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基于高通量测序的RNA修饰检测技术

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摘　要：转录后修饰广泛存在于各种RNA分子中，对RNA发挥功能至关重要。目前，RNA上的化学修饰已达到160余种(https://iimcb.genesilico.pl/modomics/)，其中甲基化修饰是最常见的修饰类型。薄层层析、高效液相色谱及质谱等传统的检测方法对RNA修饰的鉴定和定量做出了重要贡献。然而，RNA修饰的精确定位以及对低丰度RNA分子的修饰检测等则依赖于近些年发展的适用于修饰检测的高通量测序技术。《生命科学》2018年发表的“RNA修饰检测技术”一文已对一些传统的RNA检测方法和部分高通量测序检测方法进行了系统的介绍。在此基础上，该文整理了目前常见的基于高通量测序的RNA修饰检测技术，并对其工作原理和应用展开介绍。

关键词：RNA修饰；高通量测序；RNA甲基化
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The detection methods of RNA modifications based on high-throughput sequencing

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Abstract: Post-transcriptional modifications widely exist in various RNA molecules and are essential for RNA function. At present, there are more than 160 kinds of chemical modifications on RNA (https://iimcb.genesilico.pl/modomics/), and methylation is the most common type of modification. Traditional RNA detection methods, such as thin layer chromatography, high performance liquid chromatography and mass spectrometry, have made

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important contributions to the identification and quantification of RNA modifications. However, the precise identification of RNA modifications and the detection of modifications on low abundant RNA molecules rely on some high-throughput sequencing based techniques, which have been developed in recent years. Traditional RNA detection methods and some high-throughput sequencing based methods have been introduced in the article “The detection methods of RNA modifications” published in this journal in 2018. Here, we summarize the popular detection methods of RNA modification based on high-throughput sequencing, and introduce their working principles and application.

Key words: RNA modification; high-throughput sequencing; RNA methylation

化学修饰使组成RNA分子的4个标准核苷酸扩展到超过160个修饰核苷酸，这大大增加了RNA结构和功能的复杂性[1-2]。RNA修饰可以调节RNA生命周期的全部过程，包括RNA转录、加工、成熟、出核等[3-5]，影响RNA稳定性和蛋白质生物合成、细胞生长、代谢、压力应激、免疫应答等多种生物功能[6-10]，并与多种疾病，如神经系统疾病、癌症、线粒体疾病、男性不育等密切相关[11-15]。RNA修饰研究开创了专门的研究领域，称为“RNA表观遗传学”(RNA epigenetics)，其已成为研究热点[16-18]。因此，建立可靠的方法来研究RNA修饰是RNA表观遗传学研究的重要组成部分。传统的RNA修饰检测方法包括二维纤维素薄层层析(two-dimensional cellulose thin-layer chromatography, 2D-TLC)、引物延伸(primer extension)、高效液相色谱(high performance liquid chromatography, HPLC)、高效液相色谱与质谱联用(coupling of high performance liquid chromatography to mass spectrometry, HPLC-MS)等。近年来，随着高通量测序技术的发展，可以同时对几十万到几百万条DNA 分子进行序列测定，这大大增加了检测效率。检测RNA修饰核苷的高通量测序技术通常包括结合抗体免疫沉淀的测序技术，如CLIP-seq (crosslinking-immunoprecipitation sequencing)、RIP-seq (RNA immunoprecipitation sequencing)、PAR-CLIP-seq (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation sequencing)、MeRIP-seq (methylated RNA immunoprecipitation sequencing)等，以及依赖化合物、化学小分子标记的测序技术等。上述部分技术的原理在2018年本刊第30卷第4期“RNA修饰检测技术”[19]一文中已有详细介绍，这里不再进行赘述。本文将针对常见的RNA修饰着重介绍基于高通量测序的检测技术。

