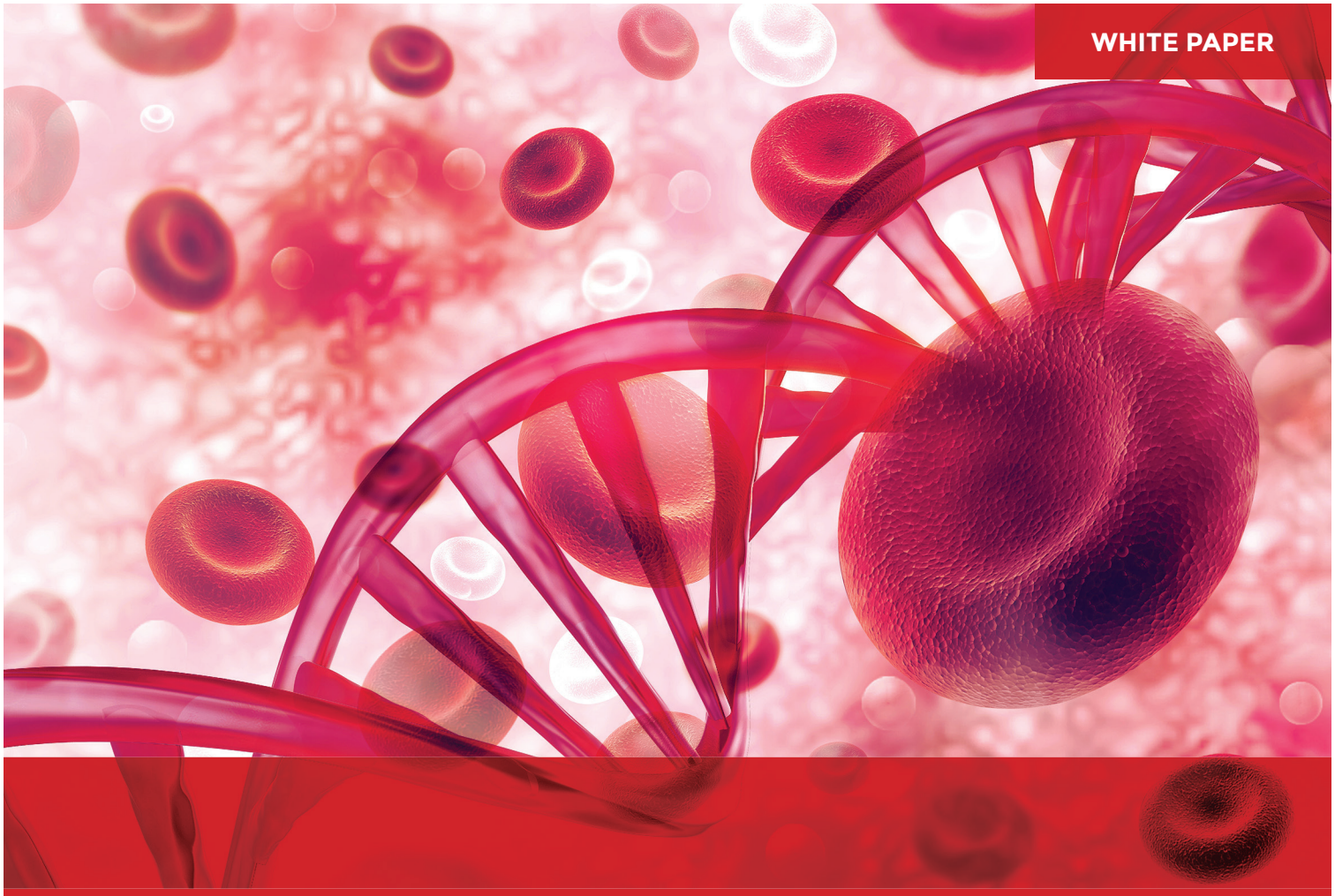
WHITE PAPER



Comparison of Yield and Size Distribution of cfDNA Extracted from Human Plasma Using the NextPrep-Mag™ cfDNA Isolation Kit and QIAamp® Circulating Nucleic Acid Kit

