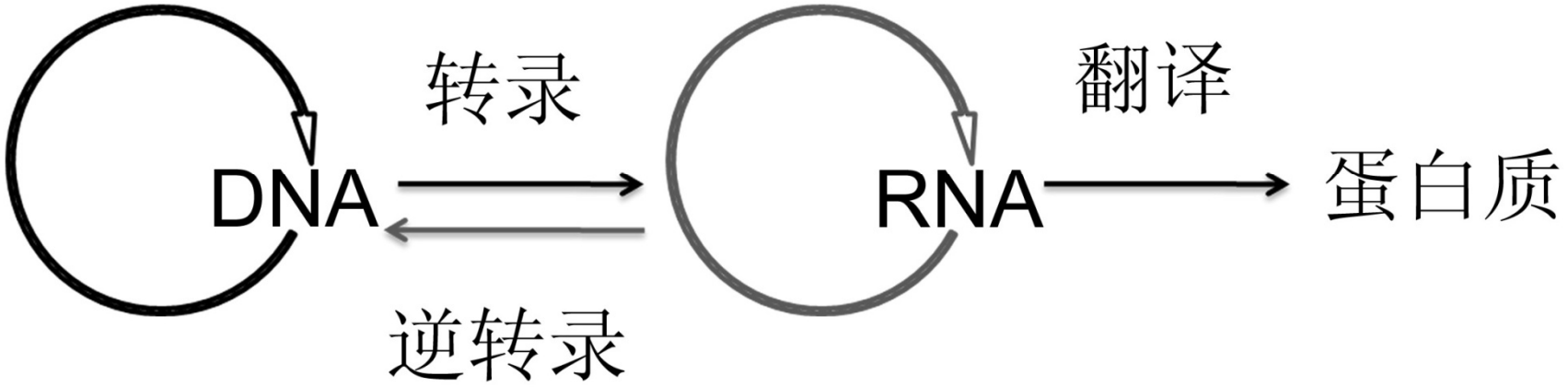
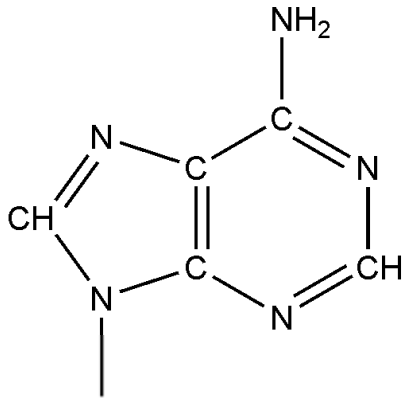
生物信息学

第二章 生物序列数据获取和检索

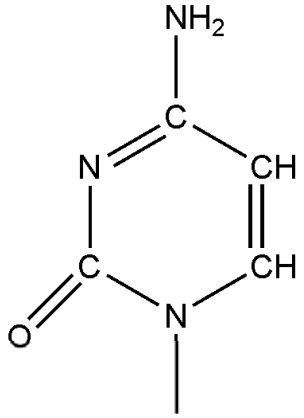
中心法则



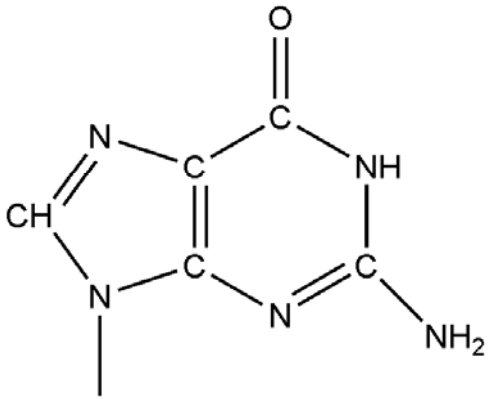
DNA结构：碱基/核苷



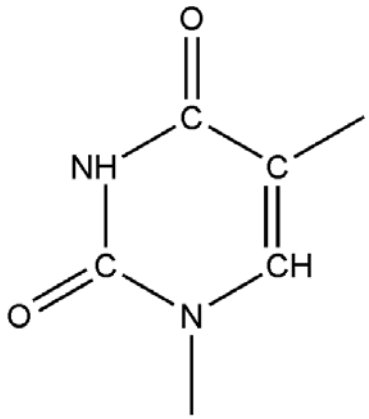
Adenine (A)



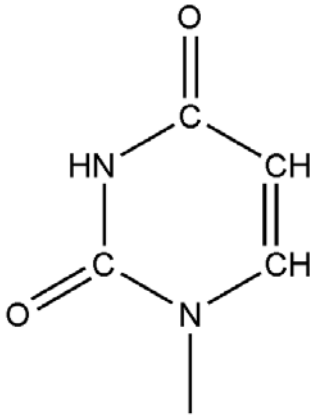
Cytosine (C)



Guanine (G)



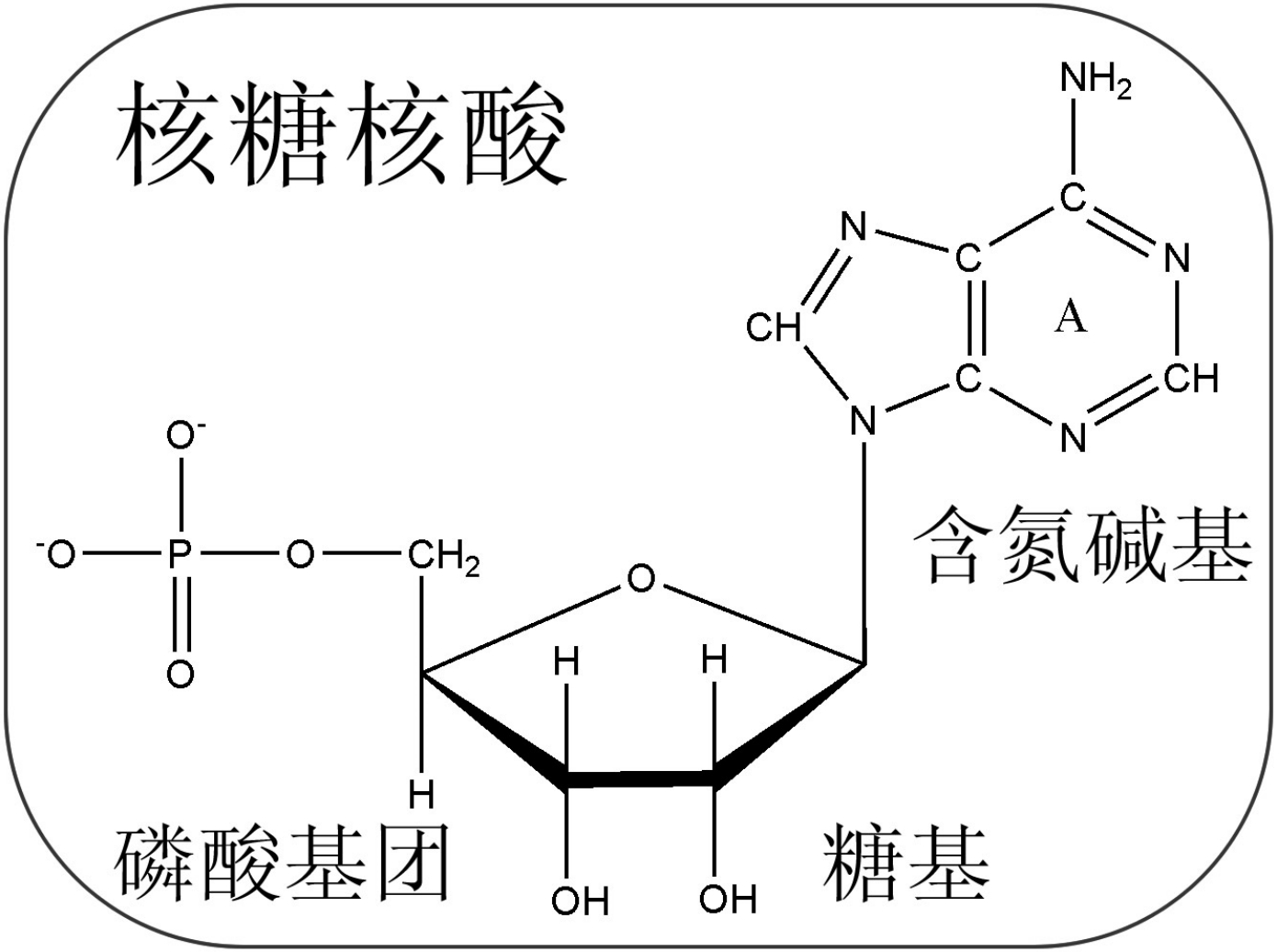
Thymine (T)



Uracil (U)

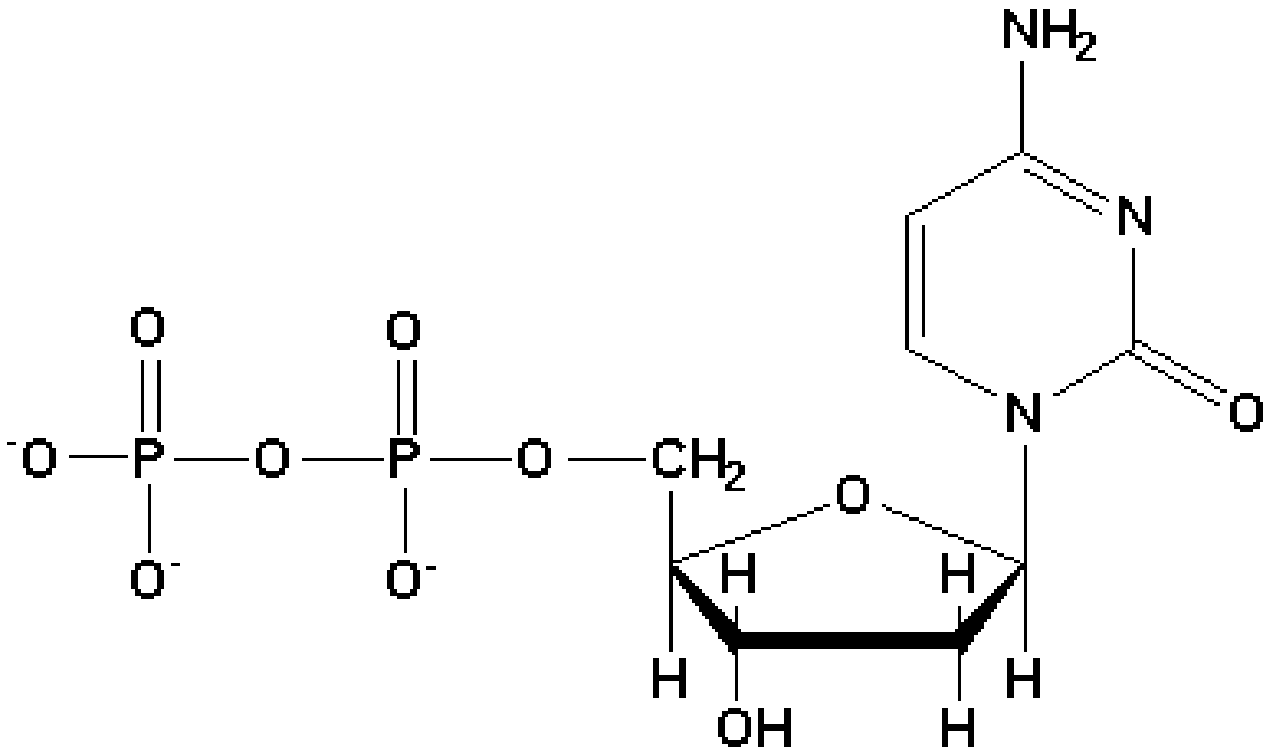
核糖核酸

Ribonucleotide



脱氧核糖核酸

Deoxyribonucleotide

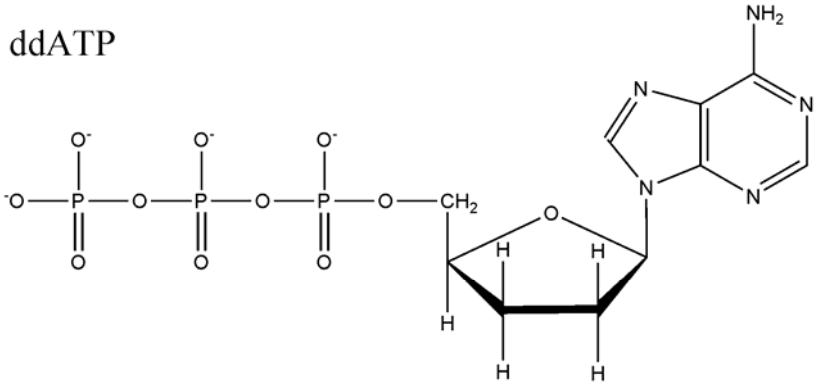


Deoxycytidine diphosphate (dCDP)

