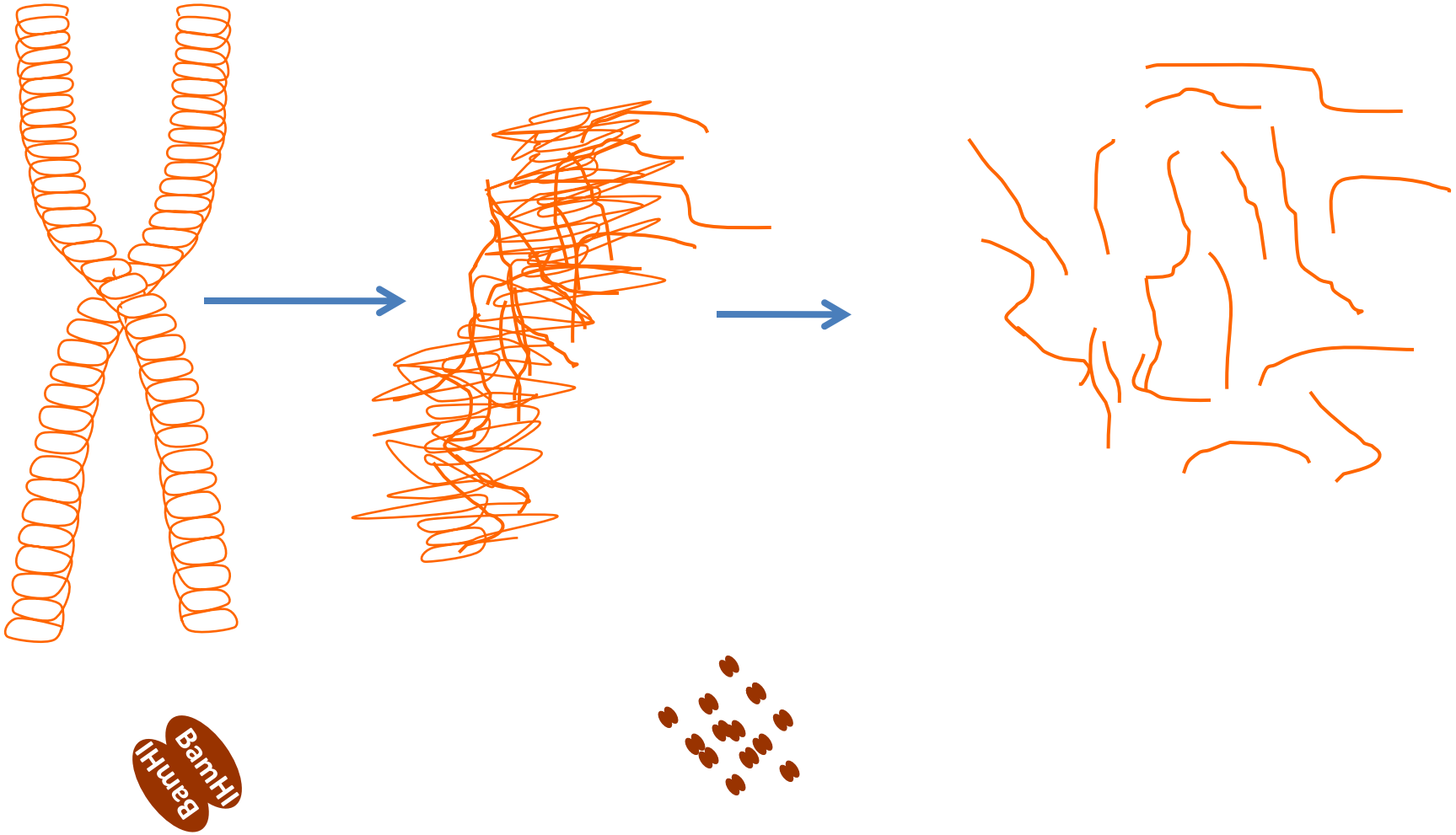
Core course
BMS361N
Genetic Engineering

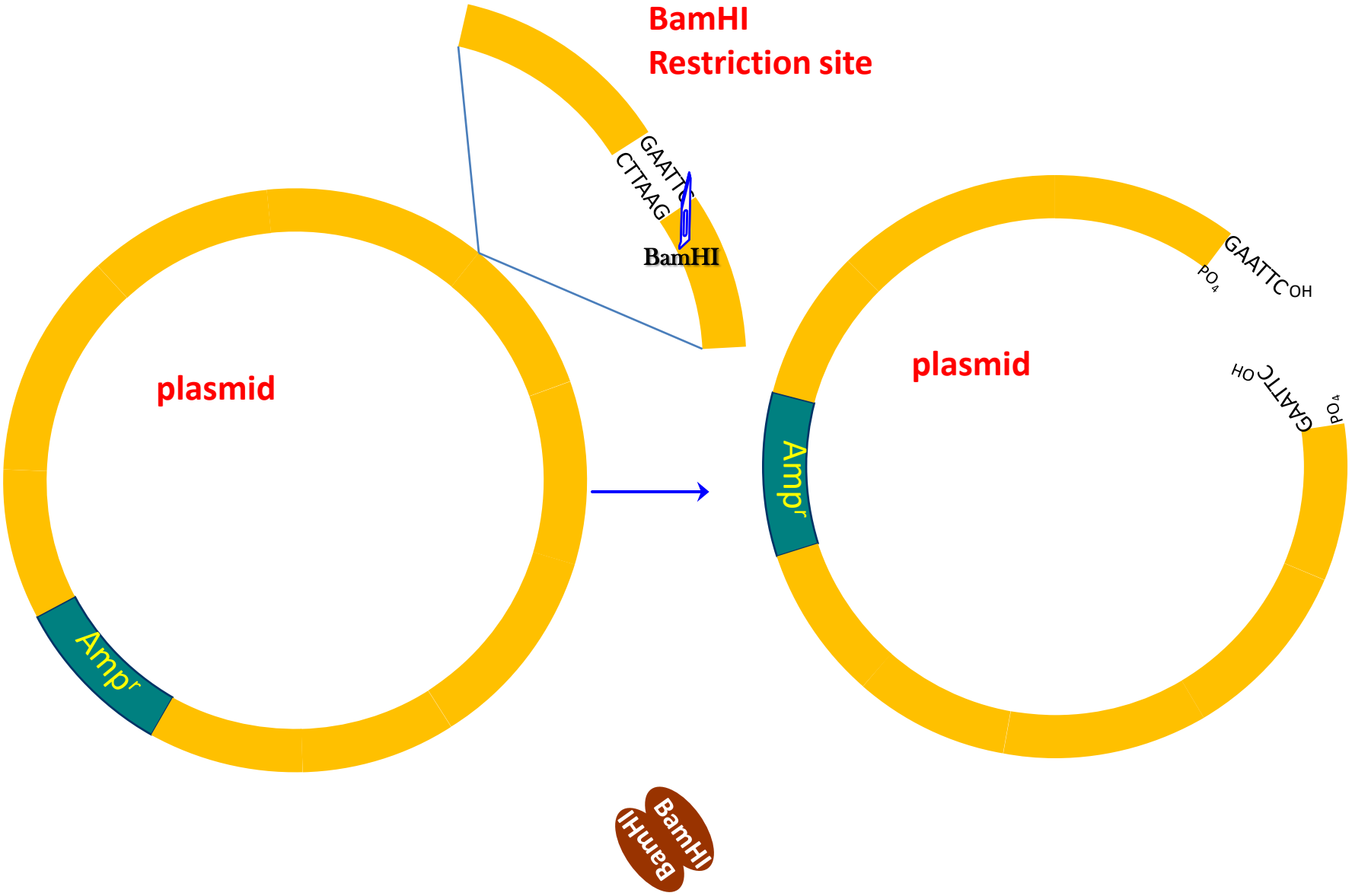
Gene Cloning

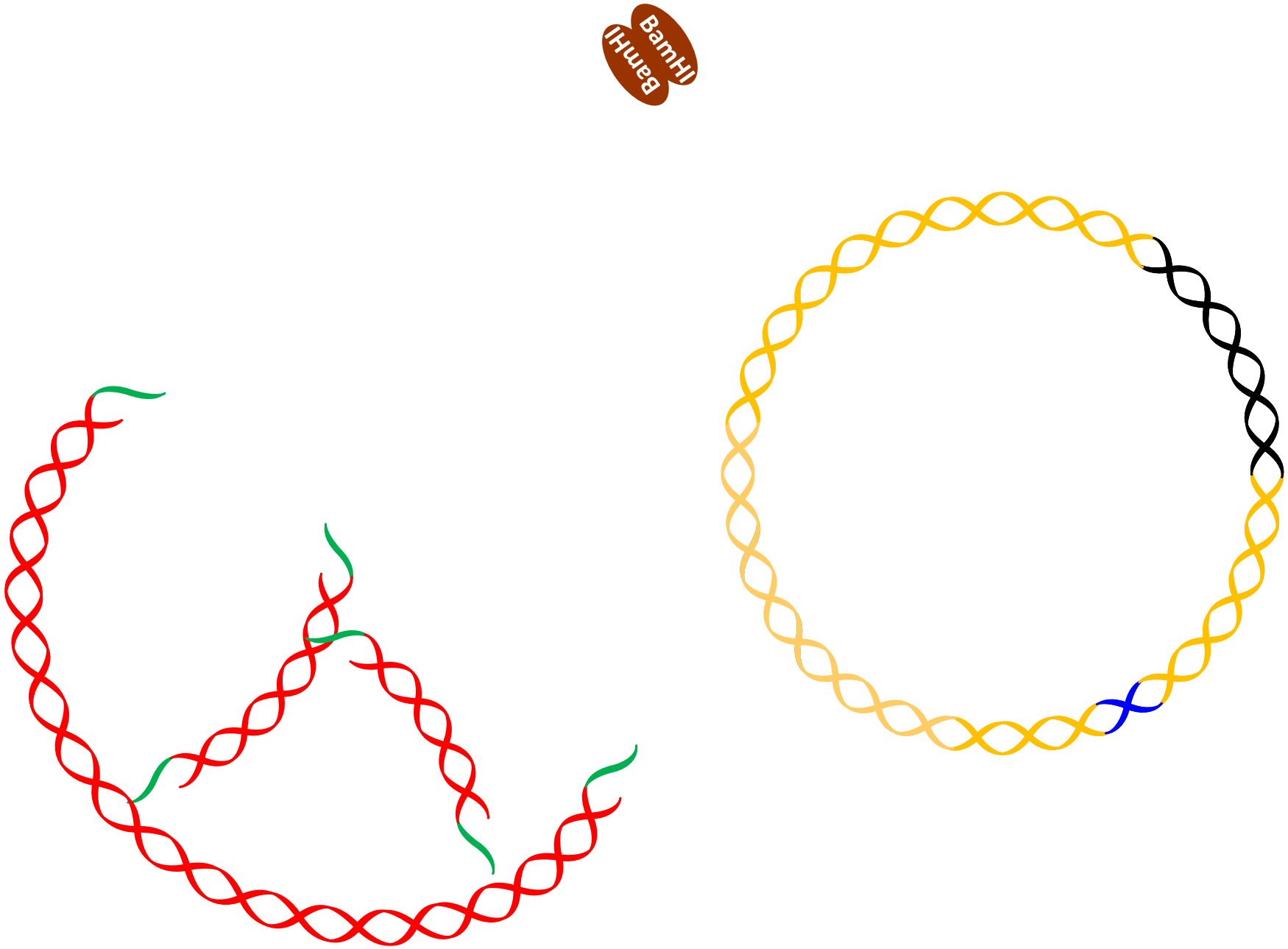
Prof. Narkunaraja Shanmugam
Dept. Of Biomedical Science
School of Basic Medical Sciences
Bharathidasan University

