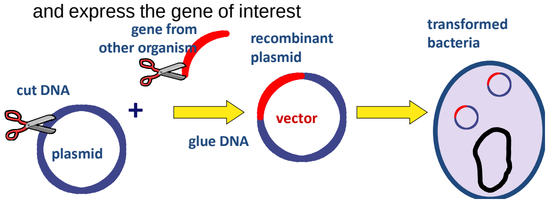
Biotechnology

Cloning

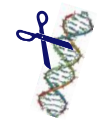
- What is it?
  - The production of multiple copies of a single gene (*gene cloning*)
- How is it used?
  - For basic research on genes and their protein products
  - To make a protein product (insulin, human growth hormone)

Transformation

- What is it?
  - The ability of bacteria to pick up naked foreign DNA from the environment
- How is it used?
  - We can engineer plasmids which bacteria will take up and express the gene of interest



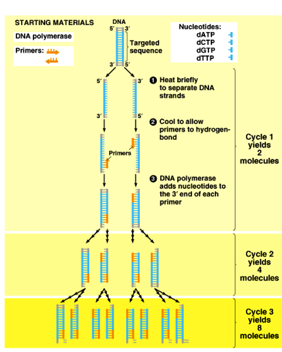
Restriction Enzymes



- What is it?
  - evolved in bacteria to cut up foreign DNA for protection against viruses other bacteria
- How is it used?
  - cut DNA at specific sequences called restriction sites which are symmetrical palindromes
  - produces protruding ends called sticky ends which will bind to any complementary DNA
  - video

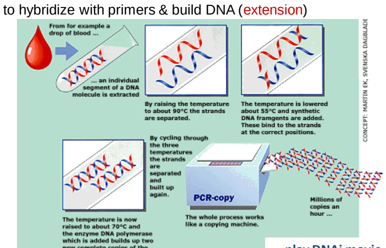
Polymerase Chain Reaction

- What is it?
  - method for making many, many copies of a specific segment of DNA



Polymerase Chain Reaction

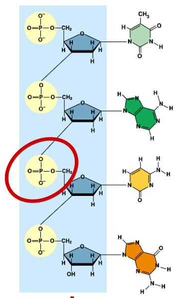
- How is it used?
  - in tube: DNA, DNA polymerase enzyme, primer, nucleotides
  - denature DNA: heat (90°C) DNA to separate strands
  - anneal DNA: cool to hybridize with primers & build DNA (extension)

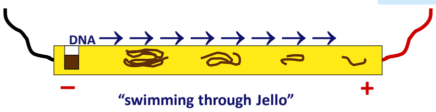


play DNAi movie

Gel electrophoresis

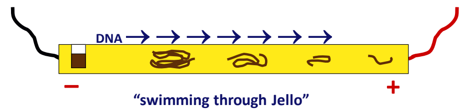
- What is it?
  - A method of separating DNA in a gelatin-like material using an electrical field



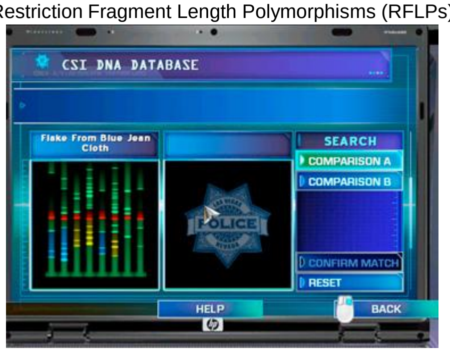


Gel electrophoresis

- How is it used?
  - size of DNA fragment affects how far it travels
    - small pieces travel farther
    - large pieces travel slower & lag behind



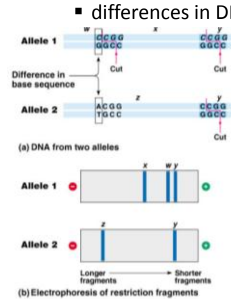
Restriction Fragment Length Polymorphisms (RFLPs)



2005-2006

Restriction Fragment Length Polymorphisms (RFLPs)

