Novel Method for High Molecular Weight (HMW) DNA Extraction

P. Giron Koetsier, Barbara Taron, Luo Sun, Danielle Freedman, Eileen Dimalanta and Eric Cantor | New England Biolabs, Inc.

INTRODUCTION

Extraction of high molecular weight (HMW) genomic DNA (gDNA) is currently a pain point for many researchers, with existing solutions taking hours, or even days, to complete. Under the Monarch® Nucleic Acid Purification product portfolio, we have developed a rapid and reliable process for extracting intact HMW genomic DNA from mammalian tissues, cultured cells, blood, and bacteria. Utilizing an optimized process that combines gentle lysis with tunable fragment length generation, followed by precipitation of extracted DNA onto the surface of glass beads, the prep proceeds rapidly and utilizes standard laboratory equipment. DNA size ranges from 50-250 kb for the standard protocol and can be adjusted to produce longer DNA up to >1 Mb. Purified DNA is recovered in high yield with excellent purity, including nearly complete removal of RNA. Prep time is approximately 30-90 minutes, depending on sample type. The purified HMW gDNA is suitable for a variety of downstream applications including long-read sequencing and genome mapping.

HIGHLIGHTS

- Rapid protocols with minimal hands-on time
- Maximal yield and purity
- Purified DNA ranges from 50 kb to > 1 Mb
- DNA size from cells, blood, bacteria and soft organ tissues can be tuned based on agitation speeds
- Standard protocols yield DNA 50-250 kb for Oxford Nanopore Technologies® (ONT) sequencing
- Highly reproducible
- Low RNA content
- RNase A, Proteinase K, microtube pestles included

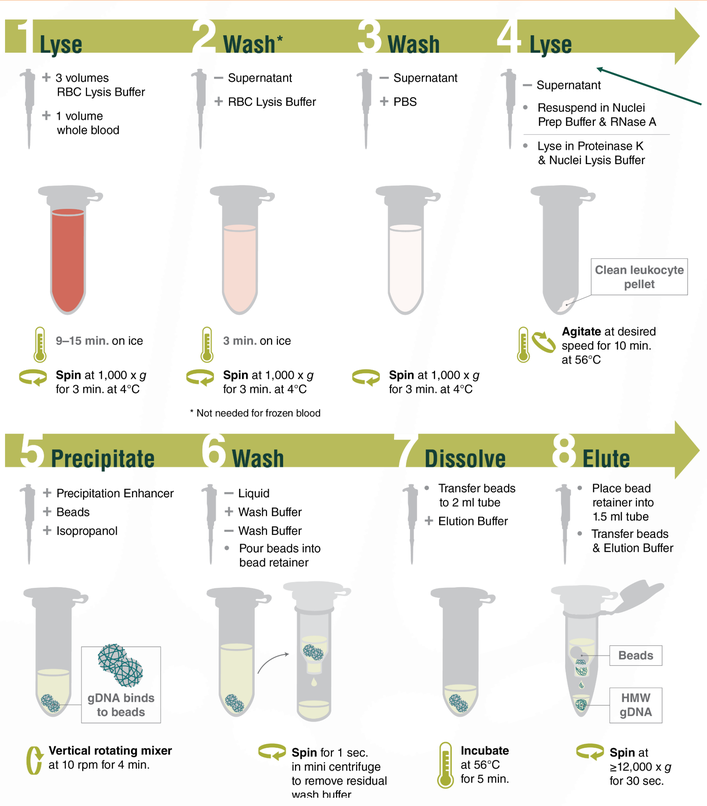
Fast workflows:

- Cells: ~30 min
- Blood: ~60 min
- Tissue: ~90 min
- Bacteria: ~90 min

High N50s:

