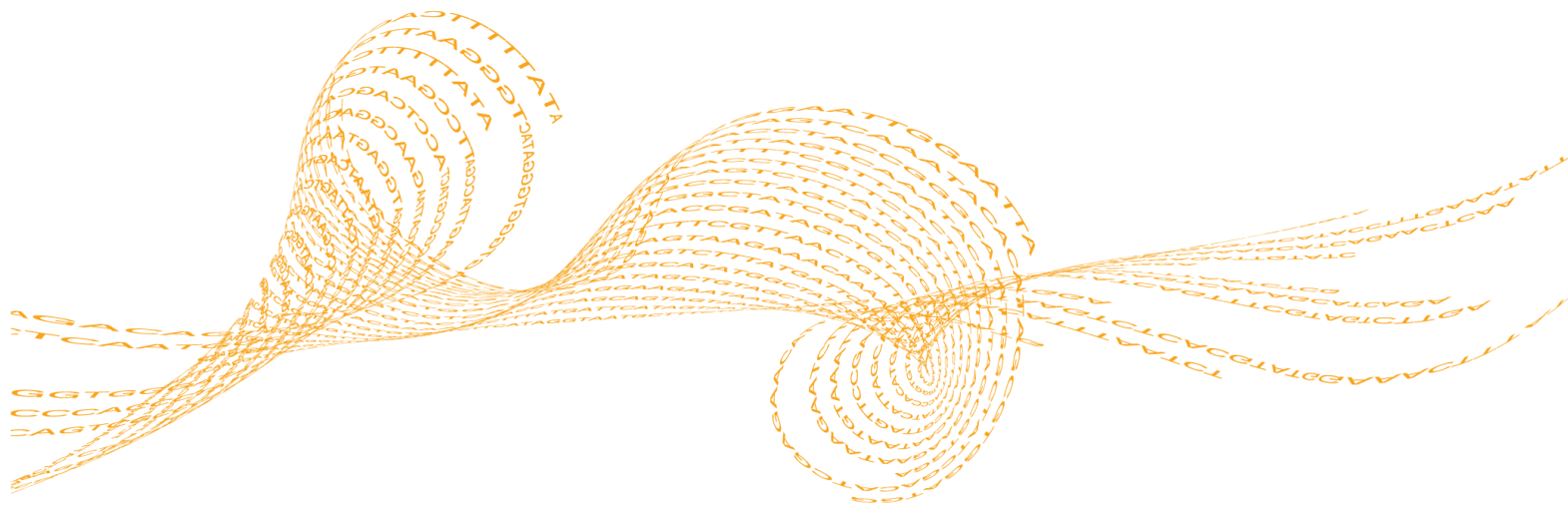


TruSeq DNA Methylation

Library Preparation Guide

Kit Contents	3
Consumables and Equipment	4
Preparation	5
Quality Control of Bisulfite-Converted DNA	6
TruSeq DNA Methylation Kit Protocol	7
Sequencing the TruSeq DNA Methylation Kit Library	16
Adding a User-Defined Barcode to the Library	17
TruSeq DNA Methylation Kit Index PCR Primers	18
Technical Assistance	19



This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY ILLUMINA IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

FOR RESEARCH USE ONLY

© 2014 Illumina, Inc. All rights reserved.

Illumina, IlluminaDx, BaseSpace, BeadArray, BeadXpress, cBot, CPro, DASL, DesignStudio, Eco, GAllx, Genetic Energy, Genome Analyzer, GenomeStudio, GoldenGate, HiScan, HiSeq, Infinium, iScan, iSelect, MiSeq, MiSeqDx, Nextera, NuPCR, SeqMonitor, Solexa, TruGenome, TruSeq, TruSight, Understand Your Genome, UYG, VeraCode, the pumpkin orange color, and the Genetic Energy streaming bases design are trademarks of Illumina, Inc. in the U.S. and/or other countries. All other names, logos, and other trademarks are the property of their respective owners.

AMPure®, Beckman, and Beckman Coulter are trademarks or registered trademarks of Beckman Coulter, Inc.

