生 物 工 程 学 报
Chinese Journal of Biotechnology
http://journals.im.ac.cn/cjbcn
DOI: 10.13345/j.cjb.210113

Feb. 25, 2022, 38(2): 411-426

·综 述·

高通量测序技术在肉类掺假检测中的应用进展

甘永琦[1]，卢曼曼[2]，赖青鸟[1]，朱斌[1]

1 广西壮族自治区食品药品检验所，广西 南宁 530021
2 广西壮族自治区农业科学院甘蔗研究所，广西 南宁 530004

甘永琦, 卢曼曼, 赖青鸟, 朱斌. 高通量测序技术在肉类掺假检测中的应用进展. 生物工程学报, 2022, 38(2): 411-426.
GAN YQ, LU MM, LAI QN, ZHU B. Application and Progress in high-throughput sequencing technology for meat adulteration detection. Chin J Biotech, 2022, 38(2): 411-426.

摘 要：肉类掺假现象普遍存在，导致严重的公共卫生风险和侵犯宗教信仰行为。快速、有效、准确和可靠的检测技术是有效监管肉类掺假的关键手段。近年来，基于高通量测序的 DNA 宏条形码技术发展迅速，具有高通量、高精度和速度快等特点，并且可以实现复杂样品中多个物种的同时检测，因而在肉类及其制品的掺假检测方面具有明显的优势。文中介绍了近 20 年来高通量测序技术的主要发展历程、DNA 宏条形码的技术特点和研究方法，综述了近年来 DNA 宏条形码技术在肉类掺假检测中的应用，讨论分析了 DNA 宏条形码技术应用于肉类掺假检测领域面临的挑战，并对该技术的未来发展趋势进行了展望。

关键词：高通量测序；DNA 条形码；宏条形码技术；肉类掺假；物种鉴定；食品安全

Application and progress in high-throughput sequencing technology for meat adulteration detection

GAN Yongqi[1], LU Manman[2], LAI Qingniao[1], ZHU Bin[1]

1 Guangxi Institute for Food and Drug Control, Nanning 530021, Guangxi, China
2 Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530004, Guangxi, China

Abstract: Adulteration in meat products is a widespread issue that could lead to serious threats to public health and religious violations. Technology that offers rapid, sensitive, accurate and reliable detection of meat species is the key to an effectual monitoring and control against meat adulteration. In

Received: February 2, 2021; **Accepted:** May 31, 2021; **Published online:** December 28, 2021
Supported by: Natural Science Youth Foundation of Guangxi, China (2018JJB130010)
Corresponding author: ZHU Bin. Tel: +86-771-5827968; E-mail: zhubin1226@sina.com
基金项目：广西壮族自治区自然科学青年基金(2018JJB130010)

recent years, high-throughput sequencing-based DNA metabarcoding technology has developed rapidly. With the characteristics of being high-throughput, highly precise and high-speed, this technology can simultaneously identify multiple species in complex samples, thus offering pronounced advantages in the surveillance of adulteration in meat and meat products. Starting with an introduction of the major developments in the high-throughput sequencing technology in the past two decades, this review provides an overview of the technical characteristics and research methods of DNA metabarcoding, summarizes the application of DNA metabarcoding technology in meat adulteration detection over the last few years, discusses the challenges of using DNA metabarcoding technology in the detection of meat adulteration, and provides future prospects on the development of this technology.

Keywords: high-throughput sequencing; DNA barcoding; metabarcoding technology; meat adulteration; species identification; food safety

动物源性食品中肉类掺假一直是影响食品质量与安全的主要问题之一，引发社会对食品欺诈行为的广泛关注。肉类掺假行为主要是为了获得产品的表观价值或降低产品的成本，在该产品中欺诈性地、有目的地替代或添加某物质的行为[1-3]，是影响及破坏食品安全秩序的重要因素之一，存在食品安全风险隐患，危及消费者的身心健康。美国食品生产协会报告指出，全球食品和消费品造假每年给产业带来的损失已超过 100–150 亿美元[4]。2008–2012 年欧盟食品和饲料快速预警系统通报的 376 件在芬兰发生的掺假事件中，大多数都是动物源性产品掺假[5]。在国内报道[6]的 1 553 个食品安全事件中，掺假最多的食品种类也是动物源性食品 (38%)。

虽然各国已制定法规对肉类和肉制品的质量和安全进行监督，如美国《联邦肉类检查法》[7]、加拿大《肉类检验法》[8]以及我国《食品安全法》[9]等，但是肉类掺假现象仍然普遍存在。一些不法商贩将相对廉价的猪、鸭、鸡和鼠[10-13]等肉类原料掺入牛羊肉制品中进行销售以谋取不正当利益，严重扰乱了市场秩序，侵犯了消费者的合法权益。检测发现混合肉制品在标签明示肉类成分方面比较混乱，部分样品检出标签中未标示的肉类成分[14]。肉类掺杂掺假行为还影响消费者的生活方式，涉及宗教、伦理等问题[15]。因此，寻找和建立有效的检验检测方法，对动物源性食品风险预警保障工作具有重要意义。

