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GENOME SEQUENCES

Complete Genome Sequence of Feline Leukemia Virus Kawakami-Theilen Strain KT-FeLV-UCD-1

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ABSTRACT This full-length genome sequence of a feline leukemia virus Kawakami-Theilen strain (designated KT-FeLV-UCD-1), produced from the chronically infected FL74-UCD-1 cell line, was obtained using high-throughput sequencing. It consisted of 8,464 bp and had a genomic organization similar to that of other gammaretroviruses, containing long terminal repeats and open reading frames for Gag, Pol, and Env.

Feline leukemia virus (FeLV) infections are widespread in cats due to horizontal species-to-species transmission (1–3) and can remain chronic or result in a variety of disorders (4). Although humans have close contact with cats, cross-species infections have not been reported (5). An FeLV vaccine for cats was developed using the FL74 feline lymphoblastoid cell line (6), originally derived by long-term laboratory culture from the Kawakami-Theilen (KT) cell line (7, 8). These cells are chronically infected with FeLV strain KT, which has attenuated virulence for cats (9). The cell line was designated FL74-UCD-1 (6), and the virus was designated KT-FeLV-UCD-1.

A high-titer virus stock of KT-FeLV-UCD-1 was prepared by harvesting and concentrating supernatant from expansion of FL74-UCD-1 cells (catalogue number CRL-8012; American Type Culture Collection, Manassas, VA). Nucleic acid was extracted from 200 μl of the virus stock using the QIAamp MinElute virus spin kit and eluted in 60 μl RNase-free water. cDNA was obtained using random primers and Superscript II enzyme (Invitrogen) to obtain cDNA, followed by purification using magnetic beads (AMPureXP beads; Beckman Coulter). Double-strand cDNA was quantified with a Qubit 3.0 fluorometer (Life Technologies) using a Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Thermo Fisher Scientific). The library was obtained using the Nextera XT DNA library preparation kit (Illumina) using 5 ng as the starting material quantity. Library quality control was checked using an Agilent 2100 bioanalyzer instrument to calculate library average size, while quantification was verified using a Qubit 3.0 fluorometer. Sequencing was performed using the HiSeq 2500 platform (Illumina) with 101-bp paired-end mode.

Read trimming and assembly were done using the CLC BIO Genomics Workbench software version 11.0.1 (Qiagen Aarhus A/S, Denmark). The total number of trimmed, paired-end reads was 505,153,300, and the average read length was 88 bp. The 8,464-bp complete viral genome sequence was obtained by mapping the raw reads (using the default parameters) to the FeLV Rickard strain (GenBank accession number NC_001940.1) as a reference genome, and the consensus sequence was extracted using default parameters. Open reading frames were identified using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/) (10). The genomic structure was similar to those of other retroviruses encoding for Gag, Pol, and Env, with 5′ and 3′ long terminal repeats (LTRs). Nucleotide sequence analysis using NCBI BLASTN indicated

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94% sequence identity with the FeLV Rickard strain. Comparative nucleotide sequence analysis indicated that the most remarkable difference was an insertion of 9 nucleotides resulting in an in-frame addition of 3 amino acids in the N-terminal leader region of the glycoGag protein (gPr80) in KT-FeLV-UCD-1 (11). Further analysis of Gag using a CLC BIO Genomics Workbench version 11.0.1 antigenicity plot (12) indicated a potential reduction in the antigenicity of N-terminus Gag in KT-FeLV-UCD-1 compared with that in the FeLV Rickard strain. The availability of the full genomic sequence of KT-FeLV-UCD-1 may help identify the attenuation determinants in this strain versus the pathogenic FeLV Rickard strain.

Data availability. The complete genome sequence of KT-FeLV-UCD-1 has been deposited in NCBI/GenBank under the accession number MT129531. The raw data were deposited under SRA accession number SRR11242952, BioSample number SAMN14273276, and BioProject number PRJNA610048.

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