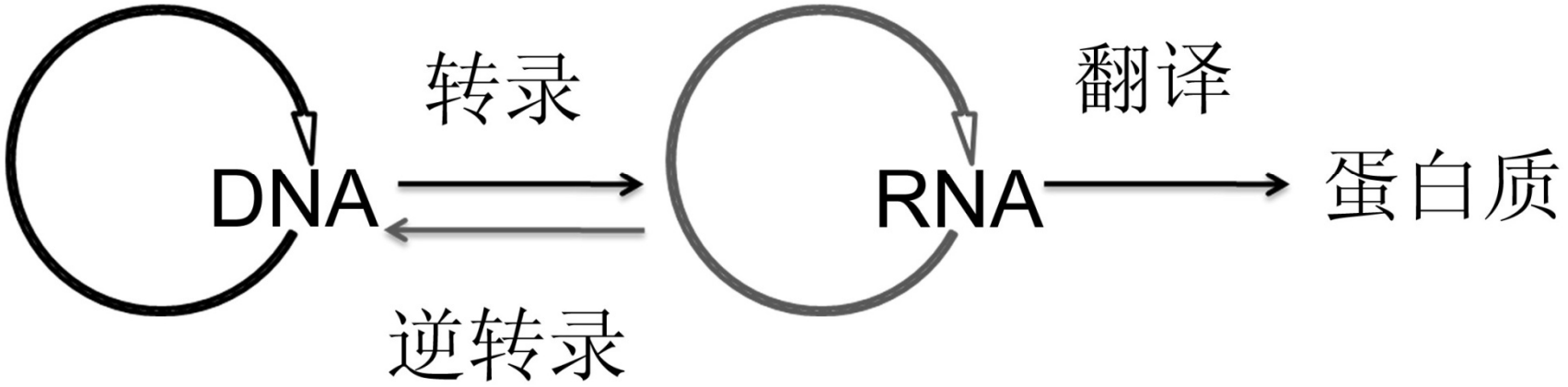
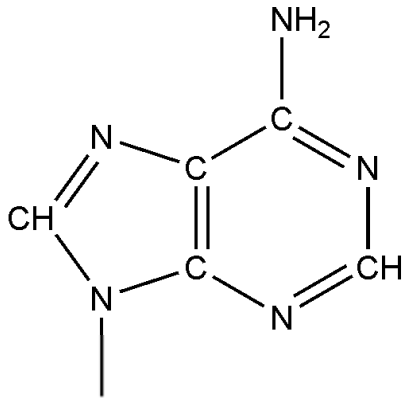
生物信息学

第二章 生物序列获取和存储

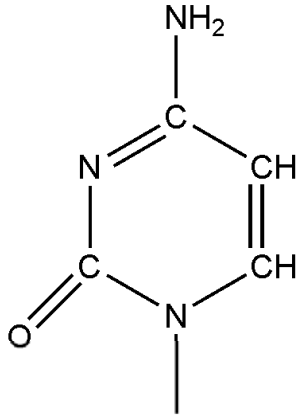
中心法则



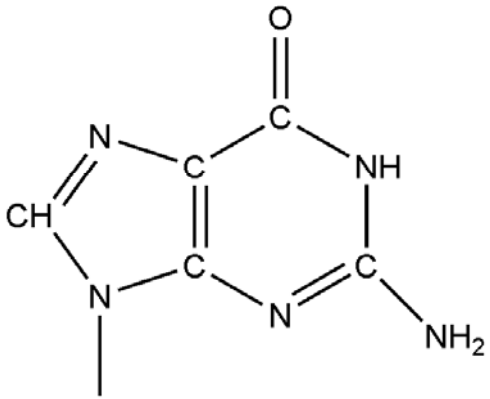
DNA结构：碱基/核苷



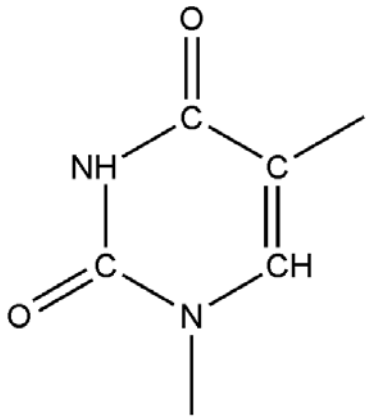
Adenine (A)



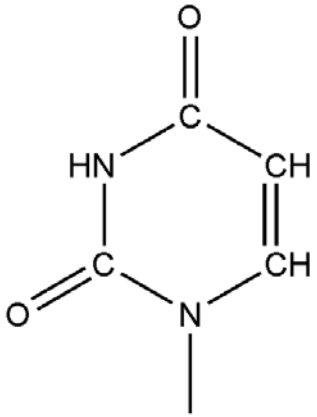
Cytosine (C)



Guanine (G)



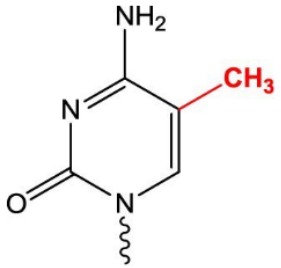
Thymine (T)



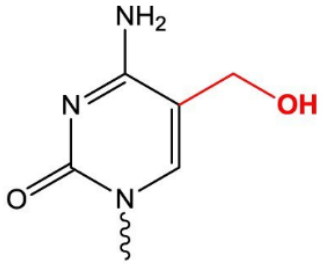
Uracil (U)

DNA修饰（~10种）

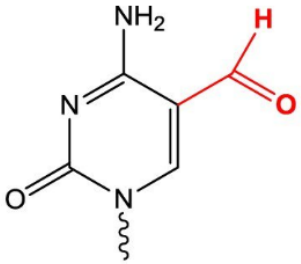
- 5-methylcytosine (5-mC)
- 5-hydroxymethylcytosine (5-hmC)
- 5-formylcytosine (5-fC)
- 5-carboxylcytosine (5-caC)
- 4-methylcytosine(4-mC)
- 6-methyladenine (6-mA)
- 8-oxoguanine (8-oxoG)
- 8-oxoadenine (8-oxoA)



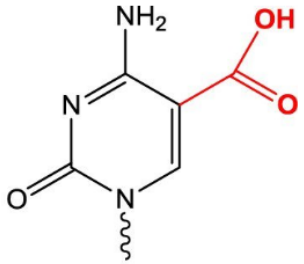
5-mC



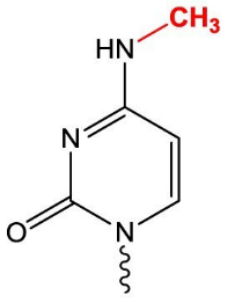
5-hmC



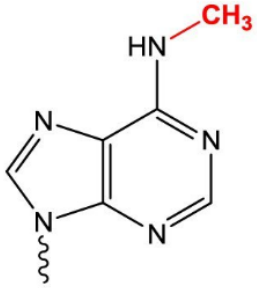
5-fC



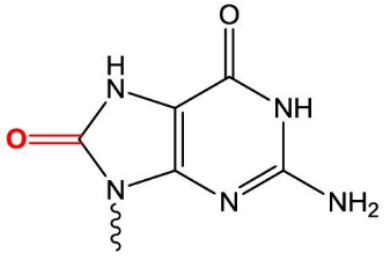
5-caC



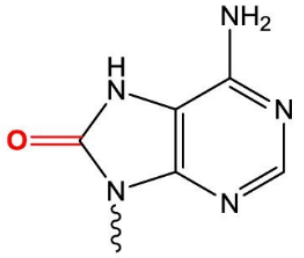
4-mC



6-mA

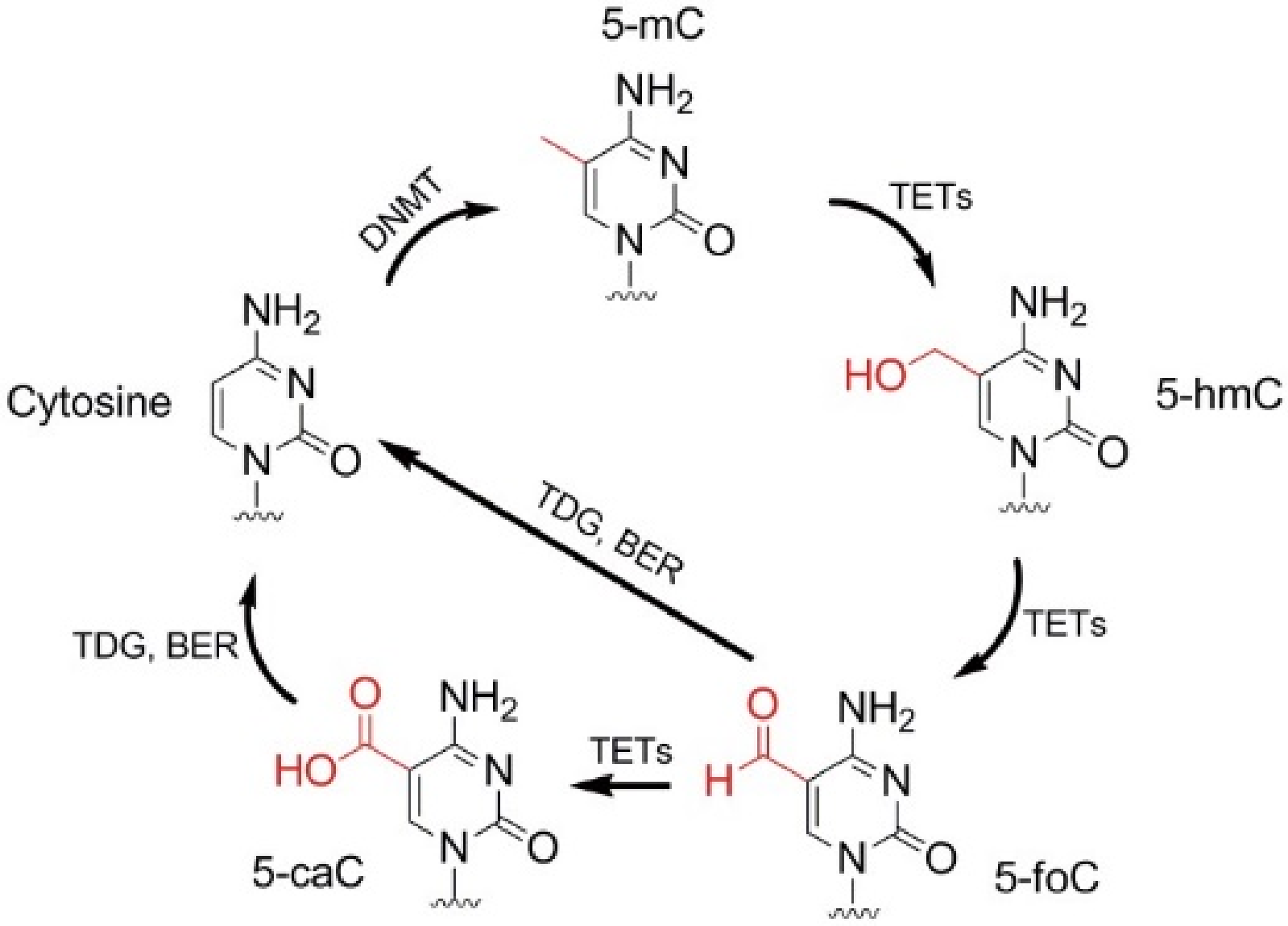


8-oxoG

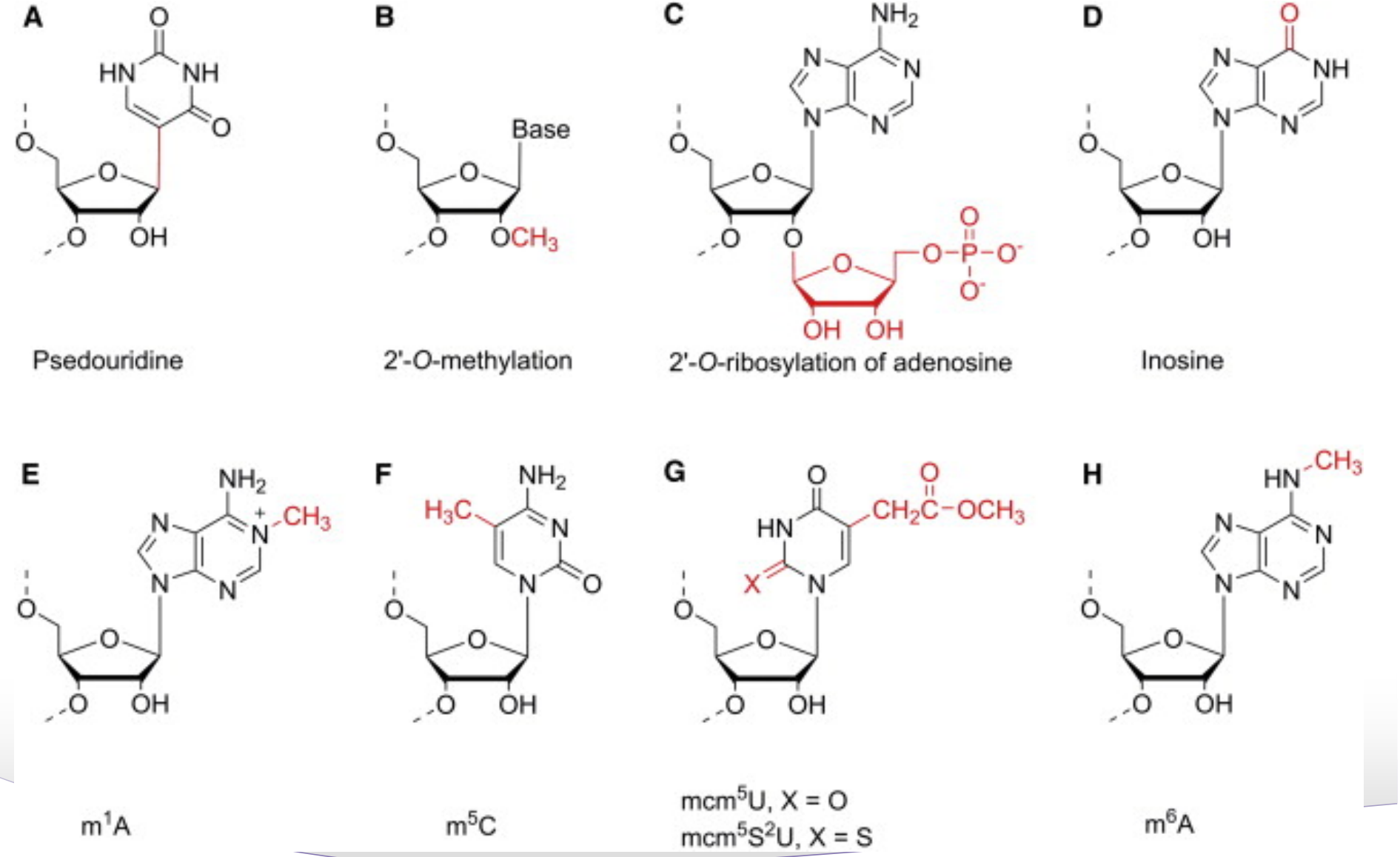


8-oxoA

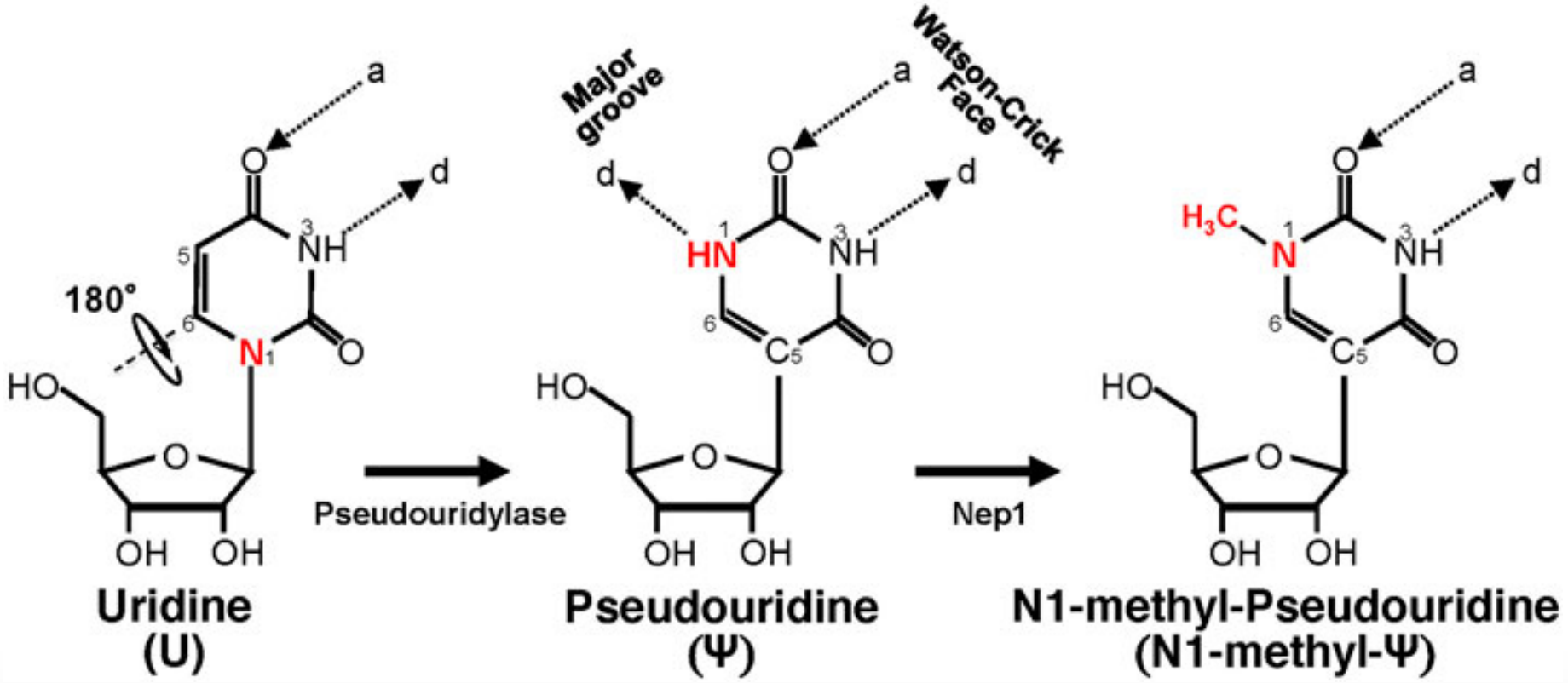
DNA修饰的催化过程


5-mC
DNMT
TETs
Cytosine
5-hmC
TDG, BER
TETs
TDG, BER
TETs
5-caC
5-foC

RNA修饰（~150种）

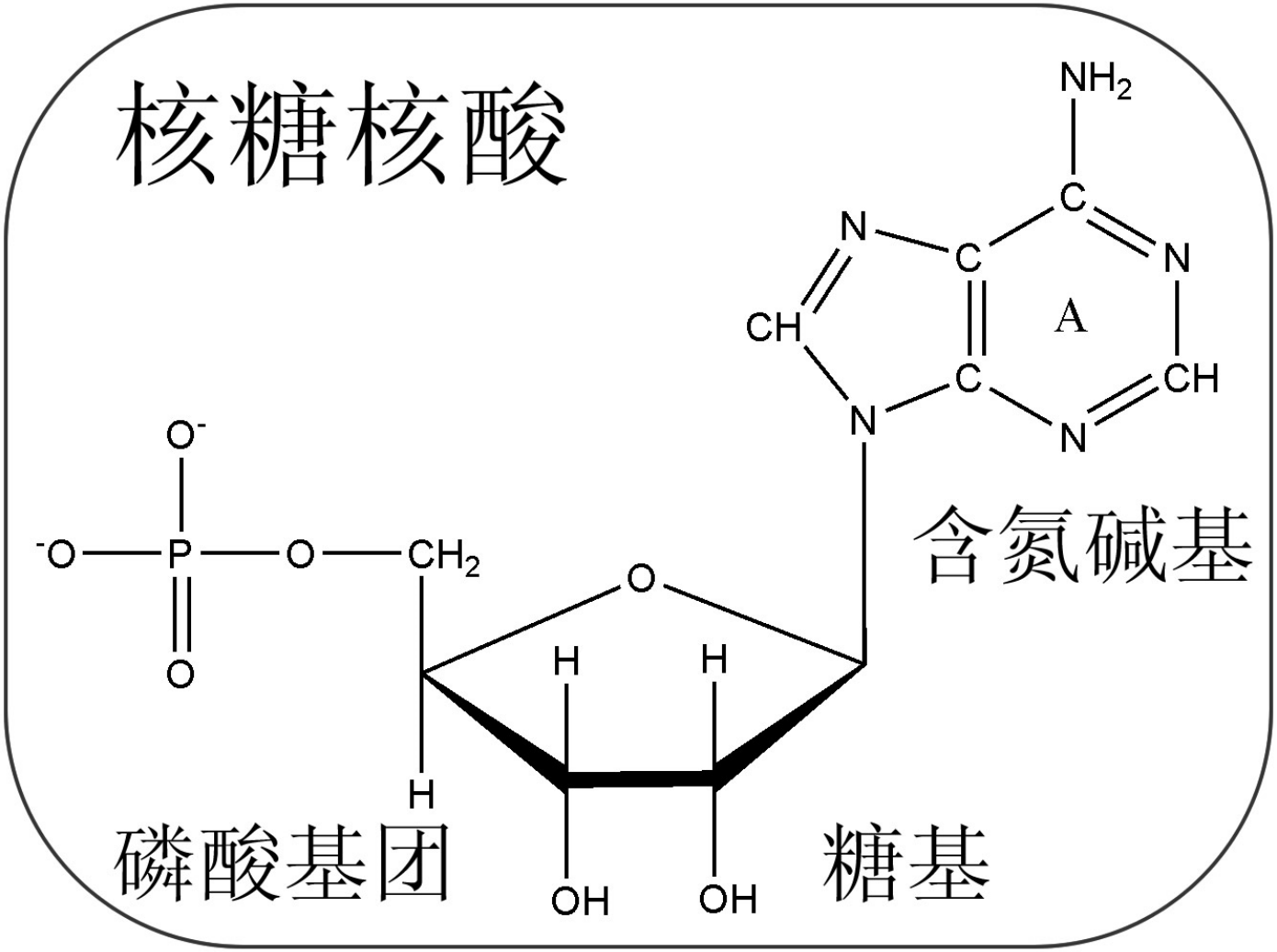

A
B
Base
C
D
Psedouridine
2'-O-methylation
2'-O-ribosylation of adenosine
Inosine
E
F
G
H
m1A
m5C
mcm5U, X = O
mcm5S2U, X = S
m6A

mRNA疫苗中的关键修饰



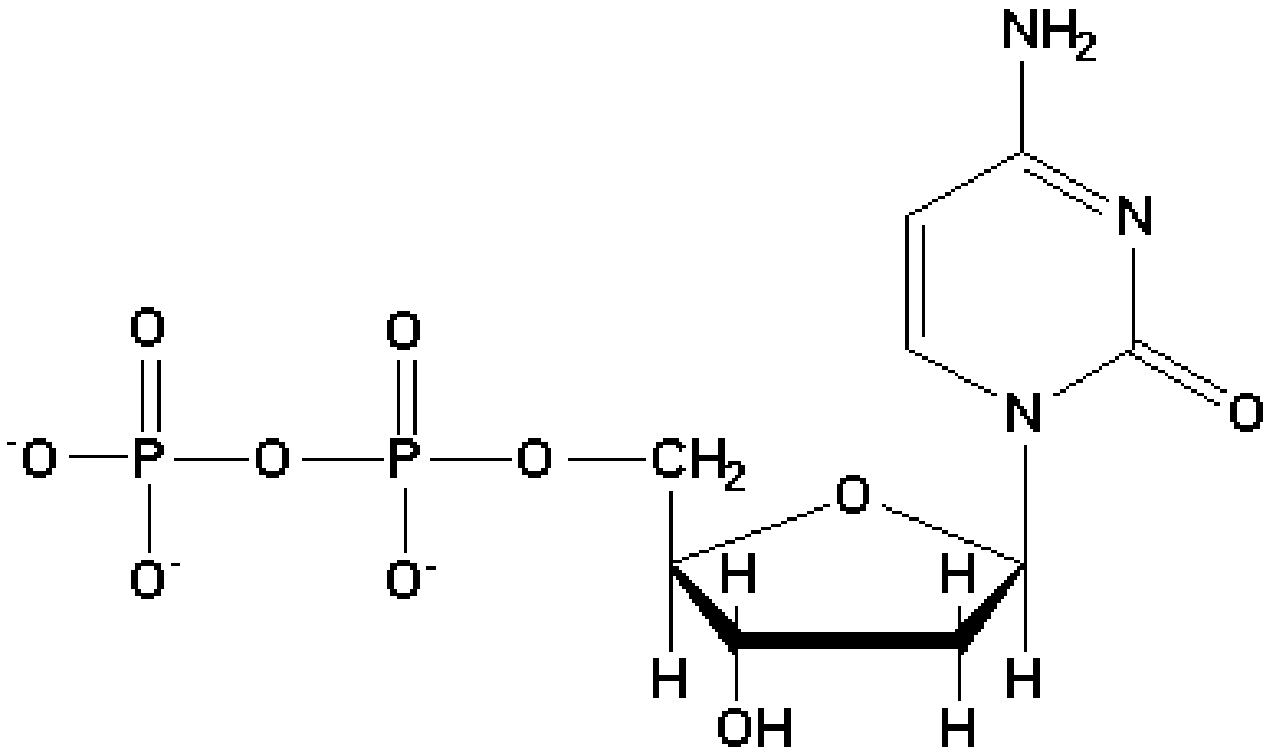
核糖核酸

Ribonucleotide



脱氧核糖核酸

Deoxyribonucleotide

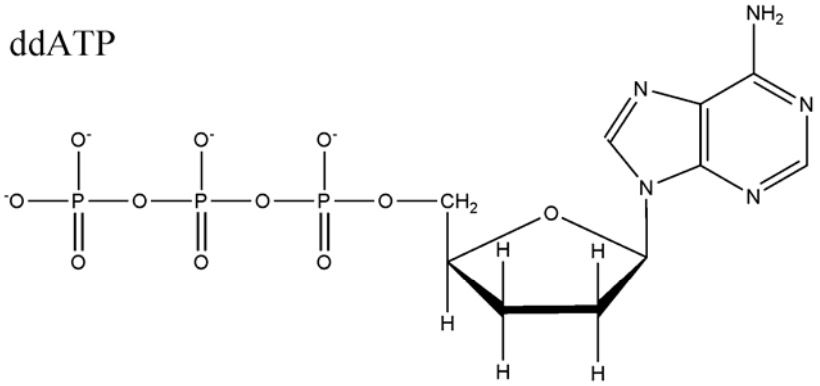


Deoxycytidine diphosphate (dCDP)

