Why different copy numbers?

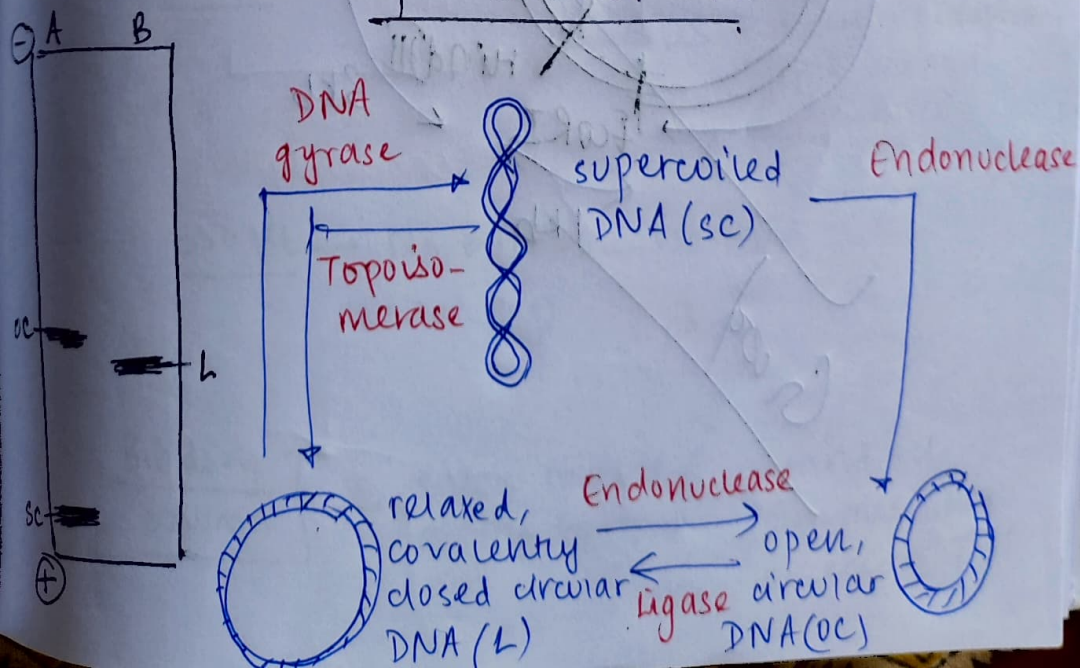
↳ to produce more plasmids & hence more antibiotic resistance genes → better survival rate.

