# Supplemental File S1

## Detailed overview of the microarray experimental designs

## & Contact Information

### Project INS-2: comparison of three different ES cell lines

**Cell lines: CGR8; E14TG2a; R1**

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**Summary of the project**

In order to identify differences in gene expression profiles between the three mouse ES cell lines used within the FunGenES consortium, RNA was prepared from undifferentiated CGR8, E14TG2a and R1 cells.

| Abbreviation | Description | Arrays |
|---|---|---|
| CGR8 | ES cell line CGR8 | 5 |
| E14TG2a | ES cell line E14TG2a | 5 |
| R1 | ES cell line R1 | 5 |

INS-2 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-671

Accession: E-TABM-671

# Project INS-1: Identification of Stat3 target genes

## Cell lines: E14TG2a; E14(T)-Stat3-ER; E14(T)-Cre-ER

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### Summary of the project

The objective of this study is to identify target genes of the STAT3 transcription factor in ES cells, making use of the hormone-dependent STAT3-ER$^{T2}$ protein. STAT3-ER$^{T2}$ is a fusion protein between STAT3 and the mutated ligand-binding domain of the human estrogen receptor. In the absence of ligand, STAT3-ER$^{T2}$ is sequestered in the cytoplasm in an inactive state by association of the ligand-free ER$^{T2}$ domain with the ubiquitous Hsp90 complex. In the presence of tamoxifen, STAT3-ER$^{T2}$ is liberated and translocates into the nucleus. We made use of the pPCAGIZ vector and episomal supertransfection in E14/T cells to express STAT3-ER$^{T2}$.

After electroporation, cells were plated at 5x10$^5$ cells per 10 cm culture dish in medium supplemented with 1,000 U/ml of LIF and cultured in the presence of 1 µg/ml of zeocine for 7 days. Resistant colonies were pooled and further propagated in selection medium for 10 days in the presence of 10 nM tamoxifen and in the absence of LIF.

STAT3-ER$^{T2}$-expressing ES cells do not require LIF for propagation in the undifferentiated state. They are strictly dependent upon tamoxifen for self-renewal. RNAs have been prepared from: (*i*) ES cells starved of LIF and tamoxifen for 24 hours; (*ii*) starved cells after re-stimulation with 1,000 U/ml of LIF for 1 hour; and, (*iii*) starved cells after re-stimulation with 100 nM of tamoxifen for 2 hours.

E14/T cells expressing Cre-ER$^{T2}$, a fusion protein between the Cre recombinase and the ER$^{T2}$ domain have been also generated and used as control cells to identify -and eliminate off-target effects of STAT3-ER$^{T2}$. Cre-ER$^{T2}$-expressing ES cells require LIF for propagation in the undifferentiated state. RNA have been prepared from: (*iv*) ES cells starved of LIF and tamoxifen for 24 hours; and, (*v*) starved cells after re-stimulation with 100 μM tamoxifen for 2 hours.

| Abbreviation | Description | Arrays |
|---|---|---|
| STAT3-ER-CTL | STAT3-ER not stimulated | 4 |
| STAT3-ER-Tax | STAT3-ER stimulated with Tax | 6 |
| STAT3-ER-LIF | STAT3-ER stimulated with LIF | 5 |
| Cre-ER-CTL | Cre-ER not stimulated | 5 |
| Cre-ER-Tax | Cre-ER stimulated with Tax | 5 |

INS-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-562

Accession: E-TABM-562

# Project CNRS-UMR-5164: Identification of LIF target genes

**Cell line: CGR8**

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**Summary of the project**

The purpose of these experiments was to identify genes the expression of which is regulated by LIF in ES cells. To this end, CGR8 ES cells were grown in DMEM medium with 10% FCS with or without 20 ng/ml recombinant human LIF.