Digesting Genomic DNA by RE



Linearization of Plasmid

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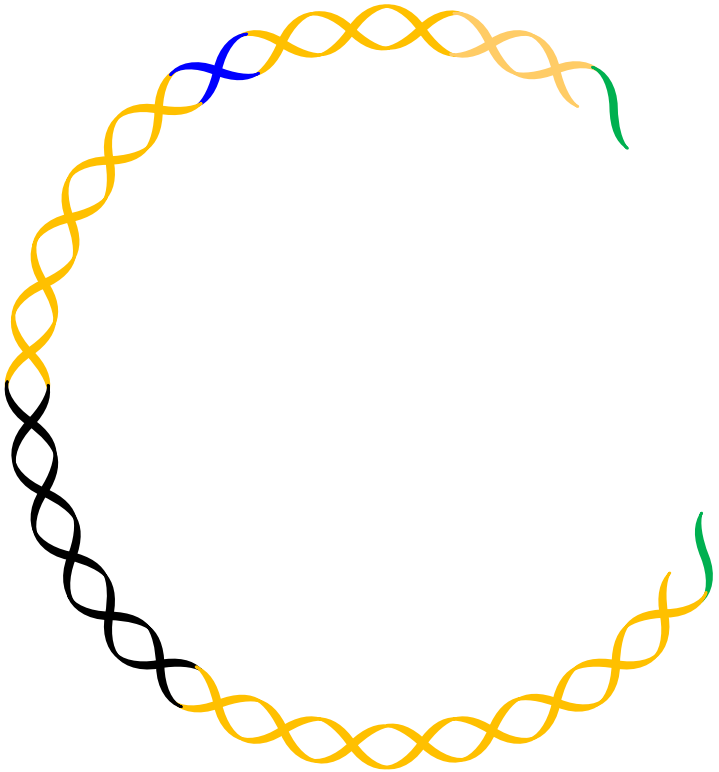
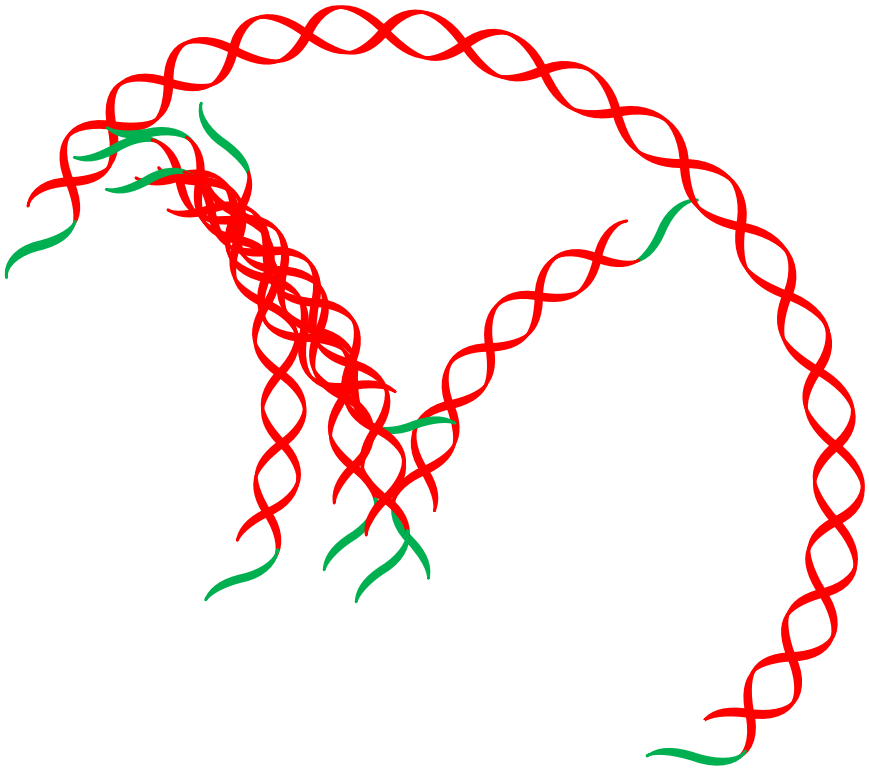
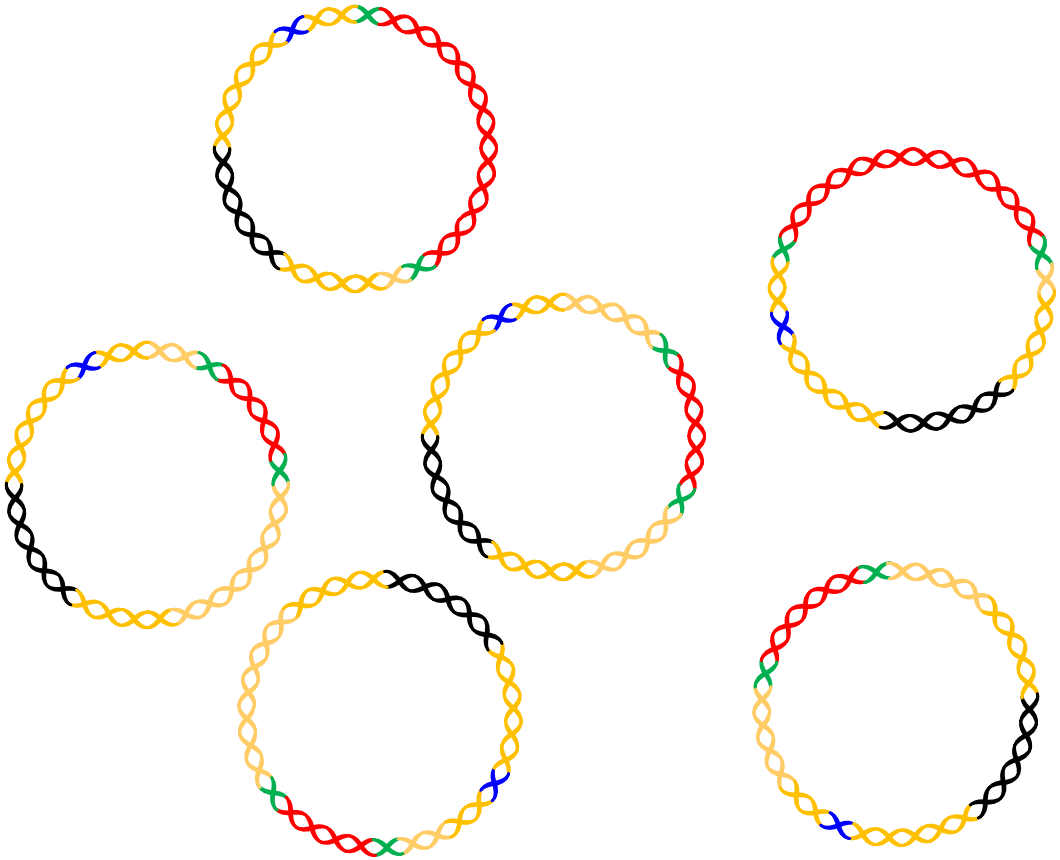
BamHI

