*Trans*NGS® DNA Library Prep Kit for MGI®

Please read the datasheet carefully prior to use.

Cat. No. KP221

Storage: at -20°C for one year

Description

The kit is designed to efficiently and quickly prepare DNA library from 1 ng to 1 μg fragmented double-stranded DNA for the MGI high-throughput sequencing platform. The samples can be fragmented dsDNA such as ultrasonic fragmented genomic DNA, enzyme digestion products, nucleic acid amplification products, chromatin immunoprecipitation DNA (ChIP DNA), formalin-fixed paraffin-embedded DNA (FFPE DNA), and can be combined with exon or other target capture reagents for exon sequencing or target capture sequencing.

Features

- Applicable to a wide range of sample types.
- High library conversion rate.

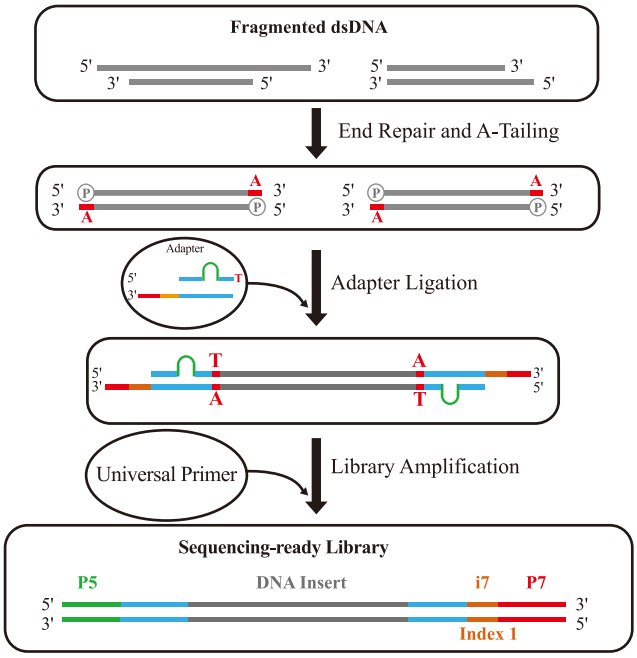
Application

- Whole genome sequencing.
- Target gene sequencing.
- Exon sequencing / other targeted capture sequencing.
- Metagenomic sequencing.
- ChIP sequencing.

