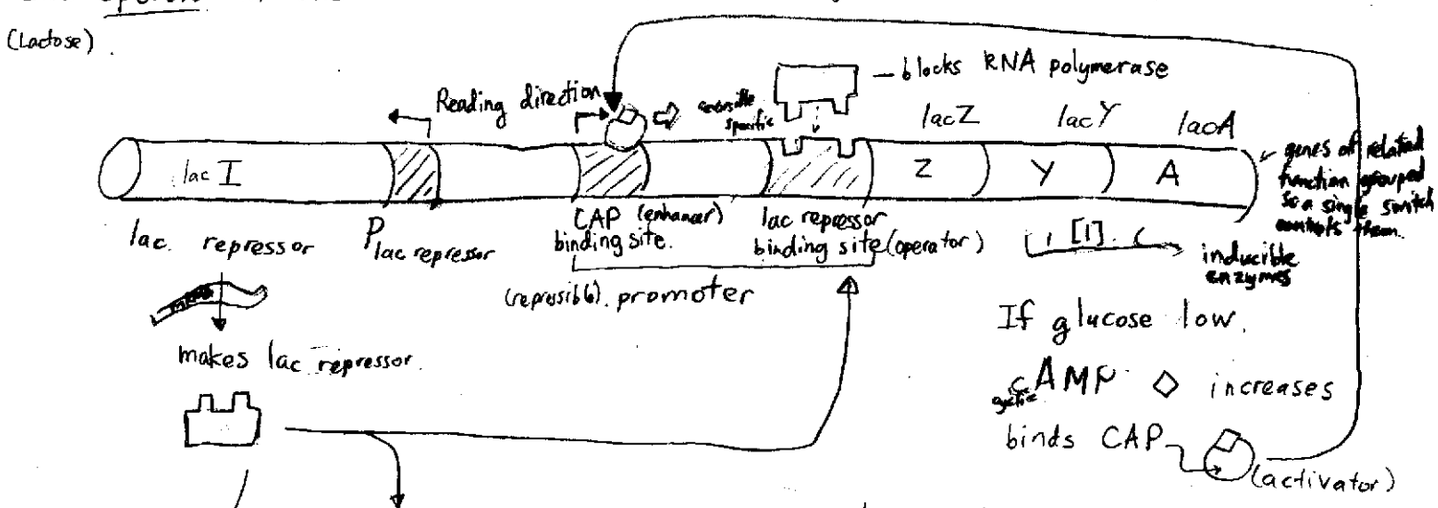
8 Regulating Gene Expression

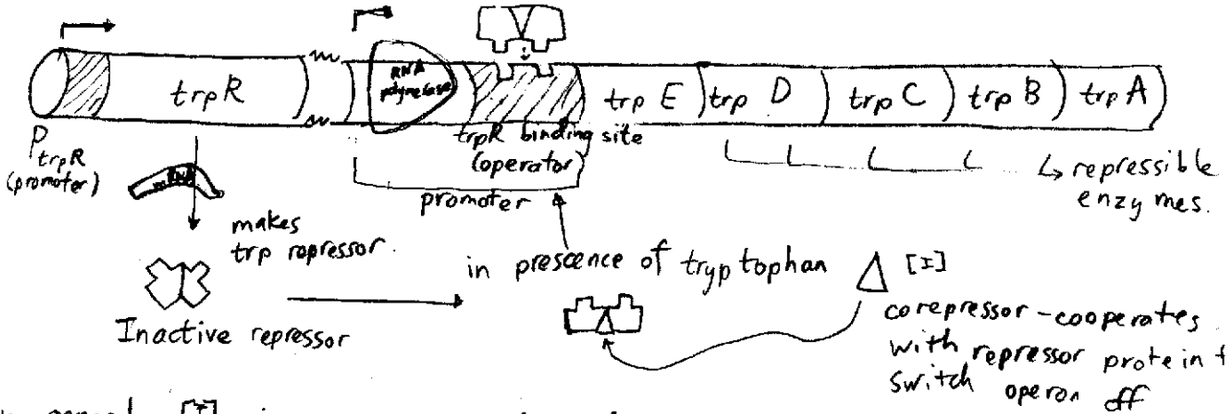
- Lac operon in bacteria – inducible operon (usually off but stimulated by [2])



Lac proteins are always produced at basal level. Only high level of production when
- CAP binds (glucose low)
- lacI doesn't bind ([allo]lactose present)

In general, [1] makes proteins that process [2]. Inducible enzymes function in catabolic pathways to break down a nutrient to simpler molecules. Produce enzyme only when nutrient available → avoid wasting energy.

