

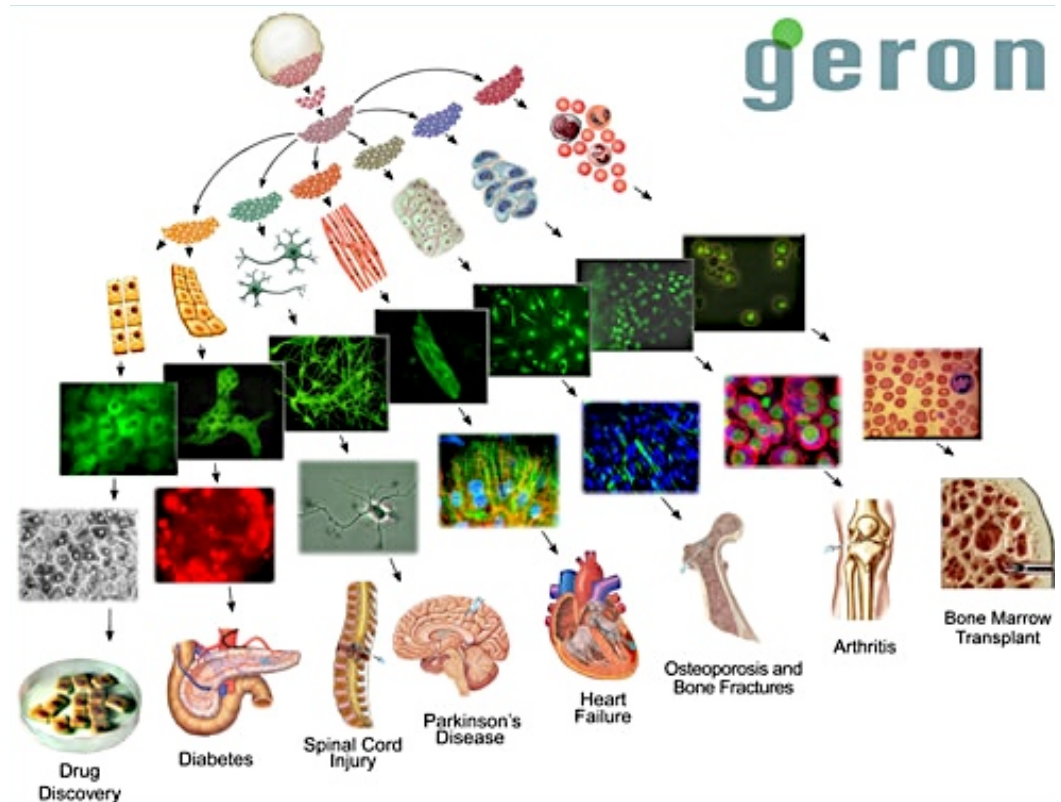
High Content Screening with hESC

The ITI LifeSciences Stem Cell Technologies Programme

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Applications of Human Stem Cells

- Toxicology - normal, human cell supply
- Drug testing - disease and patient-specific cells
- Drug discovery
- Basic research
- Therapy





Life Sciences *real possibilities*

- Intermediary Technology Institute
- Foresight and invest in emerging technologies and area
- 3 funding area: Life Science, TechMedia and Energy
- Scottish Enterprise (Scottish Government) funded

Goal: To commercialise research

Stem Cell Technologies Programme



- 3 years (2007-2010)
- £9.5 million (~\$15 million)
- Multi-disciplinary
- Academic & industrial
- Multi-centre

Programme Partners

Cellartis AB

Cell supply:
Automation,
QA/QC control



Life Sciences

Funding

**Programme
Management**

IP capture

Uni of Dundee

Scottish hit Discovery Unit

Screening:

Screen validation

Screening

Hit discovery

Compound retesting

Uni of Glasgow

Basic Science:

Screen design & development

Marker identification

'Hit' testing

Deconvolution of activity

Heriot Watt Univ

Edinburgh

Medicinal Chemistry:

Chemical synthesis

Patent Exemplification/SAR

Programme Goal

To address major technical hurdles for the eventual clinical use of hESC

- **Cell proliferation**
 - Sufficient numbers
 - Stability
- **Differentiation**
 - Early - committed progenitors (mesoderm, definitive endoderm)
 - Late - cardiomyocyte, hepatocyte, etc.
- **Scale-up**
 - Robust manufacturing processes
- **Quality**
 - GLP (commercial), GMP (therapeutic)

Technologies required for hESC screening

Cellartis

- Quality & consistent cell supply
 - Automation

U of G

- Basic biology to develop screen parameters
- Basic biology to identify and test markers
- High content analysis of markers
 - Data management

