Healthy control 3 – Cell line ID: 302 (Age of sampling: 68)

This cell line was obtained from StemBANCC, reprogramming was done with Sendai virus.

1) KaryoStat™ report (ThermoFisherScientific):

Project Summary:

- University of Luxembourg (Client) is interested in services provided by the Life Technologies Corporation in the analysis of seven (7) client-provided samples using the KaryoStat™ assay.

Service Description:

- The KaryoStat™ assay allows for digital visualization of chromosome aberrations with a resolution similar to g-banding karyotyping. The size of structural aberration that can be detected is > 2 Mb for chromosomal gains and > 1 Mb for chromosomal losses. The KaryoStat™ array is optimized for balanced whole-genome coverage with a low-resolution DNA copy number analysis, the assay covers all 36,000 RefSeq genes, including 14,000 OMIM® targets. The assay enables the detection of aneuploidies, submicroscopic aberrations, and mosaic events.

Materials & Methods:

Genomic DNA purification

Cells were prepared according to the Genomic DNA Purification Kit (Catalog #: K0512) and quantified using the Qubit™ dsDNA BR Assay Kit (Catalog #: Q32850)

GeneChip® Preparation

250 ng total gDNA was used to prepare the GeneChip® for KaryoStat™ according to the manual, and is an array that looks for copy number variants and single nucleotide polymorphisms across the genome.

1. KaryoStat™ analysis of KS-3669 revealed the sample originated from a female individual
2. No chromosomal aberrations were found when comparing against the reference dataset (Figure 5)

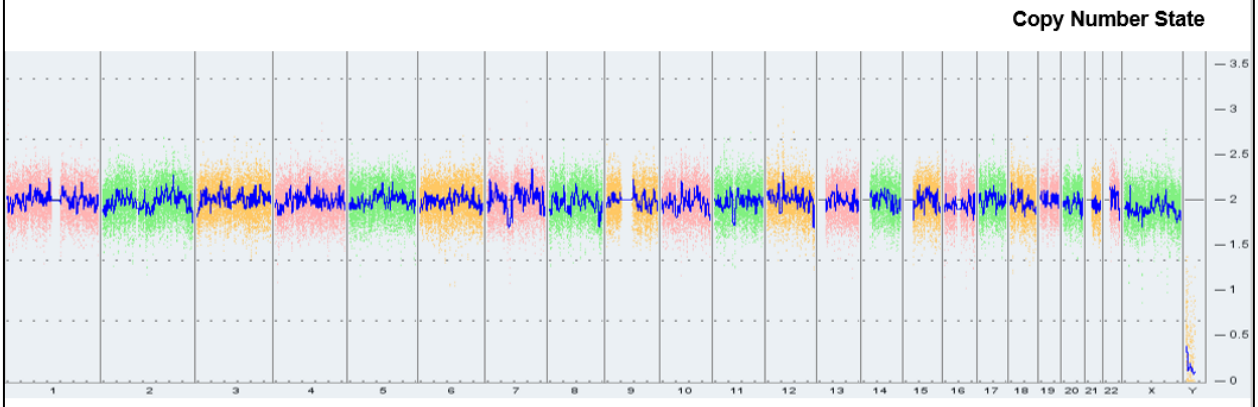
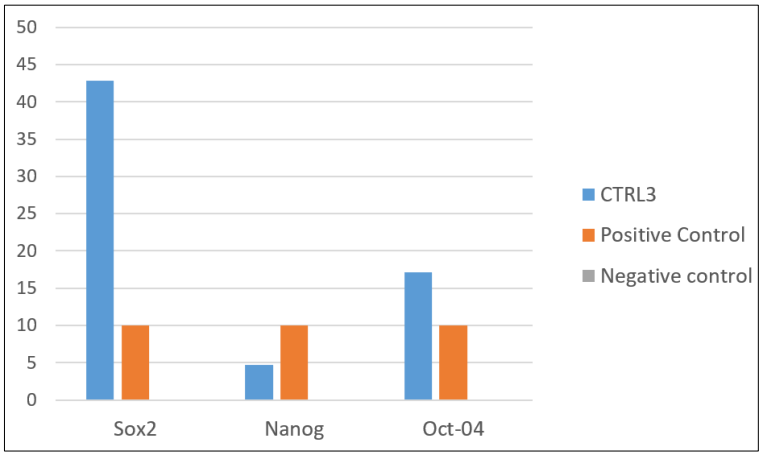