- Cells: 40-55 kb
- Blood: 40-55 kb
- Tissue: 35-45 kb
- Bacteria: 40-48 kb

WORKFLOW: CELLS & BLOOD



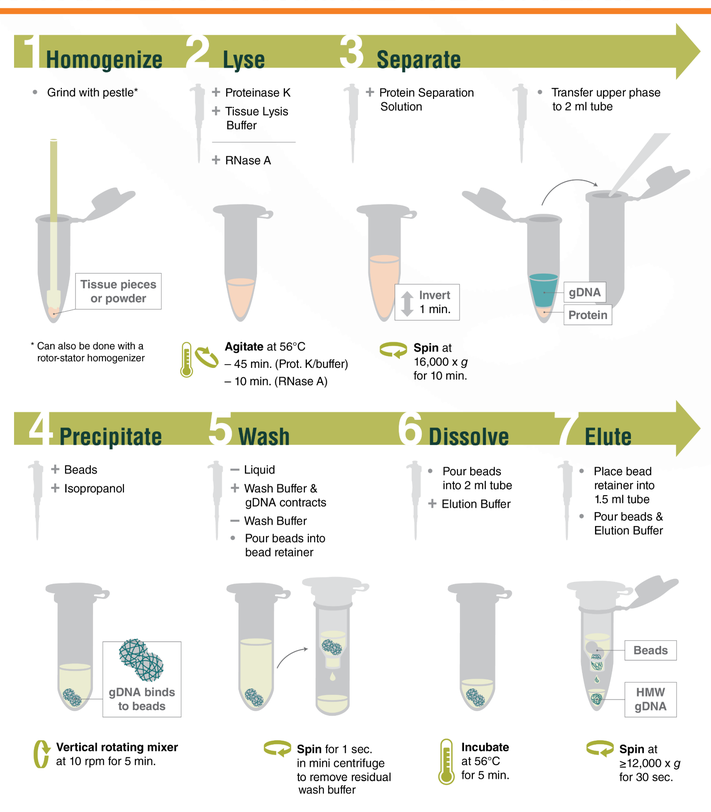
Cell workflow begins here (step 4)
Total protocol: ~30 min

- Validated for fresh and frozen cells
- Input range 1 x 10^5 - 1 x 10^7 cells
- Employs separate cytosolic lysis approach for efficient RNA removal

Erythrocyte lysis: 30 min.
Total protocol: ~60 min.

- Validated for fresh and frozen blood
- Input range 100 µl – 2 ml
- Compatible with both non-nucleated and nucleated (e.g. bird) blood samples
- Compatible with with various anticoagulants and PAXgene® Blood DNA tubes
- Erythrocyte lysis employed for best-in-class N50s

WORKFLOW: TISSUE & BACTERIA

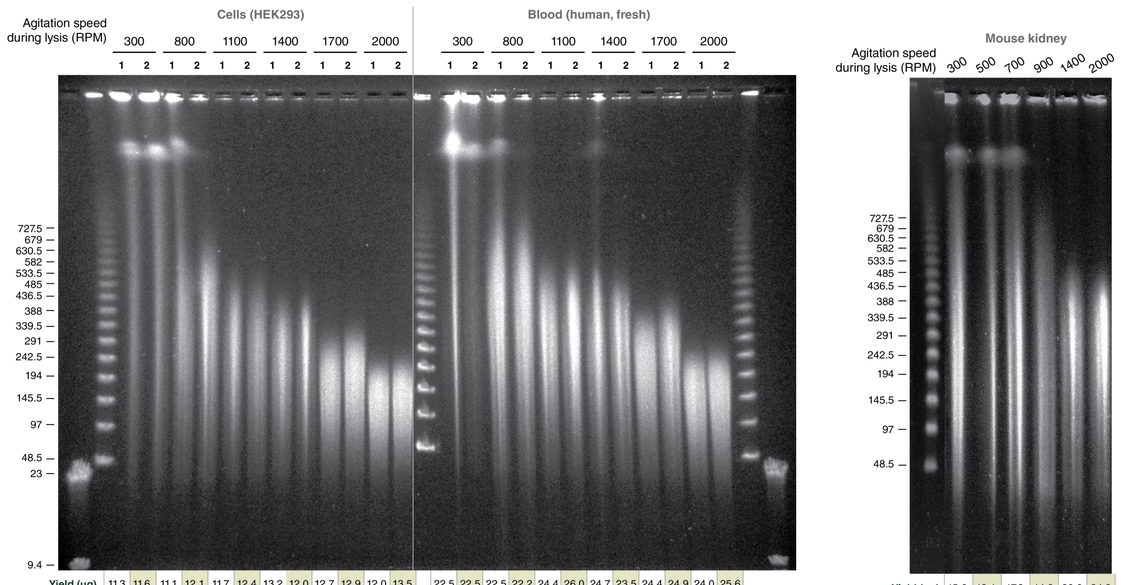


Tissue lysis: ~60 min.
Total protocol: ~90 min.

