The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Kathleen A. Hill**
DEPARTMENT:	**Biology**
ADDRESS:	**333 Western Science Centre**
PHONE NUMBER:	**1 519 661-2111 81337**
EMERGENCY PHONE NUMBER(S):	**1 519 857-8694**
EMAIL:	**khill22@uwo.ca**

Location of experimental work to be carried out :

Building : **Western Science Centre** Room(s): **329**
Building : Room(s):
Building : Room(s):

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **NSERC**
GRANT TITLE(S): **Mutation Showers across the Mouse Genome**
UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Justin Mayers	**jmayers**	**Sept 2010**
Anita Prtenjaca	**aprtenja**	**Fall 2010 renewal**
Andrea Wishart	**awishar**	**Jan 2010**
Susan Eitutis	**seitutis**	**Sept 2010**
Anson Li	**al248**	**May 2010**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

The Biological Agents are E. coli and lambda phage. Stocks are stored in the -80 freezer. Stocks are used in the Western Science Centre Room 329 and the Molecular Genetic Unit Core Facility [incubators; shaking incubators]. Cultures are grown in incuabtors [in liquid and agar cultures] and used specifically in the Big Blue transgenic mutation detection system [Stratagene]. Cultures to be disposed of are placed in biohazard bags and removed with the weekly environmental waste pick up to the Western Science Centre [Thursdays 9am BGS loading dock]. In the laboratory all plasticware in contact with the cultures is placed in the labelled disposal containers [marked biohazard waste]. This waste is disposed using the environmental waste pick up service. Glassware and large plastic assay trays in contact with the bacterial cultures are bleached [10% bleach in water] overnight and the glassware washed and autoclaved prior to reuse.

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

The following is the URL for the product protocol involving the use of E. coli and lambda phage.

http://www.biocompare.com/Articles/ApplicationNote/26/A-Positive-Selection-Assay-For-Mutation-Analysis-In-Big-Blue-Animals.html

E. coli host strain G1250 (a specialized hfl – version of Stratagene's XL1-Blue MRA cells

The Big Blue transgenic mouse mutation detection system permits the detection of mutations that occurred in the mouse. Big Blue mice are transgenic for a viral genome [lambda phage]. This genome sequence is used as a mutation target in the mouse. It is retrieved from the mouse DNA and used to infect lab strain bacteria [E. coli]. Bacteria that die from mutant viral sequences are visible plaques on culture plates. Bacteria that are viable and dividing become a confluent lawn on the culture plates. The researchers harvest the plaques [dead zones] to retrieve the mutant viral sequence. This assay requires the overnight growth of the bacteria in shaking liquid cultures and also culturing of the bacteria in agar on assay trays.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO

If YES, please give the name of the species ______________________

What is the origin of the microorganism(s)? ______________________

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli G1250	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**16 L**	**Stratagene**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Lambda Phage	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**500 ml**	**Stratagene**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ______________________

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐ YES ☒ NO
(If NO, please proceed to Section 3.0)

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☐ No		Not applicable
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☐ YES ☒ NO If **NO**, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☐ YES, complete table below ☐ NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☒ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☐ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Mus musculus**

7.3 AUS protocol # **2009-033**

7.4 List the location(s) for the animal experimentation and housing. **Health Sciences**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☒ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☐ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☒ NO

8.3 If **YES**, please specify the animal(s) used:

♦ Pound source dogs	☐ YES	☐ NO
♦ Pound source cats	☐ YES	☐ NO
♦ Cattle, sheep or goats	☐ YES, species	☐ NO
♦ Non-human primates	☐ YES, species	☐ NO
♦ Wild caught animals	☐ YES, species & colony #	☐ NO
♦ Birds	☐ YES, species	☐ NO
♦ Others (wild or domestic)	☐ YES, specify	☐ NO

8.4 If no live animals are used, please specify the source of the specimens:
Local Abbattoirs - pig and cow eyes are the speciments

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

10.2 If YES, please give the name of the species.

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree…)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Kathleen A. Hill ***Date:*** March 20, 2012

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ☐ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
- ☐ YES, location and date of most recent biosafety inspection:
- ☐ NO, please certify
- ☒ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0110**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:

flush the injury with running water
immediately report the incident to the supervisor and Western's Health Services

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Kathleen A. Hill** ***Date:*** **March 20, 2012**

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

A Positive Selection Assay For Mutation Analysis In Big Blue® Animals

Agilent Technologies

Cost-effective and easy-to-use / Select-cII™ mutation assay kit

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

Mark J. Dycaico • Gabriela M. Tobal
Stratagene

Stratagene now offers an alternative way to perform mutational analysis in the Big Blue® transgenic rodent mutagenesis assay syste that selects for mutations in the cII gene of coliphage lambda. The I Select-cII™ mutation assay kit uses an hfl¾ E. coli *strain and selective temperature conditions to provide an environment where only lambda* cII¾ *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 focusing on a second genetic region for studying tissue-specific mutant frequencies and mutational spectra.*

