

A streamlined CGE-UV workflow for high-resolution analysis of single-stranded oligonucleotides up to 200+ nt

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The analytical complexity of single-stranded oligonucleotides continues to increase, from guide RNA [gRNA] purity assessment for gene editing to characterization of poly[A] tails in mRNA-based therapeutics. These workflows require confident purity, integrity and sizing results across a broad application space, including separation of full-length product from closely related species, consistent performance across different oligonucleotide lengths and efficient implementation with minimal method optimization. The Oligo 200 nt Purity and Integrity kit is designed to deliver resolution, reproducibility and workflow simplicity for oligonucleotide analysis using a capillary gel electrophoresis with UV detection [CGE-UV] workflow. The kit covers a broad size range of 10 nt to 200+ nt and provides single-nucleotide resolution from 50/51 nt through 140/141 nt, with resolution [R] > 0.7.

Beyond analytical performance, the kit is designed for workflow simplicity, using a ready-to-use separation gel and pre-assembled bare-fused silica [BFS] capillary cartridges to streamline setup, reduce preparation time and support operation on both the PA 800 Plus system and the BioPhase 8800 system.

Key features of the Oligo 200 nt Purity and Integrity kit

- **Broad size range with single-nucleotide resolution:** Separates single-stranded oligonucleotides from 10 nt to 200+ nt and resolves seven $n/n-1$ peak pairs from 50/51 nt through 140/141 nt, with all measured R values > 0.7.
- **Reproducible performance:** Maintains consistent separation across 64 injections, 2 testing sites, 2 systems, 2 kit lots, 2 cartridges, and 2 days, with resolution %RSD < 6%.
- **High sensitivity:** Detects the 15 nt standard at an LOQ of 1 pg/ μ L across 8 capillaries, with a mean S/N of 10.2.
- **Streamlined workflow:** Uses a ready-to-use gel and pre-assembled BFS cartridges to simplify setup, reduce hands-on preparation, and support consistent implementation.
- **Platform flexibility:** Supports method transfer between the BioPhase 8800 system and PA 800 Plus system, with comparable calibrated migration time and resolution performance.

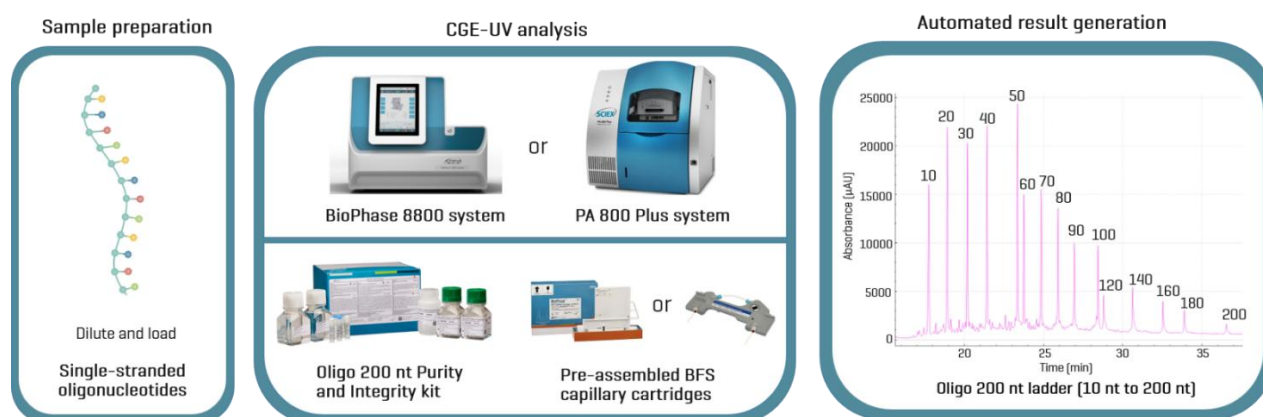
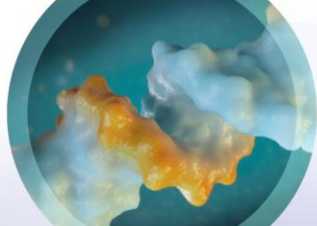


Figure 1. Workflow for oligonucleotide purity and integrity analysis. Oligonucleotide samples are analyzed by CGE-UV on the BioPhase 8800 system or the PA 800 Plus system using the ready-to-use separation gel supplied in the Oligo 200 nt Purity and Integrity kit together with pre-assembled BFS cartridges. No gel reconstitution or cartridge assembly is required, and results are generated through automated data process.



Introduction

Oligonucleotide therapeutics and related nucleic acid products, including single-guide RNA (sgRNA), guide RNA (gRNA) and poly[A]-tail constructs, require analytical methods that provide confident purity, integrity and sizing information across increasingly complex sequence designs and product profiles.¹⁻³

Current methods often rely on LC-based separation, which can present challenges in achieving sufficient resolution, robustness, and standardized performance across a broad oligonucleotide size range of 10 nt to 200 nt, particularly for longer oligonucleotides.⁴ These limitations can make it difficult to distinguish closely related analytes and impurities, generate reproducible results, and transfer methods efficiently from development to QC.

The Oligo 200 nt Purity and Integrity kit addresses these challenges with a streamlined CGE-UV workflow designed for high-resolution analysis of single-stranded oligonucleotides up to 200 nt.^{5,6} The workflow delivers single-nucleotide resolution across an extended size range, with resolution of $n/n-1$ peak pairs from 50/51 nt through 140/141 nt and reliable detection up to 200 nt, supporting confident characterization of sgRNA, gRNA, and poly[A] tail length distributions.⁷ The proprietary separation gel is supplied ready to use, with no reconstitution or dilution required, and pre-assembled BFS capillary cartridges streamline setup, reduce assay variability, and minimize method development. Designed for use on both the BioPhase 8800 system and the PA 800 Plus system, the workflow supports method continuity as teams scale from analytical development toward QC, providing reliable and reproducible data for mRNA therapeutic and gene editing programs.

