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Whether your research involves genotyping, pathogen testing, or biomarker discovery, a common challenge is processing large cohorts of samples while still maintaining accurate, reliable results.

Information presented in this issue of the VWR bioMarke magazine will show you how you can increase your throughput and reduce your cost per sample, without compromising the quality of your results.

FROM SAMPLE TO SEQUENCING
ISSUE 2

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Next-generation sequencing formalin-fixed paraffin-embedded quality control with Agilent

OVERVIEW

Formalin-fixed paraffin-embedded (FFPE) tissue represents a valuable sample source for molecular cancer research. These samples, which number in the hundreds of millions, provide a contextual snapshot of the tissue at a specific timepoint and stage of normal biology or disease. With today's high resolution technologies, such as next generation sequencing, greater information content may be extracted from these samples, including signals from low frequency alleles that could easily be missed or dismissed as artifact. There are challenges in processing FFPE samples for this type of analysis. DNA derived from FFPE is oftentimes highly fragmented, cross-linked with protein and has a high proportion of single-stranded DNA. These features of FFPE DNA make it challenging for adaptor ligation and amplification, steps that are critical for successful preparation of sequencing libraries, impacting the overall library complexity and in turn, decreasing the sensitivity of variant calling and increasing the rate of false negatives.

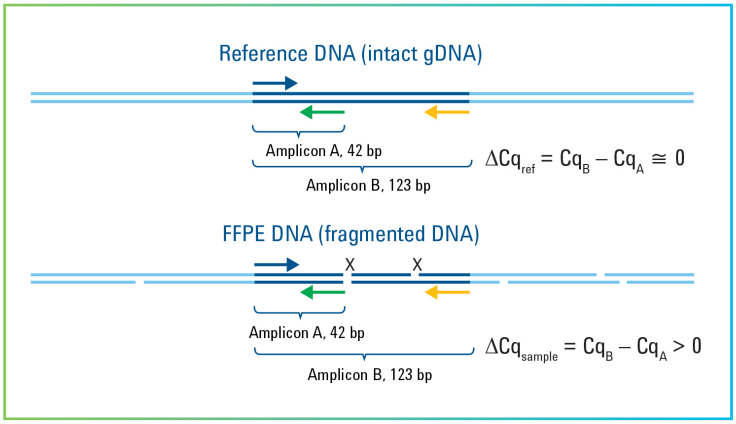


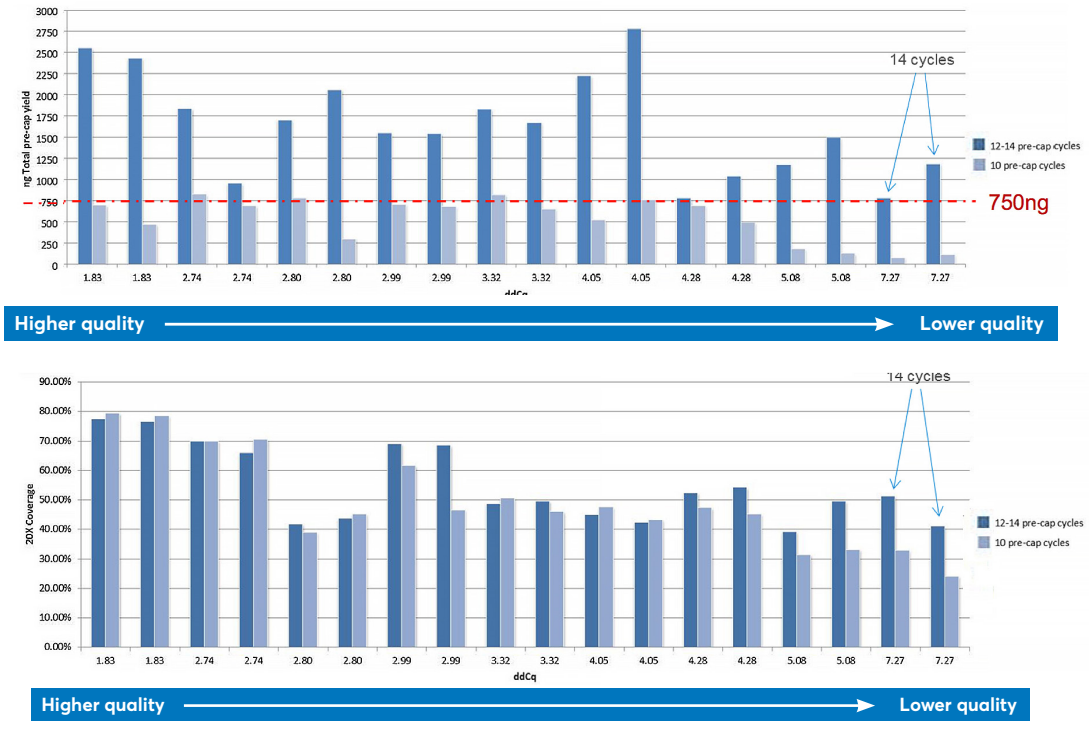
FIGURE 1. Sample quality is determined based on the ΔΔCq between the sample and the reference. Briefly, amplification of two differentially sized amplicons is assessed. The ΔCq of the sample is the difference between the Cq of amplicon B (123bp) and the Cq of amplicon A (42bp). The quality score or ΔΔCq is then calculated as the difference between the ΔCq of the sample and the ΔCq of the reference. Quantitation, on the other hand, is based on the Cq of amplicon A alone.

ACCURATE QUALIFICATION AND QUANTITATION OF AMPLIFIABLE DNA

The Agilent NGS FFPE QC kit is a qPCR-based assay that enables functional DNA quality assessment of input DNA prior to preparation of next generation sequencing libraries. This kit enables assessment of the integrity of DNA as well as accurate quantitation of amplifiable template going into library preparation. Sample integrity is assessed using two primer pairs that generate differently sized amplicons, a 42bp and a 123bp. This difference in the amplicon sizes allow for discrimination between samples that have sufficient intact amplifiable DNA and those that have a higher degree of fragmentation, effectively eliminating the need for agarose gel electrophoresis. In addition, since the assay is qPCR-based, functionality of the FFPE DNA as template for PCR is also assessed, allowing for the increased probability of successful preparation of next generation sequencing libraries (Figure 1).

OPTIMIZED LOW INPUT LIBRARY PREP WORKLOW FOR IMPROVED COMPLEXITY AND TARGET COVERAGE

Sample pre-qualification is not sufficient to increase the probability of successful preparation of sequencing libraries. To maximize the information output from FFPE samples, the



SureSelect^{XT} protocols have been optimized, providing specific recommendations on amplification of pre-capture libraries, as well as the amount of sequencing to allocate per library based on the sample quality. These modifications ensure that there is suficient representation of the molecules present in the starting sample going into the hybridization step which is critical to efficient enrichment of the targets. In addition, once these targets are enriched, the recommendations on sequencing depth should enable enough reads to ensure deep target coverage whether the starting sample is of higher or lower quality, for sensitive and accurate variant detection (Figure 2).

FIGURE 2. The Agilent NGS FFPE QC protocol provides recommendations to optimize preparation of enriched libraries for sequencing based on the quality score (ΔΔCq). Optimizations for lower quality FFPE samples include increasing ampliication cycles for the pre-capture library to ensure sufficient template molecules are introduced into the hybridization, and increasing sequencing depth to enable better target coverage.

COMPLETE CANCER RESEARCH SOLUTIONS FROM SAMPLE TO DATA

Optimized workflows are critical to providing comprehensive variant detection and reduced turn-around time from sample to data. The Agilent NGS FFPE QC kit completes the sample to sequencing worklow for FFPE samples. Combined with the efficient SureSelect^{XT} workflows that generate high complexity libraries appropriate for highly heterogenous samples, capture libraries optimized for enrichment of targets relevant in cancer research such as the ClearSeq Comprehensive Cancer panel and the SureSelect Human All Exon V6 + COSMIC, deep target coverage is achieved. For data analysis, SureCall enables analysis of paired tumor and normal samples, typical in cancer research, allowing for sensitive variant profiling of FFPE samples. (Figure 3).

Qualify Samples	Library Prep	Optimized baits for Target Enrichment	Data Analysis
Agilent NGS FFPE QC Kit	SureSelectXT Low Input	ClearSeq Comprehensive Cancer SureSelect Human All Exon V6 + COSMIC	SureCall

FIGURE 3. The Agilent NGS FFPE QC kit is part of the complete worklow solution that enables optimal data generation from even low quality FFPE samples. Complementary solutions for library prep, enrichment and data analysis are available providing support from sample pre-qualification all the way to variant calling.

US ONLY

Description	Size	Cat. No.
Agilent NGS FFPE QC Kit	16 Samples	76335-750
Agilent NGS FFPE QC Kit	96 Samples	76335-752

These products are not currently available in Canada. Please contact your VWR Life Science Specialist for more information about similar products available in your region.

Assessment of cell-free nucleic acid isolation efficiencies for liquid biopsy research

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INTRODUCTION

There is significant potential for liquid biopsy analysis to revolutionize clinical disease diagnoses and prognoses. Commonly, patient blood plasma is collected and analyzed for cell-free nucleic acids (cfNA) which consists of cell-free DNA (cfDNA) and cell- free RNA (cfRNA) molecules. These nucleic acids originate from necrotic and apoptotic cells from various tissues, or are secreted by live cells contained in microvesicles, such as exosomes, to communicate with cells at distant sites.[1,4] Cell-free nucleic acid abundance can be influenced by an individual's health, the level of nucleases in the blood, and the physiological clearance rate of cfNA by the liver and kidney.[5] Certain populations of cfNA are protected from degradation in the circulatory system by binding to proteins such as histones and RNA-processing proteins like AGO2.[2,6] Exosomes actively secreted from living cells also protect cfNA in blood and are found to have physiological functions, such as increasing cell growth or reprogramming target cells at distant sites during cancer metastasis.[7,8]

The presence of certain cell-free nucleic acids correlate with various health conditions and have the potential to enhance the efficacy of clinical diagnosis by identifying specific diseases based on cfNA profiles. The relatively non-invasive nature of simple blood draw or biological fluid collection allows for straightforward disease monitoring, which may otherwise require complicated, site-restrictive, and invasive surgical biopsies. Repeated sampling over time by liquid biopsy is practical and near painless for both patients and physicians and will likely

facilitate more effective and widespread screening during routine health exams. All-in-all, these analyses would provide a more comprehensive view of patient health status for physicians to evaluate during diagnosis and treatment.

Plasma cfDNA has already had a profound impact on prenatal genetic testing. Currently, maternal plasma cfDNA allows for prenatal genetic testing of fetal genetic material that is shed into the maternal blood stream.[9] This test is significantly safer and easier compared to amniocentesis, which poses a higher health risk for both the mother and the fetus. The success of using fetal cfDNA profiles to diagnose genetic diseases prompted scientists to survey the potential of plasma cfDNA biomarkers for the diagnosis and monitoring of other diseases such as cancer and cancer staging.[3] Active ongoing academic efforts, and overall investment of more than $1.3 billion USD into biotech companies such as Grail, Guardant Health and Foundation Medicine[10-12], are bringing routine liquid biopsy screening closer to reality.

However, there still exist challenges to face in the field of liquid biopsy. Although the amount of cfNA can significantly increase during certain diseases,[5] typical cfDNA and cfRNA levels in healthy individuals are low. Values range from 1–100ng per 1mL of plasma, with an average of 30ng, which poses several technical challenges to analysis. First, the low amount of bulk cfNA currently necessitates large sample volumes can improve by techniques including Next-Generation Sequencing and qPCR have much room to improve.[1, 13-16] The Zymo Research *Quick- cf*DNA/*cf*RNA™ Serum & Plasma Kit is capable of much higher yields from even lower sample volumes, and can improve read alignment of small cfRNA by up to 100-fold, as well as increase the diversity of detectable species by up to 4-fold.

METHODS

Plasma samples, reagents and kits

All plasma samples were purchased from Cardinal Biologicals. All plasma samples are derived from bloods collected in K2EDTA blood tubes and double centrifuged (1,900 x g for 30 minutes to separate plasma from blood, then another 1,900 x g for 30 minutes to further clear plasma from residual cells or debris) prior to shipping. Plasma samples were stored at -80°C. Prior to use, plasma samples were thawed at ambient temperature and centrifuged at 12,000 x g for 15 minutes at ambient temperature to remove any visible cryoprecipitate.

Spike-in microRNA cel-miR-39-3p mature sequence was obtained from mirbase.org, purchased from Integrated DNA Technologies, solvated in DNase/RNase-free water (Zymo Research), and stored at -80°C prior to use. Spike-in used 2.5 x 10^8 copies of cel-miR- 39-3p per sample. The 100bp DNA ladder (Zymo Research) was diluted in DNA Elution Buffer (Zymo Research) and stored in -20°C prior to use. A total of 50ng of 100bp DNA ladder spike-in was used per sample.

*Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit was obtained in-house (Zymo Research). All kit components were stored at the manufacturer's recommended temperature (ambient) prior to use. Nucleic acids were eluted in 30µL, then immediately stored at -80°C. Eluates were thawed on ice prior to use.

QIAamp Circulating Nucleic Acid Kit was purchased from Qiagen. All kit parts were stored at the recommended temperatures (ambient and 4°C) prior to use. This kit contains several different protocols specific for isolation of cfDNA, cfRNA and input volumes, and in this short the 'Protocol: Purification of Circulating microRNA from 1mL, 2mL, or 3mL Serum, Plasma, or Urine' was used to compare cfRNA recovery yield comparison. All steps were followed as the manufacturer recommended. Nucleic acids were eluted in 30µL, then immediately stored at -80°C. Eluates were thawed on ice prior to use.

miRNeasy Serum/Plasma Advanced Kit was purchased from Qiagen. All kit parts were stored at the manufacturer's recommended temperatures (ambient and 4°C) prior to use. All steps were followed as the manufacturer recommended. Nucleic acids were eluted in 30µL, then immediately stored at -80°C. Eluates were thawed on ice prior to use.

MagMax Cell-Free Total Nucleic Acid Isolation Kit was purchased from Applied Biosystems. All kit parts were stored at the manufacturer's recommended temperatures (ambient, 4°C, and -20°C) prior to use. All steps were followed as the manufacturer recommended. Nucleic acids were eluted in 30µL, then immediately stored at -80°C. Eluates were thawed on ice prior to use.

Real-time quantitative PCR assessment of cell-free RNA recovery

Protocol from published open access methods were followed to generate primer sequences and RT-qPCR data.[19,20] Briefly, samples were thawed on ice and 3µL of sample input was used for poly-A tail elongation and reverse transcription reactions. GoTaq® qPCR Master Mix (Promega) was used for 10µL qPCR reactions in duplicate per sample, with primers generated using an open access software.[19] The primer sequences for hsa-miR-16-5p are (Forward) 5´ - CGC AGT AGC AGC ACG TA and (Reverse) 5´ - CAG TTT TTT TTT TTT TTT CGC CAA. The primer sequences for cel-miR-39-3p are (Forward) 5´ - GTC ACC GGG TGT AAA TCA G and (Reverse) 5´ - GGT CCA GTT TTT TTT TTT TTT TCA AG. The CFX96 Touch™ Real-Time PCR Detection System (BioRad) platform was used. Biological duplicates were used to calculate

average threshold cycle and sample standard deviation values. Sample copy numbers were calculated using positive controls with known amount of copy numbers.

Fluorescence-based quantifications and capillary gel electrophoresis

For quantification of total small RNA yield by fluorescence-based quantification, Qubit™ microRNA Assay Kit and Qubit® 3.0 instrument (Thermo-Fisher) were used per manufacturer's recommendations. For qualitative assessment of DNA separation efficiency and yield, the High Sensitivity D1000 Kit and 2200 TapeStation (Agilent Technology) were used per manufacturer's recommendations.

RNA sequencing library generation and sequence analysis

Plasma samples from three donors were used to isolate cell-free RNA using *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit (Zymo Research), MagMax Cell-Free Total Nucleic Acid Isolation Kit (Thermo-Fisher Scientific), QIAamp Circulating Nucleic Acids Kit (Qiagen) and miRNeasy Serum/Plasma Advanced Kit (Qiagen). Libraries were prepared from cfRNA extracted from 200µL of plasma per library. The RealSeq-Biofluids Library Preparation Kit (SomaGenics) was used to generate RNA library preps. A total of 17 PCR cycles were used for the amplification step. Library quantification was done using the KAPA Library Quantification Kit (Roche). Library preps were pooled for sequencing on HiSeq 1500 v4 System (Illumina) for Zymo Research samples and the MiniSeq System (Illumina) for SomaGenics samples. Reads were trimmed and filtered (retained ≥15 nt length) using Cutadapt [-a TGGAATTCTCGGGTGCCAAGG -O 3 -q 20 -m 15].[21] Resulting reads were then mapped using either STAR22 (Small RNA-seq single-end pipeline) or Bowtie2 with vsl option.[23] Small RNA types were profiled using YM500v3 database.[24]

RESULTS

Assessment of cell-free RNA yields

To compare cell-free RNA recovery efficiencies between currently available solutions, the following kits were tested: The Zymo Research *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit, the Qiagen QIAamp Circulating Nucleic Acid Kit (CNA; microRNA protocol), and the Qiagen miRNeasy Serum/Plasma Advanced Kit (miR Adv). These three kits were used to isolate cfRNA from the same plasma sample. A range of input volumes were isolated, including the maximum input volume for each kit. Quantification of RNA yield was assessed by the Qubit™ microRNA Assay Kit (Figure 1A).

The Zymo Research *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit consistently recovered higher total small RNA over all ranges of input volumes when compared to the CNA and miR Adv protocols. Roughly linear recovery of total cfNA yield (~2-fold for 0.5–1mL and ~3-fold for 1 –3mL) was observed using the *Quick-cf*DNA/*cf*RNA™ technology. Both the miR Adv and CNA kits yielded low total cfNA levels and did not demonstrate linear recovery with sample volume input. Of note, a decrease in total yield was observed for the miR Adv kit when higher volume of sample was extracted. These results demonstrate higher total recovery of small RNA using the Zymo Research *Quick-cf*DNA/ *cf*RNA™ Kit than the CNA and miR Adv kits.

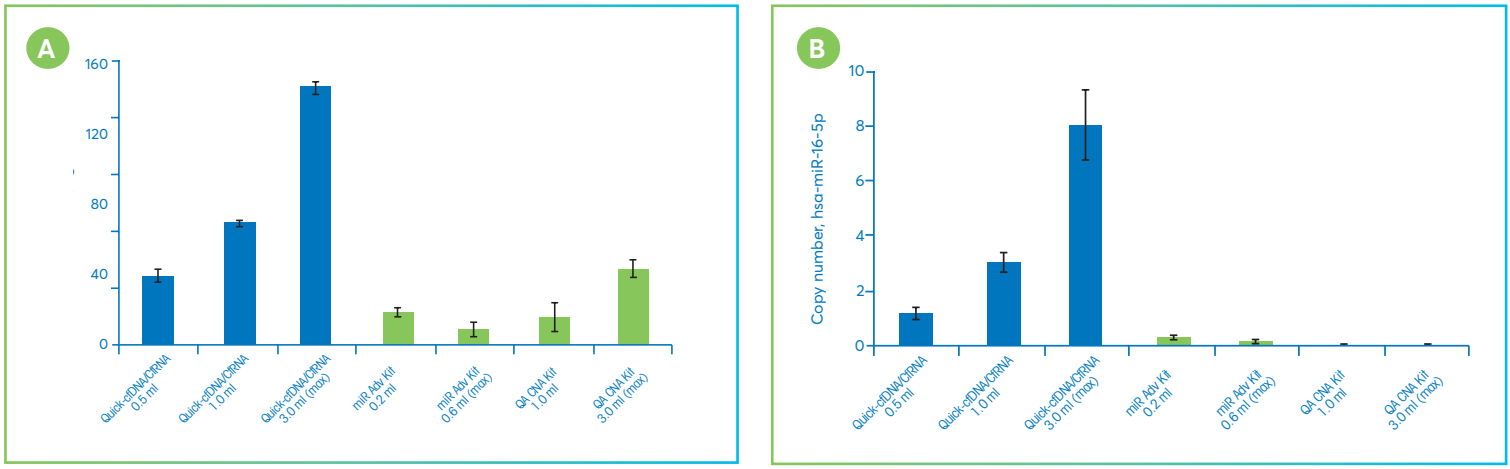

FIGURE 1: Efficient and linear isolation of cell-free RNA. Total cell-free RNA was purified from plasma (single donor, 61y-F) using three different commercial kits, The *Quick-cf*DNA/*cf*RNA™ Kit (Zymo Research), miRNeasy Serum/Plasa Advanced Kit (miR Adv Kit, Qiagen) and QIAamp Circulating Nucleic Acids Kit (QA CNA Kit, Qiagen). **(A)** Total cell-free small RNA yield from each extraction kit was assessed using Qubit quantification method specific for small RNAs. Bars represent average total yield recovered in nanogram (ng). **(B)** Quantification of microRNA hsa-miR- 16-5p isolated by the different extraction kits was assessed using RT-qPCR. Bars represent average copy numbers (x 10^9). For both graphs, error bars indicate sample standard deviations from two independent extractions.

