

HIGH THROUGHPUT HIGH MOLECULAR WEIGHT DNA EXTRACTION FROM HUMAN TISSUES FOR LONG-READ SEQUENCING



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HMW Tissue DNA Extraction

Sample Homogenization



Covaris cryoPREP CP02

Freeze Tissue in tissueTUBE 5 Min

Cryofracture using CryoPREP 5 Min

Wash Tissue Pellet 15 Min

Lyse 60 Min

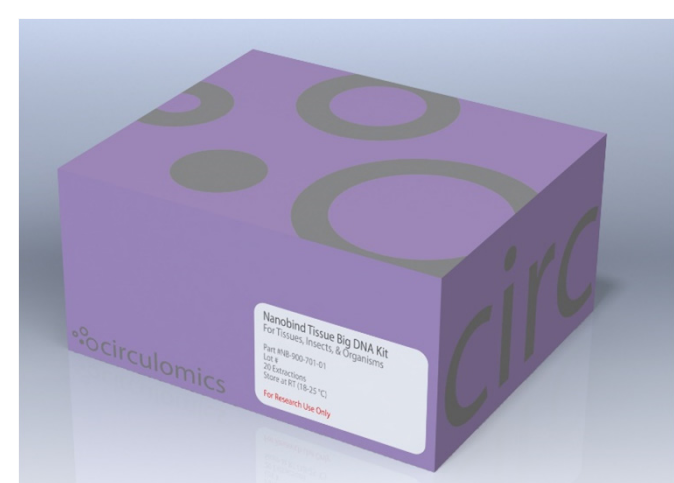
Pellet Debris 5 Min

Bind DNA 15 Min

Wash x3 5 Min

Elute 10 Min

HMW DNA Extraction



Circulomics Nanobind Tissue Big DNA Kit

Covaris CryoPREP



Covaris tissueTUBE

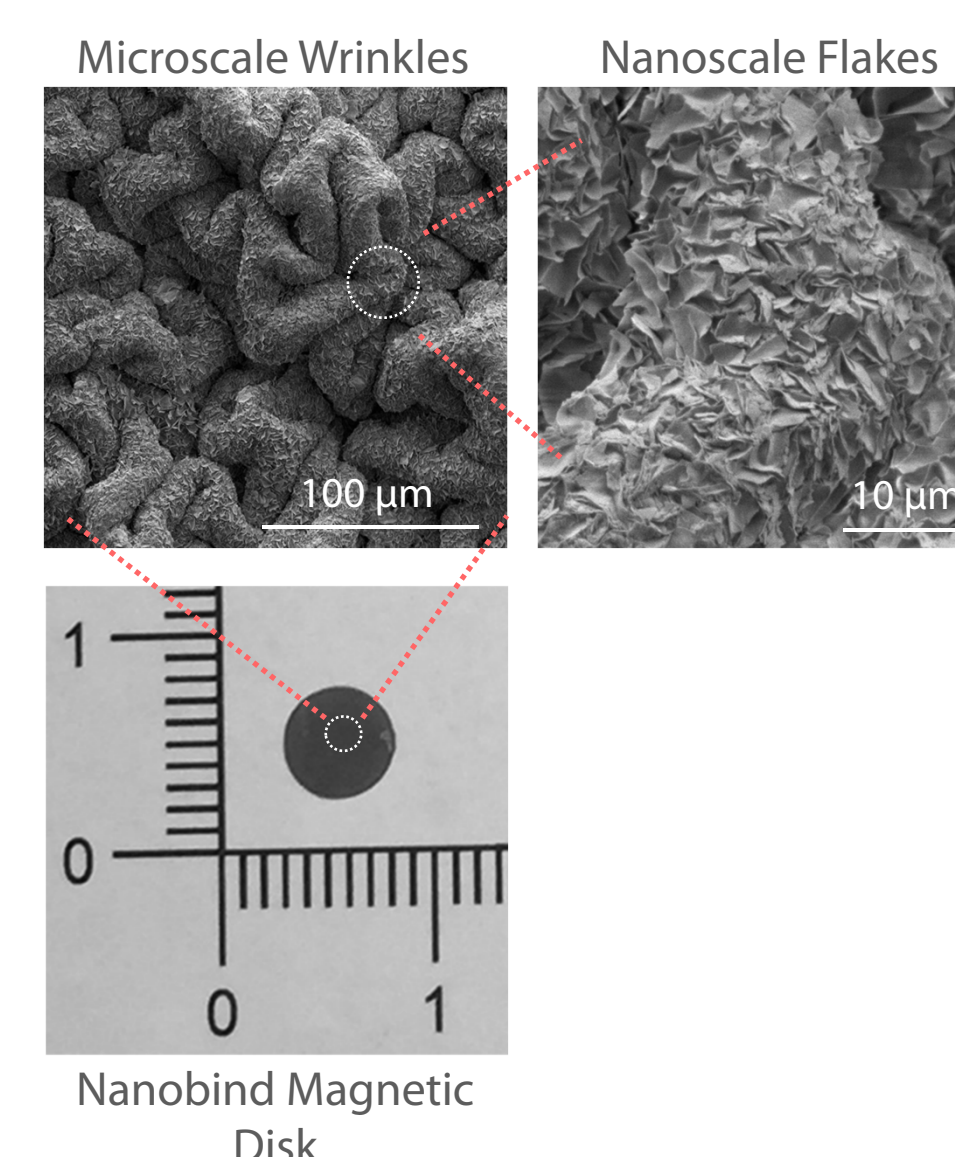
- Tissue samples are frozen and cryofractured within tissueTUBE
- Disposable tube prevents cross-contamination
- Different size tubes to accommodate wide range of inputs (15 mg – 2 g)



