US 20250263674A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2025/0263674 A1**

WANG et al. (43) **Pub. Date: Aug. 21, 2025**

(54) **PLASMID-BASED REVERSE GENETICS SYSTEM FOR TILAPIA LAKE VIRUS (TILV)**

(71) Applicant: **City University of Hong Kong**, Hong Kong (CN)

(72) Inventors: **Xingxing WANG**, Hong Kong (CN); **Nikolaus OSTERRIEDER**, Hong Kong (CN)

(21) Appl. No.: **18/442,171**

(22) Filed: **Feb. 15, 2024**

Publication Classification

(51) **Int. Cl.**

C12N 7/00	(2006.01)
C12N 5/071	(2010.01)
C12N 5/077	(2010.01)

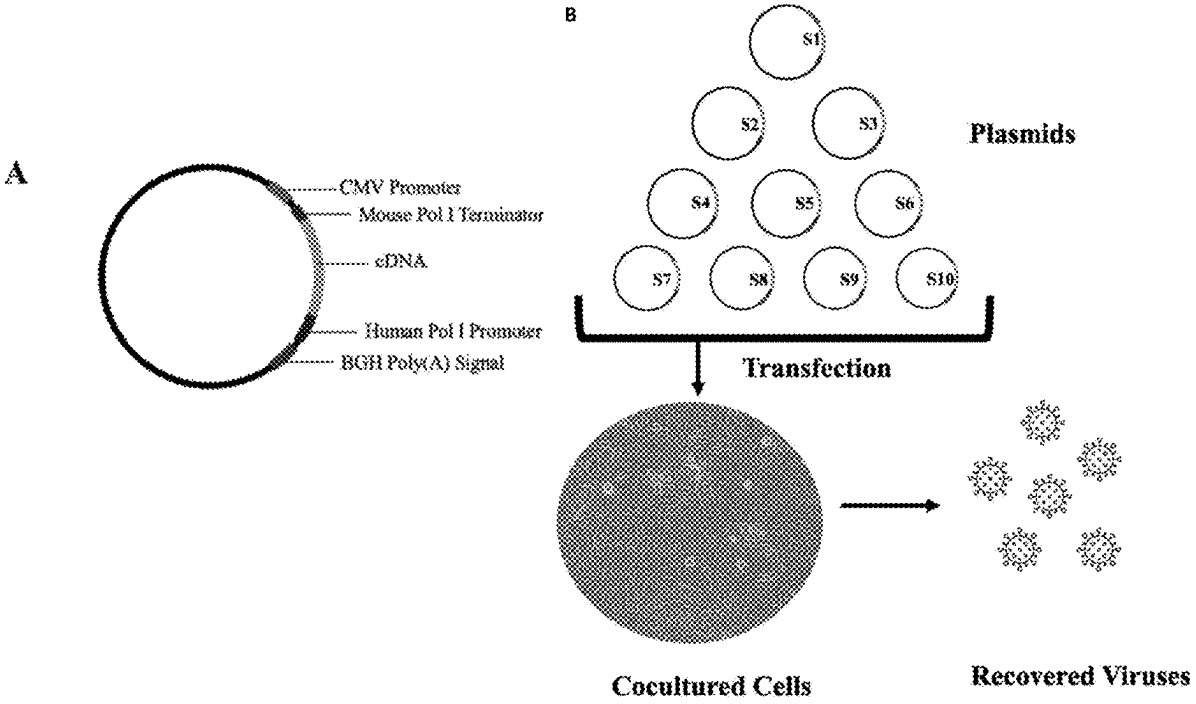
(52) **U.S. Cl.**

CPC ***C12N 7/00*** (2013.01); ***C12N 5/0656*** (2013.01); ***C12N 5/0686*** (2013.01); *C12N 2760/00021* (2013.01); *C12N 2760/00051* (2013.01)

(57) **ABSTRACT**

The present disclosures relate to the establishment of a plasmid-based reverse genetic system for TiLV and use of the system to rescue recombinant and reporter TiLVs. The present disclosures also relate to the plasmids used for the rescue of TiLV and the recombinant and reporter TiLVs generated using the methods disclosed herein.

Specification includes a Sequence Listing.



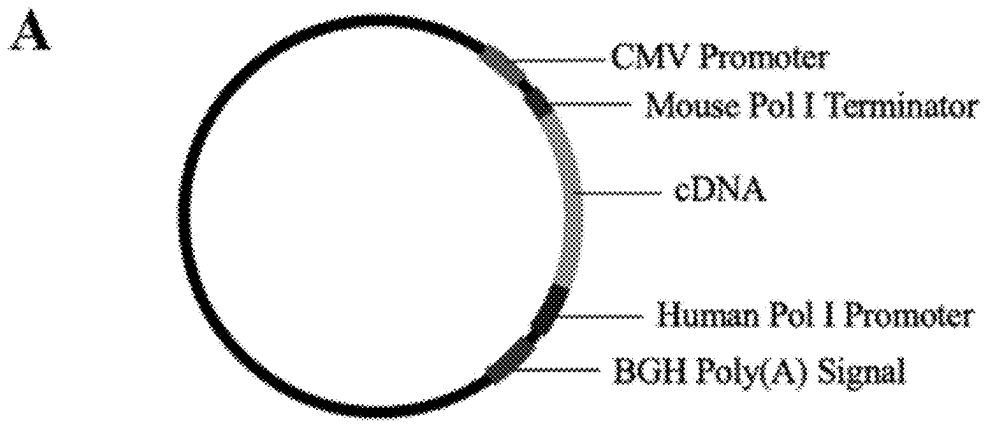

A
CMV Promoter
Mouse Pol I Terminator
cDNA
Human Pol I Promoter
BGH Poly(A) Signal

Fig. 1

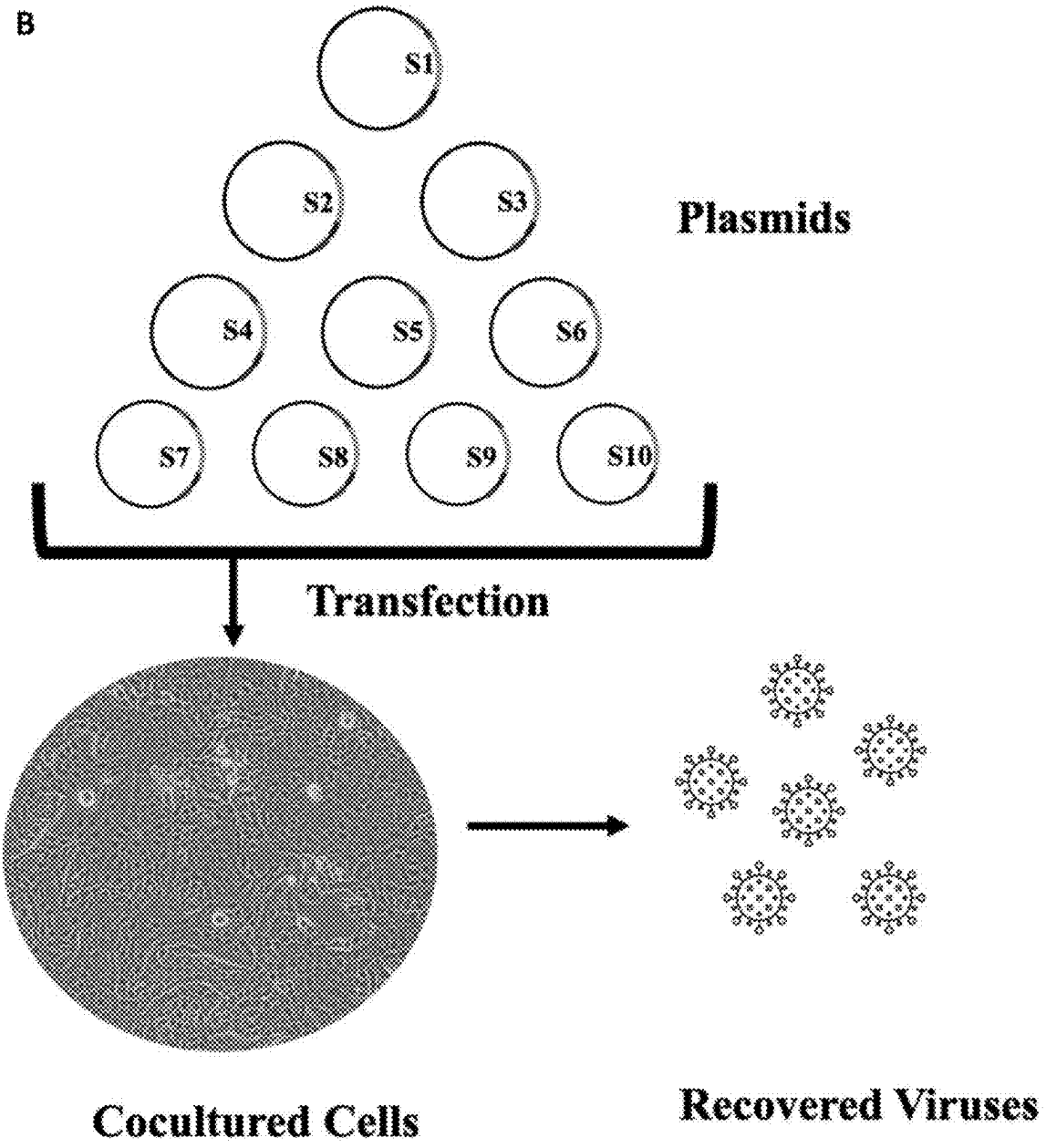


Fig. 1 (Continued)

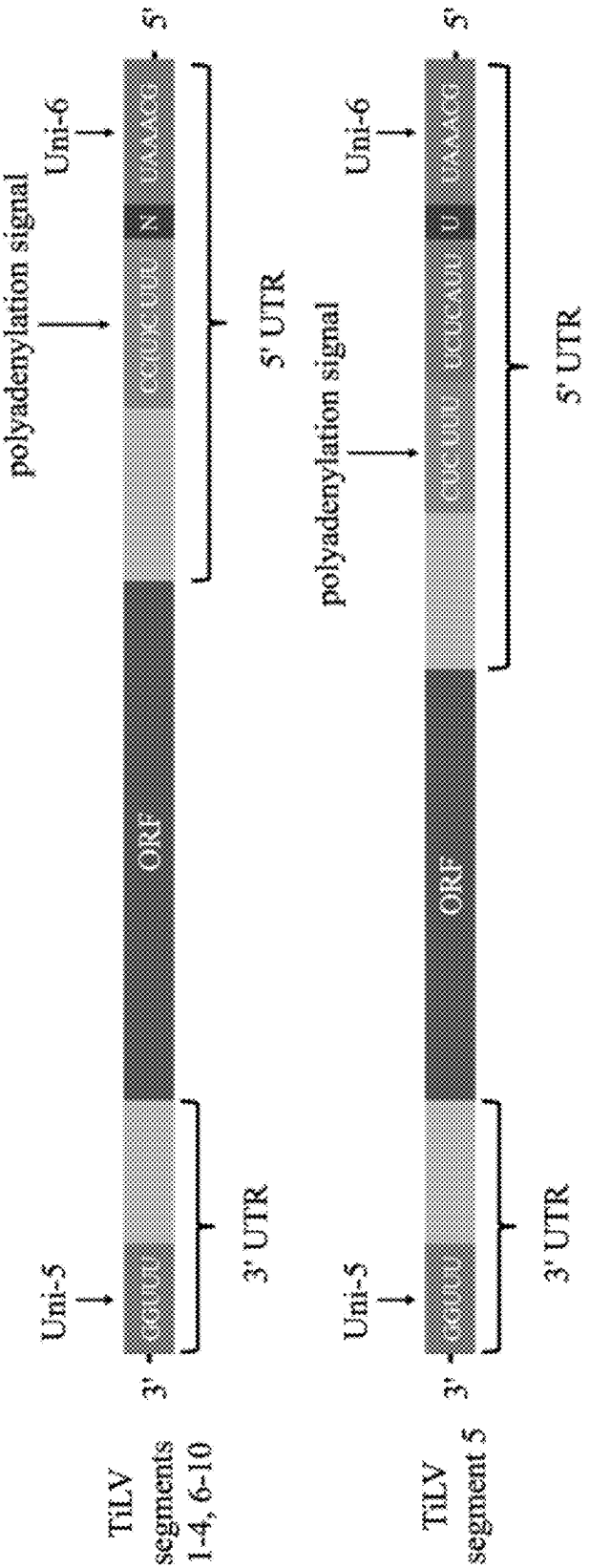

TiLV segments 1-4, 6-10
3'
Uni-5
3' UTR
ORF
5' UTR
polyadenylation signal
Uni-6
N
5'
TiLV segment 5
3'
Uni-5
3' UTR
ORF
5' UTR
polyadenylation signal
Uni-6
U
5'

