中国营销中心

高通量测序技术在乳制品安全方向的应用

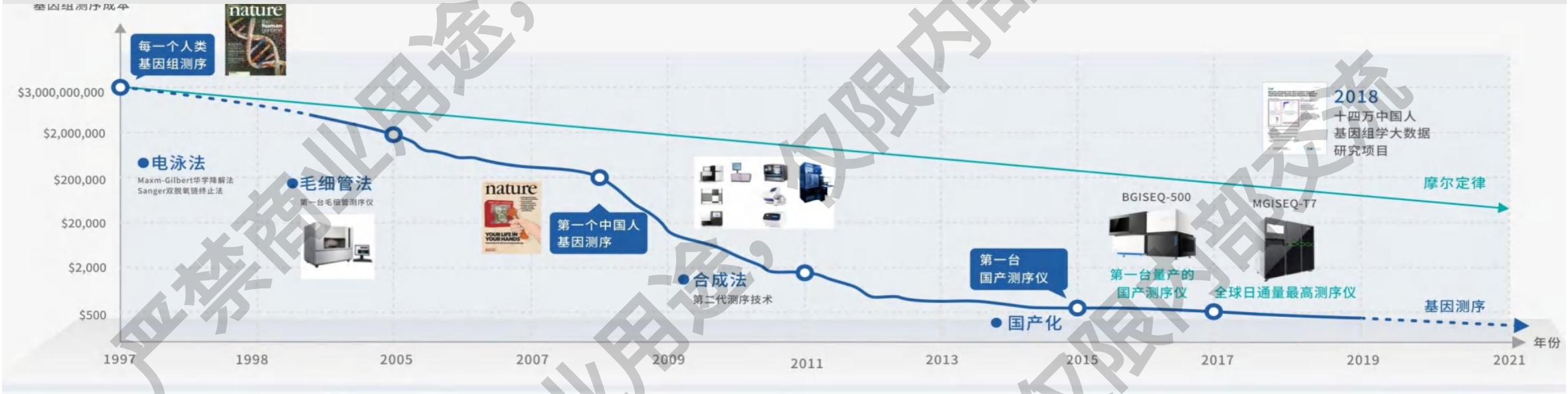
徐媛

2021.06.28

高通量测序技术介绍

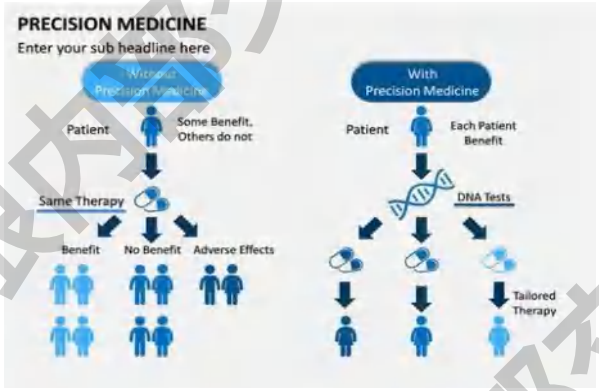
高通量测序又名下一代测序（Next Generation Sequencing，NGS）、大规模平行测序（Massive Parallel Sequencing，MPS）。

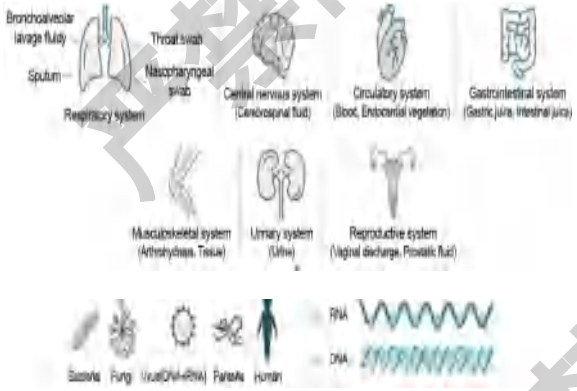
它最大的特点是能一次并行对上百万条DNA分子进行序列测定，**大大提高了测序的效率、降低了测序的成本。**

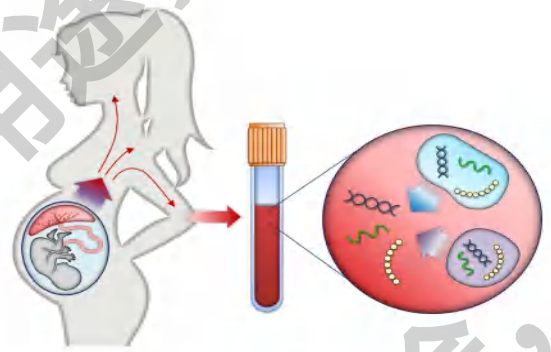


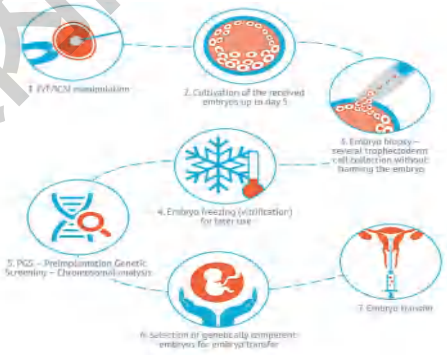
高通量测序技术应用

从科研转到具体的应用







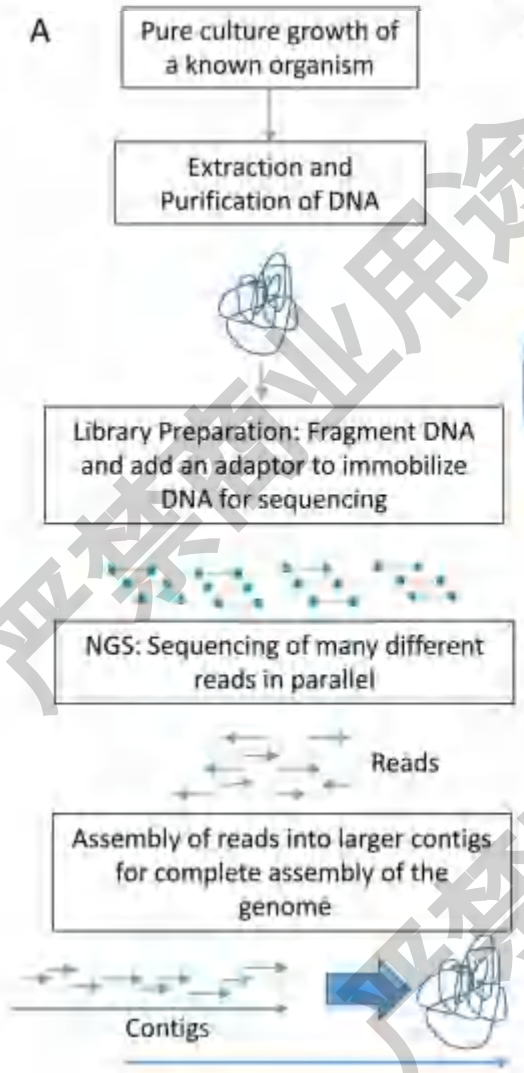


高通量测序技术在乳品安全方面的应用

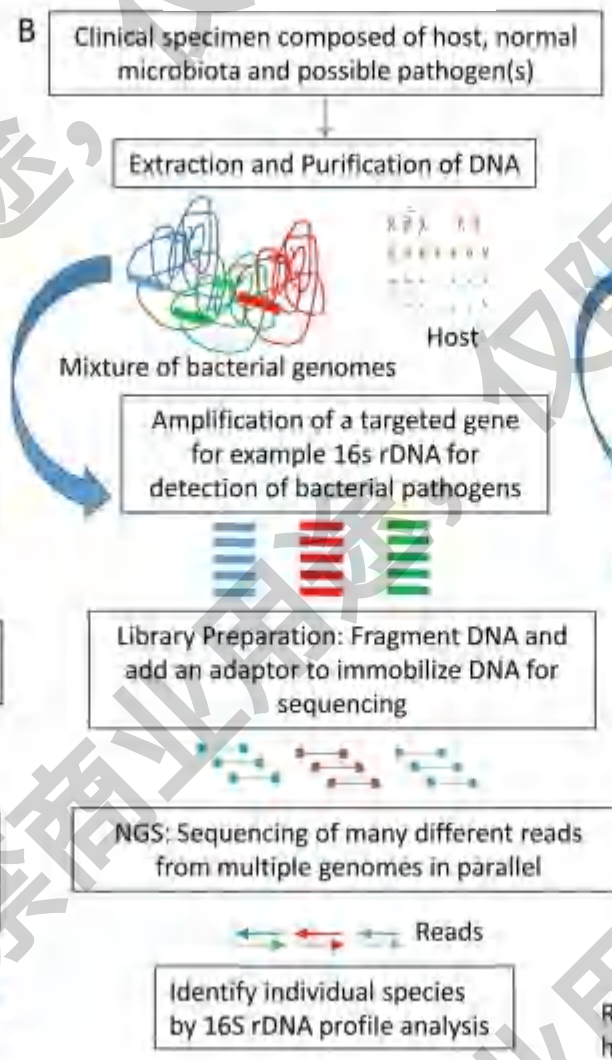
- 污染菌的精准鉴定
- 污染菌的溯源（环境、原材料等）
- 益生菌的安全评估
- 环境&样品中微生物的研究

高通量测序技术

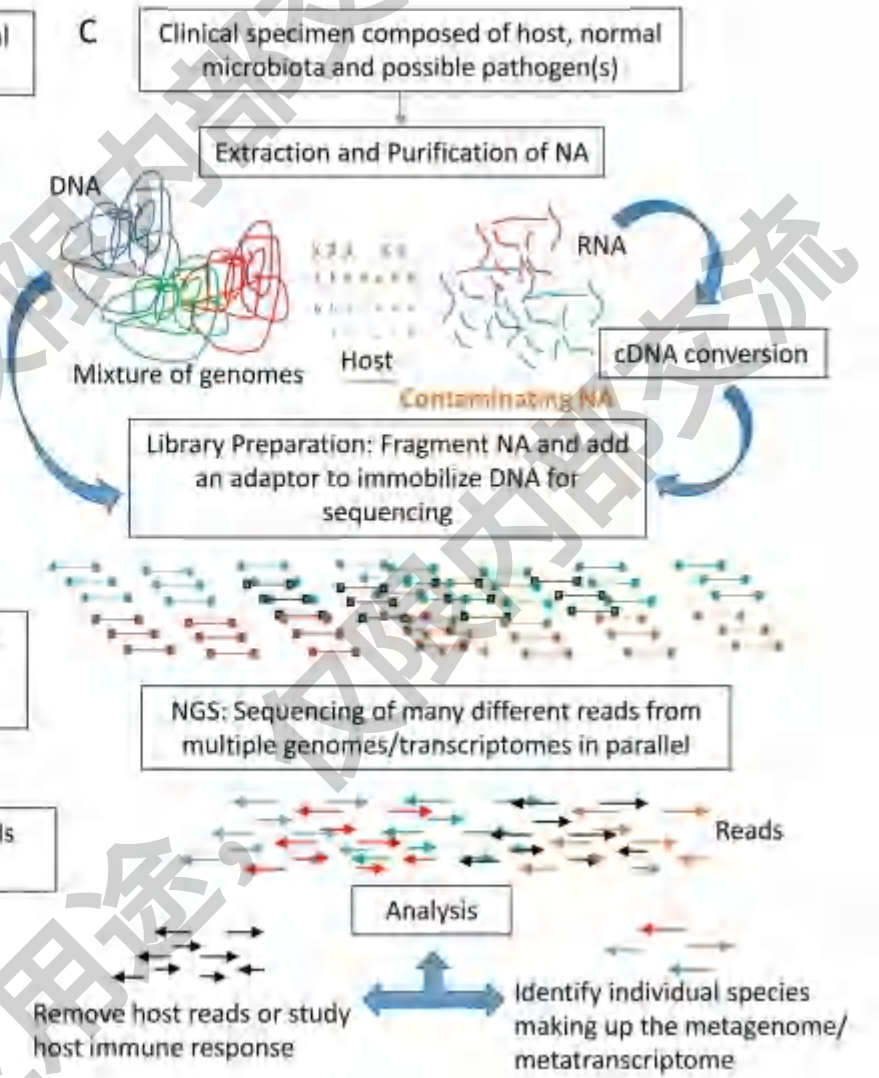
全基因组测序



靶向测序



宏基因组测序



高通量测序技术

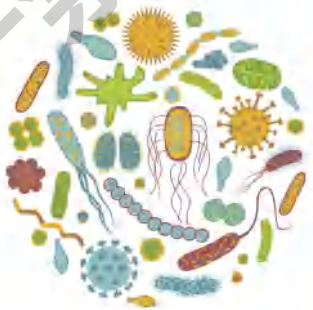
宏基因组测序

针对样本中所有的病原菌进行测序，获取基因组序列信息菌株鉴定，耐药和毒力基因检测

样本类型：环境样本

检测序列：所有微生物全部核酸序列

优势：无需培养、无需预判，可检测混合感染、快速、通量高、灵敏度高



靶向测序

针对感兴趣的病原体进行测序，获取靶标病原菌的基因组序列菌株鉴定，分型，耐药和毒力基因检测

样本类型：环境样本

检测序列：所有微生物部分核酸序列

优势：无需培养、可检测混合感染、快速、通量高、灵敏度高

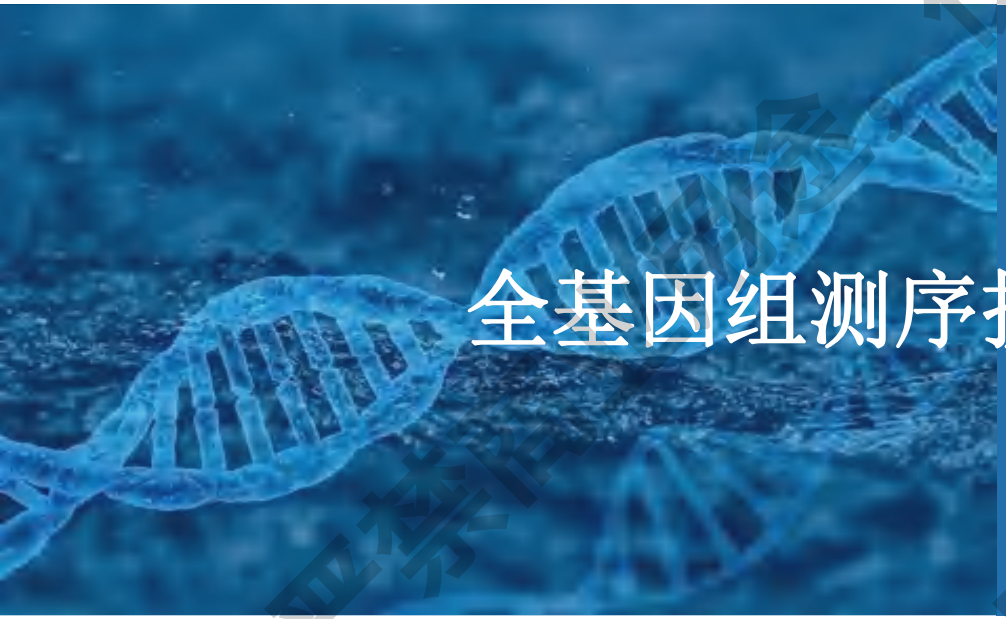
全基因组测序

仅针对一种病原进行测序，获取该病原的全基因组序列信息菌株鉴定、分型、溯源、耐药和毒力基因检测

样本类型：培养的纯菌

检测序列：单菌全部核酸序列

优势：通量高、精准鉴定和分型、溯源



全基因组测序技术用于污染菌的分型和溯源

乳制品生产过程中的生物危害

Table 1
RASFF alerts from 2015 to 2019 categorised under biological, chemical, physical contaminants and inadequate controls.

