Application of the Experion™ Automated Electrophoresis System DNA Assays to VNTR Analysis, SNP Typing, and Bacterial 16S rRNA Gene Analysis

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Introduction

The Experion automated electrophoresis system is a microfluidics-based electrophoretic system that can be applied to high-speed analysis of DNA fragments. In this report we evaluate the performance of the Experion system in terms of reproducibility and detection limit for DNA fragments. We apply the Experion system to three types of commonly performed DNA analyses: variable number of tandem repeats (VNTR) analysis, SNP typing of human aldehyde dehydrogenase type 2 gene (*ALDH2*), and 16S rRNA gene analysis of a bacterial community by amplified rDNA restriction analysis (ARDRA).

Materials and Methods

DNA Samples

DNA molecular standards used in this work were HincII digested double-stranded DNA φX174 (Toyobo Life Science) and the EZ Load™ DNA molecular mass ruler (Bio-Rad Laboratories, Inc). Human genomic DNA was extracted from cheek cells of volunteers using InstaGene™ matrix (Bio-Rad). DNA samples for ARDRA were extracted from isolated bacteria and an environmental bacterial community using conventional methods (Johnson 1991).

VNTR Analysis

VNTR analysis was performed on the D1S80 locus. A D1S80 DNA sequence was amplified using 0.50 µM of specific primers (Kasai et al. 1990) (D1SF, forward: 5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3' and D1SR, reverse: 5'-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3'), 200 µM dNTP, and 0.5 U AmpliTaq Gold (Applied Biosystems Inc.) in 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$. Forty cycles of 30 sec at 94˚C, 30 sec at 65˚C, and 4 min at 72˚C were carried out in an iCycler® thermal cycler (Bio-Rad). The PCR products were analyzed with the Experion system using the Experion DNA 1K analysis kit.

SNP Analysis of Human *ALDH2* Gene

SNP typing was performed on the human *ALDH2* gene using restriction fragment length polymorphism (RFLP) (Nachamkin et al. 2001). The exon 12 sequence of the *ALDH2* gene was amplified using 0.2 µM of specific primers (forward: 5'-CAAATTACAGGGTCAACTGCT-3' and reverse: 5'-CCACACTCACAGTTTTCTCTT-3'), 200 µM dNTP, and 0.5 U Discoverase (Invitrogen Corporation) in 50 mM Tris-SO_4, pH 8.0, 18 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$. Forty cycles of 15 sec at 94˚C, 15 sec at 58˚C, and 60 sec at 72˚C were carried out in an iCycler iQ® real-time PCR detection system. The PCR products were digested with the restriction endonuclease MboII followed by analysis with the Experion system using the Experion DNA 1K analysis kit.

ARDRA of the 16S rRNA Gene

The nearly full-length 16S rRNA gene sequence was amplified using 0.2 µM of specific primers (27f, forward: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1525r, reverse: 5'-AAGGAGGTGWTCCARCC-3'), 200 µM dNTP, and 0.5 U Discoverase (Invitrogen) in 50 mM Tris-SO_4, pH 8.0, 18 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$. Forty cycles of 30 sec at 94˚C, 30 sec at 58˚C, and 60 sec at 72˚C were carried out in an iCycler thermal cycler. The PCR products were digested with restriction endonucleases (HaeIII, AluI, and Sau3AI), followed by analysis with the Experion system using the Experion DNA 1K analysis kit. ARDRA is based on the RFLP technique (Nachamkin et al. 2001).

Results and Discussion

Performance of the Experion system

Resolution, Accuracy and Reproducibility — The fragment resolution, sizing accuracy, and reproducibility of the Experion system were evaluated using a HincII digest of double-stranded DNA φX174.

The Experion DNA 1K assay sizing resolution range for DNA fragments is between 15 and 1500 bp, which is sufficient for RFLP analysis. Particularly for the ~200 bp DNA fragments, the Experion system exhibited sufficient resolution to detect size differences of less than 10 bp. Fragments of 291, 297, 335, 345, and 392 bp were clearly separated by electrophoresis on the Experion DNA 1K analysis kit while they appear as a single band on an agarose gel (Figure 1). The separation and analysis using the Experion system was performed in about 50 min, compared to agarose gel electrophoresis which takes about 120 min (Figure 2). The sizing accuracy, intra-chip deviation (%CV), and inter-chip deviation (%CV) were ±1.03%, 0.62%, and 1.06%, respectively, indicating very good accuracy and reproducibility.

