BioReliance

Toxicology Services

Big Blue® Transgenic Rodent (TGR) Mutation Assay

BioReliance's Big Blue® Transgenic Rodent (TGR) Gene Mutation Assay utilizes a novel transgenic animal model created and bred for specific use in an assay defined by OECD Test Guideline 488, "Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays." This test system permits measurement of mutations in any tissue including germ cells following positive in vitro genetic toxicology results or positive tumor findings where a mutagenic mode of action is suspected.

Big Blue® mice and rats have been bred to have multiple copies of recoverable lambda phage shuttle vectors integrated into every cell of their body, resulting in three animal models:

- Big Blue® C57Bl/6 (Transgenic Homozygous Mice)
- Big Blue® B6C3F1 (Transgenic Heterozygous Mice)
- Big Blue® Fisher 344 (Transgenic Homozygous Rats)

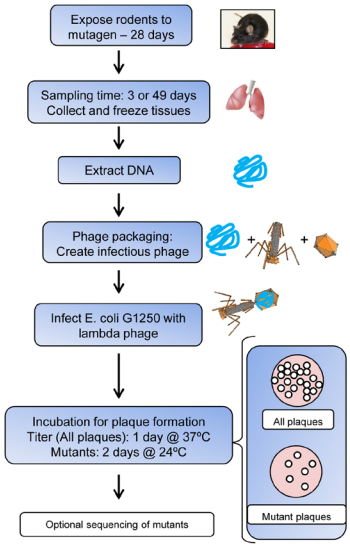
Why the Big Blue® Transgenic Rodent Mutation Assay

The Big Blue® TGR assay provides a definitive measure of mutations in any somatic cell or germ cell in rats or mice. Somatic tissues represent site of contact or tumor target, whereas, germ cells may contain mutations that can be carried on to offspring. Most other in vivo assays measure DNA damage, but DNA damage does not always result in mutations. As such, the Big Blue® TGR assay:

- Addresses mutagenic mode of action
- Is valuable as a secondary tier assay to investigate positive results from in vitro genotoxicity assays
- Is included in various regulatory guidelines and expert recommendations and has gained regulatory approval

How Big Blue® Works

- Multiple lambda phage shuttle vectors in every cell
- Gene in vector records and reports DNA mutations
- Initially mutant plaques were blue, hence "Big Blue"
- Today the *cII* gene is used to measure temperature sensitive mutants
- Animals are dosed for 28 days
- Routes: oral, inhalation, dosed water, dosed feed
- Collect and freeze tissues
- Extract DNA from tissues
- Cut vector DNA from genomic DNA
- Create infectious lambda (λ) phage particles
- Adsorb phage onto E. coli, plate onto agar plates
- If phage replicate in cell, a plaque is formed and counted
- Only *cII* mutant phage form plaques at 24ºC
- Wild type and mutant phage form plaques at 37ºC
- Mutant frequency - ratio of mutants per total phage



Fully Qualified, Commercial Assay

- Co-developed with NIEHS
- Validated in the 1990s
- Re-qualified to OECD design standards

Accepted Guidance

- OECD TG 488 finalized in 2011
- Follow up to positive findings
- Recommended by ECHA
- Recommended by EFSA
- Included in ICH M7

Why BioReliance?

- Over 25 years experience with same Study Director
- Available in mice or rats
- Custom extraction of tissues
- Inhalation Studies
- Rapid study initiation
- Control of animal breeding

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Frequently asked questions

What does Big Blue data look like?

Data are reported as the frequency of *cII* mutant phage (plaques) per million plaques screened. The table shows representative MF data for mouse (yellow) and rat (blue). Mean and standard deviation data are shown for background vehicle controls for liver and bone marrow. In addition liver and bone marrow MF from animals treated with the mutagens ethyl nitroso urea (ENU; direct acting) and benzo (a) pyrene (BaP; requires liver metabolism) are shown. The data show good reproducibility and significantly increased MF in both slow dividing (liver) and fast dividing (bone marrow) tissues with both ENU and BaP.

