



PRODUCT INFORMATION

**Thermo Scientific™ MuSeek
Library Preparation Kit for Ion Torrent™**

Cat. no. 4480829

For 10 rxns

Lot _____

Exp. ____



Store below -70°C before opening


For barcoded DNA fragment library generation use with:
Thermo Scientific™ MuSeek Barcode Set 1, Ion Torrent™
compatible (Cat. No. 4487356)

Publication Number MAN0009943

Revision A.0

CONTENTS	page
COMPONENTS OF THE KIT	3
STORAGE.....	3
DESCRIPTION	4
TECHNOLOGY OVERVIEW	4
PREPARATION OF BARCODED LIBRARIES	7
IMPORTANT NOTES	7
PROTOCOL	10
A. Fragmentation reaction.....	10
B. Adaptor addition	12
C. Size selection and library quantification.....	14
D. Sequencing	16
TROUBLESHOOTING.....	17

COMPONENTS OF THE KIT

Component	Cap color	Amount	Important Notes
MuSeek Enzyme Mix	Red	10 μ L	Store below -70 °C. Avoid repeated freeze-thaw cycles.
MuSeek Fragmentation Reaction Buffer	Yellow	100 μ L	
MuSeek Stop Solution	Orange	30 μ L	Contains SDS, store at room temperature.
Control DNA	White	4 μ L	50 ng/ μ L cl857 <i>Sam7</i> Lambda DNA, size 48.5 kb.
MuSeek Adaptor Addition Primer Mix	Purple	40 μ L	
MuSeek Adaptor Addition Reaction Buffer	Green	1 mL	Contains 2x reaction buffer with dNTPs.
Phusion™ Hot Start II High-Fidelity DNA Polymerase	Blue	40 μ L	
MuSeek Sequencing Primer	White	300 μ L	<p>After receiving this kit, we recommend you to place the MuSeek Sequencing Primer vial into the same box with your other sequencing reaction reagents (into your Ion Sequencing Kit box).</p> <p> <u>This primer must be used ONLY when sequencing non-barcoded MuSeek libraries. For barcoded MuSeek libraries use original sequencing primer, provided in the Ion Sequencing Kit.</u></p>

STORAGE

The kit is shipped on dry ice. **Upon arrival, open the kit and place MuSeek Enzyme Mix for storage below -70 °C**, avoid repeated freeze-thaw cycles. The Stop Solution can be stored at room temperature, and the remaining kit components at -20 °C.

DESCRIPTION

Thermo Scientific™ MuSeek Library Preparation Kit for Ion Torrent™* is designed for generation of high-quality genomic DNA libraries for sequencing with the Ion Personal Genome Machine™ (PGM™) and Ion Proton™ 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 the platform-specific adaptors are added using a robust and accurate Thermo Scientific Phusion™ High-Fidelity DNA polymerase. Starting with only 100 ng of the sample the same protocol can be used to generate 100-400 bp insert libraries.

The kit contains components sufficient for 10 fragmentation and the subsequent adaptor-addition polymerase chain reactions. The kit also contains a MuA-specific sequencing primer that is required in the PGM sequencing runs when non-barcoded libraries are prepared by this method.

