EBiSC

European Bank for induced pluripotent Stem Cells

# European Bank for Induced Stem Cells

The EBiSC - European Bank for induced pluripotent Stem Cells project has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115582, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. www.imi.europa.eu

efpia
European Federation of Pharmaceutical Industries and Associations

imi
Innovative Medicines Initiative

# Today's lesson…….

- EBiSC
  - What is it?
  - What has been done so far?

- Industrialisation of hiPSC
  - The things we need to remember.
  - The things we need to do (exemplar).

# Mission

EBiSC

## Standardise hiPSC

- Procurement
- Banking
- Quality Control
- Distribution

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# At a Glance – the Public Private Partnership

http://www.ebisc.org

- Pfizer co-ordinates
- Roslin Cells Ltd manages

- Janssen Pharmaceutica NV
- UCB Biopharma SPRL
- H. Lundbeck A/S
- Novo Nordisk A/S
- AstraZeneca AB
- Bayer
- University of Edinburgh
- Charite University Medicine Berlin
- University of Newcastle Upon Tyne
- Klinikum Der Universitaet Zu Koeln
- The Hubrecht Institute
- University College London
- Universitaetsklinikum Bonn

- WT Sanger Institute

- ARTTIC
- Definigen Ltd
- Bioneer A/S
- Douglas Connect

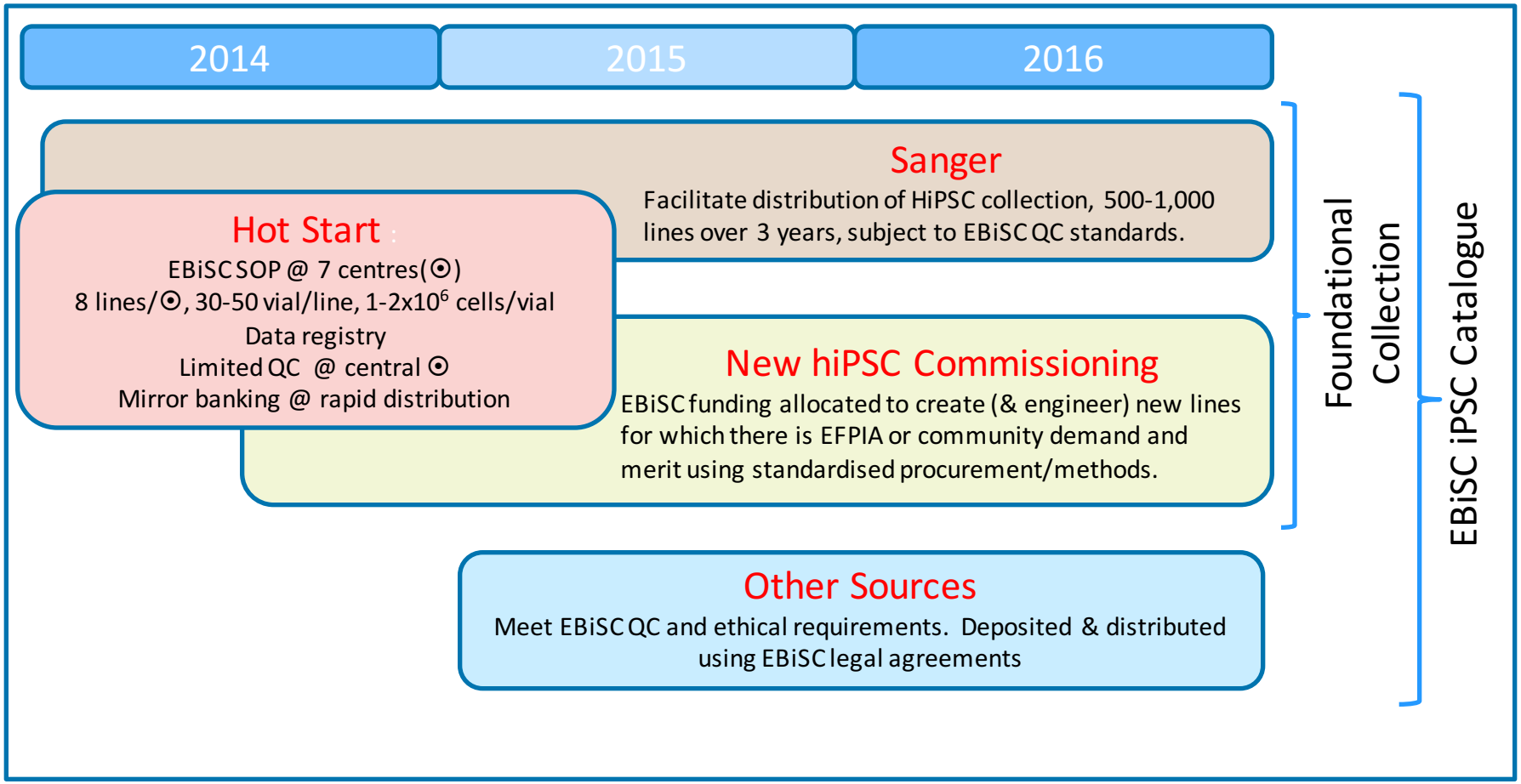
- Fraunhofer
- EMBL – EBI
- Dept. Health
- Instituto de Salud Carlos III


