Instruction manual for GenNext™ Shin-RamDA-seq™ Single Cell Stranded Kit 202303

GenNext™ Shin-RamDA-seq™ Single Cell Stranded Kit

RML-101 96 reactions
RML-101T 24 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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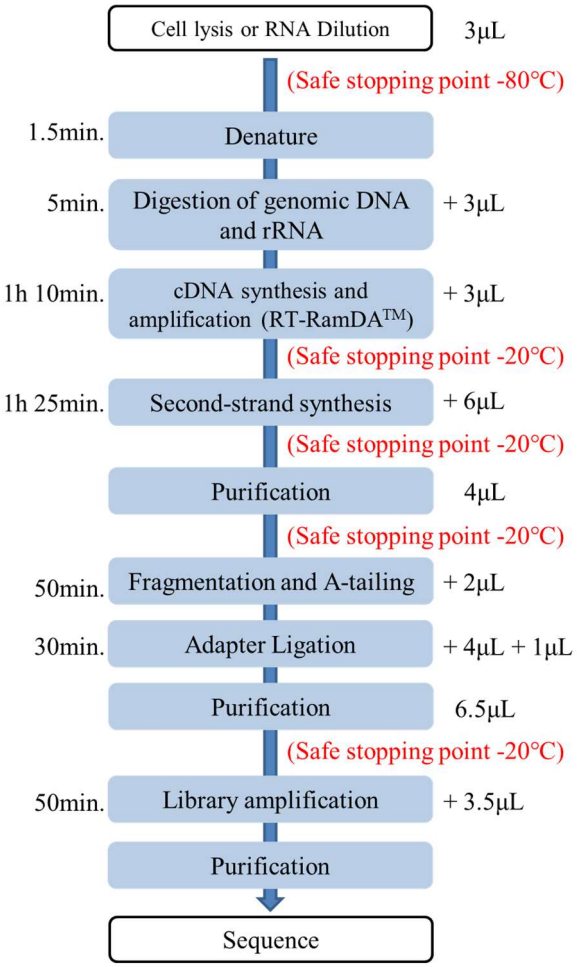
FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

The GenNext™ Shin-RamDA-seq™ Single Cell Stranded Kit contains the reagents for cDNA synthesis and library preparation from single cells or trace amounts of RNA. Subsequent strand-specific RNA-Seq analysis can be performed on the generated libraries. This kit uses Reverse Transcription with Random Displacement Amplification (RT-RamDA™); see method reference (1). RT-RamDA™ is a novel cDNA amplification method that utilizes the strand displacement activity of reverse transcriptase. This kit can not only detect poly(A) RNA but also non-poly(A) RNA with high sensitivity. Therefore, the RT-RamDA™ method can detect more genes than conventional technology. This kit does not include index reagents or magnetic beads.



Process Workflow

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[2] Components

Features

1. Obtaining cDNA library from a single cell or small amount of input RNA

1–100 cells or 10 pg–1 ng total RNA

2. Analyzing full-length cDNA

cDNA that covers the entire length of the target RNA of 10 kb or more can be prepared.

3. Detecting various RNA species

◇ Identification of isoforms and alternative splicing

◇ Detection of poly(A) RNA and non-poly(A) RNA (histone RNA and lncRNA)

◇ Detection of nuclear RNA (pre-mRNA and lncRNA)

4. Stranded specific (directional) RNA sequencing

Strand-specific RNA-Seq data reflects the cell transcriptome

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The kit contains the following reagents used for 96 (RML-101) and 24 (RML-101T) reactions: All reagents should be stored at −20°C.

Important: Do not store any solutions once mixed. All reagent cocktails should be prepared shortly before use.

