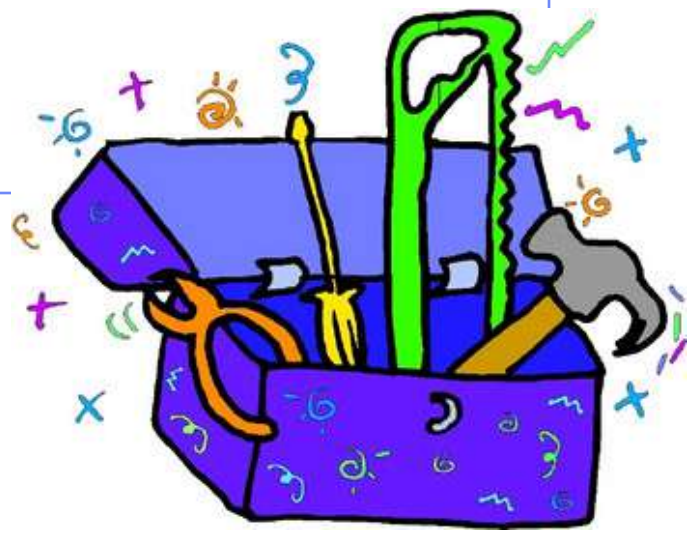
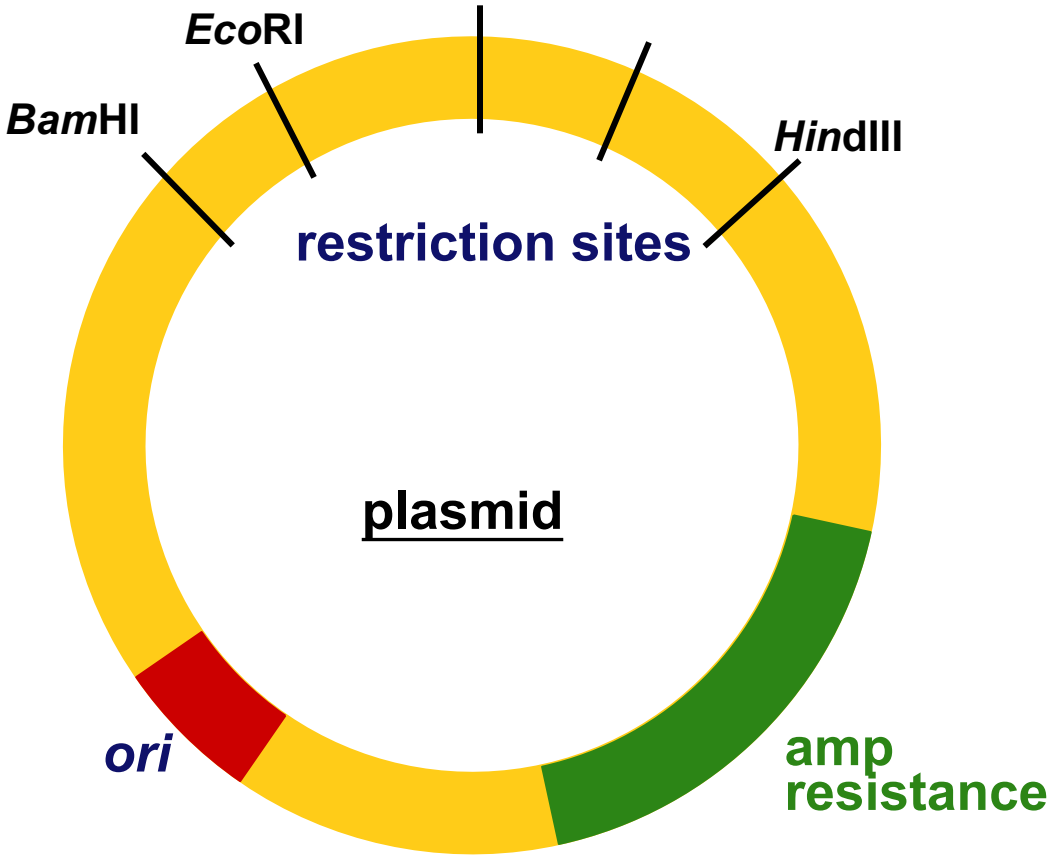
A Little More Advanced Biotechnology Tools

