BHARATHIDASAN UNIVERSITY

Program: M.Sc., Biomedical Science

Course Code : 18BMS47C10

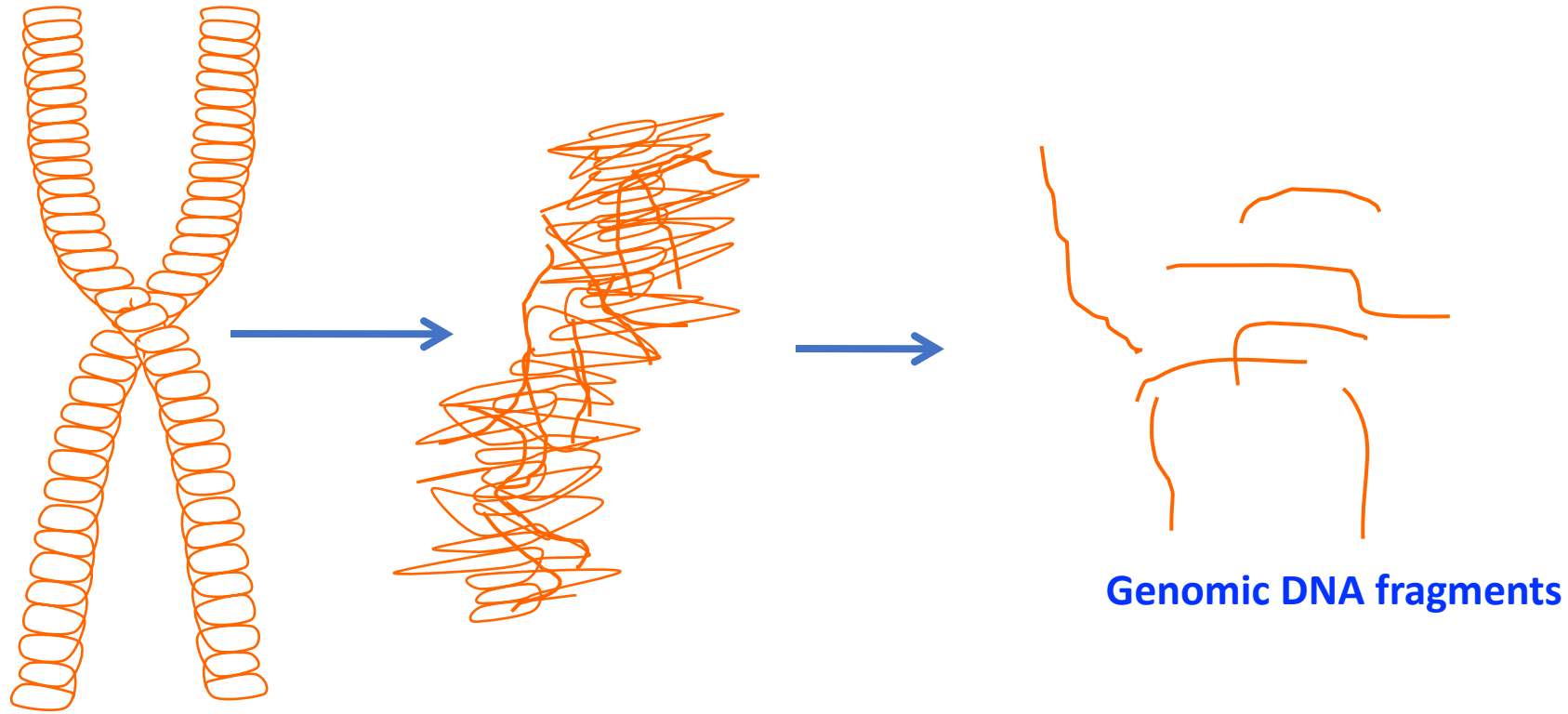
Course Title : Genetic Engineering

DNA Cloning

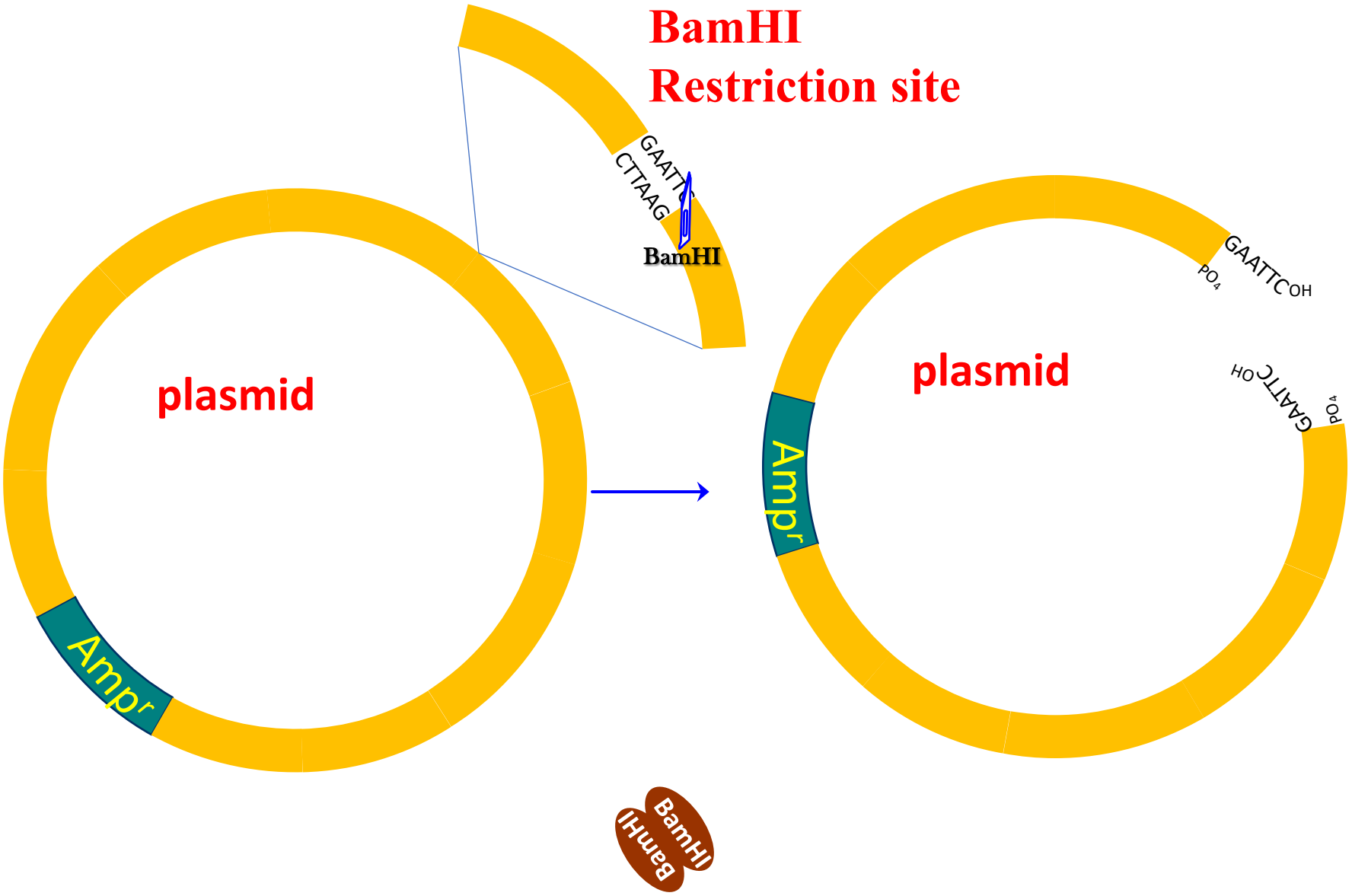
Prof. Narkunaraja Shanmugam

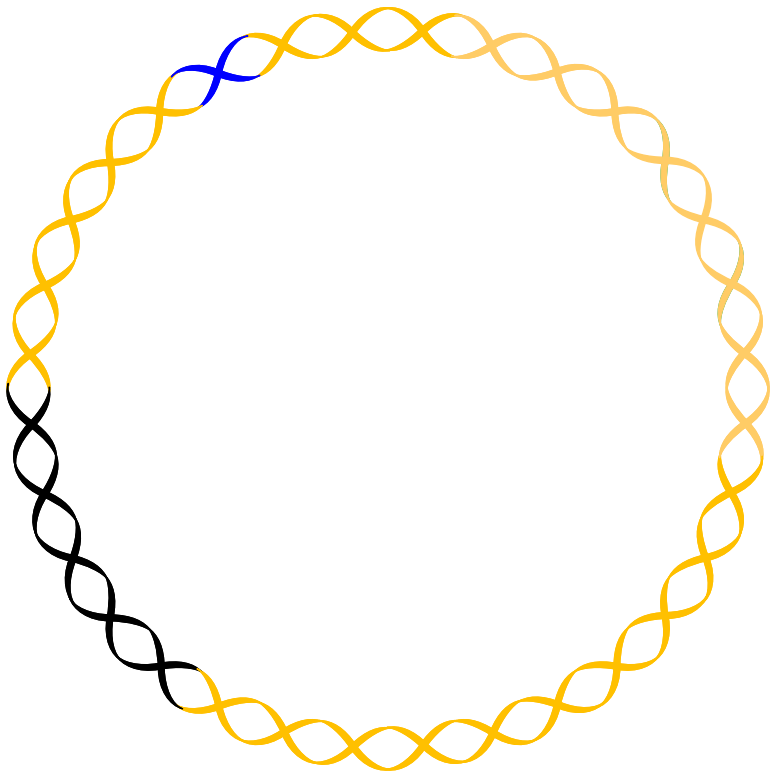