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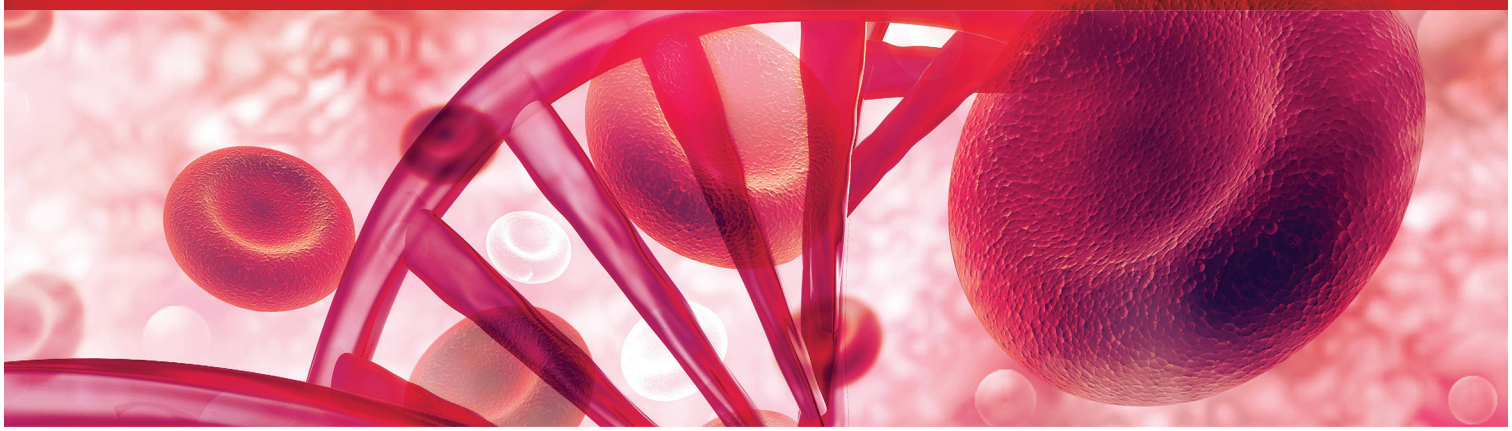


Introduction

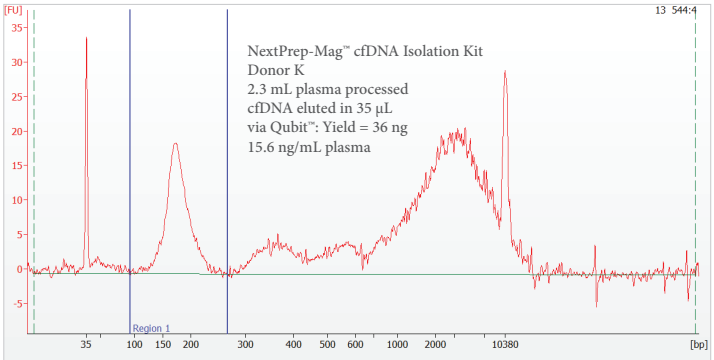
There has been a recent surge of interest in analysis of DNA recovered from cell-free biological fluids. Clinical research studies have demonstrated the feasibility of detecting genetic variants associated with recurrence of cancer after treatment, in cell-free DNA (cfDNA) extracted from blood plasma and urine. A substantial fraction of cfDNA is recovered as fragments of ~170 bp and multimers thereof, which is thought to reflect the association of cfDNA with the histone proteins that comprise nucleosomes. Larger fragments may also be recovered, even after removal of contaminating cells. The experiments reported below were carried out to compare yields and size distribution of cfDNA extracted using the NextPrep-Mag™ cfDNA Isolation Kit and QIAamp® Circulating Nucleic Acid Kit. Neither product is intended for the diagnosis, prevention, or treatment of disease.

