bionano

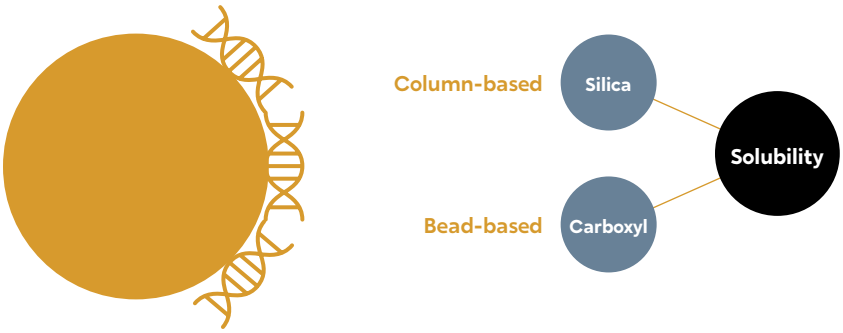
Ionic® Purification System for FFPE Samples

Why We Developed a New Approach to Nucleic Acid Purification

The commonly used bead- and column-based extraction technologies have followed the same fundamental workflow for over 20 years. This workflow uses ethanol, chaotropic salts, and other solutions to bind nucleic acid to a silica membrane or surface-labeled bead, which is then washed prior to the nucleic acid being stripped off the solid support into an elution buffer. During this typically laborious process, the nucleic acid may be denatured, dehydrated, and fragmented. The eluate is also susceptible to contamination from wash buffers or beads.

Traditional Purification

Separation Principle



Process and Characteristics

- **Surface-based → loss and damage**
- **Wash solution contamination**
- **Risk of bias** (e.g., GC content)

Disadvantages	Consequences
Incomplete binding to or removal from the solid support	• Nucleic acid loss compromises data quality when sample input quantity is limited • Recovery can be biased by fragment length or GC content • For researchers, reduced biological insight • For clinicians, less actionable information and false negatives
Contamination from wash buffers and bead coatings	• Low purity leading to false negatives and compromised data
Workflow with multiple hands-on steps	• Throughput bottleneck and potential errors; excessive use of disposable tips and labware

Isotachophoresis, a Superior Approach to Nucleic Acid Separation

Isotachophoresis (ITP) separates and concentrates charged molecules in solution solely based on their electrophoretic mobility. Biological samples are gently lysed and added to the Ionic® Fluidic Chip. An electric field is then applied to the chip and the nucleic acid is isolated in its natural, native form. The nucleic acid is not denatured or dehydrated, and there's no binding to, or stripping from, fixed surfaces. The result is a higher yield of pure nucleic acid that is less fragmented and free from bead or wash buffer contamination.

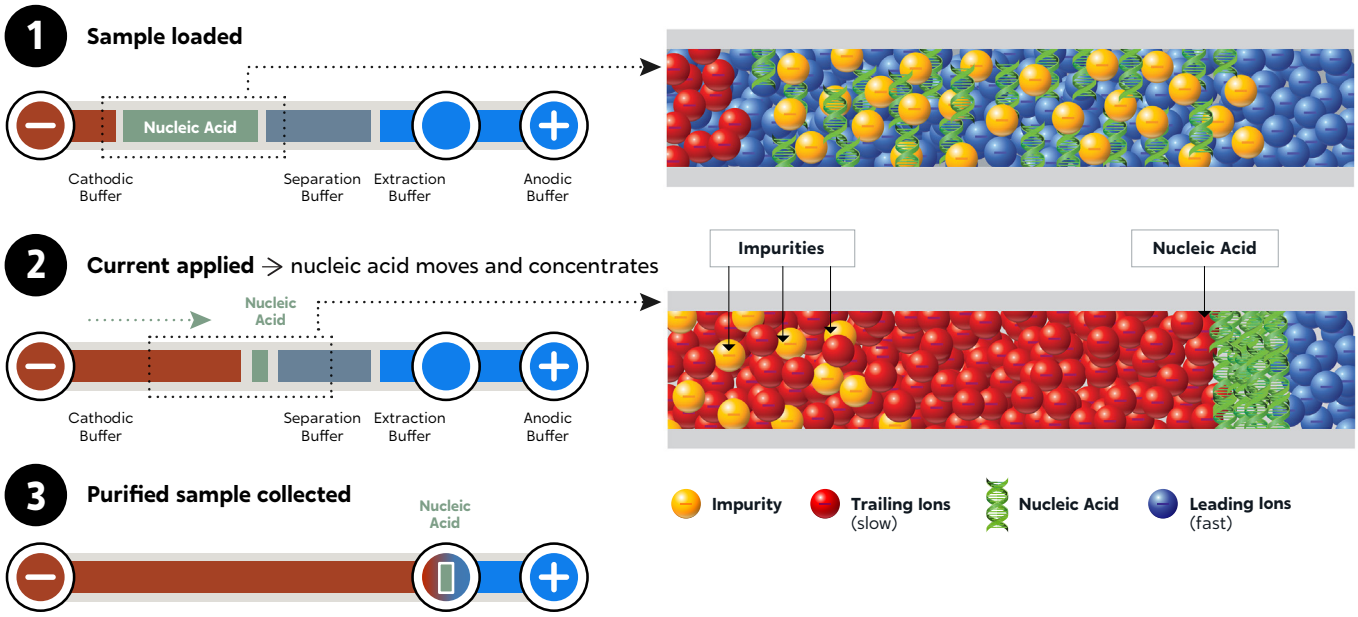
Isotachophoresis

Separation Principle

Process and Characteristics

- **Solution-based → high yield and integrity**
- **High purity**
- **Best representation of sample**
- **Result = better data**

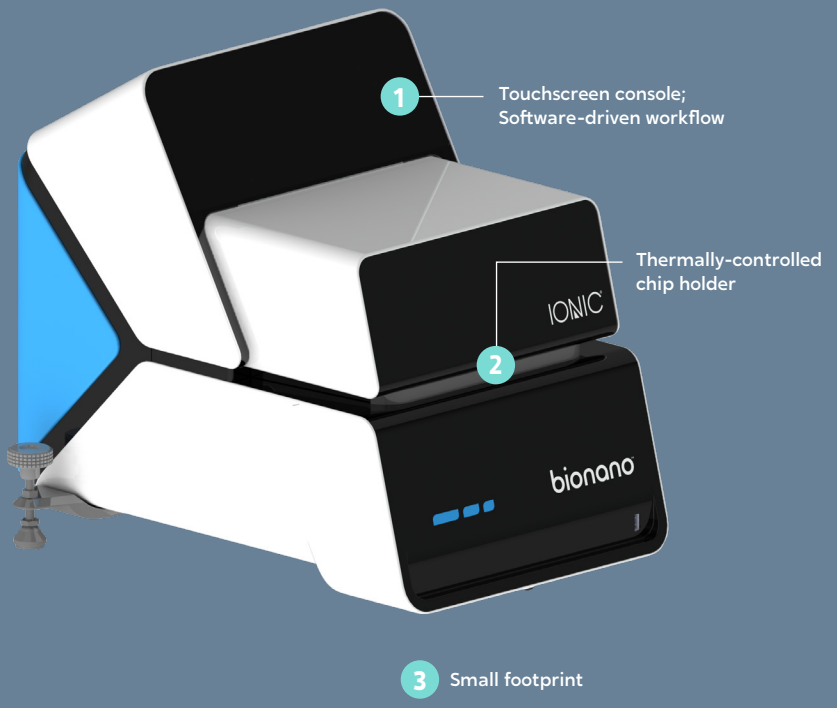
Simple, Automated Charge-based Sample Prep in Solution

