

Screen BAC Clones Quickly and Efficiently, Without *Not I*

Constructing a BAC library typically requires setting-up multiple ligations. Before transforming and plating en masse, a small portion of each ligation is transformed and assessed for "quality". This initial assessment involves screening 10 to 100 transformants for the appropriate insert size (>100 kb) and the percentage of non-recombinant or "empty" clones (optimally less than 6 %).

After the BAC library is completed, a final, much more intensive, screen is required to accurately determine the average insert size and size distribution of the clones. Hundreds of clones need to be screened, depending upon the size of the genome and the library.

Typically *Not I*, a rare-cutting restriction endonuclease, is used for both the initial and final screens. Here we describe how the ligation can be screened in only 4 hours with the Colony Fast-Screen™ Kit (Size Screen) and how the clones can be sized more efficiently by digestion at a single *cos* site with Lambda Terminase.

Not I takes time

One of the drawbacks of using *Not I* is that the technique takes about 3 days. One needs to grow the overnight culture, miniprep the DNA, digest the DNA (3 to 15 hours), and run the pulsed-field gel. When screening a ligation, time is critical because prolonged storage of the ligation results in decreased transformation efficiencies.

Quick screen without digestion

Using the Colony Fast-Screen Kit (Size Screen), also a component of EPICENTRE's CopyControl™ BAC Cloning Kits, researchers can screen the initial ligation in less than 4 hours. Briefly, a colony pick is transferred into a microcentrifuge tube or microtiter well containing EpiLyse™ Solution and vortexed. EpiBlue™ Solution is added and the lysate is electrophoresed in an agarose gel (Figure 1). The resulting single band of supercoiled BAC DNA is sized by comparison with EPICENTRE's BAC-Tracker™ Supercoiled DNA Ladder. Recently improved, the ladder contains 7 distinct bands of supercoiled DNA that range in size from 8 to 165 Kb.

Multiple bands with *Not I*

Not I digestion of purified BAC DNA releases the insert from the vector, opti-

Figure 1. The Colony Fast-Screen™ Kit (Size Screen) can be used to estimate the size of a BAC clone in less than 4 hours. Colonies from 3 different BAC clones (Lanes 1-3) were processed using the Colony Fast-Screen Kit (Size Screen) and analyzed by agarose gel electrophoresis. M, Improved BAC-Tracker™ Supercoiled DNA Ladder.



mally resulting in only two bands, the vector band and the insert band, which are sized by pulse-field gel electrophoresis. However, since *Not I* recognizes an 8-bp GC-sequence (GC▼GGCCGC), multiple *Not I* sites occur in GC-rich regions of an insert or in libraries made from GC-rich genomes. The sugarcane genome, for example, is relatively GC-rich and *Not I* digests typically generate up to 8 insert bands per BAC clone.¹ Adding up multiple bands to size the clones is tedious and inefficient.

Linearize each BAC clone to a single band

Lambda Terminase cleaves a BAC vector at a unique *cos* site resulting in linearization of the BAC clone and a single band on a gel. EPICENTRE's Lambda Terminase is highly purified to provide reproducible cleavage with minimum nonspecific nicking. The following protocol has been optimized for digesting BAC DNA with Lambda Terminase.

X μ l sterile water
 100-150 ng BAC DNA
 2 μ l 10 mM ATP
 2 μ l 10X Reaction Buffer
 0.15 U Lambda Terminase (diluted in 1X Reaction Buffer)*
 20 μ l Total reaction volume

Incubate at room temperature* for 30 minutes.

* We found that adding Lambda Terminase to the reaction as the final component and incubating the reaction at room temperature are critical steps for efficient digestion.

Figure 2 compares *Not I* and Lambda Terminase digests of several different BAC clones. When using Lambda Terminase, the size of each BAC is determined by estimating the size of a single band rather than making size estimations for multiple fragments. Both the Colony Fast-Screen

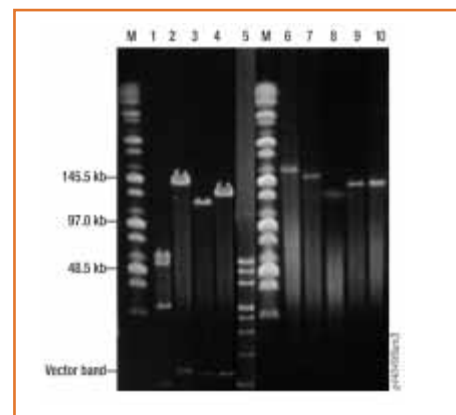


Figure 2. Digesting BAC clones with *Not I* often results in multiple DNA fragments while Lambda Terminase digests always result in one band. DNA from 5 randomly chosen BAC clones was digested with *Not I* (Lanes 1-5) or Lambda Terminase (Lanes 6-10) and analyzed by pulse-field gel electrophoresis. M, PFG Marker.

Kit and Lambda Terminase provide time-saving alternatives to *Not I* digestion.

For more information on the CopyControl™ BAC Cloning Kits, go to the website: www.epicentre.com/ccbac.asp

Reference

- Tomkins, J.P. et al. (1999) *Theor. Appl. Genet.* **99**, 419.

www.epicentre.com/cfs.asp

Colony Fast-Screen™ Kit (Size Screen)

Screen the size of clones.

FS08250 1 Kit
 Sufficient reagents to screen 250 colonies.

Contents:

EpiLyse™ Solution
 EpiBlue™ Solution

www.epicentre.com/bactracker.asp

BAC-Tracker™ Supercoiled DNA Ladder

BT010950 50 Gel Lanes
 (500 μ l)

www.epicentre.com/lambda_terminase.asp

Lambda Terminase

LT4450 2 U/ μ l 50 U
 LT44200 2 U/ μ l 200 U

Includes 10X Reaction Buffer and 10 mM ATP Solution.