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< Short Communication >

Whole genome sequencing of foot-and-mouth disease virus using benchtop next generation sequencing (NGS) system

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Abstract

In countries with FMD vaccination, as in Korea, typical clinical signs do not appear, and even in FMD positive cases, it is difficult to isolate the FMDV or obtain whole genome sequence. To overcome this problem, more rapid and simple NGS system is required to control FMD in Korea. FMDV (O/Boeun/SKR/2017) RNA was extracted and sequenced using Ion Torrent's bench-top sequencer with amplicon panel with optimized bioinformatics pipelines. The whole genome sequencing of raw data generated data of 1,839,864 (mean read length 283 bp) reads comprising a total of 521,641,058 (≥Q20 475,327,721). Compared with FMDV (GenBank accession No. MG983730), the FMDV sequences in this study showed 99.83% nucleotide identity. Further study is needed to identify these differences. In this study, fast and robust methods for benchtop next generation sequencing (NGS) system was developed for analysis of Foot-and-mouth disease virus (FMDV) whole genome sequences.

Key words : FMDV, Next generation sequencing, NGS, Whole genome sequencing

INTRODUCTION

Foot-and-mouth disease virus (FMDV) belongs to the Aphthovirus genus of the Picornaviridae family and causes a highly devastating vesicular disease in cloven-hoofed animal species (Alexandersen et al, 2003). FMDV is divided into seven immunologically distinct serotypes, A, O, C, Asia 1, and South African Territories (SATs) 1, 2, and 3. FMDV type O is the pandemic serotype and is grouped into eight topotypes: Cathay, Middle East-South Asia (ME-SA), Southeast Asia (SEA), Europe-South America (Euro-SA), Indonesia-1 and -2 (ISA-1 and -2, respectively), East Africa (EA), and West Africa (WA), based on 15% nucleotide differences (Knowles and Samuel, 2003). The viral genome is about 8.3 kb long and enclosed in a protein capsid. The capsid comprised 60 copies each of the four structural proteins (VP1-VP4); the VP1-3 proteins are located on the surface, while VP4 is internal (Brito et al, 2017).

Until now, FMDV genome sequencing has been performed by analyzing a part of a gene of conventional sequencing methods (Lee et al, 2011) but recently applying bench-top NGS to enable whole genome (Moon et al, 2016). Moreover, since October 2018, Korea has established bivalent (O and A serotype) vaccination strategies to control FMDV. After vaccination, there are some cases have rapid kit and PCR positive but isolation and sequencing is negative. Therefore, fast and robust methods for bench-top next generation sequencing (NGS) system were needed (Rothberg et al, 2011) (Fig. 1). The purpose of this study is to develop simple and rapid sequencing system for FMDV isolated from Korea (O/Boeun/SKR/

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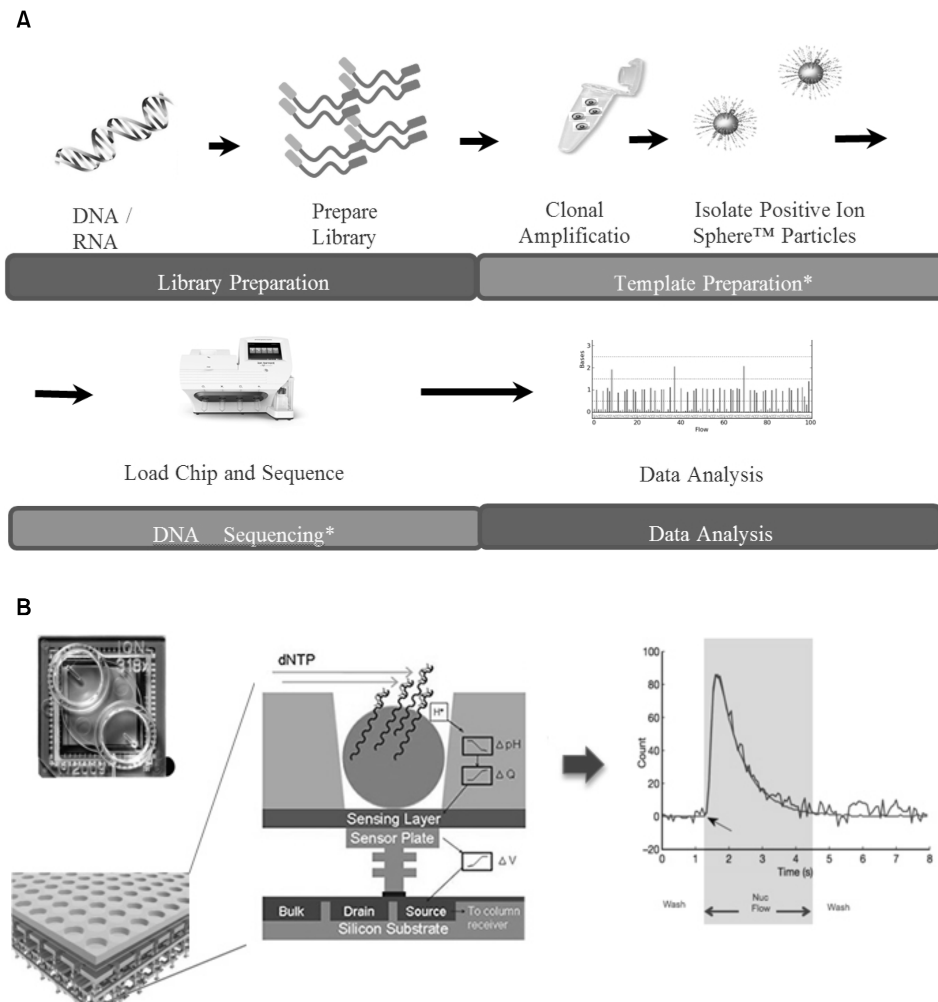

