

INTERNATIONAL STEM CELL INITIATIVE-2

PARTICIPATION UNDERTAKING

THIS AGREEMENT is made by and between:

MEDICAL RESEARCH COUNCIL whose principal office is situated at 20 Park Crescent, London W1N 4AL (hereinafter called "MRC") on behalf of the International Stem Cell Forum¹ (hereinafter the "ISCF")

and

"**INSERT INSTITUTION NAME HERE**" being the host institution/participating laboratory (hereinafter the "INSTITUTION")

and

"**INSERT NAME OF PRINCIPLE INVESTIGATOR HERE**" being the participating scientist employed at the INSTITUTION (hereinafter the "INVESTIGATOR").

- A The International Stem Cell Forum (ISCF as hereinafter defined) has agreed to jointly sponsor a comparative study of the different human embryonic stem (ES) cell isolates worldwide, to reach a consensus about different defined media formulations and their ability to maintain human ES cell cultures and to devise standardised protocols for maintenance under defined conditions. The study will also establish a program to maintain and develop the ISCI human ES cell Registry on an ongoing basis to provide an international qualification process for new human ES cells. In addition there will be comparison of the genetic stability of different cultures maintained in different laboratories and identification of common minimal applicons and other common genetic changes that may occur in human ES cultures on prolonged passage.(the "PROJECT" as hereinafter defined in Schedule 1). The UK Medical Research Council (MRC) will underwrite the costs of the PROJECT incurred by the Central-Resource Laboratories in the first instance and call up contributions from ISCF agencies. The participating laboratory will be responsible for all costs associated with the preparation and shipping (of) the cytogenetics and DNA samples and any other related costs
- B The PROJECT will be organised on a Hub-and-Spoke principle. Participating laboratories with existing human ES lines will undertake specified assays and provide material which may be distributed to 'Central Resource Laboratories' (CRL) assigned to undertake specified assays. In particular some designated CRL will undertake a primary assessment of media for the culture of hES cells. Others will undertake karyotype, DNA array and Gene expression studies as designated by the CO-ORDINATOR (as hereinafter defined).
- C Professor Peter Andrews, an employee of the University of Sheffield, UK acting on behalf of the ISCF (hereinafter the "COORDINATOR") shall be in charge of the financial and
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administrative coordination of the project and will manage the scientific coordination of the PROJECT via a project steering group (hereinafter the "PROJECT STEERING GROUP").

- D The said PROJECT will be undertaken in accordance with the PROJECT PLAN (Schedule 1) and in due course detailed work plans as prepared by the PROJECT STEERING GROUP.
- E The data generated from the PROJECT will be published in the scientific literature and in an open access data REGISTRY (as hereinafter defined) on the International Stem Cell Forum web site and any intellectual property rights contained therein will therefore be in the public domain and freely available for use by anyone.
- F The INVESTIGATOR and INSTITUTION wish to participate in the PROJECT.

1 DEFINITIONS

- (a) "PROJECT PLAN" means the study plan approved by the International Stem Cell Forum and the PARTICIPATING LABORATORIES and CRLs, details of which are attached at Schedule 1.
- (b) "PROJECT" means the comparative study of the different hES isolates worldwide as described in the PROJECT PLAN.
- (c) "COORDINATOR" means Professor Peter Andrews, University of Sheffield, UK, the coordinating principal investigator.
- (d) "CELL LINES" means the hES isolates available to the PROJECT.
- (e) "MATERIAL" means any materials supplied by the PARTICIPATING LABORATORY to the COORDINATOR.
- (f) "REGISTRY" means the database of results of the PROJECT that will be published on the ISCF web site and in the scientific literature. PROJECT data will therefore be in the public domain
- (g) KNOWLEDGE means the results, including information, arising from the project, as well as copyright or other intellectual property rights attaching to the results following applications for, or the issue of registration of, patents, designs and models, additional certificates or other similar forms of protection.
- (h) ISCF means the International Stem Cell Forum comprising representatives from Australia, Canada, Czech Republic, China, Finland, France, Germany, Italy, Israel, Japan, JDRF, South Korea, Singapore, Spain, Sweden, Switzerland, The Netherlands, UK, USA.
- (i) PARTICIPATING LABORATORY(IES) shall mean the INVESTIGATOR's laboratory within the INSTITUTION.
- (j) "REFERENCE MATERIALS" refers to Antibodies, standard RNA preparations and standard cell lines supplied by the central resource laboratories