Biological contaminants	F	Chemical contaminants	F	Physical contaminants	F	Inadequate controls	F
Pathogenic micro-organisms	249	Industrial contaminants	6	Foreign bodies	43	Packaging defective/incorrect	4
Non-pathogenic micro-organisms	16	Mycotoxins	6			Adulteration/fraud	5
		Allergens	7			Poor or insufficient controls	3
		Food additives and flavourings	5			Organoleptic aspects	3
		Heavy metals	2			Labelling absent/incomplete/incorrect	3
		Legal veterinary products	3				
T	265		29		43		18

F = frequency of notification and T = totals.

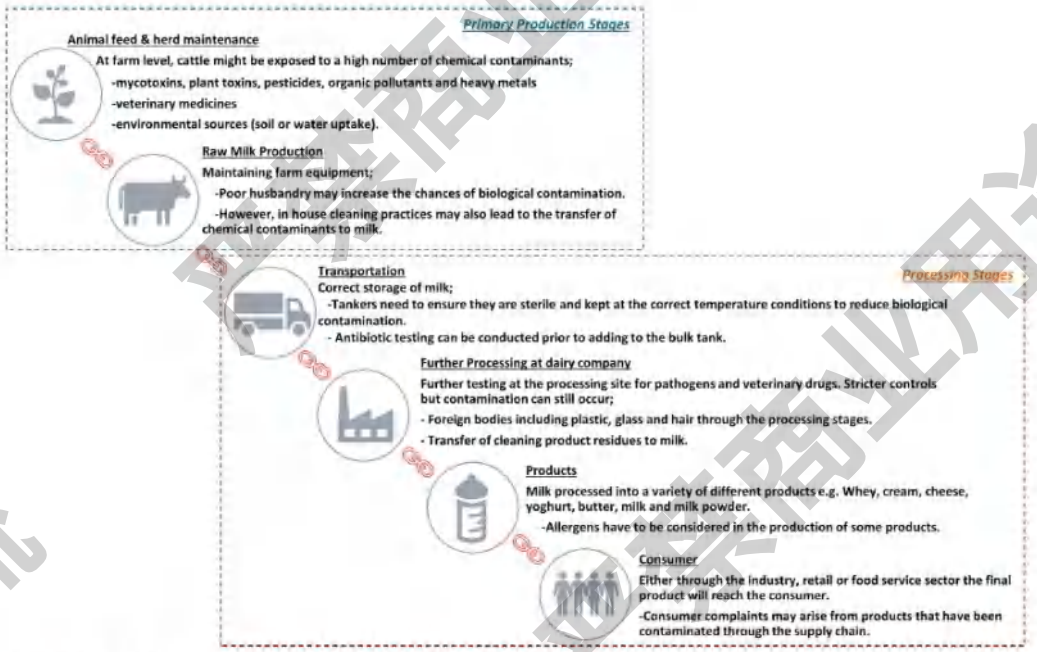
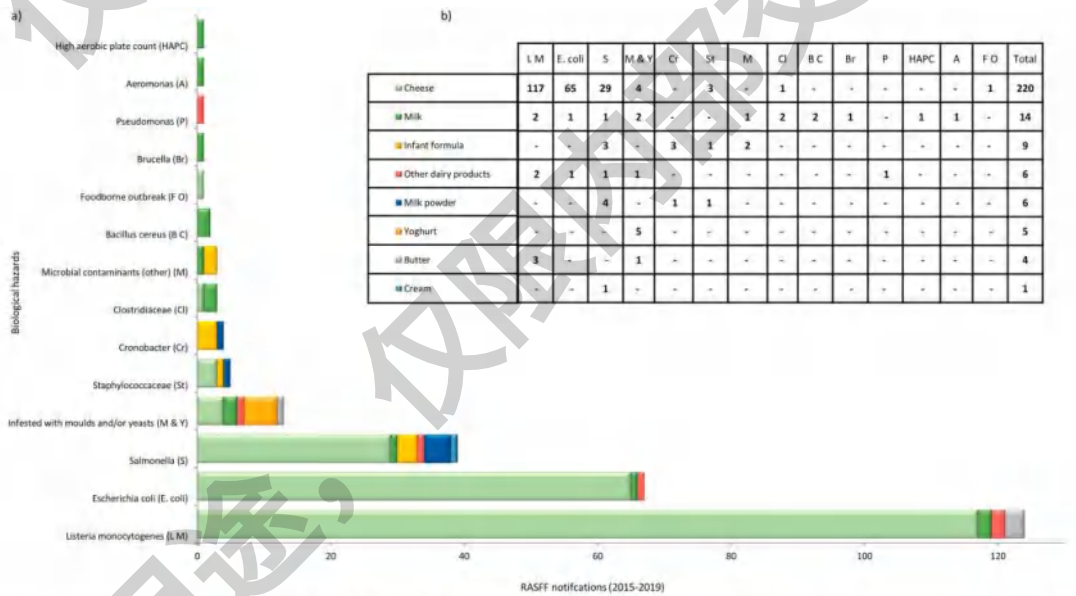


Fig. 1. Simplified dairy supply chain identifying areas of contamination in the primary and processing stages (based on information detailed in van Asselt et al., 2017).

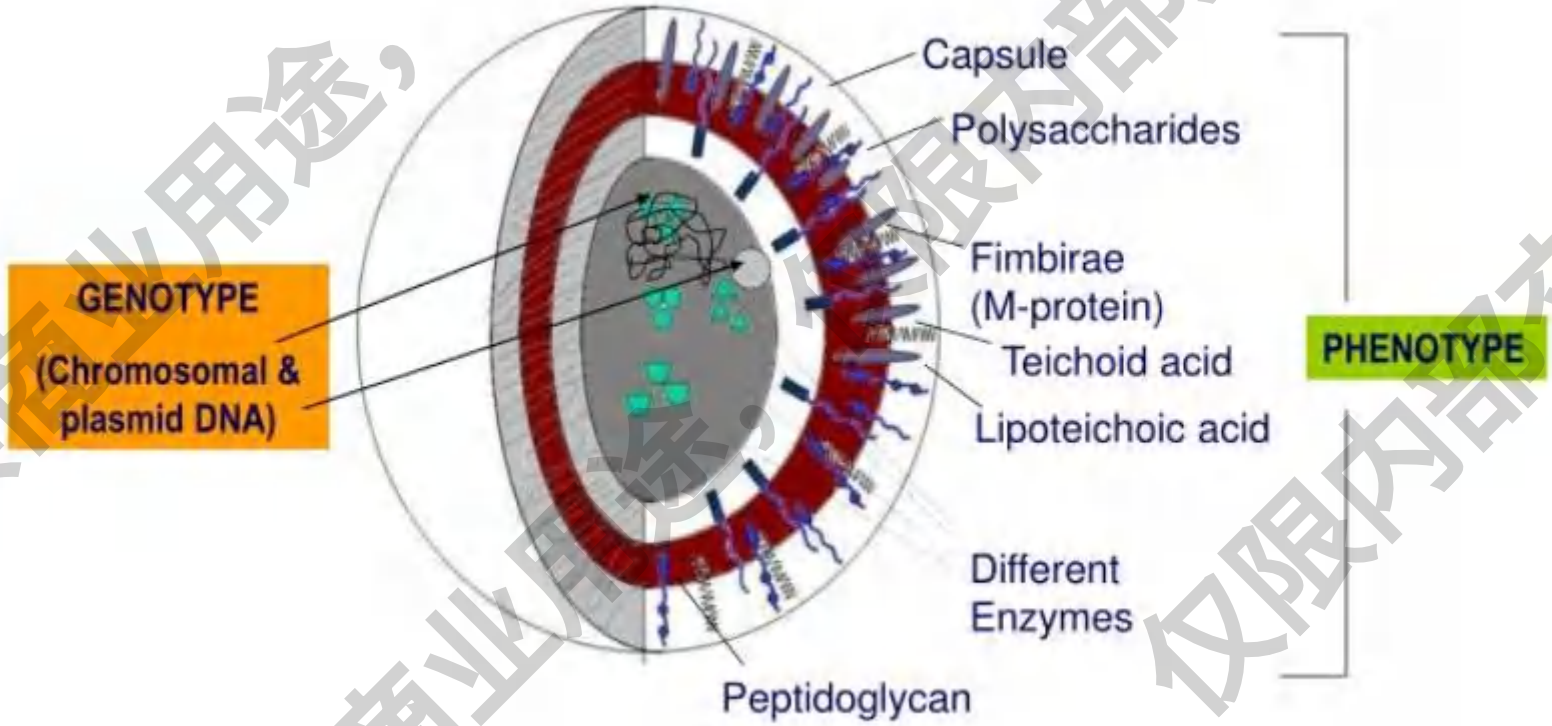


	L M	E. coli	S	M & Y	Cr	St	M	Cl	B C	Br	P	HAPC	A	F O	Total
Cheese	117	65	29	4	-	3	-	1	-	-	-	-	-	1	220
Milk	2	1	1	2	-	-	1	2	2	1	-	1	1	-	14
Infant formula	-	-	3	-	3	1	2	-	-	-	-	-	-	-	9
Other dairy products	2	1	1	1	-	-	-	-	-	-	1	-	-	-	6
Milk powder	-	-	4	-	1	1	-	-	-	-	-	-	-	-	6
Yoghurt	-	-	-	5	-	-	-	-	-	-	-	-	-	-	5
Butter	3	-	-	1	-	-	-	-	-	-	-	-	-	-	4
Cream	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1

Fig. 2. Biological hazards associated with milk and milk products from RASFF from 2015 to 2019, a) Bar chart showing the total notifications for each hazard and b) cross-referencing table showing the commodities and hazards.

https://doi.org/10.1016/j.gfs.2020.100447

微生物分型方法



微生物分型方法

	Typing System	Typeability	Reproducibility	Discrimination	Ease of interpretation	Ease of performance
Phenotype	Serotyping	Most	Good	Fair	Good	Fair
	Phage typing	Most	Fair	Fair	Fair	Poor
	Antibiotic susceptibility	All	Fair	Poor	Excellent	Excellent
	MLEE	All	Excellent	Good	Excellent	Good
Genotype	REA	All	Good	Good	Poor	Excellent
	Ribotyping	All	Excellent	Fair	Good	Good
	PFGE	All	Good	Good	Excellent	Fair
	Restriction digests of PCR Products	All	Excellent	Good	Excellent	Good
	RCR based on repeated sequences	All	Good	Good	Good	Good
	RAPD	All	Fair	Good	Fair	Good
	Sequencing	All	Excellent	Excellent	Excellent	Fair

WGS测序技术用于微生物基因分型的优势

沙门有2000多个血清型，但是基于血清型的分类并不能代表所有的菌株特异性

基于测序技术的分子分型方法			
基于Sanger测序技术		基于WGS测序技术	
Legacy MLST	rMLST	cgMLST	wgMLST
7 Loci	51 Loci	3,002 Loci	21,065 Loci
Conserved housekeeping genes	Ribosomal genes	A soft subset of core genes from wgMLST	All pan-genomic coding sequences from 537 completed/ representative genomes
Conserved low resolution	Highly conserved medium resolution	Variable high resolution	Ultravariable extreme resolution
Different scheme for each species	Single scheme across tree of life	Different scheme for each genus	Different scheme for each genus
STs: 3,929 eBGs: 360	rSTs: 5,454 reBGs: 337	cgSTs: 96,108	wgSTs: 112,409

Note: The composition of 334 reBGs is consistent with the composition of eBGs. Three reBGs exist that do not correspond to one or more eBGs.

https://doi.org/10.1371/journal.pgen.1007261.t001

WGS测序技术用于微生物基因分型的优势

01

准确性、灵敏度高

- 区分污染源
- 确定那种成分最初被污染、缩小调查范围
- 确定意外媒介

02

数据可共享

- 提升不同实验室、不同工厂的应对经验

03

数据可重复、重现

- 不同实验室的数据具有可比性

04

可对菌株特征分析

- 抗生素耐药性
- 毒力分析
- 对清洁剂的敏感性分析
- 黏附能力分析等

应用案例

完整案例：

1、2016年法国一家芝士工厂在芝士中检测到低含量（<10 CFU/g）的李斯特菌

2、当时，食物中发现的李斯特菌cgMLST分析并没有在人的分离培养物中检测到相同的菌株

3、3个月后22个人分离培养物检测出与芝士工厂李斯特菌相同cgMLST的菌株

4、一对一的研究，确定了病例与食用芝士的关联性

5、进一步的食物分析，确定了被污染的芝士是这次事件的源头

6、同期PFGE的分析灵敏度低于cgMLST,确定了30例人的分离培养物与芝士的关联性

结论：

1、WGS应替代PFGE用于李斯特菌的分子分型

2、通过这项研究，2017年开始，在法国cgMLST正式取代PFGE用于李斯特菌的监控

Food Testing

In May 2016, an investigation was conducted on a specific cheese type from a major cheese producer in France and identified low-level (<10 CFU/g) *L. monocytogenes* contamination of the final product. The firm issued a nationwide withdrawal of 30 tons of cheese, but no recall was issued according to national regulatory criteria. Food isolates were sequenced at the NRCL and did not have the same CT as any human isolate at that time. In the following 3 months, consistent with the 3-month shelf-life of the implicated product, 22 human isolates had the same CT (L1-SL1-ST1-CT2056; FR083 cluster-alert) (Table 2) and were identified nationwide. Case–case analysis confirmed a significant association between illness and consumption of the implicated product ($p = 0.00001$). Subsequent food testing confirmed that the implicated cheese was the source for this outbreak. During the same period, PFGE identified 30 human isolates that matched food strains (IVb-011001–160602). However, with cgMLST typing, the analysis was restricted to isolates that were closely related genetically, not just to those that were of the same PFGE profile. This analysis enabled the statistical association to be defined more precisely. This retrospective outbreak was the largest identified in France since 2000.

