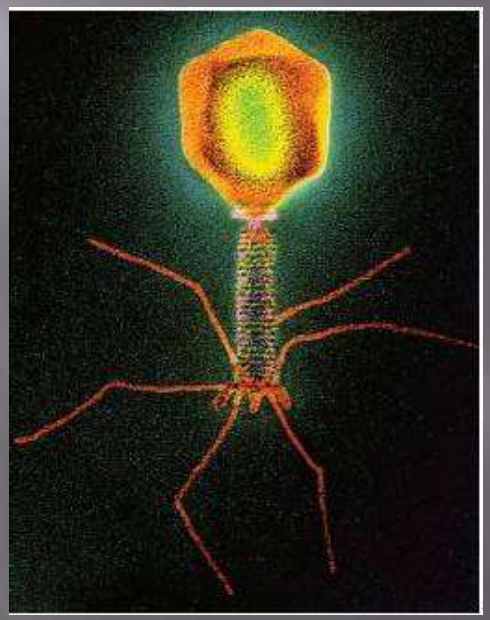
SIMULATED RNA INTERFERENCE IN THE T4 BACTERIOPHAGE LYTIC CYCLE



Undergraduate Research Day 2010

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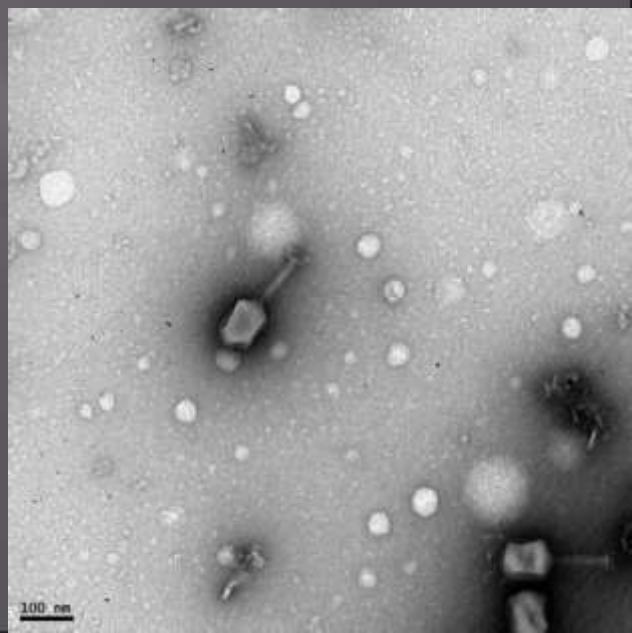
Photo taken from http://www.scienceclarified.com/images/uesc_02_img0070.jpg