2 The PARTICIPATING LABORATORY hereby agree to:

- 2.1 secure and provide to the COORDINATOR, for approval by the PROJECT STEERING GROUP, written evidence to demonstrate that the owner of the CELL LINE(S) (normally the employer of the originator) has granted permission to the use of the CELL LINES for the purpose of the PROJECT. A proforma document is provided in Schedule 2;
- 2.2 provide documentary evidence to the COORDINATOR of a) informed (anonymised) donor consent and b) approval from a local research ethics committee as proof that the cell lines were derived in accordance with national ethical and regulatory requirements. If the original consent form is not available it will be acceptable to provide a letter of confirmation from the IVF clinic that the embryo from which the cell line was derived was ethically sourced. Where such documentary evidence is not in English it must be accompanied by a verified translation into English.
- 2.3 abide by relevant national guidelines and legislation in conducting the PROJECT.
- 2.4 undertake their duties in the PROJECT as specified in the PROJECT PLAN and provide all MATERIAL and results to the COORDINATOR or to the UK Stem Cell Bank as directed by the COORDINATOR;
- 2.5 identify one or more persons who shall direct the work, ensure that the tasks assigned are correctly performed, and use reasonable endeavours to carry out the part of the PROJECT specifically assigned to them by the COORDINATOR.
- 2.6 all data generated in the PROJECT being deposited in the data REGISTRY without restriction or condition, after approval by the PROJECT STEERING GROUP, which will be part of the ISCF web site. PROJECT data will thereby be in the public domain.
- 2.7 provide to the COORDINATOR all the data required to meet the PROJECT objectives. The completed REGISTRY will show all recruited lines with a full set of characterisation data.
- 2.8 conduct its work for the PROJECT in research grade facilities using procedures that conform to good research practice and comply with all applicable safety regulations.
- 2.9 inform the COORDINATOR of any event liable to substantially affect the PROJECT and any circumstance affecting the conditions of participation.
- 2.10 complete and return this Participation Undertaking and all associated documentation to the COORDINATOR.
- 2.11 ensure that information of a confidential nature, including but not limited to results of the PROJECT, information relating to consents/approvals, commercially confidential information, shall be maintained in confidence. Research data originating from the PROJECT shall not be divulged outside the PARTICIPATING LABORATORIES, and CRLs until the final version approved by the PROJECT STEERING GROUP is published in the data REGISTRY as provided for in 2.6.
- 2.12 ensure that any papers submitted for publication acknowledge the PARTICIPATING LABORATORIES, and CRLs, and the ISCF.

3 USE OF MATERIALS

- 3.1 The PARTICIPATING LABORATORY undertakes (a) not to use material supplied by the UK Stem Cell Bank or other members of the PARTICIPATING LABORATORIES, and CRLs, for work not forming part of this agreement and (b) to return or destroy material received and not used on the project unless permission to retain material has been agreed with the owner.
- 3.2 All the MATERIAL provided by the PARTICIPATING LABORATORY will only be used for the PROJECT as described in the attached Schedules, and will not be used for any other purposes without the expressed agreement of the PARTICIPATING LABORATORY. Once the PROJECT is completed a sample of the MATERIAL may be saved in an Archive for retesting in case of any future dispute. Any MATERIAL not required for the PROJECT or for that Archive will be destroyed or, if explicitly requested, returned to the PARTICIPATING LABORATORY.

4 LIABILITY

- 4.1 The INSTITUTION shall be solely liable for any claim, damage, liability or loss arising from its work. In no event shall other participants, members of the ISCF, the Medical Research Council (MRC) and the Central Resource Laboratories (CRLs) be liable for any claim, damage, liability or loss arising from PARTICIPATING LABORATORY's use of CELL LINES.
- 4.2 The PARTICIPATING LABORATORY recognises that the CRLs, ISCF, MRC and the COORDINATOR offer no guarantee that the analysis of the results will not provide any hitherto unknown information that may affect the value or application of the CELL LINES. The UK Stem Cell Bank will retain a small sample of the original MATERIAL for confirmatory testing should the need arise.

5 LAW

- 5.1 The validity, construction and performance of this Agreement shall be governed by English law subject to the non-exclusive jurisdiction of the English Courts.

