EditCo-Supplied PGP1 iPS Cell Line

This cell line was derived from primary skin fibroblasts sourced from Dr. George Church, as part of the Personal Genome Project (PGP), which aims to distribute resources freely to improve the scientific process. The PGP1 cell line is highly characterized using sequence data and medical information. The samples have been consented for public posting and commercial use of Personally Identifying Genetic Information (PIGI), which allows open-access of extensive genetic data, such as whole-genome SNP arrays, whole-exome sequences, and whole-genome sequences.

The PGP1 cells were reprogrammed using a non-integrating Sendai viral approach (CytoTune™ -iPS 2.0 Sendai Reprogramming Kit). The PGP1 cell line is described in George Church's patent: Methods for increasing efficiency of nuclease-mediated gene editing in stem cells (WO2017184674A1).

PGP-1 cells have no commercial restrictions to distribute or use under the Open-MTA

Supporting Data on PGP1 iPS Cells

This section contains data confirming genetic stability, pluripotency, and purity of the PGP1 iPS cell line that EditCo supplies for Engineered Cells projects. Please note that these data provide information surrounding the parental cell line only and are not provided for individual Engineered Cells projects. Quality control assessments for individual projects are covered in the next section.

Please note, in February 2022, Thermo Fisher updated their karyotyping service from KaryoStat™ to KaryoStat+™. With this update, the resolution for detecting chromosomal gains improved from > 2 Mb to > 1 Mb. As a result, EditCo detected the presence of the common 20q11.21 amplification, a sub-chromosomal abnormality on chromosome 20, in our PGP1 parental cell bank. As of July 2022, EditCo clonally isolated a karyotypically normal PGP1 subpopulation and expand this population for its internal cell bank. However, due to the propensity for the PGP1 cell line to acquire this mutation, EditCo cannot guarantee that this abnormality will not reappear over time in customers' cell cultures.

To learn more specifically about the **20q11.21 amplification abnormality**, please visit our product information Help Center article on PGP1 karyotype.

1. Genomic Stability

1a. KaryoStat+™

An array-based technique for assessing the genomic stability of pluripotent stem cells. The method uses a GeneChip probe array that contains 100s-1,000s of copy number and SNP markers that are uniformly spaced across the genome. The array is optimized for whole-genome coverage with a low-resolution DNA copy number analysis. The assay enables the detection of aneuploidies, submicroscopic aberrations, and mosaic events. The size of structural aberration that can be detected is >1Mb for chromosomal gains and >1Mb for chromosomal losses.

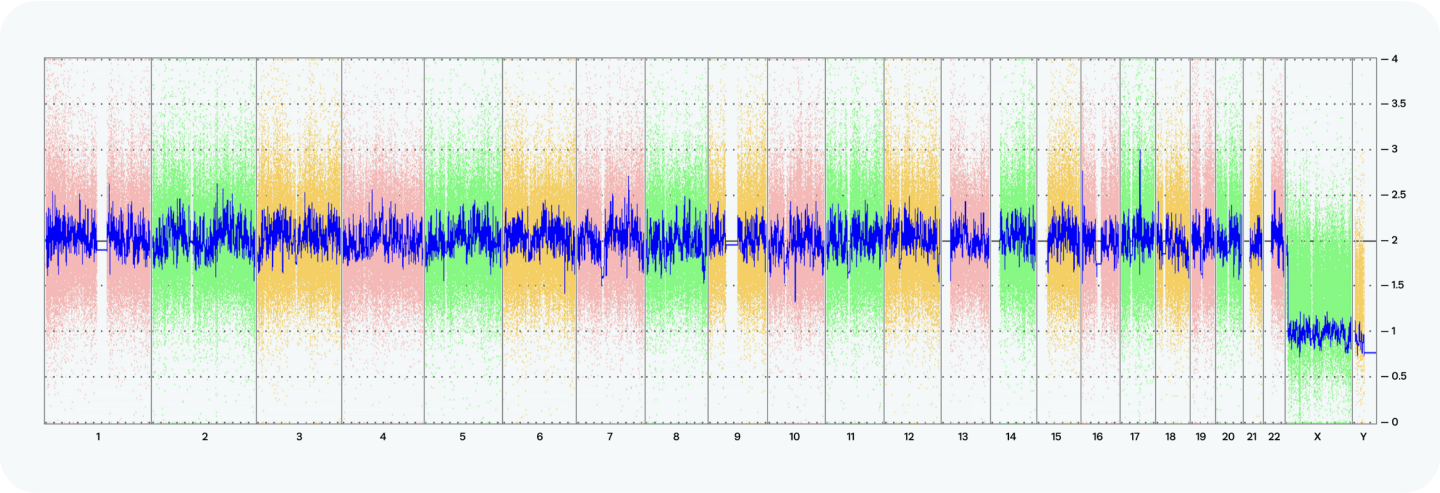
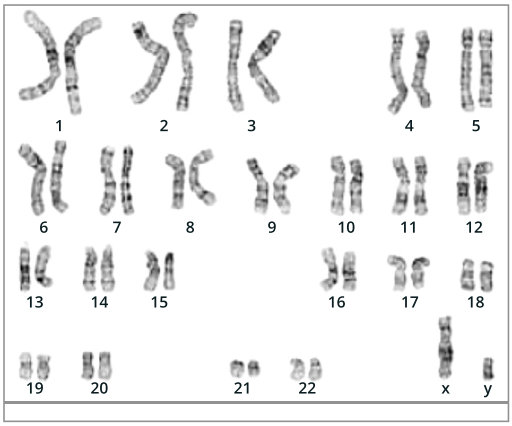


