

Why different copy numbers?

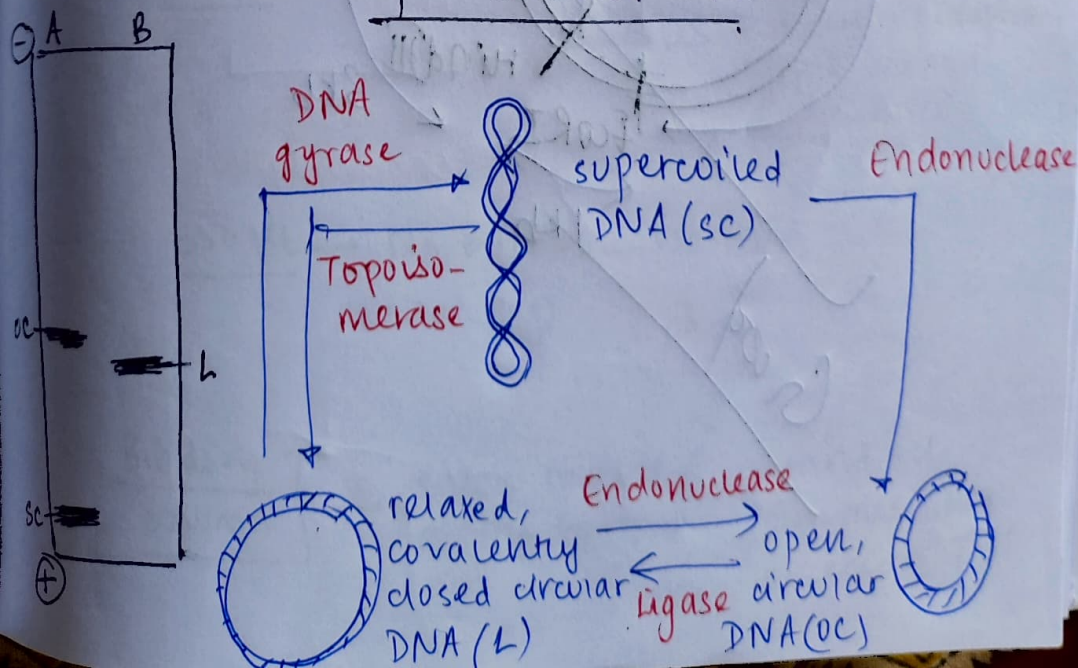
↳ to produce more plasmids & hence more antibiotic resistance genes → better survival rate.

Plasmid curing:

↳ antibiotic resistance gene present → but no antibiotic selection pressure present in media

slowly plasmids disappear from these population

Interconversion of supercoiled, relaxed covalently closed circular DNA and open circular DNA