GenNext™ Shin-RamDA-seq™ Single Cell Stranded Kits (Cat. No. RML-101 and RML-101T)

	RML-101	RML-101T
①Lysis Buffer	240 µL	60 µL
②Lysis Enhancer	240 µL	60 µL
③RNase Inhibitor	22 µL	6 µL
④Nuclease free water	960 µL	240 µL
⑤RT-RamDA™ Buffer	240 µL	60 µL
⑥gDNA Remover	54 µL	14 µL
⑦rRNA Remover	30 µL	8 µL
⑧RT-RamDA™ Enzyme Mix	54 µL	14 µL
⑨RT-RamDA™ Primer Mix	54 µL	14 µL
⑩2nd strand synthesis Buffer	330 µL	83 µL
⑪2nd strand synthesis Enzyme	55 µL	14 µL
⑫2nd strand synthesis Primer Mix	275 µL	69 µL
⑬Fragmentase	120 µL	30 µL
⑭End Repair and A-tailing Buffer	96 µL	24 µL
⑮End Repair and A-tailing Enzyme	24 µL	6 µL
⑯Ligation Solution	480 µL	120 µL
⑰Library Amplification Master Mix	300 µL	75 µL
⑱Library Amplification Primer Mix	120 µL	30 µL

Important: Do not substitute any reagent in other TOYOBO kits for the reagents in this kit, even if the reagents have the same.

Option: This kit can be used for depletion of rRNA from human or mouse total RNA preparations.

NSR Primer Set for human samples (Cat. No. NSR-101)

1st NSR Primer Mix for human	54 µL
2nd NSR Primer Mix for human	275 µL

NSR Primer Set for mouse samples (Cat. No. NSR-102)

1st NSR Primer Mix for mouse	54 µL
2nd NSR Primer Mix for mouse	275 µL

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Additional Materials Required

· Thermocycler

· Index kit
(Recommend) TruSeq DNA CD Indexes (96 Indexes, 96 Samples), (Illumina, Inc. Cat. no. 20015949)
(Recommend) IDT for Illumina – TruSeq DNA UD Indexes v2(96 Indexes,96 Samples), (Illumina, Inc. Cat. no. 20040870)

· SPRI (Solid Phase Reversible Immobilization) paramagnetic beads
Agencourt AMPure XP Beads (Beckman Coulter, Cat. no. A63880 or A63881)

· TE Buffer pH 8.0 (10 mM Tris-HCl, 1 mM EDTA)

·10 mM Tris-HCl pH 8.0

· Magnetic rack/stand for magnetic bead separation

· Ethanol (80%; freshly prepared)

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[3] Protocol

This kit can be used with cells or purified total RNA. Start with Section 1A when using fluorescence activated cell sorter (FACS) to obtain the cells, Section 1B when using non-FACS cells, and Section 1C when using purified total RNA. Thereafter, proceed with the denaturation in Section 2.

Sample		Cell lysis or RNA Dilution	Denaturation
Cells	FACS	Section 1A	Continue with Section 2 for all samples.
	Non-FACS	Section 1B	
Purified RNA		Section 1C	

Important: Avoid contamination, please keep your workspace clean.

1. Cell lysis or RNA dilution

A. *When acquiring cells with FACS*

(1) Prepare the cell lysis cocktail using the volumes listed in the table below. The volumes used should be scaled depending on the number of reactions to be performed.

Cell lysis cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
①Lysis Buffer	1	22	110
②Lysis Enhancer	0.95	20.9	104.5
③RNase Inhibitor	0.05	1.1	5.5
④Nuclease-free water	1	22	110
Total	3	66	330

*An extra 10% in volume has been added here to account for pipetting error.

(2) Dispense 3 µL of cell lysis cocktail per well into a 96-well plate or 8-tube strip. Dispense on ice, and immediately after dispensing, seal with a qPCR seal or thermocompression seal.

(3) Store the plate on ice or at 4°C until sorting. Perform cell sorting according to the FACS user manual and the parameters recommended by the manufacturer.

(4) After sorting, seal the plate or tubes and centrifuge briefly to collect the solution at the bottom of the wells/tubes.

(5) Proceed immediately to the next step (Section 2) or store the samples at −80°C.

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B. *Cell acquisition using methods other than FACS*

When obtaining the cells by manual picking, etc., the volume of the cell sample should be 1 µL or less.

(1) Prepare the reagents required for cell lysis in one tube per reaction using the volumes listed in the table below, and then add the cell samples.