快速、准确和高效的检测方法是肉类掺假检测的基本要求。DNA 检测方法特异性强、灵敏度高，结果不易受到食品加工方式的影响，并且 DNA 片段显示出比蛋白质更好的热稳定性，可作为加工肉类真实性测定的标记[16]。虽然肉类组织和物种中 DNA 含量的变化会影响肉类的定量，但基于 DNA 的 PCR 及其衍生技术由于其敏感性、简单性和可靠性，是检测肉类和肉制品掺假最常用的技术。近几年高通量测序技术发展迅速，具有成本低、通量高和速度快等优点，还可以同时检测样品中多个物种，因而已成为肉类成分鉴定的新方法。

1 高通量测序技术

在过去的 20 年里，基因测序技术经历了一代测序、二代测序和三代测序的发展，在规模、通量以及应用上都有了极大进步。高通量测序技术 (high-throughput sequencing, HTS) 又称下一代测序技术 (next generation sequencing,

NGS)，以协同方式逐步进行酶促 DNA 反应、碱基测序与数据收集，可以同时完成数万条到数十亿条 DNA 模板的测序[17]。自 2005 年第一台二代测序平台罗氏 454 焦磷酸测序仪[18]诞生以来，测序方法和技术不断更新换代 (表 1)，目前市场主流测序平台主要有 Illumina 和 Ion Torrent[19]。

1.1 二代测序技术

在此期间，Illumina 技术的进步在很大程度上为测序通量的巨大增长和成本的降低奠定了基础 (图 1)。因此，Illumina 测序仪目前在 HTS 市场占据主导地位。在进行测序时，首先克隆扩增流动槽表面的带接头 DNA 片段[20]。然后使用循环可逆终止技术读取碱基。该技术通过荧光标记的 3′-O-叠氮甲基-dNTPs 阻断聚合反应，再去除未结合的碱基并完成荧光成像以检测加入的碱基[21]。最后使用电荷耦合器件 (charge-coupled device, CCD) 扫描流动槽，切除荧光标记和 3′端阻断基团。通过碱基结合、洗涤、成像和切割，每次完成模板链中一个碱基的测序。在所有 Illumina 测序仪中，总错误率低于 1%，最常见的错误类型是替换[22]。

表 1 不同测序平台的比较

Table 1 Comparison of different sequencing platforms

Sequencing platform	Technical principle	Technical characteristics	Application field
Roche 454	Pyrophosphate synthesis sequencing	Longer read length in single sequence, higher cost and higher sequencing error rate	Whole genome sequencing, metagenome sequencing, targeted gene sequencing, exome sequencing, methylation sequencing, transcriptome sequencing
Illumina	Fluorescence reversible termination of synthesis sequencing	Shorter read length in single sequence, lower cost and lower sequencing error rate	Whole genome sequencing[23], metagenome sequencing[24], exome and large panel sequencing[25], methylation sequencing[26], transcriptome sequencing[27], targeted gene sequencing[28], single-cell sequencing[29], miRNA and small RNA sequencing[30], chromatin immunoprecipitation sequencing[31], cell-free sequencing and liquid biopsy analysis[32]
Ion Torrent	Simple chemical signal sequencing	Simple, fast, low cost, low throughput	Genome sequencing[33], targeted gene sequencing[34], exome sequencing[35], transcriptome sequencing[36], methylation sequencing[37], miRNA and small RNA sequencing[38], metagenome sequencing[39], chromatin immunoprecipitation sequencing[40], cell-free sequencing and liquid biopsy analysis[41]
Pacific Biosciences	Single molecule fluorescence signal sequencing	Long sequence reads, high cost, low throughput, high sequencing error rate	*De novo* sequencing or large genome sequencing[42], structural variation detection[43], transcriptome sequencing[44], methylation sequencing[45], single-cell sequencing[46], metagenome sequencing[47], targeted gene sequencing[48]
Oxford Nanopore	Single molecule electrical signal sequencing	Long sequence reads, simple operation, low cost, low throughput, high sequencing error rate	*De novo* sequencing or genome sequencing[49], targeted gene sequencing[50], transcriptome sequencing[51], methylation sequencing[52], metagenome sequencing[53], single-cell sequencing[54], structural variation detection[55]

