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High-throughput screen for genes predominantly expressed in the ICM of mouse blastocysts by whole mount *in situ* hybridization

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Abstract

Mammalian preimplantation embryos provide an excellent opportunity to study temporal and spatial gene expression in whole mount *in situ* hybridization (WISH). However, large-scale studies are made difficult by the size of the embryos (~60 μ m diameter) and their fragility. We have developed a chamber system that allows parallel processing of embryos without the aid of a microscope. We first selected 91 candidate genes that were transcription factors highly expressed in blastocysts, and more highly expressed in embryonic (ES) than in trophoblast (TS) stem cells. We then used the WISH to identify 48 genes expressed predominantly in the ICM and to follow several of these genes in all seven preimplantation stages. The ICM-predominant expressions of these genes suggest their involvement in the pluripotency of embryonic cells. This system provides a useful tool to a systematic genome-scale analysis of preimplantation embryos.

Keywords

preimplantation embryo; whole mount *in situ* hybridization; High-throughput screen; hybridization chamber; ICM; TE

Introduction

Preimplantation development encompasses the period from fertilization to implantation, and is marked by a number of critical events, including the degradation of maternally stored RNAs, zygotic genome activation (ZGA), compaction, and blastocyst formation (reviewed in (Edwards, 2003)). From the viewpoints of developmental potency (potential), fertilized eggs are the ultimate totipotent cells, giving rise to all cell types. The loss of totipotency occurs during preimplantation development, marked by the segregation of two distinct cell lineages in the blastocyst: the inner cell mass (ICM), which gives rise to the embryo proper and is thus pluripotent, and the trophectoderm (TE), which contributes to the trophoblast portion of the placenta and is thus lineage-restricted (Fig. 1B). Genes that are important for cellular

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Supplementary Materials

- (1) A blueprint of WISH Aluminum Chamber.
- (2) Step-by-step WISH protocol.
- (3) ES versus TS microarray data.

pluripotency, such as *Pou5f1/Oct4* (Pesce and Scholer, 2000) and *Nanog* (Chambers et al., 2003; Mitsui et al., 2003), are predominantly expressed in the ICM, and thus, the identification of genes expressed in the ICM will be an important first step towards understanding the cellular potency. Whether the emergence of such asymmetry between the ICM and TE originates from an earlier event, such as fertilization, is still controversial (Gardner, 2001; Hiragi and Solter, 2004; Piotrowska et al., 2001).

Large-scale systematic analysis holds great promise for understanding preimplantation embryos as a whole (Ko, 2001). A large number of cDNA clones have been identified from mouse preimplantation embryos and mapped to the mouse genome (Ko et al., 2000; Sharov et al., 2003; Solter et al., 2002). Microarray analysis of the preimplantation embryos has provided global picture of expression changes during preimplantation mouse development (Hamatani et al., 2004; Tanaka and Ko, 2004; Wang et al., 2004; Zeng et al., 2004). The knowledge of genes expressed in preimplantation mouse embryos has increased dramatically. However, because RNA samples are taken from homogenized tissues, spatial information is lost, and thus, questions of their asymmetric expression cannot be directly addressed. WISH allows localization of gene transcripts in the individual cell, enabling the study of the heterogeneity of cells and/or their polarity at very early stages of the embryo, in which no morphological differences are seen among cells.

Large-scale *in situ* hybridizations have been performed on mouse intestine (Komiya et al., 1997), E9.5 embryos (Gitton et al., 2002; Neidhardt et al., 2000), and E9.5 and E10.5 embryos (Reymond et al., 2002), and mouse brain as well as on other species, such as *Drosophila* (Tomancak et al., 2002), Zebrafish (Kudoh et al., 2001), *Xenopus* (Gawantka et al., 1998), Medaka Fish (Quiring et al., 2004), Chick retina (Shintani et al., 2004), Ascidian (Mochizuki et al., 2003), Chicken embryos (Bell et al., 2004). A robotic workstation is available, but due to its larger filter pore size (35 μm) it cannot be used for small embryos, such as mammalian preimplantation embryos. Due to the technical difficulty of handling small embryos, WISH data for mouse preimplantation embryos is scarce even with small-scale methods based on individual genes. During the pipetting procedure, embryos are often lost. This has been addressed by using a microcentrifuge tube, which was cut at the bottom and attached to a 20 μm pore membrane (Newman-Smith and Werb, 1995). The method has successfully circumvented laborious micropipetting work, but the microtubes were made by hand each time and were not suited for parallel processing. While a pore size of 20 μm is necessary for achieving efficient drainage without special instruments, much smaller pores are preferable to maintain the best morphology of small samples. As a result, transwell with pore size 12 μm which are originally designed for cell culture were introduced into WISH (Hanna et al., 2002) to retain embryos. Although, solution changes were achieved by manually transferring the transwell from one well to another, it is difficult to have good buffer exchange through smaller pores without the assistance of a special device. Here we report the development of a chamber system that utilizes both the transwell inserts for parallel processing and capillary action for gentle buffer exchanges. Using this method, we have identified 48 genes that are expressed predominantly in the ICM.

