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Colony Fast-Screen™ Kit (Restriction Screen)

Cat. No. FS0472H

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1. Introduction

The Colony Fast-Screen™ Kit (Restriction Screen) provides an easy and rapid method to screen the size and orientation of cloned DNA. Just like the other Colony Fast-Screen Kits (Size and PCR Screens), there is no need for overnight cultures or plasmid minipreps. The Colony Fast-Screen Kit Restricti-Lyse™ solution lyses cells in an environment which is not inhibitory to restriction enzyme activity. Thus, the Colony Fast-Screen Kit (Restriction Screen) is very convenient because it enables the use of any restriction enzyme for restriction analysis. Recombinant clone-containing *E. coli* colonies can be screened in 25 minutes using the standard protocol, or in as little as 10 minutes with the accelerated protocol.

The Colony Fast-Screen Kit (Restriction Screen) contains sufficient reagents for screening 200 colonies.

Applications

- Identify desired clones (size and orientation) from a cloning experiment or in a library.[1]
- Identify desired clones when insert is too small to be shown by total clone size screening.[2]
- Release DNA for restriction analysis in minutes, without overnight cultures or minipreps.

2. Product Specifications

Storage: Store the Restricti-Lyse Solution at 4°C. The Gel Loading Solution may be stored at –20°C, 4°C or room temperature.

Quality Control: The Colony Fast-Screen Kit (Restriction Screen) is function-tested with a restriction digest analysis of plasmid clones grown in *E. coli*.

3. Kit Contents

Desc.	Quantity
Restricti-Lyse™ Solution	2 ml
Gel Loading Solution	400 µl

4. Related Products

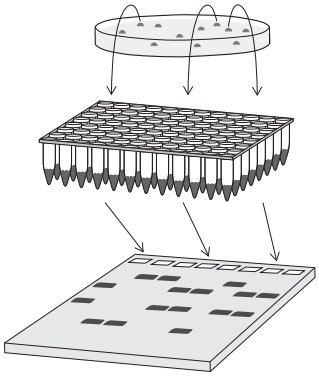
The following products are also available:

– Colony Fast-Screen™ Kit (Size Screen)
– Colony Fast-Screen™ Kit (PCR Screen)
– Fast-Link™ DNA Ligation Kit
– CopyControl™ Fosmid Library Production Kit
– CopyControl™ BAC Cloning Kit
– CopyControl™ cDNA, Gene & PCR Cloning Kits

5. Colony Fast-Screen Kit (Restriction Screen) Procedure

Rapidly screen the size and orientation of recombinant clones

The Colony Fast-Screen Kit (Restriction Screen) is used to prepare restriction-ready DNA without the need for overnight cultures or DNA isolations. The procedure can go from "colony-to-gel loading" in 25 minutes using the standard protocol, or in as little as 10 minutes with the accelerated protocol (page 4). Resultant accelerated protocol restriction bands stain less intensely than when using the standard protocol, but in most cases yield a clearly discernable pattern. An overview of the screening process and example agarose gels are shown in Figs. 1 & 2. Plates can be stored at 4°C for up to one month before being used. The Colony Fast-Screen Kit (Restriction Screen) procedure cannot be used on *end*A$^+$ *E. coli* strains. See "Additional Considerations".



1. Pick colony into a microcentrifuge tube or microtiter well.
2. Add Restricti-Lyse™ Solution, vortex, and heat at 100°C. (60 seconds – standard protocol, 10 seconds – accelerated protocol.)
3. Perform restriction digestion on the entire tube of the lysed cells. (15 minutes – standard protocol, 5 minutes – accelerated protocol.)
4. Gel electrophoresis and staining.

Figure 1. The size and orientation of cloned DNA can be determined in 25 minutes (or in as little as 10 minutes with the accelerated protocol) using the Colony Fast-Screen™ Kit (Restriction Screen).

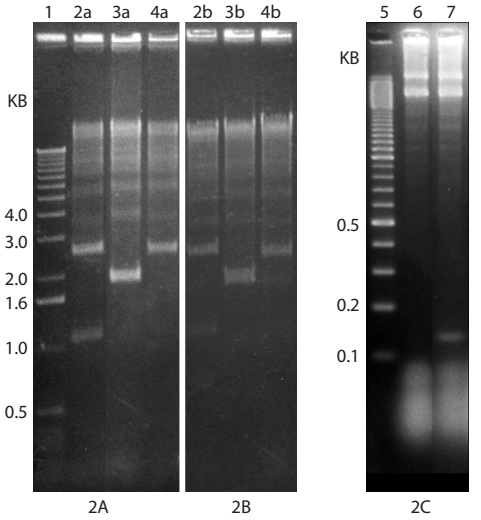


Figure 2. Examples of cloned DNA size and orientation determination. A) Standard Protocol, B) Accelerated Protocol and C) High resolution agarose gel.

Figure 2A. Potential clones cut with *Sca* I, screened for orientation using the Standard Protocol and a 1% agarose gel. Lane 2a has insert cloned into one orientation (2.5 kb and 1.1 kb bands), Lane 3a has insert cloned into the opposite orientation (1.8 kb doublet), Lane 4a has recircularized vector (2.4 kb band).