If the volume of the cell sample is less than 1 µL, adjust the amount of water added when preparing the cell lysis reaction.

	1 Reaction (µL)
①Lysis Buffer	1
②Lysis Enhancer	0.95
③RNase Inhibitor	0.05
Sample	~1.0
④Nuclease-free water	X (adjust as needed based on sample volume)
Total	3

For example, if your cell sample volume is 1 µL, prepare the cell lysis cocktail using the volumes listed in the table below, and dispense 2 µL of the cell lysis cocktail per well into a 96-well plate or 8-strip tube. Thereafter, add 1 µL of your cell samples.

Cell lysis cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
①Lysis Buffer	1	22	110
②Lysis Enhancer	0.95	20.9	104.5
③RNase Inhibitor	0.05	1.1	5.5
Total	2	44	220

*An extra 10% in volume has been added here to account for pipetting error.

(2) Seal the plate or tubes and then centrifuge briefly to collect the solution at the bottom of the wells/tubes.

(3) Proceed immediately to the next step (Section 2) or store the samples at −80℃.

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C. *When using purified total RNA*

(1) Prepare the reagents necessary for RNA dilution in one tube per reaction using the volumes listed in the table below and then add the RNA samples.
If the volume of the RNA sample is less than 1 µL, adjust the amount of water.

	1 Reaction (µL)
①Lysis Buffer	1
②Lysis Enhancer	0.95
③RNase Inhibitor	0.05
Sample	~1.0
⑧Nuclease-free water	X (adjust as needed based on sample volume)
Total	3

For example, if the RNA sample volume is 1 µL, prepare the RNA dilution cocktail using the volumes listed in the table below, and dispense 2 µL of the RNA dilution cocktail per well into a 96-well plate or 8-strip tube. Thereafter, add 1 µL of the RNA samples.

RNA dilution cocktail

	1 Reaction (µL)	20 Reactions (µL) *	100 Reactions (µL) *
①Lysis Buffer	1	22	110
②Lysis Enhancer	0.95	20.9	104.5
③RNase Inhibitor	0.05	1.1	5.5
Total	2	44	220

*An extra 10% volume was added here to account for pipetting error.

(2) After dispensing, seal the tubes and centrifuge briefly to collect the solutions at the bottom of the wells or tubes.

(3) Proceed immediately to the next step (Section 2) or store the samples at −80°C.

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2. Denaturation

(1) Centrifuge the sample plate or tubes at 4℃ and incubate them according to the instructions in table below.

Note: When using frozen samples, first thaw the samples at 4℃ and then briefly centrifuge them before proceeding to heat denaturation.

Step	Temperature	Time
Denature	70℃	1.5 min
Hold	4℃	∞

3. Digestion of genomic DNA and ribosomal RNA

(1) Prepare a cocktail of the reagents necessary for genomic DNA removal using the volumes listed in the table below. The volumes used should be scaled depending on the number of reactions to be performed.

Note: When using control RNA such as ERCC RNA, adjust the volume of nuclease-free water added.

gDNA and rRNA removal cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
⑤RT-RamDA™ Buffer	0.3	6.6	33
⑥gDNA Remover	0.45	9.9	49.5
⑦rRNA Remover	0.25	5.5	27.5
④Nuclease-free water	2	44	220
Total	3	66	330

*An extra 10% in volume has been added here to account for pipetting error.

(2) Add 3 µL of the gDNA and rRNA removal cocktail per well to each reaction from previous step. Centrifuge briefly and incubate at the following temperatures.

Step	Temperature	Time
Digestion	30℃	5 min
Hold	4℃	∞

(3) Proceed immediately to the next step after this reaction.

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4. cDNA synthesis and amplification reaction (RT-RamDA™ reaction)

(1) Prepare a cocktail of the necessary reagents for the RT-RamDA™ reaction in a single tube using the volumes listed in the table below. The volumes used will need to be scaled depending on the number of reactions you wish to perform.

