*Trans*NGS® ATAC-Seq Library Prep Kit for Illumina®

Please read the datasheet carefully prior to use.

Version No. Version 1.0

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Catalog No. KP171
Version No. Version 1.0
Storage: at -20℃ for one year.

Description

The *Trans*NGS® ATAC-Seq Library Prep Kit for Illumina® is developed for the Illumina high-throughput sequencing platform and is suitable for constructing ATAC libraries from mammalian cells. The resulting libraries can be used for Single-Read or Paired-End sequencing. This kit utilizes a novel transposase enzyme technology for library construction targeting chromatin open regions, with experiments completed in only 3 hours. It requires minimal cell input, providing high-resolution sequencing profiles with starting cell amounts ranging from 50 to 50,000 cells.

Features

- Time saving and simple operation.
- Minimal cell input requirement. Library construction can be completed with as few as 50 cells.

Applications

Preparing chromatin open region fragments into short fragment libraries suitable for the Illumina high-throughput sequencing platform.

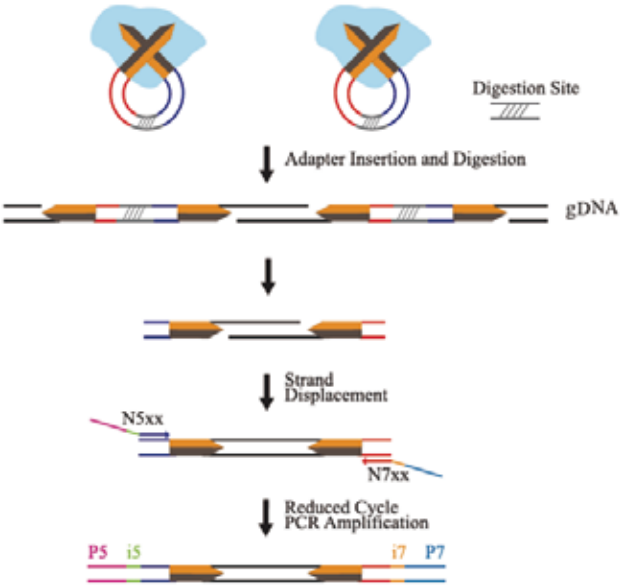
Kit content

Component	KP171-01(12 rxns)	KP171-02(96 rxns)
Cell Lysis Buffer	24 µl	192 µl
Lysis Enhancer	12 µl	96 µl
4×Insertion Buffer	90 µl	720 µl
Insertion Enhancer 1	12 µl	96 µl
Insertion Enhancer 2	12 µl	96 µl
Tn5-50 Enzyme Mix	48 µl	384 µl
4×Tn5 Digestion Buffer	120 µl	960 µl
TransNGS ATAC-Seq Library Amplification SuperMix I（2×）	300 µl	4×600 µl
TransNGS ATAC-Seq Library Amplification SuperMix II（2×）	300 µl	4×600 µl
Library Elution Buffer	600 µl	5 ml
Nuclease-free Water	1 ml	2 × 5 ml
Tn5 Storage Buffer	600 µl	5 ml

Initial Sample Requirement

It is recommended to use 50-50,000 cells for the ATAC-seq library construction experiment. Ensure that the proportion of live cells is greater than 95%, and dead cells can be identified by Trypan Blue staining. Adherent cells and tissue chunks should be digested into a single-cell state as much as possible. If the species is unique or the number of cells falls outside the specified range, appropriate adjustments to the cell lysis system and transposase insertion volume may be necessary.

Schematic Diagram of Library Construction Principle:



Library Construction

5'-AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-XXXXXXXX-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC ATCTCGTATGCCGTCTTCTGCTTG-3'

i5: Index 2, 8 bases;

i7: Index 1, 8 bases;

-XXXXXXXX-: Insert sequence

Operational Steps (5000-50000 cells)

Self-prepared Materials Required: 1×PBS solution, freshly prepared 80% ethanol, *MagicPure*® Size Selection DNA Beads (Catalog number: EC401), *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog number: KI101).

