

# European Bank for induced pluripotent Stem Cells



## About EBiSC The EBiSC Poster Book

Information on EBiSC

[www.ebisc.eu](http://www.ebisc.eu)

Access to the EBiSC iPSC Catalogue

<https://cells.ebisc.org>



innovative  
medicines  
initiative



Contact us

[ebisc@eurtd.com](mailto:ebisc@eurtd.com)

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# EBiSC: Who We Are



**Project Coordinator**  
Tim Allsopp  
(Pfizer-Neusentis, UK)



**Manager of the Public Partners**  
Aidan Courtney,  
(Roslin Cell Sciences, UK)



**Work Package 1**  
**WP1.1** Governance and Policy  
**WP1.3** Communication and  
Dissemination: Aidan Courtney  
(Roslin Cell Sciences, UK) and  
Beate Kreisel (ARTTIC, France)  
**WP1.2** Assessing the Market  
as a whole



**Work Package 2**  
**WP2.1** Procurement and Sustainability  
of iPSC line production: Paul de Sousa  
(Roslin Cell Sciences, UK)



**WP2.2** Central & Secondary  
iPSC Facilities: Julie Holder  
(Roslin Cell Sciences, UK)



**Work Package 3**  
Bio-engineering and Automation:  
Heiko Zimmermann,  
(Fraunhofer Institute for  
Biomedical Engineering, Germany)

- EFPIA companies
- SME's
- Universities, research organisations,  
public bodies, non-profit groups



## Work Package 4

**WP4.1** Ethics & Engagement:  
Responsible Research & Innovation:  
Carol George and  
Shawn Harmon  
(University of Edinburgh, UK)



## WP4.2 Training:

Glyn Stacey  
(National Institute for Biological  
Standards and Controls-NIBSC, UK)



## Work Package 5

Quality Control and  
Characterisation:  
Glyn Stacey (NIBSC, UK)

## Work Package 6

**WP6.1** Storage and Distribution:  
Bryan Bolton (European Collection  
of Cell Cultures-ECACC, UK)



**WP6.2** Validating the End  
Product: Christian Clausen  
(Bioneer, Denmark)



## Work Package 7

**WP7.1/WP7.2** Information  
Management Systems Develop-  
ment and Operation Helen  
Parkinson (EMBL-European  
Bioinformatics Institute, UK)



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# EBiSC: Project Timeline

		Document Details Document Title: Culture and Maintenance of Induced Pluripotent Stem Cells Document Owner: Brite Type Expiration: N/A Status: Final
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## July 2014

- Standard Operating Procedures and Forms for iPSC line production approved
- First commissioning of new iPSC lines for the Foundational Collection is approved

## Oct 2014

First iPSC lines deposited from non-consortium partners

## Aug 2014

- University of Newcastle become the first EBiSC depositor to create and ship a cell line to Edinburgh facility
- EBiSC demonstrates ability to re-consent donors of existing iPSC lines to enable their deposition in the bank

## June 2014

- First Hot Start lines from Roslin Cell Sciences begin expansion in Edinburgh
- Standardised format for cell line nomenclature, tracking and labelling agreed with all parties in Edinburgh



## May 2014

First EBiSC Cell Processing and Quality Control training course and workshop delivered

## Jan 2014

Project kick-off

## March 2014

First 'benchmark' protocols for cell processing distributed to iPSC depositor centres



## Dec 2014

- Gap analysis completed to identify target diseases for development of EBiSC catalogue beyond the Foundational Collection

- Consortium Material Deposit Agreement effective

- EBiSC distributes its first cell lines to Academic and EFPIA industry partners thus demonstrating end-to-end capacity of EBiSC operation

- EBiSC practical training course on hiPSC culturing

## Mar 2015

Cell processing and QC initiated at Babraham Central Facility



## Jan 2015

Fraunhofer-IBMT proving lab established at Babraham

## Nov 2014

The lease for the Babraham Central Facility is agreed and the first employee is recruited

## June 2016

ISSCR 2016 Annual Meeting in San Francisco: EBiSC Focus Session and Meet-up Hub



## May 2016

EBiSC practical training course in iPSC culture, preservation, QC and differentiation

## November 2016

EBiSC training course on reprogramming



## March 2016

EBiSC Catalogue Launch, <https://cells.ebisc.org>



## October 2016

EBiSC booth at the ESGCT/ISSCR International Symposium Florence



## June 2015

ISSCR 2015 Annual Meeting in Stockholm: EBiSC Meet-up Hub

## July 2015

Performance Qualification of Babraham Central Facility complete. Ready for EBiSC production.