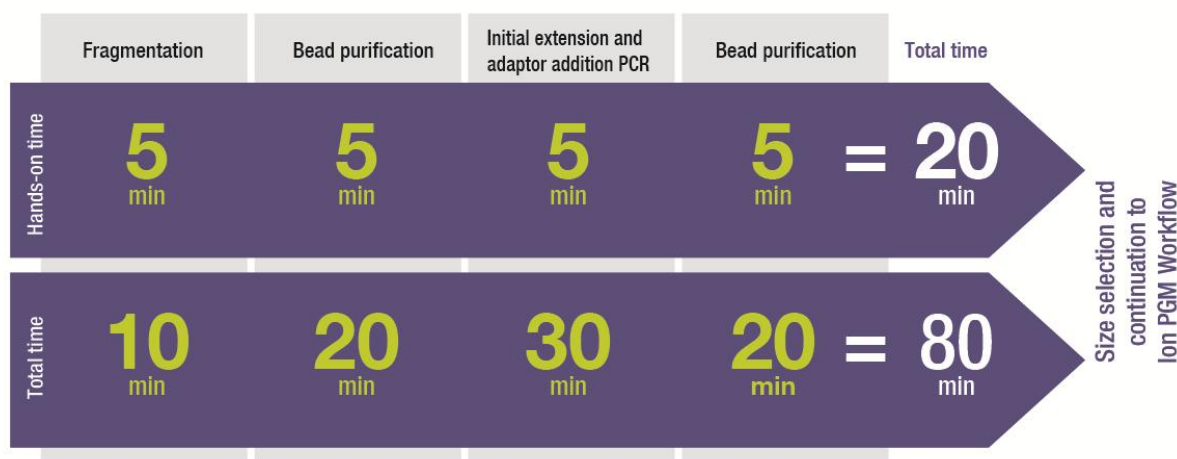


Figure 1. Overview of the workflow.

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. PGM-specific adaptors are added to the fragments in a subsequent adaptor-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 adaptor 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).

* Patent pending.