Better Plasmids



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

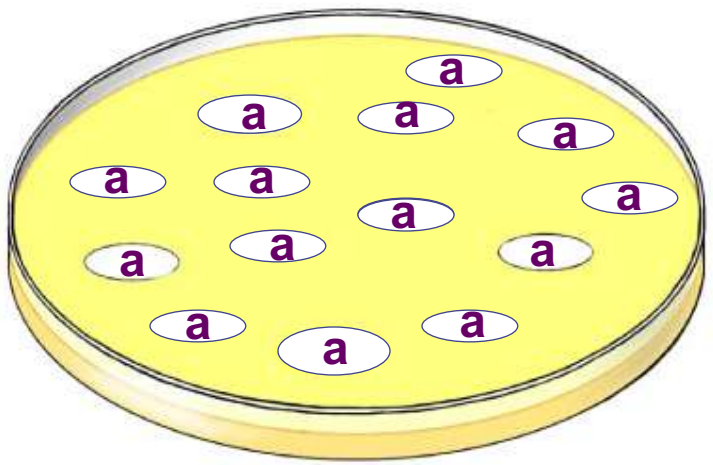
Selection for plasmid uptake

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

all bacteria grow

LB plate

only transformed bacteria grow

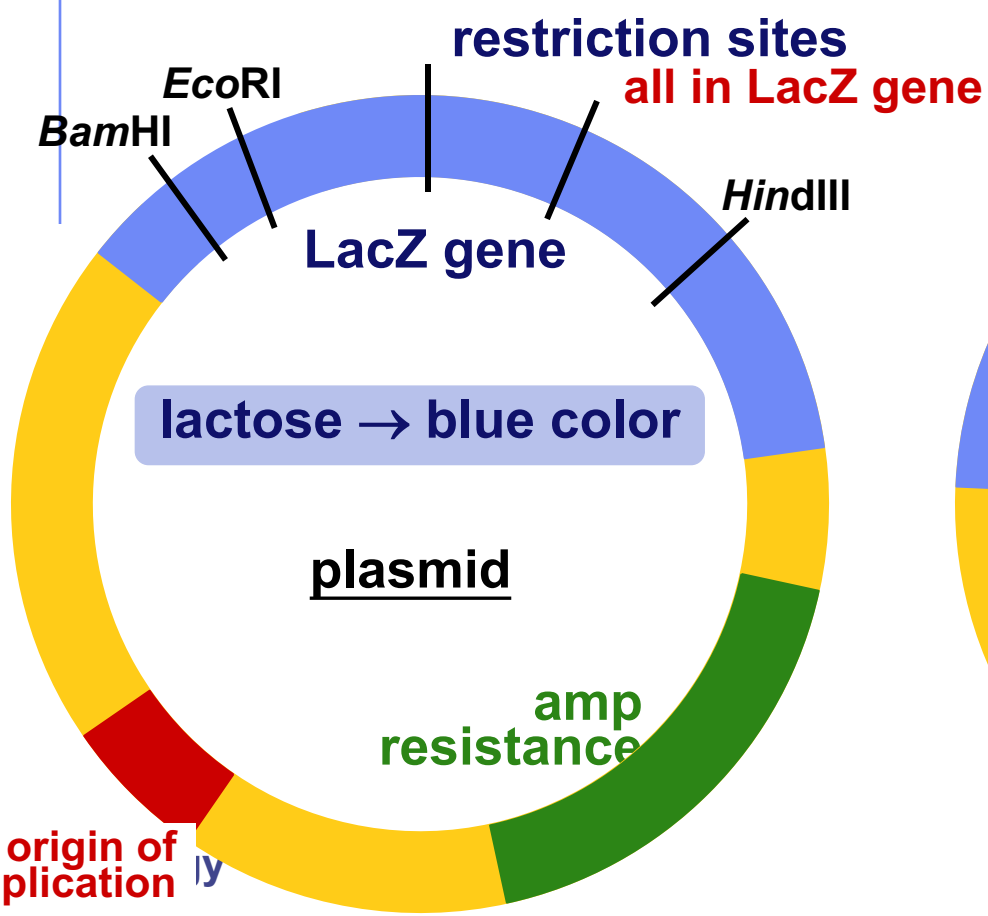


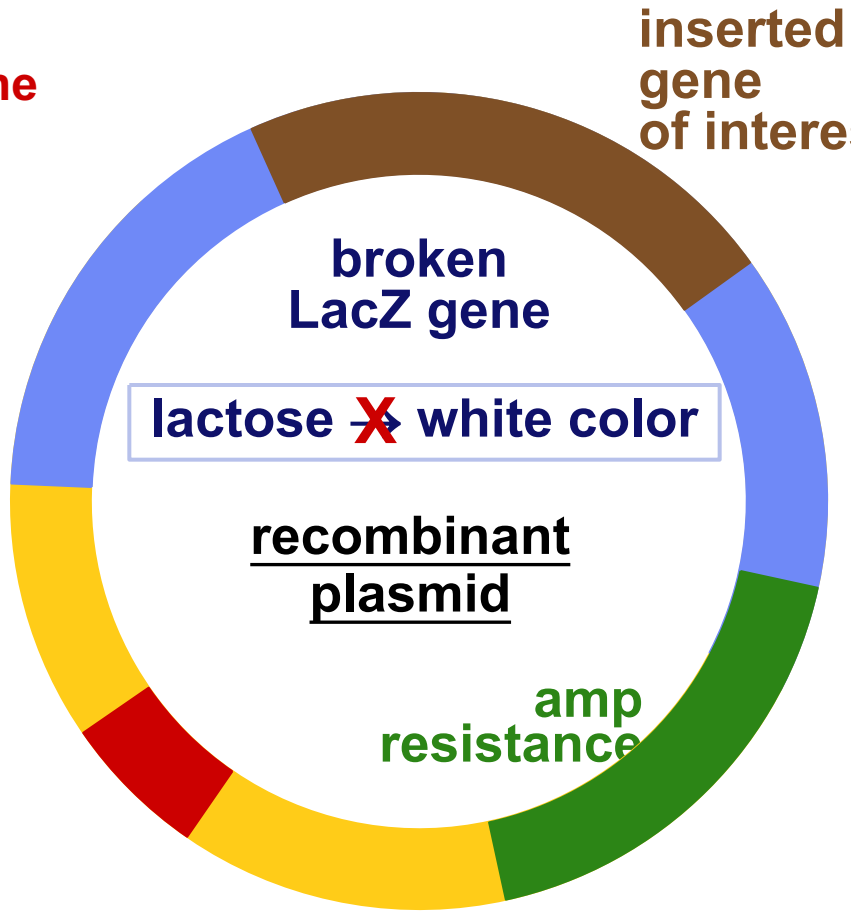
LB/*amp* plate

cloning

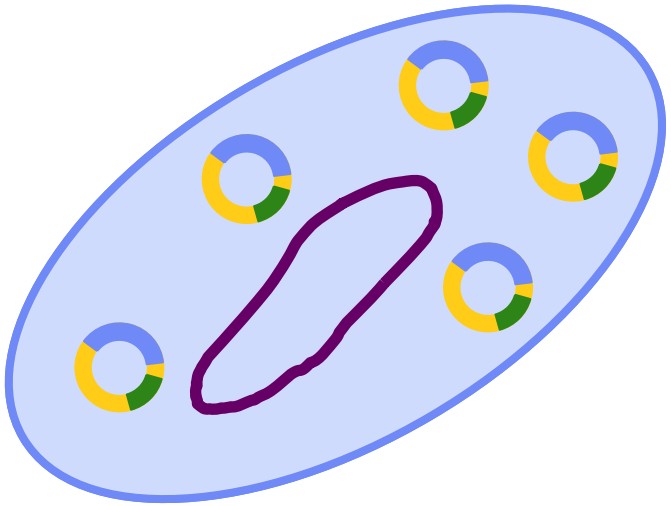
Need to screen plasmids

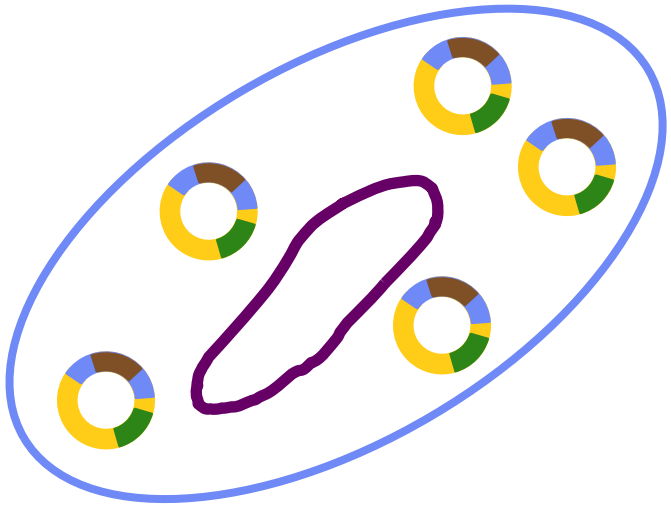
- Need to make sure bacteria have recombinant plasmid