Methods

Materials: [The Oligo 200 nt Purity and Integrity kit](#) (P/N: 5321745), containing Oligo 200 nt Purity and Integrity gel, Oligo 200 nt test mix (50 nt to 141 nt), Single-stranded Oligo standard (15 nt), Oligo 200 nt Purity and Integrity sample buffer (20×), CE Grade Water and Acid Wash/Regenerating Solution (0.1 N HCl), was obtained from SCIEX (Marlborough, MA). The Oligo 200 nt ladder (10 nt to 200 nt; P/N: 5322662) was also

obtained from SCIEX. BioPhase sample and reagent plates (4, 4, 8; P/N: 5080311) and BioPhase BFS capillary cartridges, 8 × 30 cm (P/N: 5080121), from SCIEX, were used for analysis on the BioPhase 8800 system. Universal vials (pack of 100, P/N: A62251), universal vial caps (blue, pack of 100, P/N: A62250), PCR micro vials (200 µL, pack of 100, P/N: 144709), and pre-assembled capillary cartridges (P/N: A55625) were also from SCIEX and used for analysis on the PA 800 Plus system. Rainin LTS filter tips were obtained from Mettler Toledo (Oakland, CA).

Storage and reagent preparation: The Single-stranded Oligo standard (15 nt), Oligo 200 nt ladder (10 nt to 200 nt) and Oligo 200 nt test mix (50 nt to 141 nt) were stored at -20°C. The Oligo 200 nt Purity and Integrity gel was stored at 2°C to 8°C, and the remaining kit components were stored at ambient temperature.^{5,6} Before sample preparation, the Oligo 200 nt Purity and Integrity sample buffer (20×) was diluted with CE Grade Water to prepare 1× sample dilution buffer. The gel was equilibrated to ambient temperature before use.

Preparation of the Single-stranded Oligo standard (15 nt)

solution: The Single-stranded Oligo standard (15 nt) was equilibrated to ambient temperature. A 1 µL aliquot of the standard was added to 799 µL of 1× sample dilution buffer and mixed by vortexing, followed by a quick spin.

Preparation of the Oligo 200 nt ladder (10 nt to 200 nt)

solution: The Oligo 200 nt ladder (10 nt to 200 nt) was equilibrated to ambient temperature. A 40 µL aliquot of the ladder was added to 760 µL of 1× sample dilution buffer and mixed by vortexing, followed by a quick spin.

Preparation of the Oligo 200 nt test mix (50 nt to 141 nt)

solution: The Oligo 200 nt test mix (50 nt to 141 nt) was equilibrated to ambient temperature. A 1 µL aliquot of the test mix was added to 799 µL of 1× sample dilution buffer and mixed by vortexing, followed by a quick spin.

Preparation of the Oligo 200 nt test mix spiked with Single-stranded Oligo standard (15 nt) as the internal standard (IS) for the reproducibility study:

The Oligo 200 nt test mix (50 nt to 141 nt) and Single-stranded Oligo standard (15 nt) were equilibrated to ambient temperature. A 2 µL aliquot of the test mix and a 1 µL aliquot of the IS were added to 797 µL of 1×

sample dilution buffer and mixed by vortexing, followed by a quick spin.

Preparation of the Oligo 200 nt test mix spiked with Single-stranded Oligo standard (15 nt) as the IS for the sensitivity study: The Oligo 200 nt test mix (50 nt to 141 nt) and Single-stranded Oligo standard (15 nt) were equilibrated to ambient temperature. A 1,000 pg/μL IS stock solution was prepared by adding 167 μL of the Single-stranded Oligo standard (15 nt) to 833 μL of 1× sample dilution buffer. The 100 pg/μL and 10 pg/μL IS solutions were prepared by 10-fold and 100-fold dilution of the 1,000 pg/μL IS stock solution, respectively, with 1× sample dilution buffer. The 1 pg/μL IS solution was prepared by 10-fold dilution of the 10 pg/μL IS solution with 1× sample dilution buffer. A 2 μL aliquot of the Oligo 200 nt test mix was added to 400 μL of each IS solution at 100, 10 and 1 pg/μL. Each solution was mixed by vortexing, followed by a quick spin. For each injection, 50 μL of the prepared solution was transferred to a sample plate well for analysis on the BioPhase 8800 system or to a PCR micro vial for analysis on the PA 800 Plus system.

CE instrument and software: [The BioPhase 8800 system](#) with UV/LIF (P/N: 5089278), equipped with a 254 nm filter (P/N: 144438), was from SCIEX and used for CGE-UV analyses. Automated data acquisition and analysis were performed using BioPhase software version 1.5 from SCIEX. Platform comparison experiments were performed on a [PA 800 Plus system](#) (P/N: A66528) equipped with a UV detector and 254 nm filter. Data were acquired and exported using 32 Karat software from SCIEX and analyzed using BioPhase software version 1.5.

CGE-UV separation conditions: The Oligo 200 nt Purity and Integrity kit application guides were followed for the BioPhase 8800 system⁵ and PA 800 Plus system,⁶ respectively. The Oligo 200 nt Conditioning and Separation method was used for capillary conditioning, gel loading and oligonucleotide separation. The method rinses the capillaries with Acid Wash/Regenerating Solution and CE Grade Water, loads the Oligo 200 nt Purity and Integrity gel and separates oligonucleotides using optimized CGE conditions. After each

sequence run, the shutdown method was used to rinse and store the cartridge according to the application guides.^{5, 6}

Results and discussion

Workflow for oligonucleotide purity and integrity analysis: As shown in Figure 1, the Oligo 200 nt Purity and Integrity kit provides a standardized CGE-UV workflow for single-stranded oligonucleotide purity, integrity and sizing analysis on either the BioPhase 8800 system or the PA 800 Plus system. Oligonucleotide samples are prepared using a simple dilute-and-load approach and analyzed using the ready-to-use separation gel supplied in the kit together with pre-assembled BFS capillary cartridges, eliminating the need for gel reconstitution or cartridge assembly. This streamlined workflow reduces manual preparation, simplifies setup, and supports consistent implementation, with automated data acquisition and processing.

Broad analytical range and high resolution: The Oligo 200 nt Purity and Integrity kit demonstrated broad analytical coverage and high-resolution separation for single-stranded oligonucleotides. Representative electropherograms of the Oligo 200 nt ladder showed detection and identification of the expected ladder peaks from 10 nt to 200 nt on the BioPhase 8800 system (Figure 2a). SCIEX CE Grade Water blanks injected before and after the ladder showed flat baselines without nonspecific peaks, supporting method specificity and indicating no observable carryover under the tested conditions.