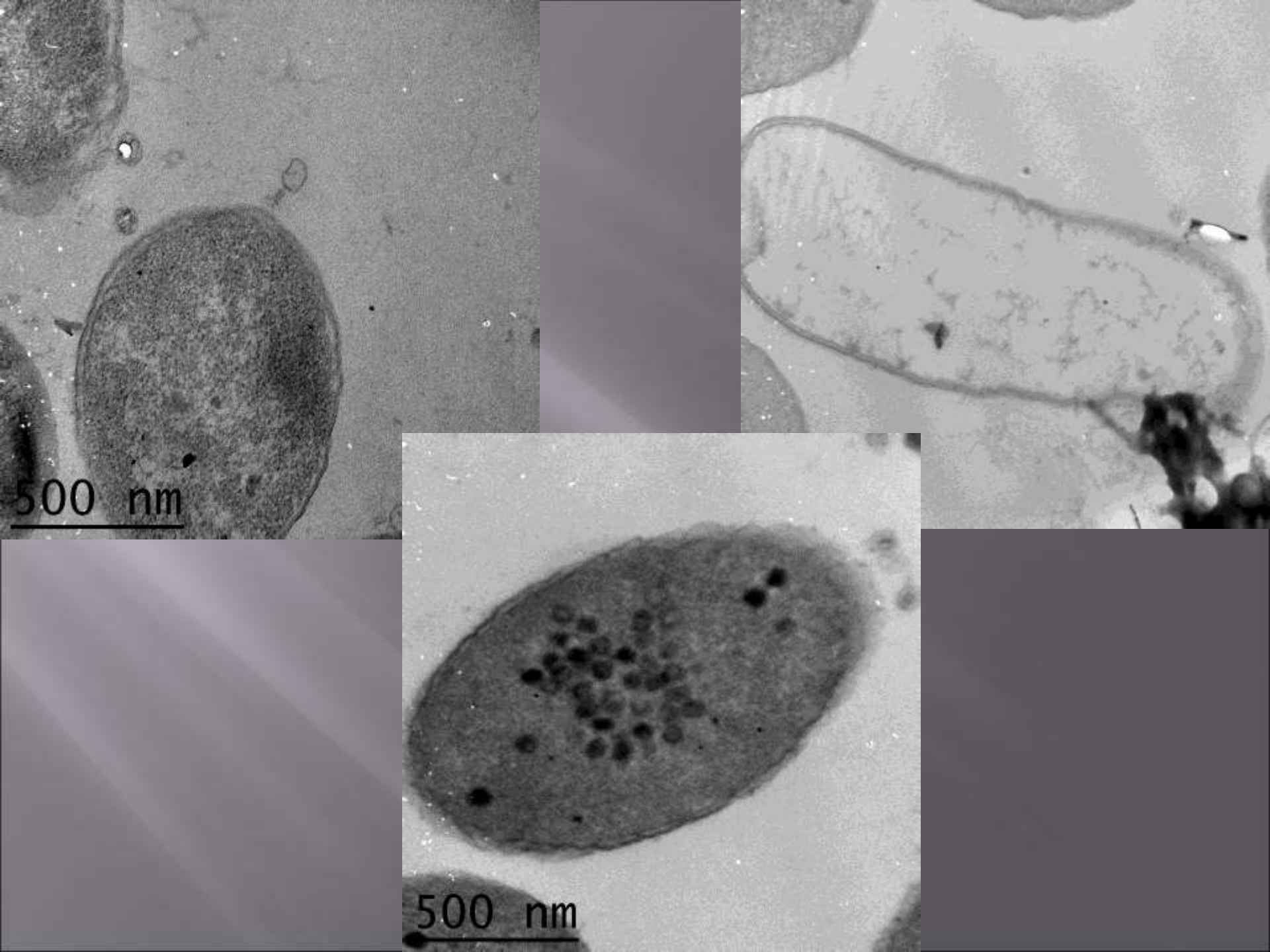
Human Virus Intro

- Influenza has caused 90,078 deaths up to this week in the 2009-2010 influenza season (CDC).
- HIV/AIDS
  - Is not just Africa. 244 cases of AIDS were reported in Wyoming in 2008 (CDC).
- No good active treatment option
  - Tamiflu is for before infection actually starts.
  - Anti-retrovirals are toxic over time, and only suppress HIV when taking the HAART treatment. Expensive.