DOI:10.3201/eid2309.170336

应用案例

Review

Whole genome sequencing uses for foodborne contamination and compliance: Discovery of an emerging contamination event in an ice cream facility using whole genome sequencing

Marc W. Allard*, Errol Strain, Hugh Rand, David Melka, William A. Correll, Leslie Hintz, Eric Stevens, Ruth Timme, Sara Lomonaco, Yi Chen, Steven M. Musser, Eric W. Brown

US Food and Drug Administration, 5001 Campus Drive, College Park, MD, USA

Fig. 1. Phylogenetic results of *Listeria* clustering environmental isolates, NCBI Pathogen detection cluster PDS000004821 (as of date of April 23, 2019). The number line corresponds to SNPs, where the branch length is proportional to the number of SNPs present. Detailed SNP differences also are available at the NCBI Pathogen

此案例表明，**低水平的散发性单核细胞增生性李斯特菌污染可诱发疾病**，通过WGS，可预测共同的来源和根本原因

高通量测序技术的应用使得FDA和冰激淋公司的行动有据可依，提前避免了更大规模的公共卫生事件

体现了各监管体系的数据共享、联合行动的优势

美国食品和药物管理局目前每年检查大约800个工厂，每次检查时收集200个环境拭子，共计160000个样本。通过这一努力，大约四分之一的公司可检测到阳性食源性病原体分离物，所有这些分离物都被测序并上传到**GenomeTrakr**数据库和NCBI病原体检测中，并在那里公开共享，包括与**PulseNet**和**USDA FSIS**以及全球公共卫生实验室共享。根据观察到的系统发育聚类，FDA可通过进一步检查、沟通和咨询来协调公共卫生响应

应用案例

雀巢工厂开始使用WGS技术用于污染菌鉴定和溯源，并发表应用实践文章

Nestlé. 优质食品，美好生活

T2-02 Practical Application of Whole Genome Sequencing for *Listeria monocytogenes* Source Tracking in the Food Industry

Wednesday, 29 March 2017: 14:15
314-316 (The Square)

Katia Rouzeau-Szynalski, Nestec Ltd- Nestle Research Center, Lausanne, Switzerland
Caroline Barretto, Nestec Ltd- Nestle Research Center, Lausanne, Switzerland
Catherine Ngom-Bru, Nestec Ltd- Nestle Research Center, Lausanne, Switzerland
Coralie Fournier, Nestle Institute of Health Sciences SA, Lausanne, Switzerland
Johan Gimonet, Nestec Ltd- Nestle Research Center, Lausanne, Switzerland
Leen Baert, Nestec Ltd- Nestle Research Center, Lausanne, Switzerland

Introduction: The utility of whole genome sequencing (WGS) for source tracking was assessed by investigating an environmental factory contamination.

Purpose: The study was conducted in order to analyze 41 *Listeria monocytogenes* factory isolates by WGS.

Methods: DNA was extracted and sequenced on an Illumina MiSeq platform. In parallel, selected isolates were sequenced with PacBio to create reference genomes. Single Nucleotide Polymorphism (SNP) calling, based on raw read mapping on a closely related reference, using the CFSAN-FDA pipeline, was performed to obtain the pairwise SNP distance matrix. The closest public available sequence data was included in the analysis to understand the biological context.

Results: From the 41 isolates, 30 isolates were grouped in 4 clusters (< 20 SNPs within one cluster) and 11 isolates could not be grouped to any of the defined clusters. One cluster consisted of 22 isolates where the other 3 clusters consisted of 2 to 3 isolates. The largest cluster consisted of isolates from and around a drain in one area of the factory. The drain was suspected of being the source of contamination by the factory quality team. The WGS results confirmed the suspicion of a drain as a source of contamination. Within this cluster, three isolates were found in other places in the factory, identifying a cross-contamination from the drain. The results showed that no raw material was linked to the drain contamination.

Significance: WGS was able to confirm the cause of contamination in the factory, which was suspected to be due to a "resident" strain with no links to recent raw materials. The application of WGS for source tracking showed to be promising; although, today, it is still a research tool, since bioinformatics pipelines evolve very quickly and the knowledge of the biological interpretation from SNP differences, in function of time and place, between isolates is scarce.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 14 March 2018
doi: 10.3389/fmicb.2018.00446

A Validation Approach of an End-to-End Whole Genome Sequencing Workflow for Source Tracking of *Listeria monocytogenes* and *Salmonella enterica*

Anne-Catherine Portmann[1], Coralie Fournier[2], Johan Gimonet[1], Catherine Ngom-Bru[1], Caroline Barretto[1] and Leen Baert[1]

[1] Nestec Ltd., Nestle Research Center, Lausanne, Switzerland, [2] Nestle Institute of Health Sciences SA, EPFL Innovation Park, Lausanne, Switzerland

- 通过WGS测序技术，分析了工厂环境中分离的41株李斯特菌
- 最大的一个cluster有菌株来自用水管道，这一证据确定了之前QC部门的怀疑
- 同一cluster有三株菌来自工厂其它环境，表明存在交叉感染
- 没有在原材料中发现与水管道中相关的菌株，有可能是管道中的常驻菌

标准法规

ICS › 07 › 07.100 › 07.100.30

ISO 23418:2022

Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance

GENERAL INFORMATION

Status : Published **Publication date :** 2022-06

Edition : 1 **Number of pages :** 45

Technical Committee : ISO/TC 34/SC 9 Microbiology

ICS : 07.100.30 Food microbiology

This document specifies the minimum requirements for generating and analysing whole genome sequencing (WGS) data of bacteria obtained from the food chain.

a) handling of bacterial cultures;

b) axenic genomic DNA isolation;

c) library preparation, sequencing, and assessment of raw DNA sequence read quality and storage;

d) bioinformatics analysis for determining genetic relatedness, genetic content and predicting phenotype, and bioinformatics pipeline validation;

e) metadata capture and sequence repository deposition;

f) validation of the end-to-end WGS workflow (fit for purpose for intended application).

国外市场现状——监管部门

USDA Food Safety and Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

- 美国食品安全署，美农业部内设负责食品安全的机构，依照美联邦肉类、禽肉、蛋制品检验法授权，负责监督管理美国产和进口的肉、禽肉和蛋制品的安全卫生。

2018年FIFS update

FSIS to Discontinue PFGE and Move to WGS for *Campylobacter* Isolates

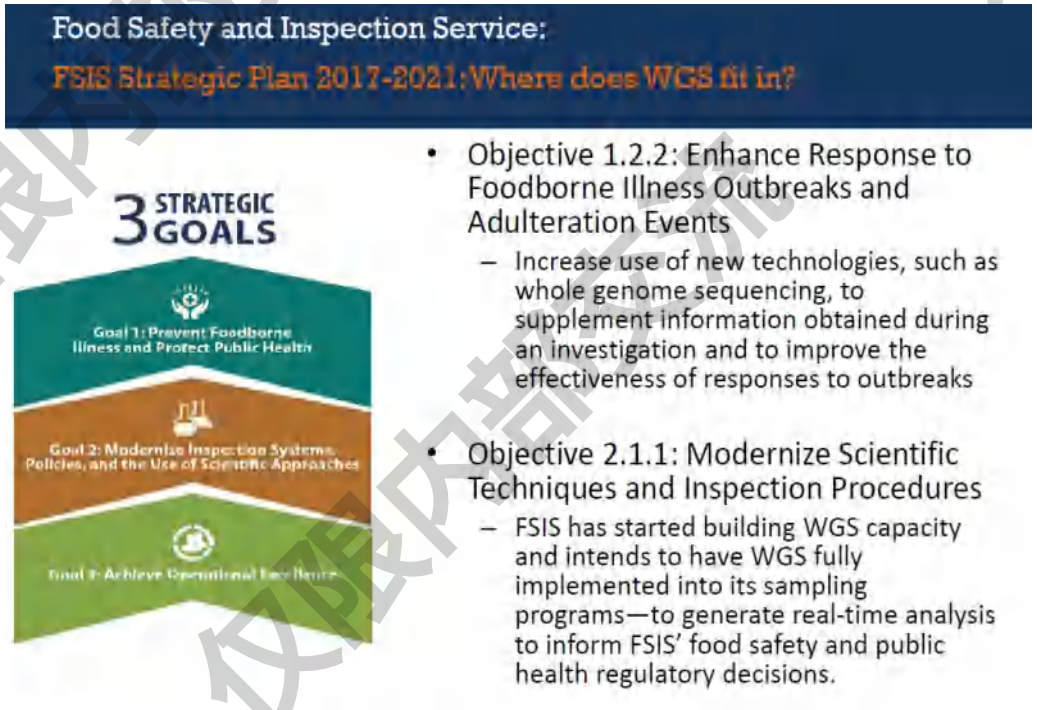
FSIS has been performing pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) in parallel for all bacterial pathogens isolated from FSIS testing programs since fiscal year 2017. Through this issuance, FSIS is announcing the suspension of PFGE analyses for *Campylobacter* isolates and transitioning to use WGS for isolate characterization. The suspension of PFGE for *Campylobacter* isolates will occur 30 days from this publication. The action is being taken in coordination with federal and public health partners in the Centers for Disease Control and Prevention (CDC) PulseNet network and takes into account the progress made in deploying the WGS capacity across state and federal partners.

2019年FIFS update

FSIS Transitions to Using WGS Data from *Salmonella* Isolates to Determine Serotype

FSIS is announcing a modernization of laboratory methods to determine serotype of *Salmonella* isolates. For all *Salmonella* positive samples collected after January 1, 2020, whole genome sequencing (WGS) data will be used to determine *Salmonella* serotype.

- 使用全基因组测序（WGS）作为肉类和家禽中沙门氏菌，弯曲杆菌和大肠杆菌的主要表征工具；



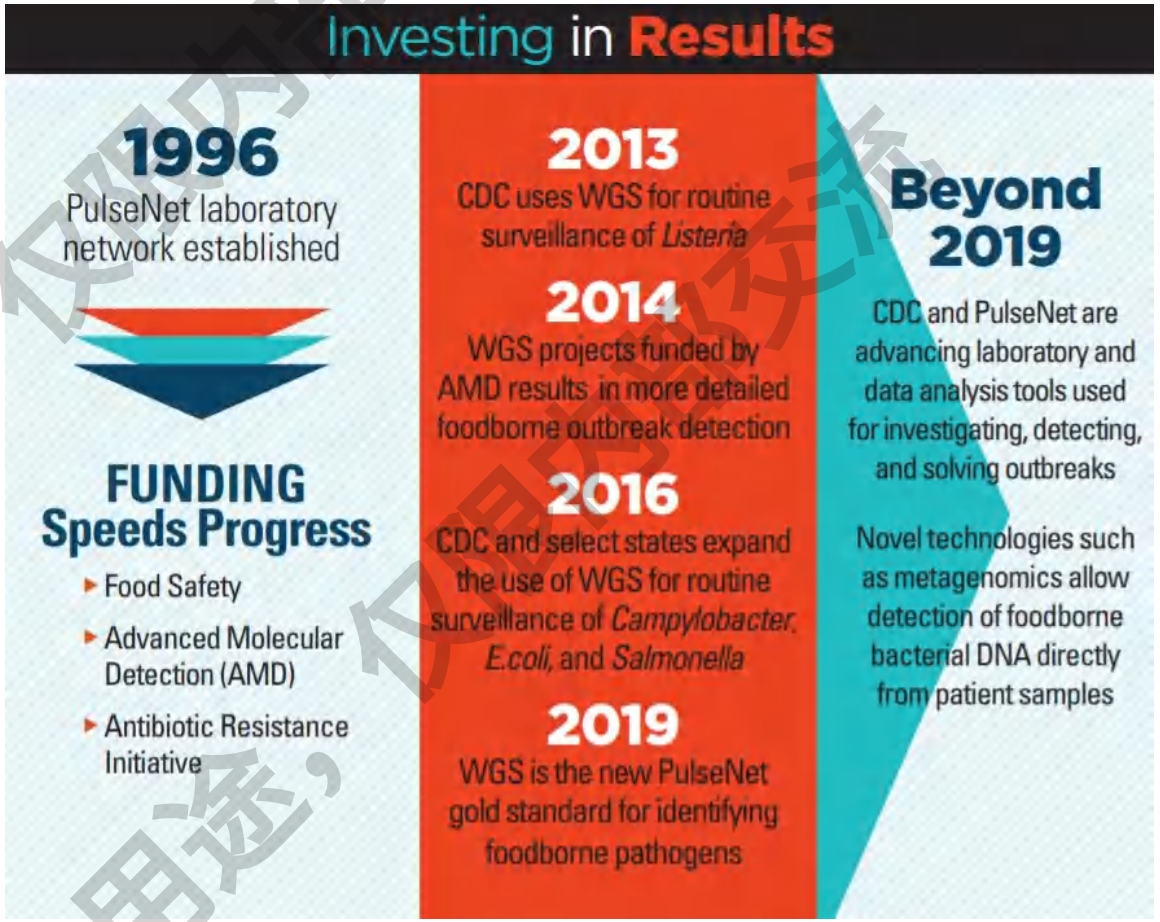
https://www.fsis.usda.gov/

国外市场现状——监管部门

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

About PulseNet

PulseNet is a national laboratory network that connects foodborne, waterborne, and One Health–related illness cases to detect outbreaks. PulseNet uses the DNA fingerprints of bacteria making people sick to detect thousands of local and multistate outbreaks. Since the network began in 1996, PulseNet has improved food safety systems through identifying outbreaks early. This allows investigators to find the source, alert the public sooner, and identify gaps in food safety systems that would not otherwise be recognized. PulseNet International performs a similar role for foodborne illnesses globally.



