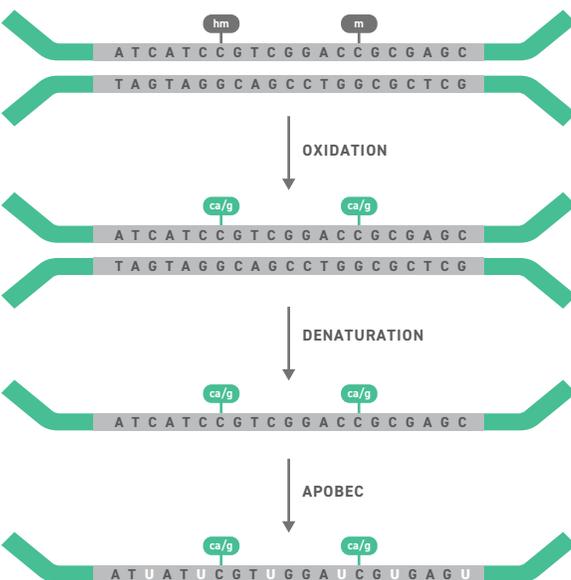# Twist EM-seq v2 Methylation Detection System

Unparalleled efficiency in NGS methylation profiling

The Twist EM-seq v2 Methylation Detection System provides a robust, end-to-end sample preparation solution for identifying methylated regions in the human genome. Library preparation employs a unique, enzymatic process that is much less damaging to DNA, requiring less sample input and resulting in higher quality, better performing libraries. Subsequently, Twist Custom Methylation Panel design provides exceptionally efficient probes for target enrichment for CpG detection. Optimized hybridization reagents add flexibility to the timing of the workflow and improve on-target rates. Whether you are investigating cellular differentiation or screening liquid biopsies for cancer, the system offers the most efficient methylation detection available.

Twist EM-seq v2 Methylation Detection System for Twist Targeted Methylation Sequencing

In partnership with New England Biolabs (NEB®), Twist Bioscience offers an updated methylation sequencing workflow that improves the quality of libraries and removes the need for damaging bisulfite treatment during prep. The workflow features enzymatic conversion of unmethylated cytosines (**Figure 1**) to identify sites of 5-methyl-cytosine (5mC) and 5-hydroxymethyl-cytosine (5hmC). Enzymatic conversion produces more intact libraries with better representation, and ultimately achieves more sensitive methylation detection. The library preparation system is suitable for downstream enrichment with Twist Methylation Panels for targeted methylome sequecing.

KEY HIGHLIGHTS

An end-to-end solution
- Convert unmethylated cytosines using a state-of-the-art, enzymatic process
- Capture with high-performance Twist probes and optimized reagents

Innovative library preparation
- Easily dropped into existing bisulfite sequencing analysis pipelines
- Superior mapping efficiency, GC uniformity, and sequencing metrics
- Detects 15% more CpGs than bisulfite method
- Less sample damage enables challenging sample inputs

Highly efficient Custom Panels*
- Sophisticated design, accurate synthesis, and detailed QC maximize capture uniformity and reproducibility
- Outstanding performance across panel sizes, target regions, and multiplexing requirements
- Easily add or enhance panel content

Optimized hybridization reagents
- Adjust hybridization timing without sacrificing performance
- Improved on-target rates with Methylation Enhancer

Figure 1. EM-seq conversion involves a series of enzymatic reactions to identify unmethylated cytosines. During the first reaction, ten-eleven translocation dioxygenase 2 (TET2) converts methylated cytosines (5mC and 5hmC) to 5-carboxycytosine (5caC) and the Oxidation Enhancer glucosylates 5hmC (5ghmC). These reactions protect 5mC and 5hmC from downstream deamination. The DNA is then denatured before APOBEC deaminates cytosines to uracils. Subsequent PCR amplification converts the modified 5mC or 5hmC into cytosines and uracils into thymines. After PCR, nucleotide representation is the same as observed for bisulfite converted DNA, making EM-seq compatible with existing analysis pipelines, for example Bismark and bwa-meth.

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Twist Standard Hybridization v2 and Fast Hybridization Systems with Custom Methylation Panels

The Twist Target Enrichment Standard Hybridization v2 and the Twist Fast Hybridization and Wash Kit provide optimal performance and maximum flexibility. Reagents and steps can be optimized to tune downstream sequencing metrics and to reduce hands-on time and pipette use. Designed with a highly sophisticated algorithm, Twist Custom Methylation Panels capture targets with exceptional efficiency across a wide range of target sizes. Together, these components ensure best-in-class hybrid capture of custom regions of interest.

