TITLE: KromaTiD Chromosome Analysis Report

Author	Michael Vernich		**Date:**
Reviewed By	Marissa Rodrigues		**Date:**
Approved By	Gretchen Pratt		**Date:**

I. Assay Information

Project Quote #	Q100000	**Performed By/Date**	MV 3/24/20
Specimen Type	IPSC		
Body Site	N/a		
Cell Line	1234		
Cell Line Gender	Male		
Passage number	12		
Study Objective	The purpose of this study is to characterize iPSCs mock and grown *in vitro,* designated for cytogenetic analysis.		

II. Cell Maintenance

Cells were grown in tissue culture flasks using culture media provided by the sponsor. Cultures were maintained at approximately 37°C in an atmosphere of approximately 5% CO2 and 95% air. Cells were cultured as indicated by the sponsor. Cultures at various cell densities were set up as needed for cytogenetic analysis.

Culture vessel	6 well plate
Media	MTESR
Density (estimated)	60% (well 1), 40% (well 2), …
Culture atmosphere	5% CO2, 95% air 37 C
Culture maintenance	SOP-XXXX

III. Chromosome Harvest

Colcemid was added at 0.1ug/mL or 10ul/mL and incubated for 3 hours. After removing EDTA, plate was rinsed with appropriate iPSC media and transferred to a 15 mL polystyrene conical tube. Samples were centrifuged at 1000rpm for 10 minutes at room temperature. Media was aspirated off and pellet was resuspended. 6mL of 75mM KCl hypotonic solution was added to the tubes and incubated for 30 minutes at room temperature.

Material	Usage information
Harvest materials (trypsin, EDTA, etc.)	**Type:** EDTA **LN:** 1234
Colcemid	**LN**: 1234 **Concentration:** 0.1 ug/ml (10 ul/ml) **Incubation time:** 3 hrs
Hypotonic	**LN:** 1234 **Solution:** standard (75 mM KCl) **Incubation time:** 30 min

IV. Cell Fixation and Preparation of Slides

1.5mL of freshly prepared fixative (3:1 methanol to acetic acid) was added to each conical and inverted several times. Samples were centrifuged at 1000rpm for 10 minutes at room temperature. 5ml of fixative was added drop wise and was left to sit at room temperature for 20 minutes. This initial Carnoy's fixation was followed by at least two washes. After the last centrifugation, the cells were resuspended in freshly prepared fixative. Drops of the final cell suspension were placed on clean slides and aged for 30 minutes at 90°C. Slides were digested in pancreatin, followed by a stain solution (3:1 Giemsa/Gurr buffer) by pouring the stain preparation on slides as to cover the entire surface. After staining for up to 1 minute, slides were washed with DI water for 1-5 seconds and air-dried.

V. Material Usage Record

Solution Type	Lot#	Exp. Date	Solution Type	Lot#	Exp. Date
HBSS	1234	12/21/20	Giemsa Stain	1234	12/21/20
Pancreatin	1234	12/21/20	Gurr Buffer	1234	12/21/20
FBS	1234	12/21/20	Permount	1234	12/21/20

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.

VI. Results

Cells Counted	20	Total Karyograms	2

Cells Analyzed	20	Average Band Resolution	450
Image File Location		Image.file.1234	

1. Chromosome Count per 20 Metaphases

Of the 20 cells counted, 16 contained cells had 46 chromosomes (80%) and 4 cells had 47 (20%). *Cells containing greater than 57 chromosomes are recorded as polyploid.* The polyploid frequency was 0%, based on the 20 metaphases counted.

