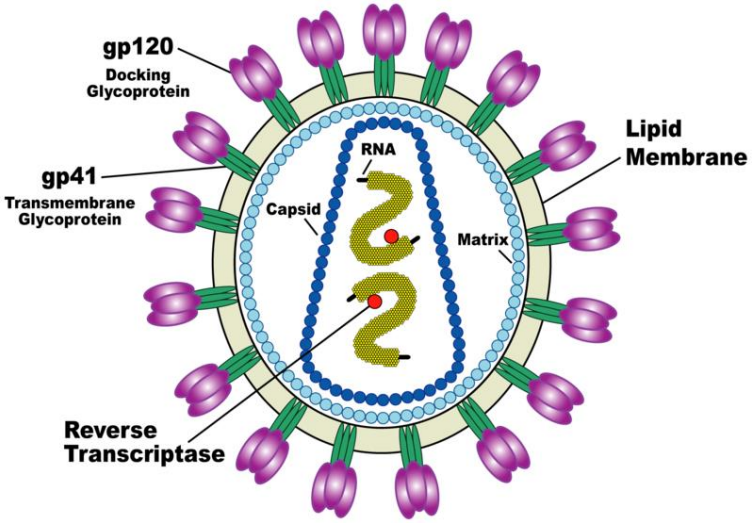
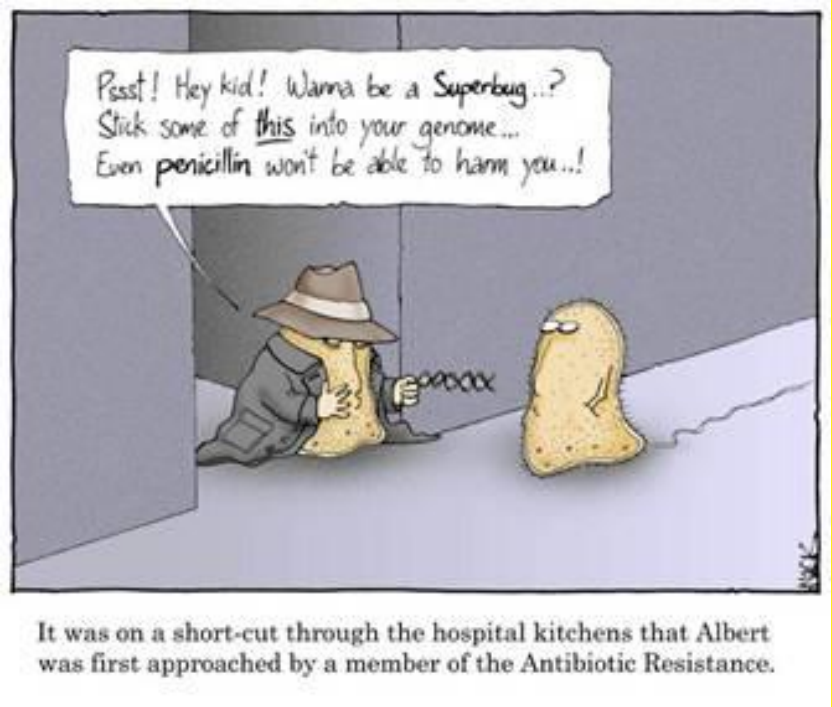
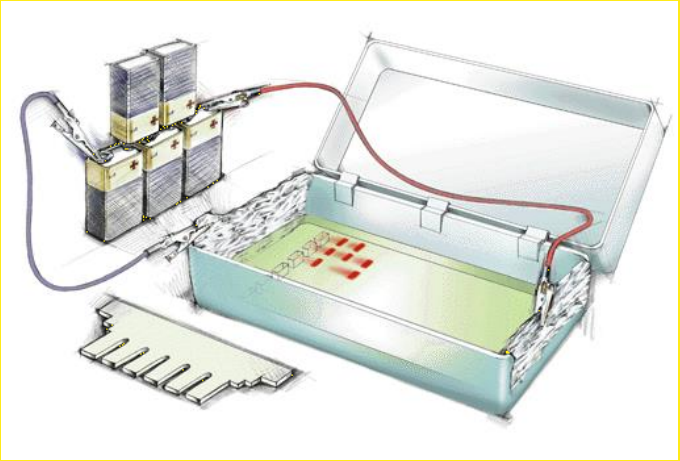
http://foodsafety.wisc.edu/assets/foodfacts_2007/wffFeb2007_clip_image002.jpg

VIRUSES, BACTERIA, and PRIONS

https://www.msu.edu/course/isb/202/ebertmay/images/HIV%20virus.png



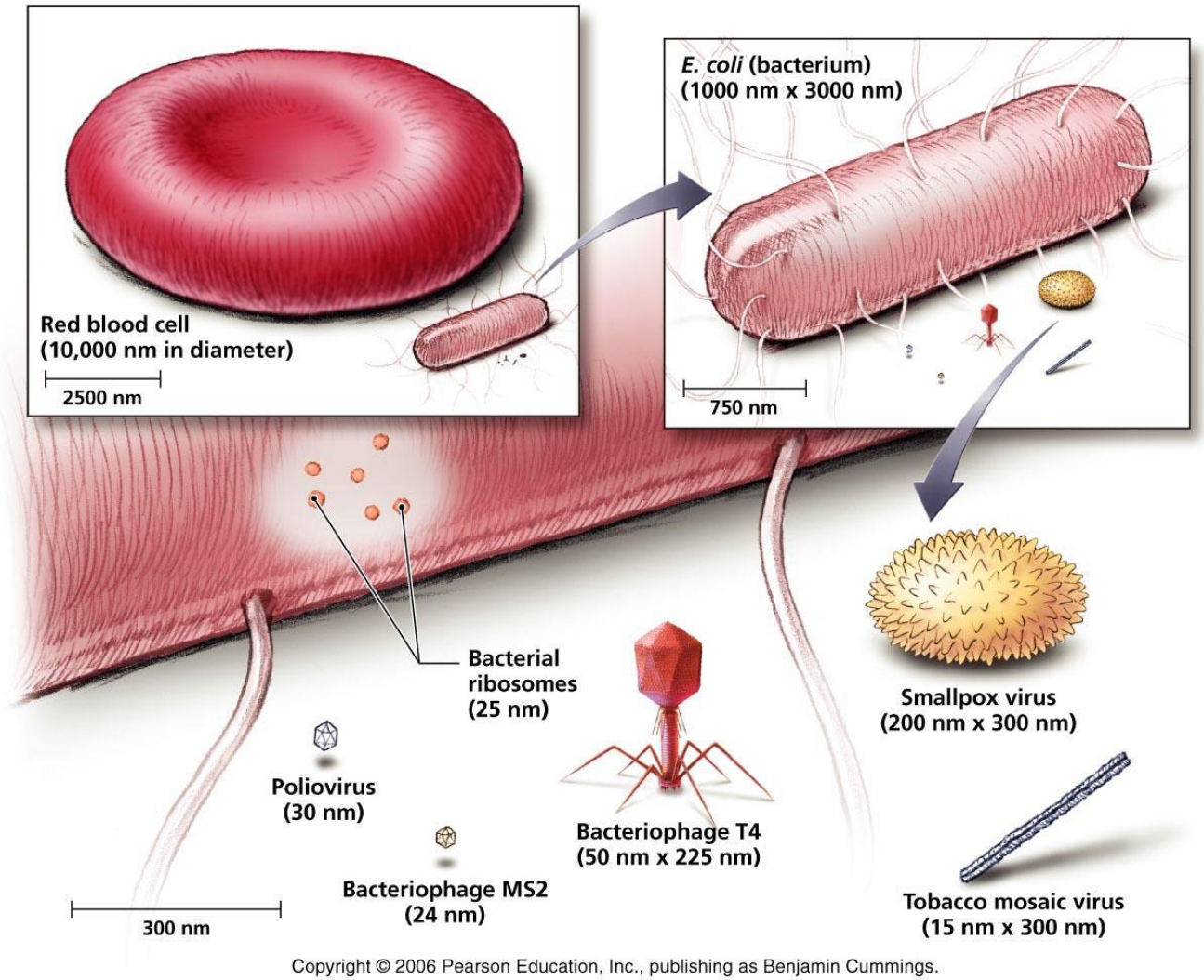




http://www.scifair.org/+images/Electrophoresis.gif

VIRUSES

- **Tiny: smaller than ribosomes**

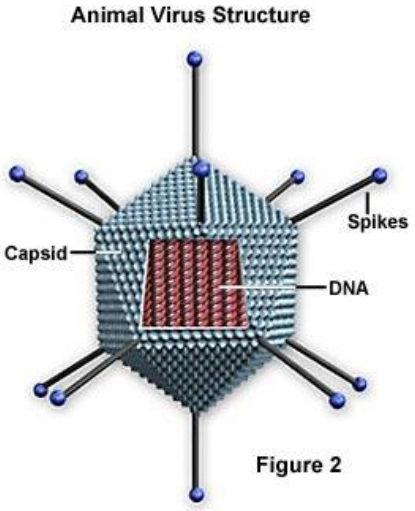


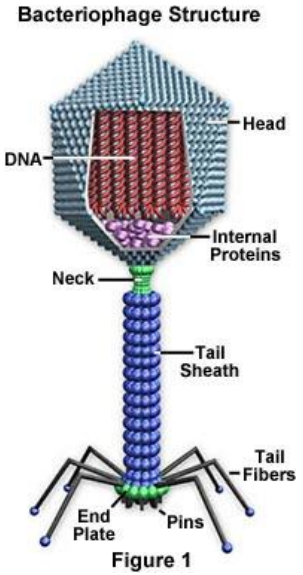
http://academic.pgcc.edu/~kroberts/Lecture/Chapter%2013/13-04_SizesOfViruses_0_L.jpg

VIRUSES

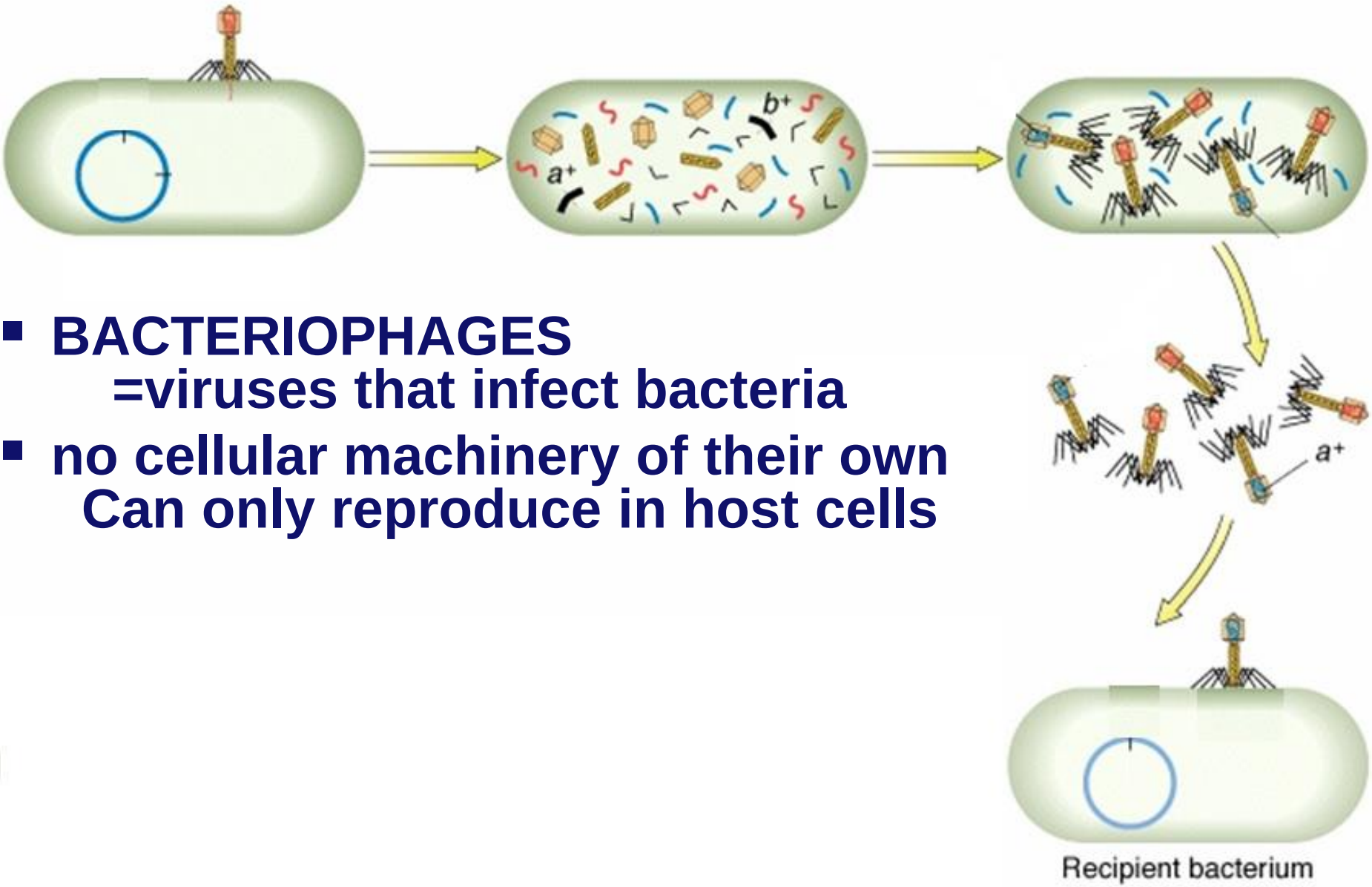
- Contain DNA or RNA
- SINGLE or DOUBLE stranded

- NUCLEIC ACID surrounded by PROTEIN coat = CAPSID
- Some have ENVELOPE outside capsid





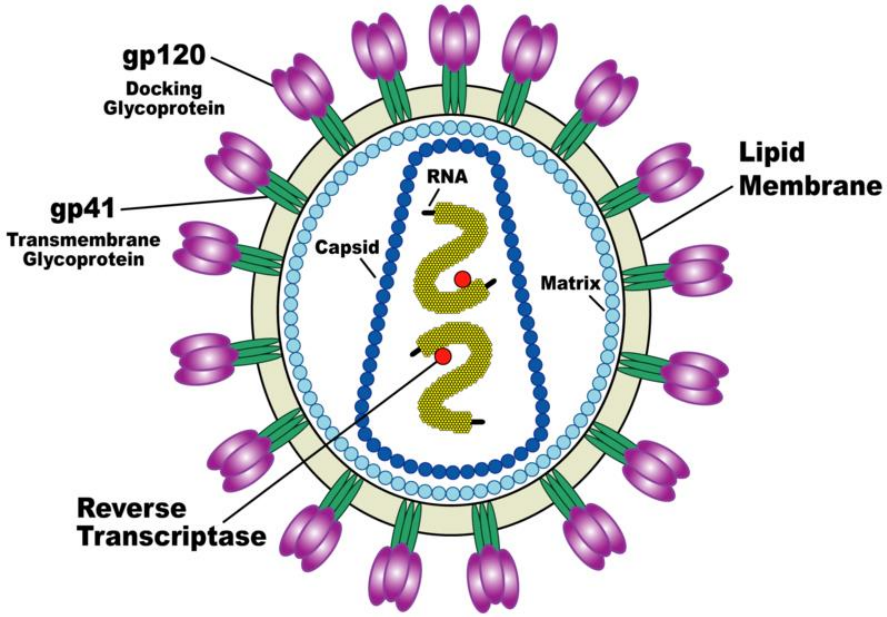
http://micro.magnet.fsu.edu/cells/virus.html



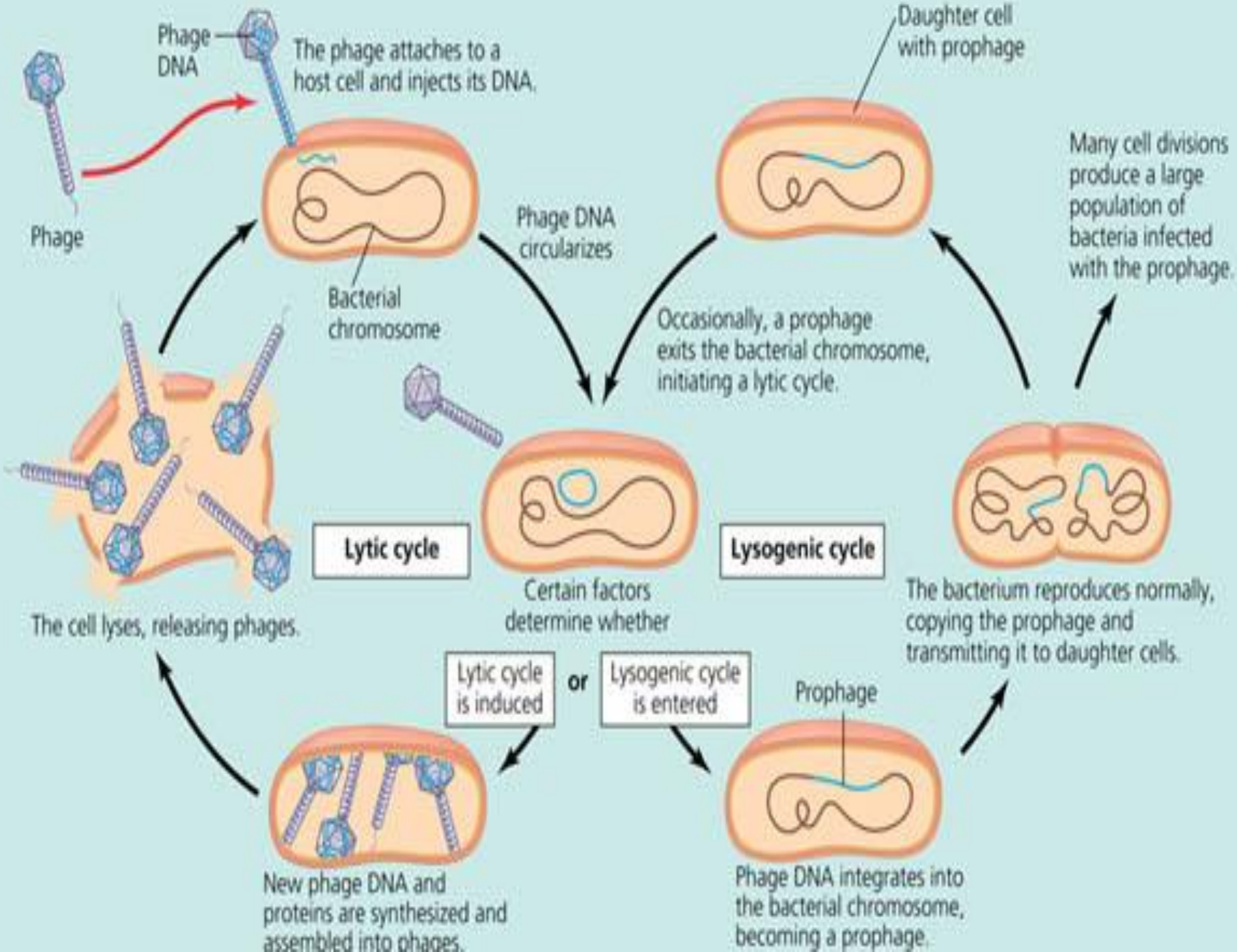
- **BACTERIOPHAGES**
  =viruses that infect bacteria
- **no cellular machinery of their own**
  Can only reproduce in host cells

HIV (Human Immunodeficiency Virus) AIDS virus

- RETROVIRUS (Contains RNA)
- Infects WHITE BLOOD CELLS
- Has REVERSE TRANSCRIPTASE
  Enzyme that can use RNA to make DNA

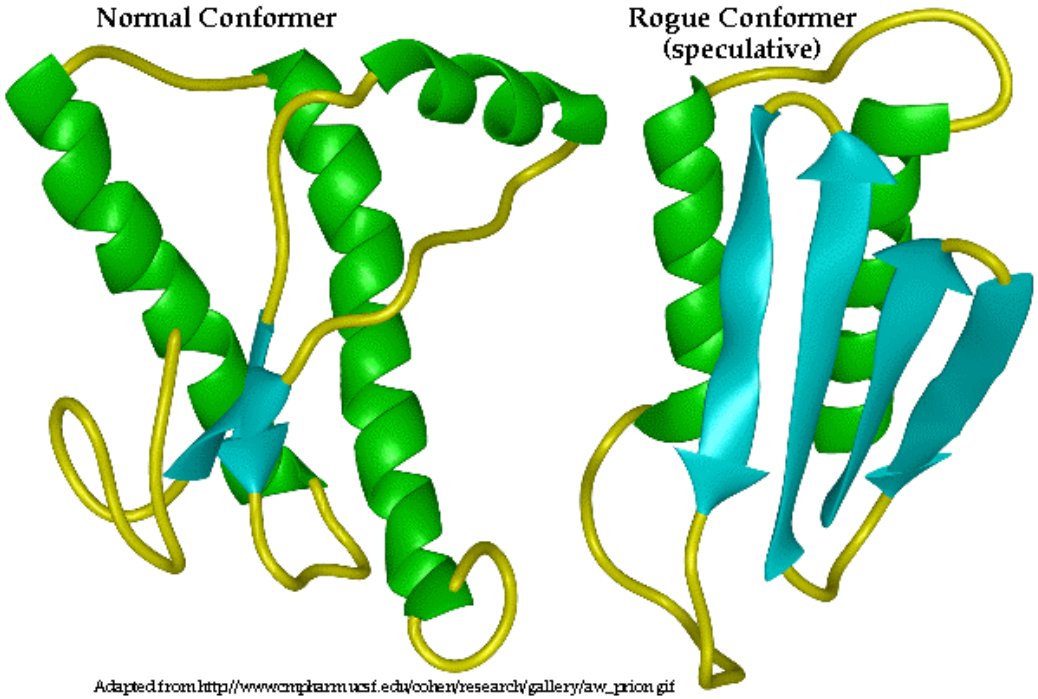


https://www.msu.edu/course/isb/202/ebertmay/images/HIV%20virus.png


Phage
DNA
The phage attaches to a host cell and injects its DNA.
Phage
Bacterial chromosome
Phage DNA circularizes
Daughter cell with prophage
Many cell divisions produce a large population of bacteria infected with the prophage.
Occasionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.
Lytic cycle
Lysogenic cycle
Certain factors determine whether
The cell lyses, releasing phages.
The bacterium reproduces normally, copying the prophage and transmitting it to daughter cells.
Lytic cycle is induced
or
Lysogenic cycle is entered
Prophage
New phage DNA and proteins are synthesized and assembled into phages.
Phage DNA integrates into the bacterial chromosome, becoming a prophage.

