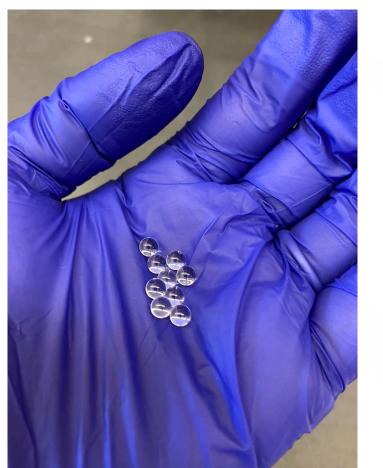
A Novel Method for High Molecular Weight (HMW) **DNA Extraction**

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INTRODUCTION

The need for very high molecular weight (HMW) DNA is growing quickly as long read sequencing and other emerging long read technologies become increasingly popular. However, the extraction of HMW DNA has been a bottleneck for these applications, and rapid, efficient and affordable HMW DNA extraction solutions are highly sought after. Here, we present a novel approach to HMW DNA extraction that utilizes large glass beads and optimized buffer chemistry, resulting in a simple workflow that enables researchers to quickly purify high quality HMW DNA from cells, blood, tissue and bacteria using 2 dedicated Monarch Kits. Purified DNA ranges from 50 kb to several megabases and size can be tuned by varying the speed at which samples are agitated during lysis. The use of glass beads enables easy handling, extremely efficient elution, high recovery, and easy dissolving of the isolated HMW DNA.