Note: When using the NSR Primer, use the 1st NSR Primer Mix for human samples or the 1st NSR Primer Mix for mouse samples instead of the RT-RamDA™ Primer Mix.

RT-RamDA™ cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
⑤RT-RamDA™ Buffer	1.5	33	165
⑧RT-RamDA™ Enzyme Mix	0.45	9.9	49.5
⑨RT-RamDA™ Primer Mix	0.45	9.9	49.5
④Nuclease-free water	0.6	13.2	66
Total	3	66	330

*An extra 10% in volume has been added here to account for pipetting error.

(2) Add 3 µL of the RT-RamDA™ cocktail solution per well to each reaction from previous step. After brief centrifugation, incubate the plate/tubes at the temperatures given in the following table.

Step	Temperature	Time
Priming 1	25°C	10 min
Priming 2	30°C	10 min
Reverse transcription and amplification	37°C	30 min
Reverse transcription 2	50°C	5 min
Inactivation	98°C	5 min
Hold	4°C	∞

(3) Proceed immediately to the next step after the RT-RamDA™ reaction or store at −20°C to −30°C.

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5. 2nd strand synthesis reaction

(1) Prepare the reagents required for the 2nd strand synthesis reaction in a tube using the volumes listed in the table below. The volumes used should be scaled depending on the number of reactions to be performed.

Note: When using the NSR Primer, use the 2nd NSR Primer Mix for human samples and the 2nd NSR Primer Mix for mouse samples instead of the 2nd strand Synthesis Primer Mix.

	1 Reaction (μL)	20 Reactions (μL)*	100 Reactions (μL)*
⑩2nd strand Synthesis Buffer	3	63	315
⑪2nd strand Synthesis Enzyme	0.5	10.5	52.5
⑫2nd strand Synthesis Primer Mix	2.5	52.5	262.5
Total	6	126	630

*An extra 5% in volume has been added here to account for pipetting error.

(2) Add 6 μL of the 2nd strand synthesis cocktail per well to each reaction from previous step. After brief centrifugation, incubate the plate/tubes at the following temperatures.

Step	Temperature	Time
Second-strand synthesis	16°C	60 min
Denaturation	80°C	15 min
Hold	4°C	∞

(3) Proceed immediately to the next step after the 2nd strand synthesis reaction or store the reactions at −20°C to −30°C.

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6. DNA Purification (1st)

(1) Prepare 1/4 × diluted AMPure XP beads prior to the purification. To collect the supernatant as diluent, separate AMPure XP beads to beads and liquid by centrifuge (10,000 rpm for 1 min) or magnetic stand.

Example: How to prepare 1/4 × diluted AMPure XP beads for eight samples

a. Place 1 × AMPure XP Beads 220 µL on a magnetic stand, and when the magnetic beads are completely absorbed, use the supernatant as a diluent.

b. Mix 180 µL of the supernatant prepared in step a with 60 µL of 1 × AMPure XP beads and use as 1/4 diluted AMPure XP beads.

(2) Add 27 µL of 1/4 × diluted AMPure XP beads to each double-stranded cDNA solution (15 µL). Centrifuge briefly, then vortex lightly or mix at 2,000 rpm for 2 min at 25°C and let stand for 5 min.

(3) The reaction tubes or 96-well plate is placed on the magnetic stand to allow the solutions to become clear. Remove the supernatant by careful pipetting.

(4) Add 150 µL of 80% ethanol per well/tube, mix briefly by pipetting the bead suspension up and down five times, and incubate the solutions for 30 s at room temperature (about 15°C to 25°C).

(5) Place the tubes or plate back onto the magnetic stand and wait until the solutions become clear. Remove the ethanol by careful pipetting.

(6) Add 150 µL of 80% ethanol per well/tube, mix briefly by pipetting five times, and incubate the solutions for 30 s at room temperature.

(7) Place the tubes or plate back onto the magnetic stand and wait until the solutions become clear. Remove the ethanol by careful pipetting.

(8) To remove any residual ethanol, leave the tubes or plate open on the magnetic stand at room temperature to dry for up to 5 min. or until the magnetic beads begin to visibly crack (if any ethanol remains, the subsequent reactions may not be successful). Adjust the drying time according to the ambient temperature and humidity in your laboratory.

