



## time-ChIP: A Method to Determine Long-Term Locus-Specific Nucleosome Inheritance

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### Abstract

Understanding chromatin dynamics is essential to define the contribution of chromatin to heritable gene silencing and the long-term maintenance of gene expression. Here we present a detailed protocol for time-ChIP, a novel method to measure histone turnover at high resolution across long timescales. This method is based on the SNAP-tag, a self-labeling enzyme that can be pulse labeled with small molecules in cells. Upon pulse biotinylation of a cohort of SNAP-tagged histones we can determine their abundance and fate across a chase period using a biotin-specific chromatin pulldown followed by DNA sequencing or quantitative PCR. This method is unique in its ability to trace the long-term fate of a chromatin bound histone pool, genome wide. In addition to a step by step protocol, we outline advantages and limitations of the method in relation to other existing techniques. time-ChIP can define regions of high and low histone turnover and identify the location of pools of long lived histones.

**Key words** Epigenetic memory, Histone variants, Chromatin dynamics, SNAP-tag, time-ChIP, Turnover

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## 1 Introduction

### 1.1 *State of the Art*

The nucleosome is the basic unit of chromatin which contributes to the maintenance of gene expression states in the form of histone variants and modifications [1]. There is evidence for such specific chromatin structures to be heritable [2–6]. To achieve this, histones can either be stably retained in chromatin in cis across multiple cell division cycles or key modifications can be copied to new molecules with high rates ensuring stability of the mark. Mathematical modeling has shown that, in fact, a bistable chromatin state can be achieved even if individual chromatin components are highly dynamic [7]. On the other hand experimental work in

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human cells has shown that replenishment of silent chromatin marks following genome duplication is a slow process that can take until the next cell cycle [8]. Much attention has been given to histone modifications and their regulation but in order to understand how histones can act as carriers of epigenetic information it is necessary to understand their association and dissociation rates in relation to the duration of the cell cycle. Histone H3 variants have a central role in epigenetic maintenance [9, 10]. Therefore, the dynamic property of these histones at genes is key to understanding the maintenance of gene expression states. We will review the currently available methods that have been used to assess histone dynamics in cellular chromatin. Two distinct types of approaches exist, one probing the behavior of bulk chromatin, the other gives insight into nucleosome kinetics at specific loci.

## **1.2 Methods to Measure Dynamics of Bulk Chromatin**

Classic experiments that assess dynamics of bulk chromatin initially exploited pulse-chase labeling of proteomes with radioactive and stable isotope-labeled amino acids followed by formaldehyde cross-linking and mononucleosome purification [11]. These experiments demonstrated that the core H3/H4 heterotetramer is more stable in chromatin than the H2A/H2B dimers whose high turnover is dependent on replication and transcription [11].

With the development of modern mass spectrometers, these pulse-chase experiments were taken a step further. Quantitative pulse SILAC (stable isotope labeling by amino acids in cell culture) experiments combined with force expression of tagged histones and purification of mononucleosomes confirmed that the H2A/H2B dimers are dynamic in chromatin but also distinguished between different H3 histone variants where H3.3 turns over faster than its canonical homolog H3.1. The authors additionally uncovered that a significant proportion of H3.3-H4 tetramers undergo splitting during the cell cycle in sharp contrast to the H3.1-H4 complex [12]. A more recent variant of a mass spectrometry-based measurement of chromatin dynamics was established by combining pulse SILAC with nascent chromatin capture. This elegant strategy uses biotin-dUTP labeling of nascent DNA coupled to strong crosslinking to purify recently replicated chromatin in synchronized cells [13]. This analysis confirmed that bulk chromatin is largely recycled onto daughter DNA during replication. By tracking turnover rates of specific, modified peptides a subsequent study determined the posttranslational modification status of histones which showed that some modifications such as H3K36me1 are acquired almost immediately after replication, while others (e.g., H3K9me3 and H3K27me3) are not fully replenished until the following cell cycle [8].

An orthogonal approach to determining bulk chromatin dynamics in living cells has been to use fluorescently tagged histones under the control of a heat-shock promoter in *Drosophila*

*melanogaster*. This approach has been used to determine the chromatin incorporation dynamics of several histone variants in cell lines. In particular, the centromeric H3 variant, CID [14] and H3.3 [15] were both shown to assemble into chromatin outside of S phase using this strategy.

A more sophisticated approach was used more recently to determine H3 histone kinetics in living flies. The system uses a histone variant fused to a fluorescent protein in a way that after induction of a recombinase, one fluorescent tag is exchanged with another (i.e., green to red switch). The approach offered both spatial (the histone fusion is under a tissue-specific Gal4 driver), and temporal control (the recombinase is induced by heat-shock). By employing this system in the *D. melanogaster* male germline asymmetric cell division was shown to strongly correlate with asymmetric distribution of the H3.1 but not the H3.3 histone variant [16].

The above strategies depend on induction of protein synthesis resulting in a relatively long temporal delay before dynamics can be assessed. To avoid this drawback, photo bleaching (fluorescent recovery after photo bleaching, FRAP) of chromatin-bound histones tagged with fluorescent proteins can be used. Determining the rate of fluorescence recovery following a bleach pulse provides a measure of the rate of histone reincorporation within the bleached nuclear area. These experiments measured recovery rates up to a few hours and confirmed that H2B turns over much faster than the core H3 and H4 histones [17].

Alternatively, photo switchable or photo activatable protein variants have been used to allow fluorescence activation of a cohort of molecules. In this case, rather than determining association kinetics, the dissociation rate of pulsed molecules is followed over time. The technology was applied to the centromeric H3 histone variant (CENP-A) to show that it has extremely slow turnover at the centromere while it is quite dynamic at other loci [18]. A similar strategy was used for the H2A histone to show that the turnover of this protein in chromatin slows down after DNA damage [19].

While fluorescence bleaching or activation techniques follow local cohorts of molecules in cells, an alternative pulse labeling method has been developed to label whole-cell populations of proteins based on the SNAP-tag. SNAP is a self-labeling enzyme derived from the human O<sup>6</sup>-alkylguanine DNA alkyltransferase, a DNA repair enzyme that de-alkylates guanines in DNA by transfer of the alkyl group to a reactive cysteine within the enzyme. This suicide-mode of SNAP is engineered to catalyze its own covalent binding to cell permeable O<sup>6</sup>-benzylguanine (BG) derivatives [20]. During this reaction a benzyl-ring is transferred to SNAP in a covalent and irreversible manner conjugating a label such as a fluorescent dye to the substrate [20–22]. We and others have extensively employed the SNAP-tag for fluorescent labeling of the centromere-bound histone H3 variant CENP-A [23–30]. By pulse labeling

total cellular CENP-A followed by a chase we can determine the turnover rate of this histone. Because cells are labeled and chased in culture before being processed for imaging, one can follow populations of cells over long time periods. While photo bleaching and photo activating methods are applicable for short timescale (fast turnover measurements) SNAP-tag pulse labeling complements these at the long timescale range. Using this strategy, we showed that CENP-A exhibits extreme stability in chromatin with no detectable turnover during multiple mitotic divisions [18, 30].

In summary, bulk biochemical methods or those based on fluorescence indicate that histones can be long lived in chromatin and that different nucleosome components have different dynamics in living cells. However, these methods do not provide any positional information or genomic spatial resolution which is restricted by the optical resolution limit. Below we describe complementary approaches that determine dynamics at specific loci, a parameter that is crucial to be able to link nucleosome stability to any role in the control of gene expression.

### **1.3 Methods to Measure Locus- Specific Chromatin Dynamics**

Defining locus-specific histone turnover is typically reliant on protocols based on chromatin immunoprecipitation (ChIP) or related chromatin pull-down methods. Initial efforts used inducible promoters in budding yeast coupled to ChIP to measure incorporation rates across the genome [31]. In this early insightful study, a Myc-tagged version of H3 histone was constitutively expressed while a second FLAG-tagged version was pulse inducible. The ratio of the ChIP signal of the two differentially tagged histones was determined genome-wide and showed that histone exchange on genes is dependent on transcription and that promoters are the regions with the highest histone incorporation rates [31]. A similar strategy was used in human cells. The H3.3 histone variant was force expressed either as an HA or FLAG-tagged version in a sequential manner to distinguish between the old and new pools of H3.3 proteins. Next, mononucleosomes were isolated and subjected to two subsequent chromatin immunoprecipitation steps for HA and Flag tags. In this way nucleosomes carrying both old and new H3.3 were purified and bound DNA was sequenced. This approach detected hybrid nucleosomes on active genes as well as on cell-specific enhancers which showed the highest degree of H3-H4 tetramer splitting events [32] as was previously shown to occur in yeast [33].

