Published in final edited form as:

Methods Mol Biol. 2013 ; 977: 21–33. doi:10.1007/978-1-62703-284-1_3.

DNase I Digestion of Isolated Nuclei for Genome-Wide Mapping of DNase Hypersensitivity Sites in Chromatin

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Summary

DNase I hypersensitivity (DHS) analysis is a powerful method to analyze chromatin structure and identify genomic regulatory elements. Integration of a high throughput detection method into DHS analysis makes genome-wide mapping of DHS sites possible at a reasonable cost. Here we describe methods for DHS analysis carried out with mouse liver nuclei, involving DNase I digestion followed by isolation of DNase I-released DNA fragments suitable for high throughput, next generation DNA sequencing (DNase-seq). A real-time PCR-based assay used to optimize DNase I digestion conditions is also described.

Keywords

DNase I hypersensitivity assay; next generation DNA sequencing; DNase-seq; chromatin structure

1. Introduction

Open chromatin regions are associated with active chromatin and can be identified by their hypersensitivity to nuclease digestion (1). Hypersensitive sites can be identified by DNase hypersensitivity (DHS) assays, in which intact, isolated nuclei are subjected to limited digestion with the enzyme DNase I. Using classical methods, DNase I cleavage sites are identified by restriction digestion followed by Southern blotting using radiolabeled genomic fragments to probe a specific genomic region of interest (2). Although widely used for many years, this method is slow and labor intensive and provides only a low resolution map of DHS sites, typically for a limited number of regulatory elements in close proximity to a single gene. Thus, classical DHS assay methods suffer from limited capacity to elucidate DHS sites that are distant (>100 kb) from their target genes (3). Classical DHS assays cannot readily be used to identify such distant DHS sites; moreover, the classical methods do not provide a global view of the overall chromatin landscape. These limitations can be overcome using high-throughput methods to identify DNA fragments generated by DHS assays, notably tiling arrays (DNase-chip) and next generation DNA sequencing (DNase-seq), (4–7). Notably, DNase-seq generates genome-wide DHS maps that identify functional DNA elements with single nucleotide resolution, as exemplified by several studies, including the ENCODE project (8) and by work from this laboratory studying global DHS sites in mouse liver (9).

Two experimental methods have been developed for high throughput DHS assays (5, 6), the main difference being how samples are processed after DNase I digestion. One method detects single cuts and requires several steps, including blunt-ending followed by ligation of a biotinylated linker, DNA shearing, and streptavidin bead affinity capture (5). The second

method detects two nearby cuts and is much simpler, requiring only purification of short DNA fragments released by DNase I digestion (6). By selecting for and sequencing released DNA fragments that are short (typically 200–400 nt), the second method (DNase fragment release method) minimizes the impact of the non-specific, random DNA cuts that inevitably occur during isolation of nuclei, but which are rarely in close proximity to each other. In contrast, increased background may result when random DNA cut sites are end labeled with biotinylated linkers using the single cut method of (5). Thus, when using the DNase fragment release method, the impact of non-specific DNA shearing during sample handling is appreciably reduced.

As originally described, both methods used tiling arrays for detection of DNase I-released fragments (5, 6). However, characterization of the released fragments can presently be carried out at substantially reduced cost using high throughput, next generation DNA sequencing (DNase-seq). Instrumentation such as the Illumina, Inc. HighSeq 2000 presently yields in excess of 150 million, 40 nt-long sequence tags from a single sequencing lane. This is sufficient to obtain high resolution genome-wide DHS maps of mouse liver for up to 5 individual DNase-seq samples, which can be multiplexed and sequenced together on a single sequencing lane using sample-specific sequence tags (bar codes).

The following protocol for DNase I digestion and fragment isolation is based on the DNA fragment release method of Sabo *et al* (6). We have found this method to be very reliable, and have successfully implemented it to generate high quality, unbiased genome-wide DHS maps of mouse liver chromatin under a number of different biological states (9). Short fragments released by DNase-I digestion of isolated nuclei are separated from the bulk of genomic DNA by sucrose gradient ultracentrifugation (Fig. 1). The size distribution of DNA fragments across the sucrose gradient is then determined by DNA electrophoresis and fluorescence imaging. DNA fragments of the desired size are then isolated, typically from a single fraction of the sucrose gradient (fraction 7 under the experimental conditions described below) (Fig. 1). Downstream steps required to generate libraries of fragments for high throughput sequencing (e.g., using standard Illumina sample preparation protocols) include a size-selection step, which further improves the signal to noise in the final DNase-seq dataset by limiting sequencing to DNase-released DNA fragments ~200–400 nt in length.