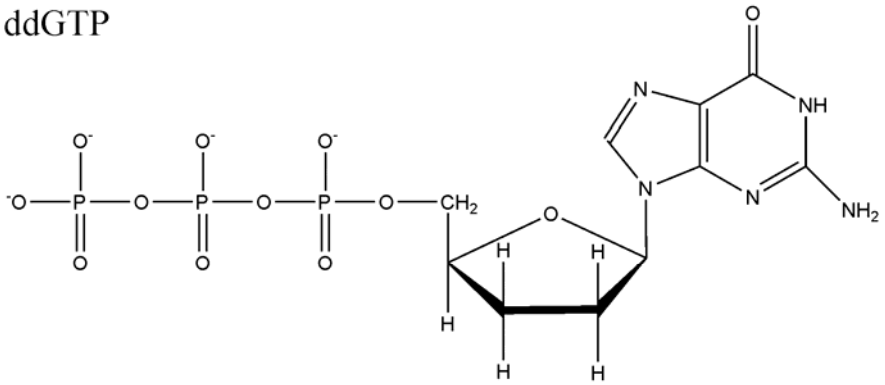
双脱氧核糖核苷酸

Dideoxyribonucleotide

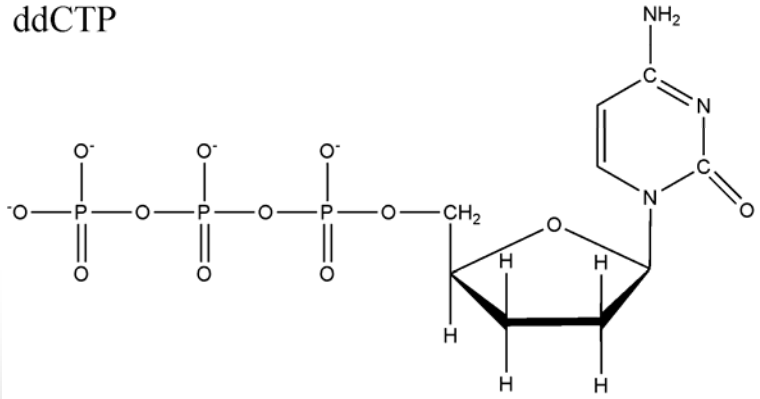
ddATP



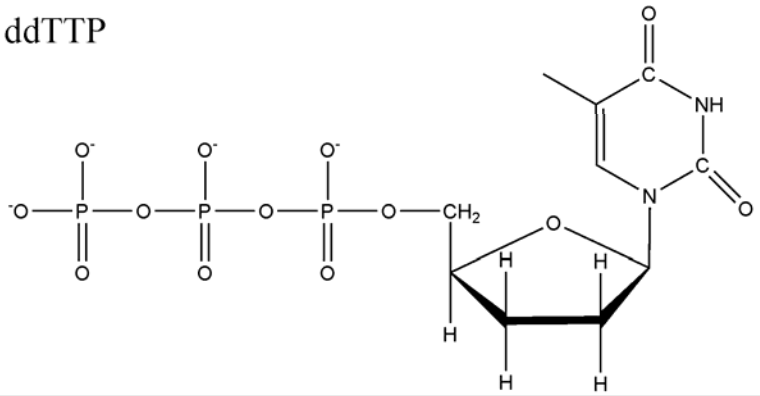
ddGTP



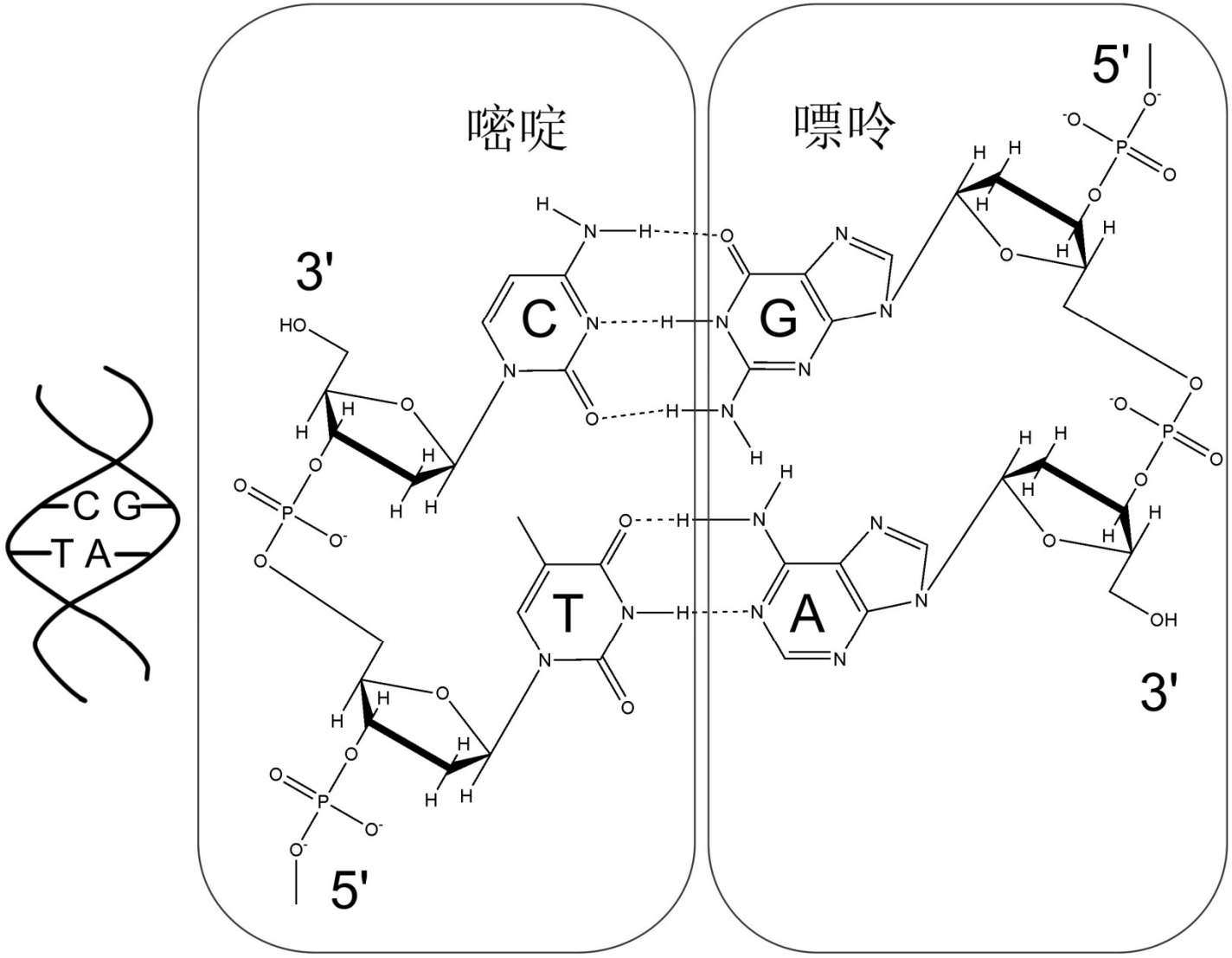
ddCTP



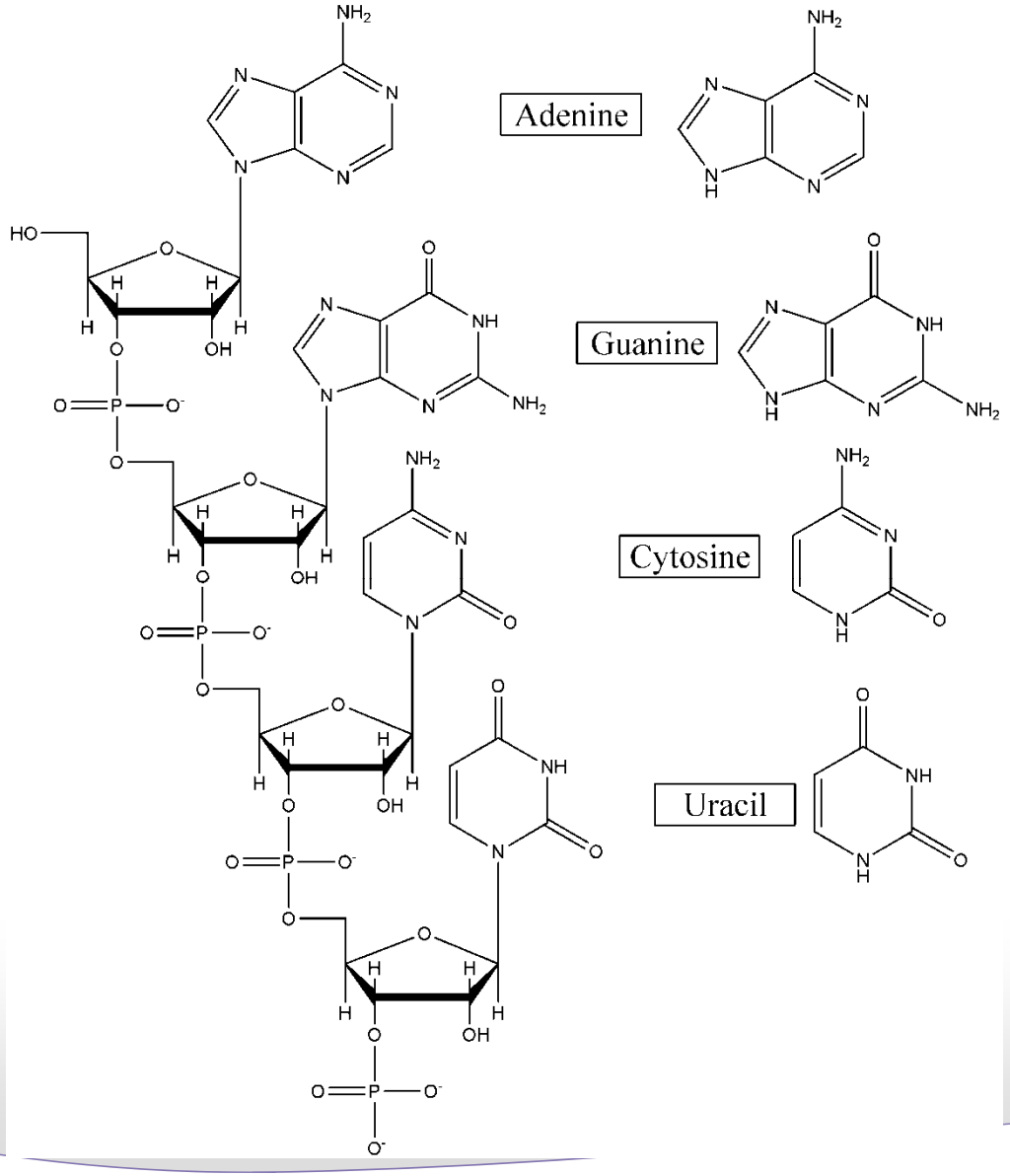
ddTTP



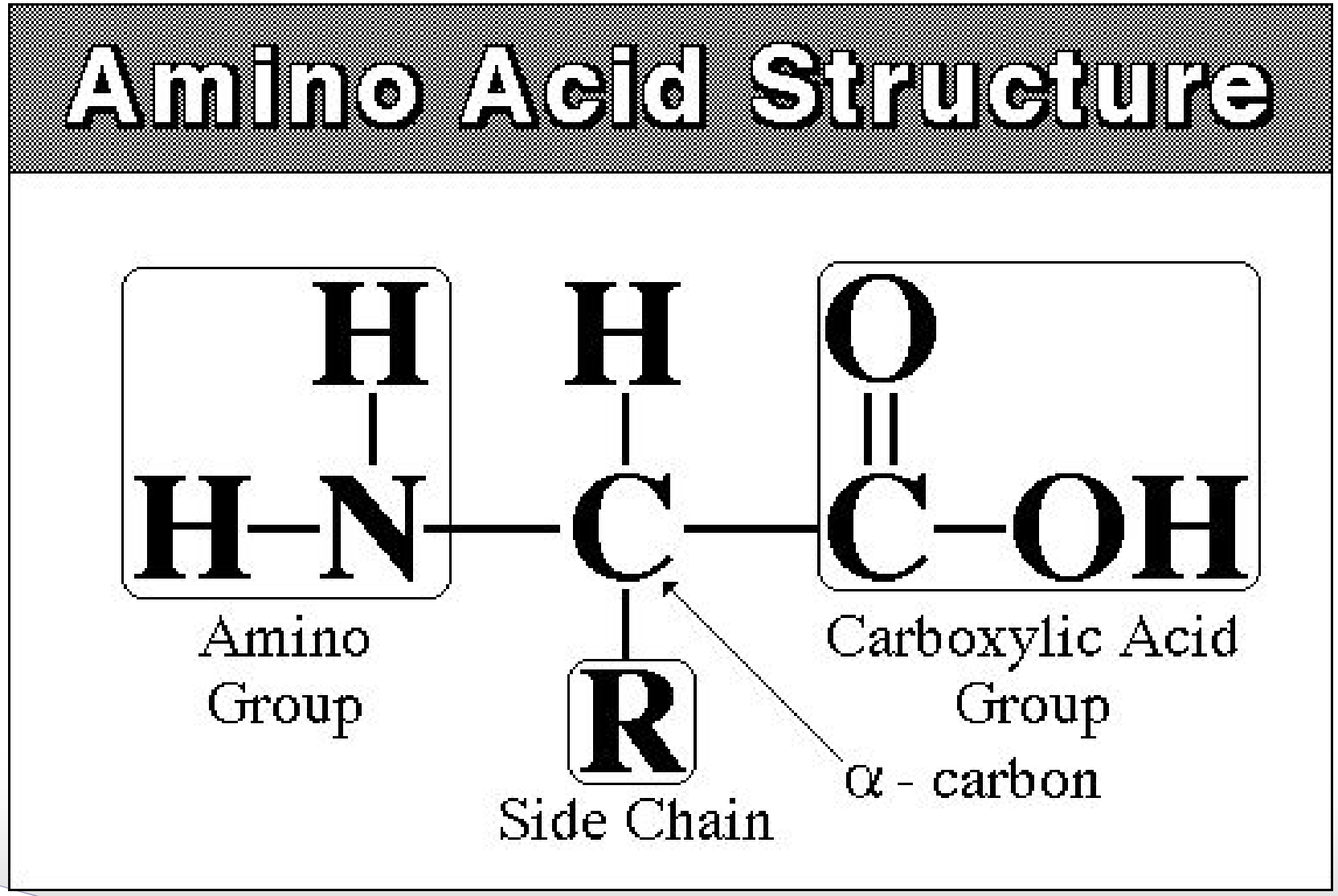
DNA的结构



RNA的结构



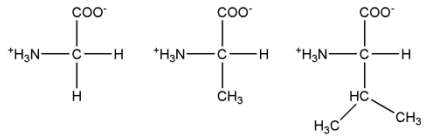
氨基酸的结构


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

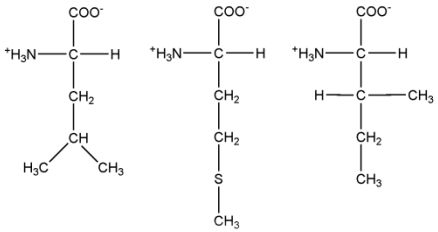
氨基酸的性质及分类

20种常见的蛋白质氨基酸

非极性脂肪酸氨基酸

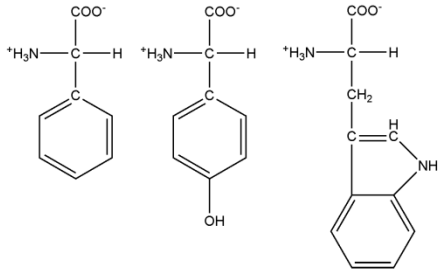


甘氨酸 Gly, G　丙氨酸 Ala, A　缬氨酸 Val, V



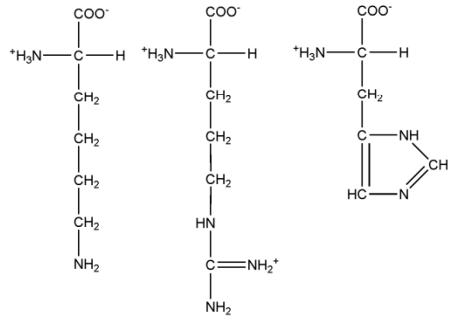
亮氨酸 Leu, I　甲硫氨酸 Met, M　异亮氨酸 Ile, I

芳香族氨基酸



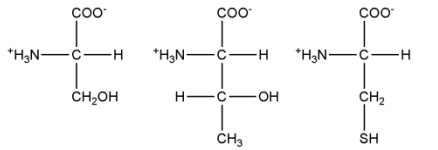
苯丙氨酸 Phe, F　酪氨酸 Tyr, Y　色氨酸 Trp, W

R基带正电荷的氨基酸

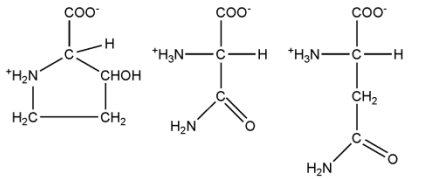


赖氨酸 Lys, K　精氨酸 Arg, R　组氨酸 His, H

R基不带电荷的氨基酸

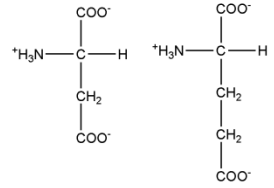


丝氨酸 Ser, S　苏氨酸 Thr, T　半胱氨酸 Cys, C



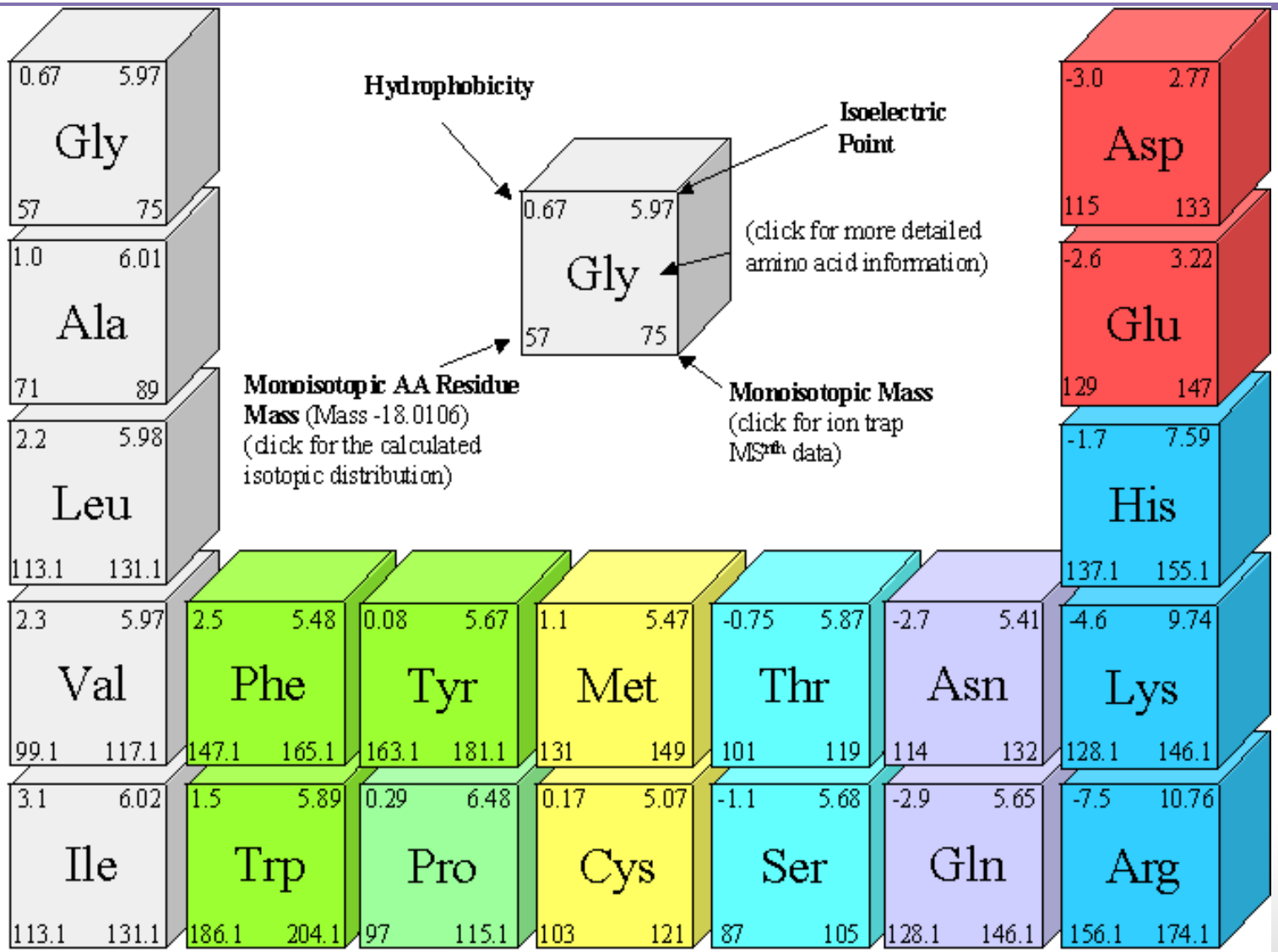
脯氨酸 Pro, P　天冬酰胺 Asn, N　谷氨酰胺 Gln, Q

