Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific MuSeek Library Preparation Kit, Illumina compatible

#K1361

12 rxns

Upon arrival store at -20 °C.
The Stop Solution can be stored at room temperature.

For indexed DNA fragment library generation use with:
Thermo Scientific™ MuSeek™ Index Set 1, Illumina™ compatible (Cat. No. #K1551)

www.thermoscientific.com/onebio

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Rev.1

COMPONENTS OF THE KIT

Component	Cap color	Amount	Important Notes
MuSeek Enzyme Mix, IL	Red	20 µL	Store at -20 °C. Avoid repeated freeze-thaw cycles.
MuSeek Fragmentation Reaction Buffer	Yellow	120 µL	
MuSeek Stop Solution	Orange	36 µL	Contains SDS, store at room temperature.
Water, nuclease-free	White	1.2 mL	
Control DNA	White	20 µL	50 ng/µL cI857 *Sam7* Lambda DNA, size 48.5 kb.
MuSeek Adapter Addition Primer Mix, IL	Purple	120 µL	
MuSeek Adapter Addition Reaction Buffer, IL	Green	150 µL	Contains 4x reaction buffer with dNTPs.
Phusion Hot Start II High-Fidelity DNA Polymerase, IL	Blue	48 µL	Store at -20 °C.
MuSeek Sequencing Primer, Read 1	White	120 µL	Concentration 30 µM
MuSeek Sequencing Primer, Read 2	White	120 µL	
MuSeek Sequencing Primer, Index Read	White	120 µL	

STORAGE

The kit is shipped on dry ice. **Upon arrival store at -20 °C**, avoid repeated freeze-thaw cycles. The Stop Solution can be stored at room temperature.

DESCRIPTION

MuSeek Library Preparation Kit, Illumina compatible, is designed for generation of high-quality genomic DNA libraries for sequencing with the Illumina MiSeq™ and HiSeq™ systems. The fast protocol utilizes MuA transposase enzyme, which catalyzes simultaneous fragmentation of double-stranded target DNA and tagging the fragment ends with transposon DNA. In a subsequent PCR step platform-specific adapters are added using a robust and accurate Thermo Scientific™ Phusion™ High-Fidelity DNA polymerase. Starting with only 50 ng or 100 ng of the sample the protocol can be used to generate 100-1,000 bp insert libraries.

The kit contains components sufficient for 12 fragmentation and subsequent adapter-addition polymerase chain reactions. The kit also provides MuA-specific sequencing primers required for sequencing libraries prepared by this method.

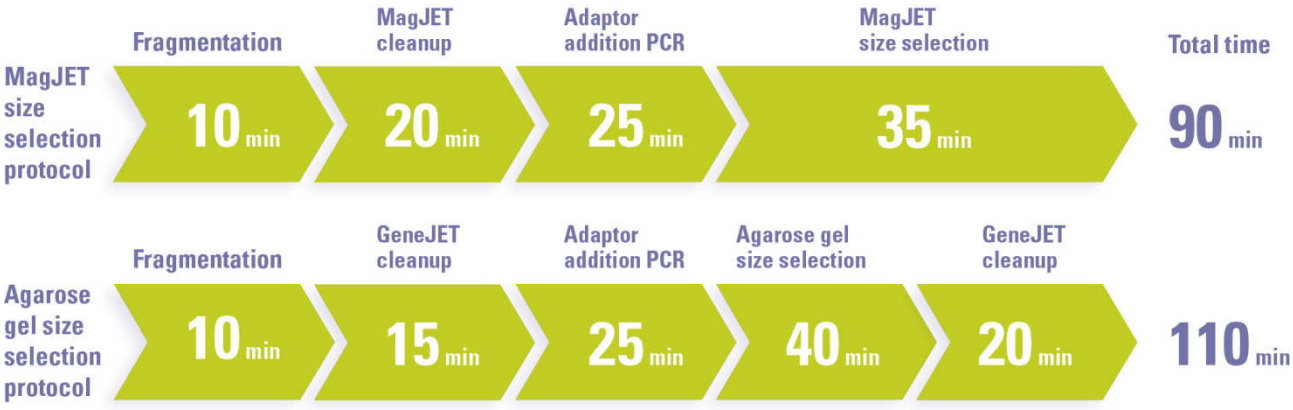

Figure 1. Workflow overview.

