Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific™ ClaSeek™ Library Preparation Kit, Ion Torrent™ compatible

#K1351, #K1352

Important! Upon arrival remove inner box with reagents and store at -20 °C. Cleanup Columns must be stored at room temperature.

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Thermo Scientific ClaSeek™ Library Preparation Kit, Ion Torrent™ compatible, is qualified by constructing DNA library from 50 ng and 1 µg of dephosphorylated and 5', 3' end overhanging DNA fragment following the main PCR-free protocol outlined in the manual. The efficiency of DNA end-conversion and adapter ligation is evaluated using Agilent 2100 Bioanalyzer. The kit is functionally validated for next generation sequencing on the Ion PGM™ System and Ion Proton™ System.

Quality authorized by: Jurgita Žilinskienė

Rev.3

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COMPONENTS OF THE KIT

Component	Cap color	#K1351 10 preps	#K1352 50 preps	Important Notes
ClaSeek End Conversion Master Mix, IT	Red	250 µL	2 × 625 µL	⚠ Store at -20 °C
ClaSeek Ligation Mix, IT	Green	350 µL	2 × 875 µL	
Adapters, IT	Purple	200 µL	2 × 500 µL	
Library Amplification Primer Mix	Blue	100 µL	500 µL	
Water, nuclease free	-	1,250 µL	2 × 1,250 µL	
Cleanup Columns (pre-assembled with collection tubes)	-	10 preps	50 preps	Store at room temperature

STORAGE

Important! Upon arrival remove inner box with reagents and store at -20 °C. Cleanup Columns must be stored at room temperature. Avoid repeated freeze-thaw cycles.

DESCRIPTION

Thermo Scientific ClaSeek Library Preparation Kit, Ion Torrent™ compatible, is designed for fast and convenient construction of amplification-free NGS fragment library from DNA sample input as low as 5 ng. The ClaSeek Library Preparation kit utilizes highly efficient NGS-optimized library construction protocol combining end-repair, cleanup and adapter addition into fast and convenient workflow. Enzyme master mixes included in the kit minimize unnecessary pipetting steps and reduce hands-on time, allowing the PCR-free library construction in as little as 60 minutes. The kit is suitable for construction of 100-, 200- , 300- or 400-base read libraries for use on Ion PGM™ and 150- or 200-base read libraries for use on Ion Proton™ System.

TECHNOLOGY OVERVIEW

The Thermo Scientific ClaSeek™ library construction technology utilizes a fast and efficient NGS library construction method by combining DNA fragment end-repair and adapter-addition steps into a convenient and fast protocol. During the initial step fragmented DNA is end-repaired, followed by fast cleanup of repair enzymes by column filtration. In next step Ion Torrent-compatible NGS adapters are added to each end of the DNA fragment. Thermo Scientific ClaSeek Library Preparation Kit can be used together with MagJET NGS Cleanup and Size Selection Kit that allows for selection of pure sequencing-ready DNA library within desired read-length interval. Alternatively the kit can be used together Thermo Scientific GeneJET NGS Cleanup Kit for cleanup prior to size selection on an agarose gel using E-Gel® iBase™ (Life Technologies, Inc.) or Pippin Prep™ (Life Technologies, Inc.) instruments. An additional high-fidelity amplification step is available for DNA library construction for sequencing on Ion Proton™ System or pooling barcoded libraries generated from low input DNA.

IMPORTANT NOTES

Input DNA requirements and general recommendations:

- The PCR-free library construction protocol is designed to be used with 5 ng-1 µg of high-quality fragmented genomic 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).
- 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. The best DNA quality is achieved after sample purification using commercial DNA purification kits, such as Thermo Scientific GeneJET™ NGS Cleanup Kit (Cat. #K0851).
- Use good laboratory practices to minimize cross-contamination of products. Where possible, perform library construction in a separate area or room.
- Perform all MagJET size selection steps using 1.5 mL Eppendorf™ LoBind™ Tubes (Eppendorf, Cat. #022431021).
- Thaw frozen reagents on ice before use, and keep them on ice until ready to use. Mix reagents thoroughly before use by vortexing or flicking the tube, especially if frozen and thawed. Minimize the time outside of -20 °C for the ClaSeek End Conversion Master Mix, IT and ClaSeek Ligation Mix, IT.

