nucleotides pppA pppT etc

DNA polymerase
Cleaves off 2 phosphates

need enzyme to make a primer
tie together w/ ligase
DNA is very tangled
Wrapped up too
topo-isomer Same structure, wrapped up differently

39

10^3 wrong

but proofreading so $1/10^8$ errors
└ 30 differences per person

2000 bp/sec
└ what unit?

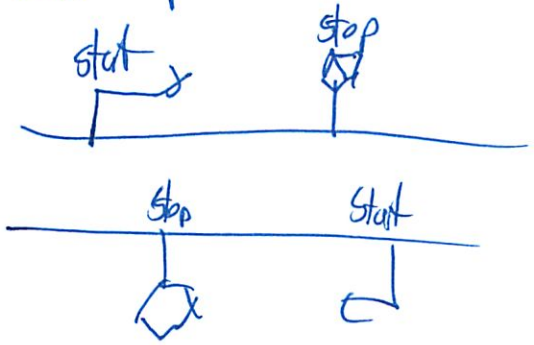
transcription

Coding + complementary

~~it fills in complementary~~

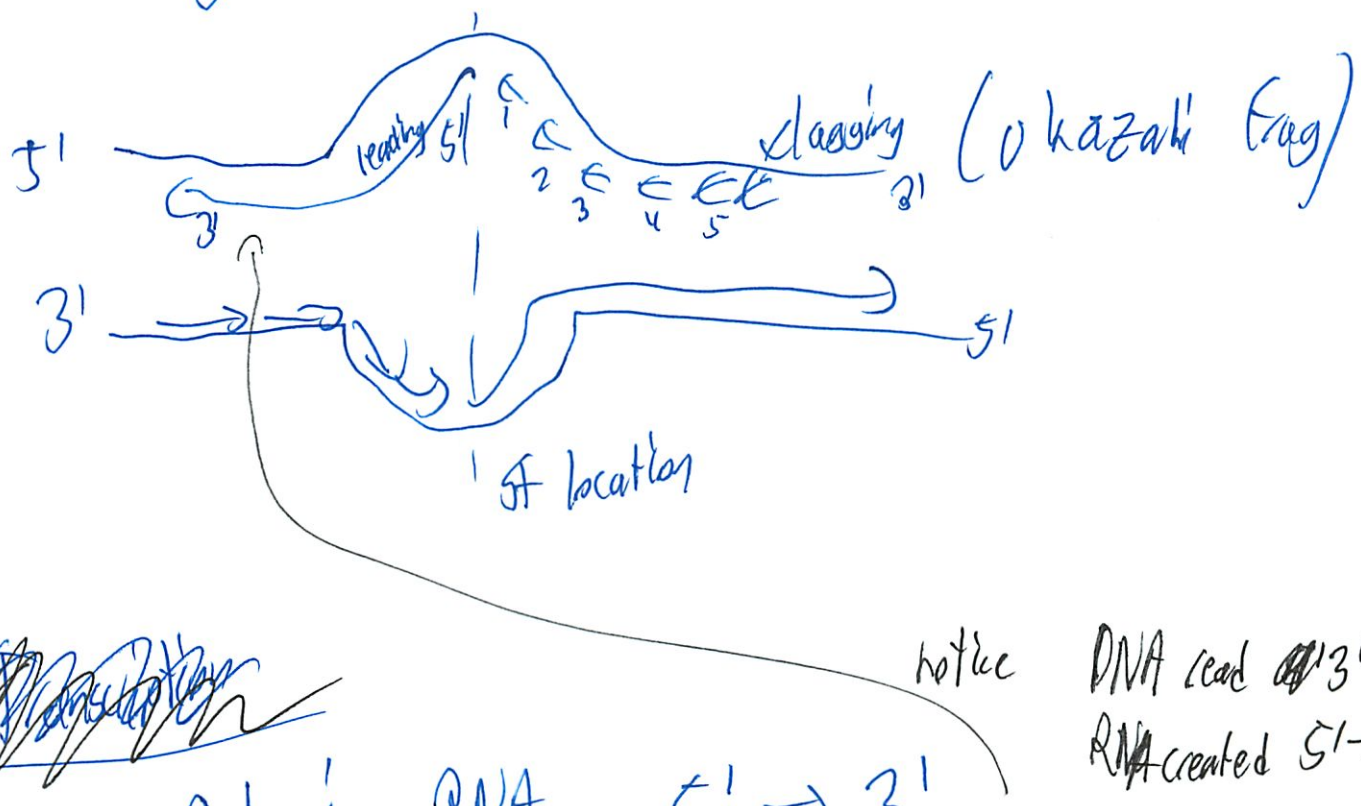
* RNA is same as coding seq *
read from complementary strand

Single stand copying
no primer needed
RNA polymerase



(40

goes towards 5'



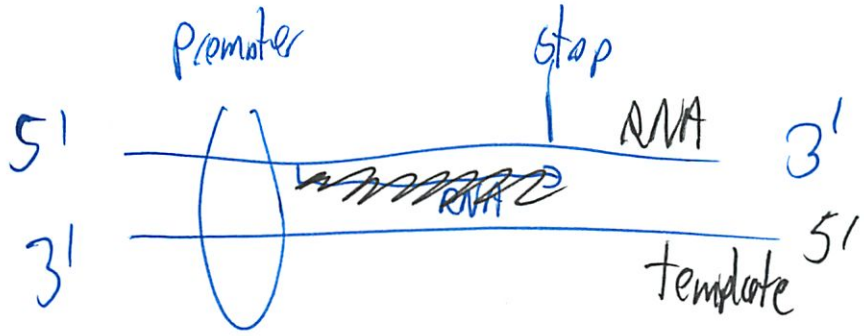
(okazaki frag)

notice

DNA read 3'-5'
RNA created 5'->3'

put in mRNA 5' → 3'

(~~diff direction~~)

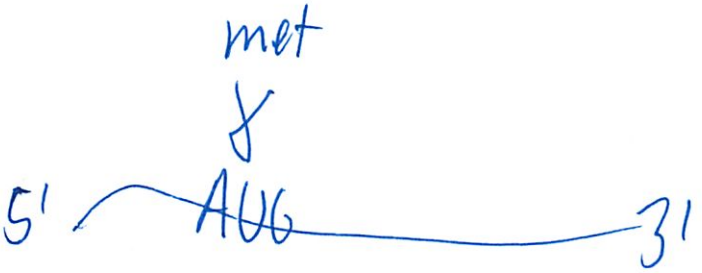


direction →

Translation

look up table

3 for each amino acid



Note table shows the codons

42

First Position	Second Position: U	Second Position: C	Second Position: A	Second Position:
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA *Stop* UAG *Stop*	
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	

met

Codons in table → 5' AUG 3'

43

Ribosome this factory that moves these

Can have a bunch in use at one

---

just stops at end

telomerase adds telomeres at end

└ the 3' end of the DNA

always TTA GGG

to prevent important info from being lost

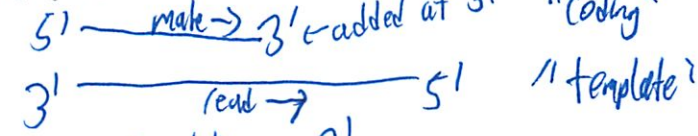
its a form of reverse transcriptase

degrades in aging

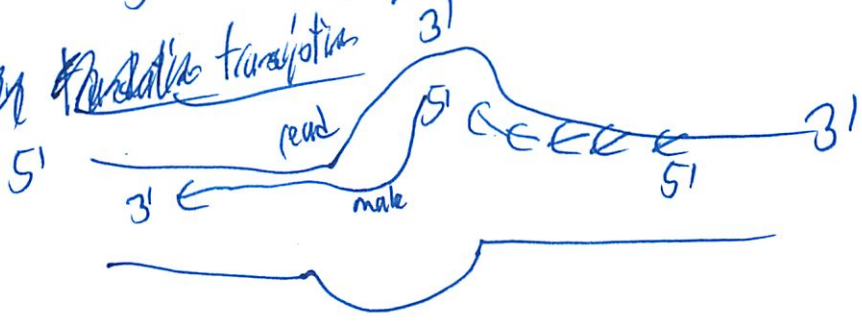
Prokaryotes have circular DNA

(make sure I remember which direction is which!)

replication

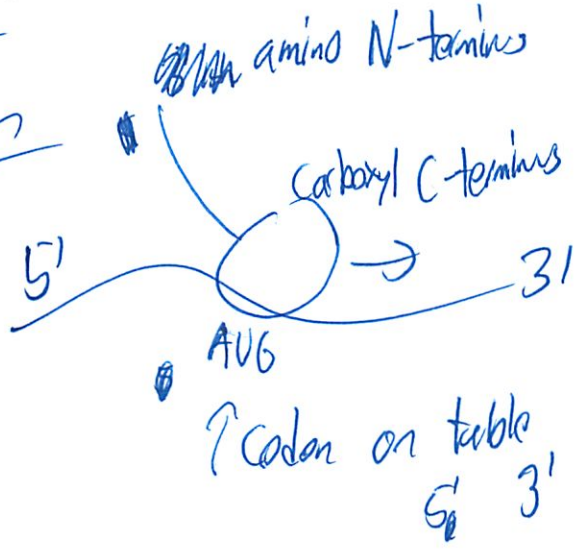


~~replication~~ ~~translation~~ transcription



~~transcription~~

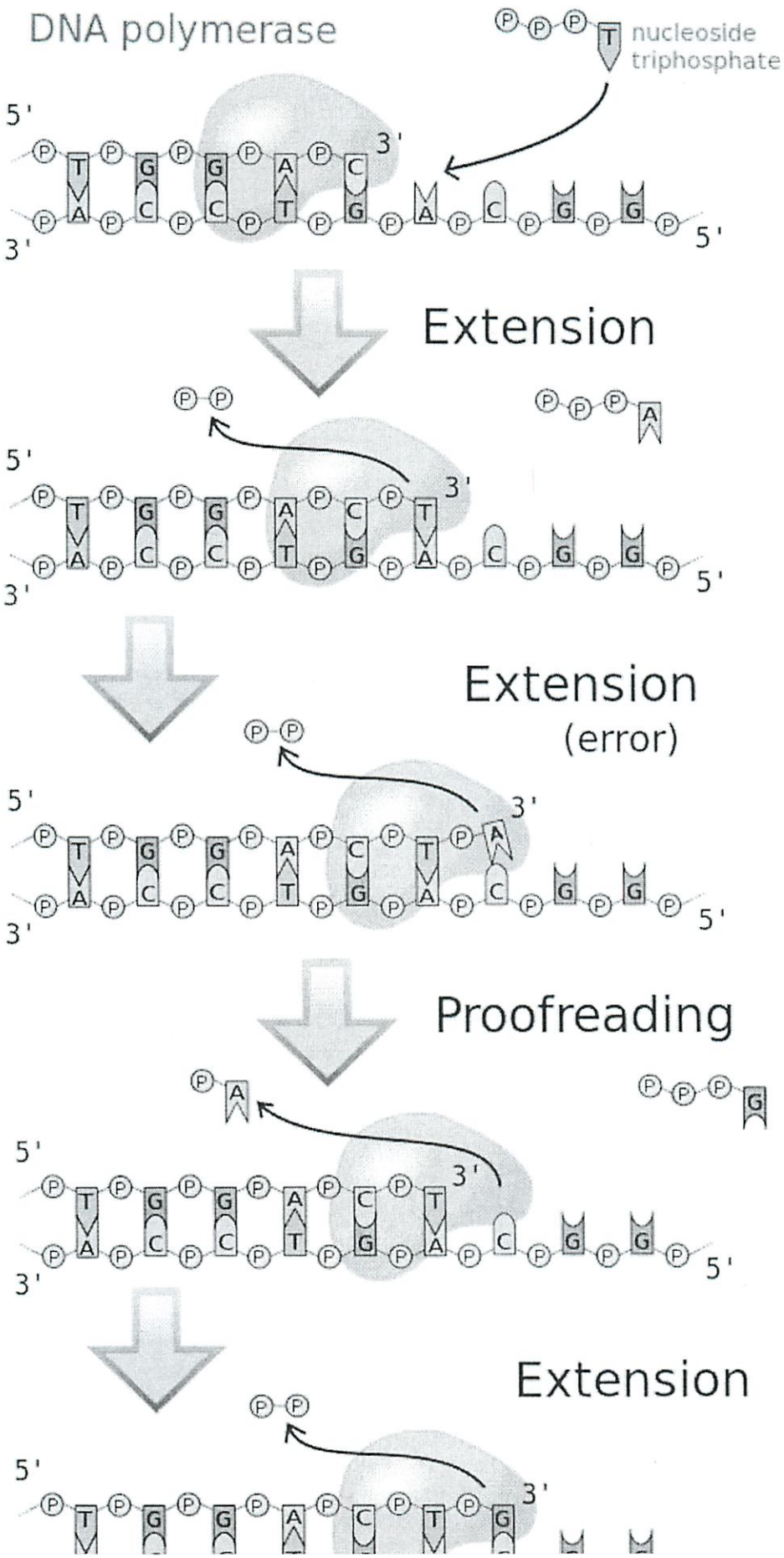
translation

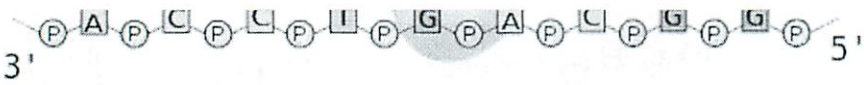


? codon on table
5' 3'

95

DNA replication




3'
A
C
C
T
G
A
C
G
G
5'

Types of mutations

Point → 1 bb

transition purine ↔ purine
pyr ↔ pyr

transversion pyr ↔ pur

missense - altered codon

nonsense - stop codon added

---

mRNA = messenger

tRNA = transfer → brings an amino acid (met, tRNA)

rRNA = ribosomal
└ attaches to start codon

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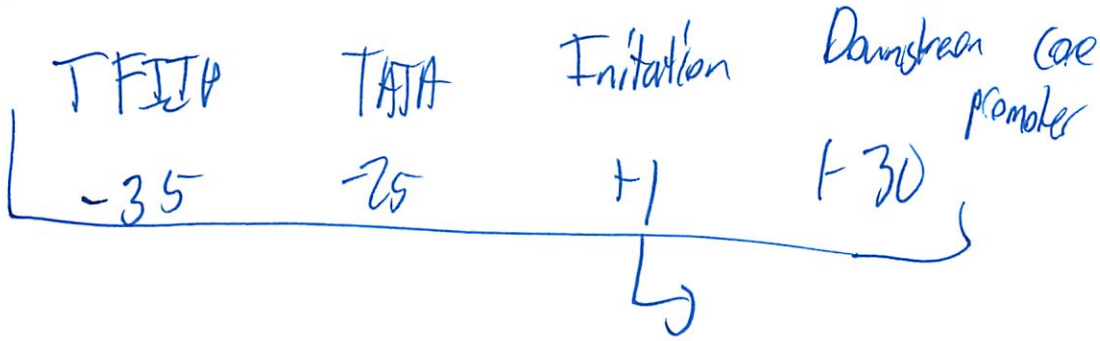
Promoter



DNA repair
Slipping

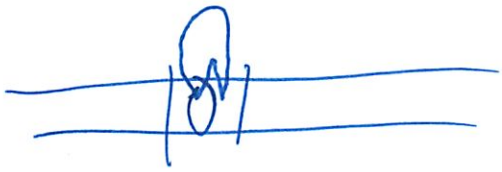
Control of transcription
(confusing lecture!)

methylation add methyl (-CH_3)
Compacts DNA
Wraps around histones

acetylation
has ⊖ charge
Causes it to dissociate from histone molecule

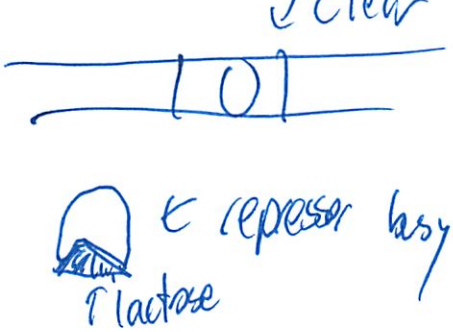
lac operon

When lactose absent repressor binds to operon



transcription blocked

When lactose present, lactose binds to repressor



CAP is transcription enhancer

trp operon

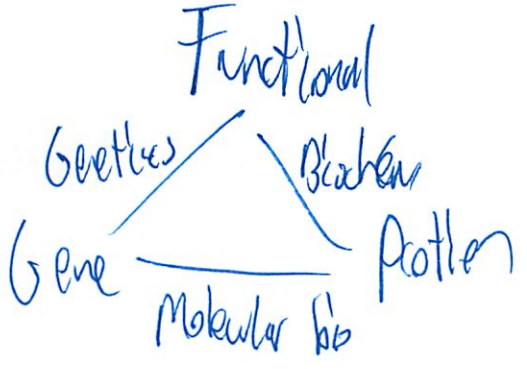
as trp accumulates, it activates the repressor, which blocks the operon, which blocks transcription of more tryptophan

---

Constitutive - always makes
↳ no control over

non inducable - never makes
↳ no matter what ~~signal~~ signal

Recombinant DNA



(9)

know what gene what function

Cut up at specific sites

Eco RI G|AATTC
CTTAA|G

(need to practice w/ this!)

Vectors

Used to artificially character foreign genetic material into a cell where it can be replicated and/or expressed

Cut open w/ ~~trigger~~ restriction enzyme

must persuade bateria to take it up

Select for vector

Lawn which have antibotic resistance make
; add antibiotic
(need to pratice!)

(51)

also need to select for the ones w/ the DNA inserted
↳ that has the color thing before, right?

Can make the ends incompatable

Libraries

make lib of DNA fragments

Used for molecular cloning

cDNA = reverse transcriptase

Finding genes

See which grow on minimal media

Or use antibodies that look for particular Compounds

Can add a bacteria promotor
↳ still need a promoter

Or gel electrophoise to measure size of insert
Size of DNA

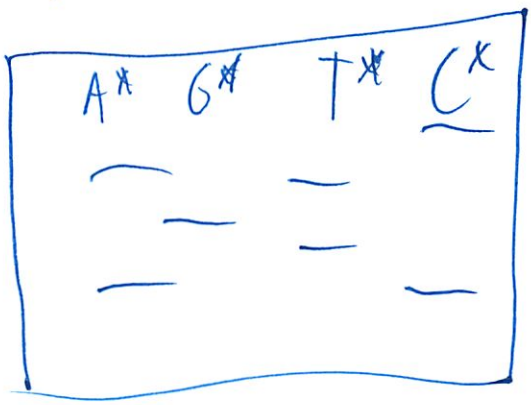
Sequencing DNA

polymerase knows what to add

dNTPs
can't add futer (no hydroxily)

line it up on the table

Sanger method →



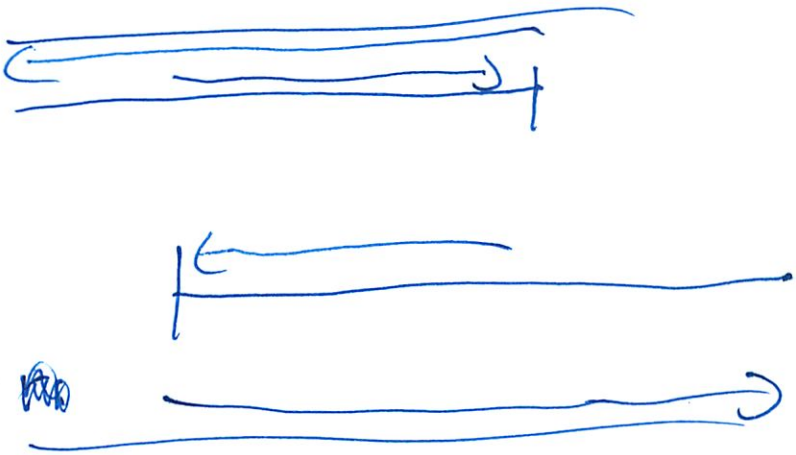
Or Fluorent die
then can run it past scanner
(so much faster!)

Sheer up into many small fragments
then computer recombines

"genome shotgun sequencing

(53)

PCR



Used to amplify a piece of DNA
to make a bunch of copies

Thermal cycling
└ do we need to know the difference?

1. Denature
   90-100 °C
   H bond broken
2. Anneal
   50-60 °C
   H bonds
3. Extend
   70-75 °C
   ideal for DNA polymerase
   w/ taq polymerase

(54)

Modern

Use Camera

100 bil bases/day

Clone by Complementation

See what grows

HGP project

hierarchial mapping

disease on a map

Correlate w/ linkages maps

(popular gv)

---

negitive selectn marker → if you have it, you die!

SNP analysis

See what is diff

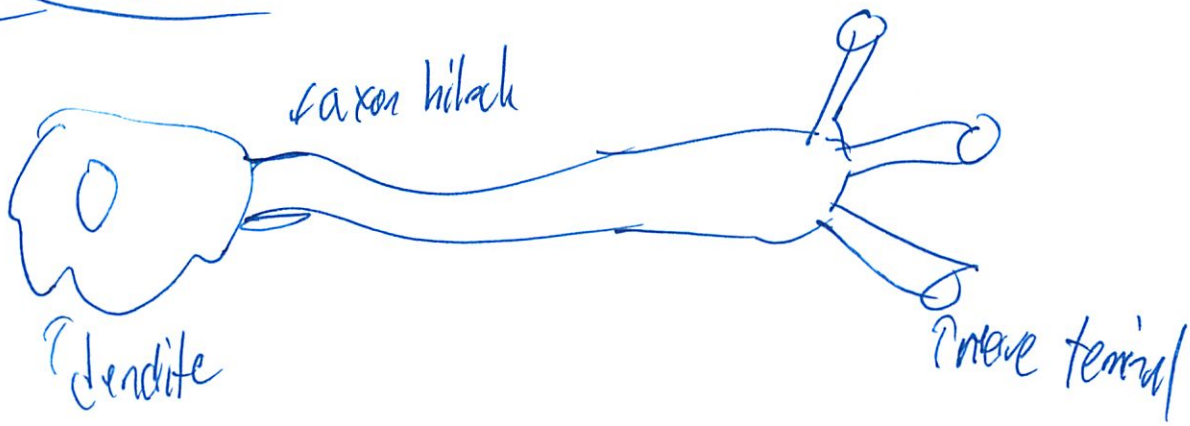
look at family tree

(need to pratice)

55

SNP array
Seq if everything in seq matches, it binds

Unit 3 Neurobio



main thing here is knowing the progression...

resting potential ~-70mv
inside (-)

Voltage gated vs ligand gated

exogenous shock to -50mv
Sodium voltage gated open
Sodium rushes in
Conc always favorable

(56)

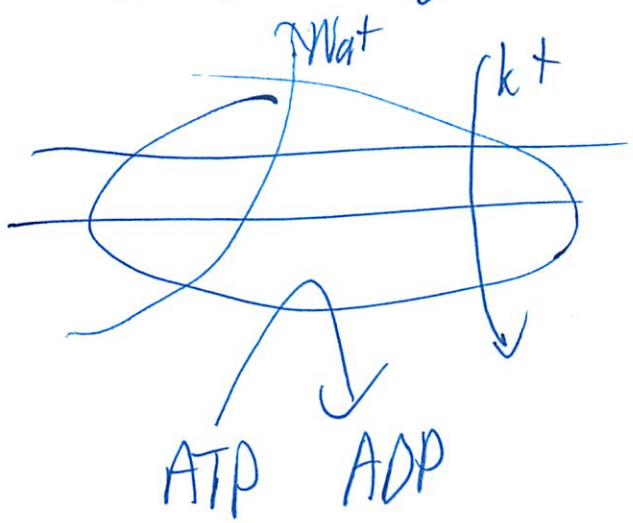
Charge favorable −50 → 0
unfavorable 0 → 50
balances +5 mV

then voltage gated potassium channel opens
potassium rushes out
conc. while the favorable

Charge 50 → 0 favorable
0 → −70 unfavorable

balances around −70

Pump maintains resting potential



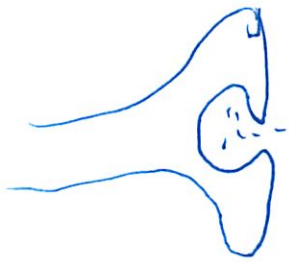
(57)

refractory period
depolarize → more ⊕
hyperpolarize → more ⊖

threshhold = 50mv

(think I will just review the review
Since more recent
Simple stuff in head
take another look at the complex stuff....)

at end Ca^{++} voltage gated
Neurotransmitter
↳ when opens
Ca rushes in
Conc much higher outside



~~receptor~~

Then the receptor is
a ligand gated Na^+ channel

rushes in when Ach binds

Get rid of Ach w/ Ach-esterase

Nerve-nerve need enough to get over the threshold

Also inhibitory w/
ligand-gate Cl^- channel

Cl^- rushes in

(They will tell ya excitory or inhibitory)

Immunology

innate
humoral
cellular

remove foreign organisms from body

innate

barrier defenses

Pagocytes - eat pathogens

Neutrophils made at injury site

macrophage

natural killers

inflaitors, interons

Complement system collection of proteins circulating in the blood...

Stasted by mast cells at injury site

histamine make blood vessels dialate

Cytokinites - regulate immune system response

(60)

Adaptive Immunity

in vertebrates only

humoral - body fluids infections in

cell-mediated - infections in body cells

lymphocytes - recognize foreign cells
use lymph nodes

immunizations induce immune response

vaccination no sign of infection

:d C
we need to know the difference

autoimmunity - body can't distinguish own body cells

B vs T cells
└ both lymphocytes

4 key features of B, T cells

1. diversity
2. high specificity + self tolerance
3. thousands made on infection
4. memory

VDJ recombination

DNA
chdict
↓ recombination
DNA dict
↓ transcription
pre-mRNA
↓ processing
mRNA
↓ translation
light-chain polypeptide

(62)

primary immune response

Secondary immune response

Cell mediated

T_H and T_C

↑ ↑

CD4+ CD8+

all

Humoral

T_H

professional

b-cells involved

So professional Ag presenting cells involved in both;

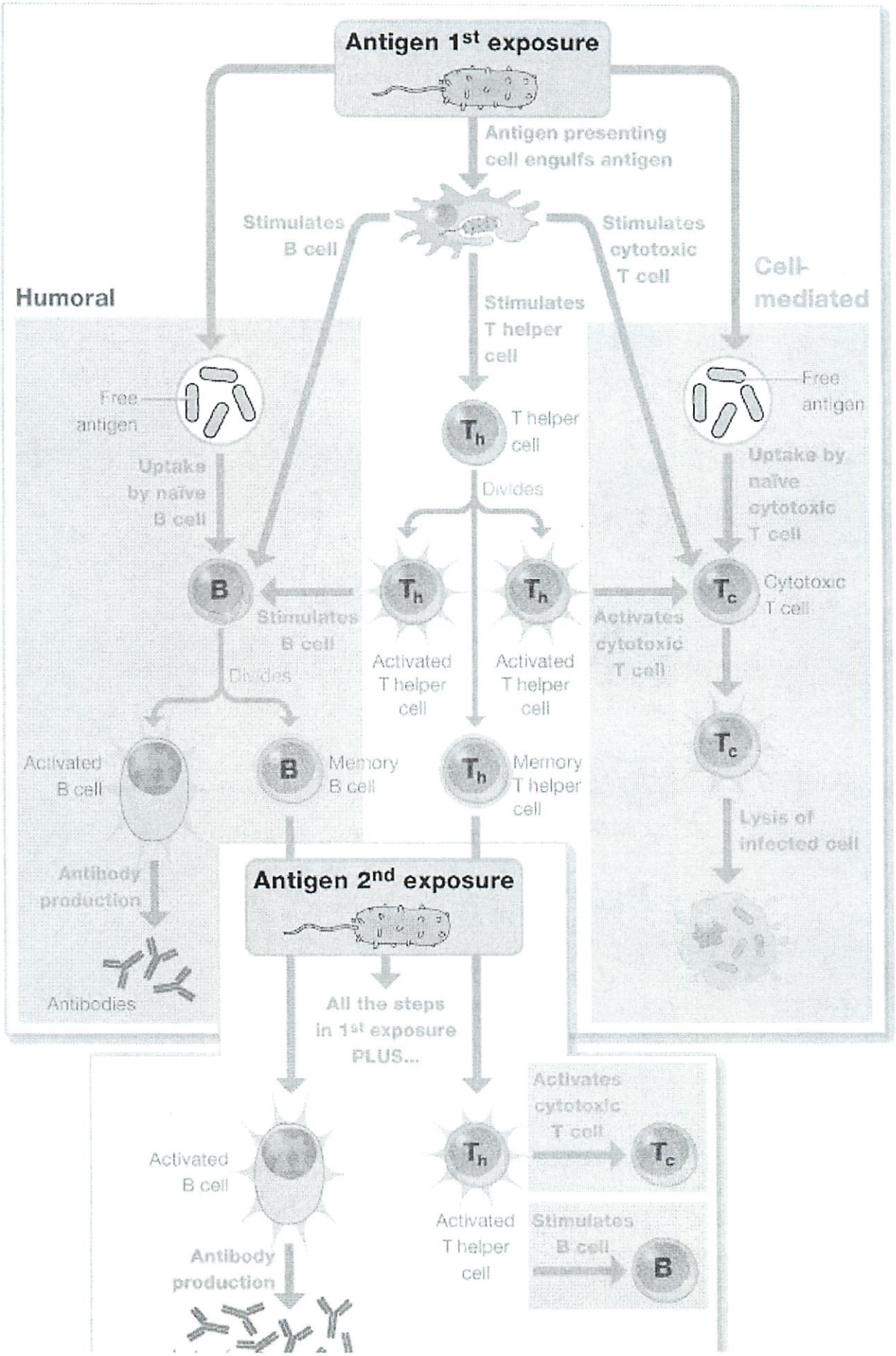
Stimulates a T_H

Which activates then either a B or T_C

---

note that antigen presenting also stimulates B, T_C

63


Antigen 1st exposure
Antigen presenting cell engulfs antigen
Stimulates B cell
Stimulates cytotoxic T cell
Stimulates T helper cell
Humoral
Cell-mediated
Free antigen
Uptake by naïve B cell
Th
T helper cell
Divides
Uptake by naïve cytotoxic T cell
B
Stimulates B cell
Activated T helper cell
Activates cytotoxic T cell
Tc
Cytotoxic T cell
Activated B cell
Memory B cell
Memory T helper cell
Lysis of infected cell
Antibody production
Antibodies
Antigen 2nd exposure
All the steps in 1st exposure PLUS...
Activates cytotoxic T cell
Activated B cell
Activated T helper cell
Stimulates B cell
Antibody production


LOTS OF
Antibodies

(64)

Humoral

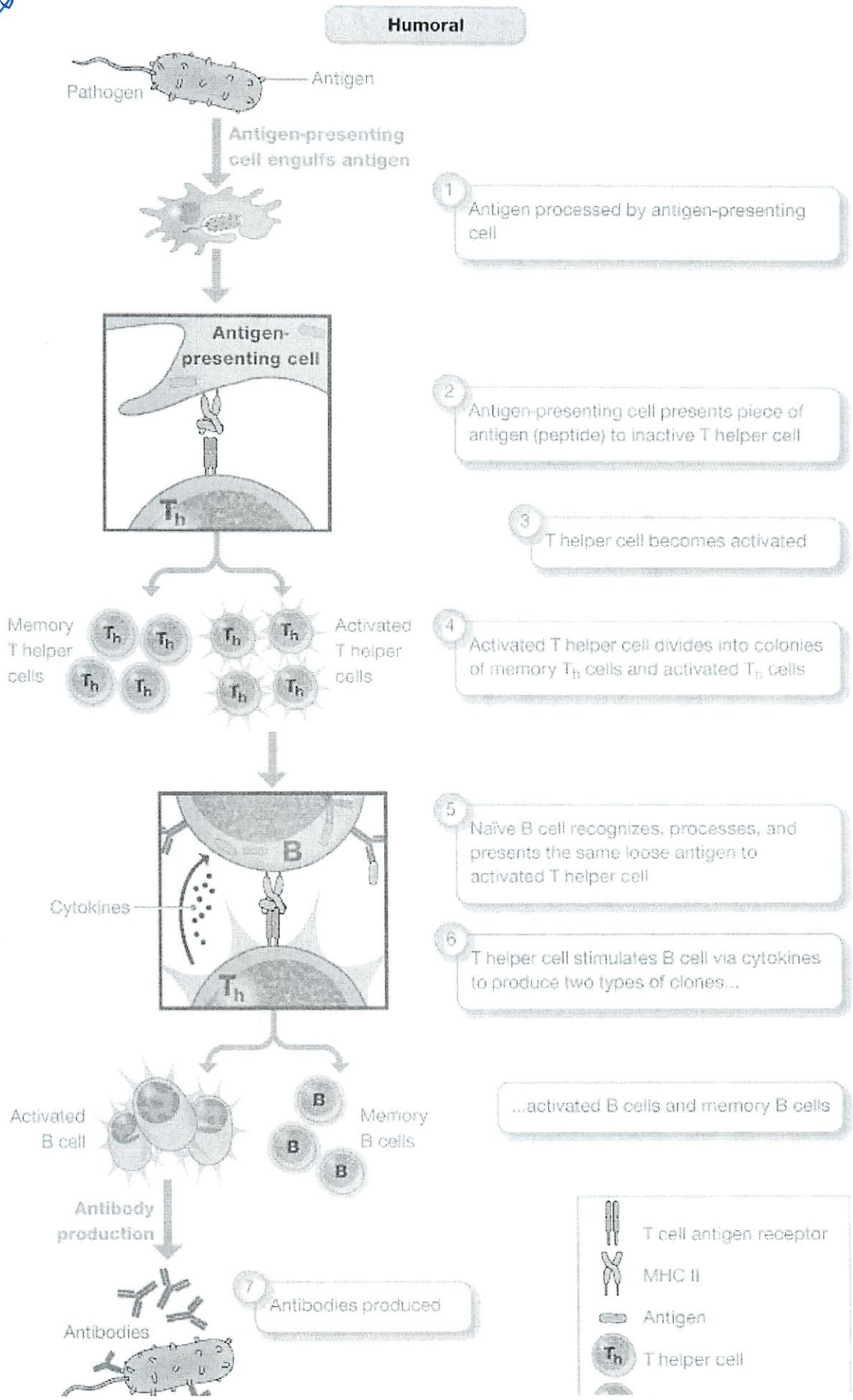
1. Antigen processed by apc Antigen presenting cell
2. Presents to inactive T_H
3. T_H activates and clonal divide — active
   — memory
4. Naive B cell recognizes same antigen + presents to T_H
5. T_H stimulates B turns active + divides

   active + memory

   ↑ ↑

   makes antibody long lasting

64b


Humoral
Pathogen
Antigen
Antigen-presenting cell engulfs antigen
1 Antigen processed by antigen-presenting cell
Antigen-presenting cell
2 Antigen-presenting cell presents piece of antigen (peptide) to inactive T helper cell
3 T helper cell becomes activated
Memory T helper cells
Activated T helper cells
4 Activated T helper cell divides into colonies of memory Th cells and activated Th cells
B
Cytokines
5 Naïve B cell recognizes, processes, and presents the same loose antigen to activated T helper cell
6 T helper cell stimulates B cell via cytokines to produce two types of clones...
Activated B cell
Memory B cells
...activated B cells and memory B cells
Antibody production
Antibodies
7 Antibodies produced
T cell antigen receptor
MHC II
Antigen
T helper cell

Cell-mediated

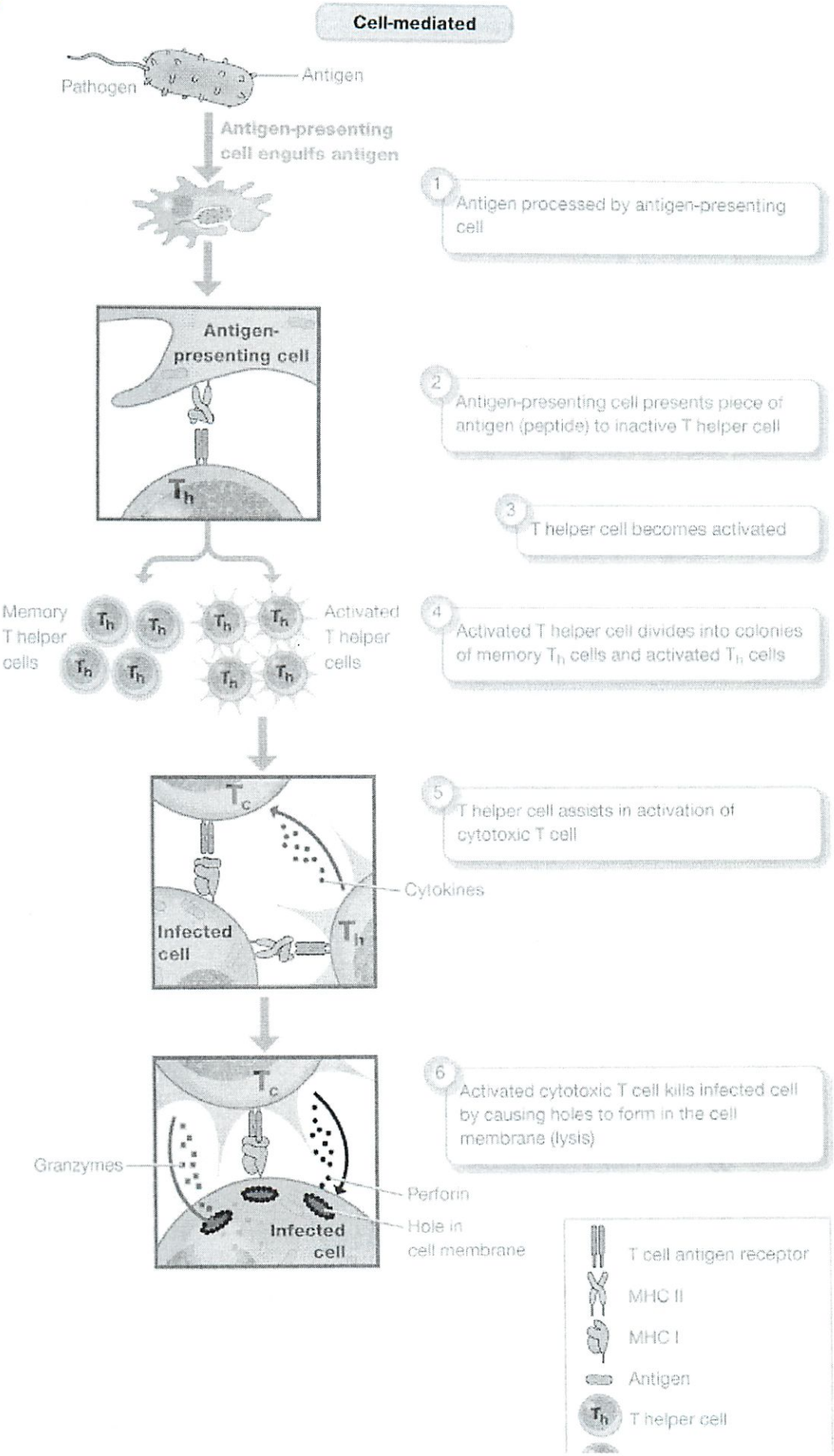
1. Antigen processed by prof Antigen presenting cell
2. Presents to inactive T_H
3. T_H activates + clonal divides - active - memory
4. T_H activates T_C
5. T_C poles hole into a infected cell
   - how does it recognize?