Five independent total RNA samples (Qiagen column) have been prepared from each cell growth condition. The proper LIF regulation has been confirmed by monitoring LIF-dependent activation of STAT3 using western blot with phospho-Tyr705 STAT3 on protein lysates prepared in parallel with each RNA preparation. The quality of RNA preparations was checked by RT-PCR with known LIF-dependent genes such as *Socs3*, *Klf4* and *JunB* (with *Hprt* serving as a non-regulated constant control).

| Abbreviation | Description | Arrays |
|---|---|---|
| h24---ser | CGR8 cells grown in DMEM/10%FCS, without LIF for 24h have been re-induced for 25 minutes with DMEM/10%FCS | 5 |
| h24---lif | CGR8 cells grown in DMEM/10%FCS, without LIF for 24h have been re-induced for 25 minutes with DMEM/10%FCS/LIF | 5 |
| h48---ser | CGR8 cells grown in DMEM/10%FCS, without LIF for 48h have been re-induced for 25 minutes with DMEM/10%FCS. | 5 |
| h48---lif | CGR8 cells grown in DMEM/10%FCS, without LIF for 48h have been re-induced for 25 minutes with DMEM/10%FCS/LIF | 5 |
| h48lifser | CGR8 cells grown in DMEM/10%FCS/LIF for 48h have been re-induced for 25 minutes with DMEM/10%FCS | 5 |
| h48liflif | CGR8 cells grown in DMEM/10%FCS/LIF for 48h have been re-induced for 25 minutes with DMEM/10%FCS/LIF | 5 |

CNRS-UMR-5164 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-667

Accession: E-TABM-667

# Project UOB-1/UOB-2: Identification of PI3-K target genes

**Cell line: E14TG2a**

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**Summary of the project**

Aim: To identify genes regulated by PI3K signaling in ES cells.

E14tg2a cells cultivated in KO-DMEM supplemented with Knock-out serum replacement (Invitrogen) in the presence of LIF were either: (i) starved for 24h in media lacking LIF, pre-incubated for 30 minutes with 5 μM LY294002 in DMSO or DMSO alone and then treated for 2h with LIF, prior to preparation of RNA samples (UOB-1 samples); or, (ii) cultured in the continuous presence of LIF, plus DMSO alone or 5 μM LY294002 for 24 to 72h prior to RNA extraction (UOB-2 samples).

**UOB-1:**

| Abbreviation | Description | Arrays |
|---|---|---|
| DMSO | DMSO + LIF 2h | 5 |
| LY | DMSO + LY294002 + LIF 2h | 5 |

UOB-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the

EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-673

Accession: E-TABM-673

**UOB-2:**

| Abbreviation | Description | Arrays |
|---|---|---|
| DMSO24h | 24h DMSO | 3 |
| DMSO48h | 48h DMSO | 3 |
| DMSO72h | 72h DMSO | 3 |
| LY24h | 24h DMSO+LY294002 | 3 |
| LY48h | 48h DMSO+LY294002 | 3 |
| LY72h | 72h DMSO+LY294002 | 3 |

UOB-2 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the

EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-674

Accession: E-TABM-674

# Project IMBB-1: Identification of genes targeted by the histone deacetylase inhibitor Trichostatin A

**Cell line: CGR8**

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**Summary of the project**

Aim: To investigate the effects of global histone deacetylation inhibition in ES cell differentiation.

CGR8 cells were cultivated in presence or absence of Trichostatin A (TSA, an HDAC inhibitor) for 0 (control), 6 and 12 hours, respectively.

| Abbreviation | Description | Arrays |
|---|---|---|
| CGR8-LIF-TSA00 | ESCs grown in presence of LIF and treated with TSA (HDAC inhibitor); 0 hours | 3 |
| CGR8-LIF-TSA06 | ESCs grown in presence of LIF and treated with TSA (HDAC inhibitor); 6 hours | 3 |
| CGR8-LIF-TSA12 | ESCs grown in presence of LIF and treated with TSA (HDAC inhibitor); 12 hours | 3 |

IMBB-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-670

Accession: E-TABM-670

# Project TUD-1: TAg effects on DOX MIB and DOX DEX induced clones

## Cell line: E14TG2a

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### Summary of the project

The purpose of the experiment was to see if changes in the expression profile of the cells caused by transient expression of T-Antigen can be reversed. Two different E14TG2a lines (ABD and GBD*) were generated: both lines carry a construct containing the SV40 Large-T Antigen under the control of the tetracycline promoter. The ABD line also contains the reverse tet-repressor fused to VP16 and Androgen Binding Domain (ABD) under the control of the CAGGs promoter. The GBD*-clone carries a construct containing the reverse tet-repressor fused to VP16 and mutated Glucocorticoid Binding Domain (GBD*) under the control of the CAGGs promoter (GBD*-clone).