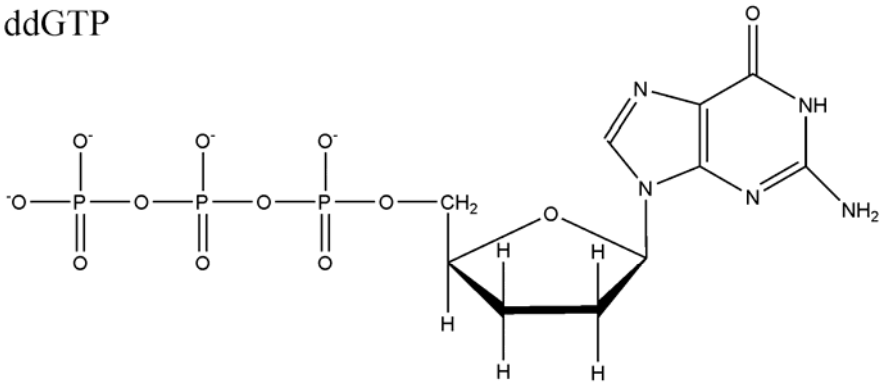
双脱氧核糖核苷酸

Dideoxyribonucleotide

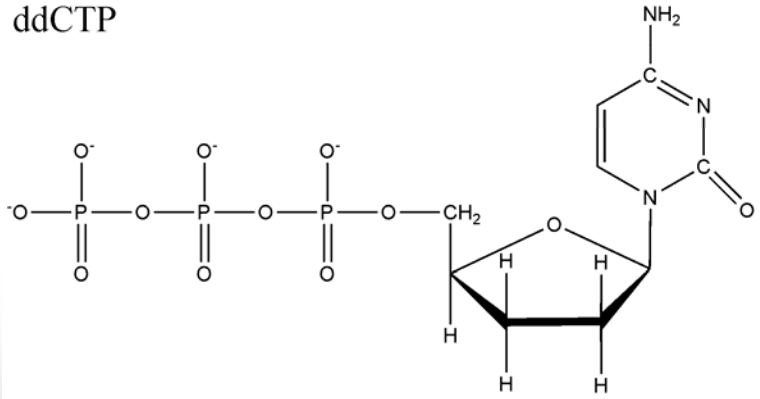
ddATP



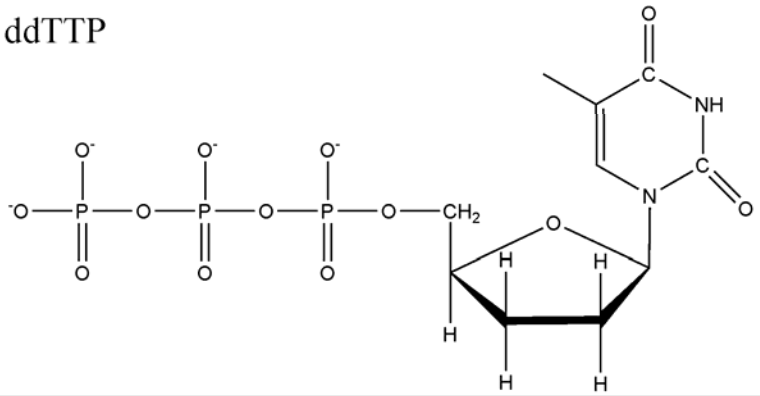
ddGTP



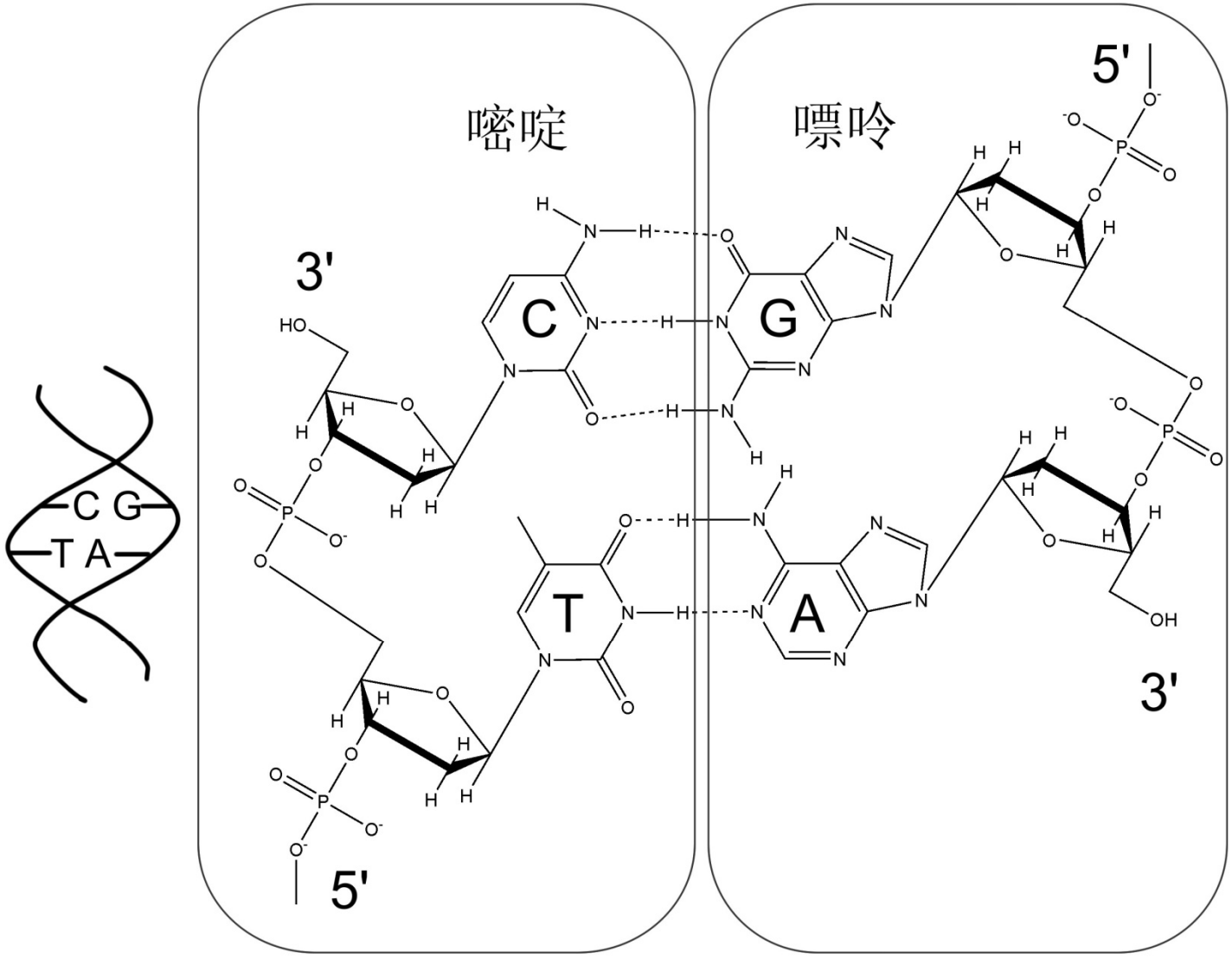
ddCTP



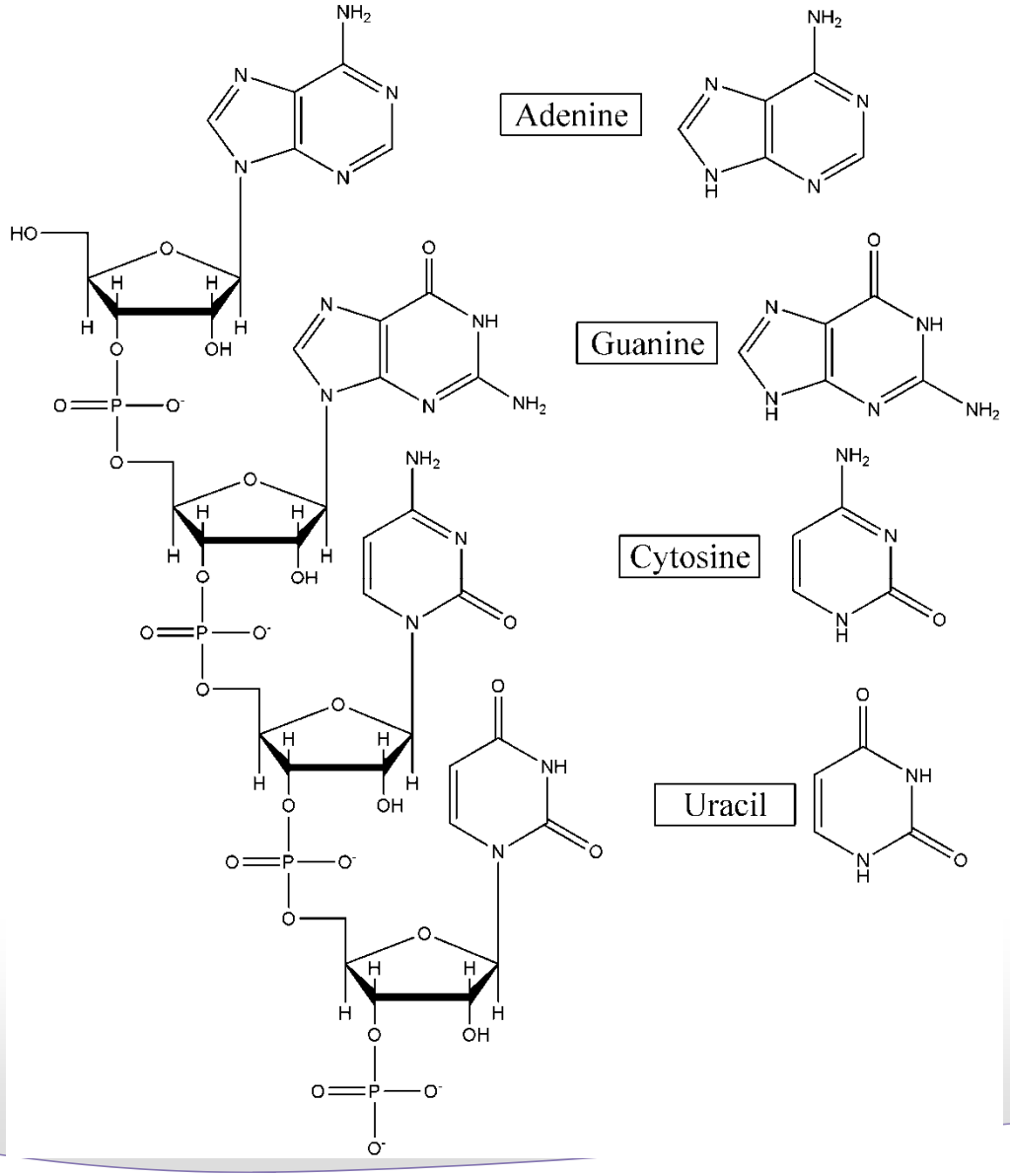
ddTTP



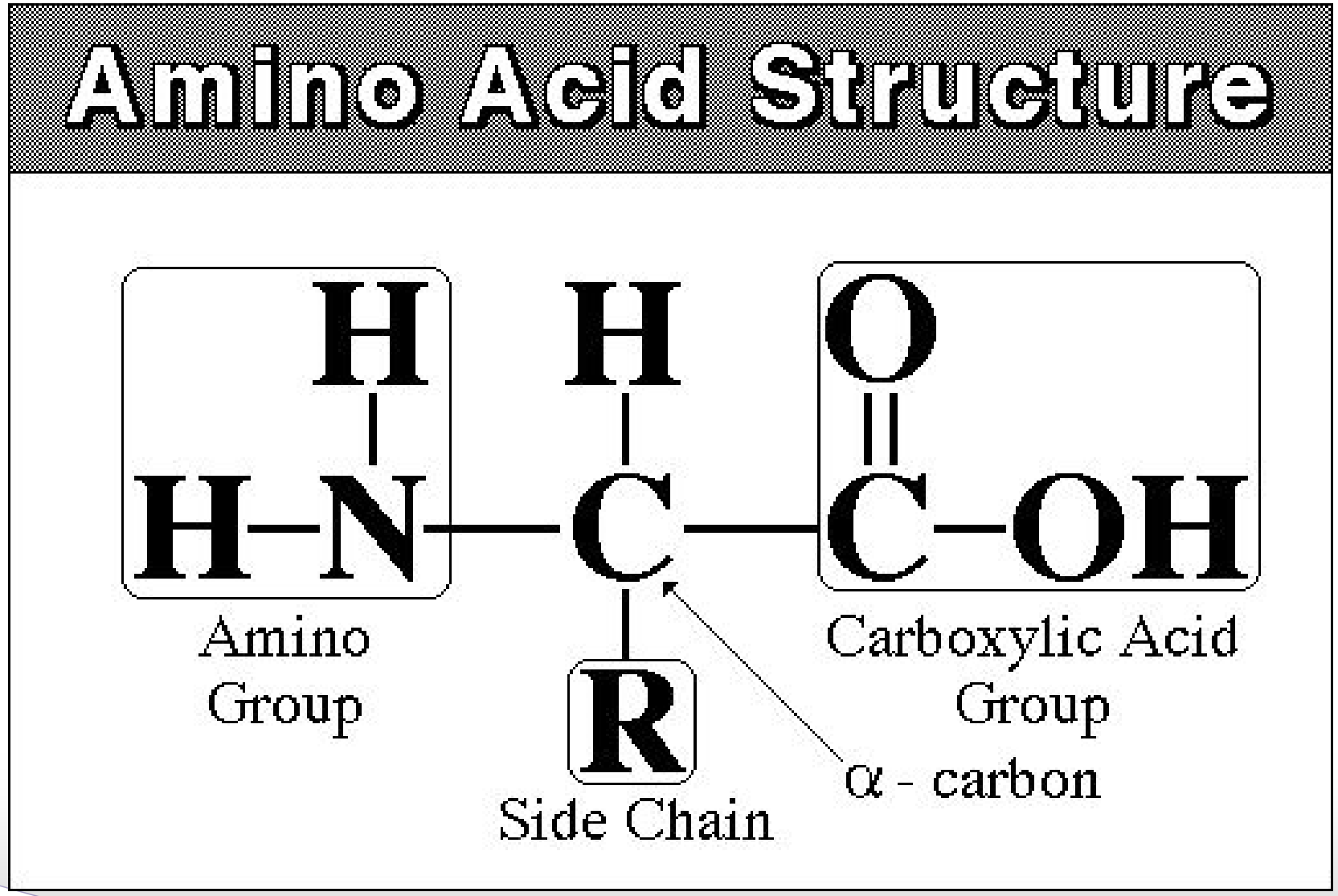
DNA的结构



RNA的结构



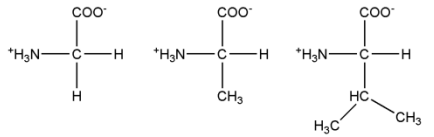
氨基酸的结构


Amino Acid Structure
H
H
O
H–N
C
C–OH
Amino Group
Carboxylic Acid Group
R
α - carbon
Side Chain

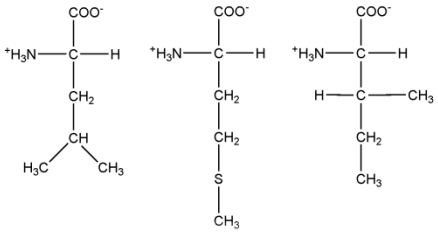
氨基酸的性质及分类

20种常见的蛋白质氨基酸

非极性脂肪酸氨基酸

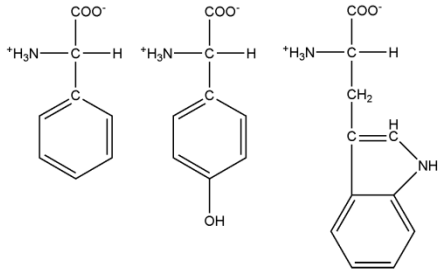


甘氨酸 Gly, G　丙氨酸 Ala, A　缬氨酸 Val, V



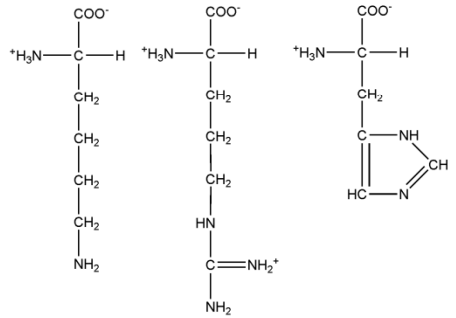
亮氨酸 Leu, I　甲硫氨酸 Met, M　异亮氨酸 Ile, I

芳香族氨基酸



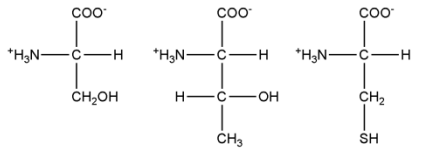
苯丙氨酸 Phe, F　酪氨酸 Tyr, Y　色氨酸 Trp, W

R基带正电荷的氨基酸

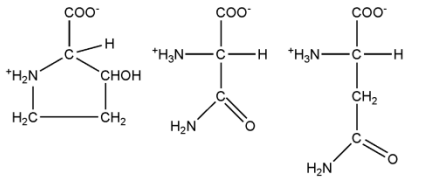


赖氨酸 Lys, K　精氨酸 Arg, R　组氨酸 His, H

R基不带电荷的氨基酸

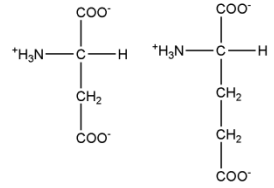


丝氨酸 Ser, S　苏氨酸 Thr, T　半胱氨酸 Cys, C



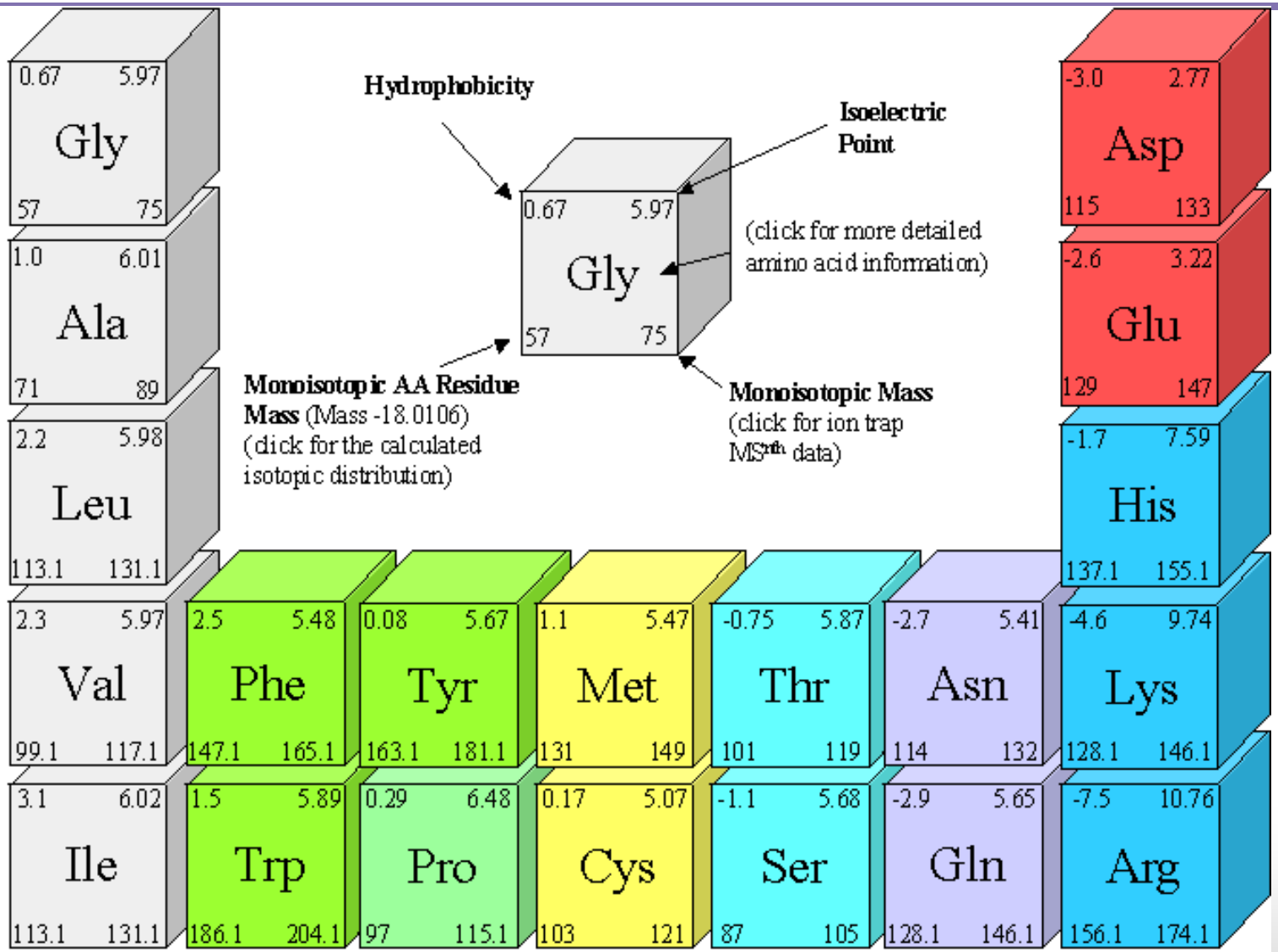
脯氨酸 Pro, P　天冬酰胺 Asn, N　谷氨酰胺 Gln, Q

R基带负电荷的氨基酸



天冬氨酸 Asp, D　谷氨酸 Glu, E

氨基酸：周期表

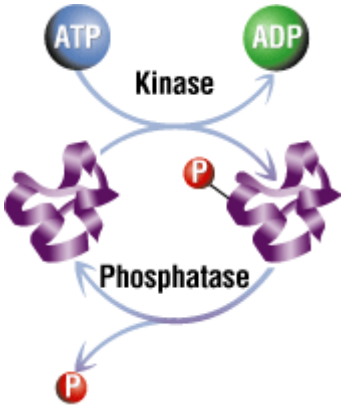


蛋白质翻译后修饰

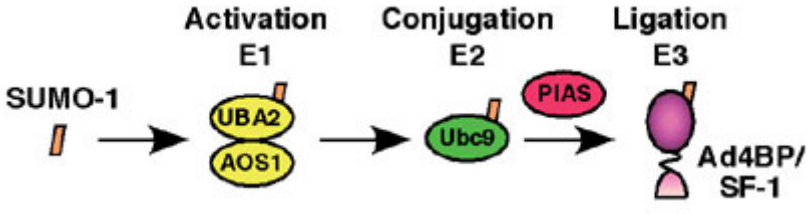
- **POST**: DNA转录为RNA，RNA翻译成蛋白质之后发生
- 生化反应：产生或破坏共价键
- 发生在氨基酸的主链或侧链上
- 通常由特定酶催化
- 可发生在15种非疏水的氨基酸上 (not F/V/I/L/A)
- 目前已发现~680种PTMs

