Quality Evaluation of RNA Using MultiNA™ Microchip Electrophoresis System

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1. Introduction

The production of mRNA vaccines and other mRNA-based drugs involves transcription synthesis, addition of an mRNA cap structure, purification, and other steps. This production process requires simple methods of checking purity and yield at the purification stage, a task often performed using high-performance liquid chromatography (HPLC) and electrophoresis. Compared to capillary electrophoresis, microchip electrophoresis offers a simpler and faster method for obtaining electrophoresis results, despite having a shorter separation length and thus lower resolution. This study investigated whether microchip electrophoresis is applicable for detecting impurities and verifying the purity of a target mRNA.

2. Experiments

2-1. RNA Sample Preparation

CleanCap FLuc mRNA (TriLink BioTechnologies) was used for degradation assessment. Analytical samples were prepared at a concentration of 100 ng/μL and subjected to heat treatment at 90 ˚C for 0, 20, 40, 60, 120, and 180 min.
CleanCap® Cas9 mRNA and CleanCap® Cre mRNA (TriLink BioTechnologies) were used for purity assessment. Cre mRNA samples (1, 5, 10, and 25 ng/μL) were each mixed with 100 ng/μL of Cas9 mRNA to prepare analytical samples.

2-2. Electrophoresis

The microchip electrophoresis device used in this study was Shimadzu'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). RNA 6000 Ladder (Thermo Fisher Scientific) was used as an RNA size standard. RNA 6000 Ladder was diluted 6-fold then mixed with the RNA marker solution from the MultiNA RNA kit at 1:1 (similar to the analytical samples). The RNA ladder solution and analytical samples (each with an RNA marker) were heated at 72 ˚C for 3 minutes then placed on ice. After heat treatment, the samples were loaded into the instrument with the separation buffer for analysis (Fig. 2). The MultiNA II software used the RNA 6000 Ladder measurements to automatically correct the mobility of the mRNA sample (Fig. 3).

Fig. 1 MultiNA™ II Microchip Electrophoresis System (a) and RNA Reagent Kit (b)

