Project *Gene Editing*

Date: 4/3/20

Customer: customer

Project Overview

In order to better understand the structural effects of inserting a sequence at a specific locus on Chr 44, an experiment was performed to assess an edited sample and associated controls. For this project, KromaTiD:

- Designed, produced, and qualified custom probes for the insert sequence and for the regions on either side of the intended insert site.
- Executed assays on three samples provided by Customer.
- Imaged 200 metaphase spreads from each of the samples provided by Customer.
- Scored 200 metaphase spreads from each of the samples for structural rearrangements.

Procedure

An assay composed of 3 targeted probes – one for the insert, and two in a separate color that bracket the edit site – plus KromaTiD's dosimetry assay was used to measure rates of structural rearrangements involving the edited chromosome, as well as the rate of background rearrangements. Customer sent three cryopreserved samples to KromaTiD for recovery to culture, dGH prep, fixation, and harvest. Specification documents SPEC-0074 describes the probes for the insert and edit site brackets.

Metaphase spreads were prepared by KromaTiD from the samples, and hybridizations performed with the dGH probes. For each sample, 200 spreads were imaged and scored for the presence of structural rearrangements, using scoring rules developed in collaboration with Customer. SOP-0082 describes the scoring rules for the assay.

Results

In all samples, some rate of rearrangement was observed. Detailed score sheets for each sample are contained in the Excel workbook referenced in Appendix A. Note that cells may have more than one event, so total cells with rearrangements does not equal the total number of different rearrangements. The results are reported for the dosimetry paint in Table 1 and Figure 1, and for the Chr44 assay in Table 2 and Figure 2.

Table 1: Summary of dosimetry paint scoring data by sample. Note that the "Normal" designation refers to both dosimetry and Chr44 assay results, such that rows may not sum to 200. Chr44 Assay data is contained in Table 2.

	Normal	Inversion / SCE	Translocation	Insertion	Total Inversion / SCE	Rate of Inversion / SCE per Cell
Fibroblast	71	129	0	0	189	0.95
Untreated iPS	79	118	0	1	189	0.95
Treated iPS	2	88	0	0	126	0.63

Figure 1: Graphical display of dosimetry scoring data breakout by sample.

