



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

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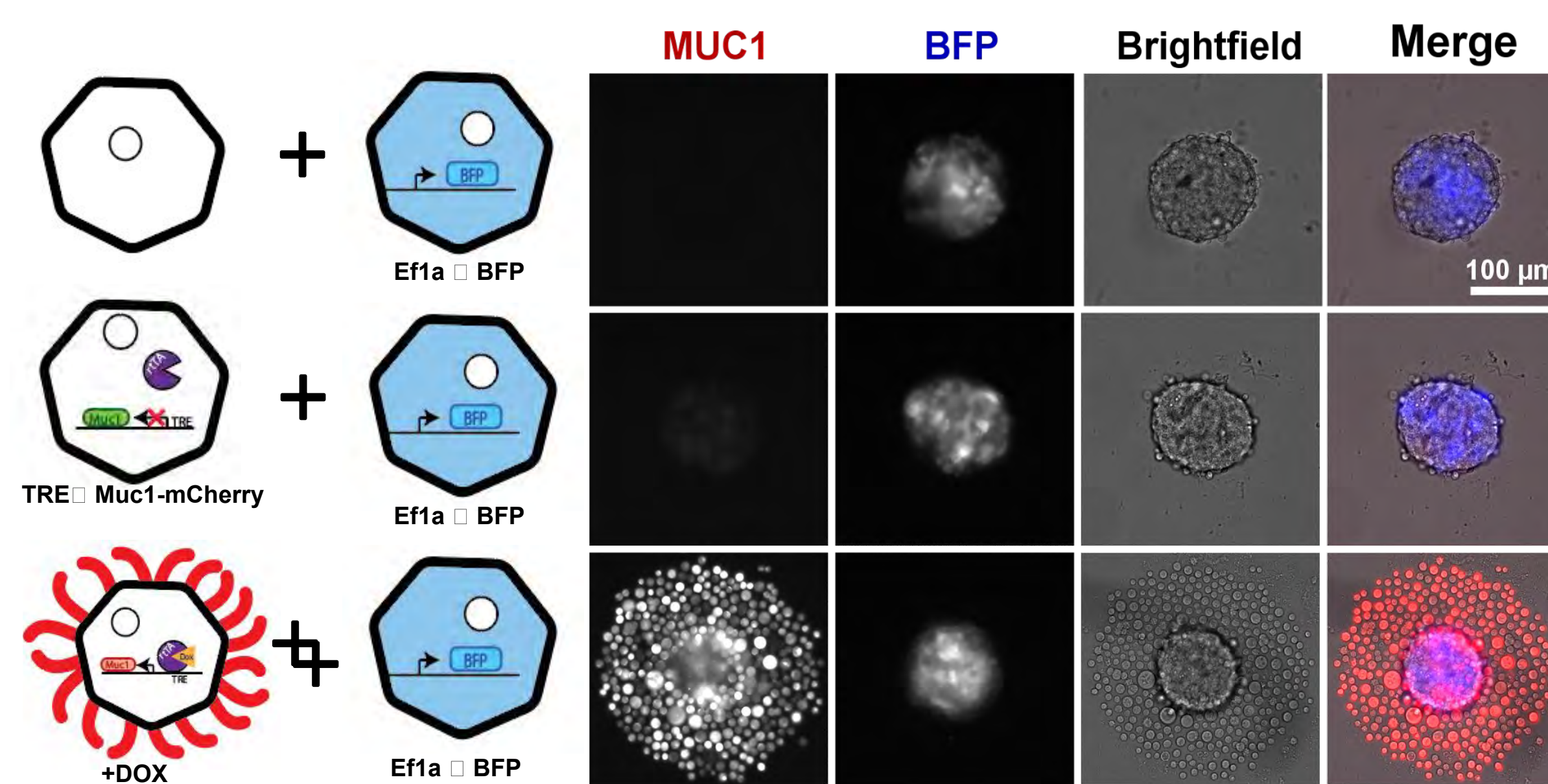


