Practical DGS protocol

PROTOCOL FOR:

Simplified DGS procedure for large-scale genome structural study

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Depending on the desired resolution and sequencing scale, different restriction enzymes can be used for genomic DNA digestion. *Pst*I is used here as an example. Among the 6-base restriction enzymes, *Pst*I has the highest restriction frequency in the human genome sequences (Chen J., et al. 2008. Scanning the human genome at kilobase resolution. Genome Research 18(5):751-762).

Procedure

Restriction digestion of genomic DNA

1. Digest genomic DNA with *Pst*I.

Genomic DNA	5–10 µg
NEBuffer 3 (10×, NEB)	10 µL
BSA (10×, NEB)	10 µL
ddH_2O	To 95 µL
PstI (20 U/µL, NEB)	5 µL

2. Incubate at 37°C for at least 4 h.
3. Evaluate digestion efficiency by loading 2 µL digested DNA on a 1% agarose gel, with an undigested DNA as a control (Figure 1).
4. Extract DNA with equal volume of phenol/chloroform and precipitate DNA.

Extracted DNA	200 µL
7.5 M NH_4OAc	100 µL
Glycogen (10 mg/mL)	1 µL
100% cold alcohol (-20°C)	850 µL

5. Incubate on ice for 1 h, spin for 15 min at 4°C, wash with 70% ethanol, centrifuge at full speed, remove ethanol, dry DNA pellet, and resuspend DNA in 30 µL ddH_2O.

Attaching PstI-MmeI-Solexa adaptor to genomic DNA fragments

The *Pst*I-*Mme*I-Solexa adaptor (synthesized, gel-purified, and double strand–formed by IDT) (Figure 2) contains Solexa adaptor A and B sequences and two *Mme*I sites closed to the mutated *Pst*I ends. A *Pst*I site is added between Solexa adaptor A and B. It can be used to remove the artificially ligated adaptor–adaptor complex.

1. Ligation

PstI-MmeI-Solexa Adaptor (200 ng/µL)	4-8 µL
Genomic DNA fragments	5-10 µg
ddH_2O	To 70 µL
Mix well; heat at 75°C for 10 min; chill on ice immediately	
10× T4 DNA Ligase Buffer (Promega)	10 µL
T4 DNA Ligase (3 U/µL; Promega)	20 µL

2. Incubate at 4°C overnight or room temperature for 3 h.
3. Extract, precipitate, and resuspend DNA in 30 µL ddH_2O.

Digestion DNA fragments with PstI to keep single adaptor A and adaptor B

Adaptor-ligated genomic DNA	28 µL
NEBuffer 3 (10×; NEB)	5 µL
BSA (10×; NEB)	5 µL
ddH_2O	To 45 µL
PstI (20 U/µL; NEB)	5 µL

1. Incubate at 37°C for at least 1 h.
2. Evaluate digestion efficiency by loading 3 µL digested DNA on a 1% agarose gel, and load undigested adaptor-ligated genomic DNA at the same time (Figure 3).
3. Recover digested products from the gel that are over 76 bp in length with QIAquick Gel Extraction Kit (Qiagen) to remove the digested adaptors, and resuspend ligation products in 90 µL ddH_2O. Check 5 µL recovered DNA on a 1% agarose gel.

Re-circularization of the purified DNA

Digestion products	80 µL
10× T4 DNA Ligase Buffer (Promega)	10 µL
T4 DNA Ligase (3 U/µL; Promega)	10 µL

1. Incubate at 4°C overnight or room temperature for 3 h.
2. Extract, precipitate, and resuspend DNA in 30 µL ddH_2O.

MmeI digestion of circulated DNA

Circulated DNA	28 µL
NEBuffer 4 (10×; NEB)	6 µL
SAM buffer (3.2 mM; NEB)	1 µL
ddH_2O	To 50 µL
MmeI (2 U/µL; NEB)	10 µL

1. Incubate at 37°C for 1 h.
2. Evaluate digestion efficiency by loading 5 µL digested DNA on 2.5% agarose gel, using *Mme*I-digested pGEM as positive control (Figure 4).
3. Recover 116 bp *Mme*I-digested products with QIAquick Gel Extraction Kit (Qiagen) and resuspend DNA in 30 µL ddH_2O.

