

Fiatal Biotechnológusok Országos Konferenciája



„FIBOK 2014”

Program és összefoglalók

MTA Szegedi Területi Bizottság Székháza

Szeged, Somogyi u. 7.

2014. március 7.

PRODUCTION OF TWIN MICE BY TETRAPLOID COMPLEMENTATION TECHNOLOGY: EFFECT OF THE HOST AND DONOR EMBRYO GENOTYPE

Erna Balogh (lucerna01@gmail.com)¹ - Renáta Fábíán¹ - Szilárd Bodó¹ - Elen Gocza¹ - Babett Bontovics¹

¹NARIC, Agricultural Biotechnology Institute, 2100 Gödöllő, Szent-Györgyi Albert str. 4

The usefulness of twin studies convincingly support the new results of heart abnormalities and diseases caused by high cholesterol level. Using twin mice in studies could substantially reduce the number of animals, which are required to obtain the relevant statistical data.

In our experiments aggregation chimeras were produced for the purpose of creation twin mice with tetraploid complementation method. Unlike clones produced by nuclear transfer, these mice are identical both nuclear as well as mitochondrial DNA level. Therefore, the tetraploid embryo complementation method to produce monozygotic twins could be a valuable tool both in biomedical and in agricultural applications.

Experiments for the creation of twins can be divided into two periods. The first period was from January to May, while the second period was in July. In July we had better results in terms of the number of fused tetraploid embryos, the number of successful transferred chimera embryos and the percentage of newborns than in the first period of the research. During the experiments the host tetraploid embryos were produced by electrofusion. In the first part of the work we set up the optimal fusion parameters and we investigated which could be the best strain for tetraploid embryo production. We used C57BL/6 albino and CD1 genotype embryos. We obtained appropriate results by fusing CD1 embryos (82,92%). Three different sets of fusion parameters were tested. We found the best developmental rate when we used 2 repetitions of a 40V, 45 μ s pulse. Later we investigated the effect of host (CD1, C57/BL6 albino) and donor (CD1/EGFP, C57BL/6) embryo genotype regarding to the number of the living newborns. Four-cell stages tetraploid CD1 and C57BL/6 albino mouse embryos were aggregated with 2 or 4 blastomeres derived from the same CD1/EGFP or C57BL/6 genotype eight-cell stages embryo. We found that the CD1/EGFP genotype mouse strain is the best as donor embryo. Examining the effect of the host embryo genotype we could not identified significant difference in the percentages of lived borne singleton mice, but according the results getting at electrofusion, the CD1 genotype mouse strain proved to be more suitable as host embryo.

Based on our results, we concluded that the best donor embryos derived from CD1/EGFP, while the best host embryos from CD1 mouse strain. These genotype embryos will be used in further experiments.

The work was supported by collaboration contract of ABC, Gödöllő, Hungary - CAMH, Toronto, Canada.

VALIDATION OF RABBIT MICRORNA SEQUENCES IDENTIFIED BY SOLID SEQUENCING IN RABBIT PREIMPLANTATION EMBRYOS AND EMBRYONIC STEM CELLS

Németh Kinga (nkinga@gmail.com)¹ - Pouneh Maraghechi¹ – Bence Lázár¹ - Babett Bontovics¹ - Elen Gocza¹

¹NARIC, Agricultural Biotechnology Institute, 2100 Gödöllő, Szent-Györgyi Albert str. 4

MicroRNAs (miRNAs) are a class of small non-coding RNAs that have an important role in the sensitive adjustment of basic functions by posttranscriptional gene regulation. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology. According to data of miRbase, more than 1000 human (*Homo sapiens*) and more than 800 mouse (*Mus musculus*) microRNAs are known, but not any till now in rabbit (*Oryctolagus cuniculus*).

We aimed to explore rabbit pluripotent stem cell specific miRNAs using SOLiD™ System Small RNA analysis of samples isolated from different embryonic stages of rabbit embryos and pluripotent rabbit stem cells, to get more insight into their potential regulatory mechanism in embryonic development. We validated these sequences by quantitative real-time PCR, and compared the expression of stem cell specific miRNAs in different cell layers of 6 dpc rabbit embryos. We also tested whether the number of reads, what we got by SOLiD™ sequencing, could be used to estimate the gene expression level.

We also analysed the expressions of ocu-miR-302 and ocu-miR-290 clusters in the cell layers of 6 dpc rabbit embryos.

We successfully identified several rabbit miRNAs and validated them by qRT-PCR. The ocu-miR-302 cluster is ESC-specific, like in human ESCs, and can play a crucial role in the maintenance of rabbit stem cell pluripotency and self-renewal. The high expression level of ocu-miR-302 cluster compared to low expression level of ocu-miR-290 cluster in rabbit pluripotent stem cells might reflect their “primed” state.

This research was funded by grants Plurabbit: OMFB-00130-00131/2010 and ANR-NKTH/ 09-GENM-010-01 to ZB, OTKA K77913 and MÖB-47-1/2009 to EG.