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Application News

No.B52

Microchip Electrophoresis

Application in Next-Generation Sequencer (NGS) Library Quality Control (QC)

Due to technological advancements in next-generation sequencers, they are being used for a wider range of applications, such as for de novo sequencing or for the analysis of mutations, exomes, and expressions. Furthermore, it is possible to select the model based on the capacity required for data analysis and data processing, which has increased their use rapidly. However, to obtain good sequencing results, regardless of the application or model, it is necessary to have a good understanding of the size distributions and concentrations in the NGS library. Therefore, quality control (QC) of that information is essential when using NGS systems.

Previously, agarose electrophoresis was used to confirm size distributions in the NGS library, and a real-time PCR system or fluorescence spectrophotometer was required to confirm concentrations.

However, the processes required, from library preparation to QC, involved a series of tedious manual operations. Therefore, there was a need for a quick, simple, and low-cost QC method. In particular, with the maturation of simultaneous multianalyte sequencing technology using index tag sequences, the number of libraries subject to quality control has been increasing, which has further increased the demand for such QC methods.

This article describes an example of mouse RNA sequencing analysis that solves this problem by using an MCE-202 MultiNA automatic electrophoresis system for NGS library QC.

Experimental Procedure

Comparison of Real-Time PCR and Concentration Quantitation Results

(1) Eight mouse total RNA samples were prepared by using a Clontech SMARTer Ultra Low RNA Kit to prepare cDNA amplification products.
(2) After sharing to the cDNA amplification products using the Illumina protocol, a TruSeq DNA sample prep kit was used to prepare the NGS library.
(3) After using a DNA-1000 kit with the MultiNA system to analyze the library, concentrations were quantitated using smear analysis software[*1].
(4) Concentration quantitation results from the MultiNA were compared with concentration quantitation results obtained from analyzing the same sample using real-time PCR (Shimadzu GVP-9600).

Sequencing with Next-Generation Sequencers

(1) A TruSeq RNA Sample Prep Kit from Illumina was used to prepare four mouse NGS library samples.
(2) After using a DNA-1000 kit with the MultiNA system to analyze the library, sizes were confirmed and quantitated using smear analysis software.
(3) Sequencing was performed using the Illumina HiSeq 1000.

Note: The four library samples could be identified by index tag sequencing. Therefore, one lane was used for sequencing.

MultiNA Reagents/Kits

• DNA-1000 Kit	Shimadzu	: P/N 292-27911-91
• SYBR® Gold	Life Technologies	: S11494
• φX174 DNA/*Hae*III Markers	Promega	: G1761

*1 Smear analysis software requires MultiNA software version 1.12 or later.

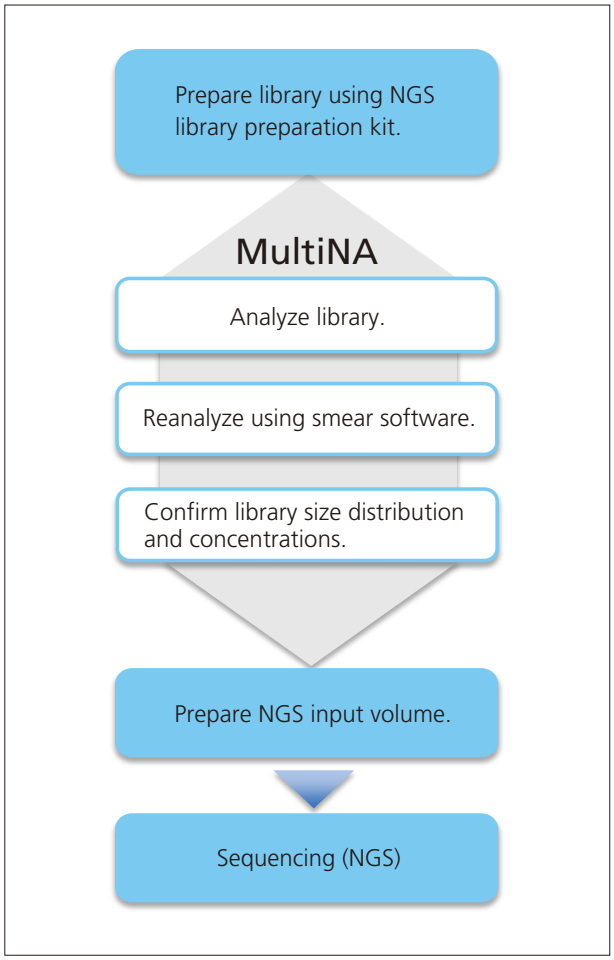

Fig. 1 NGS Library Quality Control Procedure

During the NGS library QC process, MultiNA's smear analysis software can be used to calculate the estimated average library size, concentrations, and mole concentrations (Figs. 2 and 3). The comparison of quantitation results with real-time PCR results shows that the concentration values quantitated using the MultiNA are relatively lower, but consistent (Table 1).
In addition, the mouse RNA sequencing results showed adequate read length and a stable read ratio between respective libraries due to index tag sequencing (Table 2).