T3050

Cells/Blood Kit

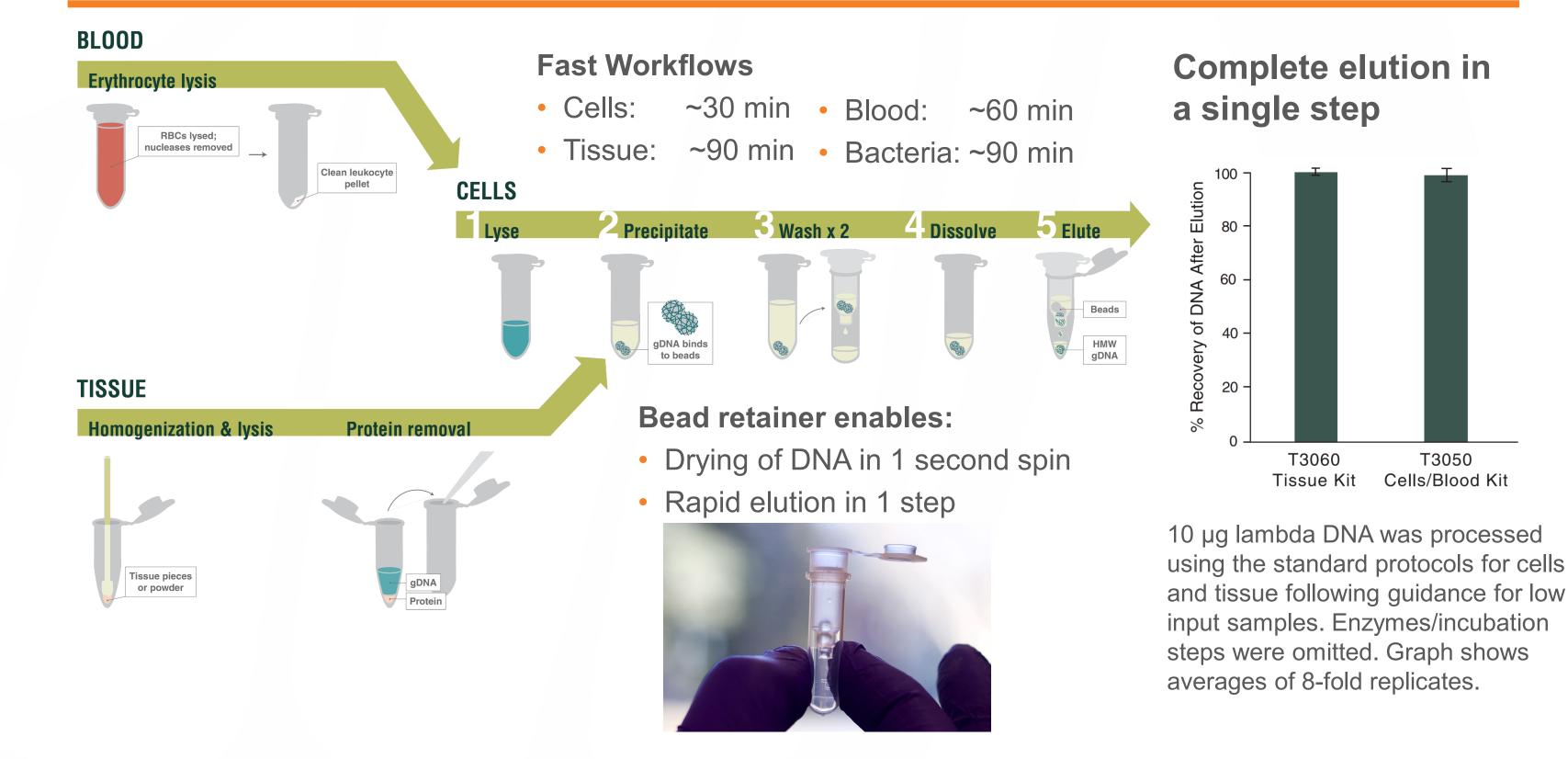
C. Higher yields for low inputs

HIGHLIGHTS

- Rapid protocols with minimal hands-on time (Cells: 30 min, blood: 60 min, tissue & bacteria: 90 min)
- Size of isolated HMW DNA is tunable and ranges from 50 kb to several Mb
- Mb sized UHMW DNA can be isolated from cells, blood, bacteria and soft organ tissues
- Complete elution in a single step
- Excellent solubility of isolated DNA
- High yields and reproducibility
- Maximal purity, very low RNA content
- Excellent performance in long read sequencing approaches



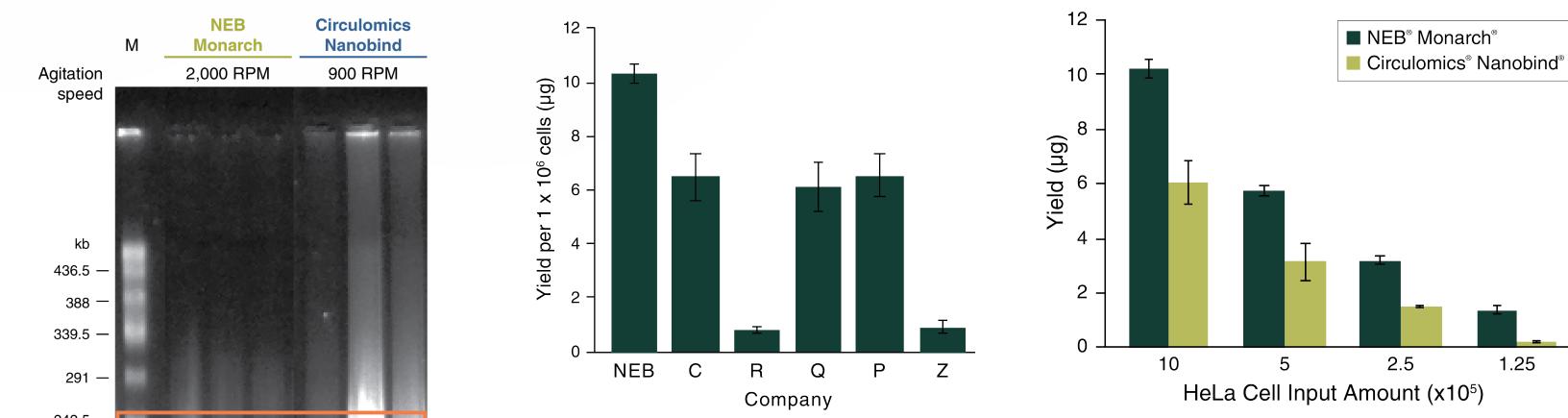
RAPID & EFFECTIVE "BIND-WASH-ELUTE" WORKFLOW



