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GENSeq™ DNA Library Prep Kit

Manual Revision v1.1
Catalog Nos. GSDL-1006, GSDL-1024, GSDL-1048, GSDL-1096

- Preparation of DNA libraries for next generation sequencing (NGS) applications.

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

GENSeq™ DNA Library Prep Kit for Illumina platforms is based on A-T ligation to prepare a library with high complexity for next generation sequencing (NGS) of genomic DNA. The kit offers the best product stability, convenience, and efficiency. It is suitable for library construction to perform whole genome or targeted sequencing, using hybridization capture system.

Recommended Application

Application	Sample Type	Recommended Input
Whole Genome Sequencing	Plant and animal gDNA	50-1000 ng
	Microbial DNA	10-1000 ng
Targeted Sequencing	Human gDNA	50-1000 ng
	FFPE DNA (Average size > 500bp)	10-1000 ng

Kit Contents and Storage

GENSeq™ DNA Library Prep Kit Catalog No.	GSDL-1006 (6 preps sample)	GSDL-1024 (12 preps x 2)	GSDL-1048 (12 preps x 4)	GSDL-1096 (12 preps x 8)	STORAGE
Number of Preps	6	24	48	96	
AT Buffer	60 µl	156 µl	312 µl	624 µl	-20°C
AT Enzyme	40 µl	104 µl	208 µl	416 µl	-20°C
Ligation Buffer	260 µl	676 µl	1352 µl	2704 µl	-20°C
DNA Ligase	20 µl	52 µl	104 µl	208 µl	-20°C
PCR Mix	250 µl	800 µl	1600 µl	3200 µl	20°C
Amplification Primer Mix	50 µl	157.5 µl	315 µl	520 µl	20°C
Nuclease-free water	1 ml	2 ml	4 ml	8 ml	4°C
TE Solution	1 ml	1 ml	2 ml	4 ml	4°C
UDI Adapter C1	12* 10 µl	12* 10 µl	12* 10 µl	12* 10 µl	-20°C
UDI Adapter C2	12* 10 µl	12* 10 µl	12* 10 µl	12* 10 µl	-20°C
UDI Adapter C3	12* 10 µl	12* 10 µl	12* 10 µl	12* 10 µl	-20°C
HighPrep™ PCR	1.5 ml	5 ml	10 ml	30 ml	4°C

Stability

All components are stable for 12 months when stored accordingly.

Safety Information

☐ Wear gloves and take precautions to avoid sample contamination.
☐ Perform all centrifugation at room temperature (+15 to +25°C) unless indicated otherwise.
☐ Unless otherwise specified, all mixing steps are listed as mix thoroughly and indicate that the sample should be mixed by either vortexing for 10 seconds or pipetting up and down 10 times.
☐ If liquid has remained in a tube's cap after mixing, gently tap or briefly spin the sample to collect the liquid into the tube's bottom, ensuring that the mixture remains homogeneous before progressing to the next step.
☐ It is recommended to perform Thermal Cycler incubations using a Thermal Cycler with a heated lid set to track 10°C above the block temperature.

Things To Know Before Starting

1. **Sample Pre-treatment**

- Samples containing high heparin/EDTA content should be supplemented with ultrapure water up to 50 µL, then purified by 1-fold volume of HighPrep™ PCR beads. Use 50 µL ultrapure water to elute DNA, repeat purification twice. For the final elution use Nuclease-free water to elute DNA.
- Use proteinase K for digestion in samples with excess protein content, then purify and recover DNA before library construction.

2. **Temperature Control**

- Reagents should be packed in small quantities to avoid repeated freezing and thawing.
- The preparation and addition of reaction system should be carried out on ice/cold metal bath to preserve enzyme activity.
- If the reaction temperature on the PCR instrument is low, don't insert the reaction tubes until the module temperature reaches the reaction temperature (programmed temperature).
- The ambient temperature of the laboratory should be stable at 20 ~ 25°C, and condition of 25°C incubation in the experimental operation step can be performed at room temperature.

3. **Size selection**

- If the constructed library fragment is small (300-400 bp) and does not require high concentration and consistency of library fragment distribution, it is recommended to use single sided size selection for fragment screening. If the library fragment is large or requires high degree of concentration and consistency of library fragment distribution, it is recommended to use double sided size selection for fragment screening.
- For FFPE DNA samples it is advised to use single sided size selection to reduce sample loss.

4. **Cycling**

- GENSeq™ supports PCR Free library construction. WGS library construction can be optimized to recommended cycle number.
- If the subsequent hybridization target acquisition experiment is carried out, the library should not over expand, and 50% of the total amount of the library should be enough for the downstream experiments.

5. **Avoid cross contamination**

- In the process of magnetic bead purification, after vortexing, the samples must be centrifuged to place all the liquid at the bottom of the PCR tube before opening the tube cover for operation. This reduces chances of cross contamination.
- The experimental area should be disinfected with 0.5% hypochlorous acid disinfectant after the completion of the experiment every day.

Workflow

Process	Time	
Fragmented dsDNA	10 min	STOP
↓		
End Repair and A-Tailing	60 min	
↓		
Adapter Ligation	15 min	
↓		
Post-ligation Cleanup	30-60 min	
↓		
Library Amplification	20 min	
↓		
Post-amplificaton Cleanup	30 min	STOP
↓		
Target Capture or Sequencing		

STOP = Safe stopping point

GENSeq™ DNA Library Prep Kit : 96 well format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Timer
- ☐ Pipettes
- ☐ Ethanol
- ☐ Nuclease-free water
- ☐ Tips
- ☐ Centrifuge Tube
- ☐ Programmable Thermal Cycler
- ☐ Microfuge
- ☐ Microcentrifuge
- ☐ Vortex Mixer
- ☐ 96 well magnetic separation device
- ☐ Qubit Fluorometer (Thermo Fisher Catalog # Q33216)
- ☐ Qubit TM dsDNA HS Assay Kit (Thermo Fisher Catalog #Q32854 - 500 rxn)

GENSeq™ Sample Requirements

Samples for DNA sequencing should be purified using a method that can deliver high-quality DNA that is as intact as possible. Sample purity should be determined spectrophotometrically by measuring the ratio of absorbance at OD 260/280 and OD 260/230. Samples should be inhibitor free and should not contain substances that may inhibit enzymatic reactions in downstream processes. Inhibitor substances include heparin, high EDTA concentration, chaotropic salts, humic acids, polysaccharides, etc.) and DNA carriers such as tRNA, acrylamide, glycogen, etc). It is also important to measure sample concentration using a DNA-specific measurement technique such as fluorometric measurement (i.e. Invitrogen Qubit, picogreen etc). This measurement is more sensitive than nanodrop or UV spectrophotometer and it provides accurate determination of true DNA concentration.

Sample Quality	**gDNA without degradation by gel electrophoresis analysis; Size of FFPE gDNA >500 bp**
Sample Quantity	**1-1000 ng; By Qubit method**
Sample Purity	**OD 260/280 = 1.8-2.0; OD 260/230 = 2-2.5**
Ambient Temperature	**20-25°C**
Ambient Humidity	**40%-60%**

Protocol

1. Fragmentation

Please refer to the laboratory conditions of your fragmentation equipment or reagent of choice. To construct a library for targeted sequencing, we recommend Covaris™ to mechanically shear DNA to obtain 150 to 350 bp fragments.

Safe stopping point: fragmented DNA may be stored at 4°C overnight or -20°C for longer.

⚠ *DNA Must be quantified after fragmentation.*

2. End Repair and A-Tailing

2.1 Thaw the End Repair & A-Tailing Buffer and End Repair & A-Tailing Enzyme on ice. Once the reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations. Spin the tube in a microfuge to collect content prior to using.

2.2 Prepare end repair and A-tailing reaction in a 0.2 ml PCR tube as indicated below. Mix well by gentle pipetting and spin down briefly.

Fragmented DNA	40 µl
End Repair & AT Buffer	6 µl
End Repair & AT Enzyme	4 µl
Total	**50 µl**

2.3 Program a thermal cycler with the conditions indicated below. Incubate the samples once the thermal cycler reaches 20°C.

