Efforts to Accelerate mRNA Process Development: From Process Development to Manufacturing Clinical Trial Materials with a Focus on Shortening Development Time and Reducing dsRNA

INTERNATIONAL mRNA HEALTH CONFERENCE

FUJIFILM 富士フイルム富山化学株式会社

○ Keita Tanaka[1,2], Shinsuke Mizumoto[1,2], Hiromi Hayashi[1,2], Shigetomo Tsujihata[1,2]

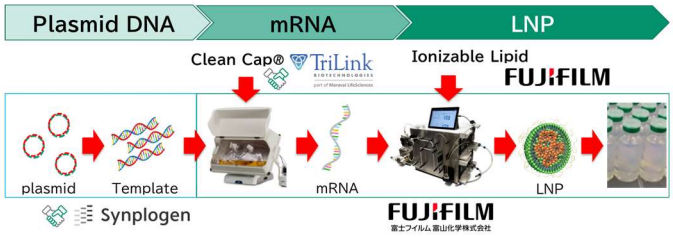
1) FUJIFILM Toyama Chemical Co., Ltd., 2) FUJIFILM Corporation

COI: All studies were based on plans and funding from the Fujifilm Group. All authors are employees of Fujifilm Toyama Chemical Co., Ltd.

Abstract

- Following the successful development of mRNA-LNP-based SARS-CoV-2 vaccines, mRNA therapeutics are emerging as promising new treatments for the prevention and treatment of various diseases. To accelerate their development, it is essential to establish manufacturing technologies for high-quality mRNA and gain a deep understanding of the manufacturing process, including LNP. This presentation focuses on mRNA, reporting on the establishment of robust manufacturing processes and the reduction of dsRNA that induces immune responses.
- For the in vitro transcription (IVT) reaction process, our proprietary, versatile IVT platform and design of experiments (DoE) allowed us to achieve reaction condition optimization in just a few weeks. Furthermore, we introduced manufacturing equipment used in GMP facilities into the laboratory, enabling rapid confirmation and validation of scalability.
- For removal of dsRNA, we established two dsRNA reduction processes: one is suppression of dsRNA generation in IVT reaction by the use of engineered T7 RNA polymerase, and another is removal of generated dsRNA through dsRNA affinity column chromatography, which resulted in a maximum of 98% dsRNA removal.
- The robust and rapid mRNA manufacturing process development technology achieved through these results is expected to enable the provision of high-quality mRNA and a significant reduction in the time required to initiate clinical trials.

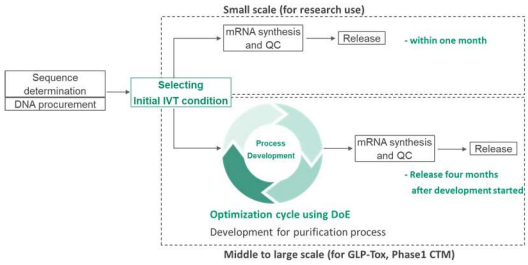
FFTC's mRNA-LNP CDMO services



- Services covering a wide range of needs from sample for research use to investigational drug and commercial manufacturing
- Supports integrated production from DNA through mRNA manufacturing to LNP formulation
- Provides proprietary lipids (GMP-compliant) to support customer LNP research and development

Our proprietary IVT platform and process development using DoE

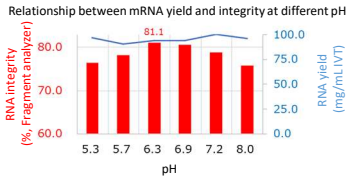
■ Our mRNA manufacturing and process development framework



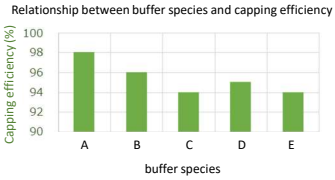
■ Understanding IVT reactions and development of selecting method of initial IVT condition

【The influence effect of pH on mRNA integrity in IVT Reactions】

- Good quality within the pH range of 6.3 to 6.9
- No difference in yield within the pH range of 5.3 to 8



- Capping efficiency varies depending on buffer species



Leveraging our expertise in IVT, we have developed a reaction condition setting technology that shortens the initial IVT reaction condition screening period.

【Selecting a pre-determined IVT reaction condition based on target mRNA information】

- Based on sequence information and our experience, IVT reactions are classified into three condition patterns, and necessary amount of each reagent is set uniformly depending on the IVT reaction pattern.
- Applicable to various types of mRNA
- **Without experimental trial and error**