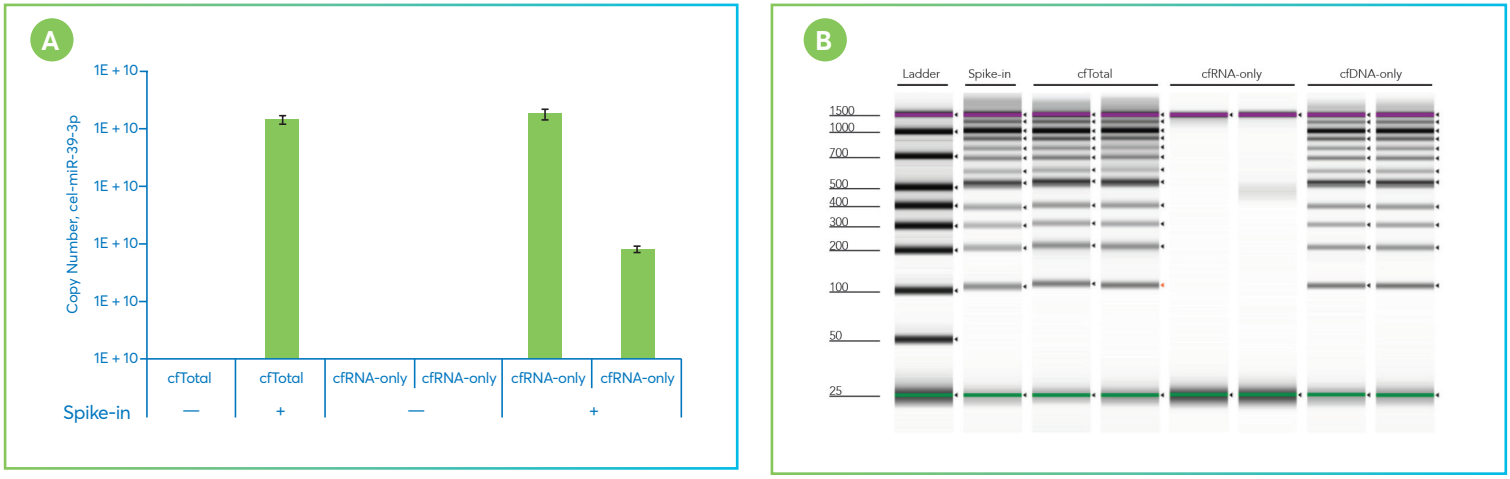

FIGURE 2: Efficient separation and recovery of spike-in microRNA and DNA by the *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit. Total cell-free nucleic acids were purified from plasma (identical donor, 39y-F) using co-elution and parallel protocols from *Quick-cf*DNA/cfRNA™ Serum & Plasma Kit. **(A)** Separation and recovery of spiked-in microRNA, cel-miR-39-3p, were quantitatively assessed using RT-qPCR. Values indicate the total average copy number +/- sample standard deviations from two independent extractions. **(B)** Separation of spike-in DNA (100 bp DNA ladder) was qualitatively assessed using capillary gel electrophoresis (TapeStation). cfTotal = eluate from 'co-purification' protocol which elutes cfDNA and cfRNA into same eluate. cfDNA-only, cfRNA-only = eluates from 'parallel purification' protocol which separates cfDNA and cfRNA into separate eluates. Spike-in = 100bp DNA ladder spike-in.

To further test the observed increases in small RNA yield, we measured the abundance level of a specific microRNA, hsa-miR-16-5p, which is present in blood plasma. After cfRNA extraction by the three kits, the total copy number of hsa-miR-16-5p was measured by RT-qPCR (Figure 1B). The copy number increased linearly with input volumes using *Quick-cf*DNA/*cf*RNA™ Kit, which was not observed when using the miR Adv kit — hsa-miR-16-5p levels surprisingly decreased when the input volume was increased 3-fold to the manufacturer's recommendations. Similarly, both low hsa-miR-16-5p copy number and an absence of linear recovery were observed for the CNA kit. This may have been caused by the inability of the CNA kit to purify very small RNAs like microRNAs, which resulted in unreliable/low RT-qPCR data. Of note, we observed that Zymo Research samples quantified ~10.7-fold more hsa-miR-16-5p than miR Adv kit at similar input volumes (0.5 mL vs. 0.6 mL). Additionally, cfRNA isolated using *Quick-cf*DNA/*cf*RNA™ Kit recovered ~515-fold more hsa-miR-16-5p than the CNA kit at the 3mL input volume.

Separation of cfDNA and cfRNA from a single sample

Current methodologies in library preparation for Next-Generation Sequencing require the separate isolation of cfDNA and cfRNA. Having the option to co-purify, or to purify cfDNA and cfRNA from the same sample in parallel, provides investigators the ability to control cfNA input into downstream applications. The *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit is optimized for separation of cfDNA and cfRNA from the same sample input. The 'parallel' protocol separates cfDNA and cfRNA into separate eluates, while the 'co-purification' protocol collects both cfDNA and cfRNA into the same eluate. To assess separation efficiency of cfRNA from cfDNA using the 'parallel' protocol, an exogenous microRNA spike-in and a DNA ladder were added to a plasma sample then subjected to the purification protocols. Quantitation of copy number was then assessed using RT-qPCR for the exogenous microRNA and capillary gel electrophoresis for the DNA ladder.

We first sought to test the capability of *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit to efficiently isolate cfRNA into cfRNA-only eluates without spilling into cfDNA-only eluates. A spike-in of 2.5×10^8 copies of an exogenous microRNA not found in mammals (cel- miR-39-3p from *C. elegans*) was added to plasma samples, mixed with digestion buffer, purified, and then recovery was assessed by RT-qPCR. This spike-in technique is typically used to normalize sample inputs and assess processing errors[17,18] Eluates from both the 'co-elution' and the 'parallel' protocols were quantified for cel- miR-39-3p copy number by RT-qPCR (Figure 2A). The input cel- miR-39-3p was efficiently recovered using both protocols. Eluates from both protocols showed greater than 80% recovery of cel-miR- 39-3p. Moreover, less than 0.003% of the microRNA was retained in the cfDNA-only eluate from 'parallel' protocol. All negative controls lacking the added microRNA had no detectable levels of the exogenous microRNA.

To assess the capability of *Quick-cf*DNA/*cf*RNA™ to efficiently isolate cfDNA without contaminating cfRNA-only eluates, a DNA ladder was spiked into a plasma sample and tracked using a capillary gel electrophoresis system (Figure 2B). Approximately 50ng of 100bp DNA ladder was added to samples during the initial digestion step. Eluates from all samples were analyzed using DNA High-Sensitivity Tape Assay on

Kit Name	Donor Detail	Total Reads	Reads Passing Filter	% Reads Passing Filter	Reads Align to hg38	% Reads Align to h38	Reads Align to miRNA Base	% Reads Align to miRBase	microRNA Species (≥5 Reads)
Zymo Research	39F	7,916,894	7,058,146	89.15	4,730,494	59.75	4,727,725	59.72	470
	61F	6,076,424	5,224,768	85.98	2,965,305	48.80	2,903,820	47.79	377
	50M	7,507802	7,224,051	96.22	5,765,842	76.80	5,719,721	76.18	367
ABI MagMax	39F	6,200,169	2,366,814	38.17	234,735	3.79	599	0.01	10
	61F	6,277,729	2,585,422	41.51	356,982	5.73	840	0.01	16
	50M	5,764,620	3,365,366	58.38	296,453	5.14	698	0.01	11
Qiagen CNA	39F	6,803,973	6,039,433	88.76	265,060	3.90	875	0.01	13
	61F	10,000,174	8,255,089	82.55	403,953	4.04	1,295	0.01	16
	50M	7,425,887	6,491,404	87.42	347,609	4.68	2,969	0.04	20
Qiagen miR Adv	39F	6,332,879	4,020,305	63.48	2,109,098	33.30	1,881,828	29.72	400
	61F	7,348,572	2,998,441	40.80	937,726	12.76	769,498	10.47	287
	50M	7,574,555	5,316,767	70.19	3,358,267	44.34	3,015,690	39.81	344

TABLE 1: RNA sequencing result comparison of RNA isolated from four commercial kits. Total cell-free RNA from a set of three different donors were obtained using four cell-free RNA isolation products: Quick-cfDNA/cfRNA™ Serum & Plasma (Zymo Research), MagMAX Cell-Free Total Nucleic Acids (ABI MagMax; Applied Biosystems), QIAamp Circulating Nucleic Acids (Qiagen CNA), and miRNeasy Serum/Plasma Advanced (Qiagen miR Adv). RealSeq-Biofluids Library Prep Kit (SomaGenics) was used to generate RNA sequencing library and analyzed on the HiSeq 1500 v4 System (Illumina). Number of reads and read quality assessments are summarized.

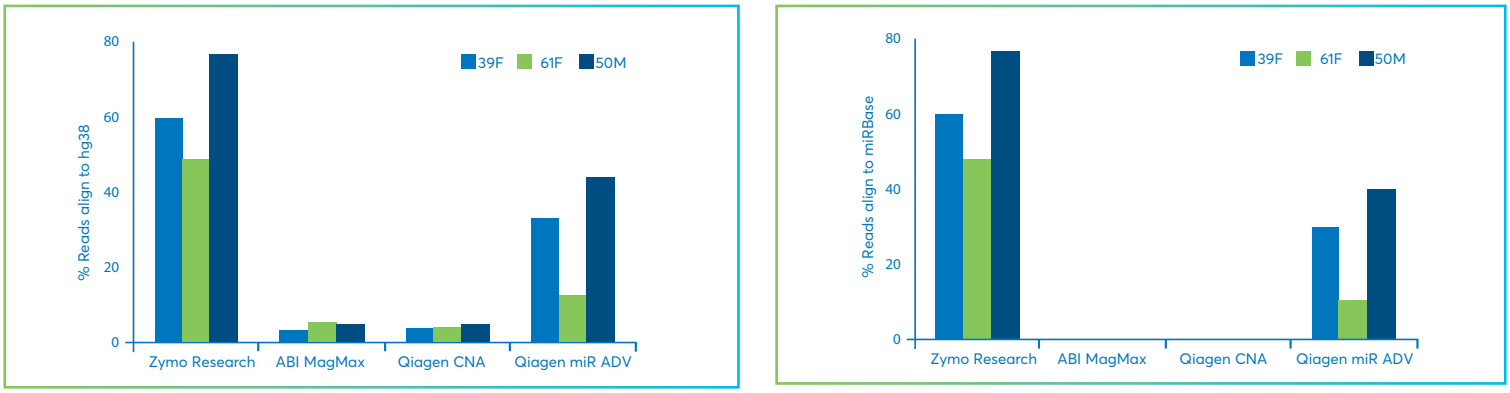

FIGURE 3: Read alignment comparison of RNA isolated from four commercial kits. Total cell-free RNA from a set of three different donors were obtained using four cell-free RNA isolation products: Quick-cfDNA/cfRNA™ Serum & Plasma Kit (Zymo Research), MagMAX Cell-Free Total Nucleic Acids (ABI MagMax; Applied Biosystems), QIAamp Circulating Nucleic Acids (Qiagen CNA), and miRNeasy Serum/Plasma Advanced (Qiagen miR Adv). RealSeq-Biofluids Library Prep Kit (SomaGenics) was used to generate RNA sequencing library and ran on HiSeq 1500 v4 System (Illumina). Values (from Table 1) indicate the percentage of reads aligning to the human genome (hg38, left) and microRNA (miRBase, right).

TapeStation 2200 (Agilent). Qualitative assessment of all DNA bands showed robust recovery of added DNA in eluates from the 'co-elution' protocol and the 'cfDNA-only' eluates from the 'parallel' protocol. The cfRNA-only eluates from the 'parallel' protocol showed no detectable level of the added DNA ladder. This result confirms efficient isolation of the cfDNA into the intended cfDNA-only eluates without contaminating the cfRNA-only eluates. Efficient separation of the smallest DNA band at 100bp size is noteworthy, since the majority of the cfDNA are in the size range of 100–240bp, with the median length of 167bp.[6] This allows for excluding post-fractionation DNase treatments on cfRNA eluates and at the same time have the option to separately and efficiently isolate cfDNA.

Capacity to produce robust cell-free RNA sequencing runs

RNA Sequencing (RNA-Seq) has become one of the most important downstream applications for RNA-based investigations. The ability to profile RNA expression as well as detecting novel targets supports RNA-Seq as a crucial research tool. With cell-free RNA as input, producing robust RNA-Seq results become difficult due to multiple challenges. First, cell-free biological fluids have very low amounts of circulating cfDNA and cfRNA, making it difficult to obtain enough material above the minimum input requirements necessary for many library preparation kits. Secondly, due to their short length, microRNAs require highly optimized isolation conditions to prevent losses during purification.

MicroRNAs, which range from 20–25 nucleotides in length, have high potential to serve as disease biomarkers from biofluids.[1,13-18] Therefore, reliable and robust isolation of microRNA from cell-free samples is of great interest. To evaluate cfRNA profiles, both Zymo Research and SomaGenics independently extracted cfRNA, generated libraries, and performed RNA-Seq data. Zymo

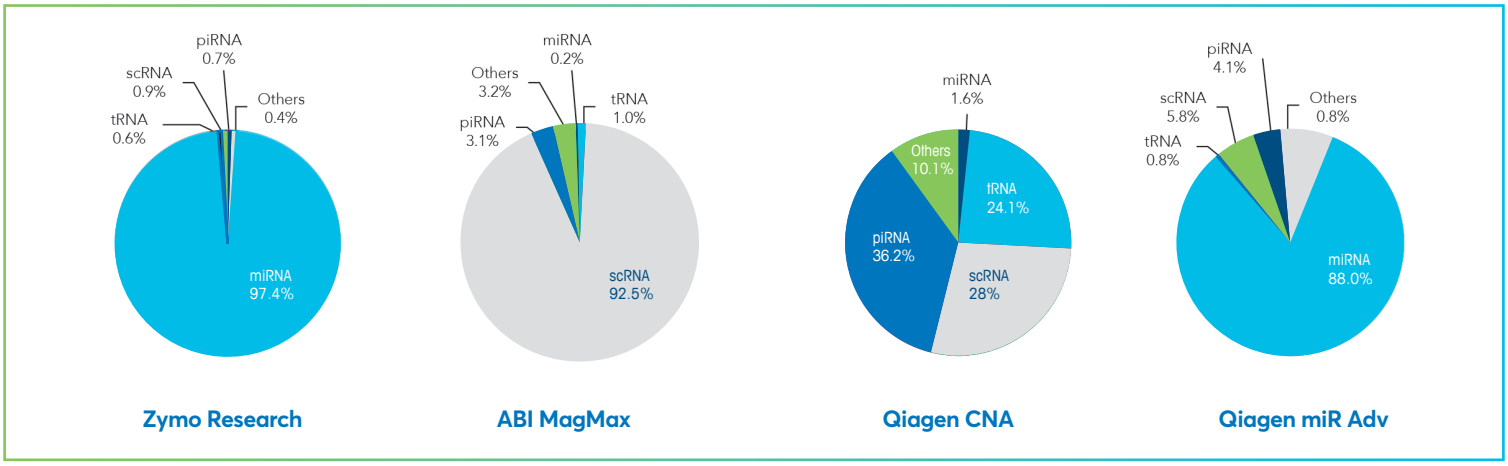

FIGURE 4: Proportions of microRNA in total cell-free RNA. Total cell-free RNAs from 50 year old male donor was obtained using four different cfRNA isolation kits: Quick-cfDNA/cfRNA™ Serum & Plasma Kit (Zymo Research), MagMAX Cell-Free Total Nucleic Acids (ABI MagMax; Applied Biosystems), QIAamp Circulating Nucleic Acids (Qiagen CNA), and miRNeasy Serum/Plasma Advanced (Qiagen miR Adv). RNA types and proportion values are represented.

Kit Name	Donor Detail	Total Reads	% Reads Passing Filter	% Reads Align to hg19	% Reads Align to miRBase	miRNAs Detected	≥ 5 Reads	≥ 10 Reads
Zymo Research *Quick-cf*RNA Serum & Plasma Kit	Donor 1	1,195,837	67.2	86.5	44.2	601	307	236
	Donor 2	1,252,927	83.7	91.6	37.3	775	420	330
	Donor 3	562,876	71.7	84.4	48.7	399	182	138
	Donor 4	1,154,655	83.9	92.8	44.2	717	382	310
	Average	1,041,574	76.6	88.8	43.6	623	323	254
Qiagen miRNeasy Serum/Plasma	Donor 1	1,092,135	70.5	67.3	0.9	232	86	49
	Donor 2	951,591	64.1	67.5	0.3	144	51	25
	Donor 3	775,414	60.7	61.7	0.3	159	45	30
	Donor 4	801,471	38.6	61.5	0.3	104	23	13
	Average	905,153	58.5	63.9	0.4	160	51	29

TABLE 2: RNA sequencing result comparison of RNA isolated from two commercial kits. Total cell-free RNA from a set of four different donors were obtained using two cell-free RNA isolation products: *Quick-cf*RNA™ Serum & Plasma Kit (Zymo Research) and miRNeasy Serum/Plasma (Qiagen). RealSeq-Biofluids Library Prep Kit (SomaGenics) was used to generate RNA sequencing library and analyzed on the MiniSeq System (Illumina). Number of reads and read quality assessments are summarized.

Research extracted cfRNA from 200µL plasma derived from three different healthy donors using four different commercially available kits. SomaGenics extracted cfRNA from 200µL plasma derived from four different healthy donors using two commercially available kits. Both prepared RNA libraries using the SomaGenics RealSeq- Biofluids Library Prep Kit. Library preparations were sequenced on the Illumina HiSeq 1500 v4 System (for Zymo Research) and Illumina MiniSeq System (for SomaGenics).

Small RNA sequencing data generated by Zymo Research, as shown in Table 1, indicates higher quality reads for *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit compared to other isolation kits. The *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit achieved an average of 4% to 44% higher usable reads than other cfRNA isolation technologies. Samples isolated by *Quick-cf*DNA/cfRNA™ Serum & Plasma Kit also achieved significantly higher aligned reads to human genome (hg38) and annotated microRNA (miRBase) databases. Zymo Research averaged 32% to 57% higher hg38 alignment and 35% to 61% higher miRBase alignment (Figure 3). Moreover, the identifications of different types of small RNAs demonstrate that samples isolated using *Quick-cf*DNA/cfRNA™ Serum & Plasma Kit contain higher proportions of microRNA than other commercial kits (Figure 4). This indicates efficient isolation all cell-free RNAs, including microRNAs, by cell-free isolation technology from Zymo Research. Observation of total number of microRNA species (defined as individual microRNA sequences with 5 or more reads) showed Zymo Research identifying a range of 61 to 393 more microRNA species compared to other isolation kits.

Independently generated small RNA isolations, library preparation and sequencing results by SomaGenics shown in Table 2 agree with data generated by Zymo Research. Higher average quality

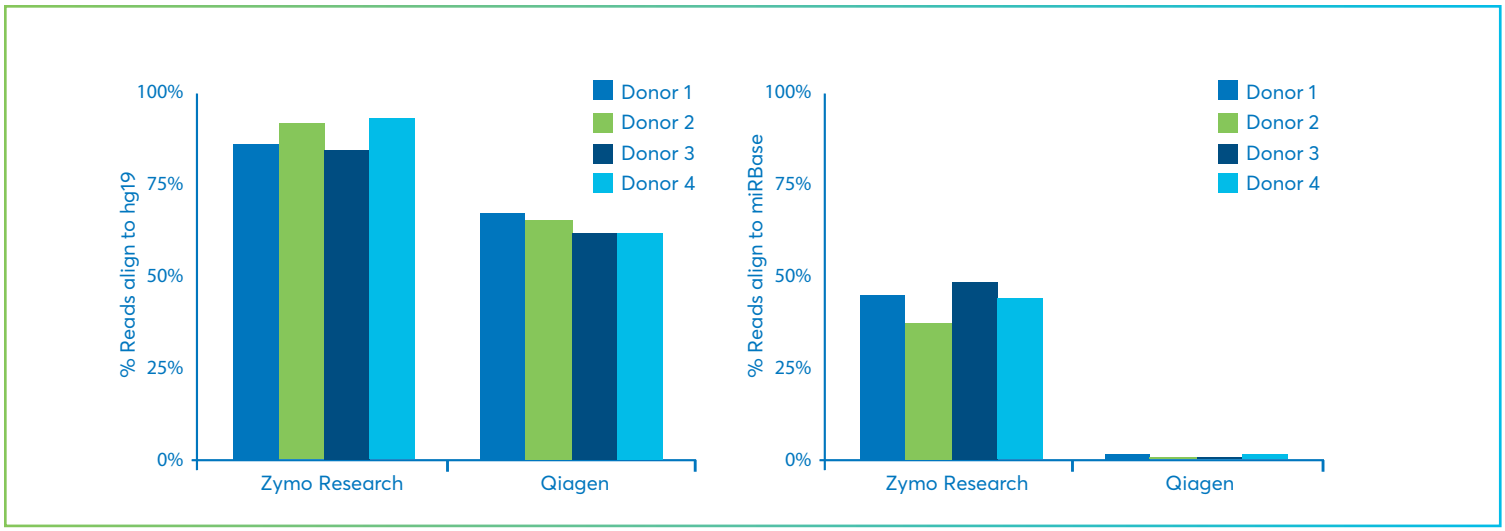

FIGURE 5: Read alignment rate comparison of RNA isolated from two commercial kits. Total cell-free RNA from a set of four different donors were obtained using two cell-free RNA isolation products: *Quick-cf*RNA™ Serum & Plasma Kit (Zymo Research) and miRNeasy Serum/Plasma (Qiagen). RealSeq-Biofluids Library Prep Kit (SomaGenics) was used to generate RNA sequencing library and ran on MiniSeq System (Illumina). Values (from Table 2) indicate the percentage of reads aligning to the human genome (hg19, left) and microRNA (miRBase, right).

reads were observed for Zymo Research (76.6%) compared to Qiagen (58.5%). Additionally, RNAs isolated using the *Quick-cf*RNA™ Serum & Plasma Kit achieved higher average percent reads (88.8%) aligning to human reference genome (hg19) compared to Qiagen (63.9%), as well as to annotated microRNAs in miRBase (43.6% and 0.4%, respectively) (Table 2, Figure 5). This confirms efficient isolation of microRNA species by cell-free RNA isolation technology from Zymo Research.