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 HindIII + EcoRI

10

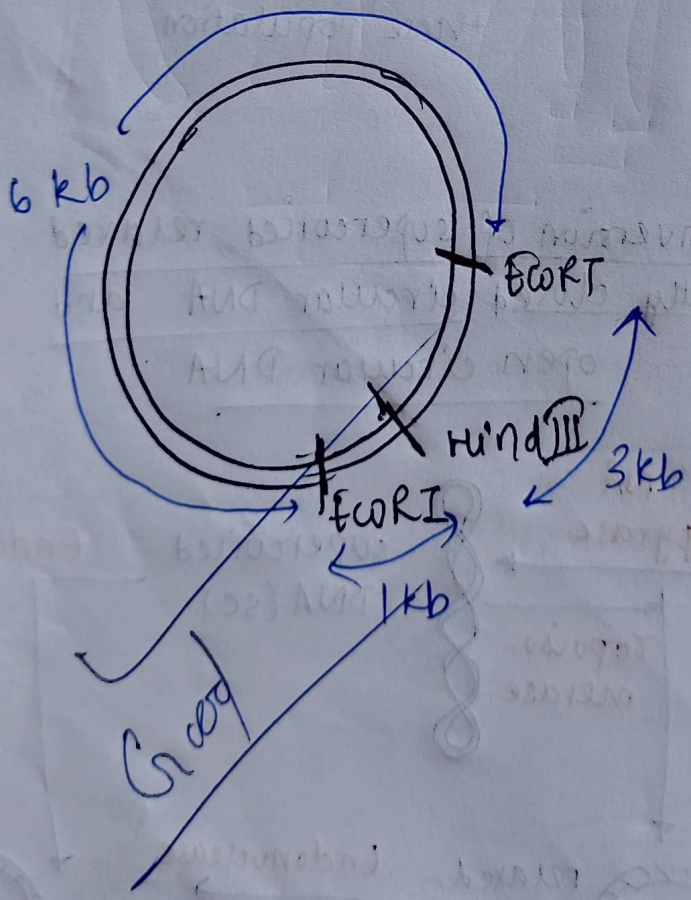
6

6

4

3

1



24/8/23

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

|

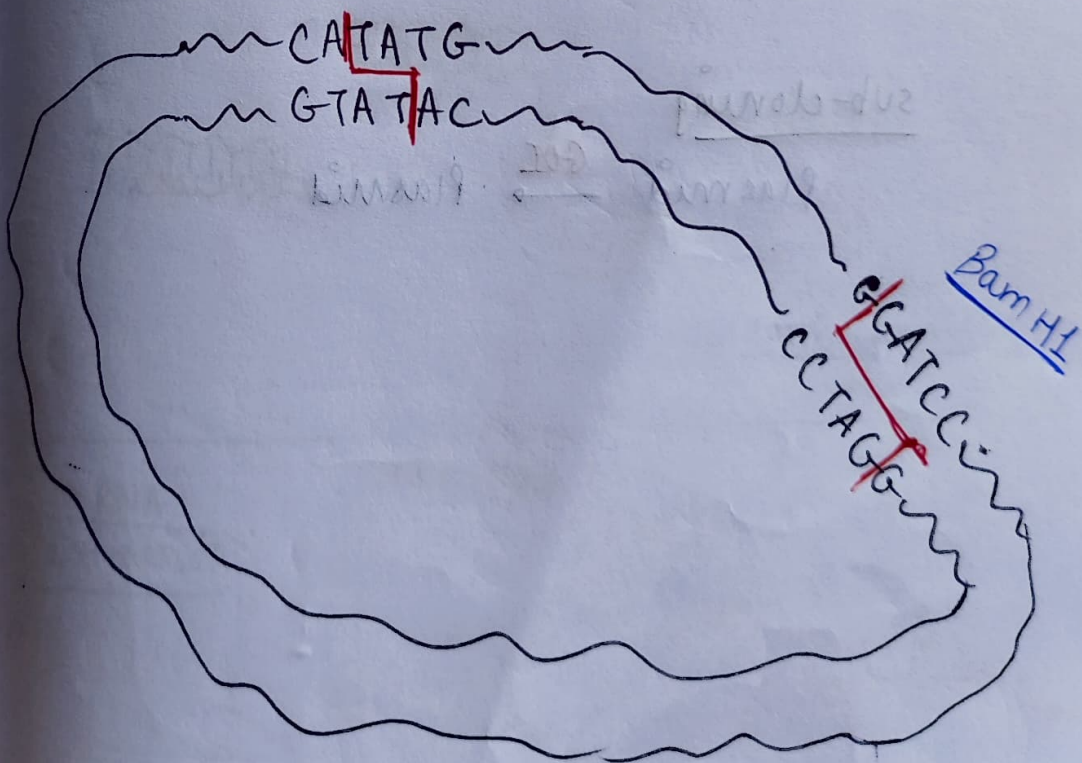
Expression Vector → you can express the gene of interest

Maintenance Vector → only to store the gene of interest → the GOI can be transferred to an expression vector

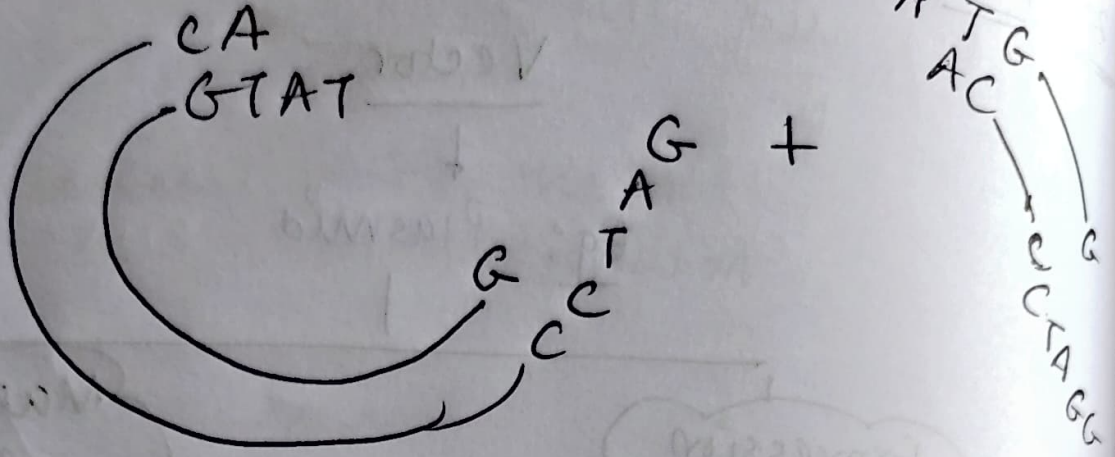
Requirements:

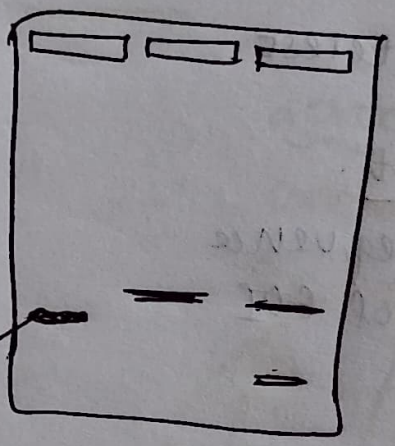
- Promoter sequence upstream of GOI
- RBS

Nde1



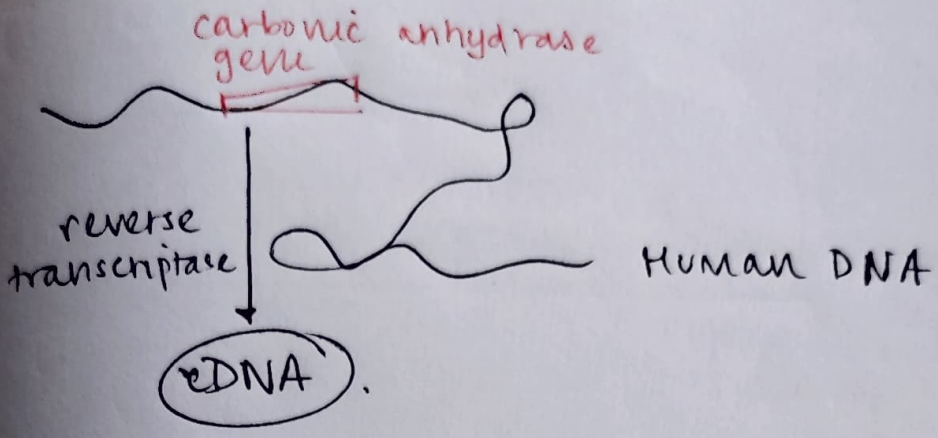
Plasmid Digestion

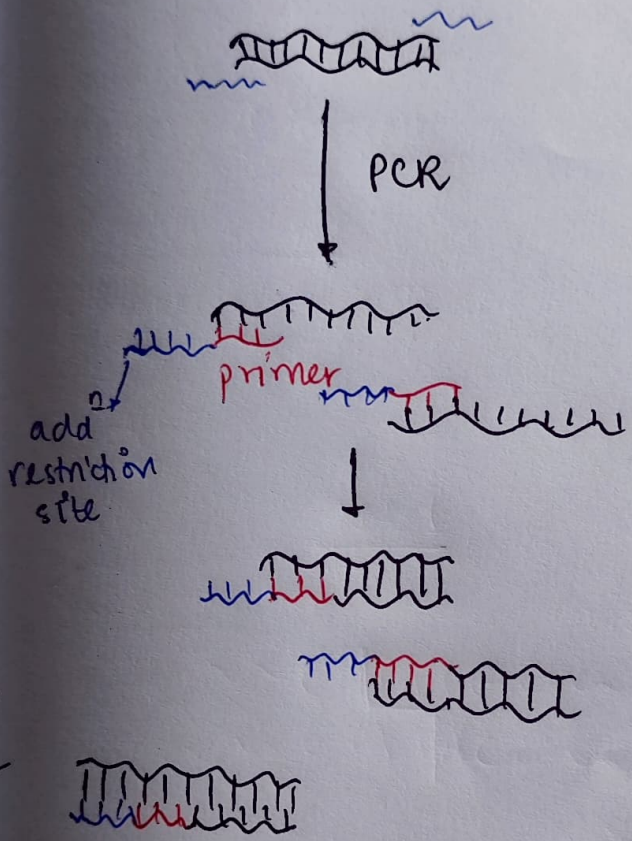




sub-cloning

Plasmid —GOI→ Plasmid





A549

alveolar epithelial cancer cell line