6 CHANGES AND AMENDMENTS

- 6.1 No addition or amendment to or modification of this Agreement shall be effective unless it is in writing and signed by the participating cell line owner/originator and agreed in writing by the COORDINATOR.

7 COUNTERPARTS

- 7.1 This Agreement may be executed in counterparts, each of which shall be an original, and such counterparts shall together constitute one and the same agreement.

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SIGNED FOR AND ON BEHALF OF MEDICAL RESEARCH COUNCIL

.....
Graham Wagner
Deputy Director Licensing and Agreements
Medical Research Council Technology

AGREEMENT BY HOST INSTITUTION AND INVESTIGATOR

ON BEHALF OF

Institution Name (in full): UNIVERSITY OF SHEFFIELD

.....

Address: Firth Court
Western Bank
Sheffield
S10 2TN

I hereby confirm that the INVESTIGATOR named below is employed by this INSTITUTION and I agree that s/he may contribute to the International Stem Cell Forum PROJECT and I understand and accept the conditions of participation as outlined above.

.....
Signed for and on behalf of the INSTITUTION
(Head of Department/Dean or equivalent)

.....
Full Name (block capitals)

.....
Position

.....
Signature of INVESTIGATOR

.....
Full Name (block capitals):

.....
Position

Stamp of the organisation:

Date:

SCHEDULE 1

Project Plan

INTERNATIONAL STEM CELL INITIATIVE-2

HUMAN EMBRYONIC STEM CELL REGISTRY

Objective

The objective is to create a registry of human ES cell lines entry on which depends on generation of standardized characterization data.

Work Program

Each PARTICIPATING LABORATORY will be asked to provide from each human ES cell line that they wish to enrol in the study the following data:

1. **Real-time PCR for a panel of markers.**
2. **Flow Cytometric analysis for a panel of antigens**, antibodies to which will be supplied by the ISCI steering committee (see appendix 2).
3. **Karyotype**

Real-time PCR for a panel of markers.

The panel of markers should be based on the genes used in the TaqMan® Human Stem Cell Pluripotency Array, Applied Biosystems, cat number 4385344 (see appendix 1). It should be noted however that whilst it is desirable to examine the panel of markers using Taqman arrays other technologies are acceptable provided they yield similar results in terms of quality and genes examined.

Flow Cytometric Analysis

A panel of antibodies supplied centrally should be used to characterize the cells. Applicants should record % cells positive and mean (or median) fluorescence for submission to the registry. Applicants where possible should save copies of the 'raw' listmode data used to generate the % positive and mean fluorescent values, this will enable retrospective analysis if problems arise with the initial data analysis.

Karyotype

A protocol for culturing in cells in colcemid and preparing them as a hypotonic treated, fixed suspension will be provided.

The PARTICIPATING LABORATORY will be responsible for all costs associated with the preparation and analysis of cell lines. The ISCI2 project will cover all costs incurred by the Central Resource Laboratories in supplying reference materials.

Appendix 1

List of genes to be examined by real-time PCR analysis with their corresponding Taqman assay number.

