

Cat. # 6148

For Research Use

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# Takara

**Takara IVTpro™  
mRNA Synthesis System (BspQ I)**

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Product Manual

v202410Da

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## I. Description

The Takara IVTpro mRNA Synthesis System (BspQ I) enables construction a template plasmid followed by high-yield generation of a desired mRNA by *in vitro* transcription (IVT). The system consists of two kits, the Cloning Kit for mRNA Template (BspQ I) (Cat. #6133) and the Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144). This system is compatible with CleanCap Reagent AG or CleanCap Reagent AG (3' OMe) cap analogs (TriLink Biotechnologies).

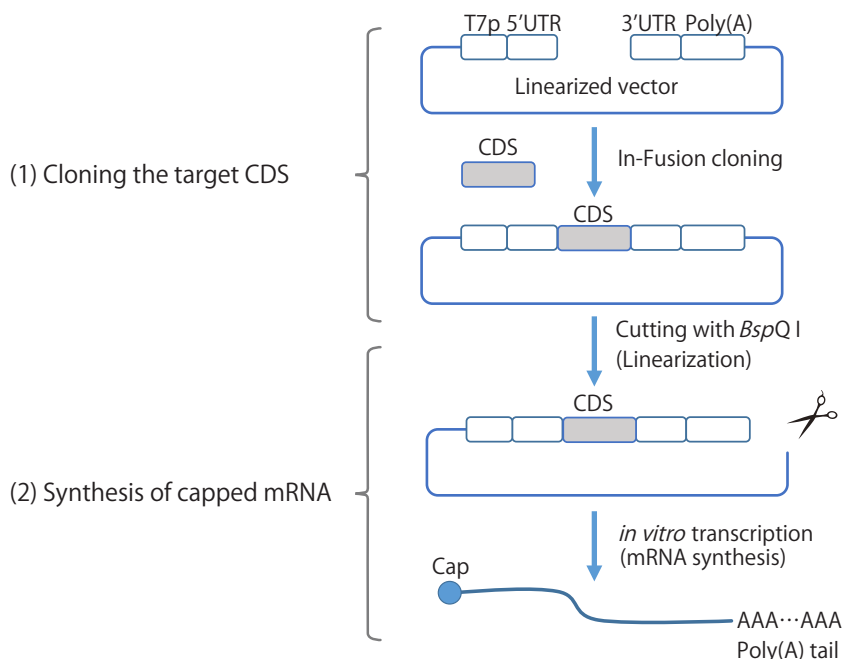


Fig. 1. Overview of the experimental flow of this product.

### (1) Cloning Kit for mRNA Template (BspQ I) (Cat. #6133)




The Cloning Kit for mRNA Template (BspQ I) allows for streamlined construction of an IVT template plasmid containing your desired coding sequence (CDS) using In-Fusion cloning. The Linearized Template Vector in the kit contains a T7 promoter, transcription start sequence (AGG), 5'-UTR (untranslated region), 3'-UTR, and a 141-base Poly(A) sequence, which are necessary for successful protein expression in mammalian cells. Unlike the Cloning Kit for mRNA Template (Cat. #6143; sold separately), the constructed plasmid is linearized at the Type IIS restriction enzyme *BspQ* I site that is immediately after the Poly(A) sequence, ensuring the mRNA synthesis template does not contain extra bases after the end of the Poly(A) sequence.

### (2) Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144)











The Takara IVTpro T7 mRNA Synthesis Kit is designed for synthesis of high-yield, high-quality mRNA through IVT. The kit uses a double-stranded DNA template containing a T7 promoter and works efficiently with CleanCap Reagent AG or CleanCap Reagent AG (3' OMe) to add a cap 1 structure to the 5' end of the mRNA, which is needed for efficient protein translation in eukaryotes. It is also possible to add a cap 1 structures to the mRNA produced with this product using Faustovirus Capping Enzyme (S17) (Cat. #2480A/B) or Vaccinia Capping Enzyme (Cat. #2460A/B) and mRNA Cap 2'-O-Methyltransferase (Cat. #2470A/B). Moreover, modified NTPs, such as pseudo-UTP, etc., can be used instead of UTP to reduce the innate immune response (Kariko, et al., 2008) of the mRNA in transfected cells without affecting mRNA yield.

