Cost-effective and easy-to-use ⬟ Select-cll™ mutation assay kit

A Positive Selection Assay for Mutation Analysis in Big Blue® Animals

Stratagene now offers an alternative way to perform mutational analysis in the Big Blue® transgenic rodent mutagenesis assay system that selects for mutations in the cll gene of coliphage lambda. The ⬟ Select-cll™ mutation assay kit uses an H1™ E. coli strain and selective temperature conditions to provide an environment where only lambda cll™ mutants form plaques. This assay not only provides a less expensive means for studying mutations in Big Blue rodents® but also complements the original Big Blue (lacI) assay by focusing on a second genetic region for studying tissue-specific mutant frequencies and mutational spectra.

Stratagene's Big Blue transgenic rodent mutagenesis assay system has been used extensively for studying mutations sustained in vivo in mammals. Big Blue transgenic rodents harbor the Big Blue ⬟LIZ shuttle vector (figure 1), which can easily be rescued from the genome for mutation analysis in an E. coli host. In the established version of the Big Blue assay, the lacI gene from E. coli, located within the ⬟LIZ shuttle vector, functions as a mutational test sequence. Rescued shuttle vector phage containing lacI™ mutations are scored as blue plaques according to a background of colorless wild-type plaques.

In addition to providing the lacI plaque color-screening assay, Stratagene is now offering an alternative method of screening for mutations in Big Blue rodents, the ⬟ Select-cll mutation assay kit. In this positive selection assay, the region encompassing the cll gene of coliphage lambda functions as the mutational test sequence. The cll gene encodes a protein that activates transcriptional promoters in lambda that are essential for lysogenization. Mutations in the cll region that lower the levels of cll protein result in a decreased ability of lambda to lysogenize. When grown under conditions that favor lysogeny, lambda prophages carrying such mutations (cll™) survive only by entering the lytic pathway of development, forming plaques. Prophages that are wild type for the cll region (cll™) integrate into the host genome and become part of the developing bacterial lawn.

The method of performing the ⬟ Select-cll mutation assay is outlined (figure 2). After rescuing the ⬟LIZ shuttle vector from the rodent genomic DNA, the packaged virions are used to infect the E. coli host strain G1250 (a specialized H1™ version of Stratagene's XL1-Blue MRA cells). The infected host cells are then plated and grown at 24°C for 48 hours, conditions that are selective for cll™ mutants (figure 2, path A). In addition, a dilution of infected G1250 cells is plated and grown under nonselective conditions (37°C overnight) to determine the total number of rescued phage screened (figure 2, path B). Because the ⬟LIZ shuttle vector contains the temperature-sensitive cl857 mutation, both cll™ and cll™ shuttle vector phages grow as plaques at 37°C. Mutant frequency is determined as the number of cll™ plaque-forming units (pfu) observed divided by the total pfu screened. The cll™ selection scheme has been used to measure mutant frequency inductions in bladder tissue from mice treated with p-cresidine, with results comparable to lacI plaque color screening.

The Big Blue Cyclist™ DNA sequencing kit can be used to rapidly sequence the cll™ mutants identified with the ⬟ Select-cll assay. The cll™ target region is approximately 300 base pairs in length, including the cll mRNA ribosome binding site and the cll protein-activated Pm promoter. The relatively short length of the cll™ target region simplifies sequencing and expedites the analysis of mutational spectra.
The λ Select-cII™ Assay

1. Treat Big Blue Rodent
2. Prepare genomic DNA from tissue of interest
3. Package genomic DNA
4. Infect G1250 Hf host and grow under selective and nonselective conditions

The first three steps (animal treatment, DNA isolation and packaging) are performed as in the lacI plaque color-screening assay. Packaged phage are then used to infect the G1250 E. coli host strain and allowed to grow at 24°C, which selects for lambda cII mutant plaques. Determination of the total number of plaques screened is accomplished by titrating at 37°C.

Conclusions
The λ Select-cII mutation assay kit offers an alternative to the lacI plaque color-screening assay for mutation analysis in Big Blue transgenic rodents. Both assays feature unique advantages. The traditional plaque color-screening assay makes use of the larger, highly characterized lacI target region and benefits from the extensive database of experimental literature published over several years. By comparison, the λ Select-cII mutation assay is more cost-effective and less labor-intensive to perform due to its selective nature and small target region. This new assay can also serve to complement plaque color screening through mutation analysis of a second genetic region within the same animal. The λ Select-cII mutation assay kit includes the G1250 selective host strain, enough Transpack® lambda packaging extract for 50 packaging reactions and λcII control mutants.

REFERENCES

** U.S. Patent No. 5,188,057

λ Select-cII™ Mutation Assay Kit #720120
cII Sequencing Primers #720122

Big Blue Transgenic Mice

STRAIN

<table>
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<tr>
<th>Strain</th>
<th>Mutation</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td>C57BL/6 inbred</td>
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<td>#720610</td>
<td>#720611</td>
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<tr>
<td></td>
<td>Big Blue Homzygous 60 copies of λLIZ per cell</td>
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<td>#523686</td>
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<td>B6C3F1 F1 hybrid</td>
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<td>TgSp53Big Blue</td>
<td>p53 allele inactivated 40 copies of λLIZ per cell</td>
<td>male</td>
<td>#720613</td>
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Big Blue Transgenic Rats

Fischer 344 inbred Homozygous 30-40 copies of λLIZ per cell | #720070 | #720071 |

Big Blue Transgenic Cell Cultures

LINEAGE

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Description</th>
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<th>Female</th>
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<tbody>
<tr>
<td>Rat 2 Embryonic</td>
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<tr>
<td>Fibroblasts</td>
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<tr>
<td>Fibroblasts</td>
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<td>#726010</td>
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Big Blue λLIZ Shuttle Vector

20 μg used in all Big Blue rodents and cell lines #746220

RecoverEase™ DNA Isolation Kit

materials and reagents for 30 isolations #720202
materials and reagents for 15 isolations #720203

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