CELL PREPS - EXCELLENT YIELDS & REPRODUCIBILITY

B. Improved yields vs

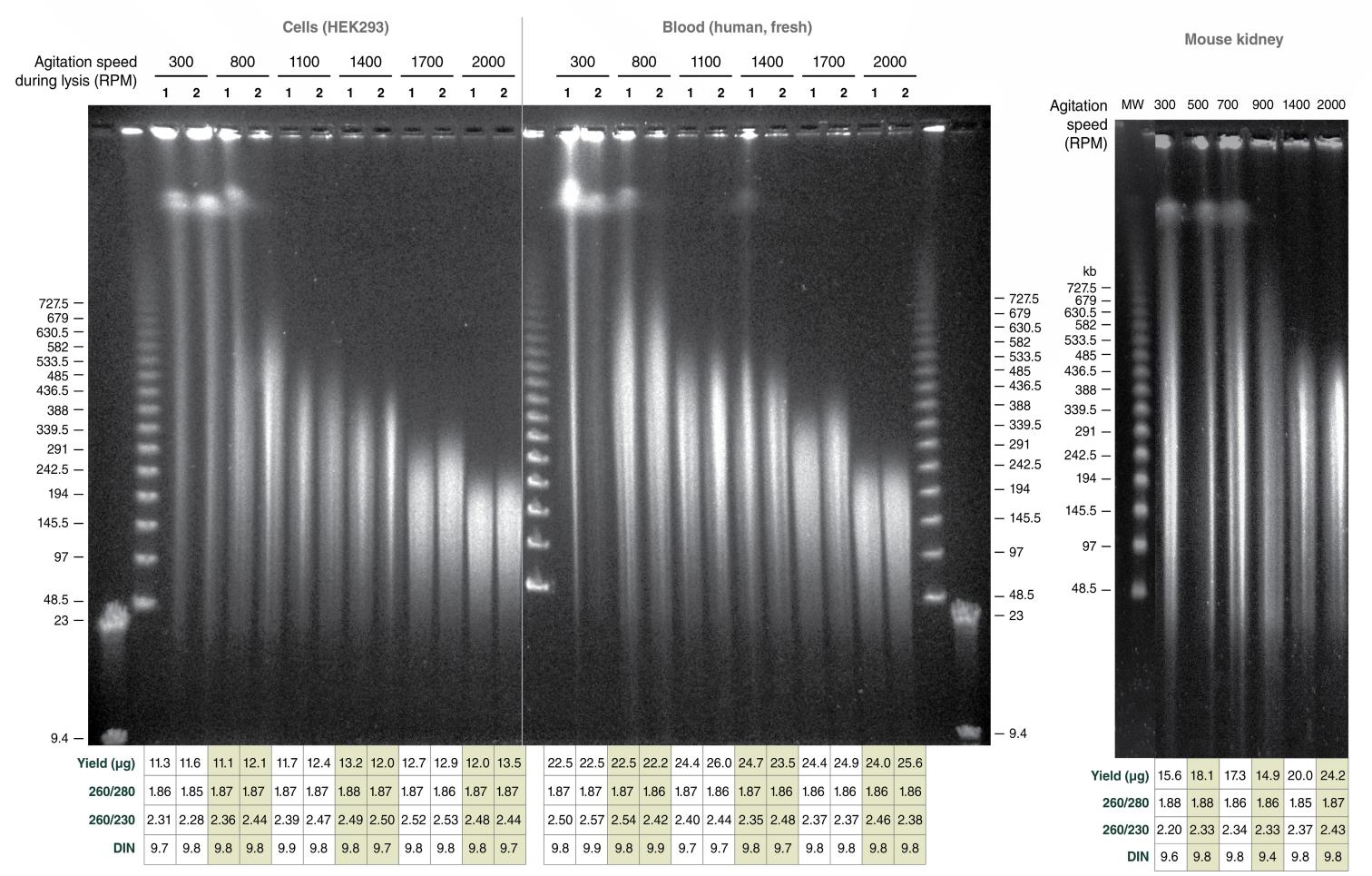
A. Improved yields, solubility, & more reproducible N50s