## II. Components

### Cloning Kit for mRNA Template (BspQ I) (Cat. #6133\*<sup>1</sup>) (10 reactions)

 Linearized Template Vector (BspQ I) (50 ng/μl)	10 μl
 FLuc Control Fragment (BspQ I) (100 ng/μl)* <sup>2</sup>	10 μl
 5X In-Fusion Snap Assembly Master Mix* <sup>3</sup>	20 μl

### Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144\*<sup>1</sup>) (20 reactions; 20 μl reaction volume)

 10X Transcription Buffer	40 μl
 10X ATP	40 μl
 10X CTP	40 μl
 10X GTP	40 μl
 10X UTP	40 μl
 10X Enzyme Mix	40 μl
 Nuclease-Free Water	1 ml x 3
 DNase I	80 μl
 Lithium Chloride Precipitation Solution	600 μl
 Positive Control Template (FLuc) (0.5 μg/μl)* <sup>4</sup>	10 μl

\*1 Each kit is also sold separately.

\*2 Control DNA fragment for In-Fusion cloning that contains a *Photinus pyralis* luciferase CDS and has been codon-optimized for expression in human cells.

\*3 Same as that in In-Fusion Snap Assembly Master Mix (Cat. #638943/638944/638947 - 638949) (Volume differs depending on the product).

\*4 Linearized plasmid template that contains a T7 promoter, 5'UTR, FLuc-CDS, 3'UTR, and Poly(A) sequence.

## III. Storage -20°C

#### IV. Materials Required but not Provided

[Reagents]

- Competent cells
  - Stellar™ Competent Cells (Cat. #636763)\*, etc.
- SOC culture
- Luria-Bertani (LB) media
- LB/kanamycin (50 μg/ml) plates
- *BspQ I* (Cat. #1227A/B)
- Cap analogs
  - CleanCap Reagent AG (TriLink BioTechnologies, Code. N-7113-1/5/10/100)
  - CleanCap Reagent AG (3' OMe) (TriLink BioTechnologies, Code. N-7413-1/5/10/100)
- Capping enzymes
  - Faustovirus Capping Enzyme (S17) (Cat. #2480A/B) or Vaccinia Capping Enzyme (Cat. #2460A/B)
  - mRNA Cap 2'-O-Methyltransferase (Cat. #2470A/B)
- Modified NTPs
  - N<sup>1</sup>-Methylpseudouridine-5'-Triphosphate
  - Pseudouridine-5'-Triphosphate
  - 5-Methoxyuridine-5'-Triphosphate
  - 5-Methylcytidine-5'-Triphosphate, etc.
- Ethanol
- 3M sodium acetate (pH 5.2)
- TE buffer (containing 0.1 mM EDTA)
- RNase-OFF® (Cat. #9037)

[Equipment]

- Micropipettes and tips
- Microcentrifuge tubes
- Constant temperature bath or thermal cycler
- Refrigerated microcentrifuge
- Spectrophotometer
  - NanoDrop (Thermo Fisher Scientific), etc.

\* Not available in all geographic locations. Check for availability in your area.

#### V. Precautions for Use

RNase contamination of the double-stranded DNA template, reagents, tubes, micropipette tips, or other materials used in the reaction can significantly decrease or digest RNA obtained with the kit. Take precautions to avoid RNase contamination by using dedicated tubes and micropipettes and wearing disposable gloves. We recommend decontaminating your work area and micropipettes with the RNase decontamination solution RNase-OFF (Cat. #9037) prior to starting the protocol.

## VI. Cloning of a Target CDS Using the Cloning Kit for mRNA Template (BspQ I) (Cat. #6133)

### VI-1. Product overview

An overview of the Cloning Kit for mRNA Template (BspQ I) (Cat. #6133) is shown in Fig. 2 A/B.