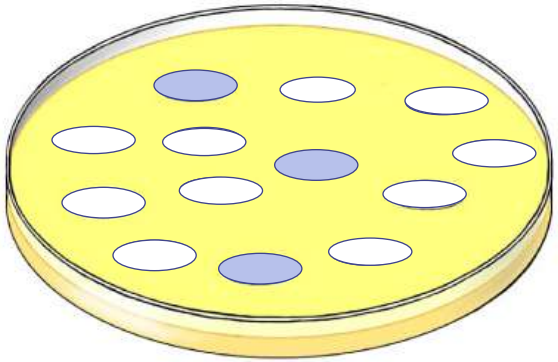
Screening for recombinant plasmid



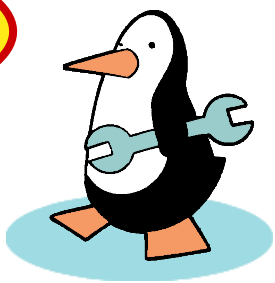


- Bacteria take up plasmid
  - Functional LacZ gene
- Bacteria make blue color

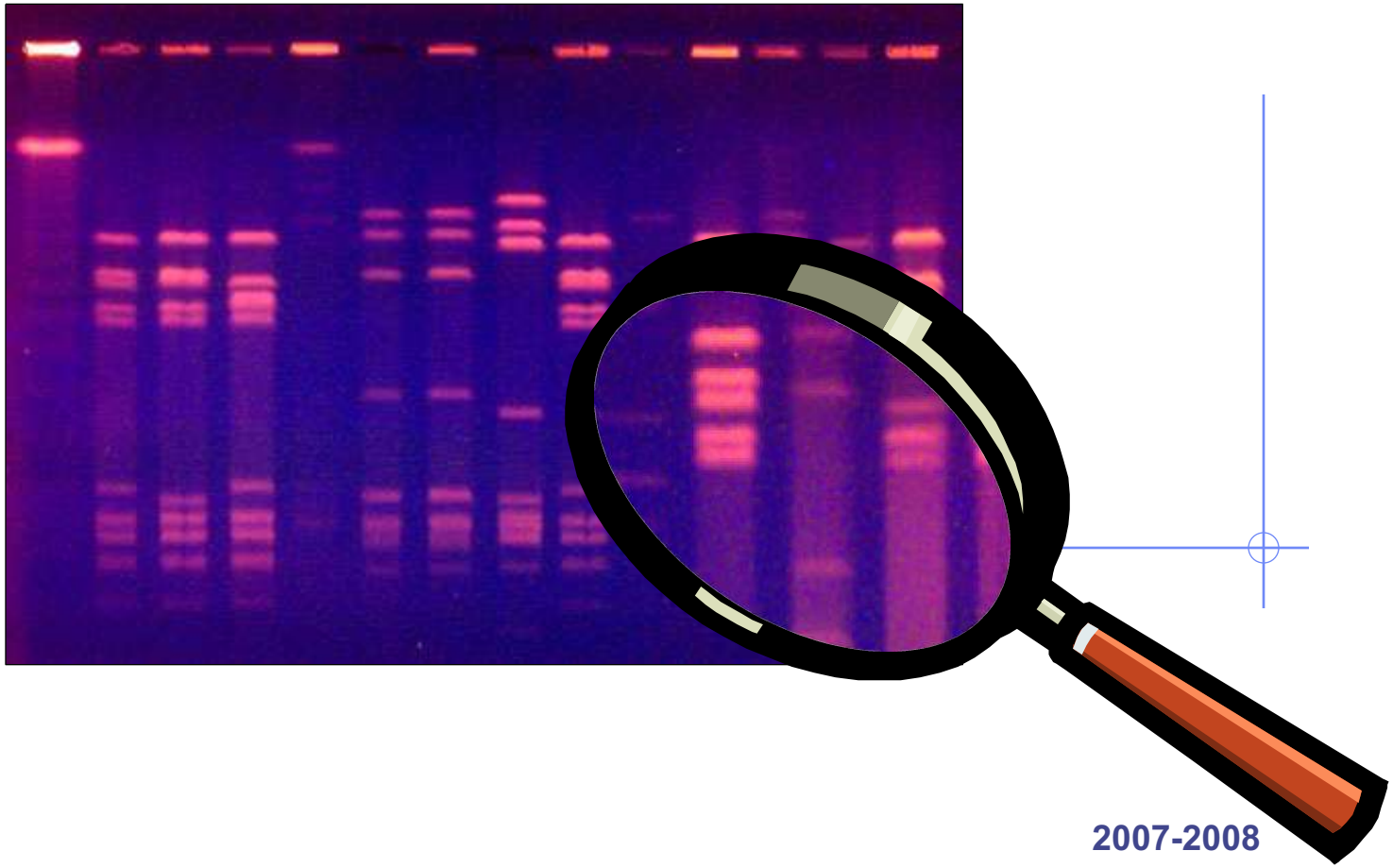
- Bacteria take up recombinant plasmid
  - Non-functional LacZ gene
  - Bacteria stay white color



Which colonies do we want?

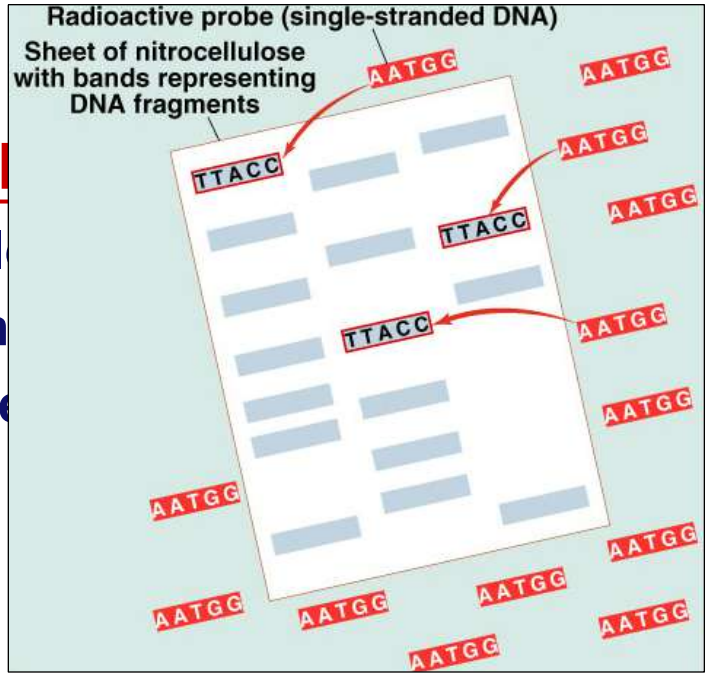


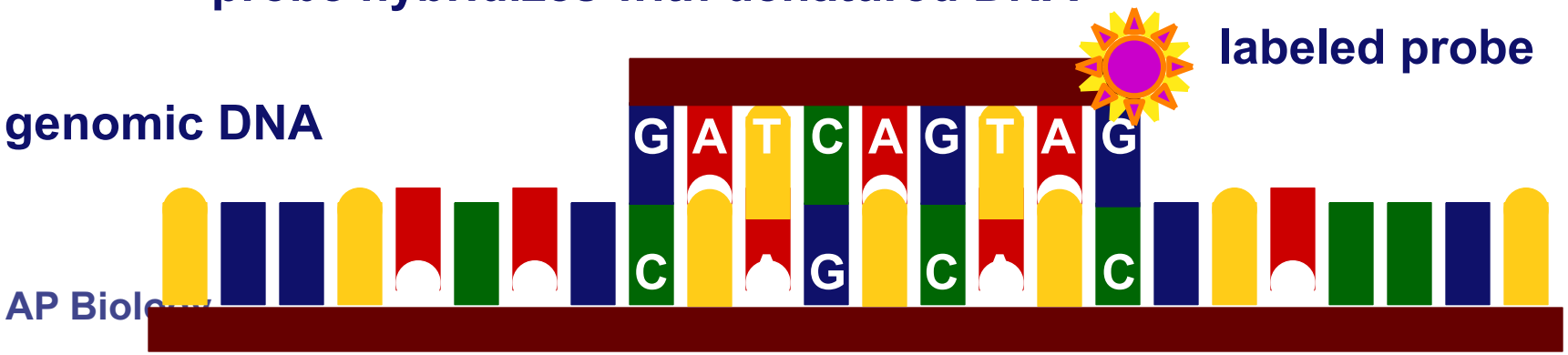
Finding your “Gene of Interest”