1. Results

1.1. Design and fabrication of WISH chamber system

To perform a high-throughput WISH for preimplantation embryos (up to 100 μm diameter), we developed a chamber system that can run multiple probes in parallel without microscope-assistance (Fig. 1A). Embryos can be placed in plastic Transwell-inserts with 8 μm pore-size membrane on the bottom. Up to 20 inserts can be placed in one aluminum chamber, which allows analysis of up to 20 different probes in parallel. The small pore size helps maintain good embryo morphology while minimizing the chance of embryo loss during the WISH procedure.

However, the small pore size makes it difficult to drain the solution through the bottom membrane. Initial design used negative air pressure by vacuum pump, resulting in poor morphology of embryos. We then devised a chamber system so that the distance between the bottom of the insert holder and the bottom of the solution container formed a small gap of 0.5 mm. This turned out to be the most effective way of draining the solution from the bottom of the transwell by capillary action. Solutions were exchanged through the port and the drainage was completed within seconds in every insert simultaneously.

One run of a WISH experiment takes only two days: one hour for the first day and six hours for the second day including the incubation time. Because multiple embryos of various stages can be processed in parallel, literally hundreds of embryos can be analyzed, and thus the collection of embryos becomes the actual rate-limiting step. Time-consuming microscopy and micropipetting are limited only to the step for transferring embryos into inserts. Overall, this new device and new protocol dramatically increased the throughput of WISH on preimplantation embryos over the conventional micropipetting method.

1.2. Test of the WISH chamber system

The WISH protocol was optimized by using antisense and sense cRNA probes for three representative genes. *Pou5f1* (*Oct3/4*) is known to be expressed specifically in ICM of blastocysts, *Krt2-8*, in TE, and *Actb*, ubiquitously. The morphology of blastocyst was maintained relatively well and all results were consistent with previous reports (Fig. 2). It should be pointed out that because the ICM forms a dense clump inside the blastocysts, for genes that are ubiquitously expressed in both the ICM and TE, the staining of the ICM would look more intense compared to TE. On the other hand, genes that appear to be stained uniformly (e.g., *Krt2-8*) are in reality expressed predominantly in TE. This makes it difficult to distinguish genes that are predominantly expressed in ICM from genes that are ubiquitously expressed. After some trial and error, we decided to use a scoring system that utilized the staining patterns of *Krt2-8*, *Pou5f1*, and *Actb* as the standards. The pictures for individual genes were always compared to the pictures of these genes.

1.3. Informatics selection of candidate genes

We used a series of informatic criteria to select candidate genes that are most likely expressed predominantly in the ICM (Fig. 1C). First, we identified 6484 genes that are highly expressed in mouse blastocysts by examining EST frequency data (Sharov et al., 2003) and microarray data (Hamatani et al., 2004). We then attempted to identify candidate genes that are expressed more highly in the ICM than in TE cells. Due to difficulty of collecting the ICM and TE from blastocysts separately, we exploited the cultured cells derived from these cell types in our previous study (Tanaka et al., 2002). Mouse ES cells are cultured from the ICM (Evans and Kaufman, 1981; Martin, 1981), whereas mouse TS cells are cultured from the TE (Tanaka et al., 1998) (Fig. 1B). Although these cells have been cultured *in vitro*, they represent the *in vitro* equivalents of the ICM and TE. Therefore, genes which are expressed more highly in ES than TS in microarray studies were good candidates for genes predominantly expressed in the ICM in blastocysts.

To supplement the data (Tanaka et al., 2002) and update to the latest microarray platform, we performed hybridizations of ES and TS RNAs onto the NIA 22K 60-mer oligonucleotide microarrays in triplicate (Carter et al., 2003a). This glass-slide microarray platform contains genes from preimplantation and stem cells (for experimental design, see ref (Tanaka et al., 2002); for all the array data set, see <http://lgsun.grc.nia.nih.gov/data>, and also Supplementary Material 3). By this criterion, we identified 1388 genes that were expressed more highly in ES cells than in TS cells. Because we were interested in transcription factors, we further narrowed down the list and identified 95 genes, which have “transcription factor activity” in GO terms

(Ashburner et al., 2000). During the course of this work, however, the assignment and GO annotation of genes have been changed and at present 65 genes out of these 95 genes can be classified as transcription factors.

1.4. Genes expressed predominantly in ICMs

Out of 95 genes, we were able to find 91 cDNA clones from the NIA mouse cDNA collection (Sharov et al., 2003). We attempted to prepare probes for these 91 genes, but two probes did not pass the quality check. After adding *Actb* and *Krt2-8* genes as controls, a total number of probes became 91. Results of the WISH using antisense probe for the 91 genes are shown in Fig. 2 and summarized in Table 1. The list included three genes (*Nanog*, *Pou5f1*, *Otx2*) which have been reported to be expressed specifically in ICM by WISH (Chambers et al., 2003; Kimura et al., 2001; Mitsui et al., 2003). When the chamber system was used on these genes, *Nanog*, and *Pou5f1* had signals only in the ICM, whereas *Otx2* showed weak, but rather ICM-predominant expression (Fig. 2).