Big Blue®C57BL/6 Mice			Big Blue®F344 Rats		
Treatment (mg/kg/day) x days	**Liver *cII* Mut Freq (x10-6)**	**BM *cII* Mut Freq (x10-6)**	**Treatment (mg/kg/day) x days**	**Liver *cII* Mut Freq (x10-6)**	**BM *cII* Mut Freq (x10-6)**
Vehicle Control Olive Oil (0) x 28	43.6 ±6.2	38.1 ±14.2	Vehicle Control Olive Oil (0) x 28	43.4 ±8.8	29.7 ±14.3
BaP (50.0) x 28	213.3*±36.32	673.7*±75.1	BaP (50.0) x 28	252.1*±118.9	269.4*±52.4
Fold Increase	4.9	17.7	Fold Increase	5.8	9.1
ENU(40.0) x 3	181.3*±37.2	405.2*±90.0	ENU(20.0) x 3	157.2*±45.3	201.5* ±31.6
Fold Increase	4.2	10.7	Fold Increase	3.6	6.8

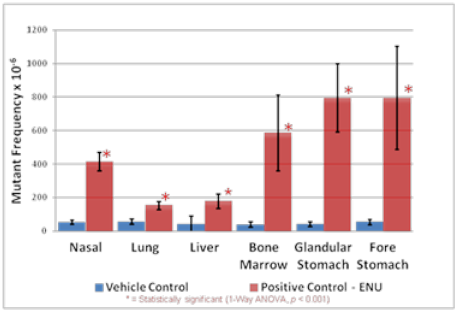
*Significant increase ($p < 0.001$)

Mouse or rat?

Big Blue® offers the advantage of using C57BL/6 or B6C3F1 mice or Fisher 344 rats. All are viewed scientifically as equivalent. Big Blue provides flexibility in selecting the appropriate species, sex and strain based on existing toxicology, toxicokinetic, metabolism or tumor data. Males are normally used unless there are data to support use of females.

What tissues should be analyzed?

Tissue selection should be based on why the study is needed and needs to consider existing mutagenicity, carcinogenicity and toxicity data. Tissue selection should consider route of exposure (based on human exposure), predicted tissue distribution and mechanism of action. Tissues should maximize chance of detection of rapidly metabolized, poorly adsorbed, or site of first contact determined by route of administration. In the absence of background information at least two tissues should be evaluated -- one slowly proliferating tissue such as liver and one rapidly proliferating tissue such as glandular stomach or bone marrow are suggested in OECD TG 488. Male germ cells (testis and cauda) should also be collected and frozen for possible later analysis if needed.



With what routes of exposure and tissues do you have experience?

All common routes of exposure have been used including oral, dosed feed, dosed water and inhalation. Tissues analyzed include: Bladder, Bone Marrow, Brain, Buccal Surface, Cauda Epididymis (Sperm), Colon, Duodenum, Forestomach, Glandular Stomach, Gingiva, Heart, Ileum, Jejunum, Kidney, Liver, Lung, Nasal Cavity, Palate, Pancreas, Spleen, Skin, Testis (Seminiferous tubules)

References

Thompson CM, Young RR, Suh M, Dinesdurage H, Elbekai RH, Harris MA, Rohr AC and Proctor DM. (2015), Assessment of the Mutagenic Potential of Cr(VI) in the Oral Mucosa of Big Blue® Transgenic F344 Rats. Environ. Mol. Mutagen. 2015 Aug;56(7):621-8. doi: 10.1002/em.21952.

Young RR, Thompson CM, Dinesdurage H, Elbekai RH, Suh M, Rohr AC and Proctor DM. (2015), A robust method for assessing chemically-induced mutagenic effects in the oral cavity of transgenic Big Blue® Rats. Environ. Mol. Mutagen. 2015 Aug;56(7):629-36. doi: 10.1002/em.21951.

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