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Protocol

DNase-seq: A High-Resolution Technique for Mapping Active Gene Regulatory Elements across the Genome from Mammalian Cells

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INTRODUCTION

Identification of active gene regulatory elements is a key to understanding transcriptional control governing biological processes such as cell-type specificity, differentiation, development, proliferation, and response to the environment. Mapping DNase I hypersensitive (HS) sites has historically been a valuable tool for identifying all different types of regulatory elements, including promoters, enhancers, silencers, insulators, and locus control regions. This method utilizes DNase I to selectively digest nucleosome-depleted DNA (presumably by transcription factors), whereas DNA regions tightly wrapped in nucleosome and higher-order structures are more resistant. The traditional low-throughput method for identifying DNase I HS sites uses Southern blots. Here, we describe the complete and improved protocol for DNase-seq, a high-throughput method that identifies DNase I HS sites across the whole genome by capturing DNase-digested fragments and sequencing them by high-throughput, next-generation sequencing. In a single experiment, DNase-seq can identify most active regulatory regions from potentially any cell type, from any species with a sequenced genome.

RELATED INFORMATION

This DNase-seq protocol was derived from methodologies originally described by Boyle et al. (2008). It has been modified to eliminate discontinued materials, reduce the amount of no-insert sequence contamination, and increase the signal-to-noise levels. An overview of the procedure is provided in Figure 1.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Alkaline phosphatase, shrimp (SAP) (1 U/µL; Roche Diagnostics)
Bovine serum albumin (BSA) (100X; New England Biolabs)
<**R**>B&W buffer (2X)
Cells for analysis (human or other mammal) in single-cell suspension culture
<**!**>Chloroform (J.T. Baker)
DNA ladder (25-bp) (Invitrogen)

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<**R**>DNA polymerase buffer
DNase I recombinant, RNase-free (10 U/µL) and incubation buffer (10X; Roche Diagnostics)
dNTPs (10 mM; Roche Diagnostics)
Dynal streptavidin beads (Dynal M-280; Invitrogen)
<**R**>EDTA (50 mM, pH 8.0)
Ethanol (70% and 100%)

Use 100% ethanol except where 70% ethanol is specified.

<**!**>Ethidium bromide (10 mg/mL)
Glycogen (20 mg/mL; Roche Diagnostics)
<**!**>IGEPAL CA-630 (Sigma)
InCert Agarose, low-melt (1%, melted in sterile 50 mM EDTA [pH 8.0]) (Lonza)

Prepare and store tubes of agarose at 4°C before the start of the experiment.

<**R**>LIDS buffer
Linkers (see Step 28 for details of preparation)
- Linker 1: annealed oligonucleotides 1a and 1b (HPLC-purified; Integrated DNA Technologies)
  - Oligo 1a: 5′-Bio-ACAGGTTCAGAGTTCTACAGTCCGAC-3′
  - Oligo 1b: 5′-GTCGGACTGTAGAACTCTGAAC-Amm-3′
- Linker 2: annealed oligonucleotides 2a and 2b (HPLC-purified; Integrated DNA Technologies)
  - Oligo 2a: 5′-P-TCGTATGCCGTCTTCTGCTTG-3′
  - Oligo 2b: 5′-CAAGCAGAAGACGGCATACGANN-3′ (N represents any of A, T, G, or C)

MmeI (2 U/µL; New England Biolabs)
NaOAc (sodium acetate; 3 M, pH 5.3)
<**!**>NaOH (0.15 M)
NEB Buffer 2 (10X; New England Biolabs)
<**R**>NEB buffer 2 for washes (1X)

Because of the large amount of buffer used in this protocol (e.g., at Step 21), we generally make 1X NEB buffer 2 in the lab.