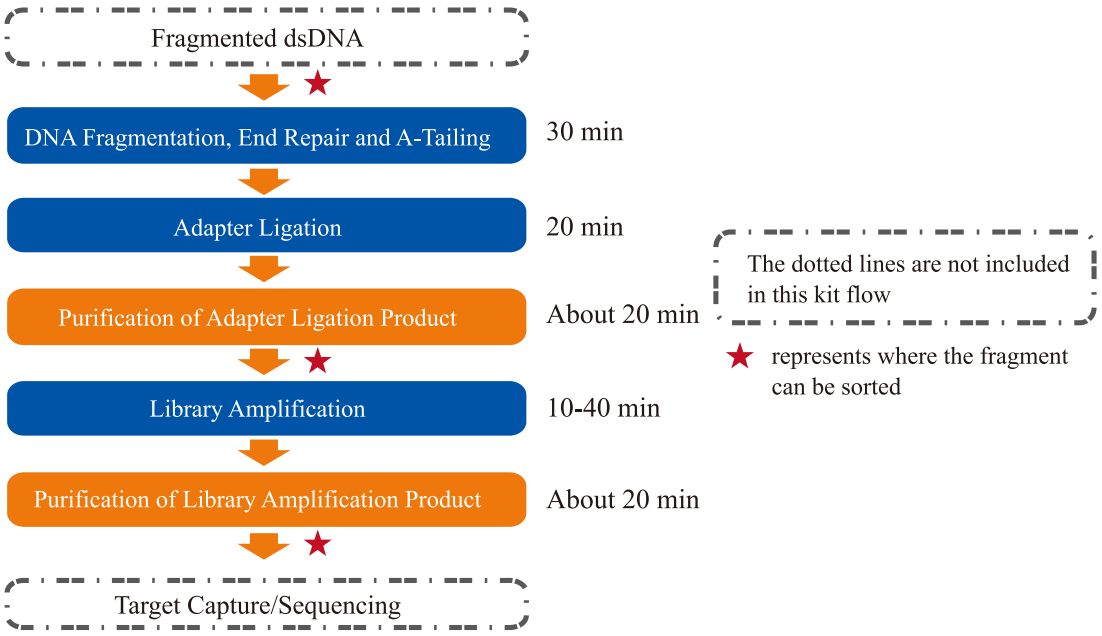
Kit Contents

Component	KP221-01 (12 rxns)	KP221-02 (96 rxns)
End-repair and A-tailing Reaction Mix	120 μl	960 μl
End-repair and A-tailing Enzyme Mix II	60 μl	480 μl
End-repair and A-tailing Enhancer	36 μl	288 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®	60μl	480μl
Library Elution Buffer	2 ml	4×4 ml
Nuclease-free Water	1 ml	5 ml

Principle Chart and Flow Chart



Principle chart of library construction
(i5 position with dotted line indicates some libraries do not have this Index.)



Flow chart of library construction

Library Structure

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

5'-GAACGACATGGCTACGATCCGACTT-XXXXXXXX-AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA

[i7]CAACTCCTTGGCTCACA-3'

i7: Index 1, 10 bases;

-XXXXXXXX-: insert sequence.

Input Sample Recommendations

- If input sample is 1 ng -1 μg genomic DNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH 8.0), the ratio of sample OD260/OD280 should be 1.8-2.0. The DNA concentration should be measured using a fluorescent dye method based on specific recognition of dsDNA, such as Qubit or the fluorescent dye PicoGreen.
- The sample input is the amount of fragmented dsDNA prepared for end repair and A-tailing. If there is loss during the fragmentation process, or fragment purification or sorting is performed after fragmentation, please quantify it accurately again before library construction.
- If the sample input contains too much metal ion chelating agents or other salts during the preparation process, it may affect the end repair and A-tailing. If the conditions are not met, the fragmented DNA needs to be purified or sorted for library construction.

Library Construction

Reagents not included in the kit: Freshly prepared 80% ethanol. *MagicPure*® Size Selection DNA Beads (Cat. No. EC401).

- The following steps do not include the fragment sorting. Fragment sorting can be performed before end repair, after adapter ligation, or after library amplification, and only needs to be performed once. Sorting is recommended after adapter ligation or library amplification.
- The reference conditions for fragment sorting using *MagicPure*® Size Selection DNA Beads (Cat. No. EC401) are shown in Appendix Table 1.
- If the sample input is ≤50 ng, it is not recommended to sort after adapter ligation. Sorting can be done after library amplification.

1. End Repair and A-Tailing

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

Component	Volume	Volume
Fragmented dsDNA	Variable (1 ng-10 ng)	Variable (10 ng-1 μg)
Nuclease-free Water	Variable	Variable
End-repair and A-tailing Reaction Mix	10 μl	10 μl
End-repair and A-tailing Enzyme Mix II	5 μl	5 μl
End-repair and A-tailing Enhancer*	3 μl	--
Total volume	60 μl	60 μl

* When the sample input is ≤10 ng, End-repair and A-tailing Enhancer need to be added.

Note: If the number of samples is greater than 1, mix the reaction reagents in one tube first and aliquot them into each reaction tube.

The efficiency of the mixed reaction reagents will decrease as the storage time increases, so it is recommended to use freshly prepared reagents.

(2) Mix by pipetting, briefly spin 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°C**).

28℃	15 min
68℃	15 min
4℃	Hold

2. Adapter Ligation

(1) Thaw *Trans*NGS® Indexed Adapter for MGI® on ice. Prepare the appropriate concentration of Adapter referring to the table below.

If dilution is required, use Adapter Dilution Buffer.

Sample Input	Adapter Dilution Factor	Adapter Concentration after Dilution
100 ng ＜x ≤ 1 μg	No dilution	20 μM
25 ng ＜x ≤ 100 ng	Diluted 2 times	10 μM
5 ng ＜x ≤ 25 ng	Diluted 10 times	2 μM
1 ng ≤ x ≤ 5 ng	Diluted 25 times	0.8 μM

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

Component	Volume
Appropriate concentration of Adapter	5 μl
Adapter-ligation Buffer II	30 μl
Adapter-ligation Enzyme	5 μl
Total volume	100 μl

Note: If the number of samples is greater than 1, mix the reaction reagents in one tube first and aliquot them into each reaction tube. The efficiency of the mixed reaction reagents will decrease as the storage time increases, so it is recommended to use freshly prepared reagents.

