Genetic Characterization and QC of a CRISPR-edited Cell Line

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Report: XXX

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1) Goal

In this study, 1 transgenic cell line with a modified *Gene 1* locus was analyzed.

The aim of this analysis was to:

1. Identify breakpoint sequences of the genomic modification(s).
2. Assess the presence of structural variants surrounding the *Gene 1* locus.

An overview of the TLA technology and technical details of the performed analyses is provided in the manual Introduction to the terminology and methods used in transgene & integration site TLA analyses & ddPCR.

2) Summary

Sample	Number of gRNA induced events	Notes
Sample 1	3	2 alleles with genomic deletions, 1 allele with a balanced genomic translocation. No wild type alleles were detected

3) Conclusion

In Sample 1, 3 different genomic deletions were detected, affecting the exon 2 and intron 2 of *Gene 1*. Additionally, an allele containing a balanced genomic translocation between the exon 2 of *Gene 1* and exon 5 of *Gene 2* on chromosome 5 was detected. Finally, no wild type alleles were found in this sample.

4) Methods

TLA, sequencing and data mapping

Viable frozen human cells were used and processed according to Solvias' TLA protocol (de Vree et al. Nat Biotechnol. Oct 2014). An overview of the TLA technology and technical details of the performed analyses is provided in the manual Introduction to the terminology and methods used in transgene & integration site TLA analyses & ddPCR.

TLA was performed with 2 independent primer sets, specific for the *Gene 1* locus (Table 1).

Table 1: Primers used in TLA analysis

Primer set	Name/Viewpoint	Direction	Binding position hg19	Sequence
1	Downstream Gene 1	FW	chr3:45,645,196	AACGTGTAGGAGCAGAC
		RV	chr3:45,645,036	CTCCTTATAGTGGAGATC
2	Upstream Gene 1	FW	chr3:45,642,918	GACGCTCCGTAGCAGAAC
		RV	chr3:45,642,903	TCTTCTGAACATTCGAAGTC

The NGS reads were aligned to the host genome. The human hg38 genome was used as host reference genome sequence.

5) Results Sample 1

Modified locus

Whole genome coverage plot

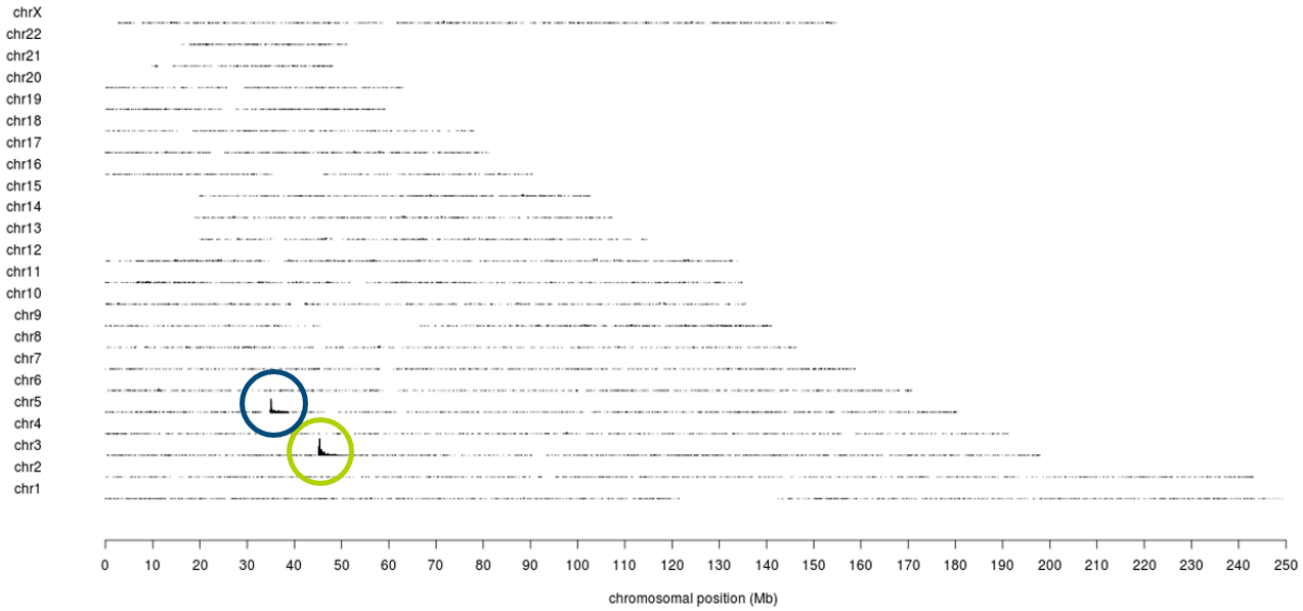

Figure 0: TLA sequence coverage across the human genome using primer set 1. The chromosomes are indicated on the y-axis, the chromosomal position on the x-axis. The *Gene 1* locus is encircled in green. The identified translocation is encircled in blue.

