RECOMBINANT MUMPS VIRUS JERYL LYNN 2 BASED VACCINE

FIELD OF THE INVENTION

The present invention provides a novel vaccine against mumps composed of highly immunogenic $rMuV^{JL2}$ (recombinant mumps virus Jeryl Lynn2 based). Further, method to develop said immunogenic composition is described in the present invention. The vaccine according to the present invention is safe, cost effective, highly efficacious and stable and consistent in terms of productivity.

BACKGROUND OF THE INVENTION

Mumps is an acute viral illness characterized by fever and swelling of the parotid gland(s) that affects typically young children and may lead to complications such as orchitis, oophoritis, pancreatitis, and meningo-encephalitis. However, approximately half of infected individuals develop classical disease. Mumps virus (MuV) is a member of paramyxoviridae family, subfamily Paramyxovirinae and genus Rubulavirus, which only infects humans [Hviid et al., 2008]. MuV has a single-stranded, negative sense RNA genome consisting of 15,384 nucleotides. The genome encodes two surface glycoproteins; fusion (F) and haemagglutinin-neuraminidase (HN), four core proteins; nucleoprotein (NP), phospho (P), matrix (M) and large protein (L), and the membrane associated small hydrophobic (SH) protein.

Historically, mumps was considered as a disease of childhood but over the past 2 decades, it has affected older children and adults in the countries where mumps immunization has been in routine use. Despite high vaccination coverage, re-emergence of mumps has been reported in a number of countries including the USA, UK, Canada, the Republic of Moldova and The Netherlands [Bernard et al., 2008; Brockhoff et al., 2010; CDC, 2010; Whelan et al., 2010; Yung et al., 2010]. In India, scanty data are available regarding the epidemiology of mumps, its incidence and antibody prevalence. A few mumps outbreaks have been reported from the States of Kerala and Maharashtra [Geeta and Kumar, 2004; John, 2004; Ghatage and Kakade, 2007]. Mumps vaccine is given to children together with measles and rubella vaccines at or after 12 months of age. Two strains of live attenuated mumps virus have been used in vaccines in the U.K., namely the Urabe strain, derived from a wild-type Japanese isolate by passage in

egg amnion and the Jeryl Lynn strain, derived by tissue culture passage of wild-type American isolate. Vaccines other than those based on live attenuated virus strains are not available for mumps.

The Jeryl Lynn (JL) mumps virus (MuV) vaccine contains two different component strains, MuVJL5 and MuVJL2 that differ considerably in their nucleotide sequences (Amexis et al., 2002).

The MuVJL vaccine has been reported to be derived from a single clinical isolate (Afzal et al., 1993), which was converted into a live-attenuated vaccine by passage of virus in non-human host cells. The complete nucleotide sequences of MuVJL5 and MuVJL2 have been reported (Clarke et al., 2000; Amexis et al., 2002) and show 414 nucleotide changes, resulting in 87 amino acid changes, between MuVJL5 and MuVJL2. This level of variation is at the same level as differences between genotypes of MuV. There are biological differences between MuVJL5 and MuVJL2. For example, MuVJL2 grows better in embryonated eggs than MuVJL5, (Amexis et al., 2002). Both MuVJL5 and MuVJL2 are non-neurovirulent in the rat neurovirulence test (Rubin et al., 1999, 2000, 2003).

It is known that MuV growth is strain- as well as host cell dependent [Afzal et al, 1990].
It is also reported that a strong intrinsic feature of MuV strains to interact specifically with Vero cell culture results in very different forms of cytopathic effect (CPE). During viral infection of cell, virus replicative cycle is accompanied by a number of biochemical and morphological changes within the cell which usually culminate with cell death. These morphological changes are referred to as the virus cytopathic effect (CPE). CPE may take several forms, e.g. syncytia formation, cell rounding, disorientation, swelling or shrinking, detachment from the surface, total cell lysis, etc. The form of CPE depends both on the virus and on the cells in which it is grown. [Vaccine 28 (2010) 1887–1892]

It is mentioned in Amexis et al., 2002 that the MAPREC (a molecular method to determine ratio sub strains of Jeryl Lynn in various Jeryl Lynn preparations) data confirmed the results from the cloning experiments, that by the first passage of Merck MVL on Vero cells, the percentage of JL2 decreased from 20 to 2% and none could be detected in subsequent

passages, while, passaging of Jeryl Lynn strain in embryonated chicken eggs (ECE) resulted in the rapid accumulation of JL2 (over 90%) and loss of JL1, suggesting that JL2 has a strong selection advantage in ECE.

It is further concluded by the Author that the rapid disappearance of JL2 during virus passaging in Vero and CEF cells, as detected by cloning and MAPREC, suggests that JL2 replicates poorly in Vero and CEF cell cultures or may require the presence of JL1 as a helper.

From the above disclosure, it is well understood that rescue model development of JL2 strain by approaching Vero cell or CEF as a host cell is difficult for further development of vaccine. Several factors such as cytopathic effect, host cell, virus strain, combination of virus strain and host cell affect in case of development of immunogenic composition against Jeryl Lynn mumps virus.

In the present invention, Inventors have developed JL2 strain based immunogenic composition which can be further formulated along with the suitable stabilizer for vaccination purpose. Such an immunogenic composition according to present invention is having higher immunogenicity, free from extraneous proteins and other agents and finally a good candidate for the development of vaccine against mumps. In addition, such vaccine is more stable than the conventional vaccine probably due to higher genetic homogenicity of the strain.

Surprisingly, Inventors of the present invention are able to develop said stable immunogenic composition by using Vero cells as a host cell. Though, Vero cell is known as not a good candidate for JL2 strain development, surprisingly Inventors of the present invention have developed novel immunogenic composition against mumps virus using mammalian cells such as Vero cells or MRC-5 cells or HEK 293T cells.

OBJECT OF THE INVENTION

In first aspect the present invention provides stable Jeryl Lynn 2 (herein after as JL2) strain that can be used to produce a vaccine against mumps.

In a preferred aspect, a vector containing full length genome of isolated JL2 strain is

described.

In second aspect, the present invention provides a rescue process of JL2 strain in which JL2 strain propagate in different host cells. Here, host cells can be suitable mammalian cell which are stable and consistent in terms of quality and productivity.

In a preferred aspect, the present invention provides a rescue composition for recombinant mumps virus JL2 strain.

In third aspect, the present invention provides JL2 strain multiplying in mammalian cells in high yields. Such mammalian cells can be Vero cell, MRC-5 cell, HEK 293T cell or others like.

In one of the aspects, the present invention provides novel immunogenic composition protecting against mumps virus.

In further aspects, the immunogenic composition according to the present invention contains genetically homogenous mumps viruses derived from the Jeryl Lynn strain.

In a fifth aspect, the present invention provides a stable vaccine comprising said immunogenic composition protecting against mumps virus.

Such vaccine can be combined with measles, rubella and/ or varicella vaccine components to make combined vaccine.

In a sixth aspect, the present invention provides method for the development of said immunogenic composition.

SUMMARY OF THE INVENTION

The present invention provides a stable immunogenic composition derived from Jeryl Lynn 2 strain that can protect against mumps virus. Such an immunogenic composition is derived by using reverse genetics methodology.

Such an immunogenic composition is made by using various mammalian cells as a host to develop a stable vaccine against mumps virus.

Further, current invention provides method for the development of said highly immunogenic and stable composition.

DETAILED DESCRIPTION OF THE FIGURES

Figure 1 depicts chemically synthesized fragments of the genome of mumps virus Jeryl Lynn strain resolved on agarose gel. There are six fragments which covers full-length of genome of mumps virus attenuated JL2 strain. Here, combination of 1+2, 3+4 and 5+6 fragments are shown. It shows that fragments are appropriately obtained after the restriction digestion.

Figure 2 depicts restriction map of $pMuV^{JL2}$.

Figure 3 depicts syncytia formation in Vero cells after P0 passage.

Figure 4 depicts a) MRC5 cells without infection (control) b) MRC5 cells infected with $rMuV^{JL2}FL$.

Figure 5 depicts Vero cells fixed by TCA staining under UV light colored by crystal violet where A and B shows $rMuV^{JL2}$ syncytia.

SUMMARY OF SEQUENCES

Sequence 1 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *NotI* and *Rsr*II. Fragment 1 obtained from the digestion has position of 1-3576 nucleotide position of the full length DNA genome. (SEQ ID NO. 1)

Sequence 2 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *Rsr*II and *Avr*II. Fragment 2 obtained from the digestion has position of 3576 – 6258 nucleotide position of the full length DNA genome. (SEQ ID NO. 2)

Sequence 3 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *Avr*II and *Xho*I. Fragment 3 obtained from the digestion has position of 6258 - 8438 nucleotide position of the full length DNA genome. (SEQ ID NO. 3)

Sequence 4 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *Xho*I and *Kpn*I. Fragment 4 obtained from the digestion has position of 8438 - 10498 nucleotide position of the full length DNA genome. (SEQ ID NO. 4)

Sequence 5 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *Kpn*I and *Xma*I. Fragment 5 obtained from the digestion has position of 10498 - 13950 nucleotide position of the full length DNA genome. (SEQ ID NO. 5)

Sequence 6 is the nucleotide sequence of the first fragment obtained by the restriction digestion using restrictions sites *Xma*I and *Nar*I. Fragment 6 obtained from the digestion has position of 13950 - 15440 nucleotide position of the full length DNA genome. (SEQ ID NO. 6)

Sequence 7 is the complete nucleotide sequence of vector $pMuV^{JL2}$. (SEQ ID NO. 7)

Sequence 8 is the complete nucleotide sequence of rescued recombinant mumps virus $rMuV^{JL2}FL$. (SEQ ID NO. 8)

Sequence 9 is the nucleotide sequence encoding nucleoprotein (NP) of the mumps virus. (SEQ ID NO. 9)

Sequence 10 is the nucleotide sequence encoding phosphoprotein (P) of the mumps virus. (SEQ ID NO. 10)

Sequence 11 is the nucleotide sequence encoding large (L) protein of the mumps virus. (SEQ ID NO. 11)

Sequence 12 is the amino acid sequence of nucleoprotein (NP) of the mumps virus. (SEQ ID NO. 12)

Sequence 13 is the amino acid sequence of phospho protein (P) of the mumps virus. (SEQ ID NO. 13)

Sequence 14 is the amino acid sequence of large protein (L) of the mumps virus. (SEQ ID NO. 14)

Sequence 15 is the nucleotide sequence of pSC6-T7 encoding T7 RNA polymerase. (SEQ ID NO. 15)

DETAILED DESCRIPTION OF THE INVENTION

In one of the embodiments, the present invention provides stable JL2 strain based immunogenic composition that can be used to produce a vaccine against mumps. The isolated JL2 strain is genetically homogenous and highly immunogenic. The isolated JL2 strain disclosed here is safe and efficient in terms of antibody production against mumps virus.

In a further embodiment, the present invention provides full length genome of JL2 strain developed from reverse transcribed m-RNA of JL2 strain. Full length JL2 genome was synthesized chemically in six different fragments to cover the total genome length (15,384 bp) of mumps virus. Such chemically synthesized genome fragments can be amplified using suitable plasmid vectors (intermediate vectors). Full length genome can be synthesized in less than or more than six fragments illustrated here using restriction digestion method.

In one of the embodiment, the present invention provides method of cloning of chemically synthesized fragments of JL2 genome in suitable vector. Trans-acting viral proteins NP (nucleoprotein), P (phosphoprotein) and L (large protein) encoding genes of mumps virus and gene encoding T7 RNA polymerase are cloned into helper plasmids.

Trans-acting viral proteins can be defined as viral proteins essentially required for viral encapsidation, transcription and translation.

In a preferred embodiment, trans-acting viral proteins according to the present invention comprise nucleotide sequences as set forth in SEQ ID NO. 9 to 11.

In a more preferred embodiment, trans-acting viral proteins according to the present invention comprise amino acid sequences as set forth in SEQ ID NO. 12 to 14.

In a further embodiment, T7 RNA Polymerase containing vector according to the present invention comprises nucleotide sequence as set forth in SEQ ID NO. 15.

In yet another embodiment, according to the present invention vectors can be selected from pBluescript, pBluescript II, pBluescript II SK (+), pUC19, pCA, pSC6 and others like. These vectors are commercially available and known to the person skilled in the art.

In a second embodiment, a vector containing full length genome of isolated JL2 strain is described.

In preferred embodiment, the present invention provides method to construct plasmid comprising the full length genome of JL2 ($pMuV^{JL2}$) by step wise cloning of chemically synthesized fragments of JL2 genome using pBluescript II SK (+) as final transcription vector.

The transcription vector comprises an operably linked transcriptional unit comprising an assembly of a genetic element or elements having a regulatory role in the expression, for example, a promoter, a structural gene or coding sequence which is transcribed into mumps RNA, and appropriate transcription initiation and termination sequences. All cloning procedures of the present invention are basically as described by Sambrook and Russel (2001).

In a third embodiment, the present invention provides a process for rescue of JL2 strain in which JL2 strain propagate in suitable host cell(s). Here, host cells can be suitable mammalian cells or non-mammalian cells which are stable and consistent in terms of quality and productivity. It is already known that passaging of Jeryl Lynn strain in embryonated chicken eggs (ECE) resulted in the rapid accumulation of JL2 (over 90%) and loss of JL1, suggesting

that JL2 has a strong selection advantage in ECE. But, ECE cells are primary cells which are not consistent and reproducible. So, it becomes difficult to maintain desired titers of JL2 stain using such primary cells.

In further embodiment, the present invention provides production of an immunogenic composition exclusively by using mammalian cell. Such mammalian cell can be selected from Vero cell, MRC-5 cell, HEK 293T cell and other similar secondary or tertiary cell lines and non-mammalian cells such as *E.coli* and others like can be used. An immunogenic composition which is stable and sufficient immunogenic at a production level is not available in the art. Therefore, the immunogenic composition using such mentioned host which can be used for vaccine preparation against mumps virus is not available in the market. The present invention is expected to satisfy the current demand.

In a preferred embodiment, the present invention provides transfection of suitable mammalian host cells using plasmid containing full length mumps JL2 genome ($pMuV^{JL2}$) and helper plasmids containing genes encoding trans-acting viral proteins NP, P, L and plasmid containing T7 polymerase gene. Transfected cells are incubated to allow syncytia formation.

In a more preferred embodiment, according to the present invention, helper plasmids encoding Nucleoprotein (NP) denoted as p_N^{JL2}, phosphor protein (P) denoted as p_P^{JL2}, large protein (L) denoted as p_L^{JL2} and plasmid encoding T7 Polymerase denoted as pSC6-T7 (SEQ ID NO. 15) are used.

Plasmids expressing trans-acting viral proteins and T7 polymerase that are used to co-transfect the host cells along with $pMuV^{JL2}$ can be defined as helper plasmids. These are essential for viral encapsidation, transcription and translation.

Syncytia can be defined as multi-nucleated enlarge cells resulting from fusion of infected cell with neighboring cells mediated by expression of viral proteins.

These syncytia are used to infect a different mammalian host cells for viral propagation (passage P0). Viral progenies are rescued after P0 passage ($rMuV^{JL2}FL$). Passage can be

defined as sub culturing of rescued virus on host cells. Following the first passage, fresh mammalian cells are infected with passage P0 for subsequent passages (P1 and P2). After the final passage, rescued viruses are stored as viral stock (master seed).