## Dec 2016

Integration of development activities for process optimisation



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# EBiSC: Automation Strategies for the Expansion and Cryostorage of iPS cells in EBiSC

## Establishment of EBiSC MirrorStorage Facility (Sulzbach, Germany)



## Establishment of Efficient Cryopreservation Procedures

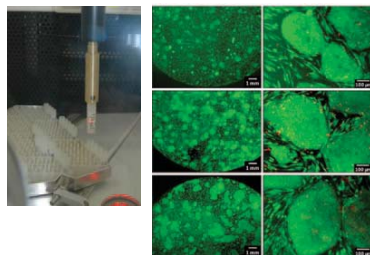
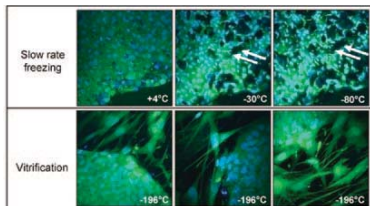
### Surface-based vitrification of hPSCs:

- High viability and recovery
- Maintained pluripotency
- Ready-to-use
- No time delay after thawing

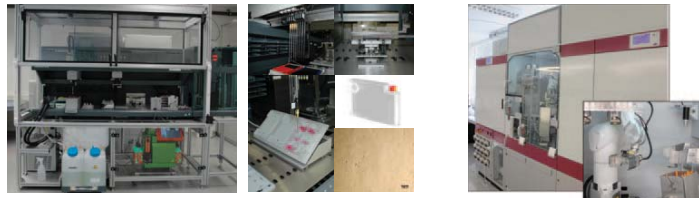


C-line™ system from Askion:

- Fully automated sample storage for closed cold chain
- Storage below -150°C
- Working temperature -80 to -110°C



## Establishment „Automation Enabling Lab“ (Babraham, UK) Automated Technologies For Large-Scale Expansion



### Automated cell culture platforms

Automated pipetting platform Freedom EVO200™ from TECAN adapted to hiPSC-expansion:

- Highly flexible and complex system
- High-throughput capacity with up to 12x Roboflask (93 cm<sup>2</sup>) passaging per 8h
- Complicated and time consuming handling
- Not recommended for complete expansion workflows

The automated cell culture system Compact Select™ from TAP Biosystems:

- Highly reproducible workflow
- Hosting of up to 90 T175 flasks, automated passaging of cells → 48 flasks/24h
- Easy and fast handling
- Recommended for EBiSC



MicroLAB Star™ from Hamilton (@UK Bonn):

- Reliable processing with high reproducibility for walkaway lab automation
- Media changes, cell seeding and coating in MTP (limited to MTP)
- Comprehensive handling due to high system variability
- Recommended for EBiSC (for small/medium scale cell production)



Biolevitator™ and Levitubes™ from Hamilton:

- Robust and reproducible
- Volume of 4x50ml
- Low shear forces
- No upscaling possible
- Further evaluation necessary

### Bioreactors



Magnetic stirrer and spinner flasks:

- Robust and reproducible
- Volume up to several litres
- High shear forces
- No sensors, not automated
- Not recommended for EBiSC



The HexaBatch™ system from HexaScreen Culture Technologies:

- Robust and reproducible
- Volume of 6x15ml
- Medium shear forces
- No upscaling possible
- Not recommended for EBiSC

### Addressing the aims of EBiSC:

- Adaptation of current protocols for scalable iPSC expansion
- Identification and installation of automation for scalable expansion of iPSC
- Establishment of innovative automated technology as e.g. closed cold chain and reliable sample identification
- Establishment of reliable biobanking infrastructure
- Establishment of efficient, standardised and reproducible protocols of high quality cryopreservation of iPSC