PRIONS

- "Misshaped" proteins
- Change the shape of other proteins they contact
- Aggregates of proteins accumulate in brain

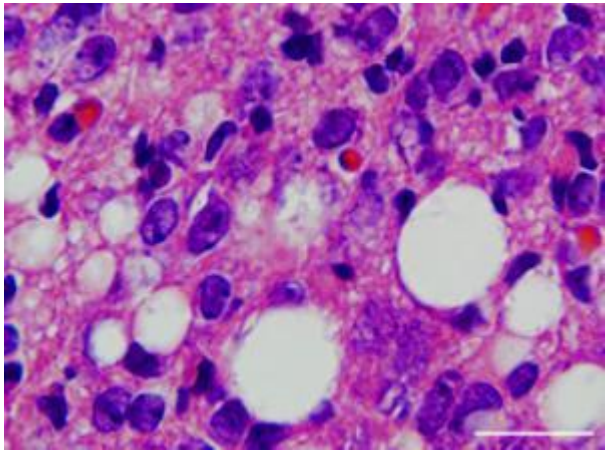


Adapted from http://www.cmpharm.ucsf.edu/cohen/research/gallery/aw_prion.gif

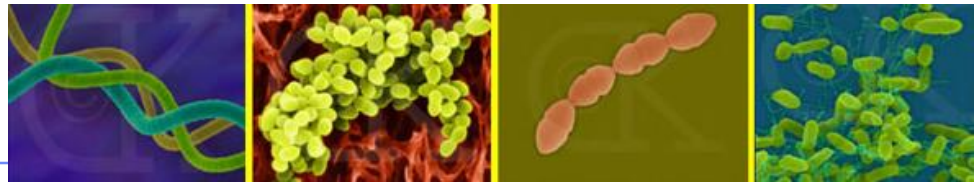
PRIONS

- Aggregates of proteins accumulate in brain
- Neurological disorders

- SCRAPIE in sheep
- BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) = “Mad Cow” disease
- CHRONIC WASTING DISEASE

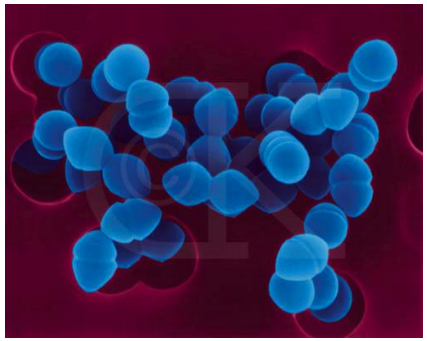


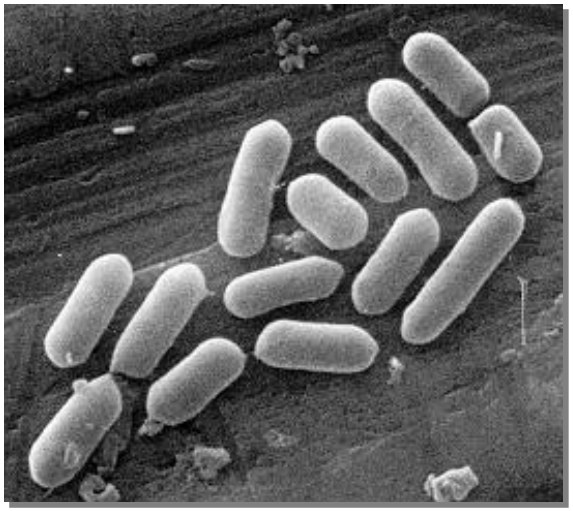
- KURU
- CREUTZFELD-JAKOB

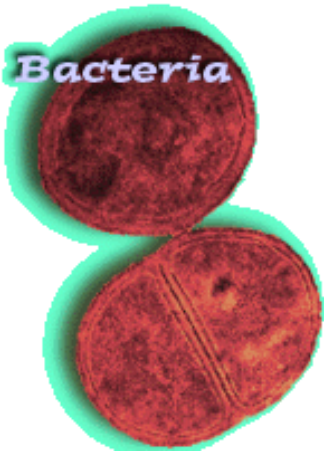


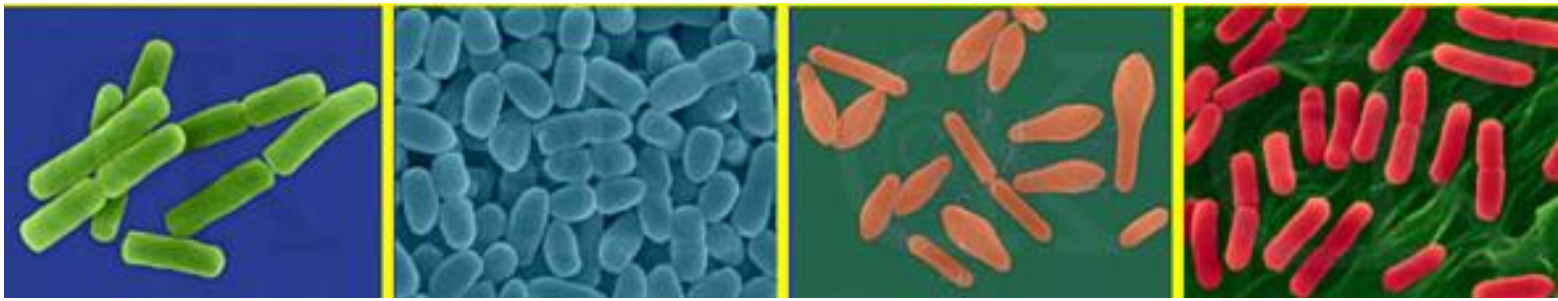
Bacteria

- Slide show by Kim Foglia (modified)
  Blue edged slides are Kim's

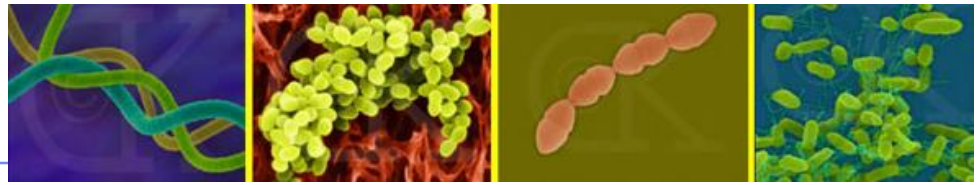




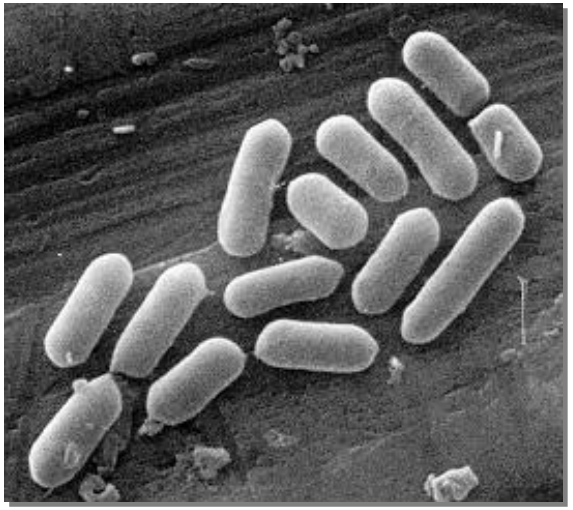


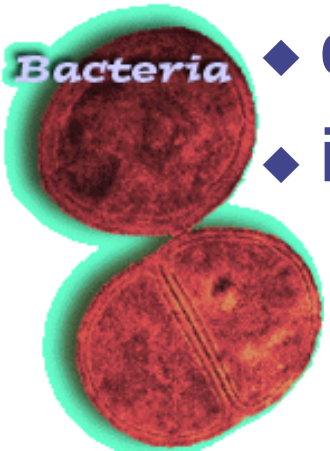


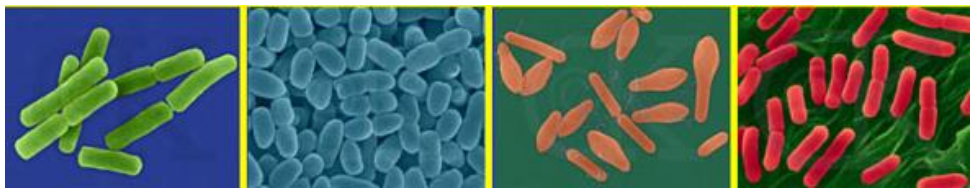
Bacteria

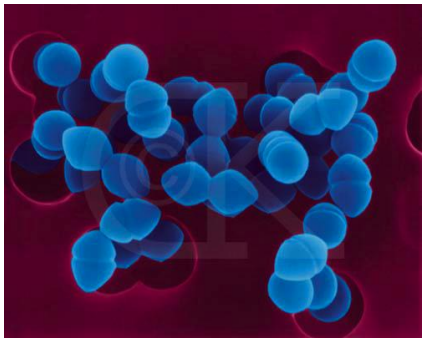


- **Bacteria review**
  - one-celled prokaryotes
  - reproduce by mitosis
    - binary fission
  - rapid growth
    - generation every ~20 minutes
    - 10^8 (100 million) colony overnight!
  - dominant form of life on Earth
  - incredibly diverse



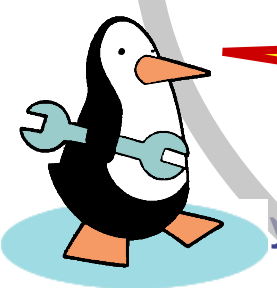




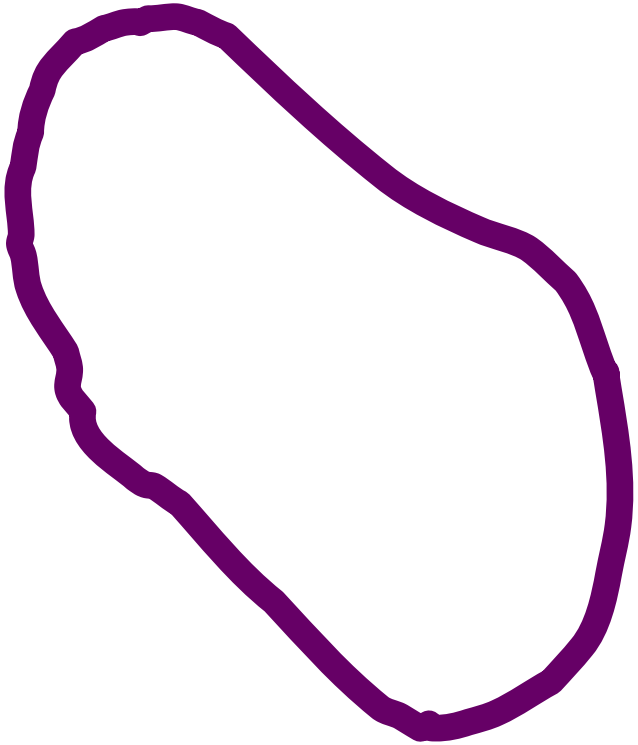


Bacterial genome

- Single circular chromosome
  - haploid
  - naked DNA
    - no histone proteins
  - ~4 million base pairs
    - ~4300 genes
    - 1/1000 DNA in eukaryote

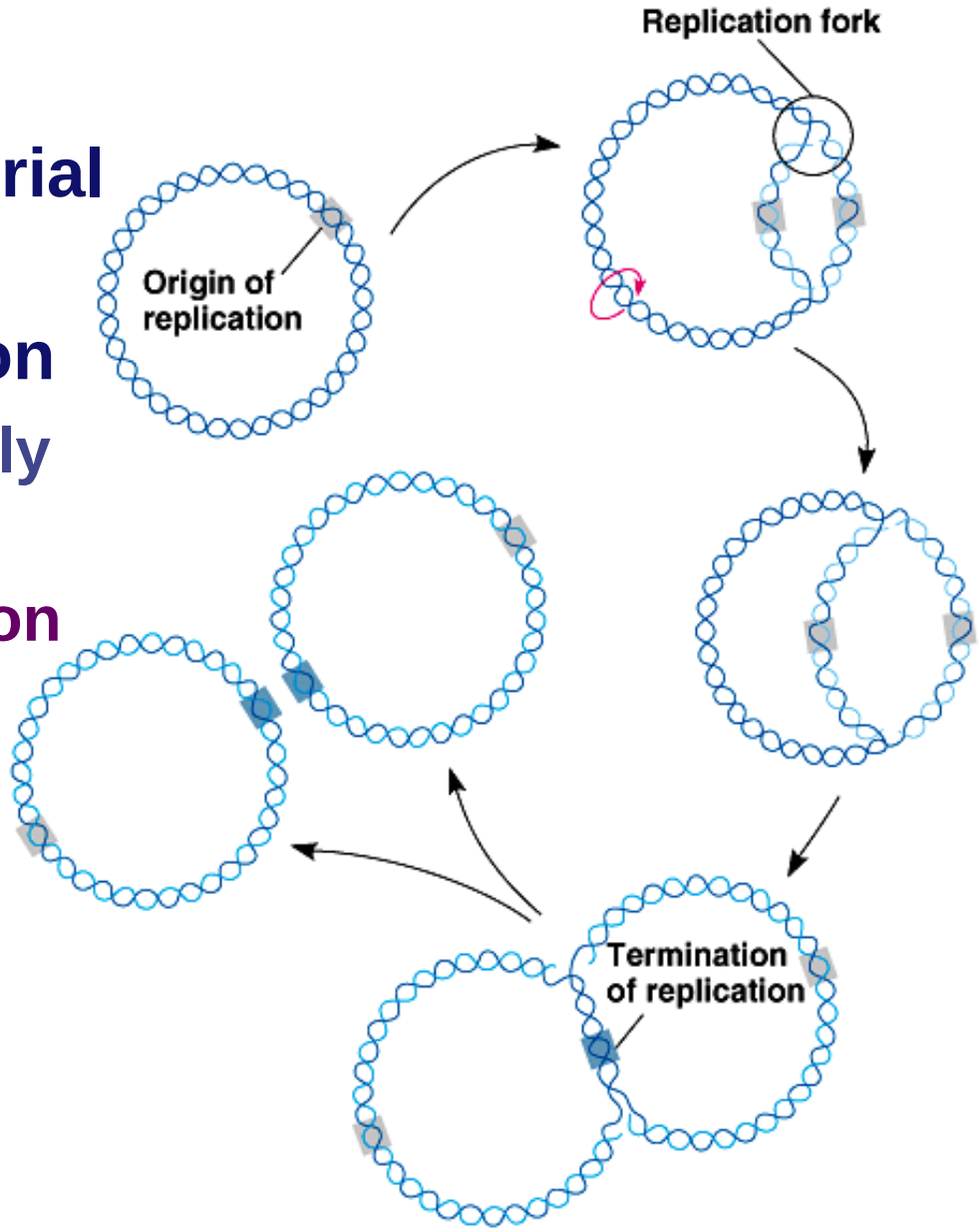


How have these little guys gotten to be so diverse??



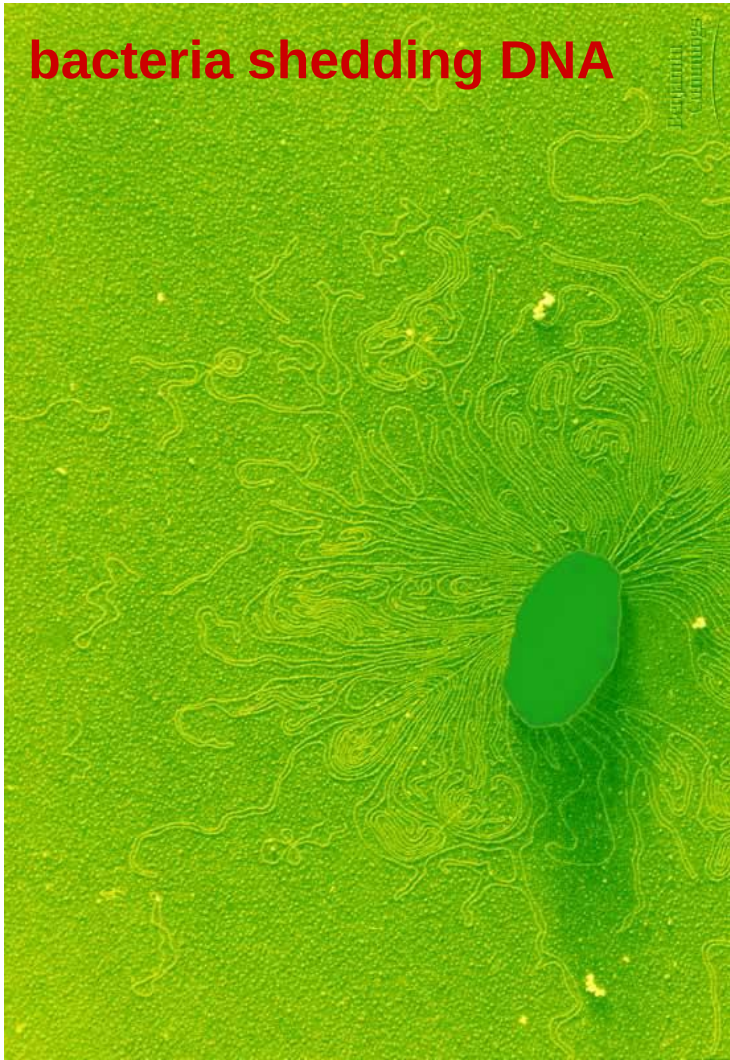
Binary fission

- Replication of bacterial chromosome
- Asexual reproduction
  - offspring genetically identical to parent
  - where does variation come from?

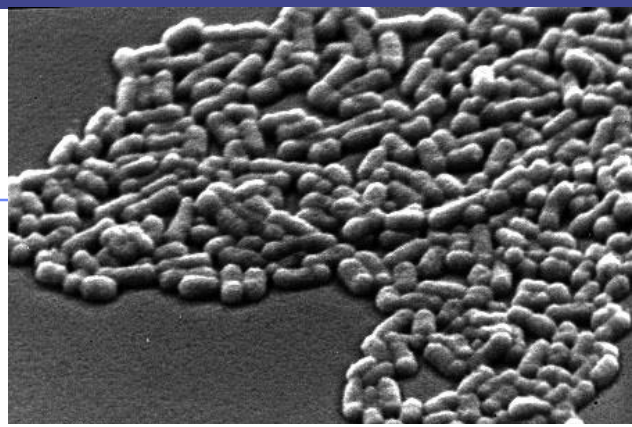


Variation in bacteria

- Sources of variation
  - spontaneous mutation
  - transformation
    - plasmids
    - DNA fragments
  - transduction
  - conjugation
  - transposons



Spontaneous mutation



- **Spontaneous mutation is a significant source of variation in rapidly reproducing species**
- **Example: E. coli**
  - **human colon (large intestines)**
  - **2 x 10^{10} (billion) new E. coli each day!**
  - **spontaneous mutations**
    - **for 1 gene, only ~1 mutation in 10 million replications**
    - **each day, ~2,000 bacteria develop mutation in that gene**
    - **but consider all 4300 genes, then:**
      4300 x 2000 = 9 million mutations per day per human host!

