nature portfolio

https://doi.org/10.1038/s41596-021-00516-2

Supplementary information

Transcription factor chromatin profiling genome-wide using uliCUT&RUN in single cells and individual blastocysts

In the format provided by the authors and unedited

Supplementary Methods:

Membrane Lysis buffer (10 mL):
Combine 500 µL of 1 M Tris HCl (pH 7.5), 100 µL of 5M NaCl, 100 µL of 1 M $MgCl_2$, 250 µL of 10% NP-40, and 9.68 mL Nuclease Free Water. Can be stored up to a year at 4°C.

Heterologous DNA spike-in production:

Here, we outline how to produce a heterologous DNA spike-in (from *S. cerevisiae*) for use in uliCUT&RUN assays. We recommend use of *S. cerevisiae* cells for the spike-in production, but other sources of eukaryotic genomic DNA can be utilized instead, provided a high-quality reference genome is available and reads aligned are uniquely mapped relative to the sample genome. The following protocol will need to be adapted for use in the chosen system for spike-in use, accordingly.

Harvest approximately six million *S. cerevisiae* cells and resuspend them in 4 mL PBS in a 15 mL falcon tube. To crosslink cells, add 100 µL 37% formaldehyde, mix well and incubate for 15 minutes at RT. Quench the crosslinking reaction, add 1 mL 2.5 M glycine and incubate for 5 minutes at RT. Next, spin the cells down at 600*g* for 4 minutes at 4°C. Discard the supernatant and wash cells by resuspending in 4 mL 1X PBS. Transfer the cell suspension to a fresh 15 mL eppendorf tube and spin down again at 600*g* for 4 minutes at 4°C. Remove the supernatant and resuspend the cells in 3 mL cell lysis buffer (10 mM Tris pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$, 0.5% NP-40) (with $CaCl_2$ and PIs) (for 10 mL buffer, add 3 µL 1M $CaCl_2$). Rotate the cells for 15 minutes at 4°C. During the rotation, aliquot 15 U of MNase into six 1.5 mL microfuge tubes. After rotation, add 500 µL of lysed cells to each tube with MNase. Incubate each sample at 37°C for 5 minutes. Stop the MNase digestion reaction by adding 10µL .5M EDTA to each sample and vortexing well. To each sample, add 2 µL RNase A and incubate for 40 minutes at 37°C with light shaking. Next, add 5 µL 10% SDS and 10µL Proteinase K to each sample and incubate at 55°C overnight.

The next morning, PCI/chloroform extract the digested genomic DNA fragments and perform a 100% EtOH/ 3M NaOAc pH 5.2 precipitation at -80°C for at least 20 minutes. Spin the precipitated DNA at max speed for approximately 20 minutes at 4°C. Wash the precipitated DNA with 70% EtOH, remove the ethanol and let the DNA pellet air dry for approximately 5 minutes. Resuspend the DNA pellet in 30µL of nuclease-free water, then load and run the sample at 100-120V for 30-45 minutes on a 1% agarose gel with an intercalating agent of preference. Once ran out, gel extract the mononulceosome band (at ~150 bp, see Supplementary Figure 2 for reference). Quantify the concentration of DNA in the sample, and dilute to 10 ng/µL for use as heterologous DNA spike-in.

Purified pA-MNase/pAG-MNase activity assessment:

Pending the antibody used, one may choose to use either pA-MNase or pAG-MNase. A list of affinity of protein A or protein G for IgG types from different species can be found here: https://www.neb.com/tools-and-resources/selection-charts/affinity-of-protein-ag-for-igg-types-from-different-species. pA-MNase or pAG-MNase can be purified after acquiring the plasmids (as described below) or pAG-MNase can alternatively be purchased (Epicypher, cat# 15-1016).

To produce purified recombinant pA-MNase, please refer to Schmid *et al* 2004[17]. This strategy uses IgG binding by protein A for purification. Importantly, now purified pAG-MNase is commercially available from Epicypher or pAG-MNase cloned into a vector containing a HIS

tag is also available from the Henikoff Group (Available through Addgene: https://www.addgene.org/123461/). The Epicypher pAG-MNase does not need to be titrated for activity unless you experience high background. The pAG-MNase plasmid available from Addgene can be used with traditional IPTG induction and standard purification over a TALON or Nickel column.