Methods

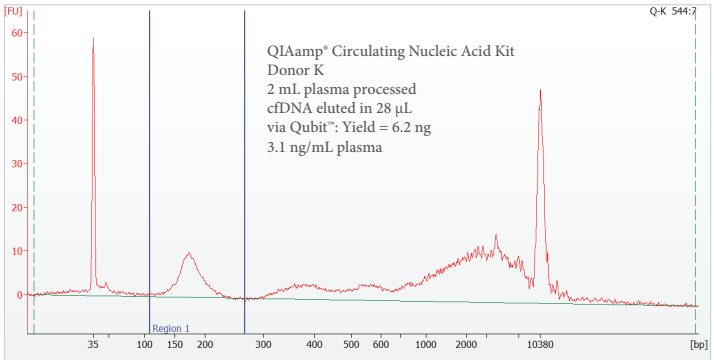
Platelet-poor plasma was obtained from healthy donors representing different ages, genders, and ethnic groups. cfDNA was extracted from plasma samples ranging from ~ 2 mL – 5 mL using the NextPrep-Mag™ cfDNA Isolation Kit, which is magnetic-bead-based, and using the QIAamp® Circulating Nucleic Acid Kit, which uses silica filters. Both products were used in accordance with their respective instructions. For samples processed using the Qiagen® QIAamp® Circulating Nucleic Acid Kit, carrier RNA was added as described in the protocol, and the lysates were passed through the silica filters using a vacuum pump and the column extenders included in the kit. The magnetic beads in the samples processed using the Bioo Scientific NextPrep-Mag™ cfDNA Isolation Kit were attracted using a DynaMag™-2 magnetic stand for microfuge tubes (Thermo Fisher Scientific®). Elution volumes were normalized based on plasma volume processed. The cfDNA was analyzed on Agilent® 2100 HS DNA chips to determine concentration and size distribution. Yields for some samples were also determined by fluorometric analysis on a Qubit™ instrument.

Results

Figure 1 shows a comparison of cfDNA yields and size distribution for cfDNA isolated from plasma samples processed from Donor K using the two kits. Samples were analyzed on an Agilent Bioanalyzer High Sensitivity DNA chip and using a Qubit™ High Sensitivity DNA kit.



From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size Distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]
94	267	138.8	27	177	13.3	653.94	5,712.6



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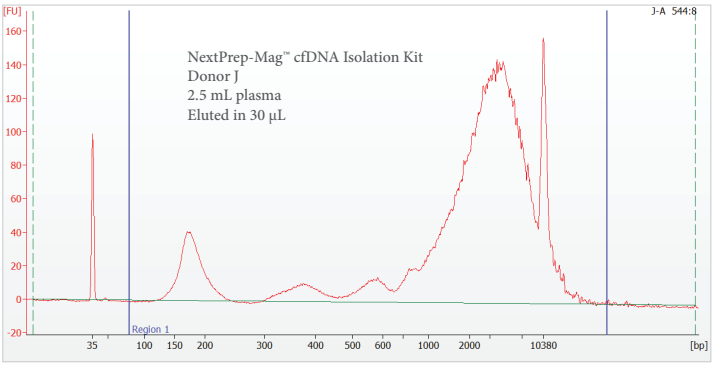
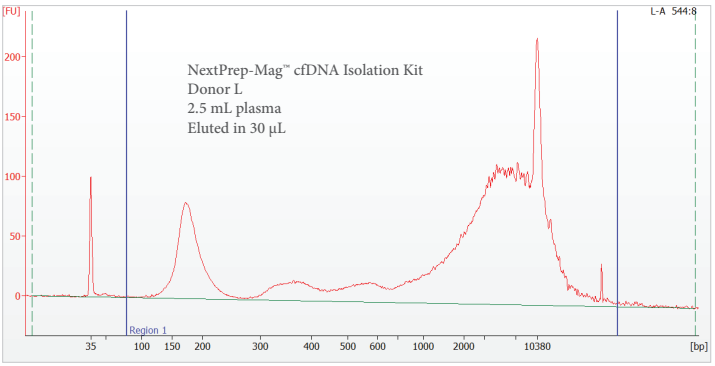
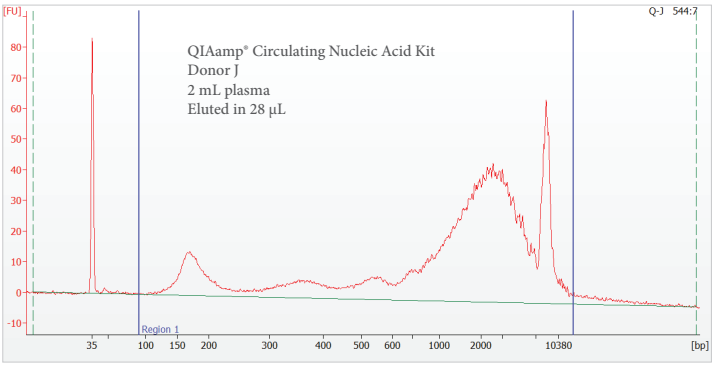
109	268	73.7	21	176	12.8	196.35	1,725.4

