



Epigenomics

M6A MODIFICATIONS AND BIOINFORMATICS

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Summary

- Epigenetics VS Epigenomics
- Epigenetic phenomena
- Contribution of epigenome landscape
- Epigenomics in RNA
- m6A modification
- m6A associated diseases
- Bioinformatic techniques for epitranscriptomics
- Detection of m6A
 - Previous techniques
 - Nowadays (bioinformatics and sequencing)
- Further approaches



Epigenetics

Study of reversible, heritable changes in gene expression that do not involve changes to the underlying DNA sequence—a change in phenotype without a change in genotype

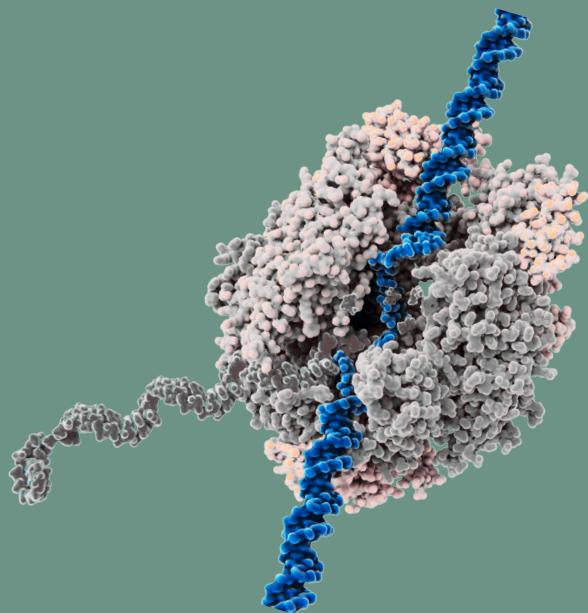
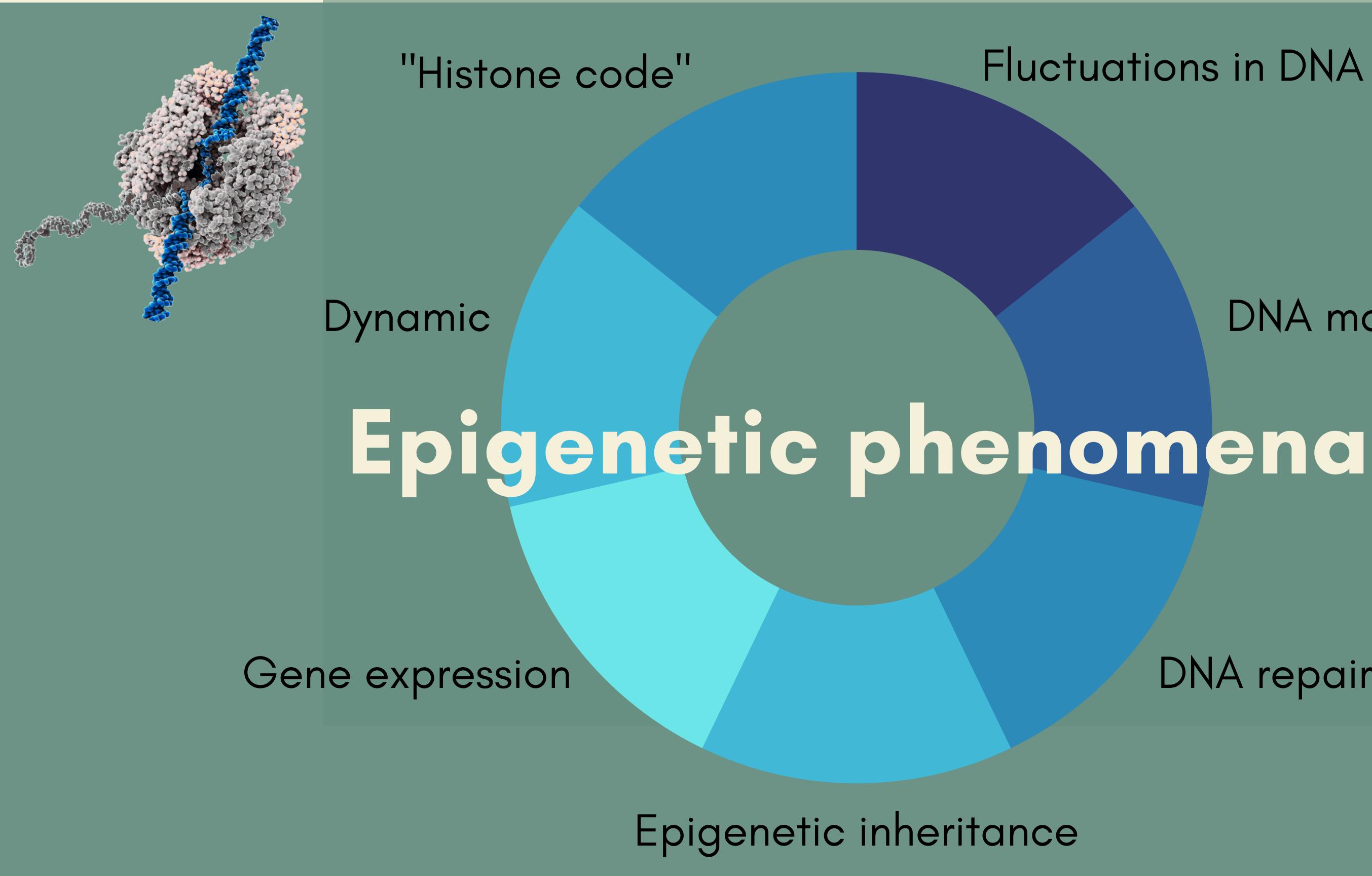
DNA methylation, chromatin remodeling, histone modification, and noncoding RNA

VS

Epigenomics

Study of the effects of chromatin structure (higher order chromatin folding and attachment to the nuclear matrix, packaging of DNA around nucleosomes, covalent modifications of histone tails , and DNA methylation) on the genetic material of a cell, known as the epigenome