Since all cells can present antigen w/ MHC 1

T_C has TCR which binds
- w/ CD8

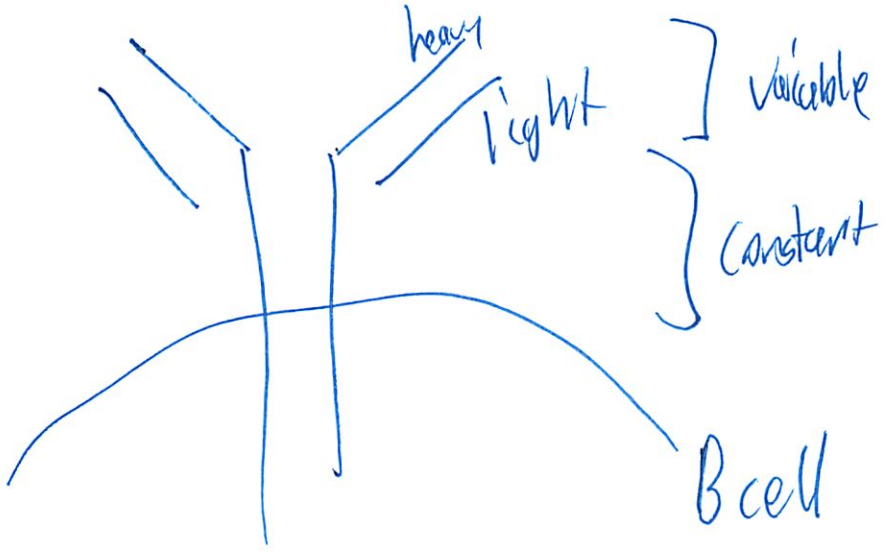
Need 2 signals for activation

65b


Cell-mediated
Pathogen
Antigen
Antigen-presenting cell engulfs antigen
1 Antigen processed by antigen-presenting cell
Antigen-presenting cell
T_h
2 Antigen-presenting cell presents piece of antigen (peptide) to inactive T helper cell
3 T helper cell becomes activated
Memory T helper cells
Activated T helper cells
4 Activated T helper cell divides into colonies of memory T_h cells and activated T_h cells
T_c
Infected cell
Cytokines
5 T helper cell assists in activation of cytotoxic T cell
Granzymes
Perforin
Hole in cell membrane
6 Activated cytotoxic T cell kills infected cell by causing holes to form in the cell membrane (lysis)
T cell antigen receptor
MHC II
MHC I
Antigen
T helper cell


T_C
Cytotoxic T cell

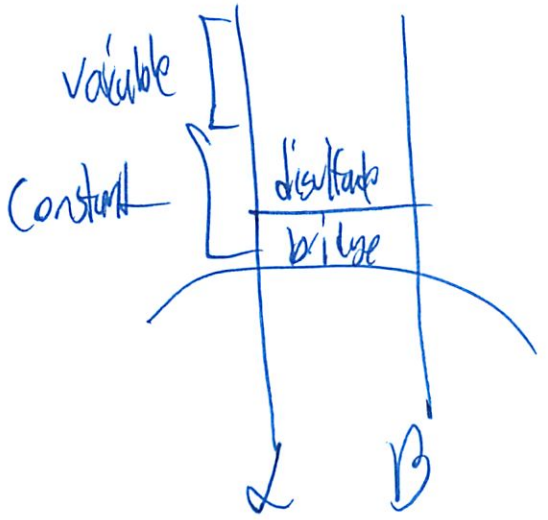
Antigen receptor



T cell Receptor (TCR) is simpler

two polypeptide chains α, β

also constant + variable regions



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immunodeficiency - lack T, B cells

Antigen recognition domains

IgG - at start
IgM } initial formed B cells
IgD
IgA } class switching
IgE } more around 1 year old

remove regions
never add I think

during VDJ
diff constant domains in heavy chain

Types of B cells

Immature produced in bone marrow

Naieve not yet activated

Plasma exposed to antigen
Secrete a lot of antibodies

Memory lives for a long time, can respond quickly

Neutrophils white blood cells
part of innate immune system
One of the 1st responders
"i did I write this word ~~B~~ 3x... "

Genomics

Function

Gene — Protien

big picture = Genomics

(this was a pointless lecture --)

Regulation

stretches of conservation

Transposons

distributors of innovation

not useless ...

linkage approach

Development / Stem Cells

Progressive Differentiation

Embryonic

Can give rise to all cells in body

Synergetic = Same genetic by

Allogenic = diff " "

Ontogeny origin + development of organism

Phylogeny study of evolution of org through molecular sequencing

the order is messed up here--

Adult

more differentiated

(20)

Cell potency

totipotency - all cells

pluripotency - can differentiate to any of 3 layers
- endoderm → stomach lining
- mesoderm → muscel, bone, blood
- ectoderm → nervous system

LiPS (induced)

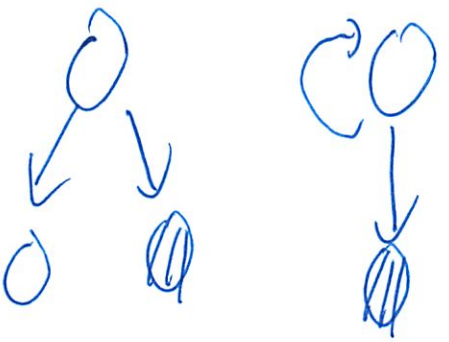
multipotency - can form multiple, but limited # of lineages

Oligo - few

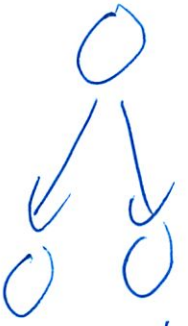
uni - one
↳ a precursor cell

12

Asymetric Division



Symetic



net gain of 1

Embronic stem cell

Cells respond to contextual signals

Chiney

Transit amplifing Cells

HSC - hempoitic stem cells...

tumors abnormal stem cells

Xengraph graph cells from 1 species to another

ectopic wrong physical location

(13)

EPO → needed to make red blood cells

Rational Medicine / Familial Hypercholesterol

Cholesterol – essential

LDL bad
HDL good

FH: lack LDL receptor
incomplete clearance

HMG CoA reductase

Unit 3

Stem cells induced by contextual signals
hard to get eggs in humans
iPS

(Dr never did org cloning or therapdic)

insert nucleus from mother into unfertalized egg

Can let it grow just long enough to get tissues

L_{50} no rejection

Virology

lots. of diff types

- some push act
- some RNA, DNA
- some reverse transcriptase

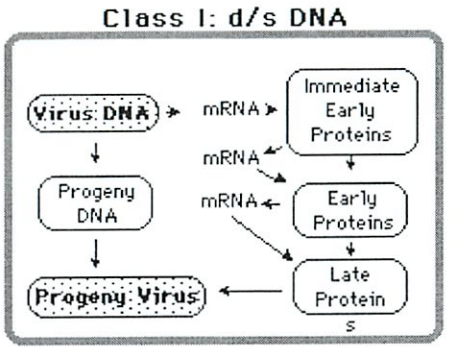
Nucleocapsid core

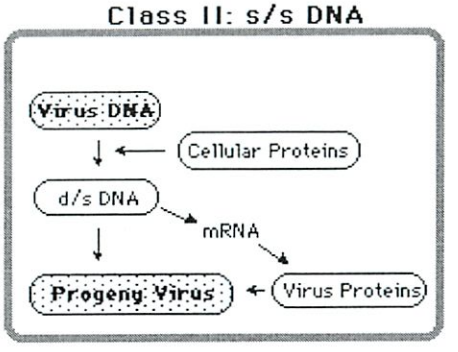
(75)

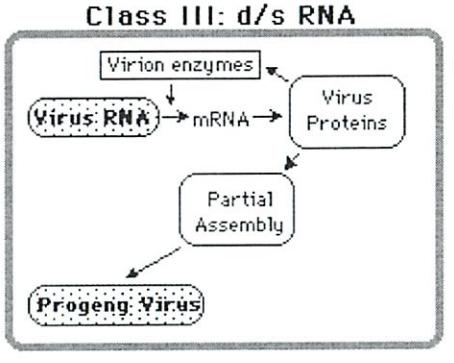
All viruses have a protien coat

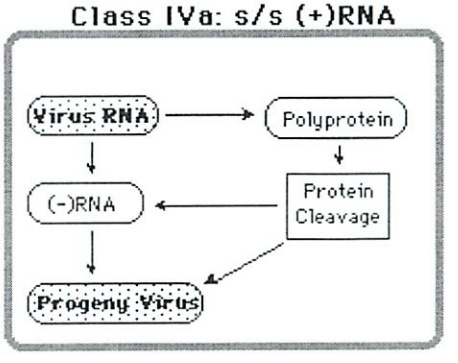
pushes at or <u>lyse</u> cell

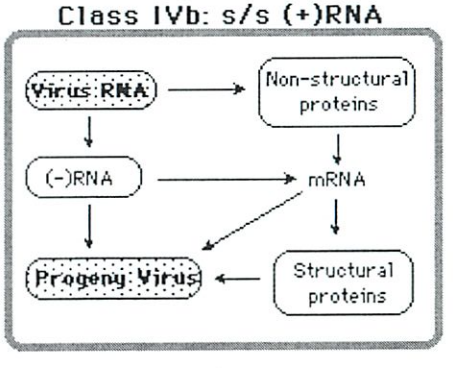
Viruses classified according to their nucleic acid genomes

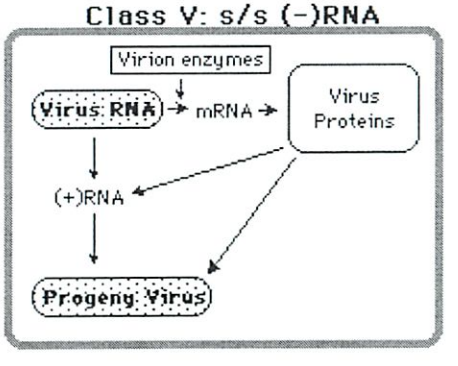


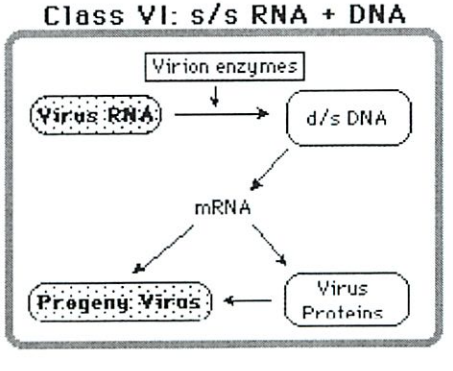


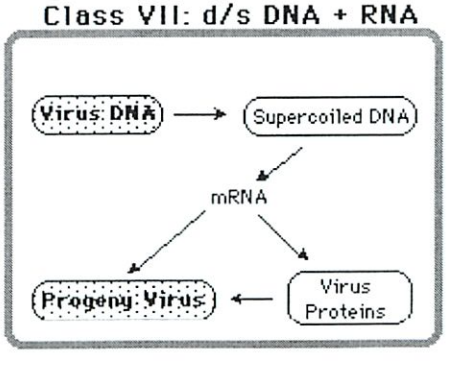












77

Types

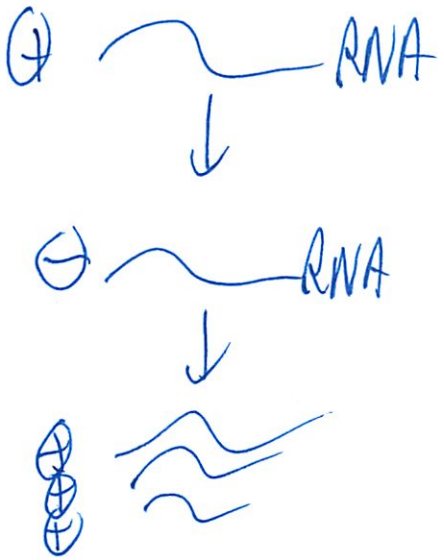
1. ds DNA
   - Uses host DNA pol
   - Uses host RNA pol

2. ss DNA

host DNA pol

make sure strand that came in gets packaged up to new capsid

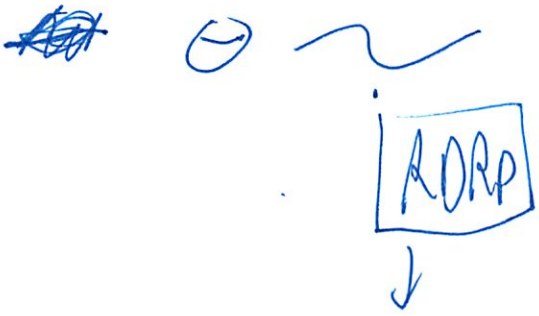
3. (+) ss RNA



RNA dep RNA polymerase
Makes RNA from RNA
Encoded already in virus

(78)

4. (-)ss RNA (anti-sense)



brings RNA dep RNA polymerase (can't encode directly)

most common

viral RNA

5. dsRNA

our bodies don't get infected

↳ plants

needs RNA dep RNA polymerase

↳ sometimes brings/ sometime encodes

6. Retroviruses

typically membrane bound

→ Reverse transcriptase RNA → DNA

→ Integrase

→ Protease

two identical (+) stranded RNAs

↳ Revese transcriptase (brought in → ds DNA ↓ integrates in host genome

---

Rase Sacoma Virs

Virs fell in light spot that allows tumor to grow

Nomally just monolayer but loses contact inhibition

Src in normal chicken DNA but mutated here

(8)

Oncogene

proto oncogene

Src acts as a kinase
4DO-5DO kinases in a normal cell

Src emits pdgm growth factor

Cancer

normally get signals from neighbor
↳ mitogenic signals

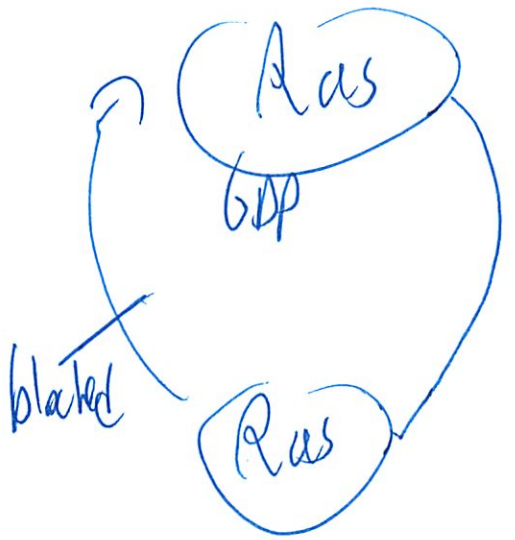
tumors are chaos

invasive cancer sends at pioneer cells
tobacco use bad
more mutagenic = more carcinogenic

81

Caused by single point mutation

GTP → GDP



So stays on signaling growth!

Retino blastoma

↳ deleted many things in gene

turned off the tumor supressing gene

82

Little can do to cure
Signal indution cascades

Gleevec inhibited Tyr-kinase of Abl
kills transit amplifing, not actual cancer cells...

Can classify tumors w/ microarray

Ames Test
test for mutagenicity
1. Start w/ mutant
bacteria his -

his media

expose this to your mutagen
2. Plate on minimal media

(83)

See how many colonies grow

Called revertant mutations

mutant → wild type

AIDS

~~Clonal expansion~~

T_H normally activates

1. Humoral → B
2. Cellular → T_c
3. Cellular → Macrophages ← new

Mutant genes can be inhibited that encode mutations in immune system

Indv can't respond to certain diseases

(84)

Can be inborne

Was common in gay men in SF

found to be a retro virus

long term disease
- 10 years

transfered through blood
- gay sex
- needles (drugs)
- blood transfusion

HIV

like a retrovirus

presented to T_H cells

but these carry the virus to other cells!

fuses to T cell and merges w/ it

and spreads its DNA

lots of protiens

Then it replicates and bds ot again

It changes to avoid viral antibodies

4 steps of life cycle drugs can target

1. Inhibit fusion
2. ~~Integration~~
2. Inhibit reverse transcription
3. Inhibit integrase
4. Integrase protease

Prions

3rd class of infectious pathogen

Can spread b/w species

TSE

- Sheep → Scrapy
- Mad Cow
- Creutzfeld Jacobs
- kuru (African tribe)

Prion and it gets stronger

Was a protien P_rP^{sc}

highly resistant to UV light

Gene P_rNP

P_rP^c is normal

↳ sensitive to protiase (breaks it down)

P_rP^{sc} is not sensitive

P_rP^c is α helix

P_rP^{sc} is β helix

replicates w/o nucleic acid

↳ by changing P_rP^c to P_rP^{sc}

the prion hypothesis

in yest

↳ reqired

87

Molecular Evolution

Can build a Phylogenetic tree

Some mutations beneficial

↳ selection mutation 3%

Some not ~> neutral mutation

humans + flies 'eyes have similar genes

Can use it to date things

Y-chromosomes + mitocondria DNA not recombines

look at African ancestors ...

Or Jewish priests -> cohens

how faithful they are

Final Lecture

Can use spelling diff to compare people
look at mutations next to it
↳ to see if from 1 mutation
or 2 seperate ~~all~~ occurances...

Sickle cell anemia

Lactase tolerance

Some regions of strong positive selection
Can trace back human history

What is a species?

RNA

pretty complicated
lots of other stuff besides encode protiens

1. Short non-coding RNAs
that interfere
destroys the mRNA that is matched

2. Long coding RNAs

Several kilobases long 1-2000 bases

never translated to protiens

Act as binders to several protiens

IPS

Reprogramming cells

Re-Review

12/18
1:30p

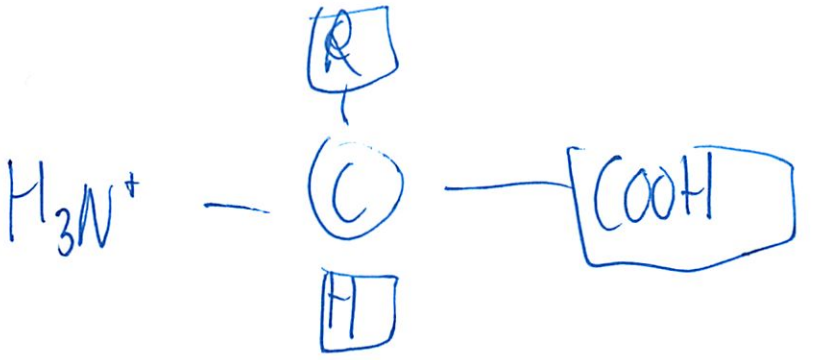
Bonds!

Atoms!

How many valence electrons

What bonds w/ what?

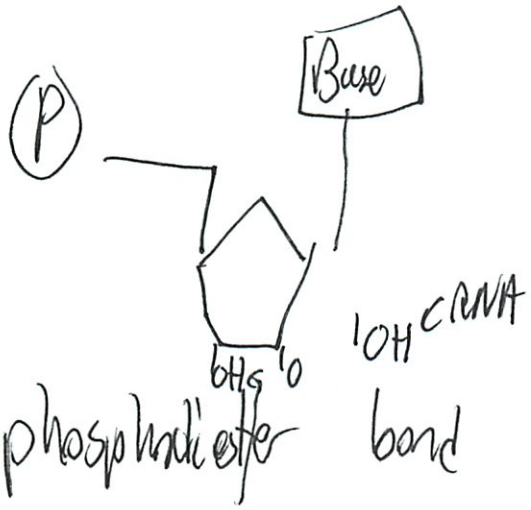
amino ____ protein ____ Carboxily



peptide bonds

(2)

Each Nucleotide



Need to do genetics
- more of a puzzle than terms to know

Meiosis
- Me; sexual (meiomic)

Mitosis
- it; cells splitting

(3)

Crossing over

enzimes
↓ activation energy

kinase → add (P) phophatase → remove (P)

know the cross names
↳ or will they tell us

~~M~~ monohybrid AA BB aa bb

dihybrid Aa Bb Aa Bb
↑ two diff genes

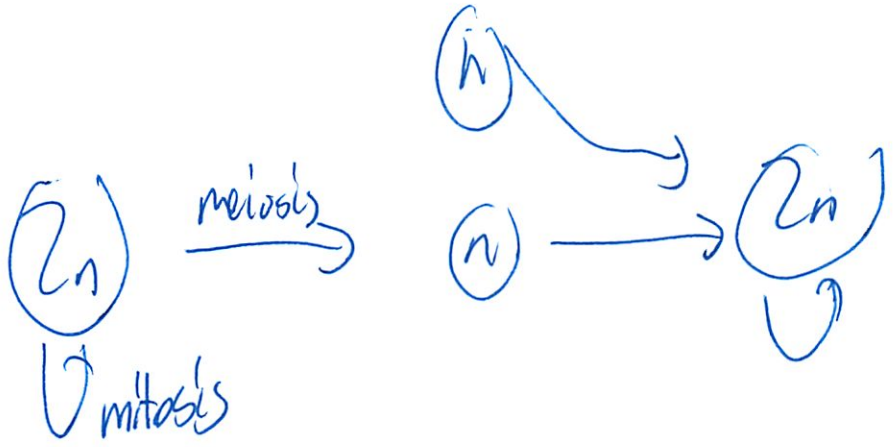
test cross (RR or Rr) rr

backcross Rr (RR or rr)

F1 cross RR rr

F2 R_ R_

9



Mutant hunt

- screen
- select

Complementation

2 strains both/w diff mutations produce same phenotype

5

Complementation (genetics)

From Wikipedia, the free encyclopedia

In genetics, **complementation** refers to a relationship between two different strains of an organism which both have homozygous recessive mutations that produce the same phenotype (for example, a change in wing structure in flies) but which do not reside on the same (homologous) gene. These strains are true breeding for their mutation. If, when these strains are crossed with each other, some offspring show recovery of the wild-type phenotype, these strains show "genetic complementation". When this occurs, each strain's haploid supplies a wild-type allele to "complement" the mutated allele of the other strain's haploid, causing the offspring to have heterozygous mutations in all related genes. Since the mutations are recessive, the offspring will display the wild-type phenotype. A **complementation test** (sometimes called a "cis-trans" test) refers to this experiment, developed by American geneticist Edward B. Lewis. It answers the question: "Does a wild-type copy of gene X rescue the function of the mutant allele that is believed to define gene X?". If there is an allele with an observable phenotype whose function can be provided by a wild type genotype (i.e., the allele is recessive), one can ask whether the function that was lost because of the recessive allele can be provided by another mutant genotype. If not, the two alleles must be defective in the same gene. The beauty of this test is that the trait can serve as a read-out of gene function even without knowledge of what the gene is doing at a molecular level.[1]

Complementation arises because loss of function in genes responsible for different steps in the same metabolic pathway can give rise to the same phenotype. When strains are bred together, offspring inherit wildtype versions of each gene from either parent. Because the mutations are recessive, there is a recovery of function in that pathway, so offspring recover the wild-type phenotype. Thus, the test is used to decide if two independently derived recessive mutant phenotypes are caused by mutations in the same gene or in two different genes. If both parent strains have mutations in the same gene, no normal versions of the gene are inherited by offspring; they express the same mutant phenotype and complementation has failed to occur.

In other words:

- If the combination of two haploid genomes containing different recessive mutations yields a mutant phenotype, then there are three possibilities:

1. Mutations occur in the same gene.
2. One mutation affects the expression of the other.
3. One mutation may result in an inhibitory product.

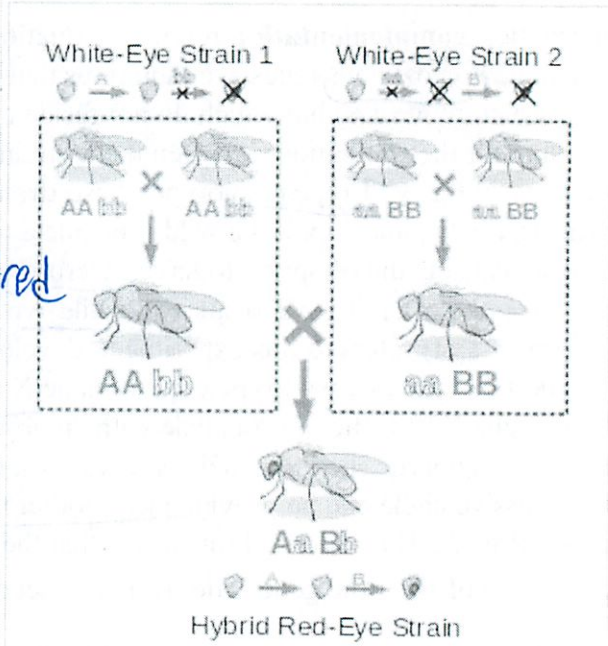
- If the combination of two haploid genomes containing different recessive mutations yields the wild type phenotype, then the mutations must be in different genes.

So to check if same/diff gene?

Contents

Example of a Simple Complementation Test

For a simple example of a complementation test, suppose a geneticist is interested in studying two strains of white-eyed flies of the species Drosophila melanogaster. In this species, wild type flies have red eyes and eye color is known to be related to two genes, A and B. Each one of these genes has two alleles, a dominant one that codes for a working protein (*A* and *B* respectively) and a recessive one that codes for a malfunctioning protein (*a* and *b* respectively). Since both proteins are necessary for the synthesis of red pigmentation in the eyes, if a given fly is homozygous for either *a* or *b*, it will have white eyes.



Example of a complementation test. Two strains of flies are white eyed because of two different autosomal recessive mutations which interrupt different steps in a single pigment-producing metabolic pathway. Flies from Strain 1 have complementary mutations to flies from Strain 2 because when they are crossed the offspring are able to complete the full metabolic pathway and thus have red eyes.

Knowing this, the geneticist may perform a complementation test on two separately obtained strains of pure-breeding white-eyed flies. The test is performed by crossing two flies, one from each strain. If the resulting progeny have red eyes, the two strains are said to complement; if the progeny have white eyes, they do not.

If the strains complement, we imagine that one strain must have a genotype aa BB and the other AA bb, which when crossed yield the genotype AaBb. In other words, each strain is homozygous for a different deficiency that produces the same phenotype. If the strains do not complement, they both must have genotypes aa BB, AA bb, or aa bb. In other words, they are both homozygous for the same deficiency, which obviously will produce the same phenotype.

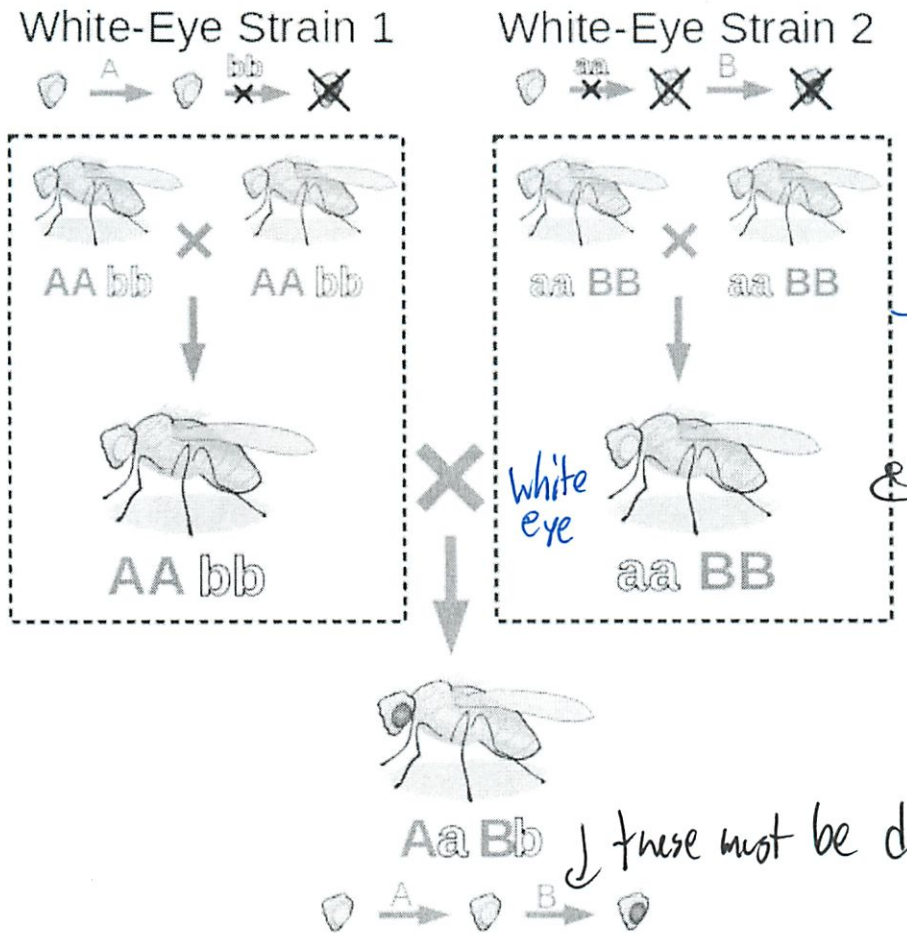
Exceptions

There are exceptions to these rules. Two non-allelic mutants may occasionally fail to complement (called "non-allelic non-complementation" or "unlinked non-complementation"). This situation is rare and is dependent on the particular nature of the mutants being tested. For example, two mutations may be synthetically dominant negative. Another exception is transvection, in which the heterozygous combination of two alleles with mutations in different parts of the gene complement each other to rescue a wild type phenotype.

See also

- blue-white screen

6



~~black diff things on~~ ~~between~~?
↳ so white eye

true breeding

these must be dominant

No both ~~one~~ A B must be present
for it to be red eye
Both are dominant
A,B shows up if only 1

(7)

true breeding = pure bred

RR × RR = RR

(; was this a diff complementation test than in class?)

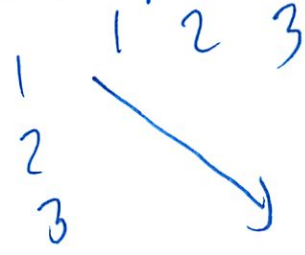
↳ No just never understood it

How many genes are in bio pathway

Do in yiest

~~If wild type → d~~

Pair each up



; but why does it say on same gene?

