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Microfluidic low-input fluidized-bed enabled ChIP-seq device for automated and parallel analysis of histone modifications

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Genome-wide epigenetic changes, such as histone modifications, form a critical layer of gene regulations and have been implicated in a number of different disorders such as cancer and inflammation. Progress has been made to decrease the input required by gold-standard genome-wide profiling tools like chromatin immunoprecipitation followed by sequencing (i.e. ChIP-seq) to allow scarce primary tissues of a specific type from patients and lab animals to be tested. However, there has been practically no effort to rapidly increase the throughput of these low-input tools. In this report, we demonstrate LIFE-ChIP-seq (Low-Input Fluidized-bed Enabled Chromatin Immunoprecipitation followed by sequencing), an automated and high-throughput microfluidic platform capable of running multiple sets of ChIP assays on multiple histone marks in as little as 1 h with as few as 50 cells per assay. Our technology will enable testing of a large number of samples and replicates with low-abundance primary samples in the context of precision medicine.

Cellular processes are regulated by a complex interplay among different layers of epigenetic information, including DNA methylation, histone modifications, nucleosome positions, and expression of noncoding RNA¹. Among these mechanisms, modifications of histones (i.e. the proteins that package the genetic material) are found in non-random patterns in the genome to form a “histone code” that specifies the states of gene expression^{2,3}. The histone code at promoters contributes to the fine tuning of expression levels, from active to poised and to inactive. At gene bodies, they distinguish between active and inactive conformations. At distal sites, histone marks correlate with levels of enhancer activity. Aberrant patterns of histone marks have been associated with cancer in a number of ways. First, previous studies reported aberrant histone marks leading to silencing of tumor-suppressor genes in a variety of human cancers⁴. Second, deregulation of histone modifications may trigger genetic changes via aberrant DNA repair/replication and gene transcription⁵. Third, large organized chromatin lysine modifications (LOCKs) defined by genomic domains enriched for

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Accession codes

Gene Expression Omnibus: LIFE-ChIP-seq data are deposited under accession number GSE 102932.

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102932>

Supporting information

Supplementary methods, supplementary figures S1–S4, table S1, file S1 (zipped LabVIEW files for microfluidic control) and movies S1–S2.

heterochromatin post-translational modifications such as H3K9me2 have been recently highlighted for their roles in cancer progression ⁶. LOCKs expand during differentiation and are lost in cancer. H3K9me2 experiences dramatic loss during epithelial–mesenchymal transition (EMT) and this is accompanied by an increase of H3K4me3 and H3K36me3. Finally, histone marks have also been proposed as potent biomarkers that predict cancer progression and reoccurrence ⁷.

The primary tool utilized for examining histone modifications is chromatin immunoprecipitation (ChIP), which applies immunoassay to capture chromatin fragments by targeting the specific type of modified histone bound to them ⁸. The purified DNA, extracted from these chromatin fragments, can be sequenced (i.e. ChIP-seq) to provide a genome-wide map of histone binding.

There are a couple of critical limitations associated with conventional ChIP-seq assays. First, they typically require a large number of cells ($\sim 10^7$ – 10^8 cells), as conventional methods are unable to efficiently adsorb chromatin of interest and remove non-specifically bound chromatin. In contrast, the sample amount generated by lab animals and patients is very limited. For example, a core needle biopsy generates a total of 10^4 – 10^5 cells. Circulating tumor cells are present at a frequency of 1–10 per ml of whole blood in patients with metastatic cancer. This sensitivity issue has hindered clinical research using patient materials and made patient stratification based on epigenomics impractical. Second, these assays typically require manual procedures for a duration of 3–4 d and are not suitable for high-throughput data production. Rapid characterization of a large number of samples is vital for use of epigenomic knowledge in clinical research and patient stratification, because 1) epigenomic profiles vary among individual subjects, cell/tissue types and disease/developmental stages; 2) there are also tens to hundreds of histone marks of interests ^{9,10}.