An elegant complementary approach named CATCH-IT (Covalent attachment of tags to capture histones and identify turnover) [34] is based on pulse labeling of endogenous proteins without the need for overexpression or tagging. The nascent proteome is pulse labeled using a substitute of methionine (azido-homoalanine, Aha). After a chase period a cycloaddition reaction of biotin is performed, followed by high salt chromatin isolation to strip any non-histone proteins and pull down of biotin labeled

nucleosomes using immobilized streptavidin. This, combined with genome-wide analysis, provides association rates of histones per locus. The results showed that histones incorporate preferentially at active genes, enhancer elements, and origins of replication, and that the rate of their turnover correlates with gene expression [34] as previously suggested and shown [31, 35, 36]. The caveat of this approach is that no specific histone variants can be analyzed. Further, because this method is based on measuring dynamics of nascent proteins at short time scales it is not suited for detection of stable pools of nucleosomes.

A methodology developed in the yeast *Saccharomyces cerevisiae* circumvents the limitation of analyzing incorporation rates and allows detection of turnover rates of ancestral pools of specific histones. This system called recombination-induced tag exchange (RITE) uses a constitutively epitope-tagged version of H3 at its endogenous genomic locus. The locus is engineered such that upon activation of Cre recombinase (controlled by  $\beta$ -estradiol) one epitope tag is genetically and irreversibly exchanged with another one [37], an approach similar to the one, described above, to monitor histone dynamics during asymmetric cell division of the *D. melanogaster* male germ cells [16]. The RITE system applied to HA/T7 tag exchange on histone H3 revealed replication independent histone replacement with preferential retention of old H3 pools at 5' ends of long poorly transcribed genes and proposed an RNA PolII dependent retrograde movement of nucleosomes on genes [38]. Interestingly, a proportion of H3 histones at these loci were shown to survive through multiple cell division cycles.

In summary, the methods described above, each with their specific advantages, have provided insight into several fundamental features of chromatin. Nevertheless, they suffer from specific drawbacks. For example, bulk (mass spectrometry or microscopy-based) measurements cannot directly link the dynamic behavior of nucleosomes to gene expression status. The locus-specific methods can be linked to gene expression but they suffer from transcription and translation delays (inducible genes). Further, when analyzing a short pulse of nascent proteins, the assay is biased to the detection of regions with fast turnover and is blind to the long-term retention of an ancestral histone pool (CATCH-IT). The RITE system offers a solution to the latter problem, however it also suffers from a delayed response to Cre induction and, as of yet, cannot be used in mammalian systems due to lack of efficiency and strict control of Cre recombinase.

#### 1.4 time-ChIP

Here, we advance and complement the existing techniques by developing and employing a novel method that allows for the detection of nucleosome retention at high resolution in human cells at long time-scales. Rather than analyzing histone incorporation rates, this method aims to measure the stability of previously

incorporated ancestral histones. The cornerstone of the method is the self-labeling SNAP-tag. As outlined above, SNAP can be pulse labeled in cells, typically by using fluorescent dyes coupled to imaging [20–22]. Here, we modify this approach by developing a pulse-chase affinity purification strategy, based on a biotin-conjugated SNAP substrate (Fig. 1a). Biotin-mediated pulse labeling of SNAP-tagged histones allows us to isolate, and directly measure, histone retention in chromatin at specific loci in human cells. We call this method time-ChIP as the pull down strategy is akin to a chromatin immunoprecipitation (ChIP) experiment with the added temporal component to determine dynamics of nucleosome occupancy. In brief, following pulse labeling of living cells, the excess label is washed out, cells are chased, nuclei are isolated, and chromatin is enzymatically fragmented. Soluble biotinylated chromatin is then isolated on immobilized streptavidin and processed for quantitative PCR or sequencing (Fig. 1b).

### 1.5 Biotinylated SNAP Substrates

SNAP biotinylation can be performed using commercially available BG-Biotin (New England Biolabs) (Fig. 2a). Recently, we have developed an improved substrate to achieve more efficient and specific SNAP biotinylation in chromatin in living cells. This optimized substrate (CP-Biotin; Fig. 2b) differs from the established BG-Biotin by its CP (chloropyrimidine) scaffold which has been shown to be more permeant to cell membranes than BG derivatives [39] and results in improved biotinylation of histones in chromatin (Fig. 2c). CP-Biotin was synthesized by reacting CP-NH<sub>2</sub> [39] with biotin amidohexanoic acid *N*-hydroxysuccinimide ester as described [21, 40, 41] (Fig. 2b) (*see Note 1* for detailed methodology).

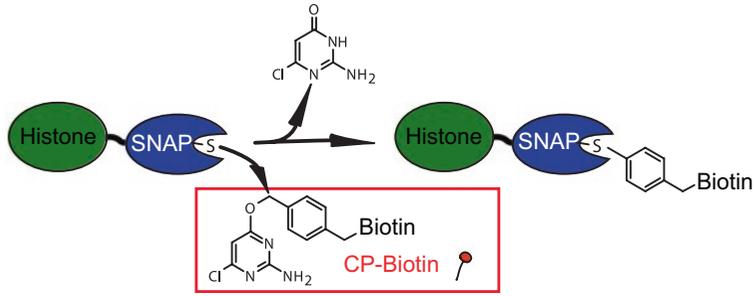
### 1.6 Outline and Initial Characterization of the Method

To perform chromatin dynamics measurements with time-ChIP, HeLa cell lines stably expressing H3.1-SNAP or H3.3-SNAP [28, 30] are *in vivo* pulse labeled with BG-Biotin (or CP-Biotin), followed by a chase period to allow for histone turnover. At specific times following pulse labeling, cells are subjected to gentle lysis to isolate nuclei. These nuclei are then MNase treated and soluble chromatin is isolated. Histone-SNAP-biotinylated nucleosomes are then captured on immobilized streptavidin beads and DNA is

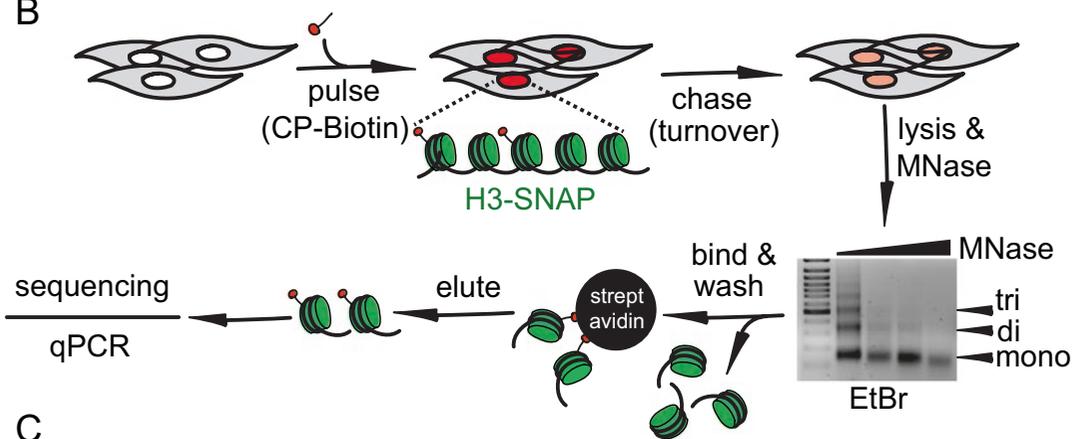
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**Fig. 1** (continued) some decays over time. At specific time points, cells are lysed, nuclei are isolated and chromatin is liberated by MNase treatment resulting in soluble chromatin of 1–3 nucleosomes in length. Biotinylated nucleosomes are isolated and purified on streptavidin beads. **(c)** CP-Biotin treatment results in a biotinylation dependent pulldown of SNAP-tagged histone. HeLa H3.3-SNAP-3XHA cells were pulse biotinylated or left untreated and soluble chromatin was isolated on streptavidin beads. Immunoblot probed for the H3.3-SNAP-3XHA protein by anti-hemagglutinin (HA) antibody. In—Input; Un—Unbound; B—Bound. **(d)** Dose-response of biotinylation. Biotinylated cells were mixed with unbiotinylated cells at indicated percentages processed as outlined in panel B and total DNA yield was quantified by PicoGreen. Genomic DNA that is recovered correlates with degree of biotinylation ( $n = 3–6$  exp.; bars are SEM)