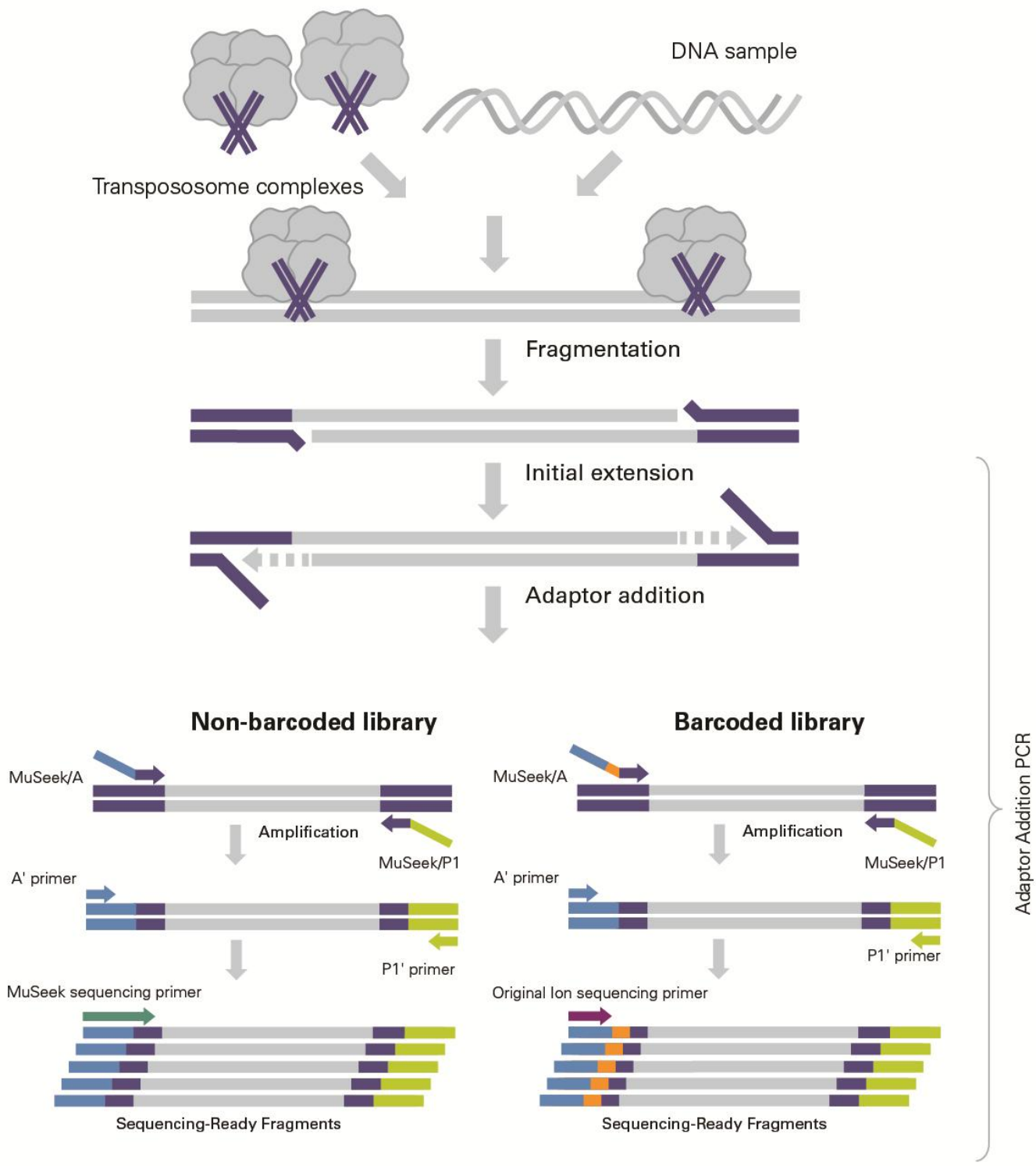


Figure 2. MuA transposase complex simultaneously fragments input DNA and tags the fragments. In a subsequent PCR, adaptor sequences are added.

Table 1. Sequences of the primers and transposon ends:

Name	Sequence
MuSeek/A	5' -CCATCTCATCCCTGCGTGTCTTCGTGCGTCAGTTCA-3'
MuSeek/A (1-16) barcoded primer*	5' -CCATCTCATCCCTGCGTGTCTCCGACTCAG barcode 1-16 TTCGTGCGTCAGTTCA-3'
MuSeek/P1	5' -CCACTACGCCTCCGCTTTCTCTCTATGGGCAGTCGGTGATTTTCGTGCGTCAGTTCA-3'
A'	5' -CCATCTCATCCCTGCGTGTC-3'
P1'	5' -CCACTACGCCTCCGCTTTCTCTCTATG-3'
MuSeek Sequencing Primer	5' -CATCTCATCCCTGCGTGTCTTCGTGCG-3'
Transposon end	5' -GTTTTCGCATTATCGTGAAACGCTTTTCGCGTTTTTCGTGCGTCAGTTCA-3' 3' -CAAAAGCGTAAATAGCACTTTGCGAAAGCGCAAAAAGCACGCAGTCAAGTCGT-5'

*The sequences of MuSeek barcoded adaptors are available at Ion Community website
<http://ioncommunity.lifetechnologies.com>.

Non-barcoded sequence structure: 5'- <i>CCATCTCATCCCTGCGTGTCTTCGTGCG</i> TCAG TTCA [Target DNA] TGAAGTACGCACGAAATCACC GACTGCC <i>CATAGAGAGGAAAGCGGAGGCGTAGTGG</i> -3'
Barcoded sequence structure: 5'- <i>CCATCTCATCCCTGCGTGTCTCCGAC</i> TCAG [Barcode 1-16] TTCGTGCGTCAGTTCA [Target DNA] TGAAGTACGCACGAAATCACC <i>GAAGTACGCACGAAATCAGTGG</i> -3'

Figure 3. Sequence structures of adaptor containing target DNA fragment (non-barcoded and barcoded).

TCAG patch is a key sequence (four different bases) that is used for Ion Torrent instrument calibration. The sequencing read starts after the key sequence, and if the fragment end is reached the read will enter the TGA... sequence of the transposon. The sequences corresponding to primer A' and complementary to primer P' are shown in italics.

PREPARATION OF BARCODED LIBRARIES

The **MuSeek Library Preparation Kit for Ion Torrent™** is compatible with generation of barcoded DNA fragment libraries when used in combination with the **MuSeek Barcode Set 1, Ion Torrent™ compatible** (Cat #K1541). The MuSeek Barcode Set 1, Ion Torrent™ compatible, provides 1-16 MuSeek Barcode Adaptor Primer Mixes, each allowing for addition of a unique Ion Torrent PGM™-compatible barcode sequence. This enables researchers to pool up to 16 fragment libraries prior to size-selection and emulsion PCR, and then conduct multiplexed sequencing analysis.