U of D

- Quality & appropriate compound libraries
- 96 and 384 formats
 - Single cell suspension & automated plating
- Kinase profiling of hit compounds (University of Dundee)

U of G

- Basic biology - Longer time-course analysis
- Basic biology - Multiparameter testing
 - Proliferation, differentiation, apoptosis

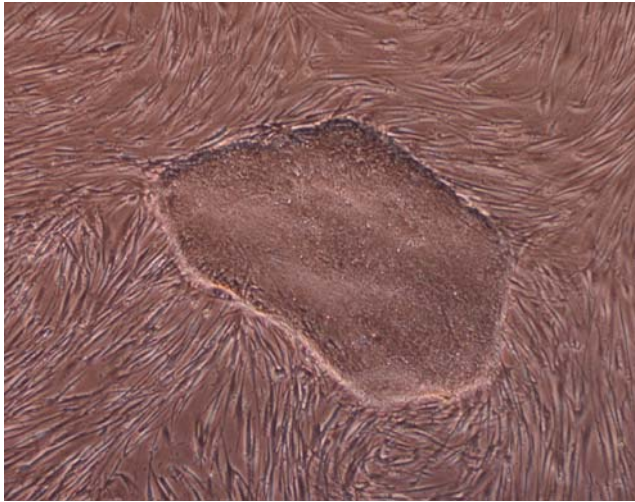
H-W Uni

- Medicinal Chemistry

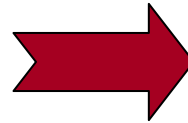
U of G

- Basic biology – Deconvolution of mechanism of action

Conversion to feeder free culture



hESC colony on a human
fibroblast feeder layer

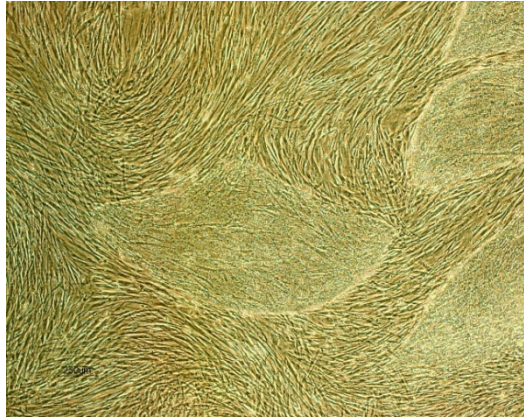


hESC monolayer

Cell lines SA121, SA461
Cells remain viable
Retain pluripotency

“Surfer” media system on fibronectin

High Quality Cell Supply



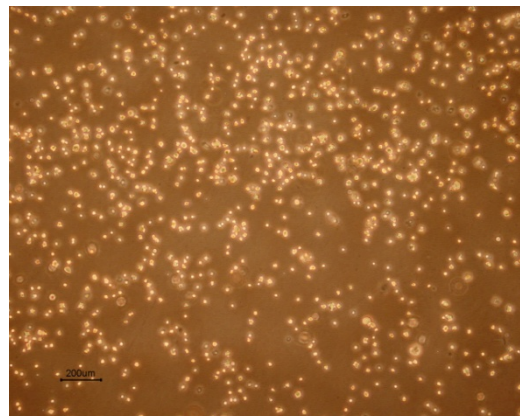
Improved media conditioning



Automation



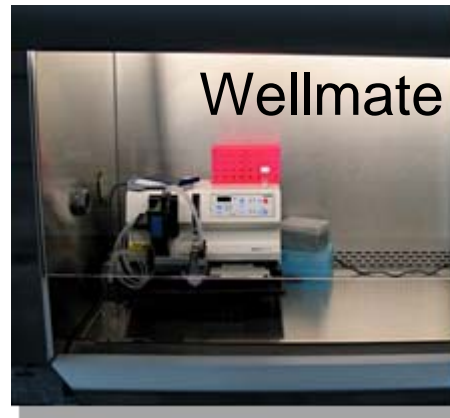
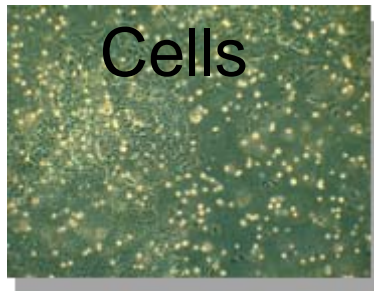
Defined culture systems



Enzymatic passaging



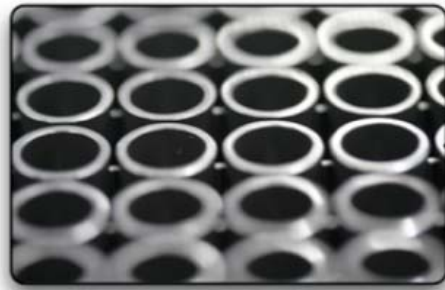
Cell Based Screening Workflow



Compound addition and downstream cell processing

Beckman Coulter BioMek FX
Thermo CytoMat Incubator/plate hotel
Bio-Tek ELX405 Plate washer
BMG FLUOstar Optima
BigNeat Robotics Enclosure