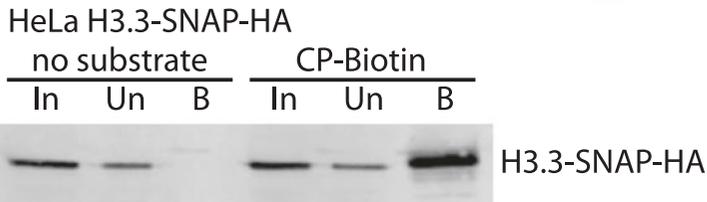
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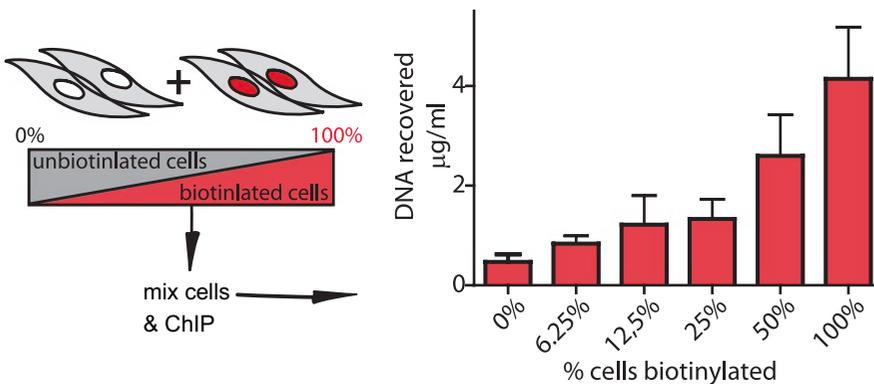
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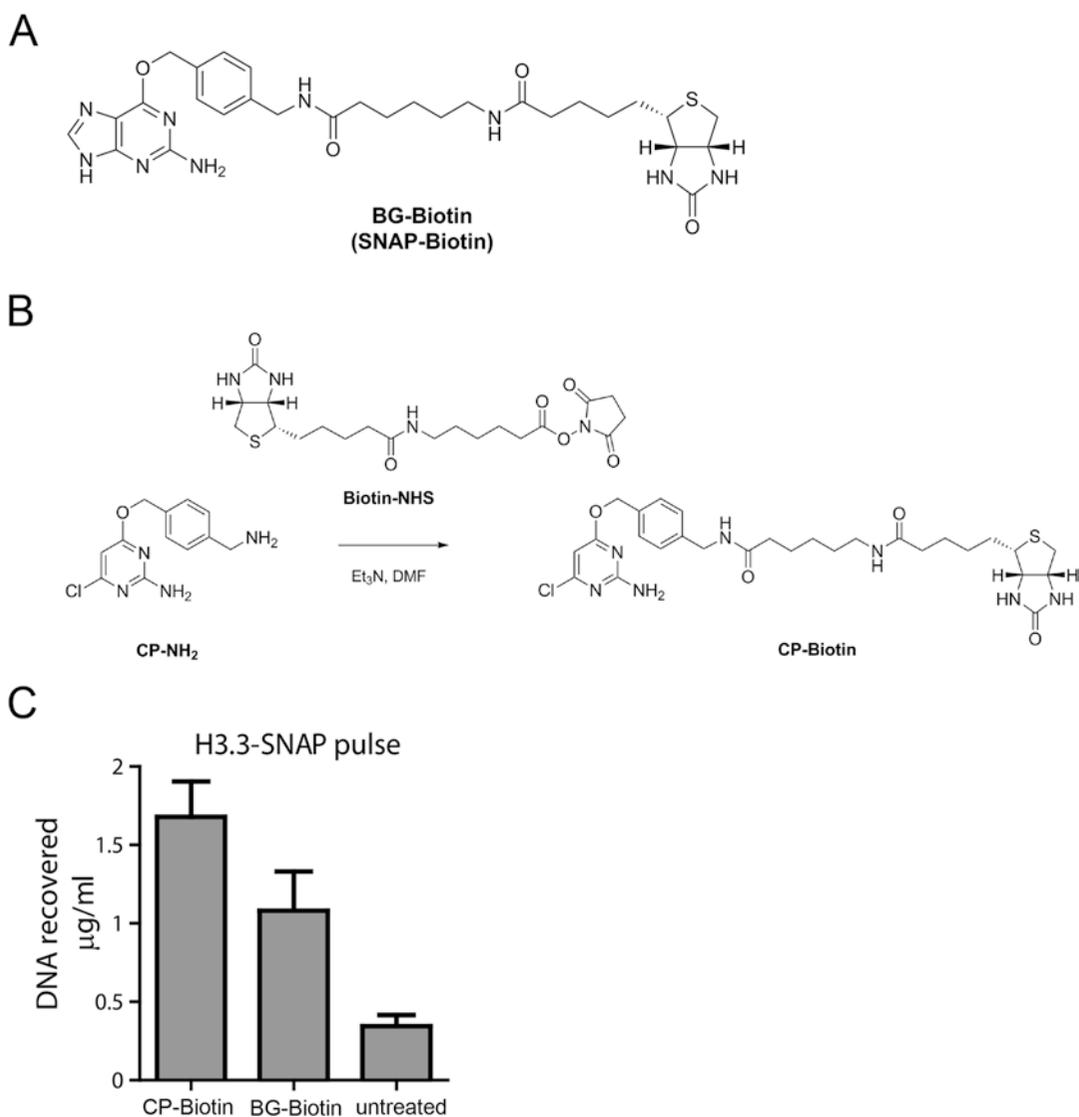
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**Fig. 1** time-ChIP, a novel method to determine chromatin retention at high resolution, genome-wide. (a) Reaction scheme of SNAP with CP-Biotin resulting in the covalent self-labeling of SNAP with biotin through a reactive cysteine (S). (b) Outline of pulse-chase time-ChIP assay. Cells expressing SNAP-tagged histone are pulse labeled with CP-Biotin. Following a chase period, the fraction of biotinylated histones retained at nucleosomes is determined by lysis and MNase digestion. The resulting DNA fragments are analyzed by qPCR and EtBr staining. (c) Western blot analysis of H3.3-SNAP-HA levels in HeLa cells treated with or without CP-Biotin. (d) Bar graph showing DNA recovered (µg/ml) versus % cells biotinylated.



**Fig. 2** Synthesis of CP-Biotin. Chemical structures of (a) BG-Biotin (commercially available as SNAP-Biotin, New England Biolabs (NEB), Ipswich, MA). (b) Benzylchloropyrimidine-NH<sub>2</sub> (CP-NH<sub>2</sub>), biotin amidohexanoic acid N-hydroxysuccinimide ester (Biotin-NHS) and CP-Biotin. CP-Biotin was synthesized by reacting CP-NH<sub>2</sub> with Biotin-NHS in the presence of anhydrous N,N-dimethylformamide (DMF) and trimethylamine (Et<sub>3</sub>N). (c) Comparison of labeling efficiency of BG-Biotin (NEB) and CP-Biotin (this study). time-ChIP pulse labeling of HeLa cells expressing H3.3-SNAP with either CP-Biotin, BG-Biotin or DMSO control. Chromatin was liberated by MNase treatment and isolated in a biotin dependent manner as described for Fig. 1. Recovered DNA was quantified by PicoGreen measurement. Averages and SEM are plotted from three experiments

purified. Quantification of biotin histone retention at specific loci is determined by quantitative PCR or sequencing (Fig. 1b). Western blot analysis shows SNAP-HA-tagged histones are specifically isolated in a biotin pulse label dependent manner (Fig. 1c). To determine the dynamic range of the assay we mix in vivo

