



PRODUCT INFORMATION

Thermo Scientific™ ClaSeek™

Library Preparation Kit, Illumina™ compatible

#K1341, #K1342




Store at -20 °C before opening

CERTIFICATE OF ANALYSIS

Thermo Scientific ClaSeek™ Library Preparation Kit, Illumina™ compatible, is qualified by constructing DNA fragment libraries from 50 ng and 1 µg of sample DNA following the main PCR-free protocol outlined in the manual. The quality of constructed libraries is evaluated using Agilent® 2100 Bioanalyzer®. The kit is functionally validated for next generation sequencing on the Illumina™ MiSeq™ and HiSeq™ instruments.

Quality authorized by:

 Jurgita Žilinskienė

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COMPONENTS OF THE KIT

Component	Cap color	#K1341 12 preps	#K1342 48 preps
ClaSeek™ End Conversion Master Mix, IL	Red	300 µL	4 × 300 µL
ClaSeek™ Ligation Mix, IL	Green	120 µL	4 × 120 µL
Water, nuclease-free	White	1250 µL	2 × 1250 µL

STORAGE

The ClaSeek™ Library Preparation Kit, Illumina™ Compatible, is shipped on dry ice and must be stored at -20 °C immediately upon arrival.

DESCRIPTION

Thermo Scientific ClaSeek™ Library Preparation Kit, Illumina™ compatible, is designed for fast and convenient construction of PCR-free NGS fragment library from a DNA sample input as low as 500 ng. In combination with the amplification protocol, the kit enables flexibility to generate NGS DNA fragment libraries from 5 ng to 1 µg sample amounts.

The ClaSeek Library Preparation Kit utilizes a highly efficient NGS-optimized library construction protocol combining the end-conversion and adapter addition steps into a simple and convenient one-tube procedure. This minimizes unnecessary pipetting steps and reduces hands-on time, allowing for PCR-free library construction in less than 70 minutes. The kit is suitable for construction of 50, 300 and 500-base read length Illumina™-compatible libraries, and is validated for use on Illumina™ HiSeq™ and MiSeq™ sequencing platforms.

TECHNOLOGY OVERVIEW

The Thermo Scientific ClaSeek library construction technology utilizes a fast and efficient NGS library construction method by combining the DNA fragment end-conversion and adapter addition steps into a convenient one-tube protocol. During the initial steps, fragmented DNA is end-repaired (5'- and 3'-overhangs are blunted, 5'-ends are phosphorylated) and a single dA-overhang is added at the 3'-end of each strand. In the following step Illumina™-compatible NGS adapters are added to each end of the converted DNA fragments. Thermo Scientific ClaSeek Library Preparation Kit can be used together with MagJET™ NGS Cleanup and Size Selection Kit that allows for selection of a sequencing-ready DNA library within a desired read length interval. Alternatively, the kit can be used together with Thermo Scientific™ GeneJET™ NGS Cleanup Kit for cleanup prior to agarose gel-based size selection and subsequent DNA extraction from gel with Thermo Scientific™ GeneJET™ Gel Extraction and DNA Cleanup Micro Kit (Cat #K0831/2). An optional high-fidelity amplification step can be performed for DNA library construction from DNA sample amounts as little as 5 ng.

IMPORTANT NOTES

Input DNA requirements and general recommendations:

- **DNA quality.** The success of DNA library preparation and reliable DNA sequencing results strongly depends on the quality and quantity of input DNA used. Proper sample handling, DNA isolation method and DNA concentration evaluation are very important to results. Residual traces of contaminating proteins, organic solvents and salts can degrade the DNA or decrease the efficiency of enzyme activities that are necessary for efficient DNA library preparation. Single-stranded DNA, RNA or free nucleotides can interfere with accurate quantification of purified DNA, especially when UV spectrometry-based methods (such as Thermo Scientific™ Nanodrop™ instrument) are used for measurement.
- **Recommendations for DNA fragmentation.** The Library construction protocol requires fragmented DNA obtained using any available physical fragmentation methods, such as sonication or nebulization. Follow supplier's recommendations to obtain fragmented DNA of desired fragment length and concentration. The quality of fragmented DNA must be evaluated by agarose gel electrophoresis or using Agilent® 2100 Bioanalyzer®. Always optimize the fragmentation protocol to obtain median fragment size of required NGS read length:

NGS library read length	50 bp or 2 × 25 bp	300 bp or 2 × 150 bp	500 bp or 2 × 250 bp
Recommended median size of fragmented DNA	200 bp	300-400 bp	500-600 bp

- The DNA library construction workflow is designed to be used with high-quality fragmented double-stranded DNA dissolved in nuclease-free water, 10 mM Tris (pH 7.5-8.5), or TE buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA).

