Compatibility of Seq-DI™ Formamide with Promega STR and Sequencing Systems

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Introduction

Formamide is used for forensic DNA and sequencing applications to denature double-stranded DNA fragments prior to capillary electrophoresis (CE). Effective denaturation is critical for proper strand separation and migration to achieve good resolution and accurate sizing of DNA fragments. The use of poor-quality formamide can result in increased background, peak broadening or artifact peaks that can obscure true allelic or sequence peaks. In forensic laboratories, such problems can complicate data interpretation and may lead to inconclusive or incorrect results. For sequencing applications, poor fragment resolution can negatively affect basecalling accuracy and read length. Thus, we recommend using only high-quality, deionized formamide to maximize data quality and reliability and to minimize artifacts and other potential problems.

This white paper presents the results of our evaluation demonstrating that Seq-DI™ Formamide (NimaGen, Nijmegen, The Netherlands) is compatible with Promega STR Systems, the ProDye® Terminator Sequencing System, and associated Matrix Standards when used with common capillary electrophoresis instruments, including the Spectrum CE System, Spectrum Compact CE System and Applied Biosystems® 3500xL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Materials and Methods

Formamide Handling and Storage

To evaluate compatibility with spectral calibration, STR analysis and DNA sequencing, three lots of Seq-DI™ Formamide (NimaGen, Cat.# SDF-025) were tested in parallel with a reference competitor formamide commonly used in forensic and sequencing workflows.

Immediately upon arrival, Seq-DI™ Formamide was dispensed into 1.5ml polypropylene tubes. An aliquot was reserved for Time 0 evaluations, and the remaining aliquots were stored at –20°C. At each subsequent time point, an aliquot from each lot of Seq-DI™ Formamide was thawed on ice immediately before being used to make a loading cocktail for allelic ladder or amplification products prior to analysis by CE. At each time point, the competitor formamide reference sample was handled in parallel. Formamide was stored at –20°C for one, three or six months. As Seq-DI™ Formamide performed comparably at each time point and across lots, data from the 6-month time point of one lot is reported here.

Spectral Calibration

Seq-DI™ Formamide or the competitor formamide was used to prepare a loading cocktail for spectral calibration of the Spectrum CE System and Spectrum Compact CE System using the 5-dye PowerPlex® 5C Matrix Standard (Cat.# DG4850) and 8-dye PowerPlex® 8C Matrix Standard (Cat.# DG5010). Spectral calibrations were also performed on the Applied Biosystems® 3500xL Genetic Analyzer using the PowerPlex® 5C Matrix Standard. Two spectral calibrations were performed per instrument, one with each formamide source. All spectral calibrations were performed as described in the appropriate technical manual (1-3).

STR Analysis

Loading cocktails were prepared with both formamides as described in the appropriate technical manual for the PowerPlex® Y23 (Cat.# DC2305, DC2320), PowerPlex® ESI 17 Fast (Cat.# DC1721, DC1720) and PowerPlex® 35GY (Cat.# DC3520) Systems (4-9). For the PowerPlex® Y23 System, Stabilizer Reagent (Cat.# DM6571) was also utilized within the loading cocktail (4,7). Loading

cocktails were heat denatured as directed in the applicable technical manual prior to electrophoresis on the Spectrum CE System and Spectrum Compact CE System.

Triplicate injections of PowerPlex® Y23, PowerPlex® ESI 17 Fast and PowerPlex® 35GY Allelic Ladders were performed on the Spectrum CE System (Cat.# CE1008) and Spectrum Compact CE System (Cat.# CE1304) to assess sizing precision, expressed as the maximum standard deviation of allelic ladder fragment sizes. To assess resolution, the PowerPlex® ESI 17 Fast Allelic Ladder was used. This ladder includes DNA fragments that differ by a single nucleotide (nt) as follows: D2S441 has 11.3 and 12 alleles, and D12S391 has 17.3 and 18 as well as 18.3 and 19 alleles. Data were analyzed using GeneMarker®HID Software for Spectrum CE Systems (available as local, network, and client, Cat. # CE3001, CE3010, CE3011, version 3.2.0; GMHID-Spectrum) as described in the technical manual (10). An analytical threshold of 100 relative fluorescence units (RFU) was used for the PowerPlex® Y23 and PowerPlex® ESI 17 Fast Systems, and 150 RFU for the PowerPlex® 35GY System with data generated using both sources of formamide.

To assess peak height, morphology, and signal-to-noise, 2800M Control human genomic DNA was amplified with three PowerPlex® systems. Pooled amplification reactions for the PowerPlex® Y23 and ESI 17 Fast Systems (both at 0.5ng DNA/25µL amplification reaction) and the PowerPlex® 35GY System (1ng DNA/25µL amplification reaction) were used throughout the study as described in the appropriate technical manual (4-9). These were injected in triplicate on the Spectrum CE System and Spectrum Compact CE System using loading cocktails made with both sources of formamide.