- Lysozyme required for bacteria preps
- Input range:
  - 2 – 25 mg tissue (2-15 mg for soft organ/DNA-rich tissue)
  - 5 x 10^8 – 5 x 10^9 bacteria
- Mb-size DNA achievable for soft organ tissue & bacteria with low agitation speed during lysis
- Pestle homogenization typically produces gDNA 50 kb to ≥ 500 kb with high agitation speed during lysis
- Needle shearing after DNA extraction improves sequencing results
- Rotor-stator homogenizer recommended for ONT sequencing of frozen tissue
- Microtube pestle recommended for fresh tissue (included in kit)

PERFORMANCE: TUNABLE LENGTH, YIELD & PURITY

Use of varying agitation speeds during lysis produces controlled fragment length of extracted HMW genomic DNA from cells, blood and soft organ tissue.



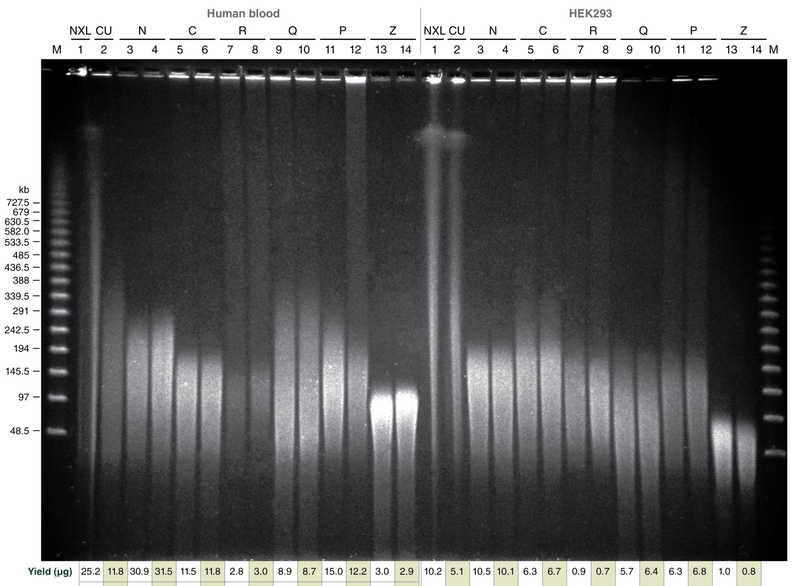
Yield (µg)	11.3	11.6	11.1	12.1	11.7	12.4	13.2	12.0	12.7	12.9	12.0	13.5	22.5	22.5	22.5	22.2	24.4	26.0	24.7	23.5	24.4	24.9	24.0	25.6
260/280	1.86	1.85	1.87	1.87	1.87	1.87	1.88	1.87	1.87	1.86	1.87	1.87	1.87	1.87	1.87	1.86	1.87	1.86	1.87	1.86	1.86	1.86	1.86	1.86
260/230	2.31	2.28	2.36	2.44	2.39	2.47	2.49	2.50	2.52	2.53	2.48	2.44	2.50	2.57	2.54	2.42	2.40	2.44	2.35	2.48	2.37	2.37	2.46	2.38
DIN	9.7	9.8	9.8	9.8	9.9	9.8	9.8	9.7	9.8	9.8	9.8	9.7	9.8	9.9	9.8	9.9	9.7	9.7	9.8	9.7	9.8	9.8	9.8	9.8

Yield (µg)	15.6	18.1	17.3	14.9	20.0	24.2
260/280	1.88	1.88	1.86	1.86	1.85	1.87
260/230	2.20	2.33	2.34	2.33	2.37	2.43
DIN	9.6	9.8	9.8	9.4	9.8	9.8

Preps were performed on aliquots of 10^6 cells (HEK293, duplicates), 500 ul 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 and purity of the individual preps are shown in the accompanying tables.

