HMW DNA Extraction From Challenging Samples for Long-Read Sequencing

11/11/2019

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Founder, CEO

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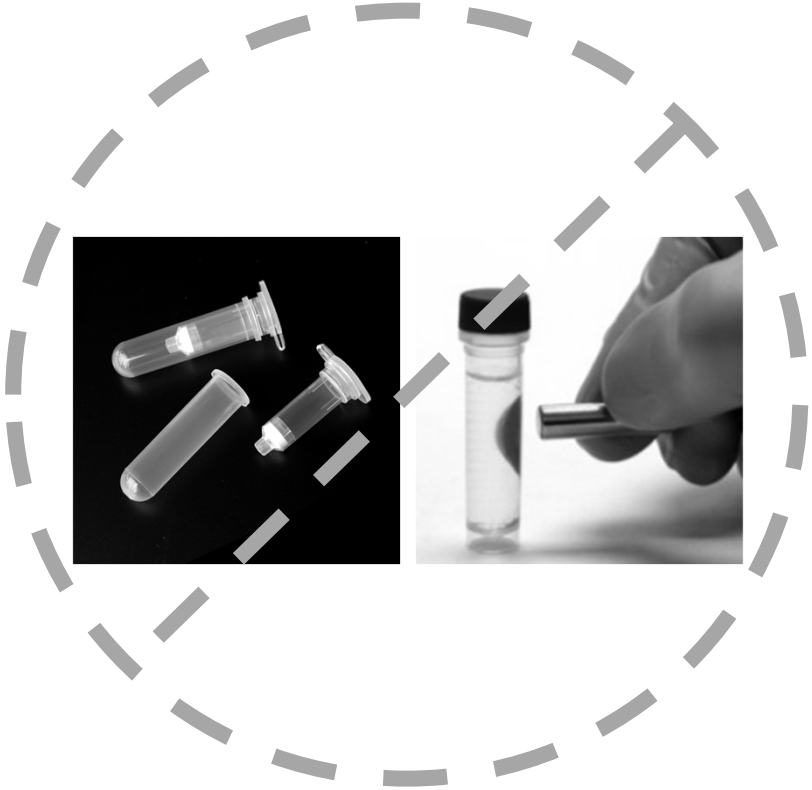
Long-Read Sequencing Requires Clean HMW DNA

Poor DNA quality leads to short read lengths and low data yield.

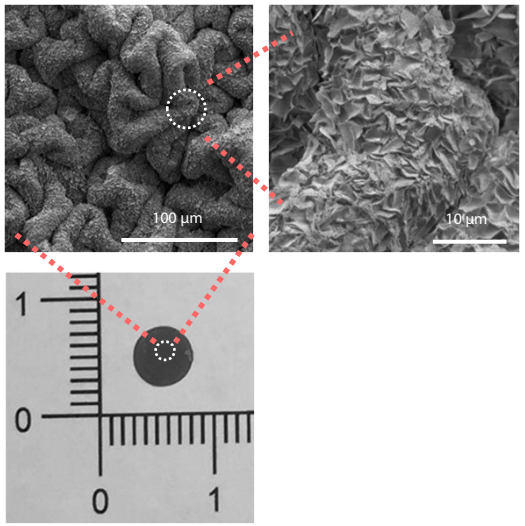


Precipitation and gel plug purification are slow and tedious.

Nanobind Magnetic Disks

New sample prep technology for HMW DNA extraction and beyond.

Longer DNA than any other product on market.



High Surface Area + Low Shear Force

Immense binding capacity

Highest DNA quality

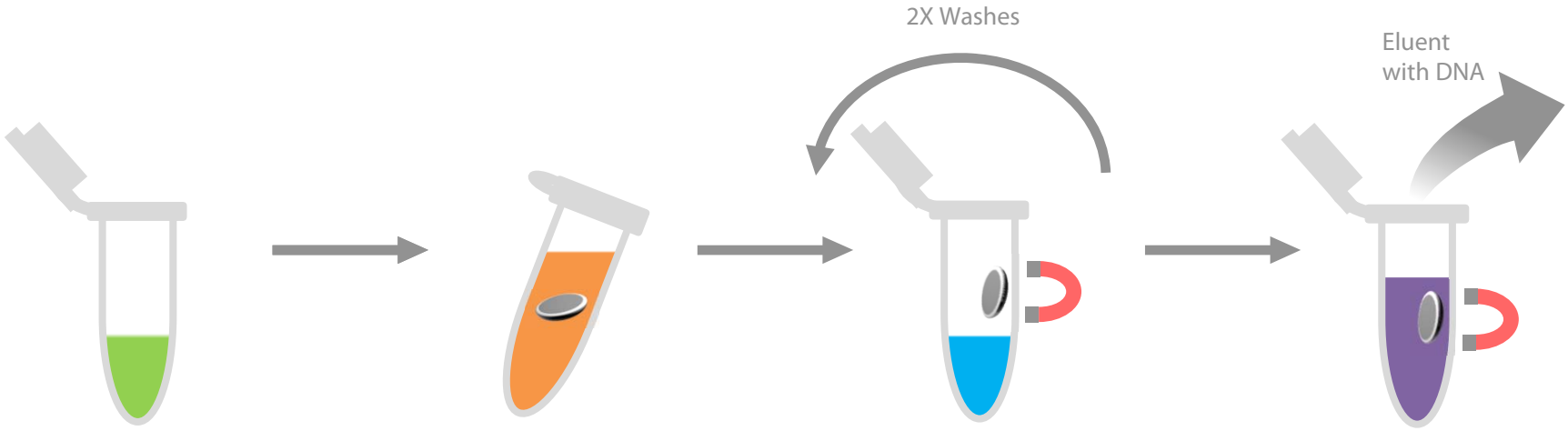
Unparalleled DNA Quality

Megabase DNA

Exceptional sequencing/mapping performance

Nanobind Protocol

Rapid 1 hour bind, wash, and elute process parallels magnetic bead extraction.



