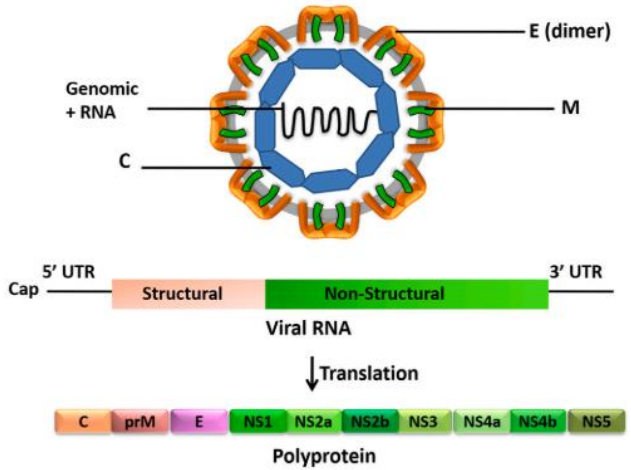
Development of novel mRNA vaccine against Japanese encephalitis virus (JEV)

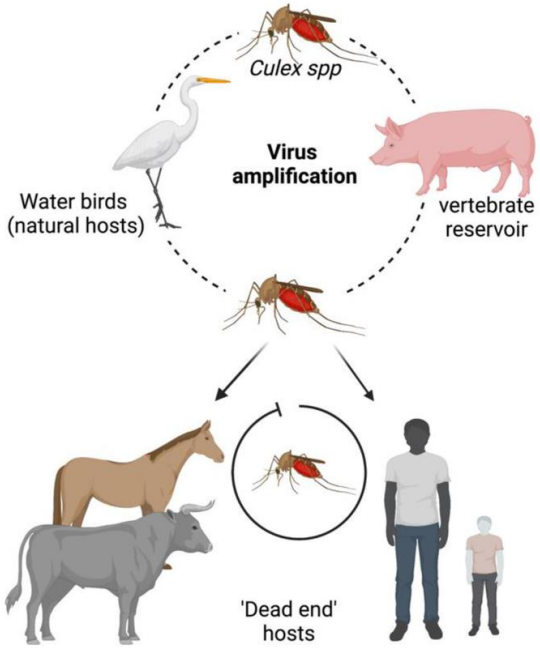
Japanese encephalitis virus

Japanese encephalitis virus



Schematic representation of the Japanese encephalitis virus genome

https://doi.org/10.1016/j.mam.2021.100994



Cycle of JEV infection and amplification

https://doi.org/10.3390/vaccines11040742

JEV: Flavivirus; Positive sense single strand (+ss) RNA virus

First case was documented in Japan in 1871

Transmission: Spread by infected mosquitoes (*Culex tritaeniorhynchus*)

Occurrence:68000 cases annually

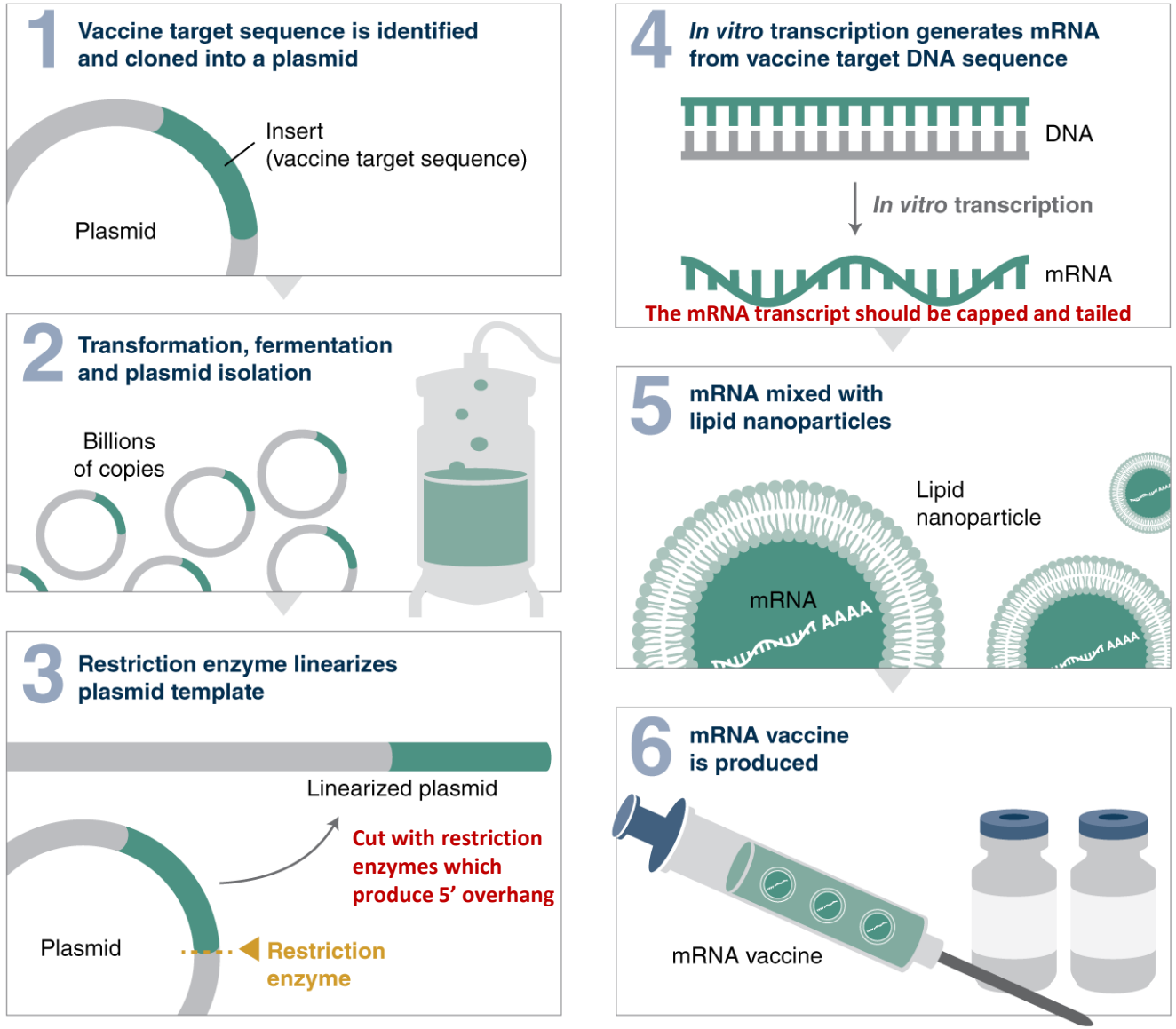
Fatality: 30%

Global geographical distribution of Japanese encephalitis

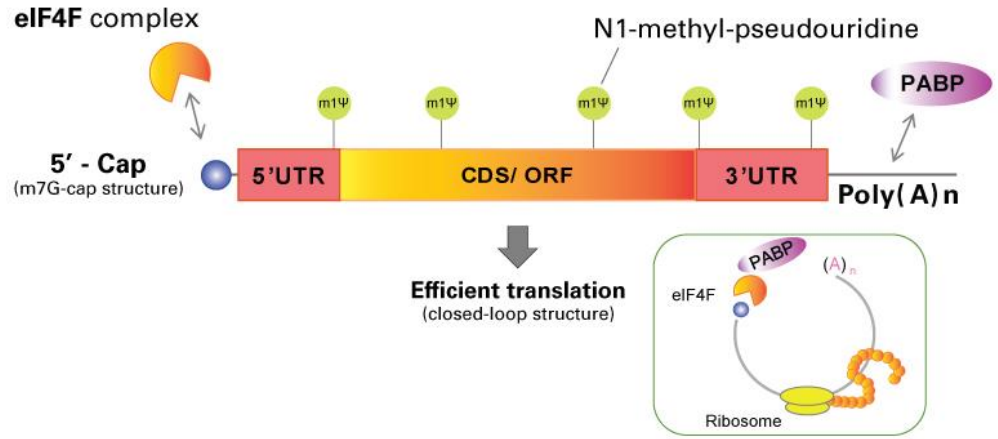
http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectious-diseases-related-to-travel/japanese-encephalitis
https://doi.org/10.1007/s40121-013-0018-2

Japanese encephalitis virus in India			
JEV Genotype	**Strain name**	**Isolation**	**Accession Number; Size (bp)**
I	JEV K94P05	Korea	AF045551; 10963
III	JEV P20778	Vellore	AF080251; 10977
V	JEV Muar	Malaysia	HM596272; 10988

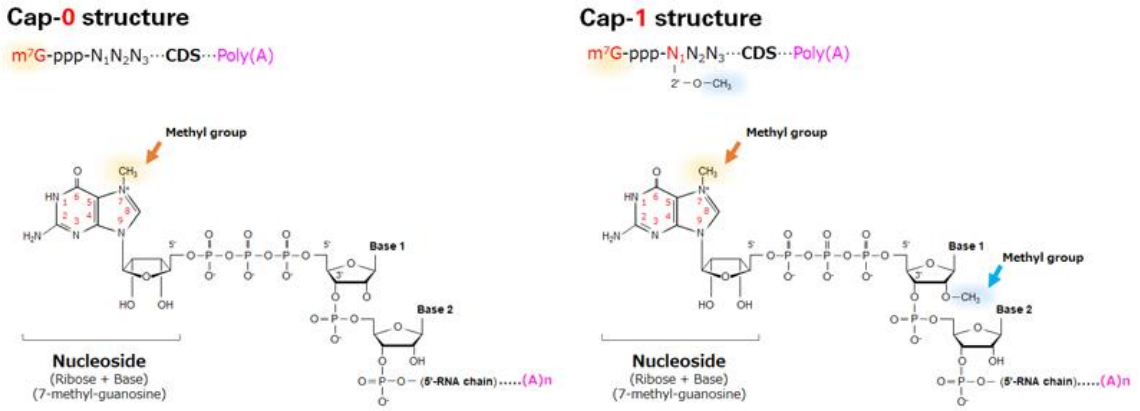
mRNA vaccine generation



Workflow for mRNA vaccine production



In vitro-transcribed mRNA structure. eIF4F = Eukaryotic initiation factor 4F, 5′ UTR = 5′ untranslated region, CDS/ORF = coding sequence/open reading frame, 3′ UTR = 3′ untranslated region, PABP = poly(A) binding protein, m1Ψ = N1-methylpseudouridine.



- The 5′ cap is guanosine with a methyl group on the 7-position; often abbreviated as **m7G**. The cap connects via triphosphate linkage (**PPP**) to the first nucleotide at the 5′ end of mRNA (the +1 position).
- In eukaryotes, the first nucleotide (+1) adjacent to the 5′ cap can be further modified to create a cap-1 structure, which suppresses immunogenicity.

5’UTR (β-globin):

AGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCA
CTAGCAACCTCAAACAGACACC

3’UTR (β-globin):

AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG
GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGG
TTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAAC
ATTTATTTTCATTGC

JEV prM-E (I)

ATGAAGCTATCAAACTTTCAAGGAAAGCTTCTGATGACCATCAACAACACGGACATTGCGGACGTCATCGTGATCCCCACCT
CAAAAGGTGAAAACAGATGTTGGGTCCGAGCAATCGACGTTGGTTACATGTGTGAAGACACCATCACGTACGAATGTCCGA
AGCTTGCCGTGGGCAACGATCCGGAAGACGTGGACTGCTGGTGCGACAATCAAGAAGTCTACGTGCAGTATGGTCGCTGC
ACACGGACCAGGCATTCCAAACGAAGCAGAAGATCCGTTTCAGTCCAAACGCATGGGGAAAGCTCACTAGTGAACAAAAA
AGAGGCTTGGCTGGATTCGACGAAGGCCACGCGATACCTCATGAAAACGGAGAACTGGATCATAAGGAACCCTGGTTATG
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GAGAAGTCACACTGGATTGTGAACCAAGGAGTGGACTGAACACTGAAGCGTTCTATGTCATGACCGTGGGTTCGAAGTCA
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GACAAACTGGCTCTGAAGGGCACGACTTATGGCATGTGTACAGAAAAATTCTCGTTCGCGAAAAATCCAGCGGACACAGG
CCATGGAACAGTTGTCATTGAGCTCACATACTCTGGAAGTGATGGTCCCTGTAAAATTCCGATTGTCTCAGTCGCGAGTTTAA
ACGACATGACCCCTGTGGGGAGGCTGGTAACAGTAAACCCCTTCGTCGCGACATCTAGCTCCAACTCAAAGGTGCTGGTTG
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CTTCGGCTCCATTGGAGGGGTATTCAACTCCATAGGGAAAGCTGTTCACCAAGTATTTGGCGGTGCATTCAGAACGCTCTTC
GGGGGAATGTCTTGGATCACACAAGGACTAATGGGGGCCTTACTTCTTTGGATGGGTGTCAACGCACGAAACCGGTCAATC
GCCCTGGCTTTTCTGGCCACGGGAGGTGTGCTCGTGTTTTTAGCGACCAATGTGCATGCC