Additional required reagents not provided with the kit:

- Enzymatic or physical DNA shearing method
- 0.2 mL and 0.5 mL thin-wall PCR tubes or plates
- LoBind™ Tubes 1.5 mL (Cat. #022431021, Eppendorf)
- Pipette tips and pipettes
- Microcentrifuge
- Thermal cycler
- Vortex mixer
- Real-time Thermocycler
- Agilent® 2100 Bioanalyzer® instrument or comparable method to assess the quality of DNA library
- Ion Library Quantification Kit (Cat. #4468802, Life Technologies, Inc.) or comparable kit for quantification of unamplified libraries
- Optional: Thermo Scientific™ MagJET™ NGS Cleanup and Size Selection Kit (Cat. #K2821) for size selection
- Optional: Thermo Scientific™ GeneJET™ NGS Cleanup Kit (Cat. #K0851) for cleanup of fragmented DNA
- Optional: Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase (Cat. # F-549S) for amplification of library prep
- Optional: E-Gel® iBase™ (Life Technologies, Inc.) or Pippin Prep™ (Life Technologies, Inc.) instruments for size selection of library fragments on agarose gels

PREPARATION OF BARCODED LIBRARIES

- ⚠ **Important!** For multiplexing several DNA libraries in one run you should substitute ClaSeek Adapters provided in the kit with barcoded Ion Xpress™ adapters: 10 µL of Ion Xpress™ P1 Adapter and 10 µL of chosen Ion Xpress™ Barcode X (1 barcode adapter per library).

PROTOCOL

A. End Repair and Adapter Addition Protocol

Note: This protocol provides instructions for amplification-free library construction using 5 ng-1 µg of high quality fragmented DNA which is sufficient for sequencing on the Ion PGM™. Additional **Amplification protocol** (**Protocol D**) is provided for library preparation and sequencing on the Ion Proton™ using low DNA input (< 100 ng), or pooling barcoded libraries prepared using low DNA input.

End conversion of fragmented DNA

1. Pipet all the reagents in the given order into a 0.5 mL thin-wall tube. **Keep the mix on ice**.

Component	Volume
Nuclease-free water	Add to 50 µL
Fragmented DNA (5 ng-1 µg)	X µL
ClaSeek End Conversion Master Mix	25 µL
Total	**50 µL**

2. Mix the contents thoroughly by vortexing briefly (3-5 seconds) and spin down to the bottom of the tube.
3. Incubate in a thermal cycler for 5 minutes at 20 °C. Return the tube into ice and proceed immediately to the cleanup step.

Cleanup after end conversion reaction

1. Transfer the End Conversion reaction mix to a supplied Cleanup Column. Centrifuge for 2 minutes at 14,000 × g at room temperature.
2. Carefully collect and transfer 45 µL of **flow-through** into a new 0.2 mL thin-wall tube. Return the tube into ice and proceed immediately to the Adapter addition step.

- **Note:** Add reaction mix directly to the center of the membrane avoiding touching column walls. A flow-through volume used in subsequent adapter addition step should not be less than 40 µL. Scaling up missing flow-through volume is unnecessary.

Addition of Sequencing Adapter

1. Pipet all the reagents in the given order into the purified End Conversion reaction mix (45 µL). **Keep the mixture on ice.** Mix the contents by vortexing (3-5 seconds) and spin down to the bottom of the tube.

Component	**Volume**
Column-filtrated End Conversion reaction mix	45 µL
Adapters*	20 µL
ClaSeek Ligation Mix	35 µL
Total	**100 µL**

⚠ *** Important Note:** For multiplexing several DNA libraries in one run you should substitute ClaSeek Adapters provided in the kit with barcoded Ion Xpress™ adapters: 10 µL of Ion Xpress™ P1 Adapter and 10 µL of chosen Ion Xpress™ Barcode X (1 barcode adapter per library). Ion Xpress™ P1 and Barcode Adapters are not supplied and must be acquired separately (Life Technologies, Inc.).

2. Incubate in thermal cycler for 5 minutes at 20 °C, 5 minutes at 60 °C and hold at 4 °C with the heated lid at 60 °C. Proceed immediately to size selection/cleanup protocol or store sample at -20 °C.

B. NGS Library Cleanup and Size Selection Protocols

I. Size Selection using magnetic beads (Main Protocol)

Library prep generated using Thermo Scientific ClaSeek™ Library Preparation Kit can be size selected with MagJET NGS Cleanup and Size Selection kit (Cat. #K2821) that enables selection of pure sequencing-ready DNA library within desired read-length interval. This protocol is available at www.thermoscientfic.com/onebio. The protocol ensures adapter removal, therefore after size selection the DNA sample can be used for direct sequencing without an additional cleanup step.