Step 1
Lyse

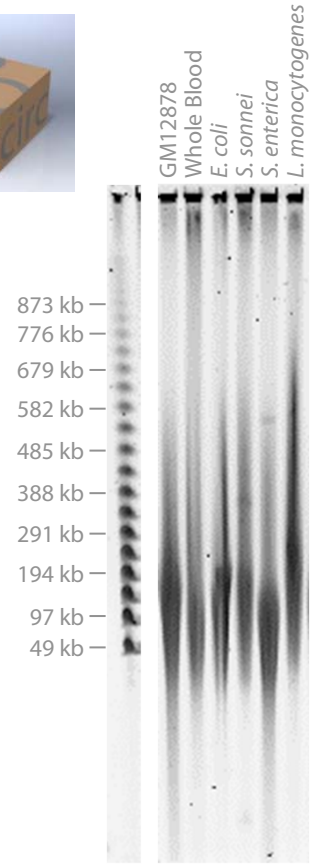
Step 2
Bind

Step 3
Wash

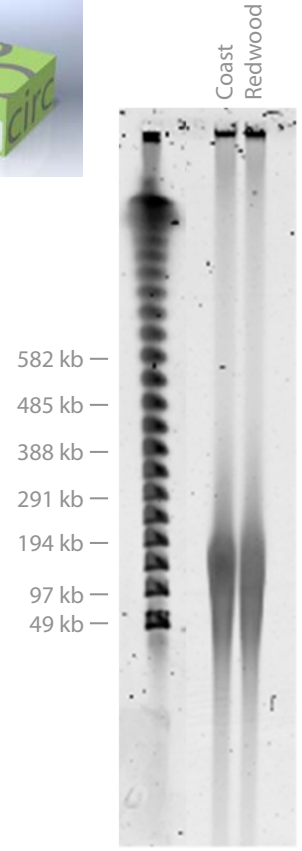
Step 4
Elute

Rapid HMW DNA Extraction (50 – 300+ kb)

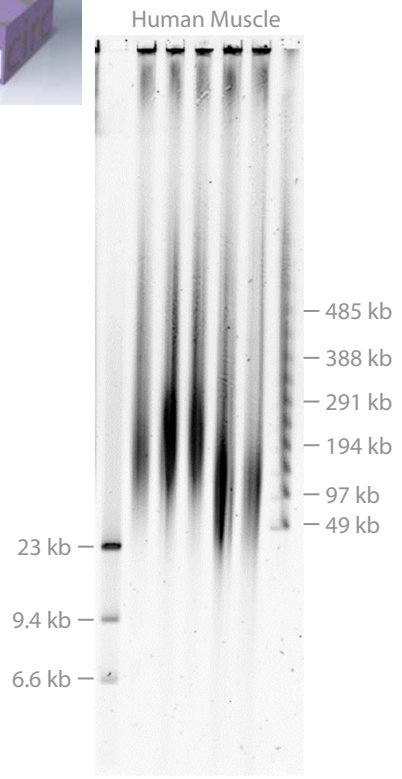
Consistent DNA size and quality across all sample types.



Nanobind CBB Big DNA Kit



Nanobind Plant Nuclei Big DNA Kit



Nanobind Tissue Big DNA Kit

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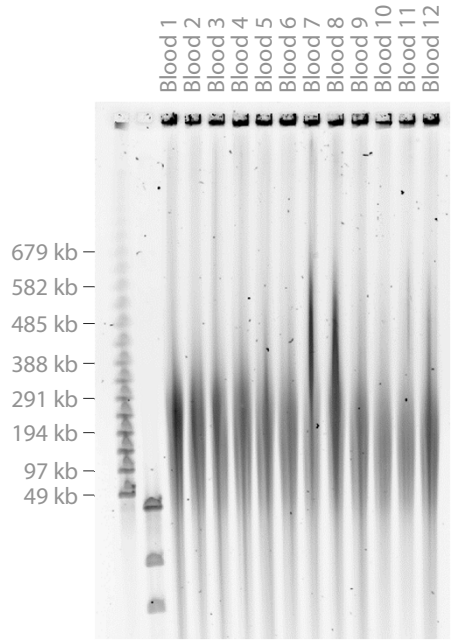
Automated Extractions on PacBio

Extract HMW DNA from 96 cell, bacteria, or blood samples in <40 minutes.

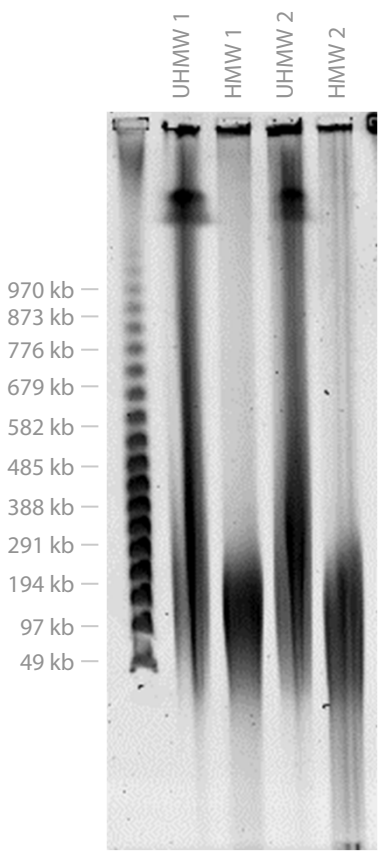
Compatible with All KingFisher Instruments

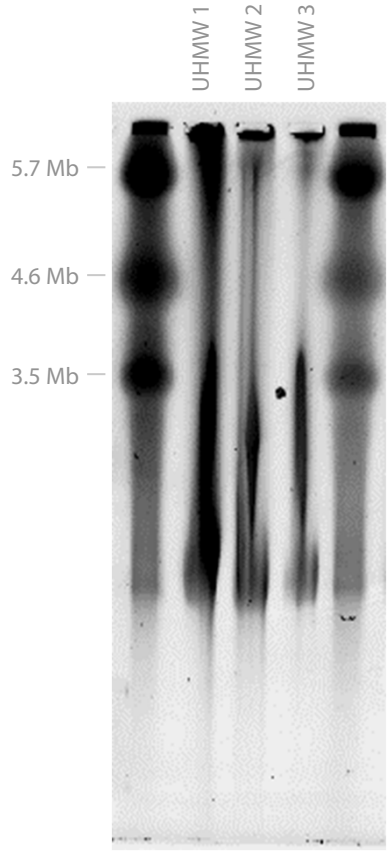


Consistent Size and Recovery

Rapid UHMW DNA Extraction (1+ Mb)

Extract megabase DNA in only 1 hr.
DNA size can only be rivaled by multi-day gel plug extraction. CBB Kit only.





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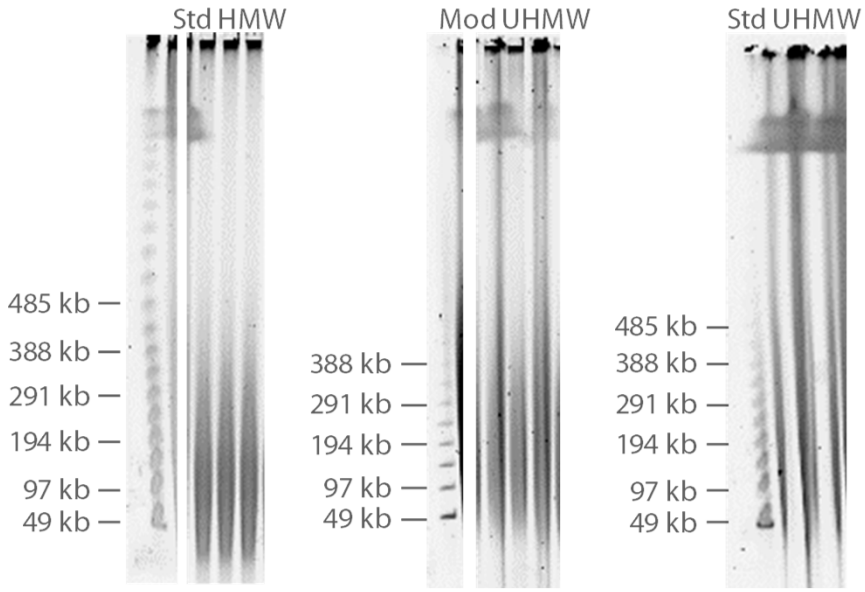
Ultra Long Reads on Oxford Nanopore

Nanobind can be used to extract megabase sized DNA for and obtain read lengths up 1.3 Mb.