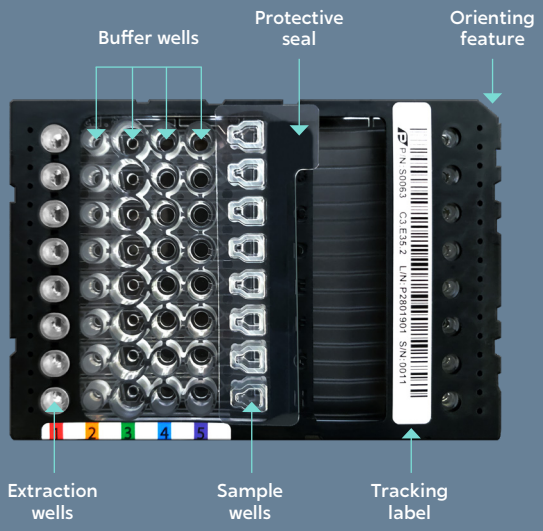


Ionic® Purification System

How it Works

The Ionic® Fluidic Chip is placed on the Ionic system and separation buffers are loaded. The chip is then primed. Next, biological samples are added into the 8 sample wells and purification using isotachophoresis begins. By applying an electric field across the length of a chip microchannel, the Ionic system separates and concentrates nucleic acid between buffers with higher and lower mobilities. Impurities fall behind the low mobility buffer and are separated from target nucleic acids. As target nucleic acids pass through the channel, an integrated sensor stops the current once nucleic acids reach the extraction well.





The Next-Gen Sample Preparation System

The revolutionary Ionic Purification System requires no binding, stripping, or washing from fixed surfaces for higher yields, higher quality nucleic acids, and ultimately, better data for your research.

- **No organic solvents**
- **No harsh, high-salt buffers**
- **No system programming**
- **No beads or repetitive washing**
- **No hands-on mixing, separation, sample transfers, or buffer exchanges**
- **No pumps, valves, or other moving parts**

Rapid Purification of Precious Samples in Just ONE Hour

Ionic System Workflow

1

Load Run Buffers

Add buffers from reagent kit –
use touchscreen to start priming

8 mins
(manual)

2

Load Samples

Load 8 samples –
use touchscreen to start the run

65 mins
(automated)

3

Get Purified Nucleic Acid

Collect samples when touchscreen displays
"Run Complete"

2 mins
(manual)

< 5 mins
Total Hands-on Time
per Sample

75 mins
Total Run-time

Simplified Nucleic Acid Preparation

The Ionic system is so different, its advantages are most readily understood in contrast with conventional nucleic acid extraction and purification methods:

Higher nucleic acid yields
No sample loss associated with binding nucleic acids to, or stripping from, fixed surfaces

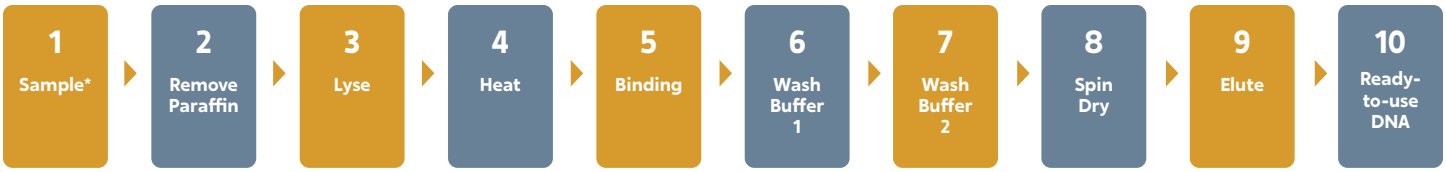
Simple workflows with fully automated separations
No columns or beads and no repetitive washing

Reduced nucleic acid fragmentation
No harsh high-salt buffers or organic solvents

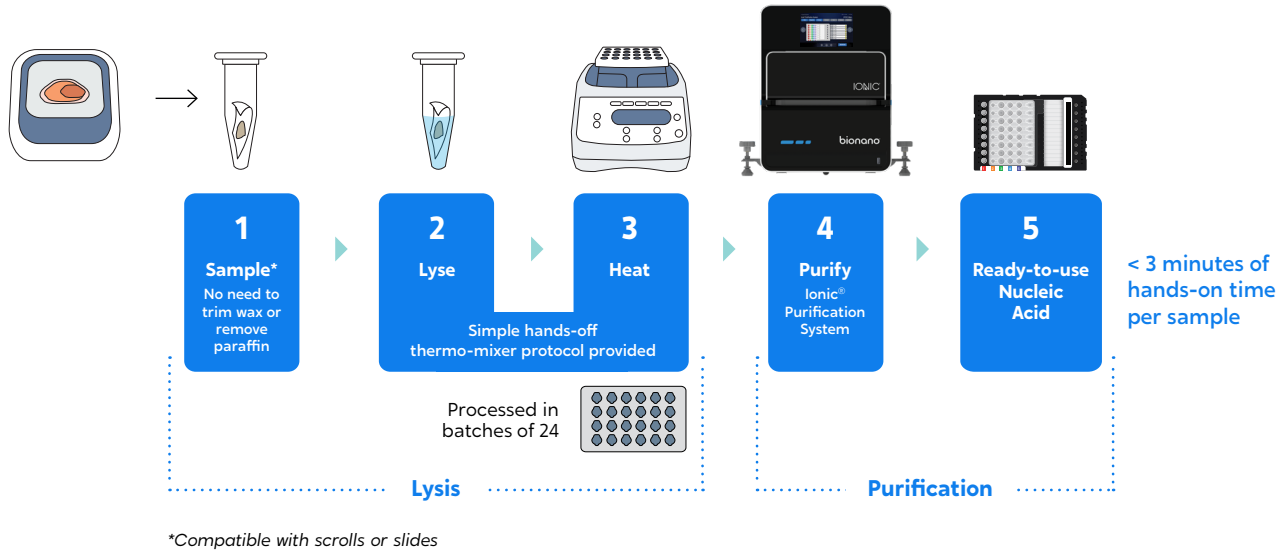
Simplified FFPE Workflow Saves Time and Money

Purigen's FFPE protocols greatly simplify the processing of FFPE samples. For example, the Ionic® FFPE to Pure DNA protocol reduces the hands-on time to less than 3 minutes per sample and enables working directly from scrolls. The protocol also eliminates the need for a separate paraffin removal step.

Column-based Kit Workflow



Isotachophoresis Workflow



Flexibility for Working with Scrolls or Slides

The Ionic system produces more DNA and RNA from FFPE samples without requiring the use of slides (slide use and microdissection is optional). The ability to obtain comparable nucleic acid yields when using scrolls (versus slide mounted FFPE slices) greatly simplifies the workflow when sample micro-dissection is not required. This allows projects to be completed faster and at a lower cost.

