

**TABLE S2** Comparison of the current techniques to map m<sup>6</sup>A modification within RNA

Technique	Description	Strengths	Limitations
meRIP-seq [117–118]	Transcriptome-wide identification of those RNAs (or fragment regions) as m <sup>6</sup> A-enriched, which bind with anti-m <sup>6</sup> A-antibody prior PCR above that of input control	<ul style="list-style-type: none"> <li>Relatively low input material is needed</li> <li>RNA library preparation is straightforward</li> </ul>	<ul style="list-style-type: none"> <li>Cannot offer single-nucleotide resolution (~200nt)</li> <li>Cannot identify multiple near located m<sup>6</sup>A residues</li> <li>Increased risk for false positive peaks</li> <li>Cannot reliably distinguish m<sup>6</sup>A from m<sup>6</sup>A<sub>m</sub></li> <li>High level of variability between replicates [122]</li> </ul>
LAIC-seq [123]	Transcriptome-wide identification of those RNAs (or fragment regions) as m <sup>6</sup> A-enriched, which bind with anti-m <sup>6</sup> A-antibody prior PCR above that of input control and at the same time are depleted from the post-IP supernatant	<ul style="list-style-type: none"> <li>Provides semi-stoichiometric information</li> </ul>	<ul style="list-style-type: none"> <li>Time-consuming method</li> <li>Titration of the antibody empirically necessary</li> <li>Spike-in (methylated and non-methylated) controls necessary</li> <li>Inability to distinguish m<sup>6</sup>A from m<sup>6</sup>A<sub>m</sub></li> </ul>
meRIP-qPCR [124]	Targeted quantification of the methylation level of a given region of interest relative to the input	<ul style="list-style-type: none"> <li>Provides semi-stoichiometric information at given specific m<sup>6</sup>A site</li> <li>Straightforward protocol</li> <li>Low input material is needed</li> </ul>	<ul style="list-style-type: none"> <li>Cannot identify stoichiometry of near m<sup>6</sup>A sites</li> <li>High level of variability between replicates</li> <li>Spike-in (methylated and non-methylated) controls necessary</li> </ul>
m <sup>6</sup> A-CLIP/IP [125]	Transcriptome-wide single nucleotide resolution identification of m <sup>6</sup> A sites after binding of the m <sup>6</sup> A with an antibody crosslinking further with the mRNA via UV. Ultimate identification of bases via induced mutation and truncation profile	<ul style="list-style-type: none"> <li>High specificity due to chemical profiles</li> <li>Capable to detect multiple m<sup>6</sup>A sites within a single transcript</li> </ul>	<ul style="list-style-type: none"> <li>No stoichiometric information</li> <li>High input (mRNA) material required</li> <li>Time-consuming method</li> </ul>
miCLIP [126]	Transcriptome-wide single nucleotide resolution identification of m <sup>6</sup> A sites after binding of the m <sup>6</sup> A with an antibody crosslinking further with the mRNA via UV. Ultimate identification of bases requires mutational signature at the site	<ul style="list-style-type: none"> <li>High specificity due to chemical profiles</li> <li>Capable to detect multiple m<sup>6</sup>A sites within a peak or adjacent sequence</li> <li>Capable to differentiate m<sup>6</sup>A from m<sup>6</sup>A<sub>m</sub></li> </ul>	<ul style="list-style-type: none"> <li>No stoichiometric information</li> <li>High input (mRNA) material required</li> <li>Time-consuming method</li> </ul>
m <sup>6</sup> A ELISA [127]	ELISA-based ready-to-use kit detecting the amount of m <sup>6</sup> A-antibody-enriched RNA	<ul style="list-style-type: none"> <li>Relatively low input material is needed</li> <li>Standardized and commercial method</li> </ul>	<ul style="list-style-type: none"> <li>Easily contaminated by the m<sup>6</sup>A in other RNA species (in case of RNA species-targeted analysis)</li> </ul>
LC-MS [128]	Digestion to single nucleotides to detect the m <sup>6</sup> A by its physiochemical features via UV	<ul style="list-style-type: none"> <li>Low input material is needed</li> <li>Straightforward and standardized protocol</li> </ul>	<ul style="list-style-type: none"> <li>Cannot identify the origin of m<sup>6</sup>A if the sample material has multiple RNA species</li> </ul>
SCARLET [129]	Quantitates m <sup>6</sup> A in a specific site of interest via RNA H-site specific cleavage, splinted ligation, ribonuclease breakdown and chromatography	<ul style="list-style-type: none"> <li>Stoichiometric information for m<sup>6</sup>A at the given single site of interest</li> <li>Accurate method</li> </ul>	<ul style="list-style-type: none"> <li>Capable to measure only single site for each transcript in each run</li> <li>Time and sample consuming method</li> </ul>
MazF [130]	Fluorescent-based site-specific quantitation of m <sup>6</sup> As near ACA sequence via MazF cleavage	<ul style="list-style-type: none"> <li>m<sup>6</sup>A stoichiometry at specific ACA contexts</li> <li>No antibody</li> </ul>	<ul style="list-style-type: none"> <li>Uncapable to measure other than ACA sites</li> </ul>
MAZTER-seq [120]	Transcriptome-wide detection of m <sup>6</sup> A sites in ACA regions via ACA-specific MazF cleavage	<ul style="list-style-type: none"> <li>Widespread mapping method, no antibody</li> <li>Stoichiometric information at ACA sites</li> <li>Low rate of false-positives</li> <li>Relatively straightforward method</li> </ul>	<ul style="list-style-type: none"> <li>Uncapable to measure other than ACA sites</li> <li>Cannot distinguish ACA sites closely adjacent with each other</li> </ul>