1 m^{6}A

N^6-甲基腺苷(N^6-methyladenosine, m^{6}A)是真核生物信使RNA (messenger RNA, mRNA)上含量最丰富的修饰[20-22]。mRNA上m^{6}A由METTL3、METTL14和WTAP等蛋白质组成的甲基转移酶复合体催化形成[23-28]，又能被去甲基化酶FTO和ALKBH5去除[29-30]。m^{6}A 修饰的检测技术在“RNA修饰检测技术”[19]一文中已有较详细的介绍，对相似的内容下文将进行简要说明。

m^{6}A-seq和MeRIP-seq将分离纯化后的mRNA切割成100~200 nt的 RNA 片段，并使用m^{6}A特异性的抗体富集含有m^{6}A修饰的片段，随后进行逆转录扩增以及高通量测序[31-32]。该方法需要大量的样本，不适用于某些珍贵的样本，如早期胚胎或病理样本。此外，由于RNA片段长度的限制，检测还无法达到单核苷酸分辨率。Schwartz等[33]通过减小测序的RNA片段，在酵母中开发了一种检测分辨率接近单核苷酸的m^{6}A免疫沉淀方法。

结合紫外线诱导的RNA-抗体交联的方法可以在细胞中以单核苷酸分辨率检测m^{6}A修饰。目前该方法分为两种：第一种是基于光活性增强的核糖核苷交联免疫沉淀(PAR-CLIP)，例如光交联辅助的m^{6}A测序方法(photo-crosslinking-assisted m^{6}A sequencing strategy, PA-m^{6}A-seq)[34]。该方法通过添加具有光活性的核苷类似物4-硫代尿苷(4-thiouridine, 4sU)并使其掺入新生 RNA转录本中，经m^{6}A特异性抗体免疫沉淀后，在365 nm紫外光下诱导经4sU 标记的含有m^{6}A的RNA与m^{6}A抗体交联，通过核糖核酸酶T1将交联的RNA消化到30 nt，并进行测序。由于4sU会在交联位点产生胸腺嘧啶到胞嘧啶突变，PA-m^{6}A-seq可有效提高m^{6}A修饰检测的信噪比。但是这种方法可能会遗漏4sU掺入位点附近的m^{6}A修饰。第二种是基于紫外交联免疫沉淀(UV cross-linking and immunoprecipitation sequencing, UV-CLIP-seq)，如m^{6}A-CLIP和miCLIP[35-36]。m^{6}A-CLIP和miCLIP都使用特异性抗体免疫沉淀包含m^{6}A修饰的RNA片段，并在254 nm紫外光下诱导其与抗

体交联，随后用蛋白酶K消化结合的抗体，回收交联的RNA。在逆转录过程中，蛋白质与RNA的交联位点会产生截断或突变，由此可以确定m^6A的准确位置。

尽管这些方法在m^6A的检测分辨率方面取得了很大的进步，但m^6A修饰在细胞内的真实含量仍然不清楚。SCARLET (site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography)可以定量检测特定位点的m^6A化学计量比[37]。m^6A-LAIC-seq (m^6A-level and isoform-characterization sequencing)在与m^6A特异性抗体免疫沉淀反应前不打断RNA，而是在免疫沉淀后通过测序方法获得完整转录本[38]。该方法能区分甲基化与非甲基化的转录本，可以定量检测m^6A。

m^6A-REF-seq和MAZTER-seq是两种基于RNA内切酶MazF的m^6A修饰检测方法[39-40]。MazF可以特异性切割RNA中ACA基序的5'端，但带有m^6A修饰的(m^6A)CA基序不会被其催化切割。基于此，对MazF处理后的mRNA片段进行建库测序，并使用经去甲基化酶FTO处理的mRNA作为阴性对照，通过分析酶切位点是在测序读长的内部还是外端来鉴定m^6A修饰位点。然而，该检测方法目前还存在一些限制：MazF识别的ACA基序只占m^6A经典的RRACH (R = A/G, H = A/C/U) 基序的16%左右，不能覆盖所有可能的m^6A位点。根据m^6A修饰的保守序列RRm^6ACH，将识别m^6A的YTH结构域与胞嘧啶脱氨酶APOBEC1融合成APOBEC1-YTH蛋白质，以此开发了DART-seq[41]。YTH结构域将融合蛋白质招募至m^6A位点，APOBEC1可催化邻近m^6A的胞苷脱氨基形成尿苷，以此为依据来检测m^6A位点。

