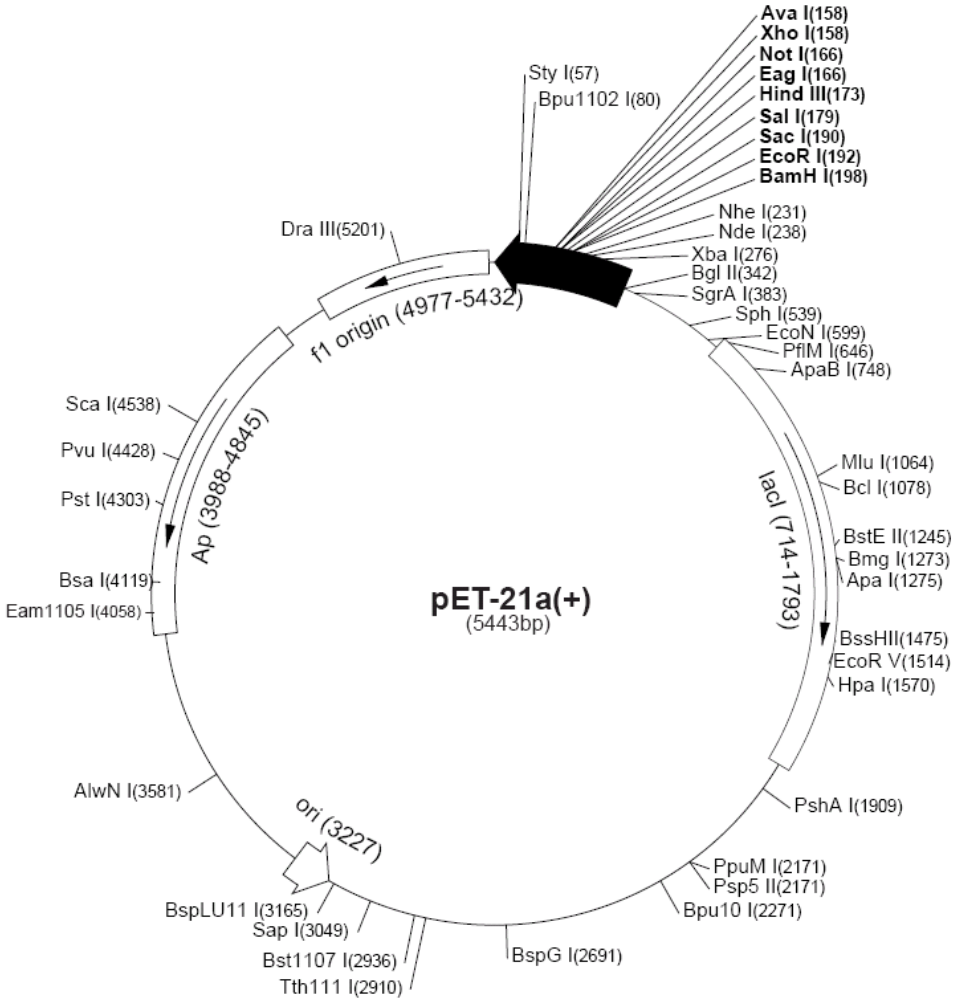
Biochemistry Experiment 2

Vector

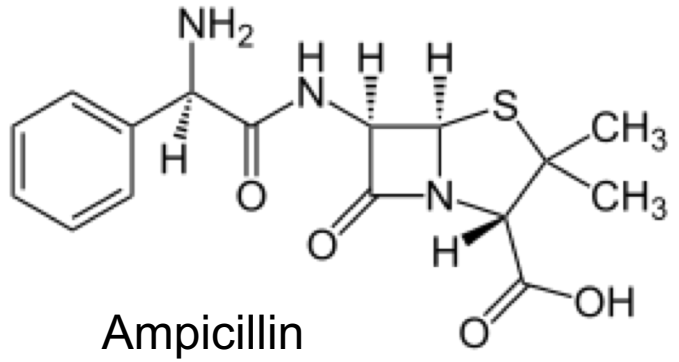
A vector is a DNA molecule used as a vehicle to transfer foreign genetic material into another cell



