PCR protocol

Goal

amplify DNA of interest for further cloning

Before you begin fill:

clone name:_____

date:_____

Template name:_____

Tm:_____

Annealing Temp:_____

Expected size:_____

Materials

- Ultra-pure water
- Primers diluted to working concentration (10µM working stocks are sufficient for most assays)
- Oligos
- DNA to be amplified
- DNA polymerase
- dNTP mix, 10 mM each of dATP, dCTP, dGTP, and dTTP

Procedure

1. Select appropriate table for reaction setup: Add the reagents to a appropriate sized tube in the order provided in the table. For large number of reactions, a mastermix without the template should be setup and aliquoted into reaction tubes. At the end, template should be added to appropriate tubes.

Amount	Component
y µL	Ultra-pure Water
5 µL	10x PCR Buffer
1 µL*	dNTPs
2.5 µL	Forward primer [10 µM]
2.5 µL	Reverse primer [10 µM]
0.5 µL	*Taq* DNA Polymerase
x µL	Template DNA (2- 10 ng)
50 µL	Total volume

2. Mix gently by hand **Do not vortex**.

3. Add 50 µL of mineral oil to the top of each tube to prevent evaporation if using a thermal cycler without a heated lid.

4. The amplification parameters:

25-30 cycles of amplification are recommended		
Initial denaturation	95 °C	30 sec
Denaturation	95 °C	30 sec
Anneal primers	60 °C	30 sec
Extension	72 °C	1 min

Final Extension	72 °C	2 min
Hold	10 °C	