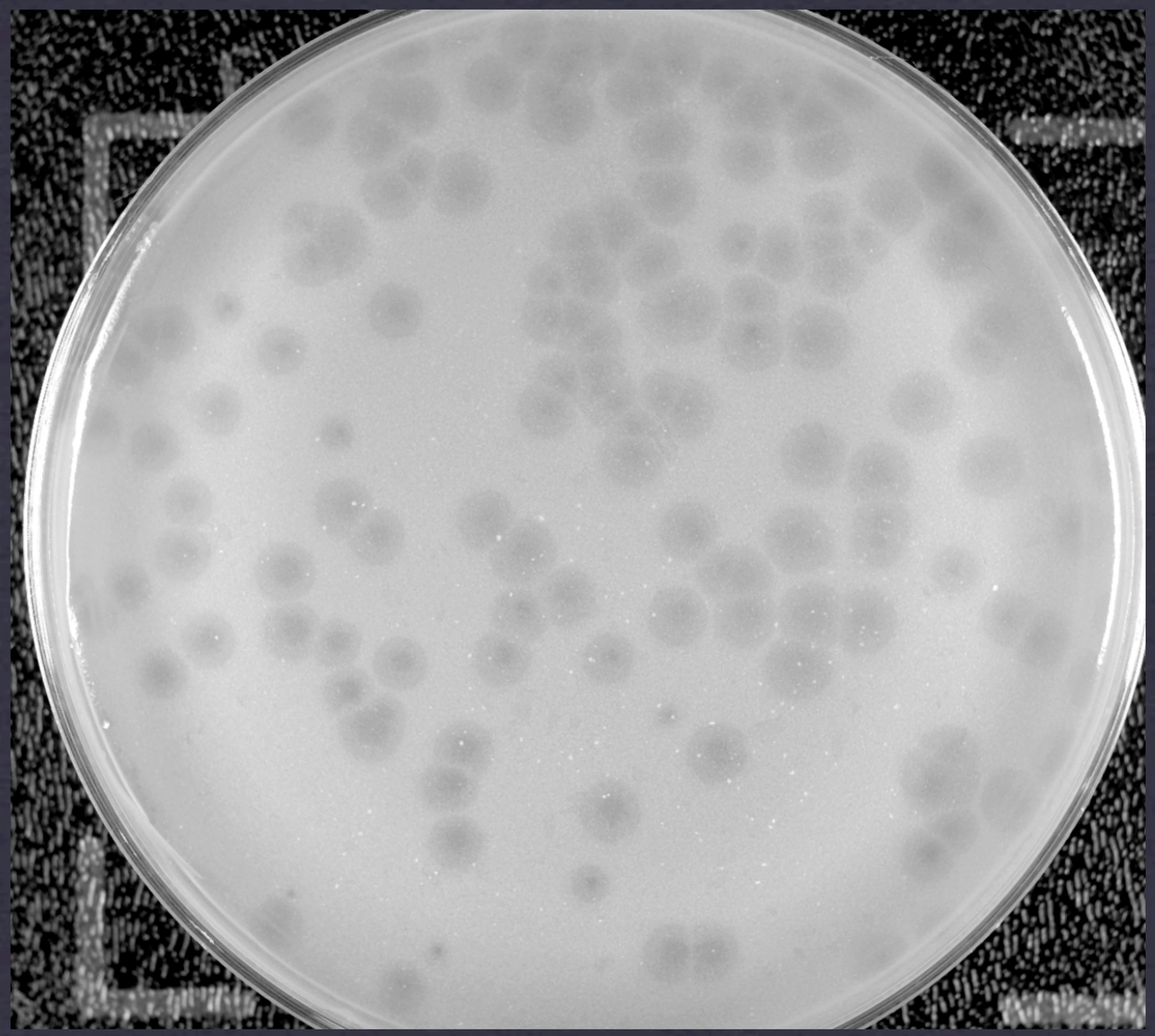
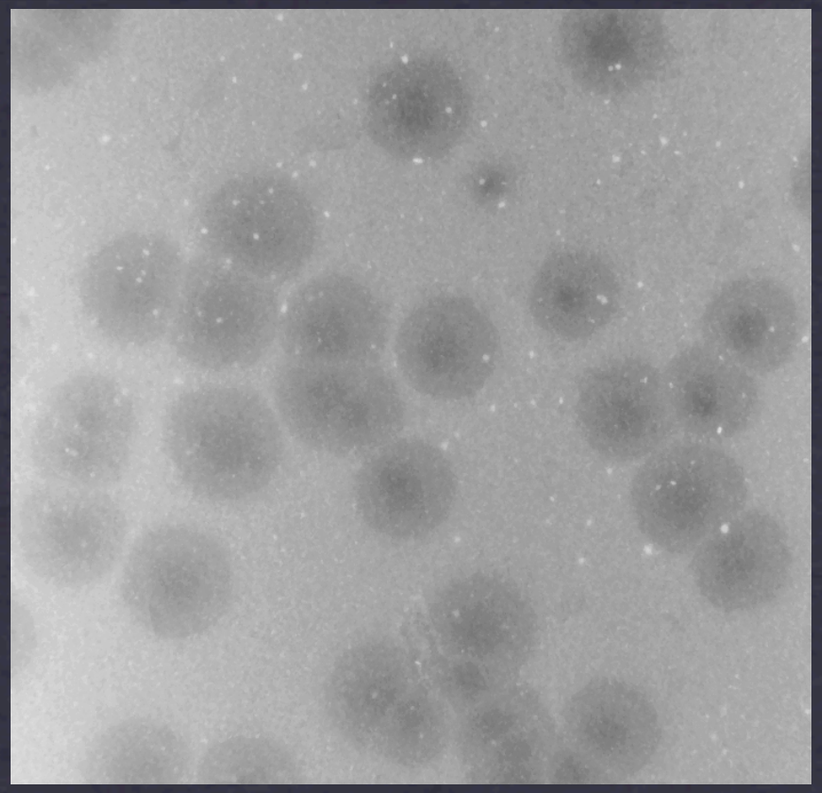
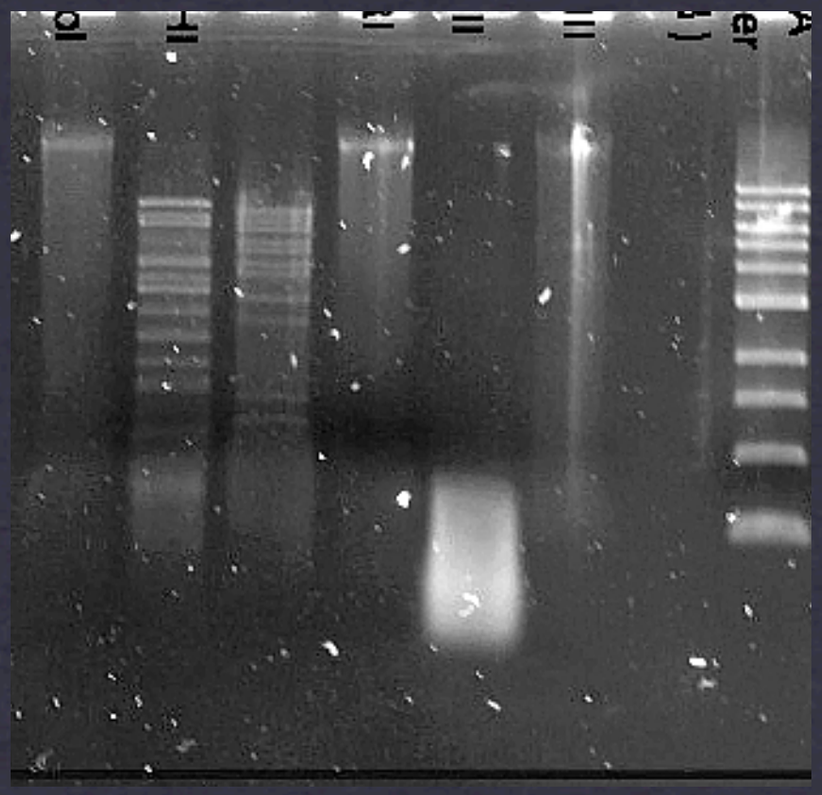
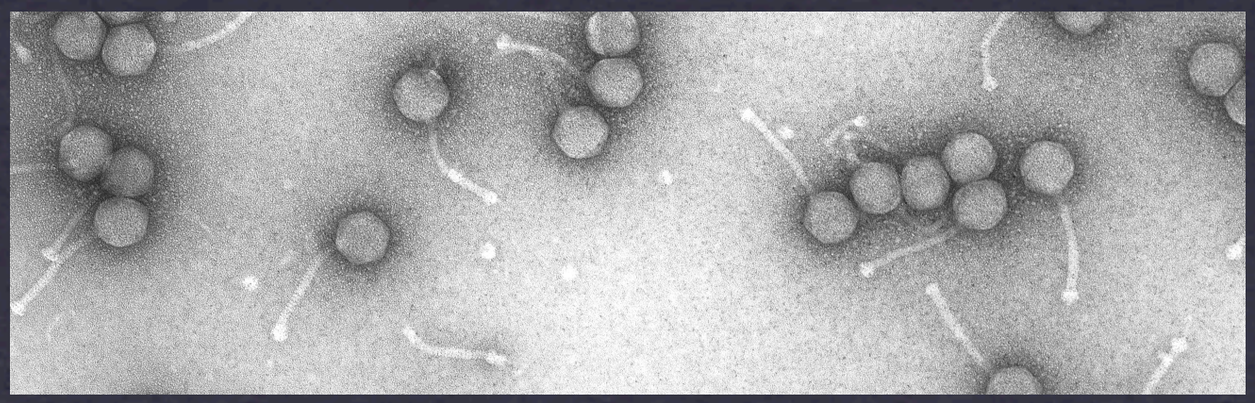