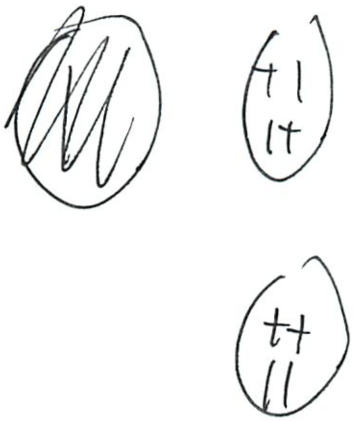
(8)

Could be on same gene

↳ then use epistasis test to test order

double mutants → see what phenotype

So in class



Can grow

Can't grow

~~it grows~~

arg^- recessive mutant

Chart

	+	arg1	arg2	arge
+	+	+	+	+
arg1	+	—	—	+
arg2	+	— on same gene	—	+
arge3	+	+	+	+

gene = Some unit of DNA

(9)

"So is it same gene or gene pathway?"

Complementating grap → same gene

but wild type "genes complement, diff genes"

So are they saying same pathway is same gene

↳ equivalent → this won't tell ya difference?

recovery of wild type

↳ "complement" mutated allell

so heterozy g

10

Percent WP

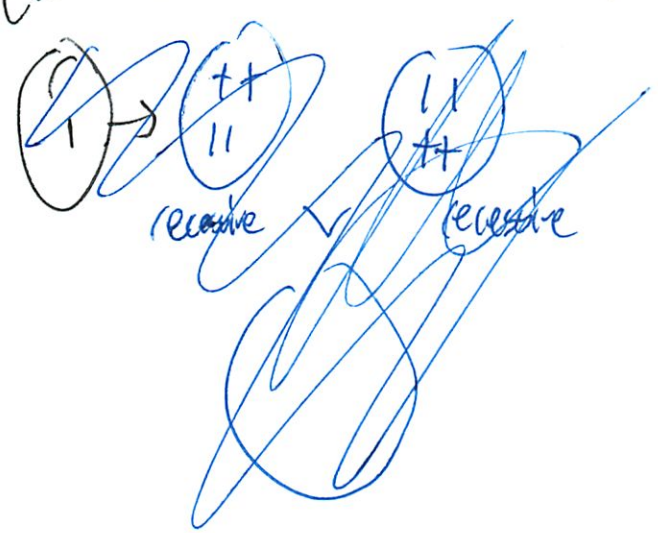
2 diff strains ↓both recessive

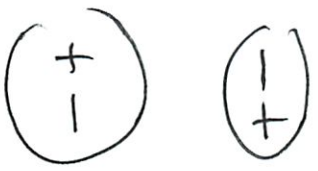
both homozygous mutation that produce same phenotype

but on diff gene

when crossed back to wild type

(so this example is diff)





Won't grow

Recessive i
Won't grow

Dani will grow

will grow

↳ recover "complement"

Go diff genotype

(11)

So what is LP test?

→ introduces pathway

(12)

If A and B are mutations in the same gene, they still produce no functional protein since while each has a different mutation they both eliminate the same function. They will not complement.

If A and B are mutations in different genes, now it is possible to get a good copy of both genes in the same organism restoring the pathway/phenotype. This is complementation.

2 mutations that both disrupt the same pathways

Complement if restore wild type

In fly red is wild type ✓ didn't think

So 2 diff mutations happened for white eyes

On diff genes

~~So when you~~

Working is dominant

So when put them together do complement and restore wild type

the other ~~ones~~ had 2 recessive or individual mutations

(13)

So we know mutations on diff genes
↳ but both on same pathway ie complement

If it didn't restore
↳ still recessive
still white

both blocked
" even more " white
not complemented
↳ which is a complementation group

items that fail to complement
* this is what throws me off

What went wrong
- and that complement means ~~[illegible]~~ will grow
- What exactly is recessive
- what is wild type
- what is expected

(19)

Epistasis test

a double mutant where one mutant masks the phenotype of another mutation

- same as mutant screening w/ replicaplating

Epistasis more than 1 gene involved in producing a trait

Or that problem where you put in order

Bonding

(do problems)

DNA

the transforming principle

DNA —transcribed→ RNA —translate→ Protein

(15)

transcription

T always attach at 3'

translation

ribosome

5' AUG 3' on table

lac

When present, blocks ~~transcription~~ repressor, ~~allows~~ allows operon, allows transcription

trp

When present, ~~di~~ activates repressor, blocks operon

(6)

Recombinant DNA

Vectors
libraries

Sea DNA
Shot gun
PCR
modern
SNP analysis

Neurobio

~~Ach~~ Ca^+ at end

release Ach

which alows Na^+ in

other inhibit

17

Immunology

- innate
- adaptive
  - humoral
  - cell mediated

all use T_H & prof antigen (MHC 2)

activate either B ← MHC 1 (attracts T_C)

or T_C

Bhas MHC 2

VDJ recombo

all have MHC ~~2~~ 1

(don't get confused)

	MHC 1	MHC 2
who has what attracts	All attract T_C	Prof cells inc b attract T_H

just confused myself → but consistent...

(18)

Antigen receptor
diff recognition domains
which are removed

types of B cells

Stem Cells ~~Embryonic~~

Synergistic

allogenic
└ will reject

Adult more differentiated

LDL bad
HDL good
└ mnemonic higher better

HMGCoA reductase

(19)

Virology

lots of diff type

(skipped over kinda fast in recitation
↳ wanted to finish

Rous Sarcoma Virus

Cancer

uncontrolled growth

~~m~~ metastasis spread

Ames test

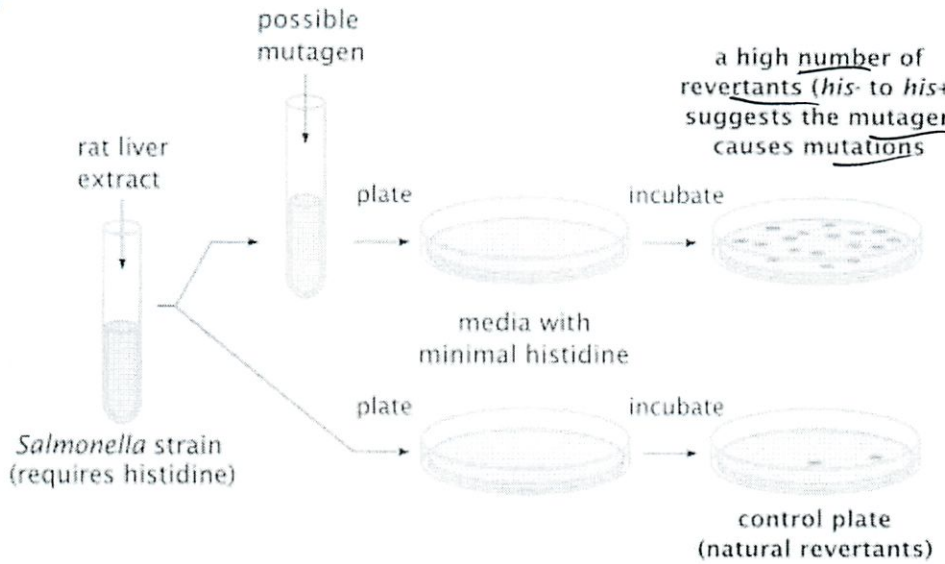
test for mutagenicity

1. start w/ bacteria his^- in his^+ media

~~Expose~~ mutate

See how many revert

20



Caused revertion back to normal (his+)

21

HIV

like a retrovirus
presents to T_H
but then takes over the the T_H

Prions

P_c / P_{sc}

β helix instead
replicates w/o DNA/RNA
by changing P_c P_c to P_c / P_{sc}

= protein infection

Stuff to Review/Practice

12/18
4:30p

Valence electrons
bonding
Groups -amine
-carboxyl

redo p-sets + quizzes
lots of bonding practice
Activation energy problems
Genetics -punnet -charts (pedigree)
-maps -epistatis
mitosis/meiosis
* DNA order/direction
lac vs trp operon
Vector questions
SNP qu

Pratice 12/18/2012

Name_____ Section_____ TA_____

2012 7.012 Problem Set 1

Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 before 4:00 PM, Thursday September 13th.

Question 1

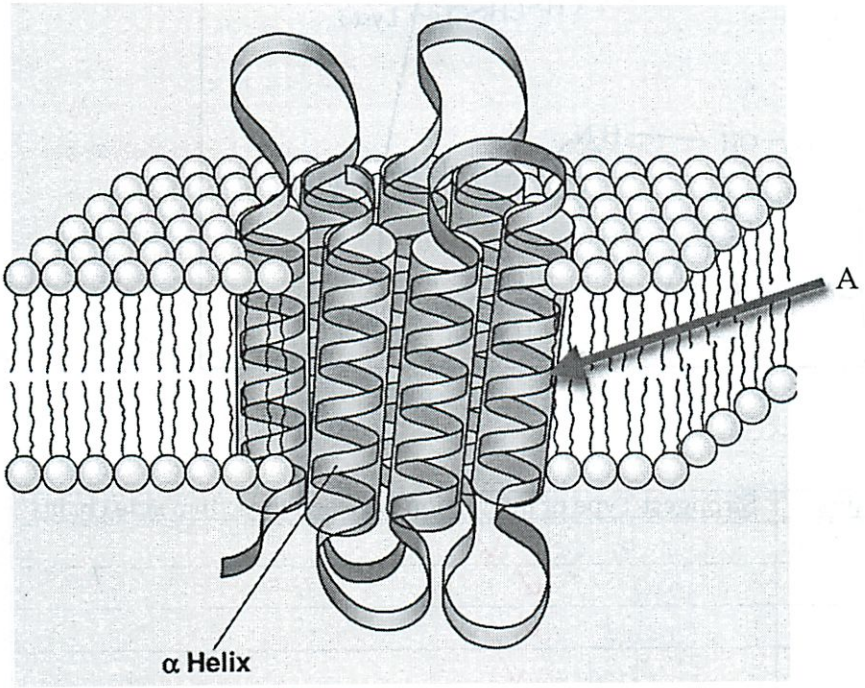
Describe the physical characteristics of the proposed first organism as it compares to modern organisms.

Single celled
Simple
mitosis only

Prothariots
no membrane bound organl
RNA

Question 2

Growth factor receptors (like that shown below) are transmembrane proteins found on the cell surface.



a) The molecules that form the **membrane** belong to what class of macromolecules?

lipid

b) Explain the important qualities/properties of the molecules listed in (a) that allow them to form membranes.

bylayer hydrophobic outside
phitic inside

c) List all of the amino acids you would expect to find at position A. Assume that the side chains of these amino acids are exposed to the membrane at this position. Explain why you made these choices. Page 7 shows the structures of the amino acids.

did not do all

i bonding qu still no good at!
Water is H_2O so nonpolar; Leu pro

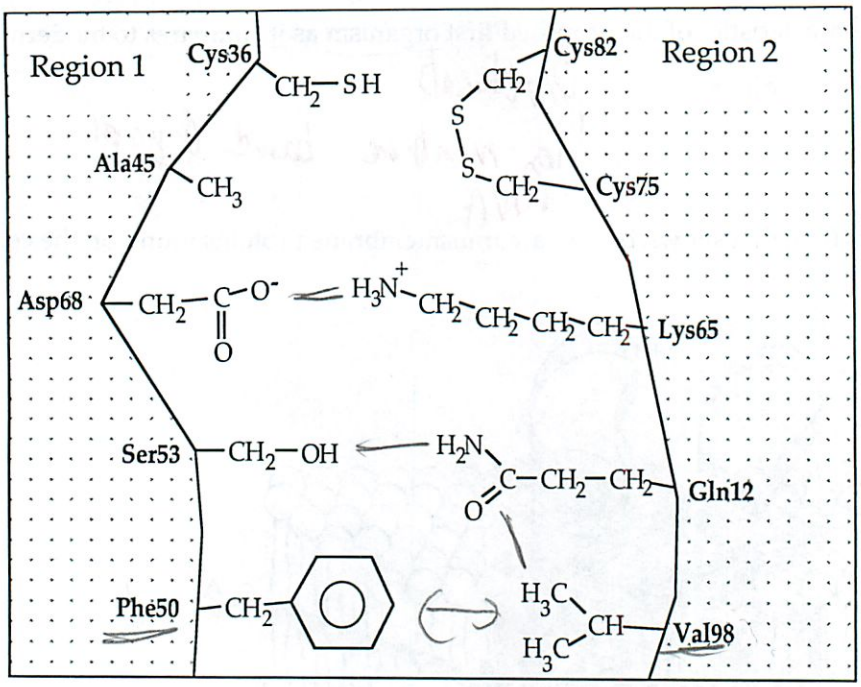
1

Name_____ Section_____ TA_____

Question 2, continued

Different regions of the protein interact in the tertiary structure of a protein.

d) Two interacting regions are shown below. In parts (i - iv) below, name the strongest type of interaction (choose from; **hydrogen bond, ionic, covalent, van der Waals**) that occurs between the side chains of the amino acids indicated.



Interacting Side chains	Strongest Type of interaction between these two side chains
i) Phe50 : Val98	van der Wall ✓
ii) Asp68 : Lys65	ionic ✓
iii) Cys75 : Cys82	covalent ✓
iv) Ser53 : Gln12	H - bonding ✓

e) Explain how Gln12 and Val98, which are far apart in the primary sequence of the protein, can be close to each other in the region of the protein diagrammed above.

folding / 2nd structure ✓ ✓ + 3rd

did Ok at bonding...

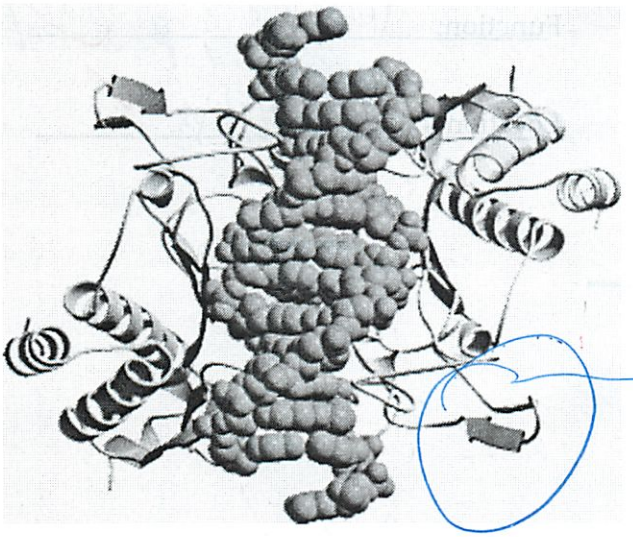
Name_____ Section_____ TA_____

Question 3

You have discovered an enzyme, enzyme E, which cleaves the phosphodiester bond in DNA. This enzyme binds to the specific double-stranded DNA sequence shown below.

```
5′ G/AATT C 3′
3′ C TTAA/G 5′
```

Once bound, the enzyme cuts both strands between the G and the A.



When you examine the gene that encodes this enzyme, you predict that the enzyme would be 305 amino acids long and weigh about 30 kilodaltons (30 kD). When you purify this enzyme you find that the **active** enzyme has a molecular weight of 60 kilodaltons (60 kD).

a) Why might the **active** purified enzyme be larger than the expected product?

1-2 copies, ✓ actually yes!

where?

b) What types of secondary structures are visible in the image above? α helixes + β sheets ✓

What bonds or forces are most important in stabilizing these secondary structures? H bonds

need to revise

What groups of the amino acids are involved in these bonds or forces? Choose from: amine groups directly attached to an α-carbon, amine groups found on side chains, carbonyl groups directly attached to an α-carbon, carbonyl groups found on side chains, and hydroxyl groups on side chains.

Side chain is the ATLG α Carbon is backbone

c) This enzyme's quaternary structure is composed of two subunits, each encoded by the same gene. Would you expect that the tertiary structure of the different subunits is the same or different? Explain.

Same since same gene

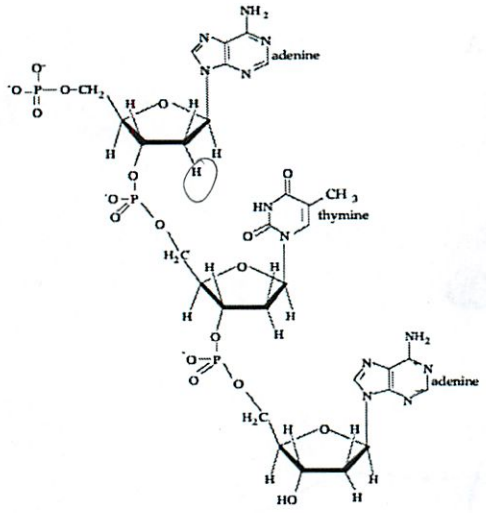
d) Assume that you can manipulate conditions in vitro such that you can induce a single subunit of this enzyme to bind and break a phosphodiester bond. How would the resulting cut DNA differ from the cut DNA generated by the intact enzyme?

it would not work
Only 1 strand cut

Name_____ Section_____ TA_____

Question 4

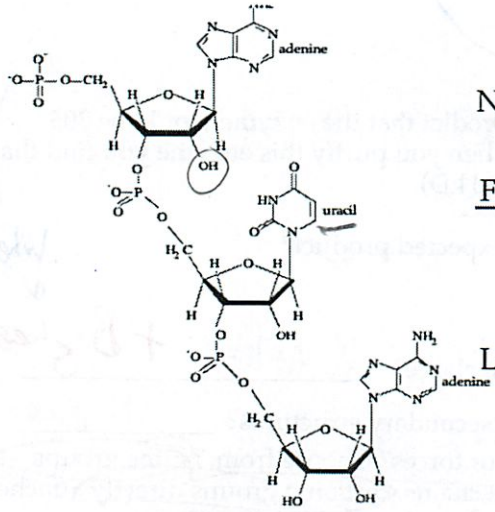
a) Name each of the following structures, give the function or functions of each, and list where in a eukaryotic cell each would be found.



Name: DNA ✓

Function: transforming principle/heredity

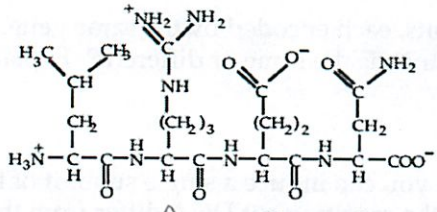
Location(s): Cell nucleus ✓



Name: RNA ✓

Function: temp copy of

Location(s): Cell ✓ Cytoplasm + nucleus



Name: Protein ✓

Function: ? Lots

Location(s): ? Everywhere

Val Arg Gln Asn

Peptide short polymers of amino acid peptides

proteins are had so much trouble earlier lots of polypeptides + cofactors

Name_____ Section_____ TA_____

Question 4, continued

b) Which of the following represent a condensation reaction? Place an X next to all that apply.

___X___ The joining of DNA fragments by DNA ligase during replication.

___X___ The formation of a peptide bond.

___X___ The formation of a glycosidic linkage to form a disaccharide.

_____ The formation of glucose from lactose.

_____ The cleavage of double-stranded DNA by a restriction enzyme.

Question 5

In this question, you will use StarBiochem, a molecular 3-D viewer, to explore the structure of several proteins and how their structures relate to their function in the cell. You will begin by importing protein structures from the Protein Data Bank by using the following instructions:

- To begin using StarBiochem, please navigate to: http://mit.edu/star/biochem/.
- Click on the Start button.
- Click Trust when a prompt appears asking if you trust the certificate.
- In the top menu, click on Import → RCSB (Protein Data Bank). Type in the four-character ID code for each protein structure listed below and select Open. Among all the structures shown, select the designated protein ID and click Open again.

Import each of the proteins listed below. The program will create a tab for each protein so once they are imported, you can navigate between proteins easily.

- 1BKV
- 1BL8
- 1EJ9
- 1H6L
- 3D9S

Explore the structure of each of the above proteins and answer the following questions. Please note that if you change the view of the protein or proteins and want to go back to a previous view, select "Reset" in the top navigation bar, and choose "reset structure" or "reset all structures".

a) Which of these proteins has a tertiary and/or quaternary structure that might allow it to act as a membrane channel to allow entry of small molecules into the cell? For each protein chosen, describe what feature or features you saw in the tertiary and/or quaternary structure that suggested to you that this is a channel protein.

b) Which of these proteins is shown binding to nucleic acid? _____
What nucleic acid is binding to this protein? _____
Describe what feature or features you saw that allowed you to identify the nucleic acid.

Name_____ Section_____ TA_____

Question 5, continued

Give a more complete description of any two of the protein(s) that you imported by exploring their structure in more detail. Determine which of the following features can be found for each of your chosen proteins. More than one may apply.

I have chosen for Protein 1:_____. This protein…

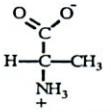
…has some alpha helix structure.	Yes or No
…has seven transmembrane alpha helices.	Yes or No
…has some beta sheet structure.	Yes or No
…has quaternary structure.	Yes or No

I have chosen for Protein 2:_____. This protein…

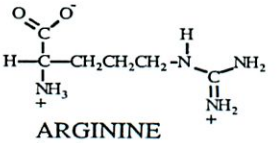
…has some alpha helix structure.	Yes or No
…has seven transmembrane alpha helices.	Yes or No
…has some beta sheet structure.	Yes or No
…has quaternary structure.	Yes or No

Name_____ Section_____ TA_____

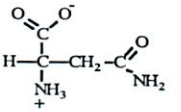
STRUCTURES OF AMINO ACIDS at pH 7.0



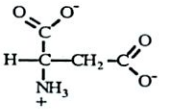
ALANINE
(ala)



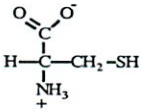
ARGININE
(arg)



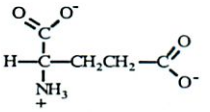
ASPARAGINE
(asn)



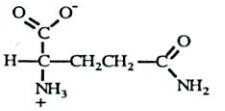
ASPARTIC ACID
(asp)



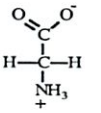
CYSTEINE
(cys)



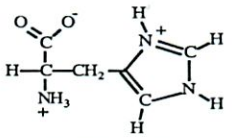
GLUTAMIC ACID
(glu)



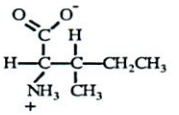
GLUTAMINE
(gln)



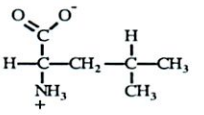
GLYCINE
(gly)



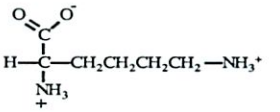
HISTIDINE
(his)



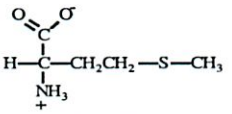
ISOLEUCINE
(ile)



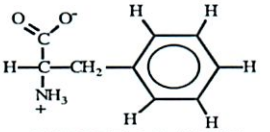
LEUCINE
(leu)



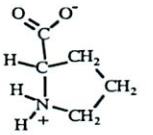
LYSINE
(lys)



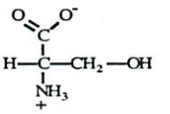
METHIONINE
(met)



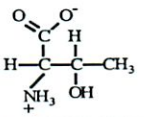
PHENYLALANINE
(phe)



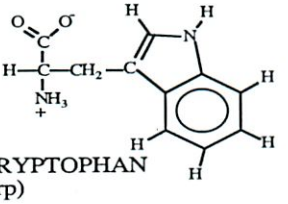
PROLINE
(pro)



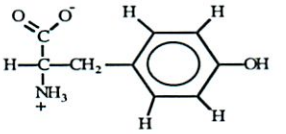
SERINE
(ser)



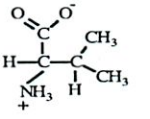
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)

Name_____ Section_____ TA_____

2012 7.012 Problem Set 2

Please print out this problem set and answer the questions on the printout. Answers to this problem set are to be turned in at the box outside 68-120 before 4:00 PM, Thursday September 27th.

Question 1

You are doing genetics experiments with the fruit fly, *Drosophila melanogaster*. In the "P" generation, you cross two true-breeding flies. The female parent is brown and wingless and the male parent is black with normal wings. All of the flies in the F1 generation are black and wingless. ∈ Dominant

Indicate the alleles associated with dominant phenotypes by a capital letter and alleles associated with recessive phenotypes by a lowercase letter. Assume the two traits you are following are autosomal. Indicate the color genotype with the letters "B" and "b" and the wing genotype with "N" and "n."

B = black
b = brown
N = wingless
n = winged

a) The genotypes of the flies in the P generation are:
bbnn female and ~~bbNN~~ BBnn male. stupid!!!

b) The genotypes of the flies in the F1 generation are: BbNn ✓

c) You cross two F1 flies and obtain 1600 offspring. List the phenotypes of the offspring in the F2 generation and predict about how many flies of each type you expect if Mendel's second law applies to these genes. ↑ good to write

	BN	bN	Bn	bn
BN	BBNN	BbNN	BBNn	BbNn
bN	BbNN	bbNN	BbNn	bbNn
Bn	BBNn	BbNn	BBnn	Bbnn
bn	BbNn	bbNn	Bbnn	bbnn

etc
900 : 300 : 300 : 100

d) You now take an F1 generation female and cross her to a true-breeding brown male that has normal wings.

i) This male's genotype is: ~~BBnn~~ bbnn. BbNn BBnn ↑ read carefully! how did I get this wrong? ↑ not parent

ii) You count 1600 offspring in the F2 generation. If you assume that the wing and the color genes assort independently, you would expect:

400 10 # of normal winged brown flies (of the genotype bbnn)
400 800 # of normal winged black flies (of the genotype BBnn, Bbnn)
400 0 # of wingless brown flies (of the genotype bbNn, bbNN)
400 800 # of wingless black flies (of the genotype BBNn, BbNn)

	Bn	
BN	BBNn	black, wingless
bN	BbNn	"
Bn	BBnn	black winged
bn	Bbnn	black winged

All very stupid mistakes!

Name_____ Section_____ TA_____

Question 2

As a plant geneticist, you have identified three traits that contribute to the taste of certain coffee beans. You have isolated strains of coffee plants that breed true for each trait. The strains produce beans that can be either 1) plain or nutty (use A or a); 2) bitter or smooth (use B or b); and decaffeinated or highly caffeinated (use D or d).

**In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lowercase letter for the allele associated with the recessive phenotype.*

You cross a true breeding nutty, smooth, decaffeinated strain to a true breeding plain, bitter, and caffeinated strain. The first (F1) generation is 100 plants:

20 nutty, bitter, caffeinated
80 nutty, smooth, caffeinated

A=nutty D=caffeinate B=smooth
a=plain d=decaffeinated b=bitter

a) Which traits are not exhibiting classic Mendelian inheritance?

1 Co dominance

b) Which traits are dominant Mendelian traits?

A

c) You cross two F1 plants:

Nutty + caffeinated

nutty, bitter, caffeinated X nutty, smooth, caffeinated

Ignore the traits that do not exhibit Mendelian inheritance. Assume that none of the Mendelian traits are linked. If there are 640 plants in the F2 generation, how many of each phenotype and genotype do you expect?

Parents aaDD AAdd

F1 AaDd

F2 AD Ad aD ad

AD
Ad
aD
ad

Same 9:3:3:1 ✓

Name_____ Section_____ TA_____

Question 3

As an undergraduate in a genetics lab your project is to study a few genes in the fly. You first look at two traits, the eyeless trait and the wingless trait, where both the wingless and the eyeless phenotypes are recessive to normal eyes and wings.

a) You cross a true-breeding normal eyed, winged female with a true-breeding eyeless, wingless male. What will be the phenotype(s) of the F1 progeny?

EeWw — eyed winged

You cross several pairs of F1 siblings and look at 320 progeny:

Eyes	wing	Number
Normal	Normal	190
eyeless	Normal	53
Normal	wingless	52
eyeless	wingless	25

b) Looking at this data, can you predict whether the eye gene is linked to the wing gene. Explain your answer.

No - its about even

You are then asked to study a few new genes in the fly. Preliminary work indicates that the mutant alleles of these genes give recessive phenotypes and the genes are not sex-linked. You first look at 2 genes, each with two alleles: "R or r" for body color and "A or a" for wing surface. The red body phenotype is dominant to the yellow body phenotype and smooth wings are dominant to crinkled wings.
You cross two true breeding parents to get F1 flies that are red with smooth wings (RrAa)

c) To determine the recombination frequency between these two genes, you perform several crosses where you cross an F1 from above (RrAa) with a yellow-bodied, crinkle-winged fly (rraa). You get the following results:

Body	Wing surface	Number
red	crinkled	4200
red	smooth	800
yellow	crinkled	809
yellow	smooth	4191

i) What is the recombination frequency between the genes for body color and wing surface?

1609 / 10,000 = 16%

ii) Explain why it is easier to calculate the recombination frequency using a test cross as compared to a F1 X F1 cross.

test cross is to recessive
So phenotype of F2 controlled by test

iii) What are the genotypes of the true breeding parents?

RRAA
rraa

Name______________________________ Section______ TA____________

Question 3, continued

You decide to turn your attention to a third gene, one that controls wing length. This gene has two alleles, "L or l" where long wings are dominant to short wings. Remember that the red body phenotype is dominant to the yellow body phenotype. You again mate two true-breeding flies:

L=long
l=short
R=red
r=yellow

red-bodied, short wing male X yellow-bodied, long wing female

RRll rrLL

*F1: All red-bodied, long wing

RrLl

d) To determine the recombination frequency between these two genes, you perform several crosses where you cross an *F1 from above with a yellow-bodied, short-winged fly. You get the following results:

Body	Wing length	Number
red	long	98
red	short	408
yellow	long	391
yellow	short	103

What is the recombination frequency between the genes for body color and wing length?

$\frac{98+103}{1000} = 20.1\%$ cM

e) Given your answers to parts (c,i) and (d), what can you say about the linkage between the gene for wing length and the gene for wing surface?

body — 16% — surface — 4% — len

Or

surface — 16% — color — 20% — wing len

are linked

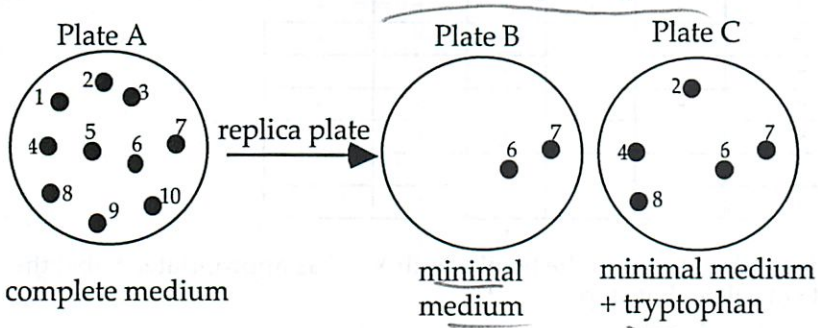
are on same chromosome

f) Based on the preceding questions, draw linear representations of the two possible **arrangements** of the three genes. Include the recombination frequency between the genes on your diagram.

Name_____ Section_____ TA_____

Question 4

You want to identify the enzymes involved in the tryptophan biosynthesis pathway by isolating yeast that fail to synthesize tryptophan (these yeast are referred to as "trp-"). You know that mutant yeast that fail to synthesize tryptophan (and thus cannot grow without addition of tryptophan to the media) are likely to be defective in one of the enzymes involved in the tryptophan synthesis pathway. You start with a population of haploid wild-type ("trp+") yeast, mutagenize it with UV light, and allow the yeast to grow into isolated colonies on plate A (see diagram). You then use the replica plating technique to transfer some yeast from each colony onto plates B and C. The contents of the growth medium are listed below each plate. Complete medium contains all nutrients; minimal medium contains nutrients sufficient to allow wild-type yeast to grow, but yeast cells with mutations in a gene for any nutrient synthesis pathway cannot grow unless the nutrient is added to the minimal growth medium. Assume that each mutant carries only a single mutation.



a) List the colonies that are trp-. ~~A-C = 1,3,5,9,10~~ C-B 2,4,8 ✓

b) Some colonies grow on plate A, but do not grow on Plate C. Give one possible explanation for the growth behavior of these colonies.

Missing other things - not trp ✓

c) You repeat this experiment multiple times and isolate eight trp- mutants. You then perform a complementation test on these mutants. The data are shown below. Briefly describe how a complementation test is performed. lol I remember

Pair each mutant together. If back to wild type → complement diff gene
If not → same gene → complementation group
✓ test on minimal media

Complementation Test Results:

In the table below, a (+) indicates growth on minimal media, a (-) indicates lack of growth on minimal media.

	m1	m2	m3	m4	m5	m6	m7	m8	WT
m1	-	+	+	+	+	+	+	+	+
m2		-	+	+	+	-	-	+	+
m3			-	+	-	+	+	+	+
m4				-	+	+	+	+	+
m5					-	+	+	+	+
m6						-	-	+	+
m7							-	+	+
m8								-	+

3,5 6,7

d) Assign the mutants 1-8 into complementation groups.

1
2,6,7
? forgot
3,5
4
8

why are some single? Leaves inside...

Name_____ Section_____ TA_____

Question 4, continued

e) You determine that the pathway for tryptophan biosynthesis involves five precursor compounds, V-Z. The growth requirements for each of the mutants are summarized in the table below, where a (+) indicates growth on minimal media supplemented with the indicated precursor, a (-) indicates lack of growth. For example, m4 will grow on minimal media supplemented with either tryptophan or compound Y, but will not grow on minimal media supplemented with V, W, X, or Z.

	Minimal media supplemented with Compounds					
	tryptophan	V	W	X	Y	Z
Mutant						
m1	+	-	-	-	-	-
m2	+	-	-	+	+	-
m3	+	-	+	+	+	-
m4	+	-	-	-	+	-
m5	+	-	+	+	+	-
m6	+	-	-	+	+	-
m7	+	-	-	+	+	-
m8	+	+	+	+	+	-
	WT	+	+	+	+	+

So what is needed to get it to grow?

right — since can bring along ✓

Draw the pathway for tryptophan biosynthesis. Fill in the blanks with V - Z as appropriate. Label the arrows with the mutants that cannot complete that step.

Z —m8→ V —3,5→ W —2,6,7→ X —4→ Y —1→ Tryptophan

can't complete step w/o extra help

Since have mutant there (need extra help)

Question 5

The following pathway is for the synthesis of the amino acid arginine, where letters represent intermediate compounds and numbers represent enzymes:

Precursor compound ⟶ X —1→ Y —2→ Z —3→ Arginine

Mutants with defective genes 1, 2, or 3 (m1, m2, or m3, respectively) will require arginine to grow on minimal medium.

a) What intermediate will build up in the following mutants?

can't move to next step Since can't produce

m1: X ✓ m2: Y ✓ m3: Z ✓

b) What intermediate(s) will the following mutants grow on? On its own

m1: Y, Z ✓ m2: Z ✓ m3: None ✓

c) What intermediate will build up in the following double mutants?