(3) Mix by pipetting, briefly spin 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 (the lid is not heated). Ligation products can be purified immediately or stored at -20°C.

3. Purification of Adapter Ligation Product

It is recommended to use 0.4 × *MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for purification. If fragment sorting is required after purification, it is recommended to use 105 μl Library Elution Buffer during elution, transfer 100 μl of the elution product to a clean 1.5 ml centrifuge tube, and perform fragment sorting.

The specific procedures for purification using 0.4 × magnetic beads are as follows:

(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 ligation product of the previous step.

(3) Mix by pipetting, and stay still for 5 minutes at room temperature.

Note: Be sure to mix thoroughly, otherwise it will significantly affect the experimental results.

(4) Place the tube on the magnetic stand and stay still at room temperature, make sure the beads settle to the magnet completely. After the solution is clarified (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 it 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. Stay still for 30 seconds at room temperature. 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: Do not heat to dry, otherwise the final yield will be affected.

(8) Remove the tube from the magnetic stand. Add 23 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 2 minutes at room temperature.

(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 PCR tube for library amplification, or store at -20°C.

Note: DNA at low concentrations is unstable. For sample input is ≤ 50 ng, it is recommended to amplify the library immediately, and it is not recommended to store at -20°C.

4. Sorting of Adapter Ligation Products (Optional)

Optional step: Purify adapter ligation products before sorting (Fragmented gDNA can be sorted in this step).

Follow the step 3 (Purification of Adapter Ligation Product). After completing step 3. (7), follow the steps below.

(1) Remove the tube from the magnetic stand. Add 105 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 5 minutes at room temperature.

(2) 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.

(3) Carefully pipet 100 μl of the eluate to a clean PCR tube.

(4) Take out the magnetic beads and stay still for 30 minutes. Vortex to mix thoroughly. Add 60 μl of magnetic beads (0.6X) to the eluent in the previous step.

(5) Mix thoroughly by pipetting or vortexing, and stay still for 5 minutes at room temperature.

Note: Be sure to mix thoroughly, otherwise it will affect the experimental results.

(6) Place the tube on the magnetic stand and stay still at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), pipet all supernatant (about 160 μl) to a clean PCR tube.

(7) Take out the magnetic beads and stay still for 30 minutes. Vortex to mix thoroughly. Add 15 μl of magnetic beads (0.15X) to the supernatant in the previous step.

(8) Mix thoroughly by pipetting or vortexing, and stay still for 5 minutes at room temperature.

Note: Be sure to mix thoroughly, otherwise it will affect the experimental results.

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

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

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

(11) Repeat step (10) once.

(12) 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.

(13) Remove the tube from the magnetic stand. Add 23 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 2 minutes at room temperature.

(14) 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.

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

Note: DNA at low concentrations is unstable. For sample input is ≤ 50 ng, it is recommended to amplify the library

immediately, and it is not recommended to store at -20°C.

5. Library Amplification

(1) Place the sterile PCR tube on ice, add reagents as follows:

Component	Volume
Purified product of the previous step	20 μl
*Trans*NGS® Library Amplification SuperMix (2×)	25 μl
*Trans*NGS® Universal Primer for MGI	5 μl
Total volume	50 μl

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

(3) Perform the following amplification procedure in a thermal cycler.

98℃	5 min	
98℃	30 sec	2-14 cycles*
60℃	30 sec	
72℃	30 sec	
72℃	3 min	
≤10℃	Hold	

For different inputs, the library yield is shown in Appendix Table 2.

6. Purification of Library Amplification Products

It is recommended to use 1.0×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401). *Trans*NGS® Library Amplification SuperMix (Cat. No. KA101) will not affect the fragment size of magnetic bead purification. Increase (to obtain libraries with shorter inserts) or decrease the bead ratio (to reduce primer residue) as needed.

If fragment sorting is required after purification, it is recommended to use 105 μl Library Elution Buffer during elution, transfer 100 μl of the elution product to a clean 1.5 ml centrifuge tube, and perform fragment sorting.

The specific steps of purification using 1.0× magnetic beads are as follows:

(1) Take out the magnetic beads from 2-8°C and stay still for 30 minutes for later use.

(2) Vortex the magnetic beads to mix well, add 50 μl magnetic beads (1.0×) to the product of the previous step.

