

# Cavitation Enhancement Increases the Efficiency and Consistency of Chromatin Fragmentation from Fixed Cells for Downstream Quantitative Applications

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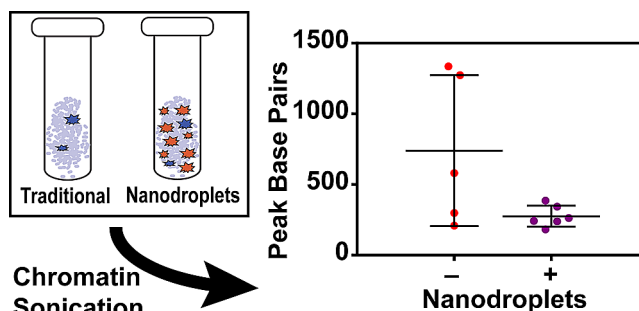
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**ABSTRACT:** One of the most sensitive, time-consuming, and variable steps of chromatin immunoprecipitation (ChIP) is chromatin sonication. Traditionally, this process can take hours to properly sonicate enough chromatin for multiple ChIP assays. Further, the length of sheared DNA is often inconsistent. In order to faithfully measure chemical and structural changes at the chromatin level, sonication needs to be reliable. Thus, chromatin fragmentation by sonication represents a significant bottleneck to downstream quantitative analysis. To improve the consistency and efficiency of chromatin sonication, we developed and tested a cavitation enhancing reagent based on sonically active nanodroplets.

Here, we show that nanodroplets increase sonication efficiency by 16-fold and provide more consistent levels of chromatin fragmentation. Using the previously characterized chromatin *in vivo* assay (CiA) platform, we generated two distinct chromatin states in order to test nanodroplet-assisted sonication sensitivity in measuring post-translational chromatin marks. By comparing euchromatin to chemically induced heterochromatin at the same CiA:Oct4 locus, we quantitatively measure the capability of our new sonication technique to resolve differences in chromatin structure. We confirm that nanodroplet-assisted sonication results are indistinguishable from those of samples processed with traditional sonication in downstream applications. While the processing time for each sample was reduced from 38.4 to 2.3 min, DNA fragment distribution sizes were significantly more consistent with a coefficient of variation 2.7 times lower for samples sonicated in the presence of nanodroplets. In conclusion, sonication utilizing the nanodroplet cavitation enhancement reagent drastically reduces the amount of processing time and provides consistently fragmented chromatin of high quality for downstream applications.



Investigating DNA–protein interactions is essential for the study of cellular processes, including gene regulation, DNA replication, DNA repair, and nucleosomal organization. Chromatin immunoprecipitation (ChIP) is a key method used to determine the genomic location of specific proteins or post-translational marks at individual loci or genome-wide.<sup>1,2</sup> A typical workflow incorporating ChIP is as follows: (1) fixation of cells with formaldehyde, which cross-links chromatin-bound proteins to DNA; (2) isolation of the cross-linked chromatin from cell nuclei; (3) lysis of nuclei and fragmentation of chromatin into 200–500 base pair (bp) fragments by sonication; (4) enrichment of the protein target and its

associated DNA sequences from the sheared lysates using antibody immunoprecipitation; (5) reversal of cross-links and isolation of DNA; and (6) downstream quantitative analysis of enriched DNA sequences by quantitative PCR (qPCR) or next-generation sequencing.

Random, unbiased fragmentation of chromatin is an important step in protocols incorporating ChIP analysis.<sup>1,3</sup> Since ChIP followed by qPCR or next-generation sequencing

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determines protein localization to specific regions of the genome, chromatin must be fragmented into 200–500 bp segments.<sup>2,4,5</sup> If the fragments are too small or degraded, the DNA sequence will be lost from downstream analyses. If the fragments are too large, it is impossible to map protein occupancy to a narrow region of the genome. Therefore, variability in the fragmentation technique between samples can bias data analyses.<sup>6</sup>