Automated microscopy and data collection



Fixed & stained
hESC



In Cell 1000
Used for primary screen

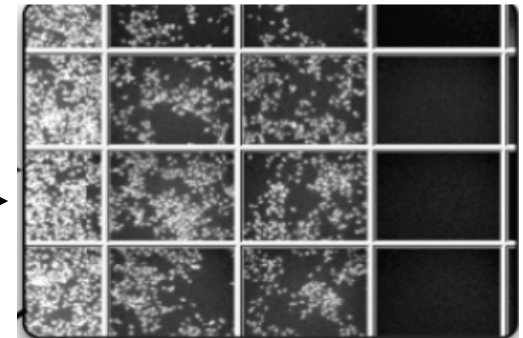
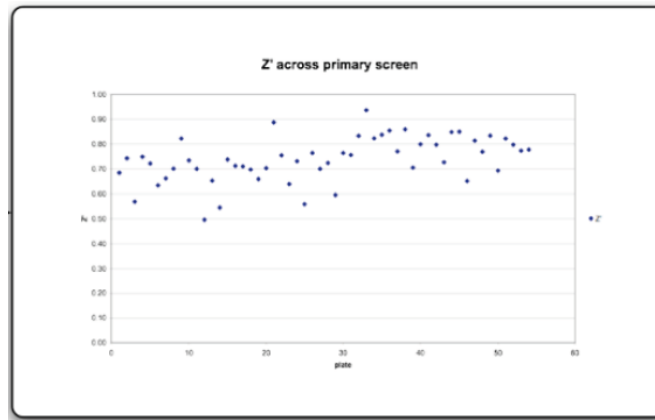


Image acquisition



Data output & upload
to database

236.000	100.000
245.000	55.000
219.000	67.000
198.000	60.000
215.000	52.000
1755.000	85.000
273.000	71.000
180.000	50.000
1515.000	88.000
219.000	61.000
253.000	95.000
302.000	93.000
1259.000	106.000
310.000	85.000
275.000	70.000
262.000	87.000

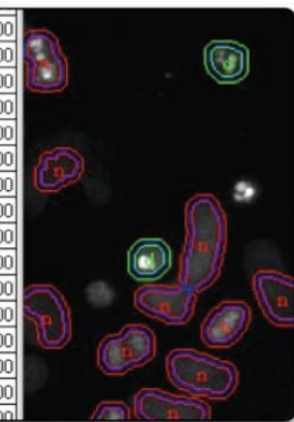


Image analysis

Compound Collections

- **Diversity set**
 - 63,362 compounds (~4,000 core fragments)
 - 15,000 compounds (~2,000 core fragments)
- **Function focused Sets**
 - Kinase, proteases, phosphatase, epigenetic enzymes, STHR family
- **Kinase Set**
 - 4110 compounds (>146 scaffolds)
- **Prestwick Library**
 - 1120 compounds, marketed, off-patent or in use compounds
- **Bioactives Set**
 - >3000 pharmacologically active agents & marketed drugs

