Phusion PCR – SOP

Phusion polymerase should be used when amplifying pieces for assembly. Colony PCR and temperature optimization studies can use the Taq polymerase Master Mix.

For a 20 uL Phusion reaction, you will need:

- 20-30 ng template DNA
- 0.5 uL Forward and Reverse Primers (at 50 uM)
- 4 uL High Fidelity Reaction buffer (5X concentration)
- 2 uL dNTPs (2mM)
- 0.6 ul DMSO
- 0.2 ul Phusion Enzyme
- Add H_2O to 20 uL

Combine all of these ingredients in a PCR tube.

The PCR Cycler should be set to:

- 2 minutes at 98°C to denature everything
- X Cycles of:
    - Denature - 15 seconds at 98°C
    - Annealing - 30 seconds at Annealing Temperature
    - Extension – 30 seconds per kilobase-pair at 72°C
- 10 minute final extension at 72°C

X should be between 20 and 30 cycles.

Add 4 uL of the 6X loading dye upon completion of the PCR. Run on an appropriate percentage agarose gel and then cut the desired pieces from the gel. Extract using the mini-prep Gel Extraction kit and then clean using the DNA Clean and Concentrator mini-prep kit.