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Generation of Full-Length Infectious cDNA Clones of Middle East Respiratory Syndrome Coronavirus

Jeong Yoon Lee, Sojung Bae, and Jinjong Myoung*

Korea Zoonosis Research Institute and Genetic Engineering Research Institute, Chonbuk National University, Jeonju 561-756, Republic of Korea

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**Corresponding author*
Phone: +82-63-900-4055;
Fax: +82-63-900-4012;
E-mail: Jinjong.myoung@jbnu.ac.kr

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Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012 and related infection cases have been reported in over 20 countries. Roughly 10,000 human cases have so far been reported in total with fatality rates at up to 40%. The majority of cases have occurred in Saudi Arabia with mostly sporadic outbreaks outside the country except for the one in South Korea in 2015. The Korean MERS-CoV strain was isolated from the second Korean patient and its genome was fully sequenced and deposited. To develop virus-specific protective and therapeutic agents against the Korean isolate and to investigate molecular determinants of virus-host interactions, it is of paramount importance to generate its full-length cDNA. Here we report that two full-length cDNAs from a Korean patient-isolated MERS-CoV strain were generated by a combination of conventional cloning techniques and efficient Gibson assembly reactions. The full-length cDNAs were validated by restriction analysis and their sequence was verified by Sanger method. The resulting cDNA was efficiently transcribed in vitro and the T7 promoter-driven expression was robust. The resulting reverse genetic system will add to the published list of MERS-CoV cDNAs and facilitate the development of Korean isolate-specific antiviral measures.

Keywords: MERS-CoV, full-length cDNA, reverse genetics

Introduction

Middle East respiratory syndrome (MERS) is also known as camel flu. The causative agent, termed MERS coronavirus (MERS-CoV), is prevalent in dromedary camels [1, 2], especially young camels which are a major reservoir for the virus. Infection with this virus in humans was first reported in Saudi Arabia in 2012 (HCoV-EMC) [3], and since then it has remained a fatal zoonotic pathogen from camels. Humans seem to be infected through direct or indirect contact with affected dromedary, but not Bactrian, camels [2, 4, 5]. Thus, MERS primarily occurs in the regions where dromedary camels reside (Africa and the Arabian Peninsula) with one exception in 2015, a large outbreak which occurred outside the Arabian Peninsula: South Korea (186 confirmed cases of infection, 38 deaths [6]). A total of 2,298 human infection cases have been reported with 811 deaths and a fatality rate of roughly 35% [7–9]. However, the mortality rate may be overestimated as individuals with minor symptoms tend to go undiagnosed especially in under-developed countries [7, 8].

The MERS-CoV outbreak in South Korea is worth noting [6, 8]. The first male patient, who had had a business trip to several MERS-CoV endemic countries, was confirmed MERS-CoV-positive on May 20th, 2015. Since then, a number of mortality and morbidity cases caused by MERS-CoV transmission, as well as 16,752 pre-emptive quarantines, ensued [6]. The situation was exacerbated by a number of factors: the South Korean government's initial failure to manage the crisis, extensive viral transmission by super-spreaders, inadequate restriction of hospital visitors, and a lack of sufficient infectious disease management capacity in hospitals, etc [7, 10, 11]. It is interesting to note that the Korean MERS-CoV isolate did not seem to be sufficiently different when compared to viral isolates already reported [9] although, albeit low, some sequence variations were

indeed present. Therefore, it appears reasonable to postulate that the super-spreading events, observed during the outbreak in South Korea, would be rather attributable to environmental factors than the host or virus-related biological characteristics [9, 12].