Kit Contents

Table 1 TruSeq DNA Methylation Kit Components

Component Name	12 rxn	24 rxn	96 rxn	Cap Color
DNA Synthesis Primer	24 μ l	48 μ l	192 μ l	Green
TruSeq DNA Synthesis PreMix	48 μ l	96 μ l	384 μ l	
100 mM DTT	6 μ l	12 μ l	48 μ l	
TruSeq Polymerase	6 μ l	12 μ l	48 μ l	
Exonuclease I	12 μ l	24 μ l	96 μ l	
TruSeq Terminal Tagging PreMix	90 μ l	180 μ l	720 μ l	Blue
DNA Polymerase	6 μ l	12 μ l	48 μ l	
FailSafe PCR PreMix E	300 μ l	600 μ l	3 \times 800 μ l	Yellow
TruSeq Forward PCR Primer	12 μ l	24 μ l	96 μ l	
TruSeq Reverse PCR Primer	12 μ l	24 μ l	96 μ l	
Nuclease-Free Water	500 μ l	1.2 ml	3 \times 1.5 ml	Clear

Storage: Store the kit at -20°C.

Consumables and Equipment

Check to ensure that you have all of the necessary user-supplied consumables and equipment before proceeding to the TruSeq DNA Methylation Kit protocol. These consumables and equipment are Illumina recommended for the TruSeq DNA Methylation Kit protocols.

Table 2 User-Supplied Consumables

Consumable	Supplier
1.5 ml microcentrifuge tubes	General lab supplier
0.2 ml or 0.5 ml microcentrifuge tubes	General lab supplier
Wide-bore pipet tips (eg, Pure 200G sterile tip)	Molecular Bioproducts, Catalog No. 3531
FailSafe PCR Enzyme	Epicentre, Catalog No. FSE51100
Optional: TruSeq Index PCR Primers	Illumina, Catalog No. EGIDX81312 (12 Indexes)
EZ DNA Methylation–Gold or EZ DNA Methylation–Lightning Kit	Zymo Research, Catalog No. D5005 or D5030
AMPure XP System	Beckman-Coulter Genomics, Catalog No. Cat. No. A63880
Freshly prepared 80% (v/v) ethanol	General lab supplier

Table 3 User-Supplied Equipment

Equipment	Supplier
Magnetic rack or stand for 1.5 ml tubes	Bangs Laboratories, Inc., Catalog No. LS001, MS002, MS003 Life Technologies, Catalog No. 12321D
Magnetic plate	Life Technologies, Catalog No. 123310
1.5 ml microcentrifuge	General lab supplier
Thermal cycler, water bath, heating block, or other temperature-controlled device	General lab supplier
Vortex mixer	General lab supplier
NanoDrop UV-Vis Spectrophotometer	Thermo Fisher
Qubit Fluorometer	Thermo Fisher

Preparation

Read this protocol closely before performing the TruSeq DNA Methylation Kit procedure.

Amount of Sample DNA

Use 50–100 ng of sample DNA in the bisulfite conversion procedure described in *TruSeq DNA Methylation Kit Protocol* on page 7. Then, use all the purified and recovered bisulfite-converted single-stranded (BC-SS) DNA in the TruSeq DNA Methylation Kit procedure beginning at *Anneal the DNA Synthesis Primer* on page 8.

Use 10 ng of genomic DNA as Control DNA without bisulfite treatment.

Sample Purity

The BC-SS DNA and untreated DNA (Control DNA) must be free of inhibitors such as guanidine salts, organics, etc.

Bisulfite Treatment of the Sample DNA

The sample DNA must be treated with bisulfite prior to the TruSeq DNA Methylation Kit procedure. Illumina recommends using the EZ DNA Methylation-Gold Kit or the EZ DNA Methylation-Lightning Kit (Zymo Research). Either kit can be used successfully for treating human DNA. Use the EZ DNA Methylation-Gold Kit with plant DNA.

The *TruSeq DNA Methylation Kit Protocol* on page 7 provides detailed information about bisulfite treatment of the sample DNA.

Follow the TruSeq DNA Methylation Kit Procedure Closely

The kit reagents have been formulated and optimized for best performance in the following conditions. Any variations in the protocol can lead to less than optimal results.

- 1 Input amount: 50–100 ng of DNA (before bisulfite treatment)
- 2 PCR cycles: 10
- 3 Use AMPure Beads for the pre- and post-PCR clean-up steps.

Control DNA (Optional)

If desired, use 10 ng of genomic DNA as nonbisulfite-treated Control DNA. There is no need to shear or fragment the Control DNA.

