Self-Amplifing RNAs (saRNAs), Replicon GFP and Replicon F-Luc

1. Description

Self-amplifying RNAs (saRNAs) also called "Replicons" are the next generation of RNA vaccines. Their advantage over conventional mRNA vaccine platforms relies on the viral replication machinery, which amplifies the mRNA of the encoded gene of interest within target cells. In recent years, saRNA vaccines have been clinically tested with the hope of reducing the vaccination dose compared to the conventional mRNA approach. Replicons induce potent humoral and cellular responses with few adverse effects upon a minimal, single-dose immunization. Delivery of replicons is achieved with virus-like replicon particles (VRPs), or in nonviral vehicles such as liposomes or lipid nanoparticles (LNPs).

2. Storage and shipping condition

Storage & Shipping: saRNAs are shipped with Dry Ice and must be stored at -80°C.

Duration of GFP protein expression

Higher percentage of GFP positive cells are detected with the classic mRNA GFP (~80%) as compared to what is observed with Replicon GFP (~50%). Intensity of fluorescent signal is higher with classic mRNA GFP at 24h but decreases rapidly while the signal is stable for weeks (up to 1 month) with the Replicon GFP.

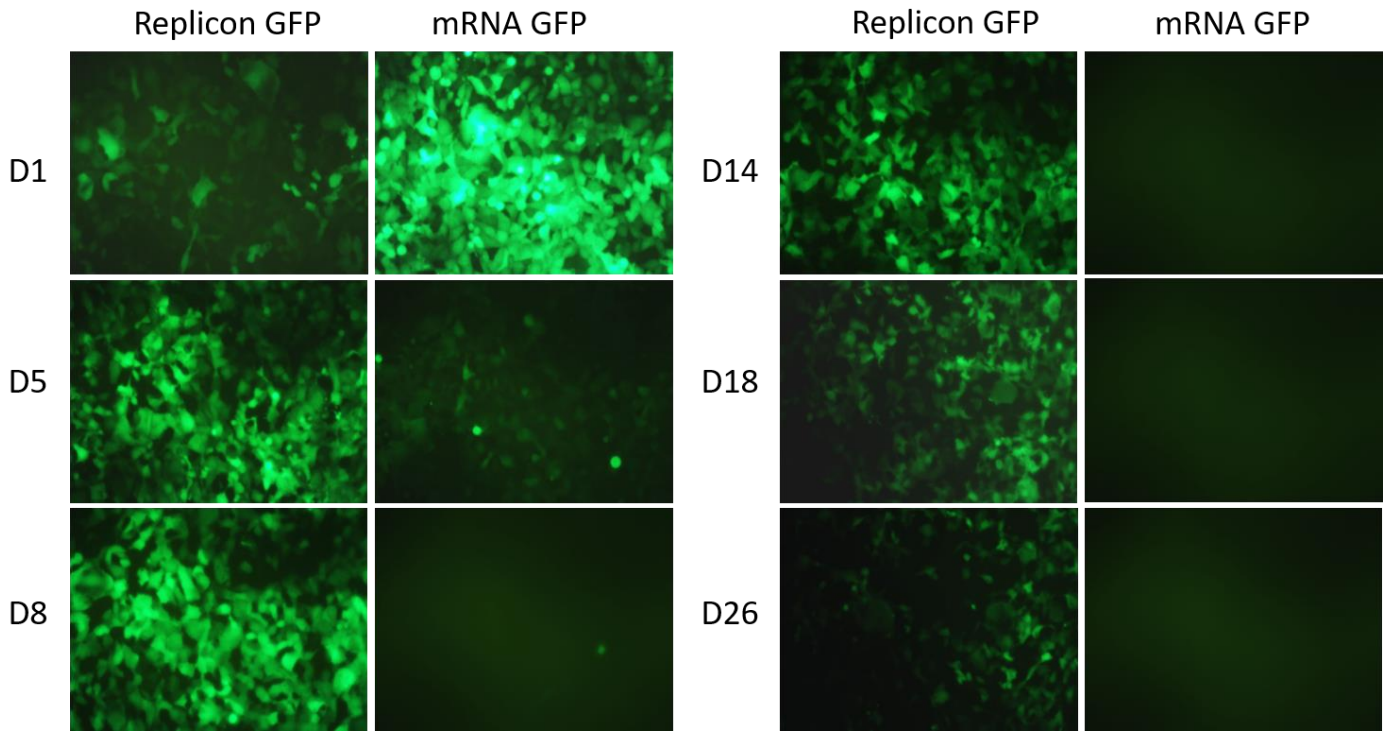


Fig. 1: GFP protein expression over time in HEK293 cells in 24-well plate cells upon lipid-based transfection with either 0,4 µg/well of Replicon GFP or 0,4 µg/well of classic mRNA GFP using NL51 transfection reagent (ratio 2:1). Photos were taken at x20 show a peak of GFP expression at 24hic mRNA GFP.