Quantitative PCR (qPCR) is used to optimize the conditions required to achieve limited DNase I digestion. qPCR can also be used to validate the final size-selected fragments prior to DNA sequencing. If one or more specific DHS sites has already been identified for the tissue or cell line under study, those sites serve as positive controls for DNase-released fragments in the qPCR validation assay. For example, for DHS analysis of mouse liver chromatin, qPCR primer pairs that map to a strong DHS site associated with the *Alb* gene (i.e., an open genomic region) can be used as a positive control (Fig. 2). qPCR primer pairs that map to an intergenic region that is closed and, therefore, insensitive to DNase I digestion, are used as a negative control. Samples validated by qPCR to show strong enrichment of DNase I-released fragments in the open, hypersensitive region compared to the closed, intergenic region are deemed suitable for library preparation and high throughput DNA sequencing. DNase digestion followed by qPCR analysis can thus be used to optimize conditions for DHS cleavage and fragment isolation. If no DHS sites are known for the tissue or cell line under study, then a preliminary DNase-seq experiment can be carried out using digestion conditions recommended below. DHS sites identified in the preliminary experiment can then be used to design qPCR primers to optimize DNase digestion conditions.

2. Materials

2.1. DHS assay

1. 37°C and 55°C water baths.
2. 2 ml Safe-Lock Eppendorf tubes or equivalents.
3. 15-ml and 50-ml conical tubes.
4. Microcentrifuge at 4°C.
5. Buffer A: 15 mM Tris-Cl, pH 8.0, 15 mM NaCl, 60 mM KCl, 1 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine 0.3 mM spermine. Store Buffer A without spermine and spermidine at 4°C. Add concentrated stock solutions of spermidine and spermine to the buffer just before use.
6. 10X DNase I Digestion Buffer: 60 mM CaCl₂, 750 mM NaCl.
7. Buffer D (Digestion buffer): mix 1 volume of 10X DNase I digestion buffer with 9 volume of Buffer A.
8. Stop Buffer: 50 mM Tris-Cl, pH 8.0, 100 mM NaCl, 0.1% SDS, 100 mM EDTA, 20 µg/ml RNase A, 0.5 mM spermidine, and 0.3 mM spermine. Store the Stop Buffer without RNase A, spermidine, and spermine at 4°C. Warm up in a 37°C water bath, and add RNase A, spermidine, and spermine just before use.
9. RQ1 DNase I (RNase-free; 1 U/µl, Promega, Madison, WI; cat. # M6101).
10. 20 mg/ml proteinase K (Sigma, St. Louis, MO; cat. # P4850)
11. 25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol saturated with 10 mM Tris, pH 8.0, 1 mM EDTA (Sigma, St. Louis, MO; cat. # P3803)

2.2. Isolation of DNase I-released fragments

1. Agarose gel electrophoresis apparatus
2. DNA ladder (100 bp DNA ladder from New England Biolabs, Ipswich, MA; cat. # N3231)
3. SYBR Green I nucleic acid staining solution, 10,000 X in DMSO (Invitrogen, Carlsbad CA; cat. # S7563).
4. Typhoon Trio laser scanner and image system (GE Healthcare Life Sciences, Piscataway, NJ) or similar instrument.
5. Qiagen PCR Purification kit (Qiagen, Valencia, CA; cat. # 28104) and vacuum manifold for DNA purification (Qiagen, Valencia, CA; cat. # 19413). Qiagen kits that have been stored for long periods of time give low yields of recovered DNA and should not be used.
6. Quant-IT high sensitivity DNA assay kit (Invitrogen, Carlsbad CA; cat. # Q33120), multi-well plates, and fluorescent plate reader for DNA concentration measurement.
7. 2X Centrifugation buffer: 40 mM Tris-Cl, pH 8.0, 10 mM EDTA, 2 M NaCl.
8. Sucrose solutions, prepared at concentrations (w/v) of 40%, 35%, 30%, 25%, 20%, 17.5%, 15%, 12.5%, and 10% in 1X centrifugation buffer. These solutions are prepared by adding sucrose to 2X centrifugation buffer; water is then added to adjust to 1X. For example, to prepare 40% sucrose solution in 1X centrifugation buffer, dissolve 400 g sucrose in 500 ml of 2X centrifugation buffer, and then use water to bring up to 1 L. The other sucrose solutions can be prepared in the same

way, or can be prepared by mixing 40% sucrose with 1X centrifugation buffer without sucrose as required.