- What is it?
  - differences in DNA between individuals

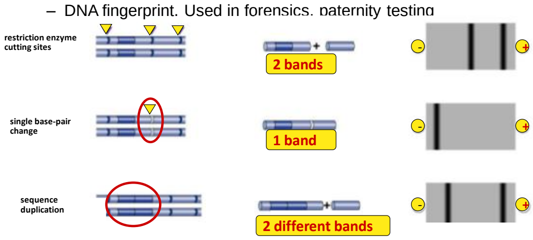


- change in DNA sequence affects restriction enzyme "cut" site
- creates different fragment sizes & different band pattern

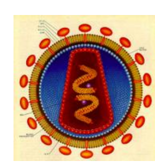
Alec Jeffries
1984

RFLPs

- How is it used?
  - many differences accumulate in "junk" DNA
  - change in DNA sequence affects restriction enzyme "cut" site
  - creates different fragment sizes & different band pattern
  - DNA fingerprint. Used in forensics. paternity testing



Chapter 19.



Viral Genetics

2005-2006

2010

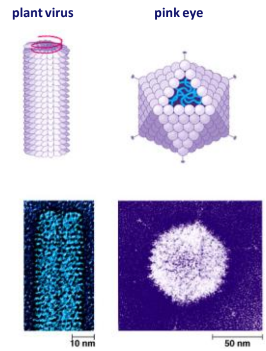
What is a virus? Is it alive?

- DNA or RNA enclosed in a protein coat
- Viruses are not cells
- Extremely tiny
  - electron microscope size
  - smaller than ribosomes
  - ~20–50 nm

1st discovered in plants (1800s)
- **tobacco mosaic virus**
- **couldn't filter out**
- **couldn't reproduce on media like bacteria**

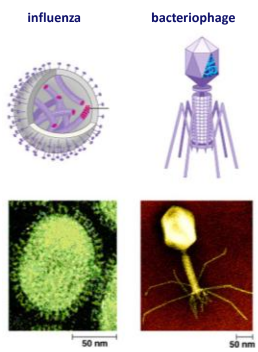
Variation in viruses

- Parasites
  - lack enzymes for metabolism
  - lack ribosomes for protein synthesis
  - need host "machinery"



Variation in viruses

- A package of genes in transit from one host cell to another



"A piece of bad news wrapped in protein"
– Peter Medawar

Viral genomes

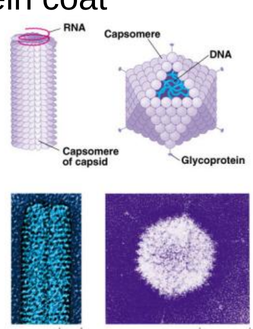
- Viral nucleic acids
  - DNA
    - double-stranded
    - single-stranded
  - RNA
    - double-stranded
    - single-stranded
  - Linear or circular
    - smallest viruses have only 4 genes, while largest have several hundred

Table 18.1 Classes of Animal Viruses, Grouped by Type of Nucleic Acid

Class*	Examples/Diseases
I. dsDNA**	
Papovavirus	Papilloma (human warts, cervical cancer); polyoma (tumors in certain animals)
Adenovirus	Respiratory diseases; some cause tumors in certain animals
Herpesvirus	Herpes simplex I (cold sores), herpes simplex II (genital sores); varicella zoster (chicken pox, shingles); Epstein-Barr virus (mononucleosis, Burkitt's lymphoma)
Poxvirus	Smallpox; vaccinia, cowpox
II. ssDNA	
Parvovirus	Roseola; most parvoviruses depend on co-infection with adenoviruses for growth
III. dsRNA	
Reovirus	Diarrhea; mild respiratory diseases
IV. ssRNA that can serve as mRNA	
Picornavirus	Poliovirus; rhinovirus (common cold); enteric (intestinal) viruses
Togavirus	Rubella virus; yellow fever virus; encephalitis viruses
V. ssRNA that is a template for mRNA	
Rhabdovirus	Rabies
Paramyxovirus	Measles; mumps
Orthomyxovirus	Influenza viruses
VI. ssRNA that is a template for DNA synthesis	
Retrovirus	RNA tumor viruses (e.g., leukemia viruses); HIV (AIDS virus)

Viral protein coat

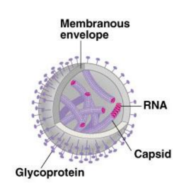
- Capsid
  - crystal-like protein shell
  - 1-2 types of proteins
  - many copies of same protein