MERS-CoV belongs to the genus betacoronavirus and is thought to be derived from bats. Its genome is ~32 kb long and single-stranded, positive-sense RNA. The MERS-CoV genome encodes 16 non-structural proteins in two open reading frames (ORF1a and ORF1b), which translates to two polypeptides: pp1a and pp1ab, respectively. Nine structural and accessory proteins, including ORF8b, are encoded at the 3′ end of the genome. Two full-length cDNAs were generated in 2013 using reverse genetic systems using the sequence of HCoV-EMC [13, 14]. These cDNAs were used successfully to generate GFP- or RFP-expressing recombinant MERS-CoVs. Here we report that a full-length cDNA clone of a Korean MERS-CoV isolate (NCBI accession number: KT029139.1) was generated by conventional cloning and Gibson assembly techniques with minimal modifications in the viral genome. At the 5′ end of the genome, two different T7 promoters were placed: strong (TAAT….TAGGG) and minimal (TAAT…TA). The T7 promoter is robust and strong, allowing efficient transcription of the downstream gene when the promoter sequence ends with 3 G's. However, as the transcription starts at the G, which is the third from the end (guanine underlined above), if the strong T7 promoter is used, then the resulting MERS-CoV RNA genome will have 3 extra G's at the 5′ end. Extra sequences at the 5′ end may seriously and adversely affect viral replication. To solve this potential problem and to generate an exact sequence of the MERS-CoV genome, we generated a full-length cDNA with the minimal T7 promoter as well. As the first base of the MERS-CoV genome is guanine (***G***ATTTAAG), the minimal T7 promoter sequence added at the 5′ end is TAAT…TA, thus transcribed RNA will have the exact sequence of the viral genome (***G***ATTTAAG), which would provide a great advantage in investigating mechanisms of MERS-CoV replication and its interaction with the host cell. In addition, development of a full-length cDNA of the Korean isolate will pave the way to understanding potential molecular roles that the virus played in the super-spreading event during the outbreak in Korea. Futhermore, by taking advantage of the reverse genetic system, effective preventive and therapeutic agents against the Korean isolate as well as similar MERS-CoV strains with pandemic potential could be rationally designed.

Materials and Methods

Competent Cells and Reagents

MAX Efficiency DH5α Competent Cells (Thermo Fisher Scientific, Waltham, USA) and NEB10-beta Competent *E. coli* (New England Biolabs, Ipswich, USA) were used for the maintenance of pUC57 and pCC1BAC vectors, respectively. T4 DNA ligase, Gibson Assembly Master Mix, restriction enzymes such as SfiI, AsiAI, BamHI-HF, StuI, MluI-HF, PacI and EagI-HF, and Quick-Load 1 kb Extended DNA Ladder were purchased from NEB. FspAI was purchased from Thermo Fisher Scientific. SalI was procured from Enzynomics (Korea). Ampicillin and kanamycine were obtained from Duchefa Biochemie (The Netherlands), and chloramphenicol was purchased from Calbiochem (Germany).

Bacteria Culture and DNA Isolation.

Antibiotics including 100 μg/ml of ampicillin, 50 μg/ml of kanamycine and 25 μg/ml of chloramphenicol were added to make LB plates or LB broth media. After culturing competent cells in 4 ml of selective media overnight, plasmid DNA was isolated using FavorPrep Plasmid DNA Extraction Mini Kit (Favorgen, Taiwan) or Dokdo-Prep Plasmid Mini-prep Kit (Elpisbio, Korea). Also, NucleoBond Xtra Midi Kit (Macherey-Nagel, Germany) was used to produce a large amount of plasmids [15–17].

Synthesis of MERS-CoV Fragments

The sequence of wild-type Korean isolate (GenBank assession: KT029139.1) of MERS-CoV (Fig. 1A) was used for fragment synthesis. The use of MERS-CoV genomic sequence was approved by the Korean Centers for Disease Control (Approval No. 16-RDM-019). The six fragments for MERS-CoV assembly were synthesized by GenScript (USA) with a few modifications: generation of MluI site, elimination of one FspAI site and T7 polymerase transcription termination site (C11267T and A2849G, respectively). The genome of MERS-CoV Korean isolate was divided into 6 fragments: M06 (1-806 (BamHI) and 25,840 (PacI) – 32000), M1 (806 (BamHI) – 7620 (Stu I)), M2 (7620 (Stu I) – 9072 (Stu I)), M3 (9072 (Stu I) – 13222 (FspAI)), M4 (13222 (FspAI) to 19196 (MluI)), M5 (19196 (MluI) – 25840 (PacI)). Poly(A) site (pA), ribozyme (Rz) and T7 terminator sequences were placed at the 3′UTR region. A plasmid named pUC57-Simple-T7p-MERS06 (pUC57S-T7p-M06) includes restriction enzyme sites for full assembly of the MERS-CoV genome. The fragments for M1, M2 were synthesized and cloned in the pUC57S vector, and the fragments for M5 were synthesized into a pUC57-Kan vector (pUC57K). M3 and M4 were unstable in pUC57 vectors. Thus, these fragments were synthesized and cloned into pCC1BAC bacmid. Each vector also includes specific enzyme sites for further cloning.