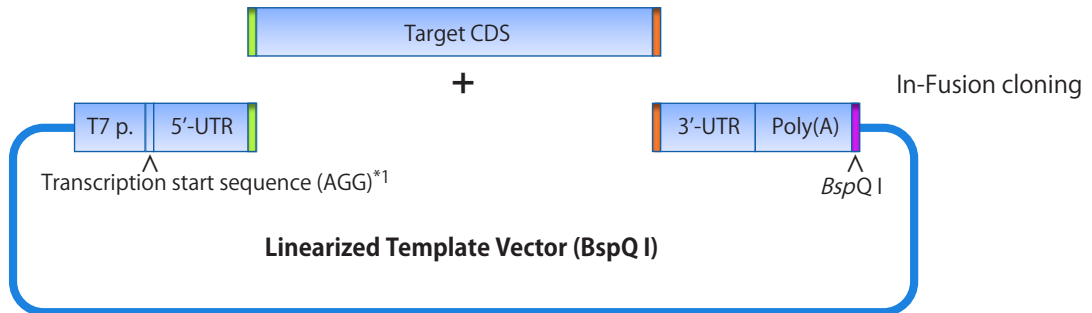


Fig. 2A. IVT template plasmid construction by In-Fusion cloning.\*2

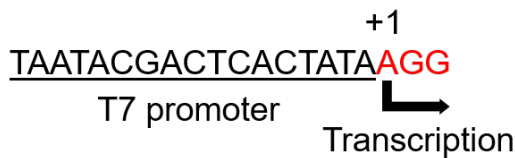


Fig. 2B. The transcription start sequence (AGG) for the IVT template when using CleanCap Reagent AG.

\*1 The transcription start sequence (AGG) is required in order to efficiently prepare mRNA with a cap structure using CleanCap Reagent AG.

\*2 For more information on In-Fusion Snap Assembly Master Mix (Cat. # 638943/638944/ 638947 - 638949), please see our website:

<https://www.takarabio.com>

## VI-2. Constructing the IVT template plasmid

### A) Design of desired gene coding sequence (CDS)

1. Obtain the CDS sequence for the desired gene.
  - The desired gene's CDS (the DNA sequence to be translated and ultimately protein, from the start codon to the stop codon) is required in order to express the gene. Even if only one region of the gene is to be expressed, including a start and stop codon is necessary (See "VI-2-B: PCR amplification of the desired CDS fragment").
  - One stop codon sequence is included on the vector sequence, but a CDS including a stop codon should be prepared in order to reliably stop translation of the target protein.
2. Optimize the CDS codons for the species of the cell types into which the mRNA will be transfected.
  - Use online tools or commercially available software.
  - When RNA is synthesized by IVT, a pseudo-UTP is often used instead of UTP to reduce the immunogenicity in mammalian cells (Kariko *et al.*, 2008). Reducing uridine (U) usage in the sequence is also important for immunogenicity reduction (Vidyanathan *et al.*, 2018 and Xia, 2021). Design the CDS considering both specific codon optimization and the frequency of uridines (U).
3. Confirm that a *BspQ I* restriction site, which is used when linearizing the final IVT template plasmid, is not present in the CDS of desired gene. If a *BspQ I* restriction enzyme site is present in the CDS, change the DNA sequence by changing the codon in the restriction sequence while keeping the amino acid sequence the same (e.g., switching an alanine codon from "GCU" to "GCC").

**Note:** We strongly recommend the use of *BspQ I* for linearization of the final IVT template plasmid.
4. Prepare the CDS of the desired gene by DNA synthesis, etc.

## B) PCR amplification of the desired CDS fragment

- Design forward and reverse primers to amplify the desired CDS that contain the 15-base In-Fusion sequence at the 5' end. In the example using FLuc below, the In-Fusion cloning sequence is shown in red, the start codon in blue, and stop codon in green (complementary strand).
  - If the desired CDS containing the In-Fusion sequence was prepared by DNA synthesis, proceed to "VI-2-C: In-Fusion cloning".

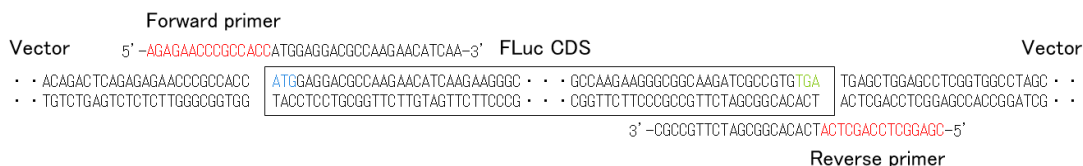



Fig. 3. Amplification of a target CDS fragment (Example: FLuc)



- Perform PCR amplification of the desired CDS using the PCR primers designed in Step 1. The resulting amplicon has a region of 15-base overlap region with both ends of the Linearized Template Vector (BspQ I).
  - For PCR amplification, we recommend using PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B), which has a very high level of accuracy, or TaKaRa Ex Premier™ DNA Polymerase (Cat. #RR370S/A/B, RR371S/A/B), which has a high PCR success rate in addition to high accuracy.
- Perform agarose electrophoresis with 5  $\mu$ l of the PCR reaction solution to confirm the amplification and quantity of the desired CDS fragment.
  - 50 to 100 ng/ $\mu$ l of purified PCR product is needed for the IVT reaction. Confirm that a sufficient quantity has been amplified.
- Purify the PCR product using a standard spin column purification kit. We recommend NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250, etc.).
  - Store at -20°C if not using immediately following purification.
  - If there are multiple amplification products perform gel extraction of the target PCR product. To obtain a single amplification product, try optimizing the PCR conditions or redesigning the primers.