m^6A-label-seq和m^6A-SEAL是两种基于化学标记的m^6A检测方法[42-43]。m^6A-label-seq通过在细胞里添加烯丙基修饰的甲硫氨酸，使得mRNA上原本是m^6A的位点形成N^6-烯丙基腺嘌呤(N^6-allyladenosine, a^6A)，a^6A在温和的碘加成条件下诱导形成1,6位环化腺嘌呤(N^1,N^6-cyclized adenosine, cyc-A)，而RNA:cyc-A在逆转录合成cDNA时会发生碱基错配。m^6A-SEAL使用m^6A的去甲基化酶FTO作为催化剂，将mRNA上化学惰性的m^6A转化为高反应活性的中间态产物N^6-羟甲基腺嘌呤(N^6-hydroxymethyladenosine, hm^6A)，然后通过二硫苏糖醇的巯基与mRNA:hm^6A发生亚胺1,2加成反应，将不稳定的hm^6A转化为更稳定的巯基加成产物N^6-二硫代糖醇甲基腺苷(N^6-dithiolsitolmethyladenosine, dm^6A)。dm^6A上的自由巯基可以通过与甲烷硫代磺酸(methanethiosulfonate, MTSEA)反应来添加各种标记，例如生物素，从而富集含有m^6A修饰的RNA片段供后续高通量测序使用。

尽管已经开发了多种m^6A修饰的检测方法，但这些方法依然存在许多亟待解决的问题。基于抗体免疫沉淀的方法可能会受到抗体的固有偏好性以及与特定RNA序列或其他修饰结合的影响[33, 36]；基于核酸内切酶的方法虽然未预先富集m^6A位点，但也具有序列偏好性，因此只能检测到部分m^6A位点；基于化学标记的方法的标记效率仍有待提高。因此，仍然需要改进上述方法或多种方法结合来检测m^6A修饰。

2 m^6Am

mRNA的5'端的第一个腺苷是2'-O-甲基化的腺苷(2'-O-methyladenosine, Am)，它可以被甲基转移酶PCIF1进一步甲基化形成N^6,2'-O-二甲基腺苷(N^6,2'-O-dimethyladenosine, m^6Am)[33, 36, 44-47]。与m^6A类似，m^6Am的N^6-甲基也可以被去甲基化酶FTO去甲基化[48-49]。由于m^6A特异性抗体可以同时识别m^6Am和m^6A，因此m^6A/MeRIP-seq和miCLIP也可用于在转录起始点检测m^6Am[36, 47]。2019年，研究人员还开发了一种更特异的m^6Am检测方法m^6Am-Exo-seq[46]，其原理是利用5'核酸外切酶去除内部含有m^6A的RNA片段，富集5'末端带有帽子修饰的包含m^6A的RNA片段，然后用抗m^6A的抗体进行免疫沉淀，再进行建库测序。然而，目前所有的m^6Am测序技术仍然依赖于抗m^6A抗体，因此还需要进一步发展无偏好、特异的m^6Am检测方法，以帮助我们更好地理解m^6Am修饰。

3 m^7G

7-甲基鸟苷(7-methylguanosine, m^7G)是一种广为人知的mRNA帽子修饰，它还普遍存在于转运RNA (transfer RNA, tRNA)中，最近在mRNA内部也发现了这种修饰[50-51]。目前已经开发了基于抗体和化学标记的测序方法来绘制m^7G修饰图谱。m^7G-MeRIP-seq通过m^7G特异性抗体免疫沉淀来实现m^7G的全转录组分析[52]。m^7G-miCLIP-seq采用交联诱导的逆转录终止和突变来检测m^7G[51]。m^7G-seq通过还原诱导的脱嘌呤反应，在m^7G位点形成一个无碱基位点，并进一步用生物素标记，然后将其富集，

RNA中标记的m^7G位点可能在逆转录过程中导致错误掺入，从而获得m^7G在转录组中的碱基分辨率图谱[52]。AlkAniline-seq以及TRAC-seq (tRNA reduction and cleavage sequencing)采用硼氢化钠对含有m^7G的RNA样品进行碱水解，使m^7G的糖苷键发生断裂，随后加入苯胺使该位置的核糖与苯胺发生β-消除反应，导致该位置RNA断裂，由此可以检测不同RNA样品中的m^7G修饰[53-54]。

