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Production of fox FGF2 for fox pluripotent stem cell culture

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Motivation and Aim: Embryonic stem cells and induced pluripotent stem cells of various non-human species are widely used to study embryonic development and differentiation. Pluripotent stem cells usually require specific growth factors such as LIF (mouse pluripotent stem cells) and FGF2 (human pluripotent stem cells). Human or mouse growth factors are successfully used for cell culture of pluripotent stem cells of different species. However, cat induced pluripotent stem cells required species-specific feline FGF2 [1], thus species-specific LIF or FGF2 may be beneficial. In our attempts to produce fox induced pluripotent stem cells we observed more colonies after partial reprogramming with human FGF2 than with mouse LIF. Thus, we decided to develop a system for fox FGF2 production for fox pluripotent stem cell culture.

Methods and Algorithms: We utilized the HEK293-EBNA1-based system [2] for protein production. EBNA1 expressing 293 c18 cell line could be used for efficient production of proteins with expression vectors bearing the Epstein-Barr virus origin of replication, oriP. Proteins could be secreted into the cell culture medium and in case of high yield be used diluted for cell culture without protein purification. To produce fox FGF2 we used pCEP-Pu-BM40-6His-Myc-fX-mLIF plasmid, a kind gift from Dr. V. Wixler, the University of Munster, Germany. We extracted RNA from the fox frontal lobe (a kind gift from Dr. Yu. Herbek, Institute of Cytology and Genetics, Novosibirsk, Russia), produced cDNA, and amplified the 465 bp coding part of fox *FGF2* gene (Gene ID: 112933647) (primers 5'-GTG GGC TAG CCG CAG CCG GGA GCA TCA CCA C-3' and 5'-GTC TGG ATC CTC AGC TCT TAG CAG ACA TTG GAA-3'). PCR-product was ligated into pCEP-Pu-BM40-6His-Myc-fX-mLIF using *Nhe*I and *Bam*HI endonucleases. We designated the plasmid as pCEP-Pu-BM40-6His-Myc-fX-foxFGF2.

Results: We transfected 293 c18 cells with pCEP-Pu-BM40-6His-Myc-fX-foxFGF2 and produced 10 clones. We have shown that the three clones with the highest growth rate all express fox *FGF2*. Next, we determined the fox FGF2 protein concentration in DMEM/F12 medium conditioned by the FGF2 producing cells using His Tag ELISA Detection Kit (GenScript, USA). The highest concentration was 19 ng/ml. Human pluripotent stem cells are cultured with 10 ng/ml, thus 19 ng/ml is most probably not enough for cell culture of fox pluripotent stem cells using a dilution of conditioned medium, protein concentration and purification are necessary

Conclusion: We produced a cell line with fox FGF2 expression. The protein yield in the conditioned medium is low for the direct usage with fox pluripotent stem cells, additional steps of concentration and purification are necessary.

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References

1. Dutton L., Dudhia J., Guest D.J. *Stem Cells Dev*. 2019;28:1299-1309.
2. Tom R., Bisson L., Durocher Y. *CSH Protoc*. 2008;pdb.prot4976.