20°C	**30 min**
65°C	**30 min**
10°C	**HOLD**

⚠ *The Thermal Cycler lid in the first program should be left open.*

3. Adapter Ligation

3.1 Once the Ligation Buffer and DNA Ligase are thawed on ice, mix them thoroughly by quick vortexing. Spin down the tube briefly in a microfuge.

3.2 Prepare Adapter Ligation reaction as indicated below in a reaction tube from step 2.3. Vortex or pipet gently to mix and spin down briefly.

Repair & AT Product	50 µL
GENSeq™ Adapter Mix (15µM)	2 µL
Ligation Buffer	26 µL
DNA Ligase	2 µL
Total	**80 µL**

⚠ *First add adapter, then add other reagents. Dilute adapter stocks to the appropriate concentration, as outlined in the following table:*

Input of fragmented DNA	Adapter stock concentration	Dilution ratio	Adapter: insert molar ratio
1000 ng	15 µM	*	2:1
500 ng	15 µM	*	10:1
250 ng	15 µM	*	20:1
100 ng	15 µM	*	50:1
50 ng	15 µM	*	100:1
25 ng	15 µM	2	100:1
10 ng	15 µM	5	100:1
5 ng	15 µM	10	100:1
2.5 ng	15 µM	20	100:1
1 ng	15 µM	50	100:1

⚠ *When the input of fragmented DNA > 500 ng, higher library yield will be achieved if higher adapter concentration is used.*

3.3 Program the thermocycler as outlined below and incubate once temperature is stabilized to 20°C:

20°C	30 min
4°C	Hold

⚠ *The Thermal Cycler lid may be left open.*

3.4 Store the rest of the reagent at -20°C.

4. Post-ligation Cleanup

4.1 Method 1: Single sided size selection

4.1.2 Remove HighPrep™ PCR Beads from 4°C storage and equilibrate at room temperature for a minimum of 30 min before using.

4.1.3 Perform the V1 volume of beads based cleanup in the same tube from step 3.3 as indicated below. Mix thoroughly by gently pipetting up and down. Allow DNA to bind to the beads by incubating the tube at room temperature for 5-10 min.

Input DNA	Volume (V1)
≥ 50 ng	40 µL
< 50 ng	30 µL

4.1.3 Centrifuge the sample briefly, then place the tube on the magnetic device to capture the beads. Incubate at room temperature for about 5 min or until the liquid is clear. Carefully aspirate and discard the supernatant and retain the beads.

4.1.4 With the tube on the magnetic device, add 150 µL of 80% ethanol. Incubate at room temperature for ≥30 sec. Make sure that the beads are completely cleared from the solution and then aspirate and discard the ethanol.

4.1.5 Repeat step 4.1.4 for a total of two 80% ethanol washes.

4.1.6 Remove any ethanol droplets from the tube by briefly centrifuging the tube then placing it back on the magnetic device. Using a 10 µL pipette or a fine pipette tip, remove all residual ethanol. Do not disturb the beads while pipetting out the ethanol.

4.1.7 Place the tube on the magnetic device and allow the beads to dry by incubating the tube at room temperature for 5 min, or until all the ethanol has evaporated. Over-drying the beads may result in bead cracking which reduces DNA yield.

4.1.8 Resuspend the beads in 21 µL of Nuclease-free water. Pipette up and down gently and incubate the tube at 25°C for 2 min for DNA elution from the beads

4.1.9 Briefly centrifuge the tube then place it on the magnetic device to capture the beads. Let sit at room temperature until the beads are completely cleared from the eluate. Transfer the clear supernatant to a new tube and put it on ice.

4.2 Method 2: Double-sided size selection (for gDNA input ≥50 ng)

Average insert DNA size	Average insert library size	Left Side Selection Volume(V1)	Right Side Selection Volume(V2)
200 bp	350 bp	30 µL	30 µL
250 bp	400 bp	24 µL	28 µL
300 bp	450 bp	20 µL	26 µL
350 bp	500 bp	18 µL	25 µL
400 bp	550 bp	15 µL	25 µL

4.2.1 Remove HighPrep™ PCR Beads from 4°C storage and equilibrate at room temperature for a minimum of 30 min before using.