Despite the importance of sonication to the success of ChIP-based assays, chromatin fragmentation remains an inefficient and inconsistent process, with few recent innovations in the development of chromatin fragmentation technology.<sup>6–8</sup> Proper chromatin fragmentation is a bottleneck in current protocols, often adding a day to processing times when handling large sample sets. To address this issue, we applied a novel cavitation enhancement reagent to improve fragmentation of chromatin from fixed cell nuclei. These biologically inert nanodroplets are composed of a liquid perfluorocarbon stabilized with a phospholipid monolayer shell.<sup>9,10</sup> The droplets have an average diameter of 250 nm. Once exposed to ultrasound energy, they vaporize into microbubbles that are ~1–5  $\mu\text{m}$  in diameter, resulting in an intense, prolonged cavitation and release of mechanical energy that enhances chromatin fragmentation.<sup>10–12</sup>

To assess the performance of the nanodroplet reagent in the sonication step of a typical protocol using ChIP, we used the chromatin *in vivo* assay (CiA).<sup>13</sup> We chose this assay as it was a previously characterized robust method that can generate significant changes to enrichment of post-translational marks at a well-defined genomic locus in mammalian cells. This system allows us to recruit chromatin modifying proteins to DNA binding elements inserted in the promoter of a specific gene and then to determine changes in the chromatin environment. Here, we recruited a fragment of heterochromatin protein 1 alpha (HP1), which stimulates heterochromatin formation, and performed ChIP of histone post-translational modifications followed by the quantitative polymerase chain reaction (ChIP–qPCR). We show that nanodroplets increase the time efficiency of chromatin fragmentation by roughly 16-fold while maintaining chromatin quality for downstream analysis by ChIP–qPCR. In practice, this method can reduce the processing time for mouse embryonic stem cells from >38 to <3 min, representing a significant advance over current techniques.

## ■ EXPERIMENTAL PROCEDURES

**Cell Culture and Infection.** The CiA mouse embryonic stem cells were cultured and infected as previously described.<sup>13</sup> Rapamycin was diluted in molecular grade ethanol and stored at  $-20^{\circ}\text{C}$ . HEK (human embryonic kidney) 293T cells were cultured as previously described.<sup>14</sup>

**Fluorescence Microscopy and Image Analysis.** Representative images of the CiA mouse embryonic stem cells were obtained using a fluorescence microscope prior to flow cytometry analysis and ChIP–qPCR. The brightness/contrast of the brightfield images was uniformly adjusted in ImageJ FIJI. The background artifact in the GFP- fluorescent images was uniformly removed in FIJI with a sliding paraboloid with a rolling ball radius of 10 pixels.

**Flow Cytometry.** Flow cytometry was performed at The University of North Carolina Flow Cytometry Core Facility. The cells were washed in 1 $\times$  PBS, trypsinized, and resuspended in FACS buffer (1 $\times$  PBS (1 $\times$  phosphate buffered saline), 0.2%

BSA, 1 mM EDTA). For each replicate, more than 100,000 cells were analyzed and gated as shown in [Figure S1 of the Supporting Information](#).

**Preparation of Fixed Cell Nuclei.** The optimal sonication time for the ChIP–qPCR protocol was determined through titration over a series of sonication time points. For consistency purposes, a fixed number of cells was treated with formaldehyde, and the nuclei were isolated, prepared for sonication, and analyzed using a standard protocol.

The mouse embryonic stem cells (mESCs) were prepared by trypsinization on 15 cm plates containing ~40 million cells (mESCs). Cells were transferred to a 15 mL conical tube and pelleted at 300g for 5 min followed by resuspension in 15 mL of 1 $\times$  PBS. Cells were centrifuged at 300g for 5 min, and the pellet was resuspended in 10 mL of fixation buffer (50 mM HEPES (pH 8.0), 1 mM EDTA (pH 8.0), 0.5 mM EGTA (pH 8.0), 100 mM NaCl). Cross-linking was performed with the addition of 1 mL of 11% formaldehyde (final concentration 1%) followed by rotation for 10 min at room temperature. To quench the formaldehyde cross-linking, 0.5 mL of 2.5 M glycine (125 mM final concentration) was added and rapidly mixed by inversion.

For the HEK 293T cells, formaldehyde was added to the medium of a 15 cm plate containing ~2 million cells to a final concentration of 1%. Plates were then placed on a plate shaker for 10 min at room temperature. To quench the formaldehyde cross-linking, glycine was added to a final concentration of 125 mM, followed by a 5 min incubation at room temperature with agitation. Cells were scraped from the plate using a rubber policeman and transferred to a 50 mL conical tube on ice. Cells were pelleted by centrifugation at 300g for 5 min, and the supernatant was discarded.