As the Spectrum CE System can hold four plates, allowing continuous CE runs approaching two days in duration, on-deck stability of STR amplification products in the presence of loading cocktail made with either formamide was assessed. Plates containing loading cocktails with amplification products and allelic ladders were left on the deck of the Spectrum CE System after initial injections and re-injected after 24, 48 and 72 hours.

To assess sensitivity of STR amplification product detection, two sets of PowerPlex® 35GY System amplifications targeting 1ng and 62.5pg of human genomic DNA were performed. Amplification products were denatured in loading cocktails containing each formamide prior to CE analysis. The data were analyzed to confirm that full profiles were present with both DNA inputs. The results were expressed quantitatively in terms of allelic peak heights for each locus.

Sanger Sequencing

ProDye® Terminator Sequencing System (Cat.# CR4324, CR4302, CR4310) was used to assess performance of Seq-DI™ Formamide for sequencing applications using the positive control template and sequencing primer included in the kit [pGEM®-3Zf(+) Vector and pUC/M13 Forward Primer (24mer)]. Template DNA was sequenced, and the resulting sequencing reactions were purified as described in the technical manual (11) before being resuspended in either formamide and analyzed in triplicate using the Spectrum Compact CE System. Performance was assessed by comparing read quality metrics including trace scores, contiguous read lengths (CRL) and QV20+ scores.

Results

Spectral Calibration

Spectral calibrations using the PowerPlex® 5C and PowerPlex® 8C Matrix Standards were performed on the Spectrum CE and Spectrum Compact CE Systems. Spectral calibration using the PowerPlex® 5C Matrix Standard was performed on the Applied Biosystems® 3500xL Genetic Analyzer. All spectral calibrations passed with comparable values with both sources of formamide on all three CE systems (Tables 1, 2 and 3). Representative multi-dye emission spectra from one capillary on the Spectrum CE System for both the PowerPlex® 5C and PowerPlex® 8C Matrix Standards are shown in Figure 1, Panels A and B, respectively. Representative electropherograms from the Applied Biosystems 3500xL Genetic Analyzer for a passing spectral calibration using PowerPlex® 5C Matrix Standard denatured in each formamide are shown in Figure 1, Panel C.

Table 1. Spectrum Calibration Report Summary Table

	Seq-DI™ Formamide	Competitor Formamide
5C Matrix		
Minimum Deconvolution Quality	0.9861	0.9858
Maximum Intra-dye Bleedthrough	0.7%	0.8%
8C Matrix		
Minimum Deconvolution Quality	0.9714	0.9617
Maximum Intra-dye Bleedthrough	1.2%	1.5%

Table 2. Spectrum Compact Calibration Report Summary Table

	Seq-DI™ Formamide	Competitor Formamide
5C Matrix		
Quality Value	0.996-0.997	0.997-0.999
Condition Number	5.9-6.1	5.9-6.1
8C Matrix		
Quality Value	0.994-0.997	0.995-0.996
Condition Number	8.1-8.2	8.0-8.3