The term "induction" means that the cells were treated for 6 days with either Mibolerone and Doxycycline (for the ABD clone) or with Dexamethasone and Doxycycline (for the

GBD* clone). This also signifies that the T-Antigen is expressed. "Deinduction" means that the cells were treated for 6 days with the above compounds and then cultured for an additional 6 days without the compounds. During the "deinduction" phase the expression of T-Antigen stops. Untreated cells means that the cells were cultured for the period of 12 days without any compounds, there is no expression of T-Antigen.

| Abbreviation | Description | Arrays |
|---|---|---|
| ABD12dnot | clone 1 (ABD) 12 days untreated | 3 |
| ABD06dyes06dnot | clone 1 (ABD) 6 days induced and afterwards 6 days deinduced | 3 |
| ABD06dyes | clone 1 (ABD) 6 days induced | 3 |
| GBD12dnot | clone 2 (GBD) 12 days untreated | 3 |
| GBD06dyes06dnot | clone 2 (GBD) 6 days induced and afterwards 6 days deinduced | 3 |
| GBD06dyes | clone 2 (GBD) 6 days induced | 3 |

TUD-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-675

Accession: E-TABM-675

# Project UKOE-1: Differentiation of ES cells to Embryoid Bodies

**Cell line: CGR8**

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## Summary of the project

CGR8 cells were cultivated as embryoid bodies (EBs) without addition of selective reagents in hanging drop cultures (day 0-7) in a time course experiment. After 7 days, EBs were plated in gelatin-coated 6-well plates. RNA was prepared from ES cells (day 0), EBs (day 1 to 7) and plated EBs (day 10).

| Abbreviation | Description | Arrays |
|---|---|---|
| 00d | ES cell line CGR8 at day 0 | 3 |
| 01d | ES cell line CGR8 at day 1 | 3 |
| 02d | ES cell line CGR8 at day 2 | 3 |
| 03d | ES cell line CGR8 at day 3 | 3 |
| 04d | ES cell line CGR8 at day 4 | 3 |
| 05d | ES cell line CGR8 at day 5 | 3 |
| 06d | ES cell line CGR8 at day 6 | 3 |
| 07d | ES cell line CGR8 at day 7 | 3 |
| 10d | ES cell line CGR8 at day 10 | 3 |

UKOE-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-672

Accession: E-TABM-672

# Project AVEF-1: EB formation and differentiation into neuronal cells

**Cell line: E14TG2a/Sox1TV2**

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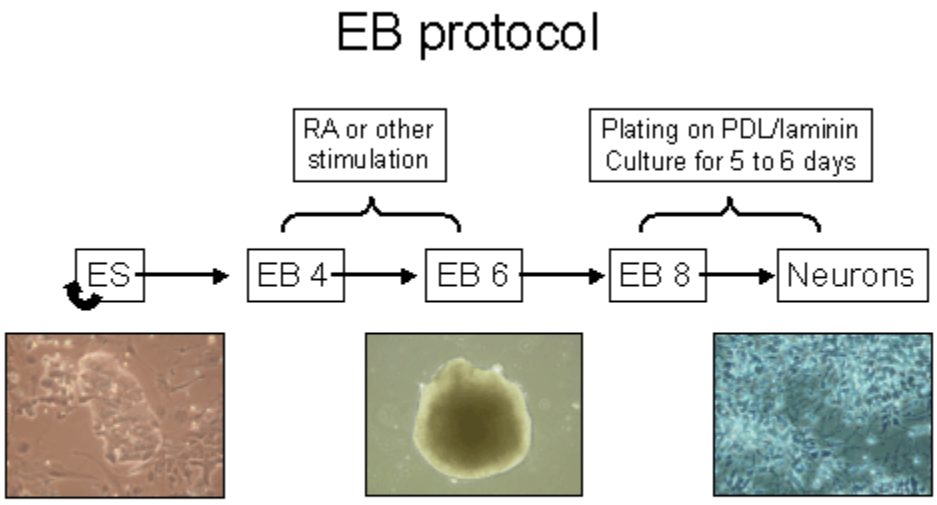
**Summary of the project**




