Many chromatin features play critical roles in regulating gene expression. A complete understanding of gene regulation will require the mapping of specific chromatin features in small samples of cells at high resolution. Here we describe Cleavage Under Targets and Tagmentation (CUT&Tag), an enzyme-tethering strategy that provides efficient high-resolution sequencing libraries for profiling diverse chromatin components. In CUT&Tag, a chromatin protein is bound in situ by a specific antibody, which then tethers a protein A-Tn5 transposase fusion protein. Activation of the transposase efficiently generates fragment libraries with high resolution and exceptionally low background. All steps from live cells to sequencing-ready libraries can be performed in a single tube on the benchtop or a microwell in a high-throughput pipeline, and the entire procedure can be performed in one day. We demonstrate the utility of CUT&Tag by profiling histone modifications, RNA Polymerase II and transcription factors on low cell numbers and single cells.
he advent of massively parallel sequencing and the dramatic reduction in cost per base has fueled a genomics revolution, however, the full promise of epigenomic profiling has lagged owing to limitations in methodologies used for mapping chromatin fragments to the genome. Chromatin immunoprecipitation with sequencing (ChIP-seq) and its variations suffer from low signals, high backgrounds and epitope masking due to cross-linking, and low yields require large numbers of cells. Alternatives to ChIP include enzyme-tethering methods for unfixed cells, such as DamID, ChEC-seq, and CUT&RUN, where a specific protein of interest is targeted in situ and then profiled genome-wide. For example, CUT&RUN, which is based on Laemmli’s Chromatin ImmunoCleavage (ChiC) strategy, maps a chromatin protein by successive binding of a specific antibody, and then tethering a Protein A/Micrococcal Nuclease (pA-MNase) fusion protein in permeabilized cells without cross-linking. MNase is activated by addition of calcium, and fragments are released into the supernatant for extraction of DNA, library preparation and paired-end sequencing. CUT&RUN provides base pair resolution of specific chromatin components with background levels that are much lower than with ChIP-seq, dramatically reducing the cost of genome-wide profiling. Although CUT&RUN can generate high-quality data from as few as 100–1000 cells, it must be followed by DNA end polishing and adapter ligation to prepare sequencing libraries, which increases the time, cost and effort of the overall procedure. Moreover, the release of MNase-cleaved fragments into the supernatant with CUT&RUN is not well-suited for application to single-cell platforms.

Here we overcome the limitations of ChIP-seq and CUT&RUN using a transposase that consists of a hyperactive Tn5 transposase—Protein A (pA-Tn5) fusion protein loaded with sequencing adapters. Tethering in situ followed by activation of pA-Tn5 results in factor-targeted tagmentation, generating fragments ready for PCR enrichment and DNA sequencing. Beginning with live cells, Cleavage Under Targets and Tagmentation (CUT&Tag) provides amplified sequence-ready libraries in a day on the bench top or in a high-throughput format. We show that a variety of chromatin components can be profiled with exceptionally low backgrounds using low cell numbers and even single cells. This easy, low-cost method will empower epigenetic studies in diverse areas of biological research.

**Results**

**Efficient profiling of nucleosomes and RNAPII with CUT&Tag.** To implement chromatin profiling by tagmentation (Fig. 1a), we incubated intact permeabilized human K562 cells with an antibody to lysine-27-trimethylation of the histone H3 tail (H3K27me3), an abundant histone modification that marks silenced chromatin regions. We incubated cells with a secondary anti-rabbit antibody to increase the local concentration of antibody bound on chromatin sites, then incubated cells with an excess of pA-Tn5 fusion protein pre-loaded with sequencing adapters to tether the enzyme at antibody-bound sites in the nucleus. The transposome has inherent affinity for exposed DNA, and so we washed cells under stringent conditions to remove un-tethered pA-Tn5. We then activated the transposome by addition of Mg++, integrating adapters spanning sites of H3K27me3-containing nucleosomes. Finally, fragment libraries were enriched from purified DNA and pooled for multiplex paired-end sequencing on an Illumina HiSeq flow-cell. The entire protocol manipulates all steps in a single reaction tube (Fig. 1b), where permeabilized cells are first mixed with an antibody, and then immobilized on Concanavalin A-coated paramagnetic beads, allowing magnetic handling of the cells in all successive wash and reagent incubation steps. For standardization between experiments, we used the small amount of tracer genomic DNA derived from the E. coli during transposase protein production to normalize sample read counts in lieu of the heterologous spike-in DNA that is recommended for CUT&RUN (see Methods section and Supplementary Fig. 1a).