1. Cell Collection and Lysis

(1) Count the processed single-cell suspension and transfer the required number of cells to a new 1.5 ml microcentrifuge tube.

(2) Add 50 µl of pre-chilled 1×PBS solution, centrifuge at 4°C, 500 g, for 5 minutes, carefully discard the supernatant, avoiding disturbing the cell pellet.

(3) Repeat step (2) once, for a total of 2 washes.

(4) Prepare the cell lysis reagent during the above washing steps (prepare and use immediately, keep on ice).

Component	Volume
Cell Lysis Buffer	2 μl
Lysis Enhancer	1 μl
Nuclease-free Water	47 μl
Total volume	50 μl

(5) Add the prepared 50 μl of pre-chilled cell lysis reagent to the cell pellet, gently pipette up and down to mix, and incubate on ice for 5 minutes.

(6) Centrifuge at 4°C, 500 g, for 10 minutes, carefully discard the supernatant.

2. Fragmentation and Termination

(1) Prepare the fragmentation Mix during the centrifugation step above, prepare and use immediately, keep on ice.

Component	Volume
4×Insertion Buffer	7.5 μl
Insertion Enhancer 1*	0.3 μl
Insertion Enhancer 2*	0.3 μl
Tn5-50 Enzyme Mix	4 μl
Nuclease-free Water	17.9 μl
Total volume	30 μl

* When preparing only one reaction, it is recommended to first dilute 1 μl of Insertion Enhancer 1 using 9 μl of Nuclease-free Water, and do the same dilution for 1 μl of Insertion Enhancer 2, then add 3 μl of each diluted Enhancer to the reaction system. Note that the amount of Nuclease-free Water should be reduced accordingly. Note that Insertion Enhancer 1 and Insertion Enhancer 2 should be completely thawed at room temperature before use.

(2) Add 30 μl of the fragmentation Mix to the nucleus pellet, mix by pipetting up and down, and incubate at 37°C for 30 minutes.

(3) Add 10 μl of 4×Tn5 Digestion Buffer to terminate the fragmentation reaction, and incubate at 50°C for 30 minutes.

3. Purification of Fragmentation Products

It is recommended to use *MagicPure*® Size Selection DNA Beads (Catalog number: EC401) for product purification, with the following specific steps:

(1) Pre -warm the DNA beads for 30mins from 2-8°C to room temperature.

(2) Vortex the magnetic beads to ensure thorough mixing. Transfer 88 μl beads (2.2×) to 40 μl product of step 2

(3) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(4) Place the 1.5 ml tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnet. Discard the supernatant.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall.

(5) Keep the 1.5ml tube on the Magnetic Stand, add 200 μl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.

Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.

(6) Repeat Step (5) once.

(7) Keep the 1.5ml tube on the Magnetic Stand with the lid open, dry the beads at room temperature.

Note: Do not heat-dry the tube, as it may affect the final yield.

(8) Remove the 1.5 ml tube from the Magnetic Stand, add 21 µl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 3 minutes.

(9) Place the 1.5 ml tube on the Magnetic Stand and wait for about 2 minutes until the solution is clear. Make sure that all magnetic beads are fully attached to the tube walls.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes.

(10) Carefully transfer 20 µl of the supernatant into a fresh PCR tube. Proceed to the amplification step next or store at -20°C.

4. Library Amplification

(1) Add the following components on ice. Make sure to use TransNGS ATAC-Seq Library Amplification SuperMix I (2×).

Component	Volume
Fragmentation output	20 µl
*Trans*NGS ATAC-Seq Library Amplification SuperMix I（2×）	25 µl
N5xx*	2.5 µl
N7xx*	2.5 µl
Total volume	50 µl

* *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog number: KI101) provides 8 types of N5xx and 12 types of N7xx. Please choose accordingly as needed.

(2) Mix thoroughly by pipetting up and down or vortexing, and spin down briefly.

(3) The PCR amplification program (two-step method) is as follows.

