PicoMaxx High Fidelity PCR System

Instruction Manual

Catalog #600420 (100 U), #600422 (500 U), and #600424 (1000 U)

Revision D.0

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PicoMaxx High Fidelity PCR System

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MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #600420	Catalog #600422	Catalog #600424
PicoMaxx High Fidelity PCR System (2.5 U/µl)	100 U	500 U	1000 U
10× PicoMaxx Reaction Buffer[a]	1 ml	2 × 1 ml	4 × 1 ml

[a] The total Mg^{2+} concentration present in the final 1× dilution of the 10× PicoMaxx reaction buffer is 2 mM.

STORAGE CONDITIONS

All Components: –20°C

ADDITIONAL MATERIALS REQUIRED

Temperature cycler
PCR tubes
PCR primers
Deoxynucleotides

Revision D.0

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INTRODUCTION

The PicoMaxx high fidelity PCR system* is a blend of cloned *Taq* and *Pfu* DNA polymerases** and Agilent's ArchaeMaxx polymerase-enhancing factor. Together with a specially optimized buffer, this enzyme blend provides the highest success rate of any PCR enzyme, even higher than other *Taq*-based blends. The PicoMaxx high fidelity PCR system reliably produces high PCR product yields on a wide variety of templates up to 10 kb. It also provides superior sensitivity by successfully amplifying samples where starting material is limited.

The PicoMaxx high fidelity PCR system is formulated with antibodies that inhibit polymerase activity until cycling begins, promoting high-specificity and reduced background. This formulation is ideal for high-throughput, multi-target amplification experiments without requiring optimization solutions or protein enhancers that can decrease PCR efficiency. The PicoMaxx high fidelity PCR system detects low-copy-number targets in complex DNA backgrounds, amplifies difficult systems with minimal non-specific amplification, and works with PCR applications that require prolonged incubations at room temperature prior to thermal cycling.

OPTIMIZATION PARAMETERS

The PicoMaxx high fidelity PCR system reliably produces high PCR product yields on a wide variety of templates up to 10 kb. The PicoMaxx high fidelity PCR system can be introduced into all existing amplification systems optimized with *Taq* or *Taq2000* DNA polymerase, with only slight optimization of reaction and cycling conditions. For other systems, optimization may be required to achieve highest product yield and specificity. Critical optimization parameters include the quality and concentration of DNA template, primers, and deoxynucleotides; primer design; the concentration of enzyme; use of the recommended reaction buffer; and cycling parameters.

DNA Template Quality and Concentration

Successful amplification is dependent upon the purity, integrity, concentration, and molecular weight of the DNA template. Isolation of intact, high molecular weight genomic DNA may be achieved by using the Agilent DNA Extraction Kit or the RecoverEase DNA isolation kit. Potential shearing of the genomic DNA template is minimized by the use of wide-bore tips for pipetting or mixing of the template. Additionally, freezing of high molecular weight templates should be avoided; storage at 4°C is recommended. The length of an intact genomic DNA template should be >50 kb.

* U.S. Patent Nos. 6,734,293, 6,444,428, 6,183,997, and 5,948,663.

** U.S. Patent No. 5,948,663.

For amplifying genomic DNA templates, use 100–200 ng of template. To amplify low-complexity targets (for example, lambda DNA or cloned DNA), use 5–20 ng of template. Excess template DNA can inhibit the PCR reaction.

Primer Design and Concentration

Primers should be ≥20 bp in length with a balanced T_m ≥60°C. The resulting high annealing temperature promotes specificity and discourages secondary structure formation. Further, primer sequences should be analyzed for potential duplex and hairpin formation as well as false priming sites in order to obtain the highest yield of specific PCR products.

We suggest using primers at a final concentration of 0.3–0.5 µM, which is equivalent to ~100–250 ng of a 20- to 30-mer oligonucleotide primer in a 50-µl reaction volume.

Deoxynucleotide Concentrations

Amplification efficiencies are influenced by deoxynucleotide (dNTP) concentrations. Insufficient concentrations of dNTPs may result in lower yields. Generally, 200 µM of each dNTP is optimal. For targets ≥5 kb, the dNTP concentration may need to be increased to 500 µM each.

Enzyme Concentration

Robust product yield requires an adequate DNA polymerase concentration. The use of 2.5 U/50-µl reaction consistently generates high yields of PCR product. Longer templates may require 5 U of PicoMaxx enzyme per 50-µl reaction for optimal results.

Reaction Buffer

For optimal yield and specificity it is essential that the provided PCR buffer is used. Suboptimal results will be achieved using other buffers. The provided 10× PicoMaxx reaction buffer contains the magnesium ion concentration that is optimal for the enzyme. Adjusting the magnesium concentration is not recommended.

Cycling Parameters

As with all PCR reactions, cycling parameters are critical for successful amplification and may require further optimization.

Extension Time

For general applications, maintain an extension time of 1.0 minute/kb of template. Longer extension times of up to 90 seconds/kb of PCR target may produce higher yields for difficult targets, such as high-complexity targets or targets > 2 kb in length.

Annealing Temperature

If using an existing primer pair, the annealing temperature may require optimization in the 10× PicoMaxx reaction buffer provided.

1. Prepare a reaction mixture for the appropriate number of samples to be amplified. Add the following components *in order* and mix gently. Table I provides an example of a reaction mixture for the amplification of a typical single-copy chromosomal target. The recipe listed in Table I is for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl.

