Determining Peptide Binding through mRNA display

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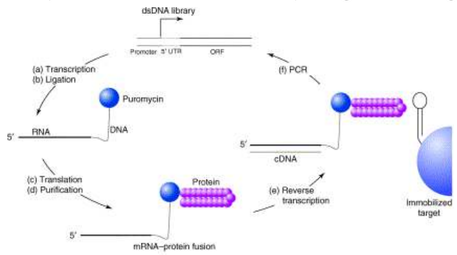
Introduction

mRNA Display

- In Professor Roberts' lab, researchers are identifying ligands that bind to specific target proteins through mRNA display

Purpose of our lab work

- Skyler and I worked on characterizing non-specific peptide binders
- Determine non-specific binding by comparing effect of different salt concentration (10mM, 200mM) and bead matrix (magnetic/agarose)

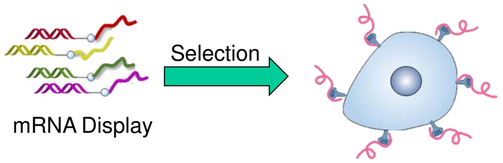


Objective & Impact of Professor's Research

mRNA display can be utilized in the future for:

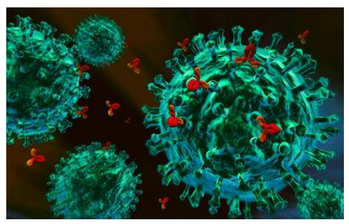
<Protein Identification>

- Identifying peptides that bind to proteins expressed on cancer cells
- Efficient cancer diagnosis/treatment



<Protein-protein interaction inhibitor>

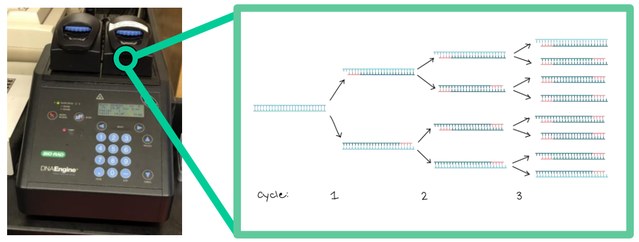
- Autoimmune diseases
- Blocking antibodies that bind to target proteins



Y-shaped antibodies end up attacking the body's own cells

Methods

1. **Polymerase Chain Reaction (PCR)**:
   - GOAL: To amplify DNA

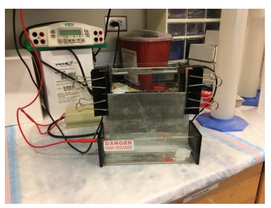


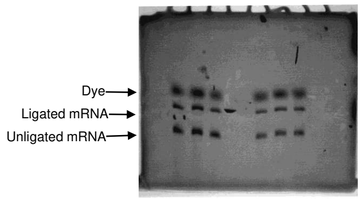
The PCR cycler was used to amplify our sample

2. **Transcription**
   - GOAL: To produce mRNA strand to mRNA strand

3. **Ligation**
   - GOAL: To ligate DNA and mRNA strands together using T4 DNA ligase

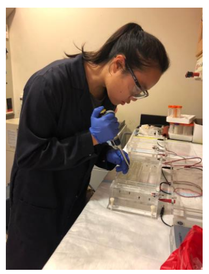
4. **Gel Purification**
   - GOAL: To purify ligated/unligated mRNA
   - Used Urea gel, Elutrap





In our gel results, the ligated mRNA at the top was separated from the unligated mRNA at the bottom

Running the Elutrap allowed us to extract our sample from the gel after gel purification



Using a pipette, we carefully removed the collected sample from the Elutrap

5. **Translation**
   - GOAL: To convert mRNA into peptides

6. **dT Purification**
   - GOAL: To purify translated peptide using dT agarose bease
   - Perform final elution to obtain sample from beads

7. **Reverse Transcription**
   - GOAL: To obtain DNA strands from mRNA

8. **Binding Assay/Selection**
   - GOAL: To select peptides that binded to the target protein

9. **Repeat starting with PCR**
   - We need to amplify the peptides that binded with our beads

The nanodrop was used to determine the concentration of our samples

We frequently used the rotation device to mix our samples evenly

Advice for Future SHINE Students

For advice for future students, I would say to be open to your mentor and Dr. Mills—they are there to help and guide you. Communication is also essential to understanding a topic that you may not be comfortable with. Ask many questions in order to expand your knowledge on the field YOU are interested in!

How This Relates to Your STEM Coursework

Working in Professor Roberts' lab has exposed me to working with different technologies, as well as helped me improve my pipetting skills and giving me a steady hand. Processes such as gel electrophoresis/running agarose gels and working with a micro-centrifuge has become much more comfortable. I am excited to bring these refined skills back to my Biotechnology class next year, as well as use my newfound knowledge of polymerase chain reaction, transcription, and translation for my AP Biology course.

Acknowledgements

I would like to thank Professor Richard Roberts, my mentor Dr. Kaori Noridomi, Skyler Brown, Dr. Mills, Dr. Herrold, and everyone else who has helped my during my SHINE experience.