Starts w/ nothing

m1,m2: X ✓ m2,m3: Y ✓ m1,m3: X ✓

d) What intermediate(s) will the following double mutants grow on?

m1,m2: Z ✓ m2,m3: — ✓ m1,m3: — ✓

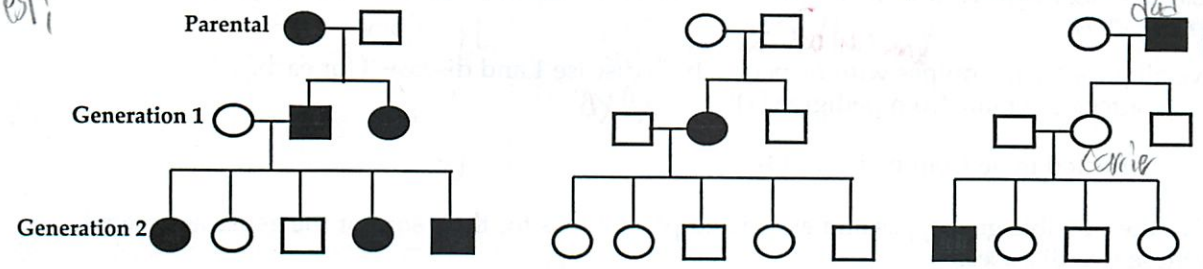
got much better :)

where did I learn this?

Name_____ Section_____ TA_____

Question 6

You are analyzing the following human pedigrees each for a specific disease. *Please note: The filled squares or circles represent a different disease phenotype. The disease allele is rare and individuals marrying into the family do not have the disease allele. Assume that no other mutation arises within the pedigree. Assume complete penetrance. Also note that each of the three families below are affected with only a single disease. They do not show either of the other two diseases and they are not carriers for either of the other two diseases.*



(Disease 1) **(Disease 2)** **(Disease 3)**

a) Which of these pedigrees (*1/ 2/ 3*) **most likely…**

i) Shows an **autosomal recessive** mode of inheritance?

ii) Shows an **X-linked** mode of inheritance?

iii) Shows an **autosomal dominant** mode of inheritance?

b) The **affected female** from pedigree (2) re-marries an affected male from pedigree (3) and they have a son and a daughter.

For disease 2, (pedigree 2): use the symbol 'A' or 'X^A' to represent the allele for the dominant phenotype and 'a' or 'X^a' for the allele for the recessive phenotype.
For disease 3 (pedigree 3): use the symbol 'B' or 'X^B' to represent the allele for the dominant phenotype and 'b' or 'X^b' for the allele for the recessive phenotype.

i) Give the genotypes with respect to both disease 2 and disease 3 for each of the following:

affected female from pedigree (2):

affected male from pedigree (3):

ii) Give the possible genotypes and associated phenotypes for their **son** for the genes associated with these two diseases.

Genotype: **Phenotype:**

iii) Give the possible genotypes and associated phenotypes for their **daughter** for the genes associated with these two diseases

Genotype: **Phenotype:**

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Question 6 continued auto somal dom female

c) The affected female from Generation 1 in pedigree (1) marries an affected male from pedigree (3) and they have a son and a daughter.

For disease 1 (pedigree 1): use the symbol 'D' or "X^D ' to represent the allele for the dominant phenotype and 'd' or 'X^d' for the allele for the recessive phenotype.
For disease 3 (pedigree 3): use the symbol 'B' or 'X^B' to represent the allele for the dominant phenotype and 'b' or 'X^b' for the allele for the recessive phenotype.

D = has
d = has not enough

know can't be DD or Dd / BB dd X^b Y

i) Give all possible genotypes with respect to both disease 1 and disease 3 for each:

affected female from pedigree (1): X^BX^B

affected male from pedigree (3): ✓

ii) Give the possible genotypes and associated phenotypes for their **son** for the genes associated with these two diseases.

Genotype: Dd X^B Y **Phenotype** has disease 1 possibly 50%
dd X^B Y not 3 50% don't get, mistake, pretty sure..

iii) Give the possible genotypes and associated phenotypes for their **daughter** for the genes associated with these two diseases.

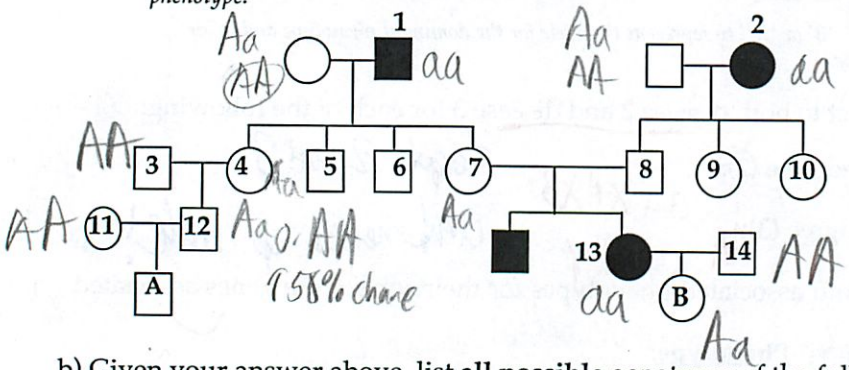
Genotype: Dd X^BX^b **Phenotype** 11 ✓
dd X^BX^b

Question 7

The following pedigree represents the inheritance pattern of a specific genetic disorder in humans.

- *The filled squares or circles represent the abnormal phenotype.*
- *The disease allele is rare and individuals marrying into the family do not have the defective allele.* ← read
- *Assume that no other mutation arises within the pedigree. Assume complete penetrance.*
- *Use the symbol 'A' or 'X^A' to represent the allele for the dominant phenotype and 'a' or 'X^a' for the allele for the recessive phenotype.*

Always make all these mistakes know what to look for but miss it!



a) State the **most likely** mode of inheritance for this disease.

autosomal recessive

b) Given your answer above, list **all possible** genotypes of the following individuals in the pedigree.

A is
A 75% a 25%
A AA Aa

B is Aa
A 87.5 a 12.5

Individuals	Genotypes
#1	aa
#4	Aa
#7	Aa
#8	Aa
#10	Aa
#12	Aa or AA

] since 1 parent has, but isn't
— we don't know 3

c) If individuals **A** and **B** have children, what is the probability that their 1st child will be affected?

A Aa 6.25 12.5% · 50% = 6.25% ✓

d) If individuals **A** and **B** have a 1st child who is affected, what is the probability that their 2nd child will be affected?

Aa aa
50 41.25 6.25

know not AA know A is Aa should have done this!! (so stupid, really should know better!)

$\frac{6.25}{6.25+41.25+6.25} = 11\%$

Try that

A is Aa

B is Aa

	A	a
A	AA	Aa
a	Aa	aa

↑ 25% ✓

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2012 7.012 Problem Set 3

Please print out this problem set and answer the questions on the printout. Answers to this problem set are to be turned in at the box outside 68-120 before 4:00 PM, Thursday October 11th.

Question 1

Briefly describe the experiments performed by each of the followings researchers, and in one sentence summarize the important findings of each experiment.

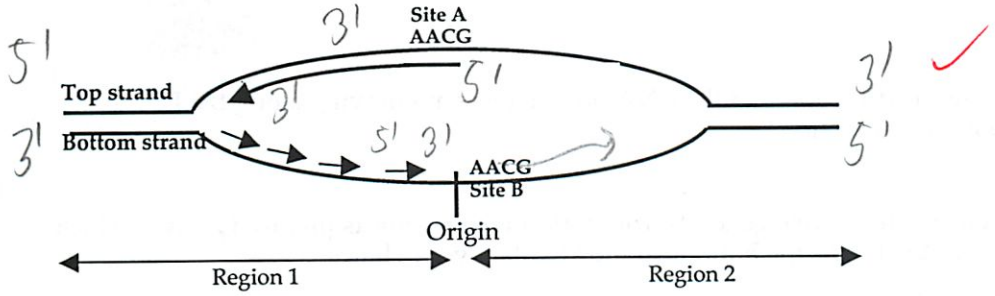
a) Frederick Griffith, 1928:

b) Oswald Avery, *et. al*, 1943-44

c) Alfred Hershey and Martha Chase, 1952

Question 2

Shown below is a schematic of replicating DNA in a bacterial cell.



a) On the diagram, label the 5′ and the 3′ ends of the parental DNA strands.

b) Which parental DNA strand (*top or bottom*) serves as a template for the synthesis of the leading strand in Region 2?

c) To which site (*A, B, or both*) can the primer **5′ UUGC 3′** bind?

its opposite
5' UUGC 3'
3' AACG 5' ← B

Coding is the strand being made

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Question 2, continued

d) The replication of which strand (*top, bottom, or both*) in Region 2 would be affected in the absence of RNA primase? Assume that replication has not yet initiated on either strand. Explain.

e) You perform DNA replication in a test tube (in vitro) using a **single-stranded linear DNA** as the template and the **appropriate DNA primer**. From the list below, circle the proteins that are required for **one round** of replication.

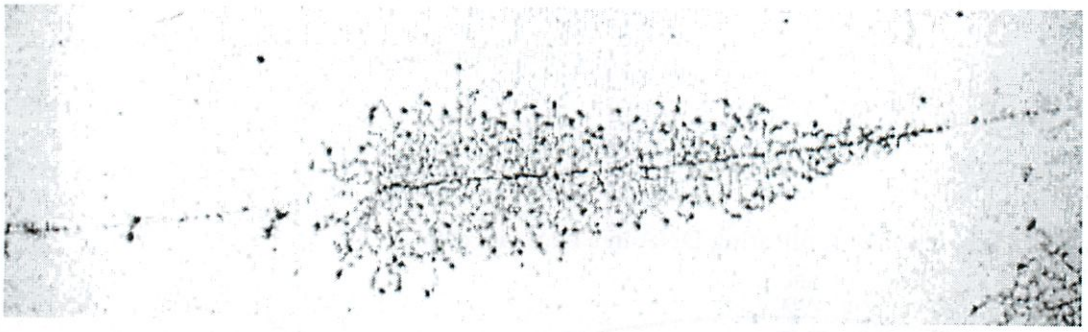
Primase **DNA polymerase** **Ribonuclease** **Topoisomerase** **Ligase**

f) Why does the DNA of a eukaryotic cell require multiple origins of replication when some prokaryotic cell genomes have only one origin of replication?

g) While studying replication you find a mutant in which the fidelity of replication has decreased by a factor of 100. You suspect that this is due to a mutation in the DNA polymerase enzyme. What specific enzymatic activity of the DNA polymerase allows it to proofread the newly replicated DNA molecule?

Question 3

Below is an electron micrograph of a single gene being transcribed. The DNA strand runs horizontally with RNA transcripts extending vertically outward.



a) Draw an arrow indicating the direction that the RNA polymerases are moving along the DNA strand. Why did you choose this direction?

b) Below is a partial sequence of the above gene. **Its orientation is the same as pictured above.** Which strand is the template strand, the top or the bottom strand? Explain your choice.

$^{5'}$ACTCGATGCTAG$^{3'}$
$^{3'}$TGAGCTACGATC$^{5'}$

c) What would be the mRNA sequence transcribed from the above sequence? Be sure to label the 5′ and 3′ ends.

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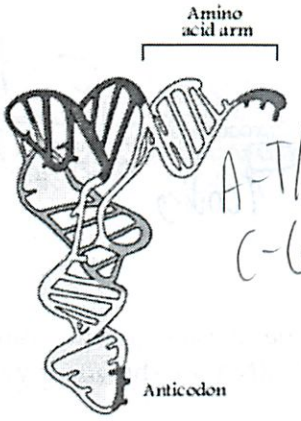
Question 4, continued

A tRNA molecule is composed of an RNA chain that folds into a 3-D shape like that shown below. At one end it has an anti-codon that base pairs with the appropriate codon on the mRNA and at the other end it has an amino acid arm that binds to a specific amino acid.

b) Below are three <u>anti-codon</u> sequences for three tRNAs, fill in the corresponding amino acid on the blanks.



A-T/U
C-G

anticodon found on tRNA	amino acid attached to tRNA
5' AGU 3' ACU 3'UCA5'	Thr
5' AUG 3' CAU 3'UAC	His
5' CUG 3' CAG GAC	Gln

✓ was ticked the 1st time

trp
y
3' ACC 5'
5' UGG

c) Give the anticodon used in the tRNA encoding trp. Be sure to label the 5' and 3'.

3' ACC 5' ✓

d) Would a substitution within a codon for trp always change the resulting protein sequence? Explain your answer.

Yes. That is the only one used ✓

e) Would a substitution within a codon for thr always change the resulting protein sequence? Explain your answer.

No Any AC* work work ✓
(ACG)

f) An aminoacyl tRNA synthetase is an enzyme that attaches a specific amino acid to the appropriate tRNAs to form an aminoacyl-tRNA. This is sometimes called "charging" the tRNA with the amino acid. Assume you have a cell with a mutation in the gene for the tryptophan aminoacyl tRNA synthetase. This mutant enzyme attaches tryptophan to tRNAs with the anticodons 5' CCA 3' and 5'GCA 3'. Explain how protein production in this cell will be altered and estimate how many different types of proteins would be affected inthis cell. Choose from: >10, 10-100, 100-1000, all or the proteins in the cell.

Pro Ala

Almost all will be ~~altered~~
(mis read)

Cys replaced

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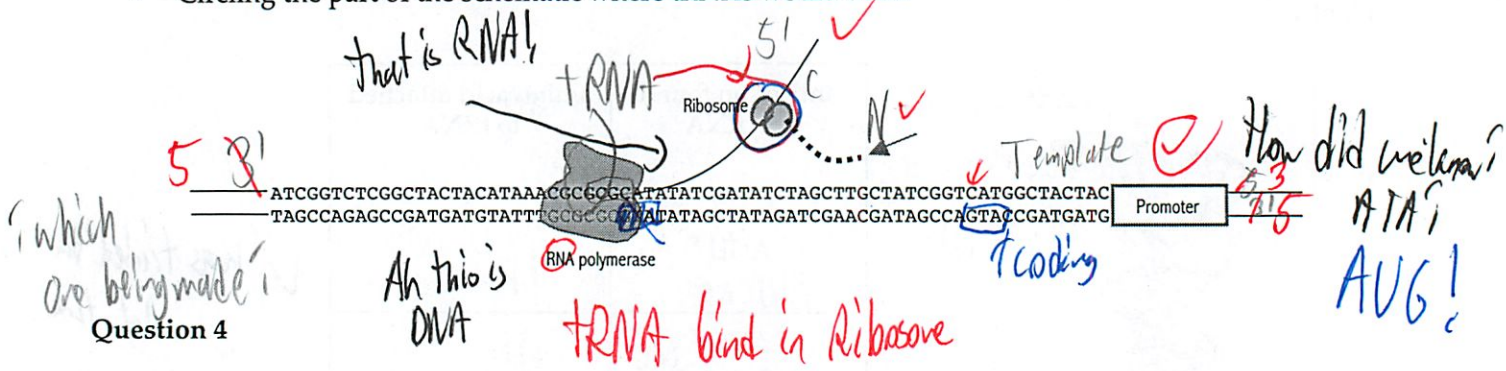
Question 3 continued

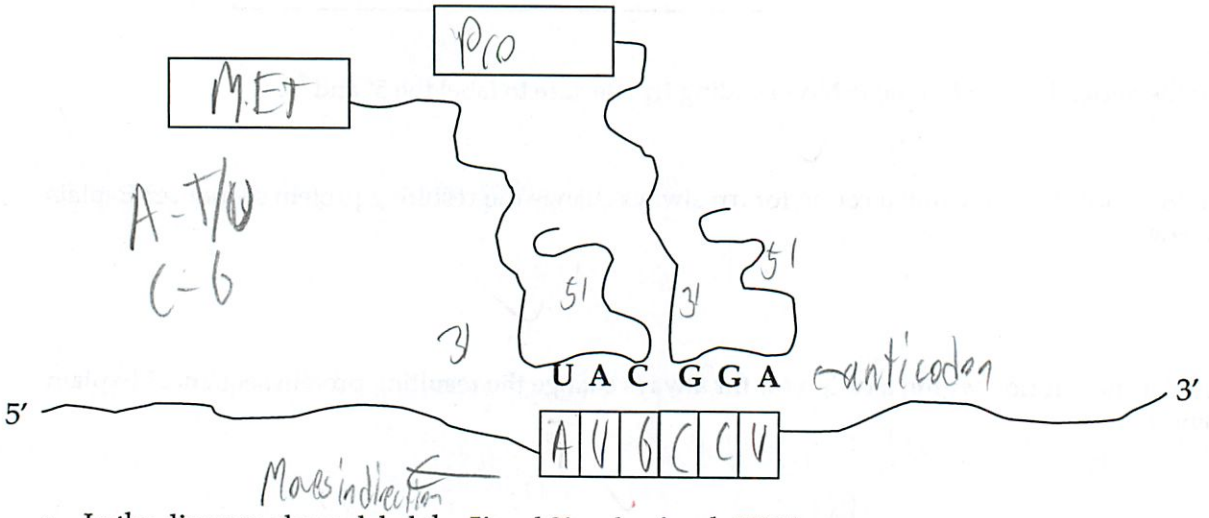
d) Complete the diagram below by…

- Labeling 5′ and 3′ on the mRNA.
- Labeling the arrow with either the N or the C to indicate the terminus of the protein.
- Boxing the 3 bases encoding the first amino acid of the protein being made.
- Labeling the template strand for transcription.
- Circling the part of the schematic where tRNAs would bind.



Question 4

a) Below is a diagram of two tRNAs and an mRNA in the active site of the ribosome during translation of the mRNA into protein. Three nucleotides from the sequence of each tRNA are shown for you.



- In the diagram above, label the 5′ and 3′ ends of each tRNA.
- In the diagram above, fill in the boxes in the mRNA with the 6 nucleotides that would be present there.
- In the diagram above, fill in the box attached to one end of each tRNA with the name of the amino acid that would be attached there.
- Which tRNA is about to transfer its attached amino acid over to the other tRNA: the tRNA on the left or the tRNA on the right?

~~right will give to left~~

left will give to right

left will leave ← when that

← 3 but when think about it - makes more sense!

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Question 5

Below is a partial sequence of a coding region, base pairs 61-102 (read left to right) of a 600 base pair open reading frame. The underlined codon indicates the correct reading frame of this gene.

5′ ATCTGGGCTAATACCGCCAACTATATAAACACCCACATTTCG 3′
3′ TAGACCCGATTATGGCGGTTGATATATTTGTGGGTGTAAAGC 5′

what is coding/template

a) What is the mRNA sequence encoded by base pairs 61-71?

AUCUGGGCUA U not T

b) What is the amino acid sequence of the peptide encoded by base pairs 61-69?

Ile Trp ~~Pro~~ Ala missed

c) How does the resulting peptide change if the sequence is altered as shown below? Also identify the type of mutation, choose from missense, nonsense, silent, frame-shift, or deletion. don't know names

i) original: 5′ ATCTGGGCTAATACCGCCAACTATATAAACACCCACATTTCG 3′
altered: 5′ ATCTGGGCTAA**C**ACCGCCAACTATATAAACACCCACATTTCG 3′

point Same Asn ~~nonsense~~ silent ✓

ii) original: 5′ ATCTGGGCTAATACCGCCAACTATA**T**AAACACCCACATTTCG 3′
altered: 5′ ATCTGGGCTAATACCGCCAACTATT**A**AAACACCCACATTTCG 3′

Stop nonsense ✓

iii) original: 5′ ATCTGGGCTAA**T**ACCGCCAACTATATAAACACCCACATTTCG 3′
altered: 5′ ATCTGGGCTAA**A**ACCGCCAACTATATAAACACCCACATTTCG 3′

missense ✓

iv) original: 5′ ATCTGGGCTAATACCGCCAACTATATAAACACCCACATTTCG 3′
altered: 5′ ATCTGGGCTAATACC------TATATAAACACCCACATTTCC 3′
(delete 6 base pairs)

AA deletion ✓

v) original: 5′ ATC--TGGGCTAATACCGCCAACTATATAAACACCCACATTTCG 3′
altered: 5′ ATCATTGGGCTAATACCGCCAACTATATAAACACCCACATTTCC 3′
(insert 2 base pairs)

frame shift ✓

d) Of the various mutations given above, which the one(s) would most dramatically affect the **function** of the protein encoded by this gene? Explain your answer.

frame shift - changes all after that

Also the nonsense

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Question 6

Shown below is a double-stranded bacterial (*E. coli*) DNA sequence coding for a hypothetical protein. Both strands are shown; the top strand reads 5′ to 3′ left to right, while the bottom strand reads 5′ to 3′ right to left. The nucleotides are numbered from 1 to 100. For this problem, transcription begins with and includes the underlined A/T base-pair (indicated with an "a") and proceeds left to right.

```
     1          a        20                  40
     |                   |                   |
5'-GTGTCCGTATAATATTGTGAGATGTTATATCCCGCCGTCAACACCATCAA-3'
   ---------+---------+---------+---------+---------+
3'-CACAGGCATATTATAACACTCTACAATATAGGGCGGCAGTTGTGGTAGTT-5'

     60         c        80                  100
     |                   |                   |
5'-ACAGGATAATCGCCTGCTGGGGCAAAGGCGGTGAAGGTAAAGGTGTTGCC-3'
   ---------+---------+---------+---------+---------+
3'-TGTCCTATTAGCGGACGACCCCGTTTCCGCCACTTCCATTTCCACAACGG-5'
```

Coding is 5′ to 3′ →
template 3′→5′

5′ AUG on coding

a) Which strand is used as a template for transcription, the top or the bottom?

Stop + think. didn't think long enough...

b) Where would the promoter be relative to base pair #1?

to the left ✓ 5′ to the start

got confused was right

c) What are the first 10 nucleotides of the resulting mRNA? Indicate the 5' and 3' ends of the mRNA.

~~5′ AUG UUA UAUC 3′~~ 5′ AUA AUA UUG U * starts at transcription start point duh!

d) What are the first 5 amino acids translated from the resulting mRNA? Indicate the amino (NH_3^+) and carboxy (COO^-) termini of the protein.

NH^+ Met etc

e) Do the underlined nucleotides TAA (indicated with the letter "**c**") encode a stop codon for this protein? Briefly explain your answer.

No frame is wrong ✓

Consider the situations in parts (f-h) independently.

f) A mutation occurs which results in the insertion of an extra G/C (top strand/bottom strand) base-pair immediately after base pair 11 (shown in bold). What effect will this insertion mutation have on transcription and translation?

nothing ✓ before it matters

g) A different mutation results in the substitution of the T/A base pair at position 30 (shown in bold and underlined) with a G/C base pair. How would this mutation affect the sequence of the protein that is produced?

~~same silent~~ Stop ! misread – stupid

h) A third mutation occurs which results in the substitution of the C/G base pair at position 42 (shown in bold italics) to a T/A base pair. How would this mutation affect the sequence of the protein that is produced?

same silent ✓

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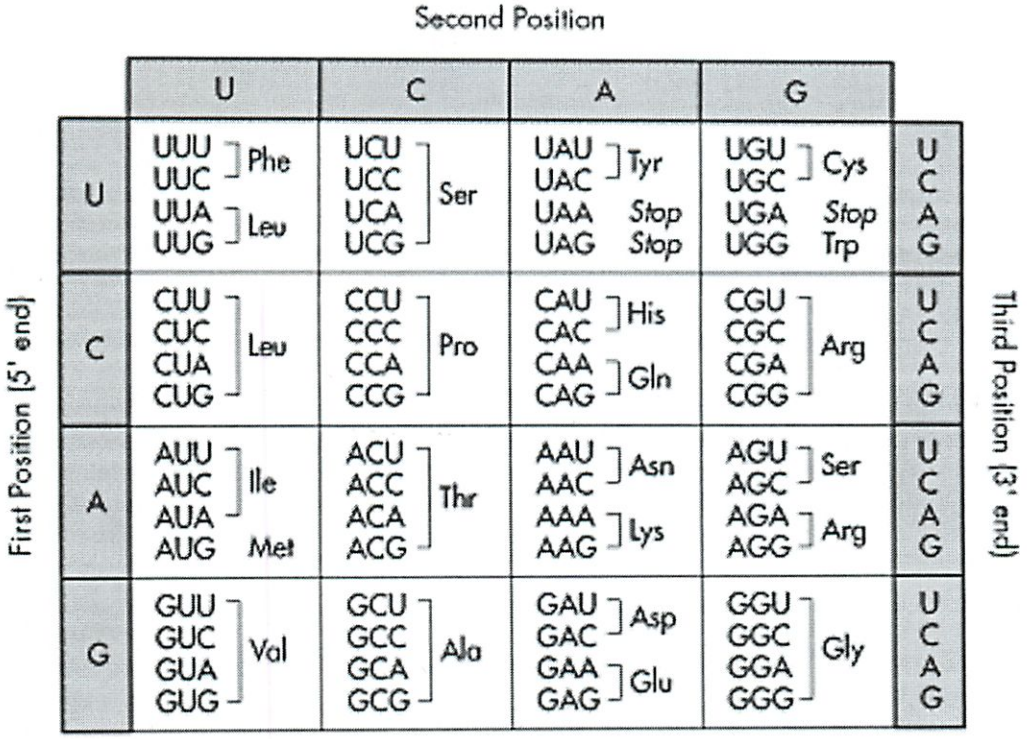
Each codon of an mRNA represents an amino acid or a stop codon as shown by the Codon Chart below.



template strand is seq that is copied

coding strand corresponds to codons that are translated into protiens

ATG is is on coding strand (AUG)

Missense point mutation
diff aminoassenid

nonsense "
but change to stop

Silent "
but no change

Jeff

12/18
11:20

OH
NH_2) polar

polar / H - bonds similar

hydrophobic / philic
↑
Don't
have
OH, NH_2
non polar (5)

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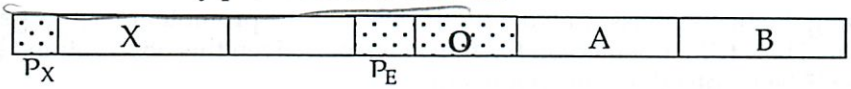
2012 7.012 Problem Set 4

Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 before 4:00 PM, Thursday October 25th.

Question 1

The following is a diagram of an inducible operon in *E. coli* and its regulatory region. Enzymes A and B are both required for the breakdown of the sugar maltose. The wild-type operon is regulated by protein X, which is continuously produced at low levels.



P_X	promoter for the regulatory protein
X	gene for the regulatory protein of the AB operon
P_E	promoter for the A and B genes
O	sequence shown to be important for transcriptional regulation by X
A	structural gene for enzyme A
B	structural gene for enzyme B

So repressor/inhibitor?

X binds to O

You have three different 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.

	without maltose		with maltose	
Cell	Amount of Enzyme A	Amount of Enzyme B	Amount of Enzyme A	Amount of Enzyme B
WT	low	low	high	high
m1	high	high	high	high
m2	low	low	low	low
m3	high	high	high	high

a) Given the data from the table, label the expression in each cell type as inducible, uninducible or constitutive.

always active

WT: inducible ✓ m1: Constitive ✓

m2: uninducable ✓ m3: " ✓

b) Based on the data shown above, does the regulatory protein X act as a repressor or an activator of the maltose operon? Explain your reasoning.

Clever. had not thought of

I can't tell

repressor. If activator would not be any loss of function mutations that caused constitutive mutations

c) A single loss-of-function mutation in which component(s) [P_X, X, P_E, O, A or B] could produce the phenotype seen in the m2 mutant? Why?

Promoter protein P_E, so less A,B even in presence of maltose

d) A single loss-of-function mutation in which component(s) [P_X, X, P_E, O, A or B] could produce the phenotype seen in m1 and m3. Explain.

inhibitor protein

Loss of function in P_X or X that prevents X1 from being expressed.

I wrote that - need to follow through

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Question 1, continued

Chromatin is a term used to describe a combination of DNA and protein. Chromatin functions to package DNA into a small volume, to prevent DNA damage, and to control gene expression. Proteins generally called histones are an important part of chromatin.

- Given that histones have an important role in chromatin, explain why they are usually basic proteins.

- When examining the histones associated with the DNA at the promoter of active genes, you find that histone H3 is trimethylated on the fourth lysine. Given this observation, describe how might histone H3 be involved in gene regulation.

Jeff Sal, Xho are same
Bam HI cuts ampicillin resistance

Question 2

A schematic of the vector p7012 is shown. The restriction enzymes listed cut only where indicated; they do not cut anywhere else in the vector or insert.

Nde I: 5′ CATATG / 3′ GTATAC

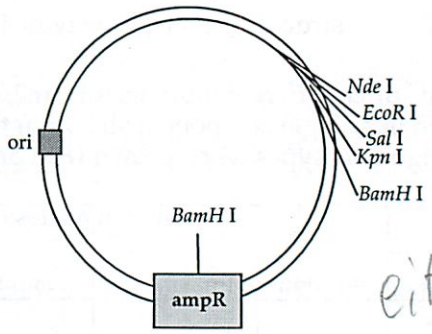
Sal I: 5′ GTCGAC / 3′ CAGCTG

EcoR I: 5′ GAATTC / 3′ CTTAAG

BamHI: 5′ GGATCC / 3′ CCTAGG

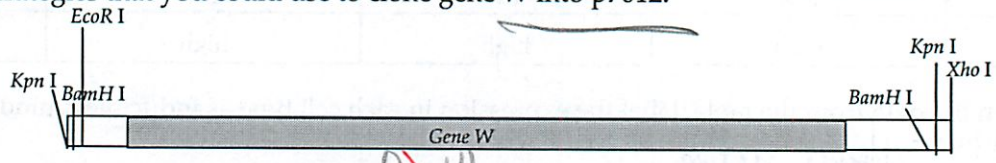
Kpn I: 5′ GGTACC / 3′ CCATGG

Xho I: 5′ CTCGAG / 3′ GAGCTC



either direction

a) A schematic of gene W is below. You want to clone all of gene W into the vector p7.012. There are three different strategies that you could use to clone gene W into p7012.



- Strategy 1 uses the restriction enzyme ___BamHI___ to cut the vector and restriction enzyme ___BamHI___ to cut Gene W.
- Strategy 2 uses the restriction enzyme(s) ___Eco___ *and* ___Sal___ to cut the vector and restriction enzyme(s) ___Eco___ *and* ___Xho___ to cut Gene W.
- Strategy 3 uses the restriction enzyme(s) ___Kpn___ *and* ___Eco___ to cut the vector and restriction enzyme(s) ___Kpn___ *and* ___Eco___ to cut Gene W.

Kpn I Kpn I Kpn I Kpn I

b) Which strategies would allow for directional cloning? (why only those?

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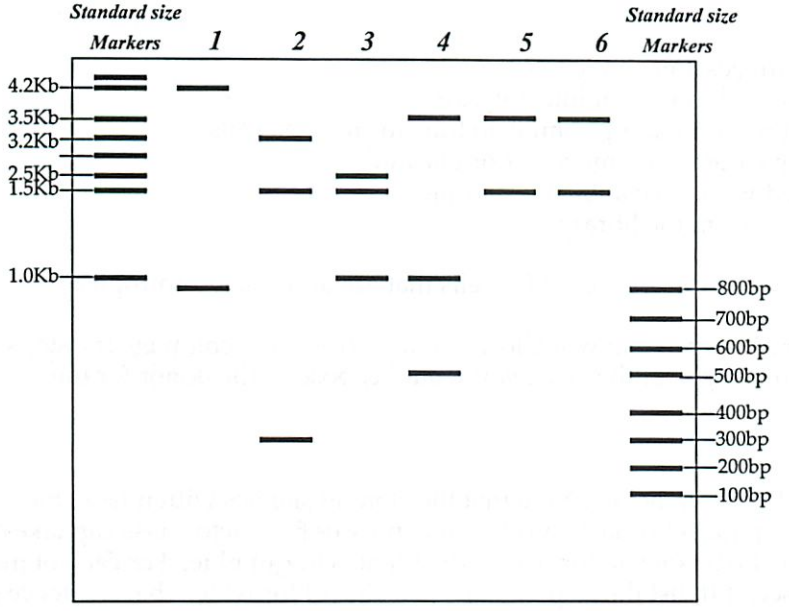
Question 2, continued

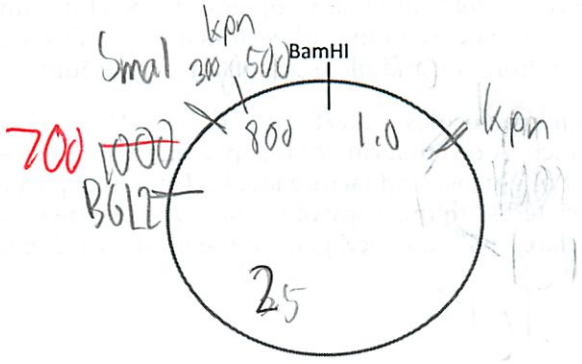
c) You are given the plasmid pSET. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis. Assume that all restriction digests were complete, i.e., each site for each restriction enzyme on each molecule of DNA was cut.



Kpn = 3.5, 1.5
Bgl = 2.5, 1.0, 1.5
Sma = 4.2, 800
Bam =

Lane	*Digest*	*Size of fragments in bp*
1	Bam*HI* and Sma*I*	4.2, 800 ✓
2	Sma*I* and Kpn*I*	2.2, 1.5, 300 ✓
3	Kpn*I* and Bgl*II*	2.5, 1.5, 1.00
4	Bam*HI* and Kpn*I*	3.5, 1.0, 500
5	Kpn*I*	3.5, 1.5
6	Bgl*II* and Bam*HI*	3.5, 1.5

- Fill in the table above, using the information from the agarose gel to determine the approximate sizes of the fragments produced in digests 1-6.
- Use your answers to determine the approximate size of pSET. pSET = 5k base pairs
- Use your answers to add the *Sma*I, *Kpn*I, *Bgl*II sites to plasmid map of pSET. On your map give the distances between each of the restriction sites.



Should have looked at class — submitted too early...

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Question 3

You have isolated two different yeast strains, strain 1 and strain 2. Each strain has a single mutation in a different gene such that neither strain 1 or strain 2 can grow in the absence of arginine. You want to clone the wild type copy of the gene or genes that are mutated in strain 1 and strain 2. To do so you plan to:

1) Obtain fragments of the entire yeast genomic DNA
2) Cut chosen vector and ligate each fragment into a vector
3) Use this pool of vectors and recombinant plasmids to transform *E. coli* cells
4) Select for *E. coli* cells that have obtained any vector or plasmid
5) Screen for *E. coli* transformed with a recombinant plasmid
6) Obtain recombinant plasmids from the library
7) transform yeast
8) Plate transformation mix ontp media and select for cells that are arginine prototrophs.