Bridge UnderGrad Science (BUGS) Summer Research Program

ABSTRACT

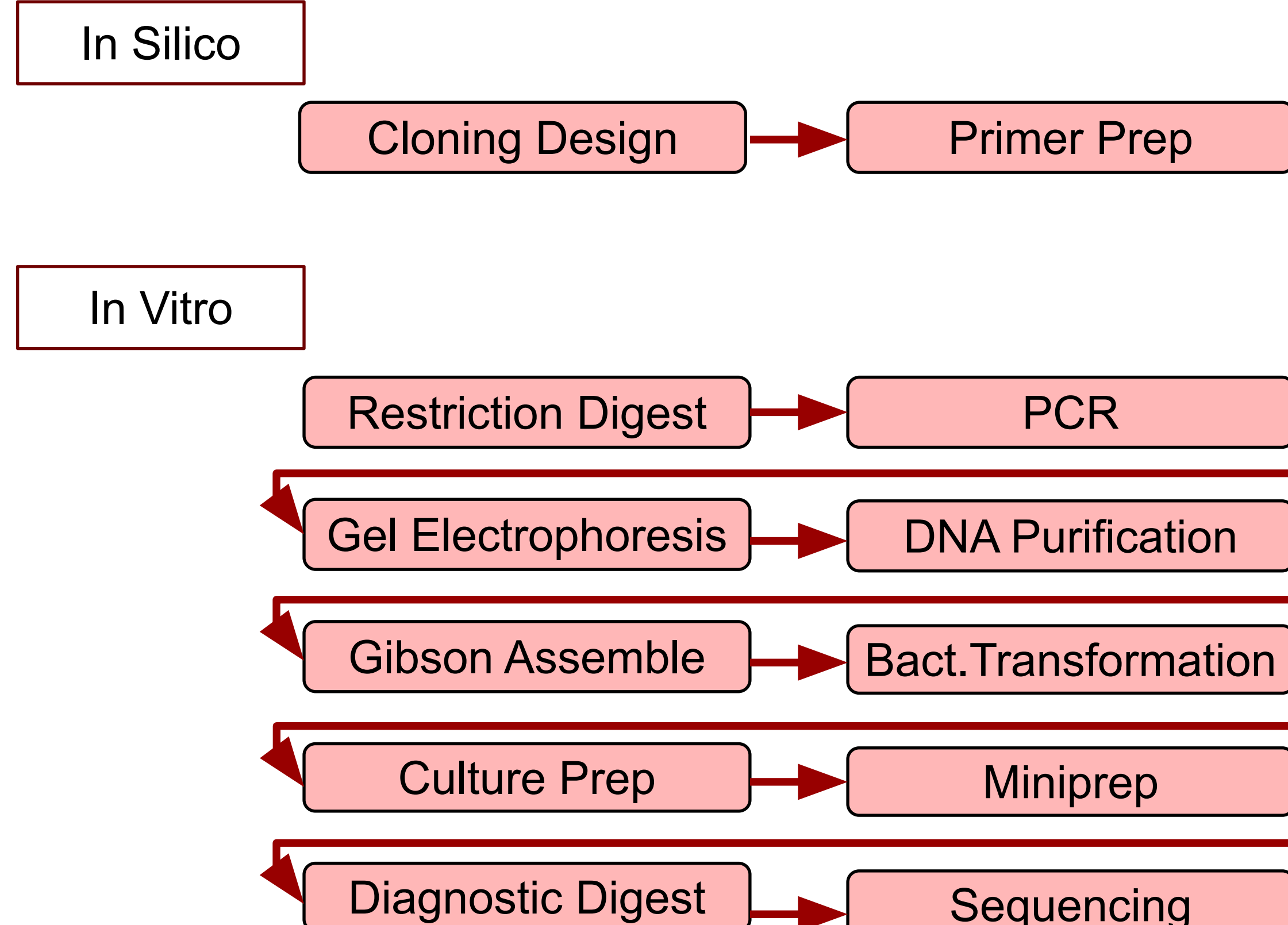
- Mucins: glycoproteins found in the glycocalyx, a gel-like shell of glycoproteins and glycolipids on the surface of all living cells.
- It plays a role in intracellular signaling and protect the epithelial surface from pathogens, however, their exact functions or structures are not completely understood.
- 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 cell-cell adhesion could allow us further control of morphogenesis.

