

Prepare DNA from Colonies in 10 Minutes for Real-Time PCR Using the Colony Fast-Screen™ Kit and FailSafe™ Real-Time PCR System

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Introduction

EPICENTRE's Colony Fast-Screen™ Kit (PCR Screen) was designed to prepare cloned DNA for screening by PCR in less than 10 minutes. This simple method allows the direct screening of clones from colonies on a plate, without growing overnight cultures and purifying DNA.¹ We wanted to verify that DNA prepared by Colony Fast-Screen could be successfully amplified with the FailSafe™ Real-Time PCR System. We compared the amplification of plasmid DNA prepared from colonies using Colony Fast-Screen to plasmids prepared from overnight cultures using a miniprep DNA purification kit.

Materials and Methods

TransforMax™ EPI300™ *E. coli* cells containing the CopyControl™ PCR Cloning Vector, pCC1™, with a 5-kb lambda DNA insert, were streaked for single colonies on LB-chloramphenicol plates or grown in liquid cultures overnight. The pCC1 vector contains the inducible *oriV* origin of replication so that cells can be maintained with a single copy of the vector or induced to high-copy numbers of the vector. To induce, cells are grown in the presence of the CopyControl™ Induction Solution.² (For more information on the CopyControl™ Cloning Systems, see page 22.) We tested both induced and uninduced colonies with the Colony Fast-Screen Kit. Overnight liquid cultures were grown induced.

Prepare DNA from colonies in less than 10 minutes

We prepared DNA from colonies using the following Colony Fast-Screen protocol:

1. Use a sterile pipette tip to pick part of a colony from the plate; transfer the cells to a microfuge tube or a microtiter plate.
2. Add 50 µl of the PCRLyse™ Solution; vortex to resuspend the cells.
3. Incubate at 99°C for 5 minutes; vortex briefly.
4. Chill on ice for 2 minutes; use 1 µl for each PCR reaction.

Plasmid DNA from overnight liquid cultures was purified using the Wizard® Plus SV Minipreps DNA Purification System (Promega) according to the manufacturer's instructions. DNA was quantitated by fluorimetry using Hoechst dye H 33258.³

Real-time PCR

After performing an initial PCR with the FailSafe™ Real-Time PCR PreMix Selection Kit, we used PreMix E for the remaining PCR reactions. The reaction amplifies a 150-bp lambda DNA segment. Reactions were set up with serial dilutions of the miniprep DNA or with DNA prepared from either induced or uninduced colonies using the Colony Fast-Screen Kit. Each 50-µl PCR reaction contained 1 µl template DNA, 1 µl FailSafe™ Real-Time PCR Enzyme Mix, 500 nM each primer, and 25 µl FailSafe™ PreMix E. Real-time PCR was performed with an initial denaturation of 95°C (2 minutes) and 35 cycles of 95°C (10 seconds), 55°C (10 seconds), 72°C (20 seconds) and analyzed on Bio-Rad's iCycler iQ Real-Time PCR Detection System.

Results

The graphs shown here are representative of data obtained from several experiments. Figure 1 shows the quantification graphs for the template DNA from induced (green) and uninduced (red) colonies prepared using the Colony Fast-Screen Kit and from 2.5 ng (blue) and 100 pg (purple) of miniprep DNA. To confirm that the reaction produced a single PCR product, without the formation of additional, nonspecific products or primer/dimers, a melt curve analysis for these reactions is shown in Figure 2. Our experiments show that 1 µl of plasmid DNA prepared with the Colony Fast-Screen Kit, even from uninduced, single-copy cells, is readily amplified in a FailSafe real-time PCR reaction.

References

1. EPICENTRE Forum 10(1), 12.
2. EPICENTRE Forum 9(1), 1.
3. Hoffman, L. and Moan, E. EPICENTRE Forum 5(4), 1.

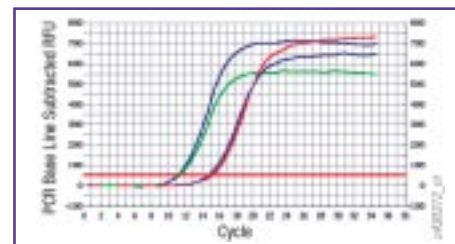


Figure 1. Quantification curves for a 150-bp real-time PCR product amplified from plasmid DNA prepared with the Colony Fast-Screen™ Kit from an induced colony (green) and an uninduced colony (red) or from miniprep DNA, 2.5 ng (blue) and 100 pg (purple).

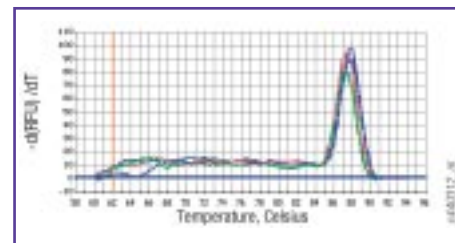


Figure 2. Melt curve analysis graphs for the reactions shown in Figure 1 indicate that no nonspecific PCR products or primer/dimers were formed in the FailSafe™ Real-Time PCR reactions.

www.epicentre.com/cfs_pcr.asp

Colony Fast-Screen™ Kit (PCR Screen)

Screen clones by PCR.

FS0322H 1 Kit

Sufficient reagents to screen 200 colonies. Thermostable polymerase and PCR primers are not included.

Contents:

PCRLyse™ Solution
Gel Loading Solution

www.epicentre.com/realtimepcr.asp

FailSafe™ Real-Time PCR PreMix Selection Kit

FSR0360 48 Reactions

Contents:

FailSafe™ PCR Enzyme Mix
12 FailSafe™ Real-Time PCR 2X PreMixes
Passive Reference Dye

FailSafe™ Real-Time PCR System

FSR03200 200 Reactions

Contents:

FailSafe™ PCR Enzyme Mix
Choice of two FailSafe™ Real-Time PCR 2X PreMixes
Passive Reference Dye