Assembly of MERS-CoV Fragments

pCC1BAC vector was cut with SalI and religated to simplify the vector (pCC1-SalI). pCC1-SalI vector was mutagenized using

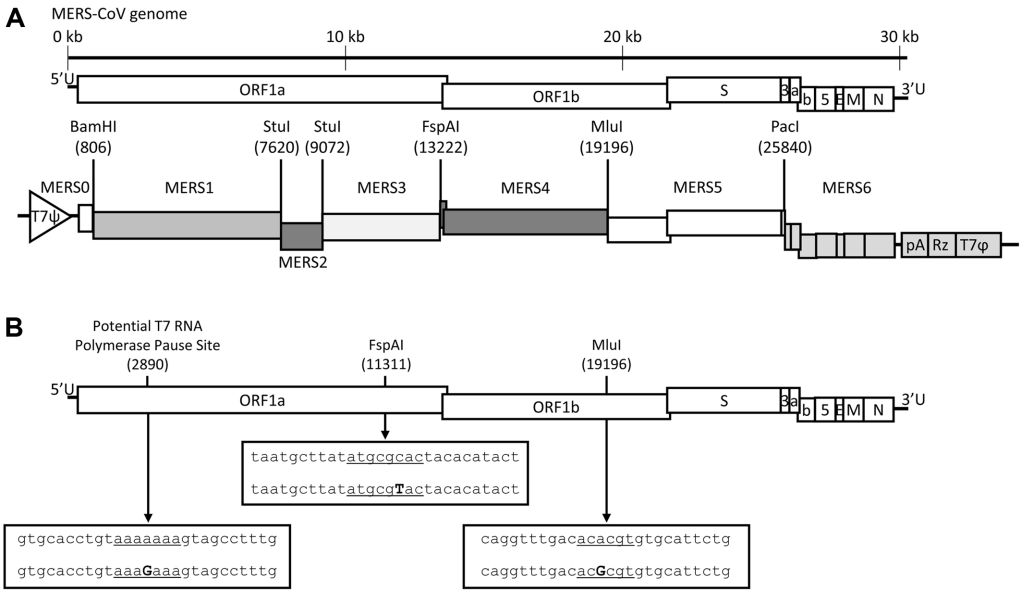

Fig. 1. Schematic of MERS-CoV genome and assembly strategy.
(**A**) MERS-CoV genome was divided into 6 fragments and synthesized. T7ψ: T7 promoter, pA: Poly(A), Rz: Ribozyme terminator, T7ϕ: T7 RNA polymerase terminator. (**B**) Nucleotide changes in the MERS-CoV genome for assembly. The underlined sections indicate the target sequences and bold letters show mutations.

primer sets (Table 1), synthesized by Macrogen, and QuickChange Site-Directed Mutagenesis Kit (Agilent) to eliminate StuI and FspAI on the vector (pCC1-SalI-SF). Conventional cloning was performed as previously described [18–22]. M06 fragment on pUC57S vector was restricted by SalI and subcloned into the linearized pCC1-SalI-SF vector by SalI using T4 DNA ligase (NEB) (pCC1-SalI-SF-T7p-M06). The M1 fragment was assembled to pUC57K-M5 vector using BamHI and PacI sites (pUC57K-M15). The M15 fragment was cleaved by BamHI and PacI and ligated into pCC1-SalI-SF-T7p-M06 vector (pCC1-SalI-SF-T7p-M0156). For Gibson assembly, the Gibson primers were designed with NEBuilder Assembly Tool v2.2.3 (nebuilder.neb.com/#!/) and synthesized at Macrogen (for primers, Table 2) . M2, M3 and M4 fragments were amplified using KOD-Neo-Plus pfu polymerase (KOD-401; Toyobo, Osaka, Japan) under the following condition: 98°C for 10 sec, 62°C for 30 sec, 68°C for 1 min for M2, 2 min for M3, and 3 min for M4. The PCR products (Fig. 1A) were purified using DokDo-Prep PCR Purification Kit (Elpisbio). M2, M3 and M4 fragments were cloned into pCC1-SalI-SF-T7p-M0156 and then linearized by StuI and MluI, using Gibson Assembly Master Mix following the manufacturer's instructions. Briefly, 10 µl of 2X Gibson Assembly Master Mix and M2, M3 and M4 fragments (1:1:1 ratio) were mixed with 100 ng of the linearized pCC1-SalI-SF-T7p-M0156 vector. After the mixture was incubated at 50°C for 1 h, 2 µl out of 20 µl were used for further transformation in 50 µl of NEB10-beta competent cells. Transformed competent cells were incubated on a LB/Chloramphenicol (25 µg/ml) selection plate at 32°C up to 2 days. Positive colonies were selected by conventional PCR targeting each junction of the inserted fragments. A full-length infectious cDNA clone of MERS-CoV, named pCC1-SalI-SF-T7p-MF, was screened out, the sequence integrity of which was validated by Sanger sequencing at Macrogen (Korea). To delete GGG nucleotides (dGGG) on T7 promoter and make pCC1-SalI-SF-T7p-dGGG-MF plasmid, mutagenesis was performed on pUC57S-T7p-M06 vector using mutagenesis primers (Table 1) and Q5 Hot Start High-Fidelity DNA polymerase under the following