PERFORMANCE: COMPARISON HMW DNA EXTRACTION KITS

Monarch HMW DNA Extraction Kit produces high molecular weight DNA with excellent yields and purity as compared to other commercially available kits



Yield (µg)	25.2	11.8	30.9	31.5	11.5	11.8	2.8	3.0	8.9	8.7	15.0	12.2	3.0	2.9	10.2	5.1	10.5	10.1	6.3	6.7	0.9	0.7	5.7	6.4	6.3	6.8	1.0	0.8
Yield per 100 µl (µg)	5.0	5.9	6.2	6.3	5.8	5.9	0.6	0.6	4.4	4.3	5.0	4.1	1.5	1.4														
RNA content (%)	0.1	8.5	1.5	1.1	8.6	9.7	0.1	0.1	0.0	0.0	8.3	7.1	0.0	0.5	1.1	4.3	1.3	1.4	0.6	1.3	0.6	0.6	0.4	0.9	42.9	40.2	0.3	0.2
260/280	1.83	1.74	1.84	1.85	1.85	1.83	1.72	1.75	1.89	1.88	1.87	1.89	1.96	2.00	1.83	1.81	1.86	1.87	1.87	1.89	1.69	1.51	1.85	1.87	1.98	1.96	1.84	1.85
260/230	2.25	1.33	2.41	2.41	2.03	1.73	9.47	5.99	2.70	2.25	2.51	2.38	2.31	2.17	2.82	2.03	2.60	2.33	1.92	2.24	2.69	2.59	2.66	2.85	2.31	2.39	1.66	2.52

1 (NXL)	NEB® Monarch® HMW DNA Extraction Kit, XL DNA protocol (agitation at 300 rpm)
2 (CU)	Circulomics® Nanobind®, UHMW protocol
3–4 (N)	NEB Monarch HMW DNA Extraction Kit, standard protocol (agitation at 2000 rpm)
5–6 (C)	Circulomics Nanobind, HMW protocol (agitation at 900 rpm)
7–8 (R)	Revolugen® FireMonkey®
9–10 (Q)	Qiagen® MagAttract HMW DNA Kit
11–12 (P)	Promega® Wizard® HMW DNA Extraction Kit • 11 blood: DNA dissolving room temperature overnight • 11 cells: DNA dissolving 1 hour at 65°C • 12 cells and blood: DNA dissolving 1 hour at 65°C
13–14 (Z)	Zymo Quick-DNA HMW MagBead Kit

- Only Monarch (cells + blood) and Circulomics (cells only) enable Mb-size DNA isolation.
- Monarch: highest yields, purity for blood & cells
- Reduced purity with Circulomics & Revolugen
- Low yields for Revolugen and Zymo
- Shearing in mag bead systems (Qiagen, Zymo)
- High level of RNA contamination with Promega
- Elution at 65°C & 80°C (Promega & Revolugen, respectively) results in partial denaturation

HMW DNA was isolated from 1x10^6 HEK293 cells and fresh human blood (input specified by manufacturer: N: 500 µl, C: 200 µl, R: 500 µl, Q: 200 µl, P: 300 µl, Z: 200 µl). Only Monarch & Circulomics provide a protocol for the isolation of XL/UHMW DNA (lanes 1 & 2). Standard Monarch samples (lanes 3-4) used agitation at 2000 rpm during lysis; Circulomics employed agitation at 900 rpm. All other data presented in duplicate following manufacturer's protocols. DNA was eluted in 100 µl (Zymo in 50 µl). Yield and purity were analyzed on Trinean Dropsense 16; XL/UHMW samples were measured as 5x replicates on a Devonix DS-11 FX+. Blood yields were normalized per 100 µl. RNA content was determined by HPLC of nucleosides after digestion of 1 µg of DNA with the Nucleoside Digestion Mix (NEB #M0649).

ONT SEQUENCING METRICS*

Table 1: Cells & Blood

	HEK293		Human blood		K562 cells	Chicken blood	Pig blood
	Single run	Single run	Single run	Single run	Multiplex	Multiplex	Multiplex
Read length N50 (kb):	**45.4**	**40.4**	**51.4**	**46.5**	**41**	**37.9**	**37.6**
Total bases (Gb):	8.1	12.2	8.1	11.6	0.37	0.41	0.41
Mean read length (kb):	21.3	19.2	24.7	21.5	16.9	16	16.6
Median read length (kb):	10.4	9.7	12.6	10.1	7.1	7	7.9
Mean read quality:	12.8	13.2	13.3	13.4	13.2	12.9	13
Median read quality:	13.2	13.7	13.8	13.9	13.6	13.4	13.5

