Isolation and Genomic Sequence Characterization of *Mycobacterium* sp. Peaches from a Soil Sample in Northeast Louisiana

John Robert Warner
ULM HHMI SEA
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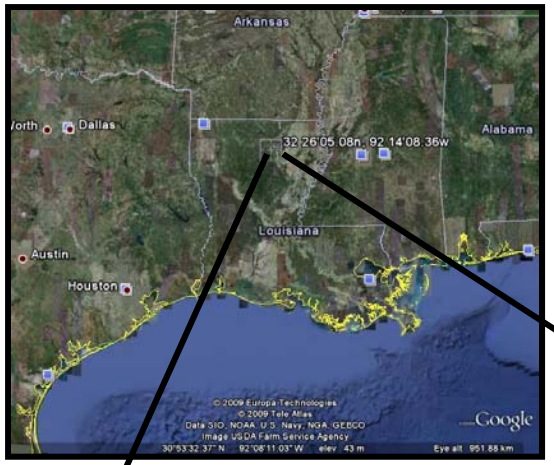
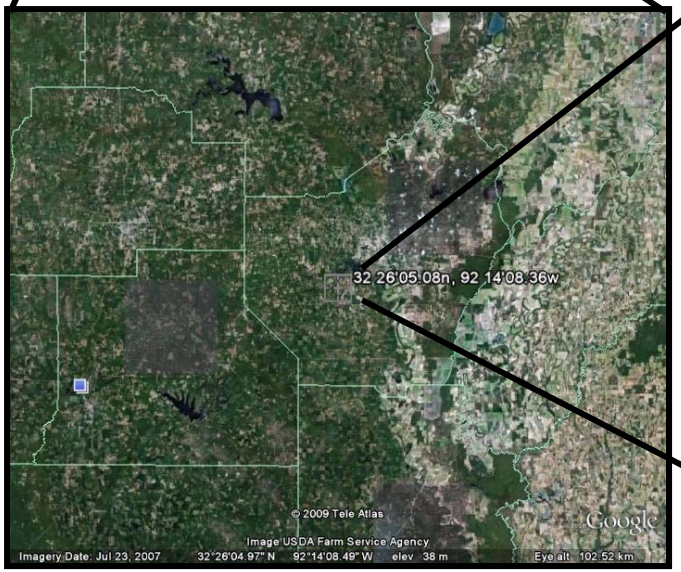
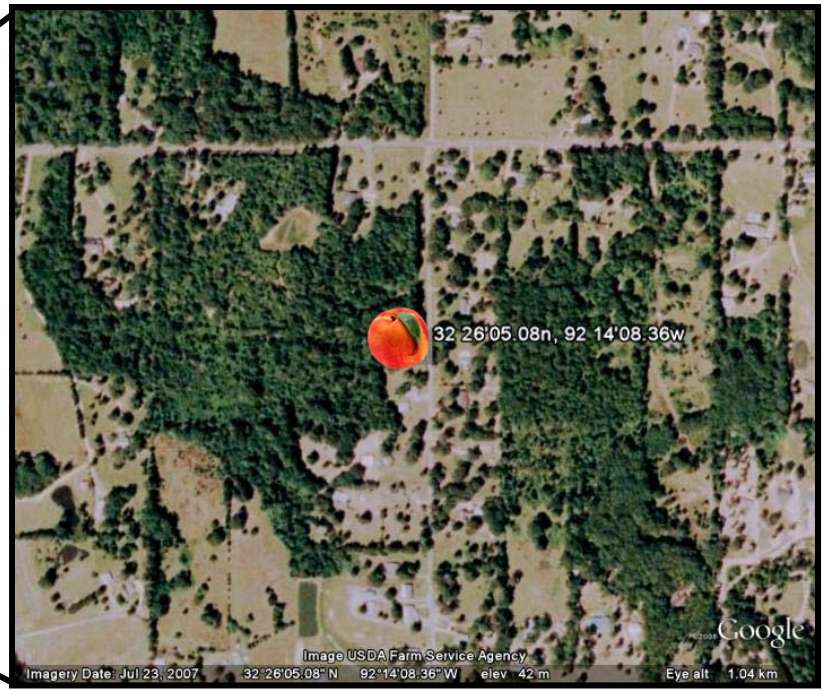




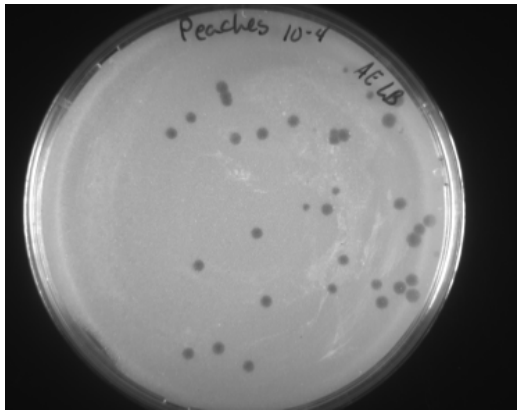


Part 1: Isolation and purification of mycobacteriophages in Northeast Louisiana

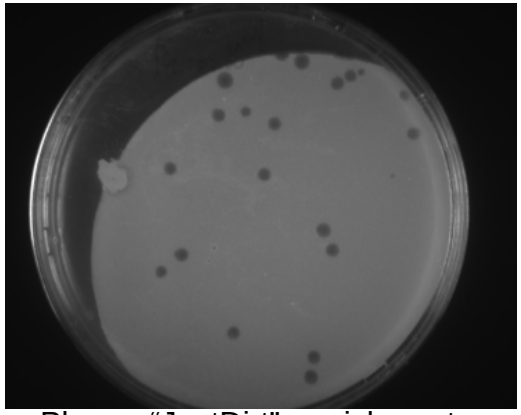
Phage Isolated

- Number of phage processed: 15
- From non-enriched samples: 3
- From enriched: 12
- Lytic: 12
  - “Large” plaque: 7
  - “Small” plaque: 5
- Lysogenic: 3

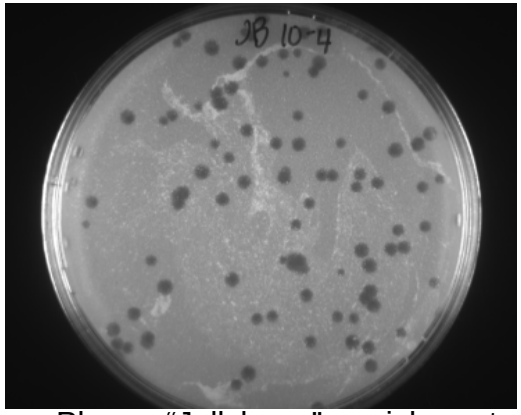
“Large Plaque” Lytic Phage



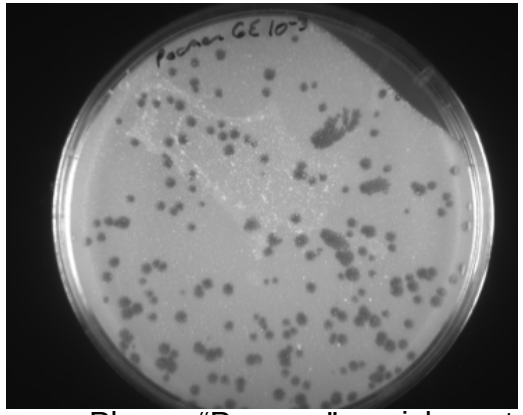
Phage: “Peaches”; enrichment
Group: Cabbit



