What do you notice about these phrases?

radar

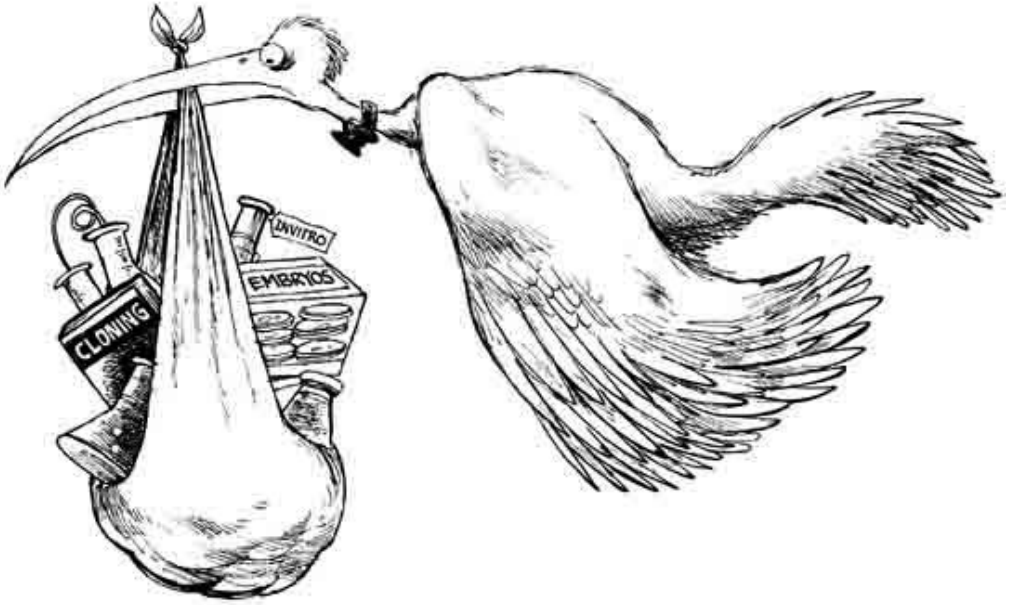
racecar

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?



Chapter 20.

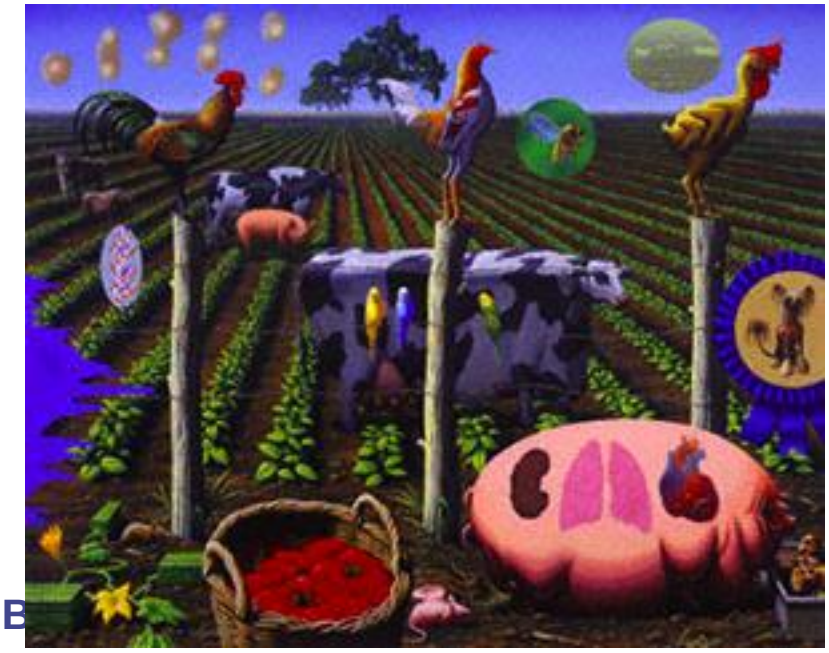
Biotechnology: DNA Technology & Genomics

The BIG Questions…

- How can we use our knowledge of DNA to:
  - diagnose disease or defect?
  - cure disease or defect?
  - change/improve organisms?
- What are the techniques & applications of biotechnology?
  - direct manipulation of genes for practical purposes

Biotechnology

- **Genetic manipulation of organisms is not new**
  - **humans have been doing this for thousands of years**
    - **plant & animal breeding**

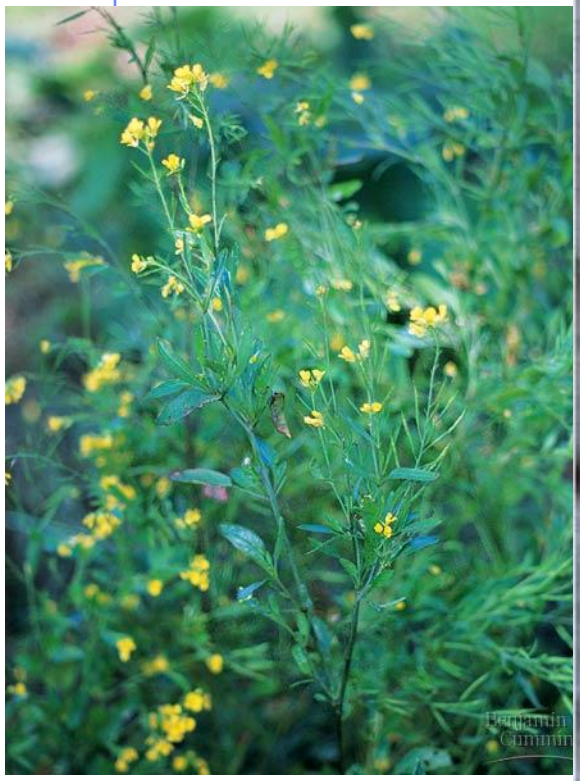


Evolution & breeding of food plants

Evolution of Zea mays from ancestral teosinte (left) to modern corn (right). The middle figure shows possible hybrids of teosinte & early corn varieties

Evolution & breeding of food plants

- "Descendants" of the wild mustard
  - Brassica spp.

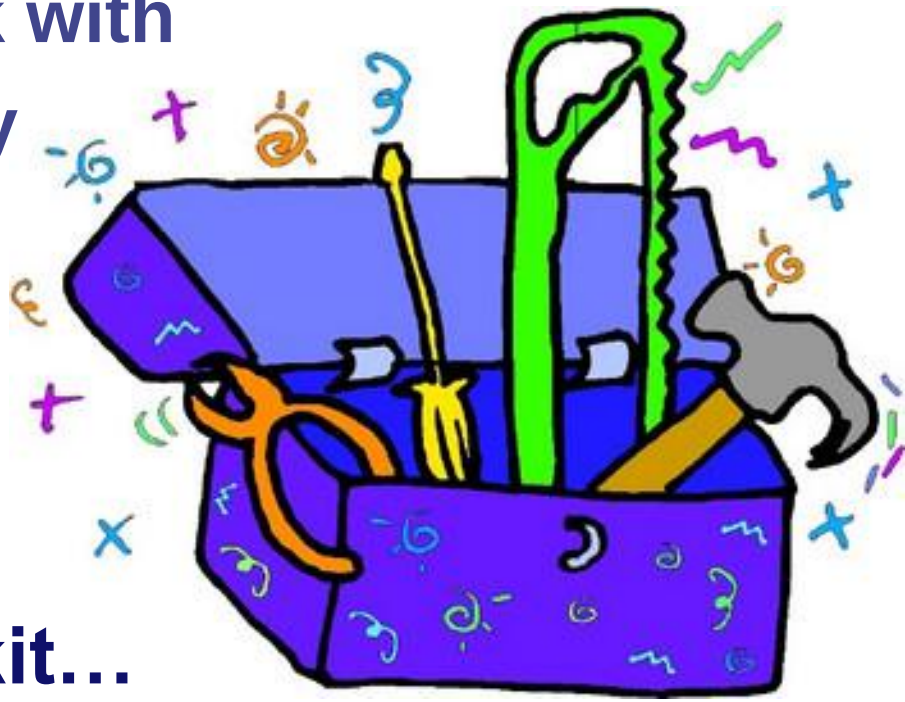


Animal husbandry / breeding

AP B

Biotechnology today

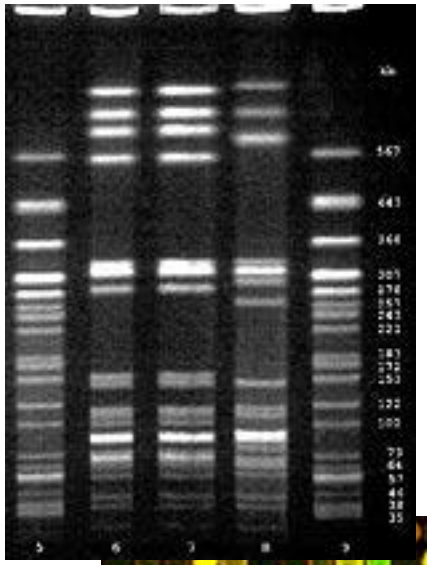
- **Genetic Engineering**
  - manipulation of DNA
  - if you are going to engineer DNA & genes & organisms, then you need a **set of tools** to work with
  - this unit is a survey of those tools…

