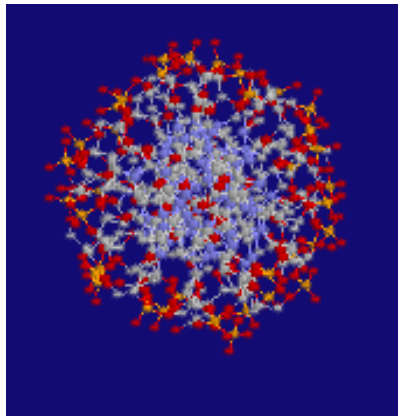
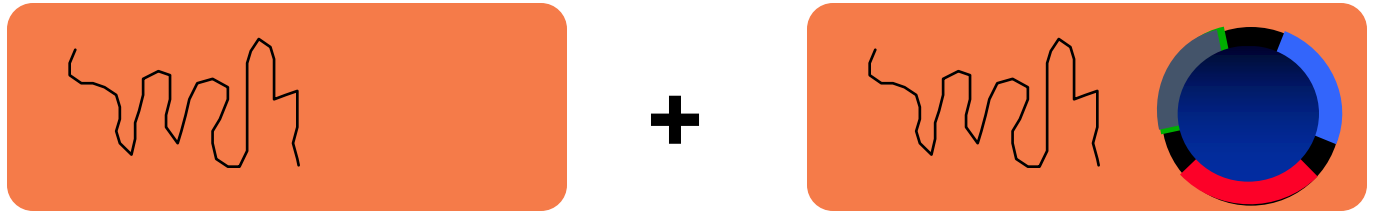
Gene Cloning 2

