

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

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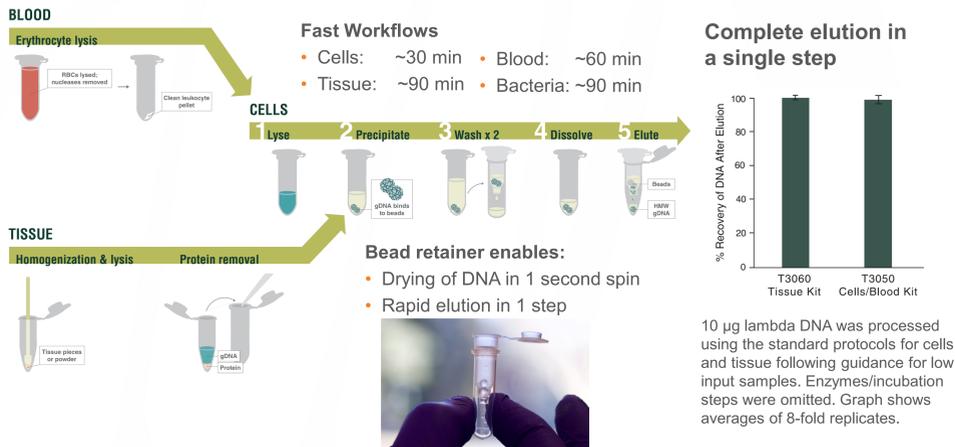


## INTRODUCTION

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.



## RAPID & EFFECTIVE "BIND-WASH-ELUTE" WORKFLOW

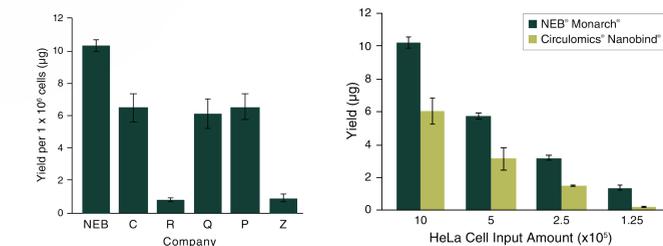
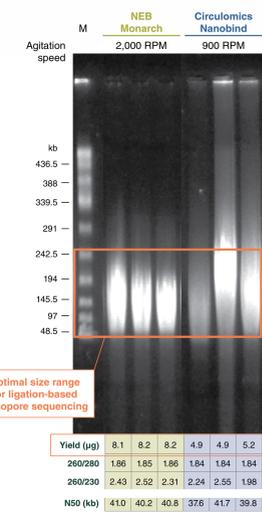


## CELL PREPS - EXCELLENT YIELDS & REPRODUCIBILITY

### A. Improved yields, solubility, & more reproducible N50s

### B. Improved yields vs other HMW workflows

### C. Higher yields for low inputs



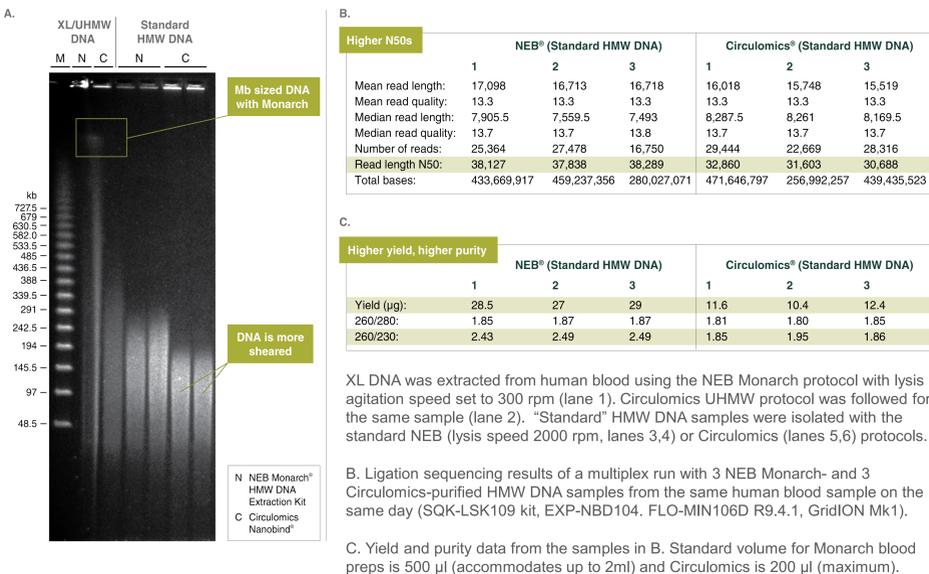
A. HMW gDNA was purified from  $0.7 \times 10^6$  K562 suspension cells with the Monarch HMW DNA Extraction Kit for Cells & Blood and the Circulomics Nanobind CBB Big DNA Kit. 400 ng of DNA was separated on a 0.75% gel (Pippin Pulse, Sage Science, 5–430 kb program. M= Lambda PFG Ladder (NEB #N0341). 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  $1 \times 10^6$  HEK293 cells or human blood (yields normalized for 100 µl) with NEB Monarch, Circulomics Nanobind (C), Revolgen 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  $1 \times 10^6$  HeLa cells and 2-fold dilutions to 5, 2.5 and  $1.25 \times 10^5$  cells.