- **origin of replication**
  particular sequence in a genome at which replication is initiated

- **multiple cloning site**
  polylinker, is a short segment of DNA which contains many (up to ~20) restriction sites

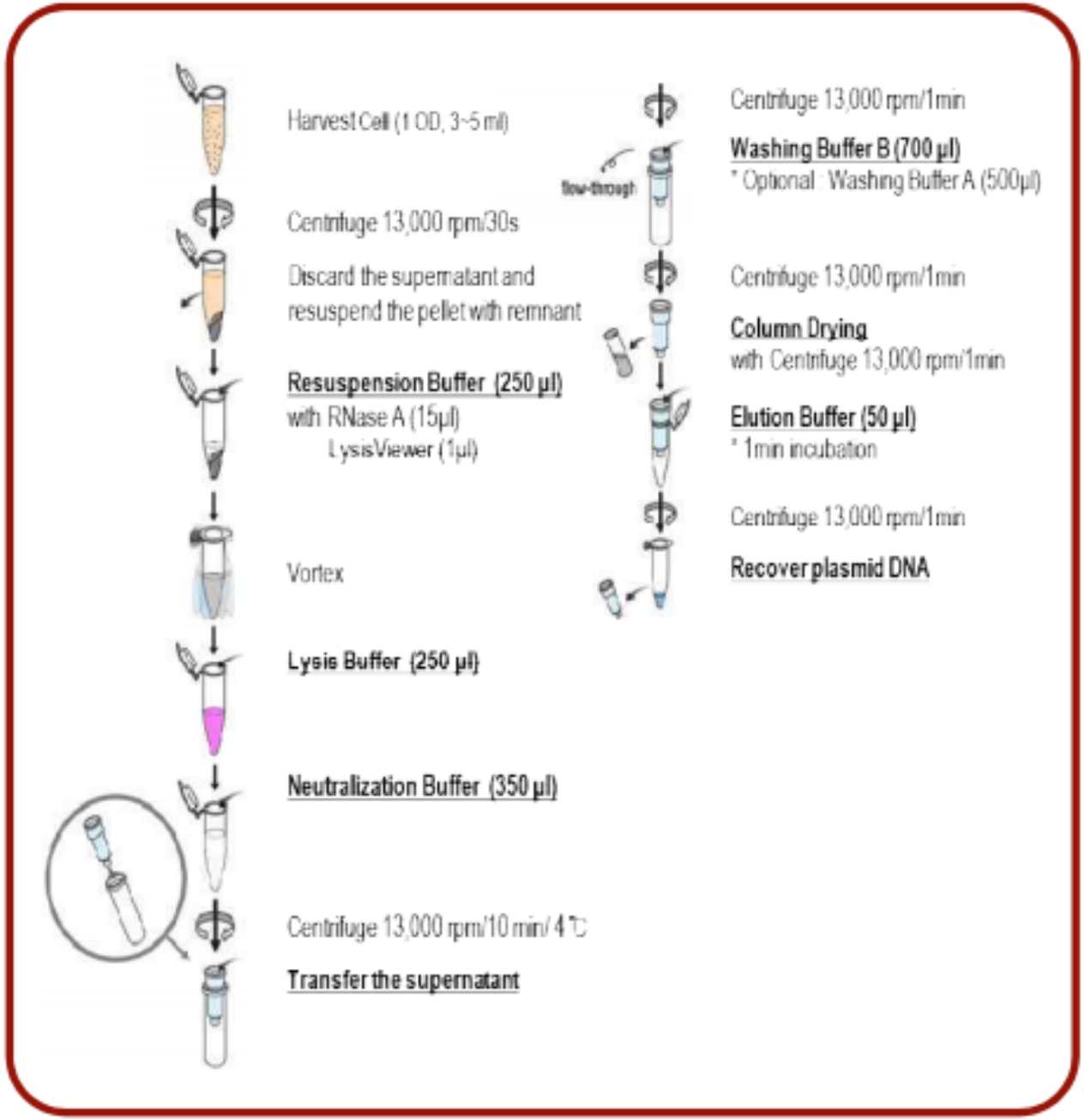
- **selectable marker**



Ampicillin

Mini-prep

- Buffer 1 : Glucose, Tris-Cl(pH 8.0), EDTA, Rnase A
  세포벽 깨기, 삼투압 유지(세포 외형 유지), RNA 분해
- Buffer 2 : NaOH, SDS(ionic detergent)
  세포막 깨기, DNA denaturation
- Buffer 3 : Potassium acetate, glacial acetic acid
  Renaturation(pH7로 복구).
- Buffer 4 : 70% EtOH
  남은 salt의 제거
- Buffer 5 : Nuclease-free water (NFW)
  Elution solution