As represented in Figure 6, Zymo Research obtained higher proportions of microRNA from total cfRNA (86.0%) compared to Qiagen (13.9%). Congruent to the small RNA-seq data, the *Quick-cf*RNA™ Serum & Plasma Kit samples achieved more diverse species of microRNA (with Zymo Research identifying 623 and Qiagen identifying 160 microRNA species that resulted in 5 or more reads). This observation is consistent with observation of the microRNA isolated using *Quick-cf*DNA/cfRNA™ Serum & Plasma Kit. Both independent extractions and small RNA-sequencing results from Zymo Research and SomaGenics indicate overall significantly better results obtained by using cfRNA isolation technologies from Zymo Research.

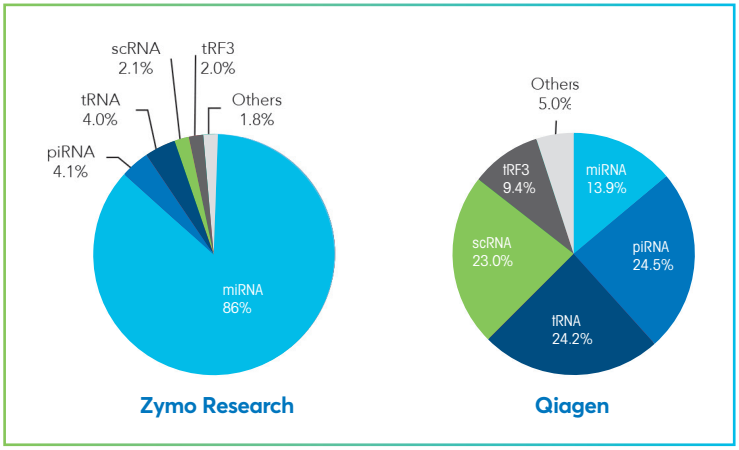

FIGURE 6: Proportions of microRNA in total cell-free RNA. Total cell-free RNAs from four donors were obtained using two different cfRNA isolation kits: Quick- cfRNA™ Serum & Plasma Kit (Zymo Research) and miRNeasy Serum/Plasma Kit (Qiagen). RNA types and proportion values are represented.

DISCUSSION

As the liquid biopsy field grows and adopts new technologies, collection and analysis of the small amounts cell-free nucleic acids in biological fluids becomes increasingly important. Currently available isolation technologies, especially for cfRNA, lack efficient recovery. This becomes an even more significant problem when surveying for rare cell-free disease biomarkers. Inability to recover these rare species during extraction will result in missed target identification and potential misdiagnosis. Additionally, lack of biomarker detection at early stages of disease progression may be partly due to inefficient extraction technologies. The analyses shown here indicate that the *Quick-cf*DNA/cfRNA™ Serum & Plasma Kit from Zymo Research had significant yield and quality improvements over the current most widely used product, the Qiagen QIAamp Circulating

Nucleic Acid Kit (Figure 1). Moreover, *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit also outperformed the Qiagen miRNeasy Serum/Plasma Advanced Kit (Figure 1). In both cases, the *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit showed high yield recovery of cfRNA as well as abundant and diverse microRNA species. Additionally, it includes the flexibility to separate cfDNA and cfRNA into separate fractions taken from the same input sample (Figure 2). This provides an advantage over Qiagen's Circulating Nucleic Acid Kit, which can process cfDNA or cfRNA from a single sample input but lacks the versatility to co-purify or fractionate the cfNA.

The ability of nucleic acid purification technologies to produce pure and consistent DNA/RNA isolations is essential to sensitive applications such as Next-Generation Sequencing. When working with biological fluids, highly effective extraction methods are required to collect total cell-free nucleic acids. Moreover, efficient isolation allows for reduced sample input needs, which is important when processing clinical samples with limited volume. As shown in Tables 1 and 2, cell-free RNA isolation technology from Zymo Research enables robust RNA sequencing results from plasma inputs as low as 200µL of starting material.

The importance of isolation technology is emphasized by comparison of extraction kits, where significant differences between isolation technologies lead to differences in yield and downstream read qualities and read alignments to established databases. Additionally, higher microRNA diversity (61 to 393 more microRNAs identified) was observed when combining the *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit with the RealSeq-Biofluids Library Prep Kit, as compared to other extraction kits. This emphasizes the importance of high cfRNA recovery rate and sensitive library preparation methods to maximize the opportunity to identify relevant rare biomarkers. Small RNA sequencing data presented in this article not only demonstrates superior results achieved by *Quick-cf*DNA/*cf*RNA™ Serum & Plasma Kit but also denotes the classic importance of using the right isolation technology to yield meaningful data. With a recent increase in research and discovery efforts to characterize cfDNA and cfRNA biomarkers in biofluids, the innovative cell-free DNA/RNA purification technologies from Zymo Research will be crucial tools to obtain consistent and reliable results.

REFERENCES

For references, please visit **vwr.com/vwrbiomarke.**

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Description	Cat. No.
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*Quick-cf*RNA™ Serum and Plasma Kit	76020-396
*Quick-cf*DNA/cfRNA™ Serum and Plasma Kit	76334-490

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Enzymatic methyl-seq:

The next generation of methylome analysis

By Louise Williams, Ph.D., Yanxia Bei, Ph.D., Heidi E. Church, Nan Dai, Ph.D., Eileen T. Dimalanta, Ph.D., Laurence M. Ettwiller, Ph.D., Thomas C. Evans, Jr., Ph.D., Bradley W. Langhorst, Ph.D., Janine G. Borgaro, Ph.D., Shengxi Guan, Ph.D., Katherine Marks, Julie F. Menin, Nicole M. Nichols, Ph.D., V. K. Chaithanya Ponnaluri, Ph.D., Lana Saleh, Ph.D., Mala Samaranayake, Ph.D., Brittany S. Sexton, Ph.D, Zhiyi Sun, Ph.D., Esta Tamanaha, Ph.D., Romualdas Vaisvila, Ph.D., Erbay Yigit, Ph.D. and Theodore B. Davis, New England Biolabs, Inc.

The identification of cytosine modifications within genomes, especially 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), is important as they are known to have an impact on gene expression. Generally, low levels of methylation near transcription start sites are associated with higher transcription levels, while genes with regulatory regions containing high levels of cytosine modification are expressed at lower levels. The ability to analyze a complete methylome is important for studying diseases, including those associated with cancer, metabolic disorders and autoimmune diseases. Unfortunately, the current technologies for investigating 5mC and 5hmC are suboptimal and do not permit a thorough evaluation of methylomes.

BISULFITE SEQUENCING

To date, the gold standard in methylome mapping has been bisulfite sequencing. In this method, DNA is chemically treated with sodium bisulfite, which results in the conversion of unmethylated cytosines to uracils, and the resulting uracils are ultimately sequenced as thymines (Figure 1). In contrast, the modified cytosines, 5mC and 5hmC, are resistant to bisulfite conversion, and are sequenced as cytosines[1]. While the preparation of bisulfite libraries is relatively straightforward, the libraries have uneven genome coverage and therefore suffer from incomplete representation of cytosine methylation across genomes. This uneven coverage is the result of DNA damage and fragmentation, which is caused by the extreme temperatures and pH during bisulfite conversion. Sequenced bisulfite libraries typically have skewed GC bias plots, with a general under representation of G- and C-containing dinucleotides and over representation of AA-, AT- and TA-containing dinucleotides, as compared to a non-converted genome[2]. Therefore, the damaged libraries do

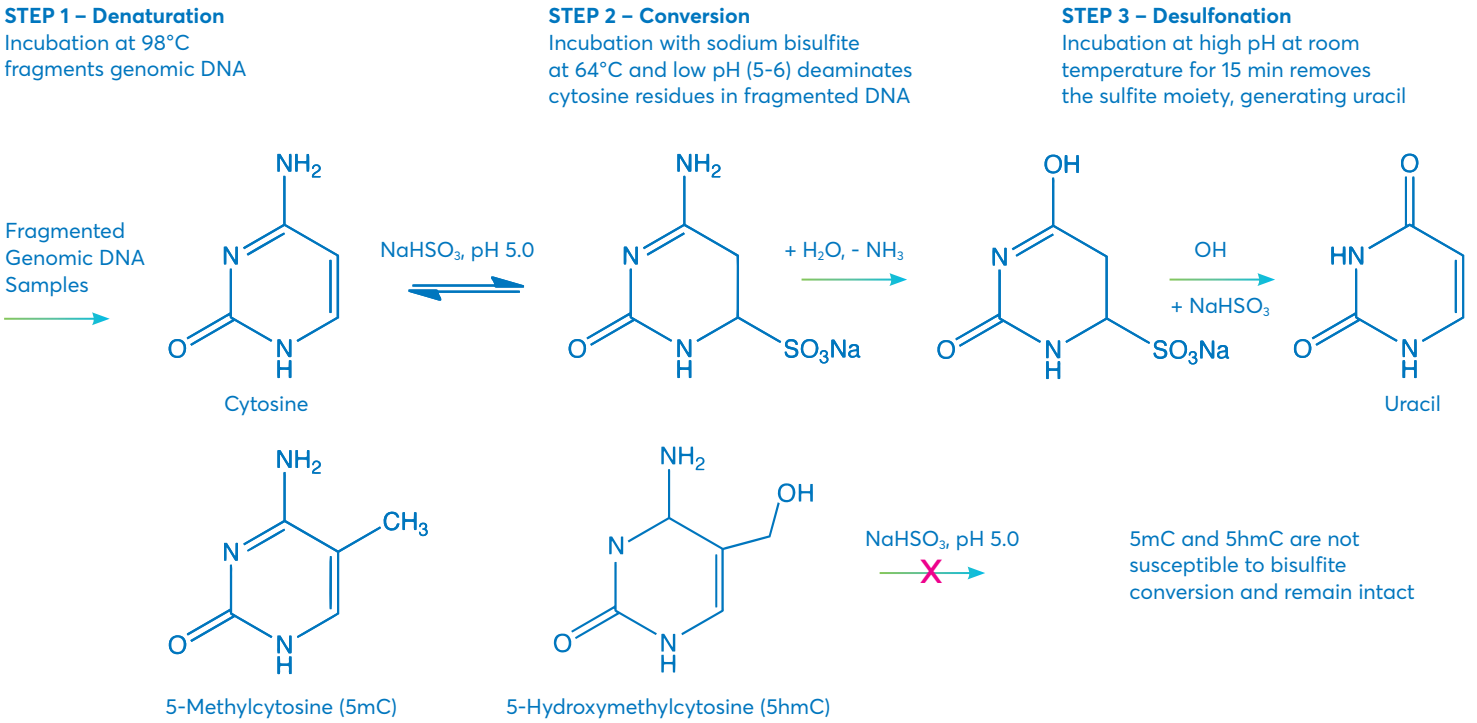


FIGURE 1: Bisulfite conversion overview. Sodium bisulfite treatment of DNA converts cytosine to 5,6-dihydrocytosine-6-sulfonate, which is converted to 5,6-dihydrouracil-6-sulfonate, and then desulfonated to uracil. In contrast 5mC and 5hmC are not susceptible to bisulfite treatment and remain intact.

Sequencing Method	Cytosine Modification	Method of Analysis	Weakness
TET-assisted bisulfite sequencing (TAB-seq)[3]	5hmC	Enzymatic treatment with T4-BGT then TET followed by bisulfite treatment	DNA damage and sequencing bias
Oxidative bisulfite sequencing (oxBS)[4]	5mC	Treatment with an oxidation reagent, followed by bisulfite treatment	DNA damage and sequencing bias
APOBEC-coupled epigenetic sequencing (ACE-seq)[5]	5hmC	Enzymatic treatment with T4-BGT and APOBEC3A	APOBEC3A not commercially available
TET-assisted 5-methylcytosine sequencing (TAmC-seq)[6]	5mC	Enzymatic treatment, followed by enrichment for 5mC regions	Enriches for 5mc-dense regions; does not currently cover entire genome

TABLE 1: Summary of alternative methods of methylome analysis

not adequately cover the genome, and can include many gaps with little or no coverage. Increasing the sequencing depth of these libraries can recover some missing information, but at steep sequencing costs. These bisulfite library limitations have driven the development of new approaches for studying methylomes.

ALTERNATIVE METHODS FOR DETECTING 5mC AND 5hmC

Additional approaches for investigating methylomes are available that either combine bisulfite conversion with another chemical modification or an enzymatic modification step, or that eliminate bisulfite conversion completely (Table 1).

5hmC can be detected using Ten-Eleven Translocation (TET)-assisted bisulfite sequencing (TAB-seq). Fragmented DNA is enzymatically modified using sequential T4 Phage ß-glucosyltransferase (T4-BGT) and then TET-dioxygenase treatments before the addition of sodium bisulfite[3]. T4-BGT glucosylates 5hmC to form beta-glucosyl-5-hydroxymethylcytosine

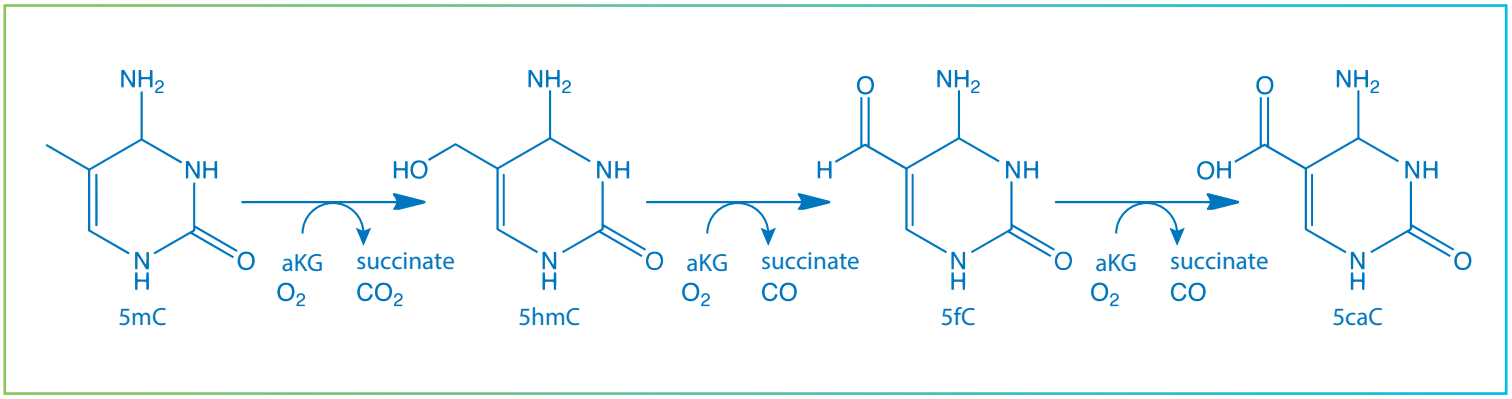


FIGURE 2: Enzymatic modification of cytosine. TET enzymes oxidize 5mC to 5hmC then 5fC and finally 5caC.

(5ghmC) and TET is then used to oxidize 5mC to 5caC (Figure 2). Only 5ghmC is protected from subsequent deamination by sodium bisulfite and this enables 5hmC to be distinguished from 5mC by sequencing.

Oxidative bisulfite sequencing (oxBS) provides another method to distinguish between 5mC and 5hmC[4]. The oxidation reagent potassium perruthenate converts 5hmC to 5-formylC (5fC) and subsequent sodium bisulfite treatment deaminates 5fC to uracil. 5mC remains unchanged and can therefore be identified using this method.

APOBEC-coupled epigenetic sequencing (ACE-seq) excludes bisulfite conversion altogether and relies on enzymatic conversion to detect 5hmC[5]. With this method, T4-BGT glucosylates 5hmC to 5ghmC and protects it from deamination by Apolipoprotein B mRNA editing enzyme subunit 3A (APOBEC3A). Cytosine and 5mC are deaminated by APOBEC3A and sequenced as thymine.

Lastly, TET-assisted 5-methylcytosine sequencing (TAmC-seq) enrichs for 5mC loci and utilizes two sequential enzymatic reactions followed by an affinity pull-down[6]. Fragmented DNA is treated with T4-BGT which protects 5hmC by glucosylation. The enzyme mTET1 is then used to oxidize 5mC to 5hmC, and T4-BGT labels the newly formed 5hmCusing a modified glucose moiety (6-N3-glucose). Click chemistry is used to introduce a biotin tag which enables enrichment of 5mC-containing DNA fragments for detection and genome wide profiling.

Libraries made from methods that combine enzymatic and sodium bisulfite identification of cytosine modifications all experience DNA damage and the inherent biases of bisulfite treatment. Furthermore, the described enzymatic methods have additional drawbacks. TAmC-seq is focused on loci and does not discriminate between methylated and unmethylated cytosines in the enriched DNA fragments. ACE-seq probes only 5hmC and requires APOBEC3A for deamination, which is not yet commercially available, making it more difficult to standardize library construction between labs.

ENZYMATIC METHYL-SEQ – A NEW APPROACH

The enzymatic methyl-seq workflow developed at NEB® provides a much-needed alternative to bisulfite sequencing. This method relies on the ability of APOBEC to deaminate cytosines to uracils. Unfortunately, APOBEC also deaminates 5mC and 5hmC, making it impossible to differentiate between cytosine and its modified forms[7,8]. In order to detect 5mC and 5hmC, this method also utilizes TET2 and an Oxidation Enhancer, which enzymatically modifies 5mC and 5hmC to forms that are not substrates for APOBEC. The TET2 enzyme converts 5mC to 5caC (Figure 2) and the Oxidation Enhancer converts 5hmC to 5ghmC[9-11]. Ultimately, cytosines are sequenced as thymines and 5mC and 5hmC are sequenced as cytosines, thereby protecting the integrity of the original 5mC and 5hmC sequence information.

The NEBNext® Enzymatic Methyl-seq Kit (EM-seq™) combines NEBNext Ultra™ II DNA reagents with these two enzymatic steps to construct Illumina® libraries that accurately represent 5mC and 5hmC within the genome. Converted libraries are amplified using NEBNext Q5U™ DNA polymerase (Figure 3). EM-seq libraries result in a more accurate representation of the methylome, with minimal DNA fragmentation or biases when compared to whole genome bisulfite sequencing (WGBS). The combination of the Ultra II reagents for library prep and the EM-seq conversion allows for lower input amounts compared to most WGBS workflows, with a range of inputs from 10 – 200ng.

EM-SEQ PERFORMANCE

Intact DNA

Several pieces of data suggest that the process of generating EM-seq libraries does not damage DNA in the same way as bisulfite sequencing. EM-seq libraries give higher PCR yields despite using fewer PCR cycles for all DNA input amounts, indicating that less DNA is lost during enzymatic treatment and library preparation, as compared to WGBS. Reduced PCR cycles, in turn, translates into more complex libraries and fewer PCR duplicates during sequencing (data not shown). EM-seq libraries also have larger insert sizes than WGBS (Figure 4, next page), which further supports the fact that DNA remains intact.

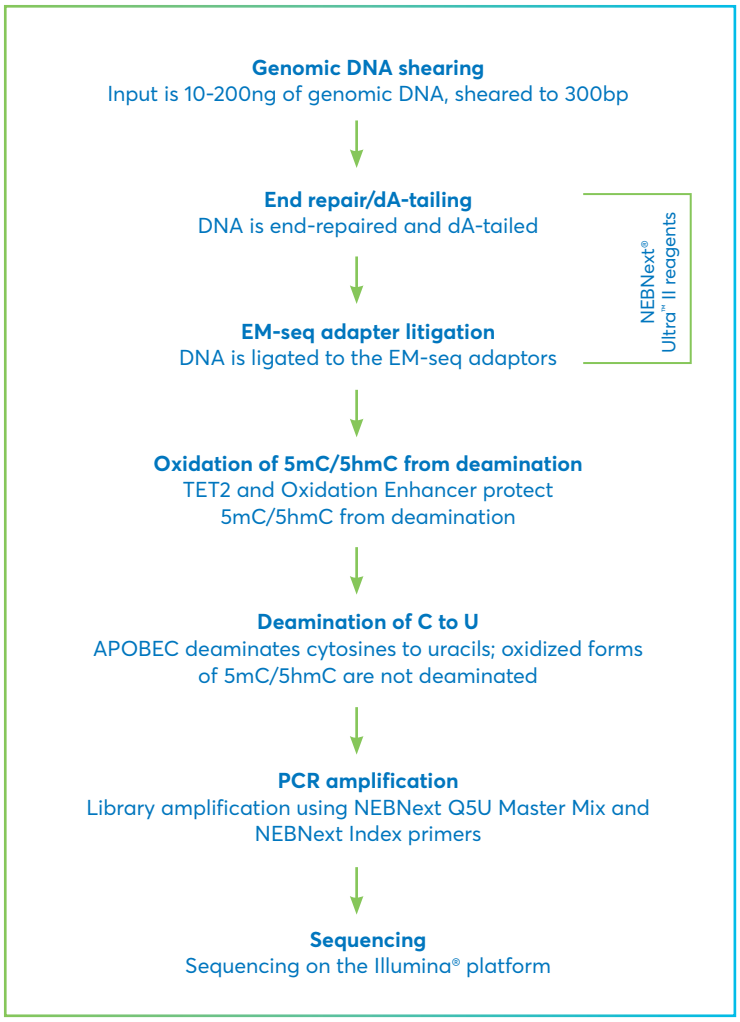


FIGURE 3: **NEBNext EM-seq Kit Workflow.** EM-seq utilizes two enzymatic steps to differentiate between modified and unmodified cytosines.

