*Trans*NGS® RNA-Seq Library Prep Kit for MGI®

Please read the datasheet carefully prior to use.

Version No. Version 2.0

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Cat. No. KP801

Storage: at -20°C for one year

Description

The kit is designed for the MGI high-throughput sequencing platform to prepare strand-specific or non-strand-specific transcriptome libraries. The applicable sample is 0.1 ng-100 ng of processed RNA (mRNA captured by mRNA magnetic beads or RNA obtained after rRNA removal) to prepare a high-quality sequencing library with high yield and complete information. The kit **completes the second-strand cDNA synthesis, end repair and A-tailing in one step, without the purification steps**, which greatly simplifies the operation process and shortens the library preparation time, and can complete the preparation of high-quality sequencing libraries within 3 hours .

Features

- High library conversion rate
- High data quality

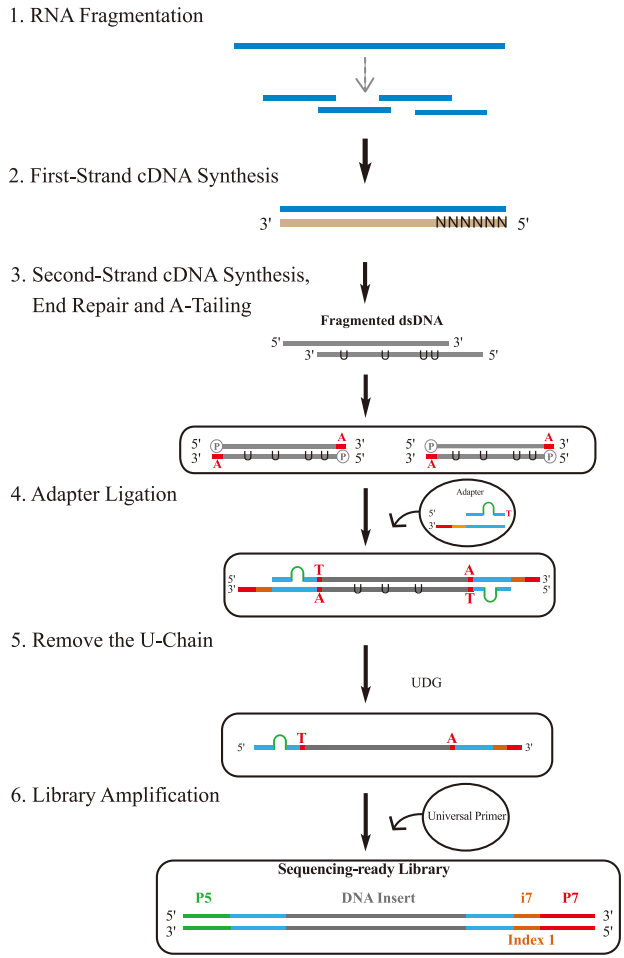
Application

- Whole transcriptome sequencing
- Gene expression analysis
- Single nucleotide variation analysis
- Variable splicing testing
- Fusion genetic testing
- Analysis of non-coding RNA and RNA precursor