4.2.2 Perform the V1 volume of beads based cleanup by following the table above. Mix thoroughly by pipetting up and down. Allow DNA to bind to the beads by incubating the tube at room temperature for 5-10 min

4.2.3 Centrifuge the sample briefly, then place the tube on the magnetic device to capture the beads. Incubate at room temperature for about 5 min or until the liquid is clear. Carefully aspirate and discard the supernatant and retain the beads

4.2.4 With the tube on the magnetic device, add 150 µL of 80% ethanol. Incubate the tube on the magnet at room temperature for ≥30 sec. Make sure that the beads are completely cleared from the solution and then aspirate and discard the ethanol.

4.2.5 Repeat step 4.2.4 for a total of two 80% ethanol washes.

4.2.6 Remove any ethanol droplets from the tube by briefly centrifuging the tube then placing it back on the magnetic device. Using a 10 µL pipette or a fine pipette tip, remove all residual ethanol. Do not disturb the beads while pipetting out the ethanol.

4.2.7 Allow the beads to dry by incubating the tube at room temperature for 5 min, or until all the ethanol has evaporated. Over-drying the beads may result in bead cracking which reduces DNA yield.

4.2.8 Thoroughly resuspend the beads in 50 µL of Nuclease-free water, and incubate the tube at 25°C for 2 min.

4.2.9 Perform the V2 volume of beads-based cleanup by following the table above. Mix the sample by pipetting up and down and incubate the tube at room temperature for 5 – 10 min to allow DNA to bind to the beads.

4.3 Centrifuge the tube briefly and place on the magnetic device. Make sure that the beads are completely cleared from the solution. Transfer the cleared supernatant to a new 0.2 ml PCR tube (50 +V2).

⚠ *At this step keep the supernatant and discard the magnetic beads.*

4.3.1 Pipette 30 µL of HighPrep™ PCR into the supernatant (from step 4.3), mix gently by pipetting up and down. Let the tube sit at room temperature for 5 – 10 min to bind DNA to the beads.

4.3.2 Place the tube on the magnetic device and allow the beads to dry by incubating the tube at room temperature for 5 min, or until all the ethanol has evaporated. Over-drying the beads may result in bead cracking which reduces DNA yield.

4.3.3 With the tube on the magnetic device, add 150 µL of 80% ethanol. Incubate at room temperature for ≥30 sec. Make sure that the beads are completely cleared from the solution and then aspirate and discard the ethanol.

4.3.4 Repeat step 4.3.3 for a total of two 80% ethanol washes.

4.3.5 Remove any ethanol droplets from the tube by briefly centrifuging the tube then placing it back on the magnetic device. Using a 10 µL pipette or a fine pipette tip, remove all residual ethanol. Do not disturb the beads while pipetting out the ethanol.

4.3.6 Allow the beads to dry by incubating the tube at room temperature for 5 min, or until all the ethanol has evaporated. Over-drying the beads may result in bead cracking which reduces DNA yield.

4.3.7 Resuspend the beads in 21 µL of Nuclease-free water. Pipette up and down gently and incubate the tube at 25°C for 2 min for DNA elution from the beads.

4.3.8 Briefly centrifuge the tube then place it on the magnetic device to capture the beads. Let sit at room temperature until the beads are completely cleared from the eluate. Transfer 20 µL of clear supernatant to a new PCR tube and put it on ice.

5. Library Amplification

5.1 Thaw 2×HiFi PCR Master Mix and Amplification Primer Mix on ice, mix well by brief vortexing. Spin down the tube in a microfuge to collect content prior to using.

5.2 Prepare each library amplification reaction as indicated below. Mix gently by pipetting up and down and briefly spin down the tube.

