

Isolation and Genomic Characterization of Mycobacteriophage sp. Hope

Marie Anderson[†], Tara Avery[†], Tiffany Bryan[†], Ashiya Buckels[†], Keithara Davis[†],
Breyon Dixon[†], Arianna Forbes[†], Zindzi George[†], Audrianna Gordon[†], Shalaina
Griffin[†], Tiffany Grigley[€], Raven Hardy[†], Lateria Haynes[†], KaTerri Kelly[†], Kinnari
Matheson[†], Jazmyn McCloud[†], Morgan McKenzie[†], Sterling Mitchell[†], Orianne
Morrison[†], Lindsay Parnell[†], Brianna Peppers[†], Christina Roberts[†], Yetta
Robinson[†], Kerona Sharpe[†], Blaire Spaulding[†], Starrissa Winters[†], Melina Zuniga[†],
Charles Hardnett[‡], and Cynthia Bauerle[†]

Departments of Biology[†], Economics[€], and Computer Science[‡], Spelman College,
Atlanta, GA 30314

Project Participants



Bio 110 Students
Fall 2008

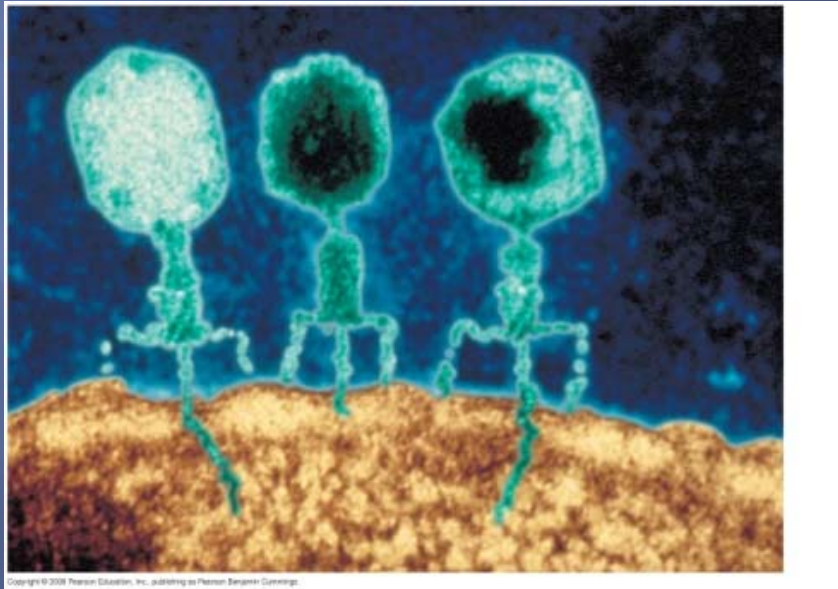


Bio 491 Students
Spring 2009

Project Background

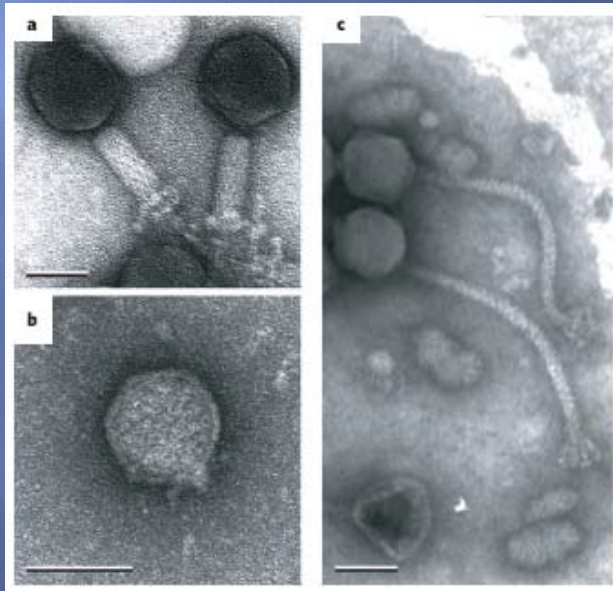
Why Bacteriophages?

