Easy-A High-Fidelity PCR Cloning Enzyme

Instruction Manual

Catalog #600400, #600402, and #600404

Revision C.0

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600400-12

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MATERIALS PROVIDED

Materials provided	Quantity [a]		
	Catalog #600400	Catalog #600402	Catalog #600404
Easy-A high-fidelity PCR cloning enzyme (5 U/µl)	100 U	500 U	1000 U
10× Easy-A reaction buffer[b]	1 ml	2 × 1 ml	4 × 1 ml

[a] Catalog #600400 provides sufficient reagents for 40 × 50-µl reactions. Catalog #600402 provides sufficient reagents for 200 × 50-µl reactions. Catalog #600404 provides sufficient reagents for 400 × 50-µl reactions.

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

STORAGE CONDITIONS

All components: –20°C

ADDITIONAL MATERIALS REQUIRED

Thermal cycler
Thin-walled PCR tubes or PCR plates[||]
PCR primers
Deoxynucleotides (Agilent Catalog #200415)

[||] For the Agilent SureCycler 8800, use Agilent tube strips and caps (Catalog #410082 and #410086) or Agilent PCR plates (Catalog #401333 for 96-well plates and Catalog #410188 for 384-well plates). If using plates, seal the reactions with adhesive film (Catalog #410186) and a compression mat (Catalog #410187)

Revision C.0

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INTRODUCTION

The Easy-A high-fidelity PCR cloning enzyme* is a proprietary thermostable DNA polymerase formulation specifically designed for improved cloning with the StrataClone PCR Cloning Kit (see Figure 1) 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 high-fidelity PCR products containing 3´-A overhangs. This enables 5-minute, high-fidelity cloning into T-vectors and U-vectors with an efficiency equivalent to that of *Taq* DNA polymerase. The Easy-A PCR cloning enzyme is as easy to use as *Taq* DNA polymerase, so special optimization is not required. Additionally, the enzyme is provided in an antibody-based "hot start" 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.

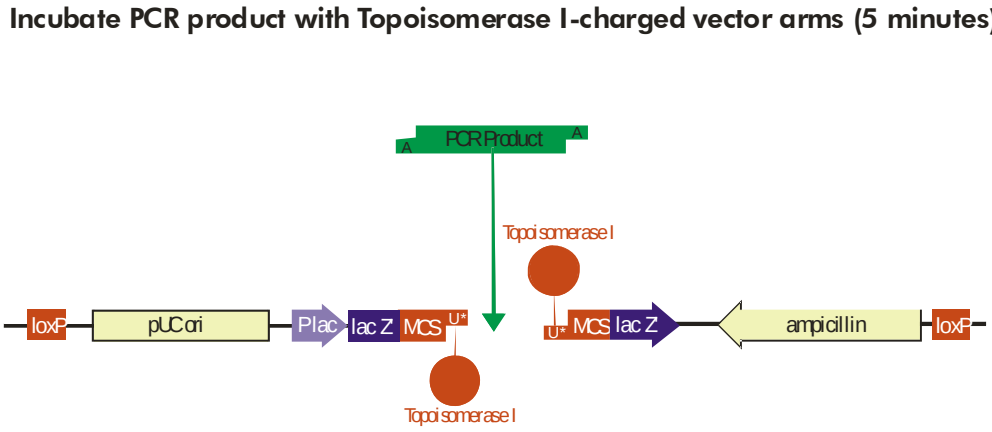

FIGURE 1 The Easy-A PCR cloning enzyme produces amplification products that are ready to clone directly into the StrataClone PCR Cloning Kit vector arms (shown), or into T-/U-vectors (not shown)

* 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.

Terminal Transferase Activity

The Easy-A PCR cloning enzyme possesses a terminal transferase activity identical to that of *Taq* DNA polymerase, which preferentially adds a single 3´-A overhang to each strand of the PCR product.[1] PCR products amplified with the Easy-A enzyme are ready to clone immediately after PCR without the post-PCR A-addition steps that are required for all other proofreading DNA polymerases.

Exonuclease Activity

Unlike *Taq* DNA polymerase, the Easy-A PCR cloning enzyme has 3´- to 5´-exonuclease activity (proofreading) for accurate amplification (see Table I). The proofreading activity of this enzyme does not affect terminal transferase activity. The Easy-A enzyme amplifies products with high fidelity, second only to Agilent's *PfuUltra/PfuUltra II* high-fidelity DNA polymerase and equivalent to *Pfu* DNA Polymerase (see Table II). The error rate of the Easy-A PCR cloning enzyme is six times lower than *Taq* DNA polymerase, and two to three times lower than other proofreading archaeal DNA polymerases.

Hot Start Capability

The Easy-A PCR cloning enzyme is formulated with a combination of antibodies that, at room temperature, effectively neutralize DNA polymerase and 3´- to 5´-exonuclease activities. Full enzyme activity is regained upon denaturation of the antibody during the initial denaturation step. Preventing priming until stringent primer annealing temperatures are reached provides reduced background and improved detection sensitivity.

