ISENET Biobanking
Stem Cell Services

Stem Cell Biobanks are an essential resource of biological samples by providing qualified and ethically sourced stem cell lines to be used in drug screening, disease modeling and regenerative medicine.

ISENET Biobanking is a research grade stem cell biobank, storing highly-controlled human and murine pluripotent cells, offering the following **Stem Cell Services**.

- **Generation of human stem cell lines** using blood (PBMC) and skin biopsy
- **Expansion, characterization, cryopreservation and distribution of** cell lines for research purposes
- **Qualification of stem cell lines** by applying the highest quality control standards
- **Embryoid bodies formation-Tripotency** (mesodem, ectoderm, endoderm)
- **Pluripotency** (immunofluorescence, flow cytometry, qPCR and WB)
- **Proliferation vs senescence determination**
- **Differentiation into**
  - ectoderm (neuronal cells) cell fate
  - mesoderm (cardiomycyte) cell fate
  - endoderm (insulin producing cells) cell fate
- **Data Sheet:** Each Cell Line stored at **ISENET Biobanking** is documented with a Data Sheet containing all QC information related to the cell line.

ISENET Biobanking is the custodian of a number of research grade human and animal stem cell lines. By participating in a number of European and National Research Projects and by collaborating with academic stem cell laboratories, **ISENET Biobanking** acquires, cryopreserves, characterizes and distributes well-documented stem cell lines. **ISENET Biobanking** applies a high quality management system by following a quality controlled procedure (figure 1).

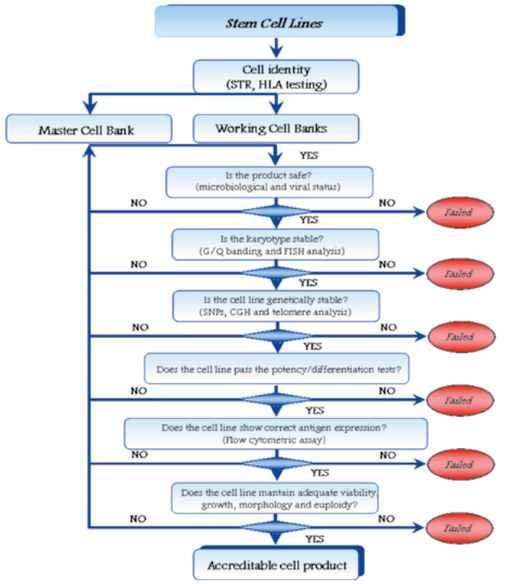


Figure 1: ISENET Biobanking acquires, cryopreserves, characterizes and distributes well-documented biospecimens following a quality controlled procedure.

Sample Traceability

ISENET Biobanking, uses state-o- the-art sample management software systems to trace all relevant data directly linked to the stored cells. The system is configured based on the tank display sample types and user defined fields. All cell stocks are linked to a unique and univocal sample ID.

Sample types stored at ISENET Biobanking

SAMPLE TYPE	SAMPLE DESCRIPTION
mESCs	mouse Embryonic Stem Cells
mNSCs	mouse Neural Stem Cells
hNCPCs	human Neural Crest Progenitor Cells
hFSCs	human Fetal Stem Cells
hIPCs	human Induced Pluripotent Stem Cells
hGSCs	human Glioma Stem Cells
hHFIBs	human Healthy Fibroblasts
hDFIBs	human Diseased Fibroblasts
HCLs	mouse and human Healthy Cell Lines
DCLs	mouse and human Diseased Cell Lines

Figure 2: The table shows the different sample types that are cryopreserved in the biobank.

Human Pluripotent Stem Cells

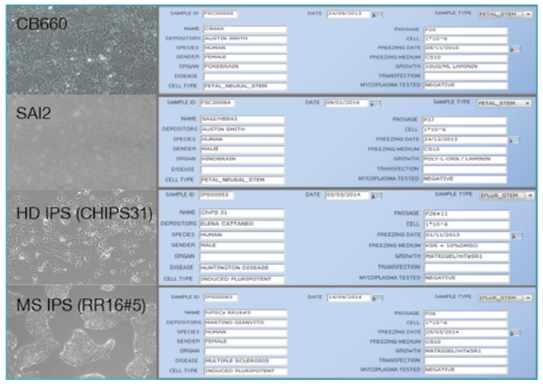


Figure 3: ISENET Biobanking cryopreserves human pluripotent stem cells (fetal and induced pluripotent) including samples derived from patients with diseases such as Huntington Disease and Multiple Sclerosis.