Table 2: Tissues & Bacteria

	Kidney	Kidney	Mouse brain	Mouse liver	Mouse muscle	B. cereus	E. coli
	Single run	Single run	Multiplex	Multiplex	Multiplex	Multiplex	Multiplex
Read length N50 (kb):	**33**	**44.6**	**40.9**	**42.3**	**37.9**	**43.4**	**48**
Total bases (Gb):	**7.1**	**4.5**	0.12	0.36	0.13	0.23	0.31
Mean read length (kb):	15.9	27.1	21.9	27.4	18.9	22.2	29.4
Median read length (kb):	8.8	23.2	14.8	23.7	10.9	13.6	23.8
Mean read quality:	13	13	12.9	13.2	12.8	12.5	12.7
Median read quality:	13.4	13.5	13.3	13.7	13.4	13.1	13.2

Table 3: Comparison of Monarch to Circulomics® Nanobind®

*Identical samples in multiplex runs	Fresh K562 Cells (7x10^5)						Fresh HEK293 Cells (2x10^6)						Human Blood (200 µl)					
	Monarch			Circulomics			Monarch			Circulomics			Monarch			Circulomics		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Read length N50 (kb):	**41**	**40.2**	**40.8**	**37.6**	**41.7**	**39.8**	**40.7**	**41.8**	**40**	**40.8**	**41.5**	**40.7**	**38.1**	**37.8**	**38.3**	**32.9**	**31.6**	**30.7**
Total bases (Gb):	**0.37**	**0.77**	**0.19**	**0.47**	**0.43**	**0.29**	**0.42**	**0.44**	**0.36**	**0.31**	**0.35**	**0.21**	**0.43**	**0.46**	**0.28**	**0.47**	**0.36**	**0.44**
Mean read length (kb):	16.9	16.4	17.4	12.5	16	13.6	18.4	18.6	17.8	18.4	18.2	17.8	17.1	16.7	16.7	16	15.7	15.5
Median read length (kb):	7.1	6.9	7.4	4.5	6.2	4.8	8.6	8	8.1	8.9	8.5	8.2	7.9	7.6	7.5	8.3	8.3	8.2
Mean read quality:	13.2	13.1	13.1	13.2	13.2	13.2	13.3	13.3	13.3	13.1	13	13	13.3	13.3	13.3	13.3	13.3	13.3
Median read quality:	13.6	13.5	13.5	13.5	13.5	13.5	13.7	13.7	13.8	13.6	13.5	13.5	13.7	13.7	13.8	13.7	13.7	13.7
Yield (µg)	**8.1**	**8.2**	**8.2**	4.9	4.9	5.2	**20.5**	**22.9**	**23.4**	15.9	15.0	17.1	11.4	10.8	11.6	11.6	10.4	12.4
260/280	1.85	1.87	1.87	1.86	1.83	1.72	1.86	1.85	1.86	1.84	1.84	1.84	1.85	1.87	1.87	1.81	1.80	1.85
260/230	2.43	2.52	2.31	2.24	2.55	1.98	2.43	2.54	2.48	2.28	2.01	2.34	**2.43**	**2.49**	**2.49**	1.85	1.95	1.86

*LSK109, FLO-MIN106D; load 800-1000 ng HMW DNA per flow cell for optimal molar ratios

CONCLUSIONS

Cells & Blood

- Workflow complete in 30 minutes (cells) and 60 minutes (blood)
- N50s between 40-55 kb
- High data quality (Q-score: 13-14) and output (>10 Gb)
- Longest reads up to >500 kb, longer reads possible with lower agitation speeds.

Tissues & Bacteria

- Workflow completed within 90 minutes
- N50s up to 45 kb from various tissues
- N50s up to 48 kb from Gram – and up to 43 kb from Gram + bacteria
- Compatible with many sample types – fibrous, fatty, & soft organ tissues, as well al insect, yeast, Xenopus.

Compared to Circulomics Nanobind:

- Considerable time savings with faster protocols and less hands-on time
- Improved yield for cell preps (up to 50% greater), better purity for blood samples
- Mb sized DNA from cells, blood, bacteria, some tissues (vs. cells and bacteria only)
- Significantly higher N50s for blood; equal or higher data quality
- Higher reproducibility due to more uniform DNA fragment size

TissueRuptor® is a registered trademark of QIAGEN GmbH; Circulomics® **and** Nanobind® are registered trademarks of Circulomics, Inc.
PAXgene® is a registered trademark of PreAnalytiX, GmbH; Oxford Nanopore Technologies® is a registered trademark of Oxford Nanolabs Ltd.