Important! When constructing barcoded DNA fragment libraries, replace the MuSeek Adaptor Addition Primer Mix from MuSeek Library preparation Kit for Ion Torrent™ with a corresponding MuSeek Barcode Adapter Primer Mix 1-16 from the Thermo Scientific MuSeek Barcode Set 1, Ion Torrent compatible (such as MuSeek Barcode Adapter Primer Mix 1). **For sequencing of barcoded DNA fragment libraries use ONLY the original Ion Torrent sequencing primer. No sequence data will be generated using MuSeek Sequencing Primer.**

IMPORTANT NOTES



FOR SEQUENCING **NON-BARCODED** LIBRARIES GENERATED WITH THIS KIT, USE ONLY THE **MUSEEK SEQUENCING PRIMER** PROVIDED WITH THIS KIT!



FOR SEQUENCING **BARCODED** LIBRARIES GENERATED WITH THIS KIT, USE ONLY THE **ORIGINAL SEQUENCING PRIMER** PROVIDED IN THE LIFE TECHNOLOGIES ION SEQUENCING KIT!



NO SEQUENCE DATA WILL BE GENERATED BY THE ION SEQUENCING PLATFORMS IF THE INCORRECT PRIMER IS USED, AS THE PRIMER BINDING SITES ARE DIFFERENT FOR NON-BARCODED AND BARCODED LIBRARIES.



Always check if the correct sequencing primer is used for non-barcoded and barcoded libraries:

	Generation of NON-BARCODED DNA fragment library	Generation of MuSeek BARCODED DNA fragment library
Required Thermo Scientific™ products	MuSeek Library Preparation Kit for Ion Torrent (Cat #K1331).	MuSeek Library Preparation Kit for Ion Torrent (Cat #K1331). AND MuSeek Barcode Set 1, Ion Torrent™ compatible (Cat #K1541).
Adaptor addition primer mix required	MuSeek Adaptor Addition Primer Mix, provided with the MuSeek Library Preparation Kit for Ion Torrent (Cat #K1331).	MuSeek Barcode Adaptor Primer Mix 1-16, provided with the MuSeek Barcode Set 1, Ion Torrent™ compatible (Cat #K1541).
Sequencing primer required	MUSEEK SEQUENCING PRIMER, provided with the MuSeek Library Preparation Kit for Ion Torrent (Cat #K1331).	SEQUENCING PRIMER, PROVIDED IN THE ION SEQUENCING KIT.

Additional required reagents not provided with the kit:

- Agencourt® AMPure® XP PCR Purification system (Beckman Coulter, Inc., Cat #A63880, #A63881, #A63882)
- 70% ethanol (required for bead purification)
- Size selection equipment [such as E-Gel® (Life Technologies, Inc.) system]
- For preparation of barcoded NGS library, a Thermo Scientific MuSeek Barcode Set 1 (Cat #K1541) is required.
- Magnetic particle processing rack

Input DNA requirements and general recommendations:

- The kit is designed to be used with 100 ng of high-quality 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). 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 (2 μ L) 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 of the below -70 °C freezer for the MuSeek Enzyme Mix.
- Mix reagents thoroughly before use by vortexing or flicking the tube, especially if frozen and thawed.
- For optimal results always prepare a fresh 70% solution before starting the purification procedure (70% ethanol is hygroscopic).

PROTOCOL

A. Fragmentation reaction

Note: Always keep the MuSeek Enzyme Mix on ice when preparing the reactions and minimize handling times outside of the -70 °C freezer. Use 100 ng of high-quality gDNA 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).

1. Pipet all the reagents, except for MuSeek Enzyme Mix, in the given order into a 0.5 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.

Component	Volume
Nuclease-free Water	Add to 30.0 μ L
MuSeek Fragmentation Reaction Buffer	10.0 μ L
gDNA	X μ L (100 ng)
MuSeek Enzyme Mix	1.0 μ L
Total	30.0 μL

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 -70 °C freezer as soon as possible.
3. **Stop the reaction** after 5 minutes by adding 3 μ L of MuSeek Stop Solution and vortexing briefly.
4. After vortexing, keep the tubes at room temperature. Do not put reaction tubes on ice, as the Stop Solution may cause precipitation.

