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Protocol SAM-Seq A.Thaliana V.1

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We use this protocol and it's working

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Abstract

Background: Epigenetic modifications, including chromatin accessibility, nucleosome positioning, and DNA methylation (5mC), are pivotal in shaping genome function. However, current short read sequencing approaches present challenges in characterising epigenetic patterns along repetitive genomic regions.
Results: We developed Simultaneous Accessibility and DNA Methylation Sequencing (SAM-seq), a robust method utilising bacterial adenine methyltransferases (m6A-MTases) to mark accessible regions in purified plant nuclei. Coupled with Oxford Nanopore Technology sequencing, SAM-seq enables high-resolution profiling of cytosine methylation and m6A-tagged chromatin accessibility along individual chromatin fibres in A. thaliana and maize. Importantly, using naked genomic DNA we uncovered significant sequence preferences of m6A-MTases, which we show must be taken into account in order to obtain reliable accessibility profiles, particularly for the analysis of highly repetitive sequences such as centromeric repeats. Using this method we found intriguing antagonism between accessibility and DNA methylation within single molecules, somatic epigenetic variation at bivalent chromatin domains, periodicity of nucleosomal strings over centromeric repeats, and high-resolution methylation profiles over nucleosomes.
Conclusions: Our study highlights the importance of considering intrinsic substrate preferences of DNA modifying enzymes for their use in chromatin profiling. Hence, SAM-seq is a robust and cross-species method to chart high-resolution accessibility and DNA methylation genome-wide. This method and findings will enable the investigation of chromatin-based regulation across plant species, with implications for the study of non-model plant species with limited genomic and epigenomic information.

Troubleshooting

Reagent Preparation

1 **Extraction Buffer (EB) 1**

To prepare 50mlm of fresh EB1 buffer

	Component	Amount	Final Concentration
	2M Sucrose	10 ml	0.4M
	1M Tris-HCl pH 8	0.5 ml	10mM
	1M MgCl2	0.5ml	10mM
	14.3M BME	17.5 µl	5mM
	+ PIs (1 tablet per 50mls)		

2 **Extraction Buffer (EB) 2**

To prepare 4ml of fresh EB2 :

	Component	Amount	Final Concentration
	Ethidium bromide	1,4 µL	5mM
	20% Triton X-100	200 µL	1%
	H20	2818,6 µL	
	1M MgCl2	40 µL	10mM
	1M Tris-HCl pH 8	40 µL	10mM
	PIs (1 mini-tablet diluted in 1 ml)	400 µL	
	2M Sucrose	500 µl	0.25M

3 **Extraction Buffer (EB) 3**

To prepare 3ml of fresh EB 3 :

	Component	Amount	Final Concentration
	1M Tris-HCl pH 8	30 µL	10mM
	1M MgCl2	6 µL	2mM
	20% Triton X-100	25 µL	0.15%
	+ Pis (1 mini-tablet diluted in 1 ml)	300 µL	
	2M sucrose	2550 µL	1.7M
	Ethidium bromide	1,05 µL	5mM
	H20 to volume	90,3µL	

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Nuclear Preparation Buffer (NPB)

To prepare 10ml of fresh NPB:

	Component	Amount	Final Concentration
	0.5M MOPS pH7	400µl	20 mM
	5M NaCl	80 µl	40 mM
	1M KCl	900µl	90 mM
	0.5 M EDTA	40µl	2 mM
	250mM EGTA	20 µl	0.5 mM
	3.5M spermidine	1.2µl	0.5 mM
	70 mM spermine	28.5µl	0.2 mM
	1 mini-tablet diluted in	1ml	1X

	Component	Amount	Final Concentration
	1ml		

5 **Plant-Tween-Wash-Buffer (PTWB)**

To prepare 3ml of fresh PTWB:

	Component	Amount	Final Concentration
	20% Tween-20	30 µl	0.2%
	1M pH7,5 HEPES-KOH	60 µl	20 mM
	5M NaCl	90 µl	150 mM
	PI Mini	300 µl	
	spermidine 3.5M	0,4 µl	0,5 mM
	H2O	2530 µl	

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Activation Buffer (AB)

To prepare 3ml of freash AB:

	Component	Amount	Final Concentration
	1M Tris-HCl pH 8	750 µl	15 mM
	5M NaCl	150 µl	15 mM
	1M KCl	3 ml	60 mM
	0.5 M EDTA	100µl	1 mM
	250mM EGTA	100 µl	0.5 mM
	3.5M spermidine	0.715 µl	0.05 mM
	BSA	50 mg	0.1%