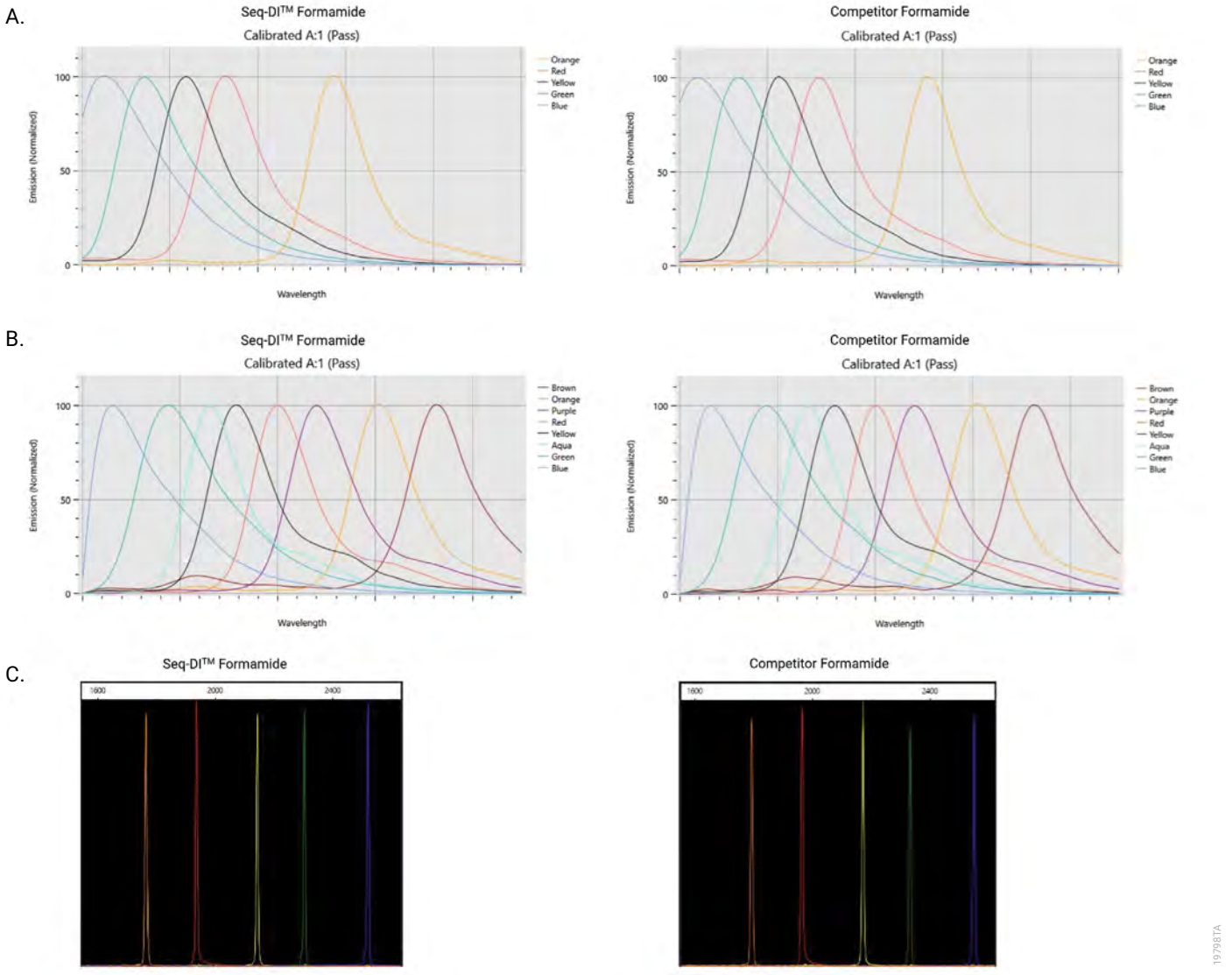


Figure 1. Spectral calibration using the PowerPlex® 5C Matrix Standard and PowerPlex® 8C Matrix Standard with each formamide. Spectral calibration results for PowerPlex® 5C Matrix Standard (Panel A) or PowerPlex® 8C Matrix Standard (Panel B) on a Spectrum CE System. Representative PowerPlex® 5C Matrix Standard electropherogram (Panel C) on an Applied Biosystems® 3500xL Genetic Analyzer.

Table 3. Applied Biosystems 3500xL Genetic Analyzer Calibration Report Summary Table

	Seq-DI™ Formamide	Competitor Formamide
5C Matrix		
Quality Value	0.994-0.999	0.992-0.997
Condition Number	10.2	10.3

STR Analysis – Allelic Ladder Sizing Accuracy, Precision and Resolution

Sizing accuracy and precision were determined by injecting the PowerPlex® Y23, PowerPlex® ESI 17 Fast and PowerPlex® 35GY Allelic Ladders on the Spectrum CE System and analyzing allelic ladder fragment sizes with GMHID-Spectrum. Sizing accuracy was confirmed by the known sample peaks falling within the correct allele bins for that sample, resulting in the correct genotype call when using GMHID-Spectrum. The maximum standard deviation (i.e., sizing precision) was 0.09nt across the three ladders prepared with Seq-DI™ Formamide and 0.12nt for ladders prepared with the competitor formamide when injected on the Spectrum CE System (Table 4). No difference in sizing precision was observed between the two formamides on the Spectrum Compact CE System (data not shown).

Table 4. Standard deviation and allele size range for PowerPlex® Y23, PowerPlex® ESI 17 Fast and PowerPlex® 35GY System allelic ladder peaks per formamide.

		Maximum standard deviation (nt)	Maximum allele size range (nt)
Seq-DI™ Formamide	ESI 17 Fast	0.08	0.20
	Y23	0.09	0.20
	35GY	0.09	0.20
Competitor Formamide	ESI 17 Fast	0.09	0.20
	Y23	0.12	0.30
	35GY	0.08	0.20

To demonstrate single-base resolution, D2S441 and D12S391 alleles (in the PowerPlex® ESI 17 Fast Allelic Ladder) that differ by a single base pair (see Methods for details) were analyzed. These alleles were all successfully resolved and called by the GMHID-Spectrum Software when utilizing either formamide (Figure 2).

