

# Single-embryo Gene Expression for Early Embryo Development



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## Application:

- Gene Expression

## Fluidigm Technology:

- BioMark System
- 48.48 dynamic array



Mylene Yao, M.D., and fellow Stanford University researchers found that *Oct4*, the master regulator of embryonic stem cell pluripotency, also has critical functions during reprogramming of the early mammalian embryo. This research may ultimately lead to improvements in human *in vitro* fertilization methods and is relevant to stem cell and cancer research.

They recently published their findings in a research paper entitled, *A Novel and Critical Role for Oct4 as a Regulator of the Maternal-Embryonic Transition*, available at <http://www.ncbi.nlm.nih.gov/pubmed/19129941>.

“Scientists from around the world are looking at how to reprogram a highly differentiated somatic cell into a pluripotent embryonic stem cell-like cell,” Dr. Yao said. “There are many different approaches with up and down sides to each; such as, how do you do this without causing side effects like cancer and how can you apply it to therapeutics? The question we ask is very different. We think the mouse, or human embryo, is a good model to study reprogramming because this is the only situ-

ation in nature where you have highly specialized cell types—the egg and sperm—that fuse together to undergo reprogramming to produce a pluripotent cell, that is capable of differentiating into many cell types.”

The Stanford scientists focused their efforts on the reprogramming mechanisms used by the em-

bryo that direct how pluripotency or totipotency is established. They picked the well-studied *Oct4* gene for its known reprogramming functions and because it is highly expressed in the oocyte and early one- to two-cell embryo stage.

“We microinjected specific antisense morpholino oligonucleotides (morpholinos) to knock down the [*Oct4*] gene,” Dr. Yao said. “This is a well established approach in other model organisms but not so much in the mouse. So we had to establish the protocols and the controls and it turned out to be a powerful technique. We were able to get very specific gene knockdown and highly reproducible results.”

Their first finding was that *Oct4* has a critical function prior to the blastocyst stage.

“That finding was new and we were very excited about it,” Dr.

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Single embryo data allowed us to identify genes that are consistently differentially regulated and to find rare outlier embryos expressing unique transcriptomes.

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## Protocol and Equipment

- BioMark™ RT-PCR system
- Fluidigm® 48.48 dynamic array
- Quake Lab single cell dynamic RT-PCR protocol
- Affymetrix® Whole Mouse Genome chip
- Nugen® protocol for amplification
- PicoPure® RNA Isolation (Molecular Devices)

Yao said. “What was known in the literature was that in the knock out mouse model, *Oct4*-deficient embryos can produce something that looks like a blastocyst...but it doesn’t continue to develop. It doesn’t make stem cells when you try to plate it because the cells are deficient or defective. In our model, because we inject morpholinos into the one-cell embryo, we are knocking down both the maternal transcripts and any embryonic transcripts that are going to be made.”

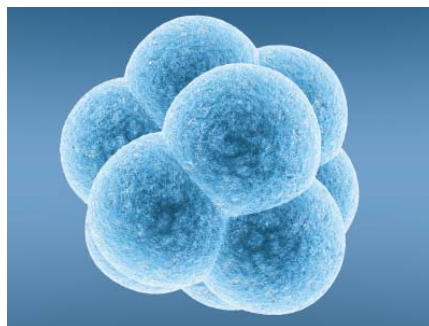
In addition to validating their finding that *Oct4* is required for embryonic development prior to the blastocyst stage, they were able to rank candidate genes for further study.

“Some genes show very little inter-embryo variation and some show very high inter-embryo variation,” she said. “The high reproducibility and low error rate of measuring relative gene expression levels of single-embryos on the BioMark system allowed us to be able to call that. [For further study], we’re proposing that the

genes showing less inter-embryo variation are more tightly controlled... and we believe that these genes are very important in the gene network. What’s interesting is when we ranked them, the top two genes with the least amount of inter-embryo variation were *Rest* and *Mta2*. These genes were just reported to have important pluripotency functions in embryonic stem cells\*. *Mta2* is also implicated in human breast cancer. So it’s really exciting. Not only were we able to validate the gene chips results, but the BioMark helps us prioritize the genes for further study.”