(9) Add 4.5 µL of 10 mM Tris-HCl (pH 8.0) to each reaction in the 96-well plate or 8-tube strips and briefly centrifuge.

(10) Vortex until the beads are completely dispersed, incubate at 25°C for 5 min., and briefly centrifuge to collect the suspension at the bottom of the wells/tubes.

(11) Place the tube or plate on the magnetic stand for 5 min or until the solutions have cleared.

(12) Transfer 4 µL of the clear supernatant to new tubes or a plate and proceed to the next step.

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7. Fragmentation and A-tailing

(1) Prepare the reagents necessary for the fragmentation and A-tailing reaction in a tube using the volumes listed in the table below. The volumes used should be scaled depending on the number of reactions to be performed.

Fragmentation and A-tailing cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
⑬Fragmentase	1	22	110
⑭ER+AT Buffer	0.8	17.6	88
⑮ER+AT Enzyme	0.2	4.4	22
Total	2	44	220

(2) Add 2 µL of the fragmentation and A-tailing cocktail to each sample (4 µL) and incubate at the following temperatures given in the table below, after brief centrifugation.

Step	Temperature	Time
Fragmentation and A-tailing	30°C	30 min
Inactivation	65°C	5 min
Hold	4°C	∞

8. Adapter ligation

(1) Dilute adapter solution (not included in the kit) to 50 nM using 10 mM Tris-HCl (pH 8.0).

Please dilute the concentrate 300 times when using the products listed in the table below.

Product Name	Cat. No.
TruSeq DNA CD Indexes (96 Indexes, 96 Samples)	20015949
IDT for Illumina – TruSeq DNA UD Indexes v2(96 Indexes,96 Samples)	20040870

(2) Add 4 µL of ⑯Ligation Solution to each reaction (6 µL) and incubate at the following temperatures after brief centrifugation.

(3) Add 1 µL of adapter solution to each reaction (10 µL) and incubate at the following temperatures given in the table below after brief centrifugation.

Step	Temperature	Time
Fragmentation and A-tailing	20°C	15 min
Hold	4°C	∞

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9. DNA Purification (2nd)

(1) Add 8.8 µL of 1× AMPure XP beads to each reaction (11 µL). Centrifuge briefly, then vortex lightly or mix at 2,000 rpm for 2 min at 25 °C and let stand for 5 min.

(2) Place the tubes or plate on a magnetic stand and allow the solutions to clear. Remove the supernatants by careful pipetting.

(3) While still on the magnetic stand, add 150 µL of 80% ethanol to each well/tube, mix briefly by pipetting five times, and incubate at room temperature for 30 s.

(4) Remove the ethanol by careful pipetting.

(5) Add 150 µL of 80% ethanol per well/tube, mix briefly by pipetting five times, and incubate the solutions for 30 s at room temperature.

(6) Place the tubes or plate back onto the magnetic stand and wait until the solutions become clear. Remove the ethanol by careful pipetting.

(7) To remove any residual ethanol, leave the tubes or plate open on the magnetic stand at room temperature to dry for up to 5 min. or until the magnetic beads begin to visibly crack (if any ethanol remains, the subsequent reactions may not be successful). Adjust the drying time according to the ambient temperature and humidity in your laboratory.

(8) Add 7 µL of 10 mM Tris-HCl (pH 8.0) to each reaction in the 96-well plate or 8-tube strips and briefly centrifuge.

(9) Vortex until the beads are completely dispersed, incubate at 25°C for 5 min., and briefly centrifuge to collect the suspension at the bottom of the wells/tubes.

(10) Place the tube or plate on the magnetic stand for 5 min or until the solutions have cleared.

(11) Transfer 6.5 µL of the clear supernatant to new tubes or a plate and proceed to the next step.

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10. Library amplification

(1) Prepare the reagents necessary for the library amplification in a tube using the volumes listed in the table below. The volumes used will need to be scaled depending on the number of reactions you wish to perform.

