TECHNIQUE

New tools for transcriptome-wide mapping of m⁶A

“m⁶A-label-seq and m⁶A-SEAL represent complementary, orthogonal techniques

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N⁶-methyladenosine (m⁶A) is the most prevalent covalent modification of mammalian mRNA and is involved in regulating post-transcriptional gene expression. Despite methodological advances, it remains challenging to directly map all m⁶A sites in the transcriptome. Now, *Nature Chemical Biology* reports two new methods that enable transcriptome-wide mapping of m⁶A independently of sequence context.

Existing methods have provided invaluable information about the m⁶A landscape but each has limitations. Antibody-based m⁶A-seq (also termed MeRIP-seq) has a low resolution of 100–200 nucleotides. Other methods indirectly infer the positions of modified adenosines from their proximity to RNA–antibody crosslinking sites (in cross-linking and immunoprecipitation (CLIP)-based approaches) or enzymatically modified sites (in deamination adjacent to RNA modification targets (DART)-seq). Approaches based on m⁶A-sensitive endoribonucleases (such as m⁶A-REF-seq or MAZTER-seq) detect only the ~16–25% of total m⁶A sites that fall within their recognition motif.

Detection of m⁶A is challenging because the N⁶-methyl group is largely unreactive and therefore difficult to chemically label. Additionally, m⁶A does not cause nucleotide mis-incorporation or termination during reverse transcription (RT), which precludes the use of mutation- or truncation-based sequencing readouts.

To address these issues, Shu et al. developed m⁶A-label-seq, a single-nucleotide resolution, transcriptome-wide approach based on metabolic labelling. Normally, m⁶A methylation enzymes transfer a methyl group from S-adenosyl methionine (SAM) to the N⁶ position of target adenosines. However, in cells cultured with a SAM analogue, Se-allyl-L-selenohomocysteine (SeAM), m⁶A sites are instead metabolized to N⁶-allyl-adenosine (a⁶A). Chemically induced cyclization of a⁶A can lead to mis-incorporations during RT, enabling detection of modified adenosines as A-to-T/C/G mutations in sequencing data.

In proof-of-concept experiments, m⁶A-label-seq detected a few thousand modified sites in each of three different cell types (human HeLa and HEK293T and mouse H2.35), a subset of which were validated by an orthogonal approach. Comparisons with published data for other single-base resolution techniques (DART-seq, m⁶A-REF-seq and miCLIP) in HEK293T cells indicated that m⁶A-label-seq is better at detecting clustered m⁶A sites. The authors suggest that the superior performance of m⁶A-label-seq reflects the limitations of indirect and sequence-dependent approaches.

Wang et al. based the second method, m⁶A-SEAL, on the ability of FTO RNA demethylase to convert m⁶A into a reactive intermediate, N⁶-hydroxymethyladenosine (hm⁶A). Subsequent treatment of hm⁶A with dithiothreitol (DTT) generated N⁶-dithiolisitolmethyladenosine (dm⁶A), which has a free sulfhydryl group that can be functionally tagged with biotin for streptavidin-based affinity purification followed by sequencing.

The authors show that m⁶A-SEAL specifically detects converted m⁶A and is highly sensitive — it can be performed on as little as 500 ng of polyA⁺ RNA. Comparisons of false-positive rates indicate that it is more reliable than MeRIP-seq, DART-seq, m⁶A-REF-seq and a number of CLIP-based methods. Although the current version of m⁶A-SEAL has a low resolution, comparable to that of m⁶A-seq, the authors hope that single-nucleotide resolution can be achieved by adopting readouts based on terminated or disrupted RT. A notable strength of m⁶A-SEAL is its flexibility. Optimizing conditions should enable the detection of other RNA modifications, such as hm⁶A or N⁶,2'-O-dimethyladenosine at the cap +1 position (cap m⁶A_m). Moreover, the sulfhydryl group can be tagged with different functional groups for different applications, such as fluorophores for imaging.

m⁶A-label-seq and m⁶A-SEAL represent complementary, orthogonal techniques that, together with existing approaches, will facilitate a fuller understanding of the distribution and biological roles of m⁶A RNA modification.

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ORIGINAL ARTICLES Shu, X. et al. A metabolic labeling method detects m⁶A transcriptome-wide at single base resolution. *Nat. Chem. Biol.* <https://doi.org/10.1038/s41589-020-0526-9> (2020) | Wang, Y. et al. Antibody-free enzyme-assisted chemical approach for detection of N⁶-methyladenosine. *Nat. Chem. Biol.* <https://doi.org/10.1038/s41589-020-0525-x> (2020)

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