Advances and Applications of Single-CellSequencing Technologies

小组成员：高帮良
冯圣宇
丁雄飞

Introduction

同一组织中的细胞往往被认为是具有相同状态的功能单位，传统的检测手段分析的是细胞群体的总体平均反应。然而通过对单个细胞的DNA或RNA进行测序，表明组织系统层面的功能是由异质性细胞构成的。单细胞测序以单个细胞为单位，通过全基因组或转录组扩增，进行高通量测序，能够揭示单个细胞的基因结构和基因表达状态，反映细胞间的异质性，在肿瘤、发育生物学、微生物学、神经科学等领域发挥重要作用，正成为生命科学研究的焦点。单细胞测序的难点是单个细胞的分离、单细胞基因组和转录组的扩增。本文主要介绍和分析了单细胞测序技术中常用的单细胞分离技术、单细胞基因组扩增技术和转录组扩增技术及其优缺点，并对当前已经取得成果的应用领域进行了阐述，为单细胞测序技术的研究与应用提供参考。

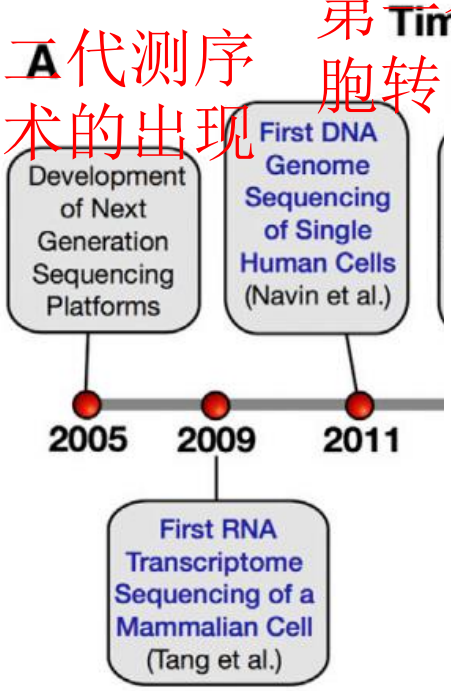
Introduction

单细胞测序萌芽于2010年，2013年才真正发展起来，2014年，单细胞测序的应用被列为《自然—方法学》(Nature Methods)年度最重要的方法学进展，2015基因组学前沿研讨会将单细胞组学单独列为一个单元，可见单细胞测序在当前基因组学前沿研究中的热度。