Display of ~8 million reads mapped to the human genome assembly shows a clear pattern of large chromatin domains marked by H3K27me3 (Fig. 2a). We also obtained profiles for H3K4me1 and H3K4me2 histone modifications, which mark active chromatin sites. In contrast, incubation of cells with a non-specific IgG antibody, which measures untethered integration of adapters, produced very sparse landscapes (Fig. 2a). To assess the signal-to-noise of CUT&Tag relative to other methods we compared it with profiling generated by CUT&RUN and by ChIP-seq for the same H3K27me3 rabbit monoclonal antibody in K562 cells. To directly compare the three techniques, we set the read depth of each dataset to 8 million reads each. Landscapes for each of the three methods are similar, but background noise

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**Fig. 1** In situ tethering for CUT&Tag chromatin profiling. **a** The steps in CUT&Tag. Added antibody (green) binds to the target chromatin protein (blue) between nucleosomes (gray ovals) in the genome, and the excess is washed away. A second antibody (orange) is added and enhances tethering of pA-Tn5 transposome (gray boxes) at antibody-bound sites. After washing away excess transposome, addition of Mg++ activates the transposome and integrates adapters (red) at chromatin protein binding sites. After DNA purification genomic fragments with adapters at both ends are enriched by PCR. b CUT&Tag is performed on a solid support. Unfixed cells or nuclei (blue) are permeabilized and mixed with antibody to a target chromatin protein. After addition and binding of cells to Concanavalin A-coated magnetic beads (M), all further steps are performed in the same reaction tube with magnetic capture between washes and incubations, including pA-Tn5 tethering, integration, and DNA purification.
dominates in ChIP-seq datasets (Fig. 2a), and it is thus appears that ChIP-seq will require substantially greater read depth to distinguish chromatin features from background. In contrast, both CUT&RUN and CUT&Tag profiles have extremely low background noise levels. As expected, very different profiles were seen in the same region for a different human cell type, H1 embryonic stem (H1 ES) cells (Fig. 2b). To more quantitatively compare signal and noise levels in each method, we generated heatmaps around genomic sites called from H3K4me1 modification for each method, where the same antibody had been
used. After sampling each dataset to 8 million reads for comparison, we found that CUT&Tag for this histone modification shows moderately higher signals compared to CUT&RUN throughout the list of sites (Fig. 2c). Both methods have low backgrounds around the sites. In contrast, ChIP-seq signal has a very narrow dynamic range that is ~1/20 of the CUT&Tag signal range, and much weaker signals across the majority of sites. To quantitatively compare methods, we displayed the average read counts for CUT&Tag, CUT&RUN and ChIP-seq datasets for the H3K4me1 histone mark around the top 10,000 peaks defined by MACS2 on an H3K4me1 ChIP-seq dataset (Fig. 2g). We found that CUT&Tag profiling gives substantially more signal accumulation at these sites, implying that CUT&Tag will be most effective at distinguishing chromatin features with fewest reads.

The transcriptional status of genes and regulatory elements can be inferred from histone modification patterns, but gene expression is directly read out by profiling chromatin-bound RNA polymerase II (RNAPII). We used an antibody to the S2p/S5p phosphorylation (S2/5p) forms of RNAPII, which distinguishes engaged polymerase20. Landscapes show enrichment of RNAPII CUT&Tag reads at many genes (Fig. 2a, Supplementary Fig. 2a), and a promoter heatmap reveals that this enrichment is predominantly at the 5’ ends of active genes21 (Fig. 2d). These results were confirmed by the observation of similar CUT&Tag patterns using antibodies to S2p, S5p and S7p forms of RNAPII (Supplementary Fig. 2a and Supplementary Fig. 3a, b).

To validate RNAPII CUT&Tag without relying upon annotations, which are typically based on mapping of processed transcripts, we chose transcriptional run-on data obtained with the base-pair-resolution PRO-seq technique, which provides direct mapping of RNAPII using a method that is unrelated to chromatin profiling22. PRO-seq maps the position of the 5’ end of engaged RNAPII as it is activated in situ, and is used to identify paused RNAPII just downstream of the transcriptional start site. Peaks were called from RNAPII S2/5p CUT&Tag and ordered using MACS2, and processed datasets from PRO-seq run-on for human K562 cells (SRA GSM148327) were aligned to the peak calls. When ordered by RNAPII CUT&Tag MACS2 score, a close correspondence between PRO-seq occupancy and RNAPII-Ser2/5p CUT&Tag occupancy is seen (Fig. 2e). Similar heat maps were obtained using antibodies to S2p, S5p, and S7p phosphorylation of the RNAPII C-terminal domain (Supplementary Fig. 3c).

CUT&Tag sensitively maps active sites in chromatin. Replicates for profiling of H3K4me1 modification by CUT&Tag are highly similar, demonstrating the reproducibility of the method (Fig. 3a). We obtained similar reproducibility when we compared H3K27me3 CUT&Tag replicates (Supplementary Fig. 2c). In previous experiments with CUT&RUN profiling, we found that H3K4me2 histone modification landscapes, which are associated with active promoters and enhancers, resemble ATAC-seq profiles18. We therefore performed CUT&Tag using an antibody to H3K4me2. An example of H3K4me2 CUT&Tag profiling to published ATAC-seq in K562 cells23 is shown (Fig. 2a). We found high occupancies for H3K4me2 at strong ATAC-seq peaks (Fig. 2f), with much higher read counts (Fig. 2h), implying that H3K4me2 profiling captures the most prominent accessible chromatin sites in the genome with greater sensitivity.