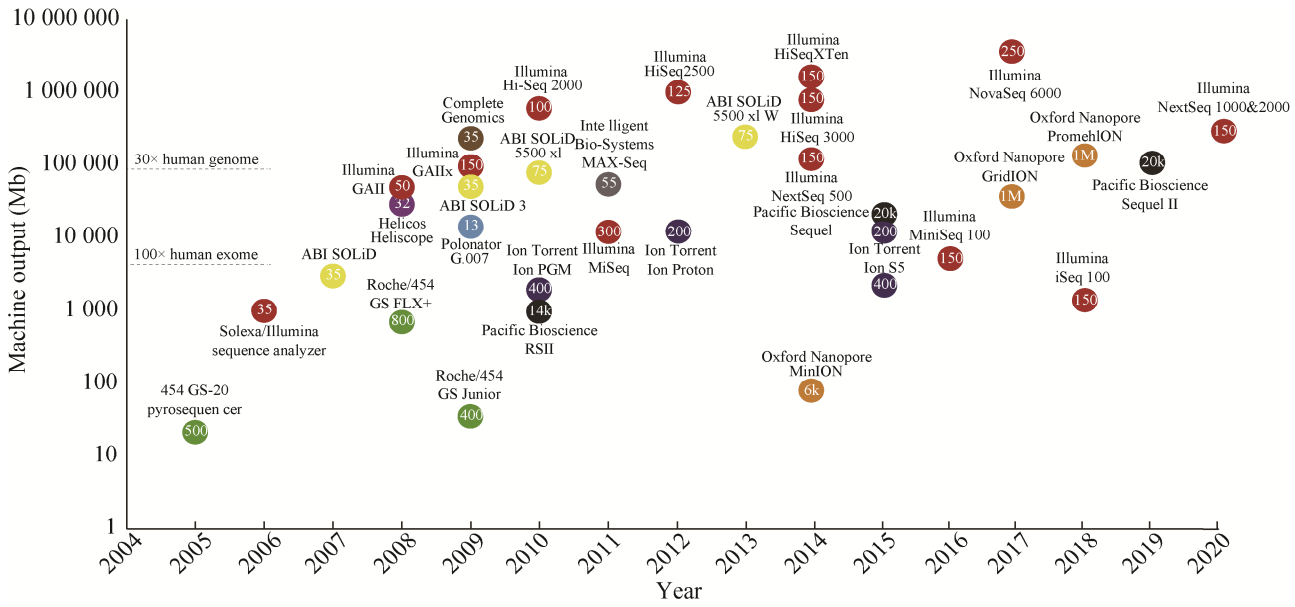

图 1　商用 HTS 仪器的大事年及比较　圆形内的数字表示当前读取长度，不同测序平台用颜色编码[56]

Figure 1　Timeline and comparison of commercial HTS instruments[56]. Numbers inside data points denote current read lengths. Sequencing platforms are color coded.

2010 年 Life Technologies 将 Ion Torrent 的半导体测序技术商业化，发布了 Ion PGM 测序仪，模板制备和测序步骤在概念上类似于罗氏 454 焦磷酸测序平台[57]。用乳液 PCR 法克隆扩增珠子表面连接的 DNA 片段。随后，将这些珠子分配到微孔中，进行边合成边测序反应。与焦磷酸测序不同，Ion Torrent 的半导体测序是检测 DNA 延伸过程中氢离子释放引起的 pH 值变化[58]。这些变化通过微孔底部的传感器检测并转换成电压信号，信号强度与结合的碱基数成正比。此外，Ion Torrent 不需要通过光学扫描区分核苷酸，极大地提高了测序速度并降低了测序成本。2015 年发布的 Ion S5 系统测序仪高度简化了靶向测序流程，支持多种不同类型的半导体芯片，目前测序长度可达 600 bp，最大数据量为 50 Gb。在所有 Ion Torrent 测序仪中，插入和缺失突变是最常见的错误类型[59]。由于结合的碱基数与产生的电压信号之间不能完全按比例缩放，因此当核苷酸同聚物重复序列超过 6 个碱基将会导致错误率增加[58]。

然而二代测序读长较短导致在进行大基因组测序时拼接困难，较长的测序时间尚不能完全满足临床快速诊断的需求[60]。在此背景下，可满足长读长和快速测序要求的三代测序 (third-generation sequencing, TGS) 平台应运而生。TGS 不依赖于 PCR 扩增技术，能够对 DNA 单个分子进行合成测序，目前的应用平台主要有单分子实时测序 (single molecule real-time, SMRT) 和纳米孔单分子测序[61-62]。

1.2　三代测序技术

单分子实时测序 (single molecule real-time, SMRT) 由 Nanofluidics 公司首创，并由 Pacific Biosciences 公司商业化。模板制备包括将单链发夹适配器连接至消化后的 DNA 或 cDNA 分子末端，生成一个带帽模板 (SMRT-bell)。通过使用链置换聚合酶，可以对原始 DNA 分子进行多次测序，从而提高准确性[63]。更重要的是，该技术避免了克隆扩增，从而允许对天然的和潜在修饰的 DNA 进行直接测序。聚合作用可持续进行，通过记录荧光信号实时读取 DNA 序列。但