http://www.uniprot.org/docs/ptmlist

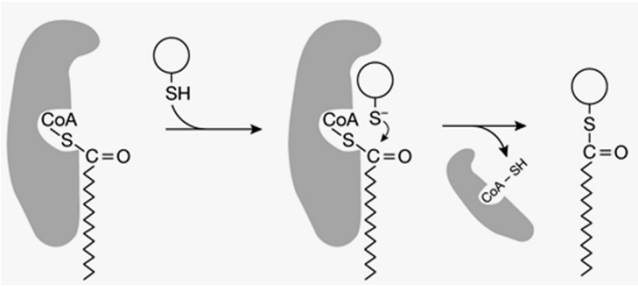
常见的蛋白质翻译后修饰



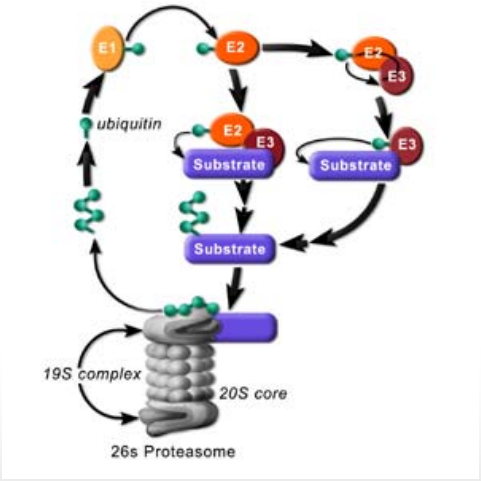
Phosphorylation



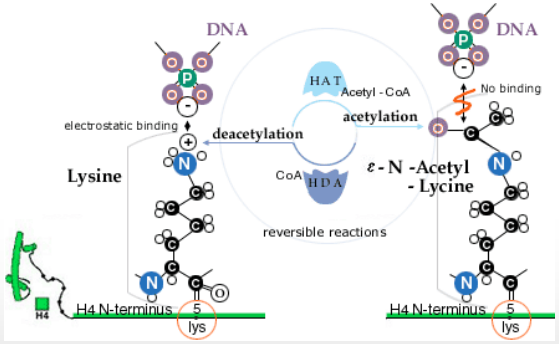
Sumoylation



Palmitoylation



Ubiquitination

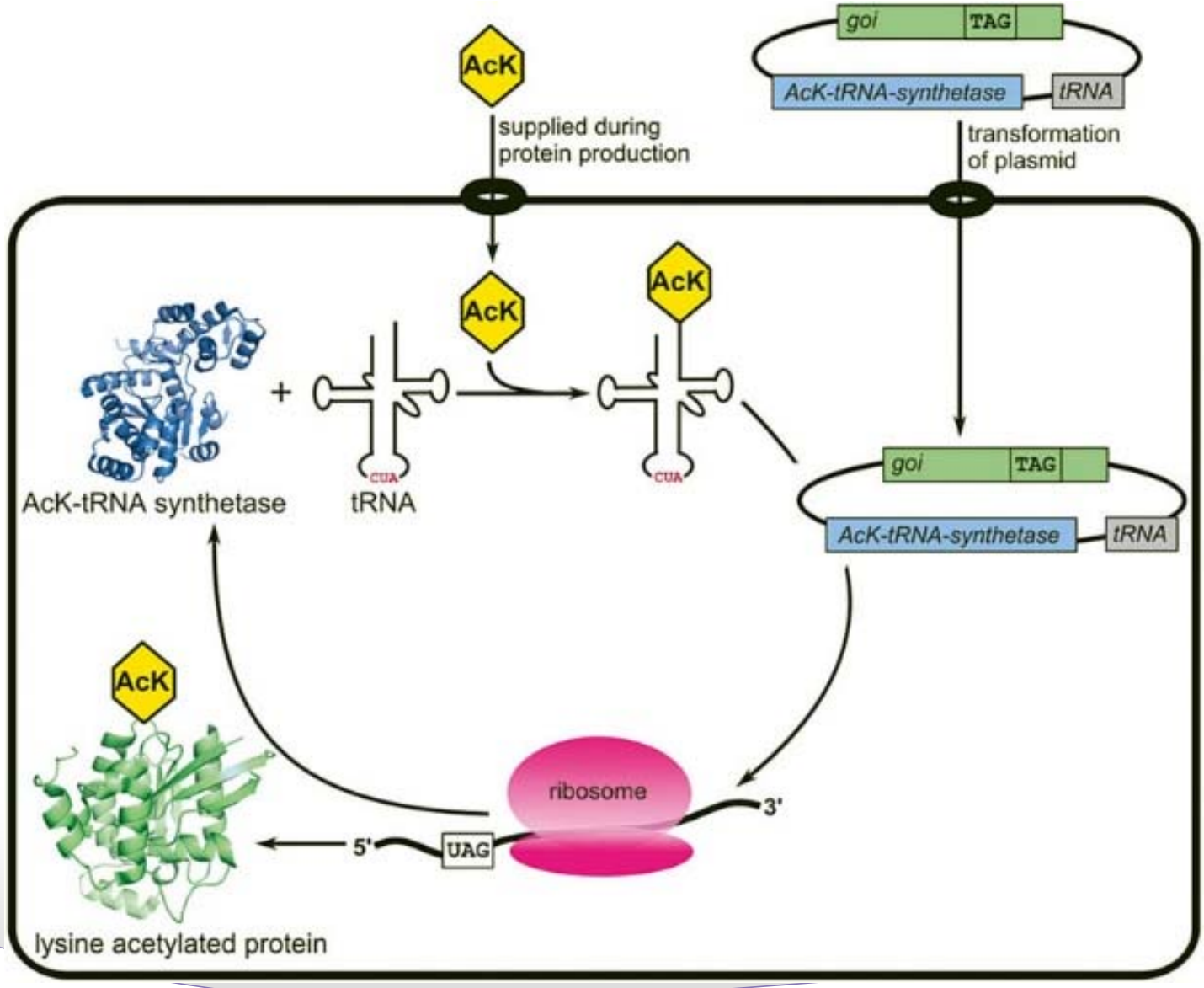


Acetylation

标准密码子（Codon）

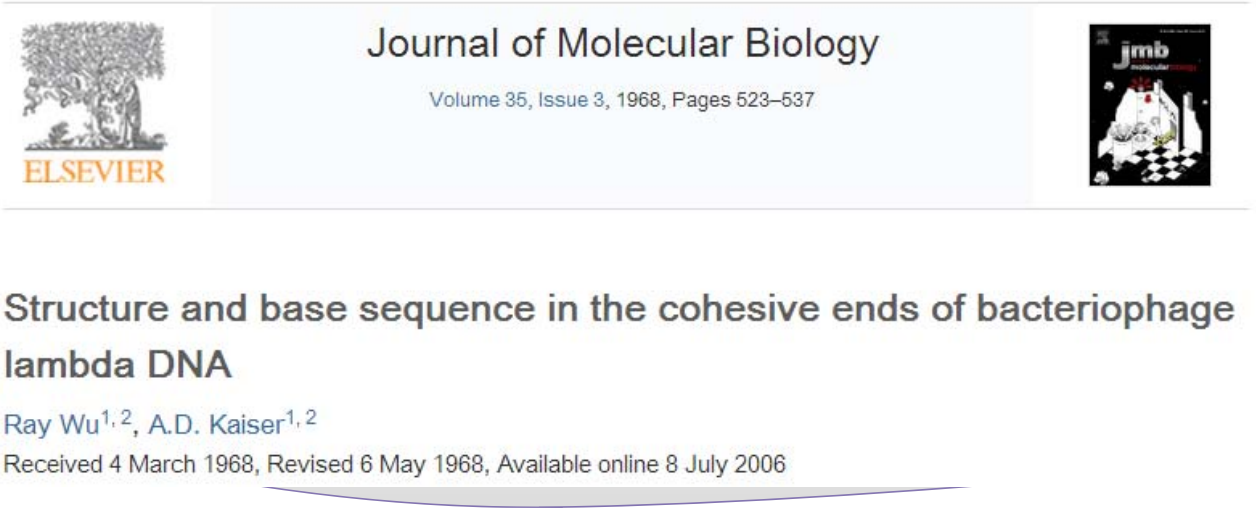
	T	C	A	G
T	TTT Phe(F) TTC .. TTA Leu(L) TTG ..	TCT Ser(S) TCC .. TCA .. TCG ..	TAT Tyr(Y) TAC TAA Ter ochre TAG Ter amber	TGT Cys(C) TGC TGA Ter opal TGG Trp(W)
C	CTT Leu(L) CTC .. CTA .. CTG ..	CCT Phe(P) CCC .. CCA .. CCG ..	CAT His(H) CAC .. CAA Gln (Q) CAA ..	CGT Arg(R) CGC .. CGA .. CGG ..
A	ATT Ile(I) ATC .. ATA .. ATG Met (M)	ACT Thr(T) ACC .. ACA .. ACG ..	AAT Asn(N) AAC .. AAA Lys(K) AAA ..	AGT Ser(S) AGC .. AGA Arg(R) AGG ..
G	GTT Val(V) GTC .. GTA .. GTG ..	GCT Ala(A) GCC .. GCA .. GCG ..	GAT Asp(D) GAC .. GAA Glu(E) GAA ..	GGT Gly(G) GGC .. GGA .. GGG ..

遗传密码子扩展


AcK
supplied during
protein production
goi
TAG
AcK-tRNA-synthetase
tRNA
transformation
of plasmid
AcK
AcK
AcK
+
AcK-tRNA synthetase
tRNA
CUA
CUA
goi
TAG
AcK-tRNA-synthetase
tRNA
AcK
ribosome
3'
5'
UAG
lysine acetylated protein

吴瑞先生：DNA测序

- Dr. Ray Wu，1928年出生于北京，2008年去世
- 康奈尔大学分子生物学与遗传学教授、植物基因工程创建人之一
- 《君子爱“生”，得之有道》
- 1968年设计DNA测序方法


ELSEVIER

Journal of Molecular Biology

Volume 35, Issue 3, 1968, Pages 523–537

Structure and base sequence in the cohesive ends of bacteriophage lambda DNA

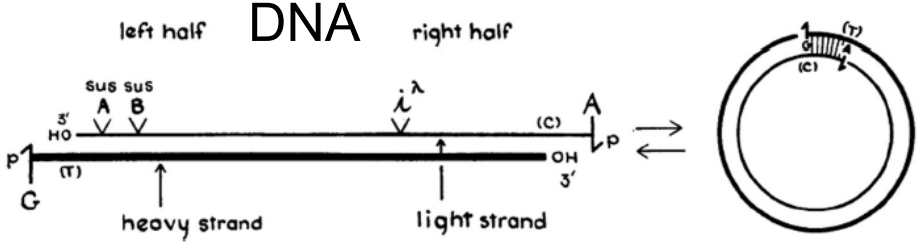
Ray Wu[1, 2], A.D. Kaiser[1, 2]

Received 4 March 1968, Revised 6 May 1968, Available online 8 July 2006

吴瑞先生：DNA测序

- 基本原理：位置特异性的引物延伸（Location specific-primer-extension principle in labeling the DNA）
- 1973年，Gilbert; 1975年，Sanger

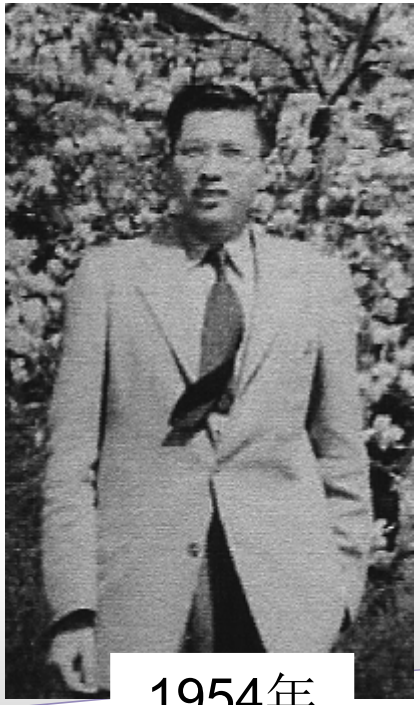
噬菌体λ DNA



Ray Wu as Fifth Business: Deconstructing collective memory in the history of DNA sequencing

Lisa A. Onaga

Studies in History and Philosophy of Biological and Biomedical Sciences 46 (2014) 1–14



1954年

51. 吴瑞先生：第五先生还是DNA测序之父？

第一次知道吴瑞先生（图一）的名字，是看了饶毅老师写的博文《君子爱“生” 得之有道》，这篇博文后来收录在《饶议科学I》里，过年期间又读过一遍。其中有一句写的很有意思：“1971年吴瑞的引物延伸，是测序的一个关键步骤，给奖是可以的”。看到这儿我笑晕了：这都哪儿跟哪儿啊？所有课本上讲的都是Sanger测序法，所以显然是Sanger的贡献最大，况且诺奖都发了，还争这个有意思吗？另外，中国的语言历来有内涵，一般来说，“可以资助”的意思就是“不可以资助”，所以饶老师写博客为华人挣功劳的心意是挺好的，但显然不符合事实，对吧？咱读这篇文章的时候就是这么想的。

吴瑞先生，1954年于宾州大学

Stud Hist Philos Biol Biomed Sci., 2014, 46:1-14

Sci China C Life Sci., 2009, 52(2):99-100

DNA测序

- 分子生物学研究中最重要的工具：确定DNA分子中的碱基组成和顺序
- 1980, Walter Gilbert & Fred Sanger: 利用DNA聚合酶测定DNA序列

1958: 胰岛素

Frederick Sanger

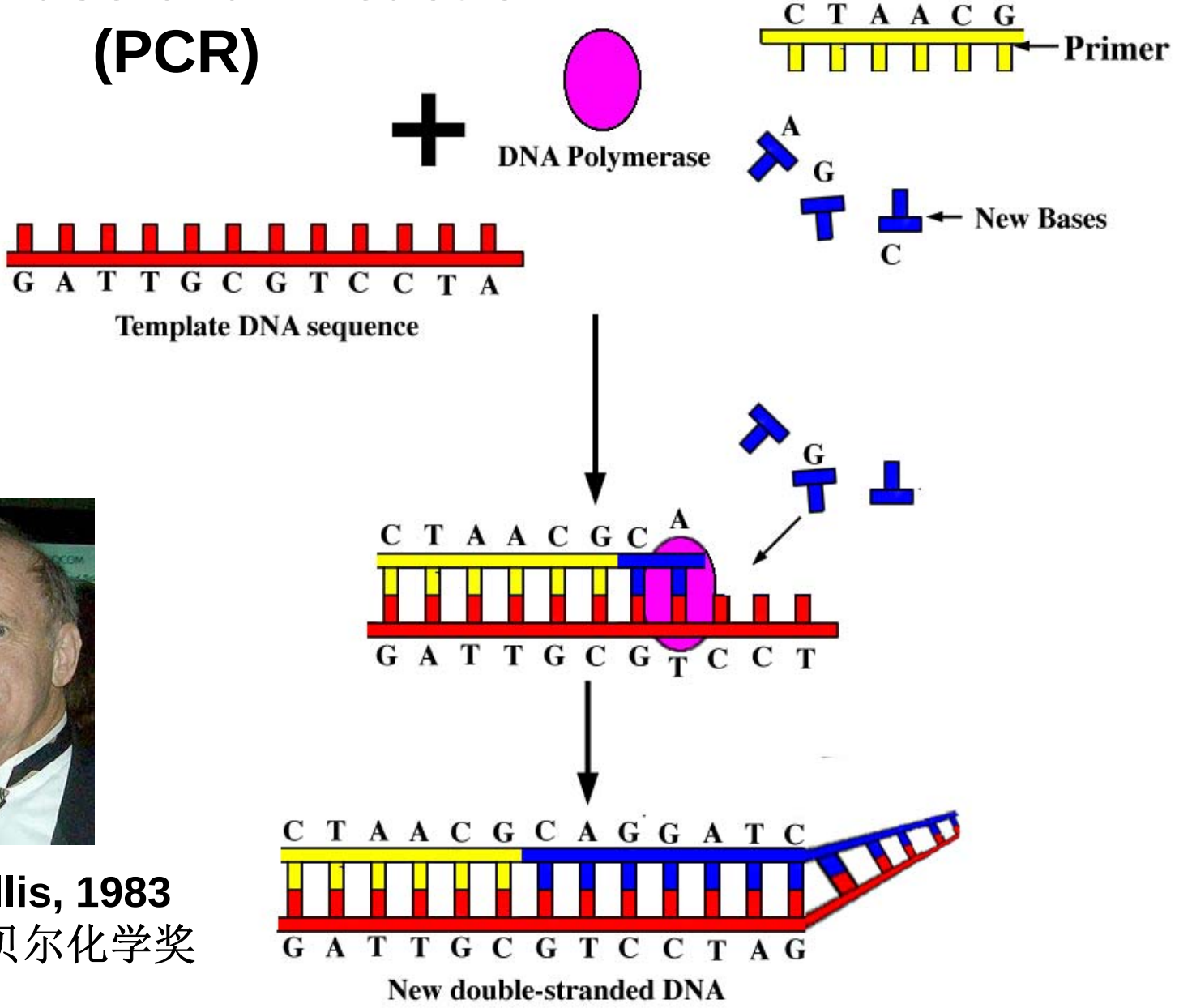
1980：DNA生化研究 & DNA测序

Paul Berg

Walter Gilbert

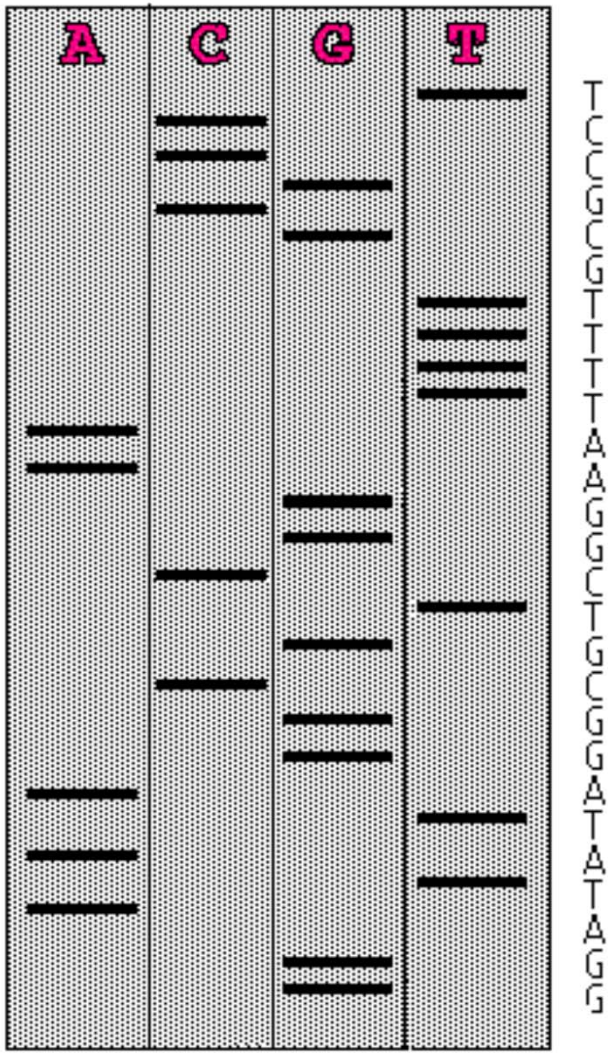
Frederick Sanger

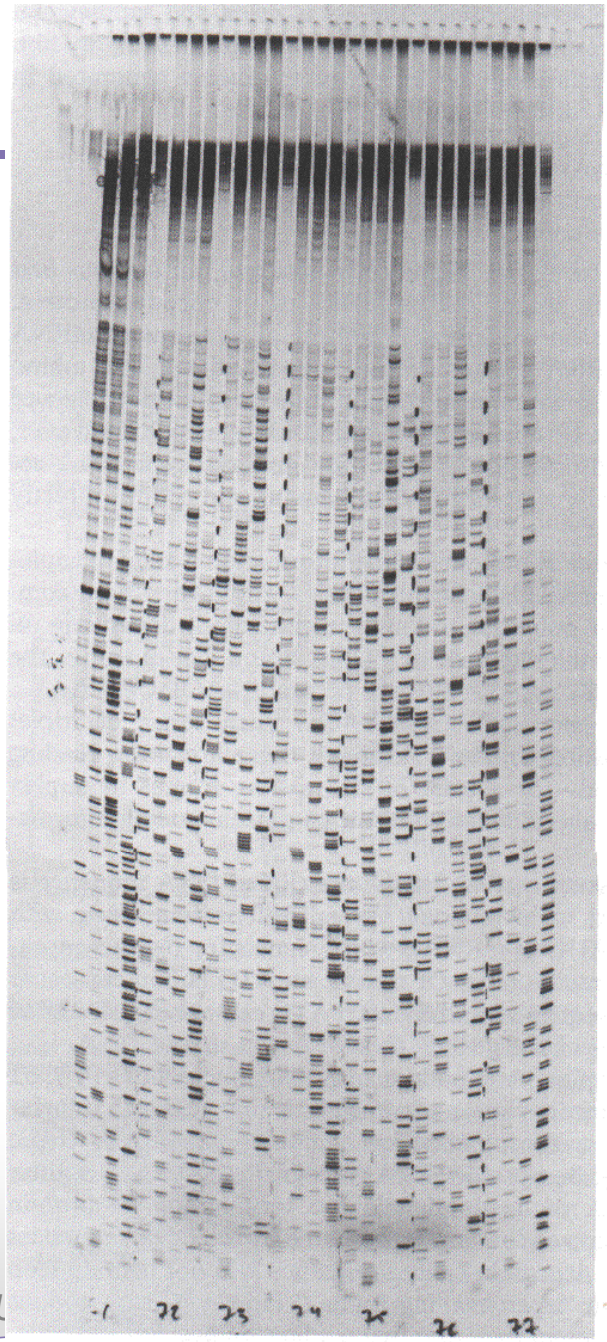
Polymerase chain reaction (PCR)



