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## Introduction

The Baylor College of Medicine Genomic and RNA Profiling (GARP) core's mission is to facilitate cutting-edge genomics research by providing state-of-the-art equipment and expert assistance in strategic and experimental planning. Fiber-seq (Chromatin Fiber Sequencing) is a single-molecule, long-read epigenomics assay that directly provides **chromatin accessibility**, **nucleosome positioning**, **CpG methylation**, **transcription factor occupancy**, and **genetic variation** simultaneously on **individual chromatin fibers**.

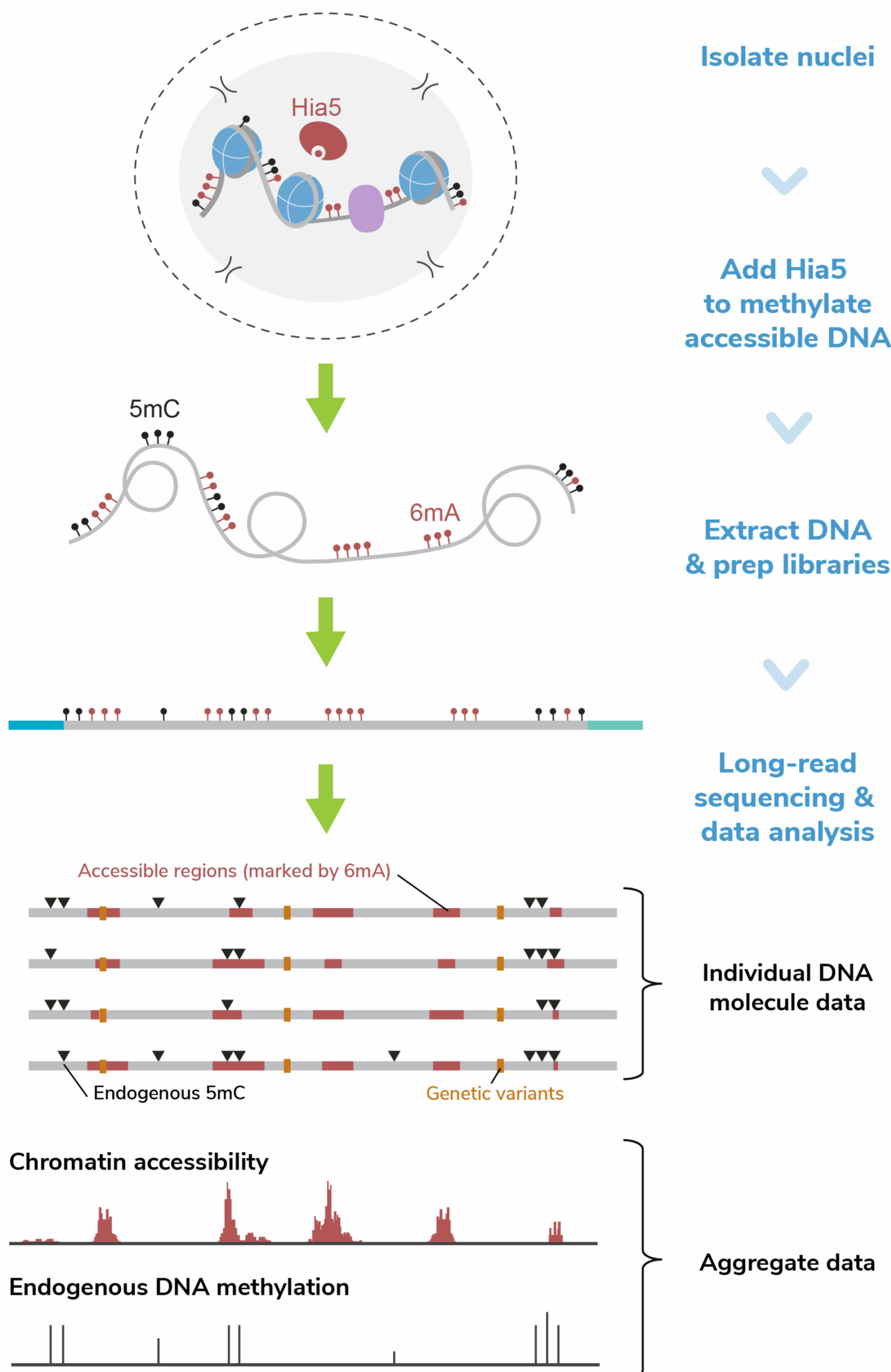
Here we validated the commercial Fiber-seq kit from EpiCypher combined with Nanopore sequencing.

