Phusion U v1.0

Introduction

Protocols for PCR reactions using Phusion U. This polymerase is used for amplification of USER fragments. Remember to keep enzymes and dNTP on ice as much as possible! If only one or two reactants are changed between the reacitons, it may be easier to make a master mix, and add the rest of the reactants in the PCR tubes

Materials

- 0.5µL Phusion U Polymerase
- 2.5µL Forward primer [10nM]
- 2.5µL Reverse primer [10nM]
- 1µL dNTP mix [10mM]
- 10µL 5x HF or GC buffer
- 0.1ng - 1µg Template DNA
- (1.5µL DMSO in case of gDNA amplification)
- MilliQ to a final reaction volume of 50µL
- Eppendorf tube
- PCR tubes

Procedure

Mix master mix

1. CRITICAL **REMEMBER CONTROLS!** (MilliQ and plasmid if available)
2. Dispense the reactants in an eppendorf tube (remember to account for varying components). Remember to mix **AT LEAST** one extra reaction.
3. Vortex
4. Spin down

Dispense master mix in PCR tubes

5. Dispense master mix in the PCR tubes to the decired reaction volume. remember to adjust volume if variable components are added afterwards
6. Add variable compenents (if any)
7. Spin down

PCR cycle (in thermocycler)

8. Keep PCR tubes on ice while the thermocycler is programmed and heats up
9. Initial denaturation: 98°C for 30s
10. **25 - 35x of:**
11. Denaturation 98°C for 10s
12. Annealing for 20 seconds (calculate annealing temperature here: http://tmcalculator.neb.com/)
13. Elongation at 72°C for ~30s/kb
14. Mix master mix
15. CRITICAL **REMEMBER CONTROLS!** (MilliQ and plasmid if available)
16. Remember to mix **AT LEAST** one extra reaction