Application Note

Evaluation of QIAxcel® Advanced System as a Tool for Next-Generation Sequencing Library Quality Control Analysis

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Introduction

Next-generation sequencing (NGS) allows in-depth study of genomics and molecular biology. Optimal sequencing performance requires careful NGS library preparation and library quality control (QC) analysis. Library QC analysis provides critical information about library quality, size and sample concentration. A poor library quality lowers sequencing efficiency, compromises accuracy and coverage, and results in increased cost and time expenses. Verifying a successful library construction prior to the costly sequencing reactions is therefore crucial and an essential component of every NGS workflow.

The QIAxcel Advanced is a capillary electrophoresis system used to separate, detect and analyze nucleic acids (DNA and RNA). It provides a faster and higher detection sensitivity as compared to traditional agarose gel electrophoresis. The QIAxcel Advanced system eliminates the need for tedious gel preparation and fully automates high-resolution capillary electrophoresis of up to 96 samples per run. Ready-to-run gel cartridges, e.g. the QIAxcel DNA High Resolution Kit allows NGS library QC analysis with a minimum of hands-on interaction, reducing manual handling errors and ensuring reproducibility of the analysis.

Here we evaluated the performance of the QIAxcel Advanced System in comparison to another capillary electrophoresis system, the Agilent Bioanalyzer 2100, in terms of NGS library size determination.

Materials and Methods

NGS Library Quality Control (QC) Analysis

A total of 90 NGS libraries of different library types were analyzed in collaboration with the Agency for Science, Technology and Research (A*STAR) at the Genome Institute of Singapore (GIS). NGS library QC was evaluated using the QIAxcel Advanced and Bioanalyzer in the laboratories at QIAGEN and A*STAR, GIS respectively.

Library quality analysis using QIAxcel: Each library was diluted at 1:5 ratio by mixing 2 µl of sample with 8 µl of sample buffer (QX DNA dilution buffer and nuclease-free water at 1:1 ratio). Libraries were then analyzed using QIAxcel DNA High Resolution Kit, using the run method OM500-10s with an injection time of 20s. QX Alignment Markers 15 bp to 5 kb and QX size marker 100 bp to 2.5 kb (2.5 ng/µl, diluted in sample buffer) were used to check the size of the libraries. Size of the libraries were determined with the QIAxcel ScreenGel 1.6.0.10 software using Distribution Analysis within a defined area of interest between 200 bp and 1000 bp, and a signal to noise ratio threshold of 50.00.

Library quality analysis using Bioanalyzer: According to the manufacturer's instructions, 1ul of sample was analyzed using the High Sensitivity DNA assay.

Statistical analysis

To compare the library size determination between the QIAxcel Advanced and the Bioanalyzer we did a linear correlation and Bland-Altmann analysis. For this, the library sizes determined by QIAxcel was plotted against those determined by the Bioanalyzer. To analyze the degree of agreement between the two systems a Bland-Altman plot was constructed by plotting the difference between the library sizes (QIAxcel – Bioanalyzer) against the mean size [(QIAxcel + Bioanalyzer) / 2].

Results and Discussion

Figure 1 illustrates the proportion of the different library types and library prep kits used in this study. The size of each library determined by both systems is summarized in Supplementary Table 1.

The linear regression analysis (Figure 2a) shows a strong correlation of the library sizes determined by the QIAxcel and the Bioanalyzer with a coefficient of correlation R2 = 0.954. The Bland-Altman analysis (Figure 2b) of the size determination of the QIAxcel and the Bioanalyzer also shows a strong agreement between the two systems with a mean difference of only 7 bp ± 19 bp with determined library sizes between 205 bp and 562 bp (Supplementary Table 1). The average percentage difference for the complete data set falls below 2% (Supplementary Table 1).

These results demonstrate that the QIAxcel Advanced system is comparable with the Bioanalyzer for NGS QC library sizing for a broad range of library types and library preparation kits.