- ❑ Bacteriophages are among the most abundant life forms in the biosphere (10^{31} !!)
- ❑ Bacteriophages display significant genetic diversity
- ❑ Phage genomes are highly mosaic and contain many novel sequences
- ❑ Small genomes are amenable to comparative genomic analysis

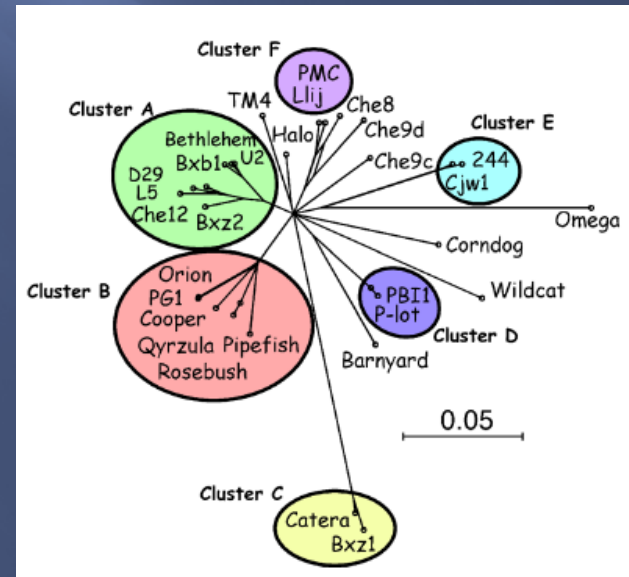


Mycobacteriophages

- ▣ Viruses that infect mycobacterial hosts (e.g. *M. smegmatis*, *M. tuberculosis*, *M. leprosoae*)
- ▣ These efforts lend themselves to possible viral therapy treatments.
- ▣ 75+ mycophage isolates classified into 9+ clusters



Suttle, C (2005) Nature, 437:3456-361



Hatfull et al, 2006, PLoS Genet 2(6): e92

Project Objective

The objective of this study is to isolate and characterize a novel mycophage in order to contribute to a growing phylogeny of diverse bacteriophage isolates from soil

Strategy

Semester: 1

Capture:

Bacteriophage were obtained from soil collected at Arabia Mountain



Tame:

Bacteriophage Hope was isolated by enrichment and purified by multiple passages on bacterial lawns



Dissect:

- Phage morphology was analyzed using electron microscopy
- Genomic DNA isolated and analyzed by restriction digestion
- Genomic DNA submitted to JGI for sequencing

Semester: 2

Information Analysis:

- Receive sequenced DNA from sequencing center
- Evaluated Genomic Quality using Sequencher



Genomic Analysis:

- Analyzed DNA information using computer programs such as Glimmer and GeneMark
- Identify genes and define genomes



Share:

- Upload genome to the SEA research archive
- Submit information to GenBank

Capture



Capture:

Bacteriophage were obtained from soil collected at Arabia Mountain

- Forested area, N.33.66°
W.084.12°
- Decaying tree log

Tame

Tame:
Bacteriophage Hope was isolated
by enrichment and purified by
multiple passages on bacterial
lawns