Microfluidics has been shown to provide a powerful platform for conducting low-input genomic ^{11–13}, transcriptomic ^{14–16}, proteomic ^{17–19}, and epigenomic analysis ^{20–26}. There have been a few different attempts to utilize microfluidics to improve the ChIP process ^{20,23,27–30}. Wu et al. developed an automated microfluidic device capable of performing ChIP-qPCR analysis using only 2000 cells ²⁷, and was able to scale their platform to 16 simultaneous reactions ²⁹. In our work, we further reduced microfluidic ChIP-qPCR sensitivity to 50 cells ²⁸. Recently, combining a packed bed with a shear stress inducing oscillating washing step, we demonstrated generating high-quality ChIP-seq data using as few as 100 cells ²⁰. Rotem et al., using a droplet microfluidic platform, was able to barcode chromatin from single cells and then perform ChIP-seq (Drop-ChIP) ²³. While useful for understanding single cell heterogeneity, Drop-ChIP yielded a low number of reads per single cell (1000 unique reads per cell) and required a large pooling of single cells (on the order of 10^3 cells) to generate datasets of sufficient quality compared to reference epigenomes. Other state-of-the-art ChIP technologies (iChIP, FARP-ChIP, LinDA, nano-ChIP) all work at 500–10,000 cells per assay range ^{31–34}. While high throughput analysis does exist in the form of AHT-ChIP ³⁵, the analysis requires 100 million cells per assay, more than traditional ChIP-seq. So far, none of the existing low-input ChIP-seq technologies allow high-throughput and simultaneous processing of multiple samples.

In this work, we demonstrate Low-Input Fluidized-bed Enabled ChIP (LIFE-ChIP), a microfluidic platform for running multiple parallel ChIP assays simultaneously by utilizing microfluidic fluidized beds. Our device permitted running 4 ChIP-seq assays on one or two histone marks in one run with as few as 50 cells per assay. We examined the histone modifications H3K4me3 and H3K27ac, which are activating and active enhancer marks, respectively. These marks were chosen due to their importance and availability of published data for comparison. We showed that our data had high reproducibility among various individual chambers and devices. Our LIFE-ChIP assay could be finished in as little as 1 h, compared to overnight ChIP process in conventional protocols. This technology paves the way to high-throughput epigenomic profiling required by precision medicine.

A ChIP-seq assay for examining histone modifications typically involves several steps^{20,28}: 1) cross-linking to fix histones to DNA sequences that they interact with; 2) sonication or enzymatic digestion to generate chromatin fragments; 3) ChIP, to adsorb chromatin fragments containing the histone of interest on immunomagnetic beads that are functionalized with an antibody targeting a specifically modified histone; 4) release of the DNA fragments (i.e. ChIP DNA) from bead surface; 5) sequencing of the DNA fragments and establishing genome-wide profile for the histone modification.

In our previous works^{20,36}, we designed a packed bed of immunomagnetic beads for highly efficient collection of ChIP DNA that enabled ChIP-seq using as few as 100 cells (MOWChIP-seq). In spite of the high adsorption efficiency at the theoretical limit, a packed bed of beads creates substantial pressure buildup in the microfluidic structure (in excess of 30 psi), and this problem would be further confounded when running multiple units in parallel is attempted. High pressure tends to break micromechanical valves involved in the device. In this study, we chose a fluidized bed design for the device, which allows for parallel reaction chambers and common inlets with low pressure buildup (<15 psi). A fluidized bed generally refers to a suspension of solid particles in fluid contained in a reactor³⁷. Our LIFE-ChIP-seq platform consisted of 7 inlet ports (all individually controlled by micromechanical valves) connected to 4 bell-shaped reaction chambers through a series of splits (Fig. 1a–b). The operation of the microfluidic system was automated by having syringe pumps and solenoid valves controlled by a custom LabVIEW program (Fig. S1). The shape created a velocity gradient that was important for generating shear force to remove surface adsorbent during washing, whilst the large volume and low velocity at the bottom of the bell shape helped retain beads.

