Protocol for a Routine Taq PCR

Introduction

All components should be mixed and spun down prior to pipetting. These recommendations serve as a starting point; in order to maximize amplification the reaction conditions may require optimization (see Taq DNA Polymerase Guidelines for PCR Optimization protocol).

Protocol

Component	***25 µl reaction***	***Final Concentration***
10X Standard Taq Reaction Buffer	*2.5 µl*	*1X*
10 mM dNTPs	*0.5 µl*	*200 µM*
10 µM Forward Primer	*0.5 µl*	*0.2 µM (0.05–1 µM)*
10 µM Reverse Primer	*0.5 µl*	*0.2 µM (0.05–1 µM)*
Template DNA	*variable*	*<1,000 ng*
*Taq DNA Polymerase**	*0.125 µl*	*1.25 units/50 µl PCR*

Gently mix the reaction and spin down in microcentrifuge.

If the thermocycler does not have a heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation.

Cycling Conditions for a Routine PCR:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	95°C	30 seconds	1
Denaturation Annealing Extension	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute per kb	30
Final Extension	72°C	5 minutes	1
Hold	4°C	∞	