Adding an Index to the TruSeq DNA Methylation Kit Library

If desired, an index or user-defined barcode can be added to the TruSeq DNA Methylation Kit sequencing library during PCR in *Amplify the Library and Add an Index (Barcode)* on page 12. If adding an index or barcode, read this part of the procedure carefully.

Quality Control of Bisulfite-Converted DNA

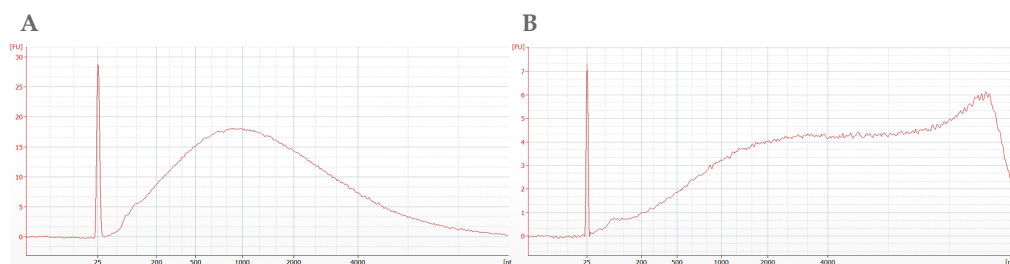
Assess the quality of the BC-SS DNA samples using an Agilent 2100 Bioanalyzer with a RNA-Pico Chip, Eukaryotic Total RNA setting. Load 1 μ l of the undiluted sample. Visually evaluate trace profiles for typical EZ-DNA Methylation–Gold or –Lightning-treated samples (Figure 1).



NOTE

DNA quantification is not required for postbisulfite-conversion steps. Optional quantification can be assessed using a NanoDrop instrument (RNA setting). Qubit quantification is not recommended due to the incompatibility of RNA/DNA standards with BC-SS DNA.