是单次读取的错误率显著偏高 (约为 11%)，主要是插入缺失引起的。然而测序错误是随机分布的，允许在增加覆盖率的情况下进行精确的一致性调用，或者围绕同一模板多次传递，即所谓的循环一致性序列[64]。通过避免克隆扩增，SMRT 测序对 GC 序列含量的敏感性也比其他平台低得多[65]。

近年来，基于纳米孔的测序作为一种新兴的单分子技术，取得了长足的发展，牛津纳米孔技术引领了该方法的开发和商业化。纳米孔测序可以采取多种形式，但主要依赖于 DNA 或单个核苷酸通过纳米孔时产生的电流变化[66]。不同碱基通过纳米孔时会产生不同强度的电流，通过检测电流的变化，识别 DNA 链上的碱基来完成测序。第一个商业化的纳米孔测序设备是 MinION，是一款小型手机大小的 USB 设备，在 2014 年的早期访问试验中首次向最终用户发布[67]。但是该技术运行故障率和错误率都很高[68]。根据数据产量不同，测序仪可分为 3 类：Flongle 数据产量为 2 Gb；MinION 和 GridION 数据产量为 30 Gb；而 PromehION 数据产量可达 180 Gb。纳米孔测序的仪器成本、尺寸、运行速度和读取长度让人们对测序未来充满期望。

2 条形码技术

2.1 DNA 条形码技术

DNA 条形码是 DNA 中的特殊区域，可以作为生物体的遗传标记。DNA 条形码通常由用于区分目标物种的中心可变部分和位于可变部分两侧的保守区组成，这使得可以使用通用引物来扩增不同物种中的条形码序列[69]。线粒体 DNA (mitochondrial DNA, mtDNA) 由于其序列变化速度很快，即使在关系密切的物种之间，也经常存在明显的差异，因此已广泛用于分子系统发育研究[70]。事实上，mtDNA 基因组的不同部分以不同的速率进化[71]。因此，选择合适的基因来评价物种划分非常关键 (图 2)。

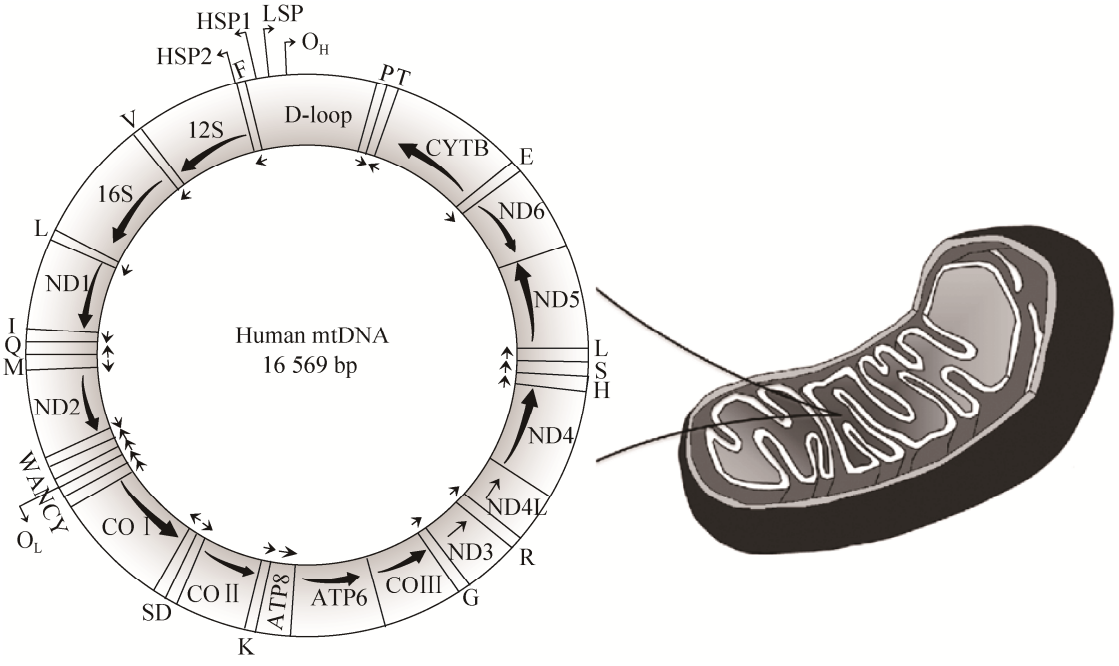

图 2 线粒体 DNA 基因组 该基因组为人类线粒体基因组的示意图，代表了动物线粒体 DNA 中常见的基因类型[72]

Figure 2 Mitochondrial DNA genome. This genome is schematic representation of the human mitochondrial genome and represents a typical gene content found in animal mtDNAs[72].