Plaque formation on *Smegmatis* lawns

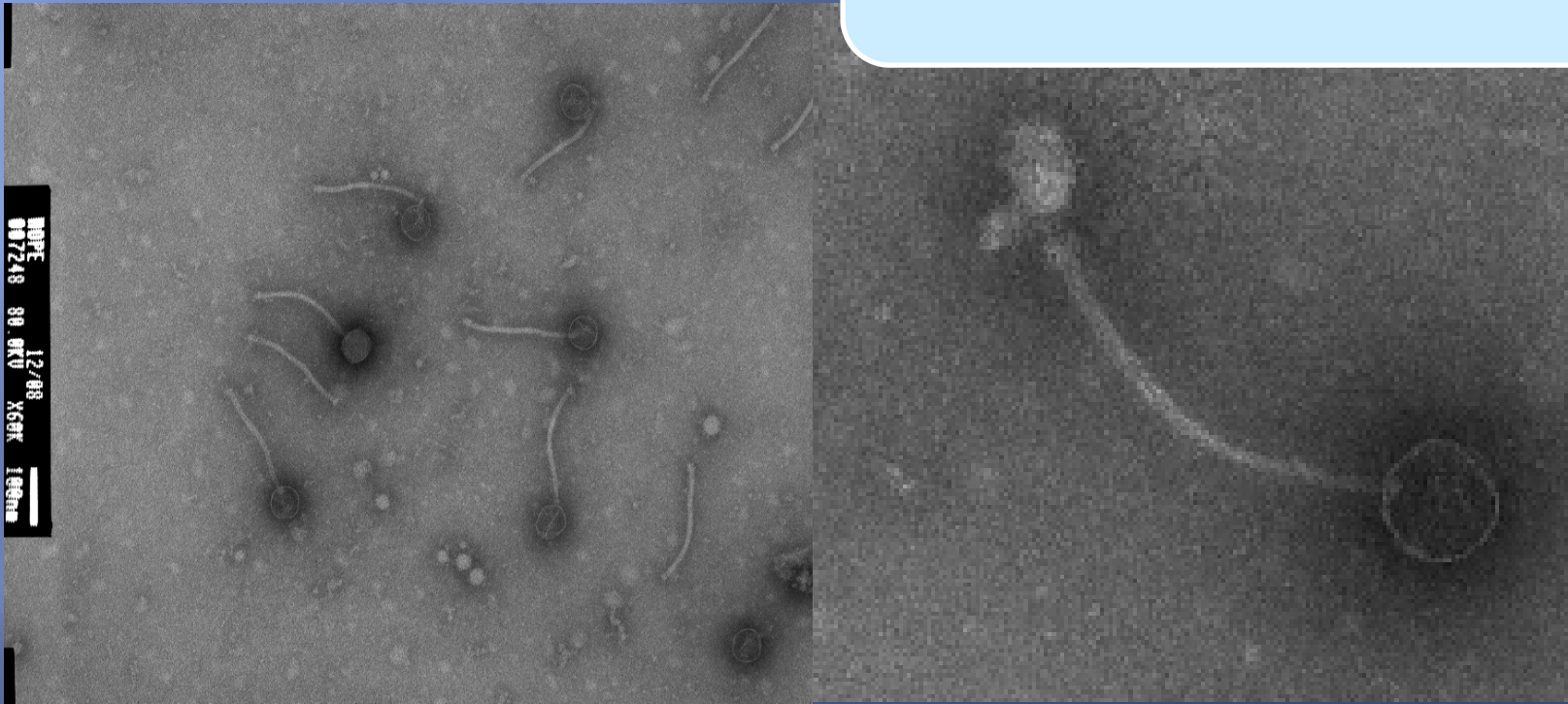


Dissect

- Tail 220 nm
- Capsid 60 nm in diameter

Dissect:

- Phage morphology was analyzed using electron microscopy



Negative staining with PTA. 60,000x magnification

Dissect

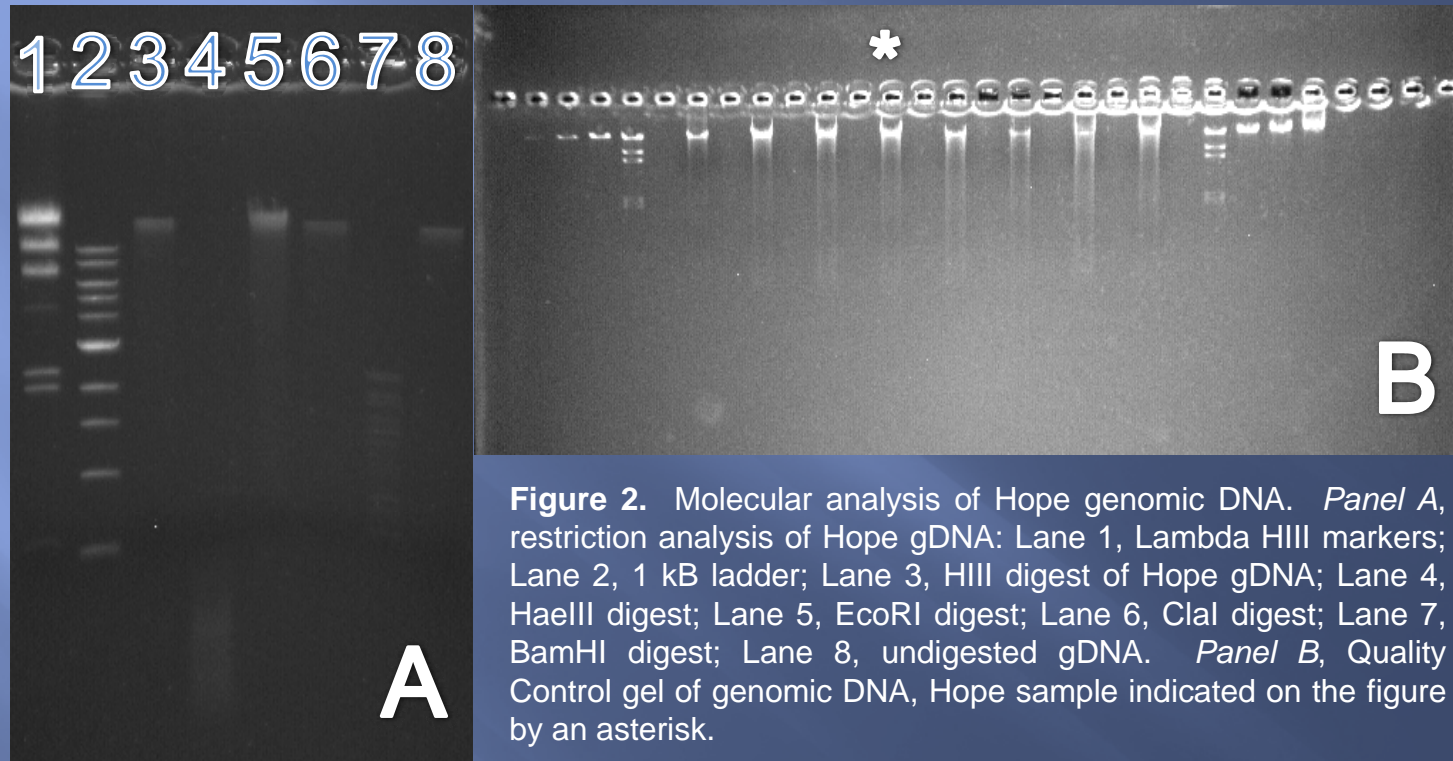


Figure 2. Molecular analysis of Hope genomic DNA. *Panel A*, restriction analysis of Hope gDNA: Lane 1, Lambda HIII markers; Lane 2, 1 kB ladder; Lane 3, HIII digest of Hope gDNA; Lane 4, HaeIII digest; Lane 5, EcoRI digest; Lane 6, ClaI digest; Lane 7, BamHI digest; Lane 8, undigested gDNA. *Panel B*, Quality Control gel of genomic DNA, Hope sample indicated on the figure by an asterisk.

Dissect:

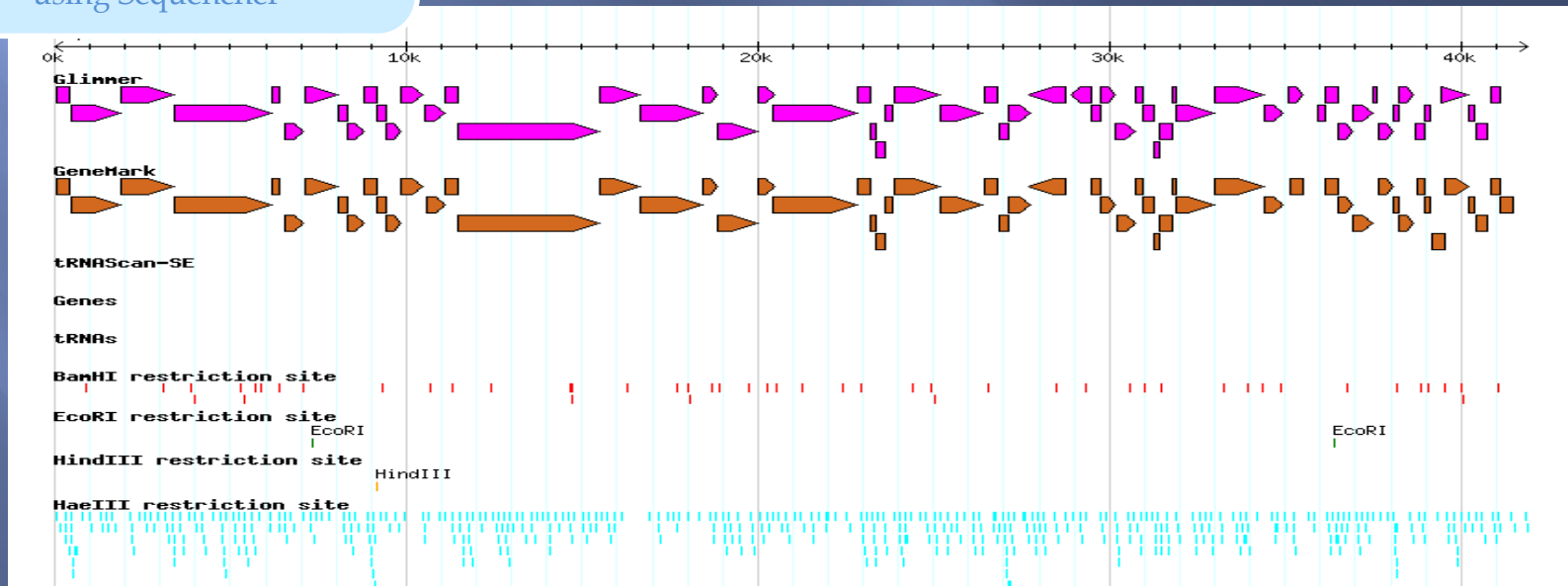
- Genomic DNA isolated and analyzed by restriction digestion
- Genomic DNA submitted to JGI for sequencing