ES cells have been allowed to form embryoid bodies in liquid culture condition onto non-adherent dishes. This step takes place during 8 days with a 48 hours retinoic acid

stimulation between days 4 and 6. During this step samples have been prepared from ES cells, 3, 4, 6 and 8 days old embryoid bodies (ES, EB3-8) with (RA) or without (CTL) retinoic acid from day 6. At day 8 of differentiation EB have been dissociated and cells have been plated on poly-D-lysine/laminin coated dishes. During the first 48 hours of plating, bFGF has been added to the culture medium in order to increase the number of neuronal progenitor cells. Samples have been prepared at the end of this step (N2 for two days neurons). After removal of bFGF, cells are allowed to differentiate during 4 more days and the last samples have been prepared from this step (N6). RNA was prepared from four independent experiments and verified by RT-PCR of reference genes.

| Abbreviation | Description | Arrays |
|---|---|---|
| 0es | ES cells | 4 |
| eb3 | 3 days old embroid bodies | 4 |
| eb4 | 4 days old embroid bodies | 2 |
| eb6ctl | 6 days old embroid bodies; without retinoic acid stimulation | 4 |
| eb6ra | 6 days old embroid bodies; with retinoic acid stimulation | 4 |
| eb8ctl | 8 days old embroid bodies; without retinoic acid stimulation | 4 |
| eb8ra | 8 days old embroid bodies; with retinoic acid stimulation | 4 |
| n2ctl | (8 +) 2 days old neurons; without retinoic acid stimulation | 4 |
| n2ra | (8 +) 2 days old neurons; with retinoic acid stimulation | 4 |
| n6ctl | (8 +) 6 days old neurons; without retinoic acid stimulation | 4 |
| n6ra | (8 +) 6 days old neurons; with retinoic acid stimulation | 4 |

AVEF-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-669

Accession: E-TABM-669

# Project CNRS-UMR-6543: Differentiation to early mesoderm (day3) and further to adipocyte precursors (day 6)

**Cell line: CGR8**

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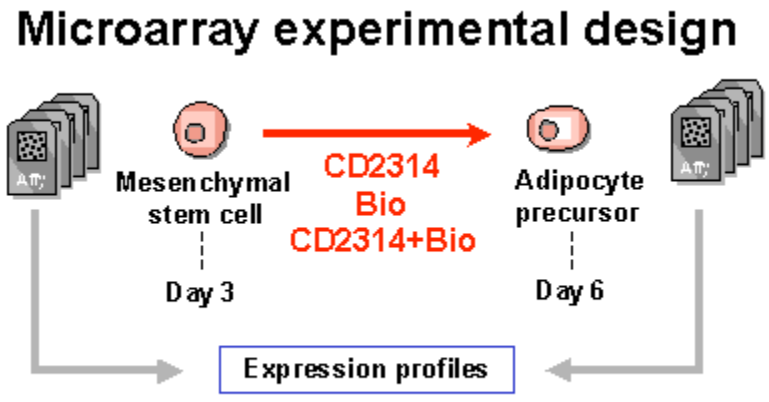
## Summary of the project

Embryoid Bodies have been treated either with CD2314 (a Retinoic Acid Receptor β agonist), Bio (a canonical wnt pathway inducer) or both between day 3-6 of differentiation and RNA samples have been prepared at day 6 and at day 11.



We have generated a high number of hanging drops, i.e., 62,100 drops, for each series of experiments and three independent series have been performed.