近十几年来越来越多的研究表明，用于动物标准条形码的基因区域是编码线粒体细胞色素 C 氧化酶亚基 I (cytochrome c oxidase subunit I, COI)基因中的 658 个碱基对区域[73]。该标记物可用于动物物种的鉴定以及发现新的或隐蔽的物种[74]。一些研究已经证实了这种方法在一些动物群体中的分辨力，如鸟类[75]、鱼类[76]、贝壳类[77]、蜘蛛类[78]、鳞翅类[79]和爬行类动物[80]。经多方共同努力，建立了一个包括数千个物种 DNA 序列的综合数据库 (http://www. barcodinglife.org)，且不断更新，并向公众开放。除了 COI 之外，其他线粒体标记也已用于系统发育或补充 DNA 条形码中的 COI，并推荐细胞色素 b (cytochrome b, Cytb) 作为确定物种界线的标记[81]。在两栖动物和软体动物中，已经提出了 16S rRNA 基因作为 DNA 条形码标记来补充 COI[82]。

除了分类学，DNA 条形码还可在其他领域发挥作用，如生态学、法医学、生物技术、食品工业、动物饮食和食品质量等。此外，食品中动物种类的鉴定正成为评估食品成分和提供给消费者适当信息的一个非常重要的问题。近年来，也有文献报道了 DNA 条形码技术在验证食品真实性方面的应用[83]。

2.2 DNA 宏条形码技术

随着基因测序技术的发展和在线数据库，如美国国立生物技术信息中心 (National Center for Biotechnology Information, NCBI)、生命条形码数据系统 (barcode of life data system, BOLD)的出现，近年来 DNA 宏条形码技术应运而生。它最初的定义是利用从环境样本中提取的 DNA 来进行高通量多物种的鉴定[84]。DNA 宏条形码技术结合了 DNA 条形码和高通量测序技术，为同时检测食物基质中的多个物种提供了理想的方法，包括产品中非预期的物种[85]。因此，它比传统的 DNA 条形码技术功能更强大。为了鉴定未知样品中的物种，首先要建立一个可靠的数据库，包含目标物种的 DNA 条形码。使用短链寡核苷酸对 DNA 提取物的条形码序列进行 PCR 扩增，然后合并来自不同样品的扩增子，在一次运行中对不同扩增子同时进行测序，并将序列与条形码数据库进行匹配，最后通过生物信息学分析得到结果 (图 3)。

在理论上几乎任何一个样本都可以通过DNA宏条形码技术进行物种识别。近年来，DNA 宏条形码方法已应用于多个领域。例如，监测水生生态系统中的鱼类[86]或土壤中的植物[87]、鉴定糖果[88]、乳制品[89]、肉类衍生产品[90]和复合或深加工海产品[91]等食品中的物种，以及测定蜂蜜[92]、益生菌[93]和中药补充剂[94]的动植物来源等。

3 在肉类掺假检测中的应用

目前用于识别肉制品掺假标志物的靶基因和 DNA 片段主要来源于 mtDNA[95]，如线粒体 D-环区、细胞色素 b (Cytb) 基因和细胞色素 c 氧化酶亚基Ⅰ、Ⅱ和Ⅲ (COⅠ、COⅡ和 COⅢ)基因，ATP 酶亚基 6 和 8 (ATPase6 和 ATPase8)基因、12S rRNA 基因和 16S rRNA 基因。因为 mtDNA 与基因组 DNA 相比具有较多优势，如排列更有效、获取更容易和不需要重组[96]等。另外，还出现新的基因组 DNA 作为检测肉制品掺假的稳定标记物，例如复制蛋白 A1 (replication protein A1, RPA1)[97]、朊蛋白 PrP (prion protein PrP, Prnp)[16]等。但是这些基于 DNA 技术的物种鉴定主要都是具有针对性的检测方法，然而在肉类掺假检测中，应识别出更多未知的肉类物种[98]。根据这一需要，开发了非目标检测的 DNA 条形码技术[99]。通过对特定基因片段进行 PCR 扩增和测序，然后在 BOLD 或 NCBI 中