**CO – Dr Tim Allsopp**

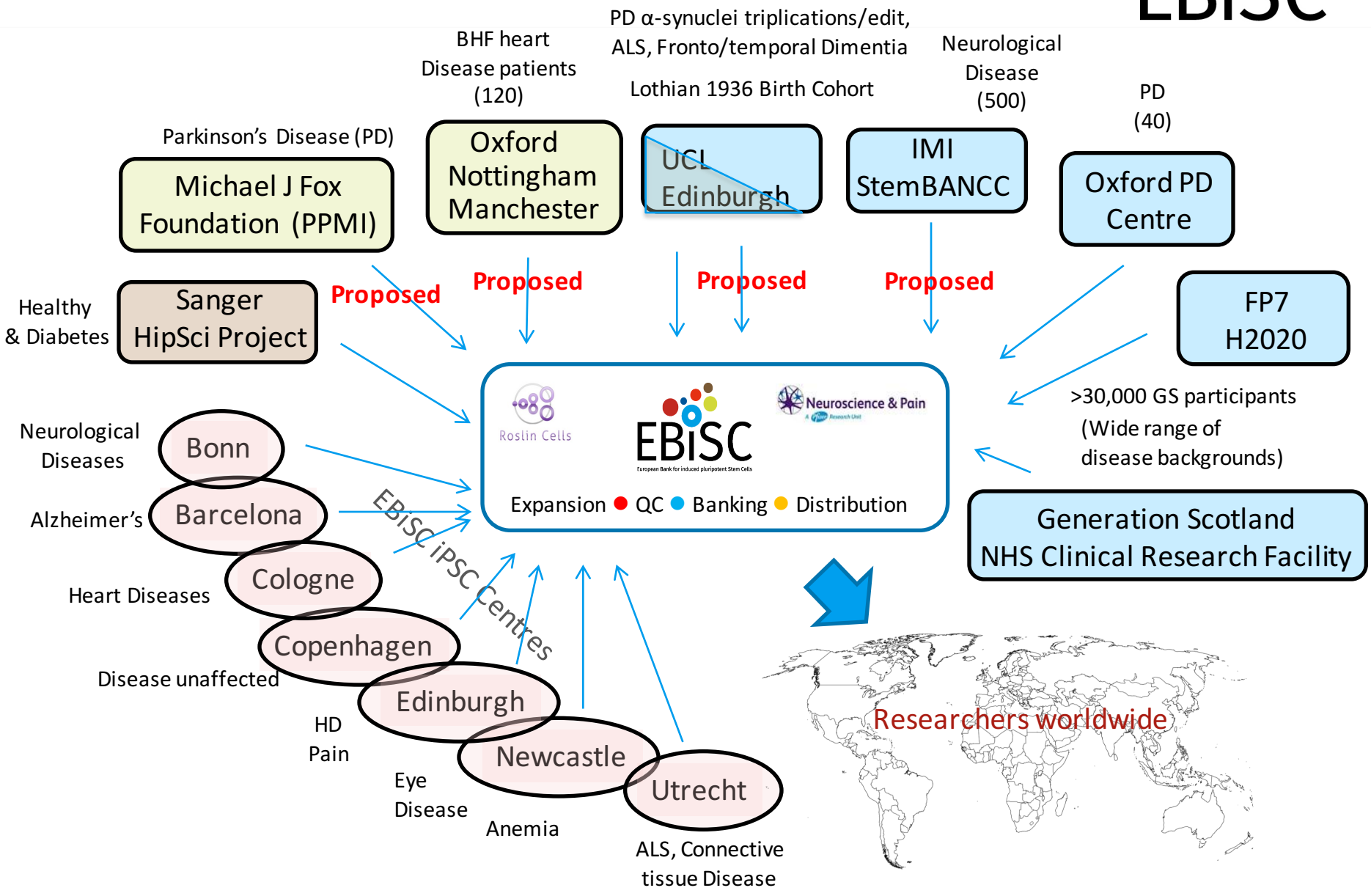
Pfizer

Neusentis

# Establishment of a Foundational Collection & Catalogue



# Disease Coverage & Catalogue Expansion to Date



# Foundational collection: Why Hot Start ?

**The EBiSC Catalogue:**


- Getting “wheels on”→ best practice via experience
- Procurement
  - No commercial restrictions.
  - Ethical prinicples.
- Centre characterisation data (hESC-Reg)
- Standardised expansion/cryopreservation/qualification.

# The Hot Start Process – *to get the wheels on*

EBiSC

**Clinic**

**At Derivation Centres**

**(Procurement)**

**Acceptance criterion & SOPs agreed/issued**

- PI&C check
- 1º tissue processing
- Donor data capture

(Traceable annonymity)

**(Derivation) Expansion**

- SOPs reinforced with training
- Feeder free
- Vitronectin or Geltrex
- E8 or mTESR1
- Passaging with EDTA

**Banking**

**Guidance Documents**

- Cryoprotectant
- At harvest cells should pooled
- Vials should be filled in numerical order
- Labels provided from RC


**Cell line data**

**Forms**

- Original data form
- General cell line information
- Banking and testing form
- Batch specific QC information