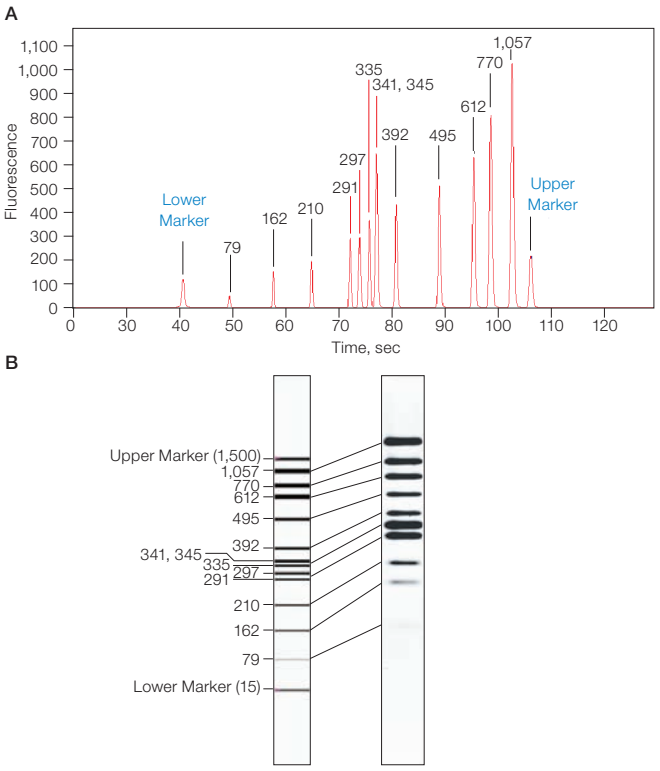

Fig. 1. Resolution of the Experion system and agarose gel electrophoresis. A, electropherogram of φX174 HincII digested DNA; **B**, comparison of the Experion system gel image and 3% agarose gel image of φX174 HincII digested DNA. The assay procedures are shown in Figure 2. The size of the bands is indicated in bp.

