New England BioLabs- Colony PCR

1. Make agar plates with transformed bacteria
2. Set up 50 µl reaction as follows in PCR tube:

COMPONENT	AMOUNT
One *Taq* Master mix	25 µl
PCR primer	200 nM
H_2O	To 50 µl

3. Transfer one individual colony with a sterile loop into the reaction tube
4. Twirl loop around until mixture becomes clody
    a. Optional: Dip sterile loop into 3 ml growth media with appropriate antibiotic to make overnight culture
    b. Optional: Use same sterile loop to streak colonies onto a new agar plate
5. Set up the following PCR program:

STEP	TEMP	TIME
Initial Denaturation	94°C	2 minutes
30 cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute pr. kb
Final hold	68°C 10°C	5-10 minutes hold

6. Load 4-6 µl of each PCR reaction on a agarose gel with the appropriate DNA ladder