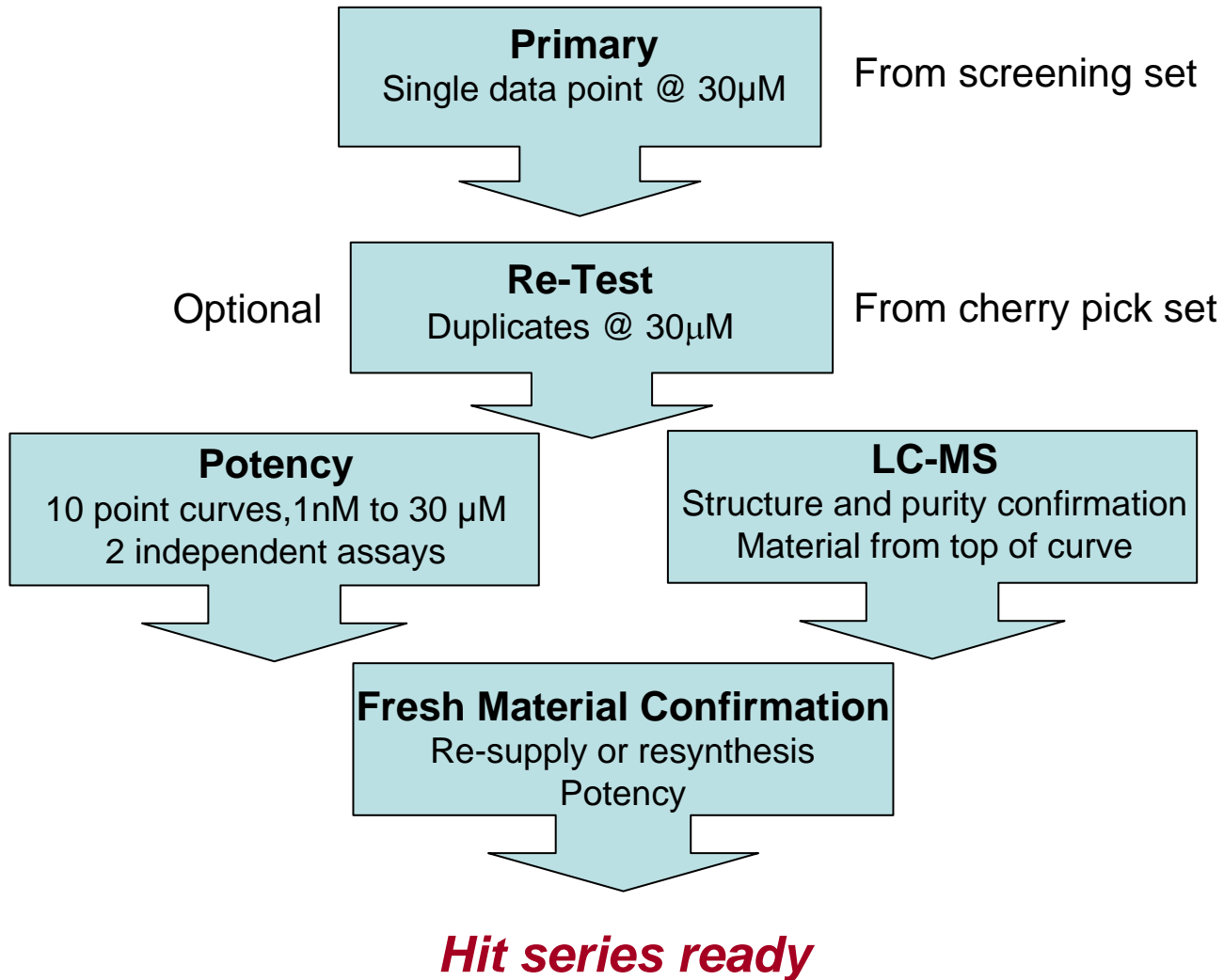
The screenshot shows a software window titled "Compound Detail - DDD0000001:01". The window contains a toolbar with various icons for file operations and navigation. Below the toolbar is a tabbed interface with tabs for "Hazard Types", "Plates", "Location History", "Notes", "Associated Documents", and "Actions". The "Plates" tab is active, showing a sub-tabbed interface with "Compound Detail", "Further Detail", "Properties", "Aliases", "Mixture", and "Handling Methods".

The main content area is divided into several sections:

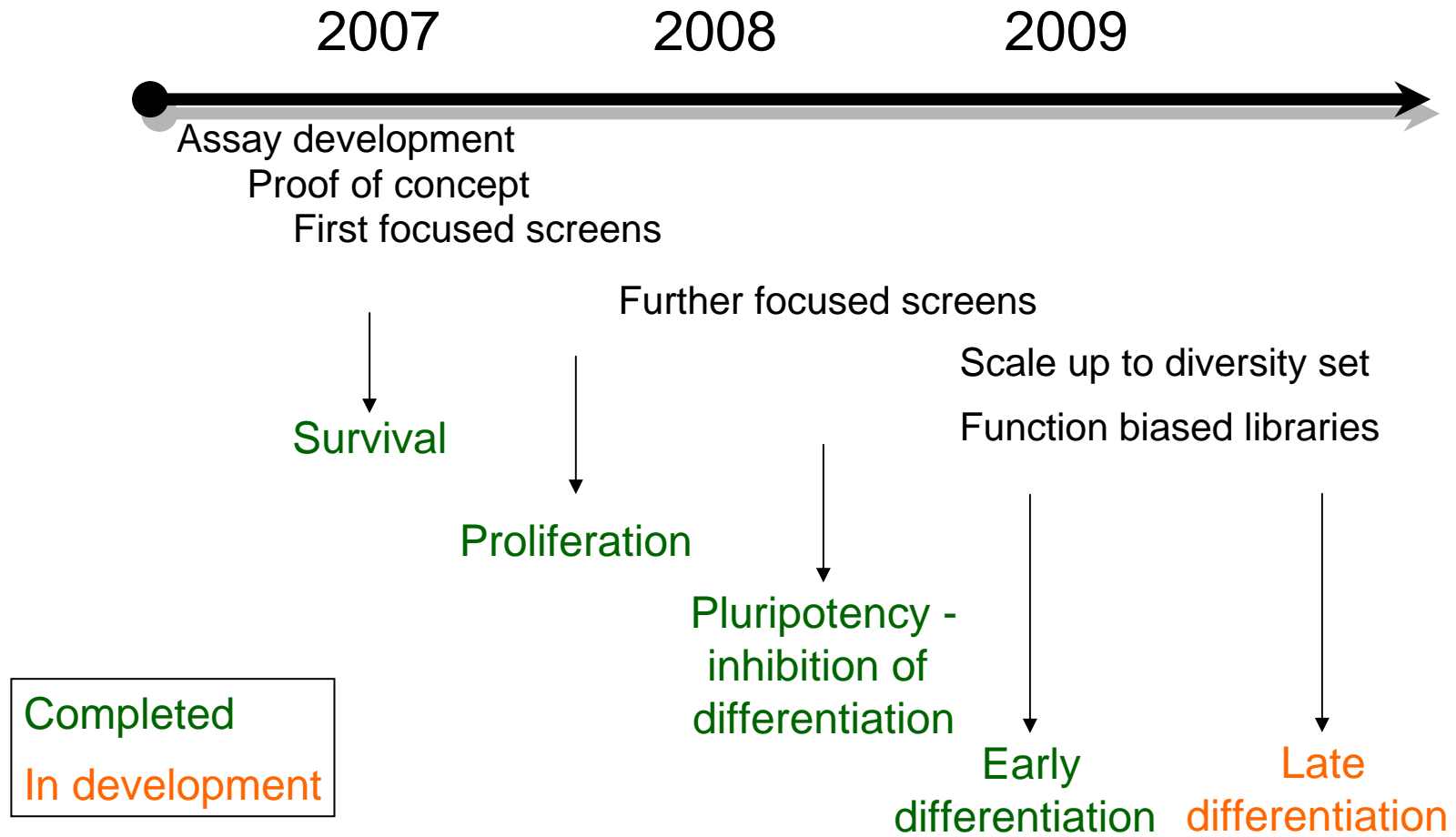
- Identification:** Object Id (DDD0000001), Parent Object Id, Batch Ref. (01), Library Id.
- Registration:** Registration Status (Unregistered), Registered By, Date.
- Structure:** A chemical structure diagram of a complex organic molecule.
- Object:** Object Status, Object Type, Object Source Type (Acquired), Object Purpose, Original Study, Originator (Jfrearson), Responsible Group (Biology).
- Supplier:** Supplier Code (ChemDiv), Compound No. (8014-8961), Batch Ref., Purchase Order, Date Received.
- Lab Notebook:** Lab Book No., Page, Page Line.
- Formula:** Parent Formula (C16H13N5OF2), Parent Mass (329.30433), Salt Code, Salt Equivs, Full Formula (C16H13N5OF2), Full Mass (329.30433), Solvate Code, Solvate Equivs.

At the bottom, it says "Created by RBRENK on 01/03/2006 at 10:50:12" and "For Help, press F1".