Figure 1. Yields and size distribution of cfDNA extracted from healthy donor's plasma with NextPrep-Mag™ cfDNA Isolation Kit (top panel) and QIAamp® Circulating Nucleic Acid Kit (bottom panel). Elution volumes were normalized for plasma volume processed. Yields were also determined by Qubit™. Donor K is 36 year-old white male.

Figure 2 shows comparison of cfDNA yields and size distribution for Donors J and L.







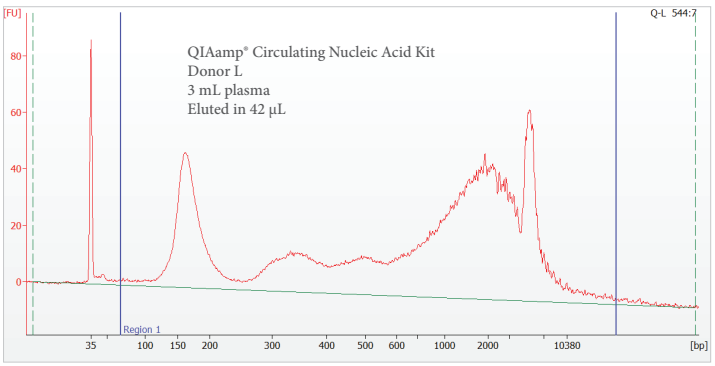
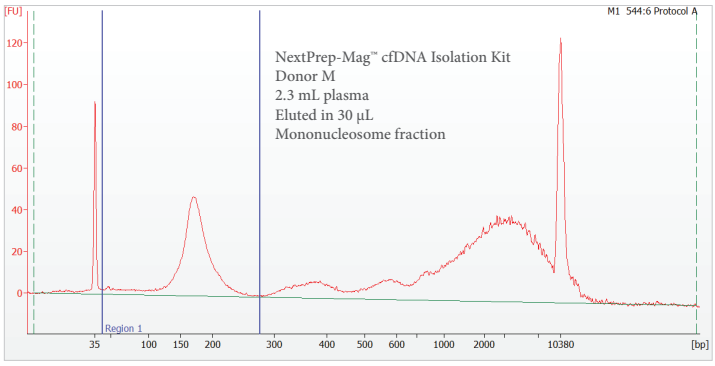
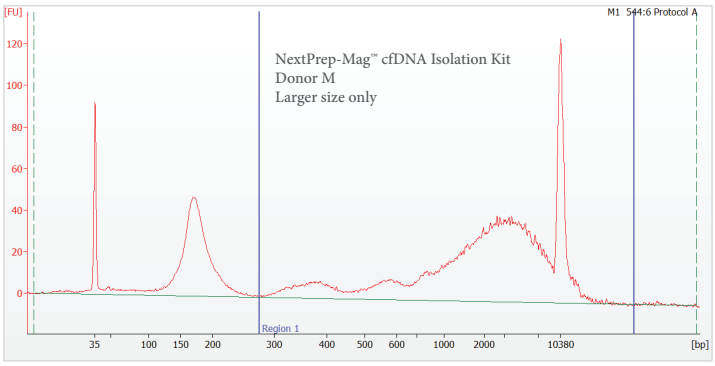
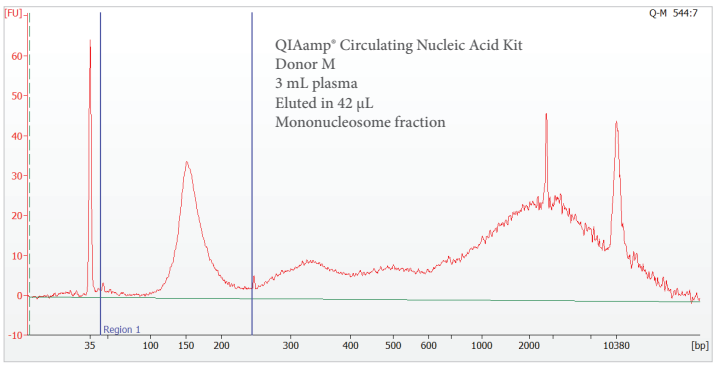