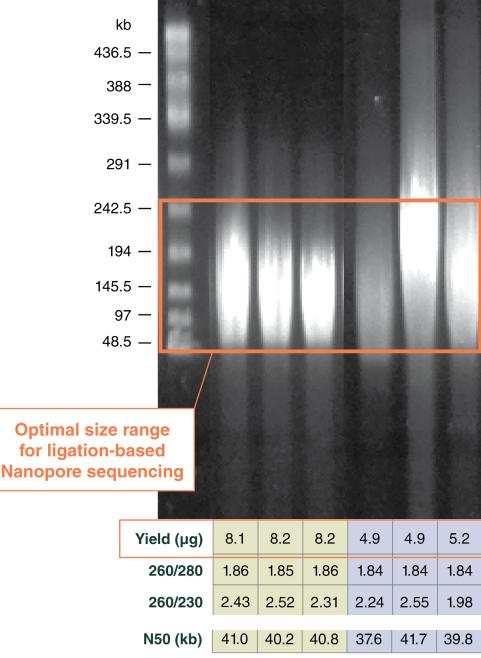
other HMW workflows

TUNABLE FRAGMENT LENGTH FOR ALL SAMPLE TYPES

Agitation speed during lysis determines fragment length of extracted HMW gDNA



Preps were performed using aliquots of 10⁶ cells (HEK293, duplicates), 500 μl human blood (duplicates), or mouse kidney (10 mg, single). Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng; kidney 300 ng) were resolved by PFGE. Yield, purity and DNA Integrity Numbers (DINs) of the individual preps are shown in the accompanying tables.



A. HMW gDNA was purified from 0.7x10⁶ K562 suspension cells with the Monarch HMW DNA Extraction Kit for Cells & Blood and the Circulomics Nanobind CBB Big DNA Kit. 400 ng of DNA was separated on a 0.75% gel (Pippin Pulse, Sage Science, 5-430 kb program. M= Lambda PFG Ladder (NEB #N0341). Barcoded libraries for ligation sequencing were prepared and analyzed on the same flow cell (SQK-LSK109, EXP-NBD104, FLO-MIN106D R9.4.1).

B. HMW gDNA was isolated from 1x10⁶ HEK293 cells or human blood (yields normalized for 100 μl) with NEB Monarch, Circulomics[®] Nanobind[®] (C), Revolugen[®] FireMonkey[®] (R), Qiagen[®] MagAttract[®] (Q), Promega Wizard[®] HMW (P), & Zymo Research[®] Quick-DNA HMW (Z).

C. HMW gDNA was purified with the Monarch HMW DNA Extraction Kit for Cells & Blood and the Circulomics Nanobind CBB Big DNA Kit from 1x10⁶ HeLa cells and 2-fold dilutions to 5, 2.5 and 1.25x10⁵ cells.

BLOOD PREPS – MAXIMAL FRAGMENT LENGTH & PURITY

Monarch is able to isolate Mb size DNA and achieve higher N50's, better purity and yield

XL/UHMW Standard Higher N50s DNA **HMW DNA NEB®** (Standard HMW DNA) **Circulomics® (Standard HMW DNA)** MNC Ν С 17,098 16,713 16,718 15,748 16,018 15,519 Mean read length: Mb sized DNA 13.3 13.3 13.3 13.3 13.3 13.3 Mean read quality: with Monarch 7,905.5 7,559.5 7,493 8,287.5 8,169.5 Median read length: 8,261 Median read quality: 13.7 13.7 13.8 13.7 13.7 13.7 16,750 25,364 27,478 22,669 28,316 Number of reads: 29,444 38,127 38,289 Read length N50: 37,838 32,860 31,603 30,688 471,646,797 256,992,257 433,669,917 459,237,356 280,027,071 439,435,523 Total bases: kb C.