Overall, out of 91 genes, 48 genes were scored for higher signals in the ICM than the TE. However, there was no gene that showed clear-cut ICM-dominant expression like *Pou5f1* (*Oct3/4*). Rather, they showed differential expression, with some expression still detectable in TE. Among these genes, 14 genes showed particularly high-contrast signals between the ICM and TE: *Csda*, *E2f5*, *Gmeb1*, *Gtf2e2*, *Gtf2i*, *Kif22*, *Mybl2*, *Nanog*, *Nmyc1*, *Smarca4*, *Sox13*, *Sox15*, *Tbp*, and *Zfp143*.

1.5. All stage analysis of selected genes

To test whether this chamber system can be applied to all preimplantation stages, we selected seven genes with apparent ICM-predominant expression. We performed WISH on embryos from unfertilized eggs to blastocysts. All-stage WISH confirmed that the seven genes that we selected indeed showed the ICM-predominant expression. The expression patterns of *Pou5f1* (Hanna et al., 2002; Pelton et al., 2002) and *Nanog* (Chambers et al., 2003; Mitsui et al., 2003) confirmed previously published results. Two new genes identified here were *Mybl2* and *Gtf2e2*. *Mybl2* (also called *B-Myb*) is myeloblastosis oncogene-like 2 and is known to play a major role during S phase (Joaquin and Watson, 2003). In the microarray analysis of preimplantation mouse development, *Mybl2* gene is grouped in Cluster 2 together with *Nanog*, *Lefty*, *Pcaf*, and *Dnmt3a* (Hamatani et al., 2004). These WISH results confirmed this microarray-based finding. It is reported that *Mybl2*^{-/-} mice die at around E4.5 - E6.5 and *in vitro* culture of *Mybl2*^{-/-} blastocyst indicates that *Mybl2* is required for ICM formation (Tanaka et al., 1999). The ICM-predominant expression of *Mybl2* is thus consistent with this null phenotype.

Gtf2e2 encodes one of the two (beta) subunits of general transcription factor IIE (TFIIE), which recruits TFIIF to the initiation complex and modulates its kinase and helicase activities (Enkhmandakh et al., 2004). Interestingly, one of the subunits of the TFIIF (*Gtf2h3*), TATA box binding protein (*Tbp*/TFIID), one of the subunits of RNA polymerase II (*Polr2i*), transcription elongation factor A (SII) 3 (*Tcea3*), and one of the TATA box binding protein (TBP)-associated factor (*Taf7*) were also identified here for ICM-predominant expression.

2. Discussion

The high-throughput WISH system described here has provided spatial and temporal expression patterns of many genes during preimplantation development. This rather simple system is expandable to increase the number of probes tested in one session. The device can be used for embryos or organs in a similar size range (~100 μm diameter) without any modification. Materials with larger size ranges, such as postimplantation mammalian embryos,

Xenopus embryos, Zebrafish embryos, can also be done, probably with slight modification. Of course, the WISH will tell us only the expression patterns of RNAs, but not proteins, and there are differences between the localizations of RNAs and proteins. For example, *Smarca4* showed ICM-predominant expression of RNAs in this study, but an earlier immunohistochemical study showed the protein localizing in both ICM and TE (LeGouy et al., 1998). Similarly, immunohistochemistry showed that the protein GTF2I2 is localized in both ICM and TE (Enkmandakh et al., 2004), but our *in situ* study showed ICM-predominant RNA localization. However, RNA localization provides a valuable entry point for further study. The *in situ* images have been incorporated into the Open Microscopy Environment (OME), which will allow web access of these images (Swedlow et al., 2003). An automatic image classification system that is being developed (I.G., unpublished) can eventually facilitate the automatic analysis of these images. The genes that have been identified for the ICM-predominant expression will be good candidates for further analysis of their role in preimplantation development and cellular pluripotency.

3. Experimental Procedures

3.1. Gene Selection and Annotation

We combined the following public database's Gene Ontology terms and eliminated redundancy in each 'U' cluster member sequences to generate Gene Ontology terms for each 'U' cluster. 1. Based on Fantom2 sequence membership in NIA Mouse Gene Index (version 1). 2. Based on InterPro domain names. 3. Based on LocusLink. We searched the above databases in April 2003.