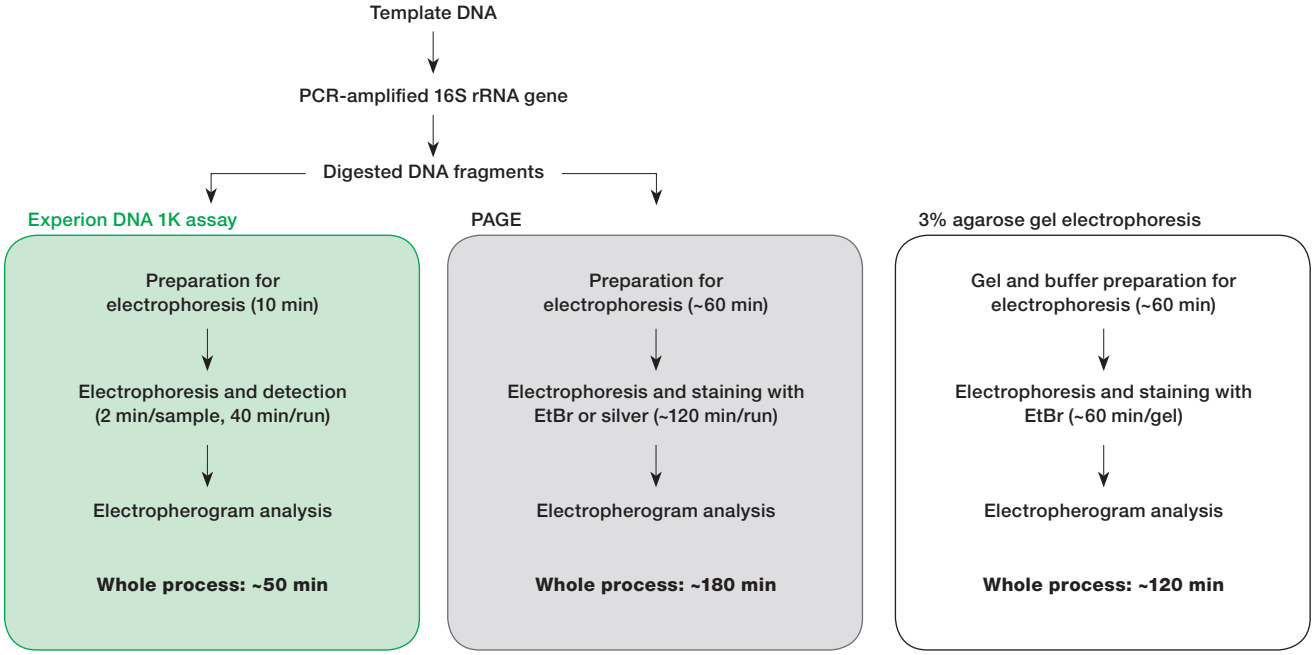

Fig. 2. Comparison of workflow and assay time. The workflow and process times for PCR-amplified DNA electrophoresis with the Experion system, polyacrylamide gel, and agarose gel are compared.

Limits of Detection — To compare the detection limits of the Experion system and agarose gel electrophoresis fivefold serial dilutions of the EZ Load DNA molecular mass ruler were analyzed with both systems. The results show that with the Experion system the limit of detection of a 100 bp fragment is 80 pg, while it is more than 400 pg on an agarose gel (Figure 3).

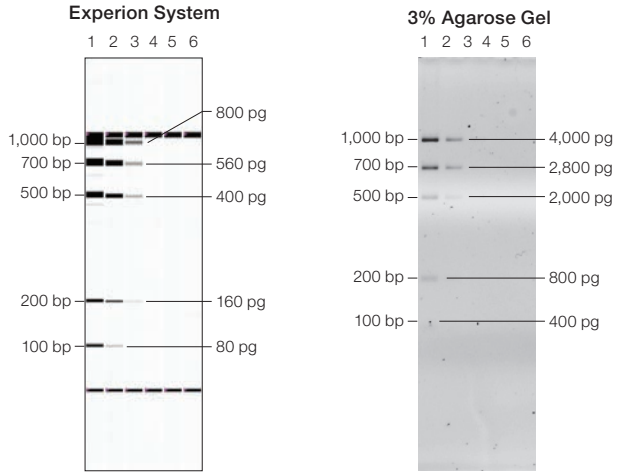

Fig. 3. Limit of detection of Experion system and agarose gel electrophoresis. Fivefold serial dilutions of EZ Load DNA molecular mass ruler run on the Experion system (left) and on a 3% ethidium bromide–stained agarose gel (right).

Evaluation of VNTR Analysis, SNP Typing, and Bacterial 16S rRNA Gene Analysis

VNTR Analysis — Typical electrophoretic profiles of amplified D1S80 DNA fragments are shown in Figure 4. The profiles were reproducible (data not shown) and coincided with those obtained on conventional PAGE analysis, indicating that data of the same quality can be obtained with the Experion assay.

SNP Genotyping — Codon 487 of the *ALDH2* gene has three kinds of SNPs, which are categorized as G/G homozygote, G/A heterozygote, and A/A homozygote. These polymorphisms are found mainly in Asian people including Japanese (Shibuya and Yoshida 1988). To assess the utility of the Experion system in SNP genotyping we performed a genotyping test on the DNA of 10 individuals. A 135 bp fragment containing codon 487 was amplified, digested with MboII, and analyzed using an Experion DNA 1K analysis kit. Since G and A peaks were completely separated, analysis with the Experion system enabled typing of these three kinds of SNPs (Figure 5) within 2 min (per sample), showing that quality data, suitable for practical high-speed analysis of SNPs, can be obtained. For DNA samples derived from 10 individuals, the SNP typing data with the Experion system correlated well with those obtained on DNA sequencing (data not shown).

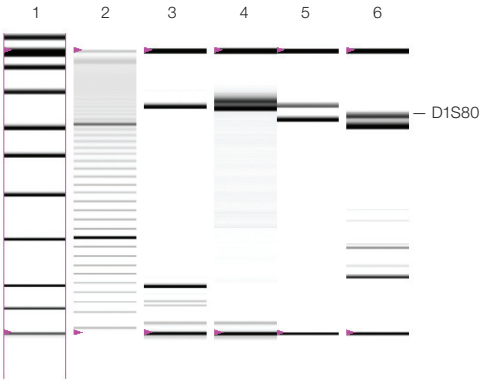

Fig. 4. D1S80 analysis using the Experion system. Lanes 1 and 2, 1K ladder and 20 bp ladder, respectively. Lanes 3–6, amplified D1S80 DNA from different individuals.