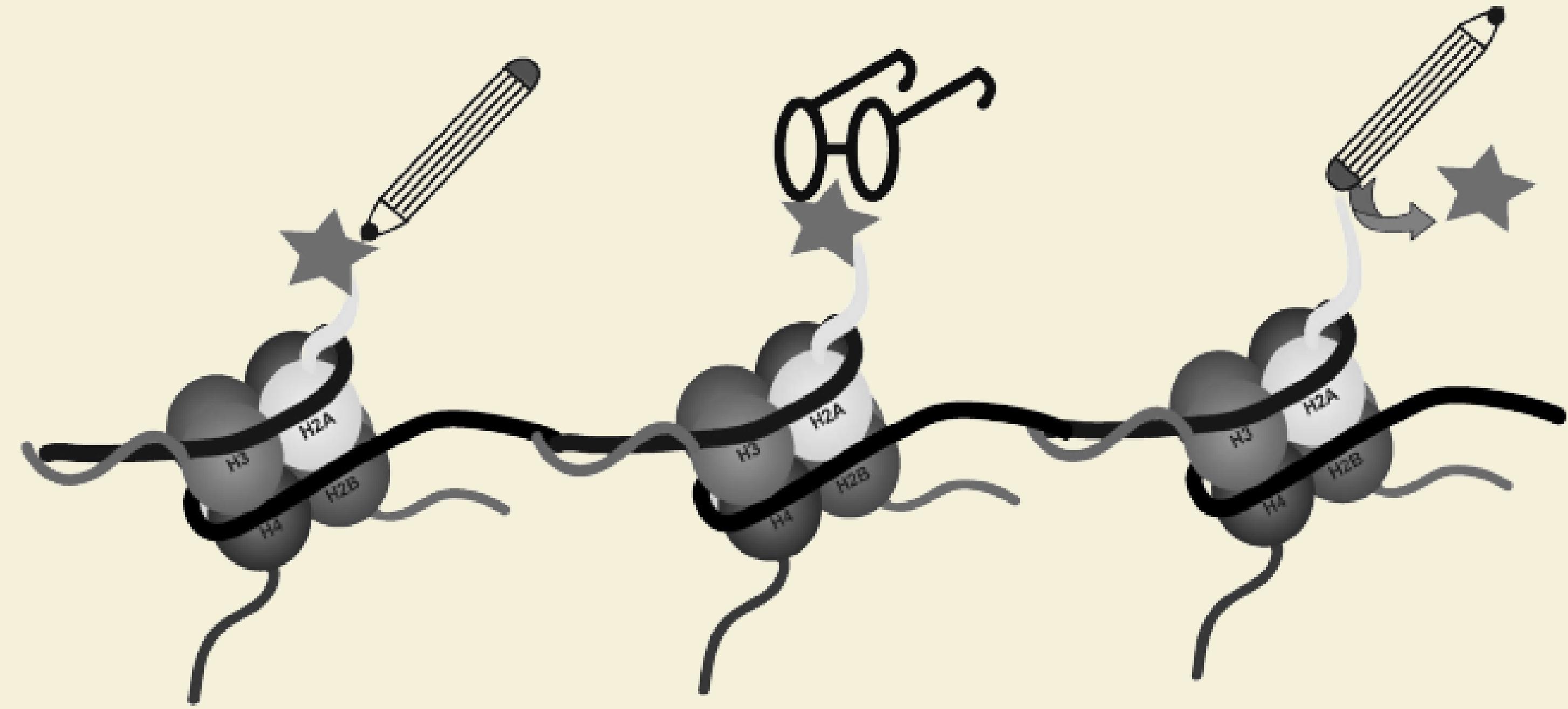
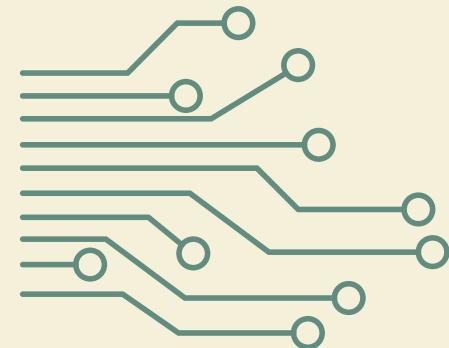


Figure 1. Writers, readers and erasers of histone covalent modifications.

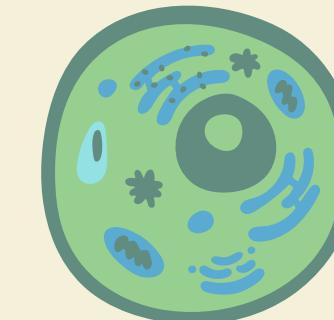
Mehta, Stuti & Jeffrey, Kate. (2015). Beyond receptors and signaling: Epigenetic factors in the regulation of innate immunity. *Immunology and cell biology*. 93. 10.1038/icb.2014.101.

How does the epigenome landscape contribute to...

Cellular circuits?



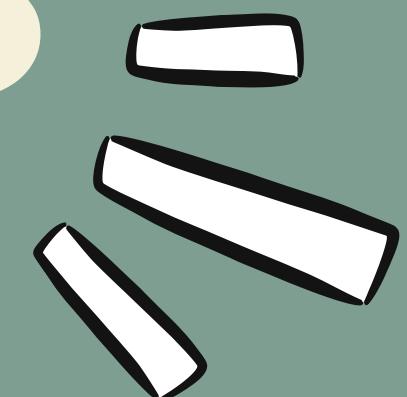
Cell lineage specification?



Start and progression of disease?



