Apr 04, 2025

Protocol for 6mA labeling and HMW DNA extraction from fresh frozen human brain samples

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlkw54wl5r/v1

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Protocol Citation: Jonas Demeulemeester, Koen Theunis 2025. Protocol for 6mA labeling and HMW DNA extraction from fresh frozen human brain samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw54wl5r/v1

Protocol status: Working
We use this protocol and it's working

Created: September 09, 2022

Last Modified: April 04, 2025

Protocol Integer ID: 69773

Keywords: HMW DNA extraction, Nanopore sequencing, PromethION, ASAPCRN, hmw dna extraction of fresh frozen brain tissue, fresh frozen human brain sample, hmw dna extraction, fresh frozen brain tissue, dna extraction, brain tissue, dna, extraction

Abstract

This protocol details the procedure of 6mA labeling and HMW DNA extraction of fresh frozen brain tissue. The protocol is inspired by Fiber-seq.

Citation

Stergachis AB, Debo BM, Haugen E, Churchman LS, Stamatoyannopoulos JA (2020)
. Single-molecule regulatory architectures captured by chromatin fiber sequencing. Science (New York, N.Y.).

https://doi.org/10.1126/science.aaz1646 LINK

Attachments

PDF

irgkbewa7.pdf
192KB

Materials

Prepare buffers (Volumes above are indicated per sample):

10x stocks of Wash buffer base (no spermidine/Tween) and Labeling buffer base (no spermidine/SAM/Hia5).

2 mL Wash buffer → take 488 µL and add Digitonin + 50XProtInh to make **Lysis buffer**.
50 µL **labeling buffer** / 250.000 cells (typically ~ 1E6 cells → 200 µL **)**.

Make [M] 25 millimolar (mM) **spermidine stocks fresh monthly** and store at -20 °C .
Add 2 µL spermidine (liquid, ~ [M] 6.38 Mass Percent) to 350 µL [M] 0.1 Mass Percent HCl and 160 µL H2O – **check pH with strips!**

	A	B	C	D
	10X Wash/Lysis base (10X-WLB) (200uL / sample)			
		Stock	Final for 10X	V (uL)
	Tris-HCl pH 7.4	1000 mM	100 mM	100
	NaCl	5000 mM	100 mM	20
	Water			880
	Total			1000

	A	B	C	D
	10X Hia5 labeling base (10X-H5B) (20uL / sample)			
		Stock	Final for 10X	V (uL)
	Tris-HCl pH 8	1000 mM	150 mM	150
	NaCl	5000 mM	150 mM	30
	KCl	1000 mM	600 mM	600
	EDTA pH 8	500 mM	10 mM	20
	EDTA pH 8	250 mM	5 mM	20
	Water			180

	A	B	C	D
	Total			1000

	A	B	C	D	E
	1X Wash buffer				
		Stock	Final	V (uL)	X5
	10X WLB	10 X	1 X	230	1150
	BSA	10%	0.10%	23	115
	Spermidine pH 7.4	25 mM	0.5 mM	46	230
	Tween-20	10%	0.10%	23	115
	Water			1978	9890
	Total			2300	11500

	A	B	C	D	E
	1X Lysis buffer				
		Stock	Final	V (uL)	X5
	Wash buffer			488	2440
	Digitonin	5%	0.02%	2	10
	ProteaseInh	50 X	1 X	10	50
	Total			500	2500

	A	B	C	D	E
	1X Hia5 labeling buffer				
		Stock	Final	V (uL)	X5
	10X-H5B	10 X	1 X	20	100
	BSA	10	0.1	2	10
	Spermidine pH 7.4	25	0.5	4	20

	A	B	C	D	E
	SAM	32	0.8	5	25
	Hia5 enzyme	250	5	4	20
	Water			165	825
	Total			200	1000

6mA labeling and HMW DNA extraction

1 Place the Dounce homogenizer and pestles On ice, chill the centrifuge to 4 °C and preheat the ThermoMixer to 37 °C.

2 **Carefully** transfer 3-4 (2 mm diameter) tissue punch biopsies (~ 25 mg) to the chilled Dounce homogenizer.

> Note
>
> Keep the Dounce homogenizer on ice during the entire disruption process.

3 Add 500 µL of the 1X Lysis buffer and let the tissue thaw for 00:01:00. 1m

4 Gently homogenize the tissue 10X with pestle A and 10X with B.

4.1 Push the tissue with the pestle firmly into the bottom of the Dounce chamber with each stroke (Down + Up = 1X).

4.2 Keep the tissue between tip of pestle and the bottom of the Dounce chamber for thorough homogenization.

4.3 Homogenate may become foamy, but this is not a cause for concern.

> Note
>
> In the next step, transfer any foam that forms.

5 Incubate On ice for 00:05:00 before adding 1000 µL of 1X Wash buffer. 5m

6 Transfer the lysate to a 2 mL Protein LoBind microcentrifuge tube.

7 Rinse the pestles and homogenizer with the remaining 500 µL 1X Wash buffer and add to the sample.

8 Pellet homogenate by centrifuging at 700 x g and 4 °C for 00:05:00. Discard supernatant. 5m

9 Resuspend the pellet in 200 µL of 1X Hia5 labeling buffer – use a 1 mL or wide bore tip.

10 Incubate on a ThermoMixer at 37 °C and 900 rpm for 00:30:00. 30m

11
- **Continue with Circulomics CBB Tissue protocol from step 8 onwards.**
- **Continue with NEB Monarch HMW.**

Nanopore sequencing (LSK-110, PromethION) 2h 5m

12 According to ONT protocol Genomic DNA by Ligation (SQK-LSK110) with the following modifications:

12.1 Start with 3 µg - 4 µg of HMW DNA in 150 uL and sheer 25x with a 26G needle or in Megaruptor to 35kb.

12.2 Adjust volumes end-prep and FFPE repair step accordingly (i.e. vol x 3), omit control strand (CS).

12.3 Extend end-prep and FFPE repair steps from 00:05:00 to 00:30:00 (i.e. 30min at 20 °C and 30 min at 65 °C). 35m

12.4 Extend ligation step to 01:00:00 at Room temperature. 1h

12.5 Elute the AMPure cleanups for 00:10:00 and 00:20:00 after the end-prep and ligation steps. 30m

12.6 This should yield a ~3x library, aim to load near the high end of the 5-50fmol range, typically ~ 8 µL .

Note

Note 1:Library prep yield is typically 30-50%.
Note 2:The amount loaded can be reduced during subsequent flushes to balance seq yield with # flushes.

Citations

Stergachis AB, Debo BM, Haugen E, Churchman LS, Stamatoyannopoulos JA. Single-molecule regulatory architectures captured by chromatin fiber sequencing.
https://doi.org/10.1126/science.aaz1646