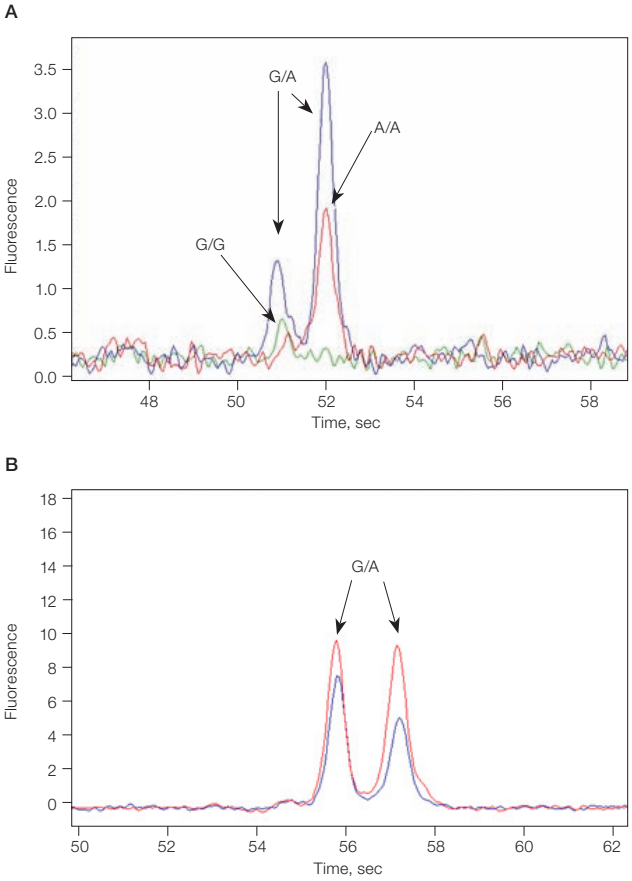

Fig. 5. *ALDH2* SNP analysis using the Experion system. A, electrophoretic profile of a MboII digested PCR fragment containing codon 487 of *ALDH2*. — G/G homozygous DNA, — G/A heterozygous DNA, and — A/A homozygous DNA; **B**, G/A heterozygous DNA from different samples.

16S rDNA Analysis — ARDRA with the Experion system was optimized and then used to identify isolated bacteria and to model the identification of a bacterium in an environmental sample. The optimized analytical procedure was performed within 2 min per sample in a total of 50 min (Figure 2), indicating this is a much quicker analytical technique than conventional PAGE (180 min). ARDRA electropherograms from the Experion system and conventional PAGE are shown in Figure 6. These data indicated that the resolution with the Experion system was the same or better than for PAGE.

PCR-amplified 16sRNA genes from *E. coli*, *B. subtilis*, and *S. aureus* were digested with HaeIII, Sau3AI, and AluI and analyzed by eletrophoresis on the Experion system and by 10% PAGE. The sizes of the fragments estimated from their electrophoretic migration pattern were compared with the sizes of the fragments deduced from the gene sequence. Results shown in Table 1 indicate that size estimation with the Experion system is very accurate.

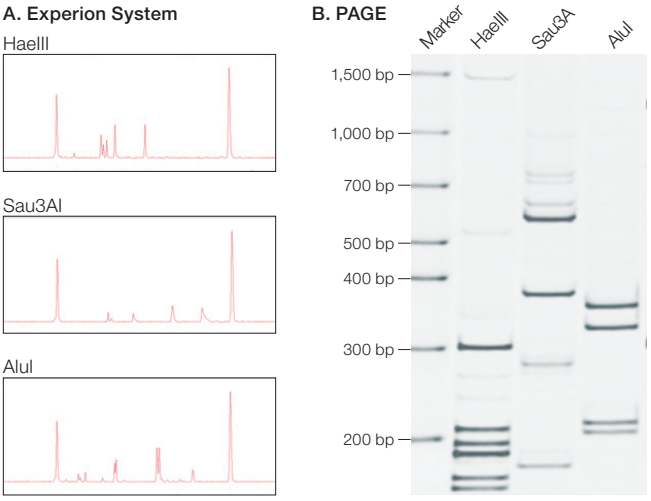

Fig. 6. Typical ARDRA profile. 16S rRNA gene from *E. coli* K12 was amplified and digested with HaeIII, Sau3AI, and AluI, followed by electrophoretic analyses with the Experion system and 10% PAGE. **A**, electropherograms obtained with the Experion system; **B**, electrophoretic profile on PAGE.