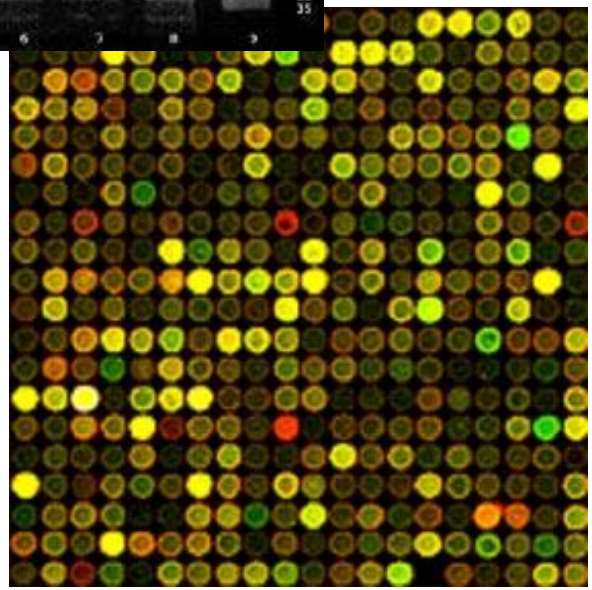


Our tool kit…

Bioengineering Tool kit

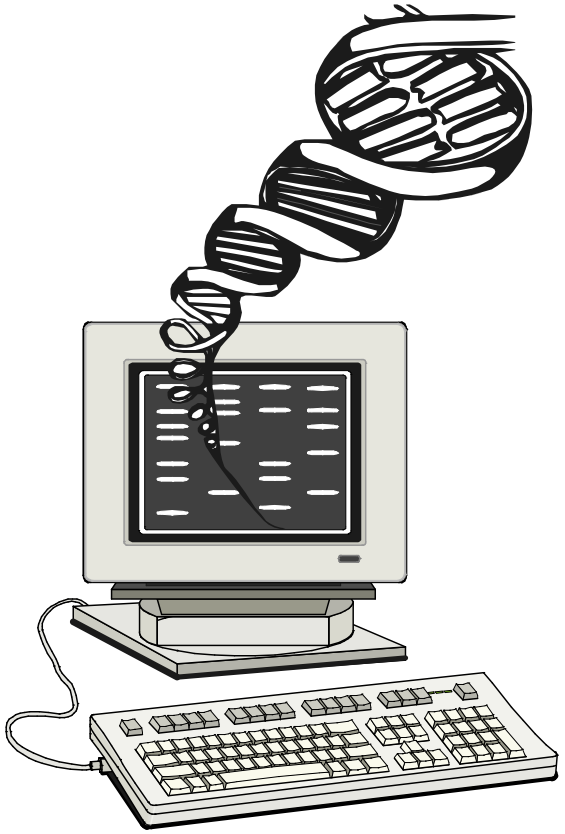
- **Basic Tools**
  - restriction enzymes
  - ligase
  - plasmids / cloning
  - DNA libraries / probes
- **Advanced Tools**
  - PCR
  - DNA sequencing
  - gel electrophoresis
  - Southern blotting
  - microarrays





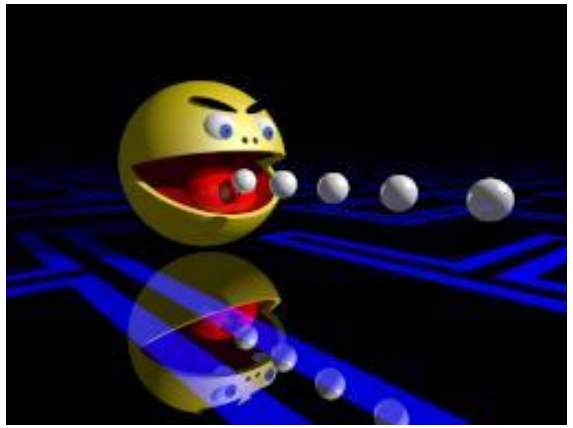
Cut, Paste, Copy, Find…

- Word processing metaphor…
  - cut
    - restriction enzymes
  - paste
    - ligase
  - copy
    - plasmids
      - bacteria
      - transformation
    - PCR
  - find
    - Southern blotting / probes



Cut DNA

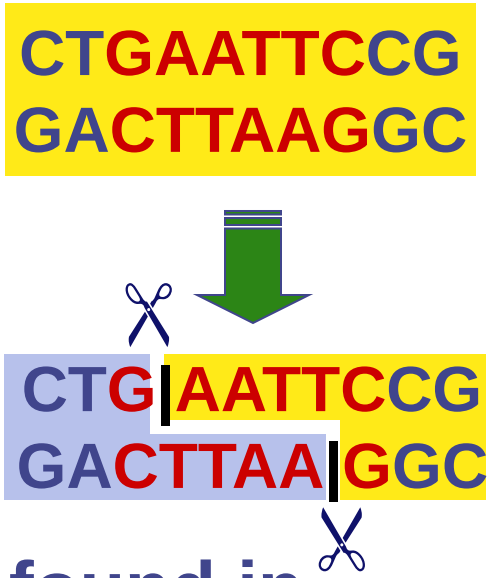
- Restriction enzymes
  - restriction endonucleases
  - discovered in 1960s
  - evolved in bacteria to cut up foreign DNA (“restriction”)
    - protection against viruses & other bacteria
      - bacteria protect their own DNA by methylation & by not using the base sequences recognized by the enzymes in their own DNA



Madam I'm Adam

Restriction enzymes

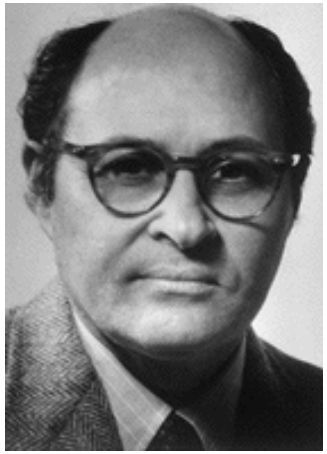
- Action of enzyme
  - cut DNA at specific sequences
    - restriction site
  - symmetrical "palindrome"
  - produces protruding ends
    - sticky ends
- Many different enzymes
  - named after organism they are found in
    - EcoRI, HindIII, BamHI, SmaI (E.coli, H. influenza, Bacillus a., Serratia m.)



