Our RNA production platform and the associated tools facilitate the development of RNA-based vaccines

Xavier Warnet, Célia Bosso-Lefèvre, Erica Cirri, Eric Mennesson, Dimitri Szymczak, Flavien Carpentier, Laetitia Emond, Alexandra Foucher, Alzbeta Komarkova, Maxime Rochet, Armelle Vindrios, Romain Goulay, Jean-François Têtu. Tebubio, France

Tebubio

Since 2021, Tebubio has developed a miniscale RNA production platform for providing RNAs (from 100 µg to 1 mg) to researchers around the world. This service is completed by offers on RNA delivery solutions using lipid nanoparticles (LNPs) and cellular tests with biomarker analysis.

Abstract

Here, we present a complete study on CAP1 capped linear RNA produced from our DNA templates. We assess the stability of two RNAs expressing GFP and the typical tumor antigen, p53, and their relative expression into antigen-presenting cells (APCs). Furthermore, we quantified the response of the APCs expressing p53 by using our biomarker multiplex analysis system.

Finally, we present our formulation platform for the encapsulation of mRNAs inside Lipid Nanoparticles (LNP), showing both information regarding the ultrastructure of the LNPs, and RNAs expression in different cellular models. This new type of service and its future developments will constitute a new addition to our pipeline of RNA production and testing for preclinical studies.

INTRODUCTION

RNA vaccines have shown their full potential in the Covid-19 pandemic. The application of these technologies for the treatment of cancers represents a significant hope, but clinical effectiveness remains to be demonstrated. For this purpose, researchers require high quality production of vaccine RNA, and accurate and cost-saving test tools that are adapted to oncology research.

To answer to this growing need for RNA, improvements in manufacturing throughput and turnaround time are required. Tebubio Contract Research Services offer an RNA production service providing µg to mg amounts of custom RNA, to be used for screening purposes in preclinical research (vaccine, therapeutic agent, personalized medicine, etc) for the pharmaceutical industries, biotechs and academics.

Construction design

We synthesized RNAs using our proprietary plasmid DNA template for linear RNAs with UTRs and poly-A tail optimized for efficient transcription and translation in mammalian cells. The T7-FlashScribe transcription kit (Cellscript, Ref. C-ASF3507) was used for the RNA synthesis.
To test the transfection efficiency, we prepared fluorescent eGFP RNAs by replacing UTPs with Andy Fluor 647-X-UTP probe (AF647, Ref. C418-T, ex/em = 650/665 nm). Transfection in cells was done using the mRNA Fect reagent (RJH Biosciences, Ref. 80-40).
RNA encapsulation was performed using the Cytiva, Precision Nanosystems Spark instrument and the GenVoy-ILM lipid mixture. Particle size and size distribution was measured by Zetasizer analyzer, Malvern Panalytical. RNA content and encapsulation efficiency was evaluated by Quant-it™ RiboGreen RNA Assay Kit (Invitrogen).

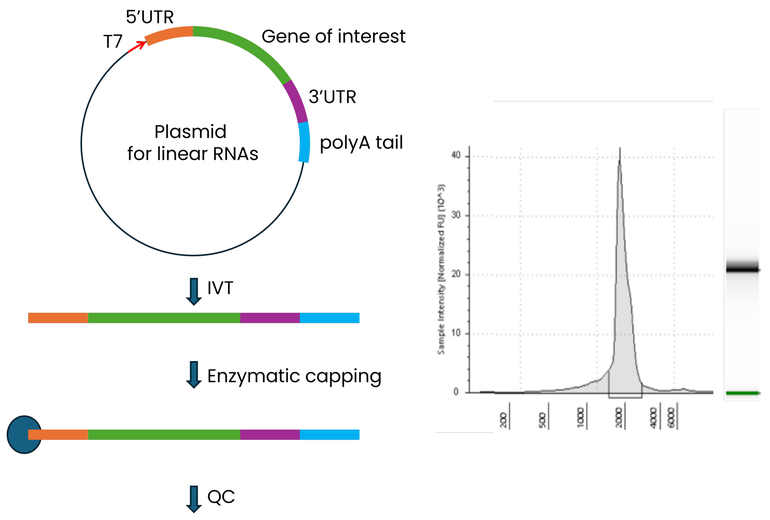

Figure 1. RNA production steps for linear RNAs. For QC step includes an electrophoresis and its densitometry profile.

eGFP & p53-eGFP expression in HEK and dentritic cell lines

The RNAs were transfected into HEK293 cells and dendritic cells (Zen-Bio, ref. SER-MODC-F). The fluorescence was checked by microscopy 24 hours after transfection in HEK293 cells and followed from 24h to 6 days after transfection in dentritic cells.

