

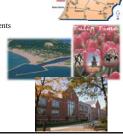


Introductions

- 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 studentsReplaced standard intro biology
 - Replaced standard intro biology lab in the fall semester

About Me...

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



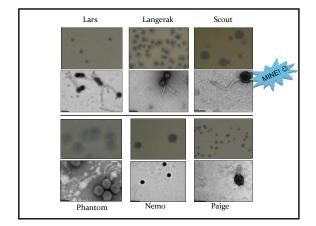




Isolation and Purification

- 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





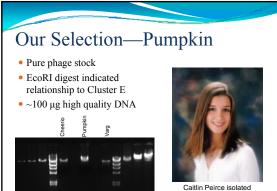
Selection of Phage

Ideal Criteria

- · Purity of Phage isolate
- Evidence of Uniqueness
- Plaque morphologyRE digest pattern
- Phage structure Quantity and Quality of DNA

Our Phage Olympics

- 20 isolates (100%)
- ~15 unique phages isolated
 Diverse plaque morphology
 19 successful RE digests confirmed
- diversity within classNo EM in time for consideration
- Only 3 had good quantity





Pumpkin – Phenotype Phage Structure Plaque morphology 1-2 mm clearing surrounded by turbid ring • 69 nm capsid

• comet shape



• 240 nm tail Siphoviridae

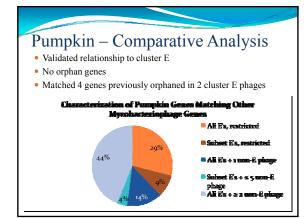
Pumpkin – Genomic Sequence Overview

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





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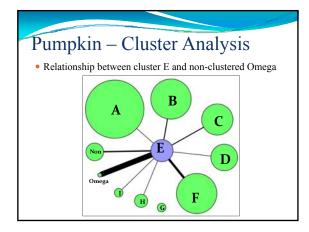
Pumpkin – Annotations

Putative functions assigned to 26 PEGs

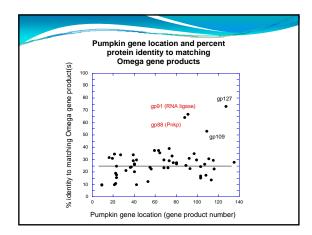
• 19% of genome

Polynucleotide Kinase/Phosphatase (Pnkp) and RNA ligase

- Demonstrated function in RNA repair and ligation
 E. coli phage T4
- Pnkp enzyme activities shown in Cjw1 and Omega
 Zhu et al., (2004) J. Biol. Chem. 279:26358-26369
- Pnkp and RNA ligase genes in all cluster E phages + Omega
- Hypothesis: Pnkp and RNA ligase used to evade bacterial
- response to viral infection?







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

Acknowledgements

- SEA Staff and HHMI NGRI Program
- Professors: Dr. Aaron Best and Dr. Joseph Stukey
- TA's: Caitlin Rice, Shirley Bradley, Nathaniel Bowerman
- Paul VanAllsburg, Hope College Computing and Information Technology
- Dr. Rob Eversole, Western Michigan University
- My Classmates

