Animal Reproduction

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Cloning, transgenesis and stem cells

Delivery of Cas9 protein/gRNA complexes using lipofectamine CRISPRMAX in mammary gland epithelial cells (bMEC)

Paula Magnelli Mangiavacchi [1], Álvaro Fabrício Lopes Rios [1], Carla Sobrinho Paes de Carvalho [1], William H. Velander [3], Gláucia Mota Bragança [1], Luis Fonseca Matos [1], Maria Clara Caldas Bussiere [1], André Luis Rios Rodrigues [2], Raquel Varella Serapião [4], Angelo José Burla Dias [1]

[1] UENF - Universidade Estadual do Norte Fluminense Darcy Ribeiro (Av. Alberto Lamego 2000, Campos dos Goytacazes - RJ, CEP: 28013602), [2] UFF - Universidade Federal Fluminense (Av. Alm. Ary Parreiras, 507, Icaraí, Niterói - RJ, 24220-000), [3] Nebraska - Nebraska University (Lincoln - Nebraska), [4] Universidade de Vassouras - Universidade de Vassouras (Vassouras, RJ)

Resumo

The bovine mammary gland epithelial cells culture (bMECs) is an efficient strategy to generate recombinant proteins, using the CRISPR-Cas9 gene editing system. The genetically modified bMEC constitutes an in vitro platform for the production of human recombinant proteins, such as human plasma fibronectin (pFN1), involved in the healing process. Therefore, genetically modified bMECs can be used for the evaluation of important factors of the animal bioreactors production, like analysis of the expression pattern of the transgene of interest, in addition to serving as a source of cell nuclei for nuclear transfer of somatic cells experiments. The aim of this study was to insert the pFN1 plasmid using purified Cas9-Nickase protein and gRNA complexes into bMEC and to establish the optimal conditions for transfection using lipofectamine CRISPRMAX. The pFN1 plasmid contains approximately 13kb, in which it includes the promoter of the BLG gene (beta lactoglobulin). For insertion of pFN1 into the bovine genome trough HDR strategy, 3'ARM (834pb) and 5'ARM regions (855pb) of ROSA26 were coupled to the plasmid, that is a common to donor vector in the bovine genome. The lipofection was performed in bMEC in the seventh passage and the pFN1+Cas9+gRNA+lipofectamine complex was prepared according to the manufacturer. Lipofected bMEC remained in culture for 96h and then were trypsinized for incorporation analysis. Qualitative analysis of the pFN1 sequence of interest was performed by RT-PCR and visualized on a 1% agarose gel. In addition to the pFN1 sequence, the housekeeping gene bGAPDH was also analyzed for positive control, and non-lipofected bMECs for negative control. All lipofected bMECs showed incorporation of pFN1 plasmid. Our preliminary results indicate that bMEC can be used to produce recombinant proteins and that delivery of Cas9/gRNA protein complexes using CRISPRMAX lipofectamine is an efficient method for the production of transgenic bMEC. The next steps of the assay are to sequence the lipofected bMECs, in addition to inducing the secretion of milk proteins and isolating the pFN1+ strains.