



**Integrated Risk Information System (IRIS) Bimonthly Public Science Meeting
Thursday, October 30, 2014**

Science Question 7: *In vivo* mutagenicity/genotoxicity studies of hexavalent chromium

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Big Blue[®] Transgenic Rodent (TGR) Mutation Assay



- *In vivo* assay to measure somatic and germ cell mutations
- Historically – limited options to measure *in vivo* mutations
- TGR Mutation assays developed and validated in 1990's
- Filled an unmet need to investigate *in vivo* mutagenic mode of action
- Uses transgenic mice and rats with recoverable lambda shuttle vector

Big Blue[®] Assay History



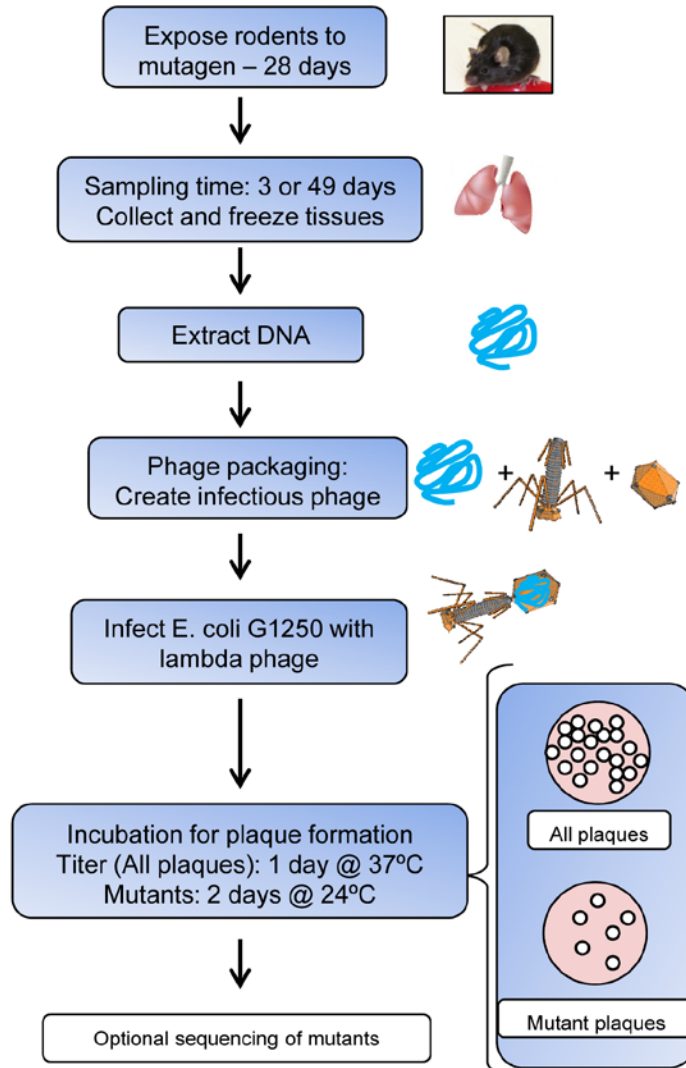
- NTP contracted BioReliance to create TGR mutation models
- BioReliance validated and commercialized the assays in 1990's
 - In absence of guidelines, little commercial interest through 2000's
- OECD Test Guideline 488 in 2011 reawakened interest and use
 - Used for ECHA, EFSA, pharmaceuticals (actives and impurities)
- BioReliance owns Big Blue[®] mice and rats
- BioReliance re-qualified Big Blue[®] to new OECD design standards

Big Blue[®] Assay History



- NTP goal – use same species/strains used by NTP for 2 year bioassays.
- Original purpose to investigate tumor MOA from NTP cancer studies
- Rat:
 - Big Blue[®] Fisher 344 rat; Homozygous
 - F344 rat used for NTP 2 year rat carcinogenicity studies
- Mouse:
 - Created in Big Blue[®] C57BL/6 mice; Homozygous
 - Breed to C3H mice to create Big Blue[®] B6C3F1 mice; Heterozygous
 - B6C3F1 used for NTP 2 year mouse carcinogenicity studies

Big Blue[®] Assay: Overview



- Dose animals
- Necropsy - freeze tissues
- Extract DNA
- Cut out shuttle vector (Transpack)
- Package into empty phage particles
- Adsorb onto *E. coli* G1250
- Plate onto 100 mm plates
- Incubate at 37°C and 24°C
 - 37°C – both *cII* wildtype and mutants give plaques
 - 24°C – only *cII* mutants produce plaques
- Count and evaluate
- Mutant frequency: ratio of mutants to total phage (plaques) screened