Figure 2. Relative yields and size distribution of cfDNA extracted from two more healthy donors' plasma with two kits. Samples processed and analyzed as described in Fig. 1. Donor J is 18 year-old black male, Donor L is 47 year-old white male.

Figure 3 shows comparison of cfDNA yields and size distribution for Donor M.







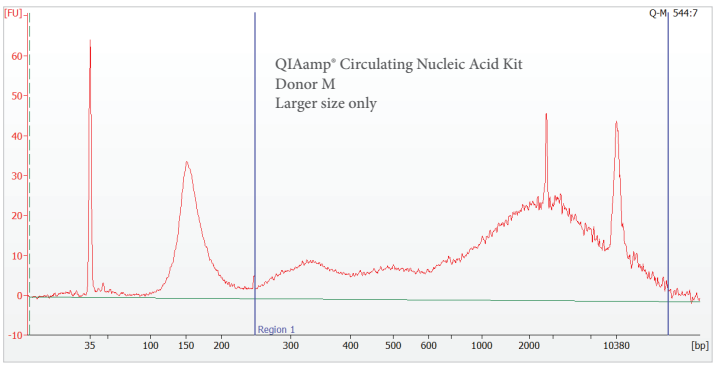


Figure 3. Relative yields and size distribution of cfDNA extracted from healthy donor M plasma with two kits. Samples processed as described in Fig. 1. Quantitation of indicated subregions is shown, as determined by Bioanalyzer software. The fraction of cfDNA derived from mononucleosomes is the peak at ~170 bp. Donor M is a 46 year-old Hispanic male.

Figure 4 shows overlay of cfDNA recovered from 5 mL plasma from Donors J and K shown in Figures 1 and 2, processed in a subsequent experiment.