4 m^1A、m^3C、m^1G

1-甲基腺苷(1-methyladenosine, m^1A)、3-甲基胞苷(3-methylcytidine, m^3C)和1-甲基鸟苷(1-methylguanosine, m^1G)影响Watson-Crick碱基配对，在逆转录过程中能导致该位点发生cDNA合成的终止或错误配对核苷酸的掺入[55-56]。这种固有属性已被用于许多逆转录结合高通量测序的方法[55, 57-58]。

4.1 m^1A

m^1A是m^6A的异构体，其普遍存在于mRNA、tRNA、核糖体RNA (ribosomal RNA, rRNA)和长非编码RNA (long non-coding RNA, lncRNA)上[59-63]。目前，m^1A抗体免疫沉淀结合高通量测序已成为检测m^1A修饰的强有力手段，包含m^1A-ID-seq[59, 64]和m^1A-seq[60]。m^1A-ID-seq利用大肠杆菌AlkB去甲基化酶对m^1A进行去甲基化处理[59, 64]，而m^1A-seq则利用m^1A的Dimroth重排反应将其部分转换为m^6A[60, 65]，但这两种方案均无法实现单核苷酸的检测分辨率。随后出现的m^1A-MAP[62]、m^1A-seq-TGIRT[61]和m^1A-miCLIP[66]通过使用特定的方法使逆转录在m^1A位点更趋向于错配或逆转录终止，从而实现单核苷酸的检测分辨率。

近年来开发的其他技术能检测m^1A位点处RNA结构、定量分析和研究不同修饰之间的影响。Structure-seq2/DMS-seq2能提供单核苷酸分辨率的RNA结构信息并识别天然甲基化的位置[67]；基于CRSIPR-Cas13a开发的m^1A荧光分析方法能定量分析RNA中的m^1A水平[68]；基于亚硫酸氢盐测序(bisulfite sequencing, BS-seq)的RBS-seq能同时检测m^1A、5-甲基胞苷(5-methylcytosine, m^5C)和假尿嘧啶(pseudouridine, ψ)修饰，为研究修饰之间的相互影响提供了途径[69]。目前，还有一些特殊的蛋白酶被用于m^1A检测技术，如基于对m^1A具有特异性的HIV-1逆转录酶[63]和对m^1A和A具有明显选择性的T3 DNA连接酶[70]。

4.2 m^3C与m^1G

目前，已在tRNA和mRNA中鉴定到m^3C [71-72]，而m^1G则主要位于tRNA和rRNA上[73-75]。基于大肠杆菌AlkB及AlkB突变体可以去除m^3C和m^1G修饰，开发了相关的测序技术[76-78]。如前所述，AlkAniline-seq不仅能检测m^7G修饰，还可以检测不同RNA样品中的m^3C修饰[53]。不仅如此，分别在野生型和编码修饰酶的基因缺失的细胞中应用RNA甲基化敏感性原位杂交MR-FISH (methylation-sensitive RNA fluorescence in situ hybridization)，可以在单细胞水平直观地检测m^1G修饰[79]。

5 2'-O-甲基化

2'-O-甲基化(2'-O-methylation, Nm)几乎存在于所有的RNA种类中[80-81]。在早期的研究中通过RNA指纹技术(RNA fingerprinting)可以对核糖甲基化进行精确的定位和定量分析[82-85]。一种可以切割RNA的脱氧核酶(DNAzymes)可以简单地识别rRNA中的2'-O-甲基化[86]。近年来，随着高通量技术的发展，通过RiboMeth-seq、Nm-seq、RibOxi-seq和2OMe-seq检测2'-O-甲基化已成为主流。