Important Notes:

- Always **perform MagJET Calibration protocol** to find the required volume of Binding Mix solution prior the size selection of your DNA libraries. **We strongly recommend using fragmented DNA** and Agilent 2100 Bioanalyzer for the Calibration protocol.
- Make sure the isopropanol **was properly mixed** with Binding Buffer before proceeding. Follow the instructions for Binding Mix preparation.
- Make sure to use **well-calibrated pipettes**.
- Only **freshly prepared Binding Mix** can be used in the procedure. Using older than 24 hours Binding Mix tends to disrupt binding conditions and to form precipitates.
- **Check the volume** of ligation reaction containing the DNA library (from the protocol A) before proceeding to size selection with MagJET protocol. **Add water or TE buffer to 100** µL if the DNA sample volume is less than 100 µL.
- Perform all MagJET size selection steps using 1.5 mL Eppendorf™ LoBind™ Tubes.
- To ensure the best DNA yields **do not** lose any magnetic beads during the procedures and **do not** shorten incubation times.
- Elute the DNA using 30 µL of Elution Buffer. Store the size selected library prep at -20 °C. Save 3 µL of the final sequencing library for fragment size distribution analysis by Agilent 2100 Bioanalyzer and DNA library quantification using qPCR.

II. Alternative Agarose Gel-based Size Selection Protocol

- The following protocol requires cleanup step using Thermo Scientific™ GeneJET™ NGS Cleanup Kit (Cat. #K0851) before size selection on agarose gel using E-Gel® iBase™ (Life technologies, Inc.), Pippin Prep™ (Life Technologies, Inc.) instruments or other comparable system.
- **The cleanup protocol** (A) using Thermo Scientific™ GeneJET™ NGS Cleanup Kit is available at www.thermoscientific.com/onebio.

Important Notes:

- **Add the indicated volume of ethanol** (96-100%) to the Pre-wash Buffer (concentrated) and Wash Buffer (concentrated) prior to the first use.
- **Check all solutions** in the kit for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37 °C, and then equilibrate to room temperature (15-25 °C).
- Elute the DNA library in 20 µL of **Elution Buffer.** Purified DNA can be stored at -20 °C.
- Proceed with the size selection on agarose gel using **E-Gel® iBase™ (Life Technologies, Inc.) or Pippin Prep™ (Life Technologies, Inc.) instruments.** Instructions for Size-selection can be found at the **web catalogue pages** at www.lifetechnologies.com and www.sagescience.com. Select the adapter ligated DNA in the proper bp range: 200 bp, 330 bp, 390 bp and 480 bp for 100-, 200-, 300- or 400-base read libraries for use on Ion PGM™ and 220 bp, 270 bp for 150- or 200-base read libraries for use on Ion Proton™ System.

C. DNA Library Qualification

Notes:

- We recommend performing fragment size distribution analysis using Agilent 2100 Bioanalyzer instrument and High Sensitivity DNA Kit (Agilent Technologies, Inc.). Before starting, dilute the samples. MagJET size-selected libraries generated from high DNA input (1 µg) should be diluted 10-fold. Analyze low input (≤ 100 ng) DNA libraries undiluted. Although the final concentration of low input (5-10 ng) libraries is outside quantitative range of the instrument it is recommend to analyze the samples to make sure the samples are not contaminated with adapters. Samples size selected using an agarose gel should be analyzed undiluted.

- Quantify the library using Ion Library Quantification Kit (Life Technologies, Inc.) to determine the template dilution factor (TDF). 20,000-fold dilution is recommended for library preps generated from 25 ng-1 µg DNA input (MagJET size selected) and 1 µg DNA input (agarose gel size-selected). For MagJET size-selected library preps from < 25 ng and agarose gel size selected library preps from 100 ng use 2000-fold dilution.

- In general, 5 ng-1 µg and 100 ng-1 µg MagJET size selected libraries do not require amplification for sequencing on Ion PGM™ and Ion Proton™, respectively. Proceed to amplification if the yield of the unamplified library is not sufficient for your experimental requirements (protocol D).

D. Optional DNA Library Amplification Protocol

- **Note:** This protocol provides instructions for amplification (enrichment) of libraries generated from 5 ng-1 µg of starting DNA sample amount.

PCR amplification of adapter-ligated DNA

1. Add 20 µL of size-selected adapter-ligated DNA library from **protocol B** into an empty thin-wall 0.2 mL tube. Add following reagents in given order. Mix the contents by vortexing (3-5 seconds) and spin down to the bottom of the tube.