Analyze

- ▣ Initial DNA sequence compiled as single contig
- ▣ Finished linear sequence 41,901 nucleotides
- ▣ Restriction site analysis corresponds with restriction analysis of isolated gDNA
- ▣ 58-59 Open Reading Frames identified by Glimmer and GeneMark
- ▣ Putative TMP orf corresponds to phage tail length

Information Analysis:

- Receive sequenced DNA from sequencing center
- Evaluate Genomic Quality using Sequencer



Analyze

- Analyze predicted ORFs (start codon, overlapping ORFs)
- Identify Shine-Delgarno sequences

Showing 41.9 kbp from Hope, positions 1 to 41,901

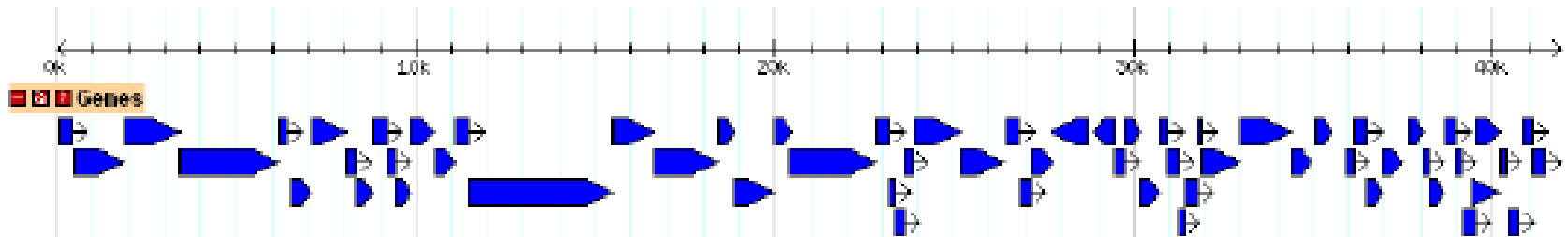
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Overview

Details

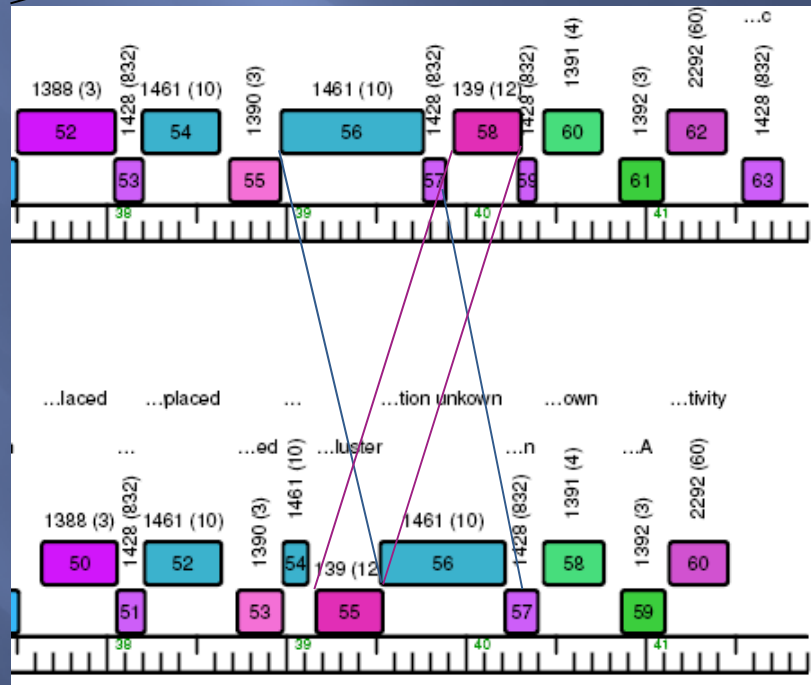
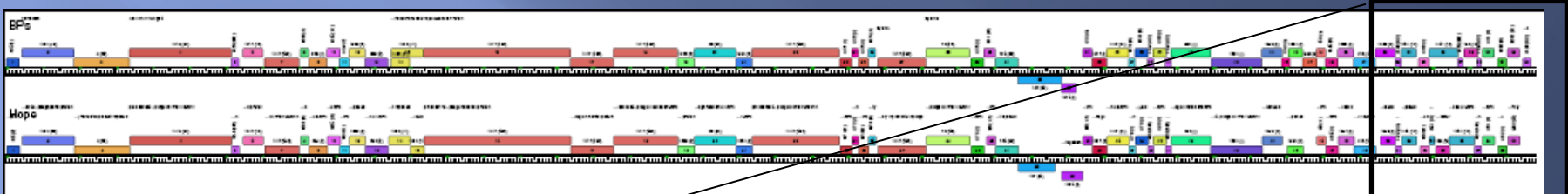


