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Colony Fast-Screen™ Kit (Size Screen)

Cat. No. FS08250

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1. Introduction

The Colony Fast-Screen™ Kit (Size Screen) provides a rapid and sensitive method to screen the insert size of plasmid, cosmid, fosmid, and BAC clones without the need to grow cultures, perform mini-preps, or restriction endonuclease digests. The Colony Fast-Screen Kit (Size Screen) can also be used to size screen deletion library clones produced using nuclease digestion.

The Colony Fast-Screen Kit (Size Screen) can be used to screen the insert sizes of both high-copy (e.g., plasmid and cosmid) clones and low-copy (e.g., BAC and fosmid) clones. **Procedure A** on page 2 describes the process for screening high-copy clones. **Procedure B** on page 3 describes the process for screening low copy clones. There are slight differences between the two procedures. Specifically, the order of addition of the EpiBlue™ and EpiLyse™ Solutions are different in the two procedures. Also, the procedure for screening high-copy clones requires a heating step that is not recommended for screening of BAC clones. Please read the appropriate procedure carefully before proceeding.

The Colony Fast-Screen Kit (Size Screen) contains reagents sufficient for screening 250 colonies.

2. Product Specifications

Storage: Store the kit at room temperature.

Quality Control: The Colony Fast-Screen Kit (Size Screen) is function-tested for detection and size analysis of plasmid clones grown in *E. coli*.

3. Kit Contents

Desc.	Quantity
EpiBlue™ Solution	2.5 ml
EpiLyse™ Solution	10 ml

4. Related Products

The following products are also available:

- Colony Fast-Screen™ Kit (Restriction Screen)
- Colony Fast-Screen™ Kit (PCR Screen)
- Fast-Link™ DNA Ligation Kit
- Fast-Link™ DNA Ligation and Screening Kit
- CopyControl™ BAC Cloning Kits
- CopyControl™ PCR Cloning Kits
- CopyControl™ Fosmid Library Production Cloning Kits
- pIndigoBAC-5 Cloning-Ready Vectors
- EpiFOS™ Fosmid Library Production Kit
- pWEB™ & pWEB-TNC™ Cosmid Cloning Kits

5. Colony Fast-Screen Kit (Size Screen) Procedure A

For Screening the Insert Size of High-Copy Plasmid and Cosmid Clones

The overall process for screening high-copy clones is described in Fig. 1.

Preparation

1. Cast an agarose gel. Add electrophoresis buffer to the anode and cathode chambers of the gel box so that the buffer comes in contact with ends of the agarose gel but does not cover the surface or fill the wells of the gel. By preparing the gel in this manner it will be easier to load and prevent loss of sample from the gel wells as cautioned about in the "Notes" in Steps 3 and 7.

Screening Process

2. Add 10 µl of EpiBlue Solution to each 1.5-ml microcentrifuge tube or well of the microplate that will be used.
3. Using a sterile toothpick gently touch the colony on the plate to collect a small amount (less than the size of a pin head) of cells from a chosen colony. Deposit the cells at the bottom of a tube or the bottom of a microplate well. Repeat the process using a fresh toothpick for each colony chosen and deposit the cells from each colony into its own tube or microplate well.

   Note: *The Colony Fast-Screen (Size Screen) process is extremely sensitive when screening high-copy clones. The most common error is to collect too large a portion of cells from a colony.*
4. Vortex the tubes or microplate vigorously for 10 seconds to resuspend the cells.
5. Add 20 µl of EpiLyse Solution into the resuspended cells.
6. Incubate the tubes or microplate at 70°C for 15 minutes.
7. After incubation, vortex each tube or plate vigorously for 10 seconds. Load 10 µl from each independently into the wells of the gel that was prepared as described in Step 1.

   Note: *A solution that is too viscous to pipette indicates that too many cells were picked from the plate in Step 3. Add an additional 20 µl of EpiLyse Solution to the highly viscous solutions, mix thoroughly and load 10 µl onto the gel well.*
8. Load supercoiled DNA size standards. Turn on the power and run the gel for 2 minutes to get the DNA into the gel. Turn off the power and carefully cover the gel with electrophoresis buffer. Turn the power back on and continue the electrophoresis.
9. Cover and store the remaining solution in each tube or microplate at room temperature for up to 1 week in the event additional gels need to be run.
10. Run the gel per standard laboratory procedure. Stain with ethidium bromide or other appropriate DNA stain.