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

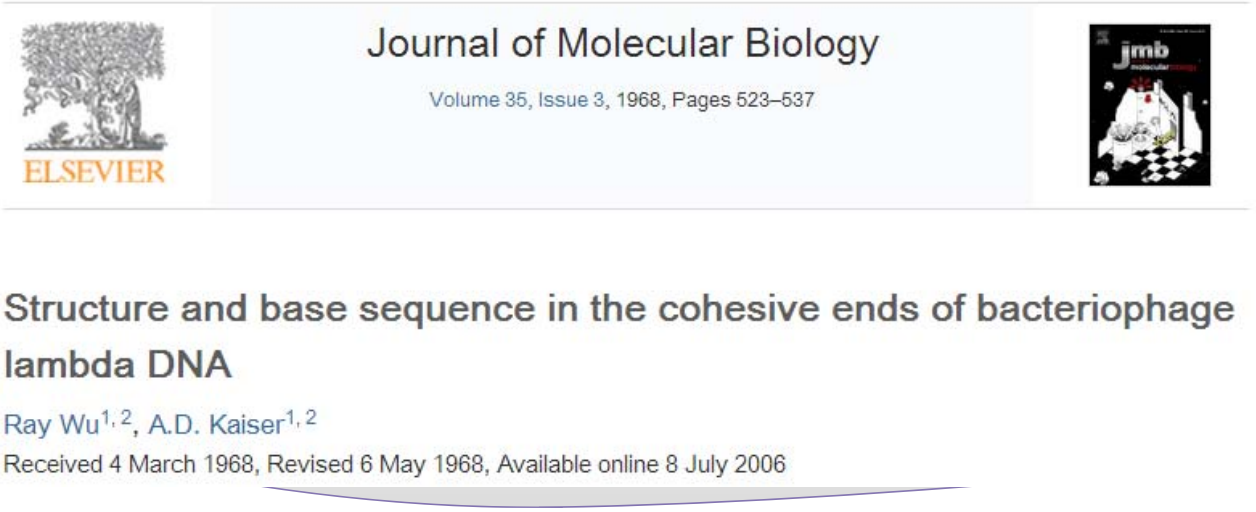


标准密码子（Codon）

	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 TAA Ter	TGT Cys(C) TGC TGA Ter 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 ..

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

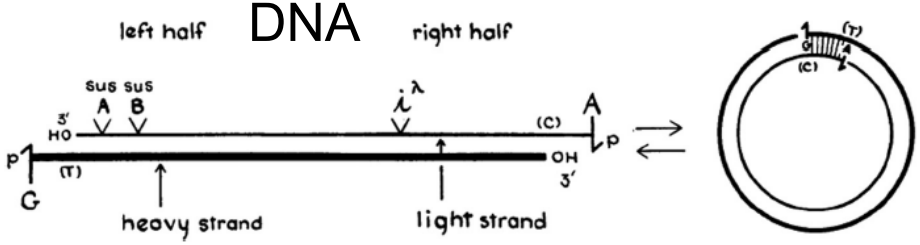
Ray Wu[1, 2], A.D. Kaiser[1, 2]

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

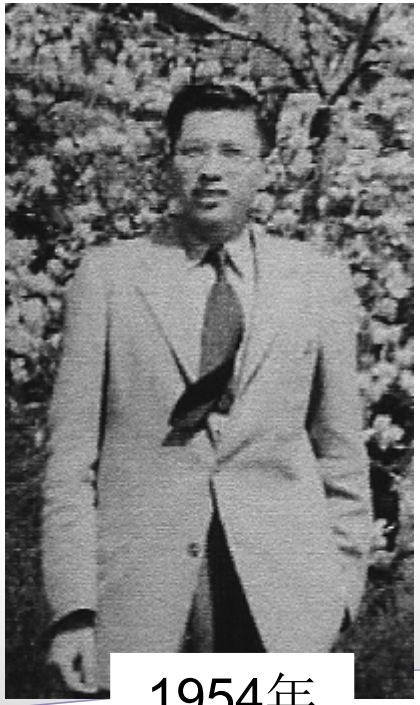
噬菌体λ DNA



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

吴瑞先生：DNA测序之父 精选

已有 10735 次阅读 2015-5-9 15:13 | 系统分类:观点评述 推荐到群组

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

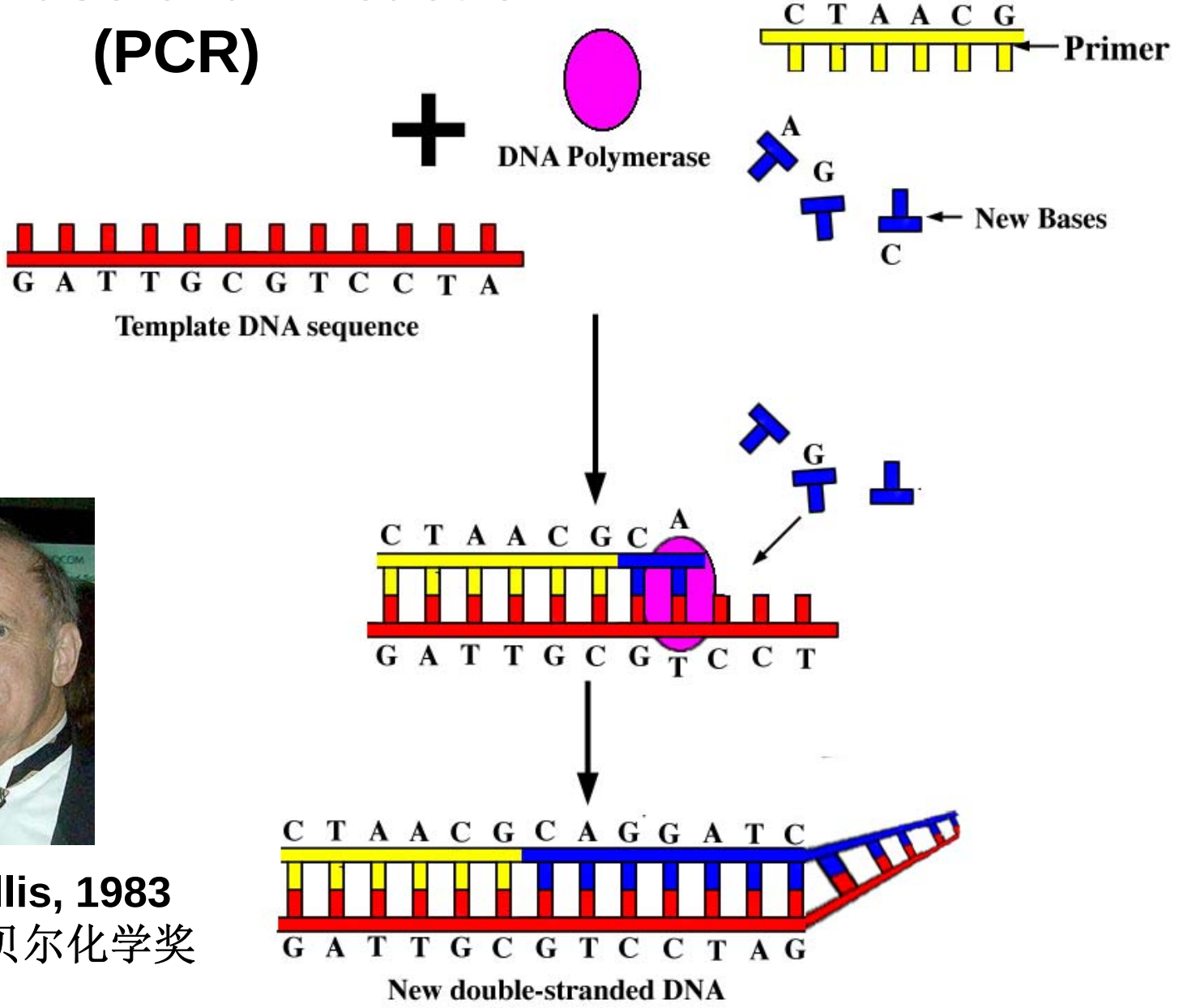
1980：DNA生化研究 & DNA测序

Paul Berg

Walter Gilbert

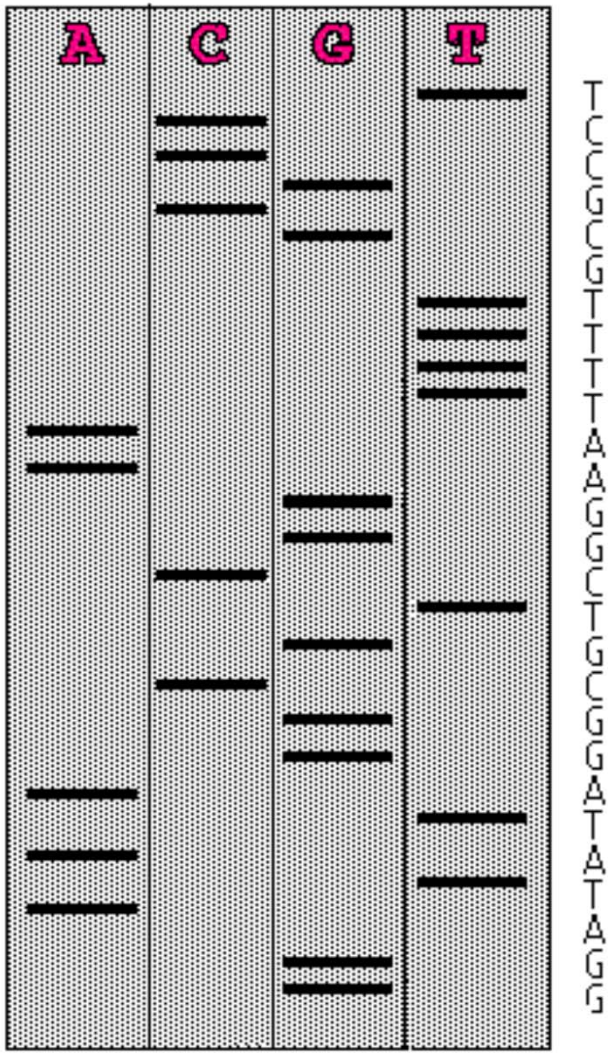
Frederick Sanger

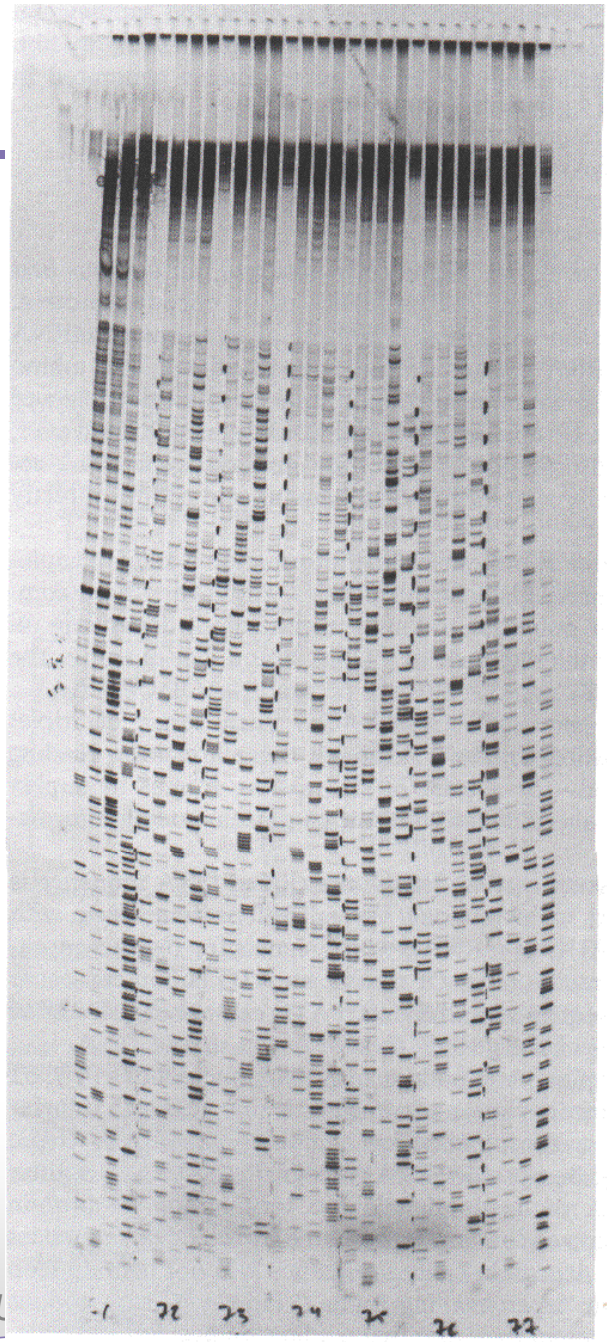
Polymerase chain reaction (PCR)



Kary Mullis, 1983
1993年诺贝尔化学奖

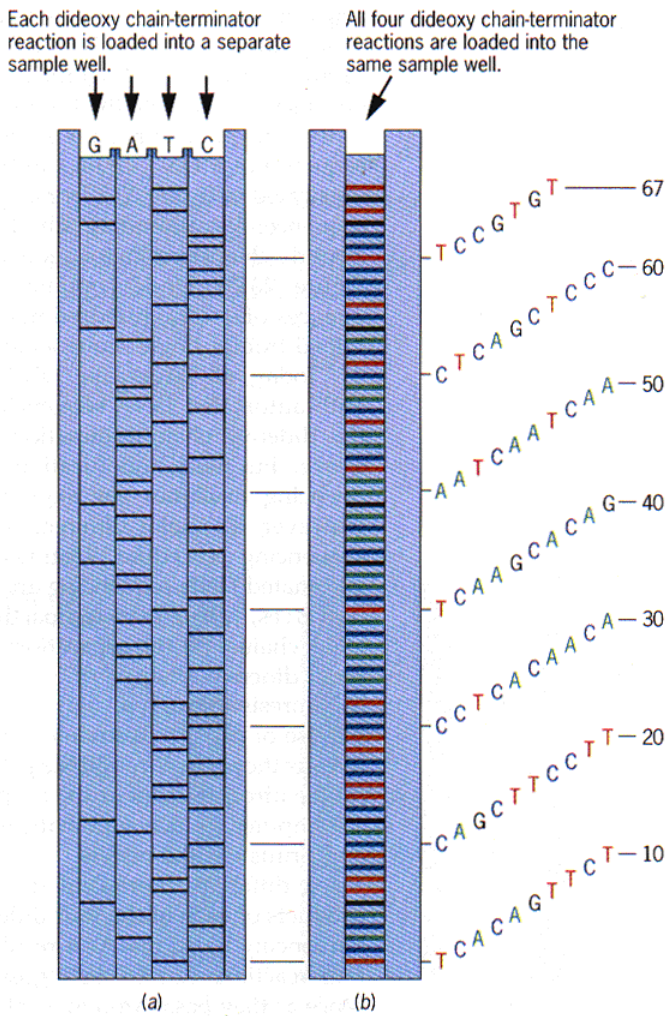
Loading each ddNTP reaction in a different lane:

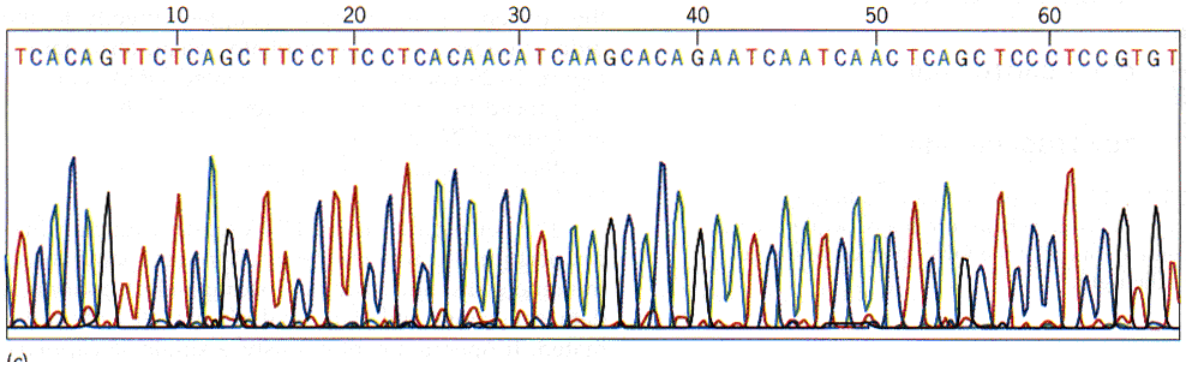


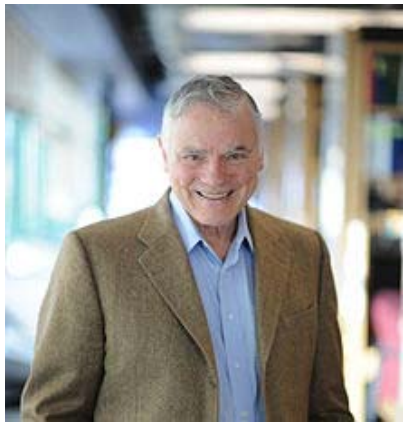


自动测序

- “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



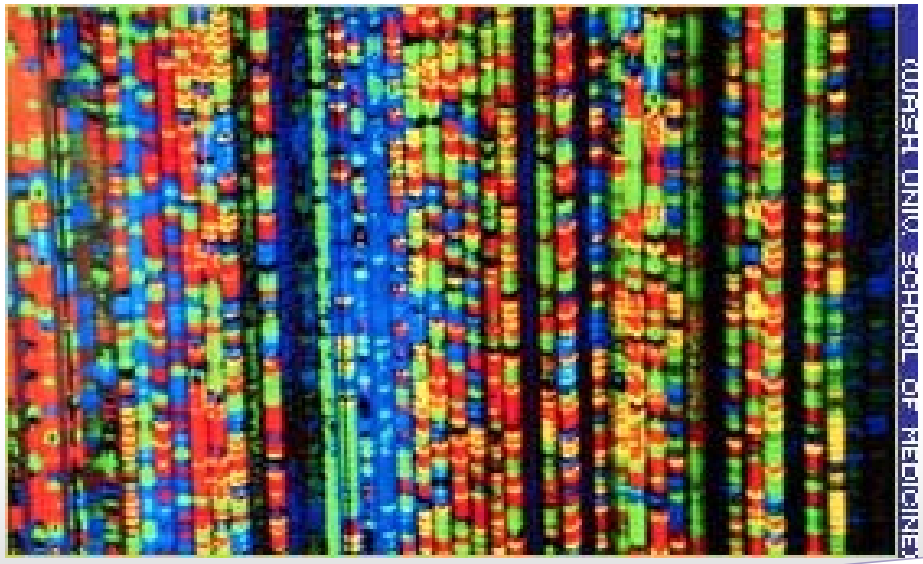
Leroy Hood

第一代测序：自动测序仪

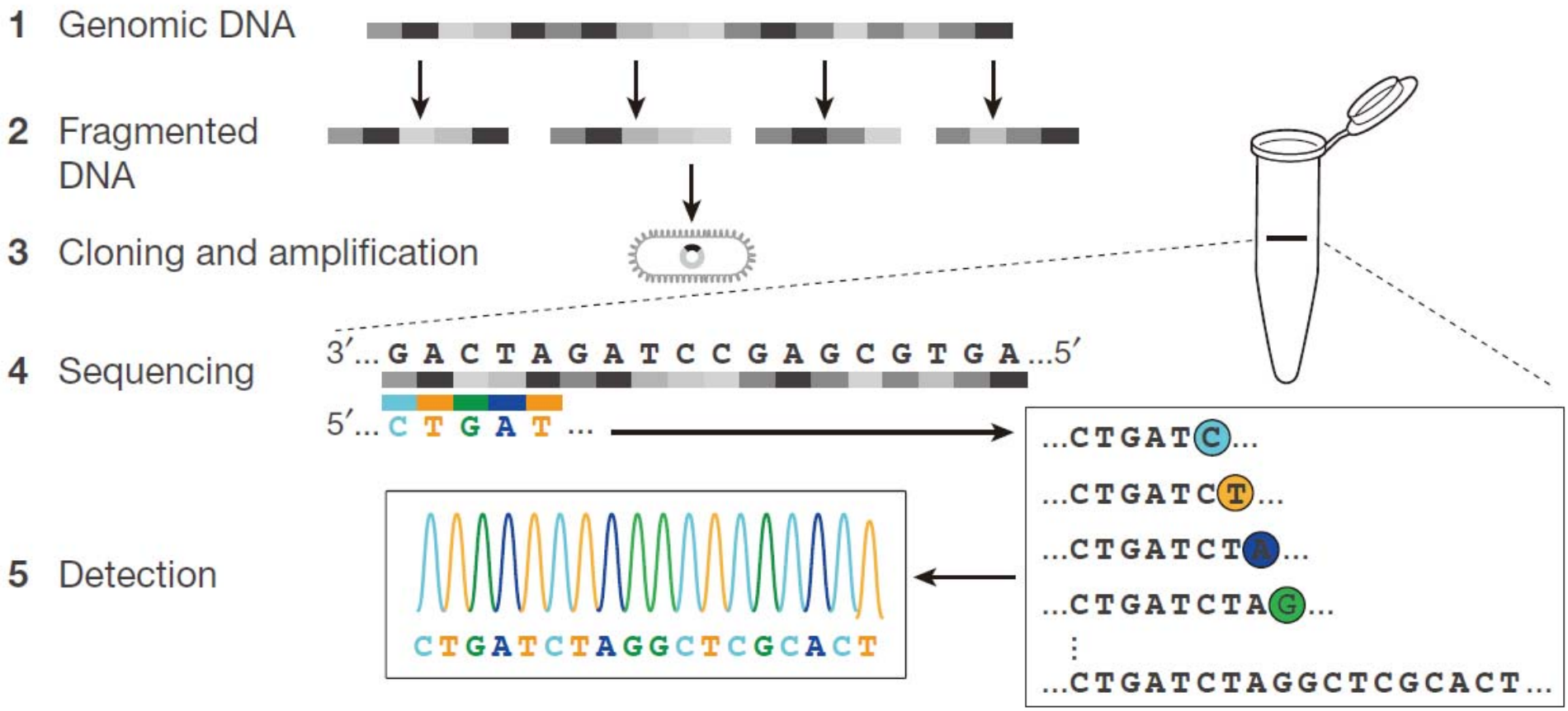
- 1986，Model 370A DNA Sequencing System
  - Applied Biosystems, Inc (ABI)
  - 1987，ABI 370
  - 利用四种颜色，可直接阅读四种碱基