High-resolution performance was further assessed using the Oligo 200 nt test mix, which contains seven $n/n-1$ peak pairs from 50/51 nt through 140/141 nt (Figure 2b). Duplicate injections showed clear separation of each peak pair on the BioPhase 8800 system, without nonspecific peaks in the bracketing blank injections. Taken together, the ladder and test mix results demonstrate that the workflow supports the analysis of oligonucleotides across the 10 nt to 200 nt size range while providing single-nucleotide resolution for closely related oligonucleotide species up to 141 nt.

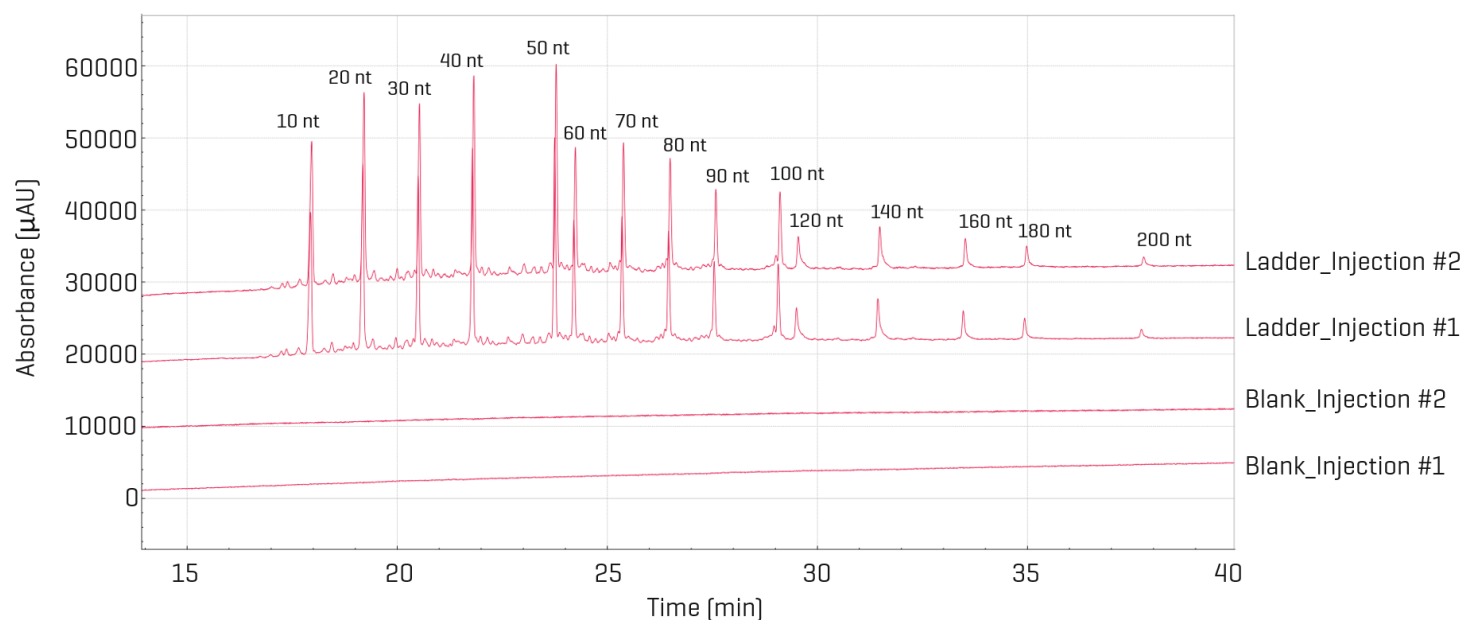


Figure 2a. Representative electropherograms of the Oligo 200 nt ladder [10 nt to 200 nt]. Duplicate injections of the ladder were analyzed by CGE-UV on the BioPhase 8800 system using the Oligo 200 nt Purity and Integrity kit, with SCIEX CE Grade Water blanks injected before and after the ladder. All expected ladder peaks from 10 nt to 200 nt were detected and identified, and no interfering or spurious peaks were observed in the blanks.