The pellets from both cell types were placed on ice for the remaining preparation steps. Cells were pelleted by centrifugation at  $4^{\circ}\text{C}$  and 1200g for 5 min, and the pellet was washed in 10 mL of 1 $\times$  PBS and recentrifuged under the same conditions. The cell pellets (without supernatant) were flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Nuclei were isolated from the cell pellet by resuspension in 10 mL of rinse buffer #1 (50 mM HEPES (pH 8.0), 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP40, 0.25% Triton X 100), incubation on ice for 10 min, and centrifugation at 1200g and  $4^{\circ}\text{C}$  for 5 min. The pellet was resuspended in 10 mL of rinse buffer #2 (10 mM Tris (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl) and centrifuged at 1200g and  $4^{\circ}\text{C}$  for 5 min. To wash residual salts from the side of the 15 mL conical tube, 5 mL of shearing buffer (0.1% SDS, 1 mM EDTA (pH 8.0), 10 mM Tris-HCl (pH 8.0)) was carefully added without disturbing the pellet. The samples were spun at 1,200g and  $4^{\circ}\text{C}$  for 3 min; the supernatant was discarded, and the wash step was repeated.

**Sonication of Fixed Nuclei.** Sonication was performed in a Covaris E110 instrument with a customized holder designed to hold glass tubes (Fisher C4008-632R) that were crimp sealed with caps (Fisher C4008-2A). Isolated cell nuclei were resuspended in 90  $\mu\text{L}$  of shearing buffer (supplemented with 1 $\times$  protease inhibitor) per 10 million mESCs and 500,000 HEK 293T cells. The resuspended cells were carefully mixed by pipetting and were transferred to the glass sonication tubes on ice.

The nanodroplet cavitation reagent (MegaShear, Triangle Biotechnology) was stored at  $-20^{\circ}\text{C}$  and was prepared by defrosting on wet ice prior to use. Briefly, this reagent is formulated by the encapsulation of liquid decafluorobutane in a

lipid shell in a buffer of phosphate buffered saline, glycerine (15% v/v), and propylene glycol (5% v/v). The encapsulation lipid was composed of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) (Avanti Polar Lipids, Alabaster, AL, USA) stabilized by 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy (polyethyleneglycol)-2000 (DSPE-PEG2000) (Avanti Polar Lipids, Alabaster, AL, USA) in a 9:1 molar ratio. Further details of the formulation process are provided by Kasoji and co-workers.<sup>9</sup>

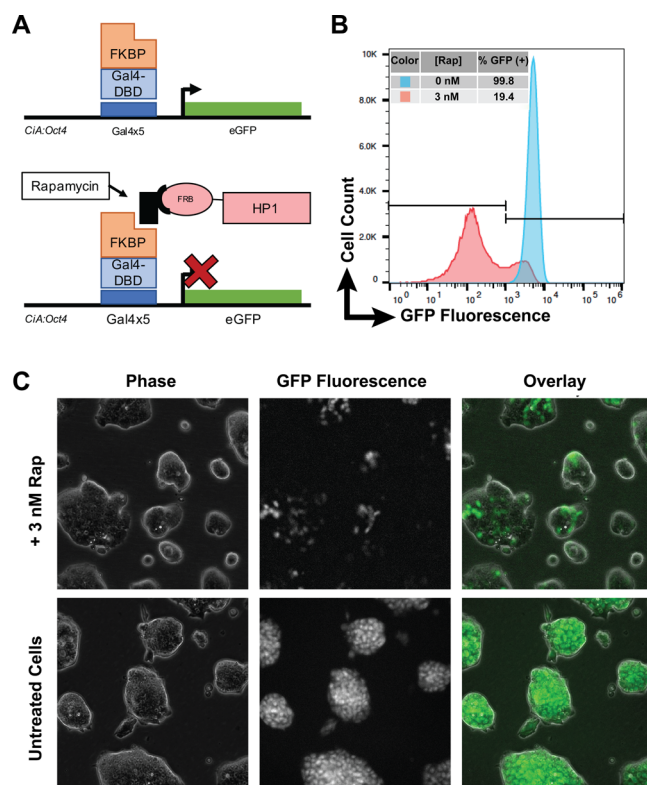
Next, 10  $\mu$ L of nanodroplets or 10  $\mu$ L of shearing buffer (traditional) was added to the appropriate tubes; the tubes were inverted for mixing, followed by a brief centrifugation to remove liquid from the sides of the tube. Samples were sonicated at 4 °C for 5 min followed by at least 5 min of rest for the total designated time with settings of a 20% duty cycle, an intensity of 8, and 200 cycles per burst.