Introduction to T4 K10

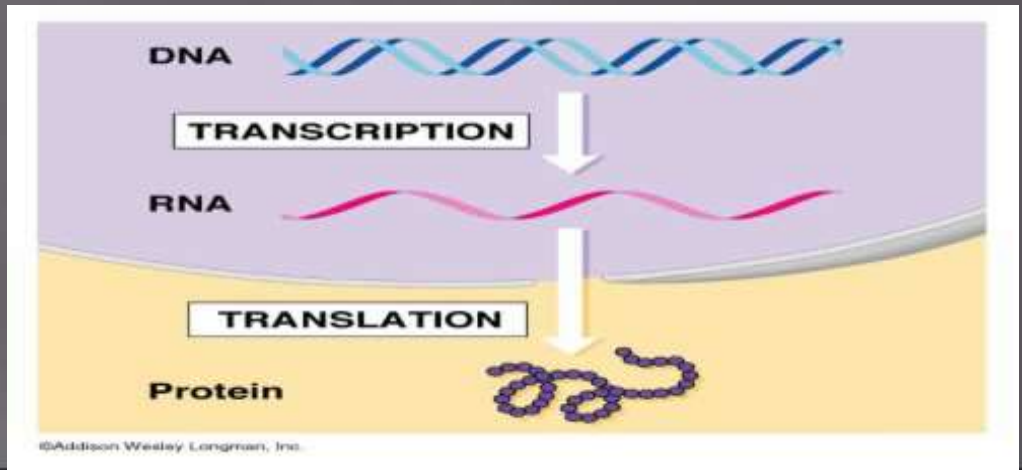
- T4 K10 is a virus that infects the *Escherichia coli* bacterium.
  - Model organism for human viruses.
- The replication of this virus is strictly lytic.
  - 100-150 virions per lysis cycle
  - *DenA* & *DenB* knockout
    - Endonuclease II & Endonuclease IV
- dsDNA virus




500 nm
500 nm

Viral Protein Production

- Transcription
  - The process from which DNA is transcribed into messenger RNA (mRNA) to then be used in translation.
- Translation
  - The process where mRNA is read by tRNA and proteins are assembled according to the specific genetic sequence of the mRNA.

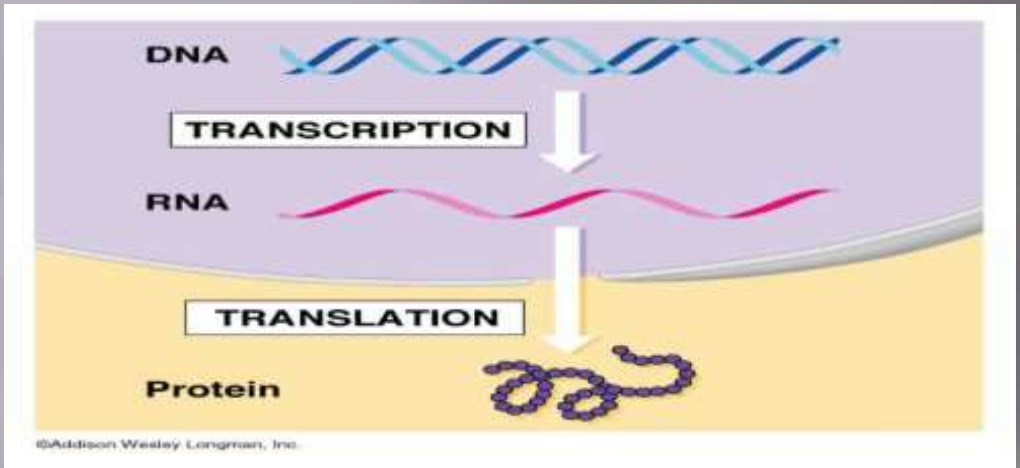


Picture taken from:
http://www.anselm.edu/homepage/jpitocch/genbio/transcrtransl.JPG

RNA Interference

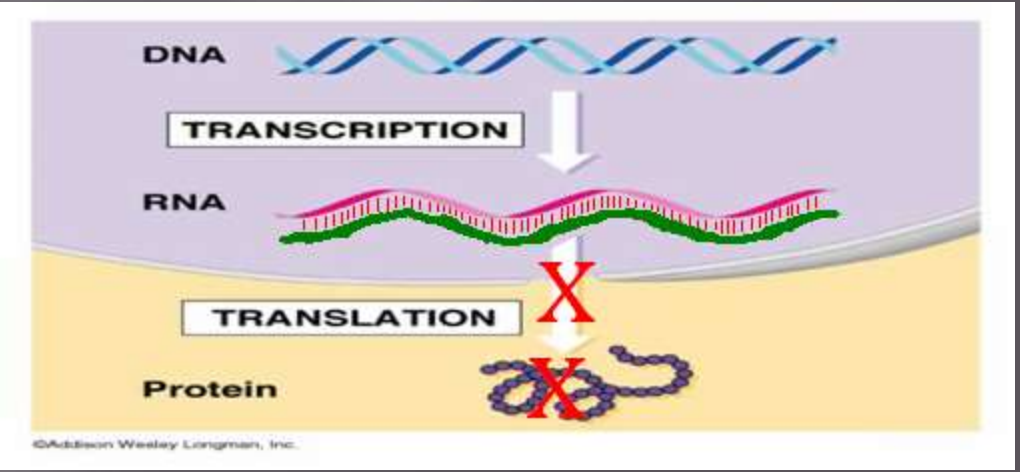
- The use of a complimentary strand of RNA to block the translation of mRNA of interest
  - By blocking the mRNA, translation of the protein product the gene codes for cannot be made
    - miRNA
    - siRNA
      - Small interfering RNA/enzyme complex that eukaryotic cells use.
        - Highly effective when targeted correctly
      - Expensive