3.2. Design and fabrication of aluminum chamber

Parallel micro WISH system consists of four aluminum parts and disposable Transwell-inserts (Corning) (Fig. 1A, 1B). Our device follows standard 24-well format and can hold up to 20 inserts. Solutions were exchanged through "port" shown in the figure. Exact dimensions of the chamber are available for its fabrication (Supplementary Material 1 or <http://lgsun.grc.nia.nih.gov/data>). Transwell-inserts were placed in the insert-holder, which was then placed in the solution container. There is a 0.5 mm gap between the bottom of the insert and the bottom of the solution container. We add 20–25 ml solution through the port of the insert-holder. The solution fills the space between the insert-holder and the solution container and then enters each transwell-insert through the microporous membrane on the bottom of inserts. Embryos in the insert were suspended in about 100 - 200 μ l solution. The whole chamber is agitated on a shaker during washing steps. We drain solutions by suction through the same port of the insert-holder. Because of the capillary pressure generated in the gap, the same negative pressure was applied to all Transwell-inserts and the drainage was completed within seconds simultaneously in all inserts.

3.3. Embryo collection

We collected one-cell embryos and blastocysts from super-ovulated mice at 0.5 dpc and 3.5 dpc, respectively. Oocytes were collected from unmated super-ovulated females. We collected *in vitro* cultured two-, four-, eight-cell stage embryos, and morula.

3.4. Whole Mount In Situ Hybridization (WISH)

For each gene in the list, we identified a cDNA clone that contains approximately 1 kb 3'-end sequence from the NIA mouse cDNA collection (Carter et al., 2003b) (<http://lgsun.grc.nia.nih.gov/cDNA>). All the EST clones are available through ATCC (individual clones) and the designated academic centers (Tanaka et al., 2000; VanBuren et al., 2002). In this mouse cDNA collection, cDNAs were cloned at the Sall/NotI site of vector pSPORT1 or

pCMV-SPORT6 (Invitrogen). We prepared the DIG-labeled RNA probes by digesting plasmids followed by *in vitro* transcription using SP6 or T7 RNA polymerase.

Preimplantation embryos were kept in a Transwell-insert (Corning, Catalogue #3422) during the entire WISH procedure. It has a polycarbonate membrane with pore size of 8.0 μm at the bottom, and thus can retain embryos and allow sufficient solution exchange with the aid of the Chamber System. The insert (6.5 mm diameter) fits to a well of a 24-well plate and a well of our custom-made aluminum chamber device (see below for more details). This chamber device enables us to change solutions simultaneously for all inserts placed in the chamber. WISH was performed essentially as described (Wilkinson and Nieto, 1993). Detailed step-by-step WISH protocol is available (Supplementary Material 2; <http://lgsun.grc.nia.nih.gov/data>). Here we highlight major steps. (1) Fix embryos in 4% paraformaldehyde in PBT (phosphate buffered saline with 0.1% Tween-20) at 4 °C for 30 min. – overnight. (2) Treat embryos with Proteinase K. (3) Fix embryos again in 4% paraformaldehyde, 0.2% EM-grade glutaraldehyde in PBT at room temperature for 20 minutes. (4) Treat embryos with prehybridization buffer (4X SSC [pH7.0], 50% deionized Formamide, 100 $\mu\text{g}/\text{ml}$ Heparin, 250 $\mu\text{g}/\text{ml}$ Yeast tRNA, 100 $\mu\text{g}/\text{ml}$ Salmon Sperm DNA, 2X Denhardt's solution, 0.1% Tween-20) typically at 60 °C (or up to ~70 °C) for 3 – 8 hrs. (5) Treat embryos with DIG-RNA probe in hybridization buffer for overnight at the same temperature used for prehybridization. (6) Wash embryos with a buffer (50% Formamide, 2X SSC, 0.1% Tween-20) at least three times at the hybridization temperature. (7) Follow the manufacturer's recommended procedure to detect DIG-labeled probes. (8) Transfer embryos suspended in PBS (phosphate buffered saline) containing 1 mM EDTA and 20% glycerol to a 24-well plate for brightfield photographing with 20X objective lens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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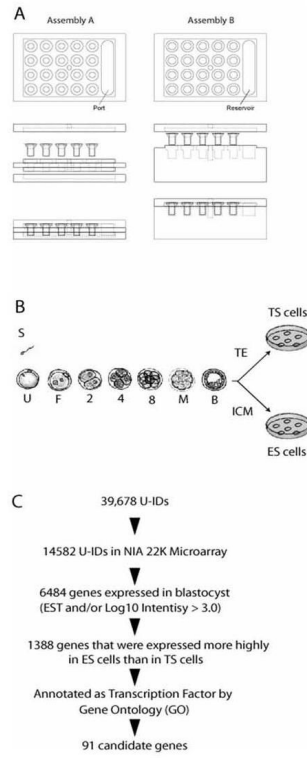


Figure 1. (A) Assembly A: Washing chamber. Assembly B: Hybridization chamber. (B) Schematic drawings of mouse preimplantation development. At blastocyst stage, there are two different cell types: Trophectoderm (TE) and Inner Cell Mass (ICM). ES cells are derived from the ICM, whereas TS cells are derived from the TE cells. (C) Bioinformatic selection of candidate genes.