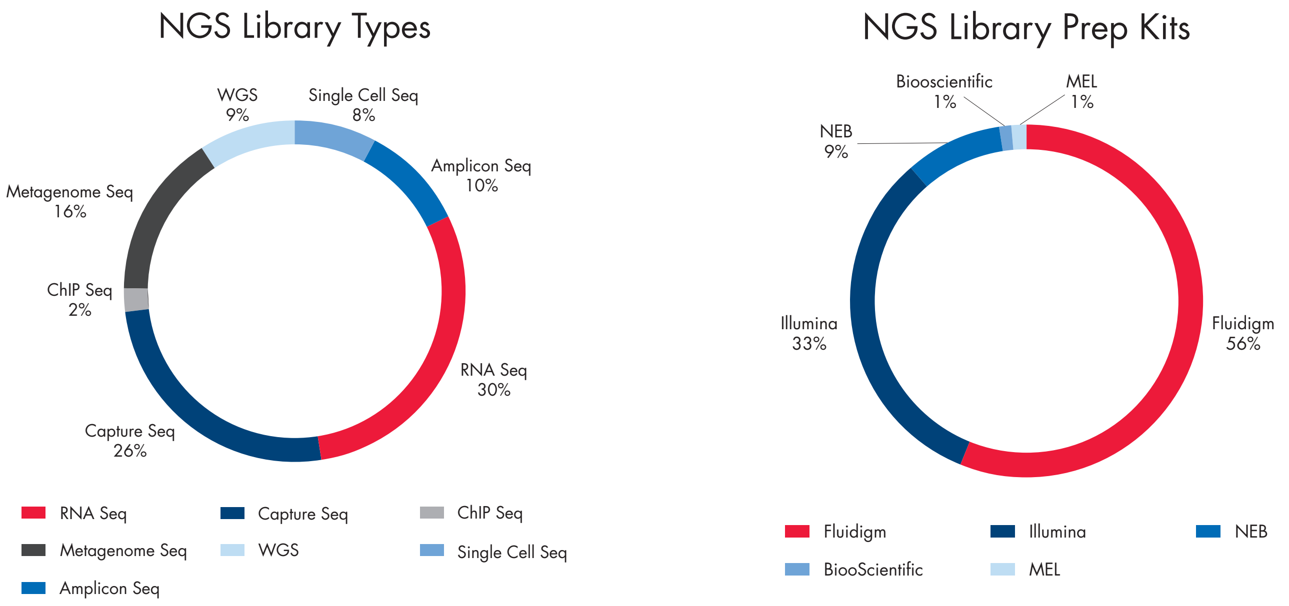

Figure 1. Distribution of left) NGS library types and right) library prep kits used in this study.