72°C	3 min*	
98°C	3 min	
98°C	8 sec	13-15 cycles
60°C	5 sec	
72°C	1 min	
≤10°C	Hold	

*This step is essential. The transposition reaction product is not a complete double-stranded DNA. It requires incubation at 72°C for 3 minutes to generate complete PCR templates.

5. Purification or Fragment Selection of Library Amplification Products

If there are no specific requirements for library length distribution, the library amplification products can be directly purified using 1.2× magnetic beads (operational steps same as previous purification step).

If fragment selection of the library amplification products is required, it is recommended to use *MagicPure*® Size Selection DNA Beads (Catalog number: EC401) for fragment selection of the products, with the following specific steps:

(1) Pre-warm the DNA beads for 30mins from 2-8°C to room temperature.

(2) Vortex the magnetic beads to ensure thorough mixing. Transfer 27.5 µl beads (0.55×) to 50 µl PCR product.

(3) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(4) Place the PCR tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnet. Transfer the supernatant into another clean 1.5 ml tube. Discard the beads.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall.

(5) Add 60 μl of beads (1.2×) into the supernatant. Mix by pipetting up and down. Incubate at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(6) Place the PCR tube on the magnetic stand and let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand. Discard the supernatant.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand, ensuring all magnetic beads are fully attached to the tube wall. Do not pipette the magnetic beads, as this may affect the final yield.

(7) Keep the PCR tube on the Magnetic Stand, add 200 μl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.

Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.

(8) Repeat Step (7) once.

(9) Keep the PCR tube on the Magnetic Stand with the lid open, dry the beads at room temperature.

Note: Do not heat-dry the tube, as it may affect the final yield.

(10) Move the PCR tube out of the Magnetic Stand, add 21 μl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 3 minutes.

(11) Place the PCR tube on the Magnetic Stand and wait for about 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes, to ensure all magnetic beads are fully attached to the tube wall.

(12) Carefully transfer 20 μl of the supernatant into a fresh EP tube. Proceed to sequencing or store at -20°C.

Operational Steps (≤5000 cells)

Self-prepared Materials Required: 1×PBS solution, freshly prepared 80% ethanol, *MagicPure*® Size Selection DNA Beads (Catalog number: EC401), *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog number: KI101).

1. Cell Collection and Lysis

(1) Count the processed single-cell suspension and transfer the required number of cells to a new 0.2 ml PCR tube. Total sample volume should be less than 1 μl and keep on ice.

(2) Prepare cell lysis reagents. First, take 1 μl of Cell Lysis Buffer and 1 μl of Lysis Enhancer, and dilute 10 times respectively (adding 9 μl of Nuclease-free Water). Then prepare the cell lysis mixture required for one reaction according to the table below. Prepare and use immediately, and keep on ice.

Component	Volume
Diluted Cell Lysis Buffer	2 µl
Diluted Lysis Enhancer	1 µl
Nuclease-free Water	2 µl
Total volume	5 µl

(3) Add the prepared pre-chilled cell lysis mixture to the cell pellet, to reach a total volume of 5 µl (e.g. adding 4 µl of cell lysis mixture to 1 µl of cell sample). Gently pipette up and down to mix, and incubate on ice for 5 minutes.

2. Fragmentation and Termination

(1) Prepare the fragmentation Mix during the incubation step above. prepare and use immediately, keep on ice.

Component	Volume
4×Insertion Buffer	3.75 µl
Insertion Enhancer 1*	0.15 µl
Insertion Enhancer 2*	0.15 µl
Tn5-50 Enzyme Mix*	2 µl
Nuclease-free Water	3.95 µl
Total volume	10 µl

* When preparing only one reaction, due to small volume, it is recommended to first dilute 1 µl of Insertion Enhancer 1 using 9 µl of Nuclease-free Water, and do the same dilution for 1 µl of Insertion Enhancer 2, then add 1.5 µl of each diluted Enhancer to the reaction system. Note that the amount of Nuclease-free Water should be reduced accordingly. Note that Insertion Enhancer 1 and Insertion Enhancer 2 should be completely thawed at room temperature before use.