Poly (A) Tail: 120 nt

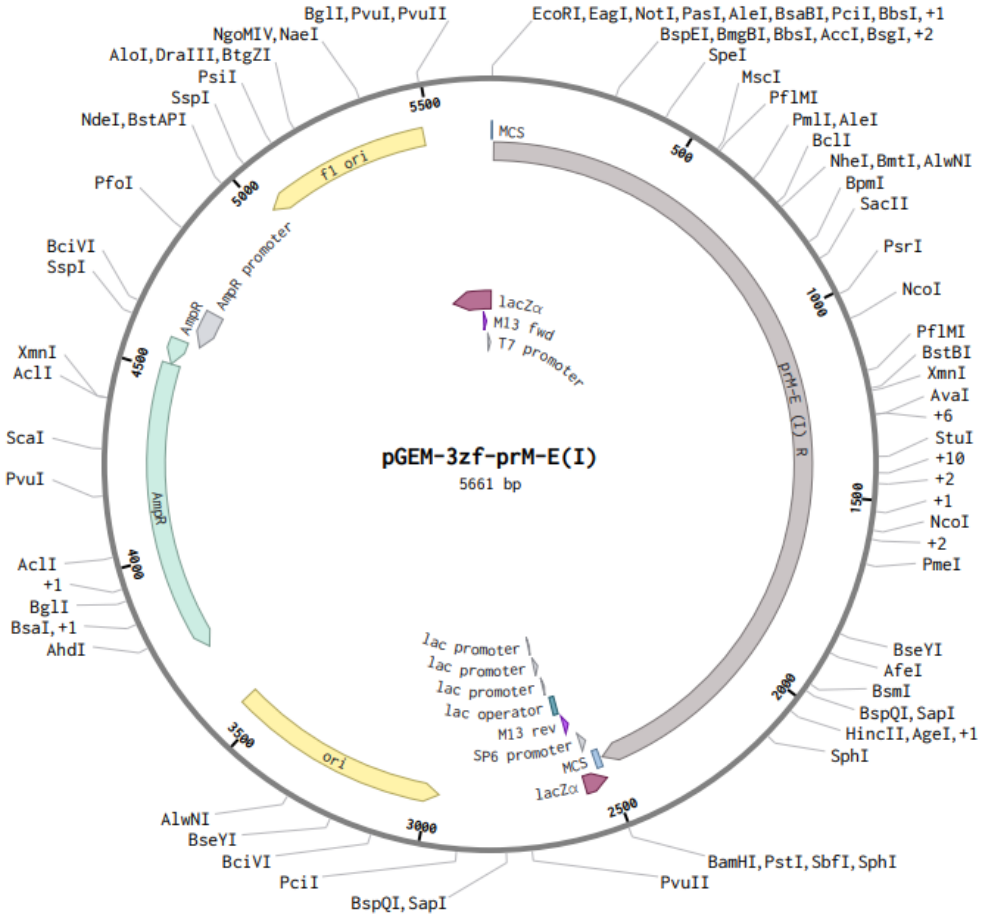
Restriction enzymes used for inserting into the plasmid: *Eco*RI and *Pst*I
Restriction enzymes used for linearization: *Bam*HI

vaccines MDPI

Article

mRNA Vaccines Encoding the HA Protein of Influenza A H1N1 Virus Delivered by Cationic Lipid Nanoparticles Induce Protective Immune Responses in Mice

Xinyu Zhuang [1,†], Yanxin Qi [2,†], Maopeng Wang [3], Ning Yu [4], Fulong Nan [5], He Zhang [1], Mingyao Tian [1], Chang Li [1], Huijun Lu [1] and Ningyi Jin [1,*]



Map of the *prM-E* gene of JEV genotype(I) inserted into pGEM-3Zf(+)

5’UTR (β-globin):

AGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCAC
TAGCAACCTCAAACAGACACC

3’UTR (β-globin):

AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGG
CCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTC
CTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTA
TTTTCATTGC

JEV prM-E (III)

ATGAAGTTGTCAAATTTCCAGGGAAAGCTTTTGATGACCATCAACAACACGGACATTGCAGACGTTATCGTGATTCCCACCTC
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CTCACCATGGGCAATGATCCAGAGGATGTGGACTGTTGGTGTGACAACCAAGAAGTCTACGTCCAATATGGACGGTGTACGC
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GGCGCACGCCACAAAACAGTCCGTTGTTGCTCTTGGGTCACAGGAAGGAAGCCTCCATCAGGCGTTGGCAGGAGCCATCGT
GGTGGAGTACTCAAGCTCAGTGAAGTTAACATCAGGCCACCTGAAATGCAGGCTGAAAATGGACAAACTGGCTCTGAAAGG
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CTGGTGACAGTGAACCCCTTTGTCGCGACTTCCAGCGCCAACTCAAAGGTGCTGGTCGAGATGGAACCCCCCTTCGGAGACT
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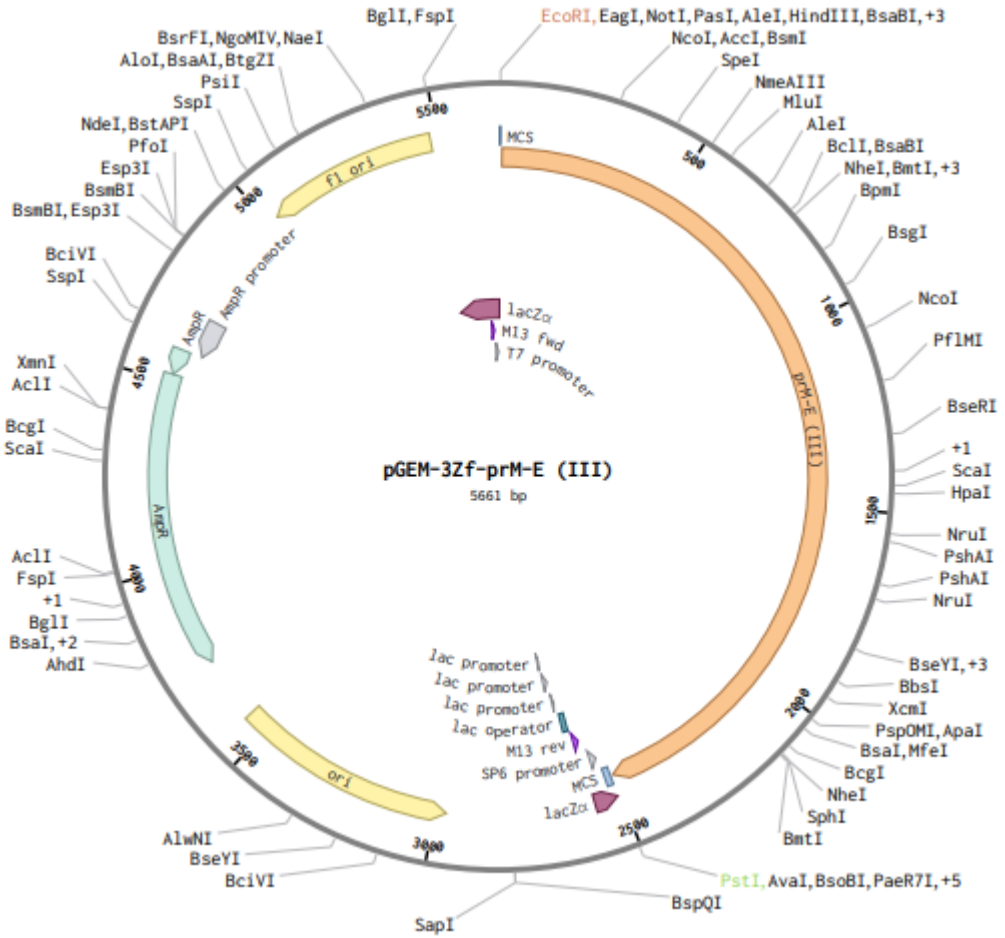
CAACGACTTTGAAGGGAGCTCAAAGACTGGCAGCGTTGGGCGACACAGCCTGGGACTTTGGCTCTATTGGAGGGGTCTTCA
ACTCCATAGGGAAAGCCGTTCACCAAGTGTTTGGTGGTGCCTTCAGAACACTCTTCGGGGGAATGTCTTGGATCACACAAGG
GCTAATGGGGGCCCTACTACTCTGGATGGGCGTCAACGCACGAGACCGATCAATTGCTTTGGCCTTCTTAGCCACAGGAGGT
GTGCTCGTGTTCTTAGCGACCAATGTGCATGCT

Poly (A) Tail: 120 nt

Restriction enzymes used for inserting into the plasmid: *Eco*RI and *Pst*I
Restriction enzymes used for linearization: *Xho*I



Map of the *prM-E* gene of JEV genotype(III) inserted into pGEM-3Zf(+)

mRNA sequence synthesis (Takara)

prM-E mRNA sequence JEV Genotype III:

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CGCCACAAAACAGUCCGUUGUUGCUCUUGGGUCACAGGAAGGAAGCCUCCAUCAGGCGUUGGCAGGAGCCAUCGUGGUGGAGUACUCAAGCUCAGUGAAGUUAACAUCAGGC
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AAAAAAAAAAAAAAAAAAAAAAAAAAA

5’UTR (β-globin):

AGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCA
CTAGCAACCTCAAACAGACACC

3’UTR (β-globin):

AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG
GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGG
TTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAAC
ATTTATTTTCATTGC

JEV prM-E (V)

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CGAAAGGGACCAATAGATGTTGGGTTCGGGCAATAGATGTGGGACACATGTGCGAGGACACAATCACCTACGAATGCCCTA
AACTTGATGCTGGTAATGACCCAGAGGACATTGACTGTTGGTGCGACAAACAAGCCGTGTATGTCCAGTATGGGCGTTGCA
CGAGGACCAGGCACTCCAGGAGAAGTAGAAGATCTGTGTCAGTGCAAACCCACGGAGAAAGCTCCCTAGTGAACAAAAA
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CGCACCCGCATACAGCTTTAACTGCCTAGGCATGGGCAACCGCGACTTCATTGAAGGAGTCAGCGGAGCCACGTGGGTAG
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AATACAGCCAGAAAATATCAAATATGAAGTTGGAGTATTTGTCCATGGAACCACAACAGCCGAGAACCATGGAAACTACTCC
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GAGAAGTCACAATGGATTGCGAGCCTCGTAGTGGATTTAACACTGAAGCATTTTATGTGCTGACCGTTGGGACTAAGTCGTT
TCTAGTCCATCGCGAATGGTTTAATGATTTGGCGCTTCCATGGCTGTCTCCATCTAGCACAAACTGGAGAAACAGAGAGATC
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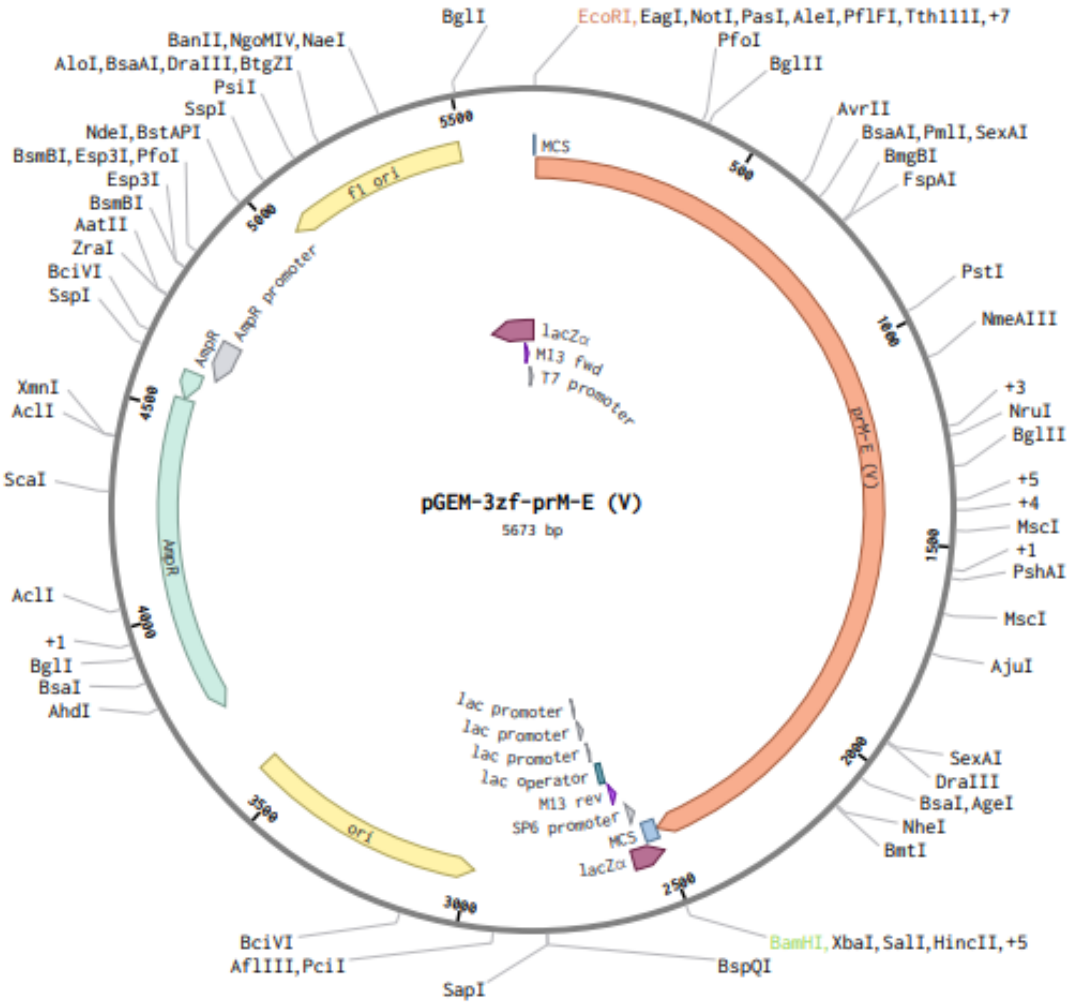
GGGTCCATTGGAGGAGTTTTTAATTCCATTGGCAAGGCCGTGCACCAGGTGTTTGGAGGAGCTTTTAGAACACTTTTTGGT
GGCATGTCTTGGATAACACAAGGATTGATGGGAGCACTGCTGCTGTGGATGGGTATCAATGCGCGAGACCGGTCGATCGCA
CTGGCCTTTCTTGCTACAGGAGGCGTGCTCTTGTTTCTGGCTACCAATGTCCACGCT

