Characterization of transgene site of integration using next generation sequencing
Background

In CAR T cell therapy using lentiviral vectors, the **vector copy number (VCN)**—the average number of integrated transgene copies per cell genome—is a critical quality control parameter. It ensures both therapeutic efficacy and safety, since too few integrations may reduce CAR expression, while too many increase the risk of insertional mutagenesis.

Summary

The protocol involves Next-Generation Sequencing (NGS)-based integration site analysis, and provides comprehensive analysis of both copy number and information on integration sites.
The method applies high-coverage NGS for spanning the whole genome. The information from the lentiviral construct is used, along with the whole genome information, to map loci of integration for each insert. Our sequencing and analysis protocols detect both left and right borders of insertion to enumerate sites of insertion. The method detects and flags potentially truncated or concatenated as head-to-head or head-to tail multimers from insertion events.

Mapping summary

Reference GRCh38 (hg38)	Coverage			
1X	99.99%			
10X	99 %			

Reads detecting insert

Reference vector	Coverage			
1X	99.99%			
10X	99 %			

Integration summary

Integration event	**Chromosome**	**Loci reference hg38**		**Attribute**	**Notes**
Event 1	17p21.31 Chr17:43044295	Left – 100bp genomic coordinates	Right 100bp genomic coordinates	Full insertion event	No mutation detected in event
Event 2	21p20.31 Chr21:434295	Left – 100bp genomic coordinates	Right 100bp genomic coordinates	Truncated	Coordinates in construct deleted <Range>
Event 3	11p2.3 Chr11:34295	Left – 100bp genomic coordinates	Right 100bp genomic coordinates	Concatemer (Head to head)	

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References

Nicholls et al., Locating and Characterizing a Transgene Integration Site by Nanopore Sequencing, *G3 Genes|Genomes|Genetics*, Volume 9, Issue 5, 1 May 2019, p1481–1486.
Yu, C. *et al.* (2023). ASIS-Seq: Transgene Insertion Site Mapping by Nanopore Adaptive Sampling. In: Saunders, T.L. (eds) Transgenesis. Methods in Molecular Biology, vol 2631. Humana, New York, NY

Appendix data

1.Flanking read of insert visualization (and supporting. BAM file) of detected insertion event with details of coverage and genomic coordinates
2.Whole genome copy number analysis and local genome copy analysis flanking insertion sites
3.Genome purity summary* (will not cover RNA species)

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