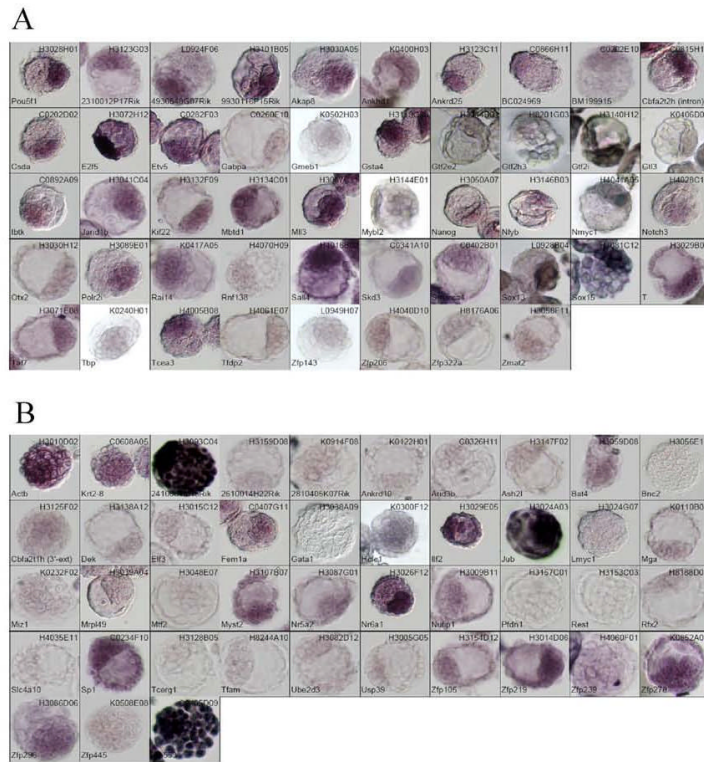


Figure 2. WISH results of 91 genes: Each image is accompanied with a gene symbol and a cDNA clone name used as a probe. Panel A, genes identified for their ICM-predominant expression (including *Pou5f1*). Panel B, genes that were not expressed in ICM-predominant manner (including *Actb* and *Krt2-8*).

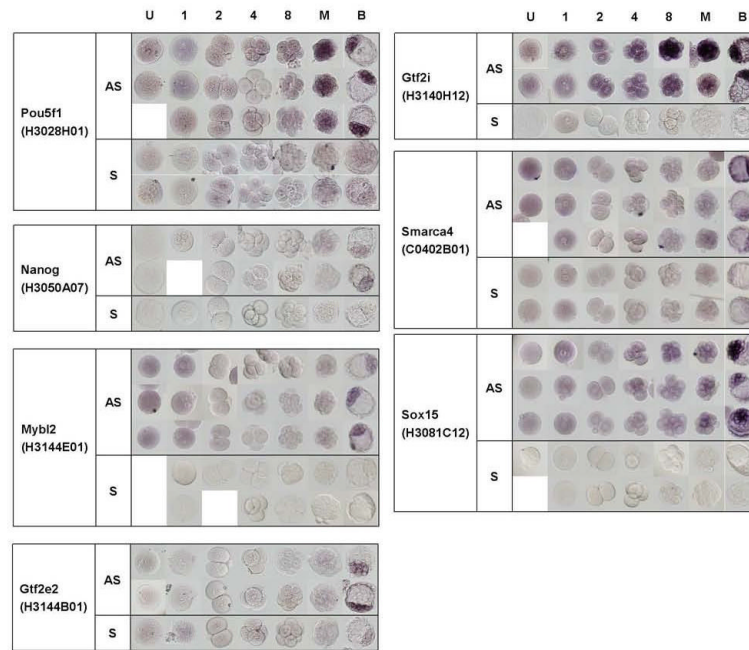


Figure 3. WISH results of 7 genes during preimplantation development. AS; antisense probe, S; sense probe

Table 1

Summary of WISH results. Clone_ID indicates cDNA clones that were used for cRNA probe preparation. U_ID is the identification of U cluster, which represents individual genes in the NIA Mouse Gene Index (Sharov et al., Genome Res., in press; <http://lgsun.grc.nia.nih.gov/geneindex4>). Log intensity is the mean value of signal intensities at log-scale measured in triplicate, which represents the expression of level of genes in mouse blastocyst (from the data in ref. (Hamatani et al., 2004)). Fold (ES/TS) indicates the ratio of signal intensities for each gene between ES cell and TS cell, averaged among biological replicates. The intensity of staining in ICM and TE in WISH are shown arbitrarily in 5 grades (-, +/-, +, ++ and +++) as the highest intensity for the amount of probe, either 1 or 5 μ l). Non specific staining is shown as NS.