2 x HiFi PCR Master Mix	25 µL
Amplification Primer Mix	5 µL
Ligated Library	20 µL
Total	**50 µL**

5.3 Amplify the library using the following cycling program:

98° C	**2 min**	**1 cycle**
98°C	**15 s**	**Number of cycles referred to in following table.**
60°C	**30 s**	**Number of cycles referred to in following table.**
72°C	**30 s**	**Number of cycles referred to in following table.**
72°C	**2 min**	**1 cycle**
4°C	**Hold**	**1 cycle**

Number of cyles required to generate

Input DNA	100 ng Library	1000 ng Library	FFPE DNA
1000 ng	**0**	**2-4**	
500 ng	**0**	**3-5**	
250 ng	**0-2**	**4-7**	
100 ng	**2-3**	**6-8**	
50 ng	**3-5**	**7-9**	
25 ng	**4-6**	**8-10**	
10 ng	**6-8**	**10-12**	
5 ng	**8-10**	**12-14**	
2.5 ng	**10-12**	**14-16**	
1 ng	**12-14**	**15-17**	**+0-3 cycles**

⚠ *Input DNA was quantified after fragmentation;*
The recommend cycle to double -sided size selection, 1-2 cycle(s) more;
Target sequencing requires to generate 600-1000 ng of library DNA.

6. Post-amplification Cleanup

6.1 Perform a bead-based cleanup of the amplified library in the library amplification tube. Add 90 µL of HighPrep™ PCR beads to 50 µL of library amplification product from step 5 and mix well by pipetting up and down multiple times. Incubate the tube at room temperature for 5 – 10 min to bind DNA to the beads.

6.2 Place the tube on the magnetic device to magnetize the beads. Incubate until the liquid is completely clear. Avoid any bead loss by carefully removing and discarding the supernatant without disturbing the beads.

6.3 With the tube on the magnetic device, add 150 µL of 80% ethanol. Incubate at room temperature for ≥30 sec. Make sure that the beads are completely cleared from the solution and then aspirate and discard the ethanol.

6.4 Repeat step 6.3 for a total of two 80% ethanol washes.

6.5 Remove any ethanol droplets from the tube by briefly centrifuging the tube then placing it back on the magnetic device. Using a 10 µL pipette or a fine pipette tip, remove all residual ethanol. Do not disturb the beads while pipetting out the ethanol.

6.6 Allow the beads to dry by incubating the tube at room temperature for 5 min, or until all the ethanol has evaporated. Over-drying the beads may result in bead cracking which reduces DNA yield.

6.7 Resuspend the beads in 20 µL of TE solution. Pipette up and down gently and incubate the tube at 25°C for 2 min for DNA elution from the beads.

6.8 Briefly centrifuge the tube then place it on the magnetic device to capture the beads. Let sit at room temperature until the beads are completely cleared from the eluate. Transfer 20 µL of clear supernatant to a new tube and store at -20°C.

6.9 Use a Qubit to quantify the library.

Safe stopping point.

Quality Control

Equipment: Agilent Bioanalyzer 2100, Qsep 100 Analyzer, or other similar equipment.

Quality Control Standard

A. Make sure there are no adapter dimers.
B. Fragment distribution should be concentrated.
C. Average library size for target sequencing is 350-450 bp

