

GBA patient PD1 – Cell line ID: 309 (Patient ID: 16281, age of sampling 66)

This is a commercially available line that was obtained from Coriell:

(https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=ND16281&Product=DNA).

Reprogramming was done with Sendai virus.

1) Karyotype report (life&brain):



Karyotype Report

Customer sample ID: S13
Internal sample ID: LU53DIVULUD100052
Date of receipt: 2017-02-24

Gender
Stated: Male
Chr. X derived: Male

Genotype identity with: S14-24.02.2017 / LUDIVULU00053

Karyotyping

Technology used: Illumina BeadArray

Product: HumanOmniExpressExome-8 BeadChip v1.3
Manifest file: HumanOmniExpressExome-8v1-3_A.bpm
Cluster file: HumanOmniExpressExome-8v1-3_A.egt

Chip barcode and segment: 200729690105 R08C01

Batch ID and 96 well position: WG1001271-MSA1 H03

Call rate: 0,998

Typing

Scanner: Illumina iScan, S/N: N234
Site of processing: Life&Brain GENOMICS, Bonn, Germany
Manufacturer: Illumina, Inc., San Diego, United States of America
Date of scan: 2017-03-22-2202

Genotype Analysis

Genome Studio: GenomeStudio V2.0.2
Genotyping module: Ver. 2.0.2

Copy Number Analysis

Algorithm applied: CNV-Partition
Version: 3.2
Software producer: Illumina, Inc., San Diego, United States of America

Noteworthy findings

No larger chromosomal aberrations to be reported.

Analyst

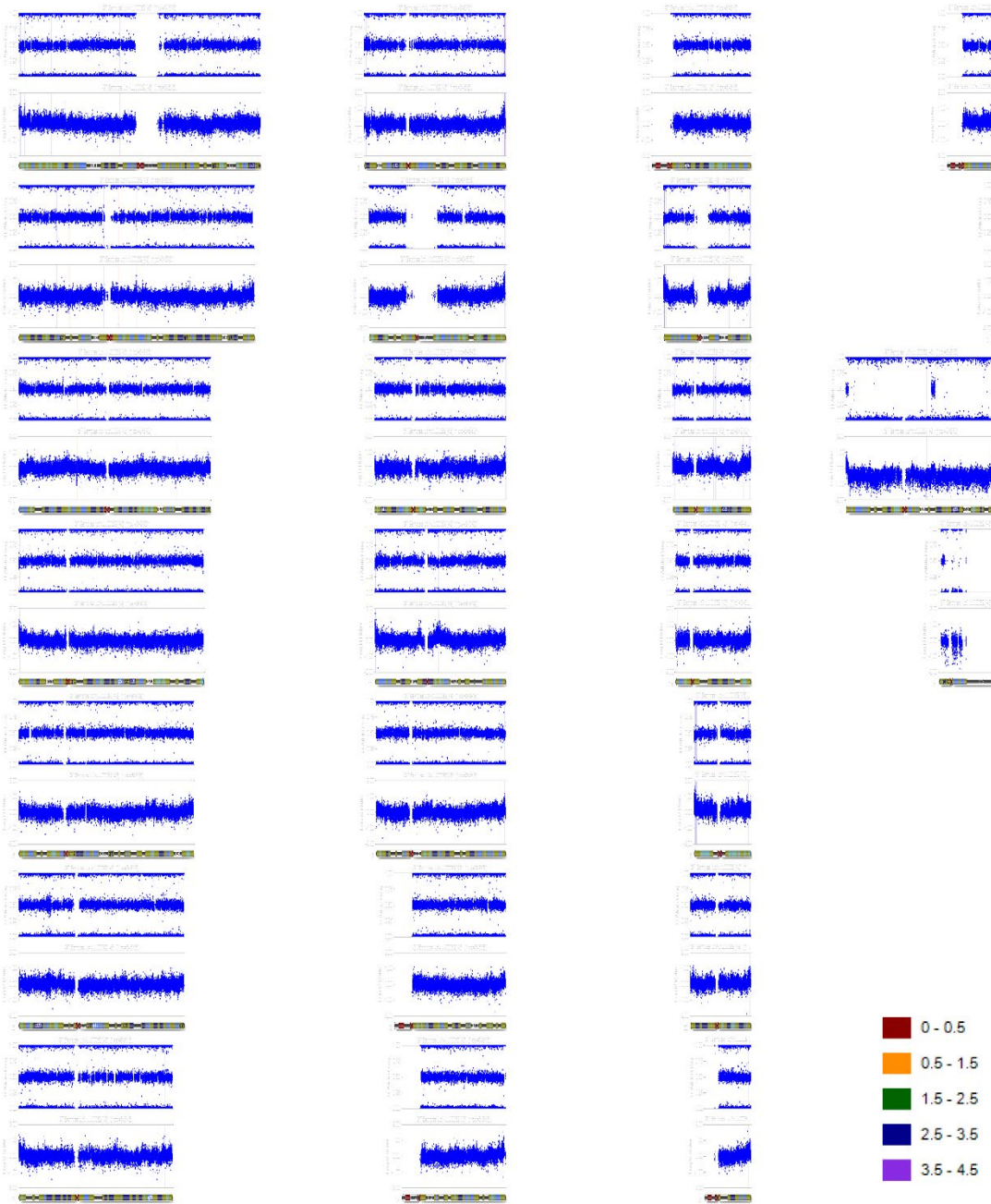
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Control Dashboard

