Custom dGH In-Site Report Part 3 ***Q23xxxx***

Date: 8/xx/23

Customer: [Customer]

Project Overview

To better understand the structural effects of editing two separate genomic loci in T-cells, including interactions between edit sites, a study was designed to investigate rearrangements in related samples. For this project, KromaTiD:

- Received three fixed samples (three time points for one sample) from [Customer] and prepared metaphase spreads from the pellets.
- Utilized targeted probes to cover each of two (2) edit sites and locate a Lentiviral insert:
  - Gene 1 Brackets, labeled in Texas Red (orange)
  - Gene 2 Brackets, labeled in 6FAM (green)
  - Gene 2 Probe, labeled in ATTO 550 (pink)
  - Custom transgene probe, labeled in ATTO 643 (yellow)
- Executed a dGH time point assessment assay on each sample provided by [Customer] to choose the best time point for analysis.
- Executed the dGH assay on the best time point from the sample.
- Imaged 200 metaphase spreads from the sample provided by [Customer].
- Scored 200 metaphase spreads from the sample for structural rearrangements.

Procedure

An assay composed of four targeted probes – one to bracket gene 2, one to cover gene 2, one to bracket gene 1 and one to detect transgene inserts – was used to measure rates of structural rearrangements involving the edited chromosomes. [Customer] prepared samples for dGH analysis, using a protocol provided by KromaTiD, and shipped them to KromaTiD for analysis. Specification documents SPEC-00xx, -00xx, -0xxx and -0xxx describe the dGH probes.

Metaphase spreads were prepared by KromaTiD, and hybridizations were performed with the assay described above. For the [sample], 200 spreads were imaged and scored for the presence of structural rearrangements, using scoring rules described in SOP-0xxx.

Results

In the [sample], a variety of rearrangements were observed **(Table 1)**. **Table 2** contains a summary of translocation events, while **Table 3** describes the rate of transgene integration. A breakout of the rearrangements by assay component is shown in **Tables 4 – 6**. A detailed score sheet is contained in the Excel workbook referenced in **Appendix A**. Also note that cells may have more than one event, so total cells with rearrangements does not equal the total number of different rearrangements.

Table 1: Summary of scoring data, totaling the number of cells in each category by sample. Note that cells may have events involving more than one edit site, so line totals may exceed 200.

Sample	Gene 2 Events	Gene 2 Bracket Events	Gene 1 Events	Insert Events	Complex	No Event
[sample]	142	37	55	91	2*	16

*Complex event #1: a cell containing an acentric fragment with Gene 2 bracket probe signal, and chromothripsis with both bracket and gene probe signal.

Complex event #2: a complex translocation involving both Gene 1 and Gene 2 as well as an unknown third chromosome.

Table 2: Summary of translocation events.

Sample	Reciprocal Translocations Gene 1/ Gene 2	Reciprocal Translocations Gene 2 Brackets / Gene 1	Other Simple Translocations	Complex Translocations
[sample]	3	0	2	1

Table 3: Summary of transgene insert events.

Sample	Cells with Inserts	Total Inserts	Insert @ Unknown	Insert @ Gene 2	Insert @ Gene 1
[sample]	91	110	109	0	1

Table 4: **Summary of Gene 2 Probe Events by sample, where each category is the total number of events.**

Sample	Trans to Brackets	Trans to Gene 1	Trans to Other	Gain (Insert)	Gain (Aneuploid)	Gain (Complex)	Loss	Truncated at Edit	SCE / Inv	Large Signal Split
[sample]	0	5	0	9	0	0	80	1	74	5

Table 5: **Summary of Gene 2 Bracket Probe Events by sample, where each category is the total number of events.**

Sample	Trans to Gene 2	Trans to Gene 1	Trans to Other	Gain (Insert)	Gain (Aneuploid)	Gain (Complex)	Loss	Truncated at Edit	SCE / Inv	Large Signal Split
[sample]	0	0	0	0	0	0	0	1	47	1

Table 6: **Breakout of Gene 1 events by sample, where each category is the total number of events. Note that "Loss" describes missing target sequence, which could be either target specific or whole chromosome (aneuploidy). Suspected allelic translocations are noted when matched SCE/inversion events occur in both homologs of the edit site.**

Sample	Trans to Gene 2	Trans to Brackets	Trans to Other	Gain (Insert)	Gain (Aneuploid)	Gain (Complex)	Loss	Truncated at Edit	SCE / Inv	Large Signal Split
[sample]	0	3	0	5	0	0	10	0	43	1

Figure 1: Cell with the expected signal pattern, and no transgene insert. [Sample] cell xx.

