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

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

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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.).

LINK

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

Attachments

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 Molarity (M)) to 350 µL [M] 0.1 Molarity (M) 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
	Total			1000

	A	B	C	D	E
	1X Wash buffer				

	A	B	C	D	E
		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
	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.
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