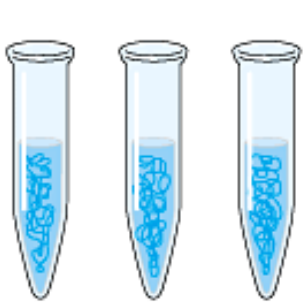
Finding your gene of interest

- **DNA hybridization**
  - find sequence of DNA using a la
    - short, single stranded DNA molecul
    - complementary to part of gene of in
    - labeled with radioactive P^{32} or fluor
  - heat treat DNA in gel
    - unwinds (denatures) strands
  - wash gel with probe
    - probe hybridizes with denatured DNA

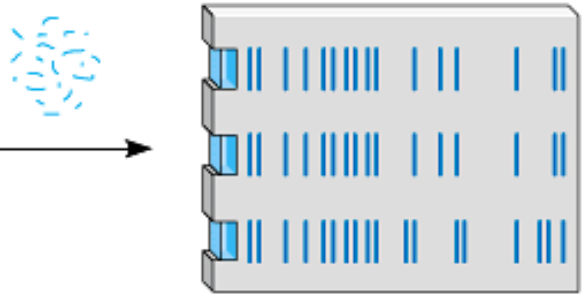




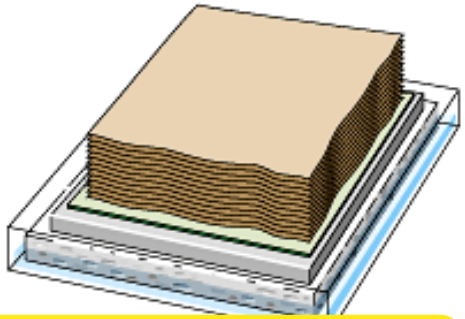
Southern blotting



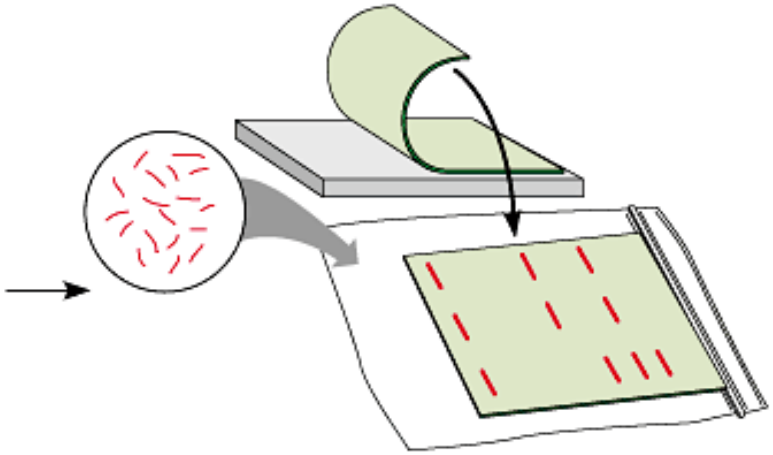
restriction digest



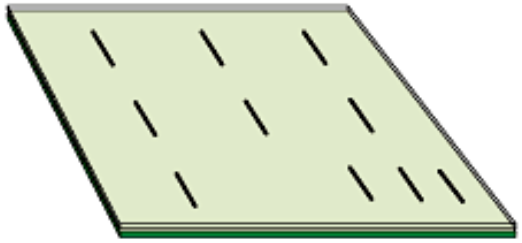
gel electrophoresis



blot DNA off of gel onto filter paper



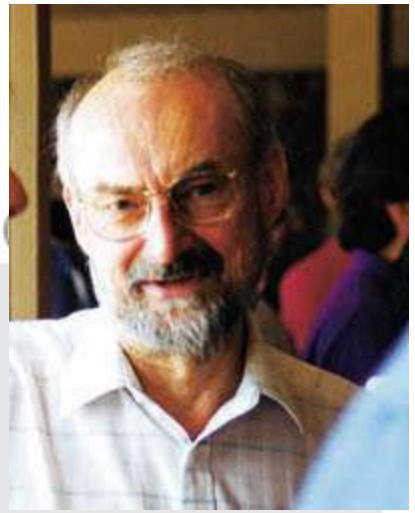