Clone_ID	U_ID	Gene Symbol	Annotation	Transcription factor?	Log Intensity (Blastocyst)	Fold (ES/TS)	Oligo ID	ICM (1 ul)	TE (1 ul)	ICM (5 ul)	TE (5 ul)	ICM predominant?
H3028H01	U017906	Pou5f1	POU domain, class 5, transcription factor 1	Yes	3.28	11.08	Z04894	++	-	++	+/-	Yes
H3123G03	U042665	2310012P17Rik	RIKEN cDNA	No	3.13	2.40	Z18099	-	-	++	+/-	Yes
L0924F06	U015681	4930548G07Rik	2310012P17 gene	Yes	4.03	1.35	Z12748	++	-	++	+	Yes
H3101B05	U036004	9930116P15Rik	4930548G07 gene	No	3.13	1.94	Z10610	-	-	++	+/-	Yes
H3030A05	U037662	Akap8	Mus musculus ES cells cDNA, RIKEN full-length enriched library, clone:C330022M23	No	2.95	1.58	Z04916	++	+/-	NS	NS	Yes
K0400H03	U018590	Ankhd1	A kinase (PRKA) anchor protein 8	Yes	3.14	1.22	Z15071	+/-	-	++	+/-	Yes
H3123C11	U030590	Ankrd25	A062A08 GGTC Gene	Yes	3.05	1.39	Z11802	+	-	++	+/-	Yes
C0866H11	U038601	BC024969	Trap Library GV03C04	Yes	3.59	1.18	Z04004	-	-	++	+/-	Yes
C0202E10	U089485	BM199915	Mus musculus cDNA clone A062A08	Yes	3.98	1.21	Z20771	+/-	-	++	+/-	Yes
C0815H11	U208874	Cbfa2l2h (intron)	ankyrin repeat domain 25	Yes	2.91	1.46	Z03694	++	+	++	+/-	Yes
C0202D02	U027935	Csda	MGC cDNA	Yes	3.72	1.26	Z01041	+	-	++	+/-	Yes
H3072H12	U003038	E2f5	core-binding factor, runt domain, alpha subunit 2, translocated to, 2 homolog (human)	Yes	3.12	1.74	Z08851	+/-	-	+++	+	Yes
C0282F03	U055077	Etv5	cold shock domain protein A	Yes	3.60	2.93	Z02758	-	-	++	+/-	Yes
C0260F10	U043544	Gabpa	E2F transcription factor 5	Yes	3.34	1.70	Z20218	N/A	N/A	+	-	Yes
K0502H03	U025592	Gmeb1	GA repeat binding protein, alpha	Yes	3.74	1.17	Z15720	+	-	++	+	Yes
H3119G08	U010777	Gsta4	glucocorticoid modulatory element binding protein 1	No	3.69	21.43	Z11620	+	-	++	+	Yes
H3144B01	U009246	Gtf2e2	glutathione S-transferase, alpha 4	Yes	3.27	1.46	Z19185	+	-	++	+	Yes
H8201G03	U006190	Gtf2h3	general transcription factor II E, polypeptide 2 (beta subunit)	Yes	3.05	1.45	Z19769	+	-	+	+/-	Yes
H3140H12	U026798	Gtf2i	general transcription factor IIIH, polypeptide 3, 34kDa	Yes	3.77	2.91	Z19056	++	-	+++	+	Yes
K0406D08	U030135	Gtl3	general transcription factor III I	No	3.64	1.47	Z09177	+	-	+	+/-	Yes
C0892A09	U031153	Ibtk	gene trap locus 3	No	3.85	1.49	Z17931	++	-	+/-	+/-	Yes
			inhibitor of Bruton agammaglobulinemia tyrosine kinase									

