A novel method for nucleic acid extraction from FFPE tissue: high compatibility and enhanced convenience with NGS applications

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Key Take Aways

- **Novel and efficient method for DNA and RNA extraction from FFPE samples**
- **No use of xylene or organic solvents → more sustainable**
- **High-quality** nucleic acids from FFPE samples → compatible with **demanding NGS applications**

Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue samples are invaluable resources for retrospective studies and diagnostic purposes. However, the extraction of processable nucleic acids is challenging due to the chemical modifications and degradation caused by fixation. We introduce a novel method for DNA and RNA extraction from FFPE tissues, designed to improve convenience and prove compatibility with downstream applications commonly used in diagnostic and research: the EchoLUTION technology.

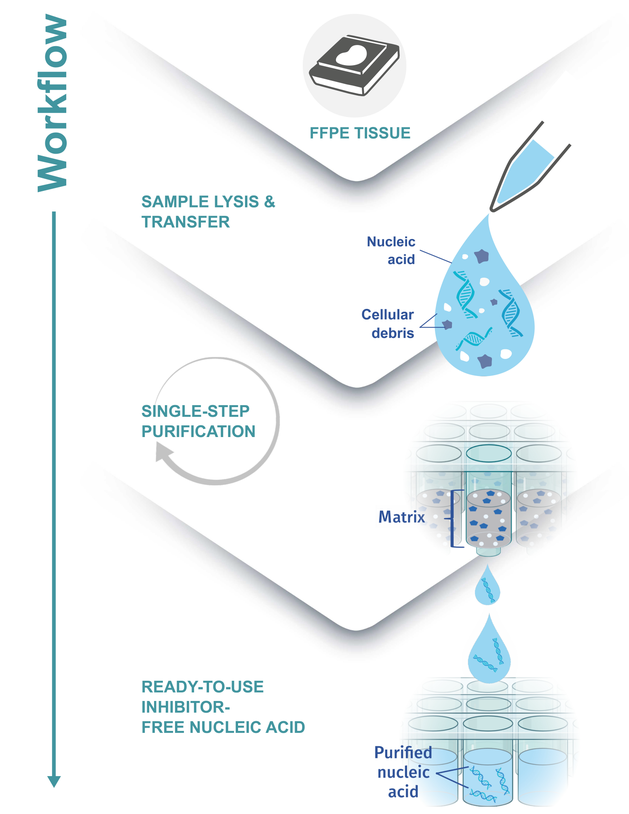
Materials and Methods

DNA and RNA extraction: We extracted the nucleic acids from highly standarized FFPE samples provided by Reference Medicine (human tumor-normal colon, brain, kidney, and breast pairs) with the EchoLUTION™ FFPE DNA Kit or the EchoLUTION™ FFPE RNA Kit, respectively.

Panel sequencing: DNA samples were subjected to gene enrichment and sequenced using the TWIST EF Library Prep 2.0 (TWIST Bioscience) with enzymatic fragmentation and a costum gene panel covering the coding sequences of 41 genes (app. 150 kb). We sequenced the library in PE101 with the Micro Reagent Kit v2 (300 cycles) and a MiSeq® System (Illumina®). The generated Fastq files were aligned using a pipeline based on GATK4 Best Practices and somatic variants were called using Mutect2.

RNAseq: We used the RNA samples extracted with EchoLUTION™ FFPE RNA Kit and a silica-based method (AllPrep® DNA/RNA FFPE Kit, Qiagen) from a brain tumor-normal pair for total RNA sequencing. Samples were subjected to rRNA depletion with RiboCop rRNA Depletion kit. The library was prepared with CORALL RNA-Seq V2 (Lexogen), and sequenced on a NextSeq® 2000 (Illumina®).