To quantify the sensitivity of H3K4me2 CUT&Tag relative to H3K4me2 CUT&RUN18, H3K4me2 ChIP-seq19, and ATAC-seq23, we downsampled reads from each method, and used MACS2 with default parameters to call peaks on each dataset. We then estimated the fraction of reads falling within the called peaks. We found that both CUT&RUN and CUT&Tag populate peaks more deeply than ChIP-seq or ATAC-seq, demonstrating that they have exceptionally low signal-to-noise (Fig. 3b). In addition, CUT&Tag more rapidly populates peaks at low sequencing depths, where ~2 million reads are equivalent to 8 million for CUT&RUN (or 20 million for ChIP-seq), demonstrating the exceptionally high efficiency of CUT&Tag. Of all the methods, only CUT&Tag reaches a fraction of 0.6 within peaks. Thus, with two histone modifications (H3K4me2 and H3K27me3), we segment the chromatin landscape into both active and silenced regions, even with relatively low sequencing depths.
To test if CUT&Tag is tractable for profiling more abundant transcription factor binding sites, we profiled the CCCTC-binding factor (CTCF) DNA-binding protein. For these experiments, we varied the stringency of wash buffers to assess displacement of transcription factors from chromatin. Under low-salt and medium-salt concentration conditions we observed read counts at CTCF sites detected by CUT&RUN and by ChiP-seq (Fig. 2a), but with additional minor peaks (Supplementary Fig. 2a). These additional peaks suggest that un-tethered pA-Tn5 contributes to coverage in these experiments. To determine if true CTCF binding sites could be distinguished from accessible features by read depth, we compared the CUT&Tag read count at high-confidence CTCF sites (defined by peak-calling on CUT&RUN data) to the CUT&Tag read count at accessible sites (defined by peak-calling on ATAC-seq data). We found that these two distributions of read counts overlap, but that of accessible sites is lower than that of CTCF sites. Based solely on read depth, we discriminate ~5600 CTCF bound sites with a 1% false discovery rate. Comparing motif enrichment in these two classes demonstrates that the high signals correspond to CTCF motifs (E-value = 2.1 × 10^{-69}), and the low signals do not.

We assessed the resolution of the CUT&Tag procedure by plotting the ends of reads centered on CTCF binding sites. This shows that CUT&Tag protects a “footprint” spanning 80 bp directly over the CTCF motif (Fig. 5c). While the segment protected from Tn5 integration is larger than the ~45 bp deduced neighboring nucleosomes (Fig. 5d).

CUT&Tag profiles low cell number samples and single cells. ChiP requires substantial cellular material, limiting its application for experimental and clinical samples. However, we and others have previously demonstrated that tethered profiling strategies

Fig. 3 Reproducibility and efficiency of CUT&Tag. a Hierarchically clustered correlation matrix of CUT&Tag replicates (R1 and R2) and with CUT&RUN and ChiP-seq profiling for the H3K4me1 histone modification. The same antibody was used in all experiments. Pearson correlations were calculated using the log2-transformed values of read counts split into 500 bp bins across the genome. b Efficiency of peak-calling between methods. Mitochondrial reads were removed from datasets from each method profiling the H3K4me2 histone modification. The remaining read counts were downsampled to varying depths, and then used to call peaks using MACS2. The summed number of reads falling within called peaks in each dataset was plotted. Source data are available in the Source Data file.

~9000 sites that includes histone gene promoters and 10% of ATAC-defined accessible sites (Supplementary Fig. 4). While this is only a fraction of the ~54,000 accessible sites defined in K562 cells, adjusting the threshold and stringency of NPAT peak calling may improve detection.

To test if CUT&Tag is tractable for profiling more abundant transcription factor binding sites, we profiled the CCCTC-binding factor (CTCF) DNA-binding protein. For these experiments, we varied the stringency of wash buffers to assess displacement of transcription factors from chromatin. Under low-salt and medium-salt concentration conditions we observed read counts at CTCF sites detected by CUT&RUN and by ChiP-seq (Fig. 2a), but with additional minor peaks (Supplementary Fig. 2a). These additional peaks suggest that un-tethered pA-Tn5 contributes to coverage in these experiments. To determine if true CTCF binding sites could be distinguished from accessible features by read depth, we compared the CUT&Tag read count at high-confidence CTCF sites (defined by peak-calling on CUT&RUN data) to the CUT&Tag read count at accessible sites (defined by peak-calling on ATAC-seq data). We found that these two distributions of read counts overlap, but that of accessible sites is lower than that of CTCF sites. Based solely on read depth, we discriminate ~5600 CTCF bound sites with a 1% false discovery rate. Comparing motif enrichment in these two classes demonstrates that the high signals correspond to CTCF motifs (E-value = 2.1 × 10^{-69}), and the low signals do not.

We assessed the resolution of the CUT&Tag procedure by plotting the ends of reads centered on CTCF binding sites. This shows that CUT&Tag protects a “footprint” spanning 80 bp directly over the CTCF motif (Fig. 5c). While the segment protected from Tn5 integration is larger than the ~45 bp protected from MNase in CUT&RUN, this indicates that the tethered transposase produces high resolution maps of factor binding sites. Similar footprints were obtained using different salt concentration washes, although 300–500 mM salt concentrations resulted in somewhat reduced signal-to-noise (Fig. 5c). The high resolution of CUT&Tag provides structural details of individual sites. For example, superposition of CTCF, H3K4me1, H3K4me2, H3K4me3, and ATAC mapping at a representative site reveals the relationship between accessible DNA, CTCF binding, and modified neighboring nucleosomes (Fig. 5d).
like CUT&RUN have sufficient sensitivity that profiling small cell numbers routinely becomes feasible\cite{32,29}. Signal improvements in CUT&Tag suggest that this method may work even more efficiently with limited samples. We first tested CUT&Tag for the H3K27me3 modification across a ~1500× range of material, from 100,000 down to 60 cells. We observed very similar high-quality chromatin profiles from all experiments (Supplementary Fig. 1b), demonstrating that high data quality is still maintained with low input material. Analyzing sample and tracer DNA in these CUT&Tag series revealed that sequencing yield is proportional to the number of cells (Supplementary Fig. 1a).