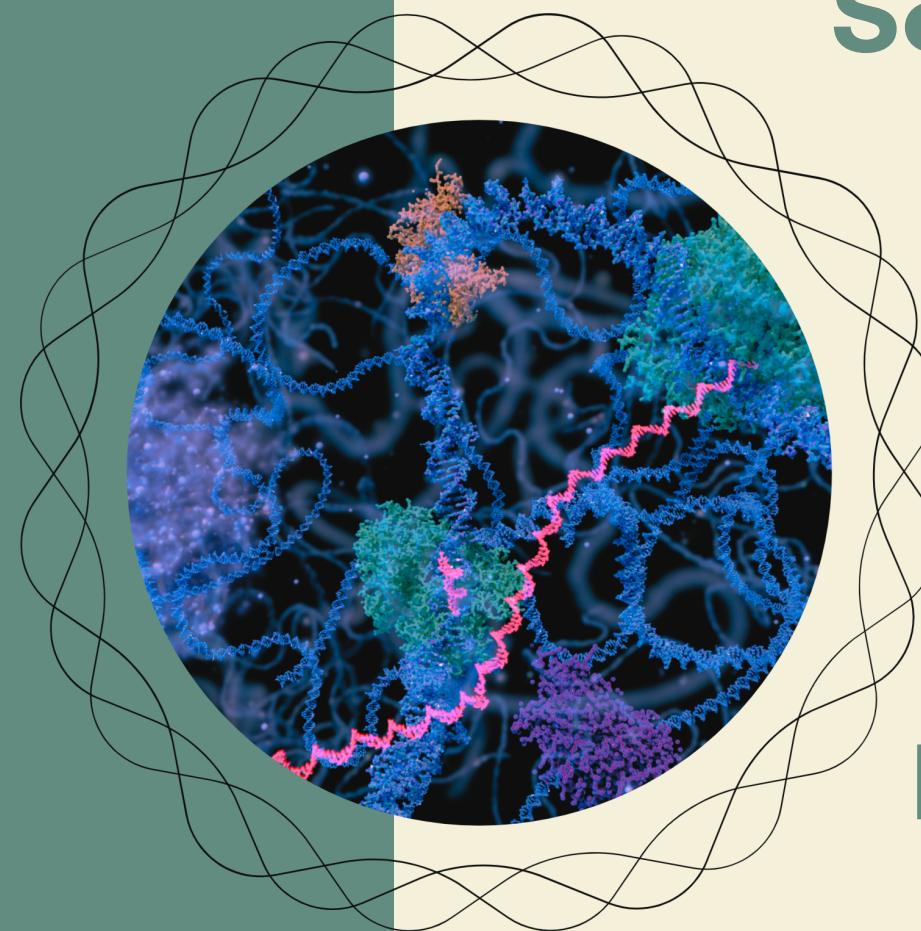
BIOINFORMATICS



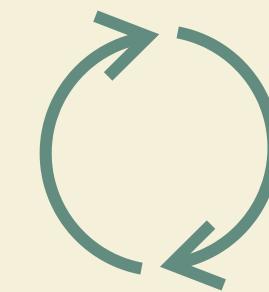
Epigenomics in RNA



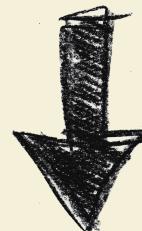
+100 posttranscriptional RNA modifications



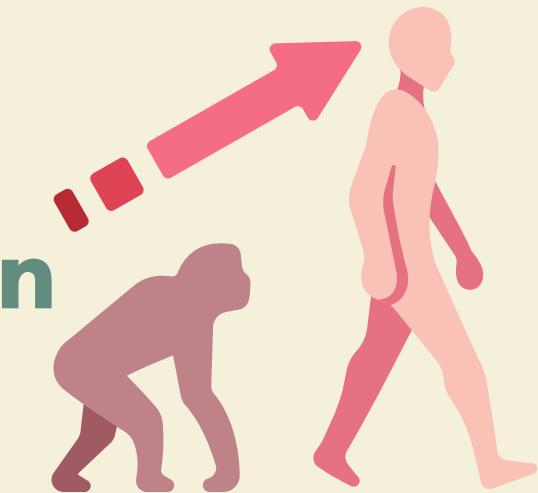
Some of them are reversible



Epitranscriptome



Evolutionary conservation



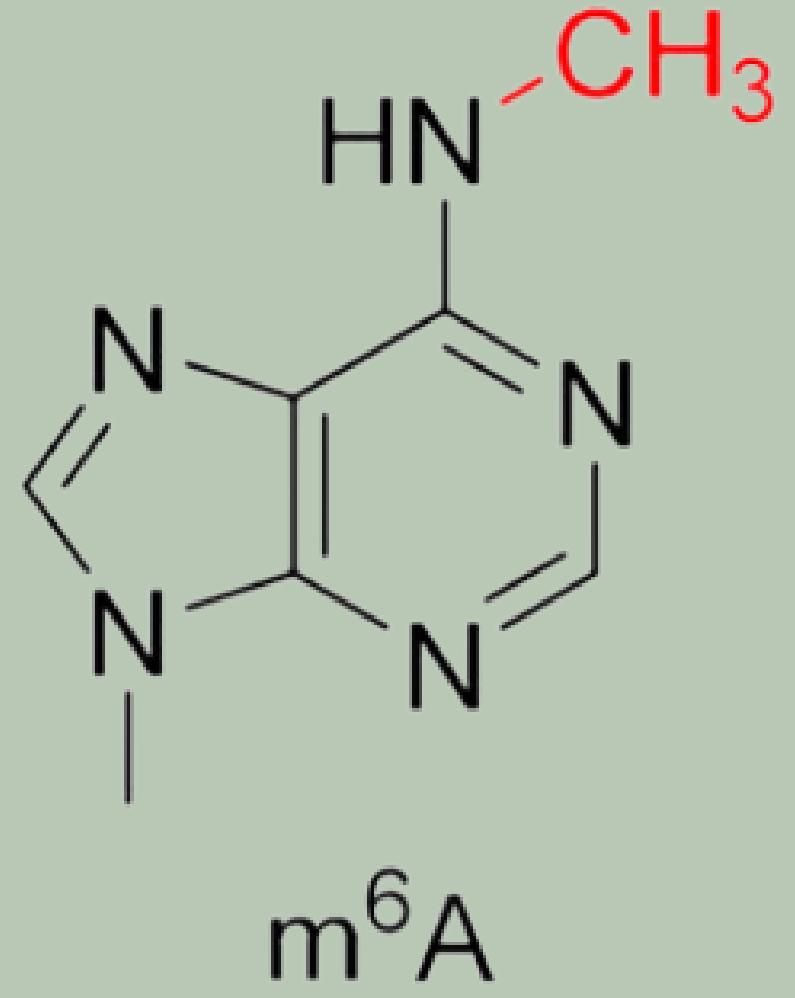
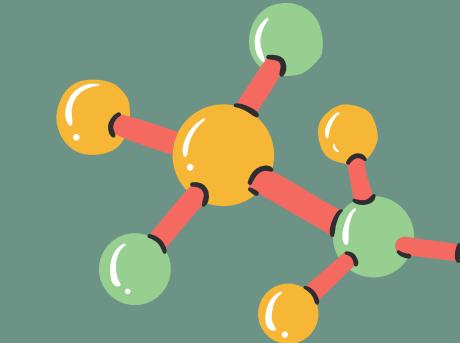


Figure 2. Chemical structure of m6A modification

Liu, N., & Pan, T. (2015). RNA epigenetics. In Translational Research (Vol. 165, Issue 1, pp. 28–35). Mosby Inc.

m6A modification



- Is the more abundant mRNA/lncRNA modification
- Can't be detected by the reverse transcriptase
- Hard to map the modifications in single nucleotide resolution
- Has effects on splice, transport, stability and immunologic tolerance of mRNA
- Mammals cells' determination of destination and differentiation of embryonic stem cells

- The m6A modification is catalyzed by an unidentified methyltransferase complex containing at least one subunit methyltransferase like 3 (METTL3)
- There are two m6A RNA demethylases: FTO gene and ALKBH5, which catalyze m6A demethylation in an α -ketoglutarate (α -KG)- and Fe $^{2+}$ -dependent manner