Ligation of DNA

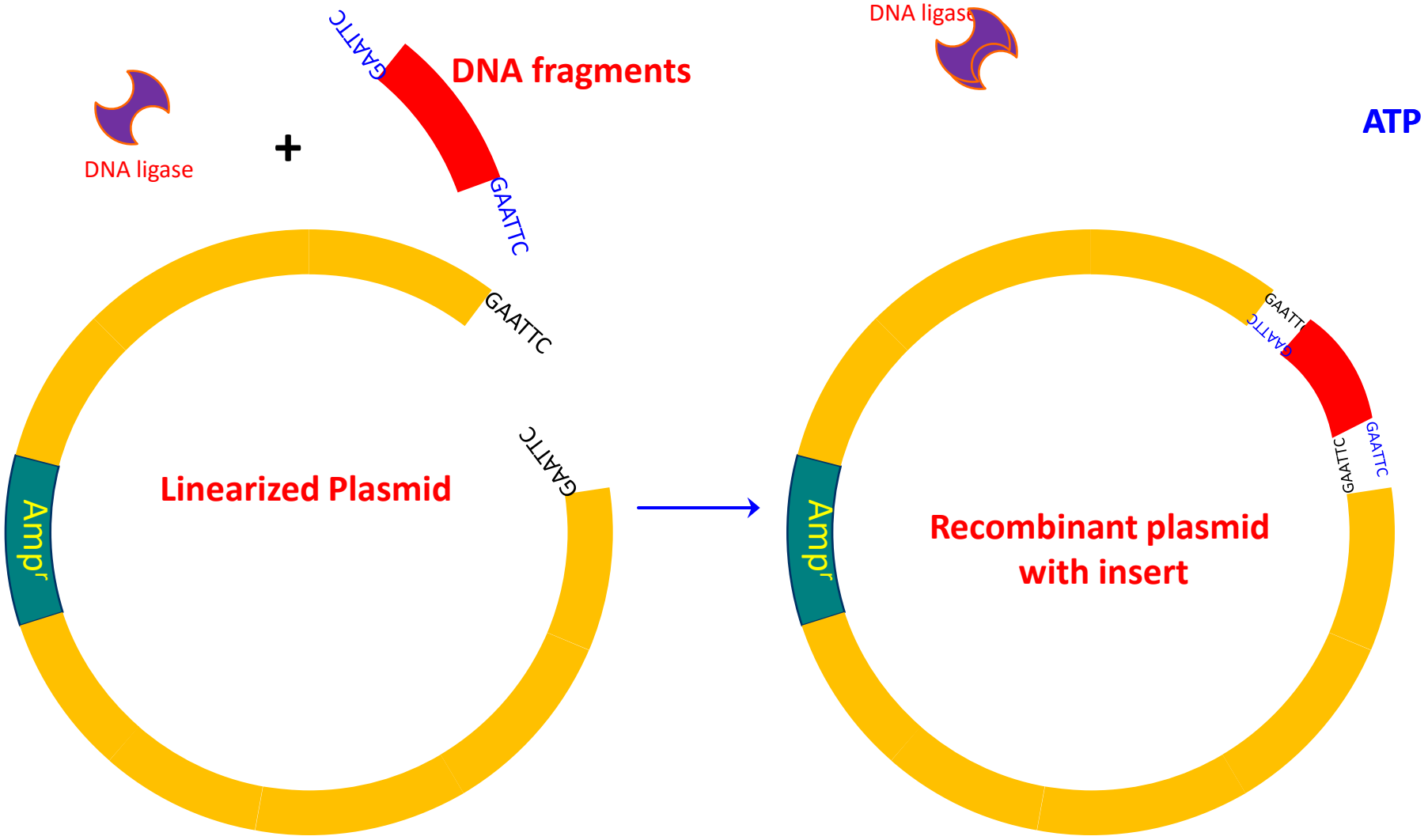




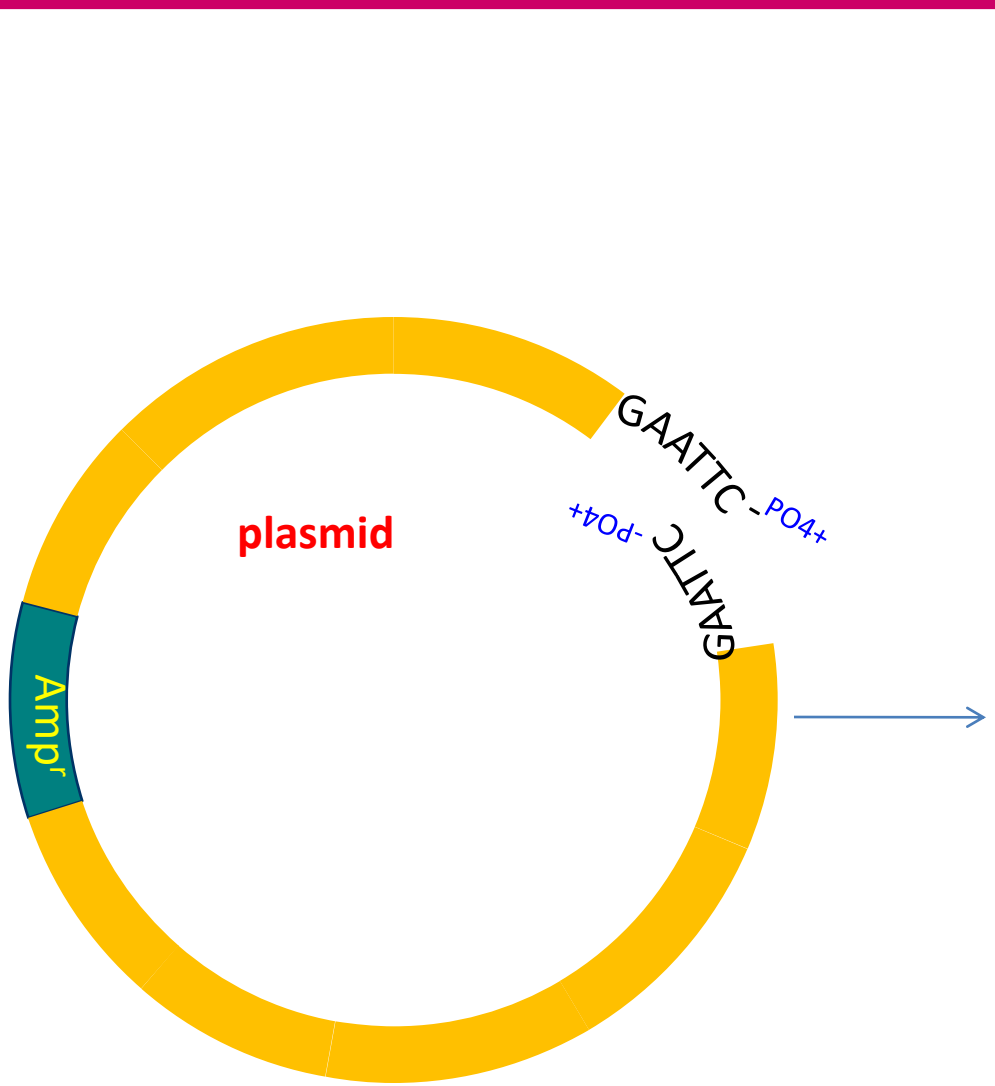
Recombinant Plasmids



Generation of Recombinant Plasmid



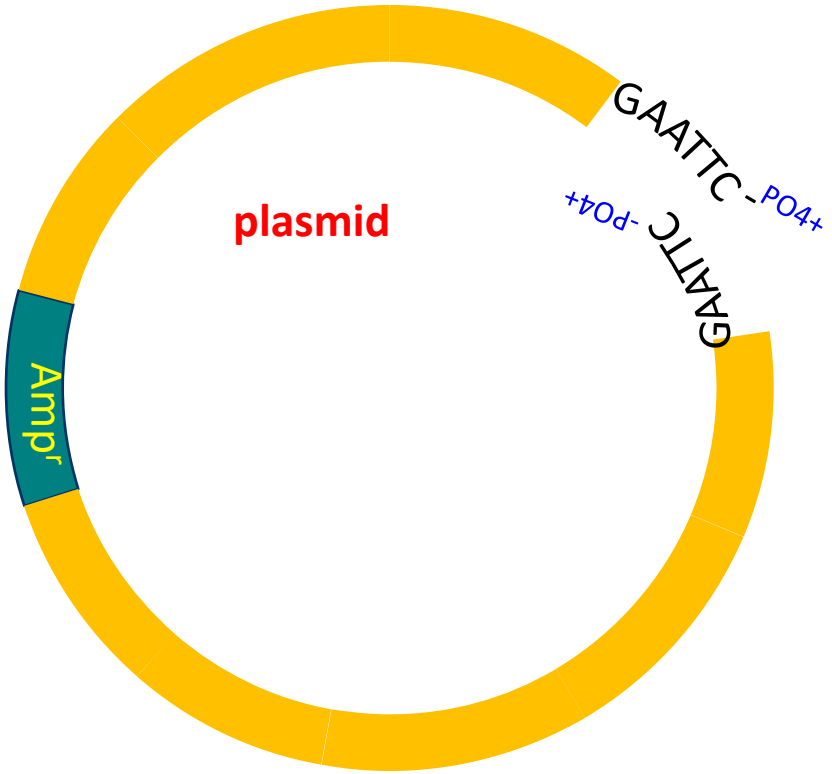
Effect of PO_4 on DNA Ligation



