Setting Up a PCR

Before gathering any materials or reagents determine the volumes of each reagent that will be needed. Fill out PCR details sheet.

Procedure

1. Get a container of ice and a PCR rack.
2. Defrost all reagents *except* Taq. Keep Taq on ice at all times.

    PCR grade water
    10 X Buffer
    dNTP mix
    forward primer @ 5pm/µl
    reverse primer @ 5pm/µl
    Magnesium ($MgCl_2$) (if necessary)

3. Briefly vortex each of the reagents *except* Taq and water. This is to ensure mixing in case of settling during freeze/thaw. Put all reagents on ice with Taq.
4. Label 0.2 ml reaction tubes and 0.5 ml tube for cocktail mix.
5. Add reagents to cocktail mix tube one by one in the order listed above. Use volumes previously determined and written on your PCR lab sheet. Check off as each reagent is added to the cocktail.
6. Vortex to mix thoroughly. (5 seconds @ maximum speed). Put on ice.
7. Add Taq to cocktail mix and vortex gently to mix with other reagents.
8. Dispense cocktail to reaction tubes. Work carefully but quickly.
9. Add DNA or water (water replaces DNA in negative controls) to reaction tubes.
10. Make sure that the lid of each reaction tube is snapped shut (sealed) or the reaction will evaporate. Flick each tube gently to ensure mixing and that the contents are at the bottom of the tube.
11. Place reaction tubes in PCR machine and start program.
12. Put all reagents away in -20°C freezer.

PCR program

1. 94° C for 2.5 min
2. 94° C for 30 s
3. 52-62° C for 45 s (depending on primer Tm)
4. 72° C for 1 – 4 min, depending on length of product (1 min/kb)
5. 72° C for 10 min
6. Hold at 4° C

Materials necessary

0.2 ml PCR tubes (individual or 8-strip)
0.5 ml microcentrifuge tube
Forward and reverse primers @ 5 pM
10X PCR Buffer
dNTPs @ 10 mM
10 and 20 µl pipette tips
5 PRIME taq polymerase
PCR grade water
Ice

Equipment necessary

PCR thermocycler
10 µl pipette
20 µl pipette
200 µl pipette