



Human Embryonic Stem Cell Gene Expression and Cell Fate:

Gene expression profiling in individual blastomeres

Embryonic stem cells (ESC) have traditionally been derived from the inner cell mass (ICM) of blastocyst embryos (day 5–6, ~120 cells). These cells are pluripotent and eventually give rise to the more than 200 cell types of a whole organism, as well as the supporting trophectoderm. Their study and use promise advances in basic research, human therapeutics, and biological screening. ESCs will be key for understanding human embryonic development and the causes of congenital birth defects, as well as for identifying and monitoring the effects of chromosomal abnormalities in early development. Stem cell research is expected to contribute significantly to advancing therapies for diseases such as Alzheimer's disease, Parkinson's disease, and diabetes mellitus; as well as for a host of single gene disorders such as cystic fibrosis and Huntington's disease. Other potential stem cell applications include the use of hESCs (human ESCs) in therapeutic drug testing and toxin screening.



Dr. Nathan Treff is the Director of Molecular Biology at Reproductive Medicine Associates of New Jersey, and Assistant Professor of Obstetrics, Gynecology, and Reproductive Sciences at the Robert Wood Johnson Medical School, UMDNJ. He completed a Ph.D. in Biochemistry at Washington State University where he earned the National Institutes of Health (NIH) predoctoral fellowship in Protein Biotechnology. Dr. Treff's work recently won a General Program Prize Paper Award at the 2007 American Society on Reproductive Medicine Annual Meeting held in Washington DC last October [1].

"The use of Applied Biosystems TaqMan® Cells-to-Ct™ Kits streamlined our procedure for studying the expression of stem cell genes in human blastomeres, embryos, and stem cells," said Dr. Treff. "The use of the kit reduced a multi-day procedure down to a single day while maintaining the accuracy and overall quality of the data. This time savings and improved efficiency has allowed us to increase the throughput of our lab 10 fold." —Nathan Treff, Ph.D.

Stem Cell Research May Provide An Embryo-Preserving Alternative

Dr. Nathan Treff and his colleagues at Reproductive Medicine Associates of New Jersey are characterizing whether single blastomeres taken from early 8-cell-stage (day 3) human embryos have begun to differentiate towards ICM and trophectoderm lineages. This research impacts the development of technologies to create ESC lines without harming embryos. A single blastomere from an 8-cell-stage embryo can provide an embryo-preserving alternative to using the ICM of a blastocyst-stage embryo as a source of pluripotent human ESCs. Reprogramming of a blastomere that is destined to contribute to the TE lineage, so that it is redirected to the ICM lineage, may also increase the efficiency of ESC line derivation from blastomeres.

Applied Biosystems Solutions for Gene Expression Profiling

The research group studies stem cell state via gene expression profiles from single blastomeres, 8-cell- and blastocyst- stage embryos, trophectoderm and ICM tissue, single blastomere-derived trophectoderm stem cell outgrowths, and ESC lines. They use the new TaqMan® Gene Expression Cells-to-Ct™ Kit to generate cell lysates from the cells of the above sources. The RNA is then reverse transcribed into cDNA directly within the cell lysate. This

cDNA is then amplified with the new TaqMan PreAmp Master Mix Kit.

The TaqMan Stem Cell Pluripotency Array is used with TaqMan Gene Expression Master Mix for quantitative real-time PCR. This panel contains a well-defined set of validated ESC gene expression markers to characterize ESC identity and assess phenotypic variations between stem cell isolates. It provides the final gene expression profile for each of these cell sources. The resulting data is analyzed using StatMiner™ Software. This whole procedure can be completed in less than a day.

Normally single cells do not provide sufficient RNA for gene expression profiling experiments. The ability to work with RNA within a cell lysate streamlines the procedure and avoids potential sample loss by eliminating the RNA isolation step. Preamplification of cDNA derived from this RNA increases representation of mRNA within the sample without introducing bias, providing more material for the subsequent PCR amplification step.

Determining Gene Signatures

The method has allowed this research group to detect specific differential gene expression in single cells and to demonstrate variation in lineage markers in blastomeres. Some of the genes that have given interesting differential

expression patterns across the cell types examined include *Oct4*, *Nanog*, *Sox2*, *Tdgf1*, *Gabrb3*, *Cdx2*, *CGB*, and *Eomes*.

Reference

1. *Fertility and Sterility* September 2007 **88**: S1
Accurate 23 Chromosome Aneuploidy Screening in Human Blastomeres Using Single Nucleotide Polymorphism (SNP) Microarrays." N. R. Treff, J. Su, J. Mavrianos, P. A. Bergh, K. A. Miller, R. T. Scott, Jr. Reproductive Medicine Associates of New Jersey, Morristown, NJ; Obstetrics, Gynecology, and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

Now Available: TaqMan® PreAmp Cells-to-Ct™ Kit

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