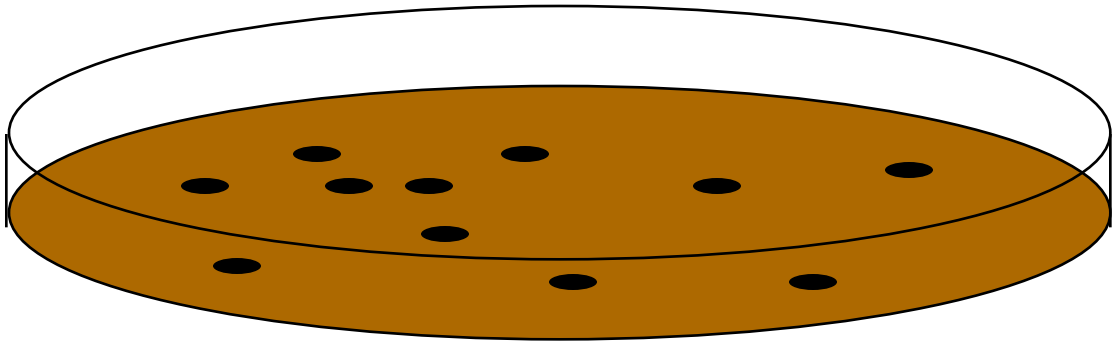


Week 10

DNA CLONING: SELECTION

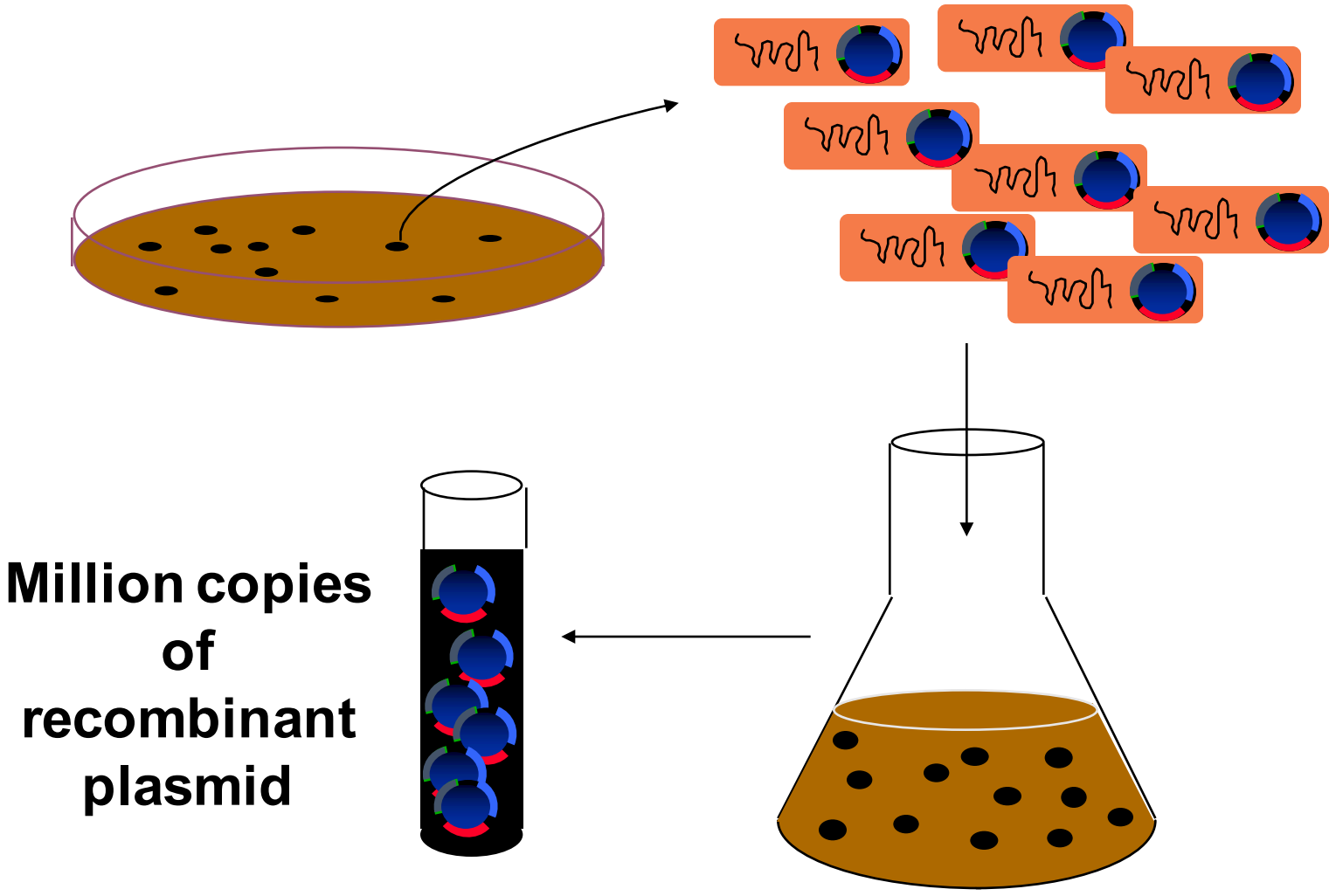


Luria Broth Agar
+
Ampicillin



ONLY ANTIBIOTIC RESISTANT (AMPICILLIN) BACTERIA (PLASMID-CONTAINING) CAN GROW

DNA CLONING: MASS PRODUCTION



DNA CLONING: PLASMIDS

PLASMID: These are double-stranded, circular extrachromosomal genetic elements, inside a bacterium which can separetely replicate from the genome

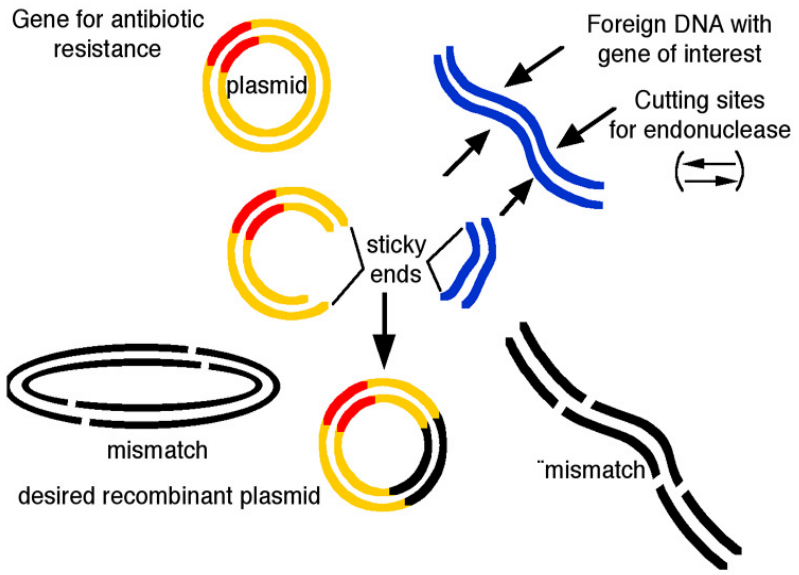
- A vector plasmid contains sequences for:
  - a **bacterial replication origin** (ori)
  - an **antibiotic resistance gene** [i.e. ampicilline resistance gene(amp)]
  - one or more special restriction digestion sites which helps the insertion of a foreign DNA fargment (MCS)
- A ***B-galactosidase*** gene losing its activity when a DNA fragment is inserted in MCS,
- **promotors** providing expression of a foreign gene in procaryotic or eucaryotic cells.