RiboMeth-seq是基于在碱性环境下，核糖的2'-OH被激活，并对邻近的磷酸二酯键进行亲核攻击而导致RNA链断裂并产生5'-OH和2',3'-环磷酸末端[87]。但在2'-O-甲基化存在的情况下，RNA的3'-磷酸二酯键得到保护而使其在碱性降解时不易断裂。通过凝胶纯化富集大小范围在20~40 nt的片段后，使用激酶活性丧失的RNA连接酶将其连接到RNA寡核苷酸接头上[87-89]。最初的RiboMeth-seq由于连接效率相对低下而需要大量的RNA样品输入。后续通过使用一些常规的高效连接方案使样品需求量显著减少，并使整个流程大大简化[90-91]。在数据处理中，由于5'端读取位点的核苷酸实际上取决于其5'邻位核苷酸的2'-OH状态，因此在数据处理过程中需要将5'端读取位点向上游移动一个核苷酸才能与3'端读取位点合并，并获取位点覆盖图，以此来检测和定量2'-O-甲基化[87-88, 92-95]。该方法适用于所有类型的RNA，并能精确定量2'-O-甲基化水平。

2'-O-甲基化的疏水基团能阻止氢键供体与核糖的配位从而保护核糖[90, 95]。基于此，开发了Nm-seq和RibOxi-seq[96-97]。正常核苷的核糖的2'和3'-OH在高碘酸盐的作用下被氧化为二醛，而2'-O-甲基化修饰的核糖则具有抵抗力。将RNA链片段化后使用高碘酸盐氧化，随后在碱性条件下进行β-消除反应以去除末端氧化的核苷酸，从而得到3'末端带有2'-O-甲基化修饰或无甲基化的RNA片段的混合物。无甲

基化的RNA片段的末端被氧化为二醛而无法与3'接头连接，而具有2'-O-甲基化修饰的末端由于具有抵抗性则能连接，最终在测序文库中选择性地富集了含2'-O-甲基化修饰末端的片段[96-97]。在Nm-seq中使用了多轮氧化-消除-去磷酸化循环进行同样的筛除和选择性富集[96, 98]。

Nm-seq与RibOxi-seq 均适用于含量丰富的RNA种类(如rRNA)的2'-O-甲基化测序[96-97, 99]。此外，Nm-seq还可以应用于较低丰度的RNA种类(如mRNA和小RNA)[96, 98]。目前，通过Nm-seq已检测到成千上万个2'-O-甲基化位点，并且Um是其中主要的2'-O-甲基化修饰[81, 97]。值得注意的是，3'末端的2'-O-甲基化修饰会降低RNA连接酶的连接效率，因此会对文库的得率产生负面影响[90, 100]。此外，实验方案中的多轮循环处理会增加样品的损失，导致样品需求量增加[90, 98]。

2'-O-甲基化不会直接影响核苷酸的碱基配对，但在低浓度的dNTP时，许多天然的RNA依赖性DNA聚合酶，如逆转录酶，会由于2'-O-甲基化修饰引起的核糖的空间位阻而使逆转录阻滞[101-102]。2OMe-seq首先在低浓度dNTP条件下进行引物延伸来获取测序文库，分析获得2'-O-甲基化修饰位点。通过与标准dNTP条件下的正常逆转录测序文库比较，可以排除一些由其他因素引起的假阳性结果。这种方法目前已应用于对rRNA的2'-O-甲基化修饰进行单核苷酸分辨率定位和相对定量[102]。

6 m^5C

5-甲基胞嘧啶(5-methylcytosine, m^5C)广泛存在于多种RNA上，包括mRNA、rRNA、tRNA、增强子RNA (enhancer RNA, eRNA)等[103]。BS-seq、MeRIP-seq、miCLIP-seq以及5-氮杂胞苷(5-azacytidine, 5-AzaC)介导的免疫沉淀测序(5-azacytidine-mediated RNA immunoprecipitation sequencing, Aza-IP-seq)已成为检测RNA:m^5C的主要手段[104]。

在亚硫酸氢盐存在时，RNA中未被修饰的胞苷经过脱氨反应形成尿苷，而被修饰成m^5C的胞苷不会发生转变。在后续的逆转录和PCR反应中，尿苷替换成脱氧胸苷并掺入新生DNA链中[105]，通过对接受亚硫酸氢盐处理和未处理的RNA测序结果进行分析即能获得RNA:m^5C修饰位点，并且能够达到单核苷酸的检测分辨率。但是，该方法也存在一些不足：(1) 碱性的反应条件使RNA分子的不稳定性增加；(2) 低丰度的RNA分子容易在反应过程中丢失；(3) 转变成脱氧胸苷之后在众多的胸腺嘧啶存在的背景下，检测的信噪比增加，使测序结果不准确；(4) 该方法不能区分m^5C与hm^5C修饰[106-107]。

