



## **ISENET STEM CELL BIOBANK** *"Banking services for your biospecimens "*

Stem Cell Biobanks are essential resource of biological samples, useful for both basic and translational research, by providing support in accessing to high quality controlled and ethically sourced stem cell lines from different origin. ISENET Stem Cell Banking Services can assist in your ES and iPS cell banking efforts.

•**Know How:** ISENET acquired know-how in stem cell banking by participating in several EU-FP7 research projects, performing characterization and banking of human and murine ES and iPS cell lines.

•**Stem Cell Banking Services:** Custodian of ES and iPS cell lines; expansion, quality control, characterization, cryopreservation and distribution.

•**Quality Controls:** ISENET uses the highest quality control standards, consisting of testing the cell lines for: identity; sterility; karyotype; aCGH, DNA methylation analysis, flow cytometry, tripotency, pluripotency and differentiation analysis.

•**Data Sheet:** Each Cell Line stored at ISENET is documented with a Data Sheet containing all QC information related to the cell line.

• **Next Generation Sequencing:** Explore the Human Genome information for clinical decision.

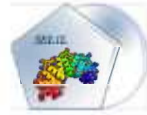


**Research-grade  
Stem cell  
BioBank for  
drug screening,  
disease modeling testing,  
& regenerative medicine**

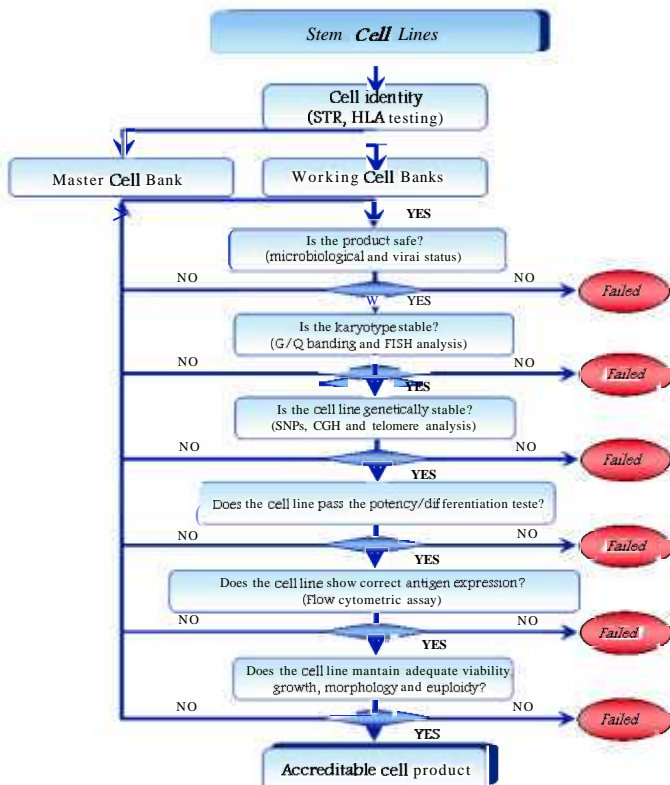
The establishment of well-characterized panels of pluripotent lines from mature cells in the body holds great potential for applications in regenerative medicine, drug screening, disease modeling, and medical treatments. While there is growing demand for new stem cell resources, our priority is to make available, in a timely manner, to the scientific community high quality research-grade pluripotent stem cells lines.

ISENET, cryopreserves human induced pluripotent stem cells that reflect the diversity of the human population with the aim to develop a **'PERSONALIZED STEM CELL BIOBANK'**.

ISENET ([www.isenet.it](http://www.isenet.it)), founded in 1996, has established its Stem Cell Bank with the objective to: store, characterize and supply ethically-approved quality controlled stem cell lines of different origin (adult, fetal, embryonic, induced pluripotent) and grade (research) of human and animal origin.



ISENET is a research-grade stem cell biobank, storing highly-controlled human and murine pluripotent cells. By participating in a number of European and National Research Projects and by collaborating with academic stem cell laboratories, Isetnet acquires, cryopreserves, characterizes and distributes well-documented several stem cell lines. ISENET applies sequentially and systematically a high quality management system by following quality control stem cell pipeline (figure 1). Cells are cryopreserved in culture medium containing 10% DIMISO and/or in Cryostore CS10, a GMP cryoreagent containing 10% DIMISO, but free of animal proteins. This DMSO-based and serum free solution gives optimal results in term of cell viability. All cell line batches are stored in liquid nitrogen containers at -196°C. Complete documentation consisting of all the results including images, generated as part of the testing is provided with the banked cells.



**Figure 1:** ISFNFT acquires, cryopreserves, characterizes and distributes well-documented biospecimens following quality control stem cell pipeline.

