Colony PCR

Colony PCR from individual colonies grown on plates, to find colonies transformed with plasmids containing insertion of the target gene.
All reagents must be kept **on ice** at all times. Assemble and mix **on ice** as well.
We are using the X7 DNA Polymerase (Common stock) and Phusion buffer 5x HF (Sotirios' lab)

Thermocycler setup

1. Heat to 95°C for 10 minutes to degrade cells and free DNA for the reaction.
2. **Initialization (hot start PCR):** Heat to 98 °C in order to activate the DNA polymerase.
3. **25-35 Cycles:**
    i. **Denaturation:** Heat chamber to denature DNA (98 °C for 5-10 sec. for X7 - Can go up to 30 sec.).
    ii. **Annealing:** Re-annealing of DNA strings/primers. Too hot and no hybridization, too cold and hybridization becomes unspecific (Use NEB Tm Calculator or similar to determine annealing temperature. 45-72°C for 10-30 sec. Typically ~3°C above the lowest Tm). Alternatively, do a temperature gradient test, where different annealing temperatures are tested to find the optimal one.
    iii. **Elongation:** dNTPs are linked by the polymerase (For X7: 72 °C, 15-30 sec per kb).
4. **Final elongation:** Makes sure all single strands are fully elongated (For X7: 72 °C for 5-10 min).
5. **Hold:** Cool down and use for short-term storage at 10 °C.

Protocol:

1. Dilute the primers 10x in an Eppendorf tube (or similar) using nuclease free H_2O.
2. Mix reagents in the listed order (make a larger quantity master mix and put 10 µl in each PCR tube)
3. Add cells by touching a colony with a pipette tip and transfer to tube.
4. Run the PCR.

Component	10 uL reaction	50 uL reaction
MQ H2O	6.7 uL	33.5 uL
5x Phusion HF or GC Buffer	2.0 uL	10.0 uL
dNTPs	0.2 uL	1.0 uL
10x diluted Forward Primer	0.25 uL	1.25 uL
10x diluted Reverse Primer	0.25 uL	1.25 uL
X7 DNA Polymerase	0.2 uL	0.5 uL