Team Project in Molecular Biology

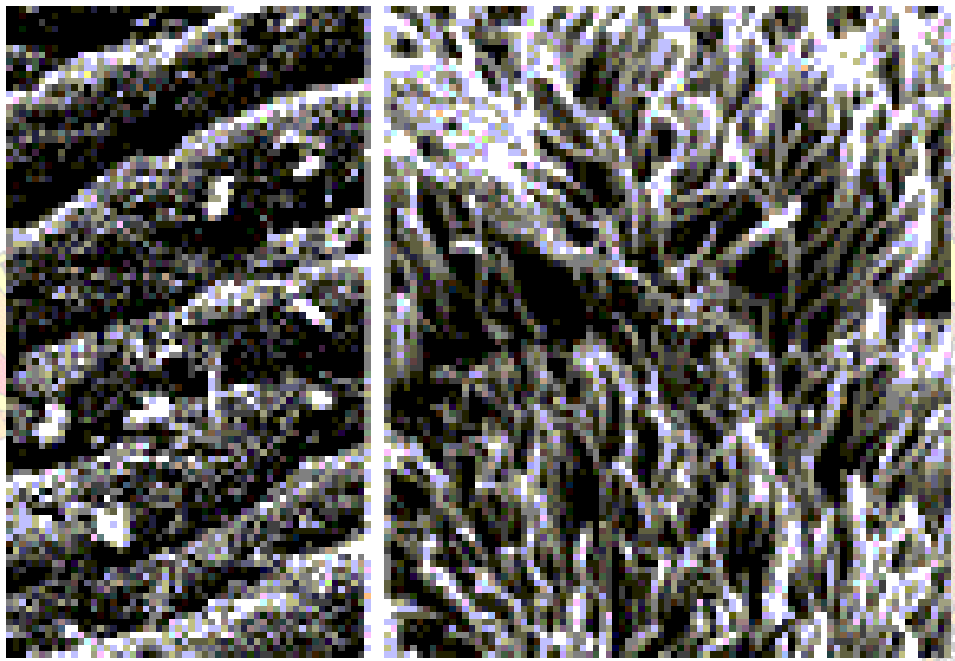
Cloning a Virulence Gene from Bordetella Avium

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**

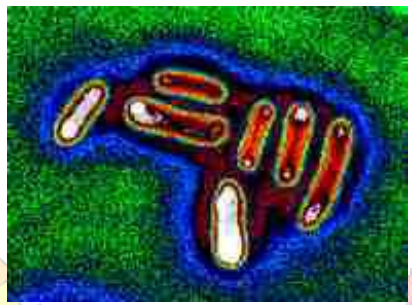
How it harms:

Colonizes the ciliated epithelium in the trachea



Why this is bad:

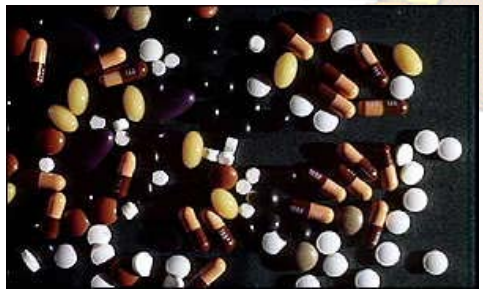
- infection causes weight loss, altered vocalization, sneezing and conjuctivitis
- makes birds susceptible to lethal infections like *E. coli*



- the sickly and dying turkeys cause the poultry industries all over the world to lose millions of dollars annually

What has been attempted:

antibiotics

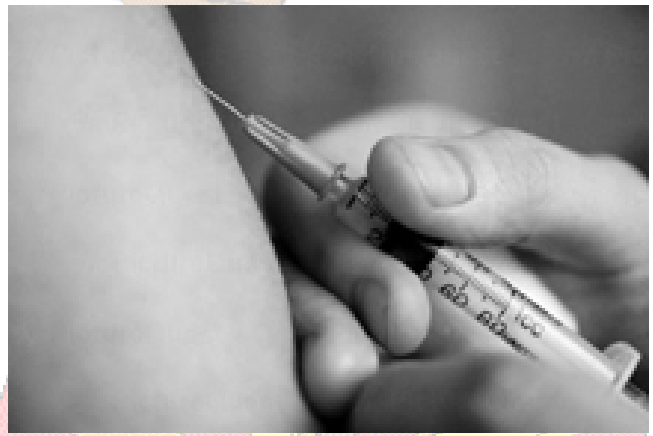


a vaccine

Neither block infection nor lessen its effects

Vaccines

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

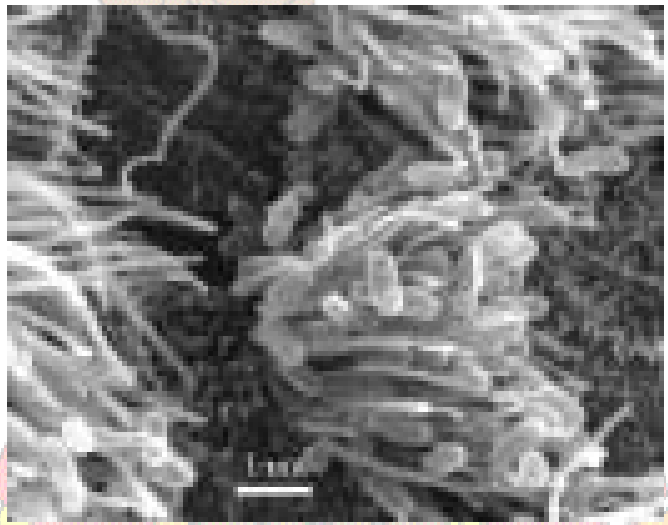


Types of Vaccines

- 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

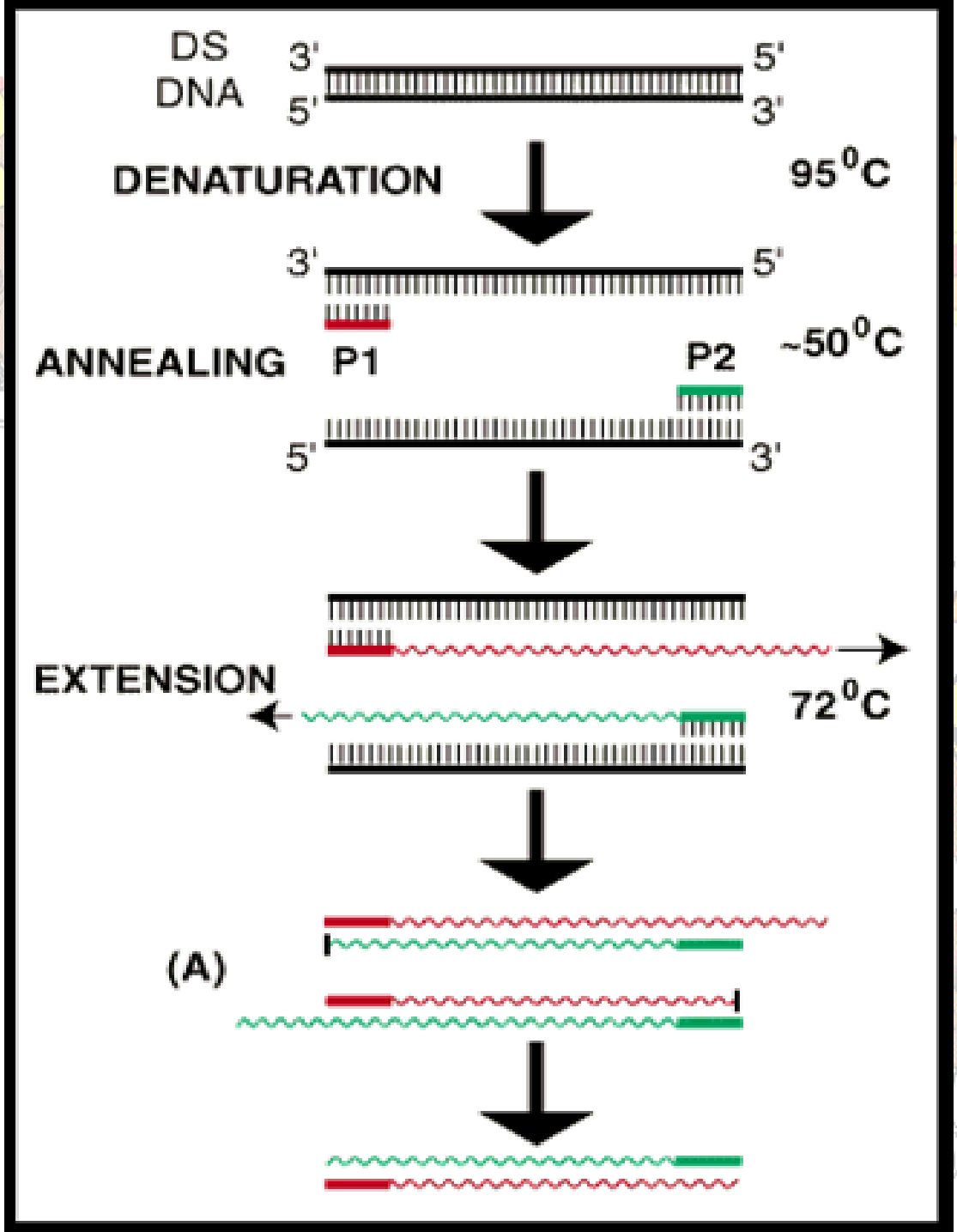
- Project from membrane and attach to ciliated tracheal cells
- Component vaccine using a fimbriae protein seems promising.
- 8 genes code for Fim protein



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)