TISSUE PREPS – HIGH SENSITIVITY & VERSATILITY

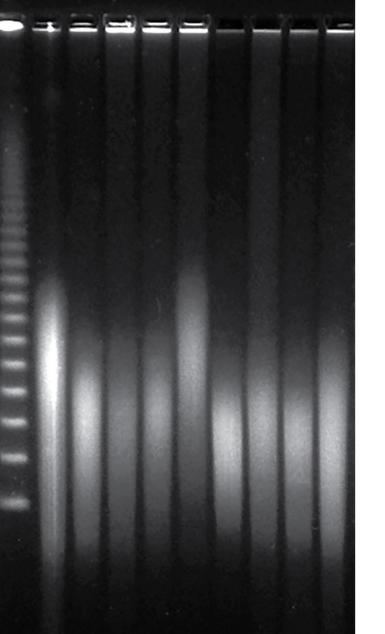
A. Process various sample types

B. Good yields for low input tissue samples

М	Rat kidney	Mouse liver	Mo _{use brain}	Mouse min.	E. coli	B. Cereus	X. Idevis	S. Cerevicio	A. degyns:	17-1-1
	= .		-	-	Ĵ	-				
b 5 9 5 5 5 5 5 5										
8 - 5 - 1 - 5 - 4 - 5 -										
7 —										
nount (mg)	10	10	20	20	2x10 ⁹	2x10 ⁸	3	4x10 ⁸	15	

Yield (µg) 20.8 20.2 16.9 8.9 14.5 4.2 5.2 12.9 6.7

260/230 2.41 2.26 2.45 2.28 2.06 1.95 2.33 2.01 2.53



MOUSE TISSUE	INPUT	YIELD (µg)	
kidney	2 mg	5.7	
liver	2 mg	5.9 (3.3*)	
brain	2 mg	2.0	
muscle	2 mg	0.4	
ear punch	1 piece	0.5	
ear punch	2 pieces	1.7 - 2.0	

A. HMW DNA isolated from insects, yeast, tadpoles, bacteria, and various animal tissues. Preps were performed according to the kit instructions with sample agitation at 2000 rpm. A modified workflow was used to process S. cerevisiae samples. 500 ng of DNA from each sample prep was resolved by PFGE. Yield and purity ratios of the individual preps are shown in the accompanying tables. M = Lambda PFG Ladder (NEB #N0341)

B. Very small tissue samples were isolated with the Monarch HMW DNA Extraction Kit for Tissue following the guidance for very low input amounts. *Liver samples were processed with the modified protocol for liver samples

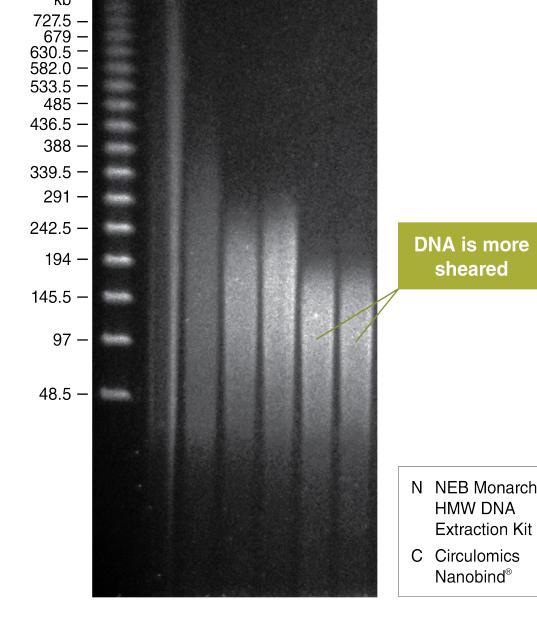
TIME SAVINGS IN CAS9 ENRICHMENT SEQUENCING

Monarch[®] HMW **DNA Extraction**

Phenol

extraction

Fast DNA extraction from tissues (90 min)