Covaris cryoPREP CP02 Automated Dry Pulverizer

- Automated piston cryofractures frozen tissue sample in seconds
- Variable power for different sample types
- Multiple impacts to achieve fine powder

Circulomics Nanobind



Nanobind Magnetic Disks

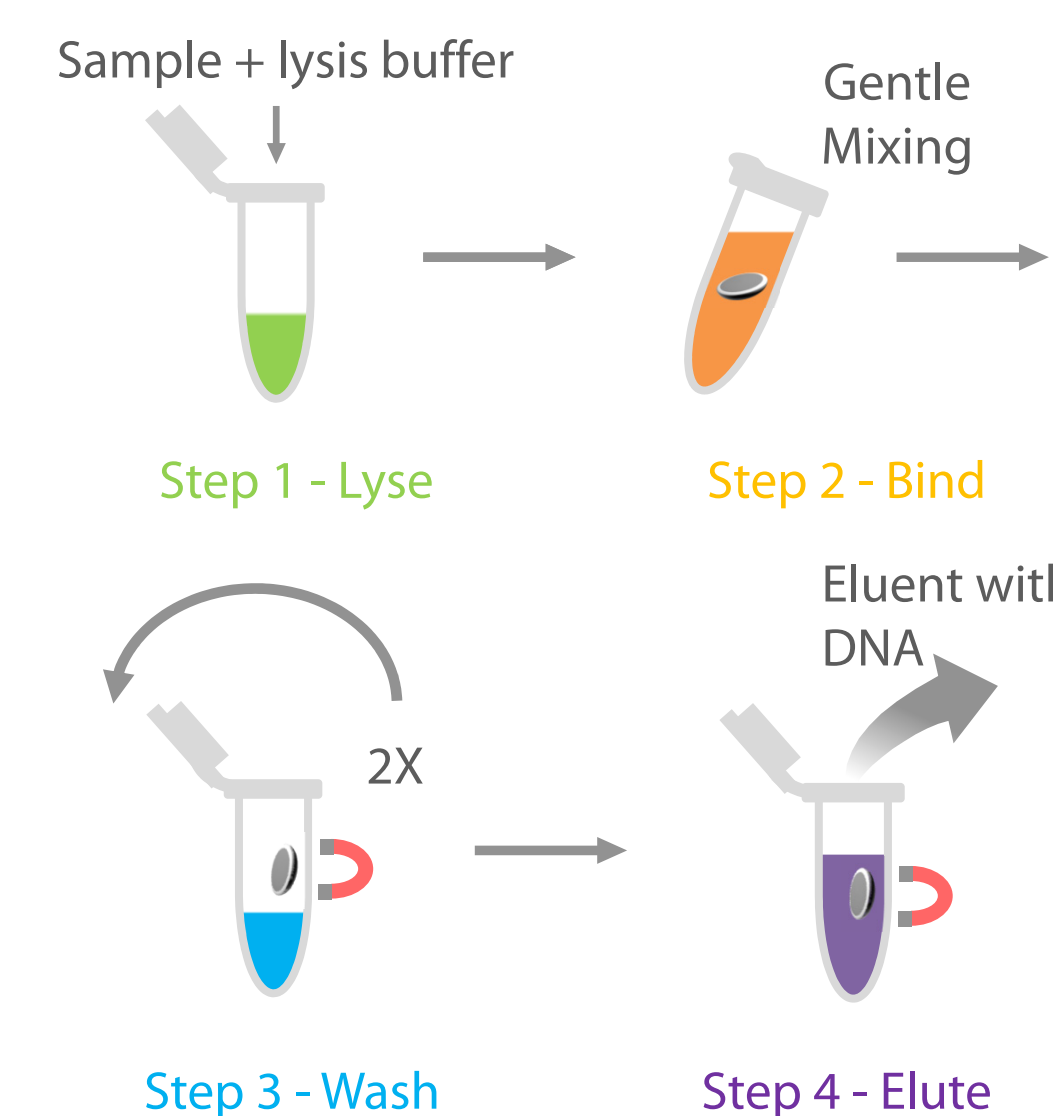
- Micro- and nanowrinkles protect DNA from shearing
- High binding capacity
- High purity for single molecule sequencing