1960s|1978

Discovery of restriction enzymes

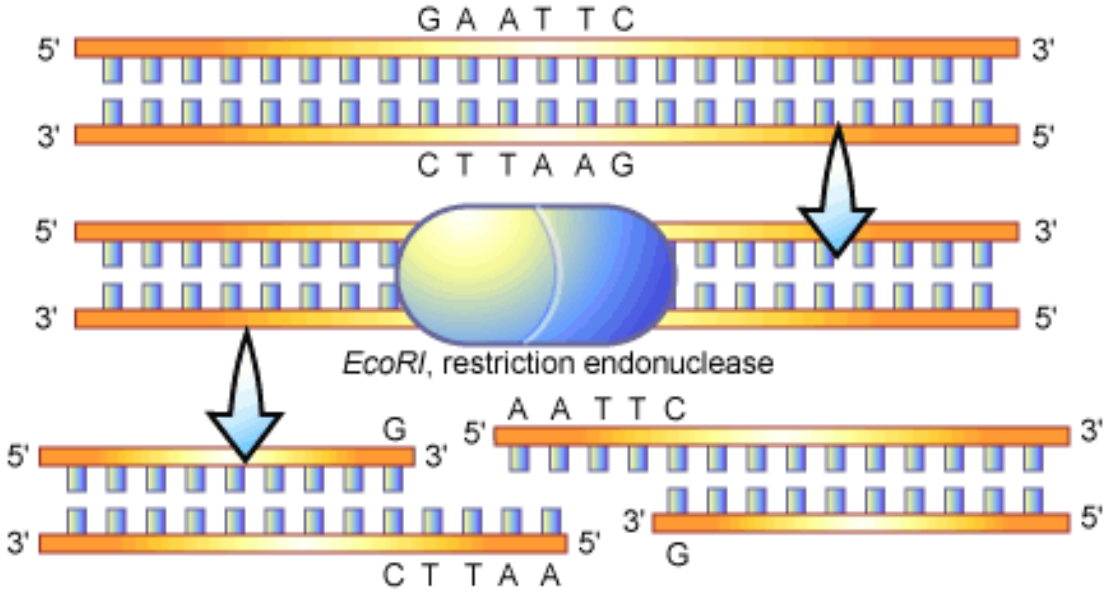
Werner Arber



Daniel Nathans

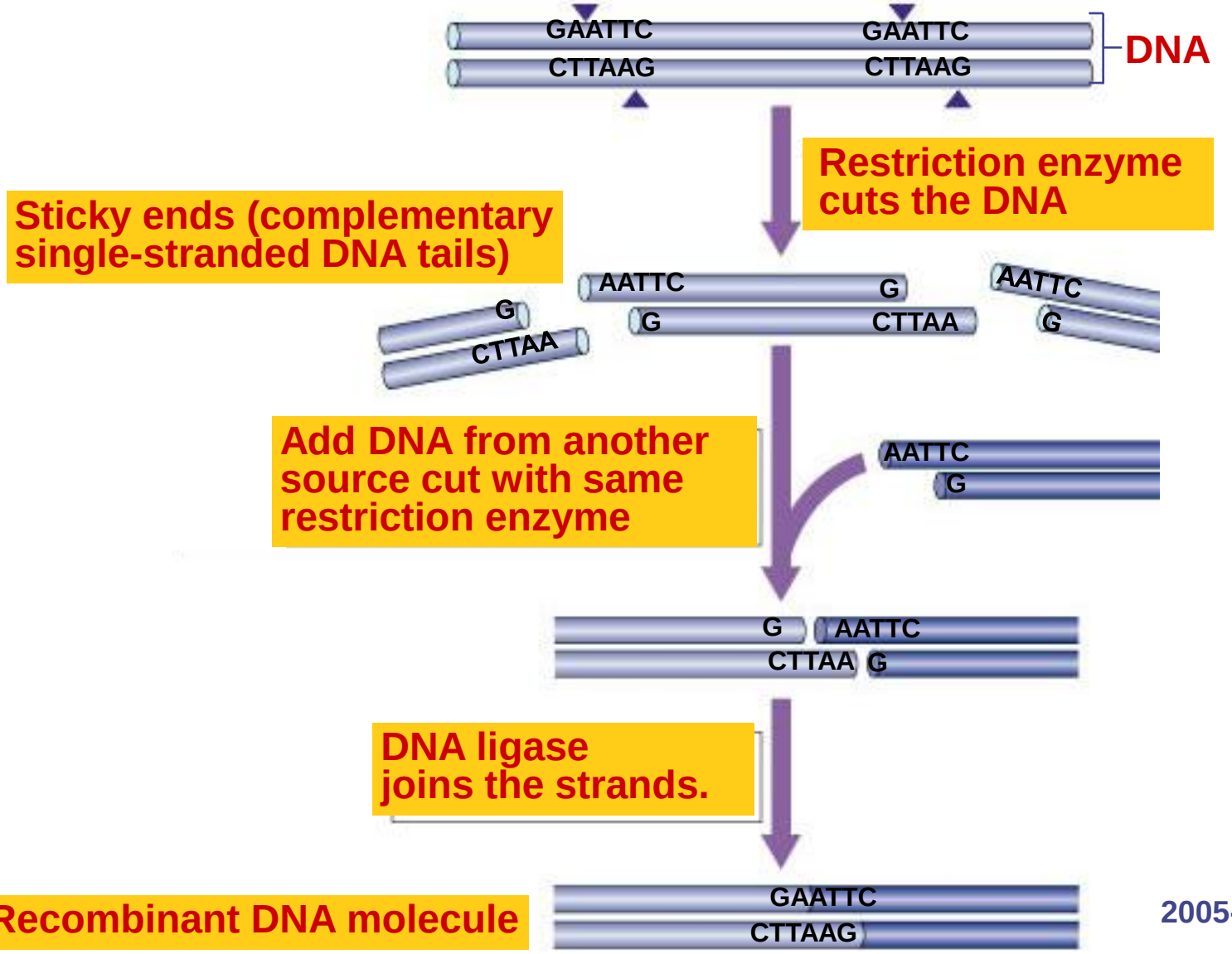
Hamilton O. Smith

Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in E. coli



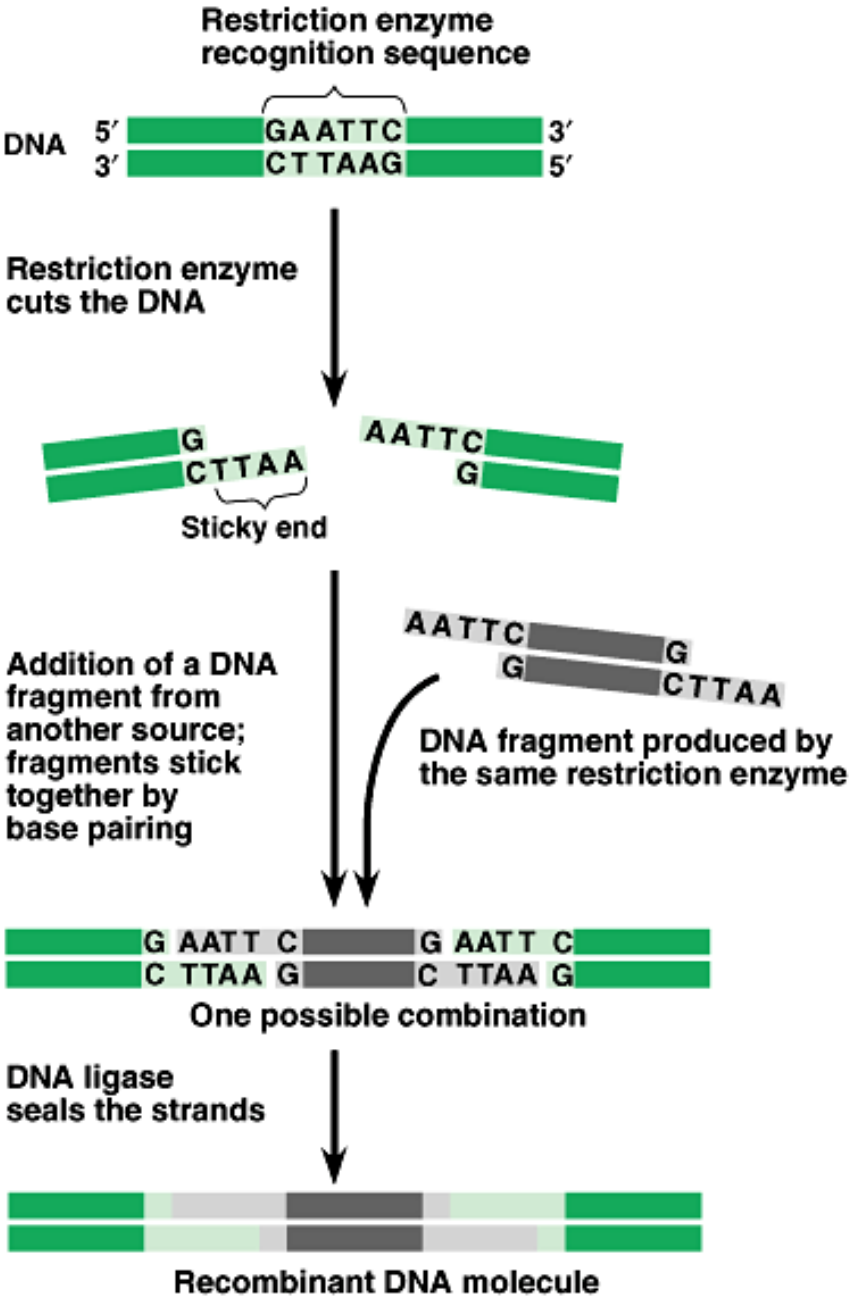
Restriction enzyme movie

Biotech use of restriction enzymes

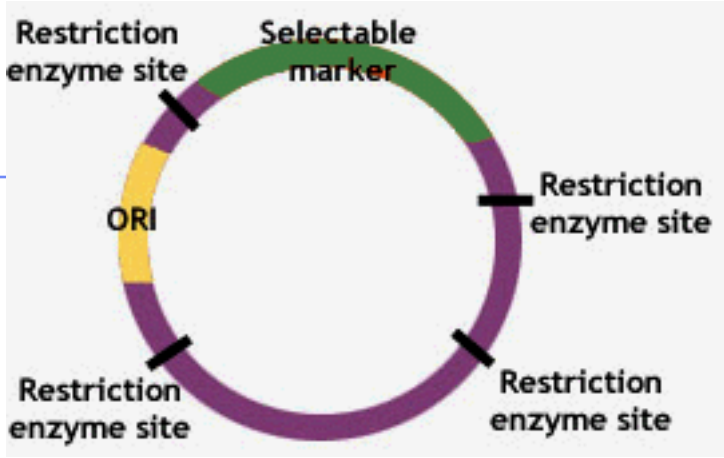


Paste DNA

- Sticky ends allow:
  - H bonds between complementary bases to anneal
- Ligase
  - enzyme “seals” strands
    - bonds sugar-phosphate bonds
    - covalent bond of DNA backbone



Copy DNA



- **Plasmids**
  - **small, self-replicating circular DNA molecules**
    - **insert DNA sequence into plasmid**
      - **vector = "vehicle" into organism**
  - **transformation**
    - **insert recombinant plasmid into bacteria**
      - **bacteria make lots of copies of plasmid**
    - **grow recombinant bacteria on agar plate**
      - **clone of cells = lots of bacteria**
    - **production of many copies of inserted gene**