PRELIMINARY RESULTS



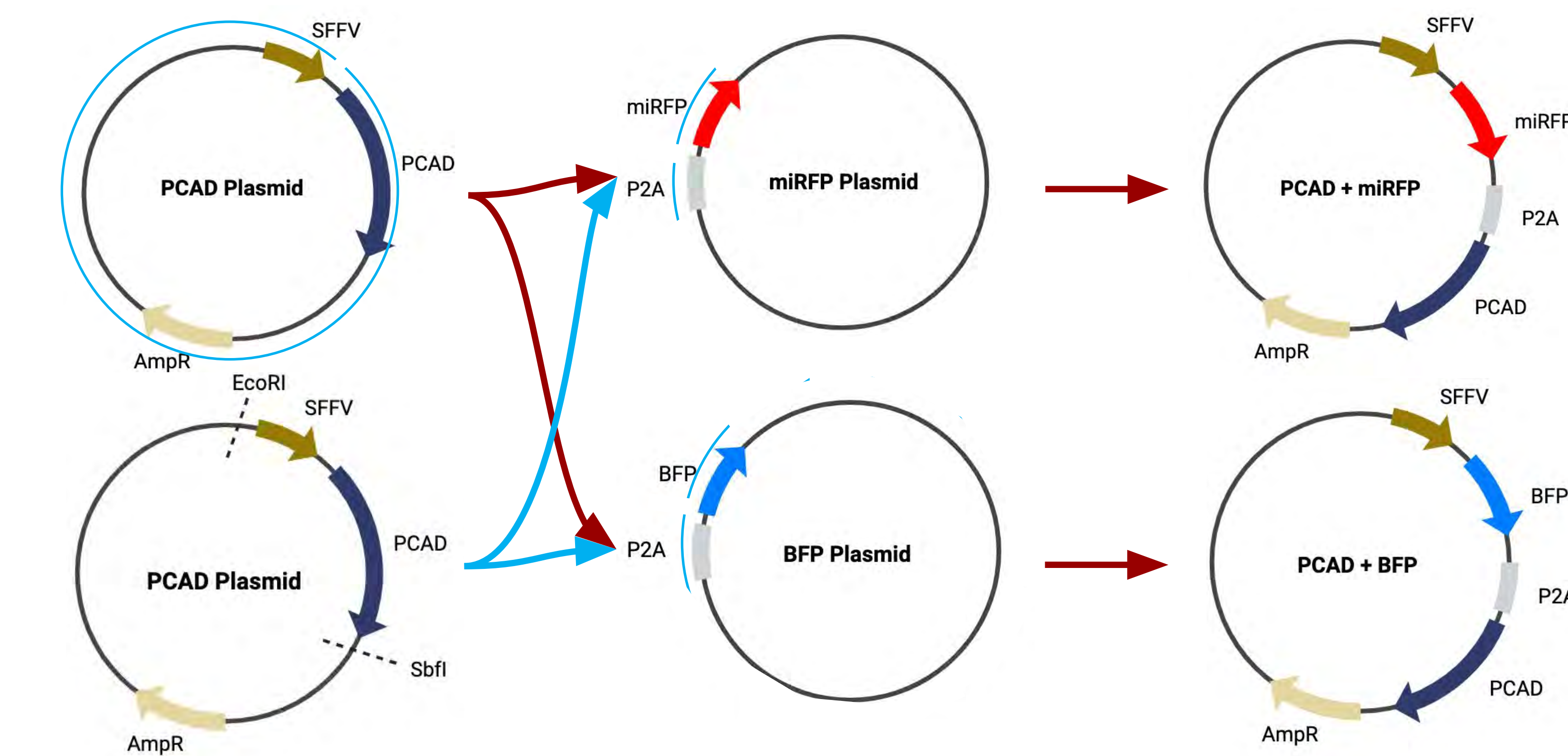
- Cells producing Mucin separate from the spheroid
- Suggests Mucins could be responsible for pushing away other cells

METHODS



METHODS CONT.