EchoLUTION technology



Results

DNA extraction

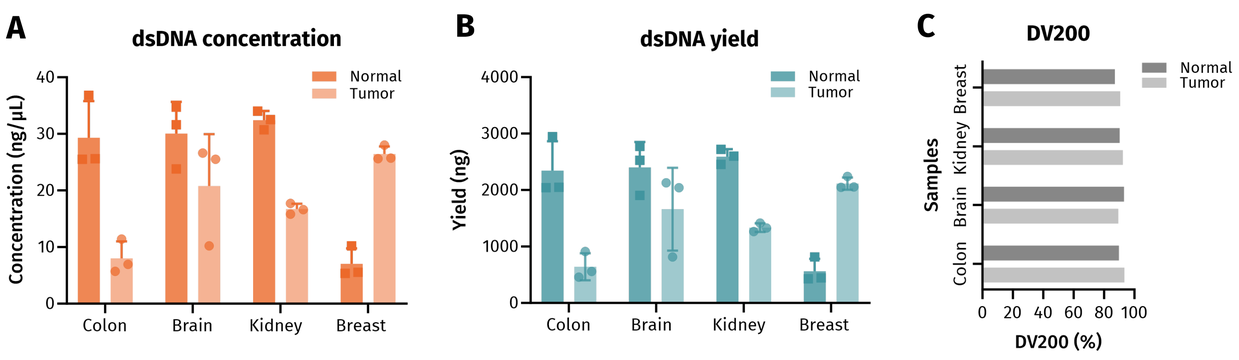


Figure 1. DNA extracted form tumor-normal pair FFPE samples. A. DNA concentration measured with fluorometry using Qubit™ (ThermoFisher Scientific®). N = 3 replicates/sample. **B.** DNA yield via flurometric measurement. *N* = 3 replicates/sample. **C.** DNA integrity represented by DV200 (%) obtained with TapeStation® 4150 System (Agilent®). Error bars represent standard deviation.

Panel sequencing

Tissue	Chr	Start	End	Ref	Alt	Gene	ExonicFunc	Total RC	RC Alt	%Alt	dbSNP	ClinVar_SIG
Colon	chr17	7674945	7674945	G	A	*TP53*	stopgain	322	33	0.10	rs397516435	Pathogenic
Brain	chr10	87961046	87961046	-	A	*PTEN*	frameshift insertion	298	135	0.48	rs786204892	Pathogenic
	chr17	7673794	7673794	C	G	*TP53*	nonsynonymous SNV	299	117	0.39	.	.
	chr17	31156011	31156011	C	-	*NF1*	frameshift deletion	242	166	0.69		
	chr17	31261733	31261733	C	T	*NF1*	stopgain	451	153	0.34	rs760703505	Pathogenic
Breast	chr3	179218303	179218303	G	A	*PIK3CA*	nonsynonymous SNV	175	55	0.31	rs104886003	Pathogenic
	chr5	112841014	112841016	ACA	-	*APC*	nonframeshift deletion	32	21	0.66	rs753952265	Uncertain significance
	chr17	7673573	7673573	T	A	*TP53*	stopgain	194	109	0.56	.	.
	chr17	7673575	7673575	G	T	*TP53*	nonsynonymous SNV	200	110	0.55	.	.

Table 1. Overview of the somatic variants found in the sequenced samples. DNA extracted from colon, brain, kidney and breast FFPE samples were used for panel sequencing via enrichment. We found somatic variants in all samples but kidney.