	Component	Amount	Final Concentration
	H2O	fill to 50 ml	
	SAM 32 mM	(add before reaction)	800µM

7 **Reagents:**

for m6A reaction
EcoGII/ rCutSmart/ SAM (M0603S New England Biolabs)
for library preparation:
NEBNext Companion Module for Oxford Nanopore Ligation Seq (E7180S New England Biolabs)
SQK-LSK110 (Oxford Nanopore Technologies)
for nanopore sequencing:
FLO-MIN106.1 or FLO-PRO002 (Oxford Nanopore Technologies)

Plant nuclei purification and permeabilization

8 Add the powder to 25 ml of Extraction Buffer (EB) 1 in a 50 ml falcon tube. Let sit on ice for 5 min.

9 Add 1% Formaldehyde for crosslinking (i.e. 675 µl Formaldehyde 37% in 25ml of EB1). Incubate 5 minutes

10 Add 1.25 M glycine to stop the crosslinking (i.e. 1.7ml of Glycine 2M per 675 µl Formaldehyde 37%)

11 Filter the solution through Miracloth into a new 50 ml falcon tube. Repeat once.

12 Filter the solution through a 0.4µm filter into a new 50 ml falcon tube.

13 Centrifuge the filtered solution for 20 minutes at 4,000 rpm at 4°C.

14 Gently remove supernatant and resuspend the pellet in 2 ml of Extraction Buffer (EB) 2. Transfer the solution into four 1.5ml Eppendorf tubes.

15 Centrifuge at 12,000 g for 10 minutes at 4°C. Repeat step 7 & 8 once

16 Remove supernatant and resuspend each pellet in 300µl of Extraction Buffer (EB) 3.

17 In a clean Eppendorf tube, add 300ul of EB3. Take the 300µl solution (resuspended pellet) from step 7 and carefully layer it on top of the clean 300µl of EB3.

18 Centrifuge for 45 min at 12,000 at 4 degrees.

19 Resuspend each chromatin pellet in 300µl Nuclear Preparation Buffer (NPB) and incubate on ice for 5 min

m6A-MTases reaction

20 Spin down nuclei at 1000g for 5 min at 4°C and resuspend in Plant-Tween-Wash-Buffer (PTWB).

21 Resuspend each pellet in 193.5 µl 1X rCutSmart, containing 5µl SAM and 1.5µl EcoGII (i.e. 3.75U for 100µl). Incubate 30 minutes at 30°C

22 Add 200 µl rSmartCut 1X + 20 µl NaCl (5M) and incubate overnight at 65 °C (or 1h30min if not crosslinked).

23 Add 5µl of RNAse A (20mg/ml) and 2µl Proteinase K. Incubate 30 minutes at 42°C.

Phenol/Chloroform extraction of genomic DNA

24 Add 1 volume of 1:1 phenol:chloroform (i.e. 400µl)

25 Centrifuge at 12,000 g for 10 minutes at 4°C

26 Recover the aqueous phase (upper) in a new LoBind Eppendorf tube

27 Add 1 volume of chloroform (i.e. 400µl)

28 Centrifuge at 12,000 g for 10 minutes at 4°C.

29 Recover aqueous phase (upper) in a new LoBind Eppendorf tube

30 Precipitate DNA by adding 1/10 volume of NaAcétate 3M pH5,2 + 2,5 volume of EtOH 100%

31 Incubate at –20°C from 45 minutes to overnight.

32 Centrifuge at 12000g for 30 minutes at 4°C to precipitate DNA.

33 Wash the pellet with 300µ EtOH 70%.

34 Remove EtOH and dry pellets.

35 Elute in 20µl TE pH 8.0

Library preparation

36 Follow the protocol provided by Oxford Nanopore technology (Genomic_DNA_by_Ligation_SQK-LSK110) with the following modification:

-Use 0.3 to 1 µg of DNA per library

-For a better library preparation increase the End-Prep step up to 20 minutes at 20°C and Ligation step incubation to at least 1h
- Elute in Tris-HCl 10 mM.
- LFB was used for final size selection step
- Quantify DNA using the Qubit dsDNA HS Assay Kit (Q33230) to check yield.

Sequencing

37 Sequence during 72h on v9.4.1 flow cell FLO-MIN106.1 or FLO-PRO002 in MinION or PromethION sequencer, respectively.