KromaTiD

Wild Type and Edited Cells Comparison *240328*

Date: 04/26/2024

Customer: Montgomery Burns

Project Overview

GENETX3000 is editing human T-Cells and testing the resulting batches against unedited controls for chromosome ploidy and structural rearrangements. For this report, KromaTiD:

- Received cryopreserved cell bullets for four samples from GENETX3000.
- Thawed, cultured, and harvested fixed cell pellets for metaphase analysis for each sample.
- Prepared metaphase slides for each sample.
- Stained and imaged metaphase slides for each sample.
- Analyzed 100 metaphase spreads from each sample and generated independent reports of genomic integrity for the four samples.
- For this pair, generated this summary report comparing the edited to the reference sample.

This report provides statistics that compare the observed frequencies of chromosomal ploidy and structural events between two human T-cell samples as measured by a G-banding assay.

Procedure

See individual sample reports (**Appendix A**) for method information. Frequency comparisons were performed using the Fisher's Exact test.

Results

The two samples compared in this summary include an unedited reference sample (Reference) and an edited sample (Treated). Independent reports for each sample (see **Appendix A**) comprise 100 cells scored using industry-standard protocols (ISCN 2020: An International System for Human Cytogenomic Nomenclature). **Appendix B** contains a list of all chromosomal variants scored and their abbreviations.

Table 1: Sample List

Sample Key	Sample Name	LIMS ID
Reference	GTX Mock 1	S018999
Treated	GTX CAR Insert 1	S019000

Table 2: Summary of Ploidy for Reference and Treated

Category	Reference	Treated	Fisher's Exact*	*p*-value significant
	# of Cells	# of Cells	*p*-value	< .05
Haploid	0	0	1.0	N/A
Diploid	100	97	0.246	No
Triploid	0	1	1.0	No
Tetraploid	0	2	0.497	No
Polyploid	0	0	1.0	N/A

Table 3: Summary of Events by Category for Reference and Treated

Category	Reference	Treated	Fisher's Exact*	*p*-value significant
	# of Cells	# of Cells	*p*-value	< .05
No Events	77	35	< 0.00001	Yes
Aneuploidy (Loss + Gain)	13	35	0.0004	Yes
Loss	11	32	0.0004	Yes
Gain	2	4	0.683	No
Acentric fragment	0	0	1.0	N/A
Additional material of unknown_origin	0	5	0.059	No
Constitutional anomaly	0	0	1.0	N/A
Chromosome break	2	1	1.0	No
Chromatid break	3	4	1.0	No
Chromothripsis	0	0	1.0	N/A
Deletion	4	24	0.0	Yes
Derivative chromosome	0	6	0.029	Yes
Dicentric	0	1	1.0	No
Double minute	0	0	1.0	N/A
Duplication	0	1	1.0	No
Endoreduplication	0	0	1.0	N/A
Fragile site	0	0	1.0	N/A
Heterochromatin (constitutive)	0	0	1.0	N/A
Homogeneously staining region	0	0	1.0	N/A
Isochromosome	0	0	1.0	N/A
Isodicentric	0	0	1.0	N/A
Insertion	0	0	1.0	N/A
Inversion	1	0	1.0	No
Marker chromosome	1	6	0.118	No
Quadriradial	0	0	1.0	N/A
Ring chromosome	0	0	1.0	N/A
Translocation	1	14	0.001	Yes
Triradial	0	0	1.0	N/A

Table 4: Number of Cells in Each Sample with Specific Events

Event Type[1]	Reference # of Cells	Treated # of Cells	Fisher's Exact* *p*-value	*p*-value significant < .05
del(16)(p12)	0	12	0.0003	Yes
-X	1	13	0.001	Yes
t(15;16)(q21;p13.3)	0	9	0.003	Yes
del(14)(q11.2)	0	5	0.059	No
add(16)(p13.3)	0	5	0.059	No
t(3;11)(p24;p11.2)	0	4	0.121	No
der(14;16)(p11.2;p13.2)	0	4	0.121	No
+M	1	5	0.212	No
del(16)(p13.2)	0	3	0.246	No
-18	0	2	0.497	No
-21	0	2	0.497	No
-11	0	2	0.497	No
+2	0	2	0.497	No
-16	0	2	0.497	No
-14	1	3	0.621	No

[1] These events are a subset of the complete event list. They are the specific karyotypic events that had the greatest difference between the two samples. See Appendix C for the comprehensive list of events.