- The DNA samples must be free of contaminating proteins, RNA, organic solvents and salts. For samples with unknown DNA quality, re-purification of DNA is highly recommended.
- For high quality gDNA purification from various sources use specialized commercial kits, such as Thermo Scientific GeneJET Genomic DNA Purification Kit (Cat. #K0721/2), GeneJET Viral DNA and RNA purification Kit (Cat. #K0821), or MagJET Genomic DNA Kit (Cat. #K2721/2), MagJET Viral DNA and RNA Kit (Cat. #K2781/2).
- Use appropriate laboratory practices to minimize cross-contamination of products. Use filtered pipette tips and if possible, perform library construction in a separate area or room.
- Thaw frozen reagents on ice before use, and keep them on ice until ready to use. Minimize the time outside of -20 °C for the ClaSeek End Conversion Master Mix, IL and ClaSeek Ligation Mix, IL.
- Mix reagents thoroughly before each use by vortexing.
- Perform all DNA library cleanup, adapter removal or size selection steps using DNA LoBind™ Tubes (Eppendorf™ Cat. #022431021).

Required reagents not provided with the kit:

- Tools for enzymatic or physical DNA shearing
- Illumina™ compatible adapters (with appropriate indices, if more than one library will be sequenced in one MiSeq™ or HiSeq™ run)
- 0.5 or 0.2 mL PCR tubes / plates
- LoBind™ Tubes 1.5 mL (Cat. #022431021, Eppendorf™)
- Pipette tips and pipettes
- Microcentrifuge
- Thermal cycler
- Vortex mixer
- Real Time Thermocycler
- Any commercially available library quantification kit, Illumina™ compatible
- Agilent® 2100 Bioanalyzer® instrument and High Sensitivity DNA Kit (Agilent Technologies Inc.) or comparable method to assess the quality of DNA library
- Thermo Scientific MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) for cleanup or size selection of DNA library of desired fragment length
- Optional: Thermo Scientific GeneJET NGS Cleanup Kit (Cat. #K0851) or Thermo Scientific™ GeneJET™ PCR purification Kit (Cat. #K0701/2) for cleanup of fragmented DNA
- Optional: Primers for DNA library amplification, Illumina™ compatible (if library amplification will be performed)
- Optional: Thermo Scientific™ Phusion Hot Start II High-Fidelity DNA Polymerase (Cat. #F-549S) for amplification of prepared DNA library
- Optional: Thermo Scientific GeneJET Gel extraction and DNA Cleanup Micro kit (Cat. #0831/2) for DNA extraction from agarose gel, if adapter-ligated DNA library size selection will be performed using an agarose gel-based method. For gel based size selection, also

needed: 50x TAE buffer, Clean Scalpels, Certified Agarose, Distilled Water, Gel Loading Dye, SYBR® Gold Nucleic Acid Gel Stain (Invitrogen), Thermo Scientific™ O'RangeRuler™ 50 bp DNA ladder (Cat. #SM0613), electrophoresis unit and power source.

PREPARATION OF BARCODED LIBRARIES

Important Note: Adapters, compatible with Illumina™ sequencing platform, are not included in this kit and should be acquired separately. Please follow Illumina™ recommendations to ensure the appropriate indexing of samples if pooled libraries will be used for sequencing.

PROTOCOL

A. End Conversion and Adapter Addition Protocol

This protocol provides instructions for amplification-free library construction using 500 ng – 1 µg of high quality fragmented DNA. For lower DNA input (5 ng-500 ng) or libraries size selected using gel method, an additional **Amplification protocol (D)** is provided.