Fig. 2

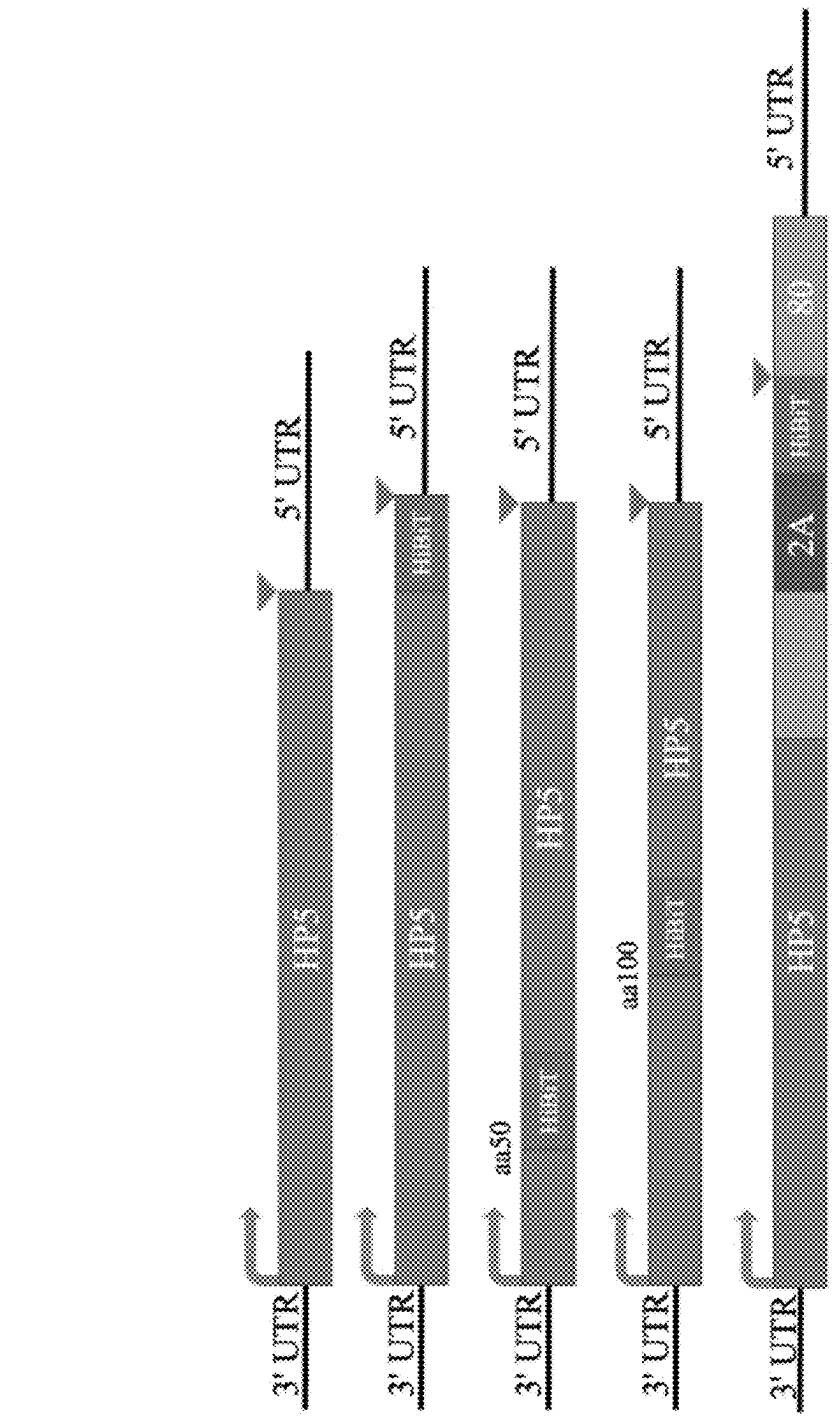

A
HP5
3' UTR
HP5
5' UTR
HP5-HiBiT
3' UTR
HP5
5' UTR
HP5-aa50-HiBiT
3' UTR
aa50
HP5
5' UTR
HP5-aa100-HiBiT
3' UTR
aa100
HP5
5' UTR
HP5-2A-HiBiT-80
3' UTR
HP5
2A
80
5' UTR

Fig. 3

B

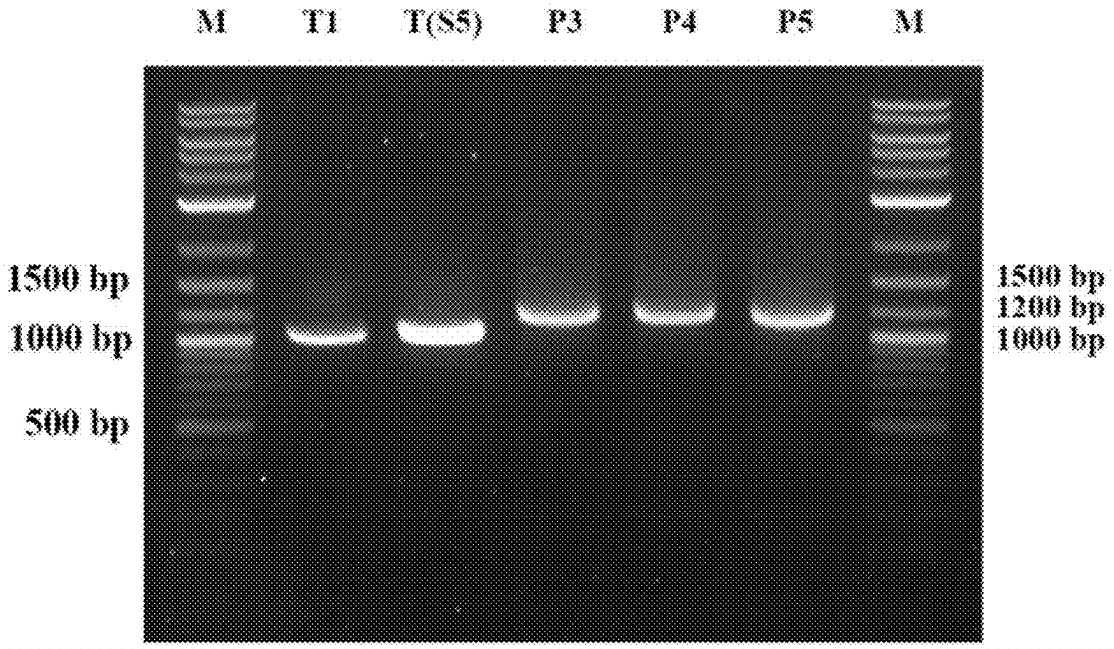


Fig. 3 (Continued)

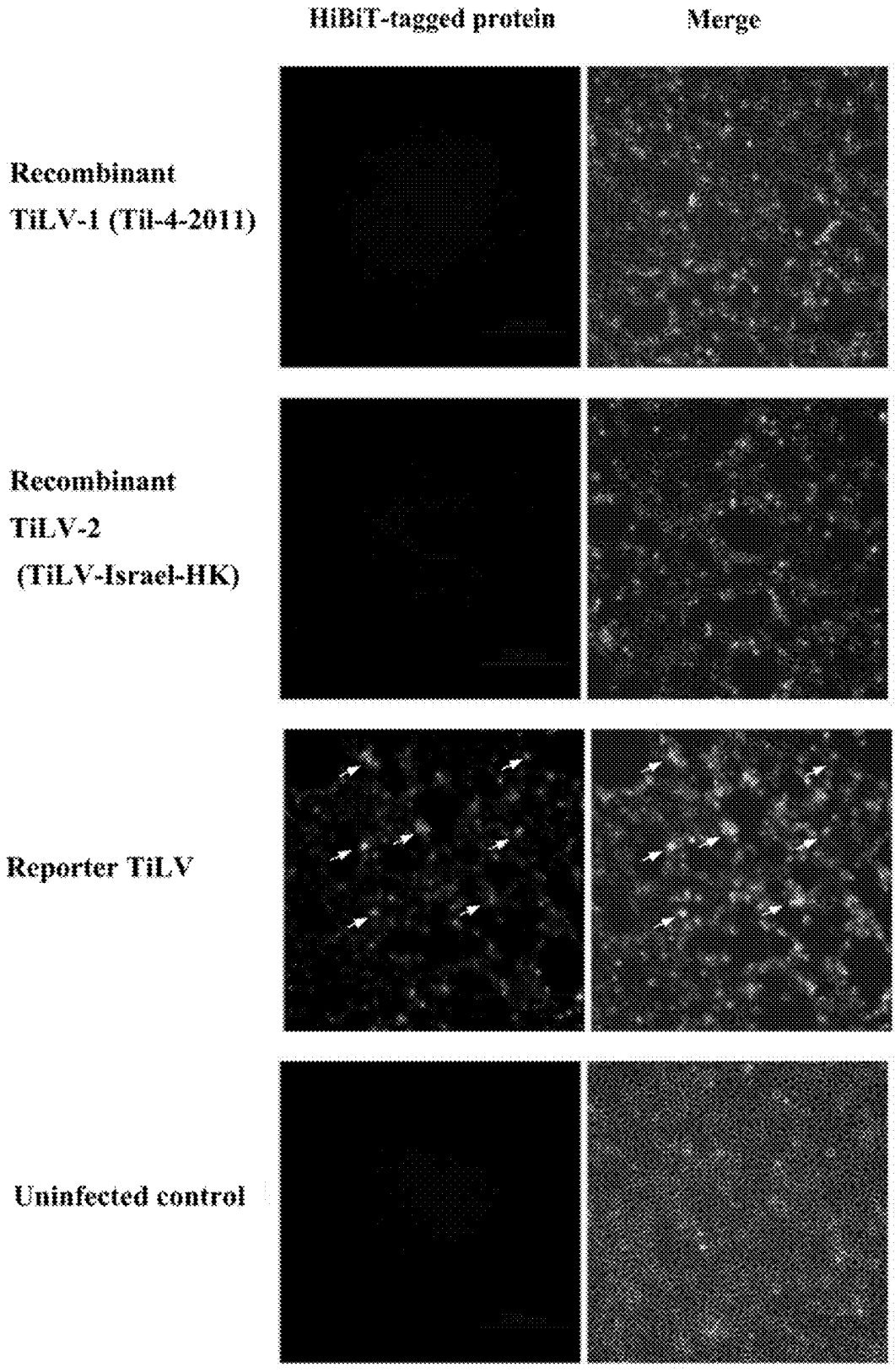

HiBiT-tagged protein
Merge
Recombinant TiLV-1 (Til-4-2011)
Recombinant TiLV-2 (TiLV-Israel-HK)
Reporter TiLV
Uninfected control

Fig. 4

PLASMID-BASED REVERSE GENETICS SYSTEM FOR TILAPIA LAKE VIRUS (TILV)

REFERENCE TO SEQUENCE LISTING

[0001] The Sequence Listing identified as Sequence_Listing_P25681US00.xml; Size: 93 kilobytes; and Date of Creation: Feb. 1, 2024, filed herewith, is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosures relate to the establishment of a reverse genetics system (RGS) for tilapia lake virus (TiLV).

BACKGROUND

[0003] Tilapia lake virus (TiLV), the etiological agent of TiLV disease, is an emerging virus first identified in Israel in 2014. It is a single-stranded, negative-sense orthomyxo-like RNA virus with ten segments, which has been assigned taxonomically to the new family Amnoonviridae, genus *Tilapinevirus,* species *Tilapinevirus tilapiae* (ICTV). Since the first description of the virus and the disease, cases have been reported in Asia, Africa, and the Americas. Recently, China, the largest producer of tilapia, reported its first natural TiLV infection. It should also be noted that tilapia is one of the most important protein sources, especially in low-to middle-income countries. TiLV can cause up to 90% mortality in tilapia, and there is no cure for TiLV infection and no commercially available vaccine. Thus, TiLV poses a severe threat to the global tilapia industry and food security.

[0004] Currently, scientific knowledge and research relating to TiLV is very limited and many important knowledge gaps need to be filled. The molecular mechanisms underlying the replication and pathogenesis of this virus remain, for the most part, unknown. Reverse genetics (RG), an approach used to generate virus entirely from cloned cDNA, is arguably the most powerful tool for studying viruses and for vaccine development. Until now, there has been no RGS for TiLV, which has hampered efforts to understand its pathogenesis and to generate novel vaccines or antiviral therapeutics.

[0005] There are several challenges in establishing a RGS for TiLV: (i) TiLV is a fish RNA virus, and recovery of infectious virus in fish-derived cells that are permissive for TiLV is difficult, primarily because systems previously employed for related viruses make use of a bidirectional transcription system, which would require knowledge of the fish RNA polymerase I promoter. However, there is currently no information available on the polymerase I promoter of cells that allow TiLV replication; (ii) The functions of most TiLV genes and proteins are unknown, which presents a significant obstacle in developing an RGS; and (iii) The TiLV genome consists of ten segments, and the large number of segments adds to the difficulty in developing of an efficient procedure to generate virus from cloned cDNA.

[0006] There is a necessity for the establishment of a RGS and use thereof for recovering TiLV.

SUMMARY

[0007] The present disclosure relates to the establishment of an RGS that allows the rescue of recombinant TiLV despite the fact that nature and functions of TiLV proteins are unknown. This is the first RGS for TiLV. Furthermore, the present disclosure relates to reporter-tagged TiLVs, which can be used to monitor virus infection both in vitro and in vivo.

[0008] In a first aspect, provided herein is a method for rescuing recombinant tilapia lake virus (TiLV), comprising:

[0009] i) preparing a plurality of expression plasmids for expressing the ten genomic segments of TiLV, wherein each of the plurality of expression plasmids comprises at least one cDNA sequence of the ten genomic segments of TiLV;

[0010] ii) introducing the plurality of expression plasmids into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection;

[0011] iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the recombinant TiLV; and optionally

[0012] iv) recovering the reassortant TiLV.

[0013] In certain embodiments, the plurality of expression plasmids are bi-directional expression plasmids which express all viral genomic RNAs (gRNAs) and viral mRNAs of TiLV.

[0014] In certain embodiments, the cDNA sequences of the ten genomic segments express all viral genomic RNAs (gRNAs) and viral mRNAs of TiLV.

[0015] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV have at least 90% identity to SEQ ID NOs:1-10, respectively.

[0016] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV have at least 90% identity to SEQ ID NOs:11-20, respectively.

[0017] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV comprise or consist of SEQ ID NO:1-10, respectively.

[0018] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV comprise or consist of SEQ ID NO:11-20, respectively.

[0019] In certain embodiments, the fish-derived cells are E11 cells derived from *Channa striatus.*

[0020] In certain embodiments, the co-cultured Vero cells and fish-derived cells are Vero E6 cells and E11 cells.

[0021] In a second aspect, provided herein is a method for producing a reporter-tagged TiLV, comprising:

[0022] i) preparing an expression plasmid comprising cDNA sequence of one genomic segment of TiLV and a reporter tag sequence that is integrated following a certain number of 3' terminal nucleotides of the open reading frame (ORF) of the segment; and preparing a plurality of expression plasmids comprises cDNA sequences of at least one of the remaining nine genomic segments of TiLV;

[0023] ii) introducing all the expression plasmids prepared in step i) into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection;

[0024] iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the reporter-tagged TiLV.