### C) In-Fusion cloning

1. Thaw  Linearized Template Vector (BspQ I) and the CDS fragment prepared in V-2-B at room temperature. Mix briefly and spin down.
2. Prepare the reaction solution as follows:

< Per reaction >

Reagent	Volume
 Linearized Template Vector (BspQ I) (50 ng/ $\mu$ l)	1 $\mu$ l
CDS fragment with In-Fusion sequence added*	100 ng
Nuclease-Free Water	x $\mu$ l
 5X In-Fusion Snap Assembly Master Mix	2 $\mu$ l
Total	10 $\mu$ l

\*If using  FLuc Control Fragment (BspQ I), use 1  $\mu$ l (100 ng).

3. Incubate at 50°C for 15 minutes.




**Note:** If not proceeding immediately to transformation, store the reaction solution on ice or at -20°C for longer storage.

### D) Transformation

For transformation, we recommend Stellar Competent Cells (Cat. #636763). Use competent cells with transformation efficiency of  $1 \times 10^8$  cfu/ $\mu$ g or higher.

1. Thaw competent cells on ice.
2. After gently mixing, transfer 50  $\mu$ l of competent cells to a microcentrifuge tube.
3. Add 2.5  $\mu$ l of reaction solution prepared in Step C, mix gently, and place on ice for 30 minutes.
4. Heat-shock competent cells at 42°C for 45 seconds.
5. Cool them on ice for 1 to 2 minutes.
6. Add 450  $\mu$ l of SOC medium and shake the culture at 37°C for 1 hour.
7. Spread 50  $\mu$ l of culture medium from Step 6 and culture medium diluted 10-fold in SOC medium on LB plates containing kanamycin. Culture overnight at 37°C.

### E) Expected results

Over 100 colonies are usually obtained from plating 50  $\mu$ l of undiluted culture using the procedure outlined above with the  FLuc Control Fragment (BspQ I). Make liquid cultures from individual colonies, isolate the plasmid (using a standard extraction method such as NucleoSpin Plasmid (Cat. #740588.10)), and confirm the sequence of the purified plasmid. Refer to the product page for the sequence information of the  Linearized Template Vector (BspQ I) and the  FLuc Control Fragment (BspQ I).

- The Poly(A) sequence may be truncated depending on the *E. coli* strain or culturing method. We recommend confirming the sequence of the colony-derived plasmid and then preparing several glycerol stocks of the verified *E. coli* clone.

The following primers can be used to verify the presence of the Poly(A) sequence:

- Poly(A) Forward primer: 5'-CCTCGGTGGCCTAGCTTCTT-3'
- Poly(A) Reverse primer: 5'-CAGGGCTCCCAACCTTACC-3'

## VII. Preparation of Template DNA for IVT

### A) Preparation of a linearized plasmid template

#### a) Restriction digest

If possible, linearize the IVT template plasmid with a restriction enzyme that will produce a 5' overhang or blunt end, as template plasmids with a 3' overhang may lead to unintended synthesis of antisense or vector sequence (Schenborn and Mierindorf, 1985). Moreover, cut the IVT template plasmid with a restriction enzyme that will not leave extra bases following the Poly(A) sequence, as this can reduce the translation efficiency of the *in vitro*-transcribed mRNA.

A final plasmid template DNA concentration of 0.5 to 1.0  $\mu\text{g}/\mu\text{l}$  is needed for IVT. Be sure to account for DNA loss following ethanol precipitation or column purification of the restriction digest reaction. Refer to the following example for restriction digest conditions.

Example: linearize the plasmid prepared in VI-2-E as follows.

< Per reaction >

Reagent	Volume
Template plasmid	50 $\mu\text{g}$
10X BspQ I Buffer	20 $\mu\text{l}$
Nuclease-Free Water	x $\mu\text{l}$
BspQ I (10 U/ $\mu\text{l}$ )	5 $\mu\text{l}$
Total	200 $\mu\text{l}$

↓

Incubate at 50°C for 3 hours.

