Protocol for a Routine *Taq* PCR Reaction

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

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	Volume (µl)	Final Concentration
Standard or ThermoPol Taq Reaction Buffer (10X)	5 µl	1X
Deoxynucleotide Solution Mix (10 mM)	1 µl	200 µM
Upstream Primer (10 µM stock)	0.5–2.5 µl	0.1–0.5 µM
Downstream Primer (10 µM stock)	0.5–2.5 µl	0.1–0.5 µM
DNA Template	determined by user	0.1-1 ng/ml plasmid DNA 1-10 µg/ml genomic DNA
Taq DNA Polymerase*	0.2 µl	0.02 units/µl
Nuclease free water	Bring reaction to a final volume of 50 µl	

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 Reaction:

Initial Denaturation	**95°C**	**30 seconds**
30 Cycles	95°C	15–30 seconds
	45–68°C	15–60 seconds

	68°C	1 minute per kb
Final Extension	68°C	5 minutes
Hold	4°C	∞