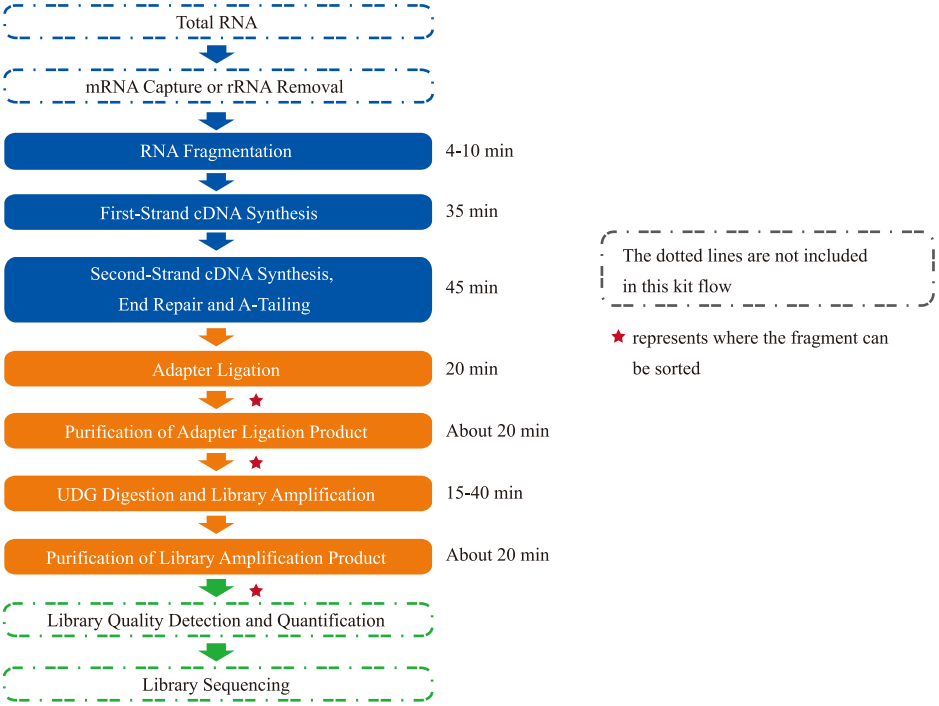
Kit Content

Component	KP801-01 (12 rxns)	KP801-02 (96 rxns)
4×RNA Fragmentation Buffer II	54 µl	432 µl
Library First-Strand Buffer II	72 µl	576 µl
Library First-Strand Enzyme Mix	36 µl	288 µl
Library SEA Buffer II （+dUTP）	300 µl	4×600µl
Library SEA Buffer II	300 µl	4×600µl
Library SEA Enzyme Mix	120 µl	960 µl
Adapter Dilution Buffer	600 µl	5 ml
Adapter Ligation Buffer II	360 µl	4×720 µl
Adapter Ligation Enzyme	60 µl	480 µl
*Trans*NGS® Library Amplification SuperMix (2×)	300 µl	4×600 µl
*Trans*NGS® Universal Primer for MGI	60 µl	480 µl
Uracil-DNA Glycosylase	12 µl	96 µl
Nuclease-free Water	2×1 ml	3×5 ml

Principle Chart and Flow Chart



Principle chart of strand-specific library construction
(No dUTP incorporation and UDG digestion steps in second-strand synthesis of non-strand-specific library)



Flow chart of strand-specific library construction

Library Structure

If *Trans*NGS® Indexed Adapter Kit for MGI® (Cat. No. KI401) is used, the single Barcode library has the following sequences:

5'-GAACGACATGGCTACGATCCGACTT-XXXXXXXX-AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA [i7]CAACTCCTTGGCTCACA-3'

i7: Index 1, 10 bases;

-XXXXXXXX-: insert sequence

Input Sample Recommendations

For total RNA quantification, it is recommended to use a fluorescent dye method based on specific recognition of RNA, such as Qubit et al. To ensure the success rate of library construction, it is recommended to use Total RNA with RIN value ≥ 8 for mRNA capture or Total RNA with RIN value > 7 for rRNA Depletion.

Library Construction Protocol

Reagents not included in the kit: Freshly prepared 80% ethanol, *MagicPure*® Size Selection DNA Beads (Cat. No. EC401), *Trans*NGS® Indexed Adapter Kit for MGI® (Cat. No. KI401).

1. RNA Fragmentation

(1) Add mRNA or RNA treated with rRNA Depletion according to the following system. Mix well by vortexing.

Component	Volume
RNA	≤12 μl
4×RNA Fragmentation Buffer II	4 μl
Nuclease-free Water	to 16 μl

*When captured mRNA is eluted or total RNA treated with rRNA Depletion is eluted, 4 × RNA Fragmentation Buffer II can be diluted to 1 × with Nuclease-free Water, RNA is eluted with 18 μl of 1× RNA Fragmentation Buffer II, and fragmented according to the step (2).