[0025] In certain embodiments, the expression plasmids are bi-directional expression plasmids which express all viral genomic RNAs (gRNAs) and viral mRNAs of TiLV.

[0026] In certain embodiments, the cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:1-10, respectively.

[0027] In certain embodiments, the cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:11-20, respectively.

[0028] In certain embodiments, the cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:1-10, respectively.

[0029] In certain embodiments, the cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:11-20, respectively.

[0030] In certain embodiments, the fish-derived cells are E11 cells derived from *Channa striatus*.

[0031] In certain embodiments, the co-cultured Vero cells and fish-derived cells are Vero E6 cells and E11 cells.

[0032] In certain embodiments, the reporter tag sequence is HiBIT, GFP, NanoLuc, or mCherry.

[0033] In a third aspect, provided herein are recombinant, reassortant and reporter TiL Vs that are produced by the method according to any of the embodiments described herein.

[0034] In a fourth aspect, provided herein are vaccines, which comprise attenuated or inactivated recombinant and reporter TiLVs described herein, any one or more of the expression plasmids described herein, and the viral proteins expressed by any one or more of the expression plasmids described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The above and other objects and features of the present disclosure will become apparent from the following description of the disclosure, when taken in conjunction with the accompanying drawings.

[0036] FIG. **1**. Schematic diagram of the reverse genetics system for the rescue of TiLV. (A) The backbone of the designed plasmid is based on the pcDNA3.1 vector and the transcription units comprising the CMV promoter, the mouse RNA polymerase I terminator, a viral cDNA of TiLV in negative-sense orientation, the human RNA polymerase I promoter, and the BGH polyadenylation signal. (B) Ten plasmids designed for the generation of viral gRNAs and viral mRNAs are transfected into co-cultured Vero E6 cells and E11 cells. Subsequently, recombinant or reporter viruses are recovered.

[0037] FIG. **2**. Schematic diagram of TiLV genome (specifically referencing the TiLV-Israel-HK strain). The genome features include a unique sequence of 5 nucleotides at the 3' terminal (uni-5), a main open reading frame (ORF) and a unique sequence of 6 nucleotides at the 5' terminal (uni-6). The polyadenylation signal sequences within the genomic segments of TiLV are indicated.

[0038] FIG. **3**. Rescue of a HiBiT-tagged reporter TiLV. (A) Schematic of the HP5-HiBiT fusions encoding the polyprotein containing HP5, HiBiT and the self-cleaving 2A peptide where indicated. The HiBiT sequence was integrated following either the 50th or 100th amino acid of HP5, represented as aa50 or aa100 respectively. The terminal 80 nucleotides of the HP5 ORF locate upstream of 2A and downstream of HiBiT. (B) RT-PCR were performed to amplify the full-length segment 5 of the reporter viruses. The PCR products were separated on 1% agarose gel. M=marker (NEB 1 kb Plus DNA Ladder), T1=recombinant TiLV-1, T (S5)=reassortant TiLV-1 (S5), P3=3rd passage of reporter virus, P4=4th passage of reporter virus and P5=5th passage of reporter virus.

[0039] FIG. **4**. Immunofluorescence assay for the detection of HiBiT-tagged viral protein in E11 cells. E11 cells were either mock-infected or infected with recombinant TiLV-1 (Til-4-2011), recombinant TiLV-2 (TiLV-Israel-HK), or reporter TiLV. After 2 days, cells were fixed and the HiBiT-tagged viral protein was identified using a mouse anti-HiBiT antibody (as the primary antibody) and the Alexa Fluor 488 goat anti-mouse IgG antibody (as the secondary antibody). The arrows point to some representative fluorescence staining in E11 cells.

DETAILED DESCRIPTION

Definitions

[0040] The use of the singular herein includes the plural (and vice versa) unless specifically stated otherwise.

[0041] As used herein, the term "about" or "around" refers to a ±10%, ±7%, ±5%, ±3%, ±1%, or ±0% variation from the nominal value unless otherwise indicated or inferred.

[0042] The term "recovering/rescuing a virus" used herein encompasses any process well known from the man skilled in the art allowing the generation of a viral clone from the genome of the virus.

[0043] "Percent identity" in the context of two or more nucleic acids or polypeptide sequences, refers to the percentage of nucleotides or amino acids that two or more sequences or subsequences contain which are the same. A specified percentage of nucleotides can be referred to such as: 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity.

[0044] A "mutation" as used herein, refers to a change in nucleic acid relative to a reference sequence (which is preferably a naturally-occurring normal or "wild-type" sequence), and includes translocation, deletion, insertion, and substitution.

[0045] The method disclosed herein produces TiLV through reverse genetics techniques. In these techniques, the viruses are produced in culture hosts using a plurality of expression plasmids which comprise cDNA sequences of TiLV genomic segments.

[0046] In a first aspect, provided herein is a method for rescuing recombinant tilapia lake virus (TiLV), comprising:

[0047] i) preparing a plurality of expression plasmids for expressing the ten genomic segments of TiLV, wherein each of the plurality of expression plasmids comprises at least one cDNA sequence of the ten genomic segments of TiLV;

[0048] ii) introducing the plurality of expression plasmids into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection;

[0049] iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the recombinant TiLV; and optionally

[0050] iv) recovering the reassortant TiLV.

[0051] The following provides detailed description of the method.

The Expression Plasmids

[0052] The plurality of expression plasmids express all the viral gRNAs and viral mRNAs for the generation of recombinant TiLV.

[0053] The expression plasmids are bi-directional expression plasmid.

[0054] In particular, a bi-directional expression plasmid contains at least two promoters which drive expression in different directions (i.e., both 5' to 3' and 3' to 5') from the same plasmid. Typically, one of the promoters is a pol I promoter and at least one of the other promoters is a pol II promoter. This is useful as the pol I promoter can be used to express uncapped viral RNAs while the pol II promoter can be used to transcribe mRNAs which can subsequently be translated into viral proteins, thus allowing simultaneous expression of RNA and protein from the same plasmid.

[0055] The pol I and pol II promoters used in the expression plasmids may be endogenous to an organism from the same taxonomic order from which the host cell is derived. Alternatively, the promoters can be derived from an organism in a different taxonomic order than the host cell.

[0056] The expression plasmids can be produced by synthesizing the cDNAs of each of the ten genomic fragments of TiLV. Then the cDNAs may be incorporated into a plasmid or other episomal construct, using conventional techniques known in the art.

[0057] The expression plasmids should encode all the genomic segments of TiLV which are necessary to produce a TiLV. Alternatively, the expression plasmid may encode one, two, three, four, five or more genomic segments.

[0058] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV have at least 90%, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NOs:1-10 or SEQ ID NO: 11-20, respectively. Typically, any mutation in the cDNA sequence does not cause termination of amino acid translation. The plurality of expression plasmids should express all viral gRNAs and viral mRNAs of TiLV in order to produce the recombinant TiLV.

[0059] In certain embodiments, the cDNA sequences of the ten genomic segments of TiLV comprise or consist of SEQ ID NO:1-10 or SEQ ID NO:11-20, respectively.

Transfection of Cells

[0060] The expression plasmids can be introduced into host cells using any technique known to those of skill in the art. For example, expression plasmids can be introduced into host cells by employing electroporation, DEAE-dextran, calcium phosphate precipitation, cationic lipid, or viral vectors. In certain embodiments, lipid-based transfection reagents such as lipofectamine 3000 are preferred. Once transfected, the host cells will begin to express the encoded viral RNA segments.

[0061] In certain embodiments, the cells for expressing the ten genomic segments of TiLV are Vero cells that are co-cultured with fish-derived cells that are susceptible to TiLV infection. For example, the Vero cells comprise Vero JCRB0111, Vero CCL-81, Vero 76 or Vero E6 cells.

[0062] In certain embodiments, the fish-derived cells that are susceptible to TiLV infection include E11, SSN-1, TiB (Tilapia brain), FHM (Fathead minnow), CIK (*Ctenopharyngodon idella* kidney), CCB (Common carp brain), and ZF4 (Zebrafish fry).

[0063] Where cells are used as a culture host, it is known that cell culture conditions (e.g., temperature, cell density, pH value, etc.) are variable over a wide range subject to the cell lines and can be adapted to the requirements of the application.

[0064] In certain embodiments, before transfection of the expression plasmids, the co-cultured cells are cultured at around 32-37° C., e.g., 33-37° C., 34-37° C., 35-37° C., 36-37° C., 32-36° C., 32-35° C., 32-34° C., or 32-33° C. Typically, the cells are cultured at about 37° C.

[0065] In certain embodiments, the cells are transfected with a total amount of 5 μg to 15 μg plasmids, with each plasmid accounting for 0.5-1.5 μg. In certain embodiments, total amount of all the plasmids for transfecting the cells is from 6 μg to 15 μg, 7 μg to 15 μg, 8 μg to 15 μg, 9 μg to 15 μg, 10 μg to 15 μg, 11 μg to 15 μg, 12 μg to 15 μg, 13 μg to 15 μg, 14 μg to 15 μg, etc.

[0066] In certain embodiments, after transfection of the plasmids, the cells are cultured at around 28° C., e.g., 30° C., 29° C., 27° C., 26° C., 25° C. or even lower temperature.

Virus Harvest

[0067] In certain embodiments, the methods also include harvesting and isolating the recombinant viruses by known methods in the art.

[0068] In certain embodiments, the recovered recombinant TiLV viruses can be passaged on fish-derived cells that are susceptible to TiLV infection, such as E11, SSN-1 and TiB.

[0069] Reporter viruses are powerful tools for monitoring virus infection in both cultured cells and their animal hosts. It has proven particularly useful for screening antiviral agents in vitro or assessing efficacy of vaccines or therapeutics in vivo. Therefore, in a second aspect, provided herein is a method for producing a reporter-tagged TiLV, comprising:

[0070] i) preparing an expression plasmid comprising cDNA sequence of one genomic segment of TiLV and a reporter tag sequence that is integrated following a certain number of 3' terminal nucleotides of the open reading frame (ORF) of the segment; and preparing a plurality of expression plasmids comprises cDNA sequences of at least one of the remaining nine genomic segments of TiLV;

[0071] ii) introducing all the expression plasmids prepared in step i) into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection;

[0072] iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the reporter-tagged TiLV.

[0073] In certain embodiments, an additional self-cleaving peptide such as 2A peptide is inserted between the ORF and the tag.

[0074] An example of integration of one tag into one genomic segment can refer to HP5-2A-HiBIT-80 in FIG. **3**A, wherein 80 nucleotides at 3' terminal of ORF of segment 5 are added to the 3' end of the HiBiT tag. It should be understood that the nucleotides to be added to the 3' end of the tag are associated with the packaging signal sequence of the segment and should encompass the packaging signal sequence specific to this segment.

[0075] In certain embodiments, the expression plasmids are bi-directional expression plasmids which express all viral gRNAs and viral mRNAs of TiLV.

[0076] In certain embodiments, the cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:1-10, respectively.

[0077] In certain embodiments, the cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:11-20, respectively.

[0078] In certain embodiments, the cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:1-10, respectively.

[0079] In certain embodiments, the cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:11-20, respectively.

[0080] In certain embodiments, the fish-derived cells are E11 cells derived from *Channa striatus*.

[0081] In certain embodiments, the co-cultured Vero cells and fish-derived cells are Vero E6 cells and E11 cells.

[0082] In certain embodiments, the reporter tag is HiBIT, GFP, NanoLuc, or mCherry. Advantageously, the reporter tag is a HiBiT tag. The reporter tags can be detected by known methods in the art.

[0083] In a third aspect, provided herein are recombinant, reassortant and reporter TiLVs which are generated by any methods disclosed herein.

[0084] The recombinant and reporter viruses are valuable tools for studying TiLV replication, pathogenesis, and host-virus interactions. They can be used to investigate the function of specific viral genes or to study the effects of viral infections on cells and organisms.

[0085] In a fourth aspect, provided herein are vaccines, which comprise attenuated or inactivated recombinant and reporter TiLVs described herein, any one or more of the expression plasmids described herein, and the viral proteins expressed by any one or more of the expression plasmids described herein.

EXAMPLES

Materials and Methods

Cells and Viruses

[0086] Vero E6 cells (ATCC, CRL-1586) and E11 cells (derived from striped snakehead fish, generously provided by Dr. Sven M. Bergmann) were cultured in MEMα medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) at the indicated temperatures under a 5% CO_2 atmosphere. The TiLV isolate (kindly provided by Prof. Eran Bacharach) was propagated in E11 cells, and the complete genome was determined as described below (this sequenced TiLV strain was named as TiLV-Israel-HK).

[0087] The ten genomic segments of tilapia lake virus isolate Til-4-2011 are shown in SEQ ID NOs: 1-10, and the ten genomic segments of tilapia lake virus isolate TiLV-Israel-HK are shown in SEQ ID NO: 11-20, respectively.

[0088] The primers for amplifying the terminals of mRNA or complementary RNA, the terminals of genomic RNA, and the full-length genomic segment of TiLV are listed in Table 1.

