RNA-Seq

a revolutionary tool for transcriptomics

冉令娟：文献翻译。苏旭美：文献翻译，演讲。向宇嘉：文献翻译，ppt制作。

目录

CONTENTS

转录组学的研究方法有两类：

第一类是基于杂交的方法，主要指微阵列技术（Microarray methods ）

第二类是基于测序的方法,包括表达序列标签技术（Expression Sequence Tags Technology，EST），RNA测序技术(RNA-seq）等

DNA微阵列技术介绍

可分为两类：

1•cDNA微阵列

2•寡核苷酸微阵列

DNA微阵列

锁定

收藏 66 0

本词条由"科普中国"百科科学词条编写与应用工作项目 审核 。

DNA微阵列（DNA microarray）又称DNA阵列或[illegible]是基因芯片（gene chip）。是一块带有DNA微阵列（micorarray）涂层的特殊玻璃片，在数平方厘米之面积上安装数千或数万个核酸探针，经由一次测验，即可提供大量基因序列相关资讯。它是基因组学和遗传学研究的[illegible]时间定量的分析大量(成千上万个)的基因表达的水平，具有快速、精确、低成本之生物分析检验能力

中文名	DNA微阵列	特　色	DNA阵列或DNA芯片
外文名	DNA microarray	类　型	临床诊断用芯片

1 DNA微阵列技术在转录组研究方面的优缺点

Hybridization-based approaches are high throughput and relatively inexpensive, except for highresolution tiling arrays that interrogate large genomes. However, these methods have several limitations, which include:reliance upon existing knowledge about genome sequence; high background levels owing to cross-hybridization; and a limited dynamic range of detection owing to both background and saturation of signals. Moreover, comparing expression levels across different experiments is often difficult and can require complicated normalization methods

优点：1·高通量
2·相对便宜

缺点：1·依靠现有的基因组序列知识
2· high background levels owing to cross-hybridization
3·检测范围有限制
4·需要更复杂的方法来比较表达的层次

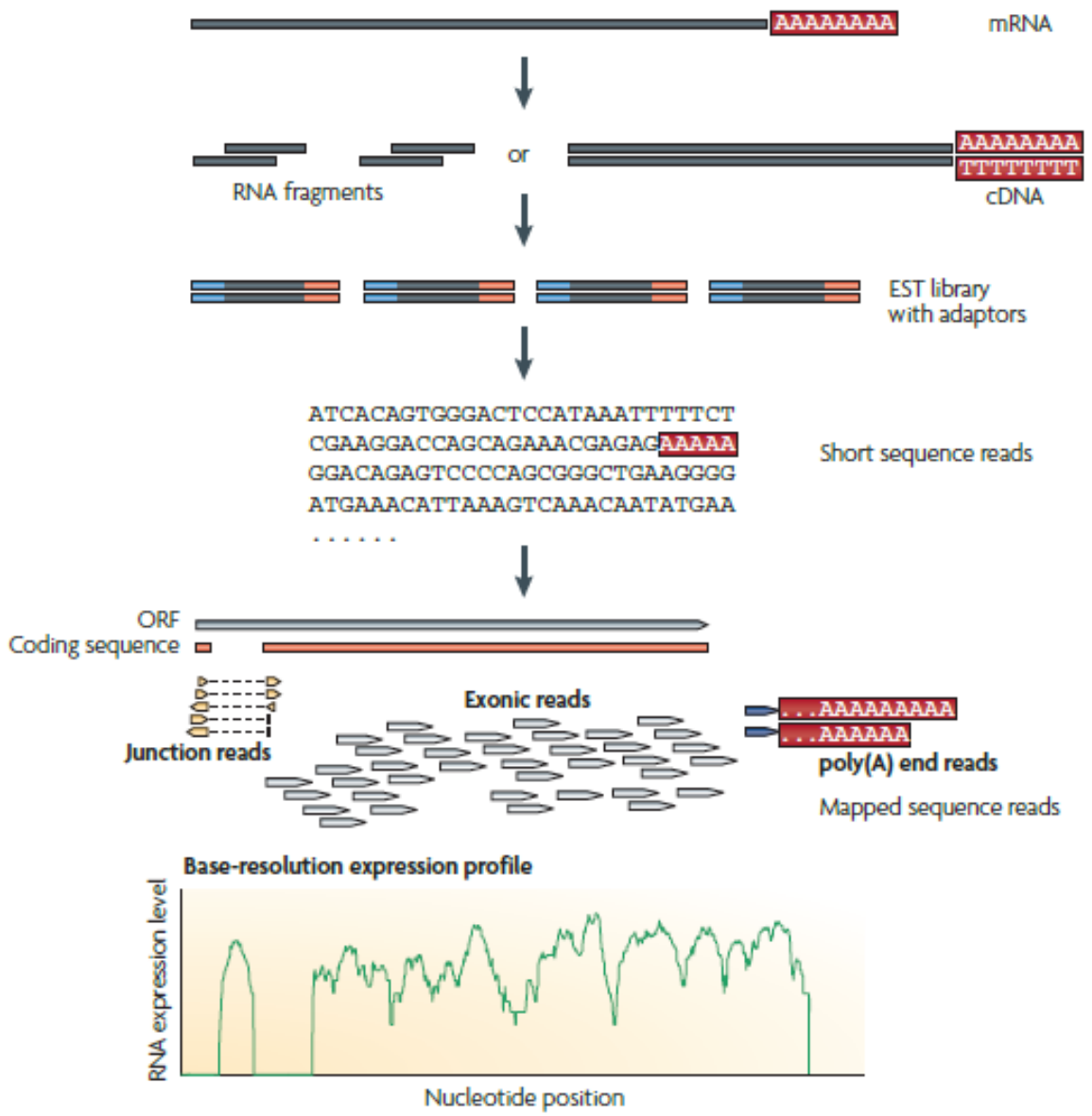
Table 1 | **Advantages of RNA-Seq compared with other transcriptomics methods**

