Recombinant Clone Screening Using the GoTaq® Hot Start Green Master Mix

ABSTRACT We describe using the new GoTaq® Hot Start Green Master Mix to screen recombinant clones from single colonies on a plate and from plasmid minipreps. The master mix formulation contains direct-to-gel loading components, which provide convenience and allow fast PCR setup. With the GoTaq® Hot Start Polymerase technology, reactions can be assembled and stored at room temperature for up to 24 hours prior to cycling.

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Whether you are using single-colony PCR or DNA minipreps, the master mix format makes reaction assembly fast.

INTRODUCTION

The GoTaq® Hot Start Green Master Mix allows easy reaction setup at room temperature and direct loading of the amplified product onto a gel. The convenient 2X mix contains all necessary components for PCR: GoTaq® Hot Start Polymerase, GoTaq® Green Reaction Buffer, 0.4 µM of each dNTP and 4 mM Mg^{2+}. To assemble reactions just add template, primers, and water. GoTaq® Hot Start Polymerase uses proprietary antibodies that render the enzyme inactive until the initial 2-minute 95 °C denaturation step of the thermal cycling protocol restores polymerase activity (1). This allows reactions to be set up at room temperature and sit at room temperature for up to 24 hours before cycling (1). To eliminate sample preparation time prior to electrophoresis, the mix contains blue and yellow tracking dyes and a compound to increase density of the sample so that it will sink into the well (2). Here we describe high-throughput screening of recombinant clones from both crude single-colony plasmid suspensions and plasmid minipreps using the GoTaq® Hot Start Green Master Mix. We show the robustness of the mix in producing amplicons of various sizes using different primer pairs with the same amplification protocol.

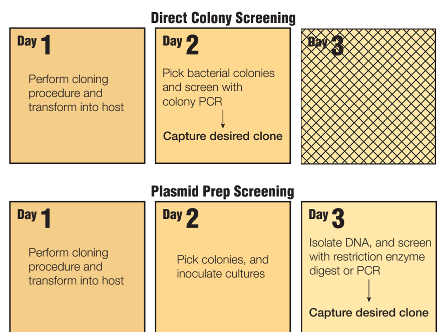

Figure 1. Using the GoTaq® Hot Start Green Master Mix to screen recombinant clones directly from colonies saves one full day.

RECOMBINANT CLONE HIGH-THROUGHPUT SCREENING

A fast and easy method is desirable when screening large sets of recombinant clones. PCR is a fast method to screen single bacterial colonies either directly or after preparing plasmid minipreps. PCR screening of colonies decreases the screening time by one full day (Figure 1). Whether you are using single-colony PCR or DNA minipreps, the master mix format makes reaction assembly fast. The hot-start technology means reactions can sit at room temperature for up to 24 hours before cycling, which can be important for high-throughput platforms.

COLONY PICKING FOR CRUDE PLASMID PREPARATION

Twenty four Flexi® clones with different backbones and protein-coding regions were transformed in *E. coli* and then plated the cells. Whole colonies, 1 mm in diameter, were picked using a pipette tip, suspended in 100 µl of Nuclease-Free Water, and incubated at 95 °C for 5 minutes. We found that 5 µl of colony suspension was needed for precise detection of the desired Flexi® clones in a 50 µl amplification.

There are various picking methods for colony PCR (3–5). Colony size and the amount of colony suspension added to reactions can have a significant impact on results (data not shown). The procedure described here allowed us to reliably detect a majority of plasmid targets using our PCR parameters. For colony PCR screening, we recommend determining the best colony-picking method for your application.

IMMEDIATE VERSUS DELAYED CYCLING

To compare the results of amplification performed immediately after reaction assembly to those of reactions that were held for 24 hours at room temperature, we picked colonies and assembled two sets of

reactions. The six primer sets we used were designed to have similar melting temperatures. The annealing temperature was determined by subtracting 4 °C from the lowest primer melting temperature. The extension time was based on 1 kb/minute for the longest PCR amplicon size. We amplified one set of reactions immediately after assembly. The second set was incubated at room temperature for 24 hours before cycling. After amplification, the samples were loaded directly onto an agarose gel for electrophoresis and visualized with ethidium bromide staining.

All reactions produced amplicons of expected size, which ranged from 0.5 kb to 3 kb (Figure 2). The yield was similar between reactions that were cycled immediately and those that were held at room temperature for 24 hours. Note that yield may vary in colony PCR as a result of amplicon size, crude template preparation or amount of template. In the method we used, the amount of template added to each reaction is not known. However, you can determine a detection limit for your application by performing serial dilutions of the colony suspension if necessary (6). Most importantly, the robustness of the GoTaq® Hot Start Green Master Mix allows a wide size range of amplimers to be amplified using different primer pairs on the same plate.

