



Complete Genome Sequence of Escherichia coli BW25113

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Escherichia coli BW25113 is the parent strain of the Keio collection comprising nearly 4,000 single-gene deletion mutants. We report the complete 4,631,469-bp genome sequence of this strain and the key variations from the type strain *E. coli* MG1655.

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Escherichia coli BW25113 is a common laboratory strain that was created in the laboratory of Barry L. Wanner and was utilized in a method taking advantage of the bacteriophage lambda red recombination system to perform gene disruptions with double-stranded PCR products (1). *E. coli* BW25113 later became the parent strain for the Keio collection, a major resource consisting of approximately 4,000 single-gene deletion mutants (2, 3). The strain and its derivatives are being used in countless laboratories for a variety of studies, including systematic phenotypic surveys (4) and synthetic biology efforts (5–7). Despite this, the complete genome sequence of this strain surprisingly remained unavailable for the scientific community.

E. coli BW25113 was obtained from the Coli Genetic Stock Center (CGSC) (strain 7636). An Illumina library was prepared from sizeselected DNA fragments of approximately 450 to 550 bp and sequenced with paired-end reads of 300 bp on a MiSeq instrument to assemble longer composite reads covering the entire insert (8). All sequences were *de novo* and reference assembled using the Roche gsAssembler version 2.6. The assemblies were merged and manually inspected before manual finishing with Sanger sequencing reads obtained from PCR products. The resulting circular chromosome (of 4,631,469 bp) was annotated by comparison with E. coli MG1655 (RefSeq accession no. NC_000913.3) using RATT (9) and manual curation. The key differences between the two organisms were accounted for in the genotype of *E. coli* BW25113 [Δ (*araD-araB*)567 Δ (*rhaD-rhaB*)568 Δ *lacZ4787* (::rrnB-3) *hsdR514 rph-1*], with the deletion of araBAD and rhaDAB and the replacement of a section of lacZ with four tandem rrnB terminators as well as a frameshift mutation in hsdR resulting in a premature translation stop codon. As noted by others (3), we observed that the strain contains the $lacI^+$ allele and not lacI^q as initially reported (1, 10). The genome sequence also confirmed the presence of the *rph-1* allele and revealed 20 substitutions as well as 11 indels (see http://bioinfo.ccs.usherbrooke.ca/BW25113.html for a complete list).

Nucleotide sequence accession number. The complete genome sequence of *Escherichia coli* BW25113 was deposited in Gen-Bank under accession number CP009273.

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REFERENCES

- Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97:6640–6645. http://dx.doi.org/10.1073/pnas.120163297.
- Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2:2006.0008. http://dx.doi.org/10.1038/msb4100050.
- Yamamoto N, Nakahigashi K, Nakamichi T, Yoshino M, Takai Y, Touda Y, Furubayashi A, Kinjyo S, Dose H, Hasegawa M, Datsenko KA, Nakayashiki T, Tomita M, Wanner BL, Mori H. 2009. Update on the Keio collection of *Escherichia coli* single-gene deletion mutants. Mol. Syst. Biol. 5:335. http://dx.doi.org/10.1038/msb.2009.92.
- Nichols RJ, Sen S, Choo YJ, Beltrao P, Zietek M, Chaba R, Lee S, Kazmierczak KM, Lee KJ, Wong A, Shales M, Lovett S, Winkler ME, Krogan NJ, Typas A, Gross CA. 2011. Phenotypic landscape of a bacterial cell. Cell 144:143–156. http://dx.doi.org/10.1016/j.cell.2010.11.052.
- Mutalik VK, Guimaraes JC, Cambray G, Mai QA, Christoffersen MJ, Martin L, Yu A, Lam C, Rodriguez C, Bennett G, Keasling JD, Endy D, Arkin AP. 2013. Quantitative estimation of activity and quality for collections of functional genetic elements. Nat. Methods 10:347–353. http:// dx.doi.org/10.1038/nmeth.2403.
- Mutalik VK, Guimaraes JC, Cambray G, Lam C, Christoffersen MJ, Mai QA, Tran AB, Paull M, Keasling JD, Arkin AP, Endy D. 2013. Precise and reliable gene expression via standard transcription and translation initiation elements. Nat. Methods 10:354–360. http://dx.doi.org/10.1038/ nmeth.2404.
- Cambray G, Guimaraes JC, Mutalik VK, Lam C, Mai Q-A, Thimmaiah T, Carothers JM, Arkin AP, Endy D. 2013. Measurement and modeling of intrinsic transcription terminators. Nucleic Acids Res. 41:5139–5148. http://dx.doi.org/10.1093/nar/gkt163.
- Rodrigue S, Materna AC, Timberlake SC, Blackburn MC, Malmstrom RR, Alm EJ, Chisholm SW. 2010. Unlocking short read sequencing for metagenomics. PLoS One 5:e11840. http://dx.doi.org/10.1371/ journal-.pone.0011840.
- Otto TD, Dillon GP, Degrave WS, Berriman M. 2011. RATT: rapid annotation transfer tool. Nucleic Acids Res. 39:e57. http://dx.doi.org/ 10.1093/nar/gkq1268.
- Lessard IA, Pratt SD, McCafferty DG, Bussiere DE, Hutchins C, Wanner BL, Katz L, Walsh CT. 1998. Homologs of the vancomycin resistance D-Ala-D-Ala dipeptidase VanX in *Streptomyces toyocaensis, Escherichia coli* and *Synechocystis*: attributes of catalytic efficiency, stereoselectivity and regulation with implications for function. Chem. Biol. 5:489–504. http://dx.doi.org/10.1016/S1074-5521(98)90005-9.