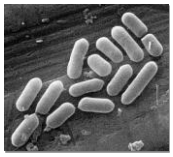
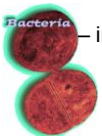
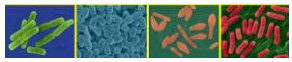
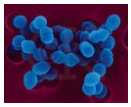
Bacteria



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

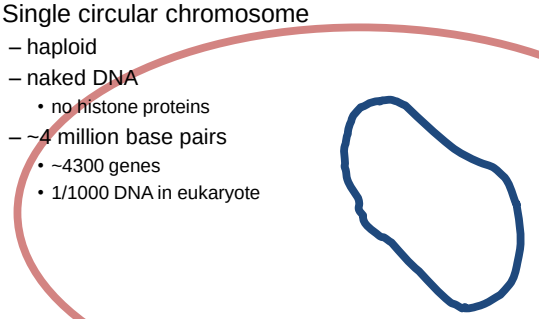






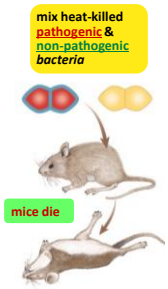
Bacterial genome

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



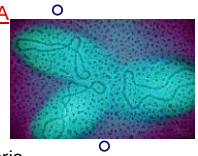
Transformation

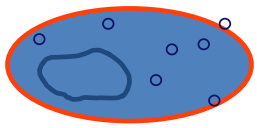
- Bacteria are opportunists
  - pick up naked foreign DNA wherever it may be hanging out
    - have surface transport proteins that are specialized for the uptake of naked DNA
  - import bits of chromosomes from other bacteria
  - incorporate the DNA bits into their own chromosome
    - express new genes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



Plasmids

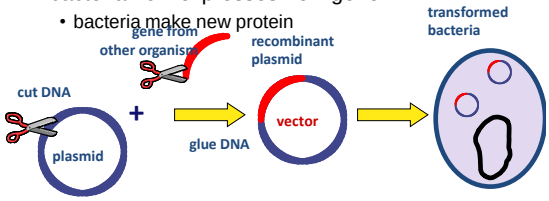
- Small supplemental circles of DNA
  - 5000 - 20,000 base pairs
  - self-replicating
  - carry extra genes
    - 2-30 genes
    - genes for antibiotic resistance
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
  - can be imported from environment





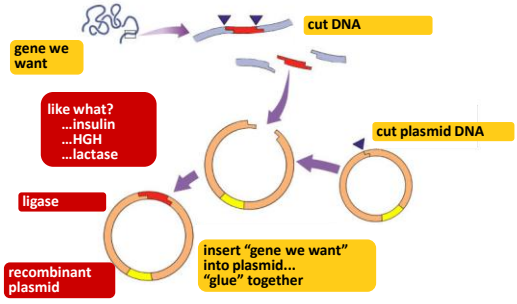
How can plasmids help us?

- 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

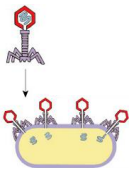


Biotechnology

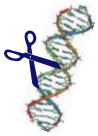
- Plasmids used to insert new genes into bacteria

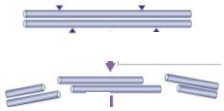


How do we cut DNA?



- 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 not using the base sequences recognized by the enzymes in their own DNA

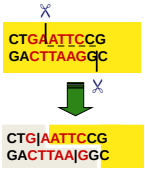




Restriction enzymes

Madam I'm Adam

- 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



Restriction enzymes

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

restriction enzyme cut site

GTAACGAATTCACGCTT
CATTGCTTAAGTGCGAA

restriction enzyme cut site

GTAACG AATTCACGCTT
CATTGCTTAA GTGCGAA

Sticky ends

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

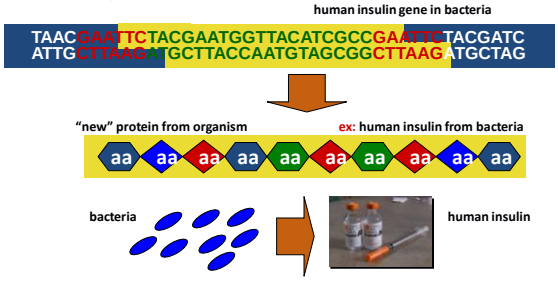
GTAACG AATTCACGCTT
CATTGCTTAA GTGCGAA — gene you want

GGACCTG AATTCCGGATA
CCTGGACTTAA GGCCTAT — chromosome want to add gene to

GGACCTG AATTCACGCTT
CCTGGACTTAA GTGCGAA — combined DNA

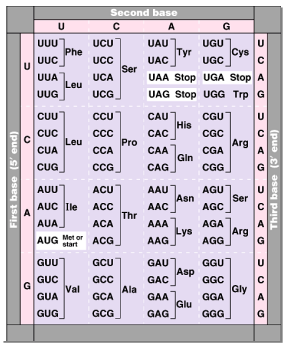
Why mix genes together?

- Gene produces protein in different organism or different individual

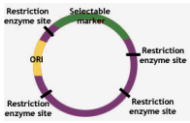


The code is universal

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



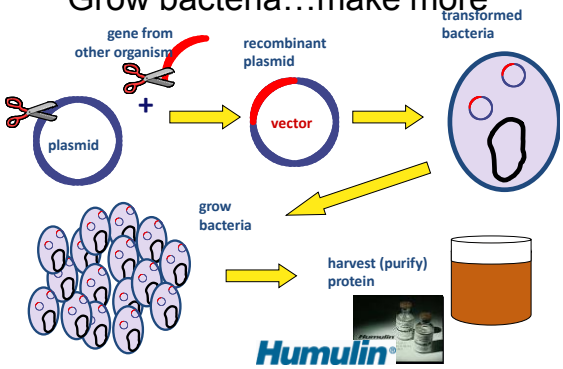
Copy (& Read) DNA



- 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

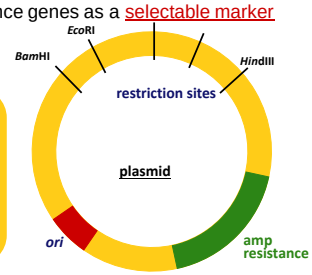
Grow bacteria…make more



Engineered plasmids

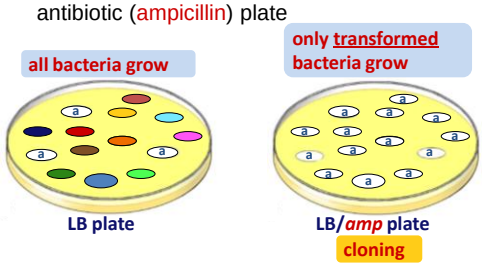
- Building custom plasmids
  - restriction enzyme sites
  - antibiotic resistance genes as a selectable marker

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



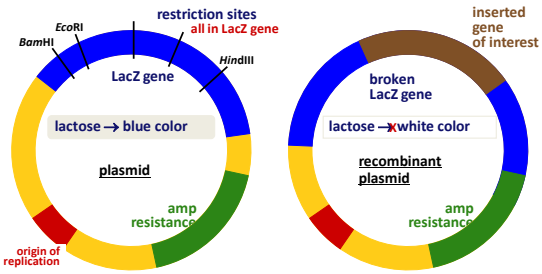
Selection for plasmid uptake

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



Need to screen plasmids

- Need to make sure bacteria have recombinant plasmid



Screening for recombinant plasmid