Modifications to Analyze Mutations in Oral Cavity



- Oral cavity not routinely evaluated in TGR assays
- Standard methods gave low yield of low quality DNA
- Methods optimized in two studies
 - Liquid nitrogen pulverization of tissue
 - Homogenization, centrifugation of nuclei, digestion, phenol chloroform extraction



Oral Mutagenesis Proof of Concept: Gingiva - Buccal



Treatment (mg/kg/day) x days	Animal Number	# Packaging	# Mutants	# Phage Screened	Mutant Frequency (x10 ⁻⁶)
Drinking water (0.00) x 28	2451	2	21	289,333	72.6
	2452	2	12	211,000	56.9
	2453	4	11	169,667	64.8
	2454	4	6	174,333	34.4
	2455	2	5	184,000	45.7
	Average ± Standard Dev	---	---	---	---

4-NQO (10 ppm) x 28	2456	2	286	252,333	1133
	2457	2	283	214,333	1320
	2458	2	213	204,333	1042
	2459	4	207	220,333	939
	2460	3	137	134,333	1020
	Average ± Standard Dev	---	---	---	---

***Significant increase (p < 0.001)**

Oral Mutagenesis Proof of Concept: Gingiva - Palate



Treatment (mg/kg/day) x days	Animal Number	# Packaging	# Mutants	# Phage Screened	Mutant Frequency (x10 ⁻⁶)
Drinking water (0.00) x 28	2451	2	7	213,333	32.8
	2452	2	16	329,333	48.6
	2453	2	12	215,000	55.8
	2454	2	15	315,667	47.5
	2455	3	13	315,667	41.2
	Average ± Standard Dev	---	---	---	---

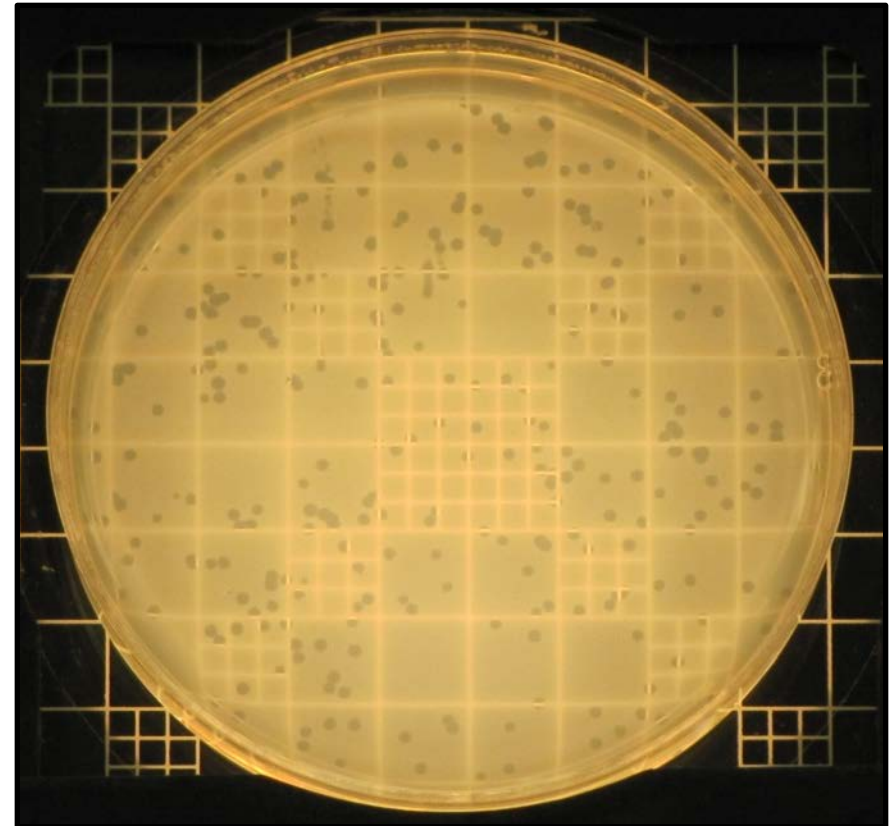
4-NQO (10 ppm) x 28	2456	2	439	203,667	2155
	2457	4	619	277,333	2232
	2458	2	682	364,667	1870
	2459	3	584	286,000	2042
	2460	2	634	207,667	3053
	Average ± Standard Dev	---	---	---	---

***Significant increase (p < 0.001)**

Big Blue[®] in Pictures



E. coli lawn with plaques



Scoring plaques

Cr(VI) in the Big Blue[®] Assay

