



Isolation and Genome Sequencing of a Novel Mycobacteriophage, Pumpkin

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Janelia Farm Research Campus
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Introductions


- Represent team from Hope College in Holland, MI
 - Undergraduate liberal arts college
- Program at Hope
 - Phage Genomics Research course
 - Honors course for 20 first-year students
 - Replaced standard intro biology lab in the fall semester

About Me...

- Hometown: Ramsey, MN
- Major: Biology
- Interest: Molecular Biology




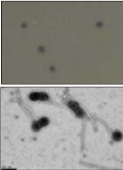
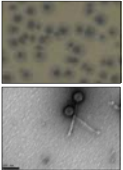
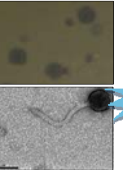
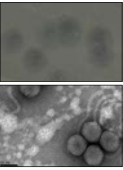
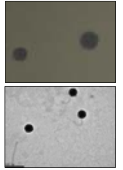
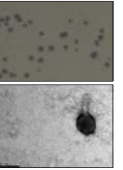
The Hope College Team



Isolation and Purification

- All soil samples collected from Holland area
- 20 phages successfully isolated (100%)
 - Students worked independently
 - 15% isolated phage by direct plating
 - 85% used an enrichment procedure (Lorenz)
- Diverse morphological and structural characteristics




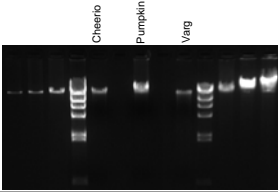
Lars	Langerak	Scout	
			MINE! ☺
			
Phantom	Nemo	Paige	

Selection of Phage

<p>Ideal Criteria</p> <ul style="list-style-type: none"> • Purity of Phage isolate • Evidence of Uniqueness <ul style="list-style-type: none"> • Plaque morphology • RE digest pattern • Phage structure • Quantity and Quality of DNA 	<p>Our Phage Olympics</p> <ul style="list-style-type: none"> • 20 isolates (100%) • ~15 unique phages isolated <ul style="list-style-type: none"> • Diverse plaque morphology • 19 successful RE digests confirmed diversity within class • No EM in time for consideration • Only 3 had good quantity
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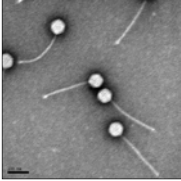
Our Selection—Pumpkin

- Pure phage stock
- EcoRI digest indicated relationship to Cluster E
- ~100 µg high quality DNA



Caitlin Peirce isolated Pumpkin from a vegetable compost pit

Pumpkin – Phenotype

<p><u>Plaque morphology</u></p>  <ul style="list-style-type: none">• 1-2 mm clearing surrounded by turbid ring• comet shape	<p><u>Phage Structure</u></p>  <ul style="list-style-type: none">• 69 nm capsid• 240 nm tail• Siphoviridae
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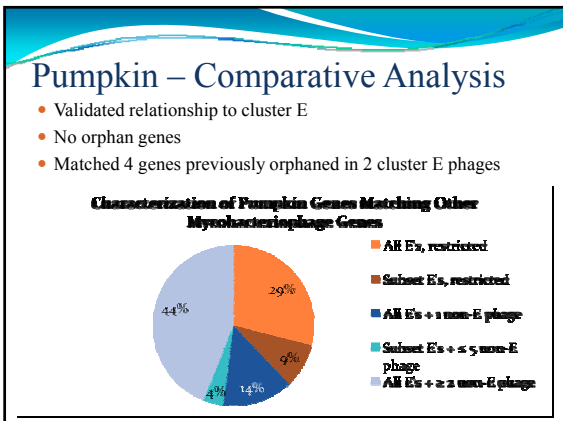
Pumpkin – Genomic Sequence Overview

