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Proficiency Establishment of the OECD 488 Big Blue® Transgenic Rodent Somatic and Germ Cell Mutation Assay

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Assessing the mutagenic potential and risk to human health and the environment of new drugs and chemicals is a global regulatory requirement. OECD 488 transgenic rodent (TGR) mutation assays are key in determining the in vivo mutagenicity risk of substances that have positive in vitro mutagenicity data. The Big Blue® rat is a suitable TGR model that allows investigation of in vivo mutation at the cII locus within the genome integrated lambda bacteriophage shuttle vector.

Gentronix conducted an initial proficiency assessment for the detection of cII locus mutation frequencies in duodenum, liver, and male seminiferous tubule derived germ cells from Big Blue® rats, and compared data with the historical laboratory, with extension of the exercise to then include glandular stomach, bone marrow and kidney. The exercise used frozen tissues banked during previously conducted Big Blue® rat studies, where a 28 day exposure period was followed by either a 3 or 28 day (somatic tissues) or 28 day (germ cells) fixation period for untreated/vehicle and N-ethyl-N-nitrosourea (ENU; positive control) treated animals. Genomic DNA was extracted from tissues using Agilent DNA RecoverEase methods, and packaged using Agilent Transpack reagents to create infectious phage particles that were then expressed using the G1250 E. coli strain plated on agar. Plates were incubated at 2 temperatures: 37°C (both wildtype and mutated phage enter the lytic cycle [packaging efficiency]) or 24°C (only cII mutated phage enter the lytic cycle), and the resulting phage plaques scored. Packaging e-ciency was demonstrated across all tissues with the mean number of phage screened from each DNA sample >200,000 in both untreated/vehicle and ENU treated animals, in accordance with OECD 488 (2022). ENU treatment significantly increased mutant frequencies over controls ($p<0.001$) for all tissues tested (mean mutation frequency fold increases of: 16, glandular stomach; 20, duodenum; 5, liver; 16, bone marrow; 8 kidney; 7, male germ cells). Absolute mutant frequencies for all tissues in the untreated/vehicle control groups were consistent with the 95% control limits of previously generated historical laboratory data. These data illustrate that Gentronix is proficient in the methods of transgene recovery from genomic DNA and in reproducing expected mutant frequencies for positive and negative controls in the Big Blue® TGR assay across a range of somatic tissues and in germ cells.

Keywords:
Transgenic rodent; mutagenicity; OECD 488.