Optimization and Evaluation of an FFPE Dual Extraction Protocol for Next-Generation Sequencing Applications

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Abstract #: 5054

Background

Formalin-fixed paraffin-embedded (FFPE) biopsies are highly valuable and widely used tissue specimens for clinical diagnostics. However, obtaining sufficient and high-quality nucleic acid material from limited FFPE samples presents a challenge for downstream molecular analysis, such as next-generation sequencing (NGS). We present an optimized sequential extraction method that generates high-quality DNA and RNA from a single set of input tissues that is automatable and operation-friendly.

Methods

8 FFPE samples were macrodissected and nucleic acid were extracted by using 4 different extraction kits. DNA and RNA yield, quality, purity and impacts on NGS assay performances were evaluated.

Overview of Extraction Workflow

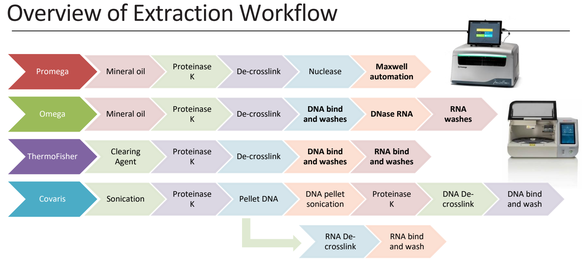
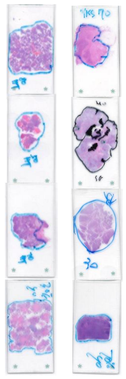


Fig 1 A: The general workflow of each kit. The Promega kit does start with a separate DNA and RNA process that ends on the Maxwell. The other 3 kits follow a sequential workflow, allowing DNA to be separated from the RNA-containing supernatant after a Proteinase K incubation time and processed independently. Omega and ThermoFisher both utilize the KingFisher.

Fig 1B: Clinical FFPE samples

Sample ID	Tumor Content %	Tumor Size (mm)	Tumor Tissue Type	Tissue Input
07	80%	27	Endometrium	1 section;10 µm thickness
08	50%	15	Lung	1 section;10 µm thickness
10	70%	16	Colon	1 section;10 µm thickness
11	30%	25	Ovary and Oviduct, Left	1 section;10 µm thickness
12*	70%	16	Colectomy	1 section;10 µm thickness
13	40%	28	Colon	1 section;10 µm thickness
14	30%	23	Ascitic Fluid (Abdomen)	1 section;10 µm thickness
15	70%	16	Lymph node, Right Flank	1 section;10 µm thickness



DNA yield (ng/µl) by Kit

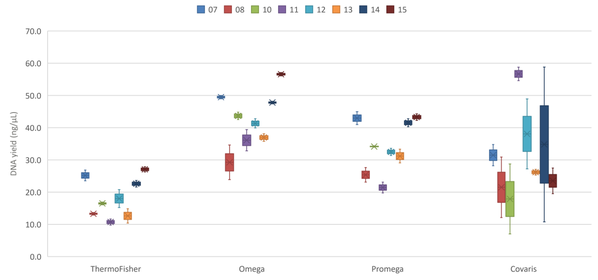


Fig 2A: Omega has the highest DNA yields across samples and good consistency.

RNA Yield (ng/µL) by Kit

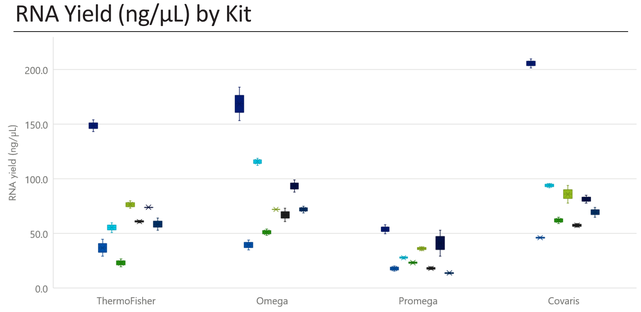


Fig 2B: Omega and Covaris have comparable RNA yields.