The GARP core currently offers ATAC-seq, whole-genome bisulfite-seq, and long-read whole-genome sequencing. Fiber-seq has the potential to support many investigations by effectively combining aspects of all three assays into a single assay. Its single-molecule resolution provides measurements beyond bulk averages and allows for haplotype phasing.

Please contact [garpcore@bcm.edu](mailto:garpcore@bcm.edu) for pricing and further information.

## Workflow

**How it works:** Fiber-seq uses the nonspecific adenine methyltransferase **Hia5** to methylate accessible DNA. Regions that are protected by nucleosomes or DNA-binding proteins are not methylated.



## Methods

### Starting material:

- 2,000,000 fresh cells of HEK293T to get the target 1,000,000 nuclei per reaction.
- 1000 ng of Hia5-labeled DNA was used for ONT library preparation.

### Reagents:

- EpiCypher CUTANA Fiber-seq Cat. # 14-2001
- ONT Ligation gDNA – Native Barcoding Kit 24 v14 (SQK-NBD114.24)

### Sequencing:

- Libraries were loaded into a PLO-PRO114M flow cell and sequenced on ONT PromethION P24

## Results

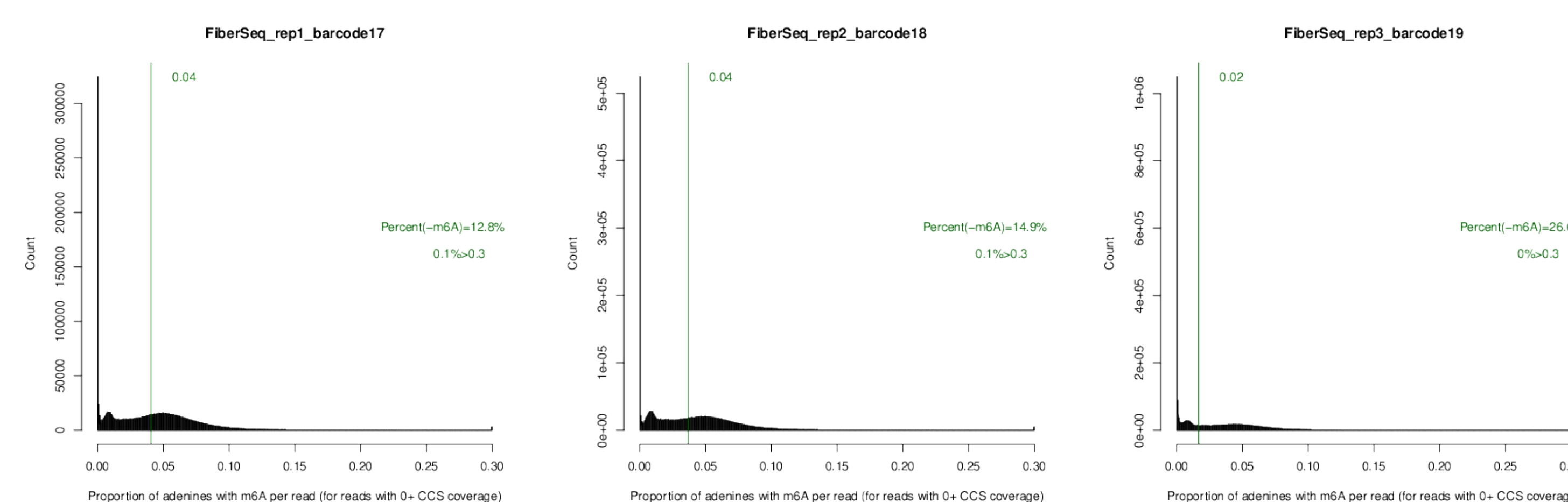
### Summary Table

| QC metric                       | Rep 1                 | Rep 2                | Rep 3                |
|---------------------------------|-----------------------|----------------------|----------------------|