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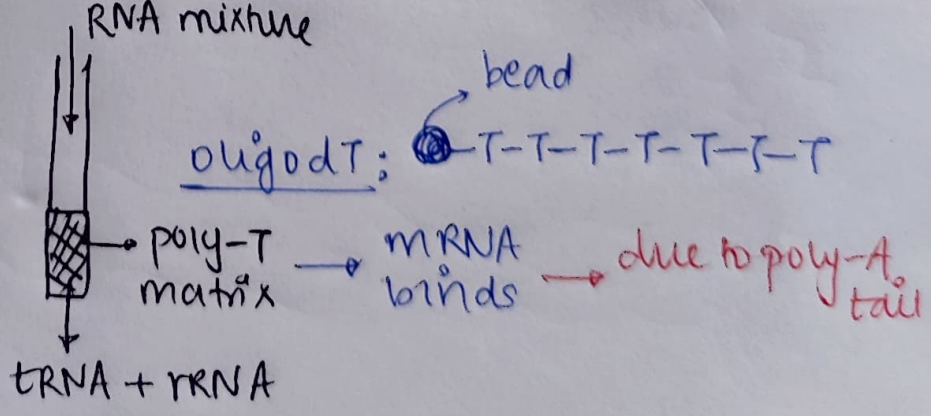
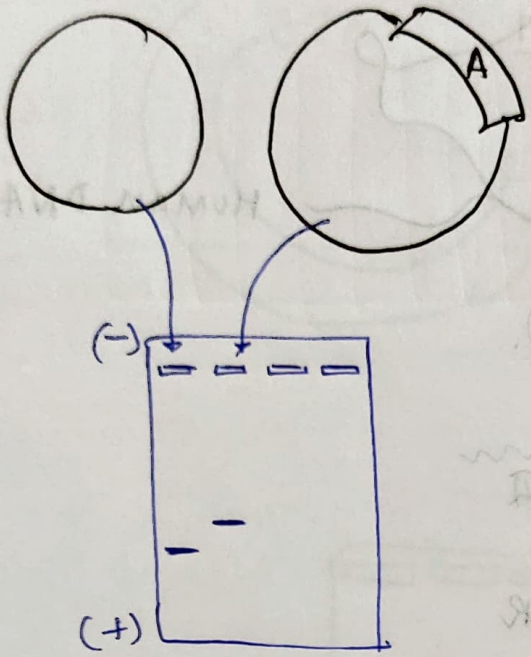
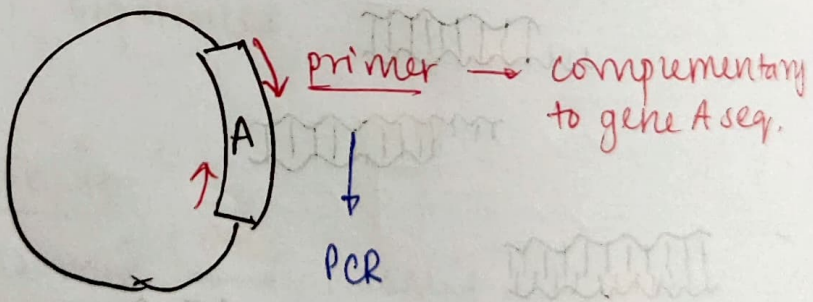
mRNA extraction :



Confirming insertion of GOI



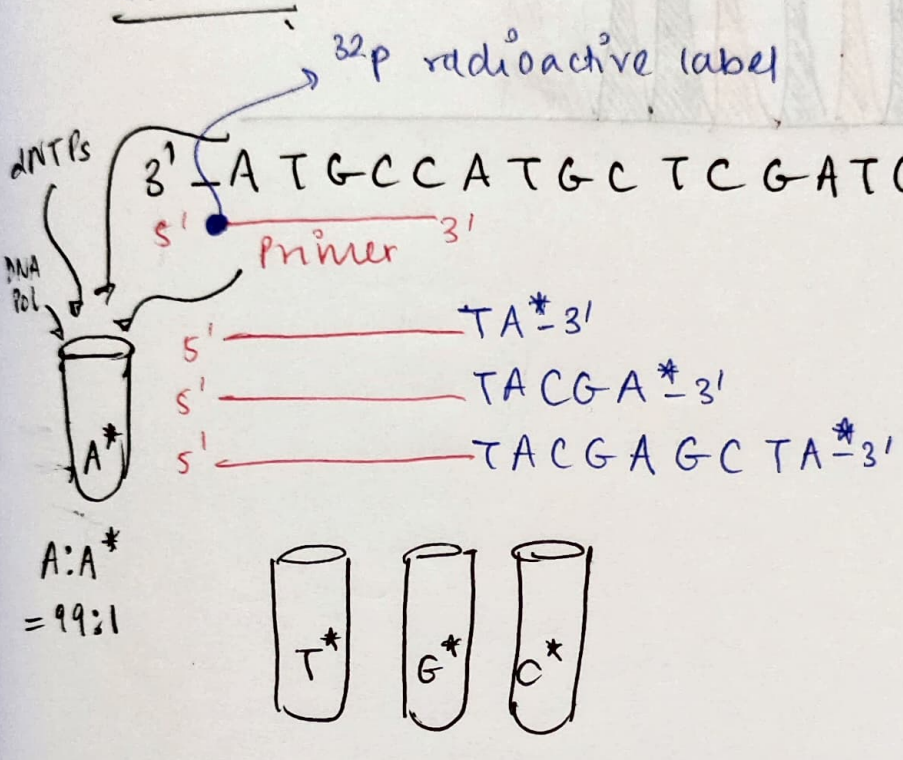
But what if the fragment we discard is of same length as the GOI?