Figure 1 Representative 2100 Bioanalyzer Profiles of Bisulfite-Treated DNA

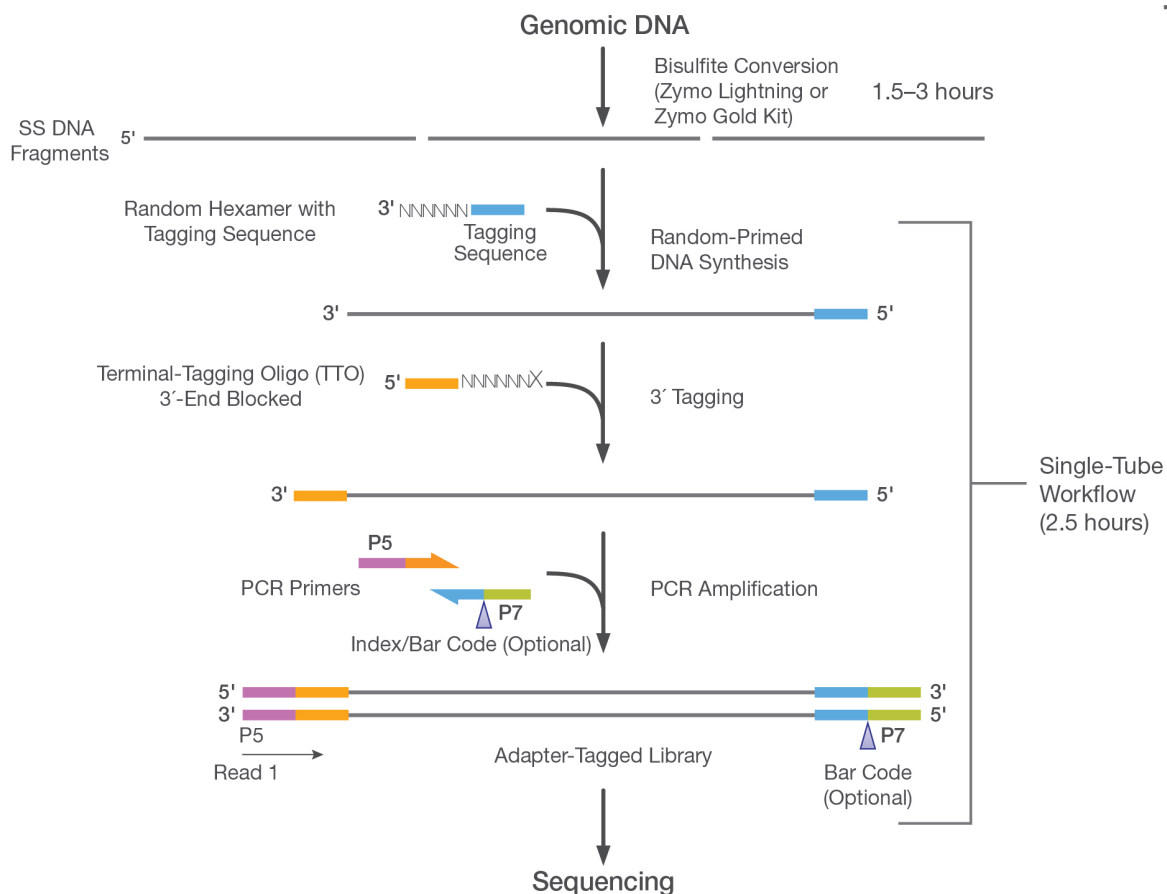


BC-SS DNA samples were assessed using an Agilent 2100 Bioanalyzer with an RNA Pico Chip. A 50 ng sample of Coriell DNA was treated with the indicated methylation kit (Zymo Research):

- A** EZ DNA Methylation–Gold Kit
- B** EZ DNA Methylation–Lightning Kit

TruSeq DNA Methylation Kit Protocol

Figure 2 Overview of the TruSeq DNA Methylation Kit Workflow



Bisulfite Treatment of the Sample DNA

The sample DNA must be treated with bisulfite before the TruSeq library prep procedure. Do not treat the Control DNA with bisulfite.

Illumina recommends using the EZ DNA Methylation–Gold Kit or the EZ DNA Methylation–Lightning Kit (Zymo Research). Either kit can be used successfully for treating human DNA.



CAUTION

Use only the EZ DNA Methylation–Gold Kit with plant DNA.

Consumables

Item	Quantity	Storage	Supplied By
0.5 or 1.5 ml microcentrifuge tubes			User
EZ DNA Methylation–Gold or EZ DNA Methylation–Lightning Kit	1 Kit		User

Procedure

- 1 Use 50–100 ng of DNA. For most accurate results, quantify the DNA using a NanoDrop spectrophotometer.
- 2 Treat 50–100 ng of the DNA following the manufacturer's protocol, except adjust the final column purification elution volume to 9 μ l.
- 3 Quantify the recovered BC-SS DNA using a NanoDrop spectrophotometer with the RNA setting. The bisulfite-treated DNA is single-stranded and contains uracil, so it resembles RNA more than DNA. **Expect ~80% recovery.**
- 4 Use the entire amount of the purified BC-SS DNA in the TruSeq DNA Methylation Kit procedure beginning at *Anneal the DNA Synthesis Primer* on page 8.

TruSeq DNA Methylation Kit Procedure

Remove all components of the TruSeq DNA Methylation Kit (except enzymes), allow them to thaw, and store them on ice. Centrifuge briefly to collect liquid at bottom of tube. Illumina highly recommends storing enzyme solutions in a benchtop cooler (-20°C) to avoid repeated freeze-thaw cycles.

Anneal the DNA Synthesis Primer

Consumables

Item	Quantity	Storage	Supplied By
PCR tubes			User
DNA Synthesis Primer	2 μ l per reaction	-20°C	Illumina

Procedure

- 1 Assemble the following reaction mixture in a PCR tube appropriate for your thermal cycler.

Reagent	Volume (μ l)
Bisulfite-treated DNA or Control DNA	9
DNA Synthesis Primer	2
Total Volume per Sample	40

- 2 Incubate at 95°C for 5 minutes in a thermal cycler with a *heated* lid.
- 3 Immediately place the reaction tube in an ice/water bath.

Synthesize DNA

Consumables

Item	Quantity	Storage	Supplied By
PCR tubes			User
TruSeq DNA Synthesis PreMix	2 µl per reaction	-20°C	Illumina
100 mM DTT	0.5 µl per reaction	-20°C	Illumina
TruSeq Polymerase	0.5 µl per reaction	-20°C	Illumina
Exonuclease I	1 µl per reaction	-20°C	Illumina

Procedure

- 1 On ice, prepare the following mastermix:

Reagent	Volume (µl)
TruSeq DNA Synthesis PreMix	4
100 mM DTT	0.5
TruSeq Polymerase	0.5
Total Volume per Sample	5

Gently but thoroughly mix the mastermix by pipetting.