CUT&Tag has the advantage that the entire reaction from antibody binding to adapter integration occurs within intact cells. The transposase and chromatin fragments remain bound together\cite{15,26}, and thus fragmented DNA is retained within each nucleus. We developed a simple strategy to generate chromatin profiles of individual cells, which we term single-cell CUT&Tag (scCUT&Tag) (Fig. 6a). We performed scCUT&Tag to the H3K27me3 modification on a bulk population of K562 cells, but with gentle centrifugation between steps instead of Concanavalin A magnetic beads. After integration, we used a Takara ICELL8 nano-dispensing system to aliquot single cells into nanowells of a 5184 well chip, identifying the nanowells that contained one and only one cell by imaging the chip. We then performed PCR enrichment of libraries in each passing nanowell using two indexed primers, and finally pooled all enriched libraries from the chip for Illumina deep sequencing to high redundancy to assess the sampling and coverage in each cell (Supplementary Fig. 6a). Libraries from each well are distinguished by unique combinations of the two indices.

The aggregate of single cell chromatin profiling closely matched profiles generated in bulk samples (Fig. 6b), with high correlations (Pearson’s r = 0.89, Supplementary Fig. 6b). Individual cells were ranked by the genome-wide number of reads, and the unique fragments are displayed in tracks for each cell. Strikingly, the majority of reads from individual cells fall within H3K27me3 blocks defined in bulk profiling, indicating high recovery in single cell chromatin profiling (Fig. 6b). A second replicate of H3K27me3 scCUT&Tag demonstrated the reproducibility of single cell profiling. Similarly, single cell profiling of the H3K4me2 modification recapitulates genomic landscapes of accessible and active chromatin (Fig. 6c). A significant fraction of reads in single cells fall within defined active and silenced chromatin features (Fig. 6d, e).

The breadth of chromatin features—from ~5 nucleosomes for H3K4me2 to hundreds in H3K27me3 domains—assists the detection of chromatin features even with sparse sampling from individual cells. To assess if chromatin features in individual cells could be used to distinguish cell types, we performed scCUT&Tag to the H3K27me3 modification in H1 cells. Again, we found that a high fraction of reads fell within domains defined by bulk profiling (Fig. 6e), with high correlations between bulk and aggregated single cell data (Pearson’s r = 0.85, Supplementary Fig. 6b). Comparing a 2 Mb region encompassing the HoxB domain reveals clear histone methylation in single cell tracks specifically in H1 cells, while this region is depleted in K562 cells (Fig. 6f). These genome-wide patterns are sufficient to discriminate single H1 cells from K562 cells with high efficiency (Supplementary Fig. 6c, d). The small fraction of K562 cells that are mis-called have the sparsest read coverage. Thus, chromatin profiling provides a method to discriminate single cell types.

### Discussion

Chromatin profiling by CUT&Tag efficiently reveals regulatory information in genomes. In contrast to RNA-seq\cite{27}, which only
measures expressed genes, chromatin profiling has the unique advantage of identifying silenced regions, which is a key aspect of establishing cell fates in development. Although methods like ATAC-seq map accessible and factor-bound sites, the specific chromatin proteins bound at these sites must be inferred from motif or chromatin profiling data. While ChIP-based methods have been extensively used in model cell line systems, the vagaries of crosslinking and fragmenting chromatin have limited chromatin profiling by ChIP-seq to an artisan technique where each experiment requires optimization. Likewise, a recently described alternative cross-linked chromatin profiling method, ChIL-seq, requires many more steps than CUT&Tag and requires 3–4 days to perform all of the steps. In contrast, the CUT&Tag procedure, like CUT&RUN, is an unfixed in situ method, and is easily implemented in a standardized approach. This, combined with the cost-effectiveness of CUT&Tag, makes it appropriate for a high-throughput pipeline that can be implemented in a core facility. It is conceivable that diverse users may provide their mixture of cells and antibody and receive processed deep sequencing files in just days. Since the first step in high-throughput CUT&Tag is antibody incubation at 4°C, samples can be accumulated overnight in a facility and then loaded together onto a 96-well plate for robotic handling, as we previously demonstrated for AutoCUT&RUN. With efficient use of reagents and better signal-to-noise, CUT&Tag requires even fewer reads per sample than AutoCUT&RUN, which is already much cheaper than commercial exome sequencing. While the ease and low cost of this pipeline is appealing, the primary virtue of automated chromatin profiling is the minimization of batch and handling effects, and thus maximum reproducibility. Such aspects are critical for clinical assays and testing for chromatin-targeting drugs.