Compared 3 different extraction protocols using Rapid Prep.



Oxford Nanopore GridION - Whale Hunting					
Nanobind Protocol	**Yield (Gb)**	**Read Length N50 (bp)**	**Reads >100 kb**	**Reads >1 Mb**	**Longest Read (Mb)**
Standard HMW	2.9	33,669	2809	0	.78
Modified UHMW	3.1	44,849	4698	2	1.1
Standard UHMW	1.9	60,734	3519	4	1.3

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Short Read Eliminator Family

Use size selective precipitation to deplete short DNA and enhance read lengths.

Fast processing. High recovery. No special equipment.

SRE XS

Up to 10 kb

Fragmented DNA + Amplicons

80% recovery

SRE

Up to 25 kb

High quality HMW DNA (>48 kb)

60% recovery

SRE XL

Up to 40 kb

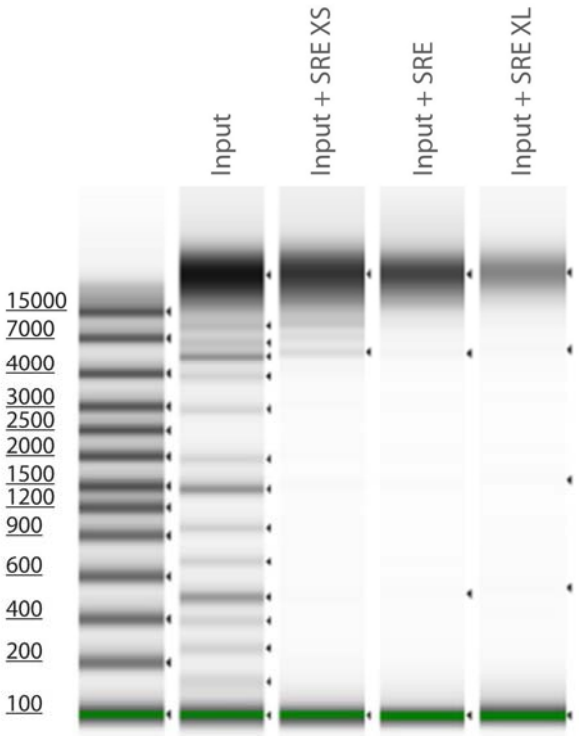
Very high quality HMW DNA (>>48 kb)

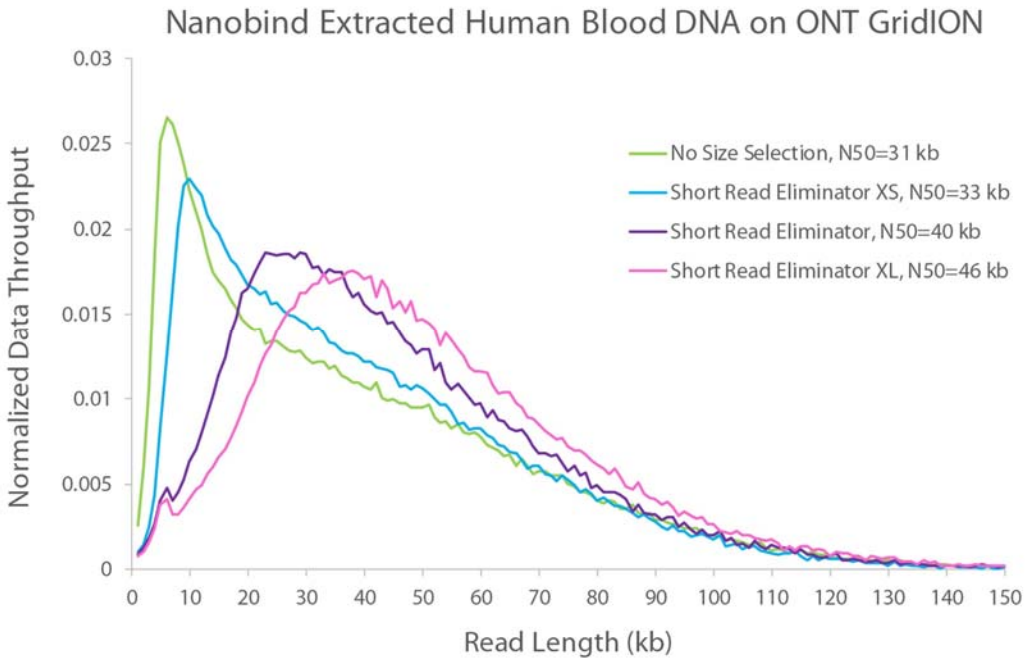
40% recovery

Selectable Cutoffs to Suit Your Sample

Enhance read length based on application.

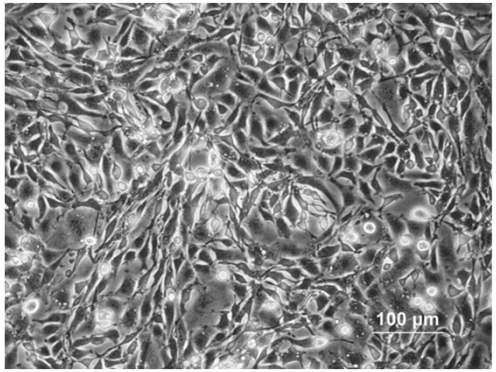
Depletion efficiency increases as DNA gets shorter.



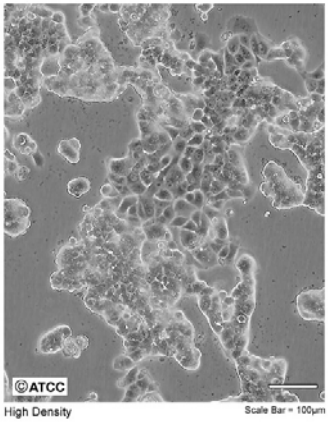


Cell Samples

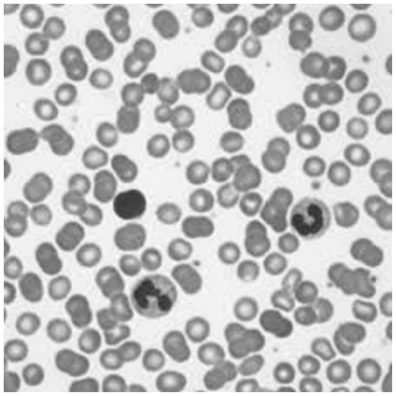
Selection of cell samples extracted using Nanobind and sequenced on PacBio or ONT.