Kary Mullis, 1983
1993年诺贝尔化学奖

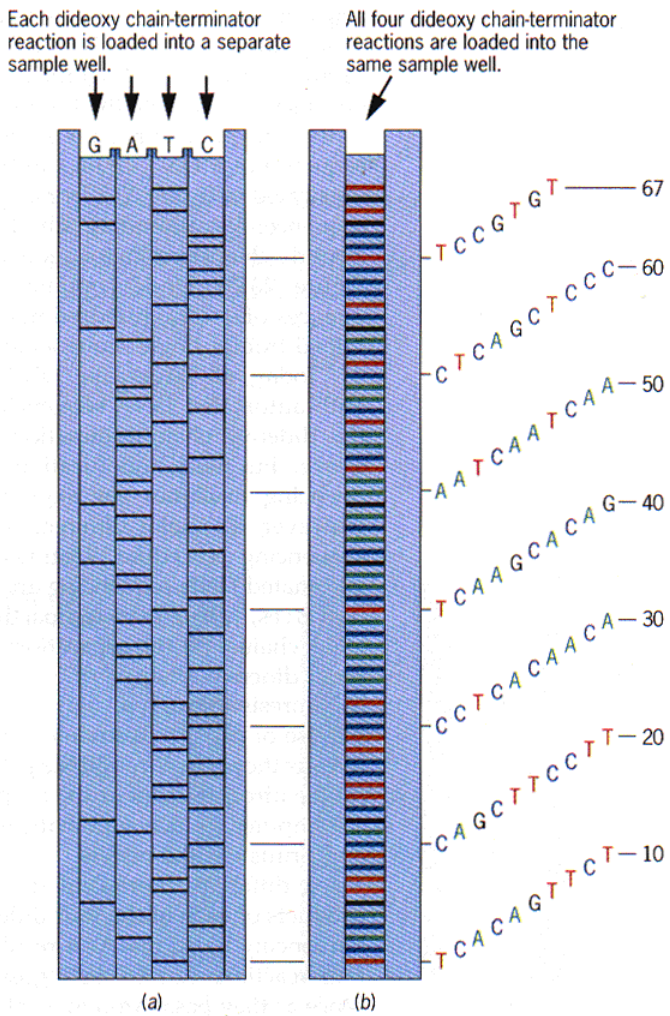
Loading each ddNTP reaction in a different lane:

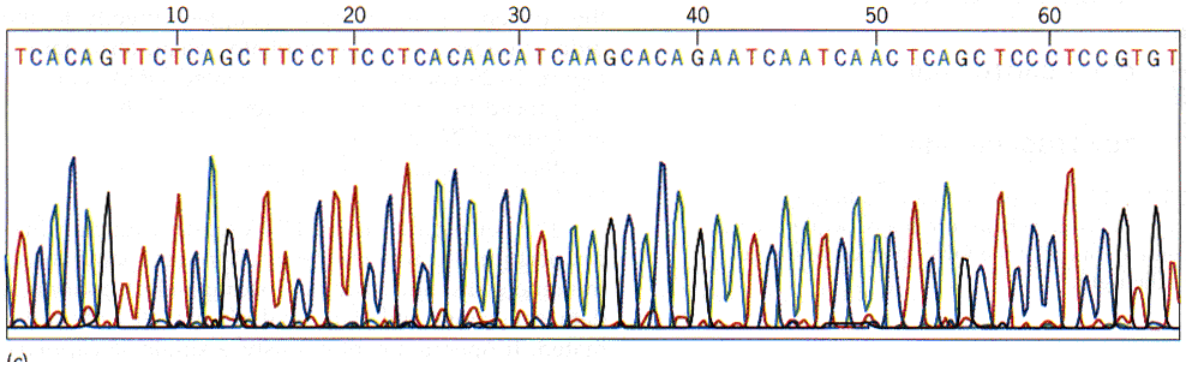


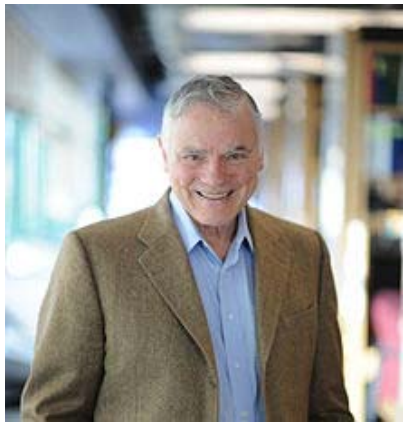


自动测序

- “假如你真想改变一门学科，发明一些新的技术，这将使你超越以前人们曾经所看到的”
- 1980s，Leroy Hood发展了为4种终止核苷酸碱基标定不同荧光颜色的方法，改进了测序技术
- 4种碱基可在同一个反应中测序，并在一个条带中排列显示
- Hood: 利用计算机收集整理数据的先驱


Each dideoxy chain-terminator reaction is loaded into a separate sample well.
All four dideoxy chain-terminator reactions are loaded into the same sample well.
G A T C
67
60
50
40
30
20
10
(a)
(b)


10
20
30
40
50
60
TCACAGTTCTCAGCTTCCTTCCTCACAACATCAAGCACAGAATCAATCAACTCAGCTCCCTCCGTGT



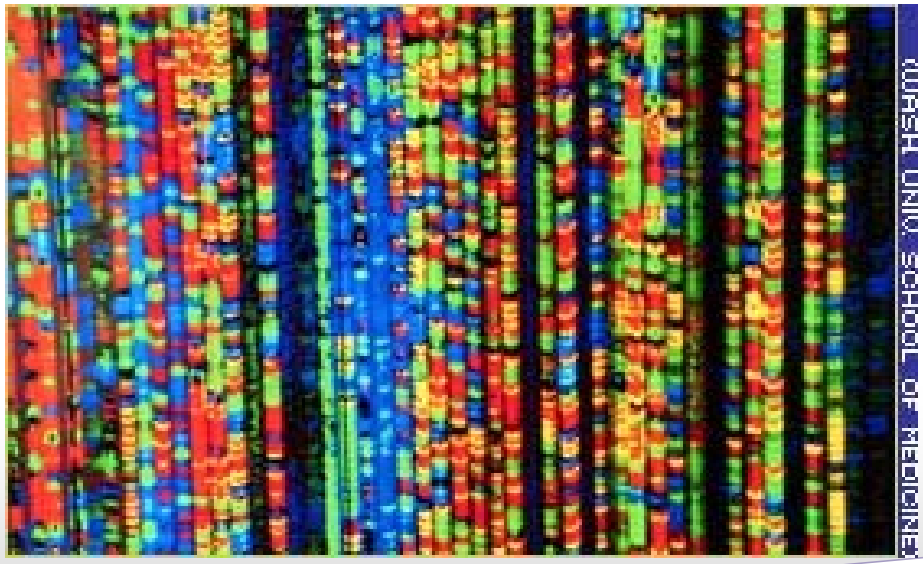
Leroy Hood

第一代测序：自动测序仪

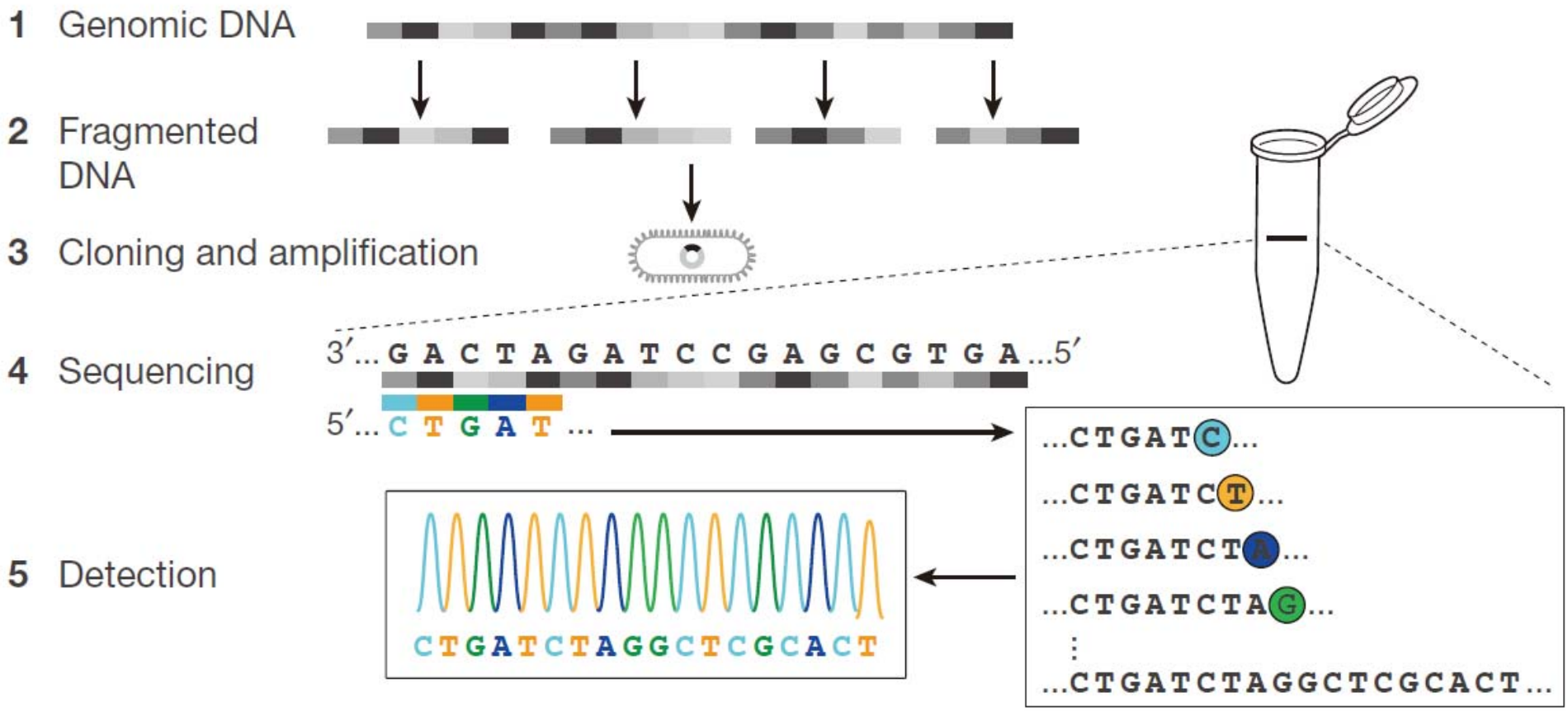
- 1986，Model 370A DNA Sequencing System
  - Applied Biosystems, Inc (ABI)
  - 1987，ABI 370
  - 利用四种颜色，可直接阅读四种碱基

Company History

Year	Company Name
1981	Genetic Systems Company (GeneCo)
1982	Applied Biosystems, Inc. (ABI)
1993	Applied Biosystems, Perkin-Elmer
1996	PE Applied Biosystems
1998	PE Biosystems
2000	Applied Biosystems Group, Applera Corp
2002	Applied Biosystems
2008	Life Technologies
2014	Thermo Fisher Scientific



第一代测序


1 Genomic DNA
2 Fragmented DNA
3 Cloning and amplification
4 Sequencing
5 Detection
3′... G A C T A G A T C C G A G C G T G A ...5′
5′... C T G A T ...
...CTGATC...
...CTGATCT...
...CTGATCTA...
...CTGATCTAG...
...CTGATCTAGGCTCGCACT...
CTGATCTAGGCTCGCACT

第二代测序

- Next Generation Sequencing, NGS
- Read: 读段

454

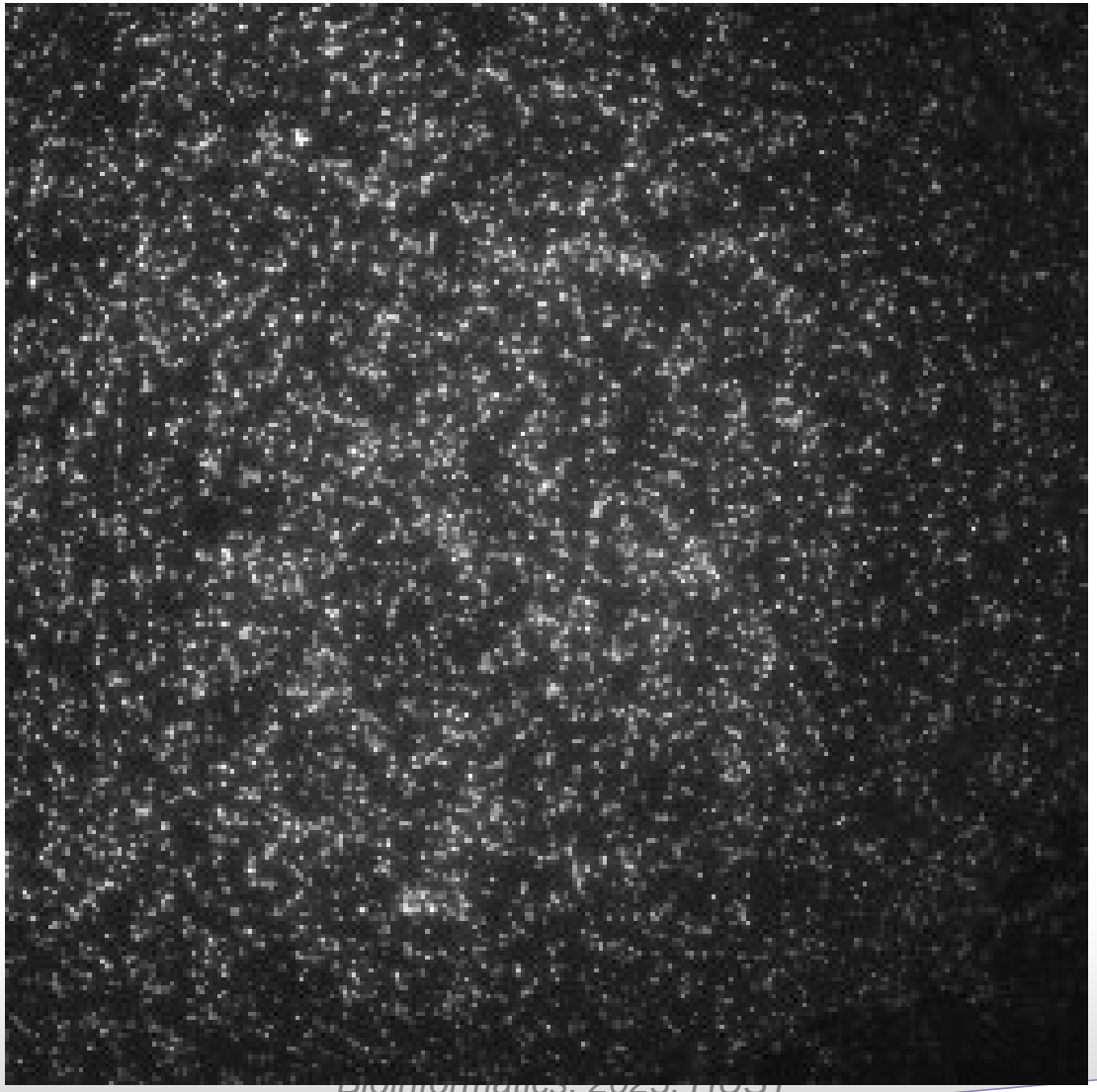
Illumina/Solexa

ABI-SOLID

纳米技术

- 每个系统的测序方式不同，但基本原理相似：
  - 将待测序的DNA切断成小的片段
  - 将每个DNA分子固定到固态材料的表面
  - 同时扩增每一个分子
  - 一次添加一个碱基，然后检测不同的信号：A, C, T, & G
  - 需要高分辨图像处理技术
    - (Solexa has 800 images @ 4 megapixels each)

Illumina/Solexa测序仪上的小区（tile）

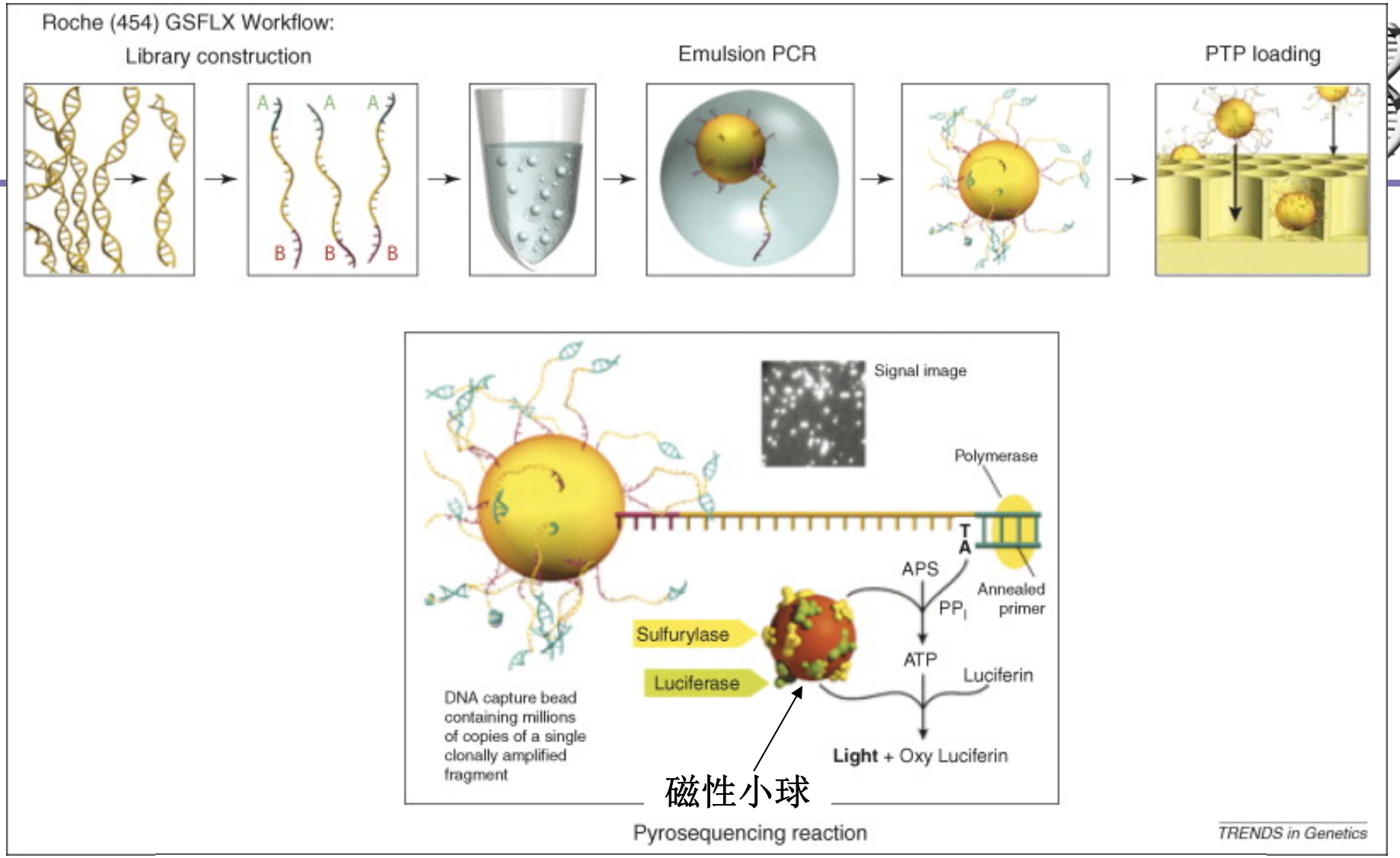


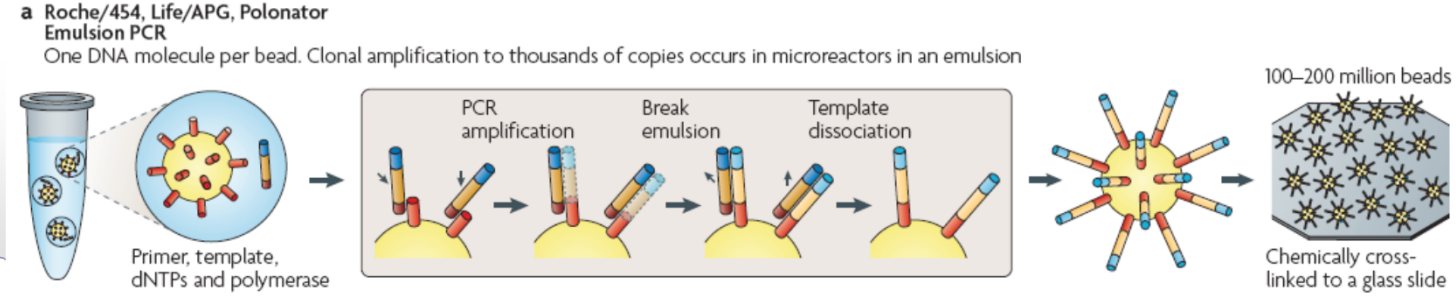
巨大容量的图像数据

- 原始图像的数据规模：TB级
  - Solexa =
  - ABI-SOLID：>
  - 454：<
- 图像数据立即被处理成亮度数据（点的位置以及亮度）
- 亮度数据需要被处理成碱基（basecall）（A, C, T, or G, 以及每一个碱基的概率分值）

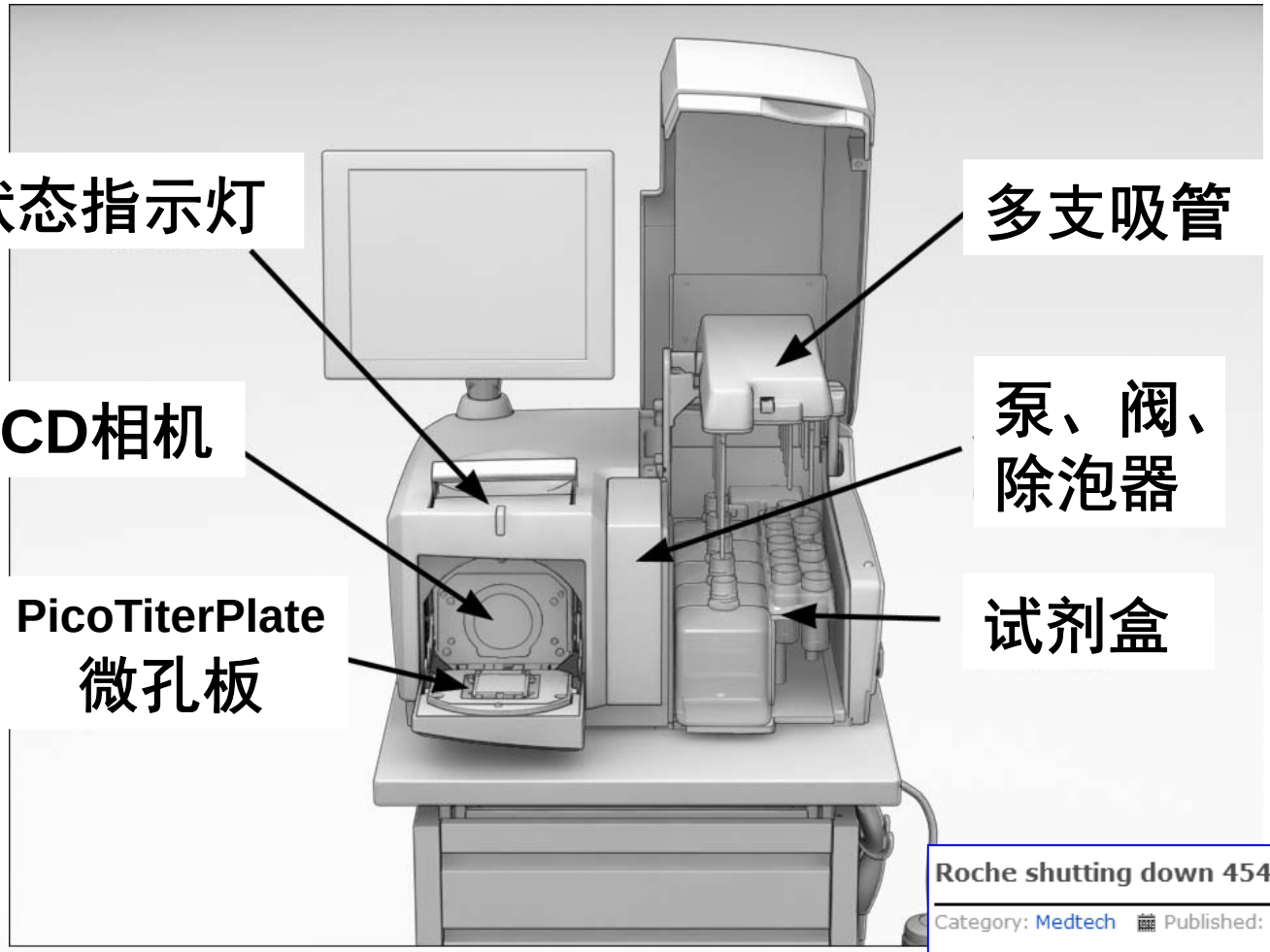
454测序原理

- 第一种的高通量DNA测序仪
- 2004年实现商业化
- 10个小时内可同时测定8个样本
- 焦磷酸测序（pyrosquencing）：
  - A. 液相反应
  - B. 一个bead上可扩增并连接百万个单一分子
  - C. 碱基上分解的焦磷酸引起发光反应，被扫描成图像
  - D. 每次扩增一个碱基
- 碱基类型的错误极少，存在插入/缺失错误
- Polonator: 原理相似，序列较短（26bp）


