

New!

Restriction-Enzyme Screen Clones from Colony to Gel in 25 Minutes or Less

Ron Meis, EPICENTRE

Because ligation reactions can generate different insert orientations, multiple inserts, and empty vectors, cloning recombinant DNA in *E. coli* usually requires a screen of the resulting colonies for the correct clone. Potential clones can be size-screened by electrophoresis of the uncut plasmid, or screened for insert size and orientation by PCR or restriction enzyme digestion. Each method has benefits and drawbacks.

The new Colony Fast-Screen Kit (Restriction Screen) allows restriction analysis of clones, from colony to gel-loading, in only 25 minutes with the standard protocol, or in as little as 10 minutes with the accelerated protocol. Briefly, the procedure consists of colony resuspension in the kit's proprietary solution, heat treatment, restriction enzyme digestion and agarose gel electrophoresis. The kit's Restricti-Lyse™ Solution lyses cells in an environment

a vector (2.4 kb). Resulting colonies were screened using *Sca* I in both the Restriction Screen standard protocol and the accelerated protocol. *Sca* I cuts in both the insert and the vector. In one insert orientation (A) a *Sca* I digest produces 2.5- and 1.1-kb fragments. In the other orientation (B), the digest produces two 1.8-kb fragments. Digestion of the recircularized vector, without an insert, gives a single 2.4-kb fragment.

The Colony Fast-Screen™ Kit (Restriction Screen) provides a significantly faster and more convenient way to screen E. coli colonies for clones by restriction analysis than ever before.

Standard protocol

For the standard protocol, each colony was vortexed in 10 µl of Restricti-Lyse Solution to resuspend the cells and incubated at 100°C for 90 seconds. After the tubes were briefly cooled at room temperature, 1 µl of 10X restriction enzyme buffer and 10 Units (1µl) of *Sca* I were added. Reactions were incubated at 37°C for 15 minutes, 2 µl of 6X gel loading buffer were added and the entire reaction

Uncut plasmid analysis

Uncut plasmid analysis is quick (just lyse the cells and run a gel) and convenient (can be performed directly on colonies, no additional overnight cultures and plasmid preps). EPICENTRE offers the Colony Fast-Screen™ Kit (Size Screen) for uncut plasmid analysis. If the orientation of the clone is not a concern, this method is very efficient.

PCR analysis

PCR analysis is convenient (can be performed directly on colonies, no additional overnight cultures and plasmid preps) and can determine the orientation of the clone. EPICENTRE offers the Colony Fast-Screen™ Kit (PCR Screen) for PCR analysis. If the necessary PCR primers are available and the extra time required for cycling is not a problem, this method provides more specific information about the clone than uncut plasmid analysis.

Restriction analysis

Restriction enzyme digest analysis uses a common tool found in most molecular biology labs (restriction enzymes) and can determine insert orientation. However, traditionally this method also required overnight cultures and plasmid preps. Now EPICENTRE offers the Colony Fast-Screen™ Kit (Restriction Screen), which allows restriction analysis directly from individual colonies.

that is not inhibitory to restriction enzyme activity.

To demonstrate the use of the kit, an insert (1.2 kb) was blunt-end ligated into

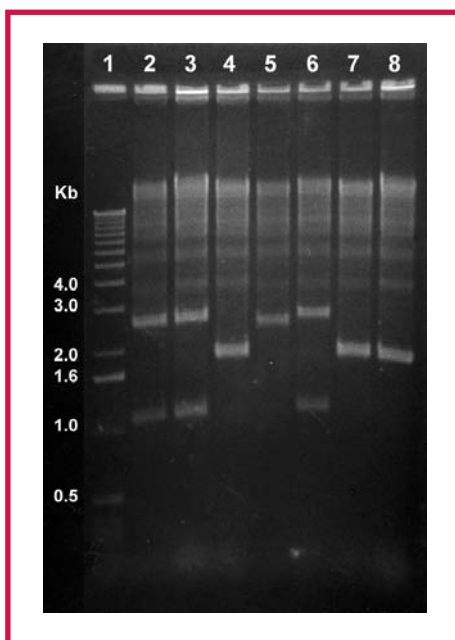


Figure 1. Results of the standard protocol with the Colony Fast-Screen™ Kit (Restriction Screen), which goes from colony to gel loading in 25 minutes. Lane 1, molecular size marker; Lanes 2 to 8, *Sca* I digests of DNA from 7 colonies prepared according to the kit's standard protocol. Lanes 2, 3, and 6 show clones in orientation A; Lanes 4, 7, and 8 show clones in orientation B; and Lane 5 is a recircularized vector.

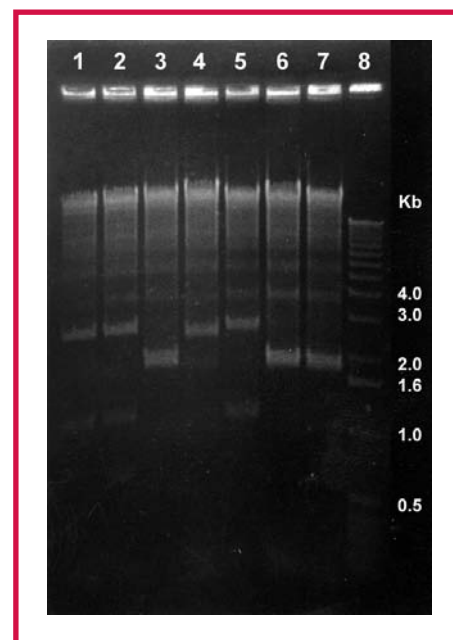


Figure 2. Results of the accelerated protocol with the Colony Fast-Screen™ Kit (Restriction Screen), which goes from colony to gel loading in 10 minutes. Lanes 1 to 7, *Sca* I digests of DNA from 7 colonies prepared according to the kit's accelerated protocol; Lane 8, molecular size marker. Lanes 1, 2, and 5 show clones in orientation A. Lanes 3, 6, and 7 show clones in orientation B. Lane 4 is a recircularized vector.

was subjected to electrophoresis on a 1% agarose gel. Figure 1 shows the results of the standard protocol.

