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C297-E075

MCE-202 MultiNA

APPLICATION 8

DNA-500
DNA-1000
DNA-2500
RNA

Accurate DNA Sizing Using the DNA-500 Reagent Kit

Using the MCE-202 MultiNA DNA-500 Reagent Kit, size analysis was conducted for a 25 bp to 100 bp sample and a 100 bp to 500 bp sample with accuracy of ±5 bp and ±5%, respectively.

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Introduction

The MCE-202 MultiNA was used to generate a size calibration curve by ladder analysis, which in turn was used to conduct high-accuracy DNA size analysis. Here we introduce an example of size analysis using the DNA-500 Reagent Kit.

Results

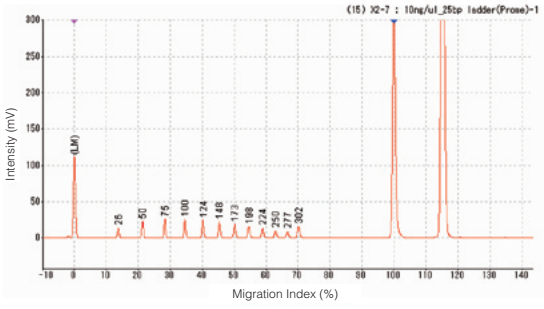
Figure 1 shows the analysis results of the 25 bp DNA Step Ladder and pBR322-Hae III digest using the MCE-202 MultiNA DNA-500 Reagent Kit. For the 25 bp DNA Step Ladder (Fig. 1 (a)), 12 peaks from 25 bp to 300 bp were detected. In addition, a 1.8 kbp peak originating from the sample was detected in the vicinity of 116% on the migration time index. Analysis of the pBR322-Hae III digest (Fig. 1 (b)) yielded 17 peaks. In this experiment, the 123 bp and 124 bp fragments were recognized as a single peak, and no peaks were detected in the low weight density range of 8 bp to 21 bp. The size calculation results for the constituent fragments are shown in Table 1. The absolute error for the 25 bp to 100 bp fragments was less than 3 bp, and the relative error for the 100 bp to 500 bp fragments ranged from -0.9% to +3.4%.

Table 1 DNA Sizing Results with the DNA-500 Reagent Kit

	Target Size	Sizing Results (n = 8)				
		Average	RE		SD	RSD
	(bp)	(bp)	(%)	(bp)	(bp)	(%)
25bp step ladder	25	26.1	4.5	1.1	0.4	1.4
	50	49.8	−0.5	−0.3	0.5	0.9
	75	75.4	0.5	0.4	0.5	0.7
	100	100.4	0.4	0.4	0.5	0.5
	125	124.5	−0.4	−0.5	0.5	0.4
	150	148.6	−0.9	−1.4	0.7	0.5
	175	173.4	−0.9	−1.6	0.5	0.3
	200	198.8	−0.6	−1.3	0.9	0.4
	225	224.5	−0.2	−0.5	0.5	0.2
	250	250.6	0.2	0.6	1.1	0.4
	275	277.0	0.7	2.0	0.9	0.3
	300	302.8	0.9	2.8	1.0	0.3
pBR322 – HaeIII digest	51	54.0	5.9	3.0	0.0	0.0
	57	57.9	1.5	0.9	0.4	0.6
	64	66.0	3.1	2.0	0.5	0.8
	80	82.4	3.0	2.4	0.5	0.6
	89	90.3	1.4	1.3	0.7	0.8
	104	107.5	3.4	3.5	0.9	0.9
	123	126.9	3.2	3.9	1.1	0.9
	184	186.6	1.4	2.6	1.5	0.8
	192	193.6	0.8	1.6	1.5	0.8
	213	215.6	1.2	2.6	1.4	0.7
	234	235.9	0.8	1.9	1.4	0.6
	267	267.9	0.3	0.9	1.0	0.4
	434	441.1	1.6	7.1	0.8	0.2
	458	467.1	2.0	9.1	1.1	0.2
	504	511.1	1.4	7.1	1.7	0.3
	540	548.1	1.5	8.1	1.2	0.2
	587	603.5	2.8	16.5	2.4	0.4

RE: Relative Error
SD: Standard Deviation
RSD: Relative Standard Deviation (= SD / Average)

(a) 25 bp DNA Step Ladder



(b) pBR322-Hae III Digest

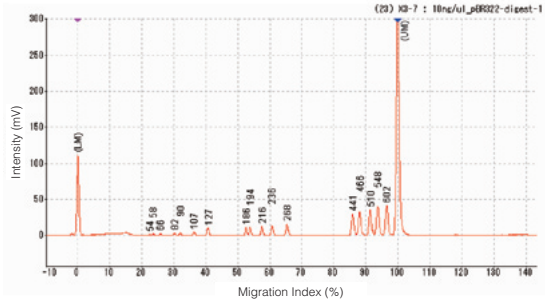


Fig. 1 Electropherogram of 25 bp DNA Step Ladder and pBR322-Hae III Digest Using the DNA-500 Reagent Kit for MultiNA

Analytical Conditions and Procedure

Instrument:	MCE-202 MultiNA	
Analysis mode:	DNA-500 on-chip mixing	
Ladder:	25 bp DNA ladder	20 ng/µL
Sample:	25 bp DNA Step Ladder	10 ng/µL
	pBR322-Hae III Digest	10 ng/µL

Reagents:

- DNA-500 Reagent Kit for MultiNA (Shimadzu) P/N 292-27910-91
- SYBR® Gold nucleic acid gel stain (Invitrogen) S-11494
- 25 bp DNA ladder (Invitrogen) 10597-011
- 25 bp DNA Step Ladder (Promega) G4511
- pBR322 – Hae III Digest (Sigma-Aldrich) D9655

Experimental Method:

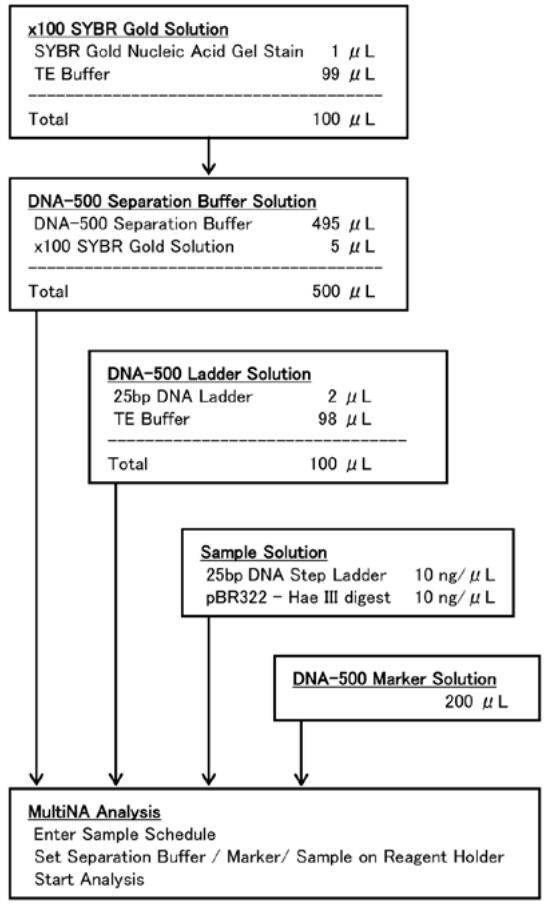


Fig. 2 Experimental Procedure (for 8 Samples)

(Note) For detailed operational information related to analysis using the MCE-202 MultiNA, please refer to the MCE-202 MultiNA Instruction Manual.

SHIMADZU

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