- eGFP RNA can be observed in most of the cells 24h after transfection
- p53-eGFP is broadly expressed in the nucleus and the cytoplasm as expected.
- Global RNAs expression at the protein level is weaker in APCs than in HEK model.
- eGFP protein is detected from 5h to 5 days post-transfection. A peak of protein of expression is observed between 24h and 48h after transfection (Table 1).

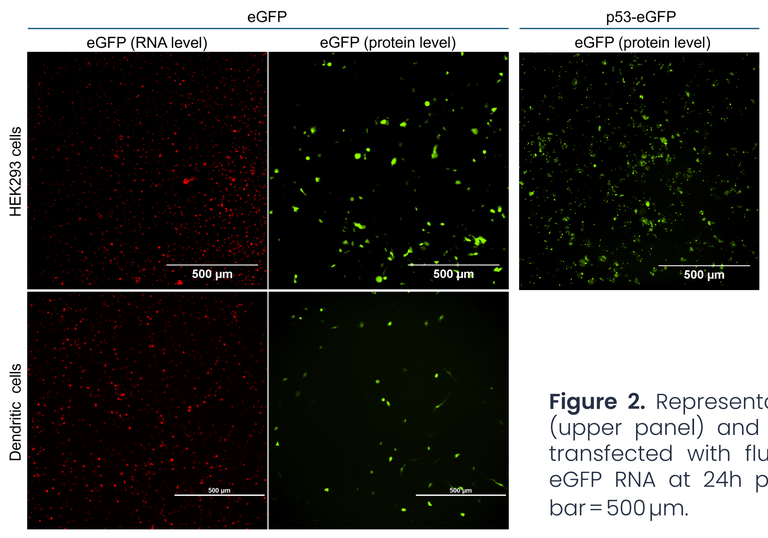

Figure 2. Representative images of HEK293 cells (upper panel) and dendritic cells (lower panel) transfected with fluorescent eGFP RNA or p53-eGFP RNA at 24h post-transfection (n=3). Scale bar = 500 µm.

A eGFP expression	5h	24h	48h	5D	6D
eGFP RNA	+++	++++	++++	+	-
AF647-eGFP RNA	++	++	++	-	-

B Fluorescent RNA	5h	24h	48h	5D	6D
AF647-eGFP RNA	+++	+++	+++	+++	-

Table 1. A: levels of eGFP protein expression after RNA transfection in dendritic cells. B: levels of fluorescent RNA (AF647) after transfection in dendritic cells.

Cytokine release post transfection with p53 in dendritic cells

RNAs were transfected in dendritic cells (Zen-Bio, ref. SER-MODC-F). 48h post-transfection, p53 expression was checked by immunofluorescent (IF) staining (Figure 3). Media were collected 48h after transfection to quantify the cytokine release by multiplex ELISA (Q-Plex™ Human Innate Immunity, Quansys Biosciences, ref. 111233HU) for the measurement of GMCSF, IFNα, IFNβ, IFNγ, IL-1α, IL-1β, IL-18 (Figure 4).

