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Instruction manual for GenNext NGS Library Prep Kit 2103 F1753K

GenNext™ NGS Library Prep Kit

LPK-101	24 reactions
LPK-101T	8 reactions
LPK-101L	96 reactions

Store at -20°C

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory safety precautions while using this kit.

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FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

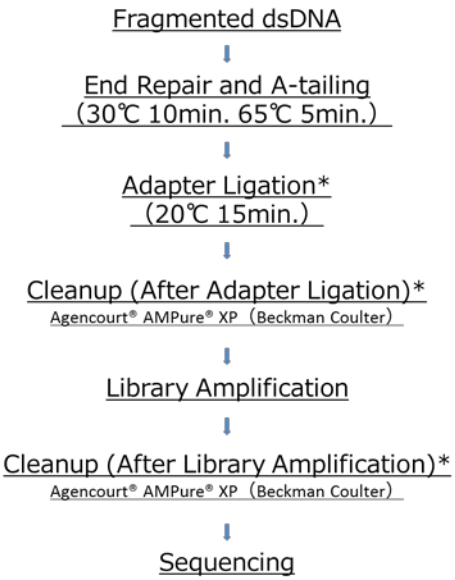
[1] Introduction

Description

GenNext™ NGS Library Prep Kit comprises the enzymes and buffers for preparing libraries for illumina® sequencing from fragmented double-stranded DNA and PCR products.

With this system, it is possible to conveniently and quickly convert a broad range (1ng - 1μg) of input amounts of DNA into libraries for illumina® sequencing. Terminal repair and 3' end adenylation of the fragmented DNA can be conducted in the end repair and A-tailing step. Platform-specific adapters are then ligated to both ends of the DNA fragments.

If required, a high-fidelity amplification step can be performed using the reagents included in the GenNext™ NGS Library Prep Kit. Library Amplification Master Mix uses a highly-accurate PCR enzyme developed using genetically-modified KOD DNA polymerase. This minimizes the influence of GC bias on amplification and can amplify various regions evenly.



Process Workflow

* Adapters and beads required for cleanup after adapter ligation and library amplification are not included.

Features

-Simple and quick operation flow

The steps from terminal repair and 3' end adenylation to adapter ligation can be conducted in the same container. Terminal repair and adenylation at the 3' end can be performed in 15 minutes. Adapter ligation can be done in 15 minutes. Library amplification can be performed in cycles of 10 seconds' annealing and 15 seconds' extension.

-A wide range of input amount

GenNext™ NGS Library Prep Kit is compatible with various inputs from 1 ng to 1 μg.

-Low bias library amplification

Library Amplification Master Mix uses a highly-accurate PCR enzyme developed using genetically-modified KOD DNA polymerase. It minimizes the influence on GC-bias-induced amplification, and it is possible to amplify various regions evenly.

[2] Components

The kits include the following reagents, which can be used for 24 (LPK-101), 8 (LPK-101T) and 96 (LPK-101L) reactions. All reagents should be stored at -20°C.

GenNext™ NGS Library Prep Kit (Code No. LPK-101, LPK-101T, LPK-101L)

	LPK-101	LPK-101T	LPK-101L
End repair and A-tailing buffer*	240 µL	80 µL	960 µL
End repair and A-tailing enzyme*	60 µL	20 µL	240 µL
Ligation solution	1,200 µL	400 µL	1,600 µL × 3
Library amplification master mix	690 µL	230 µL	1,380 µL × 2
Library amplification primer mix	138 µL	46 µL	552 µL

* Do not store the mixed solution.

End repair and A-tailing buffer

End repair and A-tailing Buffer is optimized to convert fragmented DNA to repaired DNA having 5´-phosphorylated, 3´-dA-tailed ends. End repair and A-tailing buffer should be used with end repair and A-tailing enzyme.

End repair and A-tailing enzyme

End repair and A-tailing enzyme is optimized to convert fragmented DNA to repaired DNA having 5´-phosphorylated, 3´-dA-tailed ends. End repair and A-tailing enzyme should be used with end repair and A-tailing buffer. Please pipette slowly as this is viscous.

Ligation solution

This solution contains DNA Ligase and optimized reaction buffer. Please pipette slowly as this is viscous.

Library amplification master mix

This is a 2× concentrated master mix containing genetically-modified KOD DNA polymerase, dNTPs (dATP, dGTP, dCTP, dTTP) and Mg^{2+}. Various regions of the genome can be amplified homogeneously even if these regions contain GC bias. The resulting amplicons are suitable for next-generation sequencing analyses.

Library amplification primer mix

The primer mix (10×) is optimized to amplify illumina® libraries flanked by the P5 and P7 flow cell sequences.

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Required materials not included

・Thermocycler

・Fragmentation equipment or reagent
GenNext™ NGS Library Prep Kit is compatible with mechanical and enzymatic fragmentation methods.