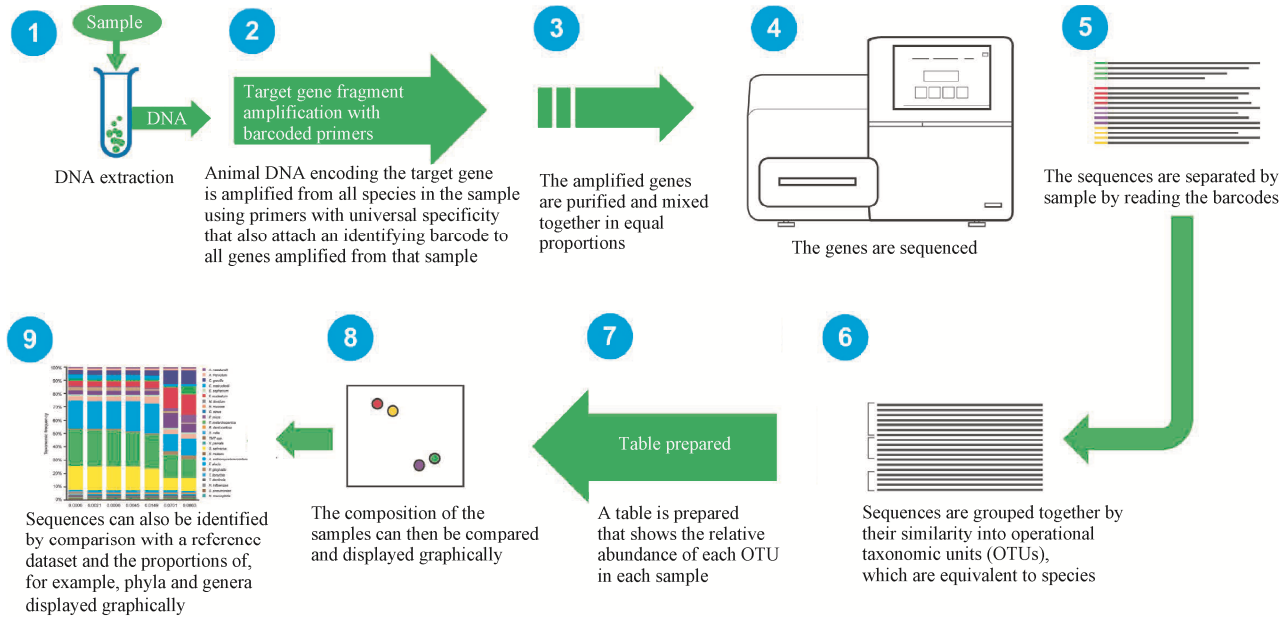

图 3　DNA 宏条形码技术鉴定物种成分的原理[100]

Figure 3　Principle of DNA metabarcoding technology for species identification[100].

搜索，可以识别出掺假的肉类物种[101]。由于 DNA 条形码方法提供了快速、准确和未知物种的识别，因此普遍认为该方法是一种很有前景的肉类掺假检测技术，并已应用于动物肉类鉴定[102-107]。

早期的 DNA 条形码技术主要依赖于动物物种 COI 和 Cytb 基因约 650 bp 区域的 Sanger 测序[108]。然而，当肉制品中存在多个掺假成分时，传统的 Sanger 测序方法会产生多个或重叠的测序峰，导致测序信息错误[109]。因此，通过构建一种 DNA 宏条形码方法，利用高通量测序技术可实现复杂样品中多个物种鉴定。在 Roche 454 平台上，Tillmar 等[110]开发了一种 DNA 宏条形码检测方法，针对 300 多种哺乳动物物种的线粒体 16S rRNA 基因区域[111]设计通用引物。在检测人工 DNA 混合物的实验中，该方法可以识别和区分 99.9%以上的哺乳动物物种并能检测混合物中的多个成分，同时也能检测低至 1%的混合样品中的次要成分。基于 PyroMark ID 平台，Abbadi 等[112]利用焦磷酸测序技术，证明线粒体 16S rRNA 和 COI 基因中的短片段可以从不同的产品中正确区分 15 种双壳类软体动物，为检测海产品中潜在的欺诈行为提供了一种替代、简单、快速和经济的工具。

利用 Ion Torrent 测序仪，Bertolini 等[113]研究了 DNA 宏条形码对 11 种肉类物种的区分能力，检测线粒体 12S rRNA 或 16S rRNA 基因中的 3 个条形码区域的识别能力，实验证明该方法可应用于 DNA 混合物中哺乳动物和鸟类物种的鉴定；Carvalho 等[114]从市场购买标识为鳕鱼的 22 种加工产品，检测其 Cytb 和 COⅡ基因，发现有 31%的产品含 2 种或 2 种以上的混合物，标签错误率为 41%；Giusti 等[105]基于 NGS 技术应用一对通用引物扩增鱼类和头足类的 16S rRNA 基因，对欧盟和非欧盟国家生产的 16 种商业鱼糜制品进行了分析，结果标签错误率为 37.5%，有 25%的产品没有在标签上标识存在软体动物；Ribani 等[115]将下一代半导体测序技术

应用于几种高度加工且复杂的肉制品和肉汤中肉类物种的鉴定。用 3 对通用引物对物种线粒体 DNA 区域的 12S rRNA 和 16S rRNA 基因进行测序并分析产品中预期的和非预期的肉类物种。检测发现烤肉串中含有非预期的猪肉成分，牛肉/猪肉酱中存在水牛的 DNA，肉汤中均检出标签中未声明的肉类。