TECHNOLOGY OVERVIEW

MuA transposase enzyme is a 75 kDa protein originating from bacteriophage Mu, and is produced as a recombinant protein in *E. coli*. Under suitable conditions, MuA transposase forms a homotetrameric complex with two 50 bp double-stranded transposon DNA ends that contain a specific MuA binding sequence. After the fragmentation reaction, the ends of target DNA are tagged with transposon sequences. Illumina specific adapters are added to the fragments in a subsequent adapter addition reaction. First, the 3'-ends of the fragmented target DNA are elongated over the 5 nt gaps generated during the transposition reaction and further extended into transposon sequences. In the initial cycles of consecutive PCR the first 16 nt of the adapter 3'-ends hybridize to transposon sequences in tagged DNA fragments. In later PCR cycles the fragments are amplified using a pair of external primers (Figure 2).

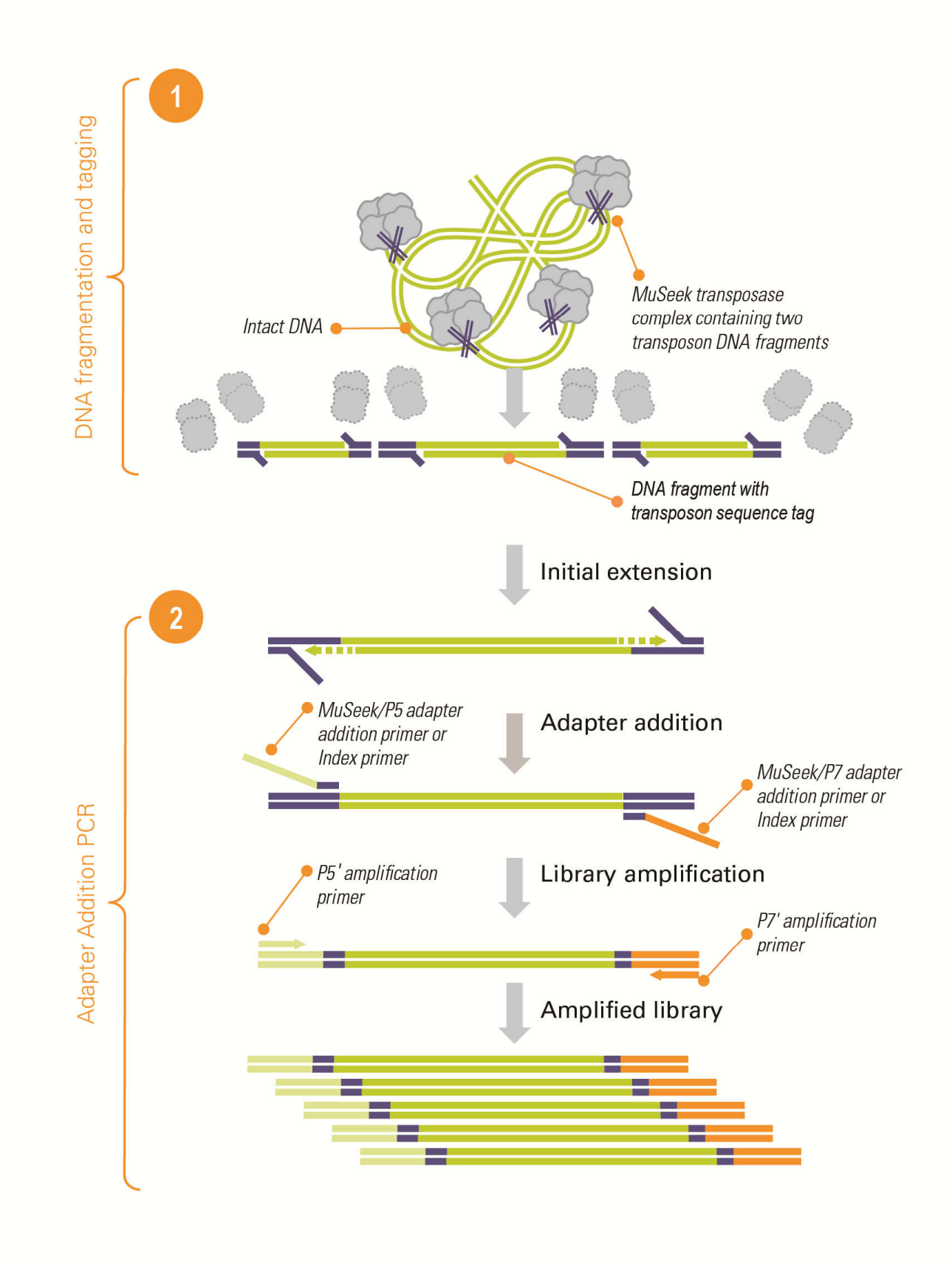

Figure 2. MuA transposase complex simultaneously fragments input DNA and tags the fragments (1). In a subsequent PCR, adapter sequences are added (2).