Table 1. Primers for mutagenesis used in this study.

Name	Sequences
pCC1-StuI-Mutagenesis-F	CTTTTTTAGAGCGCCTTGTAAGCTTTTATCCATGCTGGTTCTAG
pCC1-StuI-Mutagenesis-R	CTAGAACCAGCATGGATAAAAGCTTACAAGGCGCTCTAAAAAAG
pCC1-FspAI-Mutagenesis-F	TTGGTTTATCAAACGTGTGCCCAGTCCATCCAGAGGGC
pCC1-FspAI-Mutagenesis-R	GCCCTCTGGATGGACTGGGCACACGTTTGATAAACCAA
T7p-dGGG-F	GATCGCTAATACGACTCACTATAGATTTAAGTGAATAGCTTGGC
T7p-dGGG-R	GCCAAGCTATTCACTTAAATCTATAGTGAGTCGTATTAGCGATC

Table 2. Primers for PCR amplification for Gibson Assembly.

Name	Sequences
Gibson-0156-M2-F	TGACCTCACTACCGCCCTACGCAGGCCTATTAACGCTACGGATAG
Gibson-0156-M2-R	AACGAACATGAGGCCTCATCTGACTGTACGC
Gibson-M2-M3-F	CAGTCAGATGAGGCCTCATGTTCGTTACGAC
Gibson-M2-M3-R	ATGTTCTATATGCGCACGGCAATAGAGACAC
Gibson-M3-M4-F	TCTATTGCCGTGCGCATATAGAACATCCTGATGTCTC
Gibson-M3-M4-R	TGGCAAATTGAACTCAGAATGCACACGCGTGTCAAACCTGCATAC

condition: 98°C for 10 sec, 62°C for 30 sec, 72°C for 4 min in 30 cycles. The deletion was confirmed by Sanger sequencing at Macrogen. pUC57S-T7p-dGGG-M06 and pCC1-SalI-SF-T7p-MF vectors were treated with SfiI and BamHI, and then linearized pCC1-SalI-SF-T7p-MF-Δ0 vector and T7p-dGGG-M0 fragment were purified using DokDo-Prep PCR Purification Kit (EBD-1004; Elpisbio). The purified pCC1-SalI-SF-T7p-MF-Δ0 vector and T7p-dGGG-M0 were ligated using T4 DNA ligase (NEB) at 16°C for overnight. The ligated product was transformed into NEB10-beta competent cells (NEB) and incubated at 32°C for overnight. Positive colonies were selected by colony PCR using T7 primer with GGG (5′-TAATACGACTCACTATAGGG-3′) resulting in no PCR reactions (data not shown), and then also confirmed by Sanger sequencing at Macrogen. The size and integrity of each fragment of pCC1-SalI-SF-T7p-dGGG-MF was examined by restriction enzyme analysis using different combinations of specific targeting enzymes for each fragment (Fig. 2B).

