High-yield Extraction of DNA and RNA Using the Ionic Purification System Generation 2 Kits

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Revision History

REVISION	NOTES
A	Initial release

Introduction

The Ionic™ Purification System uses isotachophoresis (ITP) to extract, purify, and concentrate nucleic acid from biological samples without binding, washing, or stripping from physical surfaces. Since nucleic acids remain in their native form, and not denatured, or dehydrated, the Ionic system is ideal for Formalin-Fixed Paraffin-Embedded (FFPE) tissue and other challenging samples with limited or low-quality material. Generation 2 (G2) kits include optimized chemistries and protocols which improve the performance of the already streamlined Ionic workflows.

Isotachophoresis Technology

The Ionic Purification System is a compact benchtop instrument that enables the automated purification of nucleic acids from a wide range of sample types including FFPE tissue, cultured or sorted cells, and fresh-frozen tissue. The Ionic system uses an innovative ITP technology to purify nucleic acids without the fragmentation or biases associated with binding to or stripping from physical surfaces. This means the nucleic acids purified on the Ionic system are more representative of the sample. Biological samples are efficiently lysed and added directly to a fluidic chip and purified on the Ionic system. To enable ITP, an electrical current is applied to the chip causing nucleic acids to migrate in solution based solely on their charge and inherent electrophoretic mobility. As nucleic acids separate from impurities they concentrate and travel through the channel of the fluidic chip to an extraction well for collection. This technology does not require organic solvents, chaotropic salts or alcohol-based wash solutions readily used in traditional methods that are known to impact downstream assays. The purified nucleic acids are therefore ideal for use in downstream assays including next-generation sequencing (NGS) and qPCR.

Simplified Workflow

The Ionic Purification System simplifies the nucleic acid isolation workflow, seen in **Figure 1**, while providing advantages over traditional methods in both quantity and quality of purified nucleic acids. A programable ThermoMixer is used to achieve deparaffinization, lysis and de-crosslinking in a simple walk-away process and up to eight samples can be purified in parallel on the Ionic system with minimal hands-on time.

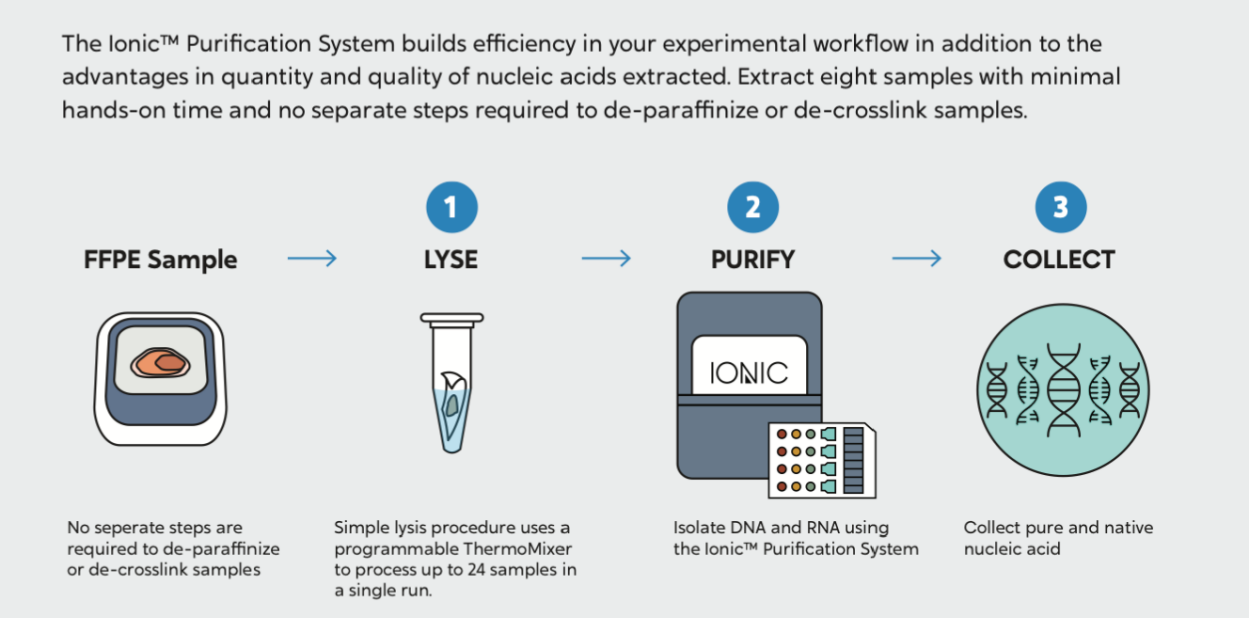

Figure 1. Workflow demonstrates the three critical steps to the Ionic G2 workflow which includes lysing the FFPE samples, purifying using the Ionic system, and collecting the final nucleic acid.