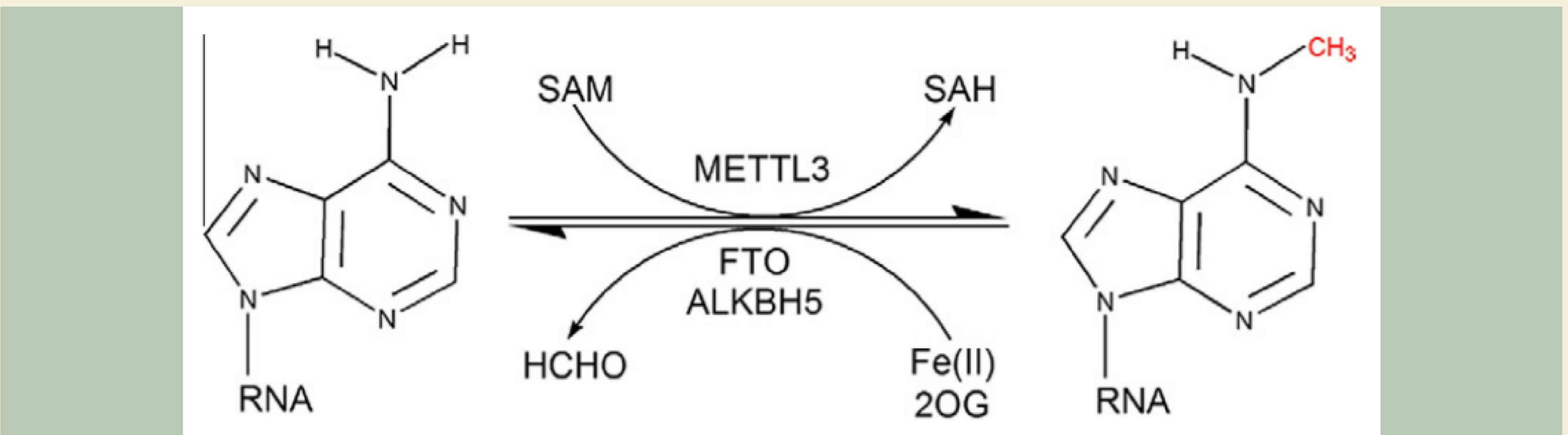


Figure 3. Reversible m6 A methylation in mRNA.

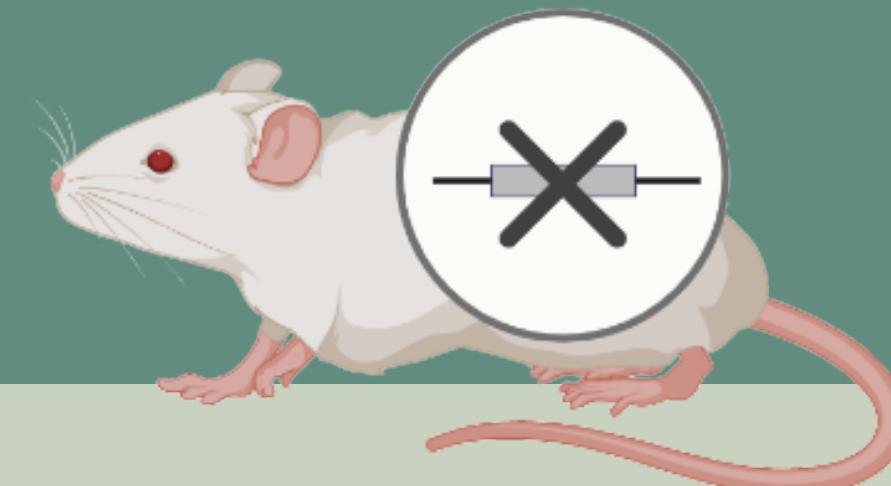
Niu, Y., Zhao, X., Wu, Y. S., Li, M. M., Wang, X. J., & Yang, Y. G. (2013). N6-methyl-adenosine (m6A) in RNA: An Old Modification with A Novel Epigenetic Function. In Genomics, Proteomics and Bioinformatics (Vol. 11, Issue 1, pp. 8-17).

m6A = FTO's substrate

FTO= CATALIZES THE OXIDATIVE REVERSAL
OF METHYLATED DNA AND RNA BASES

GWAS → FTO gene

FTO knockout -> slimmer



→ Diabetes and
obesity

FTO wild-type-> fatter



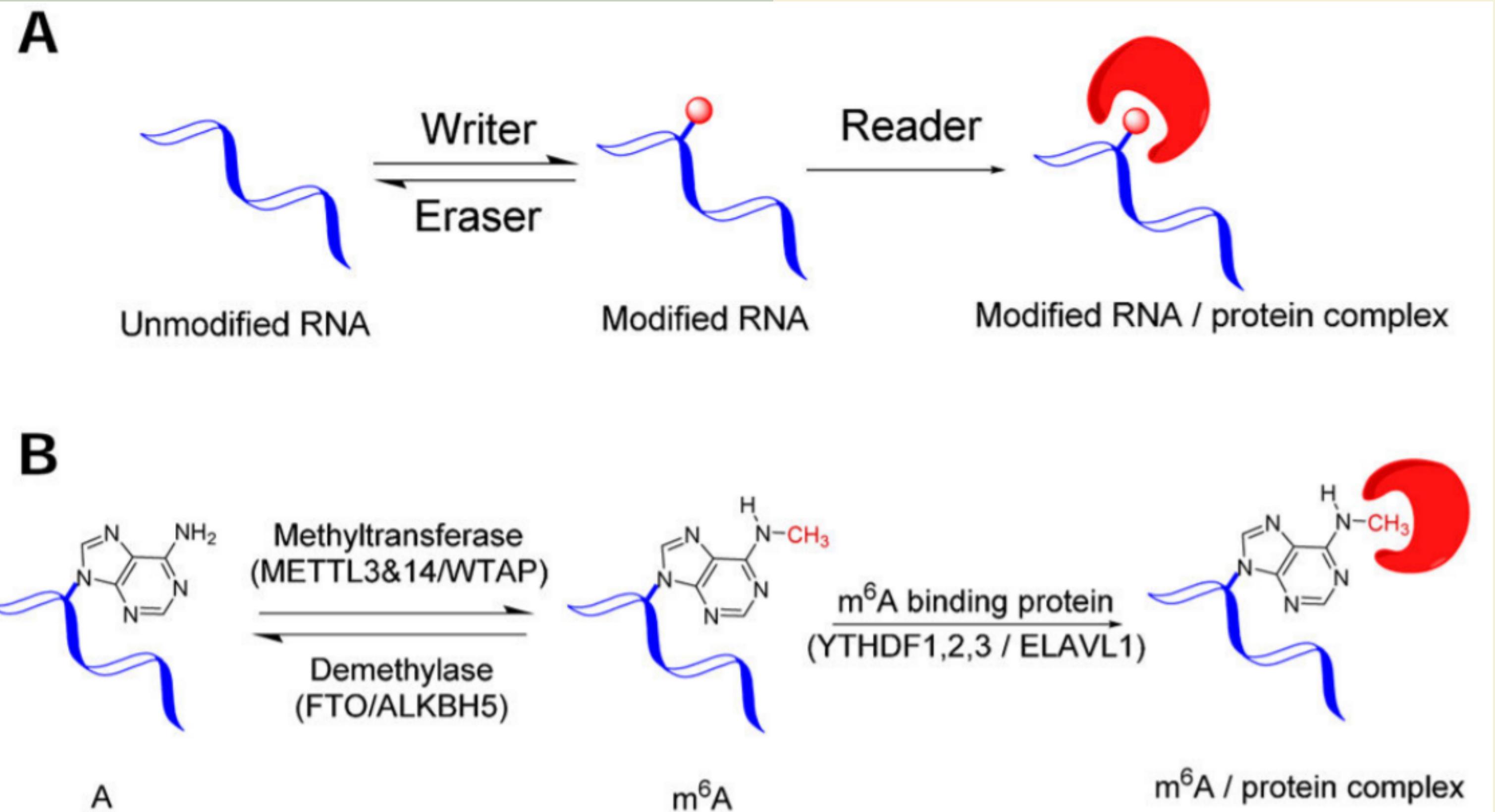
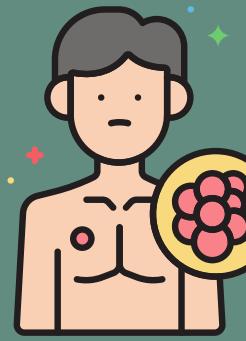


