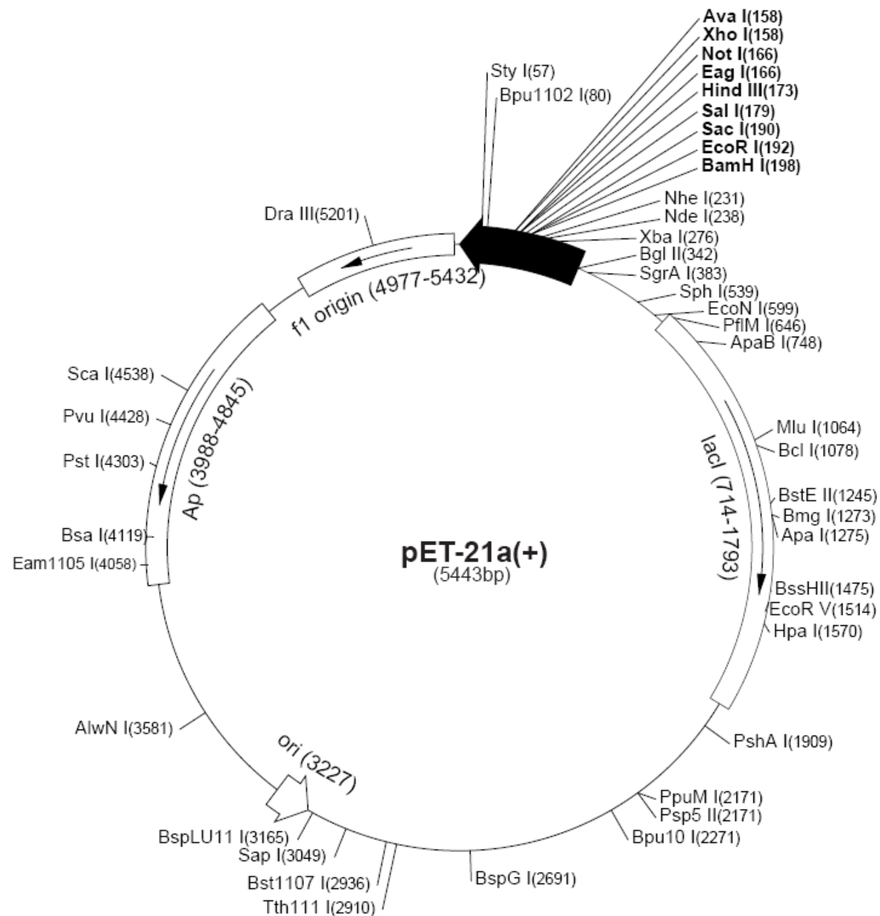
Exp2.
Plasmid Prep and Restriction

Vector

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



- Origin of replication (ori)

: particular sequence in a genome at which replication is initiated

- Multiple cloning site (MCS)

