Easy-A High-Fidelity PCR Master Mix

Instruction Manual

Catalog #600640 (100 reactions), #600642 (400 reactions)

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

600640-12

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Easy-A High-Fidelity PCR Master Mix

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #600640[a]	Catalog #600642[b]
Easy-A 2× master mix (0.1 U/µl)[c]	2.5 ml (250 U)	4 × 2.5 ml (1000 U)

[a] Catalog #600640 provides enough PCR reagents for 100, 50µl PCR reactions.
[b] Catalog #600642 provides enough PCR reagents for 400, 50µl PCR reactions.
[c] The total Mg^{2+} concentration present in the final 1× dilution of the 2× Easy-A master mix is 2 mM. The total dNTP concentration present in the final 1× dilution is 800 µM (200 µM of each dNTP).

Storage: The Easy-A high-fidelity PCR master mix should be stored at –20°C upon receipt. Store the PCR master mix at 4°C after thawing. Once thawed, full activity is guaranteed for 3 months.

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INTRODUCTION

The Easy-A high-fidelity PCR master mix*, is a 2× formulation of the Easy-A high-fidelity PCR cloning enzyme*, an optimized PCR reaction buffer, magnesium, and dNTPs. The Easy-A high-fidelity PCR cloning enzyme is a proprietary thermostable DNA polymerase preparation specifically designed for improved cloning with the StrataClone PCR Cloning Kit or with the TOPO TA Cloning® vector and other T-/U-vectors.‖ The Easy-A PCR cloning enzyme possesses both terminal transferase and exonuclease activities, generating PCR products containing 3´-A overhangs with a higher rate of accuracy. This enables 5-minute, high-fidelity cloning into T-vectors and U-/U*-vectors with an efficiency equivalent to that of *Taq* DNA polymerase. Additionally, the enzyme is provided in an antibody-based hotstart format for increased PCR sensitivity and yield from a variety of templates, while allowing room temperature setup. The Easy-A enzyme amplifies targets up to 10 kb from plasmid DNA and up to 5 kb from genomic DNA.

PCR PROTOCOL

1. Add the PCR reaction components in order while mixing gently. Table I provides an example reaction mixture for the amplification of a typical single-copy chromosomal target. The recipe listed is for one reaction and must be adjusted for multiple samples.

TABLE I Reaction Mixture for a Typical PCR Amplification

Component	Amount per reaction
Distilled water (dH_2O)	22 µl
Primer #1 (100 ng/µl)[a]	1 µl
Primer #2 (100 ng/µl)[a]	1 µl
DNA template (100 ng/µl)[b]	1 µl
Easy-A 2× master mix	25 µl
Total reaction volume	50 µl

[a] Primer concentrations between 0.2 and 0.5 µM are recommended (this corresponds to 100–250 ng for typical 18- to 25-mer oligonucleotide primers in a 50-µl reaction volume).
[b] The amount of DNA template required varies depending on the type of DNA being amplified. Generally 50–100 ng of genomic DNA template is recommended. Less DNA template can be used for amplification of lambda (1–30 ng) and vector (0.1–10 ng) PCR targets or for amplification of multicopy chromosomal genes (10–100 ng).

2. Aliquot 50 µl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes or standard 0.5-ml microcentrifuge tubes.
3. Perform PCR using optimized cycling conditions. Suggested cycling parameters are indicated in Table II.
4. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.
5. Use 2 µl of PCR product for cloning into the StrataClone PCR Cloning Kit vector arms. Use 0.5–1.5 µl of PCR product for cloning into T-/U-vectors, following the manufacturer's recommendations.

*,‖ See *Endnotes*.

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TABLE II PCR Cycling Parameters for a Typical PCR Amplification[a,b]

Segment	Number of Cycles	Temperature	Duration
1	1	95°C	2 minutes
2	30	95°C	40 seconds
		Primer T_m – 5°C[c]	30 seconds
		72°C	1 minute for targets ≤ 1 kb; 1 minute per kb for targets >1 kb and ≤ 5 kb
3	1	72°C	7 minutes

[a] Thin-wall PCR tubes are highly recommended.

[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] The annealing temperature may require optimization. Typically annealing temperatures will range between 55° and 72°C.[1]

TROUBLESHOOTING

Observation	Solution(s)
No product or low yield	Increase extension time to 2 minutes per kb of PCR target.
	Use cosolvents such as DMSO in a 1–10% (v/v) final concentration for GC-rich templates.
	Lower the annealing temperature in 5°C increments.
	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.
	Remove extraneous salts from the PCR primers and DNA preparations.
	Use the recommended primer concentrations between 0.3 and 0.5 µM (corresponding to 100–200 ng for typical 18- to 25-mer oligonucleotide primers in a 50-µl reaction volume).
	Check the melting temperature, purity, GC content, and length of the primers.
Multiple bands	Increase the annealing temperature in 5°C increments.
Artifactual smears	Reduce the extension time.

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.

ENDNOTES

* U.S. Patent Nos. 6,734,293, 6,444,428, 6,183,997.

‖ Use of these cloning vector products may require licenses from third parties in certain countries.

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MSDS INFORMATION

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