Exonuclease and Ligation-Independent Cloning (ELIC)

Adapted from Koskela et al. 2004

- The protocol that follows describes a new type of molecular cloning. Linear double-stranded DNA with homology are mixed in water, undergo homologous recombination, and are readily transformed into *E. coli*.

Protocol:

Step 1: Prepare Gene of Interest

PCR Amplification

Reaction Mix (40 µL total)

- 20 µL KAPA HiFi
- 17.5 µL nuclease-free water
- 0.75 µL forward primer at 20 mM concentration
- 0.75 µL reverse primer at 20 mM concentration
- 1 µL template DNA [Note: for negative control, replace with 1 µL nuclease-free water.]

Reaction Conditions:

- KAPA HiFi protocol with modifications:
  - Program: HOTLID 105, 30 || VOLUME 40 || TEMP 95.0, 180 || TEMP 98.0, 20 || TEMP 58, 15 || TEMP 72.0, 45 || GOTO: 2, 26 || TEMP 72.0, 300 || TEMP 12.0, 0 || END

DPNI Digest

Reaction Mix:

- 1 µL DPNI enzyme
- 40 µL PCR mix (from above)

Reaction Conditions:

- 37°C for 1 hr
- 80°C for 20 min (heat inactivation)

Prepare Ethidium Bromide Gel

- Prepare a 1% agarose EtBr gel while DPNI digest is running.
- Gel Mix: Mix 75 mL TBE, 0.75 g agarose and microwave for 1 minute until powder is dissolved. Let cool 5 minutes. Add 0.75 µL 10,000x EtBr.

Gel Electrophoresis to Check For Bands

- Add 0.5 µL loading dye to the 3 µL digested DNA and 3 µL negative control.
- Load the gel with 2 µL ladder, digested DNA, and negative control.
- Run at 150 V for 30 mins.
- Image under UV light.

PCR Purification

Follow Qiagen PCR purification kit instructions with the remaining PCR mix.

Nanodrop to Check DNA Concentration

- Wipe Take3 plate with KimWipe.
- Pipette 3 µL of nuclease-free water in center of black circle.
- Insert cuvette into nanodrop machine and press "blank."
- Pipette 3 µL of DNA in center of black circle.
- Insert cuvette into nanodrop machine and press "measure."

Step 2: Prepare Backbone

KpnI/HindIII Digest

Reaction Mix (40 µL total):

- 4 µL 10x CutSmart Buffer
- 2 µL KpnI enzyme
- 2 µL HindIII enzyme
- 1000 ng DNA [Note: for negative control, replace with equal volume nuclease-free water.]
- Nuc-free H_2O to bring volume up to 40 µL

Reaction Conditions:

- 37°C for 1 hr
- 80°C for 20 min (heat inactivation of HindIII, no heat inactivation for KpnI)

Grow up 5 mL *E. coli* liquid culture in LB min broth (about 3 hours prior to transformation).

Prepare SYBR Safe Gel

- Prepare a 1% agarose SYBR Safe gel while digest is running. After the digest, you will need to immediately load the reaction into a gel and run it.
- Gel Mix: Mix 75 mL TBE, 0.75 g agarose and microwave for 1 minute until powder is dissolved. Let cool 5 minutes. Add 7.5 µL 1000x SYBR Safe.

CIP Digest

Reaction Mix:

- Add 1 µL CIP enzyme to double digested DNA from above.

Reaction Conditions:

- 37°C for 15 mins
- IMPORTANT: CIP cannot be heat inactivated. Immediately after CIP digest is finished, run the gel or place mixture in freezer.

Gel Electrophoresis

- Add 7 µL loading dye to each reaction.
- Load the gel with 3 µL ladder, 48 µL digested backbone, and 48 µL negative control.
- Run at 150 V for 30 mins.
- Check bands under blue light.
- Cut out pZE21G band with scalpel, weigh gel slice, and proceed with gel purification.

Gel Purification

Follow Qiagen kit instructions.

Allow EB buffer to sit for 5 minutes before centrifuging to remove all traces of salt.

Step 3: Transform plasmid into bacteria

Mix Vector and Insert

Reaction Mix (47 µL):

Note: Recommended ratio of vector to insert is 4:1.

- 90 ng vector
- 113 ng insert
- Nuclease free water to bring volume up to 47 µL

Electroporation

Standard protocol:

- Wash pellet twice with cold milliQ water, then resuspend in 47 µL plasmid mix from above.

- Pull out all media on first wash.
- Shock at 1800 V.
- Recover 1-2 hrs in 1 mL LB min broth.

Plate Cells

- Transfer cultures to Eppendorf tubes. Spin down.
- Discard supernatant and resuspend pellet in 100 µL fresh LB min broth.
- Plate 100 µL on selective LB min plates.

References:

Koskela, E. V. & Frey, A. D. "Homologous Recombinatorial Cloning Without the Creation of Single-Stranded Ends: Exonuclease and Ligation-Independent Cloning (ELIC)." *Mol. Biotechnol.* **2014** 233–240.