EcoRI HindIII

10 ———

HindIII +
EcoRI

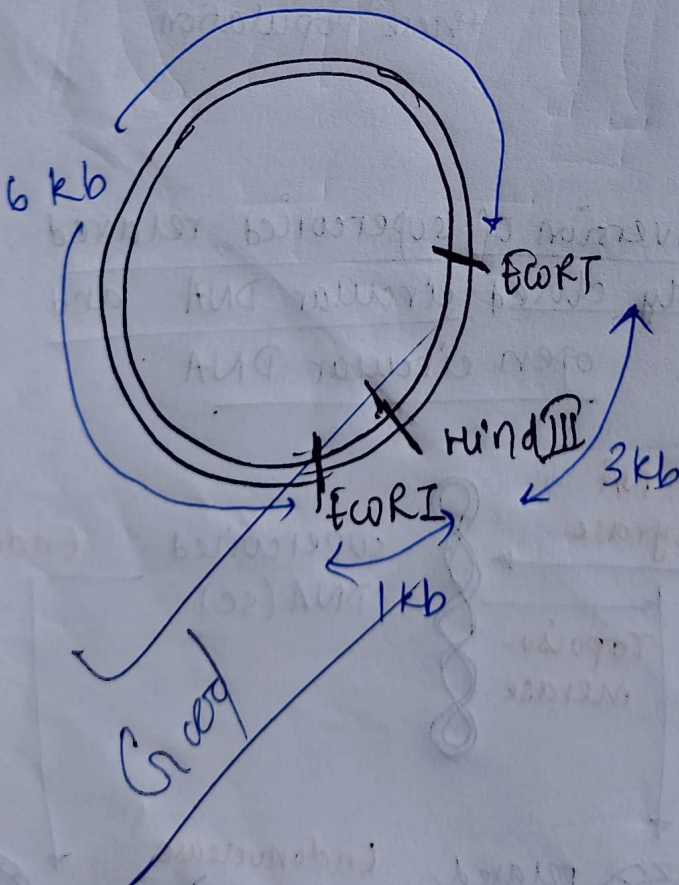
6 ———

6 ———

4 ———

3 ———

1 ———



21/8/23

Plasmid DNA Isolation:

↳ remove chromosomal DNA → remove RNA

E. coli DH5α

↳ lack some nuclease → less chances of nucleic acid damage

BN21

↳ lack some protease → protein purification

2 mL of overnight culture

Alkali lysis

250 μL → P1 Buffer ← Suspension Buffer
RNase

250 μL → P2 Buffer ← NaOH ✓ denature DNA & protein
SDS ✓ dissolve phospholipid being a detergent
pH ↑

350 μL → N3 Buffer — Na-OAc
(Renaturing) pH ↓

Binding column

silica matrix at the bottom

bind the plasmid DNA at acidic pH

PE Buffer - 70% ethanol
- wash

EB buffer - brings the DNA
in the solution

PB Guanine solⁿ
protein-solubilizing agent

28/8/23

Vector

Eg: Plasmid

Expression Vector

you can express the gene of interest

Requirements:

- Promoter sequence upstream of GOI
- RBS

Maintenance Vector

only to store the gene of interest

the GOI can be transferred to an expression vector

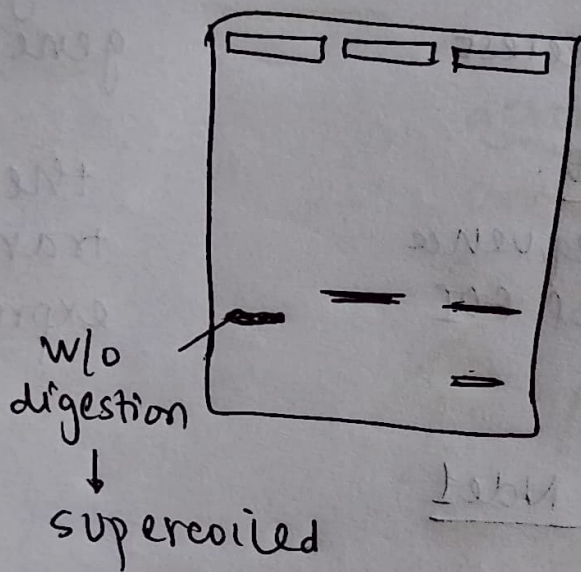
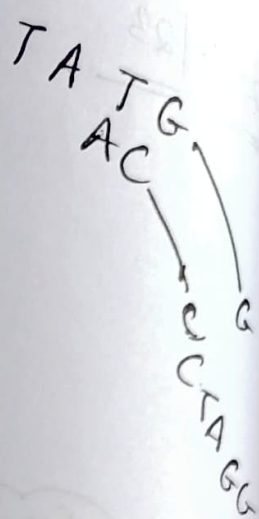
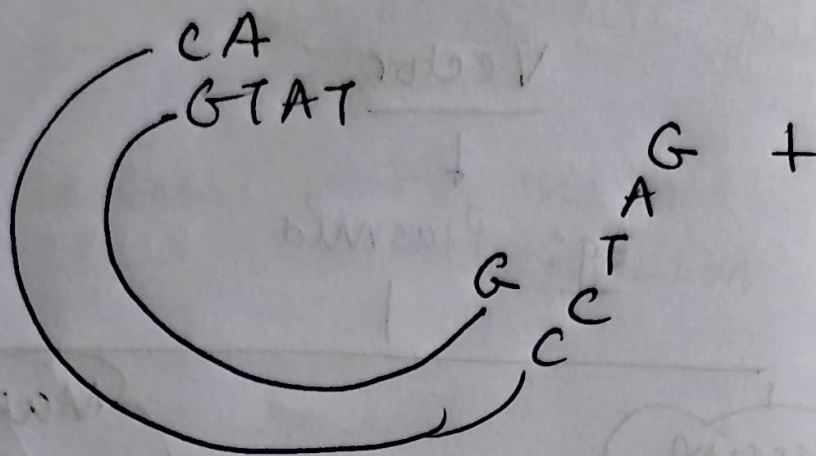
NdeI