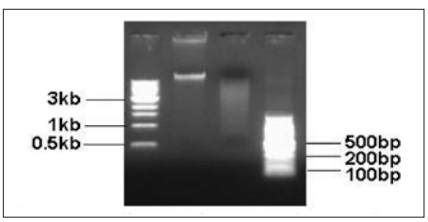

Figure 1. Lane 1–4 (from left to right): 1-kb DNA marker, undigested genomic DNA, PstI-digested genomic DNA, 100-bp DNA marker.

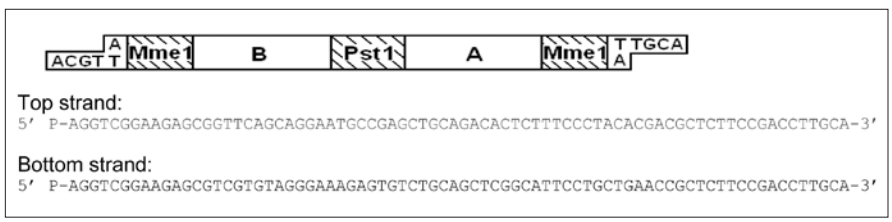

Figure 2. The structure of the adaptor.

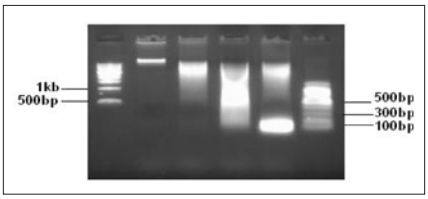

Figure 3. Lane 1–6 (from left to right): 1-kb DNA marker, undigested genomic DNA, PstI-digested genomic DNA, adaptor-ligated genomic DNA, PstI-digested adaptor-genomic DNA, 100-bp DNA marker.

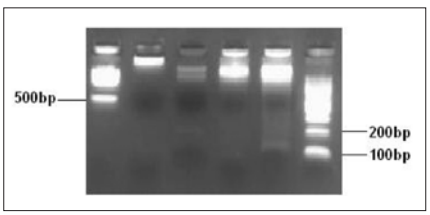

Figure 4. Lane 1–6 (from left to right): 1-kb DNA marker, pGEM, MmeI-digested pGEM, circulated adaptor-ligated DNA, MmeI-digested circulated adaptor-ligated DNA (116-bp adaptor-ditag), 100-bp DNA marker.

Blunt the Tag-Adaptor-Tag fragments

Tag-Adaptor-Tag	30 µL
NEBuffer 2 (10×; NEB)	5 µL
BSA (10×; NEB)	5 µL
1 mM dNTPs (Promega)	5 µL
ddH_2O	To 47 µL
T4 DNA Polymerase (3 U/µL, NEB)	3 µL

1. Incubate at 12°C for 15 min, add 1 µL of 0.5 M PH7.5 EDTA, and inactivate at 75°C for 20 min.
2. Extract, precipitate, and resuspend DNA in 20 µL ddH_2O.

Form ditags by Tag-Adaptor-Tag fragments ligation

Blunted DNA	20 µL
10× T4 DNA Ligase Buffer (Promega)	3 µL
ddH_2O	To 27 µL
T4 DNA Ligase (3 U/µL; Promega)	3 µL

1. Incubate at 15°C for 4–18 h.

PCR amplification of ditags with Solexa primers

Solexa PCR forward primer:
5 -AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3

Solexa PCR reverse primer:
5 -CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-3

Sequencing Primer1:
5 -ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3

Sequencing Primer2:
5 -CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-3

1. Pre-PCR test

	1×
Circulated ditag-adaptor	1 µL
5× Phire Reaction Buffer (NEB)	4 µL
10 mM dNTPs (Promega)	1 µL
Solexa forward (10 ng/µL)	1 µL
Solexa reverse (10 ng/µL)	1 µL
Phire Hot Start DNA Polymerase (NEB)	0.4 µL
ddH_2O	To 20 µL

PCR conditions: 98°C for 30 s, 35 cycles of 98°C for 5 s, 60°C for 5 s; and 72°C for 20 s; 72°C for 1 min, 4°C hold. Use adaptor-ligation products as positive control.