PREPARATION OF INDEXED LIBRARIES

The MuSeek Library Preparation Kit, Illumina compatible, is suitable for generation of indexed DNA fragment libraries when used in combination with the MuSeek Index Set 1, Illumina compatible (Cat #K1551). The MuSeek Index Set 1 provides 4 and 6 MuSeek Indices, allowing construction of 24 unique dual-indexed libraries for multiplexed sequencing analysis. A list of index sequences is provided for generating sample sheets to demultiplex the samples (see Table 2).

Table 1. Sequence structure of MuSeek indexed library:

5' [P5 sequence] **[MuSeek Index M5XX]** [Sequencing primer hybridization region]
[Target DNA] [Sequencing primer hybridization region] **[MuSeek Index M7XX]**
[P7 sequence] 3'

Table 2. MuSeek index sequences for demultiplexing:

MuSeek Index 1 (i7)	Sequence for sample sheet	MuSeek Index 2 (i5)	Sequence for sample sheet
M701	CTCTTCTC	M501	CTACATGC
M702	TCTACGCT	M502	GATAGCGT
M703	GACTGACT	M503	ACGGCTAA
M704	CGATCTTC	M504	AAGAGGAG
M705	GAGATTCG		
M706	ATCCTCGA		

Input DNA requirements and general recommendations:

- The kit is designed to be used with 50 or 100 ng of high-quality genomic DNA dissolved in nuclease-free water, 10 mM Tris-HCl, pH 7.5-8.5 or TE buffer (10 mM Tris-HCl, 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, repurification of DNA is highly recommended. The best DNA quality is achieved after sample purification using commercial DNA purification kits, like Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (Cat. #K0721, #K0722).
- For construction of NGS library from PCR amplicons in fragmentation reaction do not use PCR products shorter than 300 bp. Due to intrinsic features of the transposon technology a ~50 bp drop off is expected in sequencing coverage from each distal end of the amplicon sequence. This can be averted by designing your amplicons to be ~100 bases larger than the desired sequencing insert.
- Control DNA is provided in the kit for introductory use of the reagents prior to using own target DNA samples. Use 100 ng or 50 ng of the Control DNA supplied in the kit, and then follow the instructions described below.

- Use good laboratory practices to minimize cross-contamination of products. Where 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 -20 °C freezer for the MuSeek Enzyme Mix.
- Mix reagents thoroughly before use by vortexing or flicking the tube, especially if frozen and thawed.

Additional required reagents not provided with the kit:

- **Cleanup**: Thermo Scientific™ MagJET™ NGS Cleanup and Size Selection Kit (Cat. #K2821) or GeneJET NGS Cleanup Kit (Cat. #K0851).
- **Size selection**: MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) or agarose gel-based method.
- **For preparation of indexed NGS library, a MuSeek Index Set 1, Illumina compatible (Cat #K1551) is required**.
- **Optional**: Magnetic particle processing rack, e. g. MagJET Separation Rack (Cat. #MR01/2).
- GeneJET Gel Extraction and DNA Cleanup Micro Kit (Cat. #K0831/2).
- Thermo Scientific™ O'GeneRuler™ 1 kb DNA ladder (Cat. #SM1133) or O'GeneRuler 1 kb Plus DNA Ladder (Cat. #SM1343).
- 6X Orange DNA Loading Dye (Cat: # R0631).

IMPORTANT NOTES

 IMPORTANT REMINDER: USE ONLY THE MUSEEK SEQUENCING PRIMERS PROVIDED WITH THIS KIT! NO SEQUENCE DATA WILL BE GENERATED IF INCORRECT PRIMERS ARE USED, AS THE PRIMER BINDING SITES ARE DIFFERENT FROM ORIGINAL ILLUMINA SEQUENCING PRIMERS.