CATATG
GTATAC

BamHI

GGATCC
CCTAGG

Plasmid
Digestion

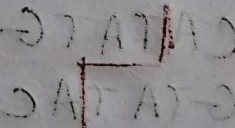


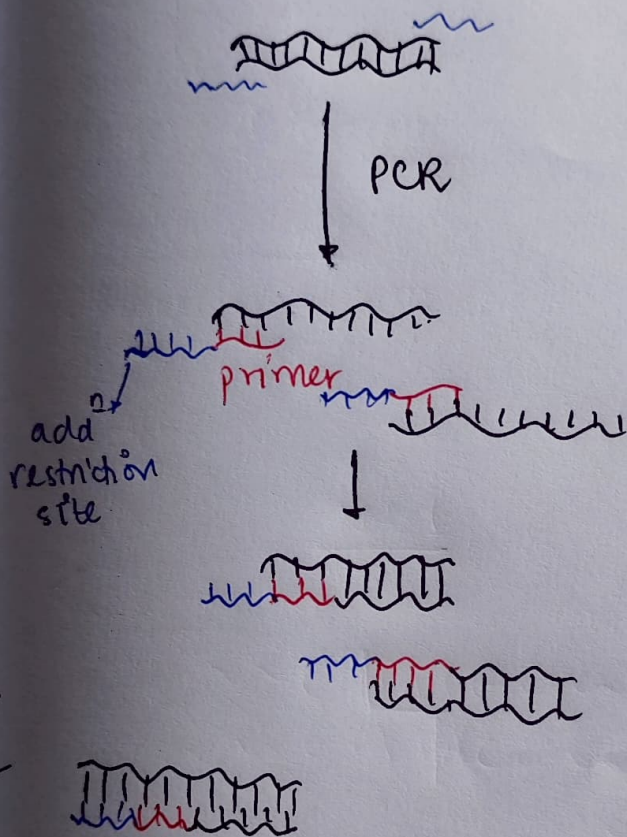
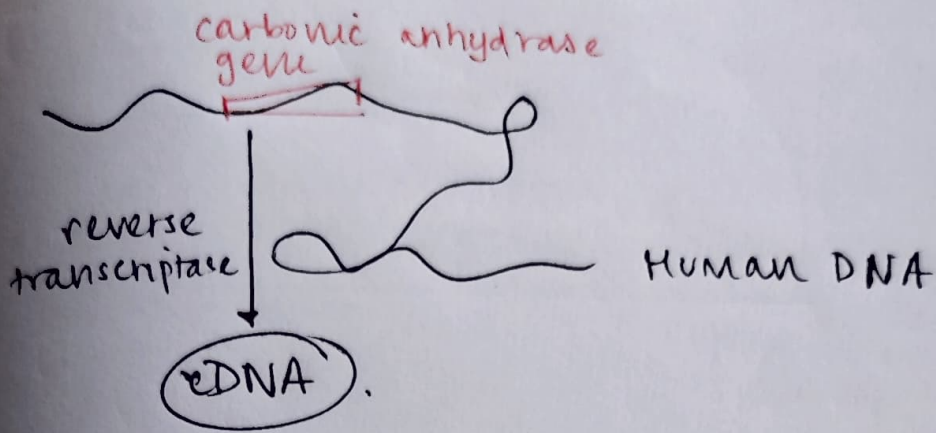
sub-cloning

plasmid

GOI

plasmid





A549

alveolar epithelial cell line ^{cancer}

mRNA extraction:

