Fragmentation of AT-rich Genomic DNA with NEBNext™ dsDNA Fragmentase

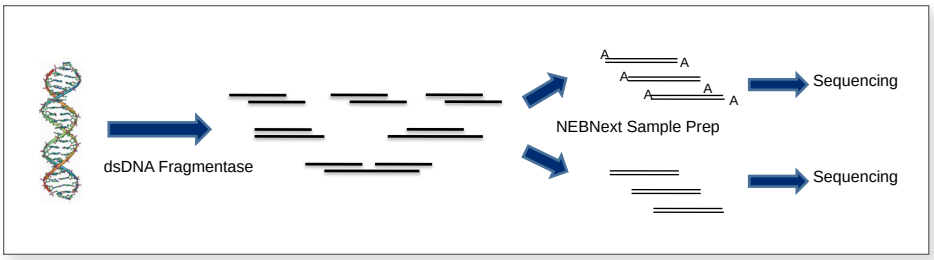

Figure 1: *NEBNext dsDNA Fragmentase generates DNA fragments suitable for next generation sequencing workflows.*

Introduction

Fragmentation of genomic DNA is required during next generation sequencing workflows as well as for creating DNA inserts for expression libraries. NEBNext dsDNA Fragmentase has been optimized to fragment DNA into size ranges compatible with Roche 454, AB SOLiD, and Illumina GAII sequencing workflows.

DNA fragmentation by physical or enzyme based methods is affected by %GC content of genomic DNA. This application note describes the use of NEBNext dsDNA Fragmentase to efficiently fragment AT-rich genomic DNA (> 60% AT). In general, shorter incubation times are required to generate 100-800 bp fragments from AT-rich genomic DNA using NEBNext dsDNA Fragmentase.

General Protocol

1. Set up the digestion reaction at room temperature using the following guidelines (note that dsDNA Fragmentase should not be added at this point):

Table I. dsDNA Fragmentase reaction conditions for AT-rich genomic DNA

Reaction Components	Starting DNA Amount				
AT-rich gDNA (µg)	1	2	3	4	5
10X Fragmentase Reaction Buffer (µl)	2	4	6	8	10
100X BSA (µl)	0.2	0.4	0.6	0.8	1
dsDNA Fragmentase (µl)	2	4	6	8	10
sterile dH_2O	variable	variable	variable	variable	variable
Final Volume (µl)	20	40	60	80	100

2. Incubate samples on ice for 5 minutes.
3. Add NEBNext dsDNA Fragmentase according to Table I.
4. Incubate at 37°C according to the recommended times below to generate the desired DNA fragment size.

Applications

- Generation of dsDNA fragments for sequencing on next generation sequencing platforms
- Generation of dsDNA fragments for libraries

Materials

- NEBNext dsDNA Fragmentase (NEB #M0348)
- 10X NEBNext dsDNA Fragmentase Reaction Buffer (NEB #B0348)
- 100X BSA (NEB #B9000)
- *Staphylococcus aureus* genomic DNA (67% AT)

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Table II. NEBNext dsDNA Fragmentase reaction times for AT-rich gDNA

Desired Fragment Size (bp)	Incubation Time (min)
600–800	10
300–600	15
100–300	20

5. Stop the reaction by adding 5 µl of 0.5 M EDTA
6. Purify DNA Sample on one column and elute in 35 µl of sterile dH_2O or elution buffer
7. End Repair. If DNA fragments are to be used for sample preparation for the Illumina Genome Analyzer or for TA cloning for library screening, add 1 µl of *E. coli* DNA ligase for Fragmentase (NEB #M0349) to the end repair reaction with > 1 µg DNA or 0.5 µl of *E. coli* DNA ligase for Fragmentase to the end repair reaction with < 1 µg DNA.

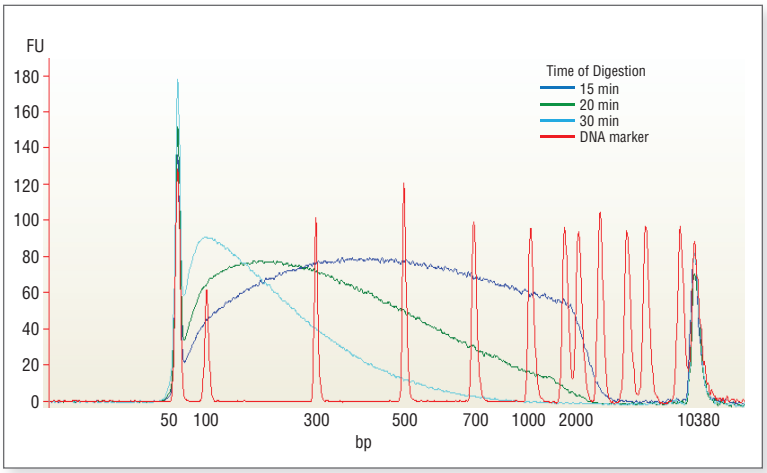

Figure 2: *Distribution of Staphylococcus aureus gDNA fragments produced by dsDNA Fragmentase and separated on an Agilent Bioanalyzer 2100.*

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Fragmentation of GC-rich Genomic DNA with NEBNext™ dsDNA Fragmentase

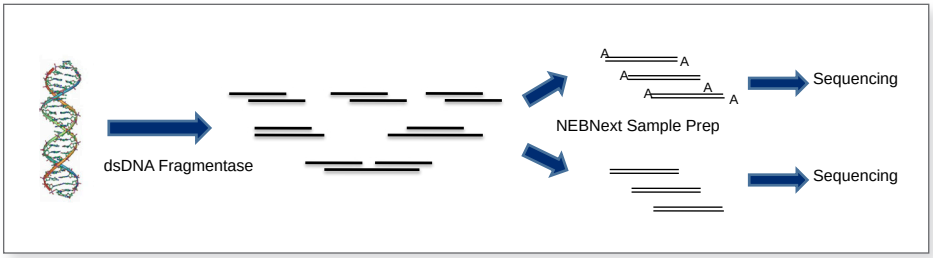

Figure 1: *NEBNext dsDNA Fragmentase generates DNA fragments suitable for next generation sequencing workflows.*

Introduction

Fragmentation of genomic DNA is required during next generation sequencing workflows as well as for creating DNA inserts for expression libraries. NEBNext dsDNA Fragmentase has been optimized to fragment DNA into size ranges compatible with Roche 454, AB SOLiD, and Illumina GAII sequencing workflows.

DNA fragmentation by physical or enzyme based methods is affected by %GC content of genomic DNA. This application note describes the use of NEBNext dsDNA Fragmentase to efficiently fragment GC-rich genomic DNA (> 60% GC). In general, longer incubation times are required to generate 100-800 bp fragments from GC-rich genomic DNA using NEBNext dsDNA Fragmentase.

General Protocol

1. Set up the digestion reaction at room temperature using the following guidelines (note that dsDNA Fragmentase should not be added at this point):

Table I. dsDNA Fragmentase reaction conditions for GC-rich genomic DNA

Reaction Components	Starting DNA Amount				
GC-rich gDNA (µg)	1	2	3	4	5
10X Fragmentase Reaction Buffer (µl)	2	4	6	8	10
100X BSA (µl)	0.2	0.4	0.6	0.8	1
dsDNA Fragmentase (µl)	2	4	6	8	10
sterile dH_2O	variable	variable	variable	variable	variable
Final Volume (µl)	20	40	60	80	100

2. Incubate samples on ice for 5 minutes.
3. Add NEBNext dsDNA Fragmentase according to Table I.
4. Incubate at 37°C according to the recommended times below to generate the desired DNA fragment size.

(see other side)

Applications

- Generation of dsDNA fragments for sequencing on next generation sequencing platforms
- Generation of dsDNA fragments for libraries

Materials

- NEBNext dsDNA Fragmentase (NEB #M0348)
- 10X NEBNext dsDNA Fragmentase Reaction Buffer (NEB #B0348)
- 100X BSA (NEB #B9000)
- *Rhodobacter sphaeroides* genomic DNA (68% GC)

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Table II. NEBNext dsDNA Fragmentase reaction times for GC-rich gDNA

Desired Fragment Size (bp)	Incubation Time (min)
600–800	40
300–600	50
100–300	60

5. Stop the reaction by adding 5 µl of 0.5 M EDTA
6. Purify DNA Sample on one column and elute in 35 µl of sterile dH_2O or elution buffer
7. End Repair. If DNA fragments are to be used for sample preparation for the Illumina Genome Analyzer or for TA cloning for library screening, add 1 µl of *E. coli* DNA ligase for Fragmentase (NEB #M0349) to the end repair reaction with > 1 µg DNA or 0.5 µl of *E. coli* DNA ligase for Fragmentase to the end repair reaction with < 1 µg DNA.

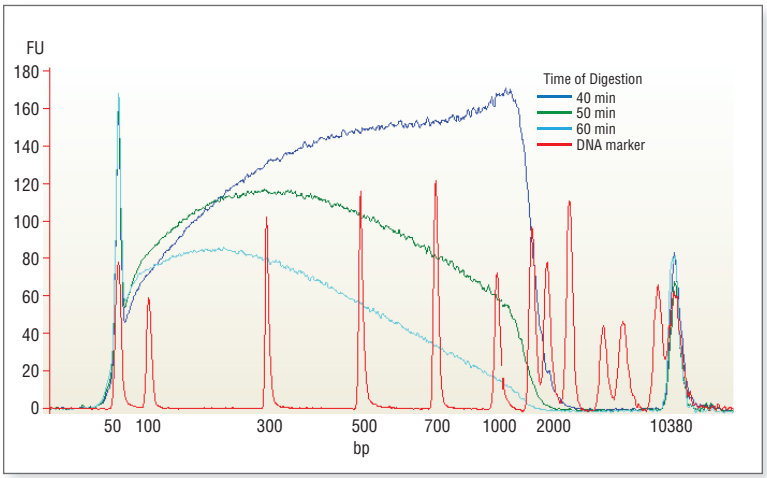

Figure 2: *Distribution of Rhodobacter sphaeroides fragments produced by dsDNA Fragmentase and separated on an Agilent Bioanalyzer 2100.*

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Fragmentation of RT-PCR and PCR Products with NEBNext™ dsDNA Fragmentase

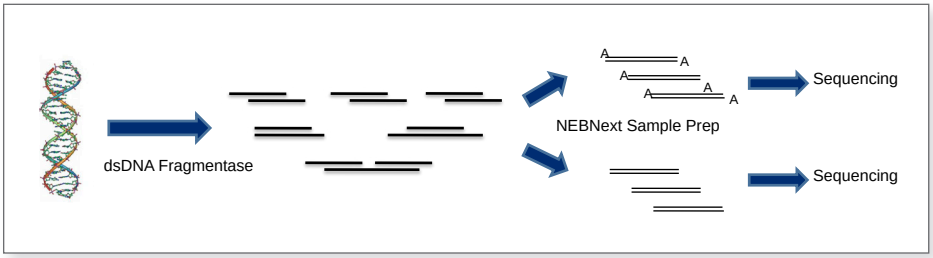

Figure 1: *NEBNext dsDNA Fragmentase generates DNA fragments suitable for next generation sequencing workflows.*

Introduction

Fragmentation of genomic DNA is required during next generation sequencing workflows as well as for creating DNA inserts for expression libraries. NEBNext dsDNA Fragmentase has been optimized to fragment DNA into size ranges compatible with Roche 454, AB SOLiD, and Illumina GAII sequencing workflows.

This application note describes the use of NEBNext dsDNA Fragmentase to efficiently fragment 1 to 6 kb RT-PCR and PCR products into 100–800 bp fragments.

General Protocol

1. Set up the digestion reaction at room temperature using the following guidelines (note that dsDNA Fragmentase should not be added at this point):

Table I. dsDNA Fragmentase reaction conditions for RT-PCR and PCR products

Reaction Components	Starting DNA Amount				
PCR or RT-PCR product (µg)*	1	2	3	4	5
10X Fragmentase Reaction Buffer (µl)	2	4	6	8	10
100X BSA (µl)	0.2	0.4	0.6	0.8	1
dsDNA Fragmentase (µl)	2	4	6	8	10
sterile dH_2O	variable	variable	variable	variable	variable
Final Volume (µl)	20	40	60	80	100

* PCR or RT-PCR products were purified by column purification

2. Incubate samples on ice for 5 minutes.
3. Add NEBNext dsDNA Fragmentase according to Table I.
4. Incubate at 37°C according to the recommended times below to generate the desired DNA fragment size.

