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| Document Code: | FORM-0068A | Document Type: | FORM |
| Revision: | 3.0 | Date Effective: | 08-27-21 |

TITLE: KROMATiD CHROMOSOME ANALYSIS REPORT

I. ASSAY INFORMATION

| | |
|-------------------------|---|
| Project Quote # | Q200401 |
| Specimen Type | iPSCs |
| Body Site | N/A |
| Sample ID | 25.1-F03 (S008113) |
| Cell Line Gender | Male |
| Passage number (or N/A) | N/A |
| Study Objective | The purpose of this study is to characterize iPS cells grown <i>in vitro</i> , 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 |

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| | |
|--|----------------------------------|
| 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 |

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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 | 220426-Gurr | 05/26/22 |
| FBS | 20J481 | 01/06/24 | Permunt | 210201-01 | 02/1/23 |

| | |
|----------------------------|--|
| Process Description | <p>A sample of fixed cells 25.1-F03 (KromaTiD Sample ID S008113) was received at KromaTiD on 4-13-22.</p> <p>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 Permunt was applied to the slides, a coverslip was placed on the slide and the slides were scanned on the microscope.</p> |
| | Analyst Initial/Date: MV 5/10/22 |

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).

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V. RESULTS

| | | | |
|----------------------------|----------------------|--------------------------------|-----|
| Cells Counted | 20 | Total Karyograms | 2 |
| Cells Analyzed | 20 | Average Band Resolution | 400 |
| Image File Location | Jax Gbanding_S008113 | | |

5.1 CHROMOSOME COUNT PER 20 METAPHASES

Of the 20 cells counted, 9 contained 46 chromosomes (45%). *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. 0 chromosome aberrations were found in the 20 cells analyzed with 0% 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 | 20 | Random loss/gain cells normalized |
| Abnormal Cells | 0 | |
| Aberration Type | N/A | |
| Aberration % | 0% | |

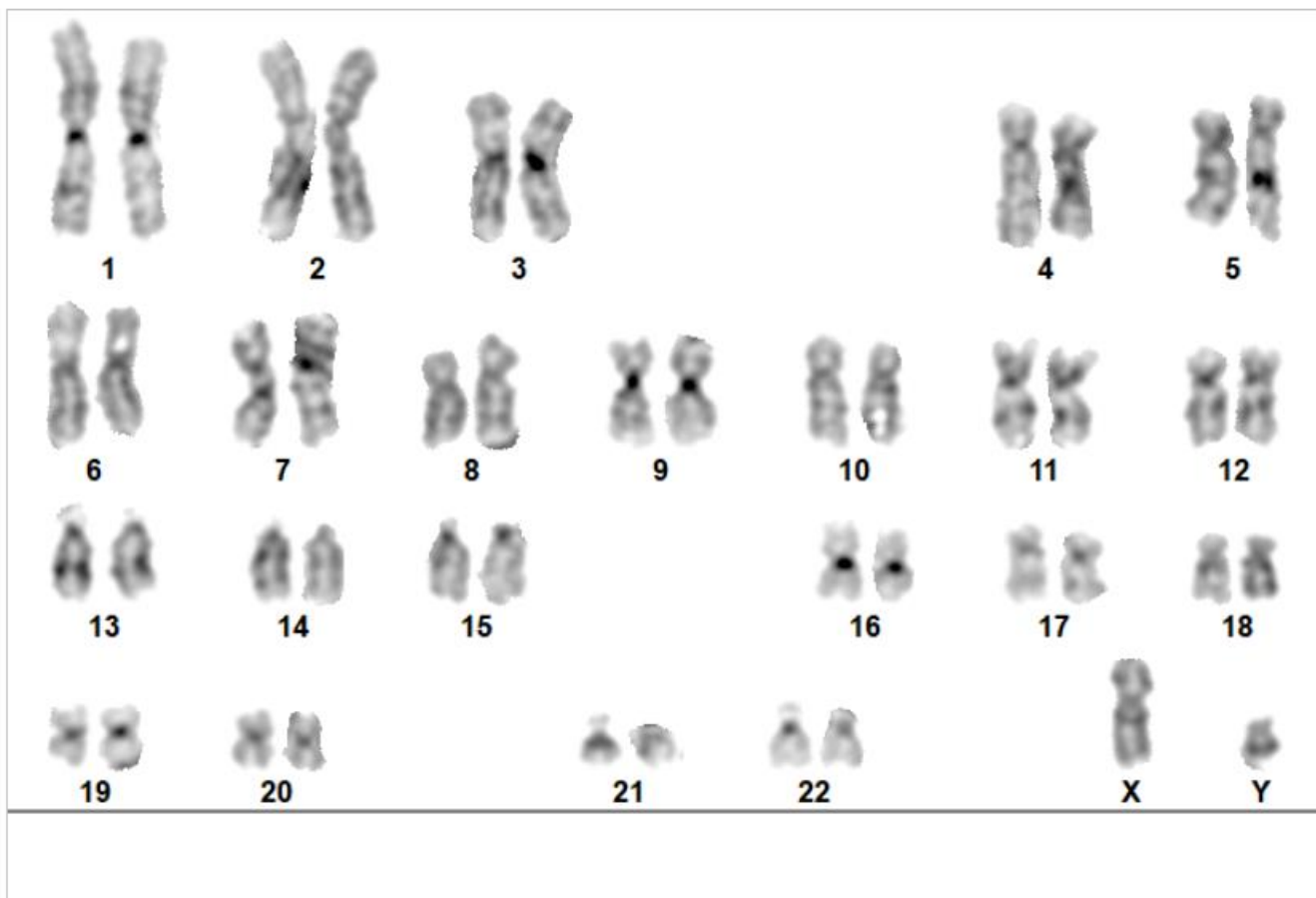
| | | | |
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5.3. INTERPRETATION/ SIGNIFICANCE:

