Supplemental information "A plug and play microfluidic platform for standardized sensitive low-input Chromatin Immunoprecipitation", Dirks et al., Genome Research 2021

Supplemental Methods

Fluorescence-Activated Cell Sorting (FACS)

Generation of *Hhex::Venus* reporter ESCs (Morgani et al. 2013), *Zscan4c::Emerald(Em)-GFP* reporter ESCs (Eckersley-Maslin et al. 2016) and *MuERV-L(MERVL)::tdTomato* reporter ESCs (Eckersley-Maslin et al. 2016) have been described before. Reporter lines were sorted (positive from negative population) using a BD FACSAria cell sorter with 5 million cells as input. Reporter positive cells were recovered from *Hhex::Venus* (51,000 cells), *Zscan4c::Emerald-GFP* (150,000 cells) and *MuERV-L::tdTomato* (500,000 cells). From the reporter positive and negative cell populations, we used 15,000 cells for MNase treatment as further described below as the standard protocol. From the MNase-treated material, we loaded the chromatin equivalent of 3,000 cells on the microfluidic chip for PnP-ChIP-seq.

Conventional ChIP and library preparation for ChIP-seq

Chromatin extracts were prepared by on-plate cell crosslinking in 1% paraformaldehyde for 8 minutes. Crosslinking was quenched using 125 mM (final concentration) freshly dissolved glycine. At room temperature, fixed cells were washed in PBS twice, then collected by scraping. Pellets were lysed and sonicated in 50 mM Tris pH 8.0, 1% SDS and fresh protease inhibitor cocktail (Roche) at a density of 15 million cells per milliliter. The cells were sonicated in a Bioruptor Pico (Diagenode) for eight to ten 30-second cycles according to instructions from the manufacturer. Proper sonication was evaluated using Agilent Bioanalyzer size checks

after decrosslinking. DNA concentrations were quantified using the Qubit HS (Thermo Fischer Scientific). The sonicated chromatin was diluted 9-fold using IP buffer (consisting of 1% Triton X-100, 1.2mM EDTA, 16.7mM Tris pH8.0, 167mM NaCl). Mouse chromatin was ChIPped using the following amounts of antibody: 1µg H3K4me3 (Diagenode C1540003), 0.2µg H3K4me1 (Diagenode C1540194), 0.5µg H3K27ac (Diagenode C15410196; lot #A1723-0041d), 1µg H3K36me3 (Diagenode pAb-192-050), 0.5µg H3K27me3 (Millipore 04-779) and 0.5µg H3K9me3 (C15410193, pAb-193-050; lot #A1671-001P) incubating overnight at 4°C while rotating. A mixture of 10µL magnetic Protein A and 10µL protein G beads (Thermo), blocked twice in IP buffer with 0.15% SDS, was next added to the ChIP. ChIPs with beads were incubated for 1 hour at 4°C and precipitated by the use of a magnetic rack. Chromatin was washed once with a buffer containing 2mM EDTA, 20mM Tris pH8.0, 1% Triton X-100, 0.1% SDS, 150mM NaCl, twice with a buffer containing 2mM EDTA, 20mM Tris pH8.0, 1% Triton X-100, 0.1% SDS, 500mM NaCl and twice with a buffer containing 1mM EDTA, 10 mM Tris pH8.0, all washes were at 4°C with 5 min rotation. Specific ChIPped DNA was eluted using 200mM NaCl, 1%SDS, 20mM Tris pH8.0 by shaking at 65°C for one hour, addition of 0.1µg/µL Proteinase K and shaking at 55°C for one hour, then shaking at 65°C for a minimum of four hours. The DNA was purified using the Qiagen Minelute kit according to manufacturer instructions. Libraries were generated using Kapa Hyper Prep on 5ng DNA according to manufacturer instructions and size selected for 300 bp in size (120 bp adaptor and 180 bp insert) using Ampure XP beads. DNA was quantified using Qubit HS and DNA fragment sizes checked using Agilent Bioanalyzer HS.

RT-qPCR and qPCR

Reverse transcription (RT) was performed according to standard protocols. Quantitative PCR (qPCR) was performed using 400nM primer and 50% v/v iQ SYBR green master mix (Bio-

rad) together with DNA in 25µL final volume. Sequences of the primers used for ChIP-qPCR: *Actin-beta* (*Actb*): fw-agtgtgacgttgacatccgt and rv-tgctaggagccagagcagta; Negative region (Neg): fw-attttgtgctgcataacctcct and Neg rv-tagcaacatcctaagctggaca. Sequences of the primers used for RT-qPCR: *Gapdh*: fw-ttcaccaccatggagaaggc and rv-ccctttttggctccaccct; *Venus*: fw-gacgacggcaactacaagac and rv-tccttgaagtcgatgcccttt; *Hhex*: fw-ctacacgcacgccctactc and rv-cagaggtcgctggaggaa; *Tmem92*: fw-ttgacctttggcctgctttc and rv-aagcggtcatttgcaggatc; *Eomes*: fw-aaattccaccggcaccaaac and rv-aaacattgtagtgggcggtg; *Serpine2*: fw-tcaagggtttgtggaagtctcggt and rv-agagctgagccaacatgggtactt; *Cdx2*: fw-ggaagccaagtgaaaaccag and rv-cttggctctgcggttctg; *tdTomato*: fw-caccacctgttcctgggg and rv-ccatgttgttgtcctcggag; *MuERVL*: fw-acaatgcaaatgtacttcctgc and rv-cttgtcggaagcctctttgc; *Lonrf3*: fw-agccactctaggcaaggtga and rv-gatctggcgctcttgttctt; *Rpia*: fw-ggaacaactggggcctct and rv-gccagcttcttagcctcctc; *emGFP*: fw-tcgtgaccaccttgacctac and rv-tccttgaagtcgatgcccttt. For RT-qPCR, the data was normalized between conditions to *Gapdh*.

