



# Title of the publication

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essentiality | conservation | transposon insertion

#### Introduction

**S** tudying the essentiality of genes helps with identifying the fundamental processes necessary for cell viability [1]. So far, scientists have studied the essential genes in organisms from different domains of life [2]. The results have led to new insights for developing new antibiotics that target essential genes of pathogenic bacteria [3, 4] and synthesising new genomes [5, 6]. Researchers have used different methods for studying the essentility of genes in prokaryotes. Baba et al. [7] have made a library of single gene deletions using phage lambda Red recombination system to screen essential genes while another group have used antisense RNA knockdowns for this purpose [8]. Another method that is widely used due to its simplicity and accuracy is transposon mutagenesis along with high-throughput sequencing [9, 10, 11, 12, 13, 14, 15]. In this method, pools of single insertion mutants are constructed using transposon mutagenesis and the effect of each mutation on the survival of mutants is evaluated by sequencing the survivors [16]. This can lead to the identification of essential

Although the essentiality of genes has been studied in a variety of organisms, there is still room to study the evolutionary conservation of essentiality. Barquist et al. [17] have used transposon-directed insertion-site sequencing to study the differentiation of the essentiality of genes in Salmonella serovars Typhi and Typhimurium which has led to divergence in their pathogenecity and host ranges. We extend this research by studying 12 bacterial strains. These include Salmonella enterica subsp. enterica serovar Typhi str. Ty2, Salmonella enterica subsp. enterica serovar Enteritidis str. P125109, Salmonella enterica subsp. enterica serovar Typhimurium str. SL1344, Salmonella enterica subsp. enterica serovar Typhimurium str. A130, Salmonella enterica subsp. enterica serovar Typhimurium str. D23580, Escherichia coli UPEC ST131, Escherichia coli ETEC CS17, Escherichia coli ETEC H10407, Citrobacter rodentium ICC168, Klebsiella pneumoniae RH201207, Klebsiella pneumoniae subsp. pneumoniae Ecl8, and Enterobacter cloacae subsp. cloacae NCTC 9394. All these strains are selected from Enterobacteriaceae family.

- 1. Mario Juhas, Leo Eberl, and John I. Glass. Essence of life: essential genes of minimal genomes. 21(10):562-568.
- 2. Hao Luo, Yan Lin, Feng Gao, Chun-Ting Zhang, and Ren Zhang. DEG 10, an update of the database of essential genes that includes both protein-coding genes and noncoding genomic elements. 42:D574-D580
- 3. Anne E. Clatworthy, Emily Pierson, and Deborah T. Hung. Targeting virulence: a new paradigm for antimicrobial therapy. 3(9):541-548.
- 4. JasonM. Peters. Alexandre Colavin. Handuo Shi, TomaszL. Czarny, Matthew H. Larson. Spencer Wong, JohnS. Hawkins, CandyH. S. Lu, Byoung-Mo Koo, Elizabeth Marta, AnthonyL. Shiver, EvanH. Whitehead, JonathanS. Weissman, EricD. Brown, LeiS. Qi, KerwynCasey Huang, and CarolA. Gross. A comprehensive, CRISPR-based functional analysis of essential genes in bacteria. 165(6):1493-1506.
- 5. Clyde A. Hutchison, Scott N. Peterson, Steven R. Gill, Robin T. Cline, Owen White, Claire M. Fraser, Hamilton O. Smith, and J. Craig Venter. Global transposon mutagenesis and a minimal mycoplasma genome. 286(5447):2165-2169.

Enterobacteriaceae is a family that includes bacteria with different host ranges and pathogenecity found in soil, water, plants, animals and humans [18]. In humans, various strains from this family can cause diarrhoea, septicaemia, urinary tract infection, meningitis, respiratory disease, and wound and burn infection [18]. Besides, they can infect poultry and livestocks and cause financial losses for farmers [18]. Here, we perform a transposon-directed insertion-site sequencing experiment to study the conservation of essentiality of genes in strains from 5 different species in this family.

{A summary of what we have done}

## Bias

Gene classes Genus specific.

Single copy.

Multi-copy.

## **Evolution of essentiality**

Case study of genes Core genes.

Accessory genes.

## Discussion

#### Materials and Methods

Clustering orthologous and paralogous genes. To study the essentiality of genes in 12 our strains, We needed to cluster sets of orthologous genes in these strain. Plenty of methods are proposed for this purpose. Altenhoff et al. have compared 15 of these methods [19] and shown that Hieranoid [20] is among three methods that keep a balance between precision and recall. We have used Hieranoid to cluster the sets of orthologous genes. In addition, we intended to study the essentiality of genes in paralogous genes. For this, we have developed a program that clusters all homologous genes using Jackhmmer from HMMER3 package [21].