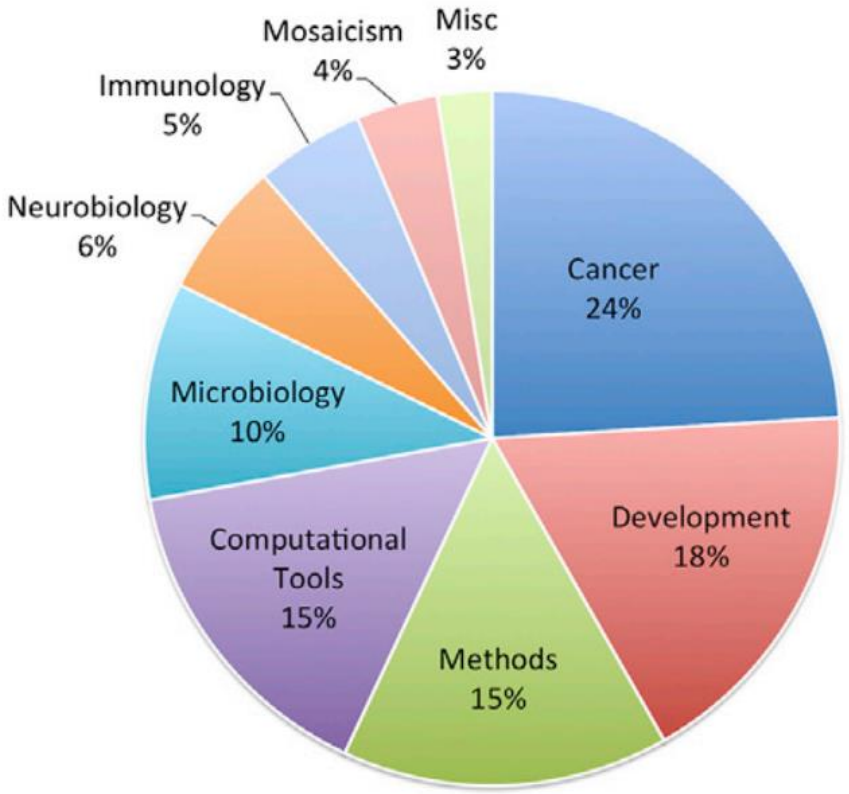
Introduction

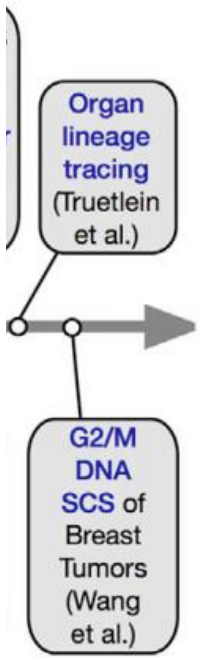
第二代测序技术的出现

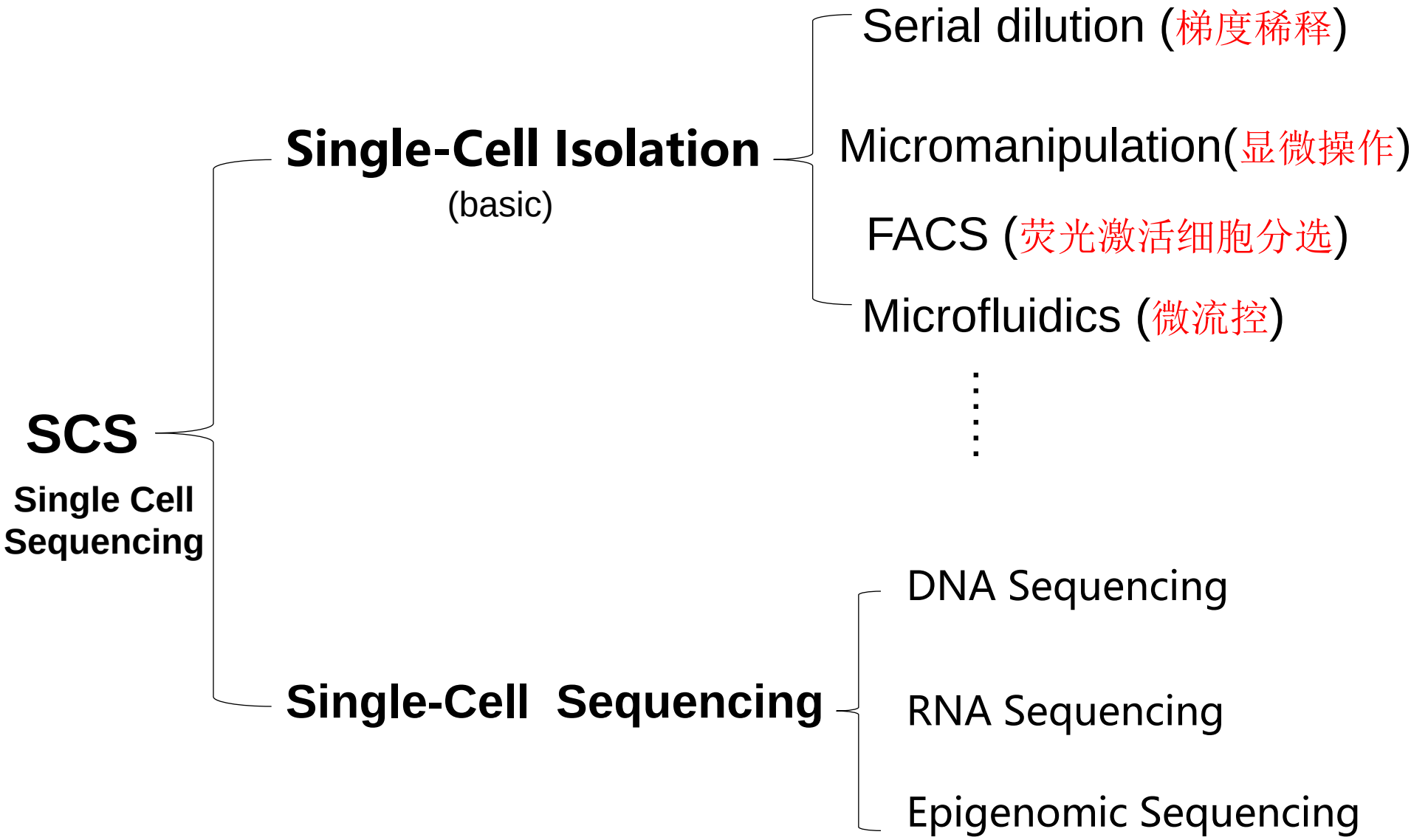
第一个人类单细胞转



第一个哺乳动物细胞转录组测序






SCS
Single Cell Sequencing
Single-Cell Isolation
(basic)
Serial dilution (梯度稀释)
Micromanipulation(显微操作)
FACS (荧光激活细胞分选)
Microfluidics (微流控)
Single-Cell Sequencing
DNA Sequencing
RNA Sequencing
Epigenomic Sequencing

Single-Cell Isolation

梯度稀释法

微流控技术

Isolation Methods	Description	Advantages	Disadvantages	Cost
Serial dilution	serial dilution to about one cell per microliter	simple approach; low cost	high probability of isolating multiple cells	$
Mouth pipetting	isolate single cells with glass pipettes	simple approach; low cost	technically challenging	$
Flow sorting	microdroplets with single cells are isolated by electric charge at high pressure	high-throughput; fluorescent markers can be used to isolate subpopulations	expensive equipment; requires operator	$$
Robotic micromanipulation	robotic-controlled micropipettes isolate single cells	high accuracy; fluorescence can be used	low throughput	$$$
Microfluid platforms	microfluidic chips isolate single cells in flow channels	high-throughput; reactions can be performed on-chip; reduced reagent costs	cell size must be uniform; expensive consumables	$$$

Isolation Methods for Rare Cells

Isolation Methods	Description	Advantages	Disadvantages	Cost