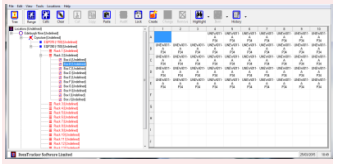
# The Hot Start Process

## At EBiSC Central

### Cell line receipt

Cell Line Registered into cryostorage

EBiSC/SOP/1
EBiSC/SOP/2
SOP/EQP/75
EBiSC/FRM/1



### Thawing of cell line

Thaw into 2 wells of a 6-well plate

EBiSC/SOP/10
EBiSC/FRM/3

- Viability
- Genetic Identity
- Genetic integrity
- Morphology

### Culture

Passage x3 in antibiotic free media

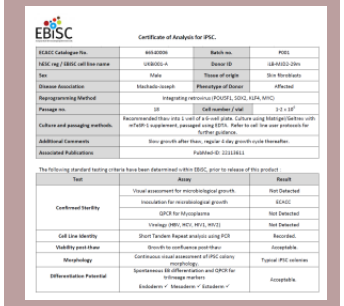
EBiSC/SOP/9
EBiSC/FRM/4
EBiSC/FRM/6

- Sterility
- Mycoplasma
- Virology
- Microorg. Growth
- Pluripotency

### Limited QC + data from ⊙

Collate all data and prepare CoA

EBiSC/FRM/14

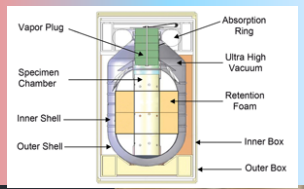


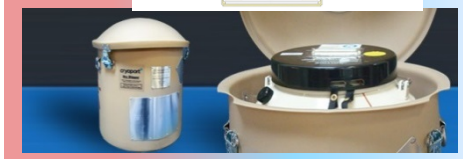

## Shipment

### Transfer of cells

Ship banks to final storage using dry shippers

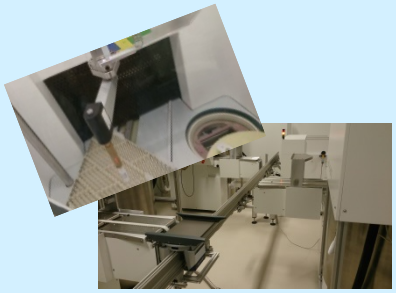
Hold 2 vials at RC as backup



# The Hot Start Process

## Final Storage



Fraunhofer IBMT

Mirror bank at Fraunhofer (IMBT)
~10 % of bank

ECACC European Collection of Cell Cultures
Operated by Public Health England

Final stage distribution at ECACC
~85 % of bank

## Customer

**Customers access EBiSC catalogue via IMS & ECACC**


Pfizer

JANSSEN PHARMACEUTICAL LTD

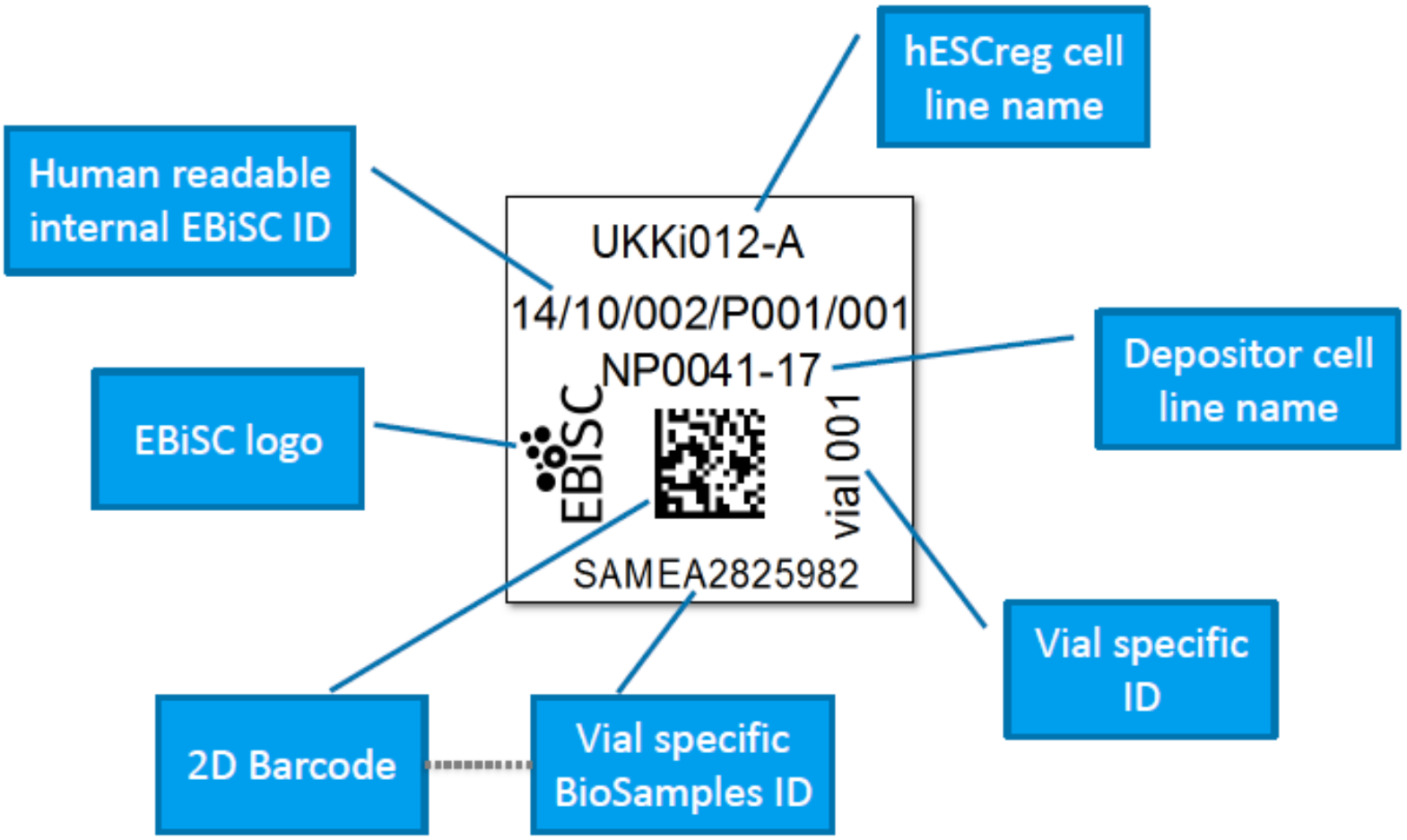
novo nordisk®

AstraZeneca

Lundbeck

ucb Pharma

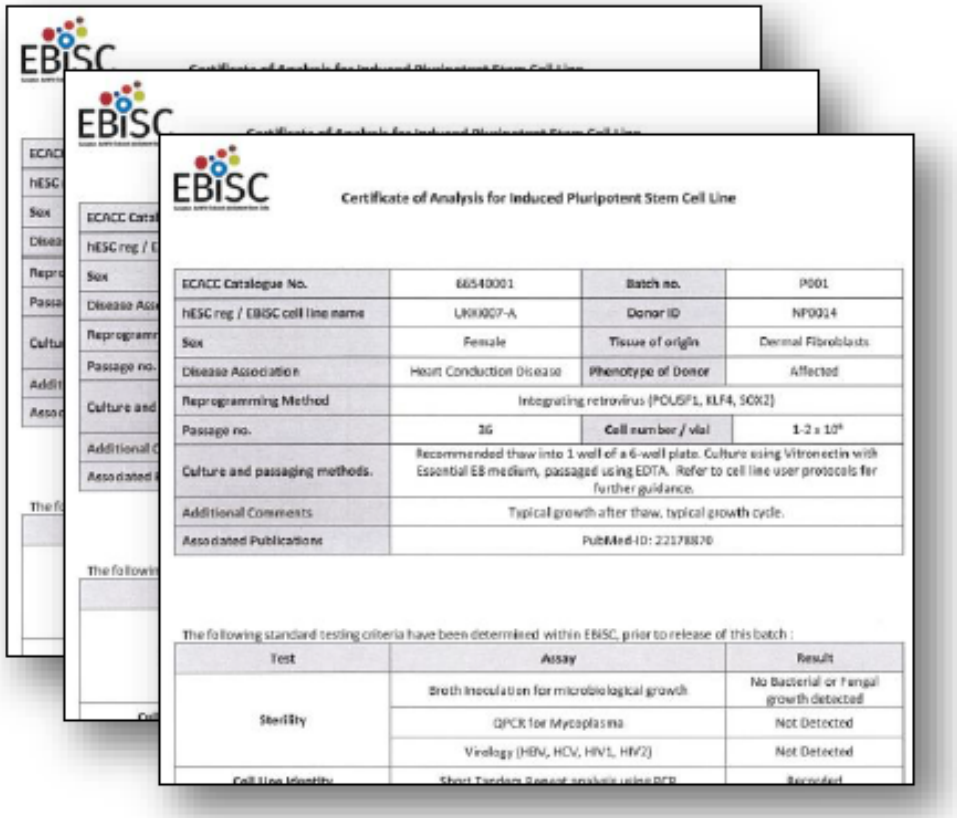
# Data management – labelling & asset tracking