RNA extraction

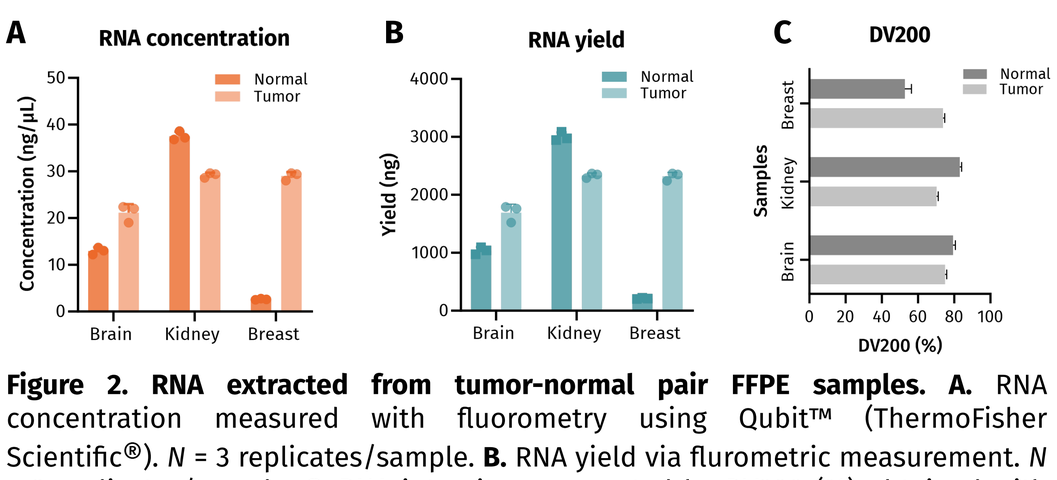


Figure 2. RNA extracted from tumor-normal pair FFPE samples. A. RNA concentration measured with fluorometry using Qubit™ (ThermoFisher Scientific®). *N* = 3 replicates/sample. **B.** RNA yield via flurometric measurement. *N* = 3 replicates/sample. **C.** RNA integrity represented by DV200 (%) obtained with TapeStation® 4150 System (Agilent®). Error bars represent standard deviation.

RNA seq

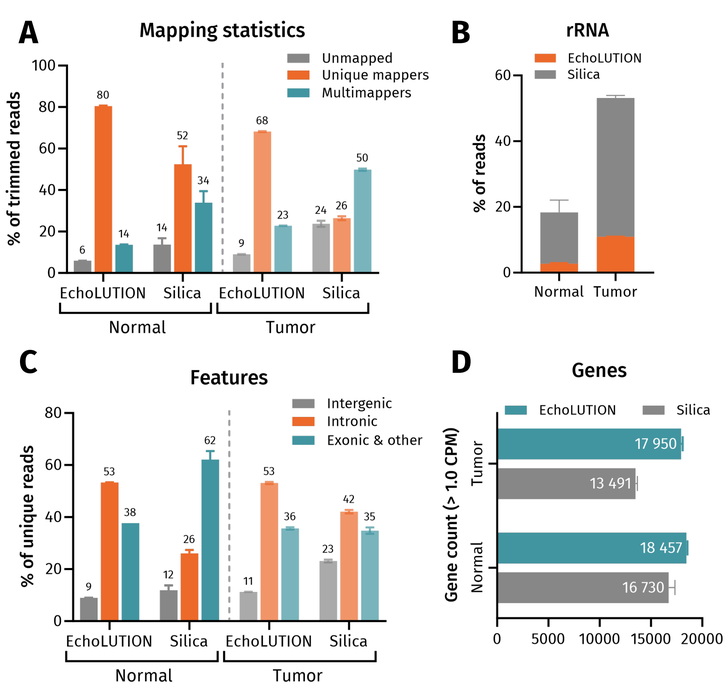


Figure 3. Transcriptome analysis of RNA isolated from brain tumor and normal FFPE tissues. A. The percentage of trimmed reads is represented from a total of 2 M reads. The number of unique mappers is higher for samples extracted with EchoLUTION than with the silica method. **B.** EchoLUTION extracted FFPE RNA results in significantly less rRNA reads in RiboCop rRNA depleted Corall V2 NGS libraries than seen with silica extracted FFPE RNA. **C.** The fraction of intronic reads is larger in EchoLUTION samples than with silica. **D.** The number of detected genes was higher with EchoLUTION than with the silica-based method. *N* = 2 biological replicates per sample. Error bars represent standard deviation.

Conclusion

We demonstrated that EchoLUTION technology offers:

1. Improved convenience in handling FFPE samples while providing high-quality nucleic acids (concentration, yield, integrity).

2. Compatibility with downstream applications such as panel sequencing and RNAseq.

Acknowledges: Reference Medicine provided the FFPE samples. Lexogen GmbH performed the RNAseq experiments.

REFERENCE MEDICINE

LEXOGEN The RNA Experts

Further information:
www.bioecho.com/FFPE