PROTOCOL

A. Fragmentation reaction

Note: Always keep the MuSeek Enzyme Mix on ice when preparing the reactions and minimize handling times outside of the -20 °C freezer. It is important to measure sample DNA concentration before the fragmentation reaction. Use 50 ng or 100 ng of high-quality gDNA dissolved in nuclease-free water, 10 mM Tris-HCl, pH 7.5-8.5 or TE buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA). Deviation from recommended DNA concentration will influence size and yield of fragmented DNA.

1. Pipet all the reagents, **except for MuSeek Enzyme Mix**, in the given order into a 1.5 mL thin-wall tube. **Keep the mixture on ice**. Mix the contents thoroughly by vortexing briefly (3-5 seconds) and spin down to the bottom of the tube.

a) For 100 ng of genomic DNA

Component	Volume
Water, nuclease-free	Add to 30.0 µL
MuSeek Fragmentation Reaction Buffer	10.0 µL
gDNA	X µL (100 ng)
MuSeek Enzyme Mix	1.5 µL
Total	**30.0 µL**

b) For 50 ng of genomic DNA

Component	Volume
Water, nuclease-free	Add to 30.0 µL
MuSeek Fragmentation Reaction Buffer	10.0 µL
gDNA	X µL (50 ng)
MuSeek Enzyme Mix	0.7 µL
Total	**30.0µL**

2. **Note: Important step! For successful DNA fragmentation avoid foaming of the reaction mixture.** Before proceeding start the vortex at maximum speed.
   Add MuSeek Enzyme Mix to the other reaction components and mix by briefly touching the vortex five times, for 1 second each time, spin-down briefly. Place the tube **immediately** at 30 °C for 5 minutes incubation. It is recommended to incubate the sample in water bath for faster heat transfer. Return the MuSeek Enzyme Mix to the below -20 °C freezer as soon as possible.

3. Stop the reaction after 5 minutes by adding 3 µL of MuSeek Stop Solution and vortexing briefly.
   Note: Make sure that Stop Solution is clear and no precipitates are seen. If present dissolve precipitates by warming Stop solution at 30 °C**.**
4. After vortexing, keep the tubes at room temperature**.** Do not put reaction tubes on ice, as the Stop Solution may cause precipitation.
   Note: The total volume of fragmented sample following addition of the Stop solution is 33 µL.

B. Fragmented DNA cleanup

- Purify fragmented DNA library using MagJET NGS Size Selection and Cleanup Kit, using **Cleanup Protocol (A)**. Make sure that all Washing Buffer is fully evaporated from magnetic particles before proceeding to elution step. Elute DNA library in **28 µL Elution Buffer**.
- Alternatively, use GeneJET NGS Cleanup Kit. Follow the **Protocol A**. **Cleanup Protocol**. Don't forget to centrifuge the empty DNA Purification Micro Column for additional 2 minutes at 14,000 × g to completely remove residual Wash Buffer. Elute DNA library in **28 µL Elution Buffer.**
- Keep 1 µL of the fragmented DNA for size distribution and concentration analysis on the Agilent 2100 Bioanalyzer.

C. Sequencing adapter addition

For a **non-indexed library:**

1. Take 23.5 µL of the purified fragmented DNA from the purification step described above and add to a reaction mixture at room temperature in 0.2 mL tube:

Component	**Volume**
MuSeek Adapter Addition Reaction Buffer	12.5 µL
MuSeek Adapter Addition Primer Mix	10 µL
Purified fragmented DNA	23.5 µL
Phusion Hot Start II High-Fidelity DNA Polymerase	4 µL
Total	**50 µL**

For **indexed** library:

1. Take 23.5 µL of the purified fragmented DNA from the purification step described above and add to a reaction mixture at room temperature in 0.2 mL tube:

Component	**Volume**
MuSeek Adapter Addition Reaction Buffer	12.5 µL
MuSeek Index Mix M5XX	5 µL
MuSeek Index Mix M7XX	5 µL
Purified fragmented DNA	23.5 µL
Phusion Hot Start II High-Fidelity DNA Polymerase, IL	4 µL
Total	**50 µL**

2. Perform temperature cycling of using the following cycling conditions:

Temperature	**Time**	**Cycles**
66 °C	3 min	1
98 °C	30 sec	
98 °C	10 sec	6
59 °C	40 sec	
72 °C	30 sec	
72 °C	1 min	1
4 °C	hold	

D. Size selection and library quantification

1. Size selecting correct DNA library fragment length

Sequencing-ready libraries prepared using MuSeek Library Preparation Kit, Illumina compatible, contain DNA fragments ranging within 150-1,500 bp. Each library fragment contains 64 bp and 62 bp length adapter sequences at both ends of the DNA molecule. When size-selecting DNA library fragments of a relevant sequencing read length, the length of adapter sequences must be taken into account. For size-selecting correct DNA library fragment peak refer to **Table 3**.