Library amplification cocktail

	1 Reaction (μL)	20 Reactions (μL)*	100 Reactions (μL)*
⑰Library Amplification Master Mix	2.5	55	275
⑱Library Amplification Primer Mix	1	22	110
Total	3.5	77	385

(2) Add 3.5 μL of the fragmentation and A-tailing cocktail to each sample (6.5 μL) and perform PCR with the following cycling conditions after brief centrifugation.

Step	Temperature	Time	No. of cycles
Denature	94℃	30 s	1
PCR	98℃ 60℃ 68℃	10 s 10 s 15 s	19*
Hold	4℃	∞	1

*Change the number of PCR cycles according to the table below.

Estimated amount of total RNA per cell/total RNA amount	Typical number of PCR Cycles
>10 pg	19
5–10 pg	19–20

*If the amount of input RNA is less than 5 pg, increase the number of PCR cycles.
Note: If the library yield is too high, the number of PCR cycles can be reduced.

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11. DNA purification (3rd)

(1) Add 10 µL of 1× AMPure XP beads to each PCR reaction (10 µL). Centrifuge briefly, then vortex lightly or mix at 2,000 rpm for 2 min at 25 °C and let stand for 5 min.

(2) Place the tubes or plate on a magnetic stand and allow the solutions to clear. Remove the supernatants by careful pipetting.

(3) While still on the magnetic stand, add 150 µL of 80% ethanol to each well/tube, mix briefly by pipetting five times, and incubate at room temperature for 30 s.

(4) Remove the ethanol by careful pipetting.

(5) Add 150 µL of 80% ethanol per well/tube, mix briefly by pipetting five times, and incubate the solutions for 30 s at room temperature.

(6) Place the tubes or plate back onto the magnetic stand and wait until the solutions become clear. Remove the ethanol by careful pipetting.

(7) To remove any residual ethanol, leave the tubes or plate open on the magnetic stand at room temperature to dry for up to 5 min. or until the magnetic beads begin to visibly crack (if any ethanol remains, the subsequent reactions may not be successful). Adjust the drying time according to the ambient temperature and humidity in your laboratory.

(8) Add 20 µL of TE Buffer per well/tube and briefly centrifuge.

(9) Vortex until the beads are completely dispersed, incubate at 25°C for 2 min. and then briefly centrifuge.

(10) Place the tubes or plate on the magnetic stand and allow to stand for 2 min. until the solutions clear.

(11) Transfer 20 µL of the clear supernatant to a new plate or tubes and proceed to the QC step or store at −20°C.

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12. Library QC

Library quantification

Library quantification can be performed using the TOYOBO GenNext™ NGS Library Quantification Kit (Cat. No. NLQ-101) or equivalent. This 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 an Agilent Bioanalyzer or equivalent. [4] Application data is shown as an example of library distribution.

13. Sequence QC

It is recommended to check the coverage uniformity before analyzing the sequence data. [4] Application data is also shown as an example of coverage.

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[4] Application data

Application 1. Library preparation from culture cell

<Method>

With GenNext™ Shin-RamDA-seq™ Single Cell Stranded Kit, cDNA and NGS libraries were prepared from averaged mouse NIH3T3 single cells (n = 4). Thereafter, RNA-Seq analysis was performed with MiSeq system (Illumine, inc.).

The library was generated using random primers included in this product or the mouse NSR primer (NSR Primer Set for mouse, NSR-102). TruSeq DNA Single Indexes Set A (12 Indexes, 24 Samples) was used as index adapter, followed by PCR amplification for 20 cycles. Library qualities were analyzed by Agilent 4200 TapeStation System (Agilent technologies, Inc.).

<Result>

10 – 60 nM of libraries were obtained, and the peak of library size was approximately 300 bp in both samples.

Analyzing the sequence data using RNA-Seq Alignment Version 2.0.2 on BaseSpace Sequence Hub (Illumina, inc.), the results suggest this kit enables RNA-Seq analysis with suppressed rRNA ration, high strand specificity, and full-length coverage across transcripts.

