

## Quality Evaluation of mRNA by Electrophoresis

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### User Benefits

- ◆ Electrophoresis operations can be performed almost entirely automatically.
- ◆ Electrophoresis completes in approximately 100 seconds per analysis.
- ◆ It is possible to detect impurities such as mRNA degradation products and off-target RNA.

### Introduction

mRNA vaccines and pharmaceuticals are prepared through processes including synthesis by transcription, addition of the Cap structure, and purification. Methods for conveniently confirming the purity and yield of mRNA during the purification process are sought, and high-performance liquid chromatography (HPLC) and electrophoresis are commonly used. Microchip electrophoresis offers a simpler and faster method for obtaining electrophoresis results compared to capillary electrophoresis, despite having a shorter separation length and thus lower resolution. We present results verifying the applicability of microchip electrophoresis analysis for confirming the purity of target mRNA and detecting impurities.

### Sample Preparation and Analysis Conditions

#### RNA sample preparation

CleanCap FLuc mRNA (TriLink BioTechnologies) was used for degradation assessment. Samples were prepared at a concentration of 100 ng/μL and subjected to thermal denaturation at 90 °C for 0 min, 20 min, 40 min, 60 min, 120 min, and 180 min to create the analytical samples.

For purity evaluation, CleanCap Cas9 mRNA and CleanCap Cre mRNA (TriLink BioTechnologies) were used. Cre mRNA was prepared at concentrations of 1 ng/μL, 5 ng/μL, 10 ng/μL, and 25 ng/μL. Cas9 mRNA at 100 ng/μL was added to each and used as the analysis sample.

#### Electrophoresis

For mRNA analysis, we used the Shimadzu Corporation's MultiNA II microchip electrophoresis system (Fig. 1a).

The analytical sample was mixed 1:1 with the marker solution (internal standard for mobility correction) included in the MultiNA RNA Kit (Fig. 1b). The RNA 6000 Ladder (Thermo Fisher Scientific) was used as the RNA size standard. The RNA 6000 Ladder was diluted 6-fold and then mixed 1:1 with the marker included in the MultiNA RNA Kit, similar to the analytical sample. The analytical sample mixed with the marker and the ladder were heated at 72 °C for 3 minutes and then placed on ice. After heat treatment, the samples were loaded into the instrument with the separation buffer for analysis (Fig. 2). The mRNA size was automatically calculated by the MultiNA II software using the electrophoresis results of the RNA 6000 Ladder (Fig. 3).

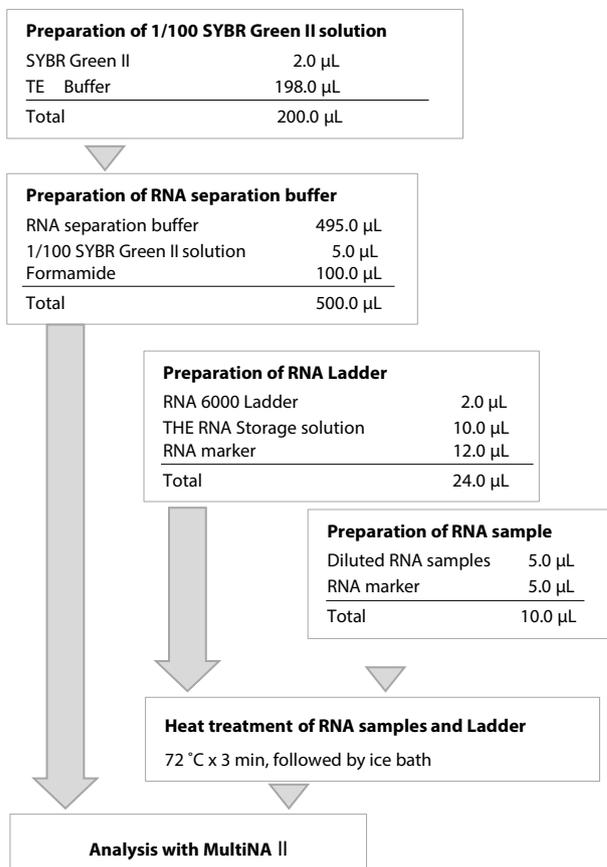


Fig. 2 Analysis procedure of RNA by MultiNA II

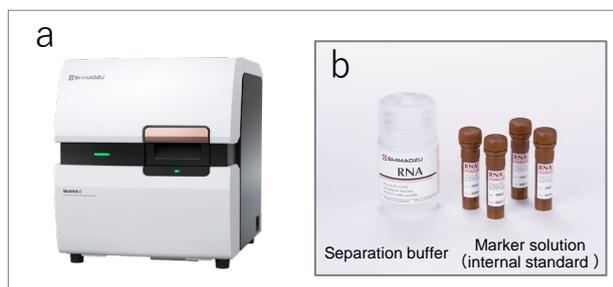


Fig. 1 Microchip electrophoresis system MultiNA™ II MCE-301 (a) and RNA Kit (b)