Dept. of Biomedical Science

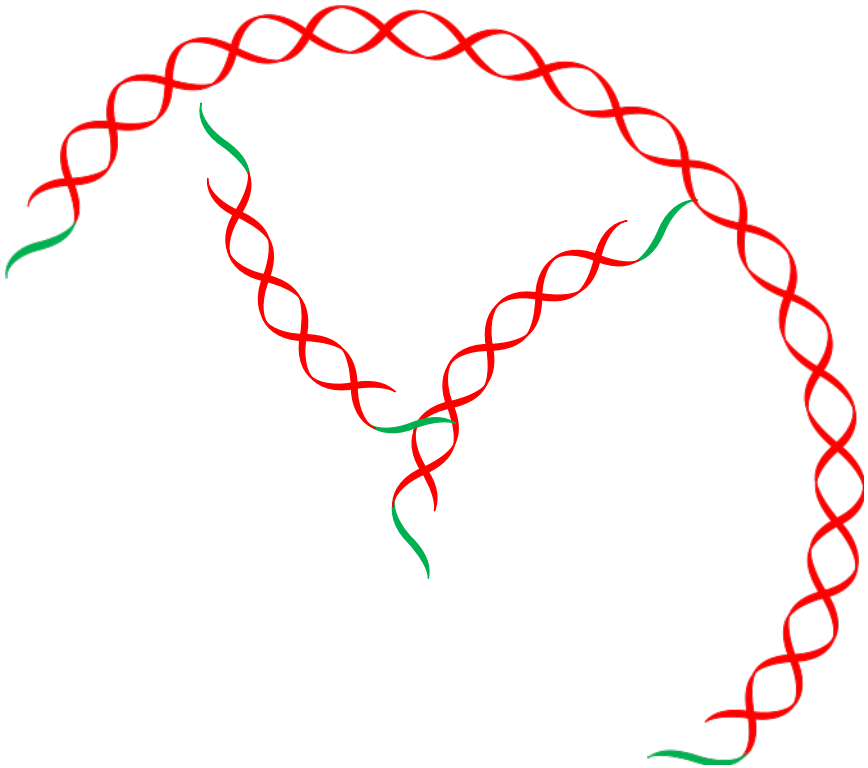
RE fragmentation of Genomic DNA



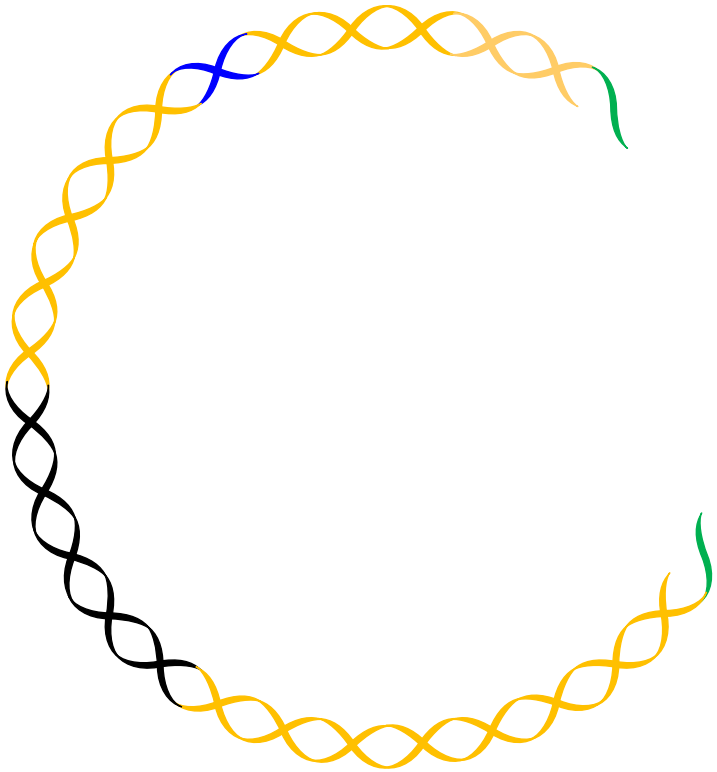
Linearization of Plasmid

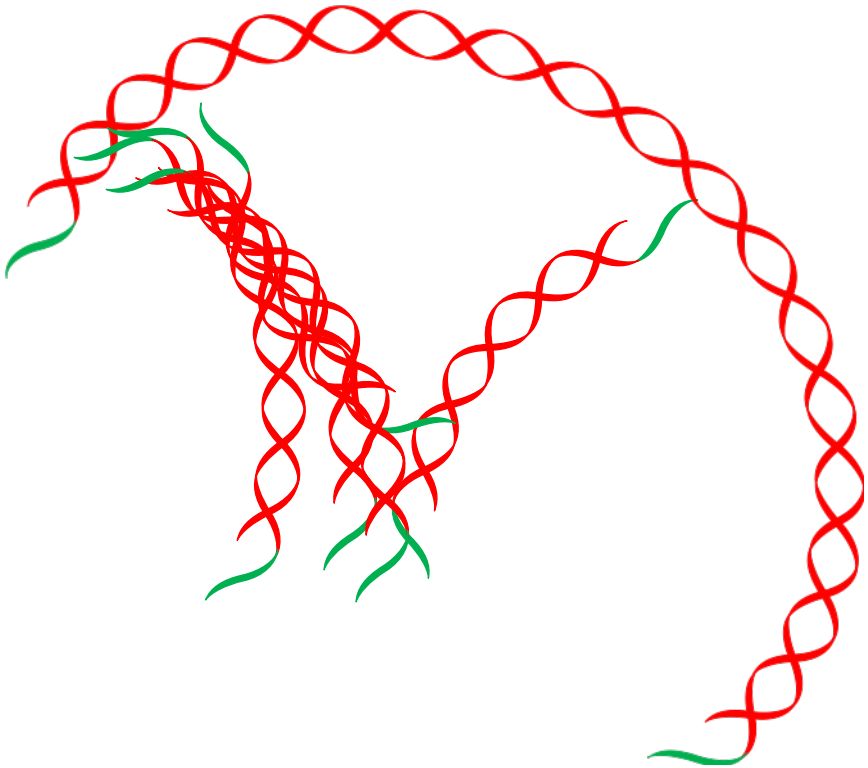




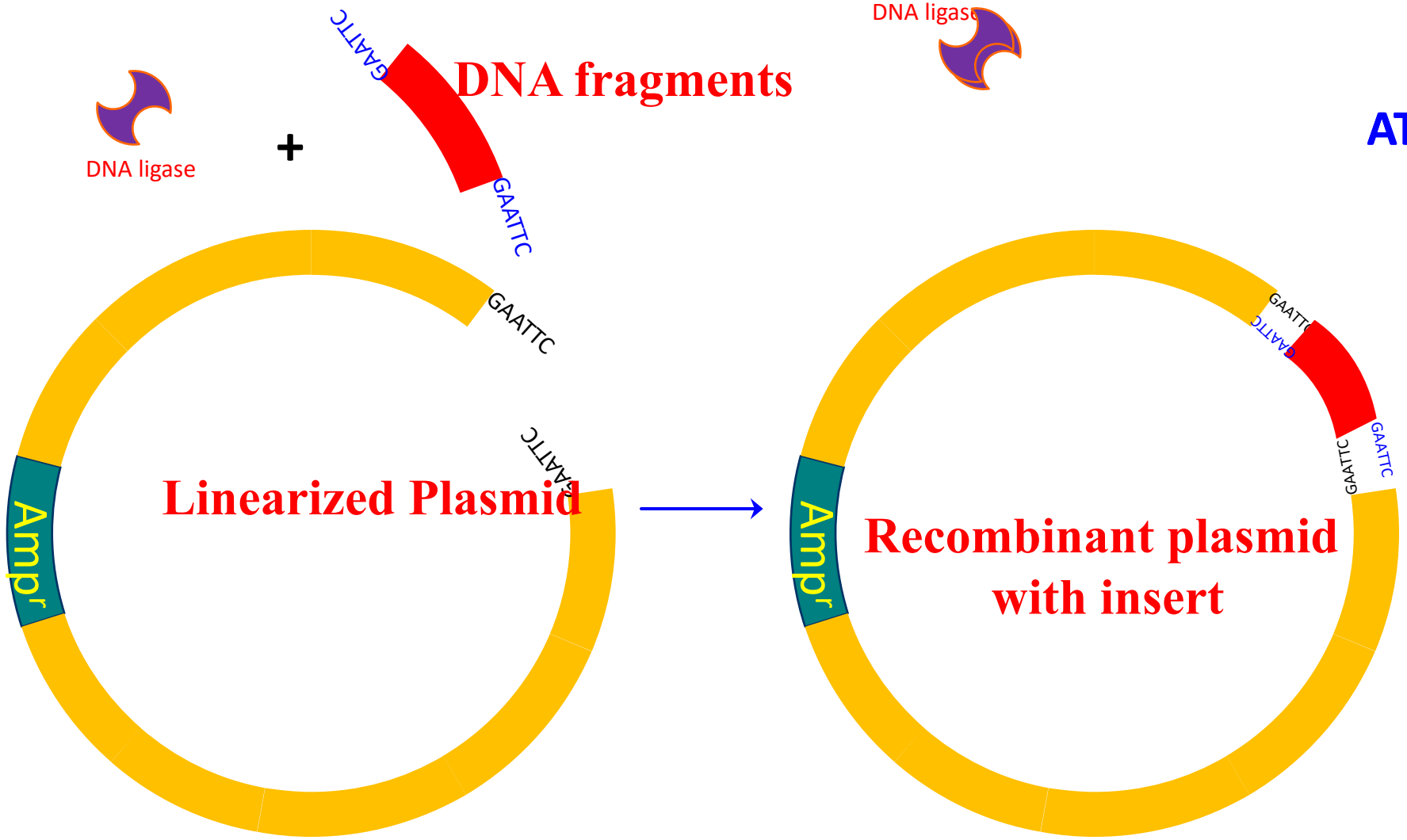