PSEUDONYM

MIRANDA PARRISH

Bacteriophage

- Bacteriophage (often shortened to “phage”) are viruses that infect bacteria
- This phage, Pseudonym, infects *Mycobacterium smegmatis* (*M. smegmatis*), a bacterium related to pathogenic *M. tuberculosis* and *M. leprae*
- Phage can be lytic or lysogenic.
- A lysogenic phage integrates its DNA into the bacterial chromosome and reproduces when the host is threatened.
- A lytic phage does not integrate its DNA

Plaque Formation

- We use a plate to see the plaques
- The phage is mixed with its host bacteria and hot top agar. The mixture is then poured onto the plate. The plate is incubated shortly after the mixture hardens.
- Without the phage, the bacteria grows into a clear lawn
- Plaques are formed when the phage kills off the cells of its host.

Soil Collection

- Collected on 09/05/2011
- 18°C at 2:00PM EST
- 37.7976 N, 85.4708 W
- The weather was cloudy and rainy. It was not raining when the sample was collected.
- The soil was collected nearby an ant colony.

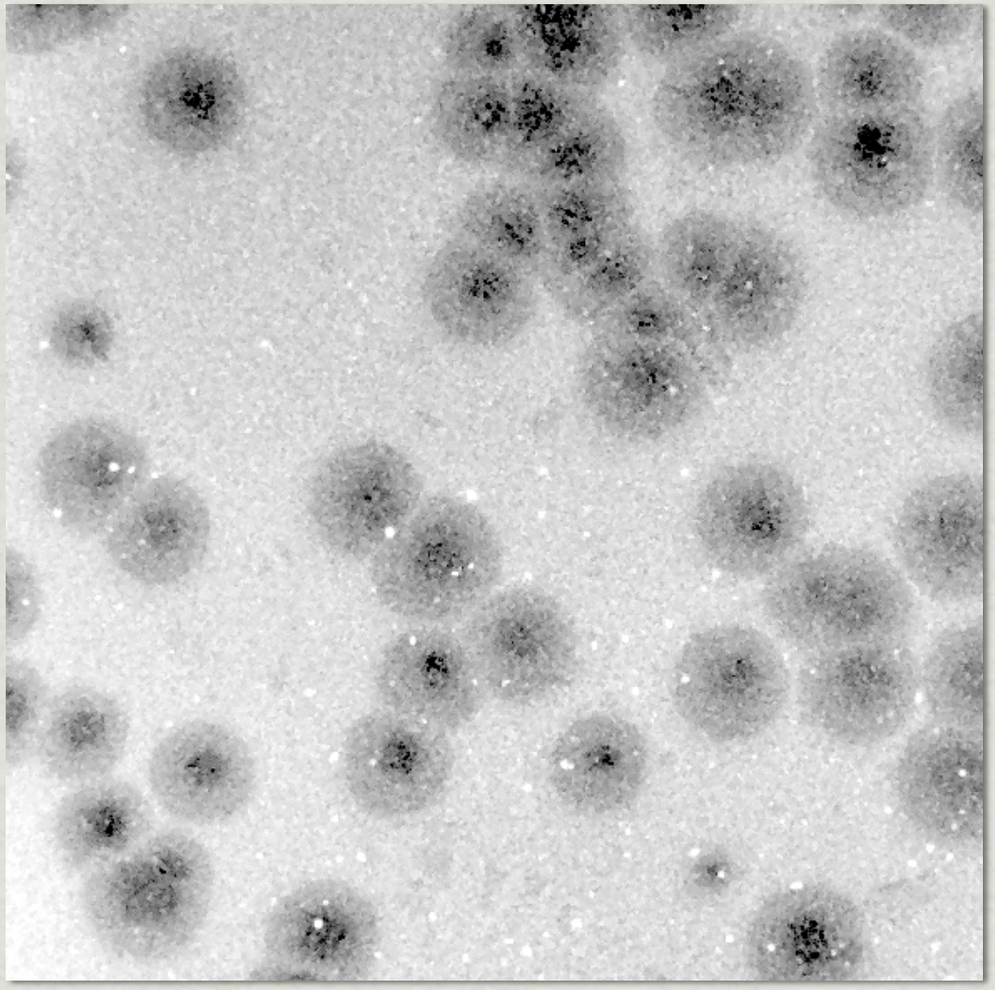

Dr
Edgewood Ct
28
Gilkey Run Rd
200 ft
100 m

Purification

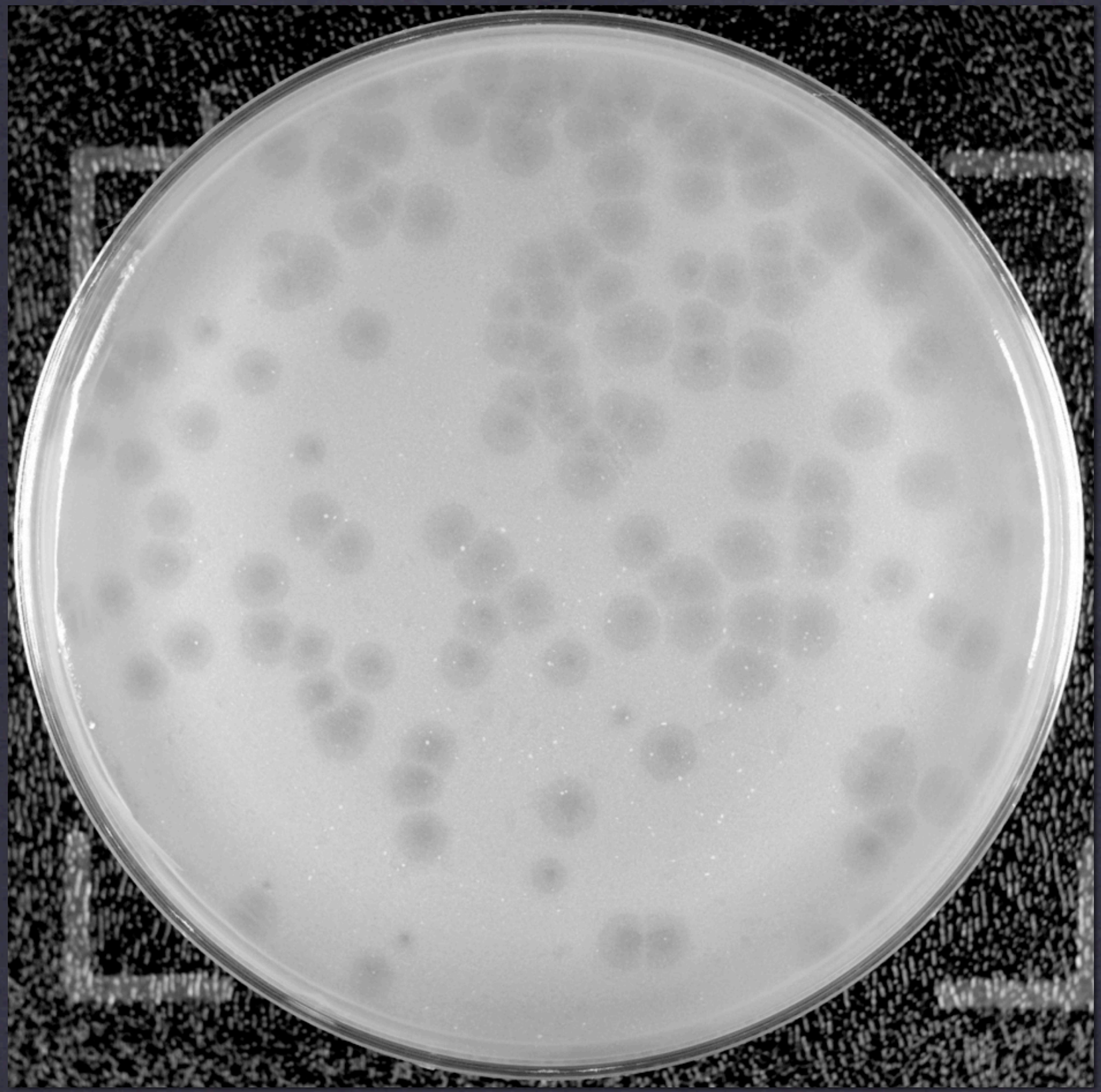
- Phage from a single plaque is plated for each round.
- Three rounds of purification were needed to isolate a single type of phage
- The phage was then concentrated in a high-titer lysate.
- Two rounds of plates were set back during the making of the lysate because the plates were placed in the fridge rather than incubator
- 10-Plate Lysate performed twice
- End titer of $1*10^9$ plaque forming units (pfu)