In another embodiment, the present invention provides rescue composition comprising following elements:

a. Transcription vector comprising full-length genome of JL2 strain.
b. An expression vector comprises isolated gene(s) encoding trans-acting protein(s) wherein trans-acting protein is selected from NP protein, P protein, L protein and combination thereof.
c. RNA polymerase for appropriate transcription of full-length genome of JL2 strain.

Here, according to the present invention, RNA polymerase is preferably T7 RNA polymerase.

In yet another embodiment, in the present invention, rescued viruses are characterized by sequencing methods known in the art, more preferably primer walking sequencing method.

In a fourth embodiment, the present invention provides novel, stable and highly immunogenic composition comprising isolated JL2 strain that can be used for the development of vaccine against mumps virus.

Such an immunogenic composition comprises genetically homogenous JL2 strain. The stable immunogenic composition according to the present invention is free from MuVJL5 component strain which is a part of conventional vaccine against mumps virus. Thus, the present invention provides stable pure and highly immunogenic composition which can protect against mump virus antigen which is conventionally protected using combination of JL2 and JL5 strains.

In a preferred embodiment, the host cell is selected from Vero cells and MRC-5 cells.

As discussed earlier, conventional JL2 strain is known to grow well in primary chicken embryo derived cells. The drawbacks of using embryonated egg cells lies in the risk of residual egg derived proteins and other unknown extraneous agents including other viruses.

Such residual egg derived proteins may be cause of inducing undesired allergic reactions in the vaccinated individuals. Moreover, presence of unknown other viruses may induce an additional risk for the vaccine. Inventors of the present invention have developed a stable, homogenous and pure isolated JL2 strain and composition comprising it using Vero cells and MRC-5 cells that can be used for the development of stable vaccine against mumps virus.

In a fifth embodiment, the present invention provides a stable vaccine comprising genetically homogenous and heat stable JL2 strain.

In a further embodiment, the present vaccine provides combination vaccine containing measles, rubella, varicella and other live virus vaccine strains along with the Jeryl Lynn vaccine of the present invention.

In a sixth embodiment, the present invention provides method to develop said immunogenic composition using conventional reverse genetics methodology. It includes cloning method to develop full–length genome of JL2 strain of mumps virus. Such a full-length genome of JL2 strain is reverse transcribed from the original m-RNA of the isolated Jeryl Lynn strain. Stable syncytia formation will take place as a resultant cytopathic effect from the reverse transcribed JL2 strain of the present invention. Such syncytium obtained from isolated JL2 strain of the present invention is genetically homogenous and highly immunogenic. Such immunogenic vaccine strain can be grown in production cell culture using different mammalian cell lines. Inventors found cell substrates such as Vero cells, MRC-5 cells or HEK 293T cells as compared to CEF primary cells which are used in classical Jeryl Lynn vaccine, are better in cell propagation in multiple passages with lower cost of production.

The immunogenic composition obtained using mentioned mammalian cell lines is consistent in terms of quality and susceptibility to produce desired titers of JL2 stain while, it is not reproducible in case of CEF primary cells.

Moreover, the immunogenic composition according to the present invention helps to avoid risk of contamination of new adventitious agents from cell substrates by using suitable stable

secondary cell line which is not in case of any primary cells. It means the immunogenic composition as described herein is commercially viable.

The method to develop said immunogenic composition according to the present invention comprising cloning strategy which includes use of a suitable vector to transfect suitable host cell.

In one of the embodiment, the present invention provides a method for producing a recombinant mumps virus comprising following step:

a. Chemical synthesis of genome sequence fragments of mumps virus attenuated JL2;
b. Cloning of each obtained gene fragment into an intermediate vector for its amplification;
c. Construction of clone of full-length DNA genome of the mumps virus attenuated JL2 strain to obtain pMuVJL2 vector through ligation each gene fragment into single vector;
d. Co-transfection of host cell with pMuVJL2 and an expression vector comprising isolated gene encoding trans-acting protein wherein trans-acting protein is selected from NP protein, P protein and L protein to negative strand of mumps virus rescue;
e. Optionally, passaging of rescued mumps virus into host cell for its expansion.

In a furthermore embodiment, the present invention provides a pharmaceutical composition comprising an immunogenic composition with suitable pharmaceutically acceptable carrier or excipient.

In one of the embodiment, the present invention provides a pharmaceutical composition comprising an immunogenic composition with suitable buffer, stabilizer, tonicity agent, surfactant and cryoprotectant.

In one of the embodiments, buffer can be selected from Phosphate-buffer, histidine-buffer, citrate-buffer, succinate-buffer, acetate-buffer, arginine buffer, phosphate buffered saline, tromethamine buffer and others like, preferably phosphate buffer.

In one of the embodiments, stabilizers can be selected from amino acid(s), sugar(s), polyol(s) and their combination.

In one of the embodiments, amino acids can be selected from arginine, glycine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophane, methionine, serine, proline, cysteine / cystine and suitable combination thereof.

In the present invention, sugars can be selected from glucose, fructose, galactose, mannose, sorbose, ribose, deoxyribose, sucrose, trehalose, lactose, maltose, raffinose and suitable mixtures thereof, preferably trehalose or sorbitol or mannitol.

Further, polyol can be selected from mannitol, sorbitol, dextran, glycerol, arabitol, propylene glycol, polyethylene glycol and suitable combinations thereof, preferably sorbitol.

In one of the embodiments, suitable tonicity agents can be selected from sugar(s), salt(s) and their combination for said pharmaceutical composition.

In one of the embodiments, suitable salts can be selected from tonicity agent such as NaCl or KCl.or others like.

In the present invention, surfactant can be selected from polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers, alkylphenylpolyoxyethylene ethers, polyoxyethylene-polyoxypropylene copolymer and sodium dodecyl sulphate (SDS) and combination thereof.

Further, cryoprotactant can be selected from sugars and others like.

In further embodiment, the present invention provides an immunogenic composition containing recombinant JL2 (rMuVJL2FL) with suitable adjuvant.

An adjuvant can be defined as a substance that is added to a vaccine to increase the body's immune response to the vaccine.

Adjuvants according to the present invention can be selected from salts of aluminium, Mono Phosphoryl Lipid (MPL) analogues such as GLA (glucopyranosyl lipid adjuvant), monatide, cytokine inducers, and squalene based adjuvants, lipophilic adjuvants and others like.

In a preferred embodiment, the present invention provides vaccines containing immunogenic composition of the present invention against Jeryl Lynn. These vaccines can be administered in conventional routes and dosages.

Analytical methods used in present invention

1. RT-PCR: Reverse transcription polymerase chain reaction (RT-PCR) is the method used for synthesis and amplification of complementary DNA by reverse transcribing the RNA. RT-PCR uses reverse transcriptase enzyme and a primer to anneal and extend a desired RNA sequence. If the RNA is present, the reverse transcriptase and primer will anneal to the RNA sequence and transcribe a complimentary strand of DNA (cDNA). This strand is then replicated with primers and Taq Polymerase, and the standard PCR protocol is followed. This protocol copies the single stranded DNA millions of times in a small amount of time to produce a significant amount of DNA. The PCR products (the cDNA strands) are then separated with agarose gel electrophoresis.

2. Agarose gel electrophoresis: Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of DNA or proteins in a matrix of agarose.

3. Primer walking sequencing method: Primer walking is a sequencing method of choice for sequencing large DNA fragments. Fragments which are too long to be sequenced in a single sequence read using the chain termination method, this method works by dividing the long sequence into several consecutive short ones. The term "primer walking" is used where the main aim is to sequence the genome.