**Note:** If the restriction digestion is incomplete and undigested IVT template plasmid remains, some of RNA synthesized by IVT will be longer than desired. Confirm complete linearization of the plasmid by agarose gel electrophoresis using 5  $\mu\text{l}$  of the reaction solution.

#### b) Ethanol precipitation

1. Add a volume of 3M sodium acetate equal to 1/10th the restriction digest reaction volume and a volume of ethanol equal to double the volume of the restriction digest reaction volume to the reaction mixture.
2. Mix well and store for at least 15 minutes at -20°C.
3. Centrifuge at maximum speed for 15 minutes to pellet the DNA.
4. Carefully remove the supernatant, add 1 ml of 70% ethanol, and centrifuge at maximum speed for 15 minutes.
5. Carefully remove the supernatant and dry the pellet.
6. Resuspend the pellet in Nuclease-Free Water or TE buffer (containing 0.1 mM EDTA) and measure the DNA concentration. If necessary, adjust until the concentration of the solution is between 0.5 to 1.0  $\mu\text{g}/\mu\text{l}$ . Store at -20°C until use.

**Note:** Fragmentation of the mRNA synthesized using the linearized plasmid template above may indicate RNase contamination. To prevent RNA degradation, perform phenol-chloroform extraction of the plasmid template after restriction digestion and then purify it by ethanol precipitation.

### B) Preparation of a PCR product template

When using a PCR product as IVT template, perform PCR amplification of the sequence from T7 promoter to the end of the Poly(A) tail. Prepare the IVT template following the same purification method described in VII-A-b.

## VIII. mRNA Synthesis and Purification Using the Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144)

### VIII-1. Preparation of the IVT reaction

1. Prepare the reagents.
  - Thaw all components except the  $\text{EM}$  10X Enzyme Mix at room temperature. Gently mix and spin down before use.
  - Briefly spin down the  $\text{EM}$  10X Enzyme Mix and place on ice until use (do not vortex).
2. Prepare the reaction solution by adding each of the following components together in the order shown at room temperature.

**Note:** It is important to add each component in the **order shown**.

mRNA synthesis reaction using CleanCap Reagent AG or CleanCap Reagent AG (3' OMe):

< Per reaction >

Reagent	Volume	Final conc.
$\text{H}_2\text{O}$ Nuclease-Free Water	x $\mu\text{l}$	
$\text{TB}$ 10X Transcription Buffer* <sup>1</sup>	2 $\mu\text{l}$	1 X
$\text{ATP}$ 10X ATP* <sup>2</sup>	2 $\mu\text{l}$	1 X
$\text{CTP}$ 10X CTP* <sup>2</sup>	2 $\mu\text{l}$	1 X
$\text{GTP}$ 10X GTP* <sup>2</sup>	2 $\mu\text{l}$	1 X
$\text{UTP}$ 10X UTP* <sup>2</sup>	2 $\mu\text{l}$	1 X
CleanCap Reagent AG* <sup>3,4</sup>	1.6 $\mu\text{l}$	8 mM
Template DNA* <sup>5</sup>	1 $\mu\text{g}$	0.05 $\mu\text{g}/\mu\text{l}$
$\text{EM}$ 10X Enzyme Mix	2 $\mu\text{l}$	1 X
Total* <sup>6</sup>	20 $\mu\text{l}$	

\*<sup>1</sup>  $\text{TB}$  10X Transcription Buffer contains spermidine. Spermidine forms a complex with nucleic acids that may precipitate out of the solution. To prevent formation of this precipitate, be sure to add the components **in the order shown**.

\*<sup>2</sup> **The concentration of each NTP is 100 mM.** When using modified NTPs, replace the corresponding NTP with an equivilant quantity.

\*<sup>3</sup> Use CleanCap Reagent AG or CleanCap Reagent AG (3' OMe) at a 4:5 molar ratio with the NTPs (final concentration 8 mM).