## BLOOD PREPS – MAXIMAL FRAGMENT LENGTH & PURITY

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



## 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

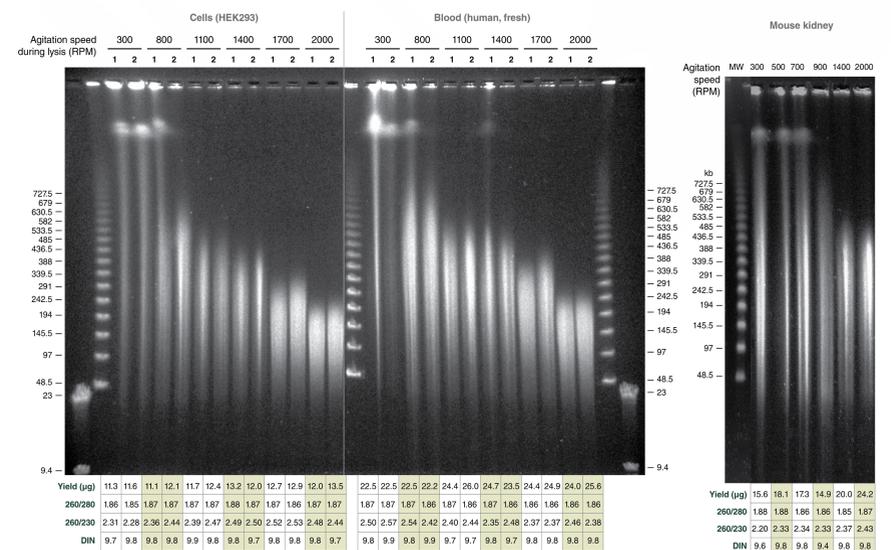
□ SQK-LSK109, FLO-MIN106D R9.4.1, GridION Mk1, run for up to 48 hrs, no reloading

## 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

## TUNABLE FRAGMENT LENGTH FOR ALL SAMPLE TYPES

Agitation speed during lysis determines fragment length of extracted HMW gDNA

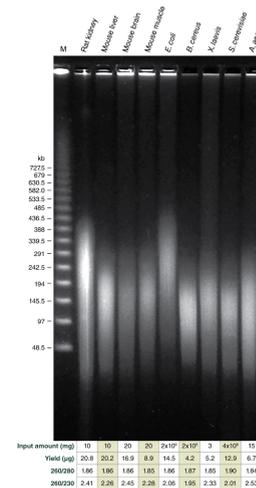


Preps were performed using aliquots of  $10^6$  cells (HEK293, duplicates), 500 µl human blood (duplicates), or mouse kidney (10 mg, single). Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 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.

## TISSUE PREPS – HIGH SENSITIVITY & VERSATILITY

### A. Process various sample types

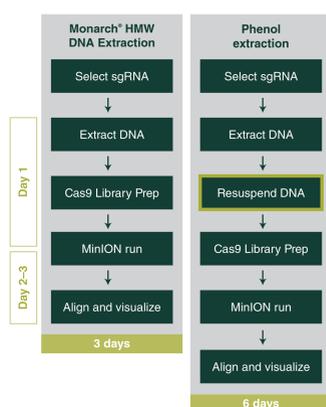
### B. Good yields for low input tissue samples



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



- Fast DNA extraction from tissues (90 min)
- 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)

## SUMMARY

- 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