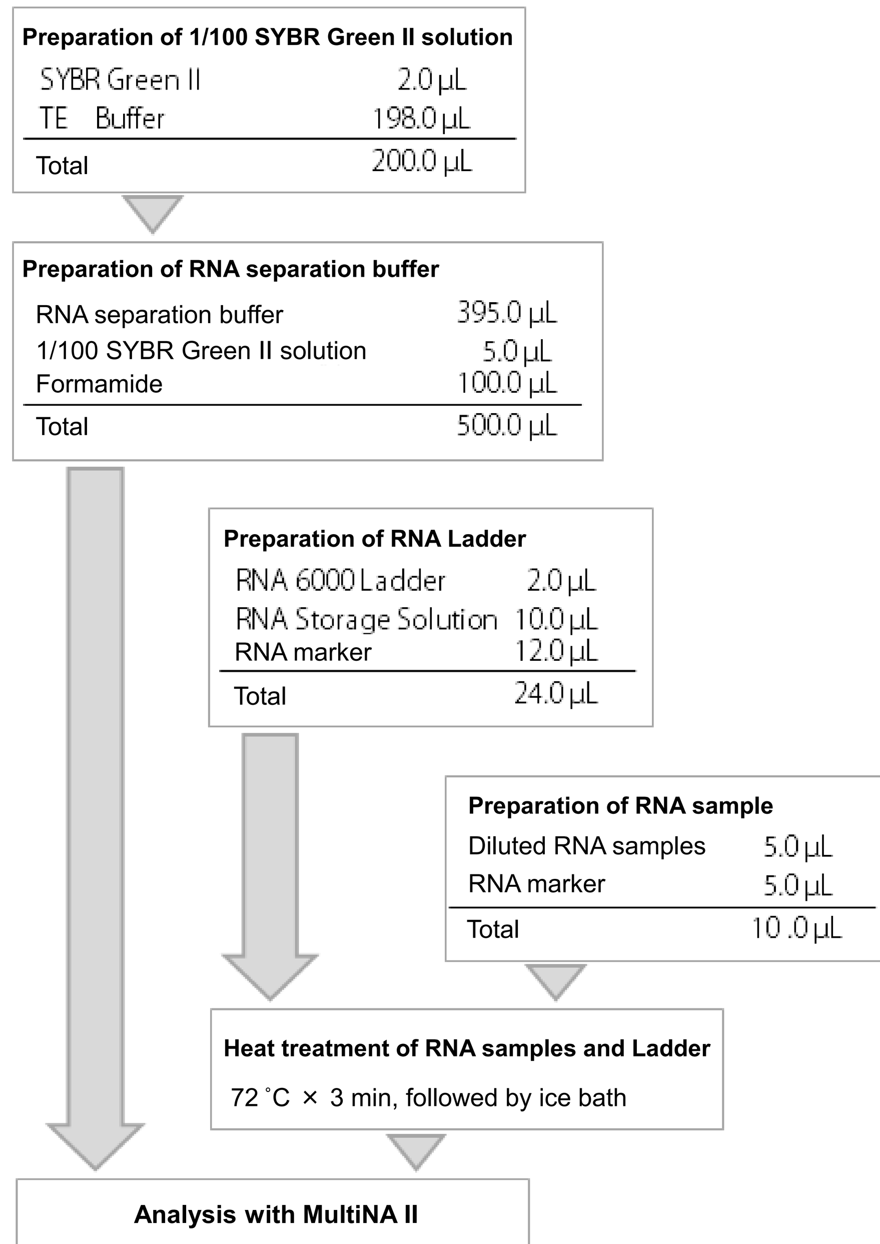


Fig. 2 Protocol for RNA Analysis Using the MultiNA II Microchip Electrophoresis System