Technology	Tiling microarray	cDNA or EST sequencing	RNA-Seq
Technology specifications			
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to 100 bp	Single base	Single base
Throughput	High	Low	High
Reliance on genomic sequence	Yes	No	In some cases
Background noise	High	Low	Low
Application			
Simultaneously map transcribed regions and gene expression	Yes	Limited for gene expression	Yes
Dynamic range to quantify gene expression level	Up to a few-hundredfold	Not practical	>8,000-fold
Ability to distinguish different isoforms	Limited	Yes	Yes
Ability to distinguish allelic expression	Limited	Yes	Yes
Practical issues			
Required amount of RNA	High	High	Low
Cost for mapping transcriptomes of large genomes	High	High	Relatively low

3·亚种大多数不能被区分

RNA-Seq方法介绍

2 RNA-seq方法介绍



A typical RnA-seq experiment. Briefly, long RNAs are first converted into a library of cDNA fragments through either RNA fragmentation or DNA fragmentation (see main text). Sequencing adaptors (blue) are subsequently added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology. The resulting sequence reads are aligned with the reference genome or transcriptome, and classified as three types: exonic reads, junction reads and poly(A) end-reads. These three types are used to generate a base-resolution expression profile for each gene, as illustrated at the bottom; a yeast ORF with one intron is shown.

该技术首先将细胞中的所有转录产物反转录为cDNA文库(利用最新的SMS技术可略去这一步，直接对RNA进行测序)，然后将cDNA文库中的DNA随机剪切为小片段(或先将RNA片段化后再转录) 在cDNA 两端加上接头利用新一代高通量测序仪测序，直到获得足够的序列，所得序列通过比对(有参考基因组)或从头组装(无参考基因组)形成全基因组范围的转录谱

1 RNA-seq优点

Taking all of these advantages into account, RNA-Seq is the first sequencing based method that allows the entire transcriptome to be surveyed in a very high-throughput and quantitative manner. This method offers both single-base resolution for annotation and ‘digital’ gene expression levels at the genome scale,often at a much lower cost than either tiling arrays or large-scale Sanger EST sequencing.