	N	NEB [®] (Standard HMW DNA)		Circulomics [®] (Standard HMW DNA)		
	1	2	3	1	2	3
Yield (µg):	28.5	27	29	11.6	10.4	12.4
260/280:	1.85	1.87	1.87	1.81	1.80	1.85
260/230:	2.43	2.49	2.49	1.85	1.95	1.86

XL DNA was extracted from human blood using the NEB Monarch protocol with lysis agitation speed set to 300 rpm (lane 1). Circulomics UHMW protocol was followed for the same sample (lane 2). "Standard" HMW DNA samples were isolated with the standard NEB (lysis speed 2000 rpm, lanes 3,4) or Circulomics (lanes 5,6) protocols.

B. Ligation sequencing results of a multiplex run with 3 NEB Monarch- and 3 Circulomics-purified HMW DNA samples from the same human blood sample on the same day (SQK-LSK109 kit, EXP-NBD104. FLO-MIN106D R9.4.1, GridION Mk1).

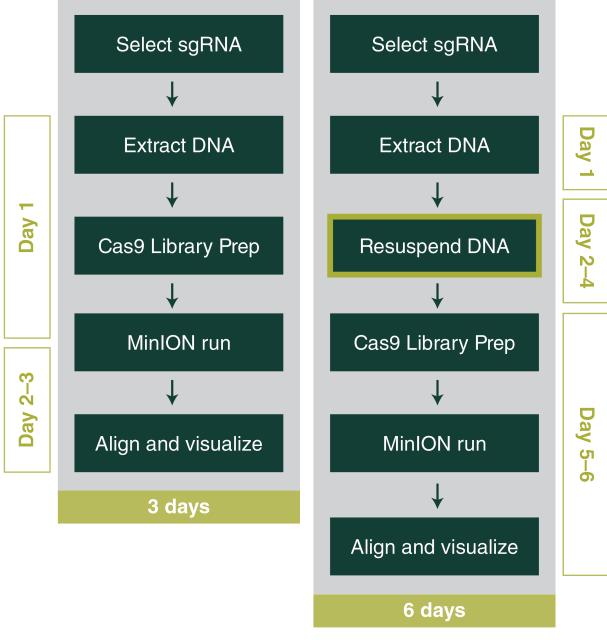
C. Yield and purity data from the samples in B. Standard volume for Monarch blood preps is 500 µl (accommodates up to 2ml) and Circulomics is 200 µl (maximum).

SINGLE RUN NANOPORE SEQUENCING DATA

Single Run Sequencing Results of HEK293, Human Blood, and Mouse Kidney

	HEK293 SAMPLE 1	HEK293 SAMPLE 2	HUMAN BLOOD Sample 1	HUMAN BLOOD SAMPLE 2	MOUSE KIDNEY SAMPLE
Mean read length (bases)	21338.9	19249.9	21522.6	24677.7	27120.7
Mean read quality	12.8	13.2	13.4	13.3	13
Median read length (bases)	10388	9702	10130	12593	23150
Median read quality	13.2	13.7	13.9	13.8	13.5
Number of reads	377687	633636	538090	327314	164000
Read length N50 (bases)	45432	40415	46542	51394	44631
Total bases	8.1 Gb	12.2 Gb	11.6 Gb	8.1 Gb	4.4 Gb

SQK-LSK109, FLO-MIN106D R9.4.1, GridION Mk1, run for up to 48 hrs, no reloading



SUMMARY

- Easily dissolvable DNA sample to sequencing in 1 day
- Time savings of up to 3 days vs. phenol extraction
- Tunable fragment length enables rapid troubleshooting
- High coverage even with small inputs (<500 ng)
- Fast workflows and increased DNA solubility offer significant time savings
- High yields in a single, efficient elution step provide more DNA from the same sample
- Good yields achievable even from low input amounts
- Tunable HMW DNA fragment length enables flexible design of experiments
- High N50s careful HMW DNA purification minimizes need for size selection
- High data amounts achievable from extremely pure and intact HMW DNA
- UHMW DNA option for all sample types empowers all long-read sequencing approaches