5. Purify the fragmented DNA by Agencourt™ AMPure™ XP PCR Purification system:

Note:

- This kit has been designed to work with Agencourt™ AMPure™ XP PCR Purification system and therefore we do not recommend using other purification systems.
- Equilibrate the beads at room temperature at least 30 minutes prior to starting the purification protocol, and mix well before pipetting.

a)	Transfer the DNA sample into a 1.5 mL tube.
b)	Add 49.5 μ L of room temperature Agencourt AMPure XP magnetic beads to the reaction (1.5x sample volume) and mix thoroughly by pipetting up and down for 10 times (avoid foaming).
c)	Incubate for 5 minutes at room temperature.
d)	Pulse-spin and place the tube in a magnetic rack for 2-4 minutes or until the solution is cleared.
e)	Aspirate and discard the supernatant carefully without disturbing the beads. Make sure that no supernatant is left.
f)	Keep the tube in the rack and add 400 μ L of freshly-prepared 70% ethanol directly onto magnetic beads, incubate for 30 seconds, and remove all the supernatant.
g)	Repeat the ethanol wash in step f).
h)	Air-dry the beads on the magnet, by opening the tube for 2-5 minutes, allowing all traces of ethanol to evaporate. Be careful not to over dry the beads (this can be seen as cracking of the beads).
i)	Remove the tube from the magnetic rack, and thoroughly suspend the beads in 25 μ L of nuclease-free water by pipetting up and down 10 times.
j)	Place the tube in the magnetic rack and let the solution clear for at least 1 minute.
k)	Collect the supernatant without disturbing the pellet into a new sterile tube. This supernatant contains the purified fragmented DNA, do not discard the supernatant.

Note: Save 1 μ L for later analysis of size distribution and concentration of purified fragments using the Agilent 2100 Bioanalyzer instrument (see C. Size selection and library quantification).

B. Adaptor addition

Note: For a **barcoded library**, replace MuSeek Adaptor Addition Primer Mix from MuSeek Library preparation kit with a corresponding MuSeek Barcode Adapter Primer Mix 1-16 from Thermo Scientific MuSeek Barcode Set 1, Ion Torrent compatible (such as MuSeek Barcode Adapter Primer Mix 1).

1. Take 20 μL of the purified fragmented DNA from the purification step described above and add to a reaction mixture at room temperature in a 1.5 mL tube:

Component	Volume
Nuclease-free Water	72 μL
MuSeek Adaptor Addition Reaction Buffer	100 μL
MuSeek Adaptor Addition Primer Mix OR Selected MuSeek Barcode Adapter Primer Mix 1-16 from Thermo Scientific MuSeek Barcode Set 1, Ion Torrent compatible (Cat #K1541)	4 μL
Purified fragmented DNA	20 μL
Phusion Hot Start II High-Fidelity DNA Polymerase	4 μL
Total	200 μL

2. Aliquot the reaction mixture into four separate 200 μL PCR tubes. (For some thermal cycler instruments it is recommended to amplify DNA in reaction volumes not higher than 50 μL).
3. Perform temperature cycling of all four reaction mixture aliquots using the following cycling conditions:

Temperature	Time	Cycles
66 °C	3 min	1
98 °C	30 sec	
98 °C	10 sec	8
60 °C	50 sec	
72 °C	10 sec	
72 °C	1 min	1
4 °C	hold	

4. Combine all four reactions into one 1.5 mL tube and purify the DNA by Agencourt AMPure XP PCR Purification system:

a)	Add 360 μ L of room temperature Agencourt™ AMPure™ XP magnetic beads to the reaction (1.8x sample volume) and mix thoroughly by pipetting up and down 10 times (avoid foaming).
b)	Incubate for 5 minutes at room temperature.
c)	Pulse-spin and place the tube in a magnetic rack for 2 minutes or until the solution is cleared.
d)	Aspirate the supernatant carefully without disturbing the beads and discard the supernatant. Make sure that no supernatant is left.
e)	Keep the tube in the rack and add 1000 μ L of freshly prepared 70% ethanol directly onto magnetic beads (make sure that all beads are covered by ethanol), incubate for 30 seconds, and remove the supernatant.
f)	Repeat the ethanol wash in step e).
g)	Air-dry the beads on the magnet, by opening the tube for 2-5 minutes, allowing all traces of ethanol to evaporate. Be careful not to over dry the beads (this can be seen as cracking of the beads).
h)	Remove the tube from the magnetic rack, and thoroughly suspend the beads in 20 μ L of nuclease-free water or 10 mM Tris-HCl, pH 8.0.
i)	Place the tube in the magnetic rack and let the solution clear for at least 1 minute.
j)	Collect the supernatant without disturbing the pellet into a new sterile tube. This supernatant contains the purified fragmented DNA, do not discard the supernatant.