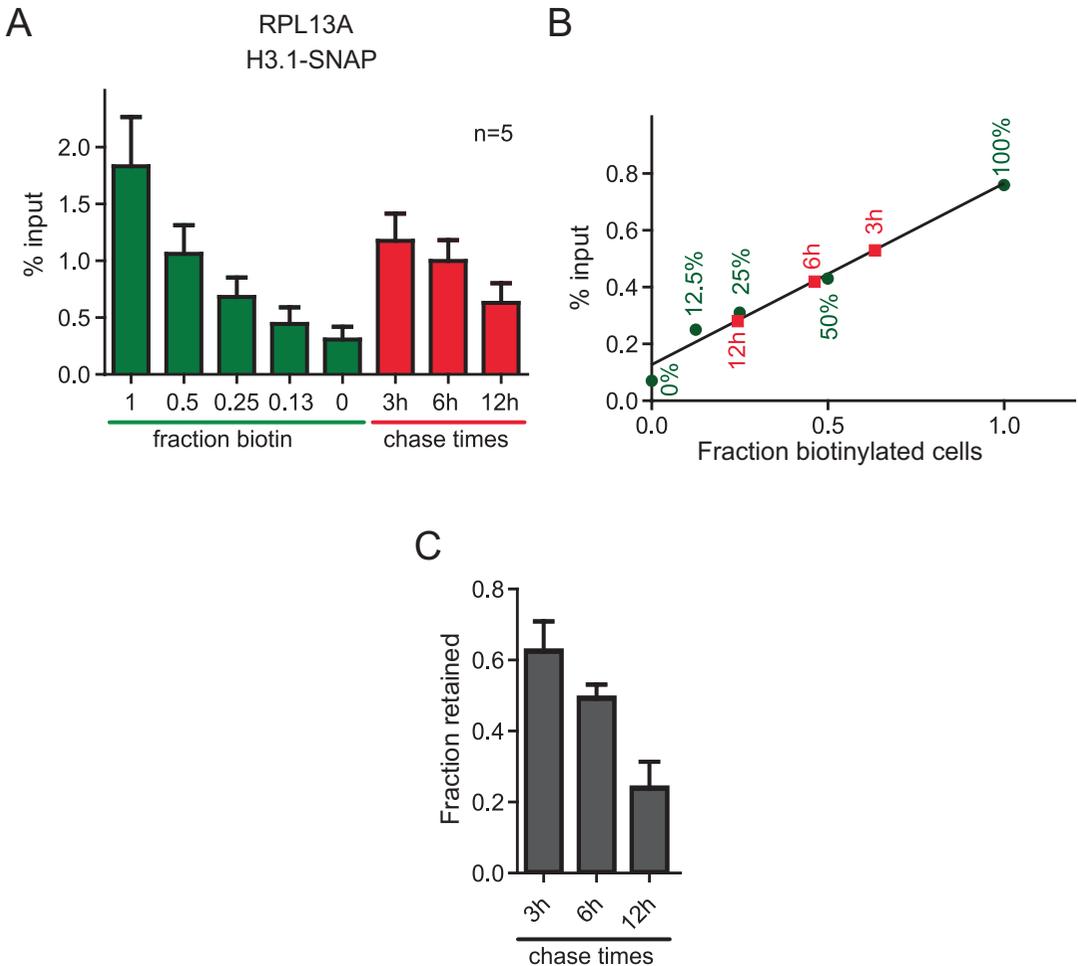
labeled HeLa H3.1–SNAP cells with unlabeled cells at different ratios. The genomic DNA that we recovered correlates with the degree of biotinylation and allowed us to detect as little as ~6% histone retention (Fig. 1d).

### 1.7 *time-ChIP-qPCR*

Analysis of biotin-dependent isolated DNA can be performed either by qPCR or sequencing methods. For qPCR we use standard SYBR green protocols with the following key adaptations in order to determine the linearity and dynamic range at which histone retention can be measured. First, a dilution series of input DNA is made to determine the linear range of the PCR reaction. Next the percentage input (% input) is calculated by subtracting the cut off cycles for the measured IP from the corrected input as presented in detail with the formula at Subheading 3.7, **step 8**. In addition to the qPCR standard curve, a time-ChIP standard curve is generated for each pulse-chase experiment to determine the dose-response of the qPCR to the fraction of biotinylated nucleosomes. This determines how much DNA is recovered at different chase time points relative to the initial pool present at the pulse. To this end, we mix known ratios of biotin-pulse labeled and non-labeled cells at the beginning of the protocol in a 2-fold ratio series. Cells are then lysed, MNase treated and subjected to biotin pulldown as described in detail in the Methods section. A linear relationship between IP values and the level of chromatin biotinylation is expected. In Fig. 3, a typical result is shown where dilution of biotin-pulse labeled cells expressing H3.1-SNAP results in a correspondingly reduced % input signal. In Fig. 3a, an example of an active gene locus (RPL13) is shown that is probed with a specific primer set hybridizing to a coding exon. Dilution of biotinylated chromatin results in a corresponding reduction in IP signal. This is used to produce a standard curve upon which chase signals are interpolated (Fig. 3b) resulting in a measure of histone retention expressed as a fraction of the signal present at steady state (Fig. 3c).

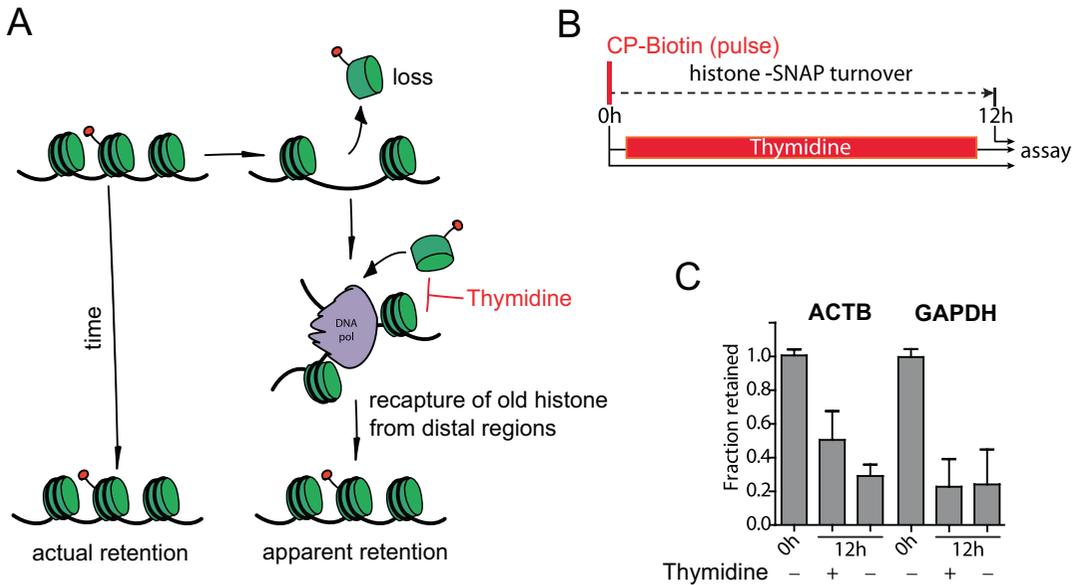
### 1.8 *H3.1-SNAP Is Locally Retained in Chromatin*

time-ChIP-qPCR measurements of ancestral H3.1-SNAP show that a proportion of H3.1 can be retained in cis on the DNA even during continued transcription and replication (Fig. 3c). Presumably DNA unwinding during these processes results in nucleosome disruption. Histone retention would therefore involve sliding or local recapture of ancestral histones. However, it is formally possible that nucleosomes are lost and recaptured distally in trans. This would result in “apparent” retention rather than actual in cis stability of nucleosomes (Fig. 4a). Such recapture of old histones in trans is unlikely because the soluble pool size is a small fraction of the total chromatin bound pool. Therefore, recapture of histones would represent a minor part of the biotin signal in chromatin. Indeed, histones with a chromatin signature are not detected in the soluble histone pool [42]. Nevertheless, we directly tested whether the apparent histone retention signal could be the



**Fig. 3** time-ChIP-qPCR analysis quantifies histone retention rates. **(a)** HeLa cells expressing H3.1-SNAP were pulse labeled and mixed with mock labeled cells at indicated ratios (1, 0.5, 0.25, etc). A proportion of pulse labeled cells were chased for 3, 6 and 12 h before being processed for histone-biotin purification. Biotin:mock labeled cell mixtures (green bars) and chase samples (red bars) were analyzed by qPCR for the active house-keeping locus RPL13A and % input was determined. IP efficiency correlates with the degree of biotinylation. Mean and SEM of five replicate experiments are shown. **(b)** Example of linear regression of time-ChIP standard curve derived from biotin:mock labeled cell mixtures. Chase time points are interpolated on the regression line to obtain corresponding “fraction biotin” values. A plot of a single replicate is shown. **(c)** For each replicate the fraction of biotin retention is determined by interpolation to the time-ChIP standard (obtained independently for each replicate). Mean and SEM of retention values are plotted for each chase time point. In this example, H3.1-SNAP is present at 62%, 48%, and 24% after 3, 6, and 12 h respectively

result of dynamic reincorporation. H3.1 assembly is strictly dependent on DNA synthesis [15, 28]. Therefore, blockage of DNA replication will prevent incorporation of histone H3.1. As a consequence, if the observed H3.1 retention would be the result of reincorporation of distally recycled old histones, then retention would be no longer observed. In contrast, treatment of cells with thymidine to prevent DNA replication-dependent turnover during a

