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Graham Hatfull's Lecture Part 3:
Mycobacteriophage genomics

Teaching Tools were prepared by Welkin Pope with Graham Hatfull.

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1. Keywords and Terms

Mycobacterium tuberculosis, Mycobacterium smegmatis, plaque, titer, direct plating, enrichment, permissive host, genome clustering, heterogeneity, genome comparison, nucleotide similarity, protein similarity, ORF, gene, pham, orpham, BRED

2. Lecture Notes

Hatfull describes work from his lab on mycobacteriophages, phage that infect mycobacteria including M. tuberculosis, the causative agent of the human disease tuberculosis.

3. Review Questions

1. If we are interested in studying Mycobacterium tuberculosis, why not just use it in the lab?
2. What characteristics describe known Mycobacteriophages (circle all that apply)? 60% G-C, 72kb average genome length, Siphoviridae, Myoviridae, 12 ORFs, 246 kb average genome length, 33% G-C, Podoviridae
3. How do we cluster bacteriophages together?
4. What is a singleton?
5. Which of the following describes bacteriophage genomes (circle all that apply)? Tightly packed with genes, many promoters, synteny of structural genes, mosaic, many operons, few genes with recognizable functions.
6. What is an orpham?
7. What is BRED and what is it used for?

4. Answers to Review Questions

1. It is infectious (unlike M. smegmatis), and it grows very very slowly!
2. 60% G-C, 72kb average genome length, Siphoviridae, Myoviridae
3. DotPlot, gene content, genome comparison
4. A mycobacteriophage that is genetically distant from other known mycobacteriophage isolates.
5. Tightly packed with genes, synteny of structural genes, mosaic, few genes with recognizable functions
6. An orpham is a gene family that contains one gene with no other close relatives by protein similarity within all the known mycobacteriophage genes in all known genomes.
7. Bacteriophage Recombination of Electroporated DNA; generating mutants in bacteriophage genomes

5. Discussion Questions

1. Why study Mycobacteriophages?
2. Why choose phages of the same host for comparative genomics?
3. Why aren't there any Podoviridae among the isolated mycobacteriophages?
4. What can we learn from comparing protein sequences instead of nucleotide sequences?
5. What is the evidence for genome mosaicism and illegitimate recombination in bacteriophage genomes?
6. Why can't we build a phylogenetic tree for bacteriophages like we do for other organisms?
7. Why is it useful to have a tool like BRED that allows for the directed generation of mutations in bacteriophage genomes?

6. Answers to Discussion Questions

1. Mycobacterium tuberculosis kills 2 million people a year, more than any other infectious agent. There has also been a recent rise of drug resistant strains.
2. Phages that infect the same host may undergo simultaneous infections in the same cell and therefore are in genetic contact with each other.
3. We believe that it may be due to the Mycobacterial cell surface. It may be too thick for a shorter tails to cross the barrier into the cell. We don't really know.
4. We can recognize conserved genes that still have amino acid similarity but no longer have nucleotide identity.
5. Two adjacent genes in one genome each have a close relative in two other genomes and they are not flanked by any matching nucleotide sequences.
6. Individual genes in phage have different evolutionary histories making it impossible to place a phage species in a distinct place/time on a phylogenetic tree.

7. We don't know the function of most phage genes, and we can use BRED to knockout or alter one gene at a time and then study the phenotype.

7. Explain or Teach These Concepts to a Friend

1. Explain how new mycobacteriophages are isolated
2. Explain why we study phages of Mycobacterium smegmatis
3. Explain what diagonal lines on DotPlots mean when genome nucleotide sequences are compared to each other.
4. Explain the difference between nucleotide similarity and protein similarity.
5. Explain why we can't build a single phylogenetic tree for bacteriophages.