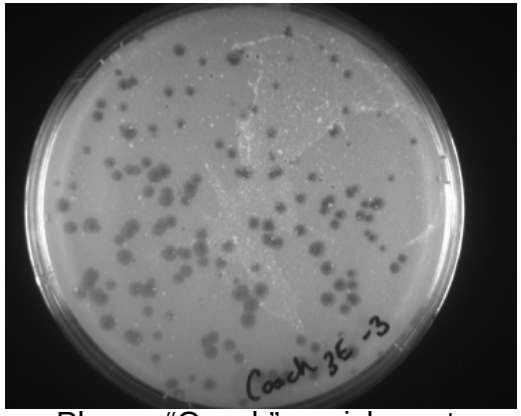
Phage: “JustDirt”; enrichment
Group: Just Dirt



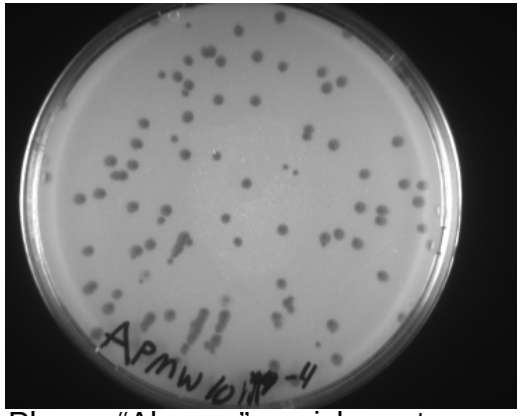
Phage: “Jellybean”; enrichment
Group: Jellybean



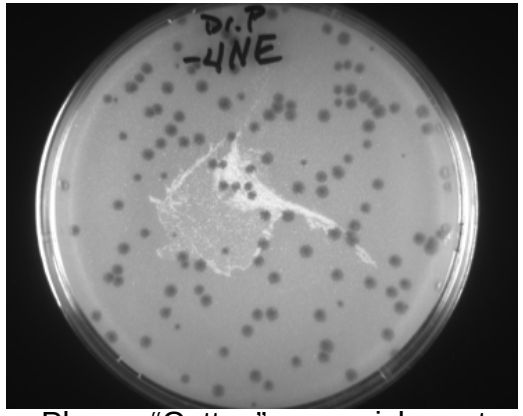
Phage: “Pacman”; enrichment
Group: Pacman



Phage: “Coach”; enrichment
Group: Coach

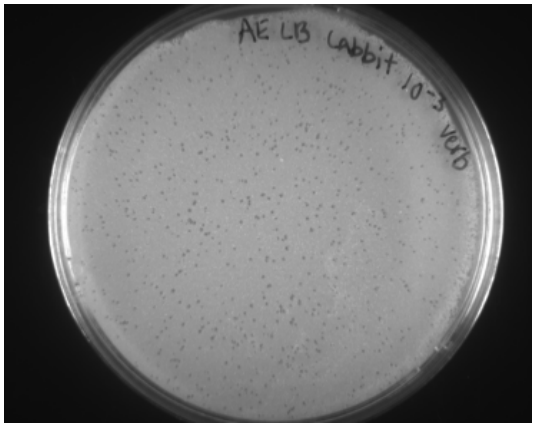


Phage: “Akroma”; enrichment
Group: Angel Phage Monster Wing

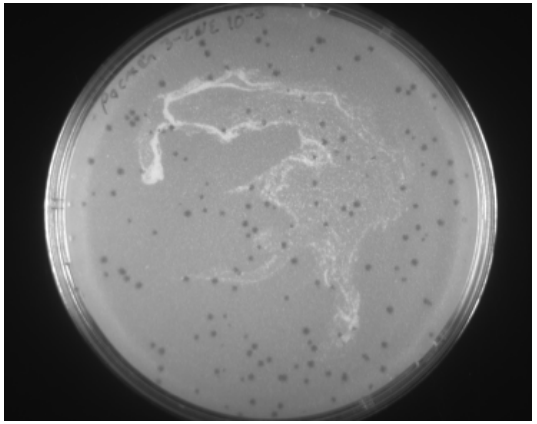


Phage: “Cotton”; no enrichment
Group: Dr. Phage

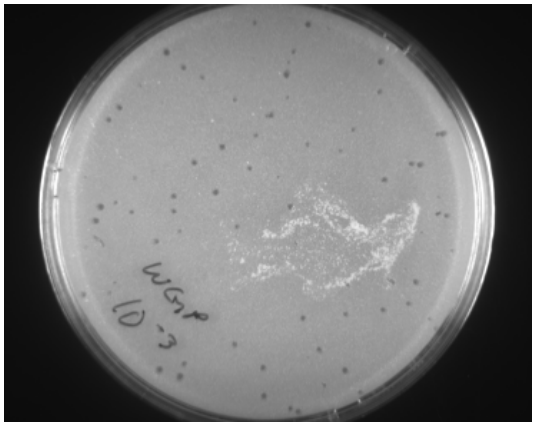
“Small Plaque” Lytic Phage



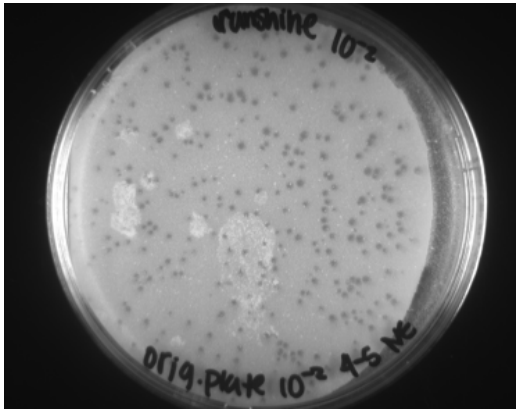
Phage: “Verb”; enrichment
Group: Cabbit



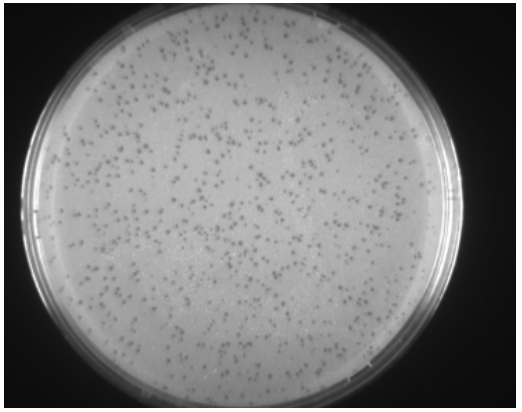
Phage: “Watchmen”; no enrichment
Group: Pacman



Phage: “WGP”; enrichment
Group: W.G.P.