・Adapters for illumina®
GenNext™ NGS Library Prep Kit is compatible with adapters in which both the cluster generation sequences and sequencing are added during the ligation step, such as those routinely used in TruSeq (illumina®) and other similar library preparation workflows.

・SPRI (Solid Phase Reversible Immobilization) paramagnetic bead
Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880, A63881) are recommended for use with the GenNext™ NGS Library Prep Kit.

・10 mM Tris-HCl, pH 8.0 - 8.5
Dilution of adapter stock in water and elution of DNA in water is not recommended.

・Magnetic rack/stand for magnetic bead separation

・80% ethanol (freshly prepared)

[3] Protocol

1. End repair and A-tailing

(1) Prepare the reaction mix in a tube or PCR plate as follows:

Component	Reaction volume (60 µL)
Fragmented dsDNA	50 µL
End repair and A-tailing buffer*	8 µL
End repair and A-tailing enzyme mix*	2 µL

* Premixes are stable for at least 24 hours at 4°C. Use the mixed solution within 24 hours.

(2) Vortex gently or mix well by pipetting. Spin down briefly and proceed immediately to the next step.

(3) Incubate the mixture as follows:

30°C, 10 minutes
65°C, 5 minutes
4°C, hold

(4) Proceed immediately with adapter ligation as described in the next protocol.

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2. Adapter Ligation

(1) Dilute adapter stocks in 10 mM Tris-HCl, pH 8.0 - 8.5, as follows:

Fragmented DNA per 50 µL End repair and A-tailing reaction	Adapter stock concentration	Adapter : insert molar ratio
1 µg	15 µM	10:1
500 ng	15 µM	20:1
250 ng	15 µM	40:1
100 ng	15 µM	100:1
50 ng	15 µM	200:1
25 ng	7.5 µM	200:1
10 ng	3 µM	200:1
5 ng	1.5 µM	200:1
2.5 ng	750 nM	200:1
1 ng	300 nM	200:1

(2) In the same plate/tubes in which end repair and A-tailing was performed, prepare the reaction mix as follows:

Component	Reaction volume (110 µL)
End repair and A-tailing reaction product	60 µL
Adapter stock	5 µL
Ligation solution	45 µL

(3) Mix well and centrifuge briefly.

(4) Incubate the mixture as follows:

20°C, 15 minutes
4°C, hold

(5) Immediately proceed to the next process (cleanup).

3. Cleanup (after adapter ligation)

(1) Perform a 0.8× SPRI-bead-based cleanup as follows:

Component	Volume (198 µL)
Adapter ligation reaction product	110 µL
Agencourt® AMPure® XP*	88 µL

* Ensure that beads are fully resuspended.

(2) Mix well by vortexing and/or pipetting up and down multiple times.

(3) Incubate the plate/tubes at room temperature for 5 - 15 minutes.

(4) Place the plate/tubes on a magnetic stand to capture the beads. Incubate until the solution is clear.

(5) Carefully remove and discard the supernatant.

(6) Add 200 µl 80% ethanol in the plate/tubes on the magnetic stand.

(7) Incubate the plate/tubes on the magnetic stand at room temperature for 30 seconds.

(8) Carefully remove and discard the ethanol.

(9) Add 200 µl 80% ethanol in the plate/tubes on the magnetic stand.

(10) Incubate the plate/tubes on the magnetic stand at room temperature for 30 seconds.

(11) Carefully remove and discard as much ethanol as possible. Be careful not to disturb the beads.

(12) Air dry the beads at room temperature for 3 - 5 minutes while the plate/tubes are on the magnetic stand with lids open.

Notes
Overdrying the beads may reduce the yield.

(13) Remove the plate/tubes from the magnetic stand.

(14) Resuspend the beads in 25 µL elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) and incubate the plate/tubes at room temperature for 2 minutes. If proceeding with size selection (optional: see [3] 6), resuspend the beads in 55 µL elution buffer.

(15) Place the plate/tubes the magnetic stand to capture the beads. Incubate until the solution is clear.

(16) Transfer the supernatant to a new plate/tubes. Purified libraries can be stored at –20°C.

4. Library Amplification

(1) Prepare a reaction mix as follows:

Component	Reaction volume (50 µL)
Library amplification master nix (2×)	25 µL
Library amplification primer mix (10×)	5 µL
Adapter-ligated library	20 µL

(2) Mix well and centrifuge briefly.

(3) Amplify using the following cycling conditions:

(amplified libraries can be stored at –20°C.)