Table 1. DNA input recommendations for PCR-free and amplified library construction:

NGS library read length	PCR-free library			Amplified library		
	50 bp or 2 × 25bp	300 bp or 2 × 150 bp	500 bp or 2 × 250 bp	50 bp or 2 × 25bp	300 bp or 2 × 150 bp	500 bp or 2 × 250 bp
Median insert size	~ 200 bp	~ 350 bp	~ 550 bp	~ 200 bp	~ 350 bp	~ 550 bp
Recommended DNA input	500 ng*-1 µg		1 µg	5 ng-1 µg		

* 0.5 µg input DNA is sufficient to prepare PCR-free libraries of ≥ 2 nM concentration. For higher concentration PCR-free libraries, 1 µg DNA input is required, or few PCR cycles for library amplification should be used.

End Conversion of Fragmented DNA

1. Thaw the reaction components on ice, mix, and briefly centrifuge. Keep on ice.
2. Pipette all the reagents in the given order into a sterile 0.5 mL or 0.2 mL thin-wall tube.

Keep the mixture on ice. Mix the contents by vortexing (3-5 seconds) and spin down to the bottom of the tube.

Component	Volume
Water, nuclease-free	Add to 50 µL
Fragmented DNA (5 ng-1 µg)	X µL
ClaSeek End Conversion Master Mix, IL	25 µL
Total	50 µL

3. Incubate the mixture in a thermal cycler (lid temperature 100 °C) for 5 minutes at 20 °C, followed by 10 minutes incubation at 72 °C, and hold reaction at 4 °C. Proceed immediately to Sequencing Adapter Addition step.

Addition of Sequencing Adapter

1. Supplement the reaction mixture (from Step 3) with Illumina™-compatible adapters*, ClaSeek Ligation Mix, IL and water. **Keep the mixture on ice.** Mix the contents by vortexing (3-5 seconds) and spin down to the bottom of the tube.

Component	Volume
ClaSeek End Conversion reaction mixture (from Step 3)	50 µL
Adapters, Illumina compatible*	X µL (to 1 µM final conc.)
ClaSeek Ligation Mix, IL	10 µL
Water, nuclease-free	Add to 70 µL
Total	70 µL

2. Incubate the mixture at room temperature (20 °C-25 °C) for 5 minutes. Proceed to size selection or adapter removal protocol or store sample at -20 °C.

Note: Ligation time could be safely extended to up to 30 minutes without negative effect on the yield of the reaction products.



***Important Note:** Illumina™ sequencing-compatible adapters are not included in this kit and should be acquired separately. Please ensure the appropriate indexing of samples if pooled libraries will be used for sequencing.

B. NGS Library Cleanup and Size Selection

DNA libraries obtained after Sequencing Adapter Addition reaction must be purified from reaction mixture before proceeding to sequencing or amplification steps, using size selection or adapter removal procedures.

I. **Size Selection or Adapter Removal using magnetic beads (Main Protocol)**

- Libraries prepared from 50 ng to 1 µg input DNA can be size selected with MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) that allows the selection of pure sequencing-ready DNA library within the desired read-length interval. The protocol is available at www.thermoscientific.com/onebio. The protocol ensures adapter removal, therefore after size selection the DNA sample can be used directly for sequencing without an additional cleanup step. For recommended median DNA library size of various read length sequencing refer to Table 2.
- For libraries prepared from less than 50 ng DNA input (5 ng-50 ng) the adapter removal procedure using MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) is recommended.

Important Notes:

- The MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) is not provided and should be obtained separately.
- Refer to MagJET NGS Size Selection and Cleanup Kit product information for detailed protocols and magnetic particle handling instruction.
- Check the volume of ligation reaction containing the DNA library (from the Sequencing Adapter Addition protocol) before proceeding to size selection or adapter removal with MagJET and add water or TE buffer to obtain 100 µL of total DNA sample volume.
- Perform all DNA library size selection or adapter removal steps using 1.5 mL Eppendorf™ LoBind™ Tubes.
- Always perform MagJET Calibration protocol (The MagJET NGS Cleanup and Size Selection Kit, Cat. #K2821) to find the required volume of Binding mix solution prior to size selection of your DNA libraries.