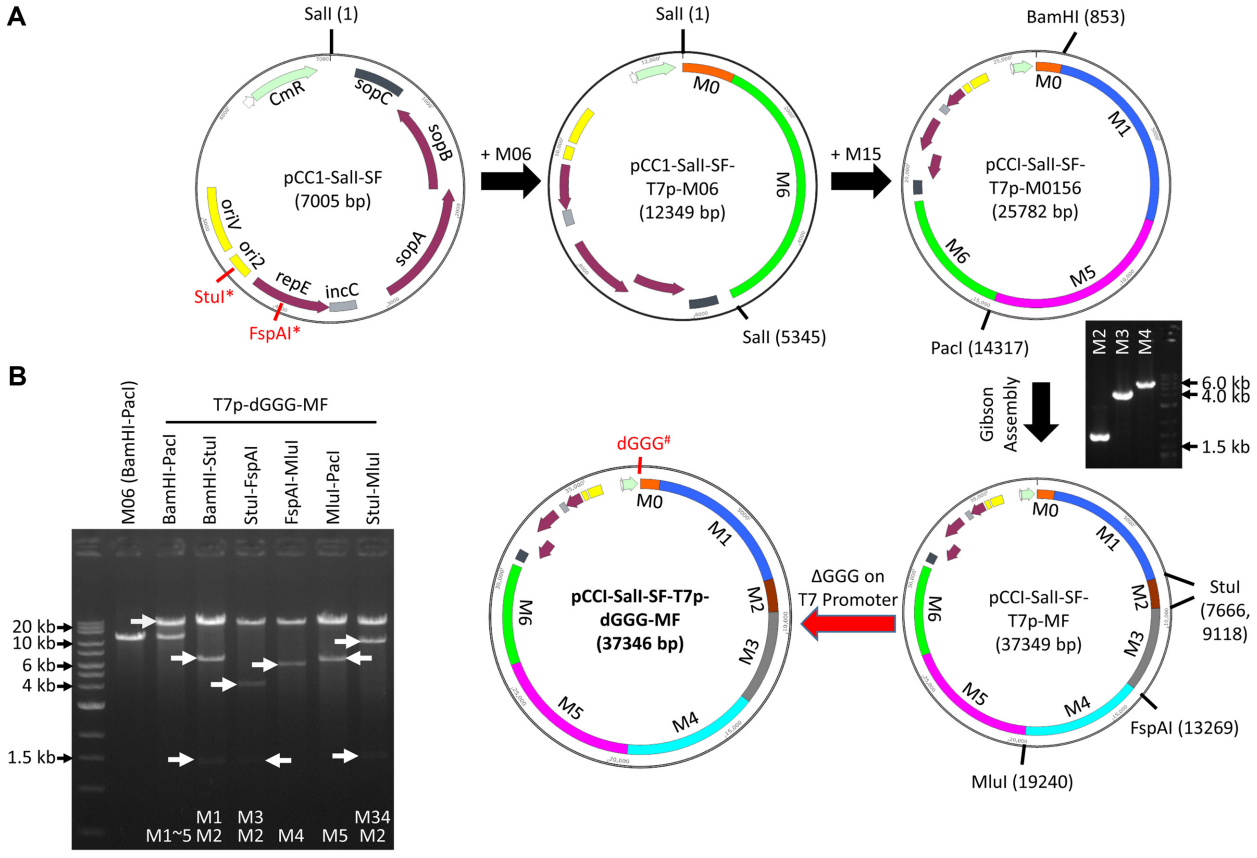

Fig. 2. Generation of a full-length infectious cDNA clone of MERS-CoV.

(**A**) Flow chart of cloning procedure to create a MERS-CoV cDNA clone: *mutagenesis for eliminating the enzyme sites; #three G's deletion on T7 promoter. (**B**) Restriction enzyme analysis for confirmation of each fragment of the MERS-CoV cDNA clone. The arrows indicate target bands for each fragment, and the target genes in size order for each lane were marked at the bottom of the gel picture (M1~5: ~25.0 kb, M1: ~6.8 kb, M2: ~1.5 kb, M3: ~4.2 kb, M4: ~6.0 kb, M5: ~6.6, M34: ~10.1 kb).

In Vitro Transcription

To linearize vectors for in vitro transcription, both pCC1-SalI-SF-T7p-MF and pCC1-SalI-SF-T7p-dGGG-MF vectors were restricted by using EagI-HF for overnight, and the linearized plasmids were purified manually by phenol/chloroform extraction. For in vitro transcription, HiScribe T7 ARCA mRNA Kit (NEB) was utilized to make synthetic RNA according to the manufacturer's instructions. Additionally, 20 U of RNase inhibitor (Promega, Madison, USA) was added in the reaction mixture. The reactions were performed at 37°C for 2 h. After DNase I treatment, synthetic mRNA purifications were also performed with LiCl provided in the kit following the manufacturer's instructions.

