



Single-nucleotide resolution of N^6 -adenine methylation sites in DNA and RNA by nitrite sequencing

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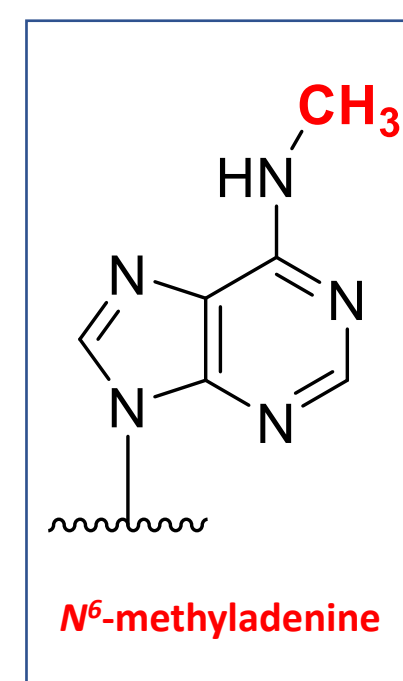
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Abstract

A single-nucleotide resolution sequencing method of N^6 -adenine methylation sites in DNA and RNA is described. Using sodium nitrite under acidic conditions, chemoselective deamination of unmethylated adenines readily occurs, without competing deamination of N^6 -adenine sites. The deamination of adenines results in the formation of hypoxanthine bases, which are read by polymerases and reverse transcriptases as guanine; the methylated adenine sites resist deamination and are read as adenine. The approach, when coupled with high-throughput DNA sequencing and mutational analysis, enables the identification of N^6 -adenine sites in RNA and DNA within various sequence contexts.

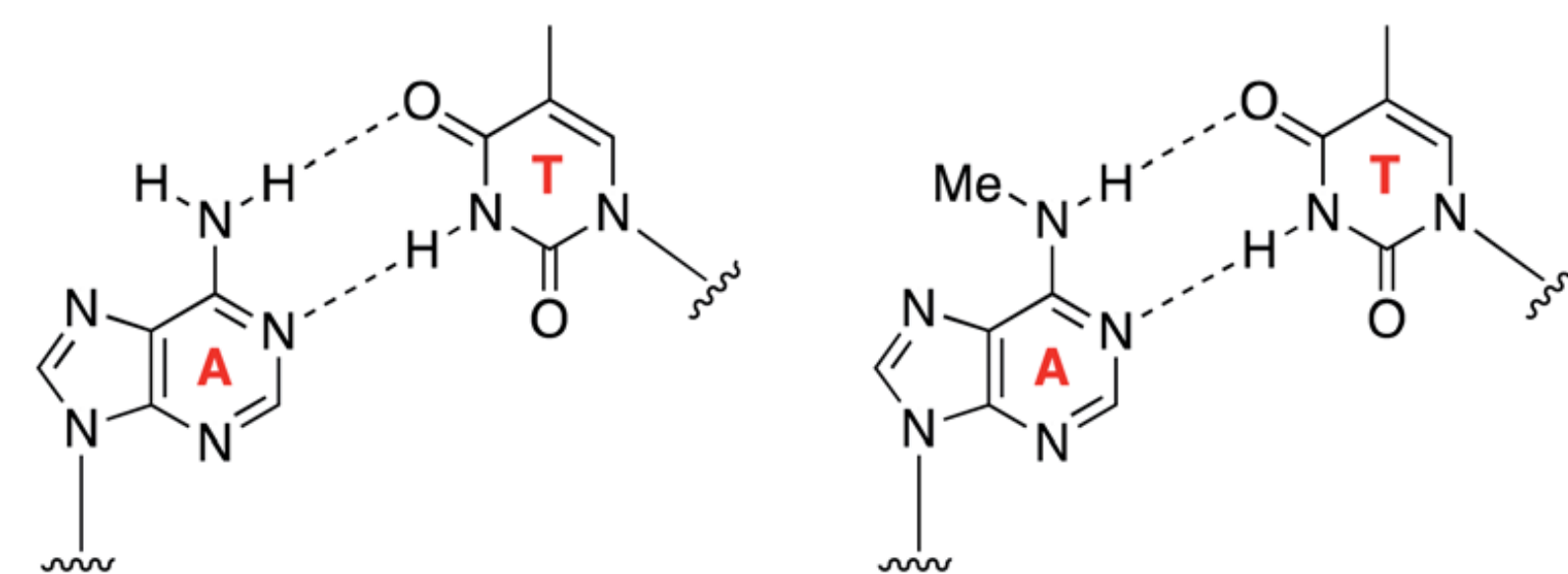
Introduction

The ability to map methylation sites in the human genome and epitranscriptome has transformed our understanding of how these modifications govern and influence a host of cellular processes and diseases. Amongst the most widely studied methylations is N^6 -methyladenine, known as 6mA in DNA and m^6A in RNA.