STR Analysis – Amplification Product Peak Height, Morphology and Signal-to-Noise Ratio

STR amplification products from PowerPlex® Y23, PowerPlex® ESI 17 Fast, and PowerPlex® 35GY Systems were denatured in loading cocktails using each formamide and analyzed using the Spectrum CE and Spectrum Compact CE Systems. For all three PowerPlex® systems, there was no difference in peak heights, peak morphology, and artifact instances between the two sources of formamide. Representative Spectrum CE System electropherograms showing peak heights and peak morphology for the PowerPlex® 35GY System are shown in Figure 3.

To demonstrate no significant difference in peak height between these two formamides at typical and low DNA inputs, the PowerPlex® 35GY System was used to amplify 1ng or 62.5pg of 2800M Control human genomic DNA; amplicons were then injected on the Spectrum CE System following heat denaturation in loading cocktails made with each formamide. At both 1ng and 62.5pg, the peak heights obtained were virtually identical between the two sources of formamide (Figure 4). With 62.5pg of DNA, peak heights were lower with either formamide (as expected). All loci had comparable peak heights, correct allele calls and no allele dropout across formamides.

To assess signal-to-noise ratios, amplification products from the PowerPlex® Y23, PowerPlex® ESI 17 Fast, and PowerPlex® 35GY Systems were denatured in loading cocktails made with each formamide and injected on the Spectrum CE System. Limit of detection (LOD), signal and signal-to-noise ratio (Signal/LOD) were calculated for each dye channel (mean ± SD of three replicates) using the GMHID-Spectrum Software. Overall signal-to-noise was comparable between the two sources of formamide across all three tested STR Systems. Data from the PowerPlex® 35GY System are shown in Table 5.

STR Analysis – Amplification Product Stability

On-deck stability of amplification products was evaluated using the PowerPlex® Y23, PowerPlex® ESI 17 Fast, and PowerPlex® 35GY Systems. Amplification products from each STR system were denatured in loading cocktail made with each formamide and injected after 0, 24, 48 or 72 hours of on-deck storage on the Spectrum CE System.

