

Regulated Bioanalysis Case Study:

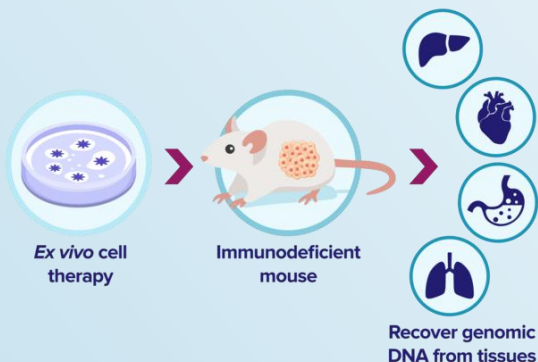
Detection of Human Alu Sequences in Rodent Genomic DNA Background

OBJECTIVE

Prior to the administration of a cell-based therapy medicinal product to human subjects, understanding of the biodistribution, persistence, and clearance of the product in a relevant animal model is essential. Biodistribution data contributes to the interpretation and design of nonclinical pharmacology and toxicology studies conducted to support early-phase clinical trials. The selected target for the detection of human genomic DNA (hgDNA) in a rodent gDNA background must feature a high species specificity to fulfill the sensitivity requirement in accordance with regulatory guidance. The highly repetitive nature of the Human Alu (hAlu) sequence chosen for PCR detection of hgDNA in the developed assay meets this standard.

METHOD

For the method qualification, the standard curve contained Rowett nude (RNU) rat/NOD scid gamma (NSG) mouse gDNA derived from liver with various amounts of human gDNA. Quality controls were made from purified matrix (genomic DNA isolated from RNU rat and NSG liver, brain and whole blood) spiked with various amounts of hgDNA in order to assess the following parameters: specificity, sensitivity, assay range, standard curve performance (slope, linearity, and efficiency), inter-assay/ intra-assay precision and accuracy.



ASSAY RESULTS

Parameters	Range
Range of the method	50.0 - 1.00×10^6 fg of hgDNA/ 100 ng of rodent gDNA*
LLOQ (Lower Limit of quantification)	100 fg of hgDNA/ 100 ng of rodent DNA
ULOQ (Upper Limit of Quantification)	1.00×10^6 fg of hg DNA / 100 ng of rodent gDNA.
Precision (CV (%))	CV (%) of fg/rxn \leq 35.0% except when \leq 100 fg/rxn (LLOQ) then \leq 45.0.
Accuracy/Relative Error (RE (%))	RE (%) of \leq 35.0, except when \leq 100 fg/rxn (LLOQ) then \leq 45.0 . RE (%) for blood \leq 45.0 for all levels.
Specificity	BLOQ/UD

* 100ng of rodent DNA is equivalent to 0.886 human cells per million rodent cells.

CONCLUSIONS

- Highly repetitive Alu elements can account for up to 10% of hgDNA and therefore serve as useful markers for tracking engineered cell therapy products in rodent studies.
- Pharmaron (Exton) Lab Services LLC has a qualified (non-GLP) qPCR method for the detection of Alu elements in NSG mouse and RNU rat gDNA background.
- The method is available to support early-stage cell therapy in RNU and NSG tissue and blood sample matrix.