Applications

- Generation of dsDNA fragments for sequencing on next generation sequencing platforms
- Generation of dsDNA fragments for libraries

Materials

- NEBNext dsDNA Fragmentase (NEB #M0348)
- 10X NEBNext dsDNA Fragmentase Reaction Buffer (NEB #B0348)
- 100X BSA (NEB #B9000)
- 1.1 kb PCR product *E. coli malE* gene
- 5.8 kb human *dnmt* RT-PCR product

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Table II. NEBNext dsDNA Fragmentase reaction times for RT-PCR and PCR products

Desired Fragment Size (bp)	Incubation Time (min)
600–800	15
300–600	20
100–300	30

5. Stop the reaction by adding 5 µl of 0.5 M EDTA
6. Purify DNA Sample on one column and elute in 35 µl of sterile dH_2O or elution buffer
7. End Repair. If DNA fragments are to be used for sample preparation for the Illumina Genome Analyzer or for TA cloning for library screening, add 1 µl of *E. coli* DNA ligase for Fragmentase (NEB #M0349) to the end repair reaction with > 1 µg DNA or 0.5 µl of *E. coli* DNA ligase for Fragmentase to the end repair reaction with < 1 µg DNA.

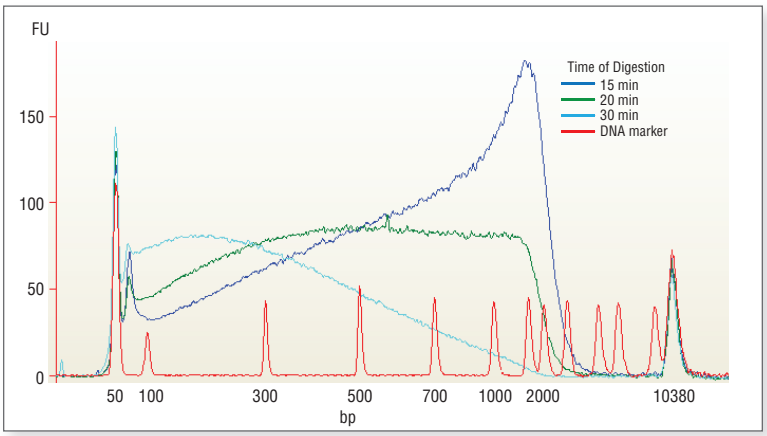

Figure 2: *Distribution of RT-PCR (5.8 kb) product fragments produced by dsDNA Fragmentase and separated on an Agilent Bioanalyzer 2100.*

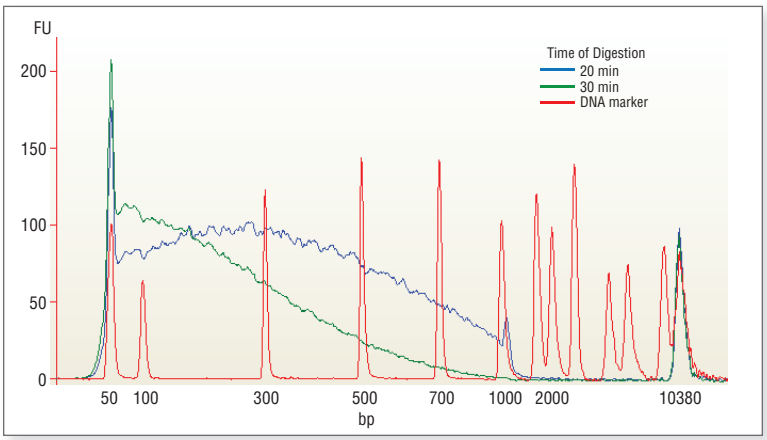

Figure 3: *Distribution of PCR (1.1 kb) product fragments produced by dsDNA Fragmentase and separated on an Agilent Bioanalyzer 2100.*

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Fragmentation of Whole Genome Amplified (WGA) DNA with NEBNext™ dsDNA Fragmentase

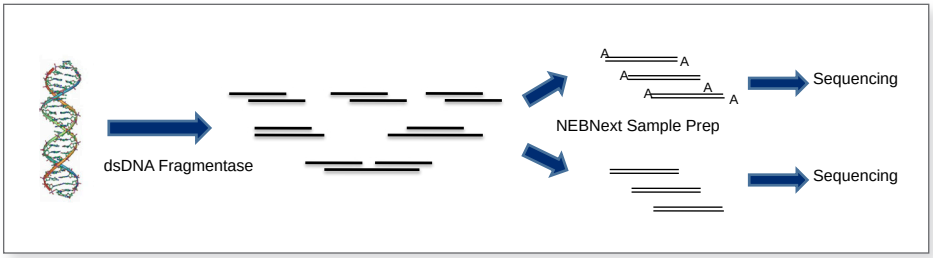

Figure 1: *NEBNext dsDNA Fragmentase generates DNA fragments suitable for next generation sequencing workflows.*

Introduction

Fragmentation of genomic DNA is required during next generation sequencing workflows as well as for creating DNA inserts for expression libraries. NEBNext dsDNA Fragmentase has been optimized to fragment DNA into size ranges compatible with Roche 454, AB SOLiD, and Illumina GAII sequencing workflows.