- trp (tryptophan) operon – repressible operon (usually on but turned off by [I])



In general, [I] is a necessary chemical. Repressible enzymes function in anabolic pathways that synthesize essential end products from raw materials. Stop production when already enough → save energy.

- Positive gene regulation – activator binds to site to stimulate gene expression (see lac operon above).

• Regulation of enzyme production (last page) vs. activity

feedback inhibition (anabolic): one enzyme in a synthesis pathway inhibited by end product [I]. ⇒[I] shuts down synthesis

* In a diploid cell, both genes are expressed, so an activator, repressor made on one affects the other.

9-1 Mendelian Genetics.

Mendel's experiment.

1. Model organism – pea plants
2. Developed control – develop true breeds
3. Experiment ○ × ◌ → ○*, ○* × ○* → ③ ◌, ...
4. Count & simplify ≈ 3:1.
5. Model + predict future outcomes.

Law of segregation: Each trait determined by 2 genes, one from each parent. Each parent gives 1 gene to each offspring.

Law of independent assortment: Different traits inherited independently [if on different chromosomes].

Dominant trait masks recessive trait.

Vocab.

- Genotype (which allele of a gene) vs. phenotype (observed trait)
- Homozygous (AA, aa) vs. heterozygous (Aa).
- Punnett Square. ⊞
- Test cross w/ homozygous recessive to determine genotype of individual

Sex-Linked Genes.

For humans:

Male ♂ XY
Female ♀ XX

X^A vs. X^a vs. Y

others are autosomes

- Sex-linked gene on sex chromosome, usually X. Inherited with sex chromosome

For genes on X:

- Female express recessive phenotype iff homozygous. Carrier if heterozygous
- Single X gene for male determines phenotype (hemizygous)
- Sex-linked disorders more likely to occur in males
  Ex. color blindness, Duchenne muscular dystrophy, hemophilia

(9-1-2) Other Patterns of Inheritance

- Codominance – both alleles affect phenotype in separate, distinguishable ways
- Incomplete dominance – heterozygous has phenotype "midway" between — lesser level of expression than homozygous dominant.
  Ex Snapdragons C^RC^R = red, C^RC^W = pink, C^WC^W = white
- Multiple alleles – ex. blood type I^A, I^B dominant, i recessive. (ii = O)
- Pleiotropy – gene with multiple effects
- Polygenic inheritance – additive effect of >1 gene on a single phenotypic character (ex. skin color)
- Epistasis – Gene at one locus (position) alters phenotypic expression of a second locus. (Us. determine if the 2nd will have any effect — ex. one controls if there's color, 2nd controls color)

For other organisms (flies),

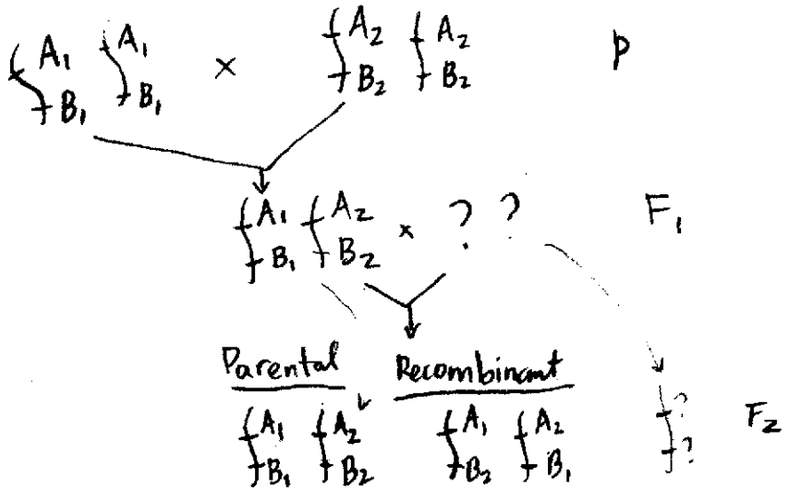
X^+ = wild type, dominant phenotype

X = recessive phenotype X.

Nondisjunction: Chromosomes or chromatids fail to separate in anaphase.

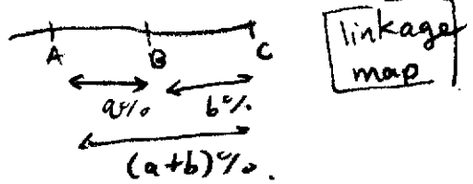
9-3 Chromosomal Basis of Inheritance.

