Genome-Scale CRISPR-Cas9 Knockout Screening of genes involved in pluripotency of Rabbit induced Pluripotent Stem Cells.

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Poster + Flash-talk

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Pluripotency refers to the ability of a stem cell to give rise to all cell types in mature organisms. Two types of pluripotent stem cells (PSCs) could be generated in vitro: embryonic stem cells (ESCs) derived from the inner cell mass of early embryos and induced pluripotent stem cells (iPSCs) resulted from reprogramming differentiated cells by overexpression of pluripotency factors. In mice, iPSCs could be stabilized in two pluripotency states depending on culture conditions: the naïve state sustained by the LIF/gp130/Stat3 pathway and the primed state supported by FGF2 and Activin A/TGFb/Smad pathways. Only mouse iPSCs in the naive state are able to colonize recipient embryos and give rise to chimeric animals. In rabbits, only primed iPSCs have been generated and until now, attempts to reprogram them to the naïve state have failed. We decide to use of Genome-Scale CRISPR-Cas9 Knockout Screening (GeCKO), a powerful tool for discovering gene functions, to find the genes that hinder or promote the production of naïve rabbit iPSCs. With this method, we will target more than 14,000 genes on the rabbit induced pluripotent stem cell line, named B19, using a GeCKO library including 84,400 single guide RNAs. For preliminary steps, a DOX-inducible Cas9 protein expression system was transfected into B19 cells. Then, we used a lentiviral delivery of gRNAs to knockout the *Kdm4a* as a testing gene to improve the system and set-up the GeCKO screening. The B19-cas9#12 cells will now be transduced with the Gecko library. After Cas9 induction and culture under naïve state conditions, cells will be sorted into two populations based on specific expression of two naïve markers (EOS-GFP and FOLR1): the naïve one selected on high expressions of EOS-GFP and FOLR1 and the primed population selected on low expressions of EOS-GFP and FOLR1. Amplification and sequencing of Cas9-targeted genes in these two cell populations will give us signaling pathways to induce or repress to reprogram primed rbiPSCs towards the naïve state.