Extract and Purify Higher Yields of Higher Quality Nucleic Acid from Limited Input Using Isotachophoresis

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INTRODUCTION

The Purigen Ionic® Purification System uses isotachophoresis (ITP) to extract, concentrate and purify nucleic acid from biological samples. ITP is a gentle, microfluidic, process that separates nucleic acid from impurities using electrophoretic mobility.

To evaluate the performance of the Ionic system for extraction of nucleic acid from limited or low input samples, we purified DNA from a set of FFPE tissue blocks and cultured cells at counts of 10,000 cells per sample or less. Replicates of each sample were purified and extracted using a commercially available column-based purification kit for comparison. We assessed the quantity and quality of the purified DNA with the Qubit dsDNA High Sensitivity Assay and Qiagen MRef multicopy reference assay.

FIGURE 1: Ionic® Purification System and Ionic® Fluidic Chip

Isotachophoresis

Using a cutting-edge technology based on isotachophoresis (**FIGURE 2**), the Ionic system separates nucleic acids freely in solution, without binding to or stripping nucleic acid from physical surfaces. Taking as little as 5 minutes of hands-on time per sample to purify nucleic acid from a range of sample types including cells and FFPE tissue, the Ionic System is a highly efficient platform that produces more nucleic acid of higher purity, consistently and reliably.

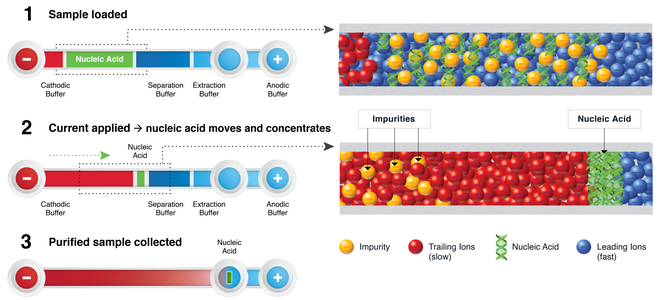

FIGURE 2: Conceptual representation of isotachophoresis in the following steps: **1)** Lysate containing nucleic acids is loaded into the microfluidic channel between a leading electrolyte containing ions with a faster electrophoretic mobility than nucleic acids and a trailing electrolyte containing ions with a slightly slower electrophoretic mobility. **2)** Electrical current is applied to the channel, causing nucleic acids to move through the solution toward the positive electrode. As this happens, the leading and trailing ions form a sharp electric field gradient, and nucleic acids focus toward the gradient. This is the ITP zone. Impurities are not collected into the zone because their mobilities are not bracketed by the leading and trailing ions. **3)** The band moves into an extraction well. The field gradient is detected by a sensor, and the system shuts down the electric current. The result is purified nucleic acid in a well accessible by a pipette tip.

RESULTS – Cultured Cell Samples

Improved yield from as few as 10 cells per sample

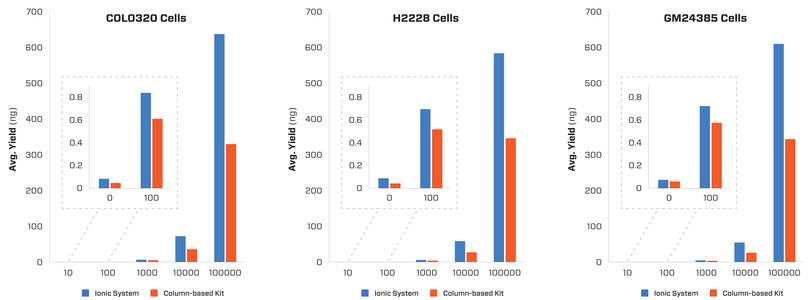

FIGURE 3: Cells from COLO320, GM24385, and H2228 cell lines were cultured and allocated to individual sample tubes in the amounts of 100,000, 10,000, 1000, 100, and 10 cells with a replicate tube at each cell amount. DNA was purified from the replicate samples using the Ionic system and a market-leading column-based kit. The DNA concentration and yield from each sample was determined using the Qiagen Multicopy Reference Assay and a standard control. Samples purified using the Ionic system had 1.5 – 2x higher yields than those from the column-based kit.

		Ave. Extract Concentration	Ave. Library Concentration (nM)	Reads After Down-sampling	Ave. % Reads Aligned	Ave. Fold-80
COLO320 (100k cells)	**Ionic System**	**12.68** (+/- 1.63)	**536.75** (+/- 136.2)	**895,698**	**97.90** (+/- 0.75)	**2.36** (+/- 0.01)
	Column-based Kit	**7.33** (+/- 0.78)	**510.75** (+/- 68.8)		**98.40** (+/- 0.45)	**2.48** (+/- 0.19)
H2228 (100k cells)	**Ionic System**	**17.25** (+/- 2.40)	**1027.5** (+/- 146.0)	**797,072**	**98.70** (+/- 0.07)	**2.41** (+/- 0.07)
	Column-based Kit	**8.23** (+/- 1.74)	**640.25** (+/- 66.3)		**98.63** (+/- 0.04)	**2.53** (+/- 0.12)
GM24385 (100k cells)	**Ionic System**	**15.73** (+/- 3.87)	**507.33** (+/- 108.3)	**381,864**	**98.57** (+/- 0.05)	**2.18** (+/- 0.02)
	Column-based Kit	**8.00** (+/- 0.78)	**567.25** (+/- 199.3)		**98.45** (+/- 0.11)	**2.27** (+/- 0.04)