TABLE 1

Primer list

Primer name	Primer sequence (5' to 3')	Purpose
PCR anchor primer	GACCACGCGTATCGATGTCGAC (SEQ ID NO: 21)	
oligo(dT)-anchor primer	GACCACGCGTATCGATGTCGACTT TTTTTTTTTTTTTV (SEQ ID NO: 22)	V = A, C, or G
S1-m&cRNA	GCAGGAATGTGCCTATAGGT (SEQ ID NO: 23)	Primers used to amplify the terminals of mRNA or complementary RNA of TiLV.
S2-m&cRNA	GCATTACGTTCTCCCGAG (SEQ ID NO: 24)	
S3-m&cRNA	GCTGGATATGTTGGAACTACCT (SEQ ID NO: 25)	
S4-m&cRNA	GCAGATAGGCGACCAGGT (SEQ ID NO: 26)	
S5-m&cRNA	GACTACAAGACCATGCTGGCCTC (SEQ ID NO: 27)	
S6-m&cRNA	GATATTGGAGAGCCGGGGAATA (SEQ ID NO: 28)	
S7-m&cRNA	GATGGTGGAGAAAAGGTACCT (SEQ ID NO: 29)	
S8-m&cRNA	GCGAAGGCCTCTCAGAAGAA (SEQ ID NO: 30)	
S9-m&cRNA	GGTGATGTCACGATGGATAGAAAAT (SEQ ID NO: 31)	
S10-m&cRNA	GGATGAGTGTGGCAGATTATTT (SEQ ID NO: 32)	
S1-gRNA	GATAGGGATTCTACTGTCAA (SEQ ID NO: 33)	Primers used to amplify the terminals of genomic RNA of TILV.
S2-gRNA	GCACCTGAGACGCTGTCACCAT (SEQ ID NO: 34)	
S3-gRNA	CGTGCGTACTCGTTCAGTATAAGTTCT (SEQ ID NO: 35)	
S4-gRNA	ACCTAGCCTTCCAGACCTC (SEQ ID NO: 36)	
S5-gRNA	GACTTCCCGTGTCAAAGCTTCTA (SEQ ID NO: 37)	
S6-gRNA	GCTCCACTTGTATTCAGTTCTGA (SEQ ID NO: 38)	

TABLE 1-continued

Primer list

Primer name	Primer sequence (5' to 3')	Purpose
S7-gRNA	GTAGGAACACCACGATTCATTGA (SEQ ID NO: 39)	
S8-gRNA	GCATTTCACGGAAATGATTGA (SEQ ID NO: 40)	
S9-gRNA	CGTCCTTAAAGTCATACTTTGCCA (SEQ ID NO: 41)	
S10-gRNA	GATATTAAGGTGCTAAGACTGC (SEQ ID NO: 42)	
S1-FL-F	CCAAACGTTATCTCTTAATTACGC (SEQ ID NO: 43)	Primer pairs used to amplify the full-length genomic segment of TiLV with the exception of segment 2.
S1-FL-R	TAATACGACTCACTATAGCAAATATTTCTCTCATTC (SEQ ID NO: 44)	
S2-FL-F	CCAAATTTTACTCTCTATTACCAAATAC (SEQ ID NO: 45)	
S2-Part-R	AGGTCCTCGTAACCCATCCA (SEQ ID NO: 46)	
S3-FL-F	CCAAATATTACCCCTTAATCCTTAATAG (SEQ ID NO: 47)	
S3-FL-R	TAATACGACTCACTATAGCAAATTTTTCCCATAAT (SEQ ID NO: 48)	
S4-FL-F	CCAAAGTTTACTCCTATTACCCAG (SEQ ID NO: 49)	
S4-FL-R	TAATACGACTCACTATAGCAAATCTTTCTCCAATT (SEQ ID NO: 50)	
S5-FL-F	CCAAATGTTTCTCTTATCTCAGAC (SEQ ID NO: 51)	
S5-FL-R	TAATACGACTCACTATAGCAAATTTTACTCTTTTT (SEQ ID NO: 52)	
S5-FL-F-2	CCAAATGTTTCTCTTA (SEQ ID NO: 53)	
S5-FL-R-2	GCAAATTTTACTCTTTT (SEQ ID NO: 54)	
S6-FL-F	CCAAATTTTACCTCTCGCATG (SEQ ID NO: 55)	
S6-FL-R	TAATACGACTCACTATAGCAAATATTTCTCTCAATC (SEQ ID NO: 56)	
S6-FL-F-2	CCAAATTTTACCTCTCGCA (SEQ ID NO: 57)	
S6-FL-R-2	GCAAATATTTCTCTCAATCAAG (SEQ ID NO: 58)	
S7-FL-F	CCAAATTTTACTCTCTTTGCATTG (SEQ ID NO: 59)	
S7-FL-R	TAATACGACTCACTATAGCAAATCTTTCTCTCATG (SEQ ID NO: 60)	
S8-FL-F	CCAAATATTACCTCATCTACACTAAC (SEQ ID NO: 61)	
S8-FL-R	TAATACGACTCACTATAGCAAATTTTTCTCATCATT (SEQ ID NO: 62)	
S9-FL-F	CCAAATTTTACTCACAAGTCCGAT (SEQ ID NO: 63)	
S9-FL-R	TAATACGACTCACTATAGCAAATCTTTCTCACGTC (SEQ ID NO: 64)	
S10-FL-F	CCAAATTTTAACCCTACTAACACCAA (SEQ ID NO: 65)	
S10-FL-R	TAATACGACTCACTATAGCAAATCTTTCCCTCTG (SEQ ID NO: 66)	

Plasmids

[0089] The cDNA of each TiLV segment (SEQ ID NO: 1-10) was synthesized and introduced into pcDNA3.1 vector at sites flanked by the 222 bp of human RNA polymerase I (Pol 1) promoter sequence and the 34 bp of murine terminator sequence. The resulting plasmids were synthesized by BGI and were named pPolI-TiLV-S1, pPolI-TiLV-S2, pPolI-TiLV-S3, pPolI-TiLV-S4, pPolI-TiLV-S5, pPolI-TiLV-S6, pPolI-TiLV-S7, pPolI-TiLV-S8, pPolI-TiLV-S9, and pPolI-TiLV-S10.The synthetic sequences of TiLV segments were identical to the TiLV reference genome (NCBI accession no: GCF_001630085.1), with G chosen for R (G or A) at nucleotide position 481, A for R at nucleotide position 661, G for R at nucleotide position 703 of segment 3.

[0090] Three plasmids were constructed based on our TiLV genome sequencing results. The synthetic sequences of TiLV segments 2, segment 5, and segment 6 in plasmids pPolI-TiLV-S2, pPolI-TiLV-S5, and pPolI-TiLV-S6 were replaced with new sequences (SEQ ID NO:12, SEQ ID NO: 15 and SEQ ID NO: 16) from the TiLV-Israel-HK strain using overlap extension PCR cloning. The resulting plasmids were named pPolI-TiLV-S2-2, pPolI-TiLV-S5-2, and pPolI-TiLV-S6-2.

[0091] The plasmids used for generating reporter virus were synthesized by BGI and was named pPolI-HP5-2A-HiBiT80 (SEQ ID NO: 67). All plasmids used for transfection were isolated by the Plasmid Maxi or Midi Kit (QIAGEN).

Determination of the Complete Viral Genome

[0092] The 5' and 3' terminal sequences of TiLV were determined by 3' RACE using the 5'/3' RACE Kit (2^{nd} Generation, Roche). Firstly, total RNA was extracted from TiLV-infected E11 cells using TRIzol Reagent (Invitrogen) and polyadenylated using Poly (A) Polymerase (NEB). The polyadenylated RNA was used for first-strand cDNA synthesis with oligo (dT)-anchor primer (See Table 1). The synthesized cDNA was then used as the template to amplify the terminal sequences with the PCR anchor primer and gene-specific primers (GSPs) (See Table 1). All target PCR products were purified by the QIAquick Gel Extraction Kit (QIAGEN) and were subjected to Sanger sequencing.

[0093] According to the sequencing results of 3' RACE, primers (See Table 1) were designed to amplify the full-length of TiLV segments. Similarly, target PCR products were purified and were subjected to Sanger sequencing.

3' RACE for Determining the Polyadenylation Signal Sequence

[0094] The 3' terminal sequences of TiLV mRNA were determined by 3' RACE using the total RNA isolated from E11 cells infected by the wild-type TiLV (TiLV-Israel-HK). Unlike the above procedure, the total RNA was not polyadenylated. All other steps are almost the same as above described.

Recovery of Recombinant, Reassortant and Reporter TiLVs From Cloned cDNAs

[0095] Vero E6 cells and E11 cells (at a ratio of approximately 4:1) were seeded in T25 flasks and cultured at 37° C. overnight. The co-cultured cells were transfected with 10 plasmids (each 1.25 μg) using 2 μL of Lipofectamine 3000 Transfection Reagent (Invitrogen) per microgram of plasmid DNA. The transfected cells were then incubated at 28° C.

Sanger Sequencing of Segments 5 and 6 for Recombinant, Reassortant and Reporter TiLVs

[0096] Recovered viruses were passaged on E11 cells. At 3 days post-infection, total RNA was isolated from E11 cells infected by the recovered viruses (at least passage 3) using the TRIzol Reagent, and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Touchdown PCR was performed to amplify the full-length of segments 5 and 6. Target PCR products were purified and were subjected to Sanger sequencing.

Next-Generation Sequencing of a Recombinant TiLV

[0097] Total RNA was isolated from E11 cells infected with recombinant TiLV-1 (Til-4-2011). The library preparation, Illumina sequencing (pair-end sequencing of 151 bp) and basic de novo assembly were done at Centre for PanorOmic Sciences (CPOS) of the University of Hong Kong.

Immunofluorescence (IF) Assay for Reporter TiLV Detection

[0098] The mouse anti-HiBiT monoclonal antibody (Promega) was used for the identification of reporter TiLV-infected cells. Cells were fixed with ice-cold methanol (Sigma) for 15 min at −20° C., then blocked with 1% bovine serum albumin (BSA, Sigma) containing 0.3% Triton X-100 (Sigma) for 1 h at room temperature. After that, the cells were incubated with the anti-HiBiT antibody at a dilution of 1:1000 in 1% BSA containing 0.3% Triton X-100 for 2 h at room temperature. After three consecutive washing steps with PBS, the cells were incubated with Alexa Fluor 488 goat anti-mouse IgG antibody (Invitrogen) at a dilution of 1:1 000 in 1% BSA containing 0.3% Triton X-100 for 1 h at room temperature. Finally, the cells were washed with PBS three times as above, and images were captured using a Nikon ECLIPSE Ti2-E inverted microscope.

Results

Development of a Reverse Genetics System for TiLV

[0099] In efforts to develop an RGS for TiLV, the susceptibility of many mammalian cell lines such as HEK293T, Calu-3, Vero E6, BHK21, and swine testicular (ST) to TiLV infection was tested. Ultimately, it is discovered that TiLV can replicate in Vero E6 cells. It is surmised, therefore, that TiLV can be rescued by transfecting Vero E6 cells. Due to the limited information available on the function of TiLV proteins, a bidirectional transcription system to generate influenza viruses was employed. The strategy involved introducing all viral genomic RNA (gRNA) segments and viral mRNAs into TiLV permissive cells using this approach.

[0100] First, a plasmid was designed in which the cDNA of each TiLV segment was inserted between an RNA polymerase I promoter and an RNA polymerase II promoter (FIG. **1**A). The insertion of the 10 TiLV cDNAs was expected to result in transcription of the 10 viral gRNAs and all viral mRNAs. Ten plasmids containing the cDNAs of the TiLV reference genome (GCF_001630085.1, TiLV isolate Til-4-2011) were synthesized and transfected into Vero E6 cells that were co-cultured with fish-derived E11 cells (FIG. **1**B, Table 2). The transfected cells were cultured at 28° C., and supernatants were collected at different time points to test for the presence of recovered viruses in E11 cells. Starting from 7 days post-transfection, it was observed transfected cells released recovered viruses in numbers sufficient for detection in the cells. Recovered viruses were then passaged on E11 cells and two methods were used to verify that the recovered viruses indeed were from cloned cDNA and represented the designed virus (Til-4-2011): (i) The full-length cDNAs of segments 5 and 6 of the recovered virus were produced by RT-PCR and sequenced. Sequence analysis confirmed that the sequences for segments 5 and 6 were identical to the cDNAs of the plasmids used for generating this designed virus, and (ii) Next-generation sequencing was performed using total RNA isolated from E11 cells infected with this recovered virus (raw sequencing reads are available at NCBI and can be accessed with BioProject No. PRJNA1010714). De novo assembly was performed and of the 15 assembled scaffolds with the highest read coverages, ten were found to be highly similar (approximately 100%) to the 10 TiLV segments (Table 3).

Taken together, these results show that the virus from cloned cDNA indeed corresponded to the designed virus (Til-4-2011).