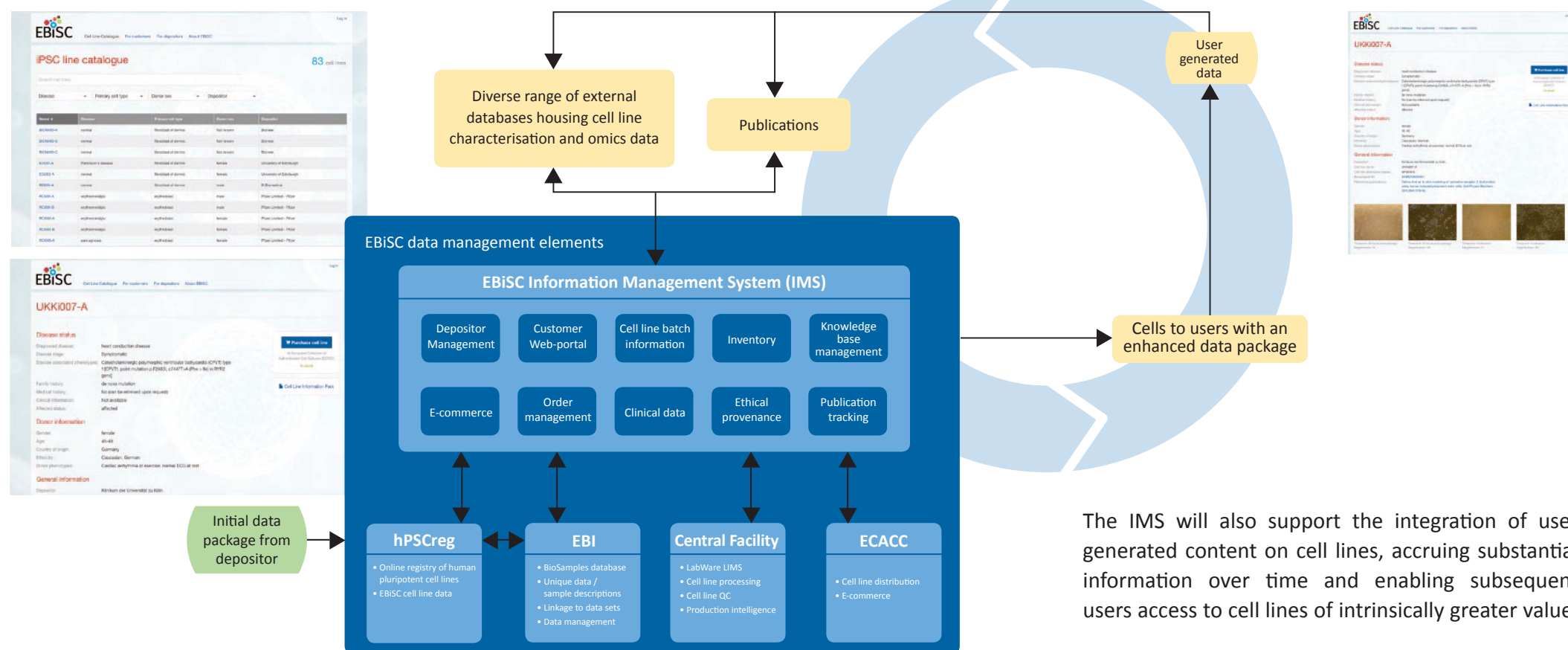
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# EBiSC: Data Management Overview

The EBiSC Information Management System (IMS) seamlessly connects the flow of data across the entire supply chain from cell line deposition through to customer order and supply. It provides a wealth of descriptive information on the cell lines and will also capture user generated data to enrich and add value to a constantly expanding knowledge base for every cell line in the bank.

