Accurate DNA Sizing Using the DNA-1000 Reagent Kit

Using the MCE-202 MultiNA DNA-1000 Reagent Kit, size analysis was conducted for a 100 bp to 1000 bp sample with accuracy of ±15%.

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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-1000 Reagent Kit.

Results

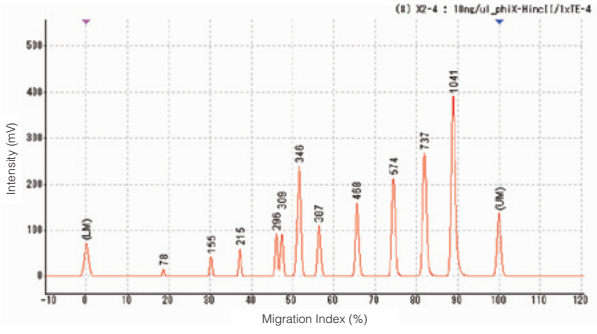
Figure 1 shows the analysis results of the ϕX174-Hinc II digest and 50 bp DNA Step Ladder using the MCE-202 MultiNA DNA-1000 Reagent Kit. For the ϕX174-Hinc II digest, 11 peaks were detected (Fig. 1 (a)). In this experiment, 335 bp, 341 bp, and 345 bp were recognized as a single peak. Analysis of the 50 bp DNA Step Ladder (Fig. 1 (b)) yielded 16 peaks from 50 bp to 800 bp. In addition, a sample-derived 1.8 kbp peak was detected in the vicinity of 96% on the migration time index. The size calculation results for the constituent fragments are shown in Table 1. The relative error for the 100 bp to 1000 bp fragments ranged from -6.0% to +9.1%.

Table 1 DNA Sizing Results with the DNA-1000 Reagent Kit

	Target Size	Sizing Results (n = 8)				
		Average	RE		SD	RSD
	(bp)	(bp)	(%)	(bp)	(bp)	(%)
ΦX174-Hinc II digest	79	79.0	0.0	0.0	0.8	1.0
	162	157.4	-2.9	-4.6	2.0	1.3
	210	217.3	3.5	7.3	2.3	1.0
	291	297.5	2.2	6.5	1.7	0.6
	297	309.6	4.3	12.6	1.6	0.5
	335	346.5	3.4	11.5	2.0	0.6
	392	387.4	-1.2	-4.6	2.7	0.7
	495	468.6	-5.3	-26.4	3.5	0.7
	612	575.0	-6.0	-37.0	3.7	0.6
	770	737.5	-4.2	-32.5	3.8	0.5
	1057	1036.3	-2.0	-20.8	6.7	0.6
50bp step ladder	50	35.1	-29.8	-14.9	1.2	3.5
	100	95.6	-4.4	-4.4	0.9	1.0
	150	142.9	-4.8	-7.1	1.1	0.8
	200	198.6	-0.7	-1.4	0.9	0.5
	250	256.5	2.6	6.5	1.1	0.4
	300	311.3	3.8	11.3	1.8	0.6
	350	361.0	3.1	11.0	2.5	0.7
	400	405.3	1.3	5.3	3.0	0.7
	450	447.1	-0.6	-2.9	3.5	0.8
	500	489.4	-2.1	-10.6	3.5	0.7
	550	534.9	-2.8	-15.1	3.7	0.7
	600	586.5	-2.3	-13.5	3.4	0.6
	650	646.0	-0.6	-4.0	3.5	0.5
	700	712.3	1.8	12.3	4.3	0.6
	750	790.9	5.5	40.9	6.6	0.8
	800	872.5	9.1	72.5	5.3	0.6

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

(a) ϕX174-Hinc II Digest



(b) 50 bp DNA Step Ladder

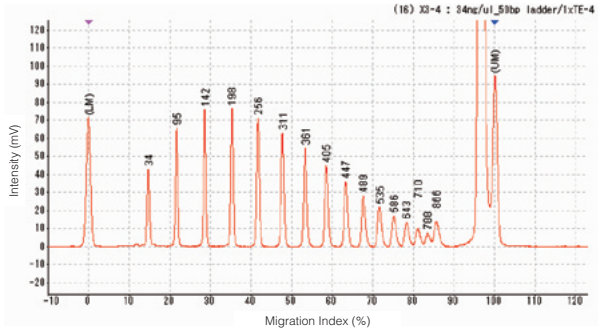


Fig. 1 Electropherogram of ϕX174-Hinc II Digest and 50 bp DNA Step Ladder Using the DNA-1000 Reagent Kit for MultiNA

Analytical Conditions and Procedure

Instrument: MCE-202 MultiNA
Analysis mode: DNA-1000 on-chip mixing
Ladder: ϕX174 DNA / Hae III Markers
Diluted 100:1 with TE buffer
Sample: ϕX174-Hinc II Digest 10 ng/µL
50 bp DNA Step Ladder 34 ng/µL

Reagents:

- DNA-1000 Reagent Kit for MultiNA
  (Shimadzu) P/N 292-27911-91
- SYBR® Gold nucleic acid gel stain
  (Invitrogen) S-11494
- ϕX174 DNA / Hae III Markers
  (Promega) G1761
- ϕX174-Hinc II Digest
  (Takara Bio) 3406A
- 50 bp DNA Step Ladder
  (Promega) G4521

Experimental Method:

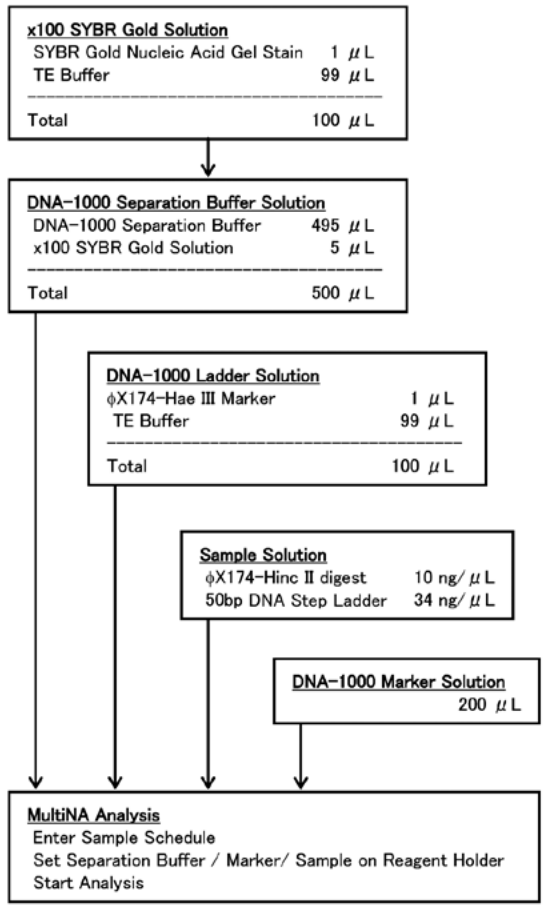


Fig. 2 Experimental Procedure (for 8 Samples)

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

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