EM-seq libraries have reduced bias

The preservation of DNA integrity is also demonstrated by the GC-bias graphs (Figure 5, next page) and the dinucleotide coverage distribution graph (Figure 6, next page). Both of these figures indicate that reduced bias is associated with the EM-seq libraries. The EM-seq libraries have a flat GC-bias distribution (Figure 5, next page) with even coverage at both GC- and AT-rich regions, and do not display a preference for any dinucleotide combination (Figure 6, next page). This is in stark contrast to WGBS, which shows a skewed GC-bias profile along with the previously mentioned dinucleotide biases. Reduced library bias improves the mapping and therefore coverage of CpGs.

CpG detection

Human DNA is methylated almost exclusively in CpG contexts. EM-seq global CpG methylation levels for human NA12878 DNA are consistent with WGBS libraries (Figure 7A, next page), indicating that EM-seq libraries accurately detect methylation. The more striking difference between EM-seq and WGBS libraries becomes apparent when the focus is shifted to CpG coverage. EM-seq libraries detect more CpGs to a higher depth of coverage than WGBS libraries (Figure 7B, next page). The ability to detect more CpGs at a greater depth also increases confidence in the data and leads to more accurately defining methylation within a region of interest. This, in turn, aids in detecting methylation changes in diseased states such as cancer. In addition, increased CpG coverage has an economic impact – with more CpGs detected using the same number of reads compared to WGBS, EM-seq represents a significant cost-savings.

Potential applications

In addition to making Illumina libraries, there are other potential applications for the EM-seq technology. Many of these applications already exist, but can now be improved upon because of the intact nature of enzymatically-converted DNA and the accuracy of CpG detection. Lower input DNA is also a driving factor for some of these applications. Converted DNA can be detected on arrays, and can be used for target enrichment, reduced representation-type libraries or amplicon detection. Different types of DNA inputs, such as low-input, cell-free DNA (cfDNA) or damaged FFPE DNA, can also be used.

CONCLUSION

Bisulfite sequencing, while commonly used, is suboptimal in detecting 5mC and 5hmC – large amounts of DNA are needed, DNA can be damaged, and sequences are biased towards AT-rich regions. Other methods that couple chemical or enzymatic treatment with bisulfite sequencing also share similar

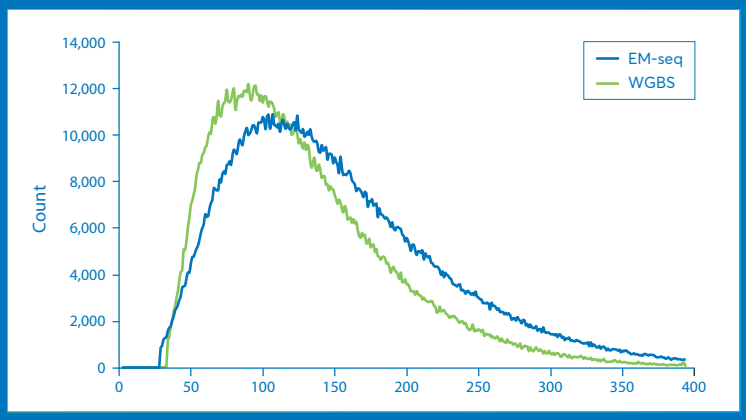


FIGURE 4: NEBNext Enzymatic Methyl-seq (EM-seq) libraries have larger inserts. EM-seq library insert sizes are larger than whole genome bisulfite sequencing (WGBS) libraries. Library insert sizes were determined using Picard 2.18.14. The larger insert size indicates that EM-seq does not damage DNA as bisulfite treatment does.

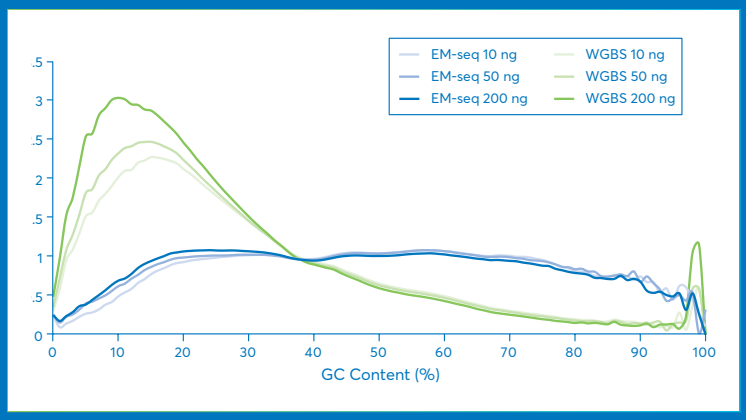


FIGURE 5: EM-seq has superior uniformity of GC coverage. GC coverage was analyzed using Picard 2.18.14 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.

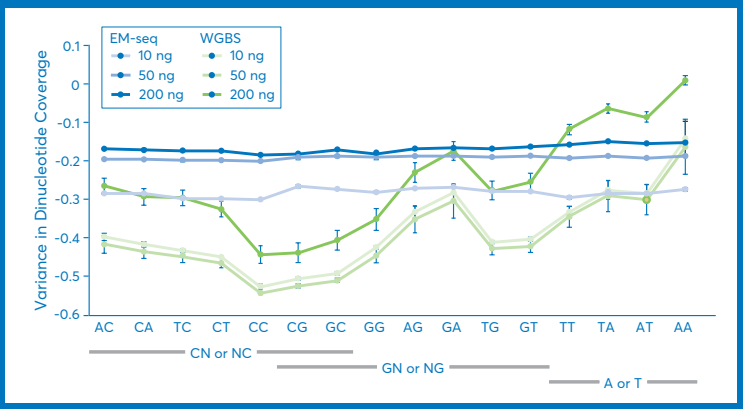
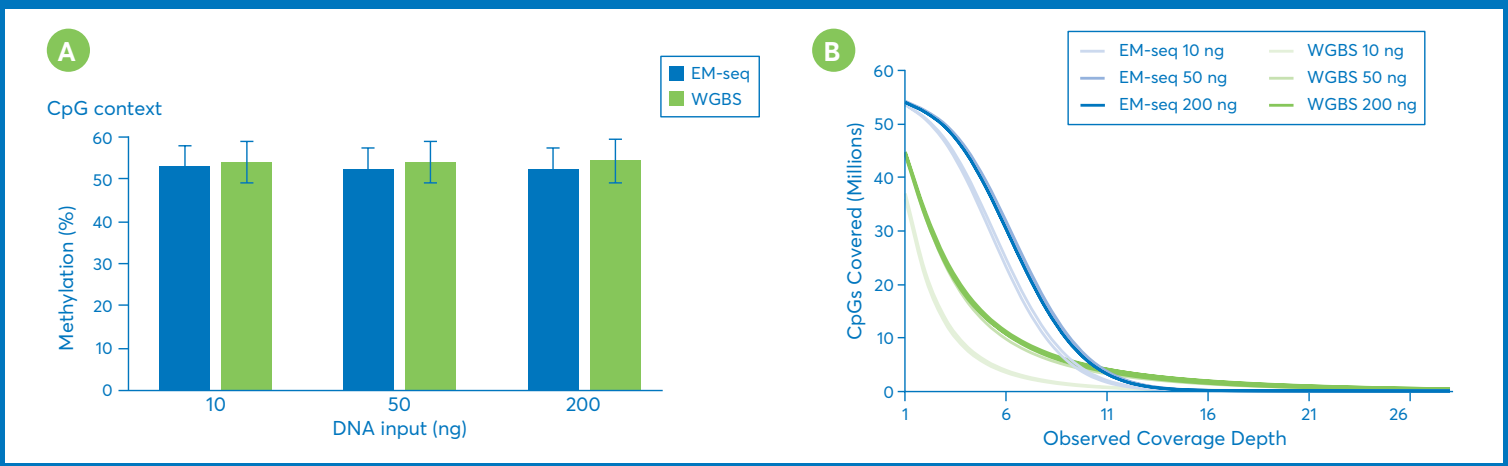


FIGURE 6: Dinucleotide coverage distribution. Dinucleotide coverage distribution for EM-seq and WGBS libraries showing the variance in coverage for dinucleotides in the reads when compared to unconverted Ultra II library dinucleotide distribution. EM-seq libraries show even coverage across all dinucleotide combinations compared to WGBS. C-containing dinucleotides are underrepresented in WGBS libraries and A/T containing dinucleotides are overrepresented.

FIGURE 7: EM-seq identifies detect more CpGs to a higher depth of coverage than WGBS. 10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). 324 million paired end reads were aligned to hg38 using bwa-meth 0.2.2.
A: Methyl Dackel was used to determine methylation levels, which were found to be similar between EM-seq and WGBS.
B: Coverage of CpGs with EM-seq and WGBS libraries was analyzed, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.



limitations. EM-seq provides the first commercially available, nonbisulfite method that comprehensively addresses the limitations of bisulfite sequencing and represents a new opportunity for more complete methylome analysis. EM-seq libraries are not damaged and have longer inserts, higher PCR yields with fewer PCR cycles, and lack biases associated with GC content. More CpGs are identified with greater coverage depth using EM-seq, as compared to WGBS. These advantages all contribute to EM-seq having more usable sequencing data when comparing the same number of reads for EM-seq and WGBS, which ultimately reduces sequencing costs. EM-seq is the only commercially available alternative to bisulfite sequencing that provides an effective method for accurate and comprehensive detection of 5mC and 5hmC across the genome, and offers a new, more accurate alternative for studying disease states.

REFERENCES

For references, please visit **vwr.com/vwrbiomarke.**

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High efficiency *E. coli* strains for phage display combinatorial peptide libraries

By Lynne Sheets, Chris Niebauer, and Kristen Terry

Phage display technology places foreign peptides and proteins on the surface of filamentous bacteriophages. This methodology is used to identify peptide ligands to a wide variety of targets by screening for the ability to bind with high affinity and specificity. It has become a cornerstone method to investigate molecular interactions involving protein surfaces.

Library construction using existing technology can generate 3×10^8 recombinants, which is adequate for coverage of the hexapeptide sequence space ($206 = 6.4 \times 10^7$). Although random peptide libraries with longer amino acid sequences have been constructed, they are of limited utility because libraries are not sufficiently large to completely explore the additional sequence space.

Other variations of phage display, such as antibody display and cDNA display, incorporate large proteins into the virion. The functional utility of these libraries is also limited by the number of transformants that can be generated using the technology available today.

New strains of *E. coli* selected for enhanced DNA uptake can improve the transformation efficiency over existing methods by approximately ten-fold ($3\text{-}5 \times 10^{10}$ cfu/µg). These improvements can dramatically increase the absolute number of recombinants for these challenging applications, significantly reducing the cost to produce and screen phage display libraries of peptides and proteins.

METHODS

The electrocompetent cells were made using a proprietary method of cell preparation developed by Lucigen, now a part of LGC, Biosearch Technologies. This method produces electrocompetent cells that have higher transformation efficiencies than that of cells produced using traditional methods.

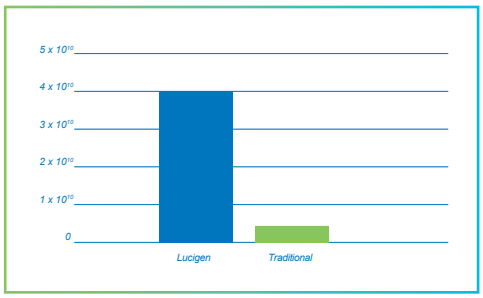
The transformation efficiency was tested by transforming 10pg of pUC19 DNA into 25µL of cells. A 1.00mm gap electroporation cuvette was used in a Bio-Rad Micro Pulser with settings of 10µF, 600 Ohms, 1800 Volts. Following the pulse, 975µL of Recovery Medium was added to the cuvette and the cells were resuspended by pipetting up and down three times.

The cells and Recovery Medium were transferred to a culture tube and placed in a shaking incubator at 250 rpm for 1 hour at 37°C. The transformed cells were diluted 1/100 and spread on YT agar plates containing carbenicillin. The plates were incubated overnight at 37°C.

GENOTYPES

ER2738 (VWR Cat. No. 95057-474 & 95057-476) F´proA+B+ lacIq Δ(lacZ)M15 zzf::Tn10(TetR)/ fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5

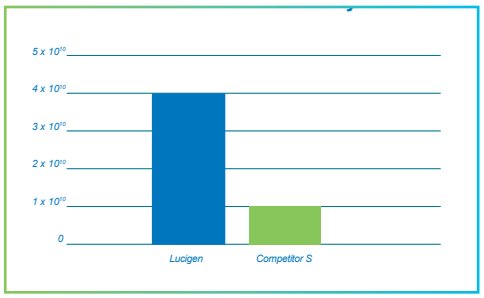
Comparison of SS320 cells transformation efficiency



Method of Preparation	Transformation Efficiency
Lucigen	4×10^{10} cfu/µg
Traditional	6×10^{9} cfu/µg

Lucigen SS320 cells were compared with cells prepared with traditional methods.

Comparison of TG1 cells transformation efficiency



Company	Transformation Efficiency
Lucigen	4×10^{10} cfu/µg
Competitor S	1×10^{10} cfu/µg

The Lucigen TG1 cells were compared with cells from competitor "S" using pUC DNA.

Comparison of phage display strains and transformation efficiencies

Cell line	Transformation Efficiency
TG1	4×10^{10} cfu/µg
SS320	4×10^{10} cfu/µg
MC1061 F-	4×10^{10} cfu/µg
ER2738	2×10^{10} cfu/µg

Transformation efficiency of Lucigen electrocompetent cells for phage display using pUC DNA.

MC1061 F- (VWR Cat. No. 89260-194 & 89260-196) araD139 Δ(araA-leu)7697 Δ(lac)X74 galK16 galE15(GalS) lambda- e14- mcrA0 relA1 rpsL150 (StrR) spoT1 mcrB1 hsdR2

SS320 (MC1061F´) (VWR Cat. No. 95057-470 & 95057-472) F´hsdR2 hsdM+ hsdS+ araD139 Δ(ara-leu)7697 Δ(lac)X74 galE15 galK16 rpsL (StrR) mcrA mcrB1

TG1 (VWR Cat. No. 95040-450 & 95040-452) supE thi-1 D (lac-proAB)D(mcrB-hsdSM)5 (rK– mK–) [F' traD36 proAB lacIqZDM15]

RESULTS

Electrocompetent cells were prepared using the Lucigen proprietary protocol (A) and traditional protocol (B). Cells were transformed with pUC DNA and plated on YT plates containing carbenicillin, X-Gal, and IPTG.

SUMMARY

– The proprietary protocol developed by Lucigen allows a greater transformation efficiency of *E. coli* strains to be achieved.
– Competent cells produced by Lucigen outperform the competition by up to 5-fold.
– Several strains are available for phage display which achieve transformation efficiencies > 2×10^{10} cfu/µg with pUC DNA.

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- Generate larger, more diverse phage display libraries with the highest efficiency TG1 cells available
- Improve Gibson® Assembly cloning efficiencies with TransforMax™ EPI300™ and E. cloni 10G strains

Optimize your Gibson® assembly cloning, as well as routine cloning and plasmid propagation with E. cloni® 10G and TransforMax™ EPI300™ strains. Choose Endura™ for cloning direct repeats, or the Phage Display ElectroCombo Pack (including TG1, SS320, and ER2738 strains) for constructing phage display libraries. Increase library complexity with the highest transformation efficiencies commercially available (up to 4 × 10^{10} cfu/µg).

Description	Size	Cat. No.
E. cloni® 10G Chemically Competent Cells (≥1 x 10^9 cfu/µg DNA)	12 Rxns	89005-078
E. cloni 10G ELITE Electrocompetent Cells (≥2 x 10^10 cfu/µg DNA)	12 Rxns	89005-074
TransforMax™ EPI300™ Electrocompetent E. coli	10 x 100 µL	75927-928
Endura™ Chemically Competent Cells (≥1 × 10^8 cfu/µg DNA)	24 Rxns	71003-034
Endura ElectroCompetent Cells (≥1 × 10^10 cfu/µg DNA)	24 Rxns	71003-038

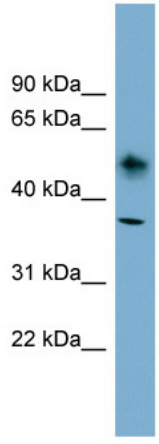
DNA sequencing made simple

Distributor
GE Healthcare

GE HEALTHCARE LIFE SCIENCES OFFERS INNOVATIVE PRODUCTS TO ENABLE HIGH QUALITY RESULTS, QUICKLY AND EASILY

- Prepare circular DNA templates for cycle sequencing, cloning and transformation in 4–6 hours
- Use amplified DNA directly for cycle sequencing without purification
- Generate microgram quantities of template DNA from picogram amounts of starting material

TempliPhi™ Kits use a unique process to efficiently prepare micrograms of circular DNA from picogram input material. The DNA templates are prepared by Rolling Circle Amplification (RCA) using bacteriophage Phi29 DNA polymerase. TempliPhi uses an isothermal method for the exponential amplification of circular DNA. Phi29 DNA polymerase is active at 30°C, enabling amplification to be performed at this temperature without the need for thermal cycling. The TempliPhi protocol requires less than 20 min of hands-on time to amplify 96 samples from bacterial colonies.



Description	Size	Cat No.
TempliPhi 100	100 rxn	89131-608
TempliPhi 2000	100 rxn	89134-006
GenomiPhi™ V2	100 rxn	95040-352
Single Cell GenomiPhi	100 rxn	10146-198
Nucleon Blood & Cultured Cells	50 rxn	95017-486

dsDNA quantitation kits

Unrivaled sensitivity and linear ranges for fluorescent detection of dsDNA

By Alexis Madrid and James Maclean, Biotium Inc., Fremont, CA

FLUORESCENCE-BASED dsDNA QUANTITATION

Precise quantification of dsDNA is essential for many genomics applications, such as next-generation sequencing and quantitative PCR. Unlike absorbance-based nucleic acid quantitation, fluorescent DNA binding dyes are highly sensitive as well as selective for double-stranded DNA, providing more accurate DNA quantitation in the presence of contaminating RNA.

Biotium offers dsDNA quantitiation kits and solutions for different instruments and sample concentration ranges. Some of our kits offer unrivaled sensitivity or linear ranges. To choose the DNA quantitation assay that is right for you, see the linear detection ranges and features table below.

KITS FOR THE QUBIT® FLUOROMETER

Features

- Designed for use with Qubit® dsDNA quantitation programs
- Direct replacements for Qubit® assay kits
- Significant cost savings compared to Qubit® kits

AccuGreen™ High Sensitivity Kit

- Designed specifically for the Qubit® fluorometer
- Linear range: 0.1-100ng dsDNA
- Green fluorescence (Ex/Em: 502/532nm)
- Replaces Qubit® dsDNA HS assay kit for cost savings

AccuGreen™ Broad Range Kit

- Designed specifically for the Qubit® fluorometer
- Linear range: 2 to 1000 ng dsDNA
- Green fluorescence (Ex/Em: 500/530nm)
- Replaces Qubit® dsDNA BR Assay kit for cost savings

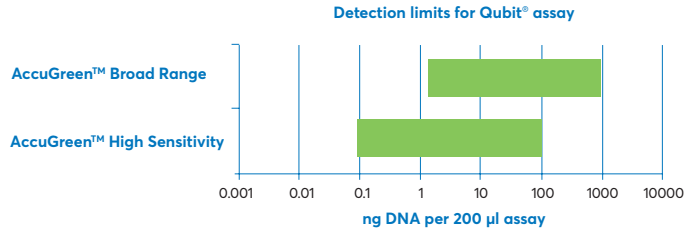


FIGURE 1: Chart comparing the linear detection ranges for AccuGreen™ kits for Qubit® fluorometer.