We have shown that CUT&Tag provides high-quality single-cell profiles using the ICELL8 nano-dispensation system, which allows for imaging prior to reagent addition and PCR. Likewise, CUT&Tag should be suitable for the 10x Genomics encapsulation system by adaptation of their recently announced ATAC-seq single-cell protocol. Adaptability to high-throughput single-cell platforms is possible for CUT&Tag because adapters are added in bulk, whereas previous single-cell adaptations of antibody-based profiling methods, including ChIP-seq, ChIL-seq, and CUT&RUN require reactions to be performed after cells are

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**Fig. 6** Chromatin profiling of individual cells. **a** Single cell CUT&Tag (scCUT&Tag) processing. All steps from antibody incubations through adapter tagmentation are done on a population of permeabilized unfixed cells. Individual cells are then dispersed into nanowells of a Takara ICELL8 chip. After verifying nanowells with single cells by microscopy, combinations of two indexed barcoded primers are added to each well and fragment libraries are enriched by PCR. Libraries from the chip are pooled for multiplex sequencing. **b** A chromatin landscape across a 500 kb segment of the human genome is shown for H3K27me3 CUT&Tag on K562 cells. Tracks from bulk CUT&Tag, aggregated scCUT&Tag, and for 956 single cells are shown. Single cells are ordered by total read counts in each cell. **c** A chromatin landscape across a 500 kb segment of the human genome for H3K4me2 CUT&Tag on K562 cells. Tracks from bulk CUT&Tag, aggregated scCUT&Tag, and for 808 single cells are shown. Single cells are ordered by total read counts in each cell. **d** Fraction of reads in single K562 cells falling within called active peaks for the H3K4me2 histone modification using stringent criteria. Narrow peaks were called using MACS2 on bulk profiling data, and reads from scCUT&Tag were assigned to those peaks. **e** Fraction of reads in single K562 and H1 cells falling within called silenced domains for the H3K27me3 histone modification. Domains were called using SEACR on bulk profiling data for each cell type, and reads from scCUT&Tag were assigned to those domains. **f** Comparison of chromatin landscapes in H1 and K562 single cells across a 2 Mb segment including the HoxB locus. Four hundred and seventy-nine single cells of each type were ordered by total read counts. Source data are available in the Source Data file.
separated. The distinct distributions of low-level untargeted accessible DNA sites and high-level CTCF-bound sites in CUT&Tag datasets suggests that by modeling the two expected underlying distributions, true binding sites can be distinguished from accessible DNA sites without using other data. An advantage of this strategy is that the statistical distinction between true binding sites and accessible features allows characterization of two chromatin features in the same experiment, where accessible DNA sites can be annotated as well as binding sites for the targeted factor. This parsing out of the low-level ATAC-seq background from the strong targeted CUT&Tag signal makes possible de novo “multiOMIC” CUT&Tag38. In the future, we expect that barcoding of adapters26 will allow for multiple epitopes to be simultaneously profiled in single cells in large numbers, maximizing the utility of single-cell epigenomic profiling for studies of development and disease.

Methods

Biological materials. Human K562 cells were purchased from ATCC (Manassas, VA, Catalog #CCL-243) and cultured following the supplier’s protocol. H1 ES cells were obtained from VCGC (CRL-1651, lot W535186). We used the following antibodies: Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) antibody (Antibodies-Online ABIN101961). H3K27me3 (Cell Signaling Technology, 9733, Lot 14), H3K27ac (Millipore, MABE647), H3K1me1 (Abcam, ab8895), H3K1me2 (Upstate 07-630, Lot 263353), H3K4me3 (Active Motif, 39195), Pol2ser2P, Polser5P, Polser5A (Millipore, No. 97,009). The Sigma-GenEP Kit, 54020), CTCF (Millipore 07-79959). The 3XFlag-pA-Tn5-Fl plasmid (Addgene plasmid # 124601) was transfected into C3013 cells (NEB) following the manufacturer’s protocol. Each colony tested was inoculated into 3 mL LB medium and growth was continued at 37 °C for 4 h. That culture was used to start a 400 mL culture in 100 µg/mL carbenicillin containing LB medium (as it is more stable than ampicillin) and incubated on a shaker until it reached O.D. ~0.6, whereupon it was chilled on ice for 30 min. Fresh IPTG was added to induce expression and the culture was incubated at 18 °C on a shaker overnight. The culture was collected by centrifugation at 10,000 rpm, 4 °C for 30 min. The bacterial pellet was frozen in a dry ice-ethanol bath and stored at −70 °C. Protein purification was performed as previously described with minor modifications. Briefly, a frozen pellet was resuspended in 40 mL chilled HEGX (5 mM HEPES-KOH at pH 7.2, 0.2 M NaCl, 1 mM Na2EDTA, 0.5 mM EDTA, 2 mM MgCl2, 0.5 mM DTT, 0.05% Spermidine, 0.2% Triton X-100) including 1× Roche Complete EDTA-free protease inhibitor tablets. The lysate was sonicated 10 times for 45 s at a 50% duty cycle with output level 7 while keeping the sample chilled and holding on ice between cycles. The sonicated lysate was centrifuged at 10,000 rpm in a Fibrelite rotor at 4 °C for 30 min. The 1 mL aliquot of chitin slurry resin (NEB, S66515) was packed into each of two disposable columns (Bio-rad 7321010). Columns were washed with 20 mL of HEGX Buffer. The soluble fraction was added to the chitin resin slowly, then incubated on a rotator at 4 °C overnight. The unbound soluble fraction was drained and the columns were rinsed with 20 mL HEGX and washed thoroughly with 20 mL of 500 µM HEPES buffer to induce expression of the expressed complex in E. coli. The chitin slurry was transferred to a 15 mL conical tube and resuspended in elution buffer (10 mL HEGX with 100 mM DTT). The tube was placed on rotator at 4 °C for ~48 h. The elute was collected and dialyzed twice in 800 mL 2X Tn5 Dialysis Buffer (100 mM HEPES-KOH pH 7.2, 0.2 M NaCl, 0.2 mM EDTA, 2 mM DTT, 0.2% Triton X-100, 20% Glycerol). The dialyzed protein solution was concentrated using an Amicon Ultra-4 Centrifugal Filter Units 30 K (Millipore UFC803024), and sterile glycerol was added to make a final 50% glycerol stock of the purified protein.

