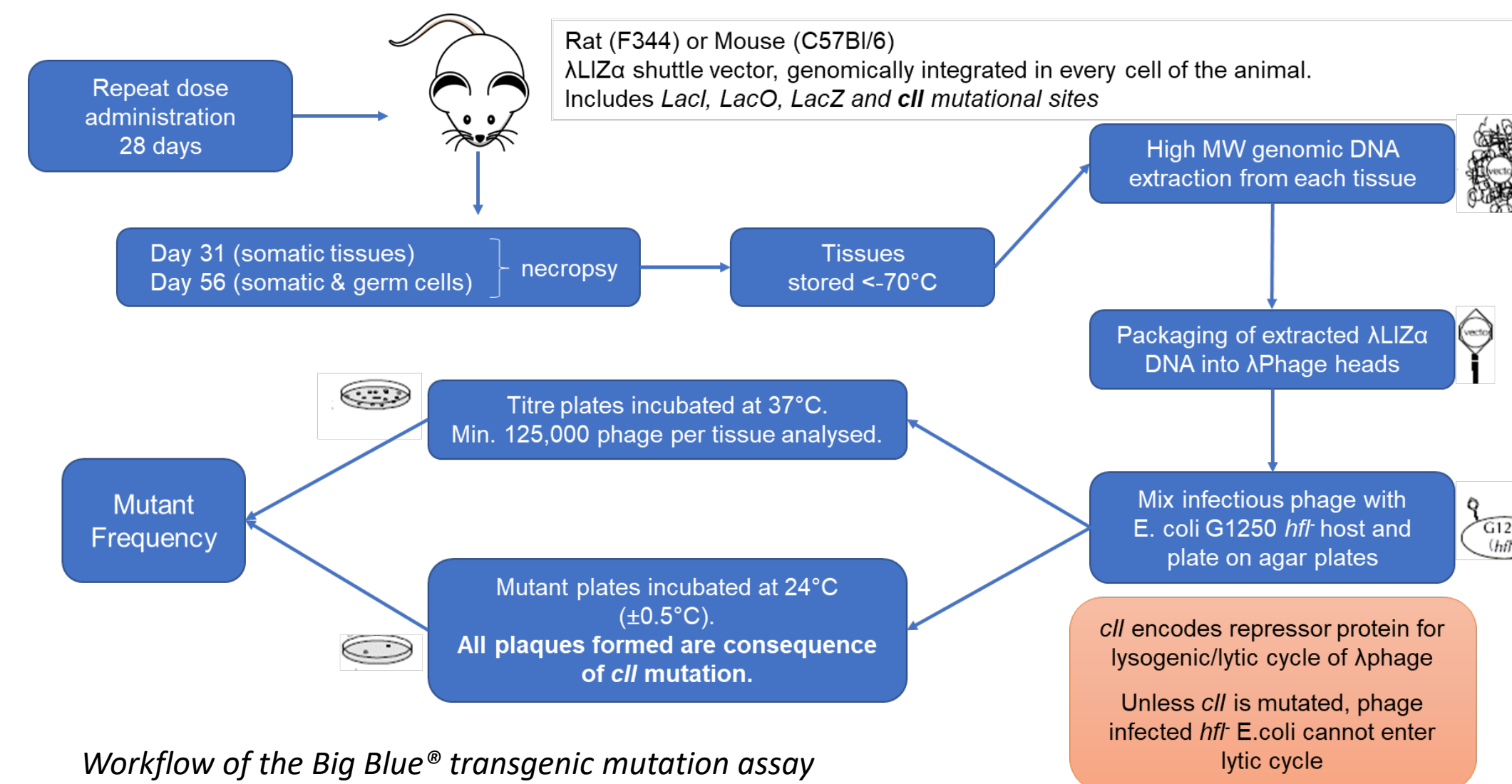


Establishing the Big Blue® assay at Gentronix

OECD 488 transgenic rodent (TGR) mutation assays are key in determining *in vivo* mutagenicity risk of substances and new laboratories looking to establish OECD 488 studies should complete a laboratory proficiency exercise. Gentronix commenced this exercise in 2021 following acquisition of the Big Blue® assay models from BioReliance, Merck KGaA (“Historic Lab”) and a large tissue bank collected from Big Blue® rats treated with either vehicle (VC) or positive control ethyl nitrosourea (ENU; 20 mg/kg). Methods for DNA extraction, phage packaging, plating, phage titre and mutant frequency scores were established at Gentronix facilities as follows:

- High molecular weight DNA extraction conducted using Recoverase reagents (Agilent Technologies) from:
 - Glandular stomach
 - Duodenum
 - Liver
 - Bone marrow
 - Kidney
 - Contents of seminiferous tubules from testes (germ cells)