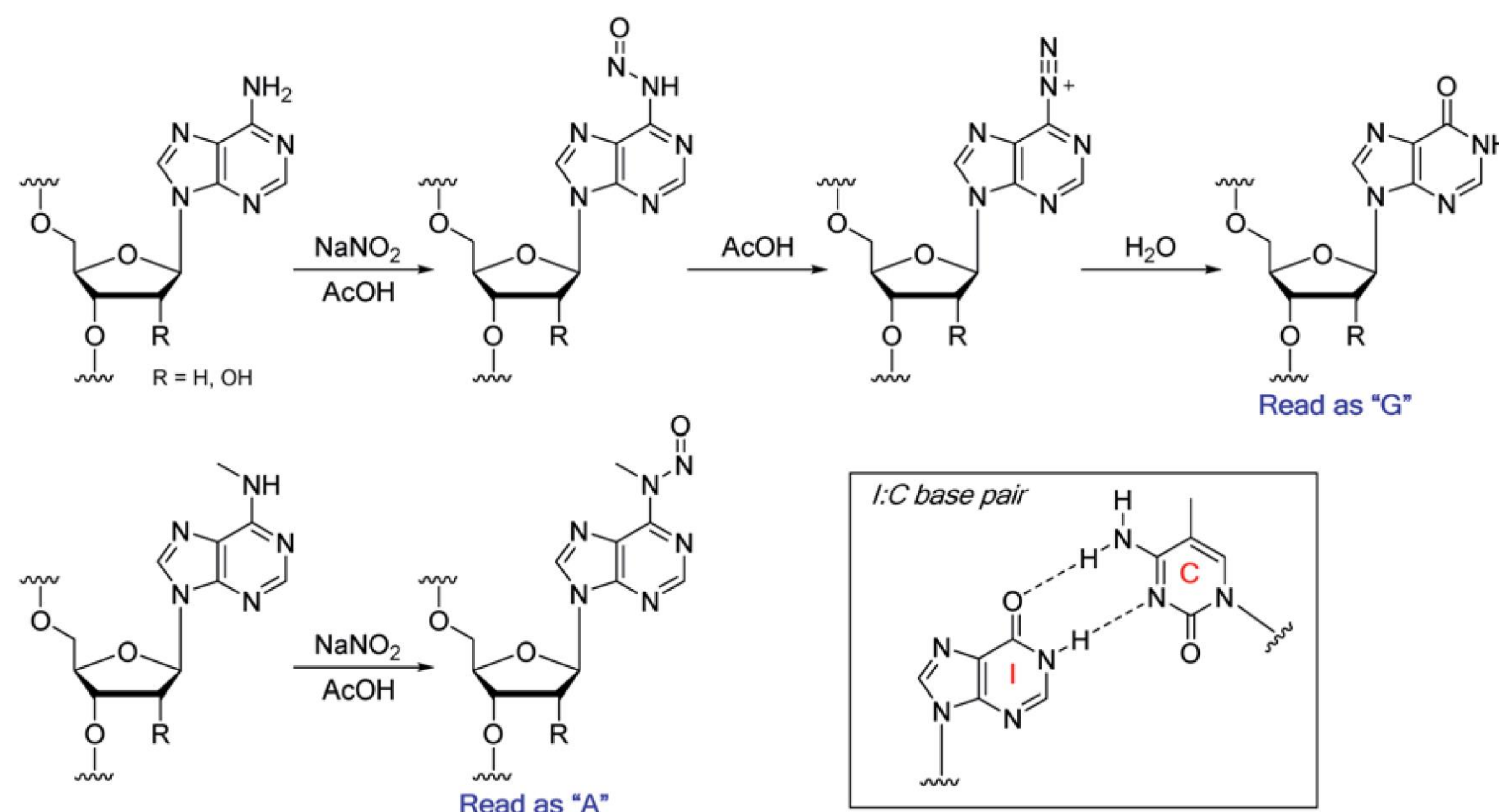
The dynamics of m^6A incorporation into RNA are regulated by “writers” (i.e., methyltransferases) and “erasers” (i.e., demethyltransferases), and can directly affect processes such as nuclear RNA export, splicing, and RNA stability. Not surprisingly, the deregulation of these dynamics and resulting aberrant levels of m^6A has been linked to obesity, immunoregulation, and cancer. 6mA is thought to play an epigenetic role in embryonic development, tumorigenesis, response to stress, neuropsychiatric disorders, and embryonic stem cell function, and it can be inherited. Understanding the role of N^6 -methyladenine in RNA and DNA requires robust single-nucleotide sequencing methods.

Due to the similar Watson–Crick–Franklin hydrogen-bonding nature of adenine and N^6 -methyladenine with thymine, these bases get read the same by polymerases and direct high-throughput sequencing has been challenging using conventional methods.