Superior Nucleic Acid Recoveries from FFPE Samples

A vast majority of clinical samples used in oncology research are stored as FFPE tissues, which often contain degraded or fragmented nucleic acid. Conventional extraction methods are labor intensive and can further damage nucleic acid during the extraction and purification process. The Ionic system simplifies and accelerates nucleic acid purification, resulting in higher yields of higher quality DNA.

Higher yields of DNA from FFPE Samples

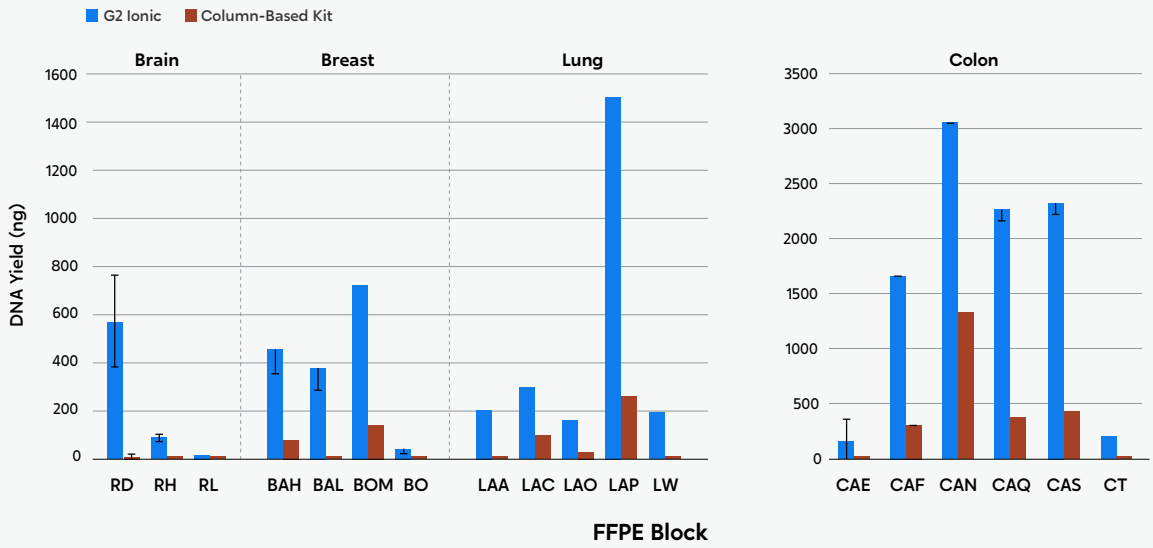


FIGURE 1: Comparison of DNA yields from consecutive sections of 18 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE DNA kit or a commercially available column-based kit. The total DNA yield for each sample was determined by multiplying the extract volume by the DNA concentration derived from the Qubit 1x dsDNA High Sensitivity assay. Error bars represent standard deviation from the average DNA yield. More DNA is recovered using the G2 FFPE DNA kit for each of the 18 FFPE blocks with expected increase in DNA yield of 3-5x on average compared to the column-based kit, with greater yield advantages for lower yielding samples.

Nucleic acid yields from scrolls using the Ionic Purification System are on average 3.5x higher when compared to yields from a column-based kit.

Higher Quality DNA vs. Column-based Kits

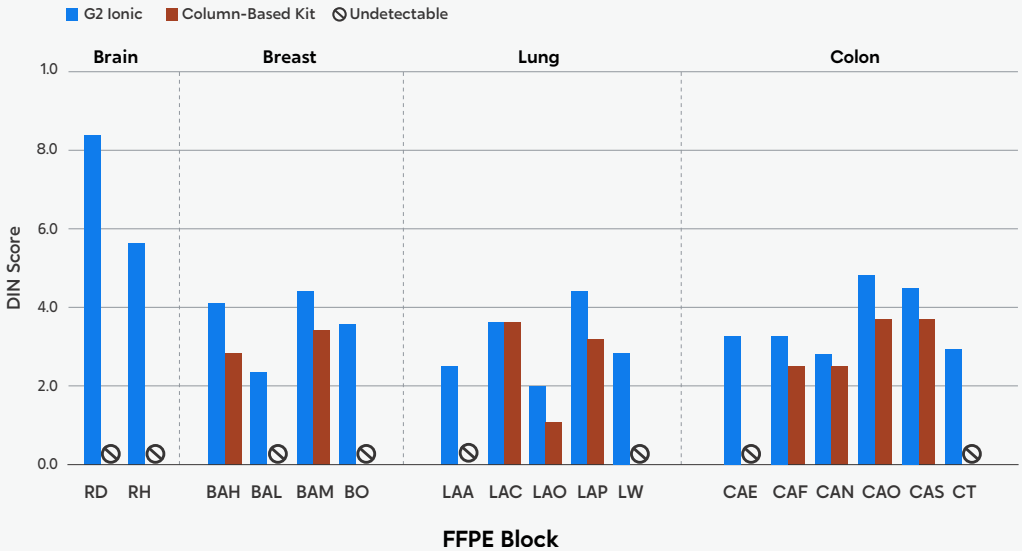


FIGURE 2: Comparison of DNA from consecutive sections of 18 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE DNA kit or a commercially available column-based kit. The DNA Intergity Number (DIN) was calculated for each extract using a Genomic DNA ScreenTape on the Agilent TapeStation. . The Ionic G2 DNA quality is typically higher than the column-based competitor, and nearly half of the column-based eluates had a concentration that was below that required for DIN score calculation.

Improved NGS Data Quality from FFPE Samples

In addition to increased yields and a greatly simplified workflow, data quality is also improved. Data below shows purified DNA from FFPE samples analyzed using the Agilent SureMASTR Tumor Hotspot sequencing panel which includes 252 amplicons ranging in size from 128–245 bps. To highlight coverage differences related to the sample purification technology, all data was normalized to a reference sample to remove the effect of coverage differences introduced by differences in target amplicon amplification efficiencies. The reference data set was generated using the average results obtained from the purification of a high-quality sample using both Ionic system and column-based techniques (as such the reference sample is not biased to either technology).

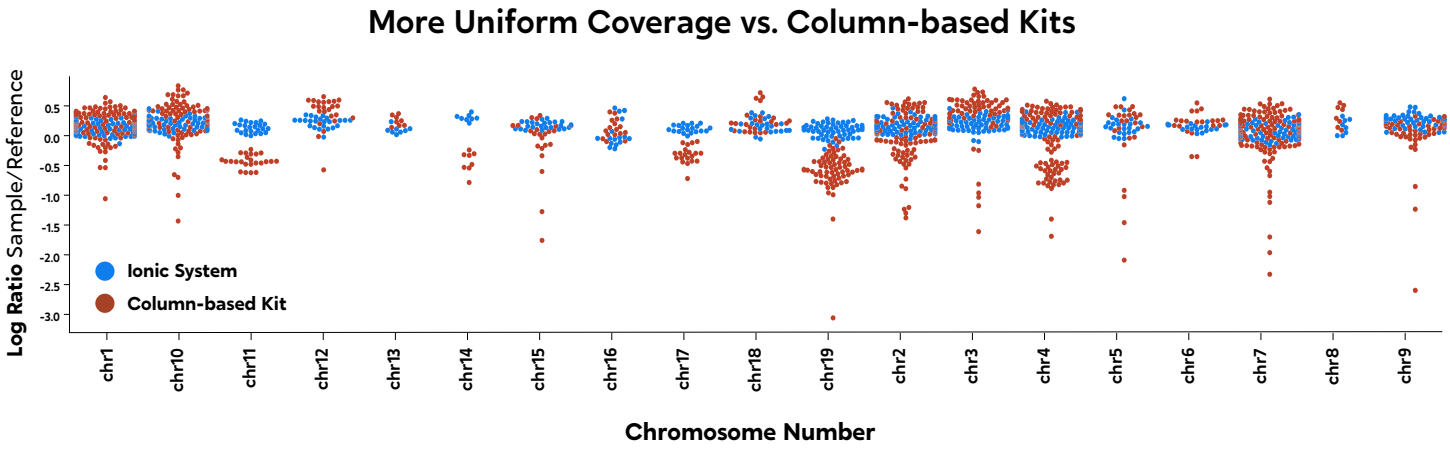


FIGURE 3: Results shown on a chromosome level. Ionic system eluates show tighter clustering more centered around the zero line. This is indicative of more uniform sequencing coverage.

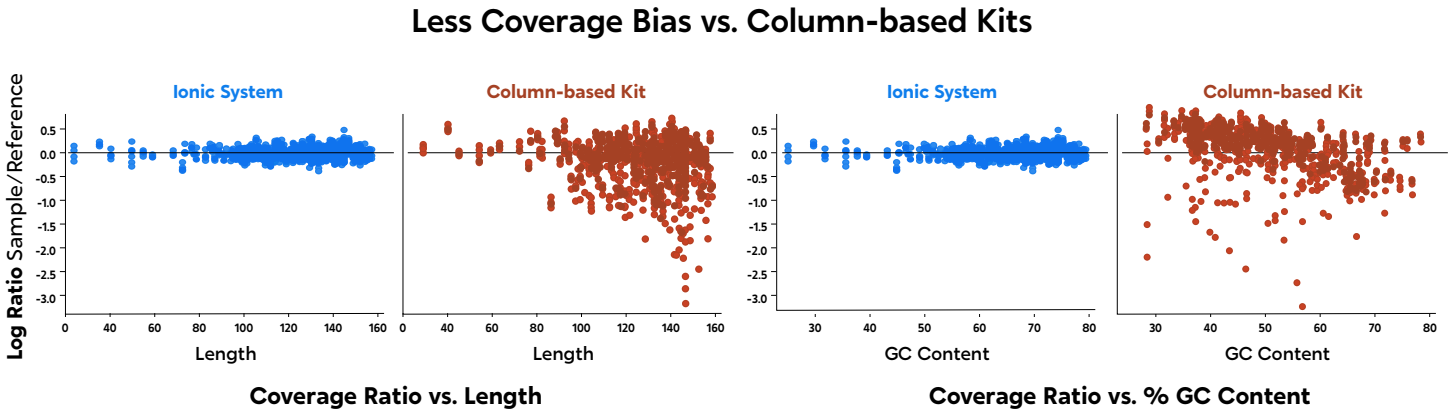


FIGURE 4: Results shown relative to amplicon length and amplicon GC content. Ionic system samples show superior uniformity for both amplicon length and GC content, with very little deviation from the expected coverage. Column-based purification shows a bias towards shorter amplicon lengths and lower GC content amplicons.

- Ionic system purification shows no bias towards amplicon length.
- Ionic system purification shows no bias towards GC content.

A Better Solution for RNA from FFPE Samples

The Ionic Purification System provides for the automated purification of RNA from FFPE tissue samples with less hands-on time than conventional bead and column-based methods. To help scientists overcome the sample preparation bottleneck commonly associated with FFPE samples, the Ionic system provides a simple workflow that co-purifies both mRNA and miRNA with higher yields versus column-based extraction kits.

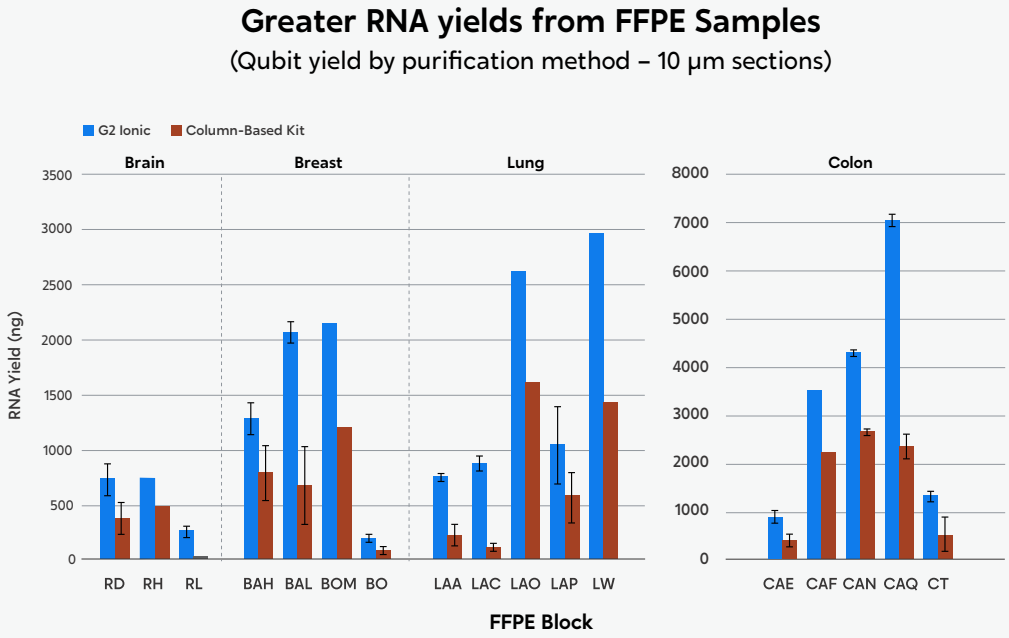


FIGURE 5: Comparison of RNA yields from consecutive sections of 17 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE RNA kit or a commercially available column-based kit. The total RNA yield for each sample was determined by multiplying the extract volume by the RNA concentration derived from the Qubit HS RNA assay. Error bars represent standard deviation from the average RNA yield. More RNA is recovered using the G2 FFPE RNA kit for each of the 17 FFPE blocks with expected increase in RNA yield of 2-3x on average compared to the column-based kit