Table 1. DNA fragment size estimation. The size of DNA fragments (in base pair number) determined experimentally by electrophoresis on the Experion system (Experion) or on a 10% polyacrylamide gel (PAGE) is compared to the predicted size based on sequence data (In silico).

HaeIII	*E.coli*	In silico	34 bp	39 bp	68 bp	154 bp	161 bp	167 bp	180 bp	205 bp	210 bp	317 bp
		Experion	—	—	67 bp	157 bp	165 bp		177 bp	207 bp		317 bp
		PAGE	—	—	112 bp	130 bp	165 bp		180 bp	205 bp		305 bp
	B. subtilis	In silico	22 bp	155 bp	309 bp	457 bp	599 bp	—	—	—	—	—
		Experion	—	155 bp	314 bp	447 bp	610 bp	—	—	—	—	—
		PAGE	—	120 bp	305 bp	375 bp	560 bp	—	—	—	—	—
	S. aureus	In silico	310 bp	1,234 bp	—	—	—	—	—	—	—	—
		Experion	312 bp	1,392 bp	—	—	—	—	—	—	—	—
		PAGE	305 bp	1,200 bp	—	—	—	—	—	—	—	—
Sau3AI	*E. coli*	In silico	7 bp	12 bp	174 bp	267 bp	424 bp	651 bp	—	—	—	—
		Experion	—	—	178 bp	266 bp	434 bp	652 bp	—	—	—	—
		PAGE	—	—	140 bp	280 bp	335 bp	560 bp	—	—	—	—
	B. subtilis	In silico	7 bp	12 bp	51 bp	119 bp	175 bp	187 bp	296 bp	695 bp	—	—
		Experion	—	—	54 bp	117 bp	176 bp	189 bp	300 bp	689 bp	—	—
		PAGE	—	—	—	—	140 bp	175 bp	295 bp	630 bp	—	—
	S. aureus	In silico	7 bp	12 bp	74 bp	119 bp	175 bp	223 bp	352 bp	582 bp	—	—
		Experion	—	—	75 bp	116 bp	175 bp	225 bp	365 bp	583 bp	—	—
		PAGE	—	—	—	140 bp	170 bp	235 bp	350 bp	530 bp	—	—
AluI	*E. coli*	In silico	75 bp	86 bp	99 bp	122 bp	207 bp	211 bp	361 bp	374 bp	—	—
		Experion	79 bp	85 bp	100 bp	—	203 bp	209 bp	363 bp	373 bp	586 bp	—
		PAGE	—	—	—	—	210 bp	215 bp	325 bp	355 bp	—	—
	B. subtilis	In silico	73 bp	173 bp	186 bp	207 bp	208 bp	265 bp	430 bp	—	—	—
		Experion	78 bp	174 bp	184 bp	205 bp	210 bp	261bp	435 bp	—	—	—
		PAGE	—	155 bp	195 bp		245 bp		385 bp	—	—	—
	S. aureus	In silico	51 bp	74 bp	84 bp	86 bp	87 bp	124 bp	209 bp	214 bp	615 bp	—
		Experion	—	—	86 bp		86 bp	122 bp	201 bp	212 bp	646 bp	—
		PAGE	—	—	—	—	—	135 bp	210 bp	215 bp	620 bp	—

To evaluate the sensitivity of 16S rDNA detection, an *E.coli* 16S rDNA-HaeIII digest was mixed with an *E.coli* 16S rDNA-AluI digest in different ratios and analyzed by electrophoresis with the Experion system. The *E.coli* 16S rDNA-AluI digest could be detected even when representing as little as 11% of the DNA present in the mixture (green line in Figure 7), indicating that specific DNA fragments might be detected in a DNA sample from an environmental bacterial community if the bacterium represents 10% of the bacterial community.