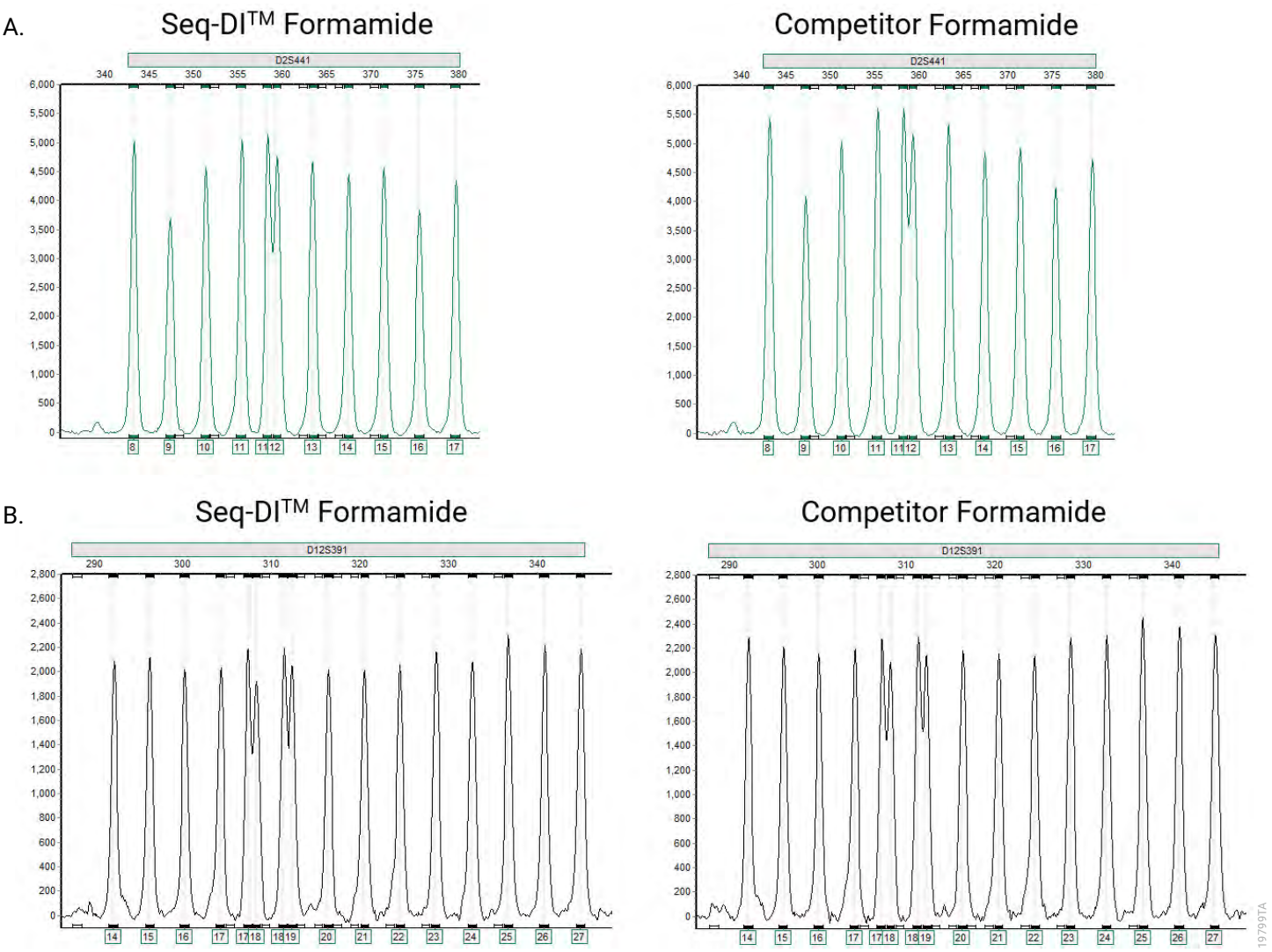


Figure 2. Single-base resolution of D2S441 and D12S391 alleles in PowerPlex® ESI 17 Fast Allelic Ladder with each formamide on the Spectrum CE system. Representative electropherograms of D2S441 (Panel A) and D12S391 (Panel B) loci.

Table 5. LOD, signal and signal-to-noise ratio for each dye channel in the PowerPlex® 35GY System per formamide.

	Seq-DI™ Formamide			Competitor Formamide		
Dye	LOD	Signal	Signal/LOD	LOD	Signal	Signal/LOD
FAM	81	3791	47	84	3697	44
JOE	93	5477	59	93	5421	58
AQA	149	4702	32	151	4591	30
3FTMR	159	3977	25	149	3870	26
3FCXR	128	4200	33	127	4115	32
TOM	93	3710	40	90	3661	41
WEN	65	3076	47	65	3015	46
Average	**110±33**	**4133±714**	**40±11**	**108±31**	**4052±713**	**40±10**