Off-target capture frequently occurs in targeted methylation sequencing workflows because the conversion process reduces the sequence complexity of the sample during library preparation. To maximize the capture of post-conversion samples, the Twist EM-seq v2 Methylation Detection System features a proprietary blocker designed specifically for methylation detection. Referred to as Methylation Enhancer, this reagent can further reduce off-target capture without affecting other performance metrics (**Figure 2**).


Figure 2. Performance of Methylation Enhancer. Addition of Methylation Enhancer effectively reduced off-target capture by 1.5 Mb Twist Alliance Pan-Cancer panel. Fast Hyb, Twist Fast Hybridization; STV2, Twist Standard Hybridization v2.

Highly Sensitive Detection of Differentially Methylated Regions

Methylation levels vary substantially across the human genome, and differentially methylated regions (DMRs) can be used to identify certain cancers. To test the effects of methylation levels on the performance of the Twist EM-seq v2 Methylation Detection System, libraries of varying methylation levels (0–100% methylation) were generated by combining hypo- and hypermethylated genomic DNA in defined ratios. This analysis showed minimal effects of methylation level on final sequencing metrics (**Figure 3**).

The Twist EM-seq v2 Methylation Detection System also captures hypo- and hypermethylated regions with high sensitivity. Figure 4 illustrates methylation detection in the *CCND2* locus. The Twist EM-seq v2 Methylation Detection System demonstrated highly sensitive detection of methylation, even at low input levels.
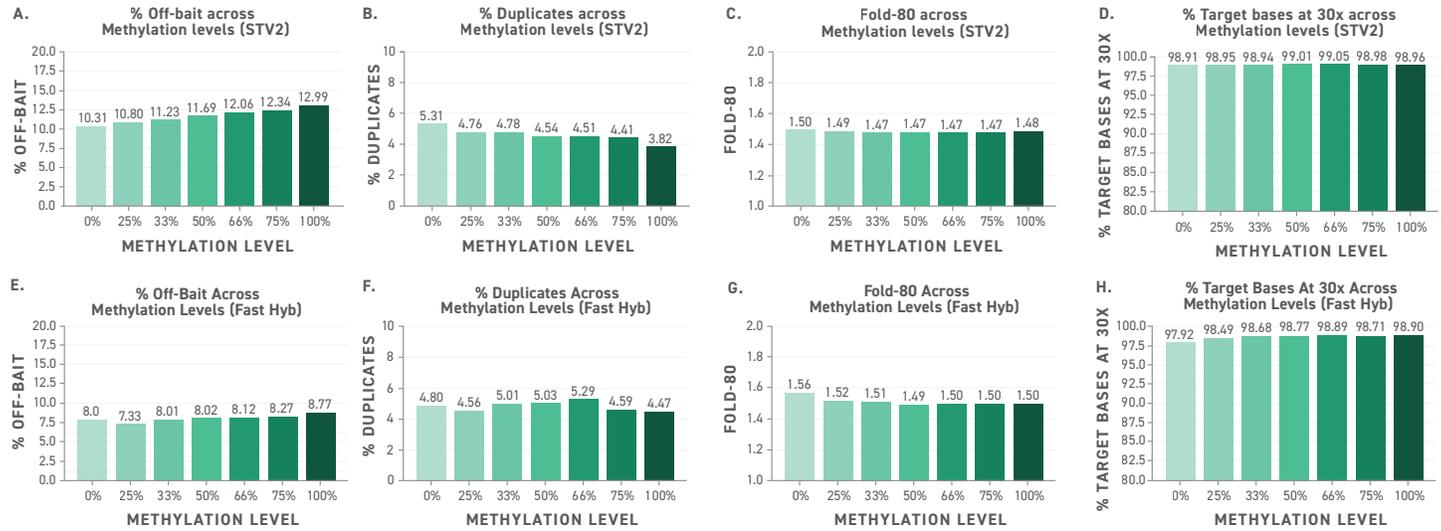
Figure 3. Detection of DMRs. The Twist EM-seq v2 Methylation Detection System efficiently captures differentially methylated regions of input DNA from 0 to 100% methylation, with minimal or no impact on sequencing metrics, including 30x coverage and uniformity (fold-80 base penalty). Fast Hyb, Twist Fast Hybridization; STV2, Twist Standard Hybridization v2.
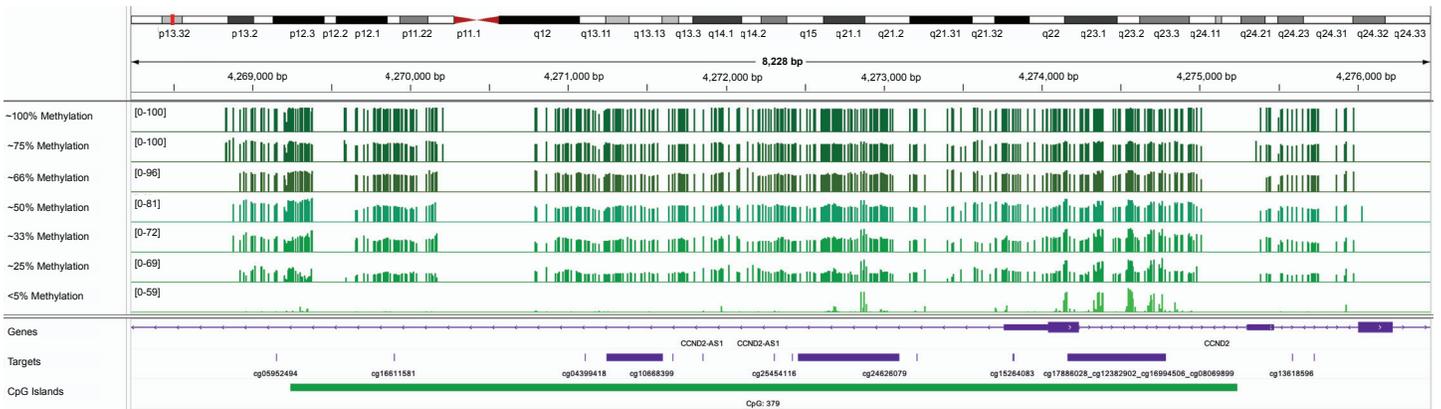