Plaques

- The plaques are about 2.4 cm
- Their centers are clear and surrounded by a more turbid area
- The plaques do not grow in to each other
- Likely lysogenic

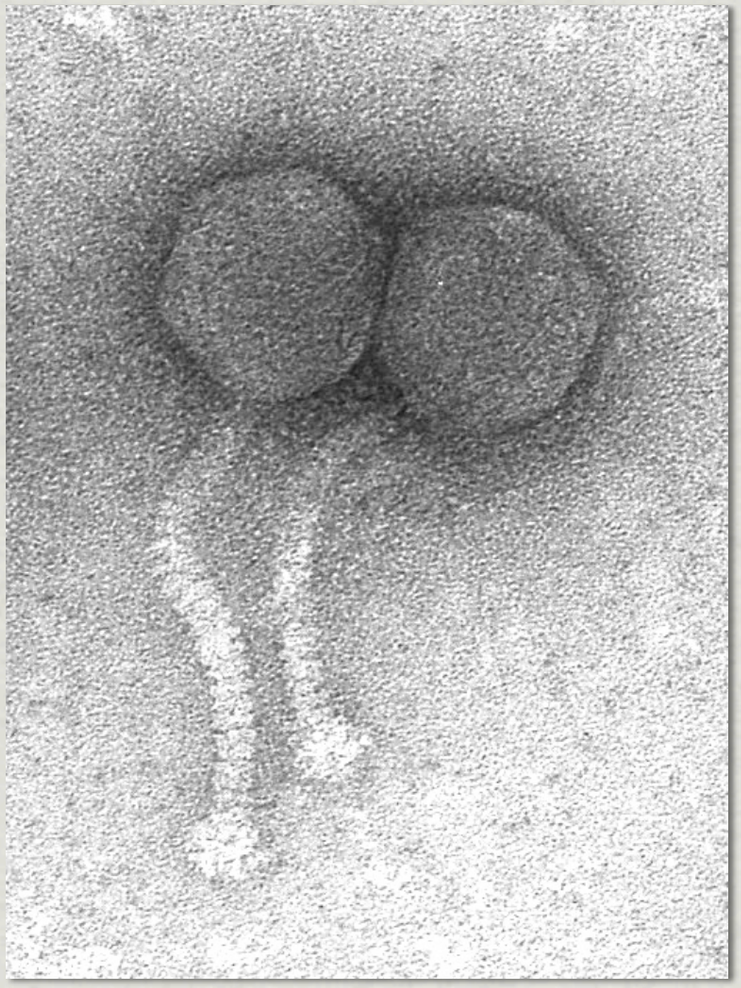


Plaque Morphology	
Plaque	**Size (cm)**
1	2.43
2	2.29
3	2.72
4	2.4
5	2.19
Average	2.406