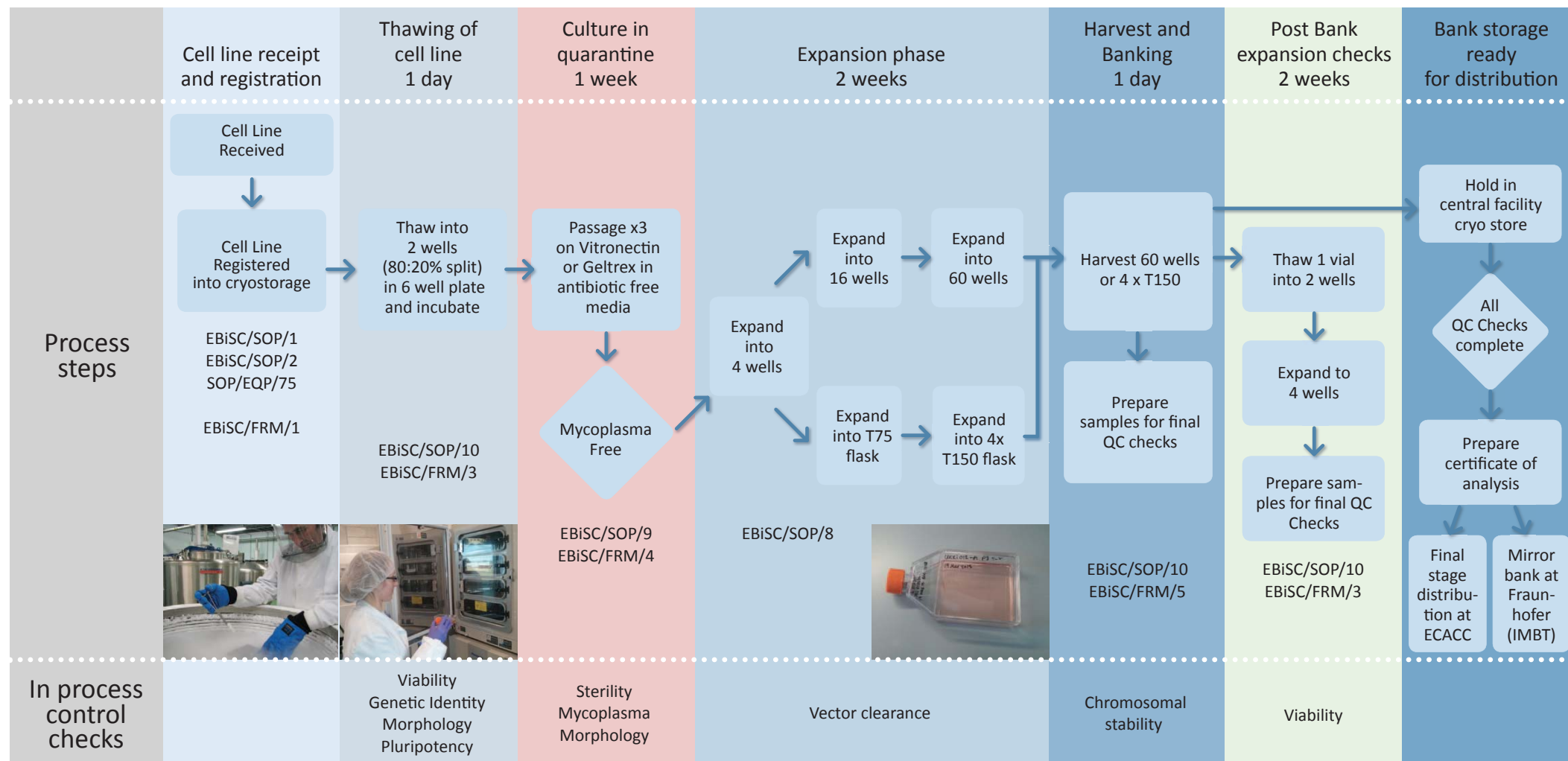


The IMS provides a web-based platform for ease of access that maximises the utility of the EBiSC resource and provides an efficient facility for purchasing cell lines quickly and at reasonable cost.

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# EBiSC: The Banking Process



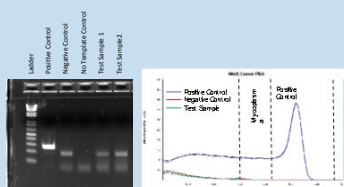


# EBiSC: Quality Control

All cell lines supplied from EBiSC are thoroughly characterised in order to sustain a collection of high quality iPSC lines. Roslin Cell Sciences, the National Institute for Biological Standards and Controls (NIBSC) and EBiSC depositor centres collaborate closely to provide a comprehensive data set for each cell line.

## Sterility

Adventitious contamination of cell lines can seriously affect cell identity, proliferation, morphology and behaviour. All lines processed within EBiSC undergo continuous monitoring for the presence of microbiological agents such as Mycoplasma and bacteria.

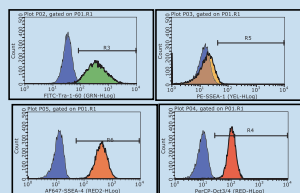


Mycoplasma testing is performed on in-process samples using endpoint PCR and cell banks for distribution are screened using the more sensitive QPCR method.

All lines also undergo broth inoculation and continuous visual assessments to detect non-specific microbiological growth.

## Cell Phenotype

Human pluripotent stem cells have a well characterised phenotype associated with the maintenance of self-renewal. A number of factors contribute to maintaining this phenotype including optimisation of cell culture conditions.