TABLE 2

Plasmids used for generating recombinant, reassortant and reporter TiLVs

recombinant TiLV-1 (Til-4-2011)	recombinant TiLV-2 (TiLV-Israel-HK)	reassortant TiLV-1 (S5)	reassortant TiLV-2 (S6)	reporter TiLV
pPolI-TiLV-S1	pPolI-TiLV-S1	pPolI-TiLV-S1	pPolI-TiLV-S1	pPolI-TiLV-S1
pPolI-TiLV-S2	pPolI-TiLV-S2-2	pPolI-TiLV-S2	pPolI-TiLV-S2	pPolI-TiLV-S2-2
pPolI-TiLV-S3	pPolI-TiLV-S3	pPolI-TiLV-S3	pPolI-TiLV-S3	pPolI-TiLV-S3
pPolI-TiLV-S4	pPolI-TiLV-S4	pPolI-TiLV-S4	pPolI-TiLV-S4	pPolI-TiLV-S4
pPolI-TiLV-S5	pPolI-TiLV-S5-2	pPolI-TiLV-S5-2	pPolI-TiLV-S5	pPolI-HP5-2A-HiBiT80
pPolI-TiLV-S6	pPolI-TiLV-S6-2	pPolI-TiLV-S6	pPolI-TiLV-S6-2	pPolI-TiLV-S6-2
pPolI-TiLV-S7	pPolI-TiLV-S7	pPolI-TiLV-S7	pPolI-TiLV-S7	pPolI-TiLV-S7
pPolI-TiLV-S8	pPolI-TiLV-S8	pPolI-TiLV-S8	pPolI-TiLV-S8	pPolI-TiLV-S8
pPolI-TiLV-S9	pPolI-TiLV-S9	pPolI-TiLV-S9	pPolI-TiLV-S9	pPolI-TiLV-S9
pPolI-TiLV-S10	pPolI-TiLV-S10	pPolI-TiLV-S10	pPolI-TiLV-S10	pPolI-TiLV-S10

TABLE 3

Summary of the top 15 scaffolds from the de novo assembly

Scaffold	Scaffold length (bp)	TiLV reference segment length (bp)	Reads coverage for assembly*	BLAST result (NCBI Accession Number)	Percentage identity from BLAST
scaffold_55556	598	548	1,069,961	Tilapia lake virus isolate Til-4-2011 segment 9 (NC_029925.1)	100%
scaffold_53218	618	465	1,048,445	Tilapia lake virus isolate Til-4-2011 segment 10 (NC_029930.1)	99.78%
scaffold_42292	733	657	848,522	Tilapia lake virus isolate Til-4-2011 segment 8 (NC_029929.1)	100%
scaffold_18957	1,254	1,044	712,218	Tilapia lake virus isolate Til-4-2011 segment 6 (NC_029928.1)	100%
scaffold_13319	1,542	1,371	509,164	Tilapia lake virus isolate Til-4-2011 segment 3 (NC_029927.1)	99.78%
scaffold_10695	1,745	1,641	487,765	Tilapia lake virus isolate Til-4-2011 segment 1 (NC_029926.1)	100%
scaffold_17261	1,326	1,250	353,432	Tilapia lake virus isolate Til-4-2011 segment 4 (NC_029922.1)	100%
scaffold_34455	853	777	305,468	Tilapia lake virus isolate Til-4-2011 segment 7 (NC_029924.1)	100%
scaffold_13226	1,548	1,471	254,358	Tilapia lake virus isolate Til-4-2011 segment 2 (NC_029921.1)	100%
scaffold_12082	1,631	NA	85,975	*Channa striata* isolate (MN057623.1)	99.88%
scaffold_262	6,388	1,099	75,816	Tilapia lake virus isolate Til-4-2011 segment 5 (NC_029923.1)	99.91%
scaffold_126	7,620	NA	43,674	*Brama japonica* voucher SIO (OP151200.1)	92.22%
scaffold_13258	1,546	NA	28,414	*Xanthia icteritia* genome assembly, chromosome: 23 (OX421953.1)	90.00%
scaffold_89093	4,22	NA	23,968	*Epinephelus fuscoguttatus* DNA (AP022677.1)	91.61%
scaffold_55040	6,02	NA	17,810	*Plectropomus leopardus* neuroblast differentiation-associated protein (XM_042483312.1)	82.53%

*No. of paired reads mapped to assembly × 151/scaffold length

[0101] To further confirm the utility of our established system, we sequenced the complete genome of a wild-type TiLV strain originating from Israel (named TiLV-Israel-HK). Since we had successfully recovered TiLV using the developed system, we hypothesized that complementary RNA (cRNA) is produced during TiLV replication. By adding poly (A) tails to the gRNA and CRNA, 3' rapid amplification of cDNA ends (3' RACE) can be used to determine the 3'-terminal sequences of gRNA and cRNA. Thus, we were in a position to determine the 5'-and 3'-terminal sequences of the TiLV genome segments. Combined with amplifying the full-length cDNA of each TiLV segment, the complete TiLV genome of TiLV-Israel-HK was assembled (SEQ ID NO: 11-20; GenBank accession numbers: OQ437054-OQ437063). Our sequence analysis revealed that segments 2, 5 and 6 have many differences when compared to the published reference genome of TiLV, while the sequences of the other seven segments were almost identical to the TiLV reference genome. Therefore, we constructed three plasmids containing cDNA based on our newly sequenced TiLV genome for segments 2, 5 and 6. We then transfected the previously synthesized plasmids (representing segments 1, 3, 4 and 7 to 10) and the three new plasmids into co-cultured Vero E6 cells and E11 cells (Table 2). Recovered viruses were passaged in E11 cells and the full-length cDNAs of segments 5 and 6 were amplified and sequenced. Sequence analysis of the amplified segments demonstrated that the new recombinant TiLV (TiLV-Israel-HK) was also successfully recovered.

Identification of the TiLV Polyadenylation Signal

[0102] The polyadenylation signal of influenza viruses is a stretch of uridines located near the 5' terminal of the genomic RNA. However, the sequence analysis of the TiLV genome did not reveal a similar pattern. This discrepancy led to further investigate the polyadenylation signal in TiLV. Given that the viral mRNAs of influenza viruses possess a poly (A) tail, it was hypothesized that the viral mRNAs of TiLV might also exhibit this feature. Finally, the 3' terminal sequences of 10

[0103] TiLV mRNAs were determined by 3' RACE. The sequence analyses revealed that the polyadenylation signal sequence for the 10 TiLV segments is either 3'-CCC UUU-5' or 3'-CUC UUU-5'. Segment 5 stands out with its unique location of the polyadenylation signal sequence, with 14 nucleotides following the polyadenylation signal. In contrast, the remaining 9 segments show more consistency, each having 7 nucleotides after the polyadenylation signal sequence (FIG. **2**).

Rescue of Reassortant TiLVs

[0104] Phylogenomic studies have provided evidence of reassortment in TiLV, particularly with regard to segments 5 and 6. To provide direct experimental proof for the generation of reassortant viruses using the developed RGS, nine plasmids containing the cDNA of Til-4-2011 were transfected with one plasmid containing the cDNA of either segment 5 or segment 6 of the TiLV-Israel-HK (Table 2). The recovery of reassortant viruses was confirmed by Sanger sequencing, which verified the presence of the TiLV-Israel-HK segments in the Til-4-2011 background. These results demonstrate that the established system allows for recovery of reassortant viruses and further confirms its utility and versatility.

Engineering of a HiBiT-Tagged Reporter TiLV

[0105] Reporter viruses are powerful tools for monitoring virus infection in both cultured cells and their animal hosts. It has proven particularly useful for screening antiviral agents in vitro or assessing the efficacy of vaccines or therapeutics in vivo. However, the insertion of a reporter gene into viral genomes, especially in the case of (segmented) RNA viruses, can negatively affect critical biological features, making the development of a reliable reporter virus challenging. We, therefore, opted for the insertion of the HiBiT tag, an 11-amino-acid peptide sequence that enables easy detection and quantification of HiBiT-tagged proteins, to generate a reporter TiLV.

[0106] Initially, we attempted to add the HiBiT sequence directly to the predicted C-terminus of the open reading frame (ORF) encoded by TiLV segment 5 (FIG. **3**A). However, despite several attempts, we were unable to generate a reporter virus. We then selected two insertion sites (FIG. **3**A) that we predicted would have minimal impact on the putative function of the viral protein, but this approach also proved unsuccessful. Given that the genome of influenza viruses contains segment-specific packaging signal sequences that are essential for vRNA incorporation into virions, we hypothesized that similar packaging signals may exist in TiLV. To test this hypothesis, we designed a plasmid (pPolI-HP5-2A-HiBiT80) in which the terminal 80 nucleotides of the ORF of segment 5 were added after the HiBiT sequence to restore the packaging signal (FIG. **3**A). Using this construct and the other nine unmodified plasmids, we could successfully generate a reporter virus. This reporter virus was passaged in E11 cells and caused obvious CPE that was similar to that observed with wild-type virus and the other rescued viruses. We employed RT-PCR to amplify the full-length segment 5 of the reporter-tagged viruses. As anticipated, gel electrophoresis imaging indicated that the size of the full-length segment 5 of the reporter TiLV is larger than that of the other unmodified rescued viruses (FIG. **3**B). This validates the successful incorporation of our modifications into the TiLV genome. Furthermore, sequencing of segment 5 from the 3^{rd}, 4^{th}, and 5^{th} passage reporter viruses revealed that all sequences were identical to the cDNA of pPolI-HP5-2A-HiBiT80 and that the 80 nucleotides repeat sequence remained intact. We concluded from the results that the generated reporter virus is stable over serial passages. In addition, we performed immunofluorescence (IF) assay for the reporter virus. As shown in FIG. **4**, the HiBiT-tagged viral protein was readily detected in E11 cells using an anti-HiBiT antibody.

[0107] In the experiments, we first tested the susceptibility of many mammalian cell lines to TiLV infection and discovered that TiLV can replicate in Vero E6 cells. Second, to address the unknown functions of TiLV genes and proteins, we introduced all viral gRNAs and viral mRNAs into Vero E6 cells. Third, to solve the problem caused by the large number of segments, we transfected Vero E6 with E11 cells, which present one of the most permissive cells for TiLV. We also increased the number of cells and the amounts of plasmids used for transfection to optimize yields.

[0108] The successful development of this TiLV RGS suggests that the replication mechanisms of TiLV are similar to those of influenza viruses. Our genome sequencing results indicate that viral cRNAs also exist during TiLV replication.

However, the exact replication mechanisms require further investigation. Additionally, TiLV exhibits some unique features. For instance, we identified a distinct polyadenylation signal sequence in the TiLV genome through sequencing the terminal sequences of viral mRNAs. This signal sequence differs from those found in other RNA viruses.

[0109] The present invention is significant in that we have successfully established the plasmid-based RGS for TiLV. In summary, the developed RGS for TiLV will contribute to further research into the replication mechanisms of TiLV and enable thorough characterization of this virus. Significantly, it will pave the way for the creation of novel vaccines and antiviral treatments.

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gtggcctact tcgttgccta tctcccaaca gcccctgctg tctccttgag gcagtgatac  180
agactcttcc cacttctctt acatttttatg acaccgtcct gtttctgaat ggcaatgcat  240
tcttccagca gattcgtgtt ccctgttccc tcaatcctag agtcggcaat tgctgagagc  300
ccgtggctag ctaccaccag gaagcagctt cctgtagctt gcagaggtag accaacaaca  360
acaccaatac tcccgtcccc ctgcgagggg ctccaacact cagcaaggac accgtcgcaa  420
aggccccaag caattgaggc aagcagctcc ctgagggtga cctggtcgcc tatctgcagc  480
ctgtggccaa tgatcagaaa tctcggaggc gggattatag cagtctgatt caacttttg  540
tagttctttc ctctttctcc tgctgctgct ttctttgcaa agtagccacc aaccagccct  600
gggcaagcac gataggaatc cccactcaga tatgggaacc attgggcgtc ctccaaagtc  660
tgaatcctct cgtcgagagc aaccaaactg acgtacctag ccttccagac ctcaatgcga  720
acacacactc ttaatagaga gttatggtcc ttcatcatct gaatcatagc tgcgtcccct  780
gaggctttta tcaccttcat ctttgagccc agtgcaggca cacaggttgc gcagtattca  840
gtccatctgc tggttaatat aaggctcctt ccgaccctca tccctgcttc tggtgccttc  900
cttgatttct tagcgttctc cctctctctc ttcttttcac tggctctcag aaccttagcc  960
tcctgaccat cgagggagtg agtgtgcatt ttaaagagaa atgcagcagc atcagatatc  1020
acactgttgt cctccctgtt aaaggaactt ccaaacacat gaccacaagc ttcgttgaaa  1080
acctcaaggt ttcttgggag ttcagcttgt acctgcgaag tgctgggtga actttcatcc  1140
attgctacct ctggtgcaac agtgctggca gctgccatac tagtctttgt agttctcacc  1200
attttcagtt tattaagtta gctattctgg gtaataggag taaactttgg             1250

SEQ ID NO: 5           moltype = RNA  length = 1099
FEATURE                Location/Qualifiers
source                 1..1099
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 5
gcaaatttta ctctttttct cagtttacca ctctatgacc taccaggaat agaagcttaa  60
ggatcaagat aatggaagca gagggacttc gtcatcctca agcgggcttt tcttcttaag  120
ccgcttctta atatagaaaa aggtcttgaa acacacaact cttagcctcc ggaatactaa  180
aactttgata ctcccataga agggcacctg cttcaacaga gaaacaagtt tactgagcaa  240
tgaaagaacc atcagcgaaa gcaccaggta atagacaaac ttgtatttct ctagcatccc  300
aaacactatt gcaagaatag cttccccaaa ggctgcaaat tgctccttga atttgcctat  360
gttatcagat ttacagacga gggatttaat cgagcaaaac tctcctagcg tcataccggt  420
gacttcccgt gtcaaagctt ctaaatactc ccaaatctca gaaacatctg cttgagctgc  480
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gtccgctagg ccgatgaccc cagcataatt gtaaacaccc atgccaattg ctactgagct  540
agcaagtaaa cacagtataa tgaggcctgc tactacaaac gacttcggct gccttgaggc  600
cagcatggtc ttgtagtcgt ccacagtggg tctggtacag tcacactgac ctggcagtac  660
ggtggaatac acctccaaat ctctgcactc gctactgtgg ctgcacctgg aaacgttgta  720
taggacaacc ccgctatctt cgtcaatcct aacttcagta gctctccaat cacctcttcc  780
ccctgctttg ggaagtttgt acccgagggc tgggaagctg ttaggtactg catgtaaccc  840
agtgcagaac gacccacccg aggatggggt tacaatgtag tgcaccccca tagtgcagga  900
gcaactttcc atcctgaact tggatgcaaa tccacgcctt atttcacttt ctatctccct  960
actttggtag gtattgttta ggtatgattg taatcgcttt atccttaaac tttcatcact  1020
tgctagtgcg caaactaggc agcttatcag gaccagcgcc tgcatagcta ttggagtctg  1080
agataagaga aacatttgg                                               1099