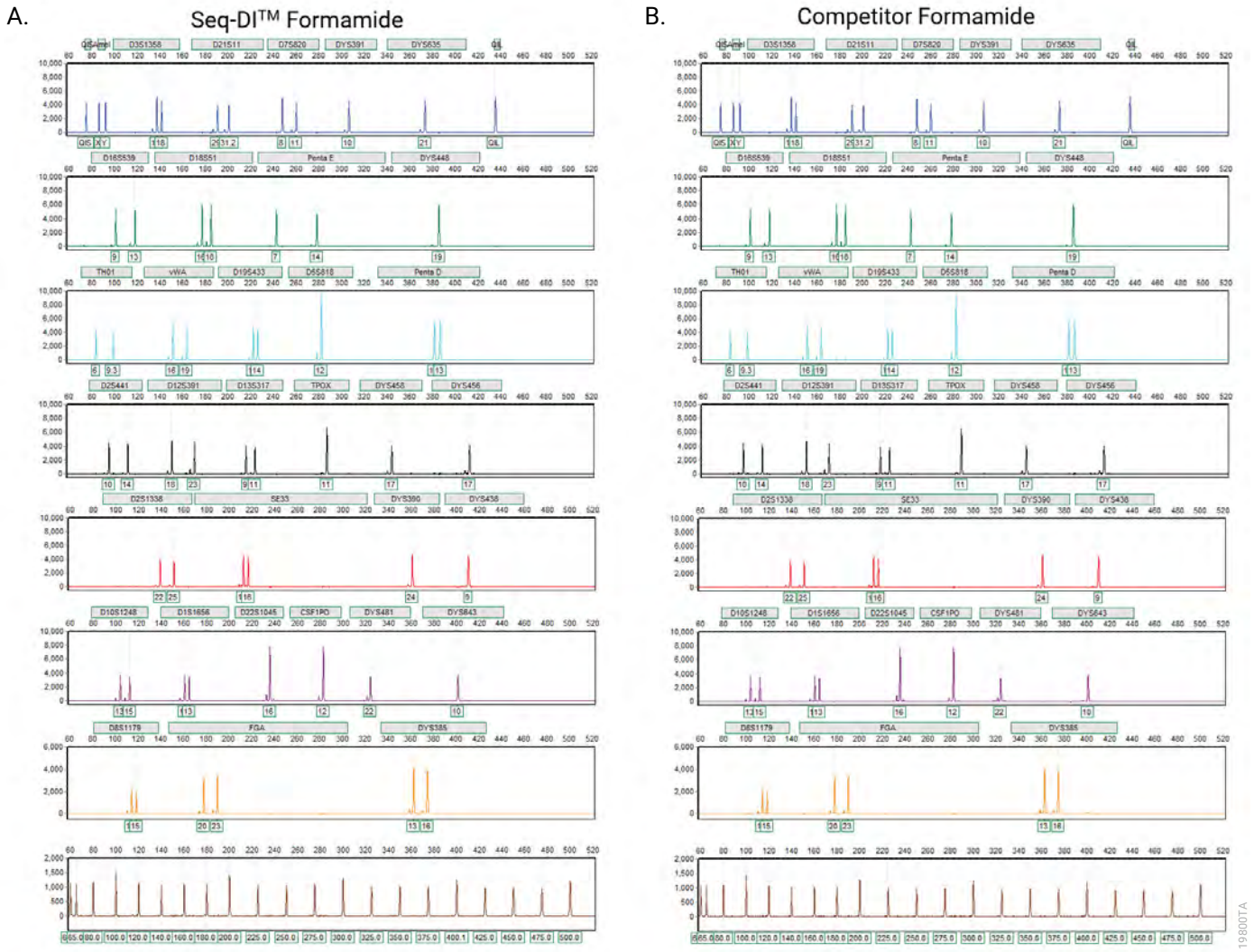


Figure 3. Representative electropherograms of PowerPlex® 35GY System amplification products with each formamide on the Spectrum CE system. Amplification products generated by amplifying 1ng of 2800M Control DNA using the PowerPlex® 35GY System were denatured in loading cocktail containing Seq-DI™ Formamide (Panel A) or a competitor formamide (Panel B), injected on the Spectrum CE System and analyzed using GMHID-Spectrum Software.

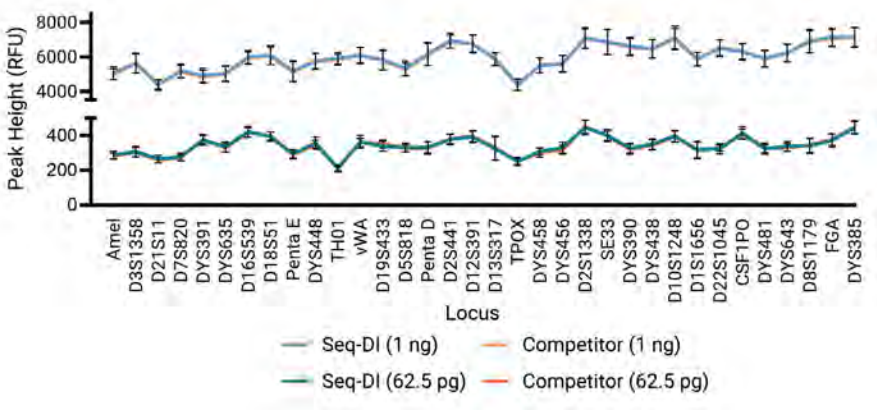


Figure 4. Peak heights for PowerPlex® 35GY System amplification with each formamide. Amplification products generated by amplifying 1ng (light colors) or 62.5pg (dark colors) of 2800M Control DNA using the PowerPlex® 35GY System were denatured in loading cocktail containing Seq-DI™ Formamide (blue) or a competitor formamide (orange) and injected on the Spectrum CE System. Values are means ± SD of three replicates.

Peak heights from each of the three STR systems remained unchanged after 72 hours of on-deck storage (Figure 5).

Sanger Sequencing

To assess Seq-DI™ Formamide performance for Sanger sequencing, purified sequencing products from the ProDye® Terminator Sequencing System were resuspended in each formamide and injected on a Spectrum Compact CE System. Trace scores, CRL and QV20+ values were comparable for purified sequencing products suspended in either formamide and injected immediately after preparation or 24 hours after preparation. No differences in average trace scores, CRLs and QV20+ values were seen between both formamides. Representative electropherograms are shown in Figure 6.

Conclusion

This evaluation demonstrates that Seq-DI™ Formamide may be used for STR analysis and Sanger sequencing applications. Seq-DI™ Formamide offers laboratories a high-quality alternative to current formamide sources without affecting data quality or reliability.