Step	Temp	Duration	Cycles
Initial denaturation	94°C	2 minutes	1
Denaturation	98°C	10 seconds	Minimum number required for optimal amplification (see below)
Annealing	60°C	10 seconds	
Extension	68°C	15 seconds	
HOLD	4°C	-	1

Recommended cycle numbers

Input DNA (into end repair and A-tailing reaction)	Cycles*
1 µg	0
500 ng	0
250 ng	0
100 ng	0-2
50 ng	3-5
25 ng	5-6
10 ng	7-9
5 ng	9-11
2.5 ng	11-13
1 ng	13-15

* The optimal number of amplification cycles may be 1 - 3 cycles higher or lower, depending on the sample type and size distribution of the input DNA.

5. Cleanup (after library amplification)

(1) Perform a 1× SPRI-bead-based cleanup as follows:

Component	Volume (100 µL)
Library amplification reaction product	50 µL
Agencourt® AMPure® XP*	50 µL

* Ensure that beads are fully resuspended.

(2) Mix well by vortexing and/or pipetting up and down multiple times.

(3) Incubate the plate/tubes at room temperature for 5 - 15 minutes.

(4) Place the plate/tubes on a magnetic stand to capture the beads. Incubate until the solution is clear.

(5) Carefully remove and discard the supernatant.

(6) Add 200 µl 80% ethanol in the plate/tubes on the magnetic stand.

(7) Incubate the plate/tubes on the magnetic stand at room temperature for 30 seconds.

(8) Carefully remove and discard the ethanol.

(9) Add 200 µl 80% ethanol in the plate/tubes on the magnetic stand.

(10) Incubate the plate/tubes on the magnetic stand at room temperature for 30 seconds.

(11) Carefully remove and discard as much ethanol as possible. Be careful not to disturb the beads.

(12) Air dry the beads at room temperature for 3 - 5 minutes.

Notes
Overdrying the beads may reduce the yield.

(13) Remove the plate/tubes from the magnetic stand.

(14) Resuspend the beads in an appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5). Incubate the plate/tubes at room temperature for 2 minutes. If proceeding with size selection (optional: see [3] 6), resuspend the beads in 55 µL elution buffer.

(15) Place the plate/tubes on a magnetic stand. Incubate until the solution is clear.

(16) Transfer the supernatant to a new plate/tubes. Purified, amplified libraries can be stored at –20°C. Proceed with size selection, library quality control or sequencing, as appropriate.

6. Size selection (optional)

If required, size selection may be performed at several points in the process workflow, for example, prior to end repair and A-tailing, after the post ligation cleanup or after library amplification. Size selection results in a narrower library size distribution, but at the cost of a much amount of library. Below is an example of selecting a library of 250 - 450 bp.

(1) Add 0.6× SPRI beads to the library as follows:

Component	Volume (80 µL)
Library	50 µL
Agencourt® AMPure® XP*	30 µL

* Ensure that beads are fully resuspended.

(2) Mix well by vortexing and/or pipetting up and down multiple times.

(3) Incubate the plate/tubes at room temperature for 5 - 15 minutes.

(4) Place the plate/tubes on a magnetic stand. Incubate until the solution is clear.

(5) Transfer 75 µl the supernatant to a new plate/tubes.

(6) Add 0.13× SPRI beads to the supernatant as follows:

Component	Volume (85 µL)
Library (supernatant)	75 µL
Agencourt® AMPure® XP*	10 µL

* Ensure that beads are fully resuspended.

(7) Mix well by vortexing and/or pipetting up and down multiple times.

(8) Incubate the plate/tubes at room temperature for 5 - 15 minutes.

(9) Place the plate/tubes on a magnetic stand. Incubate until the solution is clear.

(10) Carefully remove and discard the supernatant.

(11) Add 200 µL 80% ethanol in the plate/tubes on the magnetic stand.

(12) Incubate the plate/tubes on the magnetic stand at room temperature for 30 seconds.

(13) Carefully remove and discard the ethanol.

(14) Add 200 µL 80% ethanol in the plate/tubes on the magnetic stand.

(15) Carefully remove and discard as much ethanol as possible. Be careful not to disturb the beads.

(16) Air dry the beads at room temperature for 3 - 5 minutes.

Notes
Overdrying the beads may reduce the yield.

(17) Remove the plate/tubes from the magnetic stand.

(18) Add the required amount (e.g., 20 µL) of 10 mM Tris-HCl (pH 8.0 - 8.5) to the plate/tubes and incubate these at room temperature for 2 minutes.

(19) Place the plate/tubes on a magnetic stand. Incubate until the solution is clear.

(20) Transfer the supernatant to a new plate/tubes. Purified libraries can be stored at –20°C.

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[4] Library QC

Library quantification

Library quantification can be performed using GenNext™ NGS Library Quantification Kit (Code No. NLQ-101) or equivalent. The kit allows the specific and accurate quantification of libraries bearing P5 and P7 adapters and can be applied to flow cell amplification.