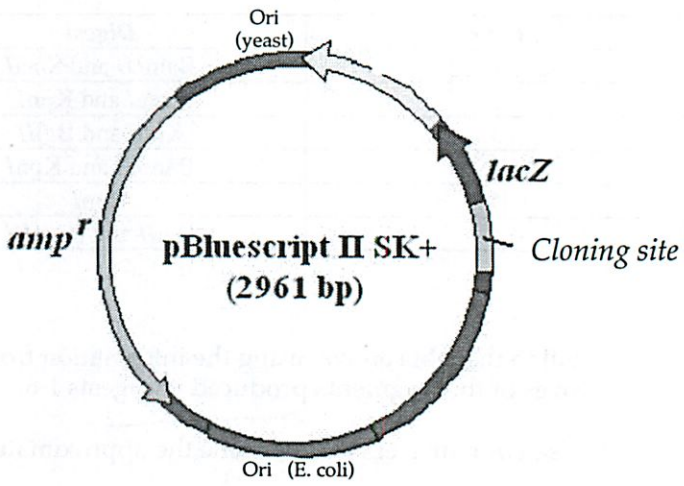
a) To construct a yeast genomic library in *E. coli* that will allow you to successfully complete the steps outlined above, what would be the **phenotype** of the yeast you would choose as the donor for the genomic DNA?

b) You choose the vector pBluescript II, shown below. Note that the cloning site lies within lacZ, the coding region of the gene that encodes β-galactosidase. A cell that expresses β-galactosidase can take a substrate called X-gal and cleave the β-1,6 linkage to form a product that is bright blue. For each of the following sequences found on pBluescript II, list the step or steps (1-8 above) for which that sequence is needed and explain the role that sequence plays.

Yeast ori:

Amp^r:

E. coli ori:



c) You digest both the yeast genomic DNA and many copies of the vector with the BamH1 restriction enzyme. You mix the genomic fragments with the cut vectors and add DNA ligase. You then transform *E. coli* cells with the ligation mix and plate on solid agar medium.

i) If one of the many vector molecules is NOT cut with BamH1, or religates without an insert, the lacZ gene remain intact. A cell that carries this plasmid will always express the lacZ gene at high levels, independent of glucose and lactose levels. Do you expect the promoter and regulatory regions associated with this copy of the lacZ gene is the same as the promoter and regulatory regions associated with the lacZ gene in the lac operon? Explain your thoughts.

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Question 3, continued

c) ii) Describe what medium you could use to distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant plasmid. Explain how this media would allow you to distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant plasmid.

d) You successfully create a yeast genomic library in E. coli cells, and obtain a pool that represents a complete set of recombinant plasmids from the library. Briefly describe how you would use this complete set of recombinant plasmids to clone by complementation the gene that can restore the yeast of strain 1 to arginine prototrophy.

e) Would it be possible to use the same library to clone by complementation the gene that can restore the yeast of strain 2 to arginine prototrophy? Explain.

f) You successfully identify a recombinant vector that restores yeast strain 1 to arginine prototrophy (clone 1). You are curious as to whether this gene can also rescue a bacterial cell that is arg– (i.e., it is also an arginine auxotroph). Give 2 reasons why clone 1 *may not* work to rescue the arg– bacterial cell.

g) Your friend suggests that you use her yeast cDNA library to attempt to restore an arg– bacterial cell to arginine prototrophy.

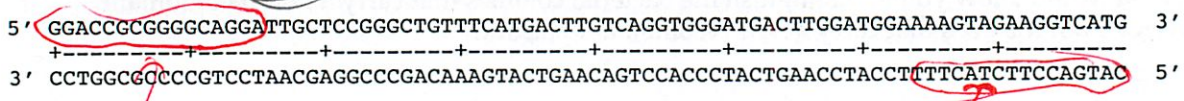
i) Briefly describe how a cDNA library is different from a genomic library.

ii) You transform arg– bacterial cells with your friend's yeast cDNA library and find a clone, clone 2, that restores the cells to arginine prototrophy. What sequence NOT found on pBluescript II would have been present on the vector that your friend used to create this library? Explain why this sequence is required.

Name_____ Section_____ TA_____

Question 4

a) Design primers, each 16 nucleotides long, which would allow you to amplify the 80 base pairs of sequence below using PCR. Label the 5′ and 3′ ends.



Primer 1:

Primer 2:

b) PCR consists of a series of 20-40 repeated temperature changes, called cycles. Each cycle of PCR involves three different steps.

- To begin, the reaction mixture is prepared. List the components that must be present in the reaction mixture for successful PCR to occur.

  Template DNA DNA primes dNTPs DNA polymerase

- In the first of the regular cycling events, the reaction is heated to 94–98 °C for 20–30 seconds. What occurs during this step?

  denatured – 2 strands of DNA broken apart

Even if don't know it – what must happen!

- The reaction temperature is then lowered to 50–65 °C for 20–40 seconds. What occurs during this step? When choosing the appropriate temperature for this step, what should you be considering?

  primes anneal single stranded template DNA

- The reaction temperature is then raised to a temperature of 68–80 °C. What occurs during this step? When choosing the appropriate temperature for this step, what should you be considering?

  DNA polymerase adds to

- If you started with a double stranded template molecule, at the completion of your PCR reaction, you will still have the original double stranded template molecule and many copies of the target DNA molecule. Will there be any other types of DNA molecules in your PCR tube. Explain.

  lots of other fragments – extend pass binding site

  did I get this one wrong earlier?

c) DNA sequencing using the Sanger method once required four different reaction mixes, but can now be carried out as single reaction.

- List the components needed for DNA sequencing using the Sanger method.

- Assume you are sequencing a single-stranded template that is 800 bp long, and your primer is 20 nucleotides long (i.e., your primer binds to nucleotides 1-20 of your template). How many different sized DNA molecules will you have when your successful sequencing reaction is complete?

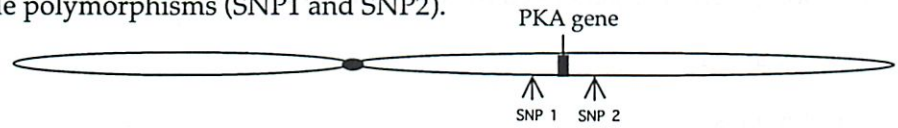
Name_____ Section_____ TA_____

2012 7.012 Problem Set 5

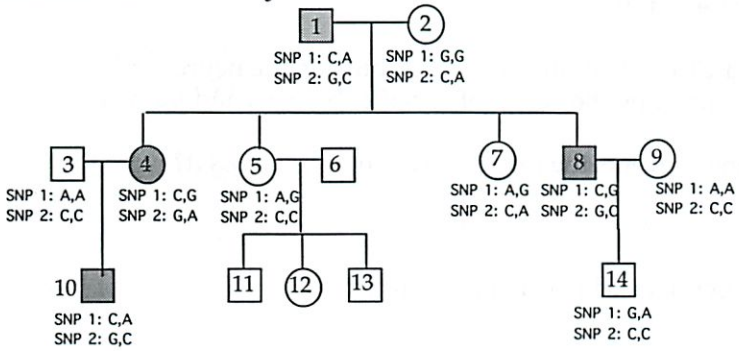
Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 by 4.00 PM, Thursday Nov 8th.

Question 1
A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single base pair in the genome differs among members of a species or paired chromosomes in an individual. By convention this base pair change is represented as one nucleotide — A, T, C, or G — of the base pair.

The chromosomal position of the PKA gene is diagrammed below, as is the location of two single nucleotide polymorphisms (SNP1 and SNP2).



The pedigree shows the inheritance of SNP1 and SNP2 associated with the PKA gene. The affected individuals are shaded. Also listed are the alleles of SNP 1 and 2 for some individuals. ***Note:*** *Assume individual 3 does not carry the disease allele.*



The two letters identify the alleles of the SNP that would be found on the "top" strand of each of the two homologous chromosomes. For example, "SNP 1: C,A" indicates that on one of the homologous chromosomes the top strand would contain a C (that is, the chromosome would have a C/G base pair in this position), while on the other chromosome the top strand would contain an A (that is, the chromosome would have an A/T base pair in this position).

a) What is the most likely **mode of inheritance** for this disease?

b) Individuals 5 and 6 have no affected children. What is the **genotype** for individual 6 at the PKA locus? *Note: Use the symbol 'A' or 'X^A' to represent the allele for the dominant phenotype and 'a' or 'X^a' for the allele for the recessive phenotype.*

c) Individuals 5 and 6 have no affected children. Can you predict the genotype for individual 6 at SNP1 and SNP2 loci (*Yes/ No*)? **Explain** why you selected this option.

d) Which allele (or alleles) of SNP 1 and SNP2 is linked to the mutant PKA gene in this family? ***Note:*** *Assume no recombination.*

SNP1: **SNP2:**

e) Briefly describe how you can use a SNP microarray to determine the SNP genotype of an Individual.

Name_____ Section_____ TA_____

Question 2

a) Circle **all** the correct options from below. The resting membrane potential of a neuron is determined by…

i. ions that can travel freely through channels in the resting neuron

ii. ions that require ATP to cross the resting membrane

iii. unequal distribution of different ions across the neuronal membrane

U All

b) Circle **all** that apply. An action potential is generated by the passage of ions through…

i. **only** the resting ion channels

ii. voltage-gated ion channels

iii. G-protein coupled receptors

iv. **only** the sodium potassium ATPase pump

✓

c) Under resting conditions the Na^+, Ca^{2+} and Cl^- concentrations are high outside the neuron, K^+ concentration is high inside and this is maintained by the action of specific channels and pumps.

i. What feature of the plasma membrane prevents the free diffusion of ions across it?

thick layer

ii. Is the resting membrane potential observed exclusively in a neuron?

iii. Complete the following table for the two channels/pumps that establish and maintain the resting membrane potential.

Channels/pumps	Ions passing through them	Default state (open/closed).	Is the ion transport active or passive? Explain.

(cells should do - not in the mood

* Don't forget about Na k pump

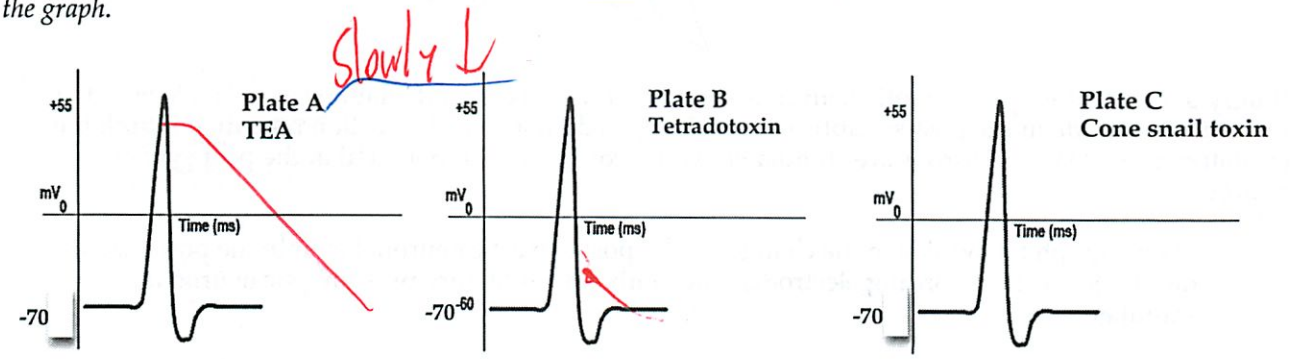
Name_____ Section_____ TA_____

Question 2 continued

d) Different neurotoxins are very often used to study neuronal function. You culture a GABA (an inhibitory neurotransmitter) secreting neuron in the presence of the following neurotoxins in three separate petri-plates (A-C)

- **A:** Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K^+ channels.**
- **B:** Neuron is treated with tetradotoxin, which **inhibits voltage gated Na^+ channels.**
- **C:** Neuron is treated with cone snail neurotoxin, which **inhibits voltage gated Ca^{++} channels.**

A normal action potential in a GABA secreting neuron that has been stimulated **in the absence of any neurotoxin** has been drawn in each panel below. Sketch the alteration in action potential following the treatment of the neuron with each neurotoxin. *Note: If there is no change please write "NO CHANGE" on the graph.*



e) Multiple sclerosis is an autoimmune disorder in which the immune system of the patient attacks and destroys the myelin sheath of a neuron. These patients show a very slow propagation of action potentials along the axons of their neurons. **Explain** why is this so.

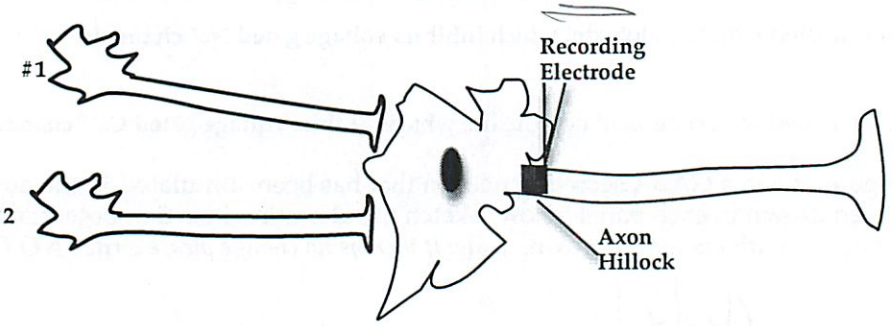
f) A functional neuron may receive both excitatory and inhibitory signals from multiple neurons at the synaptic junctions. In a post- synaptic neuron, where are the signals from all the pre- synaptic excitatory or inhibitory synapses integrated and the decision to fire an action potential made? **Circle** the correct option from the following choices. **Explain** why you selected this option.

Cell Body *Axon Hillock* *Myelin Sheath* *Synaptic Cleft*

Name_____ Section_____ TA_____

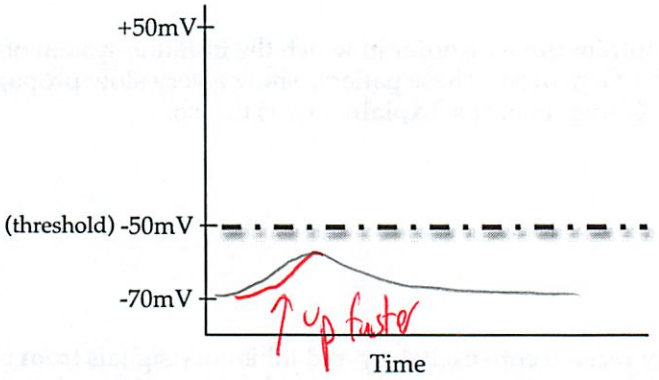
Question 2 continued

g) The following question refers to an experimental design depicted below. There are **two excitatory pre-synaptic neurons** that independently converge on a post-synaptic neuron. The two pre-synaptic neurons can be stimulated individually. In the absence of any stimulation, the recording electrode in the post-synaptic neuron measures the membrane potential as -70mV.

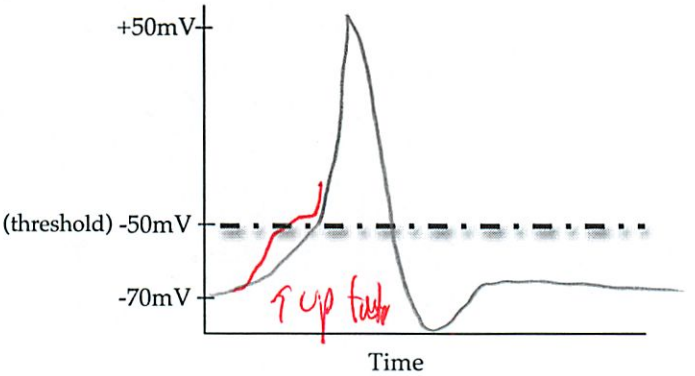


If **only one** excitatory pre-synaptic neuron is stimulated, you record a deviation from -70mV with the recording electrode in the post-synaptic neuron, but you do not record an action potential. If **both** the excitatory pre-synaptic neurons are stimulated, you record an action potential in the post-synaptic neuron.

i. On the graph below sketch the changes in the post-synaptic neuronal membrane potential, as measured by the recording electrode, when **only one** excitatory pre-synaptic neuron is stimulated.



ii. On the graph below sketch the changes in the post-synaptic neuronal membrane potential when **both** the pre-synaptic neurons are stimulated.



did I get that right?

Name_____ Section_____ TA_____

Question 3

Dopamine is one of the neurotransmitters in the mammalian brain that regulates mood, cognition and locomotion. Dopamine is commonly associated with the reward system of the brain. Dopamine can be an excitatory or inhibitory neurotransmitter, depending on the dopaminergic receptor that it binds to. It is derived from the amino acid tyrosine. At dopaminergic synapses, the released neurotransmitter is taken back into the pre-synaptic cell for re-use.

a) Beginning with the stimulation of the pre-synaptic neuron, list the steps, in order, that result in a release of neurotransmitter. Include any relevant channels, ions and molecules specific for this process.

b) Clozapine, an anti- psychotic medication interferes with the binding of dopamine to the receptor. You are studying an excitatory dopaminergic synapse where the post-synaptic cell has receptors for dopamine. If you treat these neurons with dopamine plus Clozapine, would the likelihood of generating an action potential in the post- synaptic neuron increase, decrease or not change relative to the treatment with dopamine alone? **Explain.**

c) The most extensively studied effect of cocaine on the central nervous system is the blockade of the the protein that binds to dopamine and pumps it out of the synaptic cleft back into the pre-synaptic neuron. What effect would cocaine have at an excitatory dopaminergic synapse?

d) Serotonin (5-hydroxytryptamine, 5-HT) is an excitatory neurotransmitter. It acts by binding to several HT receptor subtypes. The 5-HT3 receptor is a Na^+ channel whereas the 5-HT-2 receptor is a G-protein-coupled receptor, which leads to the opening of Ca^{2+} ion channels.

i. As the amount of serotonin is increased, **circle** the option that may change: *Amplitude of action potential/ frequency of action potential/ threshold potential.* Provide an **explanation** for the option that you have circled.

Name_____ Section_____ TA_____

Question 3 continued

ii. Complete the following table for each of the treatments. Assume that serotonin is present at these synapses. ***Note:*** *Consider each treatment independently.*

Treatment	**Action potential in the post-synaptic neuron is *more likely or less likely* to occur compared to untreated synapses? Explain your choice.**
Prozac, which inhibits the re-uptake of serotonin from the synapse	
Kentasarin blocks the binding of 5-HT3 receptor to 5-HT	

Question 4

a) The immune system is comprised of different cell types such as the *mast cells, macrophages, helper-T (T_H), cytotoxic-T (T_C), memory B and plasma B cells.* From the choices provided, list **all** the cell type(s) that would…

i. Participate in the **innate immune response.**

ii. Bind directly to an **antigen circulating** in the blood stream.

iii. Secrete large amount of antibody in response to an infection.

iv. Provide **protective immunity** against **second exposure** to the **same antigen.**

v. Show rearrangement of specific gene(s).

b) The diverse array of both TcR and antibodies is generated by DNA rearrangement. In addition to the DNA rearrangement, name the **three major processes** that contribute to the generation of the TcR and antibody diversity.

c) Circle **all** correct options from the following choices. The **innate immune response…**

i. Occurs **only** following the **first exposure to an antigen.**

ii. Occurs **only** following the **subsequent exposure the same antigen.**

iii. Occurs in response to **all infections.**

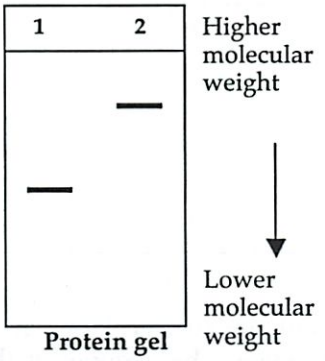
iv. Is **non-specific** unlike the adaptive immune response.

Name_____ Section_____ TA_____

Question 4 continued

d) You have purified a novel protein, which you call **Protein R.** You want to develop antibodies against this protein. You inject Protein R into a rabbit and after a month you draw some blood from this rabbit and determine that the rabbit's immune system has produced antibodies against Protein R. You wait for one month and then inject Protein R again into the same rabbit. You observe a stronger immune response with the second injection than you did in response to the first injection. Why is the primary immune response **slower** and **weaker** compared to the secondary immune response?

e) During the primary and secondary response to an antigen, the B cells of the immune system **produce membrane bound (i.e. IgM class)** and **secreted (i.e. IgG) antibodies.** You isolate the antibodies produced against Protein R, resolve them on a **protein gel** based on their molecular weight and obtain a profile as shown in the schematic below.



i. Which class of antibodies (*secreted IgG/ membrane bound IgM*) is present in lane 1 of the protein gel?

ii. Identify the B cell-type (*mature/ memory/ plasma cells*) that is responsible for producing antibodies shown in…

- Lane 1 of the protein gel.
- Lane 2 of the protein gel.

iii. If you compare the structure of the IgM and IgG antibodies that are produced against Protein R…

- would you expect these antibodies to have the **same or different** <u>variable</u> regions? **Circle** the correct option and **explain** why you selected this option.

- would you expect these antibodies to have the **same or different** <u>constant</u> regions? **Circle** the correct option and **explain** why you selected this option.

Name_____ Section_____ TA_____

Question 4 continued

f) Complete the table for the following cell types.

Cell types	**Cell-surface proteins participating in the cell-cell interactions** ***(CD4/ CD8/MHC-I/ MHC-II/TcR/antibody)***	**Briefly describe their role in the humoral immune response**
T_H cells		
Antigen presenting cells (APC)		
Macrophages		

g) All somatic cells types in our body are said to have the same set of genes although each cell type may express a unique set of genes that provides it with unique functions. However, the genome of the mature T and the B cells is slightly different compared to the genome of the remaining cells in an individual. **Explain** why this may be so.

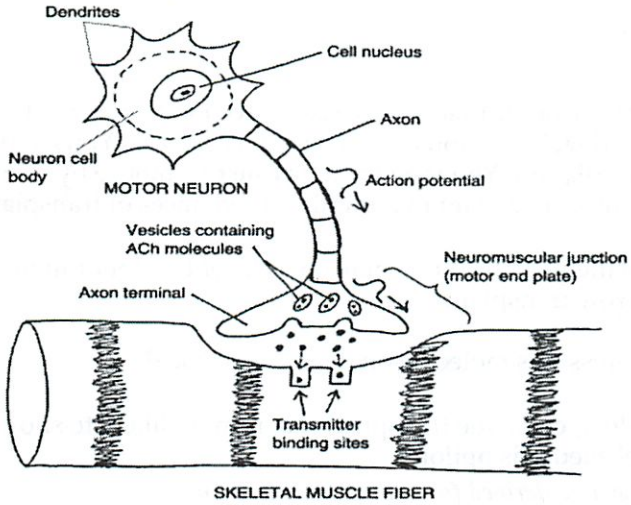
Name_____ Section_____ TA_____

2012 7.012 Problem Set 6

Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 by 4.00 PM, Wednesday Nov 21st. .

Question 1

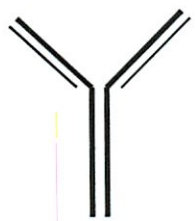
The following is a schematic of an excitatory neuromuscular junction. In this schematic, the axon is pre-synaptic and the muscle is post-synaptic. The excitatory neurotransmitter is acetylcholine (ACh). ACh binds to acetylcholine receptors (AChR), which act as **ligand-gated Na^+ channels**. An influx of Na^+ through the ligand-gated channels results in muscle contraction. Shortly after its release, the neurotransmitter is degraded by the acetylcholinesterase enzyme.



Myasthenia Gravis, is an autoimmune disease in which the immune system of the patient produces antibodies that bind to the patient's own AChR. These antibodies can either degrade the AChR or prevent the binding of ACh to AChR.

a) Autoimmune diseases, like Myasthenia Gravis, are a result of self-reacting T *and/or* B cells. Briefly describe how the self-reacting T or B cells are eliminated during the development of immune system in normal individuals.

b) The following is a schematic of an antibody molecule.



i. **Circle** the region of the antibody that interacts with the AChR.

ii. **Box** the region of the antibody that mediates the degradation of AChR.

c) You decide to use an inhibitor of the acetylcholinesterase enzyme to treat the patients suffering from Myasthenia Gravis. **Explain** how this may help alleviate some symptoms of this disease.

Name_____ Section_____ TA_____

Question 1 continued

d) You decide to make a monoclonal antibody against the AChR.

i. Briefly describe the major steps that you would follow to make this antibody in a mouse.

ii. In terms of binding to the same protein antigen, how would a population of monoclonal antibody against AChR differ from the polyclonal antibodies raised against the same antigen?

e) You are studying a genetic disorder that shows a recessive mode of inheritance and the affected individuals lack B cells. You develop a mouse model for this disease. You destroy the bone marrow of an affected mouse by UV irradiation. You then try to rescue this mouse by doing a bone marrow transplant from a donor mouse. You want to minimize the chances of transplant rejection.

i. Which surface molecule(s) on the bone marrow cells of the donor mouse are critical for the success of bone marrow transplant?

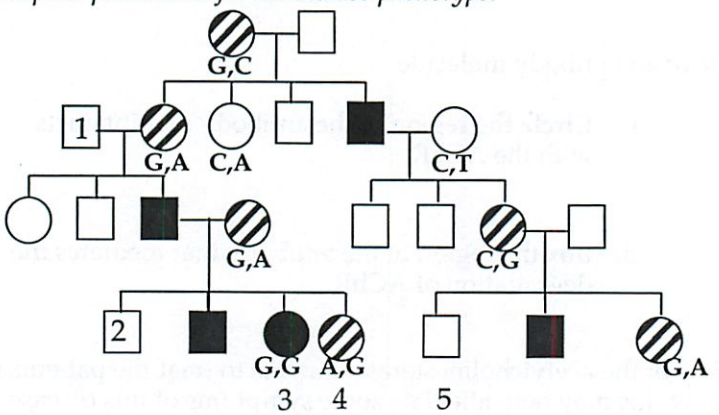
ii. Which cell types express this molecule on their cell surface?

iii. From the choices below, circle the transplant that is more likely to show a higher success rate. **Explain** why you selected this option.

- *Allogenic i. e. bone marrow derived from the patient's sibling.*
- *Syngenic i.e. bone marrow derived from the monozygotic twin of the patient.*

Question 2

Below is the pedigree of a family with a disease that is related to a mutation in Gene D. All the individuals that show the disease phenotype are shaded and the carriers are striped. Also listed are the alleles of a SNP *(A, G, T, C)* for some individuals. ***Note:*** *You may assume that this SNP is tightly linked to Gene D. Assume complete penetrance for the disease phenotype.*



Name_____ Section_____ TA_____

Question 2 continued

a) What is the **most likely** mode of inheritance of this disease?

b) Identify the allele of the SNP that is tightly linked with the disease allele. [ ]

c) What is the genotype at the Gene D locus of Individual 2? ***Note:*** *Use the letter "D" or X^D to represent the allele assocaited with the dominant phenotype and 'd" or X^d to represent the allele associated with the recessive phenotype.*

Genotype of Individual 2: [ ]

d) Individual 4 in this pedigree marries individual 5. They have a son and a daughter.

i. Give **all** of the possible genotypes of the son at the **Gene D locus.**

ii. Give **all** of the possible **SNP genotypes** of the son.

iii. Give **all** of the **possible genotypes** of the daughter at the **Gene D locus.**

iv. Give **all** of the **possible SNP genotypes** of the daughter.

Question 3

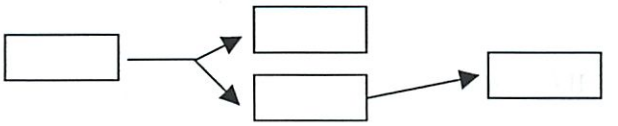
Stem cells are found in all multi-cellular organisms. They can undergo mitotic cell division to form cell types that can differentiate into diverse specialized cells. Stem cells are believed to have immense therapeutic potential.

a) A stem cell is known to divide asymmetrically. When a stem cell divides asymmetrically, what are the two possible fates of its daughter cells?

b) Four human embryonic cell types, originally prepared from the **SAME embryo,** were tested for their potency **in vitro**. Based on the data below, complete the table by ranking the potency of these cell types.

Cell types	Cell types differentiated in vitro	Potency from 1-4 (1=most potent and 4= least potent).
A	motor	
B	motor, sensory, lateral, hippocampal	
C	sensory, lateral, hippocampal	
D	motor, sensory	

c) Draw a lineage tree for the cell types A-D using the information in the table above.



Name_____ Section_____ TA_____

Question 3 continued

d) Do each of these cell types have the same DNA (*Yes/ No*)? **Explain.**

e) Induced pluripotent stem (iPS) cells hold great promise since they have the potential to differentiate into multiple cell types.

i. Which cell types do you start with while making iPS cells?

ii. If you would like to generate new kidney tissue for a patient would you start with iPS cells or the commercially available embryonic cells? Provide a brief explanation for the choice that you made.

f) Stem cells exist in most organs including bone marrow. Describe **one** experiment to prove that stem cells exist in the bone marrow.

Question 4

a) Spermatogonia are cells produced in the testes, which can be isolated from adult mice. When injected into an early embryo, spermatogonia survive and their descendent cells can be found in diverse organs. However, spermatogonia injected into an adult survive only if injected into the testes, these spermatogonia become only spermatozoa. Why do identical spermatogonia seem to have different potency when injected into an embryo rather than an adult?

b) Describe the differences, in terms of procedure and of result, between "reproductive" and "therapeutic" cloning.

c) Organismal cloning proves that the nucleus of an adult cell contains all of the genetic material necessary to generate every cell type in an organism. Could you create a mouse by organismal cloning if the adult cell you began with was a…

i. **mature B cell?** If yes, then predict what the phenotype of the organism would be as it develops from a newborn to an adult mouse. If no, explain why not.

ii. **gut epithelial cell from the intestinal lining?** If yes, then predict what the phenotype of the organism would be as it develops from a newborn to an adult mouse. If no, explain why not.

iii. **mature enucletated red blood cell** *(yes/ no)?*

Name_____ Section_____ TA_____

Question 5
Familial hypercholesterolemia (FH) is a genetic disorder where individuals heterozygous for the disease allele show a decreased expression of LDL receptors (LDL-R) and individuals homozygous for the disease allele do not express LDL-receptors.

a) Based on what you have learned from Prof. Lander's lecture, briefly explain how a decrease in LDL-R expression results in an increased blood cholesterol level.

b) Give an experiment to prove that the liver cells isolated from an FH patients show a loss-of-funtion mutation in the LDL-R gene.

c) The treatment regimen for individuals heterozygous for the disease allele with high levels of cholesterol includes a low cholesterol diet, treatment with bile resins and inhibitor of HMG CoA reductase. Briefly explain…

i. why the dietary restriction can help reduce cholesterol **only** by 10% and not more.

ii. why inhibitors of HMG CoA reductase are **most efficient** in reducing cholesterol.

d) You isolate the embryonic cells from a mouse homozygous for the disease allele of the LDL-R gene (-/-) and infect them with a viral vector that has a wild type allele of LDL-R. You then select cells that now have wild- type copy of LDL-R and re-introduce them back into the developing (-/-) embryos. These transgenic embryos are transplanted into a female mouse to obtain newborns. You then trace the location and expression of the LDL-R gene in the newborn mice by adding a blue color dye that specifically binds to the LDL-R. ***Note:*** *You may assume that the level of expression of the LDL-R gene correlates with the intensity of the blue color in the cells. In wild-type mice, the dye stains only the liver cells.*

When you add the dye you find that most of cells in the newborn mice, including the liver cells, turn blue. Based on these results, you modify the virus vector containing LDL-R gene, reinsert it into the embryonic cells and obtain newborns by following the same steps that were described above. When you add the dye you find that only the liver cells turn blue and the color is of the same intensity as in the liver cells of wild-type mice. In addition, these mice do not show the manifestations of the disease.

What modification could be made to the viral vector such that the introduced LDL-R gene was only expressed in the liver cells of newborns instead of being expressed in all the cells?

Name_____ Section_____ TA_____

Question 6
You are studying coat color in mice. You isolate the cells from a developing embryo (at the blastula stage/8-cell stage) that is produced by the fusion of gametes from mice that have white coat color (genotype = aa) which is recessive to black coat color. You then re-introduce them into the developing embryo that is produced by the fusion of gametes from mice that have black coat color to obtain newborns.

a) Give **all** the possible genotypes of the cells in the newborn obtained from this strategy? Briefly **explain** why you selected this genotype. *Note: Use the uppercase A to represent the allele responsible for the dominant phenotype and lowercase a to represent the allele responsible for the recessive phenotype.*

b) You allow the mouse obtained from the strategy outlined above to mate with a female mouse that has the black coat color (genotype: AA). Do you expect all the mice from this mating experiment to have a black coat color (*Yes/ No*)? **Explain** why you selected this option.

Question 7 (This questions is optional and will NOT be graded)
Acute myeloid leukemia (AML), is a cancer of the white blood cells, characterized by the rapid proliferation of abnormal cells which accumulate in the bone marrow and interfere with the production of normal blood cells. Acute lymphoblastic leukemia (ALL), is a different form of leukemia, though the two leukemias can be difficult to distinguish clinically.

In this problem, you will learn how to use microarray data to judge which type of tumor a patient has. (This method of diagnosis is currently one of the most cutting-edge ways to diagnose a patient with a specific type of cancer.) You gather about 20 patients with ALL and 20 patients with AML. You take tumor samples from these patients and extract mRNA samples from the tumors. You allow the mRNAs from the tumors to hybridize to DNA chips, on which each spot contains a probe for a different human gene. What you find is that some genes are expressed at very high or very low levels in ALL tumors as compared to AML tumors. Other genes are expressed at very high or very low levels in AML tumors as compared to ALL tumors. This means that each type of tumor can be assigned a signature pattern of expression of genes, and then unknown tumors can be diagnosed by examining their signature patterns. *Go to http://mit.edu/star/biogene/docs/. Click on the "Instructions for Problem Set" link, and print out those instructions. Then return the Star-Biogene home and begin by clicking the "Start Biogene" button. Use the instruction sheet to view the original data used in the following exercises.*

a) What do genes shown in green indicate?

b) What do genes shown in orange indicate?

c) What are the four genes that show the best correlation with presence of ALL? (List them by their descriptions.)

Name_____ Section_____ TA_____

Question 7 continued

d) What are the four genes that show the best correlation with presence of AML? (List them by their descriptions.)

e) Is the gene session pattern of **U18271_cds3_s_at** strongly correlated with the presence of AML or ALL? *(see "how to answer question e)" on page 2 of the instruction sheet)*

f) What is the name of the gene product encoded by **U18271_cds3_s_at?** *(see "how to answer question f) and g)" on page 3 of the instruction sheet)*

g) What chromosome arm and position does the gene **U18271_cds3_s_at** occupy? *(see "how to answer question f) and g)" on page 3 of the instruction sheet)*

h) Look in the list of gene descriptions for "terminal transferase." With respect to only the expression of **terminal transferase mRNA**, which tumor sample behaves the least like the other tumors in its class? *(see "how to answer question h)" on page 4 of the instruction sheet)*

i) Which AML sample is least like the other AML samples? *(see "how to answer questions i) and j)" on page 4 of the instruction sheet).*

j) A patient comes in with a tumor. After taking samples of the patient's tumor and running the appropriate tests you find that the tumor had high levels of expression of Nek3, and low levels of LPAP and CAMK4. Given this information and the clustering data, which type of tumor would you most likely diagnose it as? *(see "how to answer question k)" on page 4 of the instruction sheet).*

Name_____ Section_____ TA_____

2012 7.012 Problem Set 7

Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 by 4.00 PM, Thursday Dec 6th.