**Extraction and Quantitation of Total DNA for Determination of Fragmentation Efficiency.** Following sonication, the cell lysate containing chromatin was transferred to a 1.5 mL microfuge tube and centrifuged at full speed (>18000g) at 4 °C for 5 min. The supernatant was transferred to a 0.2 mL PCR tube, and the pellet was resuspended in 100  $\mu$ L of shearing buffer and transferred to another PCR tube. Next, 2  $\mu$ L of 100  $\mu$ g/ $\mu$ L RNase (Qiagen) was added to each pellet and supernatant sample, followed by incubation at 37 °C for 30 min. Protein was removed by addition of 2  $\mu$ L of 20 mg/mL proteinase K (Worthington), and samples were mixed by inversion and briefly centrifuged. Samples were incubated at 55 °C for 30 min, followed by incubation at 65 °C overnight to reverse the formaldehyde cross-links (~14 h). DNA was extracted from both pellet and supernatant samples using silica matrix columns (Zymo Research ChIP DNA Clean and Concentrator kit, D5201) according to the manufacturer's instructions and was eluted in 25  $\mu$ L of 10 mM Tris (pH 8.0). The concentration of DNA in the pellet and supernatant samples was determined using fluorometry (Qubit dsDNA High Sensitivity Assay Kit, Invitrogen). To visualize the DNA and determine fragmentation efficiency, electrophoresis was performed on 9  $\mu$ L (36% of total volume) of eluted supernatant DNA per lane loaded on a 1.2% agarose gel. Average peak fragment size was determined using the Agilent TapeStation 2200 HS D1000 and D5000 kits according to the manufacturer's instructions.

**ChIP Assay.** ChIP-qPCR was performed using the ChIP-IT High Sensitivity kit (Active Motif #53040) according to the manufacturer's instructions, except that inputs were purified using a clean and concentrate column (Zymo Research #D5205), and the ChIP samples were eluted twice with 30  $\mu$ L each for a total of 60  $\mu$ L of elution buffer for the H3K4me3 and H3K9me3 ChIP assays. For each ChIP assay, the equivalent of 5 million cells was used for mESCs and 1 million cells for HEK 293T cells. These samples were analyzed by qPCR using primers and methods previously described (Table S1).<sup>15</sup> The antibodies used included G9a (Abcam #40542), H3K4me3 (Active Motif #39915), and H3K9me3 (Active Motif #39161).

## RESULTS AND DISCUSSION

**Chromatin *in Vivo* Assay as a System for Testing the Quality of Chromatin Fragmented in the Presence of Nanodroplets.** The chromatin *in vivo* assay (CiA) is designed to control the chromatin environment at a specific gene locus (Figure 1A).<sup>13</sup> This platform is ideal for testing the quality of