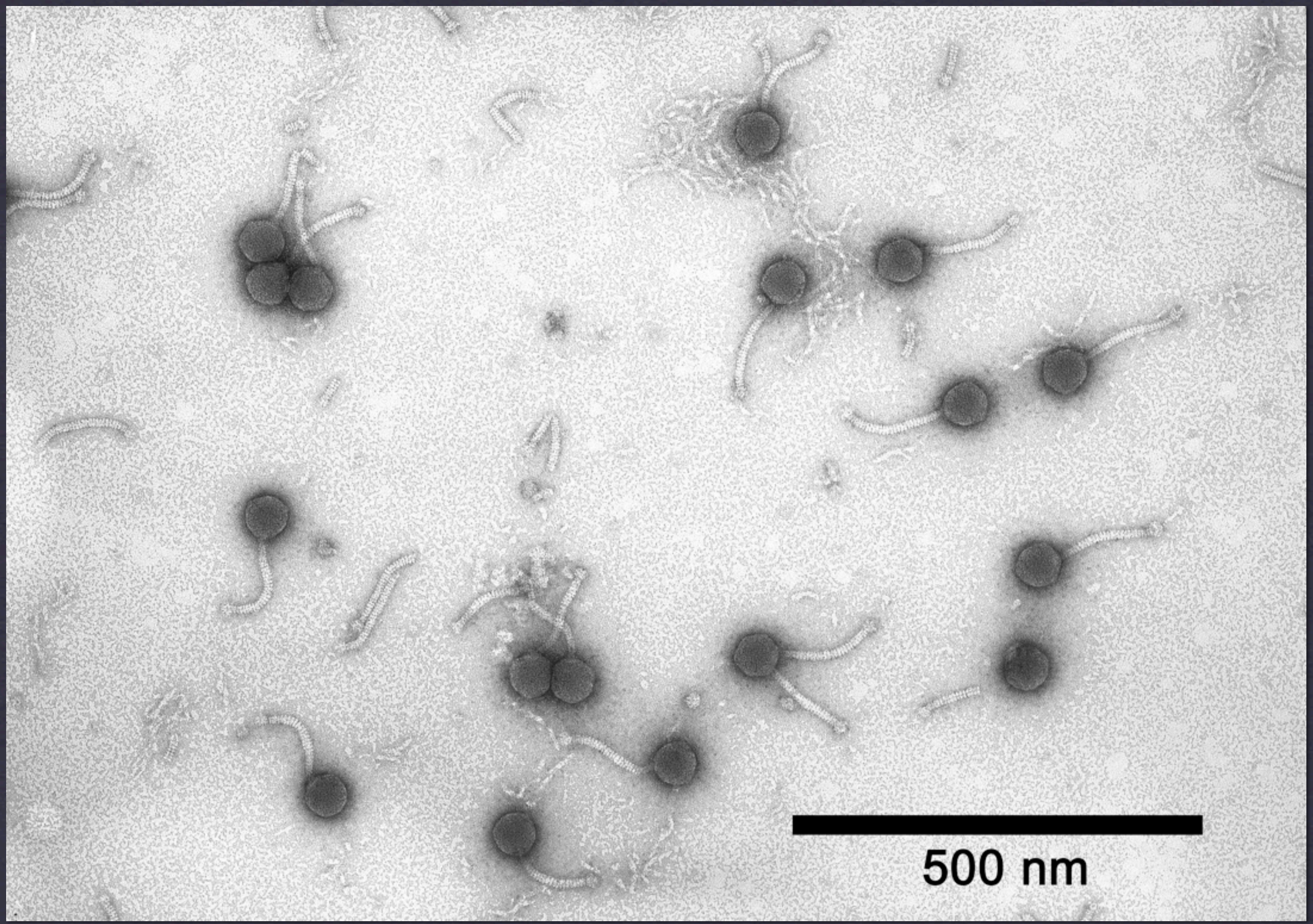


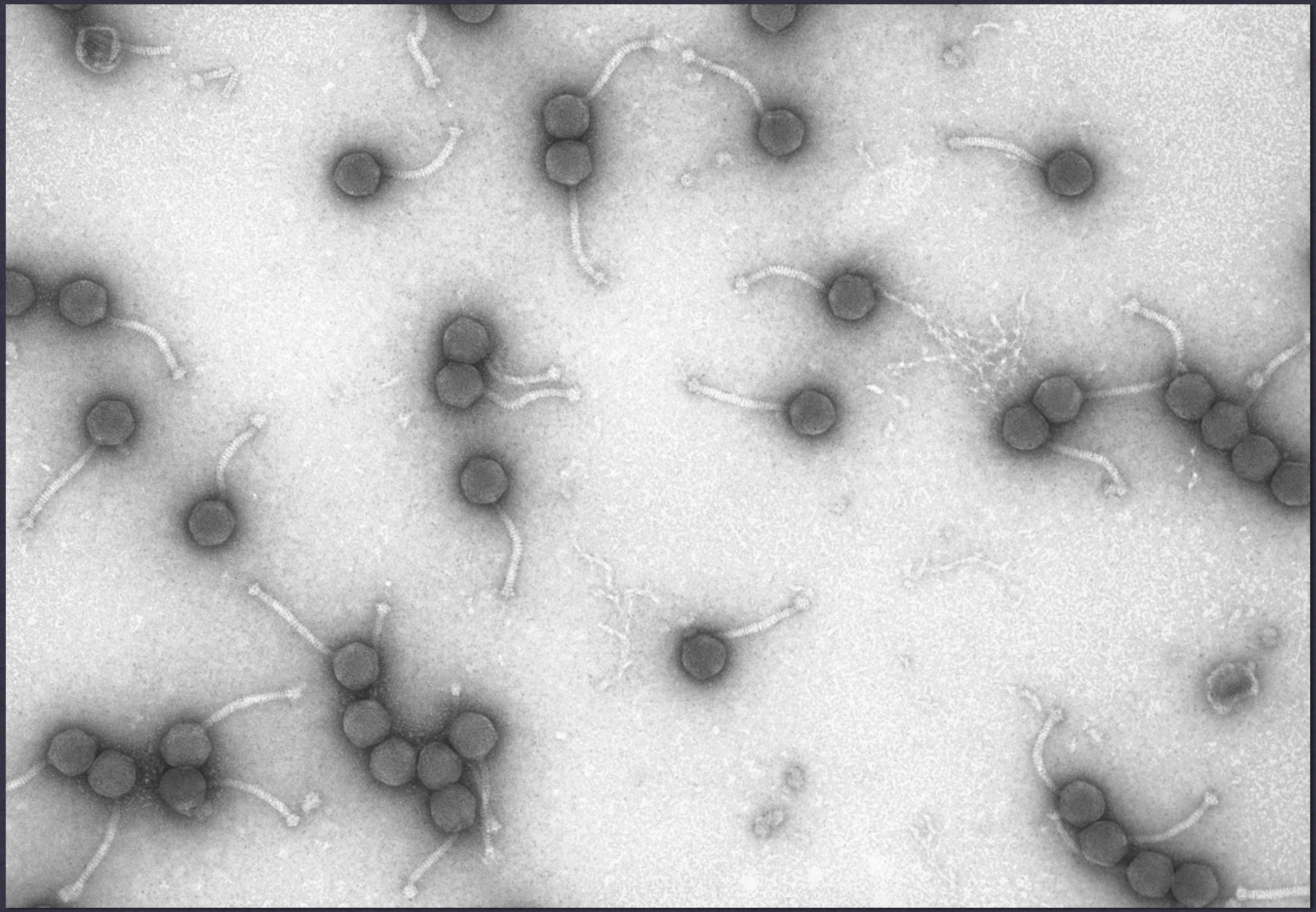
Morphology

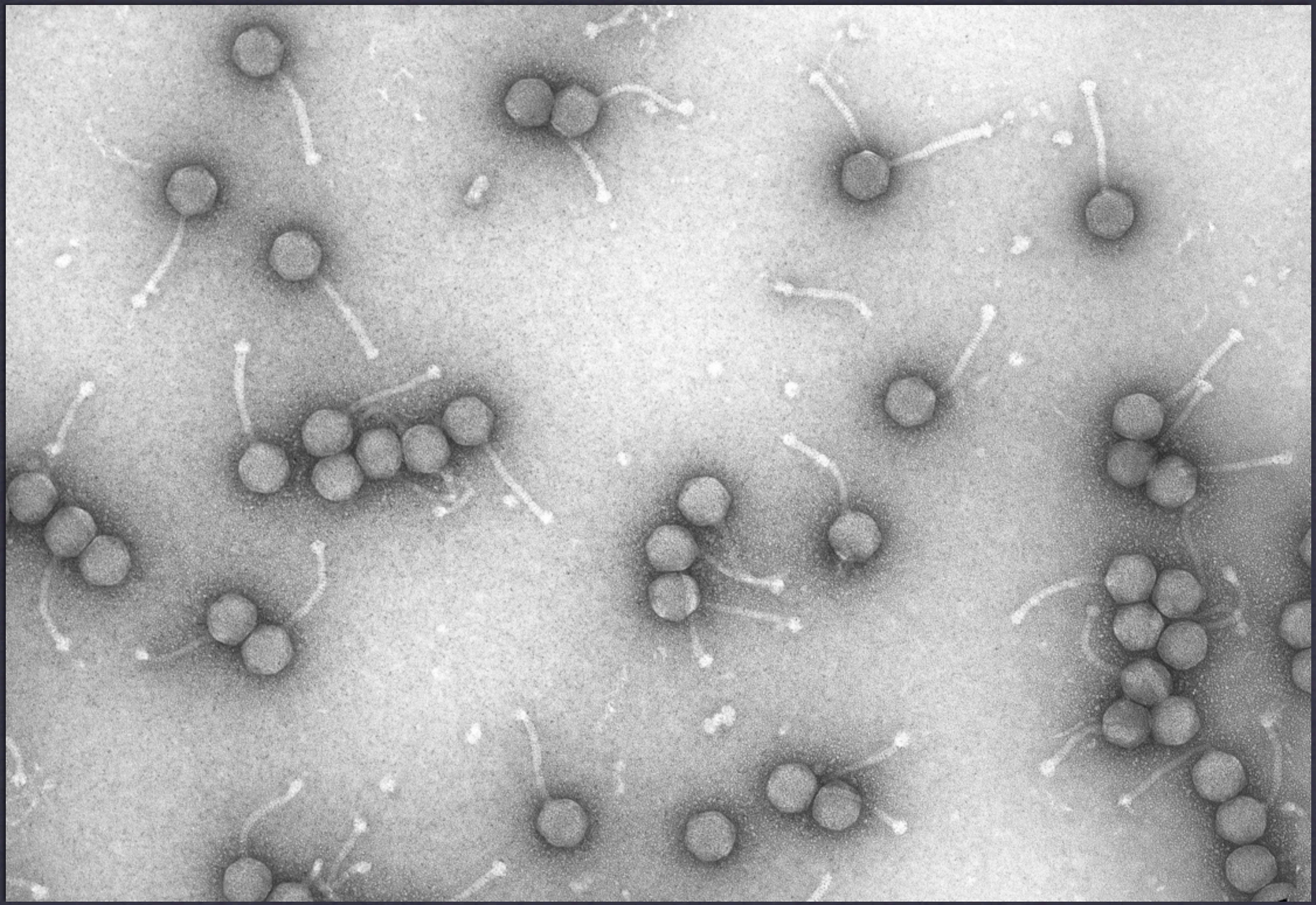
- A sample of the 10-plate lysate was concentrated further so that the phage could be photographed under an EM microscope
- They phages tend to clump together at the heads
- Tails show no attraction to each other
- The heads are about 56.45 nm
- The tail lengths are about 144.65 nm
- The tail widths are about 4 nm



Pseudonym Morphology			