Question 1

You are studying the following four different viruses.

- Type A is an **enveloped, minus stranded** RNA virus.
- Type B is an **enveloped, plus stranded** RNA virus (no viral proteins are packaged in the virion).
- Type C is an **enveloped, plus stranded retrovirus**, reverse transcriptase is packaged in the virion
- Type D is a **non-enveloped double stranded** DNA virus.

a) Which of these viruses (*Type A/ B/ C/ D*) is likely to have the **lowest mutation rate? Explain** why you selected this option.

b) You analyze the genome of each virus and are surprised to find that each has 33% adenine (A) in its genome.

i. Based on this information, you can predict the % of remaining bases (*T/ G/ C/U*) in the genome for which virus(es)?

ii. In the table below, give the percentage of each appropriate base (*T/ G/ C/ U*) found in the genome of the virus(es) you selected in part (i).

VirusType	*Base*	*A*	*T*	*G*	*C*	*U*
	% in the viral genome					

c) You successfully transduce a eukaryotic cell line with each of the above viruses in four separate plates. You isolate the viruses from the infected cells in each plate and use them to infect fresh eukaryotic cells that are being incubated with **actinomycin D** (*inhibits transcription by blocking **only** the **host** RNA polymerase)* or **anisomycin** (*host ribosome inhibitor*). Complete the following table for each of the treatments.

Treatment	**Virus**	**Virus formed (*Yes/No*)?**
Actinomycin D	Type A	
	Type B	
	Type C	
	Type D	
Treatment	**Virus**	**Virus formed (*Yes/ No*)?**
Anisomycin	Type A	
	Type B	
	Type C	
	Type D	

d) Which of the above virus(s) (*Type A/ B/ C/ D*) **must integrate** its genome in the host cell? Give all possible options and explain why you selected each.

Name_____ Section_____ TA_____

Question 2

The discovery that cancer could be caused by a virus was a major one. However, the subsequent discovery that Rous Sarcoma Virus (RSV), a cancer-causing virus discovered in chickens, encoded a mutant form (v-src) of a normal cellular gene (c-src) was even more surprising. Rous sarcoma virus (RSV) is a retrovirus that also has a + stranded RNA genome that encodes four genes; gag (encodes the capsid protein), pol (encodes the reverse transcriptase), env (encodes the envelope glycoprotein) and src (encodes a tyrosine kinase enzyme).

a) Given the information, reverse transcriptase is considered which of the following?

- A DNA directed RNA polymerase
- A RNA directed DNA polymerase
- A RNA directed RNA polymerase

b) Why is it essential that the RSV encodes Reverse transcriptase?

c) What are two major classes of genes involved in the development of cancer? For each, describe the type of mutation that is associated with cancer, and how this mutation would promote tumor formation.

d) The Human papilloma virus (HPV) has been implicated as a risk factor for cervical cancer. The E7 protein of HPV binds to pRB protein preventing it from binding to the host transcription factor E2F, which is now free to bind to the promoters of genes that promote cell cycle. In contrast, another HPV protein, namely E6 binds to p53 targeting it for destruction by proteosomes thus removing the block on the host cell's entry into the cell cycle.

i. Would you classify E7 as an oncogene or a tumor suppressor gene? **Explain** why?

ii. Would you classify E6 as an oncogene or a tumor suppressor gene? **Explain** why?

Name_____ Section_____ TA_____

Question 2 continued

e) Each of the five genes given below, when mutated, can result in a transformed phenotype in the mutant cells. In the final column, give the phenotype (*normal or transformed*) of a diploid cell that has the two alleles given. ***Note:*** *A description of each gene is given.*

ras: encodes a protein, which is active in its GTP bound form and inactive in its GDP bound form. When active it promotes cell division.
cyclin D: encodes a protein that interacts with a CDK (cyclin dependent kinase), and promotes cell division.
erb-B2: encodes an epidermal growth factor receptor which is active when dimerized. It promotes cell division when activated.
p16: encodes a protein that inhibits cyclin-dependent kinase.
WT1: encodes a protein that inhibits progression through the cell cycle.

Gene	Class	Status of allele 1	Status of allele 2	Phenotype
ras	Proto-oncogene	Mutation such that protein cannot hydrolyze GTP to GDP	Wild-type	
Cyclin D	Proto-oncogene	Mutation that results in deletion of entire gene	Wild-type	
erb-B2	Proto-oncogene	Mutation such that the receptor protein constitutively dimerizes	Mutation that results in the deletion of 120 base pairs in intron 5	
p16	Tumor suppressor	Point mutation that results in truncated protein of 20 amino acids	Wild-type	
WT1	Tumor suppressor	Mutation in promoter that prevents RNA polymerase from binding	Mutation that results in the deletion of 4 base pairs in the coding region 20 base pairs after the start codon	

Question 3

Cancer is caused by the accumulation of two or more mutations in the same cell that affects its proliferation and survival.

a) Why does a person's chance of having cancer increase with age?

b) Cell lines are often used to test the oncogenic potential of viruses. If cancer is a multi-step process, why can the introduction of a single active viral oncogene transform these cells?

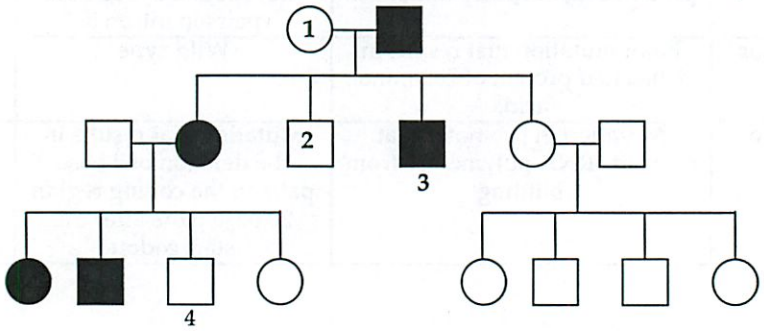
Name_____ Section_____ TA_____

Question 3 continued

d) Briefly describe what an Ames test is and how it may be used to evaluate the mutagenic potential of a chemical agent. Do you think you can evaluate the mutagenic potential of any carcinogen using Ames test (*Yes/ No*)? Justify your answer.

Question 4

The following human pedigree shows the mode of inheritance of the predisposition to colon cancer.
Please note: *The shaded squares and circles represent the individuals who eventually develop cancer.*



a) Looking only at the pedigree, the predisposition to this disease appears to have what mode of inheritance?

b) If you check the genotype of the tumor cells from individual 3, you find that they are homozygous for the disease allele (-/-). However, if you check the genotype of the blood cells from this individual, you find that they are heterozygous for the disease allele (+/-). **Explain** why the genotype with respect to the disease allele in the blood cells is different from that in the tumor cells isolated from individual 3.

c) For individual 4, the blood samples are heterozygous, carrying both the wild-type allele and a mutant allele of the gene associated with this type of cancer. However, this individual **did not develop** cancer. **Explain** why.

d) One example of a tumor suppressor gene is the Retinoblastoma (Rb) gene . The wild-type pRB protein binds and inhibits the activity of the transcription factor E2F. At the appropriate time in the cell cycle, pRB is phosphorylated (inactive state) and E2F becomes available to act as a transcription factor that stimulates cell division. If a cell has lost the functional pRB and E2F proteins, would you expect cell division (*Yes/ No*)? **Explain** your choice.

Name_____ Section_____ TA_____

Question 5

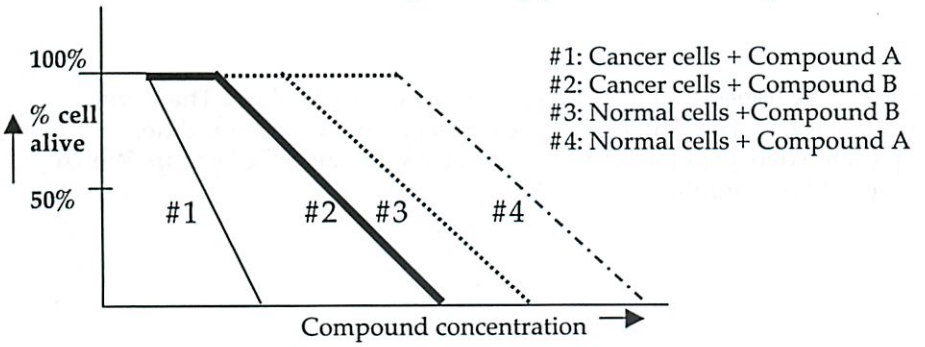
a) Radiation therapy can be used to treat tumors. Briefly **explain** how radiation therapy works to treat a tumor.

b) Chemotherapeutic drugs often have side effects such as diarrhea, constipation, mouth sores, hair loss, nausea, and blood-related side effects.

i. Chemotherapeutic drugs have a wide range of structures and functions, yet many elicit the same side effects. **Explain** why the side effects are the same for a variety of different drugs.

ii. Describe what is meant by the "therapeutic window" of a drug used in chemotherapy, and how it relates to the side effects seen in a patient.

iii. Prior to being used for treatment, each chemotherapeutic drug is extensively screened. During drug screening you identify two compounds A and B that have the potential to kill cancer cells and normal cells as shown by the following graph. Which compound (*A/B*) is a better candidate for cancer treatment? **Explain** why you selected this option.



iv. **Explain** how the use of following drugs may prevent cancer cell growth and /or cell proliferation.

Drug	Target of drug	How is cancer cell growth and / or proliferation prevented?
Vincristine	Microtubule inhibitor	
VEGF inhibitor	Inhibits blood vessel formation	

c) Her-2 receptor is encoded by the Her-2 proto-oncogene and is a member of the epidermal growth factor (EGF) family of receptor tyrosine kinases. Her-2 gene amplification is correlated with aggressive forms of breast cancer that respond better to treatment with herceptin than other non-aggressive forms of breast cancer. **Explain** why this is so.

Name_____ Section_____ TA_____

Question 6 (This question is optional and will NOT be graded)
Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.

a) HIV specifically infects the T- helper (T_H) cells of the human immune system. If HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely interacting proteins during HIV infection?

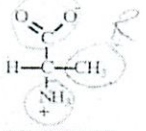
b) Why the HIV infected cells remain undetected by the host immune system for several years?

c) Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV?

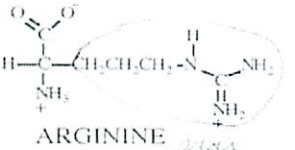
d) In recent years, therapies have been developed to fight AIDS using nucleotide analogs. The drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine. However, in AZT the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT?

TA 10/9 Name or initials MEP

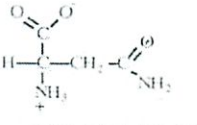
STRUCTURES OF AMINO ACIDS at pH 7.0



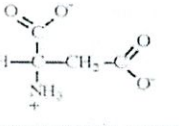
ALANINE
(ala) NP



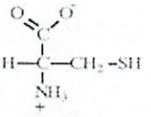
ARGININE
(arg) polar p+



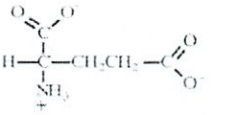
ASPARAGINE
(asn) polar



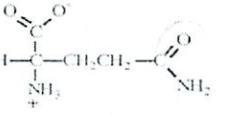
ASPARTIC ACID
(asp) polar −



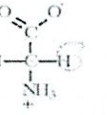
CYSTEINE
(cys) NP



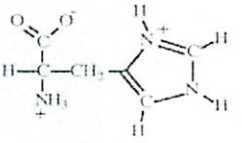
GLUTAMIC ACID
(glu) p −



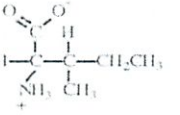
GLUTAMINE
(gln) polar



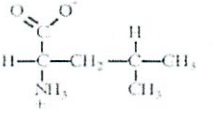
GLYCINE
(gly) NP



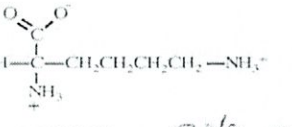
HISTIDINE
(his) polar +



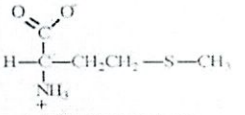
ISOLEUCINE
(ile) NP



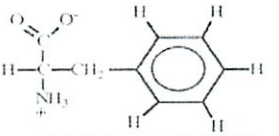
LEUCINE
(leu) NP



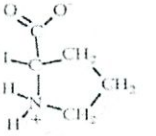
LYSINE
(lys) polar +



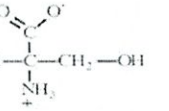
METHIONINE
(met) NP



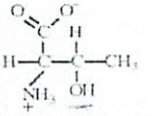
PHENYLALANINE
(phe) NP



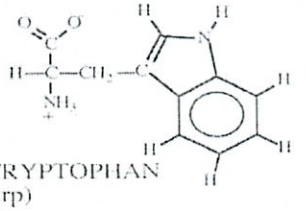
PROLINE NP
(pro)



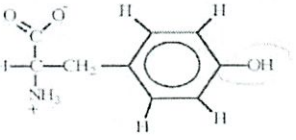
SERINE
(ser) polar



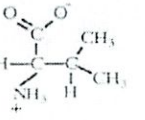
THREONINE
(thr) polar



TRYPTOPHAN
(trp) NP



TYROSINE
(tyr) semi polar



VALINE
(val) NP

? polar uncharged

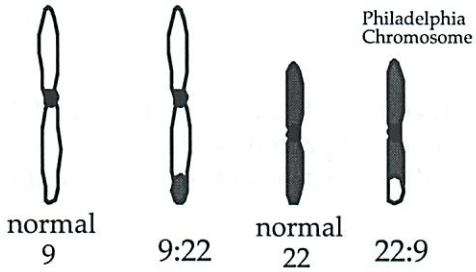
Yahoo Answers

Alanine: Neutral non-polar
Arginine: Basic polar
Asparagine: Neutral non-polar ← Disagrees w/ TA
Aspartic Acid: Acidic polar
Cysteine: Neutral Slightly polar
Glutamic Acid: Acidic polar
Glutamine: Neutral polar
Glycine: Neutral non-polar
Histidine: Basic polar
Isoleucine: Neutral non-polar
Leucine: Neutral non-polar
Lysine: basic polar
Methionine: Neutral non-polar
Phenyl-alanine: Neutral non-polar
Proline: Neutral non-polar
Serine: Neutral polar
Threonine: Neutral polari
Tryptophan: Neutral, slightly polar
Tryosine: Neutral polar
Valine: Neutral non-polar

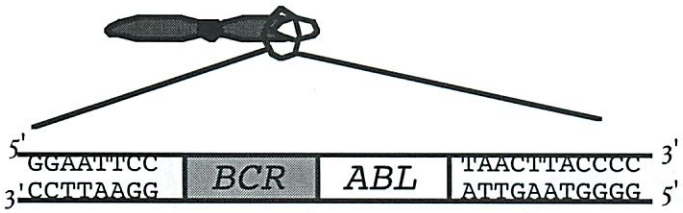
Practice Final

Question 1

The Philadelphia chromosome, results from a translocation event where pieces of chromosomes 9 and 22 switch. No DNA is lost, it is just rearranged. A schematic representing the chromosomes involved in this translocation is shown below. Please note that each of the chromosomes as drawn represents a single double-stranded DNA molecule.



Chronic Myeloid Leukemia (CML) is the cancer associated with the Philadelphia chromosome. The Philadelphia chromosome translocation creates a novel gene by attaching a portion of the coding region of the *ABL* gene (normally found on chromosome 9) to a portion of the *BCR* gene (normally found on chromosome 22). This fusion gene can be transcribed and translated to create a novel protein, the Bcr-Abl protein



You want to design PCR primers to quickly determine if a cell is carrying the Philadelphia chromosome.

a) Circle the best pair of primers for amplification of the Philadelphia chromosome.

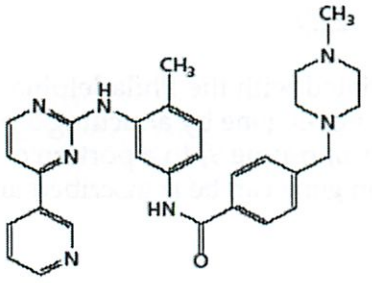
- 5′ CCTTAAGG 3′ and 5′ ATTGAAT 3′
- 5′ GGAATTCC 3′ and 5′ TAACTTA 3′
- 5′ TAAGTTA 3′ and 5′ GGAATTCC 3′
- 5′ ATTGAAT 3′ and 5′ CCTTAAGG 3′

b) Would you categorize the gene encoding the Bcr-Abl protein as a tumor suppressor gene, an oncogene or a proto-oncogene? Explain.

Question 1, continued

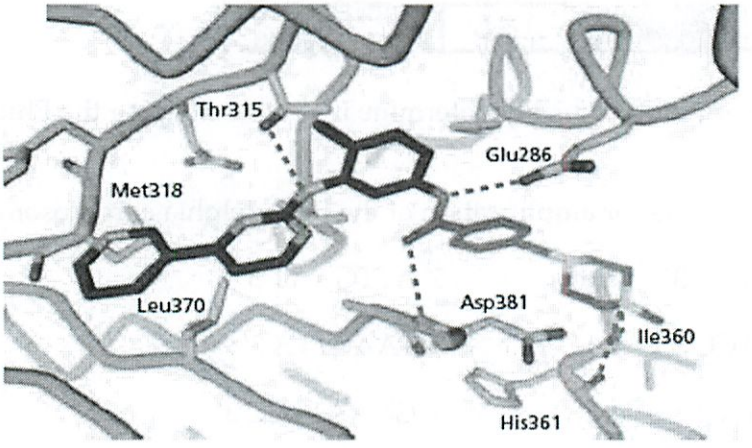
c) The Bcr-Abl protein functions as a tyrosine kinase. Many drugs that effectively inhibit tyrosine kinases are known. Most of these drugs are not useful therapeutics against CML due to a variety of side effects. Explain why there are so many side effects associated with these drugs.

d) A drug, Gleevec, has been introduced as a treatment for CML. Gleevec is a small molecule that fits into the catalytic site of the Bcr-Abl protein and prevents enzyme function. A drawing of Gleevec interacting with the Bcr-Abl kinase is shown below. For this question, the pH = 7.0.



Gleevec®
(imatinib mesylate)

A table of the amino acids can be found on the last page of this exam.



i) What is the strongest type of interaction that occurs between **Thr 315** of the Bcr-Abl enzyme and Gleevec as indicated by the dashed line in the diagram above?

ii) What is the strongest type of interaction that occurs between **Met 318** of the Bcr-Abl enzyme and Gleevec as diagramed above?

iii) What is the strongest type of interaction that occurs between **Glu 286** of the Bcr-Abl enzyme and Gleevec as indicated by the dashed line in the diagram above?

Question 1, continued

e) There are two variants of the Bcr-Abl protein such that …

- the Bcr-Abl enzyme has **Ala** at 315 instead of **Thr.** Individuals with this substitution have CML that is resistant to treatment with Gleevec.
- the Bcr-Abl enzyme has **Trp** at 315 instead of **Thr.** Individuals with this substitution also have CML that is resistant to treatment with Gleevec.

i) What type of interaction could now occur between **Ala 315** of the Bcr-Abl enzyme and Gleevec?

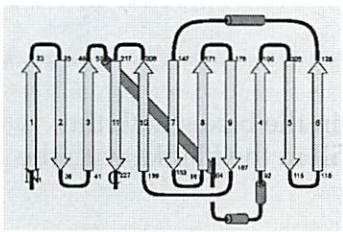
ii) What type of interaction could now occur between **Trp 315** of the Bcr-Abl enzyme and Gleevec?

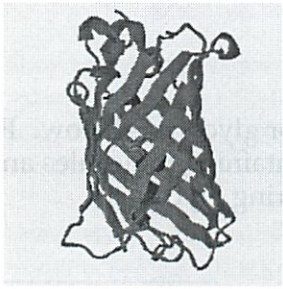
f) The CML of individuals with the Bcr-Abl enzyme that has **Ala** at 315 instead of **Thr** will respond to Gleevec, but at much, much higher doses. However, the CML of individuals with the Bcr-abl enzyme that has **Trp** at 315 instead of **Thr** is impervious to Gleevec at any concentration. Explain the difference in the resistance to Gleevec of each of these two variants.

Question 2

a) Each of the three diagrams below represent the GFP protein.

i) Label each diagram with the level of protein structure it portrays (primary, secondary, tertiary, quaternary).





MSKGEELFTGVVPVLVELDGDVNGQ
KFSVSGEGEGDATYGKLTLNFICTTG
KLPVPWPTLVTTFSYGVQCFSRYPD
HMKQHDFFKSAMPEGYVQERTIFYK
DDGNYKTRAEVKFEGDTLVNRIELK
GIDFKEDGNILGHKMEYNYNSHNVY
IMGDKPKNGIKVNFKIRHNIKDGSVQ
LADHYQQNTPIGDGPVLLPDNHYLS

_____ _____ _____

ii) Which secondary structural element is most prevalent in GFP?

Question 2

Imagine that you are a researcher working in a laboratory. You are interested in characterizing three specific proteins: X, Y and Z. In an attempt to characterize these proteins you fuse the gene encoding protein X to the gene for green fluorescent protein (GFP) such that now protein X fluoresces green. You also create a fusion protein for Y such that it fluoresces red and a fusion protein for Z such that it fluoresces orange. You then express all of these fusion proteins in eukaryotic cells.

b) When you look at cells using fluorescence microscopy you find that the medium in which control cells are grown is colorless. The medium in which the cells containing the fusion proteins are grown appears red. Why might this be?

c) You use these tagged proteins to follow the migration of proteins through different organelles of the cell.

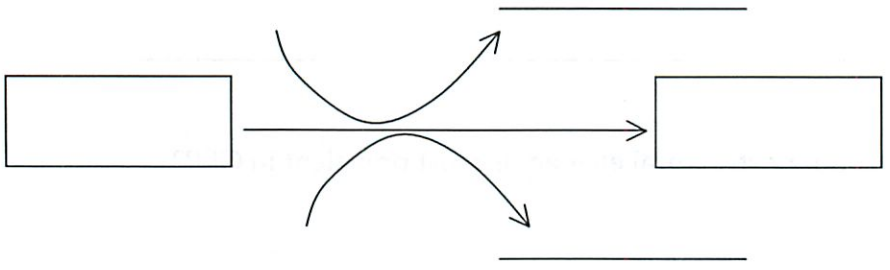
i) What are the organelles through which a protein traverses if this protein is a membrane receptor protein?

ii) Will the ligand-binding domain of the receptor protein project towards the extracellular space or cytoplasm?

d) You learn that protein Z is an enzyme that catalyzes an important step of glycolysis.

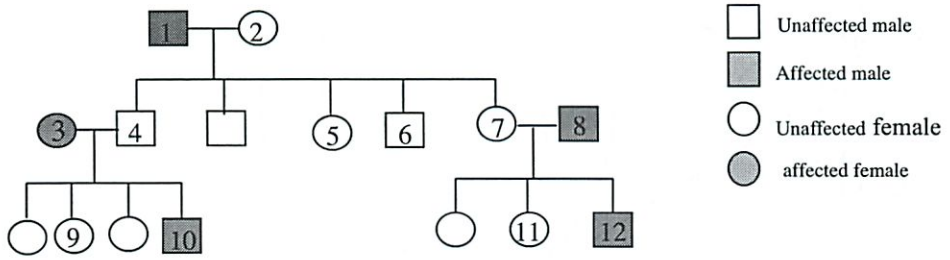
i) In which compartment of a eukaryotic cell would you first see the orange fluorescence associated with protein Z if the fluorescent tag is on the N terminus of protein Z?

ii) Complete the **overall** schematic for glycolysis below. Fill in the boxes with the **names** of the appropriate carbon-containing molecules and fill in the blanks with the **names** of the products generated during glycolysis.



Question 3

You are studying a genetically inherited disease. The pedigree for a family with this disease is shown below. Assume complete penetrance, but make no assumptions regarding the genotype of individual 8.



a) Which of the following **mode or modes** of inheritance are consistent with this disease?

Autosomal dominant Autosomal recesive X-linked dominant

X-linked recessive Y-linked

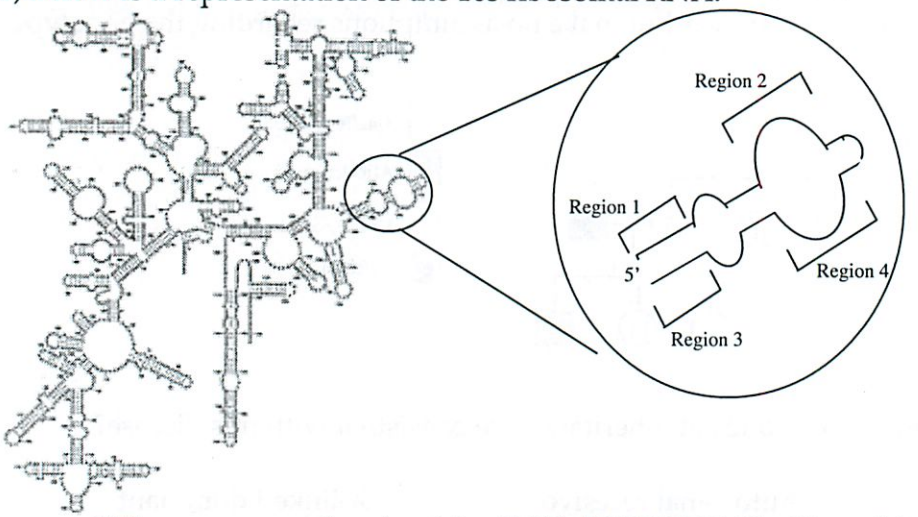
Question 4

The evolutionary relationship between organisms is often determined by comparing 16s ribosomal RNA sequences.

a) What is a ribosome composed of? What is it used for?

b) When the 16s ribosomal RNAs of organisms are compared, one sees some regions of absolute conservation, *i.e.*, the sequence in these regions is identical for all organisms examined. Other regions of the 16s ribosomal RNA sequences vary between organisms but are uniform in length, where other regions vary in both sequence and length. Explain why these conserved regions exist if mutations occur randomly throughout the genome.

c) Below is a representation of the 16s ribosomal RNA.



i) The sequence of region 1 is 5′ UACGUCCGA 3′. Given this can you deduce the sequence for region 3? If so, give the sequence and label the 5′ and 3′ ends. If not, explain why not.

ii) The sequence of region 2 is 5′ CGGAAUGCU 3′. Given this can you deduce the sequence for region 4? If so, give the sequence and label the 5′ and 3′ ends. If not, explain why not.

d) The gene that encodes the ribosomal RNA is transcribed, but the RNA produced is not translated. Give one example of another gene found in all cells that is transcribed but not translated.

Question 4, continued

The seeds from the castor bean plant, *Ricinus communis*, are poisonous to many species, due to a toxic protein called ricin. Ricin specifically and irreversibly inactivates eukaryotic ribosomes.

a) What is the function of a ribosome?

b) The eukaryotic ribosome is composed of two different ribosomal RNAs and 33 different proteins. How many genes are required to form the ribosome? Explain why you chose this number.

c) The ribosome associates with two other types of RNA: mRNA and tRNA.

- How many different types of mRNA molecules could be found in a cell

  Less than 10 between 10 – 25 between 26 – 100

  between 100 – 1000 more than 1000

- How many different types of tRNA molecules could be found in a cell?

  Less than 10 between 10 – 25 between 26 – 100

  between 100 – 1000 more than 1000

You imagine several possible therapeutic uses for ricin, and would like to produce it in large quantities. You first want to develop a castor bean plant that produces large seeds (the source of ricin) and matures quickly. You cross a true-breeding plant with large seeds and slow growth to a true-breeding plant with small seeds and fast growth. All of the resulting plants have small seeds and grow slowly.

Question 4, continued

d) What are the genotypes of the two true-breeding parental plants? Use the nomenclature outlined below.

- In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.
- For the seed size use (i.e., large or small) use D or d to designate the alleles.
- For the growth (i.e., fast or slow) use G or g to designate the alleles.

Parent	Genotype
large seeds and slow growth	
small seeds and fast growth	

e) You then cross two of the F1 plants that have small seeds and grow slowly. If these two genes are unlinked, about how many total offspring will you need to obtain 100 plants that have large seeds and are fast growing?

f) You find that the two genes are linked and plan to determine the map distance between the seed size gene and the growth gene. You test cross an F1 plant to a plant with large seeds that is fast growing.

- What are the phenotypes and associated genotypes of the <u>non-recombinant</u> progeny?

- What are the phenotypes and associated genotypes of the <u>recombinant</u> progeny?

In its active form, ricin is composed of two different glycosylated polypeptide chains that are linked by a single disulfide bond. It is processed in the ER and Golgi and stored in a sub-cellular compartment You find several different mutant castor bean plants that do not make active ricin. **Each mutant is homozygous for a single mutation.**

Mutant 1: The DNA encoding the N terminus of polypeptide 1 is deleted, neither polypeptide is glycosylated or found in the storage compartment.

Mutant 2: One promoter is mutated and no mRNA for either polypeptide is present.

Mutant 3: A stop codon is created after amino acid 255 of Polypeptide chain 1. mRNA for both polypeptide chains is detected, but only polypeptide chain 1 protein is present.

Mutant 4: A gene encoding a protease normally localized to the Golgi is deleted. mRNA for both polypeptide chains is detected, but no active ricin is made.

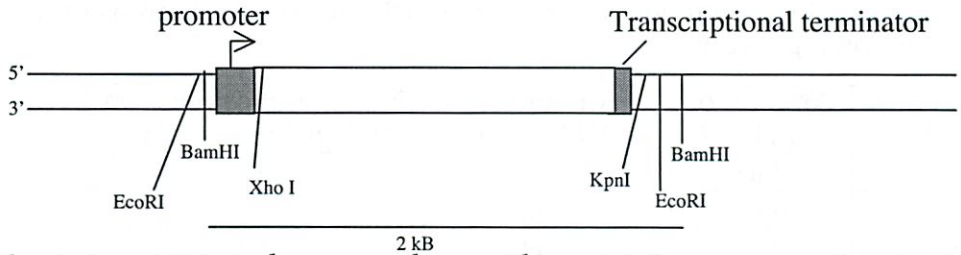
g) What do these mutants, when considered together, suggest about the DNA encoding ricin?

Question 5

You hope to clone the ricin gene and later use it as a research tool to allow cell-type specific killing. First you want to amplify the gene encoding ricin by the polymerase chain reaction (PCR). Below is DNA sequence that flanks the gene.

```
5′  ... TCAAGAGGATCCCCG[ Gene for ricin ]CTCGGAATTAACCCTCAC  3′
    ---------+-----                     -------+---------+
3′  ... AGTTCTCCTAGGGGC[ Gene for ricin ]GAGCCTTAATTGGGAGTG  5′
```

You successfully amplify the ricin gene from the castor bean genomic DNA as shown below:



You plan to insert it into the vector shown. The restriction enzymes listed cut only where indicated; they do not cut anywhere else in the vector or insert.

Nde I:
5' CATATG
GTATAC

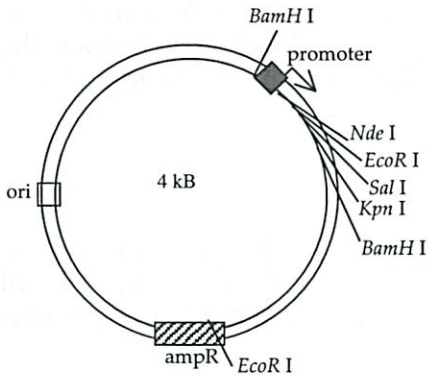
Sal I:
5' GTCGAC
3' CAGCTG

EcoR I:
5' GAATTC
3' CTTAAG

Kpn I:
5' GGTACC
3' CCATGG

Xho I:
5' CTCGAG
3' GAGCTC

Bam HI:
5' GGATCC
3' CCTAGG



The distance between the two Bam HI sites is 200 base pairs.

Question 5, continued

You digest both the ricin gene and the vector with BamHI and ligate the two together. After ligation, you transform bacteria with the DNA.

a) What must be the phenotype of the bacterial cells prior to transformation?

b To select the cells that obtained a plasmid during transformation, you would plate the transformation mix on solid agar media containing what compound?

To ligate the ricin gene into the vector, you prepare the following tubes of the digested fragments and DNA ligase. After ligation, the mixture in each tube is used to transform bacteria. You see the following results:

Tube	Amount of plasmid DNA	Amount of ricin DNA	Amount of DNA ligase	Number of colonies on transformation plate
1	0 ng	2 ng	2 ng	0
2	2 ng	0 ng	2 ng	50
3	2 ng	2 ng	2 ng	50
4	2 ng	12 ng	2 ng	500

c) You isolate plasmid DNA from the colonies generated by transformation tube 2. The plasmid contained in all 50 colonies is the same. Given **all** the data in the table above, what would be the size of the plasmid isolated from these 50 colonies? Explain your answer.

d) You successfully clone the ricin gene into the plasmid using BamHI. Assuming that the promoter shown on the drawing of the plasmid is a bacterial promoter, give two reasons why you would **not** expect that ricin protein could be made from this plasmid in bacterial cells?

e) You successfully clone the ricin gene into the plasmid using BamHI. Assume that the promoter shown on the drawing of the plasmid is a bacterial promoter and that you can insert this plasmid into castor bean cells. Under the appropriate conditions, would you expect that ricin protein could be made from this plasmid when inserted into castor bean cells? Explain your answer. (NOTE: Ricin is not toxic to castor bean cells.)

Question 5, continued

f) Assume that the promoter shown on the drawing of the plasmid is a mouse promoter. To construct a plasmid that could allow expression of the ricin gene in mouse tissue culture cells,

i) What enzyme(s) might you use to cut the plasmid?

ii) What enzyme(s) might you use to cut the ricin gene?

iii) If the enzymes that you chose for i and ii above cut every DNA molecule, how many different types of plasmids could be produced by the ligation of vector with one insert?

Question 6

Consider that you are a cancer specialist who is monitoring a prospective study on patients suffering from Non Hodgkin's lymphoma (NHL). NHL can be of two major types: aggressive (fast growing) and non aggressive (slow growing).

a) During your study you conclude that treatment of the patients that have the aggressive form of NHL is more effective and gives a better prognosis when compared to the non-aggressive NHL. Why might this be?

b) You observe that NHL patients can have mutations in the genes shown below. Classify the following genes either as oncogene or tumor suppressor gene. Also state whether you expect the NHL cells to be homozygous or heterozygous for a mutation in that gene.