## The Research Experiment

To find out what the *Oct4* pluripotency regulator does at the one- to two-cell stage, the researchers did gene chip experiments on the Affymetrix whole mouse genome chip and then Fluidigm’s BioMark™ System for Gene Expression Analysis.



The researchers ran a total of three independent experiments, each with an *Oct4* knockdown sample and an uninjected sample.

Each sample was derived from 20 embryos pooled together. Following RNA extraction using the PicoPure® kit (Molecular Devices, they amplified half the pooled

embryo sample with the Nugen Pico Ovation® kit to prepare the samples for gene chip experiments. They repeated these steps for another three pairs of samples comprising *cyclin A2* knockdown embryos and uninjected controls, as a positive control.

“We picked 42 genes to validate on the BioMark system,” Dr. Yao said. “We picked them to represent different functional categories.”

“We used the Fluidigm 48.48 dynamic array to validate differential gene expression between knockdown and control samples,” Dr. Yao explained.

Of the 42 genes, three were removed from the study due to technical difficulties, but expression changes of 39 of the genes were measured. Of those, 87% showed altered expression due to the *Oct 4* knockdown. “We were very happy to be able to validate our results,” Dr. Yao said. “That level of scrutiny is very important for a strategy that is not widely used in the mouse model.”

Prior to using the BioMark high-throughput system, which can test as little material as a single cell against 96 genes, the group used conventional RT-PCR practices.

“Our single-embryo data allowed us to go beyond simply validating our gene chip data,” the researchers wrote in their paper. “Methods using samples comprised of pooled cells or embryos, generate relative gene expression that represents an average of all cells assayed, but they cannot discern between genes that are consistently differentially regulated versus those with a tendency towards stochastic changes; similarly, rare

## Glossary

**Genotype/phenotype distinction** – Genotype is the descriptor of the genome, which is the set of physical DNA molecules inherited from the organism's parents. The phenotype is the descriptor of the phenome, the manifest physical properties of the organism, its physiology, morphology and behavior.

**Oct4** – an abbreviation of Octamer4, is a embryonic stem cell pluripotency regulator. It is also known as POU5F1.

**Oocyte** – or egg, is the most advanced developmental stage of a female germ cell, which comprises only half of the chromosome copies. The natural function of the egg is to generate new progeny by fusing with a sperm to make an embryo.

**Pluripotency** – refers to “having more than one potential outcome.”

**Somatic cells** – any cells forming the body of an organism, as opposed to germline cells. In mammals, germline cells (also known as “gametes”) are the spermatozoa and ova that fuse during fertilization to produce a cell called a zygote. Every other cell type in the mammalian body is a somatic cell: internal organs, skin, bones, and blood are all made up of somatic cells.

**Totipotency** – the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues.

**Morpholino oligonucleotide** – A Morpholino oligo specifically binds to its selected target site on RNA to block access of cell components to that target site. This property can be exploited to block translation, block splicing, block miRNAs or their targets, and block ribozyme activity.

**Gene knockdown** – techniques by which the expression of one or more of an organism's genes is reduced, typically either through genetic modification (a change in the DNA of one of the organism's chromosomes) or by treatment with a short DNA or RNA oligonucleotide with a sequence complementary to either an mRNA transcript or a gene.

outlier embryos expressing unique transcriptomes are not recognized. By analyzing quantitative expression data at the single-embryo level, we were able to make this discrimination.”

Dr. Yao said prior to using the BioMark system, they only tested three genes from pooled samples. “In the conventional system, we would have to pool, say 10 embryos per sample to test two to three genes, and for genes not highly expressed, we'd have to pool 20. Semi qPCR is able to do it, but to see differential expression levels that are statistically significant, you'd have to repeat the experiment many more times or you'd have to pool many more samples.”

