May 27, 2025

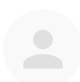
EPI-clone: Clonal tracing with somatic epimutations

Nature

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l29dzjv1y/v1

Michael Scherer[1], Chelsea Szu Tu[2], Indranil Singh[3], Martina Braun[2], Alejo Rodriguez-Fraticelli[3], Lars Velten[2]

[1]German Cancer Research Center (DKFZ); [2]Centre for Genomic Regulation;
[3]Institute for Research in Biomedicine


Lars Velten
Centre for Genomic Regulation

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l29dzjv1y/v1

Protocol Citation: Michael Scherer, Chelsea Szu Tu, Indranil Singh, Martina Braun, Alejo Rodriguez-Fraticelli, Lars Velten 2025. EPI-clone: Clonal tracing with somatic epimutations. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l29dzjv1y/v1

Manuscript citation:
Scherer et al., Clonal tracing with somatic epimutations reveals dynamics of blood aging. Nature 2025

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 have successfully used this protocol on >20 human and >10 mouse samples.

Created: March 03, 2025

Last Modified: May 27, 2025

Protocol Integer ID: 123691

Keywords: DNA methylation, Single-cell genomics, Lineage tracing, Haematopoiesis, methylation profiling with sctam, methylation profiling, targeted single cell dna, single cell dna, using somatic epimutation, cell lineage, somatic epimutations this protocol, single cell, somatic epimutation, data on the methylation state, clonal information, methylation state, clonal information from these data, cell state, clonal, cell

Funders Acknowledgements:
European Hematology Association
Grant ID: EHA Research Grant
Asociación Española Contra el Cáncer
Grant ID: AECC lab grant

Abstract

This protocol describes the EPI-Clone method for single-cell lineage tracing using somatic epimutations (Scherer et al., 2025). This method is based on targeted single cell DNA-methylation profiling with **scTAM-seq**. We here provide targeting panels for mouse and human hematopoiesis, instructions for running scTAM-seq, and instructions for data analysis. This protocol results in data on the methylation state of 100s of CpGs in 10,000s of single cells. Data analysis routines provided extract cell state and clonal information from these data.

Materials

For experiments with human cells: **Human targeting panel**, ordered from mission bio. See "before you start" section for notes.

- Human_panel_update.xlsx
- This panel consists of CpGs with differential methylation between blood progenitor states (DMC) as well as CpGs with high inter- or intra intervidual variability (IIH, IMC) and several genotyping amplicons targeted at CH mutations. See column "Amplicon_class"
- The 200 most information rich CpGs are marked in the column "Information rich site"

For experiments with mouse cells: **Mouse targeting panel**, ordered from mission bio. See "before you start" section for notes.

- Mouse_panel.xlsx
- The panel contains CpGs with high methylation in different cell states (HSC, MPP1-4), as well as CpGs with intermediately methylation (IMR) or locally uncorrelated methylation (WSH) in HSCs. Furthermore, the panel contains amplicons without a HhaI cutsite as a control. See column "Amplicon_class".

Further list of equipment and reagents

- **Antibody panel.** See "before you start" section for notes.
- **150 units/µl** HhaI from *New England Biolabs* (available upon request)
- Tapestri Single-Cell DNA Core +4 Kit v3 (PN: MB03-0092)
- Tapestri Single-Cell DNA Core -20 Kit v3 (PN: MB03-0091)
- Tapestri Single-Cell DNA Bead Kit v3 (PN: MB03-0093)
- Tapestri Protein Staining Kit v3 (PN: MB03-0094)
- Tapestri Single-Cell DNA Cartridge Box v3 (PN: MB03-0099)
- All required third-party consumables and reagents listed in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide**

For working with Totalseq-B antibodies only (e.g. mouse cells)

- Molecular grade Tris-HCl
- Molecular grade EDTA
- Molecular grade NaCl
- (for mouse/TotalSeq-B only): custom Antibody Tag Primer, Biotin Oligo, and Protein Library Indices, see TotalSeqB_oligos.xlsx 9KB

Troubleshooting

Before start

Familiarize yourself with the **scTAM-seq method** as well as EPI-Clone.