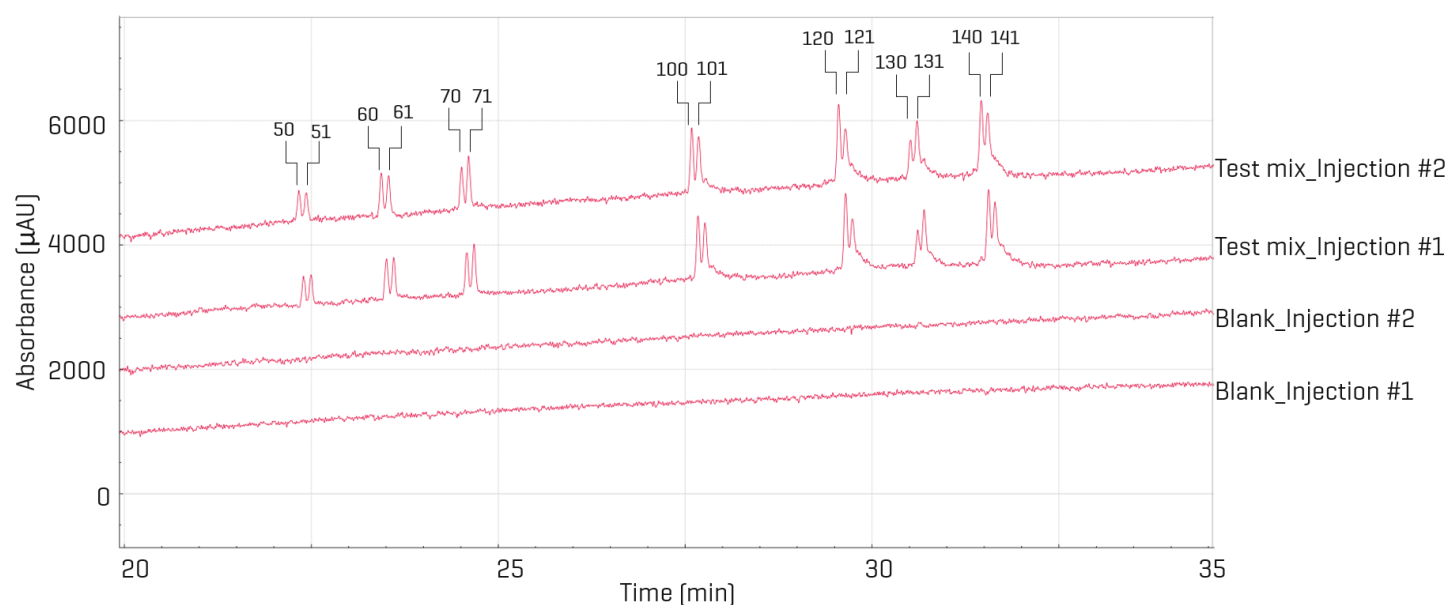


Figure 2b. Representative electropherograms of the Oligo 200 nt test mix [50 nt to 141 nt]. Duplicate injections of the test mix were analyzed by CGE-UV on the BioPhase 8800 system; SCIEX CE Grade Water blanks were injected before and after. All seven n and n-1 peak pairs, from 50/51 to 140/141, are clearly separated, demonstrating single-nucleotide resolution of single-stranded oligonucleotides up to 141 nt. All peak labels in the figure are given in nucleotides [nt].

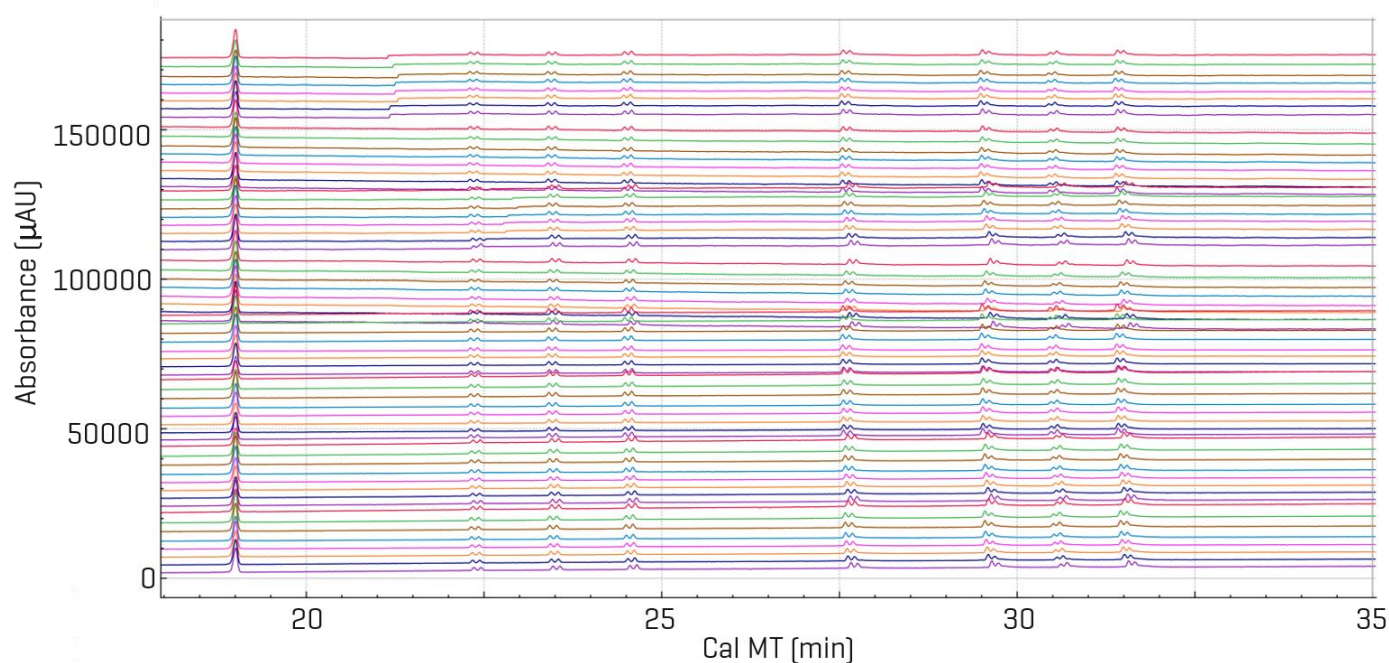


Figure 3. Reproducibility of separation across sites, systems, kit lots and cartridges. Overlaid electropherograms of injections #1, #2, #11 and #12 of the Oligo 200 nt test mix (50 nt to 141 nt) spiked with the Single-stranded Oligo standard (15 nt) as the IS. Data were acquired across 2 sites, 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges and 2 days ($n = 64$). The top 32 traces correspond to injections #1, #2, #11 and #12 from all 8 capillaries at site 1 using BioPhase 8800 system #1, kit lot #1 and cartridge #1 on day 1. The bottom 32 traces correspond to injections #1, #2, #11 and #12 from all 8 capillaries at site 2 using BioPhase 8800 system #2, kit lot #2 and cartridge #2 on day 2. Traces were overlaid using calibrated migration time [Cal MT], calculated by normalizing the migration time [MT] of each peak to the MT of the IS. The closely aligned peak profiles demonstrate reproducible separation performance across the study variables.

Reproducible single-oligonucleotide resolution separation:

Reproducibility of single-oligonucleotide resolution separation was evaluated using the Oligo 200 nt test mix spiked with the Single-stranded Oligo standard (15 nt) as an IS. The intermediate precision study included 64 injections acquired across 2 testing sites, 2 analysts, 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges, and 2 days to assess robustness across common sources of variability. Representative overlaid electropherograms from injections #1, #2, #11, and #12 showed close alignment of the peak profiles after calibration to the migration time [MT] of IS [Figure 3], demonstrating reproducible separation of the test mix across sites, systems, kit lots, and cartridges.

Use of the Single-stranded Oligo standard (15 nt) as the internal standard [IS] for normalizing the migration time improved migration time reproducibility of each test mix peaks. As exemplified for the 141 nt peak in Figure 4, migration times varied across the 64 injections, whereas IS-calibrated migration times [Cal MT] showed tighter clustering. This improvement was consistent across all 14 peaks in the Oligo 200 nt test mix (50 nt to 141 nt), as summarized in Table 1. Before calibration, migration time [MT] %RSD values ranged

from 0.99% to 1.08%; after IS calibration, Cal MT %RSD values decreased to 0.07% - 0.17%. These results demonstrate that IS calibration minimizes run-to-run migration time variability and supports reliable peak identification across different sites, systems, kit lots and cartridges.