- Genes located on chromosomes.
- Genes located close together on chromosome are <u>linked</u>, and usually inherited together.
- <u>Crossing over</u> causes maternal & paternal chromosomes to exchange a segment. The closer two genes are on a chromosome, the less likely that they will be separated during crossing over.
- <u>Parental genotype</u> - a chromosome has same combination of genes as a parent:



- <u>Recombination frequency</u> = $\frac{\text{recombinant}}{\text{total}}$ [centimorgans = cM]

Approximately additive, reveals relative position of genes on chromosome. If unlinked, RF = 50%.



Relationship between genotype & phenotype.

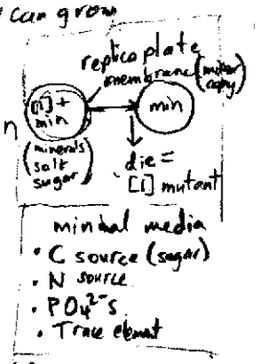
- Genes produce proteins affecting phenotype.
- A different allele may produce...
  - different protein w/ different effect → ex different amino acid
  - malfunctioning protein... / recessive.
  - no protein → ex. stop codon.
  - something that interferes w/ the protein made by the other allele (dominant disorder)
- Sometimes level (2 vs. 1) of expression important. (Note: XX) ↳ Barr body

Meiosis

- Given 2 chromosomes, 4 possible haploid daughter cells, but only 2 at a time.
- Contribution to evolution
  - → Independent assortment of chromosomes
  - → Crossing over.
  - → Random fertilization

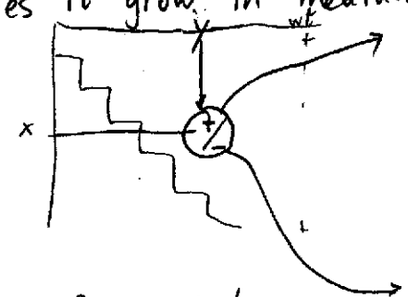
(0-1) Bacterial Genetics – Experiments

(xxx^- / xxx^+ — can't grow w/o xxx / can grow)

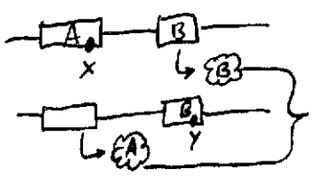


determine which mutations are on the same gene.

1. Genetic screen – mutagenize lots of cells, take the mutants that grow in a medium with the nutrient in question [1] but not without it (auxotrophs)
2. Complementation test – for each pair of mutants, make a diploid cell carrying the two mutations.
3. Does it grow in medium without [1]?



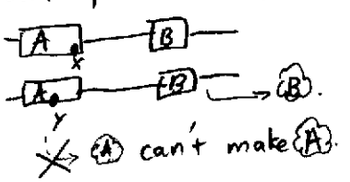
If can grow, x & y complement each other. Mutations are in different genes



→ both made (needed to process [1]).

[– 's form equivalence relation!

If can't, mutations are in the same gene.



If a gene CAN'T be complemented by wild type then it is dominant to wild type; else it is recessive.

* To determine the order that genes (enzymes) act in the synthesis of compound A_n. (Epistasis test)

$$A_0 \xrightarrow{1} A_1 \xrightarrow{2} A_2 \cdots \rightarrow A_{n-1} \xrightarrow{n} A_n$$

Make double (or n-tuple) mutant cells (on same chromosome) with different combinations of mutations (different genes) and examine phenotype (which intermediates are present/accumulate)

- If genes 1, … x-1 ok but x mutated, then A_{x-1} builds up
- If genes x+1, .. n ok but x mutated, need any of A_x, … A_n to grow
- Complementation: $A_0 \rightarrow A_1 \cdots A_{x-1} \not\rightarrow \cdots$
  $\cdots \not\leftarrow A_{x-1} \rightarrow \cdots \rightarrow A_n$

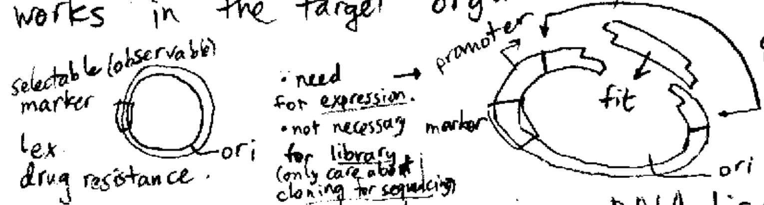
* Complementation test for phages.