Within EBiSC, cell line phenotype is assessed by quantifying the expression of self-renewal markers SSEA4, TRA-1-60, OCT3/4 and a lack of SSEA1 expression using flow cytometry. Morphology and confluency checks are also carried out on a daily basis by visual inspection to assess colonies for tight, defined borders and smooth surfaces.

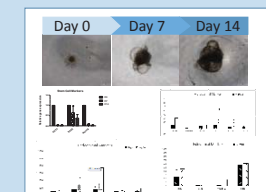
## Next Generation

Assessing colony morphology and confluency by eye is time-intensive and, even when performed by an extensively trained operator, is subject to human bias giving variable results. EBiSC will seek to adopt new technologies such as automated imaging systems which will allow far greater standardisation and higher throughput.



## Pluripotent Potential

Pluripotency is defined as the ability for a cell line to form all post-embryonic lineages. This is determined within EBiSC by spontaneous differentiation of iPSC lines to early germ layer populations. Quantitative PCR is used to assess the expression levels of early germ layer markers, with up-regulation indicating functional pluripotency.



## Next Generation

EBiSC is actively evaluating alternative technologies to assess pluripotent potential, for example, the use of microfluidic array plates.

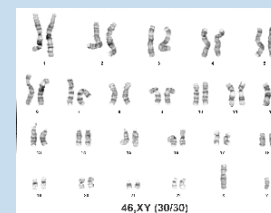
## Next Generation

EBiSC will evaluate and adopt new technologies such as KaryoLite BoBs which offers increased throughput over G-Banding and is more cost effective.

## Chromosomal Stability

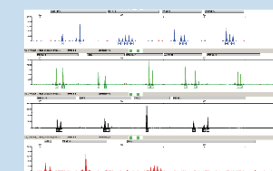
Chromosomal abnormalities such as deletions or insertions of genetic material may affect cell identity, proliferation and response to stimuli.

Within EBiSC, karyology by G-banding is used to visualise chromosomal structure and to detect genomic abnormalities such as inversions, deletions, duplications and translocations.



## Genetic Identity

EBiSC will assess and record the genetic identity of each iPSC line to detect and prevent the spread of any cross contamination of cell lines. This will be done by recording a genetic fingerprint for each line by assessment of Short Tandem Repeats (STRs). STR loci are varied numbers of sequence repeats found in highly polymorphic stretches of DNA. They are detected by amplification of a number of these loci and subsequent high resolution, size-based separation of amplicons.



As part of the EBiSC Quality Control regime a variety of cell line related data is also recorded or generated where required. This can include:

- Donor information and disease background
- Cell processing protocols and reprogramming methodology
- Nomenclature and identities for traceability
- Confirmation of genetic abnormalities

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## Central EBiSC Operations

Create banks of ~ 50 vials  
1-2 million cells per vial

hESCreg name — Name: WTSli071-A  
EBiSC internal name — Batch: P001  
Depositor's Cell Line ID — Cat.Nr: 66540183  
2D Barcode encoding vial —  
Specific BioSamples ID —  
Vial specific BioSamples ID — SAMEA4093990



## Item TRACKER Software

- Sample management software that allows reliable tracking of vial location

## Shipping of vials

- Use of validated dry shippers
- Cooling to -150 maintained for at least 10 days
- Data logger included to monitor maintenance of correct temperature
- Wide neck ensures whole boxes can be loaded without the need to manually pick vials into different containers

## Retrieve vials

- 2 vials kept at Roslin Cell Sciences as back up
- The bulk is split between two final cryopreservation facilities

90 %

10 %

## Final Storage and Distribution at ECACC



## Automated Cryopreservation at EBiSC Mirror Bank – Fraunhofer IBMT



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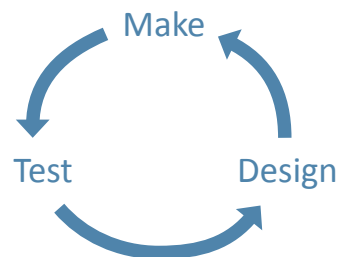
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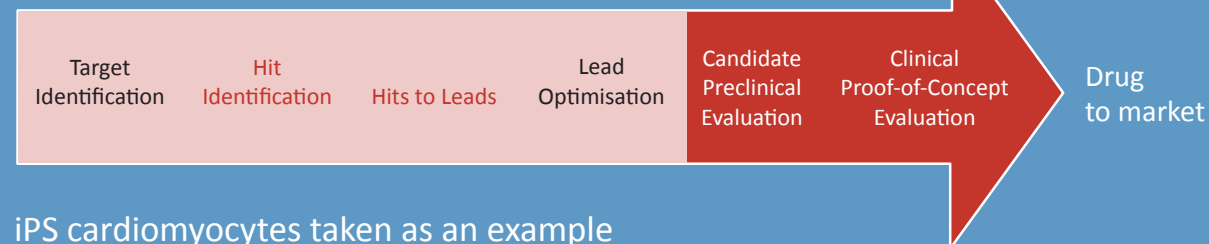