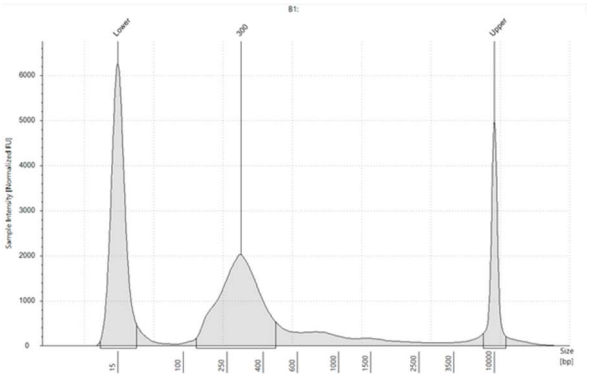

Figure 1. Library size with random primer

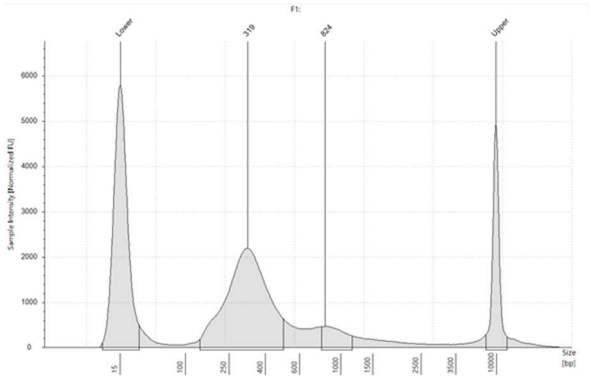

Figure 2. Library size with NSR primer

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Table 1. Fundamental data of RNA-Seq

	Mapping	rRNA	Strand Analysis
Shin-RamDA-seq™ Kit (Random primer)	91.54%	28.57%	97.24%
Shin-RamDA-seq™ Kit (NSR primer)	90.50%	11.47%	96.94%

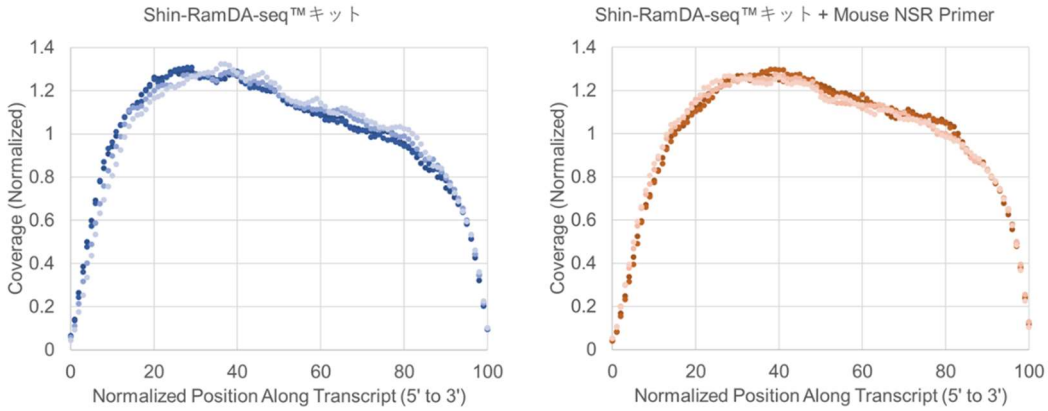

Figure 3. Average coverage for all genes

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Application 2. Full-length and strand analysis

<Method>

Using this kit, cDNA and NGS libraries were prepared from averaged human myeloid K562 single cells (n = 4). Thereafter, RNA-Seq analysis was performed with MiSeq system (Illumine, inc.).

The library was generated using the mouse NSR primer (NSR Primer Set for mouse, NSR-102). TruSeq DNA Single Indexes Set A (12 Indexes, 24 Samples) was used as index adapter, followed by PCR amplification for 20 cycles. Library qualities were analyzed by Agilent 4200 TapeStation System (Agilent technologies, Inc.).