Gene Analysis:

- Analyzed DNA information using computer programs such as Glimmer and GeneMark

Discover

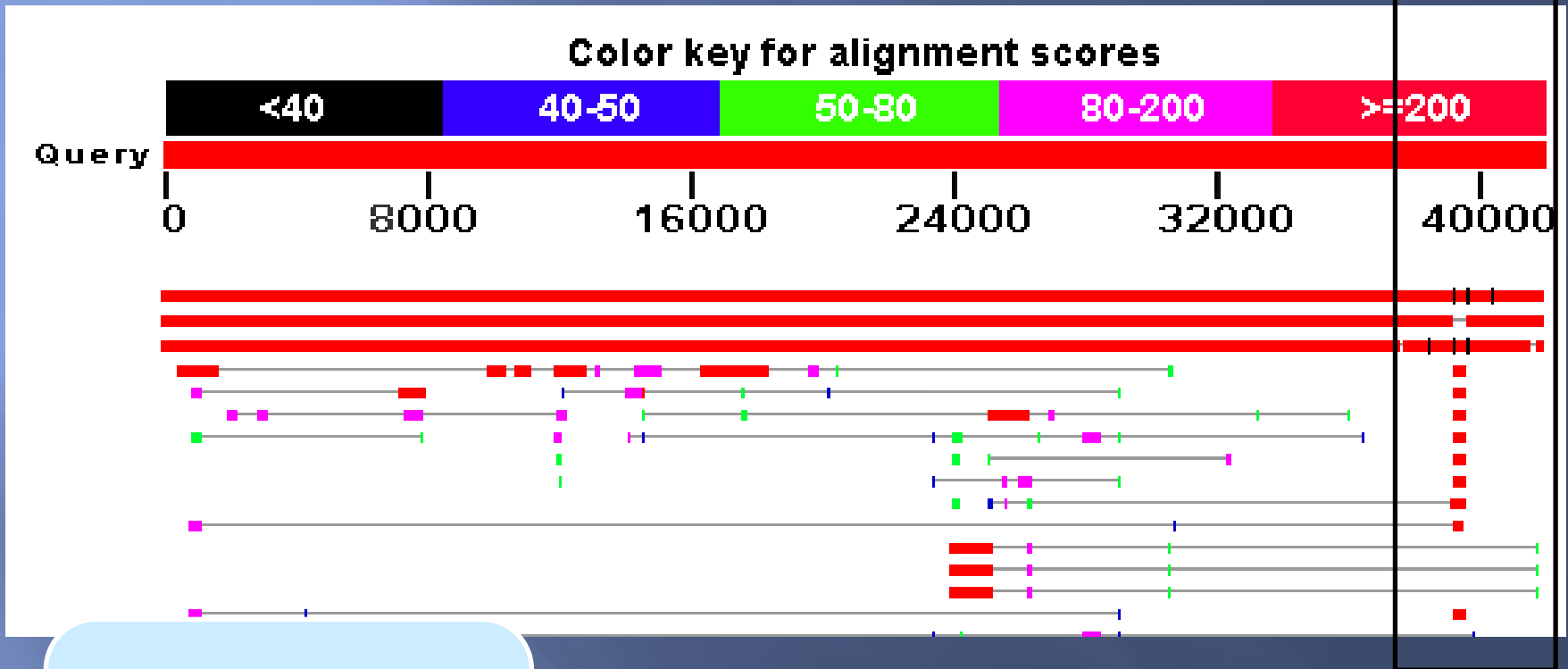
Hope (Sept 2009, GA) displays 100% nucleotide sequence identity with BPs (Mar 2008, PA):



Repositioning of a 440 nucleotide module (gp 55)

Discover

BLAST of full length Hope genome:

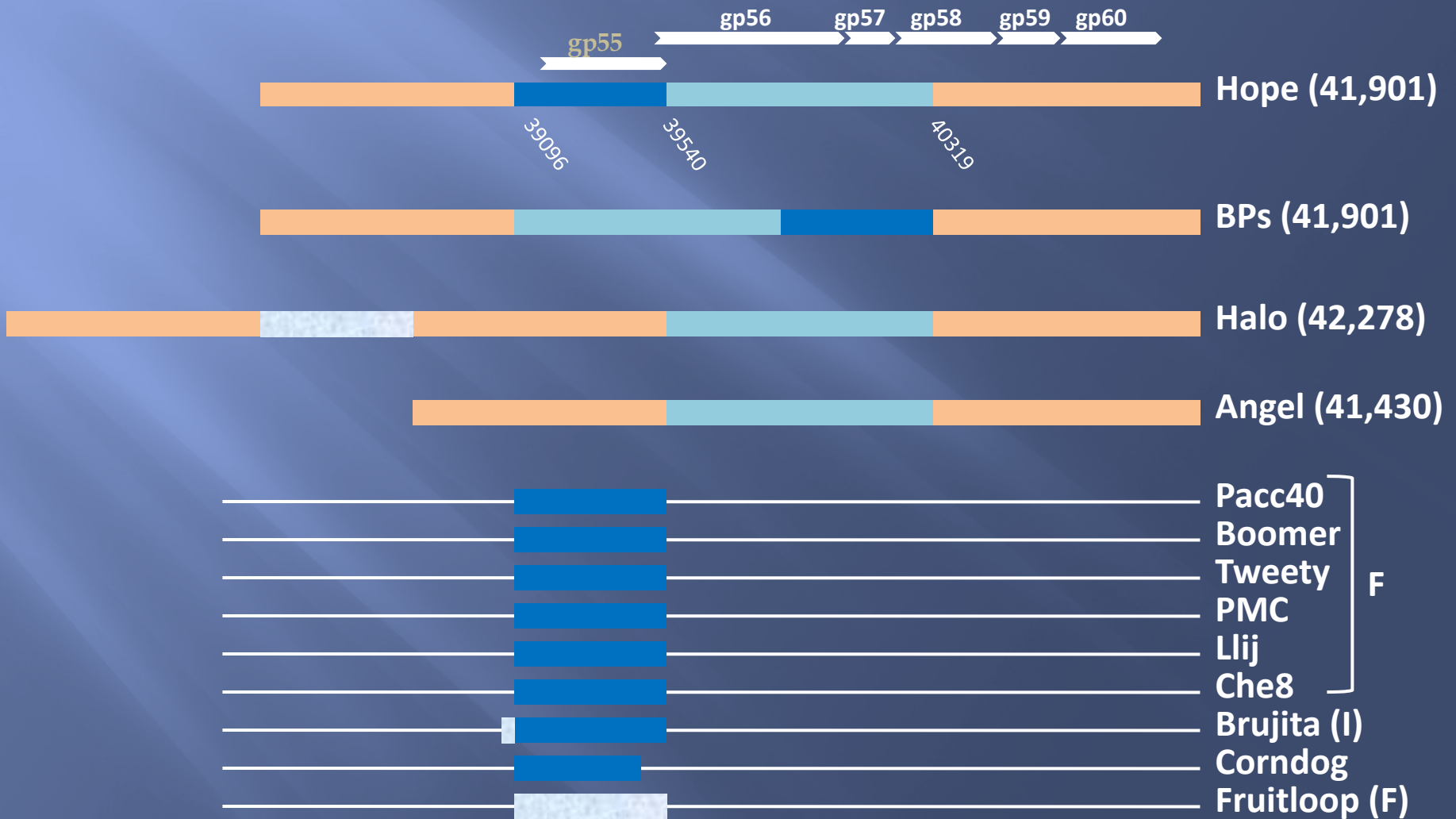


Comparative Genomic Analysis:

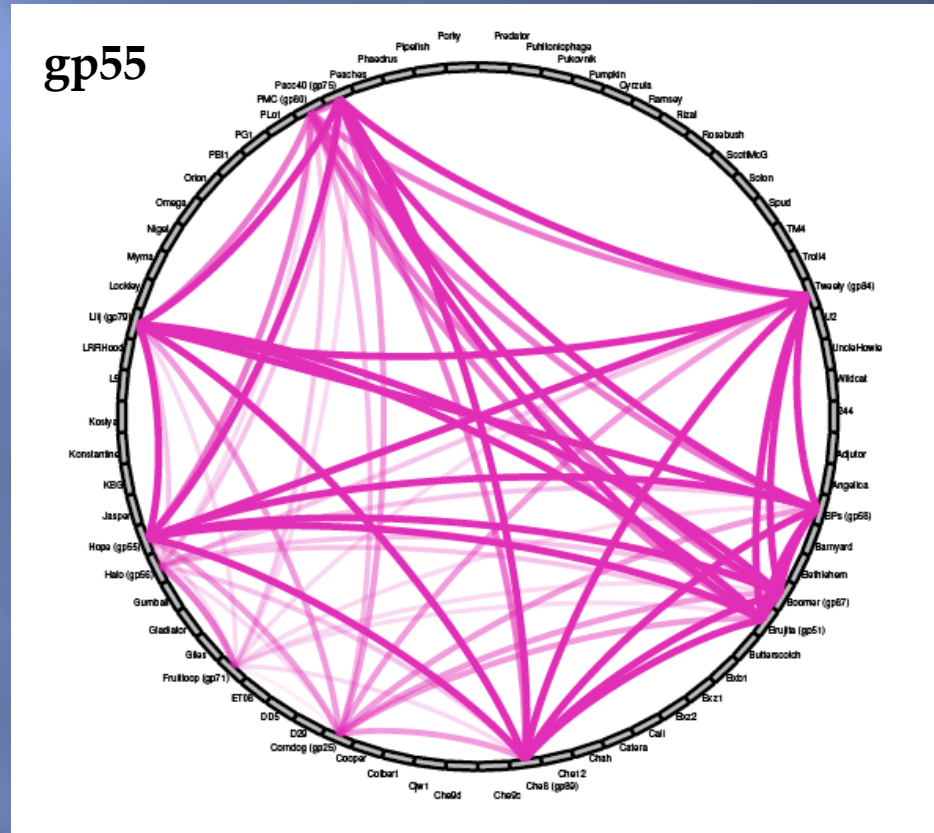
- Define novel genome
- Examine phylogenetic relationships