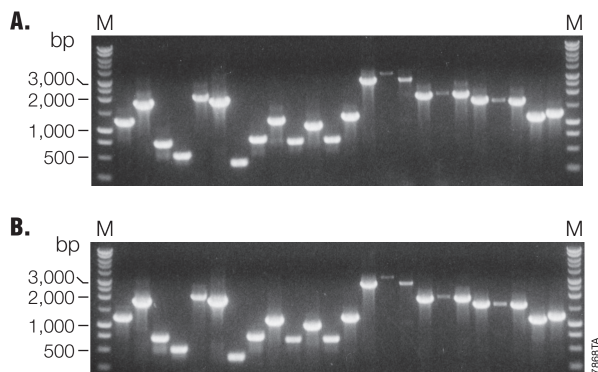

Figure 2. Amplification from colony suspension. Twenty four Flexi® plasmids with various Flexi® Vector backbones and inserts were transformed into JM109 cells. Colonies were picked, suspended in 100 µl Nuclease-Free Water and heated at 95 °C for 5 minutes. Primers flanking the insert region (0.2 µM each), GoTaq® Hot Start Green Master Mix and 5 µl of colony suspension were assembled at room temperature in a 50 µl reaction in 96-well plates. Six different primer pairs were used. **Panel A.** Reactions cycled immediately after preparation. **Panel B.** Reactions incubated on the lab bench for 24 hours at room temperature before cycling. Cycling parameters starting with a room-temperature instrument: 1 cycle (2 minutes at 95 °C), 30 cycles (10 seconds at 95 °C, 20 seconds at 50 °C, and 4 minutes at 72 °C), and 1 cycle (5 minutes at 72 °C). Lanes M, BenchTop 1kb DNA Ladder (Cat.# G7541).

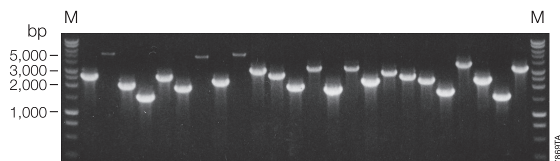

Figure 3. Amplification from DNA minipreps. Twenty four Flexi® plasmids with various protein-coding regions were isolated using Wizard® SV 96 Plasmid DNA Purification System (Cat.# A2255). DNA was diluted 1:200, and 2 µl was added to each reaction. Total DNA concentrations ranged from 0.3 ng to 1.2 ng per well. Primers flanking the insert (0.2 µM each), GoTaq® Hot Start Green Master Mix and Nuclease-Free Water were added to diluted DNA for 50 µl reactions in 96-well plates. Cycling parameters: 1 cycle (2 minutes at 95 °C), 30 cycles (10 seconds at 95 °C, 20 seconds at 50 °C, and 5 minutes at 72 °C), and 1 cycle (5 minutes at 72 °C). Lanes M, BenchTop 1kb DNA Ladder (Cat.# G7541).

RECOMBINANT CLONE SCREENING WITH DNA MINIPREPS

DNA minipreps from recombinant clones also can be screened with the GoTaq® Hot Start Green Master Mix. We isolated 24 Flexi® Vector clones with different protein-coding regions from bacterial cultures. After template dilution, reactions were set up at room temperature and amplified using an extension time of 5 minutes to ensure the longest amplicon was full-length (based on 1 kb/minute extension and the longest expected amplicon size). The reactions were loaded directly onto an agarose gel for electrophoresis and visualized with ethidium bromide staining. With this technique, we successfully amplified fragments ranging in size from 1.5 kb to 5.2 kb (Figure 3).

SUMMARY

GoTaq® Hot Start Green Master Mix can be used to successfully screen recombinant clones starting with either crude colony preparations or DNA plasmid minipreps in a high-throughput method. The mix is robust enough to amplify a wide range of fragment sizes using different primer pairs and the same cycling parameters. From single-colony suspensions, amplicons of 3 kb or smaller can be amplified. Amplicons up to 5.2 kb in length have been amplified using plasmid miniprep DNA. The GoTaq® Hot Start Green Master Mix formulation with both direct gel loading and hot-start polymerase technology is convenient to use and can save valuable time in your clone-screening application.

ACKNOWLEDGMENTS

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ORDERING INFORMATION

Product	Size	Cat.#
GoTaq® Hot Start Polymerase*	100 u	M5001
GoTaq® Hot Start Green Master Mix*	100 rxn	M5122
GoTaq® Hot Start Colorless Master Mix*	100 rxn	M5132

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* Additional sizes available.

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