Accelerated protocol

With the accelerated protocol, each colony was resuspended by pipetting in 10 μ l of Restricti-Lyse Solution and incubated at 100°C for only 10 seconds. The Sca I digest was set up as in the standard protocol, but incubated at 37°C for only 5 minutes. The reaction was prepared and electrophoresed as for the standard proto-

col. Figure 2 shows the restriction digest results using the accelerated protocol. The resulting DNA bands from the accelerated protocol are generally discernable, but have less DNA and stain less intensely than the standard protocol.

Conclusion

The Colony Fast-Screen Kit (Restriction Screen) provides a significantly faster and more convenient way to screen *E. coli* colonies for clones by restriction analysis than ever before.

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Colony Fast-Screen™ Kit (Restriction Screen)

Screen the size and orientation of clones.

FS0472H 1 Kit

Sufficient reagents to screen 200 colonies.

Colony Fast-Screen™ Kit (Restriction Screen) Identifies Subclones with 124-bp Fragment

Judith Meis, EPICENTRE

The Colony Fast-Screen™ Kit (Restriction Screen) was used to screen colonies directly for the presence of a small restriction enzyme fragment, quickly and easily, without growing overnight cultures or purifying plasmid DNA. Here pUC19-based clones with a 97-bp insert were screened for the presence of a 124-bp restriction fragment by a double restriction enzyme digest.

Methods

Ligation and transformation

A blunt 97-bp fragment was ligated into the *Sma* I site of pUC19 using the Fast-Link™ DNA Ligation Kit. One microliter of the ligation reaction was electroporated into TransforMax™ EC100™ Electrocompetent *E. coli* and the cells were plated and grown on LB-ampicillin overnight.

DNA preparation and restriction screening

DNA was prepared for restriction screening by transferring most of a large colony (about 2 mm) into 10 μ l of the kit's Restricti-Lyse™ Solution. Part of the colony was also touched to a new LB-ampicillin grid plate for future propagation. Cells were resuspended by vortexing and lysed by incubating at 100°C for 1 minute. Restriction buffer (1 μ l of 10X buffer), *Eco*R I (1 Unit), and *Xba* I (1 Unit) were added directly to the prepared DNA samples. To ensure a complete double digest with only 1 Unit of each enzyme, the digestion reactions were incubated at 37°C for 1 hour, rather than the typical 15 minutes needed with 5 to 10 Units of

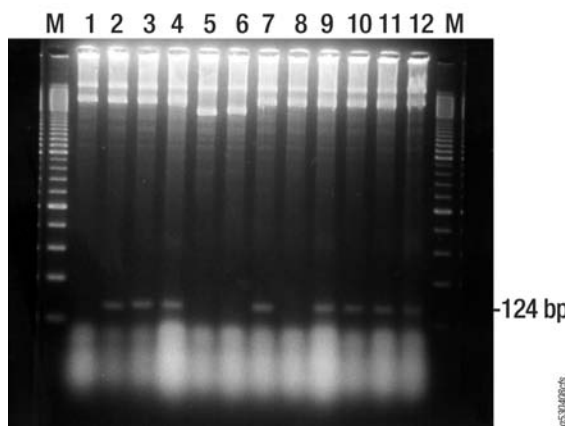


Figure 1. Electrophoresis of restriction digests of 12 plasmid DNA samples prepared with the Colony Fast-Screen™ Kit (Restriction Screen).

DNA prepared from 12 large colonies was double-digested with *Eco*R I and *Xba* I. Samples were run on a 3% agarose gel to identify clones containing the desired 124-bp fragment. M, 100 bp DNA Ladder. Lanes 2, 3, 4, 7, 9, 10, 11, and 12 show positive clones.

enzyme. Loading buffer was added directly to the samples, and they were assayed on a 3% agarose gel and stained with SYBR® Gold.

Results

Twelve large colonies were screened for the expected 124-bp *Eco*R I/*Xba* I fragment containing the desired 97-bp insert. Large colonies were used to increase the amount of DNA and more readily detect the small fragments. Figure 1 shows that 8 of the 12 colonies screened contain the insert. The Colony Fast-Screen Kit (Restriction Screen) prepares enough DNA to quickly and directly screen plasmids for even small restriction fragments, without overnight cultures and mini-plasmid preps.

SYBR® Gold is a registered trademark of Molecular Probes, Inc.

www.epicentre.com/fastlink.asp

Fast-Link™ DNA Ligation Kit

LK11025 25 Ligations
LK0750H 50 Ligations
LK6201H 100 Ligations

Contents:

Fast-Link™ DNA Ligase
Fast-Link™ 10X Ligation Buffer
10 mM ATP

TransforMax™ EC100™ Electrocompetent *E. coli*

EC10005 5 X 100 μ l
EC10010 10 X 100 μ l
Includes pUC19 control DNA.

TransforMax™ EC100™ Chemically Competent *E. coli*

CC02810 10 X 50 μ l
Includes pUC19 control DNA.