xGen™ Adaptase™ Module

REVISION HISTORY

Version	Release date	Description of changes
2	April 2022	Adaptase adapter name update
1	December 2021	Initial release

Table of contents

OVERVIEW

The Adaptase™ technology simultaneously performs tailing and ligation to incorporate a stubby P7 adapter to the 3' ends of single-stranded DNA, and the protocol takes about 30 minutes to complete. The xGen™ Adaptase™ Module contains enough reagents for the preparation of 96 libraries, including 10% overage volume.

Use the xGen Adaptase Module together with the Single-Cell Methyl-Seq Library Preparation Protocol described in Appendix A in this guide. Further description of this protocol and its use with single nucleus methylcytosine sequencing (snmC-Seq) can be found in the publication by Luo, et. al. "Single Cell Methylomes Identify Neuronal Subtypes and Regulatory Elements in Mammalian Cortex" *Science*, 2017 [1] and the updated methods published for snmC-Seq 2, also by Luo, et. al. "Robust single-cell DNA methylome profiling with snmC-seq2" *Nature Communications*, 20 September 2018 [2].

When performing a full, single-cell workflow, use the instructions provided in the Appendix A of this protocol as a guideline. It is based on the original snmC-Seq workflow. For best results, see the updates published in the subsequent snmC-Seq2 publication, such as use of H-random primers (G-depleted) to reduce artifacts and increase library complexity, and an increase in the multiplexing capacity with additional, validated, in-line barcodes.

Because Adaptase performs with high efficiency and is template independent, this module can support numerous upstream and downstream research applications. In the Appendix A, a method for single-cell methylation sequencing is described, but the kit is not limited to this application alone.

Important: This xGen Adaptase Module Kit includes only the Adaptase reagents. Additional components and reagents for your desired research application must be purchased separately.

Supported applications

- Cataloging cell populations within heterogeneous tissues
- Assessing normal tissue for regulation of cellular mechanisms
- Exploring across species to identify evolutionary conservation of epigenomic regulation

CONSUMABLES AND EQUIPMENT

Consumables from IDT—Kit contents

	Component	Volume 96 rxn	Storage
Adaptase	• Buffer G1	211 µL	–20°C
	• Reagent G2	211 µL	
	• Reagent G3	132 µL	
	• Enzyme G4	52 µL	
	• Enzyme G5	52 µL	
Controls	1 (20 bases)	15 µL	
	2 (50 bases)	15 µL	
	3 (70 bases)	15 µL	
Other reagents	Low EDTA TE	24 mL	Room temperature

Consumables from other suppliers

Item	Supplier	Catalog number
MicroAmp® EnduraPlate™ Optical 96-well Clear GPLE Reaction Plate with Barcode	Thermo Fisher Scientific	4483348
MicroAmp clear adhesive film	Thermo Fisher Scientific	4306311
96-well PCR plates	Various suppliers	Varies
DNA LoBind® tubes, 1.5 mL	Various suppliers	Varies
PCR tubes, 0.2 mL	Various suppliers	Varies
Aerosol-resistant, low-retention pipettes and tips, 2–1000 µL	Various suppliers	Varies

Equipment

Item	Supplier	Catalog number
Centrifuge with 96-well plate compatibility	Various suppliers	Varies
Microcentrifuge	Various suppliers	Varies
Programmable thermal cycler	Various suppliers	Varies
Vortex	Various suppliers	Varies

GUIDELINES

Reagent handling

Store the xGen Adaptase Module reagents at –20˚C, except for the Low EDTA TE which should be stored at room temperature.

For all non-enzyme reagents, thaw on ice, then briefly vortex to mix well. Remove enzyme tubes from –20°C storage and place on ice 10 minutes before use. Attempting to pipette enzymes at –20°C may result in reagent shortage. Spin all tubes in a microcentrifuge to collect contents before opening.

To create the Master Mix, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Add reagents in the order listed ON ICE, then pulse-vortex to mix and briefly centrifuge.

Avoid cross-contamination

Important: To reduce the risk of DNA and/or library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed.

Also, use these best practices while performing this protocol:

- Clean lab areas and equipment using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change tips between each sample.

Automation of workflow

This protocol is readily automatable. A 10% overage volume of reagents is supplied to accommodate for automation. Contact us at applicationsupport@idtdna.com if you require additional reagent overage volume or would like to learn about our custom packaging options.

