MANAGING OF A STEM CELL BIOREPOSITORY

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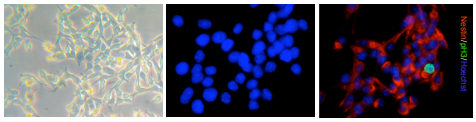
Stem cells have acquired a great deal of attention as they promise to be new vehicles for cell and gene therapy. It is then important to optimize culturing conditions, cryostorage protocols and monitoring systems to obtain ready to use cells for clinical applications. BioRep is a partner in two European Financed projects (FP6 and FP7) with the aim to establish a cell bank hosting neural derived stem cells and define the proper procedures to limit the unreproducibility of their use.
BioRep regularly performs a series of tests to assess the presence of contaminants in the cultures including the presence of viral particles. Cells are cryopreserved and banked in liquid nitrogen tanks connected to a central monitoring alarm system. Routinely, the cells are assessed for their pluripotency status and differentiation capabilities, chromosomal stability and viability.
A set of standard operating procedures (SOP) have been generated relating to each step of stem cell culturing, processing and storage with the objective to guarantee the freezing of cells with highly reproducible characteristics. Using GMP freezing medium free of animal proteins (Cryostor™ CS10) in addition to a controlled rate cooling system it is possible to achieve remarkable cell viability and low apoptotic level.
Joining the stem cell biology expertise of the academy with the technology standards of the industry has allowed the development of reagents and processes to generate safe and effective stem cell lines. In this way, several neural stem cell lines have been established and made available to the scientific community.

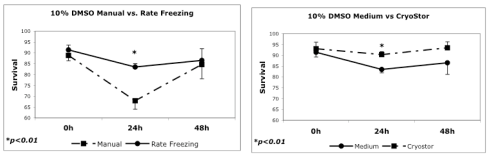
The generation of a neural stem cell bank at BioRep represents a fundamental source of highly controlled biomaterial that fulfil the most stringent standards. Presently BioRep is storing several neural stem cell lines:

- *Mouse and Human Neural derived stem cells* (University of Milan, Italy, Prof. E. Cattaneo)
- *Human Glioblastoma stem lines* (University of Edinburgh, UK, Prof A.Smith)

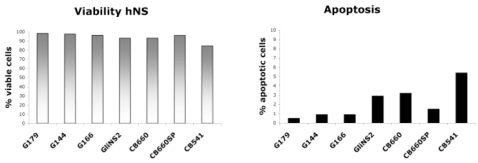
Neural Stem Cells (NSCs) are cultured at high cell density and checked for bacterial, fungal, mycoplasma and viral contamination; cells are maintained in pluripotent status and in active proliferation.



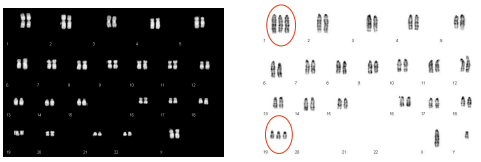
Cryopreservation of NSCs with controlled rate freezer in GMP freezing medium free of animal proteins (Cryostor™ CS10, *BioLife Solutions*) showed superior cell viability compared with standard manual freezing protocol.



We are able to achieve ~90% of viability at 24 hours post-thawing and < 5% of apoptosis in several human Neural Stem Cell lines.



Human NSCs after expansion and cryopreservation maintain a normal karyotype (left panel) while mouse NSCs tend to accumulate chromosomal aberrations after extensive *in vitro* culture (right panel).



Acquisition

Login of sample in the BioRep database and barcoding

Expansion

In vitro culturing (antibiotic-free) up to ~4x10^7 cells performing quality control tests:

- ***Sterility***
- ***Duplication time***
- ***Pluripotency***
- ***Karyotyping***

Cryopreservation

Freezing aliquots of ~1x10^6 cells in glass ampules in Cryostor™ CS10 with a controlled rate freezer and storage in liquid nitrogen

Recovery

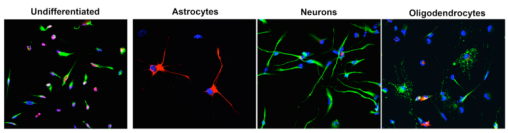
Thawing and in vitro culturing performing quality control tests:

- ***Sterility***
- ***Duplication time***
- ***Viability and Apoptosis level***
- ***Pluripotency***
- ***Differentiation***
- ***Karyotyping***

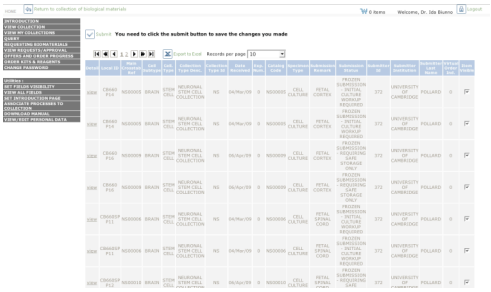
Distribution

Shipment of frozen samples after appropriate MTA approval

NSCs after expansion and cryopreservation maintain their normal capability to differentiate *in vitro* in the three neural types: astrocytes, oligodendrocytes and neurons.



Testing results are published in a report and consultable through our online catalogue (CRB). The CRB is our link with outside users, accessible by protected registration to view and request the material available in the biobank.



Additional Services

CELL MICROARRAY (CMA)
CMA evolved from TMA with the exception that **cells are pelletted**. Cells are grown in culture, fixed, suspended in agarose and embedded in paraffin.

- Immunocytochemistry analysis (IC) of any type of cells
- High-throughput screening of hundreds of cell samples on a single slide
- Several slides can be generated from a paraffin recipient block ready to be assayed with different markers