Results

Gene Synthesis and Vector Modification for MERS-CoV cDNA Assembly

The MERS-CoV genome was divided into 6 fragments for gene synthesis as the size of the genome is too large to be synthesized in a single fragment (~ 30 kb, Fig. 1A). Fragments M06, M1, M2, and M5 were stably maintained in high-copy-number vectors, either pUC57-Simple or pUC57-kan. However, fragments M3 and M4, were unstable in those vectors (data not shown), thus they were cloned in pCCI bacmid, which is a single copy vector. This is consistent with the previous findings by the Enjuanes [13] and Baric group [14]. Similar regions of the MERS-CoV genome were not stably propagated in regular *E. coli* strains (DH5α and Top10). The regions were either split into smaller fragments for assembly in a single reaction [14] or synthesized in a longer fragment for conventional cut-and-paste cloning [13]. To facilitate the assembly of the full-length genome of MERS-CoV, 3-nucleotide silent mutations were incorporated (Fig. 1B): 1) a potential T7 RNA polymerase pause site was eliminated by replacing A with G (n.t. 2890). A stretch of single nucleotide may disrupt T7 DNA-dependent RNA polymerase-driven transcription [14]. 2) There are two naturally occurring FspAI sites in the MERS-CoV genome, one of which was removed by C to T substitution at n.t. 11,311. The MluI site was generated by A to G silent mutation at n.t. 19,196. Thereby, assembly of the full-length cDNA was attempted by taking advantage of 4 unique restriction sites (BamHI (806), FspAI (13,222), MluI(19,196), and PacI (25,840)) and 2 StuI sites (7,640 and 9,072).

Generation of a Full-Length Infectious cDNA Clone of MERS-CoV

To efficiently generate a full-length cDNA clone, the pCCI vector was first modified to remove StuI and FspAI sites in the vector backbone by incorporating synonymous codons (Fig. 2A). In addition, there are three SalI sites in the original vector between CmR and sopC, spanning roughly 1 kb sequences (data not shown). The vector was cut with SalI and self-ligated to generate pCCI-SalI-SF (Fig. 2A, the first plasmid map). M06 fragment, which contains T7 promoter at the 5′ end and polyA tail, ribozyme and T7 termination sequence at the 3′ end, was cloned into the pCCI-SalI-SF vector, generating pCCI-SalI-SF-T7p-M06 using Sal I site. Subsequently, M15 fragment was cloned using BamHI and PacI, termed pCCI-SalI-SF-T7p-M0156. However, a number of attempts to clone M3 and M4 fragments into the M0156 vector, either single or in combination, failed likely due to toxicity and instability of those fragments. Thus, we took advantage of Gibson assembly, which allows cloning of multiple fragments in a single reaction. M2, M3, and M4 fragments were individually amplified and added into a Gibson reaction together with the linearized M0156 vector, generating pCCI-SalI-SF-T7p-MF, MERS-CoV full-length (Fig. 2A), which were further manipulated to remove 3 guanines at the end of the T7 promoter (Fig. 3A). The resulting T7p-dGGG-MF construct will produce the viral RNA with the exact 5′ end as that of the genome of wild-type MERS-CoV. To examine the integrity of the full-length cDNA, T7p-MF was subjected to restriction analysis using varying combinations of enzymes (Fig. 2B). As expected, inserts of the right size were detected. Subsequently, the whole genome was verified by Sanger sequencing.

In Vitro Transcription of the MERS-CoV cDNA Clone

Two different T7 promoter-harboring MERS-CoV cDNAs were generated in this study (Figs. 2 and 3A). T7 promoter, ending with 3 G's, is a strong promoter (Fig. 3A, top panel) and will allow optimal transcription in vitro. However, as the transcription starts at the first G of the 3 G's at the end of the T7 promoter, the extra 3 G's precede the exact viral genome. The presence of extra nucleotides, especially C's or G's, would adversely affect viral genome replication by disturbing secondary structures of 5′ UTR. On the other hand, the minimal promoter without 3 G's at the 3′ end of the promoter may show less transcription capacity, but it would allow production of viral genomes with the exact 5′ end (Fig. 3A, bottom panel). We tested both constructs for RNA production by in vitro transcription. The pCCI-T7p-MF or pCCI-T7p-dGGG-MF vectors were first linearized to allow efficient in vitro transcription. In vitro transcription reactions were performed according to the manufacturer's instructions (details in Materials and Methods). As the MERS-CoV genome is approximately 32 kb long, we tested