To generate the pA-Tn5 adapter transposome, 16 µL of a 100 µM equimolar mixture of pA-MNase and pA-MNase-B oligonucleotides were mixed with 100 µL of 5.5 µM pA-Tn5 fusion protein. The mixture was incubated on a rotating platform for 1 h at room temperature and then stored at −20 °C. The complex is stable at room temperature, with no detectable loss of potency after 10 days on the benchtop (Supplemental Fig. 5A), and without noticeable loss of data quality (Supplemental Fig. 5B). Unexpectedly, this extended room temperature incubation resulted in a ~2–10-fold decrease in the number of tagged E. coli fragments (Supplemental Figure 5C), which can be used as a calibration standard within a series using a constant amount of pA-Tn5. This observation suggests that the E. coli DNA that co-purifies with pA-Tn5 is subject to tagmentation both during room temperature incubation and during tagmentation itself. This was seen with pre-incubated complexes and subsequent trapping of tagged pA-Tn5-bound DNA within the cell. In support of this interpretation, we note that E. coli carry-over DNA suitable for calibration is also released by pA-MNase in CUT&RUN reactions during digestion32. A likely explanation for the trapping of these different fusion protein-bound DNAs within cells is that the protein–DNA interaction domains are specific for IgG bind non-specifically to cellular proteins, whereupon with elevation of dilution cation results in MNase digestion and release (pA-MNase) or tagmentation (pA-Tn5).

CUT&Tag for bench-top application. Cells were harvested, counted and centrifuged for 3 min at 8000 × g at room temperature. Aliquots of cells (60–500,000 cells) were washed twice in 1.5 mL Wash Buffer (20 mM HEPES pH 7.5; 150 mM NaCl 0.5 mM Spermadine; 1× Protease inhibitor cocktail) by gentle pipetting. Concanavalin A coated magnetic beads (Bangs Laboratories) were prepared as described6 and 10 µL of activated beads were added per sample and incubated at RT for 15 min. We observed that binding cells to beads at this step increases binding and recovery. The unbound supernatants were discarded. The bead-bound cells were resuspended in 50–100 µL Dig-wash Buffer (20 mM HEPES pH 7.5; 150 mM NaCl 0.5 mM Spermadine; 1× Protease inhibitor cocktail; 0.05% Digitonin) containing 2 mM EDTA and a 1:50 dilution of the appropriate primary antibody. Primary antibody incubation was performed on a rotating platform for 2 h at room temperature (RT) or overnight at 4 °C. The primary antibody was removed by placing the tube on the magnet stand to cold and pulling off all of the liquid. To increase the number of Protein A binding sites for each bound antibody, an appropriate secondary antibody (such as Guinea Pig anti-Rabbit IgG antibody for a rabbit primary antibody) was diluted 1:50 in 50–100 µL of Dig-wash buffer and cells were washed at RT and 4 °C. The cells were washed with RT Dig-wash buffer 2×-3× for 5 min in 0.2–1 mL Dig-Wash buffer to remove unbound antibodies. A 1:120 dilution of pA-Tn5 adapter complex (~0.04 µM) was prepared in Dig-med Buffer (1× Digitonin, 20 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM Spermadine, 1× Protease inhibitor cocktail). After removing the liquid on the magnet as described, the beads were resuspended in 100 µL of HEPES Buffer and 100 µL of 0.5 µM Tris pH 8 was added, the vortexed, quickly spun and held at 5 min. Tubes were placed on a magnet stand to cold, then the liquid was carefully withdrawn. Without disturbing the beads, beads were washed twice in 1 mL, 80% ethanol. After allowing to dry ~5 min, 30–40 µL of 10 mM Tris pH 8 was added, the vortexed, quickly spun and allowed to sit for 5 min. Tubes were placed on a magnet stand and the liquid was withdrawn to a fresh tube. To amplify library, 21 µL DNA was mixed with 2 µL of a universal i5 and an i7 barcode, digested with different enzymes and amplified using 1× volume of 25 µL NEBNext HiFi 2× PCR Master mix was added and mixed. The sample was placed in a Thermocycler with a heated lid using the following cycling conditions: 72 °C for 5 min (gap filling); 98 °C for 30 s × 14 cycles of 98 °C for 10 s and 63 °C for 30 s; final extension at 72 °C for 1 min and hold at 8 °C. Post-PCR purification was performed by adding 1× volume of Inactivate Protease K (Beckman Counter), and libraries were incubated with beads for 15 min at RT, washed twice gently in 80% ethanol, and eluted in 30 µL 10 mM Tris pH 8.0. A detailed, step-by-step protocol can be found at https://www.protocols.io/view/bench-top-cut-amp-tag-wnudlwe/abstract.