Translation Interference



Normal Protein Production

RNA Interference



Picture taken and adapted from:
http://www.anselm.edu/homepage/jpitocch/genbio/transcrtransl.JPG

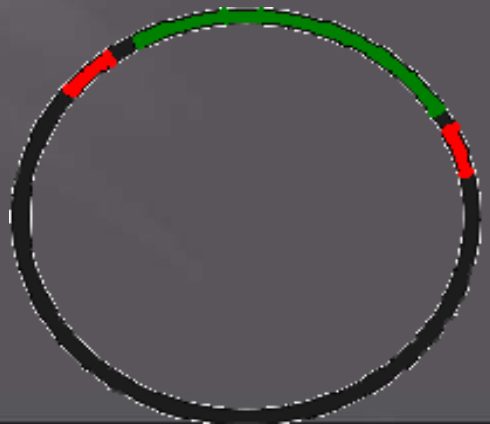
Project Goals

- Clone complimentary gene *t* (T-Holin) pseudogene into an expression vector
- Express complimentary mRNA during an active T4 infection to interfere with lysis
- Characterize the RNA interference effects phenotypically

Cloning Methods

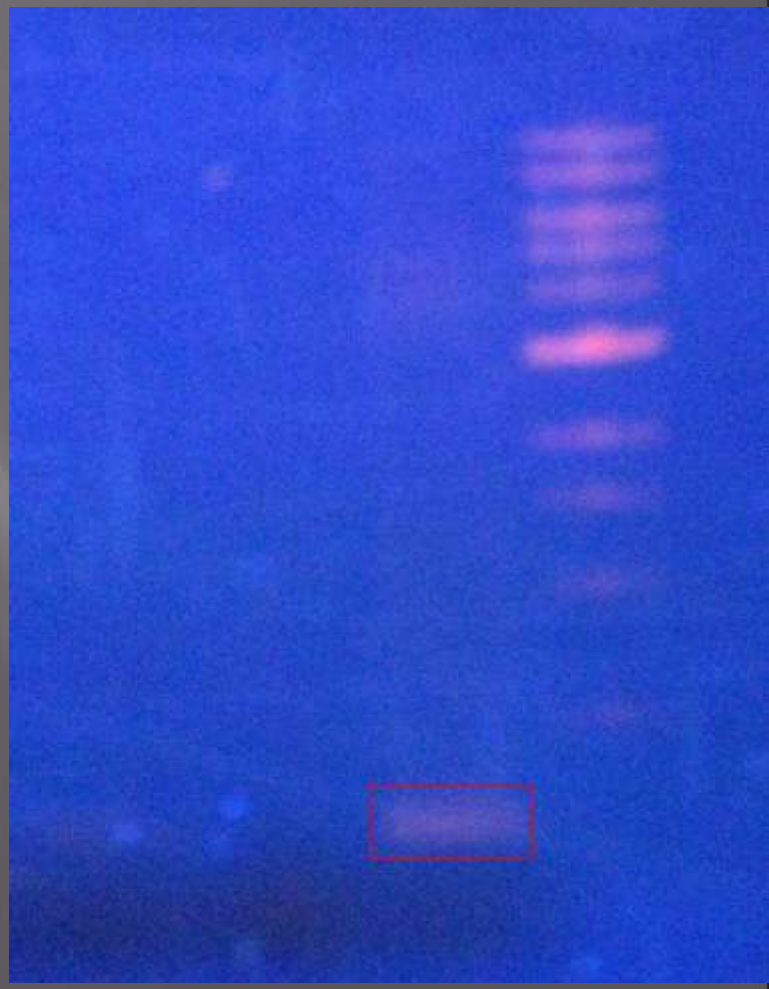
- Amplified the first ~120bp of gene *t* via polymerase chain reaction (PCR)
- Cut out and gel purified 120bp bands and TOPO cloned into pET expression vector (Novagen)
- Confirmed complimentary insert with double reverse primer PCR