   Note *If the tube of 10× PicoMaxx reaction buffer contains a visible precipitate, heat the tube to 37°C for 2–3 mintues to redissolve the contents. Vortex briefly to mix. This heating step does not impact PCR results.*

TABLE I

Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification

Component	Amount per reaction
Distilled water (dH_2O)	40.6 µl
10× PicoMaxx reaction buffer[a]	5.0 µl
100 mM dNTP mix (25 mM of each dNTP)[b]	0.4 µl
DNA template (100 ng/µl)[c]	1.0 µl
Primer #1 (100 ng/µl)[d,e]	1.0 µl
Primer #2 (100 ng/µl)[d,e]	1.0 µl
PicoMaxx high fidelity PCR system	1.0 µl (2.5 U)
Total reaction volume	50 µl

[a] The total Mg^{2+} concentration present in the final 1× dilution of the 10× PicoMaxx reaction buffer is 2 mM.

[b] For targets ≥5 kb, the dNTP concentration may need to be increased to 500 µM each.

[c] The amount of DNA template required will vary depending on the type of DNA being amplified. Generally 100–200 ng of genomic DNA template is recommended. Less DNA template (typically 5–20 ng) can be used for amplification of lambda or plasmid PCR targets.

[d] Primer concentrations between 0.3 and 0.5 µM are recommended (generally 100–250 ng for typical 20- to 30-mer oligonucleotide primers in a 50-µl reaction volume).

[e] For best results, design primers to have similar melting temperatures (typically between 60° and 80°C).

2. Immediately before thermal cycling, aliquot 50 µl of the reaction mixture into the appropriate number of sterile 0.2-ml or 0.5-ml thin-wall PCR tubes or standard microcentrifuge tubes.

3. Perform PCR using the optimized cycling conditions in Table II.

TABLE II

PCR Cycling Parameters[a,b]

Segment	Number of cycles	Temperature	Duration
1	1	95°C	2 minutes
2	30–35	95°C	40 seconds
		Primer T_m – 5°C[c]	30 seconds
		72°C	1 minute/kb PCR target
3	1	72°C	10 minutes

[a] Thin-wall PCR tubes are highly recommended. These PCR tubes are optimized to ensure more efficient heat transfer and to maximize thermal-cycling performance.

[b] Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers; therefore, each manufacturer's recommendations for optimal cycling parameters should be consulted.

[c] If using existing primer pairs, the annealing temperatures may require optimization in the 10× PicoMaxx reaction buffer provided.

4. Analyze the PCR amplification products on a 1.0–4.0% (w/v) agarose gel by electrophoresis.

TROUBLESHOOTING

Observation	Suggestion(s)
No product or low yield	Ensure that 10× PicoMaxx reaction buffer is used.
	Increase the amount of PicoMaxx enzyme up to 5 U per 50-µl PCR reaction.
	Increase extension time to 90 seconds per kb of PCR target.
	Use intact and highly purified DNA templates.
	Increase the amount of full-length intact DNA template, adjust the ratio of primer versus template, and/or increase the number of cycles up to a maximum of 40 cycles to optimize yield of the desired product.
	Remove extraneous salts from the PCR primers and DNA preparations.
	For targets ≥5 kb, increase the dNTP concentration to 500 µM each.
	Check the melting temperature, purity, GC content, and length of the primers.
	Use the recommended primer concentrations between 0.3 and 0.5 µM (generally 100–250 ng for typical 20- to 30-mer oligonucleotide primers in a 50-µl reaction volume).
	Primer pairs with matching primer melting temperatures (T_m) and complete complementarity between the primer and template are recommended.
	If using existing primer pairs, the annealing temperatures may require optimization in the 10× PicoMaxx reaction buffer provided.
	Denaturation times of 30–60 seconds at 94–95°C are usually sufficient, while longer denaturation times may damage the DNA template. Use the shortest denaturation time compatible with successful PCR on the thermal cycler.
	Use thin-wall PCR tubes. These PCR tubes are optimized to ensure ideal contact with the block to permit more efficient heat transfer and to maximize thermal-cycling performance.
Artifactual smears	Increase the annealing temperature in 4°C increments.
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used.
Multiple bands	Increase the annealing temperature in 4°C increments.
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used.
	Use Perfect Match PCR enhancer to improve PCR product specificity.

REFERENCES

1. Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (1990). *PCR Protocols: A Guide to Methods and Applications.* Academic Press, New York.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.

PicoMaxx High Fidelity PCR System

Catalog #600420, 600422, 600424

QUICK-REFERENCE PROTOCOL

- Prepare reaction mixtures according to the table below, mix gently, and place in thin-walled PCR tubes.

Component	Amount per reaction
Distilled water (dH_2O)	40.6 µl
10× PicoMaxx reaction buffer	5.0 µl
100 mM dNTP mix (25 mM of each dNTP)	0.4 µl
DNA template (100 ng/µl)	1.0 µl
Primer #1 (100 ng/µl)	1.0 µl
Primer #2 (100 ng/µl)	1.0 µl
PicoMaxx high fidelity PCR system	1.0 µl (2.5 U)
Total reaction volume	50 µl

- Perform PCR using the cycling conditions according to the following table:

Segment	Number of cycles	Temperature	Duration
1	1	95°C	2 minutes
2	30–35	95°C	40 seconds
		Primer T_m – 5°C	30 seconds
		72°C	1 minute/kb PCR target
3	1	72°C	10 minutes

- Analyze the PCR amplification products by gel electrophoresis.