# EditCo-Supplied BX-32 iPS Cell Line

EditCo supplies the following iPS cell line from an ethically sourced female donor that has been reprogrammed by BrainXell from blood-derived endothelial progenitor cells (EPCs) using blood episomal reprogramming:

| Synthego ID | Cell Line | Donor Sex | Donor Age | Donor Race/Ethnicity |
|---|---|---|---|---|
| BX-32 | BX-32 | Female | 29 years | Asian/Chinese |

In contrast with other methods, episomal-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.

## Supporting Data

This section contains data confirming the genetic stability, pluripotency, and purity of the BX-32 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

## Stem Genomics iCS-digital™

A Digital Droplet PCR-based technique for assessing the genomic stability of pluripotent stem cells.

Thanks to the high level of performance offered by digital PCR (200 bp and 20% mosaicism) combined with an in-depth analysis of most recurrent abnormalities in hPSCs, the iCS-digital™ PSC range of tests can detect sub-karyotyping abnormalities traditional methodologies would miss. The iCS-digital method uses a 24-probe test that captures over 93% of recurrent defects in hPSCs, including the 20q11.21 amplification that accounts for 25% of recurrent abnormalities in hPSCs worldwide.

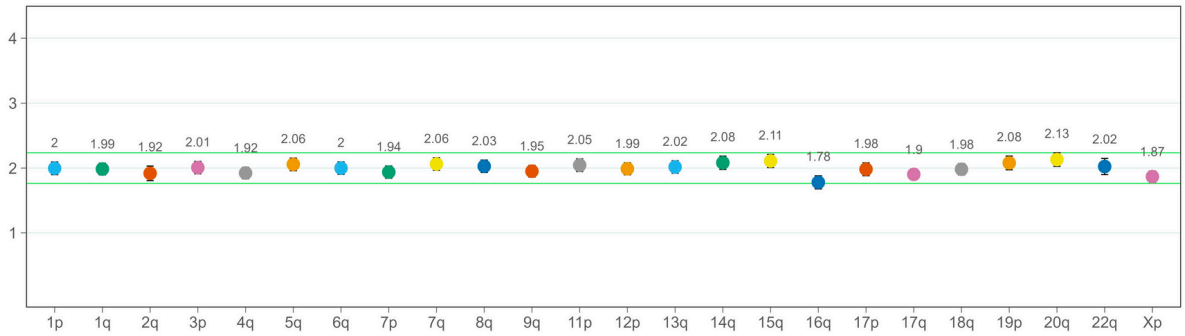


**Figure 1.** Stem Genomics iCS-digital **indicates** BX-30 **cells are genomically stable.*** Stem Genomics iCS-digital results for wild type BX-32 cells indicate that genomic integrity is maintained.

The iCS-digital test allows for the detection of Copy Number Variations (CNVs) of one kilobase (kb) or larger that are present at an abnormal copy number in comparison with a reference genome. Normal copy number should be equal or close to the value of 2 at all the 24 recurrent regions that we analyze (except for the Xp position since it depends on the sex of the cell line studied: XX or XY). However, due to intrinsic variation caused by multiple factors (DNA concentration, quality, etc.), some samples will present higher copy number fluctuation than others over the 24 positions. The interpretation method takes into account the difference in CNV fluctuation observed among samples. More specifically, the statistical analysis is based on normal distribution and is adapted to the overall variability of each sample in an independent manner. P-values arethen assigned to each probe and the detection of anomalies is calculated based on their specific p-values and CNVs. The confidence limits (p-value threshold) are displayed as green lines on each graph. A sample is considered normal by default if its copy number values are strictly between 1.88 and 2.2, or if its p-values are strictly above 0.05. A trend (Trend to loss or Trend to gain), corresponds to a position detected with a p-value between 0.01 and 0.05. Trends are not anomalies but are defined as suspicion of anomalies. It could be linked to the quality of the samples, to the run, or the limit of sensitivity of the test (<20% mosaicism). In these cases we advise to keep an eye on the samples involved and potentially re-test them few weeks/passages later. An anomaly (CNV = Loss or Gain) is detected if a position presents a p-value strictly below 0.01.