9. PB buffer for DNA purification (Qiagen, Valencia, CA)
10. 1X TAE buffer: 40 mM Tris-acetate, pH 8.0, 1 mM EDTA,
11. NaCl, 5M.

2.3. Validation of DHS sample quality by qPCR

1. qPCR primer sets targeting known DHS sites and as well as genomic regions that are insensitive to DNase I.
2. Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA; cat. # 4368702, or equivalent)
3. qPCR instrument, ABI 7500 (Applied Biosystems, Foster City, CA) or equivalent.
4. Genomic DNA template and DNase-released DNA fragments.

3. Methods

Sections 3.1 to 3.3 describe DNase I digestion carried out on a scale of 30 million mouse liver nuclei, divided into six 2-ml microcentrifugation tubes, each containing a 1 ml reaction volume (see Note ¹). DNase I treatment of purified genomic DNA (in place of isolated nuclei) is used to generate control samples, which are used to define the background level of intrinsic hypersensitivity of genomic DNA; DNase fragmentation of these control samples requires a much lower concentration of DNase I than the DNase-digested nuclei (see Note ²). The quality of the DHS samples obtained can be assessed by qPCR analysis using one or more known DHS sites as positive controls (see Note ³).

The use of an optimal DNase I concentration and digestion time is critical for the success of DHS-seq analysis. Section 3.4 describes a protocol that can be used to optimize DNase I digestion conditions using a DHS site cleavage assay, modified from McArthur *et al* (12). This protocol is based on the concept that DNase digestion at a DHS site destroys the ability to PCR amplify a genomic region that spans the DHS site. The qPCR amplicon should therefore be several hundred bp in length, and must span the DHS site used to optimize the DNase digestion conditions with respect to DNase concentration and/or time of digestion. Several DNase I concentrations are typically tested in parallel using digestion time and reaction conditions that are identical to the full-scale digestion protocol described in Sections 3.1 and 3.2. Alternatively, the DNase I concentration may be fixed and the incubation time may be varied. This optimization protocol requires fewer nuclei and is less time-consuming than the full-scale DNase fragment release assay. Once an optimal DNase I

¹-In general, one batch of DNase-digested samples containing a total of ~30 million mouse liver nuclei will generate 10 to 30 ng of purified, DNase I-released fragments, which is more than sufficient for high throughput sequencing on the Illumina sequencing platform (e.g., HiSeq 2000 instrument).

²-Control DHS samples (i.e., digestion of purified, chromatin-free genomic DNA) are required for downstream data analysis. The amount of DNase I required for control sample DNase digestion is much lower than that required for digestion of chromatin, typically requiring only 0.3 to 1.0 unit of DNase I per ml. It is important that the extent of digestion of control genomic DNA samples vs. chromatin samples should be similar in terms of the DNA size distribution obtained after sucrose step gradient fractionation. Multiple DNase I concentrations should therefore be tested with genomic DNA samples to determine the optimal digestion conditions. In our experience, sucrose step gradient fraction 7 is used (Fig. 1), as this fraction primarily contains released DNA fragments < 1 kb in size. After digestion, the control DHS assay continues in exactly the same way as in the experimental DHS assay.

³-High quality DHS sample show >10-fold enrichment of known DNase-releasable fragments (positive control region) compared to DNase I insensitive regions (negative control region) when assayed by qPCR. qPCR using genomic DNA as template is also carried out to quantify any differences in qPCR primer efficiency, which must be taken into account when calculating the enrichment of positive control region sequences in the preparation of released fragments.

concentration (and/or digestion time) is identified by qPCR validation, a larger scale DNase digestion can be performed to purify released DNA fragments for sequencing. As an alternative to the above DHS site cleavage assay for optimization of the DNase digestion conditions, optimization can be carried out using the qPCR-based fragment release assay described in Section 3.3. This involves comparison of the qPCR signal for release of DNA fragments from a known hypersensitive region to the qPCR signal for a non-hypersensitive control (background) genomic region.

