Team Project in Molecular Biology

Cloning a Virulence Gene from Bordetella Avium
What is bordetellosis?

• a contagious upper respiratory tract infection caused by the bacterium *Bordetella avium*
• related to whooping cough in humans
• affects birds worldwide like chickens, finches, macaws, ostriches, and most prominently…TURKEYS
How it harms:
Colonizes the ciliated epithelium in the trachea
Why this is bad:

- infection causes weight loss, altered vocalization, sneezing and conjunctivitis
- makes birds susceptible to lethal infections like *E. coli*
- the sickly and dying turkeys cause the poultry industries all over the world to lose millions of dollars annually
What has been attempted:

- antibiotics
- a vaccine

Neither block infection nor lessen its effects
Vaccines

- A killed or weakened pathogen
- Stimulate the immune system to produce antibodies
- Protect from later infection
Types of Vaccines

- Inactivated vaccines
- Live, attenuated vaccines
- Toxoid vaccines
- Conjugate vaccines
- Recombinant vector vaccines
- **Subunit (or component) vaccines**
- What we are trying to make
**B. avium Fimbriae**

- Project from membrane and attach to ciliated tracheal cells
- Component vaccine using a fimbriae protein seems promising.
- 8 genes code for Fim protein
Polymerase Chain Reaction

- First step for vaccine
- Need a large amount of Fim protein
- PCR is used to amplify genes
- Method used to make copies of gene coding for Fim protein

Thermo-cycler for polymerase chain reaction (PCR)
Designing Primers

- Bind to beginning and end of gene
- Vital to replication process
- Made primers for 3 possible *fim* genes

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>FimA</td>
<td>5’-CAC CGC GGC AGA CGG CAC GAT CAC TAT TC-3’</td>
<td>5’-CCT GTT ACG CCT CAG GGG TAC ATG ACA G-3’</td>
</tr>
<tr>
<td>Fim2</td>
<td>5’-CAC CGC CGA CGG GCT GAT CAC CAT C-3’</td>
<td>5’-GGG GCC ACG TCA GGG ATA AAC G-3’</td>
</tr>
<tr>
<td>Fim3</td>
<td>5’-CAC CGC GGC AGAVCGG CAC GAT CAC TAT TC-3”</td>
<td>5’-CCT GTT ACG CCT CAG GGG TAC ATG ACA G-3’</td>
</tr>
</tbody>
</table>
Ligation

- Creating phosphodiester bonds between two pieces of DNA
- Replicated *fim* gene from PCR was combined with plasmid/cloning vector
- A commercially prepared plasmid that enables gene expression
Transformation

• Ligated plasmid moved to *E. coli* cells
• Positive control: *E. coli* and plasmid without extra gene
• Negative control: only *E. coli* cells
Miniprep and Restriction Digestion

• Tested to see if \textit{fim} gene was ligated in correct orientation
  – Broke \textit{E.coli} cell walls and isolated the plasmid
  – Restriction enzyme \textit{BclI} added
  – Incubation
  – 1.2\% agarose gel made to analyze the restriction digest.
  – Compared gel band lengths with predicted lengths
Protein Induction

- A repressor binds to the *lac* operon in the plasmid
- Inducer- IPTG binds to the repressor
- The repressor releases the DNA
- T7 RNA polymerase can bind to the promoter
- Transcription of the Fim protein is now possible
Induction

• Selection for *E. coli* with correctly oriented plasmids, as determined by restriction digest

• Incubated a single colony in BHI broth for each gene

• Added isopropylthiogalactose (IPTG)—the inducer—to final concentration of 1 mM
Harvesting Cells

- Centrifuge cells and resuspend in lysis buffer

- Frozen in liquid nitrogen and thawed for 2 minutes to break cell walls and release proteins
Protein Gel Electrophoresis

• Similar to agarose gel for DNA
• Proteins run on acrylamide gel
• Gel stained using Daiichi silver stain II
PCR Results

fimA

DNA standard

1000 bp

500 bp

fim2

fim1

1  2  3  4  5

fim3

1000 bp

500 bp

DNA Standard
## Transformation Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>50</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0</td>
</tr>
<tr>
<td>FimA</td>
<td>7</td>
</tr>
<tr>
<td>Fim2</td>
<td>30</td>
</tr>
<tr>
<td>Fim3</td>
<td>20</td>
</tr>
</tbody>
</table>
Protein Gel Electrophoresis Results

18.4 kD

14.2 kD

Induced Genes

Non-induced Genes
Why was the protein not produced?

Possibility 1

- Did not test enough colonies
- Frame shift in gene inserted into plasmid may have occurred

- Evidence:
  - The desired protein was not expressed

- What can we do?
  - Complete test digestion with more colonies

- However, does not explain digestion band pattern
Possibility 2

- Enzyme used for test digestion was bad and so correct cloning could not be detected
- However, does not explain why protein was not expressed
- How can we tell?
  - Enzyme did not cut many of the plasmids tried
- What can we do?
  - Use a different enzyme
Possibility 3

- Ligation process did not succeed
- Evidence
  - Digestion should have shown two cuts at the appropriate lengths
  - The desired protein was not expressed
- What can we do differently?
  - Use a different cloning vector or a new batch
  - Repeat ligation process under different conditions
Conclusions

• Goal was to produce fimbrial protein for a vaccine
• PCR: Successful
• Ligation: Undetected
• Protein Expression: Unsuccessful
• Future Goals: Retry cloning and eventually create vaccine
Imagine a World Without Turkeys
Acknowledgements

• Thank you Dr. Temple, Karen, and everyone else who helped us on this project!