TITLE: KROMATID CHROMOSOME ANALYSIS REPORT

I. ASSAY INFORMATION

Project Quote #	Q200401
Specimen Type	iPSC
Body Site	N/A
Sample ID	078-PFN-C/G A09 (S005738)
Cell Line Gender	Male
Passage number (or N/A)	N/A
Study Objective	The purpose of this study is to characterize iPS cells grown *in vitro*, designated for cytogenetic analysis.

II. CELL MAINTENANCE

Culture vessel	N/A
Media	N/A
Density (estimated)	N/A
Culture atmosphere	N/A
Culture maintenance	N/A

Culture Maintenance Process Description	N/A
	Analyst Initial/Date: N/A

III. CULTURE HARVEST

Culture Harvest Process Description	N/A
	Analyst Initial/Date: N/A

Material	Usage information
Harvest materials (trypsin, EDTA, etc.)	Type: N/a LN/ Exp. Date: N/a
Colcemid	LN/ Exp. Date: N/a Concentration: 0.1 µg/ml (10 µl/ml) Incubation time: N/a
Hypotonic	LN/ Exp. Date: N/a Solution: N/a Incubation time:
Fixative	Prepared Fresh, day-of-use

IV. STAINING

Solution Type	Lot#	Exp. Date	Solution Type	Lot#	Exp. Date
Isoton II Diluent	4710610	07/12/22	Wright Stain	210817-Wright	08/17/22
Pancreatin	SLCD9444	01/11/23	Gurr Buffer	211007-Gurr	11/7/21
FBS	19J079	11/4/22	Permount	210201-01	02/1/23

Process Description	A sample of fixed cells labeled 078-PFN-C/G A09 (KromaTiD Sample ID S005738) was received at KromaTiD on 10-5-21. The fixed cells were washed twice with fixative (prepared fresh day-of-use) and the O.D. was adjusted. Drops of the final cell suspension were placed on clean slides and aged for 60 minutes at 90°C. Slides were digested in a pancreatin solution with Isoton II diluent. The enzymatic reaction was then stopped by rinsing with FBS, followed by application of a stain solution (3:1 Wright/Gurr buffer) which was poured on the slides so that it covered the entire surface. After staining for up to 1 minute, slides were washed with de-ionized water for 1-5 seconds and air dried. The mounting medium Permount was applied to the slides, a coverslip was placed on the slide and the slides were scanned on the microscope.
	Analyst Initial/Date: MV 10-07-21 through 10-19-21

TEST DESCRIPTION:

G-banding with trypsin treatment and Giemsa stain (GTG-banding) is used in cytogenetics to produce a visible karyotype by staining metaphase chromosomes. This technique allows each chromosome to be distinguished by its characteristic banding pattern. G-banding is useful in assessing structural abnormalities in individual chromosomes, as well as extra or missing chromosomes within a cell. Industry-standard protocols for scoring and describing results were used (ISCN 2016: An International System for Human Cytogenomic Nomenclature).

V. RESULTS

Cells Counted	20	Total Karyograms	2
Cells Analyzed	20	Average Band Resolution	475
Image File Location		Jax Gbanding_S005738	

5.1 CHROMOSOME COUNT PER 20 METAPHASES

Of the 20 cells counted, 16 contained 46 chromosomes (80%). *Cells containing greater than 57 chromosomes are recorded as polyploid.* The polyploid frequency was 0%, based on the metaphases counted.

5.2 CHROMOSOME ABERRATION DATA

The chromosome aberration data via G-band for the 20 metaphases examined is summarized in attached case report cell list. 1 chromosome aberration was found in the 20 cells analyzed with 5% of the cells aberrant.