wash filter with labeled probe



expose filter paper to X-ray film

Southern blotting

Edwin Southern



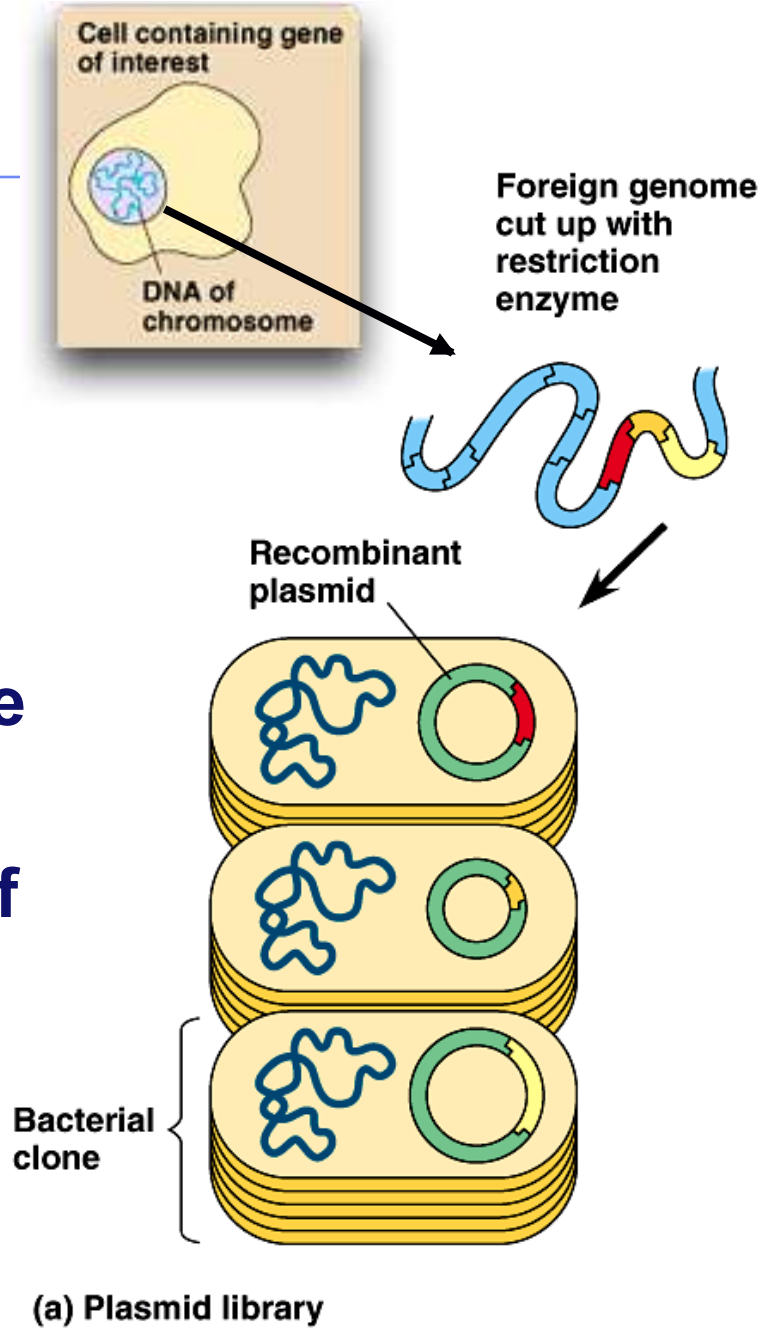
gel of genomic DNA

Southern blot IDing one gene

Southern blot illustration

DNA libraries

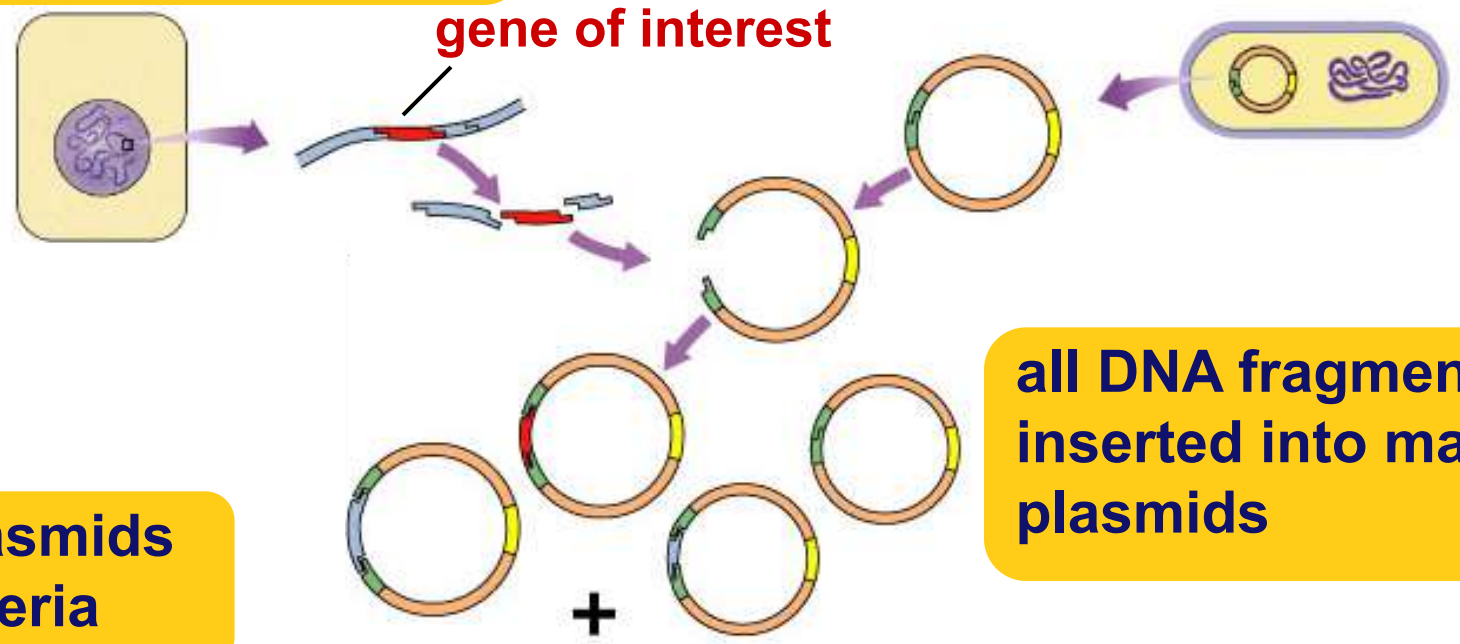
- Cut up all of nuclear DNA from many cells of an organism
  - restriction enzyme
- Clone all fragments into many plasmids at same time
  - "shotgun" cloning
- Create a stored collection of DNA fragments
  - petri dish has a collection of all DNA fragments from the organism



Making a DNA library

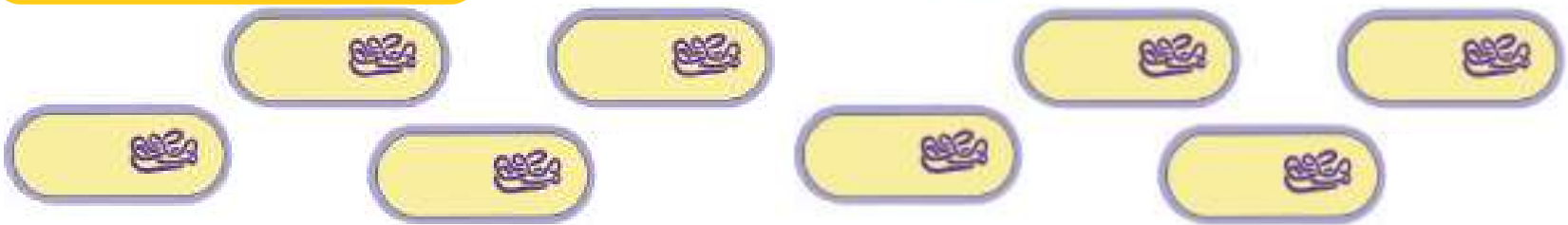
1 all DNA from many cells of an organism is cut with restriction enzymes