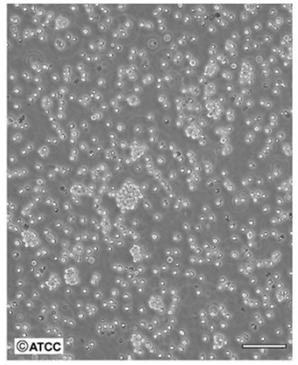
CHO



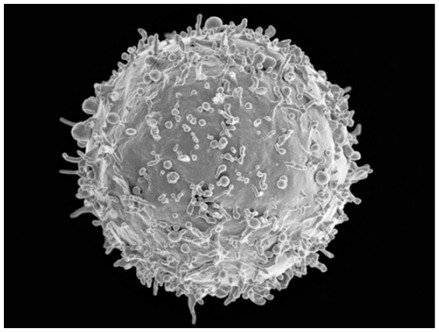
MCF-7



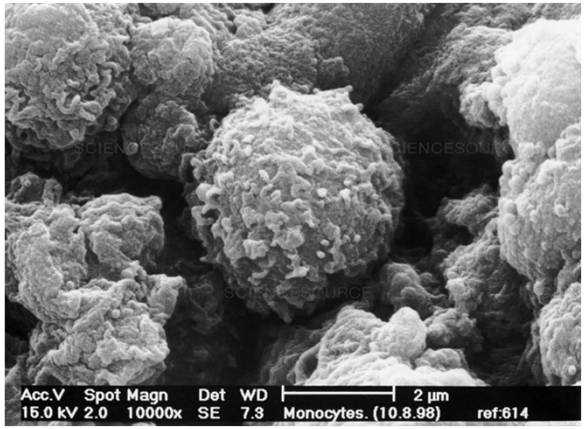
Whole Blood



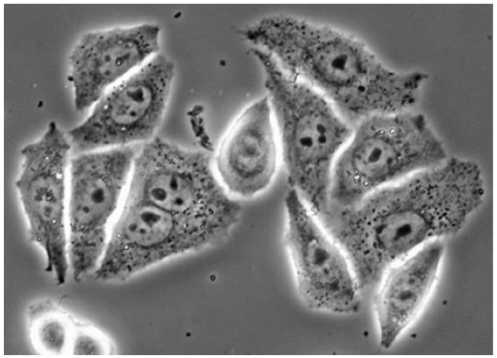
Jurkat



GM12878



PBMC



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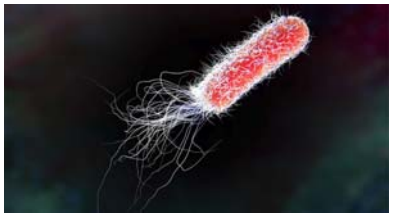
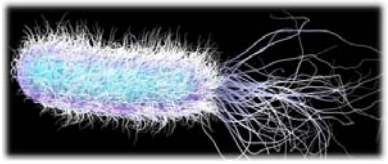
Microbial Samples

Selection of microbes extracted using Nanobind and sequenced on PacBio or ONT.

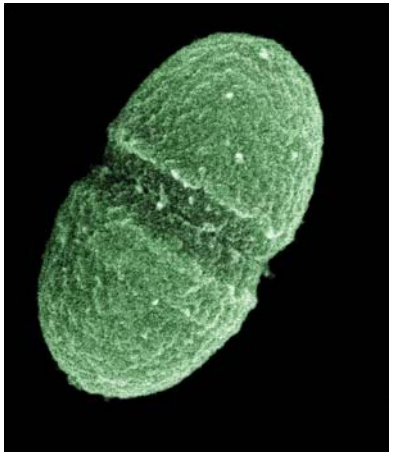
L. monocytogenes



E. coli K12



P. aeruginosa

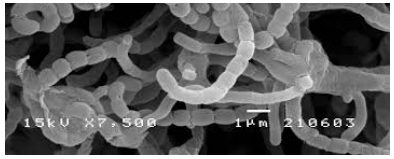


E. faecalis

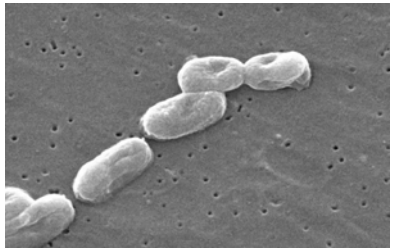
S. sonnei

B. cereus

C. diptheriae

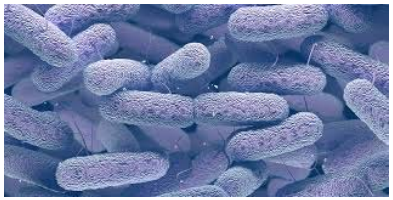


S. rapamycinicus



B. cepacia

S. enterica



K. pneumoniae

S. Aureus (25923 / BAA-44)

B. subtilis

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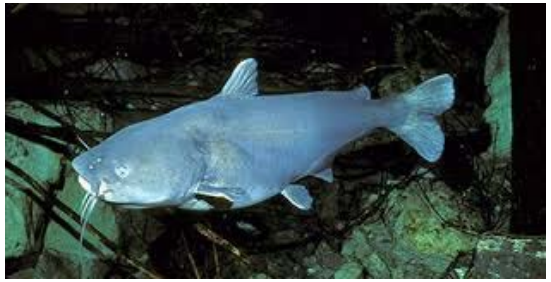
Blood Samples

Selection of blood samples extracted using Nanobind and sequenced on PacBio or ONT.



Plant Samples

Selection of plants extracted using Nanobind and sequenced on PacBio or ONT.