Choosing the right **CpG targeting panel** is a critical step in scTAM-seq.

- In the context of **mouse blood and immune cells** we obtained high resolution on clones and cell states with our mouse panel of 663 amplicons (see materials). We also showed that the same set of CpGs can resolve cell state and clones in the context of **mouse endothelia**.
- In the context of **human blood and immune cells** we developed a modular panel of 665 amplicons (see materials). This panel contains both amplicons targeting CpGs for methylation profiling, and amplicons targeting hotspots of clonal hematopoiesis mutations for genotyping. Genotyping amplcons should be left out if not of interest, since they tend to consume >80% of sequencing reads. For the CpG targets, based on experience with this panel, we have identified the 200 most information rich sites. It is possible to leave out the remaining CpG targeting amplicons without losing resolution. It is essential to always include control amplicons without a HhaI cutsite.
- For **other tissues**, we have no data. For users interested in other tissues, we would recommend to check ibulk methylome data of their tissue if the CpGs targeted here display intermediate methylation (i.e. are not full methylated or unmethylated). If that is the case for a majority of CpGs, our CpG panel may be used. Alternatively, a new panel can be designed, by selecting intermediately methylated CpGs in the tissue of interest and/or CpGs with differential methylation between cell states of interest. Our manuscript details how the mouse hematopoiesis panel was constructed. We provide a **CpG selection pipeline**. Users interested in designing a new CpG panel should make sure to include a sufficient (>40) number of control amplicons without a HhaI cutsite as controls.

Choosing the right **surface antibody panel** is also important.

- In the context of **mouse tissues**, antibodies will need to be ordered in Totalseq-B format, and adaptations need to be made to the default mission bio protocol (see optional steps in protocol)
- In the context of human tissues, we recommend working with the **TotalSeq-D human heme oncology cocktail**

Finally it is important to understand the **limitations** of the computational method for clone identification. In the current EPI-Clone version, only cells belonging to expanded clones can be assigned to their clone of origin. Cells belonging to very small clones (<0.25% of cells in post-transplant, and <1% in native hematopoiesis) can be identified as not belonging to expanded clones, but their clonal identity cannot be inferred with the cell numbers typically obtained from a scTAM-seq run. This is especially relevant to studies of healthy young human donors, where the clonal diversity is so large that no clones >1% might exist.

Run mission bio (human cells - TotalSeq-D antibodies)

1 Follow the steps outlined in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide**

2 Add 5 ul of **150 units/µl Hhal** from *New England Biolabs* to 300 ul of Barcode Mix in step 4.8. It is essential to use highly concentrated Hhal (available from NEB upon request).

3 Continue with the steps outlined in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide** until you have reached step 5.1 under section "5. Targeted PCR Amplification".

4 Set up the PCR program as follows if your DNA panel contains more than 300 amplicons. If you are working with a custom panel with less than 300 amplicons, adjust the annealing/extension times according to the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide**

	A	B	C	D	E
	Step	**Ramp**	**Temperature**	**Time**	**Cycle**
	0	4C/s	22C	HOLD	
	1	4C/s	37C	30min	
	2	4C/s	98C	6min	
	3	1C/s	95C	30sec	
	4	1C/s	72C	10sec	
	5	1C/s	61C	9min	
	6	1C/s	72C	20sec	back to step 3 for 10 aditional cycles
	7	1C/s	95C	30sec	
	8	1C/s	72C	10sec	
	9	1C/s	48C	9min	
	10	1C/s	72C	20sec	back to step 7 for 12 additional cycles
	11	4C/s	72C	2min	
	12	4C/s	4C	HOLD	

	A	B	C	D	E

5 Run the PCR program and skip step 0 to step 1.

6 Follow the steps as described in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide** until you have reached step 7.2 under section “7. Library PCR”.