# EBiSC: Utilisation of iPSCs in Drug Discovery and Development

## Cycle of drug manufacture



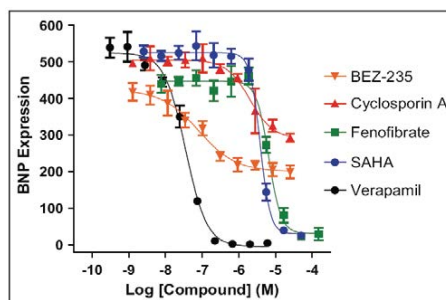
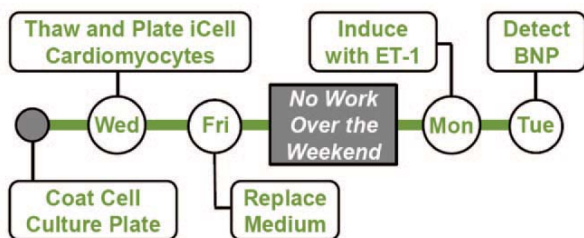
## iPSC utilisation



## Aspirational goal for iPSC in R&D

- in vitro assay
- Predictive of risk in man
- Capable of driving SAR
- Short loop time
- Low cost
- Avoid animal studies

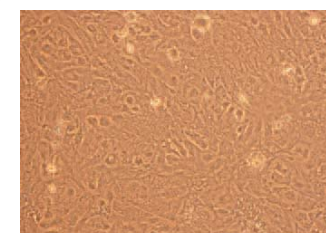
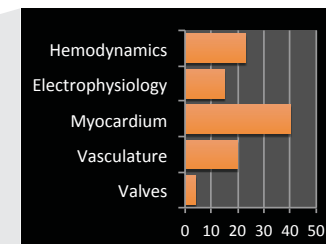
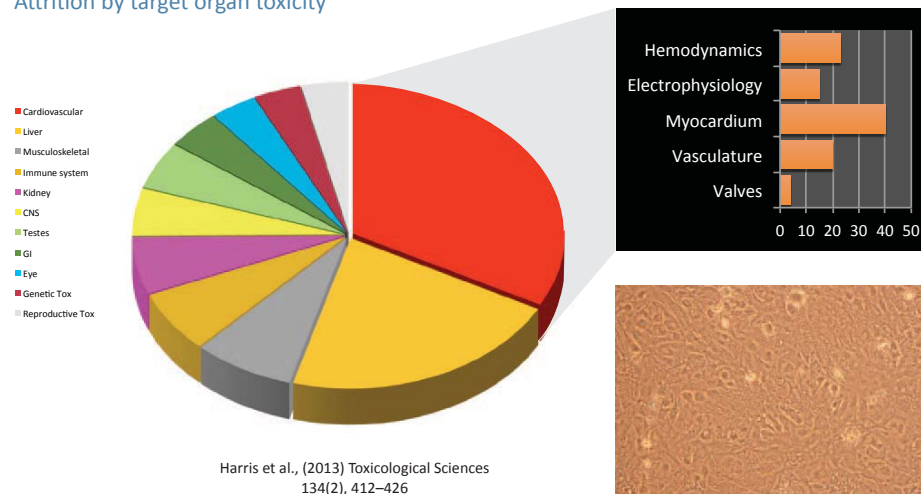
## Utilisation of iPSCs in R&D efficacy assays



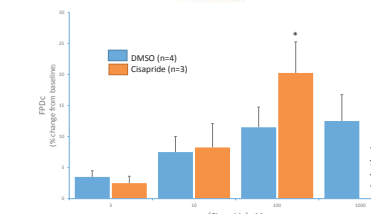
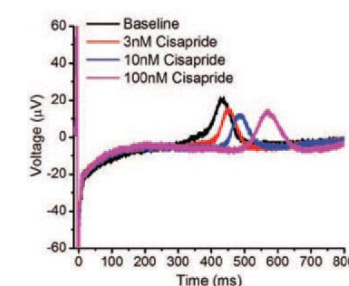
Carlson et al., (2013)  
J. of Biomolecular  
Screening 18  
1203-1211