- DNA packaged using Transpack reagents (Agilent Technologies)
- λ LIZ α shuttle packaged into λ bacteriophage heads
- Infect *E. coli* G1250 *hfl* host
- Plated on agar and incubate at:
 - 24°C (± 0.5 °C) – *cII* mutants only
 - 37 °C (± 2 °C) – total phage titres
- Score plates: minimum of 2 packagings used to achieve $\geq 200,000$ phage per tissue per group
- Mutant frequency determined and ENU data compared with VC (Figure 1)
- Gentronix results compared with Historic Lab data (Table 1)

Detecting *in vivo* mutation in somatic and germ cells

Aim: Demonstrate proficiency under OECD 488 by detecting induced *in vivo* *cII* mutations in rapidly and slowly dividing tissues and germ cells following exposure to reference *in vivo* non-mutagenic and mutagenic (ENU) substances.

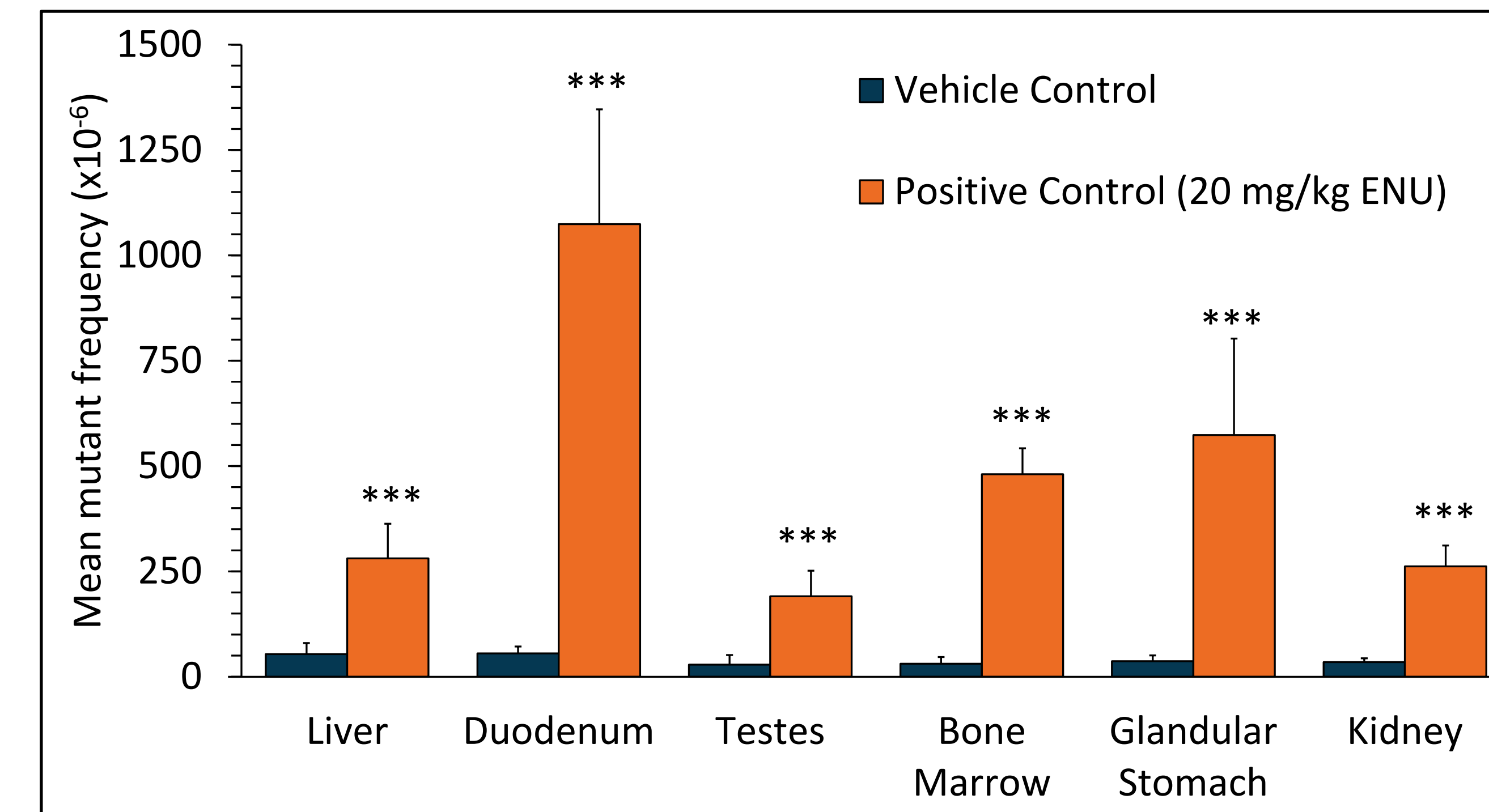


Figure 1. Detection of induced mutation across both somatic tissue and germ cells for reference mutagen in the Big Blue® male F344 rat. *** = $p < 0.001$ (Student's t-test vs vehicle control)