Fragment Amplification w/ PCR



Restriction Digest

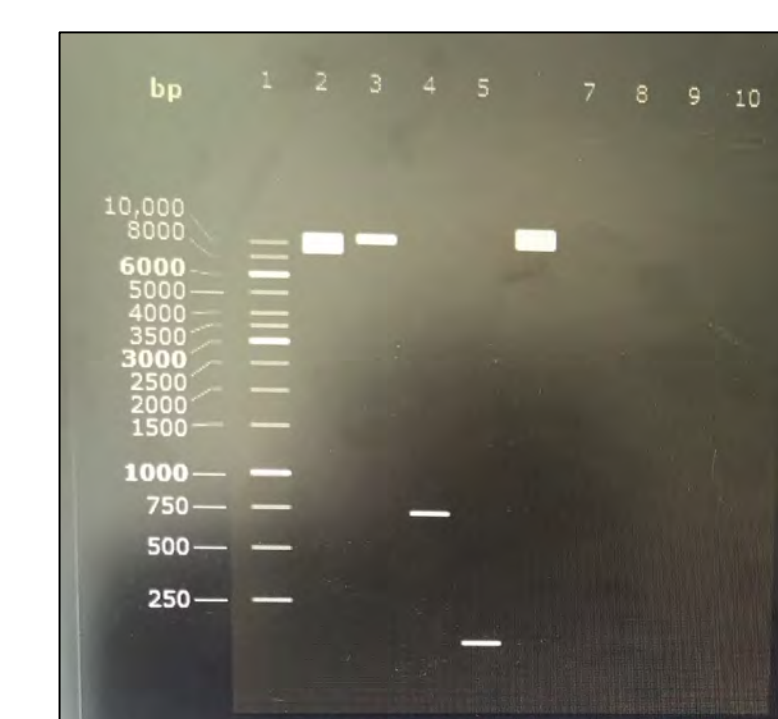
Backbone

Fragments

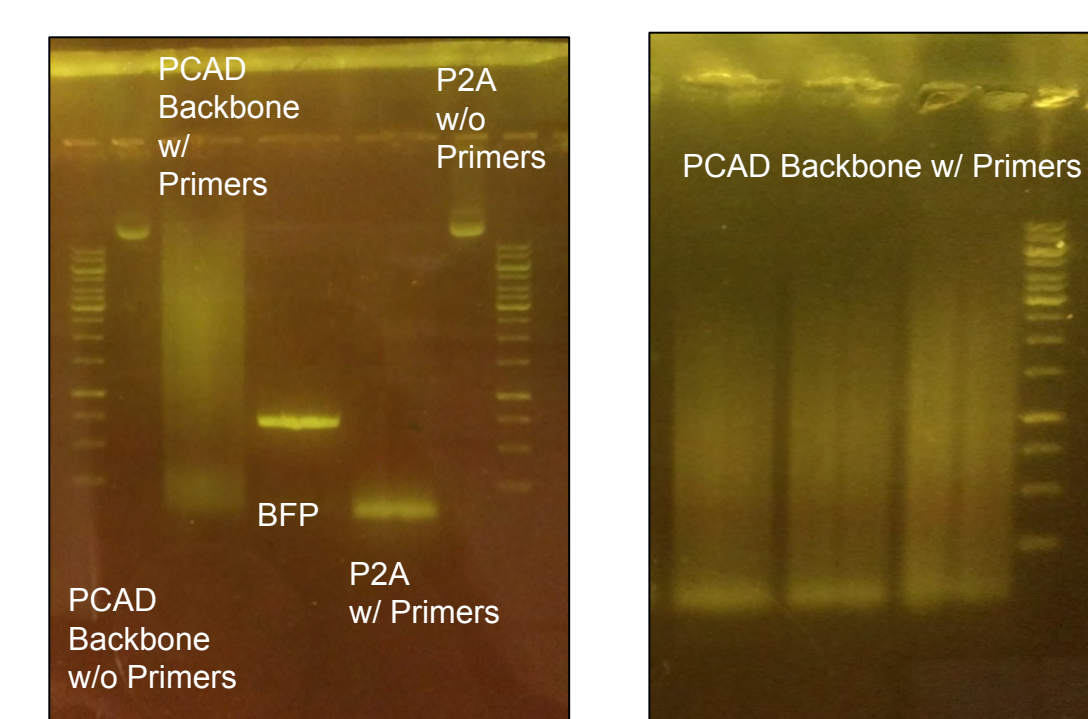
Recombinant Plasmid

RESULTS

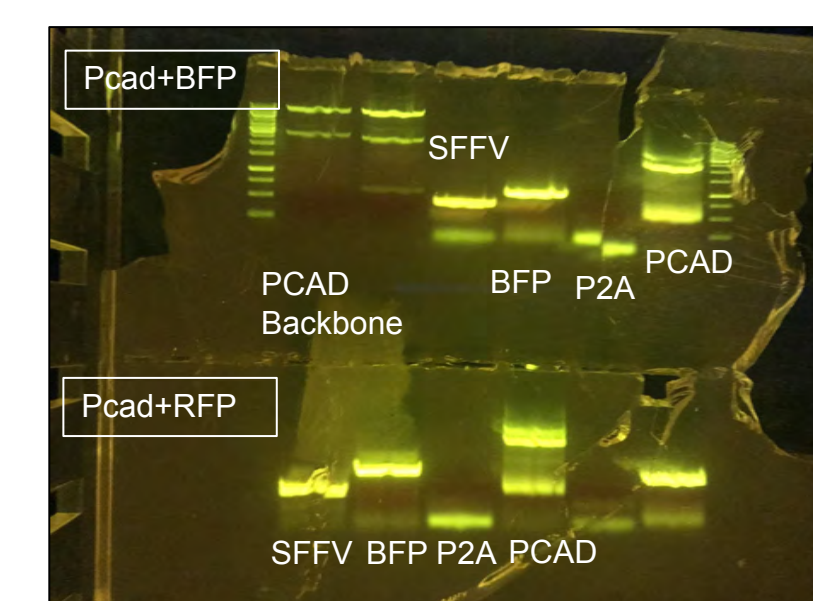
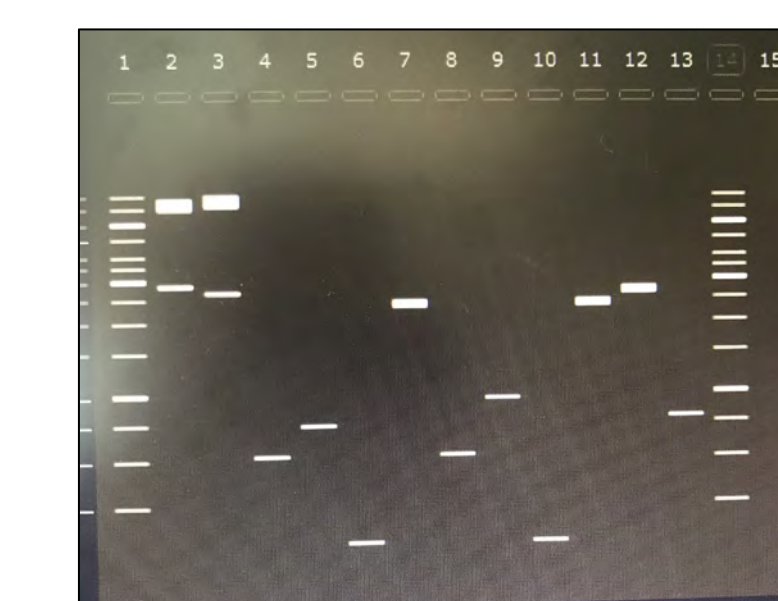
Gel Simulation