(3) Pipet to mix thoroughly. Stay still 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 clarified (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: Do not heat to dry, otherwise the final yield will be affected.

(8) Remove the tube from the magnetic stand. Add 23 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 5 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.

Appendix

Sequence of *Trans*NGS® Adapter for MGI®:

-s- indicates phosphorothioate bond, - Ⓟ indicates phosphorylation

Table 1 Recommended conditions for *MagicPure*® Size Selection DNA Beads

Average Library Size (bp)		~320	~470	~670
Insert Size (bp)		~200	~350	~550
Sorting before end repair	1st beads ratio (DNA Beads: DNA)	1.0×	0.7×	0.55×
	2ed beads ratio (DNA Beads: DNA)	0.25×	0.2×	0.15×
Purify adapter ligation products before sorting	1st beads ratio (DNA Beads: DNA)	0.9×	0.68×	0.56×
	2ed beads ratio (DNA Beads: DNA)	0.2×	0.15×	0.12×
Purify library amplification products before sorting	1st beads ratio (DNA Beads: DNA)	0.85×	0.65×	0.57×
	2ed beads ratio (DNA Beads: DNA)	0.15×	0.1×	0.1×

Note: Size selection only needed to be carried out once. For the accuracy of fragment sorting, it is recommended that the volume of sample before fragment sorting should be exactly 100 μl. The difference in the ratio of magnetic beads at the three optional positions is caused by the different sequence sizes at both ends of the insert. Due to differences in the fragment sizes distribution of different samples, when the same conditions are used for sorting, the fragment sizes of the obtained products will also be different.

Table 2 Recommended cycles for 100 ng/1 μg library output with different inputs

Input	Recommended cycles*	
	100 ng library	1 μg library
1 μg	3**	3**
500 ng	3**	3 -4
100 ng	3**	5-6
50 ng	2-3	6-7
10 ng	6-7	9-10
5 ng	7-8	10-11
1 ng	10-11	13-14

* The recommended cycles in this table are empirical values for library construction using high-quality dsDNA derived from the human genome with an average size of 300 bp that was fragmented by ultrasound. There is no fragment sorting in library construction. If the DNA purity is poor or the DNA damage is severe, increase the cycles appropriately.

** Due to the adapter design of the MGI platform, library amplification must be performed before circularization, so at least 3 cycles are required to convert the vast majority of library dsDNA into circularizable dsDNA.

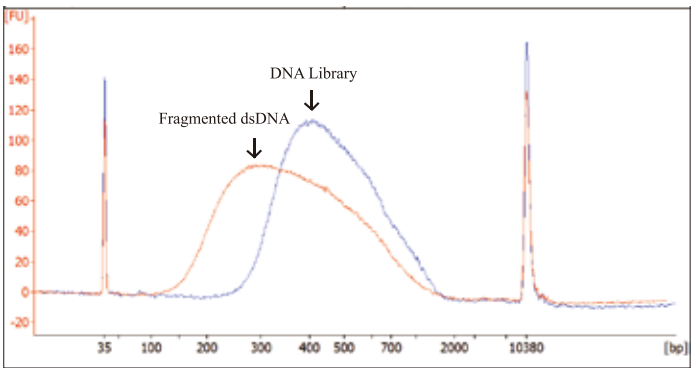


Figure 1 DNA size distribution before and after library construction
Red line-Human gDNA fragmented by sonication;
Blue line-Library constructed without size selection.

Notes

- To obtain better sequencing data, it is recommended to sort the fragments after adapter ligation or library amplification.
- Vigorous shaking should be avoided during mixing of the reaction solution to prevent the enzyme activity from decreasing which will result in a decrease in library construction efficiency.
- If magnetic beads are used for purification or fragment sorting, they should be well mixed during elution. The well-mixed magnetic beads should be uniformly suspended, free of visible particles, and no settlement after standing for 2 minutes.
- Samples with a concentration less than 1 ng/μl are recommended to be stored in a low-adsorption centrifuge tube or an ordinary centrifuge tube with 1× *Trans*NGS® Library Dilution Buffer (Cat. No. KB101) to prevent normal centrifuge tube from absorbing nucleic acid samples which will reduce the concentration of effective samples.
- The greater the number of library amplification cycles, the higher the duplication rate of the sequencing data., the less valid data. Therefore, it is recommended to use less amplification cycles on the basis of satisfying downstream applications.

Version number: V4-202111

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