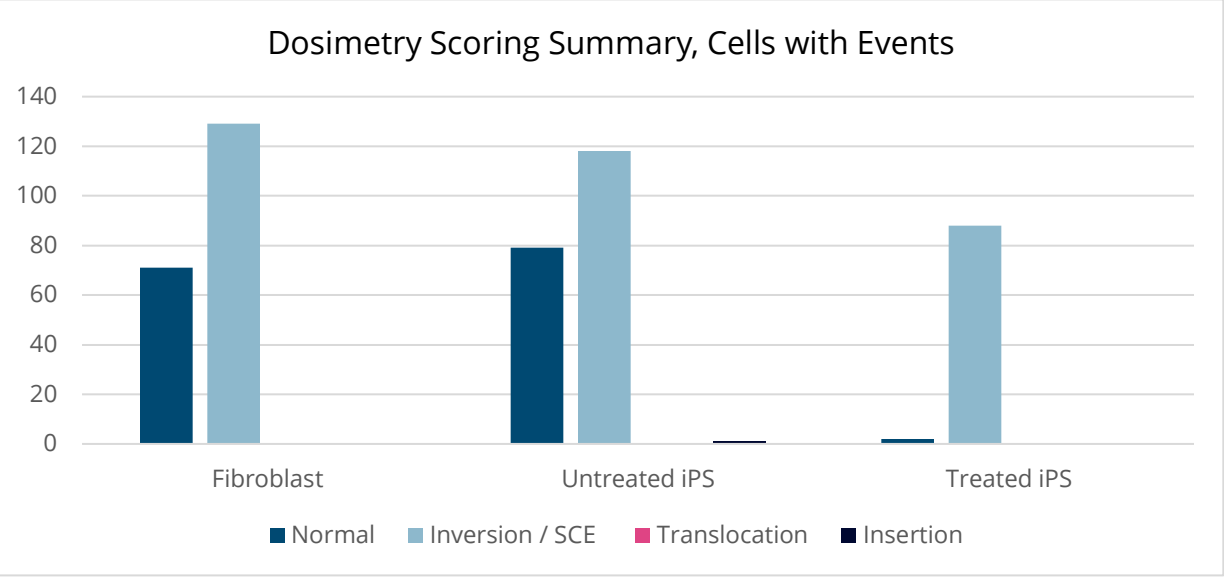
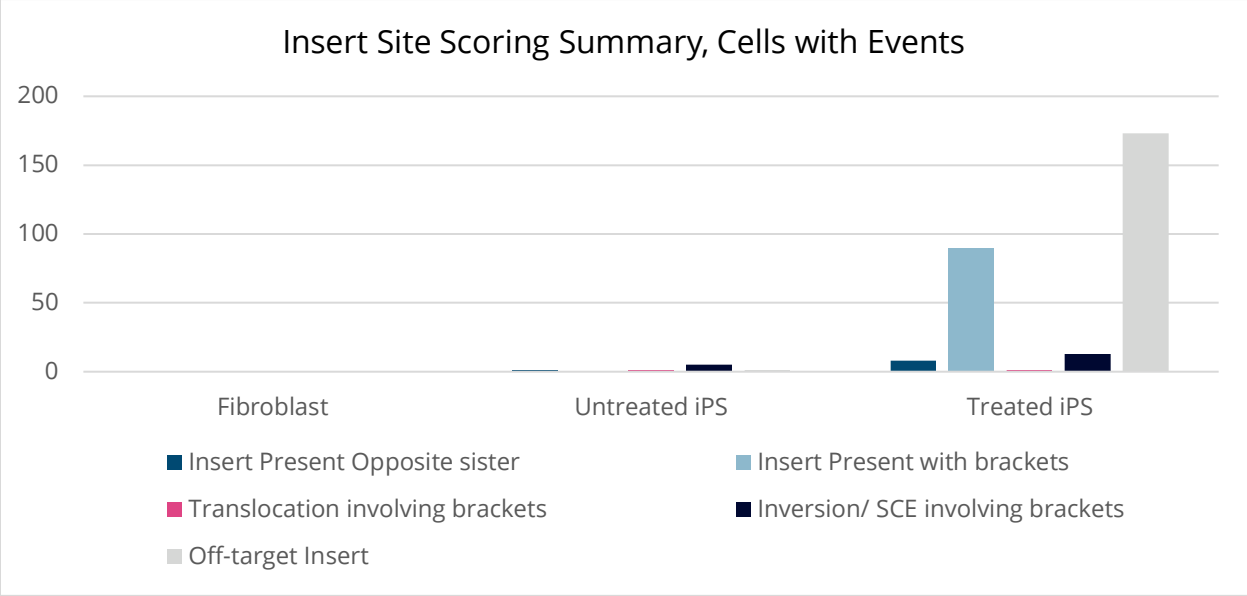


Table 2: Breakout of Chr 44 assay scoring data, separating types of rearrangements prevalent in each sample. Data represent the number of cells with a given type of rearrangement.

	Insert Present Opposite sister	Insert Present with brackets	Translocation involving brackets	Inversion/ SCE involving brackets	Off-target Insert	Total Off-targets	Rate of Off-Targets per Cell
Fibroblast	0	0	0	0	0	0	0.00
Untreated iPS	1	0	1	5	1	2	0.01
Treated iPS	8	90	1	13	173	215.5	10.78

Figure 2: Graphical display of Chr44 assay scoring data breakout by sample.



As a further division of the off-target inserts, 10 or fewer copies appeared in 88 cells, and 11 or more appeared in 85 cells.

Figure 3: Example of a normal cell (fibroblast control).