4. Multiple sequence analysis: Multiple sequence analysis is a method used for analysis of

biological sequences to determine the region of similarity and difference between the sequences which are aligned for analysis.

EXAMPLES

The following non-limiting examples describe rescue of JL2 from genomic and structural plasmids co-transfection to suitable mammalian cell. Further, isolation of pure and genetically homogenous JL2 strain by using limiting dilution method is described herein below. It will be appreciated that other immunogenic compositions with different host cells can be prepared and such immunogenic compositions are within the scope of a person skilled in the art and are to be included within the scope of the present invention.

Example 1: Construction of the clone of full-length DNA genome of the mumps virus attenuated JL2 strain

Generation of Full length cDNA of mumps JL2

Six genome sequence fragments were chemically synthesized (GeneArt-Thermo Fisher Scientific) to cover the total genome length (15,384 bp) of the mumps virus. Subsequently, each genome fragment and vector was cut by specific restriction endonuclease (New England Bio Labs) and cloned in an intermediate vectors. Each fragment was verified by resolving them on agarose gel as shown in Figure 1. Fragments obtained after restriction digestion have been described below:

Fragment 1: pos. 1 – 3576 *Not*I-*Rsr*II (SEQ ID NO.1);
Fragment 2: pos. 3576 – 6258 *Rsr*II-*Avr*II (SEQ ID NO. 2);
Fragment 3: pos. 6258 - 8438 *Avr*II-*Xho*I (SEQ ID NO.3);
Fragment 4: pos. 8438 - 10498 *Xho*I-*Kpn*I (SEQ ID NO. 4);
Fragment 5: pos. 10498 - 13950 *Kpn*I-*Xma*I (SEQ ID NO. 5);
Fragment 6: pos. 13950 - 15440 *Xma*I-*Nar*I (SEQ ID NO. 6)

Restriction sites mentioned in the present example are well known to the skilled person. A person skilled in the art can use alternative restriction sites as per his requirement.

Cloning of obtained fragments into transcription vector and ligation of fragments

Chemically synthesized and restriction endonuclease treated fragments were incorporated into the plasmid by stepwise cloning procedures, using a modified pBluescript II SK (+) as final vector. Progressive ligation of the fragments to each other through compatible cohesive ends led to the definitive $pMuV^{JL2}$ plasmid. Vector map of $pMuV^{JL2}$ plasmid has been depicted in Figure 2. The plasmid $pMuV^{JL2}$ has nucleotide sequence as set forth in SEQ ID NO. 7. Helper plasmids containing genes for structural proteins of mumps virus and plasmid containing T7 RNA polymerase gene were generated by cloning in suitable vector. Here, pSC6 vector has been used for cloning of genes encoding trans-acting proteins and T7 RNA polymerase. Cloning as described in the present application has been carried out as described in Sambrook and Russel (2001).

Example 2: Rescue of attenuated mumps Jeryl-Lynn 2 virus

Commercially available HEK 293T cells were transfected by $pMuV^{JL2}$ together with helper plasmids p_N^{JL2}, p_P^{JL2}, p_L^{JL2} and pSC6-T7. HEK 293T cells were seeded into a 35mm well to reach ~ 50-70% confluence when being transfected. Confluence is the term commonly used as an estimate of the number of adherent cells in a culture dish or a flask, referring to the proportion of the surface which is covered by cells monolayer. 4 hour before transfection, the medium was replaced with 3 ml DMEM containing 10% FCS. All recombinant plasmids were prepared according to the QIAGEN plasmid preparation kit. The kit for the Ca^{2+} phosphate co-precipitation of DNA was from Invitrogen.

Cells were co-transfected with the plasmids in the following final concentration:

p_N^{JL2}	0.5 µg,
p_P^{JL2}	0.1 µg,
p_L^{JL2}	0.5 µg,
pSC6-T7	1.0 µg,
$pMuV^{JL2}$	5.0 µg

Mentioned plasmids diluted in H2O were added in an Eppendorf tube containing 2M $CaCl_2$, the mix was added to another Eppendorf tube containing HEPES buffer under shaking conditions, and was incubated 30 min at room temperature (RT). The co-precipitates were added drop wise to the culture and the transfection was carried out at 37°C and 5% CO2 for about 18 hour. Subsequently, the transfection medium was replaced with 3.0 ml of DMEM without FCS. Transient expression of the T7 polymerase permits the efficient protein expression of T7-promoted genes, finally led to negative strand of mumps virus rescue. Transfected cells were monitored for about 15 days till syncytia formation. Syncytia formation from single plaque has been shown in Figure 3. Single syncytia was picked up to infect WHO-approved Vero cells to establish passage 0 (P0) of the virus.

The rescued recombinant attenuated virus is named as **rMuV^{JL2}FL**. The nucleotide sequence of rMuV^{JL2}FL is as set forth in SEQ ID NO. 8.

Analytical sequencing of rescued mumps virus

After about 15 days post transfection, as soon as first syncytia appeared, single clone was aspirated under the microscope and subsequently used for characterization. Characterization was carried out by sequencing of the rescued rMuV^{JL2}FL virus genome. Viral RNA from rMuV^{JL2}FL infected Vero cells has been prepared following Qiagen RNeasy Mini kit. Starting from 2.0 µl of extracted viral RNA, reverse transcription and amplification have been sequentially carried out, following Qiagen OneStep RT-PCR kit instructions.

34 couples of primers, covering the full length genome sequence (15,384 bp) have been designed in order to amplify overlapping PCR fragments. Purified PCRs have been sequenced following a primer walking sequencing procedure.

Value Read sequences have been assembled covering the entire rMuV^{JL2}FL viral genome. 6 point mutations have been detected in the L gene of the rescued rMuV^{JL2}FL with respect to the sequence of the original plasmid pMuV^{JL2}, and confirmed by multiple sequence analysis.

Nucleotide position	Position of mutation in codon	$pMuV^{JL2}$ (Synthetic)	$rMuV^{JL2}FL$ (Rescued)	Amino acid change
10441	3	T	C	None
11467	3	T	C	None
11603	1	T	C	Silent
11809	3	T	C	None
12884	1	A	G	Ser to Gly
12981	2	A	G	Gln to Arg

Table-1: Point mutations and amino acid change in the L gene of the rescued $rMuV^{JL2}FL$

Example 3: Expansion of rescued mumps JL2 virus

P0 passage of the rescued $rMuV^{JL2}FL$ virus was propagated in WHO-approved Vero cells. Vero cells, maintained as monolayers in DMEM supplemented with 5% FBS and when confluence reached at 70-80%, Vero cells have been infected by P0 passage of the rescued $rMuV^{JL2}FL$ virus using 0.5-1.0 mL of the rescued virus in a 5.0 mL total volume of OPTIMEM. Cells were incubated for 1 hour at 37°C and 5% CO2. After incubation, the inoculum was replaced by DMEM + 5% FBS. Infected cells have been split after 2 days, and 90-100% syncytia formation was observed after 5-dpi. Cell-free (CF) and cell-associated (CA) fractions have been collected to obtain lab-scale viral stocks, reaching titers between 1×10^4 pfu/mL in the collected cell-free fractions (CF) and 1×10^6 pfu/mL in the collected cell-associated fractions (CA).

Example 4: Preparation of master cell bank of rescued mumps JL2 virus and production of single virus harvest

Rescued mumps virus from P0 passage was further passaged till passage 2 (P2) in Vero cells to obtain sufficient master seed.

Titers obtained after each passage in Vero cells is given below:

P0 passage: titers between 1 x 10^3 pfu/mL (CF, Cell-free fraction) - 1 x 10^4 pfu/mL (CA, Cell-associated fraction).

P1 passage: titers between 1 x 10^4 pfu/mL (CF, Cell-free fraction) - 5 x 10^4 pfu/mL (CA, Cell-associated fraction).