Clone_ID	U_ID	Gene Symbol	Annotation	Transcription factor?	Log Intensity (Blastocyst)	Fold (ES/TS)	Oligo ID	ICM (1 ul)	TE (1 ul)	ICM (5 ul)	TE (5 ul)	ICM predominant?
H3041C04	U000832	Jarid1b	jumonji, AT rich interactive domain 1B (Rbp2 like)	Yes	3.27	2.22	Z07056	+/-	-	++	+/-	Yes
H3132F09	U029275	Kif22	kinesin family member 22	No	2.67	1.75	Z18589	-	-	++	-	Yes
H3134C01	U013196	Mbd1	mbt domain containing 1	No	3.09	1.62	Z18681	-	-	++	+/-	Yes
H3057C11	U026010	Mll3	myeloid/lymphoid or mixed-lineage leukemia 3	Yes	3.12	1.48	Z07970	-	-	++	+/-	Yes
H3144E01	U002779;U095436;U165175	Mybl2	myeloblastosis oncogene-like 2	Yes	3.89	1.38	Z19198	++	-	+++	+	Yes
H3050A07	U007377;U125018	Nanog	Nanog homeobox	Yes	3.55	4.64	Z07563	++	-	++	-	Yes
H3146B03	U032063	Nfyb	nuclear transcription factor-Y beta	Yes	3.40	1.41	Z12544	-	-	+	-	Yes
H4041A05	U033813	Nmyc1	neuroblastoma myc-related oncogene 1	Yes	3.09	2.73	Z21669	+	-	NS	NS	Yes
H4028C12	U037659	Notch3	Notch gene homolog 3 (Drosophila)	Yes	4.00	1.22	Z20375	+	-	+	-	Yes
H3030H12	U035481	Orx2	orthodenticle homolog 2 (Drosophila)	Yes	3.25	4.59	Z04978	-	-	++	+/-	Yes
H3089E01	U007921	Polr2i	Mus musculus cDNA clone MGC:73656 IMAGE:3466417, complete cds.	Yes	3.39	1.88	Z10011	-	-	++	+/-	Yes
K0417A05	U036007	Rai14	retinoic acid induced 14	Yes	3.58	1.69	Z19497	-	-	++	+/-	Yes
H4070H09	U018467	Rnf138	ring finger protein 138	No	3.43	1.32	Z16641	-	-	+	-	Yes
H4016B02	U023697	Sall4	sal-like-4 (Drosophila)	No	3.86	3.04	Z13538	+++	+	+++	+	Yes
C0341A10	U008471	Skd3	suppressor of K+ transport defect 3	Yes	3.72	1.28	Z02265	+/-	-	+	-	Yes
C0402B01	U010150	Smarc4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	Yes	3.55	1.67	Z06449	++	+/-	++	+/-	Yes
L0928B04	U021771	Sox13	SRY-box containing gene 13	Yes	3.48	2.66	Z05552	++	+/-	+++	++	Yes
H3081C12	U012826	Sox15	SRY-box containing gene 15	Yes	3.49	2.27	Z09444	++	+	+++	+	Yes
H3029B05	U017472	T	brachyury	Yes	3.93	1.48	Z06600	++	+/-	++	+	Yes
H3071E08	U038350	Taf7	TAF7 RNA polymerase II, (TBP)-associated factor	Yes	3.47	2.56	Z14353	+/-	-	++	+/-	Yes
K0240H01	U017529	Tbp	TATA box binding protein	Yes	3.20	1.21	Z09055	+	-	+/-	-	Yes
H4005B08	U004965	Tcea3	transcription elongation factor A (SII), 3	Yes	3.08	6.22	Z14874	+	-	++	+/-	Yes
H4061E07	U043020	Tfdp2	transcription factor Dp 2	Yes	2.58	1.66	Z03781	-	-	+	-	Yes
L0949H07	U008625	Zfp143	zinc finger protein 143	Yes	3.09	1.29	Z09829	+	-	+/-	-	Yes
H4040D10	U043572	Zfp206	zinc finger protein 206	Yes	4.41	1.21	Z02924	-	-	+	-	Yes
H8176A06	U072774	Zfp322a	zinc finger protein 322a	No	3.52	1.73	Z04195	N/A	N/A	+	-	Yes
H3058F11	U018598	Zmat2	zinc finger, matrix type 2	No	3.13	1.47	Z14005	-	-	+	-	Yes
H3010D02	U026912	Actb	actin, beta, cytoplasmic	No	4.92	0.09	Z06684	+++	++	N/A	N/A	No
C0608A05	U036682	Krt2-8	cyokeratin endo A	No	3.06	3.82	Z10249	NS	NS	NS	NS	No
H3093C04	U025552	2410081M15Rik	RIKEN cDNA 2410081M15 gene	No	3.06	3.82		NS	NS	NS	NS	No