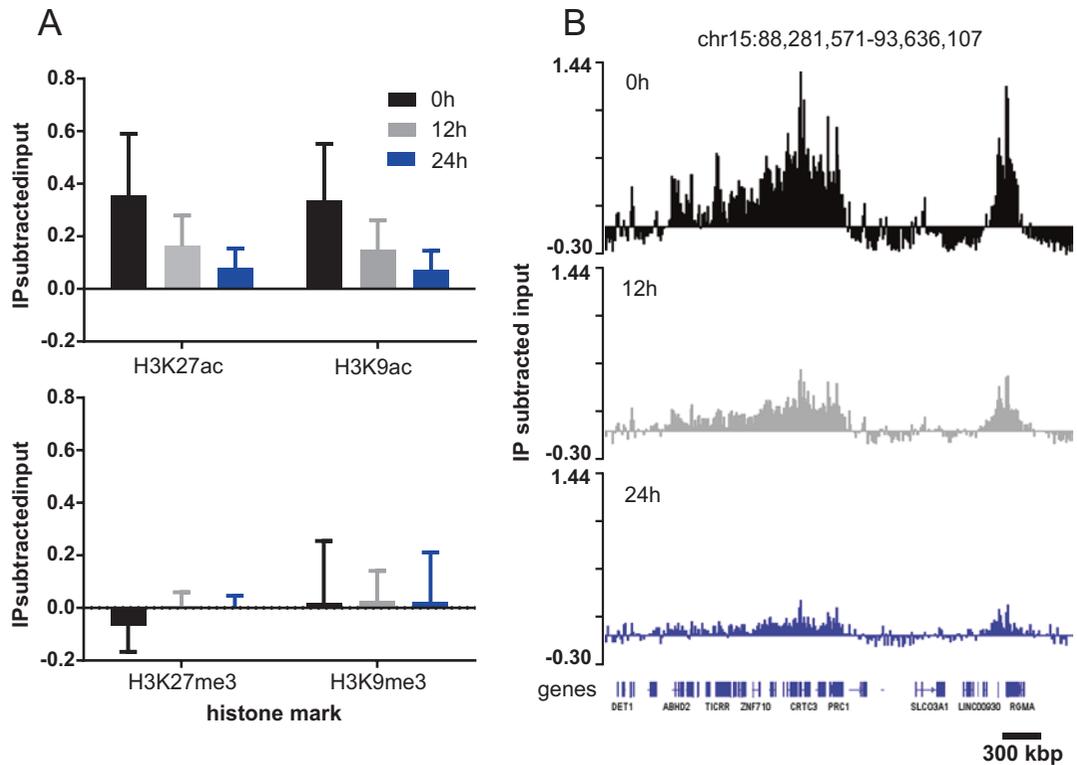


**Fig. 4** H3.1-SNAP is locally retained in chromatin. **(a)** Possible scenarios of nucleosome retention or loss. Biotinylated histones may be detected either following stable retention of nucleosomes or potentially by reincorporation during DNA replication of old histones, excised from other regions of the genome. **(b)** Thymidine prevents DNA replication. H3.1-SNAP cells were treated with thymidine for the duration of the chase period (12 h) preventing replication coupled assembly of H3.1 chromatin. **(c)** time-ChIP-qPCR of H3.1-SNAP cells for ACTB and GAPDH genes for indicated times with or without thymidine-induced blockage of DNA replication. Averages and SEM are plotted from at least three experiments

12 h H3.1-SNAP chase did not result in an elevated histone turnover (Fig. 4b, c), indicating that H3.1 histones, once incorporated, are locally retained in chromatin.

### 1.9 time-ChIP-Seq

To uncover histone dynamics genome wide we coupled our time-ChIP method to high-throughput sequencing. As a proof of concept experiment, we decided to perform the measurement for the H3.3 histone variant as it presents a known characteristic distribution across the genome linked to gene activity [35]. At each time point (0, 12, and 24 h) and for an input sample we sequenced approximately 200 million reads, 50 bp length in single end mode. We mapped the data to GRCh38 reference genome, combined the reads into 1000 bp bins, normalized to read count, and subtracted the input signal. We next intersected the data with active (H3K9ac, H3K27ac) and inactive (H3K9me3, H3K27me3) parts of the genome and found faster exchange to correlate with active marks, indicating that our H3.3-SNAP fusion protein is behaving as expected (Fig. 5a). We also note that, in general, there is less H3.3 accumulation on silent chromatin domains. Direct visualization of H3.3 occupancy as a function of genomic loci, across time shows gradual loss of H3.3 (Fig. 5b). We have recently employed this approach in the context of mouse embryonic stem cells to



**Fig. 5** time-ChIP coupled to high-throughput sequencing. Cells expressing H3.3-SNAP were biotinylated and chased for 0, 12 and 24 h in culture. Biotinylated H3.3-SNAP chromatin was fragmented by MNase, isolated and associated DNA was purified and subjected to Illumina sequencing (EMBL, Heidelberg, Germany). The reads were mapped to GRCh38 and normalized to read count. The input signal was subtracted from the pull down data. **(a)** Processed data for time points 0, 12 and 24 h for four different genomic domains: H3K27ac, H3K9ac representing active chromatin and H3K27me3, H3K9me3 representing silent chromatin. **(b)** Visual representation of time-ChIP-seq data for a genomic window showing histone H3.3-SNAP enrichment and turnover

map H3.3 dynamics genome wide [43]. In that study, we confirmed fast turnover of H3.3 at promoters and enhancers as previously reported [32, 34]. Interestingly, we found that regions of Polycomb activity reduce H3.3 dynamics, correlating with transcriptional silencing. We additionally uncovered novel regions of the genome with rapid histone turnover linked to cellular differentiation which may point to previously uncharacterized enhancers [43]. Overall these results show that the method can be combined with high-throughput sequencing to gain insight into locus-specific chromatin dynamics.

### 1.10 time-ChIP

#### Summary and Comparison with Existing Methods

In this work we describe a novel method we name time-ChIP, capable of measuring local histone dynamics and inheritance with both quantitative PCR as well as high-throughput sequencing. The method has several key advantages:

1. The creation of a labeled pool is not dependent on de novo transcription and/or translation as the existing pools of protein are labeled. However, there is a delay of approximately 1 h due to the SNAP labeling procedure.
2. Both association and long-term dissociation kinetics can be measured when used in quench-chase-pulse or pulse-chase setup, respectively (*see Note 2*).
3. time-ChIP is based on the use of genetically encoded tags allowing specific histones or histone variants to be assessed.
4. It is applicable to mammalian systems and was already used to determine histone dynamics in differentiating mouse ES cells [43].

The main disadvantage of time-ChIP is low efficiency of biotin labeling (due to limited membrane permeability of the substrate) which requires compensation with relatively high cell numbers. We have addressed this problem, in part, by developing an enhanced biotin label (CP-Biotin, Fig. 2).

In Table 1 we list an extensive comparison of time-ChIP with existing methods and outline the specific features of each strategy.

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## 2 Materials

### 2.1 Specialized Equipment

1. Dounce homogenizer, 15 mL volume, tight pestle.
2. Orbital shaker.
3. Magnetic stand designed for 1.5 mL tubes.

### 2.2 Culture Media

1. HeLa cell culture medium: DMEM (Dulbecco's Modified Eagle's medium High Glucose w/o L-Glutamine w/o Sodium Pyruvate), 10% newborn calf serum, 2 mM L-Glutamine, 100 µg/mL Penicillin, 100 µg/mL Streptomycin.

### 2.3 Buffers

1. Dulbecco's Phosphate-buffered saline (D-PBS) w/o Calcium, w/o Magnesium, cell culture grade.
2. Cell homogenization buffer: 3.75 mM Tris-HCl pH 7.5, 20 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 0.05 mM Spermidine, 0.125 mM Spermine, 0.1% Digitonin (recrystallized from 50% purity, *see Note 3* for recrystallization protocol), 1 mM PMSF, protease inhibitor cocktail.
3. Washing buffer A: 20 mM HEPES sodium salt pH 7.7, 20 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, protease inhibitor cocktail.
4. Washing buffer B: 20 mM HEPES sodium salt pH 7.7, 20 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, protease inhibitor cocktail, 300 mM NaCl.