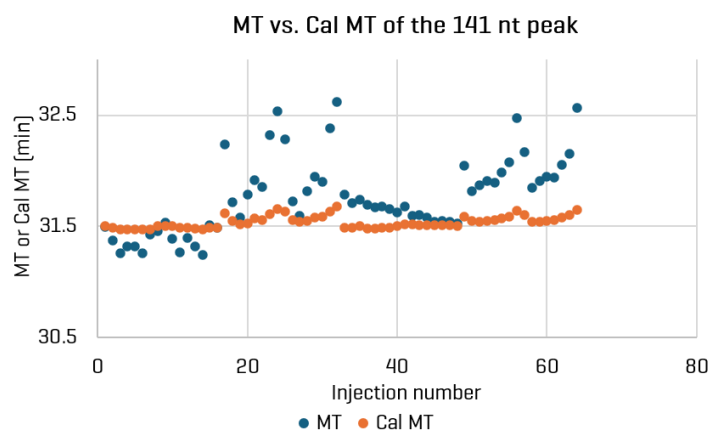


Figure 4. Migration time [MT] and IS-calibrated migration time [Cal MT] of the 141 nt peak. Values are plotted for each of the 64 injections acquired across 2 sites, 2 BioPhase 8800 systems, 2 kit lots and 2 cartridges, illustrating the effect of internal standard normalization on run-to-run migration time variability.

Table 1. Descriptive statistics of migration time and IS-calibrated migration times. Mean, standard deviation (SD), minimum [min], maximum [max] and %RSD of the migration time [MT] and IS-calibrated migration time [Cal MT] of the Single-stranded Oligo standard [15 nt, used as the internal standard, IS] and the 14 peaks of the Oligo 200 nt test mix (50 nt to 141 nt) were calculated from 64 injections acquired across 2 sites, 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges and 2 days. IS calibration reduced %RSD from up to 1.08% to <0.17% for all peaks.

Peak	MT of the 14 peaks in the test mix (n=64)					Cal MT of the 14 peaks in the test mix (n=64)				
	Mean MT [min]	SD	Min [min]	Max [min]	%RSD	Mean Cal MT [min]	SD	Min [min]	Max [min]	%RSD
50 nt	22.48	0.22	22.13	23.03	0.99%	22.32	0.02	22.30	22.36	0.07%
51 nt	22.58	0.22	22.23	23.13	0.99%	22.42	0.02	22.40	22.46	0.08%
60 nt	23.59	0.24	23.23	24.18	1.00%	23.42	0.02	23.39	23.48	0.09%
61 nt	23.69	0.24	23.32	24.28	1.00%	23.52	0.02	23.49	23.57	0.09%
70 nt	24.68	0.25	24.29	25.29	1.02%	24.50	0.02	24.46	24.56	0.10%
71 nt	24.77	0.25	24.38	25.39	1.02%	24.59	0.03	24.56	24.66	0.10%
100 nt	27.78	0.29	27.33	28.50	1.05%	27.58	0.04	27.53	27.68	0.14%
101 nt	27.87	0.29	27.42	28.60	1.05%	27.67	0.04	27.62	27.77	0.14%
120 nt	29.76	0.32	29.27	30.54	1.07%	29.54	0.05	29.48	29.66	0.15%
121 nt	29.85	0.32	29.36	30.64	1.07%	29.63	0.05	29.57	29.76	0.16%
130 nt	30.73	0.33	30.22	31.55	1.07%	30.50	0.05	30.44	30.64	0.16%
131 nt	30.82	0.33	30.31	31.64	1.07%	30.59	0.05	30.53	30.72	0.16%
140 nt	31.68	0.34	31.16	32.53	1.08%	31.45	0.05	31.38	31.59	0.17%
141 nt	31.77	0.34	31.24	32.62	1.08%	31.54	0.05	31.47	31.68	0.17%