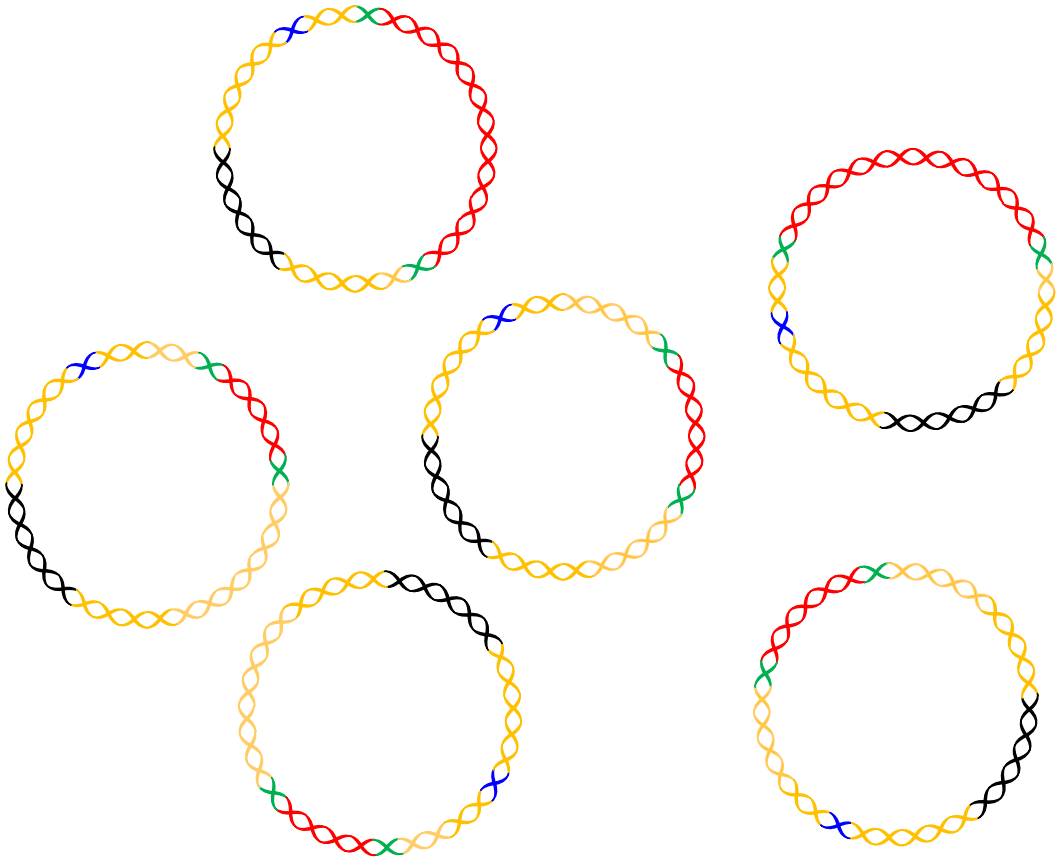

BamHI
BamHI

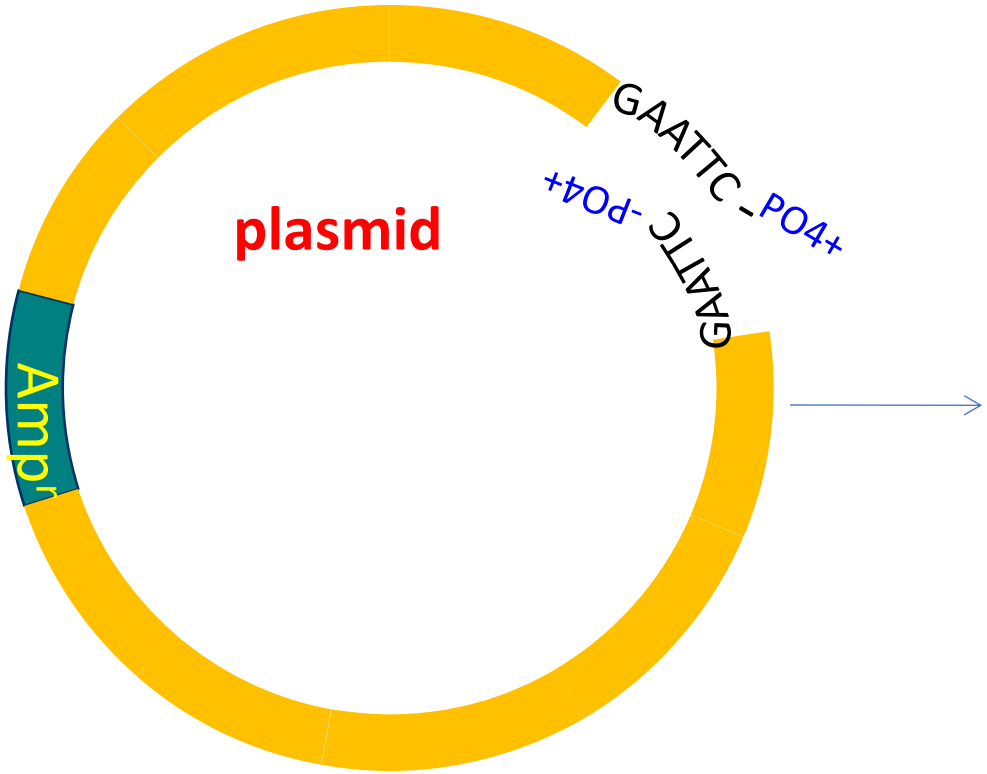




Generation of Recombinant Plasmid

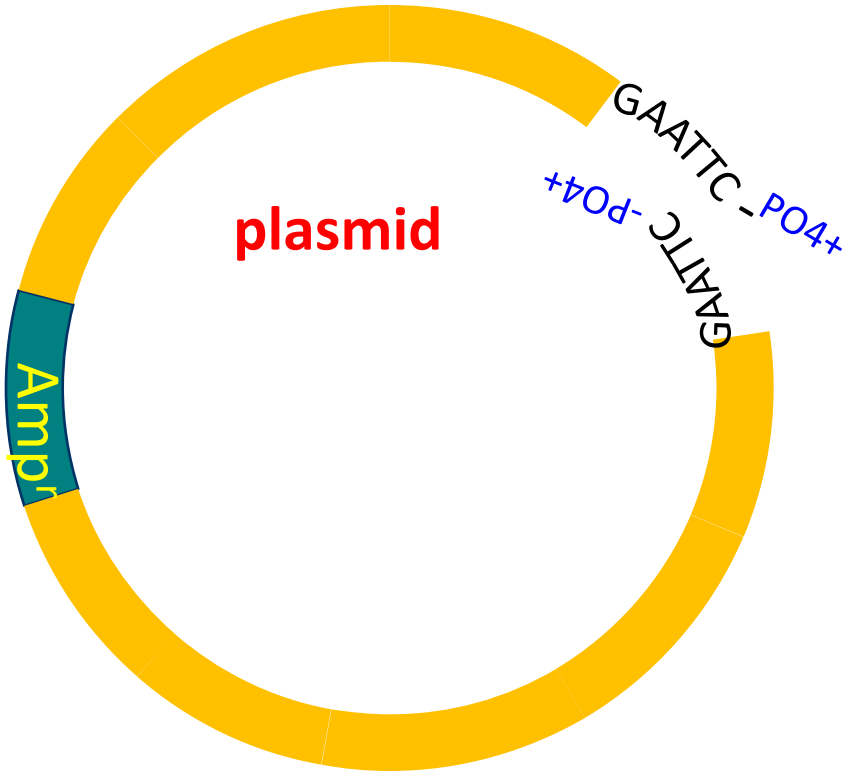






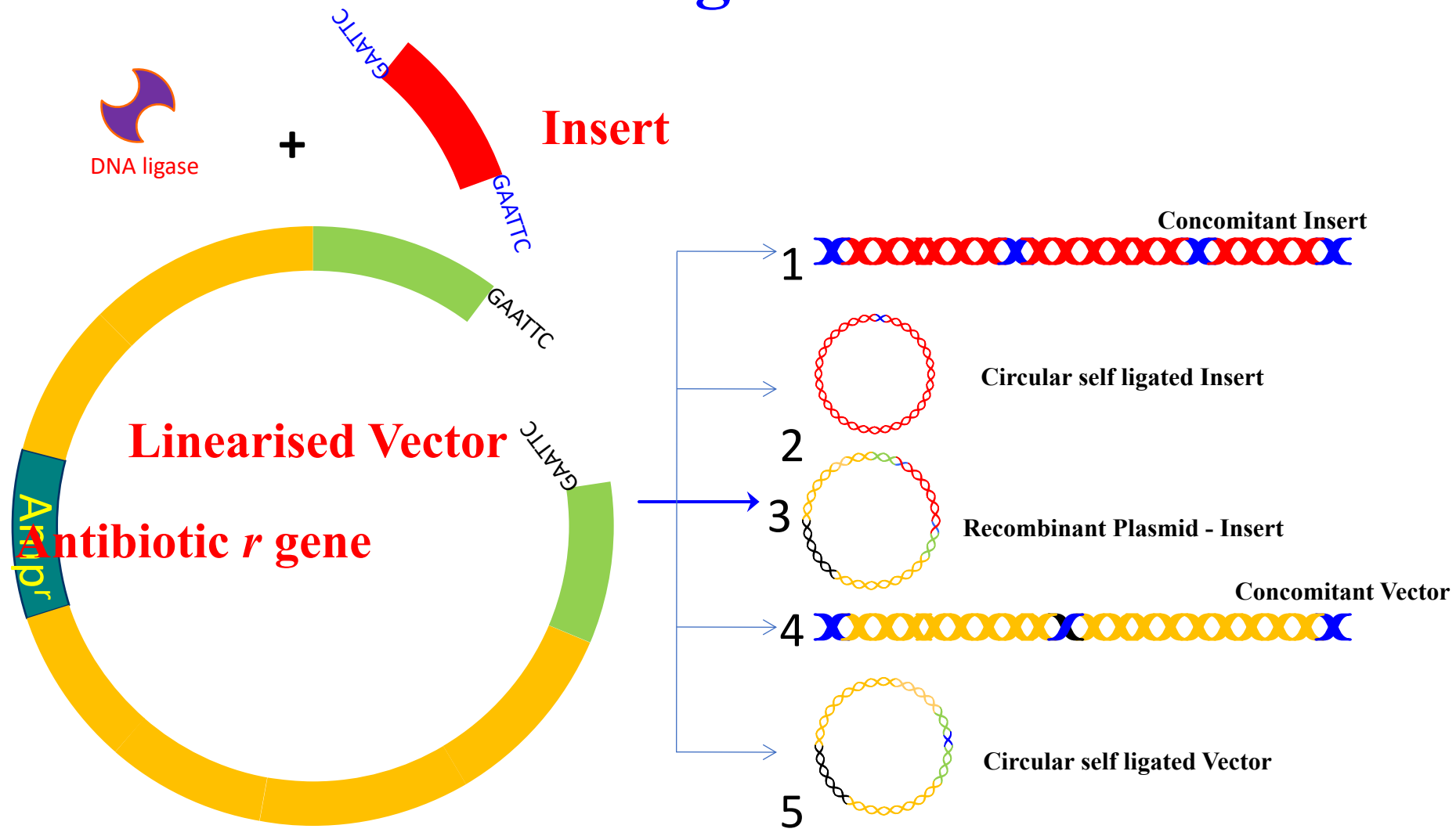
De-PO_4 plasmids can not be ligated due to absence of PO_4 Moiety