RNAs have been isolated and controlled for quality. Then, regulation of mesodermal differentiation for each condition and each series of experiments has been investigated by RT-PCR.

| Abbreviation | Description | Arrays |
|---|---|---|
| day03 | CGR8 cells at day 3; not stimulated | 3 |
| day06-0 | CGR8 cells at day 6; not stimulated | 3 |
| day06-bio | CGR8 cells at day 6; stimulated with BIO | 3 |
| day06-cd | CGR8 cells at day 6; stimulated with CD2314 | 3 |
| day06-cd-bio | CGR8 cells at day 6; stimulated with CD2314 and bio | 3 |
| day11-0 | CGR8 cells at day 11; not stimulated | 3 |
| day11-bio | CGR8 cells at day 11; stimulated with BIO | 3 |
| day11-cd | CGR8 cells at day 11; stimulated with CD2314 | 3 |
| day11-cd-bio | CGR8 cells at day 11; stimulated with CD2314and BIO | 3 |

CNRS-UMR-6543 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-668

Accession: E-TABM-668

# Project IPK-1: Differentiation of ESC to multilineage progenitors and further to committed pancreatic progenitors

**Cell lines: R1; R1-PAX4**

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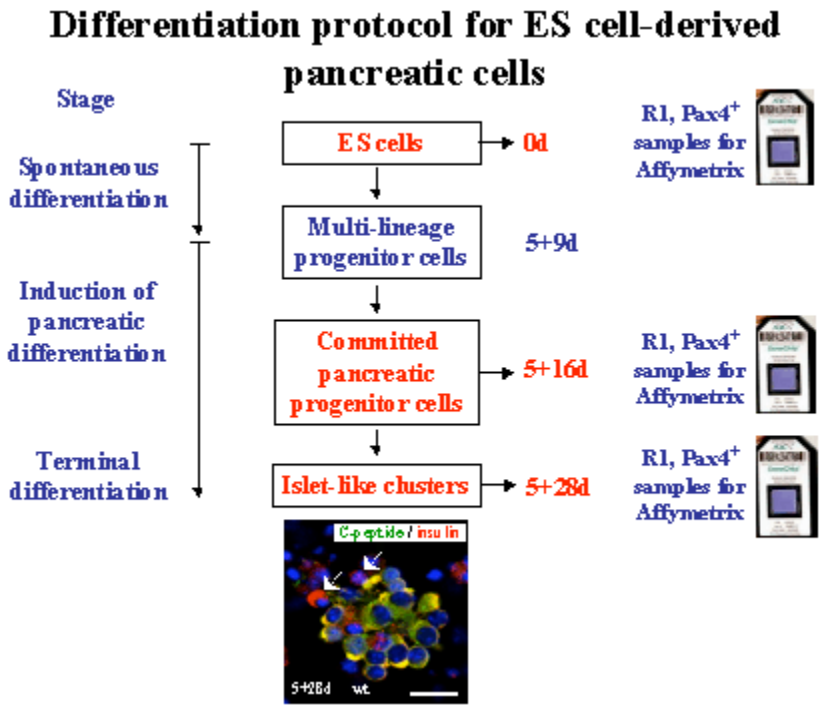
## Summary of the project




To characterize potential pancreatic progenitors during the process of induced pancreatic differentiation, wild-type R1 cells and R1 cells stably expressing the pancreatic control

gene Pax4, RNA samples of undifferentiated cells, pancreatic precursor and differentiated cells were prepared.

| Abbreviation | Description | Arrays |
|---|---|---|
| R1-ESC-1 | R1; ES cells (untreated) | 5 |
| R1-5-09d-2 | R1; 5 + 9 days | 5 |
| R1-5-16d-3 | R1; 5 + 16 days | 5 |
| R1-5-28d-4 | R1; 5 + 28 days | 5 |
| Pax4-ESC-1 | R1-PAX; ES cells (untreated) | 5 |
| Pax4-5-16d-3 | R1-PAX; 5 + 16 days | 5 |
| Pax4-5-28d-4 | R1-PAX; 5 + 28 days | 5 |

IPK-1 raw data (Affymetrix cel files of type Mouse 430_2) have been stored in the EMBL EBI ArrayExpress database:

http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-493

Accession: E-TABM-493

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