SEQ ID NO: 6           moltype = RNA  length = 1044
FEATURE                Location/Qualifiers
source                 1..1044
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 6
gcaaatattt ctctcaatca agcacttaaa actgtacctg ggcatttcgc ccacacgaca  60
ggacatatag tgtcacatgt atttattgat tttacagcag gatcttactg ttactggtgg  120
tgatatcgaa agatcaatta caggattcaa tctacgcatt tttgtgtagc tgagaaaagt  180
acgagctaga agataagcat ttgacttctt ataagcaact tcatcctgca tcgcggacca  240
ctcgctgatg agctggtgac gctttatttc ggctggtagt aagacttcca ctgcggaaaa  300
gtggcacttg tatggggagt gattctcctc cagtgactca actaatatcc aacactgttt  360
tgtactattg tctctgcagc tccacttgta ttcagttctg aagccgtttg gcgtcatata  420
tgttctatct atagtacgct tacagtttgt acaaagacca cgcgacatta gcatacaggt  480
gctaaagatg ggcctcctct tcgaaaggca cagcgatgtc tcaaacagtt tagagcttat  540
ttttatagta cgtattttac ctcggcccgg tattccccgg ctctccaata tcttgtcggt  600
tacaggctcc agttcttttc tcgatattcc cgggcacaaa acaaatcctc caagcataaa  660
gttcagcgtc ggattacatc gtttctcgac aaagttgcag ttcagatgat ggagttcccc  720
ttttgatccc tccaaatcat acatgtagta tggagaccca cctgagtcta tgacttcgtt  780
gctaccatca agtcccagaa ccaccttctg tactgctgga tccagataat cactactcga  840
atatgaacct tcaacagaat atttgtcccc gtcaataact acgcttgacg tgtagtttga  900
tctcccataa aaccgccaca tattgtccac gcattgatcg cttccgctaa agagtgttga  960
aaacaaagta agggttctta tcaccctaag ccaactcatt ggataatcct gtagataaaa  1020
atgcatgcga gaggtaaaat ttgg                                         1044

SEQ ID NO: 7           moltype = RNA  length = 777
FEATURE                Location/Qualifiers
source                 1..777
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 7
gcaaatcttt ctctcatgct accatcctta gtgaacggta ctgagtacca taagaagctc  60
tcagaccaat tatccctgct ttcaaaagta attagttaac ttagaaaggc ctccccaata  120
agacaacgaa acagctgggt aattagagtt caaacgtgat tcctttaggg attggcacta  180
acccaactct gtggtaggaa caccacgatt cattgacatc agttgcaccc ttacttatag  240
cctctaggta tgtggcaacc ctgctcaact cagtcagtgg tcgagtgctg gtcaccaacg  300
ctgatctctt ggtccaatgt ggaaactttg gggatggcgc acagagccag tacttcgctt  360
cccttctagt gtagaaggta gttgaacaga gatgcatgtc ccctttgact aaataggctc  420
ctttcgggcc tcttactgct gtaccctcct gggcaagtac ccttctgact ggcaatccgg  480
ggctcacttc atacttccat ttcaaaggta ccttttctcc accatcggtt gggtgactcg  540
tcaatacaat ccaggttgtg ccatttgtat tgggtgtaaa cccagttgca caaaagaacc  600
atacgttcga ggcaccagag taatgctcta ggagcagtcg atggaacagc ccagtctgac  660
ttccgctgtc cttggggagg gtgtttttgc gcgtgataat tctctcaagc tcaccaatct  720
tgtaggacat tgtgtctatt actatacggt atgcaatgca aagagagtaa aatttgg     777

SEQ ID NO: 8           moltype = RNA  length = 657
FEATURE                Location/Qualifiers
source                 1..657
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 8
gcaaattttt ctcatcatta cacaaatgga gtagcttacc tccctgggga aaatgacact  60
gaagactcac tttttattta agcatttcac ggaaatgatt gatagcagca gaagtgtctg  120
ccttaagggc catcctgtca tcttgtgaac cttcagtgtc cttcaaagca gttgccagga  180
cctcctcgag aaaagcgagc gcagtgggta tgtcgacaca gttaatccca gggagcttaa  240
gcttcatagc ttgtttggca atatccaggt actgcttccg attgaattca aatctttgac  300
ccttgcggtt tgttattata attgcgtcga ctgtcacact gactcgcctc gagattcgtc  360
ccagcctttc ctctatccgc tttcgggtca tgccggcgtg cttaacaagc aggtgtttaa  420
actttgtttc ttctgagagg ccttcgccaa ttgttaggga aagcaaatcc ggacctgcat  480
tgacaagaaa ctccagagca actacagtgt tgactgtgtc tctagtcact gtcatgccgt  540
tgagcgtgaa atcgtagagc ttcccttggc cctctcttag tgttgggatt tgagccatac  600
ttattcctgg atatgctgtc caattggaaa tgttagtgta gatgaggtaa tatttgg     657

SEQ ID NO: 9           moltype = RNA  length = 548
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FEATURE                 Location/Qualifiers
source                  1..548
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 9
gcaaatcttt ctcacgtcct taaagtcata ctttgccaga gctgaagctt atccattttt  60
actaaagcta aattattaat gtcacatggt tcactacaat gttctagctg aggaatcagt  120
aggttcgcgg agcttcaatt gcccctggac cgccctcata aagttctatc gccagccatg  180
tcagatatcc tccacatgac ccttcttggt gcctgccttt ccgatgttga agtgtgcaaa  240
attccgtgct ccgttactac ttggacccag ctccctatca ccattgcact ctcctgccaa  300
gttggctgac tcagtgtcat taactctgag atctctgaac attctagaca atgttttcga  360
tccaggatcc agacttgacc aggttgtgat gtcttgccgc cgcagcacaa gctccaaggg  420
gggtaatggc acaaagtgac gtacgaccga ctcatctctg tcaagattac tgacacagaa  480
tctgtatttt ctatccatcg tgacatcacc aagcggaaaa agtaatcgga cttgtgagta  540
aaatttgg                                                          548

SEQ ID NO: 10           moltype = RNA  length = 465
FEATURE                 Location/Qualifiers
source                  1..465
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 10
gcaaatcttt ccctctgaca ccctgtatag ttagcgttgg cctgtggata cgaacgaaat  60
cagaaccgat attaaggtgc taagactgca cgtcaagaga cttctttccg aaatcttcgg  120
aaaatcgaga taggtcactc tgcccatcat cctctctgtc ccttctgttt ttgggattga  180
aatcaaccct agcccactct gggattgcag aatcacagtc gtccatctcg aggtcgactt  240
cgtcacccca ctctatatta tcttcaccgc tctcgtcagc accataccct tcattcttcc  300
aacttcgctt ctttgaagca gctttcttgc ccttcttgat cttccgactt cttagtacta  360
aacatcctga gctctcagcc cccgagtcac tgtcacttga caaataatct gccacactca  420
tcctggctta tagctatatt tggtgttagt agggttaaaa tttgg                  465

SEQ ID NO: 11           moltype = RNA  length = 1641
FEATURE                 Location/Qualifiers
source                  1..1641
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 11
ccaaacgtta tctcttaatt acgcactatt actgtactac cataaggtat gtgggcattt  60
caagaaggag tttgcaaagg taacctgtta tcaggcccga cctcaatgaa ggcaccggat  120
tcagcagcga gggagtcatt agacagagcg tctgaaatca tgacaggaaa atcgtacaat  180
gctgtccaca ctggggactt aagcaagctg cctaatcagg gagaaagtcc actgaggata  240
gtcgattccg acctttattc agaaaggagt tgctgttggg ttatagagaa ggagggcaga  300
gttgtatgca aaagtaccac gctcacccgc ggtatgacgg gcctgttgaa cacaacaagg  360
tgtagttctc catctgagct catatgtaag gttttgacag tagaatccct atctgaaaag  420
ataggtgaca cgagcgtcga ggagttactt tctcatggca ggtactttaa gtgcgcactt  480
cgcgaccagg agaggggtaa acccaagagc agagctatct ttctgtcaca tccattcttc  540
agattgcttt cctctgtagt agagacgcac gctagatctg tgctgtcaaa ggtctcagca  600
gtgtacaccg ctactgctag tgcagaacaa cgggctatga tggccgcaca ggttgtagag  660
tcaagaaaac atgttcttaa tggcgactgt actaagtata atgaggcaat cgacgcagac  720
acactgctaa aagtgtggga tgcaataggc atggggtcaa tcggagtcat gctcgcttac  780
atggtgcgca ggaaatgcgt tctcattaaa gacactctag tagagtgtcc aggaggtatg  840
ttgatgggaa tgtttaacgc aactgccacc ttggcattgc aggggacgac tgacagattc  900
ctgtctttca gcgacgactt tataacatcg tttaactcgc ctgctgaatt acgcgagata  960
gaggacctgc ttttcgcaag ctgtcataac ttgtcgctaa agaagagtta catttcagtt  1020
gcctcactgg aaataaactc gtgtaccctc actagggacg gtgacctagc cacagggttg  1080
ggttgtactg ctggtgtccc tttcagggga ccacttgtga ctctgaaaca gactgcagct  1140
atgttatctg gcgctgttga ctcaggagtt atgccattcc actcagcaga acgtctgttc  1200
cagataaagc agcaggaatg tgcctatagg tataacaacc ccacttacac aacgaggaat  1260
gaggacttcc tccccacatg cctgggaggg aagactgtaa ttagctttca atctctactg  1320
acttgggatt gccacccatt ttggtaccag gtrcaccccg atggcccaga cactatagat  1380
cagaaagtcc tgtctgtcct tgcttcaaag actcgcagaa ggagaacccg actggaggct  1440
ctctcagact tggacccccc ggtccctcat aggctcctcg tatcagagtc agacgttagc  1500
aagatcagag cagctaggca ggctcacttg aagtccttag gcttggaaca acccacaaac  1560
tttaactatg ctatttataa agcagtccag cccaccgctg ggtgctaagt aactatatag  1620
gcgaatgaga gaaatatttg c                                            1641

SEQ ID NO: 12           moltype = RNA  length = 1470
FEATURE                 Location/Qualifiers
source                  1..1470
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 12
ccaaatttta ctctctatta ccaaatacat ttacttctga aaatgagtca atttgagaaa  60
tcattcaagg gcagaactga ggtcacaata accgaatatc gctctcatac tgtcaaagat  120
gtgcacagaa gcttacttac ggctgacaag tctctaagga agtcattctg ttttaggaac  180
gccctaaacc agttcttgga taaagatttg cctcttttgc ccattcggcc aaaattagag  240
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tccagggttg ctgtgaaaaa gtctaagctg aggagtcagc tgtcgttcag acccggtttg  300
actcaggagg aagcaattga tctttacaac aagggctatg atggtgacag cgtctcaggt  360
gccttgcaag acagggtagt caatgagcct gtagcttact cgagtgcaga taatgacaaa  420
tttcacaggg gcttagcggc tctagggtac actttggctg atagagcatt tgatacatgc  480
gaatccggct tcgtgagagc aattcctacc actccatgcg ggttcatatg ttgtgggcca  540
ggttctttca aagattcact tggatttgtg ataaaaatcg gcgaattctg gcacatgtat  600
gacgggttcc aacacttcgt cgctgtcgag gatgctaagt tcctagcaag taagtctcct  660
tcgttttggt tggcaaaacg tcttgcaaag aggctgaatc tggtcccaaa agaggatcca  720
tctatagcag cagctgagtg cccttgtagg aaagtgtggg aagctagttt tgctagggca  780
cctactgcac tagatccatt tggaggcagg gccttctgcg accagggttg ggtgtaccac  840
agggacgtag ggtatgcaac agctaaccac atatcacagg aaacactttt tcaacaagcg  900
ctttcagtga ggaaccttgg accgcaaggt agtgcaaatg tctcaggctc aatacatacc  960
gccctggaca ggctcagagc agcgtacagt aggggggacgc ccgcctctag atctatactg  1020
caagggcttg caaatctcat cacacctgta ggtgaaaact ttgaatgcga tctcgacaag  1080
aggaagctca atataaaggc attacgttct cccgagaggt acattacgat agagggcctg  1140
gttgtaaacc tggacgatgt ggttagaggg ttctaccttg acaaggcgaa ggtcactgtt  1200
ctctcgagat caaagtggat gggttacgag gaccttcctc agaaacctcc gaacggtaca  1260
ttttactgta gaaagaggaa ggcaatgctt ctcatctcat gtagtccagg cacgtacgca  1320
aagaagcgaa aagtggcagt gcaggaggat cgctttaaag atatgagggt tgagaatttc  1380
cgggaggtag cggaaaatat ggatctaaat cagtagggtt tcttggcaaa agccttcact  1440
atatatatgg taataatgag aaagatttgc                                   1470

SEQ ID NO: 13          moltype = RNA  length = 1371
FEATURE                Location/Qualifiers
source                 1..1371
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 13
ccaaatatta ccccttaatc cttaatagac cgttaacttt cttttgaaat ggactcgcgg  60
tttgcacagc taactggggt tttctgtgac gatttcactt atagcgaagg gagccgaagg  120
ttcctaagtt cttacagtac agtagagaga cgtccaggag tccccgtaga gggtgactgt  180
tatgactgtt tgaagaataa gtggattgcc tttgagctgg aaggccagcc gcggaaattt  240
ccaaaggcaa cagttcgttg cattttgaac aatgatgcta catacgtttg ctctgagcaa  300
gagtaccagc agatttgtaa ggtacaattc aaggattatt tggagatcga cggggttgtt  360
aaagttgggc acaaggcatc ctacgatgct gagctaaggg aacggctatt ggaactacca  420
catccaaaga gtggcccgaa gcctcgtatt gagtgggtgg caccacccag acttgcggac  480
atatccaagg aaacagctga gctaaagagg caatatggat tcttcgagtg ctcaaagttc  540
ctcgcctgcg gtgaggagtg tggtcttgac caagaggcaa gagaacttat actgaacgag  600
tacgcacgtg atagagaatt tgagttccgc aatggagggt ggatacaaag gtatacagtt  660
gcttctcaca agcctgctac acagaagata ttacctctac cggctagtgc tccacttgct  720
cgtgagcttt tgatgttgat tgctagaagc acaactcagg cagggaaagt actgcatagc  780
gataatacca gcatactagc tgtaccggtc atgcgcgact ctggaaagca cagtaaaagg  840
agaccaaccg cctccactca ccacttagtt gtaggtctaa gtaaacctgg ctgtgaacac  900
gattttgagt ttgacgggta cagggcagct gtgcatgtga tgcacctaga tcccaagcaa  960
tcggctaata taggggagca agactttgtg agtacccgag aaatttacaa gctggatatg  1020
ttggaactac ctcccataag taggaagggt gatctggaca gagctagtgg tcttgagaca  1080
agatgggacg tcatcttact tctggaatgc ctcgactcta caagggttag ccaagcagtg  1140
gctcaacatt ttaataggca ccggctagca cttagcgtct gtaaggacga gttcaggaaa  1200
ggctaccagc tggcttctga gataaggggt acaataccct taagctcact ttattattca  1260
ctttgtgcag taagattgcg gatgacagta cacccatttg cgagatgatc gctttcgacg  1320
ccttcgctaa aggttacgac gttctaatag aggattatgg gaaaaatttg c            1371