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TABLE S2 Cont'd (1)

Technique	Description	Strengths	Limitations
2D-TLC [131]	RNase T1 cleavage coupled with a 2D thin-layer chromatography to quantify m <sup>6</sup> A in GAC regions; normalized to the total RNA adenosine content	<ul style="list-style-type: none"> <li>Accurate; cannot identify m<sup>6</sup>A residues in AAC sites of rRNAs</li> </ul>	<ul style="list-style-type: none"> <li>Cannot identify m<sup>6</sup>As other than those preceded by guanosine (G)</li> <li>Radioactivity-requiring time-consuming method</li> </ul>
m <sup>6</sup> A-deoxyribozymes [132]	Ribozymes optimized to cleave RNA with m <sup>6</sup> A in DRACH sequence	<ul style="list-style-type: none"> <li>Measurement of m<sup>6</sup>A in the canonical consensus sequence</li> </ul>	<ul style="list-style-type: none"> <li>Required designing dedicated deoxyribozyme for each site of putative interest</li> </ul>
4SedTTP-RT [133]	RT-dependent truncation profile while 4Sed-TTP is used instead dTTP nucleotide during cDNA synthesis to pinpoint m <sup>6</sup> A sites	<ul style="list-style-type: none"> <li>Potential to identify each m<sup>6</sup>A irrespective of the surrounding motif sequences</li> <li>No required input</li> </ul>	<ul style="list-style-type: none"> <li>High false-positive rate (background truncation)</li> <li>High false-negative rate (eraser depletion necessary as a parallel control)</li> </ul>
Tth polymerase [134]	Detection of m <sup>6</sup> A residues by <i>Tth</i> DNA polymerase with a primer extension as it prefers dTTP incorporation opposite A as compared to m <sup>6</sup> A	<ul style="list-style-type: none"> <li>Relatively straightforward and validated</li> </ul>	<ul style="list-style-type: none"> <li>Cannot identify multiple m<sup>6</sup>As within a transcript</li> <li>Low throughput</li> <li>Reaction time and RNA concentration affect the dTTP incorporation efficiency opposite m<sup>6</sup>A</li> </ul>
RT-KTQ polymerase [135]	Detects m <sup>6</sup> A residues by recording m <sup>6</sup> A-induced increases in mis-incorporation rate by the RT-KTQ (a KlenTag DNA RT polymerase)	<ul style="list-style-type: none"> <li>Hold potential to detect every m<sup>6</sup>A site at a single nucleotide resolution without sequence motif dependence</li> <li>Relatively simple and stoichiometric</li> </ul>	<ul style="list-style-type: none"> <li>Poor to detect m<sup>6</sup>A residues at the transcript 5'-end</li> <li>High rate of false-positive results</li> </ul>
T3/T4 DNA ligase-qPCR [136]	Measurement of relative reduction in ligation efficiency of two probes designed to bind a site of interest close to m <sup>6</sup> A when it is present by qPCR	<ul style="list-style-type: none"> <li>Potential to measure stoichiometry</li> <li>Straightforward protocol and validated</li> </ul>	<ul style="list-style-type: none"> <li>Ligation efficiency can vary</li> <li>Low throughput</li> </ul>
SELECT [121]	Measurement of relative reduction in ligation efficiency at the nick site formed by <i>Bst</i> DNA polymerase as m <sup>6</sup> A is present during elongation	<ul style="list-style-type: none"> <li>Provides m<sup>6</sup>A stoichiometry</li> <li>Simple protocol and validated for specific mRNA sites</li> </ul>	<ul style="list-style-type: none"> <li>High rate of false-positive results</li> <li>Low throughput</li> <li>Two selective steps: 1) <i>Bst</i> and 2) ligation efficiencies</li> </ul>
m <sup>6</sup> A melting-qPCR [137]	m <sup>6</sup> A is detected by assessing the melting conditions as DNA oligo has been allowed prior to hybridize with sample RNA with or without m <sup>6</sup> A	<ul style="list-style-type: none"> <li>Relative stoichiometry at a given site</li> <li>Validated for some rRNA and snRNA sites</li> </ul>	<ul style="list-style-type: none"> <li>Sensitivity is low</li> </ul>
Nanopore [119,138]	Detection of characteristic m <sup>6</sup> A and A disruptions to the basic signal induced by the flow of current from a membrane-embedded nanopores while the sample RNAs travel through	<ul style="list-style-type: none"> <li>No need for complex library preparation</li> <li>No shortcomings and bias from PCR</li> <li>Provides opportunity to assess m<sup>6</sup>A in the native context of the transcript (e.g. isoform)</li> <li>Can measure the number m<sup>6</sup>As within a transcript in single nucleotide resolution</li> <li>Can provide stoichiometry</li> </ul>	<ul style="list-style-type: none"> <li>Current disruption by m<sup>6</sup>A little different from A</li> <li>Cannot distinct m<sup>1</sup>A from m<sup>6</sup>A; accuracy ~90%</li> </ul>
SMRT-seq [139]	Detects m <sup>6</sup> A as altered incorporation of labelled nucleotides during cDNA synthesis	<ul style="list-style-type: none"> <li>Information relative to m<sup>6</sup>A residue(s) with the transcript features (e.g. isoform)</li> </ul>	<ul style="list-style-type: none"> <li>High base error rate</li> <li>Low sensitivity</li> </ul>