While IDT does not supply automated liquid handling instruments, or consumables, our automation team collaborates with automation solution providers and customers to develop automated scripts with liquid handling platforms routinely used with our kits in NGS library preparation. Contact us at applicationsupport@idtdna.com to discuss automating your xGen Adaptase Module kit with your automated liquid handling system.

PROTOCOL

Perform Adaptase reaction

1. Preheat thermocycler to 95°C for denaturation of the samples.
2. Use the table below to preassemble the Adaptase Master Mix on ice. Mix thoroughly and pulse-spin to collect contents.

   Note: Do NOT add the Adaptase Master Mix to the samples until you have run the denaturation program. Keep the Adaptase Master Mix on ice until step 5 below.

Component	Volume per reaction (µL)
• Low EDTA TE	4.25
• Buffer G1	2.0
• Reagent G2	2.0
• Reagent G3	1.25
• Enzyme G4	0.5
• Enzyme G5	0.5
Total volume	10.5

3. Add 10 µL of each sample to a low nucleic acid binding PCR plate, or 0.2 mL PCR tube.

   Note: Samples should be suspended in Low EDTA TE.

4. Vortex the Adaptase Master Mix for 10 seconds, then briefly centrifuge.
5. When the thermal cycler has reached 95°C, add sample tubes and run the following thermal cycler program (lid heating ON):

Run the denaturing program

Step	Temperature*	Time
1	95°C	3 min

* Set the lid temperature to 105°C.

6. After denaturing the samples, immediately place on ice for two minutes and pre-heat thermal cycler to 37°C. Proceed immediately to the next step, adding the Adaptase Master Mix, to preserve the maximum amount of ssDNA substrate.
7. Add 10.5 µL of Adaptase Master Mix to each sample. Pipette mix, or mix by gentle vortexing. Briefly centrifuge to collect contents.
8. Place the samples in the preheated thermal cycler, then run the following Adaptase program:

Adaptase program

Step	Temperature*(°C)	Time
1	37	30 min
2	95	2 min
3	4	Hold

* Set the lid temperature to 105°C.

9. After the Adaptase program reaches 4˚C, you can proceed with your intended downstream application.

EXPECTED RESULTS

This kit includes three Adaptase control substrates to use when evaluating reagent performance. Each control is an ssDNA substrate with a 5′ stubby Illumina® P5 adapter sequence, and a 3′ sequence that is:

- 20 bases for Control 1
- 50 bases for Control 2
- 70 bases for Control 3

The Adaptase controls are provided at a 3 µM concentration.

Note: Do not combine substrates; if combined, they will concatemerize.

Example experiment

For the experiment illustrated below, 1 µL of each control substrate was added to individual Adaptase reactions with 9 µL Low EDTA TE. The protocol was followed for Denaturation, Adaptase, and 6 cycles of PCR amplification using the PCR conditions recommended in Appendix A. A post-PCR bead cleanup, using 40 µL beads (ratio: 0.8X), was eluted in 20 µL Low EDTA TE. Products were run on a high-sensitivity Bioanalyzer® chip (Agilent). Peaks were visible at ~170 bp, 200 bp, and 220 bp. Individual controls, or all three at once, can be used to test the Adaptase Module performance. The 20 bp substrate produces a lower yield due to size selection during the 0.8X SPRI cleanup.