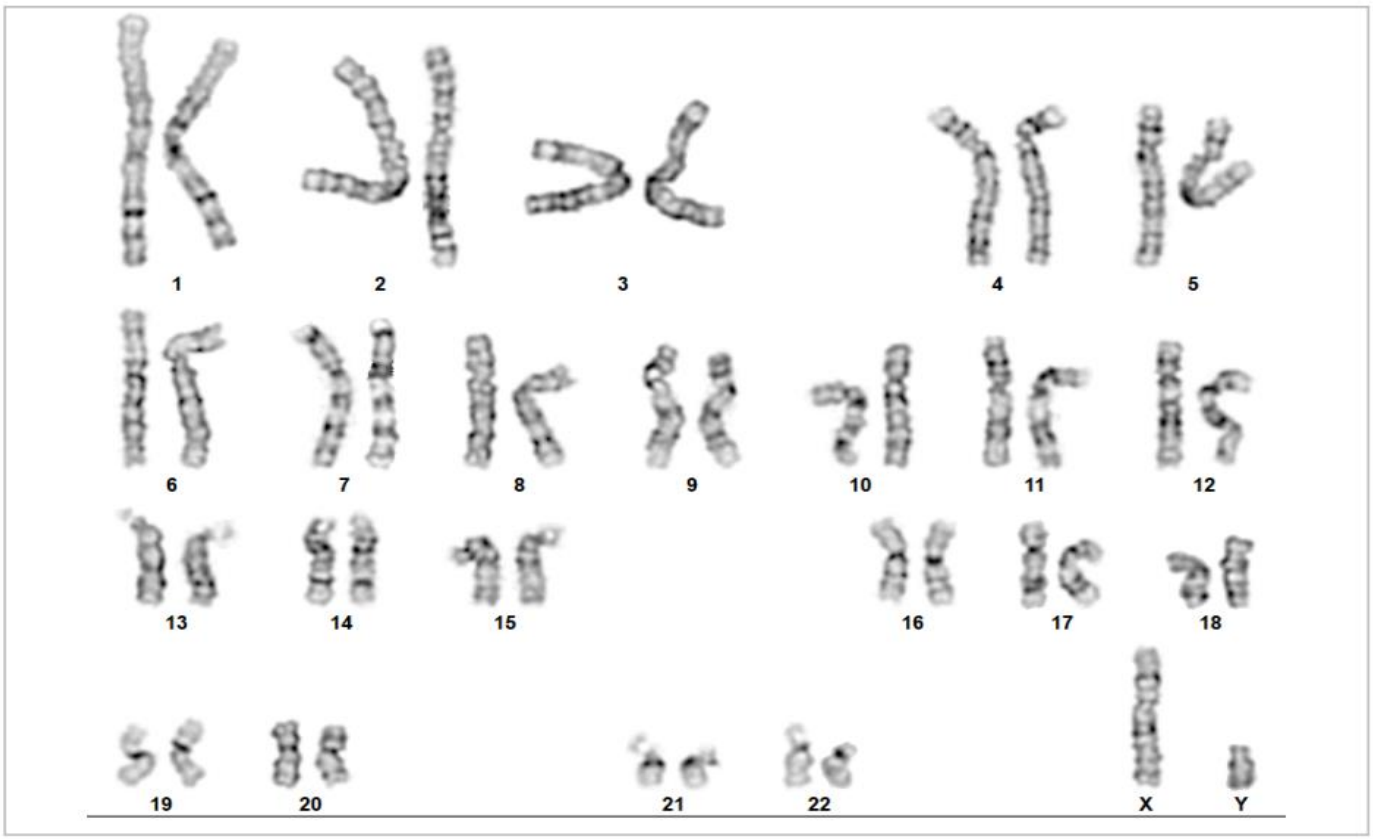
*Note: Cells with aneuploidy gain/loss were found to be non-clonal, and therefore not included in the aberration data below.

Tech Summary		Additional Comments
Karyotype	46,XY[20]	Normal Male Karyotype
Cells Analyzed	20	
Normal Cells	19	Random loss/gain cells normalized
Abnormal Cells	1	
Aberration Type	+Marker	Non-clonal
Aberration %	5%	

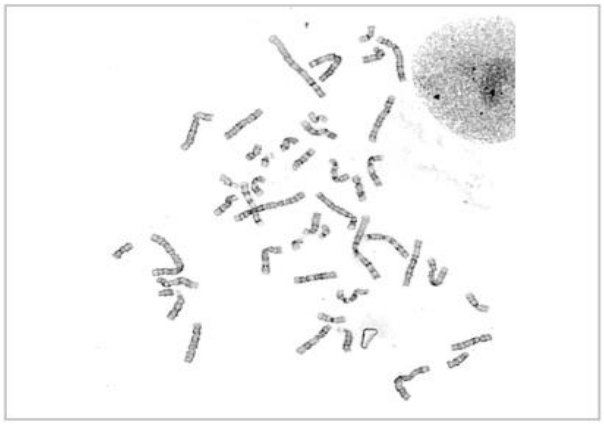
5.3. INTERPRETATION/ SIGNIFICANCE:

G-banded chromosome analysis of metaphase cells designated as 078-PFN-C/G A09 (KromaTiD Sample ID S005738) show a normal male karyotype 46,XY[20].
The other abnormalities/aberrations detected were non-clonal, and were designated as low level mosaicism or random gain/loss.