国外市场现状——监管部门

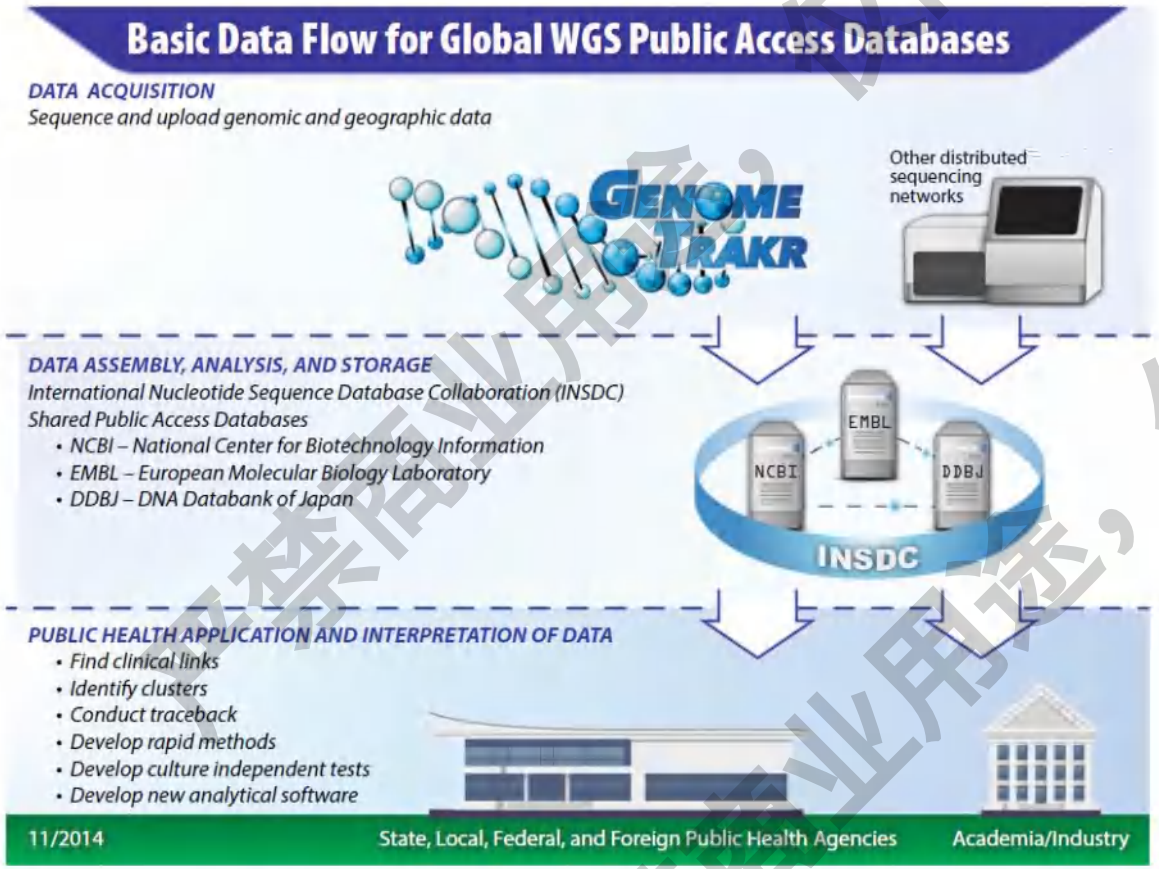
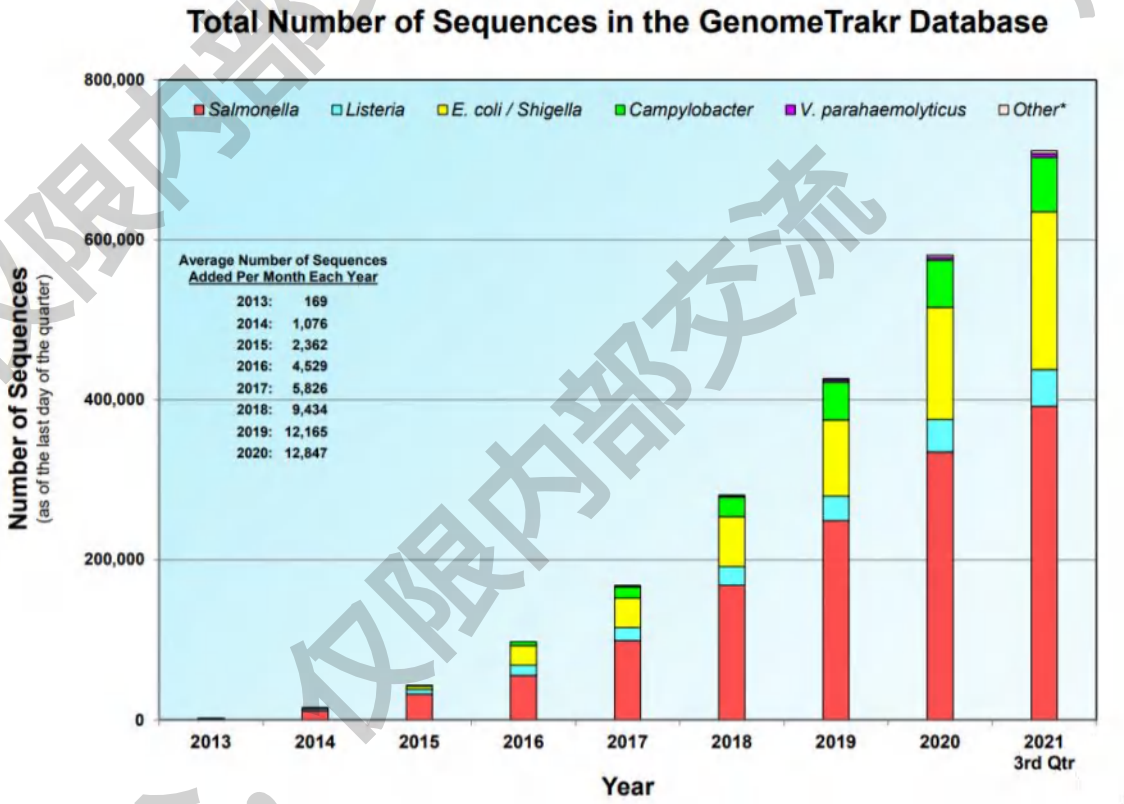


Fig. 1 GenomeTrakr data flow overview



国内市场现状——监管部门

国家食品安全风险评估中心
China National Center for Food Safety Risk Assessment

国家食品安全风险评估中心简介

国家食品安全风险评估中心（以下简称食品评估中心），成立于2011年10月13日，是经中央机构编制委员会办公室批准，直属于国家卫生健康委员会的公共卫生事业单位。

成立食品评估中心是党中央、国务院加强食品安全工作的重要举措，也是深入贯彻落实《食品安全法》，有效提升我国食品安全风险管理科学水平的重要基础建设。

信息窗

中国首个基于全基因组测序技术的食源性疾病分子溯源网络建成并投入使用

食源性疾病是全球范围内重要的食品安全问题，早期发现和查明病因是防控食源性疾病的重要保证。随着新一代基因组测序技术的发展，基于全基因组测序（WGS）的分子分型技术在食源性疾病聚集性病例识别和暴发溯源调查中已显示出极大的应用价值和发展潜力，逐渐成为国际研究热点，欧美相关国家已相继开展研究和布局。

在国家重点研发计划"食品安全关键技术研发"重点专项的支持下，国家食品安全风险评估中心与中国农业大学和北京中科助腾科技有限公司合作，以国家食源性疾病分子溯源网络（TraNet）为基础，首次建成了基于WGS分型技术的新型食源性疾病分子溯源网络，是我国首个实现国家、省、市三级实际应用的分子溯源网络。目前网络已经在泰国肠炎沙门氏菌暴发病例的跨省溯源、冷冻饮品中单核细胞增生李斯特氏菌的跨省追踪等事件调查中得到成功应用。网络的建成和运行，为中国食源性疾病暴发的快速调查和精准溯源提供了技术支撑。

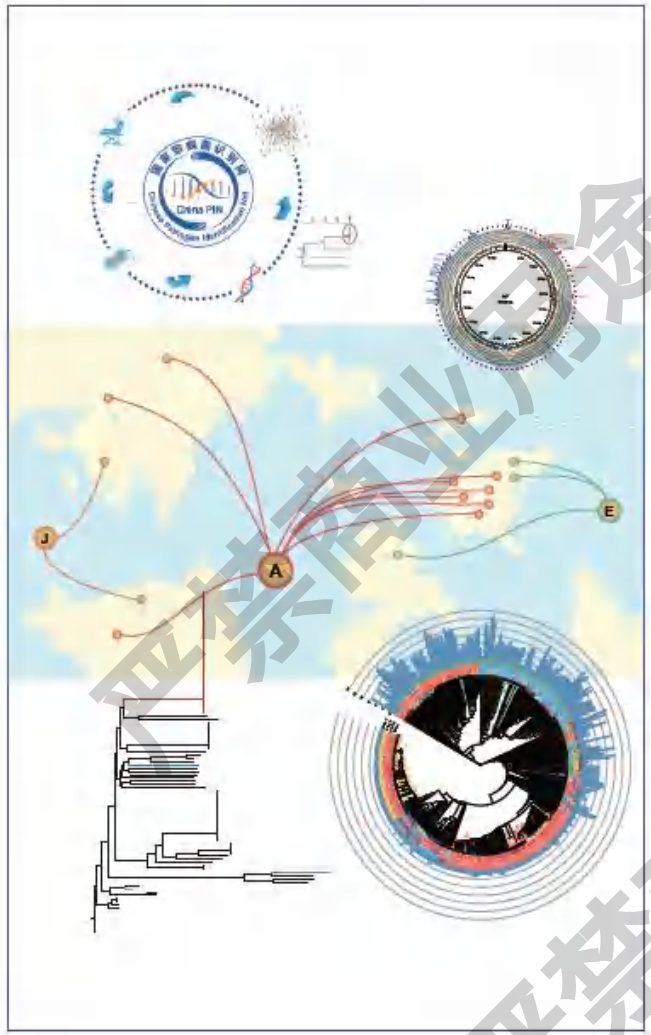
（来源：科技部）

2015年国家食品安全风险评估中心启动了《国家食源性致病微生物全基因组测序数据库》的构建工作，以应用于病因食品的快速准确识别，确保在技术上的国际话语权。

在国家重点研发计划“食品安全关键技术研发”重点专项的支持下，国家食品安全风险评估中心与中国农业大学和北京中科助腾科技有限公司合作，以国家食源性疾病分子溯源网络（TraNet）为基础，**首次建成了基于WGS分型技术的新型食源性疾病分子溯源网络，是我国首个实现国家、省、市三级实际应用的分子溯源网络**

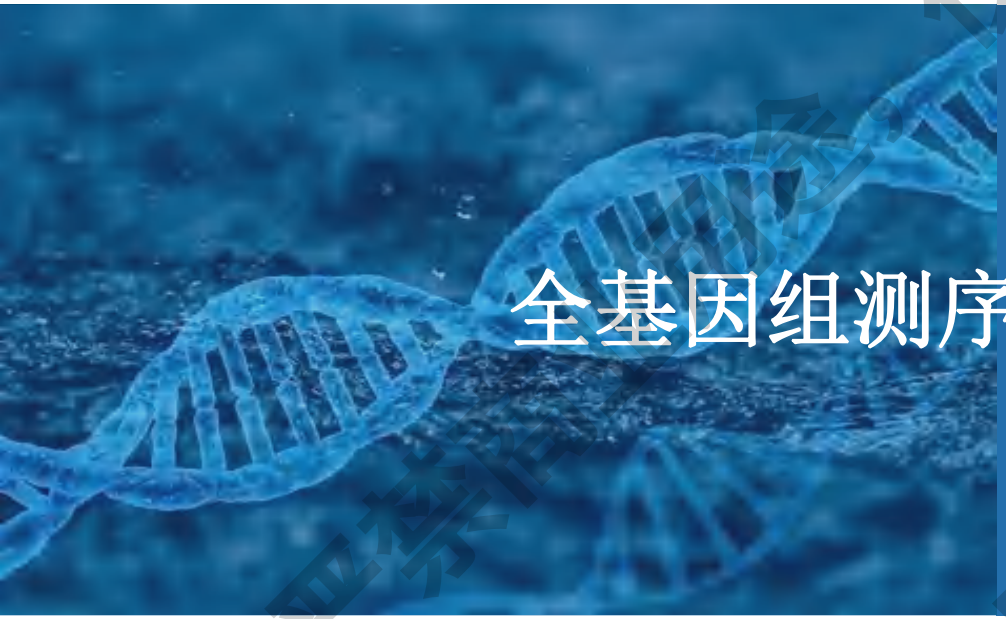
目前网络已经在泰国肠炎沙门氏菌暴发病例的跨省溯源、冷冻饮品中单核细胞增生李斯特氏菌的跨省追踪等事件调查中得到成功应用。

国内市场现状——监管部门



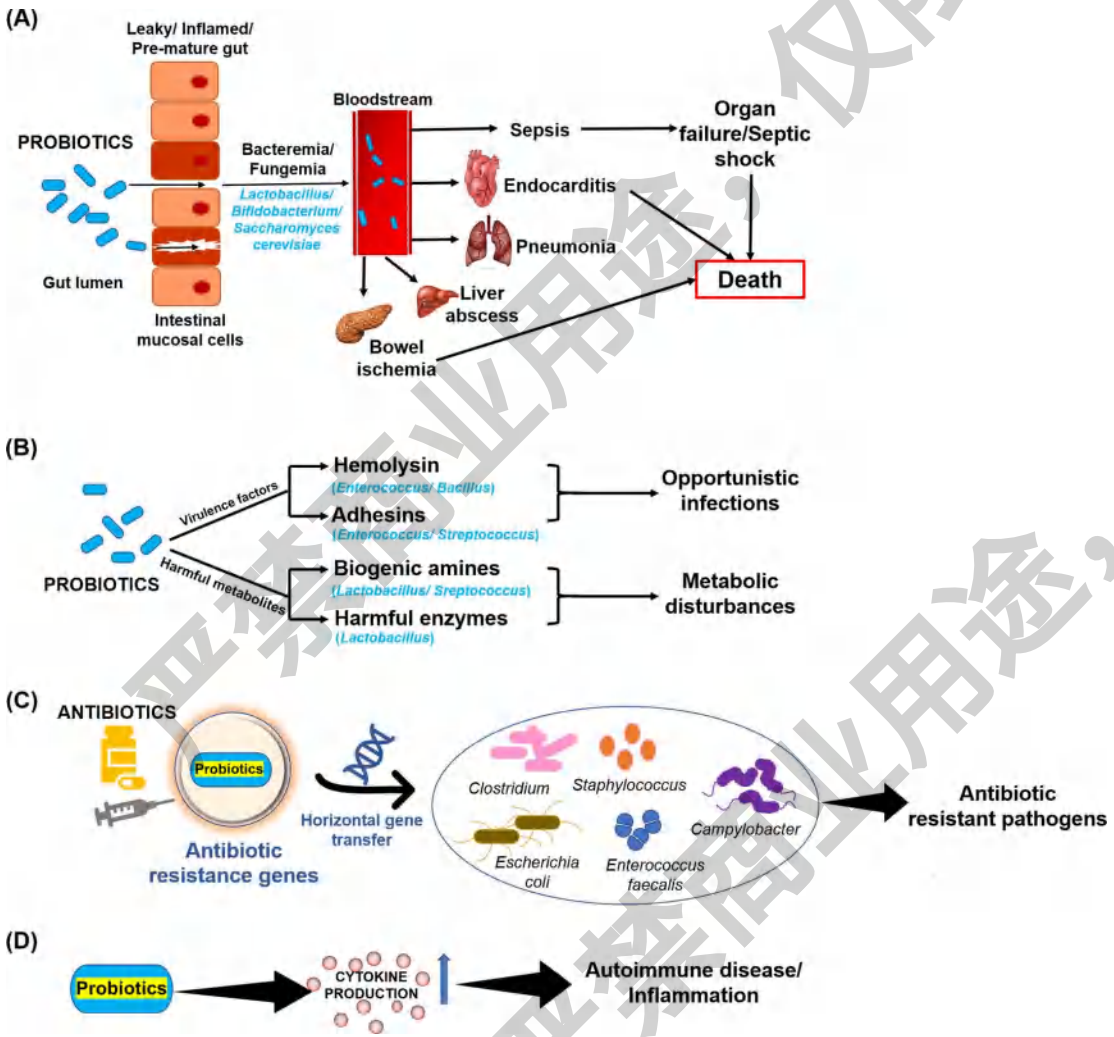
2017年，国家致病菌识别网（Chinese Pathogen Identification Net, 简称China Pin）建立。依托国家、省、市、县四级疾控中心的实验室，通过收集病原菌的基因数据，建立以实验室为基础、基因数据和基础流行病学数据为支撑的“实验数据互联网”模式，来实现细菌性传染病病原检测鉴定、病原和流行特征分析、暴发疫情早期发现、病原学监测预警、溯源及风险评估等。

病原微生物全基因组测序技术、宏基因组测序技术在病原体识别、分子分型、耐药监测、溯源分析等方面发挥了重要的作用。在国家致病菌识别网的介绍文章中，提到“这些数据通过网络信息系统上传至省、市和地市级实验室的中心数据库中，用于建立各级实验室病原菌比较分析和追溯数据库。结合流行病学数据和实验室数据，网络实验室可对病原体分型聚类进行检测，并提出可能的聚集病例建议，为开展流行病学调查、发现潜在的疫情以及疫情的溯源提供数据支持，为细菌性传染病的监测和防控奠定坚实的技术基础。”



全基因组测序技术用于益生菌安全性评估

乳制品中益生菌安全性评估的重要性



(A)肠道完整性受损会导致益生菌易位：在渗漏、发炎或未成熟的肠道中，益生菌（酿酒酵母、乳酸杆菌、，双歧杆菌）可能穿过肠粘膜进入血液或重要器官，引起全身或局部感染

(B)毒力因子和益生菌的有害代谢产物可能分别导致机会性感染和代谢紊乱

(C)抗生素耐药基因从益生菌水平转移到肠道病原菌，反之亦然，可能导致抗生素耐药病原体的发展

(D)通过诱导细胞因子产生的过度免疫反应可能导致自身免疫性疾病或炎症。

https://doi.org/10.1016/j.biopha.2018.12.