NEB Buffer 4 (10X; New England Biolabs)
<**!**>Phenol (Invitrogen)
<**!**>Phenol:chloroform:isoamyl alcohol (25:24:1; Invitrogen)
Phosphate-buffered saline (PBS) without Mg^{2+}/Ca^{2+} (1X, pH 7.2; GIBCO) (prechilled)
Phusion DNA Polymerase (2 U/µL) and Phusion HF Reaction Buffer (5X) (both from Finnzymes)
Primers
- Custom Illumina/Solexa sequencing primer:
  - 5′-CCACCGACAGGTTCAGAGTTCTACAGTCCGAC-3′
- Polymerase chain reaction (PCR) primers:
  - primer 1: 5′-CAAGCAGAAGACGGCATACGA-3′
  - primer 2: 5′-AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA-3′

<**R**>RSB buffer (prechilled)
<**!**>S-Adenosylmethionine (SAM) (500 µM; New England Biolabs)
T4 DNA ligase (5 U/µL) and ligation buffer (10X) (both from Roche Diagnostics)
T4 DNA polymerase (New England Biolabs)
<**R**>TBE buffer (0.5X)

Where 1X TBE is called for, double the concentrations in the 0.5X TBE.

<**R**>TE buffer (1X, pH 8.0)
<**R**>Tris-Cl (10 mM, pH 8.0)
<**!**>Trypan blue (GIBCO/Invitrogen)
UltraPure Agarose (Invitrogen)
UltraPure Low Melting Point (LMP) Agarose (Invitrogen)
Yeast chromosome PFG molecular weight marker (New England Biolabs)

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Equipment

- Centrifuges
  - Benchtop centrifuge for 1.5-mL microcentrifuge tubes
  - Centrifuge with a swing-out bucket rotor with cooling system, for 15-mL and 50-mL polypropylene tubes
- CHEF disposable plug molds (Bio-Rad)
- Conical tubes, 15- and 50-mL
- Container for staining gels
- DNA sequencer (Solexa/Illumina)
- Green screened plug caps (Bio-Rad)
- Ice and ice box
- Incubator preset to 37°C
- Magnetic stand (Dynal) for bead separation (Invitrogen)
- Microcentrifuge tubes, 1.5-mL and 0.5-mL (e.g., Eppendorf)
- Micropipettor and tips
- Microscope
- Needle, 21-gauge
- Pulsed-field gel (PFG) electrophoresis (CHEF) equipment (Bio-Rad)
- Razor blade (VWR Scientific)
- Rotator
- Shaker
- Spatulas (metal)
- Spin-X filter (Fisher)
- TBE PAGE gels (4%-20%, precast by Bio-Rad) and electrophoresis equipment
- Thermal cycler
- Vortex mixer
- Waterbaths or heating blocks preset to 37°C, 55°C, 65°C, and 75°C
- Wide-bore pipette tips (tips clipped off with razor or scissors)

METHOD

Isolation and DNase I Digestion of Nuclei to Isolate High-Molecular-Weight DNase-Treated DNA

Isolation of Nuclei

1. Centrifuge 50 million human or other mammalian cells in single-cell suspension at 900 rpm for 5 min in 50-mL tubes. Use vacuum aspiration or a pipette to carefully remove the supernatant (do not pour off liquid). Flick to resuspend the cell pellet in residual liquid.

   Because the pellet is loose, leave a 1 mm-thick supernatant layer over the pellet during washes to reduce cell loss.

   See Troubleshooting.

2. Wash the cells twice with cold 1X PBS (50 mL of PBS for each wash). If starting with two or more tubes of cell culture, combine cells into one tube for the first wash.

3. Resuspend the final cell pellet in 500 µL of cold RSB buffer by gentle flicking and transfer to a 15-mL conical tube (keep on ice). Rinse the 50-mL tube with 500 µL of cold RSB buffer to collect any remaining cells, and combine the rinse with the first 500 µL of cell suspension.

4. Slowly pour 14 mL of lysis buffer (cold RSB buffer + 0.1% IGEPAL CA-630) into the 15-mL tube containing the cell suspension. Invert five to 10 times. Make sure there is no detectable precipitation at this step.

5. Check cells after lysis by staining with Trypan blue and examining under a microscope.

   If lysis has been effective, at least 99% of the cells should be stained.

   See Troubleshooting.