Coastal Redwood

Lettuce

Grape

Lavender

Rapeseed

Rice

Maize

Pepper

Hyacinth

Giant Sequoia

Tomato

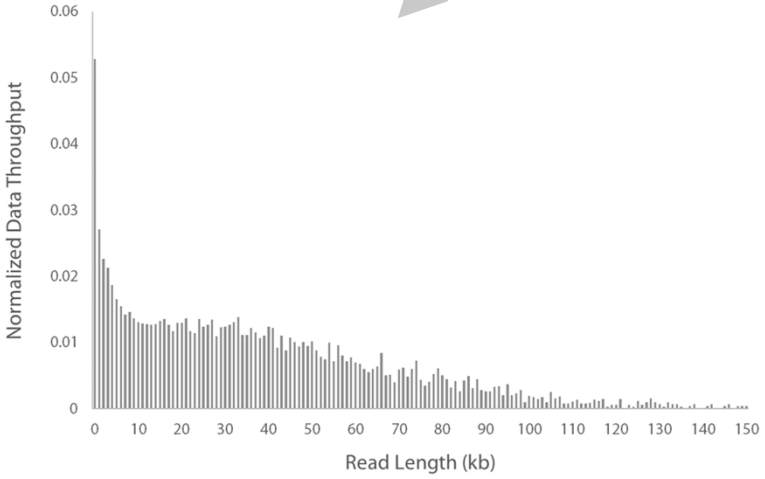
New Direct Tissue Lysis Chemistry

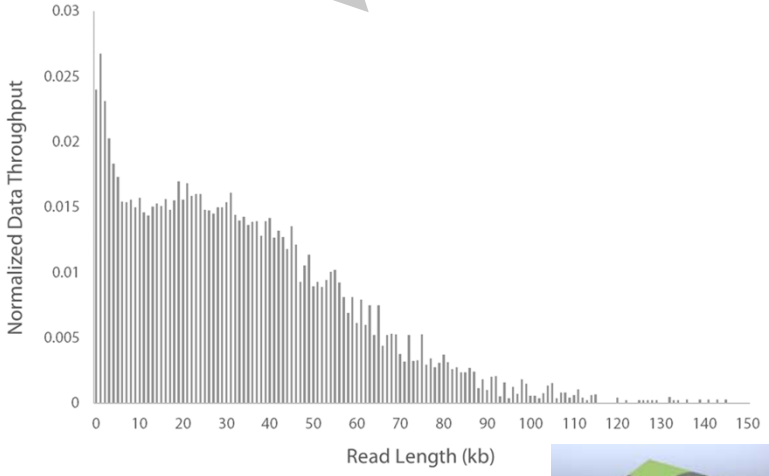
Updated Plant Kit will include chemistries for both Nuclei Preps and Direct Tissue Lysis.

Plant Nuclei Prep
1g Tissue
4 hours
N50 = 33.4 kb

Baby's Breath

Direct Plant Lysis
0.1 g Tissue
2 hour
N50 = 30.2 kb





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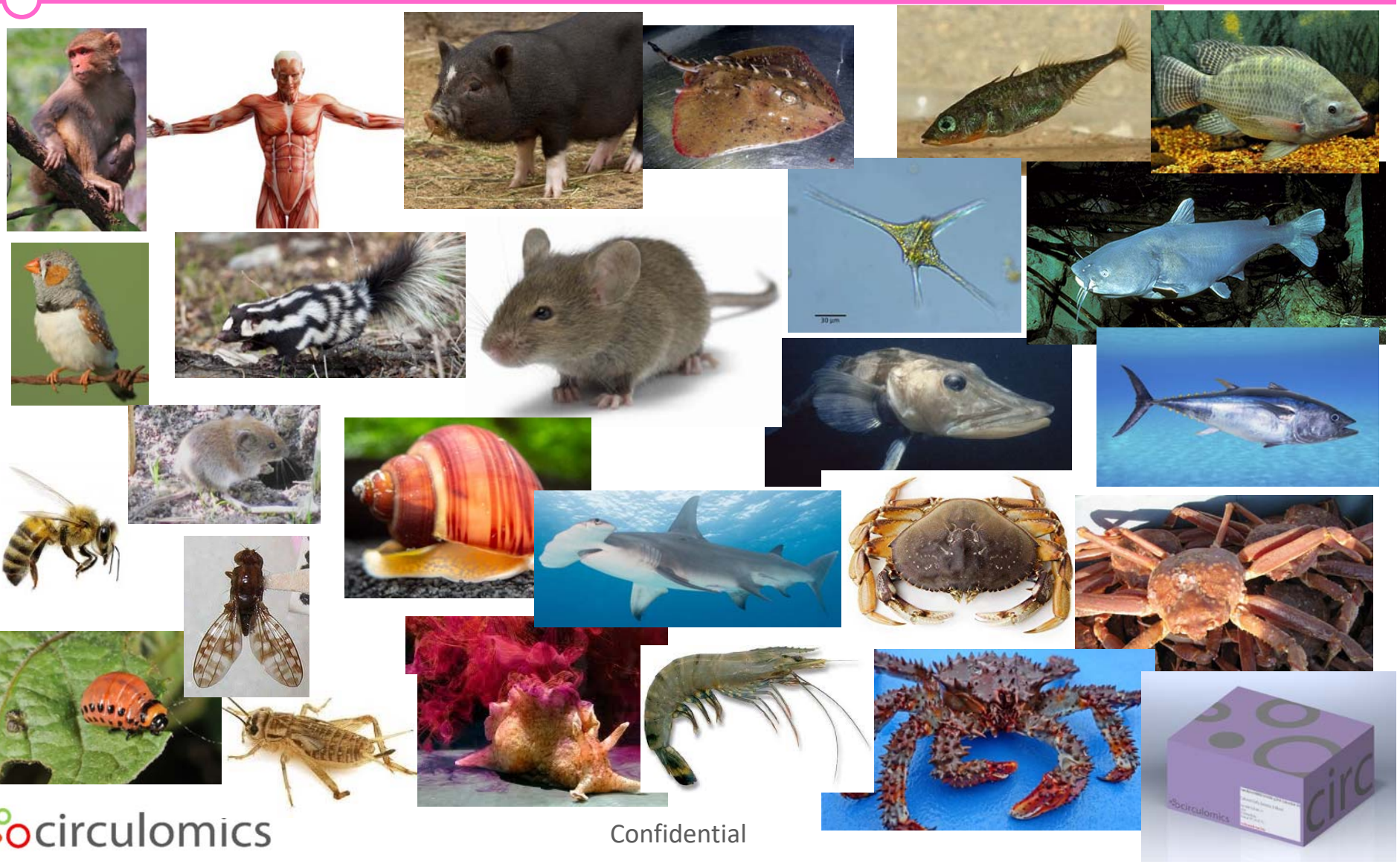
Tissue / Organism Samples

Selection of tissue and organisms extracted using Nanobind and sequenced on PacBio or ONT.



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Sample Quality

For long reads, DNA quality is paramount.

For long DNA, sample quality is paramount

Sample Quality

Type
Age
Ischemic
Preservation
Pre-analytical

DNA Quality

Size
Purity
Yield
Damage

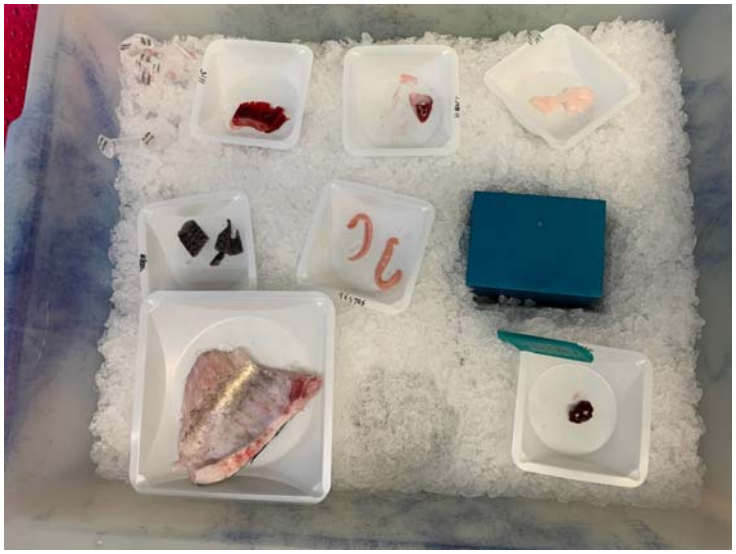
Sequence Metrics

Read Length
Throughput
QV

Nile Tilapia Sequencing

Tissue type affects DNA yield, quality, and sequencing performance.