2. Chromosome Aberration Data

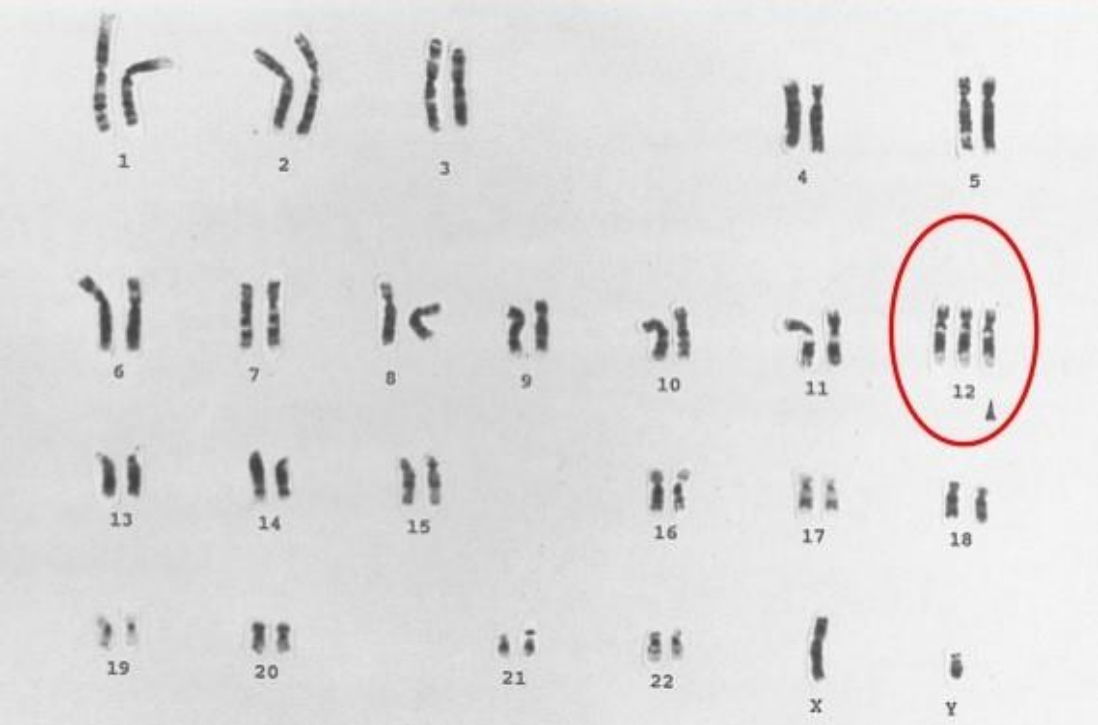
The chromosome aberration data for the 20 metaphases examined is summarized in attached spread sheet. 4 chromosome aberrations were found in the 20 cells analyzed with 20% of the cells aberrant

3. Interpretation/ Significance:

Aneuploidy, an abnormality in chromosome number, is frequently reported in in vitro cultured PSCs, including iPSCs and ESCs. Recurrent gains of specific chromosomes account for more than half of the total karyotype abnormalities, with trisomy 12 being the most common in both hESCs and hiPSCs.

Genetic and epigenetic variations in iPSCs come from different sources. Some of the variations may be inherited from donor somatic cells, induced or selected by the reprogramming process, or accumulated during culturing; others may simply reflect the innate genetic and epigenetic stability of the pluripotent state of iPSCs. Although each variation is not relevant to the functionality of iPSCs, certain variations may change the properties of iPSCs and their derivatives.

4. Representative Images



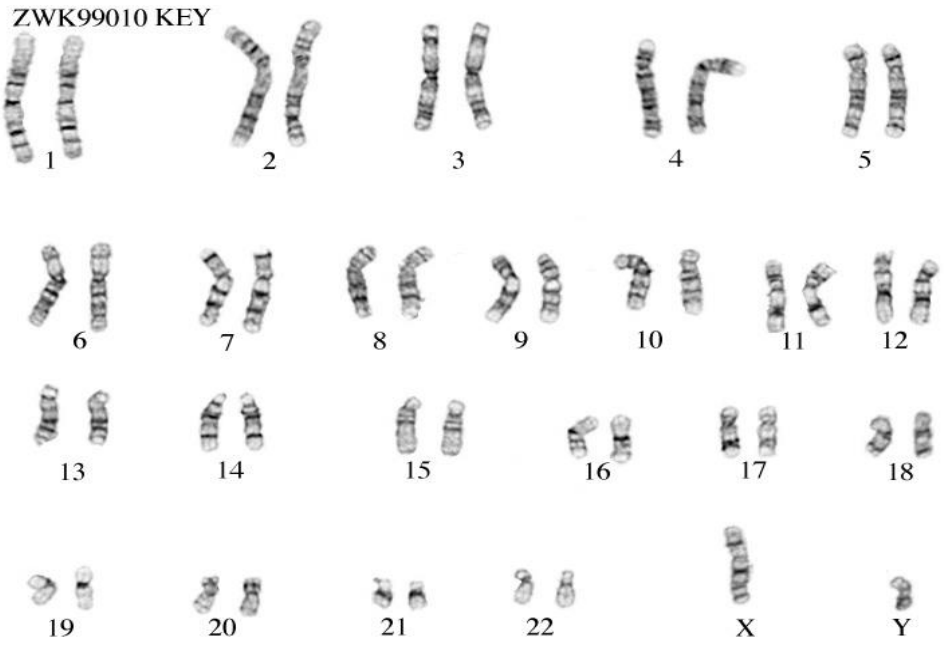
Case: IPSC sample_ Slide: 1___Cell: 7___ Image: 32___

3/29/2020

Technologist: M. Vernich, CG(ASCP)CM

Chromosome Count: 47

Karyotype: Human Male - 47,XY,+11



Case: IPSC sample__ Slide: 2___Cell: 12__ Image: 8___

3/29/2020

Technologist: M. Vernich, CG(ASCP)CM

Chromosome Count :46

Karyotype: Human Male – 46,XY

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".*

Reviewed By/ Date: _____