5’ PO_4 is must for ligation

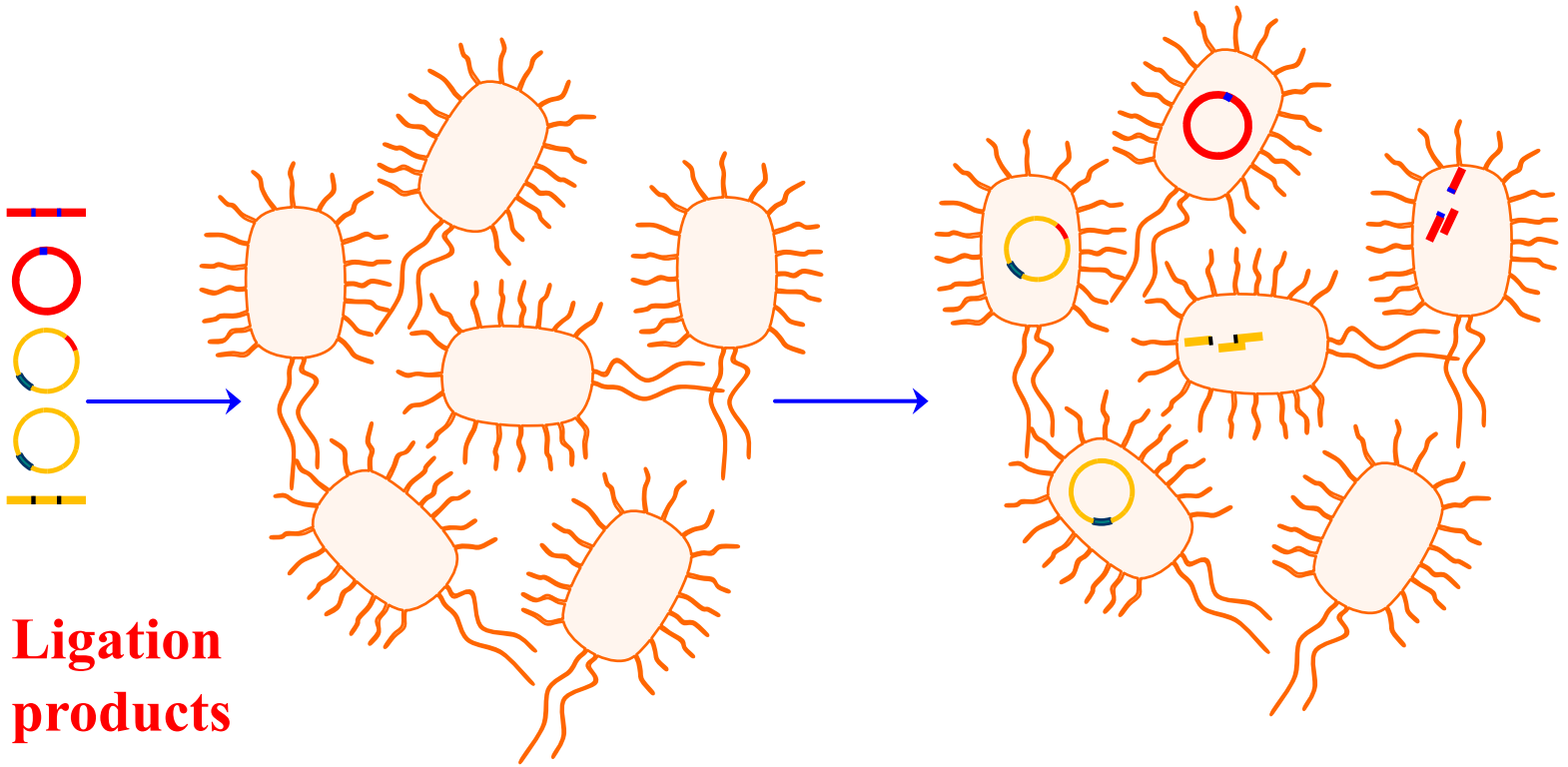


If we remove this 5’ PO_4 ligation may not taking place

Possible Ligation Events



Transformation of Recombinant plasmid into *E. coli*

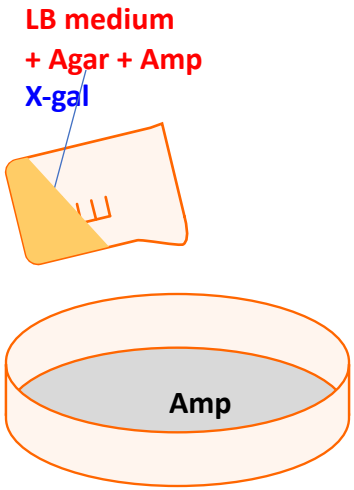


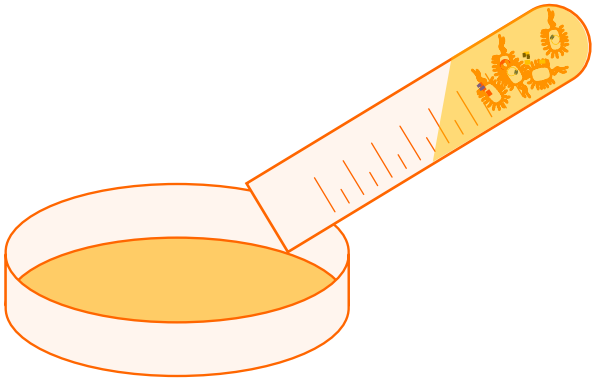
Ligation products

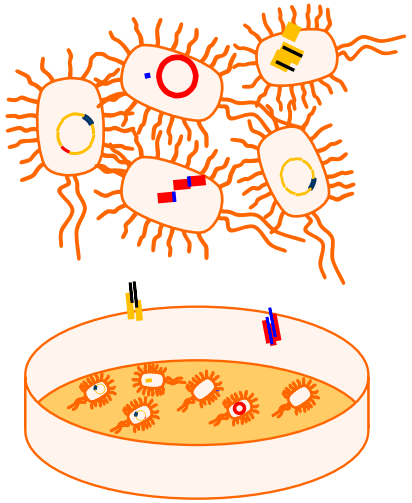
E. Coli host

Transformed *E. Coli*

Agar Plate Preparation for Screening





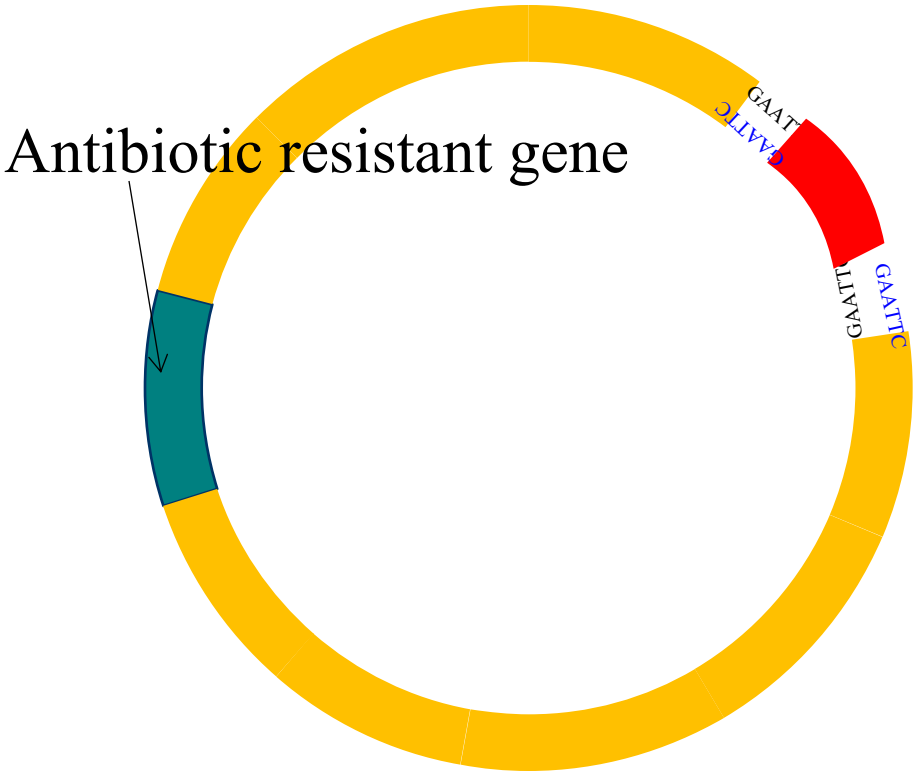


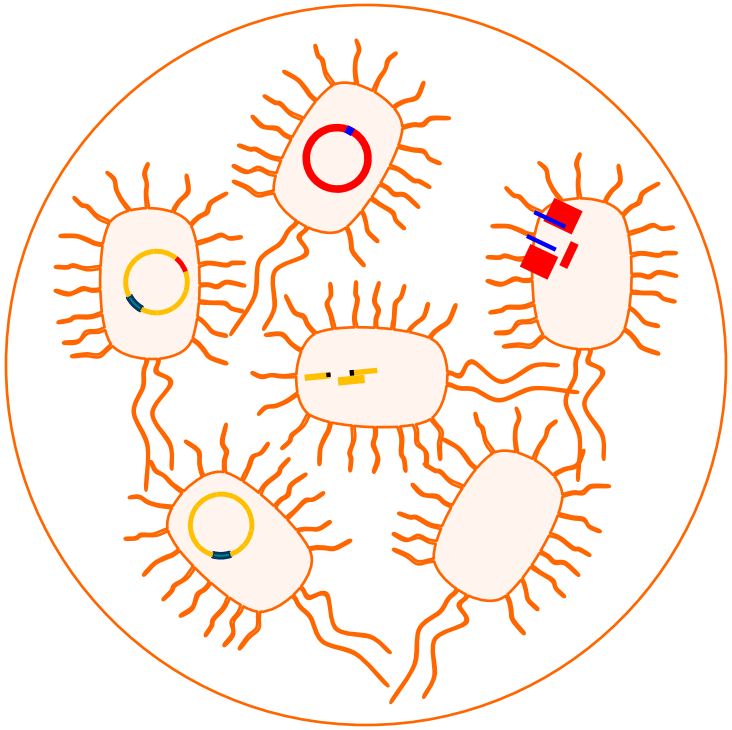
Incubated @ 37°C
overnight

Preparation of Agar Plates

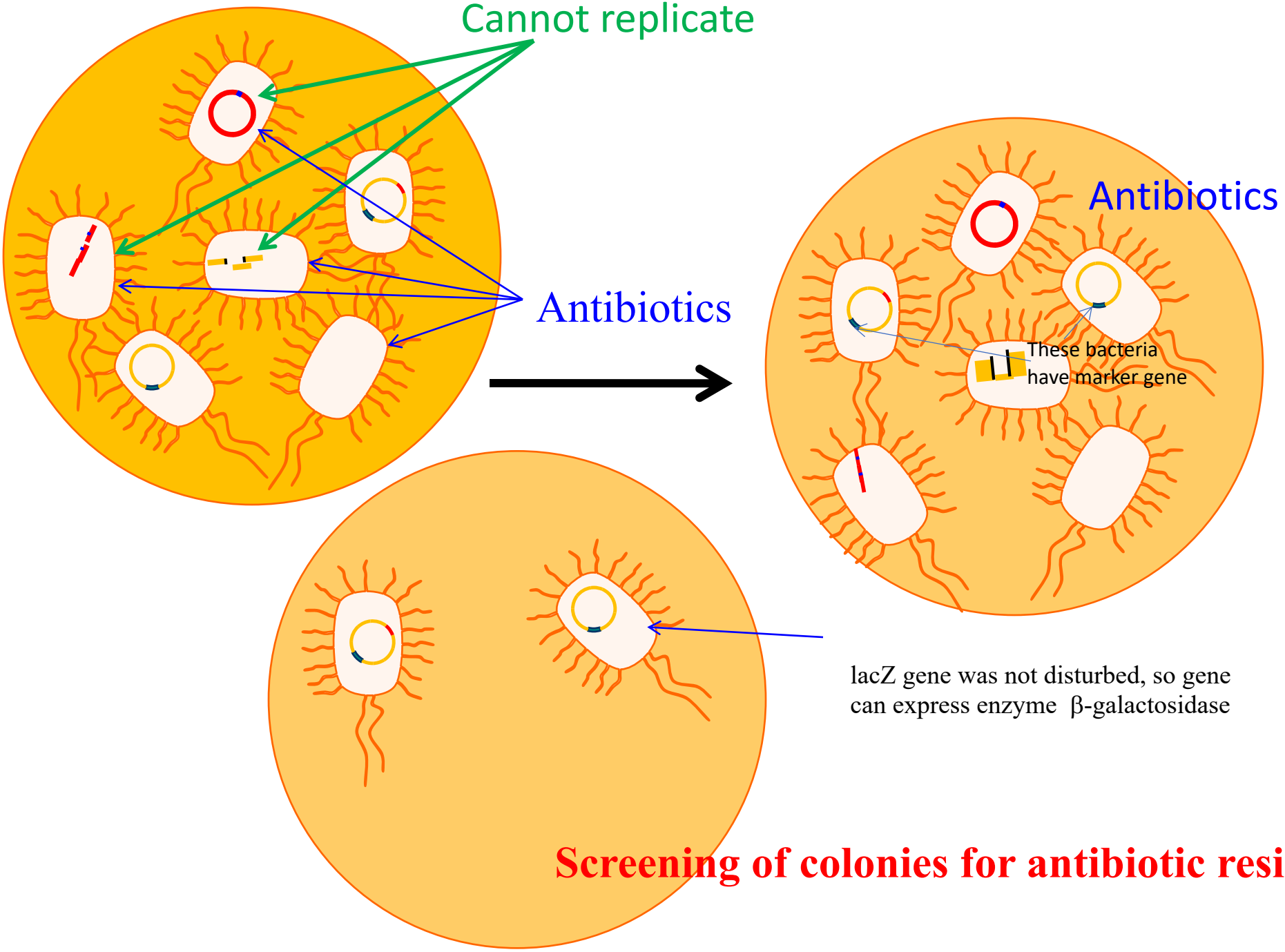
Transformed *E.coli* are plated containing Antibiotic drug

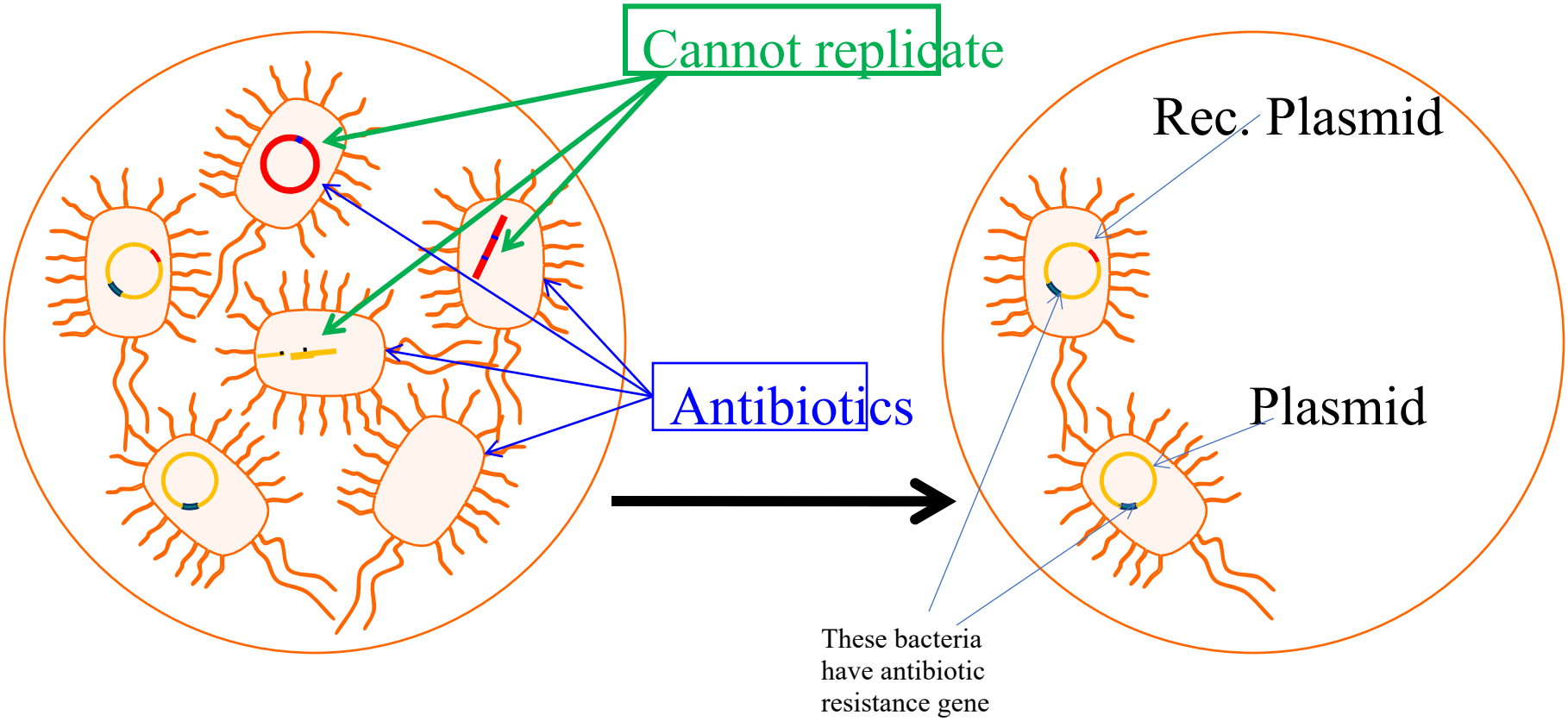
Antibiotic Drug will kill Bacteria except bacterium Bearing plasmid