Broad Applications

- HMW DNA for long-read sequencing and optical mapping
- Size selection purification for NGS library preparation
- Diverse sample types including cells, blood, plants, tissues, and insects.

Rapid Magnetic Purification

- Rapid <1 hour bind, wash, and elute process
- 1 disk per tube
- HMW protocols generate 50 – 300+ kb sized DNA
- UHMW protocols generate 50 kb – 1+ Mb sized DNA
- Manual processing using magnetic rack
- Automation compatible

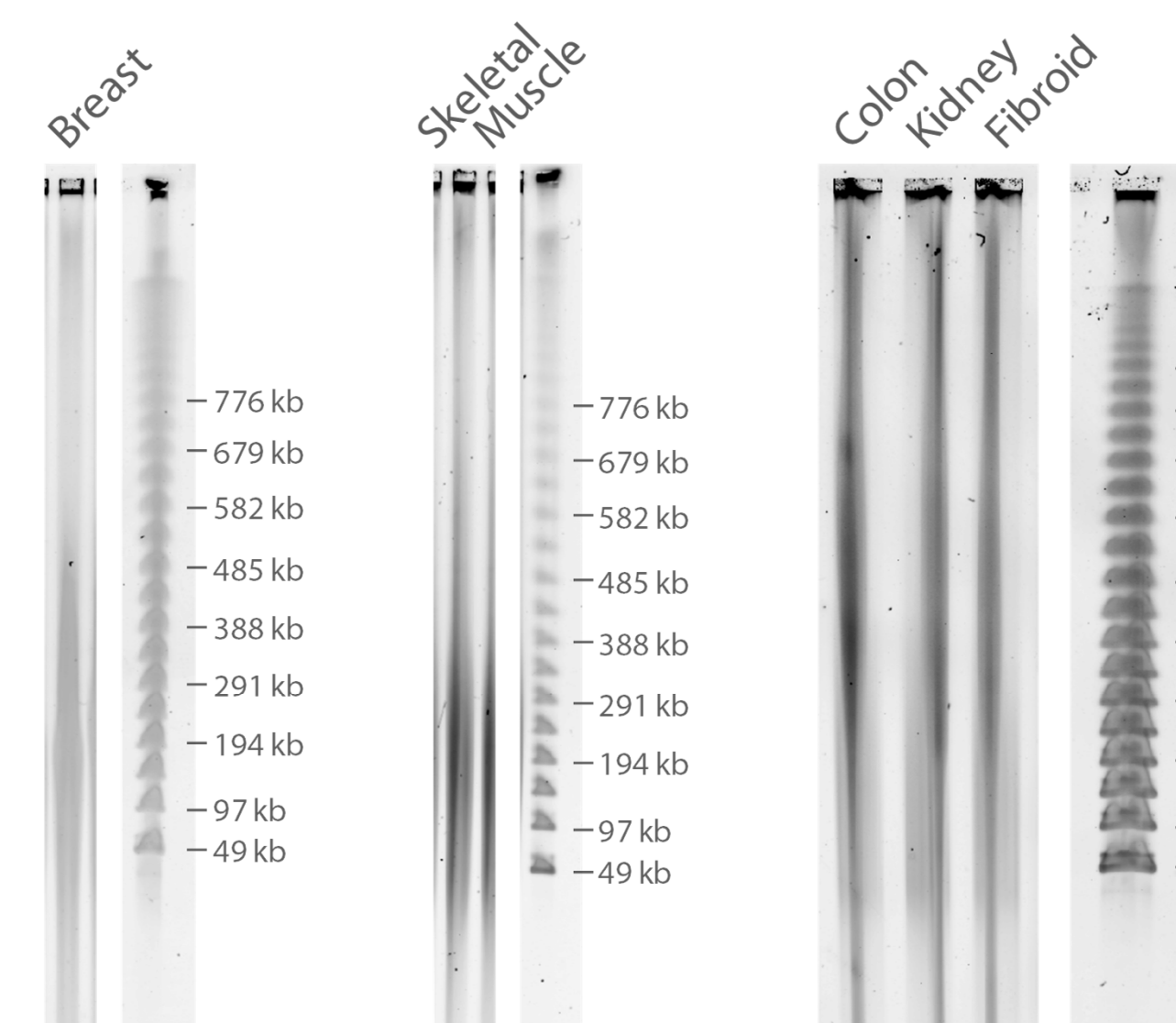
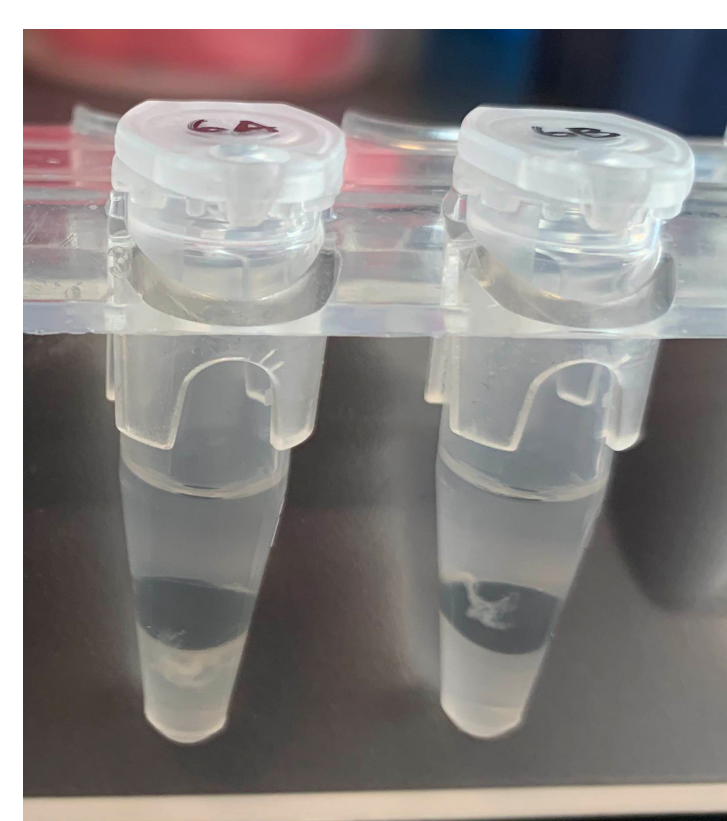


The high-throughput method for extracting HMW DNA from human tissue can be completed in 2h and is easily scaled to large numbers of samples.