High-throughput CUT&Tag. For high-throughput 96-well microplate application, cells were first permeabilized and incubated with the primary antibodies before continuing with CUT&Tag barcoding. Two different protocols were used. The first protocol was used for 20 µL of human embryonic stem cells. A volume of 25 µL NEBNExt HiFi 2× PCR Master mix was added and mixed. The sample was placed in a Thermocycler with a heated lid using the following cycling conditions: 72 °C for 5 min (gap filling); 98 °C for 30 s × 14 cycles of 98 °C for 10 s and 63 °C for 30 s; final extension at 72 °C for 1 min and hold at 8 °C. Post-PCR purification was performed by adding 1× volume of Inactivate Protease K (Beckman Counter), and libraries were incubated with beads for 15 min at RT, washed twice gently in 80% ethanol, and eluted in 30 µL 10 mM Tris pH 8.0. A detailed, step-by-step protocol can be found at https://www.protocols.io/...
DNA sequencing and data processing. The size distribution of libraries was determined by Agilent 4200 TapeStation analysis, and libraries were mixed to achieve equal representation as desired aiming for a final concentration as recommended by the manufacturer. Paired-end Illumina sequencing was performed on the barcoded libraries following the manufacturer’s instructions. Paired-end reads were aligned using Bowtie2 version 2.2.5 with options: --local--very-sensitive-local-no-unal-no-mixed-no-discordant-presh33 -110 -X 700. Because of the very low background with CUT&Tag, typically 3 million paired-end reads suffice for nucleosome modifications, even for the human genome. For maximum economy, up to 96 barcoded samples per 2-lane flow cell can be pooled for 25 x 25 bp sequencing. For peak calling, parameters used were macs2 callpeak—input (file —p 1 e-5 —f BEDPE/BEDPA(Paired End vs. Single End sequencing data) —keep-dup all —n out_name.

Single-cell CUT&Tag. Approximately 50,000 exponentially growing K562 cells were processed by centrifugation between buffer and reagent exchanges in low-retention tubes throughout. Centrifugations were performed at 600xg for 3 min in a swinging bucket rotor for the initial wash and incubation steps, and then at 300xg for 3 min after pA-Tn5 binding. Cells were collected and washed twice with 1 mL Wash Buffer (20 mM HEPES, pH 7.5; 150 mM NaCl; 0.5 mM Spermidine, 1x Protease inhibitor cocktail) at room temperature. nuclei were isolated by permeabilizing cells in NP40-Digitonin Wash Buffer (0.01% NP40, 0.01% Digitonin in wash buffer) and resuspended in 1 mL of NP40-Digitonin Wash buffer with 2 mM EDTA. Antibody was added at a 1:50 dilution and incubated on a rotor at 4°C overnight. Permeabilized cells were then rinsed once with NP40-Digitonin Wash buffer and incubated with anti-Rabbit IgG antibody (1:50 dilution) in 1 mL of NP40-Digitonin Wash buffer on a rotator at room temperature for 30 min. Nuclei were then washed 3x for 5 min in 1 mL NP40-Digitonin Wash buffer to remove unbound antibodies. For pA-Tn5 binding, a 1:100 dilution of pA-Tn5 adapter complex was prepared in 1 mL NP40-Dig-med-buffer (0.01% NP40; 0.01% Digitonin, 20 mM HEPES, pH 7.5, 0.30 mM NaCl, 0.5 mM Spermidine, 1x Protease inhibitor cocktail), and permeabilized cells were incubated with the pA-Tn5 adapter complex on a rotator at RT for 1 h. Cells were washed 3x for 5 min in 1 mL NP40-Dig-med-buffer to remove excess pA-Tn5 protein. Cells were ligated in 150 µL NP40-Dig-med-buffer and incubated at 37°C for 1 h. Tagmentation was stopped by adding 50 µL of 4x Stop Buffer (40 mM EDTA and 2 mg/mL DAPI) and the sample was held on ice for 30 min. The SMARTer ICL8 single-cell system (Takara Bio USA, Cat. #640009) was used to perform single cell protocols described for scATAC-seq12. DAPI-stained nuclei were visualized under the microscope and if there were clumps, they were strained through 10 micron cell strainers. Cells were counted using a hemacytometer and diluted to 28 cells/µL in 0.5× PBS and 1× Second Diluent (Takara Bio USA, Cat. # 640196). Cells were dispensed into a SMARTer ICELL8 350 v chip (Takara Bio USA, Cat. #640019) at 7.32 µM using ICELL8 program for the default software settings. Additional single cells were manually selected for imaging using the imaging filter, which notes single-cell containing wells and control wells, was generated. Since cells were stained only with DAPI, they were unrelated proteins. Cells were resuspended in 150 µL Tagmentation buffer (10 mM MgCl2 in 10 micron cell strainers. Cells were counted using a hematocytometer and diluted at 10067 (2013).