Data Source: The results obtained by OZB, Marseille, France.

FACS analysis of GFP expression in HEK cells upon transfection with either Replicon GFP or classic mRNA GFP shows enhanced expression of GFP protein over time when using the Replicon GFP as compared to classic mRNA GFP. Higher percentage of GFP positive cells are detected with the classic mRNA GFP (~80%) as compared to what is observed with Replicon GFP (~50%). Intensity of fluorescent signal is higher with classic mRNA GFP at 24h but decreases rapidly while the signal is stable for weeks with the Replicon GFP. HEK293 cells, 24-well plate

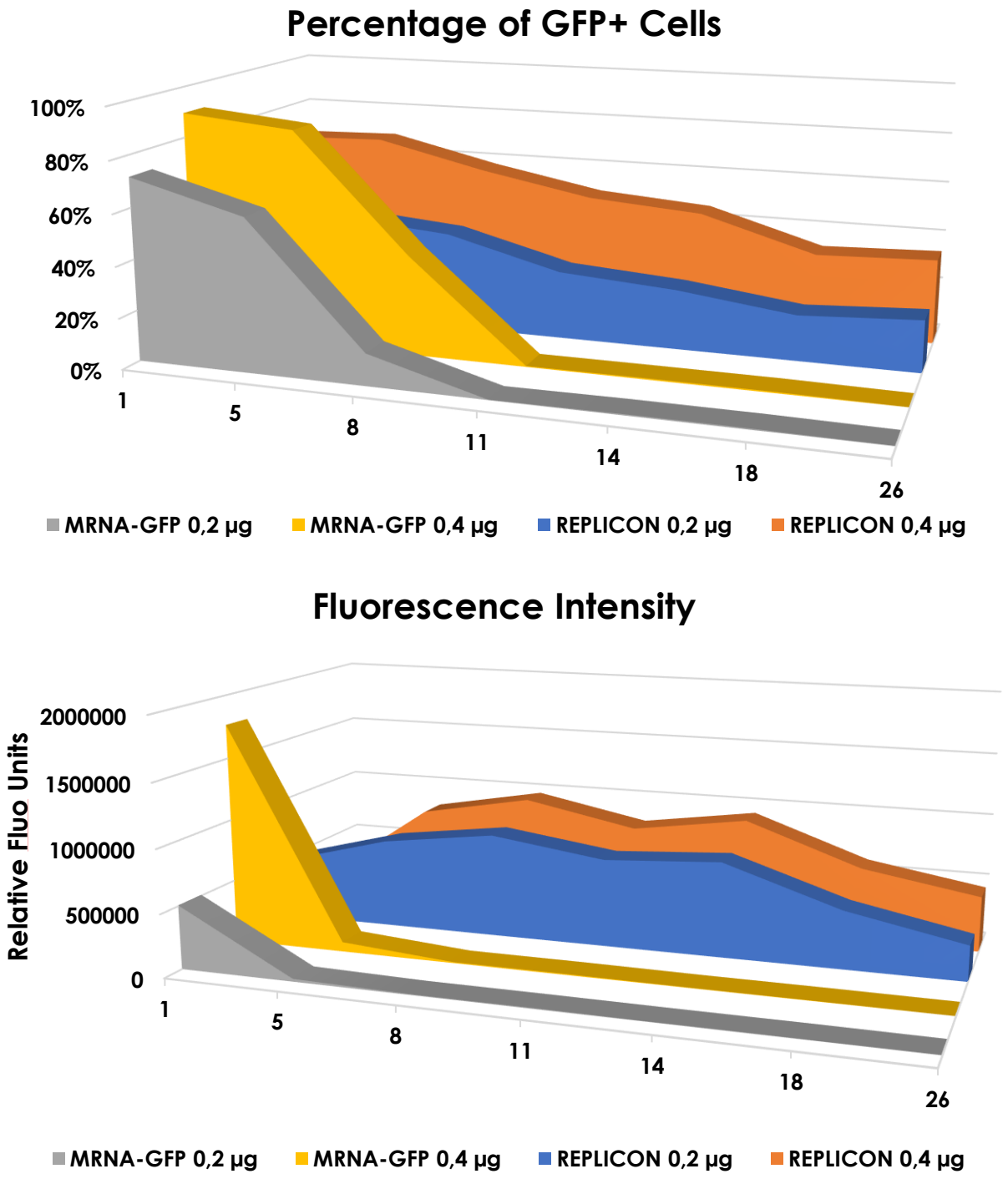


Fig. 2: Lipid-based transfection (NL51; 2:1) of mRNA GFP (0,2 and 0,4 µg/well) versus Replicon GFP (0,2 and 0,4 µg/well) over time.

Data Source: The results obtained by OZB, Marseille, France.

Validation of the replicase action by RT-PCR