| FIRE QC reads                   | 1.214 M               | 1.548 M              | 1.174 M              |
| Bases represented               | 19.53 Gb              | 23.93 Gb             | 18.40 Gb             |
| Fiber length, median (IQR)      | 10.38 kb (5.09–22.21) | 9.82 kb (4.96–20.95) | 9.85 kb (4.97–21.44) |
| m6A ratio, median (IQR)         | 2.77% (2.11–3.54)     | 2.73% (2.08–3.48)    | 2.50% (1.90–3.19)    |
| m6A/fiber, median (IQR)         | 163 (78–358)          | 154 (75–334)         | 141 (69–310)         |
| Nucleosome length, median (IQR) | 156 bp (140–175)      | 156 bp (140–176)     | 158 bp (142–179)     |
| Nucleosomes/fiber, median       | 46                    | 44                   | 43                   |
| MSP length, median (IQR)        | 29 bp (8–49)          | 28 bp (8–49)         | 26 bp (4–47)         |
| MSPs/fiber, median              | 47                    | 44                   | 43                   |
| FIRE peaks, 1% FDR              | 21,301                | 26,347               | 17,323               |

### Sequencing Performance

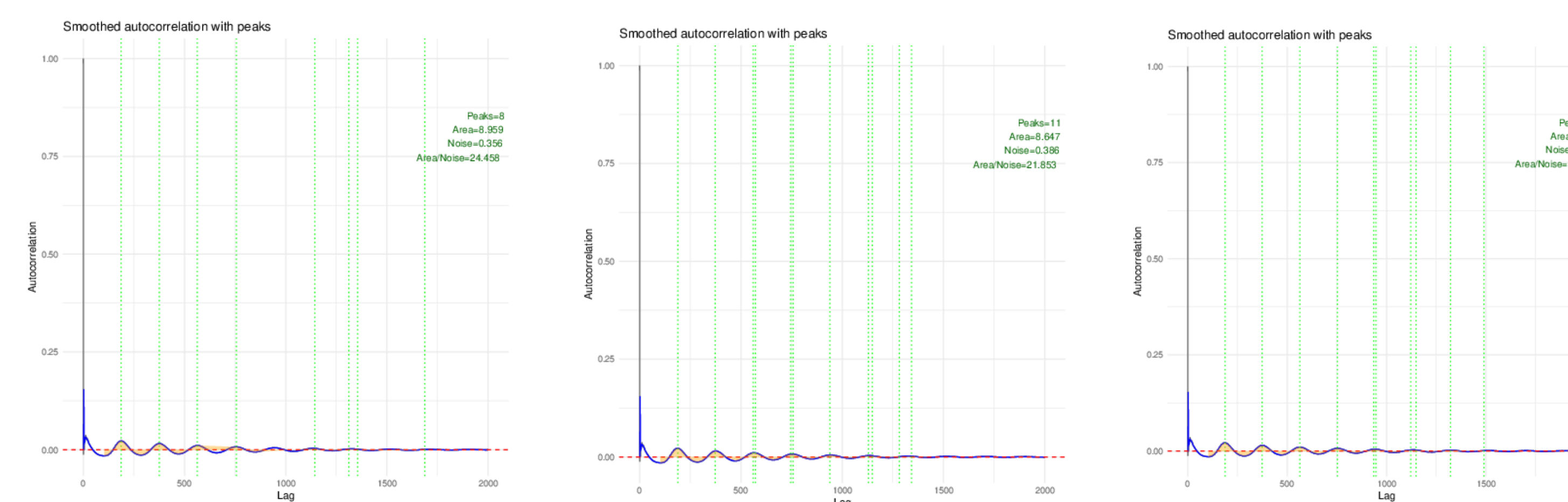
All replicates generated substantial sequencing yield (21.6-26.5 Gb; 7.0x, 8.5x and 8.2x) with similar long-read performance (N50 ≈24-25 kb), indicating successful ONT library preparation and sequencing across samples.