7 Set up 2 × 50 ul reaction for the DNA Library. For the Protein Library, carry on with the User Guide.

8 In step 7.8, under “DNA Library Cleanup II”, instead of adding 50 ul of nuclease-free water to the sample tube, combine the two 50 ul DNA Library PCR reactions

9 Proceed with the rest of the protocol as instructed in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide**.

Running mission bio (mouse cells – TotalSeq-B antibodies)

10 Follow the steps outlined in the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide.**

11 Stain the cells with TotalSeq-B antibodies as you would with TotalSeq-D antibodies

12 Continue with the steps from the **Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide.**

13 Resuspend all lyophilized custom oligos/primers in nuclease-free water to 100 uM concentration.

14 In section “4. Barcode Cells” under step “4.8 Prepare Barcoding Mix”, replace 2 ul of the Antibody Primer Tag from 4C, Protein Staining Kit with 2 ul of 30 uM of our custom Antibody Tag Primer (sequence: ACTCGCAGTAGTCTTGCTAGGACCGGCCTTAAAG) and add 5 ul of 150 units/µl HhaI from New England Biolabs to 300 ul of Barcode Mix.

15 Follow steps 4-6 from the previous section “Running mission bio (human cells – TotalSeq-D antibodies).

16 Once you have reached “Protein Library Cleanup I” in section “6. Cleanup PCR Products” of the User Guide, **do not use** the 2X Wash Buffer and Biotin Oligos from the +4C, Protein Staining Kit.

17 As a replacement for the 2X Wash Buffer, prepare 2X Binding & Washing Buffer using nuclease-free water as follows using Molecular Grade reagents:

- Tris-HCl (final concentration: 10 mM, pH 7.5)
- EDTA (final concentration: 1 mM)
- NaCl (final concentration 2M)

18 Replace all 2X Wash Buffer usage with 2X Binding & Washing Buffer.

19 To isolate antibody tags in step 6.44, use 2 uL of 5 uM of the custom Biotin Oligo: /5Biosg/GTGACTGGAGTTCAGACGTGTG/3C6/

20 Carry on with the User Guide until you have reached step 7.2 under section “7. Library PCR”.

21 As with the human cells in the previous section, set up 2 × 50 ul reaction for the DNA Library.

22 For the Protein Library, rather than adding 10 ul of the Protein Library Indices, add 5 ul of 4 uM custom Protein Index i7 Primer and 5 ul of custom Protein Index i5 Primer as listed in Table S3 of the EPI-clone paper to the Protein Library PCR reaction in step 7.2.

23 Proceed with the rest of the protocol as instructed in the User Guide.

Sequencing

24 Sequence to the following depth:

- DNA library: expected number of cells (usually around 10,000) x number of amplicons (for EPI-clone mouse panel, 663) x 70 (464 M reads for mouse EPI-clone)
- Protein library: expected number of cells (usually around 10,000) x number of antibodies (EPI-clone mouse panel: 20) x 700 (140 M reads for mouse EPI-clone)

Bioinformatic analysis

25 Process the raw sequencing files (fastq) using the scTAM-seq pipeline as described here: https://github.com/veltenlab/EPICloneProcessing

26 Generate Seurat objects from the pipeline output using this file: https://github.com/veltenlab/EPI-clone/blob/main/processing_vignette.Rmd

27 Perform cell state annotation. See https://github.com/veltenlab/EPI-clone/blob/main/figures/Figure1/Figure1.Rmd for an example.

28 Execute EPI-clone using the script available from here: https://github.com/veltenlab/EPI-clone/blob/main/EPIClone_vignette.Rmd

Protocol references

Bianchi, A., Scherer, M., Zaurin, R. *et al.* scTAM-seq enables targeted high-confidence analysis of DNA methylation in single cells. *Genome Biol* **23**, 229 (2022). https://doi.org/10.1186/s13059-022-02796-7