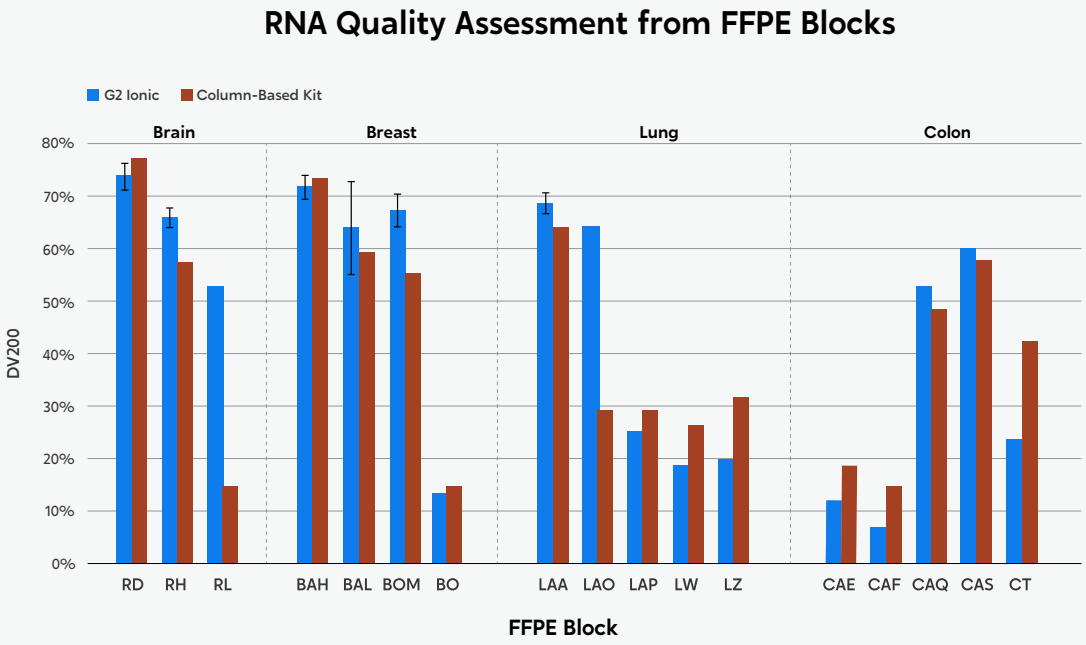


FIGURE 6: Comparison of RNA quality from consecutive sections of 17 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE RNA kit or a commercially available column-based kit. All RNA extracts were normalized to 1.5 ng/ul input concentration and the DV200 (percentage of RNA fragments >200 bp) was calculated using a High Sensitivity RNA ScreenTape on the Agilent Tapestation. For higher quality FFPE blocks (DV200 >30%) the G2 Ionic RNA quality is consistent with or higher than the column-based kit. Since the Ionic purification process co-extracts short RNAs ,such as miRNA, the G2 Ionic DV200 values may appear lower than the column-based kit for especially poor quality FFPE blocks (DV200 <30%).

Get Higher miRNA Yields from FFPE Samples

For researchers studying either the relationship between gene expression and microRNA expression or focusing purely on microRNA expression in FFPE tissue samples, the Ionic system provides more miRNA than the market-leading column-based miRNA kit. More impressive, is that no additional steps are required. The Ionic FFPE to Pure RNA kit produces both mRNA and more miRNA from FFPE samples in a single, simple workflow.

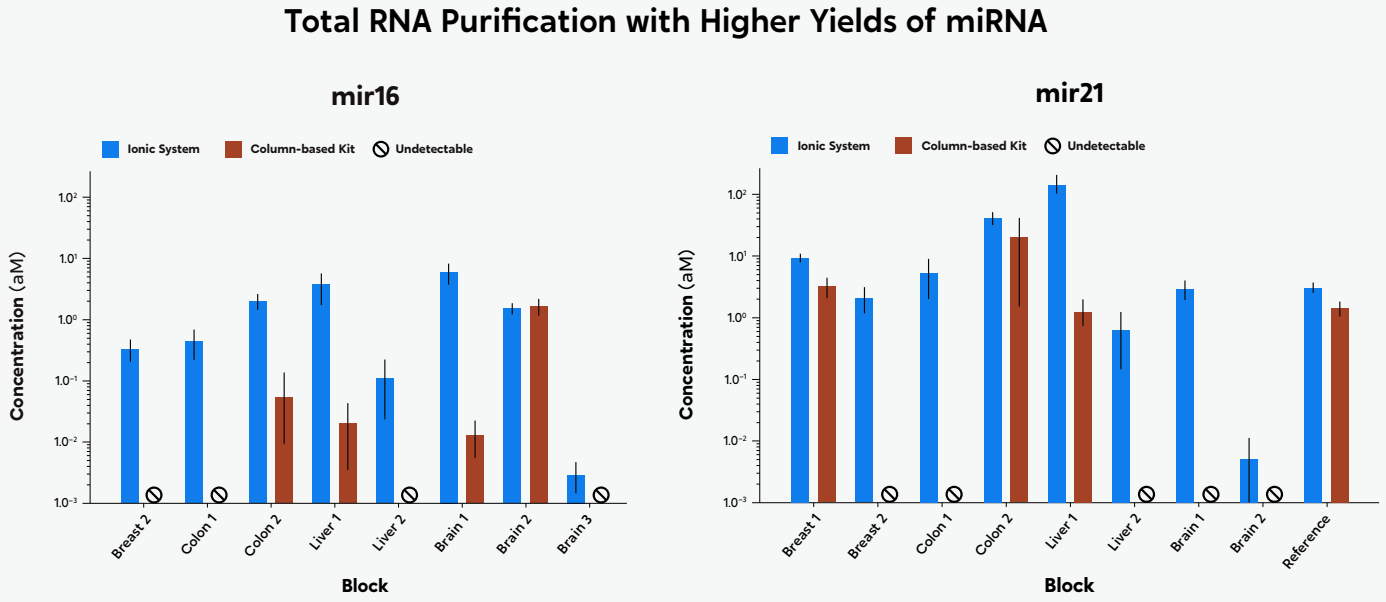


FIGURE 7: RNA from replicate sections of 8 FFPE sample blocks were purified using either the Ionic FFPE to Pure RNA Kit or a column-based miRNA extraction kit. The extracted and purified samples from each kit were analyzed by qPCR and the Applied Biosystems TaqMan Advanced miRNA Assays for miR-16 and miR-21. The concentration of the target miRNA represented in each sample was extrapolated and plotted against the tissue type of the source FFPE sample block. The Ionic system produced samples with a higher concentration of miRNA in all but one of the samples tested. For several samples the column-based miRNA extraction kit did not yield a detectable amount of miRNA.

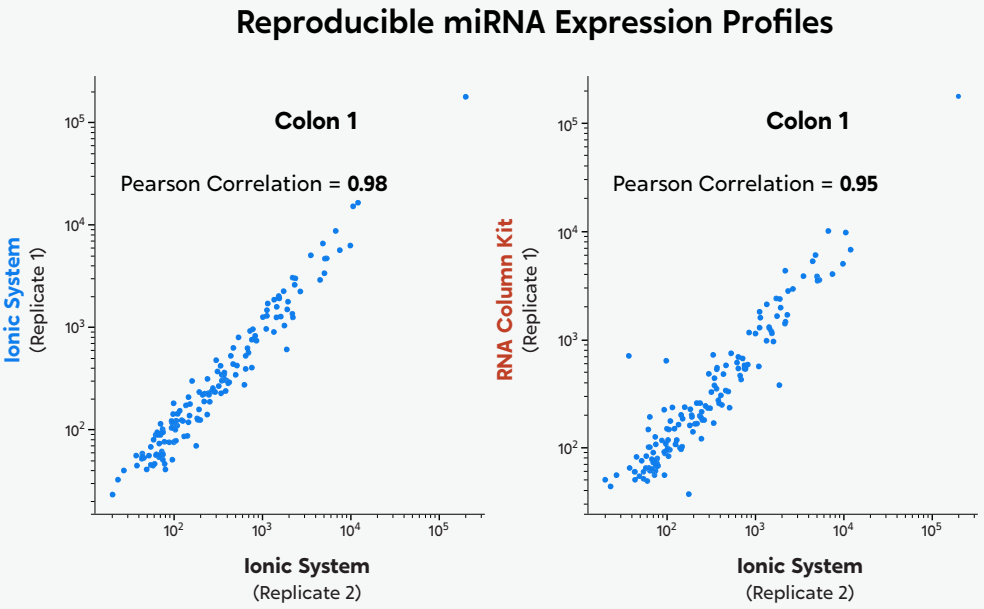


FIGURE 8: Samples from "Colon 1" of FIGURE 9 were analyzed for miRNA expression using the NanoString nCounter Human miRNA panel. The level of miRNA expression between replicate samples purified using the Ionic system has a Pearson correlation of 0.98. The level of miRNA expression between replicate samples purified using the Ionic system and the column-based miRNA kit has a Pearson correlation of 0.95. This analysis indicates a high reproducibility of miRNA expression across replicate samples purified using the Ionic system that is comparable to that of the column-based miRNA extraction kit.