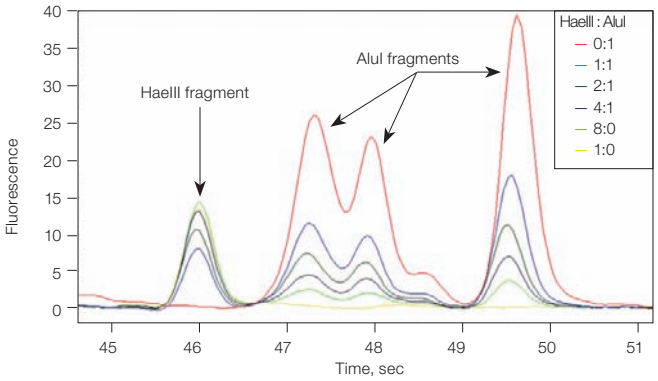

Fig. 7. Limit of detection in the ARDRA using the Experion system. An *E. coli* 16S rDNA HaeIII digest was mixed with an *E. coli* 16S rDNA AluI digest in different ratios (inset) and electrophoresed with the Experion system.

To assess the use of the Experion system to identify a bacterium in an environmental bacterial community, various amounts (0.4, 10, and 50 ng) of *E.coli* 16S rDNA-Sau3AI digest were spiked into 20 ng of a 16S rDNA-Sau3AI digest from a microbial community prior to analysis with the Experion system (Figure 8). The spiked DNA was detectable as a peak, indicating the possibility of identifying a specific bacterium in an environmental bacterial community by using the Experion system as well as conventional 16S rDNA analytical techniques including denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1993), and single-strand conformational polymorphism (SSCP) (Oto et al. 2006).

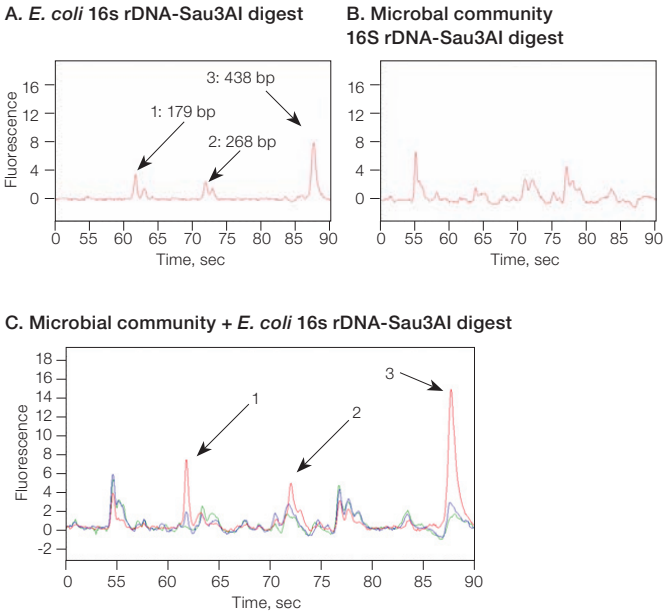

Fig. 8. Detection of target bacteria in a microbial community. Amplified 16S rDNA from *E.coli* digested with Sau3AI was spiked into 20 ng of microbial community DNA in different amounts (— 0.4, — 10, and — 50 ng). **A**, electropherogram of *E.coli* 16S rDNA; **B**, electropherogram of microbial community 16S rDNA; **C**, electropherogram of mix of *E.coli* and bacterial community 16S rDNA. **1**, **2**, and **3** indicate different peaks derived from the amplified *E.coli* 16S rDNA.

Conclusion

With the Experion system, it is possible to perform high-speed VNTR analysis, SNP typing, and bacterial 16S rRNA gene analysis. In particular, high-speed ARDRA with the Experion system may be a powerful tool for analyzing not only isolated bacteria but also microbial communities. Additionally, the Experion system is preferable for DNA length-based analyses, including sizing of PCR products and RFLP. Although the analytical formats such as the matrix concentration, buffer conditions, and running time are fixed, the Experion system is superior to gel electrophoresis in speed, resolution, detection limit, and simplicity.

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