Phage	**Head (nm)**	**Tail Length(nm**	**Tail Width (nm)**
1	52.92	152.88	4.12
2	52.92	141.12	4.12
3	58.80	135.24	4.12
4	58.80	152.88	4.12
5	58.80	141.12	3.53
Average	56.45	144.65	4.00


500 nm





DNA Isolation

- Cell debris, proteins, and other materials were removed from a sample of the lysate.
- The sample was then placed under a spectrophotometer
- Concentration of 136.4 ng/µl
- 260/280 ratio of 1.88
- 260/280 indicates that there is no excess proteins

Gel Electrophoresis

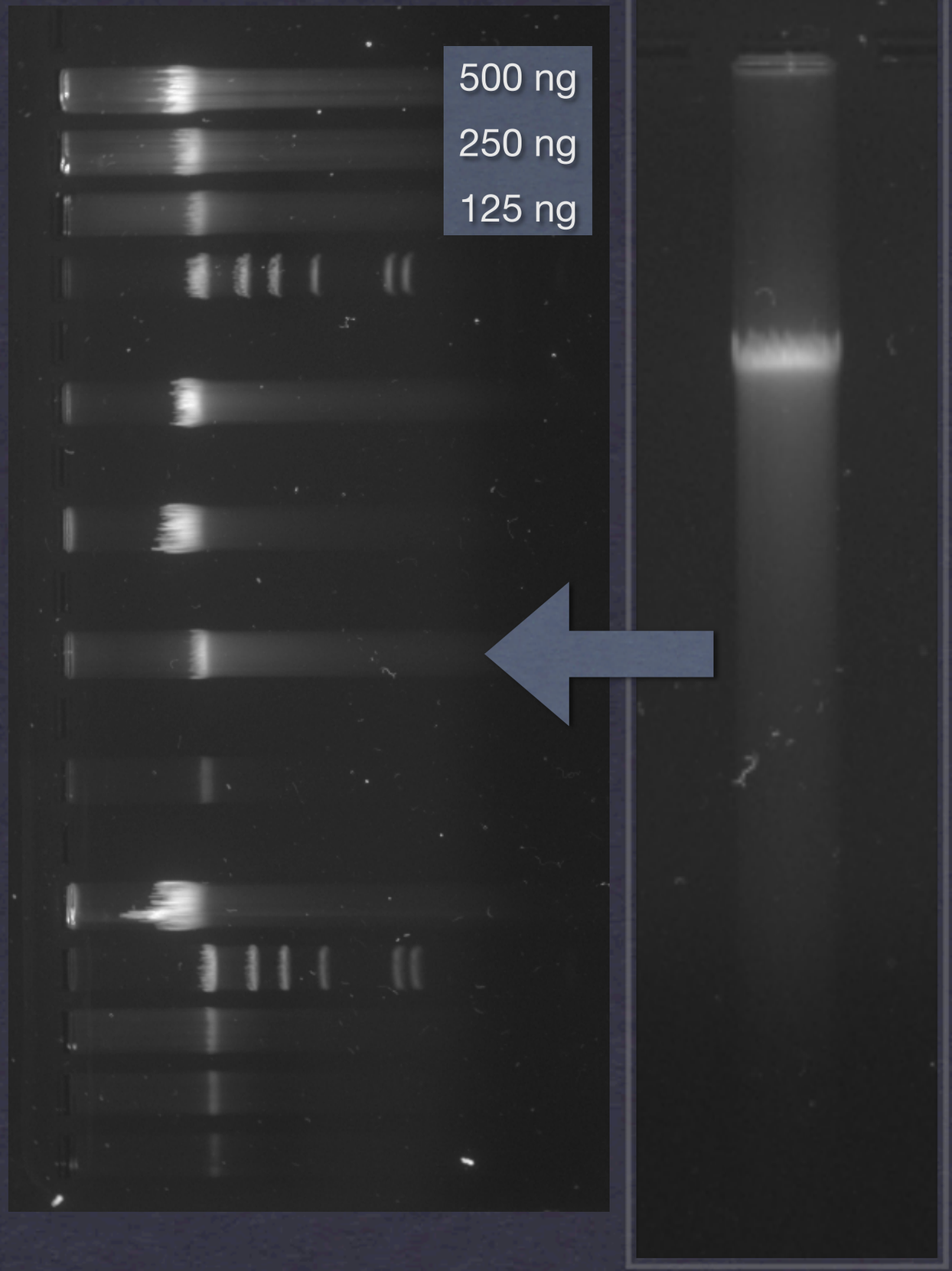
- Gel electrophoresis is used to determine the size of DNA fragments
- A solution of porous agarose is poured into the chamber and allowed to harden. A comb is placed in it to allow for wells
- DNA and dye is placed in the well
- When the electricity is turned on, the DNA and dye migrates towards the positive end of the devise
- The smaller the fragment, the farther it migrates
- DNA can be cut with enzyme prior to adding it to the wells