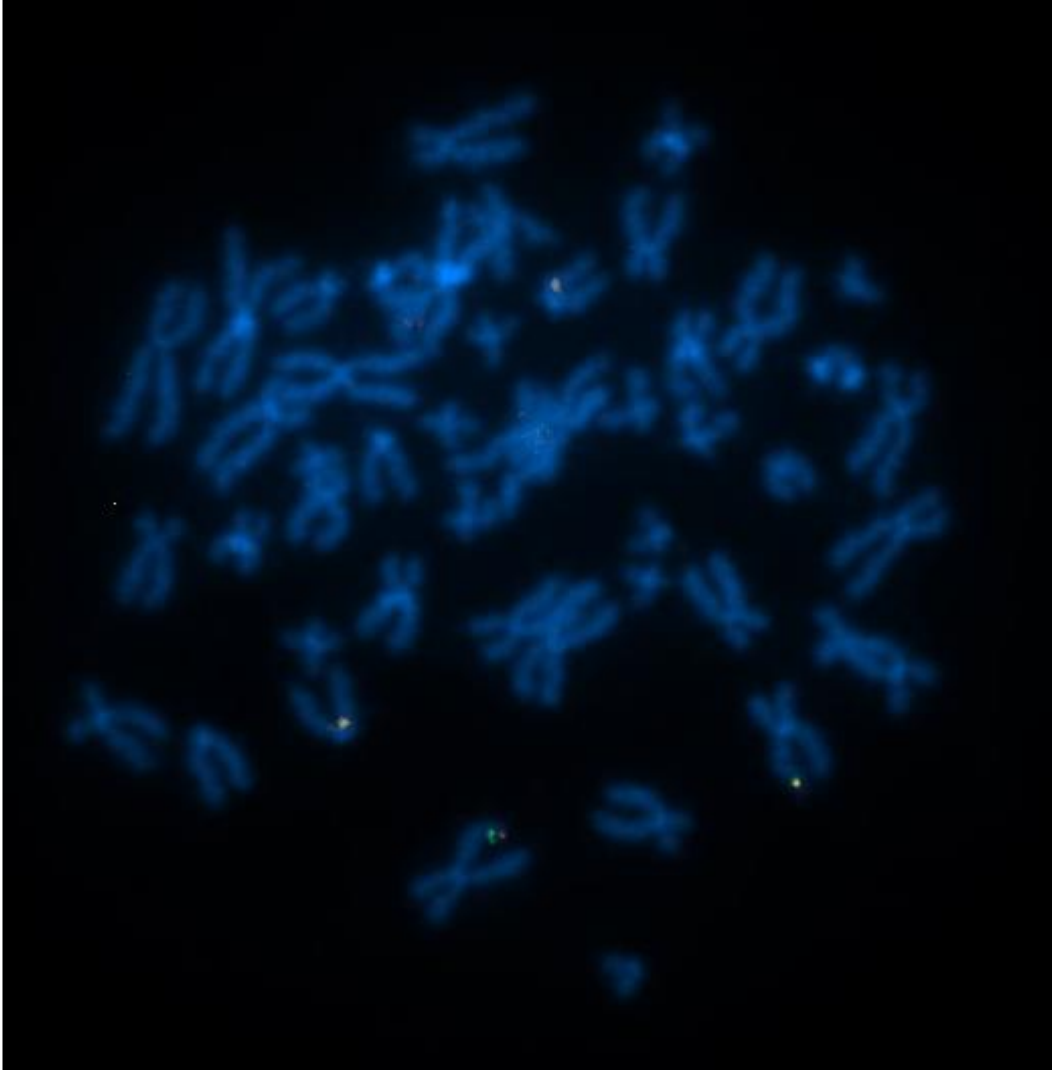


Figure 2: Cell with one transgene insert (circled). [Sample] cell xx.