乳制品中益生菌安全性评估的重要性

Table 2
Risks associated with rampant dietary intake of probiotics.

Category	Microorganisms	Population	Mechanisms	References
Transmissible antibiotic resistance genes	*Lactobacillus, Pediococcus* and *Leuconostoc* sp.	–	Vancomycin resistance → *Staphylococcus aureus*	[109]
	Lactobacillus sp.	–	Vancomycin resistance → *Enterococcus faecalis*	[110]
	Lactobacillus sp.	–	Broad spectrum antibiotic resistance (vancomycin, streptomycin, gentamicin, aztreonam, and ciprofloxacin) → *Staphylococcus* sp.	[26,111]
Systemic infection	*Lactobacillus rhamnosus* GG	Pre-term infant with short gut syndrome	*Lactobacillus* bacteremia	[112]
	L. rhamnosus GG	Critically ill children with antibiotic related diarrhea	Sepsis	[113]
	L. rhamnosus GG	11-month old infant with short gut syndrome	*Lactobacillus* bacteremia	[114]
	L. rhamnosus GG	17-year-old boy with ulcerative colitis	*Lactobacillus* bacteremia	[115]
	Lactobacillus sp.	58-year-old immunocompetent with mechanical ventilation	*Lactobacillus* bacteremia and sepsis	[116]
	Three strains of *L. rhamnosus*	24-year-old female cardiosurgical patient	Probiotic sepsis	[29]
	Bifidobacterium longum	74-year-old man with polymetastatic prostatic adenocarcinoma	*Bifidobacterium* bacteremia	[117]
	B. longum subspecies infantis	Pre-term infants	*Bifidobacterium* bacteremia	[118]
	B. longum	Low birth-weight infants	*Bifidobacterium* bacteremia	[119]
	B. breve	2-year-old boy with Philadelphia chromosome-positive acute B-cell lymphoblastic leukemia	*Bifidobacterium* sepsis	[120]
	E. coli NISSLE strain 1917	Pre-term infants	Severe sepsis	[41]
	Saccharomyces cerevisiae	48-year-old diabetic with multiple co-morbidities	Multiple organ failure and septic shock in association with toxic megacolon and probiotic fungemia	[121]
	S. cerevisiae var. *boulardii*	Immunocompromised 73-year-old patient on chemotherapy	Fungemia	[25]
	S. cerevisiae var. *boulardii*	8-year-old boy with respiratory distress (Intensive care unit patient)	Fungemia	[122]
	S. cerevisiae var. *boulardii*	Premature neonate receiving nutrition enterally	Fungal septicemia	[123]
	S. cerevisiae var. *boulardii*	Critically ill patients	Fungemia	[82]
	L. plantarum	30-year-old male with rheumatic valve disease	Endocarditis	[124]
	L. casei	53-year-old immunocompetent patient	Endocarditis	[125]
	L. jensenii	47-year-old immunocompetent patient	Endocarditis	[126]
	L. paracasei	77-year-old male patient with prostate cancer	Endocarditis	[127]
	L. acidophilus	48-year-old male with heart disease and dental manipulations	Endocarditis	[45]
	L. rhamnosus	> 65-year-old patient with hemorrhagic telangiectasia (HHT)	Endocarditis	[128]
Localized infection	*L. casei*	60-year-old with renal transplant patient	Intra-abdominal abscess	[129]
	L. paracasei	65-year-old diabetic patient	Bacteremia and liver abscess	[47]
	L. rhamnosus	11-month-old female with trisomy 21 with respiratory viral infection	Probiotic associated pneumonia	[48]
Metabolic disturbances	*Lactobacillus* spp.	–	Unregulated bile salt hydrolase (BSH)' activity	[64]
	L. lactis	–	Biogenic amine production	[63]
Allergic response	*L. rhamnosus* GG	Children (up to 4 years)	Allergic rhinitis and more serious asthma	[68]
	L. acidophilus LAVRI-A1	Infants (6 and 12 months)	Atopic sensitization	[72]
	L. rhamnosus GG	Pregnant (4-6 weeks before expected delivery) and breastfeeding mothers (up to 2 years)	Wheezing bronchitis; atopic sensitization	[130]
Bowel ischemia	*L. acidophilus, L. casei, L. salivarius, L. lactis, B. bifidum, and B. lactis*	Critically ill patients (acute pancreatitis)	Increased local oxygen demand	[55]

https://doi.org/10.1016/j.biopha.2018.12.

乳品中益生菌的评估

- 2020年，国家市场监督管理总局《保健食品原料用菌种安全性检验与评价技术指导原则》

3 对拟评价微生物菌种需提交的基本资料要求

3.1 基本信息

3.2 菌种分类学资料

3.3 菌种鉴定资料

3.4 菌种生长环境条件资料

3.5 诱变菌种

3.6 生产相关信息

3.7 国内外安全性评价资料综述

3.8 其他国家批准资料

3.9 其他需要说明的信息。

4 评价方法

4.1 全基因组测序（Whole Genome Sequencing, WGS）

4.1.1 基因测序

4.1.2 基因序列分析

4.2 动物致病性试验

4.3 产毒试验

附录A 保健食品原料用细菌致病性检验方法

附录B 保健食品原料用丝状真菌的致病性检验方法

附录C 保健食品原料用酵母的致病性检验方法

乳品中益生菌的评估

IDF第462号公报《益生菌株水平鉴定-指导性文件》是国际乳品行业益生菌株水平分型方法权威性指南。

ABSTRACT

Probiotics are specific microbial strains that have a beneficial effect on the health of the host and are defined as "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".

In 2002, a joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food proposed the first guideline for the evaluation of probiotics in food. One of the points was to use accurate taxonomy of the microorganism and identify it at the strain level. Strain identification is expected to be done using a reproducible genetic method or a unique phenotypic trait.

Various typing methods with different technical features, costs and feasibilities are available for this purpose. While Whole Genome Sequencing (WGS) is progressively replacing Pulsed Field Gel Electrophoresis (PFGE) as the gold standard of strain identification in research as well as the clinical, pharmaceutical, food, feed and industrial fields, it is not yet fully accessible to all laboratories with respect to the cost and/or technical expertise required. Depending on the purpose of identification, WGS is not always needed. For example, routine analysis for quality control of probiotic food products requires high throughput techniques within a short time frame.

- 益生菌的益生特性仅在菌株水平表现，而非在菌种或更高水平的分类单元中表现。益生菌的鉴定、功能和安全性评价需要在株水平进行
- WGS数据可以提供全面的数据，用于株水平的鉴定和特征分析。如：功能特征（如，糖利用等）、安全特征（耐药基因、毒力基因等）
- WGS数据可以用来跟踪菌株传代过程中是否存在遗传性漂移

益生菌评估公共数据库

毒力数据库

毒力基因数据库-VFDB（数据全面，更新及时）

毒力/致病岛数据库-PAIDB（主要针对李斯特、大肠、金葡和肠球四类）

生物防御数据库-MvirDB

产毒基因分类

黏附与入侵

分泌系统

毒素

铁摄取等

耐药基因数据库

CARD（数据全面，更新及时）

ResFinder (获得性耐药基因预测)

Argannot

NDARO等

耐药机制

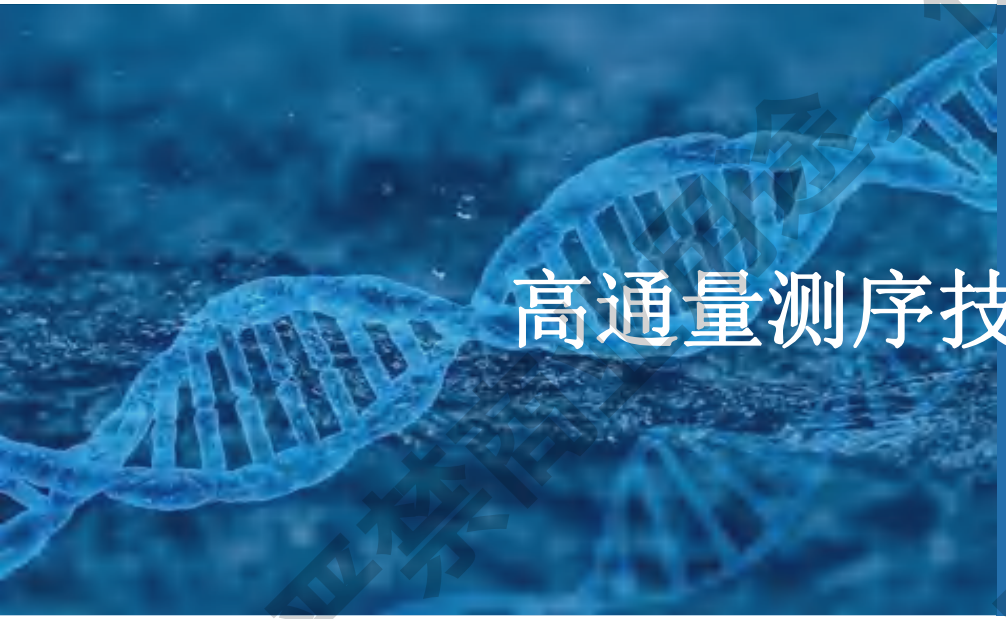
染色体介导的耐药

质粒介导的耐药

抗菌药物作用靶点的改变

产生修饰或降解抗菌的酶

微生物主动外排机制



高通量测序技术在乳品领域应用发展趋势

高通量测序技术对于病原微生物的检测

宏基因组测序

针对样本中所有的病原菌进行测序，获取基因组序列信息菌株鉴定，耐药和毒力基因检测

样本类型：环境样本

检测序列：所有微生物全部核酸序列

优势：无需培养、无需预判，可检测混合感染、快速、通量高、灵敏度高

靶向测序

针对感兴趣的病原体进行测序，获取靶标病原菌的基因组序列菌株鉴定，分型，耐药和毒力基因检测

样本类型：环境样本

检测序列：所有微生物部分核酸序列

优势：无需培养、可检测混合感染、快速、通量高、灵敏度高

全基因组测序

仅针对一种病原进行测序，获取该病原的全基因组序列信息菌株鉴定、分型、溯源、耐药和毒力基因检测

样本类型：培养的纯菌

检测序列：单菌全部核酸序列

优势：通量高、精准鉴定和分型、溯源

应用案例

MICROBIAL GENOMICS

RESEARCH ARTICLE

Grützke *et al.*, *Microbial Genomics* 2021;7:000552

DOI 10.1099/mgen.0.000552

Direct identification and molecular characterization of zoonotic hazards in raw milk by metagenomics using *Brucella* as a model pathogen

Josephine Grützke[1,*], Mayada Gwida[2], Carlus Deneke[1], Holger Brendebach[1], Michaela Projahn[1], Alexander Schattschneider[1], Dirk Hofreuter[1], Maged El-Ashker[3], Burkhard Malorny[1] and Sascha Al Dahouk[1,4,*]

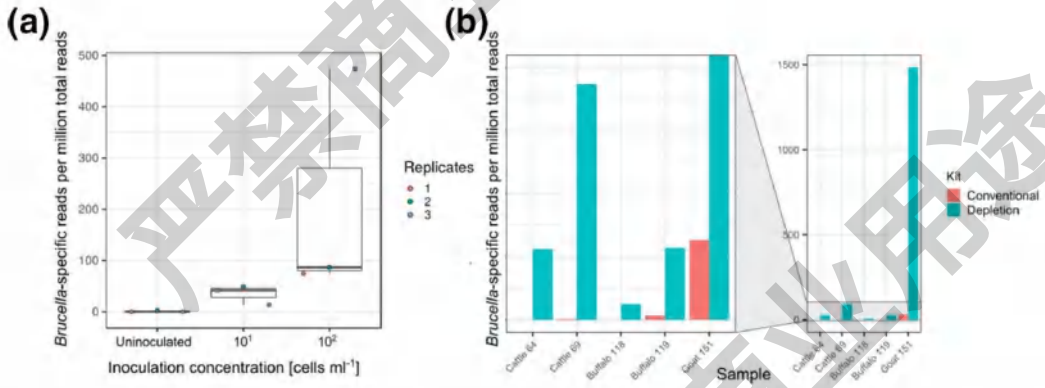
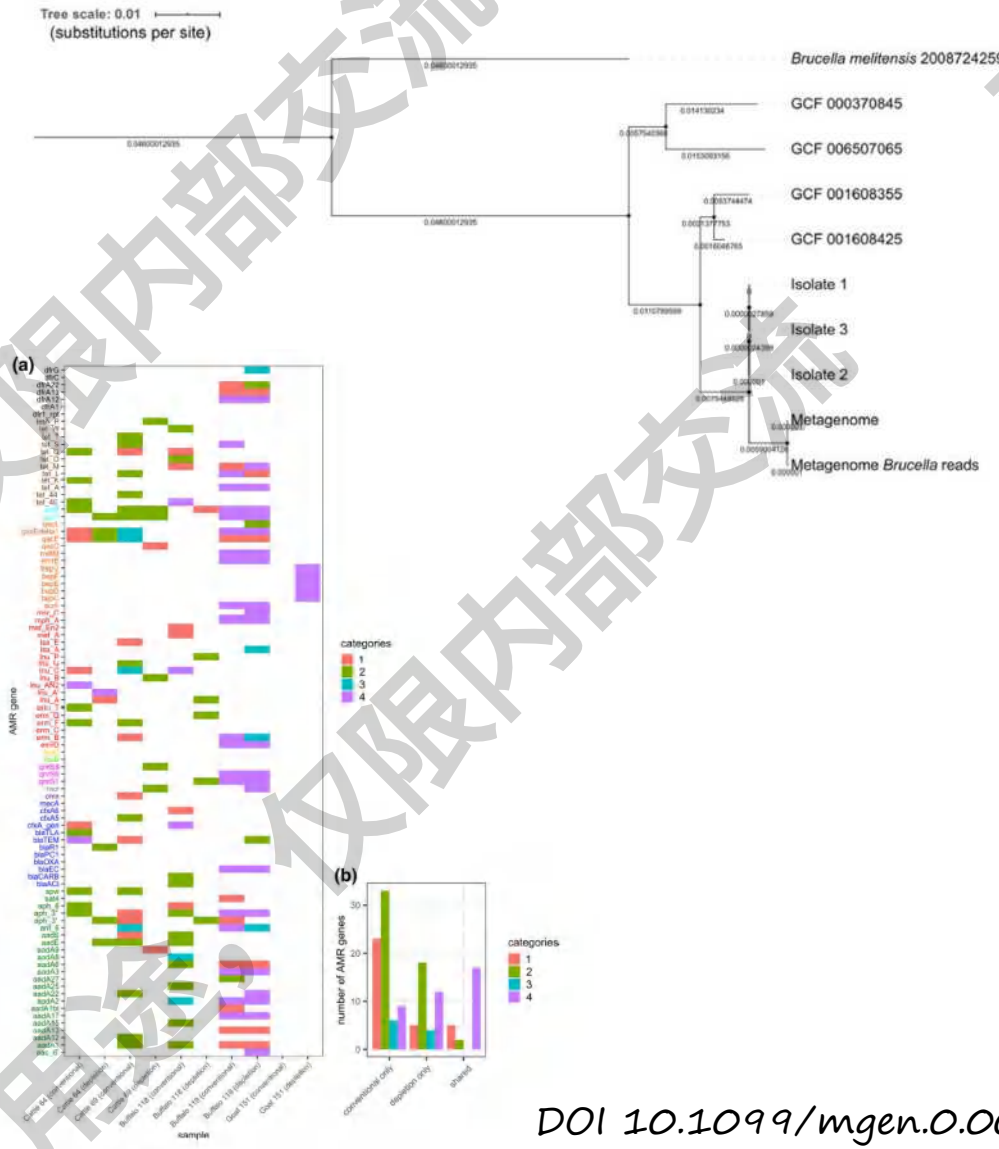
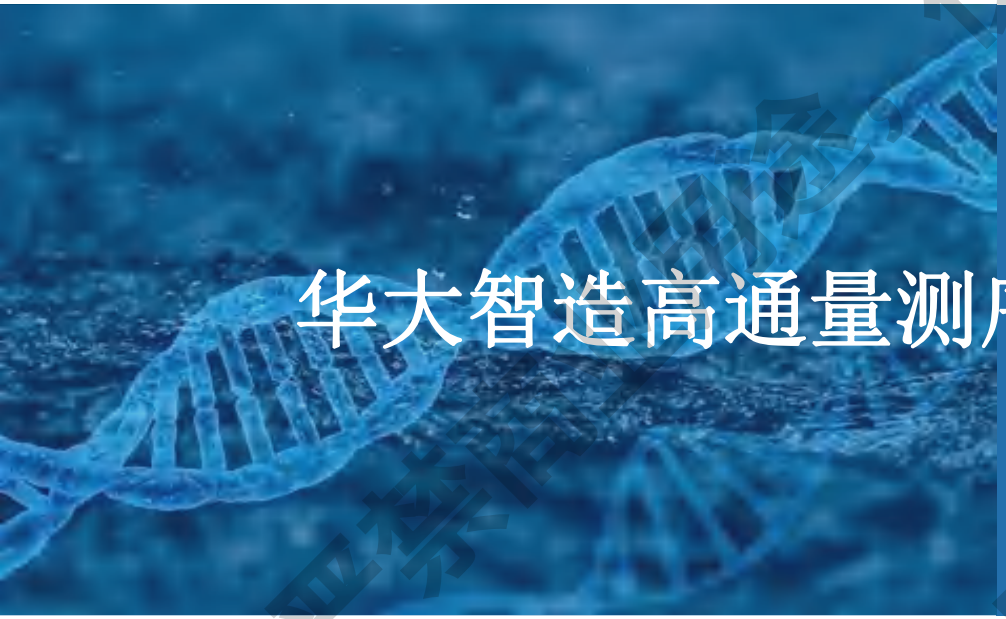


