Immortalization of mammalian cells by genetic engineering for cellular agriculture applications

MEP-student: Jordy Elfering
Email: jelfering@tudelft.nl

Supervisors: Dr. Joshua Flack

Project term: September 2024 – May 2025

Description:

Mammalian cell phenotypes must be enhanced in order to be broadly applicable for cellular agriculture processes. In this endeavour, it is essential to first create genetically stable, immortalized cells lines. In unmodified primary mammalian cells, continued proliferation eventually leads to senescence (Figure 1). This senescence is induced by the expression of cell cycle inhibitory factors such as p16, that arrest cell growth [1], [2]. One approach to achieve immortalization is thus the removal of the p16 stress response [1], [2] (Figure 1). Although 'spontaneous adaption' has in the past been used to immortalize cell lines, possibilities here seem limiting, especially with regards to control of the process and the range of phenotypes it can produce. Genetic engineering, on the other hand, offers a lot of potential, as it allows for precise genomic changes. Various systems, including CRISPR/Cas, can be used for this engineering [3].

The goal of this project is the immortalization of bovine skeletal muscle satellite cells through the knockout of p16 using CRISPR/Cas9-mediated genome editing. I will design and vectors for the editing of p16. I will also optimize DNA/mRNA delivery into cells by electroporation, which will be followed by single cell cloning to create p16KO cell line(s). Finally, I will characterize these cell lines with respect to long term proliferation, differentiation performance, transcriptomics, etc. (Figure 2).

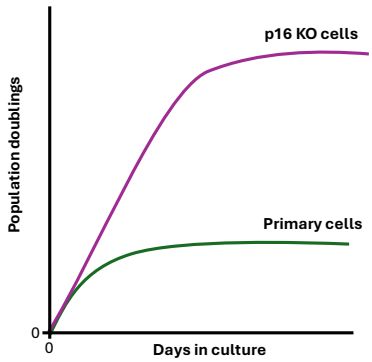

Figure 1: Suggested population doublings against time of primary and p16 KO muscle satellite cells.

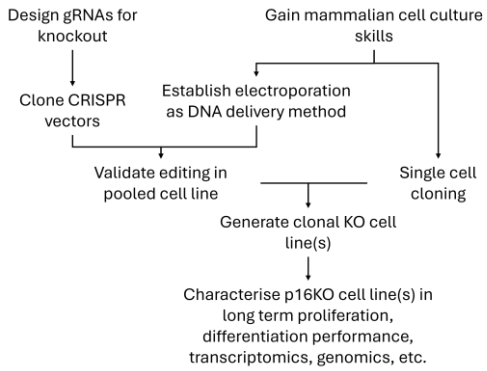

Figure 2: Overview of proposed project steps.

References:

[1] E. Soice and J. Johnston, "Immortalizing Cells for Human Consumption," *Int J Mol Sci*, vol. 22, no. 21, p. 11660, Oct. 2021, doi: 10.3390/ijms222111660.

[2] F. Giglio *et al.*, "A Glance into the Near Future: Cultivated Meat from Mammalian and Insect Cells," *Small Science*, Jul. 2024, doi: 10.1002/smsc.202400122.

[3] D.-K. Lee *et al.*, "Unlocking the potential of stem cells: Their crucial role in the production of cultivated meat," *Curr Res Food Sci*, vol. 7, p. 100551, 2023, doi: 10.1016/j.crfs.2023.100551.