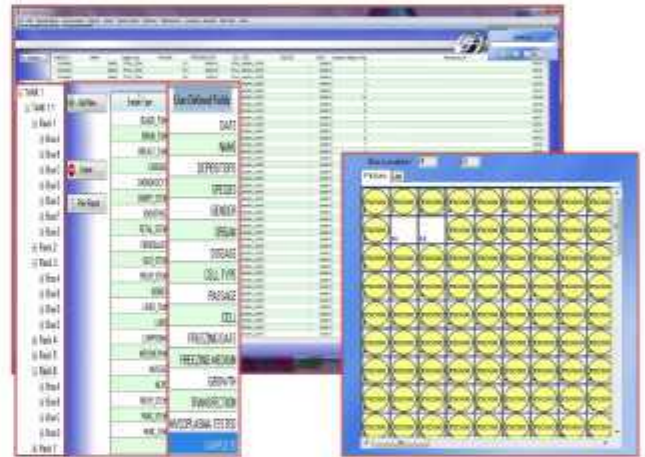
### Sample types stored at ISENET

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 3:** The table shows the different sample types that are cryopreserved in the biobank. ISENET cell line catalogue is available at [www.isenet.it](http://www.isenet.it)

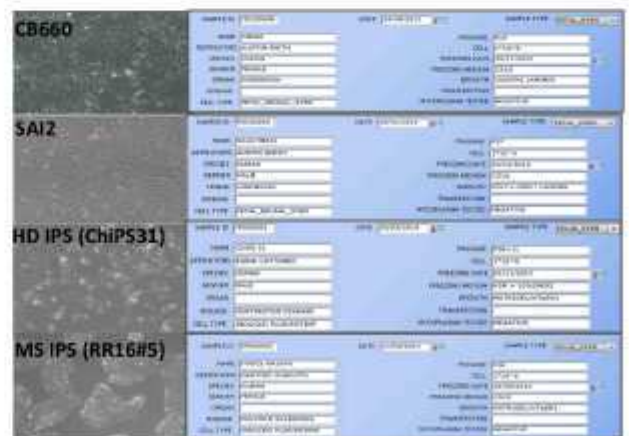
### Sample Traceability

ISENET uses the sample management software (Freezerworks) to trace all relevant data directly linked to the physical cell stocks. The software has been set up based on the tank display (figure 2), sample types and user defined fields. All cell stocks are linked to a unique and univocal sample ID.



**Figure 7:** Cell stocks are managed by Freezerworks according to their Sample ID and Sample type, linked to all associated data and information regarding their origin, phenotype, disease, STR diversity, genotype, original tissue source, epigenetic signature, gene expression profiles, pluripotency status etc.

### Human Pluripotent Stem Cells



**Figure 4:** ISENET 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 understands the importance of assuring the quality of the stem cell lines for scientific research and performs all the analyses on commissioned lines as a service to the community.

### 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.

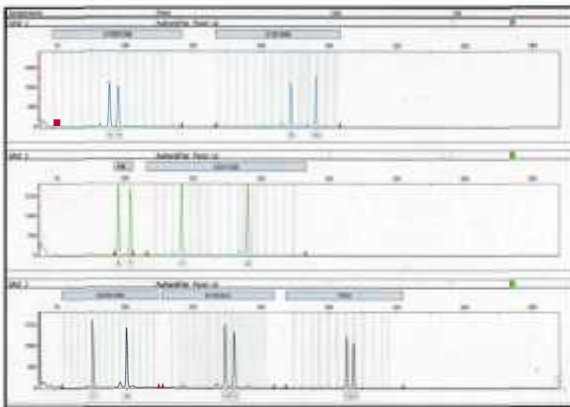


Figure 5: STR profiling analysis performed on the human fetal stem cell line SAI2, p41 using the AuthentiFiler PCR Amplification Kit.

### 2. Sterility and Mycoplasma Testing

ISENET 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).

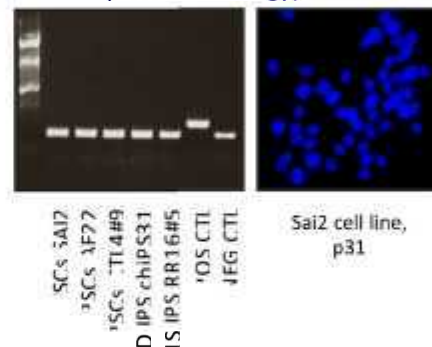


Figure 6: Human pluripotent stem cell lines are negative for mycoplasma screening by PCR (Venor GeM One Step kit) and Hoechst staining (Mycofluor Mycoplasma Detection Kit).

### 3. Genetic/Genomic Stability

ISENET 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 7: QFQ-banding karyotype analysis of MS-IPSCs (RR16#5, p27) and aCGH analysis performed on SAI2, p41 and H9-hNPCs, p25.