Quality Control Assays

ISENET Biobanking understands the importance of assuring quality of stem cell lines for scientific research.

1. Cell Line Authentication/Identity

Cell identity testing is performed on DNA on each cell line and is re-performed after cell line cryopreservation, before distribution.

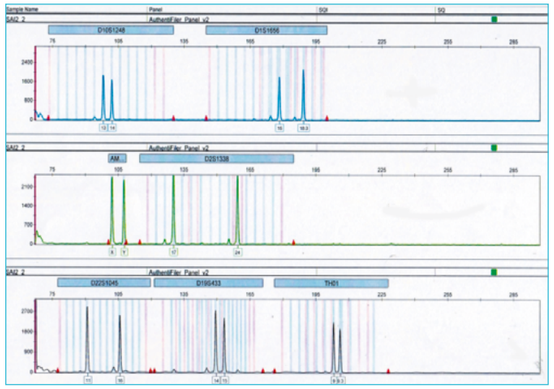


Figure 4: STR profiling analysis performed on the human fetal stem cell line SAI2, p41 using the AuthentiFiler PCR Amplification Kit.

2. Sterility and Mycoplasma Testing

ISENET Biobanking performs sterility tests pre/post thawing to assess the presence of contaminants in the cultures. These include mycoplasma (PCR analysis, bacterial growth in nutrient media for 3 weeks and indirect DNA staining), bacterial, fungal and viral contamination (NAT technology).

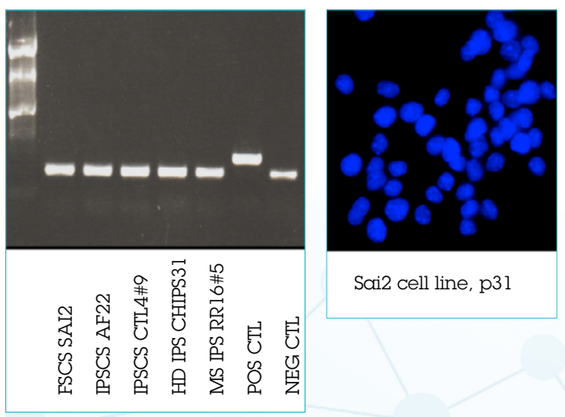


Figure 5: Human pluripotent stem cell lines are negative for mycoplasma screening by PCR (Venor GeM One Step kit) and Hoechst staining (Mycofluor Mycoplasma Detection Kit).

3. Genetic and Genomic Stability

ISENET Biobanking performs standard karyotyping (QFQ-banding) in order to detect major chromosomal aberrations (greater than 10 Megabases) and translocations. High-throughput array Comparative Genomic Hybridization (aCGH) is carried out to identify copy number variations (CNVs) at genomic level.

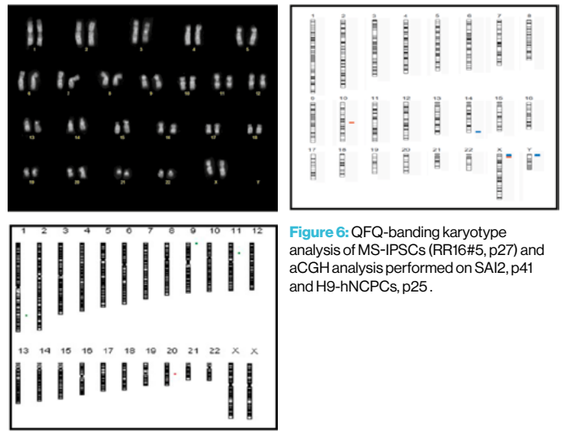
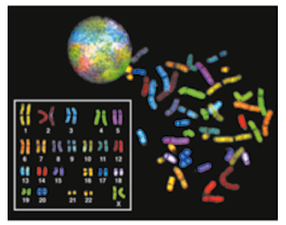


Figure 6: QFQ-banding karyotype analysis of MS-IPSCs (RR16#5, p27) and aCGH analysis performed on SAI2, p41 and H9-hNCPCs, p25 .

Spectral Karyotyping (SKY)



Spectral-Karyotyping (SKY) is a technique based on the simultaneous visualization of all human chromosomes in different colors. Through state-of-the-art imaging and optical microscope technologies, SKY provides an accurate and simple easy detection of structural and numerical chromosomal aberrations.

