Cloning Constitutive Fluorescent PCAD Plasmids to Investigate the Effects of Mucin Expression on Cell-Cell Adhesion in Multicellular Spheroids of Mammalian Cells

Neo Phuchane, Zoe Shappell, Leonardo Morsut
Dept of Stem Cell Biology and Regenerative Medicine, Bridge Institute, University of Southern California, Los Angeles, CA, USA

ABSTRACT

- Mucins: glycoproteins found in the glycocalyx, a gel-like shell of glycoproteins and glycolipids on the surface of all living cells.
- It plays a role in intracellular signaling and protect the epithelial surface from pathogens, however, their exact functions or structures are not completely understood.
- Mucins are anchored to the cell membrane, leading the polymer chains to extend from the cell, creating a “brush” [1].
- Increasing our understanding on mucin’s effects on cell-cell adhesion could allow us further control of morphogenesis.

PRELIMINARY RESULTS

- Cells producing Mucin separate from the spheroid
- Suggests Mucins could be responsible for pushing away other cells

METHODS

In Silico

- Cloning Design
  - Primer Prep

In Vitro

- Restriction Digest
- PCR
- Gel Electrophoresis
- DNA Purification
- Gibson Assembly
- Bact. Transformation
- Culture Prep
- Miniprep
- Diagnostic Digest
- Sequencing

RESULTS

- Smear PCAD band
- BFP and P2A were clear
- Re-ran PCAD amplified 58, 60, 62°C
- Still no clear PCAD bands

- Created backbone with restriction digest instead of PCR
- Assembled recombinant plasmid
- Transformed and plated but no colonies

- No BFP for PCAD+BFP or PCAD for PCAD+RFP
- Very faint band for PCAD for PCAD+BFP and FRP for PCAD+RFP
- SFFV and P2A consistently clear

REFERENCES


CONTACT US

nphuchane@gmail.com
shappel@usc.edu, morsut@usc.edu
bridge.usc.edu/bugs