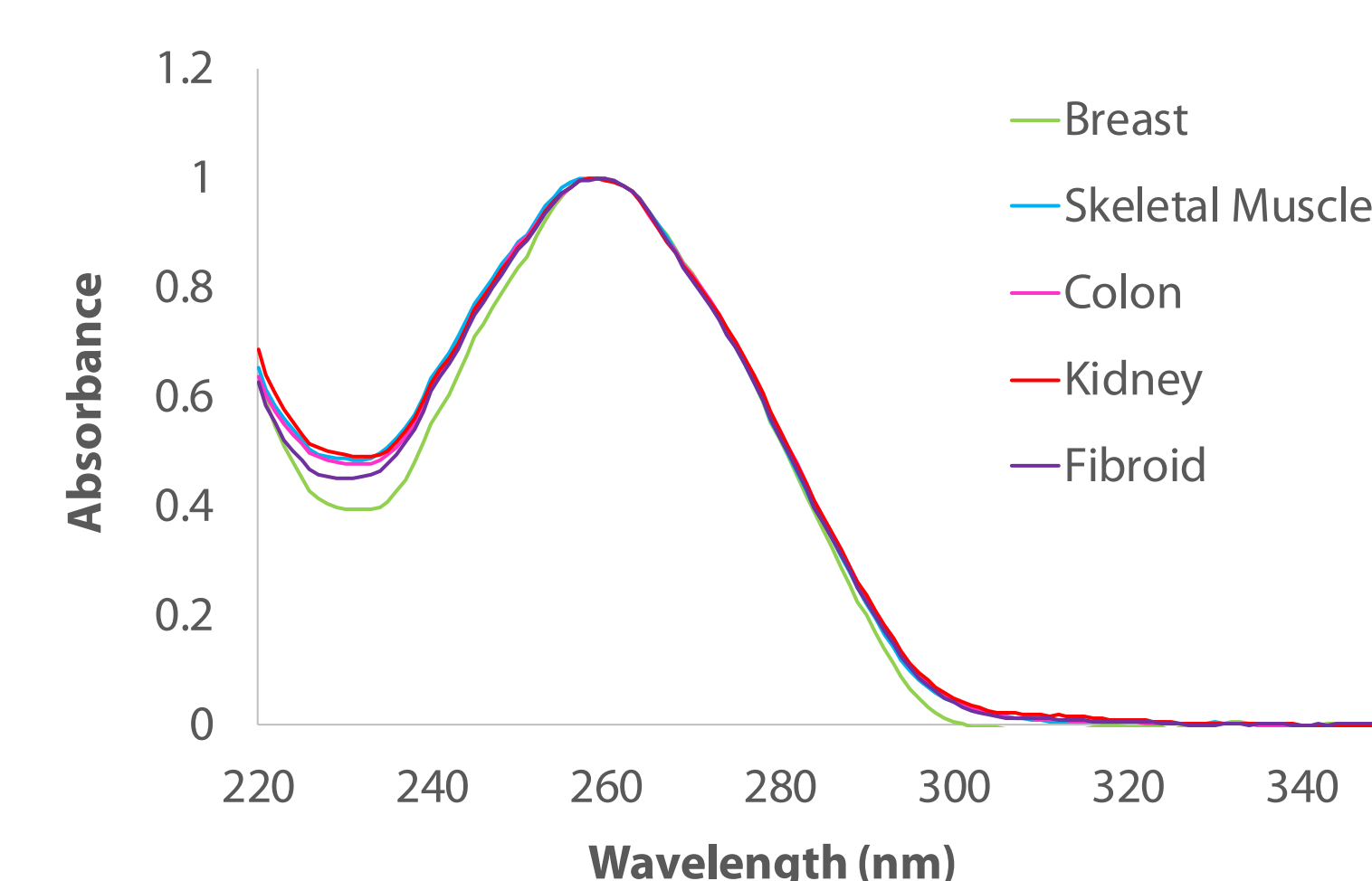
Tissue samples are frozen in Covaris tissueTUBEs and rapidly cryofractured using the cryoPREP Automated Dry Pulverizer.

Circulomics Nanobind Tissue Big DNA Kit is then used to extract HMW DNA from the tissue powder using a simple bind, wash, and elute process.

DNA Extraction Results – Size, Yield, and Purity



Sample	Sample Input (mg)	260/280	260/230	dsDNA (ng/μL)	Total DNA (μg)
Breast	35	1.85	2.06	590	44.2
Skeletal Muscle	26	1.90	2.05	90.6	6.8
Colon	35	1.85	2.09	530	39.8
Kidney	48	1.83	2.03	416	31.2
Uterine Fibroid	35	1.88	2.19	447	33.5



Tissue DNA bound to the Nanobind disk during binding step.

HMW DNA surpassing 300+ kb was obtained from a variety of human tissue types.