Fig. 1. (A) Workflow of Ion torrent PGM system; (B) Sensor, well and chip architecture. Ions get released when DNA bases are matched. Sequence output is generated by the detection system (Rothberg et al, 2011).

2017) using the benchtop NGS system.

MATERIALS AND METHODS

FMDV isolate (O/Boeun/SKR/2017) was used in this study. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, USA) as per the manufacturer's instructions. Libraries were generated using the Ion Xpress Plus fragment library kits (Thermo Fisher Scientific, USA) and the Ion Xpress Barcode Adapters kits (Thermo Fisher Scientific, USA) with adaptors 1～16 as described previously (Daum et al, 2011). Chemical shearing was performed using 50 ng cDNA. Briefly, DNA shearing was performed in a 50 ul total reaction volume by combining 5 ul Ion Shear Plus 10x reaction buffer, 10 ul enzyme, and 35 ul cDNA template. The reaction mixture was incubated at 37°C for 8 min in hot block, the reaction was terminated using 5 ul Ion Shear stop buffer. Sheared DNA was purified using Ampure XP beads (Beckman Coulter, USA) with a Dynal magnetic bead stand (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. Following sheared cDNA, barcodes were ligated to the amplicons using the Ion Xpress Barcode Adaptors Kit (Thermo Fisher Scientific, USA). Amplification was performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, CA, USA), the program consisting of an initial denaturation of 5 min at 95°C was, 15s of denaturation at 95°C, 60s of annealing at 58°C and 1 min of extension at 70°C for 8 cycles. Following amplification, libraries were purified and eluted in 25 ul low TE buffer using the MinElute reaction cleanup kit (QIAGEN, USA) according to the manufacturer's instructions. The concentration and purity of li-