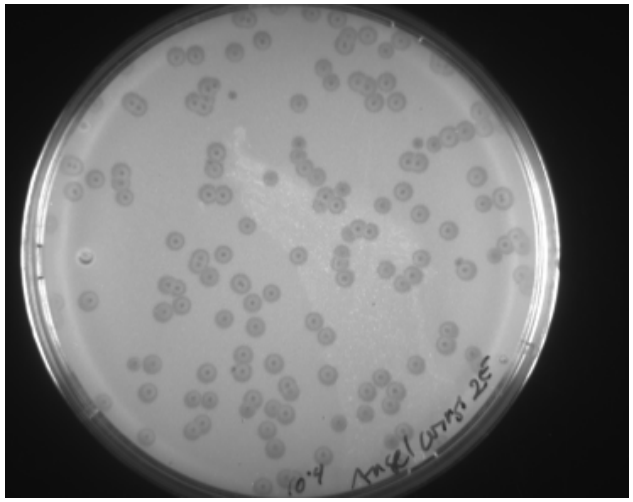


Phage: “Sunshine”; no enrichment
Group: Sunshine

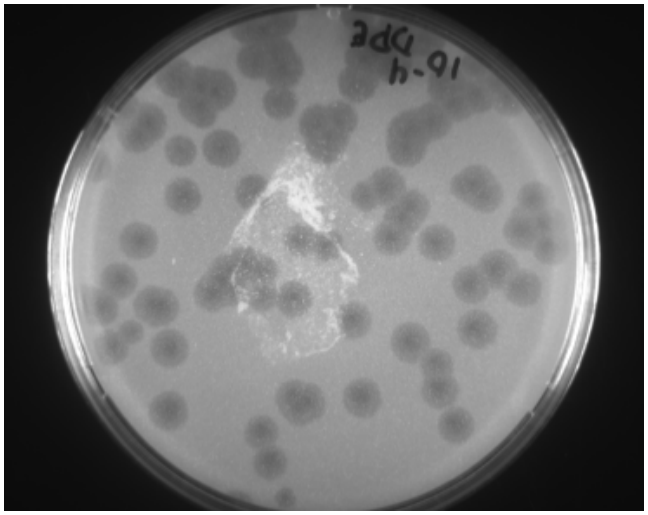


Phage: “Honesty”; enrichment
Group: Nerds!

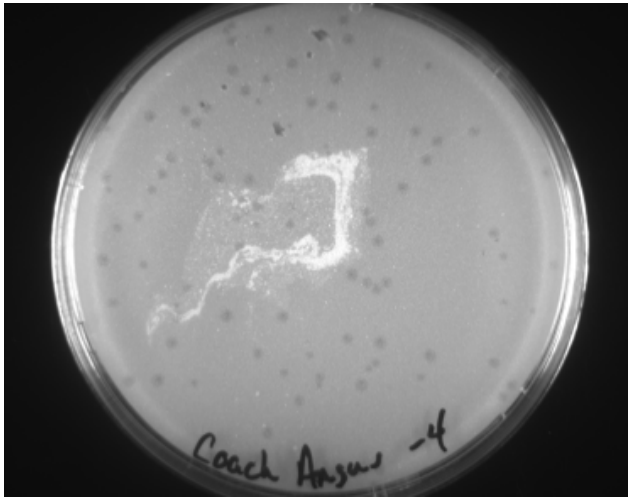
Lysogenic Phage



Phage: “Angelwings”; enrichment
Group: Angel Wings

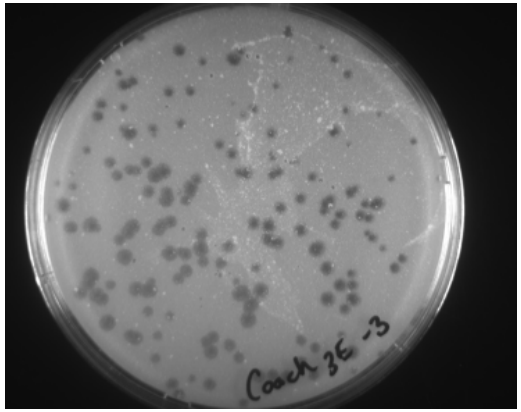


Phage: “Pumato”; enrichment
Group: Dr. Phage

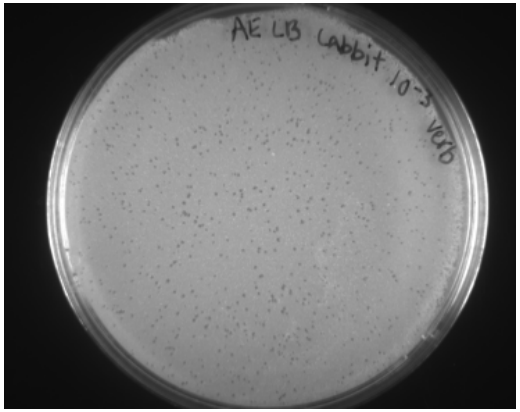


Phage: “Angus”; enrichment
Group: Coach

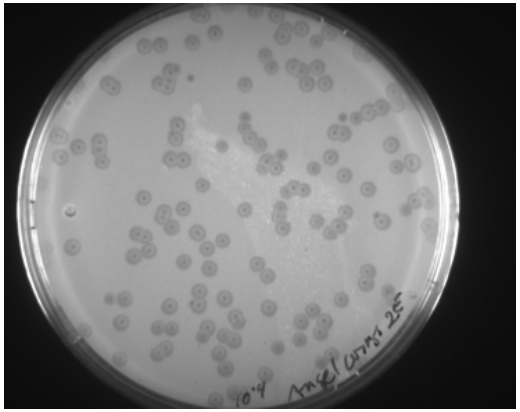
“Same Enrichment, Different Plaque”→ Different Phage?



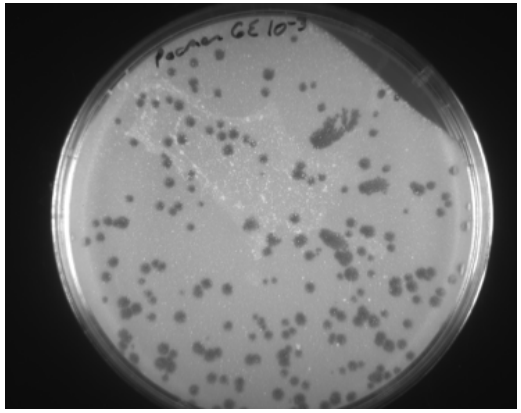
“Coach”



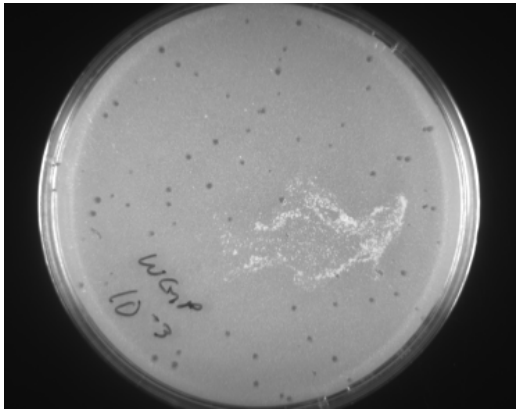
“Verb”



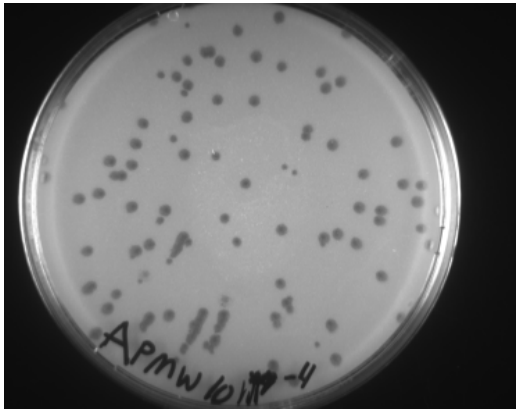
“Angelwings”