### m6A Accessibility Labeling

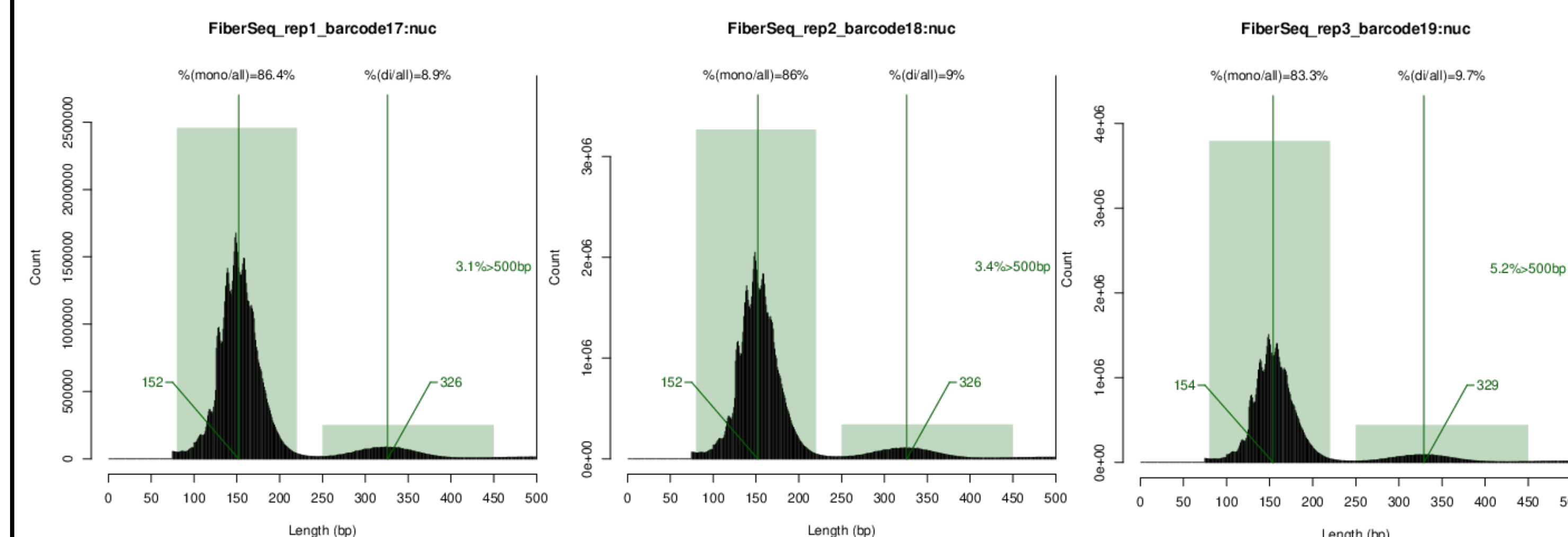


- The **m6A-per-read distribution plots** summarize the percentage of adenines that were labeled by Hia5 per read.
- Rep1 and Rep2 show similar distributions with relatively high per-read m6A labeling, indicating effective marking of accessible chromatin and robust recovery of chromatin architecture.
- In contrast, Rep3 exhibits a broad shift toward lower m6A levels across individual reads, demonstrating reduced labeling efficiency.
- This finding is consistent with the lower overall m6A/AT rate observed in Rep3 (1.66% versus 4.07% and 3.67% in Rep1 and Rep2)