Poly (A) Tail: 120 nt

Restriction enzymes used for inserting into the plasmid: *Eco*RI and *Bam*HI

Restriction enzymes used for linearization: *Bam*HI



Map of the *prM-E* gene of JEV genotype(V) inserted into pGEM-3Zf(+)

GAATTCGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGAAGCTATCAAACTTTCAAGGAAAG
CTTCTGATGACCATCAACAACACGGACATTGCGGACGTCATCGTGATCCCCACCTCAAAAGGTGAAAACAGATGTTGGGTCC
GAGCAATCGACGTTGGTTACATGTGTGAAGACACCATCACGTACGAATGTCCGAAGCTTGCCGTGGGCAACGATCCGGAAG
ACGTGGACTGCTGGTGCGACAATCAAGAAGTCTACGTGCAGTATGGTCGCTGCACACGGACCAGGCATTCCAAACGAAGCA
GAAGATCCGTTTCAGTCCAAACGCATGGGGAAAGCTCACTAGTGAACAAAAAAGAGGCTTGGCTGGATTCGACGAAGGCC
ACGCGATACCTCATGAAAACGGAGAACTGGATCATAAGGAACCCTGGTTATGCTTTCCTGGCGGCGGCACTTGGATGGATG
CTTGGCAGCAACAGTGGCCAACGTGTGGTGTTCACTATTCTCTTGCTGTTGGTCGCTCCGGCTTACAGTTTTAACTGTCTGGG
AATGGGGAATCGGGATTTCATAGAAGGAGCCAGTGGAGCCACGTGGGTGGATCTGGTGTTAGAAGGAGATAGCTGTTTGA
CAATTATGGCAAACGACAAACCAACACTAGATGTCCGCATGATCAACATTGAAGCTAGCCAACTTGCTGAAGTCAGGAGTTA
CTGTTATCACGCTTCAGTCACTGACATTTCAACGGTGGCTCGATGCCCCATGACTGGAGAAGCCCACAACGAAAAACGTGCT
GACAGCAGCTACGTGTGCAAACAAGGCTTTACTGACCGCGGATGGGGAAATGGATGTGGACTTTTCGGGAAAGGAAGCAT
TGACACATGCGCAAAATTTTCTTGTACCAGTAAGGCCATTGGAAGAATGATCCAACCAGAGAACATCAAGTACGAGGTTGG
CATATTCGTGCACGGAACCACCACCTCGGAAAACCATGGGAATTACTCAGCGCAAGTAGGAGCGTCTCAAGCAGCAAAGTT
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CCTCCTCCCCTTCCTGCACCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGGATCCCTGCAG

Restriction enzymes used for inserting into the plasmid: *Eco*RI and *Pst*I
Restriction enzymes used for linearization: *Bam*HI

The sequence has been sent to TWIST Bioscience for synthesis (excluding the restriction sites used for ligating the insert into the plasmid; *Eco*RI and *Pst*I)

Cell

Article

Modified mRNA Vaccines Protect against Zika Virus Infection

Justin M. Richner,[1,9] Sunny Himansu,[2,9] Kimberly A. Dowd,[3] Scott L. Butler,[2] Vanessa Salazar,[1] Julie M. Fox,[1] Justin G. Julander,[4] William W. Tang,[5] Sujan Shresta,[5] Theodore C. Pierson,[3] Giuseppe Ciaramella,[2,*] and Michael S. Diamond[1,6,7,8,10,*]

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[2]Valera LLC, a Moderna Venture, 500 Technology Square, Cambridge, MA, 02139, USA
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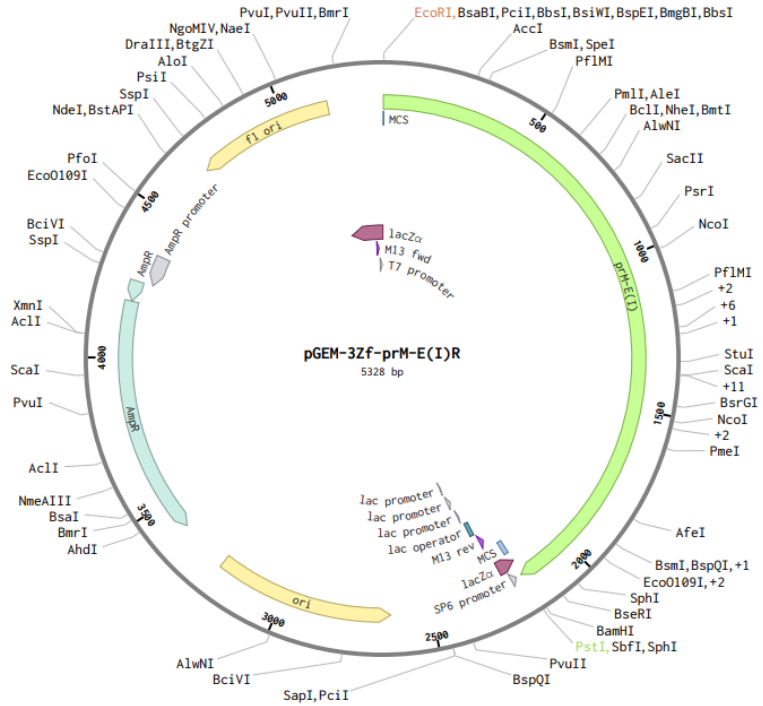
http://dx.doi.org/10.1016/j.cell.2017.02.017



Map of the *prM-E* gene of JEV genotype(I) inserted into pGEM-3Zf(+)

GAATTCGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGAAGTTGTCAAATTTCCAGGGAAAGCTTTT
GATGACCATCAACAACACGGACATTGCAGACGTTATCGTGATTCCCACCTCAAAAGGAGAGAACAGATGTTGGGTCCGGGCAATCG
ACGTCGGCTACATGTGTGAGGACACTATCACGTACGAATGTCCTAAGCTCACCATGGGCAATGATCCAGAGGATGTGGACTGTTGG
TGTGACAACCAAGAAGTCTACGTCCAATATGGACGGTGTACGCGGACCAGGCATTCCAAGCGAAGCAAAAGATCCGTGTCGGTCCA
AACACATGGGGAGAGTTCACTAGTGAATAAAAAAGAGGCTTGGCTGGATTCAACGAAAGCCACACGATACCTCATGAAAACCGAG
AATTGGATCATAAGGAATCCTGGCTATGCTTTCCTGGCGGCGATACTCGGCTGGATGCTTGGCAGCAACAACGGTCAACGCGTGGT
ATTCACCATCCTCCTGCTGTTGGTCGCTCCGGCTTACAGTTTCAACTGTCTGGGAATGGGCAATCGTGACTTCGTAGAAGGAGCCAG
CGGAGCCACCTGGGTGGACTTGGTGTTAGAAGGAGACAGCTGCTTGACAATTATGGCAAACGACAAACCAACATTGGACGTCCGCA
TGATCAACATCGAAGCTAGCCAACTTGCTGAGGTCAGAAGTTACTGCTATCATGCTTCAGTCACTGACATCTCGACGGTGGCTCGGT
GCCCCACGACTGGAGAAGCCCACAACGAGAAGGGAGCTGATAGTAGCTATGTGTGCAAACAAGGCTTCACCGATCGTGGGTGGGG
TAACGGATGCGGACTTTTTGGGAAGGGAAGCATTGACACATGTGCAAAATTCTCCTGCACCAGTAAAGCGATTGGGAGAACAATCC
AGCCAGAAAACATCAAATACGAAGTTGGCATTTTTGTGCATGGAACCACCACCTCGGAAAACCATGGGAATTATTCAGCGCAAGTTG
GGGCGTCCCAGGCGGCAAAGTTTACAGTAACACCCAATGCTCCTTCGACAACCCTCAAACTTGGTGACTACGGAGAAGTCACACTG
GATTGTGAGCCAAGGAGTGGATTAAACACTGAAGCGTTTTACGTCATGACCGTGGGGTCAAAGTCATTGTTGGTCCACAGGGAATG
GTTCCATGATCTCGCTCTCCCTTGGACGTCCCCTTCGAGCACAGCGTGGAGAAACAGAGAACTCCTCATGGAATTTGAAGAGGCGCA
CGCCACAAAACAGTCCGTTGTTGCTCTTGGGTCACAGGAAGGAAGCCTCCATCAGGCGTTGGCAGGAGCCATCGTGGTGGAGTACT
CAAGCTCAGTGAAGTTAACATCAGGCCACCTGAAATGCAGGCTGAAAATGGACAAACTGGCTCTGAAAGGTACAACCTATGGCATG
TGCACAGAAAAATTCTCGTTCGCGAAAAACCCGGCGGACACTGGTCACGGAACAGTTGTCATCGAACTTTCCTACTCTGGGAGTGAT
GGCCCTTGCAAAATTCCGATTGTCTCCGTTGCGAGTCTTAATGACATGACCCCCGTCGGGCGGCTGGTGACAGTGAACCCCTTTGTC
GCGACTTCCAGCGCCAACTCAAAGGTGCTGGTCGAGATGGAACCCCCCTTCGGAGACTCCTACATCGTAGTTGGAAGGGGAGACAA
GCAGATCAACCACCATTGGCACAAAGCCGGAAGCACGCTGGGCAAGGCCTTTTCAACGACTTTGAAGGGAGCTCAAAGACTGGCA
GCGTTGGGCGACACAGCCTGGGACTTTGGCTCTATTGGAGGGGTCTTCAACTCCATAGGGAAAGCCGTTCACCAAGTGTTTGGTGG
TGCCTTCAGAACACTCTTCGGGGGAATGTCTTGGATCACACAAGGGCTAATGGGGGCCCTACTACTCTGGATGGGCGTCAACGCAC
GAGACCGATCAATTGCTTTGGCCTTCTTAGCCACAGGAGGTGTGCTCGTGTTCTTAGCGACCAATGTGCATGCTTGATAATAGGCTG
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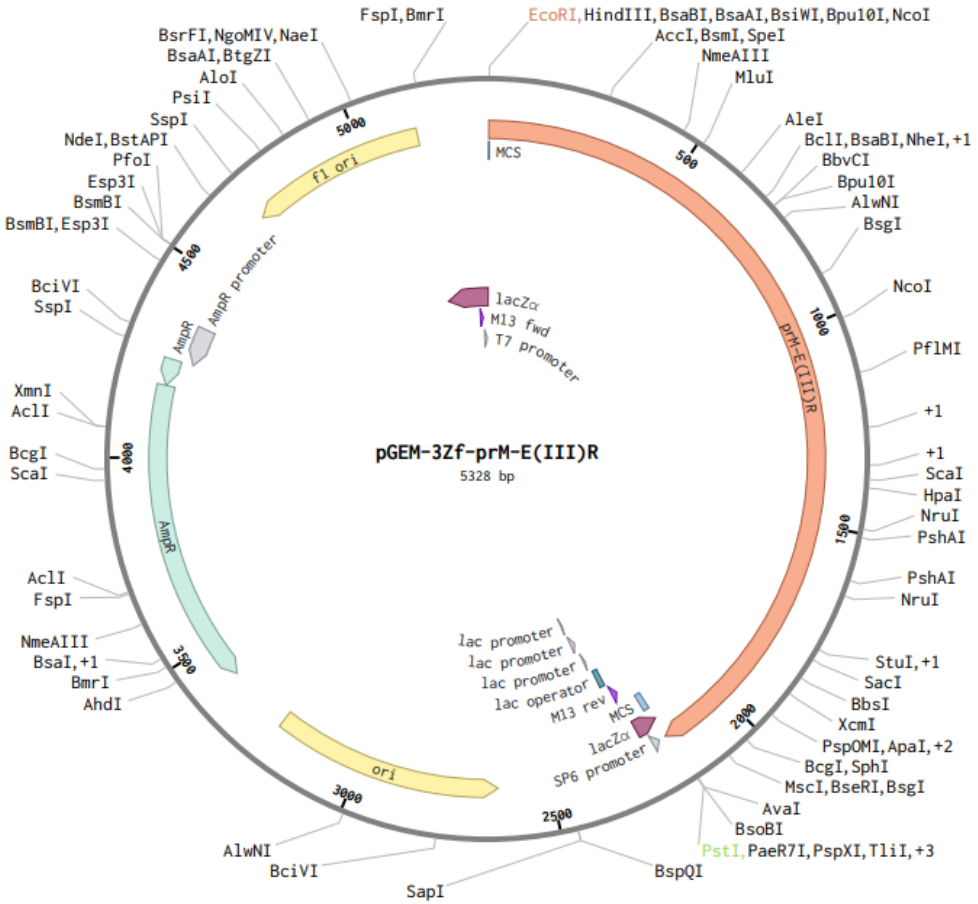
AAGTCTGACTCGAGCTGCAG