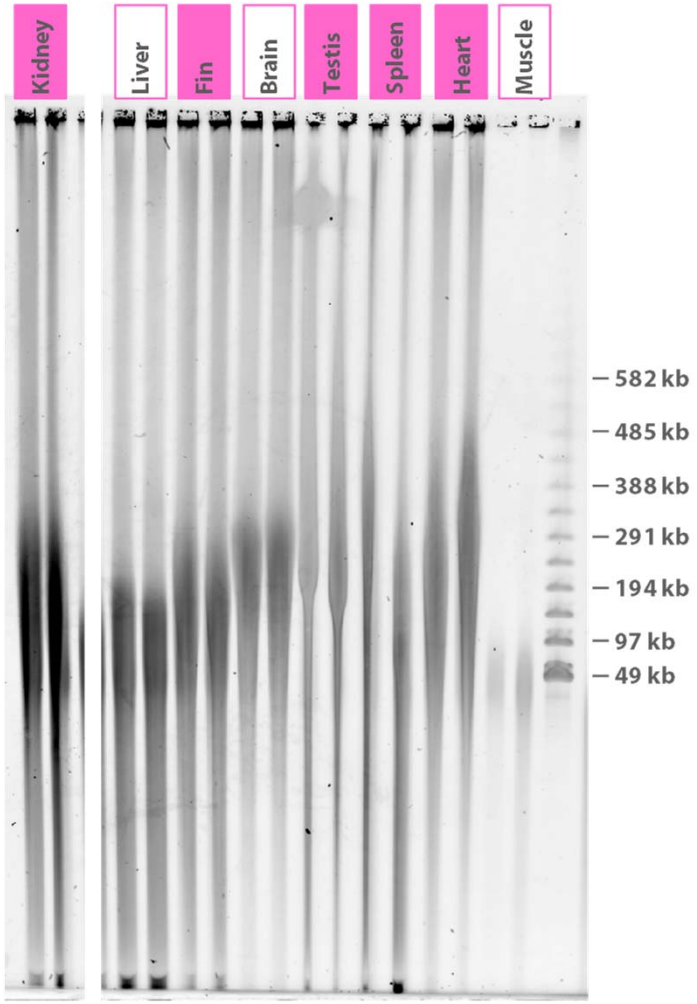
Extract from many tissue types and pick the best. Faster and better than focusing on 1 tissue.



Fish Sequencing

All samples had high UV purity. Yield and DNA size varied across sample type.

Picked 5 tissues with highest yield for sequencing.

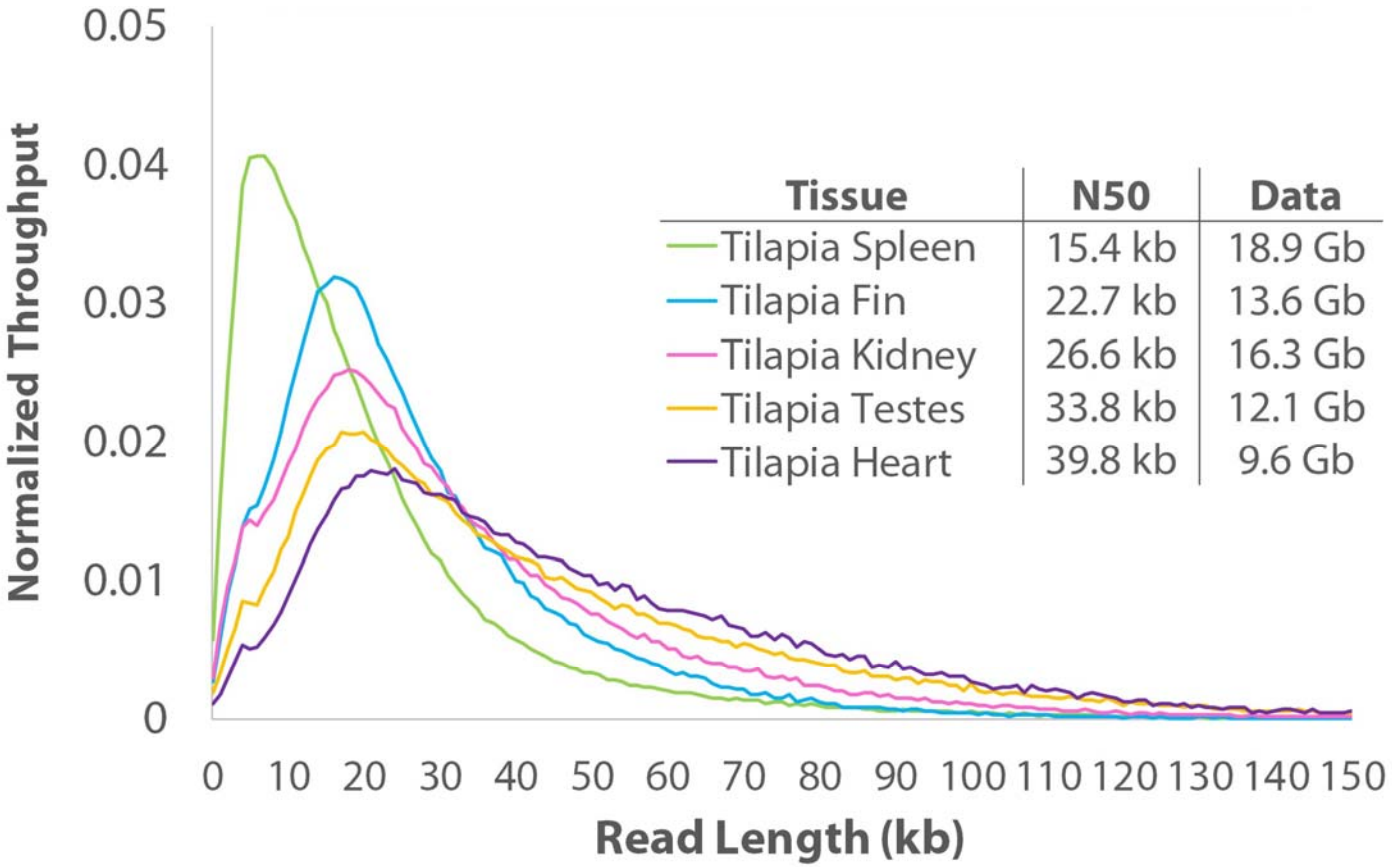


Sample Name	A260/A280	A260/A230	dsDNA (µg)
Kidney A	2.22	1.86	16.1
Kidney B	2.24	1.85	28.1
Liver A	1.94	1.85	4.4
Liver B	1.54	1.87	1.9
Fin A	2.12	1.81	8.0
Fin B	2.13	1.82	11.6
Brain A	1.83	1.90	2.5
Brain B	2.07	1.87	4.5
Testes A	2.31	1.86	41.9
Testes B	2.35	1.86	40.7
Spleen A	2.31	1.86	54.6
Spleen B	2.33	1.87	48.6
Heart A	2.01	1.86	7.6
Heart B	2.18	1.86	13.1
Muscle A	2.43	.32	0.0
Muscle B	1.95	.37	0.1

Tilapia Tissues on ONT GridION

Heart and blood generate consistent sequencing results across many fish species.