<Result>

Millefy（https://github.com/yuifu/millefy, see reference (2)）was used as the coverage tool for single-cell RNA-Seq analysis. The results below show that this kit enables the entire length analysis of *NEAT1*, a representative long non-coding RNA gene, and highly specific directional analysis of *RBM18* gene and *MRRF* gene.

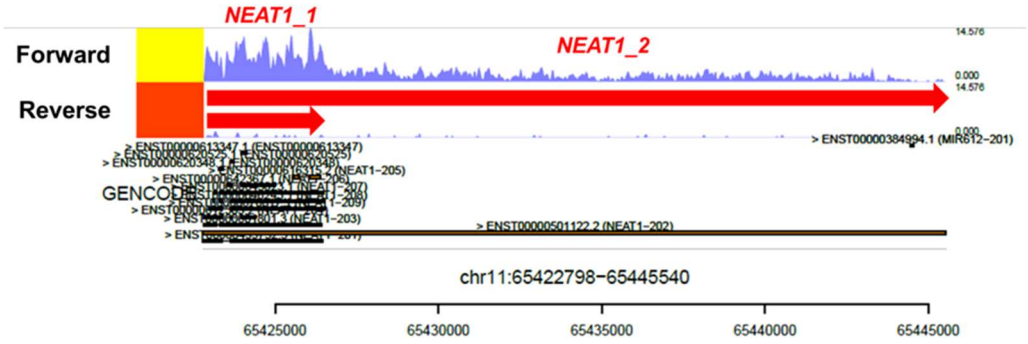

Figure 4. The coverage of *NEAT1* gene

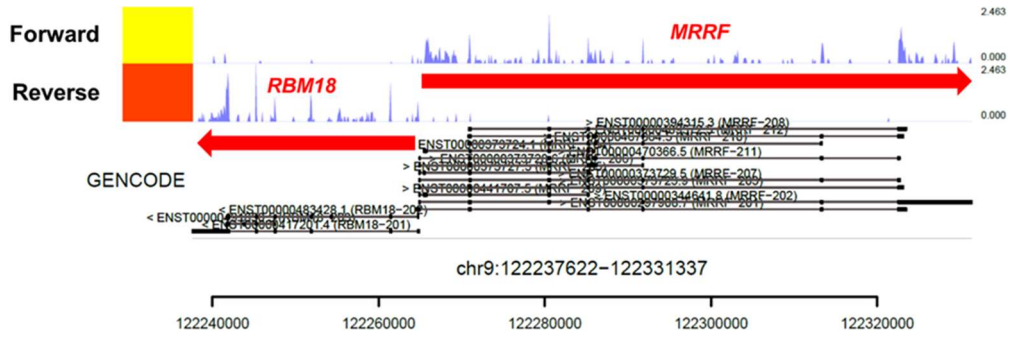

Figure 5. The coverage of *RBM18* and *MRRF* genes

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[5] Troubleshooting

Problem	Cause	Solution
Low cDNA yield	RNA is degraded	· Check that the RNA has not degraded. · Check that RNase has not contaminated the tips or tubes used. · Perform all cell lysis and RNA dilution steps on ice.
Low library yield	Ethanol remaining after purification	· If ethanol remains on the magnetic beads after washing, subsequent enzyme reactions may be hindered. Make sure that the magnetic beads are dry. · If the humidity in the laboratory is high, it may be difficult to dry the beads. It is recommended that the humidity in the work area be 55% or less.

[6] Related products

Product name	No. of reactions	Catalog No.
NSR Primer Set for human simples	96	NSR-101
NSR Primer Set for mouse simples	96	NSR-102
GenNext™ NGS Library Quantification Kit For the quantification of NGS libraries prepared with Illumina instruments	500	NLQ-101
GenNext™ RamDA-seq™ Single Cell Kit	96	RMD-101

References
(1) Hayashi T., et al. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. Nature Communications. 9：619 (2018)

(2) Ozaki H., et al. Millefy: visualizing cell-to-cell heterogeneity in read coverage of single-cell RNA sequencing datasets. BMC Genomics 21:177 (2020)

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