TABLE I

Potential PCR Cloning Errors Using *Taq* DNA Polymerase versus Easy-A High-Fidelity PCR Cloning Enzyme

PCR Product Size	Clones with Errors[a]	
	Easy-A high-fidelity PCR cloning enzyme	*Taq* DNA polymerase
250 bp	0.65 %	4.0 %
500 bp	1.3 %	8.0 %
1000 bp	2.6 %	16.0 %
2500 bp	6.5 %	40.0 %
5000 bp	13.0 %	80.0 %

[a] The calculated percentage of mutated PCR products after amplification of target sequences of various sizes for 20 effective cycles (2^{20}- or 10^6-fold amplification), based on measured enzyme error rates. Some PCR products will exhibit more than one error.

TABLE II

Comparison of Thermostable DNA Polymerases Using a *lacI*OZα-Based Fidelity Assay[a]

Thermostable DNA polymerase	Error rate[b]	Percentage (%) of mutated 1-kb PCR products[c]
PfuUltra/*PfuUltra II* high-fidelity DNA polymerases	4.3×10^{-7}	0.9
Pfu/Herculase II/*PfuTurbo* DNA polymerases	1.3×10^{-6}	2.6
Easy-A high-fidelity PCR cloning enzyme	1.3×10^{-6}	2.6
Tgo DNA polymerase	2.1×10^{-6}	4.3
Deep Vent$_R$® DNA polymerase	2.7×10^{-6}	5.4
Vent$_R$® DNA polymerase	2.8×10^{-6}	5.6
PLATINUM® *Pfx*	3.5×10^{-6}	5.6
KOD DNA polymerase	3.5×10^{-6}	5.6
Taq DNA polymerase	8.0×10^{-6}	16.0

[a] Fidelity is measured using a published PCR forward mutation assay that is based on the *lacI* target gene.[2]

[b] The error rate equals mutation frequency per base pair per duplication.

[c] The percentage of mutated PCR products after amplification of a 1-kb target sequence for 20 effective cycles (2^{20}- or 10^6-fold amplification).

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

TABLE III

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

Component	Amount per reaction
Distilled water (dH_2O)	41.1 µl
10× Easy-A reaction buffer[a]	5.0 µl
dNTPs (25 mM each dNTP)	0.4 µl
DNA template (100 ng/µl)[b]	1.0 µl
Primer #1 (100 ng/µl)[c]	1.0 µl
Primer #2 (100 ng/µl)[c]	1.0 µl
Easy-A high-fidelity PCR cloning enzyme (5 U/µl)	0.5 µl (2.5 U)
Total reaction volume	50 µl

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

[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).

[c] 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).

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. See *Additional Materials Required* for plasticware recommendations.

3. Perform PCR using optimized cycling conditions. Suggested cycling parameters are indicated in Table IV.

4. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

5. Use 0.5–1.5 µl of PCR product for cloning into T-/U-vectors, following manufacturer's recommendations.

TABLE IV

PCR Cycling Protocol [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	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 (Agilent Catalog #410082 [tube strips] and #410086 [tube cap strips]). These PCR tubes are optimized to ensure more efficient heat transfer and to maximize thermal-cycling performance.

[b] The provided cycling protocol has been optimized for the Agilent SureCycler 8800. 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.

TROUBLESHOOTING

Observation	Suggestion
No product or low yield	Increase extension time to 2 minutes per kb of PCR target
	If using the Agilent SureCycler 8800 to run PCR in plates sealed with film and a compression mat, decrease the denaturation time during cycling to 3–10 seconds whenever the reaction volume is <50 µl.
	Ensure that 10× Easy-A reaction buffer is used.
	Increase the amount of the Easy-A high-fidelity PCR cloning enzyme up to 5U per 50-ul PCR reaction.
	Use cosolvents such as DMSO in a 1–10% (v/v) final concentration for GC-rich templates
	Consider using the adjuncts [e.g., use 1–2 U of Perfect Match PCR enhancer or a low concentration (1–5%) of formamide].
	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.
	Lower the annealing temperature in 5°C increments.
	Remove extraneous salts from the PCR primers and DNA preparations.
	Use the recommended primer concentrations between 0.2 and 0.5 µM (corresponding to 100–250 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.
	Use thin-walled PCR tubes for the SureCycler 8800 (Agilent Catalog #410082 and #410086). These PCR tubes are optimized to maximize thermal-cycling performance.
	Use non-skired PCR plates for the SureCycler 8800 (Agilent Catalog #401333 [96-well] and #410188 [384-well]). Seal the plates with adhesive film (#410186) and a compression mat (#410187).
	Increase the total Mg^{2+} concentration above 2 mM.
Multiple bands	Increase the annealing temperature in 5°C increments.
	Use Perfect Match PCR enhancer (Agilent Catalog #600129) to improve PCR product specificity.
Artifactual smears	Decrease the amount of the Easy-A high-fidelity PCR cloning enzyme.
	Reduce the extension time utilized.

REFERENCES

1. Hu, G. (1993) *DNA Cell Biol* 12(8):763-770.
2. Cline, J., Braman, J. C. and Hogrefe, H. H. (1996) *Nucleic Acids Res* 24(18):3546-51.
3. 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

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