Map of the *prM-E* gene of JEV genotype(III) inserted into pGEM-3Zf(+)

Restriction enzymes used for inserting into the plasmid: *Eco*RI and *Pst*I
Restriction enzymes used for linearization: *Xho*I

mRNA synthesis through In-vitro transcription kit

CELLSCRIPT™
RNA for Translation in Cells

T7-FlashScribe™ Transcription Kit

Cat. Nos. **C-ASF3507**

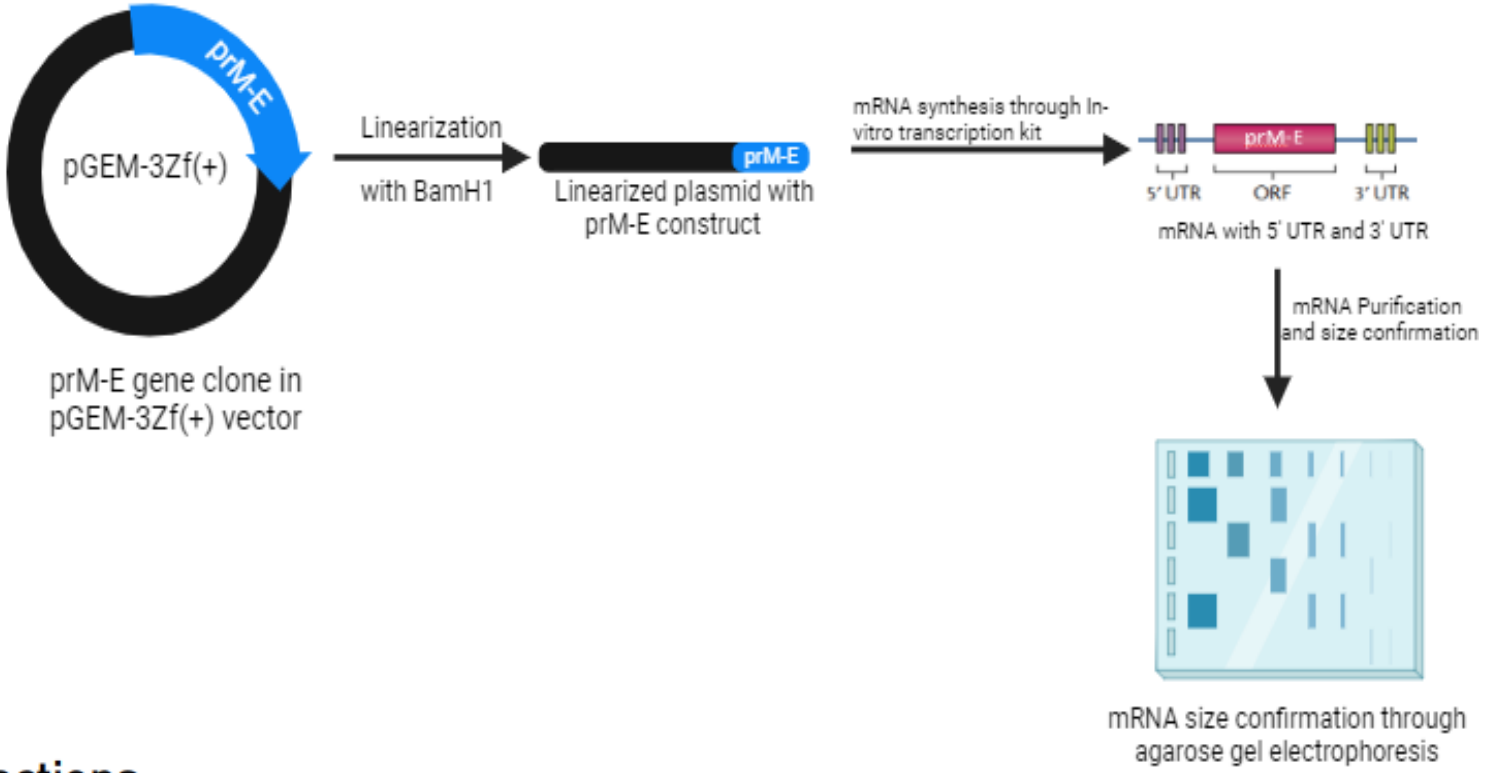
INTRODUCTION

The T7-FlashScribe™ Transcription Kit is specially formulated to enable users to obtain the maximum possible yields of RNA from an *in vitro* transcription (IVT) reaction in just 30 minutes. The standard 30 minute, 20 µl reaction will yield up to 180 µg of RNA from 1 µg of the control template. These yields are made possible by the high-performance properties of the T7-FlashScribe enzyme.

The T7-FlashScribe Transcription Kit produces exceptionally high yields of either long or short transcripts. The standard reaction can be scaled up to produce milligram amounts of RNA.

T7-FlashScribe IVT RNA can be processed into mRNA (5'-end capped and 3'-end poly(A) tailed) through the use of CELLSCRIPT's ScriptCap™ m^{7}G Capping System, ScriptCap 2'-O-Methyltransferase Kit and A-Plus™ Poly(A) Polymerase Tailing Kit (available separately).

CELLSCRIPT also offers the INCOGNITO™ line of transcription kits for the production of pseudouridine- & 5-methyl-cytosine-containing (GAΨC and GAΨ5mC) IVT RNA. It has been shown that Ψ-mRNAs and Ψ5mC-mRNAs are translated into protein at higher levels and induce lower innate immune responses in human and other mammalian cells that express various RNA sensors compared to corresponding unmodified mRNAs.[1-4]



MEGAclear™ Kit
Purification for Large Scale Transcription Reactions

Catalog Number AM1908

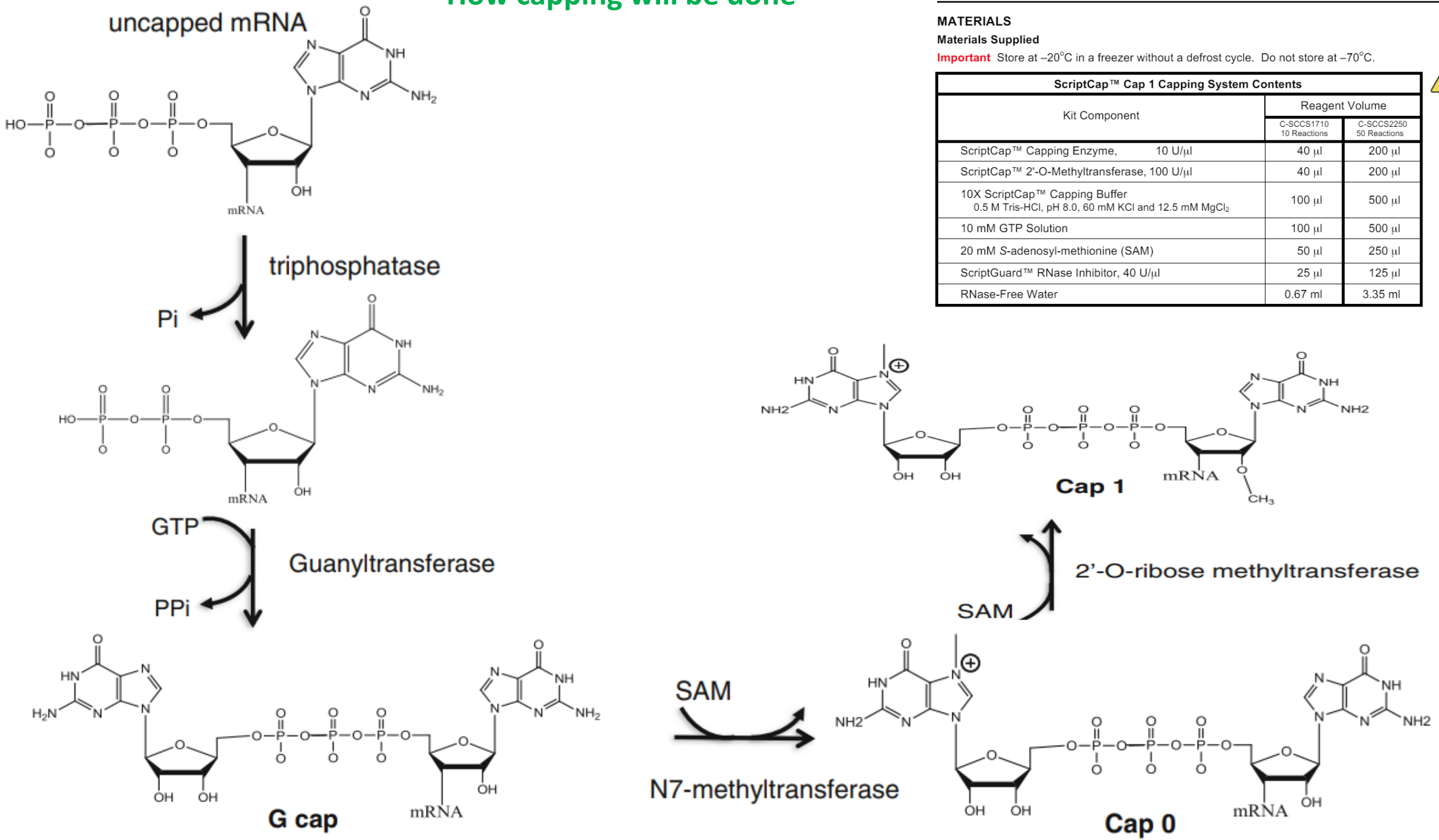
Publication Number 1908M **Revision** C

Product description

The MEGAclear™ Kit is designed for rapid high-throughput purification of RNA from enzymatic reactions such as in vitro transcription. The process is simple and fast, and it recovers from 1 ng to 500 µg of RNA efficiently. The MEGAclear™ Kit is appropriate for purification of ssRNA larger than 100 nt and dsRNA larger than 200 bp.

At this level we will have purified mRNA with 5’ and 3’ UTR
Next step is capping of mRNA at 5’ position

How capping will be done



CELLSCRIPT

ScriptCap™ Cap 1 Capping System

MATERIALS

Materials Supplied

Important Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

ScriptCap™ Cap 1 Capping System Contents		
Kit Component	Reagent Volume	
	C-SCCS1710 10 Reactions	C-SCCS2250 50 Reactions
ScriptCap™ Capping Enzyme, 10 U/µl	40 µl	200 µl
ScriptCap™ 2'-O-Methyltransferase, 100 U/µl	40 µl	200 µl
10X ScriptCap™ Capping Buffer 0.5 M Tris-HCl, pH 8.0, 60 mM KCl and 12.5 mM $MgCl_2$	100 µl	500 µl
10 mM GTP Solution	100 µl	500 µl
20 mM *S*-adenosyl-methionine (SAM)	50 µl	250 µl
ScriptGuard™ RNase Inhibitor, 40 U/µl	25 µl	125 µl
RNase-Free Water	0.67 ml	3.35 ml

Anal Bioanal Chem
DOI 10.1007/s00216-016-9605-x

RESEARCH PAPER

Label-free analysis of mRNA capping efficiency using RNase H probes and LC-MS

Michael Beverly[1] · Amy Dell[1] · Parul Parmar[1] · Leslie Houghton[1]