2. Size selection recommendations

a) Size Selection using MagJET magnetic beads

DNA libraries prepared from 50 ng or 100 ng input DNA can be size selected using the MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821/2) following size selection recommendations provided in the product manual. The protocol is available at www.thermoscientific.com/onebio. After size selection the DNA sample can be used directly for sequencing without an additional cleanup step. For recommended DNA fragment library size selection ranges refer to Table 3. Elute the DNA from each tube with 15 µL Elution Buffer. 1 µL of eluate should be left for analysis on Bioanalyzer.

Important Notes:

- The MagJET NGS Cleanup and Size Selection Kit (Cat. #K2821) is not provided and should be obtained separately.
- Always perform MagJET Calibration protocol (The MagJET NGS Cleanup and Size Selection Kit, Cat. #K2821) to identify the required volume of Binding Mix prior to size selection of your DNA libraries.

Table 3. Target DNA library fragment size for size selection procedure:

Sequencing platform	Selected Read Chemistry	Average Library length	Recommended size selection range
MiSeq	50-cycle	**~ 300 bp**	250-350 bp
	150-cycle	**~ 400 bp**	350-450 bp
	300-cycle	**~ 540 bp**	480-600 bp
	500-cycle	**~ 765 bp**	680-850 bp
	600-cycle	**~ 865 bp**	780-950 bp
HiSeq 2500	50-cycle	**~ 300 bp**	250-350 bp
	200-cycle	**~ 440 bp**	380-500 bp

b) Agarose gel-based size selection protocol

This protocol provides recommendations for DNA library preparation from 50 ng or 100 ng of sample DNA using agarose gel-based size selection protocol. No additional cleanup step of amplified 50 µL DNA library mix (Step C, Sequencing adapter addition) is required prior to loading to agarose gel.

Agarose gel set-up

Prepare 200 mL 1 % agarose gel in 1X TAE buffer, prestained using preferred dye (e. g. ethidium bromide or GelRed™):

a) Add 2 g of agarose powder to 200 mL of 1X TAE buffer, microwave the gel solution until agarose powder fully dissolves, let cool for 5-7 minutes, and add dye to the 200 mL solution and mix well by swirling. For the best results use high quality agarose powder, e.g., Thermo Scientific™ TopVision™ Agarose (Cat. #R0491/2)

b) Add the entire gel mix into a gel tray, insert a 12 or 16 well comb and let the gel set. Recommended gel dimensions to fit the entire sample volume into one well: 9 cm × 17 cm (W × L), gel thickness ~1.2 cm, well width ~0.8 cm.

c) Fill the electrophoresis unit with 1X TAE buffer to the maximum fill mark and load the gel.

Important notes:

- It is not recommended to use agarose gel prestained with SybrGold, as SybrGold impairs DNA fragment library migration in precast gels. Perform gel staining after DNA electrophoresis. Stain the gel mildly shaking it for 30 minutes in a 1X SybrGold solution in 1X TAE. The staining solution can be reused 2-3 times and should be stored at 4°C protected from the light.
- With GelRed both precast gels or staining after the electrophoresis can be used. When staining after the gel electrophoresis, shake the gel mildly 30 minutes in a 3X GelRed solution in water. The staining solution can be reused 2-3 times and should be stored at room temperature protected from the light.
- Gels can be stained using 0.5 µg/mL ethidium bromide solution in water or 1X TAE buffer. Submerge the gel and mildly shake for 30 minutes. Rinse the gel briefly with deionized water before viewing under UV. The staining solution can be reused several times and should be stored at room temperature protected from the light.