Component	Volume
Adapter-ligated DNA	20 µL
Water, nuclease-free	47 µL
5x Phusion HF Buffer	20 µL
dNTP 10 mM	2 µL
Library Amplification Primer mix	10 µL
Phusion Hot Start II High-Fidelity DNA Polymerase 2U/µL	1 µL
Total	**100 µL**

⚠ **Acquire separately the following Thermo Scientific products:** Phusion Hot Start II High-Fidelity DNA Polymerase, 2U/µL (Cat. #F-549S). dNTP Mix, 10 mM each (Cat. #R0191).

2. Aliquot the reaction mixture into 2 separate thin-wall 0.2 mL tubes (for some PCR instruments it is recommended to amplify DNA in reaction volume not higher than 50 µL).

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

Temperature	Time	Cycles
98 °C	30 sec	
98 °C	20 sec	4 cycles for 100 ng-1 µg of input DNA 8 cycles for input DNA below 100 ng
60 °C	20 sec	
72 °C	40 sec	
72 °C	60 sec	
4 °C	hold	

Cleanup of amplified Library

- Purify amplified DNA library using MagJET NGS Size Selection and Cleanup Kit (Cat. #K2821), **Cleanup Protocol** at www.thermoscientific.com/onebio. Elute DNA library in 30 µL Elution Buffer.
- Alternatively use Thermo Scientific™ GeneJET™ NGS Cleanup Kit (Cat. #K0851), **Cleanup Protocol (A)** at www.thermoscientific.com/onebio. Elute DNA library in 30 µL Elution Buffer.

Qualification of amplified library prep

- Estimate the quality of prepared DNA library by analysis on Agilent™ 2100 Bioanalyzer® and perform library quantification by qPCR.

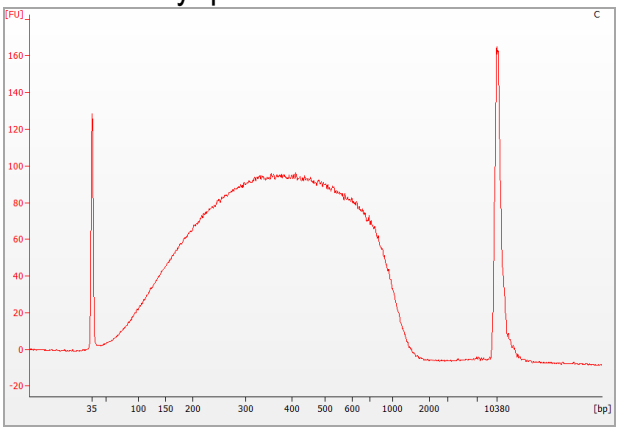

Figure 1. Relative size distribution of fragmented *E. coli* genomic DNA.
Fragment size distribution was analyzed by the Agilent 2100 Bioanalyzer instrument using the High Sensitivity DNA Kit (Agilent Technologies, Inc.); 5 ng of fragmented gDNA was used for analysis.