G-banded chromosome analysis of metaphase cells designated 25.1-F03 (KromaTiD Sample ID S008113) shows 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

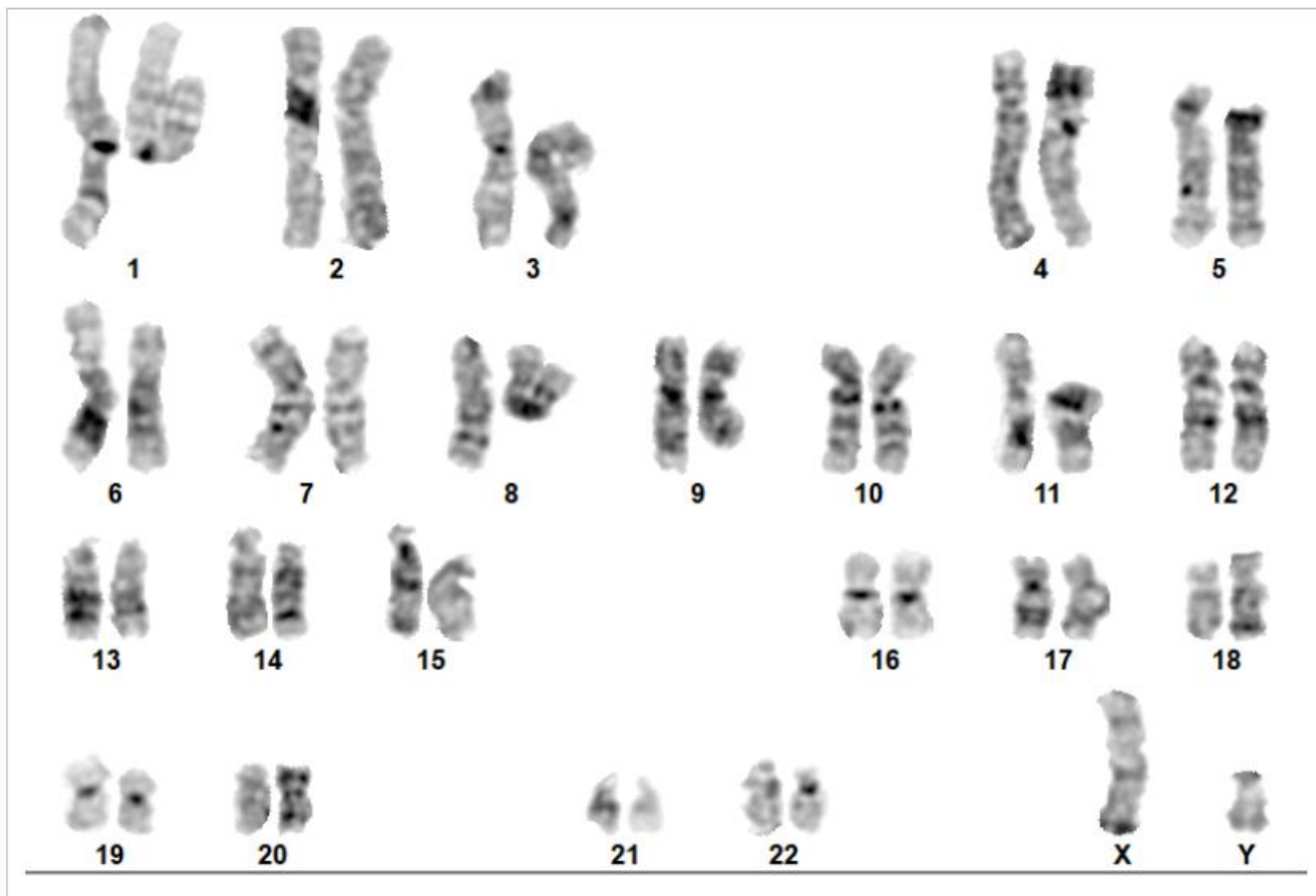
Cell Notes:



Label - Slide/Cell: S008113 - 1/36

X,Y: 11.4 , 21.5

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Cell Results: Karyotyped: 46,XY

Cell Notes:



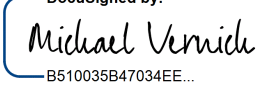
Label - Slide/Cell: S008113 - 3/38

X,Y: 5.3 , 40.9

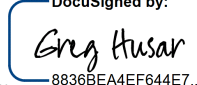
Report Date: Tuesday, May 31, 2022

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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.*

Completed By/Date:  **Michael Vernich**
Cytogenetics Supervisor

DocuSigned by: 5/31/2022
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Approved By/Date:  **Gregory Husar**
Operations Manager

DocuSigned by: 5/31/2022
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Table 1: Chromosome Analysis for sample 25.1-F03 (S008113). 20 cells were analyzed.

| # | Slide | Cell | Coordinates | Results | Analysis State | State By |
|--------------------------------------|-------|------|---------------|--|----------------|----------|
| Slide: Name: 1 Label: S008113 | | | | | | |
| 1 | 1 | 9 | 19.29 X 16.13 | Karyotyped: 40,XY, -8, -12, -14, -18, -20, -21 | Karyotyped | kstone |
| 2 | 1 | 20 | 11.11 X 18.72 | Karyotyped: 46,XY | Karyotyped | kstone |
| 3 | 1 | 21 | 11.43 X 18.81 | Karyotyped: 47,XY, +3 | Karyotyped | kstone |
| 4 | 1 | 24 | 13.14 X 19.98 | Karyotyped: 43,XY, -12, -13, -17 | Karyotyped | kstone |
| 5 | 1 | 31 | 16.09 X 20.47 | Karyotyped: 45,XY, -6 | Karyotyped | kstone |
| 6 | 1 | 36 | 11.35 X 21.53 | Karyotyped: 46,XY | Karyotyped | kstone |
| 7 | 1 | 42 | 17.74 X 24.10 | Karyotyped: 46,XY | Karyotyped | kstone |
| 8 | 1 | 109 | 9.56 X 34.39 | Karyotyped: 45,XY, -18 | Karyotyped | kstone |
| 9 | 1 | 113 | 10.62 X 34.56 | Karyotyped: 46,XY | Karyotyped | kstone |
| 10 | 1 | 132 | 8.56 X 36.21 | Karyotyped: 45,XY, -20 | Karyotyped | kstone |
| 11 | 1 | 165 | 13.58 X 46.61 | Karyotyped: 46,XY | Karyotyped | kstone |
| Slide: Name: 2 Label: S008113 | | | | | | |
| 12 | 2 | 6 | 9.11 X 10.93 | Karyotyped: 46,XY | Karyotyped | kstone |
| 13 | 2 | 19 | 10.55 X 14.01 | Karyotyped: 43,XY, -3, -14, -18 | Karyotyped | kstone |
| 14 | 2 | 41 | 15.75 X 16.66 | Karyotyped: 46,XY | Karyotyped | kstone |
| Slide: Name: 3 Label: S008113 | | | | | | |
| 15 | 3 | 23 | 3.93 X 37.15 | Karyotyped: 44,XY, -8, -8 | Karyotyped | kstone |
| 16 | 3 | 35 | 12.70 X 40.58 | Karyotyped: 44,XY, -8, -16 | Karyotyped | kstone |
| 17 | 3 | 38 | 5.27 X 40.90 | Karyotyped: 46,XY | Karyotyped | kstone |
| Slide: Name: 5 Label: S008113 | | | | | | |
| 18 | 5 | 53 | 14.91 X 43.49 | Karyotyped: 46,XY | Karyotyped | kstone |
| Slide: Name: 6 Label: S008113 | | | | | | |
| 19 | 6 | 2 | 12.41 X 13.58 | Karyotyped: 44,XY, -14, -15 | Karyotyped | kstone |
| 20 | 6 | 38 | 11.85 X 43.24 | Karyotyped: 43,XY, -9, -16, -21 | Karyotyped | kstone |