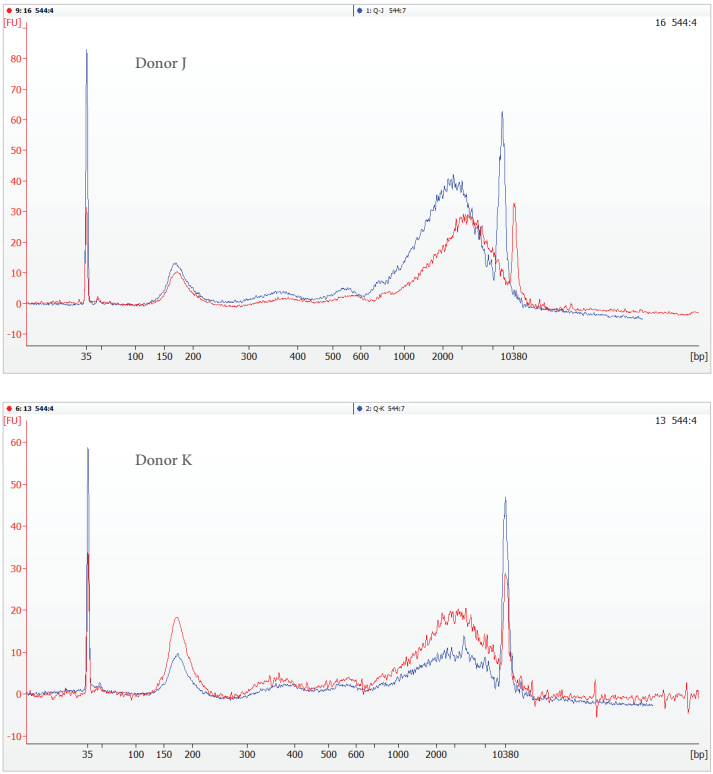


Figure 4. Overlays of cfDNA extracted using Bioo Scientific and Qiagen® kits. cfDNA was extracted from the same two healthy donors (J and K) shown in Figs. 1 and 2, but at a later time and from larger volume of plasma (5 mL). cfDNA was eluted in 60 µL. Top panel shows overlays for Donor J. Blue trace is Qiagen® sample and red trace is Bioo Scientific sample. Bottom panel shows overlays for Donor K. Blue trace is Qiagen® sample and red trace is Bioo Scientific sample.

Figure 5 shows overlays of cfDNA extracted from healthy donors N and P.

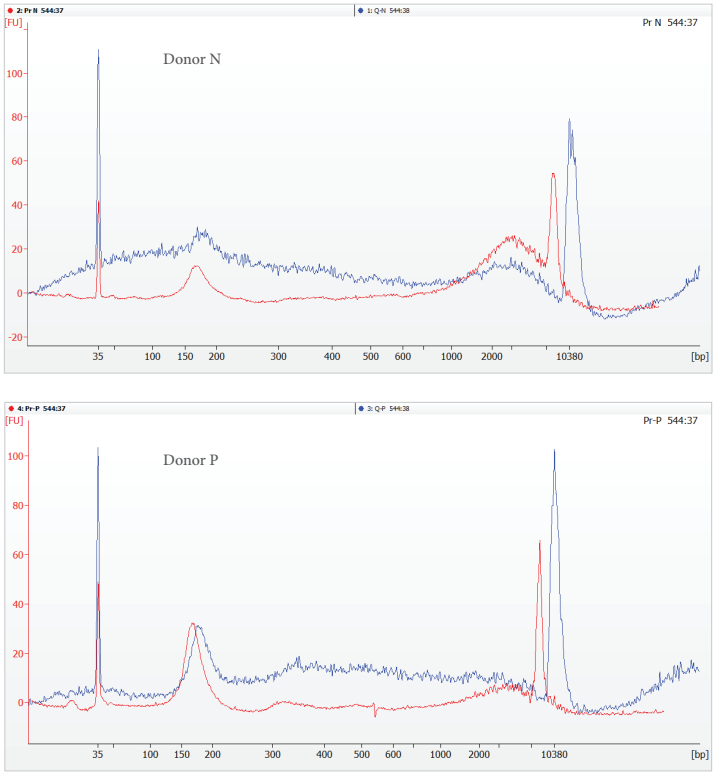


Figure 5. Overlay of cfDNA traces extracted from healthy Donors N and P. Plasma volumes of 3 mL were processed and eluted in 36 µL for all preps. Blue traces represent samples extracted using Qiagen® Circulating Nucleic Acid Kit. Red traces' samples extracted using NextPrep-Mag™ cfDNA Isolation Kit. Donor N is 33 year-old white male. Donor P is 52 year-old black female.

Figure 6 shows overlays of cfDNA extracted from donors R and S.