Sample_ID / Sentry_Label	Category	Control (BeadType)	Section 1 X	Section 1 Y	State
LUDIVULU00052 / 200729690105_R08C01	Staining	DNP (High) (27630314)	34852	307	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Staining	DNP (Bgnd) (29619375)	447	312	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Staining	Biotin (High) (41666334)	443	19567	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Staining	Biotin (Bgnd) (34648333)	287	402	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Extension	Extension (A) (17616306)	42611	386	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Extension	Extension (T) (14607337)	44979	335	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Extension	Extension (C) (12613307)	1803	30701	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Extension	Extension (G) (11603365)	2176	30242	Notable/OK
LUDIVULU00052 / 200729690105_R08C01	Target Removal	Target Removal (31623323)	1239	217	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Hybridization	Hyb (High) (19612319)	2203	28335	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Hybridization	Hyb (Medium) (20636378)	510	17520	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Hybridization	Hyb (Low) (23617335)	2351	5380	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Stringency	String (PM) (32629312)	29762	582	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Stringency	String (MM) (33668307)	4431	260	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non-Specific Binding	NSB (Bgnd) (26619332)	397	260	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non-Specific Binding	NSB (Bgnd) (27624356)	347	206	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non-Specific Binding	NSB (Bgnd) (25617343)	382	341	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non-Specific Binding	NSB (Bgnd) (24616350)	345	196	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non- Polymorphic	NP (A) (34633358)	19042	489	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non- Polymorphic	NP (T) (16648324)	15968	306	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non- Polymorphic	NP (C) (43641328)	790	17419	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Non- Polymorphic	NP (G) (13642359)	674	16501	OK/OK
LUDIVULU00052 / 200729690105_R08C01	Restoration	Restore (28637363)	372	450	OK/OK