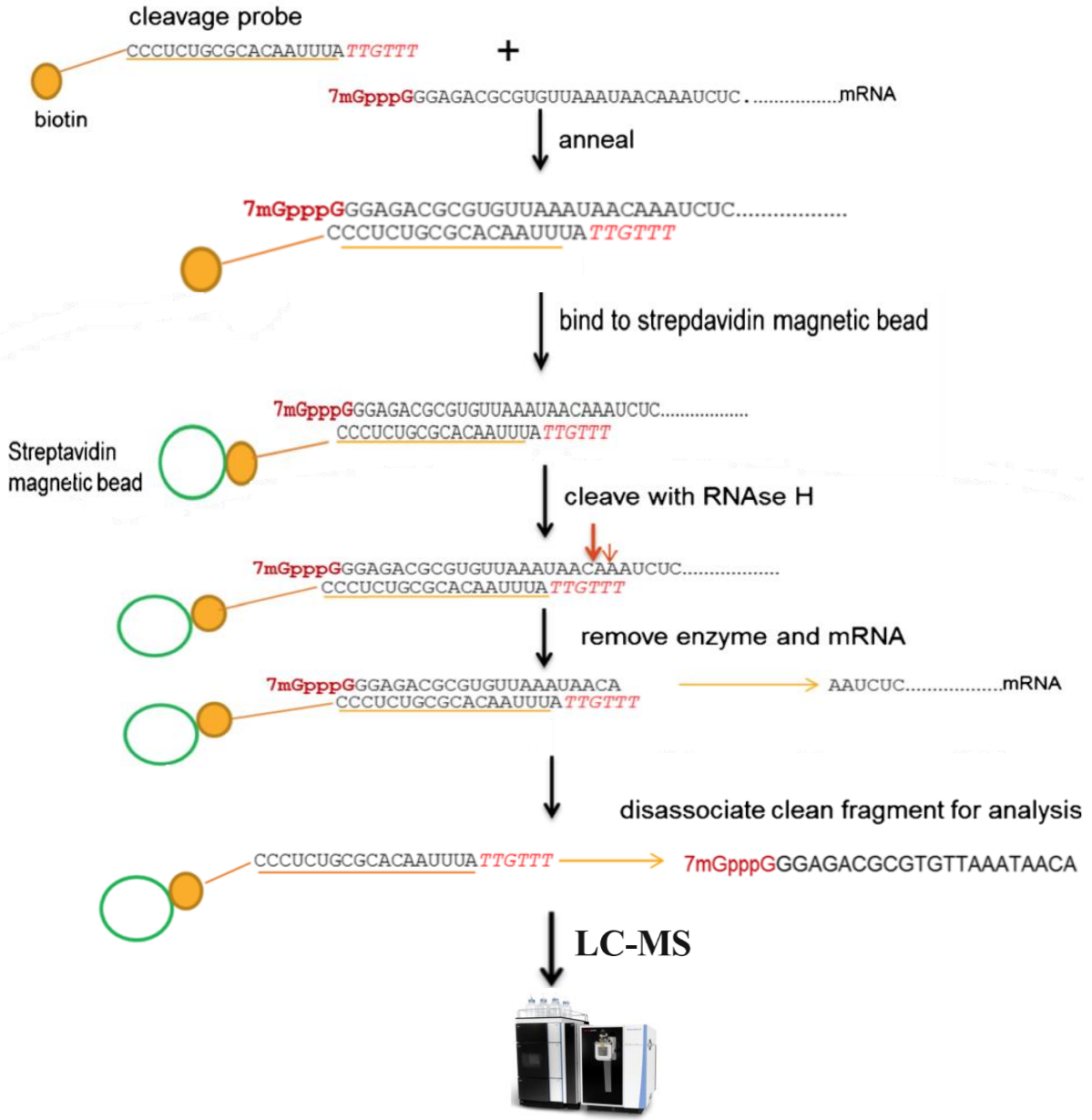
Materials required for assay

- RNase H cleavage probe conjugated with biotin
- RNase H
- RNase H reaction buffer
- RNA 5' pyrophosphohydrolase (RppH) with NEbuffer2
- Streptavidine-coated magnetic beads
- NaOH
- NaCl
- Tris-HCl
- EDTA
- Methanol
- Hexafluroisopropanol
- Triethylamine

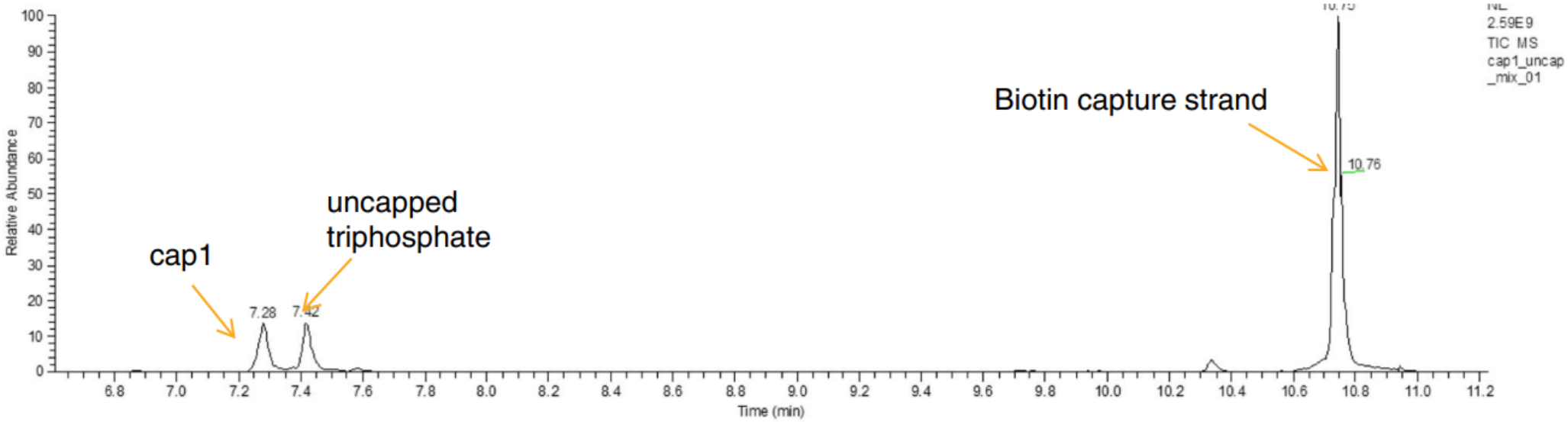
Instruments

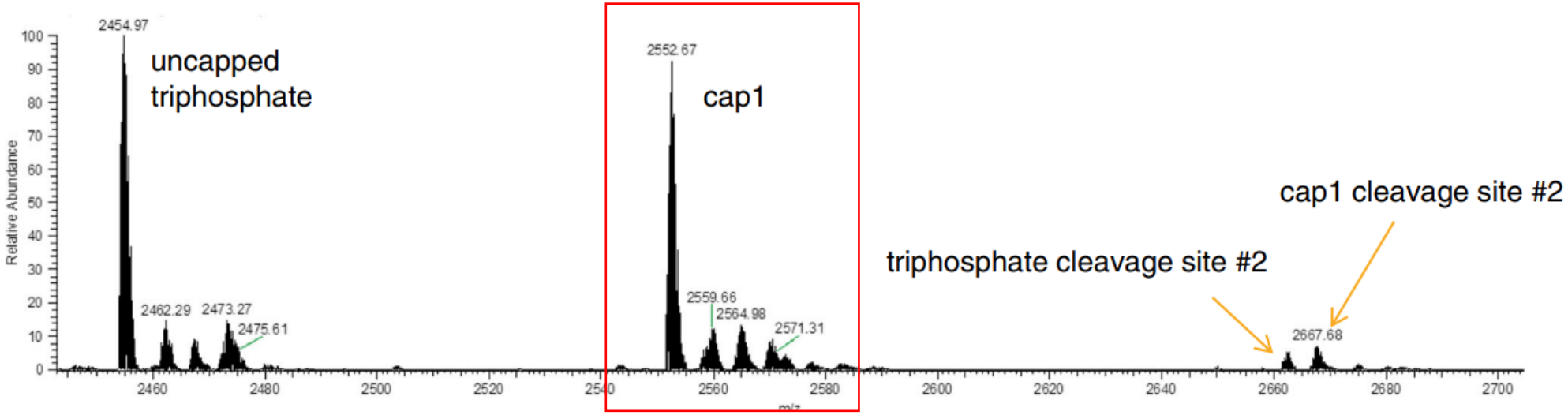
- Vacuum dryer
- LC-MS

Steps involved in analysis of mRNA capping



LC-MS Analysis





Polyadenylation of mRNA

CELLSCRIPT™
RNA for Translation in Cells

A-Plus™ Poly(A) Polymerase Tailing Kit

Cat. No. **C-PAP5104H**

INTRODUCTION

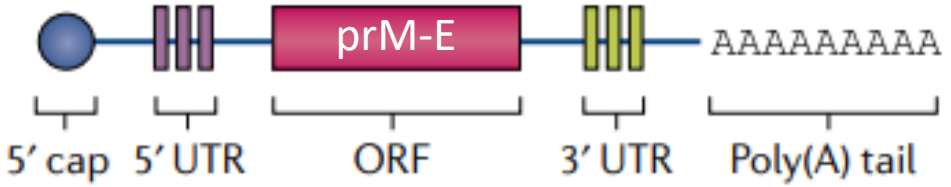
The A-Plus™ Poly(A) Polymerase Tailing Kit uses ATP as a substrate for template-independent addition of adenosine monophosphates to the 3'-hydroxyl termini of RNA. The standard protocol produces a poly(A)-tail length of ~150 b on 40-60 µg of RNA. Polyadenylation increases the stability of RNA in eukaryotic cells and enhances its ability to be translated after transfection or microinjection.[1-3] A Poly(A) tail is useful to provide a priming site for first-strand cDNA synthesis in certain applications, and can be used to end-label[4] or quantify[5] mRNA.

MATERIALS

Materials Supplied

Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

A-Plus™ Poly(A) Polymerase Tailing Kit Contents (50 reactions)	
Kit Component	Volume
A-Plus™ Poly(A) Polymerase, 4 U/µl in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA and and 0.1% Triton® X-100.	100 µl
10X A-Plus™ Poly(A) Tailing Buffer 0.5 M Tris-HCl, pH 8.0, 2.5 M NaCl and 100 mM $MgCl_2$.	500 µl
10 mM ATP	500 µl
RNase-Free Water	2 x 1.4 ml



mRNA

This is full mRNA which will act as vaccine candidate

NIH Public Access
Author Manuscript
Methods Enzymol. Author manuscript; available in PMC 2009 August 28.

Published in final edited form as:
Methods Enzymol. 2008 ; 448: 483–504. doi:10.1016/S0076-6879(08)02624-4.

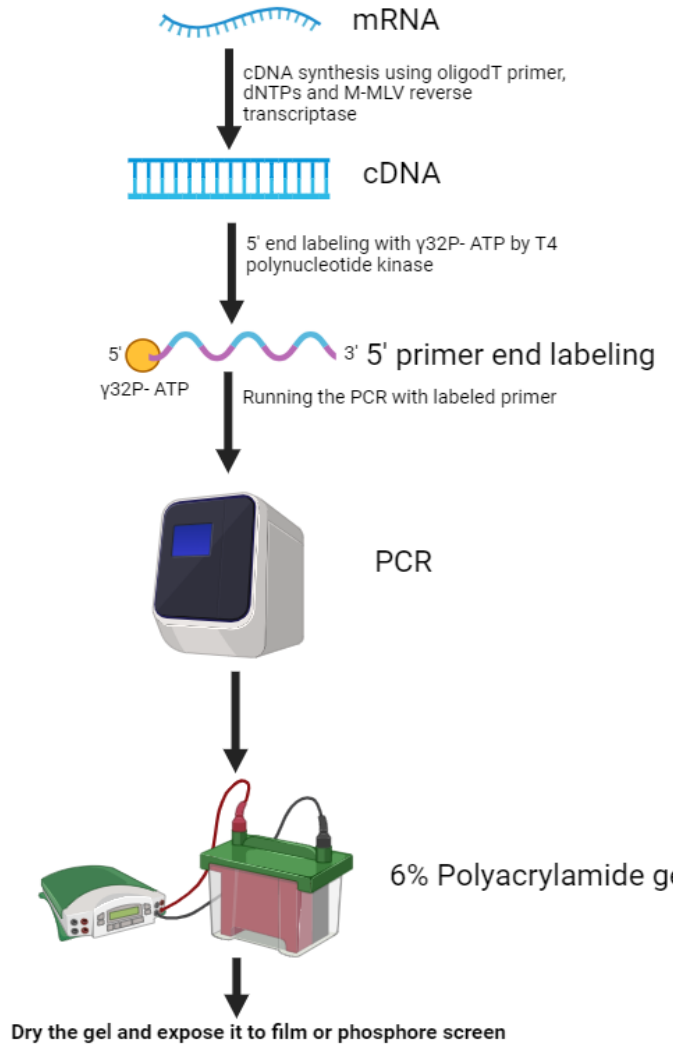
Assays for determining poly(A) tail length and the polarity of mRNA decay in mammalian cells

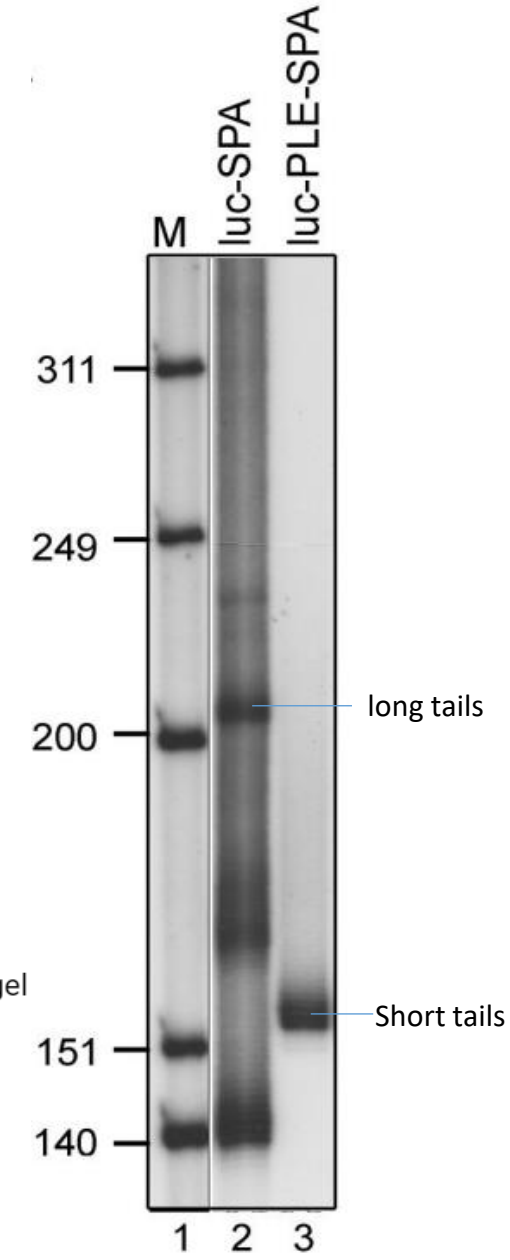
Elizabeth L. Murray and **Daniel R. Schoenberg***
Department of Molecular and Cellular Biochemistry and the RNA Group, The Ohio State Universit
Columbus, OH 43210-1218