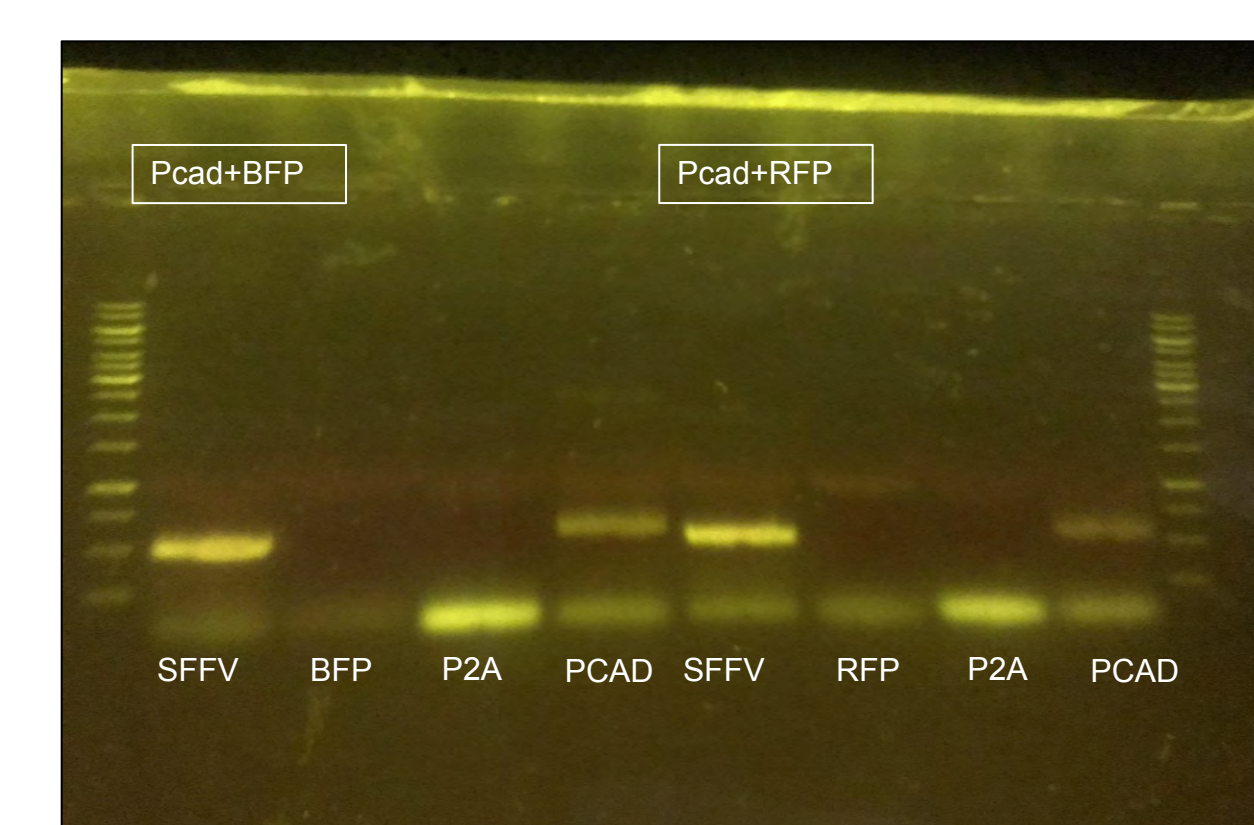
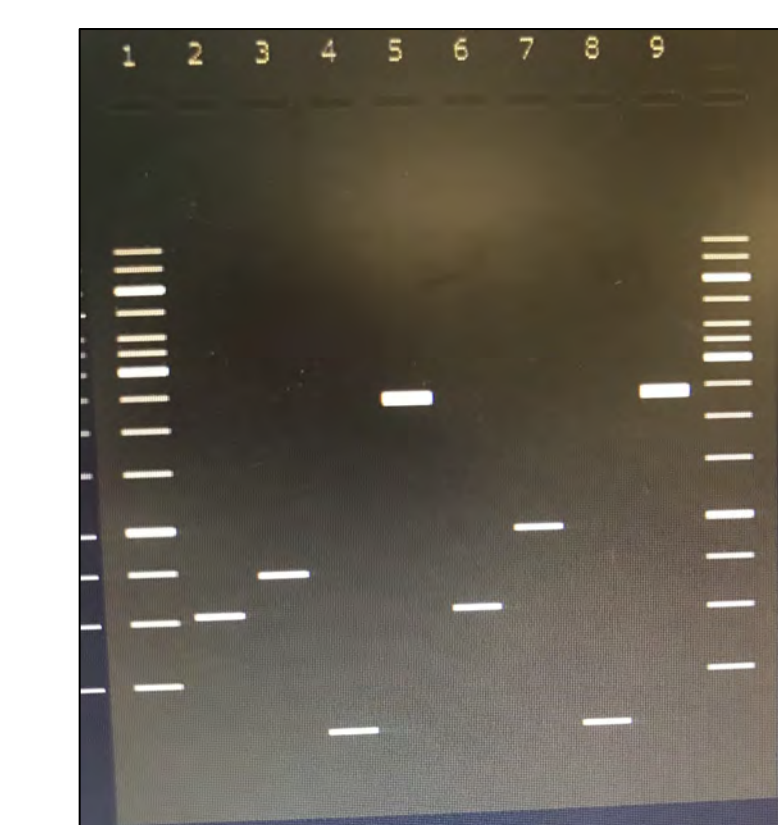
Gel Results



