* Assemble the following components in a 0.5 ml pcr tube:

50 ng of vector (10 µl of 1 in 50 dilution)

10 pmol of forward primer (1 µl of 1 in 10 dilution)

10 pmol of reverse primer (1 µl of 1 in 10 dilution)

1 µl Pfu polymerase (add last, on ice)

5 µl Pfu 10x buffer

1 µl dNTP mix

1 µl MgCl2

31 H2O

50 µl total

* Mix tube well by flicking, keep on ice
* Place tube in PCR machine, ensure that lid is tightly closed, start the following program:

1) 95 °C, 30 sec;

2) 95 °C, 30 sec; *denaturation*

3) 45 °C, 1 min; *annealing*

4) 72 °C, 12 min; *extension, 1-2 min/kbp*

5) Go to 2), repeat x25;

6) 68 °C, 7 min; *final extension*

7) Hold at 4 °C (or 10 °C )

*Note:* ***A****. temperature at step 3 can vary, should be 5°C lower than the lowest primer template melting temperature (use oligo-calculator).* ***B.*** *extension time at step 4 can vary for optimization.* ***C.*** *Other trouble shooting methods can involve varying concentration of MgCl*2 and adding DMSO for high GC-content primers.

* Add 1 µl Dpn1 to degrade any methylated native DNA, incubate at 37°C for 1 hr.
* Keep at 4°C, use this to transform into *E.* coli immediately. If not, keep tube at -20°C until ready for transformation.