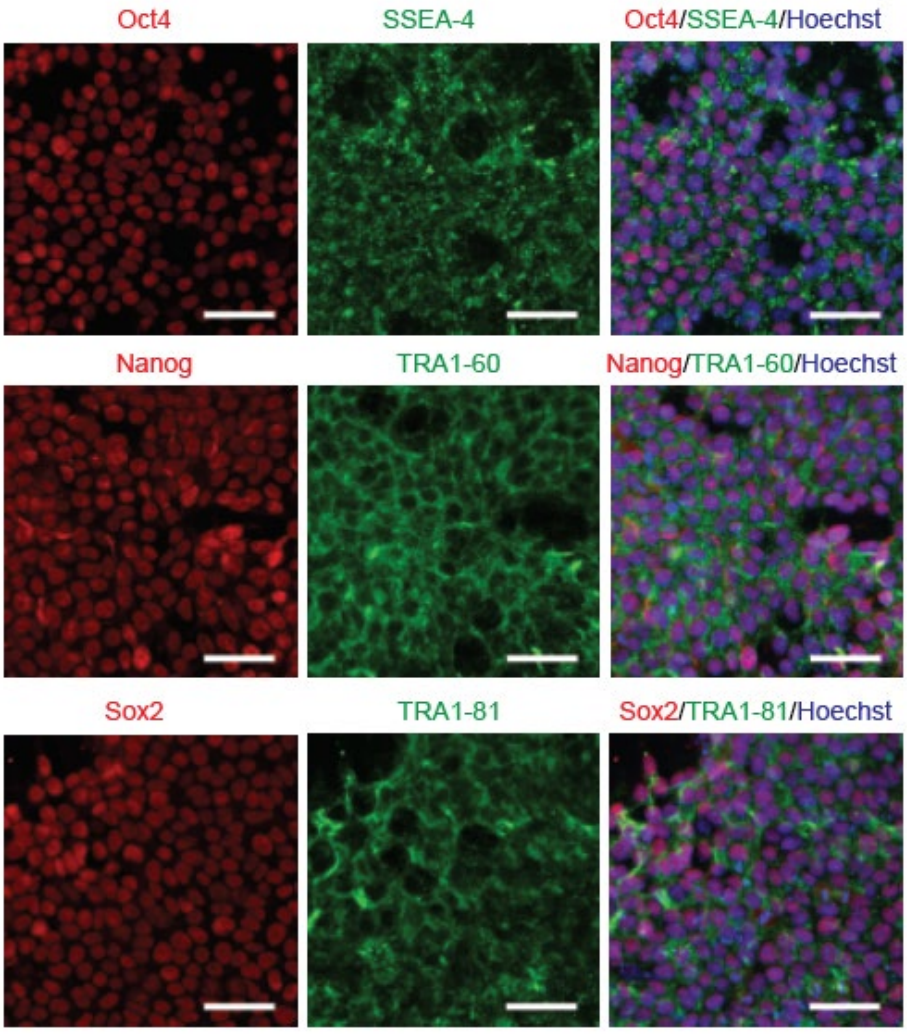
Figure 5: Whole genome view. The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the log2 ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state (CN = 2). A value of 3 represents chromosomal gain (CN = 3). A value of 1 represents a chromosomal loss (CN = 1). The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue signal represents the normalized probe signal which is used to identify copy number and aberrations (if any).

Disclaimer: This assay was conducted solely for the listed investigator/institution. The results of this assay are for research use only.

2) **Expression of pluripotency markers via qPCR**:
The abundance of pluripotency markers (SOX2, NANOG and OCT4) as measured by qRT-PCR. The results are relative to a reference commercial IPS line, and compared to a fibroblast line as a negative control



3) **Expression of pluripotency markers via Immunocytochemistry**:
Immunofluorescence staining showed high expression of six pluripotency markers: Oct4 (Red), SSEA-4 (Green), Nanog (Red), TRA-1-60 (Green), Sox2 (Red) and TRA1-81 (Green). Nuclei were counterstained with Hoechst (blue), (scalebar 50 µm).



Antibodies used for immunofluorescence staining:

Antibody	**Dilution**	**Source**	**Ref.-No.**
SOX2	1:200	R&D systems	AF2018
OCT4	1:400	Abcam	ab19857
NANOG	1:100	Millipore	AB5731
SEEA4	1:25	Millipore	MAB4304
TRA-1-60	1:25	Millipore	MAB4360
TRA-1-81	1:25	Millipore	MAB4360

4) Absence of the N370S mutation in the *GBA* gene:

Screening was done by extracting genomic DNA from blood samples using the GenElute™ Blood Genomic DNA Kit (Sigma, NA2020-1KT), PCR reactions were carried out using GoTaq® G2 Hot Start Master Mix (M7423, Promega). Primer sequences were F: TGTGTGCAAGGTCCAGGATCAG, R: ACCACCTAGAGGGGAAAGTG, which do not amplify the *GBA* pseudogene. Sample was sent for sequencing to Microsynth Seqlab.