Once purified recombinant pA-MNase or pAG-MNase is acquired, a nuclease activity assay should be performed. Cells should be lysed with membrane lysis buffer (10 mM Tris pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$, 0.5% NP-40, 300 nM $CaCl_2$). and incubated with a dilution series of recombinant pA-MNase (0U-30U, increasing in increments of 2-5U) alongside a dilution series of commercially available MNase (e.g., Takara cat# 2910A). Allow DNA digestion to proceed for ~5 min at 37ºC and chelate the reaction with EDTA. Purify digested DNA using standard RNase A and Proteinase K treatment followed by PCI and chloroform extraction and run the DNA product on an agarose gel. By visual comparison with the commercially available pA-MNase (as seen in Supplementary Figure 2), determine the endonucleolytic activity of the recombinant pA-MNase.

A traditional immunoprecipitation (IP) experiment can be conducted to test the IP capacity of the purified pA-MNase in comparison to commercially available protein A.

Finally, a pilot experiment must be performed using to determine the optimal amount of pA-MNase to be used in a uliCUT&RUN experiment. Perform a series of CUT&RUNs with a serial dilution series of pA-MNase (1:50, 1:100, 1:200, 1:400, and 1:800), construct libraries, and compare fragment distributions by Fragment Analyzer.

Compare fragment size profiles between dilutions to determine the optimal pA-MNase dilution to perform uliCUT&RUN in your sample. Once a batch of purified pA-MNase is validated, the activity does not need to be tested for every experiment as long as the recombinant protein is maintained at appropriate temperatures in appropriate storage conditions.

qPCR-based approach for library amplification cycle optimization

A quantitative approach may be necessary to determine the optimal number of PCR cycles for library amplification, as described for ATAC-seq protocols[45]. To do so, perform the library amplification steps above as described, but only perform 5 cycles of amplification. After 5 cycles of amplification, take the library out of thermocycler and place on ice. Construct the following master mix for each sample: 5 µL of pre-amplified library DNA, 2 µL nuclease-free water, 0.25 µL 25 nM i5 universal primer, 0.25 µL 25 nM i7 sample-specific index primer, and 7.5 µL SYBR Green master mix (total volume: 15 µL). On a qPCR machine, run the library enrichment PCR program for 25 cycles. For each sample, plot the Rn value vs number of cycles. Identify at which cycle the sample reaches 1/3 of maximum Rn value to determine the optimal number of PCR cycles for library amplification (N). With the remaining 45 µL of library PCR, run the PCR for N-5 cycles to complete the library amplification and proceed as described[45].

Alternative library preparation with inline or Y-shaped barcode adapters

Here, we outline an alternative library preparation protocol if the user prefers to use inline barcode adapters (user-generated as they are no longer available for purchase) or Y-shaped adapters (e.g., NEB TruSeq adapters, Illumina cat# 20015964 and 20015960). Of note, we observe a significantly higher occurrence of adapter and primer dimer with the use of these adapter types, and so additional purification steps need to be taken to maintain the quality of the final sequencing library (see Supplementary Figure 1). Finally, an additional alternative for

library preparation is using the NEBNext Ultra II library build kit (NEB cat# E7645S/L) with the NEBNext adapters described for steps 52-53. For preparation with the kit, users should follow manufacturer instructions.

Conduct the procedure up until step 53 as described. Perform the Adapter ligation step as described but add 2.5 µL of 1.5 µM PE/TruSeq adapter mix in place of the NEB stem-loop adapters. Perform AMPure XP purification as outlined. Conduct Library Enrichment as described, but use 5 µL of sample-specific primers (P5/P7 Illumina primers) in place of NEBNext adapters and primers.