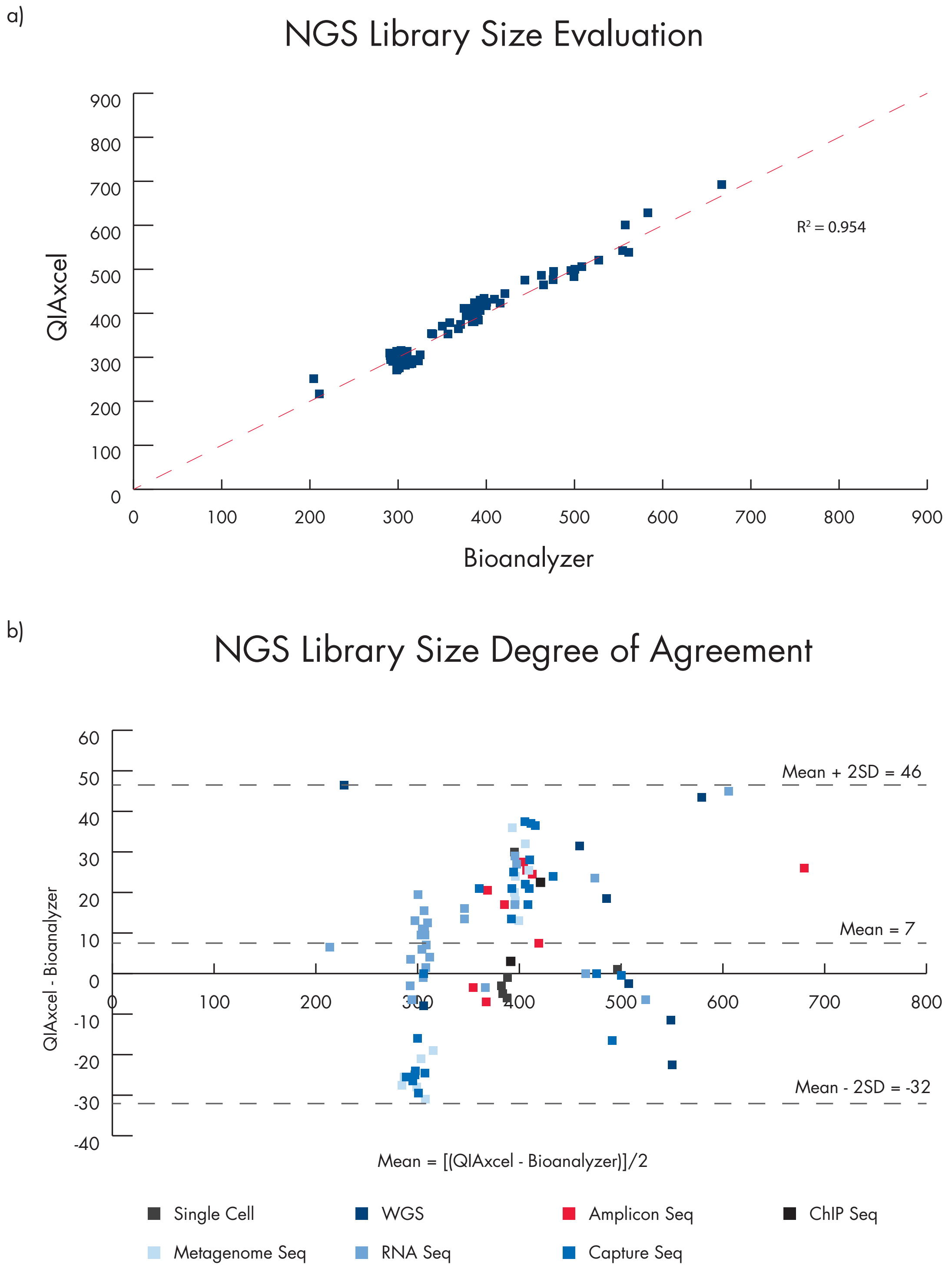

Figure 2. The Bland-Altman analysis of the library sizes determined by QIAxcel and Bioanalyzer.

a) Library sizes calculated by QIAxcel and Bioanalyzer were plotted on the line of equality for comparison. b) Degree of agreement between QIAxcel and Bioanalyzer measurements. *SD: standard deviation.

Conclusion

This Application Note demonstrates that QIAxcel Advanced System can be used for NGS library quality control (QC) analysis for various library types, such as libraries for Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES), ChIP seq, RNA seq and metagenomic seq. QIAxcel Advanced System produces comparable NGS library size calling to the Bioanalyzer. It also demonstrates consistency in NGS library size determination regardless of the library type and library preparation kit used. With its fast processing time, ready-to-run cartridges and the ability to analyze up to 96 samples automatically, the QIAxcel Advanced is a superior system in respect of efficiency, reproducibility and cost per sample for NGS library QC analysis.

Supplementary

Supplementary Table 1. Summary of the library calculated by Bioanalyzer and QIAxcel.

Library Name	Library Type	Library Prep Kit	Size on Bioanalyzer (bp)	Size on QIAxcel (bp)	Difference in size determination (bp)	Percentage difference (%)
Sample 1	RNA-seq (mRNA)	BiooScientific Directional RNAseq kit - single 6bp	368.5	365	-3.5	-0.95
Sample 2	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	384	381	-3	-0.78
Sample 3	Metagenome-seq	Fluidigm 384 Kit - single 8bp	310	285	-25	-8.06
Sample 4	Capture-seq (Index_UMI)	Fluidigm 384 Kit - single 8bp	308	292	-16	-5.19
Sample 5	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	386	381	-5	-1.30
Sample 6	Capture-seq (Index_UMI)	Fluidigm 384 Kit - single 8bp	319.5	295	-24.5	-7.67
Sample 7	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	391	385	-6	-1.53
Sample 8	WGS	NEBNext Ultra II - single 8bp	204.5	251	46.5	22.74
Sample 9	10xGenomics single cell 3' (R1:26bp)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	299.5	293	-6.5	-2.17
Sample 10	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	389	388	-1	-0.26
Sample 11	Metagenome-seq	Fluidigm 384 Kit - single 8bp	323	292	-31	-9.60
Sample 12	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	380	410	30	7.89
Sample 13	Capture-seq (Index_UMI)	Fluidigm 384 Kit - single 8bp	315.5	286	-29.5	-9.35
Sample 14	Capture-seq (Index_UMI)	Fluidigm 384 Kit - single 8bp	310	285	-25	-8.06
Sample 15	Capture-seq (whole exome)	Illumina Nextera XT Index Kit v2 - dual 8bp	499.5	483	-16.5	-3.30

