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Complete Genome Sequence of the *Autographa californica* Multiple Nucleopolyhedrovirus Strain E2

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Many vectors that are commonly used in the baculovirus/insect cell system (BICS) are derived from the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) strain E2. To facilitate work with these vectors, we sequenced the E2 genome, compared it to that of the AcMNPV C6 strain, and found that they are very similar overall.

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The baculovirus/insect cell system (BICS) (1, 2) is used to produce relatively high levels of correctly folded recombinant proteins with eukaryotic posttranslational modifications for diverse applications (3–8). Baculoviral vectors used in the BICS are almost always derived from *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) strains C6 (vector including BacPAK/flashBAC, Bac-N-Blue, and BaculoGold) or E2 (vectors including BestBac, BaculoDirect [9], Bac-to-Bac [10], and MultiBac).

The AcMNPV C6 genome sequence was reported in 1994 (11), which provided valuable information for the production and screening of baculoviral vectors derived from this strain. In contrast, only partial sequences are available for AcMNPV E2. Thus, to facilitate the production and screening of baculoviral vectors derived from E2, we determined its complete genome sequence.

The E2 virus used for this purpose was derived from stocks originally acquired from the laboratory of Max D. Summers at Texas A&M University (12). The virus was amplified in Sf9 cells (13), viral genomic DNA was extracted from infected cells (14), and the DNA was subsequently sequenced using the Ion Torrent system (Invitrogen) by PrimBio. DNA sequences were assembled *de novo*, and the resulting contigs were assembled manually. Gaps were closed by Sanger sequencing of PCR amplimers, and the sequences of the larger homologous region (HR) repeat elements and questionable regions were confirmed by Sanger sequencing of PCR amplimers, some of which were cloned. Finally, the complete genome sequence was manually curated and annotated.

Overall, the AcMNPV E2 genome was colinear with and very similar to that of the C6 reference sequence (accession no. NC_001623.1), with the same genes and genetic elements distributed in the same order across a slightly larger genome (133,966 versus 133,894 bp). The difference in genome size was largely due to an additional repeat in the E2 homologous region 2 (HR2) element (15), which also represented the largest individual difference between the genomes. A comparison of the C6 and E2 HR2 elements also revealed further differences, including several insertions, deletions, and single nucleotide polymorphisms (SNPs). Another region of substantial genetic variation between E2 and C6 was found to be the HR4b element. These results are consistent with the previously reported observation that HRs are hot spots of genetic variability among baculovirus strains (16).

In addition to the differences noted above, we found several dozen SNPs and other changes between the E2 and C6 open reading frames (ORFs). In cases in which the E2 gene product differed from the C6 gene product, the E2 gene product was generally identical to homologues from other closely related baculoviruses, such as *Plutella xylostella* MNPV (17) or *Rachiplusia ou* MNPV (18). Furthermore, there were several instances in which the E2 sequence did not match the C6 whole-genome reference sequence but matched other partial C6 sequences in GenBank, indicating that the C6 reference sequence might be erroneous at those positions. This is in agreement with a report that the C6 reference sequence is incorrect in several regions, as determined by resequencing (19).

Nucleotide sequence accession number. The complete genome sequence of AcMNPV E2 was deposited with GenBank under accession no. KM667940.

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REFERENCES

1. **Jarvis DL.** 2009. Baculovirus-insect cell expression systems. Methods Enzymol. **463:**191–222. http://dx.doi.org/10.1016/S0076-6879(09)63014-7.
2. **Geisler C, Jarvis DL.** 2009. Insect cell glycosylation patterns in the context of biopharmaceuticals, p 165–191. *In* Walsh G (ed), Post-translational modifications in the context of biopharmaceuticals, 1st ed. Wiley-VCH Verlag, Weinheim, Germany. http://dx.doi.org/10.1002/9783527626601.ch7.
3. **Gillette WK, Esposito D, Taylor TE, Hopkins RF, Bagni RK, Hartley JL.** 2011. Purify first: rapid expression and purification of proteins from

XMRV. Protein Expr. Purif. **76:**238–247. http://dx.doi.org/10.1016/j.pep.2010.12.003.