Table 2. Recommended DNA library size for various read length sequencing on Illumina platform instruments:

Median Insert Size	Median Library Size	Target Read Length
~ 200 bp	320 bp	2 × 25 or 50-base read
~ 350 bp	470 bp	2 × 150 or 300-base read
~ 550 bp	670 bp	2 × 250 or 500-base read

II. Agarose Gel-based Size Selection Protocol

This protocol provides recommendations for DNA library preparation from 0.5-1 µg of sample DNA using an alternative agarose gel-based size selection protocol. PCR amplification of gel size-selected DNA libraries is recommended.

	<p>Library Cleanup before gel size selection</p> <p>a) Purify an adapter ligated DNA library using the MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) (Cleanup protocol, A) or the GeneJET NGS Cleanup Kit (Cat. #K0851), (Cleanup protocol). Elute sample in 25 µL of Elution Buffer.</p>
	<p>Agarose gel set-up</p> <p>b) Prepare 150 mL 2% agarose gel in 1x TAE, prestained using SybrGold™*: add 3 g of agarose powder to 150 mL of 1x TAE buffer, microwave the gel solution until agarose powder fully dissolves, let cool for 5-7 minutes, add 15 µL SybrGold™ to the 150 mL solution and mix well by swirling (the dilution factor of SybrGold™ into agarose/TAE should be 10,000-fold). Pour the entire gel mix into the gel tray insert a 12 or 16 well comb and let the gel set.</p> <p>Recommended dimensions for the electrophoresis unit: 12 cm × 14 cm (W × L), 800 mL buffer volume.</p> <p>*Note: It is important to pre-stain the gel with SybrGold™. Using other staining dyes or staining the gel after running will result in slower DNA migration in comparison to the ladder. This will result in size-selection of incorrect DNA library fragment size.</p>
	<p>c) Fill the electrophoresis unit with 1x TAE Buffer to the maximum fill mark.</p>
	<p>d) Add 5 µL of 6x DNA Loading Dye to each DNA library sample of 25 µL and mix.</p>
	<p>e) Add 15 µL Water, nuclease-free, and 5 µL of 6x DNA Loading Dye to 10 µL of Thermo Scientific™ O'RangeRuler™ 50 bp DNA ladder (Cat. #SM0613) and mix.</p>
	<p>Loading sample</p> <p>f) Load 15 µL of ladder into the first well of the gel. Leave a gap of one empty well and load a library sample (2 × 15 µL) into two adjacent wells of the gel, 15 µL each. Correspondingly, leave a gap of one empty well before loading another library sample or a ladder.</p> <p>Note: Flanking the library on both sides with the ladder can make the library excision easier.</p>

Run the gel at 120 V constant voltage for ~ 120 minutes. View the gel on a UV transilluminator.

Using a clean scalpel excise a band from the gel spanning the width of two wells and ranging in size from 300-400 bp, 450-550 bp or 650-750 bp (depending on the desired library insert length).

g)

Size Selection Options:

Median insert size	~ 200 bp	~ 350 bp	~ 550bp
Target slice location on the gel	300-400 bp	450-550 bp	650-750 bp

Gel-purification

h)

Extract the size-selected DNA library from the gel using Thermo Scientific GeneJET Gel Extraction and DNA Cleanup Micro Kit (Cat. #K0831/2). Please note that one column is designed for DNA purification from ~ 200 mg gel slice. If the gel slice is larger than 200 mg, split it across two column purifications according to the protocol recommendations. Elute the DNA from each column with 11 μ L Elution Buffer, then combine elutions for a total of 20 μ L. If gel slice is \leq 200 mg, proceed with one column purification and elute sample in 22 μ L of Elution Buffer.

C. Optional DNA Library Amplification Protocol

This protocol provides instructions for amplification (enrichment) of adapter-ligated DNA libraries generated from 5 ng to 1 µg of starting DNA sample using Thermo Scientific™ Phusion™ Hot Start II High-fidelity DNA polymerase (Cat. #F-549S)*.

PCR amplification of adapter-ligated DNA

1. Add 10** µL of purified adapter-ligated DNA library into a clean thin-wall 0.2 mL tube. Add the following reagents in the given order. Mix the contents by vortexing (3-5 seconds) and spin down to the bottom of the tube.