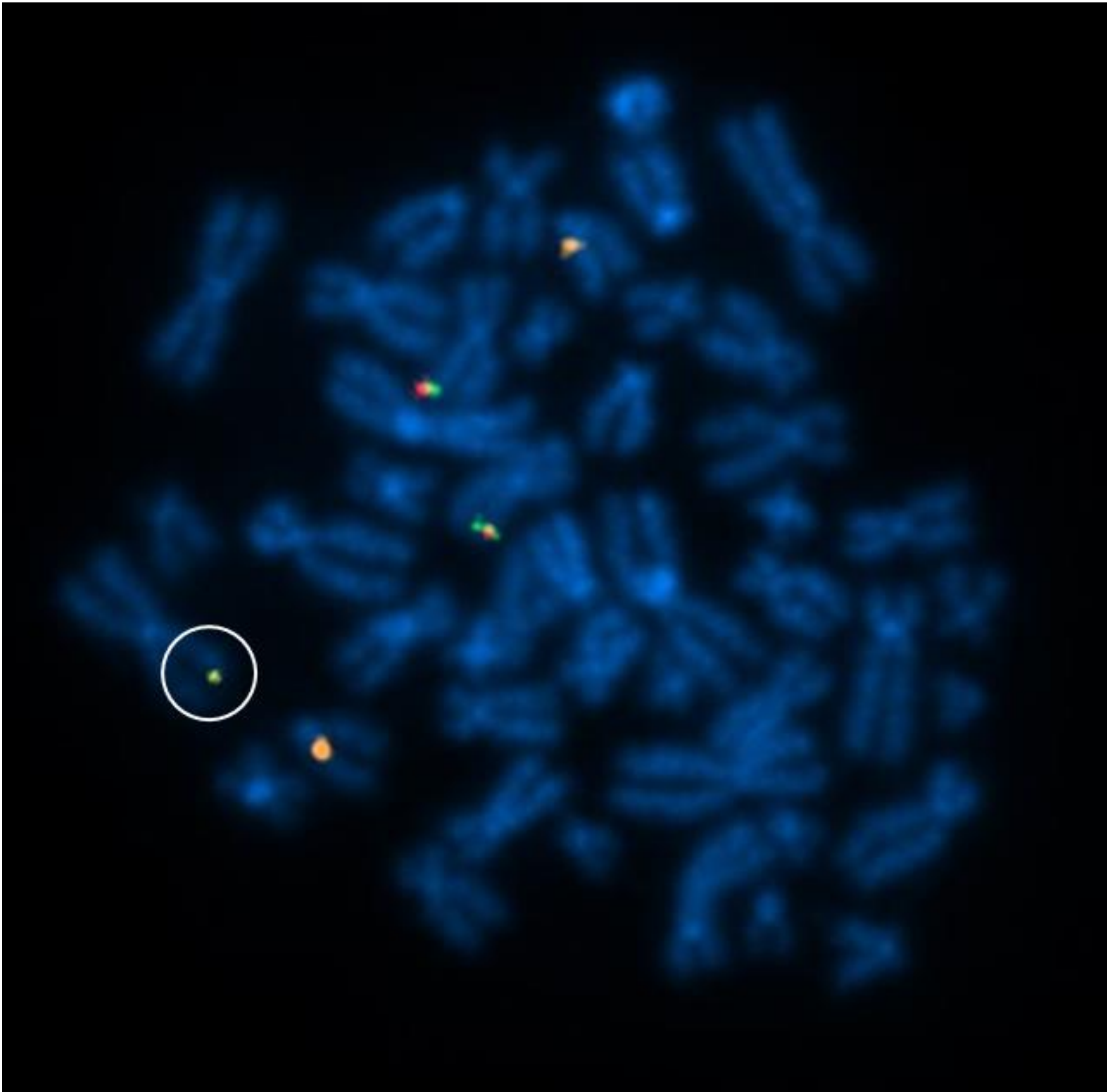


Figure 3: Cell with a translocation of the Gene 2 site to Gene 1 with a corresponding transgene insert (circled). The p arm of Chr00 is lost from the spread. The right panel has the Gene 2 bracketing probe (green) and Gene 1 probe (orange) layers removed to clearly visualize the Gene 2 and transgene probes. [Sample] cell xx.