*If mRNA capture is performed, it is recommended to add 200 μl of freshly prepared 80% ethanol (prepared with RNase-free water) after the second wash with WB33 and discarding the supernatant in order to avoid the effect of WB33 on subsequent experiment, take care not to disturb the beads, incubate at room temperature for 30 seconds, discard the supernatant, and air dry at room temperature until the magnetic beads have just cracked.

(2) Place the tube in a thermal cycler and perform the following incubation steps (with heated lid set at 105°C):

The RNA fragmentation procedure (insert size: 200 bp-350 bp) is as follows:

Temperature	Time
94℃	6 min
4℃	Hold

For inserts of other size, the fragmentation conditions are shown in Table 1:

Table 1 Fragmentation conditions for RNA insert sizes

RNA insert sizes	Temperature	Time
150-200 bp	94℃	7-10 min
200-350 bp	94℃	4-6 min
⩾ 350 bp	85℃	4-8 min

(3) Remove the sample immediately after the sample temperature drops to 4°C, and perform the first-strand cDNA synthesis. **It is not recommended to pause**.

* For fragmentation operation with magnetic beads, remove the sample immediately after the sample temperature drops to 4°C, place the tube on magnetic stand for 5 minutes, carefully transfer 16 μl of the supernatant into a new RNase-free PCR tube, and perform the first-strand cDNA synthesis immediately. **It is not recommended to pause**.

2. First-Strand cDNA Synthesis

(1) Place the PCR tube on ice, and add the following components in sequence:

Component	Volume
Fragmented RNA	16 μl
Library First-Strand Buffer II	6 μl
Library First-Strand Enzyme Mix	3 μl
Total volume	25 μl

(2) Mix by pipetting several times, briefly centrifuge to collect the liquid on the wall of the tube.

(3) Place the tube in thermal cycler and perform the following incubation steps (**with heated lid set at ≥85°C**). The first-strand synthesis procedure is as follows:

Temperature	Time
25℃	10 min
42℃	15 min
70℃	10 min
4℃	Hold

When the reaction is complete, take out the sample and put it on ice. It is recommended to proceed to the next step immediately.

3. Second-Strand cDNA Synthesis, End Repair and A-Tailing

(1) Add the following reagents to the First Strand Synthesis reaction in sequence on ice:

Component	Strand-specific library	Non-strand-specific library
First-Strand Product	25 μl	25 μl
Library SEA Buffer II （+dUTP）	25 μl	-
Library SEA Buffer II	-	25 μl
Library SEA Enzyme Mix	10 μl	10 μl
Total volume	60 μl	60 μl

(2) Mix by pipetting several times, briefly centrifuge to collect the liquid on the wall of the tube.

(3) Place the tube in thermal cycler and perform the following incubation steps **(with heated lid set at ≥80℃)**. The second-strand synthesis procedure is as follows:

Temperature	Time
16℃	30 min
68℃	15 min
4℃	Hold

4. Adapter Ligation

(1) Thaw the *Trans*NGS® Indexed Adapter for MGI® on ice, dilute Adapter to appropriate concentration with Adapter Dilution Buffer referring to the table below.

Total RNA Input	Adapter Dilution Factor
1 μg-5 μg	Diluted 5 times
200 ng-999 ng	Diluted 10 times
50 ng-199 ng	Diluted 25 times

The quality and input of Adapter directly affect the efficiency and quality of library construction. It is recommended to use TransGen Adapter. Increasing input of Adapter can increase the library output at a certain extent, but too high will lead to residues of Adapter or Adapter dimer (which can be removed by purification or sorting); insufficient input of Adapter will affect the ligation efficiency and lead to poor library quality (such as the formation of chimeras due to self-ligation of insert fragments).

