Cat. # 6137

For Research Use

TaKaRa

# DNA Fragmentation Kit

Product Manual

v202103Da

# Table of Contents

## I. Description

This kit is designed to perform random fragmentation of genomic DNAs and other long-chain dsDNAs by enzyme treatments without any special apparatus such as a sonicator, and then to blunt the obtained DNA fragment. Blunt-end fragments may be inserted into blunt-end vectors. The reaction may be stopped after fragmentation is complete if no blunting is required.
This kit may also be used to perform the pretreatment in methylated DNA enrichment and high-speed DNA sequence analysis.

## II. Components (for 20 preps)

| | | | |
|---|---|---|---|
| 1. | Enzyme- 1 | 20 µl | |
| 2. | Dilution Buffer- 1 | 1,040 µl | |
| 3. | A solution | 20 µl | |
| 4. | B solution | 50 µl | |
| 5. | Stop Solution | 400 µl | |
| 6. | 150 mM $MgCl_2$ | 40 µl | |
| 7. | Dilution Buffer- 2 | 200 µl | |
| 8. | Enzyme- 2 | 20 µl | |
| 9. | 0.5 M EDTA | 50 µl | |
| 10. | $dH_2O$ | 1 ml | x 10 |

## III. Storage

-20℃

**Note:** Components 3, 4, 6, 9 and 10 may be stored at 4℃.

## IV. Materials Required but not Provided

### 1. Reagents

- Electrophoresis loading buffer
  We recommend using a loading buffer that contains a dye (e.g., Orange G) that would not overlap with bands for fragments that are 100 - 1,000 bp on the electrophoresis gel. When using BPB or Xylene Cyanol, please be aware that they overlap with DNA fragments in size.

### 2. Apparatus

- Thermal Cycler (at least 1; 2 are preferred)

## V. Precautions for Use

This section describes precautions for using this kit. Be sure to read them before use.

1. Handle all reagents on ice. Mix reactions on ice as well. Make sure the temperature in reaction tubes does not rise.
2. When checking fragment sizes, RNA contamination may affect the accuracy of size-distribution analysis. Therefore, DNA samples should be treated with RNaseA followed by cleanup using a procedure such as phenol/chloroform treatment before use in reactions.

   General genomic DNA purification kits such as NucleoSpin Tissue (Cat. #740901.50) or NucleoSpin Blood (Cat. #740951.50) may be used to prepare genomic DNA.
3. Use a DNA solution with an EDTA concentration of no more than 1 mM.

## VI. Protocol

### 1. Fragmentation

A) Determine reaction volume recommended

Genomic DNA ≤ 100 ng: 10 µl reaction volume
Genomic DNA 100 ng - 1 µg: 20 µl reaction volume

**Note:** For samples of 1 µg or more, increase the number of reaction tubes based on a reaction unit of 1 µg/20 µl or scale the reaction volume up to 5 µg/100 µl.

B) Set one thermal cycler to 16℃ and the other to 70℃. If you are using only one thermal cycler, set it to 16℃.

C) Place the following reagents, except genomic DNA, into a 0.2 ml PCR tube on ice and mix well. Then add the genomic DNA. Mix well by pipetting or tapping without creating bubbles, and then spin down. Do not use vortex.

| Reagent | Volume |
|---|---|
| Dilution Buffer-1 | 1.9 µl |
| A solution | 1 µl |
| B solution | 1 µl |
| Genomic DNA | 1 µg |
| $dH_2O$ | x µl |
| Total | 19 µl |

**Note:** Reagent volumes provided are for 1 µg genomic DNA (1 µg/20 µl reaction x 1 tube). If you are using less genomic DNA, adjust volumes accordingly.

D) Dilute Enzyme-1 following the method below.

**Note:** Use diluted Enzyme-1 within 10 min after preparation; do not store for reuse.

[Enzyme-1 Dilution method]

- Add reagents to a 1.5 ml microtube on ice in the order shown below and mix.

| Reagent | Volume |
|---|---|
| $dH_2O$ | 450 µl |
| Dilution Buffer-1 | 50 µl |

- Vortex gently and spin down.
- Next, add 1 µl of Enzyme-1. Mix gently by pipetting 10 times without creating bubbles. Then gently invert 5 times to mix followed by spin down. Do not tap or vortex.

E) Make sure the thermal cycler temperature is 16℃. Add 1 µl of diluted Enzyme-1 (from Step 1-D) to the reaction mixture (prepared in Step 1-C). Mix by pipetting several times and then transfer immediately to the thermal cycler set at 16℃ to begin the reaction. Let the reaction run for 5 - 8 min.

**Note:** Perform reagent addition and mixing as close to the surface of ice as possible.

F) To stop the reaction, add 20 μl of the Stop solution to the tube as it stands in the thermal cycler. Mix by pipetting 2 - 3 times, place the tube on ice and mix a few more times. If using one thermal cycler, adjust the temperature setting to 70℃.