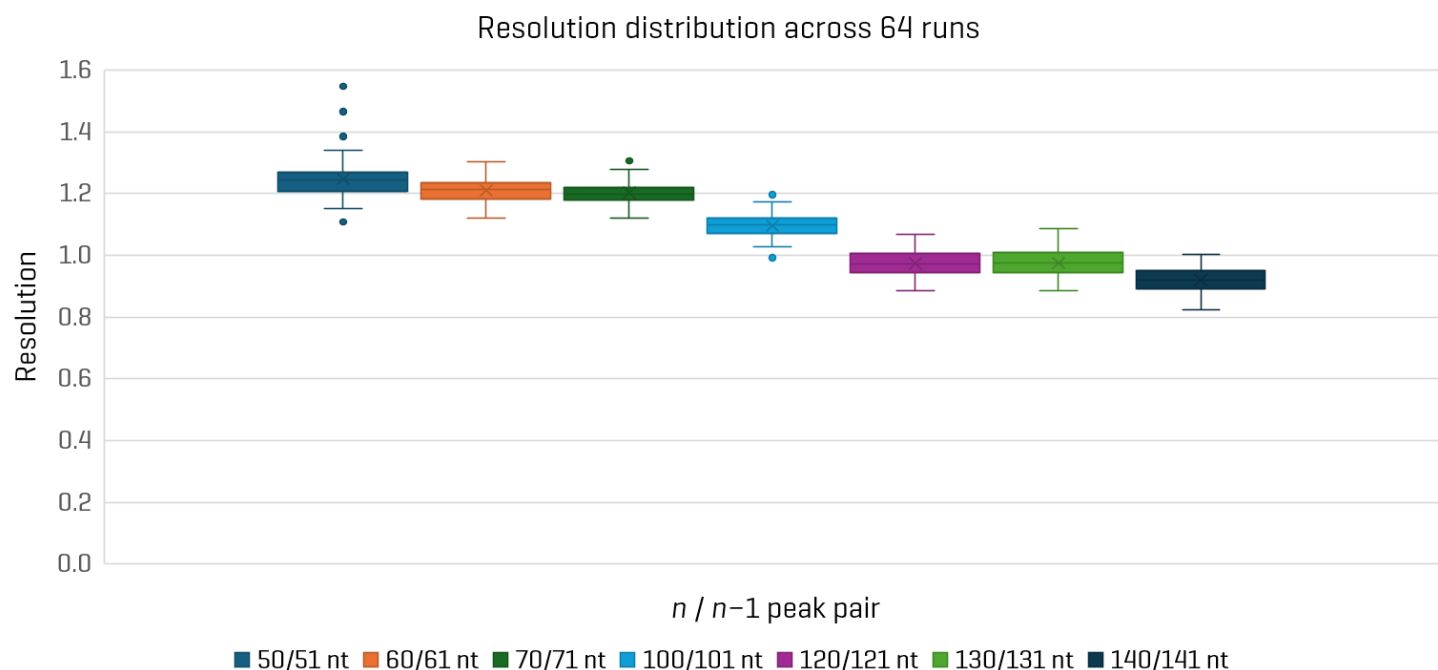


Figure 5. Resolution of the seven $n/n-1$ peak pairs in the Oligo 200 nt test mix [50 nt to 141 nt]. Box plot distribution of resolution values determined from 64 injections acquired across 2 sites, 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges and 2 days. Mean resolution decreased gradually with increasing oligonucleotide length, from 1.25 for the 50/51 nt pair to 0.92 for the 140/141 nt pair. All peak pairs obtained resolution values > 0.7, confirming single-nucleotide resolution up to 141 nt.

Table 2. Resolution of the $n/n-1$ peak pairs in the Oligo 200 nt test mix (50 nt to 141 nt). Mean, SD, %RSD, median, minimum and maximum resolution values were determined from 64 injections acquired across 2 sites, 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges and 2 days. All peak pairs obtained resolution $R > 0.7$.

Resolution of $n/n-1$ peak pairs in the Oligo 200 nt test mix (50 nt to 141 nt) ($n = 64$)							
Peak pair	% size difference	Mean	SD	Median	Min	Max	%RSD
50/51 nt	1.96%	1.2	0.1	1.2	1.1	1.5	5.3%
60/61 nt	1.64%	1.2	0.0	1.2	1.1	1.3	3.1%
70/71 nt	1.41%	1.2	0.0	1.2	1.1	1.3	3.3%
100/101 nt	0.99%	1.1	0.0	1.1	1.0	1.2	3.1%
120/121 nt	0.83%	1.0	0.0	1.0	0.9	1.1	4.2%
130/131 nt	0.76%	1.0	0.0	1.0	0.9	1.1	4.1%
140/141 nt	0.71%	0.9	0.0	0.9	0.8	1.0	4.4%

Resolution reproducibility was evaluated for the seven $n/n-1$ peak pairs in the Oligo 200 nt test mix across the same 64-injection study. As shown in the box plot of Figure 5 and summarized in Table 2, all peak pairs obtained excellent resolution ($R > 0.7$), confirming reproducible single-oligonucleotide separation from 50/51 nt through 140/141 nt. Mean resolution decreased gradually with increasing oligonucleotide length, from 1.2 for the 50/51 nt pair to 0.9 for the 140/141 nt pair, consistent with the smaller proportional

size difference between adjacent oligonucleotides at longer lengths. Resolution %RSD values ranged from 3.1% to 5.3% across the seven peak pairs, indicating consistent resolving performance across the multi-site, multi-system reproducibility study. Even for the most challenging 140/141 nt pair, the minimum resolution was 0.8 with a %RSD of 4.4%, demonstrating that the method maintained reproducible single-nucleotide resolving power throughout the study.

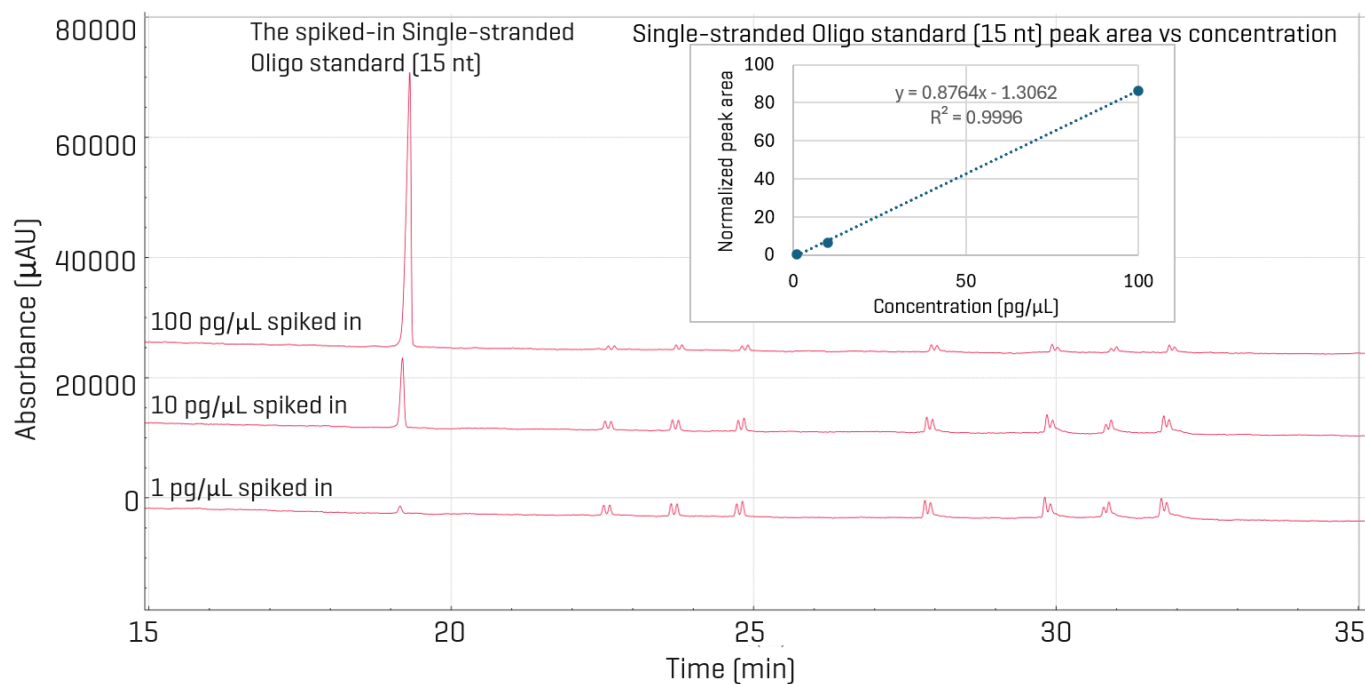


Figure 6a. Sensitivity of the Single-stranded Oligo standard (15 nt) spiked into the Oligo 200 nt test mix (50 nt to 141 nt). The standard was spiked at 1, 10 and 100 pg/ μ L and analyzed by CGE-UV on the BioPhase 8800 system. The standard was detected and integrated at all 3 levels. Inset: normalized peak area of the standard versus concentration over the 1 to 100 pg/ μ L range.