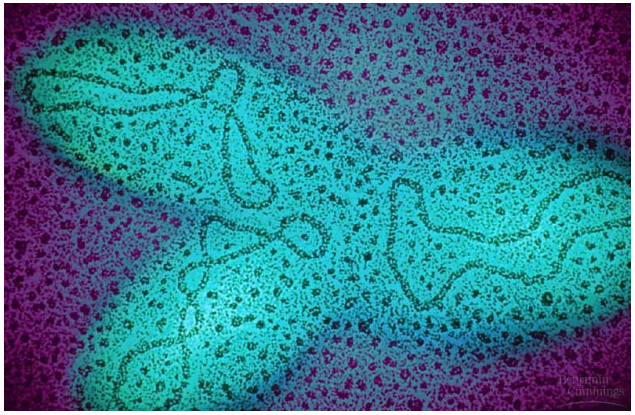
Transformation

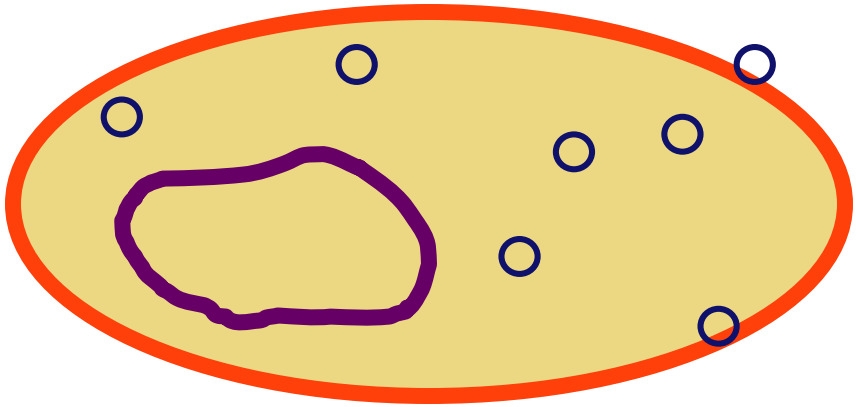
- **Bacteria are opportunists**
  - pick up naked foreign DNA wherever it may be hanging out
    - have surface transport proteins that are specialized for the uptake of naked DNA
  - import bits of chromosomes from other bacteria
  - incorporate the DNA bits into their own chromosome
    - express new genes
    - transformation
    - form of recombination

mix heat-killed pathogenic & non-pathogenic *bacteria*

Plasmids

- **Small supplemental circles of DNA**
  - 5000 - 20,000 base pairs
  - self-replicating
  - carry extra genes
    - 2-30 genes
    - genes for antibiotic resistance
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
  - can be imported from environment



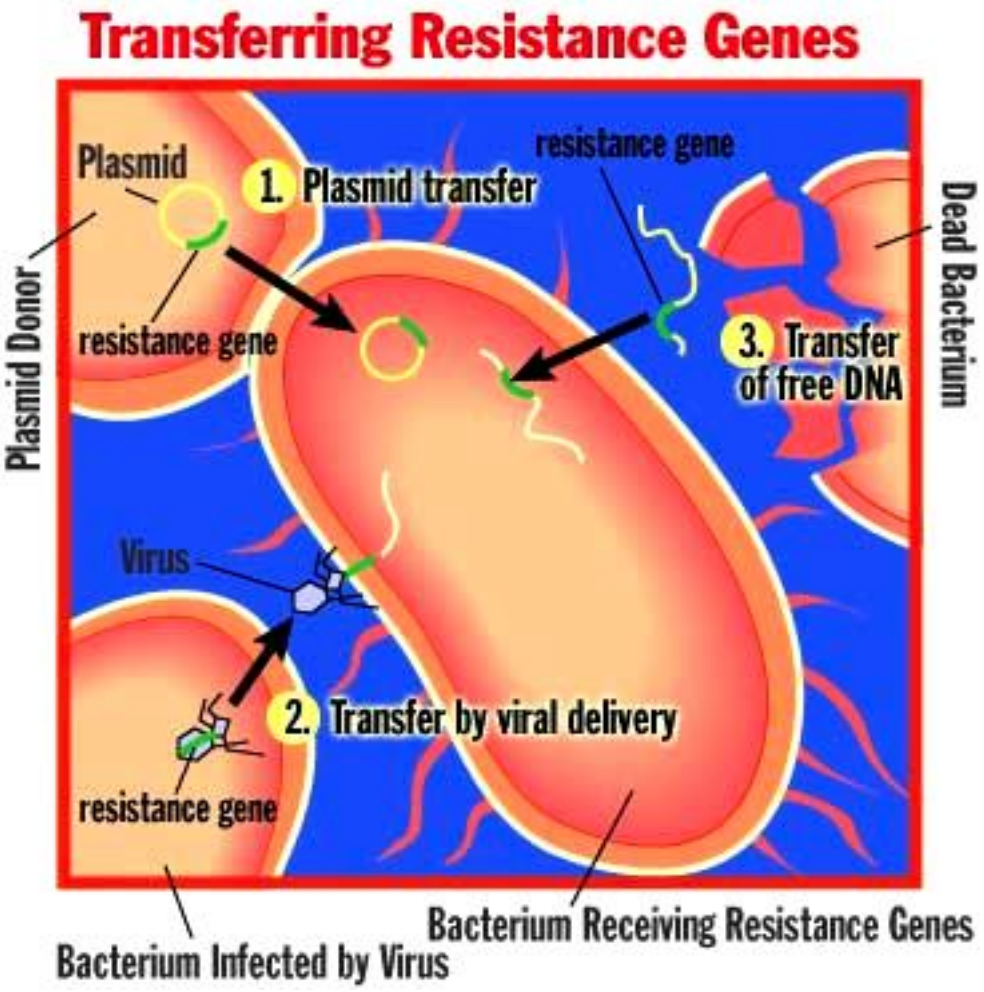


It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

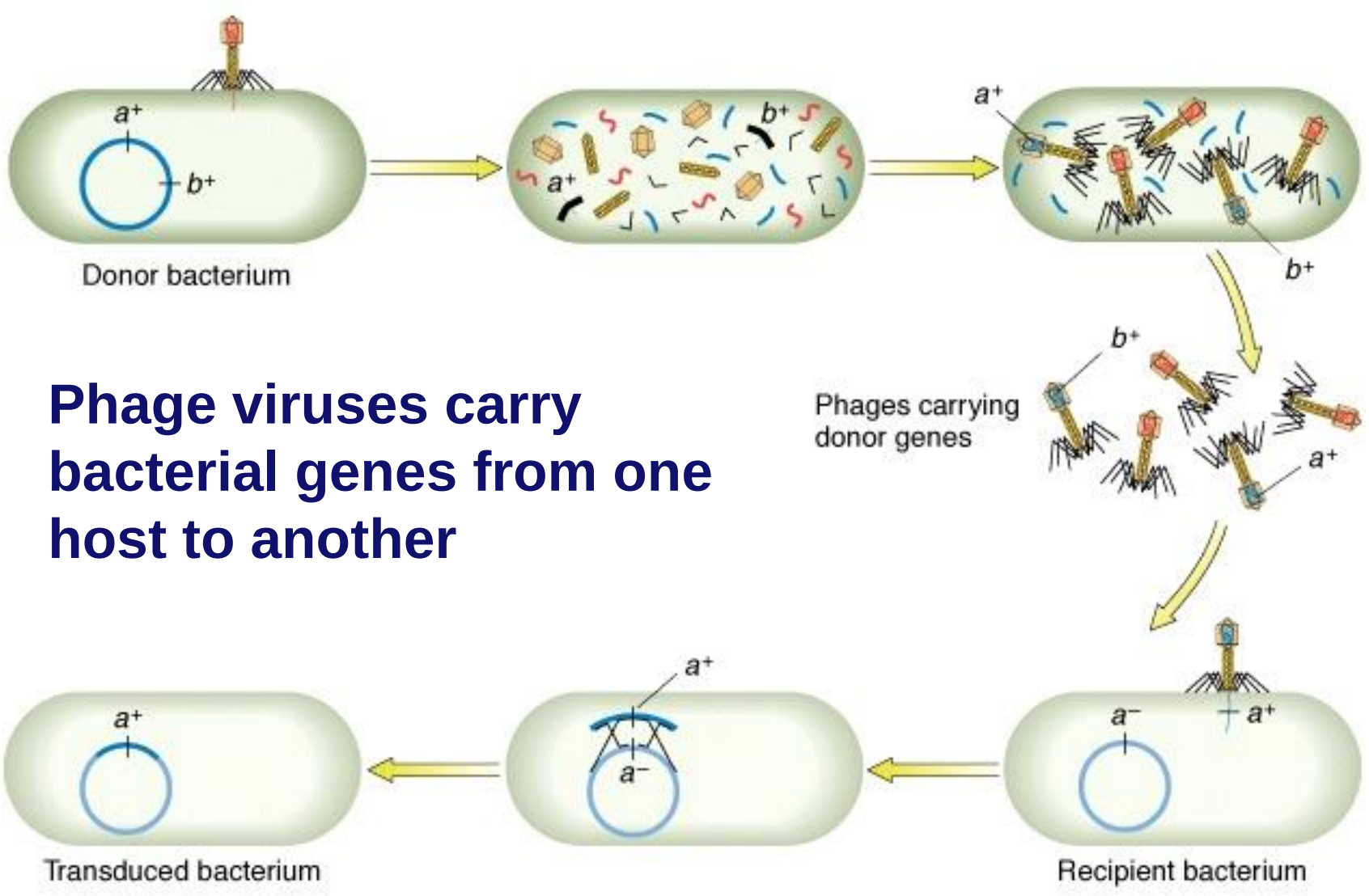
- **Genes for antibiotic resistance = R Plasmids**
- **Role in rapid evolution**
- **Method for spreading "antibiotic resistance"**

Plasmids & antibiotic resistance

- **Resistance is futile?**
  - 1st recognized in 1950s in Japan
  - bacterial dysentery not responding to antibiotics
  - worldwide problem now
    - resistant genes are on plasmids that are swapped between bacteria



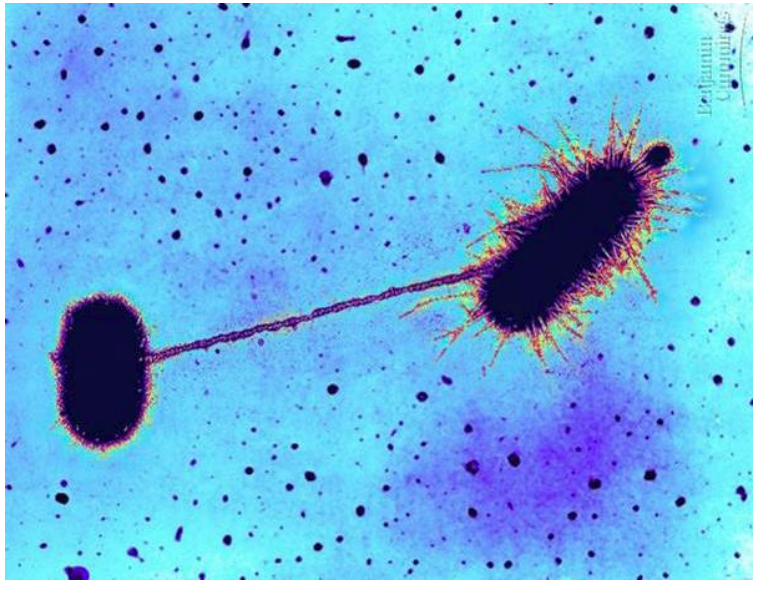
TRANSDUCTION with viruses



Phage viruses carry bacterial genes from one host to another

Conjugation - Bacteria "sex" Animation

- **Direct transfer of DNA between 2 bacterial cells that are temporarily joined**
  - **results from presence of F (fertility) plasmid**
  - **"male" extends sex pilli and attaches to "female" bacterium**
  - **cytoplasmic bridge allows transfer of DNA**

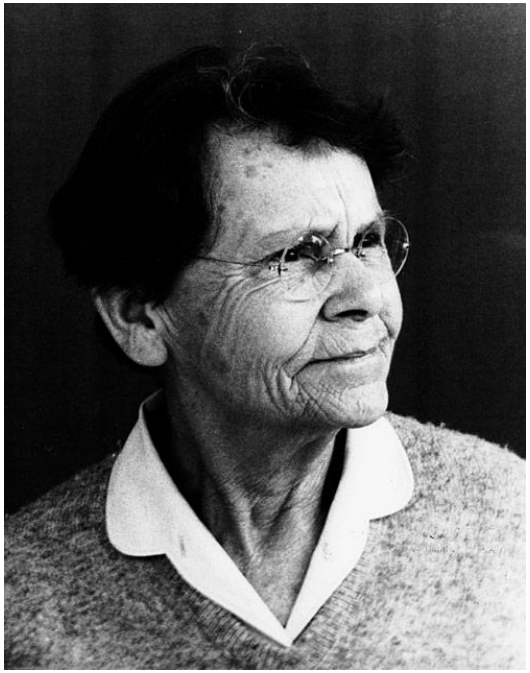


AP

TRANSPOSONS (Transposable elements)

- "Jumping" genes
- Can move from one place to another
- 1st described by Barbara McClintock in corn
- Can move genes to new site

http://www.osti.gov/accomplishments/images/mcclintock_05.jpg



http://www.nature.com/nature/journal/v443/n7111/images/443521a-i1.0.jpg

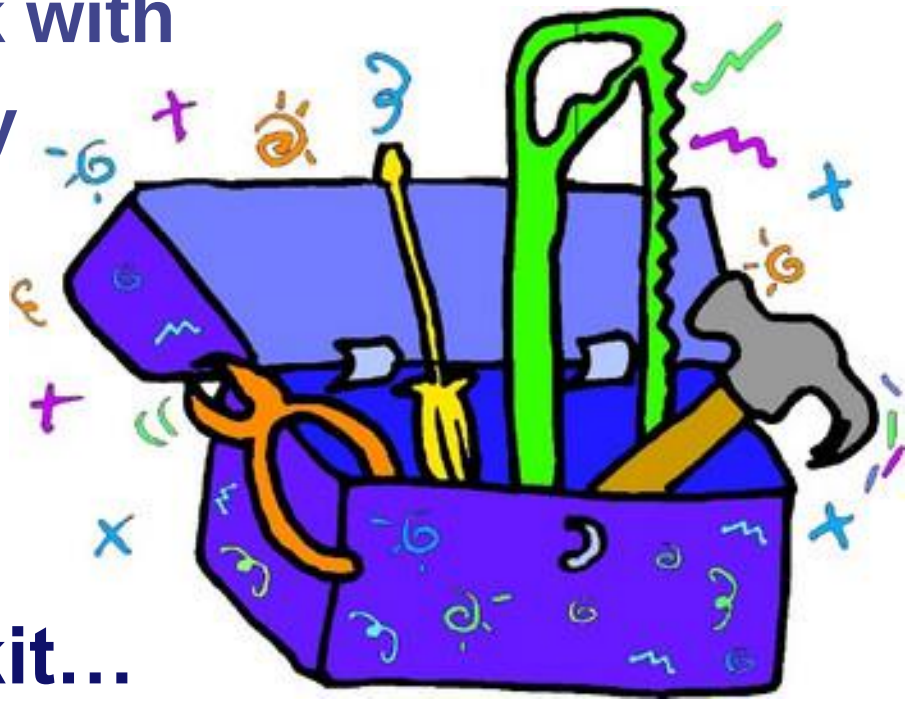
Biotechnology

Slide show by Kim Foglia (modified)

Blue edged slides are Kim's

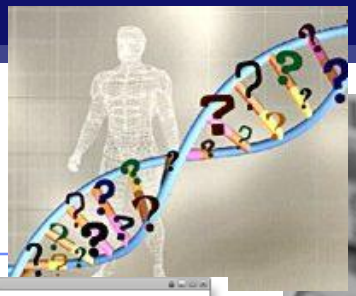
Biotechnology today

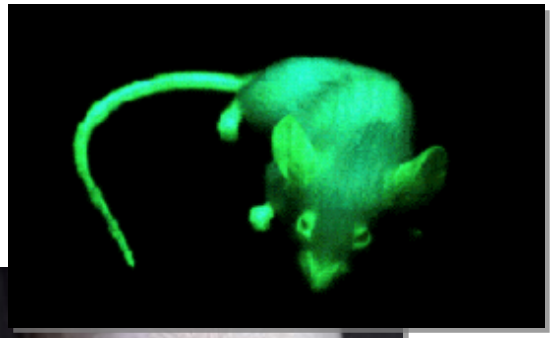
- **Genetic Engineering**
  - manipulation of DNA
  - if you are going to engineer DNA & genes & organisms, then you need a **set of tools** to work with
  - this unit is a survey of those tools…

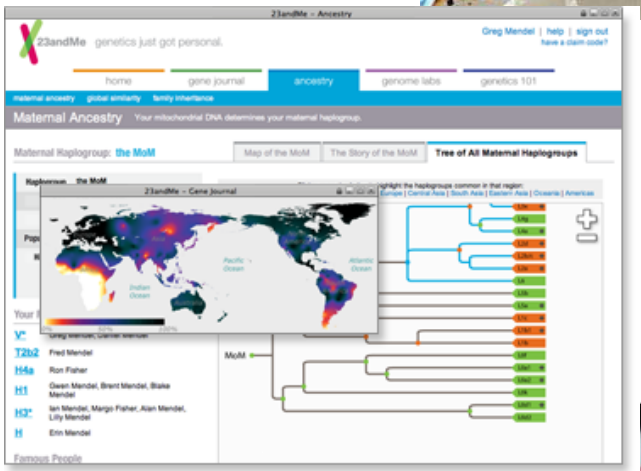


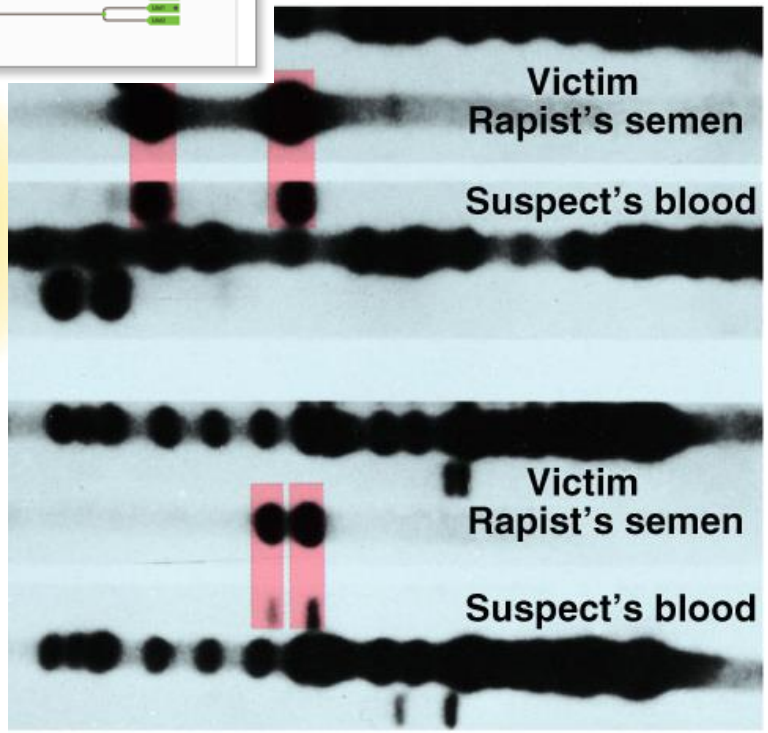
Our tool kit…

A Brave New World

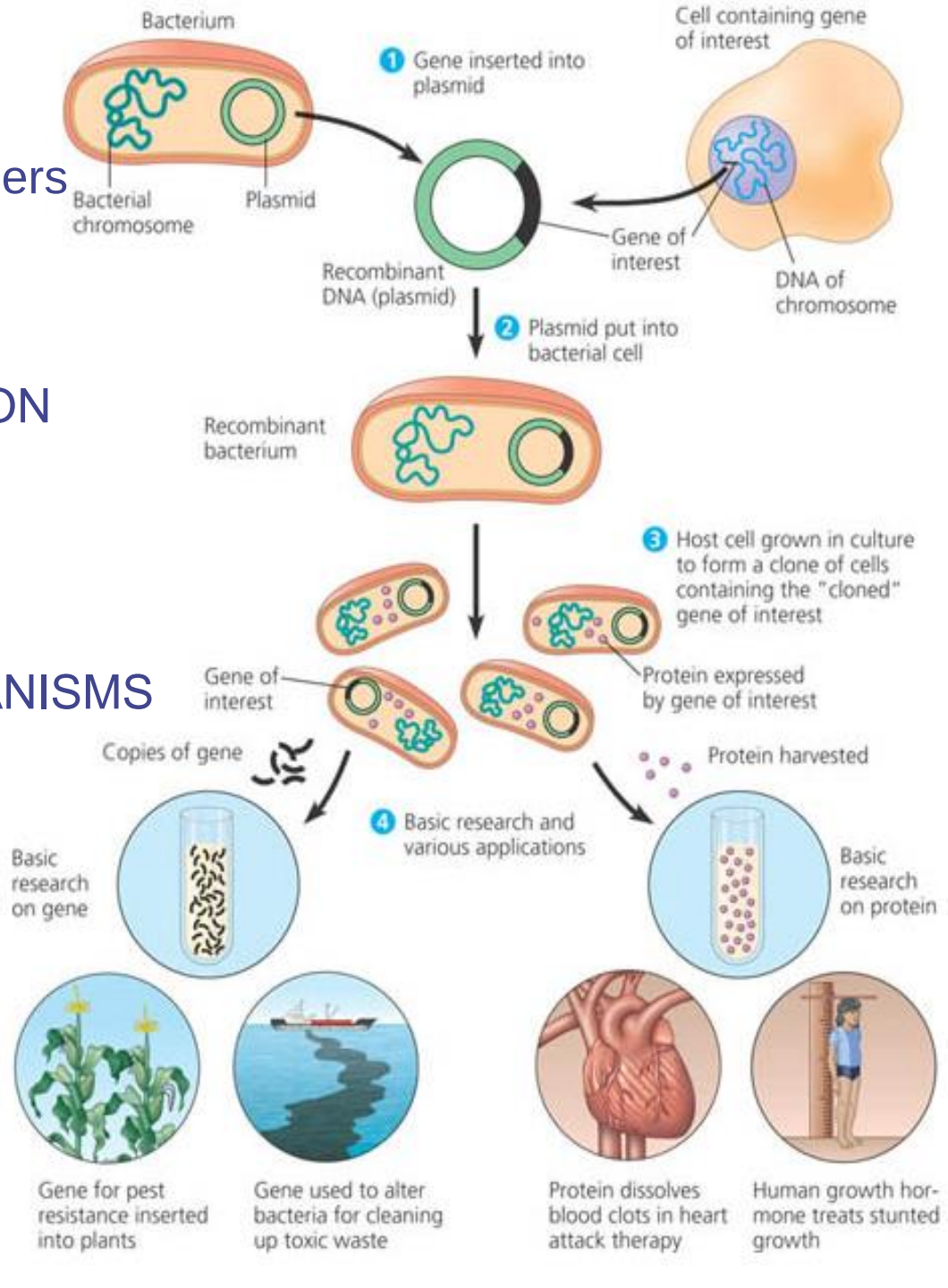








- DIAGNOSIS OF DISEASE
  - Virus detection; ID genetic carriers
- GENE THERAPY
  - ID mutant genes;
  - purify genes
- PHARMACEUTICAL PRODUCTION
  - Bacterial production of insulin,
  - Human Growth hormone, etc
- FORENSICS
  - Crime scene analysis
- GENETICALLY MODIFIED ORGANISMS
  - “Golden” rice (Vitamin A)
  - Bt-corn-resists insect pests
  - Toxic cleanup bacteria

