DNA Digestion with DNase I

Steven J. Burgess

Abstract

Protocol for DNase I digestion of nuclei for 'double hit' DNase-SEQ analysis adapted from doi:10.1038/nmeth.2762

As a general guide DNA should show moderate to light smearing after completing the protocol as seen the gel image in Figure 1.

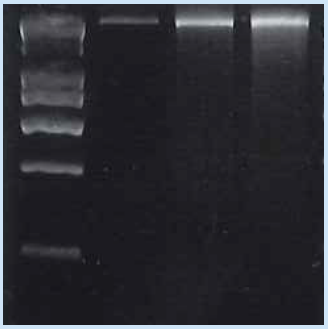

Figure 1: A representative 2% (w/v) LMP agarose gel of DNAse I treated nuclei (~2x10^{8}) from *Sorghum bicolor.* To each sample the following amounts of DNase I were added: none, 7.5U, 12.5U.

Over-digested samples will result in a high background signal from unspecific cutting of DNA and should be avoided. An example of an over-digested sample is provided in lane 5 of Figure 2.

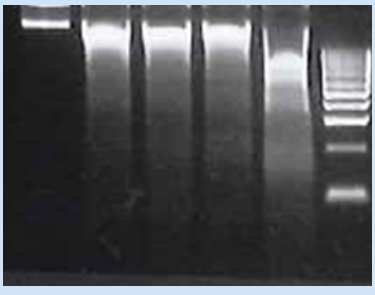

Figure 2: A representative 2% (w/v) LMP agarose gel of DNAse I treated nuclei (~2x10^{8}) from *Sorghum bicolor.* To each sample the following amounts of DNase I were added: none, 5,U 7.5U,10U, 12.5U.

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Gel fragments from ~50-600bp should be excised and DNA purified. From experience taking only smaller fragments (50-200 bp) caused difficulties in library preparation.

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Materials

cOmplete™, Mini, EDTA-free (Protease Inhibitor) #11836170001) by Roche
Qiaquick gel extraction kit 28704 by Qiagen
DNase I, RNase free EN0525 by Thermo Fisher Scientific
RNase A R4642-10MG by Sigma-aldrich
GlycoBlue Coprecipitant AM9515 by Thermo Fisher Scientific
UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) 15593031 by Thermo Fisher Scientific
UltraPure™ Low Melting Point Agarose 16520050 by Thermo Fisher Scientific

Protocol

Step 1.

Before the experiment make up stocks and store at 4°C.

Buffer A (1L)

Component	[Stock]/M	Volume/mL	[Final]/mM
TRIS-HCl pH 8.0	1	15	15
NaCl	5	3	15
KCl	1	60	60
EDTA pH 8.0	0.5	2	1
dH_2O		up to 1L	

10x Buffer D (10 mL)

Component	[Stock]/M	Volume	[Final]/mM
$MgCl_2$	1	0.6	60

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NaCl	5	0.2	100
$CaCl_2$	1	0.1	10
TRIS-HCl pH 8.0	1	4	400
dH_2O		up to 10 mL	

Stop Buffer (1L)

Component	[Stock]	Volume/mL	[Final]
TRIS-HCl pH8.0	1M	50	50mM
NaCl	5M	20	100mM
SDS	20% (w/v)	5	0.1% (w/v)
EDTA pH8.0	0.5M	200	100mM
dH_2O		up to 1L	

Buffer Preparation

Step 2.

On the day of the experiment make the following buffers:

Buffer A+

Component	[Stock]	Volume/µL
Spermine	0.1M	30
Spermidine	0.1M	100
DTT	1M	10
PIC*	100x	200
NP-40		40
Buffer A		20

*PIC=Protease inhibitor cocktail (1 tablet of Roche EDTA-free complete protease inhibitor is resuspended in 500 µl)

1x Buffer D

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Component	Volume/mL
10x Buffer D	1.5
Buffer A+	13.5

Stop Buffer Stock

Component	[Stock]	Volume
Spermine	0.1M	30
Spermidine	0.1M	100
RNAse A	10mg/mL	20
Stop Buffer Stock		Up to 10mL

Prepare nuclei

Step 3.

Spin down nuclei at 1500g for 10 mins. Wash once with buffer A and re-suspend in 1x Buffer D to a concentration of $4x10^8$ nuclei/mL

NOTES

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Note: The amount of nuclei required for each reaction may well vary between species, a concentration of ~4 x 10^8 worked well for Sorghum bicolor, Setaria italica, Zea mays and Brachypodium distachyon.

Preparing Reaction

Step 4.

Prepare enzyme mixtures in 2 mL centrifuge tubes:

Final Enzyme Concentration/U	Amount of Buffer D/µL;	Amount of DNAseI [1U/µL]/µL
0	0	500
2.5	2.5	497.5
7.5	7.5	492.5
12.5	12.5	487.5

NOTES

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Note: From experfience a range of enzyme concentrations should be used due to variability in digestion outcome. Manual inspection of the resulting digestions dictates which samples to choose

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for further processing.

Pre-Incubation

Step 5.

Incubate nuclei and enzyme mixtures at 37°C

DURATION
00:01:00

Digestion

Step 6.

Add 500 µL of nuclei to 500 µL of enzyme mix. Mix by inverting and incubate at 37°C.

Stopping the reaction

Step 7.

Stop the reaction by addition of 1 mL of stop buffer and incubate tubes at 55 °C.

DURATION
00:15:00

Proteinase K digestion

Step 8.

Add proteinase K [20mg/mL] to each tube and incubate at 55 °C.

AMOUNT
2 µl Additional info:
DURATION
01:00:00

DNA extraction part I

Step 9.

Add 1:1 (v/v) phenol:chloroform:isoamylalcohol (25:24:1) to each reaction, hand shake vigorously. spin down 14,000rpm in microcentrifuge tube.

DURATION
00:15:00

Step 10.

DURATION
01:00:00

Step 11.

Spin down 14,000 rpm in a microcentrifuge

DURATION
00:15:00

Step 12.

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Dispose of ethanol solution and wash pellet in 1 mL 70% ethanol (v/v). Invert tube 3-5 times and spin down 14,000 rpm in microcentrifuge.

Step 13.

Step 14.

DNA extraction part V

Step 15.

Perform gel extraction using QIAGEN QIAquick gel extraction kit, quantify the amount of DNA using a Qubit prior to library construction.

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