Mini-prep protocol

1. Pick up a single colony from fresh cultured LB agar plate(contains antibiotics) and inoculate the cell into the 1-5ml of fresh LB liquid media(contains antibiotics) at 37℃ with shaking for 12-16hr
2. Harvest 3 – 5 ml of bacteria culture by centrifugation at 13,000 rpm for 10 sec at RT and discard supernatant
3. Resuspend peleted bacterial cell thoroughly in **250 µl of Buffer 1** by vortexing until no clumps remain. (Buffer1은 ice에서 유지)
4. Add **250 µl of Buffer 2** to resuspended cells and mix by inverting the tube 10 times. DO NOT VORTEX and incubate for 3 min at RT.
5. Add **350 µl of Buffer 3** and gently mix by inverting the tube 10 times.
6. Centrifuge at 13,000 rpm for 10 min. While waiting for the centrifugation, insert a column into collection tube.
7. After centrifugation, transfer **supernatant** promptly into the column. Wait for 1min.
8. Centrifuge at 13,000 rpm for 15 sec. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.
9. Add 700 µl of **Buffer 4**, centrifuge at 13,000 rpm for 15 sec. Discard filtrate in the collection tube and place the spin column back in the same collection tube.
10. Add 500 µl of **Buffer 4**, centrifuge at 13,000 rpm for 1 min. Discard filtrate in the collection tube and place the spin column in the new collection tube.
11. Centrifugation the tube at 13000rpm, for 4 min.
12. Put the column into a clean, **Ep tube**. Add **30 µl** of NFW. Centrifuge at **1600 rpm** for 2.5 min.
13. Centrifugation the tube at 13000rpm, for 1min

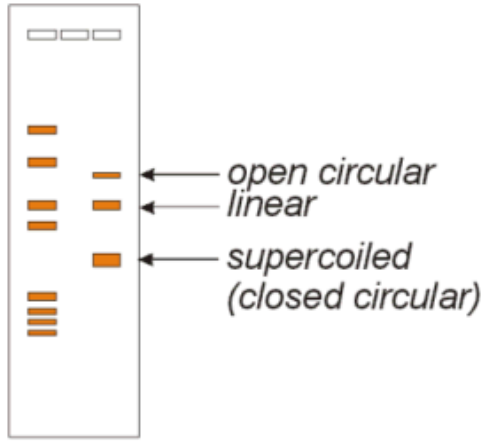
Restriction Enzyme

- **Restriction Enzyme**

  - Sequence-specific endonuclease

  - enzyme that cuts DNA at recognition site sequence(Palindrome)

<Plasmid DNA migration pattern>



ex)

EcoR I

"Sticky or cohesive end"

E *Escherichia* (genus) **co** *coli* (species) **R** RY13 (strain) **I** First identified

Sma I

"Blunt end"

S *Serratia* **ma** *marcescens* **I**

- **1unit :** 1ug DNA를 50ul 반응 용액에서 최적온도, 최적완충용액 조건에서 1시간만에 자를 수 있는 효소량

  (star acitivity, enzyme activity 고려)

Experiment

-Restriction enzyme digestion

Restriction enzyme should be on the Ice

1. Make follow reaction mixture and incubate at 37 ℃ for 2hr.

	Vector (mini-prep)	PCR product
DNA	16 ul	16 ul
Restriction buffer IV	2 ul	2 ul
Xho1 (20U/μL)	1 ul	1 ul
EcoR1 (20U/μL)	1 ul	1 ul
Total	**20 ul**	**20 ul**

** Well mixing by tapping & spin-down