P2 passage: titers between 1 x 10^4 pfu/mL (CF, Cell-free fraction) - 1 x 10^6 pfu/mL (CA, Cell-associated fraction).

Such master seed is stored at a temperature between -150°C to -196°C. This master seed will be used to produce working seed for the preparation of commercial vaccine for human use. Working seed obtained from the master seed is used for production of single virus harvest in Vero cells. Working seed obtained from the master seed can be used for production of single virus harvest in MRC5 cells. Production of single virus harvest in MRC5 cell is also illustrated herein below.

Production of single virus harvest in MRC5 cells

Propagation of cells

The media was removed and cells were washed with MEM EBS media and TrypLE solution was added (Trypsin replacement) at 1ml/ T75 cm2 and 10ml/RB 850 cm2 and was incubated at 35±2°C. Cells were re-suspended after detachment in fresh medium and were seeded into new Culture flasks and roller bottles. The fully confluent T75 flask was split at a ratio of 1:4 to 1:16in T 75, T 175 and RB 850 roller bottles respectively in subsequent passages depending upon the batch size. Cells were split once a week and after attaining sufficient confluence. Subsequently, cells were used for virus inoculation.

Single virus harvest production

Single virus harvest – Mumps was produced by infecting MRC-5 cells with working virus seed and incubating at 33 ± 2°C for 60-120 minutes for virus adsorption. After adsorption, each infected roller bottle was replenished with 250-300mL of Creek's Minimal Medium (CMM) and incubated at 33±2°C at 0.5 rpm for 40 to 70 hrs.in roller apparatus. After completion of the incubation, all the roller bottles were observed for clarity and detachment of monolayer. Monolayer was washed with plain MEM media and replenished with 100-200 mL

plain MEM media. The bottles were again incubated at 33 ± 2 °C at 0.5 rpm for 5 to 13 days in roller apparatus. At the end of incubation all the roller bottles were observed for the clarity and presence of cytopathic effects and harvest was collected from all roller bottles in a single container. Human serum albumin was used as stabilizing agent for collected Virus harvest. The same was then distributed in individual pre sterilized PETG bottles as single harvest. The Bulk thus obtained was clarified and diluted to required concentration to produce Mumps vaccine. MRC5 cells without infection (control) and MRC5 cells infected with $rMuV^{JL2}FL$ are shown as (a) and (b) respectively in Figure 4.

The Produces Mumps Vaccine (JL) was tested for potency and found to be in range of $10^{4.12}$/ml to $10^{4.57}$/ml $CCID_{50}$ (Cell Culture Infectious Dose which infect 50% of the cultured cells) tested by cell culture method.

Example 5: Identification and quantification of mumps JL2 strain by plaque assay and in situ immunofluorescence

Virus titration by plaque assay

Serial ten-fold dilutions of virus preparations were carried out using OPTIMEM to a final volume of 0.5 ml. Each dilution was added on 35 mm Vero cell cultures. After 1 h of virus adsorption, the inoculum was removed and the infected cells were overlaid with 2.0 ml of DMEM containing 5% FCS and 1% low melting point agarose (LMP agarose). After 5 days of incubation at 37°C and 5% CO2, cultures were fixed with 1ml of 10% TCA for 1 h, then UV cross-linked for 30 min. After removal of the agarose overlay, cell monolayers were stained with crystal violet dissolved in 4% ethanol, washed with water and the plaques were counted under the inverted microscope. Microscopic image is given as Figure 5.

In situ immunofluorescence

The various ten-fold dilutions of master seed were prepared from the master seed virus in MEM (minimum Essential Medium) medium containing 2% FBS (Fetal Bovine Serum). Subsequently, Vero cell suspension was prepared at a concentration range between 0.15-0.20 million/mL. This cell suspension was poured in 96-well plate over above virus dilutions and kept it at 37 °C with 5% CO2. The plate was incubated under same condition for ten days.

After ten days, the cell supernatant from the plate was discarded and washed with PBS (Phosphate Buffer Saline). The cells in plate were fixed with acetone methanol fixation buffer. Anti-Mumps IgG antibodies conjugated with FITC (fluorescein isothiocyanate) was added in working dilution of 1:50. Virus titer was determined by counting the stained cells under fluorescence microscope using Spearman Karber formula.

The Spearman-Kärber formula:

$$\log 10 \text{ (end-point dilution)} = x0 - \frac{d}{2} + d\sum\frac{ri}{ni}$$

x0 = (log10 of the lowest dilution with all wells positive) d = log10 of the dilution step, one in this case ni = number of replicates, six in this case ri = number of positive wells

Example 6: Formulation of Immunogenic composition

Required quantity of Single harvest Of Mumps Vaccine was calculated to get the target titer of 1000 $CCID_{50}$. Similarly required quantity of raw material (Stabilizer) was calculated to formulate the vaccine of desired batch size according to the composition of finished product. Stabilizer 176 (Combination mixture of Gelatin Sorbitol & other amino acids) & M199 (diluent) as per calculated quantity was transferred in to a sterile container and to that calculated amount of Single harvest Of Mumps Vaccine was added and mixed.

The final formulation is as described below:

Material Name	Single dose Qty. / dose	Used as
Single harvest Mumps	≥ 1000 $CCID_{50}$	Antigen
Stabilizer 176	0.25 ml	Stabilizer
Medium – M199	Q.S.	Diluent

The final formulation was filled 0.5ml/vials are half stoppered and lyophilized with a standard cycle where quick freezing to -60^0 C, primary drying was done at -40^{0}C and secondary drying at 37 ^{0}C . The round intact cake was found which reconstitutes in less than 1 minutes and moisture test comes under 1.6% - 2 %.

The Produced Mumps Vaccine (JL) Vaccine was been tested for potency and found to be in range of 10^3/dose to $10^{3.57}$/ml $CCID_{50}$ tested by cell culture method.

The immunogenic composition of the present invention is tested for determination of immunogenicity of rescued mumps virus by standard immune assay methods. Examples of such immune assay method are ELISA (Enzyme Linked Immunosorbent Assay) and other standard methods used for determination of mumps virus neutralizing antibody. Such assay can be used to determine immunogenicity of rescued virus in non-primates or primates. Such studies can be useful to determine optimum dose and dose regime for the said vaccine. The immunogenic composition of the present invention having rescued mumps virus is found to be suitable for further vaccine preparation. The process for producing rescued mumps virus according to the present invention does not involve use of any element from any other viral or non-viral pathogenic entity. Thus, the rescued mumps virus is stable and homogenous and therefore devoid of pathogenic particles other than mumps virus. Therefore rescued mumps virus according to the present invention is suitable candidate for vaccine development.