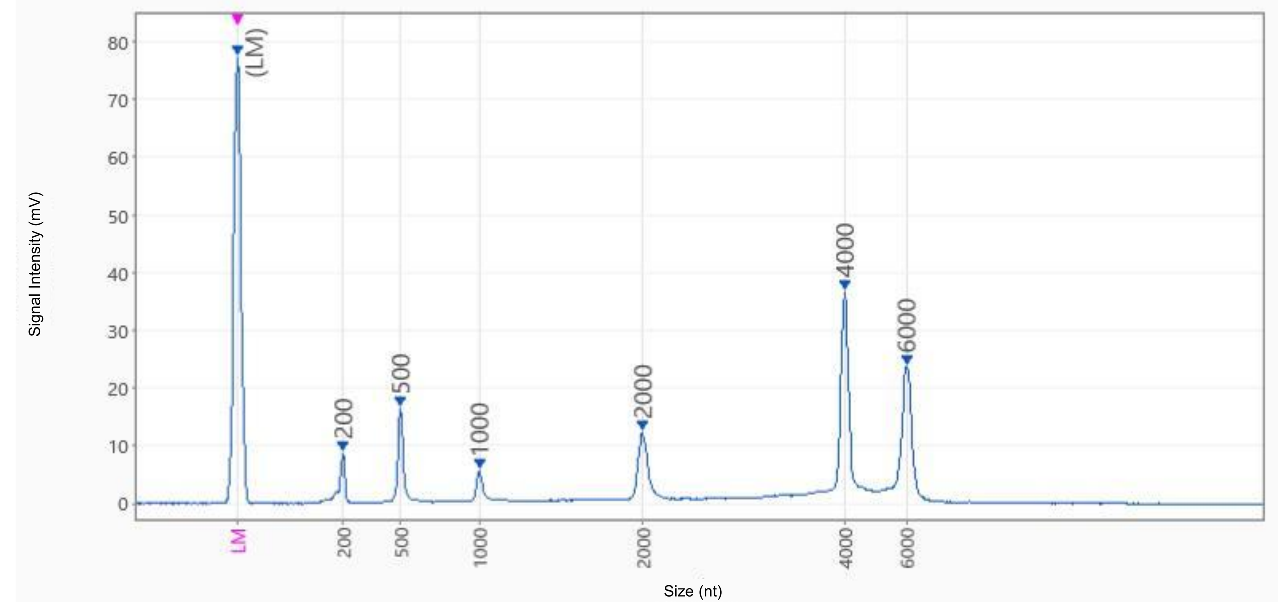


Fig. 3 Electropherogram of RNA-6000 Ladder Measured on MultiNA System

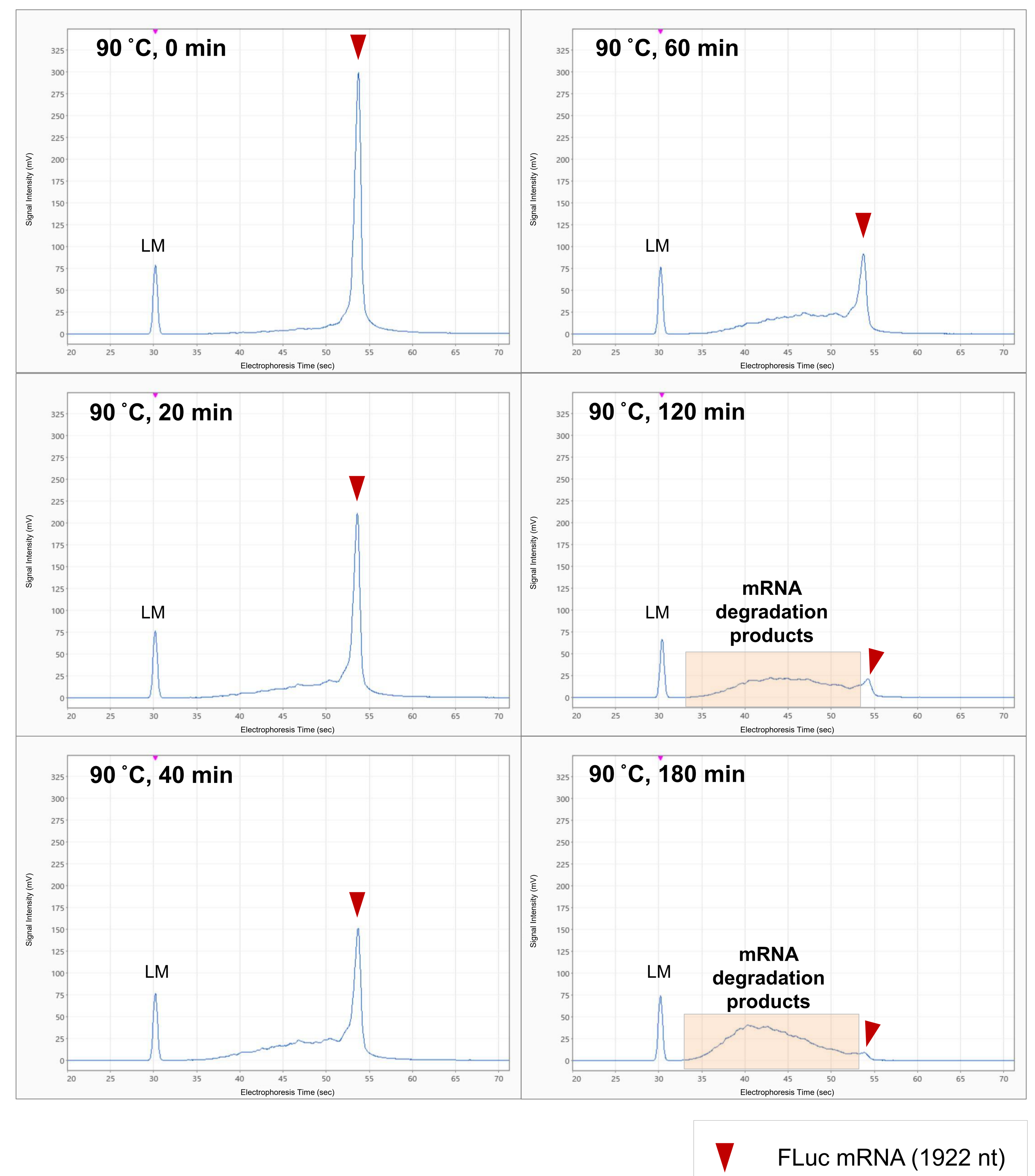