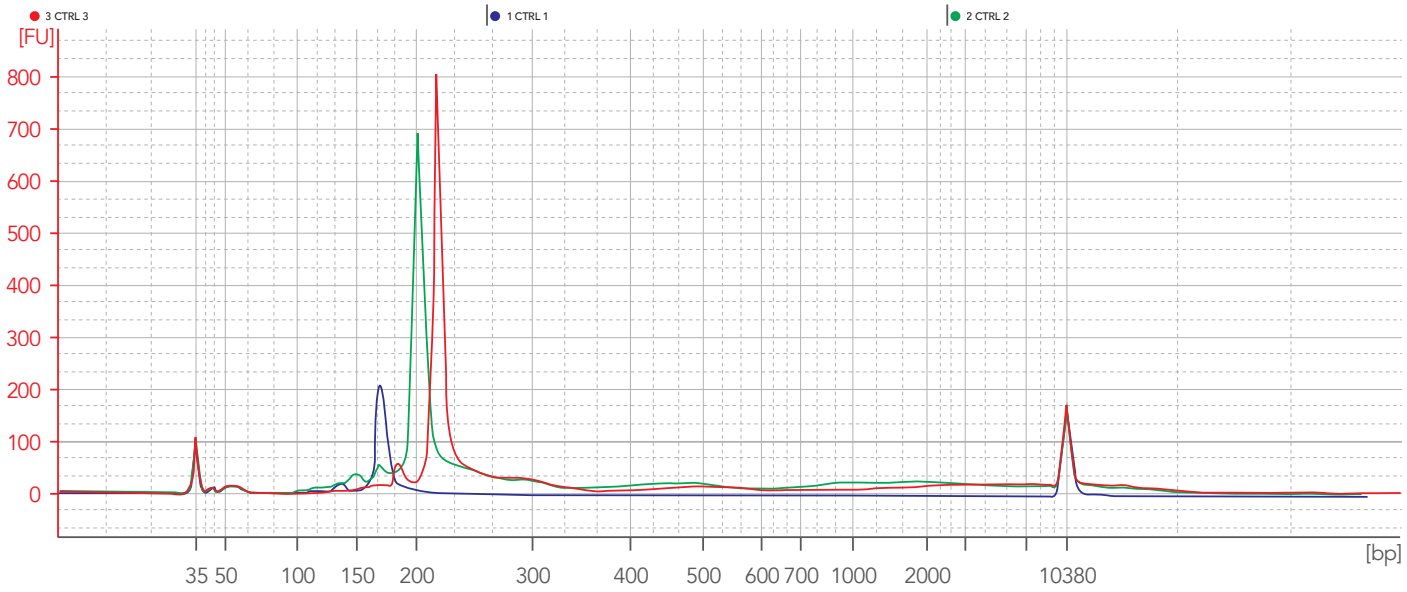

Figure 1. xGen Adaptase Module Bioanalyzer results. Expected results for xGen Adaptase Module, produced from control substrates with 3′ sequences of 20 bases (n=1), 50 bases (n=1), or 70 bases (n=1) in length. Samples were run on the Bioanalyzer with an HS DNA chip.

Appendix A: Single-cell Methyl-Seq (snmC-Seq) workflow

Overview

After completing single cell/nucleus isolation, lysis, and bisulfite conversion, the major steps to perform this protocol are:

Random priming. The random priming step generates a uracil-free strand, incorporates R1 Stubby Adapter to the 5′ ends of primer extension products, and reduces fragment size to ~400 bases for compatibility with Illumina® sequencing. Optionally, indexed, random primers can be used for downstream multiplexing capability.

Cleanup. An enzymatic step using Exonuclease I and Shrimp Alkaline Phosphatase is performed to digest unused, random primers and inactivate dNTPs, followed by a magnetic bead-based cleanup step such as AMPure (Beckman Coulter) or SPRISelect (Beckman Coulter).

Adaptase. The Adaptase reaction simultaneously performs tailing and ligation of R2 Stubby Adapter to the 3′ ends in a template-independent manner.

Indexing PCR. PCR is used to incorporate sample indexes and sequences necessary for Illumina® sequencing. Indexing primers must be custom ordered; see Appendix B.