- eGFP is expressed in the whole cells while p53-eGFP is mainly located in nucleus (Figure 3)
- Multiplex analysis enabled the quantification of IFNα and IL-18 released by dendritic cells (Figure 3)
- IFNα secretion in the medium is strongly promoted in the cells transfected with eGFP RNAs while it is slightly increased in cells transfected with p53-eGFP RNA as compared to control cells.
- We observe a similar patern of release with IL18

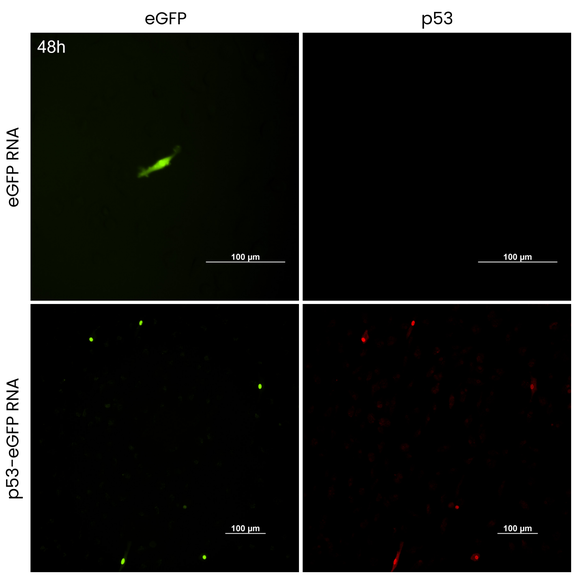

Figure 3. Immunofluorescent staining in dendritic cells transfected with eGFP, p53, and p53-eGFP RNAs (n=3). The cells were fixed in 4% paraformaldehyde and p53 stained by p53 Polyclonal Antibody (ThermoFisher Scientific, ref. PA5-27822). Scale bar = 100 µm.

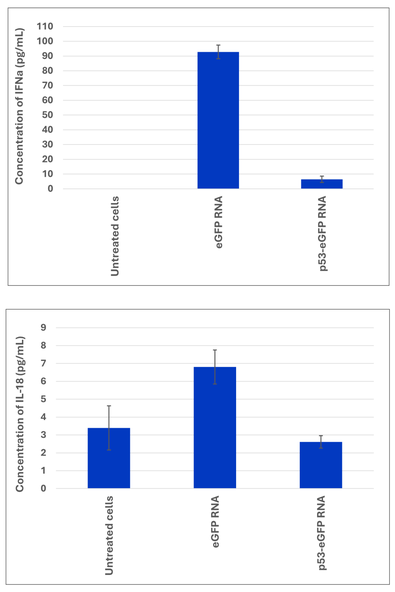

Figure 4. Multiplex quantification of cytokines IFNα and IL-18 released by dendritic cells (n=3).

LNP formulation

Two different mRNAs (eGFP and Luciferase) were encapsulated using Spark intrument (Cytiva, Presicion Nanosystems) and the GenVoy-ILM lipid mixture.

- The LNPs obtained were of similar size and displayed, mostly, monodisperse behavior as assessed by DLS.
- Encapsulation efficiency was similar between the two mRNA.
- We also evaluated mRNA payload in LNPs studying the effect of mRNA concentration on both LNP size and on encapsulation efficiency.

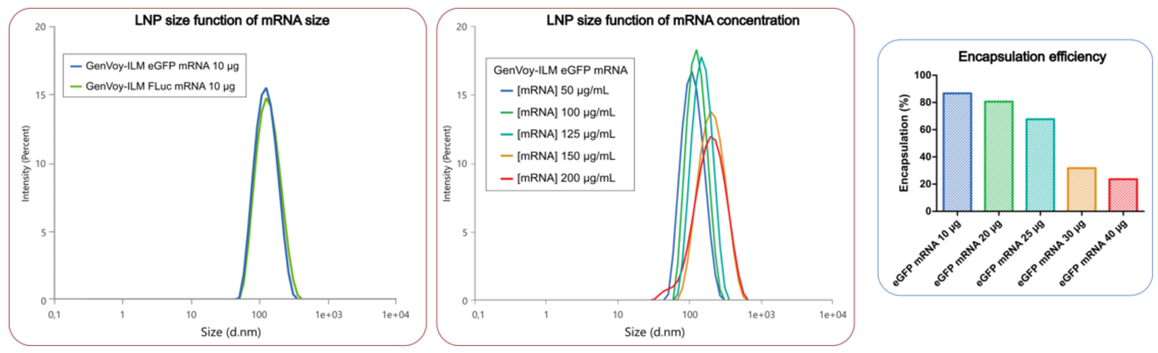

Figure 5. DLS profiles of mRNA-LNP containing eGFP-mRNA or FLuc (left pannel), and with different mRNA-eGFP concentrations during encapsulation (middle pannel). The impact of mRNA concentration in encapsulation efficiency is also presented (right pannel).