As shown in Figure 0 and Figure 1, the coverage is observed across the *Gene 1* locus on chromosome 3. Coverage is also observed on chr5 (encircled in blue in Figure 1), which indicates a translocation between chr5 and chr3 as described below. Similar results were obtained with primer set 2.

Locus-wide coverage

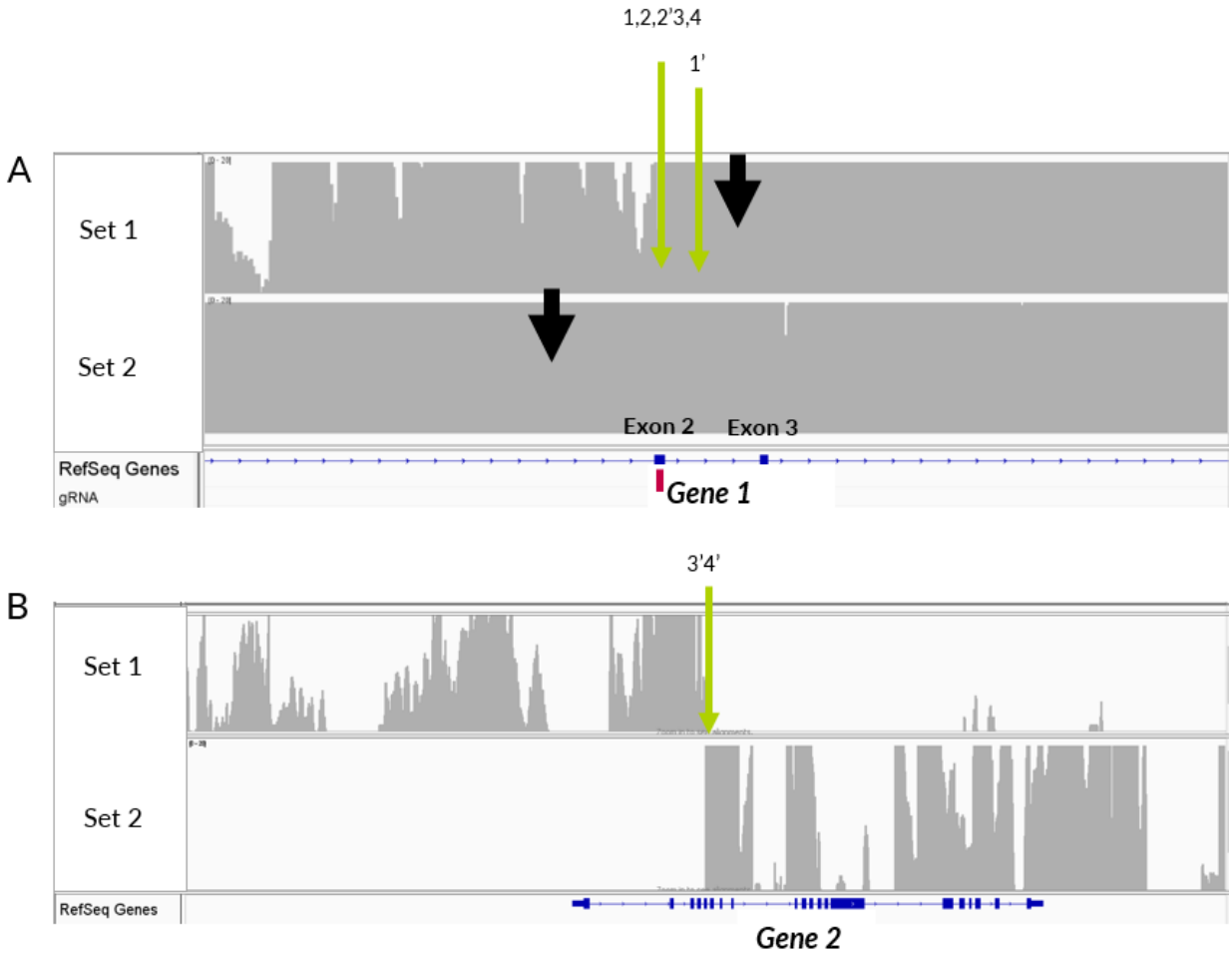

Figure 1: TLA sequence coverage (in grey) across the *Gene 1* locus on chr3 (A) and the *Gene 2* locus on chr5 (B). The black arrows represent the primer sets binding location. The green arrow indicates the location of the identified breakpoint sequences, the numbers correspond to the breakpoints described below. The red bar below *Gene 1* exon 2 indicates the sgRNA position. The Y-axes are limited to 20x.

Coverage is observed across the *Gene 1* and the *Gene 2* loci as shown in figure 1. One-sided coverage on chromosome 5 (Figure 2B) indicates the presence of the balanced genomic translocation with *Gene1* on chromosome 3.

