Supporting engineered cell therapy: targeted and accurate assessments of gene editing outcomes with Xdrop® and long-read sequencing

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Characterizing CAR cassette integration patterns is vital to determine the genotoxicity risk of engineered cells intended for therapeutic applications. Xdrop enables enrichment of integrated CAR cassette DNA, including the flanking genomic regions, for targeted long-read sequencing. Using Xdrop, we accurately identify CAR cassette integration sites in lentivirus-transformed T cells and validate the insertions via Sanger sequencing of PCR-amplified cassette–genome border regions.

We here also present initial results from a new Xdrop enrichment method that provides 8- to 12-kb long sequencing reads with a very low frequency of DNA amplification artifacts. This new Xdrop protocol facilitates precise and accurate analyses of CRISPR gene editing outcomes and unintended on-target editing resulting in rearrangements. This method could be powerful for the analysis of heterogeneously edited cell populations.

Conclusion

Xdrop-based workflows are proven to validate cell and gene engineering from just 5–10 ng DNA, enabling you to confirm or reveal:

- CAR cassette integrations
- Unintended and intended on-target CRISPR edits
- Gene cassette integrity
- Off-target cassette integration

Results

The Xdrop enrichment method reveals the sample heterogeneity

3 replicate enrichments using 10 ng DNA.
Each enrichment primarily yields unique integration sites.

- Provides high confidence level thanks to long reads
- Allows validation of integration sites
- Allows assessment of cassette integrity and rearrangements

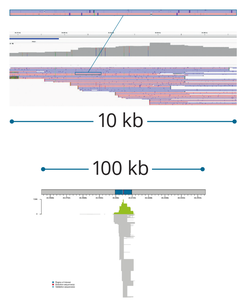
Clear insight into the distribution of CAR-T cassettes in the genome

The method identifies ~1000 CAR-T cassette insertion sites using just 30 ng DNA (~5000 cells).

The analysis reveals a high insertion rate in gene regions.

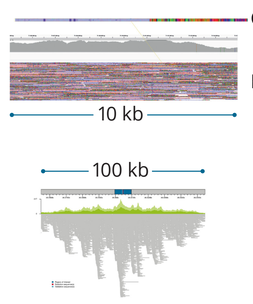
New Xdrop enrichment approach for CRISPR-engineered cells

Coverage of ~20 kb region with 99% chimera-free reads
- Shows integration sites
- Validates edits
- Finds off-target integration
- Reveals unintended on-target edits



- High fraction of 8- to 12-kb reads
- Covers ~20-kb region

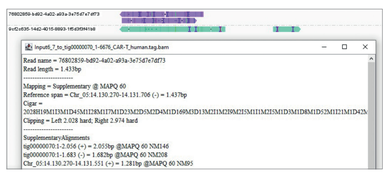
Alternative method for coverage of ~100 kb region
- 40% of reads chimera-free



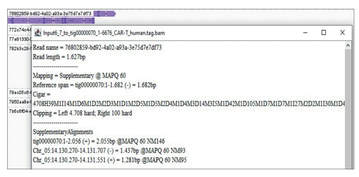
- 5-6 kb reads
- Covers ~100-kb region

Example of a candidate CAR-T cassette insertion site

Alignment of 2 reads to Chromosome 5

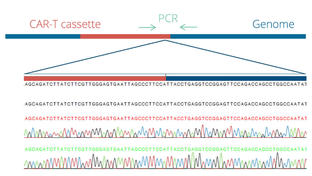


The 2 reads aligned to the CAR-T cassette



Sanger sequencing use to validate PCR-amplified borders

PCR of border and Sanger sequencing



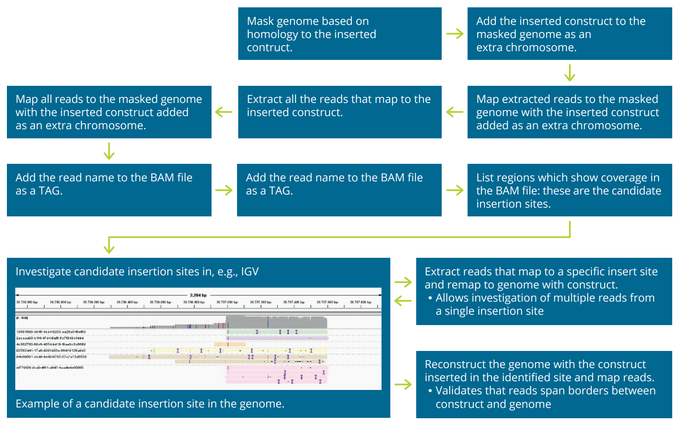
Location of integration site validated via Sanger sequencing

Position	Sites (% of validated)
Intron	64
Within 5 kb upstream of gene	9
Within 5–15 kb upstream of gene	18
Within 5 kb downstream of gene	9

Xdrop workflow and analysis

Enrichment of long DNA molecules containing CAR cassettes

Bioinformatics analysis procedure



03/2022