Nanofilters	size discrimination on nanofabricated filters	cells are selected by size exclusion	cells can adhere to filters during backwash	$
MagSweeper	rotating magnet with EpCAM antibodies	high enrichment of rare cells	biased toward markers used for isolation	$$
Laser-capture microdissection	cells are cut from a tissue section slide with lasers under a microscope	spatial context is preserved	cell slicing; UV damage to DNA/RNA	$$$
CellSearch	magnets with nanoparticles conjugated to antibodies enrich surface markers	high-throughput	biased toward markers used for isolation	$$$
CellCelector	robotic capillary micromanipulator	high-throughput	expensive system and large footprint	$$$
DEP-Array	microchip with dielectropheretic cages	high sensitivity for isolating rare cells	time-consuming; low-throughput; cells are deposited into large final volumes	$$$$

This table summarizes the advantages and disadvantages of single-cell isolation methods for abundant populations and rare subpopulations.

ns）

微流控技术需

求。扩

增，续

的测胞，

本文量

细胞

Sequencing Methods

- Single-Cell DNA Sequencing Methods
- Single-Cell RNA Sequencing Methods
- Single-Cell Epigenomic Sequencing Methods

Single-Cell DNA Sequencing Methods

The development of DNA SCS methods has proven to be more challenging than RNA. This is due to the fact that a single cell contains only two copies of each DNA molecule but thousandsof copies of most RNAmolecules.