5.4 REPRESENTATIVE IMAGES:



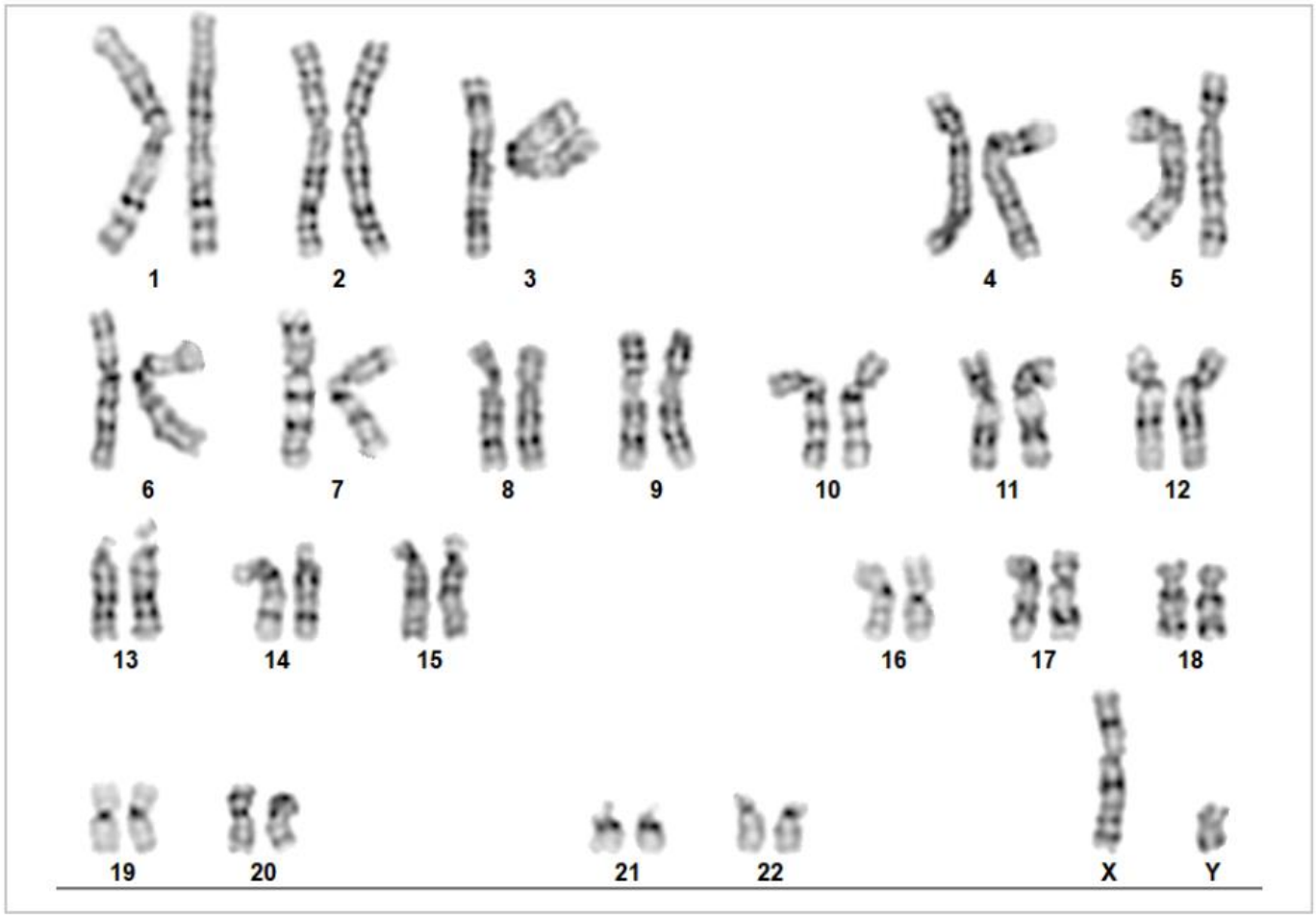
Cell Results: **Karyotyped: 46,XY**



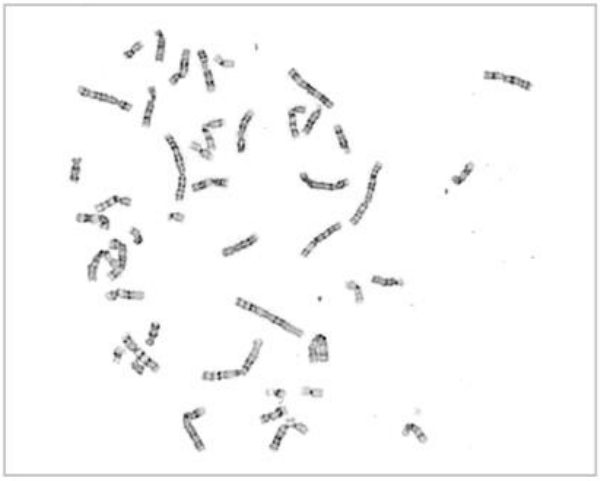
Cell Notes:

Label - Slide/Cell: **S005738_10/7 - 1/10**

X,Y: **9.3 , 15.7**



Cell Results: **Karyotyped: 46,XY**



Cell Notes:

Label - Slide/Cell: **S005738_10/7 - 1/30**

X,Y: **7.5 , 21.6**

Limitations: *This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is >3-10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. Detection of heterogeneity of clonal cell populations in this specimen is limited by the number of metaphase cells analyzed, documented above as "number of cells counted". Results are for Research Use Only, and should not be used for clinical purposes.*

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APPENDIX A: CASE REPORT FOR SAMPLE S005738

Table A-1: Chromosome Analysis for sample 078-PFN-C/G A09 (S005738). 20 cells were analyzed.

#	Slide	Cell	Coordinates	Results	Analysis State	State By
Slide: Name: 1 Label: S005738 10/7						
1	1	1	14.50 X 10.42	Karyotyped: 47,XY, +22	Karyotyped	mvernich