**Table 1**  
**Comparison of methods to analyze histone dynamics**

Feature		Applicable to animal cells						Other comments	References
Method	Timescale	Association kinetics	Dissociation kinetics	Histone variants	Locus specific	Genetic perturbation	Specialized small molecules		
Pulse labeling with radioactive and stable isotopes	Minutes to days	Yes <sup>b</sup>	Yes	No	No <sup>i</sup>	No	Radio and density labeled amino acids	Early method to study chromatin dynamics	[11]
Pulse labeling with stable isotopes combined with mass spectrometry	Minutes to days	Yes <sup>b</sup>	Yes	Yes	No	No	Density labeled amino acids, inducers of gene expression, biotin-dUTP <sup>k</sup>	Variations of the method exist with diverse purification steps	[8, 12, 13]
Florescence measurements after photo bleaching or photo switching	Seconds to minutes	Yes	Yes <sup>e</sup>	Yes	No <sup>i</sup>	Yes	No	Photo toxicity and bleaching limits the time scale at which dynamics can be measured	[17–19]

SNAP-based fluorescent pulse labeling	Minutes to hours	Yes <sup>d</sup>	Yes <sup>e</sup>	No	Yes	Yes	BG- or CP-fluorophore	Ideal for assessing slow dynamics based on imaging. Resolution limited	[21–30]
Covalent attachment of tags to capture histones and identify turnover (CATCH-IT)	Minutes to hours	Yes <sup>b</sup>	Yes <sup>f</sup>	No	Yes	Yes	Aha <sup>1</sup> , biotin-alkyne	Assay is designed to detect fast dynamics, stable chromatin is not detected	[34]
Pulsed expression of tagged histones	Hours to days	Yes <sup>c</sup>	Yes <sup>h</sup>	Yes	Yes	Yes	Inducers of gene expression, $\beta$ -estradiol <sup>m</sup>	RITE <sup>l</sup> uses an engineered, small molecule controlled Cre recombinase	[14–16, 31–33, 35]

(continued)

**Table 1**  
(continued)

Feature										
Method	Timescale	Association kinetics	Dissociation kinetics	Histone variants	Locus specific	Genetic perturbation	Applicable to animal cells	Specialized small molecules	Other comments	References
time-ChIP	Hours to days <sup>a</sup>	Yes <sup>d</sup>	Yes <sup>e</sup>	Yes	Yes	Yes	Yes	BG- or CP-biotin	Requires relatively high cell numbers due to low efficiency of biotin labeling	[43], this study

<sup>a</sup>Faster than pulsed expression of tagged histones

<sup>b</sup>Association dynamics is dependent on the protein translation rate

<sup>c</sup>Association dynamics is dependent on transcription and translation rates

<sup>d</sup>When combined with quench-chase-pulse assay

<sup>e</sup>When photo switchable proteins are used

<sup>f</sup>Dissociation dynamics is limited to the recently incorporated histone pool potentially biasing to fast dynamics

<sup>g</sup>When combined with pulse-chase assay

<sup>h</sup>Steady state cannot be reached with recombination induced tag exchange (RITE)

<sup>i</sup>There is no certainty that the measured histones constitute chromatin

<sup>j</sup>RITE shown only in yeast but equivalent Cre-mediated tag exchange system has been used in *Drosophila melanogaster* [16]

<sup>k</sup>Biotin-dUTP is used for nascent chromatin capture [13]

<sup>l</sup>Aha, azidohomoalanine is a methionine substitute

<sup>m</sup>When using recombination induced tag exchange (RITE) to control Cre recombinase [37, 38]

5. Washing buffer C: 20 mM HEPES sodium salt pH 7.7, 20 mM KCl, 5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, protease inhibitor cocktail, 500 mM NaCl, 0.5% NP-40 (nonyl phenoxypolyethoxyethanol).
6. Beads blocking buffer: 20 mM HEPES sodium salt pH 7.7, 20 mM KCl, 5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, protease inhibitor cocktail, 300 mM NaCl, 0.05% NP-40, 50 mg/mL bovine serum albumin, 200 µg/mL yeast tRNA.
7. RNA removal buffer: 10 mM Tris-HCl pH 7.4, 100 mM NaCl, 10 mM EDTA, 0.5% SDS.
8. TE buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA.

## 2.4 Solutions

1. BG-Biotin (SNAP-Biotin, New England Biolabs) dissolved in DMSO at a concentration of 4 mM stored at  $-80^{\circ}\text{C}$  long term, working stocks are kept at  $-20^{\circ}\text{C}$  (although kept frozen for long-term storage, we do not find the substrate to be temperature sensitive in the short term, hours-days).
2. CP-Biotin: dried compound dissolved in DMSO at a concentration of 4 mM stored at  $-80^{\circ}\text{C}$  (not commercially available).
3. 300 mM  $\text{CaCl}_2$ .
4. 500 mM EGTA.
5. 10% nonyl phenoxypolyethoxyethanol (NP-40).
6. Phenol:chloroform:isoamyl alcohol (25:24:1) solution pH 8.
7. Chloroform:isoamyl alcohol (24:1).

## 2.5 Enzymes

1. Trypsin, cell culture grade.
2. Micrococcal nuclease (MNase) (Roche or equivalent).
3. RNaseA, DNase free.

## 2.6 Antibodies

1. Primary antibody: anti-HA, clone HA11 (Bio-Legend or equivalent).
2. Secondary antibody: anti-mouse IRDye 800CW (Li-COR or equivalent).

## 2.7 Other Materials

1. Streptavidin Magnetic Beads (Pierce or equivalent).
2. MinElute Reaction Cleanup Kit (QIAGEN).
3. Quant-iT PicoGreen dsDNA assay kit (Invitrogen).
4. PerfeCTa SYBR Green FastMix ROX (Quanta Biosciences).
5. NEBNext ChIP-Seq Library Prep Reagent Set for Illumina (NEB).

## 3 Methods

### 3.1 General Considerations

All solutions and equipment in contact with living cells should be sterile and aseptic techniques should be used throughout the procedure.

HeLa cell lines expressing histone-SNAP fusions were cultured in HeLa cell culture medium at 37 °C and 5% CO<sub>2</sub>. Cells were grown to a maximum confluency of 80%. Proliferating cultures were maintained by washing with D-PBS, trypsinization and dilution into fresh culture medium.

DNA vectors containing the SNAP-tag and the protocols for retroviral mediated transduction, monoclonal selection and characterization of cell lines are described in detail in Bodor et al. [29].

Here, we describe a native time-ChIP protocol with chromatin solubilization by MNase treatment. Please note that an alternative protocol with formaldehyde crosslinking and sonication is also applicable (*see Note 4*).

### 3.2 SNAP Pulse-Chase

All operations at this stage of the protocol, including centrifugation steps, should be performed at room temperature to avoid cell stress.

The pulse labeling procedure can be performed with either commercially available BG-Biotin or an improved CP-Biotin label developed by Ivan Correa (New England Biolabs, Ipswich, MA), not commercially available (*see Note 1*).

1. Grow, harvest, and pellet by centrifugation ( $500 \times g$ , 5 min)  $5 \times 10^8$  cells expressing a histone-SNAP fusion of choice. Cell counts can be determined by a Scepter 2.0 Handheld Automated Cell Counter or other methods of choice.
2. Resuspend the cell pellet in 4 mL culture medium supplemented with 10  $\mu$ M BG-Biotin (or the same concentration of CP-Biotin).
3. Incubate the cell suspension for 1 h at 37 °C in a water bath. To ensure proper labeling, mix cells every 10 min.
4. Add 20 mL medium and centrifuge at  $500 \times g$  for 5 min.
5. Wash 2 times with 10 mL culture medium; centrifuge  $500 \times g$  for 5 min between each suspension step.
6. Resuspend the cell pellet in 30 mL culture medium.
7. Count and collect  $5 \times 10^7$  cells. Incubate for 30 min at 37 °C in a water bath, mixing every 10 min (this step allows excess BG-Biotin to be released from cells).
8. Centrifuge the cells at  $500 \times g$  for 5 min.
9. Snap freeze the cell pellet in liquid nitrogen and store at  $-80$  °C until further use (*see Subheading 3.3*).

10. For chase experiments, re-seed the remainder of the labeled cells (all minus  $5 \times 10^7$  collected in Subheading 3.2, step 7) in fresh medium and allow to proliferate for the desired time. Typical time points include 0, 6, 12, 24, 48 h after pulse labeling.
11. At each time point collect  $5 \times 10^7$  cells and harvest as described for Subheading 3.2, steps 8 and 9.

*(protocol can be suspended at this point)*

### 3.3 Soluble Nucleosome Preparation

All subsequent operations should be performed for all samples (pulse and chase time points) in parallel. All steps should be performed at 4 °C or on ice unless stated differently.