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<211> 549
<212> PRT
<213> Mumps Virus

<400> 12

Met Ser Ser Val Leu Lys Ala Phe Glu Arg Phe Thr Ile Glu Gln Glu
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Leu Gln Asp Arg Gly Glu Glu Gly Ser Ile Pro Pro Glu Thr Leu Lys
20 25 30

Ser Ala Val Lys Val Phe Val Ile Asn Thr Pro Asn Pro Thr Thr Arg
35 40 45

Tyr Gln Met Leu Asn Phe Cys Leu Arg Ile Ile Cys Ser Gln Asn Ala
50 55 60

Arg Ala Ser His Arg Val Gly Ala Leu Ile Thr Leu Phe Ser Leu Pro
65 70 75 80

Ser Ala Gly Met Gln Asn His Ile Arg Leu Ala Asp Arg Ser Pro Glu
85 90 95

Ala Gln Ile Glu Arg Cys Glu Ile Asp Gly Phe Glu Pro Gly Thr Tyr
100 105 110

Arg Leu Ile Pro Asn Ala Arg Ala Asn Leu Thr Ala Asn Glu Ile Ala
115 120 125

Ala Tyr Ala Leu Leu Ala Asp Asp Leu Pro Pro Thr Ile Asn Asn Gly
130 135 140

Thr Pro Tyr Val His Ala Asp Val Glu Gly Gln Pro Cys Asp Glu Ile
145 150 155 160

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Glu Gln Phe Leu Asp Arg Cys Tyr Ser Val Leu Ile Gln Ala Trp Val
                165                 170                 175

Met Val Cys Lys Cys Met Thr Ala Tyr Asp Gln Pro Ala Gly Ser Ala
            180                 185                 190

Asp Arg Arg Phe Ala Lys Tyr Gln Gln Gln Gly Arg Leu Glu Ala Arg
        195                 200                 205

Tyr Met Leu Gln Pro Glu Ala Gln Arg Leu Ile Gln Thr Ala Ile Arg
    210                 215                 220

Lys Ser Leu Val Val Arg Gln Tyr Leu Thr Phe Glu Leu Gln Leu Ala
225                 230                 235                 240

Arg Arg Gln Gly Leu Leu Ser Asn Arg Tyr Tyr Ala Met Val Gly Asp
                245                 250                 255

Ile Gly Lys Tyr Ile Glu Asn Ser Gly Leu Thr Ala Phe Phe Leu Thr
            260                 265                 270

Leu Lys Tyr Ala Leu Gly Thr Lys Trp Ser Pro Leu Ser Leu Ala Ala
        275                 280                 285

Phe Thr Gly Glu Leu Thr Lys Leu Arg Ser Leu Met Met Leu Tyr Arg
    290                 295                 300

Asp Leu Gly Glu Gln Ala Arg Tyr Leu Ala Leu Leu Glu Ala Pro Gln
305                 310                 315                 320

Ile Met Asp Phe Ala Pro Gly Gly Tyr Pro Leu Ile Phe Ser Tyr Ala
                325                 330                 335

Met Gly Val Gly Thr Val Leu Asp Val Gln Met Arg Asn Tyr Thr Tyr
            340                 345                 350

Ala Arg Pro Phe Leu Asn Gly Tyr Tyr Phe Gln Ile Gly Val Glu Thr
        355                 360                 365

Ala Arg Arg Gln Gln Gly Thr Val Asp Asn Arg Val Ala Asp Asp Leu
    370                 375                 380

Gly Leu Thr Pro Glu Gln Arg Thr Glu Val Thr Gln Leu Val Asp Arg
385                 390                 395                 400

Leu Ala Arg Gly Arg Gly Ala Gly Ile Pro Gly Gly Pro Val Asn Pro
                405                 410                 415

Phe Val Pro Pro Val Gln Gln Gln Gln Pro Ala Ala Val Tyr Glu Asp
            420                 425                 430
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Ile Pro Ala Leu Glu Glu Ser Asp Asp Asp Gly Asp Glu Asp Arg Gly
        435                 440                 445

Ala Gly Phe Gln Asn Gly Val Gln Val Pro Ala Val Arg Gln Gly Gly
    450                 455                 460

Gln Thr Asp Phe Arg Ala Gln Pro Leu Gln Asp Pro Ile Gln Ala Gln
465                 470                 475                 480

Leu Phe Met Pro Leu Tyr Pro Gln Val Ser Asn Ile Pro Asn Asn Gln
                485                 490                 495

Asn His Gln Ile Asn Arg Ile Gly Gly Leu Glu Asn Gln Asp Leu Leu
            500                 505                 510

Arg Tyr Asn Glu Asn Gly Asp Ser Gln Gln Asp Ala Arg Gly Glu His
        515                 520                 525

Gly Asn Thr Phe Pro Asn Asn Pro Asn Gln Asn Ala Gln Leu Gln Val
    530                 535                 540

Gly Asp Trp Asp Glu
545
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<210> 13
<211> 391
<212> PRT
<213> Mumps Virus

<400> 13

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Met Asp Gln Phe Ile Lys Gln Asp Glu Thr Gly Asp Leu Ile Glu Thr
1               5                   10                  15

Gly Met Asn Val Ala Asn His Phe Leu Ser Ala Pro Ile Gln Gly Thr
            20                  25                  30

Asn Ser Leu Ser Lys Ala Ser Ile Ile Pro Gly Val Ala Pro Val Leu
        35                  40                  45

Ile Gly Asn Pro Glu Gln Lys Asn Ile Gln His Pro Thr Ala Ser His
    50                  55                  60

Gln Gly Ser Lys Ser Lys Gly Arg Gly Ser Gly Val Arg Ser Ile Ile
65                  70                  75                  80

Val Pro Pro Ser Glu Ala Gly Asn Gly Gly Thr Gln Ile Pro Glu Pro
                85                  90                  95

Leu Phe Ala Gln Thr Gly Gln Gly Gly Ile Val Thr Thr Val Tyr Gln
            100                 105                 110
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Asp Pro Thr Ile Gln Pro Thr Gly Ser Tyr Arg Ser Val Glu Leu Ala
115 120 125

Lys Ile Gly Lys Glu Arg Met Ile Asn Arg Phe Val Glu Lys Pro Arg
130 135 140

Thr Ser Thr Pro Val Thr Glu Phe Lys Arg Gly Gly Pro Gly Ala Ala
145 150 155 160

Ala Gln Gly Gln Thr Ile Gln Glu Glu Gly Ile Asp Gly Asn Gly Ala
165 170 175

Ser Ala Gly Ser Lys Glu Arg Ser Gly Ser Leu Ser Gly Ala Thr Leu
180 185 190

Tyr Ala His Leu Ser Leu Pro Gln Gln Asp Ser Thr Pro Ala Asn Val
195 200 205

Gly Ile Ala Pro Gln Ser Ala Thr Ser Ala Asn Glu Ile Met Asp Leu
210 215 220

Leu Arg Gly Met Asp Ala Arg Leu Gln His Leu Glu Gln Lys Val Asp
225 230 235 240

Lys Val Leu Ala Gln Gly Ser Met Val Thr Gln Ile Lys Asn Glu Leu
245 250 255

Ser Thr Val Lys Thr Thr Leu Ala Thr Ile Glu Gly Met Met Ala Thr
260 265 270

Val Lys Ile Met Asp Pro Gly Asn Pro Thr Gly Val Pro Val Asp Glu
275 280 285

Leu Arg Arg Ser Phe Ser Asp His Val Thr Ile Val Ser Gly Pro Gly
290 295 300

Asp Val Ser Phe Ser Ser Arg Glu Glu Pro Thr Leu Tyr Leu Asp Glu
305 310 315 320

Leu Ala Arg Pro Val Ser Lys Pro Arg Pro Ala Lys Gln Thr Lys Pro
325 330 335

Gln Pro Val Lys Asp Leu Ala Gly Arg Lys Val Met Ile Thr Lys Met
340 345 350

Ile Thr Asp Cys Val Ala Asn Pro Gln Met Lys Gln Ala Phe Glu Gln
355 360 365

Arg Leu Ala Lys Ala Ser Thr Glu Asp Ala Leu Asn Asp Ile Lys Lys
370 375 380

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Asp Ile Ile Arg Ser Ala Ile
385                 390


<210>  14
<211>  2261
<212>  PRT
<213>  Mumps Virus

<400>  14

Met Ala Gly Leu Asn Glu Ile Leu Leu Pro Glu Val His Leu Asn Ser
1               5                   10                  15

Pro Ile Val Arg Tyr Lys Leu Phe Tyr Tyr Ile Leu His Gly Gln Leu
            20                  25                  30

Pro Asn Asp Leu Glu Pro Asp Asp Leu Gly Pro Leu Ala Asn His Asn
        35                  40                  45