4. Epigenetic/Epigenomic Profiling

ISENET Biobanking performs whole genome methylation profiles of cell lines during extended passages and differentiation under appropriate conditions. Epigenomic differences often involve cancer related genes and onco-microRNAs with negative effects on the function, stability, differentiation potential and safety.

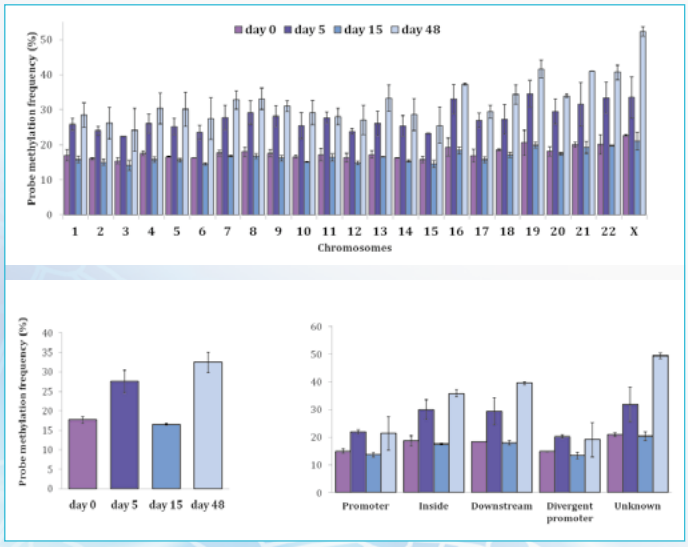


Figure 7: Global Methylation Levels of the human Embryonic Stem cell line H9 during striatal differentiation.

5. Tripotency, Pluripotency and Differentiation Assays

Immuno-fluorescence in combination with qRT-PCR (data not shown) technologies are used to verify tripotency, pluripotency and differentiation capacity of the stem cell lines under appropriate conditions. Pluripotency and differentiation are tested before and after cryopreservation both at early and late phases.

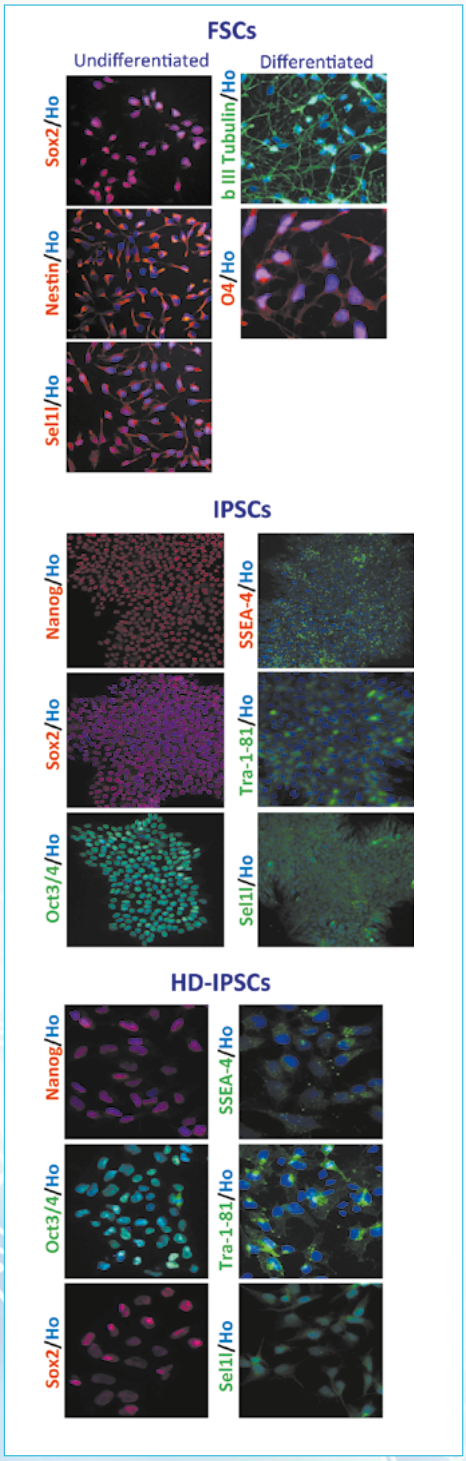


Figure 8: Immunofluorescence analysis performed on feta neural stem cells (SAI2, p49), IPSCs (CTR4#9, p29) and HD-IPSCs (ChiPS31, p43) showing pluripotency and/or positivity to differentiation markers.

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