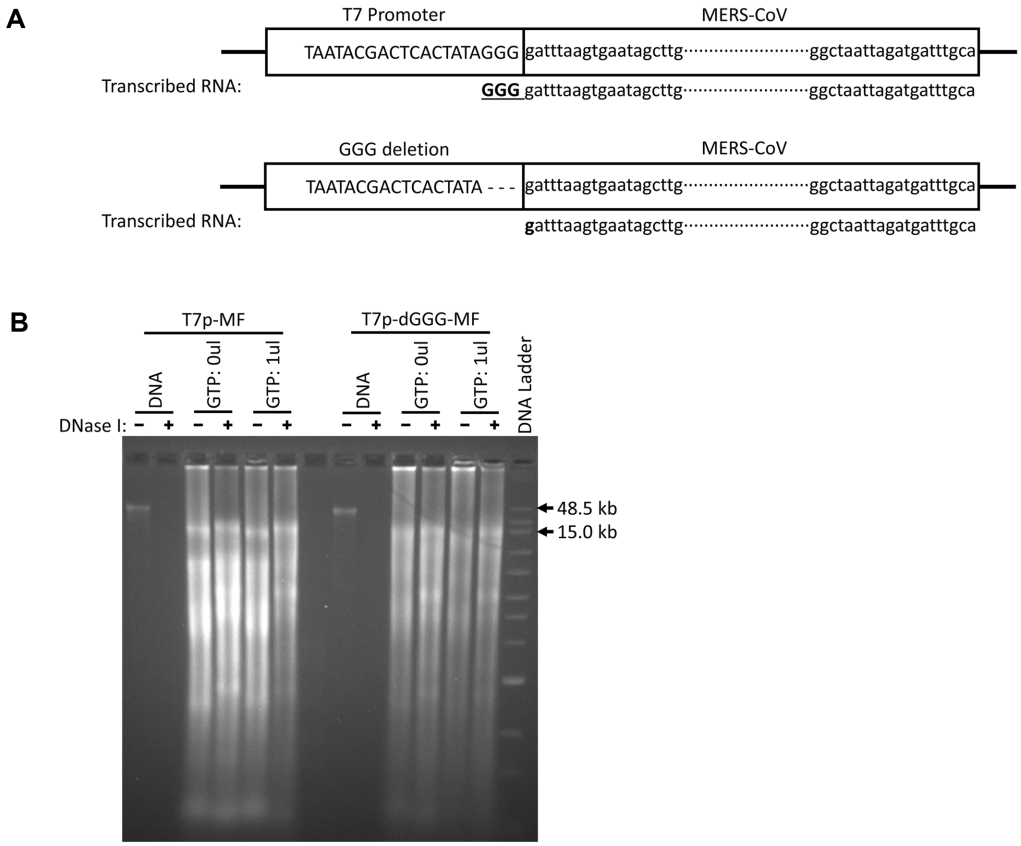

Fig. 3. In vitro transcription of the full-length infectious cDNA clone of MERS-CoV.
(**A**) Deletion of three G's on T7 promoter. The underlined section emphasizes three G's on strong T7 promoter. The bold section shows the last start nucleotides of 5′ end on synthetic mRNA by T7 RNA polymerase. (**B**) In vitro transcription of the full-length infectious clones of both pCC1-SalI-SF-T7p-MF and pCC1-SalI-SF-T7p-dGGG-MF.

whether additional GTP would increase the yield. Of note, with or without extra GTP, RNA yield was comparable (Fig. 3B; lane 3 vs lane 5 (T7p-MF); lane 10 vs lane 12 (T7p-dGGG-MF)). A few different sizes of RNAs seem to have been apparently produced, which is common for in vitro transcription reactions [23]. In addition, DNase I treatment degraded the DNA templates with RNAs unaffected. As expected, the RNA yield from the T7p-dGGG-MF was lower than that from the T7p-MF (Fig. 3B, right panel). However, it is most likely that T7p-dGGG-MF-driven RNA will better recapitulate the biology of MERS-CoV than that of T7p-MF.

