

EasyStart™ PCR Mix-in-a-Tube Provides Increased Specificity for a Wide Range of Genomic DNA Templates

ABSTRACT

EasyStart tubes, a new product from Molecular Bio-Products, inc., were tested to determine their ability to amplify genomic DNA of various lengths and from a range of species. These results were compared to those from standard PCR using the same template DNA to determine the efficacy of this new product. In each instance, EasyStart routinely produced better results than standard PCR.

INTRODUCTION

Often times, commercially available PCR products have the capability to only amplify DNA of certain lengths. EasyStart, however, has the ability to amplify fragments from as small as a few base pairs to fragments over 2kb. With its pre-aliquoted MgCl₂ (2mM), PCR buffer, dNTPs, and dH₂O, EasyStart provides a virtually contamination-free, one-step PCR protocol for amplifying almost any DNA template.

EasyStart's innovative design allows for increased accuracy and yield in PCR while decreasing the time and contamination risk associated with PCR. When working with genomic DNA, this heightened specificity is particularly important due to the difficulty of amplifying this class of DNA.

OBJECTIVE

To prove EasyStart's ability to amplify various lengths of DNA fragments, six different genomic DNA templates were used ranging from 409 bp to 2.5 kb. In addition, EasyStart's results were then compared to those when amplifying the same fragments via standard PCR.

MATERIALS AND METHODS

In each experiment, three 50 µl EasyStart

reactions and three 50 µl standard PCR reactions were performed on various genomic DNA templates utilizing specified primers and 1 Unit of Taq DNA Polymerase (Perkin-Elmer). The EasyStart reactions were performed according to the recommended protocol from Molecular Bio-Products; standard PCR reactions were performed using a mineral oil overlay and reagents from Perkin-Elmer following protocols listed in [PCR Protocols](#) (Innis, et.al.).

Thermal cycling consisted of one minute at 94°C followed by 30 cycles: 94°C, one minute; 56°C, one minute; 72°C, one minute.

The resulting PCR products were analyzed on a 1% agarose gel, run at 100V for 30 minutes.

Experiment #1

Drosophila hsp70 gene (1 kb) was amplified using primers H705 and H703¹.

Experiment #2

In this experiment, Rat p53 pseudogene was the target DNA. This 443 fragment was amplified using RP5 and RP3 primers².

Experiment #3

The 2.5 kb long Zea Mays polyubiquitin (Corn genomic DNA) fragment was amplified using AC1382 and AC3583³.

Experiment #4

Wheat genomic DNA, *Triticum rubisco L* (1446 bp), was amplified using primers RU5 and RU3⁴.

Experiment #5

Chick ovalbumin (1.5 kb), a difficult gene to copy, was amplified using primers OV5 and OV3⁵.

Experiment #6

Human BRCA1 (409 bp), the oncogene studied in many breast cancer research centers, was amplified using primers BR5 and BR3⁶.

RESULTS

In each experiment, EasyStart tubes provided equal or superior results when com-

pared with the standard PCR reactions performed on the same DNA templates. In fact, the 2.5kb Zea Mays template could only be amplified using EasyStart tubes (*figure 3*).

While EasyStart maintained highly specific results, most of the standard PCR reactions were complicated by primer dimerization. This problem was particularly visible in the Zea Mays, *Triticum rubisco*, and chicken ovalbumin samples (*figures 3-5*).

Overall, EasyStart provided significantly better specificity and yield with a much simpler protocol.

DISCUSSION

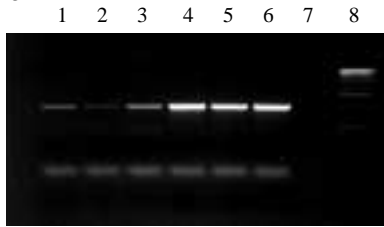
When compared to standard PCR, EasyStart gives superior results regardless of the template size. This improved accuracy is a result of the reduced contamination risk and facilitated hot start reaction in each EasyStart tube. By preventing the competitive side reactions which can detract from PCR results, EasyStart significantly improves reaction yield with routine and hard-to-copy DNA.

Additionally, these tests confirm that EasyStart's standard MgCl₂ concentration of 2mM is optimum even with this wide range of DNA samples. Nearly all reaction conditions can be satisfied with this amount and if higher concentrations are required, additional MgCl₂ can be added to the upper layer reagent mix.

Because routine PCR requires the addition of many more reagents, there is a greater potential for contamination. EasyStart solves this problem with its pre-aliquoted, non-specific reagents. Furthermore, standard PCR does not have the specificity and yield of EasyStart, therefore mis-primers, primer dimers, and premature annealing can easily distract from the target DNA.

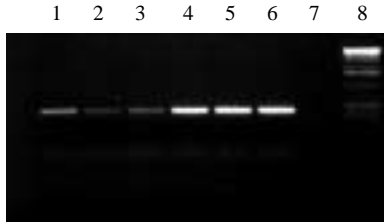
From these experiments, it is clear that overcoming the specificity problems associated with PCR of genomic is simple with EasyStart.

Figure 1



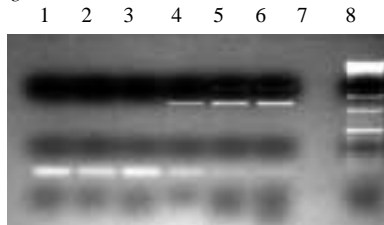
Drosophila hsp70 (1kb): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

Figure 2



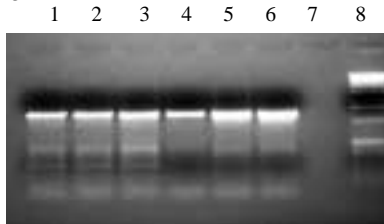
Rat p53 pseudogene (443bp): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

Figure 3



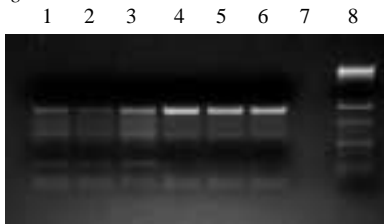
Zea Mays polyubiquitin (2.5kb): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

Figure 4



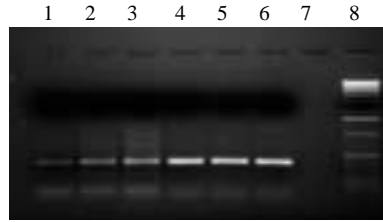
Triticum rubisco L (wheat genomic DNA: 1446bp): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

Figure 5



Chicken ovalbumin (1.5kb): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

Figure 6



Human BRCA1 (409bp): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder

REFERENCES

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