Loading samples

a) Add 10 µL of 6X Orange DNA Loading Dye (Cat: # R0631) to 50 µL of your sample and mix.
b) Load 5 µL of O'GeneRuler 1 kb (Cat. #SM1133) or O'GeneRuler 1 kb Plus DNA Ladder (Cat. #SM1343) into the first well of the gel. Leave a gap of one empty well and load **Adapter Addition reaction mix from Step C containing DNA library premixed with loading dye into**. Correspondingly, leave a gap of one empty well before loading another library sample or a ladder.
c) Run the gel at 6 V/cm voltage for ~ 30-45 minutes. View the gel on a UV transilluminator.

Notes:

- It is recommended to load the whole DNA library sample into one well of the gel. If the sample does not fit into one well, use no more than 2 wells for one DNA library sample.
- Flanking the library on both sides with the ladder can make the library excision easier.
- Load the DNA library sample directly after adapter addition reaction, no additional cleanup of adapter addition reaction mix is required.

Size selection

Excise the target DNA band from the gel using a clean scalpel. Target the recommended DNA fragment length corresponding to selected read chemistry (**Table 3**). DNA band excision interval should span 100 bp – 180 bp in length, depending on target region (there are fewer DNA molecules of higher fragment length in the same size of the gel slice as compared to shorter fragment range).

Notes:

- Minimize exposure of the gel to UV.
- Save the gel after band excision for additional excision in case incorrect library size was selected.

Recommended DNA library size selection ranges for use with Illumina™ MiSeq™ v2 (1-3), MiSeq™ v3 (4-5) and HiSeq™ 2500 (6-7) reagent chemistries. M – O'GeneRuler 1 kb Plus DNA Ladder (Cat. #SM1343). 1% agarose gel, prestained with ethidium bromide.

Gel-purification

Extract the size-selected DNA library from the gel using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Cat. #K0831/2). 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 the column with 15 µL Elution Buffer. Keep 1 µL of eluate for analysis on Agilent Bioanalyzer™.

Notes:

- If two columns are used to purify one DNA library sample, elute DNA in each column using 8 µL Elution Buffer and pool into one sample. Keep 1 µL of pooled eluate for analysis on Agilent Bioanalyzer™.

3. Evaluation of prepared sequencing library

Verify the size distribution of prepared DNA library by performing analysis on the Agilent 2100 Bioanalyzer instrument using High Sensitivity DNA Kit (Agilent Technologies Inc.). Analyzed DNA library samples after size selection should have a 2-fold dilution in nuclease-free water. Typical example of an analyzed library is demonstrated in **Figure 4**.

It is highly recommended to perform qPCR quantification of prepared 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 8.0 with 0.05% Tween 20. Use 10,000-fold dilution for PCR-free library quantification and 1,000,000-fold dilution for amplified library quantification.

