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

1. Prepare the following 50 µl reaction in a 0.5 ml PCR tube on ice:

   ** Due to the difficulties in pipetting small volumes of enzyme, Taq DNA Polymerase can be diluted in Diluent F (NEB #B8006S) or 1X reaction buffer. For example, 1 µl of Taq DNA Polymerase is mixed with 4 µl of diluent and 1 µl of that mixture is added to the reaction. Enzyme diluted in Diluent F can be stored at -20°C for future use.*

Component	***25 µl reaction***	***50 µl reaction***	***Final Concentration***
10X ThermoPol or Standard Taq Reaction Buffer	*2.5 µl*	*5 µl*	*1X*
10 mM dNTPs	*0.5 µl*	*1 µl*	*200 µM*
10 µM Forward Primer	*0.5 µl*	*1 µl*	*0.2 µM (0.05–1 µM)*
10 µM Reverse Primer	*0.5 µl*	*1 µl*	*0.2 µM (0.05–1 µM)*
Template DNA	*variable*	*variable*	*<1,000 ng*
*Taq DNA Polymerase**	*0.125 µl*	*0.25 µl*	*1.25 units/50 µl PCR*
Nuclease-free water	*to 25 µl*	*to 50 µl*	

2. 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.

3. Cycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	