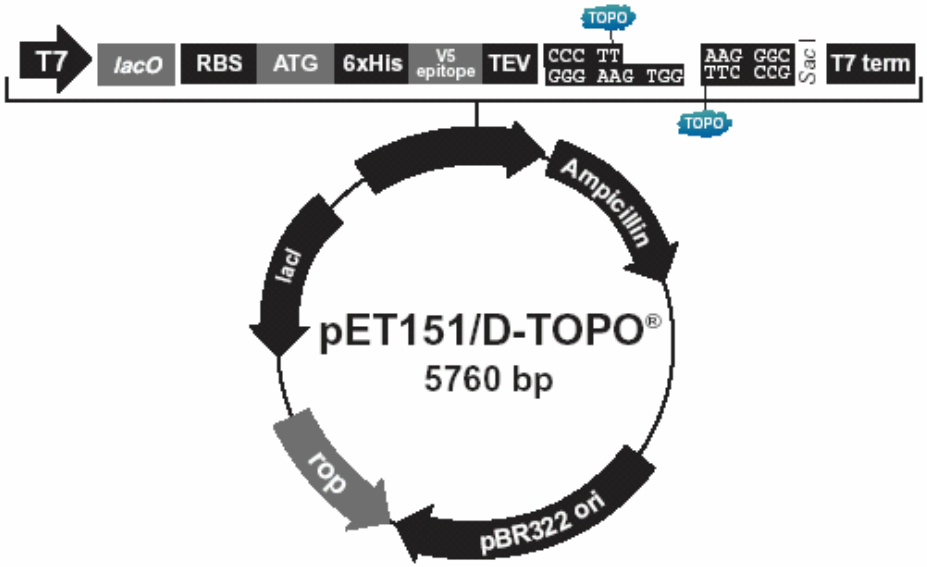

DS
DNA
3'
5'
5'
3'
DENATURATION
95⁰C
3'
5'
ANNEALING
P1
P2
~50⁰C
5'
3'
EXTENSION
72⁰C
(A)

Designing Primers

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

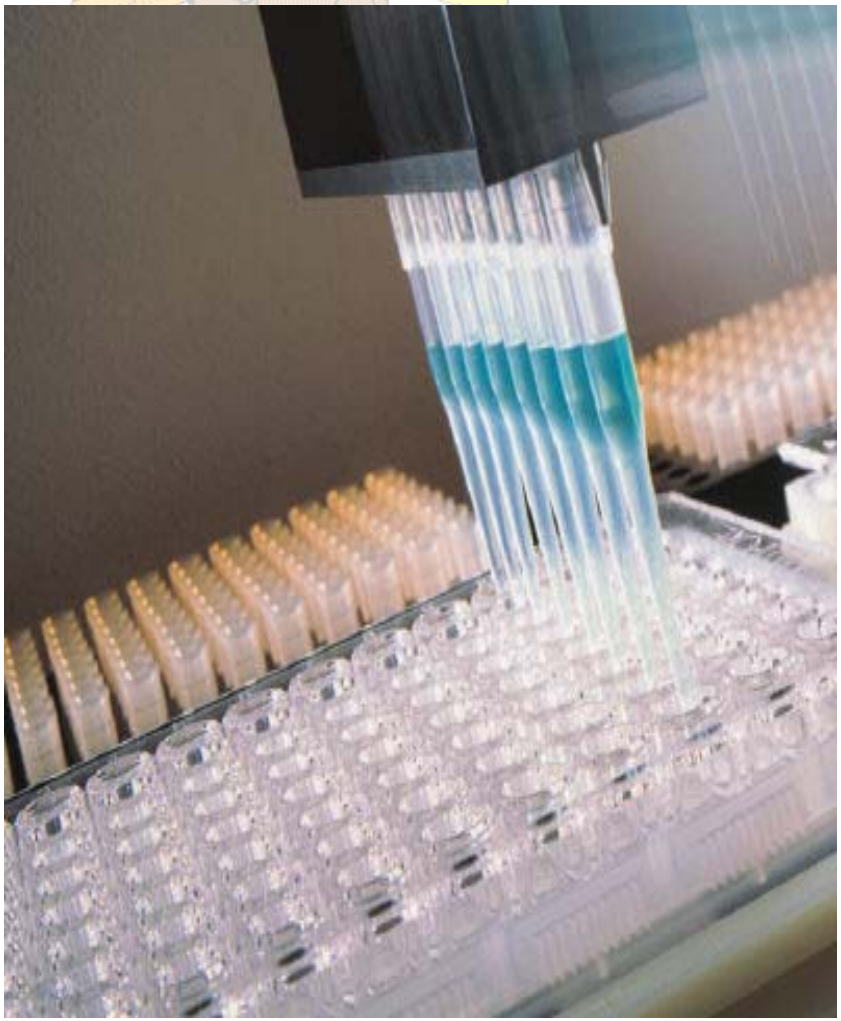
Primer Name	**Forward Sequence**	**Reverse Sequence**
FimA	5'-CAC CGC GGC AGA CGG CAC GAT CAC TAT TC-3'	5'-CCT GTT ACG CCT CAG GGG TAC ATG ACA G-3:
Fim2	5'-CAC CGC CGA CGG GCT GAT CAC CAT C-3'	5'-GGG GCC ACG TCA GGG ATA AAC G-3'
Fim3	5'-CAC CGC GGC AGAVCGG CAC GAT CAC TAT TC-3"	5'-CCT GTT ACG CCT CAG GGG TAC ATG ACA G-3'