# How do customers benefit?

## *Cell Line & Data Assets*

- Vial of cells
- Access and Use Agreement
- Terms and conditions
- Instructions for use
- Culture manuals, SOPs
- Certificates of analysis
- Clarification on QC applied
- Managed access to data
- Customer feedback system



Certificate of Analysis for Induced Pluripotent Stem Cell Line

| | | | |
|---|---|---|---|
| ECACC Catalogue No. | 66540001 | Batch no. | P001 |
| hESC reg / EBiSC cell line name | UKKi007-A | Donor ID | NP0014 |
| Sex | Female | Tissue of origin | Dermal Fibroblasts |
| Disease Association | Heart Conduction Disease | Phenotype of Donor | Affected |
| Reprogramming Method | Integrating retrovirus (POU5F1, KLF4, SOX2) | | |
| Passage no. | 16 | Cell number / vial | 1-2 x $10^6$ |
| Culture and passaging methods. | Recommended thaw into 1 well of a 6-well plate. Culture using Vitronectin with Essential E8 medium, passaged using EDTA. Refer to cell line user protocols for further guidance. | | |
| Additional Comments | Typical growth after thaw, typical growth cycle. | | |
| Associated Publications | PubMed ID: 22178870 | | |

The following standard testing criteria have been determined within EBiSC, prior to release of this batch :

| Test | Assay | Result |
|---|---|---|
| Sterility | Broth inoculation for microbiological growth | No Bacterial or Fungal growth detected |
| | QPCR for Mycoplasma | Not Detected |
| | Virology (HBV, HCV, HIV1, HIV2) | Not Detected |
| Cell Line Identity | Short Tandem Repeat analysis using PCR | Recorded |

# The Hot Start – State of Play

| Number of Centres that have: | All 8 lines | 3-7 lines | 0-2 lines |
|---|---|---|---|
| Specified contribution | 6 | 1 | 0 |
| Sent lines to EBiSC central (RC) | 4 | 3 | 0 |
| Completed Depositor forms | 4 | 2 | 1 |
| Completed Banking & Testing forms | 4 | 3 | 0 |
| Supplied P&IC information | 1 | 3 | 4 |
| Signed EBiSC MDA | 3 | 0 | 4 |

# Culturing Cell Lines at Roslin Cells: Our experience

| Performance of 47 Hot Start cell lines processed so far | | | |
|---|---|---|---|
| **Recovery after thaw** | **Recovered well** | **Were difficult to recover** | **Were not viable** |
| | 41 | 5 | 1 |
| **Cell morphology** | **Very good iPS morphology** | **Good iPS morphology** | **Poor iPS morphology** |
| | 26 | 14 | 7 |
| **Levels of differentiation in culture** | **Low to Medium** | **High differentiation** | |
| | 40 | 7 | |
| **Sterility issues** | **Had no issues** | **That were contaminated** | |
| | 44 | 3 | |
| **Cell identity issue** | **Had no issues** | **Wrong identity** | |
| | 40 | 7 | |

# The Hot Start – Lessons learned

- Depositor concerns re: competitor access.
- √ Feeder-free culture conditions.
- Training → SOP compliance.
- √ Biosample ID label.
- Interface with hESCreg and EBI.
- √ Laboratory Information Management System.
- Member state restrictions on hiPSC distribution.

# The Hot Start - EBiSC Control Lines

| hESC reg | UKKi012-A | UKBi005-A |
|---|---|---|
| Local cell line ID | NP0041-17 | LB-31-rl |
| Derivation centre | Cologne | Bonn |
| Disease / Control | Healthy Control | Healthy Control |
| Gender | Male | Female |
| Reprogramming method | Episomal | Retroviral |
| No. vials currently banked. | 30 | 70 |
| HotStart | Initiated 24-Feb-15. EBiSC QC in process. | Expanded bank completed. EBiSC QC in process. |
| Derivation centre QC | Submitted, some aspects still in progress. | Data in hESCreg |
| Comments | Initial HotStart shows typical morphology and growth. | Female control line, previously requested by WP6.2. |

Well characterised lines for use within the EBiSC consortium to establish consortium-wide SOPs and as a basis for gene editing to generate novel isogenic disease lines

# RBi001-A control line

| Test | Result |
|---|---|
| **Derivation centre** | R-Biomedical |
| **Disease** | Healthy control |
| **Gender** | Male |
| **Reprogramming method** | Episomal |
| **Passage number** | P17 |
| **Post-bank Inoculation for microbiological growth.** | Not contamination detected |
| **Post-bank Mycoplasma QPCR** | Not mycoplasma detected |
| **Viral Screening (HIV1, HIV2, HEP-B and HEP-C)** | Negative |
| **Viability post-cryopreservation (post-bank)** | Pass |
| **Differentiation Potential** | Neuronal lineage confirmed |
| **Phenotype** | Normal |
| **Post-bank morphology (EBiSC/FRM/2)** | Normal |
| **Post-bank STR** | Recorded |
| **Post-bank Flow Cytometry (Roslin Cells)** | Pass |
| **STR match between fibroblasts and post-bank thaw.** | Pass |
| **Karyology** | Normal |
| **Episomal Clearance** | Clear |

# hiPSC -Underpinning Presumptions

EBiSC

- Cell/tissue functional maturity.