WGA: 单细胞全基因组扩增技术

原理

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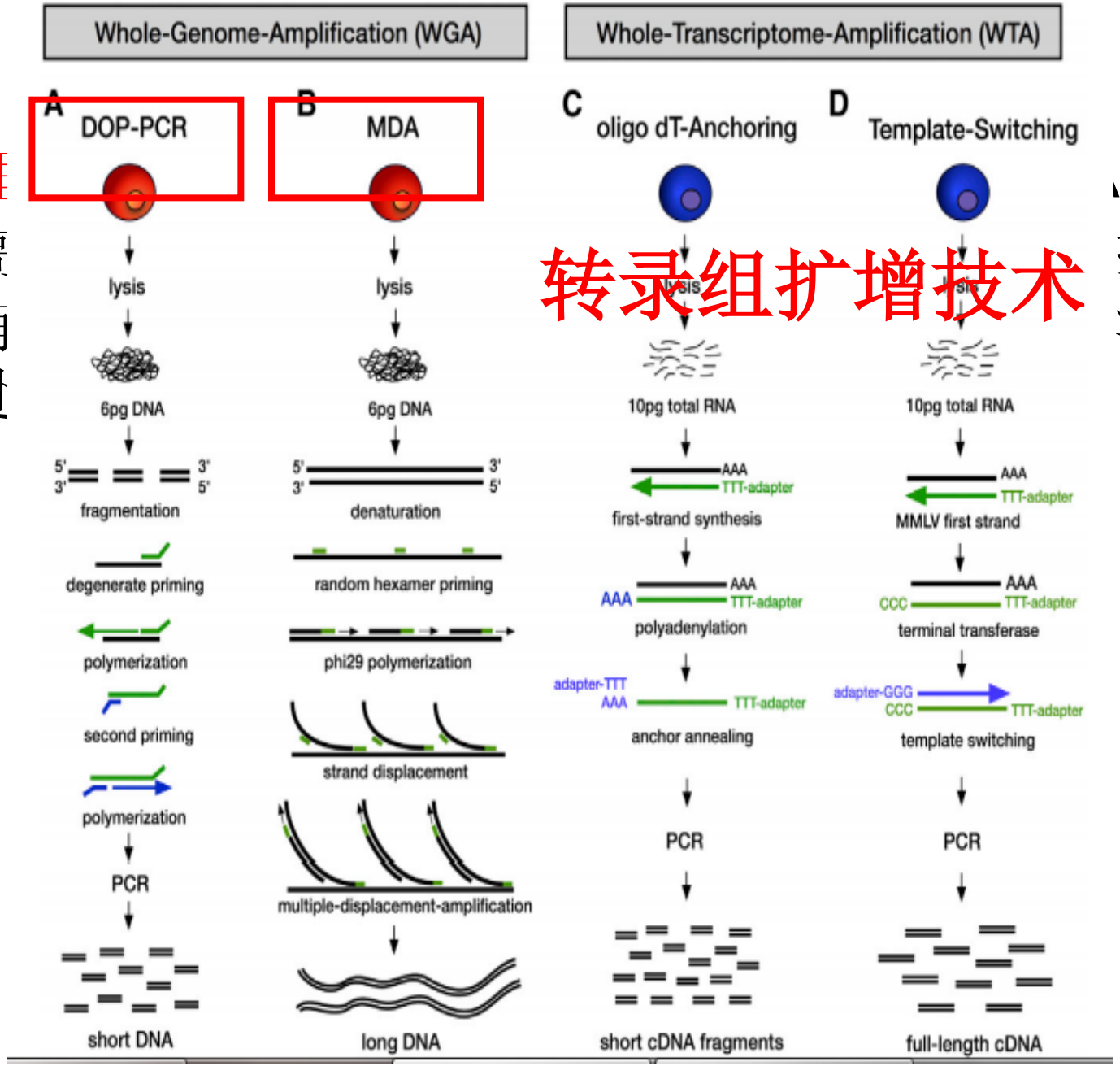
行高