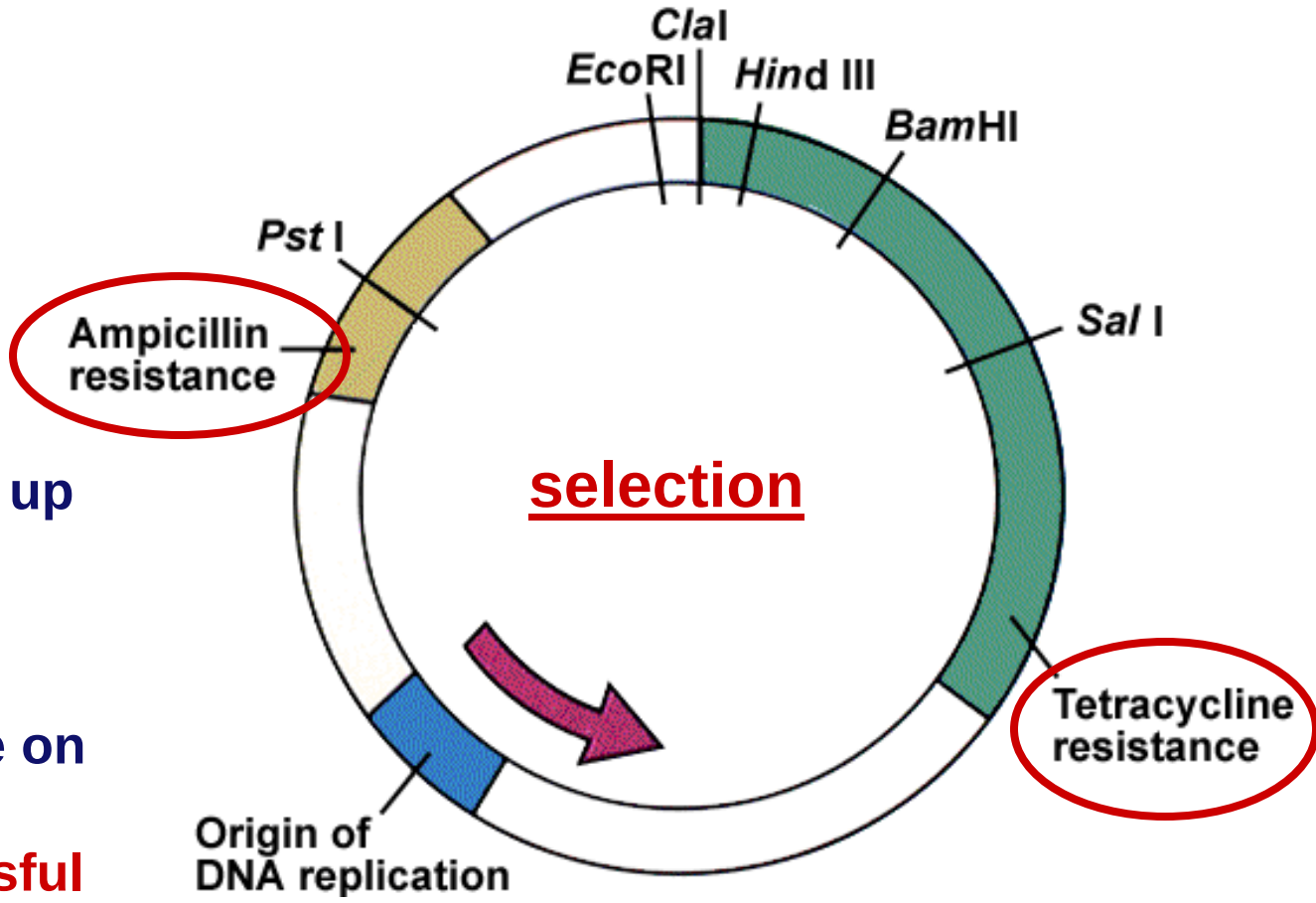
DNA → RNA → protein → trait

Recombinant plasmid

- Antibiotic resistance genes as a selectable marker
- Restriction sites for splicing in gene of interest

Selectable marker

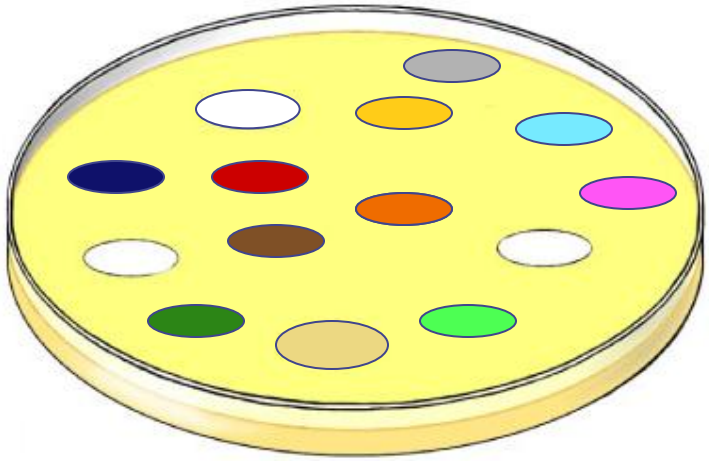
- Plasmid has both "added" gene & antibiotic resistance gene
- If bacteria don't pick up plasmid then die on antibiotic plates
- If bacteria pick up plasmid then survive on antibiotic plates
- selecting for successful transformation



Selection for plasmid uptake

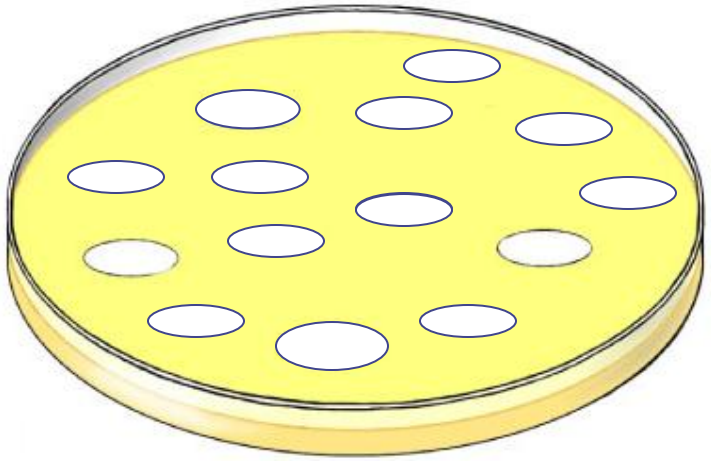
- **Ampicillin becomes a selecting agent**
  - **only bacteria with the plasmid will grow on amp plate**