2. Check 5 µL on a 2.5% agarose gel to ensure positive amplification of the 160-bp fragments.

3. Large-scale PCR (20 wells per sample)

	1×	20×
Circulated ditag-adaptor	1 µL	20 µL
5× Phire™ Reaction Buffer (NEB)	4 µL	80 µL
10 mM dNTPs (Promega)	1 µL	20 µL
Solexa forward (10 ng/µL)	1 µL	20 µL
Solexa reverse (10 ng/µL)	1 µL	20 µL
Phire™ Hot Start DNA Polymerase (NEB)	0.4 µL	8 µL
ddH_2O	To 20 µL	To 400 µL

PCR conditions: 98°C for 30 s, 35 cycles of 98°C for 5 s, 60°C for 5 s; and 72°C for 20 s, 72°C for 1 min, 4°C hold.

4. Combine all PCR products into two 1.6-mL Eppendorf tubes (200 µL/tube).

5. Extract, precipitate, and resuspend DNA in 100 µL ddH_2O.

6. Load the entire DNA on a 2.5% agarose gel, excise, and purify the ditag fragments (about 160 bp) that contain ditags and Solexa PCR primers using QIAquick Gel Extraction Kit (Qiagen); resuspend in 50 µL Buffer EB (Qiagen).

7. Check the purified DNA by gel and quantify by optical density (OD). The purified ditag DNA templates are ready for Solexa sequencing reaction. 500–1000 ng will be sufficient for Solexa sequencing collection.

Reagents

- NEBuffer 2 [Cat. no. B7002S; New England BioLabs (NEB), Ipswich, MA USA]
- NEBuffer 3 (Cat. no. B7003S; NEB)
- NEBuffer 4 (Cat. no. B7004S; NEB)
- BSA (Cat. no. B9001S; NEB)
- PstI (Cat. no. R0140S; NEB)
- S-adenosylmethionine (SAM) (Cat. no. B9003S; NEB)
- MmeI (Cat. no. R0637L; NEB)
- T4 DNA Polymerase (Cat. no. M0203L; NEB)
- Phire Hot Start DNA Polymerase (Cat. no. F-120S; NEB)
- 5× Phire Reaction Buffer (Cat. no. F-524S; NEB)
- Glycogen (Cat. no. 10901393001; Roche Diagnostics Corporation, Indianapolis, IN USA)
- Alcohol, ethyl (Cat. no. AB00138; American Bioanalytical, Natick, MA, USA)
- T4 DNA Ligase (Cat. no. M1804; Promega U.S., Madison, WI, USA)
- 10× T4 DNA Ligase Buffer (Cat. no. C1263; Promega U.S.)
- 10 mM dNTP Mix (Cat. no. U1515; Promega U.S.)
- Agarose, LE, Analytical Grade (Cat. no. V3121; Promega U.S.)
- QIAquick Gel Extraction Kit (Cat. no. 28704; Qiagen Inc., Valencia, CA, USA)
- PstI-MmeI-Solexa Adaptor [Integrated DNA Technologies Inc. (IDT), Skokie, IL, USA]
- Solexa forward/reverse primers (IDT)
- Phenol:chloroform (Cat. no. 0883; Amresco Inc., Solon, OH, USA)
- Ammonium acetate (NH_4OAc) (Cat. no. 0103; Amresco Inc.)
- EDTA (Cat. no. 0105; Amresco Inc.)
- ddH_2O (made in-house)

Equipment

- 1.6-mL Ultra Clear Graduated Micro Tube (Cat. no. 3445; Biotix, Inc., San Diego, CA, USA)
- GeneAmp PCR System 9700 (Cat. no. N8050200; Applied Biosystems Inc., Foster City, CA, USA)
- Centrifuge (Cat. no. 5415C; Eppendorf North America, Westbury, NY, USA)
- BioPhotometer (Cat. no. RS232C; Eppendorf North America)
- Digital Dry Bath (Cat. no. D1100; Labnet International, Inc., Woodbridge, NJ USA)
- Gel XL Ultra (Cat. no. E0145A; Labnet International, Inc.)

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