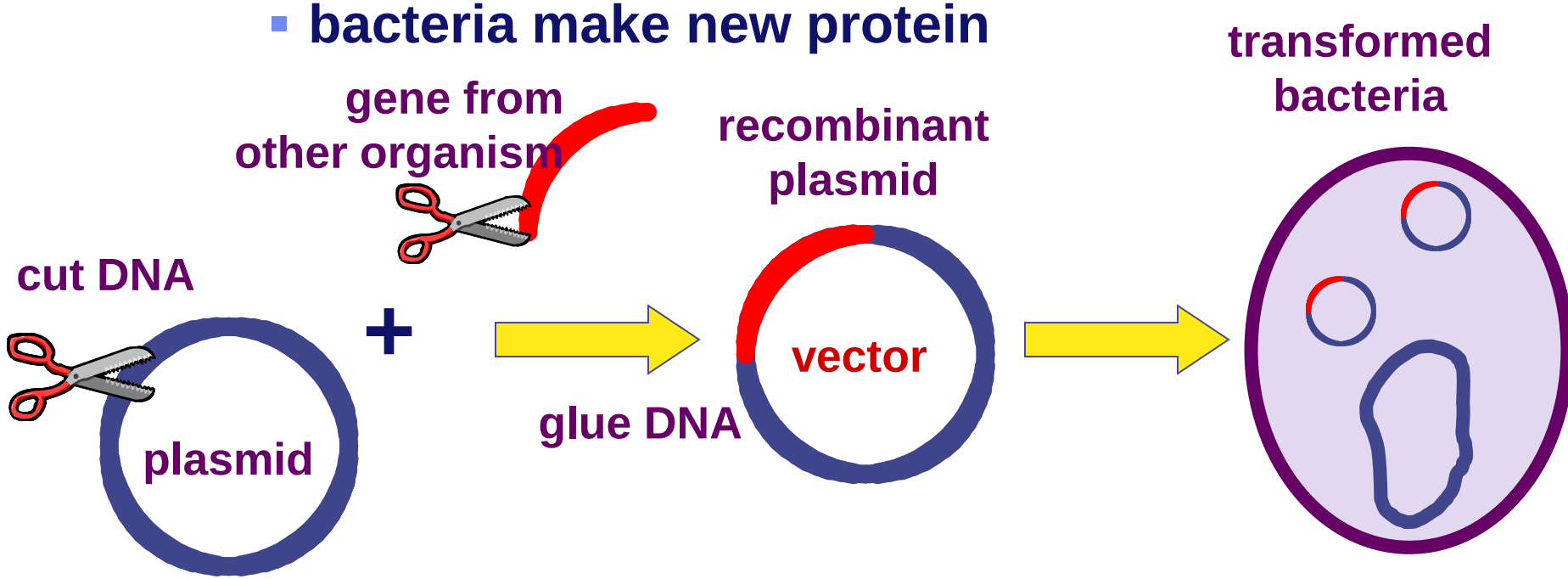


Uses of genetic engineering

- **Genetically modified organisms (GMO)**
  - **enabling plants to produce new proteins**
    - **Protect crops from insects: BT corn**
      - corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)
    - **Extend growing season: fishberries**
      - strawberries with an anti-freezing gene from flounder
    - **Improve quality of food: golden rice**
      - rice producing vitamin A improves nutritional value

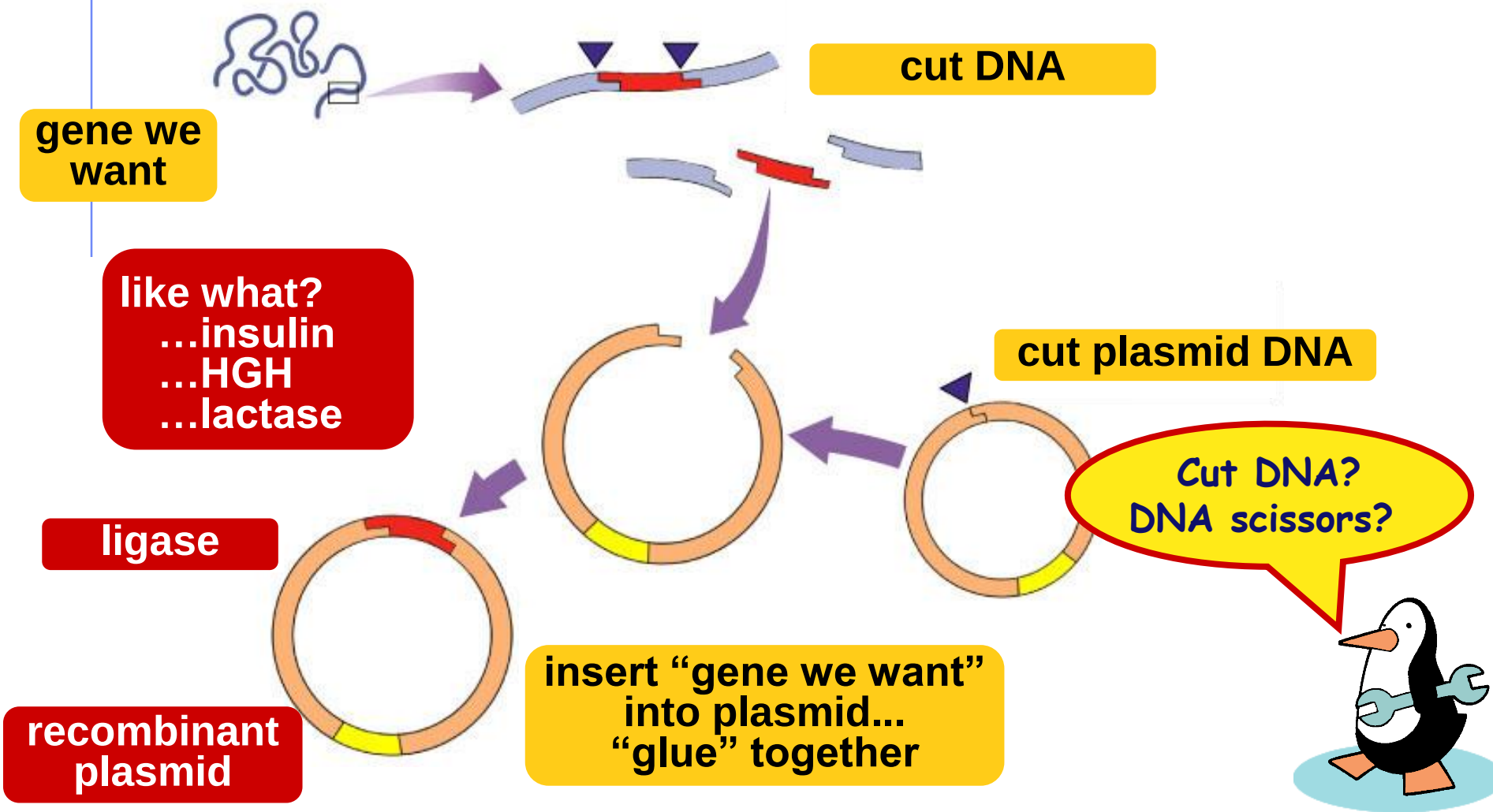
How can plasmids help us?

- A way to get genes into bacteria easily
  - insert new gene into plasmid
  - insert plasmid into bacteria = vector
  - bacteria now expresses new gene
    - bacteria make new protein

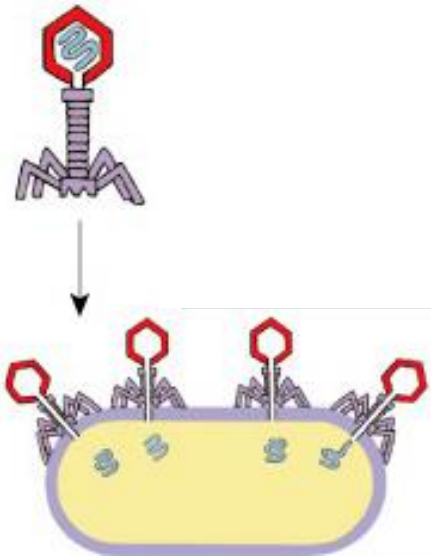


Biotechnology

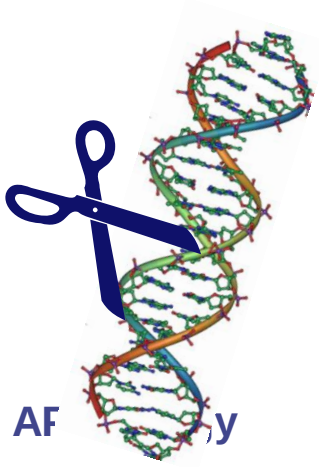
- Plasmids used to insert new genes into bacteria

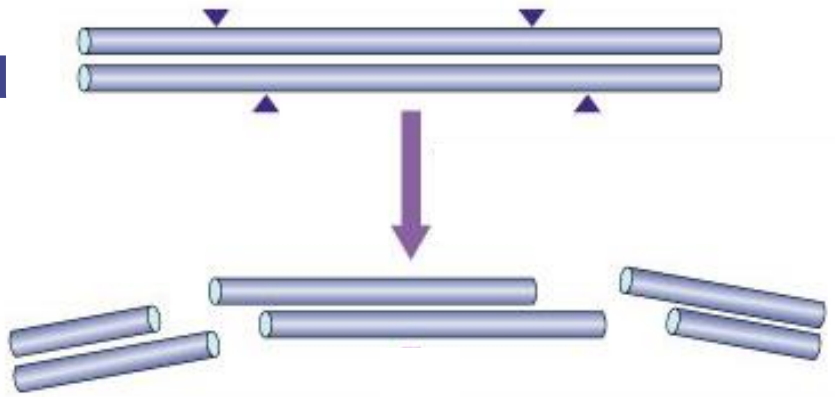


How do we cut DNA?



- **Restriction enzymes**
  - **restriction endonucleases**
  - **discovered in 1960s**
  - **evolved in bacteria to cut up foreign DNA**
    - **"restrict" the action of the attacking organism**
    - **protection against viruses & other bacteria**
      - **bacteria protect their own DNA by methylation & by not using the base sequences recognized by the enzymes in their own DNA**





What do you notice about these phrases?

radar

racecar

Madam I’m Adam

Able was I ere I saw Elba

a man, a plan, a canal, Panama

Was it a bar or a bat I saw?

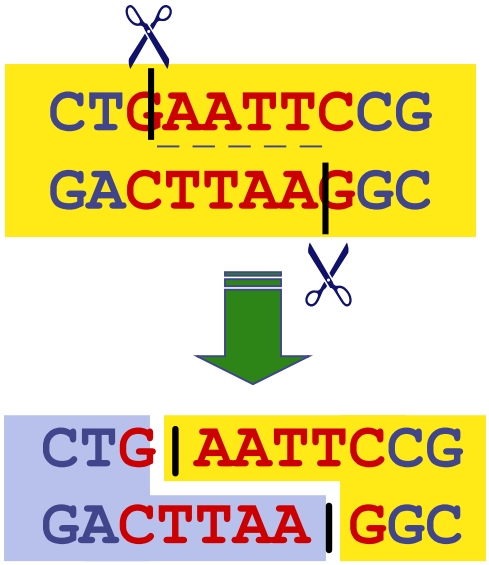
go hang a salami I’m a lasagna hog

palindromes

Madam I'm Adam

Restriction enzymes

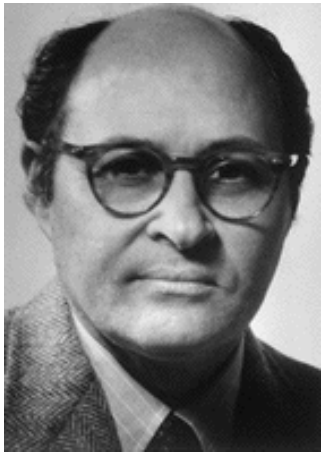
- Action of enzyme
  - cut DNA at specific sequences
    - restriction site
  - symmetrical "palindrome"
  - produces protruding ends
    - sticky ends
    - will bind to any complementary DNA
- Many different enzymes
  - named after organism they are found in
    - EcoRI, HindIII, BamHI, SmaI



1960s | 1978

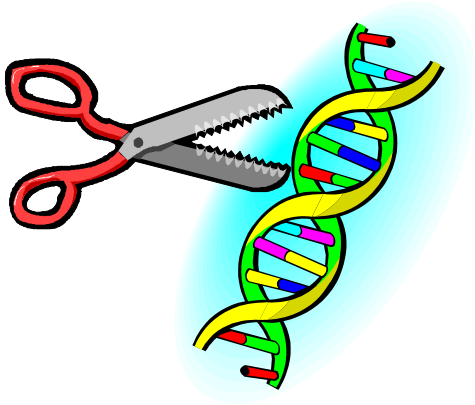
Discovery of restriction enzymes

Werner Arber



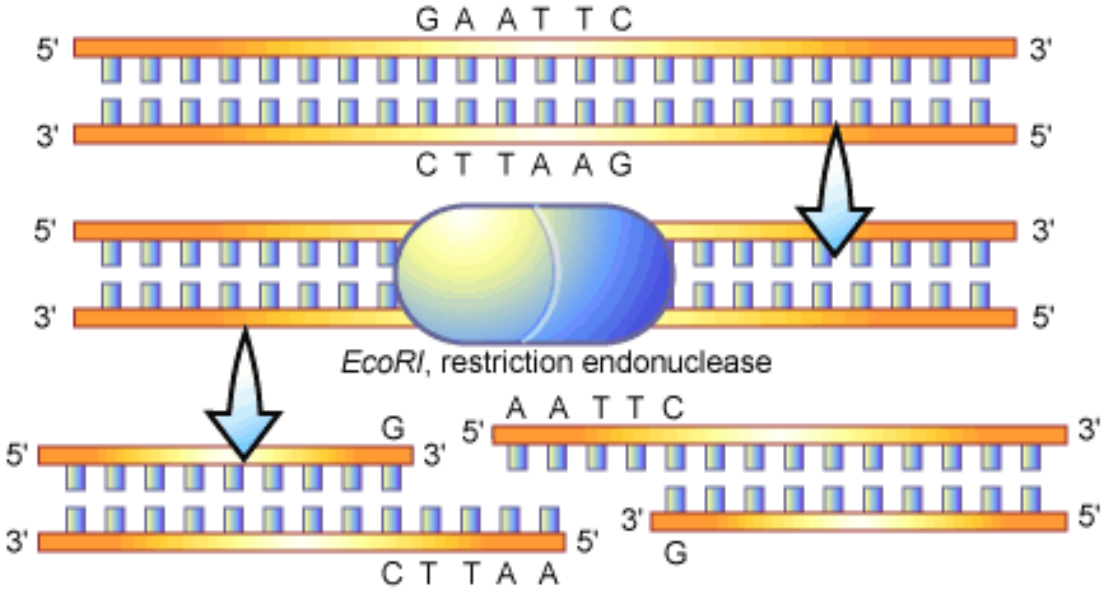
Daniel Nathans

Hamilton O. Smith



Restriction enzymes are named for the organism they come from:

EcoRI = 1st restriction enzyme found in E. coli

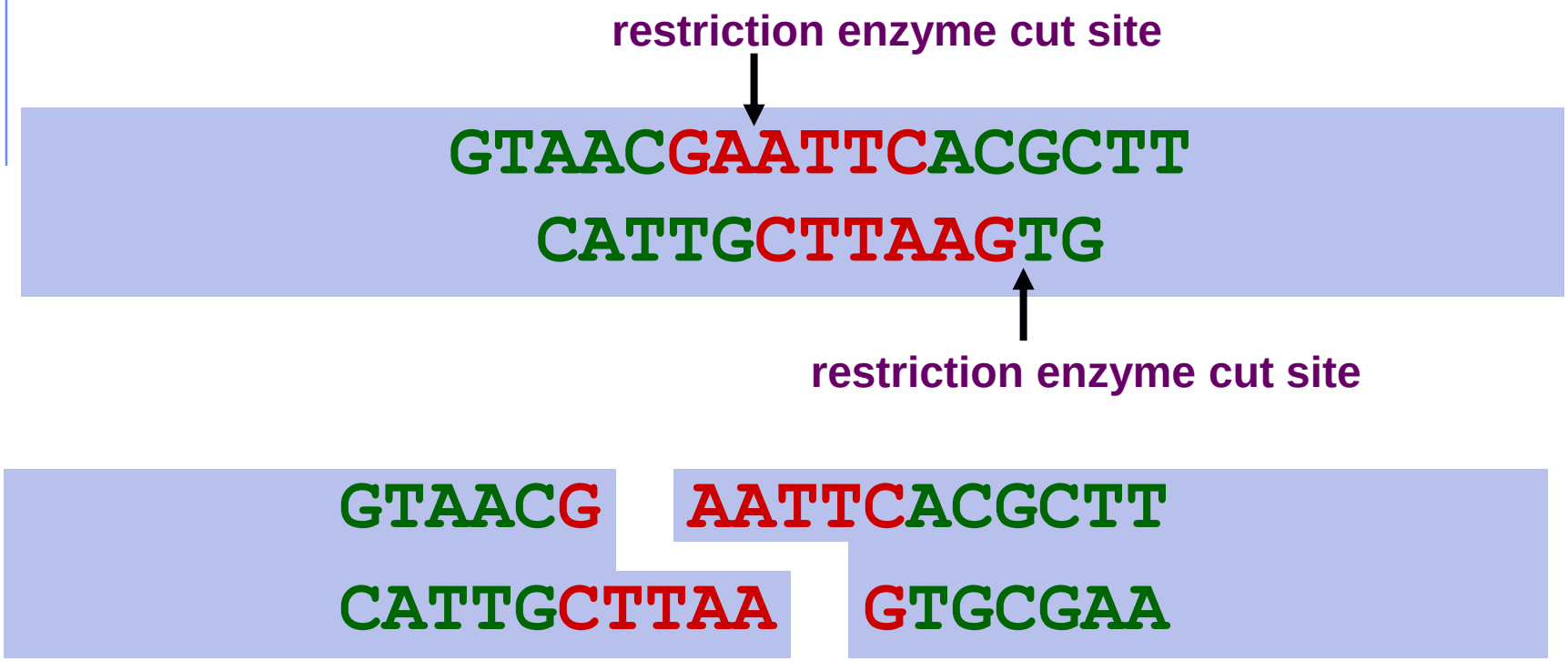


RESTRICTION ENDONUCLEASES

- Different enzymes recognize different sequences
- Different kinds of DNA cut with same enzyme will have the same “sticky ends” and can be joined

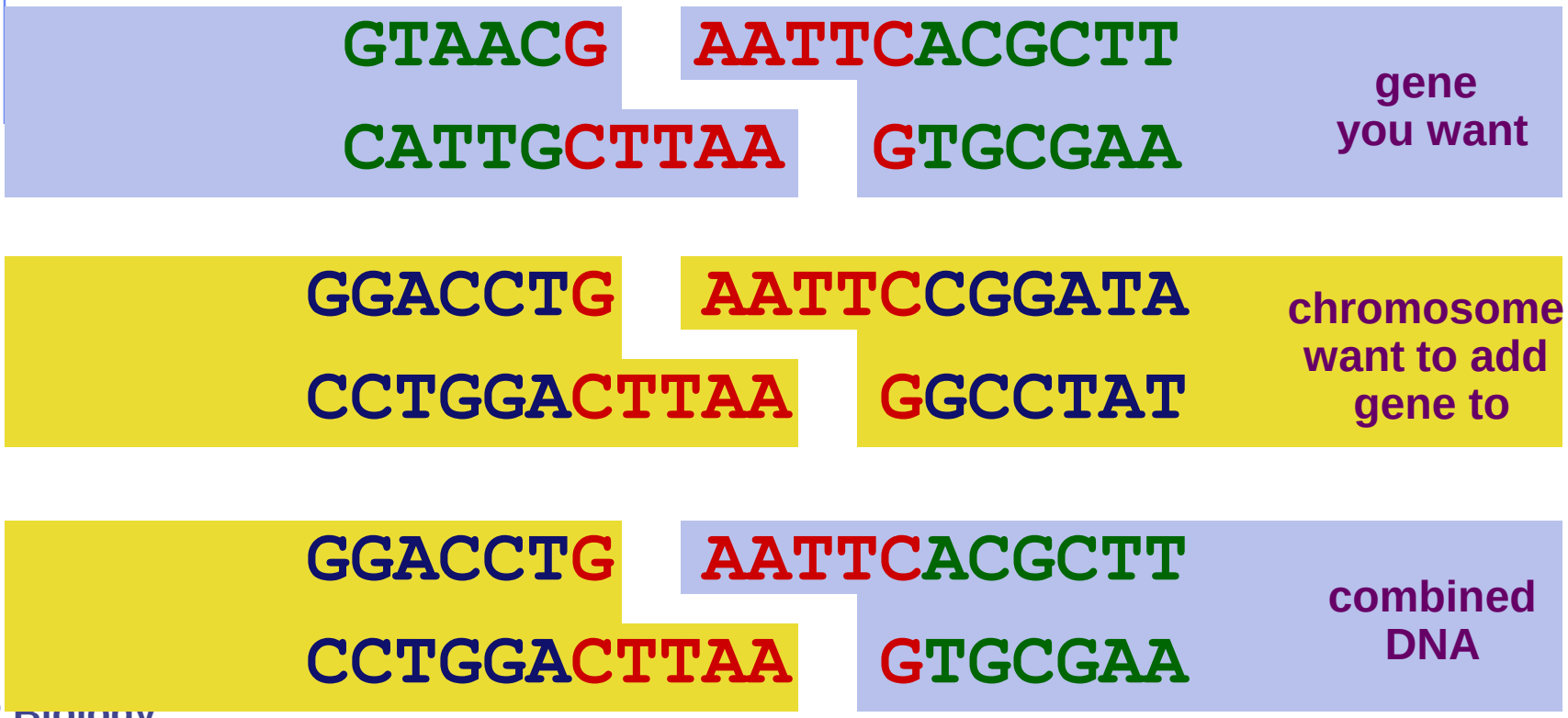
Restriction enzymes

- Cut DNA at specific sites
  - leave "sticky ends"

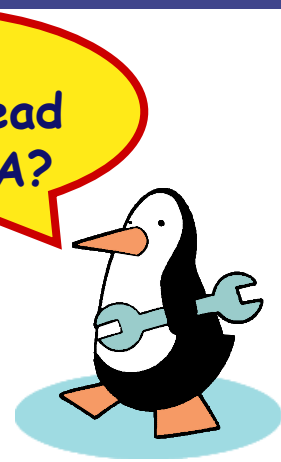


Sticky ends

- Cut other DNA with same enzymes
  - leave “sticky ends” on both
  - can glue DNA together at “sticky ends”



Why mix genes together?