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

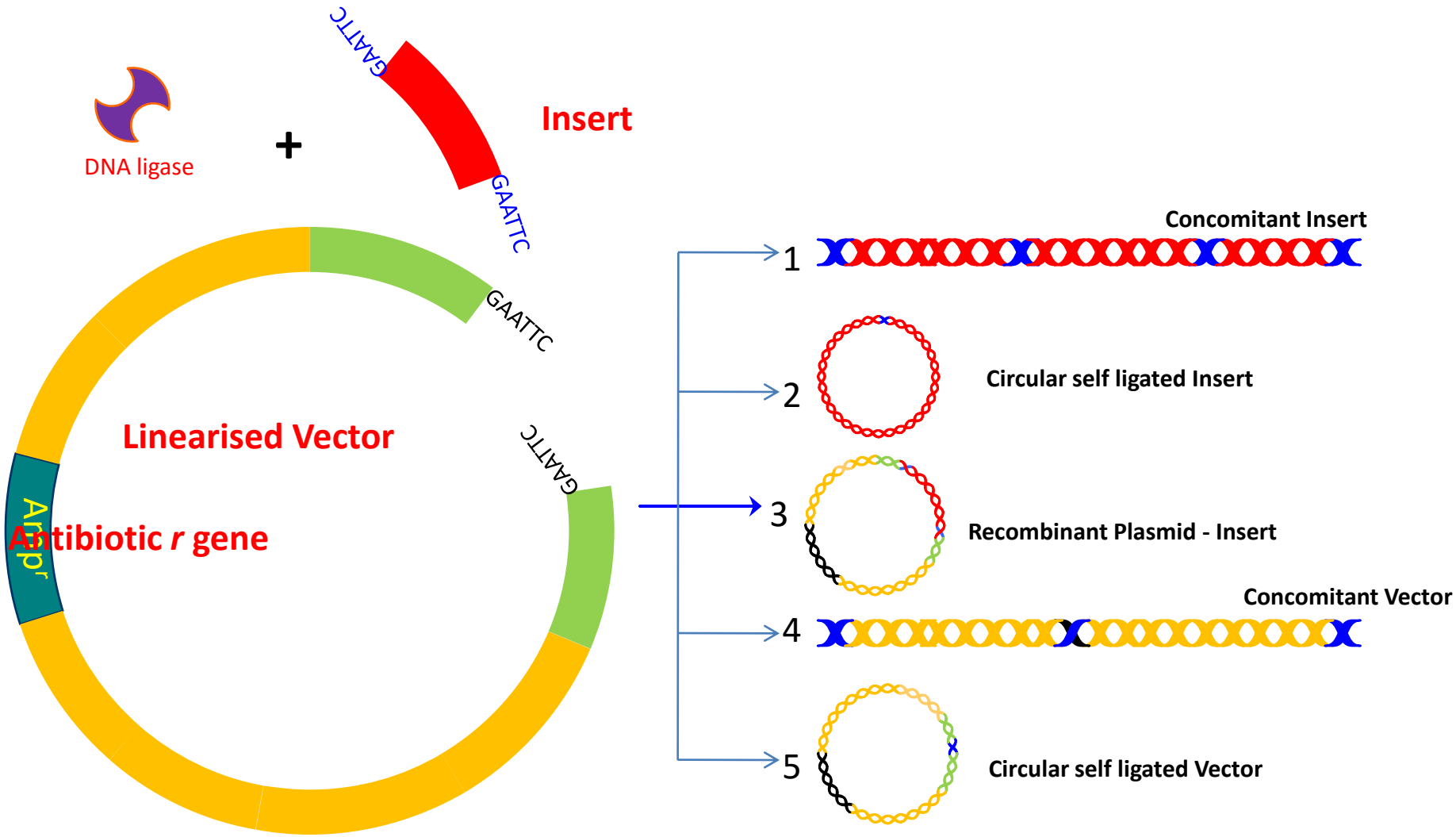
Effect of PO_4 on DNA Ligation

5’ PO_4 is must for ligation

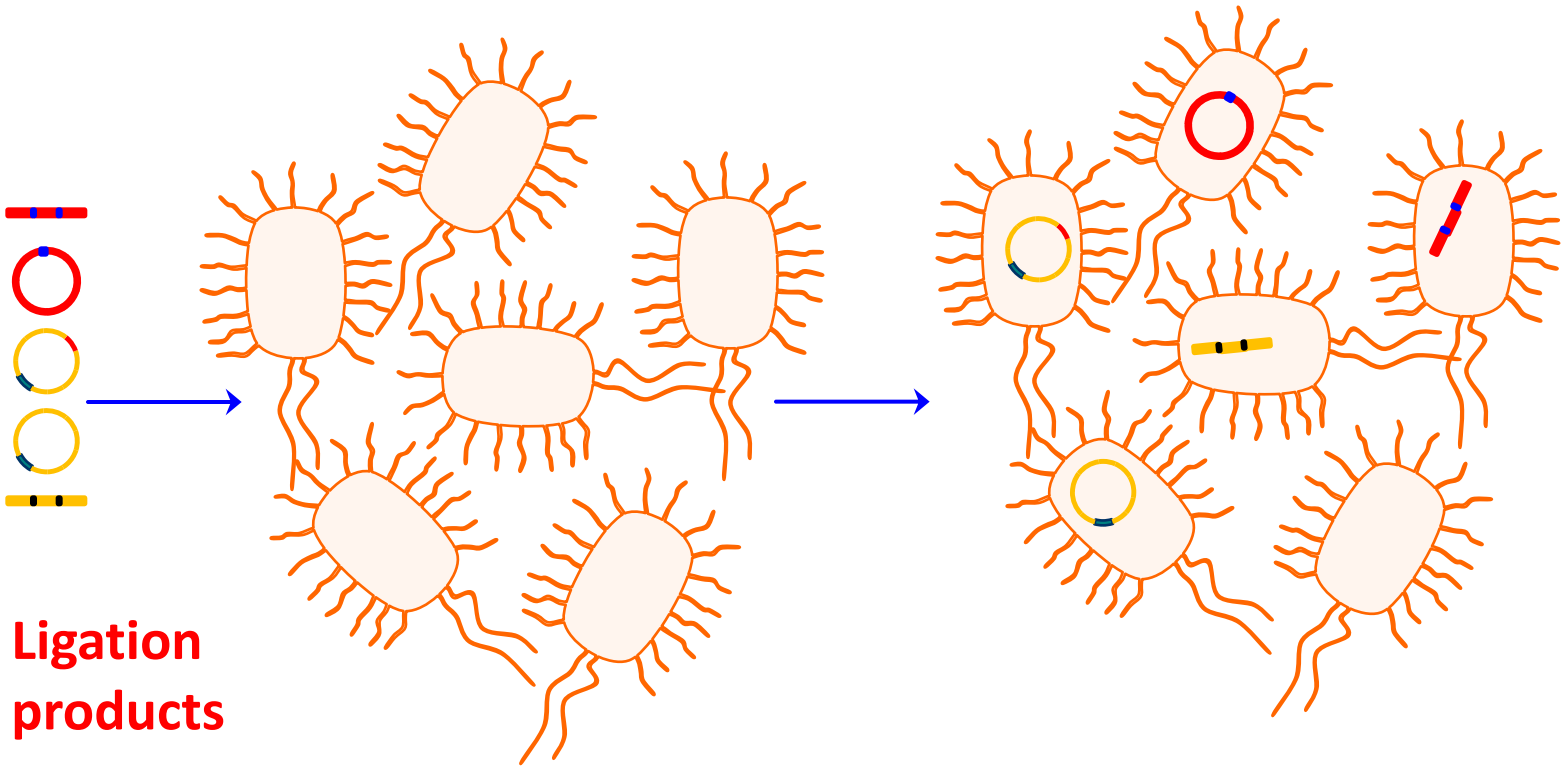


If we remove this 5’ PO_4 ligation may not taking place