### Inference of Chromatin Architecture

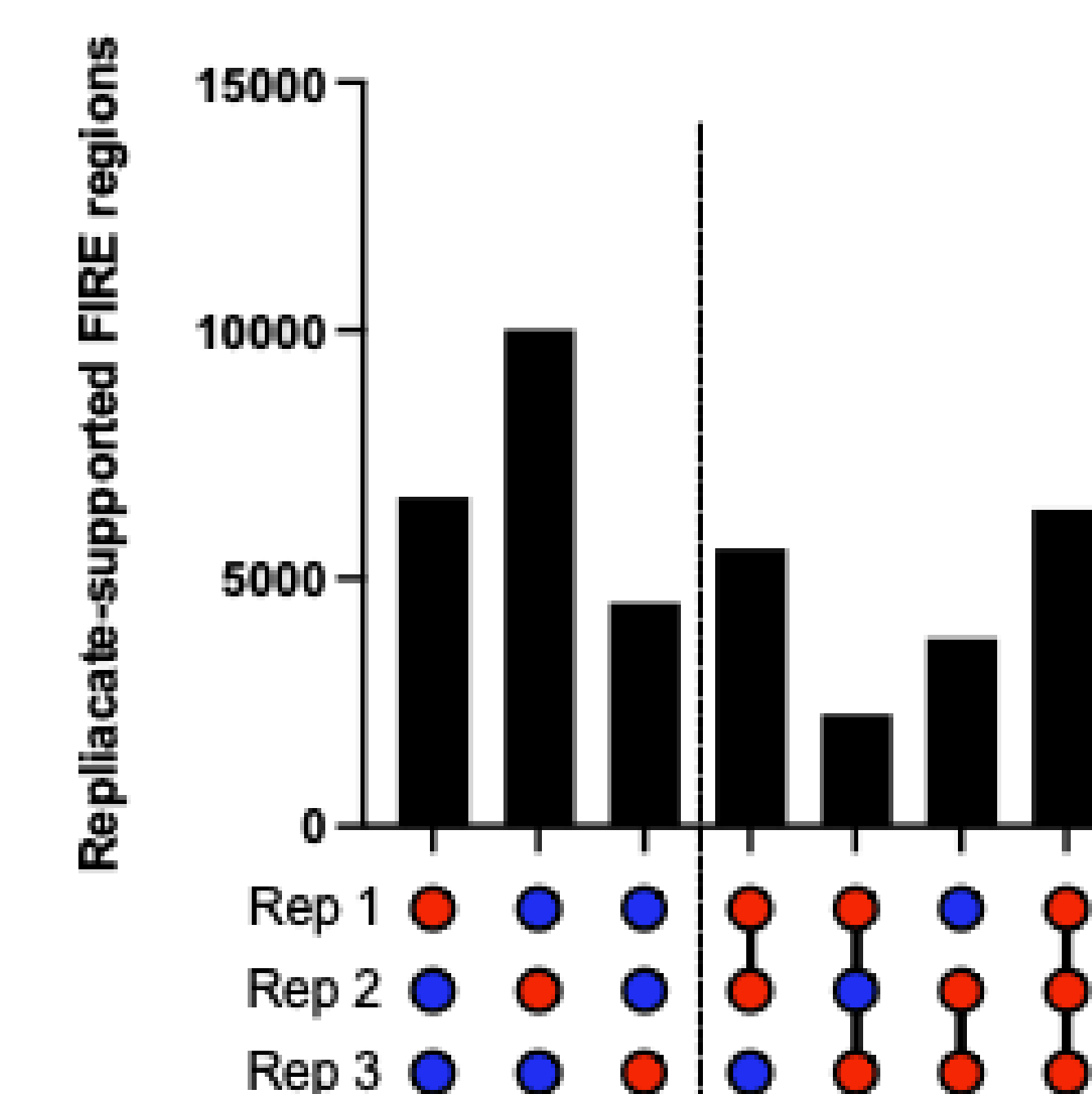


- The Hia5 labeling successfully recovered nucleosome-scale chromatin architecture in all three replicates.
- Nucleosome spacing appears broadly consistent across replicates.
- Rep3 has reduced chromatin signal quality, consistent with its lower m6A labeling rate and lower number of detected FIRE peaks