1·数字化信号：直接测定每个转录本片段序列，单核苷酸分辨率的精确度，同时不存在传统微阵列杂交的荧光模拟信号带来的交叉反应和背景噪音问题。

2·高灵敏度：能够检测到细胞中少至几个拷贝的稀有转录本。

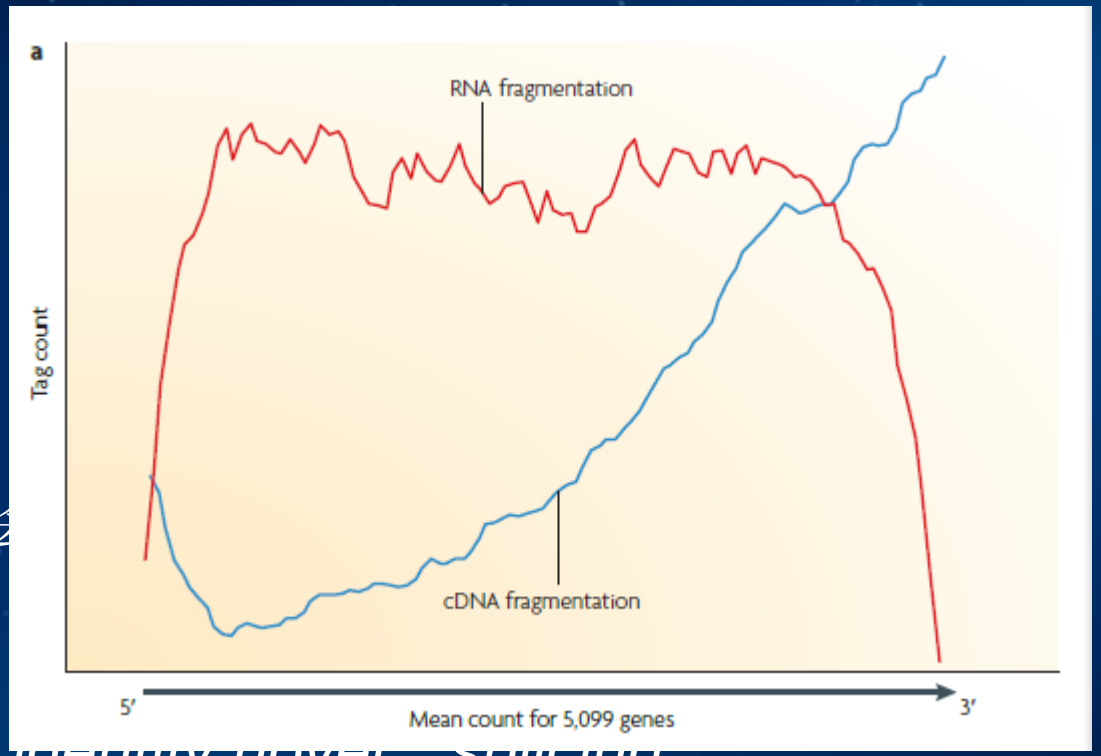
3·全基因组分析：可以对任何物种进行全基因组分析。无需预先设计特异性探针，因此无需了解物种基因信息，能够直接对任何物种进行转录组分析。同时能够检测未知基因，发现新的转录本，并精确地识别可变剪切位点及cSNP，UTR区域。

4·检测范围：高于5个数量级的动态检测范围，能够同时鉴定和定量稀有转录本和正常转录本。

2 Challenges for RNA-Seq

1·Library construction
构建cDNA文库时较为复杂

2·大的RNA分子需要打断成小片段，任何方法都会
果有偏差



3·develop computationally simple methods to identify novel splicing events that take place between two distant sequences or between exons from two different genes.提出更简单的方法来识别发生在两条序列或者不同基因外显子之间的splicing events

4 'Coverage versus cost. the larger the genome, the more complex the transcriptome, the more sequencing depth is required for adequate coverage.

5·等等，，，此处不列举了

2 RNA - seq在转录组研究中的更多应用

New transcriptomic insights

1· *Mapping gene and exon boundaries.准确的定位基因和外显子的边界*

2·Extensive transcript complexity

3·Novel transcription.发现更多的转录组相关组件

4·Defining transcription level 更精确的确定转录组的表达层次

2 对RNA-seq技术的未来展望

Although RNA-Seq is still in the early stages of use, it has clear advantages over previously developed transcriptomic methods. The next big challenge for RNA-Seq is to target more complex transcriptomes to identify and track the expression changes of rare RNA isoforms from all genes. Technologies that will advance achievement of this goal are pair-end sequencing, strand-specific sequencing and the use of longer reads to increase coverage and depth. As the cost of sequencing continues to fall, RNA-Seq is expected to replace microarrays for many applications that involve determining the structure and dynamics of the transcriptome.

尽管RNA-Seq仍处于使用的早期阶段，但它相对于以前的转录组学方法具有明显的优势。RNA-Seq的下一个大挑战是针对更复杂的转录组来识别和追踪所有基因中罕见RNA亚型的表达变化。推进实现这一目标的技术是对端测序，链特异性测序和使用更长的读段以增加覆盖和深度。随着测序成本的不断下降，RNA-Seq预期取代微阵列，并且在确定转录组的结构和动力学的方面将有许多应用。

谢谢

THANK YOU FOR WATCHING