Note: Save 1 μ L for later analysis of size distribution and concentration of fragments after adaptor-addition PCR by using the Agilent 2100 Bioanalyzer instrument (see **C. Size selection and library quantification**). Typically from 200 μ L of PCR reaction mixture at least 100 fmol of DNA (in the fragment length interval of 297-363 bp) are generated as measured by the Agilent 2100 Bioanalyzer instrument.

C. Size selection and library quantification

a) Size-selecting correct DNA library fragment length

Sequencing ready libraries prepared using MuSeek Library Preparation Kit for Ion Torrent™ contain DNA fragments of ~ 150-1,500 bp in length. Each fragment includes adaptor sequences on both ends, 93 base pairs in total for non-barcoded library. When size-selecting libraries of a relevant sequencing read length, the length of adaptor sequences must be taken into account. For size-selecting correct DNA library fragment peak refer to **Table 2**.

b) Size selection recommendations

To perform emulsion-PCR and subsequent sequencing reaction efficiently, size-selection by the E-Gel® Agarose Gel Electrophoresis System using an E-Gel® SizeSelect™ 2% Agarose Gel is recommended. Instructions can be found in the E-Gel Technical Guide and E-Gel SizeSelect Agarose Gels Quick Reference, which are available at the web catalogue at www.lifetechnologies.com. Save 1 µL of the final sequencing library for fragment size distribution and concentration analysis by Agilent 2100 Bioanalyzer instrument.

c) Evaluation of prepared sequencing library

Collect all the samples saved from the fragmentation reaction, adaptor-addition PCR and size selection for analysis using the Agilent 2100 Bioanalyzer instrument. Before starting, dilute the samples after the fragmentation reaction by 2-fold and the samples after adaptor-addition PCR by 4-fold. The samples after size selection by E-Gel® should not be diluted. Analyze all the samples together by the 2100 Bioanalyzer instrument using the High Sensitivity DNA Kit (Cat #5067-4626). Typical example of an analyzed library is demonstrated in **Figure 3**.

d) Recommendations for the size-selection of barcoded DNA libraries

Mix all barcoded libraries at equal molar amounts before E-Gel® size selection so that the final mix volume is larger than 25 µL. We recommend calculating molarity of individual barcoded libraries only within intervals of interest which are distinct for 100-400 bp library sizes (see **Table 2**), such as for 200 base reads, calculate molarity within the region of 297 to 363 bp, which corresponds to subsequent target E-Gel® size selection peak size ($\pm 10\%$).