* When the initial cell count is significantly lower than 5000, Tn5-50 Enzyme Mix can be diluted using the Tn5 Storage Buffer provided in the kit according to experimental requirements. The dilution factors for Tn5-50 Enzyme Mix corresponding to different cell counts are detailed in the table below.

Initial cell count	Recommended Tn5-50 dilution factor
5000	No dilution
1000	5-fold dilution
500	10-fold dilution
100	50-fold dilution
50	100-fold dilution

(2) Add 10 µl of the fragmentation Mix to the product of step 1 (3), mix by pipetting up and down, and incubate in a PCR machine at 37°C for 30 minutes.

(3) Add 5 µl of 4×Tn5 Digestion Buffer to terminate the fragmentation reaction. Total volume now is 20 µl Pipette up and down and incubate in a PCR machine at 68°C for 30 minutes.

3. Library Amplification

(1) Add the following components on ice. Make sure to use TransNGS ATAC-Seq Library Amplification SuperMix II (2×).

Component	Volume
Fragmentation output	20 μl
TransNGS ATAC-Seq Library Amplification SuperMix II（2×）	25 μl
N5xx*	2.5 μl
N7xx*	2.5 μl
Total volume	50 μl

* *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog number: KI101) provides 8 types of N5xx and 12 types of N7xx. Please choose accordingly as needed.

(2) Mix thoroughly by pipetting up and down or vortexing, and spin down briefly.

(3) The PCR amplification program (two-step method) is as follows.

72°C 3 min*
98°C 3 min
98°C 5 sec
60°C 10 sec
18-25 cycles*
72°C 1 min
≤10°C Hold

*This step is essential. The transposition reaction product is not a complete double-stranded DNA. It requires incubation at 72°C for 3 minutes to generate complete PCR templates.

* If the initial cell count is low, the amplification cycle can be extended accordingly.

4. Purification or Fragment Selection of Library Amplification Products

If there are no specific requirements for library length distribution, the library amplification products can be directly purified using 1.2× magnetic beads (operational steps same as previous purification step).

If fragment selection of the library amplification products is required, it is recommended to use *MagicPure*® Size Selection DNA Beads (Catalog number: EC401) for fragment selection of the products, with the following specific steps:

(1) Pre-warm the DNA beads for 30mins from 2-8°C to room temperature.

(2) Vortex the magnetic beads to ensure thorough mixing. Transfer 27.5 μl beads (0.55×) to 50 μl PCR product.

(3) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(4) Place the PCR tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnet. Transfer the supernatant into another clean 1.5 ml tube. Discard the beads.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall.

(5) Add 60 μl of beads (1.2×) into the supernatant. Mix by pipetting up and down. Incubate at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(6) Place the PCR tube on the magnetic stand and let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand. Discard the supernatant.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand, ensuring all magnetic beads are fully attached to the tube wall. Do not pipette the magnetic beads, as this may affect the final yield.

(7) Keep the PCR tube on the Magnetic Stand, add 200 µl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.

Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.

(8) Repeat Step (7) once.

(9) Keep the PCR tube on the Magnetic Stand with the lid open, dry the beads at room temperature.

Note: Do not heat-dry the tube, as it may affect the final yield.

(10) Move the PCR tube out of the Magnetic Stand, add 21 µl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 3 minutes.

(11) Place the PCR tube on the Magnetic Stand and wait for about 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes, to ensure all magnetic beads are fully attached to the tube wall.

(12) Carefully transfer 20 µl of the supernatant into a fresh EP tube. Proceed to sequencing or store at -20°C.

Appendix

Reference of library output (Hela cell, without fragment selection)

Initial cell count	50000	10000	5000	1000	500	100	50
Tn5-50 dilution factor	/			5-10		50-100	
PCR cycles	15 cycles			20 cycles		25 cycles	
Library output (ng)	>1200	>800	>800	>1200	>1100	>1600	>1200

Reference of library output (Hela cell, without fragment selection)