## Utilisation of iPSCs in R&D toxicity assays

Attrition by target organ toxicity



## Dose response of cisapride in iPS cardiomyocytes

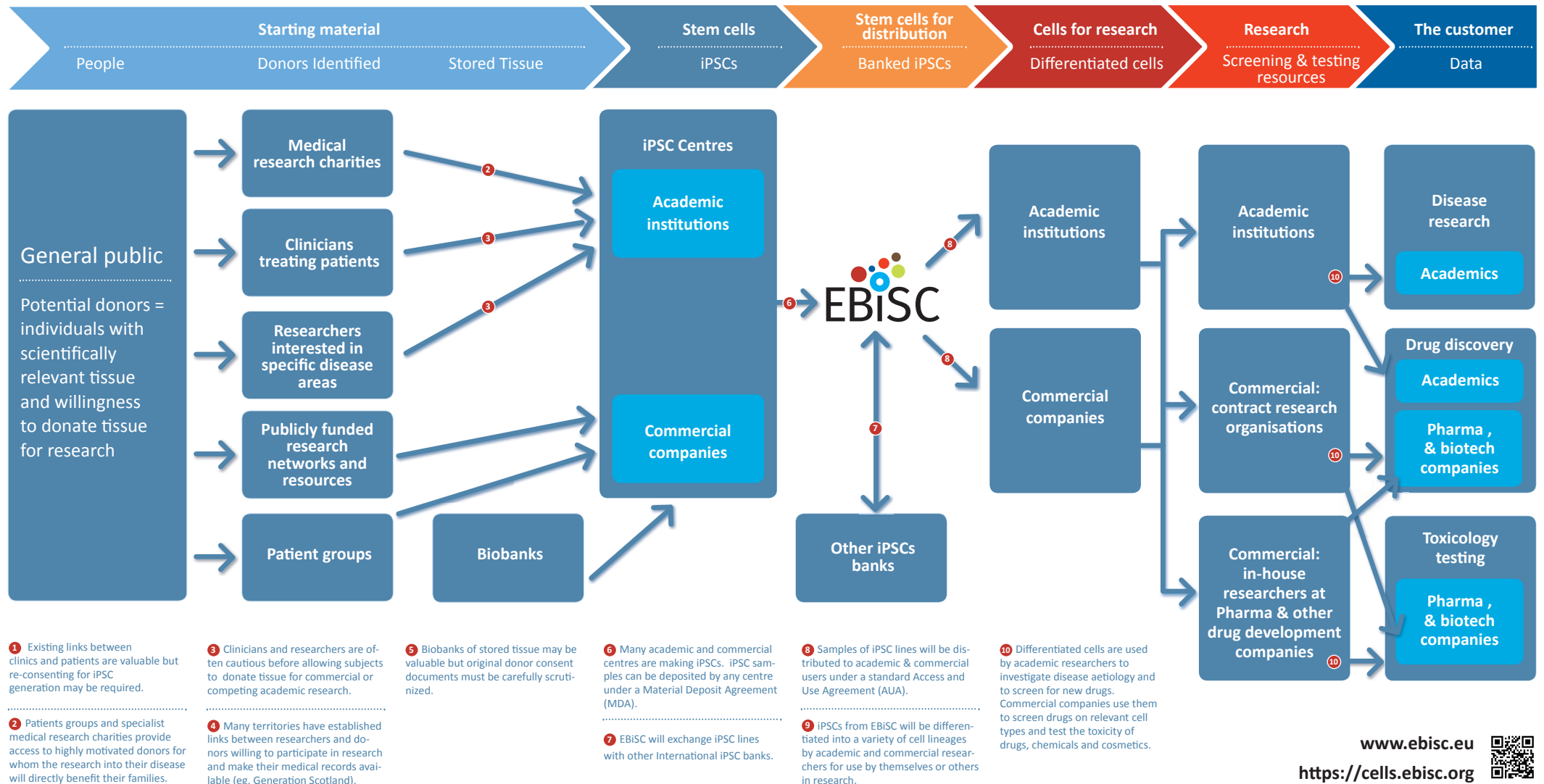


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# EBiSC: Supply chain



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<https://cells.ebisc.org>



# The EBiSC Catalogue

EBiSC launched its online catalogue of induced pluripotent stem cells (iPSCs) on 23 March 2016. The iPSC lines deposited by EBiSC project partners as well as leading external iPSC labs are available to academic and commercial scientists for use in disease modelling and other forms of pre-clinical research. The growing catalogue is being added to in three phases which shall in future cover a large number of disease representative and control lines.

