CUTANA™ CUT&RUN Library Prep Kit with Primer Set 1

Catalog No	14-1001	**Pack Size**	48 Reactions
Lot No	24122005-81	**Kit Version**	v1

DESCRIPTION

The CUTANA™ CUT&RUN Library Prep Kit offers high fidelity library generation for Illumina® sequencing by harnessing the power of New England Biolabs® best-in-class NEBNext® reagents. The kit offers a streamlined protocol specifically optimized for high sensitivity CUT&RUN applications, including those with low cell inputs. Included are all necessary reagents to perform end repair, adaptor ligation, combinatorial dual indexing for multiplexing up to 48 samples, and DNA cleanup with SPRIselect reagent from Beckman Coulter, Inc. If additional multiplexing is desired, this kit can be used in tandem with Primer Set 2 (EpiCypher 14-1002) for up to 96 samples. Pairing this kit with the EpiCypher ChIC/CUT&RUN Kit (EpiCypher 14-1048) affords users a cells-to-sequencing solution for chromatin mapping experiments with all the necessary controls and validated reagents to ensure confidence in obtaining high quality data.

KIT CONTENTS

Item	Cat. No.	Item	Cat. No.
8-strip Tubes	10-0009p	Adapter for Illumina®	18-1000p
SPRIselect reagent from Beckman Coulter, Inc.	21-1405p	Ligation Mix	15-1020p
0.1X TE Buffer	21-1025p	Ligation Enhancer	15-1021p
End Prep Enzyme	15-1019p	U-Excision Enzyme	15-1023p
End Prep Buffer	21-1012p	Hot-Start 2X PCR Master Mix	15-1022p

Multiplexing Primers	Primer Set 1 includes i5 primers 1-8 and i7 primers 1-6

TECHNICAL INFORMATION

Storage	OPEN KIT IMMEDIATELY and store components at room temperature and -20°C as indicated (see Kit Manual for full instructions). Stable for 6 months upon date of receipt.
Instructions for Use	See the included Kit Manual and Quick-Start Card or find them in “Documents & Resources” at epicypher.com/14-1001.

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0060), H3K27me3 (EpiCypher 13-0055), or 0.125 µg of CTCF (EpiCypher 13-2014) antibodies using the ChIC/CUT&RUN Kit v4 (EpiCypher 14-1048). Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ CUT&RUN Library Prep Kit. Both kit protocols were adapted for high throughput Tecan liquid handling. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 8.1/7.6 million reads (IgG Rep 1/Rep 2), 30.6/12.9 million reads (H3K4me3 Rep 1/Rep 2), 9.5/8.9 million reads (H3K27me3 Rep 1/Rep 2), and 6.8/8.5 million reads (CTCF Rep 1/Rep 2). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

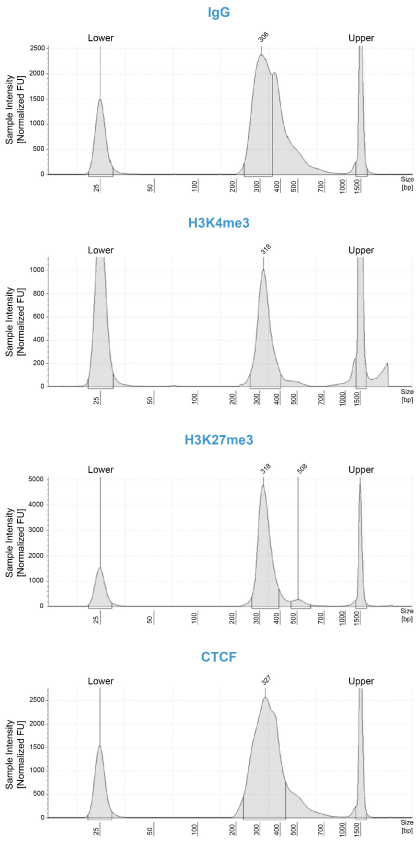

FIGURE 1 CUT&RUN DNA Fragment Size Distribution Analysis. CUT&RUN was performed as described above. Library DNA was analyzed by Agilent TapeStation®, which confirmed that mononucleosomes were predominantly enriched in CUT&RUN (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters). Peaks at ~380 bp correspond to the SNAP-CUTANA™ K-MetStat Panel of spike-in controls (EpiCypher 19-1002).

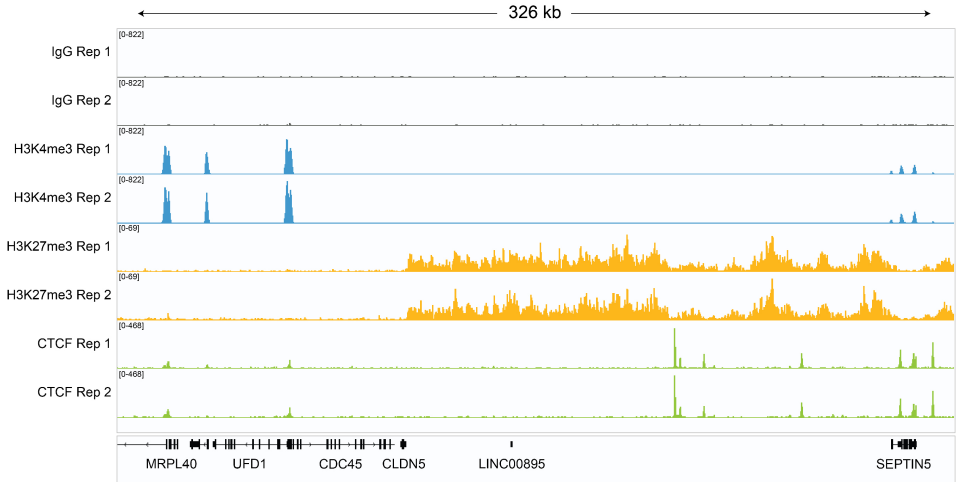

FIGURE 2 Representative Gene Browser Tracks. CUT&RUN was performed as described above. A representative 326 kb window is shown for two replicates ("Rep") of IgG, H3K4me3, H3K27me3, and CTCF antibodies, demonstrating the robustness and reproducibility of the workflow with a variety of targets. Sequencing libraries prepared with the CUTANA CUT&RUN Library Prep Kit produced the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).