Materials required for assay

- OligodT primer/adapter
- mRNA specific primer (Primer should be located 100bp upstream of poly A tail that will give size between 100-300 bases.
- Strand buffer
- DTT
- dNTPs
- RNase Inhibitor
- M-MLV reverse transciptase
- Polynucleotide kinase buffer
- γ32P-ATP
- T4PNK
- Reagents for PCR
- Reagents to run polyacrylamide gel
- Film or phosphor screen

Steps involved in assay





mRNAs with short tails yield a compact band while mRNAs with long tails yield PCR products of a variety of lengths which appear as a smear on the gel

Transfection of mRNA into cell line

npj | vaccines

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ARTICLE OPEN

Check for updates

Rational development of a combined mRNA vaccine against COVID-19 and influenza

Qing Ye[1,6], Mei Wu[1,6], Chao Zhou[1,6], Xishan Lu[2,6], Baoying Huang[3,6], Ning Zhang[1], Hui Zhao[1], Hang Chi[1], Xiaojing Zhang[2], Dandan Ling[2], Rong-Rong Zhang[1], Zhuofan Li[2], Dan Luo[1], Yi-Jiao Huang[1], Hong-Ying Qiu[1], Haifeng Song[2], Wenjie Tan[3]✉, Ke Xu[4], Bo Ying[2] and Cheng-Feng Qin[1,5]✉

Cell

CellPress

Article

A Thermostable mRNA Vaccine against COVID-19

Na-Na Zhang,[1,2,6] Xiao-Feng Li,[1,6] Yong-Qiang Deng,[1,6] Hui Zhao,[1,6] Yi-Jiao Huang,[1,6] Guan Yang,[3,6] Wei-Jin Huang,[4,6] Peng Gao,[5] Chao Zhou,[1] Rong-Rong Zhang,[1] Yan Guo,[1] Shi-Hui Sun,[1] Hang Fan,[1] Shu-Long Zu,[1] Qi Chen,[1] Qi He,[3] Tian-Shu Cao,[1] Xing-Yao Huang,[1] Hong-Ying Qiu,[1] Jian-Hui Nie,[4] Yuhang Jiang,[5] Hua-Yuan Yan,[5] Qing Ye,[1] Xia Zhong,[5] Xia-Lin Xue,[5] Zhen-Yu Zha,[5] Dongsheng Zhou,[1] Xiao Yang,[3] You-Chun Wang,[4,*] Bo Ying,[5,*] and Cheng-Feng Qin[1,7,*]

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[3]State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China
[4]Division of HIV/AIDS and Sex-Transmitted Virus Vaccines, Institute for Biological Product Control, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, China
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[7]Lead Contact
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https://doi.org/10.1016/j.cell.2020.07.024

Emerging Microbes & Infections
https://doi.org/10.1080/22221751.2023.2192815

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RESEARCH ARTICLE

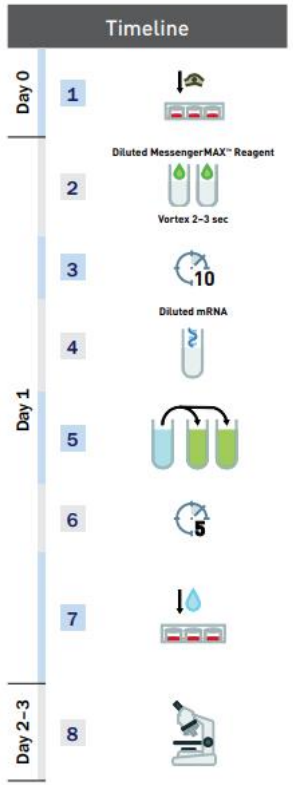
OPEN ACCESS Check for updates

Rational development of multicomponent mRNA vaccine candidates against mpox

Rong-Rong Zhang[a]†, Zheng-Jian Wang[a]†, Yi-Long Zhu[b,c]†, Wei Tang[a]†, Chao Zhou[a]†, Suo-Qun Zhao[a], Mei Wu[a], Tao Ming[a], Yong-Qiang Deng[a], Qi Chen[a], Ning-Yi Jin[b], Qing Ye[a], Xiao Li[b] and Cheng-Feng Qin[a,d]

[a]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, People's Republic of China; [b]Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Changchun, People's Republic of China; [c]Academicians Workstation of Jilin Province, Changchun University of Chinese Medicine, Changchun, People's Republic of China; [d]Research Unit of Discovery and Tracing of Natural Focus Diseases, Chinese Academy of Medical Sciences, Beijing, People's Republic of China

Lipofectamine MessengerMax reagent transfection protocol (Thermo Fischer)



Steps
Seed cells to be 70–90% confluent at transfection
Dilute MessengerMAX™ Reagent in Opti-MEM™ Medium (2 tubes) – Mix well
Incubate
Prepare Diluted mRNA master mix by adding mRNA to Opti-MEM™ Medium – Mix well
Add Diluted mRNA to each tube of Diluted MessengerMAX™ Reagent (1:1 ratio)
Incubate
Add mRNA-lipid complex to cells
Visualize/analyze transfected cells

Procedure Details (Two Reaction Optimization)			
Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Opti-MEM™ Medium	5 µL × 2	25 µL × 2	125 µL × 2
Lipofectamine™ MessengerMAX™ Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
Incubate diluted MessengerMAX™ Reagent in Opti-MEM™ Medium for 10 minutes at room temperature.			
Opti-MEM™ Medium	10 µL	50 µL	250 µL
mRNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg
Diluted mRNA	5 µL	25 µL	125 µL
Diluted Lipofectamine™ MessengerMAX™ Reagent	5 µL	25 µL	125 µL
Incubate for 5 minutes at room temperature.			
Component (per well)	96-well	24-well	6-well
mRNA-lipid complex	10 µL	50 µL	250 µL
mRNA	100 ng	500 ng	2500 ng
Lipofectamine™ MessengerMAX™ Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
Incubate cells for 1–2 days at 37°C. Then, analyze transfected cells.			

mRNA transfection and protein expression

For immunofluorescence staining, HEK293T cells were seeded in 24-well plates at 4×10^5 cells per well and cultured at 37 °C in 5% CO_2 for 12 h. mRNA encoding HA was transfected into HEK293T cells using Lipofectamine MessengerMAX Reagent (Thermo Fisher Scientific). Transfected cells were fixed with cold methanol/acetone (7:3) 48 h post-transfection and incubated with primary antibody (rabbit polyclonal antibody for H1N1-HA, GeneTex, GTX127357, 1:400) at 37 °C for 1 h. Cells were then washed with PBS three times and then incubated with secondary antibody conjugated to Alexa Fluor 488 (Proteintech, SA00013-2, 1:400). HA-positive cells were examined using a PerkinElmer High Content Analysis System Operetta CLS and processed using Harmony 4.9 software.

48hr post transfection cells will be fixed with cold methanol/acetone (7:3)

⬇

Incubate with primary antibody

⬇

Wash the cells with PBS three time

⬇

Incubate with secondary antibody conjugated with alexa fluor 488

⬇

Imaging with microscope

LNP Formulation of mRNA

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[4]Division of HIV/AIDS and Sex-Transmitted Virus Vaccines, Institute for Biological Product Control, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, China
[5]Suzhou Abogen Biosciences Co., Ltd., Suzhou 215123, China
[6]These authors contributed equally
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RESEARCH ARTICLE

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Rational development of multicomponent mRNA vaccine candidates against mpox

Rong-Rong Zhang[a]†, Zheng-Jian Wang[a]†, Yi-Long Zhu[b,c]†, Wei Tang[a]†, Chao Zhou[a]†, Suo-Qun Zhao[a], Mei Wu[a], Tao Ming[a], Yong-Qiang Deng[a], Qi Chen[a], Ning-Yi Jin[b], Qing Ye[a], Xiao Li[b] and Cheng-Feng Qin[a,d]

[a]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, People's Republic of China; [b]Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Changchun, People's Republic of China; [c]Academicians Workstation of Jilin Province, Changchun University of Chinese Medicine, Changchun, People's Republic of China; [d]Research Unit of Discovery and Tracing of Natural Focus Diseases, Chinese Academy of Medical Sciences, Beijing, People's Republic of China

LNP Formulation of the mRNA

The mRNA vaccine encoding HA protein of H1N1 was prepared in LNP formulations. Briefly, a lipid mixture including ionizable lipids, 1-,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol and PEG-lipid (molar ratios of 50:10:38.5:1.5) was combined with 20 mM citrate buffer (pH 4.0) containing mRNA at a ratio of 1:2 through a T-mixer. The formulation were then diafiltrated in 10 × volume of PBS (pH 7.4), reduced to the desired concentrations through a tangential flow filtration membrane with 100 kD, passed through a 0.22 mm filter, and stored at 2–8 °C until use. All formulations were tested for particle size, distribution, RNA concentration and encapsulation. The combined mRNA vaccine candidate (AR-CoV/IAV) was developed by mixing ARCoV and ARIAV under the same LNP-mRNA vaccine platform. Empty LNPs were utilized as placebo.

T-mixer (medimix)

Steps involve in LNP formulation

ionizable lipids, DSPC, cholesterol and polyethylene Glycol (PEG)-lipid (molar ratio of 50:10:38.5:1.5)

➕

Combined with 20mM citrate buffer (pH 4.0) containing mRNA at a ratio of 1:2 and mixed with T-mixer

⬇

The formulation will be diafiltrated in 10x volume of PBS

⬇

Reduce to desired concentration through tangential flow filtration membrane with 100kD

⬇

Pass through 0.22µM filter

⬇

stored at 2-8

LNP Formulation of mRNA

viruses

MDPI

Article

Protective Immune Responses Induced by an mRNA-LNP Vaccine Encoding prM-E Proteins against Japanese Encephalitis Virus Infection

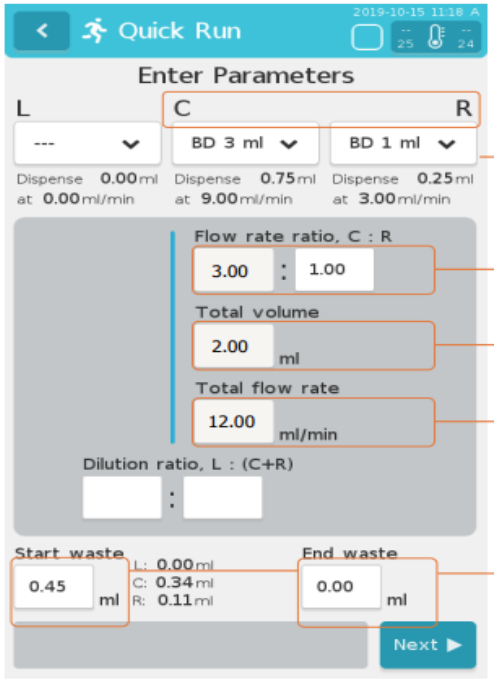
Tao Chen [1,2,3], Shuo Zhu [1,2,3], Ning Wei [1,2,3], Zikai Zhao [1,2,3], Junjun Niu [1,2,3], Youhui Si [1,2,3], Shengbo Cao [1,2,3,*] and Jing Ye [1,2,3,*]

1 State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China; ct@webmail.hzau.eud.cn (T.C.); zhushuo@webmail.hzau.edu.cn (S.Z.); weining@webmail.hzau.edu.cn (N.W.); zikaizhao@hotmail.com (Z.Z.); njj@webmail.hzau.edu.cn (J.N.); youhui@mail.hzau.edu.cn (Y.S.)
2 Laboratory of Animal Virology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
3 The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan 430070, China
* Correspondence: sbcao@mail.hzau.edu.cn (S.C.); yej@mail.hzau.edu.cn (J.Y.)

2.2. Generation of mRNA and mRNA-LNP

The mRNA contained 5′ and 3′ UTR and a poly-A tail was produced from a linearized DNA template with a T7 in vitro transcription kit (Cellscript Madison, WI, USA), and pseudouridine was used in place of uridine. Then, the mRNA was enzymatically capped. According to the protocol, the mRNA was dissolved in an aqueous buffer and combined with GenVoy ILM (Precision Nanosystems, Vancouver, BC, Canada) at a flow ratio of 3:1 through a microfluidic mixer (Precision Nanosystems, Vancouver, BC, Canada). The solvent was removed by centrifugation at 2000× *g* using a 100 kDa ultrafiltration tube (Milipore, Billerica, MA, USA) and the size and PDI of mRNA-LNP was measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS (Malvern, Westborough, MA, UK). The concentration of mRNA-LNPs was measured by an Invitrogen's Quant-iT Ribogreen RNA assay kit (Invitrogen, Eugene, OR, USA).

NanoAssemblr Ignite



Settings on instrument

Genovy-ILM reagents (cytiva)

Component	Mol%
PNI Ionizable Lipid	50
DSPC	10
Cholesterol	37.5
PNI stabilizer	2.5
PNI formulation buffer	

- Working Genvoy-ILM- 12.5mM in anhydrous ethanol
- Working RNA concentration- 0.17mg/ml in PNI formulation buffer

- It will be mixed in the ratio of 3:1 (working RNA solution:working lipid mix)
- Atleast 1.5ml of prepared RNA working solution and atleast 0.5ml of prepared Genvoy-ILM solution so total 2ml of RNA-LNP formulation will be made.