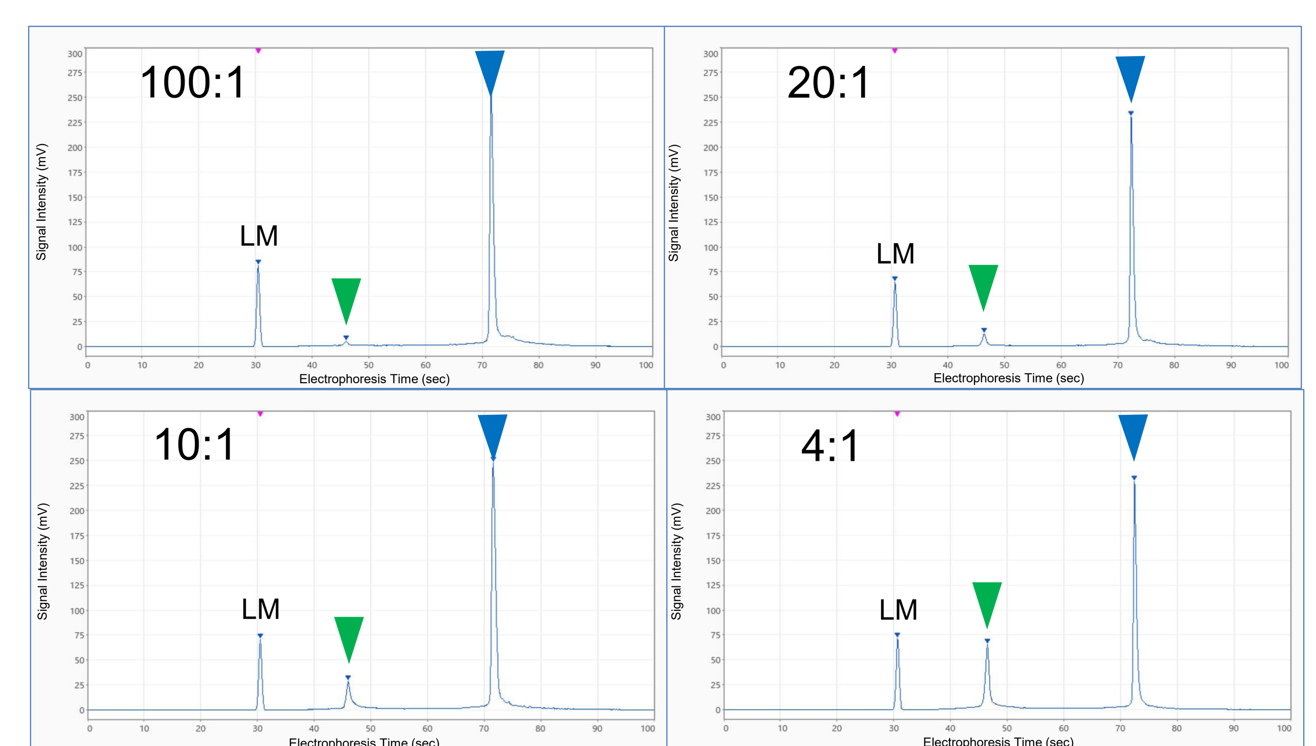


Fig. 4 Results of Degradation Assessment Using the MultiNA II Microchip Electrophoresis System