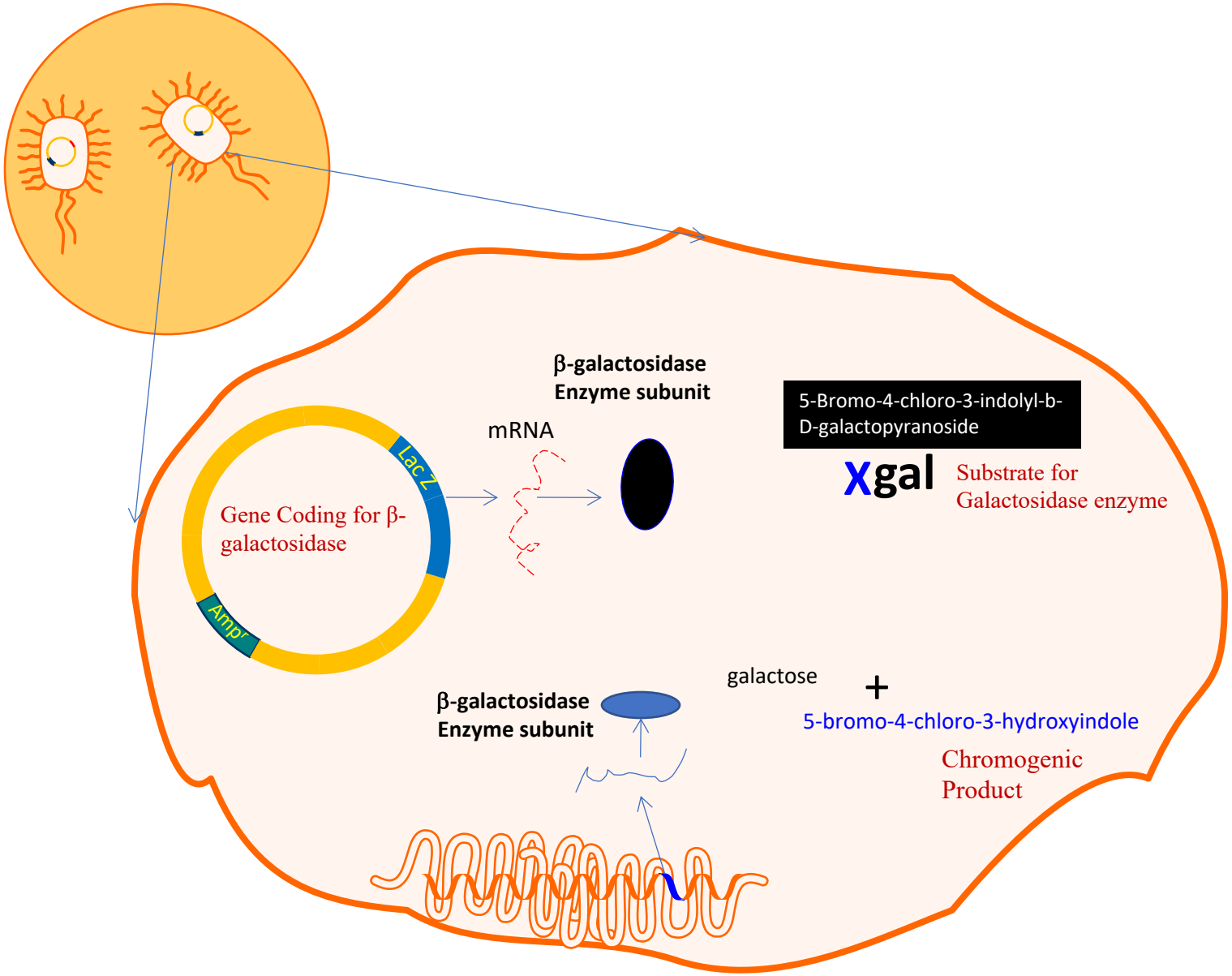
Antibiotic Drug will kill Bacteria except bacterium Bearing plasmid

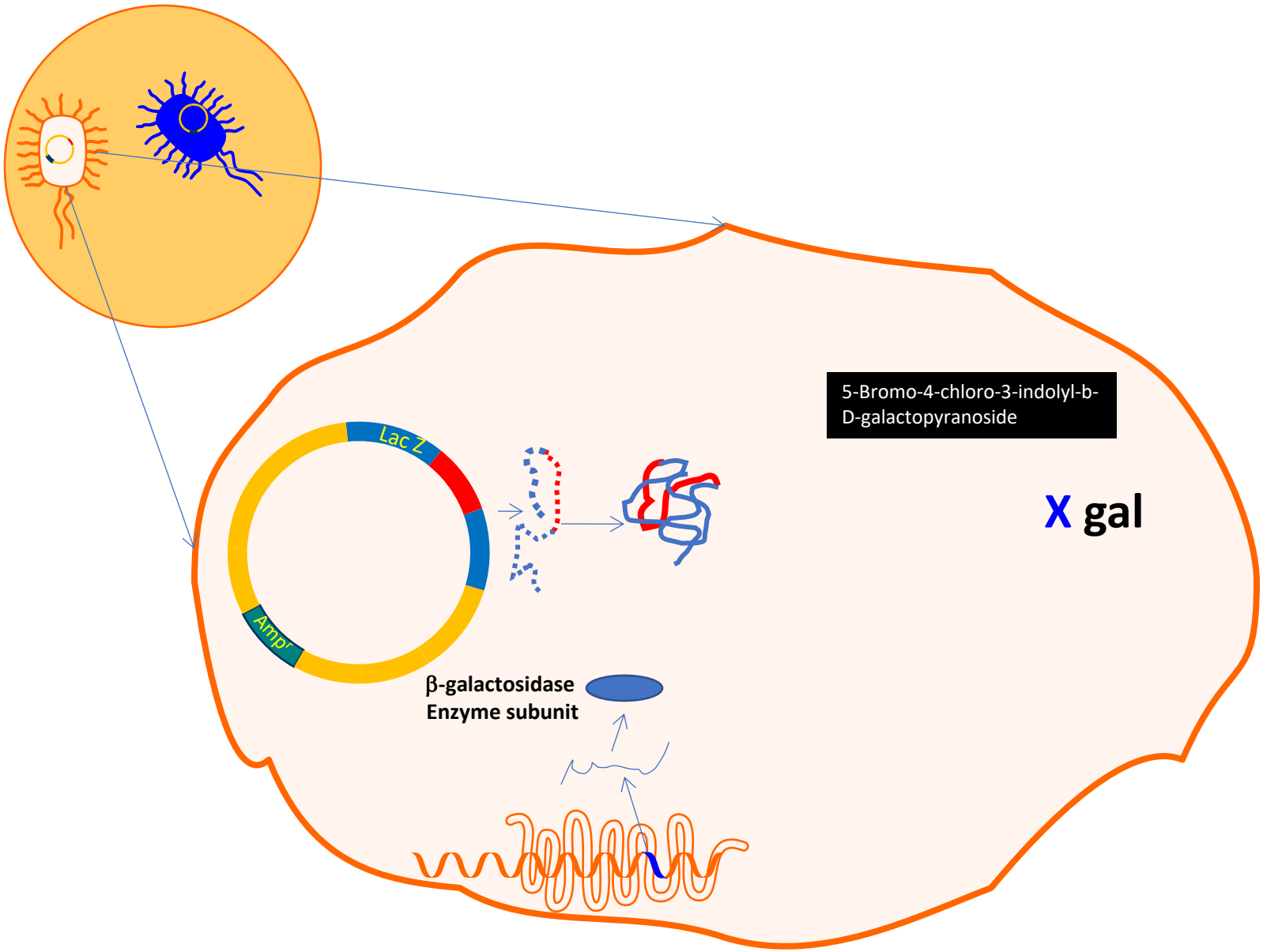

Cannot replicate
Antibiotics
Antibiotics
These bacteria
have marker gene
lacZ gene was not disturbed, so gene
can express enzyme β-galactosidase
Screening of colonies for antibiotic resi

