






# Genome Sequences of Mycobacteriophages Findley, Hurricane, and TBond007

Sanghamitra Saha,<sup>a</sup> Wenbo Yan,<sup>b</sup> Jacqueline M. Washington,<sup>b</sup> William B. Davis,<sup>c</sup> W. Brad Barbazuk,<sup>d</sup> Rosa Medellin,<sup>a</sup> Aissa Mendez,<sup>a</sup> Jacqueline R. Ramos,<sup>a</sup> Rachna Sadana,<sup>a</sup> Miranda Edmonds,<sup>c</sup> Guanqiao Feng,<sup>d</sup> Maria Ilyas,<sup>d</sup> Joshua Kaiser,<sup>d</sup> Stephen J. Monroe,<sup>d</sup> Thinh V. Pham,<sup>d</sup>  Steven G. Cresawn,<sup>e</sup> Rebecca A. Garlena,<sup>f</sup> Ty H. Stoner,<sup>f</sup> Daniel A. Russell,<sup>f</sup>  Welkin H. Pope,<sup>f</sup> Deborah Jacobs-Sera,<sup>f</sup>  Graham F. Hatfull<sup>f</sup>

Department of Biology, University of Houston-Downtown, Houston, Texas, USA<sup>a</sup>; Department of Biology and Chemistry, Nyack College, Nyack, New York, USA<sup>b</sup>; School of Molecular Biosciences, Washington State University, Pullman, Washington, USA<sup>c</sup>; Department of Biology and Genetics Group, University of Florida, Gainesville, Florida, USA<sup>d</sup>; Department of Biology, James Madison University, Harrisonburg, Virginia, USA<sup>e</sup>; Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA<sup>f</sup>

**ABSTRACT** We report here the genome sequences of three newly isolated phages that infect *Mycobacterium smegmatis* mc<sup>2</sup>155. Phages Findley, Hurricane, and TBond007 were discovered in geographically distinct locations and are related to cluster K mycobacteriophages, with Findley being similar to subcluster K2 phages and Hurricane and TBond007 being similar to subcluster K3 phages.

A large collection of completely sequenced mycobacteriophages reveals their considerable genetic diversity, and the mycobacteriophages can be grouped into more than 30 genomic types based on overall nucleotide sequence similarity (1). One of these groups, cluster K, contains temperate phages with broad host ranges that infect both *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* (2).

Phages Findley, Hurricane, and TBond007 were isolated from geographically dispersed locations in New York, Washington, and Texas, respectively, using *M. smegmatis* mc<sup>2</sup>155 as the host. Electron microscopy shows that all three phages are morphologically members of the family *Siphoviridae*. After isolation and purification, double-stranded DNA was sequenced using Illumina MiSeq, and single-end reads of 140 bp were assembled using Newbler to yield a major contig for each with at least 1,000-fold coverage, except for the contig of Hurricane, which has 54-fold coverage. The genomes of Findley, Hurricane, and TBond007 are 58,150 bp, 61,318 bp, and 61,145 bp long, respectively, and coverage analysis shows they possess *cos* ends with 10- to 11-nucleotide single-stranded 3' extensions. All three phages have a G+C content of 67%, which is similar to that of *M. smegmatis*.

Protein-coding genes were identified using Glimmer (3) and GeneMark (4), followed by manual inspection and revision using DNA Master (<http://cobamide2.bio.pitt.edu/computer.htm>). From a comparative analysis using previously described parameters (5), Findley was assigned to cluster K2, and both Hurricane and TBond007 were assigned to cluster K3 (2, 6, 7). Ninety-four to 100 protein-coding genes are predicted for each phage, accounting for 92.5 to 94.4% of the genome-coding capacities. Protein-coding genes with assigned functions constitute 36 to 51% of the total genomes. No tRNA genes were identified in any of the three phages. BLASTN analysis shows that Hurricane is closely related to TBond007, sharing 95% nucleotide identity, whereas Findley only shares 75% nucleotide identity with the other two phages; the strongest regions of similarity are in the genome left arms containing the virion structure and assembly

**Received** 10 September 2017 **Accepted** 11 October 2017 **Published** 9 November 2017

**Citation** Saha S, Yan W, Washington JM, Davis WB, Barbazuk WB, Medellin R, Mendez A, Ramos JR, Sadana R, Edmonds M, Feng G, Ilyas M, Kaiser J, Monroe SJ, Pham TV, Cresawn SG, Garlena RA, Stoner TH, Russell DA, Pope WH, Jacobs-Sera D, Hatfull GF. 2017. Genome sequences of mycobacteriophages Findley, Hurricane, and TBond007. *Genome Announc* 5:e01123-17. <https://doi.org/10.1128/genomeA.01123-17>.

