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A309E Cloning, Transgenesis and Stem Cells

Optimization of RNA concentration for genome editing by CRISPR in rabbit zygotes

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Site-specific genetic modification aiming to delete (knock-out) a gene provides an unequivocal answer to elucidate the function of such particular gene in the whole organism. Site-specific genetic modification has been achieved by homologous recombination, generally in embryonic stem cells, which has made the mouse the most widely used mammalian model. However, the mouse model is not appropriate to study some biological functions or to recapitulate some human diseases. As an example, ZP4, one of the four proteins of the zona pellucida in humans and rabbits, is not present in mice, so its function remains elusive due to the lack of a knock-out model. The use of the newly developed site-specific endonucleases, such as CRISPR, allows site-specific genetic modification in zygotes, being a suitable technique for genetic modification in domestic mammalian species. The aim of this experiment has been to determine the optimal concentration of the two components of the CRISPR system (Cas9 mRNA and gdRNA) for genome editing following microinjection of rabbit zygotes. Capped polyadenylated Cas9 mRNA was produced by *in vitro* transcription from BstBI digested pMJ920 plasmid. A gdRNA was designed against the first exon of rabbit ZP4 gene, cloned into the plasmid px330, amplified by PCR adding T7 promoter and *in vitro* transcribed. Rabbit zygotes were obtained from the oviduct 14 hours after mating. Immediately after collection, zygotes were microinjected into the ooplasm with approximately 10 picoliters of three different combinations of Cas9 capped polyadenylated mRNA and gdRNA: 1) 300 ng/µl Cas9 and 150 ng/µl of gdRNA (300:150), 2) 150 ng/µl Cas9 and 50 ng/µl gdRNA (150:50) and 3) 100 ng/µl Cas9 and 25 ng/µl gdRNA (100:25). Following microinjection, embryos were cultured in TCM199 supplemented with 5% FCS at 38.5ºC in a 5% CO2, 5% O2 and 90% N2 water saturated atmosphere. CRISPR components did not affect preimplantation development, as all embryos surviving microinjection (~90%) developed to the blastocyst stage. At the blastocyst stage, the zona pellucida was removed and blastocysts were individually stored at -20 ºC. Blastocysts were digested in 8 µl of a 100 µg/ml proteinase K buffered solution and 2 µl of the lysate were used to amplify the genomic sequence including the CRISPR target site. PCR products were purified and sequenced to determine genome edition around the target site. All combinations were similarly effective in generating insertion/deletions around the target site: in the groups 300:150 and 150:50 all blastocysts analysed (6/6 in both groups) were edited, whereas in the group 100:25 only one blastocyst out of six was not edited. In conclusion, CRISPR system constitutes an effective means for genome editing in rabbit zygotes and the ooplasm microinjection of 100 ng/µl capped polyadenylated Cas9 mRNA and 25 ng/µl gdRNA achieves high genome editing efficiencies.

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