all bacteria grow



LB plate

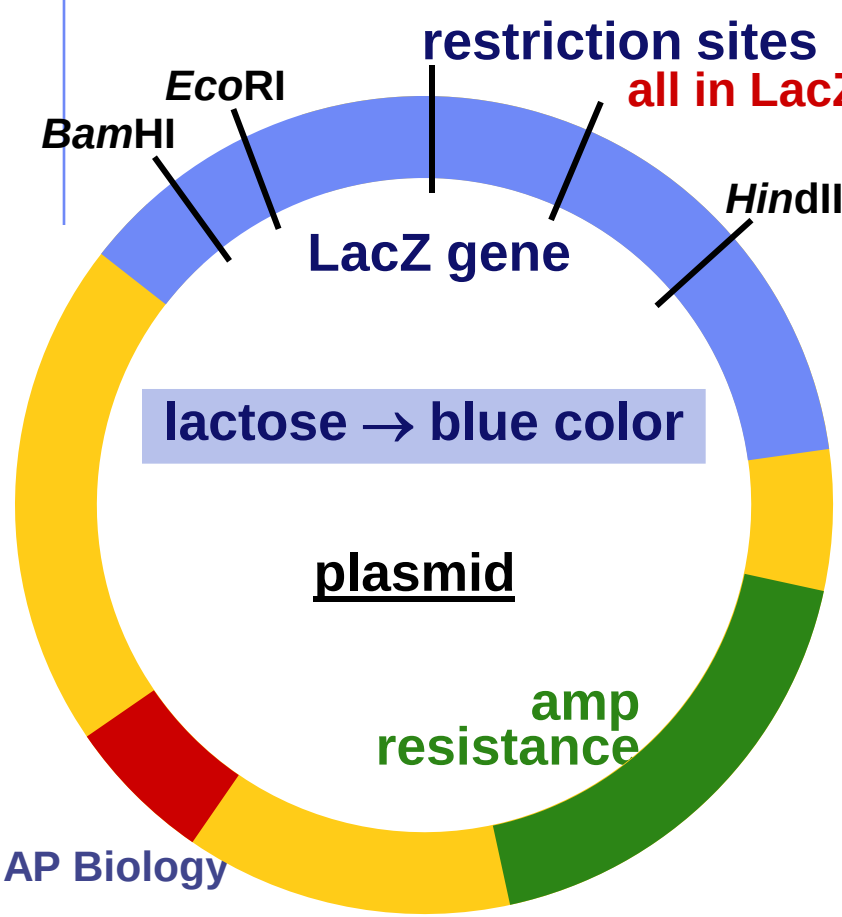
only transformed bacteria grow

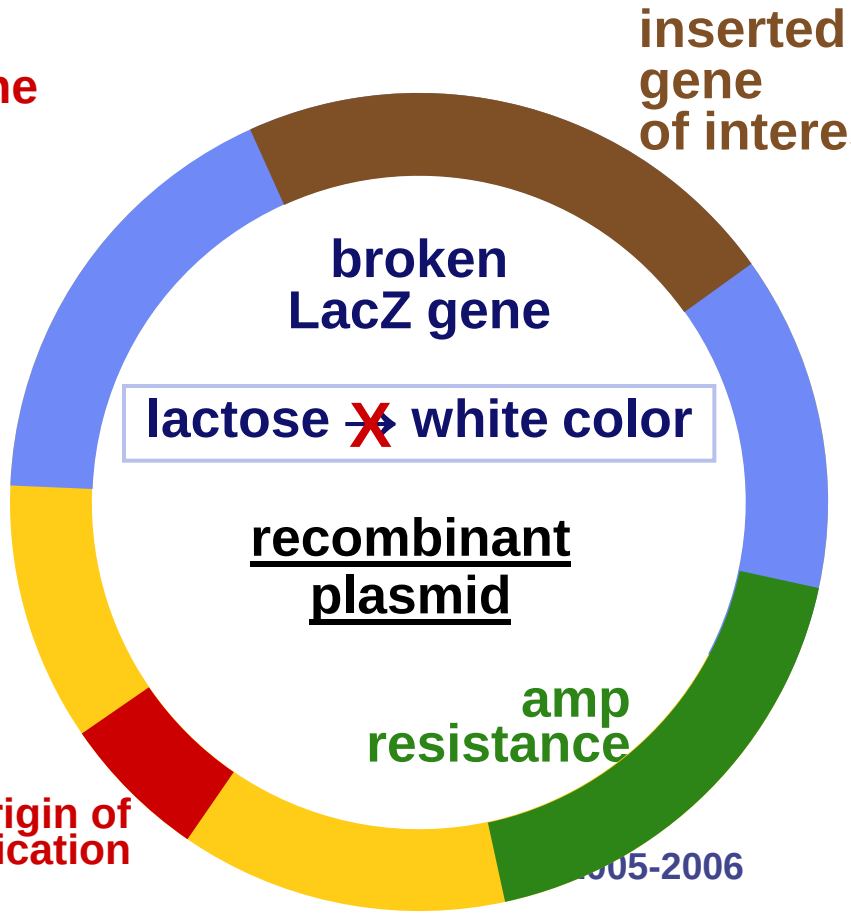


LB/amp plate

Need to screen…

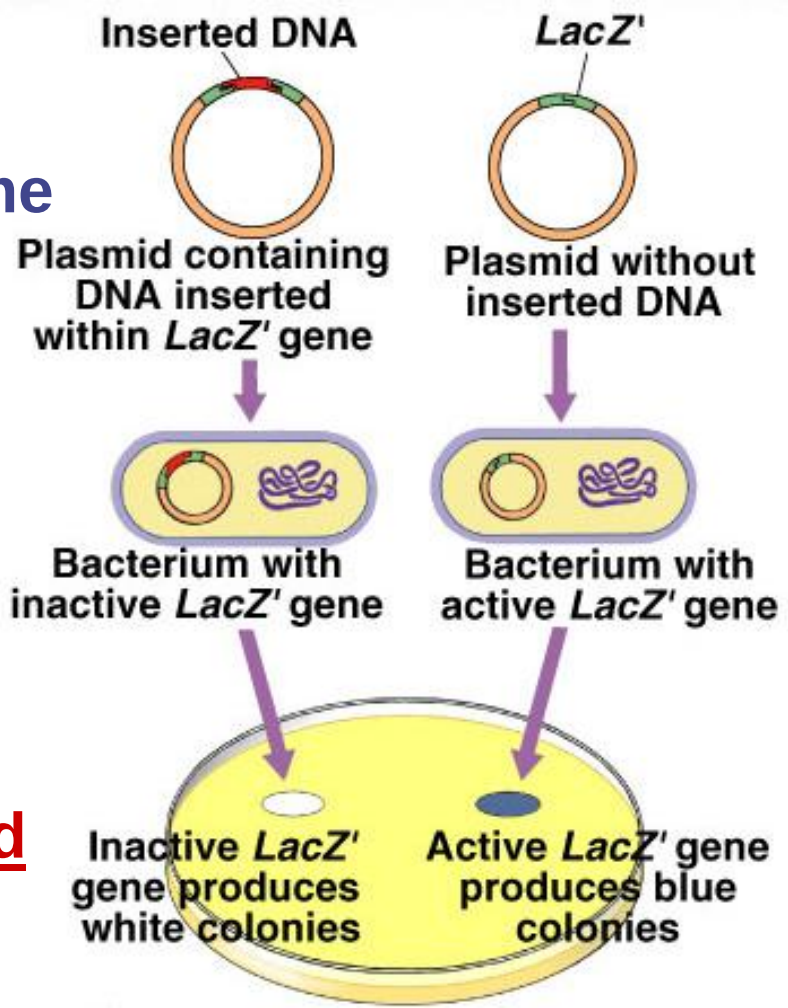
- Need to make sure bacteria have recombinant plasmid





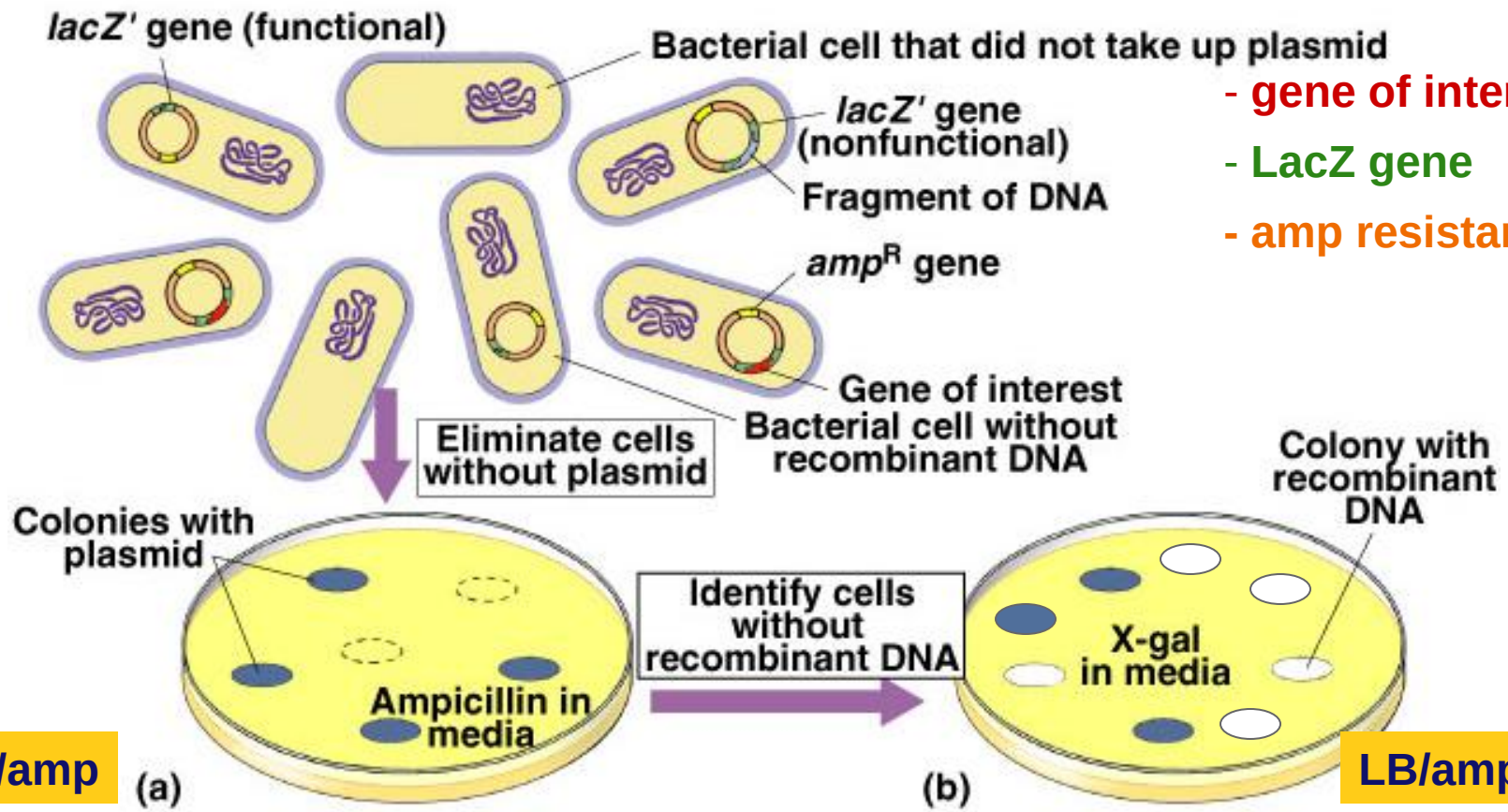
LacZ is a screening system

- **Make sure inserted plasmid is recombinant plasmid**
  - LacZ gene on plasmid produces digestive enzyme
    - lactose (X-gal) → blue
    - blue colonies
  - insert foreign DNA into LacZ gene breaks gene
    - lactose (X-gal) ~~→~~ ~~blue~~
    - white colonies
  - white bacterial colonies have recombinant plasmid



We want these!!

Amp selection & LacZ screening



- gene of interest
- LacZ gene
- amp resistance

LB/amp

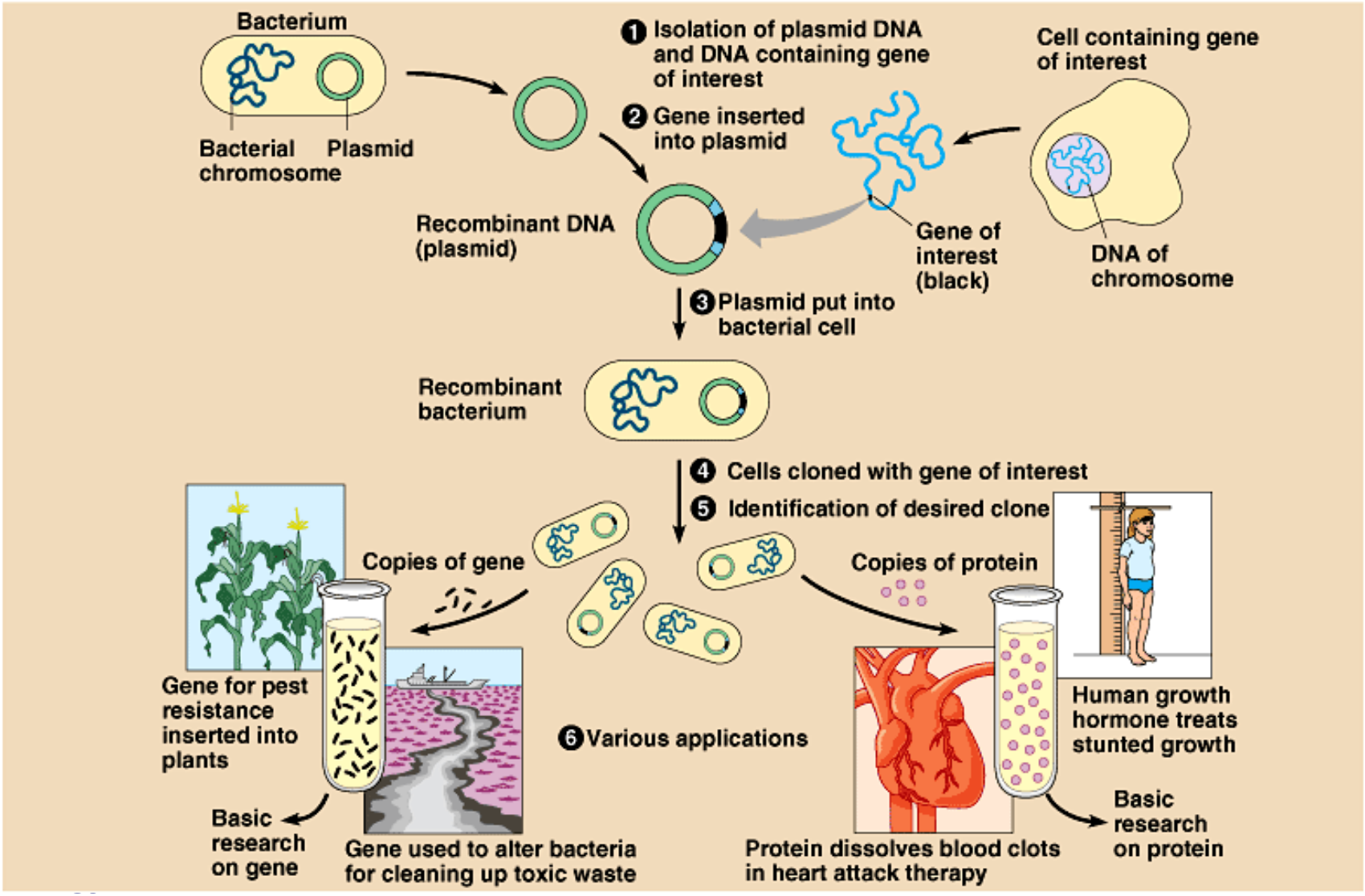
LB/amp/Xgal

Cells that did not take up the plasmid are not resistant to ampicillin and do not form colonies on media containing this antibiotic.