Vector Types

- Plasmids: 15 kb capacity.
- Bacteriophages (Lambda phage): 25 kb capacity.
- Cosmid vectors: 35-45 kb capacity.
- Bacterial originated Artificial Chromosomes (BAC): 50-300 kb capacity.
- Yeast originated Artificial Chromosomes (YAC): 300-1500 kb capacity.
- Human originated Artificial Chromosomes (HAC): Greater than 2000 kb capacity.

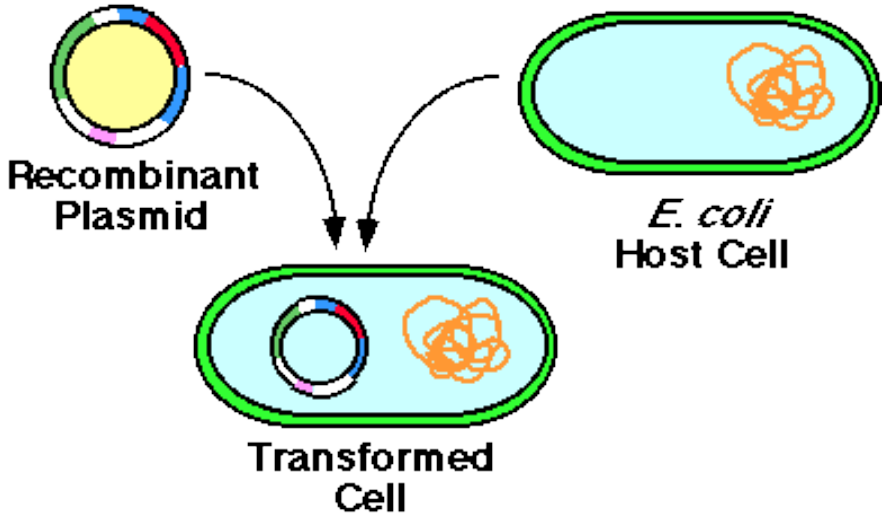
DNA Cloning, II

- Bacterial plasmids are cut (extra-chromosomal small circular DNA structures) with the same RE.
- By this way, a DNA fragment could be inserted into plasmid DNA and a recombinant DNA molecule is formed.



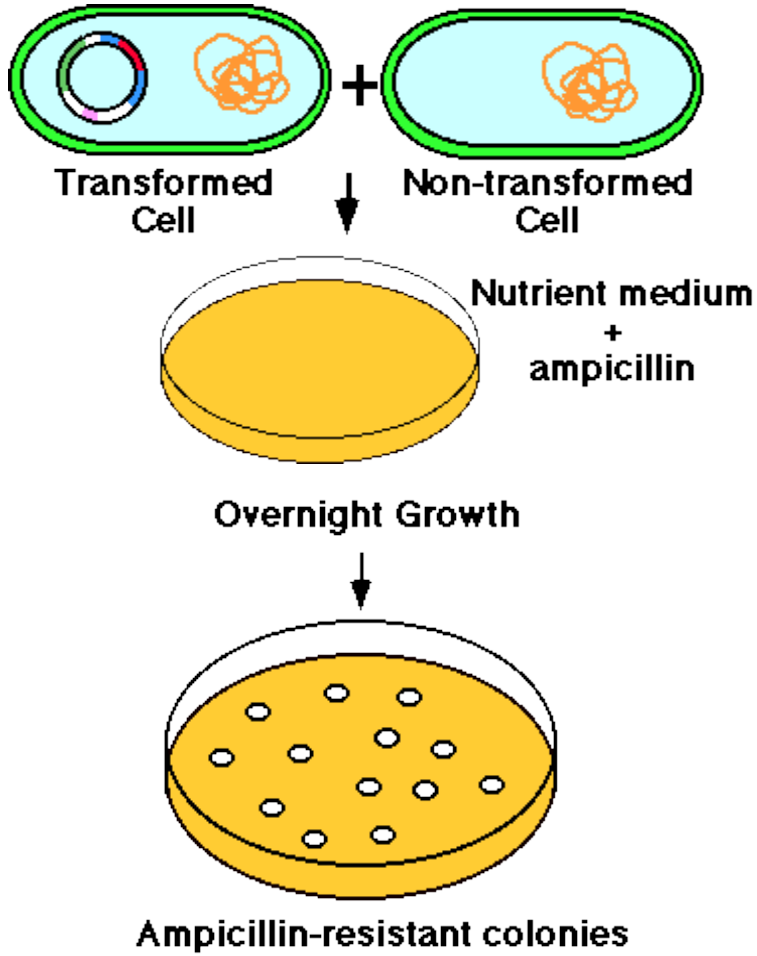
DNA Cloning, III

- These recombinant plasmids are electropolated (put) into competent (which could take plasmids) bacteria.
- This transport or transfer process is called transformation.