Characterization of mRNA-LNP formulation

- Particle size
- Zeta potential

DLS analyzer (e.g. Malvern Zetasizer)

Malvern Zetasizer

RNA concentration in encapsulation

Encapsulation efficiency can be calculated with

Quant-it™ RiboGreen Assay (Thermo Fisher Scientific)

Standard curve will be generated with serial dilution of working RNA

↓

The concentration of unencapsulated RNA ($U_{[RNA]}$) is measured by adding RiboGreen reagent to wells containing intact LNPs

↓

The total amount of RNA ($T_{[RNA]}$) in each sample is measured by adding RiboGreen reagent to wells containing LNPs that have been disrupted using 0.5% Triton X-100 in 1 × TE buffer

↓

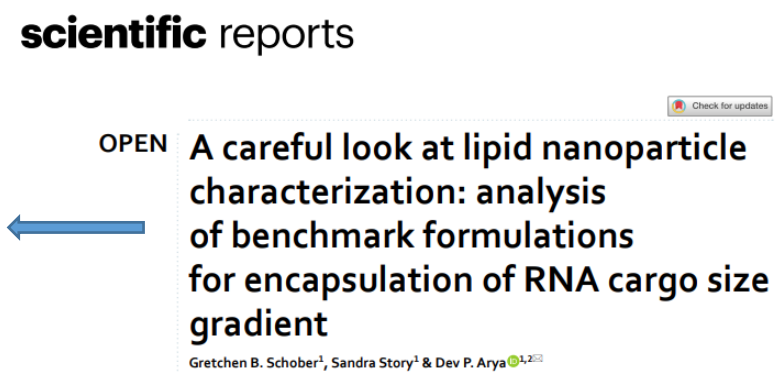
Encapsulated RNA ($E_{[RNA]}$) is calculated by subtracting the unencapsulated concentration of RNA from the total concentration of RNA

↓

$$E_{RNA} = T_{RNA} - U_{RNA}$$

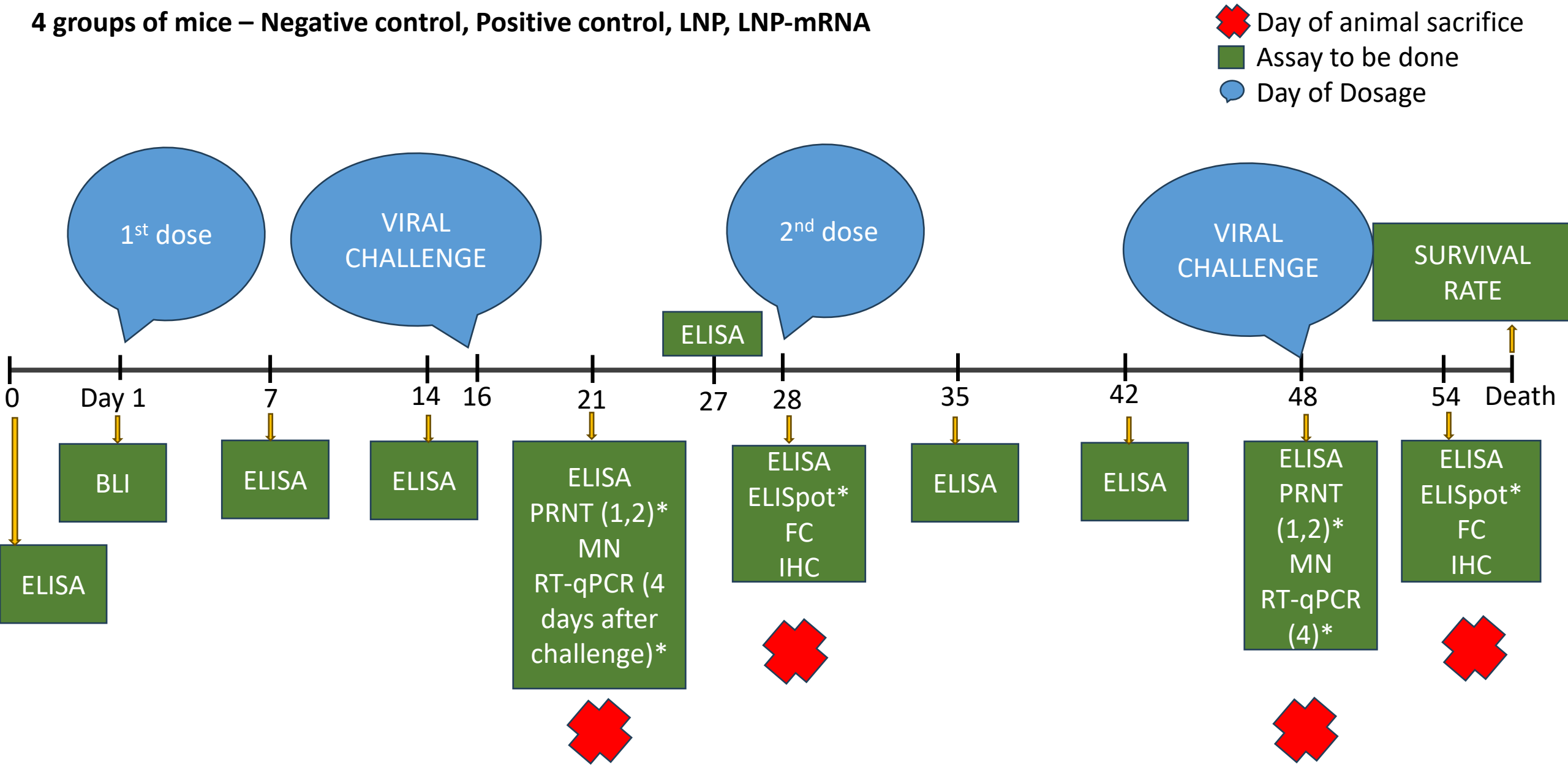
$$EE\% = E_{RNA} / T_{RNA} \times 100$$

Excitation wavelength -485nm
Emission wavelength- 528nm



Sr. NO.	ASSAY	SAMPLE TYPE	REASON
1.	PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT)	MICE SERUM SAMPLE Organs (Brain)	To study Neutralizing Ab Titer Virus Titer
2.	ELISA	MICE SERUM SAMPLE	To study Ab Titer (IgG)
3.	ELISpot assay	Splenocytes	To quantify various cytokines level (IFN-γ, TNF-α, IL-2, IL4, IL-6, CCL2, CCL5)*
4.	Flow cytometry (FC)	Splenocytes	To quantify CD4+ & CD8+ Level in spleen, Dendritic cell maturation
5.	MN Assay	MICE SERUM SAMPLE	To study microneutralization
6.	RT-qPCR	Tissue sample (Brain)	To quantify Viral Load
7.	BIOLUMINISCENCE (BLI)	NA	To study distribution and thermostability of LNP-mRNA
8.	H&E/IHC staining	Tissue sample	To study the neural cell distortion level (gliosis and cell morphogenesis)
9.	MICE SURVIVAL RATE	Observation till death	Overall effectiveness of vaccine

4 groups of mice – Negative control, Positive control, LNP, LNP-mRNA



Plaque reduction neutralization assay

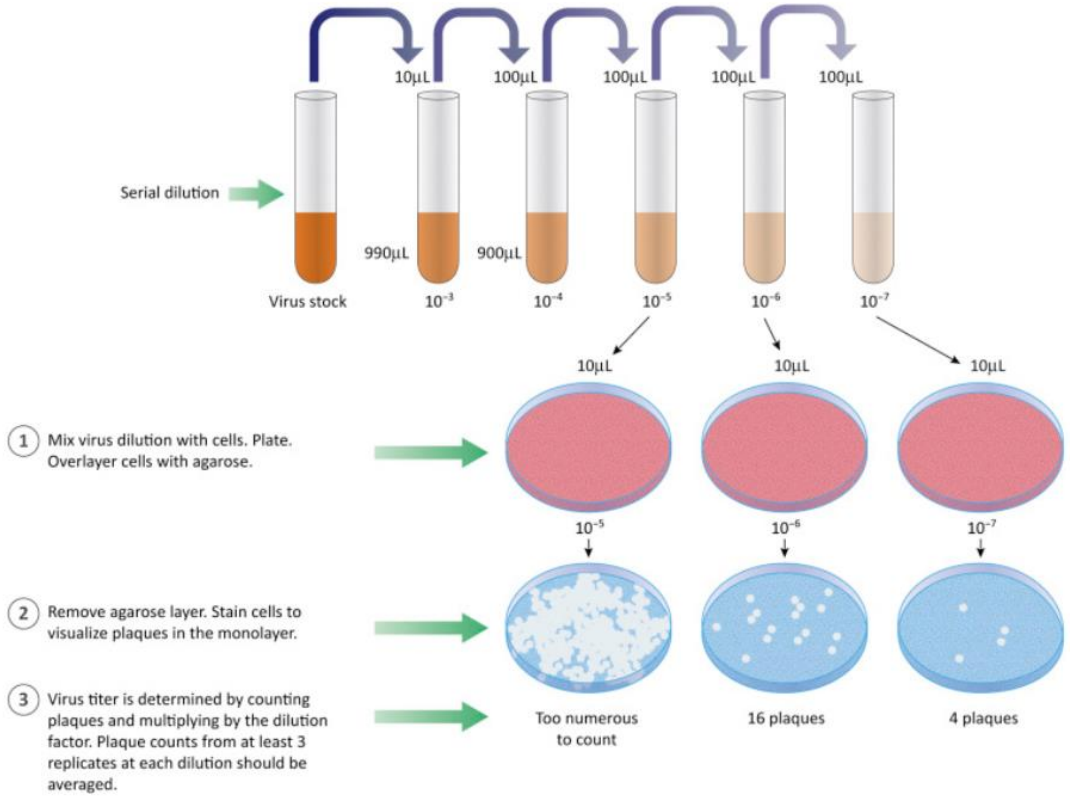
Article | Open access | Published: 05 August 2020

A replication-defective Japanese encephalitis virus (JEV) vaccine candidate with NS1 deletion confers dual protection against JEV and West Nile virus in mice

Na Li, Zhe-Rui Zhang, Ya-Nan Zhang, Jing Liu, Cheng-Lin Deng, Pei-Yong Shi, Zhi-Ming Yuan, Han-Qing Ye & Bo Zhang

Requirements

1. BHK21 cells/ Vero cells
2. Serum sample from mice
3. Methyl cellulose
4. Cell specific media
5. FBS
6. Formaldehyde
7. Cell culture plates



FLOW CYTOMETR

Requirements

1. FACSCalibur flow cytometer
2. Splenocytes from mice
3. 96 well microtiter plate(round bottom)
4. Peptide mixture (antigen specific)
5. Brefeldin A/GolgiPlug™
6. LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (L34966, Invitrogen, MA, USA)
7. PBS
8. Fluorescently Conjugated Ab to CD4, CD8
9. Zombie UV3 fixable viability Kit (STAIN DAED CELLS)

Cell. 2020 Sep 3;182(5):1271-1283.e16. doi: 10.1016/j.cell.2020.07.024. Epub 2020 Jul 23.

A Thermostable mRNA Vaccine against COVID-19

Na-Na Zhang [1], Xiao-Feng Li [2], Yong-Qiang Deng [2], Hui Zhao [2], Yi-Jiao Huang [2], Guan Yang [3], Wei-Jin Huang [4], Peng Gao [5], Chao Zhou [2], Rong-Rong Zhang [2], Yan Guo [2], Shi-Hui Sun [2], Hang Fan [2], Shu-Long Zu [2], Qi Chen [2], Qi He [3], Tian-Shu Cao [2], Xing-Yao Huang [2], Hong-Ying Qiu [2], Jian-Hui Nie [4], Yuhang Jiang [5], Hua-Yuan Yan [5], Qing Ye [2], Xia Zhong [5], Xia-Lin Xue [5], Zhen-Yu Zha [5], Dongsheng Zhou [2], Xiao Yang [3], You-Chun Wang [6], Bo Ying [7], Cheng-Feng Qin [8]

nature › npj vaccines › articles › article

Article | Open access | Published: 05 June 2023

Analyzing immune responses to varied mRNA and protein vaccine sequences

Hyeong-Jun Park, Yoo-Jin Bang, Sung Pil Kwon, Woori Kwak, Sang-In Park, Gahyun Roh, Seo-Hyeon Bae, Jae-Yong Kim, Hye Won Kwak, Yongkwan Kim, Soyeon Yoo, Daegeun Kim, Gyochang Keum, Eun-Kyoung Bang ✉, So-Hee Hong ✉ & Jae-Hwan Nam ✉