Results

- Robust detection of background (VC) and ENU-induced mutations across a range of somatic tissues from Big Blue® rats.
- Robust detection of background (VC) and ENU-induced mutations in male germ cells derived from testes from Big Blue® rats.
- Mutant frequencies of the VC groups were consistent with published data.
- Statistically significant increase in mutant frequency detected in all tissues treated with ENU compared with respective VC-treated tissues.

Comparison of vehicle and positive control *cII* mutation data between Gentronix and Historic Lab

Table 1	Mutant frequency ($\times 10^{-6}$) data at the <i>cII</i> locus of the Big Blue® F344 rat – VEHICLE CONTROL											
	Duodenum		Liver		Testes (Male Germ Cells)		Bone Marrow		Glandular Stomach		Kidney	
	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab
Mean	55.2	40.7	53.6	41.9	28.7	20.7	30.9	29.4	37.0	30.6	34.9	28.4
SD	16.7	16.8	26.1	19.7	22.6	10.4	15.8	13.2	13.8	12.0	9.0	13.8
95% Control Limits	21.8 – 88.6	7.1 – 74.3	1.4 – 105.8	2.5 – 81.3	0.0 – 73.9	0.0 – 41.5	0.0 – 62.5	3.0 – 55.8	9.4 – 64.6	6.6 – 54.6	16.9 – 52.9	0.8 – 56.0
Range	28.7 – 98.7	9.6 – 105.5	12.0 – 117.3	5.0 – 120.2	6.8 – 72.8	7.6 – 54.5	9.6 – 58.7	6.8 – 71.7	19.4 – 61.1	5.2 – 69.9	14.4 – 44.6	8.6 – 67.1
N	38	101	35	208	17	25	10	115	10	62	10	37

Table 1	Mutant frequency ($\times 10^{-6}$) data at the <i>cII</i> locus of the Big Blue® F344 rat – POSITIVE CONTROL (ENU 20 mg/kg)											
	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab	Gentronix	Historic Lab
	Mean	1073.9	831.0	280.7	210.6	190.9	126.6	480.5	388.2	573.7	504.5	261.8
SD	272.7	171.3	82.4	90.0	60.5	50.1	61.8	127.7	228.8	249.6	49.7	35.3
95% Control Limits	528.5 – 1619.3	488.4 – 1173.6	115.9 – 445.5	30.6 – 390.6	69.9 – 311.9	26.4 – 226.8	356.9 – 604.1	132.8 – 643.6	116.1 – 1031.3	5.3 – 1003.7	162.4 – 361.2	54.5 – 195.7
Range	614.8 – 1665.2	439.3 – 1285.3	157.4 – 456.2	60.8 – 614.1	137.7 – 340.4	39.5 – 197.7	400.0 – 563.7	143.3 – 1030.6	331.3 – 1026.0	200.9 – 1452.7	179.9 – 340.8	84.5 – 233.1
N	39	74	30	169	10	18	10	100	12	36	10	15
Fold Increase	19.5	20.4	5.2	5.0	6.7	6.1	15.6	13.2	15.5	16.5	7.5	4.4

Aim: Demonstrate successful laboratory transfer by comparing mutant frequency data with Historic Lab.

Results

- As Table 1 shows, both somatic and germ cell tissues mutant frequencies are highly comparable between Gentronix and Historic Lab.
- Mean mutation frequencies for each tissue type are consistent.
- 95% control limits in both VC and ENU groups for each tissue type are also consistent.

Importantly, for Gentronix, there is a clear separation between VC and ENU control ranges.

CONCLUSION

Following completion of a successful laboratory method transfer, work to establish proficiency of the OECD 488 Big Blue® TGR somatic and germ cell mutation assay at Gentronix has now been successfully completed in the Big Blue® rat. Mutant frequency data generated to date has been highly comparable to the Historic Lab data across 5 somatic tissue sites (glandular stomach, duodenum, liver, bone marrow & kidney) and in male germ cells, for both vehicle control and reference mutagen exposed tissues. As such, Gentronix is considered proficient for the conduct of OECD 488 Big Blue® transgenic rat mutation studies.