3.2 Verifying cloning with colony PCR

Adapted from UiOslo_Norway 2018:

http://2018.igem.org/wiki/images/3/32/T--UiOslo_Norway--Prot1.pdf

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

Component	Amount
One *Taq* Master Mix	25 ul
PCR primer	200 nM
H_2O	Fill till tube volume is equal to 50 uL

3. Transfer one individual colony with a sterile lop into the reaction tube
4. Twirl loop until mixture becomes cloudy.
    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 PCR program:

Step	**Temperature**	**Time**
Initial denaturation	94°C	2 minutes
30 cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute per kb.
Final hold	68°C 10°C	5-10 minutes hold

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