(2) Place the PCR tube completed in the previous step on ice, and add the following reagents in sequence:

Component	Volume
Reaction product of previous step	60 μl
Adapter: Appropriate concentration of Adapter	5 μl
Adapter Ligation Buffer II	30 μl
Adapter Ligation Enzyme	5 μl
Total volume	100 μl

It is recommended to mix the Adapter and the reaction product of the previous step first, and then add the mixture of Adapter Ligation Buffer II and Adapter Ligation Enzyme.
(3) Mix by pipetting several times, briefly centrifuge to collect the liquid on the wall of the tube.
(4) Place the tube in thermal cycler and incubate at 20°C for 20 minutes. Ligation products can be purified immediately or stored at -20°C.

5. Purification of Adapter Ligation Products

It is recommended to use 0.4×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for purification of product.
Note: If the RNA input is small, or the RNA integrity is low, it is recommended to sort the ligation products directly (without purification). See step 6.2 for the sorting conditions.
(1) Take out the magnetic beads from 2-8°C and stay still for 30 minutes at room temperature before use.
(2) Vortex the magnetic beads to mix well, add 40 μl magnetic beads (0.4×) to the product of the previous step.
(3) Pipet to mix well, and stay still for **5** minutes at room temperature.
Note: Insufficient mixing will significantly affect the experimental results.
(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield.
(5) Add 200 μl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.
(6) Repeat step (5) once.
(7) Air dry the beads at room temperature while the tube is on the magnetic stand.
Note: It is advisable to dry until the beads lose luster but are not cracked; do not heat to dry, otherwise the final yield will be affected.
(8) Remove the tube from the magnetic stand. Add 105 μl Nuclease-free Water. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.
(9) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.
(10) Carefully transfer 100 μl of the eluate to a clean PCR tube for sorting, or store at -20°C.
Note: DNA at low concentrations is unstable, it is recommended to amplify the library immediately, and it is not recommended to store at -20°C.

6.1. Sorting after Purification of Adapter Ligation Products (Followed by Step 5)

It is recommended to use *MagicPure*® Size Selection DNA Beads (Cat. No. EC401). According to the selection conditions in Table 2, taking the library size of 430 bp as an example:
(1) Take out the magnetic beads from 2-8°C and incubate for 30 minutes at room temperature before use.
(2) Vortex the magnetic beads to mix well, pipet 65 μl beads (0.65×) to the product of the previous step.
(3) Pipet to mix well, and incubate for 5 minutes at room temperature.
Note: Insufficient mixing will significantly affect the experimental results.

(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), pipet 160 µl supernatant into a new 1.5 ml centrifuge tube, and discard the beads.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final selection effect.

(5) Add 15 µl beads (0.15×) to the supernatant again.

(6) Pipet to mix well, and incubate for 5 minutes at room temperature.

Note: Insufficient mixing will significantly affect the experimental results.

(7) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.

(8) Add 200 µl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.

Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(9) Repeat step (8) once.

(10) Air dry the beads at room temperature while the tube is on the magnetic stand.

Note: Do not heat to dry, otherwise the final yield will be affected.

(11) Remove the tube from the magnetic stand. Add 22 µl Nuclease-free Water. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.

(12) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(13) Carefully transfer 19 µl or 20 µl of the eluate to a clean PCR tube for the next step of the library amplification or store at -20°C.

Table 2 Reference table for fragment selection using *MagicPure*® Size Selection DNA Beads

Sorting conditions for adapter ligation products			
Average product size(bp)	~280	~430	~630
Insert size (bp)	~200	~350	~550
First volume ratio (DNA Beads: DNA)	0.75×	0.65×	0.54×
Second volume ratio (DNA Beads: DNA)	0.20×	0.15×	0.12×

6.2. Direct Sorting of Adapter Ligation Products (Followed by Step 4)

With low RNA input, or low RNA integrity, direct sorting of the ligation product is recommended(without purification).