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



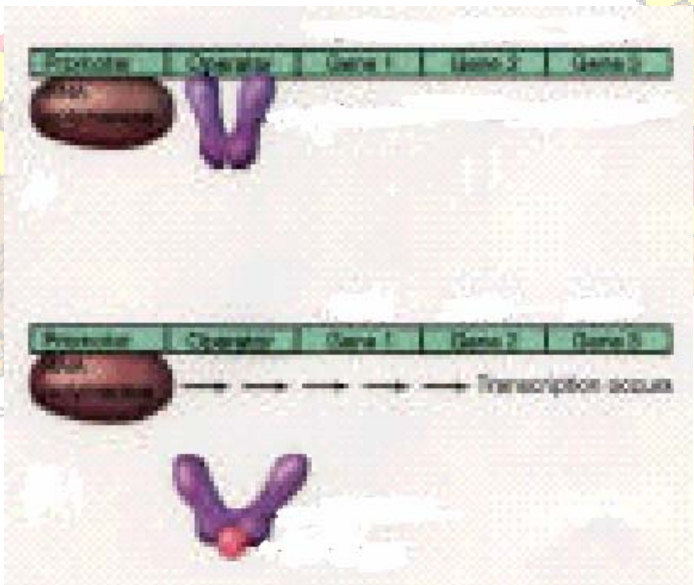
- Ligated plasmid moved to *E. coli* cells
- Positive control: *E. coli* and plasmid without extra gene
- Negative control: only *E. coli* cells

Miniprep and Restriction Digestion

- Tested to see if *fim* gene was ligated in correct orientation
  - Broke *E.coli* cell walls and isolated the plasmid
  - Restriction enzyme *BclI* added
  - Incubation
  - 1.2% agarose gel made to analyze the restriction digest.
  - Compared gel band lengths with predicted lengths

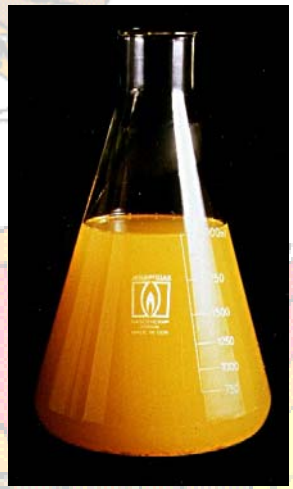
Protein Induction

- 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



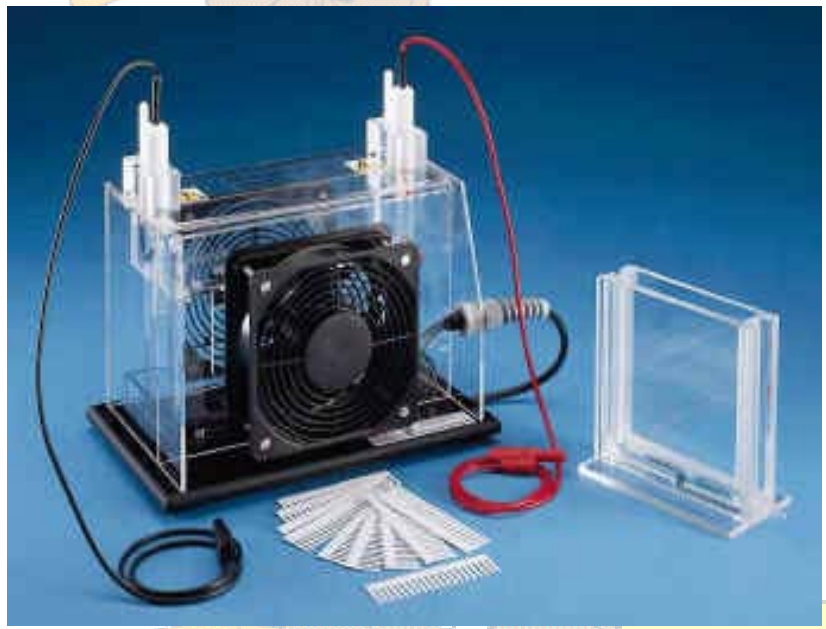
Harvesting Cells

- Centrifuge cells and resuspend in lysis buffer

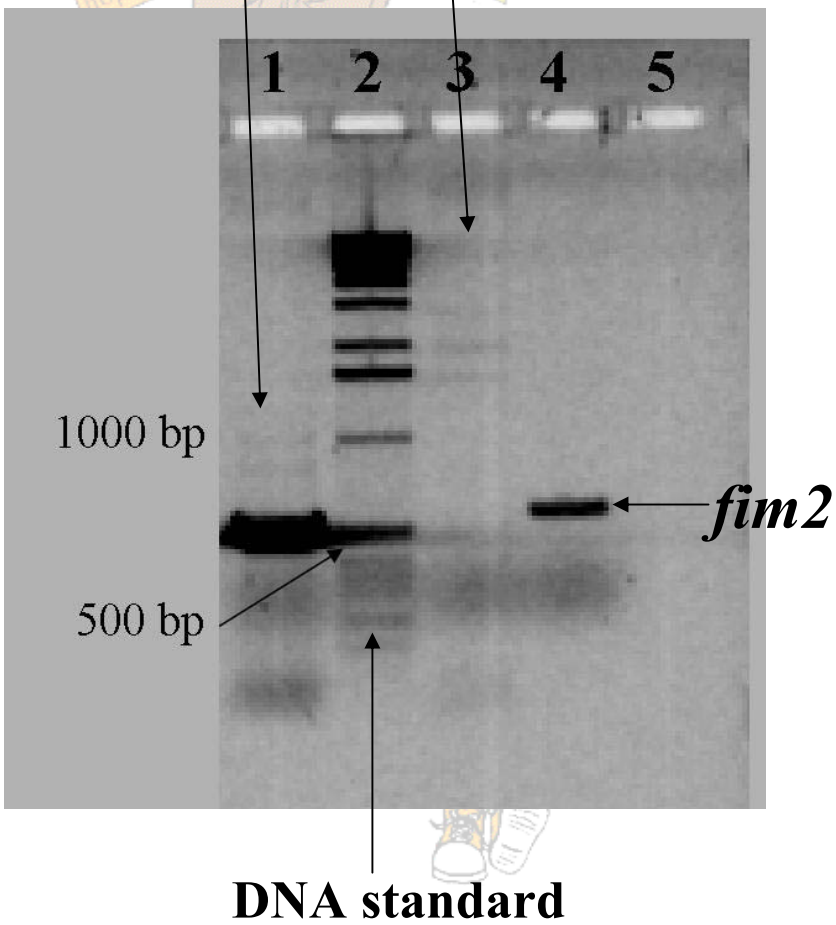
- Frozen in liquid nitrogen and thawed for 2 minutes to break cell walls and release proteins