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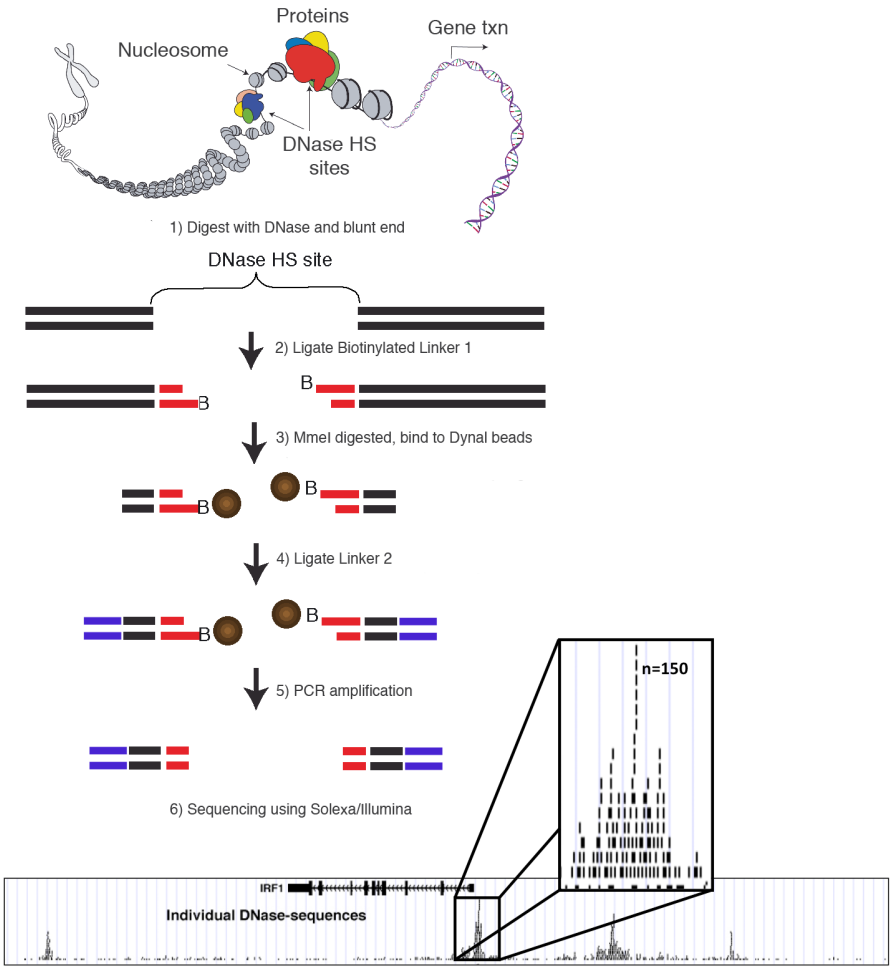

FIGURE 1. Flowchart of DNase-seq protocol. Briefly, cells are lysed with detergent to release nuclei, and the nuclei are digested with optimal concentrations of DNase I. DNase I-digested DNA is embedded in low-melt gel agarose plugs to reduce additional random shearing. DNA (while still in the plugs) is then blunt-ended, extracted, and ligated to biotinylated linker 1 (red bars). Excess linker is removed by gel purification. Biotinylated fragments (linker 1 plus 20 bases of genomic DNA) are digested with MmeI and captured by streptavidin-coated Dynal beads (brown balls). Linker 2 (blue bars) is ligated to the 2-base overhang generated by MmeI, and the ditagged 20-bp DNAs are amplified by PCR and sequenced by Illumina/Solexa. (For color figure, see doi: 10.1101/pdb.prot5384 online at www.cshprotocols.org.)

6. Centrifuge immediately at 500*g* for 10 min at 4°C to pellet nuclei, and remove supernatant completely. Do not leave any supernatant on the pellet.

   Proceed with Step 7 during centrifugation.

DNase I Digestion and Embedding DNA into Agarose Plugs

In the following steps, be sure to follow the instructions for mixing (e.g., if the directions specify "flick" or "invert," do not vortex or pipette the material).

