PCR (Hot start, KAPA biosystems)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Ice
- 0,5ml eppendorf tubes
- Sterile H2O
- 5 x KAPA Taq HotStart Buffer
- 10mM dNTP mix
- 10µM Forward primer
- 10µM Reverse primer
- 5U/µl KAPA Taq HotStart DNA polymerase
- Template DNA

Procedure

Master Mix (25µl reactions):

1. Keep all competents on ice. Everything can be melted in rt. Keep the polymerase in the freezer until you need it and return it immeadiately after use.
2. **16,75 µl** H2O (Calculate the amount of water based on how much DNA you use)
3. **5µl** 5 x Buffer
4. **0,75 µl** 10mM dNTP mix
5. **0,75 µl** 10µM forward primer
6. **0,75 µl** 10µM reverse primer
7. **0,5 µl** KAPA HiFi HotStart DNA Polymerase
8. Use <100 ng genomic DNA (10 – 100 ng) and <1 ng less complex DNA (0.1 – 1 ng) per 50 µl reaction as first approach. -> use **0,5 ng DNA** for 25 ul reaction

   Make a **1 ng/ul dilution** of your template DNA and use **0,5 ul** of it.

   For multiple reactions (gradient etc): multiply this by the amount of sample +1
   (E.g. You need to make 3 different PCR reactions with the same primers. Multiply all components of the Master mix by 4 and use for the reaction)

   NOTE! DNA polymerase CAN NOT BE VORTEXED. Mix the mastermix by pipetting back and forth, tapping the tube or by inverting. If inverting, give the tube a quick spin after mixing.

PCR program:

1. 95° *C - 3 min*
2. 98° *C - 30 sec*
3. X° *C - 30 sec **
4. 72° *C - 1 min/kb*
5. 72° *C - 5 min*
6. 4° *C - forever*

*Choose the temperature in step 3. according to your primers' anneling temperature.

9. Repeat steps 2.-4. 35 times