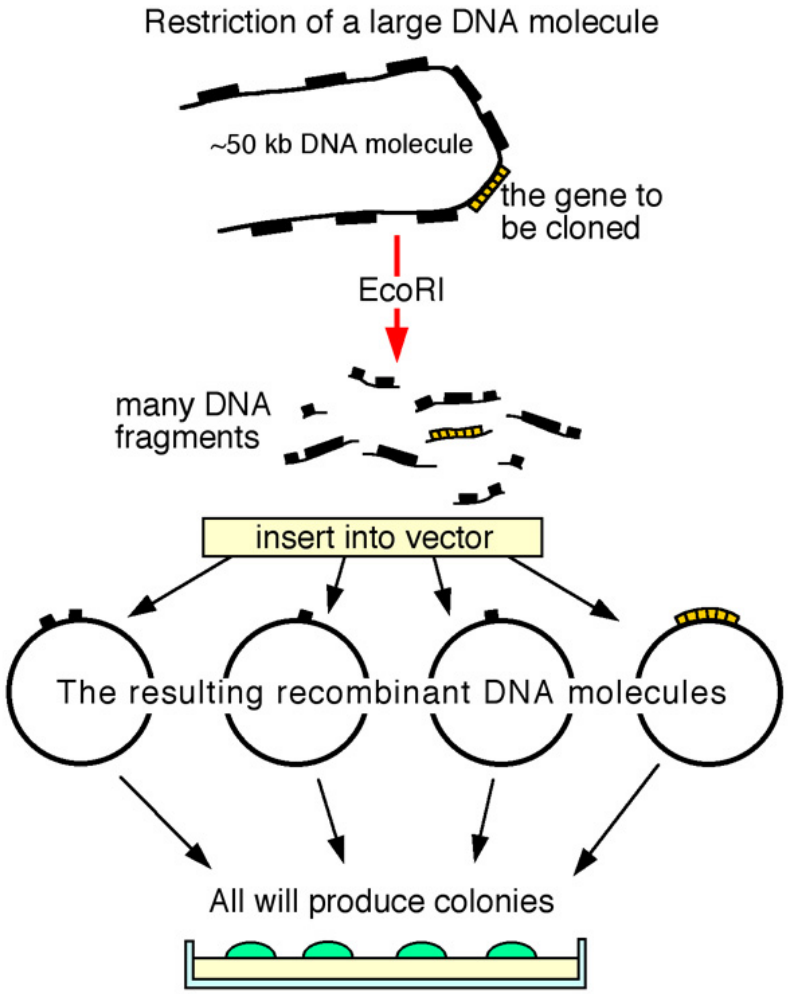


DNA Cloning, IV

- These plasmids carry antibiotic resistance genes.
- By this way, while bacteria carrying resistance genes can grow on antibiotic containing media other bacteria are eliminated and die, thus only transformed bacteria can live and replicated on the media.



DNA Cloning V

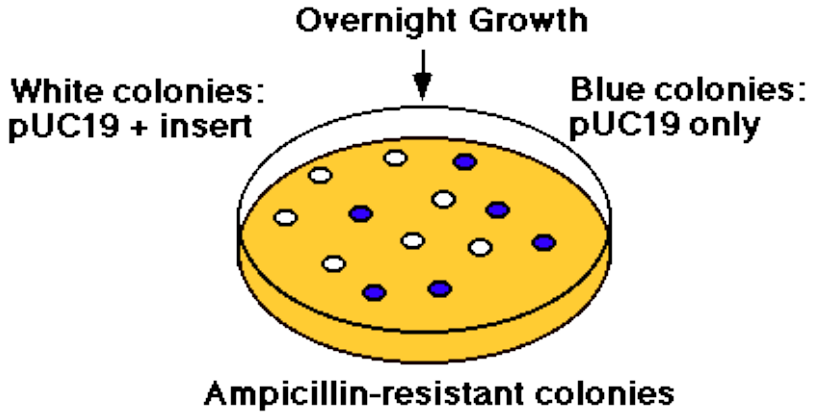


- Transformed bacteria grow on medium as colonies
- In a bacterial colony, each bacterial cell has the same plasmid thus the same DNA!
- In bacterial cells fronm different colonies have different plasmids thus different DNA fragments!