7. During centrifugation in Step 6, prepare tubes for the DNase I digestion series, as follows:

   i. Use 1X DNase incubation buffer to make five concentrations (0.01, 0.03, 0.1, 0.3, and 1 U/µL) of recombinant DNase I in five microcentrifuge tubes (1.5-mL); keep tubes on ice.

   When diluting DNase, make sure dilutions are extensively mixed.

   ii. Label seven empty microcentrifuge tubes #1-#7 and place on ice.

   iii. Pipette 12 µL of the indicated concentration of DNase into each of tubes #3-#7. Do not add DNase to tubes #1 and #2.

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Tube #	Concentration of DNAse I to add (U/μL)	Volume to add (μL)	Final units of DNase per tube (U)
#1 and #2	N/A	0	0 (empty)
#3	0.01	12	0.12
#4	0.03	12	0.4
#5	0.1	12	1.2
#6	0.3	12	4.0
#7	1	12	12

8. Suspend nuclei (lysed cells) in 840 μL of cold 1X DNase incubation buffer and mix by flicking. The pellet should appear white and fluffy and should be suspended completely.

9. Slowly pipette 120 μL of nuclei suspension into tubes #1-#7 by using wide-bore pipette tips. Gently swirl to resuspend nuclei before each transfer.

10. Keep tube #1 on ice, and incubate tubes #2-#7 for 15 min in a 37°C water bath.

11. Melt several tubes of InCert low-melt agarose at 75°C. Once they are completely melted, transfer to 55°C.

    Agarose will be used in Step 13.

12. Slowly pipette 330 μL of 50 mM EDTA into each of tubes #1-#7 to stop the reactions, and invert tubes five times to mix. Keep tubes at room temperature until all have been mixed, then equilibrate for 1 min at 55°C.

13. Slowly pipette 450 μL of InCert low-melt agarose (from Step 11) into each tube. Invert four times to mix.

14. Pipette mixtures into CHEF disposable plug molds using wide-bore pipette tips. Let sit for ~5 min at 4°C to solidify. The volume of each plug is ~80 μL.

    Low-melt gel agarose plugs are used to stabilize high-molecular-weight DNA and protect against random shearing.

15. Carefully release plugs into 50 mL of LIDS buffer in a 50-mL conical tube, place a green screened plug cap (for washing plugs) between each tube and cap, and shake horizontally at 50-60 rpm for 1-2 h at room temperature. Keep plugs with different DNase1 concentrations in separate conical tubes.

16. Replace with fresh LIDS buffer and incubate tubes overnight at 37°C, horizontal without shaking.

17. Wash plugs five times with 50 mL of 50 mM EDTA per tube, 1 h each wash, shaking at 60 rpm at room temperature. Prior to the fourth and fifth EDTA washes, wash the filters and lids with water to remove residual detergent from the filter and lid threads. Make sure that no detergent bubbles are left in the tubes after the fifth washing.

18. Store the plugs at 4°C in 50 mM EDTA.

    Plugs can be stored indefinitely under these conditions.

Identifying Optimal DNase Digestion by PFG Electrophoresis

19. To perform PFG electrophoresis:

    i. Prepare a 1% UltraPure Agarose gel in 0.5X TBE.

    ii. Load one-third of a plug from each DNase concentration into the gel by sliding each plug piece into a separate well using two metal spatulas.

    iii. Use yeast chromosome PFG marker to size smearing patterns.

    iv. Set PFG unit to keep 0.5X TBE running buffer chilled at 16°C.

    v. Set running parameters as follows: 20-60 sec switch time; 18 h running time; 6 V/cm (180 V total).