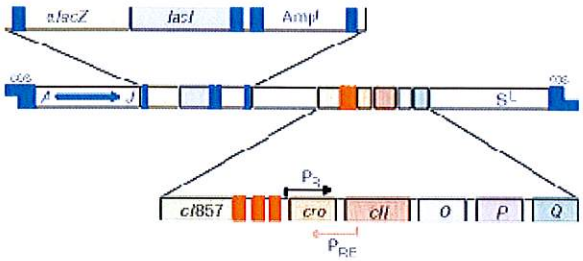

figure 1

Stratagene's Big Blue transgenic rodent mutagenesis assay system has been used extensively for studying mutations sustained in v in mammals.[1,2] Big Blue transgenic rodents harbor the Big Blue ILIZ 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 ILIZ shuttle vector, functions as a mutational test sequence. Rescued shuttle vector phage containing *lacI* ¾ 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 l Select-cII mutation assay kit. In this positive selection assay, the region encompassing the *cII* gei 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 (lc*II* $^-$) survive only by entering the lytic pathway of development, forming plaques. Prophages that are wild type for the *cII* region (lc*II* $^+$) integrate into the host genome and become part of the developing bacterial lawn.

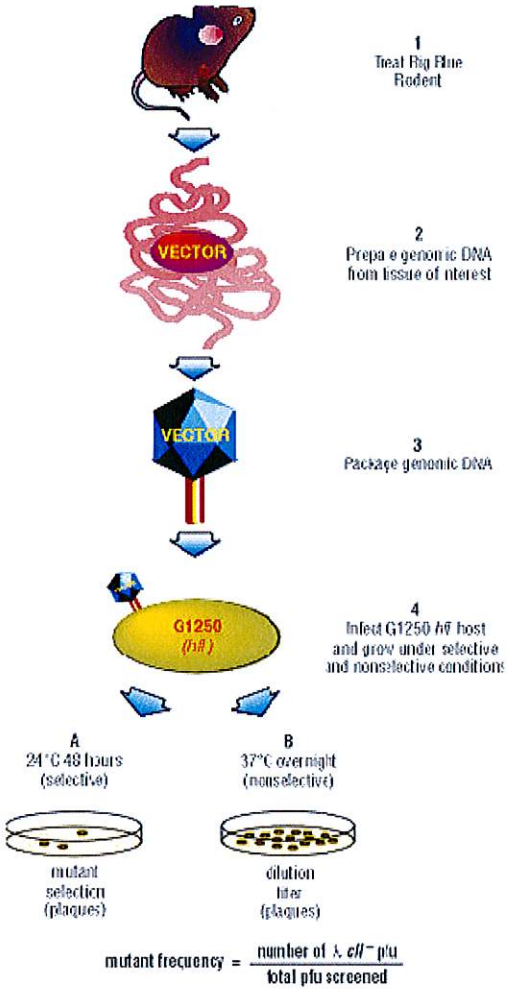

figure 2

The method of performing the l Select-cII mutation assay is outlined (**figure 2**). After rescuing the ILIZ 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 lc*II* $^-$ mutant (**figure 2**, path A). In addition, a dilution of infected G1250 cells is plated and grown under nonselective conditions (37°C overnight) t determine the total number of rescued phage screened (**figure 2**, path B). Because the ILIZ shuttle vector contains the temperature-sensitive *cI* 857 mutation, both lc*II* $^+$ and lc*II* $^-$ shuttle vector phages grow as plaques at 37°C. Mutant frequency is determined as the number of lc*II* $^-$ plaque-forming units (pfu) observed divided by the total pfu screened. The lc*II* $^-$ 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 col screening.[4]

The Big Blue Cyclist® DNA sequencing kit can be used to rapidly sequence the lcII⁻ mutants identified with the l Select-cII assay. Th *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.

Conclusions

The l 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 yea By comparison, the l Select-cII mutation assay is more cost-effective and less labor-intensive to perform due to its selective nature a 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 l Select-cII mutation assay kit includes the G1250 selective host strain, enough Transpac lambda packaging extract** for 50 packaging reactions and lcII⁻ control mutants.

REFERENCES

1. Mirsalis, J.C., Monforte, J.A., and Winegar, R.A. (1995) *Annu. Rev.* Pharmacol. Toxicol. 35: 145-164.
2. Gorelick, N.J., and Mirsalis, J.C. (1996) *Environ. Mol. Mutagen.* 28: 434-442.
3. Dycaico, M.J., et al. (1994) *Mutat. Res.* 307: 461-478.
4. Jakubczak, J.L., et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 9073-9078.
5. Schwarz, E., et al. (1978) *Nature* 272: 410-414.
6. Place, N., et al. (1984) *J. Mol. Biol.* 180: 865-880.
7. Rogers, B.J., et al. (1995) *Mutat. Res.* 327: 57-66.
8. Young, R.R., et al. (1995) *Mutat. Res.* 327: 67-73.

* 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

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