# 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** BX-30 **cells.***

| Sample ID | PluriTest Result | PluriCor | NovelCor |
|---|---|---|---|
| Wild type BX-32 | Pass | 29.39207 | 1.344068 |
| iPSC Control | Pass | 37.90687 | 1.229926 |
| non-iPSC Control | Fail | -45.36237 | 2.718222 |

These tests included wild-type BX-32 cells 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.

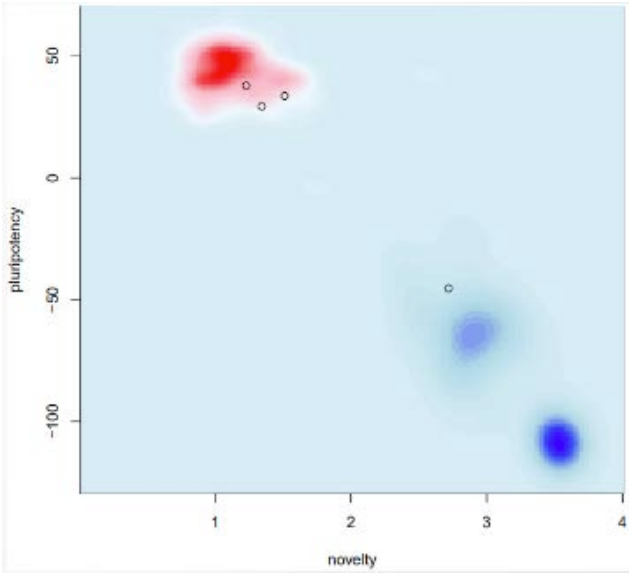


**Figure 3. Pluripotency plot.***
The pluripotency plot provides a visual representation of the tested samples in the analysis. PluriTests were conducted on the wild type and control cells indicated in Table 1, as well as edited clones from a different parental line (data excluded from table for brevity). 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.

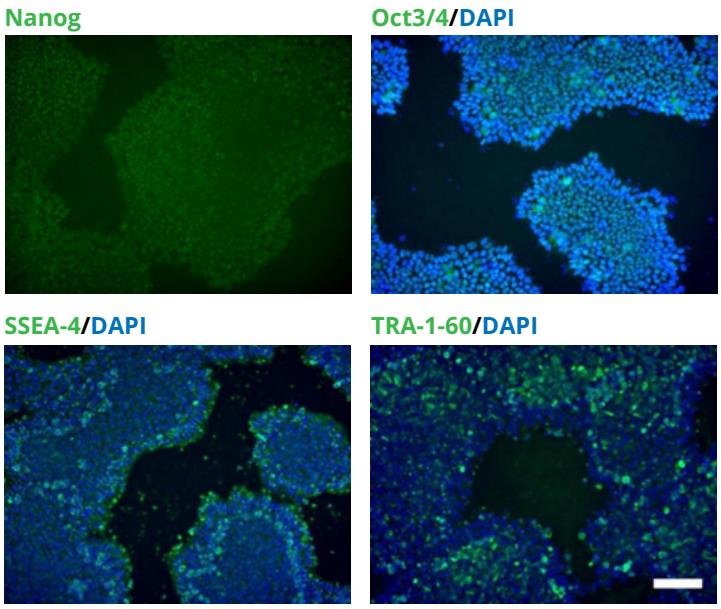


**Figure 4. BX-32 cells are positive for standard pluripotency markers.**
BX-32 cells were stained for pluripotency markers Nanog, Oct3/4, SSEA-4 or TRA-1-60 (green) and DAPI (blue). Scale bars: 100 µM

# 3. Sterility

## 3a. Sterility Test

Comprehensive testing of cell cultures for bacterial and fungal contamination.

**Table 2. IDEXX sterility analyses and results for** BX-3**2 cells.**

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

# EditCo's Quality Control Analyses

All Engineered Cellsprojects usingthe BX-32 iPScelllineinclude 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) | Stem Genomics iCS-digital ™ | Engineered iPS Cell Clones |
| Pluripotency (optional add-on) | PluriTest™ | Engineered iPS Cell Pools and Clones |