Methods

Device fabrication

The microfluidic system consisted of 4 reaction chambers connected to 7 inlet channels (I1–I7) with successive splits. There were two-layered micromechanical valves³⁸ to close/open each and every inlet and split. The bell-shaped reaction chambers had a major axis of 9 mm and a minor axis of 3.5 mm and included microscale pillars (with a diameter of 140 μm) to prevent collapse. The reaction chambers had a depth of 50 μm , while the parts that formed fully closed valves had a depth of 25 μm .

The chip was manufactured using two-layer soft lithography³⁸ in polydimethylsiloxane (PDMS, RTV615, Momentive Advanced Materials) Photomasks were prepared using LayoutEditor and printed on Mylar transparencies at high resolution (10,160 DPI) by FineLine Imaging. The photomasks were used to fabricate 2 separate master molds using photolithography on silicon wafers (3 inch, university wafer). The control layer and a part of the fluidic layer (some channels and reaction chambers) were fabricated using SU-8 2025 (MicroChem) to create rectangular channels (depth ~50 μm). The fully closed valves of the fluidic layer were fabricated by spinning 2 layers of AZ 9260 (EMD Performance Materials) each at 1500 RPM for 30 s, with a 10 min wait between the spins to allow evaporation of solvent (depth ~ 25 μm). The fluidic layer master was then heated to 130 °C for 60 s to round the AZ channels.

Prepolymer PDMS was mixed in a ratio of 5:1 prepolymer to crosslinking agent, degassed, and cast as a bulk layer (~5 mm thick) on the fluidic layer mold, while a degassed 20:1 ratio of PDMS was spun onto the control layer mold at 1750 RPM for 30 s. After partial curing at 75 °C for 12 min, the fluidic layer casting was removed from the silicon master and inlet and outlet ports were punched using a 1.5 mm core punch (Harris Uni-Core). The fluidic layer was laid on top of the control layer (that was still attached to its master), aligned and cured at 75 °C for 1 h. The multilayer device was then removed from the mold and punched, using a 1.5 mm core punch. The PDMS device and a glass slide (50 mm $\times$ 75 mm, Corning), precleaned with dish soap, were treated by plasma (Harrick Plasma) then bonded together irreversibly. The glass bound PDMS chips were cured at 75 °C for 30 min to strengthen bonding and reduce air bubbles before being used.

Cell culture

GM 12878 cells were obtained and cultured as described previously²⁰. GM cells were cultured in RPMI 1640 supplemented with 15% FBS, 100 U penicillin, and 100 mg/mL streptomycin at 37 °C in a humid incubator with 5% CO₂. The cells were passaged every 2 d in order to maintain log phase growth.

Chromatin shearing

Sonicated chromatin was prepared as previously described²⁰. Sample containing 10⁶ cells was used as the starting material and centrifuged at 1,600 g for 5 min and washed twice with 1 ml cold PBS. Cells were crosslinked by adding 1 ml freshly made 1% formaldehyde and incubated at room temperature for 5 min on a shaker. Crosslinking was promptly terminated by adding 50 μl of 2.5 M glycine and shaking for 5 min. The pellet was resuspended in 130 μl Covaris buffer (10 mM Tris-HCl, pH 8.1, 1 mM EDTA, 0.1% SDS and 1 $\times$ protease inhibitor cocktail) and sonicated using the following conditions. Using Covaris M 220 (Covaris), the sample was sheared at 4 °C with 75 W peak incident power, 5% duty factor, and 200 cycles per burst for 12 min. This resulted in sheared chromatin with lengths ranging primarily from 150 to 600 bp (Fig. S3). The sheared sample was centrifuged in a 4 °C centrifuge at 14,000 g for 10 min, and the supernatant was transferred to a new tube. Sheared chromatin was diluted with IP buffer (20 mM Tris-HCl, pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% (w/v) sodium deoxycholate, 0.1% SDS, 1% (v/v) Triton X-100, with 1% freshly added PMSF and PIC) and aliquoted into 25 μl samples containing

250, 500, 1500, and 5,000 cell samples and stored at -80°C . Select chromatin samples were purified by ethanol precipitation for fragment analysis using HS D1000 tapes on TapeStation 2200 (Agilent). Before use, 20% was taken out as an input sample and the remaining sample was diluted to the appropriate volume according to experimental procedure (30–120 μl).

