SLIC Cloning

Reference: Li *et al*, Nature Methods, 2007 "Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC"

Materials:
T4 DNA polymerase (T4 DNAP): NEB (# M0203S)
RecA: NEB (# M0249S)
T4 ligase buffer (containing ATP): NEB (sold together with T4 ligase # M0202S)
100x BSA: NEB (sold together with many enzymes)

Primer design:
Add 30 bps from the vector sequence to 5' of the PCR primers to ensure annealing.

Insert:
PCR amplify the insert in a 50 uL rxn, gel purify product, elute in 30 uL water.

Vector:
Double digest the vector: 2 ug vector in 50 uL rxn, 1-2 hr at desired temperature, gel purify, elute in 30 uL water. Or, PCR amplify the vector backbone – especially if you do not want to rely on restriction sites.

SLIC cloning:
1. T4 DNAP treatment
   - Make master T4 RNAP solution for 12 rxns:

     60 uL
     1.2 uL BSA (10mg/ml from NEB)
     12 uL 10x T4 ligase buffer
     1 uL T4 DNAP stock
     45.8 uL H2O

   - 5 uL of master solution + 5 uL eluted DNA
   - RT 30min; then stop rxn by adding 1 uL 10 mM dCTP and more to ice

2. Annealing
   - Make master RecA solution:
     1) Dilute RecA: 1 uL RecA + 5 uL ddH2O
     2) Take 1 uL diluted RecA, add 1 uL BSA + 10 uL T4 ligase buffer + 88 uL water

   - 6 uL master RecA + 2 uL treated vector + 2 uL treated insert
   - 37 C 30 min; then move to ice ready for transformation

3. Transformation
Use 5-10 uL to transform 100 uL Mach1 competent cells. Do not use any short-cut transformation methods.

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