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

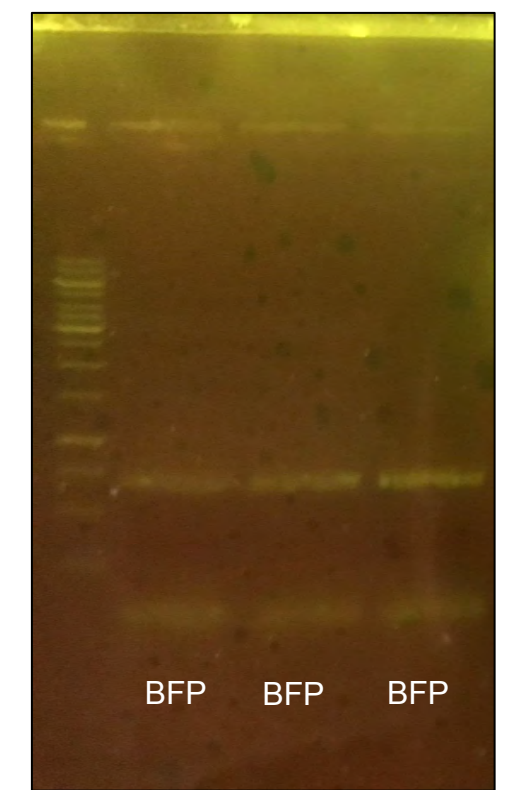
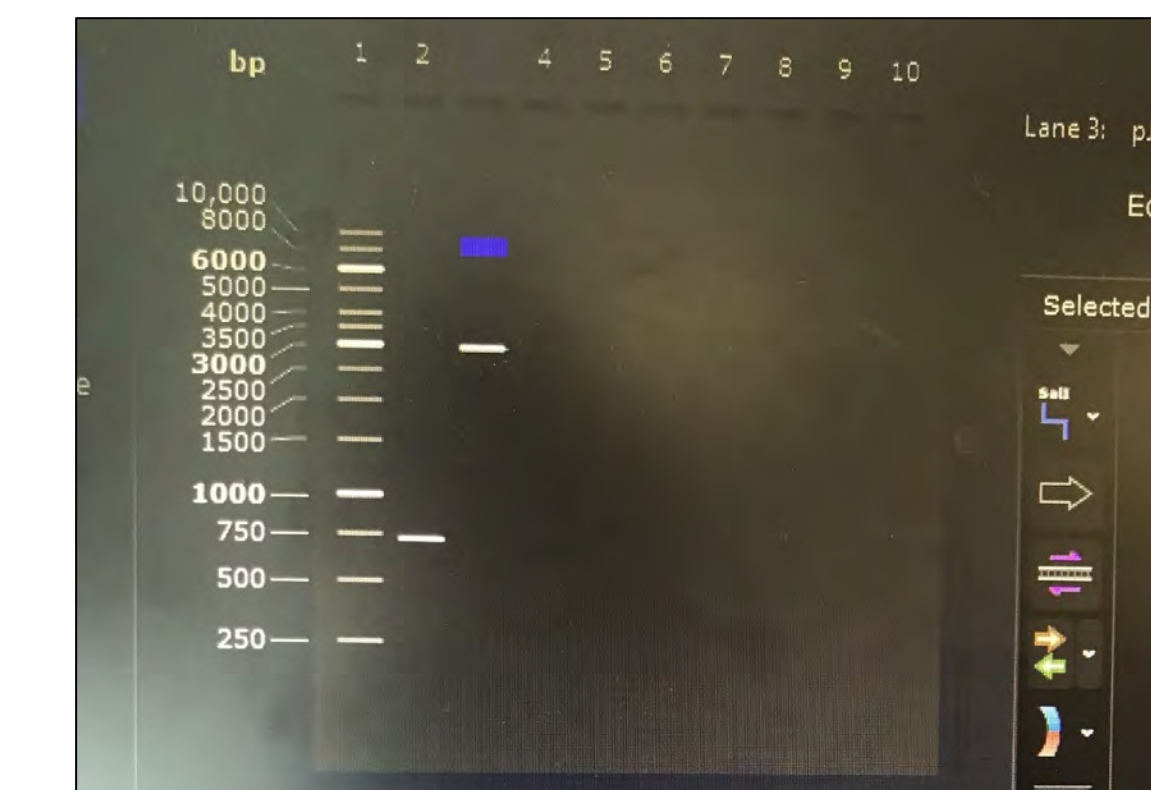