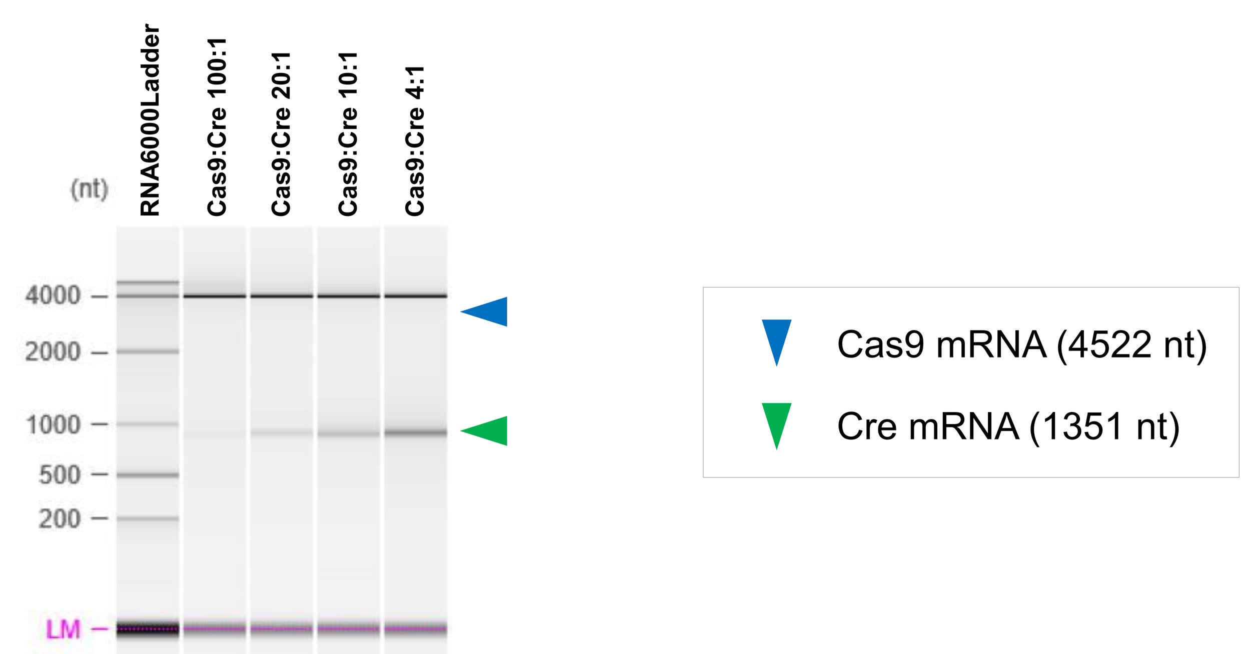


Fig. 5 Results of Purity Assessment Using the MultiNA II Microchip Electrophoresis System

3. Results and Conclusions

Degradation Assessment

FLuc mRNA samples heated at 90 ˚C for 0, 20, 40, 60, 120, and 180 min were used to analyze samples at various degradation levels using the MultiNA II system. The results showed that the longer the heating time, the smaller the main FLuc mRNA peak (1922 nt) detected by the system. The results also showed that as heating time progressed, the peak area of mRNA degradation products increased on the lower molecular weight side of the main peak (Fig. 4).

Purity Assessment

Sample mixtures containing Cas9 mRNA (4522 nt) at a fixed concentration (100 ng/μL) and Cre mRNA (1351 nt) at a range of relative concentration ratios (100:1, 20:1, 10:1, and 4:1) were analyzed using the MultiNA system. The results showed that both were detected as distinct peaks. In particular, Cre mRNA in the 100:1 mixture was detected as a distinct peak despite having a concentration of only 1 ng/μL, and was also visible as a band in the gel image (Fig. 5).

These results demonstrate that the MultiNA II microchip electrophoresis system is an effective tool for evaluating mRNA degradation and purity.
Microchip electrophoresis provides lower resolution and accuracy compared with capillary electrophoresis. However, the MultiNA II system produced results simply and quickly and only needed around 100 seconds per run, showing that the system could potentially be used for quality control of synthetic mRNA production.

We have no financial relationships to disclose for this presentation.