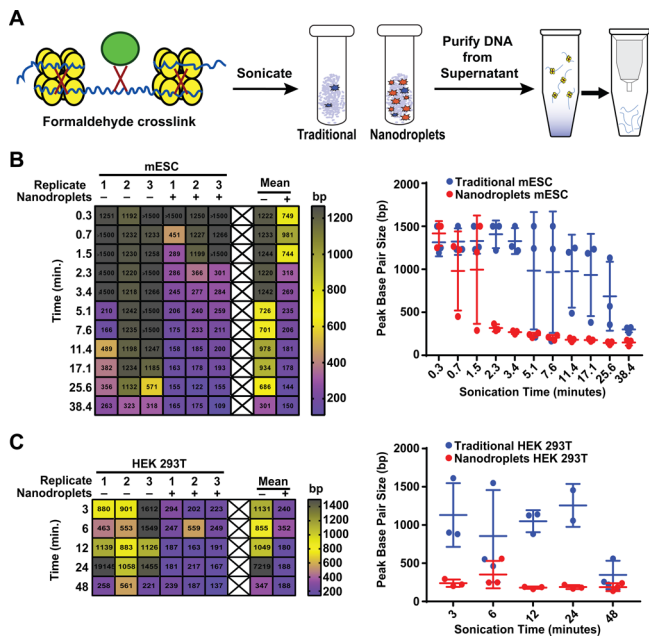
**Figure 1.** Establishment of the CiA system as a tool for assessing chromatin changes. Mouse ES cells were infected with constructs expressing FKBP-Gal4 and FRB-HP1. Rapamycin (a chemical inducer of proximity) brings together FKBP and FRB at the Gal4 domain upstream of eGFP (A) as shown in cartoon form. (B) Flow cytometry results of infected cells treated with 3 nM rapamycin. (C) Fluorescent images of infected cells treated with 3 nM rapamycin.

nanodroplet-fragmented chromatin because it uses well-defined methods to induce enrichment of post-translational marks at the CiA:Oct4 locus in mESCs. In this system HP1 $\alpha$  is rapidly tethered using the chemical inducer of proximity (CIP) rapamycin.<sup>13</sup> When HP1 $\alpha$  is recruited to the CiA:Oct4 promoter region it induces histone methyltransferases (HMT) to catalyze trimethylation on histone H3 lysine-9 (H3K9me3).<sup>16–19</sup> This post-translational mark will then recruit endogenous HP1 enzymes to propagate heterochromatin formation mostly symmetrically across the promoter and gene body of the targeted allele.<sup>20</sup> These domains lead to chromatin compaction and gene repression.<sup>21</sup> ChIP-qPCR can be used to quantify the extent of H3K9me3.<sup>13</sup> In our system CIP-rapamycin-mediated recruitment of HP1 $\alpha$  for 5 days results in a complete heterochromatin domain formed at the CiA:Oct4 target locus marked by H3K9me3.<sup>13</sup> In this report, we used this well-characterized system to investigate the quality of chromatin fragmented using nanodroplets.

Our experimental system compares enrichment by ChIP-qPCR of H3K9me3 at the CiA:Oct4 locus between two conditions, euchromatin (no CIP-HP1 $\alpha$ ) and heterochromatin (CIP-HP1 $\alpha$  recruitment for 5 days). To confirm that recruitment of HP1 $\alpha$  results in the expected gene repression, the cells were exposed to 3 nM CIP-rapamycin for 5 days and GFP expression was quantified and imaged. Flow cytometry results revealed a decrease in GFP positive cells in CIP-HP1 $\alpha$ -treated samples from 99.8% to 19.4% (Figures 1B and S1). Cells were also imaged with fluorescence microscopy, which

confirms the flow cytometry data (Figure 1C). Taken together these results show that recruitment of HP1 $\alpha$  causes gene repression at the CiA:Oct4 locus, as previously described.<sup>13</sup>

**Determining Efficiency of Nanodroplet-Mediated Chromatin Fragmentation Using Fixed mESCs and HEK 293T Cells.** To determine if cavitation enhancement could alleviate the sonication bottleneck in sample processing for ChIP, we measured chromatin fragmentation efficiency in the presence or absence of nanodroplets in two different cell lines. Cell nuclei were prepared from formaldehyde cross-linked mESCs or HEK 293T cells, sonicated in the presence (nanodroplets) or absence (traditional) of the nanodroplet reagent, followed by purification of total soluble DNA, which was used to measure chromatin fragmentation efficiency (Figure 2A, see Experimental Procedures). DNA was visualized