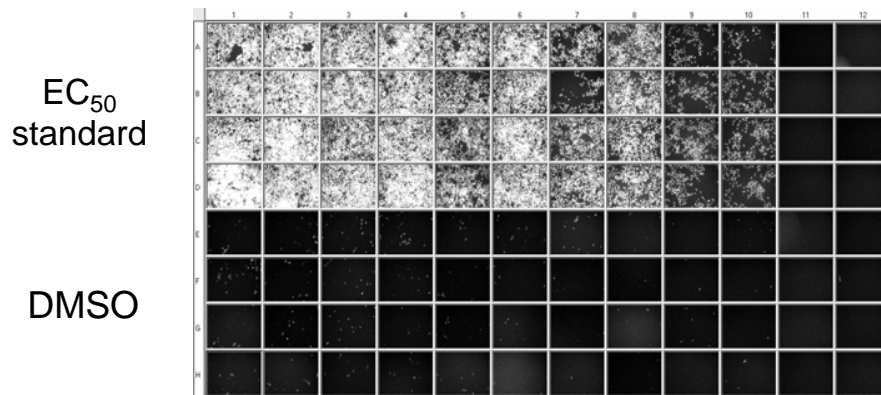
Hit Discovery & Validation Workflow



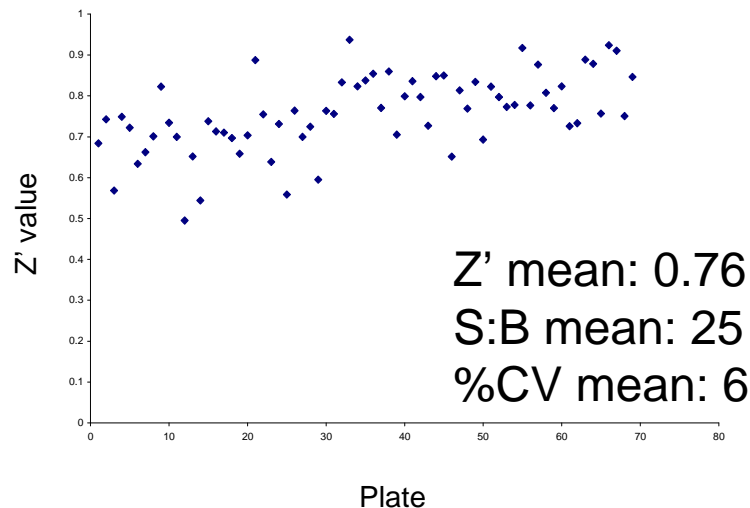
Screening Plan



1. Disaggregated Cell Survival Screen



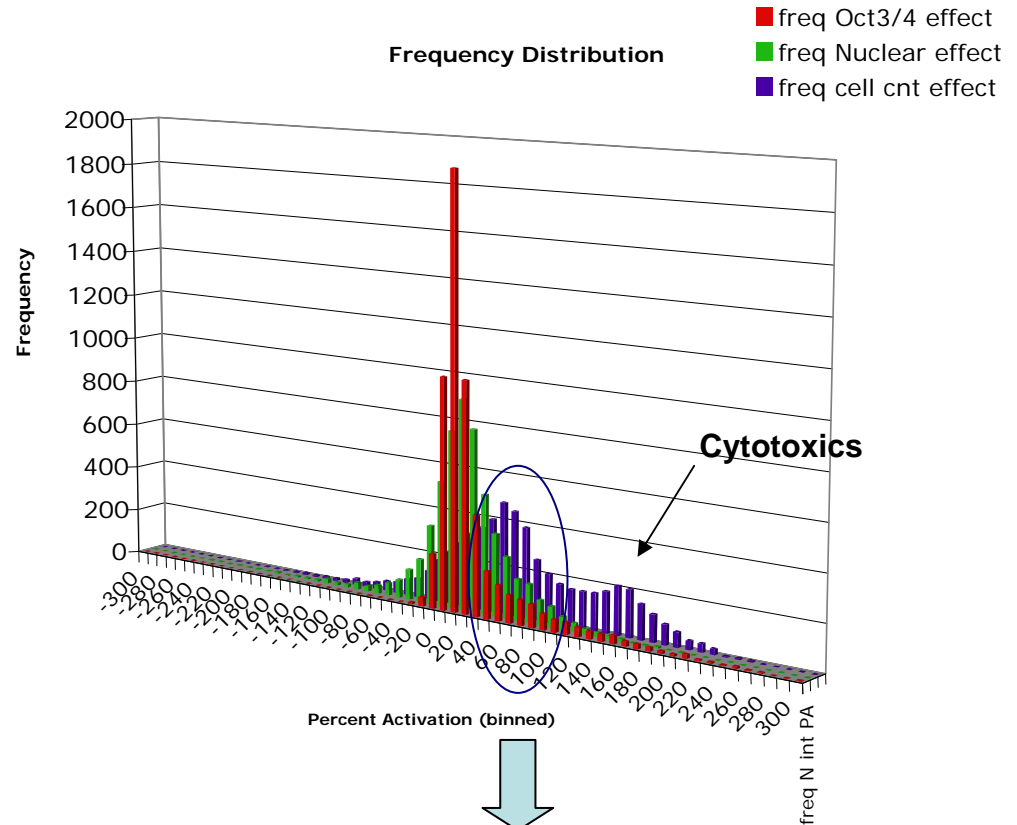
DNA stained and “live” cells counted



- hESC highly sensitive to disaggregation *in vitro*
 - major issue for routine culture and microtitre plate seeding
- kinases involved in permissive survival as single cells
- ~21,000 compounds screened
 - Diversity, kinase & prestwick sets
- Multiple series
 - 3 equivalent to standard
 - Total of 7 hits (EC₅₀ 2-20 μM)
- MOA indicated through *in vitro* profiling

2. Inhibiting differentiation *in vitro*

- Stimulus that promotes proliferation, Oct3/4 down-regulation affects nuclear morphology
- Screen for antagonists
 - kinase set
 - Prestwick set
- Multi-parametric HCS
- 4 hit compounds



Actives of interest inhibit:

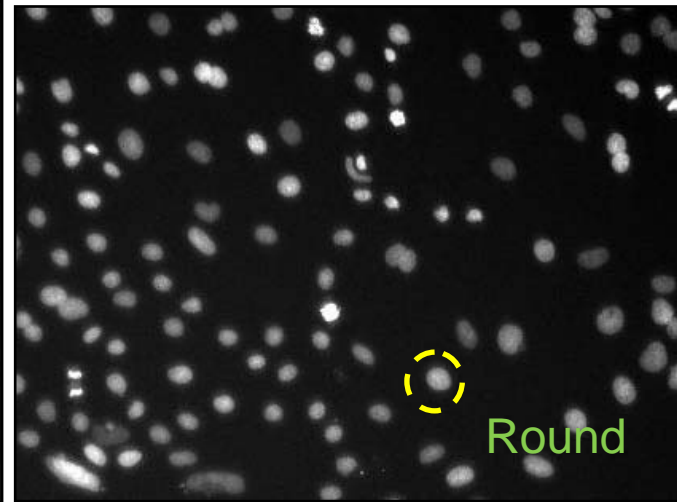
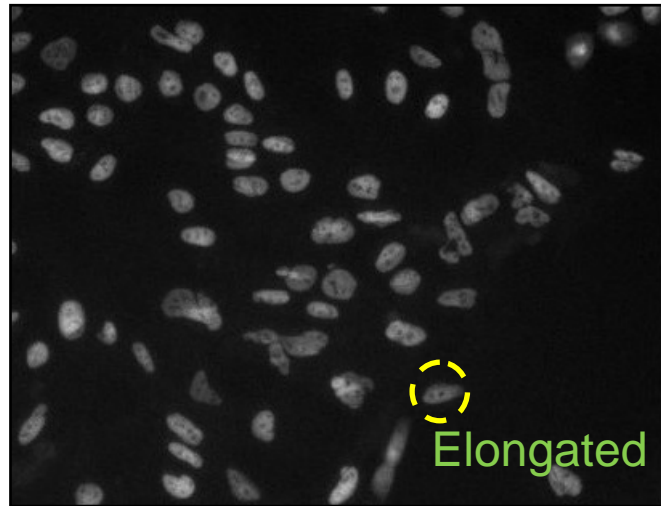
- growth
- nuclear shape change
- Oct3/4 loss

Cell count, nuclear morphology & Oct4 level

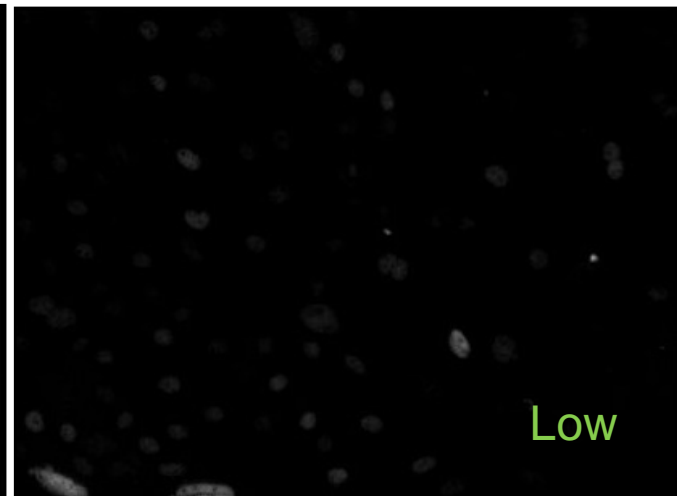
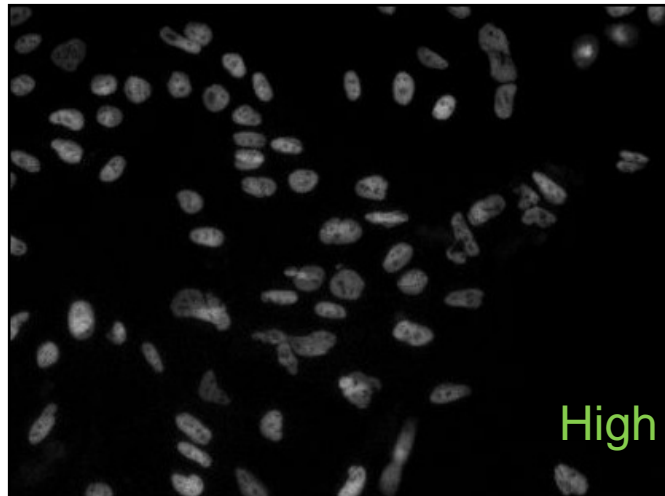
control