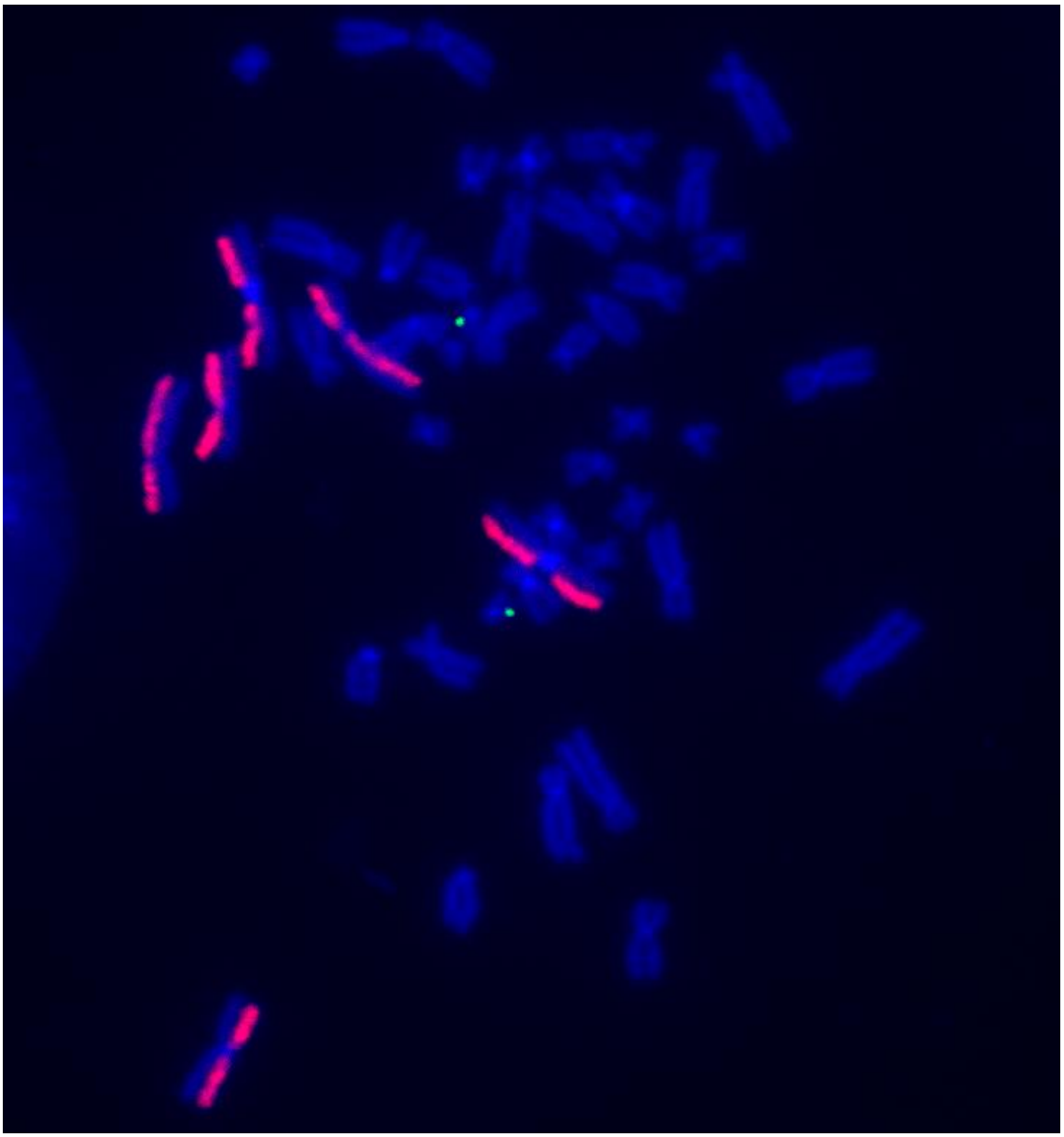


Figure 4: The cell from the unedited iPS sample where an insert of one of the dosimetry paints occurred on another chromosome.