20. Compare gel pattern to Figure 2. Optimal amounts for DNase-seq are 0.4 U, 1.2 U, and 4.0 U.

    The smearing patterns (50-100 kb to 1 Mb in size) seen in the lanes for the 0.4 U, 1.2 U, and 4.0 U DNase amounts are usually ideal for making DNase-seq libraries from mammalian cells. Because DNase HS sites are

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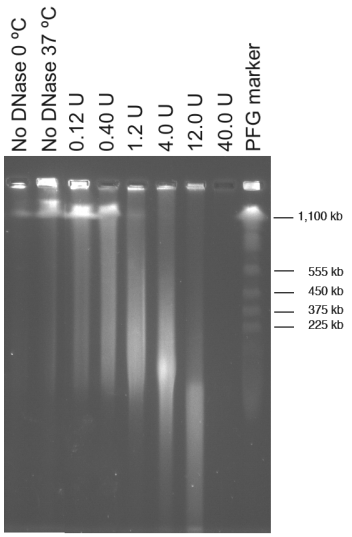

FIGURE 2. PFG picture of DNaseI-digested DNA isolated from human umbilical vein endothelial cells (HUVEC). An ideal PFG pattern displays gradual and consistent changes in high-molecular-weight DNA fragment sizes as DNase I concentrations increase.

not binary, but instead represent a continuum of signal intensities, the optimal size should include multiple DNase concentration patterns to capture both the strongest and the weaker DNase sites. The smear size ranging from ~1 Mb to 50-100 kb typically generates high-quality DNase data. We have found that using more heavily digested DNA results in lower signal to noise. DNase concentrations to achieve optimal smearing sizes may differ for each cell line and therefore must be determined empirically for each cell type. Use of this protocol on smaller eukaryotic genomes (such as yeast) may require different optimal smearing patterns.

See Troubleshooting.

Blunt-Ending DNase-Digested Ends

Because DNase I nicks DNA and leaves single-strand overhangs, these overhangs need to be blunt-ended by T4 DNA polymerase before ligating to blunt-ended linker 1.

21. Wash the EDTA-soaked plugs twice with 50 mL of 1X NEB buffer 2 per tube, 1 h for each wash, shaking at 60 rpm at room temperature.

This step removes EDTA from the plugs.

22. Remove all traces of liquid from the 50-mL conical tubes and push plugs to the bottom of the tubes.

23. To polish the DNA ends, proceed as follows:

i. Mix the plugs in the 50-mL conical tubes with T4 DNA polymerase and other reagents as indicated.

Reagent	Volume
DNA plug	80 µL
NEB Buffer 2 (10X)	12 µL
dNTPs (10 mM)	5 µL
T4 DNA Polymerase	6 µL
Water	99.2 µL
BSA (100X)	2 µL
Total volume	204.2 µL

ii. Incubate for 4 h at room temperature. Do not mix plugs from different optimal DNase concentrations (they will be combined in Step 29).

iii. Shake the reactions occasionally.

24. Rinse the plugs quickly with 1X TE buffer to remove residual enzyme.

25. To extract DNA from the plugs:

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i. Transfer plugs to 1.5-mL microcentrifuge tubes. Add 500 µL of 1X TE buffer and heat for 15 min at 65°C to melt. Flick every 5 min to make sure agarose is dissolving.

ii. Extract each tube using equal volumes of a series of phenol, phenol:chloroform:isoamyl alcohol, and chloroform.

iii. Precipitate the DNA with 1 µL of 20 mg/mL glycogen, 50 µL of 3 M NaOAc, and 1.0 mL of ethanol.

26. Pellet the DNA by centrifuging at 16,000*g* for 10-15 min at 4°C, wash the pellet with two volumes of 70% ethanol, centrifuge again, and remove all residual liquid. Let dry for <4 min.

Longer drying times may cause difficulty in dissolving the DNA pellet.

27. Resuspend the DNA pellet in 40 µL of 10 mM Tris-Cl (pH 8.0).

DNase-seq Library Construction

28. Anneal oligos to make linker 1 and linker 2:

Stock linkers are at a concentration of 25 pmol/µL.

i. Anneal oligo 1a and 1b (to make linker 1) and oligo 2a and 2b (to make linker 2) in 1X NEB buffer 2.

ii. Heat for 5 min at 95°C.

iii. Slowly cool to room temperature. Make fresh or store aliquots at –20°C.