Clone_ID	U_ID	Gene Symbol	Annotation	Transcription factor?	Log Intensity (Blastocyst)	Fold (ES/TS)	Oligo ID	ICM (1 ul)	TE (1 ul)	ICM (5 ul)	TE (5 ul)	ICM predominant?
H3159D08	U028390	2610014H22Rik	Mus musculus cDNA clone IMAGE:5704432, partial cds.	Yes	3.30	1.19	Z19782	-	-	+/-	-	No
K0914F08	U023323	2810405K07Rik	RIKEN cDNA 2810405K07 gene	Yes	3.32	1.56	Z15697	-	-	+/-	-	No
K0122H01	U029585	Ankrd10	ankyrin repeat domain 10	Yes	3.50	1.91	Z01774	-	-	-	-	No
C0326H11	U108385	Arid3b	AT rich interactive domain 3B (Bright like)	No	3.60	1.50	Z02467	-	-	+/-	-	No
H3147F02	U029672	Ash2l	ash2 (absent, small, or homeotic)-like (Drosophila)	Yes	3.83	1.46	Z02226	-	-	+/-	-	No
H3059D08	U065275,U017890	Bat4	Mus musculus adult male epididymis cDNA, RIKEN full-length enriched library, clone:9230110P17	Yes	3.56	1.16	Z08070	-	-	+	+/-	No
H3056E10	U025224	Bnc2	basonuclin 2	No	3.14	1.31	Z13933	N/A	N/A	-	-	No
H3125F02	U215470,U183035	Cbfa2l1h (3'-ext)	CBFA2T1 identified gene homolog	Yes	3.51	1.29	Z18223	-	-	+/-	+/-	No
H3138A12	U034741,U134034	Dek	DEK oncogene (DNA binding)	Yes	3.65	1.90	Z12228	-	-	+	+	No
H3015C12	U021793	Elf3	E74-like factor 3	Yes	3.55	1.37	Z06047	-	-	+	+/-	No
C0407G11	U018129,U012414	Fem1a	feminization 1 homolog a (C. elegans)	Yes	3.70	1.23	Z01940	-	-	+	+/-	No
H3038A09	U039236	Gata1	GATA binding protein 1	Yes	4.48	1.29	Z13445	-	-	N/A	N/A	No
K0300F12	U039463	Hcf1	host cell factor C1	No	3.69	1.33	Z00572	-	-	NS	NS	No
H3029E05	U003494	Ilf2	interleukin enhancer binding factor 2	No	3.71	1.60	Z06628	-	-	++	+	No
H3024A03	U035555	Jub	ajuba	No	3.19	2.43	Z06379	NS	NS	NS	NS	No
H3024G07	U042574	Lmyc1	lung carcinoma myc related oncogene 1	Yes	3.41	1.25	Z06413	-	-	-	-	No
K0110B04	U002325	Mga	MAX, gene associated	Yes	3.85	1.77	Z19295	-	-	+/-	-	No
K0232F02	U018914	Miz1	Mus musculus Msx-interacting-zinc finger (Miz1), mRNA	Yes	2.66	1.92	Z06850	+/-	+/-	+/-	+/-	No
H3039A04	U019023	Mrp149	mitochondrial ribosomal protein L49	No	3.15	1.50	Z06929	-	-	+/-	-	No
H3048E07	U005938	Mtf2	metal response element binding transcription factor 2	Yes	4.03	4.22	Z07461	-	-	-	-	No
H3107B07	U033300	Myst2	MYST histone acetyltransferase 2	Yes	3.45	1.23	Z11023	-	-	++	+	No
H3087G01	U021824	Nr5a2	nuclear receptor subfamily 5, group A, member 2	Yes	2.94	3.28	Z09889	-	-	+	+/-	No
H3026F12	U022570	Nr6a1	nuclear receptor subfamily 6, group A, member 1	Yes	3.50	2.49	Z06529	+	+/-	+++	++	No
H3009B11	U015867	Nurfp1	nuclear fragile X mental retardation protein interacting protein	No	3.29	1.58	Z05791	-	-	+	+/-	No
H3157C01	U038334	Ptdn1	prefoldin 1	Yes	3.67	1.33	Z13037	-	-	-	-	No
H3153C03	U042648	Rest	RE1-silencing transcription factor	Yes	3.05	2.21	Z19589	-	-	-	-	No
H8188D01	U037958	Rfx2	regulatory factor X, 2 (influences HLA class II expression)	Yes	3.77	1.60	Z14719	-	-	+/-	-	No

Clone_ID	U_ID	Gene Symbol	Annotation	Transcription factor?	Log Intensity (Blastocyst)	Fold (ES/TS)	Oligo ID	ICM (1 ul)	TE (1 ul)	ICM (5 ul)	TE (5 ul)	ICM predominant?
H4035E11	U001816,U067822	Slc4a10	solute carrier family 4, sodium bicarbonate cotransporter-like, member 10	Yes	3.30	1.31	Z21184	-	-	-	-	No
C0234F10	U043484	Sp1	trans-acting transcription factor 1	Yes	3.49	1.87	Z01040	-	-	++	+	No
H3128B05	U043672	Tceerg1	transcription elongation regulator 1 (CA150)	Yes	4.04	1.37	Z18367	-	-	-	-	No
H8244A10	U031911	Tfam	transcription factor A, mitochondrial	Yes	2.66	1.22	Z02399	-	-	+/-	-	No
H3082D12	U003889	Ube2d3	UBIQUITIN-CONJUGATING ENZYME E2-17 KDA 3 (EC 6.3.2.19)	No	2.28	1.39	Z09504	-	-	+	+/-	No
H3005G05	U027470	Usp39	ubiquitin specific protease 39	No	3.20	1.33	Z05697	-	-	+/-	-	No
H3154D12	U089830,U011188	Zfp105	zinc finger protein 105	Yes	3.40	2.58	Z19647	+/-	-	++	+	No
H3014D06	U035530	Zfp219	zinc finger protein 219	Yes	3.58	1.77	Z05989	-	-	+++	++	No
H4060F01	U043940	Zfp239	zinc finger protein 239	Yes	3.02	2.90	Z03653	-	-	+	+/-	No
K0852A06	U012241	Zfp278	zinc finger protein 278	Yes	3.45	2.02	Z04975	+/-	-	++	+	No
H3086D06	U007769	Zfp296	zinc finger protein 296	No	3.82	3.71	Z09785	+/-	-	++	+	No
K0508E08	U031463	Zfp445	zinc finger protein 445	Yes	3.27	1.71	Z21112	+/-	-	+/-	+/-	No
C0405D09	U008795	Zfp553	zinc finger protein 553	No	3.18	1.56	Z02818	NS	NS	NS	NS	No