MeRIP-seq通过m^5C特异性抗体结合高通量测序获取m^5C在RNA中修饰丰度的信息[108-109]。该检测方法能维持RNA的稳定性，并能富集低丰度RNA再进行后续的检测。但是，该方法无法达到单核苷酸检测精度，存在一定的局限性。

m^5C特有的miCLIP-seq利用RNA甲基转移酶NSun家族催化中心保守的半胱氨酸残基(结构模块IV-Cys)突变，使酶与RNA底物间形成不可逆的蛋白质-RNA复合物[110]。该方法在不需要进行紫外交联的情况下，就可以通过免疫共沉淀的方法富集RNA底物，随后进行逆转录PCR及高通量测序。该方法具有较高的特异性，能够达到单核苷酸的检测分辨率。

5-AzaC作为胞苷的类似物，在转录时掺入新生转录本，并特异性地将m^5C甲基转移酶捕获在RNA上，形成共价复合物。在逆转录以及后续过程中，该共价键断裂，开环的5-AzaC被读成G [111]。比较处理前后的样本可以确定RNA中m^5C修饰位点。这种方法能避免低丰度的RNA:m^5C 测序丢失，同时能够特异性鉴定m^5C甲基转移酶的催化位点。但是，这种方法容易导致一些不稳定的m^5C修饰位点的丢失。

7 hm^5C

m^5C的氧化产物5-羟甲基胞苷(5-hydroxymethylcytidine, hm^5C)在RNA上也有分布[103, 112]。目前，可以采用过氧化钨盐氧化测序法(peroxotungstate oxidation sequencing, WO-seq)检测RNA中的hm^5C修饰[113]。过氧化钨盐能将hm^5C氧化为三羟基化的胸腺嘧啶 (trihydroxylated-thymine, ^{th}T)。随后在逆转录合成过程中，使用耐热的逆转录酶TGIRT (thermostable group II intron reverse transcriptase)，将^{th}T转化为T。该检测方法能达到单核苷酸分辨率，但由于其基于碱基置换原理，可能导致检测背景信号高。

8 ψ

假尿嘧啶(pseudouridine, ψ)是RNA中发现的第一种化学修饰并且是丰度最高的RNA修饰[114]，约占总尿嘧啶的7%~9%[115]，在多种RNA，如mRNA、tRNA、rRNA和核小RNA (small nuclear RNA,

snRNA)中均有分布[116-119]。该修饰的检测方法在“RNA修饰检测技术”[19]一文中已有详细介绍，对相似的内容下文将简要说明。

化合物CMC (*N*-cyclohexyl-*N′*-*β*-(4-methylmorpholinium) ethylcarbodiimide)可以与ψ反应形成稳定的复合物，在碱性条件下(pH = 10.4)稳定存在，并且，在逆转录过程中，CMC的加成影响ψ与其他碱基互补配对，导致截短DNA片段的产生[120]。在1993年时，基于CMC开发了引物延伸方法检测ψ修饰[121]。在此基础上，近年来，针对逆转录引物设计策略进行了改进，开发了CLAP方法(CMC-RT and ligation assisted PCR)[122]。通过使用位点特异性的引物进行PCR，在同一样品中生成两种分别对应于修饰和未修饰的位点的不同PCR产物，通过凝胶电泳可以鉴定某个具体的位点是否带有ψ修饰。这种方法可以对携带ψ修饰的低丰度RNA进行定量，但其应用范围仅限于已知的带有ψ修饰的RNA片段。

随着高通量测序技术的发展，现已开发了多种基于CMC及其衍生物的测序方法。在PSI-seq、ψ-seq和Pseudo-seq中，将mRNA与CMC反应后进行碱处理，并对片段化的RNA进行建库和高通量测序，可以在全转录组范围内以单核苷酸的分辨率检测ψ修饰位点[119,123-124]。但上述方法并不能定量检测ψ，且由于缺少富集含ψ修饰的RNA片段的过程，容易导致携带ψ修饰的低丰度RNA种类的丢失。