Bead preparation

Protein A coated superparamagnetic Dynabeads (Invitrogen) were utilized for immunoprecipitation of sheared chromatin. 10 μl of bead suspension was washed twice with 100 μl freshly prepared blocking buffer (made by mixing 467 μl filtered PBS and 33 μl of 7.5% Bovine Albumin Fraction V solution) in each wash. The beads were incubated for 2 h on a rotator at 4°C in 300 μl blocking buffer containing 5, 10, or 20 μl of H3K4me3 antibody (07–473, Millipore) for 50, 100–300, and 1000 cells per ChIP assay, respectively. For the dual-bead experiment, for each bead type ($\sim 5\ \mu\text{l}$), half the volume of all reagents mentioned above were used in the preparation. The beads for H3K27ac profiling were coated with 2.5 μl of antibody (ab4729, Abcam) in the 150 μl blocking buffer. After bead coating, the beads were then washed twice in 100 μl IP buffer, before being resuspended in 50 μl IP buffer for on-chip use.

Device operation

The microfluidic system shown in Figure S1 was comprised of a microfluidic chip, a computer running a custom LabVIEW program (File S1), which controlled a set of 14 solenoid valves (ASCO) and 3 syringe pumps (Chemyx), and a gas cylinder which provided a pressure source. The gas pressure used throughout this experiment was 30 psi. The microfluidic chip was placed on an Olympus microscope IX71 with a CoolSNAP HQ2 camera (Photometrics) for visual monitoring of experiments. The solenoid valve system was connected to the microfluidic device with PFA high purity tubing (IDEX). Prior to the experiment the control layer channels were filled with water. Washing buffers were loaded into syringes, while small-volume solutions ($<200\ \mu\text{l}$) were loaded into the tubing connected to water filled syringes with an air gap to separate solution from water. The syringes were loaded onto the computer-controlled syringe pumps and connected to the microfluidic device through PFA tubing.

The fluidic layer was evacuated of air by flowing IP buffer (20 mM Tris-HCl, pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.1% Sodium deoxycholate, 0.1% SDS, 1% (v/v) Triton X-100, with 1% freshly added PMSF and PIC) through the channels at a flow rate of 10 $\mu\text{l}/\text{min}$. After all air bubbles were evacuated from the fluidic layer, a $1/2 \times 1/8 \times 3$ inch magnet (K&J Magnetics) was positioned under the reaction chambers with upper edge of the magnet aligned with position 2 (Fig. S2). Operation was performed as demonstrated in Fig. 1c. The beads were loaded into 4 chambers using the following method. The bead suspension (6 $\mu\text{g}/\mu\text{l}$) was flowed into one of 4 reaction chambers at a flow rate of 8 $\mu\text{l}/\text{min}$ while the valves alternated flow among the chambers in the order of chamber 1-3-2-4 at 4 s intervals to ensure even loading. Each loading cycle took 16 s and we finished loading all 4 chambers in 20 cycles.

In the dual-bead experiment, the loading of anti-H3k4me3 and anti-H3K27ac beads occurred in two separate steps. The two types of beads were loaded from different inlets. The bead suspension was flowed into the device at a rate of 8 $\mu\text{l}/\text{min}$ into 2 chambers on the same side with loading alternating between the two chambers at 4 s intervals for 20 cycles (i.e. each chamber had a total loading time of 80 s). The channels were purged with IP buffer between loading steps to ensure no cross-contamination.

Beads were visually inspected to ensure even loading in each chamber. The magnet was repositioned, with the lower edge aligning with position 3 in Fig. S2. The chromatin was loaded at a flow rate of 1 $\mu\text{l}/\text{min}$. To ensure even loading of chromatin among chambers and proper fluidization, chromatin was initially loaded using the following method. Chromatin solution was directed into one of 4 reaction chambers while the on-chip valves alternated flow among the chambers in the order of chamber 1-3-2-4 with intervals varying from 8 to 0.25 s for a total of 104 s (cycle 1: 8 s per chamber, cycle 2: 6 s per chamber, cycle 3: 4 s per chamber, cycle 4: 3 s per chamber, cycle 5: 2 s per chamber, cycle 6: 1 s per chamber, cycle 7–8: 0.5 s per chamber, cycle 9–12: 0.25 s per chamber). After these 12 loading cycles, we opened all chambers to load chromatin into them simultaneously. This rotating loading procedure was performed every 15 min of loading to reduce bead settling.