  *Embryonic vs adult equivalence?*

- Physiological and disease relevance

  *Factor co-dependence? Environment, age?*

- Practical value

  *Prospective? Retrospective? Corrective?*

- Cost/benefit value

  *Efficacy? Affordability?*

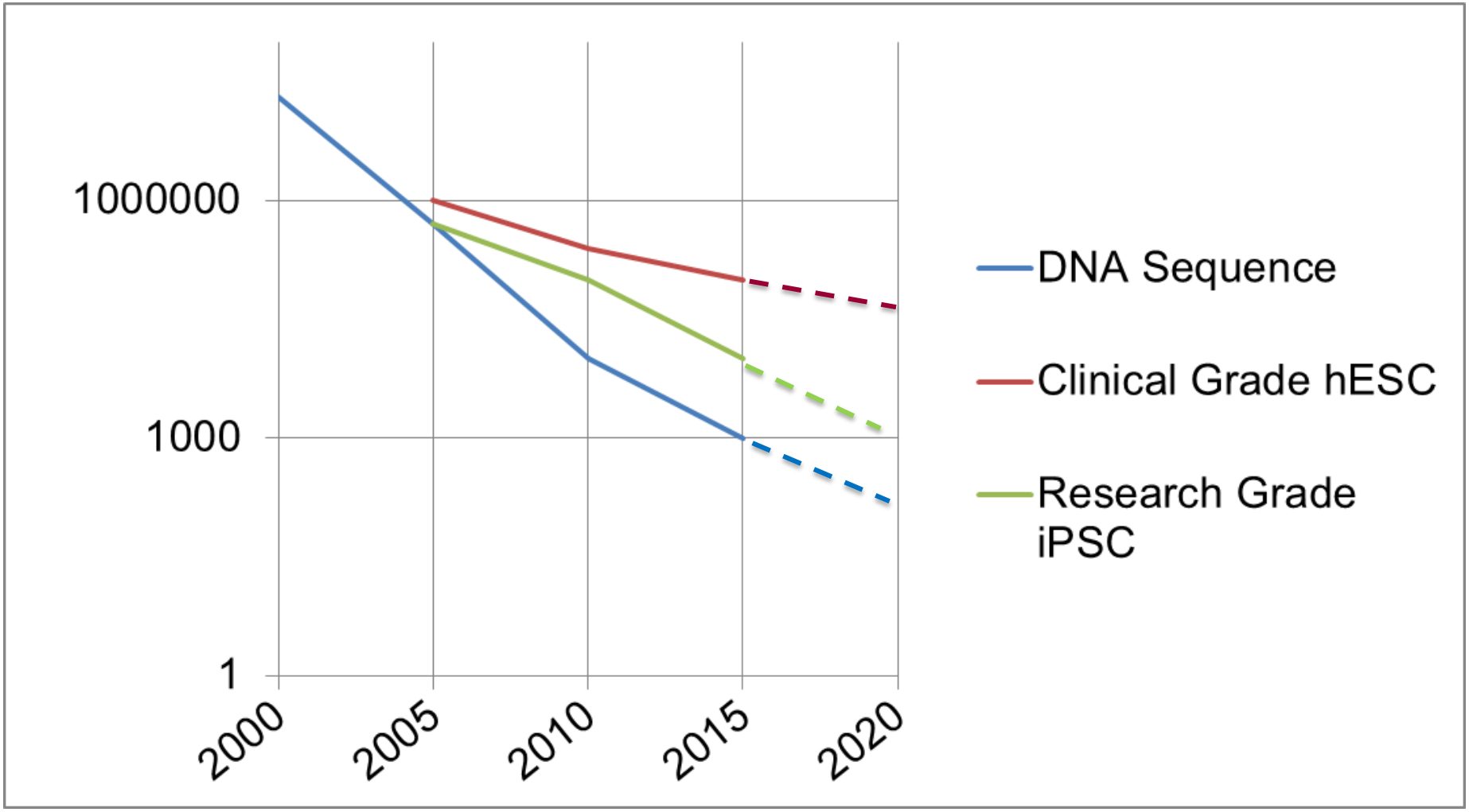
# Which of us is representative enough, and when?

*"The People's Monarch"*
*By Helen Marshall*
*Pier II Gatwick South Terminal*

What do we need to do…..?

# Need to drive cost out of hiPSC production....

EBiSC



# Development of industrialised screening (On the road)

EBiSC

DropTech®.eu

SEVENTH FRAMEWORK PROGRAMME

European Commission

**Aim:** Establishment of an automated hanging drop (HD) human iPSC based version of a mouse ES test (EST) for developmental toxicity.

(Seiler & Spielmann 2011, Nature Protocols 6; 961)