\*<sup>4</sup> An mRNA cap can instead be added through an enzymatic reaction. For more information, please see the data sheets for the following products:

- Faustovirus Capping Enzyme (S17) (Cat. #2480A/B) or Vaccinia Capping Enzyme (Cat. #2460A/B)
- mRNA Cap 2'-O-Methyltransferase (Cat. #2470A/B)

\*<sup>5</sup> Use a template that has an AGG transcription start codon. The optimal amount of template differs depending on the size and type of the template, but it usually ranges from 0.5 to 2  $\mu\text{g}$ . For  $\text{PC}$  Positive Control Template (FLuc), use 2  $\mu\text{l}$  (1  $\mu\text{g}$ ).

\*<sup>6</sup> Scale-up as needed.

3. Mix well and incubate at 37°C for 2 hours.
  - Adjust the reaction time depending on the target length and desired RNA yield.
  - A white precipitate may form at the end of the reaction, which is thought to be magnesium pyrophosphate produced when released pyrophosphate reacts with the magnesium in the reaction solution. It does not affect the subsequent operations. Proceed to “VIII-2. DNase I treatment”.

#### VIII-2. DNase I treatment

After the reaction in VIII-1-3, add 4  $\mu$ l of  $\ominus$  DNase I, mix gently, and incubate at 37°C for 15 minutes.

#### VIII-3. Purification by LiCl precipitation

The LiCl precipitation method can effectively remove unincorporated NTPs and proteins. However, RNA cannot be efficiently recovered if the RNA is shorter than 300 bases or if the RNA concentration is less than 0.1  $\mu$ g/ $\mu$ l. In these cases, purify the synthesized RNA by phenol-chloroform extraction followed by ethanol precipitation or spin column purification (using NucleoSpin RNA Clean-up (Cat. #740948.10/.50/.250)). RNA purified by these methods can be used in transfection, electroporation, and microinjection experiments.

1. Thaw the  $\oplus$  Lithium Chloride Precipitation Solution at room temperature. Mix well, and if a precipitate is observed, warm the solution at 37°C. If the precipitate persists, use as is. The precipitate does not affect the subsequent steps.
2. Add 30  $\mu$ l of  $\oplus$  Nuclease-Free Water and 30  $\mu$ l of  $\oplus$  Lithium Chloride Precipitation Solution to the IVT reaction solution treated with DNase I in VIII-2 to stop the reaction.
3. After mixing well, place at -20°C for at least 30 minutes.
4. Centrifuge at 4°C for 15 minutes at maximum speed to pellet the RNA.
5. Carefully remove the supernatant and wash the pellet with 1 ml of 70% ethanol.
6. Centrifuge again at 4°C for 15 minutes at maximum speed to pellet the RNA.
7. Carefully remove the supernatant.
8. Air-dry the pellet and dissolve in 100  $\mu$ l of  $\oplus$  Nuclease-Free Water.
  - Note:** Do not over-dry the pellet, as it can make resuspension difficult.
  - Note:** Depending on the RNA yield, it may take time for the pellet to dissolve. Let stand at room temperature or 4°C, mix as needed, and check for dissolution.
9. After the pellet dissolves, measure the RNA concentration with a spectrophotometer (NanoDrop, etc.). If not using the RNA sample immediately, store at -20°C or lower.


**Note:** Presence of residual unused NTPs, cap analogs, or the template DNA will affect the OD measurement. Measure a sample that has been purified by the method described above.

**Note:** If necessary, use denaturing agarose/acrylamide gel or a Bioanalyzer (Agilent) to confirm the length and purity of the RNA obtained.

## IX. Troubleshooting

If you are having trouble inserting the desired CDS into the linearized vector by In-Fusion cloning, please refer to the troubleshooting guide in the In-Fusion Snap Assembly Master Mix User Manual (Cat. #638943/638944/638947 - 638949), available on our website at <https://www.takarabio.com>.

Other troubleshooting issues:

Problem	Cause	Solution
RNA yield is less than expected	RNase contamination of template DNA	Perform phenol-chloroform extraction of the template DNA after restriction digest and purify by ethanol precipitation.
	Amount of template DNA is inappropriate	Check the amount of DNA by agarose electrophoresis. If there is a discrepancy with the measured OD value, try purifying the template DNA again.
	Reaction time was insufficient	Increase the IVT reaction time.
	RNA is shorter than 300 bases or has a concentration of 0.1 $\mu\text{g}/\mu\text{l}$ or less	Rather than purifying RNA by the LiCl precipitation method, purify by phenol-chloroform extraction followed by ethanol precipitation or spin column.
	RNA pellet loss	Carefully remove the supernatant, using a micropipette with the smallest possible tip.
	Insufficient RNA dissolution/elution	Dissolution may take time depending on the RNA yield. Allow the solution to stand at room temperature or 4°C, mix as needed, and then measure the RNA after it has completely dissolved. If it still does not dissolve, add more solvent. When the spin column method is used for purification, a single round of elution may not be sufficient. It is strongly recommended that you perform the elution twice (e.g., 50 $\mu\text{l} \times 2$ ).
	RNase contamination of reagents or equipment during processing	Take extreme care to avoid RNase contamination by using dedicated tubes and tips for the reaction and wearing disposable gloves. Use RNase-OFF (Cat. #9037) to decontaminate the work bench and equipment.
RNA longer than the target size is observed	Reagent deterioration	Keep enzymes on ice. Avoid excessive agitation and freezing-thawing. If at least 100 $\mu\text{g}$ of RNA cannot be obtained with the  Positive Control Template (FLuc), please purchase a new Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144).
	Insufficient linearization of the template plasmid	Perform restriction digestion on the template plasmid again, and confirm complete linearization of the plasmid with agarose electrophoresis.
RNA shorter than the target size is observed	Insufficient RNA denaturation	Use a denaturing agarose gel or acrylamide gel to perform electrophoresis.
	The CDS contains a sequence similar to the transcription termination signal of the T7 RNA Polymerase	If possible, change the nucleic acid sequence to replace the codons in a manner that maintains the original amino acid sequence.
Fragmented RNA below the target size is observed	RNase contamination	Take extreme care to avoid RNase contamination by using dedicated tubes and tips for the reaction and wearing disposable gloves. Use RNase-OFF (Cat. #9037) to decontaminate the work bench and equipment.

## X. References

- 1) Karikó, K. *et al.* Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther J Am Soc Gene Ther.* (2008) **16**: 1833-1840.
- 2) Vaidyanathan, S. *et al.* Uridine Depletion and Chemical Modification Increase Cas9 mRNA Activity and Reduce Immunogenicity without HPLC Purification. *Mol Ther Nucleic Acids.* (2018) **12**: 530-542.
- 3) Xia, X. Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines. *Vaccines (Basel).* (2021) **9**: 734.
- 4) Schenborn, E. T. and Mierendorf, R. C. A novel transcription property of SP6 and T7 RNA polymerases: dependence on template structure. *Nucleic Acids Res.* (1985) **13**: 6223-6236.

## XI. Related products

Takara IVTpro™ mRNA Synthesis System (low dsRNA) (Cat. #6131)  
Cloning Kit for mRNA Template (BspQ I) (Cat. #6133)  
Takara IVTpro™ T7 mRNA Synthesis Kit (low dsRNA) (Cat. #6134)  
Takara IVTpro™ mRNA Synthesis System (Cat. #6141)  
Cloning Kit for mRNA Template (Cat. #6143)  
Takara IVTpro™ T7 mRNA Synthesis Kit (Cat. #6144)  
Faustovirus Capping Enzyme (S17) (Cat. #2480A/B)  
Vaccinia Capping Enzyme (Cat. #2460A/B)  
mRNA Cap 2'-O-Methyltransferase (Cat. #2470A/B)  
PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)  
TaKaRa Ex Premier™ DNA Polymerase (Cat. #RR370S/A/B)  
TaKaRa Ex Premier™ DNA Polymerase Dye plus (Cat. #RR371S/A/B)  
Stellar™ Competent Cells (Cat. #636763)\*  
*E. coli* HST08 Premium Competent Cells (Cat. #9128)  
NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)\*  
NucleoSpin Plasmid (Cat. #740588.10/.50/.250)\*  
BspQ I (Cat. #1227A/B)  
NucleoSpin RNA Clean-up (Cat. #740948.10/.50/.250)\*  
In-Fusion® Snap Assembly Master Mix (Cat. #638943/638944/638947 - 638949)  
RNase-OFF®(RNase decontamination solution) (Cat. #9037)

\* Not available in all geographic locations. Check for availability in your area.

PrimeSTAR is a registered trademark of Takara Bio Inc.  
In-Fusion is a registered trademark of Takara Bio USA Inc.  
RNase-OFF is a registered trademark of PureBiotech, LLC.  
IVTpro and Ex Premier are trademarks of Takara Bio Inc.  
Stellar is a trademark of Takara Bio USA Inc.

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**NOTE:** This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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