Discover

3' end of Hope genome:

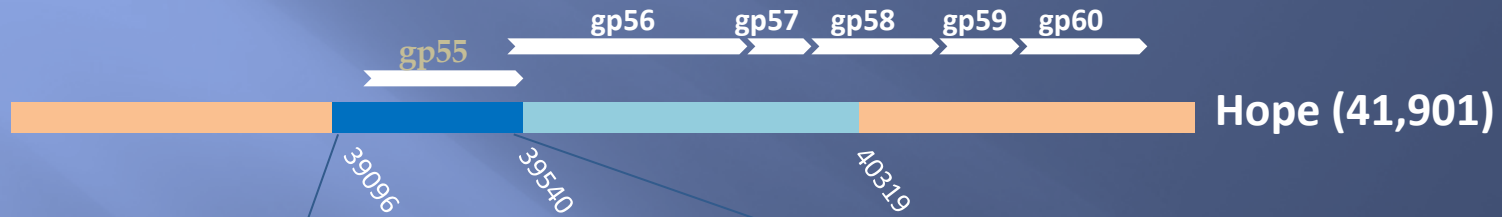


Discover



Gp55 is highly conserved among 12 mycophage including representatives from clusters G, F and I

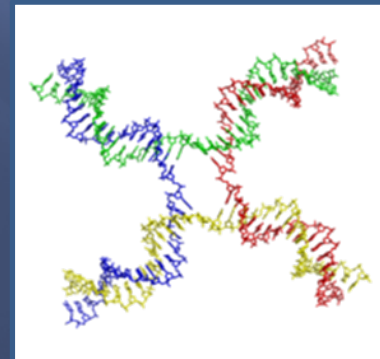
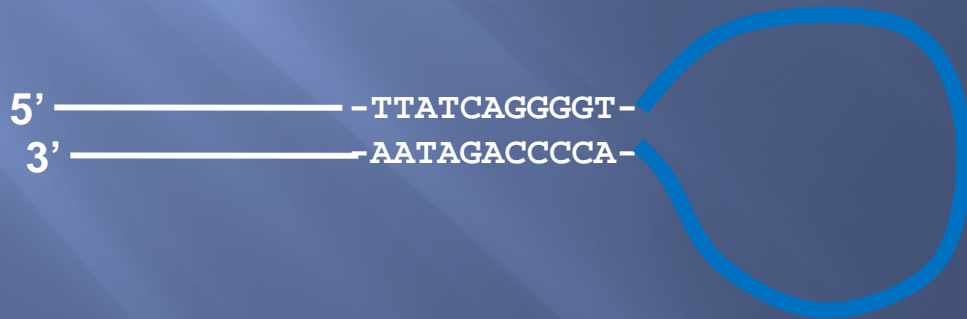
Homologous Recombination ?



TTATCAGGGGT
AATAGTCCCA

ACCCAGATAA
TGGGTCTATT

Inverted Repeats



Holliday junction
Recombination intermediate

Conclusions/Discussion

- Hope is a member of the Siphoviridae family of mycobacteriophages, and displays significant nucleotide sequence identity with G cluster mycophages
- Hope displays a novel genomic arrangement at the 3' end compared to BPs, Halo, and Angel
- Hope genome contains a highly conserved 440 bp module that bears a putative gene coding sequence (gp57) and is observed in diverse mycophage genomes
- Mycophage Hope genome contains genetic elements consistent with the presence of a RecE homologous recombination mechanism

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Deborah Jacobs-Sera, UPitt

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