CeU-seq (N_3-CMC-enriched pseudouridine sequencing)是利用CMC的衍生物N_3-CMC实现ψ修饰特异性标记与富集的方法[125]。N_3-CMC仅与ψ形成复合物，随后通过叠氮化合物将生物素添加至N_3-CMC与ψ的复合物上，并以此富集含ψ修饰的RNA。CeU-seq可以在全转录组范围内检测ψ修饰的位点和丰度，具有较高的灵敏度。

9 I

次黄嘌呤核苷(inosine, I)，也称肌苷，是由腺苷经过脱氨反应(A-to-I)形成的修饰核苷，这种修饰过程也被称为RNA编辑[126]。I存在于多种RNA上，如mRNA、tRNA、rRNA[127-129]。限制性核酸内切酶RNase T1能够特异性地作用于鸟苷及肌苷的3'末端，并能够切割两者与相邻核苷之间的磷酸二酯键。而用乙二醛或硼酸盐处理RNA分子后再加入RNase T1，只有肌苷与相邻核苷之间的磷酸二酯键会发生断裂。随后，通过变性的聚丙烯酰胺凝胶电泳检测不同大小的RNA片段。但是该方法只能定性检测I修饰[130]。限制性片段长度多态性(restriction fragment length polymorphism, RFLP)结合RT-PCR、PCR及限制性酶切，对PCR产物进行特殊的酶切反应后，进行琼脂糖凝胶电泳或聚丙烯酰胺凝胶电泳，可以检测携带肌苷修饰的RNA分子[131]。

逆转录酶会将肌苷识别为鸟苷，在逆转录及cDNA合成的过程中，携带肌苷修饰的位点具有腺嘌呤到鸟嘌呤的变化，通过高通量测序即可检测出I修饰及其具体位点[132]。近年来，基于上述原理，陆续开发了一些高通量测序方法检测I修饰，如挂锁探针(padlock probe)检测法以及氰乙基化(cyanoethylation)检测法[133-134]。挂锁探针分别与基因组DNA和cDNA进行杂交，随后进行连接环化，通过PCR扩增以及测序后，在基因组DNA检测出腺嘌呤，而cDNA相应的位置则是鸟嘌呤，表明转录本RNA在该位点存在I修饰。该方法具有较高的检测灵敏度[133]。氰乙基化能与I结合，在逆转录过程中抑制了碱基互补配对，导致cDNA无法在该位点进行延伸，而未处理的样品在该位点的测序结果为鸟嘌呤。通过比对处理以及未处理的测序结果，即可获得RNA上I修饰的位点[134]。

10 总结与展望

近十年，得益于高通量测序技术的发展，RNA修饰的检测达到了前所未有的精确性和高通量。本文总结了用于检测十余种常见RNA修饰的高通量测序技术，而对于较复杂的RNA修饰，除了传统的检测手段，仍需开发特异性抗体或化学小分子用于建立高通量测序检测方法。目前二代测序技术仍依赖逆转录以及PCR扩增，这些过程可能引起错误率增加，同时还受到测序长度较短的限制，具有一定的系统偏差。第三代测序技术具有单分子测序、无需PCR 扩增、读取长度增加、测序错误随机及无系统偏差等特点，已被应用于DNA和RNA测序，但对RNA修饰的测序有待进一步研究。截至目前，已经开发了包括PacBio单分子实时测序SMRT技术(single molecule amplification and sequencing technology)和牛津纳米孔测序在内的第三代测序方法[135-138]，实现了DNA和RNA的长片段读取和单分子测序。除了更长的读取长度，SMRT和纳米孔测序也允许直接读出DNA和RNA修饰，但其较高的错误率和不成熟的碱基识别阻碍了目前的实际应用。2020年有研究将上述某些测序方法与第三代测序相结合，开发了可以提供高精度的应用于DNA的表观遗传测

序——lrTAPS[139]。随着技术的发展，未来将开发新的更高效和精确的高通量测序方法，并且第三代测序可能逐步应用于检测RNA修饰，推动RNA表观遗传学的发展。

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