### 4. Epigenetic/Epigenomic Profiling

ISENET performs whole genome methylation profiles of the 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 of the cells. Whole genome methylation analysis is performed by using the human CpG Island Microarray Kit Ix244K (Agilent Technologies).

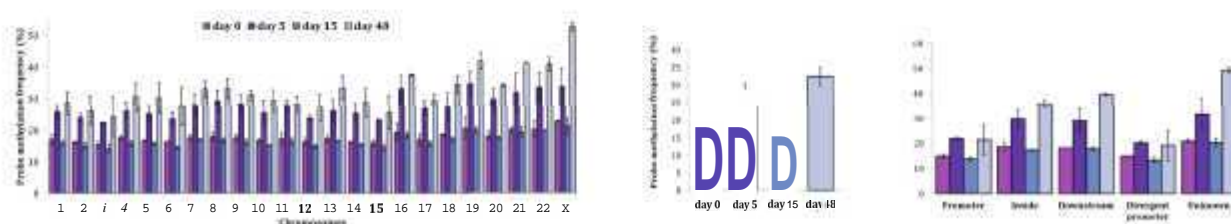


Figure 8: Global Methylation Levels of the human Embryonic Stem cell line H9 during striatal differentiation.



## 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 passages.

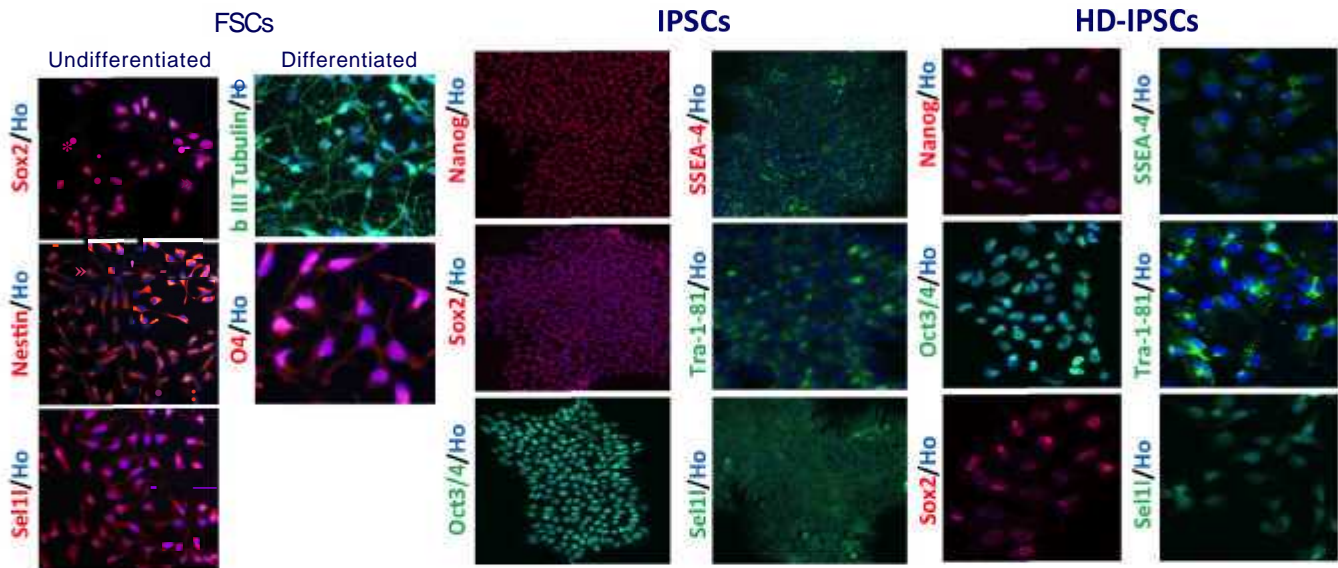


Figure 9: Immunofluorescence analysis performed on feta neural stem cells (SAI2, p49), iPSCs (CTR4#9, p29) and HD-IPSCs (ChiPS31, p43) showing pluripotency and/or positivity to differentiation markers.

## Next-generation sequencing

ISENET can perform next-generation high-throughput DNA sequencing to provide solutions tailored specifically to genome and transcriptome exploration.

### Genome

- **ChIP -sequencing (ChIP-seq)** allows genome-wide mapping of sequence specific protein-DNA interactions
- **Whole Exome sequencing** is aimed at sequencing and analyzing only the transcribed and coding regions of the genome

### Transcriptome

- **Whole Transcriptome Analysis (WTA)** explores the entire population of RNA transcripts, coding and non-coding, in a quantitative and unbiased manner. It is a suitable technique for genome-wide RNA expression profiles studies.
- **Small RNA sequencing (Small RNA)** identifies in a quantitative manner the entire small RNA population in a sample with higher sensitivity, specificity and a wider dynamic range than microarray technology.