Figure 4. Highly sensitive methylation detection. Detection of methylation is possible across a wide range of methylation levels and targets.

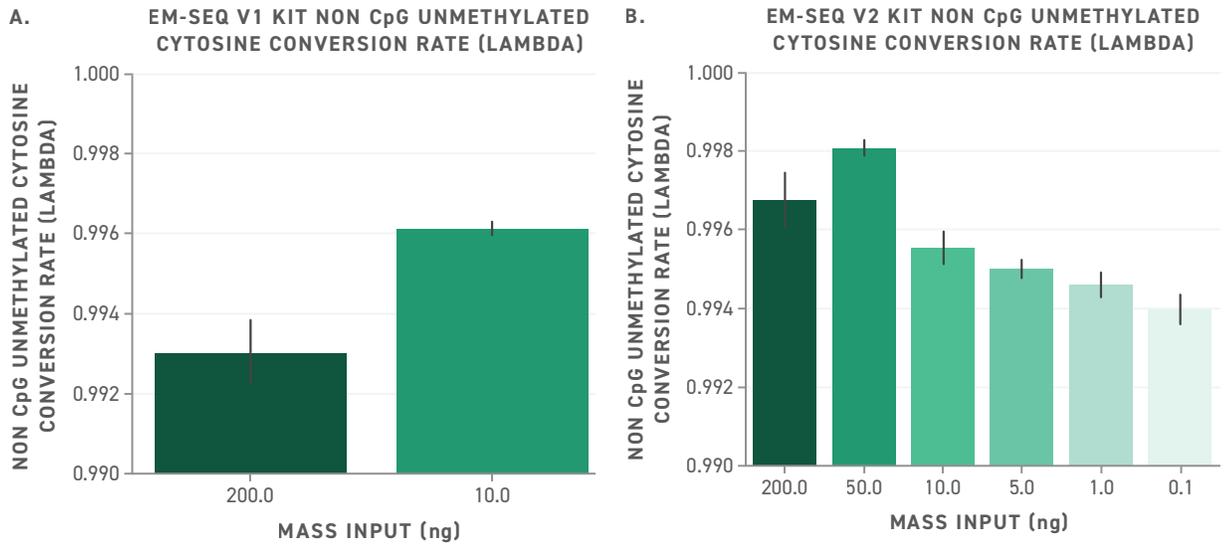# v2 Methylation Detection System Performance

The Twist EM-seq v2 Methylation Detection System is replacing the Twist NGS Methylation Detection System and supports a wider mass input range for library preparation into either WGS or target enrichment. Additionally, the Twist EM-seq v2 Methylation Detection System is compatible with both Twist Standard Hybridization v2 and Twist Fast Hybridization target enrichment. This updated system offers a shorter workflow and improved performance for both WGS and target enrichment applications with large and small methylation panels.