- Phage lives = bacteria dies. Look for plaques (holes of dead bacteria)
- Temperature-sensitive mutations — phage only makes plaque at certain temperatures.
- Similar techniques for double mutant. ← can find recombination frequency, relative position

(10-2) Recombinant DNA → combine DNA sequences from 2 different sources

Gene cloning Recombinant DNA – Overview

1. Cut DNA at defined site with restriction enzymes.
2. Cut a vector (bacterial plasmid — DNA different from circular chromosome) at a unique defined site (Use a vector b/c it has an origin of replication that works in the target organism)



• Normal cell has no marker (no drug resistance).
• Cell w/ nonrecombinant vector has marker & makes X (blue)
• Cell w/ recombinant vector has marker & doesn't make X (white)
OR cut w/ r.e. & use gel electrophoresis
⇒ recombinant has ≥2 pieces
OR sequence

3. Join the DNA molecules with DNA ligase.
4. Transform the DNA into an organism
5. Select for cells that have acquired the vector (apply drug).

<u>Restriction enzyme</u> – recognizes & cuts particular DNA sequence.
ex. for bacterial immune system

ex. EcoRI

$$\begin{array}{l} -G_{OH}^{3'} \quad {}^{5'}_{PO_4}AATTC- \\ -CTTAA_{PO_4}^{5'} \quad {}^{3'}_{OH}G- \end{array}$$

sticky end

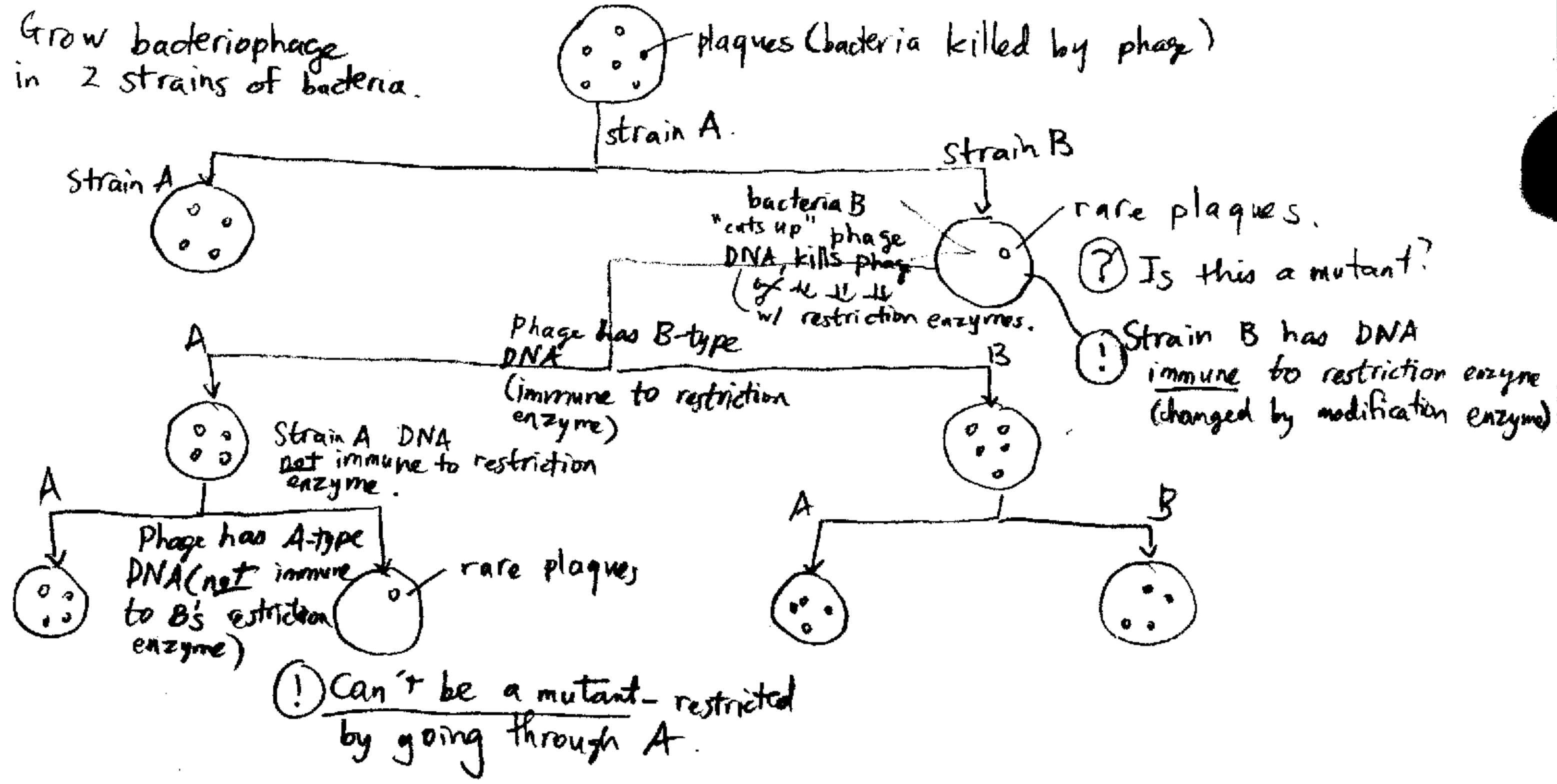
Modification Enzyme – shield own DNA sequence from restriction enzyme

