

Sub Cloning (including Transformation)

I. Making Competent Bacteria:

1. Grow bacteria in 25ml. LB, 37° C, O/N
2. Inoculate 250-800ml. warm LB, grow 2-3 hr. (until OD600=0.5)
3. Spin down bacteria in 250-ml. sterile tube, 4.3 K rpm., 15 Min. at 4°C in J6B centrifuge,(keep bacteria on ice from step 3-7).
4. Resuspend pellet carefully by pipeting (avoid making bubbles) in 30ml. of TfBI (pH.5.8, adjust by adding drop by drop of 1MCHCl, don't over triturate. Then filter sterilize):

30mMKOAc

50mM MnCl₂

100mMRbCl (Sigma R2252, FWt=120.9)

10mM CaCl₂

15% (V/V) glycerol

Leave on ice for 10 min..

5. Spin down as above.
6. Resuspend pellet carefully by pipeting (avoid making bubbles) in 4-8 ml. of TfBII (pH.7.0, filter sterilized):

10mM NaMOPS, or HEPES (pH.7.0)

10 mMRbCl (Sigma R2252, FWt=120.9)

75mM CaCl₂

15% (V/V) glycerol

7. Aliquot into 200 ul. in 1ml. tubes, freeze in liquid N₂., store at -70°C,

II. DNA

1. Cut plasmid DNA with proper restriction enzyme

2. Separate the DNA fragments on 1% LMP agarose TAE gel 120 volts, with cold TAE.
3. Isolate the proper DNA fragments (vector and insert) from LMP gel using sterile Pasteur pipet, transfer to Eppendorf tubes, such DNA LMP samples can be stored at -20°C for years.

III. Ligation reaction in 10-20 ul. Reaction at 16°C for 4-8 hrs. (usually 1uL. Vector +7uL. Insert DNA both in LMP): Use Promega 10Xlig (Z ATP) buffer or 5X lig. Buffer:

250mM Tris 7.6	<u>10X Klenow Kinase B</u>
50mM MgCl₂	700 mM. Tris 7.6
25% Peg8000	100mM MgCl₂
5mM ATP	50 mM B-ME
5mM DTT	1mM EDTA
	10X Annealing-Klenow B:
	ABOVE + 500 mL. NaCl

IV. Transformation:

1. Thaw the competent bacteria on ice for 10 min., in the meantime; dilute 3X (add 25mL. H₂O to 10mL. Lig. Rxn) and heat lig. mixture at 65°C for 7 min., vortex to mix well.
2. Add the competent bacteria to the diluted lig. Mixture, incubate on ice for 10-15 min.
3. Heat shock 2 min. at 42°C , put back on ice for 1 min. add 0.3-0.5mL. LB, incubate at 37°C for 30-45 min.
4. Plate onto Ampicillin-LB plate, grow 37°C O/N.
5. Grow bacteria in 5ml. Amp-TB, miniprep. Plasmid DNA, restriction digestion to identify the desired clones.