Data and Results

FFPE to RNA

Consecutive FFPE tissue sections from brain, breast, colon and lung were processed using the standard Ionic Generation 1 (G1) and G2 FFPE to RNA protocols and compared to column- and bead-based extraction methods (see **Figures 2** and **3**). Briefly, FFPE tissue sections are combined with mineral oil and a lysis cocktail containing Proteinase K followed by deparaffinization, lysis, and de-crosslinking using a programmable ThermoMixer according to **Table 1**. Lysates are centrifuged, separated from mineral oil and DNase treated. RNA is purified on the Ionic Purification System in ~60-70 minutes with the appropriate G1 or G2 purification protocol. Column-and bead-based competitor eluates were generated according to the manufacturer's recommendations.

Table 1. Programmable Thermomixer parameters**.**

Ionic Kit	Deparaffinization	Lysis	De-Crosslinking
Ionic G2 FFPE to DNA	65°C, 5 min, 1000 rpm	56°C, 1 hr, 1000 rpm	70°C, 8 hr, 1000 rpm
Ionic G1 FFPE to DNA	65°C, 10 min, 1600 rpm	56°C, 1 hr, 1000 rpm	70°C, 8 hr, 1000 rpm
Ionic G2 FFPE to RNA	65°C, 5 min, 1000 rpm	60°C, 1 hr, 500 rpm	70°C, 1 hr, 0 rpm
Ionic G1 FFPE to RNA	65°C, 5 min, 1600 rpm	60°C, 30 min, 1000 rpm	80°C, 30 min, 0 rpm

G2 CHEMISTRY IMPROVES IONIC RNA YIELD BY 1.4X; OUTPERFORMS COMPETITORS BY ~2.5-3X

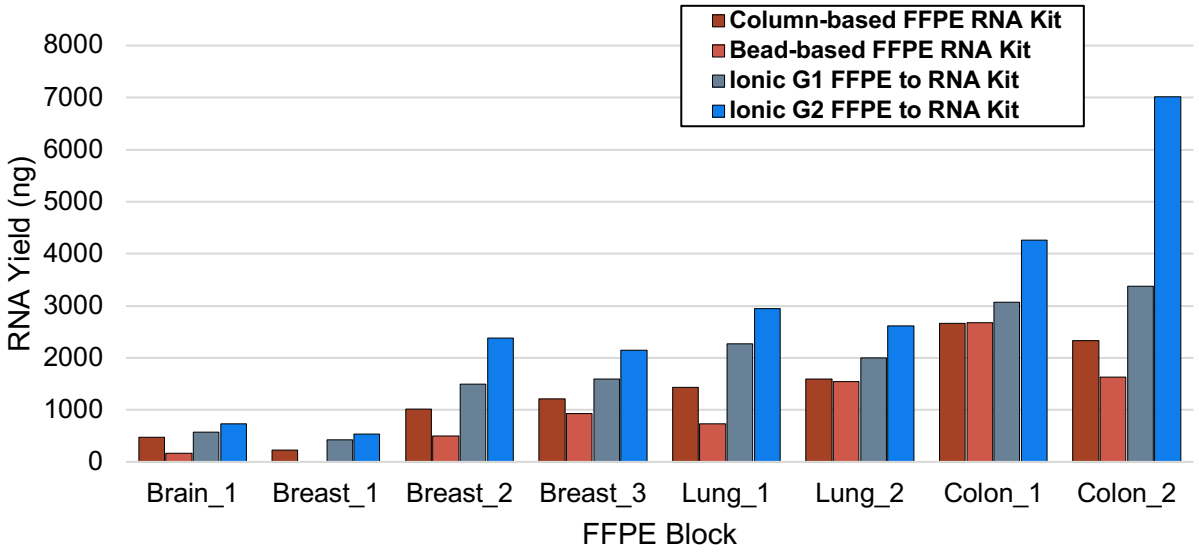

Figure 2. Comparison of RNA yields from consecutive FFPE tissue sections purified on the Ionic Purification System using the G1 and G2 FFPE to RNA kits, or commercially available extraction kits. The total RNA yield for each sample was determined by multiplying the extract volume by the RNA concentration derived from the Qubit HS RNA assay. The G2 kit consistently provides higher RNA yields compared to the G1 kit (1.4x) and each of the traditional extraction methods (~2.5-3x).