Library quality control

Assess the quality of the library using a capillary electrophoresis device such as Agilent BioAnalyzer or equivalent.

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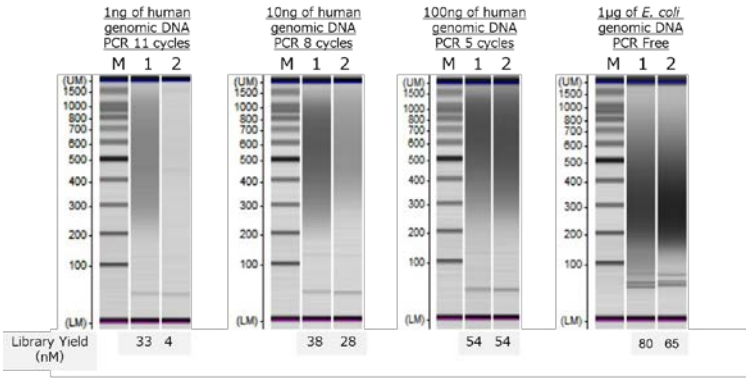
[5] Application data

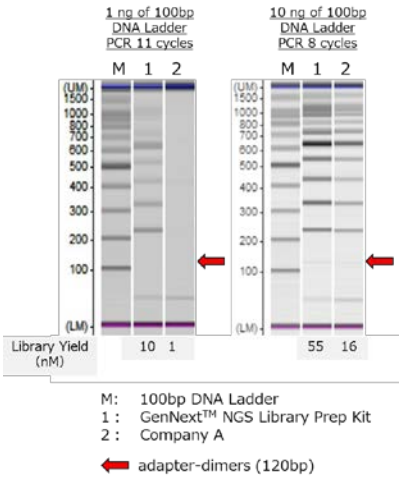
1. Example of library quantification and quality control

Libraries were prepared from fragmented human genomic DNA, *E. coli* genomic DNA, or 100 bp DNA ladder with the GenNext™ NGS library prep kit or another company's Kit (Company A).

Libraries were amplified using 0-11 cycles of PCR and the size distribution checked using a MultiNA (Shimadzu Corporation). Library quantifications were performed using a GenNext™ NGS library quantification kit (code NLQ-101).

There was no difference in the distribution of libraries between the GenNext™ NGS library prep kit and the other company's kit (Company A). For most illumina® sequencing platforms, 2 - 4 nM for each library is the preferred starting concentration for denaturation and dilution guidelines. These data illustrate that the GenNext™ NGS library prep kit achieved sufficient adapter-ligated library yields, even with low input amounts of DNA.





Adapters of approximately 60 bp are ligated to both ends of the insert DNA fragments, so the total size of DNA fragments will increase by approximately 120 bp.

2. Example of next generation sequencing results

Libraries were prepared from 1µg or 1ng of *E. coli* genomic DNA with the GenNext™ NGS library prep kit or another company's library construction kit (Company A). Libraries prepared from 1ng DNA were amplified using 12 PCR cycles. Sequencing was performed on an illumina® MiSeq® and data analyzed using CLC Genomics Workbench (QIAGEN / CLC bio). Sequencing reads were down-sampled to 1 million per library prior to analysis.

These data illustrate that GenNext™ NGS library prep kit enables high quality sequence data.

Libraries from 1 µg DNA with no amplification step

	% Mapped	% Nucleotide differences
GenNext™ NGS Library Prep Kit	93.14	5.65
Company A	93.01	5.89

Libraries from 1ng DNA with amplification step (PCR 12cycles)

	% Mapped	% Nucleotide differences
GenNext™ NGS Library Prep Kit	92.31	5.77
Company A	89.64	5.99

% Mapped: The percentage of reads mapped to reference.
% Nucleotide differences: The percentage of Nucleotide differences in reads relative to reference.

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[6] Troubleshooting

Symptom	Cause	Solution
Presence of adapter dimers	Low-quality adapter	- Avoid excessive freezing and thawing of adapter stock solutions. - Dilute and store adapters in 10 mM Tris-HCl (pH 8.0 - 8.5).
	Non-optimal adapter concentration	- The optimal concentration of adapter should be determined by preliminary tests: try the recommended adapter concentration (see [3] 2). - If the yield of the library is sufficient, repeat the purification process or perform size selection (see [3] 6).
Low yield	Incorrect ratio of added magnetic beads reagent and library solution	- Ratio of SPRI magnetic bead reagent and library solution used for purification greatly influences size distribution and yield. Please check whether the liquid volume proportion is accurate.
	Overdrying the magnetic beads	- Overdrying the magnetic beads may reduce the yield. Air dry the beads at room temperature within 5 minutes.

[7] Related products

Product name	Package	Code No.
NGS Library quantification for illumina®'s instrument **GenNext™ NGS library quantification kit**	500 reactions	NLQ-101

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