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Fast-ATACseq protocol – Stanford Bone Marrow TMC

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We use this protocol and it's working

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Abstract

Frozen CD34$^+$ enriched cells from mobilized peripheral blood were thawed and suspended in cell-staining media with TruStain FC blocker for 10 min at room temperature prior to staining. Next, the cocktail of surface antibodies was added (**Table S1**), and cells were stained in the dark for 30 min on ice and then washed in cell-staining media. Prior to data acquisition, cell suspensions were spiked with 7-AAD (BioLegend) to label non-viable cells. The sorted populations (**Figures S3B and S5D**) were used for bulk ATAC-seq analysis. For bulk Fast-ATAC-seq, cells were processed as described previously (**M.R. Corces et al., Nat. Genet., 48 (2016)**). Briefly, approximately 50,000 FACS-purified cells were pelleted and used for the transposition reaction for each technical replicate, followed by DNA purification. Amplification and purification of the transposed fragments were performed as described previously (**J.D. Buenrostro et al., Curr. Protoc. Mol. Biol., 109 (2015)**) with modified indexing primers (**J.D. Buenrostro et al., Nature, 523 (2015)**). Libraries were quantified using qPCR. All Fast-ATAC libraries were sequenced on an Illumina NovaSeq through Novogene. Each population analyzed included two technical replicates and two biological replicates.

Troubleshooting

Details

1 Table S1 (novel EMP and MP FACS Panel with the cocktail of surface antibodies, Figures S3B and S5D): https://doi.org/10.1016/j.celrep.2025.115913

2 ATAC-seq protocol:
M.R. Corces et al., Nat. Genet., 48 (2016) - **10.1038/ng.3646**
J.D. Buenrostro et al., Curr. Protoc. Mol. Biol., 109 (2015) - **.1002/0471142727.mb2129s109**
J.D. Buenrostro et al., Nature, 523 (2015) - **10.1038/nature14590**

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