RNA DV200 by Kit

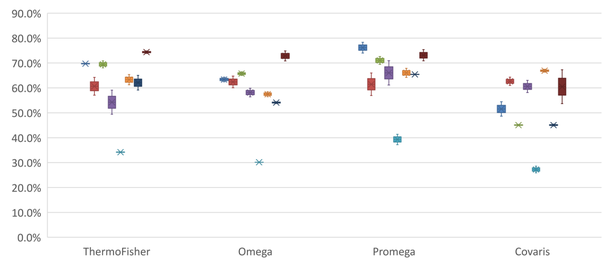


Fig 2C: Promega had slightly longer RNA fragments

TSO500HT Feasibility Data

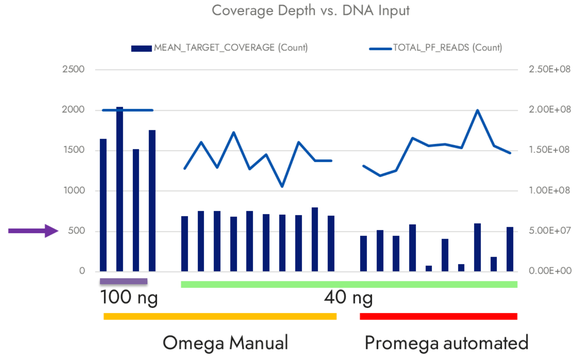


Fig 3: Omega manual DNA extraction at 40 ng input had higher library complexity than Promega

Comparison between Omega and Covaris kit

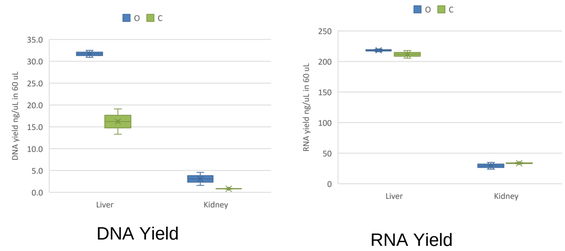


Fig 4A: Omega had a higher DNA yield. RNA yield is comparable

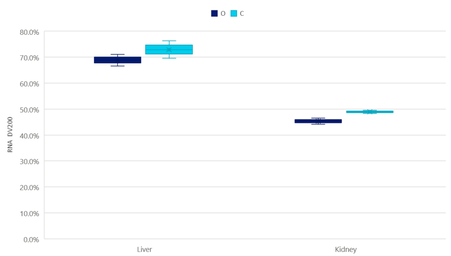


Fig 4B: DV200 is comparable between Omega and Covaris kit

Deparaffinized Slides

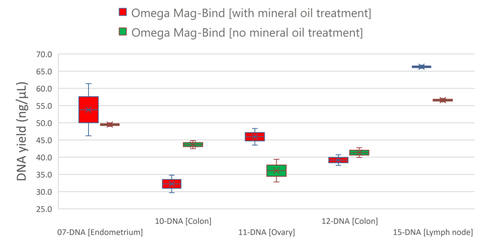


Fig 5A: Omega kit showed there is no significant differences with using mineral oil versus no mineral oil treatment prior to the proteinase K digestion on deparaffinized tissue samples.

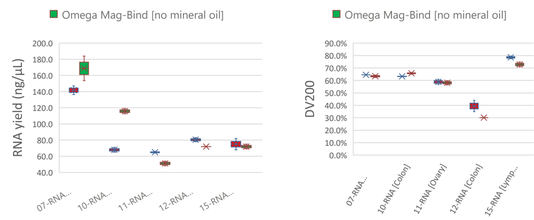


Fig 5B: Omega kit showed there is no significant differences with using mineral oil versus no mineral oil treatment prior to the proteinase K digestion on deparaffinized tissue samples.

Key findings

An optimized sequential extraction method generates high-quality DNA and RNA from a single set of input tissues that is automatable and operation-friendly. This workflow performs well with reduced FFPE tissue input and efficiently supports various high-throughput clinical NGS applications.

Conclusions

An optimized FFPE extraction method allows more clinical biopsy samples to be tested with different NGS workflows, providing a better diagnostic value for patient care.

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