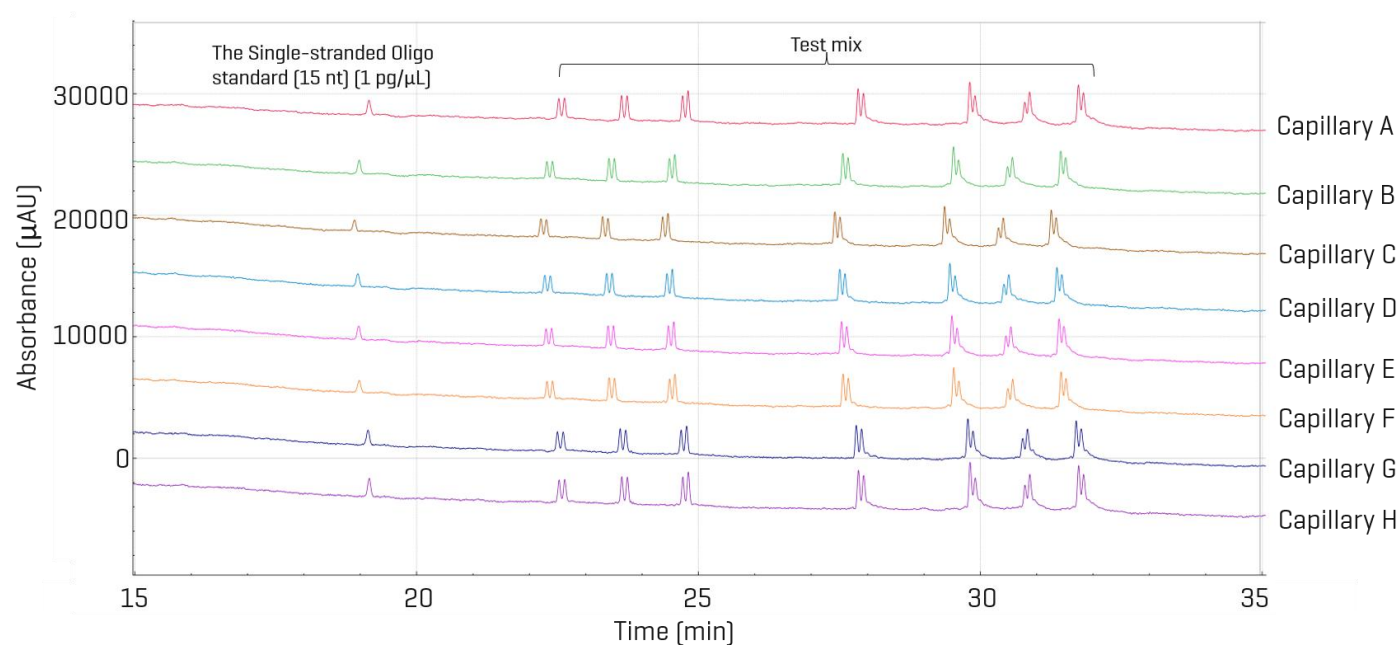


Figure 6b. Overlaid electropherograms of the test mix spiked with 1 pg/μL Single-stranded Oligo standard (15 nt), acquired simultaneously in the 8 capillaries of a BioPhase 8800 cartridge. The internal standard peak and the test mix profile were consistently detected in all 8 capillaries, illustrating uniform sensitivity and separation performance across the capillary array.

Sensitivity assessment: Sensitivity of the CGE-UV workflow was evaluated by spiking the Single-stranded Oligo standard (15 nt) into the Oligo 200 nt test mix at 1, 10 and 100 pg/μL and analyzing the samples on the BioPhase 8800 system. As shown in Figure 6a, the 15 nt standard was detected and integrated at all 3 concentration levels. The normalized peak area, calculated by normalizing the 15 nt peak area to the 141 nt peak area in the same electropherogram, showed linearity with $R^2 = 0.9996$. At the lowest tested concentration of 1 pg/μL, overlaid electropherograms from the 8 capillaries showed consistent detection of the 15 nt standard together with the Oligo 200 nt test mix profile (Figure 6b). Table 3 further demonstrates uniform low-level detection across the capillary array, with a - average migration time of 19.16 min and %RSD of 1.82%, and an average peak-to-peak signal-to-noise ratio [S/N P-P] of 10.2 with %RSD of 11.3%. These results support reliable detection of the Single-stranded Oligo standard (15 nt) at 1

pg/μL and indicate an approximate limit of quantitation (LOQ) of 1 pg/μL under the tested conditions.

Table 3. Migration time MT and S/N P-P of the Single-stranded Oligo standard (15 nt) spiked at 1 pg/μL. Values were determined in each of the 8 capillaries. The mean S/N of 10.20 [%RSD 11.26%] supports an approximate limit of quantitation [LOQ] of 1 pg/μL.

1 pg/μL spiked-in Single-stranded Oligo standard (15 nt)		
	MT [min]	S/N P-P
Capillary A	19.15	10.8
Capillary B	19.98	11.0
Capillary C	18.90	8.5
Capillary D	18.96	9.7
Capillary E	18.97	10.1
Capillary F	18.98	8.9
Capillary G	19.14	10.8
Capillary H	19.16	11.9
Average	19.16	10.2
%RSD	1.82%	11.3%

Table 4. Migration time [MT] of the Oligo 200 nt test mix on the PA 800 Plus system and the BioPhase 8800 system. Average MT and Average calibrated MT [Cal MT], each with its %RSD, for the 14 peaks of the test mix (50 nt to 141 nt), determined from 2 injections on the BioPhase 8800 system and 4 injections on the PA 800 Plus system. Calibration reduced MT variability to $\leq 0.10\%$ RSD on both systems, and the calibrated migration times agreed closely between the two platforms, supporting method transfer.