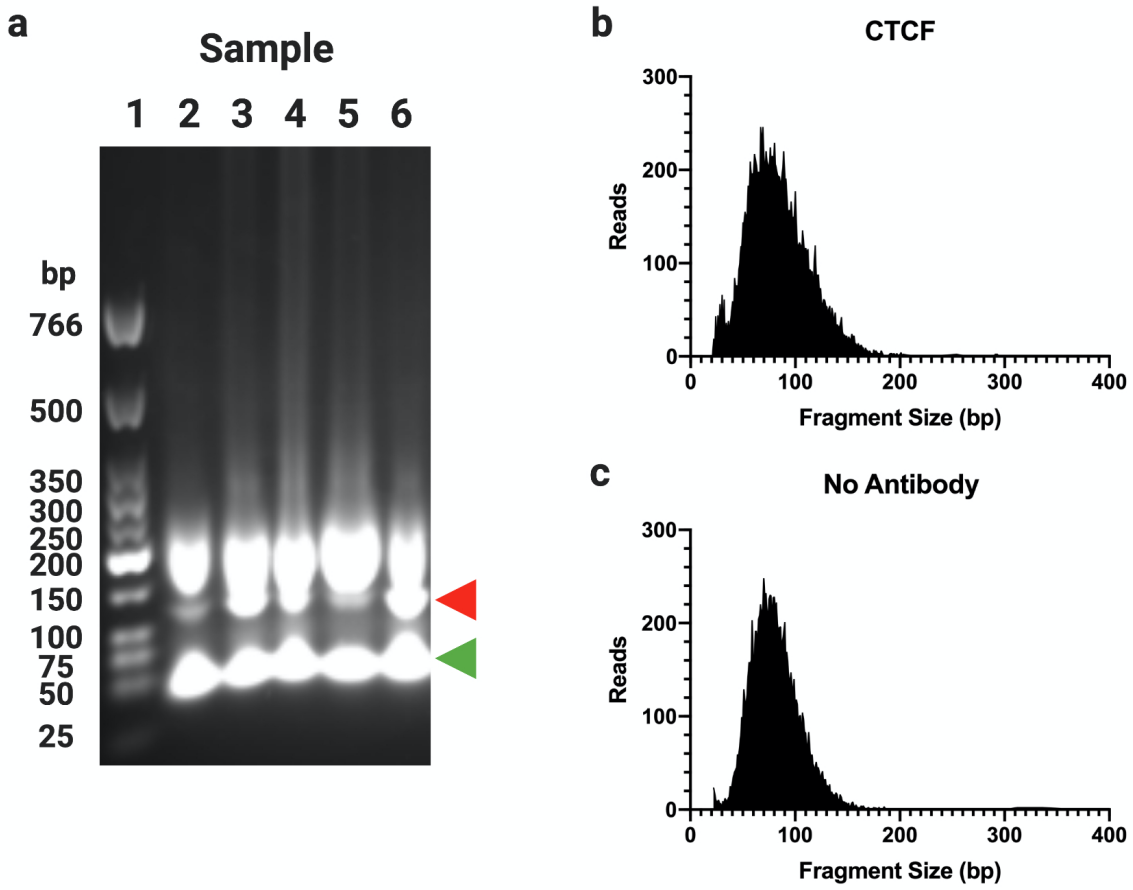
Immediately after Library Enrichment, transfer the samples (50 µL total) to 1.5 mL microfuge tubes. To each sample, add 5 µL 3M NaOAc pH 5.2 (0.1X), 5 µL glycogen (20 mg/mL), and 100 µL 100% ethanol (2X). Mix well by vortexing. Incubate at -20°C for at least 20 minutes (or overnight). Centrifuge the samples at 16,000*g* for 30 min at 4°C. Carefully remove the supernatant without disturbing the DNA pellet, add 1 mL 80% ethanol, and spin the samples at 16,000*g* for 5 min at 4°C. Carefully remove the supernatant without disturbing the DNA pellet. Briefly spin the samples in a myfuge and remove any residual ethanol. Let the samples sit with lids open to air-dry for ~2 min. Resuspend the samples in 10 µL nuclease free water, and store on ice.