Distribution	94%
From	170 bp
To	898 bp
Average BP	391.38 bp

Adapter name	i5 index (HiSeq® 2000/2500, MiSeq®, NovaSeq® systems)	i5 index (HiSeq 3000, 4000, HiSeq X-ten, NextSeq®, MiniSeq® systems)	i7 index (all Illumina systems)
GENSeq™ Adapter Index 1	ATATGCGC	GCGCATAT	CTGATCGT
GENSeq™ Adapter Index 2	TGGTACAG	CTGTACCA	ACTCTCGA
GENSeq™ Adapter Index 3	AACCGTTC	GAACGGTT	TGAGCTAG
GENSeq™ Adapter Index 4	TAACCGGT	ACCGGTTA	GAGACGAT
GENSeq™ Adapter Index 5	GAACATCG	CGATGTTC	CTTGTCGA
GENSeq™ Adapter Index 6	CCTTGTAG	CTACAAGG	TTCCAAGG
GENSeq™ Adapter Index 7	TCAGGCTT	AAGCCTGA	CGCATGAT
GENSeq™Adapter Index 8	GTTCTCGT	ACGAGAAC	ACGGAACA
GENSeq™ Adapter Index 9	AGAACGAG	CTCGTTCT	CGGCTAAT
GENSeq™ Adapter Index 10	TGCTTCCA	TGGAAGCA	ATCGATCG
GENSeq™ Adapter Index 11	CTTCGACT	AGTCGAAG	GCAAGATC
GENSeq™ Adapter Index 12	CACCTGTT	AACAGGTG	GCTATCCT
GENSeq ™Adapter Index 13	ATCACACG	CGTGTGAT	TACGCTAC
GENSeq™ Adapter Index 14	CCGTAAGA	TCTTACGG	TGGACTCT
GENSeq™ Adapter Index 15	TACGCCTT	AAGGCGTA	AGAGTAGC
GENSeq™ Adapter Index 16	CGACGTTA	TAACGTCG	ATCCAGAG
GENSeq™ Adapter Index 17	ATGCACGA	TCGTGCAT	GACGATCT
GENSeq™ Adapter Index 18	CCTGATTG	CAATCAGG	AACTGAGC
GENSeq™ Adapter Index 19	GTAGGAGT	ACTCCTAC	CTTAGGAC
GENSeq™ Adapter Index 20	ACTAGGAG	CTCCTAGT	GTGCCATA

Adapter name	i5 index (HiSeq® 2000/2500, MiSeq®, NovaSeq® systems)	i5 index (HiSeq 3000, 4000, HiSeq X-ten, NextSeq®, MiniSeq® systems)	i7 index (all Illumina systems)
GENSeq™ Adapter Index 21	**CACTAGCT**	**AGCTAGTG**	**GAATCCGA**
GENSeq™ Adapter Index 22	**ACGACTTG**	**CAAGTCGT**	**TCGCTGTT**
GENSeq™ Adapter Index 23	**CGTGTGTA**	**TACACACG**	**TTCGTTGG**
GENSeq™ Adapter Index 24	**GTTGACCT**	**AGGTCAAC**	**AAGCACTG**

⚠ *Dual-index sequencing on a paired-end flow cell follows different workflows, depending on the Illumina system.*

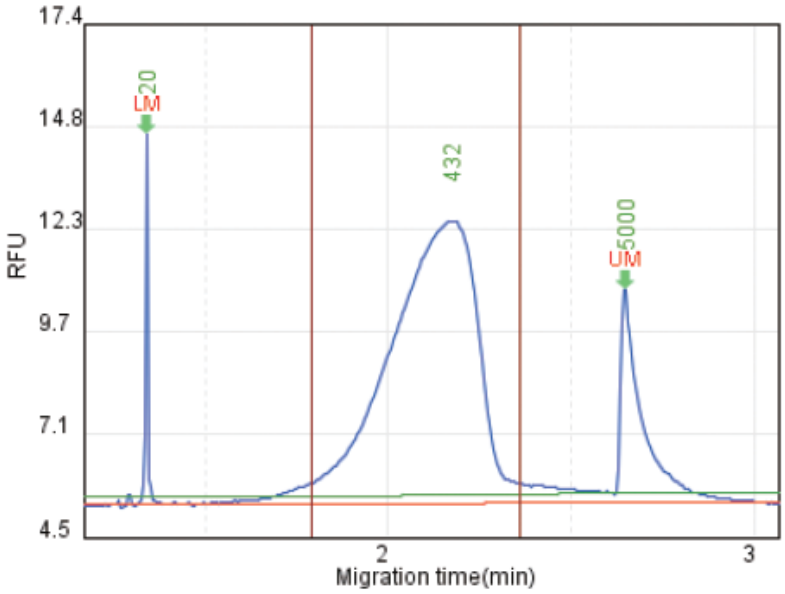

Figure 1. The size of libraries prepared with the GENSeq™ DNA Library Prep Kit

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