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

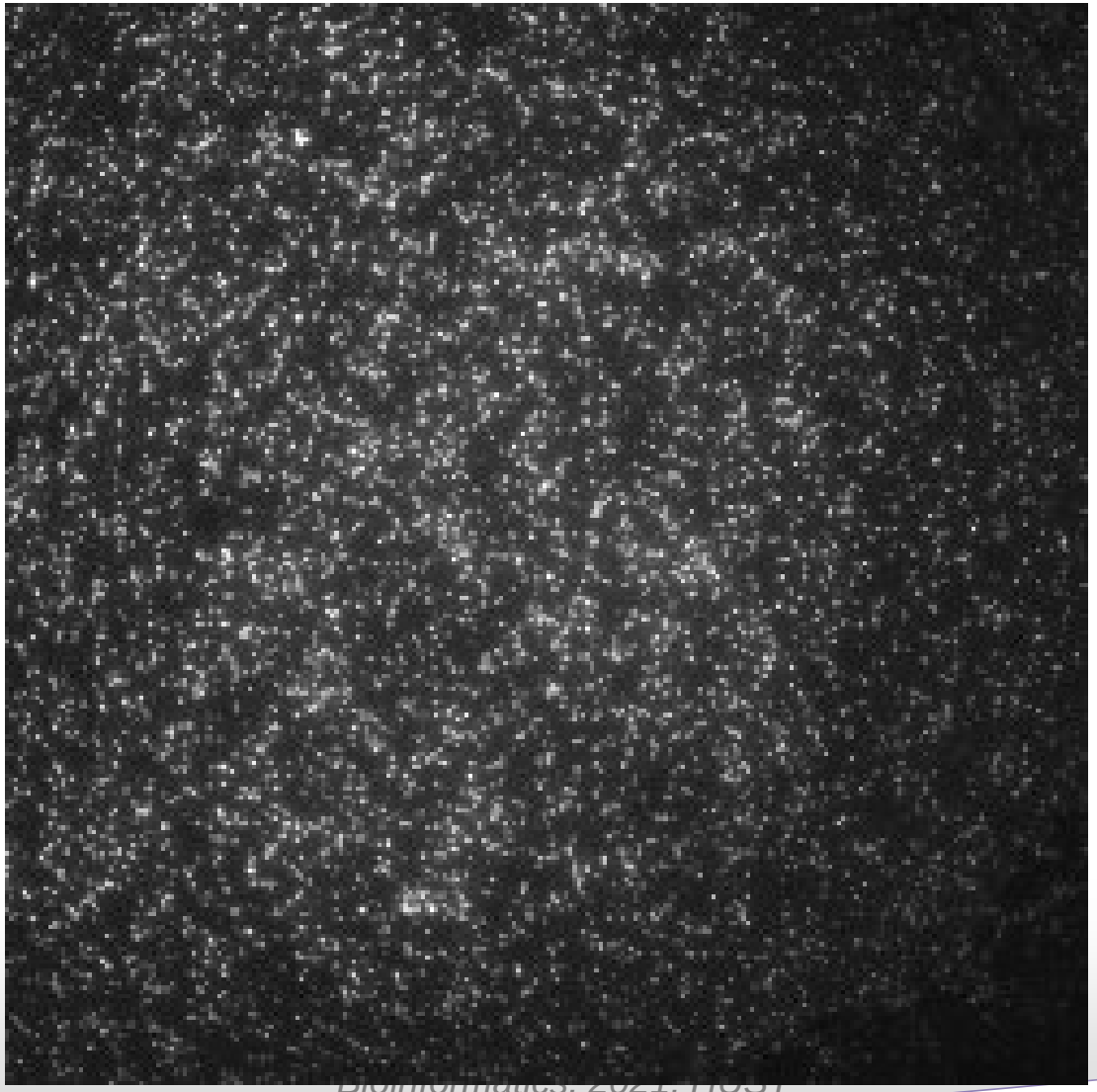
Illumina/Solexa

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）

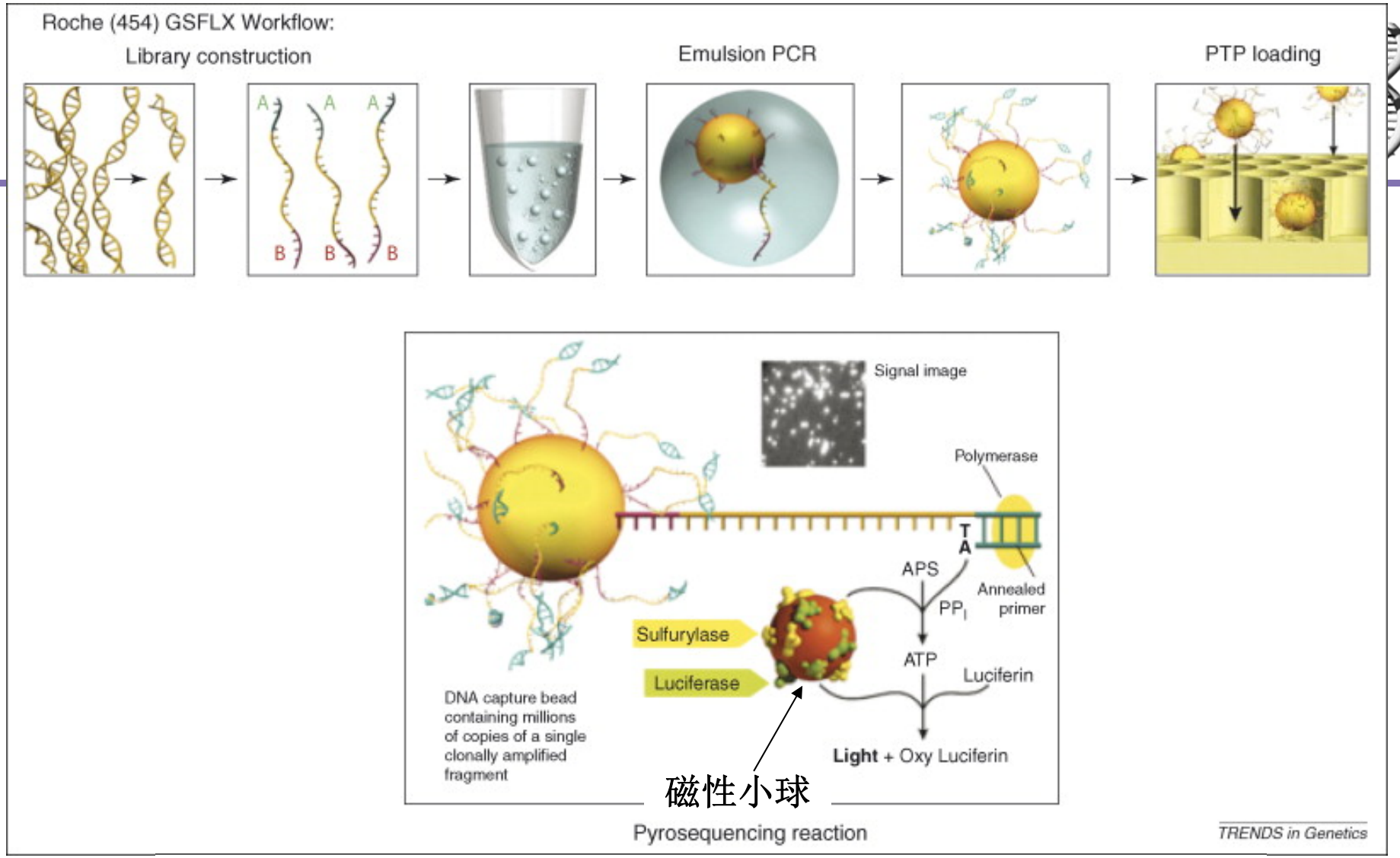


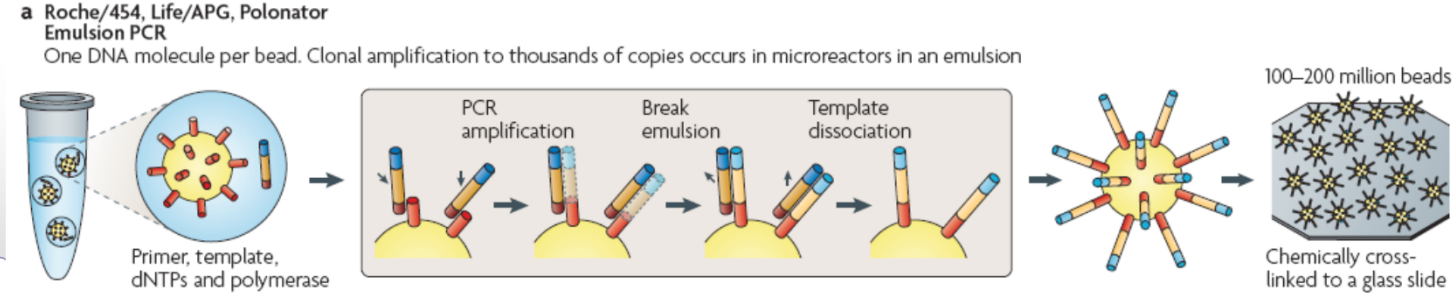
巨大容量的图像数据

- 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）

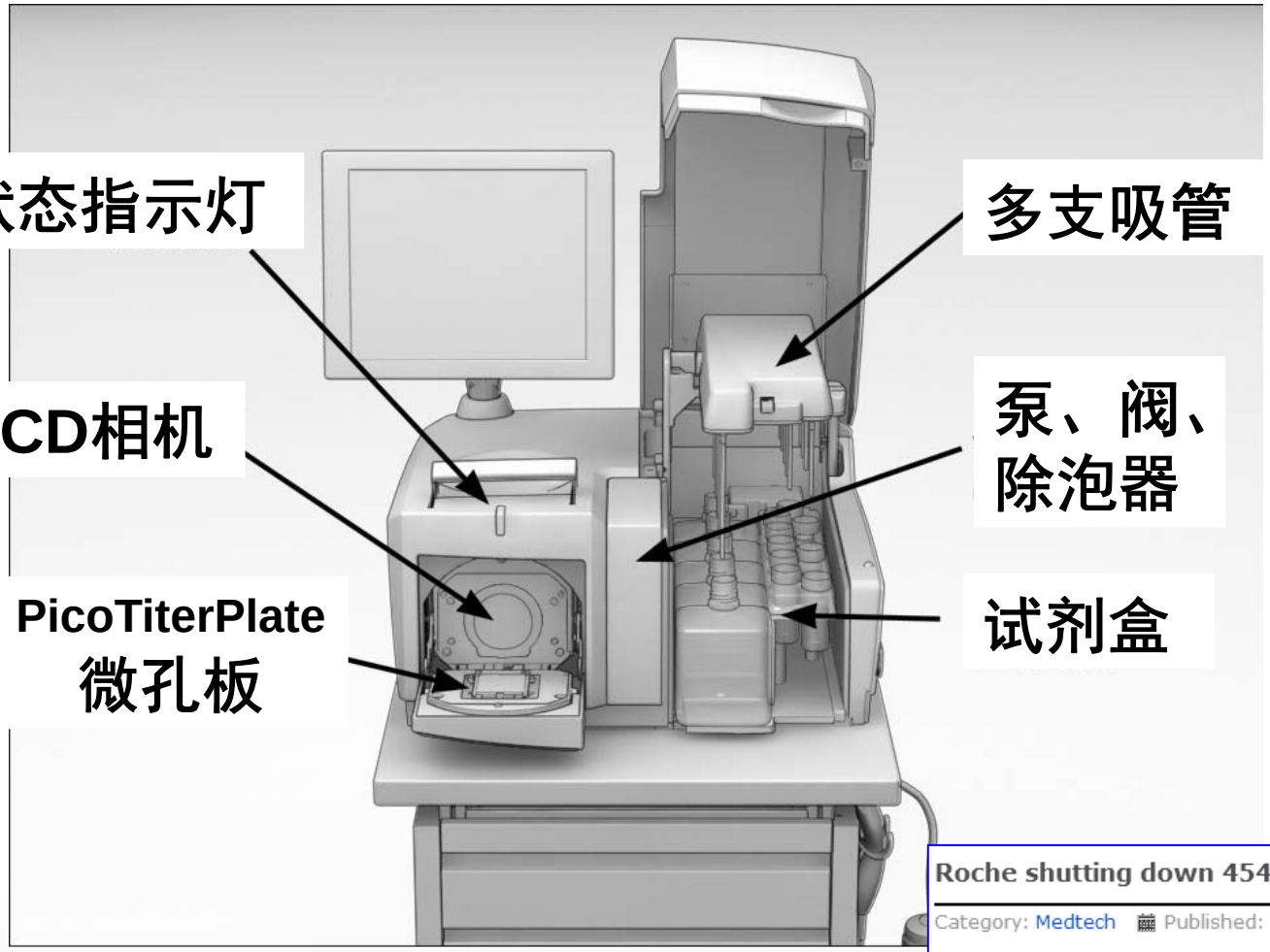
454测序原理

- 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

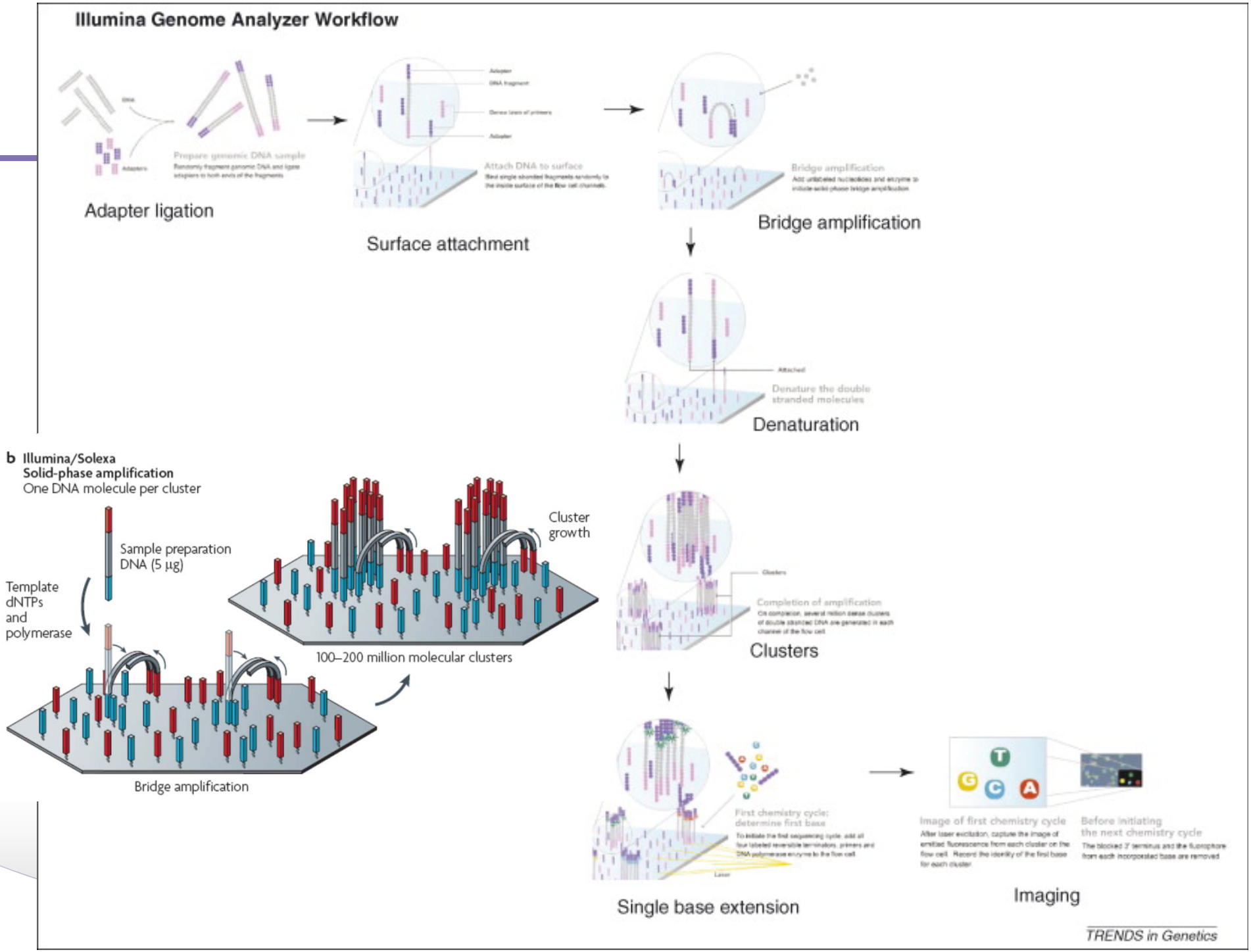
Category: Medtech Published: 21 October 2013

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

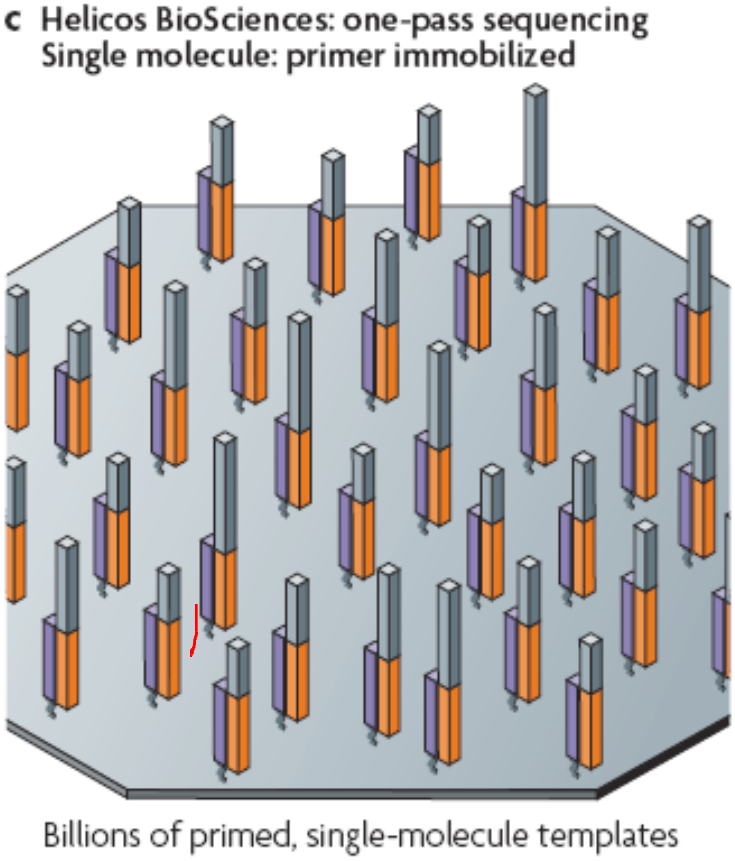
The 454 sequencers will be phased out in mid-2016, and the 454 facility in Branford, Conn., will be closed "accordingly," Roche said in a statement e-mailed to GenomeWeb Daily News.