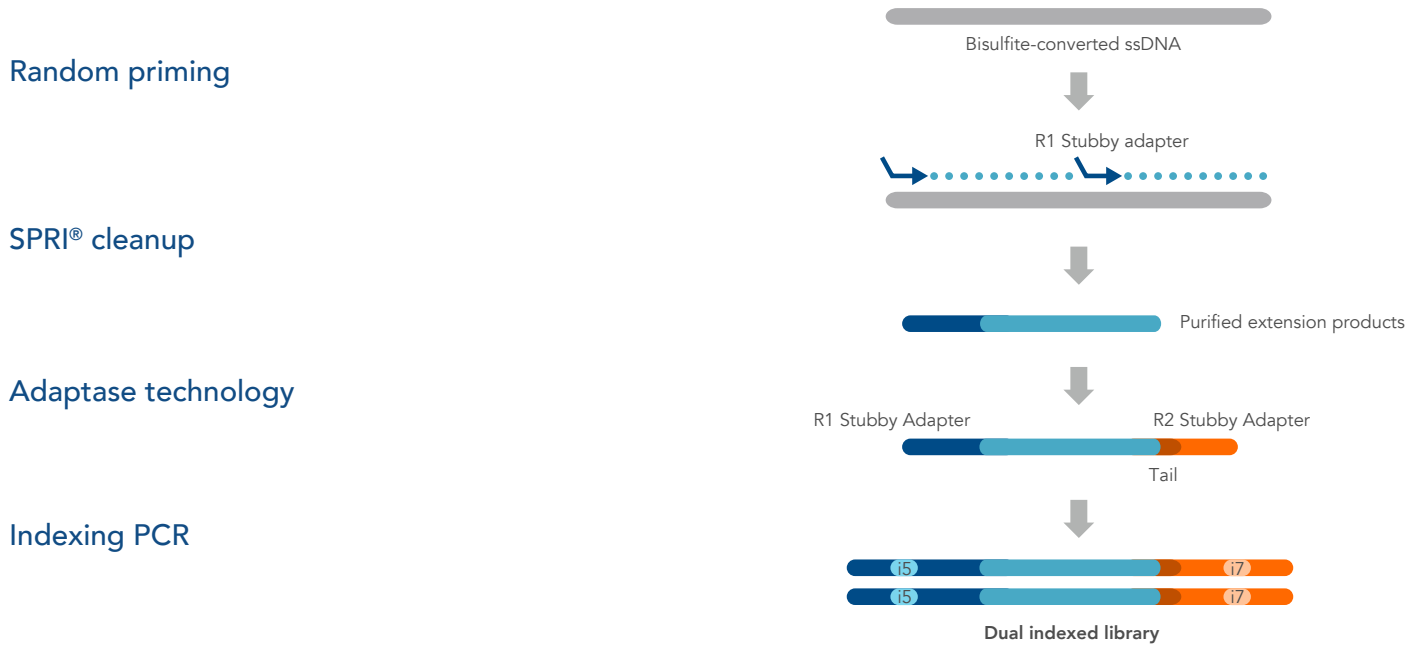

Figure 2. The xGen Adaptase Module workflow. Dual indexed libraries are produced from ssDNA in these main steps: 1) Random priming incorporates an R1 Stubby Adapter and creates fragments of the correct size for sequencing; 2) SPRI cleanup with magnetic beads; 3) addition of the R2 Stubby Adapter to the 3′ end of the fragment using Adaptase; and 4) indexing PCR.

Consumables from other suppliers

Item	Supplier	Catalog number
Purification beads: SPRISelect® OR AMPure®	Beckman Coulter	B23317/B23318/ B23319 A63880/A63881/ A63882
Digital electrophoresis chips and associated reagents (choose one):		
Experion® DNA 1K Analysis Kit	Bio-Rad	700-7107
High Sensitivity DNA Kit	Agilent	5067-4626
High Sensitivity D1000 ScreenTape®, or equivalent	Agilent	5067-5584
KAPA® Biosystems Library Quantification Kit–Illumina/Universal	Roche	KK4824
KAPA® HiFi HotStart ReadyMix	Roche	KK2602
EZ-96 DNA Methylation-Direct™ Kit (deep-well format)	Zymo Research	D5023
MicroAmp® EnduraPlate™ Optical 96-well Clear GPLE Reaction Plate with Barcode	Thermo Fisher Scientific	4483348
MicroAmp® Clear Adhesive Film	Thermo Fisher Scientific	4306311
Random primer(s) with a P5 adapter 5' tail sequence (see **Appendix B**)		
Klenow® Exo-DNA Polymerase (high concentration 50 U/µL), supplied with Blue Buffer	Enzymatics	P7010-HC-L
Exonuclease I (20 U/µL)	Enzymatics	X8010F
Shrimp Alkaline Phosphatase	New England BioLabs	M0371
Custom P5 and P7 indexing PCR primers (see **Library amplication primers**)		
Electrophoresis apparatus for agarose gels		
QIAGEN® QIAquick® Gel Extraction Kit, or equivalent	Qiagen	28704
dNTPs	Various suppliers	Varies
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851 or Q32584
96-well PCR plates	Various suppliers	Varies
DNA LoBind® tubes, 1.5 mL	Various suppliers	Varies
PCR tubes, 0.2 mL	Various suppliers	Varies
Serological pipettes (5–25 mL)	Various suppliers	Varies
50 mL conical tubes	Various suppliers	Varies
Aerosol-resistant, low-retention pipettes and tips, 2–1000 µL	Various suppliers	Varies
Absolute ethanol (200-proof)	Various suppliers	Varies
Nuclease-free water	Various suppliers	Varies

Consumables from other suppliers (continued)