Stimulated

DAPI
Nuclear
morphology



Oct4



A growing portfolio of hES cell-active small molecules from screens

- **Single Cell Survival**
 - Multiple series identified
 - Kinase MOA
 - Best hit $\sim 2\mu\text{M}$ EC_{50}
- **Proliferation**
 - Dominant *in vitro* growth promoting agents identified
- **Antagonist of differentiation**
 - Multiple singletons identified
 - Best activity $< 1\mu\text{M}$

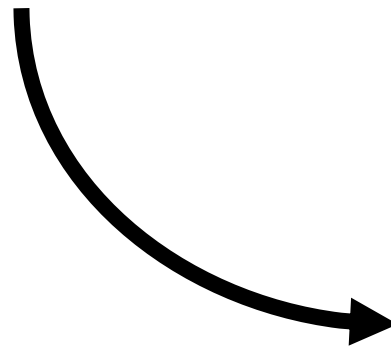
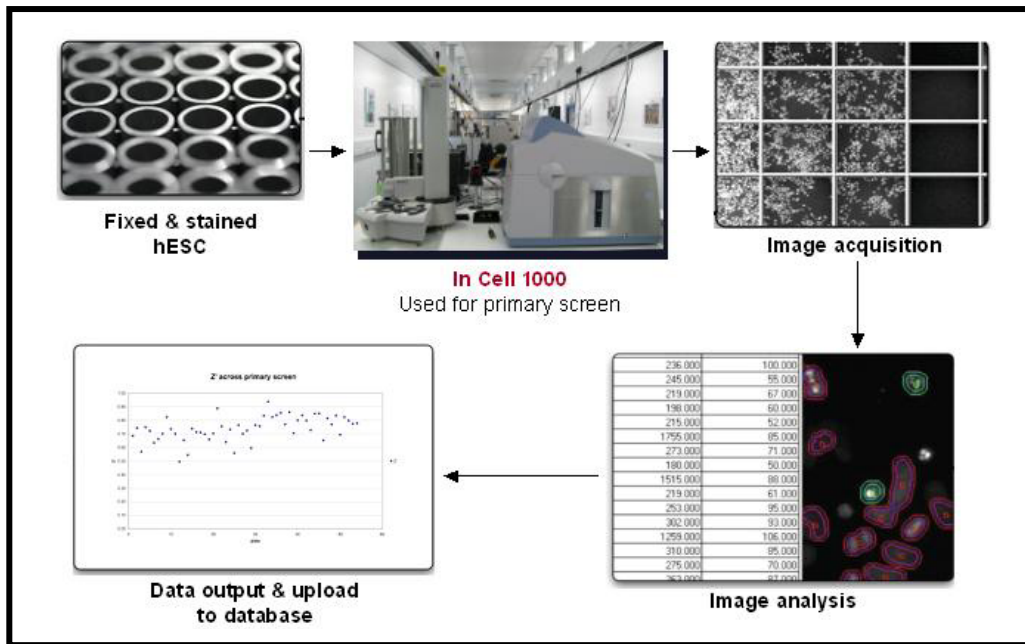
Development of a screen for cardiogenic compounds

Challenges:

1. **Time scale** - long differentiation required for acquisition of phenotype
2. **Monolayer** not EBs necessary for screen
3. **Efficiency** - most protocols <5% cardiac cells
4. **Cell survival** with established protocols eg. ActivinA

Pre-treat in bulk & re-plate for screening

Screen for cardiogenic compounds



Functionally mature cardiomyocytes

The block displays electrophysiological data and a fluorescence image of functionally mature cardiomyocytes. The top trace shows a series of action potentials with a scale bar of 5 nA and 10 ms. Below it, a legend indicates: Voltage (0.2 V), Current (0 nA), and Charge (20 nC). The bottom trace shows a similar action potential with a scale bar of 0.05 nA and 3 s. To the right, a fluorescence image shows green-stained cells.

Successes to date

1. **Feeder-free** culture - “Surfer” media / fibronectin / enzymatic passage
2. Compound that **maintains pluripotency**
3. Compounds that **permit survival** of hESC
4. Compounds that **inhibit differentiation** (spontaneous or stimulated)
5. Compounds that **induce early non-trophectoderm differentiation**
6. Generation of a pre-treated **cardiogenic mesoderm population for screening** for cardiac differentiation factors



Life Sciences **real possibilities**

Thanks to:

ITI Life Sciences

Programme Manager: Fergus McKenzie

Cellartis AB

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Scott McRae

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Miles Houslay

Graham Milligan

Andy Baker

Nicole Kane

Pete Burton

Jane Gilmour

Angela McCahill

John McAbney

Alexandra Kaupisch

Heriot-Watt Univ

David Adams

Rob Allcock

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Paul Andrews

Melissa Becroft

Martyn James