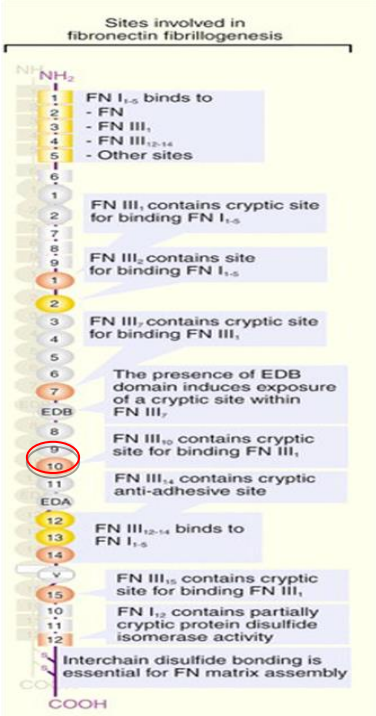
Manuscript Title: The Production of Fibronectin Domains Harvested from *Escherichia Coli*

Author: Sherly Lande and Faculty Mentor: Dr. Karuri W. Nancy
Institution: Illinois institute of Technology
Department of Chemical and Biological Engineering Theme: Health

ABSTRACT: Fibronectin (FN) is crucial for cell adhesion, growth, migration, and differentiation. Moreover, FN plays a major role in wound healing, blood clotting, embryogenesis, tissue repair, and disrupting diseases such as cancer and fibrosis. The purpose of this research project is to increase the production of fibronectin domains in *E. Coli* by increasing the concentration of Isopropyl β-D-1- thiogalactopyranoside (IPTG) during culture. We test different IPTG concentrations: 0.0, 0.3, 0.6 and 0.9 milimolar (mM) and culture for 2 hours with a 200 rpm shaking. More fibronectin domains are produced as IPTG concentrations increase. To reiterate, in this research *E. Coli* produces more 42 KDa FN domains when IPTG concentration is 0.6 and 0.9 mM during induction, and the maximum FN domains produced is at 0.9 mM.

I. INTRODUCTION

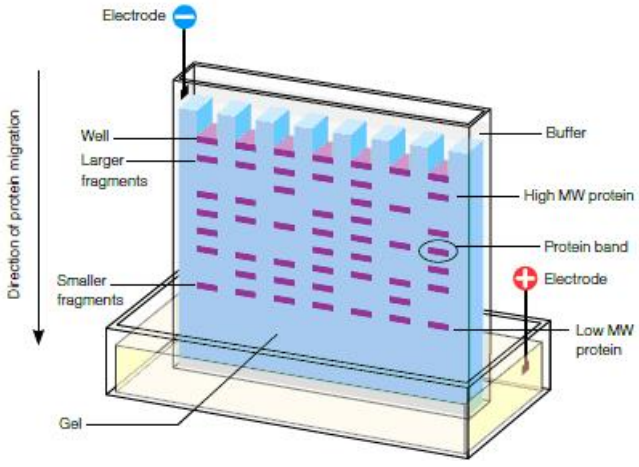
Fibronectin (FN) is important for cell adhesion, growth, migration, and differentiation. Additionally, FN is crucial for wound healing, blood clotting, embryogenesis, tissue repair, and disrupting diseases such as cancer and fibrosis [1]. Dr. Karuri's laboratory uses FN domains in their research. The purpose of this research project is to increase the production of fibronectin domains in *E.Coli* by increasing the concentration of Isopropyl β-D-1- thiogalactopyranoside (IPTG) during culture.



Structure of FN [3]

II. METHODS

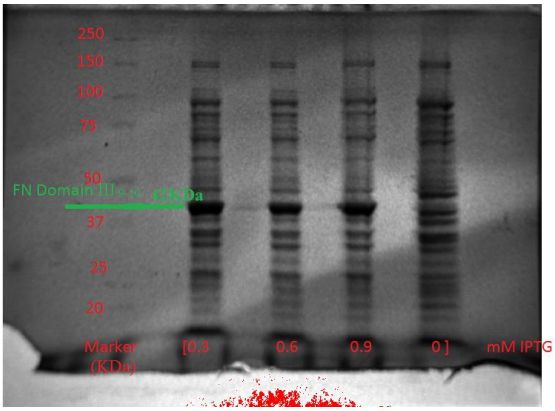
An overnight *E. Coli* suspension culture in lysogeny broth– ampicillin (LB-Amp); culture conditions: 37 °C and 200 revolutions per minute (rpm). A 30 minute culture of 1:10 dilution in LB-Amp of overnight culture; culture conditions: 37°C and 200 rpm. Induced protein production by adding 0.3, 0.6 and 0.9 mM IPTG, and a solution without IPTG, which is the control. Then, grow for 2 hours at 37°C with shaking (200 rpm). Harvest of proteins: Isolation of the bacteria by centrifugation, lysing and resuspension in electrophoresis buffer.



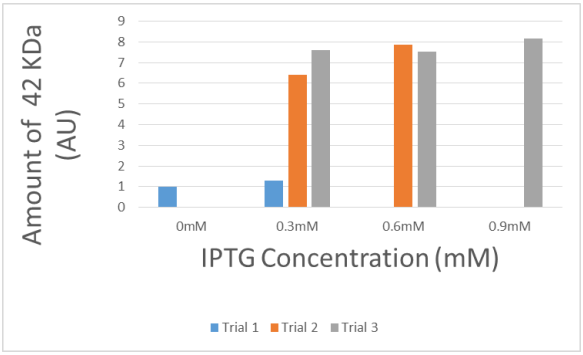
Analysis of electrophoretic protein separation in a polyacrylamide gel [4]

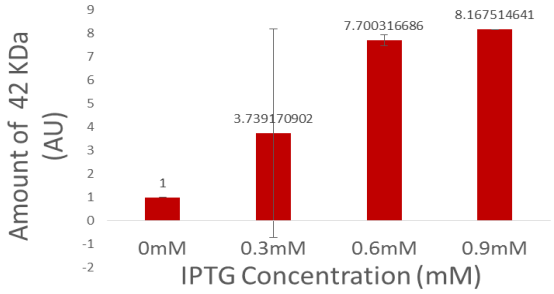
III. RESULTS

Gel Electrophoresis



Relationship between IPTG concentration and proteins produced





- *E. Coli* produces more FN domains as IPTG concentration is increased.
- Maximum FN domains produced is at 0.9 mM IPTG.

IV. DISCUSSION

- In future experiment, will vary induction time and temperature, and revolution speed of bacterial culture.

V. CONCLUSION

Literature uses 0.3 mM of IPTG during induction. This research shows more FN domains will produce when IPTG concentration is 0.6 and 0.9 mM.

VI. ACKNOWLEDGEMENTS

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Thanks to Dr. Karuri for guiding me though this project.

VII. REFERNCES

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[2] Evans, Lauren. “Using Image J to quantify protein bands on a PAGE gel.” Maryville College. Online video clip. YouTube. YouTube, 27 Mar. 2014. Web. 1 Mar. 2016.

[3] Pankov, Roumen, and Kenneth M. Yamada. "Fibronectin at a glance." Journal of cell science 115.20 (2002): 3861-3863.

[4] Schematic of electrophoretic protein separation in a polyacrylamide gel. Protein Electrophoresis Methods. Bio Rad. Web. 5, Mar.2016.