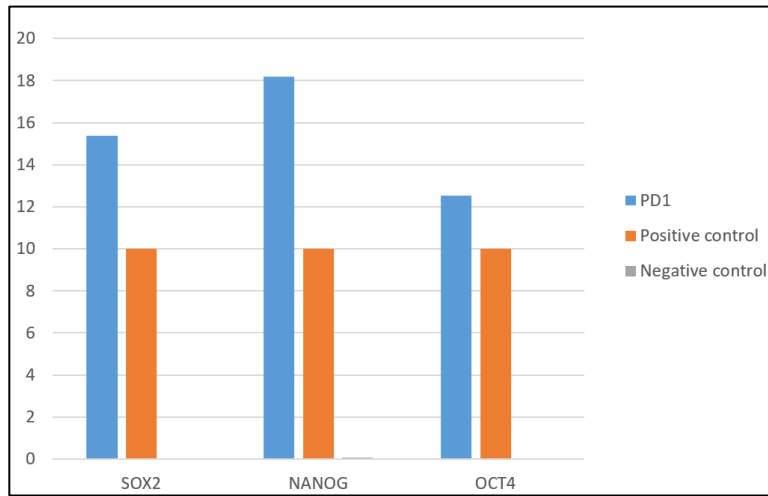
All „Notable“ tagged probes are within specs. The karyogram can be evaluated.

Karyogram



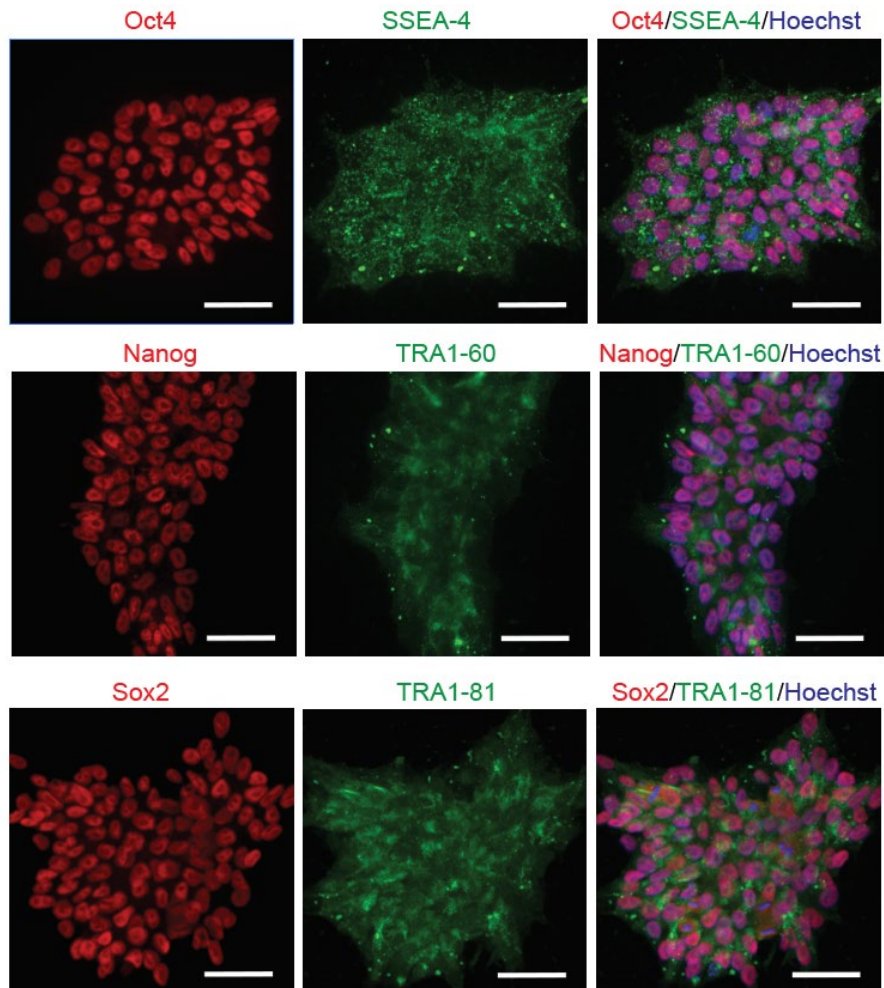
2) Expression of pluripotency markers via qPCR:

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 μ m).



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) Presence 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.