Simultaneous Extraction of RNA and DNA from FFPE

The Ionic® FFPE Complete Purification Kit is used with the Ionic system to enable the automated purification of DNA and RNA, including microRNA from FFPE tissue samples. The kit provides a protocol, Ionic® Fluidic Chips and reagents to enable the Ionic system to automate DNA and RNA purification using an innovative isotachophoresis technology. Samples are prepared for purification on the Ionic system using a simple lysis procedure that can be automated using a programmable thermomixer without any need for micro-dissection or de-paraffinization using harsh chemicals.

Comparison of Total Hands-on Time vs. Manual Methods

	IONIC	Manual Bead-based	Manual Column-based
Lysis time	1.5 hrs	Overnight	1 hr
RNA isolation	2 hrs	2 hrs	2.5 hrs
Lysis time	1.7 hrs	3 hrs	3.5 hrs
Total time	5.2 hrs	13 hrs	6.5 hrs
Total hands-on time	1.5 hrs	6 hrs	7 hrs

TABLE 1: In a study conducted by a third-party genomic services lab, this table shows 3 extraction methods that were used to compare the hands-on time and total time to extract and purify RNA and DNA from 8 samples. Replicate 10 µm sections of FFPE samples were extracted and purified using either the Ionic system, a market-leading manual bead-based kit, or a manual column-based kit.

Six adjacent sections of a 10 µm thickness were harvested from 6 FFPE tissue blocks containing brain, breast, colon, or lung tissue. DNA and RNA were extracted and purified from 4 of the 6 sections using the published workflow for the Ionic FFPE Complete Purification Kit. DNA and RNA were extracted and purified from the remaining sections using the published workflow for either a market-leading manual column-based kit or a market-leading manual bead-based kit.

The average estimated time to process 8 samples through the Ionic FFPE Complete Purification kit was 5 hours and 12 minutes with a hands-on time of 1 hour and 30 minutes **(TABLE 1)**. This results in 11.25 minutes of hands-on time per sample to extract both DNA and RNA. The estimated time to process 8 samples through the column-based kit was 7 hours with most of that time being hands-on. This results in a hands-on time of 52.5 minutes per sample. Using a similar calculation, the hands-on time for the manual bead-based approach was 45 minutes per sample.

Improvement to DNA Yield with Comparable RNA Yield

The simplified workflow of the Ionic FFPE Complete Purification Kit provides simultaneous extraction and purification of FFPE samples without compromising yield.

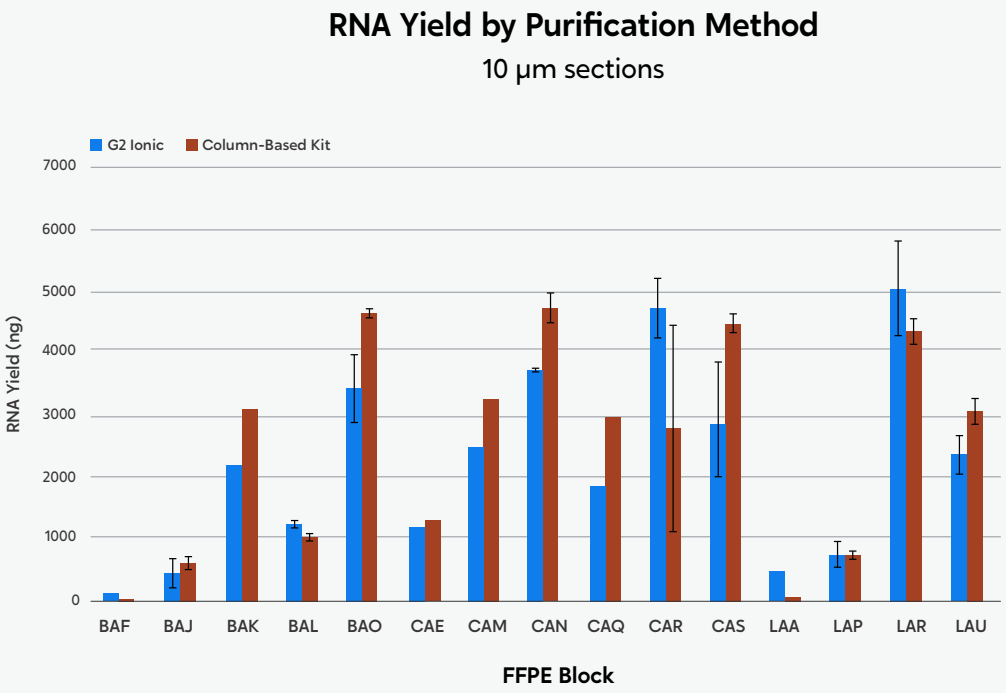


FIGURE 9: Comparison of RNA yields from consecutive sections of 15 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE Complete kit or a commercially available column-based kit. The total RNA yield for each sample was determined by multiplying the extract volume by the RNA concentration derived from the Qubit HS RNA assay. Error bars represent standard deviation from the average RNA yield. A similar or higher amount of RNA is recovered using the G2 FFPE Complete kit for 8 of the 15 FFPE blocks with an expected RNA yield increase of ~15% on average compared to the column-based kit