技术。

只有

测序

和处



Distinguishing Technical Errors from Biological Variations

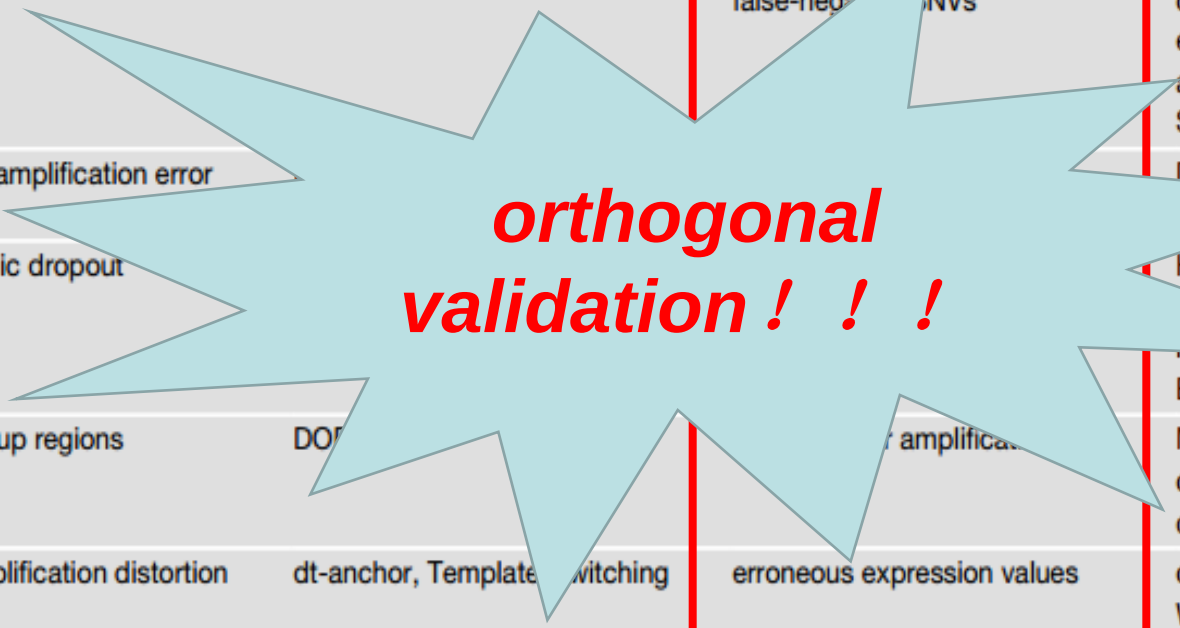
naive users often interpret these technical errors as extensive biological heterogeneity.

At the current state of technology, most SCS methods introduce extensive technical errors and variability into data sets. Unfortunately, naive users often interpret these technical errors as extensive biological heterogeneity. (难以区分技术错误导致的差异性还是本身细胞存在的差异性)

Table 2. Technical Errors Associated with Single-Cell Sequencing

	Technical Artifact	Amplification Method	Error Type	Description
WGA	chimeric molecules	MDA	false-positive inversions	3′ and 5′ ends of newly synthesized molecules hybridize together during MDA leading to inversions
	coverage nonuniformity	MDA, DOP-PCR, MALBAC	copy number a[illegible]errations, false-nega[illegible]SNVs	Under and over amplifications of the genome can lead to erroneous copy number abberations and false-negative SNVs
	FP amplification error			DNA polymerase introduces [illegible]FP errors
	allelic dropout			Heterozygous (AB) variants [illegible]go dropout during WGA [illegible]ozygous (AA or BB) genotypes
	pileup regions	DO[illegible]	[illegible] amplifica[illegible]	Massive over-amplifications of focal genomic regions occur during DOP-PCR
WTA	amplification distortion	dt-anchor, Templat[illegible]witching	erroneous expression values	over/under amplification during WTA leads to erroneous expression values
	transcript dropout	dt-anchor, Template-Switching, UMI	false-negative unexpressed genes	failure to amplify a transcript during WTA
	3′ bias	dt-anchors	failure of RT polymerase to fully synthesize the first cDNA strand	strong bias toward amplification of 3′ end of RNA transcripts

This table lists the common technical errors that arise during WGA and WTA in single-cell sequencing experiments.



Applications

- Microbiology
- Neurobiology
- Tissue Mosaicism
- Germline Transmission
- Embryogenesis
- Organogenesis
- Immunology
- Cancer Research
- Clinical Applications
- Computational Methods

Microbiology（微生物学）

However,bacteria and other microorganisms often have only femtogramsof DNA and RNA,（DNA和RNA的含量非常少）making it even more challenging to amplify than mammalian cells.