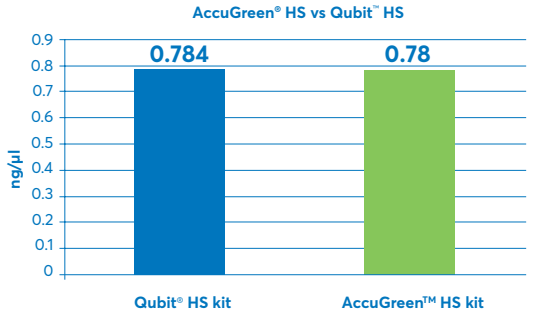


FIGURE 2: A genomic DNA sample was quantified using both the Qubit® HS kit and the AccuGreen™ HS kit using a Qubit® 3 fluorometer. A nearly identical result was obtained with each kit.

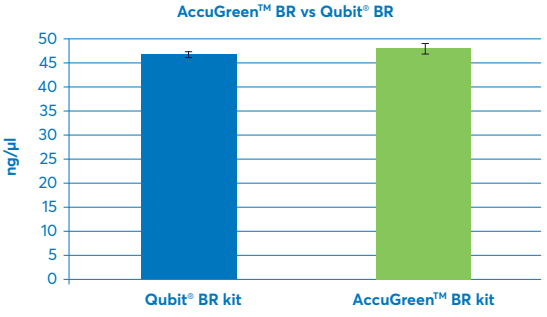


FIGURE 3: A genomic DNA sample was quantified using both the Qubit® BR kit and the AccuGreen™ BR kit using a Qubit® 3 fluorometer. A nearly identical result was obtained with each kit.

KITS FOR FLUORESCENCE MICROPLATE READERS

Features

- Allows quantitation of many samples and replicates at once for better accuracy
- Microplate readers have the highest sensitivity and adjustable excitation and emission settings
- The AccuBlue® NextGen assay is the most sensitive DNA quantitation assay on the market, allowing accurate detection down to 1pg of DNA (instrument dependent)
- The AccuClear® assay is extremely versatile, with a very broad dynamic range, allowing quantitation of nearly any sample

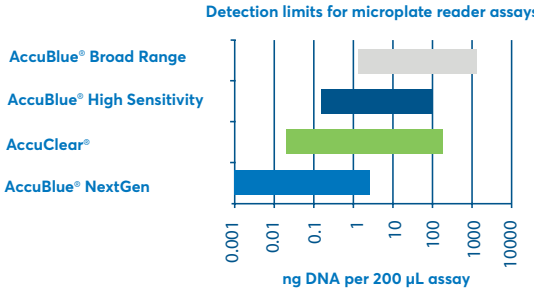


FIGURE 4: Chart comparing the linear detection ranges for dsDNA quantitation kits for fluorescent microplate reader.

AccuClear® Ultra High Sensitivity Kit

- Linear range: 0.03 to 250ng dsDNA
- Very high sensitivity and dynamic range
- Green fluorescence (Ex/Em: 468/507nm)
- Designed for fluorescence microplate reader
- Spectrally compatible with Qubit, QuantiFluor-P, or Nanodrop spectrofluoromter (may not be compatible with quantitation programs)

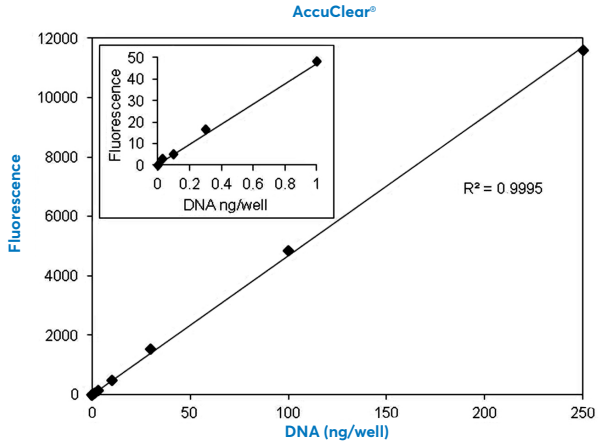


FIGURE 5: Standard curve of calf thymus DNA assayed using the AccuClear® Ultra High Sensitivity kit and read on a microplate reader (Ex/Em 460/507nm). Inset shows the lower end of the titration.

AccuBlue® NextGen Kit

- Linear range: 1 to 3000pg dsDNA (depending on plate reader capability)
- Unrivalled sensitivity for ultra-low amounts of DNA
- Green fluorescence (Ex/Em: 460/507nm)
- Designed for fluorescence microplate reader
- Spectrally compatible with Qubit, QuantiFluor-P, or Nanodrop spectrofluorometer (may not be compatible with quantitation programs)

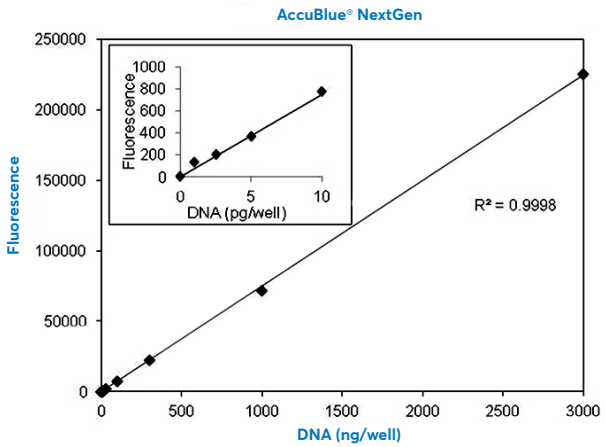


FIGURE 6: Standard curve of calf thymus DNA assayed using the AccuBlue® NextGen kit and read on a microplate reader (Ex/Em 460/507nm). Inset shows the lower end of the titration.

AccuBlue® High Sensitivity Kit

- Linear range: 0.2 to 100ng dsDNA
- Non-toxic, non-mutagenic for safer handling and easy disposal
- Green fluorescence (Ex/Em: 500/530nm)
- Designed for fluorescence microplate reader
- Spectrally compatible with Qubit, QuantiFluor®-P or NanoDrop spectrofluorometer (may not be compatible with quantitation programs)

AccuBlue® Broad Range Kit

- Linear range: 2 to 2000ng dsDNA
- Blue fluorescence emission (Ex/Em: 350/460nm)
- Designed for fluorescence microplate reader

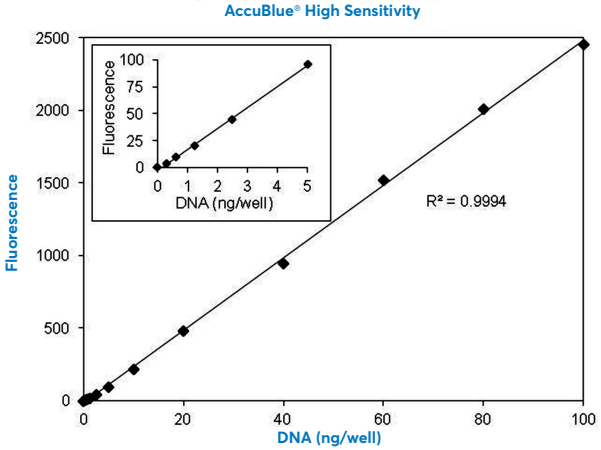


FIGURE 7: Standard curve of calf thymus DNA assayed using the AccuBlue® High Sensitivity kit and read on a microplate reader (Ex/Em 485/530nm). Inset shows the lower end of the titration.

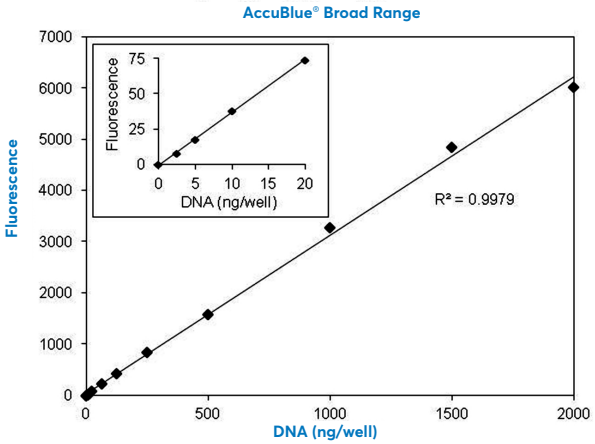
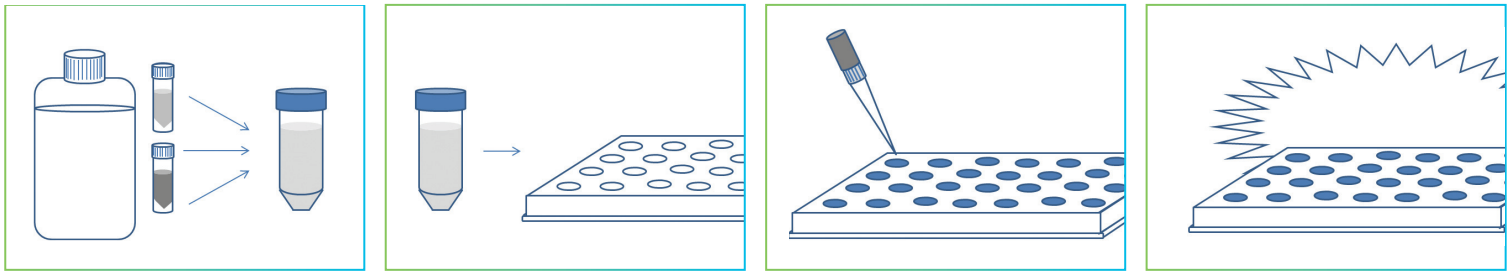


FIGURE 8: Standard curve of calf thymus DNA assayed using AccuBlue® Broad Range Kit and read on a microplate reader (Ex/Em 350/460). Inset shows the lower end of the titration.

ACCUBLUE® DNA QUANTITATION ASSAYS



01. Prepare quantitation working solution
02. Add working solution to wells
03. Add standards or samples to wells
04. Measure fluorescence using plate reader

Description	Size	Cat. No.
AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)	500, 100 assays	75845-716; 75845-718
AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)	500, 100 assays	76204-710; 76204-712
AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA standards	1000 assays	89427-078
AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA standard	4000 assays	89427-080
AccuBlue® NextGen dsDNA Quantitation Kit with 1 DNA standard	1000, 200 assays	76204-714; 76204-716
AccuBlue® High Sensitivity dsDNA Quantitation Kit with DNA standard	200 assays	89493-590
AccuBlue® High Sensitivity dsDNA Quantitation Kit with 8 DNA standards	1000 assays	89139-008
AccuBlue® Broad Range dsDNA Quantitation Kit with DNA standard, trial size	200 assays	89493-594
AccuBlue® Broad Range dsDNA Quantitation Kit with 9 DNA standards	1000 assays	89139-010

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Rapid and accurate quantification of Illumina NGS libraries using the Q Real-Time qPCR instrument

Achieve 60% faster time to reliable results

By Peter Bartholomew, Hongbo Liu, Eleanor Kolossovski, Brian Komorous, and David Schuster - Quantabio, 100 Cummings Center Suite 407J, Beverly, MA 01915

INTRODUCTION

Accurate quantification of the number of amplifiable library molecules is a critical factor for obtaining high quality read data with next generation sequencing technologies. The high sensitivity, broad dynamic range, and specificity of qPCR to quantify library molecules that are suitable for the bridge PCR, provide significant advantages over methods for total DNA quantification. However, these advantages are often offset by the time to result, requirement for inclusion of absolute DNA standards in every qPCR run, and errors associated with dilution of libraries, so that reportable results are within the linear dynamic range of the technology. Here we describe the application of a new Real-Time quantitative PCR instrument, the Q from Quantabio, to simplify reliable library quantification with faster run times.

FEATURES OF THE Q REAL-TIME QUANTITATIVE PCR INSTRUMENT

Magneticinduction technology

- Rapidly heats reactions held in a unique spinning aluminium rotor

Superior temperature uniformity of ±0.05°C

- Eliminates well position effects associated with traditional Peltier block-based Real-Time cyclers

Ultra-fast data acquisition

- Robust, fixed optical path allows simultaneous acquisition of all channels, with no need for reference dyes or crosstalk compensation

Scalable and wireless

- Up to 10 Q instruments can be operated from a single workstation wirelessly via Bluetooth®, enabling processing of 480 samples simultaneously

Portable and compact

- The compact size and 4.5 pound weight of the Q allows easy portability with no need for calibration
- Occupies 1/4 the bench space required for other cyclers on the market

Powerful software

- User friendly Q-qPCR software for advanced automated statistical analysis, including relative quantification, absolute quantification, genotyping and allelic discrimination

MATERIALS & METHODS

Trials were conducted on a Q using DNA standards, primer, and SYBR® Green SuperMix from the PerfeCTa® NGS library quantification kit for Illumina sequencing platforms (VWR Cat. No. 10029-558). PerfeCTa SYBR Green FastMix® (VWR Cat. No. 101414-276) was also used where noted.

RESULTS

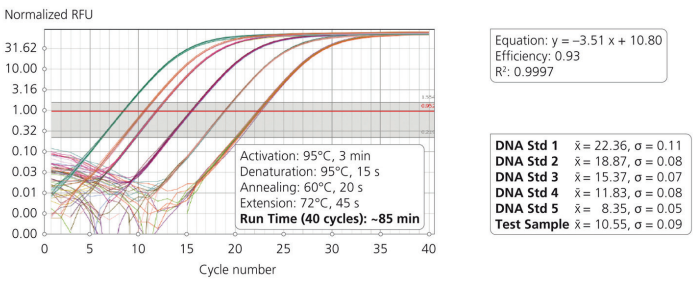


FIGURE 1: Reliable results across runs. Pre-diluted 426bp DNA standards were amplified in a typical three-step cycling protocol that completes in approximately 85 minutes. Results show the highly repeatable Cq values obtained across four distinct trials.

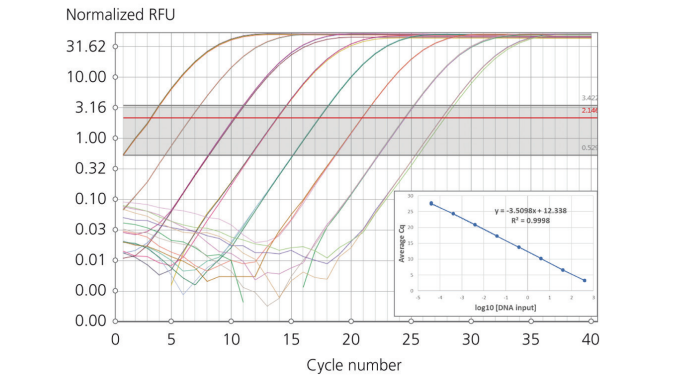


FIGURE 2: Samples from multiple NGS libraries were pooled into a test sample with average fragment size of 450bp. A 10-fold dilution series was prepared and assayed on the Q. Analysis of the resulting amplification curves showed high efficiency and sensitivity across an 8 log dynamic range.

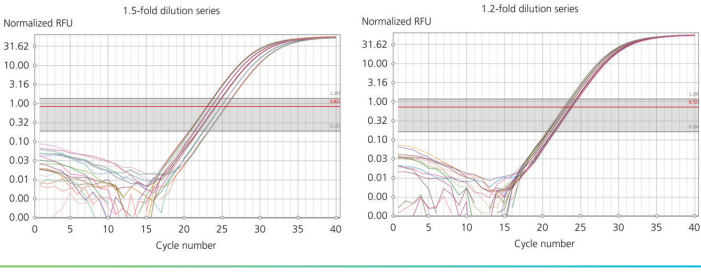


FIGURE 3: A range of dilution series were prepared from the 426 bp unknown test sample for qPCR analysis on the Q. Results show the ability of the Q to clearly distinguish samples in both a 1.5-fold and 1.2-fold dilution series.

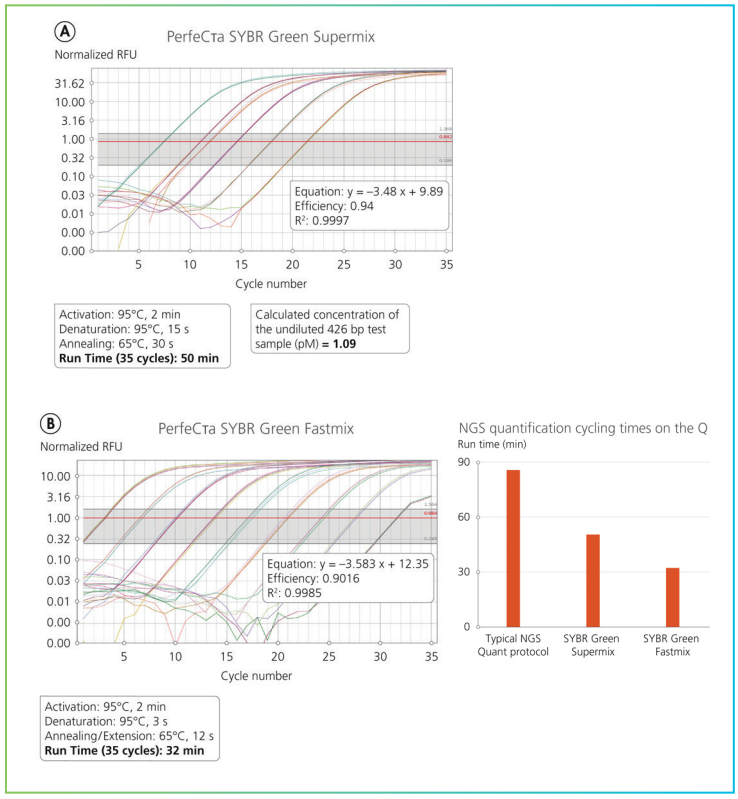


FIGURE 4:
Accurate quantification with rapid cycling. Using PerfeCTa SYBR Green SuperMix, along with the 426 bp DNA standards and the identically sized unknown test sample as templates, high efficiency amplification and accurate quantification was achieved with a 50 minutes run time (A). Furthermore, when we swapped in PerfeCTa SYBR Green FastMix, and included a dilution series of pooled libraries in the tests, we could achieve high efficiency amplifications with run times as short as 32 minutes (B).

CONCLUSIONS

Together, the results presented clearly establish the suitability of the Q Real-Time PCR instrument and PerfeCTa NGS quantification kit for quantification of NGS libraries of various sizes and GC contents. The clear benefits provided by the Q for NGS quantification include:

- Highly precise measurements across multiple trials (Figure 1).
- High efficiency amplifications under varied cycling conditions (Figure 2).
- Exceptional quantitative sensitivity for distinguishing down to 1.2-fold differences (Figure 3).
- Reliable results and performance from run times 60% shorter than typical cycling protocols (Figure 4).

Description	Cat. No.
Q 2-channel PCR instrument	76175-392
Q 4-channel PCR instrument	76175-394

Simple, high quality, and cost-effective library preparation solutions for NGS

By Yi Jin, Hongbo Liu, Marissa Bolduc, David Bays, Eleanor Kolossovski, Brian Komorous, and David Schuster — Quantabio

Quantabio's sparQ DNA Library Prep Kit uses optimized chemistry that combines end-repair and dA-tailing into a single step, followed by direct ligation of adapters (Figure 1). The unique formulation and simplified workflow greatly reduce the total processing and hands-on time, while ensuring high library yield and quality.

