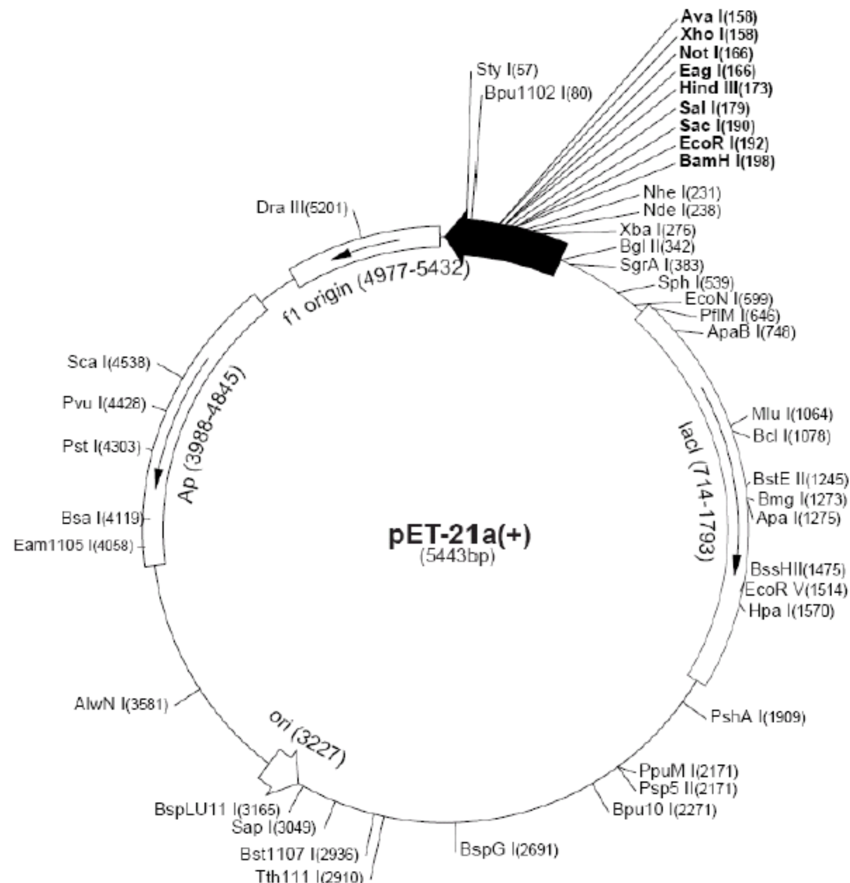
Exp6.
DNA Purification
(mini-prep, RE digestion, gel extraction)

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)

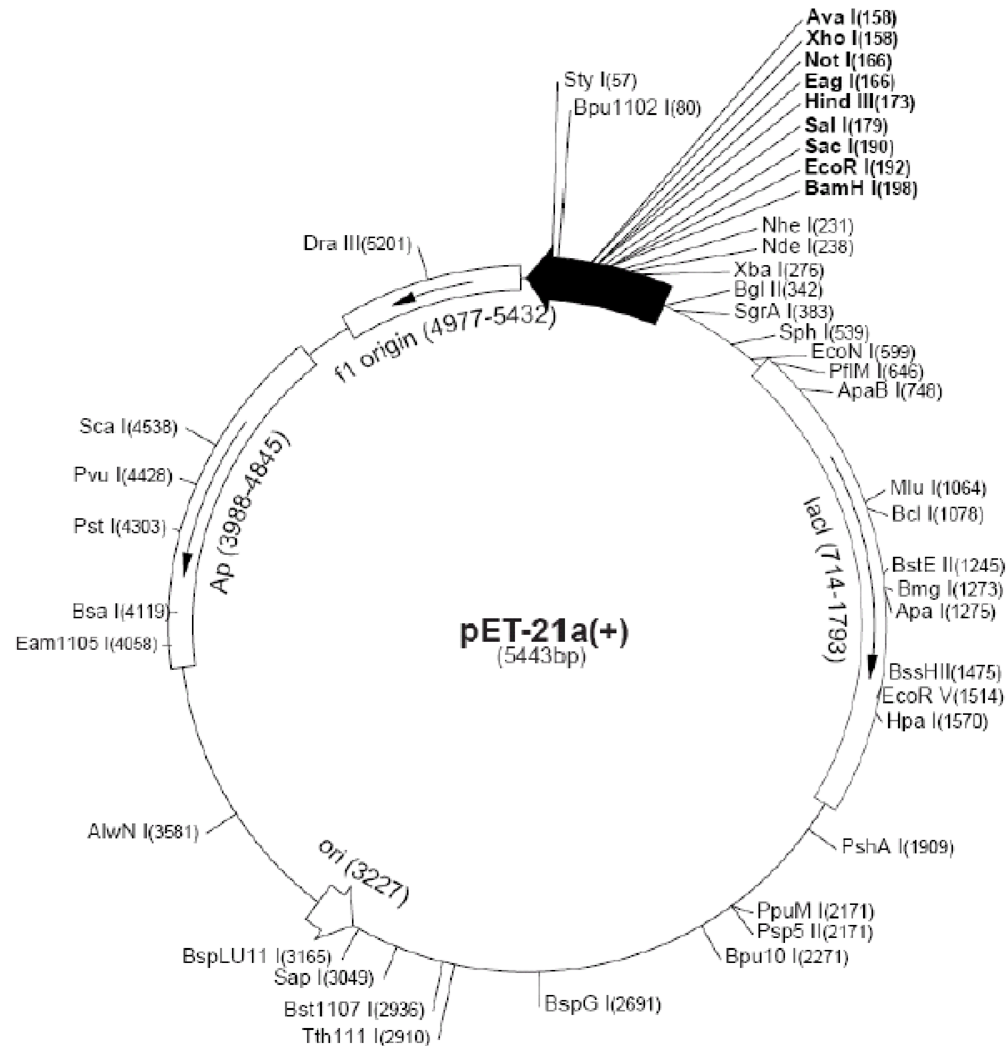
: polylinker, is a short segment of DNA which contains many (up to ~20) restriction sites