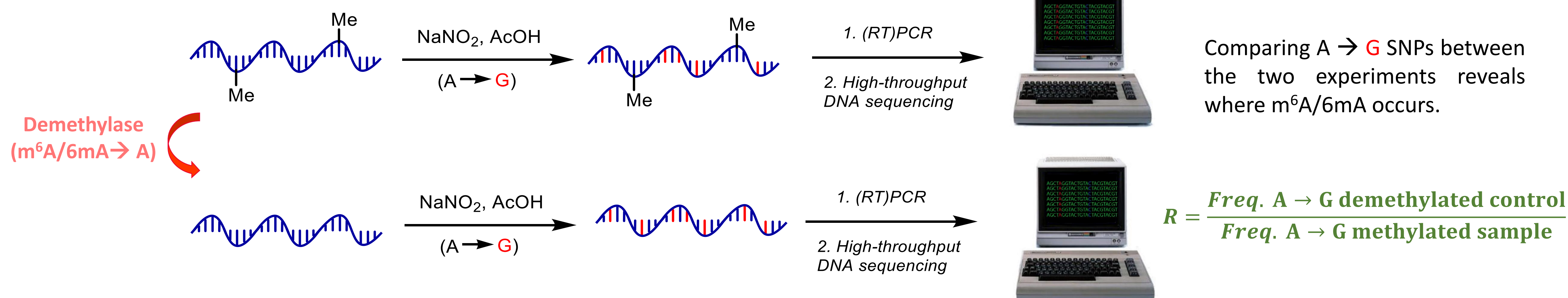


Approach

In the presence of acid under aqueous conditions, nitrite forms reactive nitrosonium ion, which reacts with aromatic amines to form nitrosamines. Subsequent dehydration to form the diazonium ion can only proceed with primary aromatic amines, as secondary aromatic amines lack the additional N–H required for dehydration. Hydrolysis of the diazonium yields the deaminated product. Accordingly, the process should be chemoselective for the primary exocyclic amine of adenine over the secondary exocyclic amine of N^6 -methyladenine seen in m^6A and 6mA. Thus, only unmethylated adenine will be hydrolyzed under these conditions to form hypoxanthine –an exchange of a hydrogen bond donor for a hydrogen bond acceptor. Polymerases are known to read hypoxanthine as guanine, resulting in an A $\rightarrow$ G transition, which can be detected by high-throughput DNA sequencing.

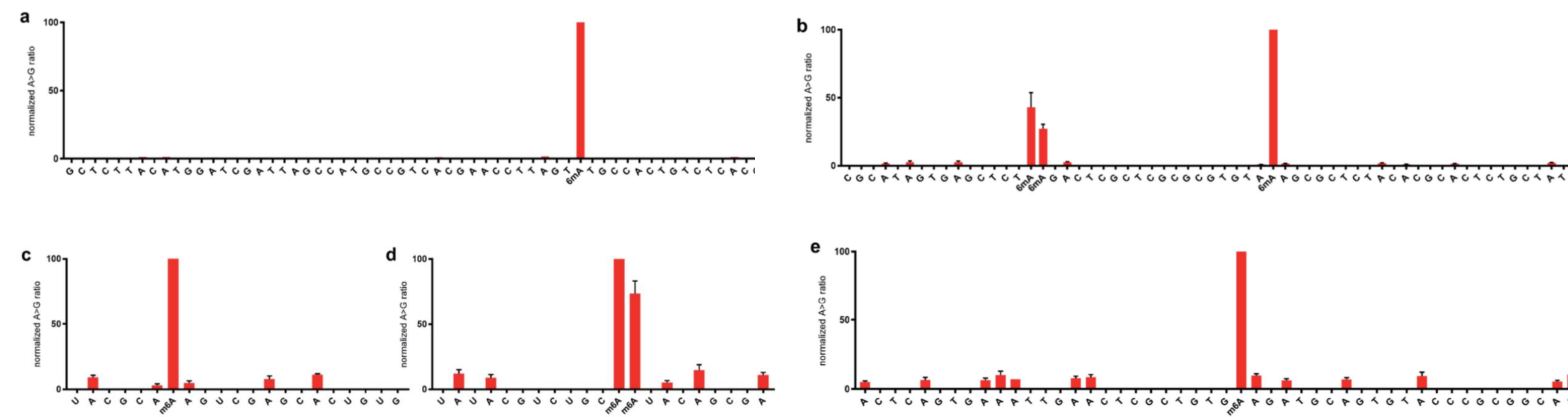


Workflow



Results

Normalized sequencing representation of the ratio of (d)A $\rightarrow$ (d)G mutation (R) at each nucleobase following treatment with 1 M sodium nitrite in the presence of acetic acid for 5 h at 22 ° C. The DNA sequences contain a single 6mA site at position 63 (a) and three 6mA sites at positions 35, 36, and 55 (b). The RNA sequences contain a single m^6A site at position 26 (c) and two m^6A sites at positions 31 and 32 (d). The 23S rRNA from *E. coli* contains a single m^6A site at position 2030 (e):



Conclusion

We have demonstrated the first chemistry-based method to facilitate the sequencing of both m^6A in RNA and 6mA in DNA. The chemistry takes advantage of the acid mediated nitrite reaction that chemoselectively deaminates adenine in the presence of N^6 -methyladenine. This results in a large increase in (d)A $\rightarrow$ (d)G transitions only at unmethylated sites. When coupled to high-throughput DNA sequencing, nitrite sequencing enables the identification of m^6A and 6mA sites at single-nucleotide resolution.