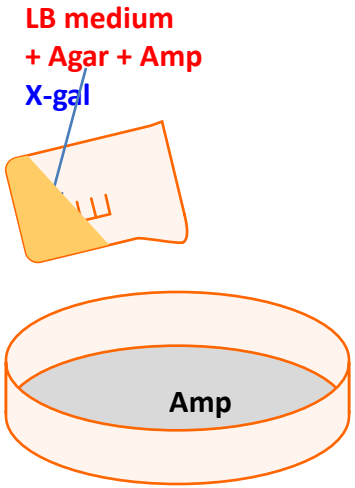
Possible Ligation Events

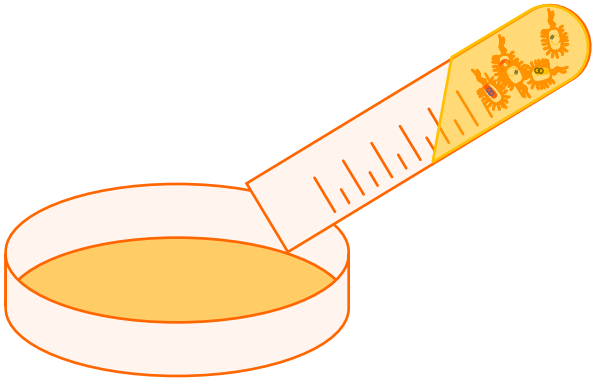


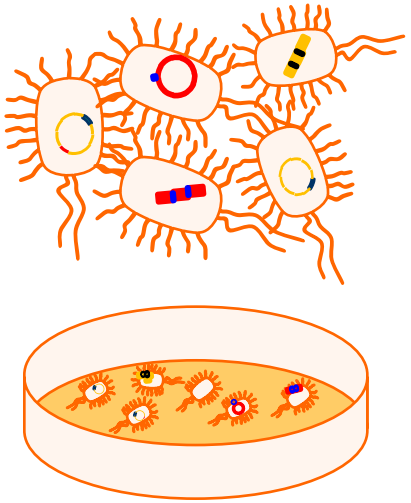
Transformation of Recombinant plasmid into *E. coli*