Figure 1. KaryoStat+™ indicates PGP1 cells are genomically stable.*
KaryoStat+™ results for wild-type PGP1 cells (passage 27) indicate that genomic integrity is maintained. The whole-genome view displays all somatic and sex chromosomes in one frame with a high-level copy number. The smooth signal plot (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). * descriptions adapted from ThermoFisher Scientific.

1b. G-Banding

A karyotyping technique that involves staining condensed chromosomes and visually assessing them for abnormalities.



Cell: *5*
Slide: *G01*
Slide Type: *Karyotype*

Total Counted: *20*
Total Analyzed: *8*
Total Karyogrammed: *4*
Band Resolution: *450 - 550*

Interpretation:
This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.

Completed by: *Kate Bird, CG (ASCP)*
Reviewed and Interpreted by: *Vanessa Horner, Ph.D., FACMG*

Figure 2. G-Banding verifies normal karyotypes of PGP1 cells.
G-banding results for wild type PGP1 cell clone (passage 49).

1c. Short Tandem Repeat (STR) Analysis

A technique that analyzes STR repeats (microsatellites) at specific loci in DNA across samples. This analysis is used to confirm cell line identity and genomic stability.

Table 1. STR analysis of wildtype PGP1 cells at passage 40, confirming the authenticity of this cell line.

Label on Tube	PGP1 p40 (76913)
Label on Report	PGP1 p40 (76913)
Concentration (ng/µL)	21.1
A260/280	1.90
Assay Date	5/29/19
File Name	STR 190530 WMR
FGA	22, 22
TPOX	8, 9
D8S1179	13, 13
vWA	18.18
Amelogenin	X, Y
Penta_D	8, 14
CSF1PO	10, 11
D16S539	11, 12
D7S820	10, 13
D13S317	8, 11
D5S818	9, 11
Penta_E	17, 17
D18S51	15, 16
D21S11	28, 30
TH01	7, 8
D3S1358	16, 19
Allelic Polymorphisms	26
Matches	76916, 76915
Comments	

2. Pluripotency

2a. PluriTest™

A pluripotency assay that compares the transcriptional profile of a sample to reference data of >450 pluripotent and non-pluripotent cell and tissue types. Samples are screened against samples in the stem cell database and given a pluripotency score (PluriCor) and novelty score (NovelCor). A positive PluriCor value indicates high similarity to the pluripotent samples in the model matrix. A high novelty score indicates that there are patterns in the tested sample that cannot be explained by the existing database of well-characterized, karyotypically normal pluripotent stem cells. A low novelty score indicates that the tested sample can be well reconstructed based on existing data from other well-characterized iPSC and ESC lines.

Table 2. PluriTest™ results for PGP1 wild type and edited cells.*

Sample ID	PluriTest Result	PluriCor	NovelCor
Wild type PGP1	Pass	39.74922	1.545877
Edited PGP1-1	Pass	37.12558	1.566561
Edited PGP1-2	Pass	36.24864	1.597517
Edited PGP1-3	Pass	36.44504	1.644794
Edited PGP1-4	Pass	39.6279	1.640673
Positive control	Pass	42.08363	1.276022
Negative (Non-iPSC) control	Fail	-44.67648	2.743842

This test included a PGP1 wild type sample (labeled PGP1) and 4 edited PGP1 clones. A “Pass” shows a clear pluripotency signature, whereas “Fail” indicates that the samples are not pluripotent. The “iPSC control” served as a positive control for pluripotency and “non-iPSC” served as a negative control for non-pluripotency.

*descriptions adapted from ThermoFisher Scientific.