TABLE 1: Libraries were generated for each sample described in **FIGURE 3** following the protocol for the AmpliSeq for Illumina Comprehensive Cancer Panel and sequenced on the Illumina MiSeq sequencer. Standard sequencing metrics show comparable results, with the Ionic System samples showing a slightly improved Fold-80 score, indicating an improved coverage uniformity.

RESULTS – FFPE Tissue Samples

Improved yield from a single 10 µM thick FFPE tissue section

Block	Sample Type	Method	Reads in BAM File Passing Mapping Quality Filters	% Reads in Covered Regions	% Duplicate Reads	Sequenced Bases in Analyzable Target Regions	Avg. Read Depth in Analyzable Target Regions	Median Read Depth in Analyzable Target Regions
C1	GM24385	Control	1.24E+07	77.00%	15.92%	1.08E+09	1,469	1,447
C2	GM24385	Control	1.23E+07	74.78%	17.08%	1.04E+09	1,416	1,416
BI	Breast	Ionic system	6.78E+06	34.44%	49.68%	2.24E+08	306	294
BI	Breast	Column kit	7.12E+06	27.00%	39.98%	1.82E+08	248	219
BQ	Breast	Ionic system	1.08E+07	39.50%	20.10%	4.46E+08	608	612
BQ	Breast	Column kit	6.23E+06	26.18%	48.65%	1.57E+08	213	200
BS	Breast	Ionic system	4.81E+06	20.42%	61.68%	5.30E+08	724	735
BS	Breast	Column kit	4.81E+06	20.42%	61.68%	9.51E+07	129	122
CK	Colon	Ionic system	9.01E+06	46.37%	27.02%	4.23E+08	577	561
CK	Colon	Column kit	7.74E+06	36.59%	37.63%	3.00E+08	409	412
CW	Colon	Ionic system	7.02E+06	44.81%	41.09%	3.05E+08	416	416
CW	Colon	Column kit	5.43E+06	10.62%	42.17%	5.30E+07	72	67
CX	Colon	Ionic system	9.00E+06	28.16%	23.65%	2.45E+08	335	330
CX	Colon	Column kit	5.68E+06	13.76%	44.15%	7.07E+07	96	88
LW	Lung	Ionic system	1.05E+07	61.29%	26.74%	6.86E+08	936	936
LW	Lung	Column kit	9.51E+06	50.96%	30.67%	5.11E+08	697	661
LX	Lung	Ionic system	1.04E+07	56.29%	27.01%	6.20E+08	846	851
LX	Lung	Column kit	8.19E+06	50.14%	40.58%	4.29E+08	586	562

TABLE 2: Summary of properties for replicate sections from 8 FFPE tissue blocks purified by the Ionic system and a column-based kit for hybrid-capture based library preparation. Purified and sheared amounts were quantified by Qubit 1X dsDNA High Sensitivity Assay. PCR cycles were performed prior to hybridization capture according to SureSelect XT HS protocol recommendations. High-quality human reference Coriell DNA was used as a "gold standard" control.

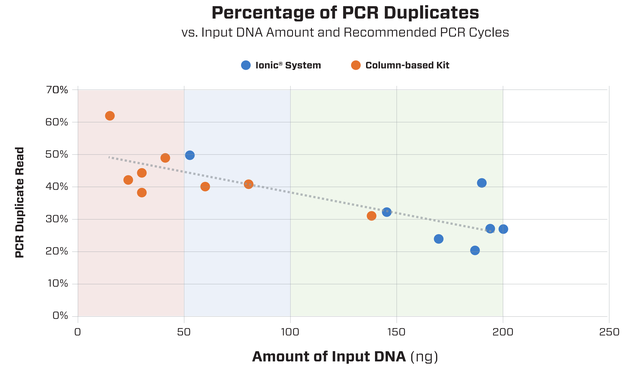

FIGURE 4: Comparison of the percentage of PCR duplicates reported for sequenced libraries from the 16 samples described in **TABLE 2** to the amount of input DNA used for each library and the recommend PCR cycles for each amount. Libraries from extracts purified using the Ionic system are represented with blue dots. Libraries from extracts purified using a column-based kit are represented with orange dots. Shading indicates the recommended number of PCR cycles with red, blue and green representing 14, 12, and 11 respectively.

Summary

- Higher yields of nucleic acid from a single FFPE tissue section or as few as 10 cells
- Higher yields and quality results in better coverage uniformity and fewer PCR duplicates in downstream NGS
- Isotachophoresis enables a simple, automated workflow for nucleic acid purification

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