Agar Plate Preparation for Screening







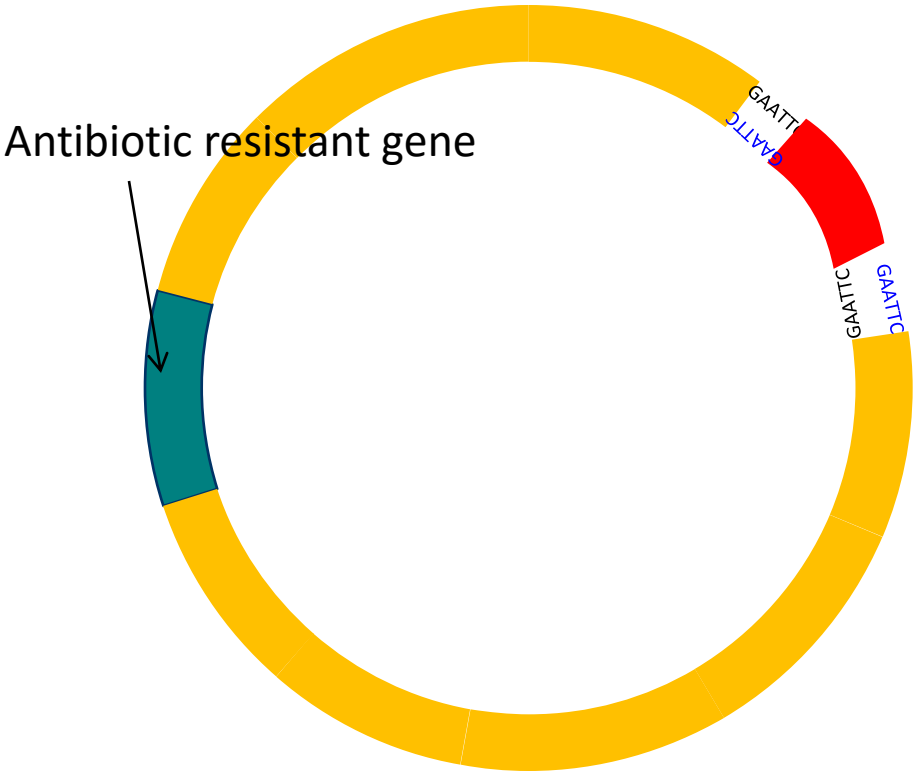
Incubated @ 37°C
overnight

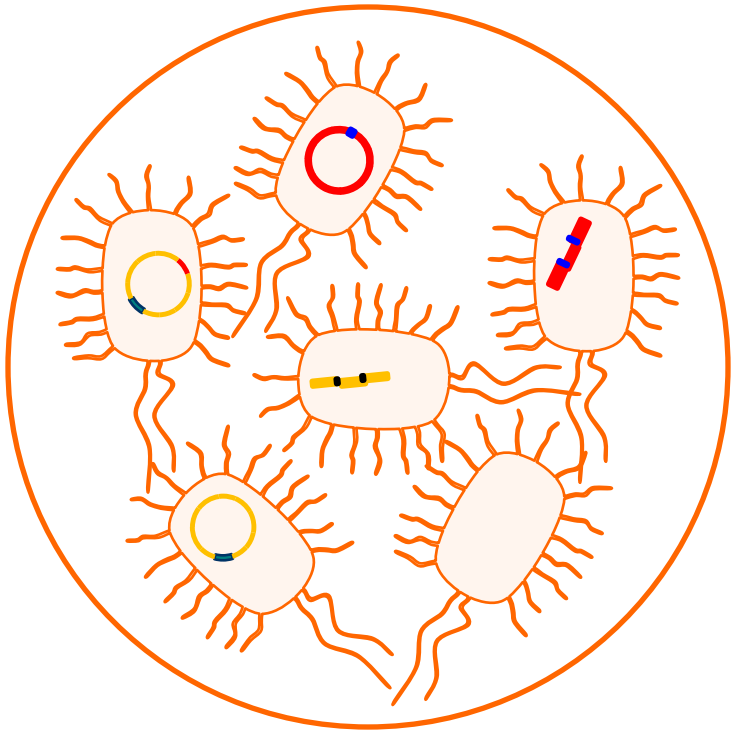
Preparation of Agar Plates

Transformed *E.coli* are plated containing Antibiotic drug

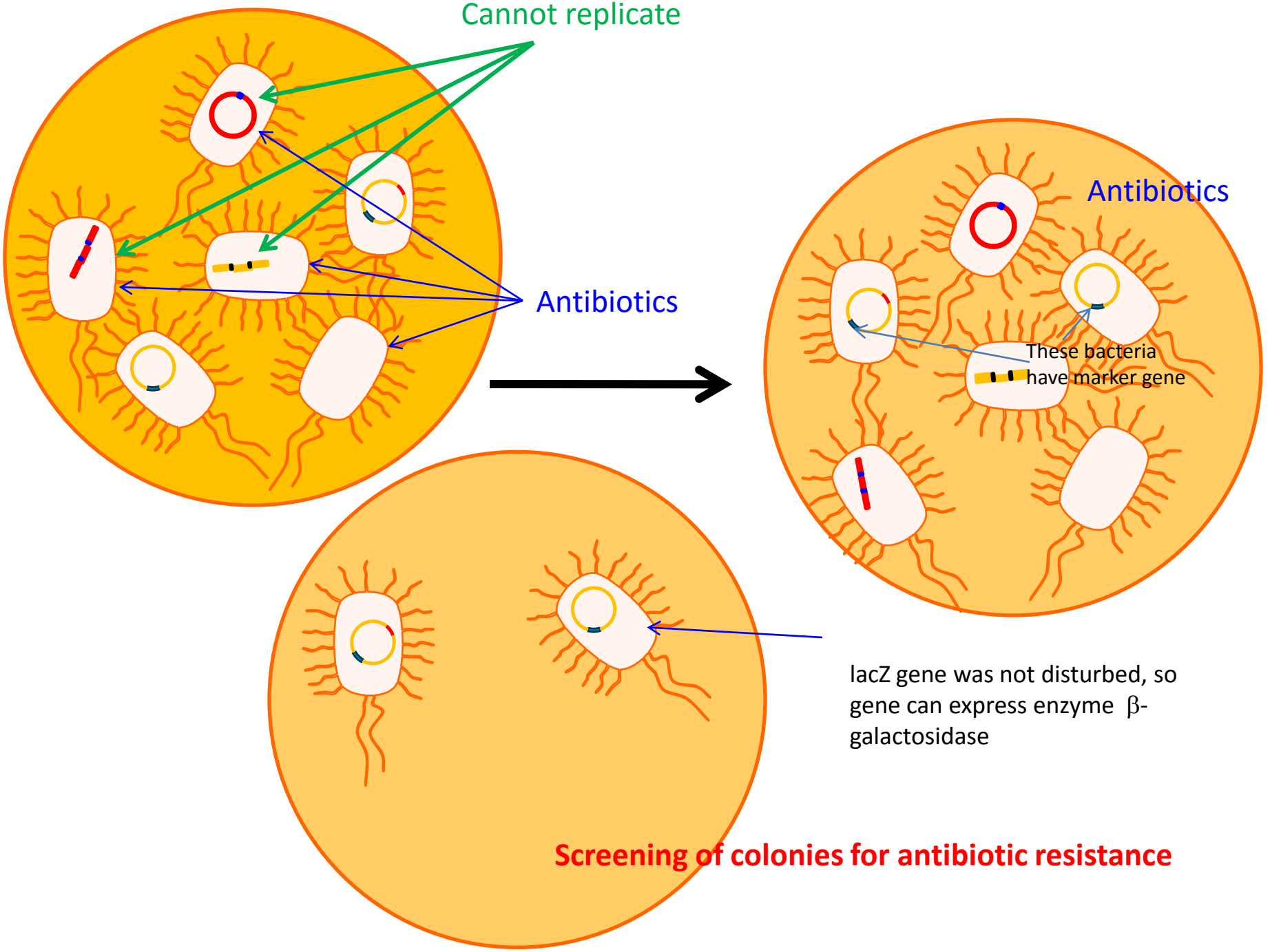
Antibiotic Drug will kill Bacteria except bacterium Bearing plasmid