**CO:** Prof. Heiko Zimmermann

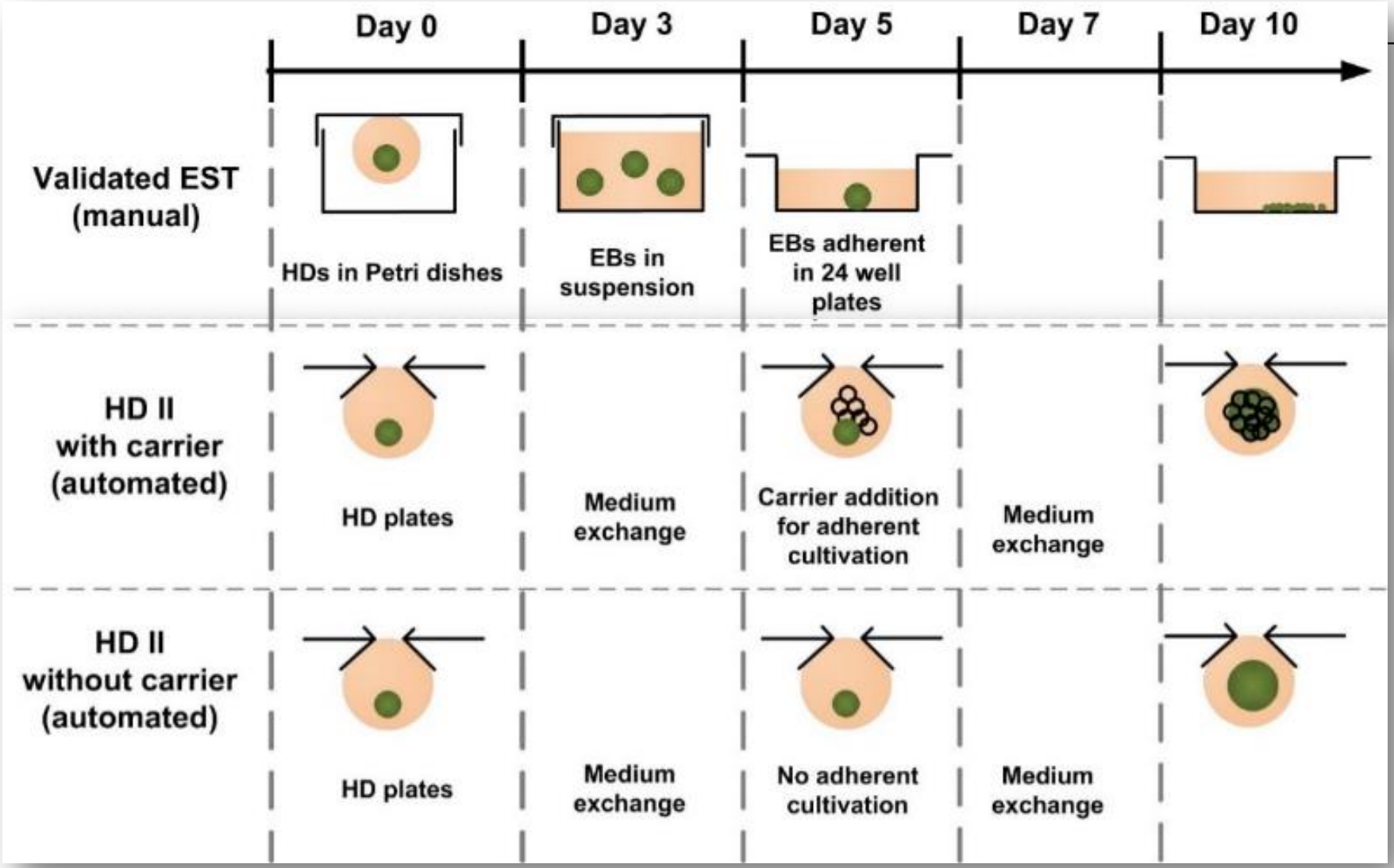
Fraunhofer IBMT

Fraunhofer IME-Screening Port

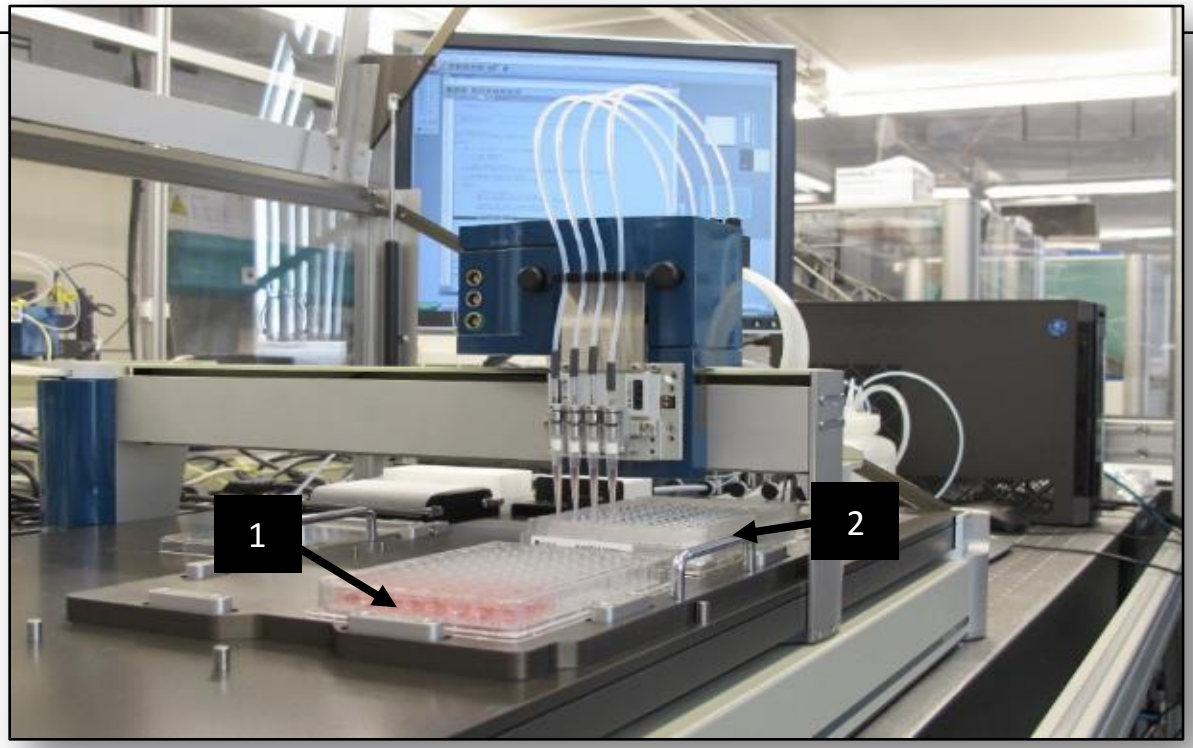
RoslinCellab

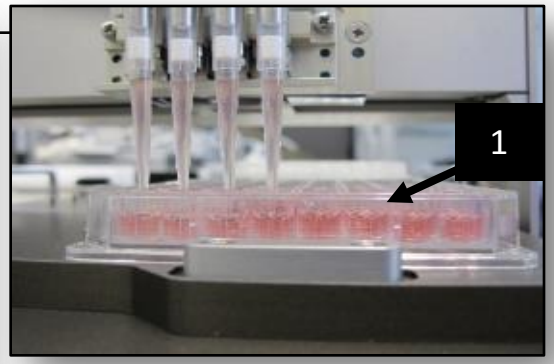
ARTTIC

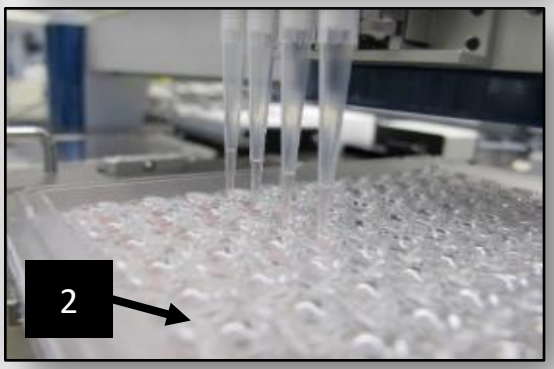
# Workflow of EST in HDs




Courtesy Dr Ina Meiser / Prof. Heiko Zimmermann