8. Papers for Journal Club

1. PLOS ONE. Vol 4, Issue 3, Pages e4870, 2009. Fluoromycobacteriophages for rapid, specific, and sensitive antibiotic susceptibility testing of Mycobacterium tuberculosis. Mariana Piuri, William R Jacobs, Graham F Hatfull
2. PLOSONE. Vol 6, Issue 1, Pages e16329, 2011. Expanding the diversity of mycobacteriophages: Insights into genome architecture and evolution. Pope WH, Jacobs-Sera D, Russell DA, Peebles CL, Al-Atrache Z, Alcoser TA, Alexander LM, Alfano MB, Alford ST, Amy NE, Anderson MD, Anderson AG, Ang AA, Ares M Jr, Barber AJ, Barker LP, Barrett JM, Barshop WD, Bauerle CM, Bayles IM, Belfield KL, Best AA, Borjon A Jr, Bowman CA, Boyer CA, Bradley KW, Bradley VA, Broadway LN, Budwal K, Busby KN, Campbell IW, Campbell AM, Carey A, Caruso SM, Chew RD, Cockburn CL, Cohen LB, Corajod JM, Cresawn SG, Davis KR, Deng L, Denver DR, Dixon BR, Ekram S, Elgin SC, Engelsen AE, English BE, Erb ML, Estrada C, Filliger LZ, Findley AM, Forbes L, Forsyth MH, Fox TM, Fritz MJ, Garcia R, George ZD, Georges AE, Gissendanner CR, Goff S, Goldstein R, Gordon KC, Green RD, Guerra SL, Guiney-Olsen KR, Guiza BG, Haghighat L, Hagopian GV, Harmon CJ, Harmson JS, Hartzog GA, Harvey SE, He S, He KJ, Healy KE, Higinbotham ER, Hildebrandt EN, Ho JH, Hogan GM, Hohenstein VG, Holz NA, Huang VJ, Hufford EL, Hynes PM, Jackson AS, Jansen EC, Jarvik J, Jasinto PG, Jordan TC, Kasza T, Katelyn MA, Kelsey JS, Kerrigan LA, Khaw D, Kim J, Knutter JZ, Ko CC, Larkin GV, Laroche JR, Latif A, Leuba KD, Leuba SI, Lewis LO, Loesser-Casey KE, Long CA,

Lopez AJ, Lowery N, Lu TQ, Mac V, Masters IR, McCloud JJ, McDonough MJ, Medenbach AJ, Menon A, Miller R, Morgan BK, Ng PC, Nguyen E, Nguyen KT, Nguyen ET, Nicholson KM, Parnell LA, Peirce CE, Perz AM, Peterson LJ, Pferdehirt RE, Philip SV, Pogliano K, Pogliano J, Polley T, Puopolo EJ, Rabinowitz HS, Resiss MJ, Rhyan CN, Robinson YM, Rodriguez LL, Rose AC, Rubin JD, Ruby JA, Saha MS, Sandoz JW, Savitskaya J, Schipper DJ, Schnitzler CE, Schott AR, Segal JB, Shaffer CD, Sheldon KE, Shepard EM, Shepardson JW, Shroff MK, Simmons JM, Simms EF, Simpson BM, Sinclair KM, Sjoholm RL, Slette IJ, Spaulding BC, Straub CL, Stukey J, Sughrue T, Tang TY, Tatyana LM, Taylor SB, Taylor BJ, Temple LM, Thompson JV, Tokarz MP, Trapani SE, Troum AP, Tsay J, Tubbs AT, Walton JM, Wang DH, Wang H, Warner JR, Weisser EG, Wendler SC, Weston-Hafer KA, Whelan HM, Williamson KE, Willis AN, Wirtshafter HS, Wong TW, Wu P, Yang Y, Yee BC, Zaidins DA, Zhang B, Zúniga MY, Hendrix RW, Hatfull GF.

3. PLOS ONE. Vol 3, Issue 12, Pages e3957, 2008. BRED: a simple and powerful tool for constructing mutant and recombinant bacteriophage genomes. Marinelli LJ, Piuri M, Swigonová Z, Balachandran A, Oldfield LM, van Kessel JC, Hatfull GF