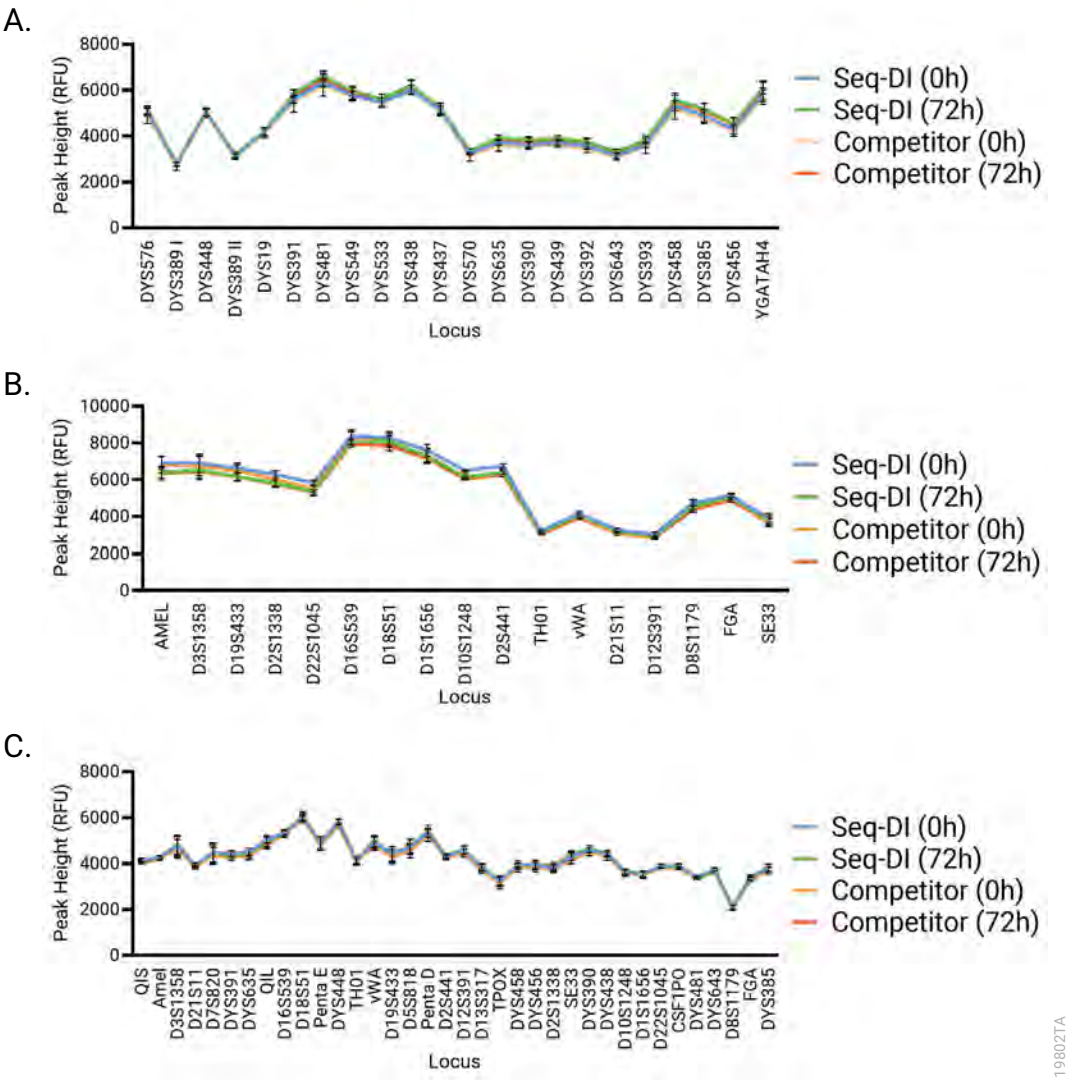


Figure 5. Stability of 2800M Control DNA amplification products stored on the deck of the Spectrum CE System in the presence of each formamide. Amplification products from PowerPlex® Y23 System (Panel A), PowerPlex® ESI 17 Fast System (Panel B), and PowerPlex® 35GY System (Panel C) were denatured using loading cocktail containing either source of formamide. Samples were injected immediately after loading the plate (0h) then stored on the deck of the Spectrum CE System for 72 hours (72h) prior to reinjection. Values are means ± SD of three replicates.