Trp Lys Ala Ile Arg Ala Glu Glu Ser Gln Val His Ala Arg Leu Lys
    50                  55                  60

Gln Ile Arg Val Glu Leu Ile Ala Arg Ile Pro Ser Leu Arg Trp Thr
65                  70                  75                  80

Arg Ser Gln Arg Glu Ile Ala Ile Leu Ile Trp Pro Arg Ile Leu Pro
                85                  90                  95

Ile Leu Gln Ala Tyr Asp Leu Arg Gln Ser Met Gln Leu Pro Thr Val
            100                 105                 110

Trp Glu Lys Leu Thr Gln Ser Thr Val Asn Leu Ile Ser Asp Gly Leu
        115                 120                 125

Glu Arg Val Val Leu His Ile Ser Asn Gln Leu Thr Gly Lys Pro Asn
    130                 135                 140

Leu Phe Thr Arg Ser Arg Ala Gly Gln Asp Thr Lys Asp Tyr Ser Ile
145                 150                 155                 160

Pro Ser Thr Arg Glu Leu Ser Gln Ile Trp Phe Asn Asn Glu Trp Ser
                165                 170                 175

Gly Ser Val Lys Thr Trp Leu Met Ile Lys Tyr Arg Met Arg Gln Leu
            180                 185                 190

Ile Thr Asn Gln Lys Thr Gly Glu Leu Thr Asp Leu Val Thr Ile Val
        195                 200                 205