Figure 4. (A) Schematic plot for general RNA modification. (B) Schematic plot for the m^6A modification. FTO and ALKBH5 are erasers. METTL3 is a writer. m^6A binding proteins are readers.

m6A associated diseases



cancer

glioblastoma

brain diseases

Alzheimer

Parkinson

heptocellular carcinoma

Gene	Function	Phenotype	Associated Diseases
METTL3	Methyltransferase	Apoptosis, development	Prostatitis, Aicardi Syndrome
METTL14	Methyltransferase	Apoptosis, development	Alcohol dependence, Alcoholism
WTAP	Scaffolding or localization	mRNA splicing	Wilms Tumor, Hypospadias, Sarcoma, Malignant Mesothelioma, Synovial Sarcoma
FTO	Demethylase	Cellular energy homeostasis	Obesity, diabetes, Polycystic Ovary Syndrome, Heart attack, Cancer, Alcoholism, Mental Disorders, Cataract, Hepatitis
ALKBH5	Demethylase	Male fertility	Hypoxia, Smith Magenis Syndrome
YTHDF1	m ⁶ A binding	mRNA stability	Pancreatic Cancer, Pancreatitis, Dermatomyositis
YTHDF2	m ⁶ A binding	mRNA stability	Leukemia, Renal Cell Carcinoma, Breast Cancer
YTHDF3	m ⁶ A binding	N/A	N/A
ELAVL1	m ⁶ A binding	Apoptosis, mRNA stability	Cancer, Leukemia, Hepatitis, Anoxia, Alzheimer's Disease, Arthritis, Endotheliitis, Prostatitis, Hypoxia, Laryngitis, Keratoconus, Pancreatitis

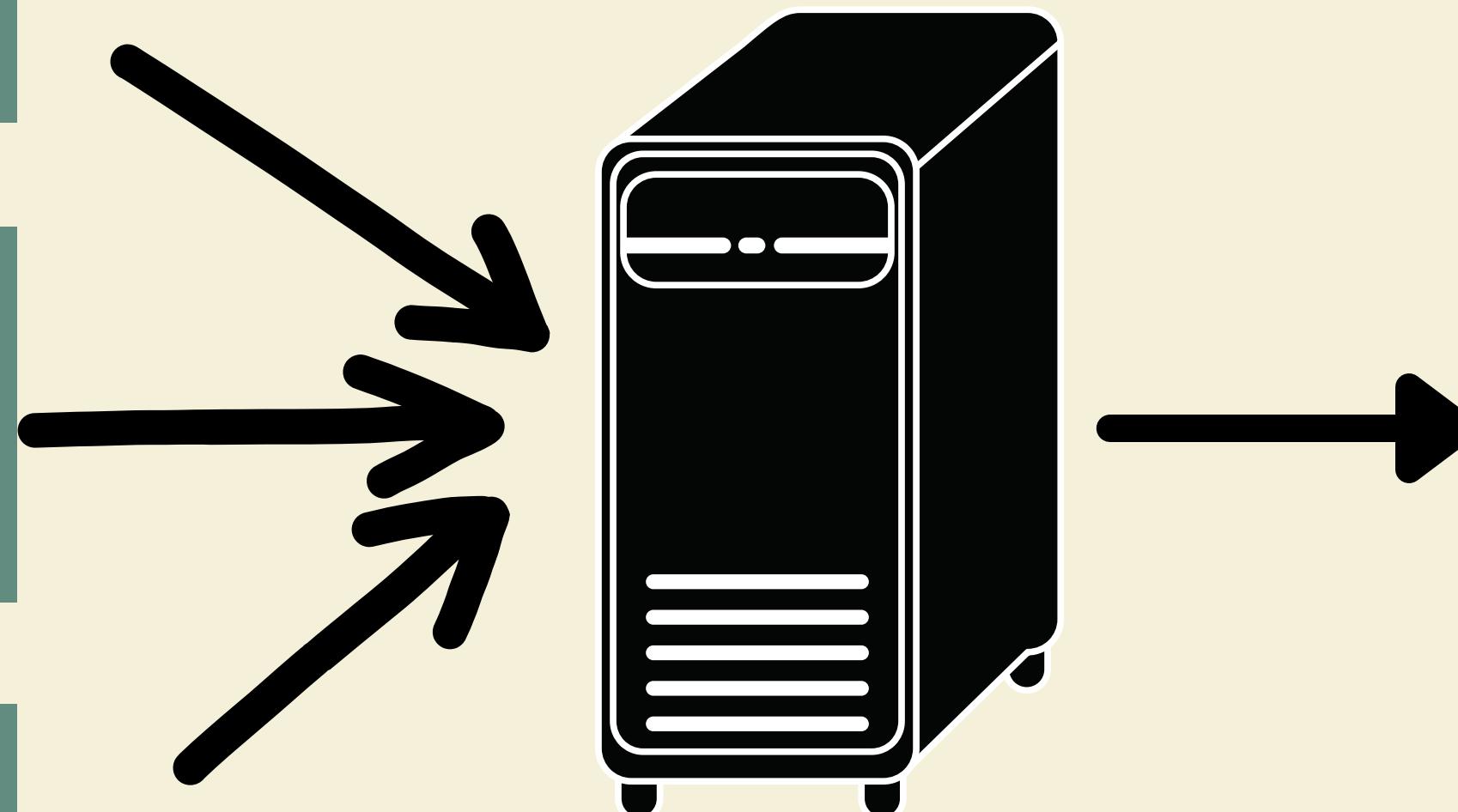
Figure 4. Human diseases associated with genes involved in m6A modification.