Dr. Yao said the time savings in using the BioMark system was significant.

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“What we accomplished on the BioMark system in 3 weeks would take more than 9 months to accomplish with conventional PCR methods.”

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“What we accomplished on the BioMark system in three weeks would take a similarly skilled and dedicated researcher more than nine months to accomplish,” she said.

## Possible Medical Application of Results

Dr. Yao said studying embryo development in mice, may ultimately help improve *in vitro* fertilization methods for treating human infertility.

“In clinical infertility, we don't understand why some couples have trouble conceiving,” she said. “According to the diagnostic tests we have available, about 10-30% don't have a cause that can be identified.

Many embryos that are cultured do not make it to the eight-cell stage by day three, or the blastocyst stage. We don't know if it is something inherent in the couples or if the problem is that *in vitro* fertilization methods are not really suited to culturing some human embryos.”

Dr. Yao explained that it is technically not feasible to do gene chip analysis on human embryos and it is good to first understand the mouse system to see if we can understand what genes are critical for the embryo development process, and to use that knowledge to investigate clinical infertility.

## Further Embryo Research Planned

Dr. Yao and her fellow researchers are now looking at the mouse embryo development at the multi-cell stage, by which time ~90% of *Oct4* knockdown embryos have arrested in development.

“We're going to do gene chip experiments at the other stages and use the BioMark to validate results and further deconstruct the gene network.”

## Biography

*Mylene W. M. Yao is assistant professor and clinician-scientist in obstetrics and gynecology, reproductive endocrinology and infertility, and reproductive biology. Dr. Yao graduated from medical school at University of Toronto in 1993, and completed her obstetrics and gynecology residency training at McGill University in 1998. She received her clinical subspecialty training in reproductive endocrinology and infertility at Brigham and Women's Hospital, Harvard University, from 1998-2001. During that time, she also trained in developmental biology as post-doctoral fellow in the laboratory of Richard L. Maas, M.D., Ph.D. After a brief time as assistant professor at Columbia University, she moved to Stanford University in 2003 to initiate her independent research on mammalian embryo development.*

## Research Summary

*We are interested to understand how the early mammalian embryo is reprogrammed to establish totipotent or pluripotent blastomeres, which subsequently undergo lineage-specific differentiation to give rise to all the cell types in the organism. Specifically, we aim to understand how key processes such as nuclear reprogramming, establishment of developmental competence, maintenance of pluripotency, and cell fate decisions are regulated at the earliest stages of mouse and human development. These questions are especially intriguing and relevant to medicine because the zygote, or one-cell stage embryo, are formed by the fusion of the sperm and egg, which are arguably the most differentiated cell types, as they only host haploid genomes. This natural process of reprogramming is relatively efficient, quick and safe from oncogenic potential. Thus, the early pre-blastocyst stages hold the key to Nature's reprogramming toolkit, which is invaluable to our efforts in developing stem cell-based therapies.*

*We have taken innovative approaches to establish both in vitro and in vivo human and mouse models, to identify the genetic and physical determinants that are critical and sufficient for optimal development. Our methods include gene knockdown strategy combined with single-embryo gene expression profiling, applying microfluidics to control physical culture parameters, and the use of clinical in vitro fertilization (IVF) outcomes analysis to direct us towards clinically relevant embryo and reproductive phenotypes. We discovered novel and critical roles of master pluripotency regulator, Oct4, in the maternal-embryonic transition, and have begun to deconstruct the gene regulatory network in the early embryo. Combined with deep phenotyping efforts in early human embryo development and clinical IVF, we are investigating early human development in a translational context that is directly relevant to human health and disease. Our mission is to contribute new and essential knowledge to advance paradigms in the fields of stem cell, cancer, and assisted reproductive technologies, and broaden our scope of human development, health and disease in the 21st century.*

## References and Related Links

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Dr. Yao's home page <http://womenshealth.stanford.edu/research/yao.html>



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