- Gene produces protein in different organism or different individual

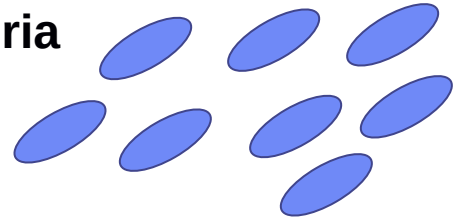
human insulin gene in bacteria

TAACGAATTCTACGAATGGTTACATCGCCGAATTCTACG
CATTGCTTAAGATGCTTACCAATGTAGCGGCTTAAGATGCTAGC

"new" protein from organism

ex: human insulin from bacteria

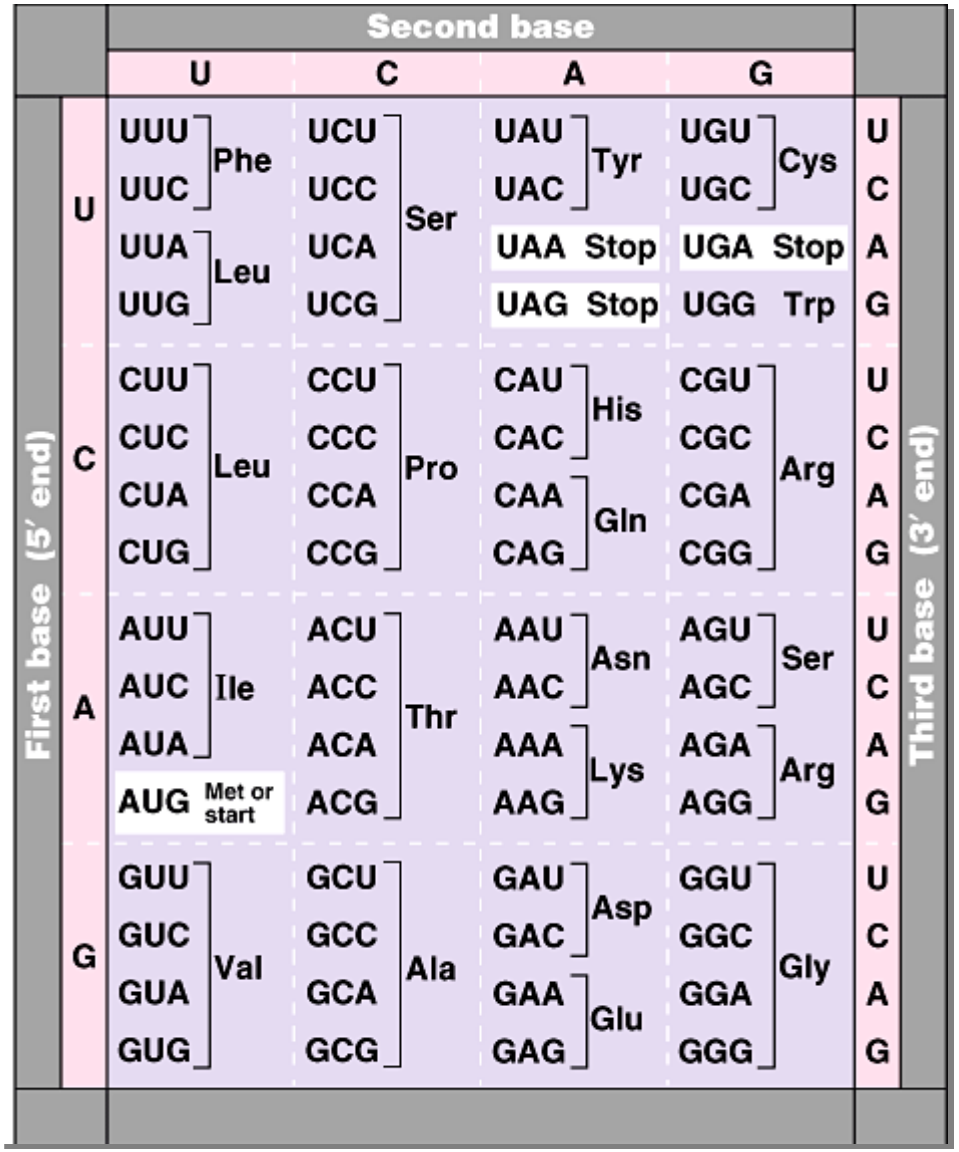
bacteria



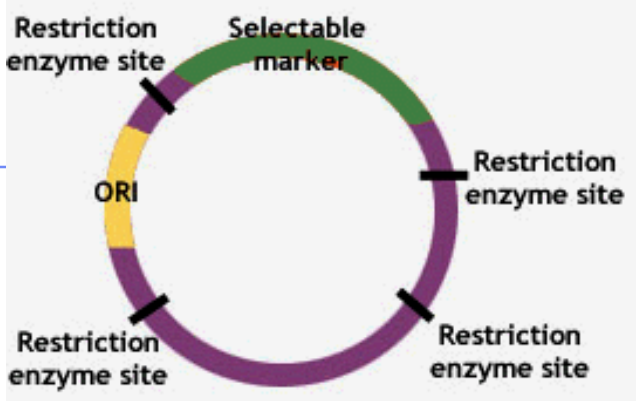
human insulin

The code is universal

- **Since all living organisms…**
  - **use the same DNA**
  - **use the same code book**
  - **read their genes the same way**



Copy (& Read) DNA



- **Transformation**
  - insert **recombinant** plasmid into bacteria
  - grow recombinant bacteria in agar cultures
    - bacteria make lots of copies of plasmid
    - "**cloning**" the plasmid
  - production of many copies of inserted gene
  - production of "new" protein
    - transformed phenotype

DNA → RNA → protein → trait

Grow bacteria…make more

gene from other organism

recombinant plasmid

transformed bacteria

plasmid

vector

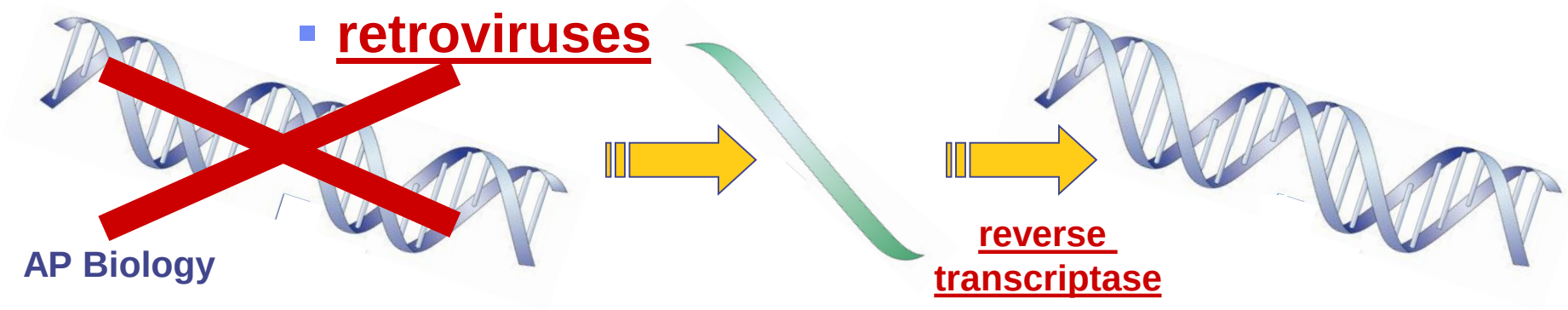
grow bacteria

harvest (purify) protein

Humulin®

How do you clean up the junk?

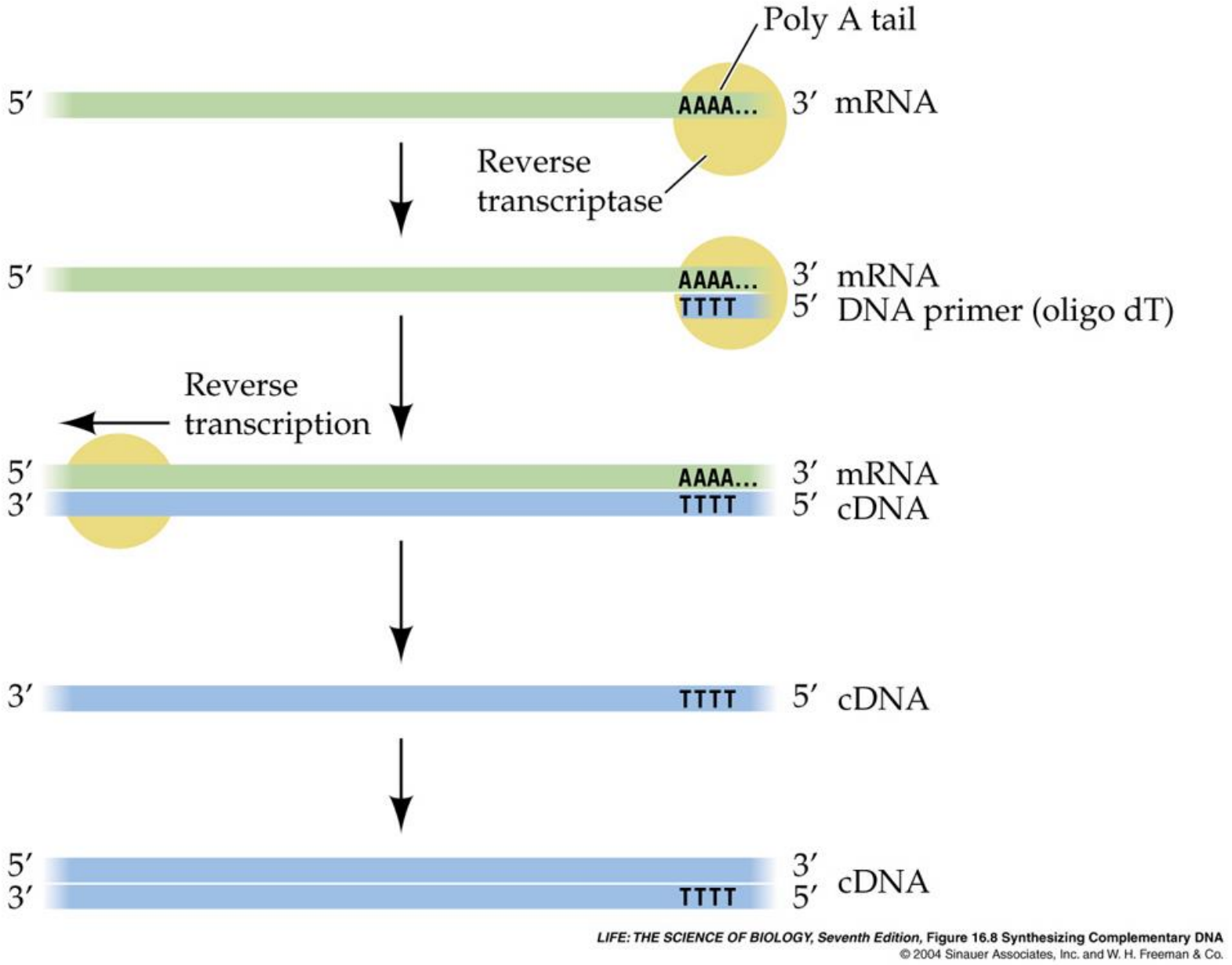
- Don't start with DNA…
- Use mRNA
  - copy of the gene without the junk!
- But in the end, you need DNA to clone into plasmid…
- How do you go from RNA → DNA?
  - reverse transcriptase from RNA viruses
    - retroviruses



REVERSE TRANSCRIPTASE

- **Found in RETROVIRUSES (RNA not DNA)**
- **Uses RNA message to make DNA**
- **Info flows in reverse RNA → DNA**

- **Can take eukaryotic RNA message after introns have been removed and change it into a DNA sequence to be read by bacteria (no RNA processing in prokaryotes)**



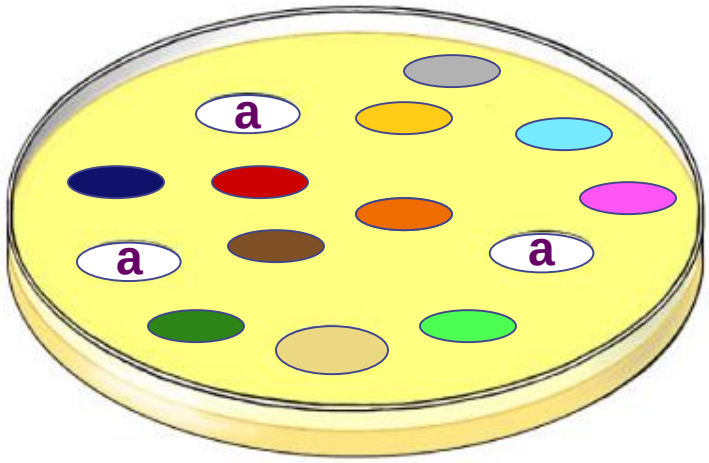
LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.8 Synthesizing Complementary DNA

http://biology200.gsu.edu/houghton/4564%20'04/figures/lecture%204/AAAreverse.jpg

Selection for plasmid uptake

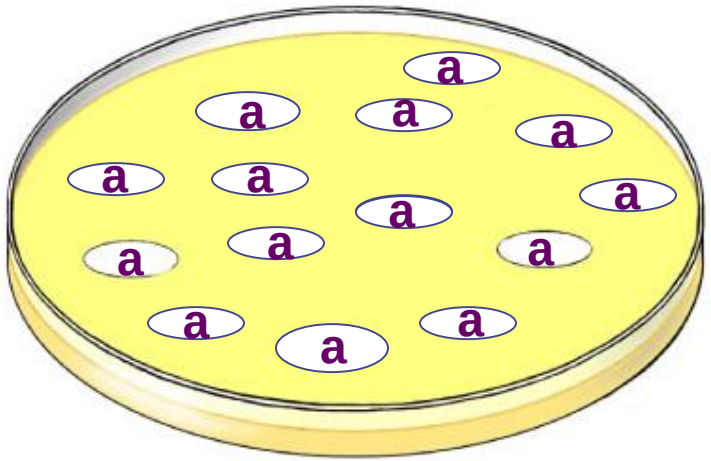
- Antibiotic becomes a selecting agent
  - only bacteria with the plasmid will grow on antibiotic (ampicillin) plate

all bacteria grow



LB plate

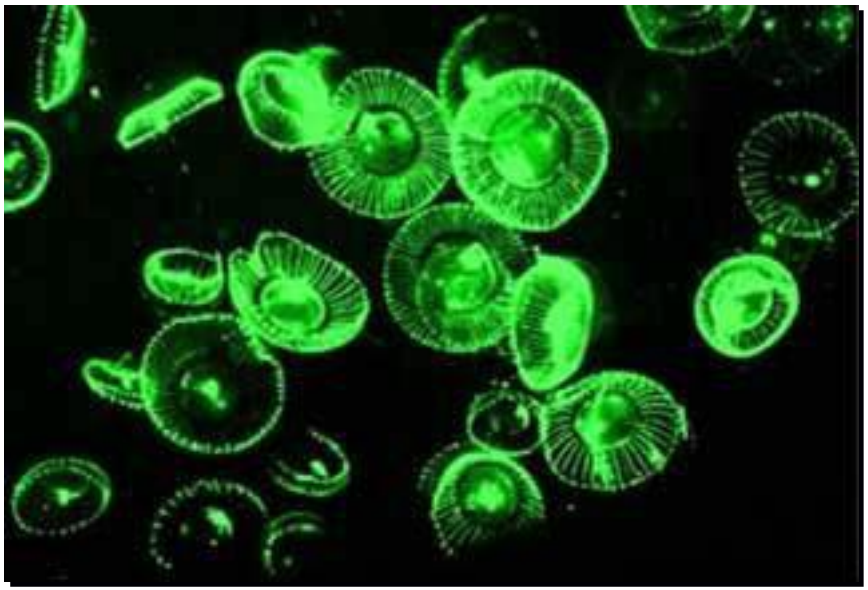
only transformed bacteria grow



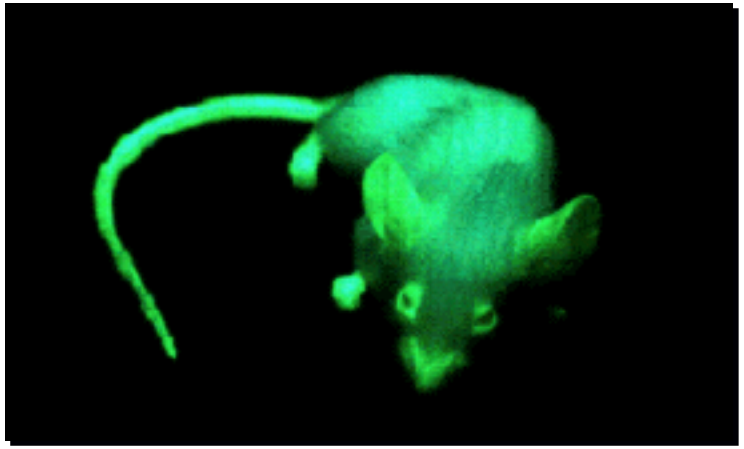
LB/*amp* plate

cloning

Green with envy??



Jelly fish “GFP”



Transformed vertebrates

http://mabryonline.org/blogs/larkin/GFP%5CGFP_aequorea_victoria-1.jpeg

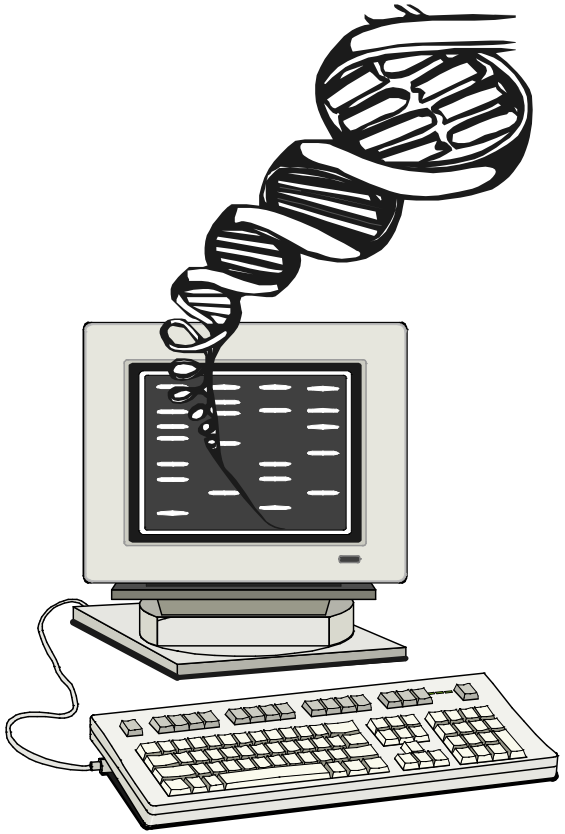
Green Fluorescent Protein (GFP)

- Genetic tool
- Originally from jellyfish
- Way to tell if gene has been incorporated

http://www.vet.upenn.edu/schoolresources/communications/publications/bellwether/61/stem_cells.html

Cut, Paste, Copy, Find…

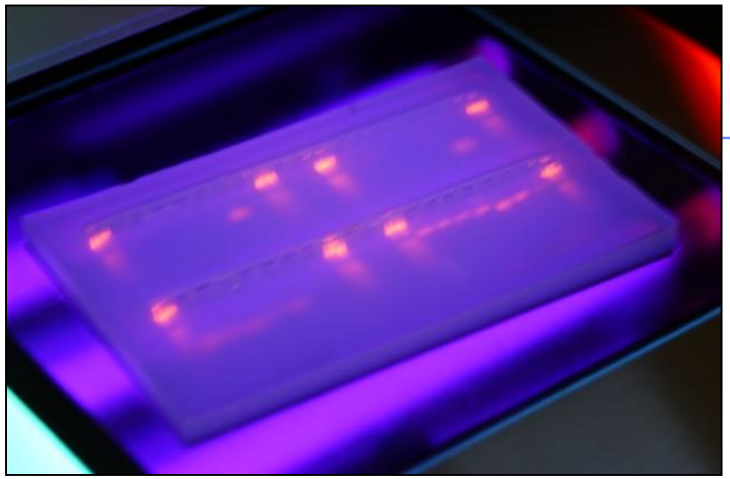
- Word processing metaphor…
  - cut
    - restriction enzymes
  - paste
    - ligase
  - copy
    - plasmids
      - bacterial transformation
    - is there an easier way??
  - find
    - ????