Cells that did not take up DNA fragments have functional *lacZ′* genes, are able to metabolize X-gal, and turn blue on media that contain X-gal.

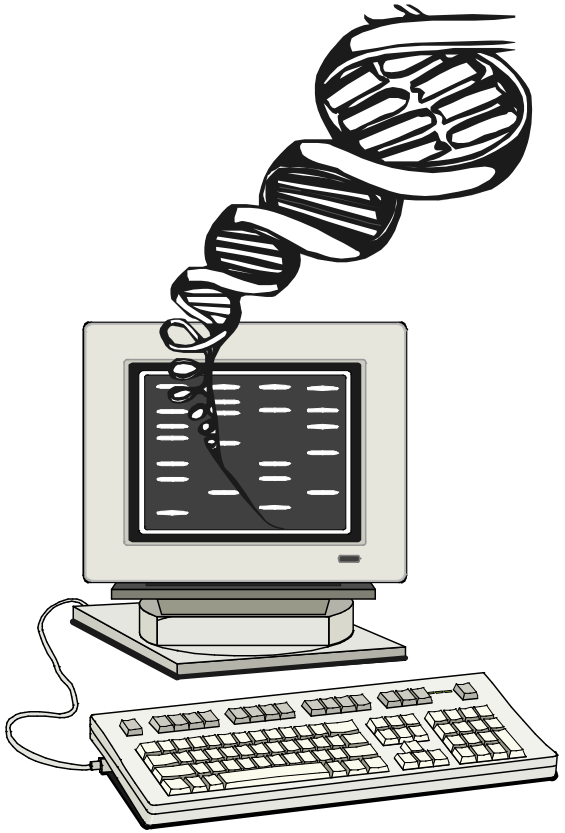
Recombinant DNA movie

Gene cloning

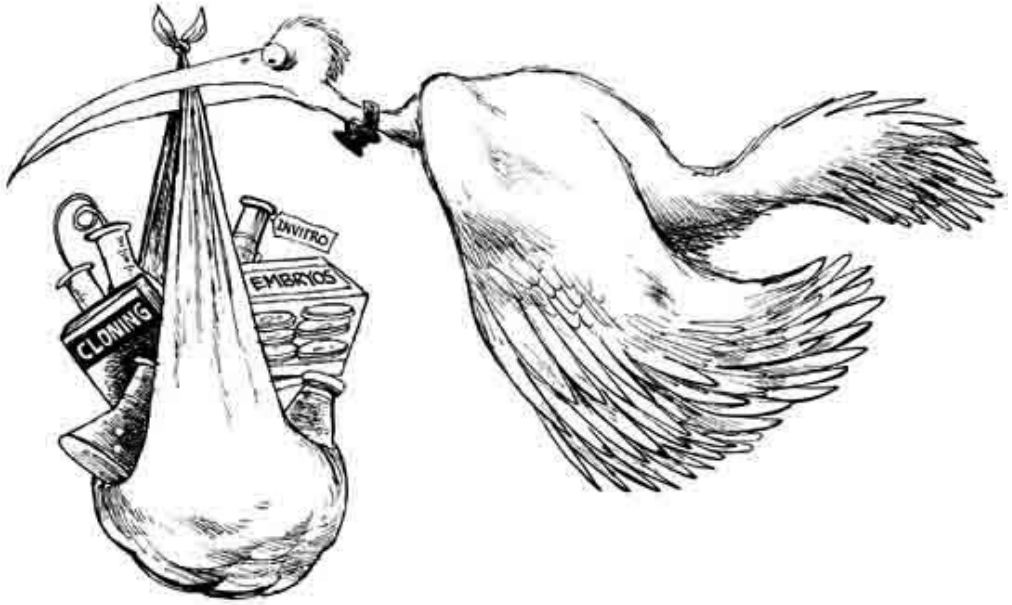


Cut, Paste, Copy, Find…

- Word processing metaphor…
  - cut
    - restriction enzymes
  - paste
    - ligase
  - copy
    - plasmids
      - bacteria
      - transformation
    - PCR
  - find
    - Southern blotting / probes



Any Questions??



Chapter 20.

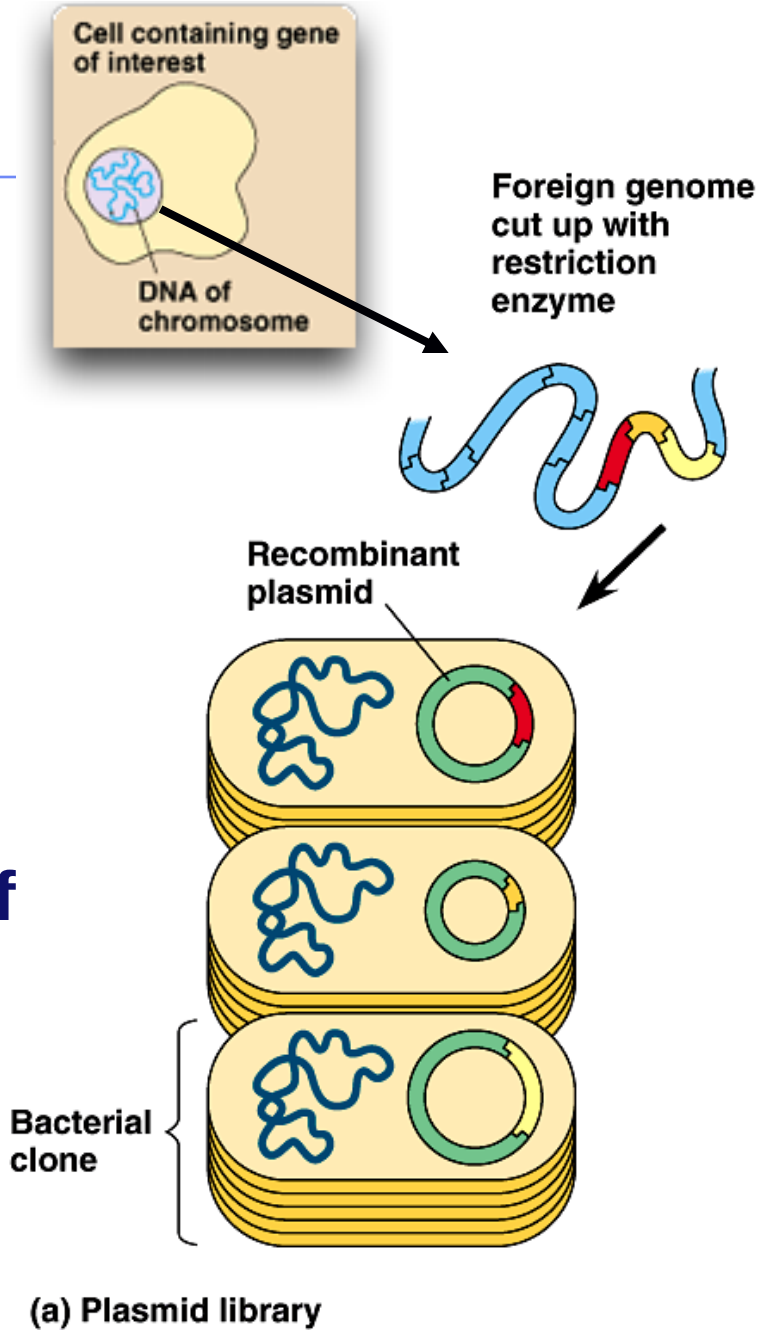
Biotechnology: DNA Technology & Genomics Part 2

What if you don't have your gene conveniently on a chunk of DNA ready to insert into a plasmid?