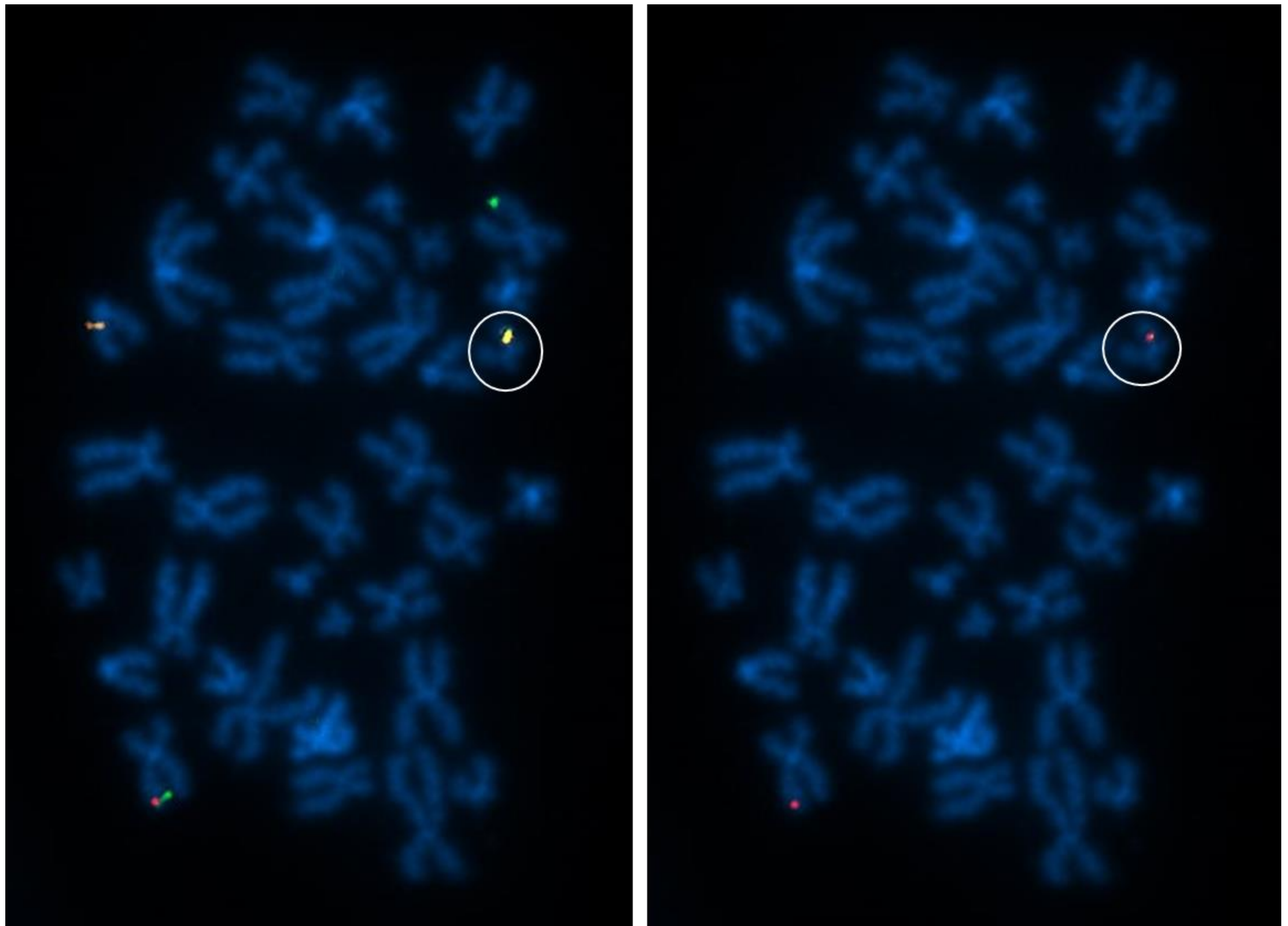
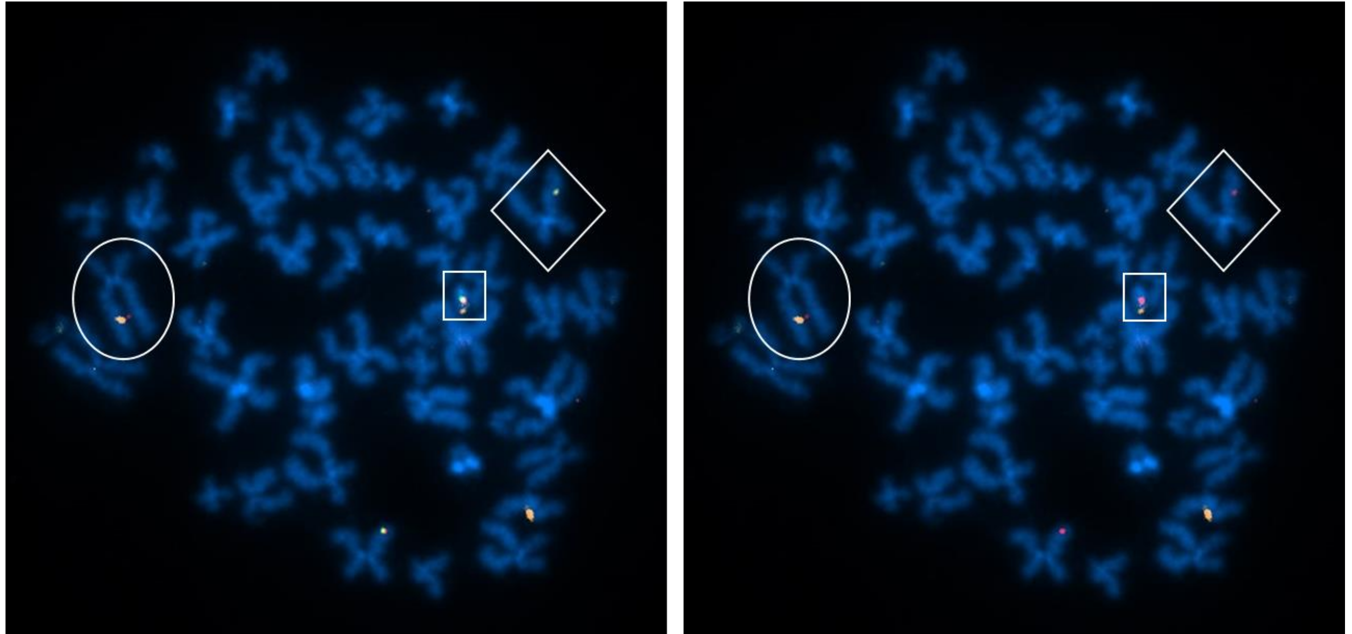


Figure 4: **Complex translocation involving Gene 2, Gene 1, and an unknown chromosome. Part of the Gene 1 probe and the Chr00q arm have translocated to a larger chromosome (circled). The centromeric portion of Chr00 is present with Gene 1 and Gene 2 probe signal (boxed). There is also Gene 2 bracket and gene probe signal on a third chromosome (diamond). The cell has one normal copy of Chr00 and one of Chr0. [Sample] cell xx.**



Discussion

The sample contained a low level of significant structural variants in the form of translocations and truncations involving the edit sites. There was transgene integration detected in 45.5% of the cells. SCE/inversion and loss of Gene 2 Probe signal were the most prevalent events.

Appendix A

Score sheet file