Fig. 1. Eukaryotic cell depletion improves the detection of *Brucella* in raw milk by metagenomic analysis. (a) Detection of *Brucella*-specific reads per million total reads in artificially contaminated raw cow's milk after eukaryotic cell depletion in three replicates. (b) *Brucella*-specific reads per million reads in milk samples from different dairy animals detected after conventional DNA extraction and DNA extraction with eukaryotic cell depletion.



DOI 10.1099/mgen.0.000552



华大智造高通量测序平台及微生物测序组合产品介绍

基于华大生态，聚焦先进制造，推动产学研全面发展

华大智造 MGI

华大生命科学研究院 BGI · Research

文章发布 3335
SCI 2864
GCNNS 501
篇均引文量 90

国家基因库 CNGB

读/写/存
10^{18}
数据存储
10M+
生物知识库

华大基因学院 BGI College

国科大105 MS+PhD
华南理工15 MS
深大20 MS，南科大15 MS
郑州大学10 MS
西北大学25 MS
哥本哈根大学10 PhD
丹麦科技大学5 PhD

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华大股份
科技服务&大数据

华大农业 BGI · Agro

生物智造
未来农业

华大智造推动 科研、产业、民生联动发展

猛犸基金会 Mammoth Foundation

恤病助医
科普教育
跨界交流

华大健康 BGI · Health

数字化人生
99+

华大医疗 BGI · MED

未来医院：
深圳1000个床位

从同步到引领，华大智造不断突破与超越

华大智造 MGI

2013

3月

收购美国Complete Genomics公司，以此打通基因测序上下游产业链

2015

5月

完全集成式“超级测序仪”Revolocity发布

10月

BGISP-100及BGISEQ-500高通量桌面型基因测序仪发布

2016

4月

华大智造正式成立

10月

BGISEQ-50高通量基因测序仪发布

2017

7月

获得ISO13485医疗器械质量管理体系认证

10月

MGISEQ-2000、MGISEQ-200高通量基因测序仪，MGISP-100自动化样本制备系统以及MGIUS-R3远程超声机器人发布

2018

2月

BGISEQ-50与MGIUS-R3揽获德国iF工业设计奖大奖

2019

1月

在第37届J.P.Morgan医疗健康大会上发布全球测序者计划

10月

DNBSEQ E，DNBelab D及DNBelab C系列等产品发布

10月

DNBSEQ-T7超高通量基因测序仪及MGISP-960高通量自动化样本制备系统发布

11月

华大智造拉脱维亚基地正式启用

2020

1月

DNBSEQ-T7测序平台获国家药监局应急审批

8月

MGISTP-7000全自动分杯处理系统发布

2021

1月

超高通量MGISP-NE384全自动核酸提取纯化仪发布

10月

- 全自动核酸提取纯化仪MGISP-NE32发布
- 发布DNBSEQ-T10 x 4RS大人群基因组学一站式平台

- 华大智造投资24亿于武汉建制造基地，预计2022年一期建成投产

蓄势与萌芽 | 突破与追赶 | 同步与超越

三大核心业务板块，全面赋能行业发展

华大智造 MGI

测序仪产品线

DNBelab D系列
模块化数字生物实验室

DNBSEQ E系列
便携式基因测序系统

灵活可定制的
cube(模块化)

MGISEQ-200
桌面型测序仪

MGISEQ-2000
全能型测序仪

DNBSEQ-T7
生产型测序仪

DNBSEQ-Tx
大人群基因组学一站式技术平台

MGIEasy/MGICare
建库试剂

实验室自动化产品线

MGISTP-3000

MGISTP-7000 MGIGlab-S系列

样本前处理

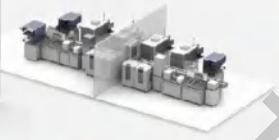
MGISP-NE32

MGISP-NE384

自动化核酸提取

MGISP-100

MGISP-960



MGIGlab-L

自动化移液工作站

MGIEasy

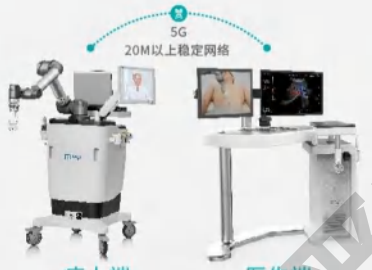
提取试剂

MGIFLP

建库测序一体机

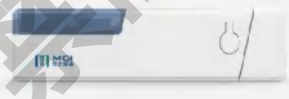
新业务

MGIUS-R3
远程超声机器人



病人端 医生端

DNBelab C4
便携式单细胞系统



BIT产品

移动实验室平台

超低温自动化生物样本库

基于DNBSEQ平台的一站式平台

STEP 1
样本前处理
Library Prep

MGISP-100

MGISP-960

MGIEasy建库试剂

第三方建库试剂

STEP 2
高通量测序
Sequencing

MGISEQ-200

MGISEQ-2000

DNBSEQ-T7

CoolMPS/StandardMPS
测序试剂

MGI ZLIMS

STEP 3
数据分析
Data Processing

MegaBOLT / Ztron

ZMART

PFI / BFI

分析软件（含第三方）

高度自动化
全流程自动化程度高达80-90%
人工干预少，节省重复劳动

高度准确性
建库过程高度自动化
DNBSEQ技术保证数据精准度

高度兼容性
支持第三方文库制备试剂盒
和第三方分析软件

MGI微生物快速识别与组装溯源平台

快速、准确、全面的对环境或临床样本MPS数据进行微生物分类鉴定，耐药基因鉴定。自动生成结果报告，适用于不明原因的传染病的诊断。

面向对象

- 突发疫情爆发
- 常见传感染监控
- 特殊传染案例分析

产品特点

- 流程自动化：对Fastq数据一键式分析，自动生成报告
- 检测类型全：覆盖超过2万种病原，涵盖所有病原类型
- 物种覆盖广：人、常见动物的病原检测均可适用
- 分析功能全：可实现DNA、RNA共检

内置物种数据库

宿主数据库

包括10种以上的宿主物种，人、猪、山羊、绵羊、小鼠等；同时，支持定制化开发。

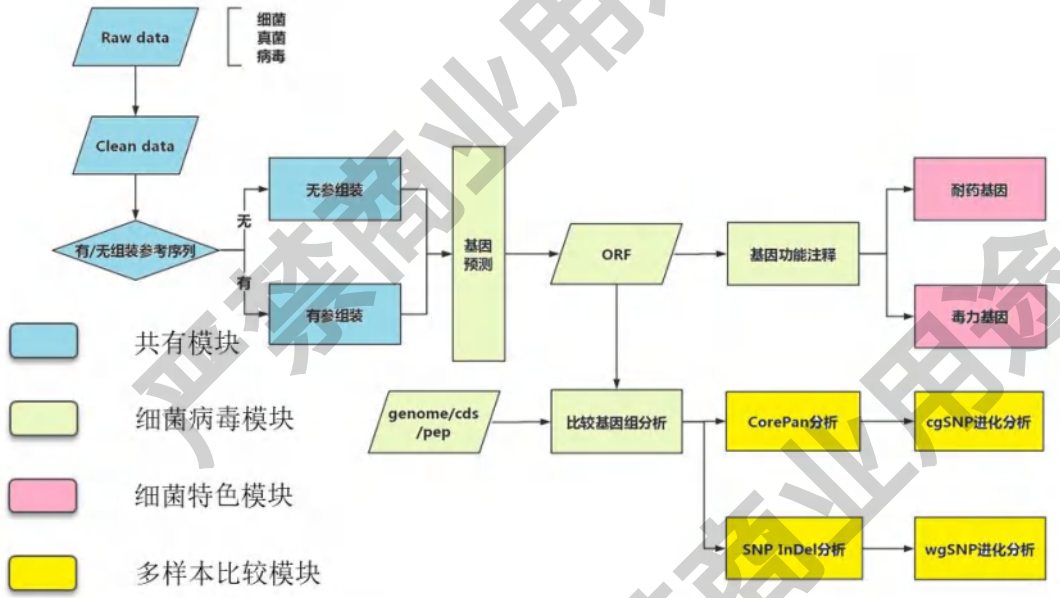
病原数据库

收录超过2.7万种微生物，含新冠病毒序列，支持定期更新。

微生物分类	病毒	真菌	细菌	古生菌	寄生虫
种水平数目	9000+	9400+	8300+	360+	640+
属水平数目	1000+	2600+	1900+	130+	360+