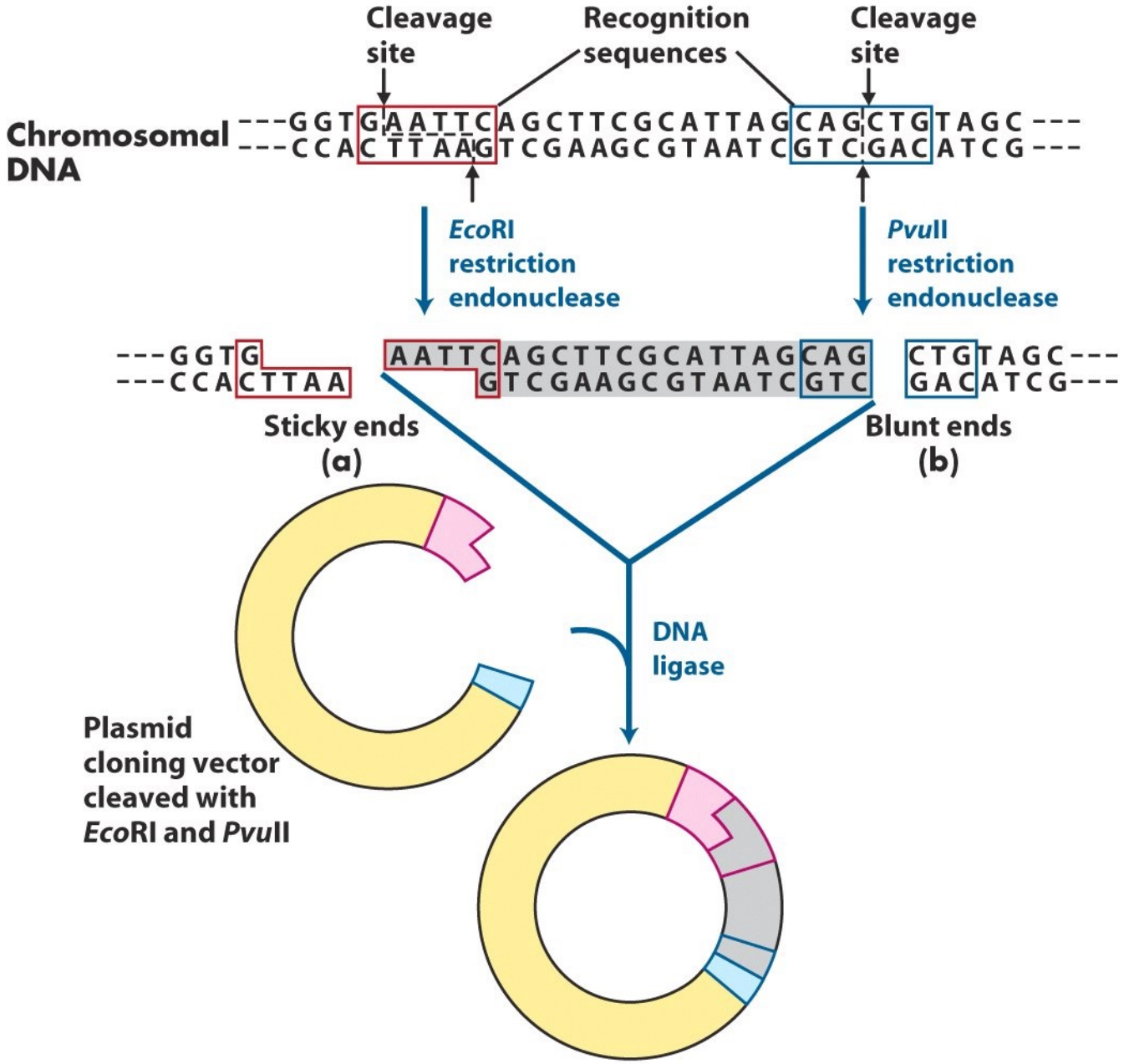


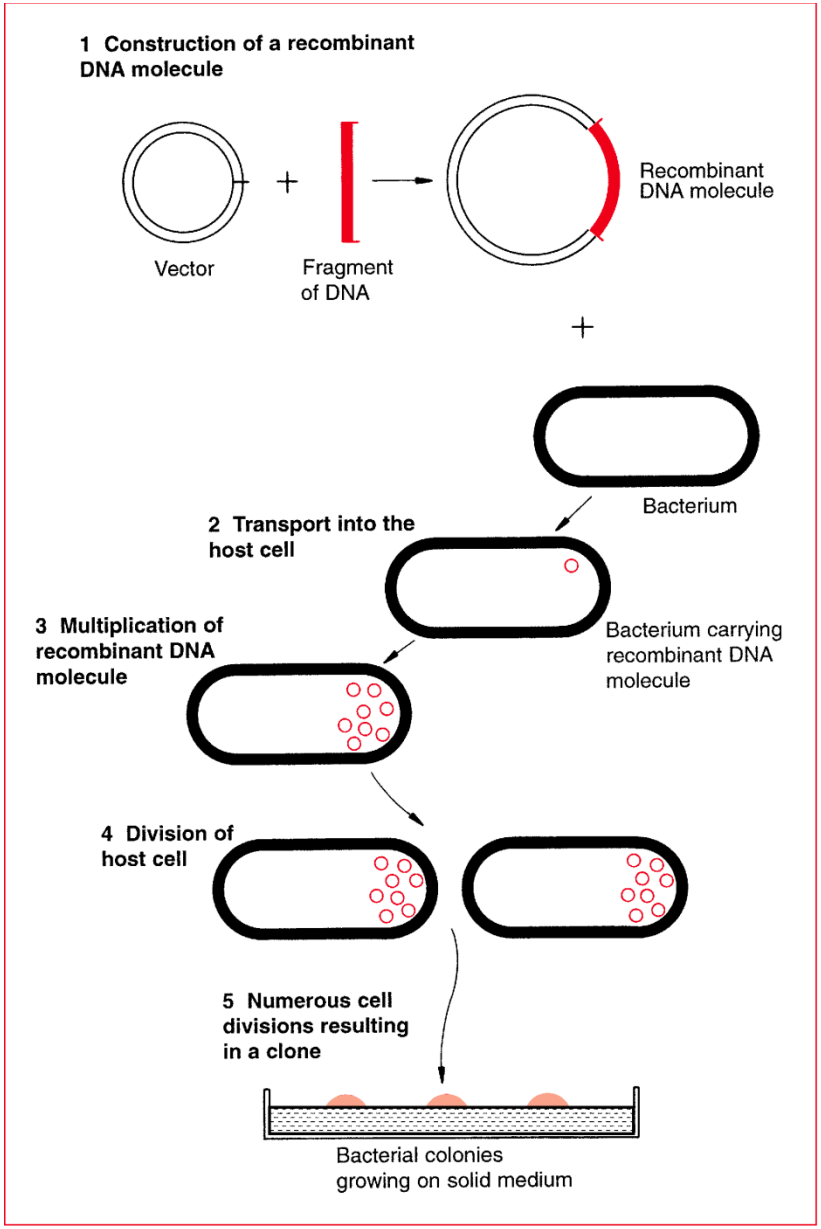
Only Antibiotic resistance bactrial colonies will grow in LB-ag containing antibiotics

Blue White Colony Selection

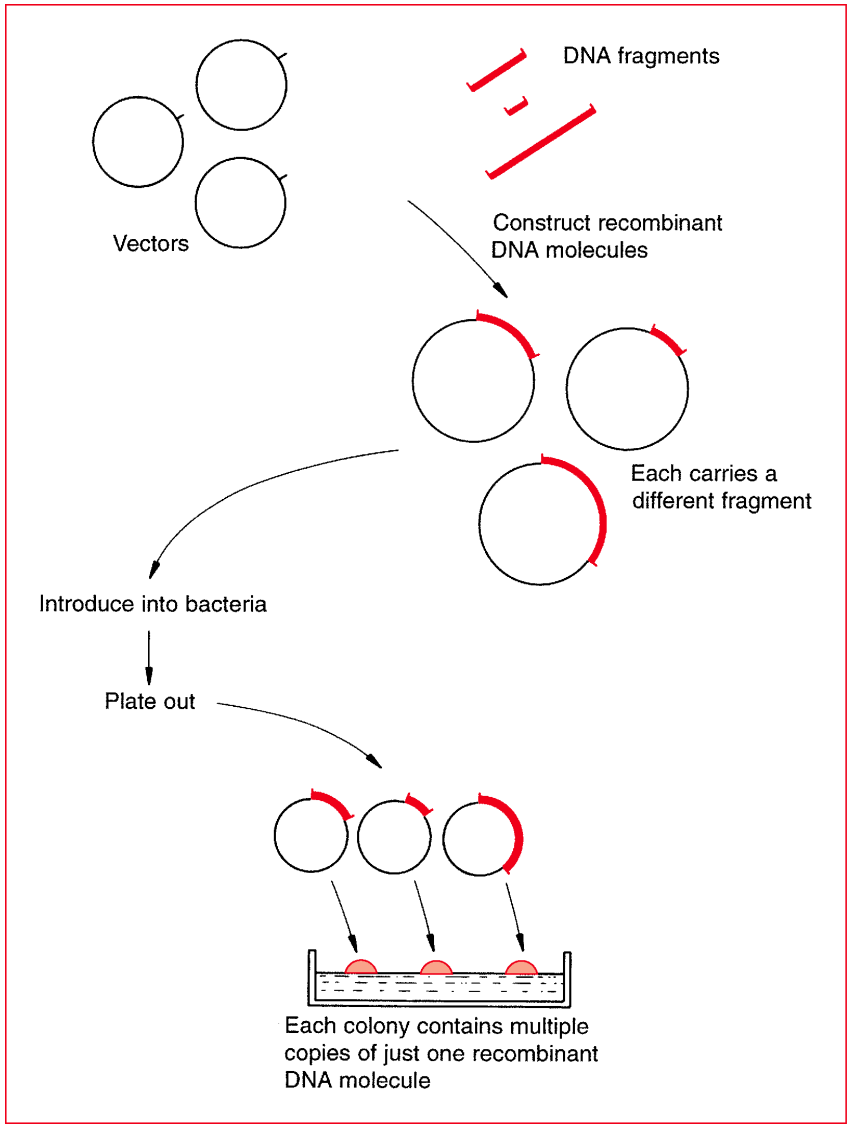



5-Bromo-4-chloro-3-indolyl-b-D-galactopyranoside
X gal
Lac Z
Amp
β-galactosidase
Enzyme subunit


Cleavage site
Recognition sequences
Cleavage site
Chromosomal DNA
---GGTGAATTCAGCTTCGCATTAGCAGCTGTAGC---
---CCACTTAAGTCGAAGCGTAATCGTCGACATCG---
EcoRI restriction endonuclease
PvuII restriction endonuclease
---GGTG
---CCACTTAA
AATTCAGCTTCGCATTAGCAG
GTCGAAGCGTAATCGTC
CTGTAGC---
GACATCG---
Sticky ends
(a)
Blunt ends
(b)
DNA ligase
Plasmid cloning vector cleaved with EcoRI and PvuII



The basic steps in gene cloning.

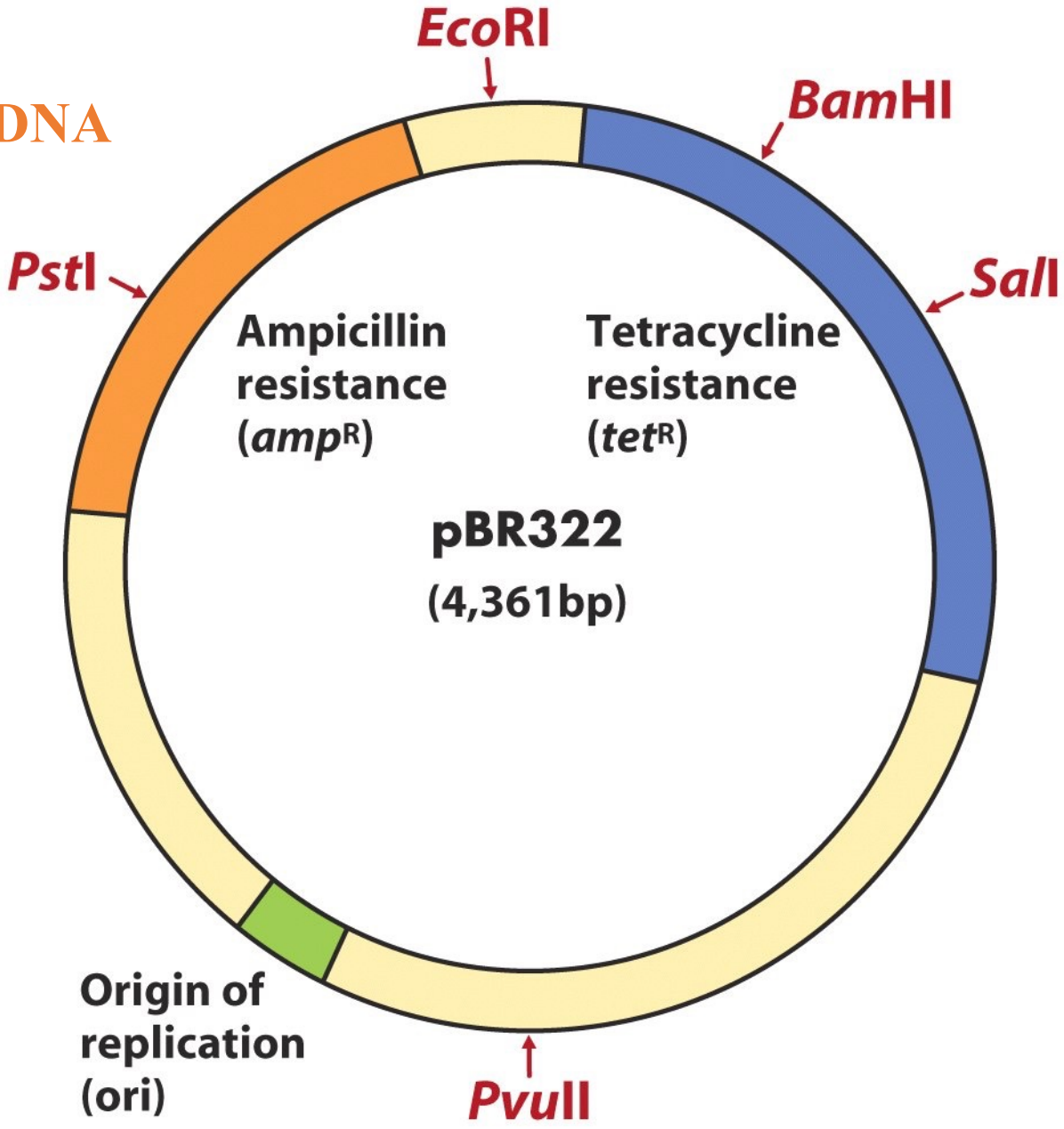


Cloning allows individual fragments of DNA to be purified.

