OneTaq master mix v1.0

Introduction

Protocols for PCR reactions using 2x OneTaq MM. This polymerase is used for routine amplification and colony PCRs. 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

- 12.5µL OneTaq Quick-Load 2X Master Mix
- 0.5µL Forward primer [10nM]
- 0.5µL Reverse primer [10nM]
- 0.1ng - 1µg Template DNA (For colony PCR suspend a colony in 20µL MilliQ using a pipet tip, and substitute the template DNA with 1µL of suspended colony)
- MilliQ to a final reaction volume of 25µL

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: 94°C for 1min
10. **25 - 35x of:**
11. Denaturation 94°C for 25s

12. Annealing for 40s (calculate annealing temperature here: http://tmcalculator.neb.com/)

13. Elongation at 68°C for ~1min/kb