- 2 Add 5 µl of the mastermix to each reaction on ice from *Anneal the DNA Synthesis Primer* on page 8 and mix by pipetting.
- 3 Incubate in a thermal cycler as follows:
 - 25°C, 5 minutes
 - 42°C, 30 minutes
 - 37°C, 2 minutes
 Pause the thermal cycler.
- 4 Remove one reaction at a time from the thermal cycler and add 1.0 µl of Exonuclease I. Mix gently but thoroughly by pipetting. Return each reaction to the thermal cycler.
- 5 Incubate in a thermal cycler as follows:
 - 37°C for 10 minutes
 - 95°C for 3 minutes
 - 25°C for 2 minutes



NOTE

During the 95°C incubation, prepare the TT mastermix as described in *Tag the DNA* on page 10.

Tag the DNA

Consumables

Item	Quantity	Storage	Supplied By
PCR tubes			User
Wide-bore pipet tips			User
TruSeq Terminal Tagging PreMix	7.5 µl per reaction	-20°C	Illumina
TruSeq DNA Polymerase	0.5 µl per reaction	-20°C	Illumina

Procedure



CAUTION

The TruSeq Terminal Tagging PreMix is a *highly viscous* solution. Mix it thoroughly before use by pipetting slowly several times.

Illumina strongly recommends using a wide-bore pipet tip when pipetting the Terminal Tagging PreMix and the TT mastermix.

- 1 On ice, prepare the TT mastermix.
For each reaction, combine on ice:

Reagent	Volume (µl)
TruSeq Terminal Tagging PreMix	7.5
TruSeq DNA Polymerase	0.5
Total Volume per Sample	8.0

Thoroughly mix the *viscous* TT mastermix by pipetting or by flicking the tube, followed by brief centrifugation to collect droplets in the tube.

- 2 Remove one reaction at a time from the thermal cycler (from *Synthesize DNA* on page 9) and add 8.0 µl of the TT mastermix. Gently but thoroughly mix the reaction by pipetting. Return each reaction to the thermal cycler and incubate:
 - 25°C for 30 minutes
 - 95°C for 3 minutes
 - Hold at 4°C

Purify the Tagged DNA


Each reaction is now in a volume of 25 µl. The DNA must be purified before PCR amplification. **Illumina recommends using a 1.6X AMPure XP Bead purification.**

If using the AMPure XP System, the purification can be done in a plate or in the microcentrifuge tubes containing the ditagged DNA from *Tag the DNA* on page 10.

Consumables

Item	Quantity	Storage	Supplied By
0.2 ml or 1.5 ml microcentrifuge tubes, or multiwell plate			User
AMPure XP System			User
80% (v/v) ethanol, freshly prepared		Room temperature	User
Nuclease-Free Water	24.5 µl per reaction	-20°C	Illumina

Procedure

- 1 Warm the AMPure XP Beads to room temperature. While the beads warm, prepare 400 µl of fresh 80% ethanol at room temperature for each sample.
 - 2 If using a plate format, transfer each ditagged DNA from *Tag the DNA* on page 10 into a separate well of the plate.
 - 3 Vortex the AMPure XP Beads until they are a homogenous suspension.
-  **CAUTION**
Make sure that the AMPure XP Beads are in a homogenous suspension before continuing.
- 4 Add 40 µl of the beads to each well of the plate or to each microcentrifuge tube containing ditagged DNA from *Tag the DNA* on page 10.
 - 5 Mix thoroughly by gently pipetting the entire volume of each well/tube 10 times.
 - 6 If using microcentrifuge tubes, transfer each 65 µl volume to a separate 1.5 ml tube.
 - 7 Incubate the plate/tubes at room temperature for 5 minutes.
 - 8 Place the plate/tubes in a magnetic stand at room temperature for at least 5 minutes, until the liquid appears clear.
 - 9 Remove and discard the supernatant from each well/tube using a pipette. Some liquid can remain in each well/tube. Take care not to disturb the beads.
 - 10 With the plate/tubes remaining on the magnetic stand, add 150–200 µl of 80% ethanol to each well/tube without disturbing the beads. Make sure that the beads are covered with 80% ethanol.
 - 11 Incubate the plate/tubes at room temperature for at least 30 seconds, then remove and discard all of the supernatant. Take care not to disturb the beads.
 - 12 Repeat steps 10 and 11 one more time for a total of two 80% ethanol washes
 - 13 After the second wash, remove the ethanol by pipetting (as much as possible without disturbing the beads) with the plate/tubes still on the magnetic stand. Remove the plate/tubes, centrifuge for 10–30 seconds, and place the plate/tubes back on the magnetic stand for 1 minute. Use a fine pipette tip to remove all the residual ethanol. Let the wells/tubes air-dry for 3 minutes on the magnetic stand.
 - 14 Add 24.5 µl of Nuclease-Free Water to each well/tube, and remove the plate/tubes from the magnetic stand.
 - 15 Thoroughly resuspend the beads by gently pipetting 10 times.