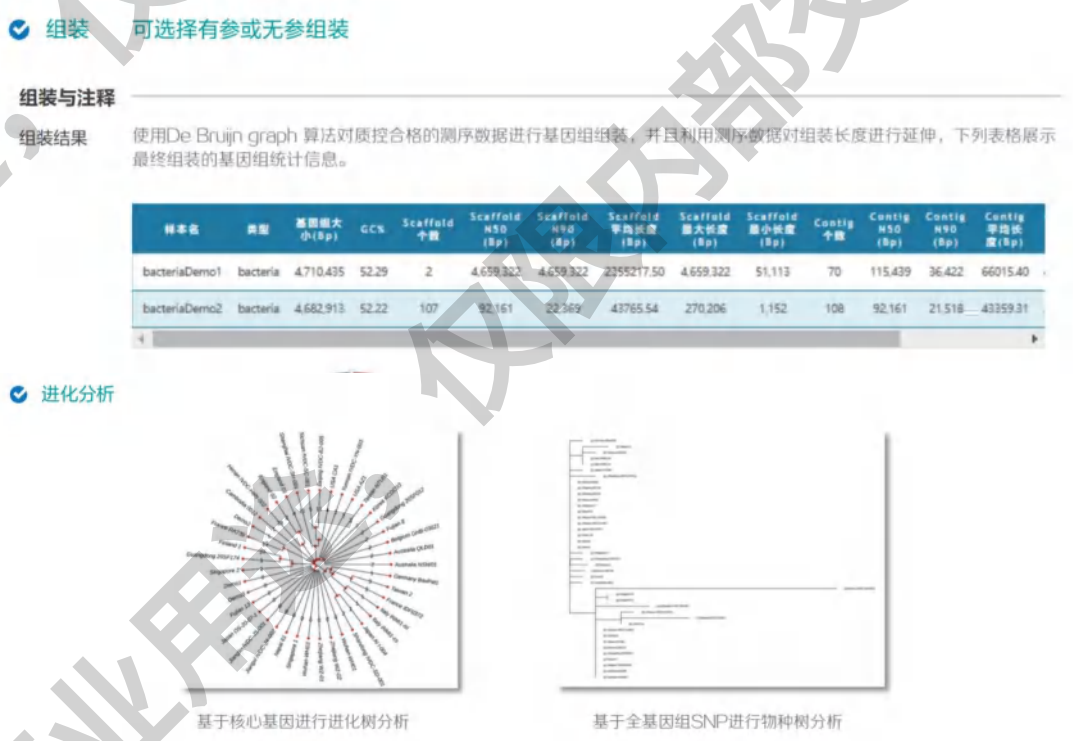
MGI微生物快速识别与组装溯源平台

开发了多个产品模块来实现从测序下机数据到基因组组装、系统进化分析，并自动计算变异检测结果供研究参考，客户可根据实际需求灵活选择分析模块。

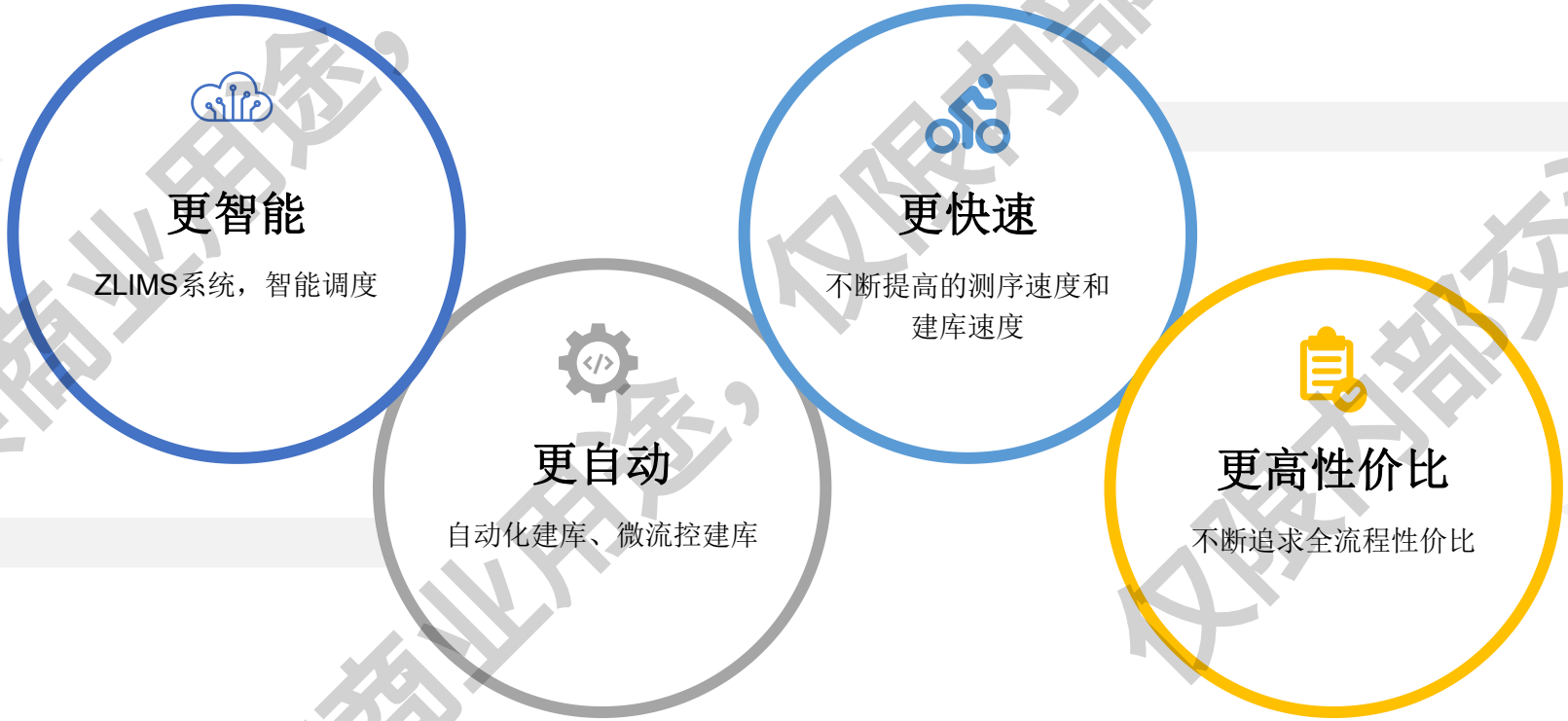


功能模块：

数据质控、组装、基因预测、变异检测、毒力基因分析、耐药基因分析、基于核心基因进行进化树分析、基于全基因组SNP进行系统进化树分析等。



华大智造产品目标



更加自动化、更快速

桌面型建库实验室

DNBelab-D4RS数字化样本制备系统

一卡建库，集成定量

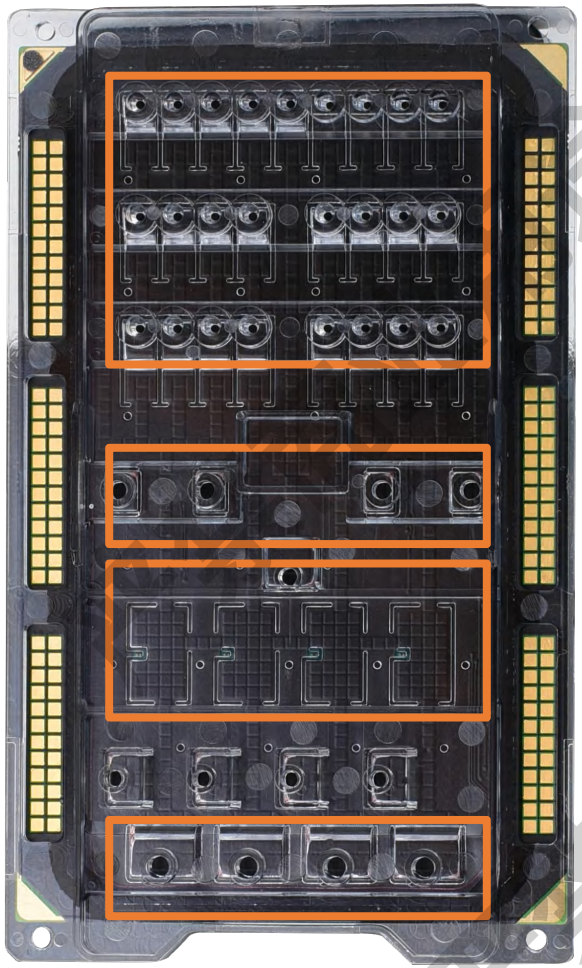
便携式基因测序仪

DNBSEQ-E5RS基因测序仪

随到随测，即开即用

更加自动化、更快速

采用数字化技术通过表面张力控制液滴运动



样本制备卡功能分区

试剂预存

试剂预存区域，4℃温度保持；根据不同的实验流程加载所需试剂至反应区

DNB出口

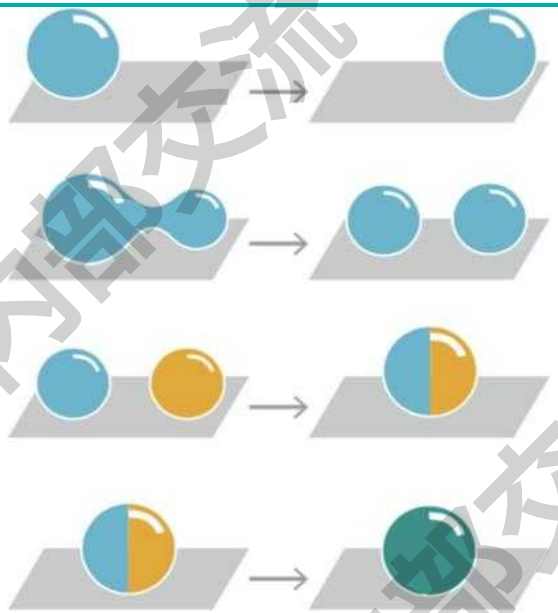
反应完成后，产物移动至DNB出口；建库完成，取出DNB

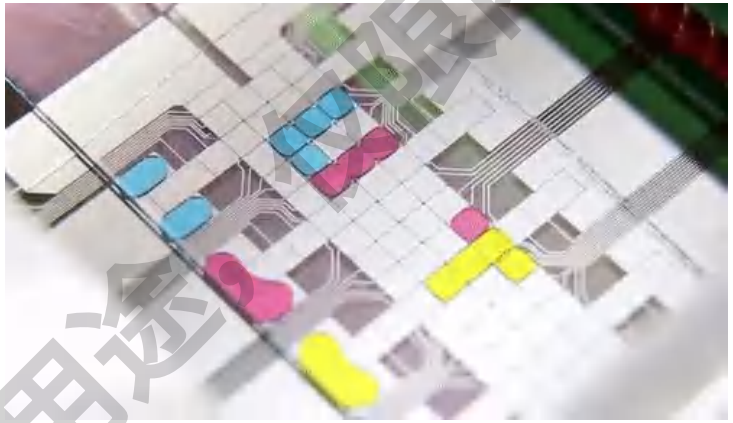
反应区

升降温、核酸纯化、反应体系混匀、DNB定量等操作

DNA入口

样本加入至样本制备卡中，移动样本至反应区进行相应操作





更加自动化、更快速

安装简单

对实验室温湿度无特殊要求
一体化测序载片，无需光路调校
开箱接线即可使用

快捷测序

10 分钟即可完成准备开始测序
集成信号采集模块直接读取序列信息
测序速度更快

微流控载片设计

测序载片采用微流控设计，与测序试剂盒直接搭配使用，测序试剂不经过仪器直接进入载片。

集成信号采集模块

集成信号采集模块直接读取碱基信号，无需传统光学系统。

集成自发光生化体系

全新设计的自发光体系，无需外界激发光源即可产生信号，仪器简洁耗能低，可移动部署。

耗材类型	Reads *	支持读长	数据量产出	运行时间	Q30 §
常规耗材	5M	SE100	0.5 GB	~8小时	>85%
快速耗材 †	5M	SE100	0.5 GB	~3.5小时 ‡	>85%

† 快速测序耗材作为新冠特供耗材使用。

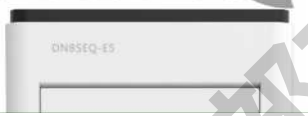
* 有效Reads的最大值根据特定标准文库运行所得，实际应用文库受样本类型、建库方式会有所波动。

‡ 运行时间均包含DNB加载及FASTQ生成时间，不包含标签测序时间。

§ 高于Q30的碱基百分比及运行时间是特定标准文库通过整个运行平均所得，实际应用表现受样本类型、文库质量、插入片段长度等影响。

华大智造基因测序仪

DNBSEQ-E5



- 无需废液桶，即用即抛
- 所用试剂量少
- 常规运行下无需清洗

更高性价比

● 电泳法

Sanger测序平板法

数据通量：10^2-10^3碱基/运行周期

$30亿/基因组

● 毛细管法

Sanger测序毛细管法

数据通量：10^3-10^6碱基/运行周期

$30亿/基因组

BT+IT规模化

● 合成法

合成测序3D法

数据通量：10^9-10^{12}碱基/运行周期

$百万~万/基因组

BT+IT规模化大科学应用

● 国产化

合成测序3D法

数据通量：10^{15}-10^{18}碱基/运行周期

￥万~千/基因组

BT+IT规模化大科学、大产业应用

华大智造DNBSEQ平台测序流程及技术原理

全方位源头性专利布局，比肩国际先进水准

累计研发投入数十亿，构筑技术及专利壁垒，境内外有效授权专利380项

自主可控的
源头性核心技术体系

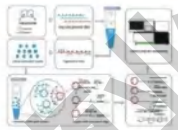
基因测序领域
- DNBSEQ测序技术
- 规则阵列芯片技术
- 测序仪光机电系统技术

新型生命数字化技术
- 关键文库制备技术
- 自动化样本处理技术
- 远程超声诊断技术

华大智造独有DNBSEQ™ 核心技术

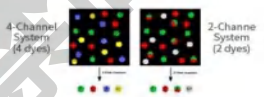
DNA模板阵列技术

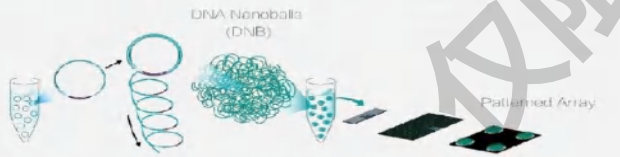
LFR和stLFR技术



DNA纳米球

2-color测序技术





准确性	重复序列率	标签跳跃
高	低	少
Precision*99.4% Sensitivity*98.8%	<3%	0.0001%~0.0004%

基于DNBSEQ™ 和stLFR 技术，发布“676”标准，
推动基因组测序进入“全高清”时代

Contig N50＞10^6

Scaffold N50＞10^7

组装全基因组数据＞6Gb

华大智造DNBSEQ平台测序流程及技术原理

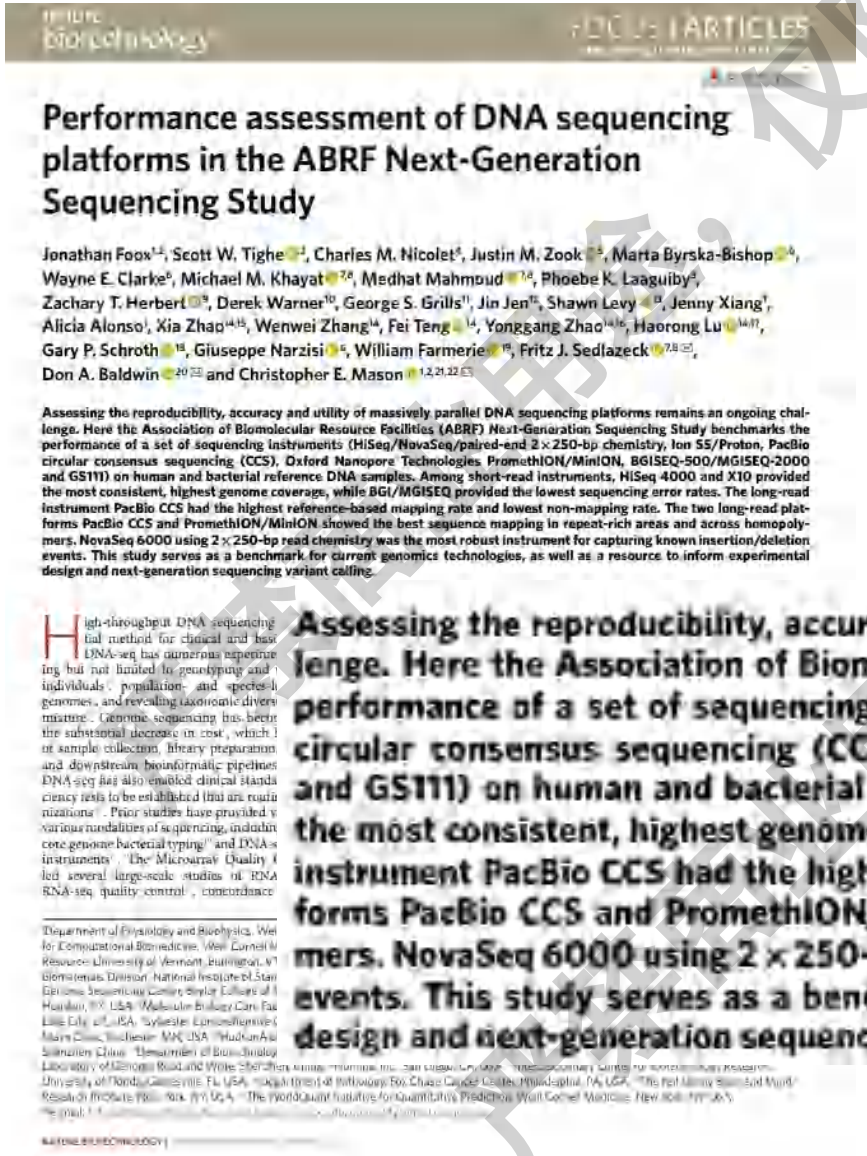
准确性-高
Increased accuracy
Precision*99.4%
Sensitivity*98.8%

重复序列率-低
Decreased duplicates
<3%

标签跳跃-少
Reduced Index hopping
0.0001%~0.0004%

"*"-MGI PCR-Free 文库对NA12878 InDel的检测精准度和灵敏度

多种测序技术性能比较分析



nature biotechnology FOCUS | ARTICLES

Performance assessment of DNA sequencing platforms in the ABRF Next-Generation Sequencing Study

Jonathan Foox[1,2], Scott W. Tighe[1,2], Charles M. Nicolet[3], Justin M. Zook[4], Marta Byrska-Bishop[5,6], Wayne E. Clarke[6], Michael M. Khayat[7,8], Medhat Mahmoud[7,8], Phoebe K. Laaguiby[3], Zachary T. Herbert[9], Derek Warner[10], George S. Grills[11], Jin Jen[12], Shawn Levy[13], Jenny Xiang[1], Alicia Alonso[1], Xia Zhao[14,15], Wenwei Zhang[14], Fei Teng[14], Yonggang Zhao[14,16], Haorong Lu[14,17], Gary P. Schroth[18], Giuseppe Narzisi[5,6], William Farmerie[19], Fritz J. Sedlazeck[7,8] ✉, Don A. Baldwin[20] ✉ and Christopher E. Mason[1,2,21,22] ✉

Assessing the reproducibility, accuracy and utility of massively parallel DNA sequencing platforms remains an ongoing challenge. Here the Association of Biomolecular Resource Facilities (ABRF) Next-Generation Sequencing Study benchmarks the performance of a set of sequencing instruments (HiSeq/NovaSeq/paired-end 2×250-bp chemistry, Ion S5/Proton, PacBio circular consensus sequencing (CCS), Oxford Nanopore Technologies PromethION/MinION, BGISEQ-500/MGISEQ-2000 and GS111) on human and bacterial reference DNA samples. Among short-read instruments, HiSeq 4000 and X10 provided the most consistent, highest genome coverage, while BGI/MGISEQ provided the lowest sequencing error rates. The long-read instrument PacBio CCS had the highest reference-based mapping rate and lowest non-mapping rate. The two long-read platforms PacBio CCS and PromethION/MinION showed the best sequence mapping in repeat-rich areas and across homopolymers. NovaSeq 6000 using 2×250-bp read chemistry was the most robust instrument for capturing known insertion/deletion events. This study serves as a benchmark for current genomics technologies, as well as a resource to inform experimental design and next-generation sequencing variant calling.