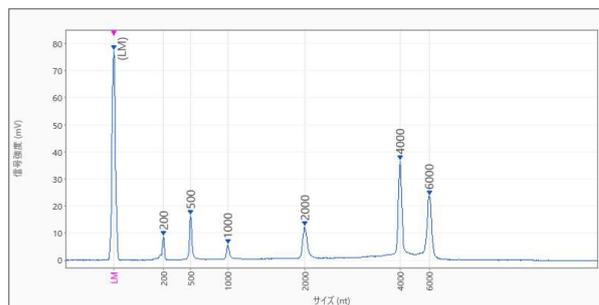


Fig. 3 Electropherogram of RNA 6000 Ladder by MultiNA II

## ■ Results

### mRNA Degradation evaluation

The extent of degradation of FLuc mRNA was measured using MultiNA II at heating times of 0 min, 20 min, 40 min, 60 min, 120 min, and 180 min at 90 °C. A decrease in the main peak at 1922 nt for FLuc mRNA was observed as the heating time increased. Additionally, an increase in peak area originating from mRNA degradation products was observed on the low-molecular-weight side of the main peak (Fig. 4).

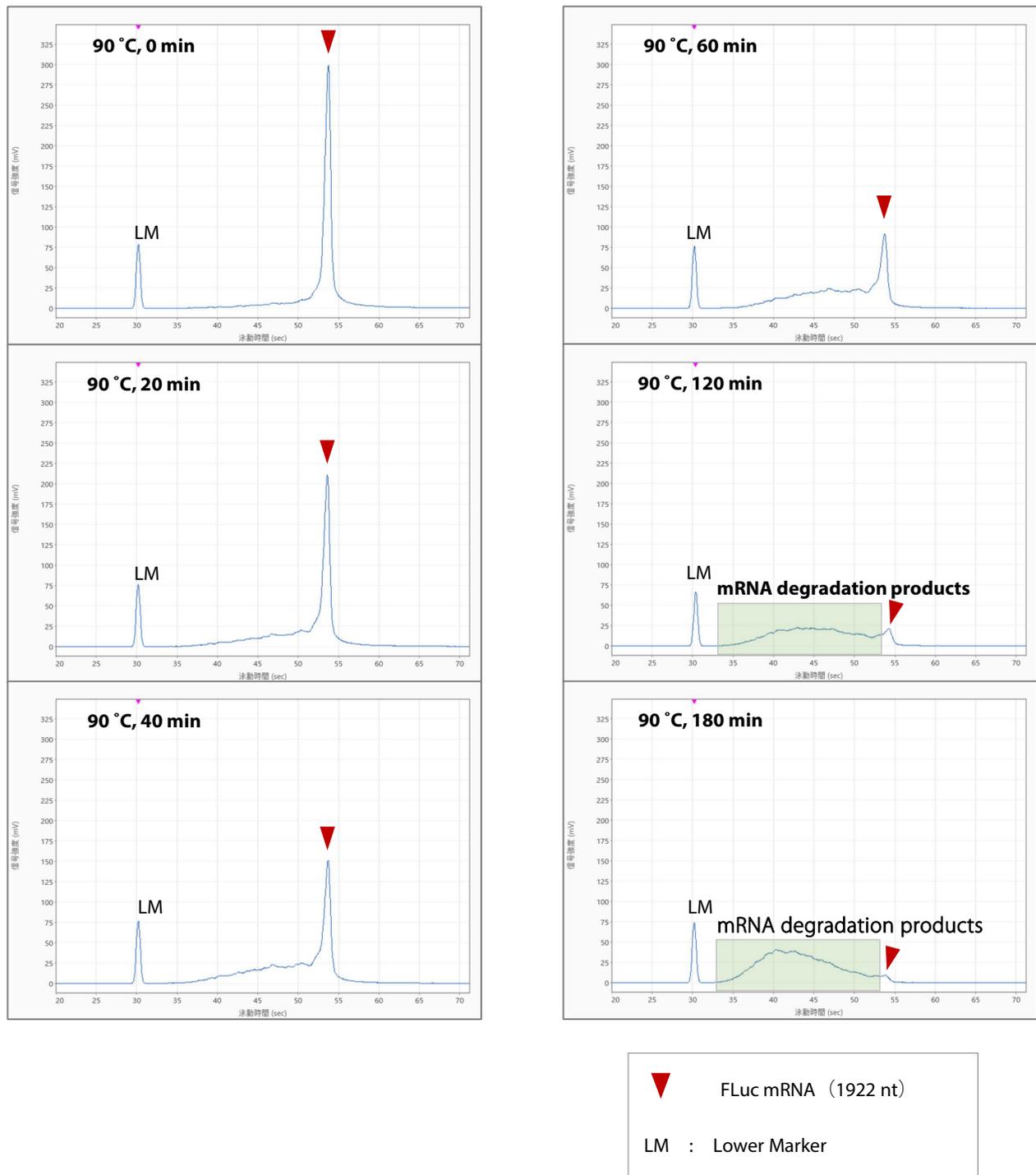


Fig. 4 mRNA degradation evaluation using MultiNA II (electropherogram)

### mRNA Purity Evaluation

Cas9 mRNA (4522 nt) was fixed at a concentration of 100 ng/μL. Cre mRNA (1351 nt) was mixed at concentration ratios of 100:1, 20:1, 10:1, and 4:1 relative to Cas9 mRNA and analyzed using MultiNA II. All samples were detected as peaks (Fig. 5). In particular, even at a concentration of 1 ng/μL, Cre mRNA was detected as a peak in the sample mixed at a 100:1 ratio. Furthermore, a distinct band was confirmed in the gel image (Fig. 6).