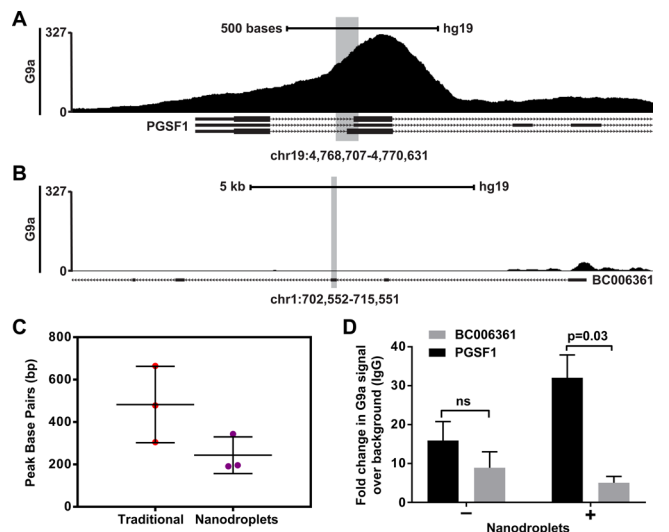
**Figure 2.** Efficiency of chromatin fragmentation in the presence and absence of nanodroplets. (A) Nanodroplets increase the number of cavitation events when exposed to ultrasound during the sonication phase of the chromatin isolation protocol. (B and C) Peak DNA fragment size (bp) after sonication was determined by Agilent TapeStation 2200 analysis for mESCs (B) and HEK 293T cells (C). Data from each replicate in base pair (bp) size at each DNA fragment peak without (–) or with (+) nanodroplets are shown as a heat map. The mean value of all three replicates is also indicated. The peak bp size for each sample was also plotted as a function of time without (blue dots) or with (red dots) nanodroplets. The mean peak bp size for each condition is indicated by a horizontal line. Error bars represent the standard deviation of 3 biological replicates except for that for HEK 293T cells (traditional) at 24 min, which represents 2 replicates.

on an agarose gel (Figure S2), and peak average fragment size was quantitated using an Agilent TapeStation instrument (Figure 2B,C). Agarose gel electrophoresis demonstrated that samples sonicated in the presence of nanodroplets reached the desired ChIP fragment size distribution of 200–500 bp in less time than traditional sonication for both cell lines (Figure S2). Quantitation of DNA size by TapeStation revealed that samples sonicated with nanodroplets reached a mean peak fragment size of  $318 \pm 34.7$  bp after just 2.3 min sonication time for mESCs (Figures 2B and S3–5) and  $239 \pm 39.4$  bp after 3 min

sonication time for HEK 293T cells (Figures 2C and S6–8). In contrast, traditional samples did not achieve the targeted 200–500 bp range until 38.4 min sonication time (mean peak fragment size of  $301 \pm 27.2$  bp) for mESCs (Figure 2B) and 48 min sonication time ( $347 \pm 152.3$  bp) for HEK 293T cells (Figure 2C). Therefore, the addition of nanodroplets to the sonication mixture decreases chromatin fragmentation time from formaldehyde cross-linked cells by 16.7-fold for mESCs and 16-fold for HEK 293T cells, both of which represent a similarly significant reduction in sample processing time.

**ChIP of G9a Lysine Methyltransferase Can Be Successfully Performed on Chromatin Fragmented from HEK 293T Cells in the Presence of Nanodroplets.**

After determining optimal times for chromatin fragmentation using either traditional or nanodroplet-mediated sonication, we tested HEK 293T cell chromatin quality by performing ChIP–qPCR for lysine methyltransferase protein G9a and an IgG control. The qPCR amplification regions were selected from previously published G9a ChIP next-generation sequencing (ChIP-seq) data (Figure 3A,B).<sup>14</sup> Chromatin preparation and