1. Before starting, chill centrifuges and following materials to 4 °C or on ice: Dounce homogenizer (15 mL with tight pestle), cell homogenization buffer, washing buffer A and washing buffer B.
2. Resuspend frozen cell pellets ( $5 \times 10^7$  cells) in 15 mL of cell homogenization buffer.
3. Lyse the cells with precooled Dounce homogenizer with 10 strokes. Be careful not to create air bubbles.
4. Centrifuge nuclei at  $500 \times g$  for 5 min and discard the supernatant.
5. Resuspend nuclei in 15 mL of cell homogenization buffer.
6. Repeat the homogenization step as in Subheading 3.3, step 3.
7. Centrifuge the lysate at  $500 \times g$  for 5 min and discard the supernatant.
8. Wash the nuclei twice with 15 mL of washing buffer A and centrifuge at  $500 \times g$  for 5 min.
9. Resuspend the pellet in 7.5 mL of washing buffer B.
10. Centrifuge nuclei at  $500 \times g$  for 10 min at 4 °C.
11. Resuspend in 500  $\mu$ L of washing buffer B and transfer to a 1.5 mL tube.
12. Add  $\text{CaCl}_2$  to a final concentration of 3 mM (5  $\mu$ L from 300 mM stock).
13. Add MNase to final concentration of 800 U/mL (the correct MNase concentration may need to be experimentally defined, see Note 5).
14. Incubate in an orbital shaker at room temperature for 1 h.
15. Add EGTA to final concentration of 5 mM to stop the reaction (5  $\mu$ L from 500 mM stock).
16. Add NP-40 to final concentration of 0.5% and mix to solubilize nuclear membranes (25  $\mu$ L from 10% stock).
17. Centrifuge the lysate at  $10,000 \times g$  for 15 min at 4 °C.

18. Transfer the supernatant (containing solubilized chromatin) to a fresh 1.5 mL tube. Store the pellet fraction as a control, *see Note 6*.
19. Determine the concentration, i.e., optical density (OD) of soluble chromatin at 276 nm. A standard spectrophotometer such as NanoDrop can be used for this purpose.
20. Normalize sample to a final concentration of 1 OD<sub>A276</sub>, with washing buffer B, ensuring a final volume of at least 400  $\mu$ L.
21. Collect a 30  $\mu$ L input sample for DNA isolation at Subheading 3.6 (this is an essential step required for downstream analysis).
22. Collect a sample of 10  $\mu$ L for agarose gel analysis of MNase treated DNA.
23. Optionally a 10  $\mu$ L sample can be taken for Western Blot analysis to determine the efficiency of the pull down in Subheading 3.5 (*see Note 7*).
24. Store the rest of the chromatin sample (about 350  $\mu$ L) at  $-80^{\circ}\text{C}$  until Subheading 3.4.
25. Purify the DNA from the sample collected in Subheading 3.3, **step 22** as in Subheading 3.6 and separate DNA fragments by standard agarose gel electrophoresis to assess the efficiency of MNase digestion. The majority of DNA should be digested to mononucleosome fragments (*see Fig. 1b*). If the DNA is under- or over-digested, MNase concentration should be optimized (*see Note 5*).

*(protocol can be suspended at this point)*

### **3.4 Purification of Biotin-Labeled SNAP-Tagged Nucleosomes**

All operations in this step of the protocol should be performed for all chase time points in parallel.

1. Transfer 20  $\mu$ L (0.2 mg) of homogenized streptavidin magnetic beads into a 1.5 mL tube per sample (pulse or chase time point). Do not allow beads to dry as this greatly reduces their binding capacity.
2. Place the tubes into a magnetic stand designed for 1.5 mL tubes to concentrate beads at the tube wall and remove the supernatant.
3. Wash the beads with 1 mL of washing buffer A for 5 min with rotation on an orbital shaker and remove the supernatant by using the magnetic rack.
4. Repeat washing step with 1 mL of buffer A as in Subheading 3.4, **step 3**.
5. Repeat washing step with 1 mL of buffer C.
6. Repeat washing step with 1 mL of buffer B.
7. Add 300  $\mu$ L of beads blocking buffer and incubate 1 h at  $4^{\circ}\text{C}$  with rotation on an orbital shaker.

8. Carefully remove the supernatant by using the magnetic rack.
9. Add 310  $\mu\text{L}$  of purified nucleosomes at a concentration of 1 OD<sub>A276</sub> (from Subheading 3.3, 24) and incubate overnight at 4 °C with rotation.
10. The next day, place the tubes on a magnetic rack, allow the beads to concentrate and collect the unbound fraction. Store this fraction as a control, *see* **Note 7**.
11. Wash the beads 3 times with 1 mL washing buffer B for 5 min at room temperature with rotation on an orbital shaker.
12. Between every wash step remove the supernatant on a magnetic rack.
13. Add 310  $\mu\text{L}$  of RNA removal buffer supplemented with 100  $\mu\text{g}/\text{mL}$  of RNaseA to the samples and 270  $\mu\text{L}$  of the same buffer to the previously collected input sample (*see* Subheading 3.3, **step 21**).
14. Incubate the samples for 10 min at room temperature.
15. Resuspend the samples with the beads and collect 10  $\mu\text{L}$  for western blot analysis. This step is required to assess the efficiency of the biotin pulldown.
16. Freeze the rest of the beads as well as the input samples (300  $\mu\text{L}$  each) in  $-80$  °C for further processing.  
*(protocol can be suspended at this point)*

### 3.5 Immunoblotting

In brief, to assess the efficiency of the biotin pulldown, samples (collected at Subheading 3.4, **step 15**) can be separated by SDS-PAGE and probed by immunoblotting using standard procedures. Our SNAP-tagged constructs carry a triple hemagglutinin (HA) epitope tag for this purpose. Samples can be separated on an SDS-PAGE gel (8–12%, depending on the expected size. For histones we typically use 12%) and transferred to Hybond PVDF membranes. Blots are probed with a monoclonal mouse anti-HA antibody at 1:2000 dilution and an anti-mouse secondary antibody at 1:10000 dilution. Fluorescence is detected and quantified with an Odyssey Image Analyzer. Alternatively, standard HRP-conjugated secondary antibodies and ECL detection on photosensitive film can be used.

*(protocol can be suspended at this point)*

### 3.6 DNA Purification

1. Add 300  $\mu\text{L}$  of phenol:chloroform:isoamyl alcohol (25:24:1) solution (use buffered phenol at pH 8, *see* **Note 8**) to samples collected in Subheading 3.4, **step 16** and mix by inverting the tubes.
2. Centrifuge at  $20,000 \times g$  for 5 min at room temperature.
3. Remove the upper aqueous phase and transfer to a new tube.
4. Add 300  $\mu\text{L}$  of chloroform:isoamyl alcohol (24:1) solution and mix.

5. Centrifuge at  $20,000 \times g$  for 5 min at room temperature.
6. Remove the upper aqueous phase and purify the DNA with Qiagen MinElute Reaction Cleanup Kit according to the manufacturer's instructions (the use of this kit is required to obtain sufficient DNA purity for subsequent high-throughput sequencing steps).
7. Elute DNA in 30  $\mu$ L TE buffer.
8. Determine the concentration of the recovered DNA with Quant-iT PicoGreen dsDNA assay kit to assess the efficiency of DNA purification.

*(protocol can be suspended at this point)*

### 3.7 Quantitative PCR

Perform all quantitative PCR reactions with technical triplicates to ensure high accuracy of the measurement.

1. Perform tenfold serial dilutions on purified input samples up to  $10^{-6}$ . This step is required to calculate the percentage input as well as to measure the linear response of the quantitative PCR reaction.
2. Prepare a master mix of PerfeCTa SYBR Green FastMix ROX (Quanta) for each desired primer pair. Account for IP sample as well as input DNA dilutions (up to  $10^{-6}$ ) with technical triplicates for each reaction for all performed chase times. Primers are used at a concentration of 300 nM. Store the master mix in the dark at 4 °C.
3. Pipette 6  $\mu$ L of master mix (containing SYBR Green FastMix and primers) for a desired number of reactions into a quantitative PCR plate.
4. To the distributed primer master mixtures pipette 4  $\mu$ L of each input dilution and pulled down DNA.
5. Seal the plate with optical sealing film. Use gloves to ensure the seal remains clean, not to perturb the qPCR measurement.
6. Centrifuge the plate at  $500 \times g$  for 30 s at room temperature to ensure PCR volume is homogeneous and concentrated at the bottom of the plate.
7. Perform a real-time PCR measurement. For our experiments we used the 7900HT Fast Real-time PCR System from Applied Biosystems.
8. Perform the quantification of percentage input by the following formula:  

$$100 \times 2^{-(C_{t_{input}} \times \text{Log}_2(\text{dilution factor}) - (C_{t_{IP}}))}$$
 where dilution factor is 10 in our experiments.