Bioinformatic techniques for epitranscriptomics

Histone modification
detection

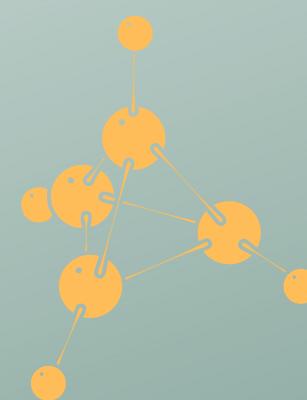
Genome-Wide methylation
profiling

Analysis of Chromatin
accessibility data

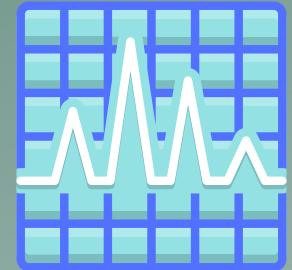


How was detected m6A previously?

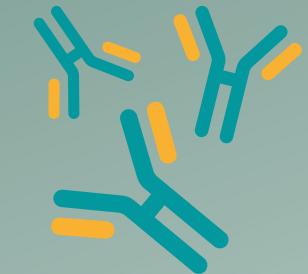
- m6A-seq
- Thin-layer chromatography
- Liquid chromatography (HPLC)
- Capillary electrophoresis



- Liquid chromatografy - mass spectrometry (LC-MS / MS)