Cloning vectors allow amplification of inserted DNA segments

E coli plasmid pBR 322

1. Origin of replication
2. Antibiotic resistance genes
3. Unique restriction sites
4. Small size



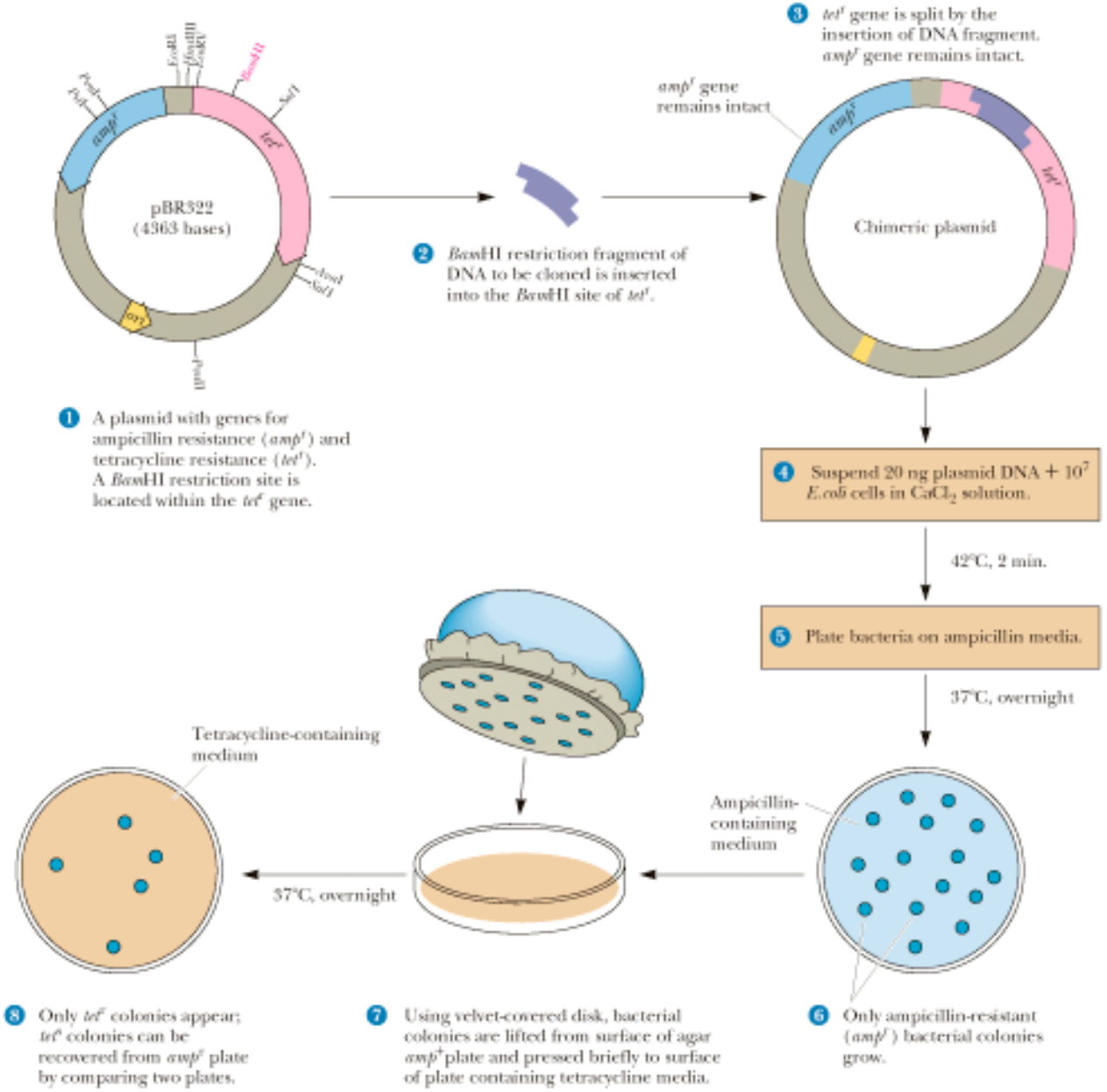
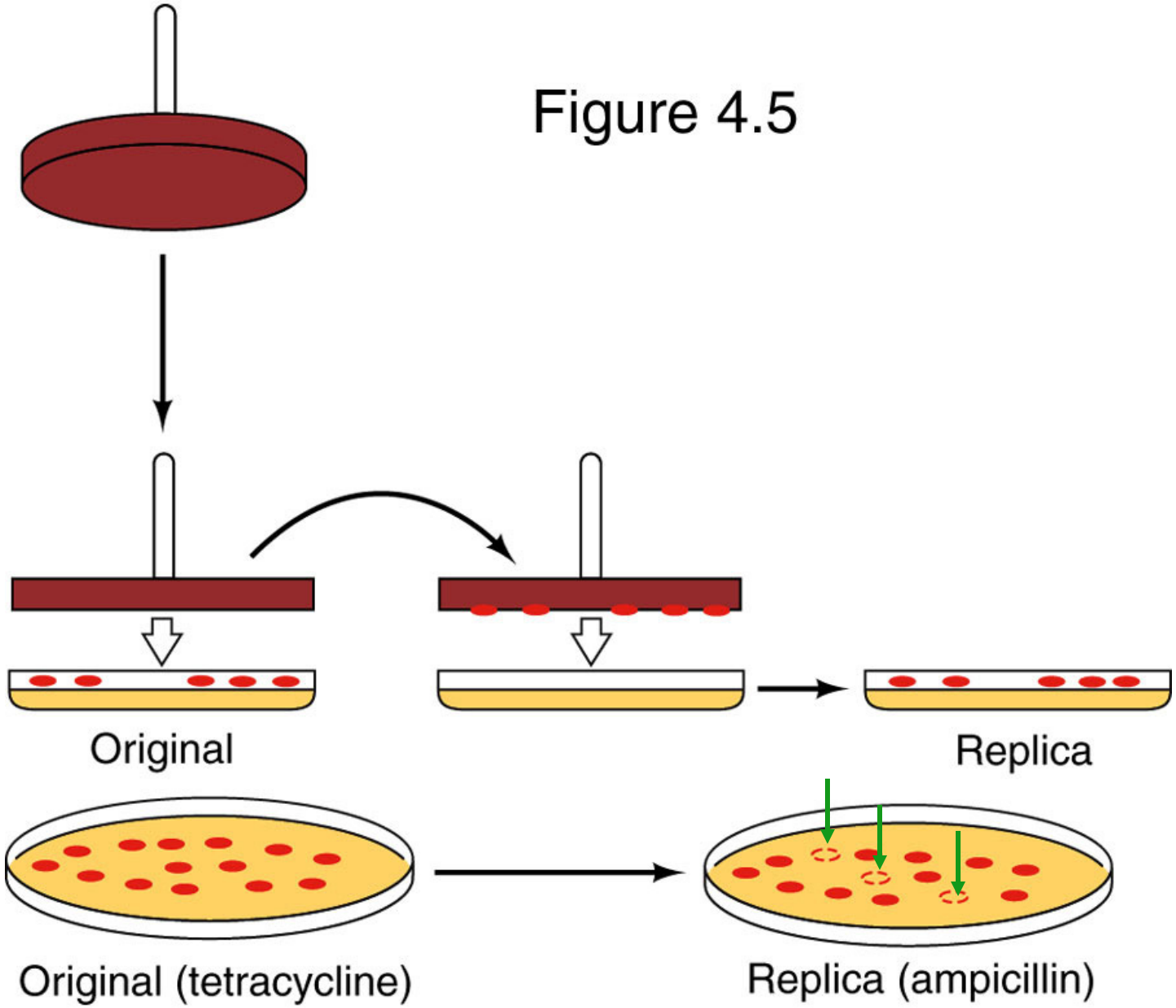
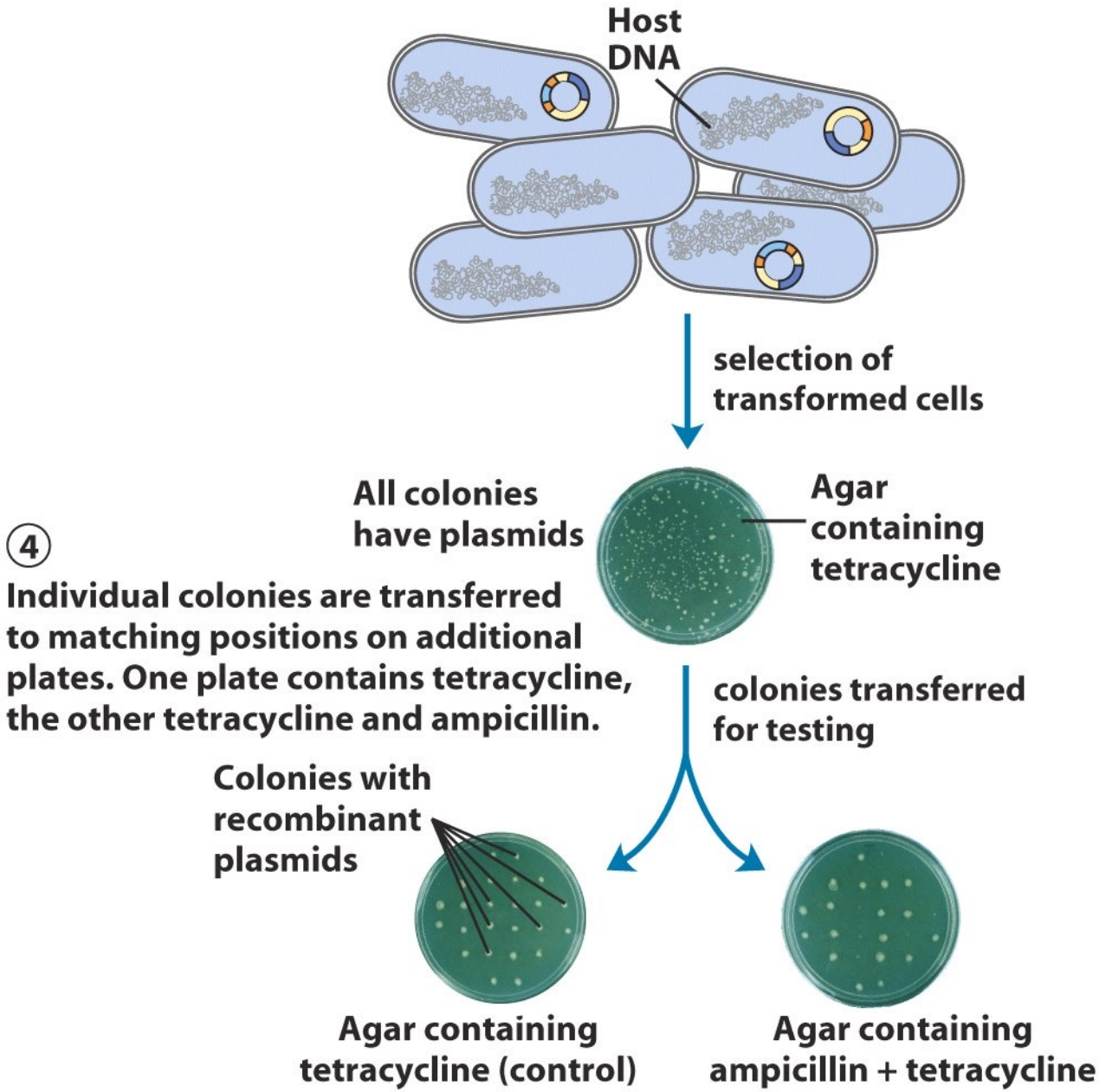

pBR322
(4363 bases)
ampr
tetr
ori
PvuII
PstI
HindIII
EcoRI
ClaI
BamHI
SalI
AccI
HincII
PvuII
ampr gene
remains intact
Chimeric plasmid
tetr
1 A plasmid with genes for ampicillin resistance (ampr) and tetracycline resistance (tetr). A BamHI restriction site is located within the tetr gene.
2 BamHI restriction fragment of DNA to be cloned is inserted into the BamHI site of tetr.
3 tetr gene is split by the insertion of DNA fragment. ampr gene remains intact.
4 Suspend 20 ng plasmid DNA + 10^7 E.coli cells in CaCl2 solution.
42°C, 2 min.
5 Plate bacteria on ampicillin media.
37°C, overnight
Ampicillin-containing medium
6 Only ampicillin-resistant (ampr) bacterial colonies grow.
7 Using velvet-covered disk, bacterial colonies are lifted from surface of agar ampr plate and pressed briefly to surface of plate containing tetracycline media.
37°C, overnight
Tetracycline-containing medium
8 Only tetr colonies appear; tets colonies can be recovered from ampr plate by comparing two plates.