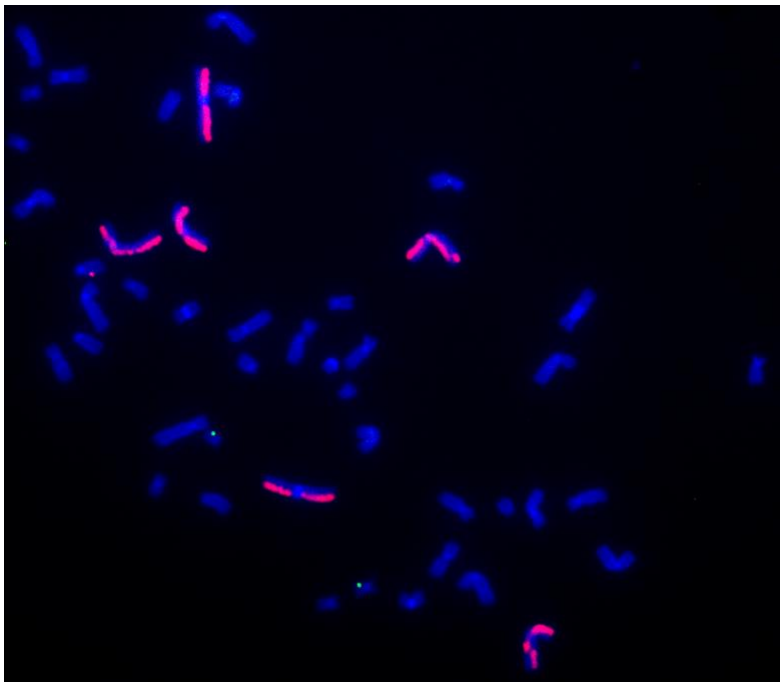


Figure 5: A cell with on target inserts in both homologs. Image left has all colors; image right has the green signal removed to better show the yellow insert probe.

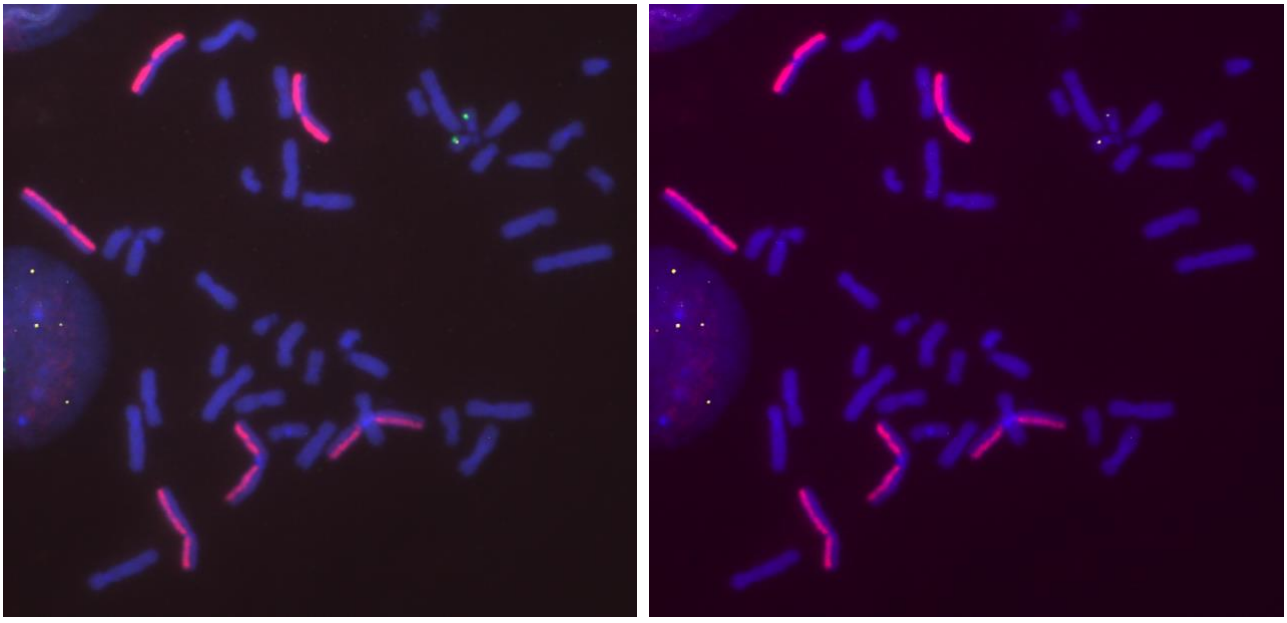


Figure 6: A cell from the edited iPS sample with a single on target insert in inverted orientation.