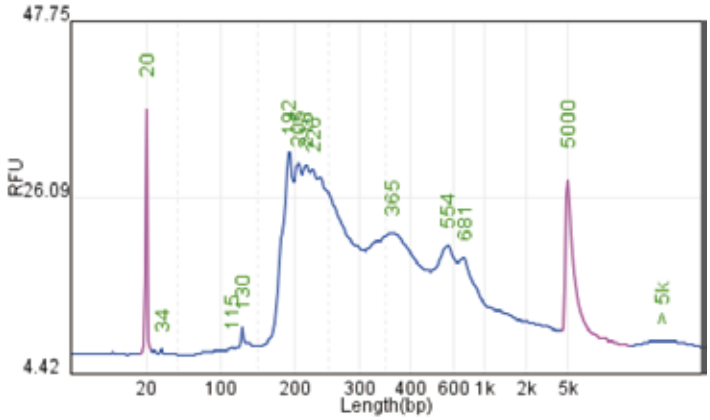


Figure 1. library peaks of 50000 cells

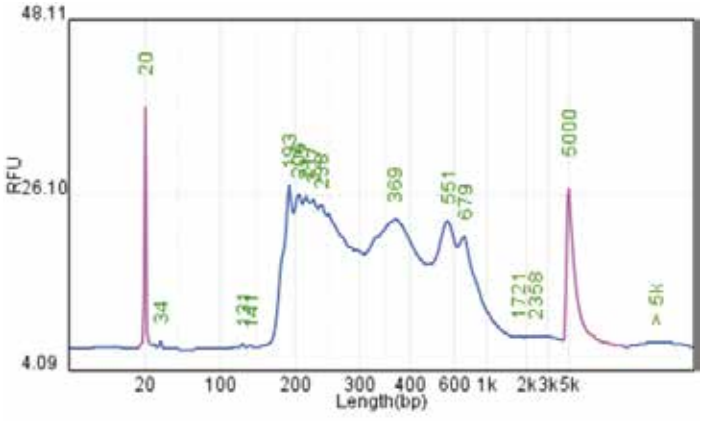


Figure 2. library peaks of 10000 cells

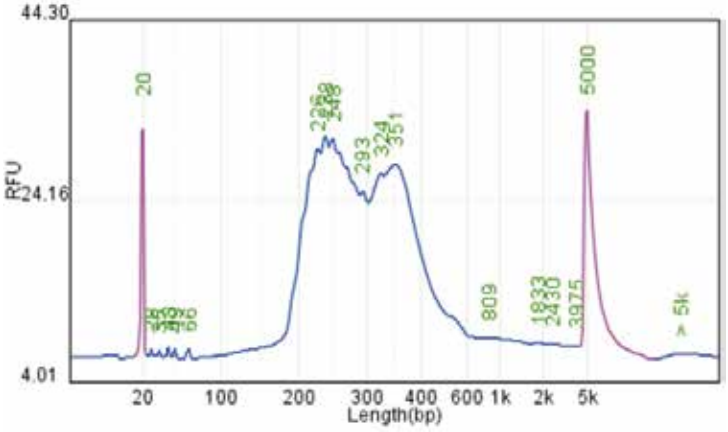


Figure 3. library peaks of 5000 cells

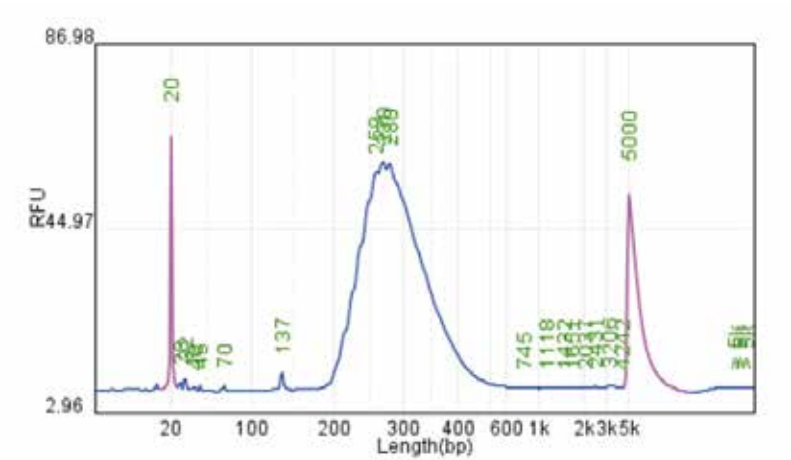


Figure 4. library peaks of 1000 cells (Tn5-50 5-fold dilution)