More Basic Biotechnology Tools

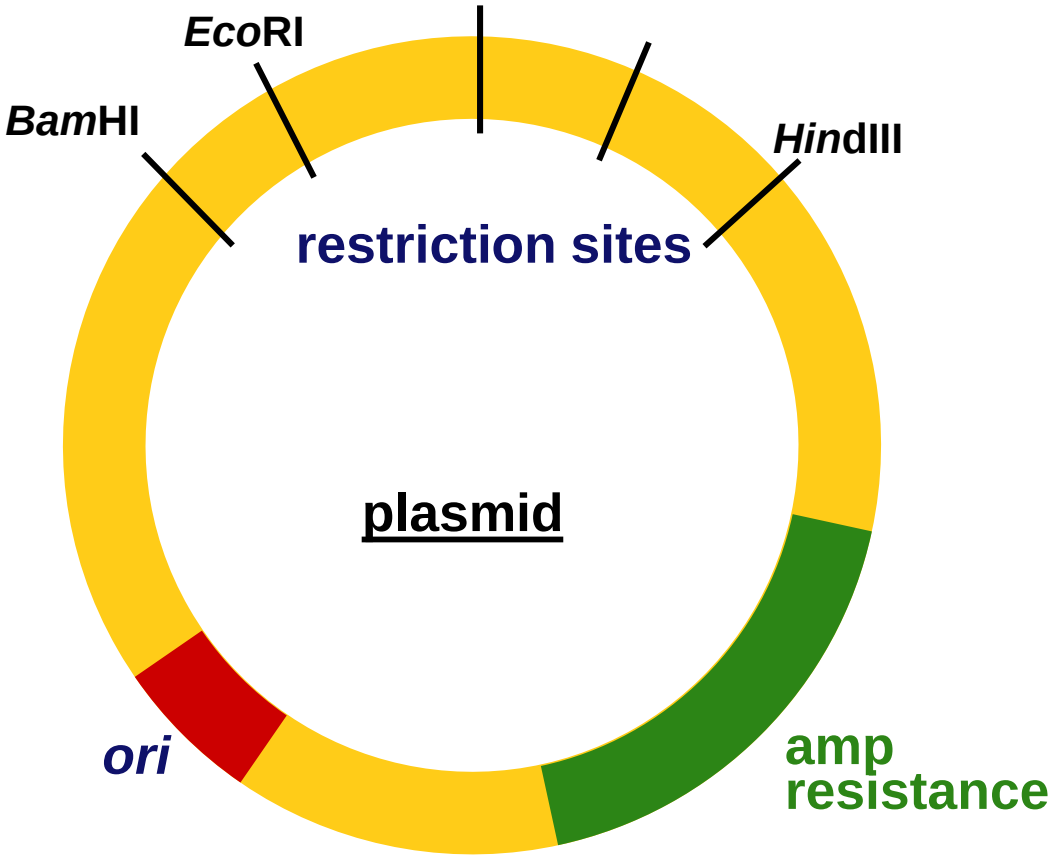
Sorting & Copying DNA

Slide show by Kim Foglia (modified)
Blue edged slides are Kim's



Engineered plasmids

- **Building custom plasmids**
  - restriction enzyme sites
  - antibiotic resistance genes as a **selectable marker**

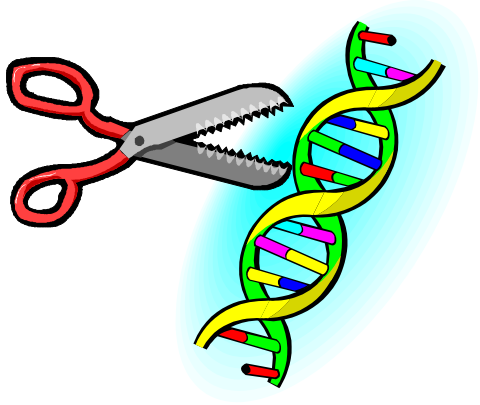


Selectable marker

- antibiotic resistance gene on plasmid
  - ampicillin resistance
- selecting for successful transformation
  - successful uptake of recombinant plasmid

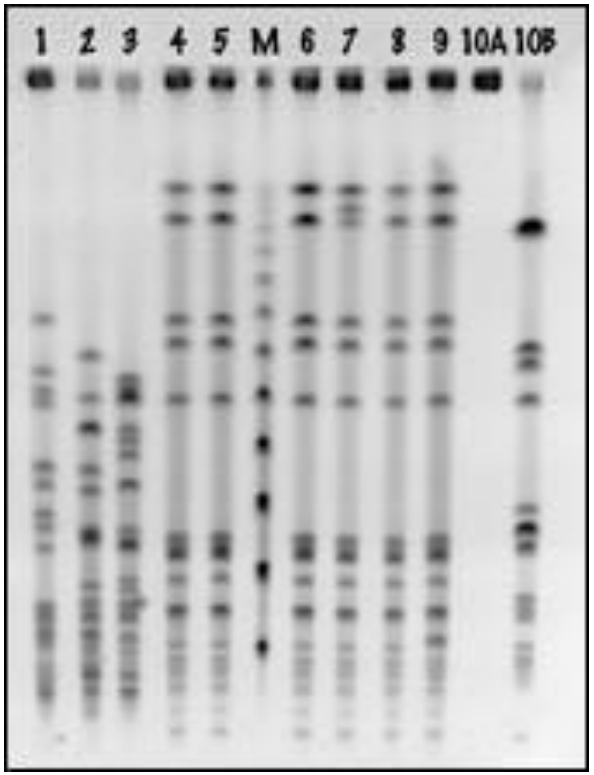
Many uses of restriction enzymes…

- Now that we can cut DNA with restriction enzymes…
  - we can cut up DNA from different people… or different organisms… and compare it
  - why?
    - forensics
    - medical diagnostics
    - paternity
    - evolutionary relationships
    - and more…



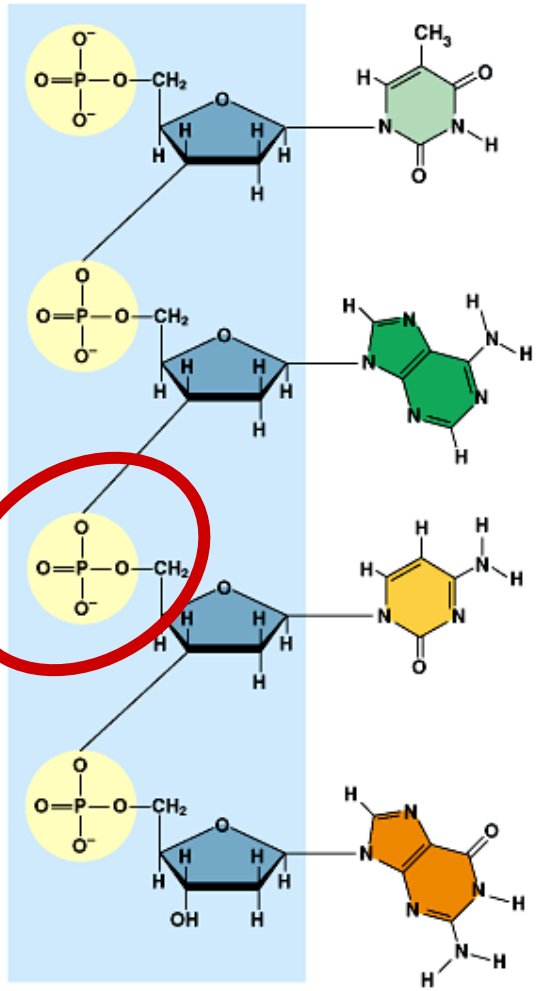
Comparing cut up DNA

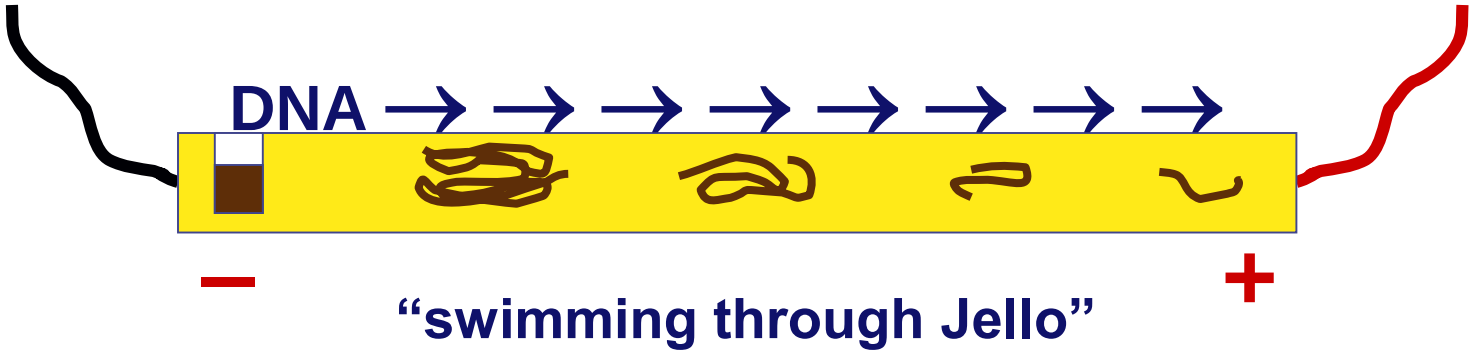
- How do we compare DNA fragments?
  - separate fragments by size
- How do we separate DNA fragments?
  - run it through a gelatin
    - agarose
    - made from algae
  - gel electrophoresis



Gel electrophoresis

- A method of separating DNA in a gelatin-like material using an electrical field
  - DNA is negatively charged
  - when it's in an electrical field it moves toward the positive side

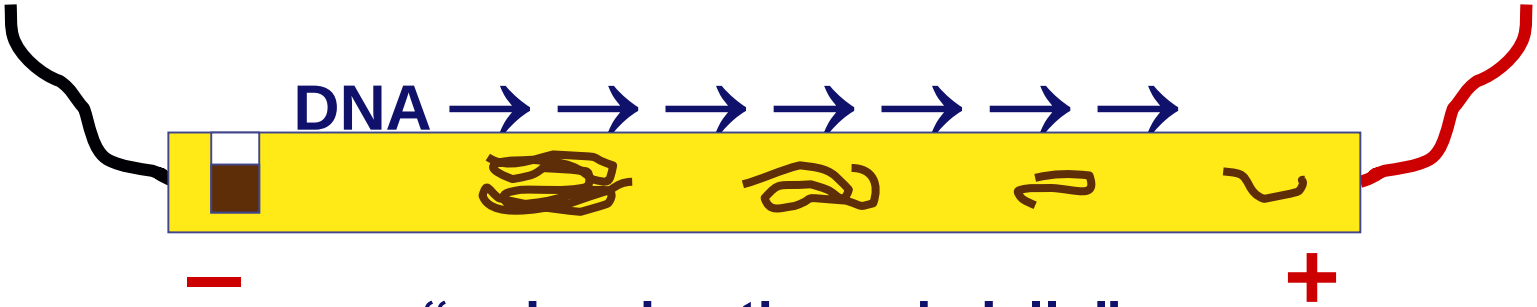




"swimming through Jello"

Gel electrophoresis

- DNA moves in an electrical field…
  - so how does that help you compare DNA fragments?
    - size of DNA fragment affects how far it travels
      - small pieces travel farther
      - large pieces travel slower & lag behind



"swimming through Jello"

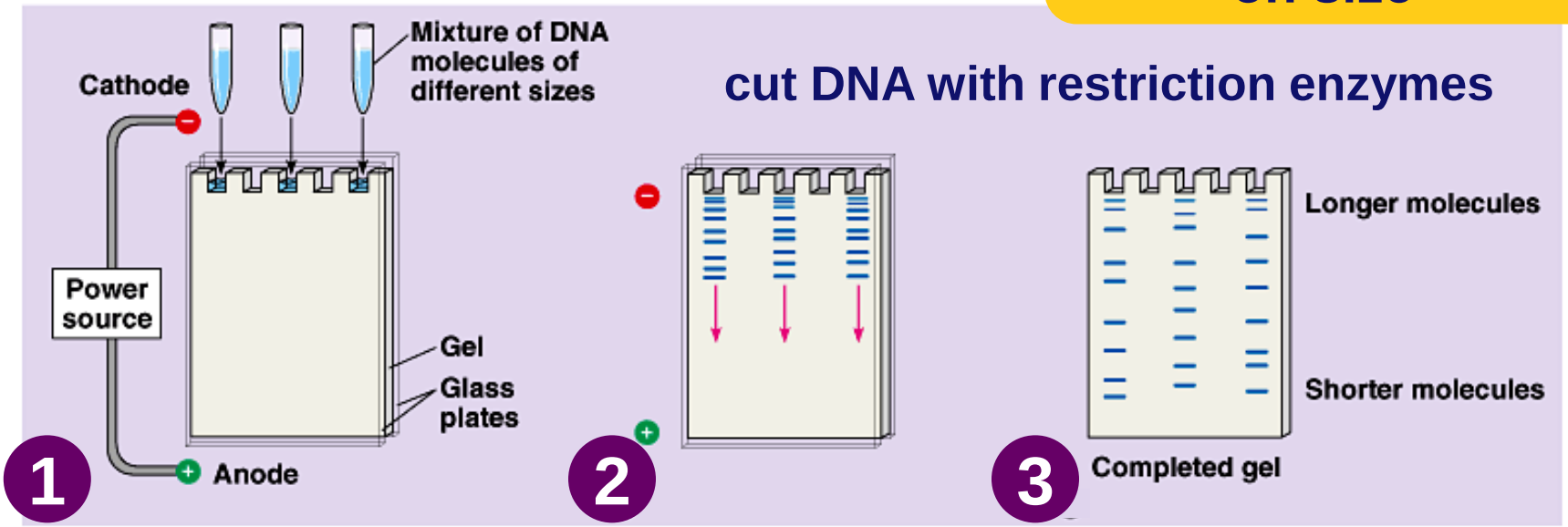
Gel Electrophoresis

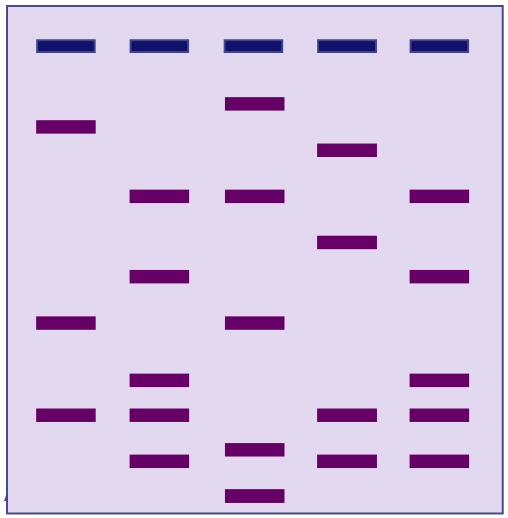
DNA & restriction enzyme

(b)

Running a gel

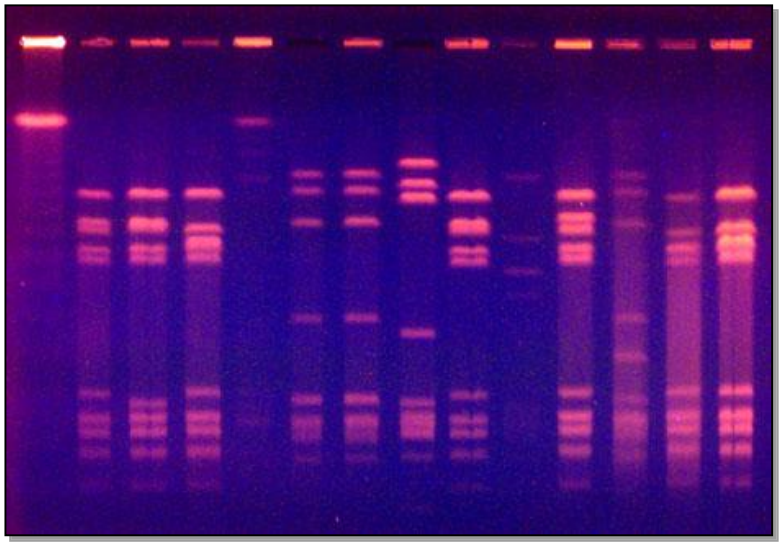
fragments of DNA separate out based on size





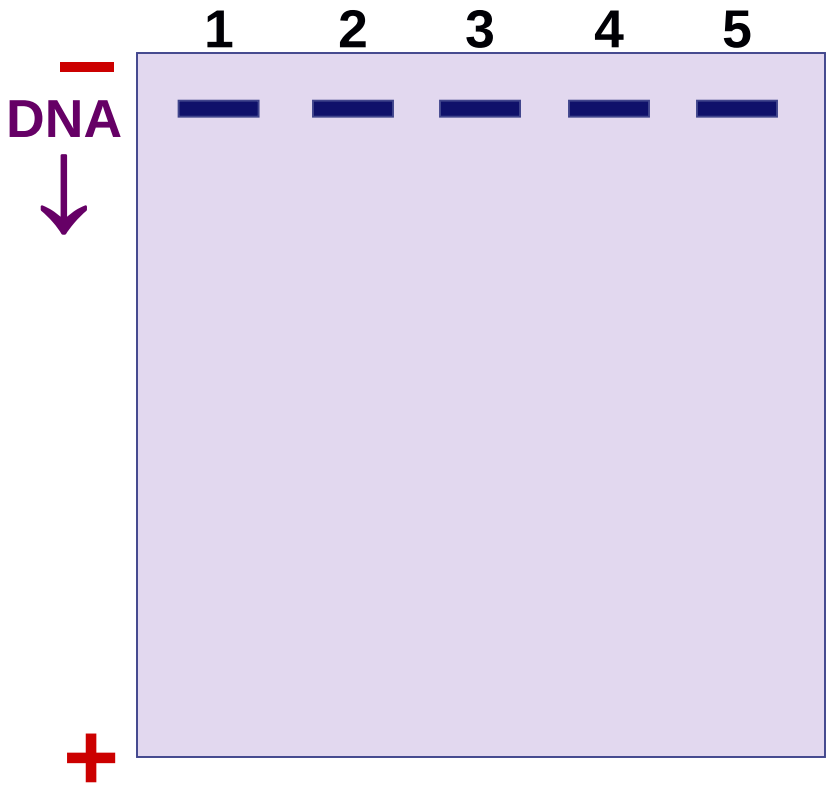
Stain DNA

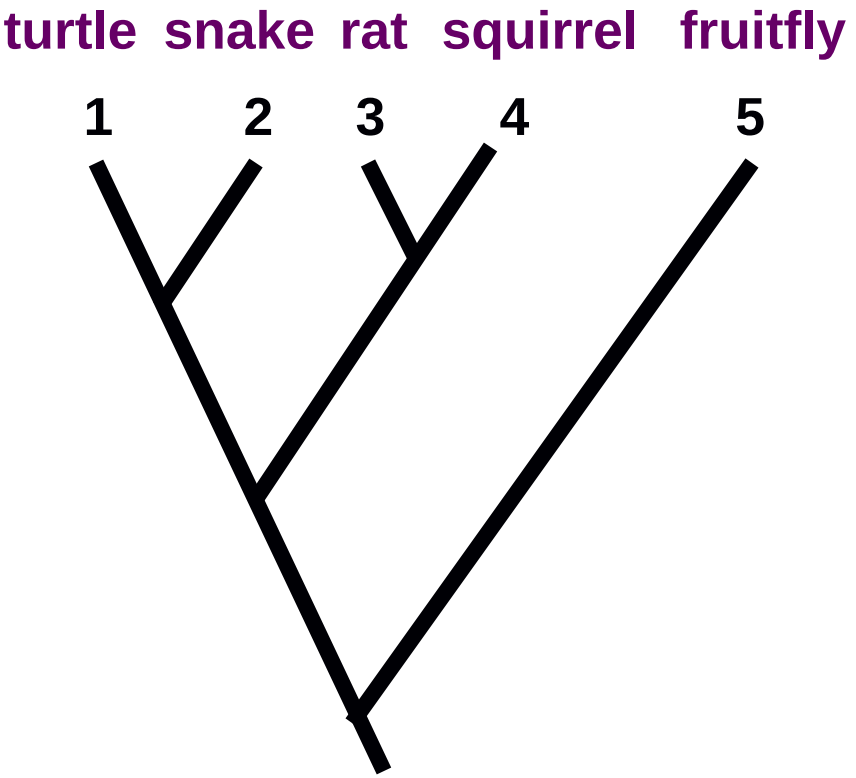
- ethidium bromide binds to DNA
- fluoresces under UV light



Uses: Evolutionary relationships

- Comparing DNA samples from different organisms to measure evolutionary relationships

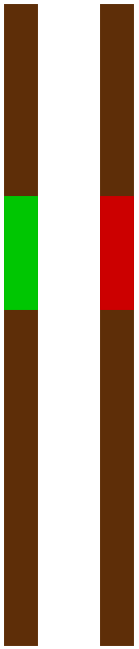




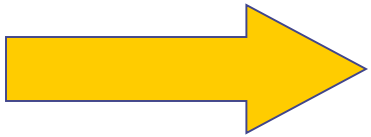
Uses: Medical diagnostic

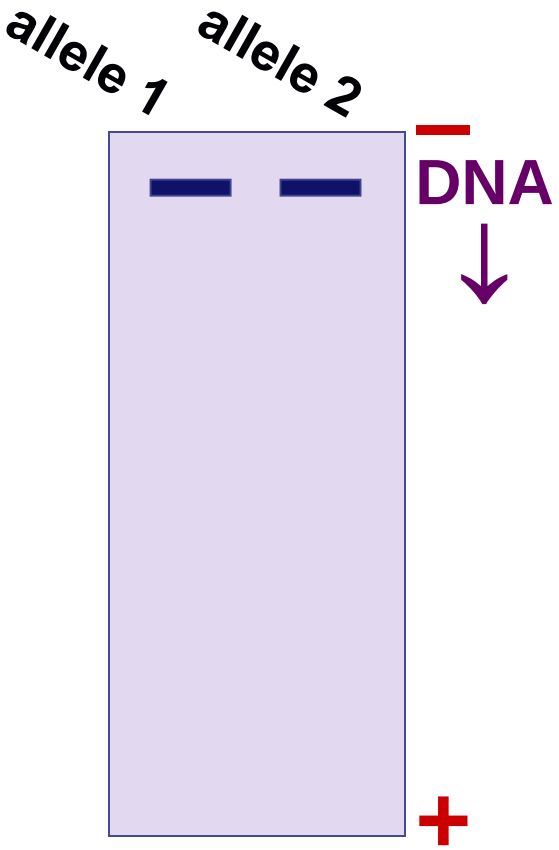
- Comparing normal allele to disease allele