Asp Thr Arg Ser Thr Leu Cys Ile Ile Thr Pro Glu Leu Val Ala Leu
    210                 215                 220
```

Tyr Ser Asn Glu His Lys Ala Leu Thr Tyr Leu Thr Phe Glu Met Val
225 230 235 240

Leu Met Val Thr Asp Met Leu Glu Gly Arg Leu Asn Val Ser Ser Leu
245 250 255

Cys Thr Ala Ser His Tyr Leu Ser Pro Leu Lys Lys Arg Ile Glu Val
260 265 270

Leu Leu Thr Leu Val Asp Asp Leu Ala Leu Leu Met Gly Asp Lys Val
275 280 285

Tyr Gly Ile Val Ser Ser Leu Glu Ser Phe Val Tyr Ala Gln Leu Gln
290 295 300

Tyr Gly Asp Pro Val Ile Asp Ile Lys Gly Thr Phe Tyr Gly Phe Ile
305 310 315 320

Cys Asn Glu Ile Leu Asp Leu Leu Thr Glu Gly Asn Ile Phe Thr Glu
325 330 335

Glu Glu Ala Asn Lys Val Leu Leu Asp Leu Thr Ser Gln Phe Asp Asn
340 345 350

Leu Ser Pro Asp Leu Thr Ala Glu Leu Leu Cys Ile Met Arg Leu Trp
355 360 365

Gly His Pro Thr Leu Thr Ala Ser Gln Ala Ala Ser Lys Val Arg Glu
370 375 380

Ser Met Cys Ala Pro Lys Val Leu Asp Phe Gln Thr Ile Met Lys Thr
385 390 395 400

Leu Ala Phe Phe His Ala Ile Leu Ile Asn Gly Tyr Arg Arg Ser His
405 410 415

Asn Gly Ile Trp Pro Pro Thr Thr Leu His Gly Asn Ala Pro Lys Ser
420 425 430

Leu Ile Glu Met Arg His Asp Asn Ser Glu Leu Lys Tyr Glu Tyr Val
435 440 445

Leu Lys Asn Trp Lys Ser Ile Ser Met Leu Arg Ile His Lys Cys Phe
450 455 460

Asp Ala Ser Pro Asp Glu Asp Leu Ser Ile Phe Met Lys Asp Lys Ala
465 470 475 480

Ile Ser Cys Pro Lys Gln Asp Trp Met Gly Val Phe Arg Arg Ser Leu
485 490 495

Ile Lys Gln Arg Tyr Arg Asp Val Asn Arg Pro Leu Pro Gln Pro Phe
500 505 510

Asn Arg Arg Leu Leu Leu Asn Phe Leu Glu Asp Asp Arg Phe Asp Pro
515 520 525

Ile Lys Glu Leu Glu Tyr Val Thr Ser Gly Glu Tyr Leu Arg Asp Pro
530 535 540

Glu Phe Cys Ala Ser Tyr Ser Leu Lys Glu Lys Glu Ile Lys Ala Thr
545 550 555 560

Gly Arg Ile Phe Ala Lys Met Thr Lys Arg Met Arg Ser Cys Gln Val
565 570 575

Ile Ala Glu Ser Leu Leu Ala Asn His Ala Gly Lys Leu Met Arg Glu
580 585 590

Asn Gly Val Val Leu Asp Gln Leu Lys Leu Thr Lys Ser Leu Leu Thr
595 600 605

Met Asn Gln Ile Gly Ile Ile Ser Glu His Ser Arg Arg Ser Thr Ala
610 615 620

Asp Asn Met Thr Leu Ala His Ser Gly Ser Asn Lys His Arg Ile Asn
625 630 635 640

Asn Ser Gln Phe Lys Lys Asn Lys Asp Asn Lys His Glu Met Pro Asp
645 650 655

Asp Gly Phe Glu Ile Ala Ala Cys Phe Leu Thr Thr Asp Leu Thr Lys
660 665 670

Tyr Cys Leu Asn Trp Arg Tyr Gln Val Ile Ile Pro Phe Ala Arg Thr
675 680 685

Leu Asn Ser Met Tyr Gly Ile Pro His Leu Phe Glu Trp Ile His Leu
690 695 700

Arg Leu Met Arg Ser Thr Leu Tyr Val Gly Asp Pro Phe Asn Pro Pro
705 710 715 720

Ser Asp Pro Thr Gln Leu Asp Leu Asp Thr Ala Leu Asn Asp Asp Ile
725 730 735

Phe Ile Val Ser Pro Arg Gly Gly Ile Glu Gly Leu Cys Gln Lys Leu
740 745 750

Trp Thr Met Ile Ser Ile Ser Thr Ile Ile Leu Ser Ala Thr Glu Ala
755 760 765

Asn Thr Arg Val Met Ser Met Val Gln Gly Asp Asn Gln Ala Ile Ala
770 775 780

Ile Thr Thr Arg Val Val Arg Ser Leu Ser His Ser Glu Lys Lys Glu
785 790 795 800

Gln Ala Tyr Lys Ala Ser Lys Leu Phe Phe Glu Arg Leu Arg Ala Asn
805 810 815

Asn His Gly Ile Gly His His Leu Lys Glu Gln Glu Thr Ile Leu Ser
820 825 830

Ser Asp Phe Phe Ile Tyr Ser Lys Arg Val Phe Tyr Lys Gly Arg Ile
835 840 845

Leu Thr Gln Ala Leu Lys Asn Val Ser Lys Met Cys Leu Thr Ala Asp
850 855 860

Ile Leu Gly Asp Cys Ser Gln Ala Ser Cys Ser Asn Leu Ala Thr Thr
865 870 875 880

Val Met Arg Leu Thr Glu Asn Gly Val Glu Lys Asp Leu Cys Tyr Phe
885 890 895

Leu Asn Ala Phe Met Thr Ile Arg Gln Leu Cys Tyr Asp Leu Val Phe
900 905 910

Pro Gln Thr Lys Ser Leu Ser Gln Asp Ile Thr Asn Ala Tyr Leu Asn
915 920 925

His Pro Ile Leu Ile Ser Arg Leu Cys Leu Leu Pro Ser Gln Leu Gly
930 935 940

Gly Leu Asn Phe Leu Ser Cys Ser Arg Leu Phe Asn Arg Asn Ile Gly
945 950 955 960

Asp Pro Leu Val Ser Ala Ile Ala Asp Val Lys Arg Leu Ile Lys Ala
965 970 975

Gly Cys Leu Asp Ile Trp Val Leu Tyr Asn Ile Leu Gly Arg Arg Pro
980 985 990

Gly Lys Gly Lys Trp Ser Thr Leu Ala Ala Asp Pro Tyr Thr Leu Asn
995 1000 1005

Ile Asp Tyr Leu Val Pro Ser Thr Thr Phe Leu Lys Lys His Ala
1010 1015 1020

Gln Tyr Thr Leu Met Glu Arg Ser Val Asn Pro Met Leu Arg Gly
1025 1030 1035

Val Phe Ser Glu Asn Ala Ala Glu Glu Glu Glu Glu Leu Ala Gln
1040 1045 1050

Tyr Leu Leu Asp Arg Glu Val Val Met Pro Arg Val Ala His Val
1055 1060 1065

Ile Leu Ala Gln Ser Ser Cys Gly Arg Arg Lys Gln Ile Gln Gly
1070 1075 1080

Tyr Leu Asp Ser Thr Arg Thr Ile Ile Arg Tyr Ser Leu Glu Val
1085 1090 1095

Arg Pro Leu Ser Ala Lys Lys Leu Asn Thr Val Ile Glu Tyr Asn
1100 1105 1110

Leu Leu Tyr Leu Ser Tyr Asn Leu Glu Ile Ile Glu Lys Pro Asn
1115 1120 1125

Ile Val Gln Pro Phe Leu Asn Ala Ile Asn Val Asp Thr Cys Ser
1130 1135 1140

Ile Asp Ile Ala Arg Ser Leu Arg Lys Leu Ser Trp Ala Thr Leu
1145 1150 1155

Leu Asn Gly Arg Pro Ile Glu Gly Leu Glu Thr Pro Asp Pro Ile
1160 1165 1170

Glu Leu Val His Gly Cys Leu Ile Ile Gly Ser Asp Glu Cys Glu
1175 1180 1185

His Cys Ser Ser Gly Asp Asp Lys Phe Thr Trp Phe Phe Leu Pro
1190 1195 1200

Lys Gly Ile Arg Leu Asp Asp Asp Pro Ala Ser Asn Pro Pro Ile
1205 1210 1215

Arg Val Pro Tyr Ile Gly Ser Lys Thr Asp Glu Arg Arg Val Ala
1220 1225 1230

Ser Met Ala Tyr Ile Lys Gly Ala Ser Val Ser Leu Lys Ser Ala
1235 1240 1245

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We claim:

1. An isolated full-length genome of mumps virus attenuated JL2 strain comprises the polynucleotide sequence having SEQ ID NO.8.
2. A vector comprising polynucleotide sequence as claimed in claim 1, and, optionally, wherein the vector is selected from pUC19, pBluescript, pBluescript II, pBluescript II SK (+), pCA or pSC6.
3. A host cell comprising vector as claimed in claim 2, and optionally, wherein the host cell is selected from a mammalian cell or a non-mammalian cell.
4. The host cell as claimed in claim 3 wherein the mammalian cell is selected from HEK 293T cell, Vero cell or MRC-5 cell, and the non-mammalian cell is selected from bacterial cell or yeast cell.
5. A mumps virus vaccine comprising full-length genome of mumps virus attenuated JL2 strain as claimed in claim 1.
6. An immunogenic composition containing full-length genome of mumps virus attenuated JL2 strain as claimed in claim 1 with suitable pharmaceutical excipients, and optionally, wherein the pharmaceutical excipients are selected from buffer(s), stabilizer(s), tonicity agent(s), surfactant(s), adjuvant(s), cryoprotectant(s) or combination thereof.
7. The immunogenic composition as claimed in claim 6 wherein
   (a) Buffer is selected from phosphate-buffer, histidine-buffer, citrate-buffer, succinate-buffer, acetate-buffer, arginine buffer, phosphate buffered saline, tromethamine buffer and mixtures thereof;
   (b) Stabilizer is selected from amino acid(s), sugar(s), polyol(s), or combination thereof;
   (c) Tonicity agent is selected from sugar(s), salt(s) or combination thereof;
   (d) Surfactant is selected from polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers, alkylphenylpolyoxyethylene ethers, polyoxyethylene-polyoxypropylene copolymer and sodium dodecyl sulphate (SDS) or combination thereof;
   (e) Adjuvant is selected from salts of aluminium, Mono Phosphoryl Lipid analogues such as glucopyranosyl lipid adjuvant, monatide, cytokine inducers, squalene based adjuvants, lipophilic adjuvants or combination thereof; and

(f) Cryoprotectant is sugar(s).

8. The immunogenic composition as claimed in claim 7 comprises stabilizers selected from amino acid(s), sugar(s), polyol(s), or combination thereof, buffer(s) or salt(s).
9. The immunogenic composition as claimed in claim 8 wherein
   - Buffer is selected from phosphate-buffer, histidine-buffer, citrate-buffer, succinate-buffer, acetate-buffer, arginine buffer, phosphate buffered saline, tromethamine buffer and mixtures thereof;
   - Amino acid is selected from arginine, glycine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophane, methionine, serine, proline, cysteine / cystine and suitable combination thereof;
   - Sugar is selected from glucose, fructose, galactose, mannose, sorbose, ribose, deoxyribose, sucrose, trehalose, lactose, maltose, raffinose and suitable mixtures thereof preferably trehalose or raffinose;
   - Polyol is selected from mannitol, sorbitol, dextran, glycerol, arabitol, propylene glycol, polyethylene glycol and suitable combinations thereof;
   - Salt is selected from NaCl or KCl.
10. A rescue composition comprising the following elements:
    a. Transcription vector comprising polynucleotide sequence as claimed in claim 1;
    b. An expression vector comprises isolated gene(s) encoding trans-acting protein(s) wherein trans-acting protein is selected from NP protein, P protein, L protein and combination thereof;
    c. RNA polymerase for appropriate transcription of polynucleotide sequence as claimed in claim 1.
11. A method for producing a recombinant mumps virus comprising the following steps;
    a. Chemical synthesis of genome sequence fragments of mumps virus attenuated JL2;
    b. Cloning of each obtained gene fragment and their combination into an intermediate vector for its amplification;
    c. Construction of clone of full-length DNA genome comprises the polynucleotide sequence as claimed in claim 1 of the mumps virus attenuated JL2 strain to obtain $pMuV^{JL2}$ vector through ligation of each gene fragment obtained from step b, into single transcription vector;

d. Co-transfection of host cell with $pMuV^{JL2}$ and an expression vector comprising isolated gene encoding trans-acting protein wherein trans-acting protein is selected from NP protein, P protein and L protein and RNA polymerase to rescue negative strand of mumps virus;

e. optionally, passaging of rescued mumps virus into host cell for its expansion;

wherein the recombinant mumps virus comprises polynucleotide sequence as claimed in claim 1.

12. The composition of claim 10 or the method of claim 11, wherein the gene(s) encoding the trans-acting protein is selected from SEQ ID NO. 9 to 11.

13. The composition of claim 10 or 12 or the method of claim 11 or 12, wherein the amino acid sequence of the trans-acting proteins are selected from SEQ ID NO. 12 to 14.

14. The method of claim 11, wherein the vector encoding RNA polymerase is as set forth in SEQ ID NO. 15.

15. The method of producing a recombinant mumps virus as claimed in claim 11 wherein

- Transcription vector or an intermediate vector is selected from pUC19, pBluescript, pBluescript II, pBluescript II SK(+), pCA or pSC6; and/or
- Host cell is selected from HEK 293T cell, Vero cell or MRC-5 cell and non-mammalian cell is selected from E. coli or suitable yeast cells.