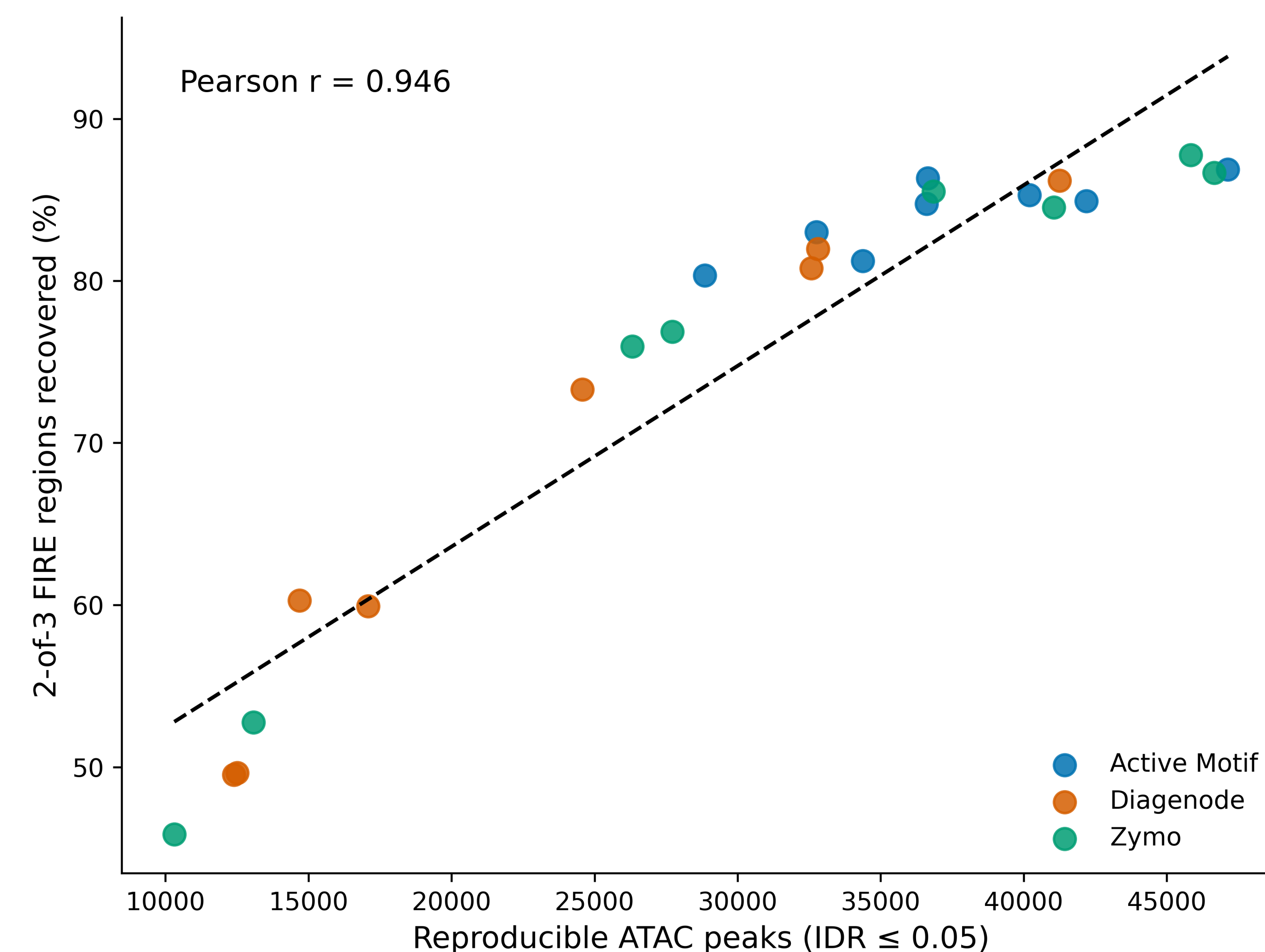
Fiber-seq recovers chromatin structure and nucleosome organization at the expected resolution with mononucleosomal peaks at 152bp.

### Reproducibility



- 18,052 regions were supported by ≥2 of 3 Fiber-seq replicates at 1% FDR.
- 3/3 contain 6,390 regions and gives the same major condition patterns.

### Correlation with ATAC-seq data



- ATAC-seq data was generated from the same cell line using three different commercial kits under different conditions.
- High-quality ATAC-seq data sets containing a large number of reproducible peaks recover a high percentage of FIRE-regions.

## Conclusion

- The EpiCypher CUTANA Fiber-seq kit, when combined with Nanopore sequencing, generated libraries with expected labeling rates and effectively recovered chromatin architecture and nucleosomal structure
- At 1% FDR and ~ 8x coverage we observed a large number of peaks unique to each replicate suggesting relatively low signal over background. Deep sequencing and stringent cut-offs should be used in the data analysis.
- Over 18,000 Fire regions were shared between two or more replicates and showed a strong correlation between Tn5 Tagmentation and Hia5 labeling.
- Replicate 3 showed a globally lower labeling rate. Future utilization of the kit will provide further insight into the robustness of the protocol.
- For pricing and sample prep guidelines please reach out to [garpcore@bcm.edu](mailto:garpcore@bcm.edu).