These results indicate that library QC using the MultiNA can be used not only to confirm size distributions, but also to simultaneously quantitate libraries under routine analysis. MultiNA is able to automatically analyze the migration and corresponding data for up to 108 libraries. In addition, it can reduce the actual working time required to obtain results to about 10 to 20 minutes. Thus, by using the MultiNA system for library QC, it is possible to perform QC quickly and easily, particularly when the number of NGS libraries increases.

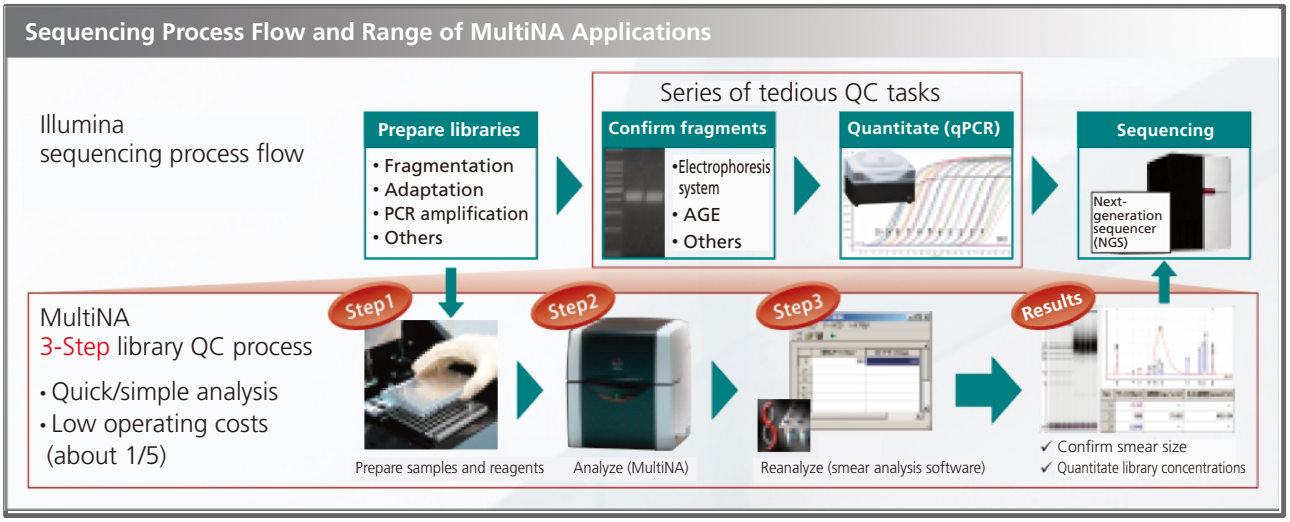

Fig. 2 NGS Process Flow and Range of MultiNA Applications in Library QC

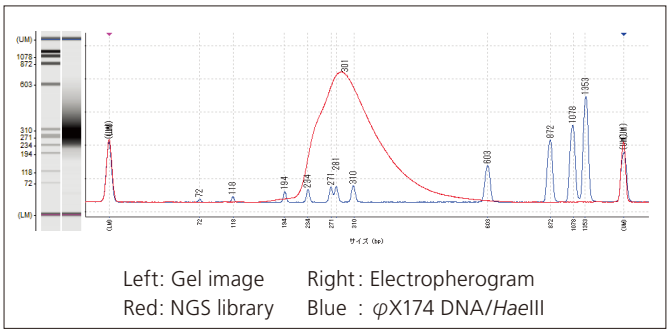

Fig. 3 Example of NGS Library Electrophoresis

Table 1 Concentration Quantitation Results for MultiNA and Real-Time PCR*2

Library No.	MultiNA [nmol/L]	GVP-9600 (q-PCR) [nmol/L]	Ratio [MultiNA/GVP-9600]
1	121.1	167.3	72.4 %
2	126.9	145.1	87.5 %
3	54.1	57.8	93.6 %
4	214.0	292.3	73.2 %
5	187.4	226.8	82.6 %
6	166.3	229.3	72.5 %
7	206.0	256.8	80.2 %
8	158.7	181.9	87.3 %
		Average	81.2% (CV 9.9%)

Table 2 NGS Results of Four Libraries When Using MultiNA for QC*2

Cluster Density	548 K / mm^2	Total Read Count	151.5 M reads
Read Count (after filtration)	143.3 M reads	≥ Q30 ratio	95.9 %
Read Ratio of 4 Libraries	21.2 - 26.2% (Four libraries per lane sorted by index tag sequencing: Theoretical value of 25 %)		

*2: Samples and data provided by Dr. Osamu Ohara.

Note 1: This Application News article is provided as an analytical example of using Shimadzu products outside the scope of normal product specifications. Therefore, we do not guarantee the indicated analytical results.

Note 2: Adequate sensitivity may not be achieved depending on the library distribution status or if the PCR process is not performed during library preparation.

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