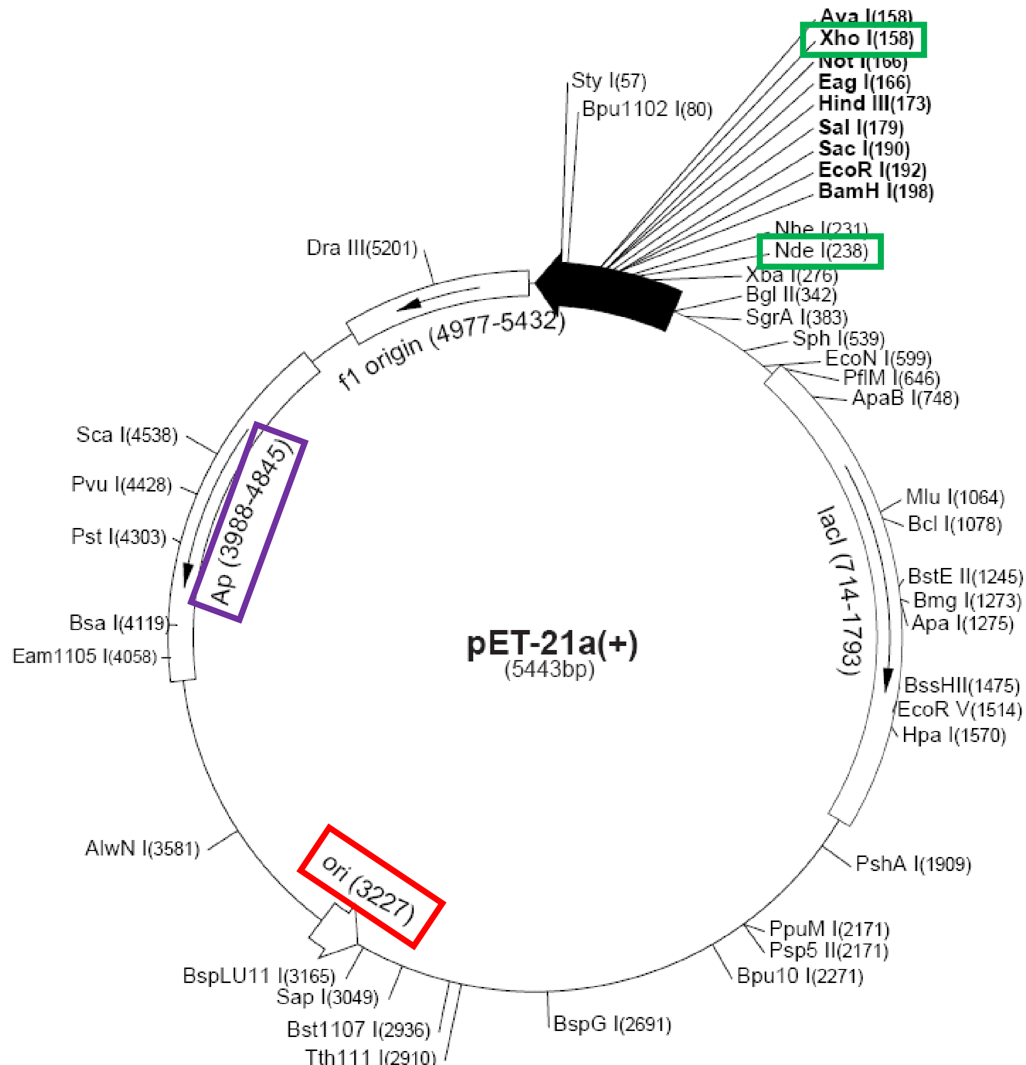
: polylinker, is a short segment of DNA which contains many (up to ~20) restriction sites

- Selectable marker

: a gene introduced into a cell that confers a trait suitable for artificial selection

: often antibiotics resistance genes

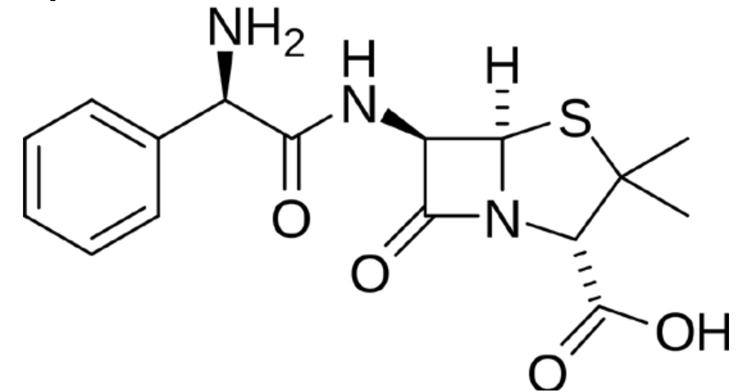
Vector : pET-21a



- Origin of replication (ori)
- Multiple cloning site (MCS)

  : Nde I , Xho I
- Selectable marker

  : Ampicillin (AmpR)



Mini-prep

- A method of plasmid DNA extraction and purification from bacteria

- Buffer S1 (Glucose, Tris, EDTA, RNase A)
  : open the cell wall, RNA degradation
- Buffer S2 (NaOH, SDS)
  : open the cell wall, denatures chromosomal DNA and proteins, and releases plasmid DNA into the supernatant.
- Buffer G3 (Potassium acetate)
  : renaturation, precipitates chromosomal DNA and protein
- Buffer PW (EtOH)
  : removes salts and other cellular components
- Buffer EB
  : plasmid elution

Mini-prep protocol

① Pick up a single colony from fresh cultured LB agar plate (contains antibiotics) and inoculate the cell into the 5mL of fresh LB liquid media (contains antibiotics) at 37′C with shaking for 12~16hr.