Have to find your "gene of interest" out of the entire genome of the organism…

DNA libraries

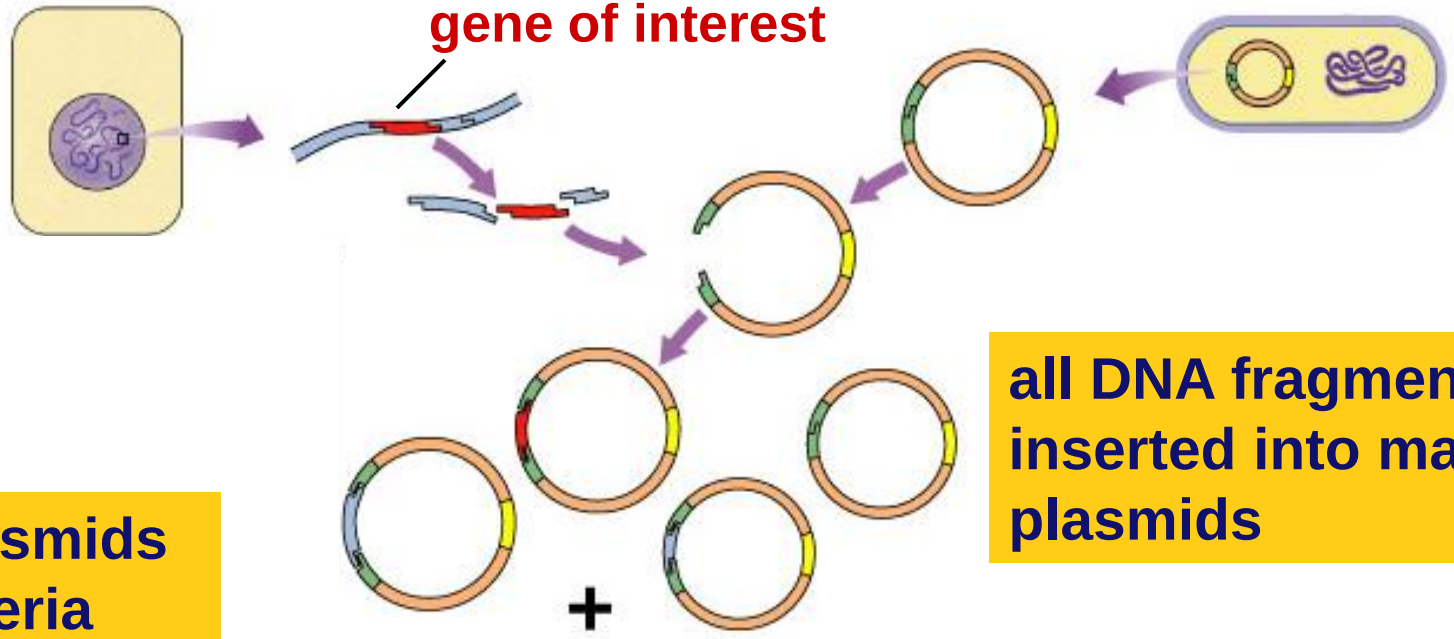
- Cut up all of nuclear DNA from many cells of an organism
  - restriction enzyme
- Clone <u>all</u> fragments into plasmids at same time
  - “shotgun” cloning
- Create a stored collection of DNA fragments
  - petri dish has a collection of all DNA fragments from the organism



Making a DNA library 1

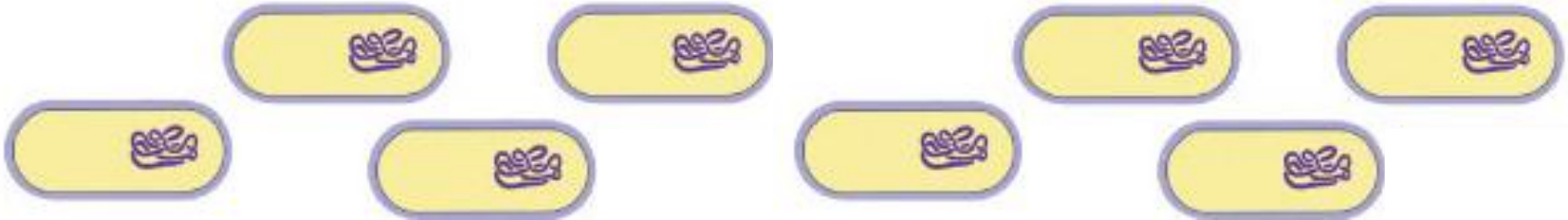
all DNA from many cells of an organism is cut with restriction enzymes

engineered plasmid with selectable marker & screening LacZ gene

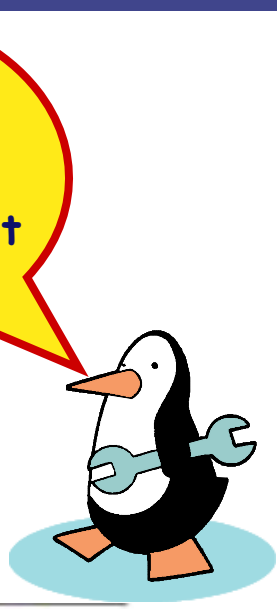


all DNA fragments inserted into many plasmids

clone plasmids into bacteria

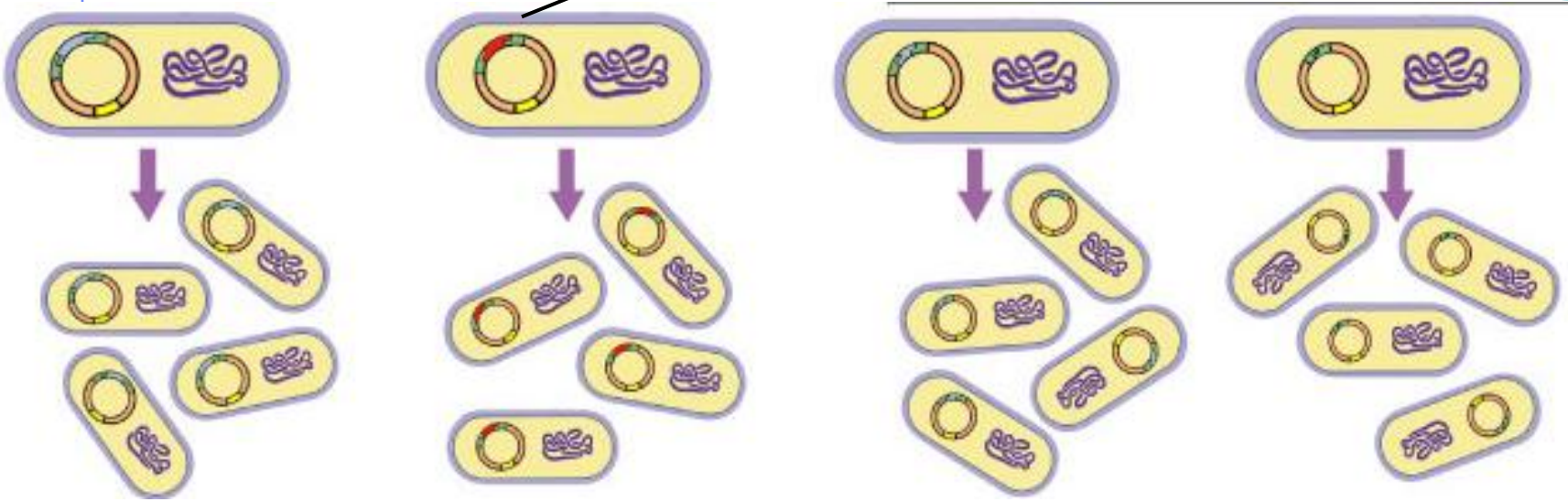


Making a DNA library 2



recombinant plasmids inserted into bacteria

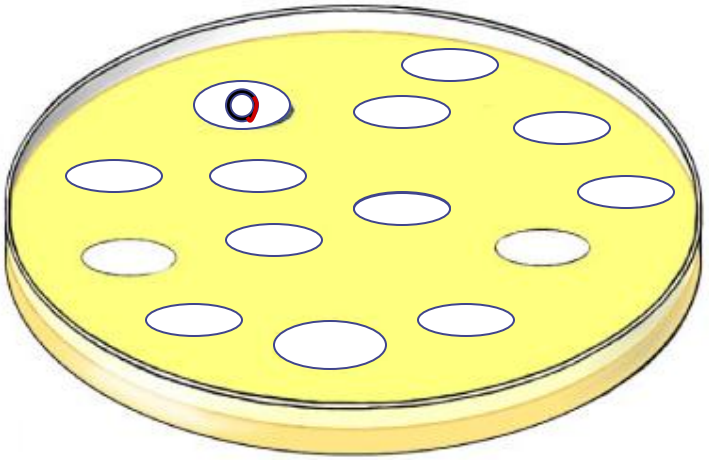
gene of interest



bacterial colonies (clones) grown on LB/amp/Xgal petri plates

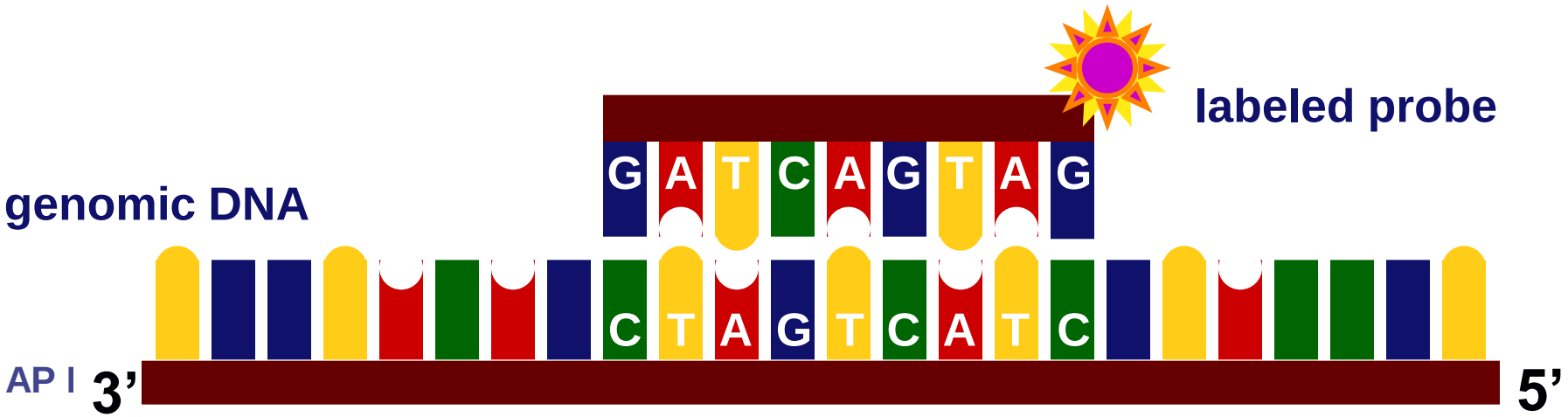
Find your gene in DNA library

- **Locate Gene of Interest**
  - to find your gene you need some of gene's sequence
    - if you know sequence of protein…
      - can guess part of DNA sequence
      - "back translate" protein to DNA
    - if you have sequence of similar gene from another organism…
      - use part of this sequence



