Analyses of gene disruption by whole-cell PCR using the GoTaq® Green Master Mix

ABSTRACT

We used GoTaq® Green Master Mix for PCR directly from yeast colonies to analyze targeted gene disruptions in S. pombe.

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Introduction

We used Promega GoTaq® Green Master Mix to check the targeted deletion of genes from the genome of fission yeast, *S. pombe*. We tested the targeted disruption of *ase1+*, encoding a conserved microtubule-bundling protein(1) (2) . GoTaq® Green Master Mix is a premixed, ready-to-use solution containing GoTaq® DNA Polymerase, dNTPs, $MgCl_2$, reaction buffers and gel loading solution.

Materials and Methods

An *S. pombe* strain was transformed with a DNA fragment carrying the *kanMX6* cassette, conferring G-418 resistance, flanked by *ase1* target sequences(1) . Colonies resistant to G-418 were screened by PCR for correct disruption of the *ase1+* gene.

S. pombe strains were maintained in YE medium(3) . The oligonucleotide primers designed to check the disruption of *ase1+* were obtained from IDT DNA technology (Coralville, IA) and are as follows:

Ase1 5´FL: 5´ ttactttcgcaggaaaaggc 3´
KanMXRev: 5´ ccggcgcaggaacactgcc 3´

We tested reactions in a final volume of either 10µl or 20µl. In our experience, the 20µl reactions worked best. For each primer, 0.5µl of a 20µM stock was added to each reaction and was diluted to 10µl with water. To this primer mix, 10µl of GoTaq® Green Master Mix was added to have a final volume of 20µl per reaction. A toothpick of cells was added to the reactions, and PCR was performed under the following conditions: 95°C for 5 minutes; 12 cycles of 95°C for 45 seconds, 60°C for 1 minute and 72°C for 2.5 minutes; 25 cycles of 95°C for 45 seconds, 52°C for 45 seconds and 72°C for 2.5 minutes. The reaction products were separated on a 1% agarose gel and visualized.

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Results

The results of the PCR are shown in Figure 1. Lanes 1, 2 and 6 contain a PCR product of ~800bp representing the disruption of *ase1+* with *kanMX6*. Lanes 9 and 10 represent positive and negative control for the disruption, respectively. The low molecular weight bands presumably represent excess primers and/or denatured DNA from the reactions. As shown in Figure 1, the GoTaq® Green Master Mix is very efficient in the amplification of DNA fragments from yeast colonies. In summary, GoTaq® Green Master Mix is easy to use, reliable, versatile and efficient in the generation of PCR products from yeast colonies.

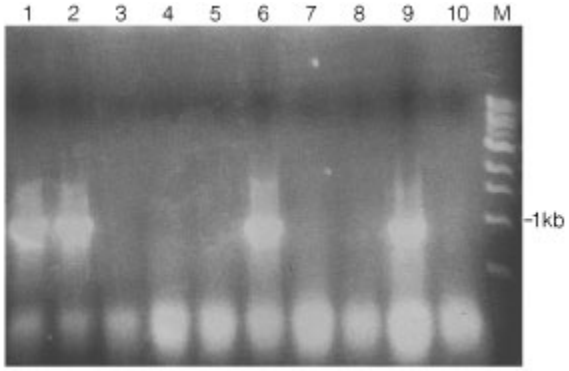

Figure 1. Agarose gel stained with ethidium bromide to detect PCR products generated from *S. pombe* colonies. Lanes 1–8, colonies that were G-418 resistant; lane 9, an *ase1*::*kanMX6* strain (a positive control for disruption); lane 10, a wildtype strain that is G-418 sensitive (a negative control for disruption); and lane M, a 1kb DNA ladder.

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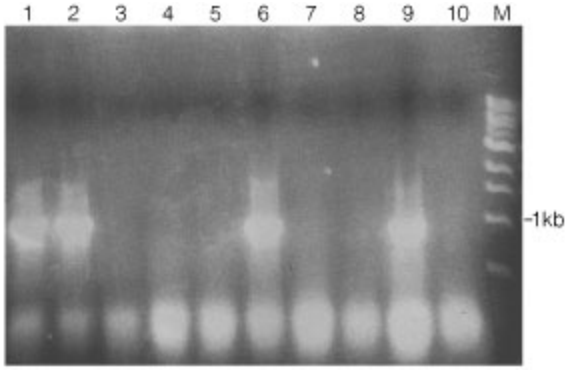
FIGURES



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