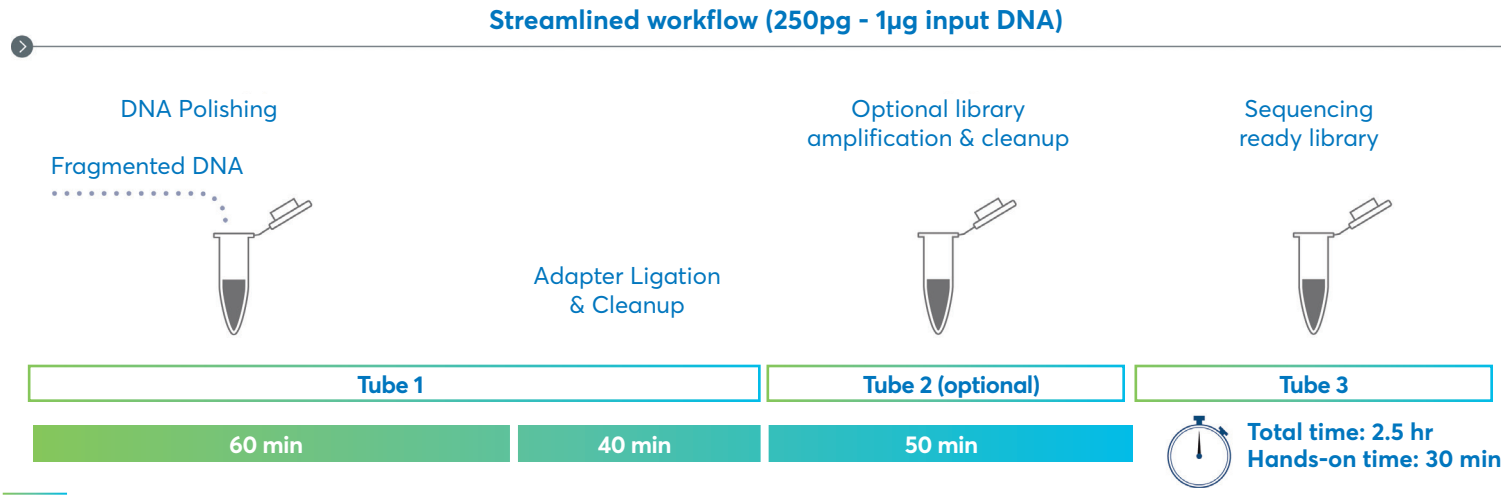


FIGURE 1: Library Prep Workflow

		1ng Input DNA		100ng Input DNA	
		Mapped Reads	Duplication	Mapped Reads	Duplication
sparQ	with Library Application	94.3%	0.07%	95.5%	0.04%
Supplier K		95.0%	0.09%	95.6%	0.04%
Supplier N		94.9%	0.07%	95.4%	0.03%
sparQ	PCR-Free			95.6%	0.03%
Supplier K				95.3%	0.02%
Supplier N				95.1%	0.02%

TABLE 1: Yield & Quality Comparisons

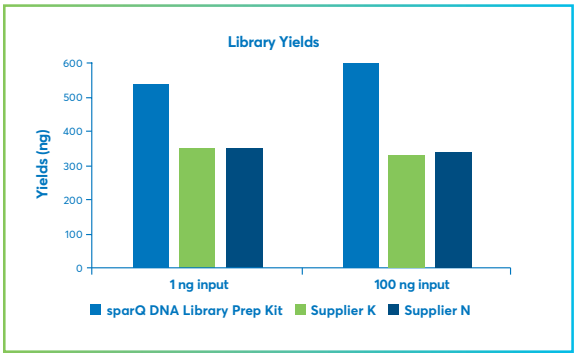


FIGURE 2

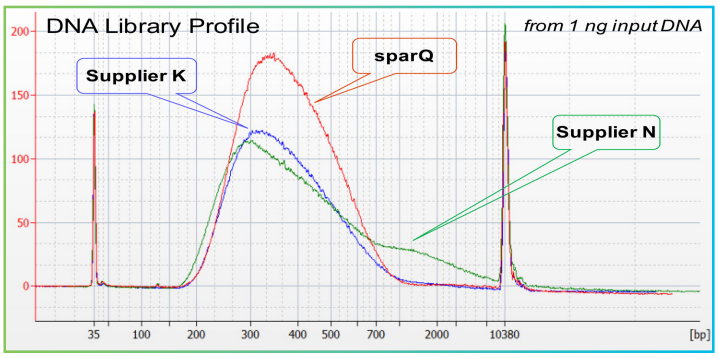
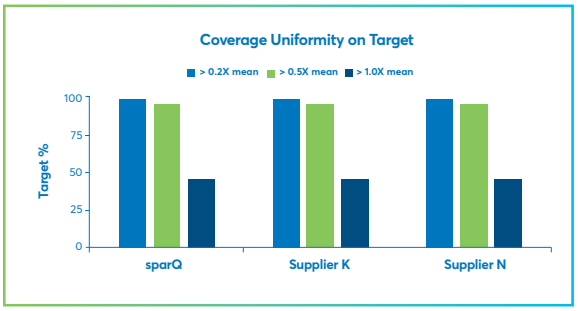
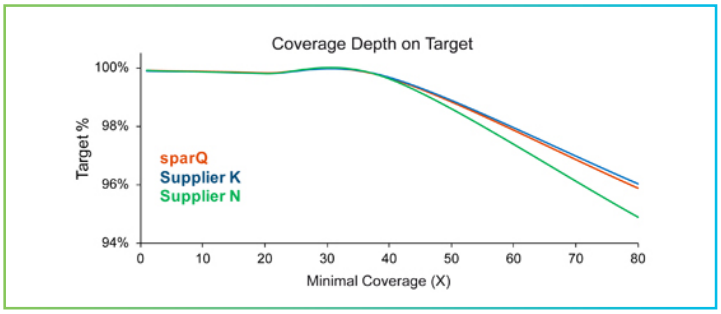


FIGURE 3





FIGURES 4 AND 5: Target enrichment application with xGen® acute myeloid Leukemia cancer panel.

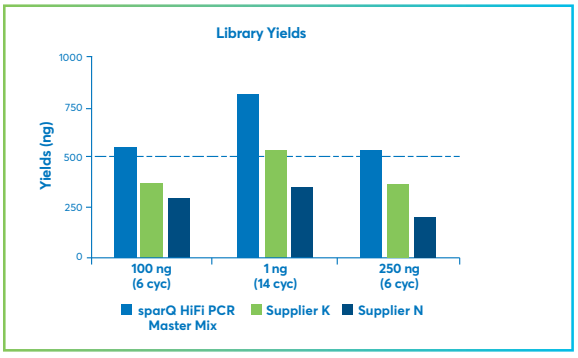


FIGURE 6

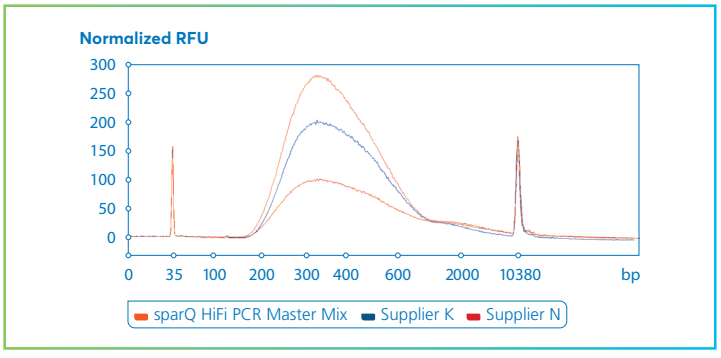


FIGURE 7: DNA libraries from 250 pg input DNA.

sparQ DNA Library Prep Kit offers significantly higher NGS library preparation efficiency and produces high quality libraries (Figures 2 & 3). Libraries were prepared with Covaris-sheared bacterial genomic DNA mix (250bp average size) and then amplified using 6 cycles for 100ng input DNA or 13 cycles for 1ng input DNA. DNA library profiles were analyzed using a high sensitivity DNA analysis kit on an Agilent Bioanalyzer. Library quality was measured by alignment percentage and duplication rate post sequencing. High quality DNA Libraries were prepared using sparQ DNA Library Prep Kit with Covaris-sheared human Coriell DNA NA12878 (300bp average size) to ensure superb performance of a downstream targeted sequencing application (Figures 4 & 5).

SUPERIOR SENSITIVITY & EFFICIENCY

Libraries were prepared from Covaris-sheared human genomic DNA with sparQ DNA Library Prep Kit prior to

library amplification. Pre-amplified libraries were then amplified using sparQ HiFi PCR Master Mix (orange), HiFi PCR Master Mix from Supplier K (blue), or Supplier N (green) with the identical amplification cycle numbers (6 cycles for 100ng input DNA, 14 cycles for 1ng input DNA, and 16 cycles for 250pg input DNA) (Figures 6 & 7). Amplified libraries were quantified with Qubit fluorometric quantitation method and qPCR-based quantification method (data not shown).

Library amplification with sparQ HiFi PCR Master Mix resulted in uniform coverage across a wide range of GC-content. Libraries were prepared by using sparQ DNA Library Prep Kit with 100ng input DNA. Coverage depth against GC content of libraries amplified by sparQ HiFi PCR MM (red) were compared to corresponding

	Duplication reads %		
	Mixed geomes	AT-rich genome	GC-rich genome
PCR-free Lib	0.03%	0.19%	0.05%
Amplified Lib	0.04%	0.18%	0.05%

TABLE 2

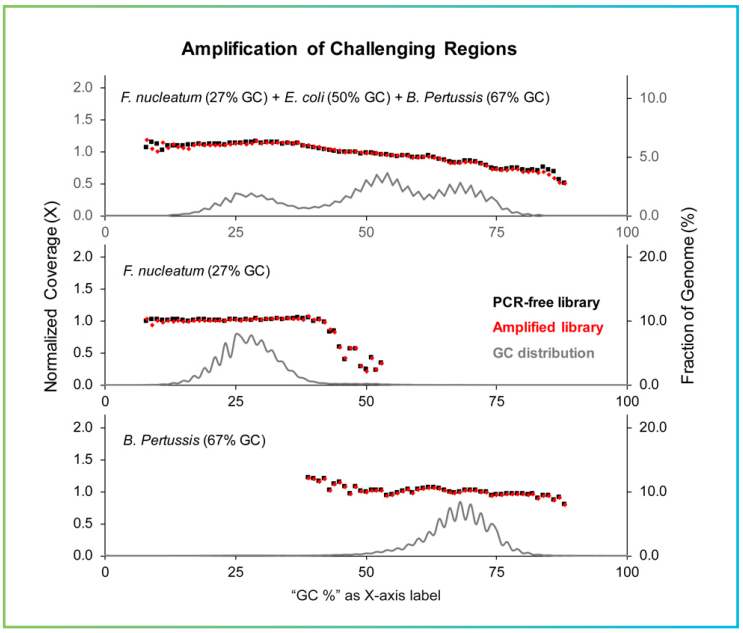


FIGURE 8: Uniform Coverage Across Challenging Genomic Regions

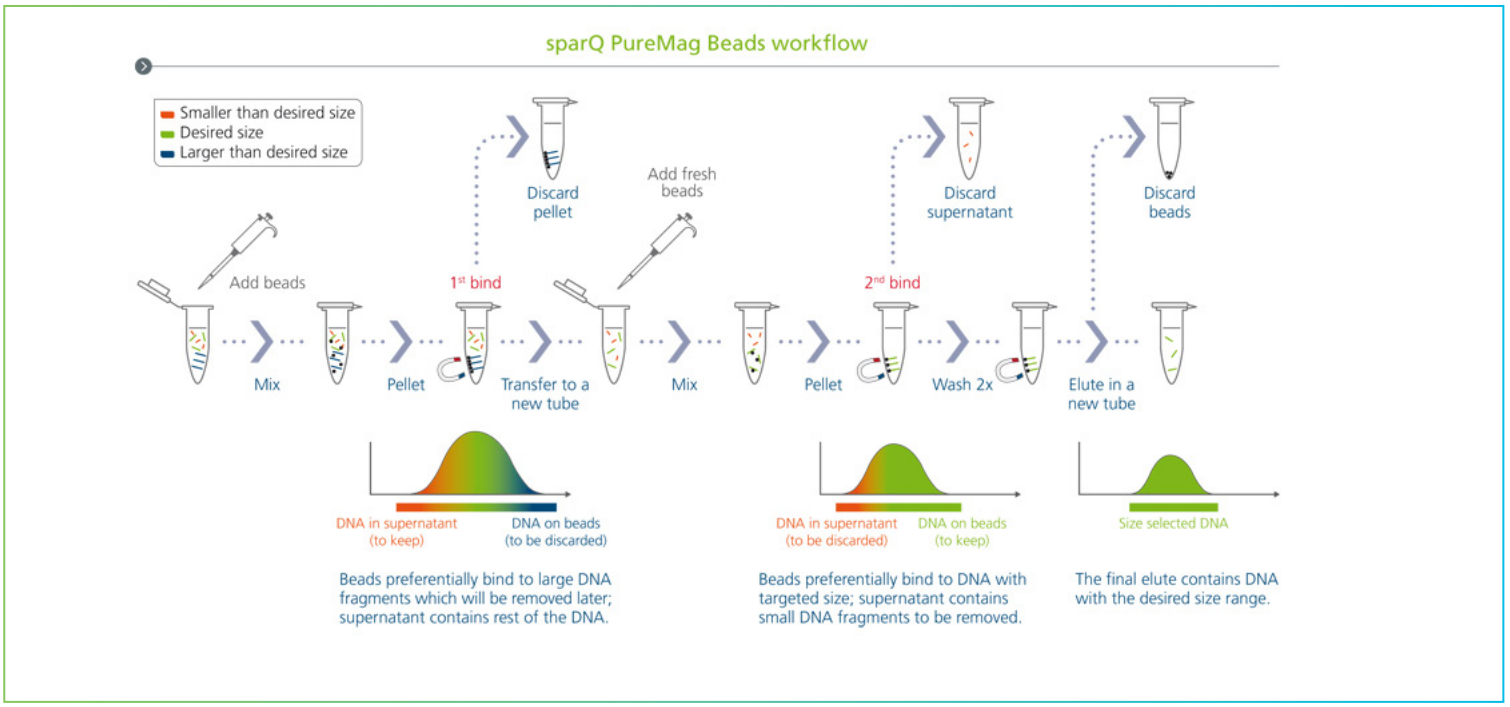


FIGURE 9: **Consistent & adjustable size selection. sparQ.** sparQ PureMag Beads takes advantage of the reversible nucleic acid-binding properties of magnetic beads for efficient nucleic acid purification. It offers a reliable and cost-effective solution for reaction cleanup and size selection, and can be seamlessly integrated into existing NGS workflows with little or no protocol changes.

libraries without amplification (black). GC-content distribution of targeted genomes is indicated by the gray line (Figure 8).

SPARQ FAST LIBRARY QUANT KIT (FOR Q)

sparQ Fast Library Quant Kit provides rapid and accurate quantification of libraries prepared for sequencing on Illumina® NGS platforms. Accurate quantification of the number of amplifiable library molecules prior to loading onto a flow cell is a critical step in the NGS workflow and it ensures optimal cluster generation and cost-effective use of sequencing capacity. The sparQ Fast Library Quant Kit uses real-time qPCR (qPCR) to specifically quantify the number of library molecules that possess the appropriate adapter tag at each end (Figures 9 & 10).

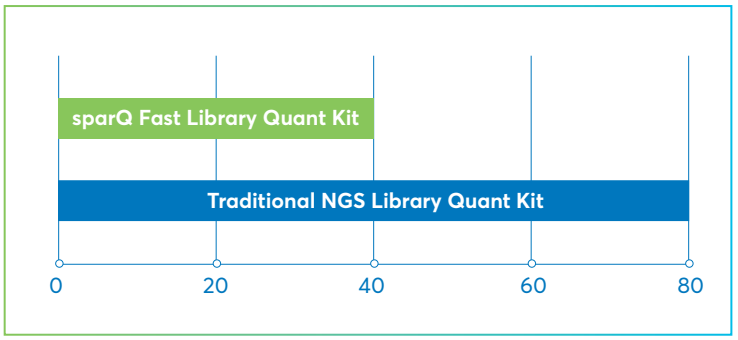


FIGURE 10: Faster run time. Comparison of average qPCR run time for library quantification. sparQ Fast Library Quant Kit uses fast cycling protocol, allowing results to be achieved in 40 minutes versus 1 hour and 20 minutes with the traditional NGS Library Quant Kit.

Fast, reliable DNA purification for NGS workflows

For more information, visit **vwr.com/quantabio**

sparQ DNA Library Prep Kit

- 2-step protocol generates sequencing ready library in 2.5 hours
- 250pg–1µg of input DNA produces unbiased libraries
- High library yields from enzymes engineered for optimal sensitivity and efficiency
- Capable of PCR-free library prep workflow
- Simple and cost-effective solution to streamline library prep (e.g., whole genome sequencing, targeted sequencing, ChIP-seq, cfDNA, etc.)

sparQ HiFi PCR Master Mix

- Increased amplification efficiency results in higher yields for NGS library amplification
- Unbiased amplification provides improved coverage across AT- and GC-rich regions

sparQ Fast Library Quant Kit (for Q)

- Faster time to results — 50% shorter run time than traditional cycling protocols
- Accurate, reliable quantification of NGS libraries of various sizes and GC-content

sparQ PureMag Beads

- Fast, reliable DNA purification and size selection in NGS workflows

Description	Size	Cat. No.
sparQ DNA Library Prep Kit	24 Reactions	**76169-150**
sparQ DNA Library Prep Kit	96 Reactions	**76169-152**
sparQ HiFi PCR Master Mix	50 Reactions	**76121-366**
sparQ HiFi PCR Master Mix	250 Reactions	**76121-368**
sparQ DNA Frag & Library Prep Kit	24 Reactions	**76183-242**
sparQ DNA Frag & Library Prep Kit	96 Reactions	**76183-244**
sparQ Fast Library Quant Kit (for Q qPCR Instrument)	50 Reactions	**76323-384**
sparQ Fast Library Quant Kit (for Q qPCR Instrument)	500 Reactions	**76323-386**
sparQ PureMag Beads	450 mL	**76302-830**
sparQ PureMag Beads	60 mL	**76302-832**
sparQ PureMag Beads	5 mL	**76302-834**
sparQ Adapter Barcode Set A (12 Single Index Barcodes)	96 Reactions	**76169-154**
sparQ Adapter Barcode Set B (12 Single Index Barcodes)	96 Reactions	**76169-156**

Low volume, high throughput DNA and protein detection on the SpectraMax ABS Plus microplate reader

By Hoang Ha, Applications Scientist, Molecular Devices

Nucleic acid and protein quantitation are essential measurements upstream of many sophisticated assays in genetics and molecular biology. Various methods have been developed to quantitate these biological components, however, the most commonly used technique is still ultraviolet (UV) spectrophotometry.

The basis of spectrophotometry is that every molecule absorbs or transmits light over a certain wavelength range, and their concentrations can be calculated by using the Beer-Lambert law (Equation 1) with preceding knowledge of the sample's molar extinction coefficient and the measurement path length.

$A = \varepsilon cL$

Equation 1: The Beer Lambert law states that absorbance (A) is equal to the measured molecule's molar coefficient (ε) multiplied by the concentration (c) and path length (L) used to measure said molecule. Re-arranging the equation allows us to use absorbance to calculate concentration.

BENEFITS

- Widen the range of quantitative experiments with a 190 to 1000nm wavelength range
- Minimize sample volume while retaining sensitivity with the SpectraDrop micro volume microplate
- Simplify and expedite experiments with SoftMax Pro software's pre-configured protocols

Nucleic acid quantitation is a very well established technique, and its basic principles have not been modified much since its inception. To calculate nucleic acid concentration, samples are measured at 260nm, and auxiliary measurements are taken at 230 and 280nm wavelengths to check for sample purity.

Protein samples can be quantitated using UV spectrophotometry as well, however, there are more accurate colorimetric assays available. The UV spectrophotometric method uses tryptophan's aromatic properties to absorb light at 280nm, but calculated protein concentrations can be skewed by varying amounts of tryptophan residues in the amino acid sequence. Alternatively, the Bicinchoninic Acid (BCA) protein assay can measure protein concentrations independent of amino acid sequence and length. The assay uses the copper-based Biuret reaction where the amino acid backbone forms a color chelate complex with copper molecules in an alkaline environment.

The SpectraMax® ABS Plus is a compact UV/Vis absorbance microplate reader ideal for these types of quantitative assays. Here, we demonstrate several different ways the ABS Plus reader, in combination with the SpectraDrop™ micro volume microplate and SoftMax® Pro software, can quantitate double-stranded DNA and bovine serum albumin (BSA) protein.

MATERIALS

- SpectraMax ABS Plus microplate reader (Cat. No. 76266-088)
- SpectraDrop micro volume microplate (Cat. No. 89230-746)
- 96-well, clear, flat bottom, polystyrene microplate
- UltraPure™ calf thymus DNA solution
- Pierce BCA protein assay kit
- Pierce™ Bovine serum albumin standard ampoules, 2mg/m
- UV-Star® 96-well microplates

METHODS

DNA quantitation

A 2-fold dilution series of dsDNA starting at 1000ng/µl was prepared from UltraPure calf thymus DNA diluted in 1x PBS. Either 2 or 4µL of sample was pipetted onto a 64-well SpectraDrop microplate, and a 0.5 or a 1.0mm coverslip was used to cover the dsDNA sample respectively. The pre-configured protocol, 'SpectraDrop DNA Quantitation', was opened in SoftMax Pro software, and the plate was read using the provided settings. A log-log curve fit was applied to the data, and a standard curve was generated using SoftMax Pro software. Additionally, a spectral scan ranging from 220 to 350nm with 4nm steps was performed on all concentrations to assess sample purity.

DNA quantitation in a cuvette or a microplate format was also compared. A 2-fold dilution series from 250 to 0.5µg/µL was transferred to a UV-transparent 96-well microplate at a volume of 200µL per well or several UV/Vis cuvettes at 1000µL. Both formats were read on the SpectraMax ABS Plus microplate reader at 260nm. A log-log curve fit was applied to each data set, and the standard curves were generated using SoftMax Pro software.

Protein quantitation

The BCA assay standard curve was prepared by diluting provided BSA according to the assay kit's protocol. 25µL of protein standards and 200µL of the BCA working reagent was transferred to a 96-well clear microplate and incubated for 30 minutes at 37°C. The 'BCA' pre-configured protocol was opened in SoftMax Pro software, and the plate was read at 562nm using the settings provided. A quadratic curve fit was applied to the data, and a standard curve was generated using SoftMax Pro software.

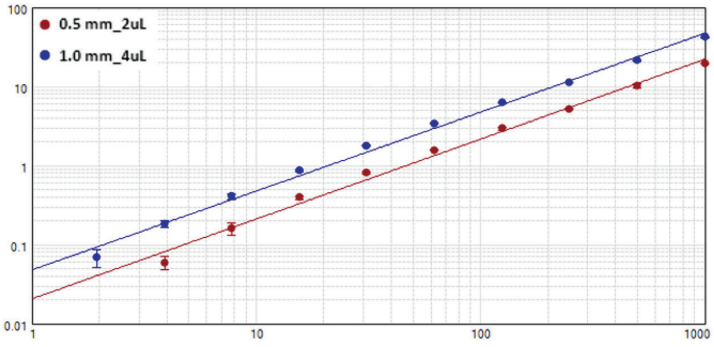


FIGURE 1: dsDNA quantitation on the SpectraDrop micro volume microplate. The ABS Plus reader with the SpectraDrop microplate could detect down to 2 and 4 ng/µl of DNA using the 1.0mm (blue) and 0.5mm (red) coverslips respectively.