GFP RNA is detected over a longer period of time when HEK cells are transfected with the Replicon GFP (>72h) as compared to classic mRNA GFP (~24h). RNA of the viral replicase from VEEV (Venezuelan equine encephalitis virus) is detected only in Replicon GFP transfected cells over at least 72h. Housekeeping genes (GAPDH and actin-B) show equal loading of samples.

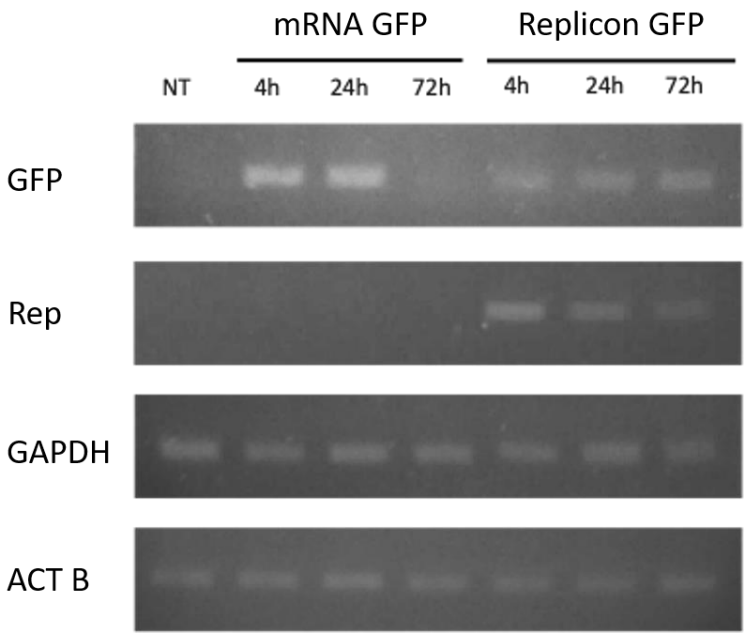


Fig. 3: Self-amplification of the synthetic RNA by RT-PCR.

Data Source: The results obtained by OZB, Marseille, France.

Duration of Luciferase activity

Firefly luciferase activity was measured using the Luc assay kit (OZ Biosciences #LUC0100) on extracts from HEK293 cells transfected with either Replicon Luc or classic mRNA Luc over time. Results show a peak of Luc activity with strong signal at 24h with classic mRNA Luc that quickly drop off to be extinguished by D5, while a duration of Luc activity is observed (up to 1 month) when using the Replicon Luc.