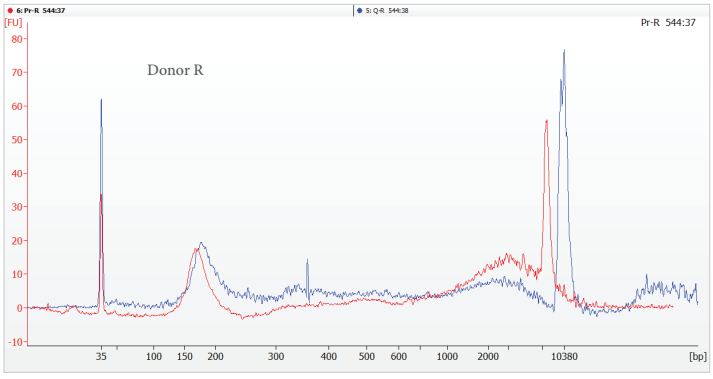
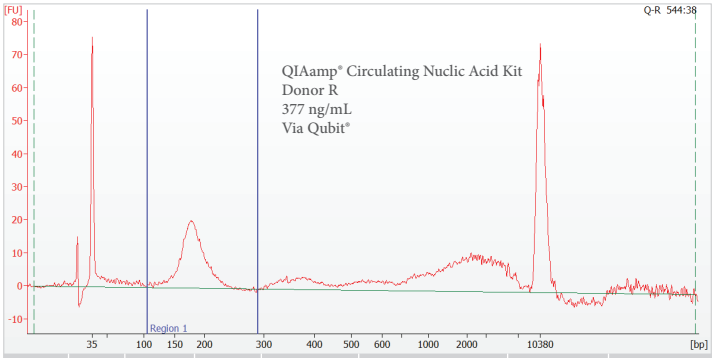
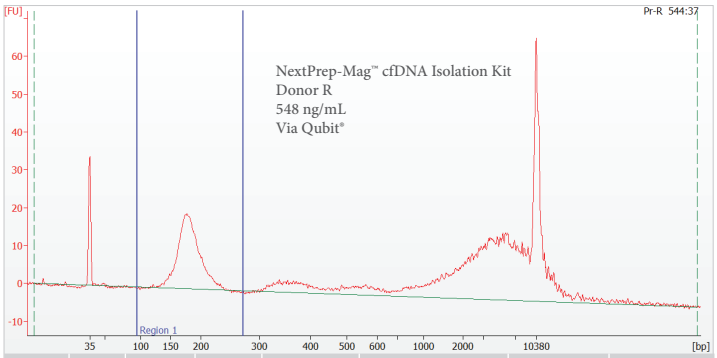


Figure 6. Overlay of cfDNA from healthy Donors R and S. cfDNA was extracted from 3 mL plasma and eluted in 36 µL. Blue traces are samples extracted using Qiagen® QIAamp® Circulating Nucleic Acid Kit. Red traces are samples extracted using Bioo Scientific NextPrep-Mag™ cfDNA Isolation Kit. Note very high MW material present in samples extracted using QIAamp® Circulating Nucleic Acid Kit (green arrow). Donor R is 57 year-old white male. Donor S is 46 year-old white male.

Figure 7 shows Bioanalyzer quantitation of mononucleosome peaks (~ 170 bp) in cfDNA samples extracted from Donor R and quantitation of the entire cfDNA size as determined using Qubit® assay.



From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size Distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]
106	290	153.3	37	180	13.1	209.10	1,796.3



From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size Distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]
95	273	135.5	34	181	10.7	296.13	2,503.4

Figure 7. Quantitation of cfDNA mononucleosome peaks via Bioanalyzer software. Concentration (pmolar and pg/µL) of mononucleosome peaks in the regions defined by the vertical blue lines is shown for cfDNA extracted from Donor R using QIAamp® Circulating Nucleic Acid Kit (left image) and NextPrep-Mag™ cfDNA Isolation Kit (right image). Concentrations are determined by comparison to the FU of the lower MW marker. Concentration of the total cfDNA (spanning the entire size range) as determined by Qubit® is shown in each image.

Figure 8 shows quantitation of cfDNA from Donors N, P, R, S extracted using Qiagen® and Bioo Scientific kits, as determined using the Qubit® High Sensitivity DNA assay. Samples were diluted 1:40 into the Qubit® reagent containing a dsDNA-specific fluorescent dye and read in the Qubit® fluorometer. Concentrations were determined based on comparison to the high and low standards included in the kit.