64-well Trial 2 | 1.0mm_4uL

1.0mm_4uL

Sample	Well	KnownConcentratio ng/uL	A260	Average A260	STDev A260	A280	A230	260:280	260:230	Co
01	H7	1000.000	41.445	41.776	0.889	21.457	17.843	1.931	2.323	
	I7		41.351			21.470	17.785	1.926	2.325	
	J7		41.207			21.322	17.663	1.933	2.333	
	K7		43.101			27.930	24.539	1.543	1.756	
02	H8	500.000	21.129	21.102	0.103	10.872	8.875	1.944	2.381	
	I8		21.029			10.808	8.813	1.946	2.386	
	J8		21.015			10.801	8.817	1.946	2.383	
	K8		21.237			11.183	9.203	1.899	2.308	
03	H9	250.000	11.329	11.282	0.041	5.859	4.691	1.933	2.415	
	I9		11.257			5.808	4.641	1.938	2.426	
	J9		11.259			5.816	4.639	1.936	2.427	
	K9		Masked			Masked	Masked	Masked	Masked	
04	H10	125.000	6.335	6.296	0.035	3.367	2.567	1.881	2.468	
	I10		6.283			3.321	2.505	1.892	2.508	
	J10		6.269			3.320	2.509	1.889	2.499	
	K10		Masked			Masked	Masked	Masked	Masked	
05	H11	62.500	3.449	3.425	0.031	1.914	1.349	1.802	2.557	
	I11		3.395			1.868	1.291	1.818	2.630	
	J11		3.401			1.884	1.311	1.806	2.594	
	K11		3.453			1.909	1.331	1.808	2.594	

FIGURE 2: SoftMax Pro software pre-configured data table. The software's pre-configured protocols simplify DNA quantitation experiments by automatically calculating absorbance ratios and concentrations of unknown samples.

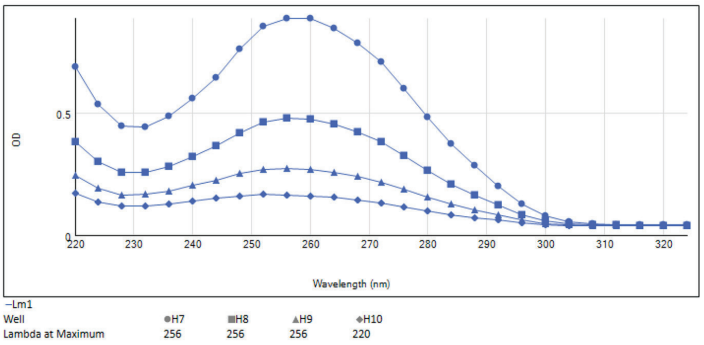


FIGURE 3: SoftMax Pro software pre-configured data table. Spectral scans were performed to assess sample purity. Four representative concentrations of DNA were scanned and only a peak at 260nm was identified, indicating pure dsDNA.

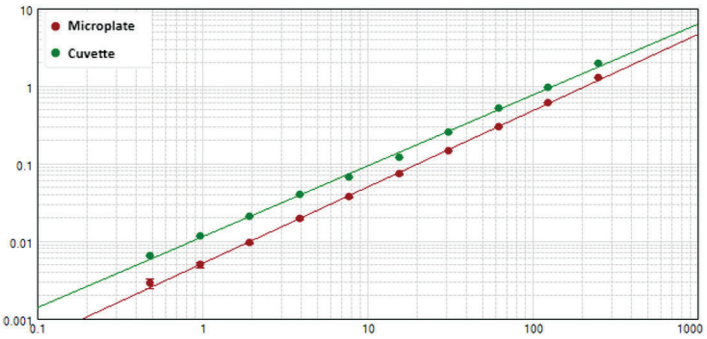


FIGURE 4: dsDNA quantitation in a 96-well microplate or cuvette. The dsDNA dilution series was detectable from 250 to 0,50ng/µl in both cuvette and microplate formats. Additionally, both dilution series demonstrated high linearity.

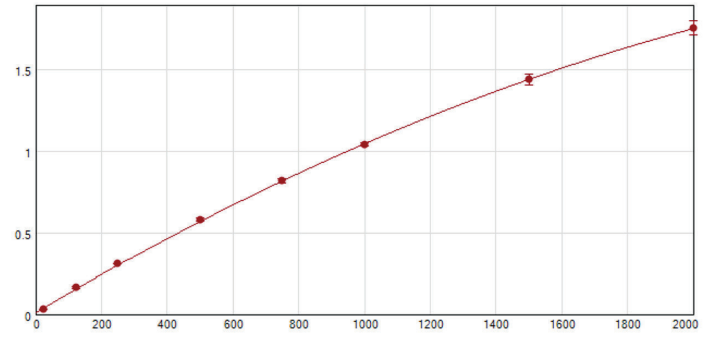


FIGURE 5: BCA Assay standard curve. The ABS Plus microplate reader was able to detect the BSA standards from the BCA assay. SoftMax Pro software was used to apply a quadratic curve fit to the data points, and a robust standard curve (r2 = 1,000) was generated.

RESULTS

The ABS Plus microplate reader was able to quantitate dsDNA concentration using both low volume and standard microplate methods. Using the SpectraDrop plate and pre-configured protocol, the ABS1Plus microplate reader could detect down to 2ng/µl using as little as 4µl of sample, and could automatically measure and calculate relevant parameters such as A_{260}/A_{280} and A_{260}/A_{230} ratios (Figure 1 and Figure 2). A spectral scan further demonstrated that the samples were contaminant-free (Figure 3).

Alternatively, the ABS1Plus microplate reader can quantitate dsDNA in a microplate format. Using a UV-transparent microplate, the reader could measure as little as 0.02 ng/µL dsDNA (Figure 4).

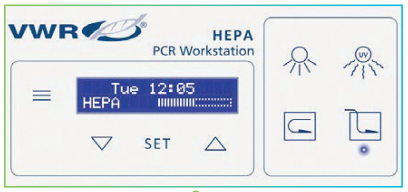
Finally, the SpectraMax ABS Plus microplate reader can be used to quantitate protein samples with the aid of the BCA assay. Following the provided protocol, BSA standards were measured, and a standard curve was generated comparable to the curve in the assay kit's literature (Figure 5).

CONCLUSIONS

The SpectraMax ABS Plus is a compact and adaptive microplate reader capable of quantitating nucleic acid and protein samples in various formats, such as cuvettes or microplates. When combined with the SpectraDrop microcolume microplate, the reader can quantitate up to 64 samples with as little as 2µl sample volume in a single read. Additionally, SoftMax Pro software's pre-configured protocols allow for optimised experimentation and faster time to results.

PCR workstation with HEPA filter

Dual-surface and air cleaning action gives a contamination-free working space for sensitive PCR-based applications



Clearly arranged control panel with display. Optional indication of UV tube run time enables well-timed replacement of tubes for constant UV intensity.

The VWR® PCR Workstation is designed as an ideal controlled environment for the manipulation of DNA and RNA, especially for the set-up of PCR assays.

The VWR PCR Workstation decontaminates the workspace by UV inactivation of airborne and surface-bound contaminants, reducing the risk of sample contamination when setting up PCR applications. The unit provides effective twin action by using UV irradiation during non-working times and by inactivating aerosol-bound contaminants with a shielded UV air recirculator during use. Finally, the HEPA system in the Pro version provides an ultra-clean, particle-free working space reducing the risk of contamination from the air that blows into the PCR chamber.

The large stainless steel work surface is 72x54cm and features four internal power outlets, providing ample space for bench top equipment. Removable shelves give additional storage space for reaction tubes, pipettes or racks in the rear panel. This means that several working steps can be combined without changing location or interrupting workflow — minimizing the risk of cross-contamination.

HIGH QUALITY MATERIALS & DESIGN GUARANTEES MAXIMUM USER-PROTECTION & SAFETY

- 8mm thick, ethanol-resistant safety panels absorb UV irradiation
- Electromagnetic safety mechanism stops UV irradiation if front panel is opened
- Work surface and rear panel made of easy to clean stainless steel
- Function indicator for UV air recirculator tube
- Displays operating time of UV tubes to enable well-timed replacement of tubes for constant UV intensity
- Off hours programmable decontamination operation
- Carbon filter to remove ozone and gases
- HEPA filter (H14 Standard) provides a barrier against dust, bacteria and mold with a filtration efficiency of 99.995% for particles down to 0.3µm. The HEPA system provides an ultra-clean, particle-free ISO Class 5 working space according to the EN 1822-1 standards

Description	Cat. No.
VWR® PCR Workstation	10783-132
VWR® PCR Workstation Pro with HEPA Filter	76289-386

Innovative power supplies for nucleic acid and protein electrophoresis applications

VWR® 300V AND 500V POWER SUPPLY

- Color touch screen control panel
- Compact footprint, takes up minimal lab space
- Four sets of output terminals compatiable with all popular gel tanks
- Ideal for horizontal DNA gels, vertical protein gels

Specifications

Voltage Output	300V (1 volt increments)	500V (1 volt increments)
Current Output	2000mA (5 mA increments)	400mA (1 mA increments)
Output Control	Constant voltage or constant current	Constant voltage or constant current
Maximum Power	300W	120W
Output Terminals	4 pairs	4 pairs
Electrical Input	115V / 60Hz or 230V / 50Hz	115V / 60Hz or 230V / 50Hz
Weight/Dimensions	1.2 kg (2.7 lbs)/ 8.9 x 12.7 x 21.6 cm (3½ x 5 x 8½")	1.14 kg (2.5 lbs) / 8.9 x 12.7 x 21.6 cm (3½ x 5 x 8½")

Description	Cat. No.
300V, 115 VAC/60Hz Input	**76196-458**
300V, 230 VAC/50Hz Input	**76196-460**
500V, 115 VAC/60Hz Input	**76196-454**
500V, 230 VAC/50Hz Input	**76196-456**

VWR® MINI POWER SUPPLY

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- Simple operation — connect gel box and select voltage to start
- Selectable, 100V or 200V output
- Ideal for basic DNA separations in agarose gels
- Compatible with standard horizontal gel boxes
- Two pairs of output jacks for running two gels at one time

Specifications

Voltage Output	100V or 200V, constant
Current Output	Maximum 200mA
Maximum Power	40 Watts
Output Terminals	2 pairs in parallel
Electrical Input	115V / 60 Hz or 230V / 50 Hz
Weight/Dimensions	0.4 kg (0.9 lbs.)/ 16 x 11.5 x 5.7 cm (5¼ x 4½ x 2¼")
Warranty	2 years

Description	Cat. No.
115 VAC/60 Hz Input	**76196-450**
230 VAC/50 Hz Input	**76196-452**

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VWR LIFE SCIENCE AGAROSE

- Agarose I™ is for routine nucleic acid analytical/preparative applications.
- Agarose RA™ is the ideal agarose for high throughput
- Agarose SFR™ (Super Fine Resolution) is a high resolution sieving agarose with excellent clarity
- Agarose 3:1 HRB™ (High Resolution Blend) is a unique mixture of agarose formulated to provide high resolution of small nucleic acids and PCR products

Size	Cat. No.
Agarose I	
25 g	**97062-248**
100 g	**97062-244**
250 g	**97062-246**
500 g	**76020-396**
Agarose RA	
25 g	**97064-256**
100 g	**97064-250**
250 g	**97064-254**
500 g	**97064-258**

Size	Cat. No.
1 kg	**97064-252**
Agarose SFR™	
25 g	**97064-138**
100 g	**97064-134**
250 g	**97064-136**
Agarose 3:1 HRB	
25 g	**97062-988**
100 g	**97062-984**
250 g	**97062-986**

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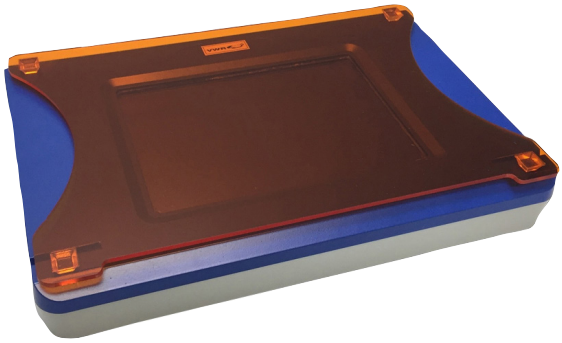
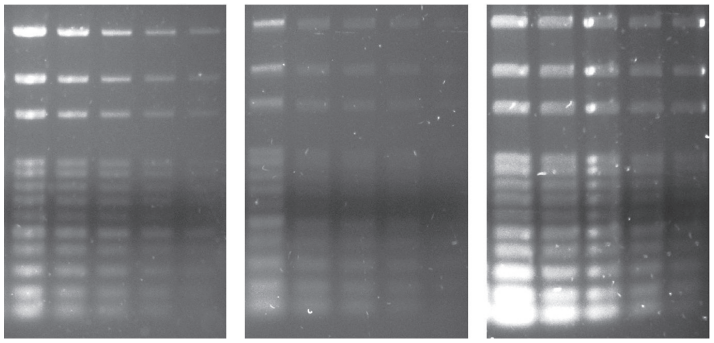
Protein A chromatography resin with enhanced alkaline stability and binding capacity for improved process economy in antibody purification.

Description	Size, mL	Cat. No.
MabSelect PrismA resin	25	**76237-724**
MabSelect PrismA resin	200	**76237-732**

A safer way to visualize your DNA





Comparison of EZ-Vision® Blue Light DNA Dye to GelGreen™ and SYBR® Green I Two-fold dilution series of identical DNA fragments were electrophoresed in 1% agarose gels. The gels were then stained with EZ-Vision Blue Light (left panel), GelGreen (middle panel) and SYBR Green I (right panel). DNA fragments were visualized using a blue light transilluminator and SYBR Green filter.

VWR® BLUE LIGHT TRANSILLUMINATOR

- Blue wavelength does not damage DNA
- Uniform illuminated surface
- 465 nm wavelength, ideal for most fluorescent DNA stains
- Amber cover is removable and can be set to an angle

VWR®'s Blue Light Transilluminator provides a safe and cost-effective alternative to UV transilluminators for viewing gels stained with dyes excited in the blue light spectrum. Blue light excitation does not damage DNA samples and these dyes can be safe alternatives to hazardous ethidium bromide. The evenly illuminated viewing area can accomodate gels up to 12 x 17 cm, and the scratch-resistant glass surface allows cutting out bands.

Electrical	Cat. No.
100 to 240VAC (Includes US type plug)	**76151-834**

EZ-VISION® BLUE LIGHT PERFOMANCE VERSUS COMPETING BLUE LIGHT STAINS

BLUE LIGHT VISUALIZATION

Fluorescent dye visualizes DNA bands using blue light excitation or standard UV transilluminator.

LESS DAMAGE TO DNA

Use with blue light excitation (410–510nm) and a green emission filter (500–600 nm) to eliminate UV-induced DNA damage and improve downstream cloning efficiency.

SAFER TO USE

Formula is both non-toxic and non-mutagenic and eliminates the need to use ethidium bromide and UV light.

FLEXIBILITY AND COMPATIBILITY

Use as an in-gel stain for immediate post-electrophoresis results or as an easy 30 minute post-stain. Compatible with standard downstream applications, including gel extraction, cloning and sequencing.

Description	Size, µL	Cat. No.
EZ-Vision Blue Light Dye, 10,000X	500	**10791-798**

Lonza

DNA electrophoresis and recovery in minutes

FLASHGEL™ SYSTEM

- Complete separation, recovery and documentation safely at the bench
- No gel preparation, band excision, purification or UV light needed
- Load samples, watch bands migrate and get data in as little as two minutes
- Allows a 5 to 20X reduction in DNA levels, saving time and money

FlashGel System consists of enclosed, disposable, precast agarose gel cassettes and a combination electrophoresis and transilluminator unit that provides visualization of both DNA and RNA cassettes. The FlashGel camera captures gel images from the FlashGel system right at the benchtop with complete gel run and image capture in just five minutes.

Description	Formulation	Cat. No.
FlashGel ™ System	Pack of 1.2% DNA Cassettes, Loading Dye, DNA Marker, Dock and Camera	95045-604
FlashGel™ DNA Kit	Includes FlashGel™ DNA Cassettes 1.2%, Loading Dye, and Marker	89400-688
FlashGel™ RNA Kit	Pack of 1.2% RNA Cassettes, Formaldehyde Sample Buffer, RNA Marker and Molecular Biology Grade Water	95015-614
FlashGel Recovery Kit	Pack of 1.2% Recovery Cassettes, Loading Dye, Recovery Buffer, FlashGel QuantLadder, Visualization Glasses	95053-314
FlashGel Device Kit	Dock, Power Supply and Camera	89400-686

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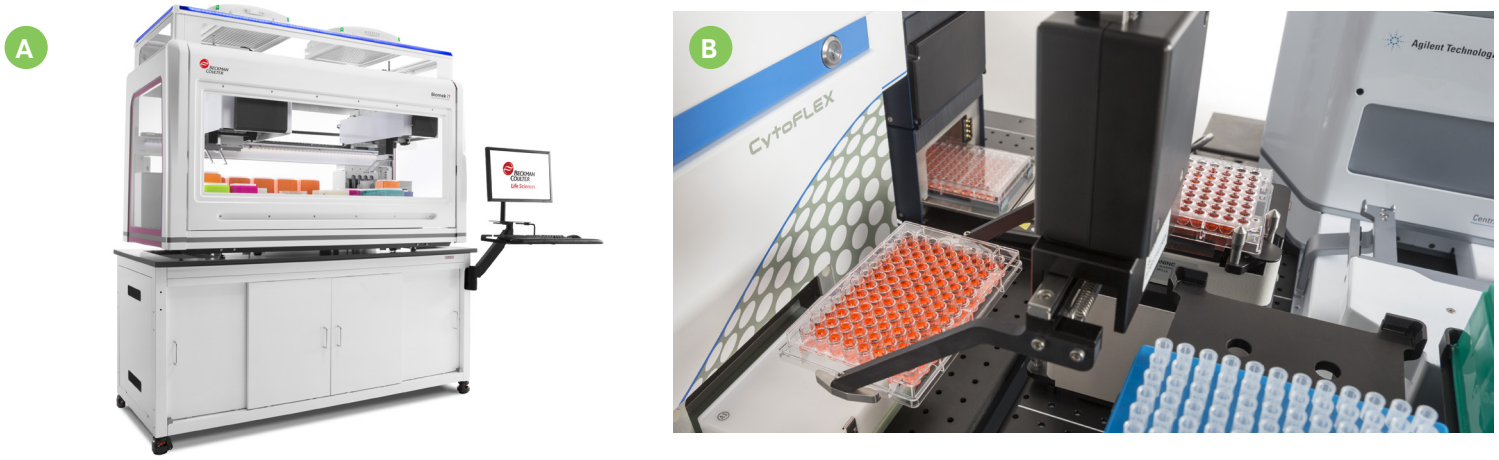
All qTOWER³ models include the qTOWER³ system, Analytik Jena's PC-based qPCRsoft software to conduct qPCR and perform analysis, 115V power cable, USB cable, and user manual.

Description	Cat. No.
qTOWER3 Real-Time PCR	75830-232
qTOWER3 G Real-Time PCR	75830-234
qTOWER3 touch Real-Time	75830-236
qTOWER3 G touch Real-Time PCR	75830-238

Fully-automated cellular analysis by flow cytometry

By Michael Kowalski, Jason Lowery, Nancy Dempsey, Tara Jones-Roe, Beckman Coulter Life Sciences, Indianapolis, IN



Flow cytometry is a widely-used and powerful tool for single-cell analysis — an essential ability for those studying heterogeneous cell populations. However, the need for cells to be in single-cell suspensions can result in a challenging sample preparation process. This can include trypsinization of adherent cells and/or centrifugation steps to remove staining reagents. Automating these steps can decrease the time at the bench, while improving reproducibility by ensuring consistent treatment (i.e., trypsin incubations) across samples. In addition, moving to a plate-based format increases the potential sample throughput.

Here we demonstrate how the Biomek i7 Automated Workstation (Figure 1A) was used to automate the complete cellular workflow for induction and analysis of apoptosis in two cancer lines. The Biomek instrument utilized its HEPA-filtered enclosure to maintain cell sterility during manipulations. In addition, the i-Series instruments enable simple and direct integrations, including the CytoFLEX Flow Cytometer configured with a plate loader (Figure 1B) used here, without the need for additional robotic transports.

FIGURE 1: **(A)** Biomek i7 Automated Workstation with HEPA filters **(B)** accessing an integrated CytoFLEX Flow Cytometer with plate loader.

We chose human leukemia (Jurkat) and colon carcinoma (HCT116) cell lines to demonstrate the workflows for both suspension and adherent cells. In both cases, 25,000 cells were plated in 96-well plates and after 24 hours, the selective tip feature of the multichannel head was used to serially dilute three compounds — staurosporine, camptothecin, and 5-fluorouracil. These apoptosis inducers were added to cells and incubated for 24-72 hours. Prior to staining, the HCT 116 cells were trypsinized, using an on-deck Peltier heating device for

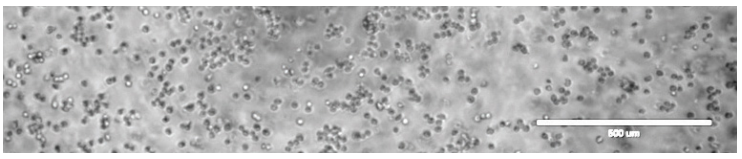


FIGURE 2: HCT116 cells following automated trypiniszation and resuspension.

incubation and the multichannel head was used for repeated pipetting to create a single-cell suspension (Figure 2). Both cell lines were incubated with Caspase-3/7 Green to identify cells undergoing apoptosis and DRAQ7 (Beckman Coulter) to label cells with compromised cellular membranes as a measure of cell death.

