CUTANA™ Fragmented Controls for DNA Methylation Sequencing

Catalog No	14-1804	**Pack Size**	1 Set
Lot No	24224001-81		

DESCRIPTION

The CUTANA™ Fragmented Controls for DNA Methylation Sequencing set includes pre-fragmented methylated pUC19 and unmethylated Lambda DNA controls optimized for assessing cytosine conversion efficiency in Enzymatic Methyl-seq (NEB® EM-seq™) when performed downstream of meCUT&RUN and Multiomic CUT&RUN workflows (CUT&RUN-EM). EM-seq is the preferred method for achieving base-pair resolution of 5-methylcytosine (5mC) from CUT&RUN DNA libraries. In traditional EM-seq, cytosine conversion controls are fragmented through sonication after mixing with genomic DNA. When CUT&RUN is used prior to EM-seq to excise chromatin regions of interest, pre-fragmented pUC19 and Lambda controls are required. CUTANA Fragmented Controls for DNA Methylation Sequencing provides a reliable and easy-to-use solution for validating conversion rates and optimizing methylation sequencing protocols in cutting-edge epigenomics experiments.

SET CONTENTS

Item	Cat No	Qty
CpG Methylated pUC19 Fragmented Control DNA	18-8001-05	20 µL
CpG Unmethylated Lambda Fragmented Control DNA	18-8002-05	20 µL
0.1X TE Buffer	21-1025-05	2 x 2 mL

RECOMMENDED ACCESSORY PRODUCTS

meCUT&RUN for DNA Methylation Sequencing

Item	Cat No
CUTANA™ meCUT&RUN Kit	14-1060-24

Multiomic CUT&RUN

Item	Cat No
CUTANA™ Multiomic CUT&RUN Controls Set	14-1802
CUTANA™ ChIC/CUT&RUN Kit	14-1048

TECHNICAL INFORMATION

Storage OPEN SET IMMEDIATELY and store components at room temperature and -20°C as indicated. Stable for 6 months upon date of receipt.

APPLICATION NOTES

Perform meCUT&RUN (EpiCypher 14-1060-24) or Multiomic CUT&RUN (EpiCypher 14-1802). Follow instructions for adding CUTANA Fragmented Controls as outlined in each respective user manual (www.epicypher.com/protocols). In brief:

- Transfer 1 ng CUT&RUN DNA to a new tube and adjust final volume to 49 µL with 0.1X TE Buffer. If CUT&RUN yields are < 1 ng, use the total amount of recovered DNA.
- In a fresh tube, combine 1 µL Methylated pUC19 DNA and 1 µL Unmethylated Lambda DNA with 98 µL 0.1X TE Buffer.
- Add 1 µL of the combined diluted Fragmented Control DNAs to the 49 µL of CUT&RUN DNA.

This diluted DNA will be the input for EM-seq conversion and library prep using the NEBNext® Enzymatic Methyl-seq v2 Kit (NEB E8015). Follow the EM-seq protocol adjustments as outlined in the meCUT&RUN (EpiCypher 14-1060-24) or Multiomic CUT&RUN (EpiCypher 14-1802) user manuals.

VALIDATION DATA

CUT&RUN-EM Methods CUT&RUN-EM was performed using the CUTANA™ Fragmented Controls for DNA Methylation Sequencing and the CUTANA™ ChIC/CUT&RUN Kit (EpiCypher 14-1048) starting with 500k K562 cells and 0.5 µg of IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0060), or H3K36me3 (EpiCypher 13-0058) antibodies. Library preparation was performed with 1 ng of DNA using the NEBNext Enzymatic Methyl-seq v2 Kit (NEB E8015). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 31.5 million reads (IgG), 21.3 million reads (H3K4me3), and 20.6 million reads (H3K36me3). Data were aligned to the hg38 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions. Validation data are representative where noted.

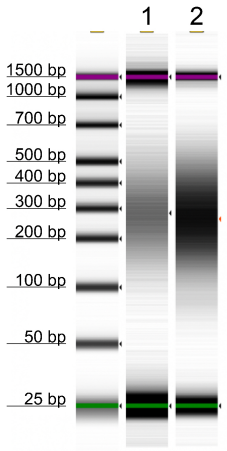

FIGURE 1 **Control DNA fragment size**. Agilent TapeStation® confirms fragmentation of pUC19 (Lane 1, 0.2 ng) and Lambda (Lane 2, 4 ng) control DNAs to target size of 200-400 bp.

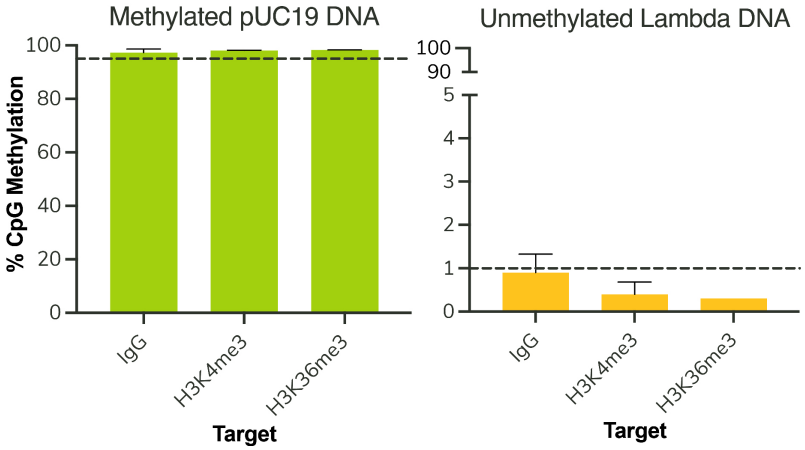

FIGURE 2 **Representative EM-seq conversion efficiency**. CUT&RUN-EM was performed as described above. Across multiple CUT&RUN targets, methylated pUC19 control DNA shows >95% methylated CpGs, as expected. Unmethylated Lambda control DNA shows <1% DNA methylation, indicating >99% EM-seq conversion efficiency.