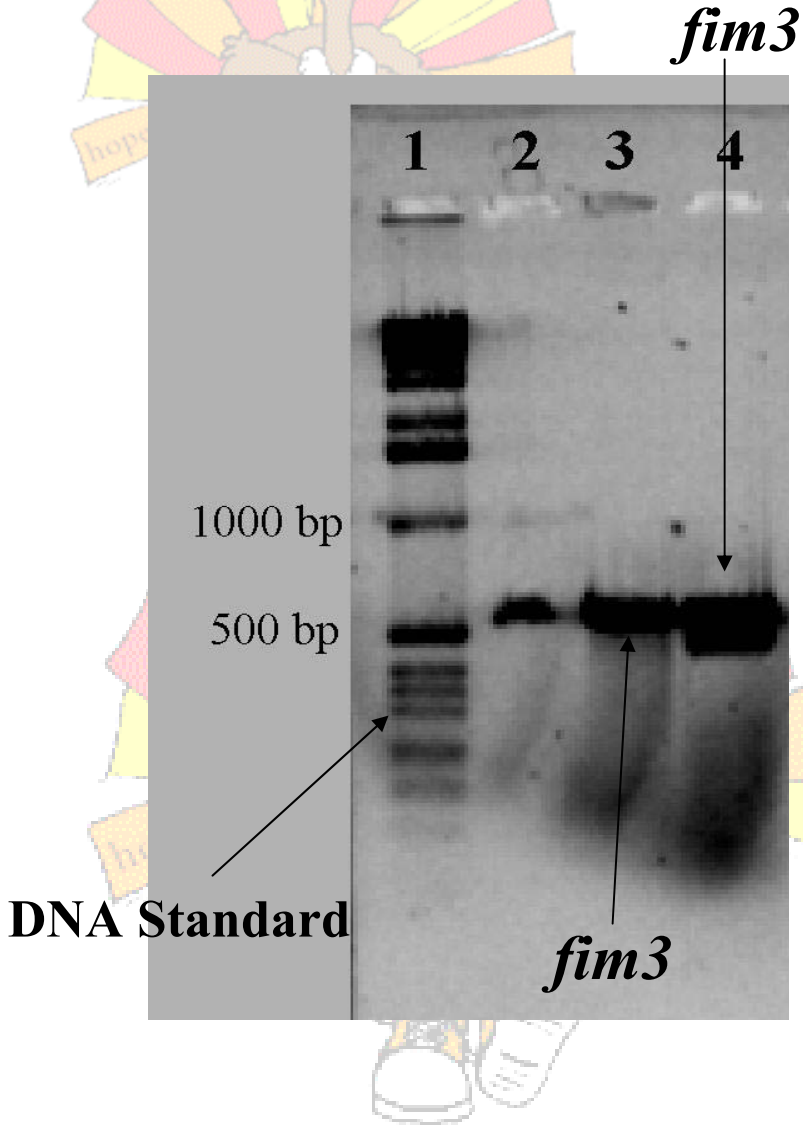
Protein Gel Electrophoresis

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



PCR Results

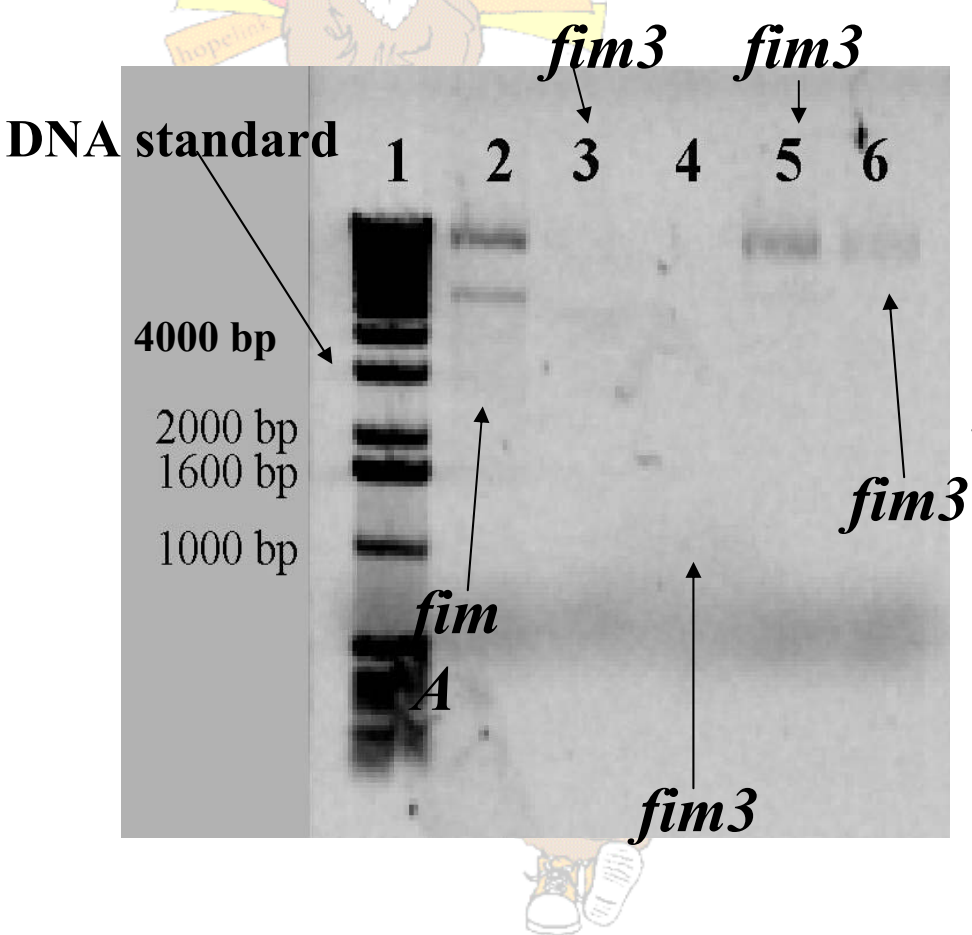

fimA
fim1
1
2
3
4
5
1000 bp
500 bp
fim2
DNA standard

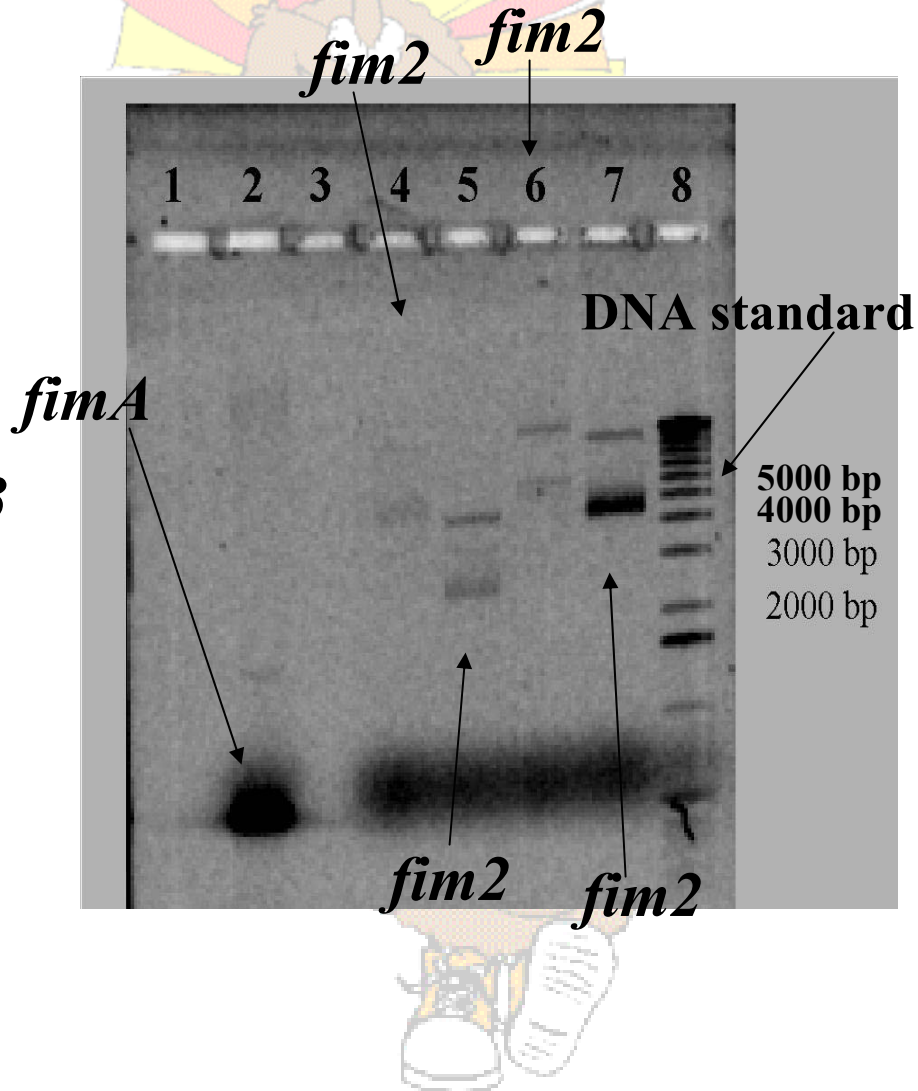

fim3
1
2
3
4
1000 bp