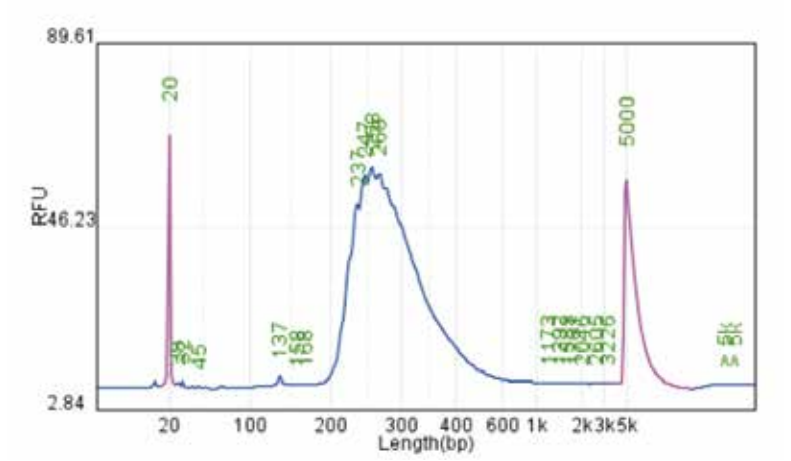


Figure 5. library peaks of 500 cells (Tn5-50 10-fold dilution)

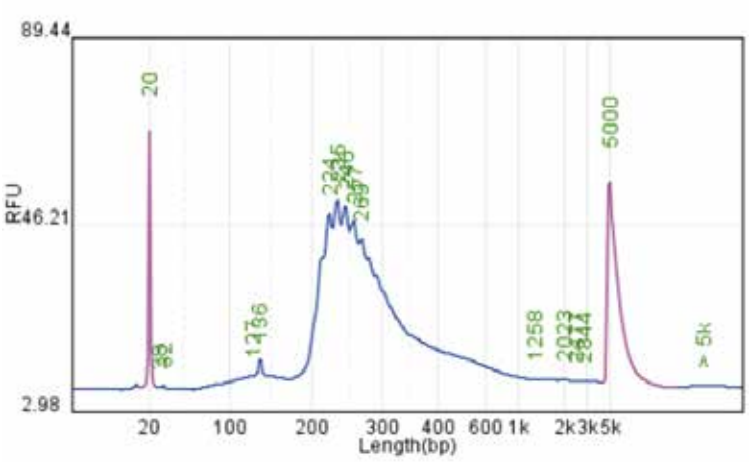


Figure 6. library peaks of 100 cells (Tn5-50 50-fold dilution)

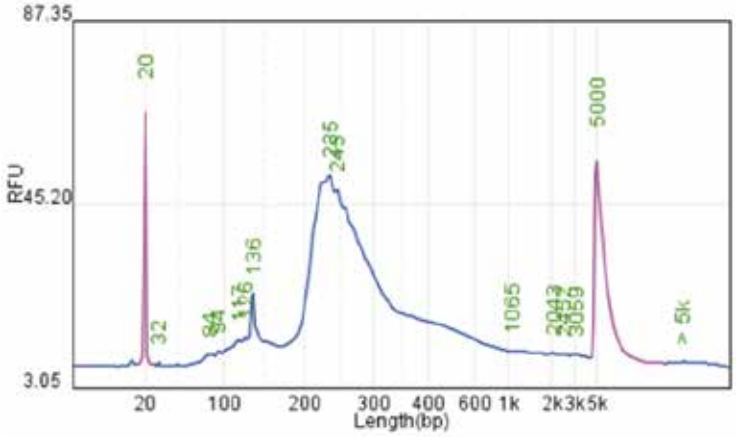


Figure 7. library peaks of 50 cells (Tn5-50 100-fold dilution)

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