Item	Supplier	Catalog number
FACS or other method for isolation and distribution of single cells	Various suppliers	Varies
Centrifuge with 96-well plate compatibility	Various suppliers	Varies
Magnetic rack for plates	Permagen	MSP750
Fluorometer (Qubit™ , Thermo Fisher) or similar input DNA quantification instrument	Various suppliers	Varies
Digital electrophoresis instrument for library size determination	Various suppliers	Varies
Quantitative PCR instrument	Various suppliers	Varies
Microcentrifuge	Various suppliers	Varies
Programmable thermal cycler	Various suppliers	Varies
Vortex	Various suppliers	Varies

Protocol

1. Before you start library preparation, prepare the Zymo lysis buffer with proteinase K by following the Zymo EZ96 manufacturer's instructions. Preload 2 µL of the Zymo lysis buffer to each well of the 96-well plate to be used for cell sorting. After sorting, heat the plate at 50°C for 20 min, again, following the direct kit manual protocol. Elute the bisulfite-converted DNA from the Zymo I-96 spin-plate with 12 µL Low EDTA TE, which will result in a 9 µL eluate for each single cell (with 3 µL excess volume).
2. Place samples in thermal cycler and run the Denaturation Program.

Denaturation Program

Step	Temperature*	Time
1	95°C	3 min

* Set the lid temperature to 105°C.

3. After the Denaturation program ends, immediately place samples on ice for 2 minutes.
4. To set up the Random priming step, briefly spin the plate, unseal, add P5L random primer to each well of the 96-well plate containing the single cell samples, and seal the plate.

Reagent	Volume (µL)	Final concentration
P5L random primer (5 µM)	1	250 nM

* Set the lid temperature to 105°C.

Note: For downstream 4-plex multiplexing, use random primers that incorporate AD002, AD006, AD008, and AD010 (see Appendix B). Alternatively, if not multiplexing, add a P5L non-indexed random primer.

5. Prepare the Random Priming Master Mix and keep on ice:

Reagent	Volume (µL)
Blue Buffer (10×)	2
Klenow exo- (50 U/µL)	1
dNTP (10 mM each)	1
H_2O	6
Total volume	**10**

6. Place samples containing the random primers in a thermal cycler and run the Denaturation Program.

Denaturation Program

Step	Temperature*	Time
1	95°C	3 min

* Set the lid temperature to 105°C.

7. After the Denaturation Program ends, immediately place samples on ice for 2 minutes.
8. Briefly spin the plate, unseal, and add 10 µL of the Random Priming Master Mix to each well of the 96-well plate. Seal the plate. Mix the reactions by gently vortexing the plate, then quickly spin the plate to collect contents.
9. Use a thermal cycler to run the Random Priming Program.

Random Priming Program

Step	Temperature (°C)	Time
1	4	5 min
	Ramp 0.1°C/s to 25	
2	25	5 min
	Ramp 0.1°C/s to 37	
3	37	60 min
4	4	Hold

10. Briefly spin the plate, unseal, and add Exonuclease I and Shrimp Alkaline Phosphatase to each well of the 96-well plate. Seal the plate. Mix the reactions thoroughly by gently vortexing the plate, then quickly spin the plate to collect contents.

Reagent	Volume (µL)
Exonuclease I (20 U/µL)	2
Shrimp Alkaline Phosphatase	1
Total volume	**3**

11. Use a thermal cycler to run the Enzymatic Digestion Program.

Enzymatic Digestion Program

Step	Temperature °C	Time
1	37	30 min
2	4	Hold

12. Briefly spin the plate, unseal, and add 18.4 µL SPRI beads (ratio: 0.8x) to each well of the 96-well plate. Seal the plate. Mix the reactions thoroughly by gently vortexing the plate, then quickly spin the plate to collect contents.

    Important: Only pulse-spin plate to collect contents. Over-centrifugation of the plate will cause beads to attach to the bottom of wells and will make it more difficult to completely aspirate the mixture of solution and beads from the wells.

13. For the purification step use a magnetic plate rack and cleanup instructions according to the bead manufacturer. If multiplexing using indexed random priming, combine the solution/beads mixture from four plates into one of the plates. Wash the beads with 200 µL 80% ethanol three times, and then elute in 10 µL Low EDTA TE. If not multiplexing, wash beads with 200 µL 80% ethanol twice, then elute in 10 µL Low EDTA TE.