References

1. Zentner, G. E. & Henikoff, S. High-resolution digital profiling of the epigenome. Nat. Rev. Genet. 15, 814—827 (2014).
2. Pollicastro, R. A. & Zentner, G. E. Enzymatic methods for genome-wide ChIP sequencing. Nat. Protoc. 9, 138—145 (2014).
3. Rhee, H. S. & Pugh, B. F. Comprehensive genome-wide protein-DNA interactions detected at single-nucleotide resolution. Cell 147, 1408—1419 (2011).
4. Skene, P. J. & Henikoff, S. A simple method for generating high-resolution maps of genome-wide protein binding. elife 4, e09225 (2015).
5. Kasinathan, S., Orsi, G. A., Zentner, G. E., Ahmad, K. & Henikoff, S. High-resolution mapping of transcription factor binding sites on native chromatin. Nat. Methods 11, 203—209 (2014).
6. Teytelman, L., Thurtle, D. M., Rine, J. & van Oudenaarden, A. Highly expressed loci are vulnerable to misreading ChIP localization of multiple unrelated proteins. Proc. Natl. Acad. Sci. USA 110, 18602—18607 (2013).
7. van Steenbergen, B., Delrow, J. & Henikoff, S. Chromatin profiling using targeted DNA adenine methyltransferase. Nat. Genet. 27, 304—308 (2001).
8. Zentner, G. E., Kasinathan, S., Xin, B., Rohs, R. & Henikoff, S. ChEC-seq kinetics discriminates transcription factor binding sites by DNA sequence and shape in vivo. Nat. Commun. 6, 8733 (2015).
9. Skene, P. J. & Henikoff, S. An efficient targeted nucleosome strategy for high-resolution mapping of DNA binding sites. Elife 6, e21856 (2017).
10. Skene, P. J., Henikoff, J. G. & Henikoff, S. Targeted in situ genome-wide profiling with high efficiency for low cell numbers. Nat. Protoc. 13, 1006—1019 (2018).
11. Schmid, M., Durussel, T. & Laemmli, U. K. ChIC and ChEC: genomic mapping of chromatin proteins. Mol. Cell 16, 147—157 (2004).
12. Meager, A. et al. High-throughput chromatin accessibility profiling at single-cell resolution. Nat. Commun. 9, 3647 (2018).
13. Zheng, X. G. et al. Massively parallel digital transcriptional profiling of single cells. Nat. Commun. 8, 14049 (2017).
14. Reznikov, W. S. Tn5 as a model for understanding DNA transposition. Mol. Microbiol. 47, 1199—1206 (2003).
15. Picelli, S. et al. Tn5 transposes and tabulation procedures for massively scaled sequencing projects. Genome Res. 24, 2033—2040 (2014).
16. Steiniger, M., Adams, C. D., Marko, J. F. & Reznikoff, W. S. De novo targeting of protein binding sites. Cell 145, 18607 (2013).
17. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods 10, 1213(2013).
18. Janssens, D. H. et al. Automated in situ profiling of chromatin modifications resolves cell types and gene regulatory programs. Epigenetics Chromatin 11, 74 (2018).
19. Landt, S. G. et al. CHIP-seq guidelines and practices of the ENCODE and modENCODE consortia. Genome Res. 22, 1813—1831 (2012).
20. Zaborowska, J., Egloff, S. & Murphy, S. The pol II CTD: new twists in the tail. Nat. Rev. Genet. 13, 771—777 (2012).
21. Consortium, E. P. An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57—74 (2012).
22. Core, L. J. et al. Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. Nat. Genet. 46, 1311—1320 (2014).
23. Liu, X. et al. In situ capture of chromatin interactions by biotinylated dCas9. Cell 170, 1028—1043 e1019 (2017).
24. Mattzolf, W. F., Gongidi, P., Woods, K. R., Jin, J. P. & Maltais, L. J. The human and mouse replication-dependent histone genes. Genomics 80, 487—498 (2002).
25. Hainer, S. J., Boskovic, A., Rando, O. J. & Fazzio, T. G. Profiling of pluripotency factors in individual stem cells and early embryos. bioRxiv p.286351 (2018).
26. Amini, S. et al. Haplotype-resolved whole-genome sequencing by contiguity-preserving transposition and combinatorial indexing. Nat. Genet. 46, 1343–1349 (2014).

27. Svensson, V., Vento-Tormo, R. & Teichmann, S. A. Exponential scaling of single-cell RNA-seq in the past decade. Nat. Protoc. 13, 599–604 (2018).

28. Harada, A. et al. A chromatin integration labelling method enables epigenomic profiling with lower input. Nat. Cell Biol. 21, 287–296 (2018).

29. 10xgenomics. https://www.10xgenomics.com/solutions/single-cell-atac.

30. Rotem, A. et al. Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state. Nat. Biotechnol. 33, 1165–1172 (2015).

31. Packer, J. & Trapnell, C. Single-cell multi-omic: an engine for new quantitative models of gene regulation. Trends Genet. 34, 653–665 (2018).

32. Meers, M. P., Bryson, T. D. & Steven Henikoff, S. A streamlined protocol and analysis pipeline for CUT&RUN chromatin profiling. BioRxiv 569129, 1–32 (2019).

33. Buenrostro, J. D. et al. Single-cell chromatin accessibility reveals principles of regulatory variation. Nature 523, 486–U264 (2015).

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Author contributions
H.S.K. and S.H. designed and performed all experiments. C.A.C and E.S.P. and T.D.B. assisted with the experiments. H.S.K., S.J.W., J.G.H., K.A., and S.H. developed algorithms and analyzed the data. H.S.K, K.A., and S.H. wrote the manuscript.

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