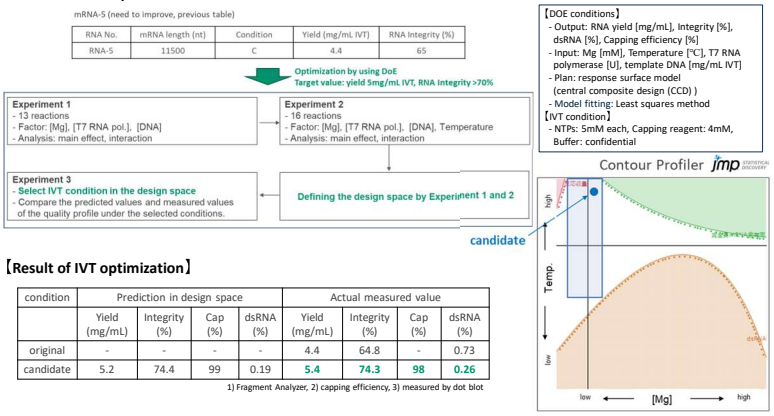
RNA No.	mRNA length (nt)	IVT Condition*	Yield (mg/mL IVT)	RNA Integrity (%)
RNA-1	860	B	4.9	90
RNA-2	900	A	5.3	96
RNA-3	3000	A	4.5	88
RNA-4	4300	B	4.6	91
RNA-5	11500	C	4.4	65

* Detailed IVT condition is undisclosed.

- We clarified that buffer type and pH are critical factors for mRNA quality
- Using our IVT platform enables rapid, moderate to high-quality synthesis of mRNA for early-stage research (no need for search for IVT reaction condition)

■ Optimization of IVT reaction condition by using DoE

【DoE-based IVT optimization flow】



【Result of IVT optimization】

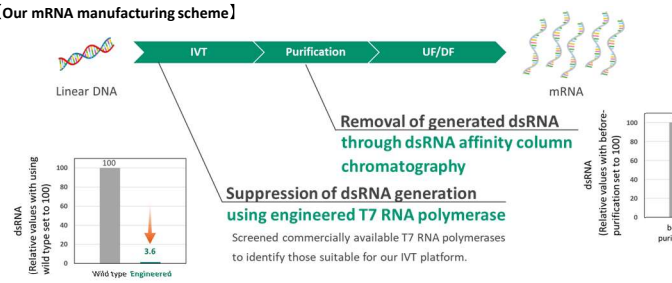
condition	Prediction in design space				Actual measured value			
	Yield (mg/mL)	Integrity (%)	Cap (%)	dsRNA (%)	Yield (mg/mL)	Integrity (%)	Cap (%)	dsRNA (%)
original	-	-	-	-	4.4	64.8	-	0.73
candidate	5.2	74.4	99	0.19	**5.4**	**74.3**	**98**	**0.26**

1) Fragment Analyzer, 2) capping efficiency, 3) measured by dot blot

- Construct design space using DoE to determine robust IVT reaction condition with fewer experiments and shorter timelines
- Complete process development, including scalability testing and downstream steps, in approximately three months

Efforts aimed at reducing dsRNA

【Our mRNA manufacturing scheme】



【Performance】 Target mRNA length: 1800 nt, IVT reaction scale: 0.675 L, Final mRNA product amount: 2.0 g

Manufacturing process sample	RNA Integrity (%)	dsRNA by DotBlot (%)	dsRNA by ELISA (%)
Post-IVT	86.5	<0.02*	upper LOQ
Post-Purification	90.1	<0.02*	0.012
Post-UF/DF (Final product)	88.9	<0.02*	0.018

* below LOQ

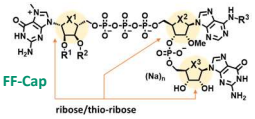
- DotBlot shows dsRNA level has been below LOQ ever since IVT.
- ELISA method suggests the effect of dsRNA affinity column chromatography on reducing dsRNA.
- **92.5%** is average recovery rate after dsRNA affinity column chromatography

- We developed the dsRNA suppression process using engineered T7 RNA polymerase and the dsRNA removal process through affinity column purification, achieving over 90% dsRNA reduction in both cases.
- We demonstrated effectiveness of our mRNA manufacturing scheme in gram scale.

New mRNA Cap analog

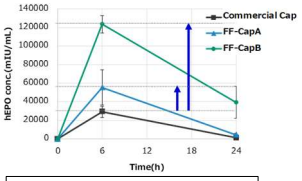
■ Fujifilm original Cap analog (FF-Cap) has high IVT reactivity and translation activity

【Structural features】



Non-natural: having one or more thio-ribose units

【*in vivo* translation】



- Animal: mouse (n=3)
- Administration: 0.1mg/kg LNP including mRNA (hEPO), single-iv
- Analysis: hEPO concentration in plasma at 6 hours and 24 hours measured by ELISA

【IVT】 Target mRNA: hEPO-mRNA (859 nt)

Cap reagent	RNA Integrity (%)	Capping efficiency (%)
Commercial Cap	82.5	97
FF-CapA	90.6	97
FF-CapB	90.8	76

- FF-Cap is promising to achieve higher level of *in vivo* translation than commercial cap

Conclusion

The results of this research propose a highly robust and rapid mRNA manufacturing process development methodology, enabling the provision of high-quality mRNA and significant reduction in development time from initiation to clinical trial entry. In addition, our company possesses advanced LNP formulation development technology and a proven track record, which is expected to contribute to accelerating mRNA-LNP drug development. Going forward, we will further refine these results and moreover enhance our CDMO services.