Table 5: Event Rates for Edit Target Chromosomes

Sample Name	Reference			Treated		
Sample ID	S018999			S019000		
Category	# Events	# of Cells	% of Cells	# Events	# of Cells	% of Cells
Chromosome 16	1	1	1	38	37	37

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KromaTiD Genomic Integrity G-Band Assay is for research use only and is not a medical diagnostic test

Appendix A:

ASGCT_240328_S018999_KTreport
ASGCT_240328_S019000_KTreport

Appendix B: Table of karyotyping abbreviations

Abbreviation	Definition
ace	acentric fragment
add	additional material of unknown origin
c	constitutional anomaly
chrb	chromosome break
chtb	chromatid break
cth	chromothripsis
del	deletion
der	derivative chromosome
dic	dicentric
dmin	double minute
dup	duplication
end	endoreduplication
fra	fragile site
h	heterochromatin,constitutive
hsr	homogeneously staining region
i	isochromosome
idic	isodicentric
ins	insertion
inv	inversion
mar or M	marker chromosome
minus sign (-)	loss
multiplication sign (x)	multiple copies of same abnormality
plus sign (+)	gain
qr	quadriradial
r	ring chromosome
t	translocation
tr	triradial

Appendix C: Complete Version of Table 4

Event Type	Reference # of Cells	Treated # of Cells	Fisher's Exact* *p*-value	*p*-value significant < .05
add(16)(p13.3)	0	5	0.059	No
chtb(1)(p)	1	1	1.0	No
chtb(10)(q)	0	1	1.0	No
chtb(16)(p)	0	1	1.0	No
chtb(16)(q)	1	0	1.0	No
chtb(2)(q)	1	0	1.0	No
chtb(4)(q)	0	1	1.0	No
chrb(10)(p)	1	0	1.0	No
chrb(3)(p)	0	1	1.0	No
chrb(6)(p)	1	0	1.0	No
del(14)(q11.2)	0	5	0.059	No
del(15)(q21)	0	1	1.0	No
del(16)(p12)	0	12	0.0003	Yes
del(16)(p12)x2	0	1	1.0	No
del(16)(p13.2)	0	3	0.246	No
del(19)(p13.2)	0	1	1.0	No
del(3)(p10)	0	1	1.0	No
del(3)(p25)	0	1	1.0	No
del(4)(p15)	1	0	1.0	No
del(6)(q15)	1	0	1.0	No
del(9)(p21)	1	0	1.0	No
del(X)(q22)	1	0	1.0	No
der(14;16)(p11.2;p13.2)	0	4	0.121	No
der(3;6)(p21.3;p21)	0	1	1.0	No
dic(6;11)(p25;p15)	0	1	1.0	No
dup(9)(q21q31)	0	1	1.0	No
+2	0	2	0.497	No
+7	0	1	1.0	No
+11	1	0	1.0	No
+X	1	1	1.0	No
inv(2)(p11.2q31)	1	0	1.0	No
-2	1	1	1.0	No
-3	1	1	1.0	No
-4	0	1	1.0	No

-6	1	2	1.0	No
-8	1	0	1.0	No
-9	0	1	1.0	No
-10	1	0	1.0	No
-11	0	2	0.497	No
-13	0	1	1.0	No
-14	1	3	0.621	No
-15	1	1	1.0	No
-16	0	2	0.497	No
-18	0	2	0.497	No
-19	1	2	1.0	No
-20	1	2	1.0	No
-21	0	2	0.497	No
-22	1	0	1.0	No
-X	1	13	0.001	Yes
+4M	0	1	1.0	No
+M	1	5	0.212	No
der(11)t(11;17)(p11.2;q21)	0	1	1.0	No
t(15;16)(q21;p13.3)	0	9	0.003	Yes
t(3;11)(p24;p11.2)	0	4	0.121	No
t(5;11)(q31;q13)	1	0	1.0	No