ARTICLE

PCR: Achieving Better Results at a Lower Cost

DNA amplification by PCR has become a universally used procedure in molecular biology. Despite the availability of other thermostable DNA polymerases, Taq DNA polymerase remains, in many cases, the best choice for routine PCR due to its proven reliability. However, many experimental variables affect the quality of PCR results, including purity and activity of the Taq preparation used. Even trace amounts of contaminating DNA can result in high nonspecific amplification backgrounds, particularly when using generic primers. Residual exo- or endonucleases can cause template degradation, truncated products, or complete loss of the target region.

Since many labs do thousands of PCR reactions per year, Taq DNA polymerase cost, as well as performance, is often an important consideration. Expiration of the core PCR patents* and related legal decisions have recently removed barriers to cost reduction in supplying Taq for research use. With these barriers removed, Lucigen has applied its expertise in enzyme manufacturing to develop EconoTaq™ DNA Polymerase as the best choice for routine PCR applications.

At less than 9¢ per unit list price (quantity discounts available), EconoTaq is the lowest priced Taq DNA Polymerase. EconoTaq's very low price is coupled with high quality and performance when compared to more expensive Taq DNA polymerase preparations from other suppliers, or to other thermostable DNA polymerases often touted as superior to Taq in PCR.

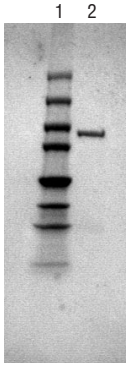

Figure 1. High purity of EconoTaq DNA Polymerase (SDS PAGE). Lane 1, broad range molecular weight markers; lane 2, Lucigen EconoTaq DNA Polymerase

EconoTaq Purity

QC specifications for EconoTaq are rigorous:

- Greater than 99% pure by SDS gel electrophoresis (see Figure 1).
- No detectable DNA contamination as determined by PCR using generic primers.
- No detectable endonuclease (nicking) activity. Incubation of 10 U of EconoTaq DNA Polymerase with 1 µg of supercoiled pBR322 DNA for 16 hours at 70ºC results in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.
- No detectable exonuclease activity. Incubation of 10 U of EconoTaq DNA Polymerase with 1 µg of HindIII-cut lambda DNA for 16 hours at 70ºC results in no smearing of bands on agarose gels.

EconoTaq Performance

As shown in Figures 2 and 3 (next page), Lucigen's EconoTaq DNA Polymerase performs as well as, or better than, more expensive Taq preparations from several other suppliers in routine PCR. EconoTaq is as effective in DNA amplification as another standard PCR enzyme, Tfl DNA polymerase (Figure 4). In this case, the background of non-specific amplification was much lower with EconoTaq (compare "+" and "–" lanes for EconoTaq and Tfl in Figure 4). EconoTaq DNA Polymerase also offers high lot-to-lot reproducibilityand reliability (Figures 2 and 4).

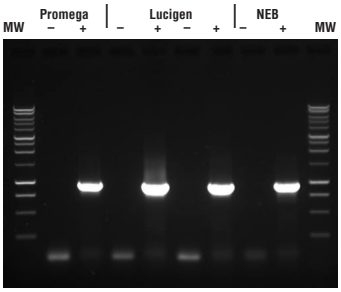

Figure 2. Taq DNA polymerase from Promega and New England Biolabs were compared to Lucigen's EconoTaq DNA Polymerase (2 different lots) in amplifying the ampicillin gene (0.8 kb) in a pUC19 vector. (–), no DNA. (+), DNA added (40 ng). MW, 1 kb ladder.

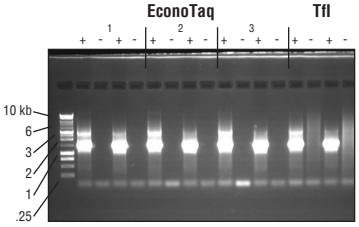

Figure 4. PCR amplification was performed under standard conditions using three different lots of EconoTaq DNA Polymerase and buffer, or duplicate reactions with TfI DNA polymerase and buffer (Promega). Reactions contained primers specific for the 16S ribosomal RNA gene, with *Bacillus* genomic DNA (+) or no DNA (-) as a template (1450 bp product expected).