3.1. DNase I digestion

1. Add spermine, spermidine, and RNase A fresh to Buffer A and to Stop Buffer (see previous chapter). Prepare Buffer D from freshly made Buffer A and 10X DNase I buffer. Warm up the required amount of Buffer D and Stop Buffer (for example 10 ml each for a 6 ml digestion vol) in 15 ml conical tubes to 37°C at least 10 min before digestion.
2. Thaw on ice an aliquot of frozen nuclei. Aliquot total amount of nuclei required (5 million nuclei/ml digestion reaction; 30 million nuclei in total for six 1-ml reactions run in parallel) into a 1.5-ml tube pre-aliquoted with 500 µl ice cold Buffer A. Mix by inverting the tube several times and spin at 500 rcf for 10 min at 4°C. Decant the supernatant and tap the tubes to loosen the nuclear pellet. Wash with Buffer A once more, remove most of the supernatant, loosen the pellet, and place the tubes on ice (see Note ⁴).
3. Aliquot the desired amount DNase I enzyme needed for each reaction, for example, 40 µl of 1U/ µl RQ1 DNase I, into each of six 2-ml tubes pre-aliquoted with 60 µl Buffer D (see Note ⁵).
4. Carry out DNase I digestion in each of 6 tubes, each containing 1-ml reaction volume and the same batch of nuclei (see Note ⁶):
 - a. Resuspend nuclei in 5.5 ml of Buffer D (prewarmed to 37°C for at least 10 min) in a 15-ml tube (master tube of the nuclei). Some clumping of nuclei may occur but should not affect the assay.
 - b. Place one of the reaction tubes in a 37°C water bath every 20 seconds, thereby staggering the start of DNase digestion for each sample. Incubate each tube for 3.0 minutes before adding the nuclei, as described in the next step.
 - c. Transfer 850 µl of suspended nuclei from the master tube of nuclei to a 2-ml prewarmed reaction tube at the precise time specified for each tube. Stop each reaction by adding 950 µl of Stop Buffer after precisely 2.0 min of digestion with DNase I.

⁴. Keep the nuclei on ice until DNase I digestion is initiated. Do not use extra *g* force to spin down the nuclear pellet. Dislodge the pellet gently by tapping after each spin, which helps prevent clumping of the nuclei.

⁵. Pre-dilution of DNase I in Buffer D will decrease the glycerol concentration and make it easier to mix the samples properly. Optimal and reproducible reaction conditions are required for a successful DHS assay, especially when DHS profiles are to be compared between two or more biological samples. If the nuclei are under-digested, the final DNA yield will be low and a greater portion of the released DNA will be derived from non-specific DNA fragmentation that occurs during preparation of the nuclei. Excessive digestion with DNase I will result in extensive release of DNA fragments from genomic regions that show little or no true hypersensitivity. We have found that at a concentration of 5 million nuclei per ml, digested for 2 min at 37°C with 40 to 60 units of DNase I per ml, works well and gives reproducible DHS maps. These reaction conditions can serve as a useful starting point when optimizing DNase I digestion conditions.

⁶. The multi-tube format presented here is also useful when optimizing DNase I digestion conditions by testing a range of DNase concentrations and/or digestion times. DNase I treatment can also be carried out using a single tube format, for example, by setting up a 6-ml reaction in a 15-ml test tube. Care must be taken to allow sufficient time to fully equilibrate the samples to 37°C before DNase I digestion is initiated, making sure that the samples are mixed thoroughly.

5. Immediately transfer each reaction tube to a 55°C water bath for 15 min, then add 10 µl of 20 mg/ml proteinase K.
6. Continue incubation at 55°C overnight.
7. Perform a phenol-chloroform extraction using an equal volume of buffered phenol/chloroform/isoamyl alcohol (i.e., 6 ml for a set of six 1-ml reactions). Store the liquid phase at 4°C or proceed to fragment isolation directly (see Note ⁷).