4. **Savitsky P, Bray J, Cooper CDO, Marsden BD, Mahajan P, Burgess-Brown NA, Gileadi O.** 2010. High-throughput production of human proteins for crystallization: the SGC experience. J. Struct. Biol. **172:**3–13. http://dx.doi.org/10.1016/j.jsb.2010.06.008.
5. **Shrestha B, Smee C, Gileadi O.** 2008. Baculovirus expression vector system: an emerging host for high-throughput eukaryotic protein expression. Methods Mol. Biol. **439:**269–289. http://dx.doi.org/10.1007/978-1-59745-188-8_19.
6. **Pushko P, Tumpey TM, Bu F, Knell J, Robinson R, Smith G.** 2005. Influenza virus-like particles comprised of the HA, NA, and M1 proteins of H9N2 influenza virus induce protective immune responses in BALB/c mice. Vaccine **23:**5751–5759. http://dx.doi.org/10.1016/j.vaccine.2005.07.098.
7. **Harro CD, Pang Y-YS, Roden RBS, Hildesheim A, Wang Z, Reynolds MJ, Mast TC, Robinson R, Murphy BR, Karron RA, Dillner J, Schiller JT, Lowy DR.** 2001. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. J. Natl. Cancer Inst. **93:**284–292. http://dx.doi.org/10.1093/jnci/93.4.284.
8. **Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF, Study, IMPACT Investigators.** 2010. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N. Engl. J. Med. **363:**411–422. http://dx.doi.org/10.1056/NEJMoa1001294.
9. **Bennett R, Welch P, Harwood S, Madden K, Frimpong K, Franke K.** Nov 2004. Expression vector comprising multiple recombination and/or topoisomerase sites for use as gene transfer tool in prokaryotic and eukaryotic cells. US patent US20040219516.
10. **Luckow VA, Lee SC, Barry GF, Olins PO.** 1993. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in *Escherichia coli*. J. Virol. **67:**4566–4579.
11. **Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD.** 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology **202:**586–605. http://dx.doi.org/10.1006/viro.1994.1380.
12. **Smith GE, Summers MD.** 1978. Analysis of baculovirus genomes with restriction endonucleases. Virology **89:**517–527. http://dx.doi.org/10.1016/0042-6822(78)90193-9.
13. **Summers MD, Smith GE.** 1987. A manual of methods for baculovirus vectors and insect cell culture procedures 1555. Texas Agricultural Experiment Station, College Station, TX.
14. **Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A.** 1991. Simplified mammalian DNA isolation procedure. Nucleic Acids Res. **19:**4293. http://dx.doi.org/10.1093/nar/19.15.4293.
15. **Cochran MA, Faulkner P.** 1983. Location of homologous DNA sequences interspersed at five regions in the baculovirus AcMNPV genome. J. Virol. **45:**961–970.
16. **Erlandson M.** 2009. Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. Virol. Sin. **24:**458–469. http://dx.doi.org/10.1007/s12250-009-3052-1.
17. **Harrison RL, Lynn DE.** 2007. Genomic sequence analysis of a nucleopolyhedrovirus isolated from the diamondback moth, *Plutella xylostella*. Virus Genes **35:**857–873. http://dx.doi.org/10.1007/s11262-007-0136-6.
18. **Harrison RL, Bonning BC.** 2003. Comparative analysis of the genomes of *Rachiplusia* ou and *Autographa californica* multiple nucleopolyhedroviruses. J. Gen. Virol. **84:**1827–1842. http://dx.doi.org/10.1099/vir.0.19146-0.
19. **Rohrmann GF.** 2013. The AcMNPV genome: gene content, conservation, and function. *In* Baculovirus molecular biology, 3rd ed. National Center for Biotechnology Information, Bethesda, MD.