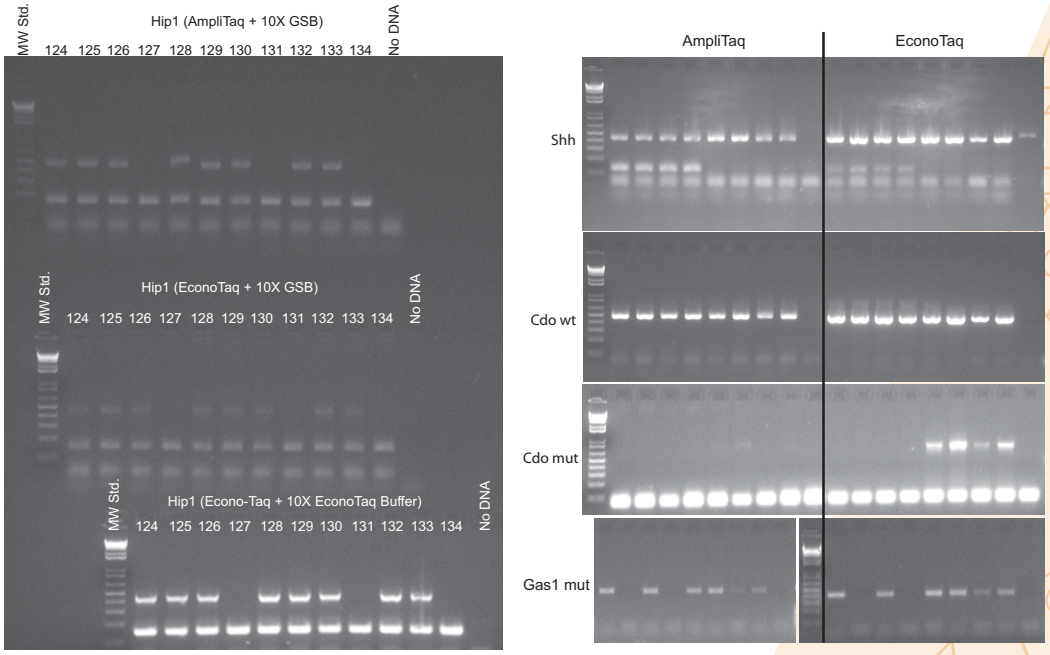

Figure 3. EconoTaq vs. AmpliTaq® (Applied Biosystems) DNA polymerase in genotyping.
All PCR reactions were performed in a RoboCycler 96 (Stratagene). Hip1 genotyping was performed using the following PCR conditions: 94°C for 1min, 35 cycles of 94°C for 30sec, 62°C for 60sec, 72°C for 90sec, and 72°C for 7min. Shh, Cdo and Gas1 genotyping were performed using the following PCR conditions: 94°C for 2min, 35 cycles of 94°C for 60sec, 65°C for 60sec, 72°C for 90sec, and 72°C for 7min. All PCR reactions contained final concentrations of 1µM of each primer, 200µM dNTPs, 1X cresol red loading dye, and 1U of the indicated Taq polymerase. Sequences for all PCR primers have been previously published (references available).
Data courtesy of Dr. Benjamin Allen, Dept. Molecular & Cellular Biology, Harvard University.

EconoTaq Specifications

Concentration: 5 units/µl. One unit catalyzes the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 70°C in 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM dGTP, dATP, dTTP dCTP (a mix of unlabeled and [^{33}P] dCTP), 10 µg of Activated Calf Thymus DNA, and 0.1 mg/ml BSA.
Storage Buffer: 10 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.1% Triton X-100, 0.1 mM EDTA, 1mM DTT, and 50% glycerol.
10X Reaction Buffer: 100mM Tris-HCl (pH 9.0), 500 mM KCl, 15 mM $MgCl_2$, and 1% Triton X-100.

Order Information

EconoTaq DNA Polymerase is provided with 10X Reaction Buffer containing the magnesium concentration optimal for routine PCR (see Specifications above).

Products	Cat. No.	Size
EconoTaq DNA Polymerase	30031-1	1,000 U

***Please Note:** Some applications in which Lucigen's EconoTaq DNA Polymerase can be used may be covered by patents issued and applicable in the United States and certain other countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application in which the product is used. The PCR process is the subject of European Patent Nos. 201,184 and 200,262 owned by Hoffman-LaRoche. Those patents expired on March 28, 2006. The corresponding PCR process patents in the United States expired on March 29, 2005. It is the sole responsibility of the buyer to ensure that use of the product does not infringe the patent rights of third parties.

FREE SAMPLE!

(US & Canada only)

A free sample (250 units; 50 reactions) of EconoTaq DNA Polymerase (includes 10X Reaction Buffer) is available on request with an order of any other Lucigen product. Simply request catalog number 30031-0. Limited to one free sample per customer.