Discussion

The first infectious cDNA clone of an RNA virus was developed by Racaniello et al in 1981 [24, 25]. The entire poliovirus genome was cloned in a plasmid and its transfection into a permissible cell line allowed recovery of infectious polio viruses. Since then, a number of reverse genetic clones of almost all virus families were generated [26]. To name just a few: calicivirus [27, 28], hepevirus [29], flavivirus [30–33], coronavirus [13, 14, 34–36], picornavirus [25] (positive-sense RNA virus), reovirus [37, 38] (double-stranded RNA virus), influenza virus [39, 40] and arenavirus [41] (negative-sense RNA virus), and retrovirus [42, 43] among many others. Infectious molecular clones have been extensively utilized to 1) manipulate viral genomes to study the functions of viral genes as well as regulatory sequences in viral replication, pathogenesis, and viral tropism [26] 2) generate reporter gene-expressing recombinant viruses to facilitate convenient detection of viral infection for the identification of antivirals and host factors that interact with invading viruses [14, 34, 44], and 3) develop attenuated [45] or replication-defective [46] vaccines.

A couple of full-length cDNAs of MERS-CoV have been generated by employing different cloning strategies. Scobey *et al*. generated a full-length MERS-CoV cDNA by utilizing

a class II restriction enzyme, BglI (GCCNNNNNGGC), which enables a directional cloning of six fragments in a single reaction [14]. To utilize BglI enzyme, the authors introduced a number of silent mutations at the junctions of the fragments as well as a mutation in a potential T7 pause site. In addition, naturally-occurring 2 BglI sites were eliminated. Of note, the fragment D was too unstable to be carried in a vector, which suggests that the region may be toxic to bacterial cells. The same phenomenon was observed by Almazan *et al*. [13] and in our studies as well. Scobey *et al*. detoured the cloning by dividing the D fragment into 2 sub-fragments while Almazan *et al*. bypassed the problem by synthesizing a bigger fragment of 10 kb. In this manuscript, we successfully solved the problem by employing a Gibson assembly [47−51], which allows efficient assembly of overlapping DNA molecules (M2, M3, M4 fragments, ~12 kb, Fig. 2B). The rest of the MERS genome was assembled by employing the same conventional cut-and-paste cloning method as that used by Almazan *et al*. Here we report that a Gibson assembly can be efficiently exploited to assemble a large RNA genome which may contain an unstable region in a vector. Currently, we are applying this Gibson assembly method to clone a flavivirus infectious clone that is known to be 'notoriously hard' to assemble [30, 31, 33]. Once established, the Gibson assembly method will prove itself to be an efficient way to generate an infectious clone of emerging and pre-emerging viral pathogens, providing a streamlined platform for the production of a cDNA.

With the aim to develop recombinant MERS-CoV viruses with deletion, which are defective in replication, we generated a full-length infection cDNA clone (Figs. 1 and 2). Replication-deficient vaccines have demonstrated both efficacy and safety [47, 52−54]. As the genome of MERS-CoV is ~ 30 kb, its in vitro transcription is not a simple task as it seems. We have tested three different in vitro transcription kits and also optimized transcription conditions. As shown in Fig. 3, with or without additional GTP, the MERS-CoV genome was efficiently transcribed with a couple of small-sized fragments, which might be byproducts of transcription due to potential T7 pause site(s) in the genome. Nevertheless, full-length RNA, of the MERS-CoV genome size, was successfully generated. Of note, transcription from the strong T7 promoter, whose sequence ends with TAGGG, was significantly higher than that from the ΔGGG T7 promoter, whose sequence ends with TA (with deletion of GGG). As the MERS-CoV genome starts with G, the first G of the MERS-CoV genome forms a minimal T7 promoter sequence (TAG). Obviously, the strong T7 promoter transcribed the downstream MERS-CoV genome (Fig. 3, left panel). However, the resulting RNA would start with GGGG. Considering the fact that additional sequences in the 5′ UTR greatly affects its secondary structure, replication of a virus usually is negatively affected by the presence of unwanted additional nucleotide in the 5′ UTR. Albeit low in quantity, the exact MERS-CoV RNA genome, transcribed from the 'minimal' T7 promoter, would be more efficient in recovering recombinant viruses when transfected into a permissible cell line.

Taken together, here we report that a full-length cDNA clone of MERS-CoV is generated by employing the combination of conventional cut-and-paste cloning technique and Gibson assembly under the minimal T7 promoter, which would allow transcription of the exact 5′ end of the MERS-CoV genome. Currently, recovery of recombinant viruses is underway by transfecting in vitro transcribed RNA. The infectious clone will greatly facilitate the development of an attenuated/replication-defective vaccine of the deadly virus.

Acknowledgment

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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