Figure 2B. Same analysis as shown in Fig. 2A except using the Accelerated Protocol.

Figure 2C. Potential clones cut with *Eco*R I and *Xba* I, screened for size using the Standard Protocol and a 3% agarose gel. Lane 6 has recircularized vector (2.68 kb band), Lane 7 has proper insert (2.66 kb and 124 bp band).

Standard Protocol

1. Aliquot 10 µl of Restricti-Lyse Solution into a 0.5 ml microcentrifuge tube or the bottom of a microtiter plate well for each colony to be screened.
2. Using a sterile micropipette tip, pick bacterial colonies which are minimally 1 mm in diameter and transfer as much of the colony as possible to the microcentrifuge tube or microtiter plate well containing the Restricti-Lyse Solution. Repeat the process using a fresh micropipette tip for each colony to be screened.

   Note: *Prior to addition of the cells to the Restricti-Lyse Solution, spot a sample of each colony onto a master plate, or inoculate a culture if desired.*
3. Stopper the tubes or cover the microtiter plate and vortex vigorously until the colony pick is completely resuspended (10-60 seconds).

   Note: *Older colonies (>4 days old) tend to be "stickier" and thus more difficult to resuspend thoroughly. Some cellular aggregates are fine.*

4. Incubate the tubes or plates at 100°C for 60 seconds in a thermocycler or water bath (incubation up to 3 minutes is acceptable). The Restricti-Lyse Solution facilitates release of restriction-ready DNA from the cells in an environment which is not inhibitory to restriction enzyme activity.
5. Briefly spin (5 seconds) each tube or plate to collect any condensate.
6. Add 1 µl of the appropriate 10X restriction enzyme buffer to each tube or well.
7. Add the desired restriction enzyme to each tube or well and incubate at the appropriate temperature for the desired length of time.

   Note: *If using 10 U of restriction enzyme, incubate 5-60 min. (10-15 minutes recommended).*
   If using 5 U of restriction enzyme, incubate 10-60 min. (20 minutes recommended).
   If using 1 U of restriction enzyme, incubate 30-120 min. (60 minutes recommended).
8. Add 2 µl of Gel Loading Solution to each tube or well and mix briefly (5 seconds) by vortexing.
9. Subject the entire reaction sample to agarose gel electrophoresis and stain the gel by your preferred method.

Accelerated Protocol

1. Aliquot 10 µl of Restricti-Lyse Solution into a 0.5 ml microcentrifuge tube or the bottom of a microtiter plate well for each colony to be screened.
2. Using a sterile micropipette tip, pick bacterial colonies which are minimally 1 mm in diameter and transfer as much of the colony as possible to the microcentrifuge tube or microtiter plate well containing the Restricti-Lyse Solution. Resuspend the cells by repeated pipetting up and down in the pipette tip (5-10 times). Repeat the process using a fresh micropipette tip for each colony to be screened.

   Note: *Prior to addition of the cells to the Restricti-Lyse Solution, spot a sample of each colony onto a master plate, or inoculate a culture if desired.*

   Note: *Older colonies (>4 days old) tend to be` "stickier" and thus more difficult to resuspend thoroughly. Some cellular aggregates are fine.*
3. Incubate the tubes or plates at 100°C for 10 seconds in a thermocycler or water bath. The Restricti-Lyse Solution facilitates release of restriction-ready DNA from the cells in an environment which is not inhibitory to restriction enzyme activity.
4. Add 1 µl of the appropriate 10X restriction enzyme buffer to each tube or well.
5. Add 10 U of the desired restriction enzyme to each tube or well and incubate at the appropriate temperature for 5 minutes.
6. Add 2 µl of Gel Loading Solution to each tube or well and mix briefly (5 seconds) by vortexing.
7. Subject the entire reaction sample to agarose gel electrophoresis and stain the gel by your preferred method.

Additional Considerations

1. **Background:** Background banding patterns due to genomic DNA restriction will increase as more restriction enzyme is used and/or as incubation times are increased. Amounts of restriction enzyme and/or incubation times may be increased with a minimal increase in genomic background when screening with a "rare-cutting" restriction enzyme.
2. **Banding Pattern:** Due to decreased band staining intensities, choose restriction enzyme screening patterns, whenever possible, with bands ≥500 bp in size. It may be necessary to increase photographic exposure times to visualize bands <500 bp in size. Alternatively, such restriction digests may be resolved on high resolution gels (ie. 3% agarose, modified agarose or polyacrylamide). See Fig. 2C.
3. **Bacterial Culture:** 20-60 µl of a saturated bacterial liquid culture may be substituted for a colony in the screening procedure. When using liquid culture, pellet cells by centrifugation (spin at least 5 minutes), discard the supernatant, resuspend cells in 10 µl of Restricti-Lyse Solution, continue with procedure as described.
4. **Streaky Gels:** Excess cellular debris (from large colonies, large culture volumes or incomplete cellular resuspension) can cause streaking on the agarose gel. If desired, this can be remedied by spinning out the cellular debris, in a microcentrifuge, prior to loading the completed reaction supernatant onto the agarose gel.

6. References

1. Meis, R. (2004) *EpicentreForum* **11** (5), 18.
2. Meis, J. (2004) *Epicentre Forum* **11** (5), 19.

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