Migration time [MT] of the 14 peaks of the Oligo 200 nt test mix (50 nt to 141 nt)								
	PA 800 Plus system (n=4)				BioPhase 8800 system (n=2)			
Peak	Average MT [min]	%RSD of MT	Average Cal MT [min]	%RSD of Cal MT	Average MT [min]	%RSD of MT	Average Cal MT [min]	%RSD of Cal MT
50 nt	22.59	0.06%	22.31	0.06%	22.58	0.12%	22.32	0.03%
51 nt	22.69	0.08%	22.40	0.03%	22.68	0.11%	22.42	0.03%
60 nt	23.71	0.09%	23.41	0.03%	23.69	0.12%	23.43	0.03%
61 nt	23.81	0.09%	23.51	0.00%	23.79	0.11%	23.52	0.00%
70 nt	24.80	0.08%	24.48	0.03%	24.78	0.11%	24.50	0.03%
71 nt	24.90	0.08%	24.58	0.03%	24.88	0.12%	24.60	0.00%
100 nt	27.93	0.09%	27.57	0.03%	27.90	0.12%	27.59	0.03%
101 nt	28.02	0.11%	27.66	0.05%	28.00	0.13%	27.68	0.05%
120 nt	29.93	0.10%	29.55	0.05%	29.89	0.14%	29.56	0.02%
121 nt	30.02	0.09%	29.64	0.05%	29.98	0.13%	29.65	0.02%
130 nt	30.91	0.09%	30.52	0.07%	30.87	0.15%	30.52	0.05%
131 nt	30.99	0.10%	30.59	0.05%	30.95	0.13%	30.61	0.05%
140 nt	31.87	0.09%	31.46	0.04%	31.82	0.15%	31.46	0.04%
141 nt	31.96	0.09%	31.55	0.07%	31.91	0.14%	31.55	0.04%

Table 5. Resolution comparison of the BioPhase 8800 and PA 800 Plus systems. Resolution of the 120/121, 130/131 and 140/141 nt peak pairs of the Oligo 200 nt test mix, determined from 4 individual injections on each system, with the mean of the 4 injections reported for each peak pair. Results for the PA 800 Plus system are shown in the first set of columns and those for the BioPhase 8800 system in the last set; peak pair labels are given in nucleotides [nt]. Resolution was comparable across the two systems, with every pair meeting the acceptance criterion of $R > 0.7$, supporting method transfer between the PA 800 Plus system and BioPhase 8800 system.

Resolution of the $n/n-1$ peak pairs on the PA 800 Plus system and BioPhase 8800 system (n = 4)						
	PA 800 Plus system			BioPhase 8800 system		
Injection	120/121	130/131	140/141	120/121	130/131	140/141
Injection 1	1.0	0.9	0.9	0.9	1.0	1.0
Injection 2	1.1	1.0	0.9	1.0	0.9	0.9
Injection 3	1.0	0.8	1.0	1.0	1.0	1.0
Injection 4	0.9	0.9	0.9	0.9	1.0	1.0
Mean	1.0	0.9	0.9	1.0	1.0	1.0

Compatible with the BioPhase 8800 system and the 800 Plus systems: Platform compatibility was evaluated using the Oligo 200 nt test mix on both the BioPhase 8800 system and the PA 800 Plus system. The same method settings, capillary chemistry and capillary length were used on both systems, supporting a consistent separation mechanism across platforms. Migration times were compared across the 14 peaks from 50 nt to 141 nt in the test mix, and resolution was

assessed for the three most challenging longer $n/n-1$ pairs: 120/121 nt, 130/131 nt and 140/141 nt.

As summarized in Table 4, migration times were closely aligned between the PA 800 Plus system and the BioPhase 8800 system. Migration time variability was low on both platforms, with %RSD values $\leq 0.11\%$ on the PA 800 Plus system and $\leq 0.15\%$ on the BioPhase 8800 system. After calibration to the

IS, the calibrated migration time was further improved with %RSD values $\leq 0.07\%$ on the PA 800 Plus system and $\leq 0.05\%$ on the BioPhase 8800 system, demonstrating that internal standard-based calibration supports reliable automated peak assignment and method transfer between platforms. Resolution performance was comparable between the two systems [Table 5]. All tested peak pairs met the acceptance criterion of $R > 0.7$ across all injections, confirming single-nucleotide resolution for the longer oligonucleotide peak pairs. The average resolution values for the PA 800 Plus system were 1.0, 0.9 and 0.9 for the 120/121 nt, 130/131 nt and 140/141 nt pairs, respectively, while the BioPhase 8800 system produced corresponding average values of 1.0, 1.0 and 1.0. These results demonstrate that the Oligo 200 nt Purity and Integrity kit delivers comparable separation performance on both platforms and supports flexible implementation and method transfer between the BioPhase 8800 system and the PA 800 Plus system while maintaining resolution performance for closely related longer oligonucleotide species. This transferability is important because the BioPhase 8800 system can support earlier-stage development, method development and medium-sized sample sets where higher sample throughput is beneficial, while the PA 800 Plus system can support transfer into QC environments where lower sample throughput is typically required.

Conclusions

- The Oligo 200 nt Purity and Integrity kit provides a standardized CGE-UV workflow for single-stranded oligonucleotide purity, integrity analysis across a broad 10 nt to 200 nt range.
- The workflow demonstrated single-nucleotide resolution for seven $n/n-1$ peak pairs in the Oligo 200 nt test mix from 50/51 nt through 140/141 nt, with R values > 0.7.
- The intermediate precision study demonstrated the separation performance was reproducible across 64 injections acquired at 2 sites using 2 BioPhase 8800 systems, 2 kit lots, 2 cartridges and 2 days, supporting method robustness across common sources of variability.
- The use of the Single-stranded Oligo standard [15 nt] as an internal reference standard for migration time, reduced migration time variability, decreasing calibrated migration time %RSD values to 0.07% - 0.17% for all tested peaks and supporting reliable peak assignment.
- The method maintained consistent resolving power across the reproducibility study, with resolution %RSD values ranging from 3.1% to 5.3% and a minimum resolution of 0.8 for the most challenging 140/141 nt peak pair.
- Sensitivity results further support workflow utility, with the 15 nt internal standard detected at 1 pg/ μ L across 8 capillaries.
- Comparable migration time and resolution performance on the PA 800 Plus and BioPhase 8800 systems supported method transfer between platforms.
- The ready-to-use gel, simple dilute-and-load sample preparation and pre-assembled BFS cartridges streamline

implementation while supporting consistent performance across sites and platforms.

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