I. Introduction

Please read the Advantage 2 PCR Kit User Manual before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

II. Protocol

A. Primer Design

Primer design is the single largest variable in PCR applications and the single most important factor in determining the success or failure of PCR reactions. *Always check your primer design before constructing or ordering primers.*

Advantage 2 PCR Kit can be used in a wide variety of PCR applications, and the constraints on primer design will vary from one application to the next. In general, primers should have a Tm of around 70°C to achieve optimal results in a two-step cycling program with a 68°C combined annealing/extension step. Therefore, whenever possible, primers should be at least 22 nucleotides (nt) long (25–30-mers are preferred) and should have a G-C content of 45–60%. Furthermore, the 3'-terminal ends of each primer should not be complementary to each other and should have a low G-C content.

B. Setting up the Reaction

Combine the following in a PCR tube on ice:

40 µl	PCR-Grade Water
5 µl	10X Advantage 2 PCR Buffer* or 10X Advantage 2 SA PCR Buffer*
1 µl	50X dNTP Mix (10 mM ea.)
1 µl	5' primer (10 µM)
1 µl	3' primer (10 µM)
1 µl	50X Advantage 2 Polymerase Mix
1 µl	DNA Template (100 ng/µl)
50 µl	**Total volume**

* Use either one of the two 10X Buffers shown. See the Advantage 2 PCR Kit User Manual for additional information.

C. Recommended Cycling Parameters

Use the following guidelines when setting up your initial experiments with Advantage PCR Products. These are general guidelines—the optimal pa-rameters may vary with different thermal cyclers and will depend on your particular primers, templates, and other experimental variables.

Target Size	**Cycle Parameters**	**Target Size**	**Cycle Parameters**
<1 kb:	• 95°C for 1 min • 25–35 cycles 95°C for 30 sec 68°C for 1 min • 68°C for 1 min	5–9 kb:	• 95°C for 1 min • 25–35 cycles 95°C for 30 sec 68°C for 6 min • 68°C for 6 min
1–5 kb:	• 95°C for 1 min • 25–35 cycles 95°C for 30 sec 68°C for 3 min • 68°C for 3 min	10–20 kb:	• 95°C for 1 min • 25–35 cycles 95°C for 30 sec 68°C for 12 min • 68°C for 12 min

D. Recommendations for Electrophoresis

Expected insert size range	Recommended % agarose	Recommended DNA size markers
0.3–1.5 kb	1.5	ΦX174/HaeIII
0.5–10 kb	1.2	1-kb DNA ladder
>5 kb	0.8	Φ/HindIII

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