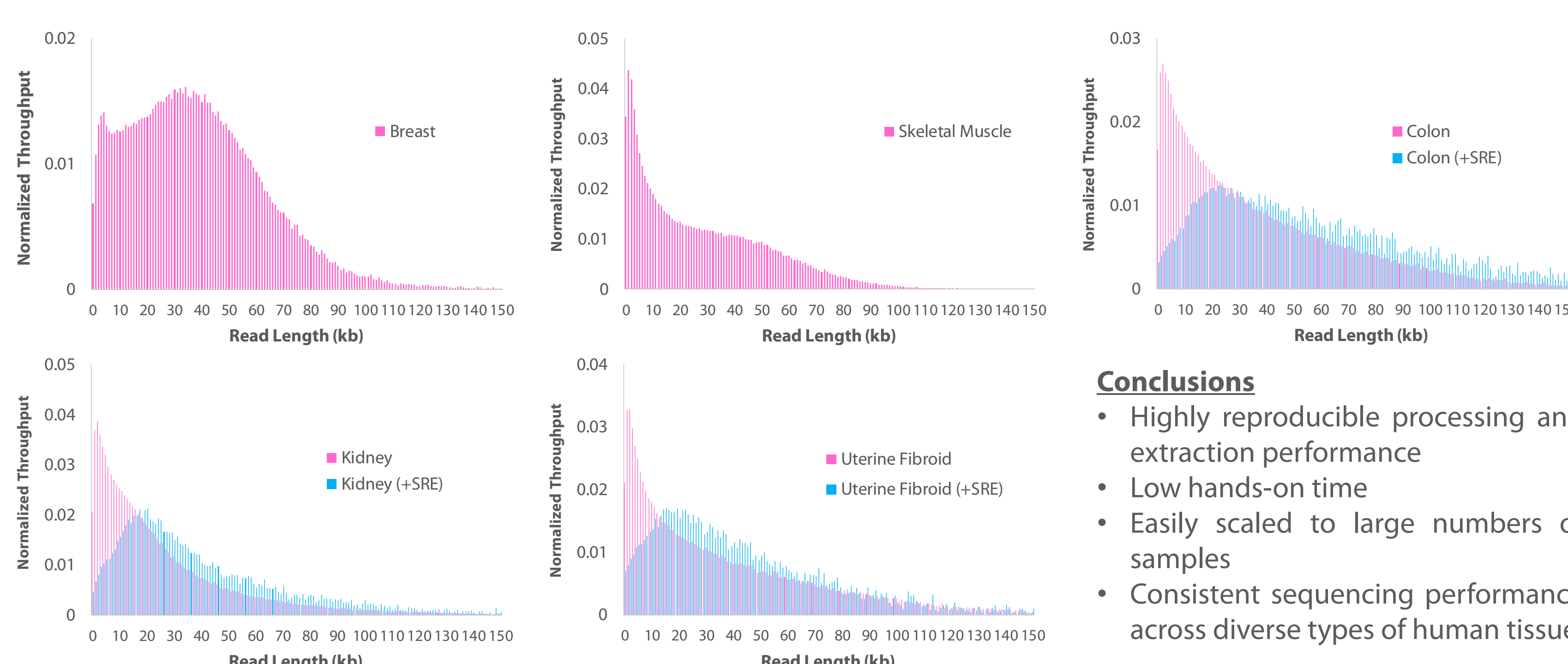
25 – 50 mg of tissue was used per extraction and resulted in ample DNA for long-read sequencing library preparation.

The extractions resulted in high purity samples with near ideal UV ratios.

Oxford Nanopore MinION Sequencing

Sample	Library Prep	Total Data	Data >100 kb	Read Length N50 (-SRE)	Read Length N50 (+SRE)
Breast	No size selection SQK-LSK109, FLO-MIN106D	6.8 Gb	137 Mb	36.2 kb	-
Skeletal Muscle	No size selection SQK-LSK109, FLO-MIN106D	8.6 Gb	87 Mb	23.1 kb	-
Colon	No size selection SQK-LSK109, FLO-MIN106D	9.6 Gb	662 Mb	28.6 kb	51.7 kb
Kidney	No size selection SQK-LSK109, FLO-MIN106D	12.1 Gb	401 Mb	18.4 kb	31.8 kb
Uterine Fibroid	No size selection SQK-LSK109, FLO-MIN106D	8.3 Gb	681 Mb	28.3 kb	36.1 kb

- HMW DNA was sequenced on Oxford Nanopore MinION using the Ligation Sequencing Kit.
- No size selection was performed on the initial 48h runs. An average of 9 Gb of data was obtained for each of the 5 tissue types.
- HMW DNA samples were then sequenced a 2nd time using Circulomics Short Read Eliminator to deplete short DNA, resulting in significantly enhanced read length N50.



Conclusions

- Highly reproducible processing and extraction performance
- Low hands-on time
- Easily scaled to large numbers of samples
- Consistent sequencing performance across diverse types of human tissue