

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  $\mu$ 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

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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.

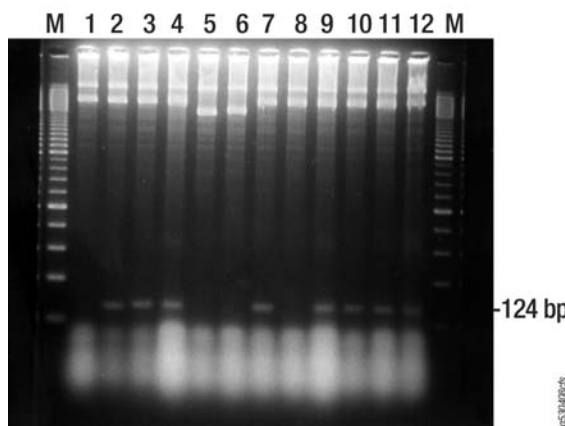
#### Methods

##### 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  $\mu$ 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  $\mu$ 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](http://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  $\mu$ l  
EC10010 10 X 100  $\mu$ l  
Includes pUC19 control DNA.

#### TransforMax™ EC100™ Chemically Competent *E. coli*

CC02810 10 X 50  $\mu$ l  
Includes pUC19 control DNA.