Kit (Q: Qiagen® B: Bioo) / Donor (N, P, R, S)	1st Elution, 1:40	Re-elution, 1:40	% in Re-elution	Yield (from 1st Elution)	Yield/mL plasma, 1st Elution
Q/N	5.95	3.63	38%	8.6 ng	2.9 ng/mL
Q/P	10.2	4.53	31%	14.7 ng	4.9 ng/mL
Q/R	9.43	4.09	30%	13.6 ng	4.5 ng/mL
Q/S	13.2	4.88	27%	19 ng	6.3 ng/mL
B/N	18.2	5.73	24%	26.2 ng	8.7 ng/mL
B/P	16.6	4.27	20%	23.9 ng	8 ng/mL
B/R	13.7	3.57	21%	19.7 ng	6.6 ng/mL
B/S	14.3	4.15	22%	20.6 ng	6.9 ng/mL

Figure 8. Qubit® quantitation of cfDNA from Donors N, P, R, and S extracted using Qiagen® QIAamp® Circulating Nucleic Acid and Bioo Scientific NextPrep-Mag™ cfDNA Isolation Kits. All were extracted from 3 mL plasma and eluted in 36 µL, then all were re-eluted in 36 µL additional elution solution. Qubit® values are for 1:40 dilutions.

Figure 9 shows cfDNA extracted from plasma from a patient with advanced colorectal cancer, using the Qiagen® and Bioo Scientific kits. The sample extracted from the Qiagen® kit shows very high molecular weight material that is not present in the Bioo Scientific-extracted sample.

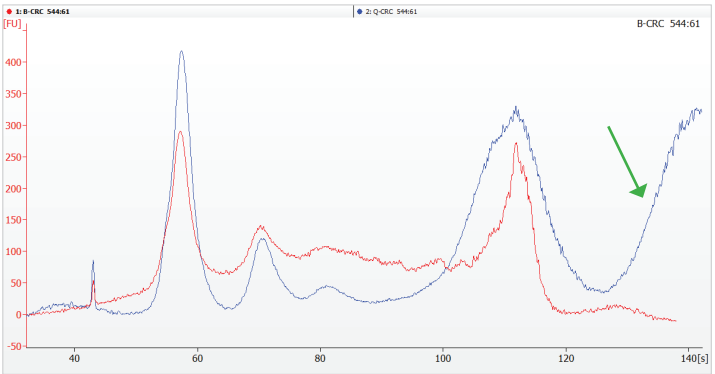


Figure 9. cfDNA from cancer patient plasma, extracted using QIAamp® Circulating Nucleic Acid Kit and NextPrep-Mag™ cfDNA Isolation Kit. Plasma from a 66 year-old male, colorectal cancer patient (Stage IV at diagnosis, high grade, with metastatic disease in lymph nodes and liver) was purchased from ProMedDx®. cfDNA was extracted from 3 mL using the QIAamp® Circulating Nucleic Acid Kit (Q-CRC, blue trace) and from 3 mL using the NextPrep-Mag™ cfDNA Isolation Kit (B-CRC, red trace). The cfDNA was eluted in 50 µL for both preps and analyzed on a HS DNA chip. The image shows an overlay of the traces, without manually setting upper and lower markers. The extracted sample from the QIAamp® Circulating Nucleic Acid Kit has very high MW material (green arrow) not seen in the NextPrep-Mag™ cfDNA Isolation Kit-extracted sample.

Discussion

Yields of plasma cfDNA are similar or higher for samples processed using the NEXTPrep-Mag™ cfDNA Isolation kit compared to samples processed using the QIAamp® Circulating Nucleic Acid Kit. In most cases size distribution is also similar, with significant amounts of high molecular weight DNA being recovered from some samples. The QIAamp® Circulating Nucleic Acids Kit recovers additional material that migrates as very high molecular weight DNA from some samples that do not contain this fraction when processed using the NextPrep-Mag™ cfDNA Isolation Kit.

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