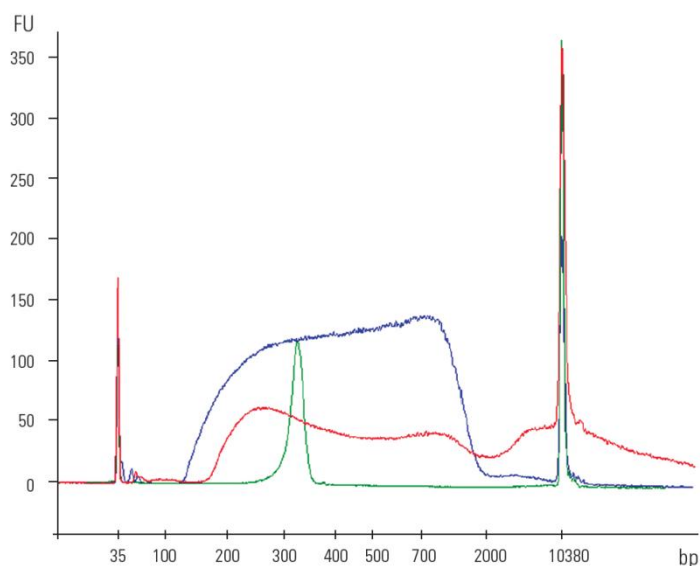
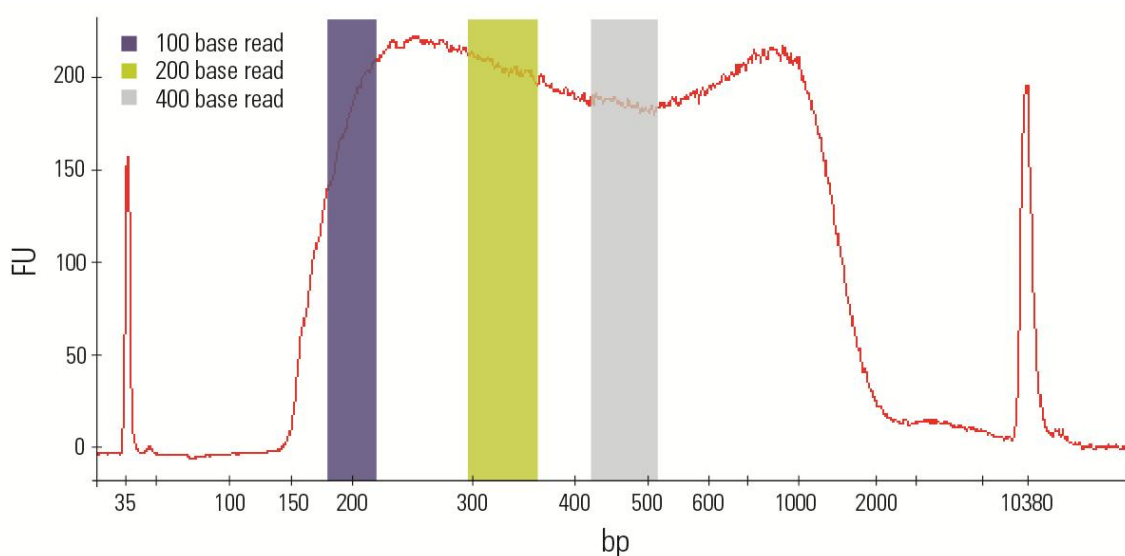


Figure 3. Relative size distribution of *E. coli* genomic DNA fragment library generated using MuSeek Library Preparation Kit. Fragment size distribution was analyzed by the Agilent 2100 Bioanalyzer instrument using the High Sensitivity DNA Kit (Life Technologies). Red – 2-fold diluted DNA fragments after fragmentation reaction; Blue – 4-fold diluted DNA fragments after Adapter Addition PCR; Green – fragments after size-selection by the E-Gel® Agarose Gel Electrophoresis System on E-Gel® SizeSelect™ 2% Agarose Gel.

Table 2. Target DNA library fragment size for size selection procedure:



Sequencing platform	Library size	Target peak size	Interval of interest for barcoded library concentration measurement (target peak \pm 10%)
Ion Torrent PGM™	100 base read	~ 200 bp	180-220 bp
	200 base read	~ 330 bp	297-363 bp
	300 base read	~ 390 bp	351-429 bp
	400 base read	~ 470 bp	423-517 bp
Ion Proton™	150 base read	~ 220 bp	198-242 bp

D. Sequencing



IMPORTANT REMINDER: SEQUENCING PRIMERS FOR NON-BARCODED AND BARCODED DNA FRAGMENT LIBRARIES ARE DIFFERENT! BEFORE LOADING A LIBRARY ONTO A CHIP, SELECT APPROPRIATE SEQUENCING PRIMER:

1. FOR SEQUENCING **NON-BARCODED** LIBRARIES GENERATED WITH THIS KIT, USE ONLY THE **MUSEEK SEQUENCING PRIMER** PROVIDED WITH THIS KIT! The addition of Control Ion Spheres™ is not required, as they do not contain the binding site for the MuSeek Sequencing Primer.
2. FOR SEQUENCING **BARCODED** LIBRARIES GENERATED WITH THIS KIT, USE ONLY THE **ORIGINAL SEQUENCING PRIMER** PROVIDED IN THE LIFE TECHNOLOGIES ION SEQUENCING KIT!