Gene mutated in NHL	Normal function of encoded protein	Proto-oncogene or tumor suppressor?	Would NHL cells be homozygous or heterozygous for a mutation in that gene?
Fas	Promotes cell death (apoptosis)		
cERB	Growth factor receptor protein		
p53	Halts the cell cycle in the G1 phase		
Bcl2	Promotes cell to enter the cell cycle		
Abl	Encodes for a tyrosine kinase that stimulates cell division		

c) None of the mutations listed above is sufficient on its own to cause a normal cell to become an NHL cell, but any one of them increases the likelihood that the cell will become an NHL cell. Why might that be?

d) The NHL patients were provided either with radiation therapy, chemotherapy or both. These patients showed signs of severe anemia so you decided to administer EPO. How could EPO help these patients?

Question 7

The immune system is often divided into the humoral and the cellular systems. The primary agents in the humoral immune system are secreted antibodies.

a) The process that produces millions of different antibody molecules from only two different genetic loci results from:

many alleles of these loci	splicing of introns and exons
meiotic recombination	DNA rearrangment

clonal expansion

b) Could the process in (a) produce antibody molecules that have the ability to recognize an antigen that has never been encountered before?

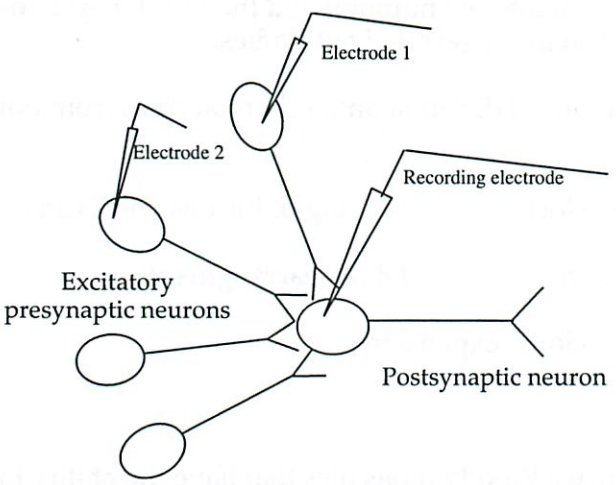
c) Briefly describe the steps involved in B cell activation. Begin with a mature B cell displaying a surface bound antibody and end with plasma B cells and memory B cells.

d) The cellular arm of the immune system employs Cytotoxic T lymphocytes and natural killer cells. Cytotoxic T lymphocytes can recognize virally infected body cells.

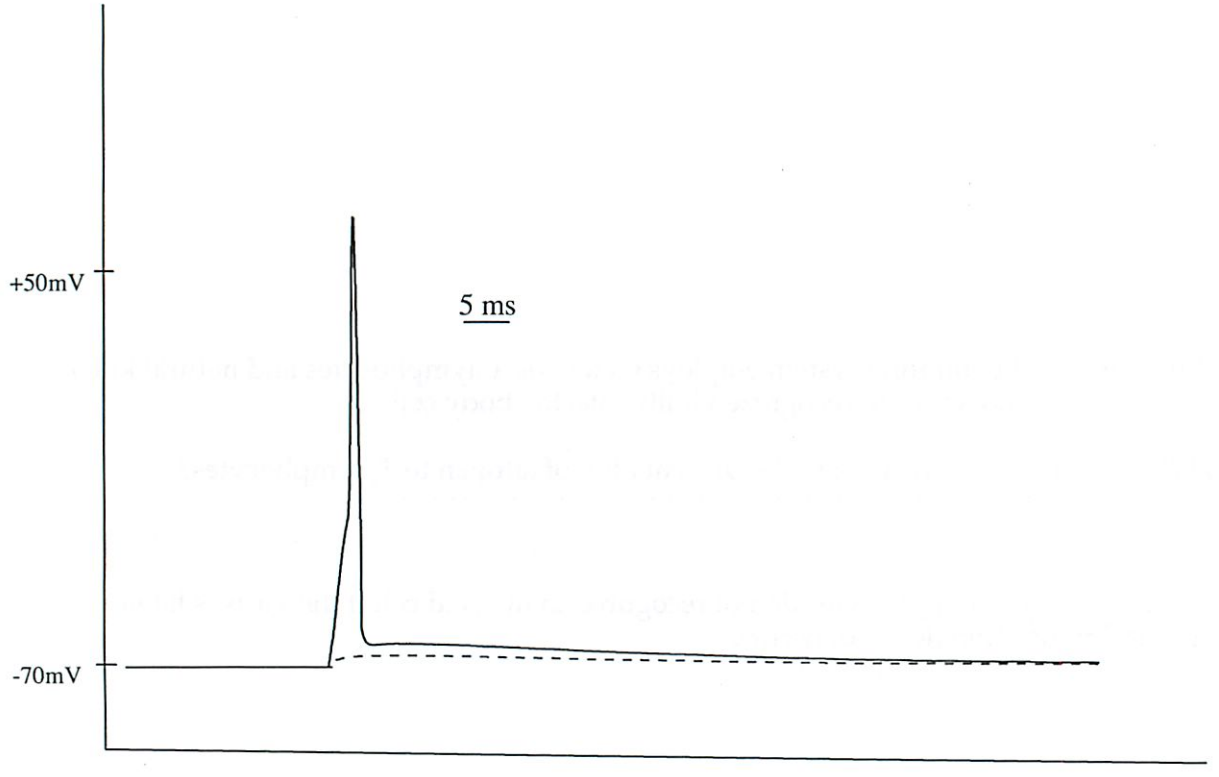
i) What proteins are involved in the presentation of antigen to T_c lymphocytes?

ii) Explain why T_c lymphocytes do not recognize an infected cell if the virus is latent (*i.e.*, viral translation does not occur).

Question 8



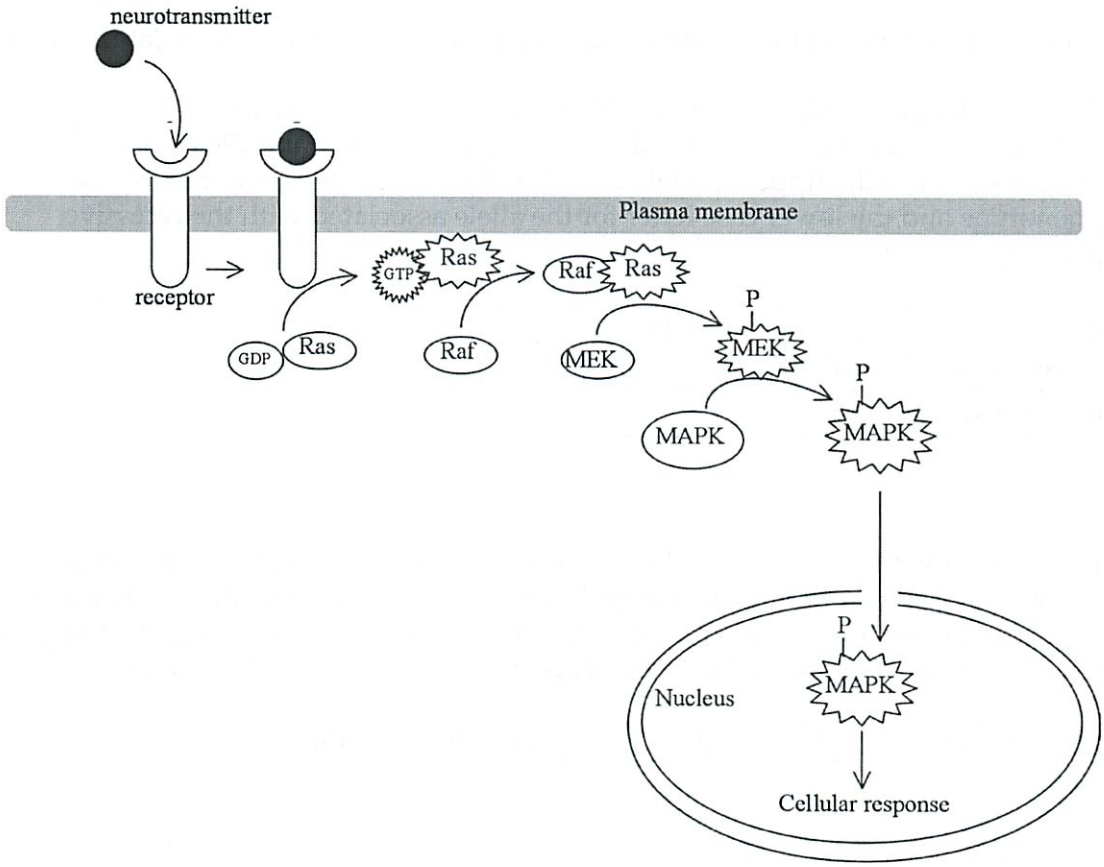
You can depolarize a presynaptic neuron by passing current into the cell through electrodes like electrodes 1 and 2. You can record a response in the postsynaptic neuron using the recording electrode. If you stimulate one of the presynaptic cells, you do not see an action potential in postsynaptic cell (shown by the dashed line). If you stimulate two of the presynaptic cells, you can record an action potential in the postsynaptic cell (shown by the solid line). You stimulate all of the presynaptic cells, and record from the post-synaptic cell. On the following figure, draw the trace you would expect.



Question 9

Your experiment confirms that semaphorin 2 is an attractive signal to these neurons. In the embryo, type W neurons form synapses with neurons expressing semaphorin 2. When these synapses are first formed, type W neurons release a neurotransmitter that binds to metabotropic receptors on the postsynaptic cell. When neurotransmitter binds the metabotropic receptors the following signal transduction cascade is activated.

(Note: Raf, MEK and MAPK are all protein kinases)



a) Describe a mutated version of the receptor protein that would always stimulate the cellular response.

b) Describe a mutated version of RAS that could not activate Raf.

c) Describe a mutated version of MEK that would prevent the cellular response.

Question 9

Tomato plants can be tall or short and have green or red fruit. You cross a true-breeding tall, green-fruited plant with a true-breeding short, red-fruited plant. All of the progeny are tall and red-fruited.

a) Which traits are recessive?

b) What are the genotypes of the two true-breeding parents? Use the nomenclature outlined below.

- For the height (i.e., tall or short) use H or h to designate the alleles.
- For the fruit color (i.e., green or red) use G or g to designate the alleles.
- In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.

Parent	Genotype
Tall, green-fruited plant	
Short, red-fruited plant	

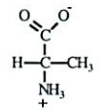
c) Then you cross the F1 plants to true-breeding short, green-fruited plants and, after analyzing 800 progeny, you calculate a map distance of 20 cM between the height and fruit color loci. What are the four genotypic and phenotypic classes you see in the 800 progeny? Given that these two genes are **linked** (20 cM apart), how many of each should you get?

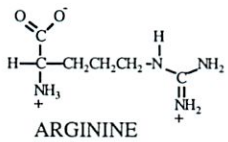
Genotypic class	Phenotypic class	How many of this type?
1.		
2.		
3.		
4.		

Resources:

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

STRUCTURES OF AMINO ACIDS at pH 7.0


ALANINE
(ala)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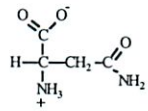
(arg)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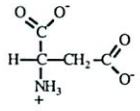
(asn)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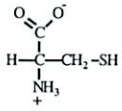
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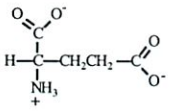
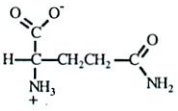
(cys)


GLUTAMIC ACID
(glu)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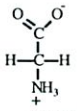
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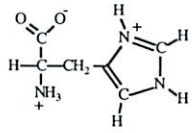
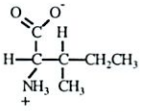
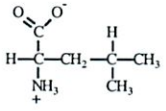

HISTIDINE
(his)


ISOLEUCINE
(ile)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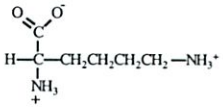
(leu)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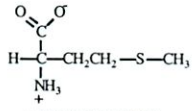
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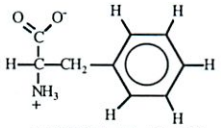
(met)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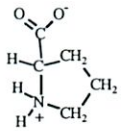
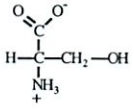
(phe)


PROLINE
(pro)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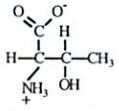
(ser)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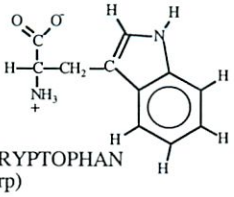
(thr)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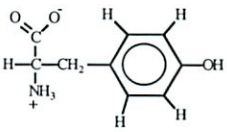
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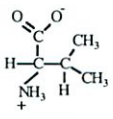
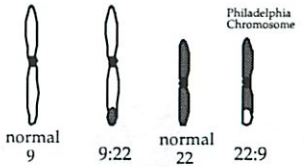
(tyr)

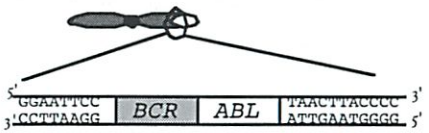

VALINE
(val)

Question 1

The Philadelphia chromosome, results from a translocation event where pieces of chromosomes 9 and 22 switch. No DNA is lost, it is just rearranged. A schematic representing the chromosomes involved in this translocation is shown below. Please note that each of the chromosomes as drawn represents a single double-stranded DNA molecule.



Chronic Myeloid Leukemia (CML) is the cancer associated with the Philadelphia chromosome. The Philadelphia chromosome translocation creates a novel gene by attaching a portion of the coding region of the *ABL* gene (normally found on chromosome 9) to a portion of the *BCR* gene (normally found on chromosome 22). This fusion gene can be transcribed and translated to create a novel protein, the Bcr-Abl protein



You want to design PCR primers to quickly determine if a cell is carrying the Philadelphia chromosome.

a) Circle the best pair of primers for amplification of the Philadelphia chromosome.

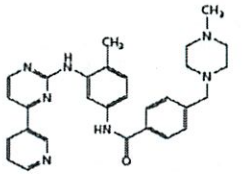
- 5′ CCTTAAGG 3′ and 5′ ATTGAAT 3′
- 5′ GGAATTCC 3′ and 5′ TAACTTA 3′
- 5′ TAAGTTA 3′ and 5′ GGAATTCC 3′
- 5′ ATTGAAT 3′ and 5′ CCTTAAGG 3′

b) Would you categorize the gene encoding the Bcr-Abl protein as a tumor suppressor gene, an oncogene or a proto-oncogene?
oncogene

Question 1, continued

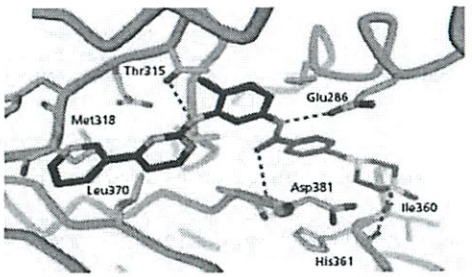
c) The Bcr-Abl protein functions as a tyrosine kinase. Many drugs that effectively inhibit tyrosine kinases are known. Most of these drugs are not useful therapeutics against CML due to a variety of side effects. Explain why there are so many side effects associated with these drugs.
All tyrosine kinases share some homology, so these drugs will inhibit lots of different tyrosine kinases. Because each kinase has a particular function in the cell, these drugs interfere with many different functions and thus you see a wide variety of side effects associated with non-specific tyrosine kinase inhibitors.

d) A drug, Gleevec, has been introduced as a treatment for CML. Gleevec is a small molecule that fits into the catalytic site of the Bcr-Abl protein and prevents enzyme function. A drawing of Gleevec interacting with the Bcr-Abl kinase is shown below. For this question, the pH = 7.0.



Gleevec®
(imatinib mesylate)

A table of the amino acids can be found on the last page of this exam.



i) What is the strongest type of interaction that occurs between **Thr 315** of the Bcr-Abl enzyme and Gleevec as indicated by the dashed line in the diagram above?

Hydrogen bond

ii) What is the strongest type of interaction that occurs between **Met 318** of the Bcr-Abl enzyme and Gleevec as diagramed above?
Van der waals forces or hydrophobic interactions.

iii) What is the strongest type of interaction that occurs between **Glu 286** of the Bcr-Abl enzyme and Gleevec as indicated by the dashed line in the diagram above?
Hydrogen bond

Question 1, continued

e) There are two variants of the Bcr-Abl protein such that …

- the Bcr-Abl enzyme has **Ala** at 315 instead of **Thr.** Individuals with this substitution have CML that is resistant to treatment with Gleevec.
- the Bcr-Abl enzyme has **Trp** at 315 instead of **Thr.** Individuals with this substitution also have CML that is resistant to treatment with Gleevec.

i) What type of interaction now occurs between **Ala 315** of the Bcr-Abl enzyme and Gleevec?
Van der waals forces

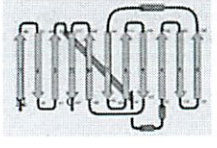
ii) What type of interaction now occurs between **Trp 315** of the Bcr-Abl enzyme and Gleevec?
Van der waals forces

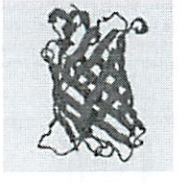
f) The CML of individuals with the Bcr-Abl enzyme that has **Ala** at 315 instead of **Thr** will respond to Gleevec, but at much, much higher doses. However, the CML of individuals with the Bcr-abl enzyme that has **Trp** at 315 instead of **Thr** is impervious to Gleevec at any concentration. Explain the difference in the resistance to Gleevec of each of these two variants.
The Ala 315 mutant can still bind to Gleevec, but less efficiently so it requires a greater concentration. Because of the size of the tryptophan side chain, the Trp 315 mutant can not bind to Gleevec.

Question 2

a) Each of the three diagrams below represent the GFP protein.

i) Label each diagram with the level of protein structure it portrays (primary, secondary, tertiary, quaternary).





MSKGEELFTGVVPVLVELDGDVNGQ
KFSVSGEGEGDATYGKLTLNFICTTG
KLPVPWPTLVTTFSYGVQCFSRYPD
HMKQHDFFKSAMPEGYVQERTIFYK
DDGNYKTRAEVKFEGDTLVNRIELK
GIDFKEDGNILGHKMEYNYNSHNVY
IMGDKPKNGIKVNFKIRHNIKDGSVQ
LADHYQQNTPIGDGPVLLPDNHYLS

secondary *tertiary* *primary*

ii) Which secondary structural element is most prevalent in GFP?
Beta sheet

Question 2

Imagine that you are a researcher working in a laboratory. You are interested in characterizing three specific proteins: X, Y and Z. In an attempt to characterize these proteins you fuse the gene encoding protein X to the gene for green fluorescent protein (GFP) such that now protein X fluoresces green. You also create a fusion protein for Y such that it fluoresces red and a fusion protein for Z such that it fluoresces orange. You then express all of these fusion proteins in eukaryotic cells.

b) When you look at cells using fluorescence microscopy you find that the medium in which control cells are grown is colorless. The medium in which the cells containing the fusion proteins are grown appears red. Why might this be?
Protein Y must be a secreted protein.

c) You use these tagged proteins to follow the migration of proteins through different organelles of the cell.

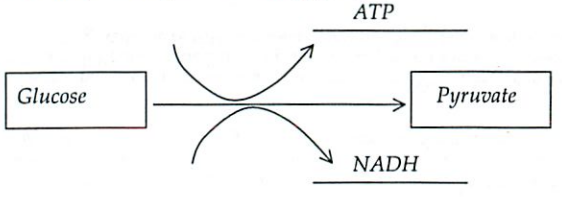
i) What are the organelles through which a protein traverses if this protein is a membrane receptor protein?
The endoplasmic reticulum and the golgi apparatus

ii) Will the ligand-binding domain of the receptor protein project towards the extracellular space or cytoplasm?
extracellular space

d) You learn that protein Z is an enzyme that catalyzes an important step of glycolysis.

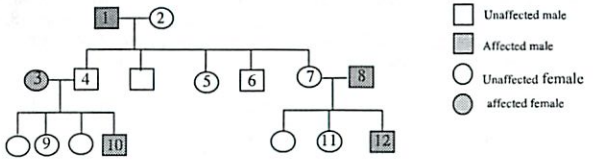
i) In which compartment of a eukaryotic cell would you first see the orange fluorescence associated with protein Z if the fluorescent tag is on the N terminus of protein Z?
In the cytoplasm

ii) Complete the **overall** schematic for glycolysis below. Fill in the boxes with the **names** of the appropriate carbon-containing molecules and fill in the blanks with the **names** of the products generated during glycolysis.



Question 3

You are studying a genetically inherited disease. The pedigree for a family with this disease is shown below. Assume complete penetrance, but make no assumptions regarding the genotype of individual 8.



a) Which of the following **mode or modes** of inheritance are consistent with this disease?

Autosomal dominant Autosomal recesive X-linked dominant

X-linked recessive Y-linked

Question 4

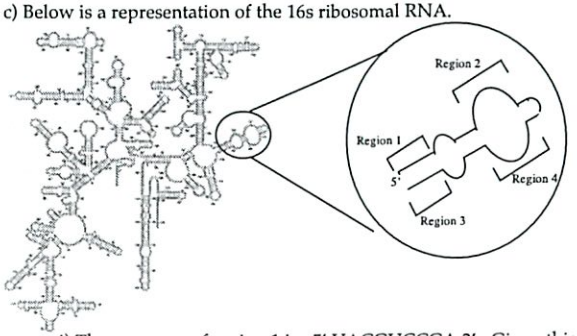
The evolutionary relationship between organisms is often determined by comparing 16s ribosomal RNA sequences.

a) What is a ribosome composed of? What is it used for?
Ribosomes are composed of to RNA sububits and many different polypeptide. They bind to mRNA and use it as a template for protein synthesis.

b) When the 16s ribosomal RNAs of organisms are compared, one sees some regions of absolute conservation, *i.e.*, the sequence in these regions is identical for all organisms examined. Other regions of the 16s ribosomal RNA sequences vary between organisms but are uniform in length, where other regions vary in both sequence and length. Explain why these conserved regions exist if mutations occur randomly throughout the genome.
Mutations do occur randomly throughout the genome, but not all mutations are tolerated equally by the cell. The conserved regions in the RNA are regions where the specific sequence is essential to the function of the rRNA . Mutations do occur in the DNA encoding these regions, but cells that suffer mutations in these region do not survive so those mutation do are not see in the evolutionary record.

Question 4, continued

c) Below is a representation of the 16s ribosomal RNA.



i) The sequence of region 1 is 5′ UACGUCCGA 3′. Given this can you deduce the sequence for region 3? If so, give the sequence and label the 5′ and 3′ ends. If not, explain why not.
3′ AUGCAGGCU 5′

ii) The sequence of region 2 is 5′ CGGAAUGCU 3′. Given this can you deduce the sequence for region 4? If so, give the sequence and label the 5′ and 3′ ends. If not, explain why not.
Can't tell as region 2 and region 4 do not form base pairs.

d) The gene that encodes the ribosomal RNA is transcribed, but the RNA produced is not translated. Give one example of another gene found in all cells that is transcribed but not translated.
Any gene encoding tRNA

The seeds from the castor bean plant, *Ricinus communis*, are poisonous to many species, due to a toxic protein called ricin. Ricin specifically and irreversibly inactivates eukaryotic ribosomes.

a) What is the function of a ribosome?
Ribosomes bind to mRNA and use it as a template for protein synthesis.

b) The eukaryotic ribosome is composed of two different ribosomal RNAs and 33 different proteins. How many genes are required to form the ribosome? Explain why you chose this number.
35 genes, one for each of the RNAs and one for each of the proteins.

Question 4, continued

c) The ribosome associates with two other types of RNA: mRNA and tRNA.

- How many different types of mRNA molecules could be found in a cell

Less than 10 between 10 – 25 between 26 – 100

between 100 – 1000 (more than 1000)

- How many different types of tRNA molecules could be found in a cell?

Less than 10 between 10 – 25 (between 26 – 100)

between 100 – 1000 more than 1000

Question 5

You imagine several possible therapeutic uses for ricin, and would like to produce it in large quantities. You first want to develop a castor bean plant that produces large seeds (the source of ricin) and matures quickly. You cross a true-breeding plant with large seeds and slow growth to a true-breeding plant with small seeds and fast growth. All of the resulting plants have small seeds and grow slowly.

a) What are the genotypes of the two true-breeding parental plants? Use the nomenclature outlined below.

- In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.
- For the seed size use (i.e., large or small) use D or d to designate the alleles.
- For the growth (i.e., fast or slow) use G or g to designate the alleles.

Parent	Genotype
large seeds and slow growth	*ddGG*
small seeds and fast growth	*DDgg*

b) You then cross two of the F1 plants that have small seeds and grow slowly. If these two genes are unlinked, about how many total offspring will you need to obtain 100 plants that have large seeds and are fast growing?
Because the ratio expected is 9:3:3:1, you would expect to need 1600 total offspring to see 100 plants that have large seeds and are fast growing.

c) You find that the two genes are linked and plan to determine the map distance between the seed size gene and the growth gene. You test cross an F1 plant to a plant with large seeds that is fast growing.

- What are the phenotypes and associated genotypes of the non-recombinant progeny?
  dGdg (ddGg): large seeds and slow growth
  Dgdg (Ddgg): small seeds and fast growth

- What are the phenotypes and associated genotypes of the recombinant progeny?
  dgdg (ddgg): large seeds and fast growth
  DgDg (DdGg): small seeds and slow growth

In its active form, ricin is composed of two different glycosylated polypeptide chains that are linked by a single disulfide bond. It is processed in the ER and Golgi and stored in a sub-cellular compartment You find several different mutant castor bean plants that do not make active ricin. **Each mutant is homozygous for a single mutation.**

Mutant 1: The DNA encoding the N terminus of polypeptide 1 is deleted, neither polypeptide is glycosylated or found in the storage compartment.

Mutant 2: One promoter is mutated and no mRNA for either polypeptide is present.

Mutant 3: A stop codon is created after amino acid 255 of Polypeptide chain 1. mRNA for both polypeptide chains is detected, but only polypeptide chain 1 protein is present.

Mutant 4: A gene encoding a protease normally localized to the Golgi is deleted. mRNA for both polypeptide chains is detected, but no active ricin is made.

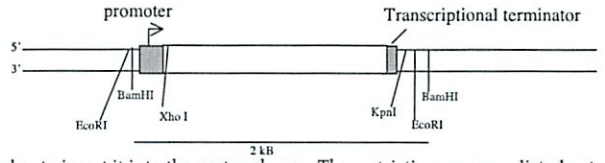
d) What do these mutants, when considered together, suggest about the DNA encoding ricin?
These mutants, when considered together, suggest that the DNA encoding ricin is a single gene that make a single polypeptide. This polypeptide is cut to make two different polypeptides that form the active ricin protein.

Question 5

You hope to clone the ricin gene and later use it as a research tool to allow cell-type specific killing. First you want to amplify the gene encoding ricin by the polymerase chain reaction (PCR). Below is DNA sequence that flanks the gene.

```
5'  ... TCAAGAGGATCCCCG[  Gene for ricin  ]CTCGGAATTAACCCTCAC  3'
            ---------+-----                -------+---------+
3'  ... AGTTCTCCTAGGGGC[                  ]GAGCCTTAATTGGGAGTG  5'
```

You successfully amplify the ricin gene from the castor bean genomic DNA as shown below:



You plan to insert it into the vector shown. The restriction enzymes listed cut only where indicated; they do not cut anywhere else in the vector or insert.

Nde I: 5' CATATG / GTATAC

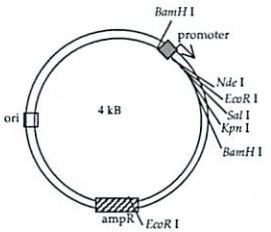
Sal I: 5' GTCGAC / 3' CAGCTG

EcoR I: 5' GAATTC / 3' CTTAAG

Kpn I: 5' GGTACC / 3' CCATGG

Xho I: 5' CTCGAG / 3' GAGCTC

Bam HI: 5' GGATCC / 3' CCTAGG



The distance between the two Bam HI sites is 200 base pairs.

Question 5, continued

You digest both the ricin gene and the vector with BamHI and ligate the two together. After ligation, you transform bacteria with the DNA.

a) What must be the phenotype of the bacterial cells prior to transformation?
These cells must be ampicillin sensitive

b To select the cells that obtained a plasmid during transformation, you would plate the transformation mix on solid agar media containing what compound?
On solid agar media that containing ampicillin.

To ligate the ricin gene into the vector, you prepare the following tubes of the digested fragments and DNA ligase. After ligation, the mixture in each tube is used to transform bacteria. You see the following results:

Tube	Amount of plasmid DNA	Amount of ricin DNA	Amount of DNA ligase	Number of colonies on transformation plate
1	0 ng	2 ng	2 ng	0
2	2 ng	0 ng	2 ng	50
3	2 ng	2 ng	2 ng	50
4	2 ng	12 ng	2 ng	500

c) You isolate plasmid DNA from the colonies generated by transformation tube 2. The plasmid contained in all 50 colonies is the same. Given **all** the data in the table above, what would be the size of the plasmid isolated from these 50 colonies? Explain your answer.
The plasmid isolated from these 50 colonies would be the vector without insert, so would be 4000-200, or 3800 base pairs long.

d) You successfully clone the ricin gene into the plasmid using BamHI. Assuming that the promoter shown on the drawing of the plasmid is a bacterial promoter, give two reasons why you would **not** expect that ricin protein could be made from this plasmid in bacterial cells?
By cutting with BamHI, you have removed the bacterial promoter from the vector. Although the insert brings in the ricin promoter, it will not be recognized in bacterial cells. The ricin gene likely has introns that would need to be spliced out before proper translation could occur, and bacterial cells do not have the capacity to splice eukaryotic

e) You successfully clone the ricin gene into the plasmid using BamHI. Assume that the promoter shown on the drawing of the plasmid is a bacterial promoter and that you can insert this plasmid into castor bean cells. Under the appropriate conditions, would you expect that ricin protein could be made from this plasmid when inserted into castor bean cells? Explain your answer. (NOTE: Ricin is not toxic to castor bean cells.)
By cutting with BamHI, you have removed the bacterial promoter from the vector. The insert brings in the ricin gene with its own promoter and this should allow expression of ricin in the bean cells

Question 5, continued

f) Assume that the promoter shown on the drawing of the plasmid is a mouse promoter. To construct a plasmid that could allow expression of the ricin gene in mouse tissue culture cells,

i) What enzyme(s) might you use to cut the plasmid? *Sal I and Kpn I*

ii) What enzyme(s) might you use to cut the ricin gene? *Xho I and Kpn I*

iii) If the enzymes that you chose for i and ii above cut every DNA molecule, how many different types of plasmids could be produced by the ligation of vector with one insert? *One.*

Question 6

Consider that you are a cancer specialist who is monitoring a prospective study on patients suffering from Non Hodgkin's lymphoma (NHL). NHL can be of two major types: aggressive (fast growing) and non aggressive (slow growing).

a) During your study you conclude that treatment of the patients that have the aggressive form of NHL is more effective and gives a better prognosis when compared to the non-aggressive NHL. Why might this be?
Many chemotherapeutic agents target rapidly dividing cells, which makes the cells of the aggressive form more susceptible?

b) You observe that NHL patients can have mutations in the genes shown below. Classify the following genes either as oncogene or tumor suppressor gene. Also state whether you expect the NHL cells to be homozygous or heterozygous for a mutation in that gene.

Gene mutated in NHL	Normal function of encoded protein	Proto-oncogene or tumor suppressor?	Would NHL cells be homozygous or heterozygous for a mutation in that gene?
Fas	Promotes cell death (apoptosis)	*tumor suppressor*	*homozygous*
cERB	Growth factor receptor protein	*Proto-oncogene*	*heterozygous*
p53	Halts the cell cycle in the G1 phase	*tumor suppressor*	*homozygous*
Bcl2	Promotes cell to enter the cell cycle	*Proto-oncogene*	*heterozygous*
Abl	Encodes for a tyrosine kinase that stimulates cell division	*Proto-oncogene*	*heterozygous*

c) None of the mutations listed above is sufficient on its own to cause a normal cell to become an NHL cell, but any one of them increases the likelihood that the cell will become an NHL cell. Why might that be?
Any of the above mutations un-regulates the cell cycle and the increases the risk that additional mutation occur.

d) The NHL patients were provided either with radiation therapy, chemotherapy or both. These patients showed signs of severe anemia so you decided to administer EPO. How could EPO help these patients?
EPO binds to a receptor on the red blood cell precursor and stimulate it to divide and produce additional red blood cells.

Question 7

The immune system is often divided into the humoral and the cellular systems. The primary agents in the humoral immune system are secreted antibodies.

a) The process that produces millions of different antibody molecules from only two different genetic loci results from:

many alleles of these loci — splicing of introns and exons

meiotic recombination — DNA rearrangment

clonal expansion

b) Could the process in (a) produce antibody molecules that have the ability to recognize an antigen that has never been encountered before?
Yes.

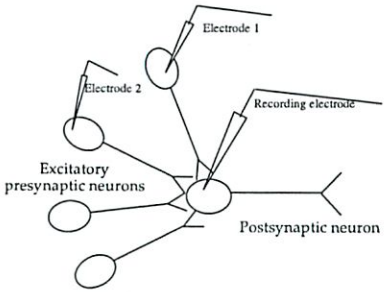
c) Briefly describe the steps involved in B cell activation. Begin with a mature B cell displaying a surface bound antibody and end with plasma B cells and memory B cells.
1) mature B cell displaying a surface bound antibody binds antigen
2) Antigen internalized and displayed on MHCII
3) T helper cell recognizes MHCII + antigen and activates B cell
4) B cell divides (clonal expansion) to produce to tpes of cells, memory B cells and plasma cells.

d) The cellular arm of the immune system employs Cytotoxic T lymphocytes and natural killer cells. Cytotoxic T lymphocytes can recognize virally infected body cells.

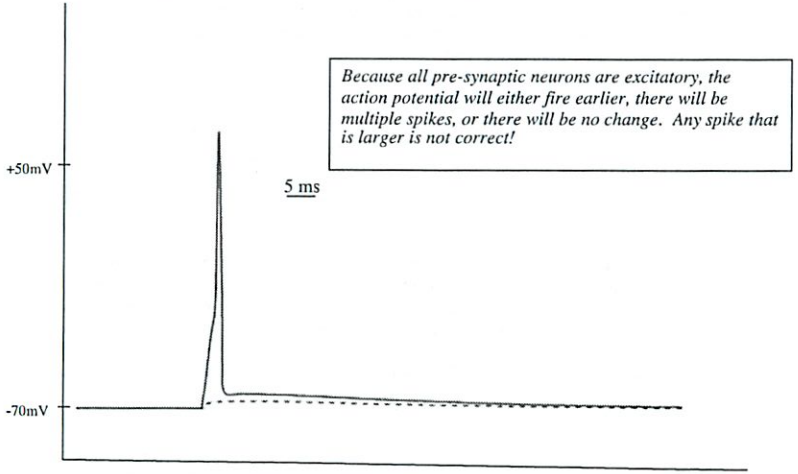
i) What proteins are involved in the presentation of antigen to T_c lymphocytes?
MHCI

ii) Explain why T_c lymphocytes do not recognize an infected cell if the virus is latent (*i.e.*, viral translation does not occur).
If the viral proteins are not being synthesized, then no viral epitomes will be displayed on the MHCI molecules, and the T_c cell will not "see" this cell as infected.