通过 Illumina MiSeq 或 HiSeq 平台，Khanyile 等[116]使用针对线粒体 16S rRNA 基因的下一代测序技术，检测从南非各地的零售店、屠宰场和加工厂收集的 161 种加工肉制品。在多数标识为牛肉的加工制品中检出含有混合来源的牛、猪和羊，其中香肠掺假的发生率最高，34%的样品由不同物种组成；Carew 等[117]使用 NGS 成功可靠地检测了大型无脊椎动物物种。通过多种扩增子检测，证实了 NGS 检测混合样本中多种物种的有效性以及 MiSeq 平台测序 DNA 条形码的有效性；Kappel 等[118]制备了 9 个含有多达 4 个物种的混合物金枪鱼样品，并对其进行针对 Cytb 基因两个短片段的 NGS 分析，结果表明，该方法可检测低至 1%的混合物种，且具有可重复性。在 3 个金枪鱼商业罐头的首次检测中发现，产品内存在不同的物种，该现象违反了欧盟法律规定；Xing 等[119]基于 NGS 的 DNA 宏条形码技术，研究 16S rRNA 基因短片段在混合物中识别哺乳动物和鸟类的能力，并对在中国销售的 27 种肉禽产品开展市场调查。结果表明，有 67%的肉禽加工产品存在贴错标签现象；Dobrovolny 等[120]基于家禽动物物种，以线粒体 16S rRNA 基因的一个区域作为条形码区域设计了一对新的引物，并将其与区分 300 种哺乳动物物种的引物组合应用于双重检测，可以识别食品中的 15 种哺乳动物和 6 种家禽，区分和检测到的成分比例下降到 0.1%，可同时对 96 个样品进行测序，因此该方法在常规分析中具有很高的应用潜力。

然而，在许多样本中，DNA 降解通常会阻碍 PCR 扩增长度超过 250 bp 的片段[121]，严重影响 DNA 质量。所以，遗传标记应足够短，以便能够从加工过的样本 (食物)、无创方式获取的样本 (皮毛、羽毛、粪便、唾液等) 和保存样本的 DNA 中精确扩增。为了克服这些问题，Meusnier 等[122]开发了一套通用引物，在条形码区扩增了一个 130 bp 的 COI 基因片段。利用 HTS 技术开发一种小型条形码方法，该方法可针对较短的 DNA 片段 (100–200 bp)[104,108]，如经高温高压处理加工的肉制品 DNA 小片段 (<200 bp)。与早期的 DNA 条形码技术相比，微型条形码具有更高的通量和灵敏度。当以小片段为目标时，它甚至适用于高度加工肉制品的肉类鉴定[105]。最近，Cottenet 等[98]在 Ion GeneStudio™ S5 平台上，应用一个商业化的 NGS 食品真实性检测流程来识别非目标肉类物种，能在低至 1%的混合物中检测和鉴定物种。成功地测试了来自欧洲和亚洲市场的 45 份绞肉，包括一些相似的物种，如野牛与水牛、马鹿与驯鹿，其中 18%的样品显示有未标识的肉类物种。该方法还适用于加工 (磨碎、煮熟和罐装) 样品的鉴定。因此，在未来的研究中，需重点研究开发更独特的条形码。

笔者所在的实验室从国内肉制品市场、自然环境中常见的动物及国家重点保护野生动物名录中遴选硬骨鱼纲、软骨鱼纲、哺乳纲、鸟纲、爬行纲等物种作为研究对象，构建包含 1 500 多个属、2 700 多个种的动物分类数据库。基于物种系统进化分析结果，针对线粒体 16S rRNA 基因的目标区域设计引物，完成引物的筛选及通用性检测，建立高通量测序方法和数据分析流程。目前正在扩大测试样本的范围，深入开展肉类掺假检测方法的特异性、灵敏度和准确性研究。

4 面临的挑战

DNA 宏条形码技术将 DNA 条形码原理与高通量测序技术相结合，可产生大量生物多样性数据。长期以来该技术一直应用于微生物领域，然而近几年在动植物群体生物多样性评估中也获得较快发展。DNA 宏条形码技术依赖于 DNA 的初始质量[123]，能够从一个样品的 PCR 产物中同时鉴别多个生物体[124]，这些限制影响了条形码标记的选择和结果的分析方法。