Cells were identified using forward and side scatter. Apoptosis and cell death stains were measured in the FITC and PC5.5 fluorescence channels respectively. Analysis plots were generated in Kaluza 1.5 software. Figure 3A shows the viable, apoptotic, and dead HCT116 cells for a high, medium, and low dose of 5-fluorouracil treatment at 48 hours, and Figure 3B shows the 48 hour dose response curve and calculated IC50s for all three compounds, illustrating the effectiveness of the automated serial dilutions. Figure 4A shows the progression of Jurkat cells through the cell death pathway over time following a treatment with 78 nM camptothecin. The change in the percentage of cells in each condition is plotted in Figure 4B. This drug time course was made simple by multichannel selective tip pipetting, which enabled the cells to be platedonce, the drug dilutions stamped into replicate wells, and one set of wells be harvested per time point.

While the reagents used here did not require washes, the ability to directly integrate microplate centrifuges (Figure 1B), plate washers, and incubators to the i-Series instruments means that antibody-based workflows that discriminate populations in a heterogeneous mixture can also be easily automated. In addition, if samples need to be processed by a tube-based flow cytometer, the Span-8 pipettors can be used to rapidly process samples in tubes or perform a final transfer from plates to tube prior to analysis. Finally, for high throughput applications SAMI EX software can be used to schedule staining and analysis workflows to ensure consistent treatment across plates.

*Data obtained during development. For Research Use Only. Not for use in diagnostic procedures.

Description	Cat. No.
CytoFLEX System, B4-RO-VO	76330-530
CytoFLEX System, B3-R1-VO	76330-090
CytoFLEX System, B2-RO-V2	76330-092
CytoFLEX System, B2-R2-VO	76330-094

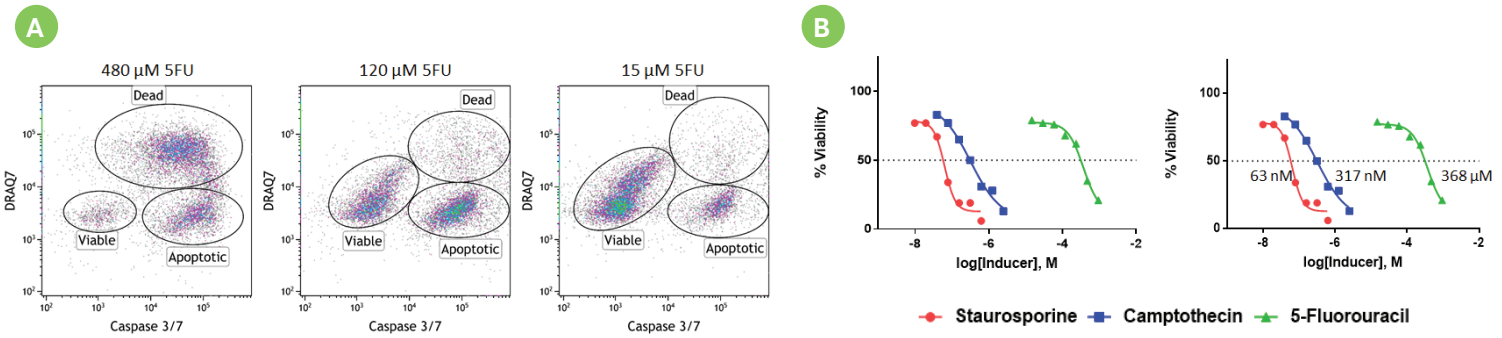


FIGURE 3: **A)** Dot plots showing HCT116 cell populations that are viable (unstained), apoptotic (caspase 3/7 positive), or dead (DRAQ7 positive) following 48 hours of 5-fluorouracil treatment. The cytotoxic effects are diminished as concentration decreases indicating effective serial dilutions. **B)** Dose response curves and IC50 values based on the percentage of viable HCT116 cells following 48-hour treatment with three apoptosis inducers.

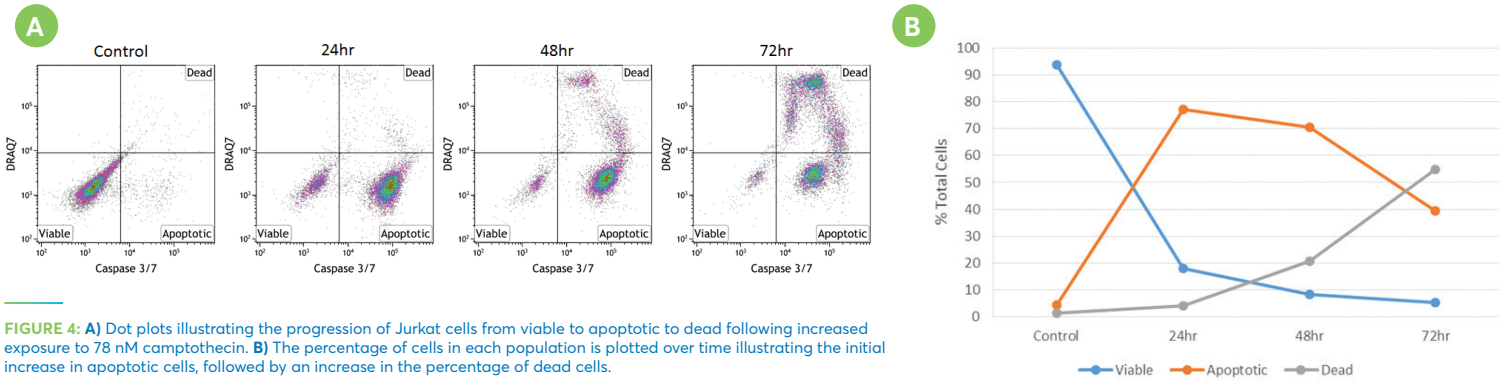


FIGURE 4: **A)** Dot plots illustrating the progression of Jurkat cells from viable to apoptotic to dead following increased exposure to 78 nM camptothecin. **B)** The percentage of cells in each population is plotted over time illustrating the initial increase in apoptotic cells, followed by an increase in the percentage of dead cells.

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Human FGF-basic (146aa)	10 µg	10771-944
Human FGF-basic (154aa)	10 µg	10778-898
Human GM-CSF	20 µg	10780-398
Human IL-2	50 µg	10779-566
Human IL-4	20 µg	10779-606
Human M-CSF	10 µg	10773-706
Human SCF	10 µg	10780-450
Human TPO	10 µg	10773-600

Mirus

Easy optimization for high efficiency, low toxicity electroporation

INGENIO® EZPORATOR® ELECTROPORATION SYSTEM

- **Performance:** deliver any nucleic acid to hard-to-transfect stem and primary cells
- **Simplicity:** use a single, universal electroporation solution across all cell types
- **Flexibility:** easily optimize electroporation parameters for each cell type

The Ingenio® EZporator® Electroporation System was developed for efficiency, simplicity, and for use with the Ingenio® Electroporation Kits and Solution. Optimization is simple and protocols for various cell types are available. Every system comes with a complimentary Ingenio® Electroporation Kit (8 reactions, 0.4cm cuvettes) and an easy-to-use manual.

Description	Size	Cat. No.
Ingenio® EZporator® Electroporation System	—	76304-532
Ingenio® Electroporation Solution and Kits		
Ingenio® Electroporation Solution	25 Reactions	10766-836
Ingenio® Electroporation Solution	100 Reactions	10766-848
Ingenio® Electroporation Kit with 0.2 cm Cuvettes	25 Reactions	10766-838
Ingenio® Electroporation Kit with 0.2 cm Cuvettes	100 Reactions	10766-850
Ingenio® Electroporation Kit with 0.4 cm Cuvettes	25 Reactions	10766-840
Ingenio® Electroporation Kit with 0.4 cm Cuvettes	100 Reactions	10766-990

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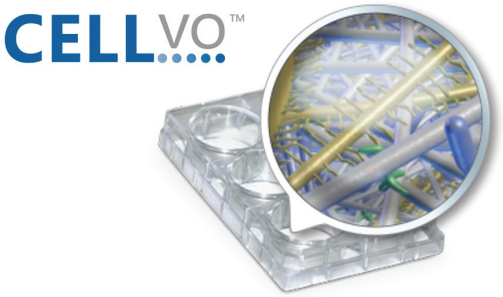
CELLVO™ MATRIX

USE IN MAMMALIAN, ADHERENT CELL CULTURE TO AVOID UNNATURAL CELL-MATRIX INTERACTIONS

- Cells grown on CELLvo Matrix express a more natural gene profile and reduced donor-donor variability
- Cells grown on CELLvo Matrix exhibit more natural responses to experimental variables
- CELLvo Matrix enhances isolation of primary cells



CELLvo Matrix is an extracellular matrix of proteins synthesized *in vitro* by bone marrow stromal cells and contains more than 150 proteins that were secreted and assembled by the cells during production. The final product is cell free with only the ECM attached to the surface of the dish providing a native microenvironment for rapid expansion of hiqh quality adherent cells...There is no place like home!

Description	Growth Area	Cat. No.	Unit
CELLvo Matrix			
6-Well Flat Bottom Plates	9.5 cm^2	**75853-938**	**Pack of 5**
T75 Vented Flasks	75 cm^2	**75853-856**	**Pack of 5**
T150 Vented Flasks	150 cm^2	**75853-858**	**Pack of 5**
CELLvo Xeno-Free (XF) Matrix			
XF-Matrix 6-Well Flat Bottom Plates	9.5 cm^2	**76183-420**	**Pack of 5**
XF-Matrix T75 Vented Flasks	75 cm^2	**76183-422**	**Pack of 5**
XF-Matrix T150 Vented Flasks	150 cm^2	**76183-424**	**Pack of 5**

CORNING

Rock the science of 3D

CORNING® SPHEROID MICROPLATES

With a novel and proprietary design, these microplates are ideal for generating and analyzing 3D multicellular spheroids in the same microplate. The Ultra-Low Attachment (ULA) coating enables uniform and reproducible 3D multicellular spheroid formation. The black opaque microplate body shields each optically clear, round well bottom from well-to-well cross talk. All plates listed here are sterile.

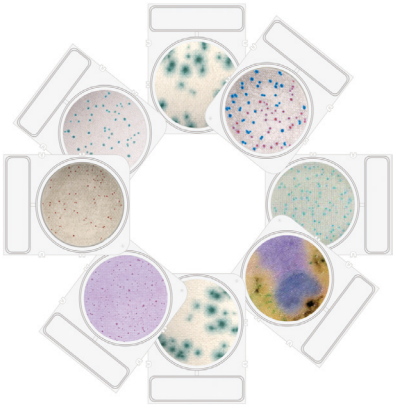
Plate Format	Working Volume, µL	Cat. No.	Case of
96-Well	75-300	**10185-094**	**5**
96-Well	75-300	**10037-558**	**50**
384-Well	20–90	**10185-096**	**5**
384-Well	20–90	**10037-556**	**50**
1536-Well	10–12	**76311-728**	**5**
1536-Well	10–12	**76210-918**	**50**

There's always a better way

COMPACT DRY™

- Rigid transparent frame with removeable lid keeps your colonies intact when subculturing
- Self-diffusing dry media tranforms into gel with the 1mL sample — no need for an extra step
- 18-month shelf life, room temperature storage, and stackable

The Compact Dry plate line presents a variety of organism-specific and total count tests, packaged up nicely in a sophisticated and sturdy cassette. This product line was designed for the end user with the intent to save technicians time while performing high-throughput colony counts on food & beverage, cannabis, cosmetic, environmental, meat, and raw material samples.



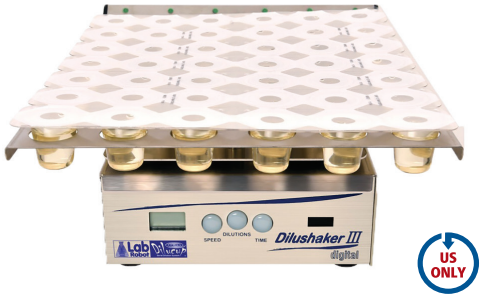
Description	Application	Cat. No.	Unit
Compact Dry EC	EC — E. coli and Coliforms	10145-954	Pack of 100
CompactDry ETB	ETB — Enterobacteriaceae	10753-986	Pack of 100
CompactDry LS	LS — Listeria spp.	10789-408	Pack of 100
CompactDry X-SA	X-SA — Staphylococcus aureus	10145-970	Pack of 100
CompactDry SL	SL — Salmonella spp.	10789-458	Pack of 100
CompactDry YM	YM — Yeast and Mold	10145-972	Pack of 100
CompactDry YMR	YMR — Yeast and Mold Rapid Test	76076-714	Pack of 100
CompactDry TC	TC — Total Plate Count	10145-968	Pack of 100

Save time and money with sterile, pre-filled dilution vials!

DILUCUP™ ELEGANCE

- The Dilushaker™ uses orbital rotations to make sure each diluent is thoroughly vortexed the same way, every time
- Simplify your serial dilution process with attached, pre-filled vials that are easy to open
- Save bench space with this small unit and refrigerator space with Dilucups that are easily stackable and store at room temperature

This automated, vortexting system maximizes lab efficiency with pre-filled, sterile, and ready-to-use dilution cups. Bringing automation to your vortexing process virtually eliminates variable results from tech to tech. The Dilucup line also reduces the occurrence of repetitive motion injuries with its ergonomic design.



US ONLY

Description	Cat. No.
Dilushaker III Digital, 3 Rows, LED, 21 Cups, 7x3	75860-388
Dilushaker III Digital, 6 Rows, LED, 42 Cups, 7x6	75860-392
Dilucup Elegance Solutions	
Dilucup Elegance BPW, 32 Trays, 7 x 3 Cups, 9 mL	75860-416
Dilucup Elegance BPW, 16 Trays, 7 x 6 Cups, 9 mL	75860-418
Dilucup Elegance MRD, 32 Trays, 7 x 3 Cups, 9 mL	75860-406
Dilucup Elegance MRD, 16 Trays, 7 x 6 Cups, 9 mL	75860-408
Dilucup Elegance NaCl, 32 Trays, 7 x 3 Cups, 9 mL	75860-412
Dilucup Elegance NaCl, 16 Trays, 7 x 6 Cups, 9 mL	75860-414

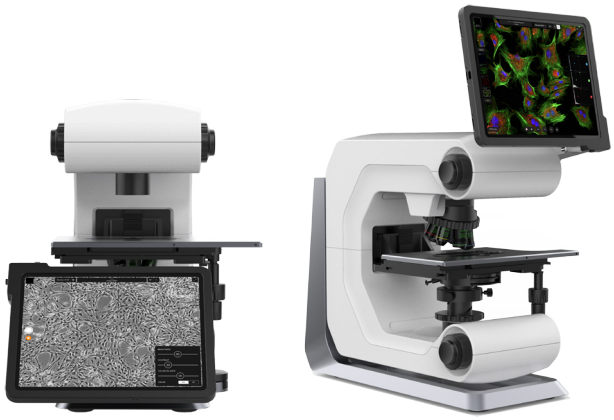
These specific products are not available in Canada. Please contact your Life Science Specialist to learn about similar options available in your region.

The world's first and only HYBRID microscope

REVOLVE4 UPRIGHT, INVERTED, BRIGHTFIELD, FLUORESCENT MICROSCOPE, ECHO

- (4) Modes Included: Upright + Inverted + Brightfield + Fluorescence
- (2) Cameras Included: BF 12mp CMOS + FL 3.2mp sCMOS
- Objectives and condensers by Olympus
- Sputter hard coated filter sets by Chroma
- Ultra high resolution 13" retina touch display
- Motorized LED Fluorescence Turret
- Capabilities of 2 instruments for the price of 1

Revolve is significantly easier to use. No longer are years of training and experience required to drive your microscope. You'll feel and appreciate Revolve's familiar app interface with responsive touch control.



Description	Cat. No.
REVOLVE4 Upright, Inverted, Brightfield, Fluorescent Microscope	76299-574

Highly efficient and cost effective

Free samples available at **vwr.com/mirusbio**

INGENIO® ELECTROPORATION SOLUTION AND KITS

ELECTROPORATION USING ANY INSTRUMENT

- **High Efficiency Electroporation —** Deliver to hard-to-transfect cell lines and primary cells
- **Compatible with Conventional Electroporation Devices —** Use your existing system including Lonza Amaxa®, Bio-Rad®, or Harvard BTX®
- **Cost Effective —** Ingenio Electroporation Solution is available as a stand-alone solution or as part of a complete kit with cuvettes and cell droppers

Ingenio electroporation solution and kits provide a universal, high efficiency, low toxicity solution for electroporation of DNA, small RNA (siRNA, miRNA, CRISPR gRNA), or Cas9 RNP into hard-to-transfect cell types. Electroporation using Ingenio affords increased gene expression in several different cell types with minimal toxicity. Ingenio is also compatible with multiple conventional electroporation instruments including Lonza Amaxa Nucleofector®, Bio-Rad Gene Pulser®, and Harvard-BTX.

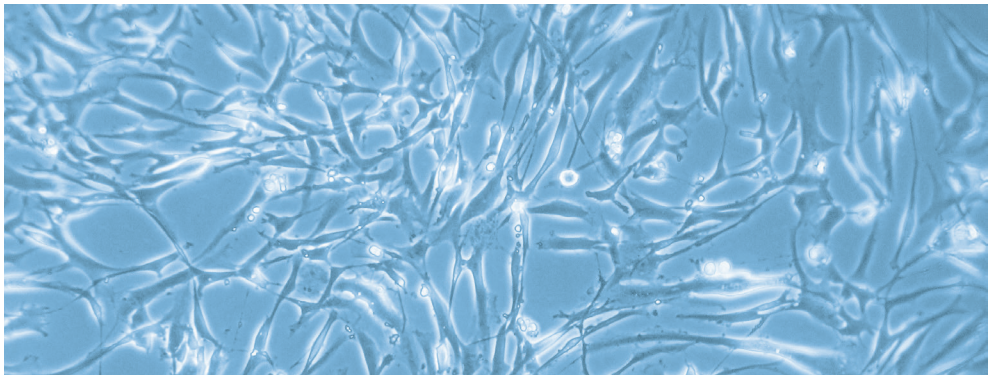
US ONLY

Description	Number of Reactions	Cat. No.
Ingenio Electroporation Solution	25	10766-836
Ingenio Electroporation Solution	50	10766-842
Ingenio Electroporation Solution	100	10766-848
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	25	10766-838
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	50	10766-844
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	100	10766-850
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	25	10766-840
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	50	10766-846
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	100	10766-990

These products are not currently available in Canada. Please contact your VWR Life Specialist to learn about similar options available in your region.

PromoCell

Reliable results for your research



MESENCHYMAL STEM CELL AND MEDIA SYSTEM

- **Defined Cells.** Each MSC batch is tested in accordance with ISCT* recommendations for CD73+/CD90+/CD105+ and CD14-/CD19-/CD34-/CD45-/HLA-DR
- **Guaranteed Performance.** Each of our MSC lots is guaranteed to grow with a doubling time of <30h through at least 10 population doublings and are also tested for differentiation into osteoblasts, adipocytes, and chondrocytes
- **Complimentary Research Tools.** PromoCell offers a comprehensive portfolio of cell biology research products including Growth & Differentiation Factors, Stem Cell Fate Regulators, Antibodies & ELISAs, Cellular Analysis, and Cell Transfection

PromoCell is a premier manufacturer of cell culture products with a complete portfolio featuring human Mesenchymal Stem Cell (hMSC) culture media, growth media, and differentiation media. All of our hMSC are isolated in accordance to the highest ethical standards and extensively quality tested including marker characterization, growth performance, and *in vitro* differentiation in our media.

* ISCT (International Society for Cellular Therapy) Cytotherapy (2006) Vol. 8, No. 4, 315-317

Description	Size	Cat. No.	Unit
Human Mesenchymal Stem Cells			
MSC from Bone Marrow (hMSC-BM)	500.000 cryopreserved cells	**10172-152**	**Each**
MSC from Umbilical Cord Matrix (hMSC-UC)	500.000 cryopreserved cells	**10172-148**	**Each**
MSC from Adipose Tissue (hMSC-AT)	500.000 cryopreserved cells	**10172-156**	**Each**
Mesenchymal Growth Media			
MSC Growth Medium 2 (serum containing)	500 mL	**75812-652**	**Each**
MSC Growth Medium DXF (defined & xeno-free)	500 mL	**10172-384**	**Each**
MSC Differentiation Media			
MSC Adipogenic Differentiation Medium 2	100 mL	**75812-654**	**Each**
MSC Osteogenic Differentiation Medium	100 mL	**10172-378**	**Each**
MSC Chondrogenic Differentiation Medium	100 mL	**10172-376**	**Each**

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- Choices from multiple suppliers
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