G) Transfer the tube from ice to the 70℃ thermal cycler.

H) Allow reaction to take place for 5 min then place the tube on ice.

**Note:** Let stand on ice for at least 2 min.

I) If you are performing fragmentation only (without blunting), add 1 μl of 0.5 M EDTA to your reaction, mix well by pipetting and then purify the fragmented DNA.

**2. Blunting**

A) Set one thermal cycler to 16℃ and the other to 70℃. If you are using only one thermal cycler, set it to 16℃.

B) Dilute Enzyme-2 (prepare immediately before use).

[Dilution method]

- Place a 0.2-ml PCR tube on ice and mix Dilution Buffer-2 and Enzyme-2 at a 9 : 1 ratio. Mix by gentle pipetting without creating bubbles.

C) Add 2 μl of 150 mM $MgCl_2$ to the fragmented DNA (from Step 1-H) and mix well by pipetting without creating bubbles. Do not vortex.

D) Add 2 μl of diluted Enzyme-2 (from Step 2-B). After pipetting several times to mix, transfer immediately to the thermal cycler set at 16℃. Allow reaction to run at 16℃ for 10 min.

**Note:** Perform reagent addition and mixing as close to the surface of ice as possible.

E) Transfer the tube on ice. If using one thermal cycler, adjust the temperature setting to 70℃.

F) Add 1 μl of 0.5 M EDTA and mix well by pipetting followed by spin down.

G) Make sure your thermal cycler is 70℃, then transfer the tube from ice to the thermal cycler and allow reaction to take place for 5 min.

H) Transfer the tube to ice.

Fragmentation check

Analyze a sample of fragmented genomic DNA equivalent to 200 - 250 ng [about 10 μl of the reaction mixture from Step 2-H] by electrophoresis in 1.5% Agarose LO3 gel.

Purification example of the fragmented DNA

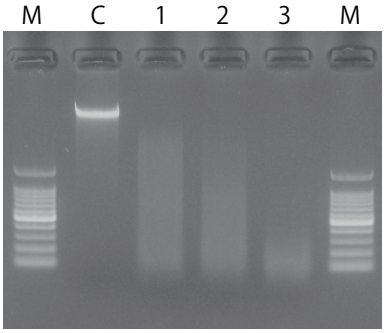
Purify the fragmented DNA by a phenol/chloroform treatment or a DNA purification kit to remove short-chain fragments, dNTPs and enzymes.

**Note:** When performing ethanol precipitation, use a coprecipitation agent [e.g., Dr. GenTLE™ Precipitation Carrier (Cat. #9094)].

## VII. Experimental Examples

### 1. Fragmentation of 1 μg of genomic DNA from *E. coli* W3110.

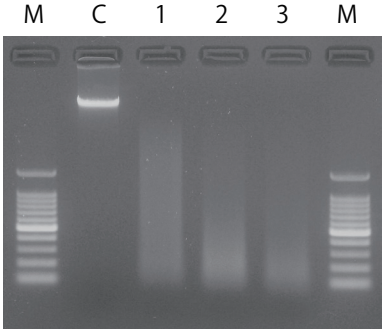
- A fragmentation reaction (16℃ : 5 min, 7 min or 9 min) and a blunting reaction were performed in accordance with the protocol.
- 11 μl aliquots of each reaction mixture were analyzed by agarose gel electrophoresis to confirm fragmentation.




Lane C : untreated genomic DNA 200 ng
1 : 16℃, 5 min
2 : 16℃, 7 min
3 : 16℃, 9 min
M : 100 bp DNA Ladder
1.5% Agarose L03 [TAKARA] (Cat. #5003)

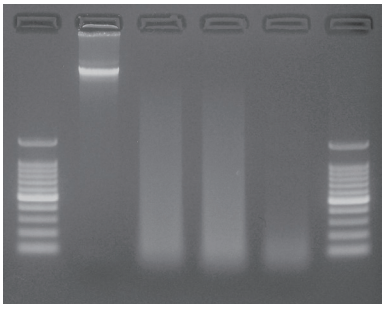
### 2. Fragmentation of genomic DNA with GC content bias

A) After a fragmentation reaction (16℃ : 5 min, 7 min or 9 min) and a blunting reaction on 1 μg of genomic DNA from *Pyrococcus furiosus* DSM 3638 (40% GC content) in accordance with the protocol, 11 μl aliquots of each final reaction mixture were analyzed by agarose gel electrophoresis to confirm fragmentation.




