

Complete Genome Sequence of a Recombinant Marek's Disease Virus Field Strain with One Reticuloendotheliosis Virus Long Terminal Repeat Insert

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Marek's disease virus (MDV) Chinese strain GX0101, isolated in 2001 from a vaccinated flock of layer chickens with severe tumors, was the first reported recombinant MDV field strain with one reticuloendotheliosis virus (REV) long terminal repeat (LTR) insert. GX0101 belongs to very virulent MDV (vvMDV) but has higher horizontal transmission ability than the vvMDV strain Md5. The complete genome sequence of GX0101 is 178,101 nucleotides (nt) and contains only one REV-LTR insert at a site 267 nt upstream of the *sorf2* gene. Moreover, GX0101 has 5 repeats of a 217-nt fragment in its terminal repeat short (TRS) region and 3 repeats in internal repeat short (IRS) region, compared to the other 10 strains with only 1 or 2 repeats in both TRS and IRS.

Marek's disease virus (MDV) is a member of the *Alphaherpesvirinae* subfamily, and its genome contains a linear double-strand DNA of about 175 kb. MDV causes Marek's disease in chickens with lymphomas (14). So far, the genome sequences of a total of 10 MDV strains have been analyzed (4, 6, 7, 8, 9, 10, 13, 16). The GX0101 strain in this report was the first recombinant MDV field strain containing the reticuloendotheliosis virus (REV) long terminal repeat (LTR) insert (17), and it is a very virulent MDV with higher horizontal transmission ability (1, 12). However, other recombinant MDV strains with the REV LTR, such as RM1, obtained in cell cultures were attenuated and did not cause tumors (2, 3, 15).

On the basis of the purified plasmid DNA of GX0101 bacterial artificial chromosome (BAC) clone (11), the complete genome of GX0101 was sequenced and analyzed with Prism 3730 sequencer (Applied Biosystems) and SeqMan software (DNASTAR Inc.). The whole-genome sequence of GX0101 consists of 178,101 nucleotides (nt), and the terminal repeat long (TRL), unique long (UL), internal repeat long (IRL), internal repeat short (IRS), unique short (US), and terminal repeat short (TRS) regions with the lengths of 12,758, 113,572, 12,741, 12,700, 11,695, and 13,134 nt, respectively. It has about 200 ORFs and 2 copies of 132-nt repeats.

The GX0101 genome contains only one REV LTR insert of 538 nt located at a site 267 nt upstream of the *sorf2* gene. In RM1, the REV LTR insertion site was further upstream of both *sorf1* and *sorf2*. In addition, RM1 obtained another REV LTR in its TRS during passage in chickens, and the REV LTR insert was not stable during passage in chickens when it was inserted into Md5 at the same site (5). In contrast, GX0101 maintained its REV LTR insert even after 20 passages in chickens.

There are 5 consecutive repeats of a 217-nt fragment in 97.3–97.6 ORF of the TRS region and 3 repeats in 86.2–86.4 ORF of the IRS region of GX0101, but only 1 or 2 copies in both TRS and IRS regions of 10 other strains.

Compared to Md5, RB1B, CU2, 584A, 648A, and CVI988/Rispens strains, GX0101 had a 486-nt deletion in its US region (corresponding to bases 164033 to 164518 of Md5) (4, 9, 16). GX0101 has the highest identity to two BAC clones PC12130-10

and PC12130-15 of United Kingdom strain C12130. Among the mutated 77 ORFs, GX0101 has 50 and 47 ORFs 100% identical to PC12130-10 and PC12130-15 but only 7 to 27 ORFs 100% identical to other strains. PC12130-10 was virulent and PC12130-15 was attenuated, although they were derived from the same strain (9).

Considering its pathogenicity, horizontal transmission ability, genomic structure, and especially its stable REV insert, analysis of the complete sequence of GX0101 will be useful in both studies of gene functions related to pathogenicity or transmission ability and understanding genomic mutations or evolutionary relationships of MDVs in different geographical areas in the world.

Nucleotide sequence accession number. The complete genome sequence of GX0101 was deposited in GenBank under accession number [JX844666](https://www.ncbi.nlm.nih.gov/nuccore/JX844666).

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (31072149) and Guangdong Joint Funds from the National Natural Science Foundation of China (U1131005).

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Received 21 September 2012 Accepted 21 September 2012

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doi:10.1128/JVI.02583-12

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