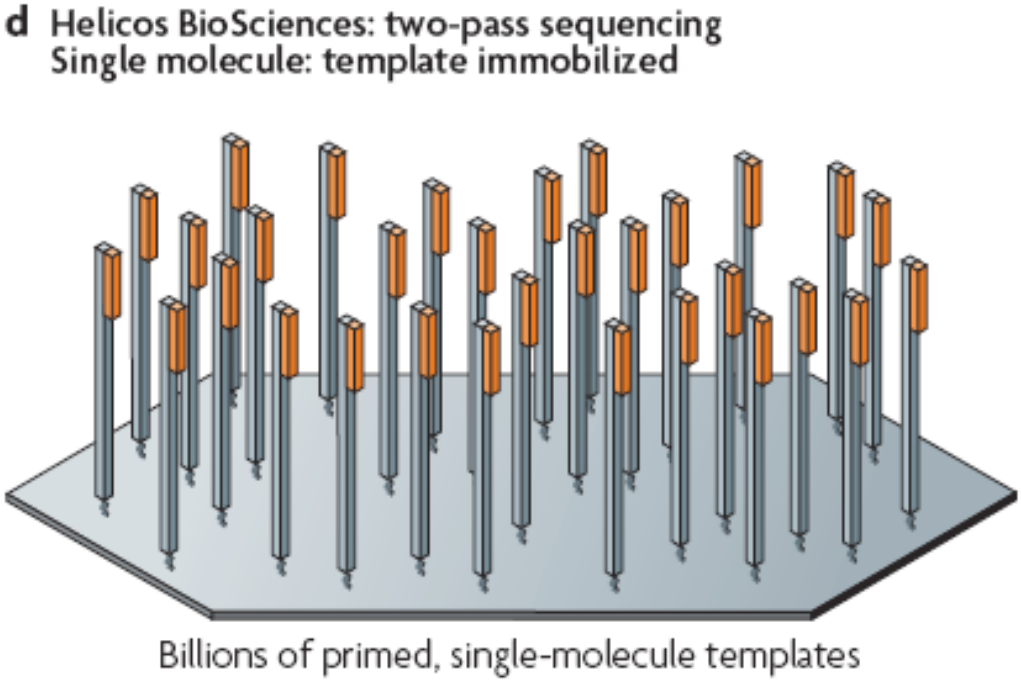
Illumina Genome Analyzer

- 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

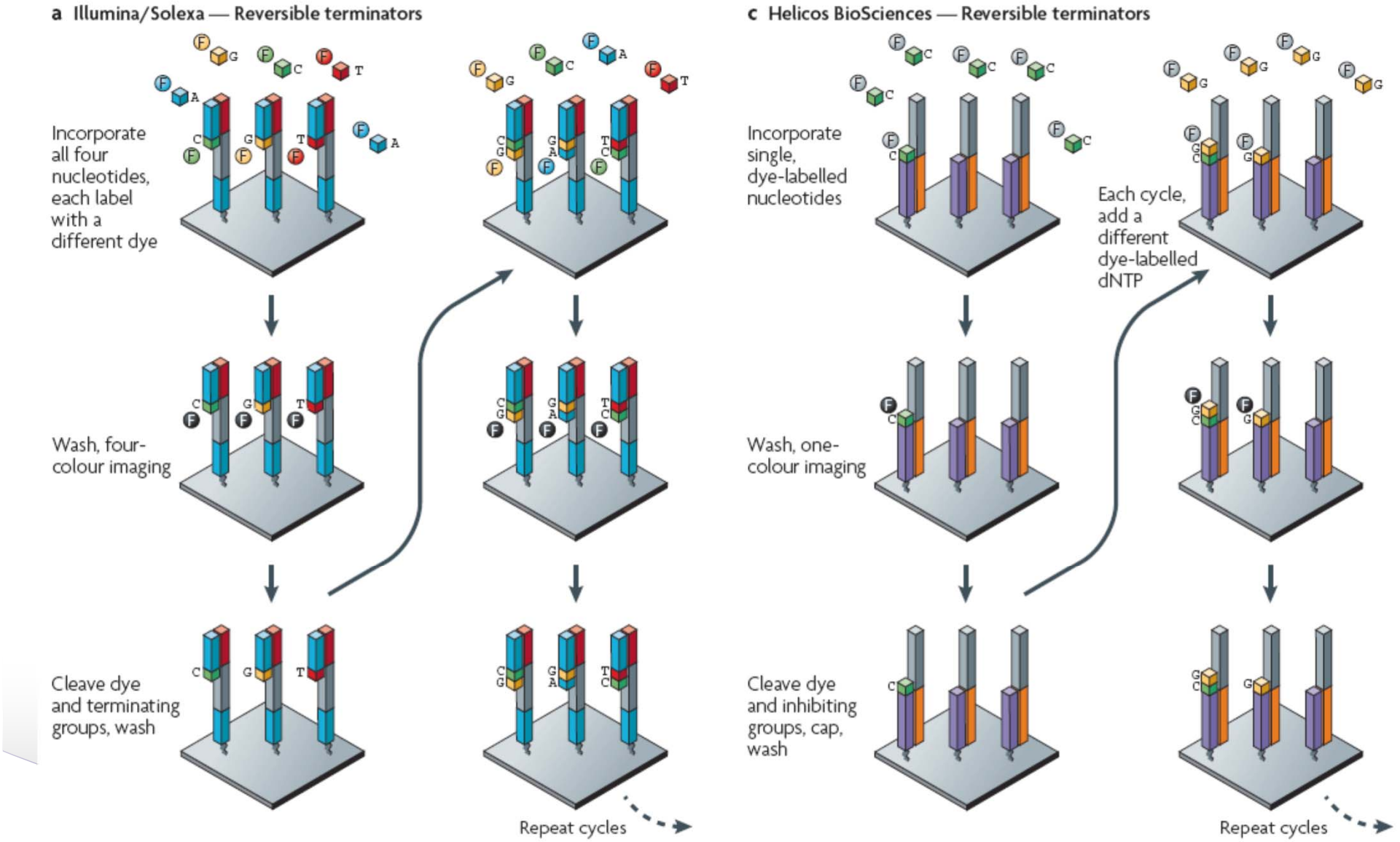




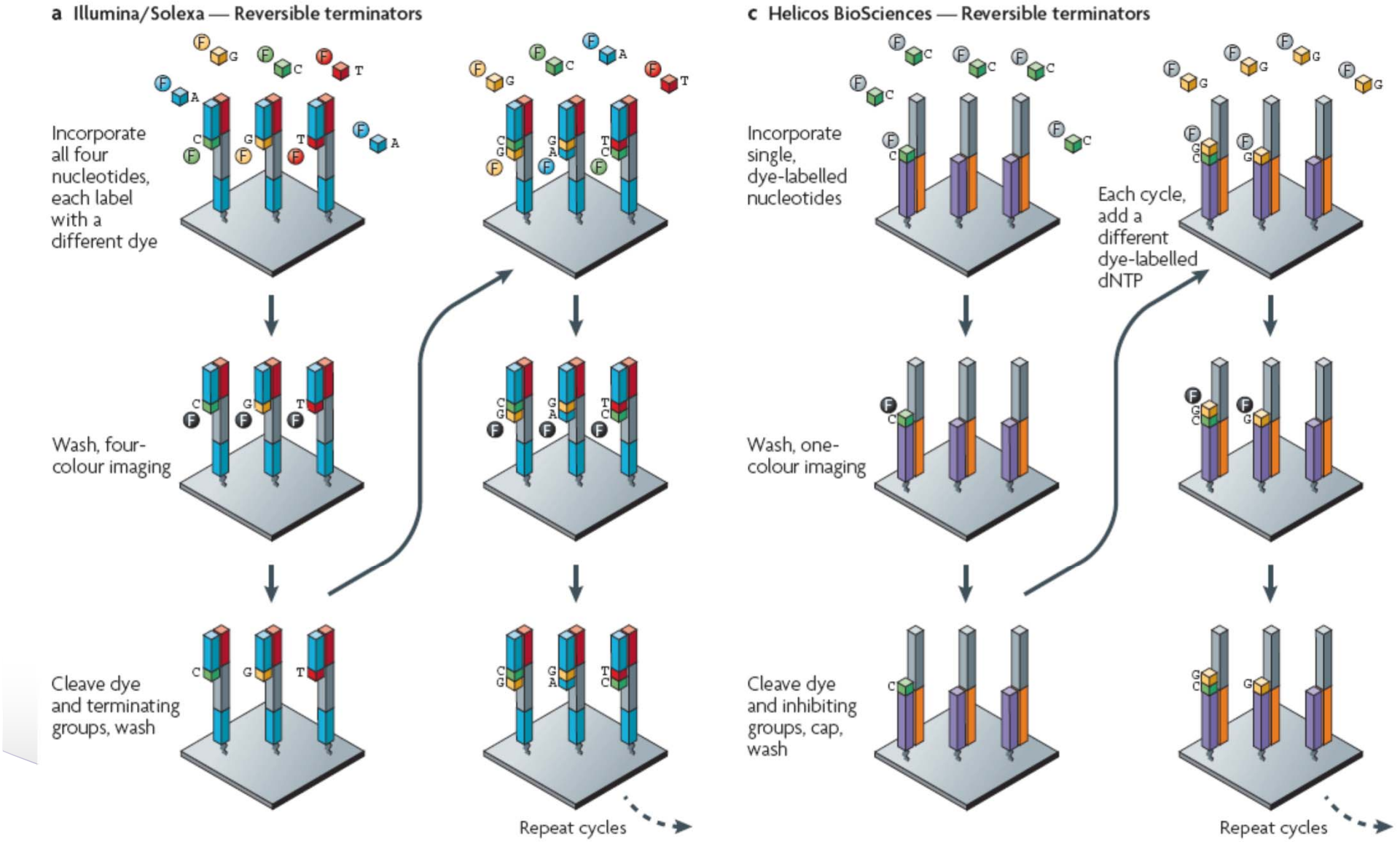
Adaptor固定，然后接模板

模板先固定，然后接**Adaptor**

Illumina/Solexa *vs*. Helicos BioSciences

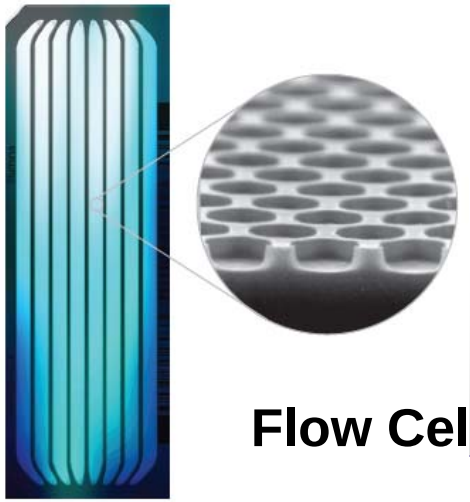


Illumina/Solexa *vs*. Helicos BioSciences



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

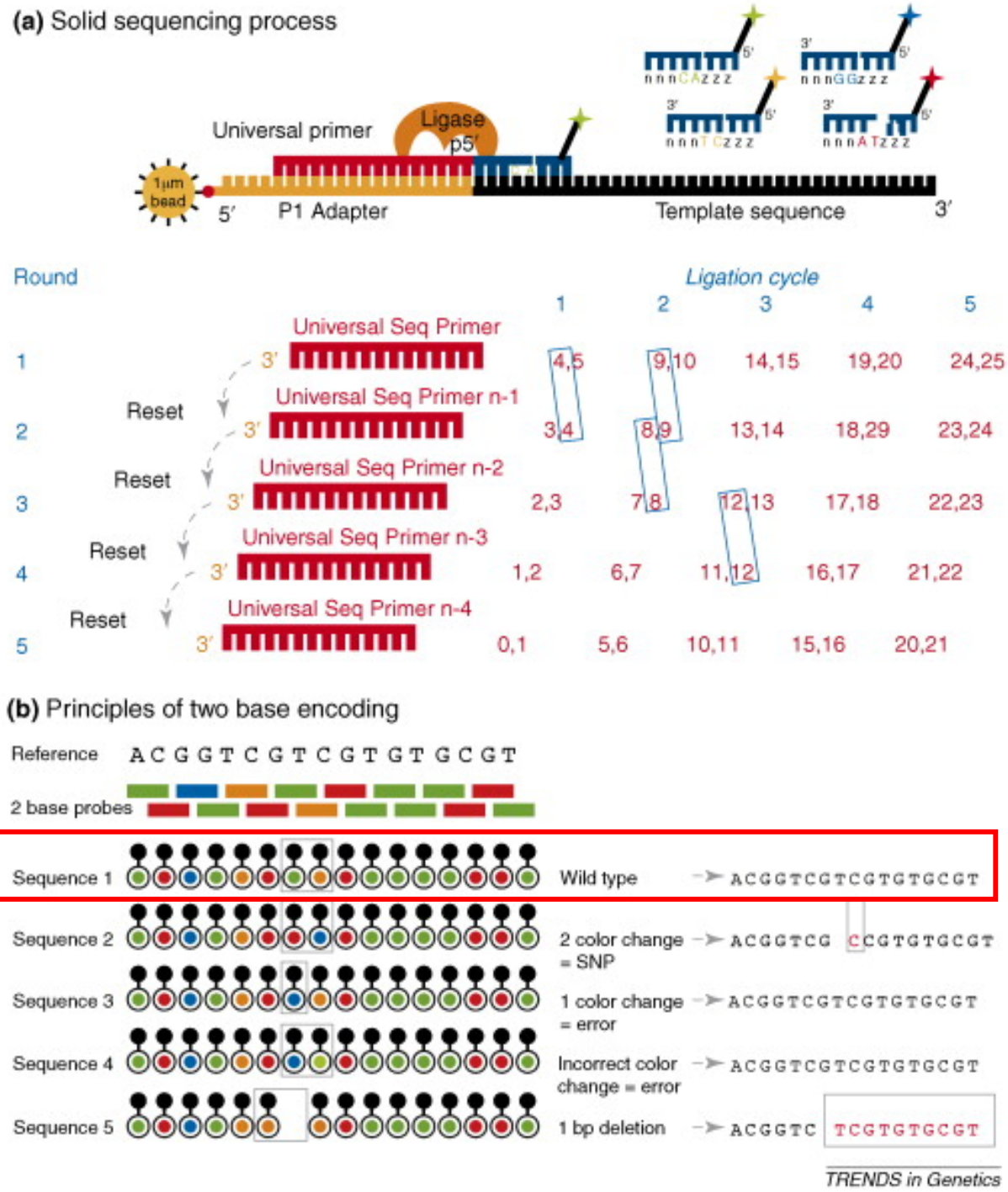
	桌面式测序仪		生产规模的测序仪	
				
	iSeq 100系统	MiniSeq系统	MiSeq系列	NextSeq系列
常用应用和方法	关键应用	关键应用	关键应用	关键应用
大型全基因组测序（人类、植物、动物）				●
小型全基因组测序（微生物、病毒）	●	●	●	●
外显子组测序				●
靶向基因测序（扩增子、基因panel）	●	●	●	●
全转录组测序				●
使用mRNA-Seq进行基因表达谱分析				●
靶向基因表达谱分析	●	●	●	
长片段扩增子测序*	●	●	●	
miRNA和Small RNA分析	●	●	●	●
DNA-蛋白质相互作用分析			●	●
甲基化测序				●
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

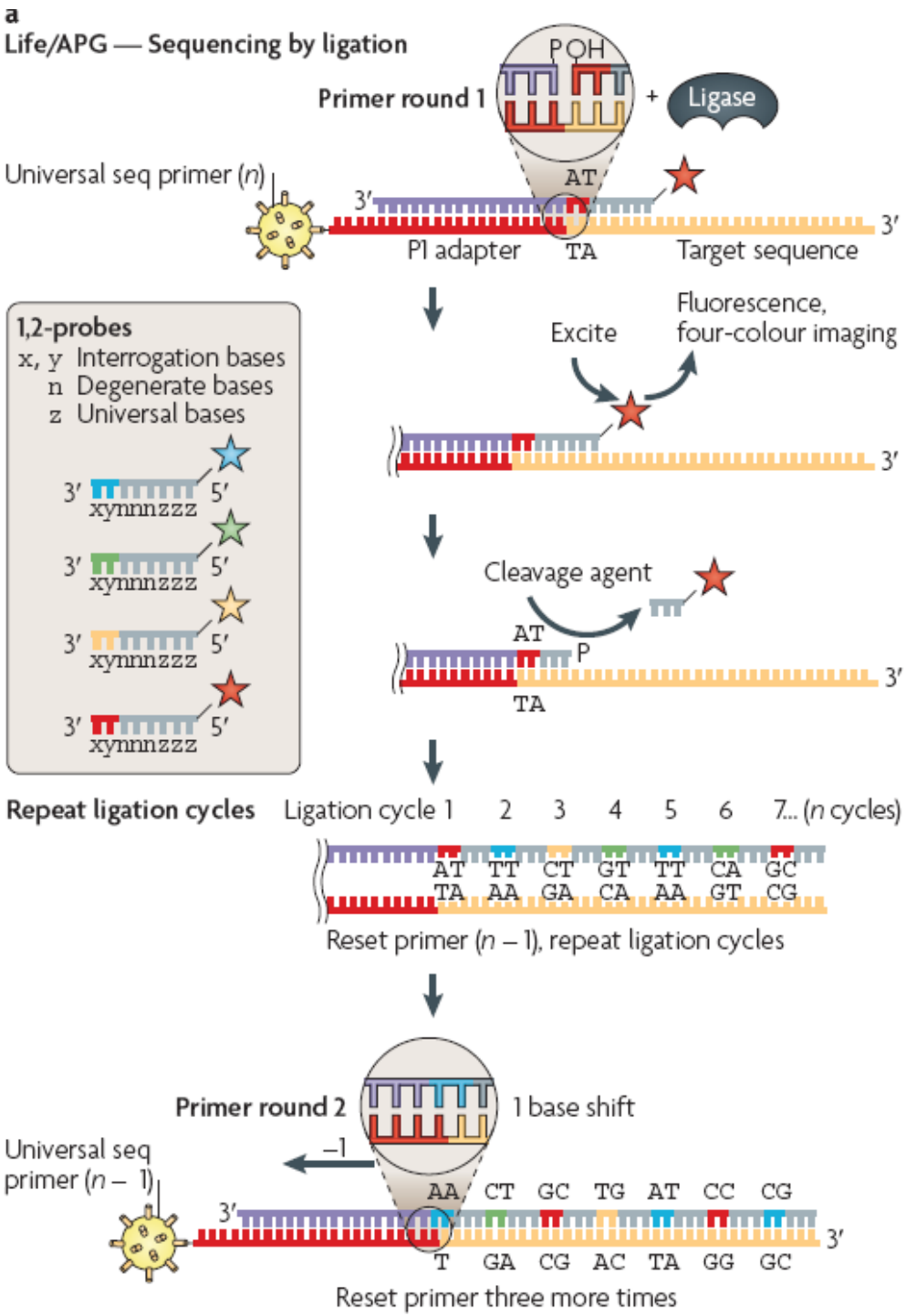
	桌面式测序仪	生产规模的测序仪		
				
	NextSeq系列	HiSeq系列	HiSeq X系列‡	NovaSeq系列
常用应用和方法	关键应用	关键应用	关键应用	关键应用
大型全基因组测序（人类、植物、动物）	●	●	●	●
小型全基因组测序（微生物、病毒）	●	●		●
外显子组测序	●	●		●
靶向基因测序（扩增子、基因panel）	●	●		●
全转录组测序	●	●		●
使用mRNA-Seq进行基因表达谱分析	●	●		●
miRNA和Small RNA分析	●	●		●
DNA-蛋白质相互作用分析	●	●		●
甲基化测序	●	●		●
鸟枪法宏基因组学测序	●	●		●
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

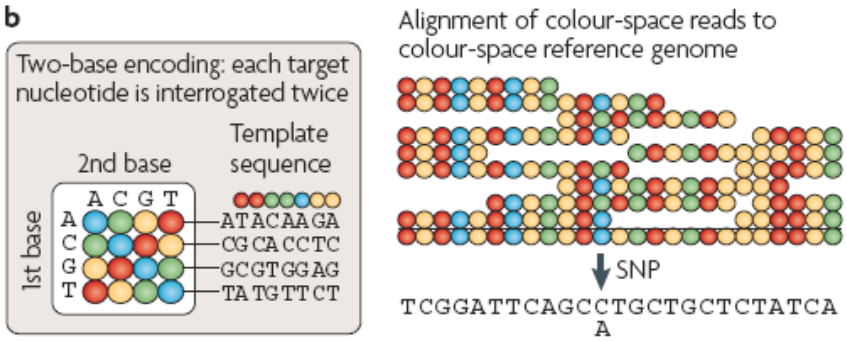
ABI-SOLID

- 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

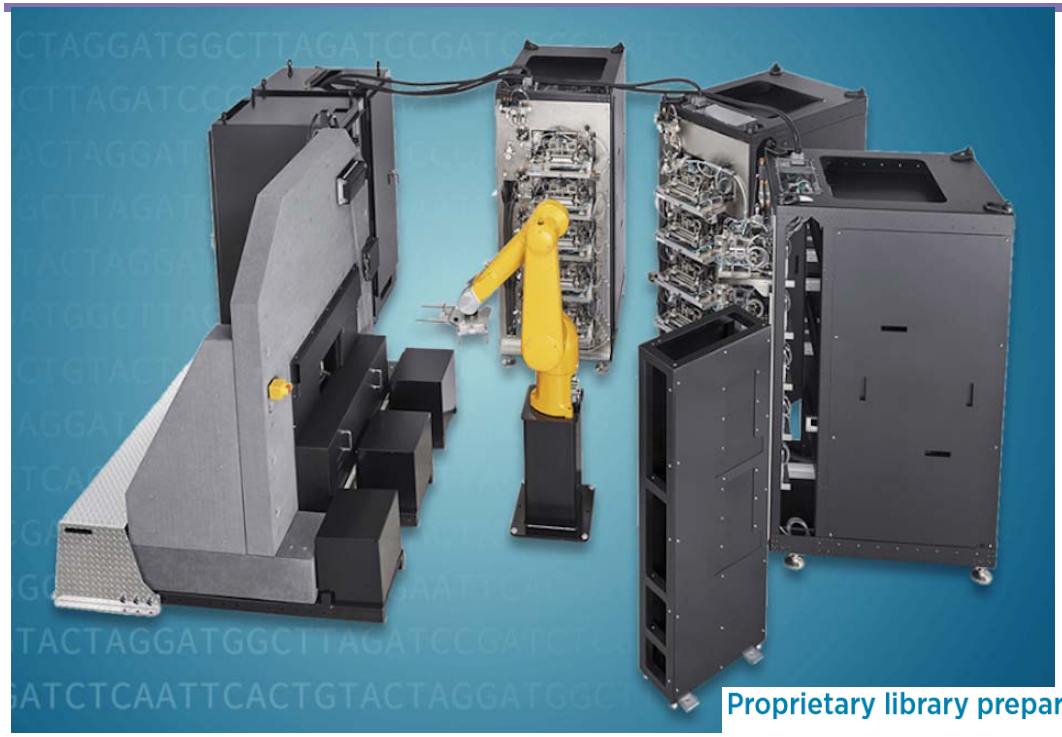

(a) Solid sequencing process
Universal primer
Ligase
p5′
1μm bead
5′
P1 Adapter
Template sequence
3′
nnnCAzzz
nnnGGzzz
nnnTCzzz
nnnATzzz
Round
Ligation cycle
1
2
3
4
5
Universal Seq Primer
Universal Seq Primer n-1
Universal Seq Primer n-2
Universal Seq Primer n-3
Universal Seq Primer n-4
Reset
4,5
9,10
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