Washing was performed with IP buffer, low salt buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% (v/v) Triton X-100, with 1% freshly added PMSF and PIC), and high salt buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% (v/v) Triton X-100, with 1% freshly added PMSF and PIC) in that order. For each washing step, we applied the following method at the start of each washing period. Washing buffer was directed into one of 4 reaction chambers while the on-chip valves alternated flow among the chambers in the order of chamber 1-3-2-4 with intervals varying from 4 to 0.25 s for a total of 50 s (cycle 1: 4 s per chamber, cycle 2: 3 s per chamber, cycle 3: 2 s per chamber, cycle 4: 1.5 s per chamber, cycle 5: 1 s per chamber, cycle 6: 0.5 s per chamber, cycle 7–8: 0.25 s per chamber). This method also included flow rate change within the first 50 s. The flow rate was slowly ramped from 1 to 5 $\mu\text{l}/\text{min}$ during the period, increasing by 1 $\mu\text{l}/\text{min}$ per 10 s. All chambers were then open after the first 50 s, and the flow rate was ramped from 5 to 20 $\mu\text{l}/\text{min}$ during the next 50 s (i.e. increasing by 3 $\mu\text{l}/\text{min}$ per 10 s). For the rest of the washing period, a constant flow rate of 20 $\mu\text{l}/\text{min}$ was used with all the chambers open. We applied this method again in the first 100 s every time when we switched to a new wash buffer. After performing all washing steps, the beads were collected from the system by removing the magnet and flowing IP buffer at a rate of 400 $\mu\text{l}/\text{min}$ for 15 s. The beads were collected via pipetting at each chamber outlet. We appended the LabVIEW file for operating the LIFE-ChIP process as File S1.

DNA purification

ChIP and input chromatin samples (beads or 10 μl solution, respectively) were incubated overnight at 65 $^{\circ}\text{C}$ with 200 μl (190 μl for input) elution buffer (200 mM NaCl, 50 mM Tris-HCl, 10 mM EDTA, 1% SDS, 0.1 M NaHCO_3) supplemented with 2 μl of 10 $\mu\text{g}/\mu\text{l}$ proteinase K. After incubation, DNA was isolated from protein debris and beads by adding 200 μl of Phenol:Chloroform:Isoamyl Alcohol 25:24:1 to the elution mix and vortexing.

After a 5 min centrifuge at 16,100 g, the supernatant (200 μ l) was removed and mixed with 750 μ l 100% ethanol, 50 μ l of 10 M ammonium acetate, and 2 μ l of 5 μ g/ μ l glycogen. The solution was vortexed and incubated at -80°C for 2 h. After incubation, the samples were centrifuged at 16,100 g at 4°C for 5 min. The supernatant was removed, and 500 μ l of 70% ethanol was added to the tube without disturbing the pellet. After a 5 min centrifuge under the same conditions, the solution was removed, and the pellet air dried. The pellet was then dissolved into 40 μ l of water for library preparation.

Library preparation

Library preparation was performed using the Swift Bio S2 library preparation kit (Swift Biosciences) using 40 μ l of purified DNA. We followed the manufacturer's instructions with minor modifications. We added 2.5 μ l of 20 $\times$ EvaGreen into the 50 μ l amplification reaction mix, and terminated amplification after samples saw a >3000 RFU increase (in a BioRad CFX Connect). After DNA purification with SPRI beads, DNA was then eluted into 7 μ l low EDTA TE buffer where 2 μ l could be used for qPCR analysis for preliminary quality control analysis, KAPA DNA quantification, and Tapestation fragment size analysis and the other 5 μ l for library pooling. Libraries were pooled at 10 nM for sequencing by Illumina HiSeq 4000 with single-end 50 nt read.