chromosome with normal allele 1



chromosome with disease-causing allele 2

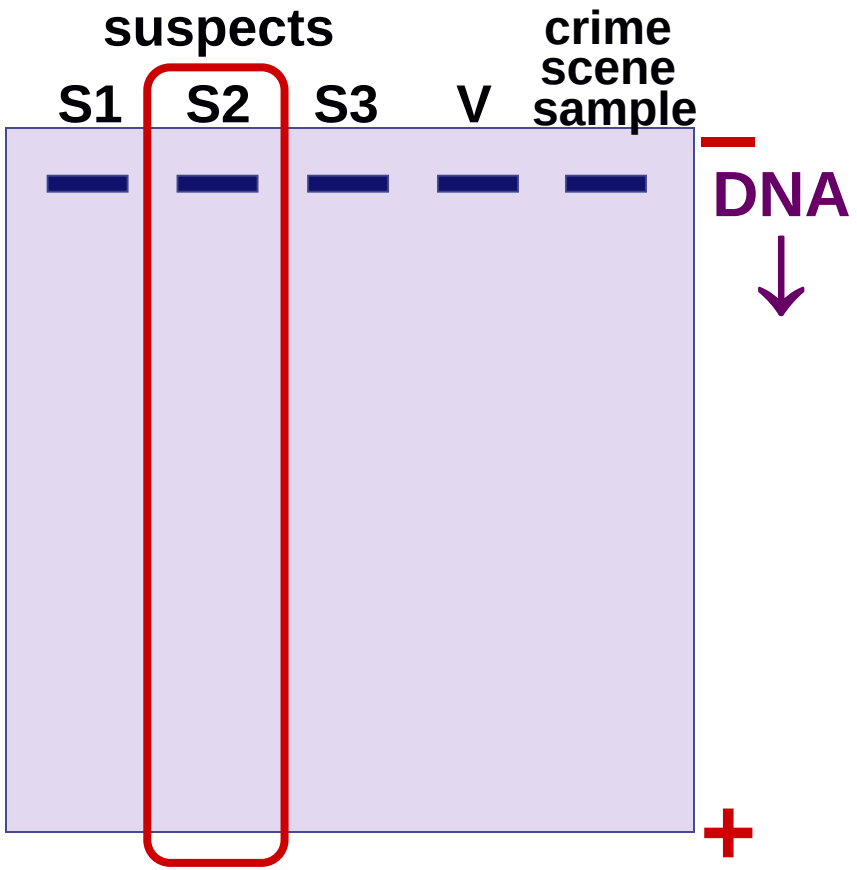




Example: test for Huntington's disease

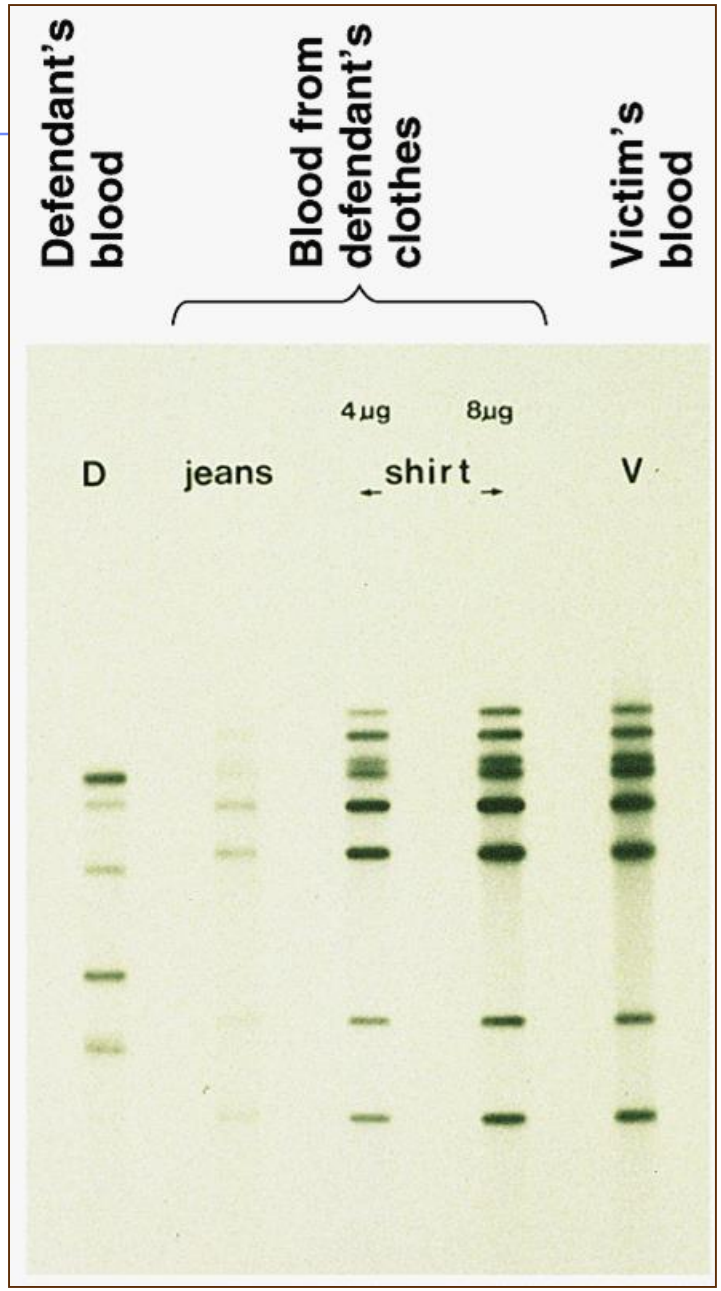
Uses: Forensics

- Comparing DNA sample from crime scene with suspects & victim



DNA fingerprints

- Comparing blood samples on defendant's clothing to determine if it belongs to victim
  - DNA fingerprinting
  - comparing DNA banding pattern between different individuals
  - ~unique patterns



Differences at the DNA level

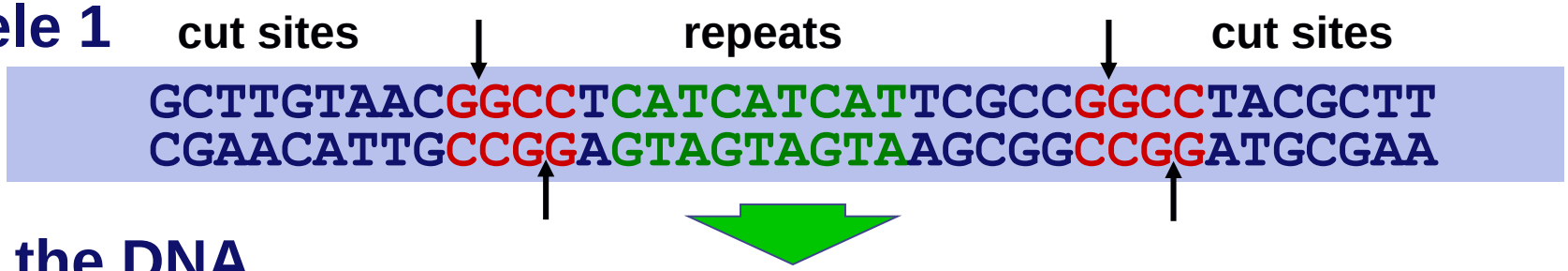
- **Why is each person's DNA pattern different?**
  - **sections of "junk" DNA**
    - **doesn't code for proteins**
    - **made up of repeated patterns**
      - **CAT, GCC, and others**
      - **each person may have different number of repeats**
    - **many sites on our 23 chromosomes with different repeat patterns**

GCTTGTAACGGCCTCATCATCATTCGCCGGCCTACGCTT
CGAACATTGCCGGAGTAGTAGTAAGCGGCCGGATGCGAA

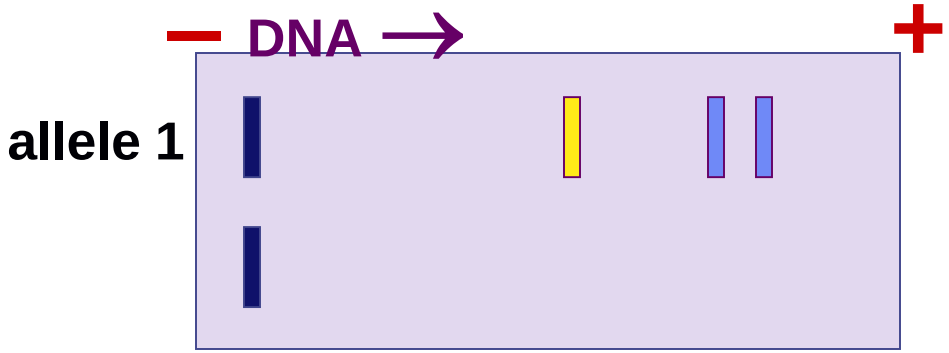
GCTTGTAACGGCATCATCATCATCATCATCCGGCCTACGCTT
CGAACATTGCCGTAGTAGTAGTAGTAGTAGGCCGGATGCGAA

DNA patterns for DNA fingerprints

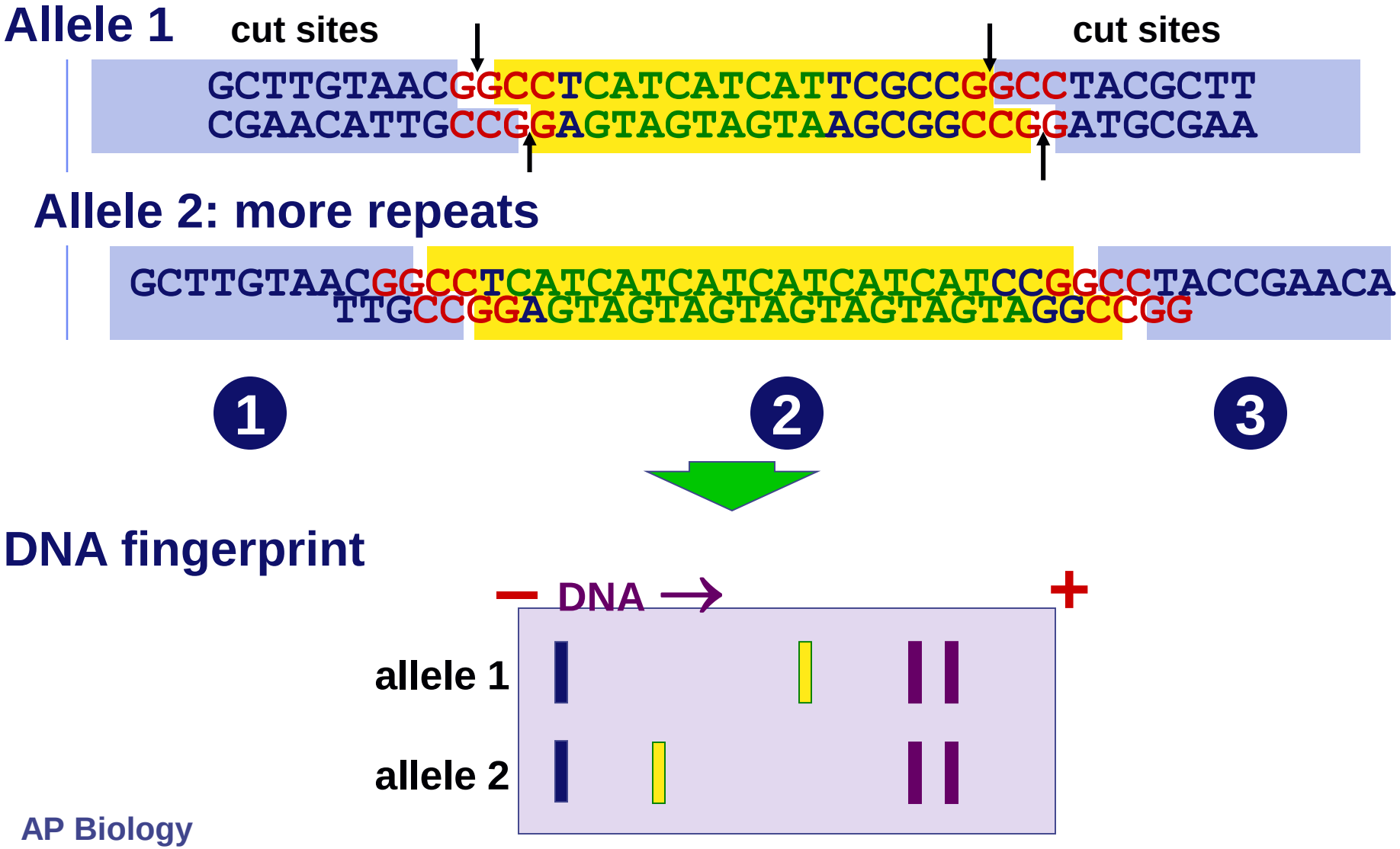
Allele 1



Cut the DNA

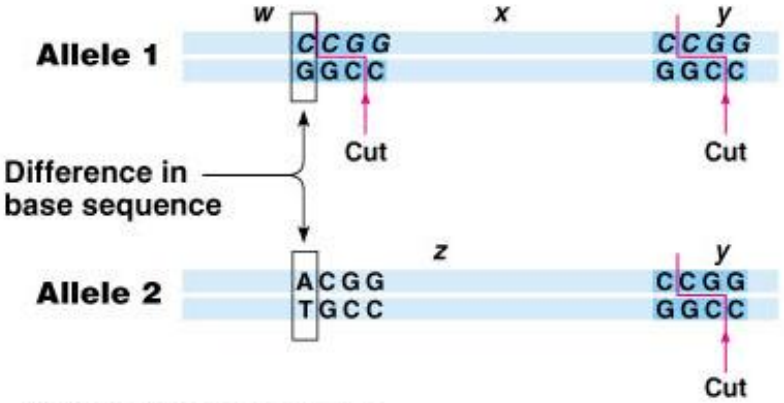


Differences between people



RFLPs

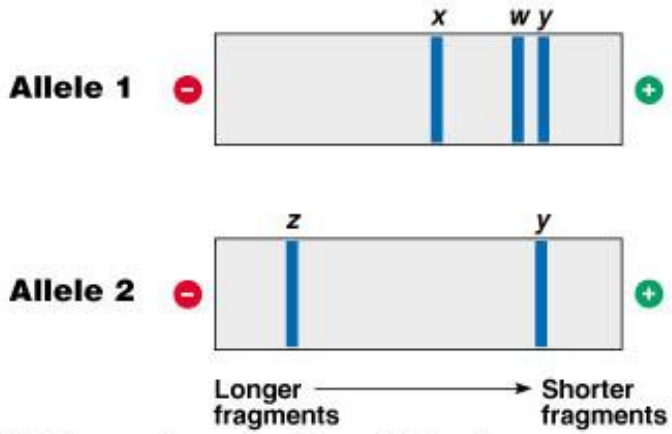
- **Restriction Fragment Length Polymorphism**
  - **differences in DNA between individuals**



(a) DNA from two alleles

(c) Completed gel

Alec Jeffries 1984

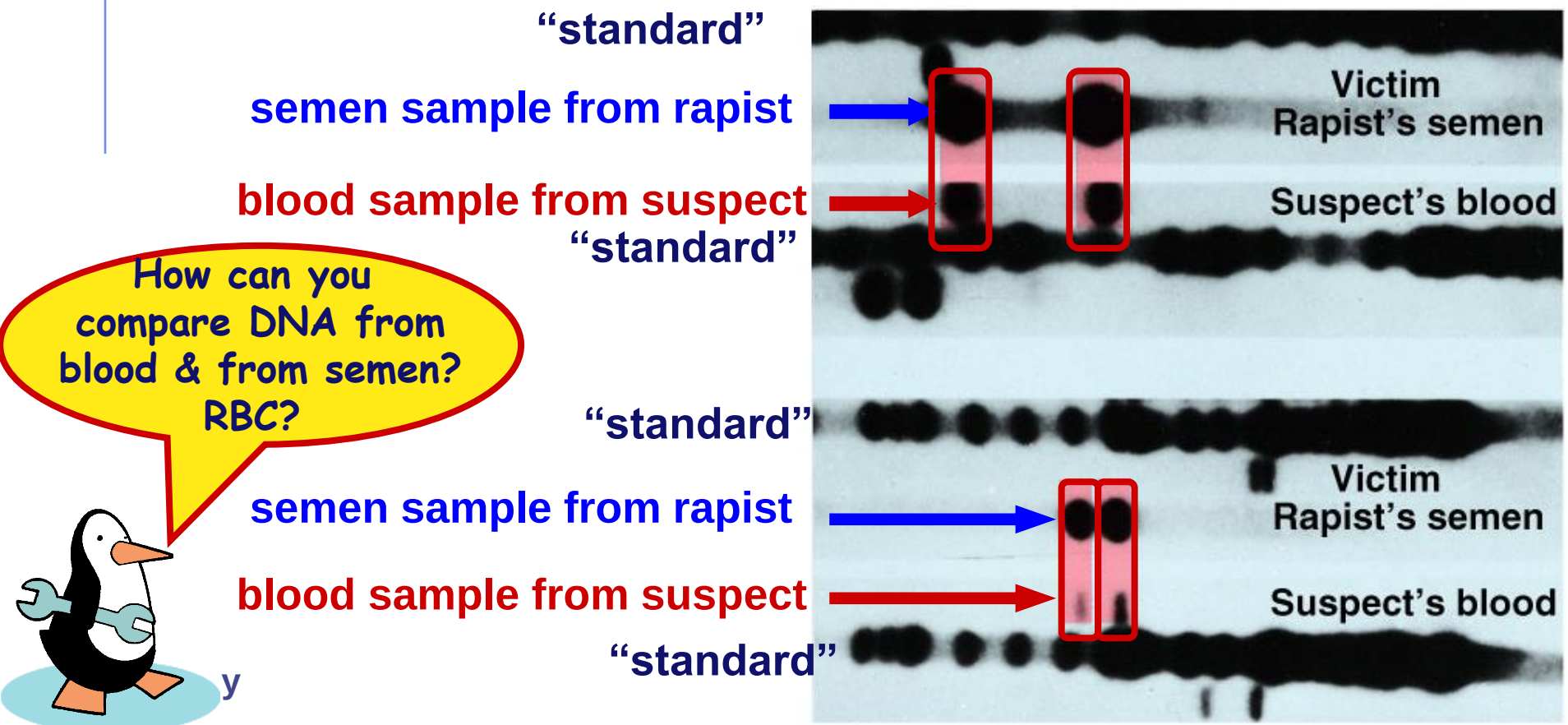


(b) Electrophoresis of restriction fragments

- **change in DNA sequence affects restriction enzyme "cut" site**
- **creates different fragment sizes & different band pattern**

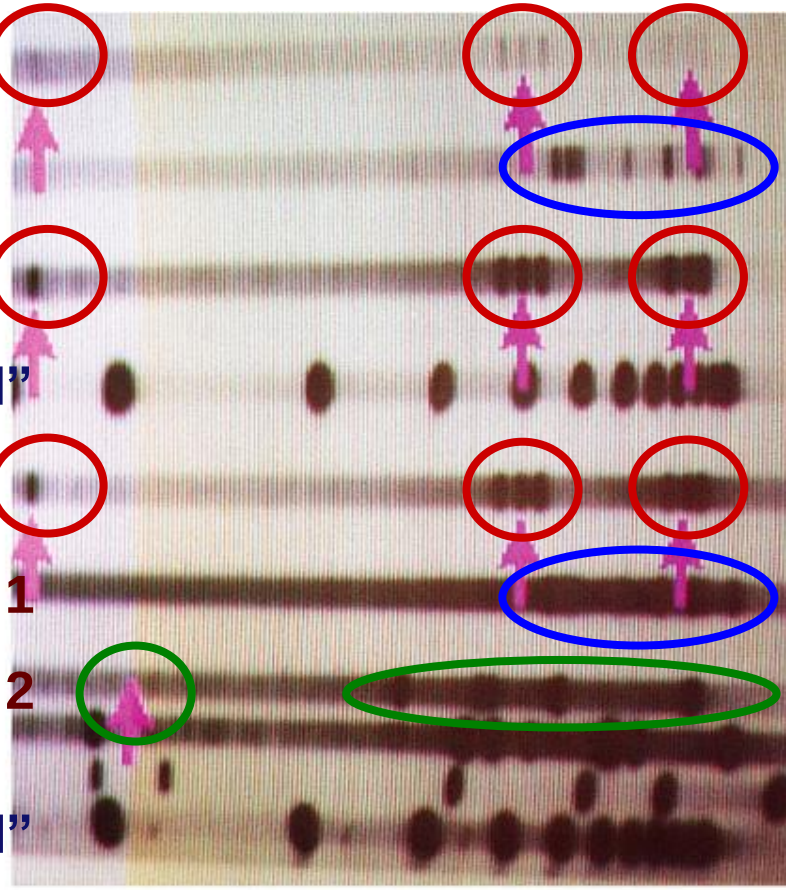
RFLP / electrophoresis use in forensics

- 1st case successfully using DNA evidence
  - 1987 rape case convicting Tommie Lee Andrews



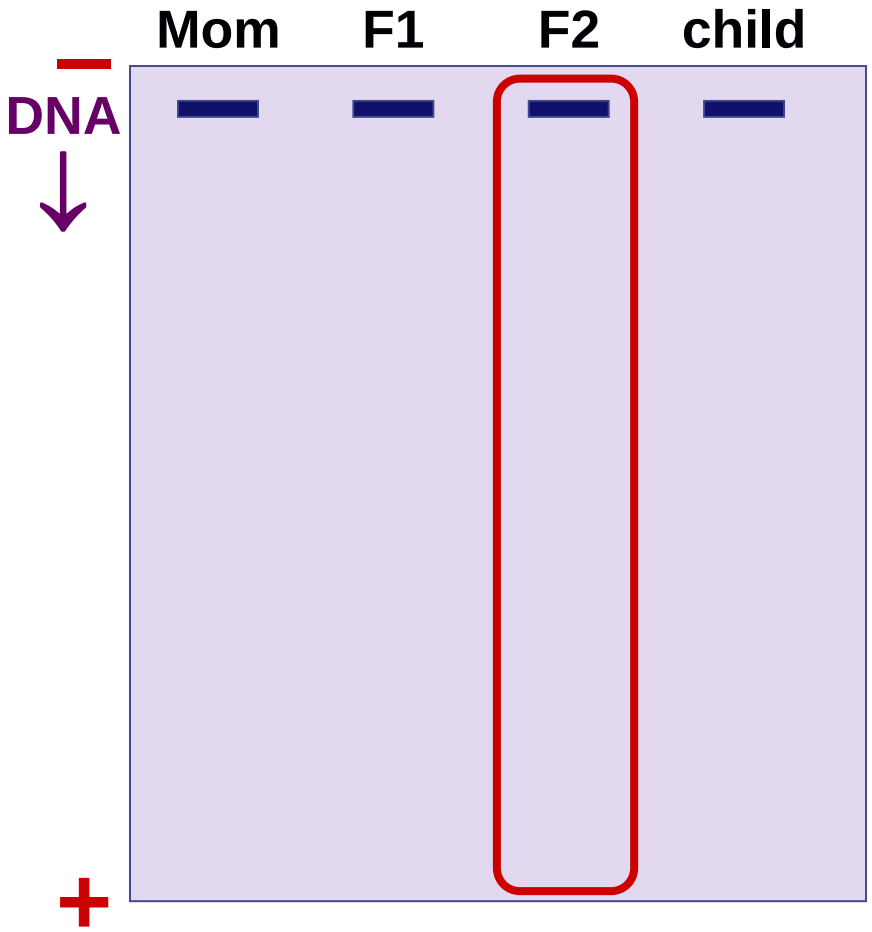
Electrophoresis use in forensics

- Evidence from murder trial
  - Do you think suspect is guilty?