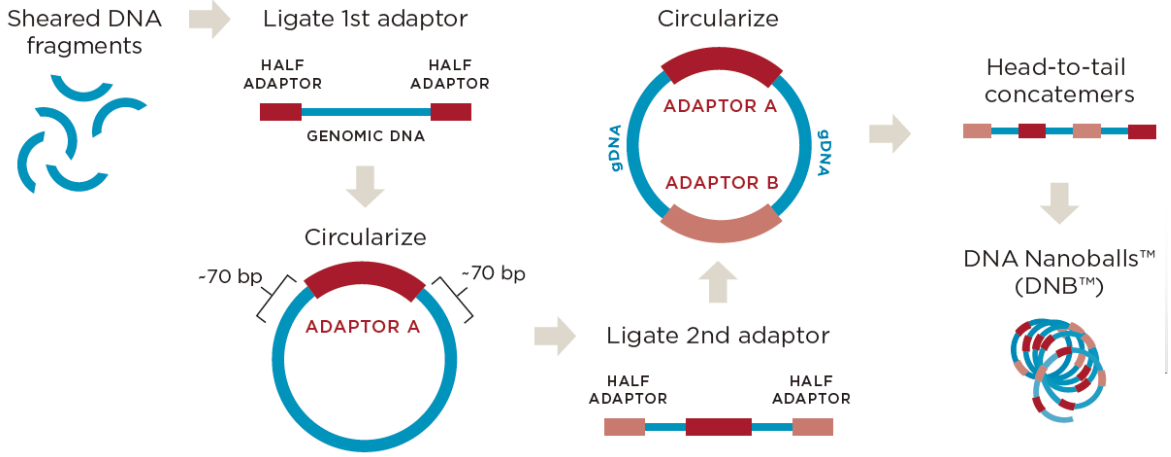

b
Two-base encoding: each target nucleotide is interrogated twice
2nd base
Template sequence
A C G T
1st base
A
C
G
T
ATACAAGA
CGCACCTC
GCGTGGAG
TATGTTCT
Alignment of colour-space reads to colour-space reference genome
SNP
TCGGATTCAGCCTGCTGCTCTATCA
A

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）

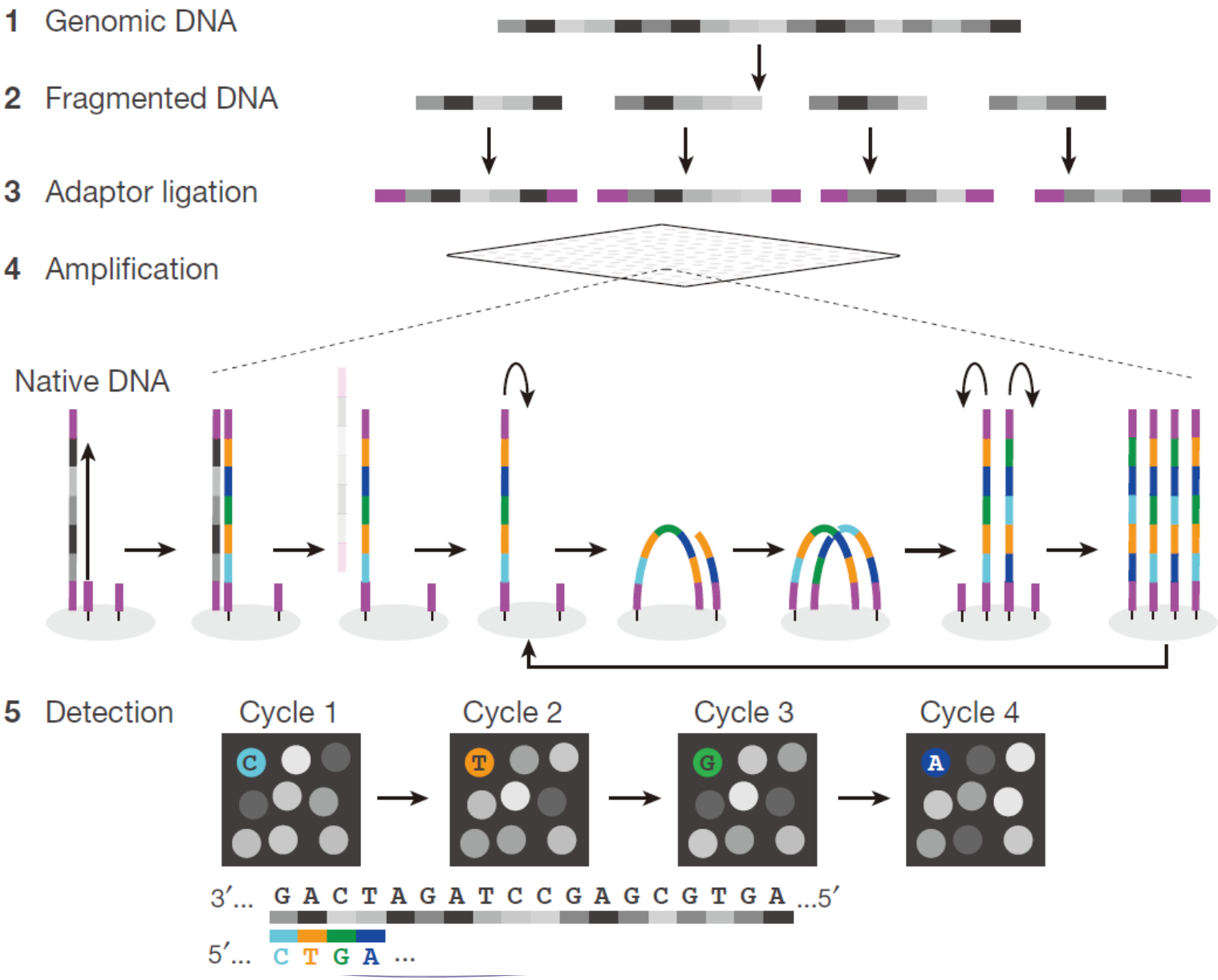
BGISEQ：桌面化测序系统

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)



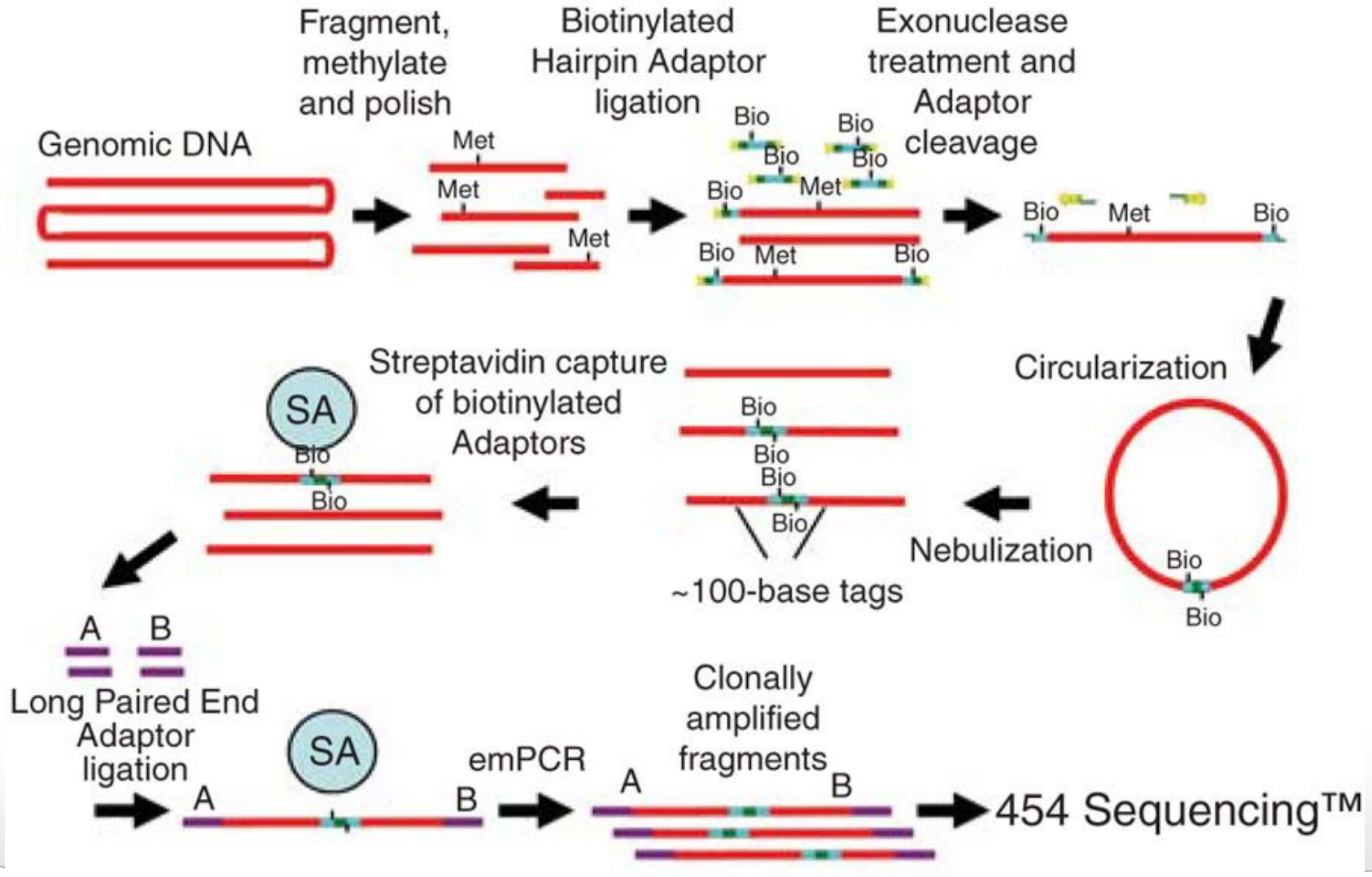
每GB数据的花费 (2008)

- 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

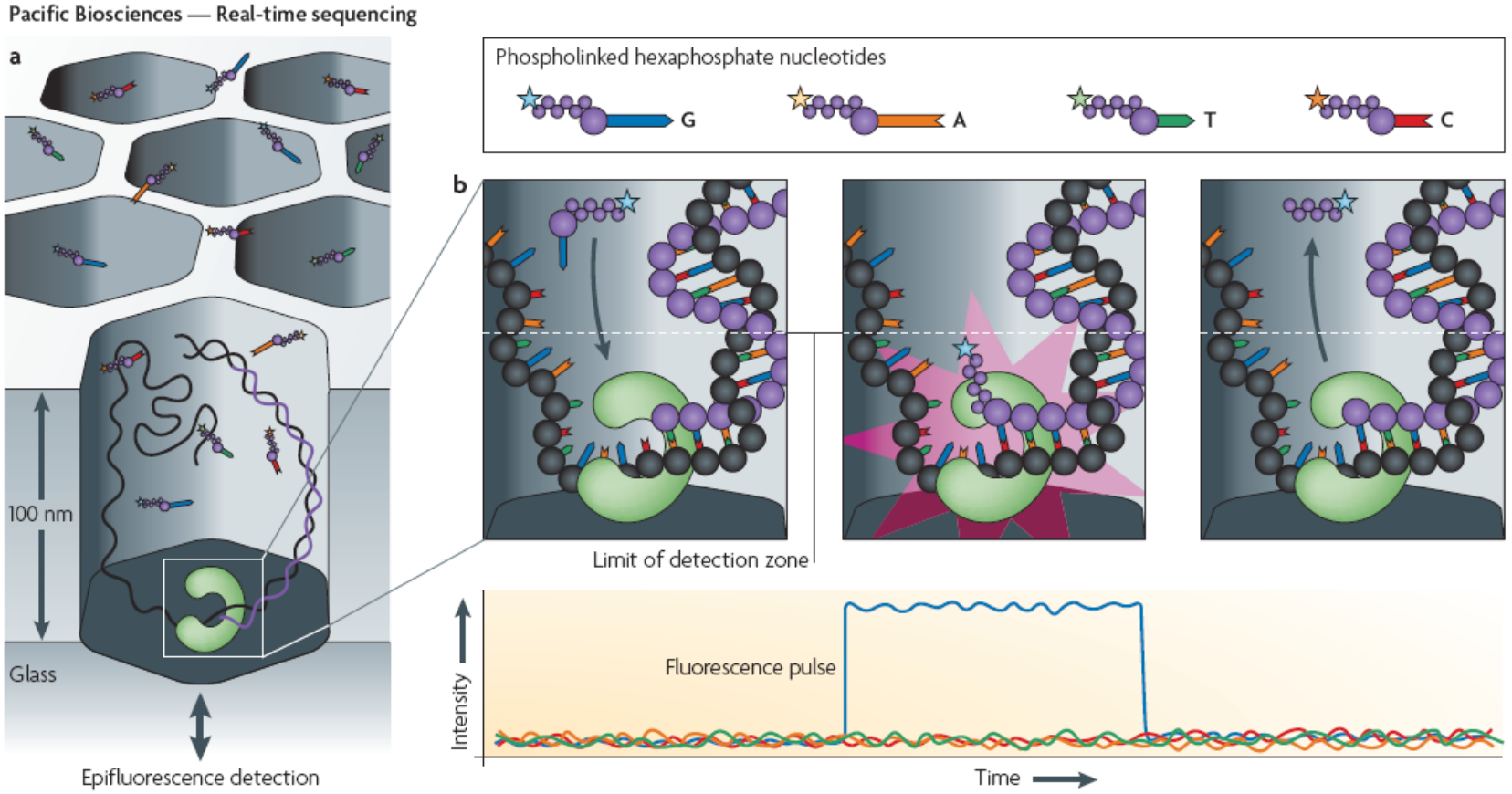
PacBio RS II

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

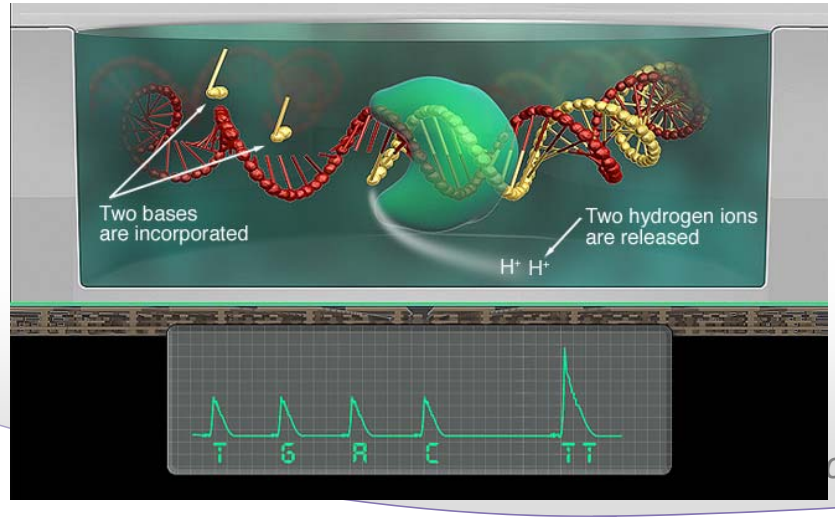
- ZMW孔（Zero-Mode Waveguides, 零模波导孔）：微孔直径小于光的波长时，光强会急剧衰减
- 孔底部仅能固定单分子DNA聚合酶和单分子DNA