Selection of transformed Bacterium by antibiotics

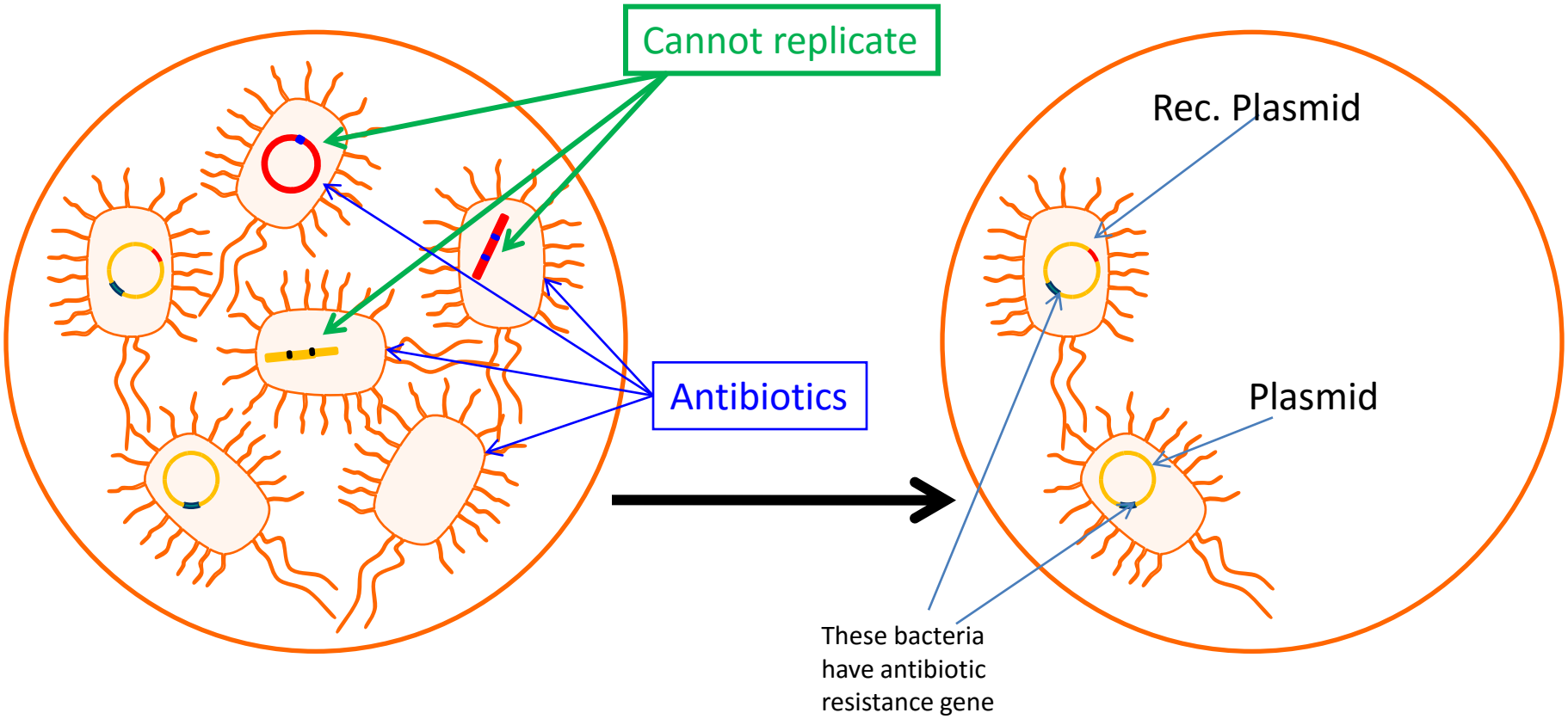




Antibiotic Drug will kill Bacteria except bacterium Bearing plasmid

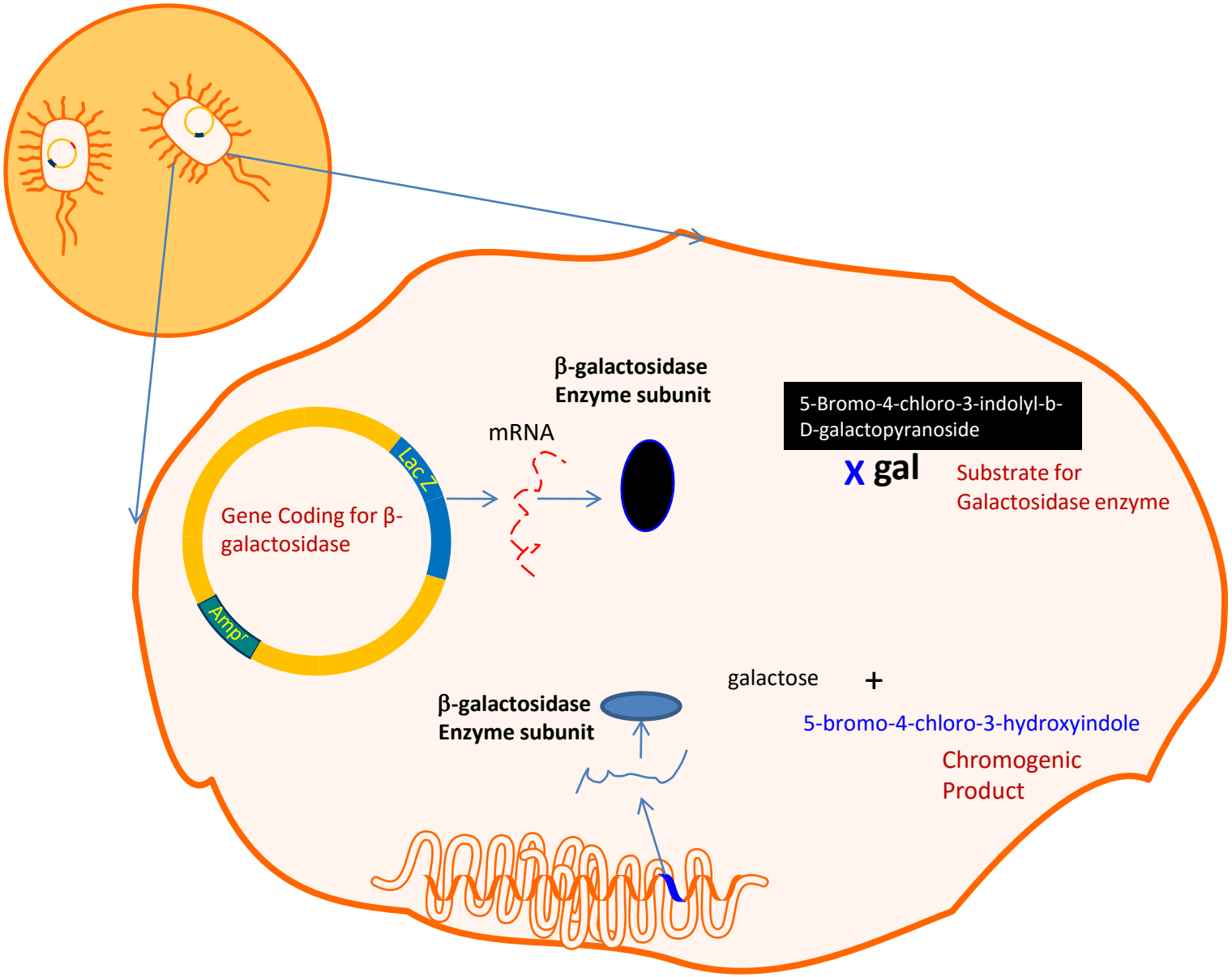

Cannot replicate
Antibiotics
Antibiotics
These bacteria
have marker gene
lacZ gene was not disturbed, so
gene can express enzyme β-
galactosidase
Screening of colonies for antibiotic resistance