500 bp
DNA Standard
fim3

Transformation Results

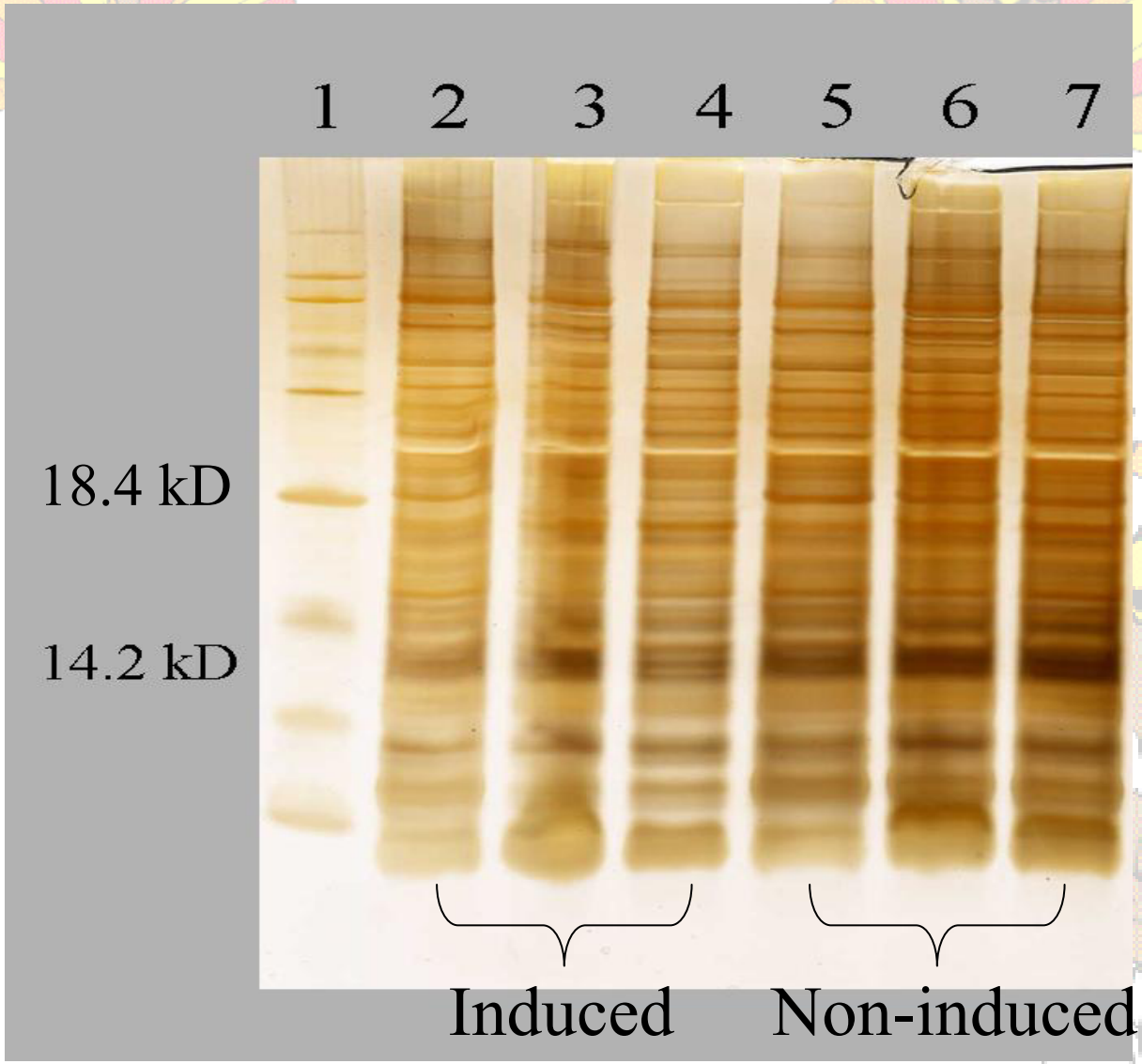
Sample	Number of Colonies
Positive Control	50
Negative Control	0
FimA	7
Fim2	30
Fim3	20

Test Digestion Results





Protein Gel Electrophoresis Results



Why was the protein not produced?

Possibility 1

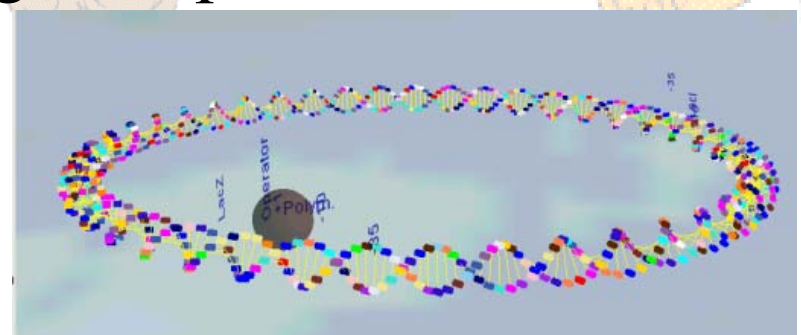
- Did not test enough colonies
- Frame shift in gene inserted into plasmid may have occurred
- Evidence:
  - The desired protein was not expressed
- What can we do?
  - Complete test digestion with more colonies
- However, does not explain digestion band pattern