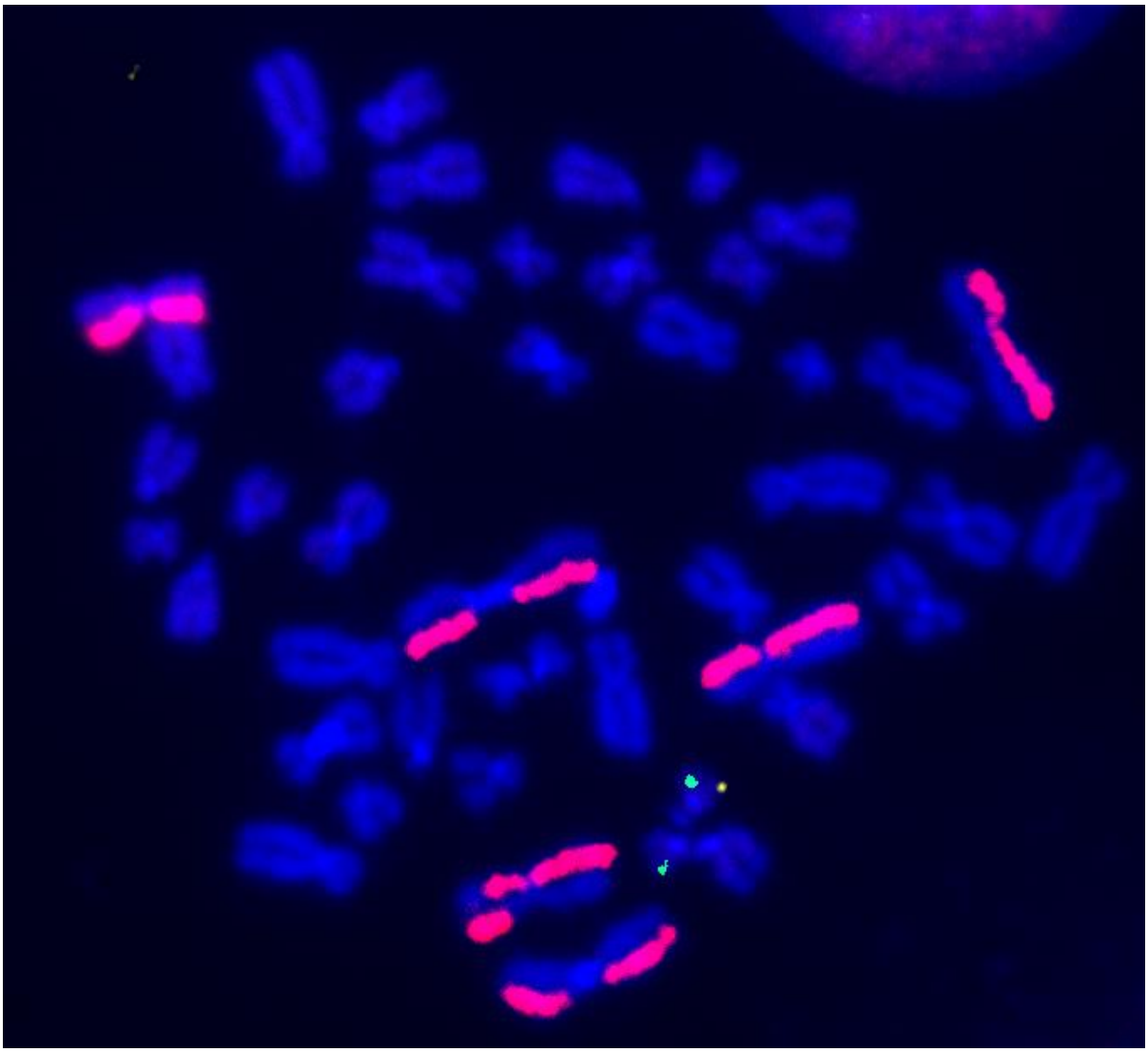
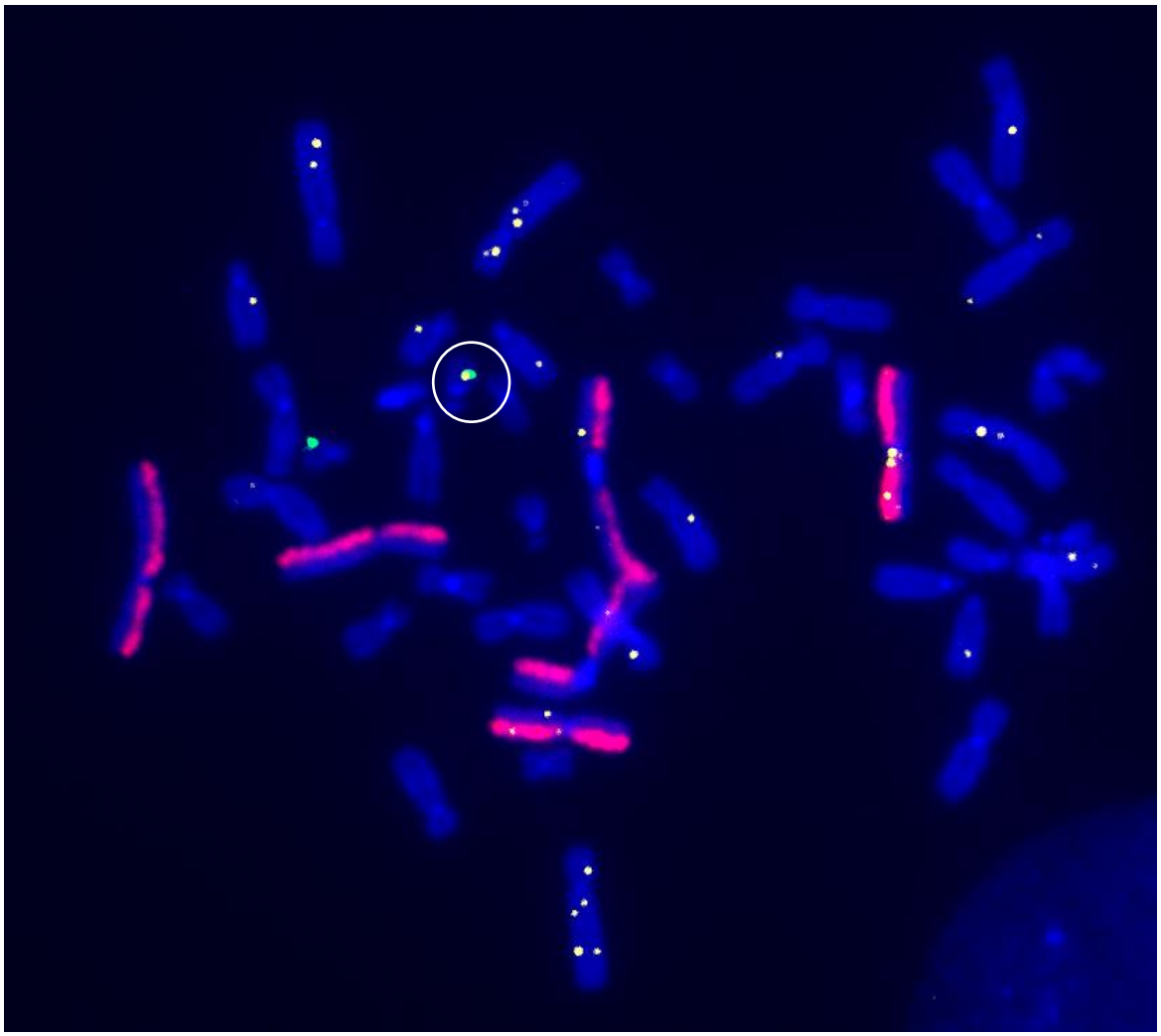


Figure 7: On- and off-target insert integration in the edited sample. One homolog of Chr44 has the insert in the expected orientation with the bracketing probes. There are also multiple off-target integrations across the genome.



Discussion

There are notable differences between the samples, and clear evidence that inserts are occurring off-target at significant rates. While the transformation of the fibroblast to iPS does not seem to generate significant change in the background rate of structural variants as measured by the dosimetry paints, editing the iPS cells introduces many copies of the insert into most cells.

Appendix A

Score sheet