

## **PCR Protocol**

## **Protocol**

1. Combine the following components in a sterile 0.5–0.6ml microcentrifuge tube. The reaction volume can be scaled as long as the final concentrations remain constant.

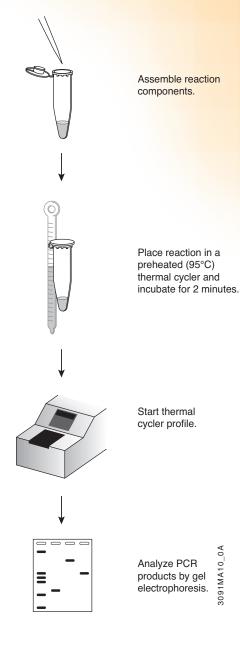
	Component	Final
Component	Volume	Concentration
MgCl <sub>2</sub> , 25mM Solution	2.0–8.0µI	1.0-4.0mM
5X Colorless GoTaq® Flexi Buffer		
<b>OR</b> 5X Green GoTaq® Flexi Buffer	10μΙ	1.0X
PCR Nucleotide Mix, 10mM each	1µI	200µM each
upstream primer	5-50pmol	0.1-1.0µM
downstream primer	5–50pmol	0.1-1.0µM
GoTaq® DNA Polymerase, 5u/μl	0.25μΙ	1.25u/50µl
template DNA	<u>variable</u>	<0.5µg/50µl
Nuclease-Free Water to a final volume of	50µI	

- 2. If using a thermal cycler **without a heated lid**, overlay the reactions with 1–2 drops of mineral oil and centrifuge briefly.
- 3. Place the reactions in a thermal cycler that has been preheated to 95°C and incubate for 2 minutes.
- 4. Start the thermal cycling program. We recommend optimizing the cycling profile for each primer:target combination.

## **Analysis**

- 1. Analyze PCR products by agarose gel electrophoresis. The products should be readily visible in an ethidium bromide-stained gel illuminated with UV light.
- 2. Store PCR products at -20°C. The PCR products can be further purified using a system such as Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

See additional protocol information in Technical Bulletin #TB254, available online at: **www.promega.com/tbs** 



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