29. Ligate DNase-treated ends to linker 1:

i. Combine reagents as indicated.

Reagent	Amount
Blunt-ended DNA (combine equal amounts from each DNA concentration)	3 µg
Ligation buffer (10X)	5 µL
T4 DNA ligase (10 U)	2 µL
Linker 1	6 µL of 25 pmol/µL (150 pmol total)
Water	to 50 µL final volume

ii. Incubate tubes overnight in a thermal cycler at 20°C.

30. Separate the unligated linkers from the ligated DNA by gel purification:

i. Prepare a 0.8% UltraPure LMP Agarose gel using 10 mM EDTA and 1X TBE.

ii. Run the reactions from Step 29 for 30-50 min at 80 V in 10 mM EDTA and 1X TBE running buffer.

31. Isolate the ligated DNA from the linkers:

i. Cut out the high-molecular-weight smear (leave the linker-only band behind).

ii. Put the gel into 500 µL of 1X TE and heat for 15 min at 65°C to melt. Gently flick every few minutes to make sure agarose is dissolving.

iii. Extract with equal volumes of a series of phenol, phenol:chloroform:isoamyl alcohol, and chloroform.

iv. Precipitate the DNA with 1 µL of 20 mg/mL glycogen, 50 µL of 3 M NaOAc, and 1 mL of ethanol.

v. Wash the DNA pellet with two volumes of 70% ethanol.

vi. Dry the pellet and resuspend the DNA in 75 µL of water.

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32. Digest the ligated DNA with MmeI:

    i. Prepare the following mixture.

Reagent	Amount
DNA	75 µL
NEB Buffer 4 (10X)	10 µL
SAM (500 µM)	10 µL
MmeI	5 µL
Total volume	100 µL

    ii. Incubate the reaction for 1.5 h at 37°C.

    iii. Add 3 µL of SAP (to prevent ligation of MmeI ends to each other). Incubate for 1 h at 37°C.

33. Treat the digestion as follows:

    i. Extract the digestion with equal volumes of phenol:chloroform:isoamyl alcohol and chloroform.

    ii. Precipitate the DNA by adding 1 µL of 20 mg/mL glycogen, 10 µL of 3 M NaOAc, and 325 µL of ethanol.

    iii. Wash the pellet with 70% ethanol.

    iv. Dry the pellet and resuspend it in 50 µL of water.

34. Prepare 100 µL of Dynal streptavidin beads for each sample.

    Use magnetic stand and vacuum aspiration for all wash steps.

    i. Wash the beads twice with 1 mL of 1X TE.

    ii. Wash the beads once with 1 mL of 1X B&W buffer. Remove wash buffer before beginning the next step.

35. Treat the washed Dynal beads as follows:

    i. Suspend the beads in a mixture of 50 µL of MmeI-digested DNA and 50 µL of 2X B&W buffer.

    ii. Incubate for 30 min at 30°C, flicking the tubes every 5 min to resuspend the beads.

    iii. Remove the supernatant and wash the beads five times with 1 mL of 1X TE.

    iv. Wash the beads once with 100 µL of 1X ligation buffer. Remove the wash buffer before the next step.

36. Ligate phosphorylated linker 2 to dephosphorylated MmeI ends:

    i. Add the following reagents to the Dynal beads (~10 µL).

Reagent	Amount
Linker 2 (25 pmol/µL)	6 µL
Ligation buffer (10X)	10 µL
T4 DNA ligase	2 µL
Water	72 µL
Total volume	100 µL

    ii. Rotate the tube for 4 h at room temperature.

37. Treat the beads as follows:

    i. Wash the beads once with 1 mL of 1X TE.

    ii. Add 500 µL of 0.15 M NaOH directly to the beads.

    This step denatures double-stranded DNA template on the beads and removes the nonbiotinylated strand to eliminate false-positive sequencing signals from mismatched base pairs at the end of linker 2.

    iii. Rotate the tube for 5 min at room temperature.