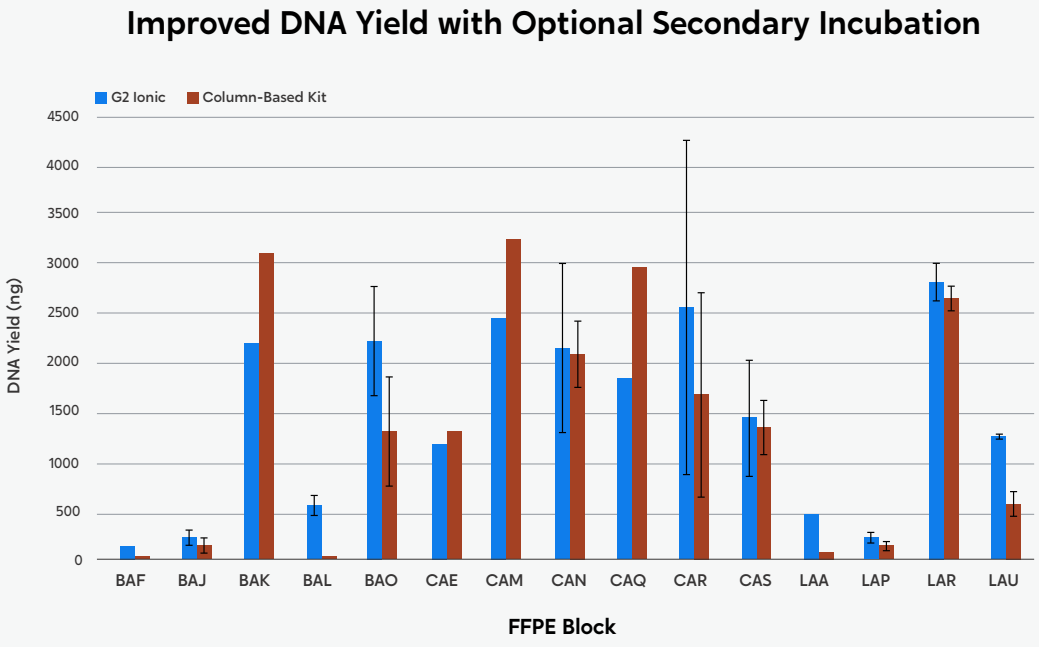


FIGURE 10: Comparison of DNA yields from consecutive sections of 15 FFPE tissue blocks purified on the Ionic Purification System using the G2 FFPE Complete kit or a commercially available column-based kit. The total DNA yield for each sample was determined by multiplying the extract volume by the DNA concentration derived from the Qubit 1x dsDNA High Sensitivity assay. Error bars represent standard deviation from the average DNA yield. More DNA is recovered using the G2 Complete kit for 12 of the 15 FFPE blocks with increased DNA yield of 1.5-2x on average compared to the column-based kit and greater yield advantages for low yielding samples.

A Better Solution for DNA Purification from Tissue Samples

The Ionic® Tissue to Pure DNA Kit provides automated purification of DNA from fresh frozen tissue samples with less hands-on time than conventional bead and column-based methods.

Comparison of Ionic Tissue to Pure DNA Kit to 3 Column-based Methods

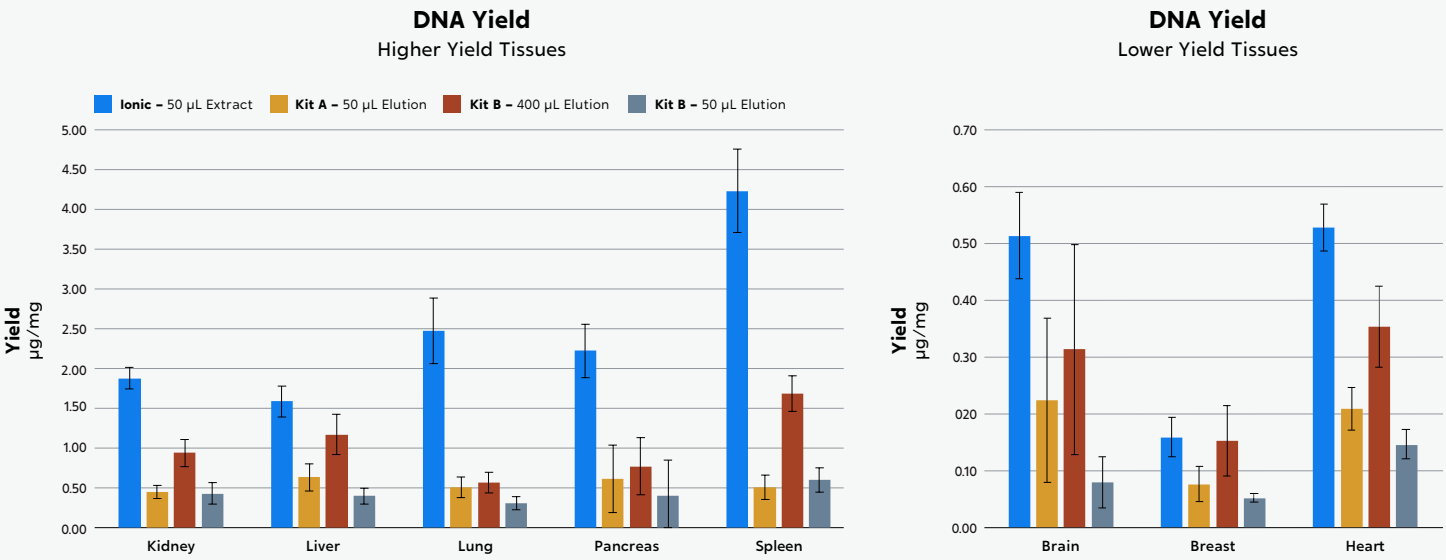


FIGURE 11: DNA extraction was performed on 8 different tissue types with four different extraction methods on a total of 632 specimens. Each tissue type/condition has an n-value ranging from 6 to 47 individual specimens. Pancreas, brain, breast, and heart specimens were derived from one donor and kidney, liver, lung, spleen were from two donors. Error Bars represent the 95% CI on the mean DNA yield (µg) per mg of tissue from replicate specimens (1-10 mg).

Linearity of Yield Across Tissue Input Amounts

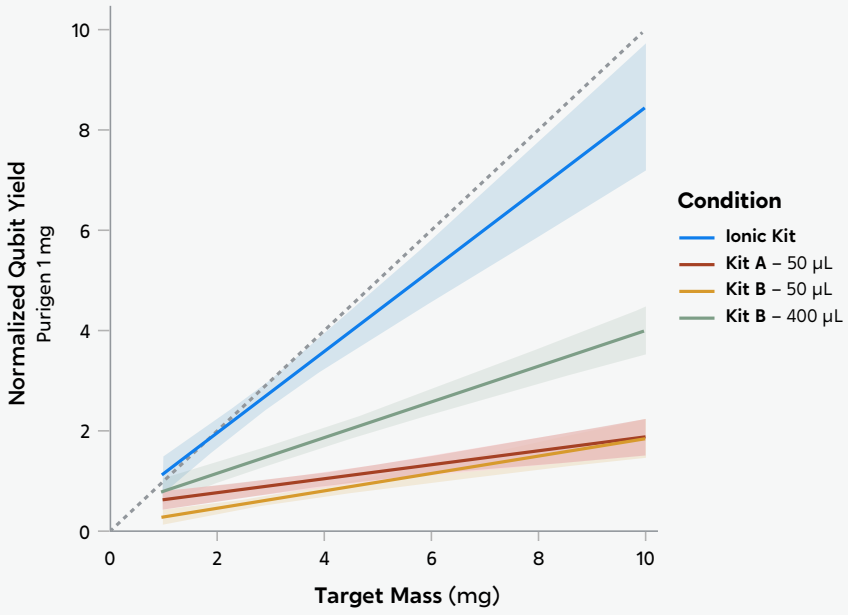


FIGURE 12: DNA yields were normalized to the Ionic kit yield for 1 mg on a by-tissue basis. The dotted line represents a theoretical linear extraction efficiency up to 10 mg tissue (assuming optimal recovery by Ionic kit at 1 mg specimens). The Ionic kit tracked the theoretical linear extraction efficiency more closely than the column-based methods.