- Antibiotics : ampicillin (Amp)

② Transfer 1mL of bacteria culture to 1.5mL tube and centrifuge at 10,000rpm for 5 min at RT. Discard supernatant using pipette.

- Labeling is needed. (Name or student number etc)

③ Resuspend palleted bacteria cell thoroughly in 250uL of buffer S1 by pipetting until no clumps remain.

- Buffer S1 contained RNase A must be stored at 4′C.

④ Add 250uL of buffer S2 and mix by inverting the tube 4 times (DO NOT VORTEX).

- Buffer S1 is strong base, do not incubate for more than 3 min.

⑤ Add 350uL of buffer G3 and immediately mix by inverting the tube 4~6 times (DO NOT VORTEX).

Mini-prep protocol

⑥ Centrifuge for 10 min.

⑦ Transfer carefully the supernatant to a spin column by pipetting. Centrifuge for 30 sec.

- Labeling is needed. (Name or student number etc)

⑧ Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.

⑨ Apply 700uL of buffer PW and centrifuge for 30 sec.

⑩ Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.

⑪ Centrifuge for an additional 1 min to remove the residual wash buffer.

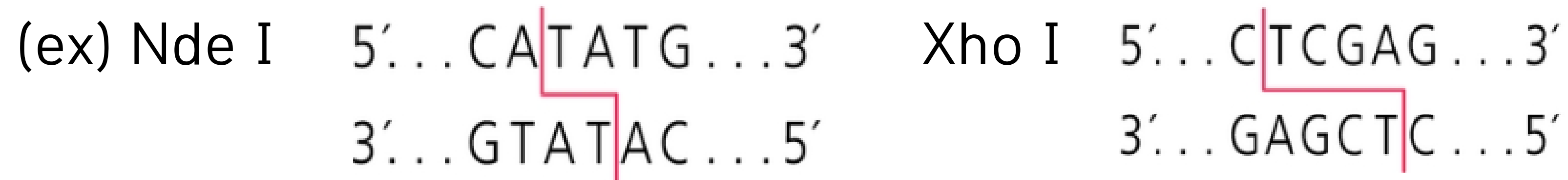
⑫ Transfer the spin column to a new 1.5mL microcentrifuge tube.

- Labeling is needed. (Name or student number etc)

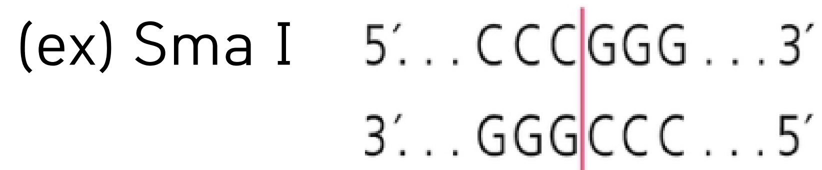
⑬ Add 50uL of buffer EB, let stand for 1 min, and centrifuge for 1 min.

Restriction Enzyme (RE)

- Sequence-specific endonuclease
- Enzyme that cuts DNA at recognition site sequence (Palindrome)
  - Sticky end (cohesive end)



  - Blunt end



- 1 units
  - The amount of enzyme that can digest 1ug of DNA (50uL reaction volume) in 1 hr with optimal buffer condition

RE digestion protocol

① Make follow reaction mixture.

- RE should be on ice.
- Well mixing by tapping and spin-down.

	Vector (mini-prep)	PCR product
DNA	16 uL	16 uL
Restriction buffer Ⅳ	2 uL	2 uL
Xho Ⅰ (20U/uL)	1 uL	1 uL
Nde Ⅰ (20U/uL)	1 uL	1 uL
Total vol.	20 uL	20 uL

② Incubate at 37′C for 2 hr.