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**TABLE S2** Cont'd (2)

Technique	Description	Strengths	Limitations
m <sup>6</sup> A-SEAL-seq [140]	FTO-assisted oxidation to unstable hm <sup>6</sup> A further stabilized via DTT-mediated thiol-addition (dm <sup>6</sup> A) and consequently biotin tagged and IP for transcriptome-wide mapping	<ul style="list-style-type: none"> <li>• No antibody needed, low input material</li> <li>• Sensitive, specific and reliable above all other m<sup>6</sup>A-seq methods in 8 tested samples</li> <li>• No m<sup>6</sup>A motif sequence dependence</li> </ul>	<ul style="list-style-type: none"> <li>• Currently detects m<sup>6</sup>A with only ~200nt resolution</li> <li>• Depends on multiple stepwise modifications with strict conditions</li> </ul>
DART-seq [141]	Transcriptome-wide mapping of m <sup>6</sup> A sites via C-U conversion in the m <sup>6</sup> AC sequence by cytidine deaminase APOBEC1 fused with an m <sup>6</sup> A YTH-binding domain (APOCEB1-YTH <sup>fusion</sup> )	<ul style="list-style-type: none"> <li>• No antibody, very low input needed</li> <li>• Potential to detect multiple m<sup>6</sup>A sites within a single transcript</li> </ul>	<ul style="list-style-type: none"> <li>• High false positive rate (steric hindrance?) [140]</li> <li>• Low sensitivity for low-abundance m<sup>6</sup>A sites <i>in vitro</i></li> <li>• Dependent on transfection efficiency of the APOCEB1-YTH<sup>fusion</sup> <i>in vivo</i></li> </ul>
PA-m <sup>6</sup> A-seq [142]	Transcriptome-wide detection of m <sup>6</sup> A sites via an anti-m <sup>6</sup> A antibody covalent UV-crosslinking after PI with consequent fragmentation by RNase T1	<ul style="list-style-type: none"> <li>• High-throughput</li> <li>• Increased resolution to meRIP-seq (~23nt)</li> </ul>	<ul style="list-style-type: none"> <li>• Dependent on m<sup>6</sup>A targeted antibody</li> <li>• No single nucleotide resolution</li> </ul>

Table modified and updated from Table 1 by Zaccara et al. [20]. Bolded horizontal lines group methods below as follows: **1) antibody-based**, **2) digestion-based**, **3) m<sup>6</sup>A-sensing and RT-based**, **4) ligation-based**, **5) hybridization-based**, **6) direct**, and **7) additional methods not listed by Zaccara et al. [20] (antibody- and C→U conversion-based)**.

**ABBREVIATIONS:** *APOBEC1*, C→U-editing enzyme APOBEC-1; *Bst*, *Bacillus stearothermophilus*; *CLIP*, UV crosslinking immunoprecipitation; *DART*, deamination adjacent to RNA modification targets; *dm<sup>6</sup>A*, N<sup>6</sup>-dithiolisitolmethyladenosine; *ELISA*, enzyme-linked immunosorbent assay; *hm<sup>6</sup>A*, N<sup>6</sup>-hydroxymethyladenosine; *IP*, Immunoprecipitation; *LAIC*, m<sup>6</sup>A-level and isoform-characterization; *MAZTER*, RNA digestion via m<sup>6</sup>A sensitive RNase; *mazF*, Endoribonuclease toxin MazF; *meRIP*, methylated RNA immunoprecipitation; *m<sup>6</sup>A-SEAL*, FTO-assisted m<sup>6</sup>A selective chemical labeling; *PA-m<sup>6</sup>A*, photocrosslinking-assisted m<sup>6</sup>A (sequencing); *qPCR*, quantitative polymerase chain reaction; *RT*, reverse transcription/transcriptase; *SELECT*, single-base elongation- and ligation-based qPCR amplification; *SMRT*, single-molecule real-time (sequencing); *Tth*, *Thermus thermophilus*; *UV*, Ultraviolet; *2D-TLC*, 2 dimensional thin layer chromatography; *4SedTTP*, 4-Selenothymidine-5'-triphosphate.