2 engineered plasmid with selectable marker & screening system



3 all DNA fragments inserted into many plasmids

4 clone plasmids into bacteria

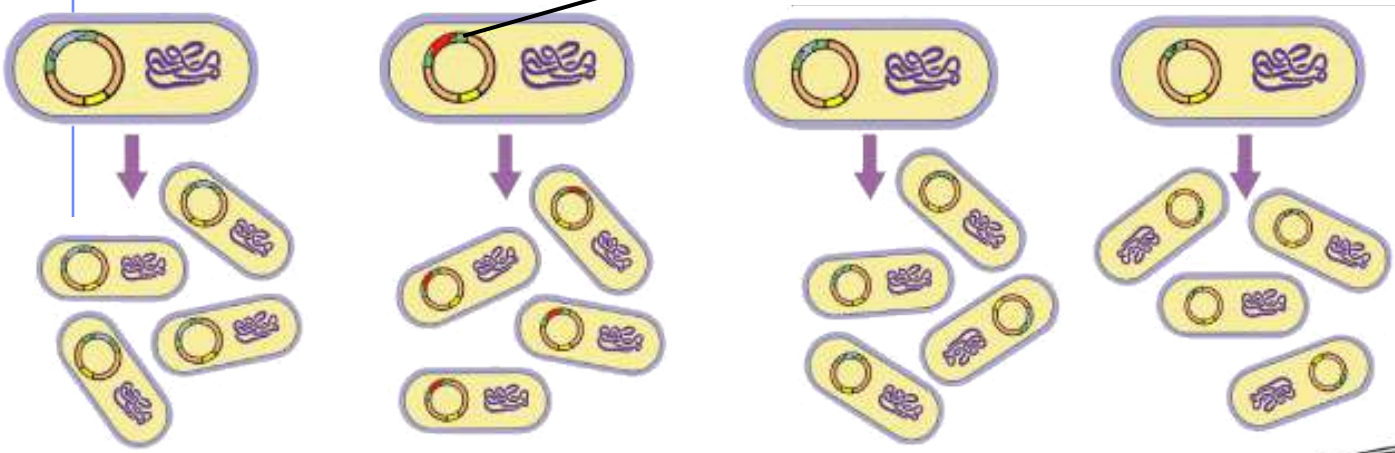


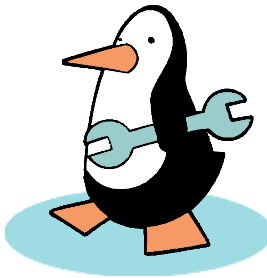
DNA library

recombinant plasmids
inserted into bacteria

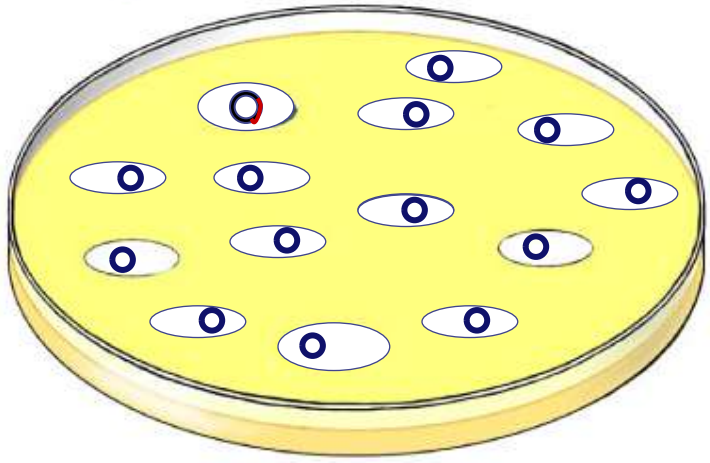
But how do we find colony with our gene of interest in it?

gene of interest



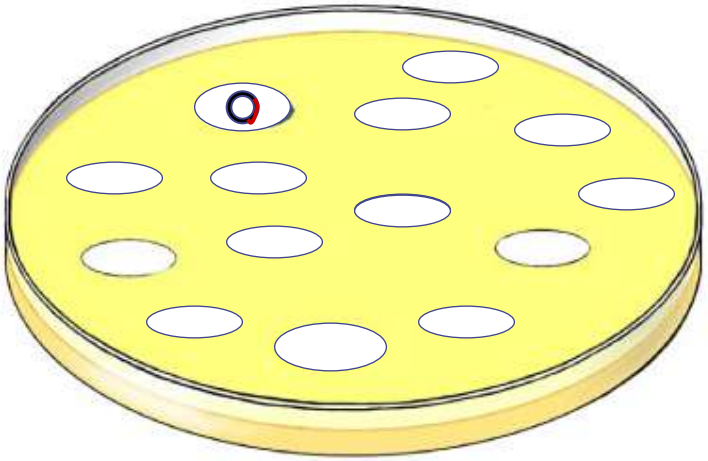


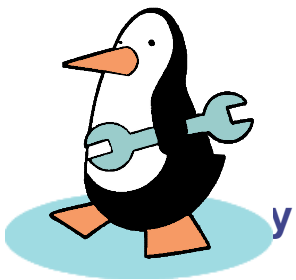
DNA Library
plate of bacterial colonies storing & copying all genes from an organism (ex. human)



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

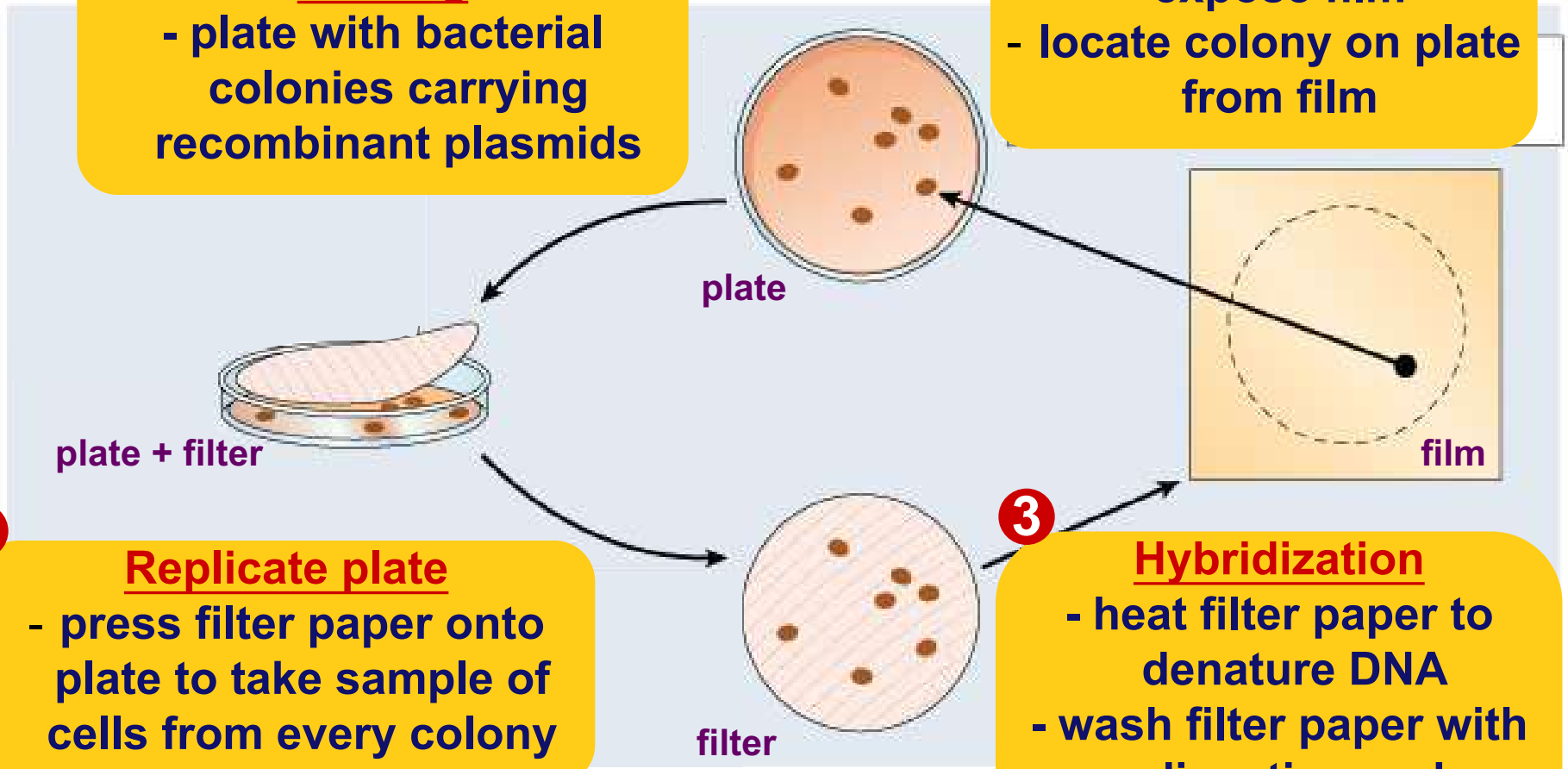




Which bacterial colony has our gene? Like a needle in a haystack!