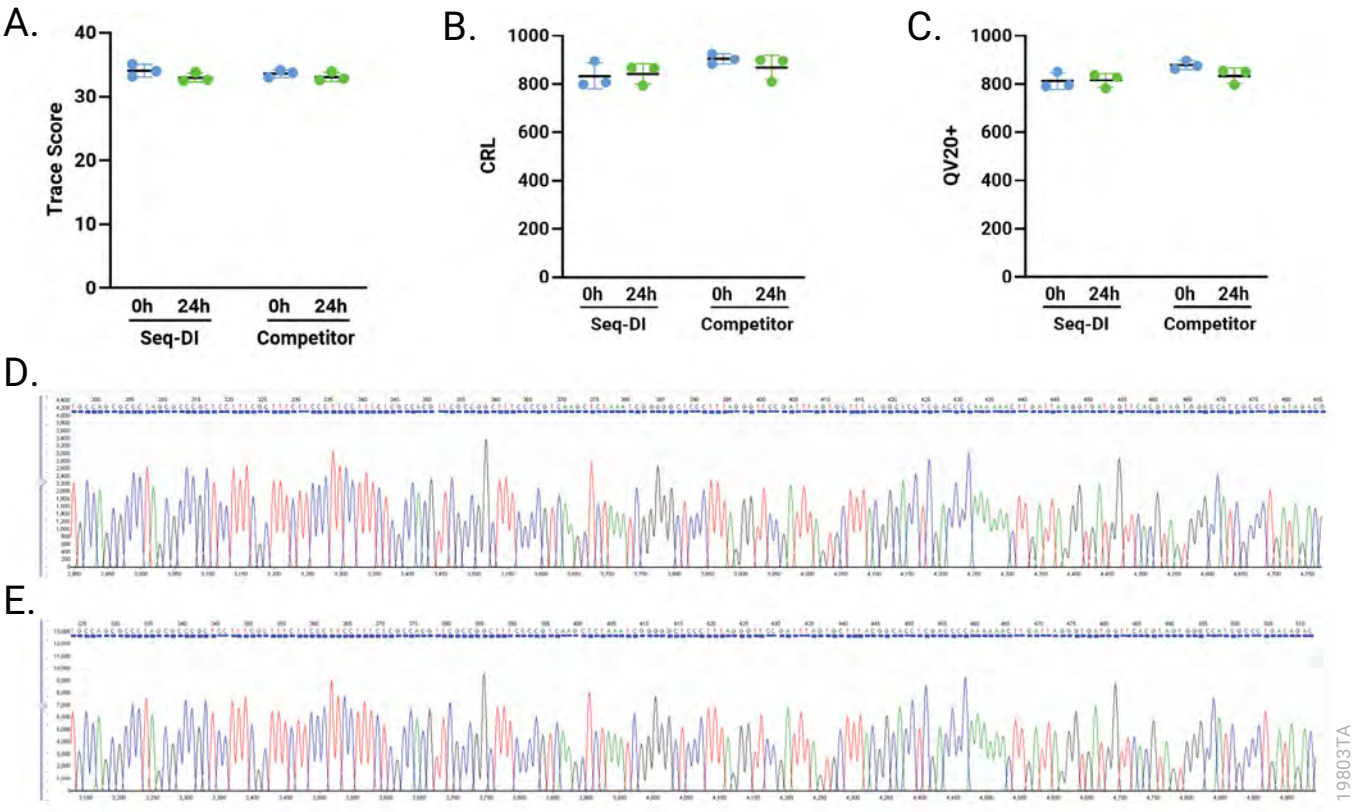


Figure 6. Results of sequencing reactions with each formamide analyzed using the Spectrum Compact CE System. Average trace scores (Panel A), average CRLs (Panel B) and average QV20+ values (Panel C) are from sequencing runs immediately after sample preparation (0h) and after 24-hour storage on the deck of the Spectrum Compact CE System (24h). Values are means ± SD of three replicates. Representative electropherograms are shown for Seq-DI™ Formamide (Panel D) and a competitor formamide (Panel E). Note that only the 300–500bp range is shown.

References

1. PowerPlex® Matrix Standards for Use on the Spectrum CE System Technical Manual #TMD068, Promega Corporation.
2. PowerPlex® Matrix Standards for Use on the Spectrum Compact CE System Technical Manual #TM062, Promega Corporation.
3. PowerPlex® 5C Matrix Standard Technical Manual #TMD049, Promega Corporation.
4. PowerPlex® Y23 System for Use on the Spectrum CE System Technical Manual #TMD078, Promega Corporation.
5. PowerPlex® ESI 17 Fast System for Use on the Spectrum CE System Technical Manual #TMD075, Promega Corporation.
6. PowerPlex® 35GY System for Use on the Spectrum CE System Technical Manual #TMD077, Promega Corporation.
7. PowerPlex® Y23 System for Use on the Spectrum Compact CE System Technical Manual #TMD060, Promega Corporation.
8. PowerPlex® ESI 17 Fast System for Use on the Spectrum Compact CE System Technical Manual #TMD057, Promega Corporation.
9. PowerPlex® 35GY System for Use on the Spectrum Compact CE System Technical Manual #TMD076, Promega Corporation.
10. GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555, Promega Corporation.
11. ProDye® Terminator Sequencing System Technical Manual #TM672, Promega Corporation.

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