Roche (454) GSFLX Workflow:
Library construction
Emulsion PCR
PTP loading
A
B
Signal image
Polymerase
T
A
APS
PPi
Annealed primer
Sulfurylase
Luciferase
ATP
Luciferin
DNA capture bead containing millions of copies of a single clonally amplified fragment
Light + Oxy Luciferin
磁性小球
Pyrosequencing reaction
TRENDS in Genetics


a Roche/454, Life/APG, Polonator
Emulsion PCR
One DNA molecule per bead. Clonal amplification to thousands of copies occurs in microreactors in an emulsion
Primer, template, dNTPs and polymerase
PCR amplification
Break emulsion
Template dissociation
100–200 million beads
Chemically cross-linked to a glass slide

GS FLX+ System (454)



读长：
最大**1000bp**
平均**700bp**

覆盖度：
>500bp: 85%碱基
>700bp: 45%碱基

通量：**700Mb**

每轮读段**1M**条

准确性：**99.997%**

每轮时间：**23**小时

Roche shutting down 454 sequencing business

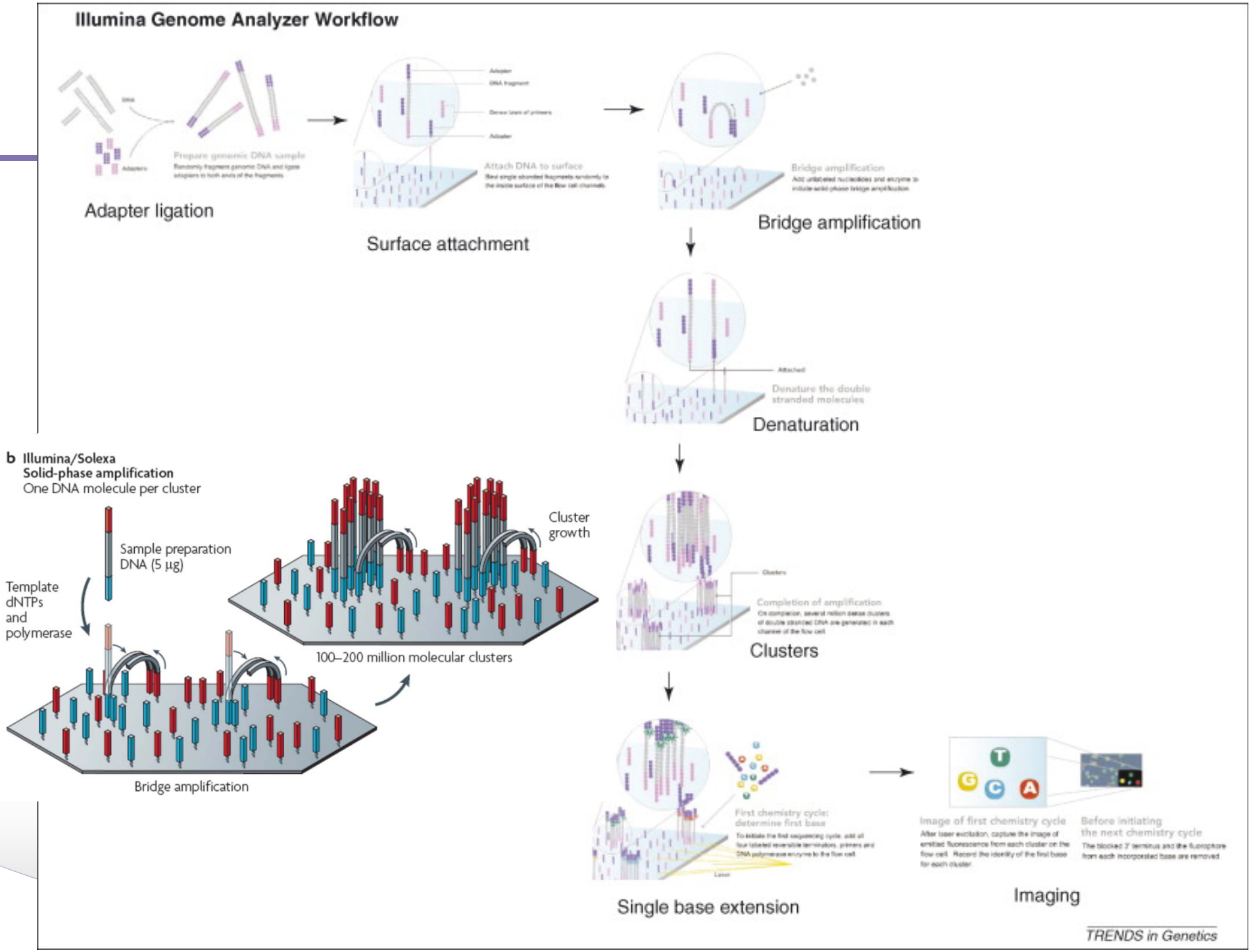
Category: Medtech Published: 21 October 2013

Roche is shuttering its 454 life sciences sequencing operations and laying off about 100 employees, the company confirmed.

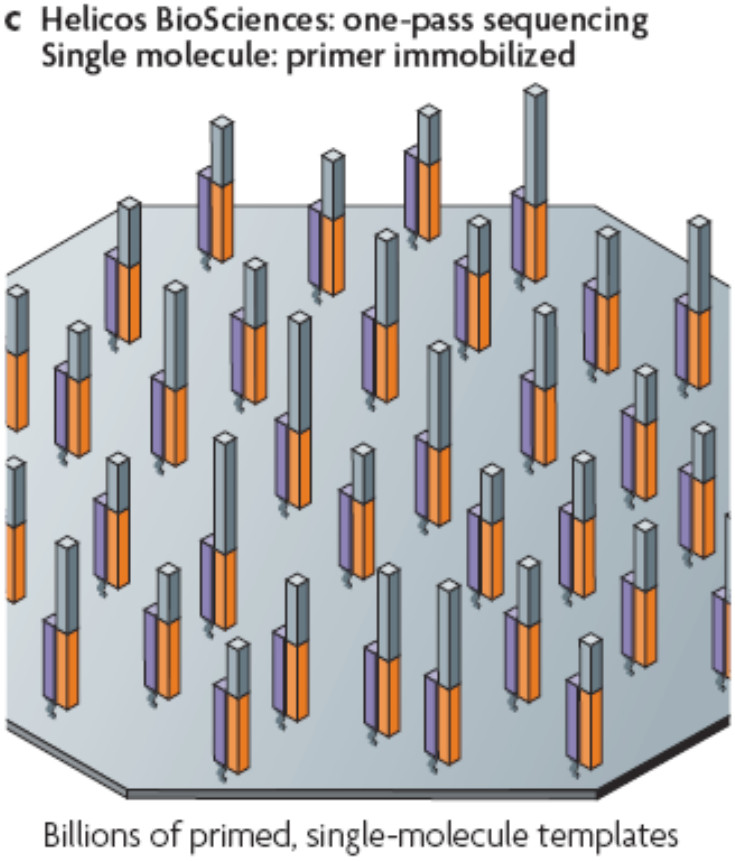
The 454 sequencers will be phased out in mid-2016, and the 454 facility in Branford, Conn., will be closed "accordingly," Roche said in a statement e-mailed to GenomeWeb Daily News.

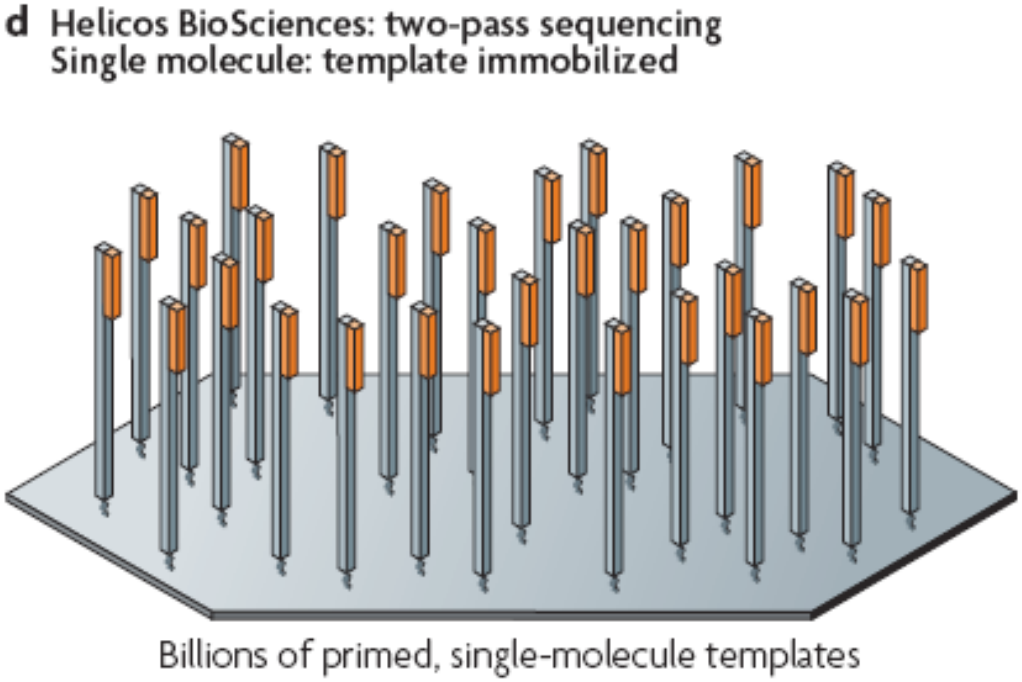
Illumina Genome Analyzer

- 最早由Solexa开发设计，现在是Illumina的子公司
- 2006年实现商业化
- 每轮实验: 7个样本/3日
- 低错误率，主要是碱基类型错误，有少量的插入/缺失
- Sequencing by synthesis


Illumina Genome Analyzer Workflow
Adapter ligation
Surface attachment
Bridge amplification
Denaturation
Clusters
Single base extension
Imaging
b Illumina/Solexa
Solid-phase amplification
One DNA molecule per cluster
Sample preparation
DNA (5 µg)
Template
dNTPs
and
polymerase
Cluster
growth
100–200 million molecular clusters
Bridge amplification
TRENDS in Genetics

Helicos BioSciences

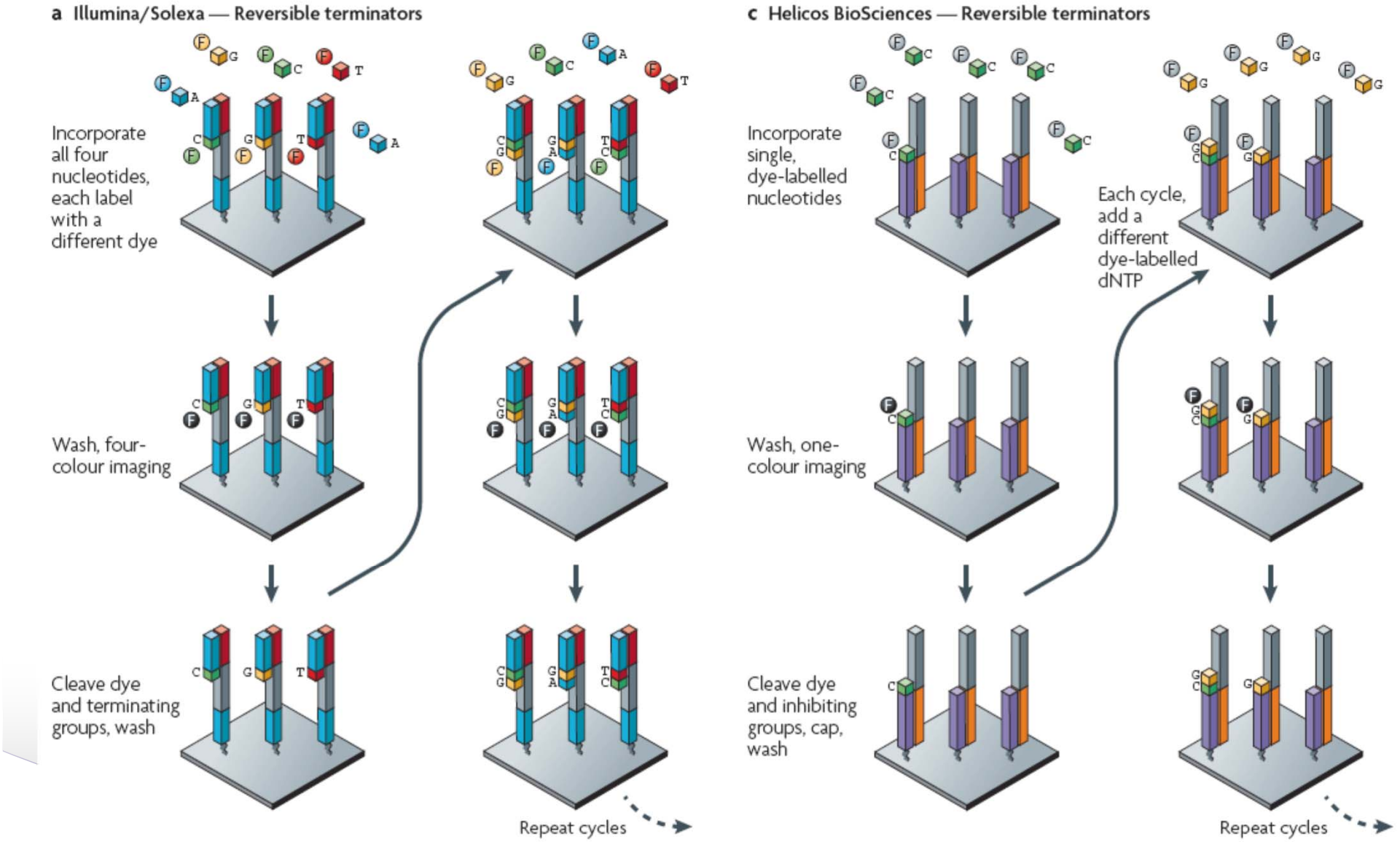




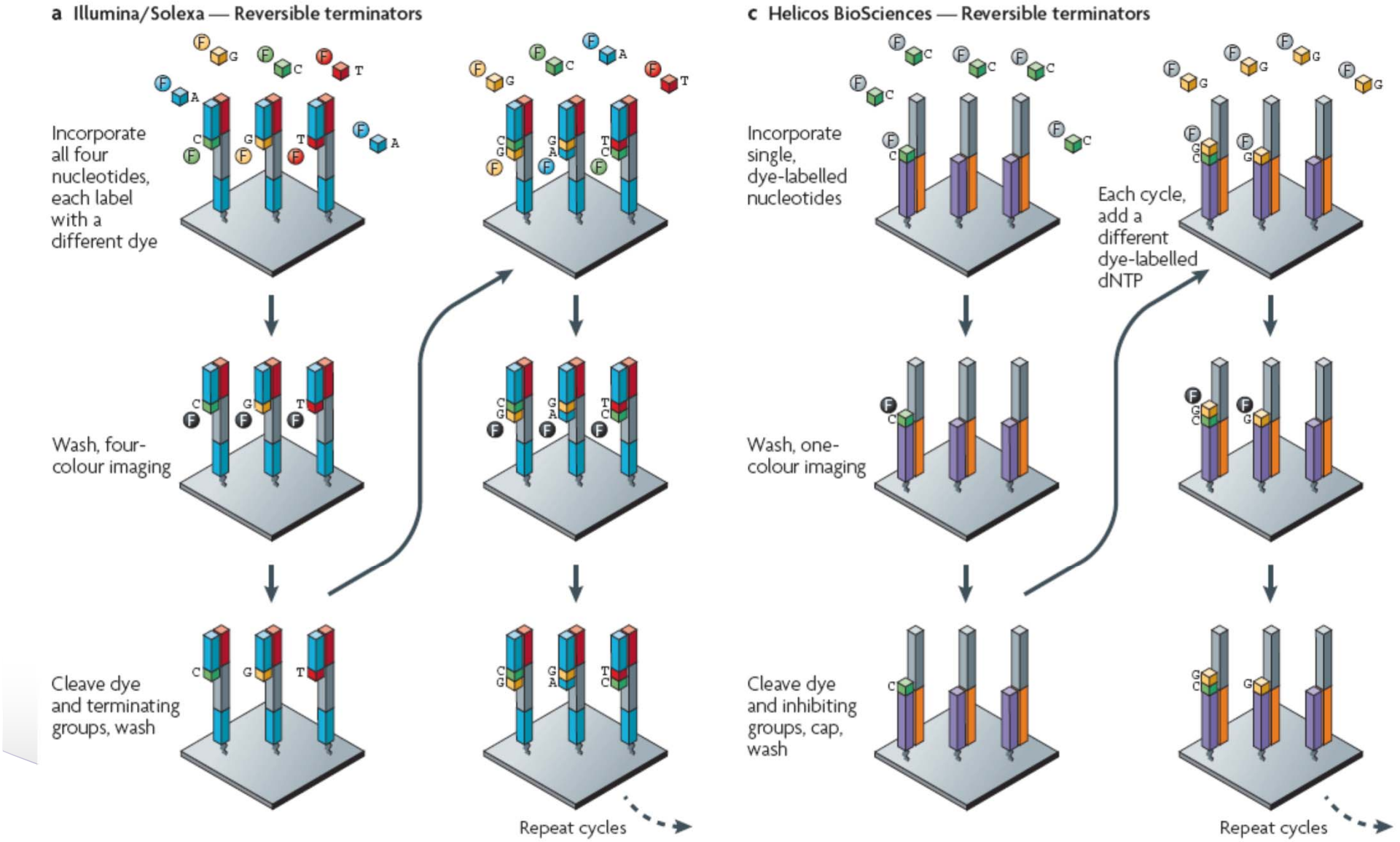
Adaptor固定，然后接模板

模板先固定，然后接**Adaptor**

Illumina/Solexa *vs*. Helicos BioSciences

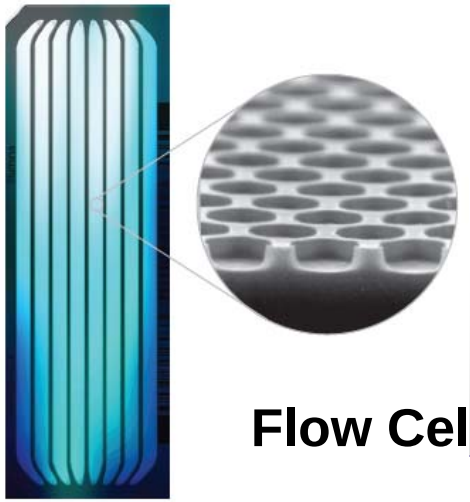


Illumina/Solexa *vs*. Helicos BioSciences



HiSeq 3000/HiSeq 4000 (illumina)

	HiSeq 3000	HiSeq 4000
Flow Cell数	1	1或2
通量/天	>200Gb	>400Gb
每轮时间	<1-3.5天	<1-3.5天
每轮测人类基因组套数	6	12



Flow Cell

HiSeq X Ten (illumina)

- 由10台HiSeq X组成
- 每年测人类基因组：>18,000个
- 单个人类基因组 (30X)：<$1,000
- 每轮通量：1.6-1.8Tb
- 每轮时间：<3天

MiSeq：第一个FDA批准的NGS设备

- 2013年11月，FDA: 美国食品和药物管理局 (Food and Drug Administration)
- “Personalized medicine”: 利用个人的遗传信息为疾病的检测、治疗和预防提供更为精准的方法
- Illumina MiSeq Dx

Illumina – 桌面式测序仪

SBS: Sequencing by synthesis

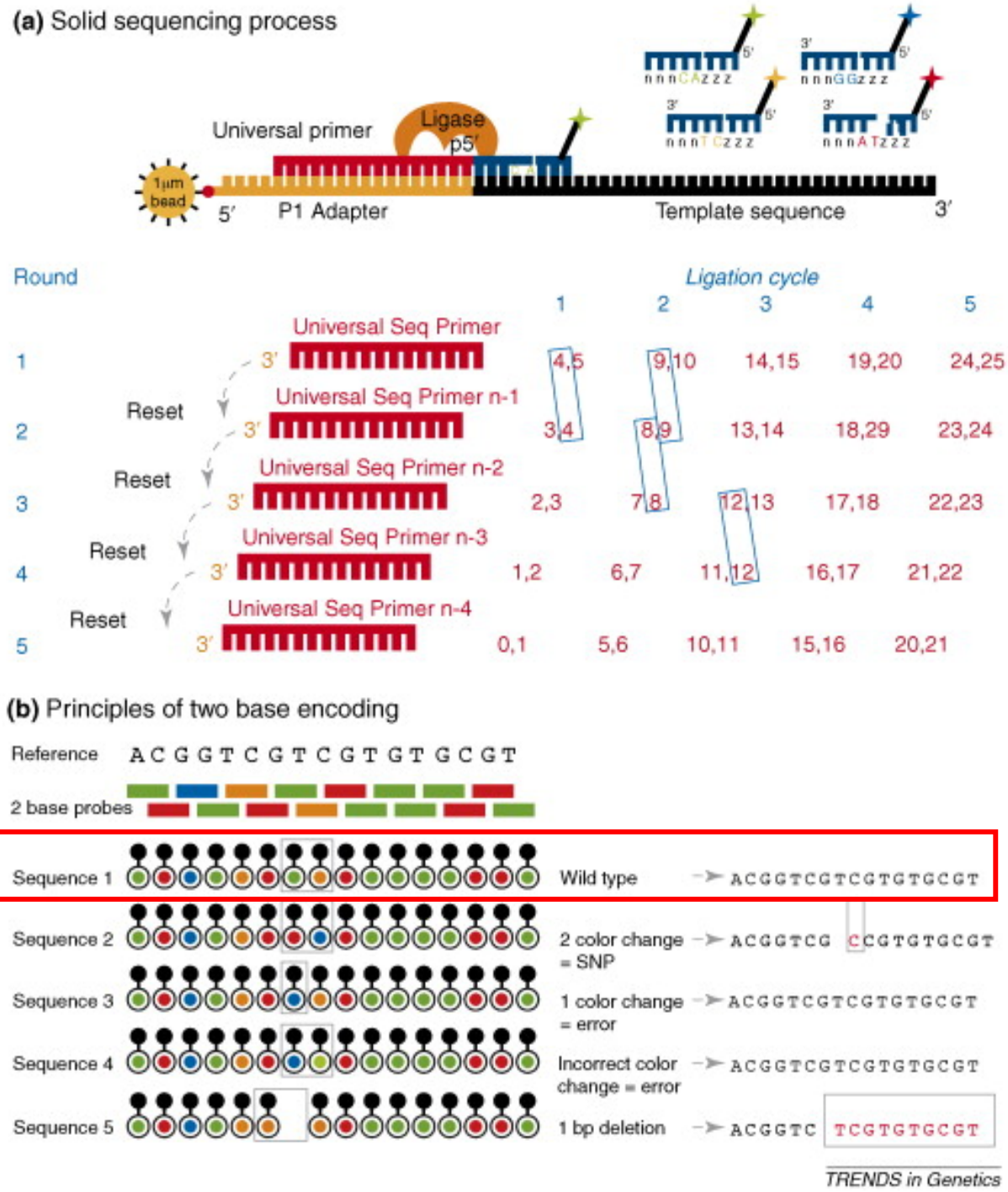
	桌面式测序仪		生产规模的测序仪	
	 iSeq 100系统	 MiniSeq系统	 MiSeq系列	 NextSeq系列
常用应用和方法	关键应用	关键应用	关键应用	关键应用
大型全基因组测序（人类、植物、动物）				●
小型全基因组测序（微生物、病毒）	●	●	●	●
外显子组测序				●
靶向基因测序（扩增子、基因panel）	●	●	●	●
全转录组测序				●
使用mRNA-Seq进行基因表达谱分析				●
靶向基因表达谱分析	●	●	●	
长片段扩增子测序*	●	●	●	
miRNA和Small RNA分析	●	●	●	●
DNA-蛋白质相互作用分析			●	●
甲基化测序				●
16S宏基因组测序	●	●	●	●
运行时间	9–17.5小时	4–24小时	4–55小时	12–30小时
最大数据产出	1.2 Gb	7.5 Gb	15 Gb	120 Gb
每次运行获得的最大read数	4 M	25 M	25 M†	400 M
最长读长	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp

Illumina – 生产规模的测序仪

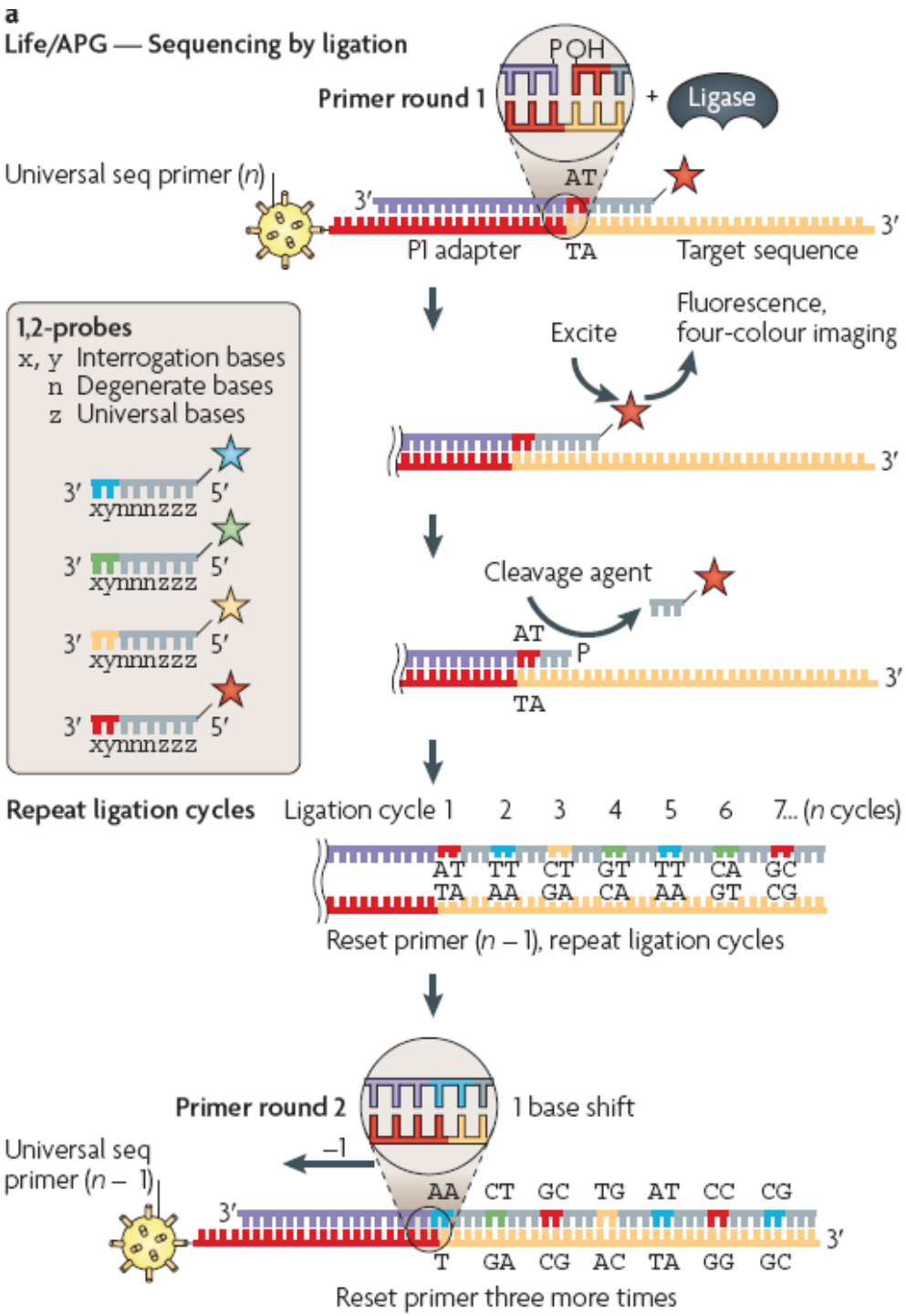
	桌面式测序仪	生产规模的测序仪		
				
	NextSeq系列	HiSeq系列	HiSeq X系列‡	NovaSeq系列
常用应用和方法	关键应用	关键应用	关键应用	关键应用
大型全基因组测序（人类、植物、动物）	●	●	●	●
小型全基因组测序（微生物、病毒）	●	●		●
外显子组测序	●	●		●
靶向基因测序（扩增子、基因panel）	●	●		●
全转录组测序	●	●		●
使用mRNA-Seq进行基因表达谱分析	●	●		●
miRNA和Small RNA分析	●	●		●
DNA-蛋白质相互作用分析	●	●		●
甲基化测序	●	●		●
鸟枪法宏基因组学测序	●	●		●
运行时间	12–30小时	<1–3.5天（HiSeq 3000/HiSeq 4000） 7小时–6天（HiSeq 2500）	<3天	19–40小时§
最大输出	120 Gb	1500 Gb	1800 Gb	6000 Gb‖
每次运行获得的最大read数	400 M	5 B	6 B	20 B**
最长读长	2 × 150 bp	2 × 150 bp	2 × 150 bp	2 × 150 bp

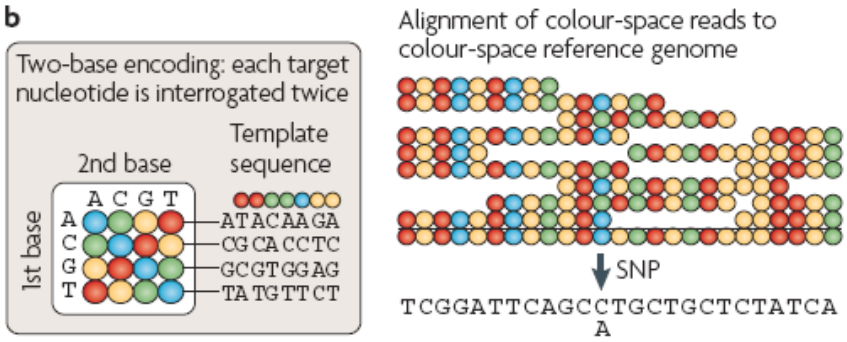
ABI-SOLID

- 2007年实现商业化
- 每周产生20GB的数据
- 每轮: 6GB
- Sequencing by ligation
- “2 based encoding”
- “color-space” data

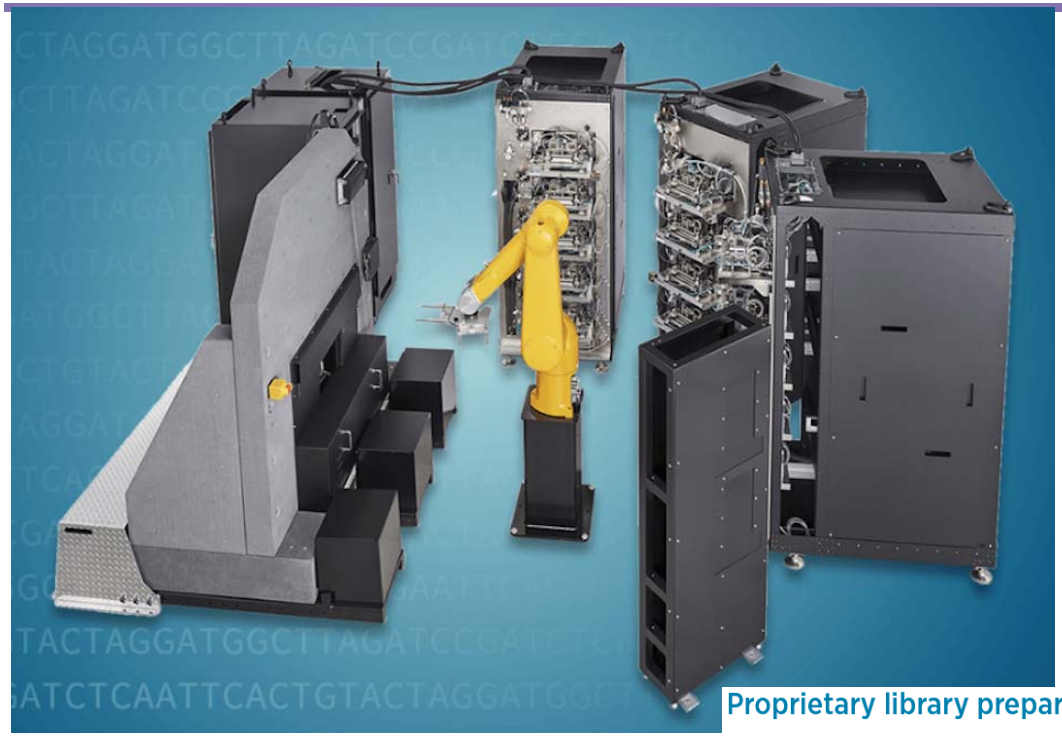

(a) Solid sequencing process
Universal primer
Ligase
p5′
1μm bead
5′
P1 Adapter
Template sequence
3′
nnnCAzzz
nnnGGzzz
nnnTCzzz
nnnATzzz
Round
Ligation cycle
1
2
3
4
5
Universal Seq Primer
Universal Seq Primer n-1
Universal Seq Primer n-2
Universal Seq Primer n-3
Universal Seq Primer n-4
Reset
4,5
9,10
14,15
19,20
24,25
3,4
8,9
13,14
18,29
23,24
2,3
7,8
12,13
17,18
22,23
1,2
6,7
11,12
16,17
21,22
0,1
5,6
10,11
15,16
20,21
(b) Principles of two base encoding
Reference
A C G G T C G T C G T G T G C G T
2 base probes
Sequence 1
Wild type
ACGGTCGTCGTGTGCGT
Sequence 2
2 color change = SNP
ACGGTCG CCGTGTGCGT
Sequence 3
1 color change = error
ACGGTCGTCGTGTGCGT
Sequence 4
Incorrect color change = error
ACGGTCGTCGTGTGCGT
Sequence 5
1 bp deletion
ACGGTC TCGTGTGCGT
TRENDS in Genetics

ABI-SOLID


a
Life/APG — Sequencing by ligation
Primer round 1
P OH
Ligase
Universal seq primer (n)
3′
AT
3′
P1 adapter
TA
Target sequence
1,2-probes
x, y Interrogation bases
n Degenerate bases
z Universal bases
3′ xynnnzzz 5′
Excite
Fluorescence, four-colour imaging
Cleavage agent
AT
P
TA
Repeat ligation cycles
Ligation cycle 1 2 3 4 5 6 7... (n cycles)
AT TT CT GT TT CA GC
TA AA GA CA AA GT CG
Reset primer (n – 1), repeat ligation cycles
Primer round 2
1 base shift
–1
Universal seq primer (n – 1)
AA CT GC TG AT CC CG
T GA CG AC TA GG GC
Reset primer three more times

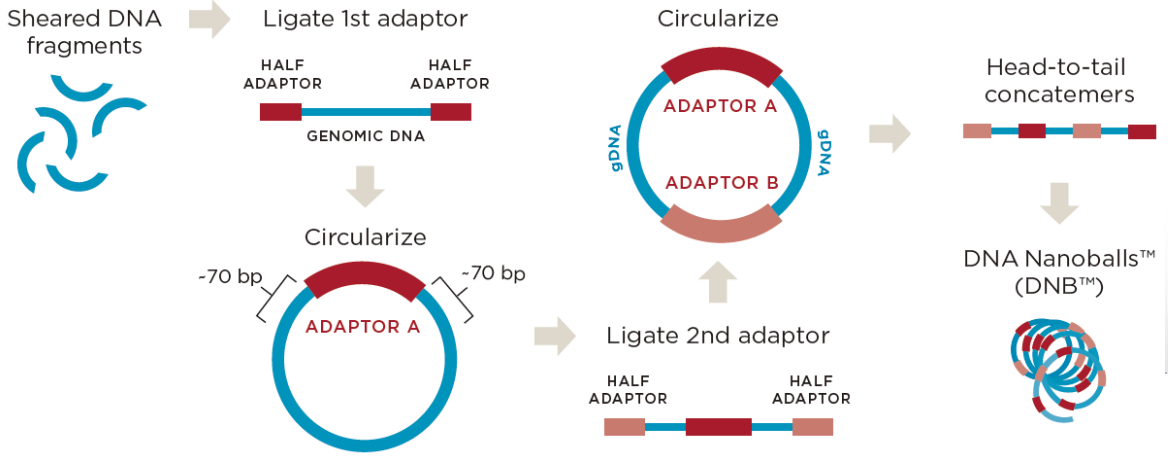

b
Two-base encoding: each target nucleotide is interrogated twice
2nd base
Template sequence
A C G T
1st base
A
C
G
T
ATACAAGA
CGCACCTC
GCGTGGAG
TATGTTCT
Alignment of colour-space reads to colour-space reference genome
SNP
TCGGATTCAGCCTGCTGCTCTATCA
A

Revolocity：Complete Genomics



IMPACT OF CORRELATION ANALYSIS ON FALSE-POSITIVE RATE		
Outcome	Without correlation analysis	With correlation analysis
SNP Sensitivity	98.5%	98.3%
SNP False-Positive Rate	12%	2.1%
Indel Sensitivity	83.4%	83.1%
Indel False-Positive Rate	26.3%	17.8%

Proprietary library preparation—the first step to quality results



华大基因（BGI）

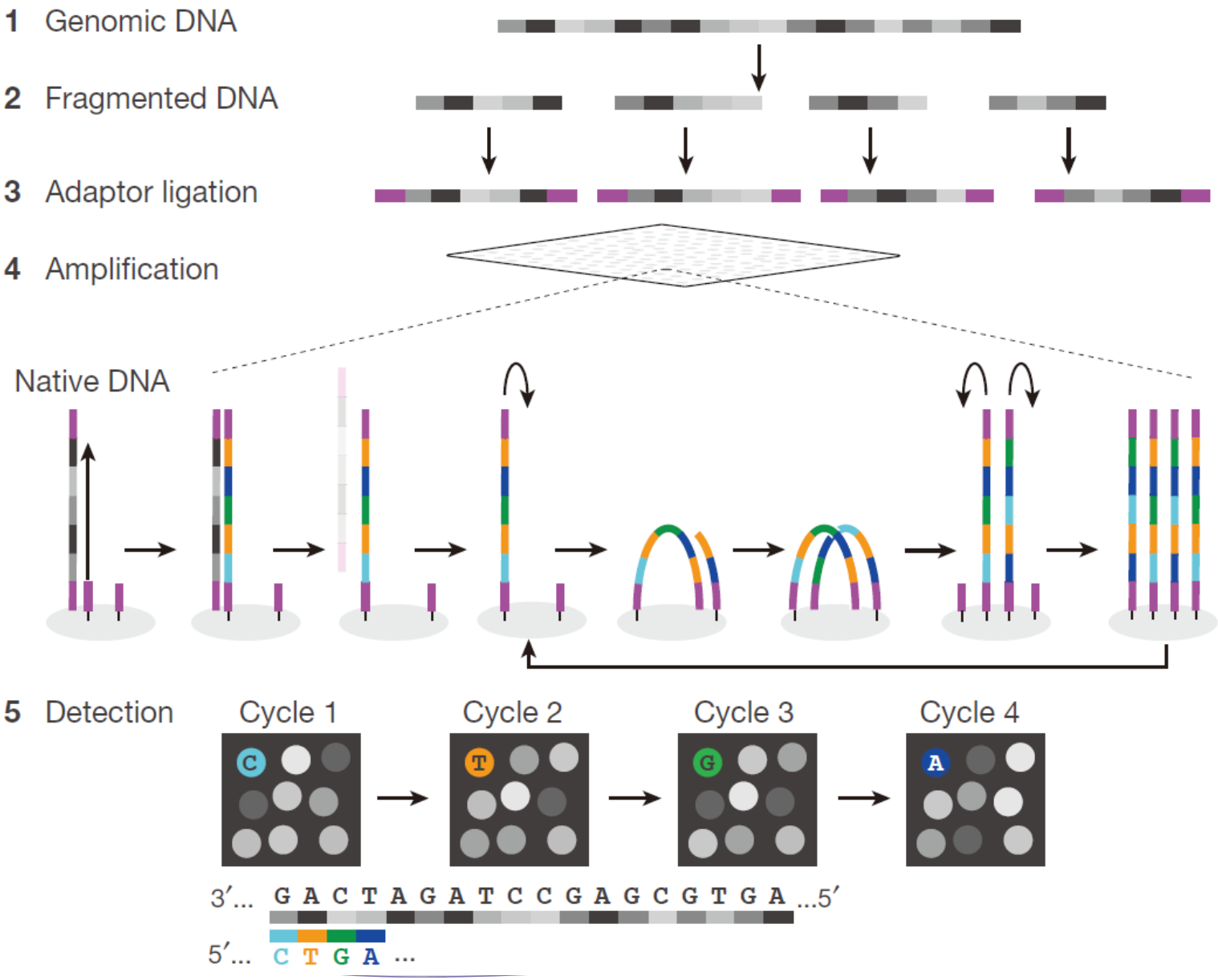
BGISEQ：桌面化测序系统

BGISEQ-500

BGISEQ-50

	BGISeq-500	NextSeq-500
通常	8-200G	25-120G
读长	50SE/50PE/100SE/100PE	75SE/75PE/150PE
质量	Q30 bases > 85%	Q30 bases > 75%/80%
运行时间	~24h	12-30h

第二代测序 (Massively parallel)



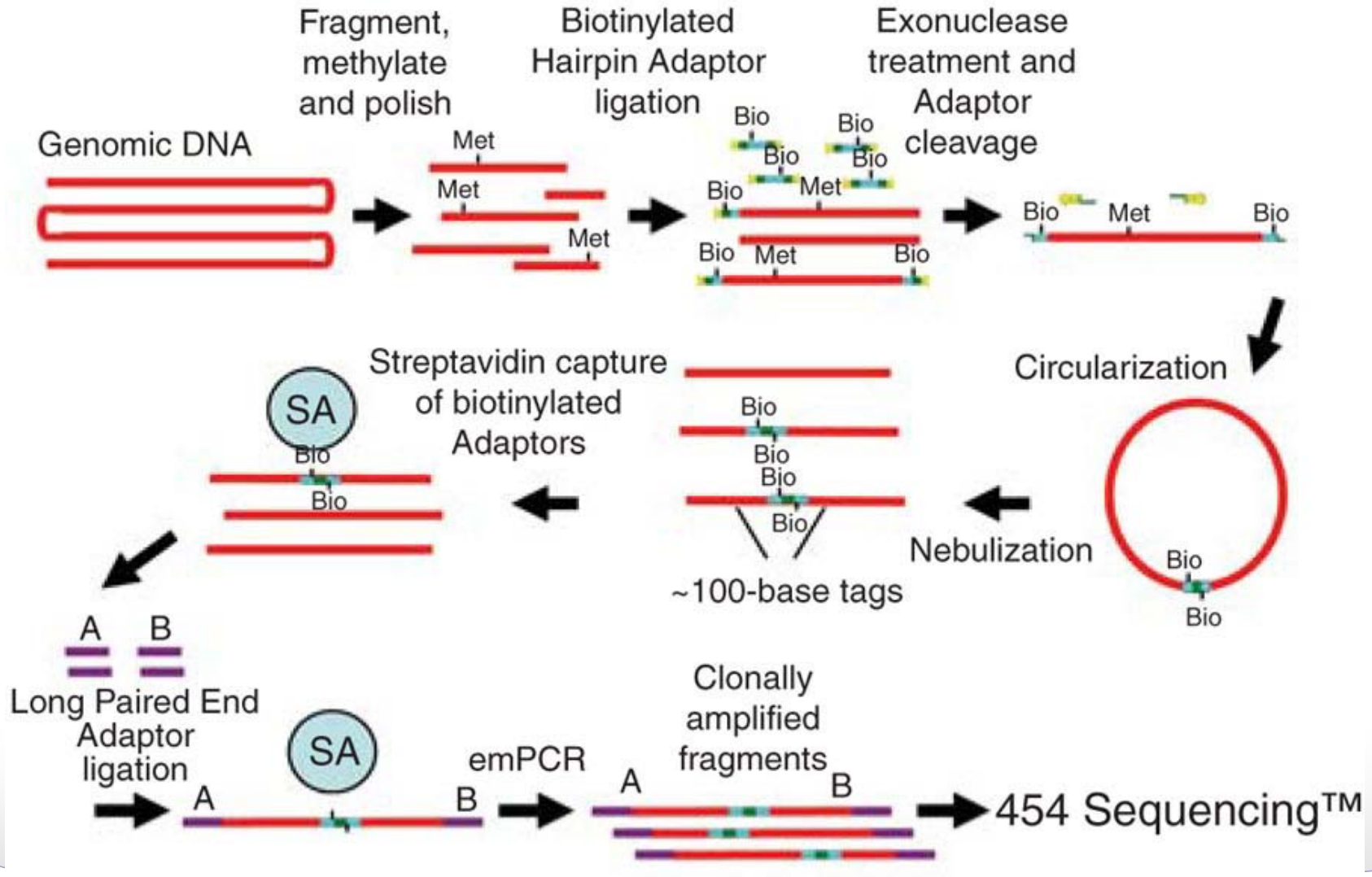
每GB数据的花费 (2008)

- Solexa: ~$6,000 per GB
- ABI-SOLID: $6,000 per GB
- 454: $85,000 per GB
- 读段越长越有价值
- 人类基因组3GB，但是1x coverage不能完成拼装
- 2015：RNA-seq, ~$1,000/GB
- 2017：RNA-seq, ~$10/GB (65~80RMB)

短读段

- 第二代测序仪产生短读段 (Solexa = 36 bp, 2008)
  - 全基因组组装困难
  - 重复区域难以确认和组装
- 需要非常多重的覆盖率
- 解决方案：
  - 新算法
  - 更长的读段

Paired-End Sequencing (3KB)



第三代测序：PacBio Systems

- The “third-generation sequencing”： long-read sequencing
- Single Molecule, Real-Time (SMRT) technology
- DNA聚合酶固定
- DNA单分子通过纳米尺度的小孔
- “real-time” sequencing

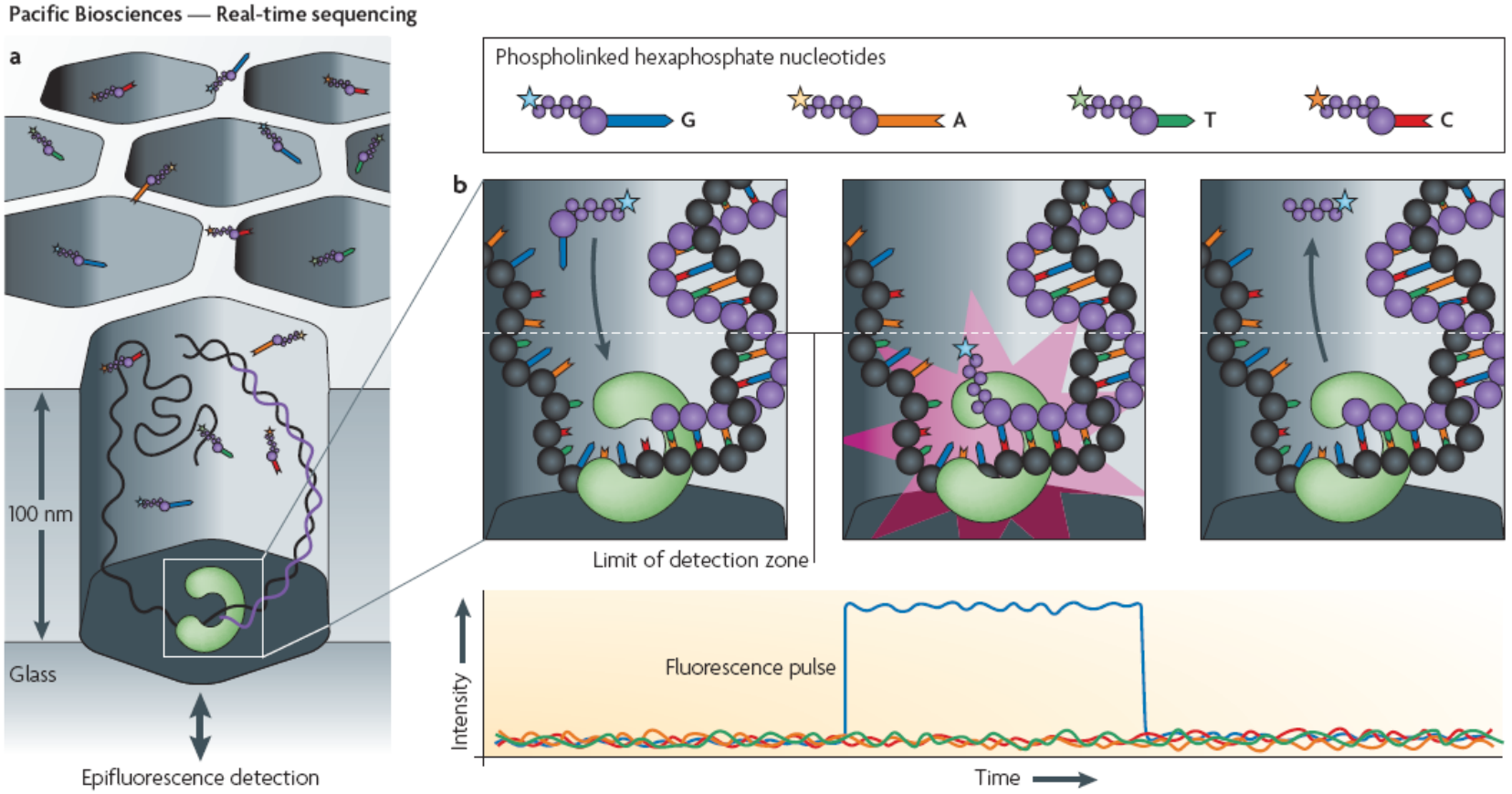
PacBio RS II

Sequel

	RS II	Sequel
平均读长	10～15kb	8～12kb
ZMWs	15万	100万
数据量 / SMRTCell	500Mb~1Gb	5～10Gb
SMRTCell No./Run	1~16	1~16
Run time / SMRTCell	0.5~6hrs	0.5~6hr
Multiplex Amplicons	384	1536