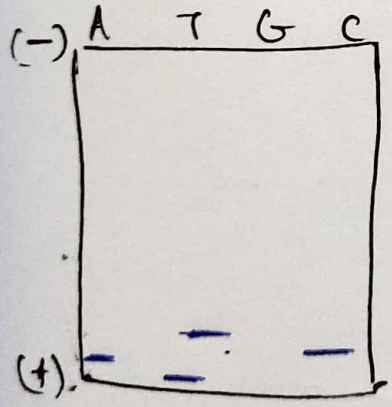
DNA Sequencing

Sanger sequencing

Classical:

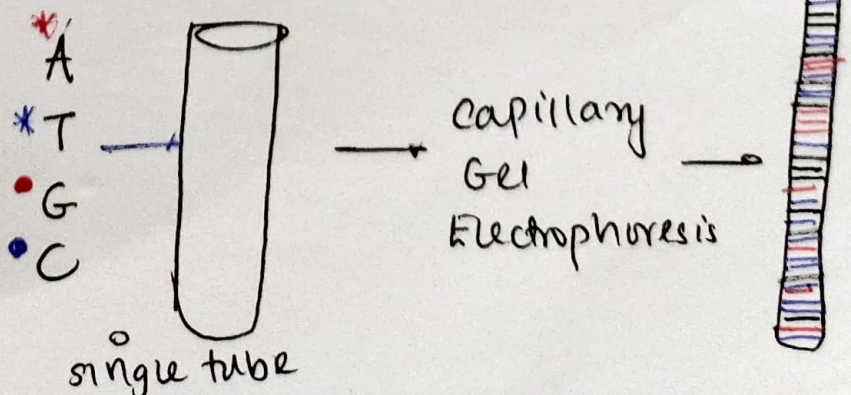


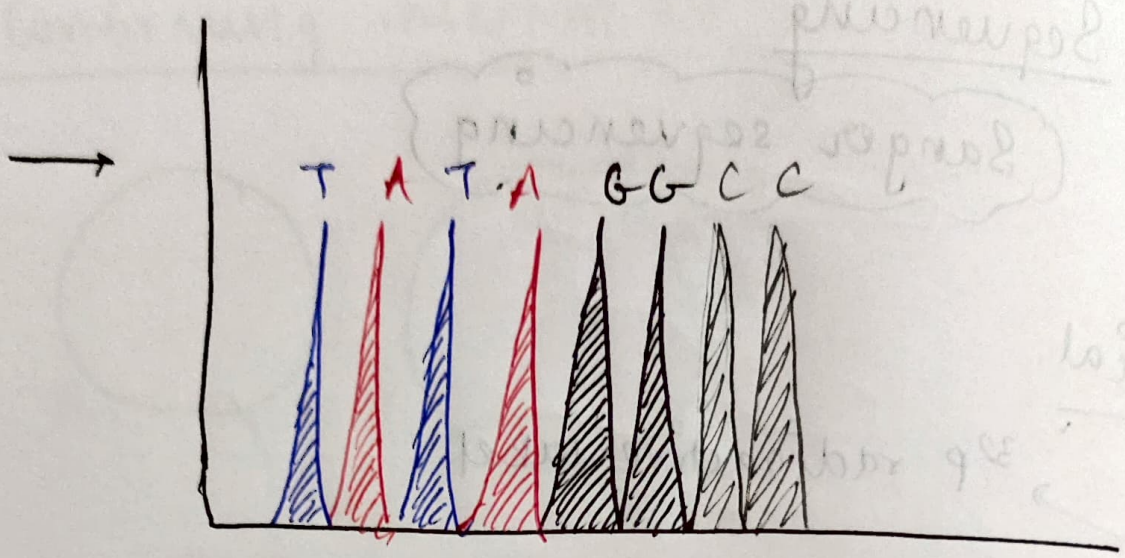
[ddNTP – dideoxyribonucleotide triphosphate]



→ use polyacrylamide gel

Nowadays:




T A T A G G C C