Microbiology

- In an early study,MDA was used to amplify DNA from the marine cyanobacterium Prochlorococcus for pyrosequencing and de novo assembly（海洋藻青菌的测序及其组装）

- In another study, Woyke et al. used FACS and MDA to perform NGS and assemble two marine flavobacteria genomes to 90% coverage (利用第二代测序技术测得海洋黄细菌的基因组，其覆盖率达到90%)

Microbiology

These studies show that SCS is complimentary to metagenomic deep-sequencing methods and can open up new avenues of investigation into microbial genomes that cannot be cultured in the lab.（可以开辟研究不能在实验室培养的微生物基因组的新途径）

Neurobiology（神经生物学）

Traditional classification has relied mainly on morphological features（形态学的特征）, single-cell RNA sequencing provides a powerful unbiased approach to classify neurons based on their transcriptional profiles.（转录谱的信息）

Neurobiology

- Pollen et al. used low-coverage single-cell RNA sequencing and microfluidics to analyze single cells from 11 brain populations, and identified Notch signaling as an important regulator of brain development（发现了在大脑发育过程中的重要调控因子）
- In another study, single-cell RNA-seq was performed in situ in spatially defined neuronal regions, which identified cell-to-cell transcriptional variation in hippocampal neurons（大脑海马体细胞与细胞之间转录组的差异）
- Several studies have also begun to investigate DNA heterogeneity in neurons.（神经元中DNA的异质性）

Neurobiology

These initial studies show that SCS provides a novel approach to classify neuronal cell types and identify an unexpected amount of DNA diversity in neuronal populations.

Germline Transmission（发育生物学）

Sperm cells and oocytes are single cells that fuse to form a zygote and transmit genomic material, and evolution has engineered this process to generate genetic variation. Single-cell DNA sequencing provides a novel approach to study the mechanisms that generate germline variation. （研究生殖细胞发育过程中的变化）

Germline Transmission

- In one of the first studies on this topic, single sperm cells were sequenced, which revealed an average of 22.8 recombination events(重组事件), 5–15 gene conversion events,(基因转换事件) and 25–36 de novo mutations in each sperm cell (精细胞)。
- a recent study used MALBAC to analyze fertilized oocytes (Hou et al., 2013). In this study oocytes from 8 individual females were analyzed,which identified 43 crossover（交叉互换） events per oocyte, a recombination rate that is 1.63 times higher than sperm(受精卵)。

Germline Transmission

- Collectively, these studies have begun to dissect the complex transcriptional regulation and epigenomic reprogramming that occurs during the earliest stages of embryogenesis

从以上的研究中，我们可以了解到，在生殖细胞的发育过程中，细胞循环、基因调控、翻译和代谢中的转录谱以一定的顺序变化着

Cancer Research（癌症研究）

- PNAS：单细胞测序技术应用于癌症无创诊断

Alternatives to Single-Cell Sequencing

SCS is not the appropriate technology to address every question in biology. In many studies, alternative approaches will provide more powerful tools for investigating population diversity and identifying rare mutations. Methods such as deep sequencing(深度测序) or multiregion sequencing（多区段测序）provide a more economical approach for resolving complexpopulation substructure

Conclusions and Future Directions

SCS methods have provided great insight into our understanding of biological diversity and rare cells that have previously been difficult to resolve in genomic data from bulk tissue samples.These tools have had a broad impact on many diverse fields of biology over the past 5 years, and several common applications have emerged:

1. delineating population diversity(描述种群的多样性)
2. tracing cell lineages(追踪细胞谱系)
3. classifying cell types(细胞类型分类)
4. genomic profiling of rare cells(稀有细胞基因组分析)

通过对单细胞测序技术的深入研究，更有助于了解细胞间的差异性与同源性，研究发育过程中细胞的分化差异以及组织环境中的协调互作，丰富了人类对自然界多样性和生物进化的认识。

但目前单细胞测序还存在着问题，例如扩增存在偏倚性，非特异性扩增，检测灵敏度不高，重复性差等。未来，随着高通量技术等的不断发展，单细胞测序技术的研究和检测会更加细致深入，应用领域也会越来越广。

thanks