Breakpoint sequences

The following breakpoint sequences were identified marking modifications to the *Gene 1* locus. The numbers of the breakpoints correspond to the numbers in Figure 2 where (1) and (1') represent two sides of one breakpoint.

1. chr3:45,644,358 (tail) fused to chr3:45,644,742 (head) with 2 bp homology (382 bp deletion)
   CTCTCTCTCACCCTTCGTACAGGGTAAGGCTGGCTGGATGAGAACTGGCCATCACCA
   GTGGCATGCATCACCAGTGGCATTTGATCAGGTACCCTAGAATCGGATCGAATCGGA
   TCGATGCCTG
   Deletion of part of exon 2 and part of intron 2 of *Gene 1*.

2. chr3:45,644,346 (tail) fused to chr3:45,644,368 (head) with 1 bp homology (21 bp deletion)
   CTCTCTCTCACCCTTCGTACAGGGTAAGGCTGGCTGGATGAGAACTGGCCATCGCTG
   GCAGGAAAGTTCCAGTGCGTGGATATGACACCTCCAGTTCCACCACCCGACAGATCT
   GAGCCTCGAGATGTAGTGACCATCCAGACCATGGTCCCCAGCTTATG
   Deletion of part of *Gene 1* exon 2.

3. chr3:45,644,360 (tail) fused to chr5:35,373,057 (head) (translocation between exon 2 of *Gene 1* and exon 5 of *Gene 2*). Please note that breakpoints 3 and 4 describe a balanced translocation between chr5 and chr3 as shown in Figure 2.
   CTCTCTCTCACCCTTCGTACAGGGTAAGGCTGGCTGGATGAGAACTGGCCATCACCA
   GTGGCATGTGCCCCCAATGGGAAGAGCTATGGACTCTCGTATTTGGTTTCCGATCCC
   CGGCGTAGAGGGGGCGTACGGTAGTGATCGGGTGCCCGTAGAGGG

4. chr5:35,373,056 (tail) fused to chr3:45,644,373 (head) (translocation between exon 2 of *Gene 1* and exon 5 of *Gene 2*). Please note that breakpoints 3 and 4 describe a balanced translocation between chr5 and chr3 as shown in Figure 2.
   GGGCTGTGGATATCCACTGCAGCTATGGAAAGTTGTTCGGCCTACACGGTGCCAACA
   GTTCTTCACTGGTGCTGGTCTCAATGGGGGCAGTGACTGTGTTACGGCTGTACGAAGC

No wild type alleles were found in this sample at the CRISPR Cas9 cut site.

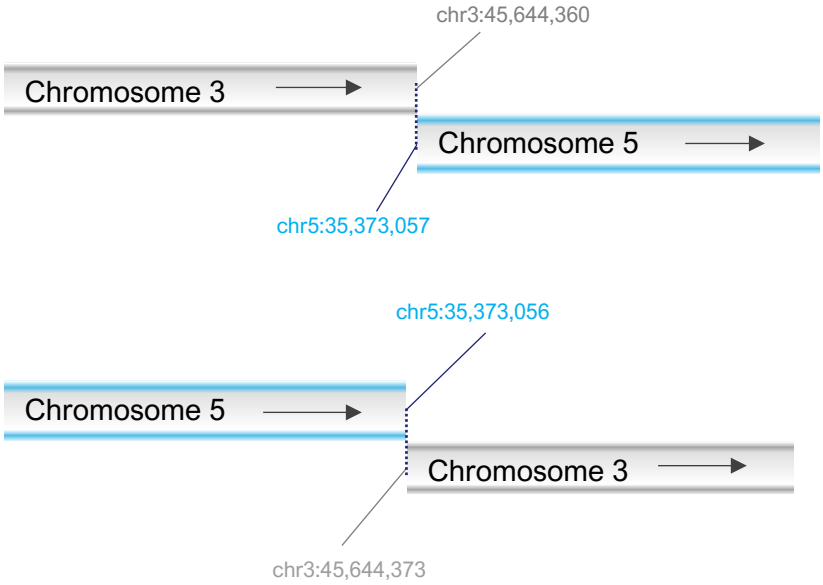

Figure 2: Schematic representation of the balanced translocation.

From this data it is concluded that the *Gene 1* locus has been modified including the deletion of part of exon 2 and part of intron 2, and a balanced translocation with the *Gene 2* locus on chromosome 5, as shown in Figure 3.

6) QC Information

Sample and study details

Sample receipt date:
Condition of sample at receipt:
Start date in the lab:
Sequencing run:
Deviations from the protocol:
TLApp version:

Study personnel

Lab technician
Data analyst
QC analysis and report

Quality control

The results are independently verified and reviewed and are an accurate and complete representation of the study. TLA processing of cells, NGS sequencing, and data analysis (except for copy number) are ISO/IEC 17025:2017 accredited by the Dutch Accreditation Council RvA, Registration number L671.

Scientific approval

Date

Signature