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iv. Wash the beads five times with 1 mL of 1X TE.

v. Resuspend the beads in 25 µL of 10 mM Tris-Cl (pH 8.0).

38. Amplify the DNase-seq product as follows:

i. Prepare the following mixture.

Reagent	Volume
Dynal bead suspension	10 µL
5X Phusion HF Reaction Buffer	10 µL
PCR primer 1 (25 µM)	0.5 µL
PCR primer 2 (25 µM)	0.5 µL
10 mM dNTP	1.25 µL
Phusion DNA Polymerase	0.5 µL
Water	27.25 µL
Total volume	50 µL

ii. Denature for 30 sec at 98°C, followed by 12 amplification cycles (10 sec, 98°C; 30 sec, 60°C; 15 sec, 72°C), followed by extension for 7 min at 72°C.

39. Perform electrophoresis:

i. Load 1 µL of the 25-bp DNA ladder into one well and 50 µL of PCR products from Step 38 into two adjoining wells of a 4%-20% TBE PAGE gel.

ii. Electrophorese for 2 h at 120 V.

iii. Pry apart the cassette and stain the gel in 1X TE + 0.5 µg/mL ethidium bromide in a clean container for 2-3 min.

A representative example of amplification is shown in Figure 3.

See Troubleshooting.

40. Prepare the 86-bp band from the gel:

i. Cut out the 86-bp band (containing linkers + insert) with a clean razor blade.

ii. Place the gel slice into a 0.5-mL microcentrifuge tube whose bottom has been punctured by a 21-gauge needle.

iii. Set this tube into a 1.5-mL round-bottom microcentrifuge tube.

iv. Centrifuge the gel slice through the hole into the 1.5-mL tube, in a microcentrifuge at full speed for 2 min.

41. Treat the gel as follows:

i. Add 100 µL of 1X NEB buffer 2 to the gel.

ii. Elute the DNA by rotating the tube gently for 2 h at room temperature.

iii. Transfer the eluate and the gel debris onto the top of a Spin-X filter. Centrifuge the filter in a microcentrifuge at full speed for 2 min to remove traces of gel.

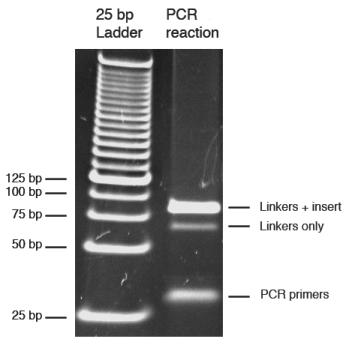

FIGURE 3. Gel picture of PCR reactions. 1 µL of 25-bp ladder and 30 µL of PCR reaction were loaded on a 4%-20% TBE-PAGE gel. The "linkers + insert" band is 86 bp, the "linkers only" band is 66 bp, and the "PCR primers" are 20-30 bases.

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42. Treat the sample as follows:
    i. Add 1 µL of 20 mg/mL glycogen, 10 µL of 3 M NaOAc, and 325 µL of ethanol. Precipitate the DNA for 30 min at –20°C.
    ii. Dry the DNA pellet in the air for 5 min.
    iii. Resuspend the pellet in 10 µL of 1X TE.
43. Check the purity of the recovered DNA:
    i. Run 1 µL of recovered DNA on a 4%-20% TBE gel.
    ii. Stain the gel with ethidium bromide.
44. Sequence libraries on a Solexa/Illumina DNA sequencer using the custom sequencing primer.

TROUBLESHOOTING

Problem: Less than 50 million cells are available for analysis.
[Step 1]
Solution: This protocol has been successfully applied to lower cell numbers. Buffer volumes, DNase concentrations, and final numbers of plugs can be reduced in proportion to the number of cells, but actual proportions need to be determined empirically for each cell type and cell number.

Problem: Cells are not adequately lysed (<90% of the cells are stained with Trypan blue) or are overlysed, which often results in precipitation and clumping at the IGEPAL CA-630 lysis step.
[Step 5]
Solution: Some cell lines are more sensitive to IGEPAL CA-630 lysis than others. Typically, we use a small number of cells (5 million) to test different concentrations of IGEPAL CA-630 (0.5%, 0.1%, 0.05%, 0.025%, and 0.01%).