Maximize Yields For WBCs, PBMCs, and Cultured/Sorted Cells

The Ionic® Cells to Pure DNA Kit supports a wide range of cell types including white blood cells (WBC) or peripheral blood mononuclear cells (PBMC) isolated from blood as well as cultured or sorted cells. The standard input range is 50k to 5 million cells. Customized protocols for as few as 10 cells are available upon request.

Higher Yields for Blood-based, Cultured, and Sorted Cells

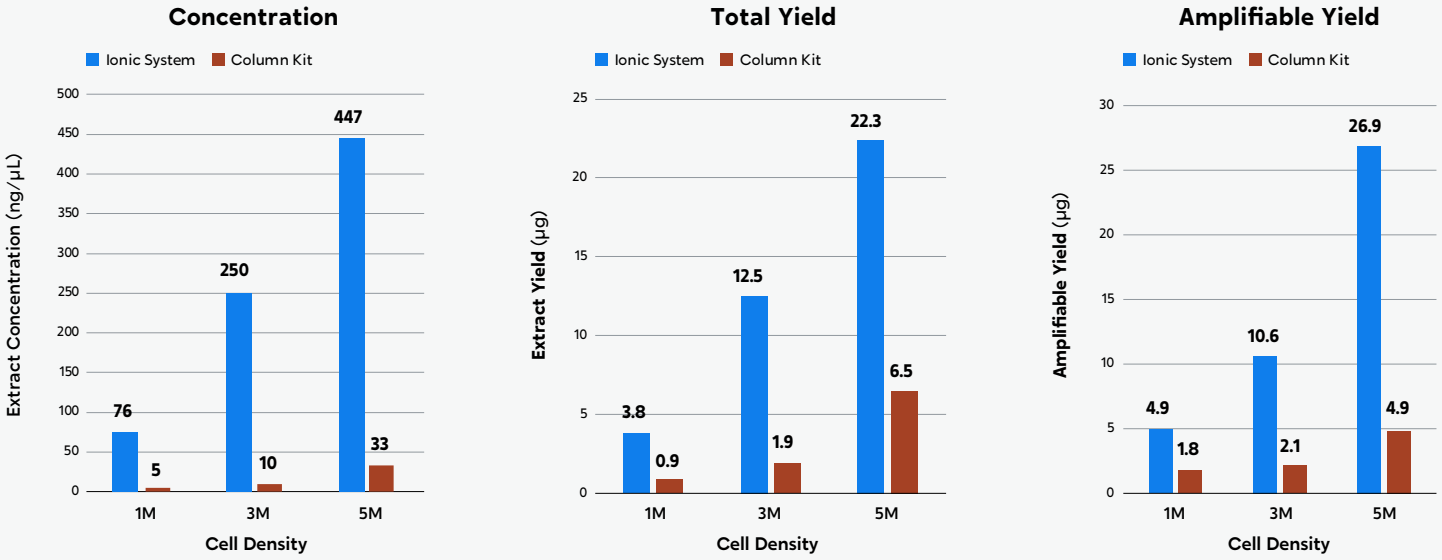


FIGURE 13: Peripheral Blood Mononuclear Cells (PBMCs) were isolated via Ficoll gradient, White Blood Cells (WBCs) were pelleted from lysed whole blood, and GM24385 cells were pelleted from culture media. Extractions were performed for each cell type at amounts ranging from 1-5 million cells then quantified via Qubit assay.

Consistent Length Profiles with Average Length Above 20k bp

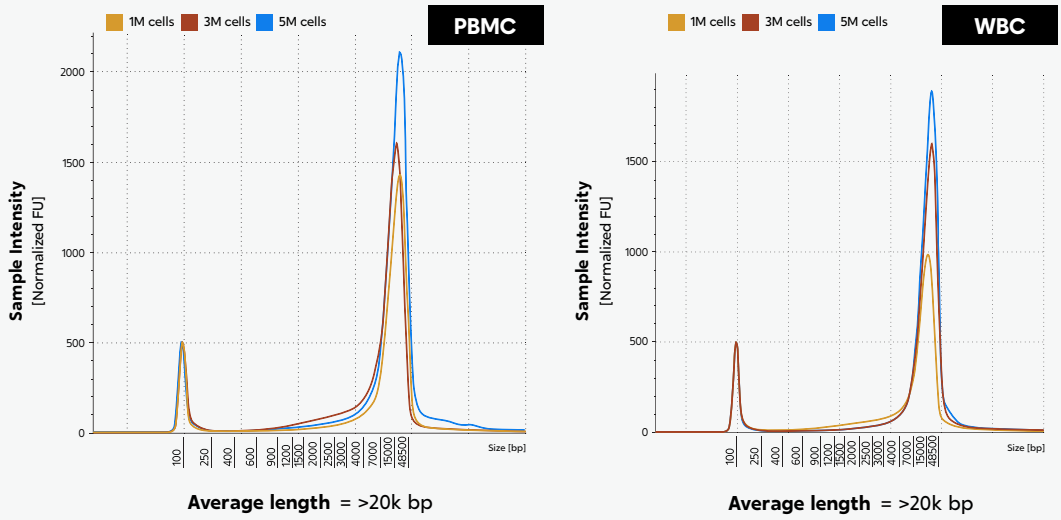


FIGURE 14: DNA Extracts from the Ionic System were evaluated on a TapeStation to determine their size profile. Size profiles were consistent across cell type and quantity.

Products

Instrument		Configuration	Part No.
	**Ionic® Purification System** **Includes:** Power cord, installation, warranty **Warranty Information** • 12 months coverage • Initial response within 8 business hours • On-site response within 3 business days • Includes parts and materials • Parts, materials • On-site labor	Standard	44001

Kits		Configuration		Part No.
	**Ionic® G2 FFPE to DNA Kit** **Includes:** Fluidic chips, room temp reagents, -20°C reagents	6-Chip Kit		90164
	**Ionic® G2 FFPE to RNA Kit** **Includes:** Fluidic chips, room temp reagents, -20°C reagents	6-Chip Kit		90167
	**Ionic® G2 FFPE Complete Kit** **Includes:** Fluidic chips, room temp reagents, -20°C reagents	6-Chip Kit		90170
	**Ionic® G2 Tissue to DNA Kit** **Includes:** Fluidic chips, room temp reagents, -20°C reagents	6-Chip Kit		90173
	**Ionic® Cells to Pure DNA (v1)** **Includes:** Fluidic chips, room temp reagents, -20°C reagents	6-Chip Kit		33005

Service Contracts	Includes	Term	Part No.
Comprehensive Service Plan	• 1 planned maintenance visit • Initial response within 8 business hours • On-site response within 3 business days • Minimum 90-day warranty on replacement parts • Parts, materials • On-site labor, per diem charges • Phone and email support • Remote support sessions • Software upgrades	12 months	44900
Planned Maintenance-only Plan	• 1 planned maintenance visit • Initial response within 12 business hours • On-site response within 5 business days • 90-day warranty on all replacement parts • 10% discount on Parts and Materials • 10% discount on on-site labor, per diem charges • Phone and email support • Remote support sessions • Software upgrades	12 months	44901
Time and Materials Service	• Hourly service work	N/A	44902

Contact your Bionano Regional Business Manager to get started.

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Call **1.858.888.7600** or contact your sales person directly.

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