PacBio Systems测序原理

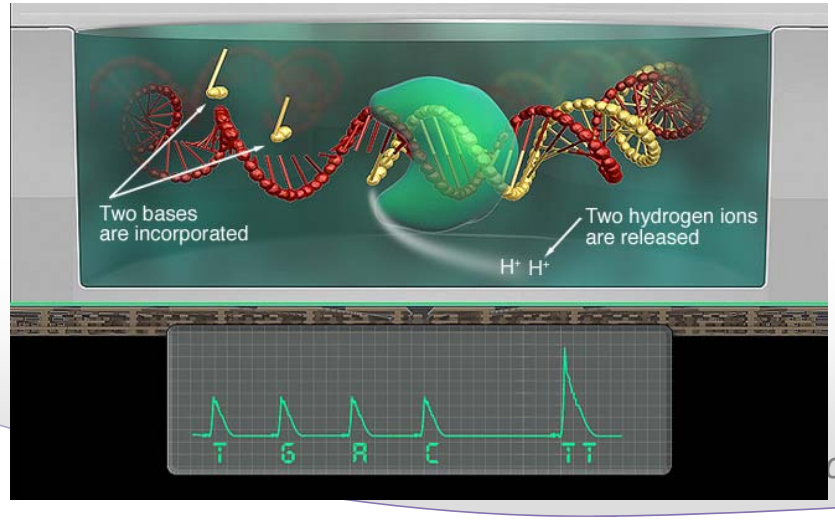
- ZMW孔（Zero-Mode Waveguides, 零模波导孔）：微孔直径小于光的波长时，光强会急剧衰减
- 孔底部仅能固定单分子DNA聚合酶和单分子DNA



Ion Torrent™ (Life Technologies)

Criteria	Ion Torrent Proton	Illumina HiSeq 2500
System Price	$243,000	$740,000
Annual Service cost (yr 2 & 3)	$19,400	$59,200
Per Gb cost	$16.67	$46.00
Per Run Gb	~ 60 Gb (est.)	120 Gb
Per Run cost	$1,000	$5520 (est)
Three year TCO @ 30% utilization	$731,800	$2,100,400
Three year TCO w/ HiSeq Upgrade	$731,800	$1,410,400
Time from library to data	8 hours	27 hours
Throughput per 40h workweek	600 Gb	480 Gb
Accuracy	99%	99%
Expected readlength at launch	200 base-pairs	150 base-pairs
Potential readlength improvement	400 base-pairs	250 base-pairs
Ease of use	+++	+++

测序原理：向 DNA 聚合物中加入 dNTP，会释放出氢离子。采用半导体测定这些氢离子引起的 pH 变化，通过同时测量数百万起此类变化，可以测定各个片段的序列

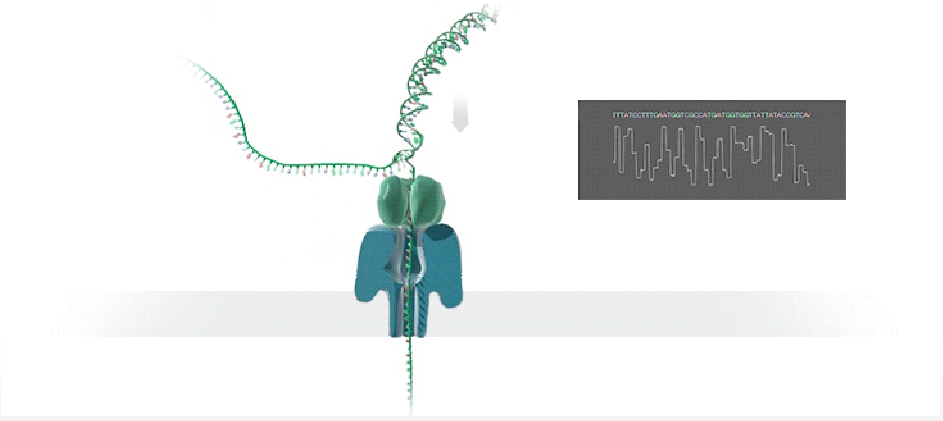


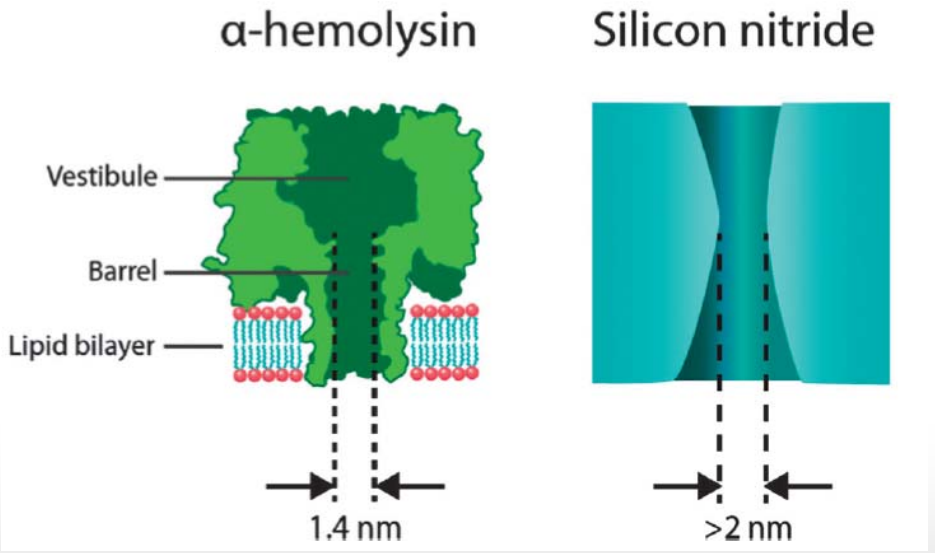
Oxford Nanopore Technologies

- MinION（100 g, USB 3.0）
  - 512个纳米孔通道，样品准备~10分钟
  - 读段>200kb，10-20Gb/48小时
- PromethION
  - 3000个纳米孔通道， 11Tb/48小时

Nanopore测序原理

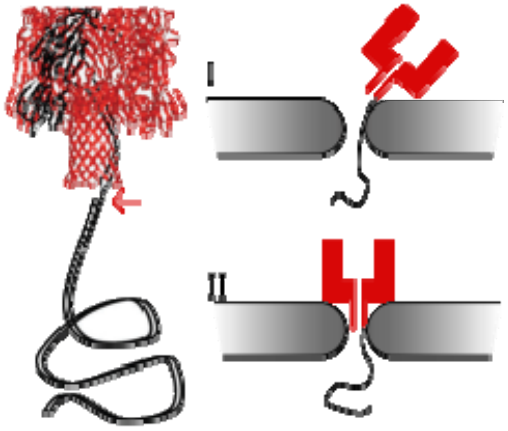
- DNA链的直径：
  - B-DNA (2.0nm); A-DNA (2.6nm)
- Nanopore类型：生物孔和材料孔

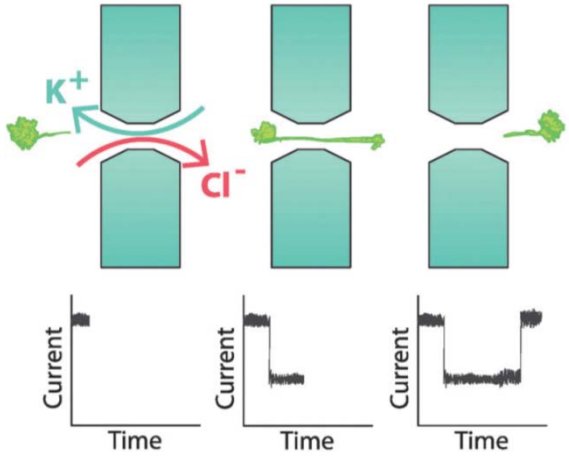




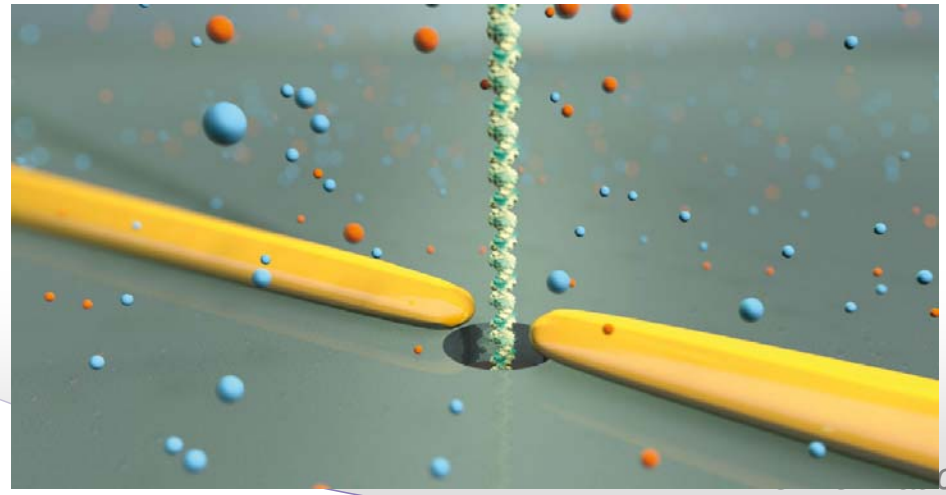
Nanopore测序原理

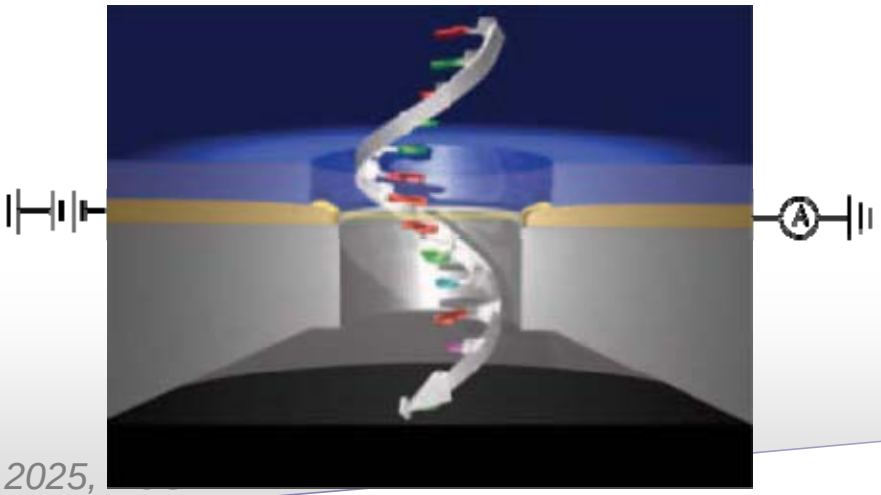
- 膜两侧加电压
  - 开孔电流
  - 阻遏电流





- 纳米孔两侧加电压





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高通量测序方法的比较

Comparison of high-throughput sequencing methods[63][64]

Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	10,000 bp to 15,000 bp avg (14,000 bp N50); maximum read length >40,000 bases[65][66][67]	87% single-read accuracy[68]	50,000 per SMRT cell, or 500–1000 megabases[69][70]	30 minutes to 4 hours[71]	$0.13–$0.60	Fast. Detects 4mC, 5mC, 6mA.[72]	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 600 bp[73]	98%	up to 80 million	2 hours	$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%	1 million	24 hours	$10	Long read size. Fast.	Runs are expensive. Homopolymer errors.
Sequencing by synthesis (Illumina)	MiniSeq, NextSeq: 75-300 bp; MiSeq: 50-600 bp; HiSeq 2500: 50-500 bp; HiSeq 3/4000: 50-300 bp; HiSeq X: 300 bp	99.9% (Phred30)	MiniSeq/MiSeq: 1-25 Million; NextSeq: 130-00 Million, HiSeq 2500: 300 million – 2 billion, HiSeq 3/4000 2.5 billion, HiSeq X: 3 billion	1 to 11 days, depending upon sequencer and specified read length[74]	$0.05 to $0.15	Potential for high sequence yield, depending upon sequencer model and desired application.	Equipment can be very expensive. Requires high concentrations of DNA.
Sequencing by ligation (SOLiD sequencing)	50+35 or 50+50 bp	99.9%	1.2 to 1.4 billion	1 to 2 weeks	$0.13	Low cost per base.	Slower than other methods. Has issues sequencing palindromic sequences.[75]
Nanopore Sequencing[76])	Dependent on library prep, not the device, so user chooses read length. (up to 500 kb reported)	~92–97% single read (up to 99.96% consensus)	dependent on read length selected by user	data streamed in real time. Choose 1 min to 48 hrs	$500–999 per Flow Cell, base cost dependent on expt	Longest individual reads. Accessible user community. Portable (Palm sized).	Lower throughput than other machines, Single read accuracy in 90s.
Chain termination (Sanger sequencing)	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	$2400	Useful for many applications.	More expensive and impractical for larger sequencing projects. This method also requires the time consuming step of plasmid cloning or PCR.

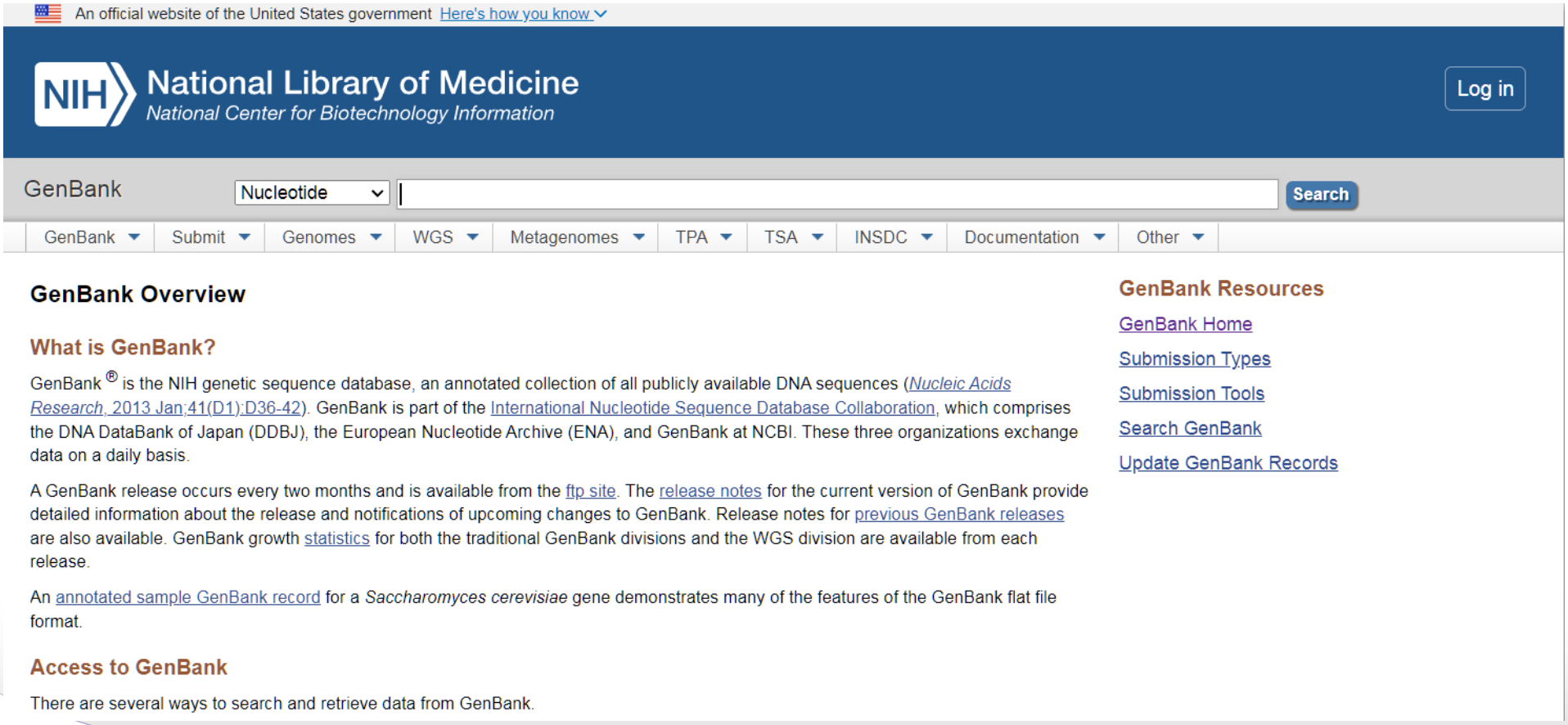
https://en.wikipedia.org/wiki/DNA_sequencing

序列数据的存储

- 核酸四大数据库：GenBank, EBI-ENA, NGDC, DDBJ
- GSA (Genome Sequence Archive)
  - Raw sequence read
- Ensembl数据库：基因组注释
- Refseq数据库
- NCBI的Gene信息数据库
- 蛋白质序列：UniProt数据库