Quality Control Gel

Performed on 11/10/2011

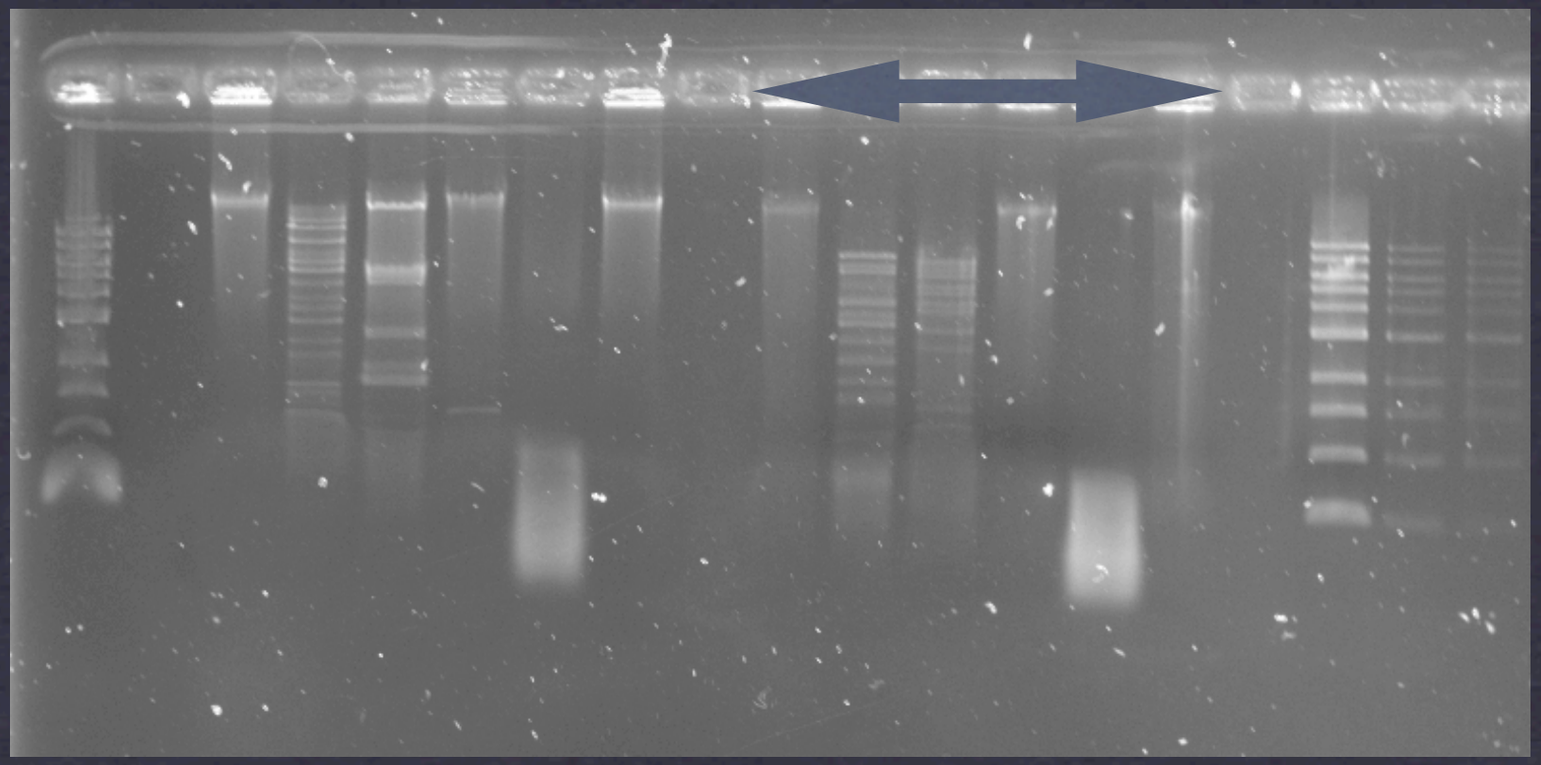
Good quality, but not as concentrated as other DNA samples in the class

