

NEB launches new kit for enzyme-based 5hmC detection at single-base resolution

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To accurately and sensitively discriminate 5hmC from un-modified cytosine and 5mC from a wide range of DNA inputs



New England Biolabs (NEB) has announced the launch of the NEBNext Enzymatic 5hmC-seq Kit (E5hmC-seq), a novel enzyme-based method for the specific detection of 5hmC sites. The gentle, enzyme-based approach enables high yields and high-quality data, with an input range of 100 pg to 200 ng.

While the biological importance of 5hmC modification is less clear than that of 5mC, the abundance of 5hmC varies significantly between tissues, suggesting that it may play a critical role in gene regulation and other biological processes.

"So far, the study of 5hmC has been hampered by the lack of precise detection methods," said Fiona Stewart, Associate Director, NEBNext Portfolio Management. "While NEBNext Enzymatic Methyl-seq (EM-seq), our gold standard for methylation detection, detects both 5mC and 5hmC, it does not distinguish between them. Additionally, bisulfite-based methods suffer from reduced data quality due to the sample fragmentation and loss of DNA that results from the damaging bisulfite treatment, thereby limiting their practical utility.

"To address these challenges, we developed the NEBNext Enzymatic 5hmC-seq Kit, which allows for the specific detection of 5hmC sites using a two-step enzymatic conversion workflow," said Stewart. "The enzymatic method minimises DNA damage

and allows for the discrimination of 5hmC from unmodified cytosine and 5mC after Illumina sequencing. Additionally, E5hmC-seq data can be subtracted from EM-seq data, allowing for precise determination of individual 5mC and 5hmC sites."

The kit includes the reagents required for E5hmC-seq conversion and library preparation compatible with Illumina sequencing; index primers for multiplexing are available separately. A conversion module is also available, for applications beyond library prep.