Selection of transformed Bacterium by antibiotics

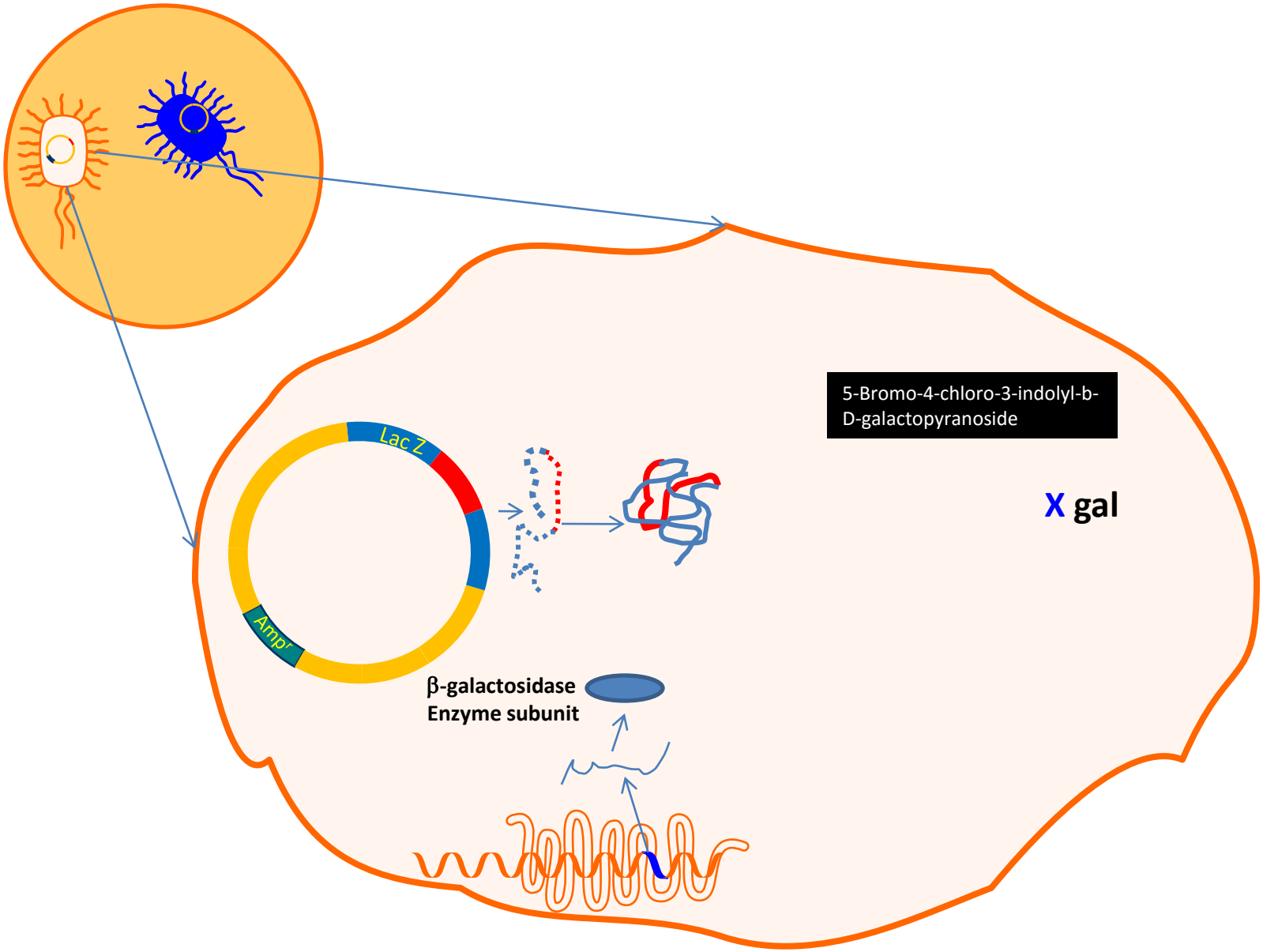


Only Antibiotic resistance bactrial colonies will grow in LB-agar containing antibiotics

Mechanism of Blue-White selection



Mechanism of Blue-White selection



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The End

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