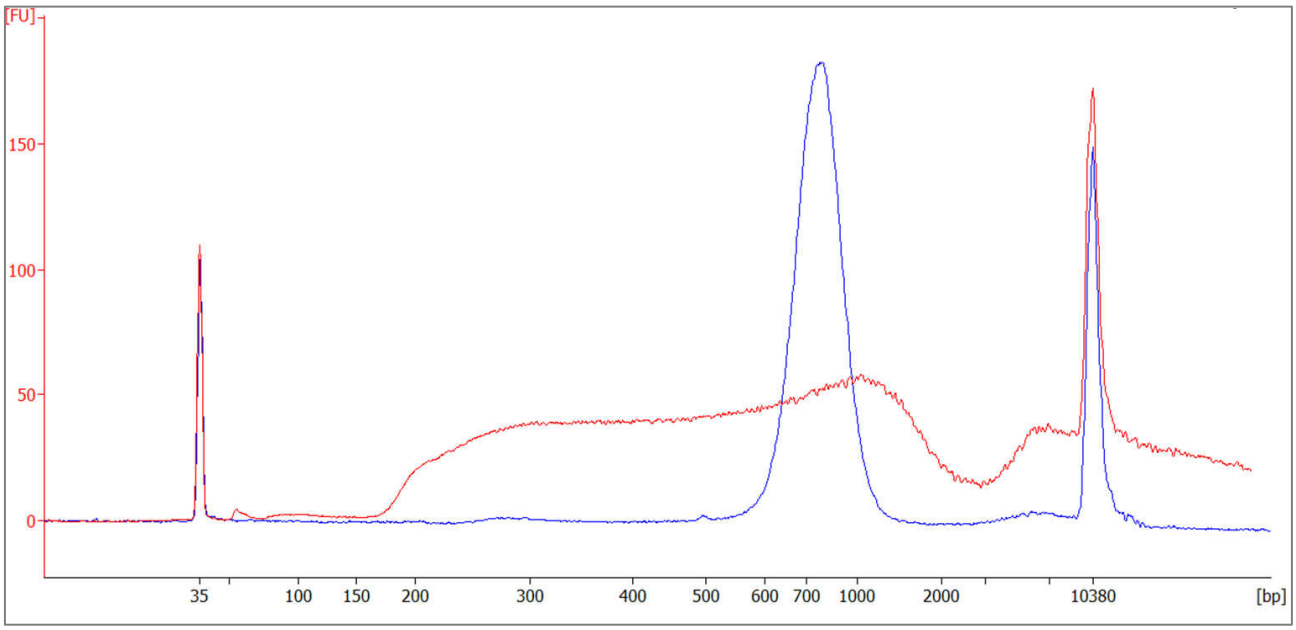

Figure 4. Relative size distribution of *E. coli* genomic DNA fragment library generated from 50 ng DNA input following MuSeek Library Preparation Kit protocol. Fragment size distribution was analyzed by the Agilent 2100 Bioanalyzer instrument using the High Sensitivity DNA Kit (Life Technologies Inc.). Red – DNA fragments after fragmentation reaction (undiluted); Blue – 2-fold diluted DNA fragments after size-selection from agarose gel and cleanup using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Cat. #K0831/2).

E. Sequencing

⚠ IMPORTANT REMINDER: USE ONLY THE MUSEEK SEQUENCING PRIMERS PROVIDED WITH THIS KIT! NO SEQUENCE DATA WILL BE GENERATED IF INCORRECT PRIMER IS USED, AS THE PRIMER BINDING SITES ARE DIFFERENT FROM ORIGINAL ILLUMINA SEQUENCING PRIMERS.

1. Custom primer preparation for sequencing run

MuSeek Library Preparation Kit provides all necessary MuSeek Sequencing Read 1, Read 2 and Index Read primers. All primer concentrations are 30 µM, the final concentration of each primer in sequencing run should be 0.5 µM. The protocol for sequencing primer preparation can be found in Illumina guide "Using Custom Primers on the MiSeq".

2. Indexing Principle

The MuSeek dual indexing method utilizes two 8 base indices, Index 1 adjacent to the P7 adapter sequence, and Index 2 adjacent to the P5 adapter sequence. By choosing different combinations of Index 1 (M501-M504) and Index 2 (M701-M706), a total of 24 unique dual-indexed libraries can be generated and used in the same sequencing run. A list of index sequences for generating sample sheets to demultiplex the samples is provided in **Table 3**.

Illumina HiSeq and MiSeq instruments use a green laser to sequence G/T and a red laser to sequence A/C. At each cycle at least one of two nucleotides for each color channel need to be read to ensure proper registration. It is important to maintain color balance for each base of the index read being sequenced, otherwise index read sequencing could fail due to registration failure. If you choose the dual index sequencing workflow always use at least two unique and compatible indices for each index (Index 1 and Index 2). The following tables illustrate possible pooling strategies.

Table 5. Example of proper and improper index combinations in the same sequencing run.

Good				Bad			
Index 2 (i5)		Index 1 (i7)		Index 2 (i5)		Index 1 (i7)	
M501	CTACATGC	M701	GAGAAGAG	M501	CTACATGC	M702	AGCGTAGA
M502	GATAGCGT	M701	GAGAAGAG	M502	GATAGCGT	M702	AGCGTAGA
M503	ACGGCTAA	M702	AGCGTAGA	M503	ACGGCTAA	M703	AGTCAGTC
M504	AAGAGGAG	M702	AGCGTAGA	M504	AAGAGGAG	M703	AGTCAGTC
	++++++++		++++++++		++++++++		--++++--

\+ = signal in both color

\- = signal missing in one color channel

Library pooling for single indexing

Samples per run	Index 2 (i5)	Index 1 (i7)
1-plex (no pooling)	Any Index 2 adapter	Any Index 1 adapter
2-plex		M701 and M702
3-plex		M701, M702, M703
4-plex		M701, M702, M703, M704
5-plex		M701, M702, M703, M704, M705
6-plex		M701, M702, M703, M704, M705, M706