14. Prepare the Adaptase Master Mix and keep on ice.

Component	Volume per reaction (µL)
Low EDTA TE	4.25
• Buffer G1	2.0
• Reagent G2	2.0
• Reagent G3	1.25
• Enzyme G4	0.5
• Enzyme G5	0.5
Total volume	10.5

15. Seal the plate, place samples in pre-heated thermal cycler, and run the Denaturation Program.

Denaturation Program

Step	Temperature*	Time
1	95°C	3 min

* Set the lid temperature to 105°C.

16. Immediately chill the samples on ice for 2 minutes.

17. Briefly spin the plate, unseal, then add 10.5 µL of the Adaptase Master Mix into each well of the 96-well plate. Seal the plate. Mix the reactions by gently vortexing the plate, then quickly spin the plate to collect contents. Place samples in a preheated thermal cycler and run the Adaptase Program.

Adaptase Program

Step	Temperature* (°C)	Time
1	37	30 min
2	95	2 min
3	4	Hold

* Set the lid temperature to 105°C.

18. Briefly spin the plate, unseal, and add PCR mixture described in the table below. Seal the plate. Mix gently by vortexing the plate, then quickly spin the plate to collect contents.

Reagent	Volume (µL)	Final concentration
Custom P5L indexing primer (100 µM)	0.3	600 nM
Custom P7L indexing primer (10 µM)	5	1 µM
KAPA HiFi Hotstart® ReadyMix	25	1X

19. Add samples to thermal cycler and run the Indexing PCR Program.

Indexing PCR Program

Step	Temperature*(°C)	Time	Cycles
1	95	2 min	
2	98 64 72	30 sec 15 sec 30 sec	17 cycles**
3	72	5 min	
4	4	Hold	

* Set the lid temperature to 105°C.

** If multiplexing using indexed random priming (4 cell library), a total of 17 cycles should be performed. If not multiplexing (single cell library), a total of 19 cycles should be performed.

20. Briefly spin the plate, unseal, and purify the amplified libraries by performing two rounds of cleanup with 0.8X SPRI beads. In the first round, add 40.6 µL of beads to 50.8 µL of sample. When eluting after the first round, use any volume between 10–50 µL of Low EDTA TE. For the second round, use a corresponding 0.8X SPRI bead volume to purify the amplified libraries. For each round of SPRI purification, wash with 80% ethanol twice.
21. If necessary, combine the libraries into a pool and load onto a 2% agarose gel for size selection to further remove adapter dimers. Cut the gel between 400 bp and 1.5 kb and then purify the library using a Qiagen QIAquick™ Gel Extraction Kit, or equivalent gel extraction product. Quantify the library concentration in the eluate, by performing a fluorometric reading, or qPCR, to determine concentration.

Appendix B: Oligonucleotide sequences

Order the following oligonucleotides from IDT. If performing cell multiplexing during the Adaptase and PCR steps, libraries will contain three indexes: a cell-specific random priming in-line index, and i5 and i7 library indexes. If not multiplexing cells at 4-plex, remove the 6-base in-line index sequence just 5' to the N sequence and order a single, random primer only.

Important: When ordering primers, specify the "custom" option for the random primers to ensure the highest quality synthesis regarding N base composition. For more information on mixed bases, see the Mixed Bases web page.

P5L indexed random primers

P5L_AD002
/5SpC3/TTCCCTACACGACGCTCTTCCGATCT**CGATGT**(N1:25252525)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1)

P5L_AD006
/5SpC3/TTCCCTACACGACGCTCTTCCGATCT**GCCAAT**(N1:25252525)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1)

P5L_AD008
/5SpC3/TTCCCTACACGACGCTCTTCCGATCT**ACTTGA**(N1:25252525)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1)

P5L_AD010
/5SpC3/TTCCCTACACGACGCTCTTCCGATCT**TAGCTT**(N1:25252525)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1)

Library amplification primers

The P5L and P7L primers are a custom length to accommodate the shorter adapter tails on the random primers. Do not use standard P5 and P7 indexing primers supplied with the xGen Indexing Kits.

The indexes that are incorporated by these primers are present in the [i5] and [i7] positions and are used for multiplexed sequencing. These are shown in bold below; any combinatorial dual index (CDI) or unique dual index (UDI) set can be alternatively used. The indexes below are standard TruSeq™ HT indexes (Illumina) that produce 96 combinatorial, dual index combinations.