ex.

$$\begin{array}{l} \quad\ \ CH_3 \\ -GAATTC- \\ -CTTAAG- \\ \quad\quad\ \ CH_3 \end{array}$$

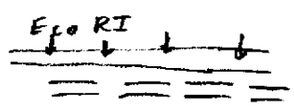
<u>Experiment</u> Existence of Restriction & modification enzymes

Grow bacteriophage in 2 strains of bacteria.

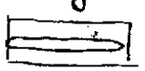


• Construction of Recombinant DNA library.

- Use restriction enzyme to cut DNA.

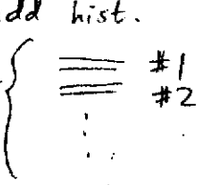


① Finding clone by complementation

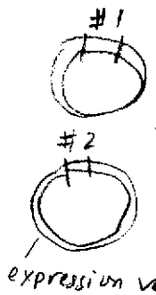


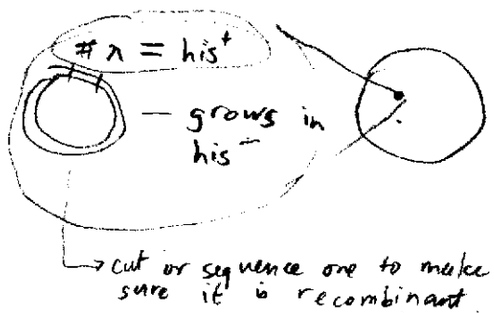
Can't grow on min glucose unless add hist.

Which gene is his⁺?



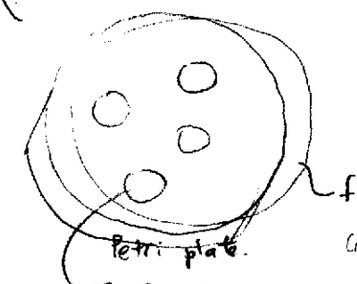
Transform all in

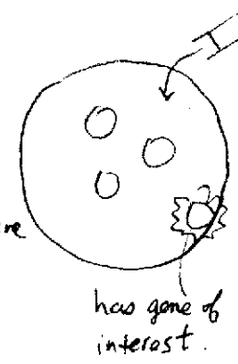




② Cloning by sequence of protein (Nucleic Acid Probe Hybridization)

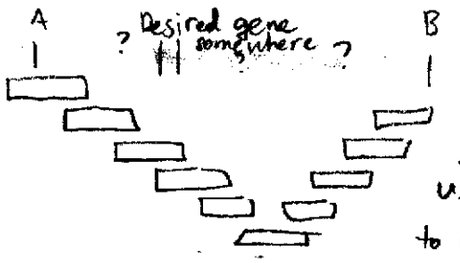
ignore ✗ - From sequence of amino acids of protein produced from gene, synthesize all possible DNA sequences.





- Need to know part of gene sequence of interest (to part of gene)
- Break open cell, DNA
- DNA sticks to filter
- Radioactive probe molecules complementary to gene of interest applied
- Probe sticks to complementary strand
- Identify by radioactivity → locate colonies w/ gene of interest

③ Cloning by position (physical mapping)

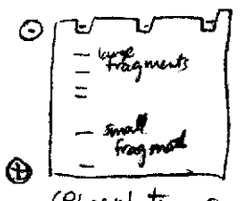


Cut w/ different restriction enzymes.

use overlapping strands to piece together entire sequence.

• Restriction map.

- Use different restriction enzymes to cut
- Gel electrophoresis - separate DNA pieces by size



- Determine relative positions of cuts by comparing lengths of resulting segments, as with recombination frequencies.