Colony Blots

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

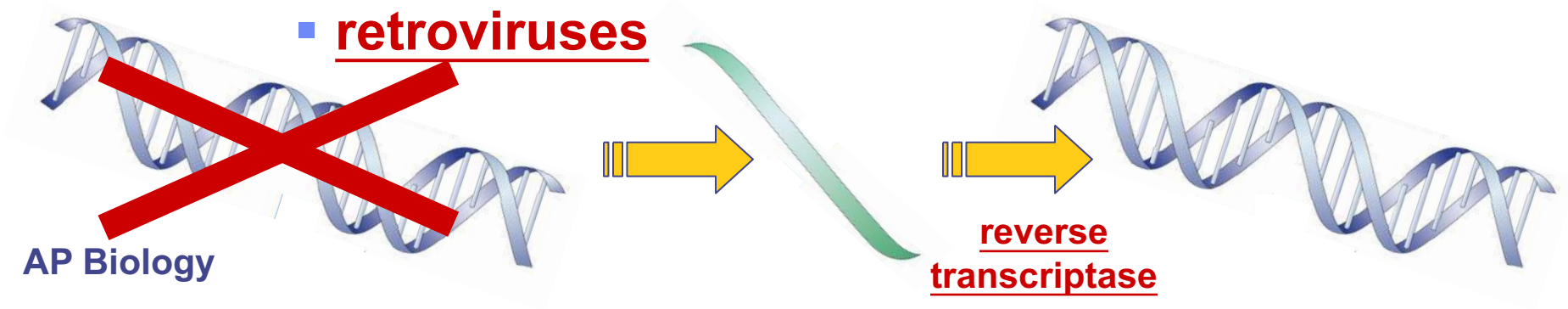
4 **Locate**
- expose film
- locate colony on plate from film

Problems…

- **Human Genome library**
  - are there only genes in there?
  - nope! a lot of junk!
  - human genomic library has more "junk" than genes in it
- **Clean up the junk*!***
  - **if you want to clone a human gene into bacteria, you can't have… introns**

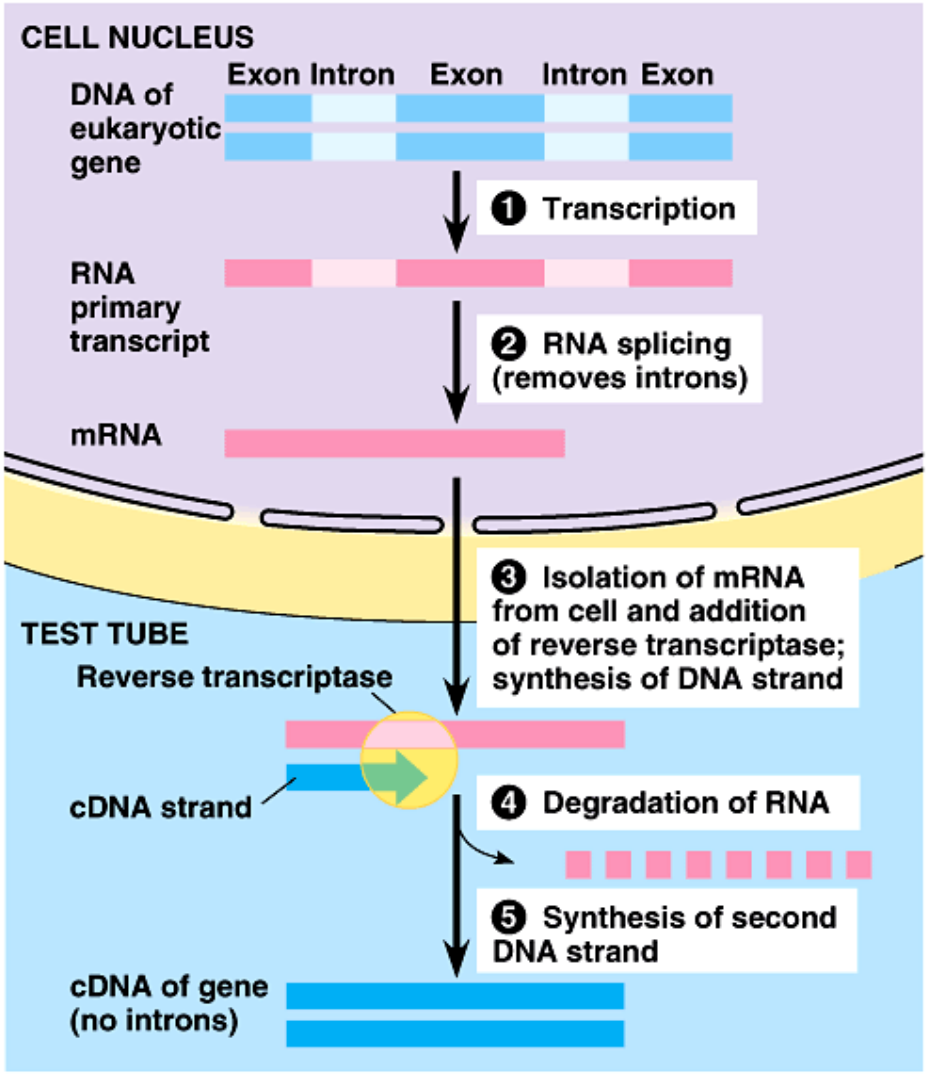
How do you clean up the junk?

- 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



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



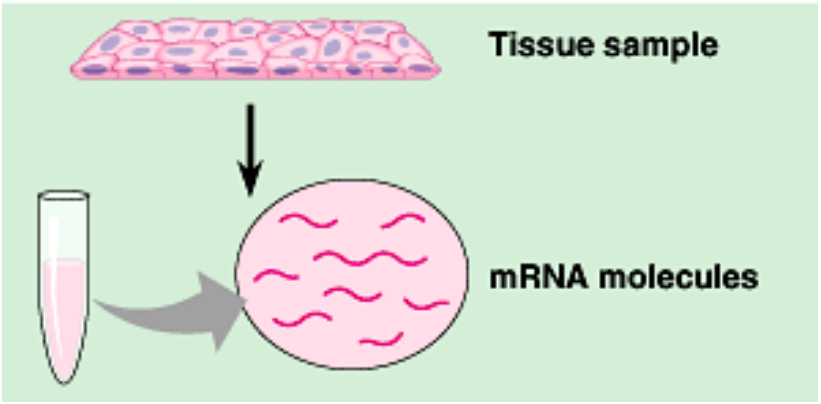
Where do we go next….

DNA → RNA → protein → trait

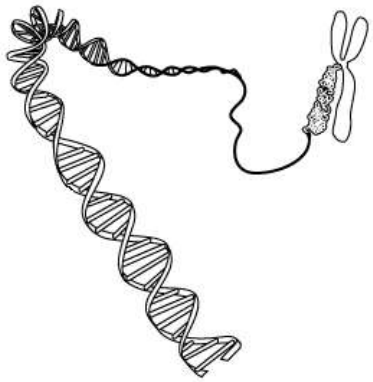
- When a gene is turned on, it creates a trait
  - ◆want to know what gene is being expressed

extract mRNA from cells
mRNA = active genes

How do you match mRNA back to DNA in cells???

