Cloning for biochemists

1. Use the iProof PCR reagents (Bio-Rad #172-5310) to amplify your favorite gene using in a 50 uL rxn.

2. Check amplification by running 2-5 uL on a gel.

3. Clean up with the Qiagen PCR purification kit (#28106), elute with 42.5 uL water.

4. Add 5 uL whatever NEB buffer you need, 0.5 uL BSA, and 1 uL each of whatever enzymes you need
    a. At the same time, set up the vector digest using 10 uL mini-prepped vector that you want to clone into and the proper reagents to 30 uL

5. Incubate 2 hr at 37 (or whatever temp your enzymes need)

6. Run digests on agarose gel to purify, cut out the vector and insert bands

7. Extract the DNA with the Qiagen gel extraction kit, elute with 30 uL water

8. Set up ligations using the Epicentre Biotechnologies Fast Link DNA ligation kit (LK6201H):

1.5 uL 10x buffer	
1 uL 10 mM ATP	
5 uL vector DNA	Incubate 1 hr at room temp
5 uL insert DNA	Kill 15 min at 70 degrees
1.5 uL water	Transform DH5α with 7.5 uL
1 uL ligase	

When it's all said and done (and if you have good competent cells), you should end up with many transformants, >75% of which represent good ligations.