npj Vaccines **8**, Article number: 84 (2023) | Cite this article

ELISpot Assay

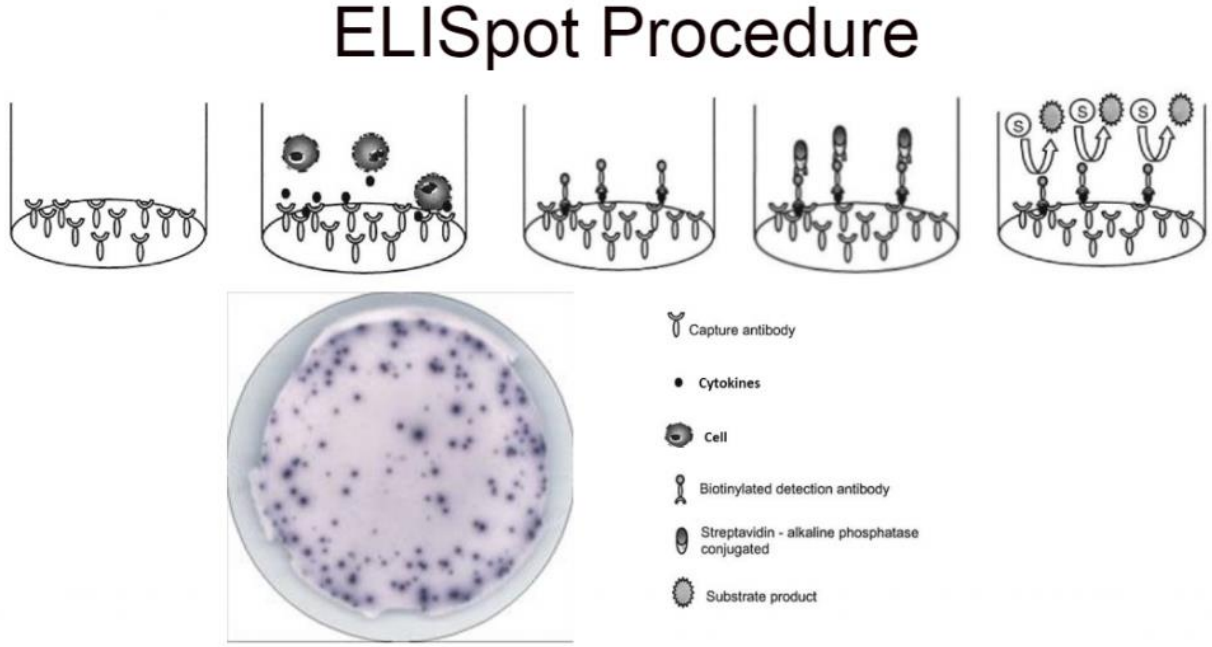
Bio Protoc. 2017 Jun 5; 7(11): e2302.
Published online 2017 Jun 5. doi: 10.21769/BioProtoc.2302

ELISPOT Assay to Measure Peptide-specific IFN-γ Production

Michelle N. Wykes[1,*] and Laurent Renia[2,3,*]

Requirements

1. Multiscreen $_{HTS}$-IP plates (PVDF membrane) (Merck, catalog number: MSIPS4510)
2. BSA, FCS
3. Rat anti-mouse IFNγ mAb
4. AEC substrate/chromogen
5. Streptavidin-horseradish peroxidase
6. Anti-IFN Ab
7. PBS
8. Peptides specific to antigen
9. Microscope or Automated ELISpot reader



ELISA

J Immunol. Author manuscript; available in PMC 2022 Jun 1.
Published in final edited form as:
J Immunol. 2021 Jun 1; 206(11): 2596–2604.
Published online 2021 May 10. doi: 10.4049/jimmunol.2100054

PMCID: PMC8165000
NIHMSID: NIHMS1688200
PMID: 33972374

Cellular and humoral immune responses in mice immunized with Vaccinia virus expressing the SARS-CoV-2 spike protein

Jake C. Harbour,[*] Zoe L. Lyski,[#*] John B. Schell,[#†] Archana Thomas,[#‡] William B. Messer,[*§¶] Mark K. Slifka,[‡] and Jeffrey C. Nolz[*‖#]

Requirements

1. ELISA plates
2. Protein specific to antigen
3. PBS
4. Tween
5. Serum sample from mice
6. Citrate buffer
7. *o*-phenylenediamine
8. anti-mouse IgG-HRP
8. Hydrogen peroxide
9. HCL
10. Plate Reader

MN Assay

nature > npj vaccines > articles > article

Article | Open access | Published: 05 June 2023

Analyzing immune responses to varied mRNA and protein vaccine sequences

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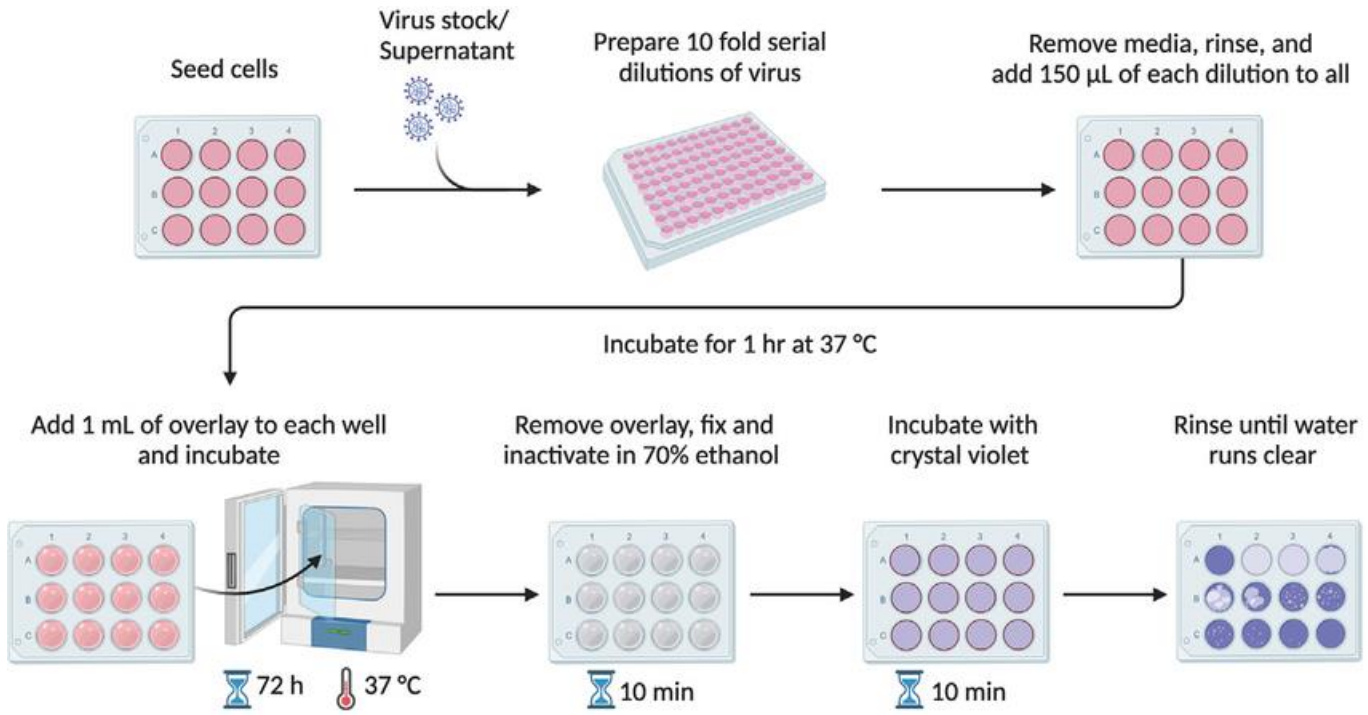
npj Vaccines **8**, Article number: 84 (2023) | Cite this article

Requirements

1. Crystal violet stain
2. Formaldehyde
3. Serum sample from mouse
4. 96-well plate
5. Vero cells
6. Cell specific media and FBS
7. Viral suspension



Bioluminiscence

(to study in-vivo distribution of LNP-mRNA and thermostability)

Cell. 2020 Sep 3;182(5):1271-1283.e16. doi: 10.1016/j.cell.2020.07.024. Epub 2020 Jul 23.

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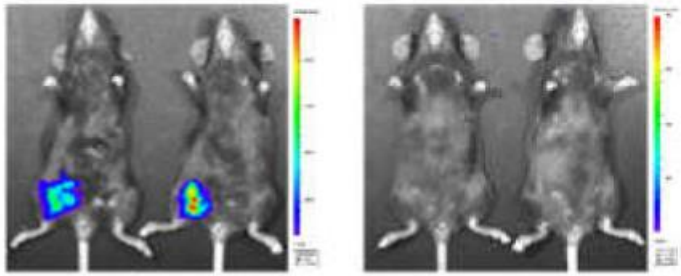
Na-Na Zhang [1], Xiao-Feng Li [2], Yong-Qiang Deng [2], Hui Zhao [2], Yi-Jiao Huang [2], Guan Yang [3], Wei-Jin Huang [4], Peng Gao [5], Chao Zhou [2], Rong-Rong Zhang [2], Yan Guo [2], Shi-Hui Sun [2], Hang Fan [2], Shu-Long Zu [2], Qi Chen [2], Qi He [3], Tian-Shu Cao [2], Xing-Yao Huang [2], Hong-Ying Qiu [2], Jian-Hui Nie [4], Yuhang Jiang [5], Hua-Yuan Yan [5], Qing Ye [2], Xia Zhong [5], Xia-Lin Xue [5], Zhen-Yu Zha [5], Dongsheng Zhou [2], Xiao Yang [3], You-Chun Wang [6], Bo Ying [7], Cheng-Feng Qin [8]

Requirements

1. Luciferase cassette in mRNA
2. Luciferase substrate
3. IVIS Spectrum instrument (for in-vivo)
4. living image 3.0 software



RT-qPCR

Requirements

1. Tissue lyser
2. Reverse transcription kit/ABscript II cDNA First Strand Synthesis kit
3. RNA extraction kit /QIAamp Viral RNA Mini Kit
4. Qrtpcr kit / One Step PrimeScript RTPCR Kit
5. JEV specific primers and probes

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mRNA Vaccines Encoding the HA Protein of Influenza A H1N1 Virus Delivered by Cationic Lipid Nanoparticles Induce Protective Immune Responses in Mice

by Xinyu Zhuang [1,†], Yanxin Qi [2,†], Maopeng Wang [3], Ning Yu [4], Fulong Nan [5], He Zhang [1], Mingyao Tian [1], Chang Li [1], Huijun Lu [1] and Ningyi Jin [1,*]

H&E /IHC staining

Requirements

1. Ethanol
2. Paraffin
3. H&E stain
4. Xylene, H2O2, Citrate buffer
5. VectaStain ABC kit (Vector Laboratories, Burlingame, CA).
6. Primary rabbit anti-occludin polycolonal antibody (pAb; Santa Cruz, Santa Cruz, CA), rabbit anti-claudin-5 pAb (Invitrogen), and rabbit anti-ZO-1 pAb (Sigma)
7. Diaminobenzidine
8. Fluroscence Microscope

nature › npj vaccines › articles › article

Article | Open access | Published: 05 June 2023

Analyzing immune responses to varied mRNA and protein vaccine sequences

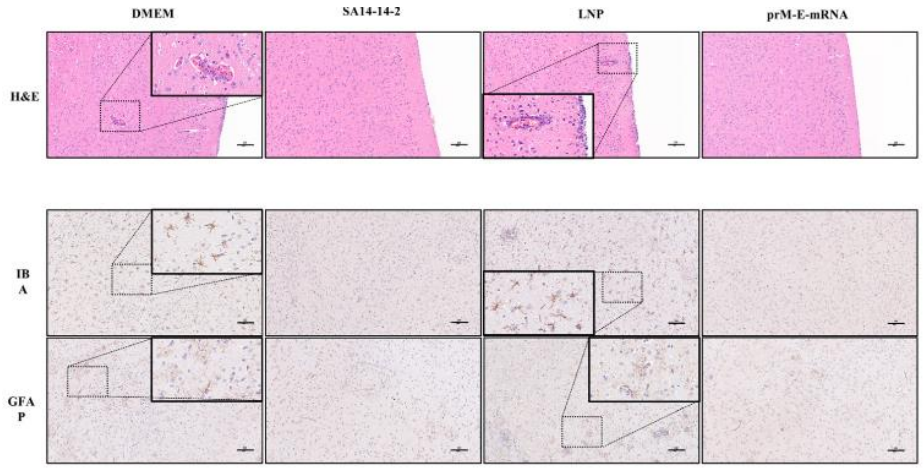
Hyeong-Jun Park, Yoo-Jin Bang, Sung Pil Kwon, Woori Kwak, Sang-In Park, Gahyun Roh, Seo-Hyeon Bae, Jae-Yong Kim, Hye Won Kwak, Yongkwan Kim, Soyeon Yoo, Daegeun Kim, Gyochang Keum, Eun-Kyoung Bang, So-Hee Hong & Jae-Hwan Nam

npj Vaccines **8**, Article number: 84 (2023) | Cite this article

J Virol. 2015 May 15; 89(10): 5602–5614. PMCID: PMC4442524
Published online 2015 Mar 11. doi: 10.1128/JVI.00143-15 PMID: 25762733

Viral Infection of the Central Nervous System and Neuroinflammation Precede Blood-Brain Barrier Disruption during Japanese Encephalitis Virus Infection

Fang Li,[a] Yueyun Wang,[a] Lan Yu,[a] Shengbo Cao,[a] Ke Wang,[a] Jiaolong Yuan,[a] Chong Wang,[a] Kunlun Wang,[a] Min Cui,[a] and Zhen F. Fu[a,b]



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6.	RT-qPCR	Tissue sample (Brain)	To quantify Viral Load
7.	BIOLUMINISCENCE (BLI)	NA	To study distribution and thermostability of LNP-mRNA
9.	MICE SURVIVAL RATE	Observation till death	Overall effectiveness of vaccine