Screening I

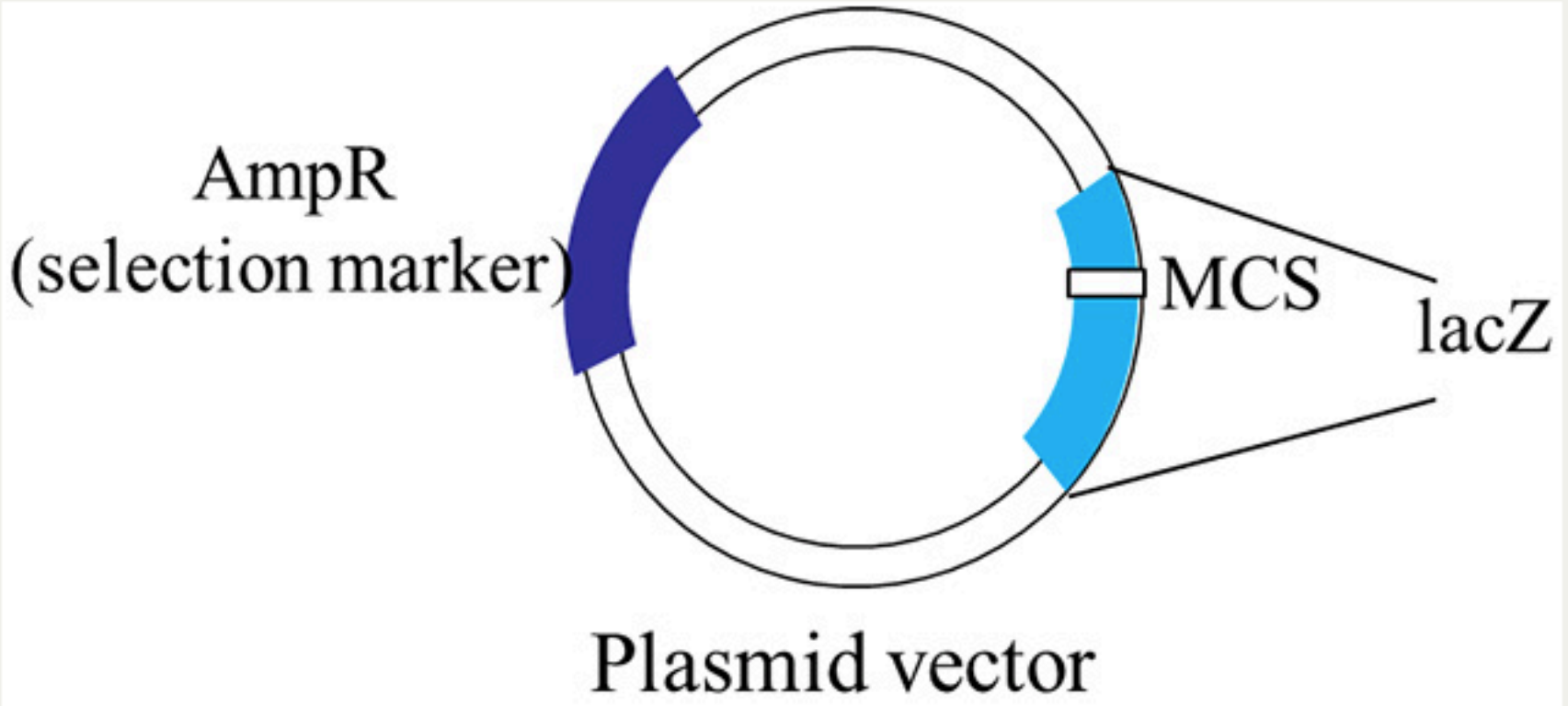
Screening:

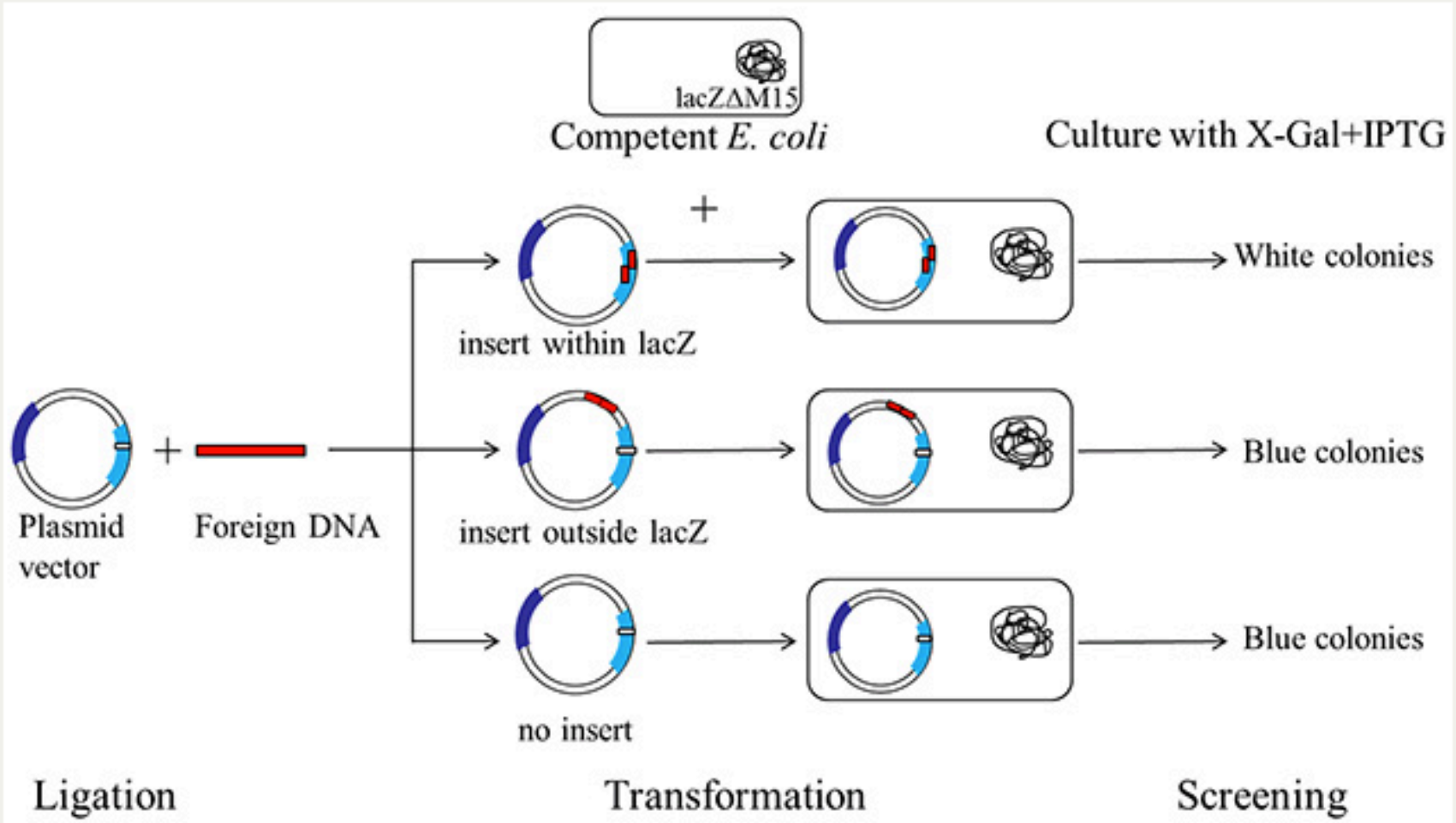
1. **Phenotypic Screening** – Protein encoded by the gene present in the plasmid changes the color of the colony.
2. **Protein expressed by a defined gene can be detected with specific antibodies**