“Pacman”

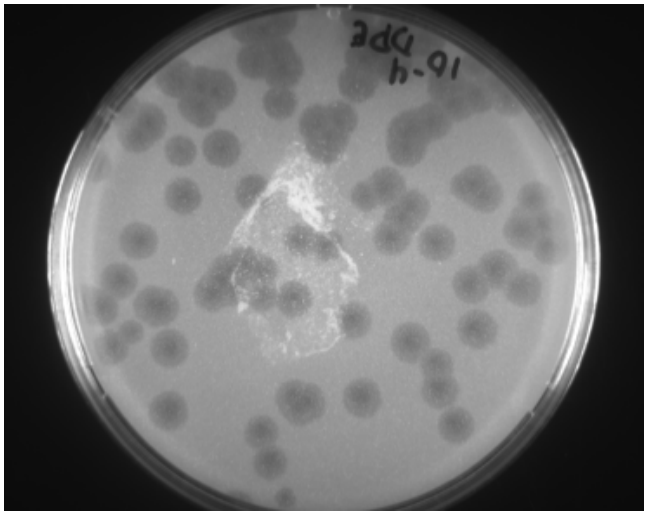


“WGP”

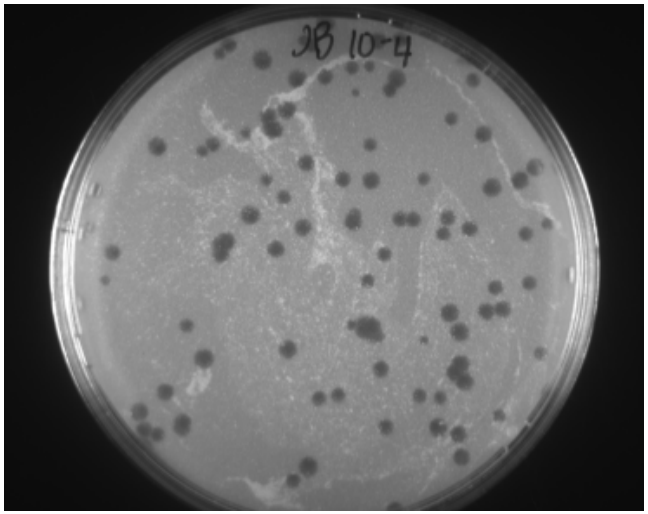


“Akroma”

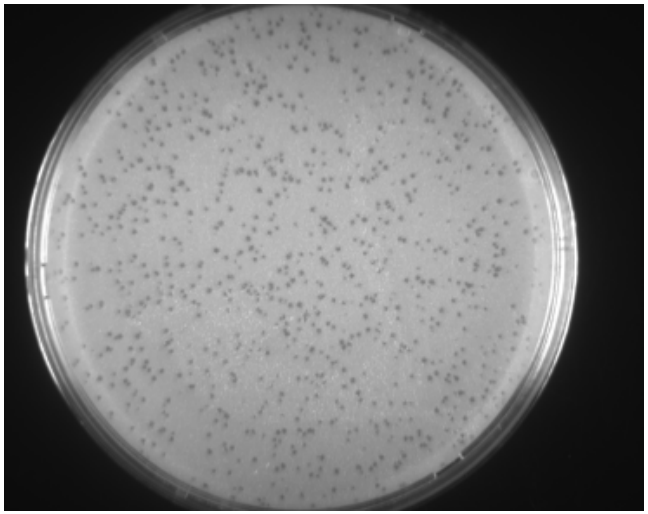
“Same Enrichment, Different Plaque”→ Different Phage?



“Pumato”

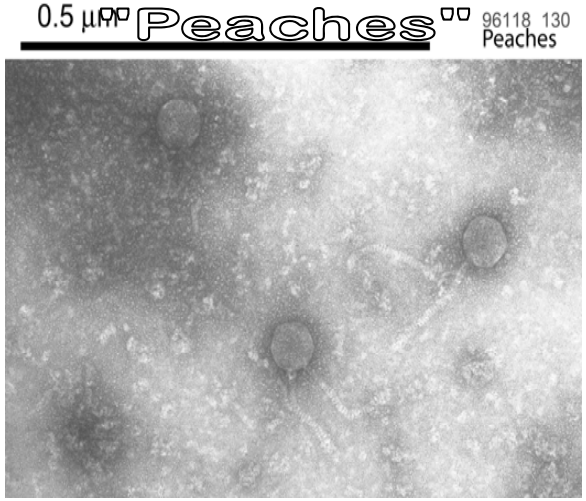


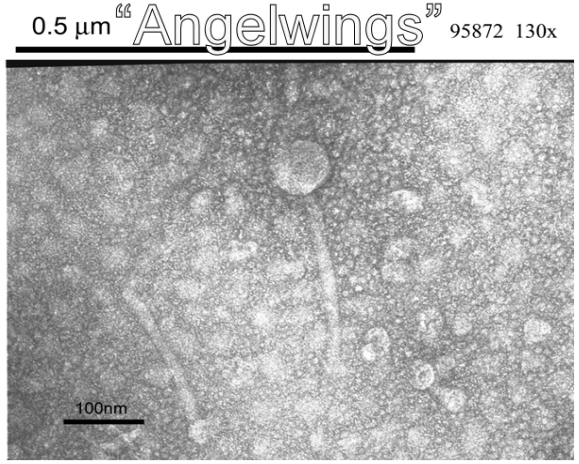
“Jellybean”

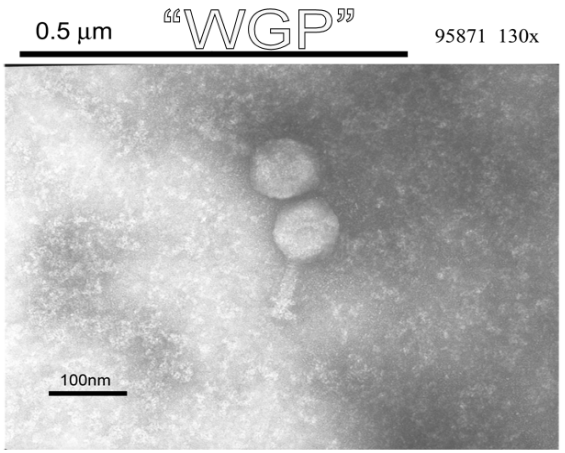


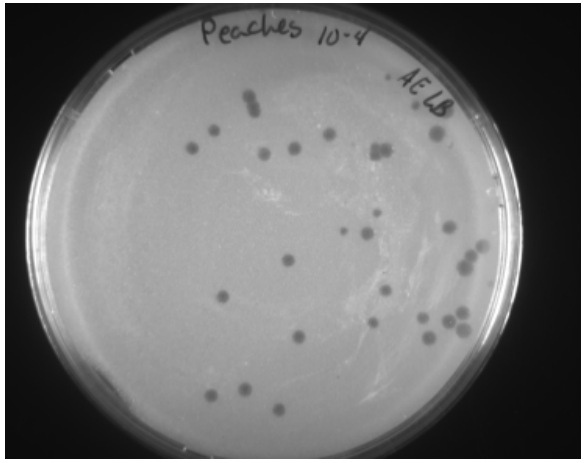
“Honesty”

