

CUTANA™ E. coli Spike-in DNA

Catalog No	18-1401	Pack Size	100 ng	
Lot No	23311006-35			

DESCRIPTION

Fragmented DNA derived from Escherichia coli (E. coli) can be used as a spike-in control for experimental normalization in Cleavage Under Targets and Release Using Nuclease (CUT&RUN). CUT&RUN normalization was originally performed with carryover E. coli DNA from MNase preparation [1], however pure preps of enzyme require post hoc DNA spike-in. CUTANA[™] E. coli Spike-in DNA contains sufficient material for 100-200 CUT&RUN reactions (range for high and low abundance targets).

TECHNICAL INFORMATION

 Storage
 Stable for 2 years at 4°C from date of receipt. After resuspending, aliquots should be stored at -20°C.

 Formulation
 100 ng lyophilized DNA. *NOTE: May not be visible

APPLICATION NOTES

Prior to opening, pellet DNA by quickly spinning in a benchtop microfuge. Reconstitute in 200 μ L DNase free water (0.5 ng/ μ L). Vortex tube on all sides to ensure complete resuspension. Quick spin in benchtop microfuge prior to use.

Use in CUT&RUN:

See full protocol: <u>www.epicypher.com/protocols</u>

1. Use with CUTANA[™] pAG-MNase for ChIC/CUT&RUN (EpiCypher 15-1016), which has very low E. coli DNA carryover.

2. Add 1-2 μ L* (0.5-1 ng) E. coli Spike-in DNA directly to the Stop Buffer, which quenches calcium-mediated pAG-MNase DNA digestion. Gently vortex to mix, and add to reactions.

***NOTE:** Based on target abundance and antibody efficiency, the amount of E. coli DNA may need to be adjusted. Aim for spike-in DNA to comprise ~1% (0.2-5%) of total sequencing reads (**Figure 2**).

3. After sequencing, align reads to the reference genome of experimental samples (e.g. human) as well as to E. coli genome.

4. Normalize data by following recommendations in the CUT&RUN Protocol: <u>www.epicypher.com/protocols</u>.

REFERENCES

[1] Meers et al. Elife (2019). PMID: 31232687

VALIDATION DATA



FIGURE 1 DNA gel data. E. coli fragment size distribution. **Lane 1:** gDNA extracted from JM101 E. coli cells (500 ng). **Lane 2:** Digested and purified CUTANA[™] E. coli Spike-in DNA (500 ng) resolved via 2% E-Gel[™] EX Agarose Gel. Migration positions of DNA molecular weight markers are indicated.

E. coli Spike-in DNA	Target	Total Reads	E. coli Reads	% E. coli Reads
	IgG	7,598,685	415,480	5.47
0.1 ng	H3K4me3	7,825,565	362,192	4.63
	H3K27me3	8,942,144	113,270	1.27

FIGURE 2 CUT&RUN sequencing data. CUTANA™ E. coli Spike-in DNA (1.0 ng) was added to CUT&RUN reactions using 500,000 K562 cells enriched for a low abundance target (H3K4me3, EpiCypher 13-0041), a high abundance target (H3K27me3, EpiCypher 13-0030), and an IgG negative control (EpiCypher 13-0042). Total numbers of paired-end sequencing reads, reads aligned to E. coli, and percentage of total sequencing reads aligned to E. coli spike-in DNA are shown. NOTE: Spike-in DNA amount should be optimized by the end user with the goal of E. coli DNA comprising ~1% (0.2 - 5%) of total sequencing reads.