G2 CHEMISTRY YIELDS HIGHER QUALITY IONIC RNA

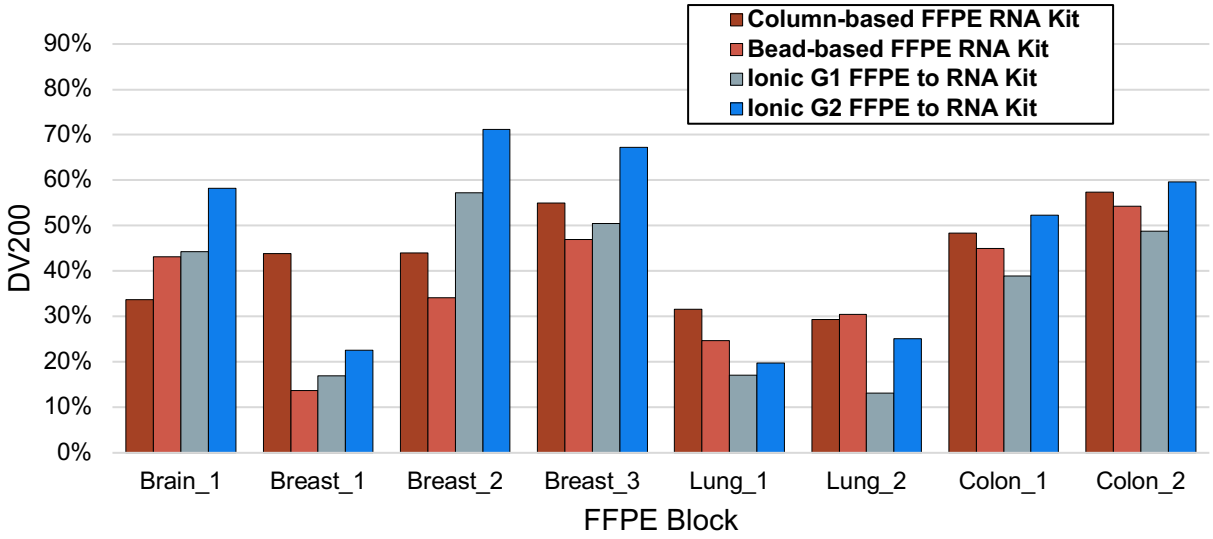

Figure 3. Comparison of RNA quality from consecutive FFPE tissue sections purified on the Ionic Purification System using the G1 and G2 FFPE to RNA kits or commercially available extraction kits. All RNA extracts were normalized to 1.5 ng/µl input concentration and the DV200 (percentage of RNA fragments >200 bp) was

calculated using a High Sensitivity RNA ScreenTape on the Agilent Tapestation. For all FFPE blocks, the percentage of G2 Ionic RNA fragments >200 bp is consistently higher than G1 reflecting the increased RNA quality associated with the improvements made in the G2 kit. For higher quality FFPE blocks (DV200>30%), the G2 Ionic RNA quality is consistent with or better than the column- or bead-based extraction methods. Since the Ionic purification process co-extracts short RNAs, such as miRNA, the G2 Ionic DV200 values can appear lower than the traditional kits for especially poor quality FFPE blocks (DV200 <30%).

FFPE to DNA

Consecutive FFPE tissue sections from breast, colon and lung were processed using the standard G1 and G2 FFPE to DNA protocols and compared to column-based extraction methods (see **Figures 4** and **5**). Briefly, FFPE tissue sections are combined with mineral oil and a lysis cocktail containing Proteinase K followed by deparaffinization, lysis, and de-crosslinking using a programmable ThermoMixer according to **Table 1**. Lysates are centrifuged, separated from mineral oil and RNase treated. DNA is purified on the Ionic Purification System in ~60-70 minutes with the appropriate V1 or G2 purification protocol. Column-based competitor eluates were generated according to the manufacturer's recommendations.