LNP samples	Size (nm)	PI	Encapsulation efficiency
FLuc mRNA 10 µg	121.93	0.13	80.4
eGFP mRNA 10 µg	116.17	0.13	84.7
eGFP mRNA 20 µg	119.07	0.14	80.6

Table 2. Quality control summary of LNP formulation (n=3) with different mRNA and mRNA concentrations.

mRNA-containing LNPs were then transfected in HEK cells, hCMEC, and fibroblasts and transfection efficiency was compared to a control transfection reagent (mRNA-fect, RJH Biosciences).

- The level of transfected RNA within HEK cells was comparable to the control.
- Transfection in fibroblast was lower but significant expression was detected in this cellular model.
- Transfection in hCMEC was barely detectable.

These results confirm the efficiency of LNPs as a transfection reagent *in vitro*. However, LNP/lipid design must be taken into consideration when designing formulation protocols to target efficiently the desired cellular model.

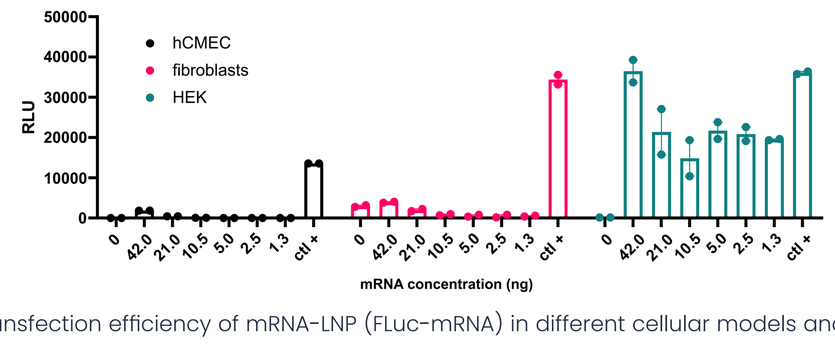

Figure 6. Transfection efficiency of mRNA-LNP (FLuc-mRNA) in different cellular models and at different mRNA concentrations.

CONCLUSIONS

- p53 expression leads to the modulation of the release of pro-inflammatory cytokine *in vitro*.
- Anti-tumoral antigen expression using Linear RNA is a promising technology for lymphocytes activation against tumor cells.
- Transfection using Genvoy-ILM kit shows transfection efficiency similar to mRNAFect in HEK293 cells. However, LNP/lipid design must be taken into consideration when designing formulation protocols to target efficiently the desired cellular model.

PERSPECTIVES

- Different types of RNAs (circular RNAs or self-amplifying RNAs) are important to consider for long term stability of the RNA.
- Tebubio already has a working circRNA template and IVT method for circular RNAs (Figure 7) production.
- Transfection using custom LNP formulation is the next step managed by Tebubio.

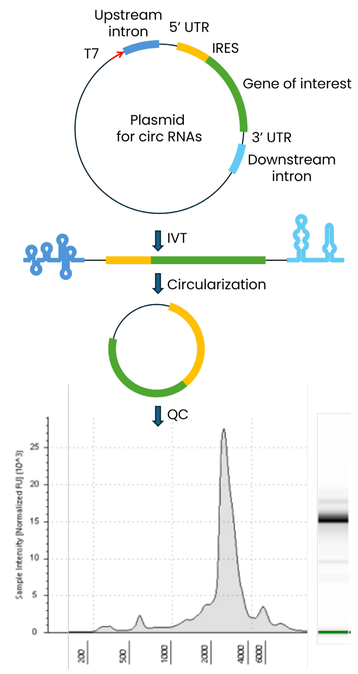

Figure 7. RNA production steps for circular RNAs. Fo QC step includes an electrophoresis and its densitometry profile.