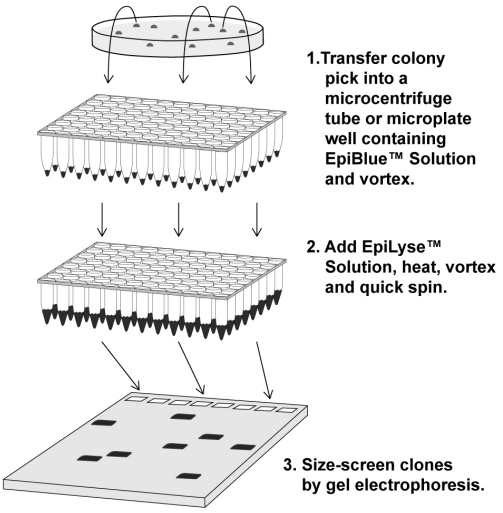

Figure 1. An overview of the process for screening the insert size of high-copy number clones.

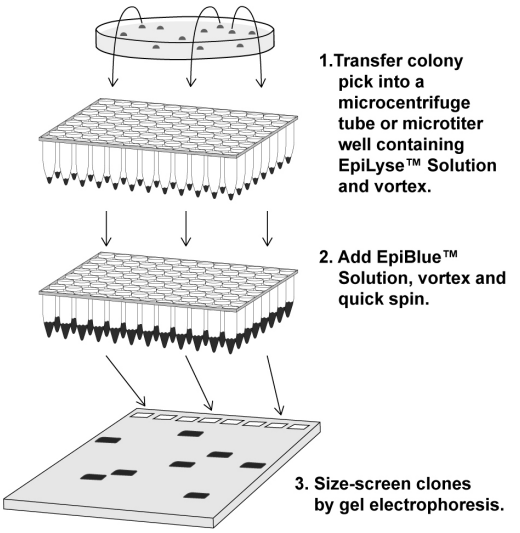

Figure 2. An overview of the process for screening the insert size of low-copy number clones.

6. Colony Fast-Screen Kit (Size Screen) Procedure B

For Screening the Insert Size of Low-Copy BAC and Fosmid Clones

For best results, colonies should be grown to at least 1 mm in size. Because of the low copy-number of the clones, it is necessary to scrape the entire colony off the plate. Therefore, if desired prepare a replica plate or other method for duplicating the clones prior to the Colony Fast-Screen (Size Screen) procedure. The overall process for screening low-copy clones is described in Fig. 2.

Preparation

1. Cast a 0.8-1% agarose gel in 1X TAE buffer. Add electrophoresis buffer to the anode and cathode chambers of the gel box so that the buffer comes in contact with ends of the agarose gel but **does not cover the surface or fill the wells of the gel**. By preparing the gel in this manner it will be easier to load and prevent loss of sample from the gel wells.

Screening Process

2. Add 25 µl of EpiLyse Solution to each 1.5-ml microcentrifuge tube or well of the microplate that will be used.
3. Using a sterile pipette tip (e.g., P200 tip) scrape the entire colony from the plate. Place the pipette tip into the EpiLyse Solution. Repeat the process using a fresh pipette tip for each colony chosen and deposit the cells from each colony into individual tube or microplate well.
4. Stir each to get all of the colony off the pipette tip. The EpiLyse Solution should become viscous as the cells lyse.
5. Vortex the tubes or microplate to evenly suspend the cell lysate.
6. Add 10 µl of EpiBlue Solution to each tube or microplate well.
7. Vortex to mix evenly. Then, centrifuge for 30 seconds to pellet the cell debris.
8. Load 25 µl from each tube or microplate well individually into the wells of the gel that was prepared as described in Step 1. Load supercoiled DNA size standards.
9. Turn on the power and run the gel at 5V/cm at 4°C for 2 minutes to get the DNA into the gel. Turn off the power and carefully cover the gel with electrophoresis buffer. Turn the power back on and continue the electrophoresis for 2-3 hours.
10. Stain the gels with a highly sensitive stain (e.g., SYBR® Gold at 1:10,000 dilution in TE buffer or water).

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