SLIC

Protocol is from David Shis' thesis.

Originally described by Li and Elledge

Cloning by SLIC is an economical strategy for ligating dsDNA. dsDNA is first treated with T4 DNA polymerase which has a 3' exonuclease domain. This creates sticky ends on either end of dsDNA. The reaction is then quenched with the addition of a single nucleotide triphosphate. dsDNA with sticky ends are then mixed together, allowing the sticky ends with significant homology to bind together. This mix is then transformed into \ecoli\ where it is assumed DNA repair machinery repairs the nicks and gaps in DNA mixture are repaired to make a functional plasmid.

Below is my working protocol. There is significant room for optimization on a case by case basis, but the protocol below has been sufficient for most of my cloning. Most of my dsDNA samples have regions of homology with a predicted melting temperature around 65°C.

SLIC is prefect for ligating two strands of dsDNA together. In such a reaction I would expect about 50-200 colonies on the final plate. One strategy for getting SLIC cloning to work on multiple fragments of DNA is to ligate some of the fragments beforehand via PCR and then using SLIC to create the final plasmid as SLIC is typically faster (45 min)than doing PCR ligation.

SLIC Protocol

Reagents

1. 10mM dGTP (can be any other dNTP)
2. T4 ligation buffer
3. NEBuffer 2.1 (NEBuffer 2 with BSA)[1]
4. At least 18l of spin column purified dsDNA
5. ~~BSA~~ [2]
6. T4 DNA polymerase

Protocol

1. For each dsDNA species, mix 18l of dsDNA with 2l NEBuffer 2.1 in a fresh eppendorf tube.
2. Add 0.5µl T4 DNA polymerase
3. Incubate at 25°C for 15 minutes
4. Add 2µl of dGTP to the reaction, place on ice
   Note: dsDNA treated with T4 DNA polymerase can be stored at -20°C for about a month
5. Mix equimolar amounts of treated dsDNA in a final reaction volume of 9µl
   Note: I usually assume each sample is at roughly the same mass concentration and scale amounts
6. Add 1µl T4 buffer
7. Incubate mix at 37°C for 30 minutes
8. Transform entire mix into competent cells - I usually transform into chemically competent cells

Footnotes

1. Though NEB says that T4 DNA polymerase retains full activity in CutSmart buffer, I believe this references the polymerase activity, not the exonuclease activity. SLIC did not work well with CutSmart buffer, but works very well with Buffer 2.1 -AJH
2. NEBuffer 2.1 already contains BSA no additional one is needed