SEQ ID NO: 14          moltype = RNA  length = 1250
FEATURE                Location/Qualifiers
source                 1..1250
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 14
ccaaagttta ctcctattac ccagaatagc taacttaata aactgaaaat ggtgagaact  60
acaaagacta gtatggcagc tgccagcact gttgcaccag aggtagcaat ggatgaaagt  120
tcacccagca cttcgcaggt acaagctgaa ctcccaagaa accttgaggt tttcaacgaa  180
gcttgtggtc atgtgtttgg aagttccttt aacagggagg acaacagtgt gatatctgat  240
gctgctgcat ttctctttaa aatgcacact cactccctcg atggtcagga ggctaaggtt  300
ctgagagcca gtgaaaagaa gagagagagg gagaacgcta agaaatcaag gaaggcacca  360
gaagcaggga tgagggtcgg aaggagcctt atattaacca gcagatggac tgaatactgc  420
gcaacctgtg tgcctgcact gggctcaaag atgaaggtga taaaagcctc aggggacgca  480
gctatgattc agatgatgaa ggaccataac tctctattaa gagtgtgtgt tcgcattgag  540
gtctggaagg ctaggtacgt cagtttggtt gctctcgacg agaggattca gactttggag  600
gacgcccaat ggttcccata tctgagtggg gattcctatc gtgcttgccc agggctggtt  660
ggtggctact ttgcaaagaa agcagcagca ggagaaagag gaaagaacta caaaaagttg  720
aatcagactg ctataatccc gcctccgaga tttctgatca ttggccacag gctgcagata  780
ggcgaccagg tcaccctcag ggagctgctt gcctcaattg cttggggcct ttgcgacggt  840
gtccttgctg agtgttggag cccctcgcag ggggacggga gtattggtgt tgttgytggt  900
ctacctctgc aagctacagg aagctgcttc ctggtggtag ctagccacgg gctctcagca  960
attgccgact cyaggattga gggaacaggg aacacgaatc ygctggaaga atgcattgcc  1020
attcagaaac aggacggtgt cataaaatgt aagagaagtg ggaagagtct gtatcactgc  1080
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ctcaaggaga cagcaggggc tgttgggaga taggcaacga agtaggccac ccatgcgcgg  1140
aaagctgcac aggctgccaa gggcccctct tagcccaagt tttctatata ttctttaaca  1200
agtcatctaa aactggtaaa ttcttagacg gtaattggag aaagatttgc             1250

SEQ ID NO: 15          moltype = RNA  length = 1098
FEATURE                Location/Qualifiers
source                 1..1098
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 15
ccaaatgttt ctcttagctc agactccaat agctatgcag gcgctggtcc tgacaagctg  60
cctagtttgc gcactagcaa gtgatgaaag tttaaggata aaacgactac aatcatacct  120
aaacaatacc taccaaagta gggagataga aagtgaaata aggcgtggat ttgcatccaa  180
gttcaggatg gagagttgct cctgcactat gggggtgcac tacattgtaa ccccatcctc  240
gggtgggtcg ttctgcactg ggttacatgc agtacctaac agcttcccag ccctcgggta  300
caaacttccc aaagcagggg ggagaggtga ttggaaagct actgaagtta ggattgacga  360
agatagtggg gttgttctat acaacgtttc caggtgcagc cacagtagcg agtgcagaga  420
tttggaggtg tattccaccg tactgccagg tcagtgtgac tgtaccagac ccactgtgga  480
cgactacaag accatgctgg cctcaaggca gccgaagtcg tttgtagtag caggcctcat  540
tatactgtgt ttacttgcta gctcagtagc aattggcatg ggtgtttaca attatgctgg  600
ggtcatcggc ctagcggacg cagctcaagc agatgtttct gagatttggg agtacttaga  660
agctttgaca cgggaagtca ccggtatgac gctaggagag ttttgctcga ttaaatccct  720
cgtctgtaaa tctgataaca taggcaaatt caaagagcaa tttgcagcct ttggggaagc  780
tattcttgca atagtgtttg gratgctaga gaaatataag tttgtctatt acctggtgct  840
ttcgctgatg gttctctcgc tactcagtaa acttgttcct ctgttgaagc aggtgccctt  900
ctatgggagt atcaaagttt tagtattccg gaggctaaga gttgtgtgtt tcaagacctt  960
tttctatatt aagaagcggc ttaagaagaa aagcccgctt gaggatgacg aagtccctct  1020
gcttccatta tcttgatcct taagcttcta ttcctggtag gtcataaagt ggtaaactga  1080
gaaaagagta aaatttgc                                                1098

SEQ ID NO: 16          moltype = RNA  length = 1044
FEATURE                Location/Qualifiers
source                 1..1044
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 16
ccaaatttta cctctcgcat gcatttttat ctacaggatt gtccaatgag ttggcttagg  60
gtgataagaa cccttacttt gttttcaaca ctctttaacg gaagcgatca atgcgtggac  120
aacatgtggc ggttttacgg gagatcaaac tacacgtcaa gcgtagtcat tgacggggac  180
aaatattctg ttgaaggttc atattcgagt agtgattatc tggatccagc agtacagaag  240
gtggttctgg gacttgatgg tagcaacgaa gtcatagact caggtgggtc tccatactac  300
atgtatgatt tggagggatc aaaaggggaa ctccatcatc tgaactgcaa ctttgtcgag  360
aaacgatgta atccgacgct aaactttatg cttggaggat ttgttttgtg cccaggaata  420
tcgagaaaag aactggagcc tgtaaccgac aagatattgg agagccgggg aataccgggc  480
cgaggtaaaa tacgtactat aaaaatagc tctaaactgt ttgagacatc gctgtgcctt  540
tcgaagagga grcccatctt tagcacctgt atgctaatgt cgcgtggtct ttgtacaaac  600
tgtaagcgta ctatagatag aacatatatg acgccaaacg gcttcagaac tgaatacaag  660
tggagctgca gagacaatag cacaaaacag tgttgggtat tagttgagtc actggaggag  720
aatcactccc catacaaatg ccacttttcc gcagtggaag tcctactacc agccgaaata  780
aagcgtcacc agctcatcag cgagtggtcc gcgatgcagg atgaagttgc ttataagaag  840
tcaaatgctt atcttcttgc tcgtactttt cttagctata caaaaatgcg tagattaaat  900
cctgtaattg atctttcgat atcaccacca gtgacagtaa gatcctgctg taaaattaat  960
aaatacatgt gacactatat gtcctatcat gtgggcgaaa tgcccaggta cagttttaag  1020
tgcttgattg agagaaatat ttgc                                         1044

SEQ ID NO: 17          moltype = RNA  length = 777
FEATURE                Location/Qualifiers
source                 1..777
                       mol_type = viral cRNA
                       organism = Tilapia lake virus
SEQUENCE: 17
ccaaatttta ctctctttgc attgcatacc gtatagtaat agacacaatg tcctacaaga  60
ttggtgagct tgagagaatt atcacgcgca aaaacaccct ccccaaggac agcggaagtc  120
agactgggct gttccatcga ctgctcctag agcattactc tggtgcctcg aacgtatggt  180
tcttttgtgc aactgggttt acacccaata caaatggcac aacctggatt gtattgacga  240
gtcacccaac cgatggtgga gaaaaggtac ctttgaaatg gaagtatgaa gtgagccccg  300
gattgccagt cagaagggta cttgcccagg aggggtacagc agtaagaggc ccgaaaggag  360
cctatttagt caaaggggac atgcatctct gttcaactac cttctacact agaagrgaag  420
cgaagtactg gctctgtgcg ccatccccaa agtttccaca ttggaccaag agatcagcgt  480
tggtgaccag cactcgacca ctgactgagt tgagcagggt tgccacatac ctagaggcta  540
taagtaaggg tgcaactgat gtcaatgaat cgtggtgttc ctaccacaga gttgggttag  600
tgccaatccc taaaggaatc acgtttgaac tctaattacc cagctgtttc gttgtcttat  660
tggggaggcc tttctaagtt aactaattac ttttgaaagc agggataatt ggtctgagag  720
cttcttatgg tactcagtac cgttcactaa ggatggtagc atgagagaaa gatttgc     777

SEQ ID NO: 18          moltype = RNA  length = 657
```

-continued

```
FEATURE                 Location/Qualifiers
source                  1..657
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 18
ccaaatatta cctcatctac actaacattt ccaattggac agcatatcca ggaataagta  60
tggctcaaat cccaacacta agagagggcc aagggaagct ctacgatttc acgctcaacg  120
gcatgacagt gactagagac acagtcaaca ctgtagttgc tctggagttt cttgtcaatg  180
caggtccgga tttgctttcc ctaacaattg gcgaaggcct ctcagaagaa acaaagttta  240
aacacctgct tgttaagcac gccggcatga cccgaaagcg gatagaggaa aggctgggac  300
gaatctcgag gcgagtcagt gtgacagtcg acgcaattat aataacaaac cgcaagggtc  360
aaagatttga attcaatcgg aagcagtacc tggatattgc caaacaagct atgaagctta  420
agctccctgg gattaactgt gtcgacatac ccactgcgct cgcttttctc gaggaggtcc  480
tggcrrctgc tttgaaggac actgaaggtt cacaagatga caggatggcc cttaaggcag  540
acacttctgc tgctatcaat catttccgtg aaatgcttaa ataaaaagtg agtcttcagt  600
gtcattttcc ccagggaggt aagctactcc atttgtgtaa tgatgagaaa aatttgc     657

SEQ ID NO: 19           moltype = RNA  length = 548
FEATURE                 Location/Qualifiers
source                  1..548
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 19
ccaaatttta ctcacaagtc cgattacttt ttccgcttgg tgatgtcrcg atggatagaa  60
aatacagatt ctgtgtcagt aatcttgaca gagatgagtc ggtcgtacgt cactttgtgc  120
cattaccccc cttggagctt gtgctgcggc ggcaagacat cacaacctgg tcaagtctgg  180
atcctggatc gaaaacattg tctagaatgt tcagagatct cagagttaat gacactgagt  240
cagccaactt ggcaggagag tgcaatggtg atagggagct gggtccaagt agtaacggag  300
cacggaattt tgcacacttc aacatcggaa aggcaggcac caagaagggt catgtggagg  360
atatctgaca tggctggcga tagaacttta tgagggcggt ccaggggcaa ttgaagctcc  420
gcgaacctac tgattcctca gctagaacat tgtagtgaac catgtgacat taataattta  480
gctttagtaa aaatggataa gcttcagctc tggcaaagta tgactttaag gacgtgagaa  540
agatttgc                                                           548

SEQ ID NO: 20           moltype = RNA  length = 465
FEATURE                 Location/Qualifiers
source                  1..465
                        mol_type = viral cRNA
                        organism = Tilapia lake virus
SEQUENCE: 20
ccaaatttta accctactaa caccaaatat agctataagc caggatgagt gtggcagatt  60
atttgtcaag tgacagtgac tcgggggctg agagctcagg atgtttagta ctaagaagtc  120
ggaagatcaa gaagggcaag aaagctgctt caaagaagcg aagttggaag aatgaaaggt  180
atggtgctga cgagagcggt gaagatarta tagagtgggg tgacgaagtc gacctcgaga  240
tggacgactg tgattctgca atcccagagt gggctagggt tgatttcaat cccaaaaaca  300
gaagggacag agaggatgat gggcagagtg acctatctcg attttccgaa gatttcggaa  360
agaagtctct tgacgtgcag tcttagcacc ttaatatcgg ttctgatttc gttcgtatcc  420
acaggccaac gctaactata cagggtgtca gagggaaaga tttgc                  465

SEQ ID NO: 21           moltype = DNA  length = 22
FEATURE                 Location/Qualifiers
source                  1..22
                        mol_type = other DNA
                        note = PCR primer
                        organism = synthetic construct
SEQUENCE: 21
gaccacgcgt atcgatgtcg ac                                           22

SEQ ID NO: 22           moltype = DNA  length = 39
FEATURE                 Location/Qualifiers
source                  1..39
                        mol_type = other DNA
                        note = PCR primer
                        organism = synthetic construct
SEQUENCE: 22
gaccacgcgt atcgatgtcg actttttttt ttttttttv                         39

SEQ ID NO: 23           moltype = DNA  length = 20
FEATURE                 Location/Qualifiers
source                  1..20
                        mol_type = other DNA
                        note = PCR primer
                        organism = synthetic construct
SEQUENCE: 23
gcaggaatgt gcctataggt                                              20
```

-continued

```
SEQ ID NO: 24          moltype = DNA  length = 18
FEATURE                Location/Qualifiers
source                 1..18
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 24
gcattacgtt ctcccgag                                              18

SEQ ID NO: 25          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 25
gctggatatg ttggaactac ct                                         22

SEQ ID NO: 26          moltype = DNA  length = 18
FEATURE                Location/Qualifiers
source                 1..18
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 26
gcagataggc gaccaggt                                              18

SEQ ID NO: 27          moltype = DNA  length = 23
FEATURE                Location/Qualifiers
source                 1..23
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 27
gactacaaga ccatgctggc ctc                                        23

SEQ ID NO: 28          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 28
gatattggag agccggggaa ta                                         22