**Copyright** © 2017 Saha et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Graham F. Hatfull, [gfh@pitt.edu](mailto:gfh@pitt.edu).

genes. All genes are transcribed in the forward direction, except for three or four genes at the center of each genome. Like other cluster K phages, all three phages contain multiple copies of 13-bp start-associated sequences (13, 16, and 17 copies in Findley, Hurricane, and TBond007, respectively), with no more than one mismatch to the consensus 5'-GGGATAGGAGCCC sequence (2). They also contain six to nine copies of the extended start-associated sequences upstream of subsets of these, as in other cluster K phages (2).

All three phages have tyrosine integrase and immunity repressor genes, indicating temperate lifestyles, as with other cluster K phages (2). Phage attachment *attP* sites were identified in Hurricane and TBond007, sharing common cores with an *attB* site overlapping the *M. smegmatis* mc<sup>2</sup>155 tRNA-Lys gene (*Msmeg\_4746*), similar to other K3 phages (2); the Findley *attP* site corresponds to an *attB* site in the transfer-messenger RNA (*tmRNA*) gene (2) and was confirmed experimentally. The Hurricane immunity repressor (gene 46; 150 amino acids) has 83.5 to 85% overall amino acid sequence identity with the repressors of other K3 phages (including TBond007), with near identity over the N-terminal 105 residues but highly divergent C termini. Further experiments are needed to determine if K1 and K3 phages share immune specificity.

**Accession number(s).** The GenBank accession numbers for Findley, Hurricane, and TBond007 are [MF140411](https://ncbi.nlm.nih.gov/nucl/MF140411), [MF373841](https://ncbi.nlm.nih.gov/nucl/MF373841), and [KX683428](https://ncbi.nlm.nih.gov/nucl/KX683428), respectively.

## ACKNOWLEDGMENTS

Funding was provided by Howard Hughes Medical Institute grant 54308198 to G.F.H.

We thank the HHMI and SEA-PHAGES program for support and Saun Dingborg for helpful discussions.

## REFERENCES

- Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. *Bioinformatics* 33:784–786. <https://doi.org/10.1093/bioinformatics/btw711>.
- Pope WH, Ferreira CM, Jacobs-Sera D, Benjamin RC, Davis AJ, DeJong RJ, Elgin SCR, Guilfoile FR, Forsyth MH, Harris AD, Harvey SE, Hughes LE, Hynes PM, Jackson AS, Jalal MD, MacMurray EA, Manley CM, McDonough MJ, Mosier JL, Osterbann LJ, Rabinowitz HS, Rhyan CN, Russell DA, Saha MS, Shaffer CD, Simon SE, Sims EF, Tovar IG, Weisser EG, Wertz JT, Weston-Hafer KA, Williamson KE, Zhang B, Cresawn SG, Jain P, Piuri M, Jacobs WR, Jr, Hendrix RW, Hatfull GF. 2011. Cluster K mycobacteriophages: insights into the evolutionary origins of mycobacteriophage TM4. *PLoS One* 6:e26750. <https://doi.org/10.1371/journal.pone.0026750>.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679. <https://doi.org/10.1093/bioinformatics/btm009>.
- Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Res* 33:W451–W454. <https://doi.org/10.1093/nar/gki487>.
- Hatfull GF, Jacobs-Sera D, Lawrence JG, Pope WH, Russell DA, Ko CC, Weber RJ, Patel MC, Germane KL, Edgar RH, Hoyte NN, Bowman CA, Tantoco AT, Paladin EC, Myers MS, Smith AL, Grace MS, Pham TT, O'Brien MB, Vogelsberger AM, Hryckowian AJ, Wynalek JL, Donis-Keller H, Bogel MW, Peebles CL, Cresawn SG, Hendrix RW. 2010. Comparative genomic analysis of 60 mycobacteriophage genomes: genome clustering, gene acquisition, and gene size. *J Mol Biol* 397:119–143. <https://doi.org/10.1016/j.jmb.2010.01.011>.
- Pope WH, Bowman CA, Russell DA, Jacobs-Sera D, Asai DJ, Cresawn SG, Jacobs WR, Hendrix RW, Lawrence JG, Hatfull GF, Science Education Alliance Phage Hunters Advancing Genomics, Evolutionary Science, Phage Hunters Integrating Research and Education, Mycobacterial Genetics Course. 2015. Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity. *Elife* 4:e06416.
- Smith KC, Castro-Nallar E, Fisher JN, Breakwell DP, Grose JH, Burnett SH. 2013. Phage cluster relationships identified through single gene analysis. *BMC Genomics* 14:410. <https://doi.org/10.1186/1471-2164-14-410>.