- 16 Incubate the plate/tubes at room temperature for 2 minutes.
- 17 Place the plate/tubes on the magnetic stand at room temperature for at least 5 minutes, until the liquid appears clear.
- 18 Transfer 22.5 µl of the clear supernatant, which contains the ditagged DNA, from each well/tube to a new PCR plate/tube.
- 19 Place the plate/tubes on ice and proceed to *Amplify the Library and Add an Index (Barcode)* on page 12 or keep at -20°C for longer-term storage.

Amplify the Library and Add an Index (Barcode)

This step generates the second strand of DNA, completes the addition of the Illumina adapter sequences, incorporates an index if desired, and amplifies the library by PCR.

Adding an Index Read

The standard TruSeq DNA Methylation Kit reaction using the Reverse PCR Primer that is included in the kit produces a nonbarcoded library.

To add:

- ▶ An Illumina index, replace the Reverse PCR Primer that is included in this kit with one of the TruSeq DNA Methylation Kit Index PCR Primers. Only the TruSeq DNA Methylation Kit Index PCR Primers (available separately; Catalog No. EGIDX81312) are compatible with TruSeq DNA Methylation Kit procedure.
- ▶ A user-defined barcode, see *Adding a User-Defined Barcode to the Library* on page 17.

Consumables

Item	Quantity	Storage	Supplied By
0.2 ml or 0.5 ml PCR tubes			User
FailSafe PCR Enzyme	1.5 U per reaction	-20°C	User
FailSafe PCR PreMix E	25 µl per reaction	-20°C	Illumina
TruSeq Forward PCR Primer	1 µl per reaction	-20°C	Illumina
TruSeq Reverse PCR Primer	1 µl per reaction	-20°C	Illumina

Procedure



NOTE

FailSafe PCR Enzyme is required for this step.

Optional: TruSeq Index PCR Primers



CAUTION

If you are adding an index or user-defined barcode to the library, do not use the Reverse PCR Primer that is included in this kit. Instead, use the index from the TruSeq Index Kit as the Reverse PCR Primer in this procedure.

- 1 In a PCR tube containing 22.5 µl of purified, di-tagged DNA from *Purify the Tagged DNA* on page 10, add on ice:

Reagent	Volume (µl)
FailSafe PCR PreMix E	25

Reagent	Volume (μl)
TruSeq Forward PCR Primer	1
TruSeq Reverse PCR Primer (or TruSeq Index PCR Primer or user-defined barcode)	1
FailSafe PCR Enzyme (1.25 U)	0.5
Total Volume per Sample	50

- Denature the double-stranded DNA at 95°C for 1 minute. Perform PCR for both the bisulfite-treated DNA and for the Control DNA as follows:
 - 10 cycles of:
 - 95°C for 30 seconds
 - 55°C for 30 seconds
 - 68°C for 3 minutes
 - 68°C for 7 minutes


Purify the TruSeq DNA Methylation Kit Library

Use the AMPure XP System to purify the TruSeq DNA Methylation Kit libraries. The AMPure XP System is optimal for removing primer-dimers that can occur during PCR. This procedure uses 1.0X AMPure XP Bead purification.

Consumables

Item	Quantity	Storage	Supplied By
0.2 ml or 1.5 ml microcentrifuge tubes, or multiwell plate			User
AMPure XP System			User
80% (v/v) ethanol, freshly prepared		Room temperature	User
Nuclease-Free Water	20 μl per reaction	-20°C	Illumina

Procedure

- Warm the AMPure XP Beads to room temperature. While the beads warm, prepare 400 μl of fresh 80% ethanol at room temperature for each sample.
 - If using a plate format, transfer each amplified library from *Amplify the Library and Add an Index (Barcode)* on page 12 into a separate well of the plate.
 - Vortex the AMPure XP Beads until they are in a homogeneous suspension.
-  **CAUTION**
Make sure the AMPure XP Beads are in a homogenous suspension before continuing.
- Add 50 μl of the beads to each well of the plate or to each microcentrifuge tube containing the amplified library from *Amplify the Library and Add an Index (Barcode)* on page 12.
 - Mix thoroughly by gently pipetting the entire volume up of each well/tube 10 times.
 - If using microcentrifuge tubes, transfer each 100 μl volume to a separate 1.5 ml tube.