Figure 4.5


Original
Replica
Original (tetracycline)
Replica (ampicillin)

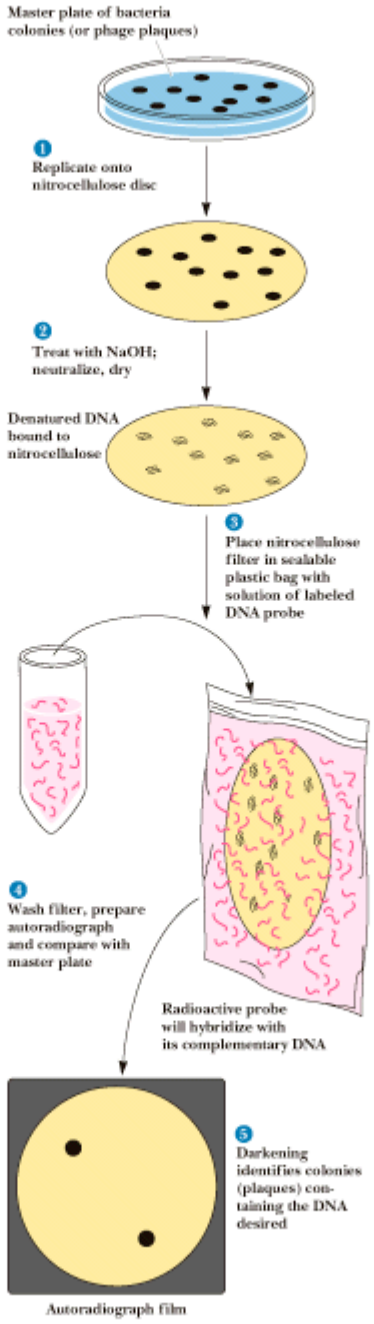

Host
DNA
selection of
transformed cells
All colonies
have plasmids
Agar
containing
tetracycline
④
Individual colonies are transferred
to matching positions on additional
plates. One plate contains tetracycline,
the other tetracycline and ampicillin.
colonies transferred
for testing
Colonies with
recombinant
plasmids
Agar containing
tetracycline (control)
Agar containing
ampicillin + tetracycline

Colony Hybridization

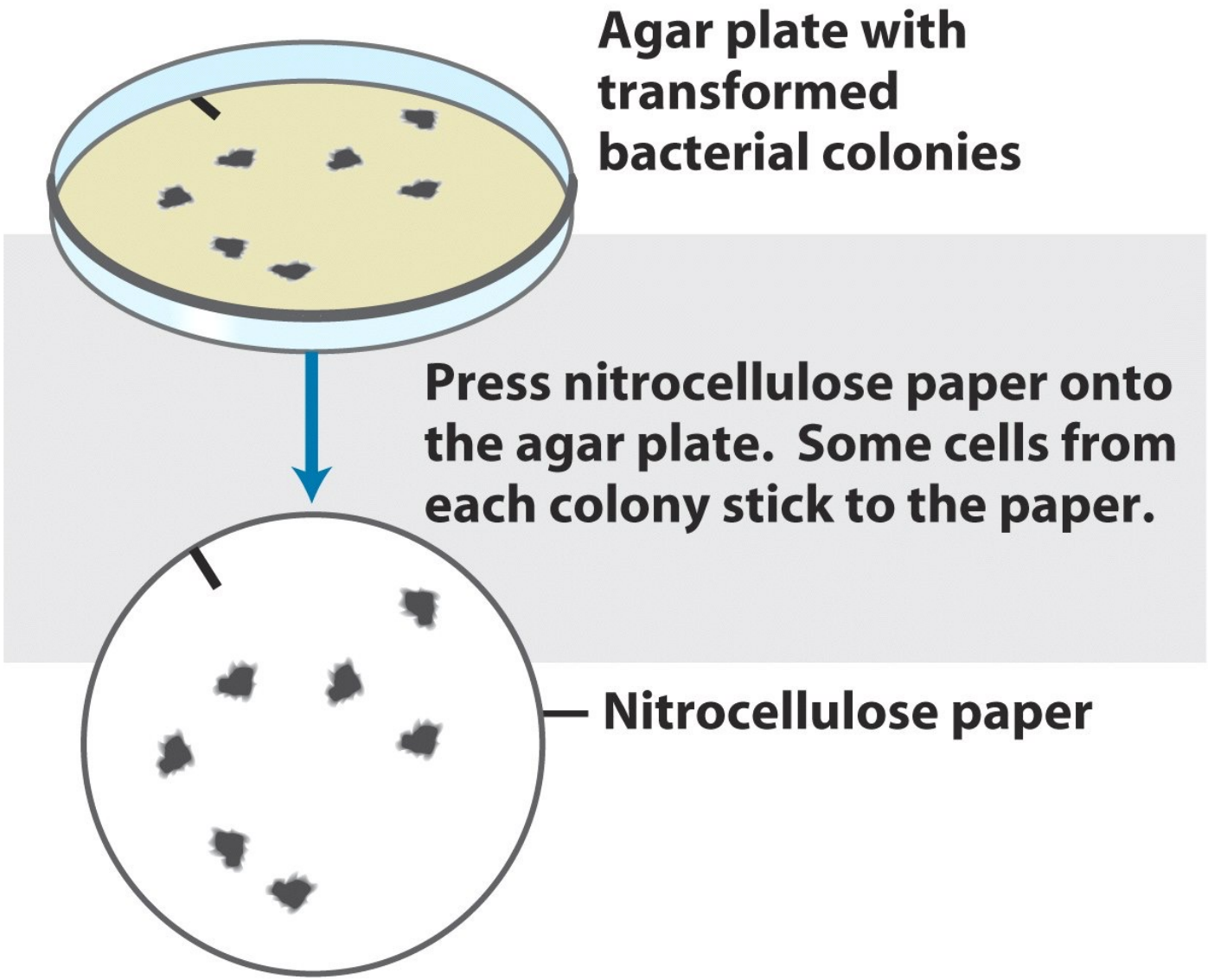
A way to screen plasmid-based genome libraries for a DNA fragment of interest

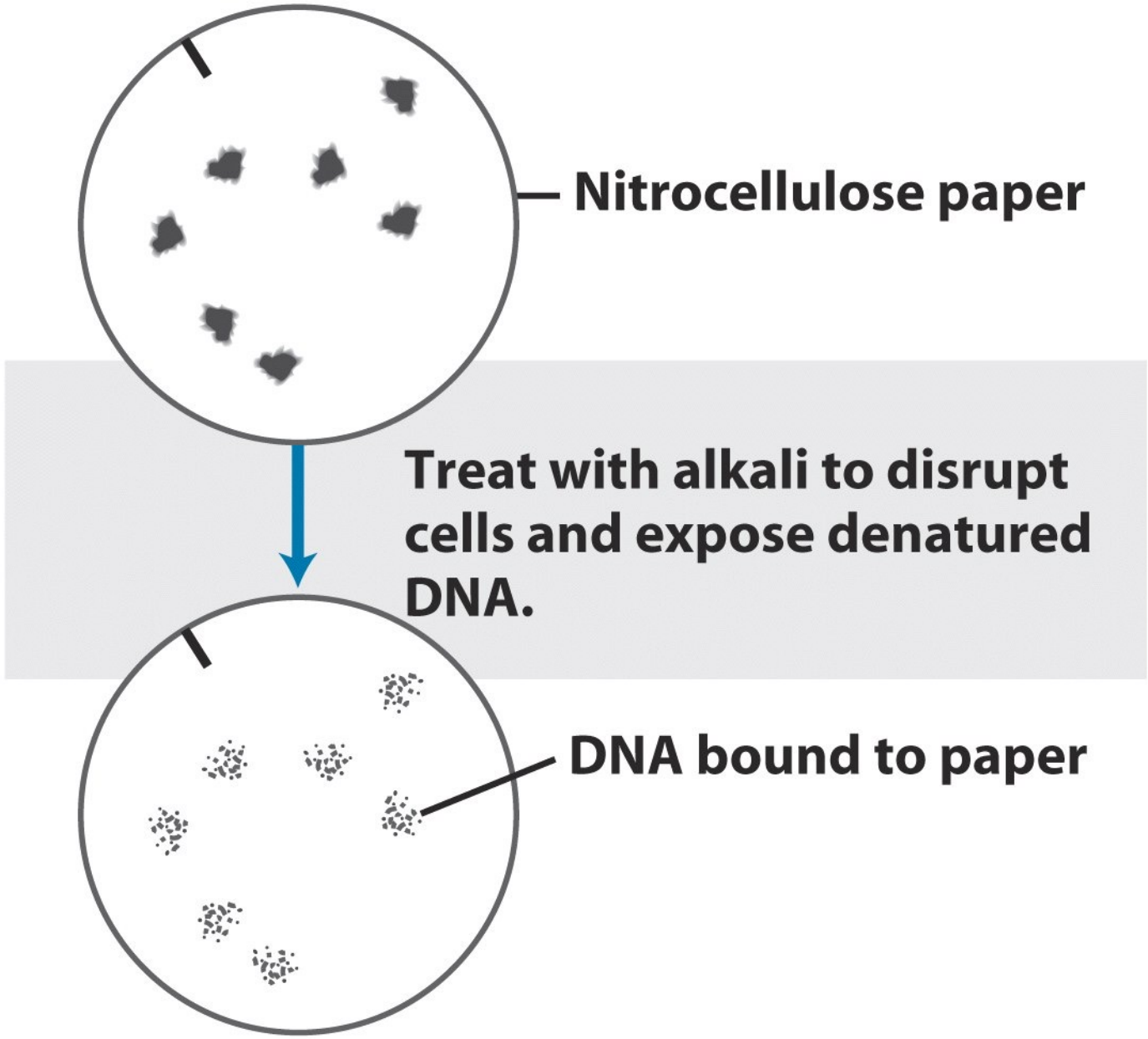
- Host bacteria containing a plasmid-based library of DNA fragments are plated on a petri dish and allowed to grow overnight to form colonies
- Replica of dish made with a nitrocellulose disk

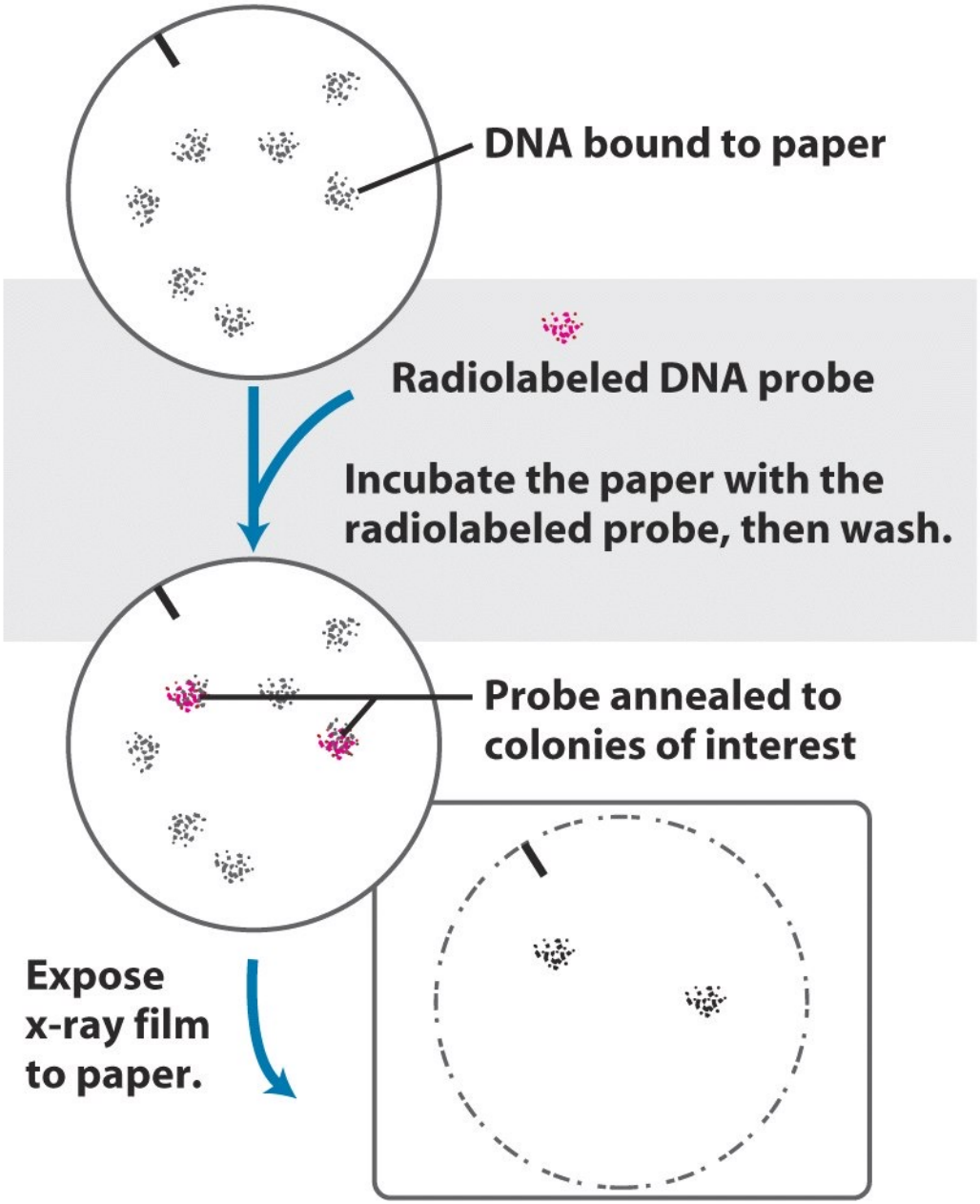
- Disk is treated with base or heated to convert dsDNA to ssDNA and incubated with probes
- Colonies that bind probe (with P-32) hold the fragment of interest



Specific sequences are detectable by hybridization




Nitrocellulose paper
Treat with alkali to disrupt cells and expose denatured DNA.
DNA bound to paper


DNA bound to paper
Radiolabeled DNA probe
Incubate the paper with the radiolabeled probe, then wash.
Probe annealed to colonies of interest
Expose x-ray film to paper.