RB PFU PCR

Standard Application

In a sterile, nuclease-free microcentrifuge tube, combine the following components:

Final Concentration

Pfu DNA Polymerase 10X Buffer with Mg^{+}	5µl	1X
dNTP mix, 10mM each	1µl	200µM each
upstream primer	5–50pmol	0.1–1.0µM
downstream primer	5–50pmol	0.1–1.0µM
DNA template	variable	<0.5µg/50µl
Pfu DNA Polymerase (2–3u/µl)	variable	1.25u/50µl
Nuclease-Free Water to final volume of	UP TO 50µl	

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Note:

1. It is critical to withhold Pfu DNA Polymerase until after the addition of dNTPs; otherwise, the proofreading activity of the polymerase may degrade the primers, resulting in nonspecific amplification and reduced product yield. Assemble on ice.

2. If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 drops

(approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reaction mix in a microcentrifuge for 5 seconds.

3. Immediately place the reactions in a thermal cycler that has been preheated to 95°C.

We recommend heating the samples at 95°C for 1–2 minutes to ensure that the target DNA is completely denatured. Incubation for longer than 2 minutes at 95°C is unnecessary and may reduce the yield due to DNA damage.

4. Start the thermal cycling program. The cycling profile given in Table 1 may be used

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as a guideline. Optimize the amplification profile for each primer/target combination. Table1 Recommended thermal cycling conditions for PfuDNA Polymerase-mediated

PCR amplification.

These guidelines apply to target sequences between 200 and 2,000bp and are

optimal for the Perkin-Elmer Thermal Cycler Model 480 or comparable thermal cyclers.

Step	**Temperature**	**Time**	**Number of Cycles**
Initial Denaturation	95°C	1–2 minutes	1 cycle
Denaturation Annealing* Extension**	95°C 42–65°C 72–74°C	0.5–1 minute 30 seconds 2–4 minutes	25–35 cycles
Final Extension	72–74°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers. See Section III for discussions on determining optimal annealing temperatures for PCR amplification.

**Allow approximately 2 minutes for every 1kb to be amplified

General Considerations

A. Enzyme Concentration

We recommend that 1.25 units of Pfu DNA Polymerase be used per 50µl amplification

reaction. The inclusion of more enzyme will increase the likelihood of primer degradation

due to the intrinsic 3′→5′ exonuclease (proofreading) activity. It is essential to withhold

Pfu DNA Polymerase from the reaction until after the addition of the dNTP mix and to

assemble components on ice.

B. Primer Design

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The sequences of the primers are a major consideration in determining the optimal temperature of the PCR amplification cycles. For primers with a high Tm, it may be advantageous to increase the annealing temperature. Higher temperatures minimize nonspecific primer annealing, increase the amount of specific product produced and reduce the amount of primer-dimer formation.

The 3′→5′ exonuclease activity may degrade primers. To overcome the degradation, longer primers with maximized GC content could be used. Primers can also be protected by introducing phosphorothioate bonds at their 3′ termini (6).

C. Extension Time

The extension rate of Pfu DNA Polymerase is lower than that of Taq DNA Polymerase. Therefore, during the extension step, allow approximately 2 minutes for every 1kb to be amplified (minimum extension time of 1 minute). For most reactions, 25–35 cycles are sufficient.

IV. Composition of Buffers and Solutions

Pfu DNA Polymerase 10X Reaction Buffer with MgSO4 (provided)

200mM Tris-HCl (pH 8.8 at 25°C)

100mM KCl

100mM (NH4)2SO4

20mM MgSO4

1mg/ml nuclease-free BSA

1% Triton® X-100

dNTP mix

10mM each of dATP, dCTP, dGTP and dTTP in water

PCR-tested dNTPs are available: PCR Nucleotide Mix and

dNTP