Component	Volume
Water, nuclease-free	Up to 50 µL
5x Phusion HF Buffer	10 µL
dNTP mix, 2 mM each	5 µL (to 0.2 mM)
P5 PCR Primer***, 10 µM	2.5 µL (to 0.5 µM)
P7 PCR Primer***, 10 µM	2.5 µL (to 0.5 µM)
Adapter-ligated DNA	10** µL
Phusion Hot Start II High-Fidelity DNA Polymerase 2U/µL	0.5 µL
Total	50 µL



Important Note:

* Phusion Hot Start II High-Fidelity DNA Polymerase, 2U/µL (Cat. #F-549S) is **not included in this kit** and should be acquired separately.

**20 µL volume is recommended when using an adapter-ligated DNA prepared from less than 50 ng input DNA.

*** P5 and P7 PCR Primers are **not included in this kit** and should be acquired separately.

2. Perform PCR using the following cycling conditions (lid heating at 105 °C):

Temperature	Time	Cycles
98 °C	30 sec	
98 °C	10 sec	6 cycles for 1 µg of input DNA 8 cycles for 500 ng of input DNA 10 cycles for 5-50 ng of input DNA
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 minutes	
4 °C	hold	

Cleanup of amplified NGS Library

- Purify amplified DNA library using Thermo Scientific MagJET NGS Size Selection and Cleanup Kit, using **Cleanup Protocol (A)**. Elute DNA library in 22 µL Elution Buffer.
- Alternatively, use Thermo Scientific GeneJET NGS Cleanup Kit. Follow the **Cleanup Protocol**. Elute DNA library in 22 µL Elution Buffer.