- 7 Incubate the plate/tubes at room temperature for 5 minutes.
- 8 Place the plate/tubes in a magnetic stand at room temperature for at least 5 minutes, until the liquid appears clear.
- 9 Remove and discard the supernatant from each well/tube using a pipette. Some liquid can remain in each well/tube. Take care not to disturb the beads.
- 10 With the plate/tubes remaining on the magnetic stand, add 150–200 μ l of 80% ethanol to each well/tube without disturbing the beads. Make sure that the beads are covered with 80% ethanol.
- 11 Incubate the plate/tubes at room temperature for at least 30 seconds, then remove and discard all of the supernatant. Take care not to disturb the beads.
- 12 Repeat steps 10 and 11 one more time for a total of two 80% ethanol washes.
- 13 After the second wash, remove the ethanol by pipetting (as much as possible without disturbing the beads) with the plates/tubes still on the magnetic stand. Remove the plates/tubes, centrifuge for 10–30 seconds, and place the plates/tubes back on the magnetic stand for 1 minute. Use a fine pipette tip to remove all the residual ethanol. Let the wells/tubes air-dry for 3 minutes on the magnetic stand.
- 14 Add 20 μ l of Nuclease-Free Water to each well/tube, and remove the plate/tubes from their magnetic stand.
- 15 Thoroughly resuspend the beads by gently pipetting 10 times.
- 16 Incubate the plate/tubes at room temperature for 2 minutes.
- 17 Place the plate/tubes on the magnetic stand at room temperature for at least 5 minutes, until the liquid appears clear.
- 18 Transfer the clear supernatant, which contains the TruSeq DNA Methylation Kit library, from each well/tube to an appropriate collection tube for assessment of library quantity and quality.

Validate Libraries

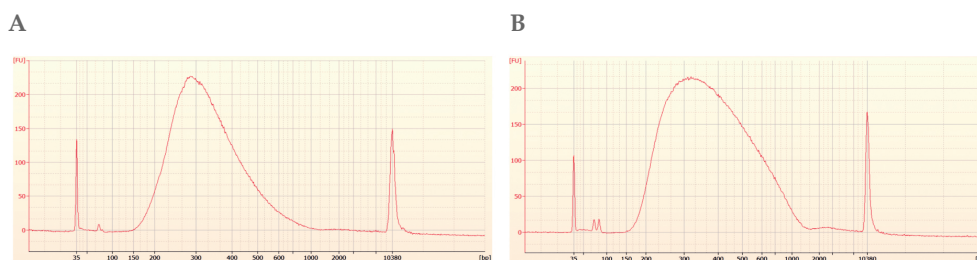
Quantify Libraries

Quantify the converted and nonconverted double-stranded DNA libraries using a fluorometric method (eg, the Qubit High-Sensitivity DNA Kit or PicoGreen) or qPCR. Expected yields will be > 3 ng/μl. A total of 3 ng is required to run a sample on an entire HiSeq flow cell (8 lanes).

Quality Control

Assess library quality using an Agilent 2100 Bioanalyzer High-Sensitivity DNA Chip (load 1 μl of the undiluted sample). Typical libraries show a broad size distribution from ~150 bp to 1,000 bp (representing median insert sizes of 260–380 bp after subtracting forward/reverse adapter sequences).

Figure 3 Representative 2100 Bioanalyzer Profiles of TruSeq DNA Methylation Kit Libraries



Bisulfite conversion of DNA was performed using the EZ DNA Methylation–Lightning Kit (Zymo Research). TruSeq DNA Methylation Kit libraries were prepared from bisulfite-treated and control DNA with 10 PCR cycles, and assessed using an Agilent 2100 Bioanalyzer with a High Sensitivity DNA Chip. TruSeq DNA Methylation Kit libraries prepared using the EZ DNA Methylation–Gold Kit for bisulfite treatment produced identical results.