pEHoT-16



pEHoT-16

pEHoT Plasmid prep gel



Double reverse primer conformation gel

Active Infection Interference Methods

- Grew pEHoT-16 transformed *E. coli* strain EC
  - With Ampicillin (50µg/mL)
  - With Ampicillin (50µg/mL) & IPTG (80µM)

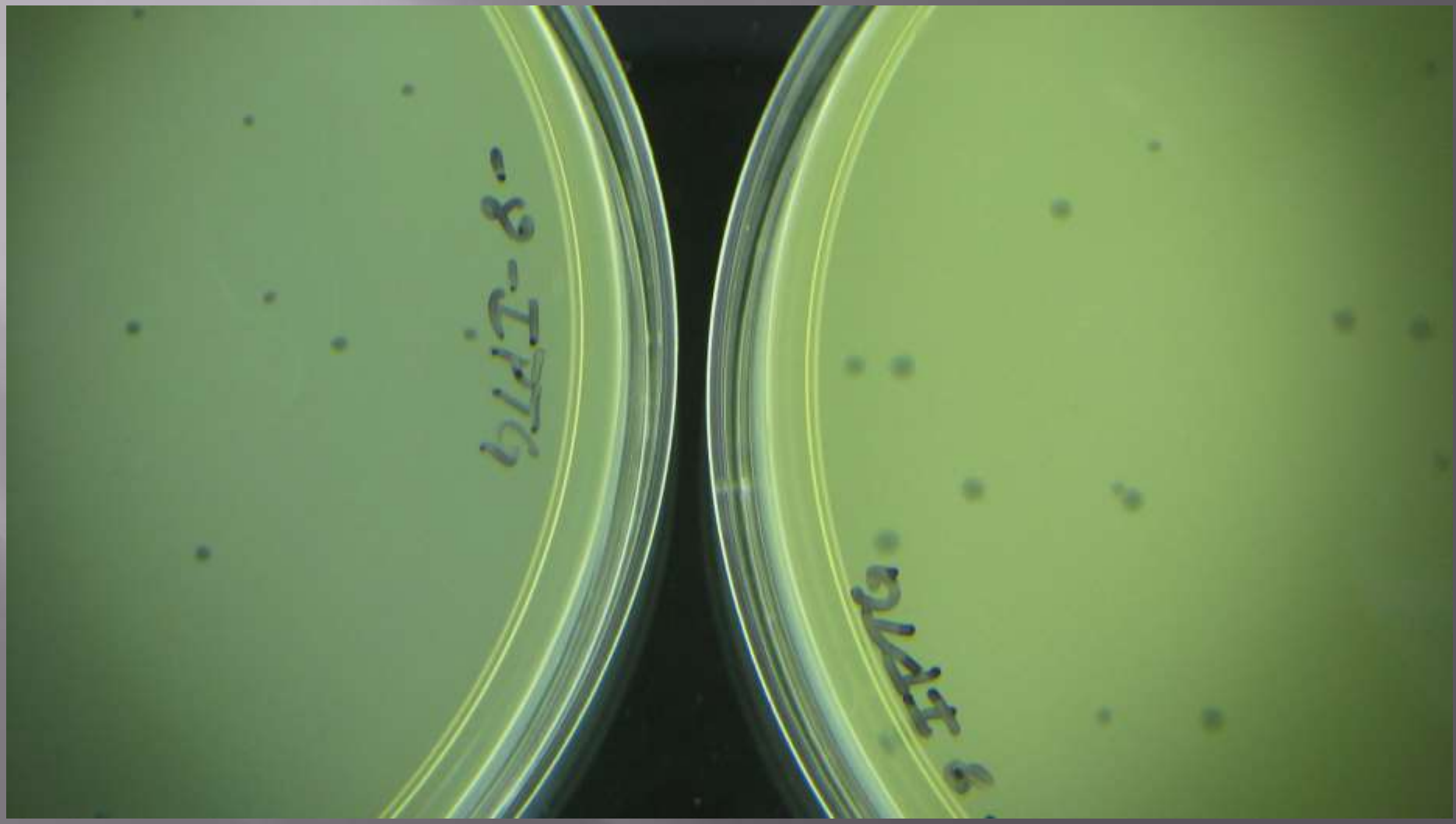
- Performed plaque assays on these cultures with T4 K10
  - With & without IPTG in top agar
    - Ampicillin in both