Lane C : untreated genomic DNA 200 ng
1 : 16℃, 5 min
2 : 16℃, 7 min
3 : 16℃, 9 min
M : 100 bp DNA Ladder
1.5% Agarose L03 [TAKARA] (Cat. #5003)

B) After a fragmentation reaction (16℃: 5 min, 7 min or 9 min) and a blunting reaction on 500 ng of *Thermus thermophilus* HB8 Genomic DNA Solution (Cat. #3071) (69% GC content) according to the protocol, 11-μl aliquots of each final reaction mixture were analyzed by agarose gel electrophoresis to confirm fragmentation.




Lane C : untreated genomic DNA 100 ng
1 : 16℃, 5 min
2 : 16℃, 7 min
3 : 16℃, 9 min
M : 100 bp DNA Ladder
1.5% Agarose L03 [TAKARA] (Cat. #5003)

**3. Cloning into blunt-end vector pUC118 *Hinc* II and insert check**

Sample: genomic DNA of *E. coli* W3110 1 μg

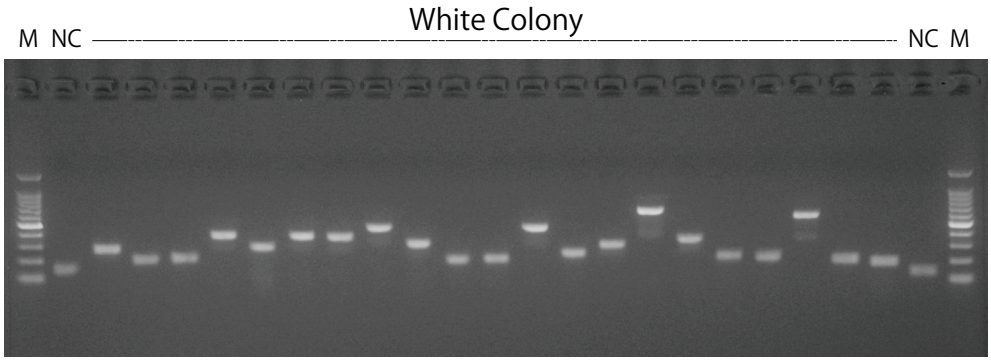
Fragmentation condition: 16℃, 8 min

- The blunt-end reaction mixture was purified by a DNA purification column yielding 25 μl of purified DNA solution.
- 5 μl of this solution was added to a ligation reaction mixture containing 25 ng of pUC118 *Hinc* II/BAP (Cat. #3322). The reaction was run at 16℃ for 30 min using Mighty Cloning Reagent Set (Blunt End) (Cat. #6027).
- Half of the ligation reaction was used to transform 100 μl of *E. coli* JM109 Competent Cells (Cat. #9052).
- After the addition of 900 μl of SOC Medium, 25 - 50 μl aliquots were seeded on LB+Amp plates, yielding white and blue colonies, respectively, at 150 - 300 colonies/plate and 35 - 70 colonies/plate.
- The following reagent and primers were used for the insert checks of the colonies.
  - SapphireAmp Fast PCR Master Mix (Cat. #RR350A)
  - M13 Primer M4 (Cat. #3832A)
  - Ladderman Sequencing Primer RV-M

PCR Reaction condition:

| | | |
|---|---|---|
| 94℃ | 1 min | |
| ↓ | | |
| 98℃ | 5 sec | ┐ |
| 55℃ | 5 sec | ├ 30 cycles |
| 72℃ | 10 sec | ┘ |

M : 100 bp DNA Ladder
NC : blue colony (no insert = about 130 bp DNA amplification product)
2% Agarose L03 [TAKARA] (Cat. #5003)



## VIII. Q & A

Q1. Fragment sizes were too small. What should I do?
A1. With Enzyme-1 being very sensitive to temperature, you should perform procedures on ice to the extent possible. Moving tubes away from ice when adding an enzyme or holding the tube may raise the temperature of reagents, resulting in excessive fragmentation. Shortening the fragmentation reaction time may sometimes improve fragment size distribution.

Q2. Why is there unsheared residual DNA?
A2. This may be attributable to unevenly mixed reaction mixture.

**Note:** It is important to mix the reactions well in procedures (such as Step 1-C or 2-C) before adding enzyme.

## IX. Related Products

TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
Agarose L03 [TAKARA] (Cat. #5003)
100 bp DNA Ladder (Cat. #3407)
*Thermus thermophilus* HB8 Genomic DNA Solution (Cat. #3071)
Alkaline Phosphatase (*E. coli* C75) (Cat. #2120A/B)
Alkaline Phosphatase (Calf intestine) (Cat. #2250A/B)
pUC118 *Hin*c II/BAP (Cat. #3322)
Dr. GenTLE™ Precipitation Carrier (Cat. #9094)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
*E. coli* JM109 Competent Cells (Cat. #9052)
SapphireAmp® Fast PCR Master Mix (Cat. #RR350A)
M13 Primer M4 (Cat. #3832A)
NucleoSpin Tissue (Cat. #740901.10/.50/.250)
NucleoSpin Blood (Cat. #740951.10/.50/.250)