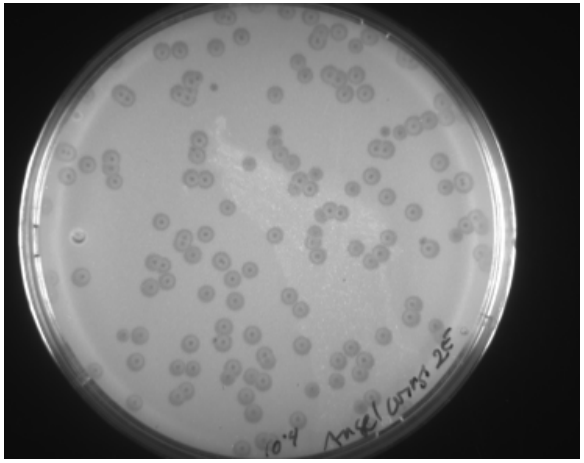
TEM structure of selected phage

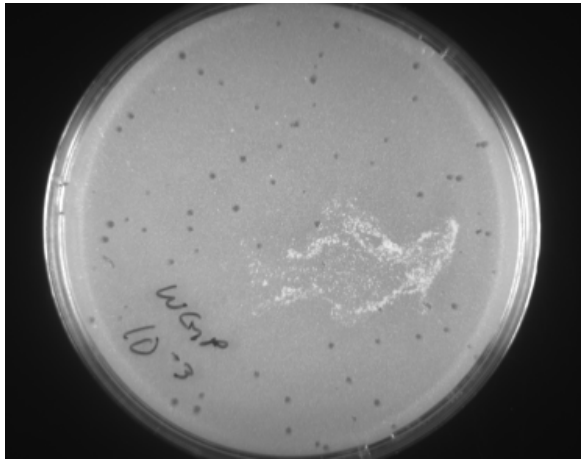












Part 2: DNA characterization of isolated phage

DNA isolation and analysis

DNA Isolation

nuclease mix treatment, phage precipitation, clean-up resin

↓

Restriction Digestion (*Bam*HI, *Cla*I, *Eco*RI, *Hae*III & *Hind*III; examine restriction profiles for unique patterns)

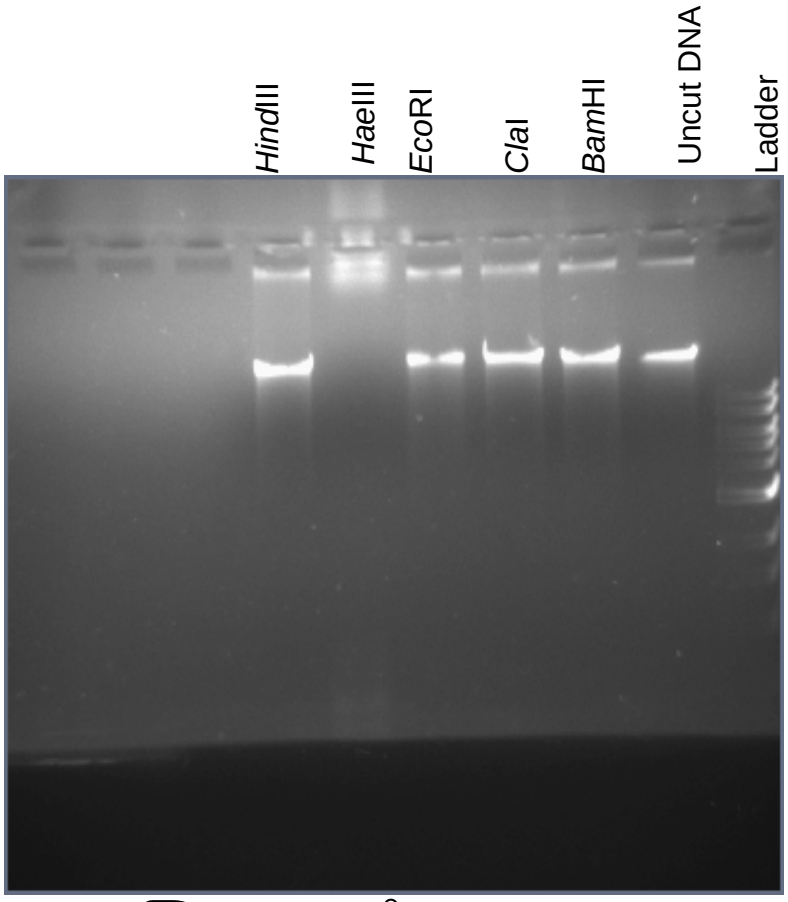
↓

Quality Control Gel on Selected Isolates

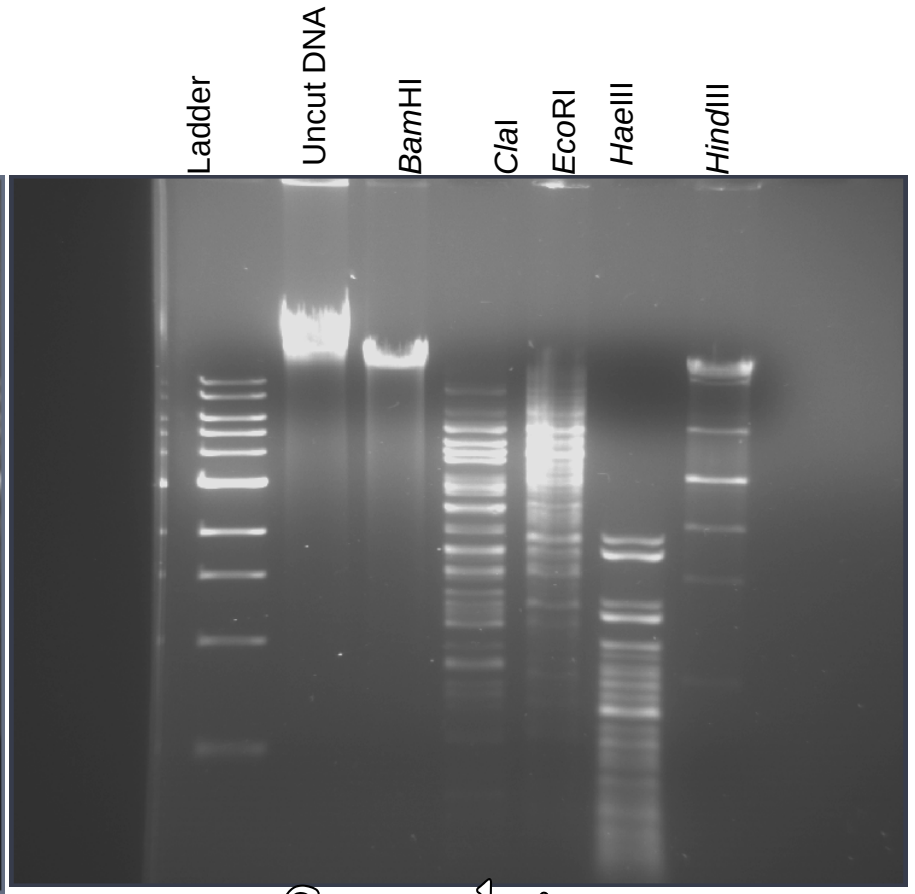
↓

Phage DNA to LANL-JGI (sequencing)