High-throughput DNA sequencing [illegible] tial method for clinical and basic DNA-seq has numerous applications including but not limited to genotyping and [illegible] individuals, population- and species-level genomes, and revealing taxonomic diversity [illegible] mixture. Genome sequencing has become the substantial decrease in cost, which has [illegible] of sample collection, library preparation [illegible] and downstream bioinformatic pipelines. DNA-seq has also enabled clinical standards [illegible] ciency tests to be established that are routinely [illegible] nizations[1–3]. Prior studies have provided [illegible] various modalities of sequencing, including [illegible] core genome bacterial typing[4] and DNA-seq [illegible] instruments[5]. The Microarray Quality Control [illegible] led several large-scale studies of RNA-seq, RNA-seq quality control[6], concordance [illegible]

Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA. [illegible] for Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA. [illegible] Resource, University of Vermont, Burlington, VT, USA. [illegible] Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD, USA. [illegible] Genome Sequencing Center, Stowers Institute for Medical Research, Kansas City, MO, USA. [illegible] Houston, TX, USA. [illegible] Molecular Biology Core Facilities, Dana-Farber Cancer Institute, Boston, MA, USA. [illegible] Life City of Hope, Duarte, CA, USA. [illegible] Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA. [illegible] Mayo Clinic, Rochester, MN, USA. [illegible] Hudson Alpha Institute for Biotechnology, Huntsville, AL, USA. [illegible] Shenzhen, China. [illegible] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY, USA. [illegible] Laboratory of Genome Research and Wuhan Supercomputing Institute, Wuhan, China. [illegible] University of Florida, Gainesville, FL, USA. [illegible] Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA, USA. [illegible] Research Institute, New York, NY, USA. [illegible] The New York Genome Center, New York, NY, USA. [illegible] The WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, NY, USA. [illegible] ✉e-mail: [illegible]

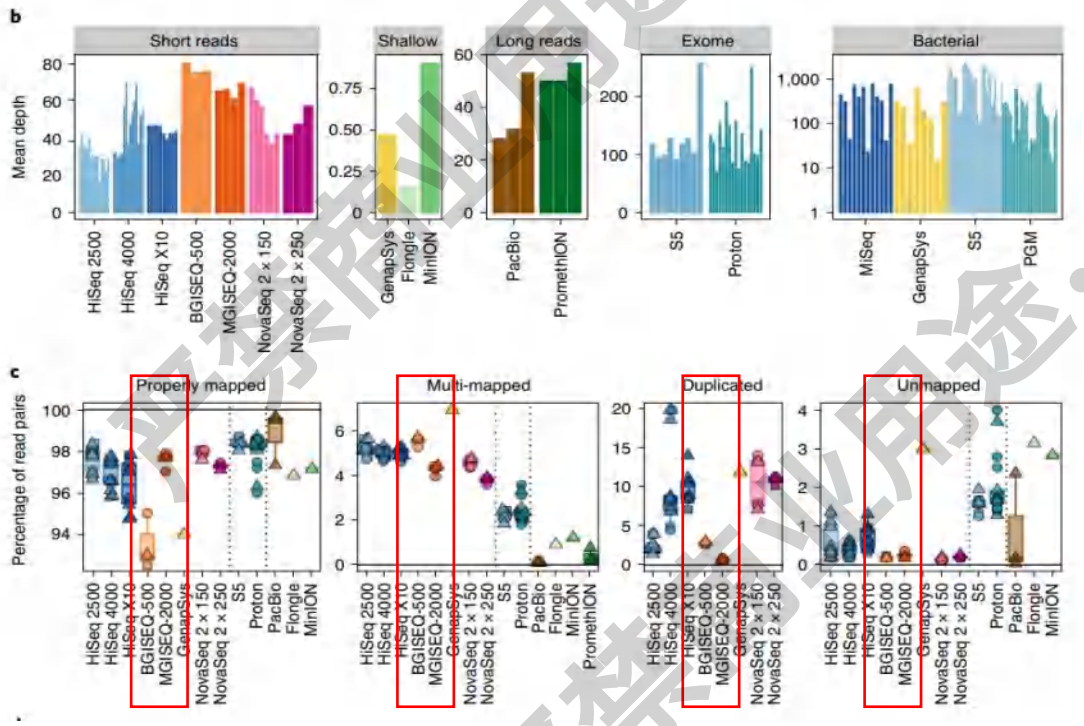
由生物分子资源设施协会（**Association of Biomolecular Resource Facilities, ARBF**）主导**Illumina、Pacific Biosciences、Thermo Fisher Scientific、BGI、Oxford Nanopore Technologies**和**Genapsys**的多款测序平台平台比较，在多个实验室对同一人类基因组家族、三个单独菌株和十种细菌的宏基因组混合物进行测序，并将各平台数据进行全方位、系统性比较，分析各个测序平台的性能差异和测序质量，以提供真实全面的参考证据。

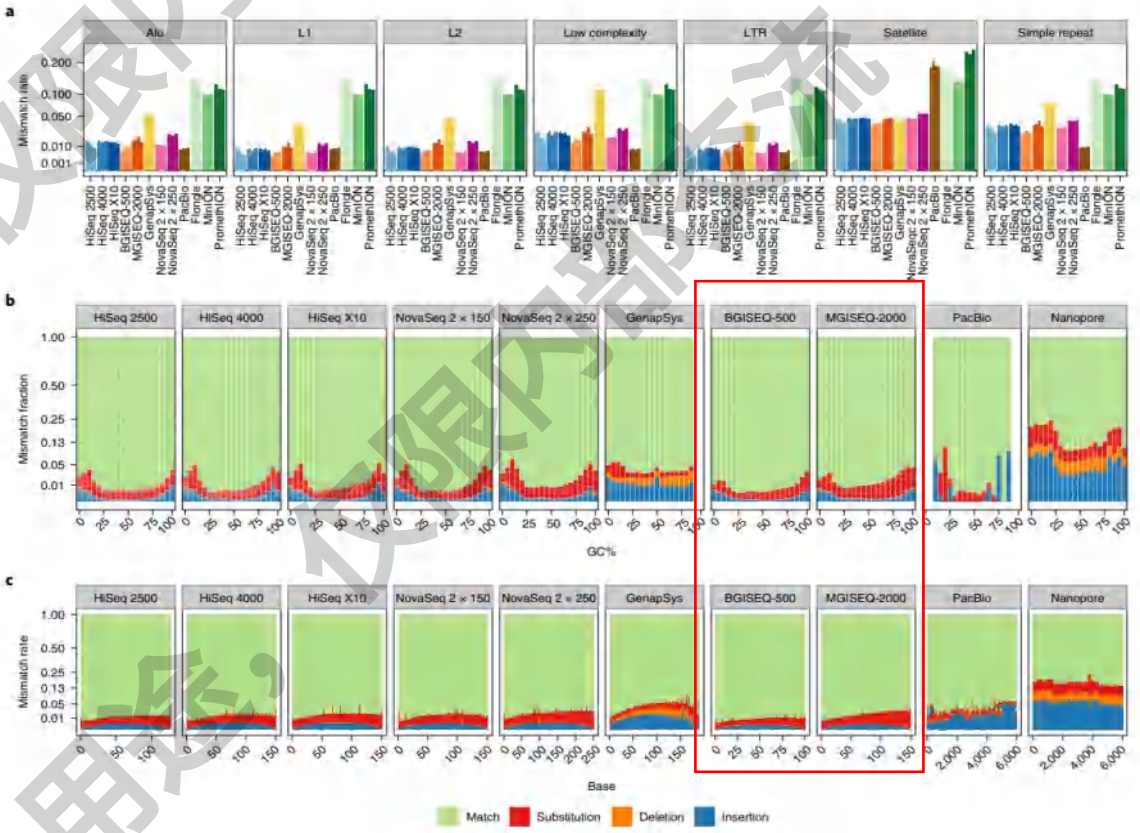
Assessing the reproducibility, accuracy and utility of massively parallel DNA sequencing platforms remains an ongoing challenge. Here the Association of Biomolecular Resource Facilities (ABRF) Next-Generation Sequencing Study benchmarks the performance of a set of sequencing instruments (HiSeq/NovaSeq/paired-end 2×250-bp chemistry, Ion S5/Proton, PacBio circular consensus sequencing (CCS), Oxford Nanopore Technologies PromethION/MinION, BGISEQ-500/MGISEQ-2000 and GS111) on human and bacterial reference DNA samples. Among short-read instruments, HiSeq 4000 and X10 provided the most consistent, highest genome coverage, while BGI/MGISEQ provided the lowest sequencing error rates. The long-read instrument PacBio CCS had the highest reference-based mapping rate and lowest non-mapping rate. The two long-read platforms PacBio CCS and PromethION/MinION showed the best sequence mapping in repeat-rich areas and across homopolymers. NovaSeq 6000 using 2×250-bp read chemistry was the most robust instrument for capturing known insertion/deletion events. This study serves as a benchmark for current genomics technologies, as well as a resource to inform experimental design and next-generation sequencing variant calling.

Jonathan Foox, et al., Nature Biotechnology. 2021.

多种测序技术性能比较分析

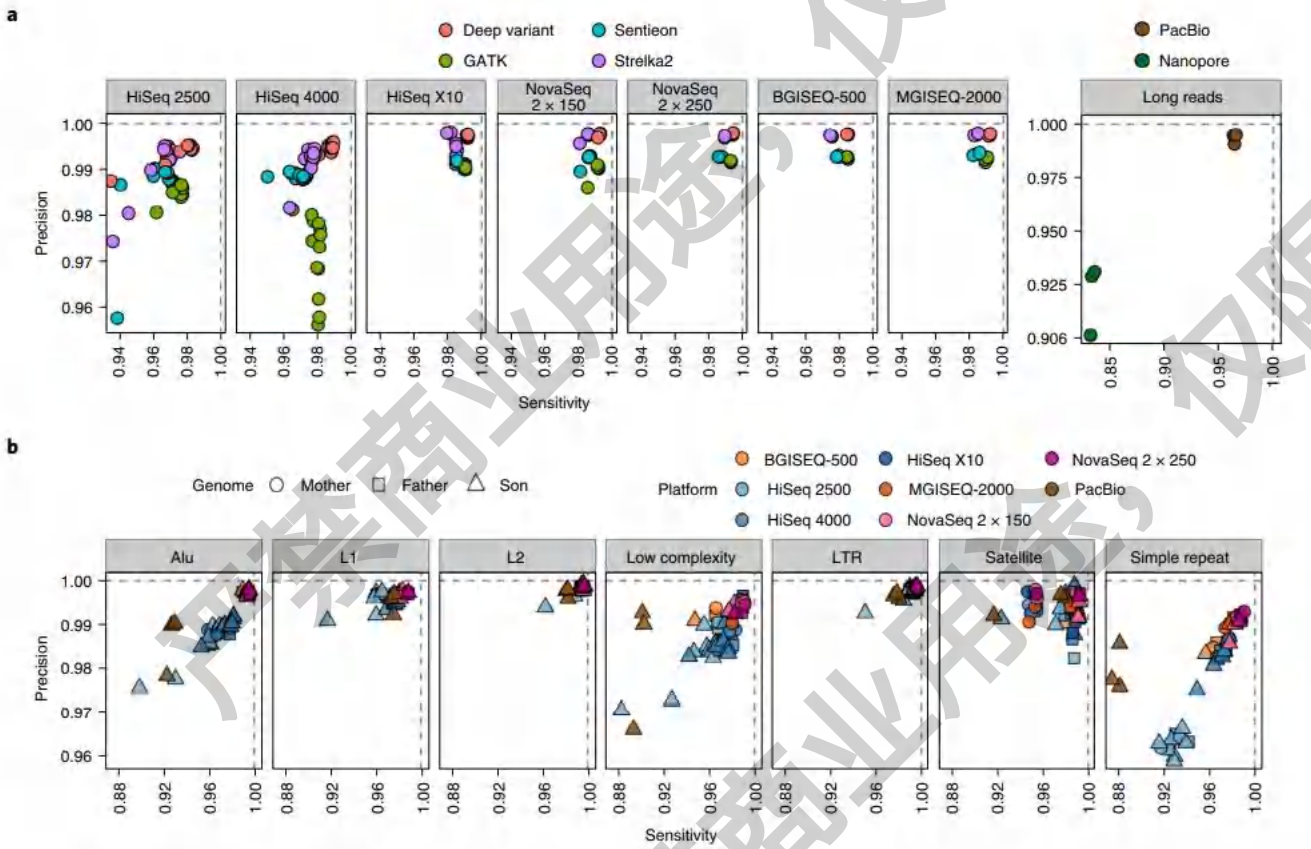
以25X对测序深度进行均一化后，**BGISEQ-500**和**MGISEQ-2000**在**Duplicated** 和**Unmapped**比例上优于其他平台，在测序错误率层面，各个平台在**GC**含量较高的区域均出现了较高的错误率。在短读长测序平台中，**BGISEQ-500**、**MGISEQ-2000**提供的测序错误率最低。





Jonathan Foox, et al., Nature Biotechnology. 2021.

多种测序技术性能比较分析



SNV和INDEL是常见的DNA突变类型。

- **在SNV的检出中， 华大智造的BGISEQ-500、MGISEQ-2000平台最为灵敏，其次是NovaSeq 2x250bp、NovaSeq 2x150bp、HiSeq 2500、HiSeq X10和HiSeq4000。**
- **在INDEL的检出中，所有平台灵敏度均达到99.5%，华大智造的BGISEQ-500、MGISEQ-2000平台和NovaSeq的检出相似，且优于其他平台。**
- **PacBio、Nanopore平台对于SNV和INDEL的捕获能力均较弱。**

Jonathan Foox, et al., Nature Biotechnology. 2021.

华大智造DNBSEQ平台发表文章

MGI 华大智造

累计发表文章1902篇

涵盖多种应用

研究物种达到接近300种

欢迎提报高通量测序应用支持计划

欢迎扫码填写，数量不限！

内部审核会根据质量及真实性进行评估，通过后获得：

1、精美礼品：电脑支架一份；

2、将针对该项目给予支持！

创新智造引领生命科技

中国营销中心

感谢你的聆听

Thanks for your time

MGI 华大智造

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