

Cost-effective and easy-to-use λ Select-cII[™] mutation assay kit

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

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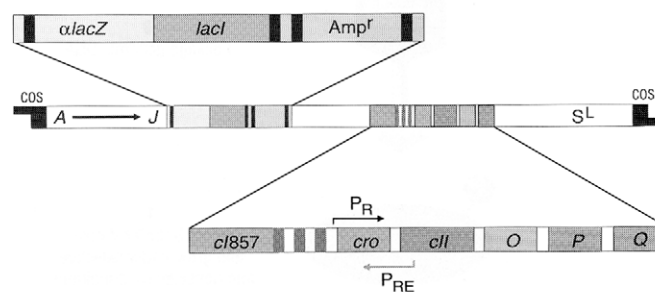
Stratagene now offers an alternative way to perform mutational analysis in the Big Blue[®] transgenic rodent mutagenesis assay system that selects for mutations in the *cII* gene of coliphage lambda. The λ Select-cII[™] mutation assay kit uses an *hfl*⁻ *E. coli* strain and selective temperature conditions to provide an environment where only lambda cII⁻ 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.^{1,2} Big Blue transgenic rodents harbor the Big Blue λ 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 λ LIZ shuttle vector, functions as a mutational test sequence. Rescued shuttle vector phage containing *lacI*⁻ mutations are scored as blue plaques against a background of colorless wild-type plaques.³

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 λ Select-cII mutation assay kit. In this positive selection assay, the region encompassing the *cII* gene of coliphage lambda functions as the mutational test sequence.⁴ The *cII* gene encodes a protein that activates transcriptional promoters in lambda that are essential for lysogenization. Mutations in the *cII* region that lower the levels of cII protein result in a decreased ability of lambda to lysogenize. When grown under conditions that favor lysogeny, lambda prophages carrying such mutations (λ cII⁻) survive only by entering the lytic pathway of development, forming plaques. Prophages that are wild type for the *cII* region (λ cII⁺) integrate into the host genome and become part of the developing bacterial lawn.

The method of performing the λ Select-cII mutation assay is outlined (figure 2). After rescuing the λ LIZ shuttle vector from the rodent genomic DNA, the packaged virions are used to infect the *E. coli* host strain G1250 (a specialized *hfl*⁻ 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 λ cII⁻