Fin is easily acquired. Spleen generates high yield but more fragmented DNA.



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Degraded Vole Samples

For some projects sample quality can be less than ideal

Fresh frozen

Trap caught, 1-2 days

Trap caught, many days

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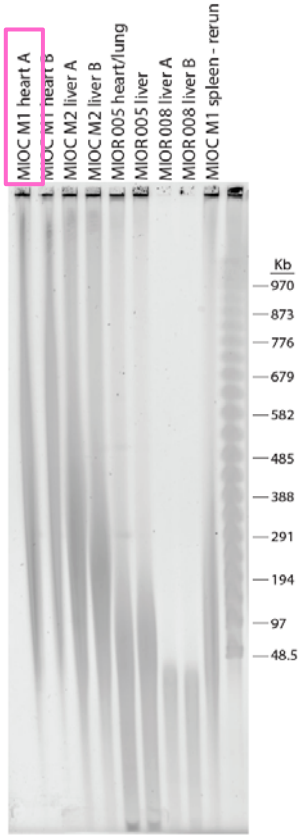
DNA Quality Varies Based on Sample Quality

Extracted from many samples and pick the best for sequencing.

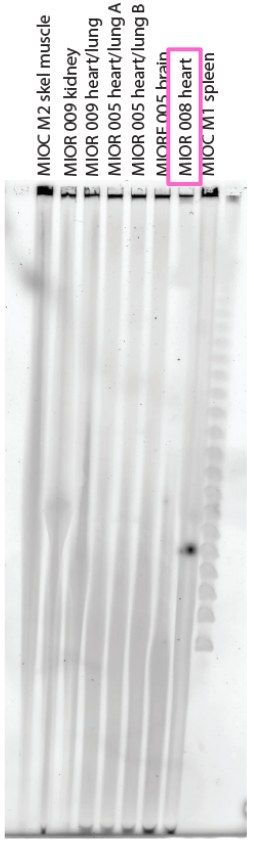
Consistent

Fresh frozen



RNALater

RNALater



Vole Samples on PacBio Sequel II CLR

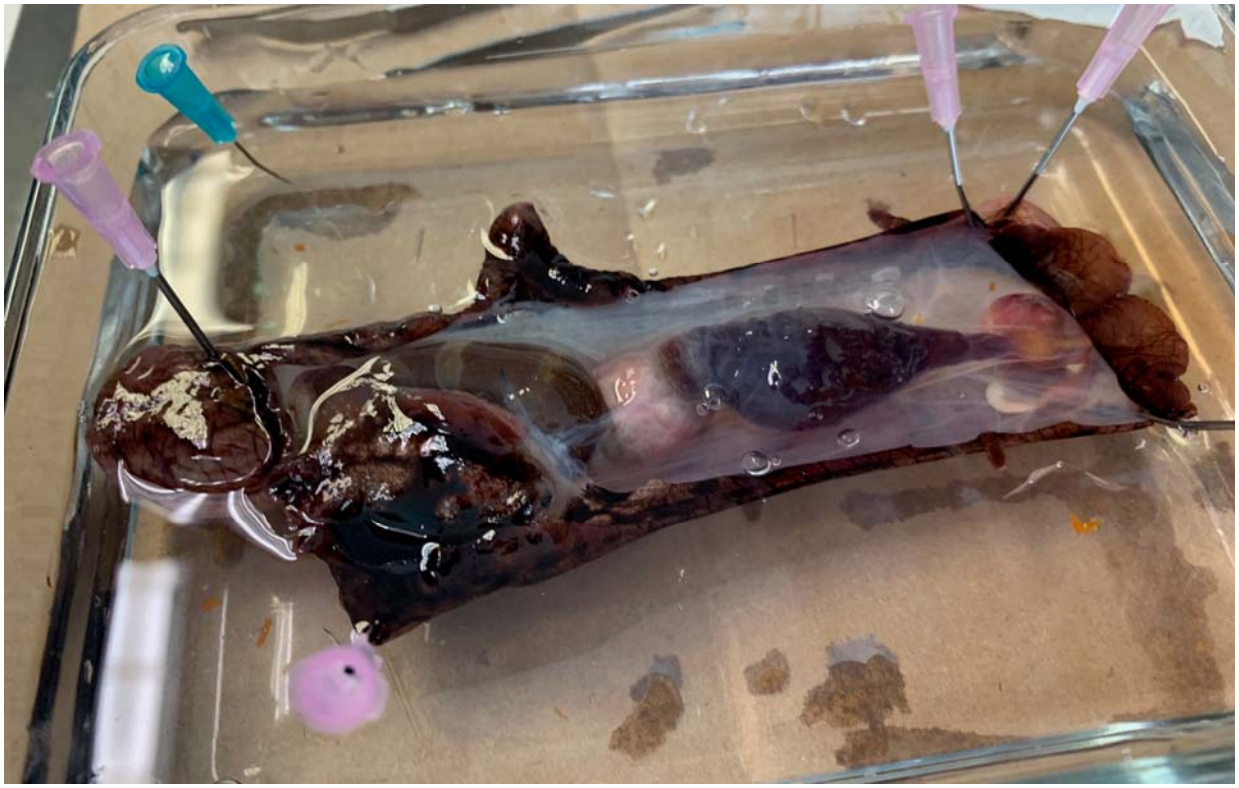
Sequencing performance shows direct correlation to initial sample quality.

	Nanobind Tissue Big DNA Kit – PacBio Sequel I/II Sequencing					
	Sample	Sample Quality	Library Prep	Subread N50 (bp)	Polymerase N50 (bp)	Total Data (Gb)
	Vole Heart	Fresh Frozen	30 kb BP	34,825	37,154	112.1
	Vole Kidney	1-2 days RNAlater	20 kb BP	24,211*	32,228*	89.0*
	Vole Heart	Many days RNAlater	No size selection	4,248	65,338	155.3

Challenging Mollusk Sample - Aplysia

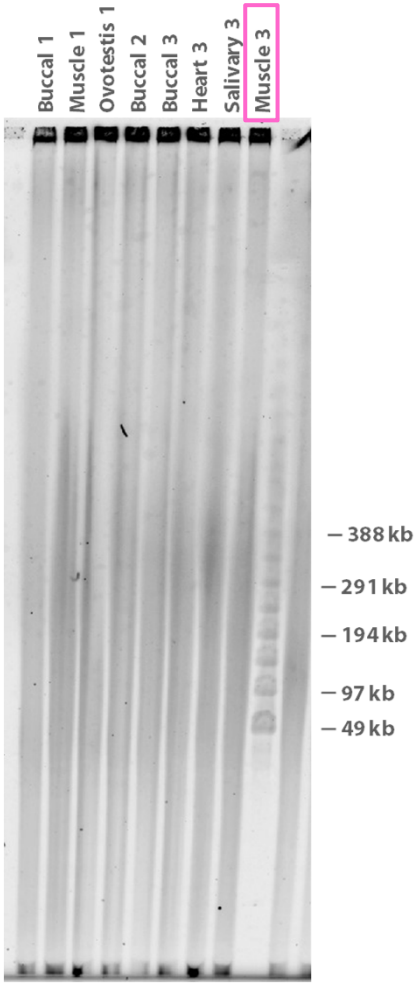
Mollusk samples can be very challenging.