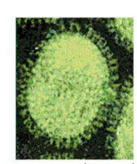
(a) Tobacco mosaic virus (b) Adenoviruses

Viral envelope

- Lipid bilayer membranes cloaking viral capsid
  - envelopes are derived from host cell membrane
    - glycoproteins on surface



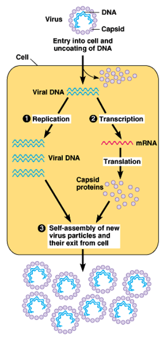
HIV



(c) Influenza viruses

Generalized viral lifecycle

- Entry
  - virus DNA/RNA enters host cell
- Assimilation
  - viral DNA/RNA takes over host
  - reprograms host cell to copy viral nucleic acid & build viral proteins
- Self assembly
  - nucleic acid molecules & capsomeres then self-assemble into viral particles
  - exit cell

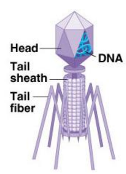


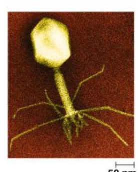
Viral hosts

- Host range
  - most types of virus can infect & parasitize only a limited range of host cells
    - identify host cells via “lock & key” fit
    - between proteins on viral coat & receptors on host cell surface
  - broad host range
    - rabies = can infect all mammals
  - narrow host range
    - human cold virus = only cells lining upper respiratory tract of humans
    - HIV = binds only to specific white blood cells

Bacteriophages

- Viruses that infect bacteria
  - ex. phages that infect *E. coli*
  - lambda phage
  - 20-sided capsid head encloses DNA
  - protein tail attaches phage to host & injects phage DNA inside

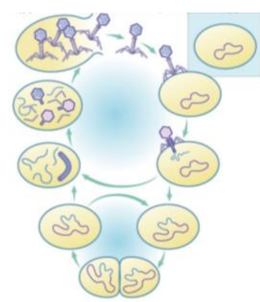




(d)Bacteriophage T4

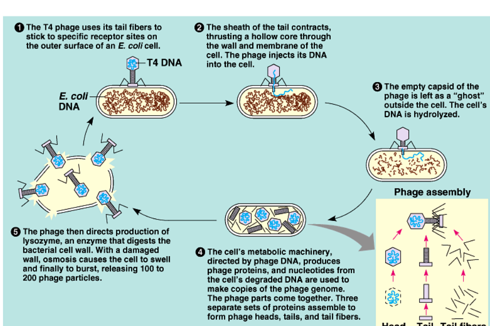
Bacteriophage lifecycles

- Lytic
  - reproduce virus in bacteria
  - release virus by rupturing bacterial host
- Lysogenic
  - integrate viral DNA into bacterial DNA
  - reproduce with bacteria



2005-2006

Lytic lifecycle of phages



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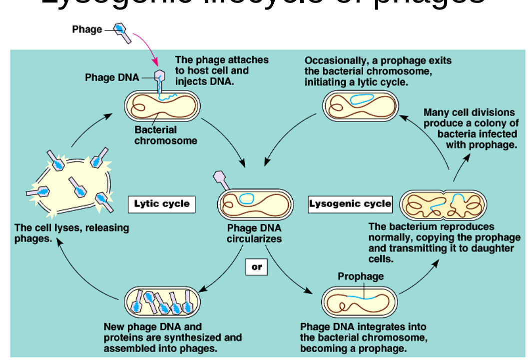
Lysogenic lifecycle of phages



Defense against viruses

- Bacteria have defenses against phages
  - bacterial mutants with receptors that are no longer recognized by a phage
    - natural selection favors these mutants
  - bacteria produce restriction enzymes
    - recognize & cut up foreign DNA
- It's an escalating war!
  - natural selection favors phage mutants resistant to bacterial defenses

RNA viruses

- Retroviruses
  - have to copy viral RNA into host DNA
    - enzyme = reverse transcriptase
    - RNA → DNA → mRNA
  - host's RNA polymerase now transcribes viral DNA into viral mRNA
    - mRNA codes for viral components
    - host's ribosomes produce new viral proteins