Restriction Digestion



Peaches

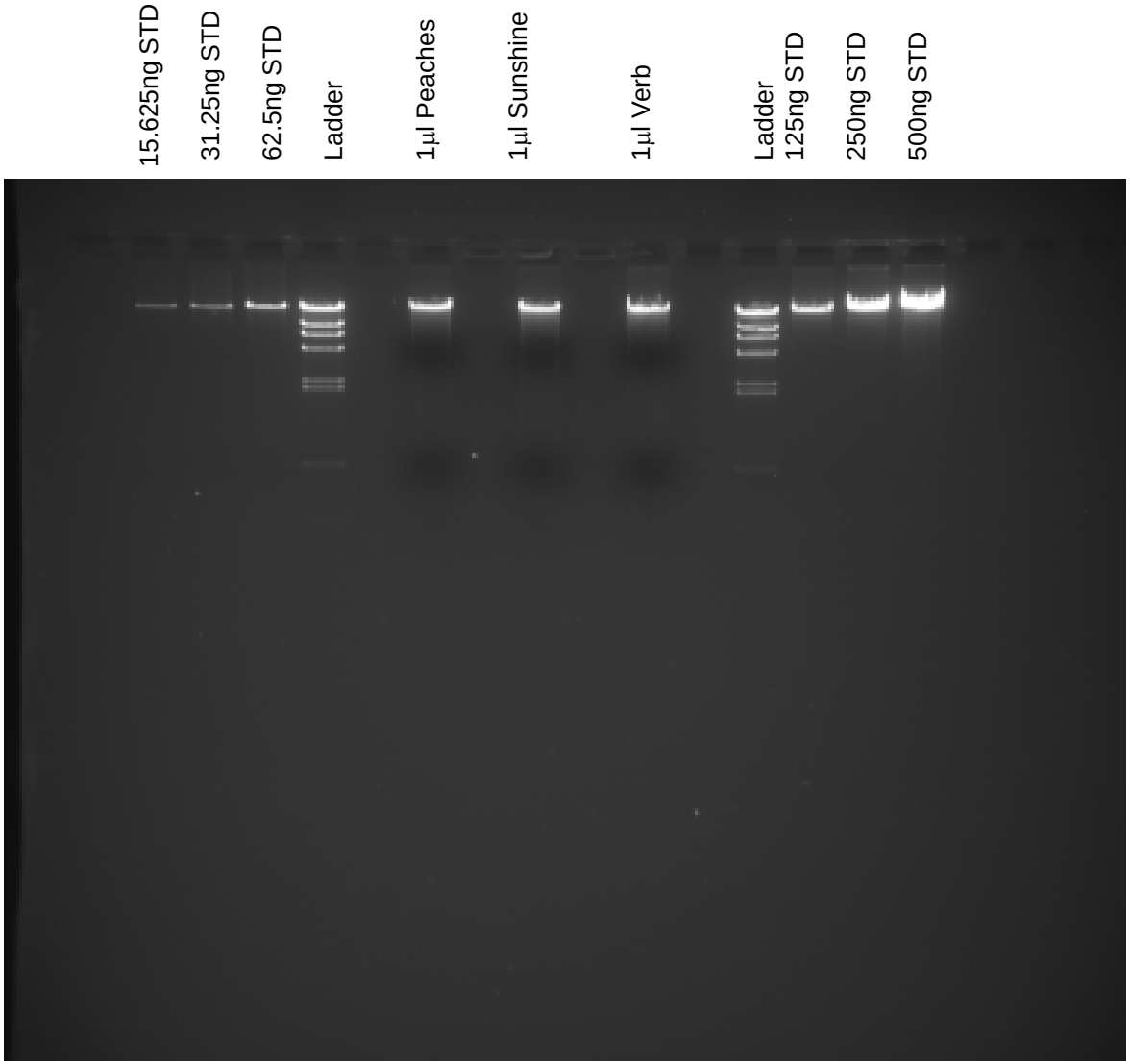


Sunshine

Restriction Analysis: Summary

- # of phage cut with BamHI: 9
- # of phage cut with ClaI: 11
- # of phage cut with HaeIII: 15
- # of phage cut with EcoRI: 4
- # of phage cut with HindIII: 3
- # of phage cut with all enzymes: 2 (Just Dirt and Pumato)
- # of phage cut with no enzymes: 0

QC gel

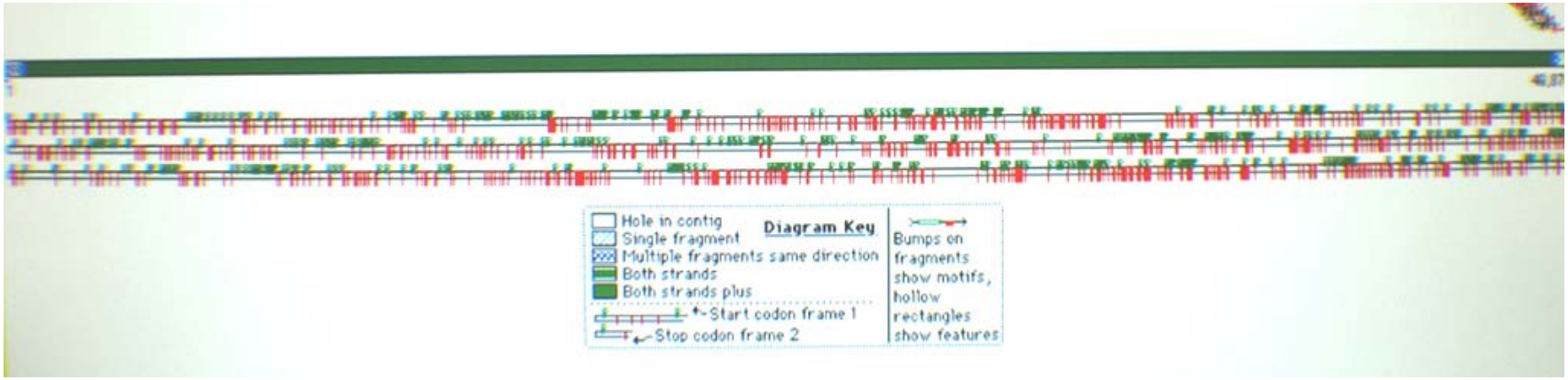

15.625ng STD
31.25ng STD
62.5ng STD
Ladder
1µl Peaches
1µl Sunshine
1µl Verb
Ladder
125ng STD
250ng STD
500ng STD

