

EditCo-Supplied 802-30F iPS Cell Line

EditCo supplies the following iPS cell line from an ethically sourced female donor that has been reprogrammed by REPROCELL® from blood-derived endothelial progenitor cells (EPCs) using mRNA (StemRNA™ 3rd-Gen technology):

EditCo ID	Cell Line	Donor Sex	Donor Age	Donor Race/Ethnicity
802-30F	RPChiPS8023G1	Female	30 years	Hispanic

In contrast with other methods, mRNA-based approaches carry no risk of integration or retention of reprogramming vectors. As demonstrated below, the genomic stability and pluripotency of the line has been thoroughly verified. In addition, whole-genome sequencing data can be provided upon request.

Supporting Data

This section contains data confirming the genetic stability, pluripotency, and purity of the 802-30F iPS cell line. Please note that these data provide information pertaining to the parental cell line only and are not provided for individual Engineered Cells orders. Quality control assessments for individual orders are covered in the next section.

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.

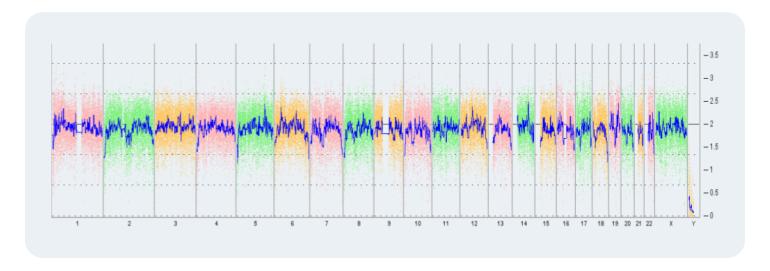


Figure 1. KaryoStat indicates 802-30F cells are genomically stable.*

KaryoStat results for wild type 802-30F cells (passage 19) 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 Thermo Fisher Scientific.

1b. G-Banding

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



Figure 2. G-banding verifies normal karyotype for 802-30F cells.

G-banding results for wild type 802-30F cell clone at passage 14.

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 iPS cell and embryonic stem (ES) cell lines.

Table 1. PluriTest™ results for wild type 802-30F cells.*

Sample ID	PluriTest Result	PluriCor	NovelCor
Wild type 802-30F	Pass	37.18372	1.282409
iPSC Control	Pass	42.6786	1.296485
non-iPSC Control	Fail	-45.97838	2.725725

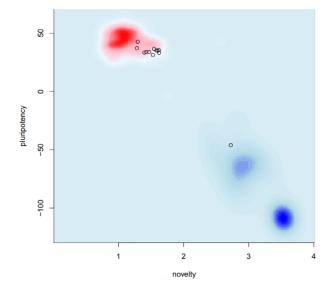
These tests included wild-type 802-30F cells (passage 19) as well as controls (iPS and non-iPS cell lines, respectively).

A "Pass" shows a clear pluripotency signature, whereas "Fail" indicates that the samples are not pluripotent.

* descriptions adapted from ThermoFisher Scientific.

Figure 3. Pluripotency plot.*

The pluripotency plot provides a visual representation of the tested samples in the analysis. PluriTests were conducted on the wild type and control cells indicated in Table 1, as well as edited clones from a different parental line (data excluded from table for brevity). 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 distributions of the pluripotent (red) and non-pluripotent (blue) samples in the reference data sets.



^{*} descriptions adapted from Thermo Fisher 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.

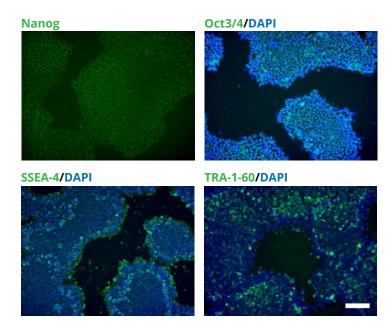


Figure 4. 802-30F cells are positive for standard pluripotency markers.

802-30F cells were stained for pluripotency markers Nanog, Oct3/4, SSEA-4 or TRA-1-60 (green) and DAPI (blue). Scale bars: $100 \mu M$

3. Sterility

3a. Sterility Test

Comprehensive testing of cell cultures for bacterial and fungal contamination.

Table 2. IDEXX sterility analyses and results for 802-30F cells.

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 802-30F iPS cell line include quality control assessments. All tests are conducted post-editing and after final cell expansion.

Table 3. Quality control assessments available for iPSC Engineered Cells orders.

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