Step 4 of the ligation products are directly sorted (without purification), and the sorting conditions need to be adjusted. There is no significant difference in the size of the main peak of the library, the yield is significantly increased, and the peak shape is slightly wider using this method (see Figure 1). Taking the sorting condition of library size 430 bp as an example, the specific sorting steps are as follows:

(1) Take out the magnetic beads from 2-8°C and incubate for 30 minutes at room temperature before use.

(2) Vortex the magnetic beads to mix well, pipet 25 µl beads (0.25×) to the product of adapter ligation.

(3) Pipet to mix well, and incubate for 5 minutes at room temperature.

Note: Insufficient mixing will significantly affect the experimental results.

(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), pipet 122 µl supernatant into a new PCR tube, and discard the beads.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final selection effect.

(5) Add 15 µl beads (0.15×) to the supernatant again.

(6) Pipet to mix well, and incubate for 5 minutes at room temperature.

Note: Insufficient mixing will significantly affect the experimental results.

(7) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.

(8) Add 200 µl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.

Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(9) Repeat step (8) once.

(10) Air dry the beads at room temperature while the tube is on the magnetic stand.

Note: Do not heat to dry, otherwise the final yield will be affected.

(11) Remove the tube from the magnetic stand. Add 22 µl Nuclease-free Water. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.

(12) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(13) Carefully transfer 19 µl or 20 µl of the eluate to a clean PCR tube for the next step of the library amplification or store at -20°C.

7. Library Amplification

(1) Place the sterile PCR tube on ice and add the following components in sequence.

Component	Strand-specific library	Non-strand-specific library
Purified product	19 µl	20 µl
Uracil-DNA Glycosylase	1 µl	-
*Trans*NGS® Library Amplification SuperMix (2×)	25 µl	25 µl
*Trans*NGS® Universal Primer for MGI	5 µl	5 µl
Total volume	50 µl	50 µl

(2) Mix by pipetting several times, briefly spin to collect the liquid on the wall of the tube.

(3) Perform the following amplification procedure in a thermal cycler **(with the heated lid set at 105°C)**.

50℃	5 min*	
98℃	3 min	
98℃	30 sec	10-16 cycles**
60℃	30 sec	
72℃	30 sec	
72℃	3 min	
4℃	Hold	

* For strand-specific libraries, amplify after incubation; for non-strand-specific libraries, directly amplify without incubation.

** For different RNA inputs, the number of amplification cycles is as follows:

Total RNA Input	Cycles	
	Strand-specific library	Non-strand-specific library
1 μg	11-12	10-11
200 ng	13-14	12-13
50 ng	15-16	14-15

8.Purification of Library Amplification Products

It is recommended to use 0.9×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401). Increase (to obtain libraries with shorter inserts) or decrease the bead ratio (to reduce primer residue) as needed.

The specific steps of purification using 0.9× magnetic beads are as follows:

(1) Take out the magnetic beads from 2-8°C and incubate for 30 minutes at room temperature before use.

(2) Vortex the magnetic beads to mix well, add 45 μl magnetic beads (0.9×) to the product of the previous step.

(3) Pipet to mix well, and incubate for 5 minutes at room temperature.

Note: Insufficient mixing will affect the experimental results.

(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield.

(5) Add 200 μl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.

Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(6) Repeat step (5) once.

(7) Air dry the beads at room temperature while the tube is on the magnetic stand.

Note: It is advisable to dry until the beads lose luster but are not cracked; do not heat to dry, otherwise the final yield will be affected.

(8) Remove the tube from the magnetic stand. Add 22 μl Library Elution Buffer*. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.

(9) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(10) Carefully transfer 20 μl of the eluate to a clean 1.5 ml centrifuge tube. The product can be stored at -20°C.

* If size selection is required after purification, it is recommended to use 105 μl Library Elution Buffer for elution, transfer 100 μl of the eluted product to a clean 1.5 ml centrifuge tube, and then perform size selection.