Part III: Genomic Characterization of Peaches

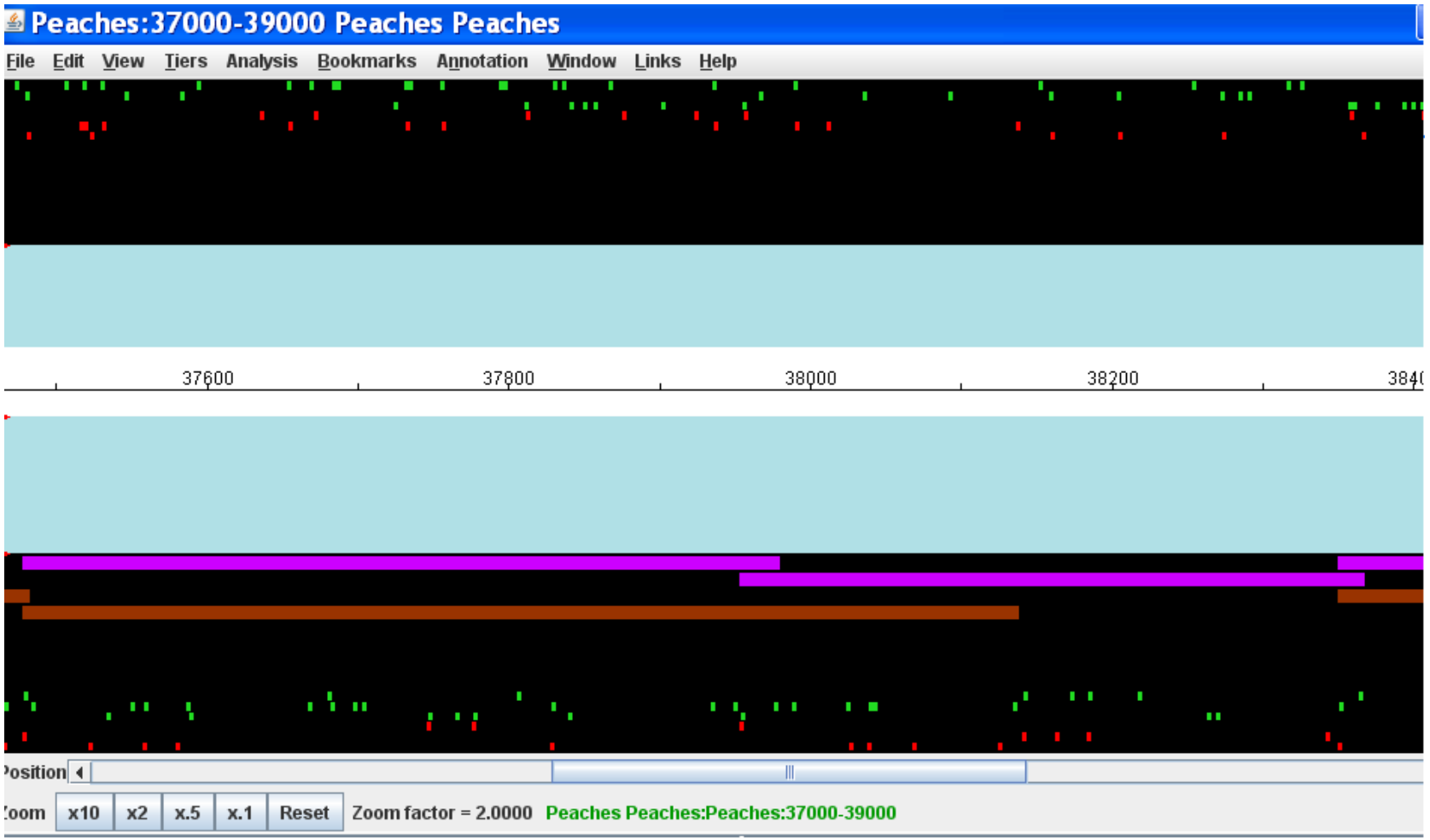
Genome information

- Size: 51, 376 bp
  - Including a 10 base pair 3’ overhang
- GC content: 63.9%
- Orphams: 10
- Sequencing summary: Draft sequence consisted 1 contig with no gaps