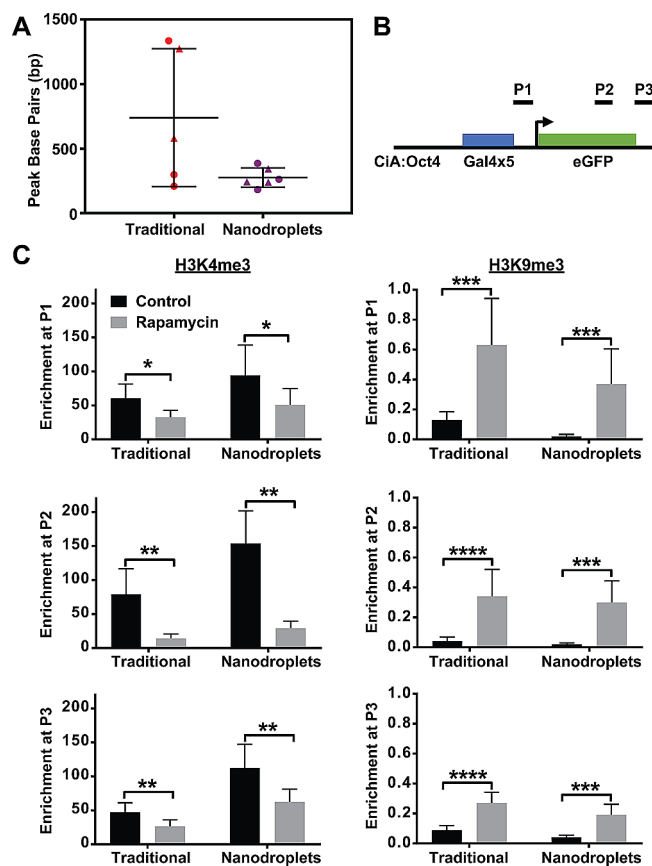
**Figure 3.** G9a ChIP from HEK 293T cells. ChIP–qPCR regions were selected from Simon and co-workers<sup>14</sup> to include a G9a positive region (A) and a G9a negative region (B). Amplicons are indicated by a gray vertical bar. (C) The peak base pair (bp) size of each input sample without (traditional, red dots; mean =  $482 \pm 180$  bp; CV = 37%) or with (nanodroplets, purple dots; mean =  $243 \pm 86$  bp; CV = 35%) nanodroplets was measured by Agilent TapeStation instrumentation. The mean peak bp size for each condition is indicated by a horizontal black line. Error bars represent the average of 3 biological replicates. (D) G9a ChIP–qPCR showing the fold change of G9a signal over IgG signal with nanodroplets ( $n = 3$ ) or without nanodroplets ( $n = 2$ ). The  $p$  values are indicated; ns indicates no significant difference.

sonication steps were performed as described (Experimental Procedures). To minimize variability, the post-sonication ChIP protocol was performed using a commercially available kit (Experimental Procedures). We determined that sonication for 3 min with nanodroplets and 48 min without nanodroplets (Figure 2C) was appropriate to fragment HEK 293T cell chromatin to a range of 200–500 bp. We used these conditions to prepare chromatin for ChIP and confirmed fragmentation by subjecting ChIP input samples to agarose gel electrophoresis (Figure S9A) and Agilent TapeStation (Figure 3C). Despite a 48 min sonication, we were unable to fragment all of the

traditionally sonicated inputs within the desired 200–500 bp range. Nanodroplet-mediated sonication produced a lower average peak bp size distribution ( $243 \pm 86$  bp) compared to that of the traditional method ( $482 \pm 180$  bp) with a similar coefficient of variation (CV) for both methods (nanodroplet CV = 35%, traditional CV = 37%). ChIP–qPCR was performed for G9a or IgG (background) at the G9a positive *PGSF1* locus (Figure 3A) and the *BC006361* G9a negative locus (Figure 3B) as determined from published G9a ChIP-seq data.<sup>14</sup> The large variation in input fragment size coupled with the long (48 min) sonication time resulted in a poor G9a ChIP signal over background for the traditional samples and no significant difference in G9a occupancy between positive and negative control regions (Figure 3D). In contrast, samples sonicated for only 3 min in the presence of nanodroplets resulted in a more robust G9a ChIP signal over background with a 6-fold difference between G9a occupancy at the positive and negative regions ( $p = 0.03$ ). Therefore, the addition of nanodroplets to the sonication of fixed HEK 293T cells results in a 16-fold decrease in sonication time compared to that of the traditional method and produces chromatin of sufficient quality to perform G9a ChIP–qPCR with a significant signal over background.