Problem: PFG electrophoresis shows that genomic DNA is not digested ideally by DNase I.
[Step 20]
Solution: The typical DNase I concentrations that we use for digestion are 0.12 U, 0.4 U, 1.2 U, 4.0 U, and 12 U, which usually create good digestion patterns. Consider the following:

1. For situations where samples are not digested enough, add higher concentrations of DNase I, use fewer cells, or increase digestion time.
2. If samples are overdigested, use less DNase I, more cells, or shorter digestion times.

Problem: The 86-bp DNA band does not appear after PCR amplification.
[Step 39]
Solution: The 86-bp DNA band contains linkers + 20 bases of insert sequence adjacent to the DNaseI cleavage site, while the 66-bp band contains only linkers. The absence of the 86-bp band may be due to various reasons, such as reduced efficiency of blunt ending, ligation, or MmeI digestion. Consider the following:

1. Repeat the blunt-ending steps (Steps 21-27).
2. Start with a larger amount of DNA (5 µg; Step 29).
3. Test the MmeI activity (Step 32).
4. Remove all traces of ethanol from precipitated DNA (Steps 26, 31, 33, 42).
5. Be careful not to lose the pellets.

DISCUSSION

Early studies support that nucleosome disrupted regions are hypersensitive to DNase I and that those regions are associated with gene activation in eukaryotic organisms (Wu et al. 1979; Wu 1980; Keene et al. 1981; Levy and Noll 1981; Gross and Garrard 1988). In the last 25 years, hundreds of DNase I

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HS sites have been identified by the traditional Southern blotting method and found to be highly correlated with a variety of active regulatory elements, including promoters, enhancers, silencers, insulators, and locus control regions. This makes DNase I HS site identification an ideal tool for detecting all types of gene regulatory elements with a single assay. The low-throughput Southern blotting assay is very informative, but it is not suitable for whole-genome analysis.

We and others have developed multiple high-throughput techniques to assay large numbers of DNase HS sites using tiled arrays (DNase-chip) or high-throughput sequencing (DNase-seq). Both methods follow the basic technique of the traditional method of digesting nuclei with optimal concentrations of DNase I to preferentially cut at open chromatin sites. For DNase-chip and DNase-seq, DNase-digested ends are enriched and either hybridized to tiled arrays or sequenced by using next-generation sequencing technologies such as Solexa/Illumina (Crawford et al. 2006; Sabo et al. 2006; ENCODE Project Consortium 2007; Xi et al. 2007; Boyle et al. 2008; Hesselberth et al. 2009; Shibata and Crawford 2009).

Although our data analysis has shown that DNase-seq is highly correlated with DNase-chip and that both methods are highly correlated with an independent quantitative PCR (qPCR) strategy (Boyle et al. 2008), the methods are distinct in many aspects. For example, DNase-seq has single-base-pair resolution of digestion sites, has a high dynamic range, and can only be applied genome-wide (unless an array capture-method is utilized). In addition, the analysis software is still relatively immature. In contrast, DNase-chip has lower resolution (300-700 bases due to shearing size), can be flexibly applied to either a local region or the entire genome based on the array design, and has a more mature set of analysis tools. Therefore, researchers need to consider several factors when they choose between DNase-seq and DNase-chip, including what specific genome portion they are interested in, the cost of sequencing versus tiled arrays, and resolution and sequencing depth required for their experimental purposes.

DNase-seq is a straightforward method that can be performed on potentially any cell type from any species with a sequenced genome. No prior knowledge is required with regard to histone modifications, transcription factor binding sites, gene annotation, or relative degree of sequence conservation between species. DNase-seq provides a good first step for identifying the location of most active gene regulatory elements. However, it does not directly disclose the biological functions of these elements. Follow-up studies, such as chromatin immunoprecipitation and/or functional assays, are needed to determine the precise function and activity associated with each regulatory region.

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