

## PCR Protocol

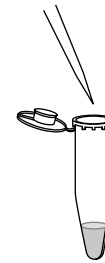
- Combine the following components in a sterile 0.5–0.6ml microcentrifuge tube. The reaction volume can be scaled as long as the final concentrations remain constant.

Component	Component Volume (25µl)	Component Volume (50µl)	Final Concentration
MgCl <sub>2</sub> , 25mM Solution	1.0–4.0µl	2.0–8.0µl	1.0–4.0mM
5X Colorless GoTaq® Flexi Buffer <b>OR</b> 5X Green GoTaq® Flexi Buffer	5µl	10µl	1.0X
PCR Nucleotide Mix, 10mM each	0.5µl	1µl	200µM each
upstream primer	2.5–25µmol	5–50µmol	0.1–1.0µM
downstream primer	2.5–25µmol	5–50µmol	0.1–1.0µM
GoTaq® DNA Polymerase, 5u/µl	0.125µl	0.25µl	0.625u/25µl 1.25u/50µl
template DNA	variable	variable	<0.25µg/25µl <0.5µg/50µl
Nuclease-Free Water to a final volume of	<b>25µl</b>	<b>50µl</b>	

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

## Analysis

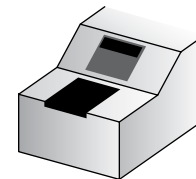
- Analyze PCR products by agarose gel electrophoresis. The products should be readily visible in an ethidium bromide-stained gel illuminated with UV light.
- Store PCR products at –20°C. The PCR products can be further purified using a system such as Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).



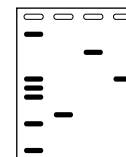
Assemble reaction components.



Place reaction in a preheated (95°C) thermal cycler and incubate for 2 minutes.



Start thermal cycler profile.



Analyze PCR products by gel electrophoresis.

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Additional protocol information is in Technical Bulletin #TB254, available online at: [www.promega.com](http://www.promega.com)