## Phase One (white):

The Foundational collection, available now.

## Phase Two (yellow):

Existing lines being deposited with EBiSC by collaborating European projects such as Stem-BANCC, HipSci and the Oxford PD Centre.

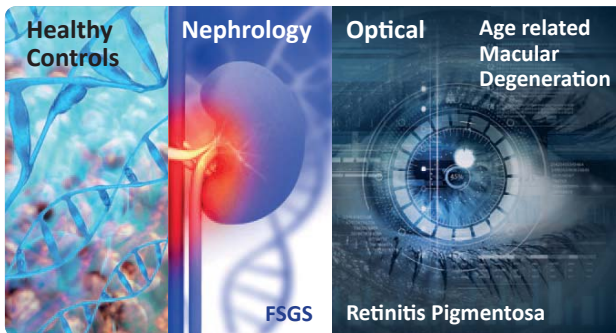
## Phase Three (orange):

New lines being reprogrammed or gene edited through activities funded by the IMI and supported by EFPIA companies.

## USERS

The growing catalogue can be viewed online at: [cells.ebisc.org](http://cells.ebisc.org)

Most lines are available immediately once the purchasing organisation has agreed to the EBiSC Access & Use Agreement.



## Parkinson's

### SNCA Triplication

**SNCA (A53T)**

**GBA (N370S)**

**GBA (L444P)**

**Lrrk2 G2019S**

**Sporadic PD**

## Huntington's

## Alzheimer's

### ApoE Isogenics

**(E4/4, 3/3, 2/2, KO)**

**MAPT (P301S)**

**MAPT (P301L)**

**CD33 (ex2 del)**

**APP (V717I)**

**TREM-2 (R47H)**

**PSEN1 (M139V)**

**PSEN1 (Y115C)**



## Autism

## Migraine

## Epilepsy

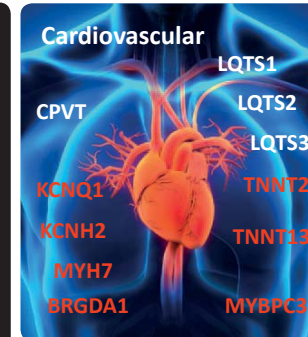
## Bipolar

## Schizophrenia

**TARDBP**  
**C9ORF72**  
**Progranulin**  
**Tau splicing mut.**

**FTD & ALS**

**Machado-Joseph**



## Cardiovascular

**LQTS1**

**LQTS2**

**LQTS3**

**CPVT**

**KCNQ1**

**KCNH2**

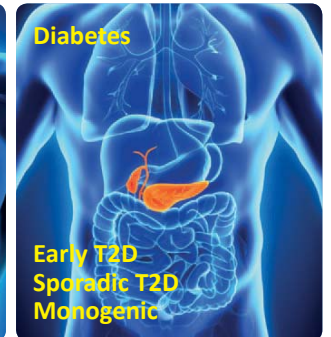
**MYH7**

**BRGDA1**

**TNNT2**

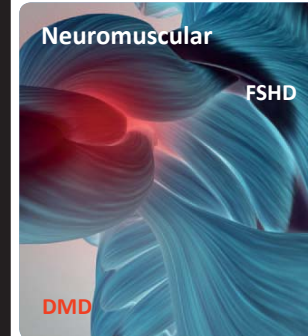
**TNNT13**

**MYBPC3**



## Diabetes

**Early T2D**  
**Sporadic T2D**  
**Monogenic**



## Neuromuscular

**FSHD**

**DMD**



## Neuropathy

**TRESK**

**GARS**

**SCN9a**

**TRPA1**

**HSP27**

**CIP**

## DEPOSITOR benefits

- Single Material Transfer Agreement
- Backup storage in case own stocks compromised
- EBiSC Quality Assured banking
- Data archiving
- Maintenance of line ownership
- Raised profile for international collaborations

<https://cells.ebisc.org>