SEQ ID NO: 29          moltype = DNA  length = 21
FEATURE                Location/Qualifiers
source                 1..21
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 29
gatggtggag aaaaggtacc t                                          21

SEQ ID NO: 30          moltype = DNA  length = 20
FEATURE                Location/Qualifiers
source                 1..20
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 30
gcgaaggcct ctcagaagaa                                            20

SEQ ID NO: 31          moltype = DNA  length = 25
FEATURE                Location/Qualifiers
source                 1..25
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 31
ggtgatgtca cgatggatag aaaat                                       25

SEQ ID NO: 32          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
```

-continued

```
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 32
ggatgagtgt ggcagattat tt                                              22

SEQ ID NO: 33        moltype = DNA  length = 20
FEATURE              Location/Qualifiers
source               1..20
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 33
gatagggatt ctactgtcaa                                                 20

SEQ ID NO: 34        moltype = DNA  length = 22
FEATURE              Location/Qualifiers
source               1..22
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 34
gcacctgaga cgctgtcacc at                                              22

SEQ ID NO: 35        moltype = DNA  length = 27
FEATURE              Location/Qualifiers
source               1..27
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 35
cgtgcgtact cgttcagtat aagttct                                         27

SEQ ID NO: 36        moltype = DNA  length = 19
FEATURE              Location/Qualifiers
source               1..19
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 36
acctagcctt ccagacctc                                                  19

SEQ ID NO: 37        moltype = DNA  length = 23
FEATURE              Location/Qualifiers
source               1..23
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 37
gacttcccgt gtcaaagctt cta                                             23

SEQ ID NO: 38        moltype = DNA  length = 23
FEATURE              Location/Qualifiers
source               1..23
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 38
gctccacttg tattcagttc tga                                             23

SEQ ID NO: 39        moltype = DNA  length = 23
FEATURE              Location/Qualifiers
source               1..23
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 39
gtaggaacac cacgattcat tga                                             23

SEQ ID NO: 40        moltype = DNA  length = 21
FEATURE              Location/Qualifiers
source               1..21
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 40
gcatttcacg gaaatgattg a                                               21
```

-continued

```
SEQ ID NO: 41          moltype = DNA  length = 24
FEATURE                Location/Qualifiers
source                 1..24
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 41
cgtccttaaa gtcatacttt gcca                                          24

SEQ ID NO: 42          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 42
gatattaagg tgctaagact gc                                            22

SEQ ID NO: 43          moltype = DNA  length = 24
FEATURE                Location/Qualifiers
source                 1..24
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 43
ccaaacgtta tctcttaatt acgc                                          24

SEQ ID NO: 44          moltype = DNA  length = 36
FEATURE                Location/Qualifiers
source                 1..36
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 44
taatacgact cactatagca aatatttctc tcattc                             36

SEQ ID NO: 45          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
source                 1..28
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 45
ccaaatttta ctctctatta ccaaatac                                      28

SEQ ID NO: 46          moltype = DNA  length = 20
FEATURE                Location/Qualifiers
source                 1..20
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 46
aggtcctcgt aacccatcca                                               20

SEQ ID NO: 47          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
source                 1..28
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 47
ccaaatatta ccccttaatc cttaatag                                      28

SEQ ID NO: 48          moltype = DNA  length = 35
FEATURE                Location/Qualifiers
source                 1..35
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 48
taatacgact cactatagca aatttttccc ataat                              35

SEQ ID NO: 49          moltype = DNA  length = 24
FEATURE                Location/Qualifiers
source                 1..24
```

-continued

```
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 49
ccaaagttta ctcctattac ccag                                          24

SEQ ID NO: 50        moltype = DNA  length = 35
FEATURE              Location/Qualifiers
source               1..35
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 50
taatacgact cactatagca aatctttctc caatt                              35

SEQ ID NO: 51        moltype = DNA  length = 24
FEATURE              Location/Qualifiers
source               1..24
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 51
ccaaatgttt ctcttatctc agac                                          24

SEQ ID NO: 52        moltype = DNA  length = 35
FEATURE              Location/Qualifiers
source               1..35
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 52
taatacgact cactatagca aattttactc ttttt                              35

SEQ ID NO: 53        moltype = DNA  length = 16
FEATURE              Location/Qualifiers
source               1..16
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 53
ccaaatgttt ctctta                                                   16

SEQ ID NO: 54        moltype = DNA  length = 17
FEATURE              Location/Qualifiers
source               1..17
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 54
gcaaatttta ctctttt                                                  17

SEQ ID NO: 55        moltype = DNA  length = 21
FEATURE              Location/Qualifiers
source               1..21
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 55
ccaaatttta cctctcgcat g                                             21

SEQ ID NO: 56        moltype = DNA  length = 36
FEATURE              Location/Qualifiers
source               1..36
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 56
taatacgact cactatagca aatatttctc tcaatc                             36

SEQ ID NO: 57        moltype = DNA  length = 19
FEATURE              Location/Qualifiers
source               1..19
                     mol_type = other DNA
                     note = PCR primer
                     organism = synthetic construct
SEQUENCE: 57
```

-continued

```
ccaaatttta cctctcgca                                                  19

SEQ ID NO: 58          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 58
gcaaatattt ctctcaatca ag                                              22

SEQ ID NO: 59          moltype = DNA  length = 24
FEATURE                Location/Qualifiers
source                 1..24
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 59
ccaaatttta ctctctttgc attg                                            24

SEQ ID NO: 60          moltype = DNA  length = 35
FEATURE                Location/Qualifiers
source                 1..35
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 60
taatacgact cactatagca aatctttctc tcatg                                35

SEQ ID NO: 61          moltype = DNA  length = 26
FEATURE                Location/Qualifiers
source                 1..26
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 61
ccaaatatta cctcatctac actaac                                          26

SEQ ID NO: 62          moltype = DNA  length = 36
FEATURE                Location/Qualifiers
source                 1..36
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 62
taatacgact cactatagca aatttttctc atcatt                               36

SEQ ID NO: 63          moltype = DNA  length = 24
FEATURE                Location/Qualifiers
source                 1..24
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 63
ccaaatttta ctcacaagtc cgat                                            24

SEQ ID NO: 64          moltype = DNA  length = 35
FEATURE                Location/Qualifiers
source                 1..35
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 64
taatacgact cactatagca aatctttctc acgtc                                35

SEQ ID NO: 65          moltype = DNA  length = 26
FEATURE                Location/Qualifiers
source                 1..26
                       mol_type = other DNA
                       note = PCR primer
                       organism = synthetic construct
SEQUENCE: 65
ccaaatttta accctactaa caccaa                                          26

SEQ ID NO: 66          moltype = DNA  length = 34
FEATURE                Location/Qualifiers
```

-continued

source 1..34
mol_type = other DNA
note = PCR primer
organism = synthetic construct

SEQUENCE: 66
taatacgact cactatagca aatctttccc tctg 34

SEQ ID NO: 67 moltype = DNA length = 6877
FEATURE Location/Qualifiers
source 1..6877
mol_type = other DNA
note = plasmid
organism = synthetic construct

SEQUENCE: 67
gacggatcgg gagatctccc gatcccctat ggtgcactct cagtacaatc tgctctgatg 60
ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg 120
cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180
ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt 240
gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata 300
tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc 360
cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt 480
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540
atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca 600
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 660
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720
aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg 780
gtaggcgtgt acggtgggag gtctatataa gcagagctct ctggctaact agagaaccca 840
ctgcttactg gcttatcgaa attaatacga ctcactatag ggagacccaa gctggctagc 900
agttaaccgg agtactggtc gacctccgaa gttgggggggg ccaaatgttt ctcttagctc 960
agactccaat agctatgcag gcgctggtcc tgacaagctg cctagtttgc gcactagcaa 1020
gtgatgaaag tttaaggata aaacgactac aatcatacct aaacaatacc taccaaagta 1080
gggagataga aagtgaaata aggcgtggat ttgcatccaa gttcaggatg gagagttgct 1140
cctgcactat gggggtgcac tacattgtaa ccccatcctc gggtgggtcg ttctgcactg 1200
ggttacatgc agtacctaac agcttcccag ccctcgggta caaacttccc aaagcagggg 1260
ggagaggtga ttggaaagct actgaagtta ggattgacga agatagtggg gttgttctat 1320
acaacgtttc caggtgcagc cacagtagcg agtgcagaga tttggaggtg tattccaccg 1380
tactgccagg tcagtgtgac tgtaccagac ccactgtgga cgactacaag accatgctgg 1440
cctcaaggca gccgaagtcg tttgtagtag caggcctcat tatactgtgt ttacttgcta 1500
gctcagtagc aattggcatg ggtgtttaca attatgctgg ggtcatcggc ctagcggacg 1560
cagctcaagc agatgtttct gagatttggg agtacttaga agctttgaca cgggaagtca 1620
ccggtatgac gctaggagag ttttgctcga ttaaatccct cgtctgtaaa tctgataaca 1680
taggcaaatt caaagagcaa tttgcagcct ttggggaagc tattcttgca atagtgtttg 1740
gaatgctaga gaaatataag tttgtctatt acctggtgct ttcgctgatg gttctctcgc 1800
tactcagtaa acttgttcct ctgttgaagc aggtgccctt ctatgggagt atcaaagttt 1860
tagtattccg gaggctaaga gttgtgtgtt tcaagacctt tttctatatt aagaagcggc 1920
ttaagaagaa aagcccgctt gaggatgacg aagtccctct gcttccatta tctggcagcg 1980
gcgcgaccaa ctttagcctg ctgaaacagg cgggcgatgt ggaagaaaac ccgggcccgg 2040
tgagcggctg gcggctgttc aagaagatta gctgaccttt ttctatatta agaagcggct 2100
taagaagaaa agcccgcttg aggatgacga agtccctctg cttccattat cttgatcctt 2160
aagcttctat tcctggtagg tcataaagtg gtaaactgag aaaagagtaa aatttgcaat 2220
aacccggcgg cccaaaatgc cgactcggag cgaaagatat acctcccccg gggccgggag 2280
gtcgcgtcac cgaccacgcc gccggcccag gcgacgcgcg acacggacac ctgtccccaa 2340
aaacgccacc atcgcagcca cacacggagc gcccggggcc ctctggtcaa ccccaggaca 2400
cacgcgggag cagcgccggg ccggggacgc cctcccggct ctagagggcc cgtttaaacc 2460
cgctgatcag cctcgactgt gccttctagt tgccagccat ctgttgtttg cccctccccc 2520
gtgccttcct tgaccctgga aggtgccact cccactgtcc tttcctaata aaatgaggaa 2580
attgcatcgc attgtctgag taggtgtcat tctattctgg ggggtggggt ggggcaggac 2640
agcaaggggg aggattggga agacaatagc aggcatgctg gggatgcggt gggctctatg 2700
gcttctgagg cggaaagaac cagctggggc tctaggggggt atccccacgc gccctgtagc 2760
ggcgcattaa gcgcggcggg tgtggtggtt acgcgcagcg tgaccgctac acttgccagc 2820
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What is claimed is:

1. A method for rescuing a recombinant tilapia lake virus (TiLV), comprising:

i) preparing a plurality of expression plasmids for expressing ten genomic segments of TiLV, wherein each of the plurality of expression plasmids comprises at least one cDNA sequence of the ten genomic segments of TiLV;

ii) introducing the plurality of expression plasmids into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection;

iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the recombinant TiLV; and optionally

iv) recovering the reassortant TiLV.

2. The method of claim **1**, wherein the plurality of expression plasmids are bi-directional expression plasmids which express all viral genomic RNAs (gRNAs) and viral mRNAs of TiLV.

3. The method of claim **1**, wherein cDNA sequences of the ten genomic segments of TiLV have at least 90% identity to SEQ ID NOs:1-10, respectively.

4. The method of claim **1**, wherein cDNA sequences of the ten genomic segments of TiLV have at least 90% identity to SEQ ID NOs:11-20, respectively.

5. The method of claim **1**, wherein cDNA sequences of the ten genomic segments of TiLV comprise or consist of SEQ ID NO:1-10, respectively.

6. The method of claim **1**, wherein cDNA sequences of the ten genomic segments of TiLV comprise or consist of SEQ ID NO:11-20, respectively.

7. The method of claim **1**, wherein the fish-derived cells are E11 cells derived from *Channa striatus*.

8. The method of claim **1**, wherein the co-cultured Vero cells and fish-derived cells are Vero E6 cells and E11 cells.

9. A method for producing a reporter-tagged TiLV, comprising:

i) preparing an expression plasmid comprising cDNA sequence of one genomic segment of TiLV and a reporter tag sequence that is integrated following a certain number of 3' terminal nucleotides of the open reading frame (ORF) of the segment; and preparing a plurality of expression plasmids comprises cDNA sequences of at least one of the remaining nine genomic segments of TiLV;

ii) introducing all the expression plasmids prepared in step i) into co-cultured Vero cells and fish-derived cells that are susceptible to TiLV infection; and

iii) culturing the cells obtained in step ii) at a temperature around 28° C., thereby producing the reporter-tagged TiLV.

10. The method of claim **9**, wherein the expression plasmids are bi-directional expression plasmids which express all viral genomic RNAs (gRNAs) and viral mRNAs of TiLV.

11. The method of claim **9**, wherein cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:1-10, respectively.

12. The method of claim **9**, wherein cDNA sequences of the total ten genomic segments have at least 90% identity to SEQ ID NOs:11-20, respectively.

13. The method of claim **9**, wherein cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:1-10, respectively.

14. The method of claim **9**, wherein the cDNA sequences of the total ten genomic segments comprise or consist of SEQ ID NO:11-20, respectively.

15. The method of claim **9**, wherein the fish-derived cells are E11 cells derived from *Channa striatus*.

16. The method of claim **9**, wherein the co-cultured Vero cells and fish-derived cells are Vero E6 cells and E11 cells.

17. The method of claim **9**, wherein the reporter tag sequence is HiBIT, GFP, NanoLuc, or mCherry.

18. A reporter TiLV that is produced by the method according to claim **9**.

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