Microfluidic IFC operation

PDMS microfluidic chips were manufactured and provided by Fluidigm® (Fluidigm Corporation, South San Francisco, CA, USA). The chips are mounted to a carrier which facilitates manual and robotic loading of microliter volumes and provides the subsequent interface for pneumatic and thermocycling control. Pressure control and thermocycling were automated using the C1™ system (Fluidigm®), and the following steps are performed in parallel for all 24 reactors on the chip. The pneumatic controller anvil is pressurized to the carrier using 30 psi of pressure. All reagent and valve control channels were dead-end filled before script operation to avoid the presence of air bubbles in the system during the workflow. The procedure is started by loading a 50µm layer of a mixture of 4.5 µm and 6.0 µm frit beads packed into the reactor (0.15 mm x 0.1 mm x 1.3 mm LxWxH) at 13 psi for 45 seconds to

block the reactor exit (5 µm drain opening size). A 1:1 mixture of 2.8 µm Protein A and Protein G (15 x 10^7 beads/mL in 30% glycerol) were packed for 10 minutes at 13 psi in the reactor to consistently achieve a column size of approximately 5-6 nl. The bead columns were washed at 11 psi of pressure using equilibration buffer (2mM EDTA, 20mM Tris pH8.0, 1% Triton X-100, 0.1% SDS, 150mM NaCl). All washing steps were performed by building up pressure on to the bead and chromatin filled reactors, with the reactor outlet closed. The buffer entry channel was then closed after which the pressure excess was released towards a waste container outside of the PDMS. This washing sequence was repeated for 15 cycles to transit approximately three reactor volumes across the bead column. Throughout the various experiments 3-8 µl chromatin in IP buffer (see Low-input microfluidic ChIP section in the main manuscript) was pushed through the 4°C pre-cooled antibody affinity columns using 8 psi (MNase) to 12 psi (sonication) of pressure during the course of 30 minutes. After loading of the chromatin, the IP buffer was cleared by repeating 20 cycles of the washing sequence with air as input. Then, the chromatin loaded column was filled with equilibration buffer using 15 washing cycles, followed by washing with High Salt wash buffer (2mM EDTA, 20mM Tris pH8.0, 1% Triton X-100, 0.1% SDS, 500mM NaCl), and finally another 20 air purging cycles. The anvil pressure was then increased to 40 psi to facilitate an increased temperature. After closing the valve downstream of the reactor, the air-dried column was dead-end filled over the course of 10 minutes using DNA extraction buffer (150mM NaCl, 30mM Tris pH 8.0, 0.1µg/µL Proteinase K (Sigma-Aldrich)) at 12 psi. All valves surrounding the reactor were closed and the temperature of the PDMS was then increased to 55°C for 20 minutes, followed by 60 minutes at 65°C. The temperature was set to 40°C for extraction and anvil pressure was relieved to 30 psi. Using DNA elution buffer (10mM Tris pH 8.5) and 300 cycles of washing, the ChIPped DNA was directed to sample specific outlets. Critical valves were locked before

release of the anvil pressure and expulsion of the microfluidic chip. Approximately 3 μL volume was consistently recovered for each sample.

ChIP-seq Repeat analysis

Repeats as covered by PnP-ChIP-seq were assayed by counting repeat-containing sequence tags in the FASTQ sequencing file. The following satellite-specific sequences were used to select for satellite tags: tggaatatggcgagaaaact; gaaaataacggaaaatgaga; aatacacactttaggacgtg; aaatatggcgaggaaaactg; aaaaaggtggaaaatttaga; aatgtccactgtaggacatg; gaatatggcaagaaaactga; aaatcatggaaaatgagaaa; catccacttgacgacttgaa; aaatgacgaaatcactaaaa; aacgtgaaaaaggagaaatg; cccactgaaggacct; cgagaaaactgaaaataacg; gaaaatgagaaatacacact; ttaggacgtgaaatatggcg; aggaaaactgaaaaaggtgg; aaaatttagaaatgtccact; gtaggacatggaatatggca; agaaaactgaaaatcatgga; aaatgagaaacatccacttg; acgacttgaaaaatgacgaa; atcactaaaaaacgtgaaaa; aggagaaatgcccactgaag; gaccttggaatatgg; ccatattccaaggtc; cttcagtgggcatttctcct; ttttcacgttttttagtgat; ttcgtcatttttcaagtcgt; caagtggatgtttctcattt; tccatgattttcagttttct; tgccatattccatgtcctac; agtggacatttctaaatttt; ccacctttttcagttttcct; cgccatatttcacgtcctaa; agtgtgtatttctcattttc; cgttattttcagttttctcg; aggtccttcagtgggcattt; ctccttttcacgtttttta; gtgatttcgtcatttttcaa; gtcgtcaagtggatgtttct; cattttccatgattttcagt; tttcttgccatattccatgt; cctacagtggacatttctaa; attttccacctttttcagtt; ttcctcgccatatttcacgt; cctaaagtgtgtatttctca; ttttccgttattttcagttt; tctcgccatattcca. To select for SineB (Alu) tags, we used the following sequence: gtggcgcacgcctttaatc.