Question 8



You can depolarize a presynaptic neuron by passing current into the cell through electrodes like electrodes 1 and 2. You can record a response in the postsynaptic neuron using the recording electrode. If you stimulate one of the presynaptic cells, you do not see an action potential in postsynaptic cell (shown by the dashed line). If you stimulate two of the presynaptic cells, you can record an action potential in the postsynaptic cell (shown by the solid line). You stimulate all of the presynaptic cells, and record from the post-synaptic cell. On the following figure, draw the trace you would expect.

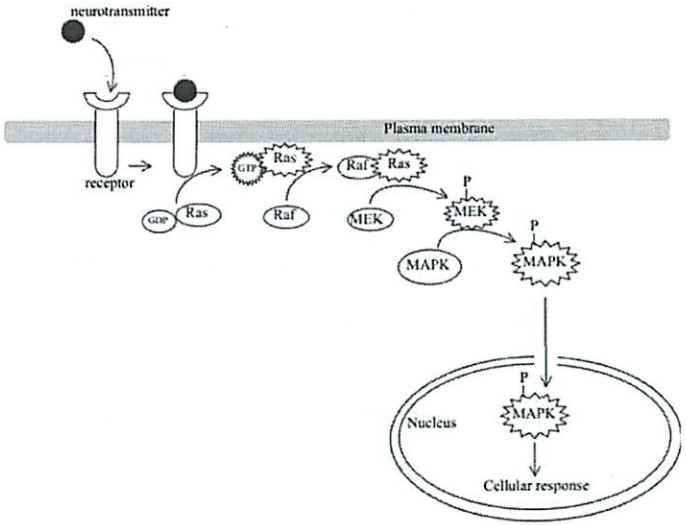


Because all pre-synaptic neurons are excitatory, the action potential will either fire earlier, there will be multiple spikes, or there will be no change. Any spike that is larger is not correct!

Question 9

Your experiment confirms that semaphorin 2 is an attractive signal to these neurons. In the embryo, type W neurons form synapses with neurons expressing semaphorin 2. When these synapses are first formed, type W neurons release a neurotransmitter that binds to metabotropic receptors on the postsynaptic cell. When neurotransmitter binds the metabotropic receptors the following signal transduction cascade is activated.

(Note: Raf, MEK and MAPK are all protein kinases)



a) Describe a mutated version of the receptor protein that would always stimulate the cellular response.
Many possible correct answers. A receptor that is independent of neutrotransmitter.

b) Describe a mutated version of RAS that could not activate Raf.
Many possible correct answers. A form of RAS that was always bound to GDP

c) Describe a mutated version of MEK that would prevent the cellular response.
Many possible correct answers. A version that could never be phosphorylated.

Question 9

Tomato plants can be tall or short and have green or red fruit. You cross a true-breeding tall, green-fruited plant with a true-breeding short, red-fruited plant. All of the progeny are tall and red-fruited.

a) Which traits are recessive?
short and green fruit

b) What are the genotypes of the two true-breeding parents? Use the nomenclature outlined below.

- For the height (i.e., tall or short) use H or h to designate the alleles.
- For the fruit color (i.e., green or red) use G or g to designate the alleles.
- In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.

Parent	Genotype
Tall, green-fruited plant	HHgg
Short, red-fruited plant	hhGG

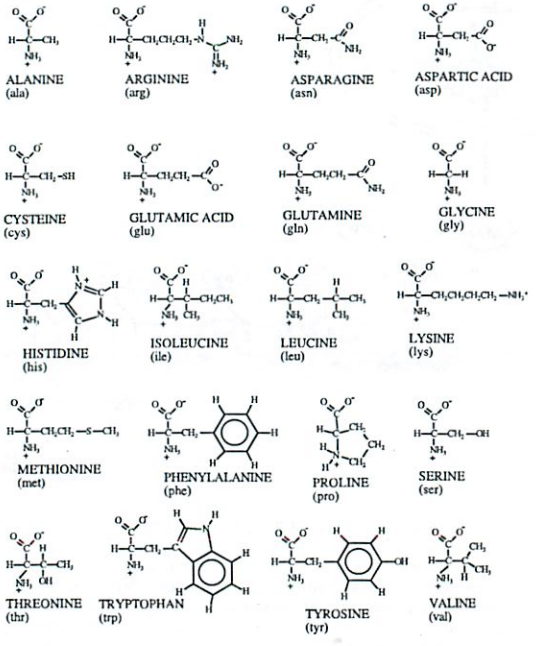
c) Then you cross the F1 plants to true-breeding short, green-fruited plants and, after analyzing 800 progeny, you calculate a map distance of 20 cM between the height and fruit color loci. What are the four genotypic and phenotypic classes you see in the 800 progeny? Given that these two genes are **linked** (20 cM apart), how many of each should you get?

Genotypic class	Phenotypic class	How many of this type?
1. Hhgg	*Tall green*	*320*
2. hhGg	*Short red*	*320*
3. HhGg	*Tall red*	*80*
4. hhgg	*Short green*	*80*

Resources:

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

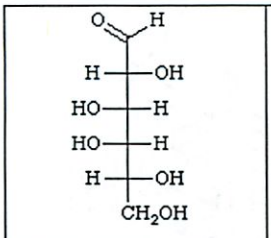
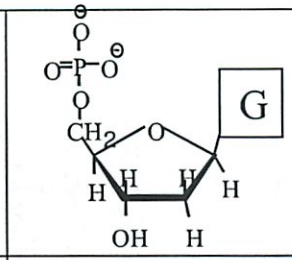
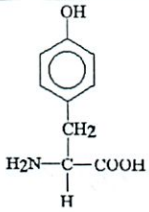
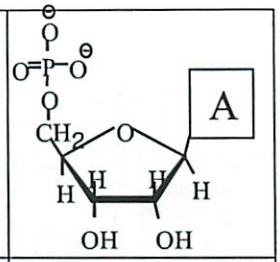
STRUCTURES OF AMINO ACIDS at pH 7.0



Practice Exam Solutions

Question 1

a) Which of the following could be used directly or indirectly as a source of energy? Circle all that apply. *A is glucose and can be used in glycolysis to produce ATP.*

			
A	B	C	D

b) ATP synthase uses an electrochemical gradient to drive the synthesis of ATP from ADP.

i) Which of the above molecules is ADP? *None, ADP = adenosine diphosphate*

ii) ATP synthase is a multimer composed of several different polypeptide chains. What is the highest level (order) of protein structure seen in ATP synthase?
Quaternary

c) You obtain a large amount of ATP synthase that has high activity at room temperature. You study the effects that different treatments have on the activity of this enzyme. Four different experiments are listed below. The activity before each treatment was high. For each experiment, the activity was measured at the end of the treatment. The sample was then returned to the pre-treatment conditions and the enzyme activity was measured again.

Experiment	Treatment	Activity of enzyme at end of treatment	Activity of enzyme after return to pre-treatment conditions
1	Heat to 90 °C for 2 minutes	none	high
2	Heat to 90 °C for 4 minutes	none	high
3	Add a protease for 2 minutes	medium	medium
4	Add a protease for 4 minutes	none	none

- Explain why 90 °C eliminates the enzyme activity and why enzyme activity is restored after experiment 1 and 2.
  Heating a protein to 90 °C disrupts the intermolecular, non-covalent bonds and forces such as ionic bonds, hydrogen bonds, and van der Waals forces. The protein loses its normal 3-dimensional shape and thus loses its function. Once returned to normal temperature, the protein can refold into its native conformation and thus activity is restored.

- Explain why there is a difference between a 2-minute and a 4-minute treatment with protease and why full enzyme activity is not restored in either experiment 3 or 4.
  Once the primary structure of the enzyme is altered, the shape and function is also destroyed. The protease acts at a certain rate, so at 2 minutes, some of the ATP synthase molecules have been destroyed, but other ATP synthase will remain intact, and a reduced amount of activity is measured. At 4 minutes, the protease has had time to destroy all of the ATP synthase molecules, so no activity remains. A return to pre-treatment conditions cannot mend the broken peptide bonds, so function is not restored.

Question 2

The protein complex, LETMEGOTHRUIN (LMGT for short), acts as a channel or pore through which charged proteins and ions can cross the phospholipid bilayer of the membrane. LMGT is composed of four polypeptides as shown in Figure A. Figure B shows a cross section of two of the polypeptides.

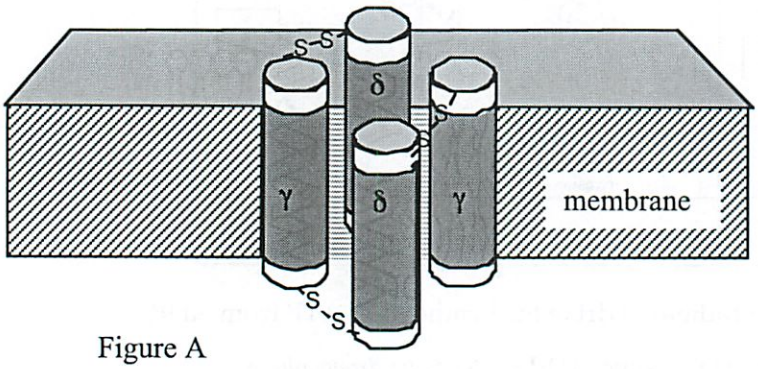


Figure A

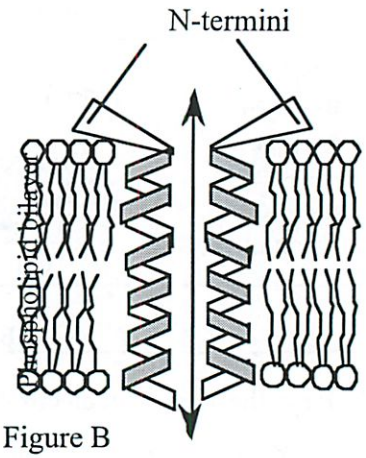


Figure B

a) In Figure B, the amino termini (N-termini) are closely associated with the phosphate groups at the surface of the phospholipid bilayer. Name three amino acids that would likely be found at the N-termini of these polypeptides.
The positively charged amino acids: Arginine, Histidine, and Lysine

c) LMGT is composed of four polypeptides (two γ polypeptides and two δ polypeptides).

i i) How many unique primary protein structures compose the LMGT protein complex?
Two

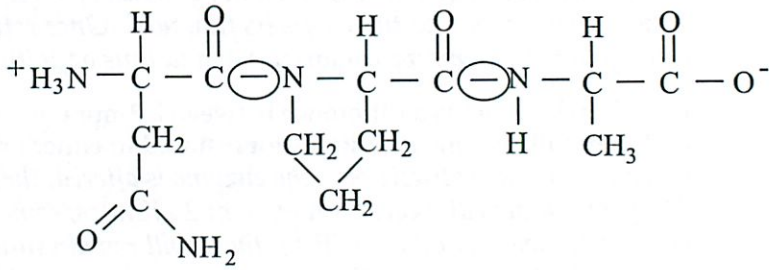
ii) What protein secondary structure is part of the LMGT protein complex?
α-helix

iii) What is the strongest type of bond that maintains the quaternary structure of LMGT?
Covalent, disulfide bond

c) Describe how the polypeptides of the LMGT protein can be surrounded by non-polar hydrocarbons but allow charged proteins and ions to transit the membrane.
The LMGT protein must have non-polar amino acids on the surfaces associated with the lipid part of the membrane and charged or polar amino acids lining the pore of the channel.

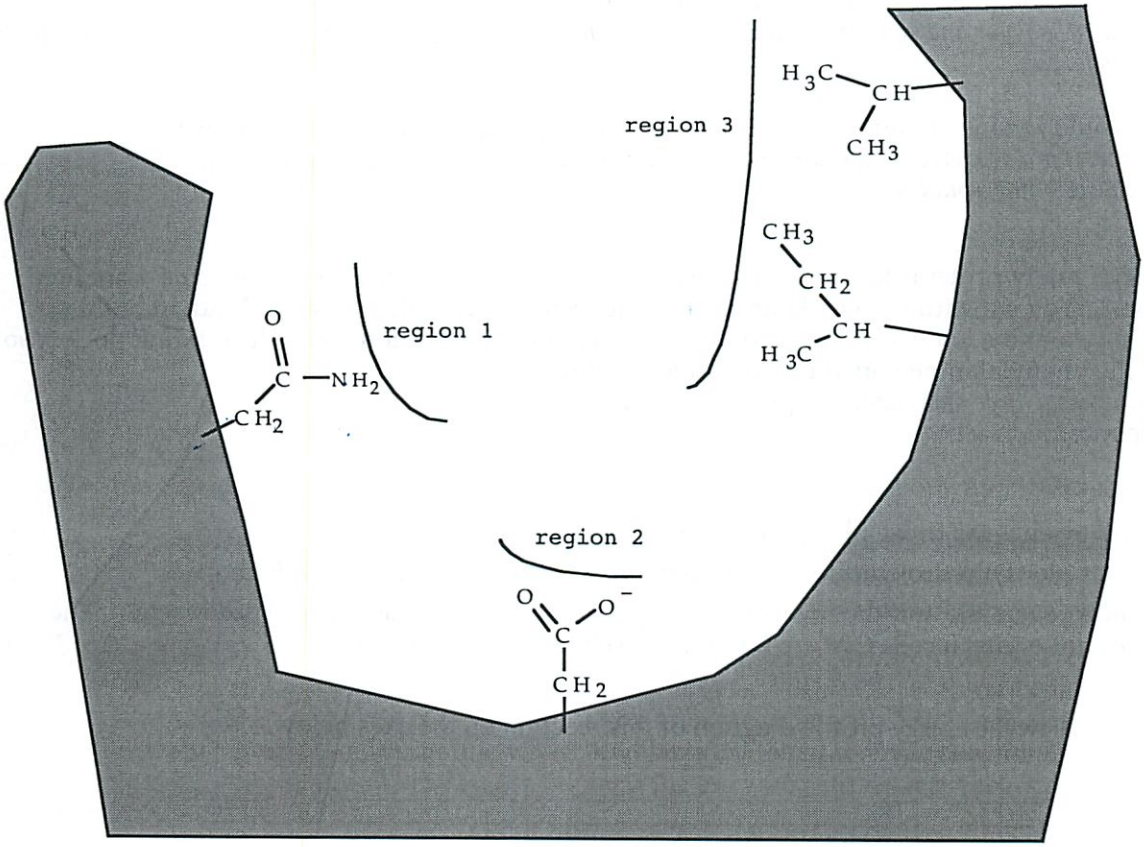
d) One of the small molecules permitted to pass through the LMGT channel is PUNY, a tripeptide. PUNY is composed of asparagine-proline-alanine with asparagine at the amino terminus.

Draw the chemical structure of PUNY.
Circle the peptide bonds.



Question 3

A drug company has isolated the protein shown in schematic below.



a) What amino acid is present in region 2? *Aspartic acid*

b) The substrate for this protein has not been identified. Given the diagram above…

i) What is the strongest interaction possible between the amino acid in region 1 and the substrate? Choose from Covalent, Ionic, or Hydrogen bonds or van der Waals forces.
Hydrogen bonds.

ii) What is the strongest interaction possible between the amino acid in region 2 and the substrate? Choose from Covalent, Ionic, or Hydrogen bonds or van der Waals forces.
Ionic bonds

iii) What is the strongest interaction possible between the amino acids in region 3 and the substrate? Choose from Covalent, Ionic, or Hydrogen bonds or van der Waals forces.
van der Waals forces

Question 3, continued

c) The drug company has asked you to design a protein that binds tightly in this pocket.

i) Would Alanine or Serine interact more strongly with region 1? Why?
Serine, it is polar and can form hydrogen bonds with region 1. Alanine would only interact with van der Waals forces

ii) Would Lysine or Glutamic Acid interact more strongly with region 2? Why?
Lysine, it is (+) charged and can form ionic bonds with region 2. Glutamic acid is also (-) charged and would repel.

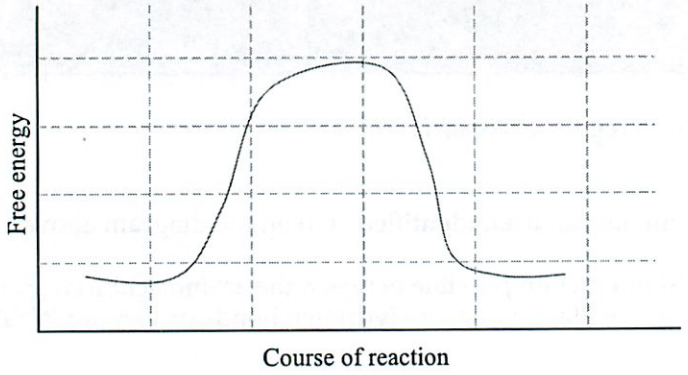
d) You design many proteins that bind tightly in this pocket. One of them has isoleucine associated with region 3. You substitute phenylalanine for isoleucine and find this prevents binding of this protein. Phenylalanine and isoleucine form the same kinds of interactions with the binding pocket, so why can't the phenylalanine version of the protein bind?
Phenylalanine has a large side chain that prevents the substrate from fitting into the pocket.
Steric hinderence.

e) The glycolytic enzyme triose phosphate isomerase, catalyzes step 5,

Dihydroxyacetone phosphate ←→ Glyceraldehde-3-phosphate.

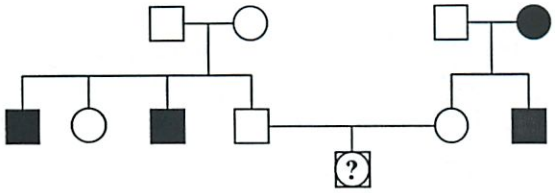
This reaction is reversible and the reverse reaction is equally as likely as the forward reaction. The reaction does not occur in cells that are missing triose phosphate isomerase.

- Draw the energy profile diagram of this reaction on the axes below.



Question 4

Which of the following modes of inheritance is consistent with the pedigree shown below. Assume complete penetrance.



Mode of inheritance	Yes/No	If yes, what is the probability of child ? being affected?
autosomal recessive inheritance	*Yes*	*2/3 X ½ X ½*
X-linked recessive inheritance	*Yes*	*½ X ½ or 0 if girl and ½ if boy.*
autosomal dominant inheritance	*No*	
X-linked dominant inheritance	*No*	

Question 5

You are working with an ornamental fish that shows two color phenotypes, red or white. The color is controlled by a single gene. These fish are hermaphrodites – meaning they can either (1) self-fertilize or (2) mate with another fish. You have three fish: fish 1, fish 2, and fish 3.

a) You set up the mating experiment #1 below using these fish. From the following statements check all that might be true (that is, are consistent with the data, allowing for reasonable statistical fluctuations), given **only** the results of experiment #1.

	Number of progeny with following phenotype	
Experiment	Red	White
#1: Fish 2 (red) with Fish 3 (red)	100	0

√ Fish 2 and fish 3 are homozygotes.
√ The red phenotype is dominant to the white phenotype.
√ The white phenotype is dominant to the red phenotype.
O Both fish 2 and fish 3 are heterozygotes.
√ Fish 2 is a homozygote, and fish 3 is a heterozygote.
√ Fish 2 is a heterozygote, and fish 3 is a homozygote.

Question 5, continued

b) You set up mating experiments #1 (same as above) and #2 below using these fish. From the following statements check all that might be true (that is, are consistent with the data, allowing for reasonable statistical fluctuations), given **both** experiment #1 and experiment 2.

	Number of progeny with following phenotype	
Experiment	Red	White
#1: Fish 2 (red) with Fish 3 (red)	100	0
#2: Fish 2 (red) with Fish 2 (red)	70	30

O Fish 2 and fish 3 are homozygotes.
√ The red phenotype is dominant to the white phenotype.
O The white phenotype is dominant to the red phenotype.
O Both fish 2 and fish 3 are heterozygotes.
O Fish 2 is a homozygote, and fish 3 is a heterozygote.
√ Fish 2 is a heterozygote, and fish 3 is a homozygote.

c) You perform experiment #3 and obtain 400 progeny. Note that fish 2 is the same fish in all three experiments. In the table below, list the phenotype or phenotypes you would see in the progeny. Then give the number of offspring expected with each phenotype.

Experiment
#3: Fish 1 (white) with Fish 2 (red)

Possible Phenotypes	**total number showing this phenotype**
red	*200*
white	*200*

Question 5, continued

While working further with this fish, you discover one other mutation (in a different gene) that leads to the recessive phenotype of long dorsal fins. We now have two phenotypes:

Color: red or white (described in part a). Alleles denoted by R and r.

Dorsal Fins: long (recessive) or short (dominant). Alleles denoted by D and d

**each trait is controlled by a single gene. For the questions below, use the upper case letter for the allele associated with the dominant phenotype.*

In experiment A, you cross true-breeding white fish with long fins to true-breeding red fish with short fins and get all red fish with short fins. You then cross the F1 fish to true-breeding white fish with long fins.

Experiment A: white fish with long fins X red fish with short fins

↓

(F1) red fish with short fins X white fish with long fins

↓

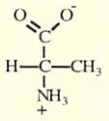
F2: ?

d) You obtain 1000 F2 offspring. Assume that the color and fin loci are 5 cM apart. In the table below, list all the possible phenotypes seen in the F2 offspring. For each phenotype given, list all the possible genotypes seen in the F2 offspring. Finally, give the number of offspring expected with each phenotype.

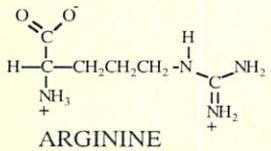
Possible Phenotypes	**Possible Genotypes**	**Total F2 showing this phenotype**
red fish with short fins	*RrDd*	*475*
red fish with long fins	*Rrdd*	*25*
white fish with long fins	*rrdd*	*475*
white fish with short fins	*rrDd*	*25*

From taking Final

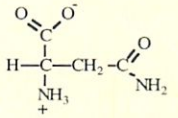
STRUCTURES OF AMINO ACIDS at pH 7.0



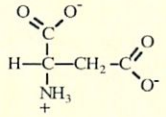
ALANINE
(ala)



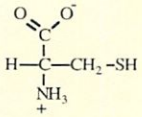
ARGININE
(arg) polar +



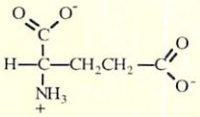
ASPARAGINE
(asn) polar



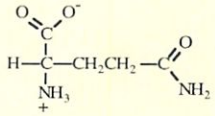
ASPARTIC ACID
(asp) polar −



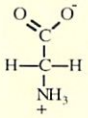
CYSTEINE
(cys) NP



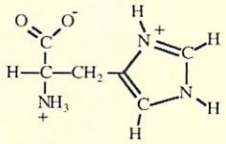
GLUTAMIC ACID
(glu) polar −



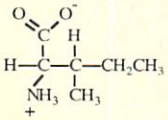
GLUTAMINE
(gln) polar



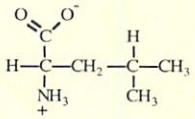
GLYCINE
(gly) polar −



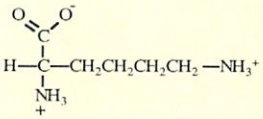
HISTIDINE
(his) NP



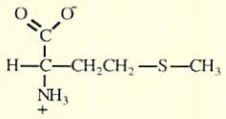
ISOLEUCINE
(ile) NP



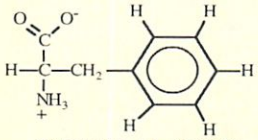
LEUCINE
(leu) NP



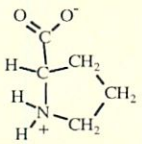
LYSINE
(lys) polar +



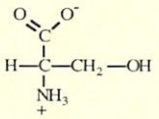
METHIONINE
(met) NP



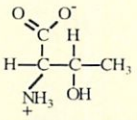
PHENYLALANINE
(phe) NP



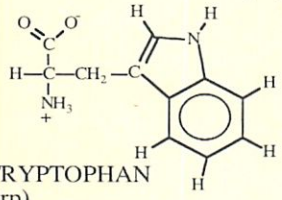
PROLINE
(pro) NP



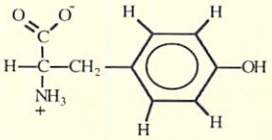
SERINE
(ser) Polar



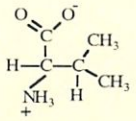
THREONINE
(thr) NP



TRYPTOPHAN
(trp) NP



TYROSINE
(tyr) Polar



VALINE
(val) NP

	U	C	A	G
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA *Stop* UAG *Stop*	UGU Cys UGC Cys UGA *Stop* UGG Trp
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly

Bio Re-study

10/1
8A

I suck at Bonds stuff

Covalent - sharing electrons

Ionic - 2 opposite charged ions

Polar = type of covalent
electrons shared unequally

y+

~~electron~~

Hydrogen electronegative attraction of H + electroneg ion
polar bond
not as strong as covalent or ionic

VdW "natural" force

Review P-set

2

Prokaryots + eucarots

lipids = membrane molecules

hydrophobic = non polar

hydrophilic = polar

└ how to tell?

Example Arg, His, Lys

has + ions

Covalent 2 of the same → CH_2

C wants 4 bonds

| w/ Mark / Prefrosh

1st 2 cols → ionic

line could rep covalent or ionic bond

if 1st + last col → ionic

right left

periodic table

(3)

= 2 electrons share

H-bonds

~~Colleges together in~~

H_2O is example

O is electro neg (right grap periodic)

So attracts 2 Hs

Amino acids - link ~~#~~ by

Carboxyl grap can bond to amnio acid

forms peptide bond

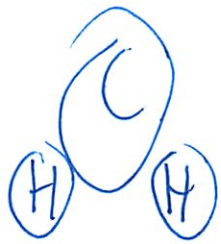
~~Hat~~ dehydration → water removed

Polypeptide chains formed

Side groups which stick out to side

+ make it different

(4) 3b)



C is bonded

needs 4

↳ 2 Ms

Substrate

Other C

So know not Covalent, Ionic

Same w/ H

VaW — when big

mass physics

ii) ~~O needs 2~~

has electron to give

↳ O needs 2

One Covalent Carbon

One to give

(5)

iii) not covalent → all H, C
each is matched

C needs 4
has 3 H
and 1 line
So has 4

VdW has a lot of mass
not H → all the Hs taken

---

don't know 1, 3

---

i) So many C, H makes can't H

H bond → N, O, F

i) is N

iii) All Cs

(6)

c) Aln nothing really

O needs 2

~~H~~ H-bonding b/w OH and electronegh

which is ~~the O~~ the O

NH_2 or

like water

Not water → dehydration

Peptide bond

1.

~~15 polar~~

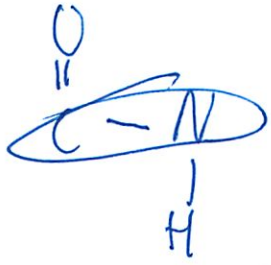
Covalent

NH_3 CO_2

↙ ↘

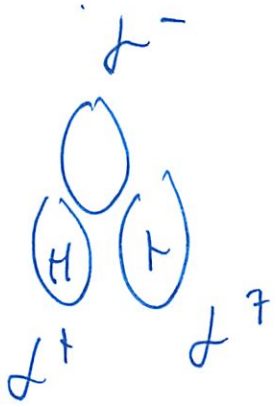
NHCO H_2O

forms b/w C, N



1)

polar electronegivity of one side



H-bonds

F, O, N are h-bond receptors
regardless of it bonded w/ H

---

ii) is the R grap interacting
everything else is stanle/c

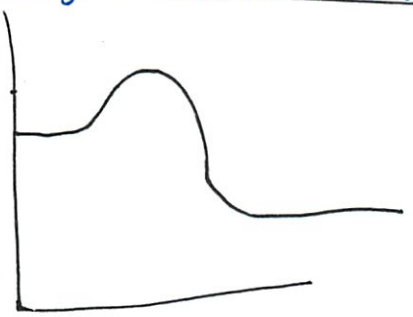
(8)

e) how it wants to be
natural space
less reactive
less volatile

then forced into space it didn't like

know it will go up since needed catalyst

~~not~~ showing reversable here



Not this since reversable is only likely

That is like having something
Non reversable
or have to add energy

Only happens when catalyst

(9)

1 b ii) multimere - several diff chains

~~4th~~ 3rd - one peptide chain
forms weird shape
all 5

2nd α, β hydrogen bonds
one chain

1st peptide bonds

c) usually when heat ~~shock~~ enzyme
↑or 3rd, 4th

breaks bond
~~polypep~~ long chains polypeptide
denatures it
~~polypeptide~~ then re folds

10

Back on my own Genetics

Think got this pretty well
Just need to review
Wish I had my charts for exam

Autosomal - recessive
Autosomal dom
) pretty normal

X-linked ~~dom~~ ~~dad / chance for dau~~
mom

dad has → will give to daughters 100%
but not sons - never 0%

mom has → 50/50 sons
50/50 s daughters

then for recessive → carrier
father only needs 1 to show - 1 slot
mom - 2 slots

(11)

father can never give to sons

~~Codominant~~

dihybrid - hetro x hetro for 2 diff traits
cross see if ind

haploid = 1 chromosome
gametes = egg + sperm cells

mitosis = split into 2 identical sets

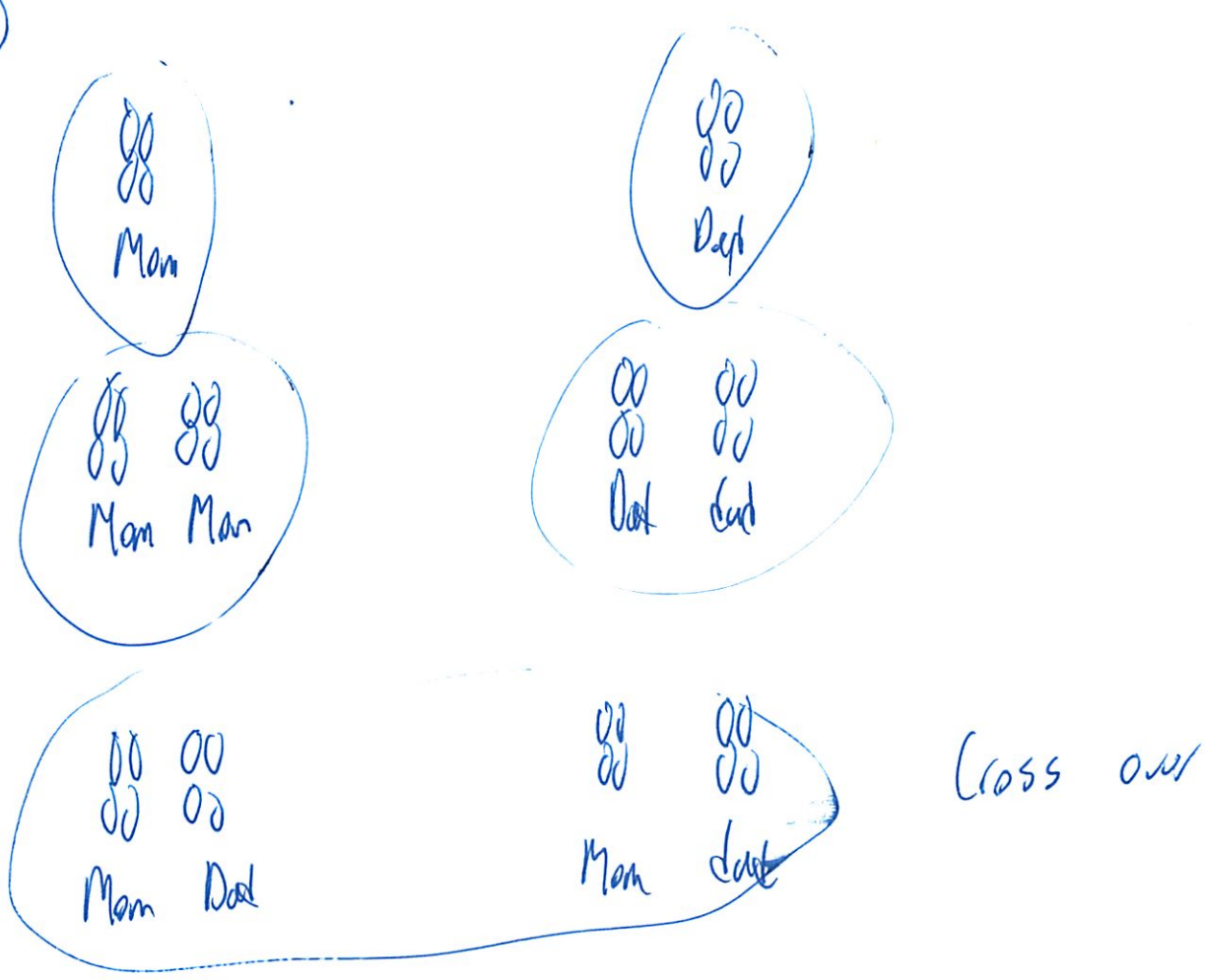
allele = version of gene

Sister chromatid = 2 identical copies

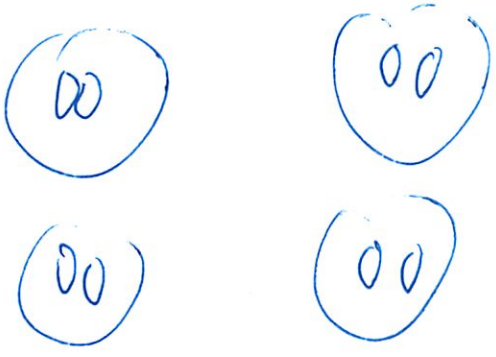
homologous chromosome = pairs w/ same characteristics but not identical

Crossing over large sections transfer

(12)



Can be DD mm
Mm Dm



(13)

monohybrid cross 2 diff alleles of interest
each parent homo / true breeding

RR
r
r

test P? ← was it PP or Pp

PP
r
lower

incomplete – pink

multiple alleles

wild type – most freq +

(4)

Linkage $\frac{\text{the 2 smallest (\# recombinants}}{\text{total \#}}$

Now try
└ on pratice exam

	Rd	rd
rD	~~RD~~ RrDd	~~rD~~ rrDd

write it properly!

Ohh shat = dominant → totally did that wrong

So we have

	RR
r	Rr Rr
r	Rr Rr

(15)

	RD	Rd	rD	rd
rd	RrDd red, short	Rrdd red long	rrDd white short	rrdd white long

all 250 kids

but there is that 5% crossover did something wrong

I don't see it

$$\frac{25+25}{1000} = 5\%$$

So $\frac{x}{1000} = 5\%$ then $\frac{x}{2}$ is # that change