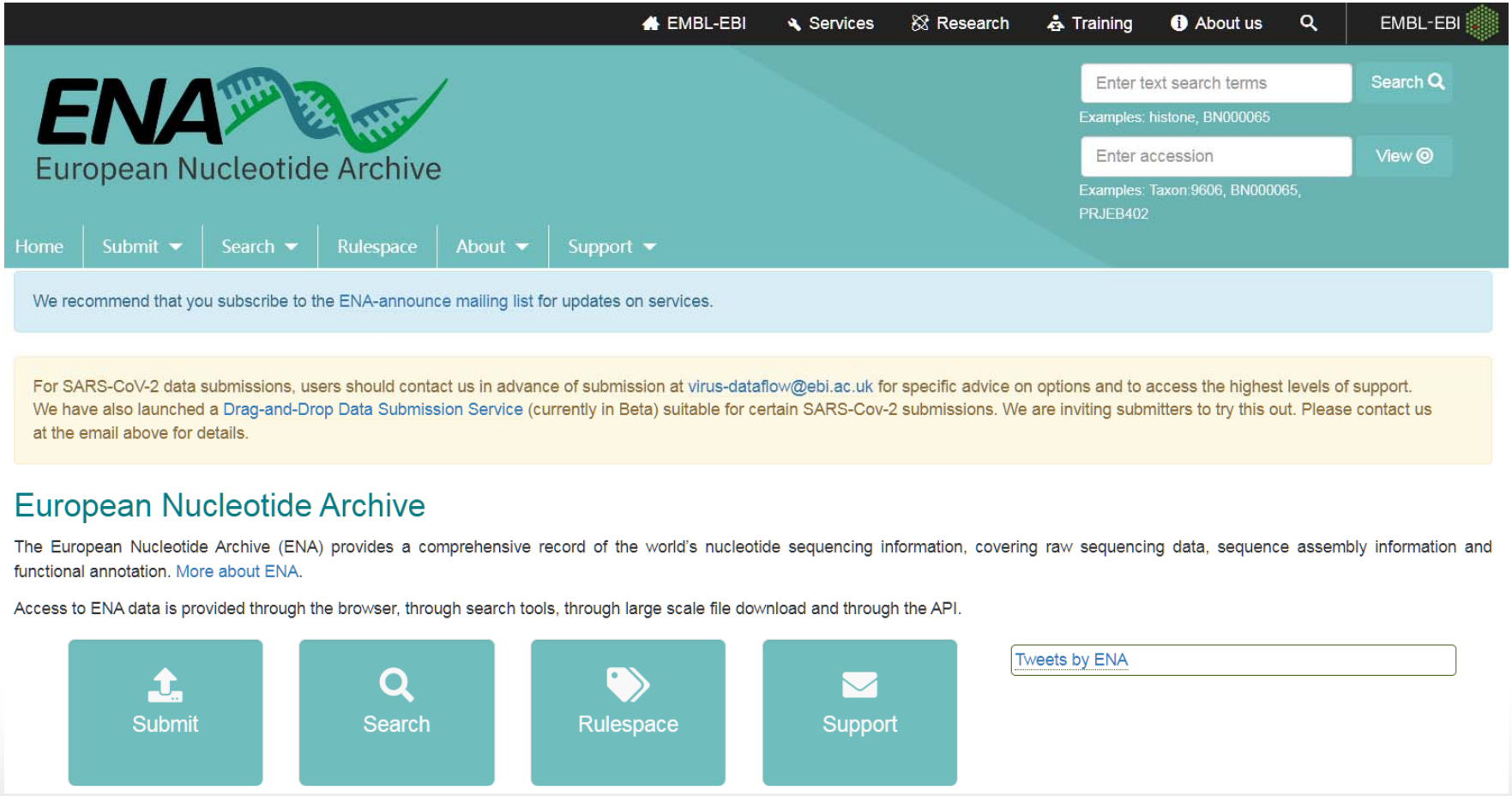
GenBank

- https://www.ncbi.nlm.nih.gov/genbank/



EBI-ENA

- https://www.ebi.ac.uk/ena/

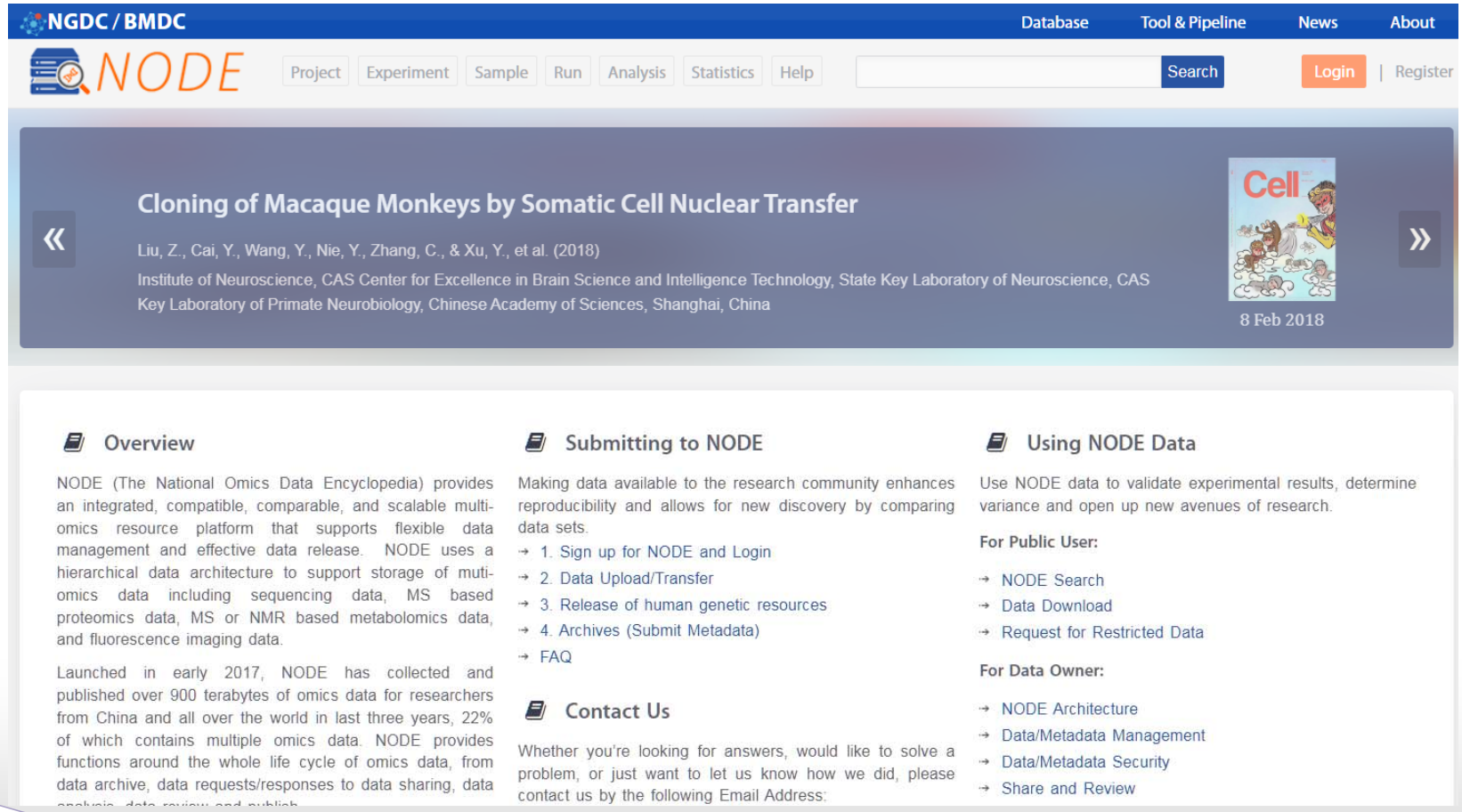


NGDC

- https://ngdc.cncb.ac.cn/

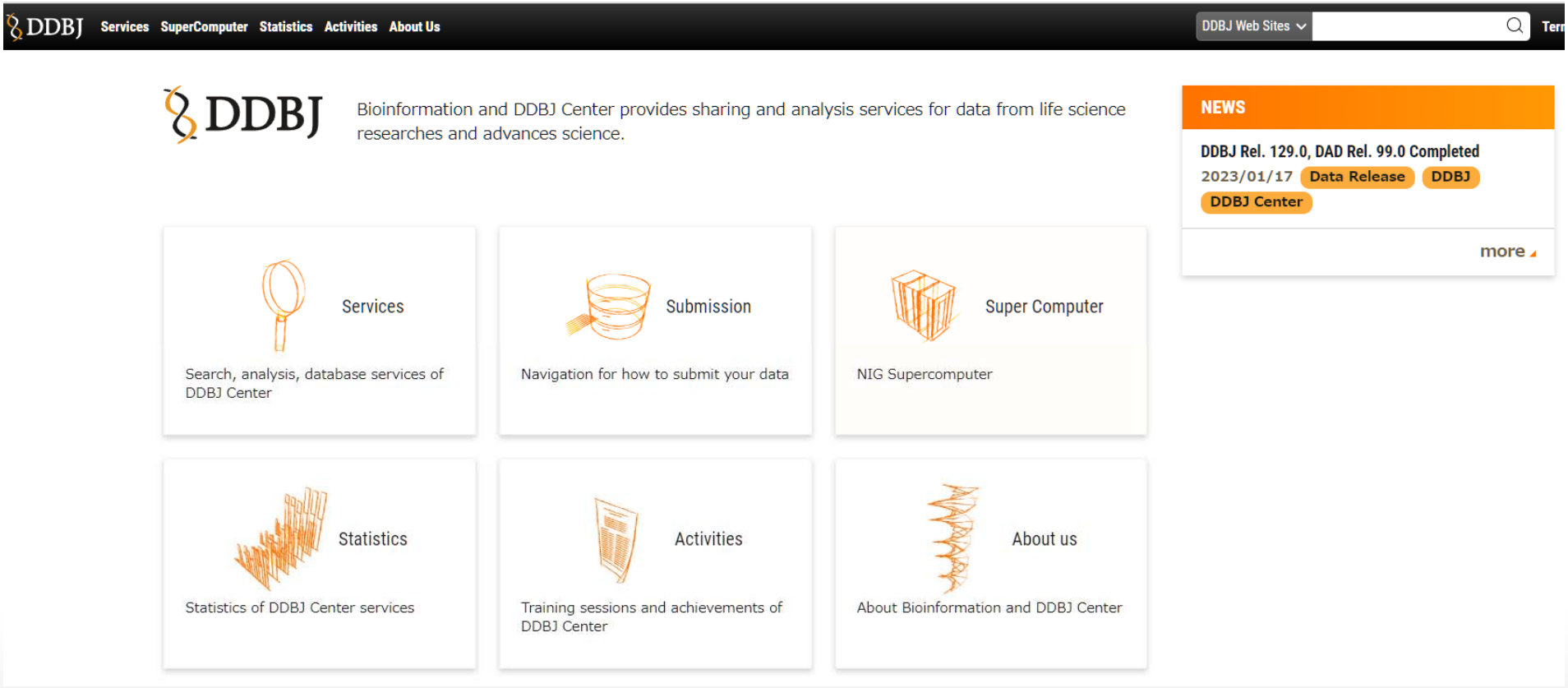
NGDC/NODE

- https://www.biosino.org/node/



DDBJ

- https://www.ddbj.nig.ac.jp/index-e.html

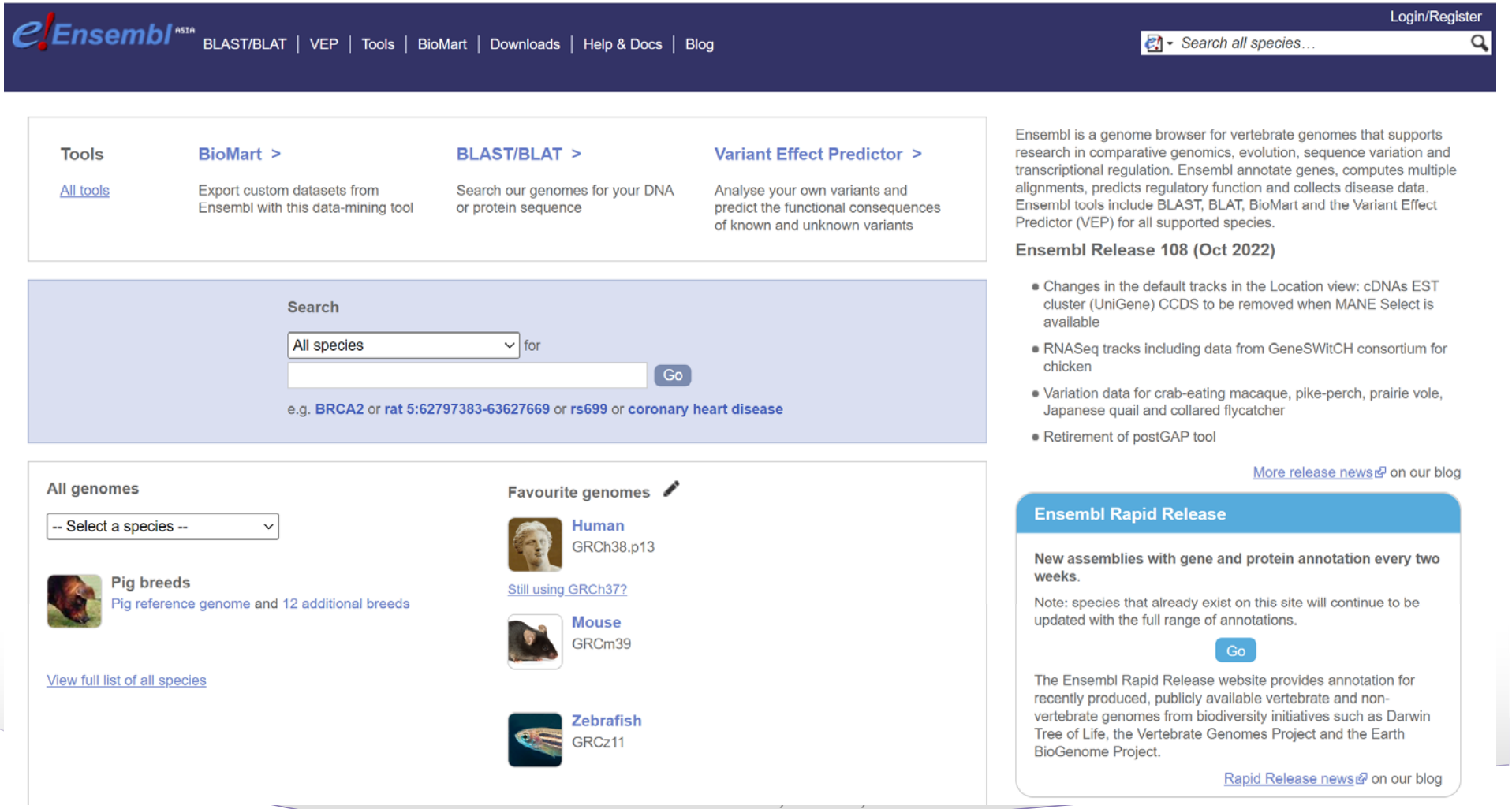


Genome Sequence Archive

- https://ngdc.cncb.ac.cn/gsa/

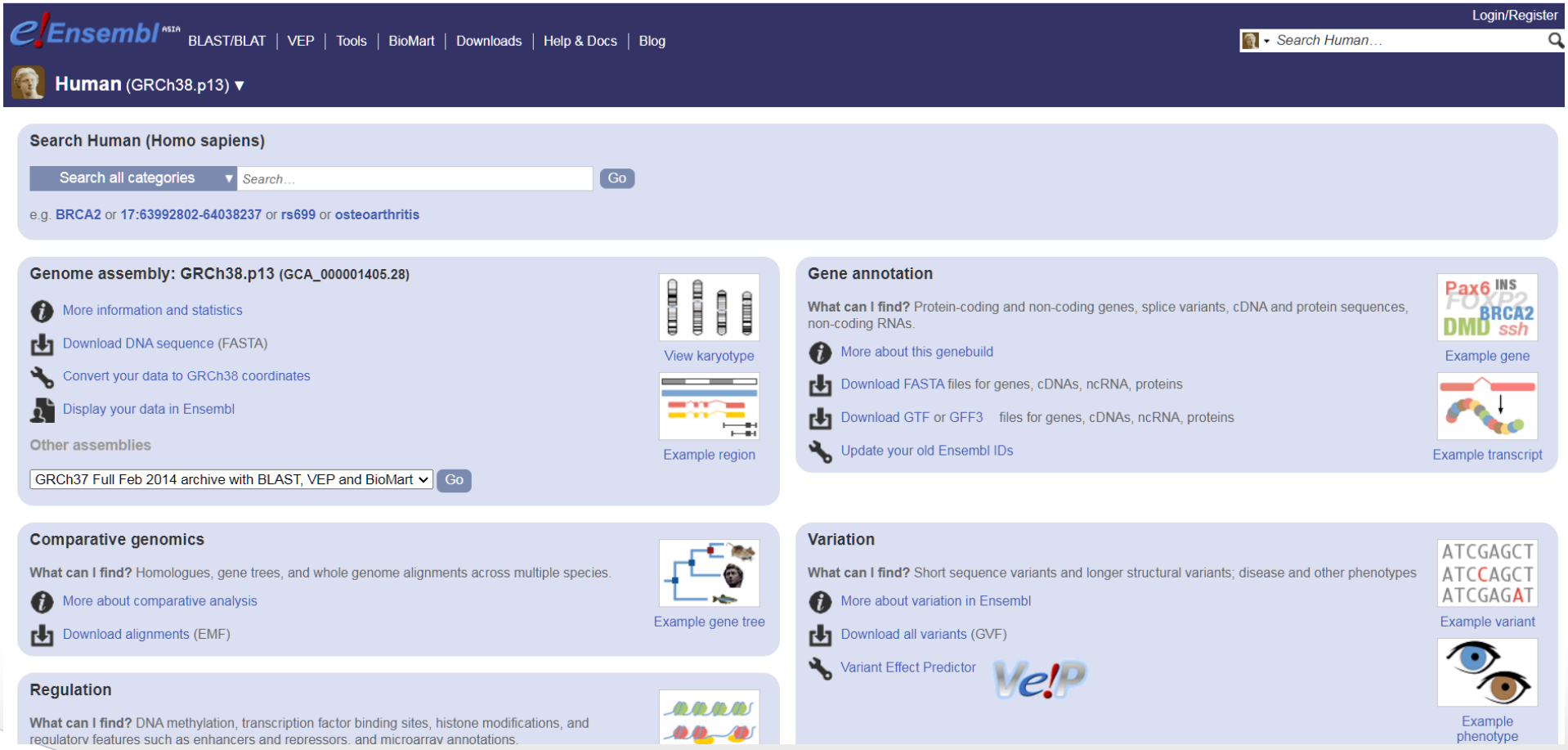
Ensembl数据库

- http://asia.ensembl.org/index.html



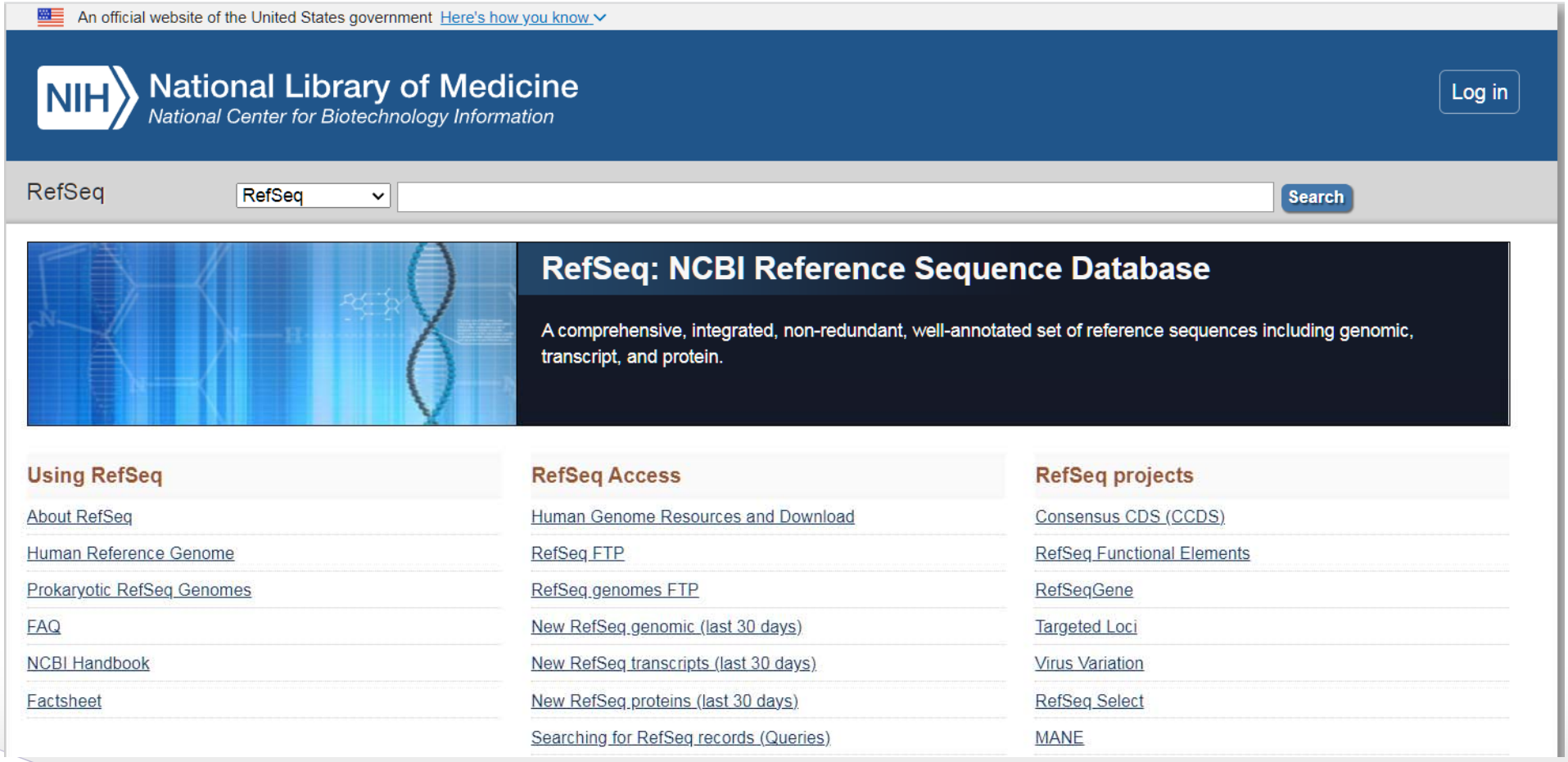
Ensembl - *Homo sapiens*

- http://asia.ensembl.org/Homo_sapiens/Info/Index



RefSeq数据库

- https://www.ncbi.nlm.nih.gov/refseq/

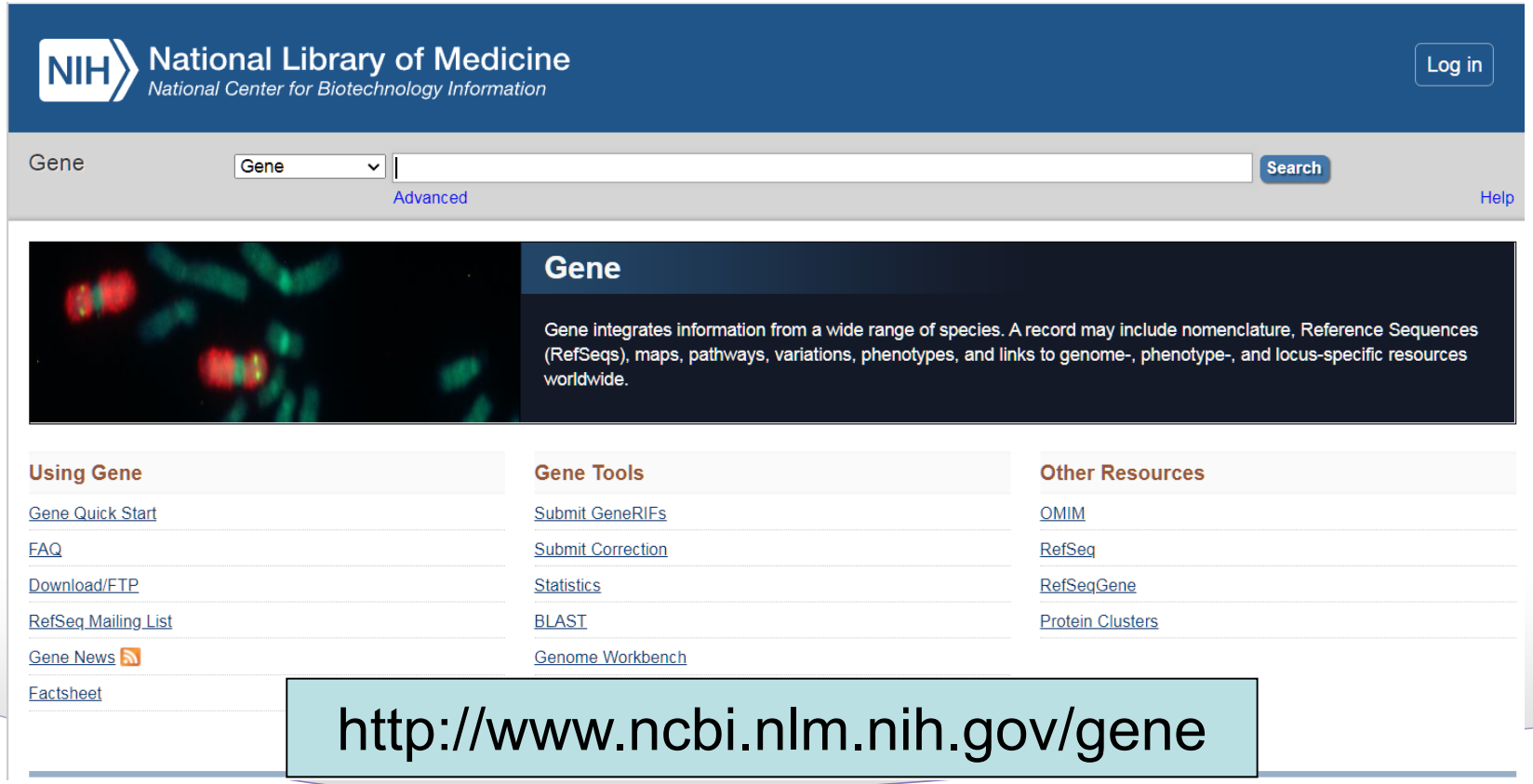


Refseq数据库

- 提供高质量无冗余完整的序列信息
- 包括基因组DNA、RNA转录本以及蛋白质等序列信息
- 序列文件的标识符：
  - **DNA/RNA序列**：NM_004336.5, XM_XXX
  - 蛋白质序列： NP_004327.1, XP_XXX
  - 非编码**RNA**：NR_135914.2
  - 染色体：NC_XXX

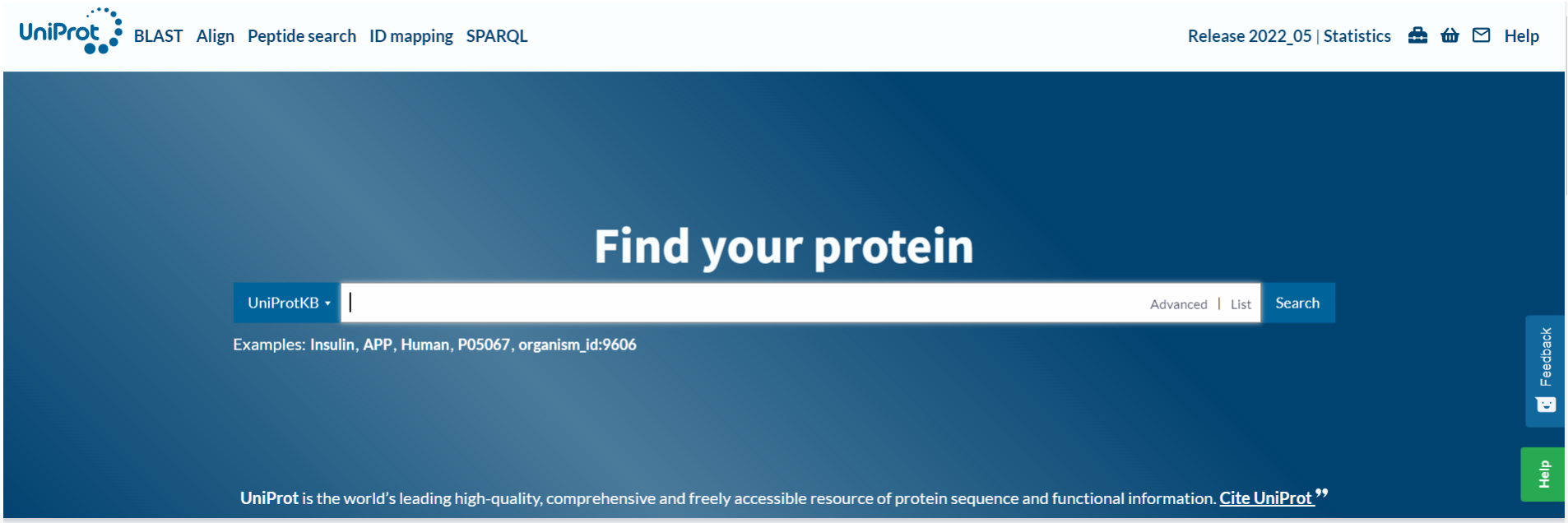
NCBI Gene

- 序列从Refseq数据库中得到
- 详尽的注释信息，包括基因在基因组的定位，基因名称、蛋白质名称，基因结构等



UniProt

- 专家审核的蛋白质序列数据与知识库
- UniProtKB: UniProt Knowledgebase



http://www.uniprot.org/

UniProt数据库

- UniProt两个字库：
  - Swiss-Prot: Reviewed
  - TrEMBL: Unreviewed
- 蛋白质标识符命名规则（先后顺序）
  - O>P>Q>A>B…
  - 标识符越靠前，注释信息越丰富