Stem Cell	POU5F1 Hs00742896_s1 XIST Hs01079824_m1 GAL Hs00544355_m1 FGF4 Hs00173564_m1 GABRB3 Hs00241459_m1 TDGF1 Hs02339499_g1 LIN28 Hs00702808_s1 TERT Hs00162669_m1 NANOG Hs02387400_g1 NODAL Hs00415443_m1 PODXL Hs00193638_m1 NES Hs00707120_s1 TFCP2L1 Hs00232708_m1 SOX2 Hs00602736_s1 CD9 Hs00233521_m1 COMMD3 Hs00201350_m1 DNMT3B Hs00171876_m1 KIT Hs00174029_m1 LIFR Hs00158730_m1 IL6ST Hs00174360_m1 PTEN Hs00829813_s1 FGF5 Hs00170454_m1 REST Hs00194498_m1 ZFP42 Hs00399279_m1 SFRP2 Hs00293258_m1 NOG Hs00271352_s1 SEMA3A Hs00173810_m1 IFITM2 Hs00829485_sH CRABP2 Hs00275636_m1 GBX2 Hs00230965_m1 LEFTY1 Hs00764128_s1 LEFTY2 Hs00745761_s1 GRB7 Hs00917999_g1 UTF1 Hs00747497_g1 FOXD3 Hs00255287_s1 IGF2BP2 Hs00538956_m1 BXDC2 Hs00217848_m1 NR6A1 Hs00265966_m1 IFITM1 Hs00705137_s1
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	GDF3 Hs00220998_m1 NR5A2 Hs00187067_m1
Trophoblast	EOMES Hs00172872_m1 CDX2 Hs00230919_m1 GCM1 Hs00172692_m1 CGB Hs00361224_gH KRT1 Hs00196158_m1
Neural	PAX6 Hs00240871_m1 NEUROD1 Hs00159598_m1 SYP Hs00300531_m1 TH Hs00165941_m1 V3 Interneurons ISL1 Hs00158126_m1 Astrocytes GFAP Hs00157674_m1 Motor neuron HLXB9 Hs00232128_m1 Oligodendrocytes OLIG2 Hs00377820_m1
Mesoderm	T Hs00610080_m1 WT1 Hs00240913_m1 Muscle MYF5 Hs00271574_m1 MYOD1 Hs00159528_m1 Blood DES Hs00157258_m1 HBB Hs00747223_g1 HBZ Hs00744391_s1 Cartilage COL2A1 Hs00156568_m1
Germ Cell	DDX4 Hs00251859_m1 SYCP3 Hs00538143_m1
Endoderm	GATA6 Hs00232018_m1 SOX17 Hs00751752_s1 GATA4 Hs00171403_m1 Parietal Endoderm FN1 Hs00277509_m1 LAMA1 Hs00300550_m1 LAMB1 Hs00158620_m1 LAMC1 Hs00267056_m1 Extraembryonic endoderm PTF1A Hs00603586_g1 FOXA2 Hs00232764_m1 Visceral Endoderm AFP Hs00173490_m1 SERPINA1 Hs00165475_m1 Endothelial CD34 Hs00156373_m1 CDH5 Hs00174344_m1 PECAM1 Hs00169777_m1 Pancreas FLT1 Hs00176573_m1 SST Hs00174949_m1 INS Hs00355773_m1

Hepatocytes	IAPP Hs00169095_m1 PAX4 Hs00173014_m1 IPF1 Hs00236830_m1 GCG Hs00174967_m1 TAT Hs00356930_m1
Control	18s Hs99999901_s1 ACTB Hs99999903_m1 GAPDH Hs99999905_m1 RAF1 Hs00234119_m1 CTNNB1 Hs00170025_m1 EEF1A1 Hs00742749_s1

Appendix 2

Panel of Antibodies to be used to Characterize cells for Submission to the ISCI registry

Antigen	Antibody	Species	Class	Expected 2102Ep expression	
				High Density	Low Density
<u>Undifferentiated ES Cell Markers</u>					
SSEA3	MC631	Rat	IgM	Positive	Positive
SSEA4	MC813-70	Mouse	IgG	Positive	Positive
TRA-1-60	TRA-1-60	Mouse	IgM	Positive	Positive
GCTM2	GCTM2	Mouse	IgM	Positive	Positive
L-ALP	TRA-2-54	Mouse	IgG	Positive	Positive
CD90(Thy-1)	Anti-Thy-1	Mouse	IgG	Positive	Positive
CD9	Anti-CD9	Mouse	IgG	Positive	Positive
<u>Differentiation Markers</u>					
SSEA1	MC480	Mouse	IgM	Negative	Positive
<u>Pan human antigens</u>					
TRA-1-85(OK(a))	TRA-1-85	Mouse	IgG	Positive	Positive
<u>Control antibody</u>					
P3X	Control IgG	Mouse	IgG	Negative	Negative

SCHEDULE 2

PROFORMA

AGREEMENT BY OWNER OF CELL LINE

I being the owner (normally the employer of the researcher who derived the cell line) of ES cell lines
(give name/no)

.....
.....
.....

.....hereby agree that this ES cell line
may be included in the International Stem Cell Forum PROJECT described in the PROJECT PLAN in
order to conduct the research necessary using the above cell line to meet the objectives of the
PROJECT. I confirm I have received and read the PROJECT PLAN.

.....
Signature of owner of cell line

.....
Full Name of owner
(block capitals)

Address (in full)

Date:.....

This agreement shall take effect as of the date set above, subject to its terms and subsist for a
period of 12 months.

INVESTIGATOR:

.....
(Name – block capitals)

.....
(Host institution)

.....

(Date)