Figure 1
Big Blue[®] λ LIZ Shuttle Vector

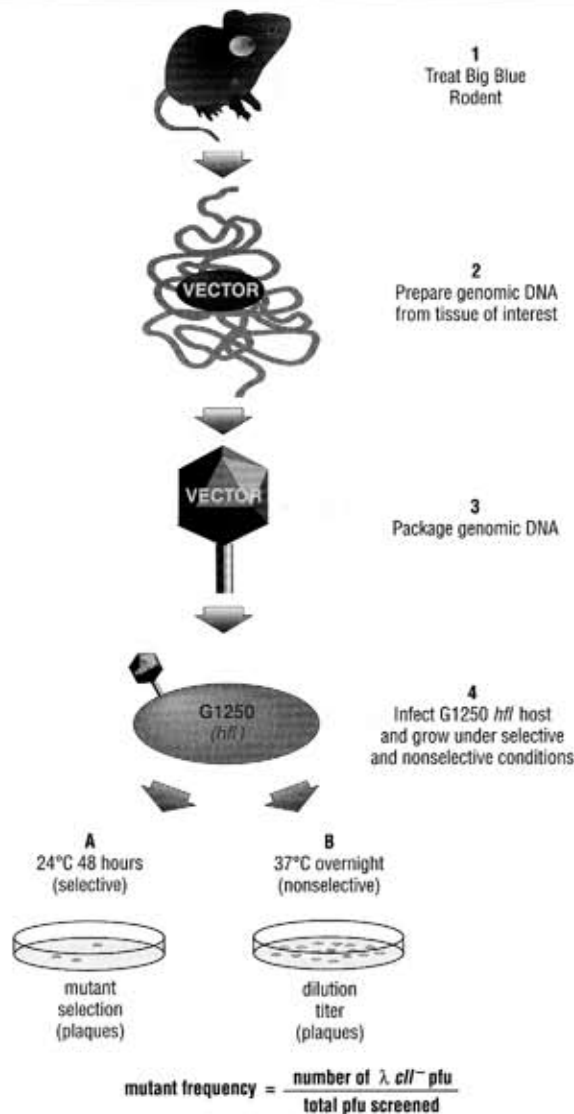


The λ LIZ shuttle vector is approximately 45.5 kb in length. The expanded regions show the areas important for both the plaque color-screening (*lacI*) and λ Select-cII (*cII*) assays. The *cII* gene is transcribed as part of a polycistronic mRNA initiated at the P_R promoter. Other genes in the cistron (*cro*, *O*, *P* and *Q*) are also shown. The P_{RE} promoter, which is responsible for establishing synthesis of the cI repressor protein, overlaps the 5' end of the *cII* gene. The P_{RE} promoter runs counter to P_R and is one of two promoters activated by the cII protein. Note that in λ LIZ, the gene for the cI repressor protein contains the temperature-sensitive *cI857* mutation, which allows titling on G1250 at 37°C (see text). Detectable mutations in the *cII* region interfere with either the translation and function of the cII protein or cII-mediated initiation of the P_{RE} promoter.

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 λ LIZ shuttle vector contains the temperature-sensitive *cI857* mutation, both λ cII⁺ and λ cII⁻ shuttle vector phages grow as plaques at 37°C. Mutant frequency is determined as the number of λ cII⁻ plaque-forming units (pfu) observed divided by the total pfu screened. The λ cII⁻ 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.⁴

The Big Blue Cyclist[™] DNA sequencing kit can be used to rapidly sequence the λ cII⁻ mutants identified with the λ Select-cII assay. The *cII* target region is approximately 300 base pairs in length, including the *cII* mRNA ribosome binding site and the cII protein-activated P_{RE} promoter.^{5,6} The relatively short length of the *cII* target region simplifies sequencing and expedites the analysis of mutational spectra.

Figure 2
The λ Select-clI™ Assay



The first three steps (animal treatment, DNA isolation and packaging) are performed as in the *lacI* plaque color-screening assay.^{7,8} 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 pfu screened is accomplished by titering at 37°C.

Conclusions

The λ Select-clI 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-clI 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-clI mutation assay kit includes the G1250 selective host strain, enough Transpack® lambda packaging extract** for 50 packaging reactions and λ cII⁻ control mutants.

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* U.S. Patent No. 5,347,075 and patents pending, European Patent No. 289121, Japanese Patent No. 2618973
** U.S. Patent No. 5,188,957

λ Select-clI™ Mutation Assay Kit		#720120
cII Sequencing Primers		#720122
Big Blue® Transgenic Mice		
STRAIN		
C57BL/6 inbred	Big Blue Hemizygous 40 copies of λ LIZ per cell	male #720010 female #720011
	Big Blue Homozygous 80 copies of λ LIZ per cell	male #929085 female #929086
B6C3F1 F1 hybrid	Hemizygous 40 copies of λ LIZ transgene	male #720020 female #720021
TSGp53/Big Blue	p53 allele inactivated 40 copies of λ LIZ per cell	male Inquire
		female Inquire
Big Blue® Transgenic Rats		
Fischer 344 inbred	Homozygous 30-40 copies of λ LIZ per cell	male #720070 female #720071
Big Blue® Transgenic Cell Cultures		
LINEAGE		
Rat 2 Embryonic Fibroblasts	CaPO ₄ transfected 50-70 copies of λ LIZ per polyploid cell	#726000
Mouse Embryonic Fibroblasts	Derived from C57BL/6 transgenic mice 40 copies of λ LIZ per cell	#726010
Big Blue® λLIZ Shuttle Vector		
20 μ g	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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