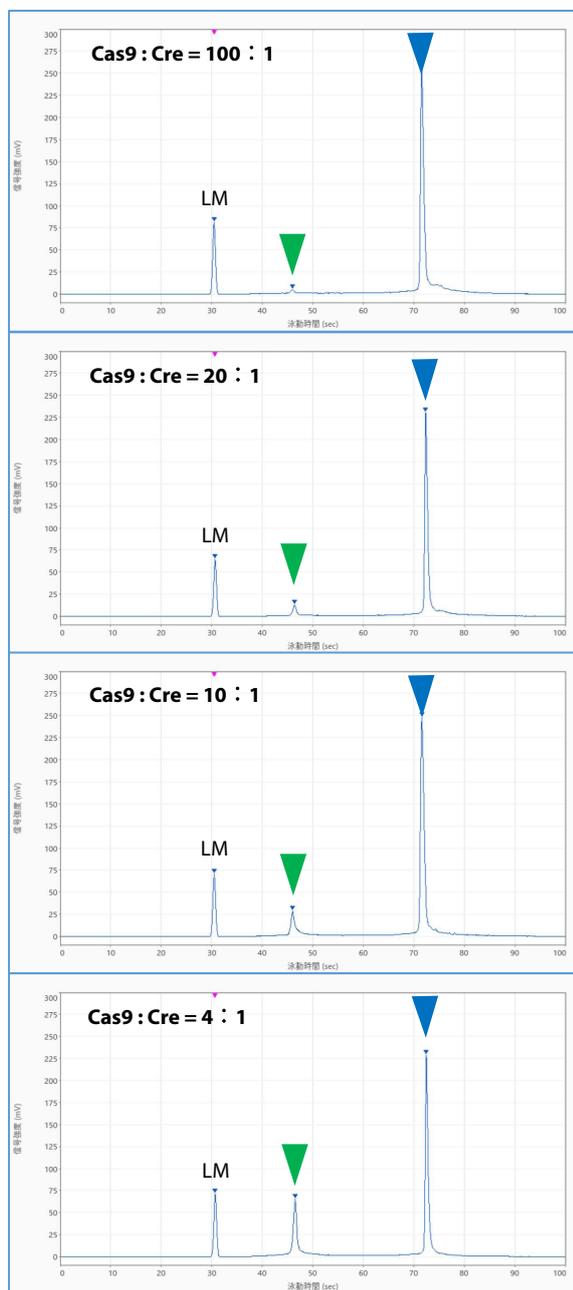


Fig. 5 mRNA purity evaluation using MultiNA II (electropherogram)

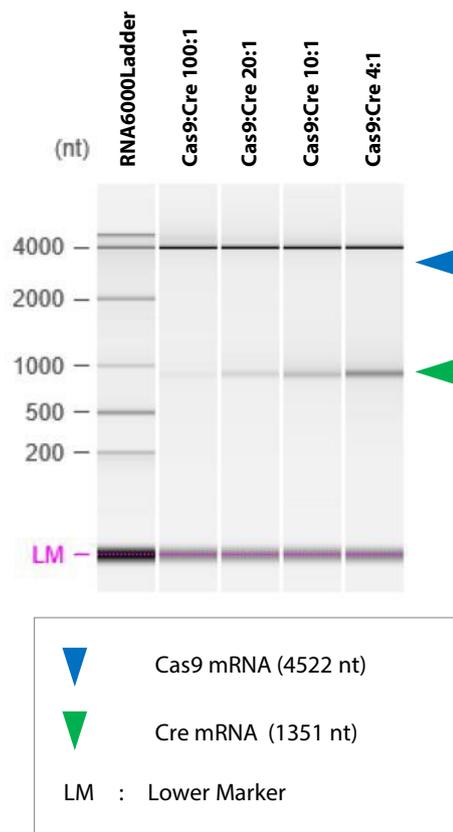


Fig. 6 mRNA purity evaluation using MultiNA II (gel image)

### Conclusions

These results demonstrate that the MultiNA II microchip electrophoresis system is an effective method for evaluating mRNA degradation and purity.

Microchip electrophoresis offers lower resolution and accuracy compared to capillary electrophoresis. However, the MultiNA II completes each analysis in approximately 100 seconds, enabling rapid and convenient result acquisition. This makes it a promising tool for process control in synthetic mRNA manufacturing.

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> **MultiNA II MCE-301**  
Microchip Electrophoresis System

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