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- Thomas J. Deerinck, Mark H. Ellisman, John Gill, Krishna Kannan, Bogumil J. Karas, Li Ma, James F. Pelletier, Zhi-Qing Qi, R. Alexander Richter, Elizabeth A. Strychalski, Lijie Sun, Yo Suzuki, Billyana Tsvetanova, Kim S. Wise, Hamilton O. Smith, John I. Glass, Chuck Merryman, Daniel G. Gibson, and J. Craig Venter. Design and synthesis of a minimal bacterial genome. 351(6280):aad6253.
- Tomoya Baba, Takeshi Ara, Miki Hasegawa, Yuki Takai, Yoshiko Okumura, Miki Baba, Kirill A Datsenko, Masaru Tomita, Barry L Wanner, and Hirotada Mori. Construction of escherichia coli k-12 in-frame, single-gene knockout mutants: the keio collection. 2-2006 0008
- H. Howard Xu, John D. Trawick, Robert J. Haselbeck, R. Allyn Forsyth, Robert T. Yamamoto, Rich Archer, Joe Patterson, Molly Allen, Jamie M. Froelich, Ian Taylor, Danny Nakaji, Randy Maile, G. C. Kedar, Marshall Pilcher, Vickie Brown-Driver, Melissa McCarthy, Amy Files, David Robbins, Paula King, Susan Sillaots, Cheryl Malone, Carlos S. Zamudio, Terry Roemer, Liangsu Wang, Philip J. Youngman, and Daniel Wall. Staphylococcus aureus TargetArray: comprehensive differential essential gene expression as a mechanistic tool to profile antibacterials. 54(9):3659–3670.
- Jeffrey D. Gawronski, Sandy M. S. Wong, Georgia Giannoukos, Doyle V. Ward, and Brian J. Akerley. Tracking insertion mutants within libraries by deep sequencing and a genome-wide screen for haemophilus genes required in the lung. 106(38):16422–16427.
- Tim van Opijnen, Kip L. Bodi, and Andrew Camilli. Tn-seq: high-throughput parallel sequencing for fitness and genetic interaction studies in microorganisms. 6(10):767– 772.
- Gemma C. Langridge, Minh-Duy Phan, Daniel J. Turner, Timothy T. Perkins, Leopold Parts, Jana Haase, Ian Charles, Duncan J. Maskell, Sarah E. Peters, Gordon Dougan, John Wain, Julian Parkhill, and A. Keith Turner. Simultaneous assay of every salmonella typhi gene using one million transposon mutants. 19(12):2308–2316.
- Beat Christen, Eduardo Abeliuk, John M Collier, Virginia S Kalogeraki, Ben Passarelli, John A Coller, Michael J Fero, Harley H McAdams, and Lucy Shapiro. The essential genome of a bacterium. 7:528.
- Andrew L. Goodman, Meng Wu, and Jeffrey I. Gordon. Identifying microbial fitness determinants by insertion sequencing using genome-wide transposon mutant libraries. 6(12):1969–1980.

- Kelly M. Wetmore, Morgan N. Price, Robert J. Waters, Jacob S. Lamson, Jennifer He, Cindi A. Hoover, Matthew J. Blow, James Bristow, Gareth Butland, Adam P. Arkin, and Adam Deutschbauer. Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons. 6(3):e00306-15.
- Benjamin E. Rubin, Kelly M. Wetmore, Morgan N. Price, Spencer Diamond, Ryan K. Shultzaberger, Laura C. Lowe, Genevieve Curtin, Adam P. Arkin, Adam Deutschbauer, and Susan S. Golden. The essential gene set of a photosynthetic organism. 112(48):E6634–E6643.
- Lars Barquist, Christine J. Boinett, and Amy K. Cain. Approaches to querying bacterial genomes with transposon-insertion sequencing. 10(7):1161–1169.
- Lars Barquist, Gemma C. Langridge, Daniel J. Turner, Minh-Duy Phan, A. Keith Turner, Alex Bateman, Julian Parkhill, John Wain, and Paul P. Gardner. A comparison of dense transposon insertion libraries in the salmonella serovars typhi and typhimurium. page gkt148.
- 18. Don J. Brenner and Noel R. Krieg. Bergey's Manual of Systematic Bacteriology: Volume Two: The Proteobacteria. Springer Science & Business Media.
- 19. Adrian M. Altenhoff, Brigitte Boeckmann, Salvador Capella-Gutierrez, Daniel A. Dalquen, Todd DeLuca, Kristoffer Forslund, Jaime Huerta-Cepas, Benjamin Linard, Ccile Pereira, Leszek P. Pryszcz, Fabian Schreiber, Alan Sousa da Silva, Damian Szklarczyk, Clment-Marie Train, Peer Bork, Odile Lecompte, Christian von Mering, Ioannis Xenarios, Kimmen Sjlander, Lars Juhl Jensen, Maria J. Martin, Matthieu Muffato, Quest for Orthologs Consortium, Toni Gabaldn, Suzanna E. Lewis, Paul D. Thomas, Erik Sonnhammer, and Christophe Dessimoz. Standardized benchmarking in the quest for orthologs. 13(5):425–430.
- Fabian Schreiber and Erik L. L. Sonnhammer. Hieranoid: hierarchical orthology inference. 425(11):2072–2081.
- Jaina Mistry, Robert D. Finn, Sean R. Eddy, Alex Bateman, and Marco Punta. Challenges in homology search: HMMER3 and convergent evolution of coiled-coil regions. 41(12):e121–e121.