Results and Discussion

We optimized the operational conditions (flow rate and magnet location) summarized in Fig. S2 for high levels of fluidization for washing and a tight packing of beads for the chromatin loading step, all while avoiding bead loss in each chamber. We eventually settled upon using magnet position 3 as it allowed for a tightly packed bed for chromatin loading and a highly fluidized bed for washing while having a large range of fluidization flow rates without bead loss. We used an inlet flow rate of 1 μ l/min (i.e. 0.25 μ l/min through each of the 4 chambers) for chromatin loading and 20 μ l/min (5 μ l/min per chamber) for washing. The 20 μ l/min flow rate for washing created beds that retained most beads with very slight variance in the flow rate among chambers which was caused by slight variability in the volume of beads in each chamber (Movie S1).

The platform was operated in several steps (Fig. 1c). First, with a permanent magnet situated with the upper edge aligned with position 2 (see Fig. S2a and 1c for reference), antibody-coated immunomagnetic beads were loaded into the 4 chambers from inlet I4 at a flow rate of 8 μ l/min while switching flow among chambers in the order of 1-3-2-4. Each loading session into one specific chamber lasted 4 s and 20 loading cycles (16 s per cycle) were used to load the chambers. Second, crosslinked and sheared chromatin (150–600 bp) (Fig. S3) was flowed from inlet I3 at 1 μ l/min into the fluidized beds in the 4 chambers simultaneously. This step lasted 30 or 120 min to allow targeted chromatin fragments adsorb on the bead surface (i.e. ChIP). Finally, 3 wash buffers (IP, low salt, and high salt) were flowed sequentially through the fluidized beds from inlets I1 and I7, I5, and I6, respectively, at a flow rate of 20 μ l/min for 10–30 min each (Movie S1). The washed chromatin-bead conjugates were collected for ChIP DNA elution (off-chip), purification, and sequencing

library preparation. Loading and washing steps were designed to keep even distribution of reagents among chambers and good fluidization of the beads (see methods for details).

Using this platform we examined tri-methylation of lysine 4 on histone H3 (H3K4me3) in GM12878 cells (a human B-Lymphocyte cell line) (all data are summarized in Table S1). We tested samples containing various numbers of cells 4000, 1200, 400, and 200 cells (equivalent to 1000, 300, 100, and 50 cells per ChIP-seq assay) (Fig. 2a). The average Pearson correlation coefficients among the data generated by 4 chambers (c1–4) were 0.958, 0.970, 0.870, 0.820 respectively, while the average correlation coefficients with ENCODE data were 0.936, 0.938, 0.892, and 0.817 respectively (Fig. 2b), showing that we were able to produce data with similar reproducibility to published ENCODE data sets (generated using tens of millions of cells), which had an average correlation of 0.937 between replicates. The LIFE-ChIP-seq data quality was also compared to those generated by MOWChIP-seq, which utilized a packed bed of beads combined with oscillatory washing²⁰ (Fig. 2c). LIFE-ChIP-seq consistently performed better than MOWChIP-seq when similar number of cells were used, in terms of the gold-standard peaks covered²⁰.

We also examined reproducibility across different devices. In Fig. 3, we compared the data collected using two devices (each with 4 chambers) with 1000, 300, and 100 cells per ChIP assay. The correlations were very similar between different chambers across the 2 devices, with lower cell numbers resulting in lower correlations between replicates. Occasionally there was one failed dataset (C3 of device 3 in this case, the only one out of more than 50 datasets). However, it appeared that our device was resistant to cross-contamination and the other 3 datasets on the same device were not affected. We conducted these experiments using small aliquots from a stock solution of sonicated chromatin. In our previous work, we have shown that it was possible to create a sonicated chromatin sample from 100 cells²⁰.