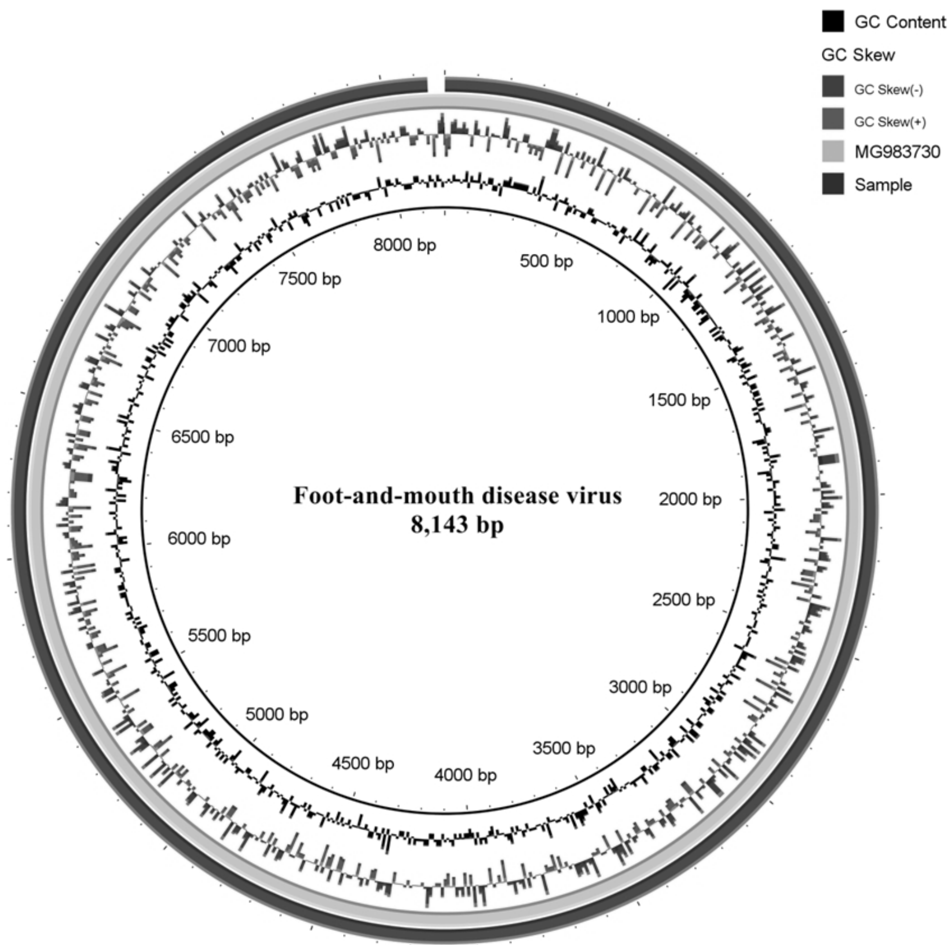

Fig. 2. The map represents the circular view of the genome sequence of Foot-and-mouth disease (FMD) virus. The circle was made by BRIG software version 0.95. The chromosome outermost circular ring shows FMD virus, followed by the second ring from reference strain (GenBank accession No. MG983730). The third circular ring towards the center represents the GC skew information in the (+) strand (green) and (−) strand (dark pink).

braries were determined using the Qubit dsDNA HS Assay Kit (Invitrogen, USA) and the Qubit 2.0 Fluorometer (Invitrogen, USA). Size selection of the libraries were selected using E-gel® iBase (Invitrogen, USA) and E-Gel® Size Select 2% Agarose Gel (Invitrogen, USA). The size selected libraries were purified with Ampure XP beads (Beckman Coulter, USA) according to the manufacturer's instructions. Libraries quantification was then performed using the Ion Library Quantitation Kit (Thermo Fisher Scientific, USA).

The final amplification of the fragment libraries was performed on the Ion One Touch 2 Instrument (Thermo Fisher Scientific, USA) with reaction mixture was consisted diluted libraries, Ion PGM Hi-Q view Enzyme Mix, Ion Sphere Particles (ISPs) and Ion PGM Hi-Q view Reagent Mix. The template positive ISPs were purified using by Ion One Touch ES instrument (Thermo Fisher Scientific, USA) with Dynabeads My One Streptavidin C1 beads (Thermo Fisher Scientific, USA). Enriched ISPs were sequenced, using the Ion PGM Hi-Q view Sequencing Kit (Thermo Fisher Scientific, USA), with Ion 316 chip, on the Ion Torrent PGM platform (Thermo Fisher Scientific, USA) according to the manufacturer's recommendation.

Raw sequence data were processed the Torrent Suite software version 5.0.2. The raw sequence data were assembled with CLC Genomics Workbench version 8.5.1 (CLC Bio, Qiagen, USA) using Foot-and-mouth disease virus (O type, GenBank accession No. MG983730). The assembly visualization was performed using BLAST Ring Image Generator (BRIG) software which allowed comparative genome analysis (Alikhan et al, 2011).

RESULTS AND DISCUSSION

Ion Torrent PGM sequencing machine provided large numbers of short read sequences that were assembled

and aligned in order to determine a consensus sequence. The whole genome sequencing of raw data generated data of 1,839,864 (mean read length 283 bp) reads comprising a total of 521,641,058 (≥Q20 475,327,721). The quality of FastQ files were checked using FastQC. CLC Genomics Workbench version 8.5.1 (CLC Bio, Qiagen, USA) was used to perform assembly on the raw data. Mapping the reads with the published genome of FMDV (GenBank accession No. MG983730, sequenced by Illumina Miseq system) as reference, using 41,026 reads created total genome length 8,140 bp. The circular genome map showing consensus sequences and corresponding to reference strain FMDV (GenBank Accession No. MG983730) is shown in Fig. 2. Compared with FMDV (GenBank accession No. MG983730), the FMDV sequences in this study showed 99.83% nucleotide identity. However, the comparative analysis between MG983730 and sequenced FMDV reveals deletion and mutation in the FMDV. Further study is needed to identify these differences.

In countries with FMD vaccination, as in Korea, typical clinical signs do not appear, and even in FMD positive cases, it is difficult to isolate the FMDV or obtain whole genome sequence. To overcome this problem, more rapid and simple NGS system is required to control FMD in Korea.

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