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

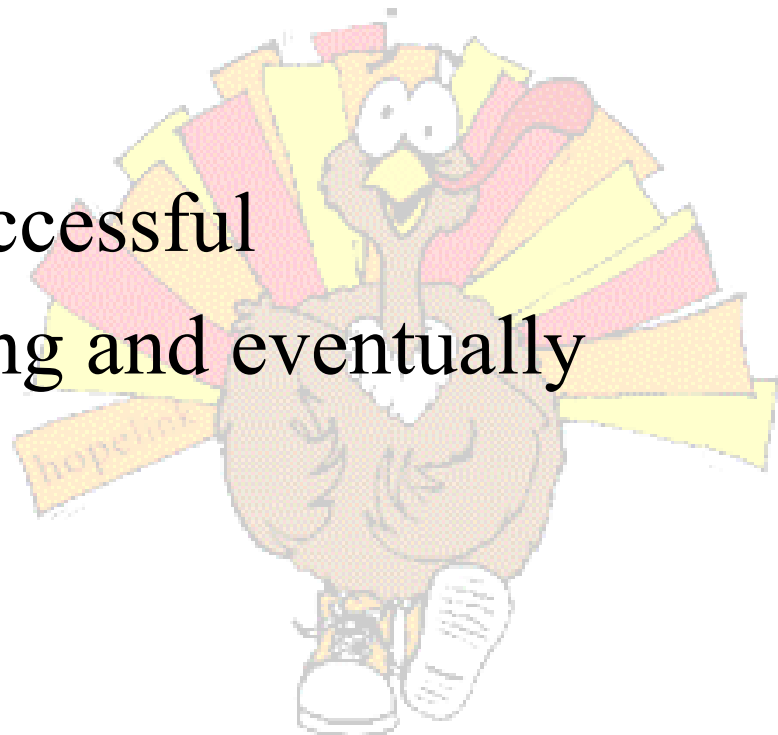
Possibility 3

- 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

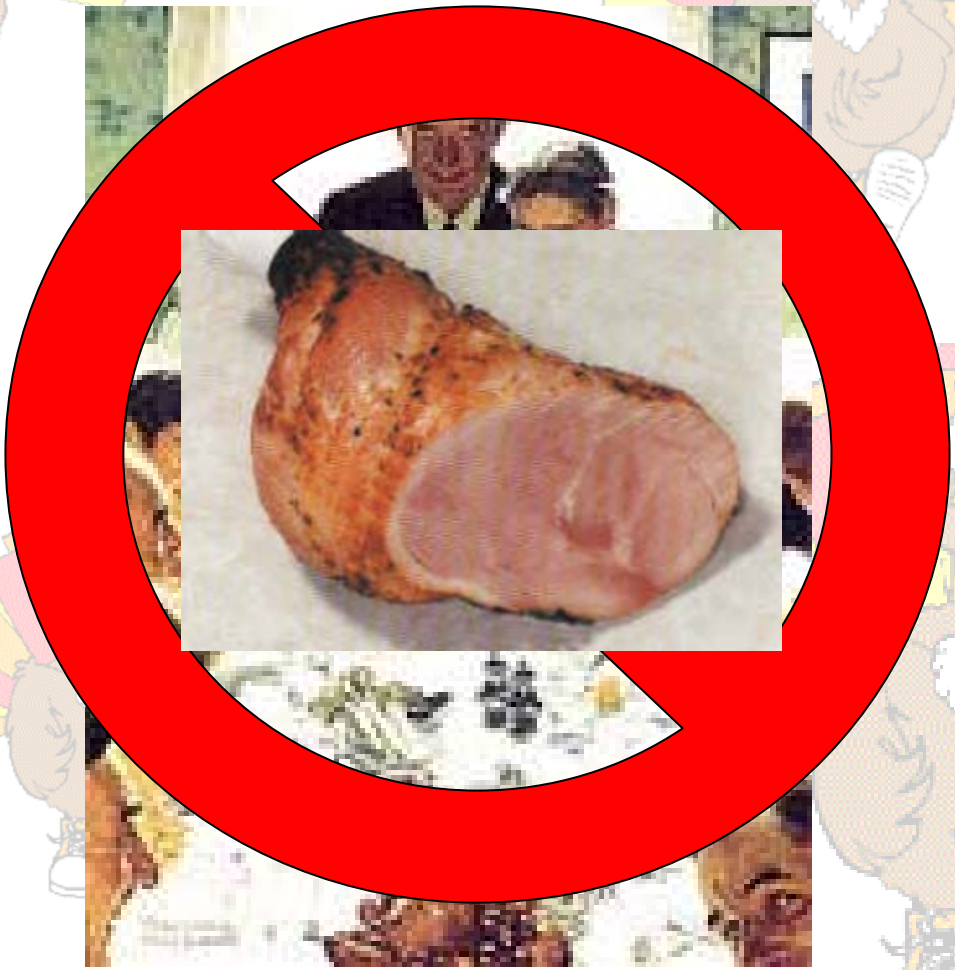


Conclusions

- 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!