The LIFE-ChIP-seq platform operation was optimized on a number of different parameters. The loading volume was optimized at the flow rate of 1 μ l/min, which was selected from the fluidized bed behavior described in Fig. S2. Thus larger input volume required longer loading time. The two conditions of 30 or 120 μ l loading volumes were created by diluting chromatin from 4,000 cells (1,000 cells per assay) to either 30 or 120 μ l respectively, while keeping all the other operational conditions identical. Generally speaking, lower chromatin concentration (associated with larger input volume) may lead to slower adsorption, depending on the adsorption kinetics. In the results (Fig. S4), we observed that 30 μ l samples indeed had a slightly higher average correlation 0.983 among the 4 replicates produced in one run on the device than that of 120 μ l samples 0.956. Additionally, the 30 μ l samples had a slightly higher correlation with ENCODE data than 120 μ l samples (0.944 vs. 0.936). The 30 min loading condition can be seen to have a higher correlation between chambers in the device than the 120 min loading.

Washing time, which was the duration for flowing each wash buffer through fluidized bed to remove nonspecifically adsorbed chromatin fragments, was another parameter that we optimized. We explored washing times varying between 0 and 30 min with 1000 cells per assay (chamber). Washing removes nonspecifically adsorbed chromatin and improves enrichment of ChIP DNA. However, excessive washing has been shown to decrease the

amount of ChIP DNA collected thus require additional amplification and result in a lower-quality sequencing library²⁰. Our LIFE-ChIP-seq data showed that no washing yielded lower-quality data (with average correlation among chambers of 0.917) which, nevertheless, yielded an average correlation of 0.915 with ENCODE data (Fig. 4). In contrast washing times from 10 to 30 min all yielded high-quality data with only a slight decrease in average correlation among chambers (from 0.974 at 10 min to 0.956 at 20 min and 0.954 at 30 min) and their correlations with ENCODE data (0.942, 0.936, 0.934 for 10, 20, 30 min, respectively) (Fig. 4). The similarities between data obtained with washing times 10–30 min suggested that the fluidized bed washing was effective for removing nonspecific chromatin molecules. Compared to previous results we obtained using packed bed reactors (i.e. MOWChIP-seq)²⁰, the fluidized bed design implemented in LIFE-ChIP decreased the possibility for physical trapping.

We also examined how the overall time of the LIFE-ChIP assay affected the results. Using the minimal conditions determined through the optimizations described above, we performed a LIFE-ChIP-seq experiment using 300 cells per assay, 30 min of loading and, 10 min of washing per buffer, which equated to a 1 h on-chip assay compared to the 3 h assay (2 h loading and 20 min per washing step) that we have set as our baseline. The 1 h assay showed a slightly higher average inter-chamber correlation than the 3 h assay (0.964 vs. 0.956) (Fig. 5), but a slightly lower average correlation coefficient with ENCODE data (0.926 vs. 0.938). Thus both conditions produced similar high-quality ChIP-seq data.

Finally, using the same device, we also demonstrated profiling two histone marks (H3K4me3 and H3K27ac) in one run. We were able to load two types of antibody-coated beads (one coated with anti-H3K4me3 and the other with anti-H3K27ac) from two separate inlets sequentially, while avoiding cross contamination (Fig. 6a, Movie S2). We directed the loading of the beads into different chambers by manipulating the micromechanical valves (Fig. 6a). We then performed parallel ChIP-seq run that generated datasets on both H3K4me3 and H3K27ac, each with two replicates, using 300 cells per assay, 120 μ l loading volume, and 20 min of washing per buffer. The results of the dual-bead experiment are summarized in Fig. 6b–c. Fig. 6b shows different features in H3K4me3 and H3K27ac at two loci. Our data revealed the same characteristics of the two marks that were also shown in ENCODE data. In general, H3K4me3 datasets (with a Pearson correlation of 0.921 between replicates) showed higher quality than H3K27ac ones (with a Pearson correlation of 0.774 between replicates) (Fig. 6c). Nevertheless, H3K4me3 replicates presented much higher correlation between themselves than with any of H3K27ac datasets.

