

Study demonstrates bovine *OCT4* and *NANOG* enhancer activities in pluripotent stem cells using fluorescent protein reporters

By Lindsay Pressman

Induced pluripotent stem cells (iPSCs) resemble embryonic stem cells (ESCs) in pluripotency but are derived from somatic cells by overexpressing reprogramming factors, including *OCT 4*, *SOX2*, *NANOG*, and *LIN28*. A research article, published by PLOS ONE by Delun Huang et al., entitled “Study demonstrates analyzing bovine *OCT4* and *NANOG* enhancer activity in pluripotent stem cells using fluorescent protein reporters”, explores the use of high transduction efficiency of lentivirus-based green fluorescence protein (GFP) reporter vector controlled by the bovine-specific *OCT4* or *NANOG* regulatory regions to facilitate mouse and human ESC/iPSC studies. The pre-implantation stage “naïve” pluripotent state represented by mouse ESCs have a dome-shaped colony morphology, have the ability for single cell colonization and depend on leukemia inhibitory factor (LIF) signaling for self-renewal. On the other hand, post-implantation stage “primed” state pluripotency is represented by mouse epiblast stem cells (EpiSCs) and human ESCs, have a flat colony morphology, are resistant to single cell colonization, and depend on basic fibroblast growth factor/Activin A signaling for pluripotency maintenance. These naïve or primed-state pluripotent ESCs and iPSCs originated from farm animals are important in the studies of livestock embryo development, animal reproduction and human disease modeling. This study reports the activity of the bovine *OCT4*-DE region in naïve vs. primed-state pluripotent cells. The lentiviral reporters were also tested in mouse iPSC generation. Lastly, the activity of

bovine *OCT4*-DE in bovine blastocysts from *in vitro* fertilization was investigated. These reporters are valuable resources for the study of bovine ESC/iPSC identification, generation, regulation and differentiation, maintenance and bovine embryo development.

The authors found that the *OCT4* and *NANOG* reporters show activity in both naïve- and primed-state pluripotency when infected in mouse and human ESCs. The bovine b*OCT4*-DE (distal enhancer), but not the proximal enhancer (b*OCT4*-PE) region is preferentially activated in naïve-state pluripotency. The b*OCT4*-DE region is silenced when naïve-state ESCs are converted into primed-state EpiSCs. Co-infecting mouse fibroblasts with the reprogramming factors for iPSC induction led to the generation of GFP positive colonies, indicating that these GFP reporters can serve as live indicators for induced pluripotent cell establishment. Additionally, it was proved that the bovine *OCT4*-DE is active in bovine blastocysts. Although through extensive research, there are still many difficulties in ESC and iPSC development in bovine, and these problems negatively impact the practical applications of bovine ESCs/iPSCs. More tools are needed to help optimize the conditions for bovine pluripotent stem cell development.

Co-author Dr. Young Tang, Assistant professor in the Department of Animal Science indicated that “These lentiviral fluorescent reporters controlled by bovine-specific pluripotent genes will help facilitate the ESC pluripotent stem cell generation.”

The view the full article, Huang D, Wang L, Talbot NC, Huang C, Pu L, Zhao X, Tian X, Zhang M, Tang Y. (2018) Analyzing bovine *OCT4* and *NANOG* enhancer activity in

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