3.2. Isolation of DNase I-released fragments

1. Add 5 M NaCl to the extracted sample to give a final concentration of 0.8 M NaCl (e.g., 1.76 ml 5M NaCl per 11.0 ml of supernatant recovered after phenol/chloroform/isoamyl alcohol extraction).
2. Prepare sucrose step gradient by carefully overlaying 3-ml cushions of each sucrose solution in each of two SW28 ultracentrifuge tubes, placing the denser gradient fractions at the bottom. Sequentially layer the following final sucrose concentrations of 40%, 35%, 30%, 25%, 20%, 17.5%, 15%, 12.5%, and 10% in 1 X centrifugation buffer.
3. Load 5.7 ml of the sample from step 1 on top of each SW28 tube filled with the sucrose step gradient. Centrifuge for 24 hr at 25,000 rpm (90,000 g) and 25°C in an ultracentrifuge.
4. The next day, carefully collect sequential 1.9 ml fractions into a set of clean numbered tubes, beginning at the top of the gradient. Store fractions at 4°C.
5. Electrophorese a 45 µl aliquot of each fraction on a 1.2% TAE agarose gel at 120 v for 1 hour, running 2 ng of a 100 bp DNA ladder in a separate lane. Stain the gel with 1X SYBR green solution (Invitrogen, Carlsbad CA) in 1X TAE for 30 min. Capture the gel images using a Typhoon Trio laser scanner according to the manufacturer's instructions.
6. Purify DNA from the sucrose fraction(s) that contain the desired size of DNA fragments using a Qiagen PCR Purification Kit according to the manufacturer's instructions. We routinely recover DNA from fraction 7 (Figure 1; also see Note ⁸). Use one Qiagen column per sample. A vacuum device is preferred over centrifugation as the Qiagen column has to be loaded multiple times.
7. Determine the DNA concentration with a Quant-IT DNA high sensitivity assay kit. Store the purified DNA samples at -20°C, or at -80°C if long term storage is required.

⁷·Avoid violent vortex during extraction. If a significant amount of jelly-like material remains in the liquid phase, or if large chunks of denatured protein are present at the interface after phenol/chloroform extraction and centrifugation, it is likely that too many nuclei were digested, which can lead to poor results. In the original protocol (6), DNase I digestion is carried out using up to 10 million nuclei per ml. However, it is preferable to use 5–7 million nuclei/ml to avoid overloading.

⁸·The rate of migration of DNA through the sucrose step gradient is DNA size-dependent, and provides a useful way to separate small DNase I-released fragments from genomic DNA that is largely uncut (10). We routinely analyze on an agarose gel a full set of sucrose gradient fractions collected from each batch of six parallel DNase I digestion reactions to insure that the desired size separation has been achieved. Because of the low DNA abundance in gradient fractions containing short DNA fragments, fluorescence staining by SYBR Green and use of high sensitivity laser scan imaging is required to visualize the DNA fragment size distribution (Figure 1). Using the methods described here, sucrose gradient fraction 7 typically contains DNA fragments less than 1 kb in size and gives excellent DNase-seq results. However, other fractions, for example, pooled fractions 6 to 8, may also be used (11). Further steps required for sample processing prior to high throughput sequencing, including adaptor ligation and library generation for Illumina sample preparation are not discussed here. Of note, there is an additional size selection step during the Illumina sample preparation protocol, where fragments 200–400 bp in length are selected for sequencing. This size selection can substantially enhance the signal to noise ratio in the final DNA sequencing data.

3.3. Validation of DHS sample quality by qPCR

1. Identify genomic sequences corresponding to known DNase I hypersensitive regions (positive controls) and insensitive regions (negative controls).
2. Use a suitable qPCR primer design program, such as Primer Express (Applied Biosystems, Foster City, CA), to design qPCR primers targeting DNase I hypersensitive and insensitive regions. The amplicon length should be kept short relative to the lengths of the released fragments (ideally about 50–60 bp) to increase the chance of detecting short fragments released by DNase digestion.
3. Use NCBI's Blat or Blast search tools to ensure the specificity of the PCR primers.
4. Perform qPCR assays based on SYBR Green-based methodology using 10 ng of genomic DNA (denoted as *g*) and 1% of the purified DHS sample (denoted as *s*).
5. Calculate the fold-enrichment adjusted for differences in primer efficiency, as follows: Fold enrichment of DHS site (denoted as **DH**) over DNase I insensitive regions (denoted as **IN**) = $2^{\text{to the power of } \{(\text{DH}_g - \text{DH}_s) - (\text{IN}_g - \text{IN}_s)\}}$, where DH_g is the qPCR threshold cycle number (Ct number) obtained for the DHS qPCR primer pair using genomic DNA as template, and IN_s is the qPCR Ct number obtained for the DNase I insensitive region qPCR primer pair using the purified, released DNA fragments as template.
6. For DNase-seq studies in mouse liver, the following qPCR primers can be used as a positive control for validation of DNase digestion. *Alb* gene DHS primers: 5'-CAATGAAATGCGAGGTAAGTATGG-3' and 5'-TCTTTAACCAATAACTGTAGATCATTAAACCA-3', corresponding to a 52 bp amplicon length. DNase I insensitive region primers located on Chr 3: 71,026,628–71,026,685 (mouse genome build mm9 and amplicon length 58 bp) can be used as a negative control (background region): 5'-GCAGCAGATGGCAAGTAATACTAAGAT-3' and 5'-CCCTTATTCTCTGAGCATTAGACAGTTATA-3'.