9. Sorting of Library Amplification Products(Optional)

For low input or low quality RNA, fragment sorting after library amplification may also be considered. It is recommended to use *MagicPure*® Size Selection DNA Beads (Cat. No. EC401). According to the selection conditions of Table 3, taking the library size of 430 bp as an example:

(1) Take out the magnetic beads from 2-8°C and incubate for 30 minutes at room temperature before use.
(2) Vortex the magnetic beads to mix well, pipet 65 µl beads (0.65×) into a PCR tube, add 100 µl product from the previous step diluted with Nuclease-free Water.
(3) Pipet to mix well, and incubate for 5 minutes at room temperature.
Note: Insufficient mixing will significantly affect the experimental results.
(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), pipet 160 µl supernatant into a new PCR tube, and discard the beads.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final selection effect.
(5) Add 15 µl beads (0.15×) to the supernatant again.
(6) Pipet to mix well, and incubate for 5 minutes at room temperature.
Note: Insufficient mixing will significantly affect the experimental results.
(7) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.
(8) Add 200 µl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.
(9) Repeat step (8) once.
(10) Air dry the beads at room temperature while the tube is on the magnetic stand.
Note: Do not heat to dry, otherwise the final yield will be affected.
(11) Remove the tube from the magnetic stand. Add 22 µl Nuclease-free Water. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.
Note: If the library needs to be stored for a long time, it is recommended to use 0.1×TE solution for elution.
(12) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.
(13) Carefully transfer 20 µl of the eluate to a clean 1.5 ml centrifuge tube. Store at -20°C.

Table 3 Reference table for fragment selection using *MagicPure*® Size Selection DNA Beads

Average product size(bp)		~280	~430	~630
Insert size (bp)		~200	~350	~550
Purify library amplification products before sorting	First volume ratio (DNA Beads: DNA)	0.75×	0.65×	0.54×
	Second volume ratio (DNA Beads: DNA)	0.20×	0.15×	0.12×

10. Library Quality Detection and Quantification

(1) It is recommended to use Agilent 2100 Bioanalyzer to assess the library quality, taking Agilent high sensitivity DNA kit (Cat.No.5067-4626) as an example. The main peak of a properly fragmented library should be between 350-470 bp, as shown in Figure 1. If there is a sharp peak around 84 bp (adapter-dimer contamination), the library

product needs to be purified again with 0.9 × *MagicPure*® Size Selection DNA Beads (Cat. No. EC401). After the library is diluted to 50 μl with Library Elution Buffer, the purification method referring to (2)-(10) in step 7. After purification, it can be assessed again with the Agilent 2100 Bioanalyzer.

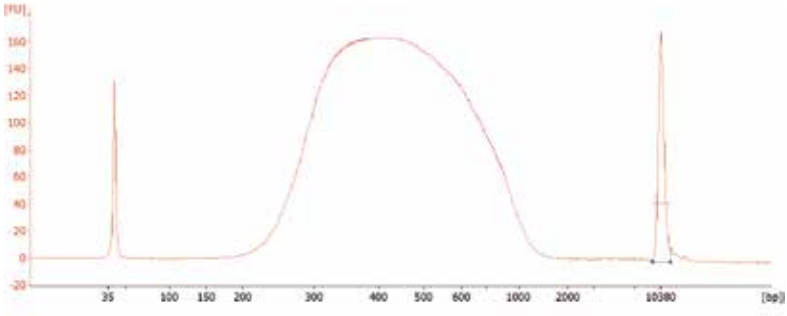


Figure 1 Library size distribution

(2)It is recommended to use the *Trans*NGS® Library Quantification Kit for MGI®(Cat. No. KQ401) to quantify the library concentration. Before quantification, please ensure that the peak shape of the library is normal and there are no obvious spurious peaks or primer-dimer contamination, otherwise the accuracy of library quantification will be affected.

For research use only, not for clinical diagnosis.

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