



VERSION 2

FEB 29, 2024

Protocol SAM-Seq A.Thaliana V.2

basile.leduque¹, Quadrana Leandro¹

¹CNRS



basile.leduque

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.

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.8epv5x1xdg1b/v2

Protocol Citation: basile.leduque, Quadrana Leandro 2024. Protocol SAM-Seq A.Thaliana. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.8epv5x1xdg1b/v2> Version created by basile.leduque

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Feb 29, 2024

Last Modified: Feb 29, 2024

PROTOCOL integer ID: 95953

Funders Acknowledgement:

European Research Council

Grant ID: 948674

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 2-Mercaptoethanol	17.5 µl	5mM
+ PIs (1 tablet per 50mls)		

2 Extraction Buffer (EB) 2

To prepare 4ml of fresh EB2 :

Component	Amount	Final Concentration
14.3M 2-Mercaptoethanol	1,4 µL	5mM
20% Triton X-100	200 µL	1%
H2O	2818,6 µL	
1M MgCl2	40 µL	10mM
1M Tris-HCl pH 8	40 µL	10mM

Component	Amount	Final Concentration
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 MgCl ₂	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
14.3M 2-Mercaptoethanol	1,05 µL	5mM
H ₂ O to volume	90,3µL	

4

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

Component	Amount	Final Concentration
70 mM spermine	28.5µl	0.2 mM
1 mini-tablet diluted in 1ml	1ml	1X

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	

6 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

7 Starting material: 1g of powder (Aerial part of ~17 days seedling)

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

- 8 Add 1% Formaldehyde for crosslinking (i.e. 675 μ l Formaldehyde 37% in 25ml of EB1). Incubate 5 minutes
- 9 Add 1.25 M glycine to stop the crosslinking (i.e. 1.7ml of Glycine 2M per 675 μ l Formaldehyde 37%)
- 10 Filter the solution through Miracloth into a new 50 ml falcon tube. Repeat once.
- 11 Filter the solution through a 0.4 μ m filter into a new 50 ml falcon tube.
- 12 Centrifuge the filtered solution for 20 minutes at 4,000 rpm at 4°C.
- 13 Gently remove supernatant and resuspend the pellet in 2 ml of Extraction Buffer (EB) 2. Transfer the solution into four 1.5ml Eppendorf tubes.
- 14 Centrifuge at 12,000 g for 10 minutes at 4°C. Repeat step 7 & 8 once
- 15 Remove supernatant and resuspend each pellet in 300 μ l of Extraction Buffer (EB) 3.

- 16 In a clean Eppendorf tube, add 300ul of EB3. Take the 300ul solution (resuspended pellet) from step 7 and carefully layer it on top of the clean 300ul of EB3.
- 17 Centrifuge for 45 min at 12,000 at 4 degrees.
- 18 Resuspend each chromatin pellet in 300ul Nuclear Preparation Buffer (NPB) and incubate on ice for 5 min

m6A-MTases reaction

- 19 Spin down nuclei at 1000g for 5 min at 4°C and resuspend in Plant-Tween-Wash-Buffer (PTWB).
- 20 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
- 21 Add 200 µl rSmartCut 1X + 20 µl NaCl (5M) and incubate overnight at 65 °C (or 1h30min if not crosslinked).
- 22 Add 5µl of RNase A (20mg/ml) and 2µl Proteinase K. Incubate 30 minutes at 42°C.

Phenol/Chloroform extraction of genomic DNA

- 23 Add 1 volume of 1:1 phenol:chloroform (i.e. 400µl)
- 24 Centrifuge at 12,000 g for 10 minutes at 4°C
- 25 Recover the aqueous phase (upper) in a new LoBind Eppendorf tube
- 26 Add 1 volume of chloroform (i.e. 400µl)
- 27 Centrifuge at 12,000 g for 10 minutes at 4°C.
- 28 Recover aqueous phase (upper) in a new LoBind Eppendorf tube
- 29 Precipitate DNA by adding 1/10 volume of NaAcétate 3M pH5,2 + 2,5 volume of EtOH 100%
- 30 Incubate at –20°C from 45 minutes to overnight.

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

32 Wash the pellet with 300μ EtOH 70%.

33 Remove EtOH and dry pellets.

34 Elute in 20μl TE pH 8.0

Library preparation

35 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

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