### 3.8 Preparation of time-ChIP-qPCR Calibration Curve

To obtain a quantitative measure of the fraction of retained nucleosomes or the turnover rate a time-ChIP standard curve should be generated.

1. Perform SNAP-labeling as in point Subheading 3.2 but without any chase period.
2. At the same time mock label a portion of cells with DMSO.
3. Mix defined numbers of in vivo biotin labeled cells with the mock treated cells in a twofold ratio series (100%, 50%, 25%, 12,5%, etc. of biotinylated cells).
4. Perform the subsequent steps: soluble nucleosome preparation, biotin-labeled SNAP-nucleosomes purification, DNA purification, quantitative PCR measurements of percentage input as described above for all twofold ratio series.
5. Make a standard curve of percent input for the different ratio mixes (as in Fig. 3b) and define linear range.
6. Interpolate the time-ChIP-qPCR data obtained in Subheading 3.7 on the time-ChIP calibration curve (*see* Fig. 3b for example) to obtain the fraction of retained nucleosomes or the turnover rates (by interpolation on the linear regression line) (*see* Fig. 3c for example).

### 3.9 Deep Sequencing

Sequencing can be performed on any platform. In our case, library preparation and sequencing was performed at the EMBL core facility in Heidelberg. DNA material from Subheading 3.6, **step 7** was used to generate sequencing libraries with NEBNext ChIP-Seq Library Prep Reagent Set for Illumina (NEB). Please note that other methods to generate sequencing libraries can be used. Sequencing was performed on a HiSeq2000, every chase time point on an individual lane, in a single end mode with 50 bp read length (200 million reads per sample).

1. After obtaining the FASTQ files resulting from sequencing, test the quality of sequencing with the FASTQC program.
2. Filter out the reads with average quality below 20 with FASTX-Toolkit.
3. Remove the sequencing adaptors with cutadapt [44].
4. Align the data for each time point to the human reference genome (GRCh38) using bowtie2 [45].
5. Remove the duplicated reads with rmdup [46].
6. Combine the sequencing reads into 1000 bp bins, normalize to read count and subtract the input sequencing data from all the time points of the chase experiment with bamcompare [47].

7. Obtain chosen genomic coordinate data from ENCODE or similar database. In our analysis we downloaded ChIP-seq peak coordinates for H3K9me3 (ENCFF712ATO), H3K9ac (ENCFF510LKP), H3K27me3 (ENCFF512TQI), H3K27ac (ENCFF392EDT) from ENCODE. For intersection analysis we used the top 10% peaks based on the peak score [48].
8. Calculate the average number of normalized reads falling inside each genomic domain and each chase time point to obtain access to dynamic properties of the selected chromatin feature (*see* Fig. 5a).
9. Generate a bigwig output file from the bamcompare program for direct visualization with genome viewer. We used Integrative Genomics Viewer (IGV) for this purpose [49], (*see* Fig. 5b).

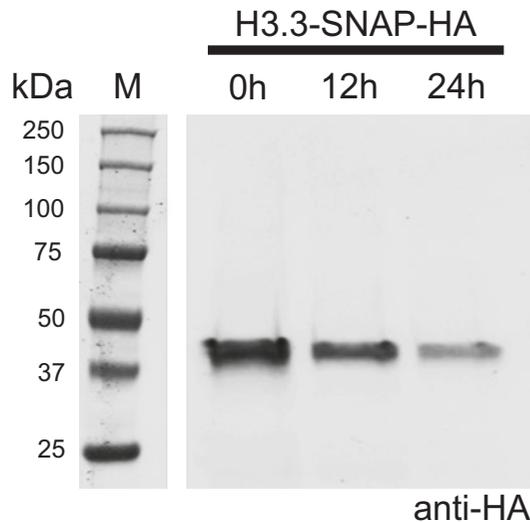
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## 4 Notes

1. Details for synthesis of CP-Biotin. CP-NH<sub>2</sub> (2.9 mg, 11.0 μmol) is dissolved in anhydrous DMF (1 mL). Biotin amidohexanoic acid *N*-hydroxysuccinimide ester (5.0 mg, 11.0 μmol) and triethylamine (1.5 μL, 11.0 μmol) is added and the reaction mixture stirred overnight at room temperature. The solvent is removed under vacuum and the product purified by reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC is performed on Agilent 1100 semi-preparative scale purification system on a VYDAC 218TP series C18 polymeric reversed-phase column (22 × 250 mm, 10 μm particle size) at a flow rate of 20 mL/min with a binary water/acetonitrile gradient and monitored by UV-visible absorbance at 280 nm. Yield: 52%. Mass spectrum is recorded by electrospray ionization (ESI) on an Agilent 6210 Time-of-Flight (TOF) system. ESI-MS *m/z* 604.2454 [M-H]<sup>+</sup> (calc. For C<sub>28</sub>H<sub>38</sub>ClN<sub>7</sub>O<sub>4</sub>S, *m/z* 604.2468).
2. In the described time-ChIP protocol we are measuring the dissociation kinetics of histones in a pulse-chase setup. By pre-incubating cells with an unconjugated SNAP substrate (SNAP-Cell Block, NEB), preexisting SNAP can be quenched. Following a period of new histone synthesis, nascent histones can be selectively biotinylated. We previously used this so-called quench-chase-pulse assay in the context of fluorescent pulse labeling of histones [23, 28, 29]. This strategy can in principle be adopted to measure the association kinetics of a chosen histone variant by time-ChIP.
3. Digitonin recrystallization. Digitonin (0.1 g, 50% purity) was dissolved in 1.3 mL of ethanol (96%) at 75 °C. Next, the solution was incubated on ice for 15 minutes and centrifuged

10,000  $\times g$  for 5 min at 4 °C. The ethanol solution was removed and the procedure was repeated two times. The pellet was dried in a vacuum concentrator for 10 min. The dried pellet was dissolved in cell homogenization buffer and used in the cell lysis procedure.

4. Here we describe a “native chromatin” time-ChIP protocol with MNase treatment as a method to liberate mononucleosomes from chromatin. Alternatively, chromatin can be formaldehyde crosslinked and solubilized by sonication. We piloted this alternative protocol and show by western blot that it can be effectively used in combination with time-ChIP (*see* Fig. 6 for more details).
5. MNase treatment should lead to chromatin samples highly enriched for mononucleosomes. This is a crucial step of the protocol and subsequent procedures depend on the quality of this digestion. A titration experiment should be performed



**Fig. 6** time-ChIP can be used with a chromatin crosslinking protocol. Cells expressing H3.3-SNAP-HA were biotinylated and chased for 0, 12, and 24 h in culture. Equal numbers of cells were collected for each time point. Biotinylated H3.3-SNAP cells were crosslinked with 1% formaldehyde, fragmented by sonication, and subjected to a pull down on streptavidin magnetic beads. The purified material was de-crosslinked by overnight incubation at 65 °C. Samples were separated by SDS-PAGE and analyzed by western blot by probing for the HA tag. We find a biotin-specific isolation of H3.3-SNAP chromatin whose signal diminished during the chase period, indicating turnover

with varying MNase concentration to determine the optimal MNase conditions to maximize the mononucleosome yield (*see* Fig. 1b for example of titration).

6. To assess the yield of soluble chromatin by MNase, collect the pellet fraction after enzyme treatment. Perform DNA isolation and compare the amounts of extracted DNA to the DNA from the soluble input fraction with Quant-iT PicoGreen dsDNA assay kit.
7. To assess the binding efficiency of magnetic beads perform a western blot to compare the amount of unbound, input and pull down fractions (*see* Fig. 1c).
8. The pH 8 of the phenol:chlorophorm:isoamyl alcohol (25:24:1) is crucial for efficient DNA isolation.

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## Acknowledgments

We thank João Mata (IGC) for technical support, Brenda Baker (New England Biolabs) for assistance with synthesis of CP-Biotin. Bianka Baying and Vladimir Benes (EMBL genecore) for NGS support. We are grateful to Alekos Athanasiadis (IGC) and EpiLab members for helpful comments on the manuscript.

This work is supported by Fundação para a Ciência e a Tecnologia (FCT) postdoctoral fellowship SFRH/BPD/117179/2016 (to WS) and doctoral fellowship SFRH/BD/33567/2008 (to MRG). Salary support to LETJ is provided by an “Investigador FCT” grant. Further support was provided by FCT grants BIA-BCM/100557/2008 and BIA-PRO/100537/2008, European Commission FP7 Program, Marie Curie Reintegration grant, an EMBO installation grant and an ERC consolidator grant ERC-2013-CoG-615638 to LETJ.

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