Library Name	Library Type	Library Prep Kit	Size on Bioanalyzer (bp)	Size on QIAxcel (bp)	Difference in size determination (bp)	Percentage difference (%)
Sample 16	10xGenomics single cell 3' (R1:26bp)	Fluidigm 384 Kit - single 8bp	496	497	1	0.20
Sample 17	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	395	417	22	5.57
Sample 18	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	393	430	37	9.41
Sample 19	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	397.5	434	36.5	9.18
Sample 20	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	396	424	28	7.07
Sample 21	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	400	417	17	4.25
Sample 22	Metagenome-seq	Fluidigm 384 Kit - single 8bp	325	306	-19	-5.85
Sample 23	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	399	420	21	5.26
Sample 24	Capture-seq (whole exome)	NEBNext Multiplex Oligos for Illumina - single 6bp	350	371	21	6.00
Sample 25	Capture-seq (whole exome)	NEBNext Multiplex Oligos for Illumina - single 6bp	500.5	500	-0.5	-0.10
Sample 26	Capture-seq (whole exome)	NEBNext Multiplex Oligos for Illumina - single 6bp	476	476	0	0.00
Sample 27	Standard RNA-seq (stranded mRNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	291	304	13	4.47
Sample 28	RNA-seq (stranded mRNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	310	314	4	1.29
Sample 29	WGS	Fluidigm 384 Kit - single 8bp	508.5	506	-2.5	-0.49
Sample 30	RNA-seq (low input mRNA)	Illumina Nextera XT Index Kit v2 - dual 8bp	583	628	45	7.72
Sample 31	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	421	445	24	5.70
Sample 32	RNA-seq (stranded mRNA)	Illumina TruSeq HT kit - dual 8bp	305	312	7	2.30
Sample 33	WGS	Illumina TruSeq HT kit - dual 8bp	310	302	-8	-2.58
Sample 34	WGS	Fluidigm 384 Kit - single 8bp	561.5	539	-22.5	-4.01
Sample 35	WGS	Illumina Nextera XT index kit - dual 8bp	476.5	495	18.5	3.88
Sample 36	WGS	Illumina Nextera XT index kit - dual 8bp	443.5	475	31.5	7.10
Sample 37	Amplicon-seq	NEBNext Multiplex Oligos for Illumina - single 6bp	358.5	379	20.5	5.72
Sample 38	Amplicon-seq	NEBNext Multiplex Oligos for Illumina - single 6bp	356.5	353	-3.5	-0.98
Sample 39	Amplicon-seq	NEBNext Multiplex Oligos for Illumina - single 6bp	377	394	17	4.51
Sample 40	Amplicon-seq	Fluidigm 384 Kit - single 8bp	400.5	425	24.5	6.12