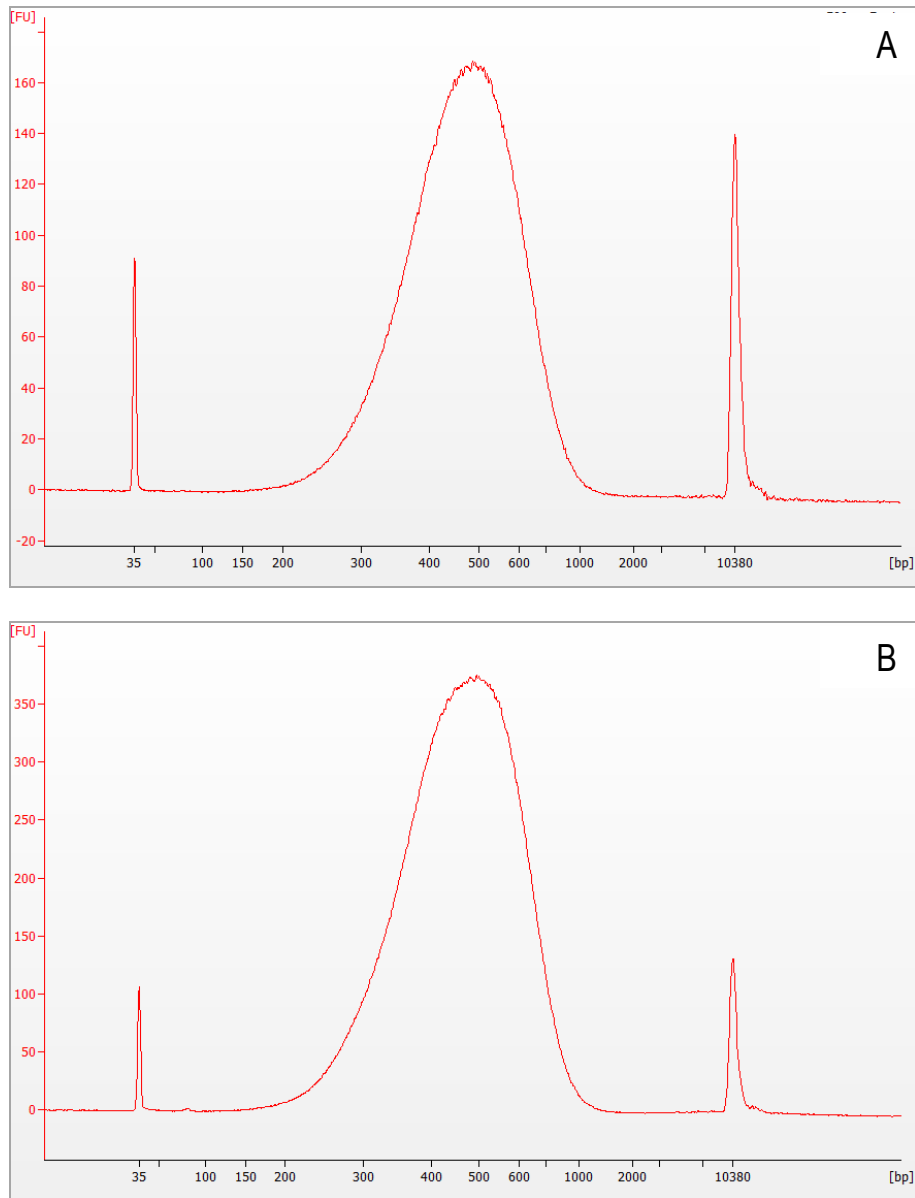


Figure 1. Relative size distribution of *E. coli* genomic DNA fragment libraries generated using ClaSeek Library Preparation Kit, Illumina™ compatible.

500 ng and 1 µg of fragmented genomic *E.coli* DNA was used for PCR-free library preparation. Size Selection of library DNA fragments for a 300 base read length sequencing was performed using MagJET NGS Cleanup and Size Selection Kit following the size selection protocol. Fragment size distribution of both libraries (undiluted samples) were analyzed on the Agilent 2100 Bioanalyzer, using the High Sensitivity DNA Kit (Agilent Technologies Inc.). **A.** DNA fragments after size selection of 500 ng input library; **B.** DNA fragments after size selection of 1 µg input library.

D. DNA Library Quantification

- It is highly recommended to perform qPCR quantification of prepared non-amplified (PCR-free) and PCR-amplified DNA libraries before proceeding to sequencing. For NGS library quantification use commercially available Illumina™ compatible quantification kits. It is recommended to dilute the DNA library sample in Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Use 10,000-fold dilution for PCR-free library quantification and 100,000-fold dilution for amplified library quantification.
- Verify the size distribution of prepared DNA library by performing analysis on Agilent 2100 Bioanalyzer instrument using High Sensitivity DNA Kit (Agilent Technologies Inc.). PCR-free DNA libraries can be analyzed undiluted. PCR-amplified libraries should be diluted 5- to 10-fold in nuclease-free water.
- The PCR-free library fragment size estimated on the Bioanalyzer is substantially larger than expected (deviation within ~ 60-100 nt). The atypical fragment migration on the chip occurs due to structural features of Illumina™ adapters, ligated to both ends of DNA fragments. If a subsequent PCR amplification step is performed, the migration of DNA library fragments becomes normal and corresponds to expected size (the median fragment size plus 120 bp of ligated 60 bp adapters at both DNA ends).

TROUBLESHOOTING

Problem	Cause and Solution												
Low DNA library yield	<p>Improperly fragmented DNA.</p> <p>DNA should be fragmented to DNA fragments of appropriate size before ligation to Illumina™ compatible adapters. The adapter ligated library is then size selected based on the selected target read length:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Median Insert Size</th> <th style="text-align: center;">Median Library Size</th> <th style="text-align: center;">Target Read Length</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">~ 200 bp</td> <td style="text-align: center;">320 bp</td> <td style="text-align: center;">50-base read library</td> </tr> <tr> <td style="text-align: center;">~ 350 bp</td> <td style="text-align: center;">470 bp</td> <td style="text-align: center;">300-base read library</td> </tr> <tr> <td style="text-align: center;">~ 550 bp</td> <td style="text-align: center;">670 bp</td> <td style="text-align: center;">500-base read library</td> </tr> </tbody> </table> <p>Optimize your DNA shearing protocol to generate the DNA fragments of correct median insert size.</p>	Median Insert Size	Median Library Size	Target Read Length	~ 200 bp	320 bp	50-base read library	~ 350 bp	470 bp	300-base read library	~ 550 bp	670 bp	500-base read library
	Median Insert Size	Median Library Size	Target Read Length										
	~ 200 bp	320 bp	50-base read library										
	~ 350 bp	470 bp	300-base read library										
	~ 550 bp	670 bp	500-base read library										
	<p>Low DNA quality.</p> <p>The quality of the input DNA has a significant impact on the yield of the resulting library. The DNA samples must be free of contaminating proteins, RNA, organic solvents and salts to ensure optimal conditions for the activity of enzymes used for library preparation. For samples with unknown DNA quality re-purification of DNA is highly recommended. High-quality DNA can be obtained using commercial DNA purification kits, such as Thermo Scientific GeneJET NGS Cleanup Kit (Cat. #K0851) or GeneJET Gel Extraction and DNA Cleanup Micro kit (Cat. #K0831/2).</p>												
	<p>Too small amount of input DNA used for PCR-free library preparation.</p> <p>Usually 500 ng of input DNA generates enough Illumina™ compatible 300-base read library to use directly for sequencing without amplification. But single stranded DNA, RNA or free nucleotides can interfere with accurate quantification of purified DNA, and if too low of an amount of dsDNA is used for the reaction, an insufficient amount of prepared library could be obtained. In such case an additional library amplification step can be performed following the prepared library cleanup with MagJET Cleanup and Size Selection Kit (Cat. #K2821).</p> <p>Refer to Table 1 on page 7 for input DNA amount recommendations for the preparation of PCR-free NGS libraries.</p> <p>For 500 base read, PCR-free library construction of 1 µg DNA input is recommended.</p>												
	<p>MagJET size selection or adapter removal protocols were not carefully followed.</p> <p>Always perform MagJET Calibration protocol to find the required volume of Binding Mix solution required prior to size selection. Before each use shake MagJET Magnetic Beads thoroughly resuspend the particles in the storage solution. Prepare fresh Binding Mix (Binding Buffer supplemented with isopropanol) before each use. Check the volume of the ligation reaction</p>												