有研究表明，生命条形码联盟 (consortium for the barcode of life, CBOL) 定义用于动植物标本分类鉴定的标准条形码标记并不完全适用于环境样本的应用[125]。首先，经典的条形码方法倾向于那些能够识别相似物种的条形码区域。为了在种水平上获得高分辨率，标准标记通常需要大于 500 bp[126]，可应用于从纯样本中提取的 DNA 分析或从活生物体中提取的微生物 DNA 宏条形码研究[127]。然而，对于动植物的宏条形码应用，研究人员通常是从生物体残骸中提取 DNA。环境中的 DNA 降解会影响大于 150 bp 的 PCR 片段回收，从而阻碍条形码的扩增[128]。其次，DNA 宏条形码需要同时扩增一个样本中所有物种的 DNA。如果这些扩增子能够代表样本中存在物种的相对丰度，那么就必须限制某些物种相对于其他物种过度扩增的风险[129]。为了解决这些问题，标准标记要求对每个主要物种使用特异引物[130]。但是，这与共同平衡扩增样本中所有物种的要求不符。因此，DNA 宏条形码方法需要选择非标准标记，筛选比较几个候选条形码，确定新的 DNA 区域，以达到该方法的限制要求。

研究人员还希望通过宏条形码方法对样品中的物种多样性进行定量分析。将测序运行中获得的读数与原始样品中的生物量相关联[131]，与 RNA 序列分析[132]以及微生物群落特征等其他应用类似。通常认为 HTS 过程中以下几个因素可能会对结果引入偏差，并导致生物量估算不准确：(1) 样本中不同组织之间细胞和 DNA 数量的变化[133]以及不同组织和物种之间 DNA 提取成功率的差异[134]。(2) 不同物种之间扩增效率的差异[135]。(3) 不同测序仪和运行之间的定量估计值的差异[136]。(4) 不同生物信息学数据分析方法得出不同的群体成分估计值[137]。但是，近年来有研究表明[138]：(1) DNA 提取并不是显著偏差的来源。以定量 DNA 为起始样本的研究与以生物量为原料的研究在定量上没有差异。但是在使用宏条形码方法之前，最好先量化目标生物体中生物量和 DNA 产量之间的关系。(2) 如果不同物种之间个体大小差异很小，那么产生的序列能够复制原始的生物量、DNA 数量或个体，且定量数据可代表生物量。如果个体大小存在显著的差异，则相对丰度与个体数量不符[139]。(3) 测序平台在定量方面没有显著差异。

然而，通常仅使用一组引物，其 PCR 产物可能会由于引物偏好性而不能反映宏条形码技术的能力。即使利用多个引物，它们也通常用于同一个测序过程，在这种情况下，则不能认为结果是独立的。目前，研究人员已验证如何在不同应用中使用标记，并且正在探索可供选择的、无偏见的方法。这包括使用多个目标标记和无 PCR 方法，这些方法可与捕获 DNA 富集相结合[140]。Ripp 等[141]建立了全食物测序 (all-food-seq, AFS) 方法，基于 Illumina HiSeq 2000 和 MiSeq 平台进行食品全基因组 DNA 的非目标深度测序。在香肠成分分析案例中，对不同物种 DNA 能够达到 1%的定量鉴别水平，还可识别来自动物、植物和微生物 DNA 的痕迹。从而实现在界的水平上所有物种高度准确

地鉴定，以及对主要成分的定量和未知食品成分的检测。Liu 等[142]证实 AFS 通过宏基因组鸟枪测序和序列 read 数来推断物种比例，只需少量样本和测序信息即可用于识别和定量复杂生物混合物 (如食品) 的成分。Hellmann 等[143]应用 AFS 分析食品中的肉类成分，发现以“旋转烤肉”标签出售的产品中，检出未标识的火鸡肉。通过分析香肠中物种的比例，与 qPCR 和 ddPCR 数据进行比较，证明在不低于 1%范围内，AFS 很容易识别和量化物种的比例。

此外，还可在测序过程中加入对照物以确保实验之间的一致性[144]。为了获得最准确的生物量估计，可以制定校正因子，以考虑混合物中分类群之间已知的生物差异 (例如，基因拷贝数差异[145-146])。

5 总结与展望

作为食品安全的主要问题之一，肉类及其制品的掺假问题近年来越来越受到人们的关注。DNA 宏条形码技术可以对未知物种进行快速、准确地识别，无疑为肉类掺假检测提供了优异的技术手段。但是目前该技术在应用中存在的影响因素仍需要开展研究确认。在进行定性分析时，识别标记的挖掘尤为重要。标记直接决定了方法的准确性，尤其是对于深度加工的肉制品，如高温加热和乳化加工，或用于区分相似的物种。在定量分析方面，虽然宏条形码技术存在诸多不确定性，我们对影响因素的了解仍然有限。但是通过测试和模拟，揭示引物选择背后的基本原理以及序列读数丰度和生物量之间的关系，或使用对照物作为质控，将有助于获得准确的生物量评估。还可以通过引入无需 PCR 扩增的单分子测序技术提高方法的准确度。随着高通量测序技术的不断发展，分子生物标记检测方法 (如 ISO 22949-1:2021[147]) 标准化的日趋完善，该技术将提供可靠的检测数据，并准确评估与类群相关的不确定性。宏条形码技术具备高灵敏度和高精度等特点，将为食品安全监管及执法机构提供有效的技术支撑。

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