This application note describes the use of NEBNext dsDNA Fragmentase to efficiently fragment whole genome amplified (WGA) DNA.

General Protocol

1. Set up the digestion reaction at room temperature using the following guidelines (note that dsDNA Fragmentase should not be added at this point):

Table I. dsDNA Fragmentase reaction conditions for whole genome amplified (WGA) DNA

Reaction Components	Starting DNA Amount				
WGA HeLa DNA* (µg)	1	2	3	4	5
10X Fragmentase Reaction Buffer (µl)	2	4	6	8	10
100X BSA (µl)	0.2	0.4	0.6	0.8	1
dsDNA Fragmentase (µl)	2	4	6	8	10
sterile dH_2O	variable	variable	variable	variable	variable
Final Volume (µl)	20	40	60	80	100

* WGA DNA was prepared by amplifying HeLa cell genomic DNA with the Qiagen REPLI-g Mini Kit according to the manufacturer instructions. WGA was purified by ethanol precipitation and suspended in dH_2O.

2. Incubate samples on ice for 5 minutes.
3. Add NEBNext dsDNA Fragmentase according to Table I.
4. Incubate at 37°C according to the recommended times below to generate the desired DNA fragment size.

Applications

- Generation of dsDNA fragments for sequencing on next generation sequencing platforms
- Generation of dsDNA fragments for libraries

Materials

- NEBNext dsDNA Fragmentase (NEB #M0348)
- 10X NEBNext dsDNA Fragmentase Reaction Buffer (NEB #B0348)
- 100X BSA (NEB #B9000)
- Whole genome amplified (WGA) HeLa cell genomic DNA (prepared by REPLI-g Mini Kit (Qiagen, Inc.)

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Table II. NEBNext dsDNA Fragmentase reaction times for whole genome amplified (WGA) DNA

Desired Fragment Size (bp)	Incubation Time (min)
600–800	15
300–600	20
100–300	30

5. Stop the reaction by adding 5 µl of 0.5 M EDTA
6. Purify DNA Sample on one column and elute in 35 µl of sterile dH_2O or elution buffer
7. End Repair. If DNA fragments are to be used for sample preparation for the Illumina Genome Analyzer or for TA cloning for library screening, add 1 µl of *E. coli* DNA ligase for Fragmentase (NEB #M0349) to the end repair reaction with > 1 µg DNA or 0.5 µl of *E. coli* DNA ligase for Fragmentase to the end repair reaction with < 1 µg DNA.

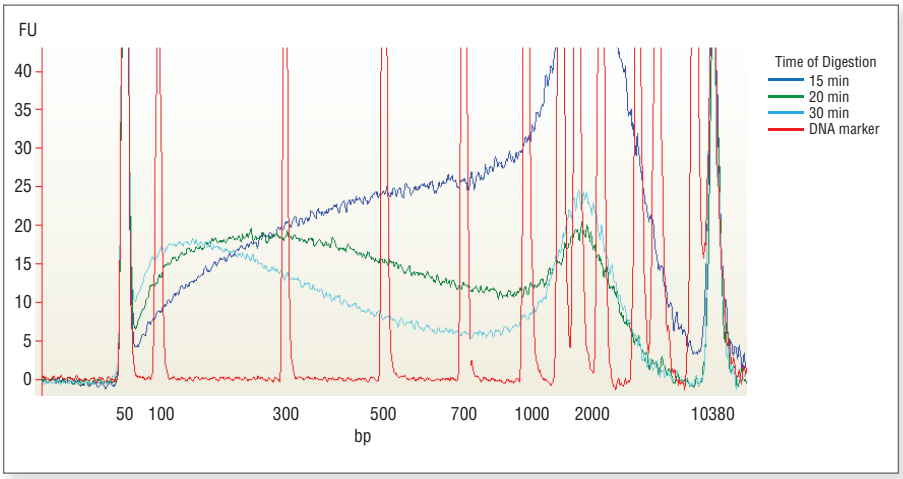

Figure 2: *Distribution of whole genome amplified (WGA) HeLa cell genomic DNA fragments produced by dsDNA Fragmentase and separated on an Agilent Bioanalyzer 2100.*

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