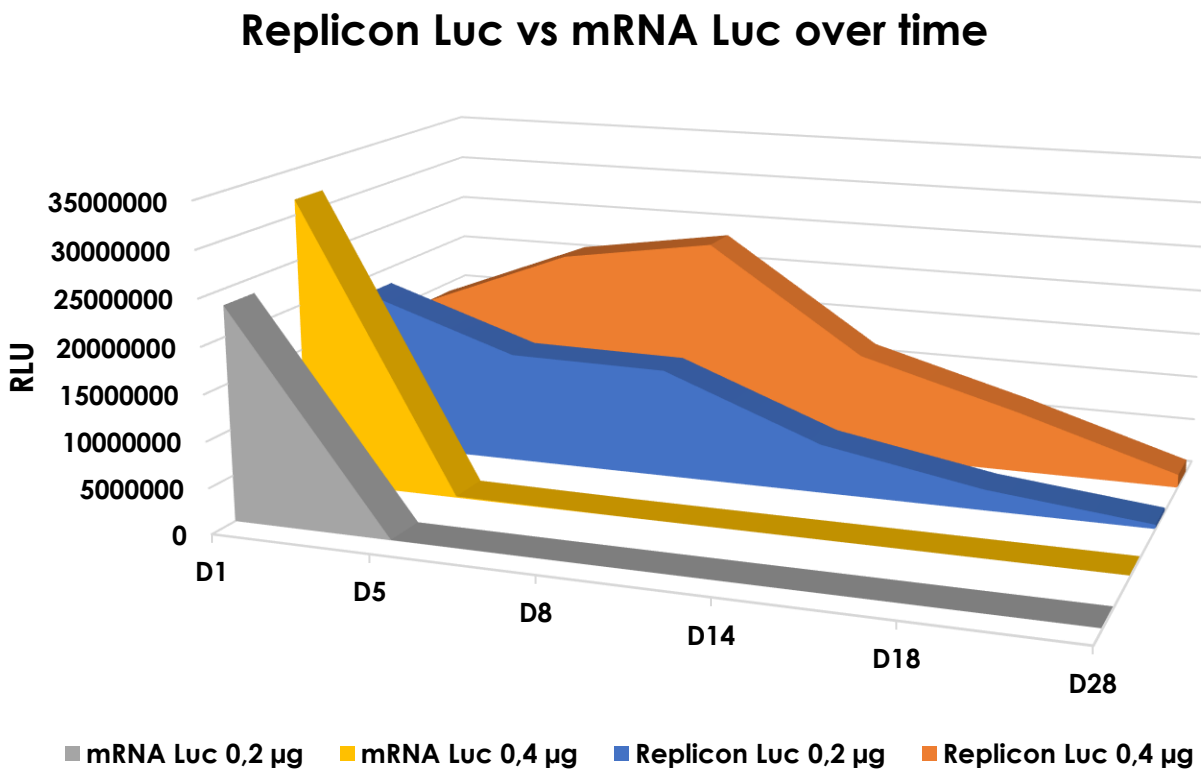


Fig. 4: HEK293 cells, 24-well plate. Lipid-based transfection (NL51; 2:1) of mRNA LUC (0,2 and 0,4 µg/well) versus Replicon LUC (0,2 and 0,4 µg/well) over time.

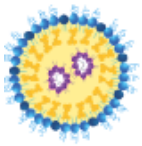
Data Source: The results obtained by OZB, Marseille, France.

OUR CUSTOM SERVICES

mRNA Synthesis

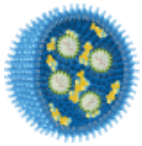
- Gene synthesis, Cloning & DNA template production.
- *In vitro* Transcription.
- Purification & Quality control.

NanOZ-LNP™ Design Platform



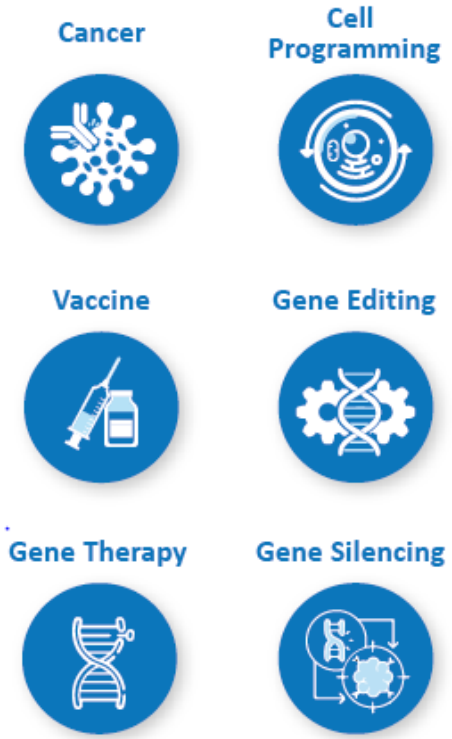
- Lipid Chemistry & Functionalization.
- Formulation Design & Manufactring.
- NanOZ-LNPs™ Custom.

Customer DNA, RNA, API



- Provide us with your molecule of interest and we will formulate it into LNPs

BIOMEDICAL APPLICATIONS



EUROPE & ASIA
OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZBIOSCIENCES
The art of delivery systems

USA & CANADA
OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com