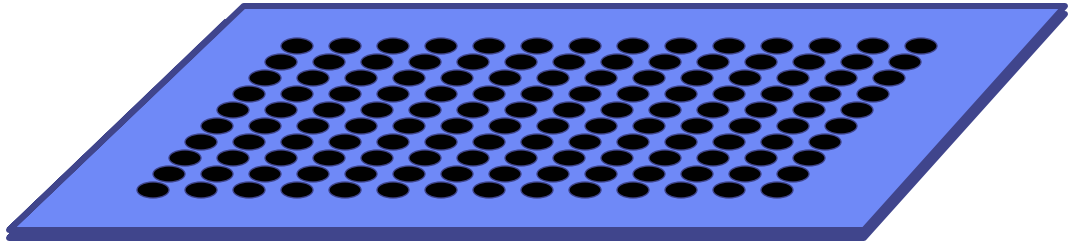


reverse transcriptase



Microarrays

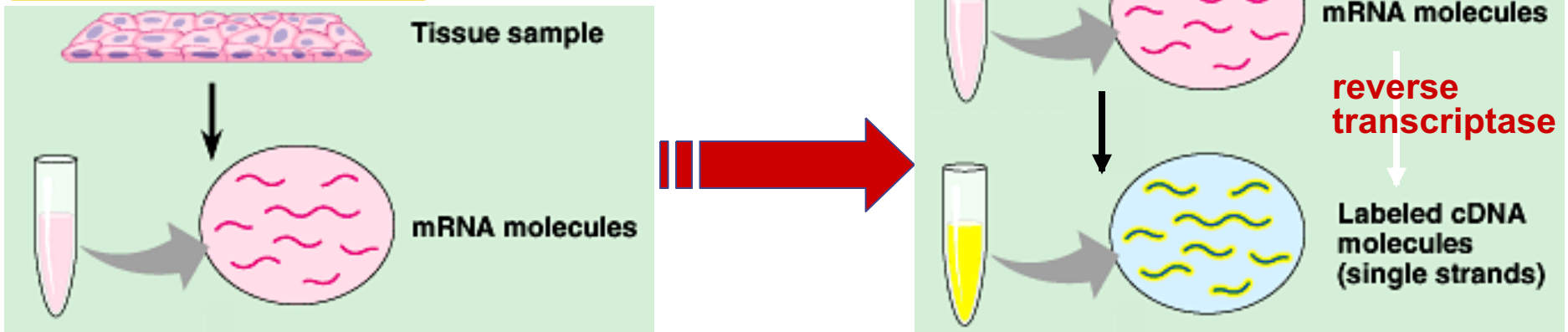
slide with spots of DNA
each spot = 1 gene



- Create a slide with a sample of each gene from the organism
  - each spot is one gene
- Convert mRNA → labeled cDNA

mRNA → cDNA

mRNA from cells



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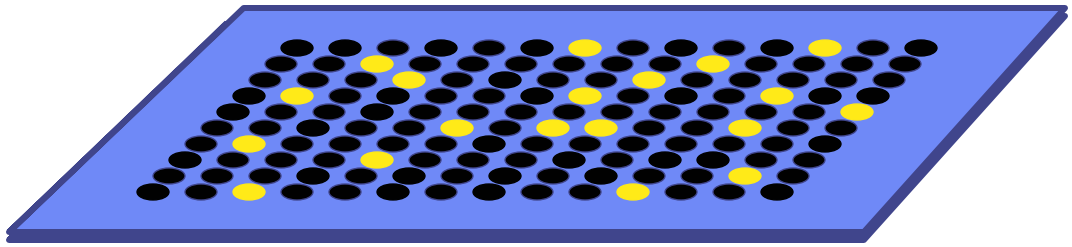
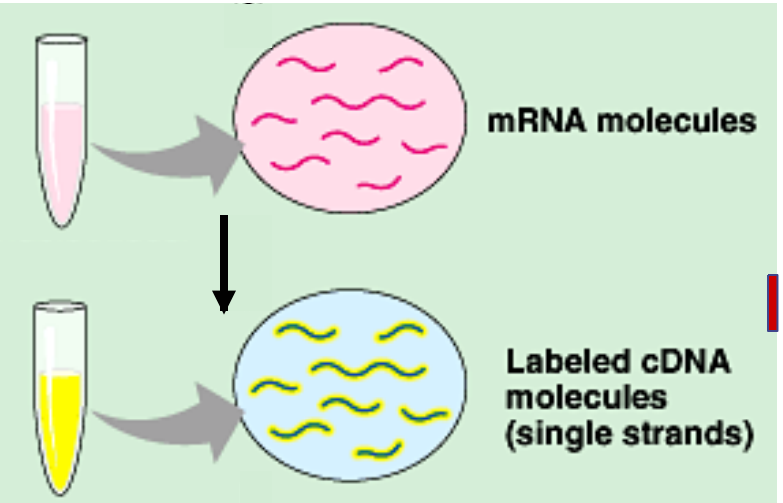
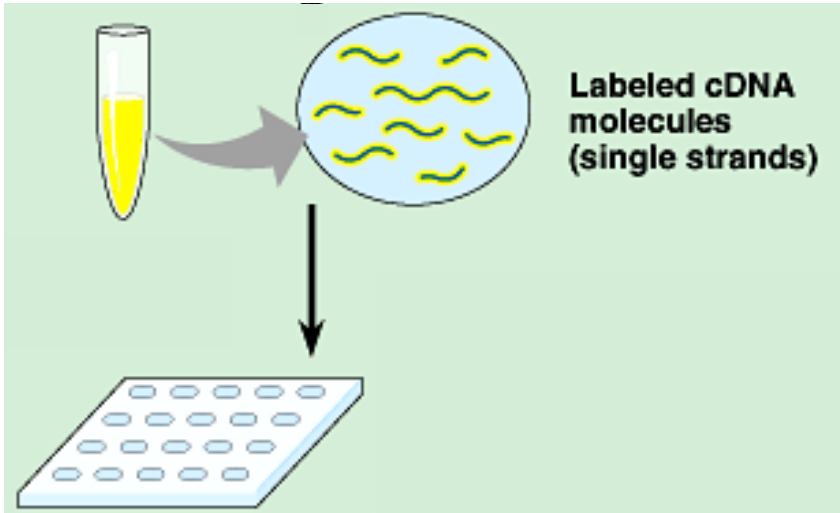


- Labeled cDNA hybridizes with DNA on slide
  - each yellow spot = gene matched to mRNA
  - each yellow spot = expressed gene

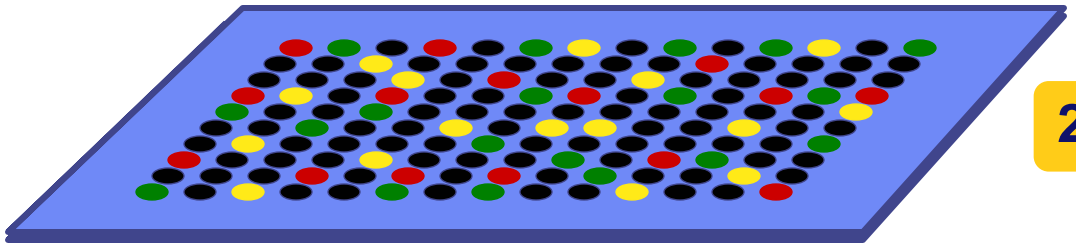
mRNA → cDNA



cDNA matched to genomic DNA



Application of Microarrays "DNA Chip"



2-color fluorescent tagging

- **Comparing treatments or conditions = Measuring change in gene expression**
- **sick vs. healthy; cancer vs. normal cells**
  - before vs. after treatment with drug
  - different stages in development
  - **Color coding: label each condition with different color**
- **red = gene expression in one sample**
  - **green = gene expression in other sample**
  - **yellow = gene expression in both samples**

I may be very selective…

But still Ask Questions!