* IPTG drives expression

Infection Interference Results

- No significant titer difference in plaque assays
  - RNAi – 2.2×10^9 PFU/mL
  - Control – 2.62×10^9 PFU/mL
- Modified plaque morphology
  - Suggests difference in replication cycle or lysis

Plaque Morphology



Future Work

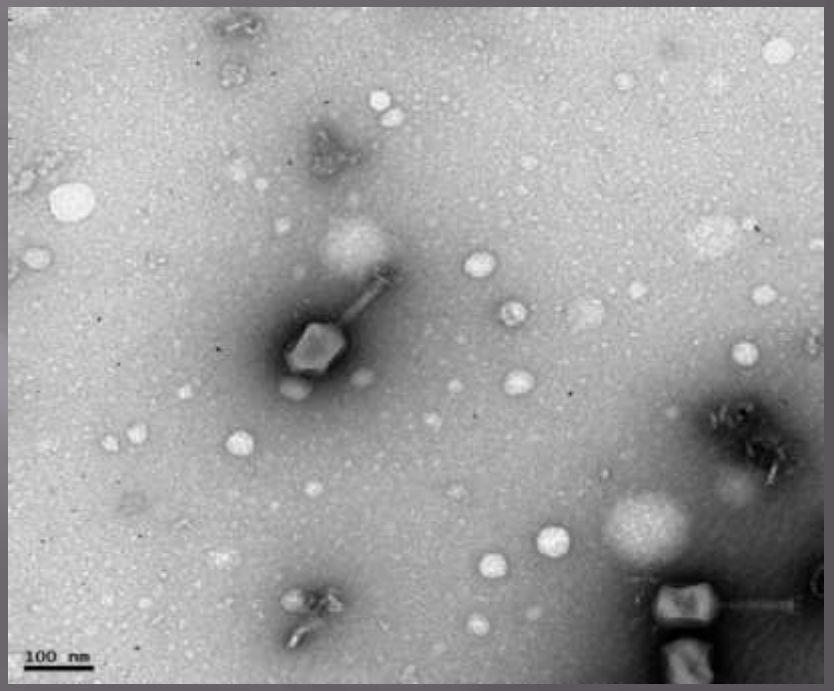
- Quantitative Results Pending:
  - One-step growth curves are in the wings
  - These tests will confirm or refute whether there is significant suppression of the lytic cycle of T4 K10 by quantitatively measuring the time it takes for a complete lytic cycle of the concerned virus and burst size.
- Temperature variations

Speculative Future Work

- Viral expression library
- Methylated plasmid DNA
- Serve as an inexpensive system to identify potential siRNA and miRNA targets

Acknowledgements

- Dr. John Willford !
- Rachel Watson
- Dr. Gerry Andrews
- INBRE Undergraduate Research Award Program



Questions?