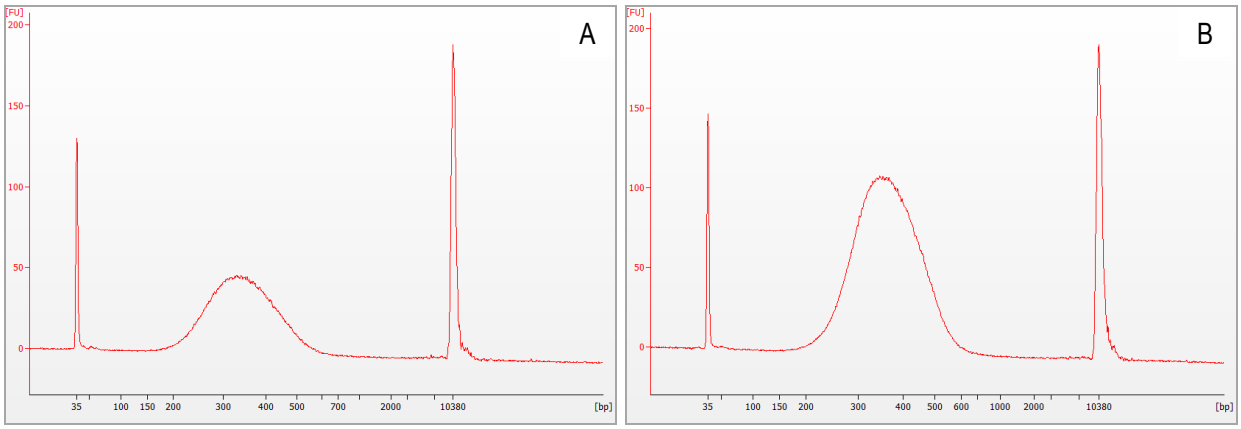

Figure 2. Relative size distribution of *E. coli* genomic DNA fragment libraries generated using Thermo Scientific™ ClaSeek™ Library Preparation Kit.
100 ng and 1 µg of fragmented DNA samples (*see* Figure 1) were used for library preparation. MagJET Size Selection protocols were used for size selection of DNA fragments with the median library size of 330 bp. Fragment size distribution was analyzed by the Agilent 2100 Bioanalyzer instrument using the High Sensitivity DNA Kit (Agilent Technologies, Inc.). **A.** DNA fragments (non-diluted sample) after size selection of 100 ng input library. **B.** DNA fragments (10-fold diluted sample) after size selection of 1 µg input library.

TROUBLESHOOTING

Problem	Cause and Solution
Low yield	**Improper DNA fragmentation.** Genomic DNA must be fragmented to appropriately sized DNA fragments which are ligated to Ion-compatible adapters. The adapter ligated library is size-selected for optimum length according to target read length:

Median Insert Size	Median library Size	Target Read Length	Ion Torrent™ System
130 bp	200 bp	100-base read library	PGM
260 bp	330 bp	200-base read library	PGM
320 bp	390 bp	300-base read library	PGM
410 bp	480 bp	400-base read library	PGM
150 bp	220 bp	150-base read library	Proton
200 bp	270 bp	200-base read library	Proton

Optimize your DNA shearing protocol to generate the DNA fragments close to the correct median insert size.

Impure DNA.

The quality of the input DNA has a significant impact on the quality of the resulting library. 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. The high-quality DNA quality can be purified using commercial DNA purification kits, such as Thermo Scientific GeneJET NGS Cleanup Kit (Cat. #K0851).

DNA loss during cleanup step.

Add reaction mix directly to the center of the membrane avoiding touching the side of the column. Avoid splashing of the reaction mixture.

MagJET size selection protocol was not carefully followed.

Before each use shake MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution. Prepare fresh Binding Mix (Binding Buffer supplemented with isopropanol) before each use. Check the volume of ligation reaction before proceeding to MagJET size selection. 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.

Low DNA input.

The final concentration of low input (5-10 ng) libraries is outside quantitative range of the Agilent 2100 Bioanalyzer. Proceed with quantification by

	qPCR. Verify the concentration of your input fragmented DNA and increase your input DNA amount or proceed to amplification if the yield of the unamplified library is not sufficient for your experimental requirements. Amplification of DNA library with Thermo Scientific™ Phusion™ polymerase will increase the TDF 5- to 16-fold (4 cycles) or 100- to 250-fold (8 cycles).
High adapter or primer contamination	**MagJET size selection protocol was not carefully followed.** 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 to settle to the magnet again. To maintain repetitive size selection results new pipette tips should be used for each sample when Binding Mix is added. Note: PCR library preps with the TDF (1.4-2.7) containing 0.7-2 nM adapters were successfully sequenced on the PGM™**.** **Agarose gel size selection protocol was not carefully followed.** Do not overload the wells when performing the size selection of 1 µg DNA library. **MagJET cleanup protocol was not carefully followed.** If short DNA fragments (40-45 bp) are observed in PCR amplified DNA library prep after MagJET cleanup use MagJET adapter removal protocol.
Size selected library is outside the range of interest	**Improper fragmented DNA library.** Make sure that your DNA fragmentation protocol generates the DNA fragments close to the correct median insert size. **MagJET size selection protocol was not carefully followed.** Always perform MagJET Calibration protocol to find the required volume of Binding mix solution prior the size selection of your DNA libraries. Make sure that the isopropanol was properly mixed with Binding Buffer before procedures. Follow the instructions for Binding Mix preparation. Only fresh-prepared Binding mix can be used in the procedure. Using older than 24 hours Binding Mix tends to disrupt binding conditions and to form precipitate. Size selection protocol is extremely sensitive to the volume of Binding Mix used. Make sure to use well-calibrated pipettes. **Agarose gel size selection protocol was not followed properly**. We recommend E-Gel SizeSelectn and Pippin Prepd users precisely follow the manuals of instruments. **High DNA input.** The peak of size selected DNA can be followed by smear of smaller or larger DNA fragments if too high DNA input was used. Reduce the DNA input.

TECHNICAL SUPPORT

Web site: www.thermoscientific.com
Support email in North America: ts.molbio@thermofisher.com
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This product is patent pending with rights co-owned by K.K. DNAFORM and RIKEN, The Institute of Physical and Chemical Research.

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.