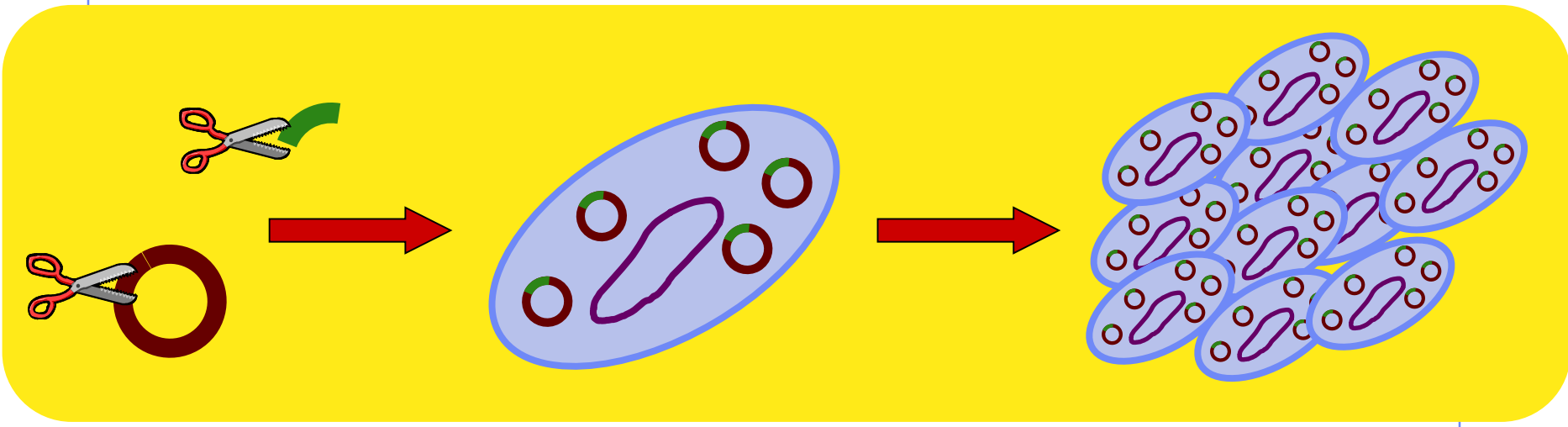


Uses: Paternity

- Who's the father?



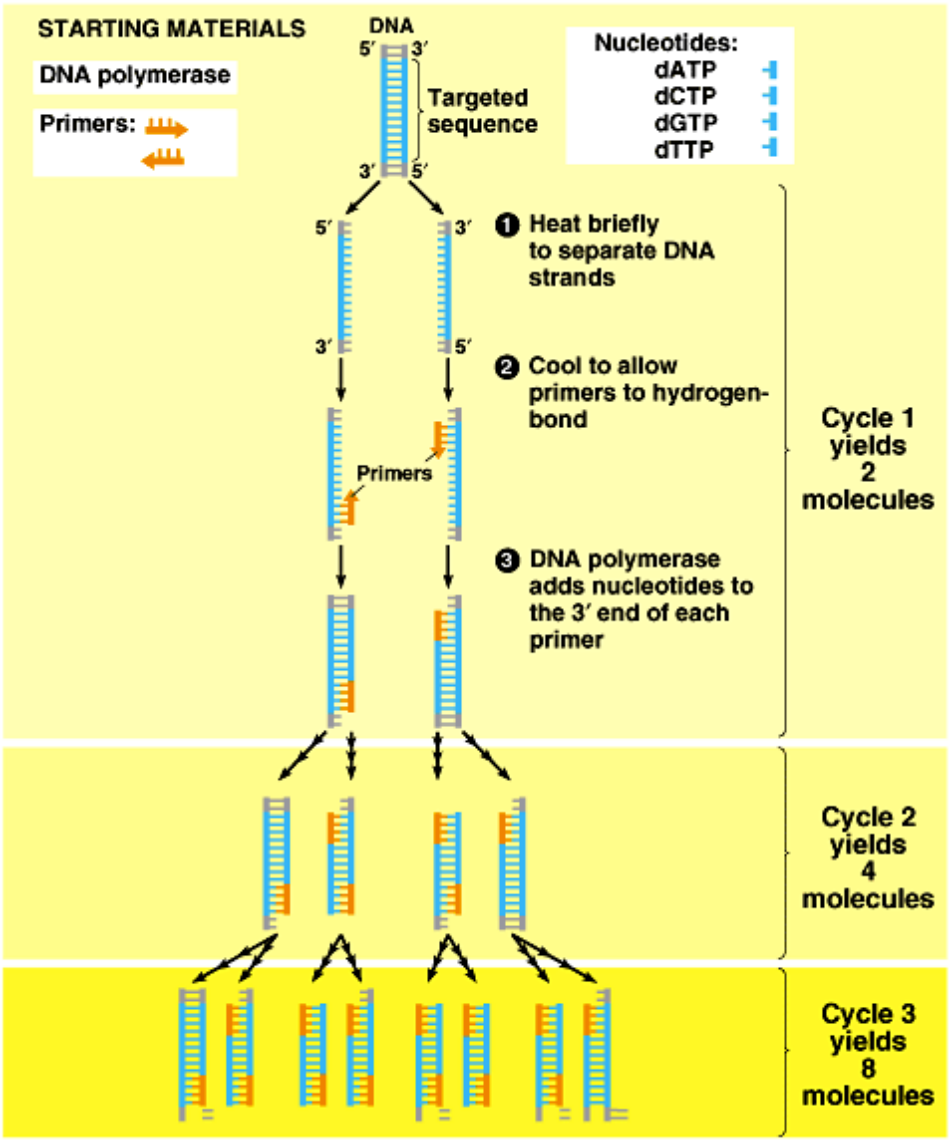
Making lots of copies of DNA



But it would be so much easier if we didn't have to use bacteria every time…

Copy DNA without plasmids? PCR!

- **Polymerase Chain Reaction**
  - method for making many, many copies of a specific segment of DNA
  - ~only need 1 cell of DNA to start



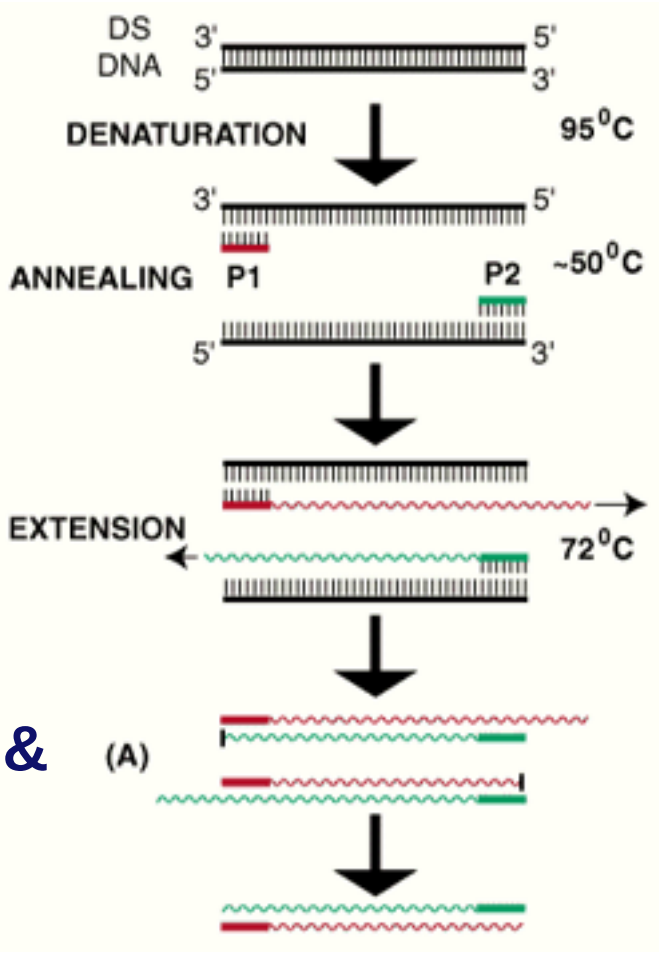
PCR process

- It's copying DNA in a test tube!
- What do you need?
  - template strand
  - DNA polymerase enzyme
  - nucleotides
    - ATP, GTP, CTP, TTP
  - primer

Thermocycler

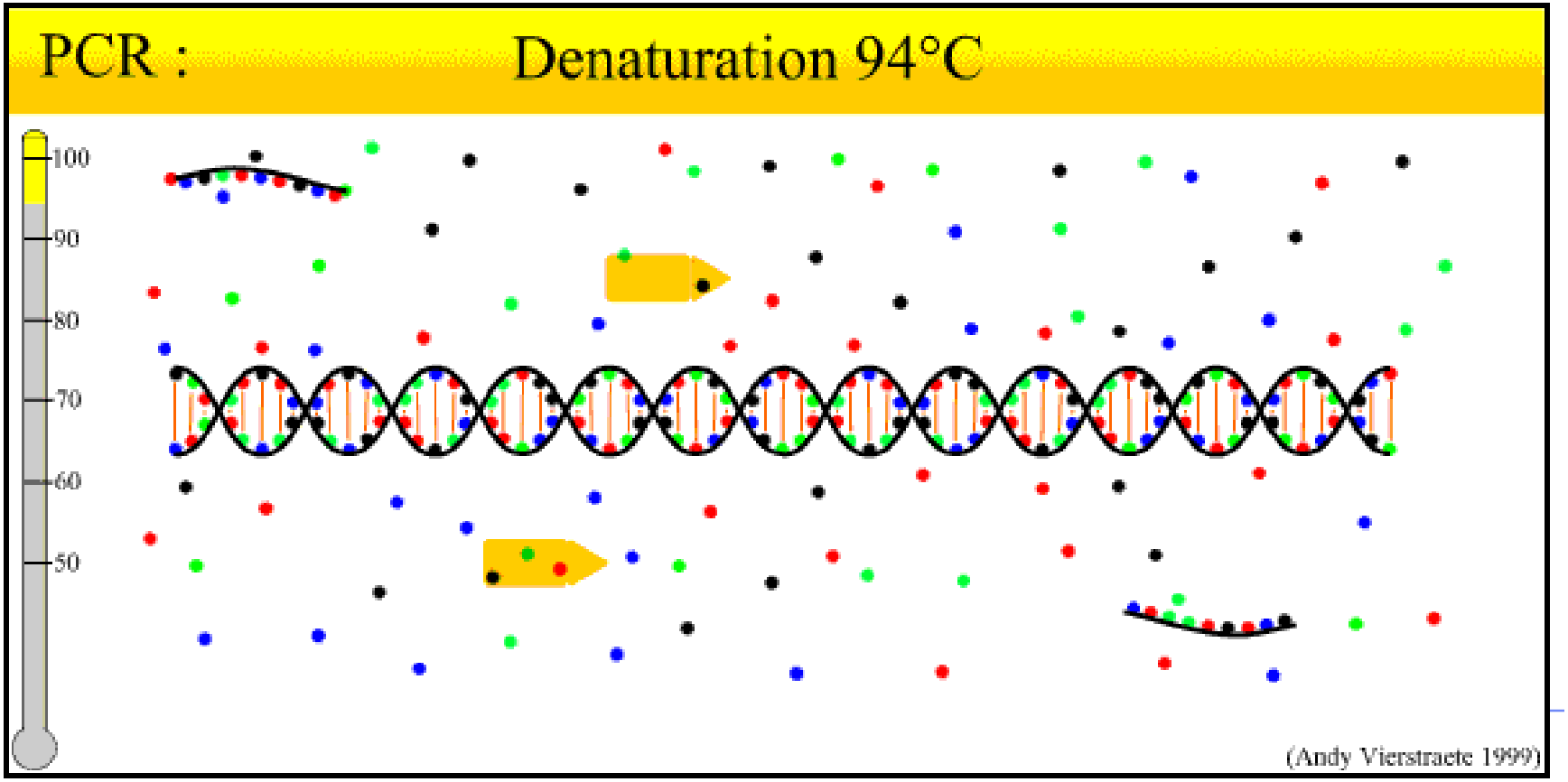
PCR primers

- **The primers are critical!**
  - need to know a bit of sequence to make proper primers
  - primers can bracket target sequence
    - start with long piece of DNA & copy a specified shorter segment
    - primers define section of DNA to be cloned

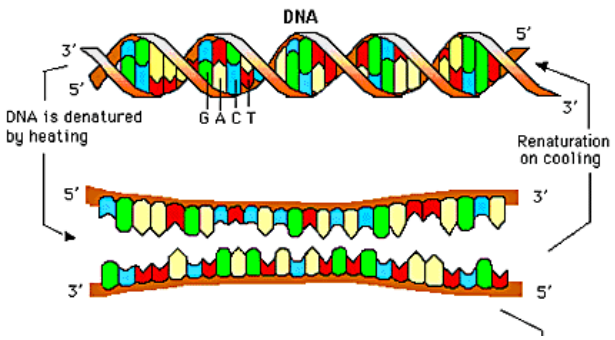


20-30 cycles
3 steps/cycle
30 sec/step

PCR movie

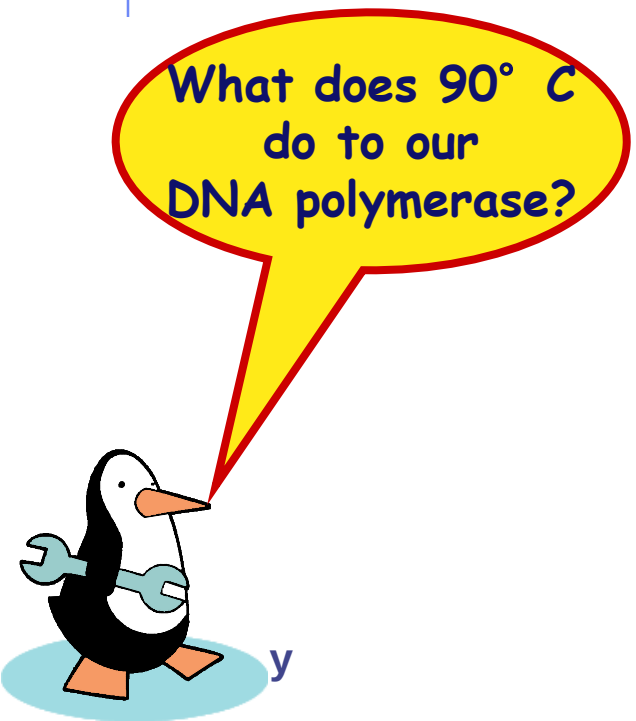


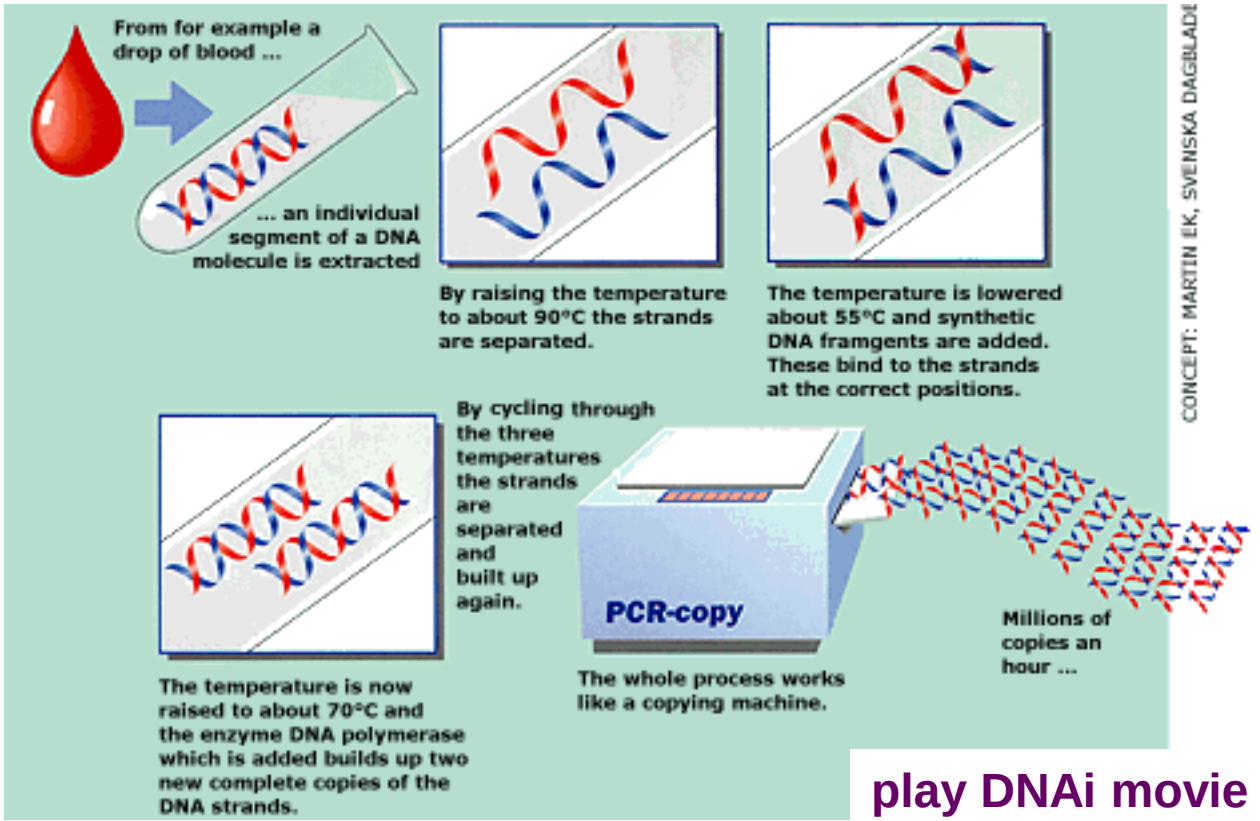
http://biology200.gsu.edu/houghton/4564%20'04/figures/lecture%204/pcranimatie.gif



PCR process

- **What do you need to do?**
  - in tube: DNA, DNA polymerase enzyme, primer, nucleotides
  - denature DNA: heat (90°C) DNA to separate strands
  - anneal DNA: cool to hybridize with primers & build DNA (extension)





play DNAi movie

The polymerase problem

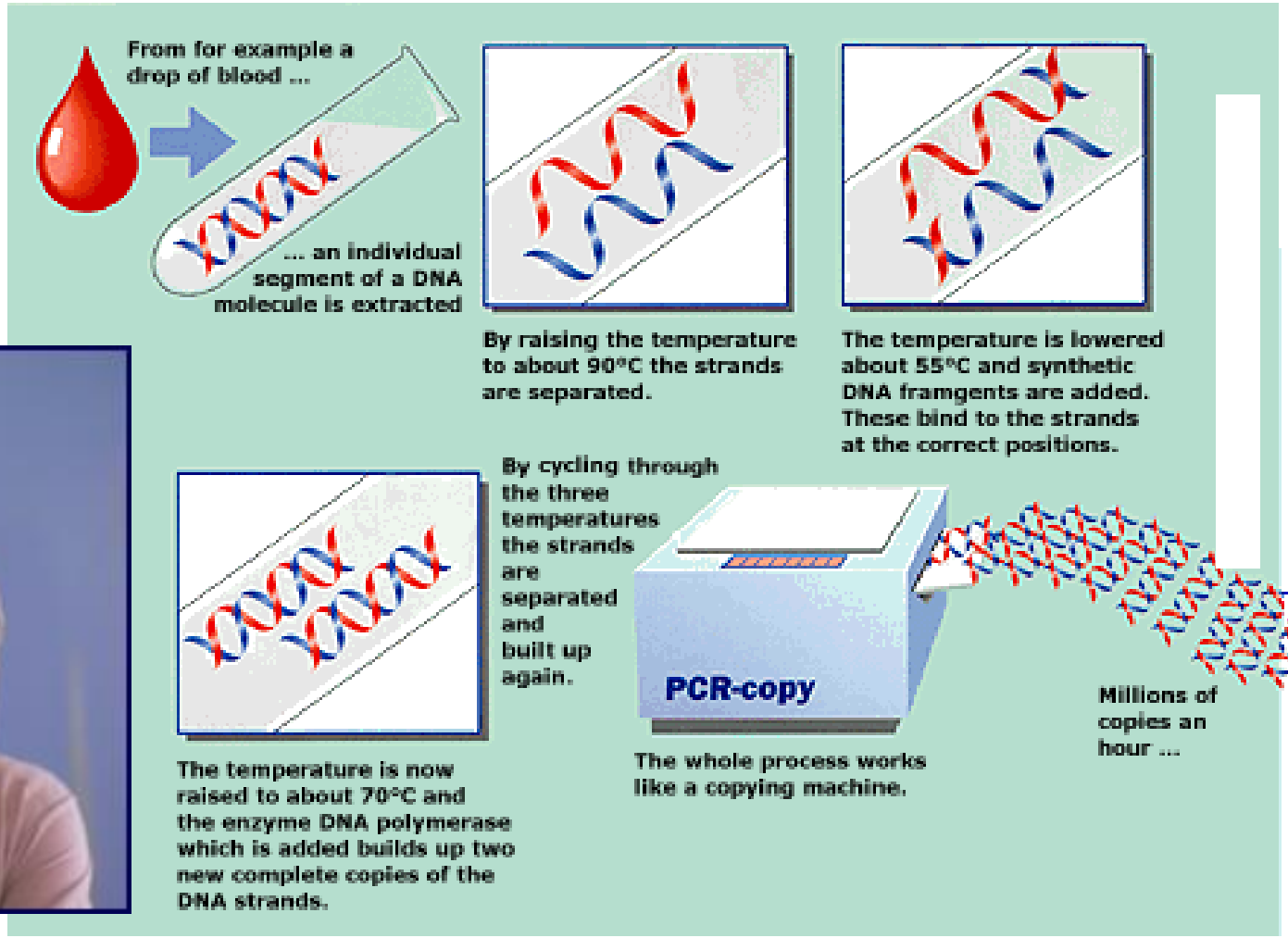
PCR
20-30 cycles
3 steps/cycle
30 sec/step

- **Heat DNA to denature (unwind) it**
  - **90°C destroys DNA polymerase**
  - **have to add new enzyme every cycle**
    - **almost impractical!**
- **Need enzyme that can withstand 90°C…**
  - **Taq polymerase**
    - **from hot springs bacteria**
      - ***Thermus aquaticus***

Kary Mullis

1985 | 1993

- ~~development of PCR technique~~
  - a copying machine for DNA



I'm a-glow!

Got any Questions?