Library pooling for dual indexing

Samples per run	Index 2 (i5)	Index 1 (i7)
9-plex	M501, M502, M503	M701, M702, M703
12-plex	M501, M502, M503	M701, M702, M703, M704
16-plex	M501, M502, M503, M504	M701, M702, M703, M704
24-plex	M501, M502, M503, M504	M701, M702, M703, M704, M705, M706

3. Preparing for sequencing on the MiSeq™(HiSeq™)

- Guidelines how to pool and dilute libraries before sequencing can be found in Illumina guide "*Preparing DNA for MiSeq*". Guidelines for HiSeq can be found in Illumina HiSeq system user guides.
- Guidelines for compiling a MiSeq and HiSeq Sample Sheet files can be found in Illumina guides "*MiSeq Sample Sheet Quick Reference guide*" and "*HiSeq 2500 System User Guide*" in Sample Sheet Overview.
- For NGS libraries prepared using the MuSeek Library Preparation kit **use only MuSeek compatible sequencing primers** provided with the kit. Apply MuSeek compatible **Read 1, Read 2 and Index** sequencing primers into dedicated reagent cartridge wells for custom primers. Refer to Illumina guide "*Using Custom Primers on the MiSeq*™" for detailed instructions on using custom primers.

Important:

- When preparing Sample Sheet in the "Assays" parameter section write in '***Nextera***'. Don't forget to add additional lines for **Custom Primers: *Read 1*, *Index* and *Read 2***.
- To enable demultiplexing of MuSeek indexed libraries the MiSeq or HiSeq compatible Sample Sheets must be prepared using custom MuSeek index sequences. **Failure to enter correct MuSeek index sequences into Sample Sheet file will result in improper sample demultiplexing**.

TROUBLESHOOTING

Problem	Cause and Solution
Low yield	**1. Excessive DNA fragmentation.** a) Target DNA was already fragmented. b) There was less than 50 ng or 100 ng of target DNA present. Check the DNA concentration and make sure that there are no other components present in solution that may cause over-estimation of DNA concentration. c) Incorrect amount of MuSeek Enzyme Mix was added due to a pipetting error. d) The fragmentation incubation time was too long. e) The Stop Solution was not added exactly after the 5 minutes incubation, or the reaction was not mixed properly after adding the Stop Solution. **2. Insufficient DNA fragmentation**. a) The target DNA is impure and contains contaminants, which inhibited the fragmentation reaction. b) Less than needed volume of the MuSeek Enzyme Mix was added into the fragmentation reaction due to a pipetting error. c) The MuSeek Enzyme Mix has not been stored below -20 °C and has lost its activity. d) The fragmentation reaction mixture was not mixed properly after adding the MuSeek Enzyme Mix. **3. The PCR Purification protocol was not followed properly.** **4. The adapter-addition reaction was not successful.** a) The adapter-addition reaction volume in PCR tubes was higher than 50 µL, which is too high for some thermal cycler instruments. b) Temperature cycling protocol was not performed according to the manual. Make sure that the initial 3 minute incubation at 66 °C is included in the protocol and that the denaturation and annealing temperatures are correct. c) Not all the required components were added to the reaction due to pipetting errors. **5. The size-selection protocol was not followed properly.** **6. Other than recommended DNA library cleanup method was chosen.** **7. Sample contaminated with RNA** a) Repurify the sample. Make sure to use RNA-free DNA purification kits.

Size selected library is outside the range of interest	**1. Improper fragmentation of DNA library.** a) Make sure that your DNA fragmentation protocol generates the DNA fragments close to the correct median insert size. **2. MagJET size selection protocol was not carefully followed.** a) Always perform MagJET Calibration protocol to find the required volume of Binding mix solution prior the size selection of your DNA libraries. b) 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. **3. Agarose gel size selection protocol was not followed properly.** a) It is not recommended to use agarose gel prestained with SybrGold, as SybrGold impairs DNA fragment library migration in precast gels. Perform gel staining after DNA electrophoresis.

TECHNICAL SUPPORT

Regarding the library preparation questions please refer to:
web site: www.thermoscientific.com
support email in North America: ts.molbio@thermofisher.com
support email in Europe: ts.molbio.eu@thermofisher.com

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TRADEMARKS