

I. List of Components

Always store any unused product at 20–22°C, either (1) back in the original foil pouch, re-sealed with desiccant; or (2) in a desiccator.

- High Fidelity PCR EcoDry Premix 8-tube strips

NOTE: Each tube contains lyophilized master mix with the following components:

- Advantage® 2 DNA Polymerase Mix (includes a hot start antibody and a proofreading enzyme)
- MgCl₂ (2 mM final conc.)
- dNTP Mix
- Reaction Buffer
- Cryoprotectant
- Stabilizers
- Optically Clear Cap Strips (8 caps/strip)

II. Protocol

1. Assemble a Primer/Template Mix

Each tube of High Fidelity PCR EcoDry Premix requires the addition of PCR-grade water, primers, and/or template in a total volume of 25 µl. Assemble a mix containing water, primers and/or template with volume sufficient for the number of PCR reactions you plan to perform, plus an additional reaction to compensate for pipetting errors.

2. Reconstitute the Premix

- a. Pipette 25 µl of the primer/template mix into each tube.
- b. Pipette up and down several times to dissolve each pellet, then cap the tubes.
- c. Briefly spin the tubes in a microcentrifuge, and begin thermal cycling using the guidelines provided below.

3. Recommended Cycling Parameters

Use the following guidelines when setting up your initial experiments. These are general guidelines—the optimal parameters may vary.

Target Size	Cycling Parameters
< 1 kb	<ul style="list-style-type: none"> • 95°C for 1 min • 25–35 cycles: 95°C for 30 sec 68°C for 1 min • 68°C for 1 min
1–5 kb	<ul style="list-style-type: none"> • 95°C for 1 min • 25–35 cycles: 95°C for 30 sec 68°C for 3 min • 68°C for 3 min

Target Size	Cycling Parameters
6–9 kb	<ul style="list-style-type: none"> • 95°C for 1 min • 25–35 cycles: 95°C for 30 sec 68°C for 6 min • 68°C for 6 min
10–20 kb	<ul style="list-style-type: none"> • 95°C for 1 min • 25–35 cycles: 95°C for 30 sec 68°C for 12 min • 68°C for 12 min

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This document has been reviewed and approved by the Clontech Quality Assurance Department.