**Chromatin Fragmented Using Nanodroplets Faithfully Recapitulates ChIP–qPCR Data for Analysis of Histone Tail Modifications in the CiA System.** Following confirmation that we could perform ChIP from HEK 293T chromatin fragmented in the presence of nanodroplets, we further tested chromatin quality from mESCs by performing ChIP–qPCR for histone post-translational modifications that undergo a dynamic change following heterochromatin formation in the CiA:Oct4 system.<sup>13</sup> Chromatin was prepared as previously described (Experimental Procedures) and sonicated for 2.3 min when nanodroplets were used and 38.4 min for the traditional method (Figure 2B). To confirm fragment size, we subjected the ChIP input samples to agarose gel electrophoresis, where they appeared to be fragmented within the desired range (Figure S9B). When fragmentation of these input samples was quantified by TapeStation, however, nanodroplet-mediated sonication produced an overall 2.7-fold reduction in variance of DNA size distribution (coefficient of variation (CV) = 27%) compared to that of the traditional method (CV = 72%) (Figure 4A). The CV values were calculated using 6 data points for nanodroplet reagent samples and 5 data points for the traditional method since one traditional sonication data point produced a peak DNA fragment size greater than 1500 bp, which is outside of the linear range of the high sensitivity TapeStation assay. The average peak fragment sizes for traditional sonication were  $614 \pm 511$  bp for the control and  $927 \pm 346$  bp with rapamycin, and for sonication with nanodroplets average peak fragment sizes were  $277 \pm 83$  bp for the control and  $275 \pm 48$  bp with rapamycin. There was no significant change in average peak fragment size after rapamycin treatment. Overall, in addition to decreasing sonication time, nanodroplets also increased fragmentation consistency by 2.7-fold between input samples in mESCs.

The ChIP assay was performed as described for HEK 293T cells. ChIP antibodies for H3K4me3 were used as a marker of active gene transcription, whereas antibodies for H3K9me3 were used to mark transcriptional repression as previously described.<sup>13</sup> ChIP was performed using three biological replicates in the presence or absence of nanodroplets, and qPCR experiments had two technical replicates for each of the three biological replicate samples using three primer sets



**Figure 4.** ChIP–qPCR results using traditional sonication compared to using nanodroplets. Control (dots) and rapamycin-treated (triangles) cells were sonicated with and without nanodroplets ( $n = 3$ ). (A) The peak base pair (bp) size of each input sample without (traditional, red; CV = 72%) or with (nanodroplets, purple; CV = 27%) nanodroplets was measured by Agilent TapeStation instrumentation. The mean peak bp size for each condition is indicated by a horizontal black line. (B) ChIP–qPCR was performed using primers positioned along the reporter gene locus as indicated. (C) Rapamycin-treated cells were compared to control cells with the traditional and nanodroplet sonication methods using antibodies for H3K9me3 and H3K4me3 (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.005$ ; \*\*\*\* $p < 0.001$ ).

spanning the CiA:Oct4 locus (Figure 4B). Following recruitment of HP1 $\alpha$  by treatment of cells with rapamycin, H3K4me3 enrichment was significantly decreased at all CiA:Oct4 locus regions tested. As expected, the opposite trend was observed for H3K9me3 levels following HP1 $\alpha$  recruitment (Figure 4C). Unlike the ChIP–qPCR results for HEK 293T cells (Figure 3D), the ChIP–qPCR results for our mESC CiA:Oct4 system<sup>13</sup> recapitulated previous findings equally well for samples treated with or without nanodroplets. Overall, our results indicate that the addition of a nanodroplet cavitation enhancing reagent to the sonication mixture does not alter the conclusions of previously published data in HEK 293T cells or the mESC CiA:Oct4 system<sup>13</sup> but does allow for a 16-fold increase in chromatin fragmentation efficiency and a 2.7-fold reduction in chromatin fragmentation variance in the inducible mESC CiA:Oct4 system.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.8b00075.

Flow cytometry gating scheme, agarose gels for sonication time titrations and ChIP input samples, histogram plots for all samples analyzed by Agilent TapeStation, and primer sequences used for ChIP–qPCR assays (PDF)

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### Notes

The authors declare the following competing financial interest(s): Authors Samantha Pattenden and Paul Dayton of this publication have equity ownership in Triangle Biotechnology, Inc., to which the following technologies used or evaluated in this paper have been licensed: nanodroplets (U.S. Patent 9,427,410 and U.S. Patent Application US14/432,747). Authors Samantha Pattenden and Paul Dayton are inventors of U.S. Patent Application US14/432,747, and author Paul Dayton is inventor of U.S. Patent 9,427,410.

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