- Created backbone with restriction digest instead of PCR
- Assembled recombinant plasmid
- Transformed and plated but no colonies

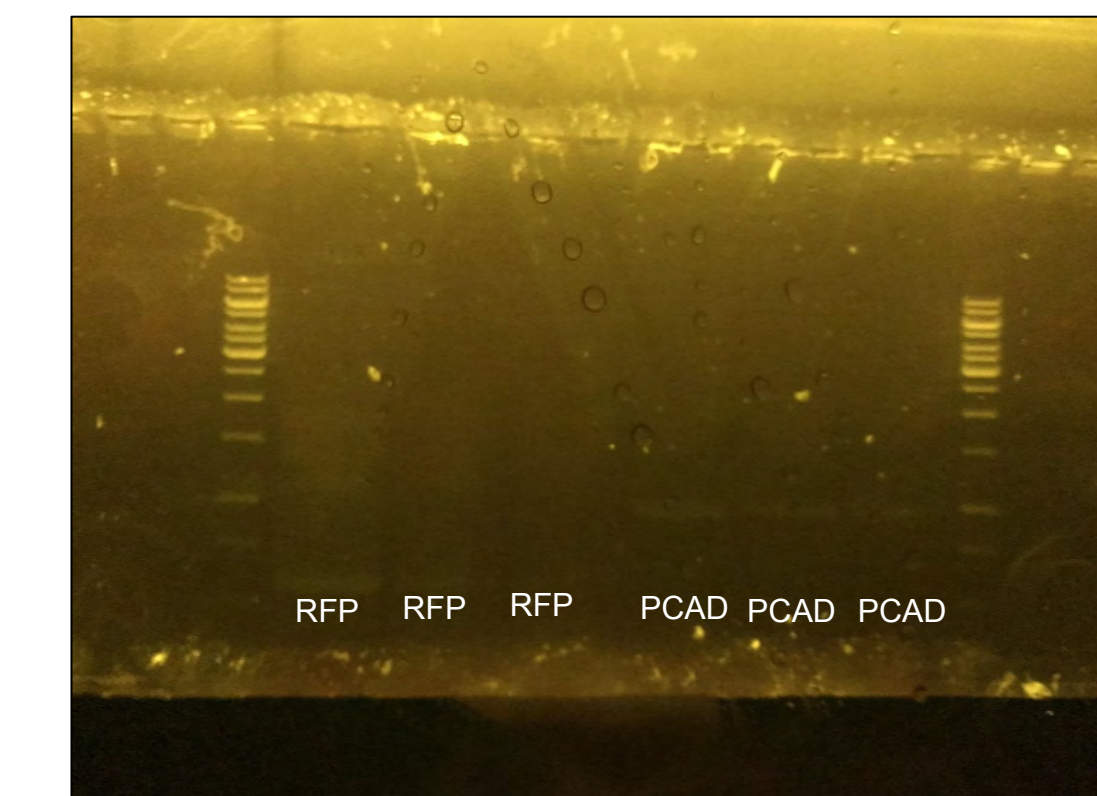


- No BFP for PCAD+BFP or PCAD for PCAD+RFP
- Very faint band for PCAD for PCAD+BFP and FRP for PCAD+RFP
- SFFV and P2A consistently clear

RESULTS CONT.