Blue-White Screening

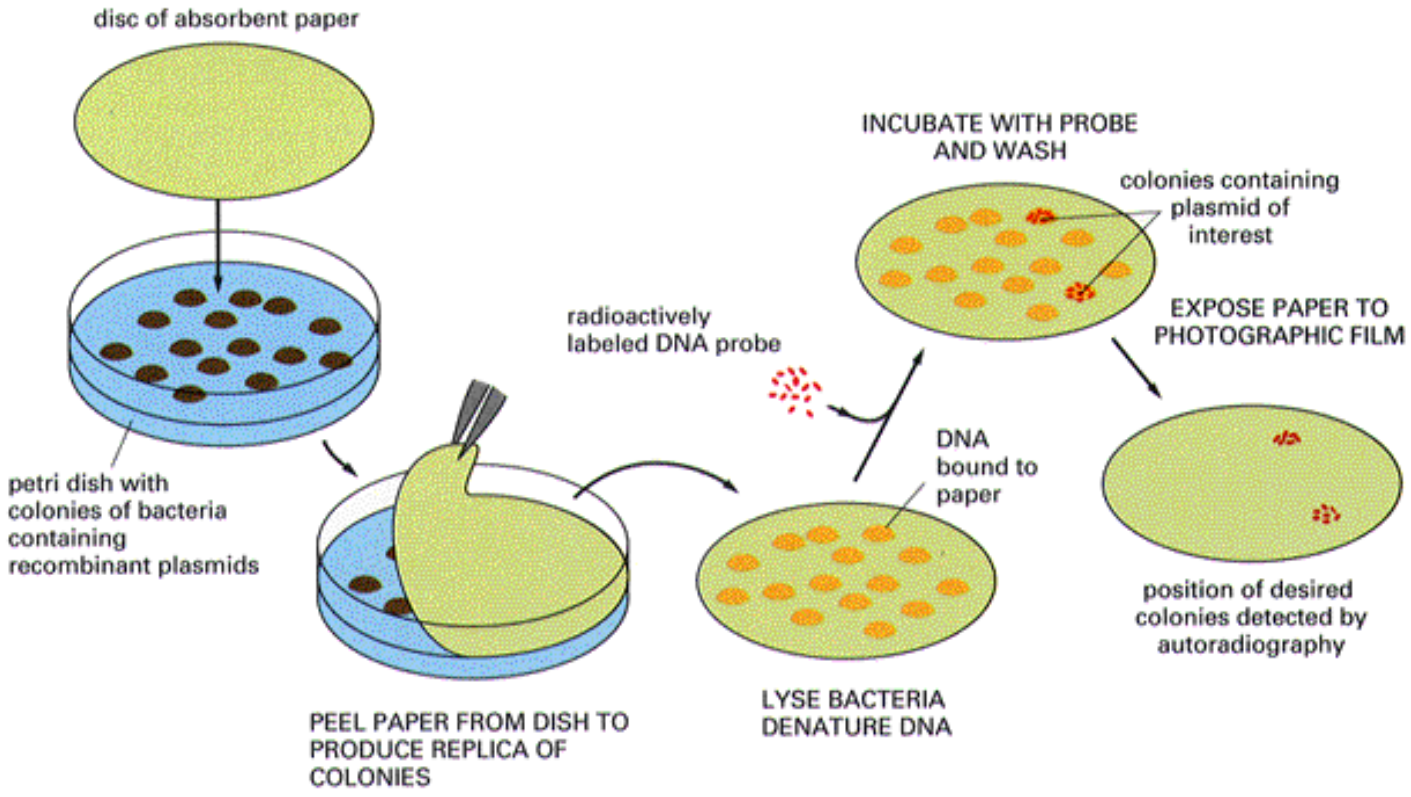
- For screening the clones containing recombinant DNA, a chromogenic substrate known as **X-gal** is added to the agar plate.
- **If β-galactosidase is produced**, X-gal is hydrolyzed to form 5-bromo-4-chloro-indoxyl, which spontaneously dimerizes to produce an **insoluble blue pigment** called 5,5’-dibromo-4,4’-dichloro-indigo.
- The **colonies formed by non-recombinant cells, therefore appear blue in color** while **the recombinant ones appear white**.
- The desired recombinant colonies can be easily picked and cultured.


AmpR
(selection marker)
MCS
lacZ
Plasmid vector


lacZΔM15
Competent E. coli
Culture with X-Gal+IPTG
+
White colonies
insert within lacZ
Plasmid vector
+
Foreign DNA
insert outside lacZ
Blue colonies
no insert
Blue colonies
Ligation
Transformation
Screening

Screening II

3. DNA sequence of a cloned gene can be detected with a DNA hybridisation probe.



Screening III

- Following the screening and isolation of colonies they can mass produced by culturing in broths!
- These can be stored at -80° C for years!

DNA Libraries

- DNA Library: In molecular biology, collection DNA fragments produced and stored in microbial populations are called DNA libraries!!!
- They are collection of living bacterial colonies transformed with different DNA fragments obtained from different organisms those form source of DNAs
- These gene libraries are screened and researchers work with colonies carrying the genes of interest!
- There are also **cDNA libraries and gene libraries** established from genomic DNA!