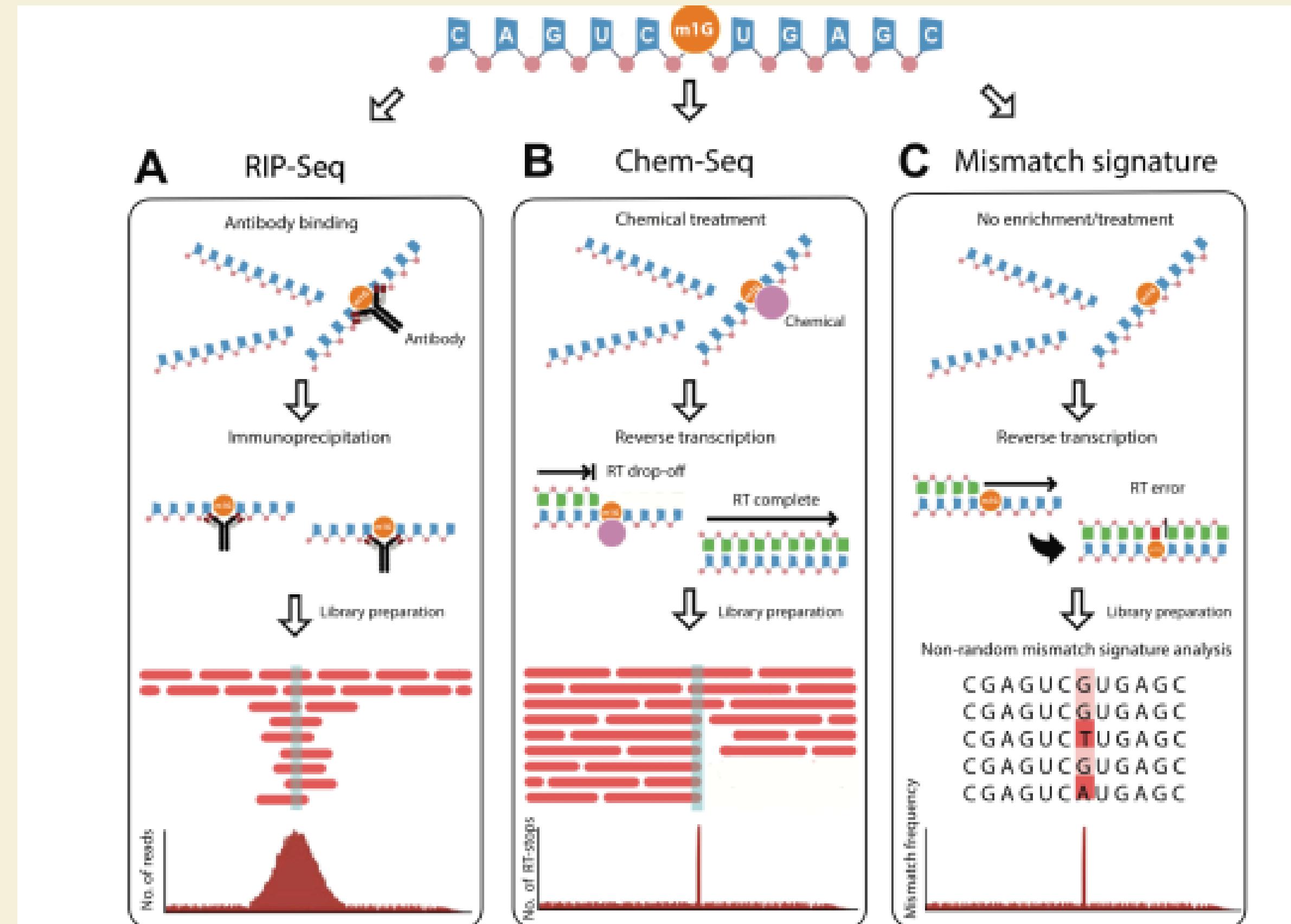


- Antibodies or chemical methods
- Immunoprecipitation



How to detect it nowadays by sequencing and bioinformatics

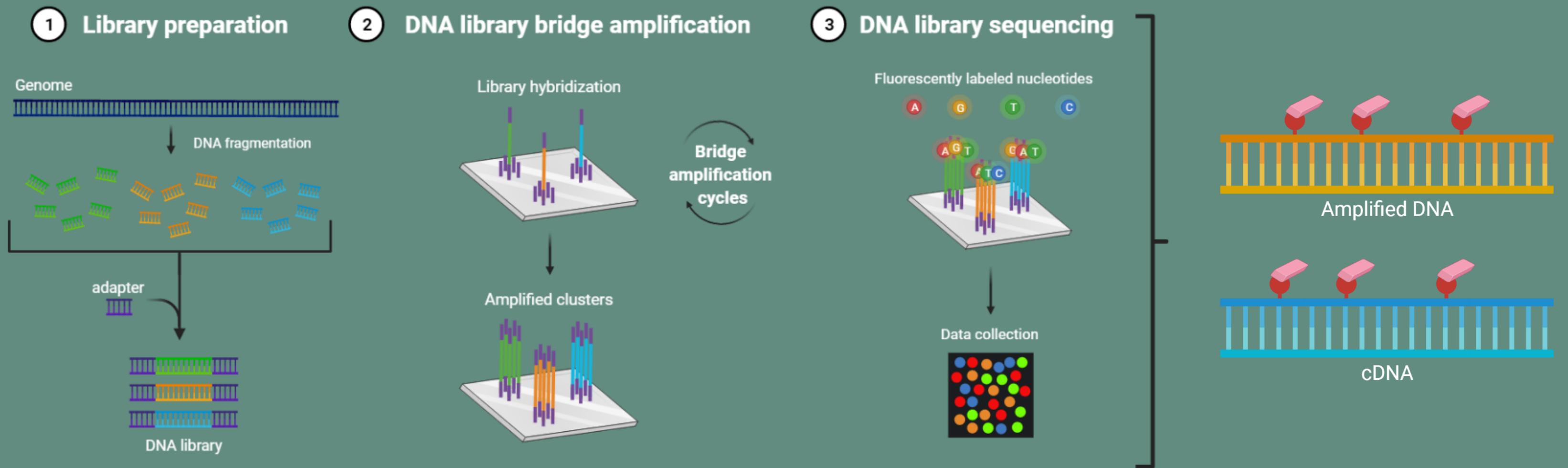
Figure 5. Current genome-wide detection methods used to identify RNA modifications. A) In the left panel schematic RIP-seq method, normalization and library preparation. B) In the middle panel, schematic Chem-seq technique to inhibit reverse transcription beyond chemically modified position. C) In the right panel, mismatch signature-based methods, which are based on the increased mismatch rates that occur upon reverse transcription at certain RNA-modified positions, are depicted.



Further approaches

Limitation: Sequence-by- Synthesis Technologies (SBS)

illumina

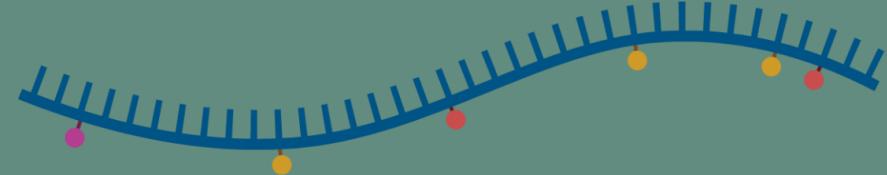


What could be the alternative when studying epigenetic modifications in RNA?

Further approaches

Alternative: Third Generation Sequencing (TGS)