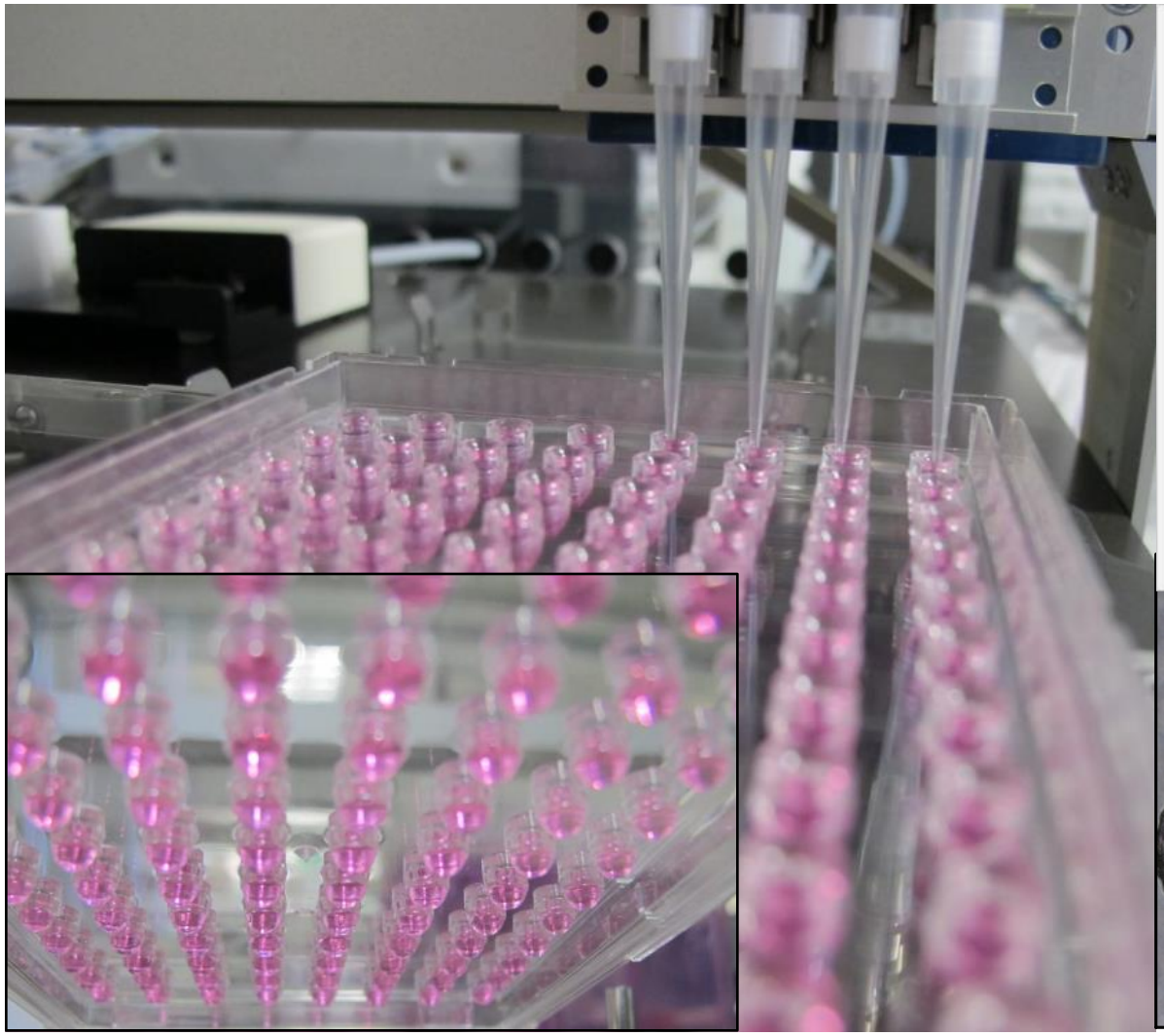



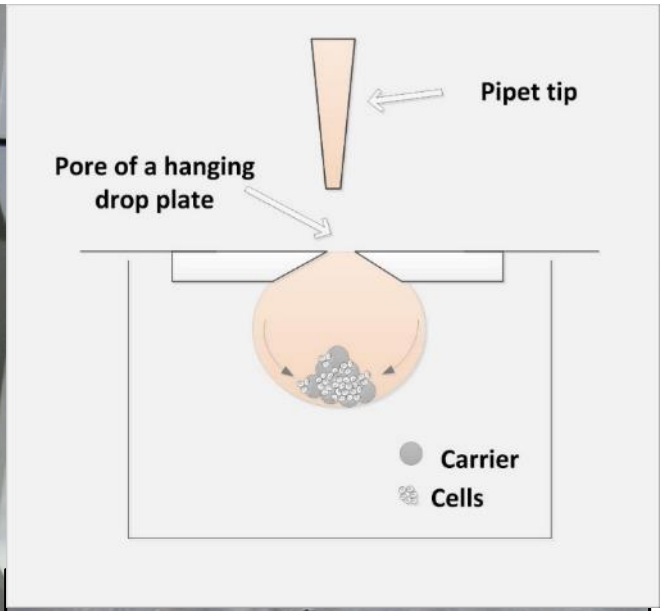

4-channel pipet robot operating an HD plate

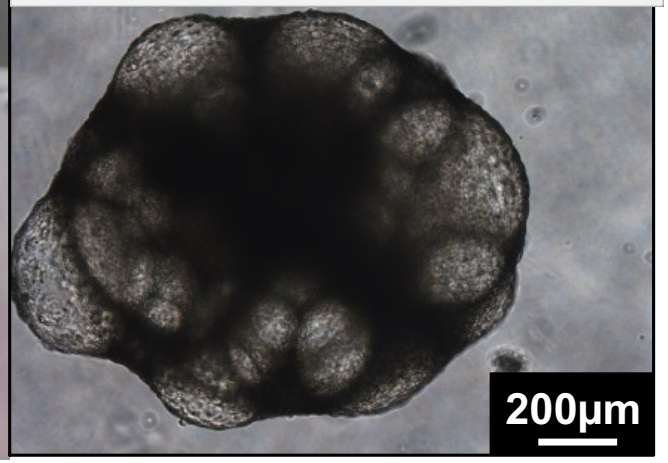
(1) Feed plate

(2) HD plate

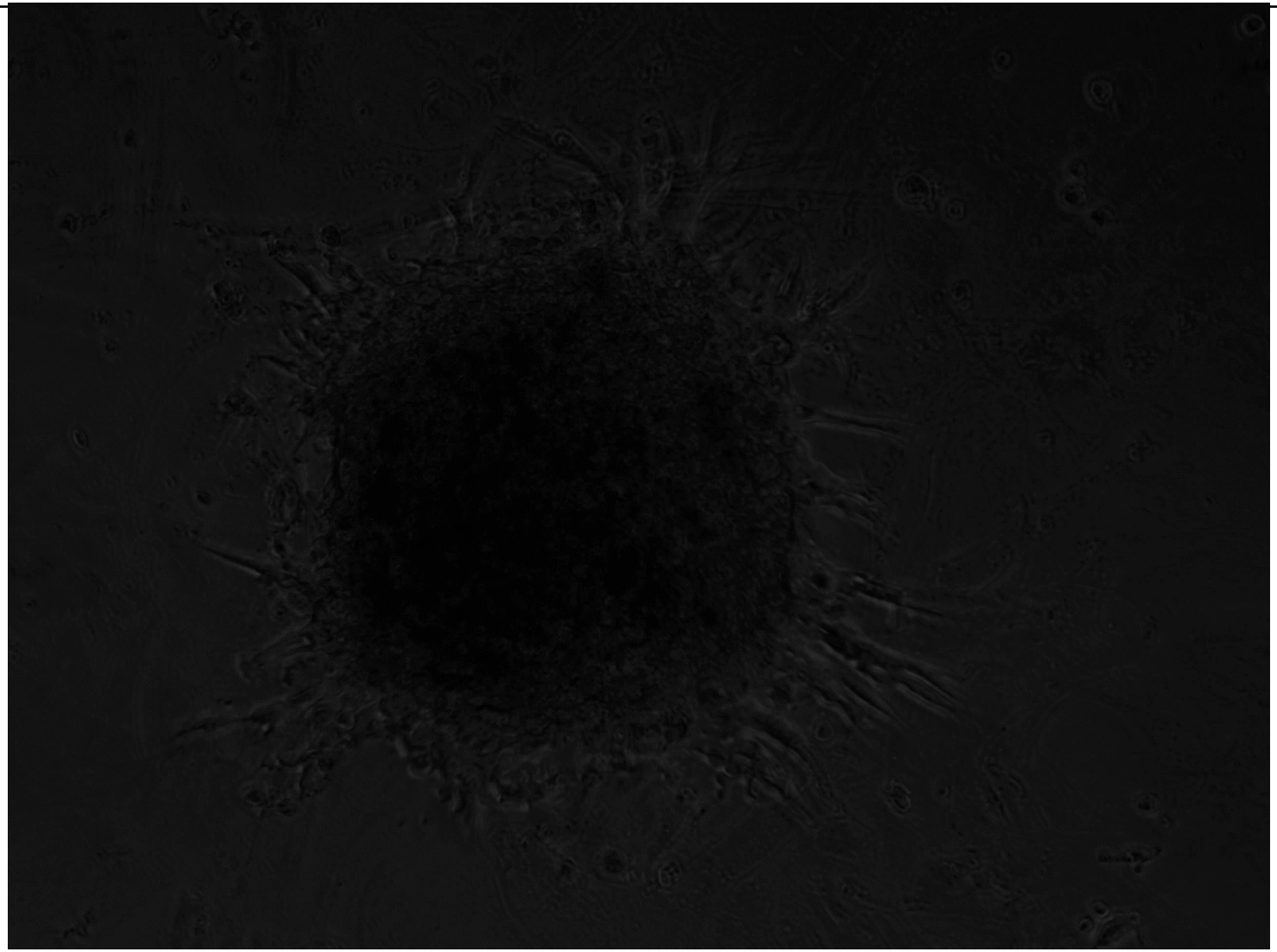
# 4-Channel Nanoplotter 2.1 operating Perfecta-3D plates (3D