Oligo annealing protocol

Resuspend dried oligos to a concentration of 100uM in 10mM Tris buffer pH 8.0

(Check the amount of DNA in the tube in nanomoles ("nm") listed on the side of the tube. Add Tris buffer to 100uM by multiplying by 10 to get the appropriate volume in uL. Example: for 27.6nm, add 276uL buffer to get a 100uM stock concentration)

Set up the annealing reaction:

10 uL of 100uM forward oligo (final rxn. conc. = 20uM of each oligo)

10 uL of 100uM reverse oligo

5 uL of NEB buffer #2

25 uL dH20

Final reaction volume: 50uL

In the microwave, heat a beaker of water to a rolling boil. Incubate the reaction in the boiling water, and let it remain in the water until the water has cooled naturally to room temperature.

Digest the annealed insert:

Take half (25uL) of the annealing reaction (store the remainder at -20C)

25uL annealed oligos

2.5uL NEB buffer 2

1uL BSA

1uL Enzyme 1 (EcoR1 or XbaI)

1uL Enzyme 2 (SpeI or PstI)

19.5 uL dH20

Final reaction volume: 50uL

Incubate 37C for 1 hour, then 80C 20 minutes. Try varying amounts of the product in the ligation reaction.