Concentration according to spectrometer: 136.4 ng

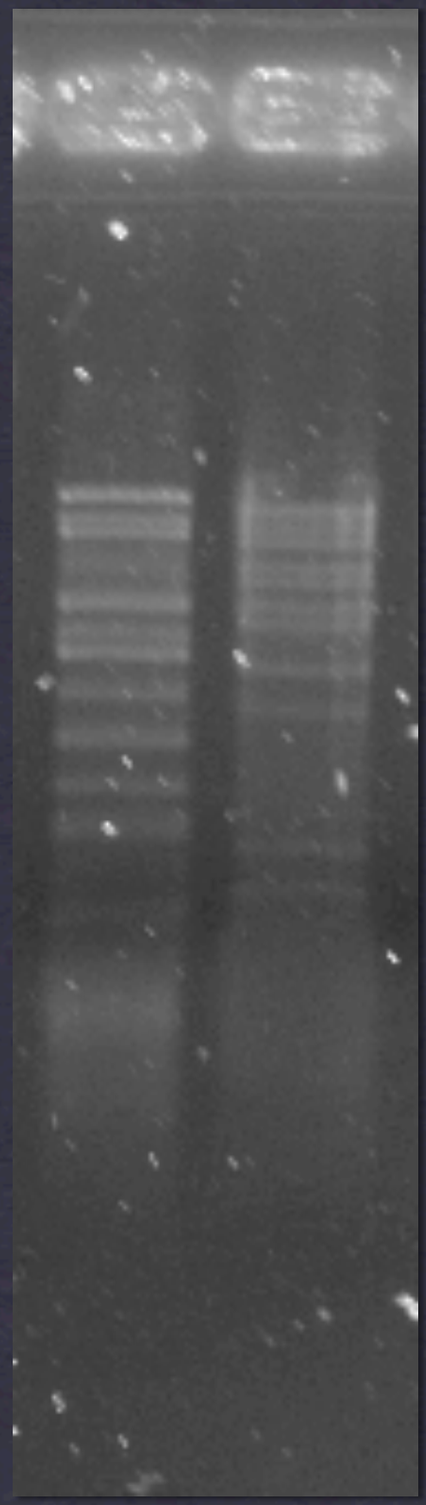


Restriction Digest

Performed on 11/10/2011



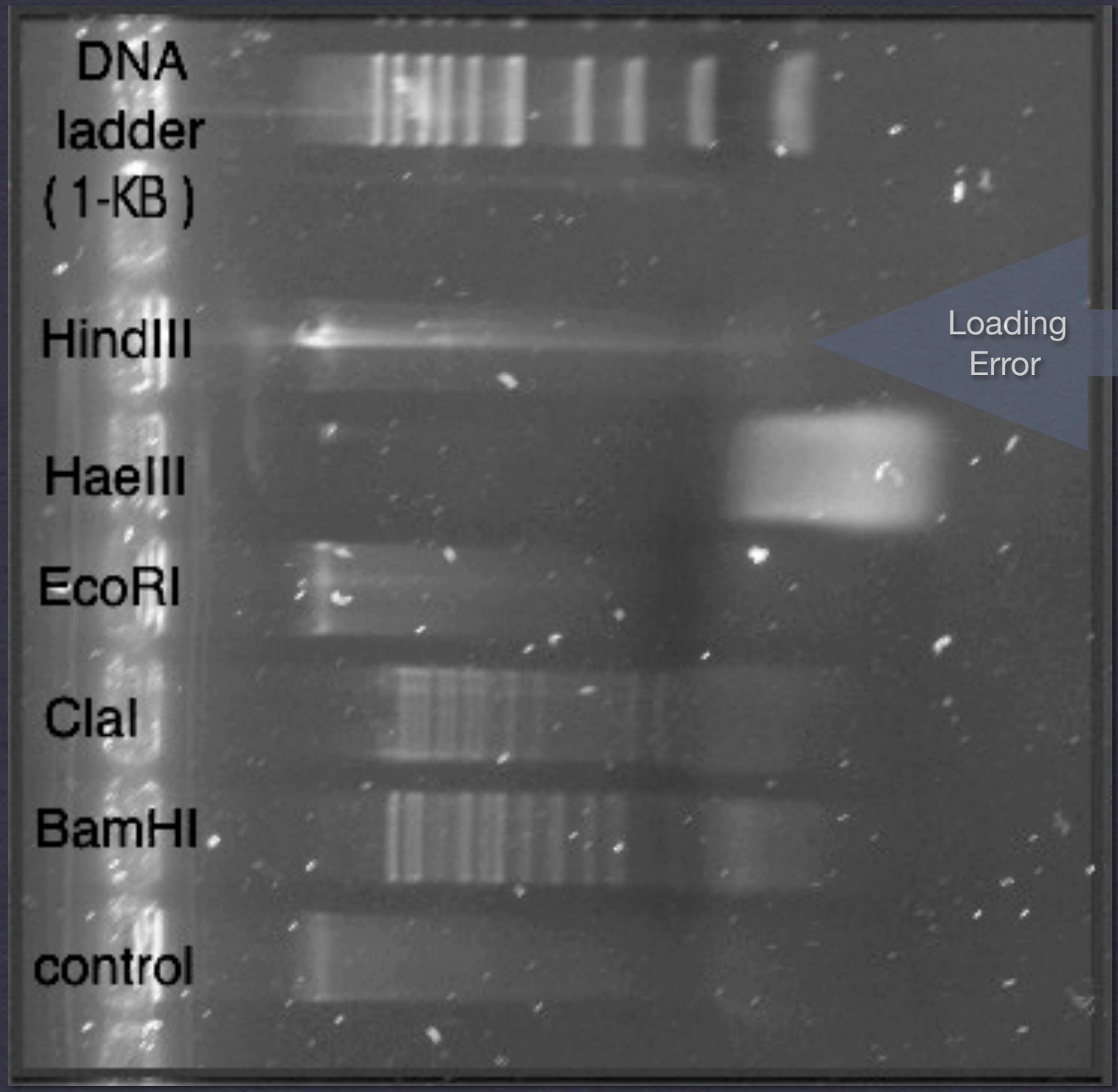
BamHI & ClaI



The enzymes ClaI and BamHI cut the DNA multiple times.

As expected of this kind of phage, HaeIII cuts the DNA into many small fragments

The enzymes HindII and EcoRI do not cut the DNA.



Pseudonym

- Isolated from enriched soil found in Bardstown, KY on a cloudy, rainy day
- DNA cut by the enzymes BamHI, ClaI, and HaeIII
- The phage tend to clump together at the heads but not the tails