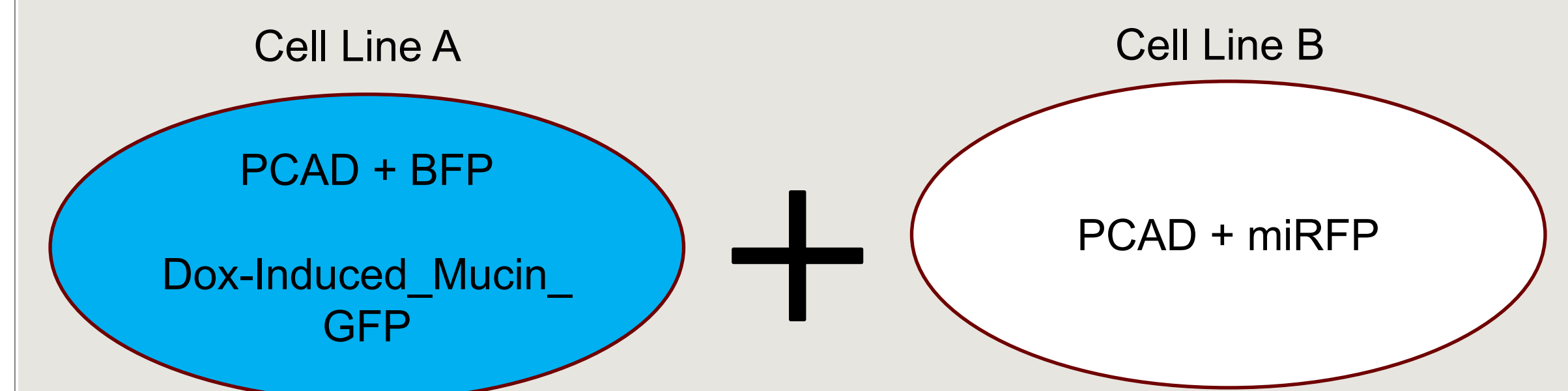
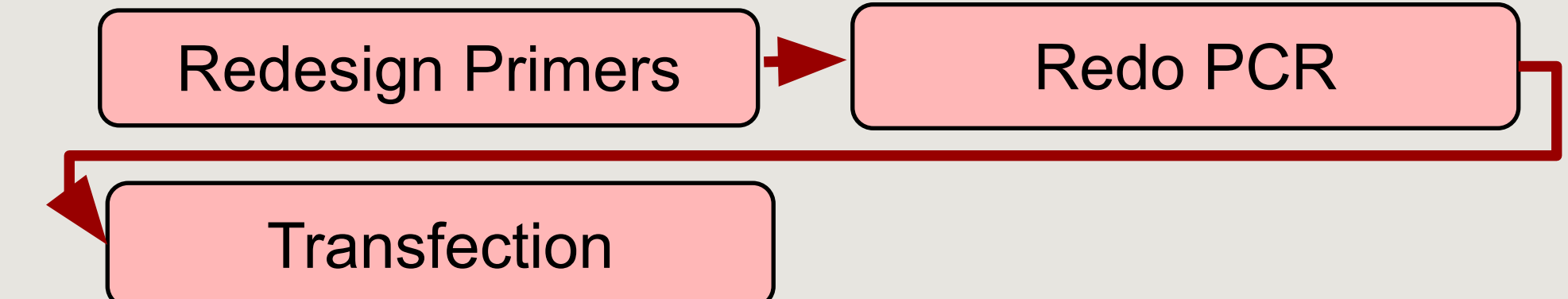


- Consistent BFP bands showing clean fragment displaying successful amplification
- BFP fragment amplified at 3 different temps



- Retrieved PCAD and FRP with different temps
- Still faint bands
- Unsuccessful amplification

FUTURE PLANS



REFERENCES

1. Shurer CR, Kuo JC, Roberts LM, Gandhi JG, Colville MJ, Enoki TA, Pan H, Su J, Noble JM, Hollander MJ, O'Donnell JP, Yin R, Pedram K, Möckl L, Kourkoutis LF, Moerner WE, Bertozzi CR, Feigenson GW, Reesink HL, Paszek MJ. Physical Principles of Membrane Shape Regulation by the Glycocalyx. Cell. 2019 Jun 13;177(7):1757-1770.e21. doi: 10.1016/j.cell.2019.04.017. Epub 2019 May 2. PMID: 31056282; PMCID: PMC6768631.

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