- A** TruSeq DNA Methylation Kit library from 50 ng of bisulfite-treated Coriell DNA
- B** TruSeq DNA Methylation Kit library from 10 ng of control untreated Coriell DNA

Sequencing the TruSeq DNA Methylation Kit Library

TruSeq DNA Methylation Kit libraries are compatible with Illumina SBS and Cluster Kits and can be sequenced on any Illumina platform.

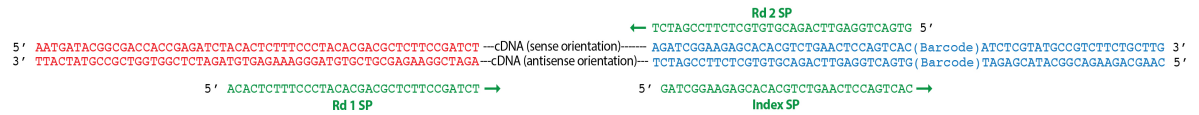


Figure 4 Sequencing the TruSeq DNA Methylation Kit Library

- Red** = Sequence incorporated by the Terminal Tagging process and PCR amplification
- Blue** = Sequence incorporated during reverse transcription and PCR amplification
- Black** = Sequence of the DNA
- Rd 1 SP** = Read 1 Sequencing Primer
- Rd 2 SP** = Read 2 Sequencing Primer
- Index SP** = First nucleotide read is that of the index or barcode

Adding a User-Defined Barcode to the Library

The Reverse PCR Primer adds a barcode in *Amplify the Library and Add an Index (Barcode)* on page 12 of the procedure. Before using a custom barcode as the Reverse PCR Primer, the oligonucleotide must be synthesized, HPLC purified, and desalted.

The user-defined Reverse PCR Primer(s) must be the following sequence:

5' -CAAGCAGAAGACGGCATACGAGAT--X--
GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'

where **X** is the desired barcode's reverse complement.

Dissolve the primer(s) to a final concentration of 10 μ M in Nuclease-Free Water.



CAUTION

The user-defined barcode sequence of the custom synthesized Reverse PCR Primer should be the reverse complement of the sequence read. For example, using the Illumina Multiplexing Index Read Sequencing Primer, the user-defined barcode sequence:

5' ...ACGTAC... will be read as ...GTACGT... 3'

Contact *Technical Assistance* on page 19 if you have questions about adding user-defined barcodes or synthesizing custom reverse PCR primers.

TruSeq DNA Methylation Kit Index PCR Primers

TruSeq DNA Methylation Kit Index PCR Primers are available separately (Catalog No. EGIDX81312). The sequence of each Index PCR Primer is:

5' CAAGCAGAAGACGGCATACGAGAT NNNNNN GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'

where NNNNNN is the reverse complement of the index sequence generated during sequencing.

Index Sequence Generated During Sequencing

Index 1 PCR Primer	5' -ATCAGC-3'	Index 7 PCR Primer	5' -CAGATC-3'
Index 2 PCR Primer	5' -CGATGT-3'	Index 8 PCR Primer	5' -ACTTGA-3'
Index 3 PCR Primer	5' -TTAGGC-3'	Index 9 PCR Primer	5' -GATCAG-3'
Index 4 PCR Primer	5' -TGACCA-3'	Index 10 PCR Primer	5' -TAGCTT-3'
Index 5 PCR Primer	5' -ACAGTG-3'	Index 11 PCR Primer	5' -GGCTAC-3'
Index 6 PCR Primer	5' -GCCAAT-3'	Index 12 PCR Primer	5' -CTTGTA-3'

Pooling Multiplexed TruSeq DNA Methylation Kit Libraries

Illumina sequencing systems use a green laser to read G/T nucleotides and a red laser to read A/C nucleotides. At least 1 nucleotide for each color channel must be read in each sequencing cycle 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.

Suggested Options for Multiplexing TruSeq DNA Methylation Kit Libraries

- ▶ Index PCR Primers 1, 4, 8
- ▶ Index PCR Primers 6, 12
- ▶ Index PCR Primers 5, 10, 11

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 4 Illumina General Contact Information

Address	5200 Illumina Way San Diego, CA 92122 USA
Website	www.illumina.com
Email	techsupport@illumina.com

Table 5 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then click **Documentation & Literature**.



Illumina

5200 Illumina Way

San Diego, California 92122 U.S.A.

+1.800.809.ILMN (4566)

+1.858.202.4566 (outside North America)

techsupport@illumina.com

www.illumina.com