2	1	2	9.70 X 11.16	Karyotyped: 46,XY	Karyotyped	mvernich
3	1	5	8.05 X 12.66	Karyotyped: 46,XY	Karyotyped	mvernich
4	1	10	9.32 X 15.72	Karyotyped: 46,XY	Karyotyped	mvernich
5	1	13	9.61 X 17.30	Karyotyped: 46,XY	Karyotyped	mvernich
6	1	18	12.58 X 17.86	Karyotyped: 46,XY	Karyotyped	mvernich
7	1	21	8.98 X 19.39	Karyotyped: 46,XY	Karyotyped	mvernich
8	1	28	9.52 X 21.94	Karyotyped: 44,Y,-X, −20	Karyotyped	mvernich
9	1	30	7.50 X 21.64	Karyotyped: 46,XY	Karyotyped	mvernich
10	1	31	9.23 X 22.45	Karyotyped: 46,XY	Karyotyped	mvernich
11	1	37	5.34 X 23.60	Karyotyped: 46,XY	Karyotyped	mvernich
12	1	42	12.78 X 29.06	Karyotyped: 46,XY	Karyotyped	mvernich
13	1	45	12.78 X 30.66	Karyotyped: 45,XY, −4	Karyotyped	mvernich
14	1	49	12.86 X 32.96	Karyotyped: 46,XY	Karyotyped	mvernich
15	1	50	10.53 X 32.03	Karyotyped: 46,XY	Karyotyped	mvernich
16	1	52	8.66 X 34.38	Karyotyped: 46,XY	Karyotyped	mvernich
17	1	64	15.53 X 38.71	Karyotyped: 46,XY	Karyotyped	mvernich
18	1	70	14.78 X 40.11	Karyotyped: 47,XY, +M	Karyotyped	mvernich
19	1	73	10.87 X 40.84	Karyotyped: 46,XY	Karyotyped	mvernich
20	1	82	14.80 X 42.39	Karyotyped: 46,XY	Karyotyped	mvernich