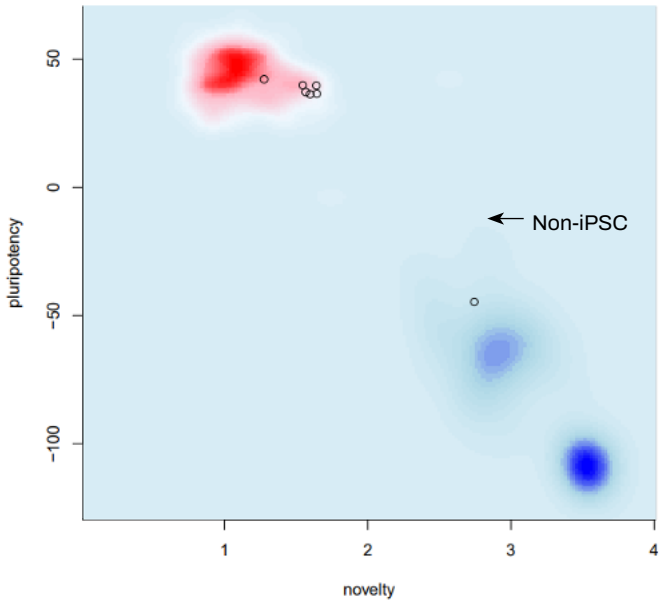


Figure 3. Pluripotency plot.*
The pluripotency plot window provides a visual representation of the tested samples in the analysis. This PluriTest was conducted on a wild type sample, 4 edited PGP1 clones, a negative control, and positive control (Table 2). The pluripotency and novelty x/y scatter plot combines the pluripotency score on the y-axis with the novelty score on the x-axis. The red and blue background hint to the empirical distribution of the pluripotent (red) and non-pluripotent (blue) samples in the reference data set. A non-iPSC sample was included in this experiment to serve as a negative control for non-pluripotency.

* descriptions adapted from ThermoFisher Scientific.

2b. Immunohistochemical Analysis

A common image-based technique for verifying iPS cell quality. This method involves the use of antibodies specific for pluripotency markers (e.g., Oct4, SSEA4, etc.) that are conjugated to fluorophores. The iPS cells are stained with the antibodies and visualized using a fluorescent microscope.

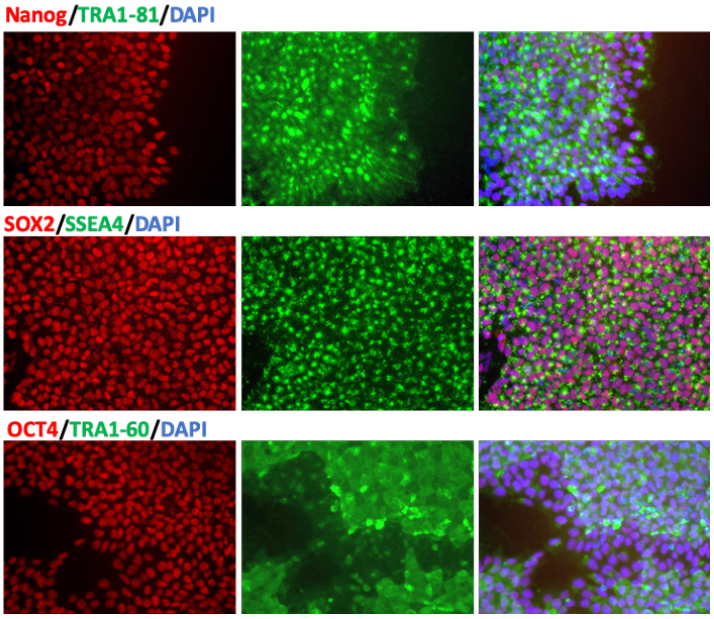


Figure 4. PGP1 cells are positive for all pluripotency markers.
PGP1 cells (passage 41) were stained for pluripotency markers (red: Nanog, SoX2, OCT4; green: TRA1-81,SSEA4, TRA1-60). The right-most column shows overlays of the pluripotency markers with DAPI (blue, marks nuclei for reference).

3. Sterility

3a. Sterility Test

Comprehensive testing of cell cultures for bacterial and fungal contamination.

Table 3. IDEXX sterility analyses and results.

Analysis	Contaminant Type	Result
PCR Evaluation	Hepatitis A Hepatitis B Hepatitis C HIV1 HIV2 HTLV 1 HTLV 2 *Mycoplasma sp.*	Negative
Microbiologic Evaluation	Bacteria Fungi	Negative

EditCo's Quality Control Analyses

All Engineered Cells projects using the PGP1 cell line include quality control assessments. All tests are conducted post-editing and after final cell expansion.

Table 4. Quality control assessments available for PGP1 Engineered Cells projects.

Assessment	Assay	Product
Mycoplasma	Luciferase-based	Edited PGP1 Pools and Clones
Sequence validation	Sanger sequencing & ICE analysis	Edited PGP1 Pools and Clones
Genomic stability (optional add-on)	KaryoStat™	Edited PGP1 Clones
Pluripotency (optional add-on)	PluriTest™	Edited PGP1 Pools and Clones

Resources

PGP cells website
Genomic information for PGP1 cells

Table 5. Cell types derived from PGP1 cells.

Cell Type	PubMed ID
Neurons	25403753
Cardiomyocytes	24813252
Brain organoids	31168097
Kidney podocytes	29995874