Extract from many tissue types and pick the best.



Aplysia DNA Quality

High quality and purity DNA was obtained from multiple tissue types

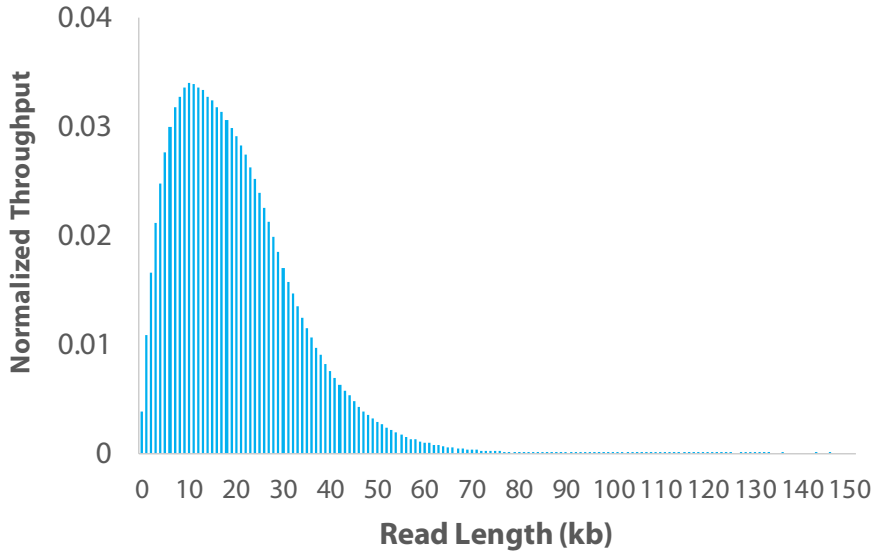


Sample Name	A260/A280	A260/A230	dsDNA (µg)
Buccal 1	1.89	1.91	4.2
Muscle 1	1.89	2.06	7.8
Ovotestis 1	1.85	2.16	46.5
Buccal 2	1.90	2.09	8.8
Buccal 3	1.86	1.86	4.2
Heart 3	1.87	2.17	14.5
Salivary 3	1.87	2.32	18.3
Muscle 3	1.87	2.07	9.1

Aplysia Muscle on PacBio Sequel II CLR

Sample generated 18 kb subread N50 and 72 Gb.

Previous attempts generated 12 kb N50.

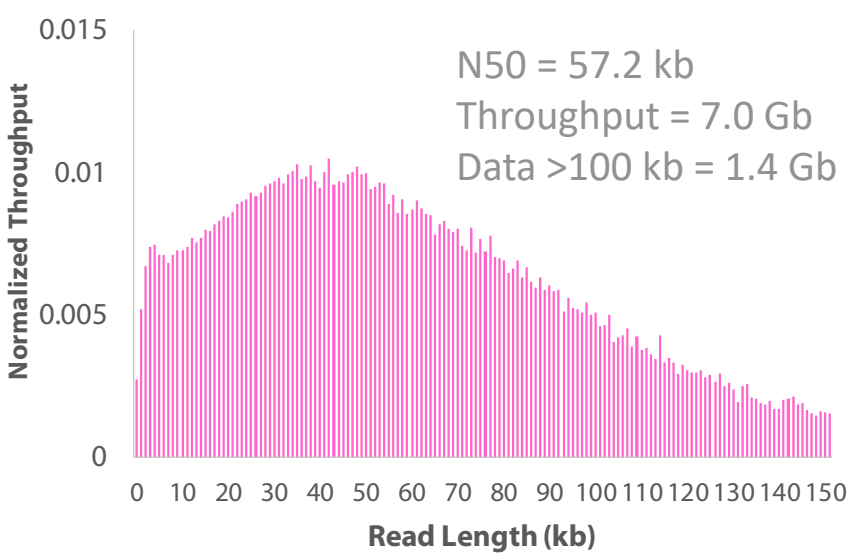


Sample	Sample Quality	Library Prep	Subread N50 (bp)	Polymerase N50 (bp)	Total Data (Gb)
Aplysia Muscle	Fresh	20 kb BP	18,151	18,868	71.6

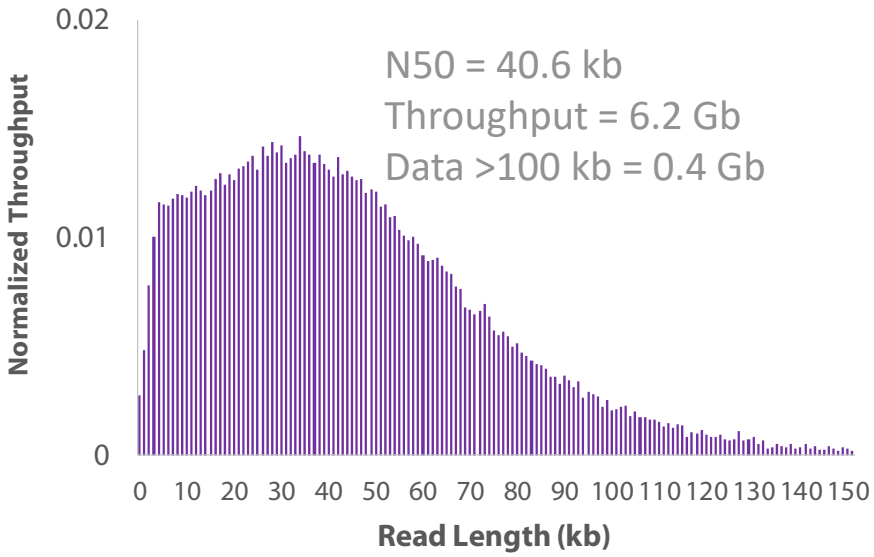
High Quality Tissues on ONT MinION

High quality tissues can generate exceptional read lengths.

Pig Brain



Pig Liver



Acknowledgements

Questions?

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Polly Campbell
Brian Couger

OSU

University of Maryland

Thomas Abrams
Luke Tallon

NIH National Institute of General Medical Sciences

NIH National Human Genome Research Institute

NIH National Institute of Environmental Health Sciences

Grand Valley State University

Charlyn Partridge

Iowa State University

Tanya Murtha