3.4 Method for optimization of DNase I digestion conditions using DHS site cleavage assay

1. Carry out DHS digestion as described in Section 3.1, steps 1 to 7, but with the following changes: a) use 25% of the reaction volume, i.e., about 1.25 million nuclei/0.25 ml digestion; and b) vary the DNase I concentration in each tube, for example, 0, 10, 20, 40, 80 and 120 U of DNase I per ml. Alternatively, the concentration of DNase I may be fixed, and a variable incubation time may be employed (e.g., 1 min to 5 min incubation with DNase I).
2. After phenol-chloroform extraction, precipitate the DNA by adding 1/10th volume of 3 M sodium acetate, pH 5.2 (Sigma, St. Louis, MO, S7899) and 2 vol of 100% ethanol.
3. Incubate the mixture at –80°C for 20 min; pellet the DNA by centrifugation using a microcentrifuge at full speed.
4. Wash the pellet with 1 ml of 70% ethanol. Spin down the pellet. Remove the ethanol and air dry for 5 min. Resuspend the pellet in 100 µl EB buffer provided in the Qiagen PCR purification kit (Note ⁹).

⁹It is critical for downstream steps that the DNA pellet be completely dissolved. Over-drying the DNA pellet makes it very difficult to dissolve. Incubation at 60°C for one hour followed by incubation at 4°C overnight may be required to completely dissolve the DNA sample.

5. Measure the DNA concentration in each dissolved DNA sample using a Quant-IT high sensitivity DNA assay kit, as described above.
6. Perform a SYBR-green based qPCR assay using 5 ng of DNA from each sample and two pairs of qPCR primers. One qPCR primer pair flanks a DNase I hypersensitive region, while the second primer pair flanks a DNase I insensitive region (Note ¹⁰). At the optimal DNase I concentration, the hypersensitive region is selectively destroyed; as a consequence, the qPCR threshold cycle (Ct) number for the primer pair that flanks the hypersensitive region increases but there is little or no change in the qPCR Ct number for the primer pair that flanks the insensitive region (12).

Acknowledgments

Supported in part by NIH grant DK33765 (to DJW).

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¹⁰. Ideally, qPCR amplicons of the DNase I hypersensitive and the DNase I insensitive genomic regions should be of similar length and extend for several hundred bp. The amplicon should cover the entire hypersensitive region in order to encompass as many DNase cutting events as possible. However, excessively long DNA amplicons will decrease the qPCR amplification efficiency and affect the assay quality. Multiple primer pairs should be tested with genomic DNA to identify primer pairs that give the best amplification efficiency and the highest specificity.

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Figure 1.

Schematic diagram of DNase-seq method. A single DHS site surrounded by heterochromatin is shown at the top. The ovals represent highly packed nucleosomes, and the arrows are DNase I cleavage sites, which occur much more frequently in DHS regions. Limited DNase I digestion releases short DNA fragments from DHS regions (red arrows, and red fragments), as well as larger fragments that result from random cutting of genomic DNA (blue arrows, and blue fragments). The short DNA fragments are then separated from larger fragments by sucrose gradient ultracentrifugation, as shown in the gel at the center. High throughput sequencing (typically using DNA purified from fraction 7) is then carried out to obtain 40 nt sequence tags, which are then mapped back to the genome. Mapped

DNase-seq data can then be uploaded to the UCSC genome browser to visualize clusters of sequence tags, which indicate a DHS site. DHS peaks such as the one shown here at the *Alb* promoter on mouse chromosome 5 typically show an asymmetric distribution of positive and negative sequence reads, with (+) strand sequences marked in blue and (–) strand sequences marked in yellow. Quantitative analysis of the full DHS dataset is then carried out using peak-calling software (e.g., (9)).

Figure 2.

DHS sites upstream of mouse *Alb* gene. Shown is a UCSC browser screen view of a 50 kb region upstream of the *Alb* promoter on mouse chromosome 5. Individual sequence tags are (green, (+) strand sequences; red, (-) strand sequences). The locations of the 10 DHS sites in view are marked relative to the *Alb* transcription start site (TSS). The DNase-seq peaks shown (i.e., DHS sites) are very sharp, generally 350-500 nt wide and corresponding to an average of two nucleosomes. The DHS sites shown coincide with regions exhibiting high cross-species conservation, as shown along the bottom track (green arrows).