G2 CHEMISTRY IMPROVES IONIC DNA YIELD BY 1.6X; OUTPERFORMS COMPETITORS BY ~4.5X

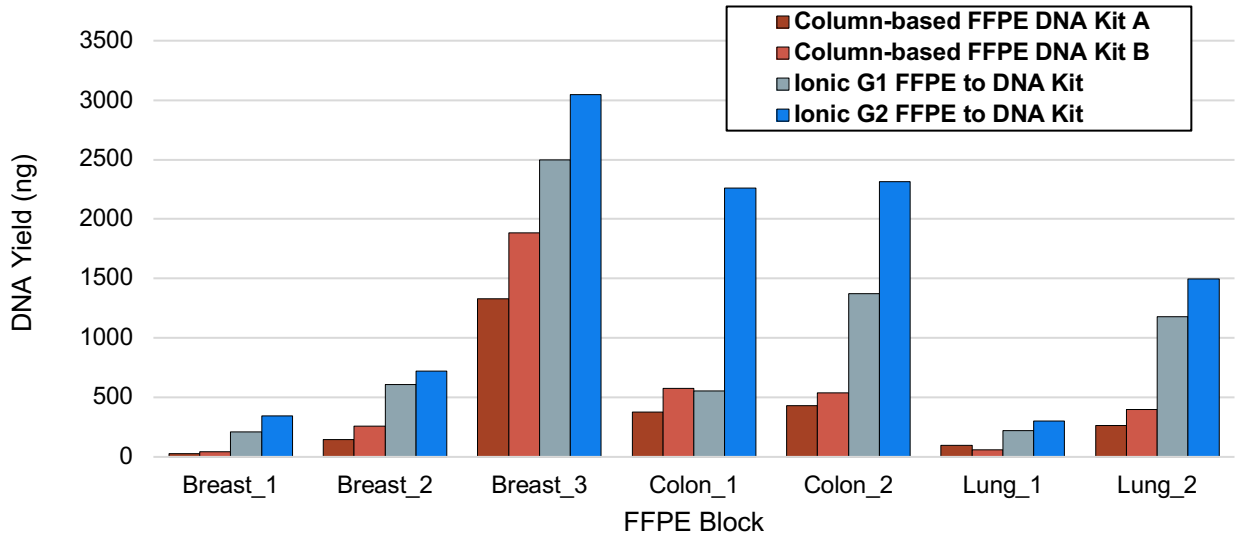

Figure 4. Comparison of DNA yields from consecutive sections of FFPE tissue blocks purified on the Ionic Purification System using the G1 and G2 FFPE to DNA kits, or commercially available column-based kits. The total DNA yield for each sample was determined by multiplying the extract volume by the DNA concentration derived from the Qubit 1x dsDNA High Sensitivity assay. The G2 chemistry provides Ionic extracts a 1.6x increase in DNA yield advantage over G1 and an advantage of ~3.5x-5x over the column-based methods.

G2 CHEMISTRY IMPROVES IONIC DNA QUALITY

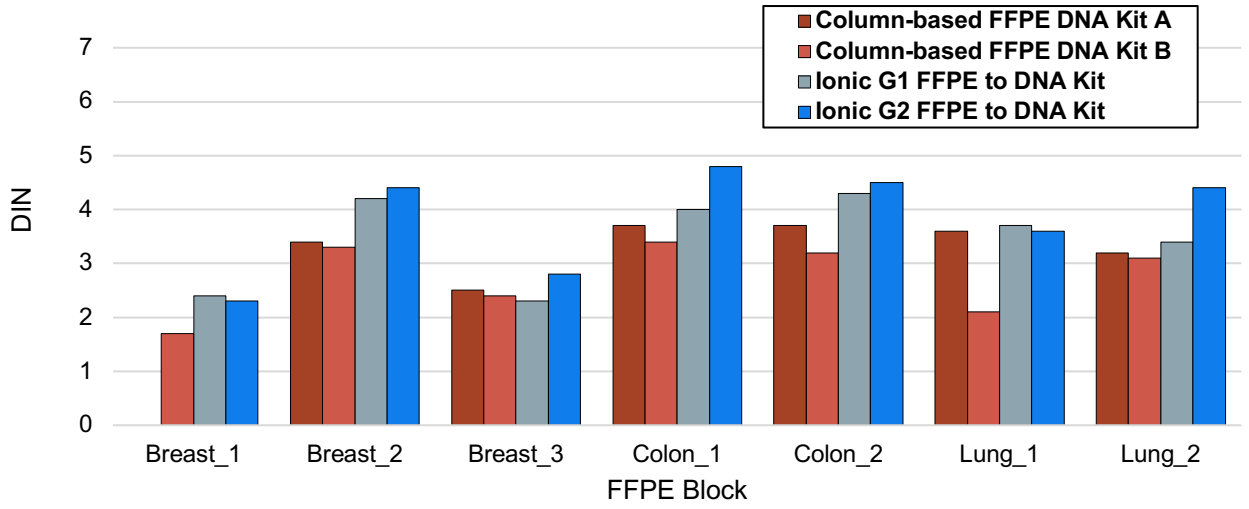

Figure 5. Comparison of DNA from consecutive sections of FFPE tissue blocks purified on the Ionic Purification System using the Ionic G1 and G2 FFPE DNA kits or commercially available column-based kits. DNA Integrity Number (DIN) was calculated for each extract using a Genomic DNA ScreenTape on the Agilent TapeStation. The DIN values for the Ionic G2 DNA extracts are consistent with or marginally better than the V1 kit and are consistently higher than the column-based kits for each comparison.

Conclusion

Direct comparisons of DNA and RNA purified from sequential FFPE tissue sections clearly show the advantages the G2 kits provide versus traditional extraction methods. The G2 kits provided outstanding yields which enables more analyses to be provided from less FFPE tissue. Along with the already simplified Ionic workflow, the G2 chemistries provide an additional 1.4x and 1.6x advantage in RNA and DNA yield while also improving nucleic acid quality over the first generation kits.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

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