	<p>before proceeding to the MagJET size selection, and adjust the total sample volume to exactly 100 μL using an appropriate amount of water or TE. Perform all size selection steps using 1.5 mL Eppendorf LoBind™ Tubes (Eppendorf™ Cat. #022431021). To ensure the best DNA yields do not lose any magnetic beads during the procedures and do not shorten incubation times described. Make sure to use well-calibrated pipettes.</p> <p>To maintain reproducible size selection results new pipette tips should be used for each sample when Binding Mix is added.</p>
<p>Adapter contamination</p>	<p>MagJET size selection or adapter removal protocol was not carefully followed.</p> <p>Pulse-spin the tube to collect droplets before placing the tube in the magnetic rack. Wait for 2-3 minutes or until the beads have formed a tight pellet. If the pellet of magnetic particles was disturbed while removing supernatant, mix the sample and let the beads settle to the magnet again.</p>
<p>Size selected library is outside the range of interest</p>	<p>Atypical PCR-free library fragment migration on the Agilent 2100 Bioanalyzer.</p> <p>It is normal that PCR-free library fragment sizes measured on the Bioanalyzer are substantially larger than could be expected (by about 60-100 nt). This is due to atypical migration of fragments on the chip due to the structural features of Illumina™ adapters, ligated to both ends of the DNA fragments. If a subsequent PCR amplification step is performed, the migration of the DNA library fragments corresponds to the expected size (the median fragment size plus 120 bp of ligated 60 bp adapters at both ends).</p> <p>Improper fragmentation of DNA sample.</p> <p>Make sure that your DNA fragmentation protocol generates the DNA fragments close to the correct median insert size.</p> <p>MagJET size selection protocol was not carefully followed.</p> <p>Make sure the isopropanol was properly mixed with Binding Buffer, by following the instructions for Binding Mix preparation. Only freshly-prepared Binding Mix can be used in the procedure. Using Binding Mix older than 24 hours tends to disrupt binding conditions.</p> <p>Size selection and adapter removal protocols are extremely sensitive to the volume of Binding Mix used. Always perform MagJET Calibration protocol to find the required volume of Binding Mix solution prior to size selection of DNA libraries. Make sure to add correct volumes of Binding Mix. Make sure to mix the components in the order described.</p> <p>Over-amplification of DNA library.</p> <p>Make sure to use as few amplification cycles as possible for library amplification (recommendations on page 12). Over-amplification of DNA library can lead to large chimeric molecules, which are observed as a smear of larger fragments along with normal the DNA library peak, when analyzed on Agilent 2100 Bioanalyzer.</p>

TECHNICAL SUPPORT

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Patent pending.

Designed and manufactured according to certified ISO 9001:2008 processes.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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