Prepare two 15 cm, 1.5% agarose (TBE) gels with the desired intercalating agent, and label as Gel 1 and Gel 2. Assemble and ready the gel electrophoresis apparatus with Gel 1. Add DNA loading dye to each sample, and load the samples into Gel 1 along with a NEB low MW DNA ladder. Run the gel at 100-120V for at least 60 minutes, allowing the loading dye front to move to the edge of the gel to achieve maximum fragment separation. From the gel and working under long-range UV light with proper safety equipment, excise each sample from ~150-650 base pairs in size, and place into 15 mL tubes. It is critical to avoid any adapter dimer (band at ~120 bp) when extracting each sample.

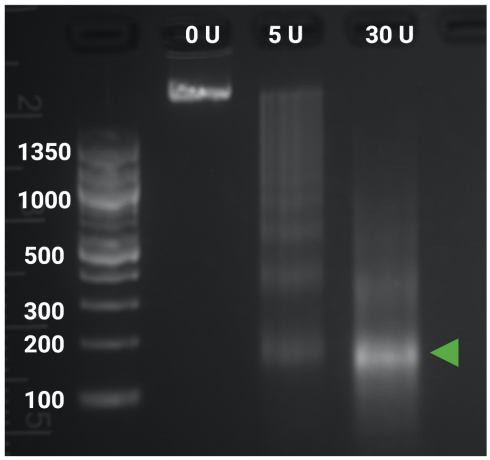
Weigh the gel pieces and add Buffer QG at a ratio of 2 µL:1 µg gel. Vortex the samples well and incubate in a 60°C water bath for ~10 minutes, vortexing every 2-3 min to ensure proper gel solubilization. Once the gel slice has been fully solubilized, add 100% isopropanol at a ratio of 1 µL:1 µg gel slice to each sample and vortex well to mix. Add 800 µL of solution to a spin column and spin at 16,000*g* for 30 seconds at RT to draw the liquid through and bind DNA to the membrane of the spin column. Dispose of the flow through and add the remaining (800 µL) of solution to the same spin column. If necessary, repeat this process until all of the solubilized gel solution has been drawn though the spin column. Add 500 µL PE buffer to the spin column, and spin at 16,000*g* for 30 seconds. Discard the flow through and perform an additional wash with 500 µL PE buffer. Perform an additional spin at 16,000*g* for 30 seconds on the empty column to remove any residual PE buffer. Transfer the spin column to a fresh 1.5 mL microfuge tube and add 20 µL nuclease-free water directly to the center of the column. Let the column sit for 2 min, and then spin at 16,000*g* for 1 min at RT to elute the DNA from the spin column. Run the samples recovered from Gel 1 on Gel 2 and perform an additional gel purification as outlined above. The samples recovered from Gel 2 can be directly prepared for submission for sequencing as above or can be stored as -20°C until needed.

Analysis of inline barcode-based sequencing libraries requires prior data processing before read mapping. Raw fastq files must be trimmed to 25 nt at both ends (with custom coding), and then de-multiplexed according to the corresponding barcodes, and barcodes trimmed. After demultiplexing, reads can then be mapped with bowtie 2 as above. See Code Availability for required coding

Supplementary Figures:



Supplementary Figure 1. Single cell uliCUT&RUN library build with inline adapters. Inline barcoded adapters or Y-shaped adapters offer an alternative to stem-loop adapter based library construction. a) Ethidium bromide stained agarose gel of 5 single cell uliCUT&RUN samples after library preparation with inline adapters. NEB low molecular weight marker is found in lane 1, with 5 libraries shown in lanes 2-6. Lanes 2 and 3 contain No antibody controls while Lanes 4-6 contain CTCF-enriched libraries. Adapter dimers (red arrowhead) and primer dimers (green arrowhead are found. b,c) Size distribution plots for uliCUT&RUN on single for CTCF (b) and No antibody (c) after sequencing.



Supplementary Figure 2. Gel image of MNase digested chromatin.
Lane 1 contains NEB 100 bp ladder. Lanes 2-4 contain equivalent amounts of yeast chromatin digested with 0 U, 5 U, and 30 U of MNase, respectively. Green arrow indicates band corresponding to mononucleosome sized DNA fragments that can be gel extracted and quantified for spike-in use.