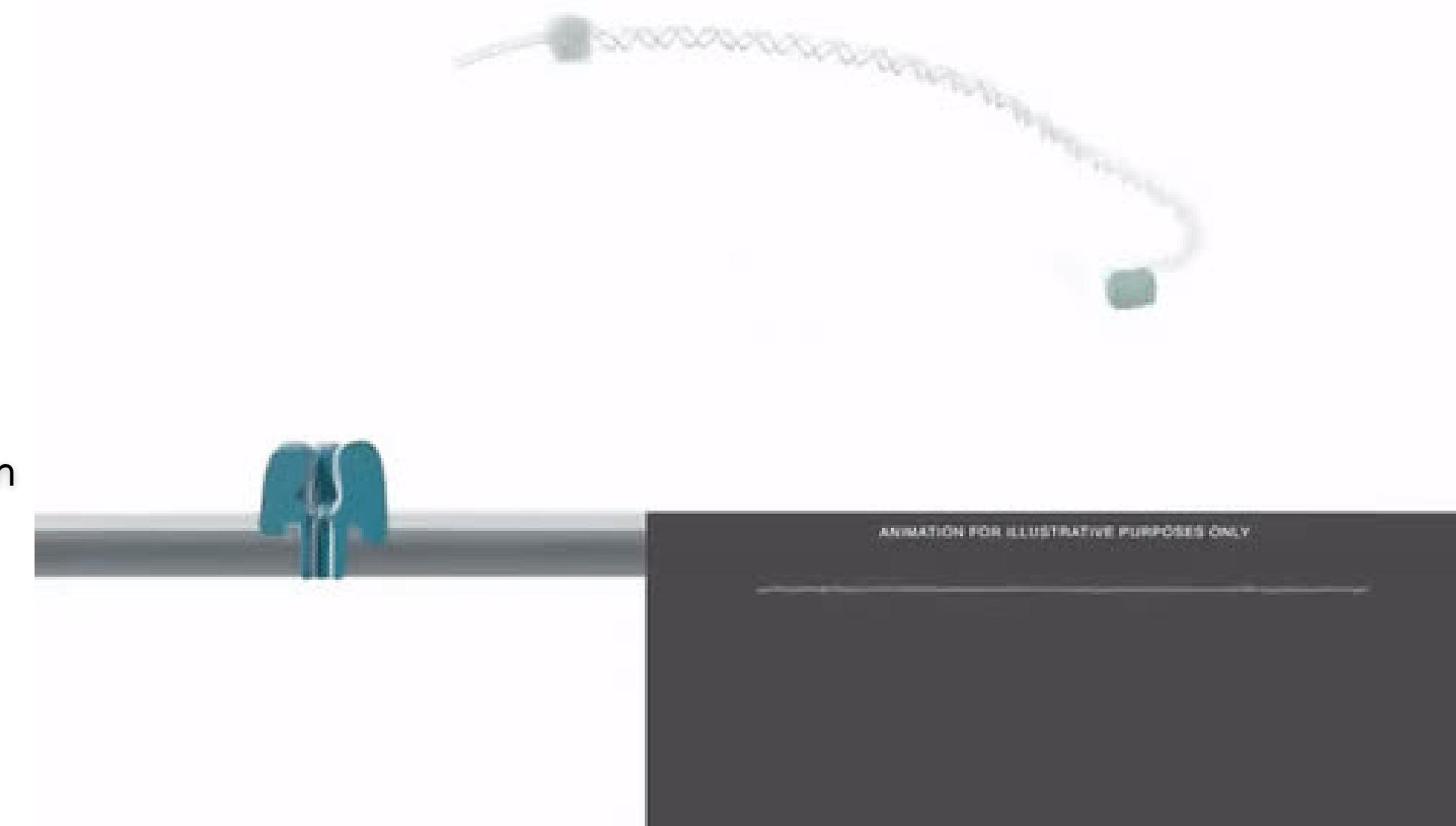
Longer reads



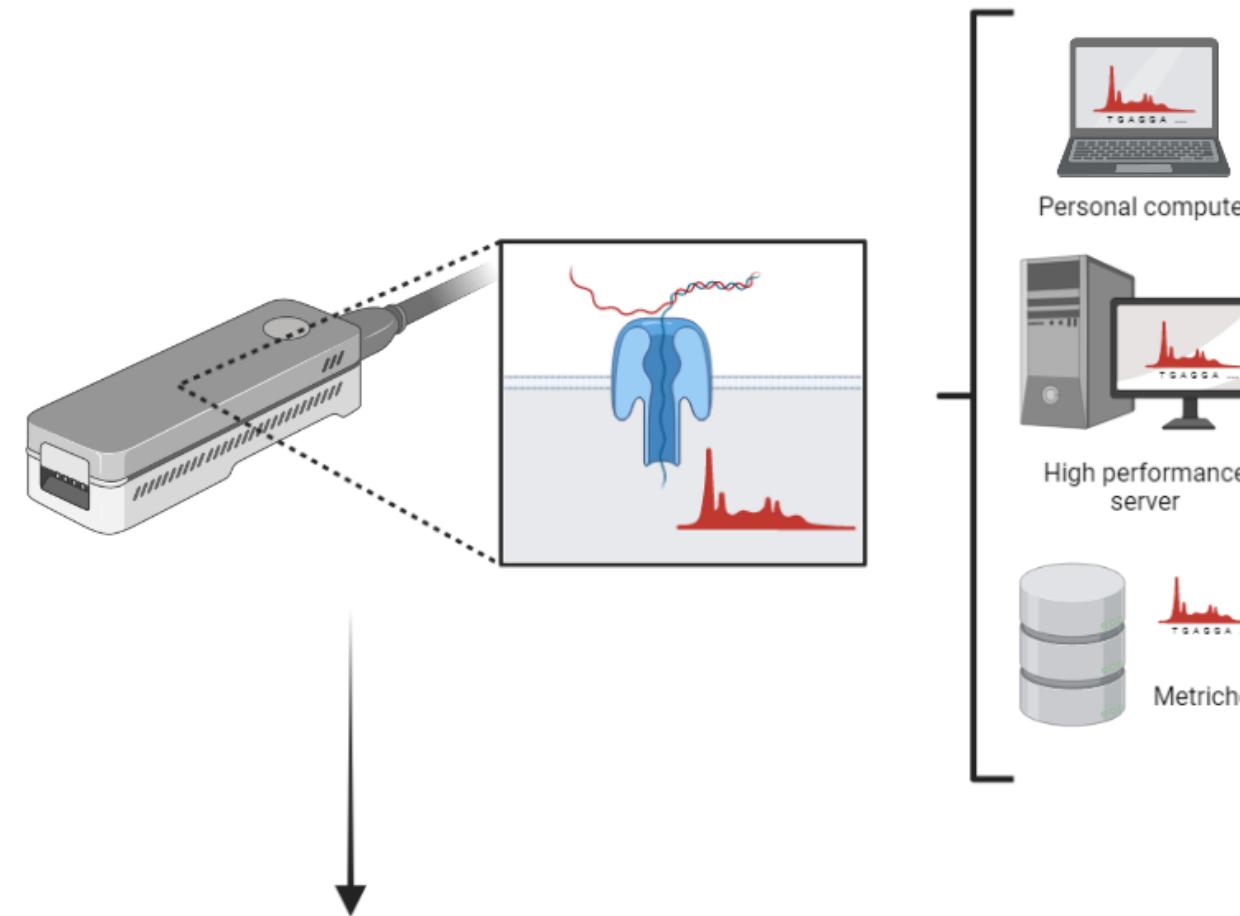
Single molecule sequencing, without amplification



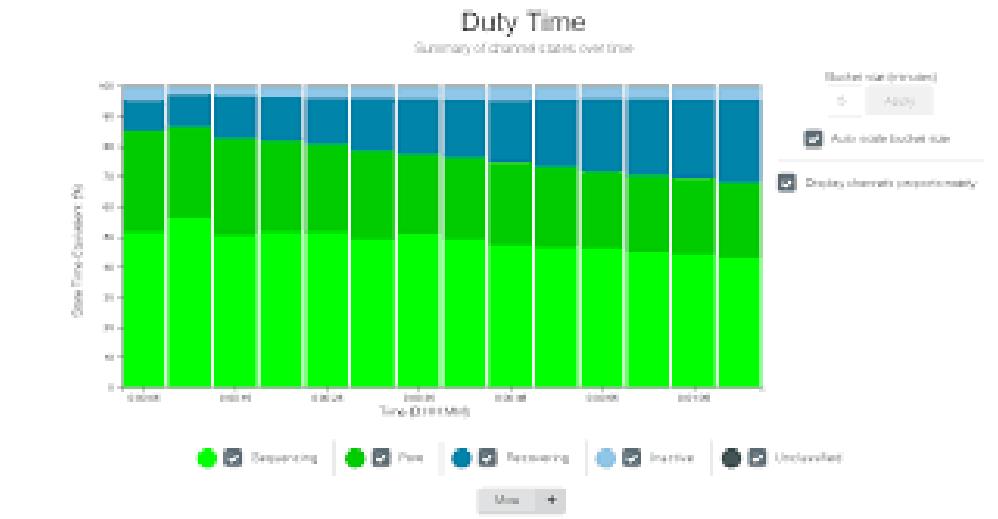
Low sample handling reduces biases introduced during SBS library preparation by fragmentation, PCR amplification or immunoprecipitation.



Further approaches

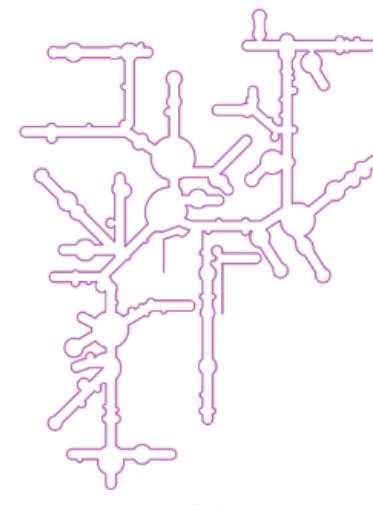


No modifications detected
Low base-calling



Decrease in the performance of each individual sequencing run.

Solutions



Training models from biological control data



More efficient nanopore protein

Base-calling with homopolymers recognition

Paired-end consensus reading

Further approaches

Third Generation Sequencing (TGS)

Distinguish whether two RNA modifications present in a given mRNA sequence actually coexist in the same RNA molecule.

Identify in which RNA transcript isoform the modification is located.

Study different diseases with biological basis or detection method related to epigenetic marks.

Rapid detection by these third-generation techniques can be a very important step.



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