P5L_D501 AATGATACGGCGACCACCGAGATCTACAC**TATAGCCT**ACACTCTTTCCCTACACGACGCTCT
P5L_D502 AATGATACGGCGACCACCGAGATCTACAC**ATAGAGGC**ACACTCTTTCCCTACACGACGCTCT
P5L_D503 AATGATACGGCGACCACCGAGATCTACAC**CCTATCCT**ACACTCTTTCCCTACACGACGCTCT
P5L_D504 AATGATACGGCGACCACCGAGATCTACAC**GGCTCTGA**ACACTCTTTCCCTACACGACGCTCT
P5L_D505 AATGATACGGCGACCACCGAGATCTACAC**AGGCGAAG**ACACTCTTTCCCTACACGACGCTCT
P5L_D506 AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCT
P5L_D507 AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCT
P5L_D508 AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCT
P7L_D701 CAAGCAGAAGACGGCATACGAGAT**CGAGTAAT**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D702 CAAGCAGAAGACGGCATACGAGAT**TCTCCGGA**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D703 CAAGCAGAAGACGGCATACGAGAT**AATGAGCG**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D704 CAAGCAGAAGACGGCATACGAGAT**GGAATCTC**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D705 CAAGCAGAAGACGGCATACGAGAT**TTCTGAAT**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D706 CAAGCAGAAGACGGCATACGAGAT**ACGAATTC**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D707 CAAGCAGAAGACGGCATACGAGAT**AGCTTCAG**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D708 CAAGCAGAAGACGGCATACGAGAT**GCGCATTA**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D709 CAAGCAGAAGACGGCATACGAGAT**CATAGCCG**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D710 CAAGCAGAAGACGGCATACGAGAT**TTCGCGGA**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D711 CAAGCAGAAGACGGCATACGAGAT**GCGCGAGA**GTGACTGGAGTTCAGACGTGTGCTCTT
P7L_D712 CAAGCAGAAGACGGCATACGAGAT**CTATCGCT**GTGACTGGAGTTCAGACGTGTGCTCTT

Appendix C: Indexed adapter sequences

The full-length adapter sequences are below. The underlined text indicates the location of the index sequences, which are 8 bp for the CDIs referenced in Appendix B. These sequences represent the adapter sequences following completion of the indexing PCR step.

Index 1 (i7) adapters

5′ GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′

Index 2 (i5) adapters

5′ AATGATACGGCGACCACCGAGATCTACACYYYYYYYYACACTCTTTCCCTACACGACGCTCTTCCGATCT 3′

For the master list of sequences, see Index Sequences Master List found on the xGen Index and Adapters page. The sequences will help with preparing your Illumina® sequencing sample sheet on the instrument of your choice.

Appendix D: Data analysis and informatics considerations

The xGen Adaptase Module adds a low-complexity, synthetic tail with an average length of 8 bases to the 3' end of each fragment during the addition of the adapter molecule. The random priming step also introduces 9 random bases at the beginning of Read 1 and a 6-base in-line index if performing multiplexed cell processing. Since both the beginning of Read 1 and Read 2 introduce synthetic sequences that may contain artificial cytosine residues, it is important to trim the synthetic sequences prior to data analysis. Also, some aligners will produce a compromised mapping rate if a synthetic sequence is present. Therefore, trim 10 bases from the beginning of Read 1 and Read 2 if performing single cell processing, and trim 16 bases from the beginning of Read 1 and Read 2 if performing multiplexed cell processing using the 6-base in-line indexing.

Further description of data analysis with single nucleus methylcytosine sequencing (snmC-seq) can be found in the publications cited in the Overview section of this protocol.

Tip: If using the 4 in-line indexes for cell multiplexing during the workflow, be aware that the sequence complexity at the start of Read 1 will be compromised and may impact data quality. This issue can be resolved by increasing the in-line indexes to 8-plex (snmC-Seq2), or by using a high-complexity spike-in such as PhiX, depending on the Illumina® sequencer used.

For additional technical support, contact us at applicationsupport@idtdna.com.

REFERENCES

1. Luo C, Keown CL, Kurihara L, et al. **Single-cell methylomes identify neuronal subtypes and regulatory elements in mammalian cortex**. *Science*. 2017;357(6351):600.

2. Luo C, Rivkin A, Zhou J, et al. **Robust single-cell DNA methylome profiling with snmC-seq2**. *Nat Commun*. 2018;9(1):3824.

xGen™ Adaptase™ Module

Technical support: applicationsupport@idtdna.com