Library Name	Library Type	Library Prep Kit	Size on Bioanalyzer (bp)	Size on QIAxcel (bp)	Difference in size determination (bp)	Percentage difference (%)
Sample 41	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	386.5	424	37.5	9.70
Sample 42	Amplicon-seq	Fluidigm 384 Kit - single 8bp	390.5	418	27.5	7.04
Sample 43	Amplicon-seq	Fluidigm 384 Kit - single 8bp	394.5	420	25.5	6.46
Sample 44	WGS	Illumina TruSeq DNA/RNA v2 kit	557.5	601	43.5	7.80
Sample 45	RNA-seq (stranded total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	297.5	291	-6.5	-2.18
Sample 46	Amplicon-seq	Illumina TruSeq Stranded Total RNA Kit - single 6bp	415.5	423	7.5	1.81
Sample 47	RNA-seq (stranded total RNA)	Fluidigm 384 Kit - single 8bp	462.5	486	23.5	5.08
Sample 48	WGS	Fluidigm 384 Kit - single 8bp	554.5	543	-11.5	-2.07
Sample 49	Metagenome-seq	Fluidigm 384 Kit - single 8bp	385	405	20	5.19
Sample 50	Metagenome-seq	Fluidigm 384 Kit - single 8bp	387	405	18	4.65
Sample 51	Metagenome-seq	Fluidigm 384 Kit - single 8bp	396.5	422	25.5	6.43
Sample 52	Metagenome-seq	Fluidigm 384 Kit - single 8bp	390	422	32	8.21
Sample 53	Metagenome-seq	Fluidigm 384 Kit - single 8bp	393	406	13	3.31
Sample 54	Metagenome-seq	Fluidigm 384 Kit - single 8bp	384	408	24	6.25
Sample 55	Metagenome-seq	Illumina TruSeq Stranded Total RNA Kit - single 6bp	375	411	36	9.60
Sample 56	Capture-seq (whole exome)	Illumina TruSeq HT kit - dual 8bp	306	306	0	0.00
Sample 57	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	308.5	282	-26.5	-8.59
Sample 58	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	310	286	-24	-7.74
Sample 59	Metagenome-seq	Fluidigm 384 Kit - single 8bp	314	293	-21	-6.69
Sample 60	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	301.5	276	-25.5	-8.46
Sample 61	Metagenome-seq	Fluidigm 384 Kit - single 8bp	298.5	271	-27.5	-9.21
Sample 62	Metagenome-seq	Fluidigm 384 Kit - single 8bp	313	285	-28	-8.95
Sample 63	Metagenome-seq	Fluidigm 384 Kit - single 8bp	299.5	274	-25.5	-8.51
Sample 64	ChIP-seq	Illumina TruSeq ChIP-Seq Sample Prep Kit - single 6bp	409.5	432	22.5	5.49
Sample 65	ChIP-seq	Illumina TruSeq ChIP-Seq Sample Prep Kit - single 6bp	390	393	3	0.77

Library Name	Library Type	Library Prep Kit	Size on Bioanalyzer (bp)	Size on QIAxcel (bp)	Difference in size determination (bp)	Percentage difference (%)
Sample 66	RNA-seq (stranded mRNA)	#N/A	465	465	0	0.00
Sample 67	RNA-seq (total RNA)	Fluidigm 384 Kit - single 8bp	387	404	17	4.39
Sample 68	RNA-seq (mRNA)	Fluidigm 384 Kit - single 8bp	338	354	16	4.73
Sample 69	RNA-seq (mRNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	306	305	-1	-0.33
Sample 70	RNA-seq (total RNA)	Fluidigm 384 Kit - single 8bp	384	411	27	7.03
Sample 71	Amplicon-seq	Fluidigm_Access Array Barcode - single 10bp	371	364	-7	-1.89
Sample 72	RNA-seq (mRNA)	Fluidigm 384 Kit - single 8bp	527.5	521	-6.5	-1.23
Sample 73	RNA-seq (mRNA)	MEL Tempo-seq assay - dual 9bp	210.5	217	6.5	3.09
Sample 74	RNA-seq (mRNA)	NEBNext Multiplex Oligos for Illumina - single 6bp	294	291	-3	-1.02
Sample 75	RNA-seq (stranded mRNA)	Illumina TruSeq HT kit - dual 8bp	339.5	353	13.5	3.98
Sample 76	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	290.5	310	19.5	6.71
Sample 77	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	303.5	316	12.5	4.12
Sample 78	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	298.5	308	9.5	3.18
Sample 79	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	302.5	312	9.5	3.14
Sample 80	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	298.5	314	15.5	5.19
Sample 81	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	299	310	11	3.68
Sample 82	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	307.5	309	1.5	0.49
Sample 83	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	301	307	6	1.99
Sample 84	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	291.5	295	3.5	1.20
Sample 85	RNA-seq (total RNA)	Illumina TruSeq Stranded Total RNA Kit - single 6bp	301.5	312	10.5	3.48
Sample 86	Amplicon-seq	Illumina TruSeq HT kit - dual 8bp	667	693	26	3.90
Sample 87	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	382	403	21	5.50
Sample 88	RNA-seq (total RNA)	Fluidigm 384 Kit - single 8bp	381	410	29	7.61
Sample 89	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	382	407	25	6.54
Sample 90	Capture-seq (whole exome)	Fluidigm 384 Kit - single 8bp	385.5	399	13.5	3.50

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor.	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips.	929002

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