Draft Assembly



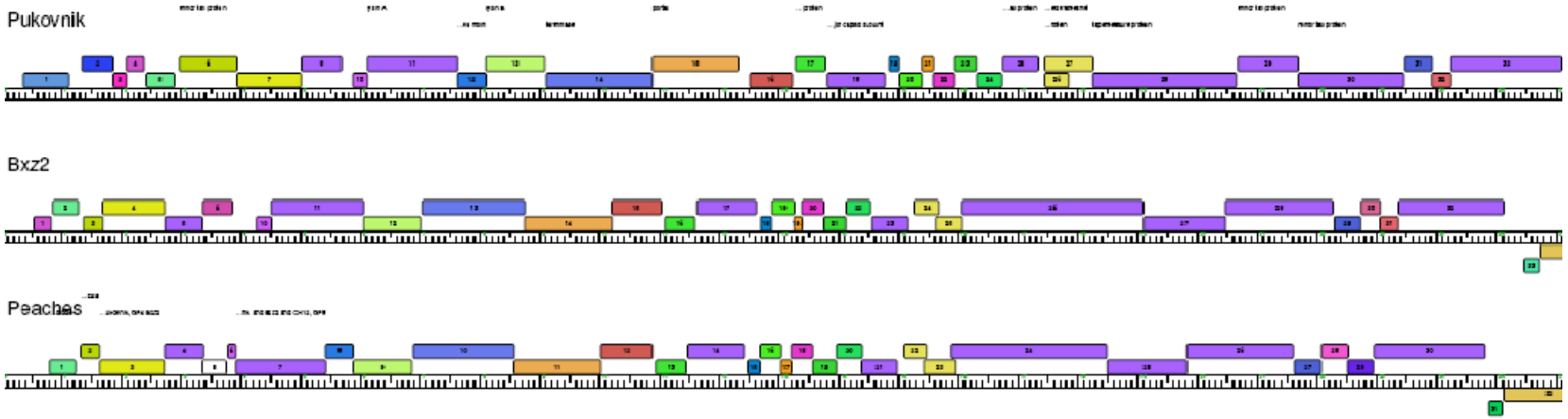
Gene Calling



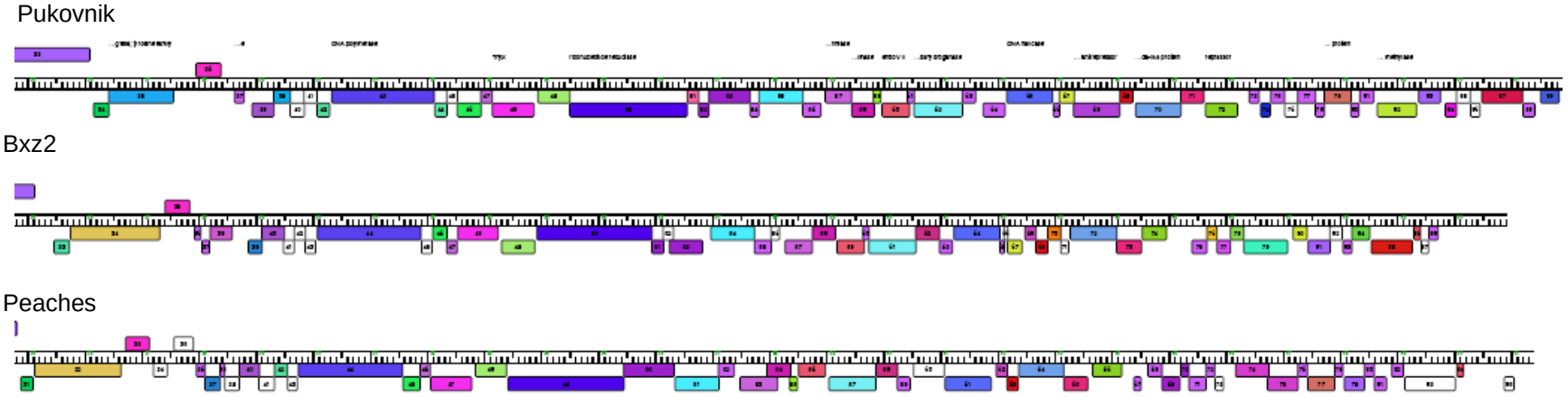
Peaches: phylogenetics

- Peaches belongs to the A2 cluster
- Most similar to Bxz2

Left Arm



Right Arm

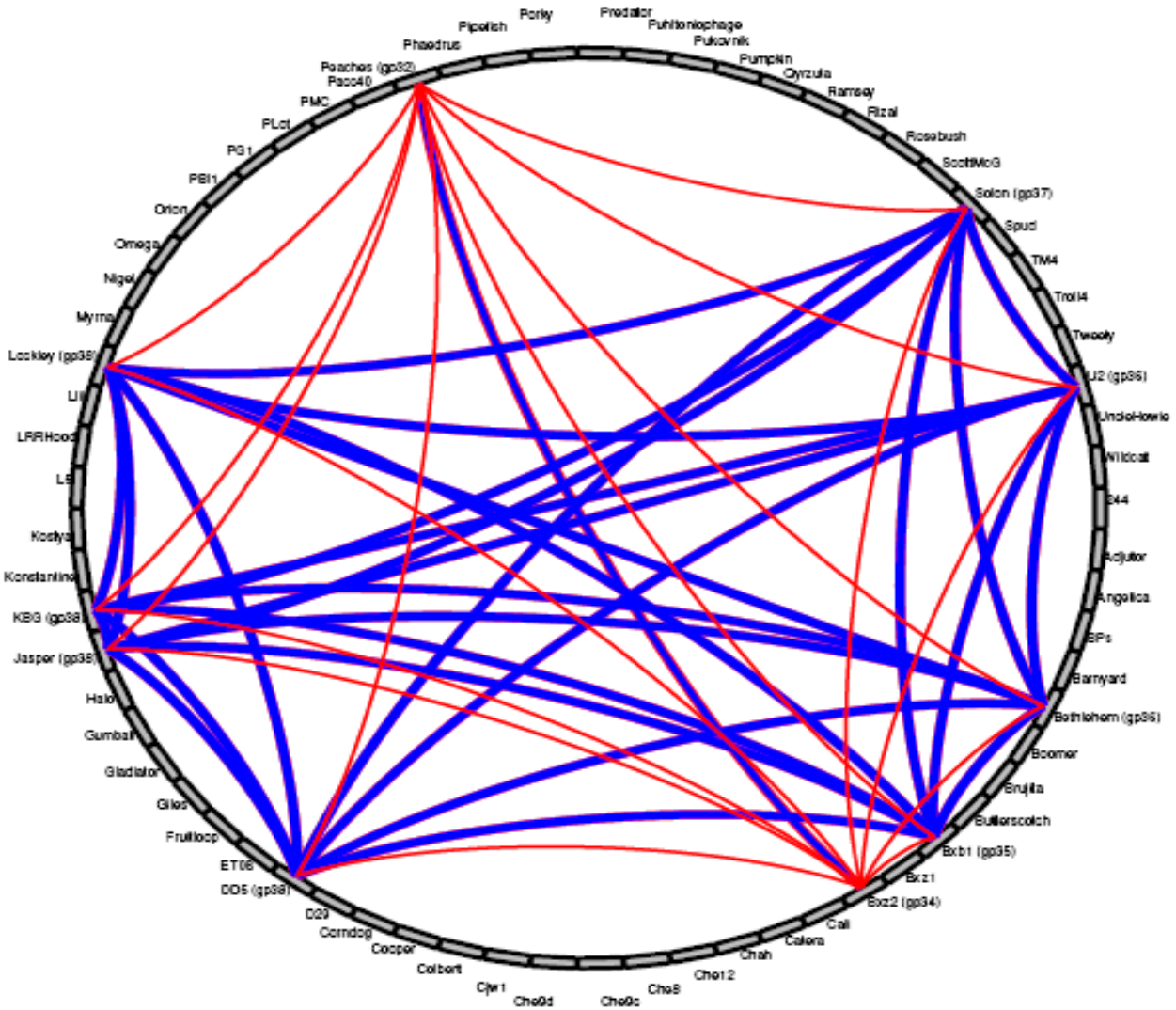


Gene annotation

Gene Prediction	Function/Similar Bacteriophage
GP3	Tail Protein/Gp7 – Pukovnik, Gp4 – Bxz2
GP7	Putative Lysozyme/Gp11 – Pukovnik, Gp8 – Bxb1
GP8	LysB/Gp12 – Pukovnik & Che12, Gp11 - D29
GP9	Peptidoglycan binding domain protein/Gp13 – Pukovnik, Gp12 – Bxz2, Gp10 - Solon
GP10	Terminase/Gp13 – Bxz2, Gp10 – Bxb1, Gp14 – Pukovnik
GP11	Portal/Gp14 – Bxz2, Gp15 – Pukovnik, Gp14 – D29
GP12	Protease/Gp15 – Bxz2, Gp15 – D29, Gp12 – Bxb1
GP13	Scaffold Protein/Gp16 – Che12, Gp16 – D29, Gp17 – Pukovnik
GP14	Capsid Head Protein/Gp17 – D29, Gp18 – Pukovnik, Gp17 – Che12
GP21	Tail Protein/Gp23 – Bxz2, Gp23 – D29, Gp25 – Che12
GP22	Fusion protein/Gp23 – KBG, Gp22 – Solon, Gp21 – U2
GP23	Fusion protein/Gp27 – Pukovnik, Gp25 – Bxz2, Gp24 – Jasper
GP24	Tape Measure Protein/Gp28 – Pukovnik, Gp22 – Bxb1, Gp24 – Solon
GP25	Minor Tail Protein/Gp27 – Bxz2 & D29, Gp25 – Solon, Gp26 – Jasper & KBG, Gp24 – Bethlehem,Gp23 – Bxb1, Gp29 – Pukovnik & Che12
GP26	Minor Tail Protein/Gp28 – Bxz2, Gp28 – KBG
GP32	S-Integrase/Gp34 – Bxz2
GP34	Putative Deaminase/Gp36.1 – D29, Gp38 – Bxz2
GP44	DNA Polymerase/Gp 43 – Pukovnik, Gp44 – Bxz2, Gp47 – Che12
GP45	DNA binding/Gp49 – Che12, Gp46 – Bxz2, D29
GP46	ThymidineX/Gp48 – Pukovnik,L5, D29
GP47	Ala/Pro Rich/Gp49 – Bxz2, Gp49 – KBG, Gp46 – Bethlehem
GP48	Ribonucleotide Reductase/Gp50 – Bxz2, Gp53 – Che12, Gp50 – D29
GP49	Reductase/Gp50 – Bxz2, Gp53 – Che12
GP54	DNA Primase/Gp58 – Bxz2, Gp58 – Pukovnik, Gp58 – D29
GP57	Hydrolase/Gp62 – Pukovnik, Gp59.2 – D29, Gp61 – Bxz2, Gp61 protein [Mycobacterium smegmatis str. MC2 155]

S-integrase

Pham 23



Acknowledgments

- Cindy Henk-LSU for Electron Microscopy
- Benji Morehead for the “Peaches” sample and additional lab support
- Dr. Allison Wiedemeier, Dr. Joydeep Bhattacharjee, and Dr. Russ Minton: Lecture instructors for SEA students
- All HHMI-SEA staff and ULM Department of Biology