

# CUTANA™ Fiber-seq Kit

Catalog No	14-2001-8rxn	Pack Size	8 Reactions
Lot No	25220001-51	Kit Version	v1

# **DESCRIPTION**

The **CUTANA™ Fiber-seq Kit** provides key reagents and a comprehensive manual to perform Fiber-seq. Key advantages include:

- Multiomic insights in one assay Fiber-seq simultaneously profiles chromatin accessibility, DNA methylation, and genetic variants, consolidating what typically requires three or more separate assays into one long-read sequencing (LRS) assay [1].
- Comprehensive solution This kit includes key reagents needed to go from cells to library prep-ready DNA.
- **User-friendly resources** The User Manual is complete with a detailed protocol, QC checks, FAQs, and expert tips to get you started, while the bench-proof Quick-Start Card provides a concise protocol for convenient reference.
- Easy workflow integration Fiber-seq can be performed in <2 hours directly upstream of routine long-read sequencing library preparation and uses reagents vetted for compatibility with library prep kits from major LRS platforms.
- Powered by Hia5 6mA methyltransferase Hia5 is the highest activity 6mA-methyltransferase available and is exclusive to CUTANA Fiber-seq Kits. This more active enzyme enables faster incubations, which results in better resolution of protein footprints [2-4].
- **Stringent quality control** Each new lot undergoes rigorous quality control checks to ensure components yield consistent results in your research.

Fiber-seq is a breakthrough approach that simultaneously maps genetic and epigenetic features from individual chromatin fibers. This single assay delivers insights that would normally require integrating data from multiple methods such as ATAC-seq, ChIP-seq, and bisulfite sequencing. This kit includes reagents to go from cells to purified genomic DNA that can be used directly in either Pacific Biosciences<sup>®</sup> (PacBio<sup>®</sup> HiFi Sequencing) or Oxford Nanopore Technologies<sup>®</sup> (ONT<sup>®</sup> Nanopore Sequencing) library preparation workflows. The recommended input for Fiber-seq is 1,000,000 native human nuclei per reaction. The Fiber-seq Kit has been successfully applied to cryopreserved and lightly cross-linked nuclei (refer to the Tech Support Center at support.epicypher.com for the most up-to-date recommendations).

# KIT CONTENTS

ltem	Cat No	Item	Cat No
gDNA Spin Columns	10-0016-09	Pre-Nuclei Extraction Buffer	21-1021-09
gDNA Collection Tubes	10-0017-09	1 M Spermidine	21-1005-09
gDNA Cell Lysis Buffer	21-1029-09	Proteinase K	15-1033-09
gDNA Binding Buffer	21-1030-09	RNase A	15-1034-09
gDNA Wash Buffer	21-1031-09	SAM	21-1034-09
gDNA Elution Buffer	21-1032-09	Hia5 for Fiber-seq	15-1032-09
Pre-1X Reaction Buffer	21-1033-09		

#### TECHNICAL INFORMATION

Storage OPEN KIT IMMEDIATELY and store components at room temperature, 4°C, -20°C, and -80°C as

indicated (see Kit Version 1 User Manual). Stable for 6 months upon date of receipt.

**Instructions for Use** See User Manual corresponding to Kit Version 1.

## Fiber-seq Methods

Fiber-seq was performed using the CUTANA™ Fiber-seq Kit starting with 2,000,000 K562 cells. Following nuclei purification, 1,000,000 nuclei were incubated with Hia5 and co-factor SAM for 10 minutes at 25°C. Genomic DNA was purified, fragmented to 10-15 kb, and prepared for sequencing on a PacBio Revio® SMRT® Cell. Data were aligned to the hg38 genome using PacBio SMRT Link tools, and Fiber-seq analysis and QC was performed using fibertools [5].

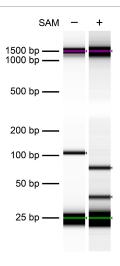


FIGURE 1 In vitro confirmation of Hia5 6mA methyltransferase activity. Hia5 MTase activity was tested in vitro using a synthetic DNA template (122 bp) containing a single GATC motif. After incubation with Hia5 in the presence (+) or absence (-) of the methyl donor SAM, DNA was treated with the methylation-sensitive DpnI restriction enzyme. DNA was then resolved via Agilent TapeStation® D1000 Screen Tape. DpnI-mediated cleavage (81 and 41 bp cut fragments) was observed only in the presence of SAM, confirming that Hia5 catalyzed creation of 6mA, enabling DpnI digestion at its restriction site (G[6mA]TC).

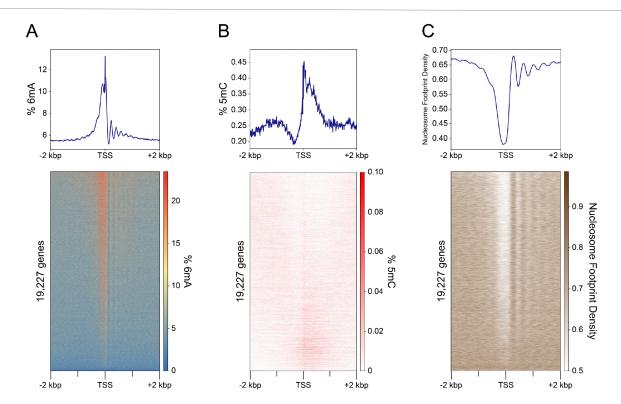


FIGURE 2 Fiber-seq genome-wide heatmaps. Fiber-seq was performed as described above. Heatmaps show aligned data for 6mA signal (A), endogenous 5mC signal (B), and inferred nucleosome positioning (called using fibertools; C) centered at transcription start sites (TSSs +/- 2 kbp). TSSs enriched for 6mA display a depletion of 5mC and well-defined nucleosome positioning as expected for active promoter regions.

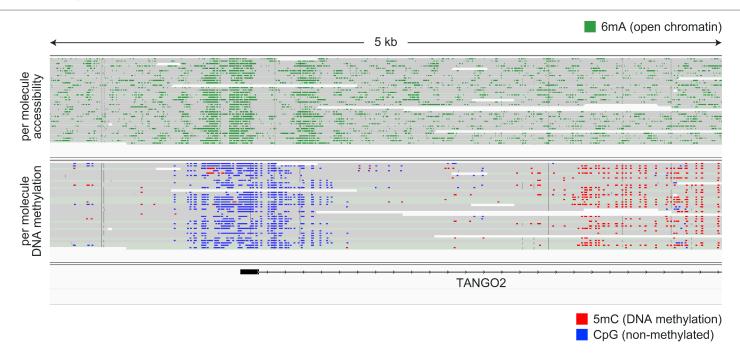


FIGURE 3 Representative locus in Fiber-seq. Fiber-seq was performed as described above. A representative locus (TANGO2) illustrates single-molecule patterns of open chromatin (6mA, green; upper track) and endogenous DNA methylation (5mC, red; lower track). Consistent with active transcription, the TANGO2 promoter region displays open chromatin and unmethylated CpGs (blue; lower track) near the TSS, with 5mC observed within the gene body.

### **REFERENCES**

- [1] Stergachis et al. Science (2020). PMID: 32587015
- [2] Tullius et al. Mol. Cell (2024). PMID: 39191261
- [3] Dubocanin et al. Cell Genom. (2025). PMID: 40147439
- [4] Vollger et al. bioRxiv (2024). DOI: 10.1101/2024.06.14.599122
- [5] Jha et al. Genome Res. (2024). PMID: 38849157

Fiber-seq is a patent-pending technology.

Relevant pending applications include PCT/US2020/017597, PCT/US2021/025644, and related patents and patent applications.

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