Conclusions

There are several distinct advantages associated with LIFE-ChIP-seq. First, this fluidized-bed based ChIP technology permits running multiple parallel assays simultaneously. Such a feature is critical for high-throughput processing of samples needed for the examination of patient materials in the precision medicine settings. The fluidized bed technology effectively alleviates pressure building associated with bead manipulation in a microfluidic system. We demonstrated producing 4 replicates from one sample and profiling 2 histone marks with 2 replicates for each. The parallel operation decreased the time averaged on each assay.

Second, LIFE-ChIP-seq is also a low-input technology. We produced high-quality data with as few as 50 cells per assay. This is comparable to or better than other state-of-the-art ChIP-seq technologies such as MOWChIP-seq²⁰ and iChIP³³. The low-input characteristic is important for profiling primary cell types with low abundance. Third, the microfluidic process of LIFE-ChIP-seq is largely automated. There is very little input from the operator during the process. Thus the technology may largely eliminate human errors and save labor. Taken together, LIFE-ChIP-seq will be a highly enabling technology for profiling epigenomes with high throughput and low input. Future integration of library preparation on the microfluidic platform will further facilitate the high throughput of the entire process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overview of LIFE-ChIP device and operation. (a) Schematic of LIFE-ChIP device. (b) A microscopic image of a LIFE-ChIP device. This was created by stitching images taken by a microscope. (c) Overview of LIFE-ChIP-seq device operation for using a single type of beads. Solid blue depicts a pressurized and closed valve while transparency indicates an open valve. The schematic omits details on the valve and flow rate conditions for loading and washing (see methods). Three steps are shown: loading of antibody-coated beads into LIFE-ChIP-seq platform containing 4 parallel chambers; flowing of the chromatin fragments through the fluidized beds (i.e. chromatin immunoprecipitation); flowing of washing buffer through the fluidized beds for removing nonspecifically bound chromatin.

Figure 2.

Summary of H3K4me3 ChIP-seq data generated using LIFE-ChIP-seq platform with various sample sizes (50–1000 cells per assay). All samples were run using 120 μ l loading volume, and 20 min per washing buffer. (a) Normalized LIFE-ChIP sequencing data at various cell numbers per assay compared with ENCODE data (ENCFF825QGB, ENCFF598WCX by Bradley Bernstein group)³⁹. (b) Pearson correlation matrix of ChIP-seq data for 1000, 300, 100, and 50 cell samples generated on single multiplexed chips compared with ENCODE data. Data generated by individual chambers are denoted as C1–C4. (c) Receiver Operating Curves for comparing LIFE-ChIP-seq to MOW-ChIP-seq data. The ROC curves were plotted using the approach described in our previous publication²⁰. MOWChIP-seq data are from ref²⁰.

Figure 3.

Inter-device and inter-chamber reproducibility of our data. All samples were run using the given cell number, 120 μ l loading volume, and 20 min per washing buffer. Two LIFE-ChIP devices were operated at each of the following cell numbers per assay: 1000, 300, and 100.

Figure 4.

LIFE-ChIP-seq data with washing conditions ranging from no washing to 30 min per washing buffer. All samples were run using 1000 cells per assay and 120 μ l loading volume. (a) Normalized LIFE-ChIP-seq tracks and (b) Pearson correlation matrix.

Figure 5.

LIFE-ChIP-seq data with different total assay times. All runs were taken using 300 cells per assay. 1 h assays were performed with 30 μ l loading volume (i.e. 30 min for loading) and 10 min per wash buffer while 3 h assays performed with 120 μ l loading volume (120 min for loading) and 20 min per wash buffer. (a) Normalized LIFE-ChIP-seq tracks and (b) Pearson correlation matrix.

Figure 6. LIFE-ChIP-seq for dual-bead loading and dual-histone-mark profiling. (a) Two-step process for loading two types of beads (targeting H3K4me3 and H3K27ac, respectively) into the system. (b) Genome browser tracks comparing our H3K4me3 and H3K27ac data to ENCODE data (ENCFF598WCX and ENCSR000AKC) at two loci in the genome³⁹. (c) Pearson correlation matrix comparing H3K4me3 and H3K27ac datasets generated in one dual-bead LIFE-ChIP experiment.