Biomatrix)





# HiPSC derived Cardiomyocyte-like outgrowth

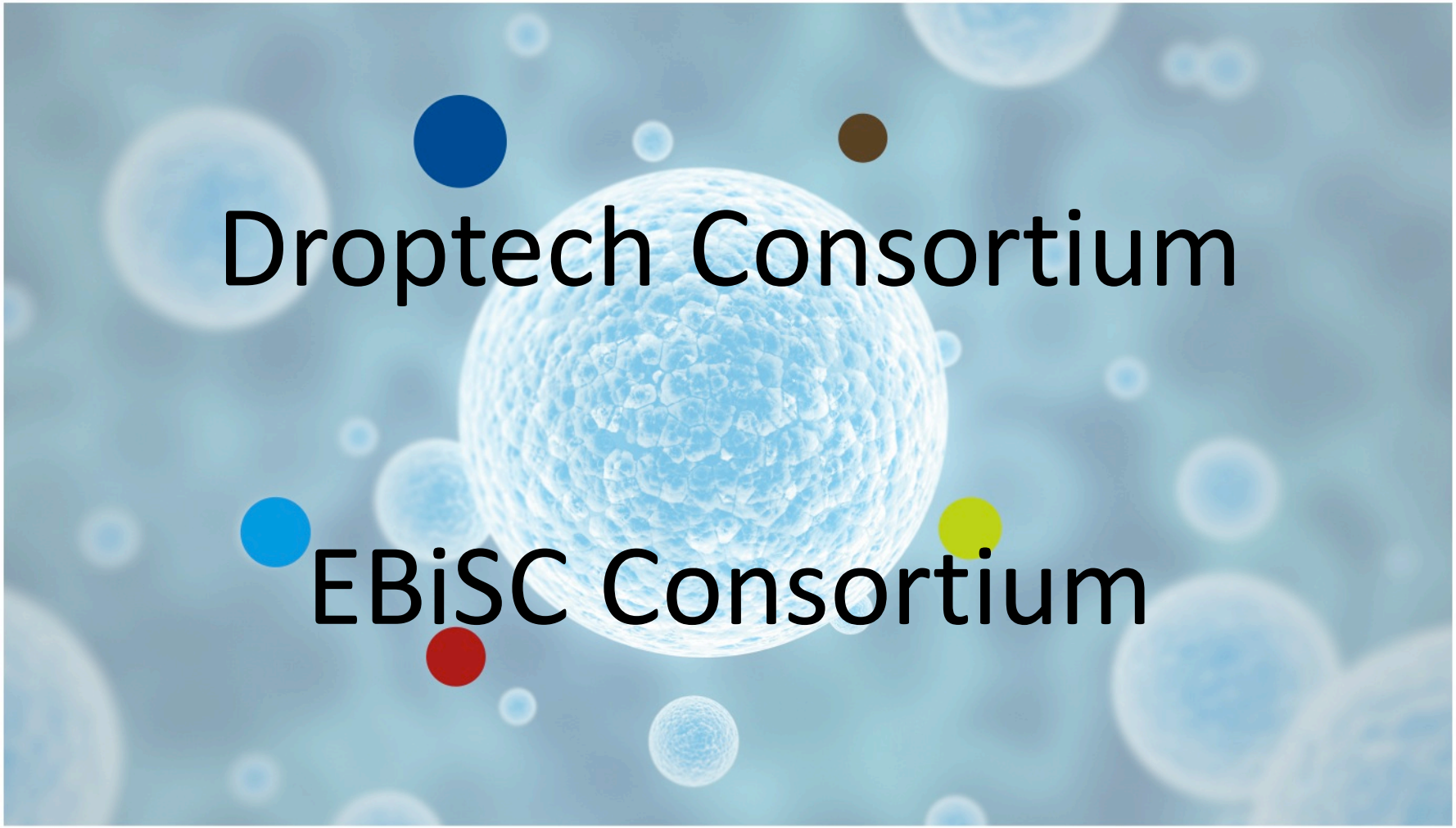


# HD automation with TECAN robot

# What the nice man said…….

- EBiSC standardisation → Industrialisation
- HiPSC lines now available.
- Automated hiPSC HD version of mEST (DROPTECH).

# Acknowldgements




Thank You.