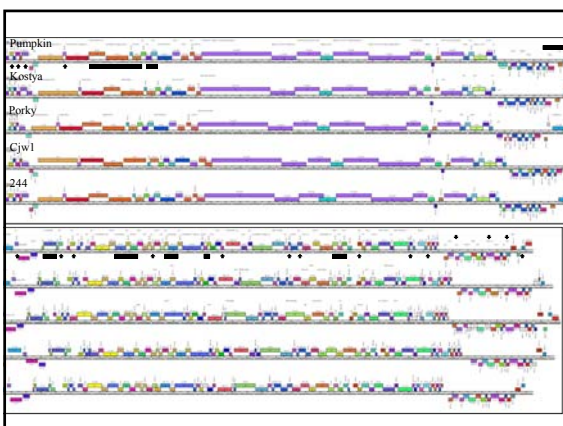
Genomic Statistics

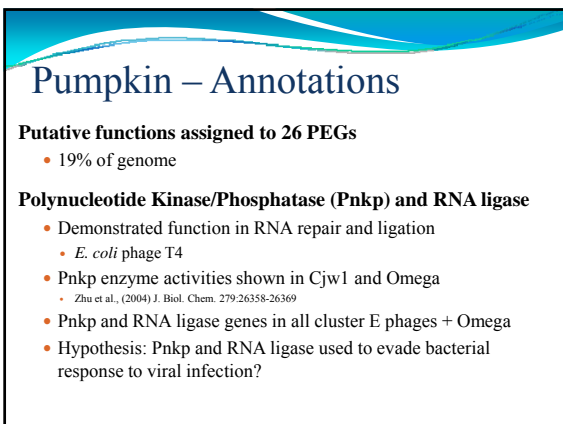
- 74,491 bp
- 63% GC
- 140 genes
 - 138 protein encoding genes (PEGs)
 - 2 tRNA genes (Arginine & Glycine)

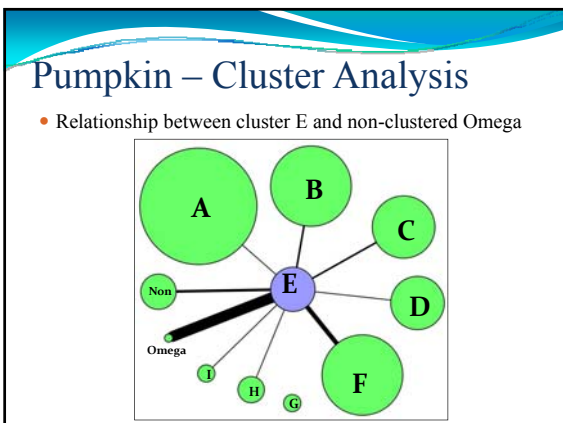
Genome Structure

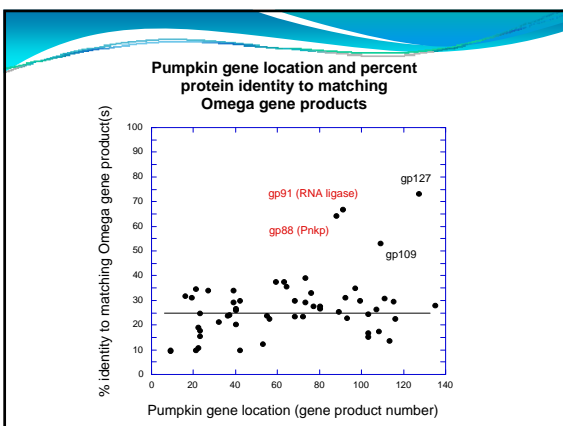
- Linear genome, cohesive ends











Conclusion

- Success isolating and characterizing
 - All 20 first-year students isolated phage
 - Diversity among class: ~15 are unique
- Selected novel phage for sequencing – Pumpkin
- Successfully analyzed complete genome of Pumpkin
 - 140 genes
 - Assigned function to 26 PEGs (19%)
 - Confirmed cluster E relationship
 - Propose relationship of Omega to cluster E