- Selectable marker

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

: often antibiotics resistance genes

Vector : pET-21a

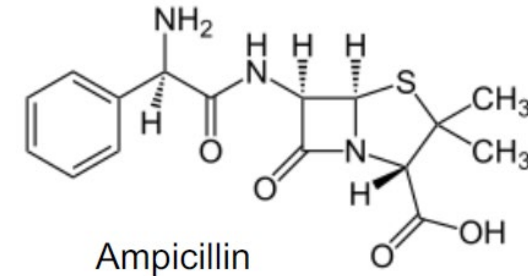


- Origin of replication (ori)
- Multiple cloning site (MCS)

: Nde Ⅰ, Xho Ⅰ

- Selectable marker

: Ampicillin (AmpR)



Mini-prep

- A method of plasmid DNA extraction and purification from bacteria

- Buffer S1 (containing Glucose, Tris, EDTA, RNase A) – 4'C storage (in fridge)
  : open the cell wall, RNA degradation
- Buffer S2 (containing NaOH, SDS)
  : open the cell wall, denatures chromosomal DNA and proteins, and releases plasmid DNA into the supernatant.
- Buffer G3 (containing Potassium acetate)
  : renaturation, precipitates chromosomal DNA and protein
- Buffer PW (containing 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 5 mL 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 1.5 mL tube and centrifuge at 15,000 rpm for 5 min at RT. Discard supernatant using pipette.

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

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

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

④ Add 250 uL 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 350 uL of buffer G3 and immediately mix by inverting the tube 4~6 times (DO NOT VORTEX).

Mini-prep protocol

⑥ Centrifuge for 10 min at 15,000 rpm.

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 at 15,000 rpm.

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.

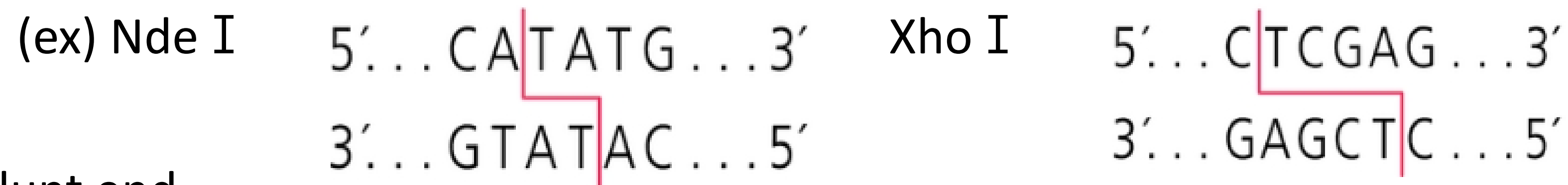
⑫ Transfer the spin column to a new 1.5mL microcentrifuge tube.
- Labeling is needed. (Name or student number etc)

⑬ Add 30 uL of buffer EB to the center of the membrane in the spin column.