Clone Libraries

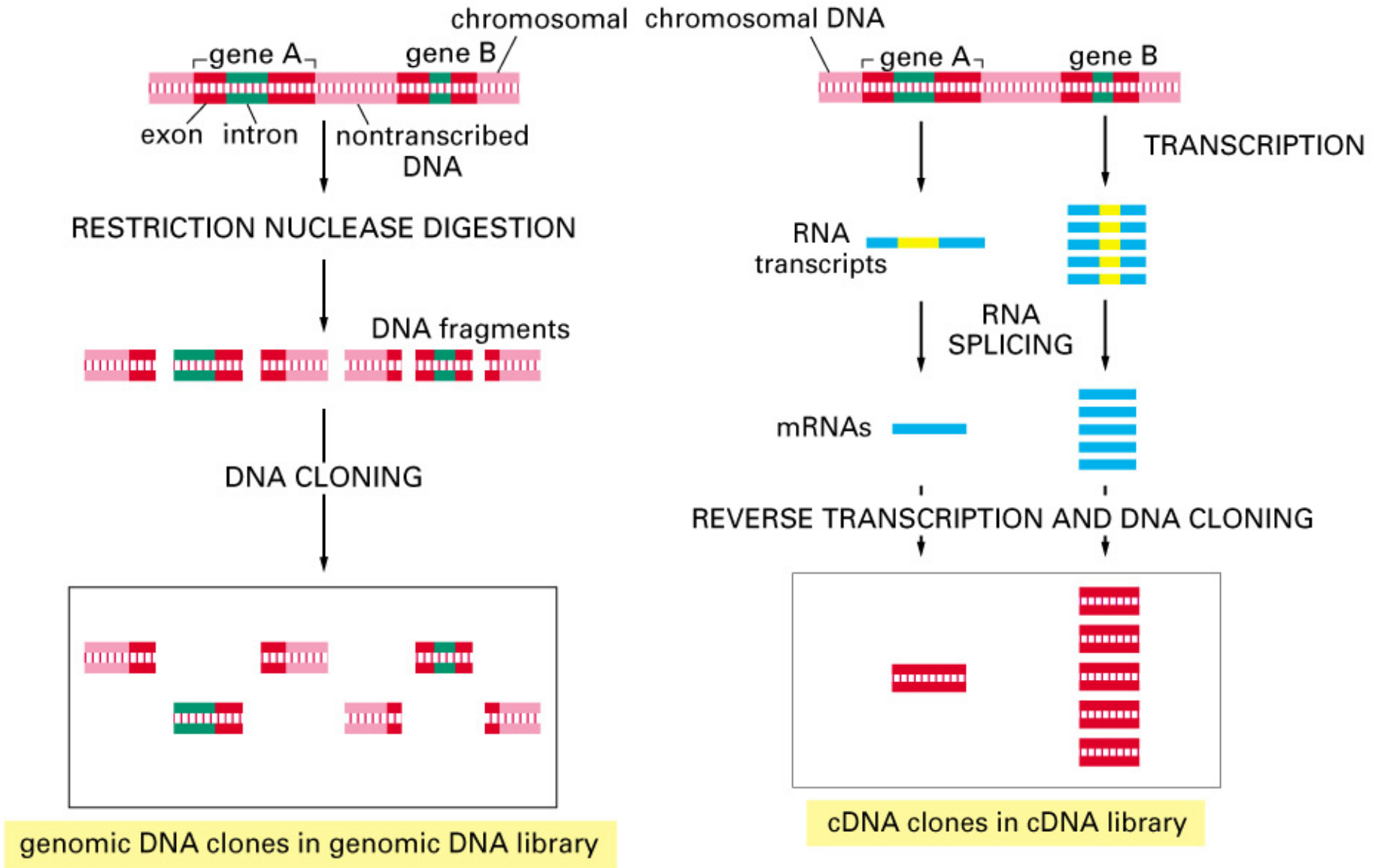


Figure 8–35 part 1 of 2. Molecular Biology of the Cell, Figure 8–35 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

cDNA ve Gene Library Applications
Why do we need them?

- For identification of new genes
- *In vitro* investigation of gene functions (cDNA molecule cloning)
- mRNA expression analysis from diverse cell and tissues
- Whole genome identification of specific organisms i.e. (human genome project or other genome projects)
- Establishment of genomic sequence resources for the production of transgenic animals
- *In vitro* investigation of regulatory sequence functions
- Investigation of genetic mutations in cancer tissues