For WGS, the relative cytosine conversion performances (converting non-CpG cytosines and protecting CpG sites from conversion) of EM-Seq v1 and EM-Seq v2 libraries were compared across the mass input ranges available for library preparation with each version of the kit. The v2 kit achieves high library yield and shows high conversion efficiency and specificity even at the lowest mass input levels **(Figures 5, 6, and 7)**.
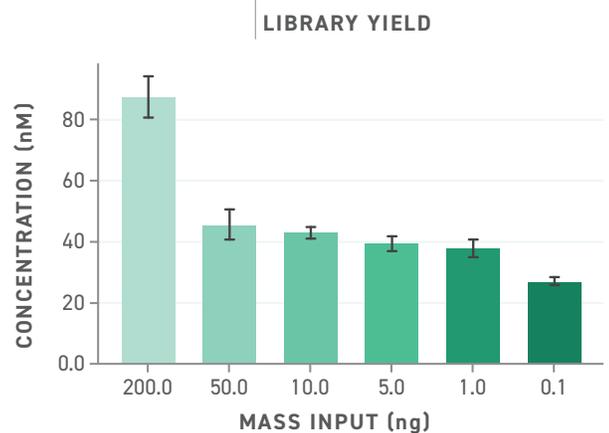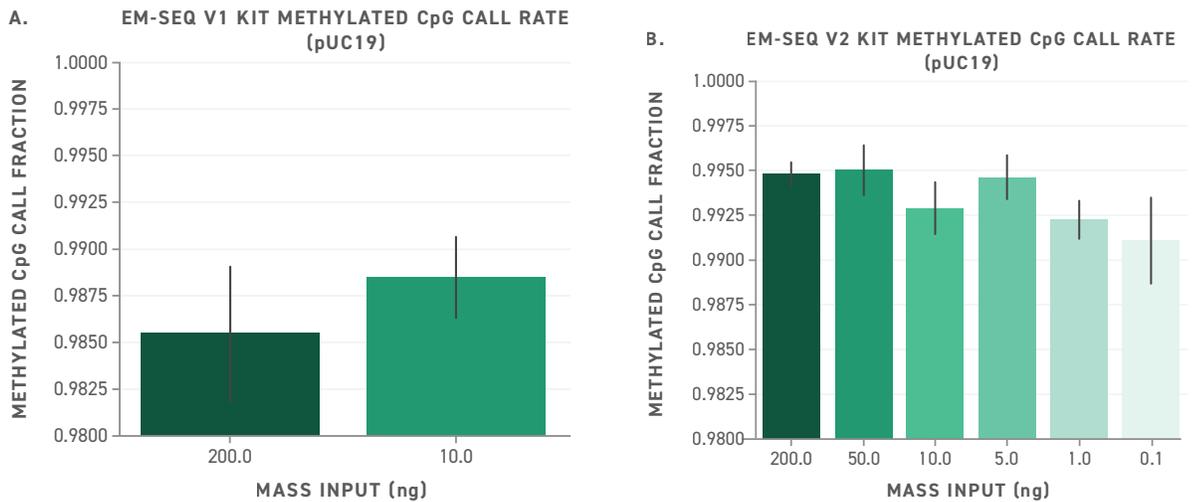

Figure 5. Yield of libraries generated with Twist EM-seq v2 Methylation Detection System. Library yields are high even with low mass input.

Figure 6. Non-CPG Conversion Rate. Conversion rates with v2 are high even with low mass input.

A.

EM-SEQ V1 KIT NON CpG UNMETHYLATED CYTOSINE CONVERSION RATE (LAMBDA)

B.

EM-SEQ V2 KIT NON CpG UNMETHYLATED CYTOSINE CONVERSION RATE (LAMBDA)

Figure 7. Called CPG Ratio. Conversion specificity with v2 is high even with low mass input.

A.

EM-SEQ V1 KIT METHYLATED CpG CALL RATE (pUC19)

B.

EM-SEQ V2 KIT METHYLATED CpG CALL RATE (pUC19)

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ORDERING INFORMATION

129335: NEBNext(R) EM-seq v2 kit for Twist Methylation, 96 rxn
129336: Twist Targeted EM-seq v2 Fast Hyb Workflow, 96x12 rxn
129337: Twist Targeted EM-seq v2 SHv2 Hyb Workflow, 96x12 rxn
127377 NEBNext® LV Unique Dual Index Primers (Plate A), 96 Samples
127378 NEBNext® LV Unique Dual Index Primers (Plate B), 96 Samples
127379 NEBNext® LV Unique Dual Index Primers (Plate C), 96 Samples
127380 NEBNext® LV Unique Dual Index Primers (Plate D), 96 Samples

Twist Custom Panels can be ordered separately. Please contact your sales representative for more information.

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