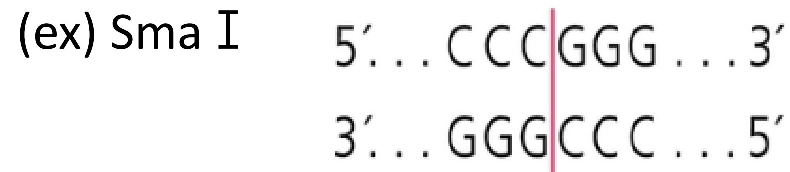
⑭ Let stand for 1 min, and centrifuge for 1 min at 15,000 rpm.

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

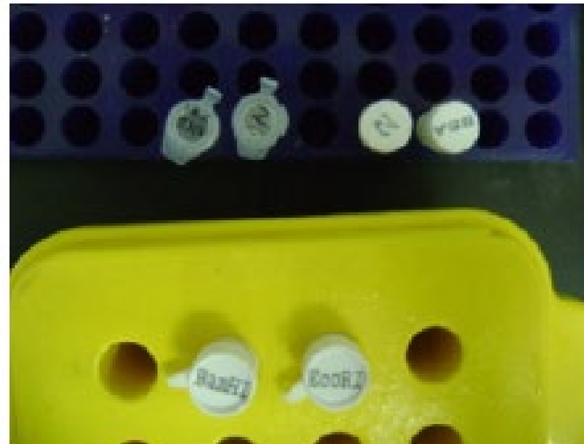
RE digestion protocol

① Make follow reaction mixture.
- RE should be on ice or cold rack.
- 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 1 hr.
- Don't forget a labeling

(your initial or student # etc)



Running an agarose gel

① Dissolve 1.5 g EB agarose powder to 100 mL 1 X TAE buffer.

- Placing plastic wrap over the top of the flask to protect evaporation of buffer.
- Microwave for 3 min until the agarose powder is completely dissolved.

② Let agarose solution cool down by stirring to about 50 ‘C.

③ Add 5 uL of SYBR safe.

- SYBR safe allows you to visualize the DNA under blue light.

④ Pour the agarose into a gel tray with the comb, and let it until it has completely solidified.

⑤ Place the gel into the gel tank and fill the tank with 1 X TAE.

⑥ Mix the sample (RE digested vector and PCR product) with 2X loading dye.

- Role of loading dye

1) a visible dye - helps with gel loading & allows you to gauge how far the DNA has migrated;

2) it contains a high percentage of glycerol → increases the density of your DNA sample causing it settle to the bottom of the gel well, instead of diffusing in the buffer.

⑦ Carefully load your samples (20 uL) and DNA marker (4 uL) into the wells of the gel.

⑧ Running gel for 130 V, 20 min.

Gel extraction (Gel purification)

- A method to extract pure DNA fragment from agarose gel.

- Buffer GB (containing chaotropic salt)

  : Enhance binding of DNA onto a membrane.

- Buffer NW (containing EtOH)

  : removes salts and other cellular components

- Buffer EB

  : DNA elution

Gel extraction (Gel purification) protocol

① Visualize your DNA fragment using blue light.

- Using the DNA ladder as a guide of the size of the DNA fragments

② Slice the desired DNA fragment from the gel using razor, and place it in a EP tube.

③ Add 400 uL of Buffer GB to the gel.

④ Incubate at 50 ‘C until the agarose gel is completely melted.

- To help dissolve gel, mix by inverting the tube every 2-3 minutes.

⑤ Add 150 uL of isopropanol to the tube and mix.

- To increase the yield.

⑥ Transfer the mixture to a spin column.

⑦ Centrifuge for 1 min at 6000 rpm. Discard the flow through and reinsert the column into the tube.

⑧ Add 750 uL of Buffer NW to the spin column. Let it stand for 5 min.

⑨ Centrifuge for 30 sec at 6000 rpm. Discard the flow through and reinsert the column into the tube.

Gel extraction (Gel purification) protocol

⑩ Centrifuge for 1 min at 15000 rpm. Transfer the spin column to a new EP tube.

- Please label your name at the side of the tube.

⑪ Apply 30 uL of Buffer EB to the center of the membrane in the spin column.

⑫ Let it stand for 1 min, and centrifuge for 1 min at 15000 rpm.