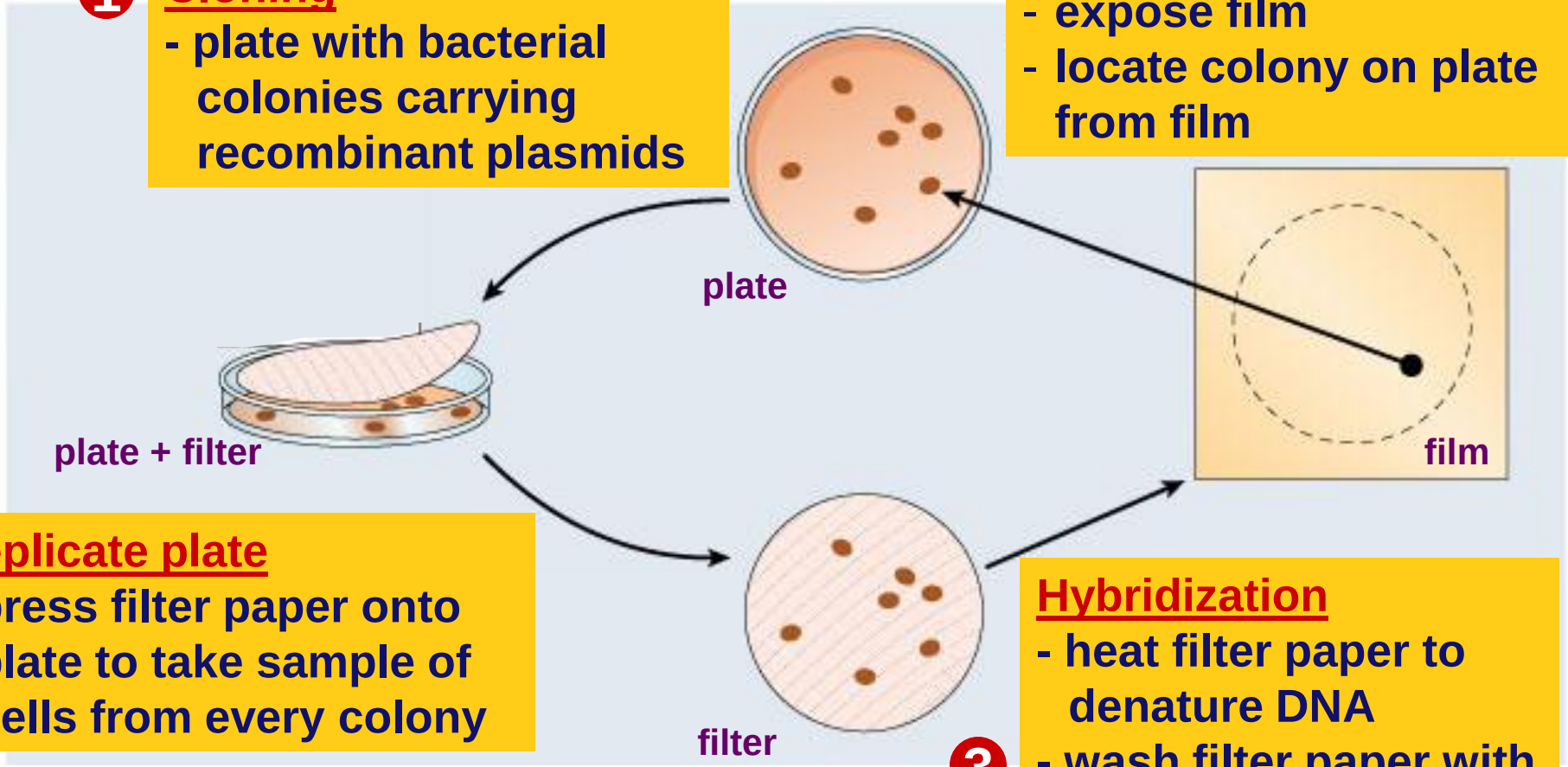
Locating your gene of interest

- **DNA hybridization**
  - find gene in bacterial colony using a **probe**
    - short, single stranded DNA molecule
    - complementary to part of gene of interest
    - tagged with radioactive P^{32} or fluorescence
  - heat treat genomic DNA
    - unwinds (**denatures**) strands
  - DNA **hybridization** between probe & denatured DNA



Hybridization

1. **Cloning**
   - plate with bacterial colonies carrying recombinant plasmids



2. **Replicate plate**
   - press filter paper onto plate to take sample of cells from every colony

3. **Hybridization**
   - heat filter paper to denature DNA
   - wash filter paper with radioactive probe which will only attach to gene of interest

4. **Locate**
   - expose film
   - locate colony on plate from film

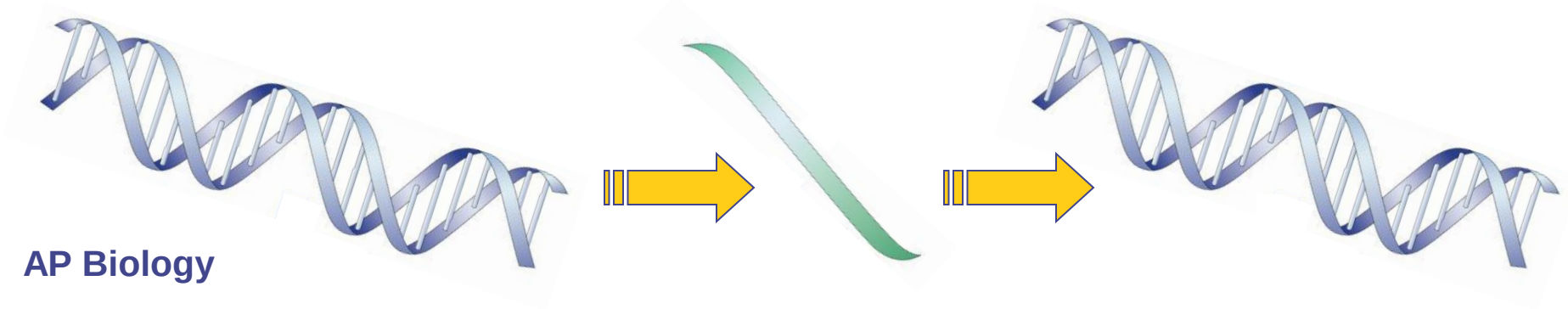
Problems…

- A lot of junk!
  - human genomic library has more “junk” than genes in it
- Introns, introns, introns!
  - if you want to clone a human gene into bacteria, you can’t have….

introns

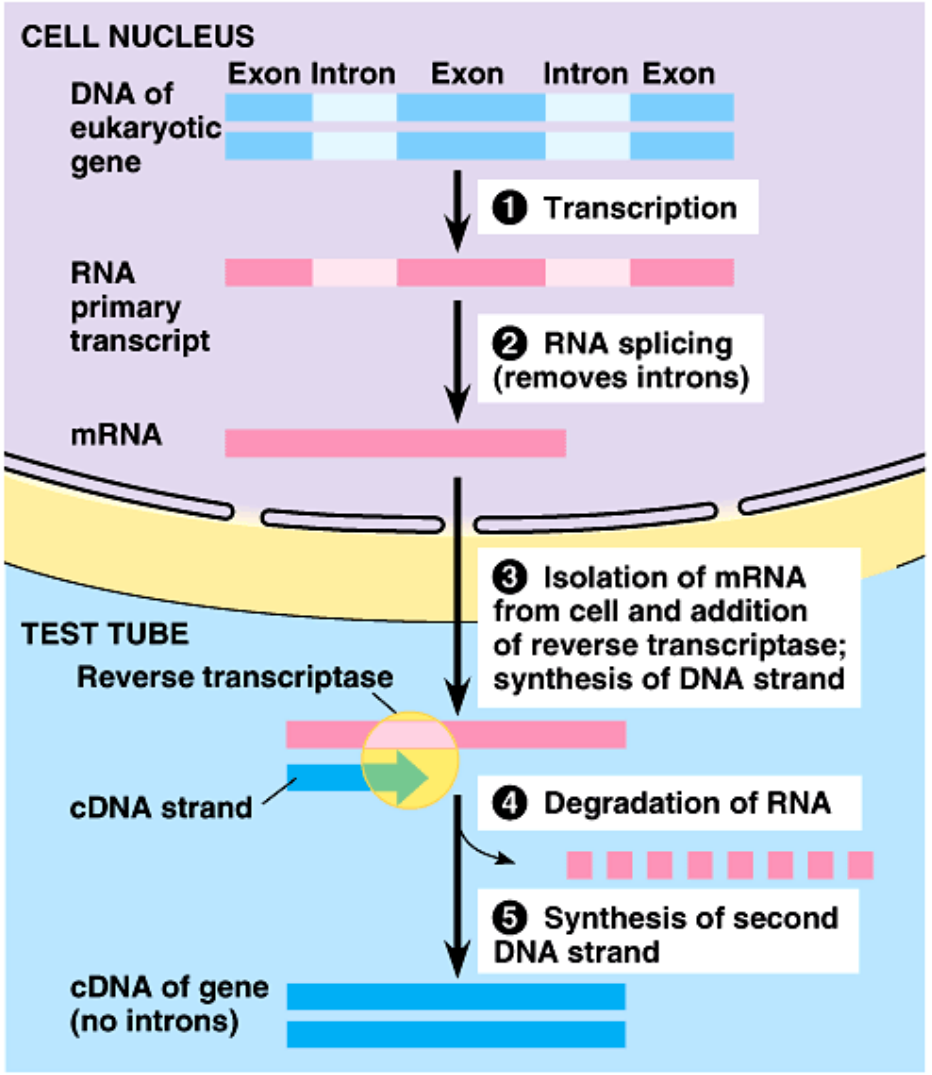
Solution…

- 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!



cDNA (copy DNA) libraries

- Collection of only the coding sequences of expressed genes
  - extract mRNA from cells
  - reverse transcriptase
    - RNA → DNA
    - from retroviruses
  - clone into plasmid
- Applications
  - need edited DNA for expression in bacteria
    - human insulin



Any Questions??