Shotgun Subcloning

I. Shearing

a. Preparing sample
   i. Make sure there is no undissolved material in the DNA sample.
   ii. You need 3-5 ug of BAC or cosmid DNA and 5-10u g for genomic DNA in 250 ul. (DNA concentration is not critical for shearing but you need enough DNA to be detected on a gel after shearing.)

b. Parameters
   i. Usually, if you want to get average insert size of 2-3kb, use speed code 9 or 10 (use 10 to get 3 kb average).
   ii. Enter your sample volume
   iii. Don't change any other numbers on the program
   iv. Run automatic running

c. Useful tips
   i. Here is a tip for removing air bubble in your sample during the shearing
   ii. After injecting your sample into the syringe, turn the silver nob halfway (until the nob is vertical) instead all the way to output when the computer says 'turn it to output'.
   iii. Right after the air bubble moved away, turn the nob to the output.
   iv. Start shearing
   v. Run a mini-gel with 10 ul of the sheared sample

II. End repairing

a. Concentration
   i. Add 1/10 vol of 3M NaOAc
   ii. Add 2.5 vol of 95% EtOH and mix
   iii. Keep the sample in freezer for 30 min
   iv. Spin at 13000rpm for 20 min

v. Rinse with 300ul of 70% EtOH
vi. Dry the pellet and resuspend in 15 ul of water

b. Enzyme reaction

5X Kinase buffer	6ul
10 mM ATP	4 ul
2.5 mM dNTP	0.56 ul
T4 Kinase (10U/ul)	0.24 ul
Klenow (4U/ul)	2 ul
T4 DNA pol. (5U/ul)	1.6 ul
DNA	15 ul

- Make the final volume to 30 ul
- Incubate at 37C for 45 min

III. Size selection

a. Gel running
   i. Run 1% agarose gel with TAE buffer
   ii. Don't run the gel too fast, use 50-80 V for 1 – 1.5 hr
b. Band isolation
   i. Cut the gel between 3 – 4 kb region
   ii. Use Qiagen gel extraction kit to elute the DNA from gel
   iii. Check the DNA conc on a mini-gel

IV. Ligation & Transformation

a. Ligation
   i. Adjust the ratio between vector and insert to 1:1.
   ii. Use pUC18/SmaI/CIP for blunt-end ligation
   iii. Mix insert, vector, buffer and ligase on ice
   iv. Use a Fast-Link™ DNA Ligation Kit (Epicentre)
   v. Incubate at room temperature for 30 min
   vi. Heat denature the enzyme at 70C for 15 min
b. Transformation
   Heat-shock transformation with 1-2 ul of the ligation.