NO SEQUENCE DATA WILL BE GENERATED BY THE PGM™ IF INCORRECT PRIMER IS USED, AS THE PRIMER BINDING SITES ARE DIFFERENT.

Library fragments prepared with this kit carry four bases of MuA transposon sequence immediately downstream of the key sequence. In order to get the best sequence alignment results from your PGM run, these bases need to be trimmed by the Torrent Server.

For sequence analysis with Torrent Browser 3.0 (or later):

- Use the example template provided for this kit (MuSeek Library Template) when planning runs using a MuSeek library. The template is found on the templates page which is held under the Plan tab. This template has defaults selected that identify the MuSeek library 3' adapter (MuSeek/P1B) and 5' sequence tag (MuSeek_5prime_tag) that will be trimmed away during data processing.

For manual upload of Barcode sequences please refer to the Torrent Browser 3.0 documentation available on the Ion Community site (<http://ioncommunity.lifetechnologies.com>)

TROUBLESHOOTING

Problem	Cause and Solution
Low yield	<ol style="list-style-type: none"> 1. DNA fragmented too much: <ol style="list-style-type: none"> a. Target DNA was already fragmented. b. There was less than 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. Too high 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. DNA not fragmented properly: <ol style="list-style-type: none"> a. The target DNA is impure and contains contaminants, which inhibited the fragmentation reaction. b. Less than 1.0 μL 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 $-70\text{ }^{\circ}\text{C}$ and has lost its activity. d. The fragmentation reaction mixture was not mixed properly after adding the MuSeek Enzyme Mix. 3. The Agencourt AMPure XP PCR Purification system protocol was not followed properly. 4. The adaptor-addition reaction was not successful: <ol style="list-style-type: none"> a. The adaptor-addition reaction volume in PCR tubes was higher than $50\text{ }\mu\text{L}$, which is too high for some thermal cycler instruments. b. The four adaptor-addition reactions were not combined before AMPure XP PCR Purification protocol. c. Temperature cycling protocol was not performed according to the manual. Make sure that the initial 3 minute incubation at $66\text{ }^{\circ}\text{C}$ is included in the protocol and that the denaturation and annealing temperatures are correct. d. Not all the required components were added to the reaction due to pipetting errors. 5. The size-selection protocol was not followed properly.

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

Regarding the sequencing questions please refer to:

web site: www.iontorrent.com

Ion community: ioncommunity.iontorrent.com

support email: ionsupport@lifetech.com

phone numbers:

In North America: 1-87-SEQUENCE (1-877-378-3623)

Outside of North America: +1-203-458-8552

NOTICE TO PURCHASER:

The purchase price of this product includes a limited, nontransferable license under U.S. and foreign patents owned by BIO-RAD Laboratories, Inc., to use this product. No other license under these patents is conveyed expressly or by implication to the purchaser by the purchase of this product.

This product is sold under license from Affibody AB, Sweden.

This product or the use of this product is covered by US patent application US20130023423A1 and corresponding counterparts. The purchase of this product includes a non-transferable license to use this product for the purchaser's internal research. All other commercial uses of this product, including without limitation product use for diagnostic purposes, resale of product in the original or any modified form or product use in providing commercial services require a separate license. For further information on obtaining licenses please contact ltvil.info@thermofisher.com

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.lifetechnologies.com for Material Safety Data Sheet of the product.

TRADEMARKS

Ion Sphere, Ion Torrent, PGM, SizeSelect, E-Gel® and Personal Genome Machine are trademarks of Life Technologies, Inc. and its subsidiaries. Agencourt and AMPure are trademarks of Beckman Coulter, Inc. and its subsidiaries.

Bioanalyzer is a trademark of Agilent Technologies Inc. and its subsidiaries.

© 2013 Thermo Fisher Scientific Inc. All rights reserved. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.