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

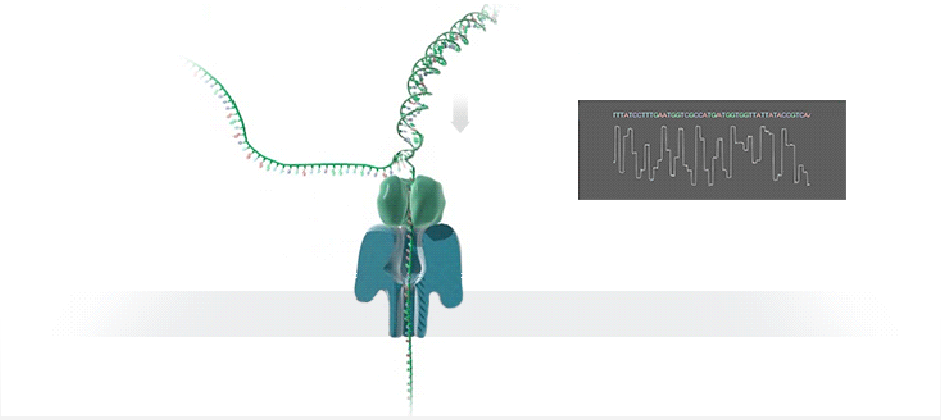


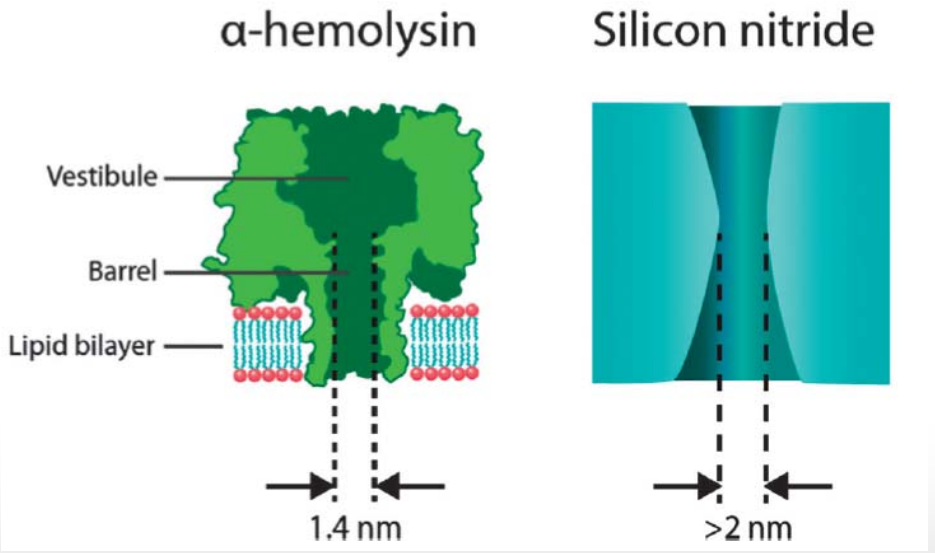
Oxford Nanopore Technologies

- **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

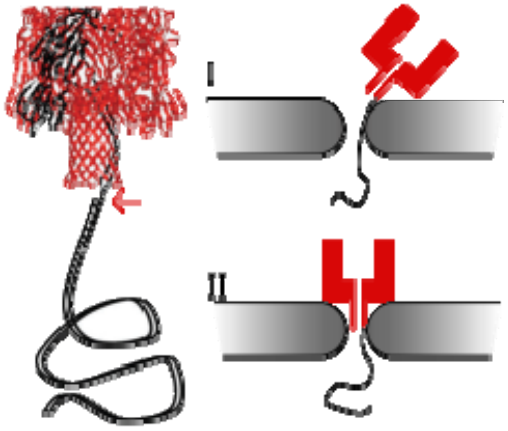
- DNA链的直径：
  - B-DNA (2.0nm); A-DNA (2.6nm)
- Nanopore类型：生物孔和材料孔

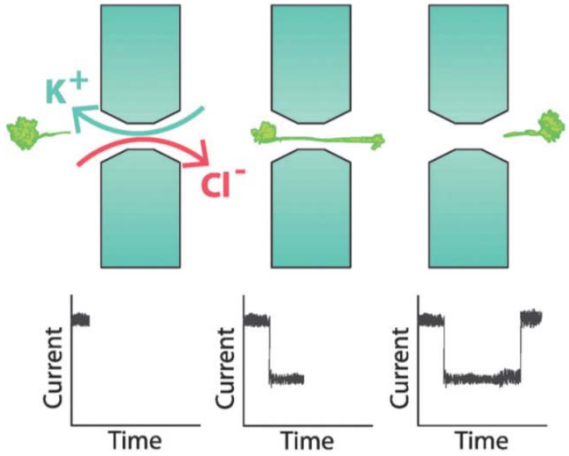




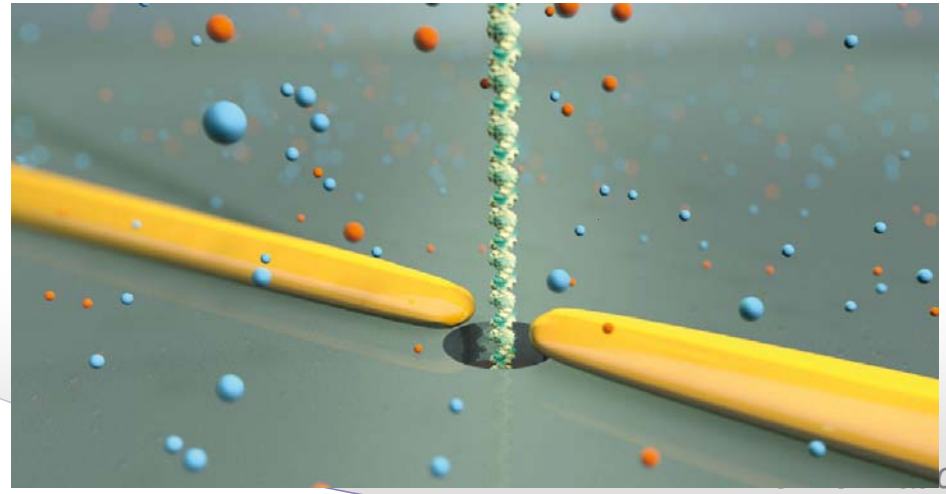
Nanopore测序原理

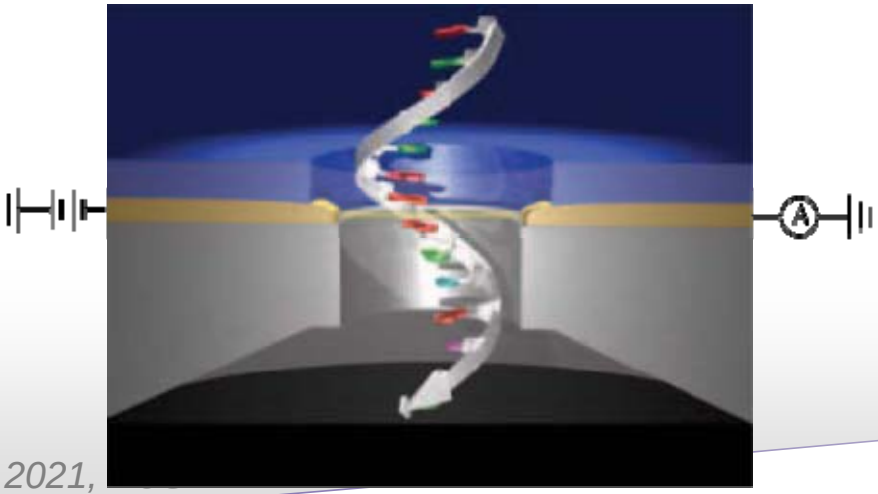
- 膜两侧加电压
  - 开孔电流
  - 阻遏电流





- 纳米孔两侧加电压





cs, 2021,

高通量测序方法的比较

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

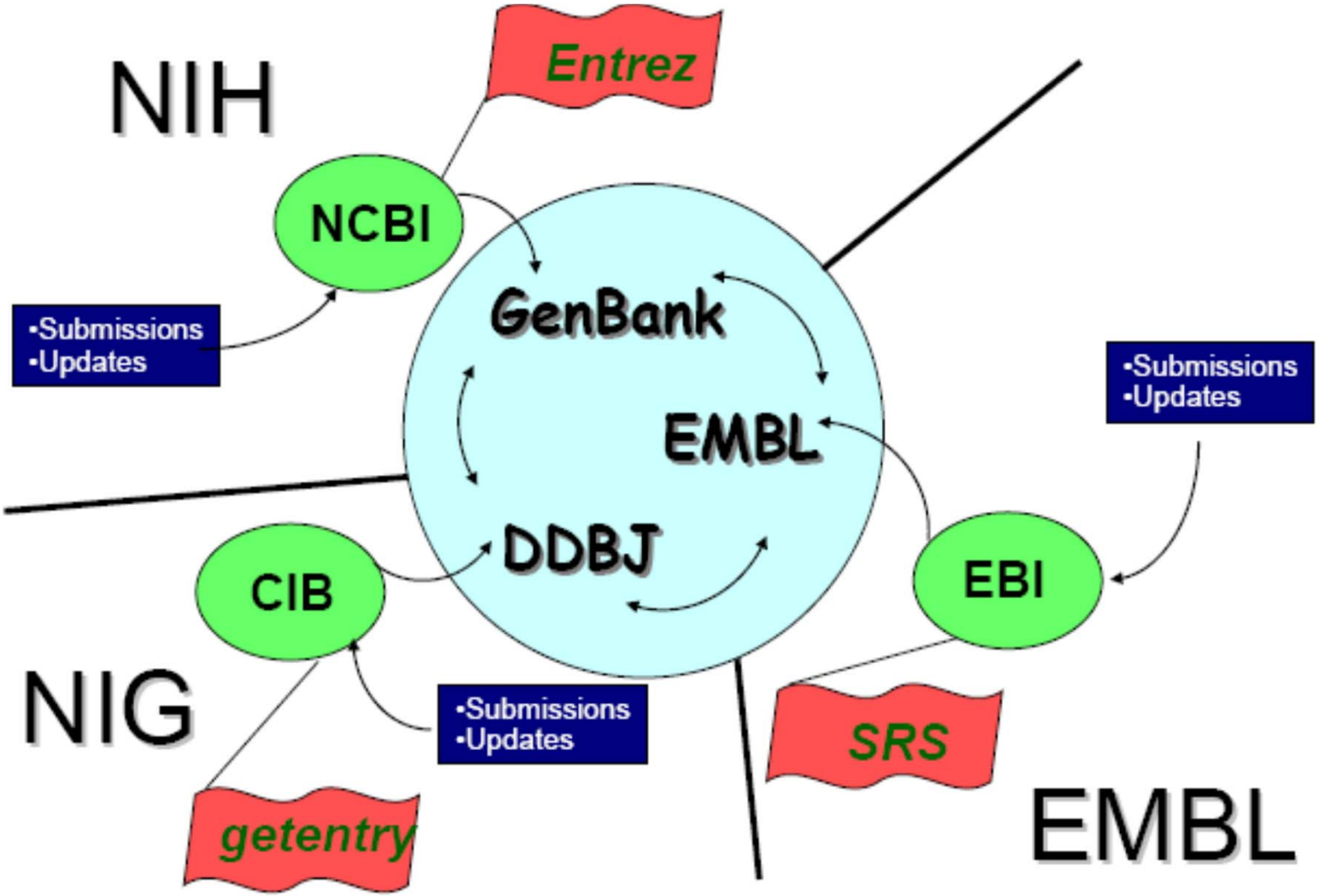
Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	10,000 bp to 15,000 bp avg (14,000 bp N50); maximum read length >40,000 bases[65][66][67]	87% single-read 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, 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

三大数据库之间的联系


NIH
Entrez
NCBI
•Submissions
•Updates
GenBank
EMBL
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CIB
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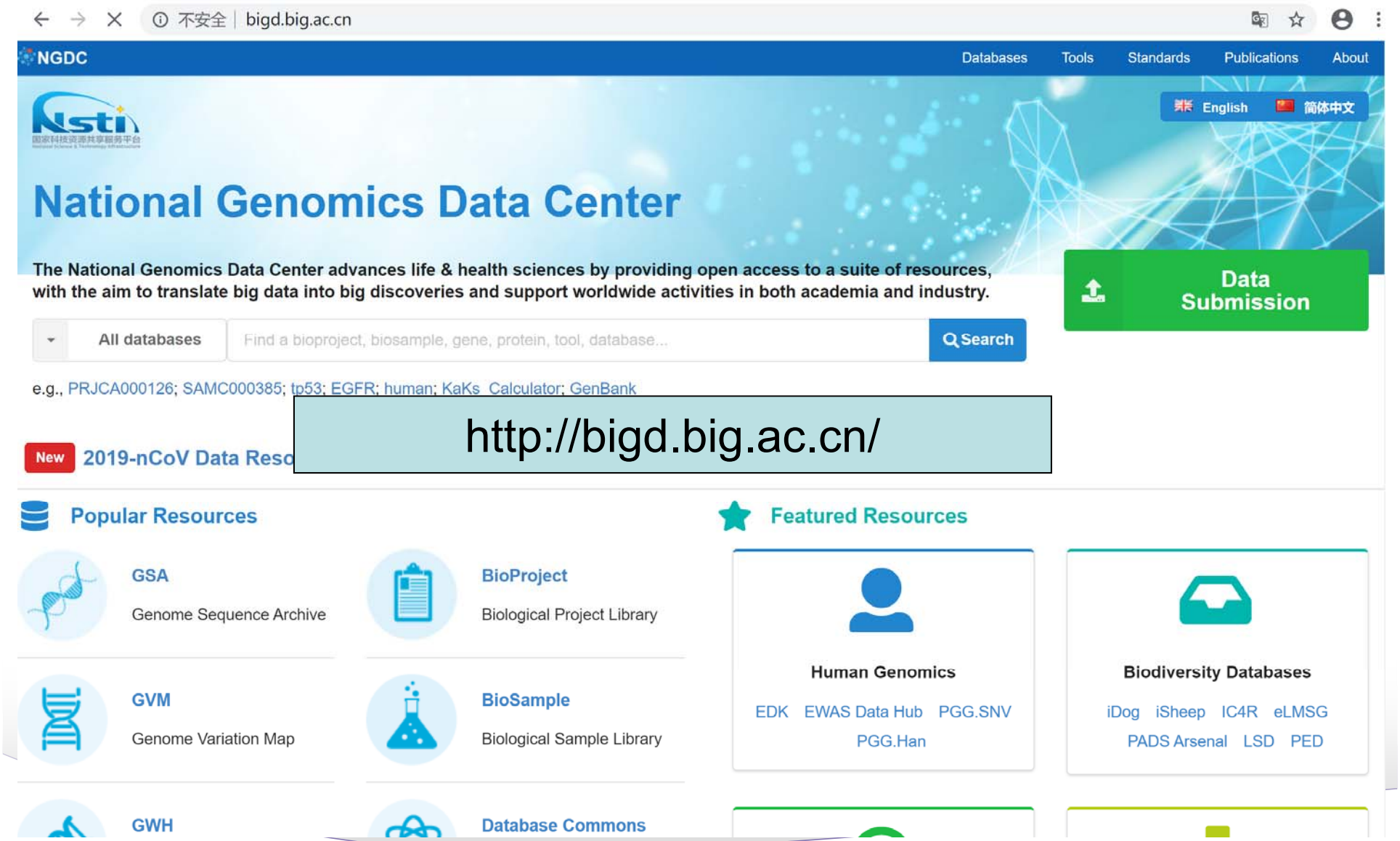
EMBL

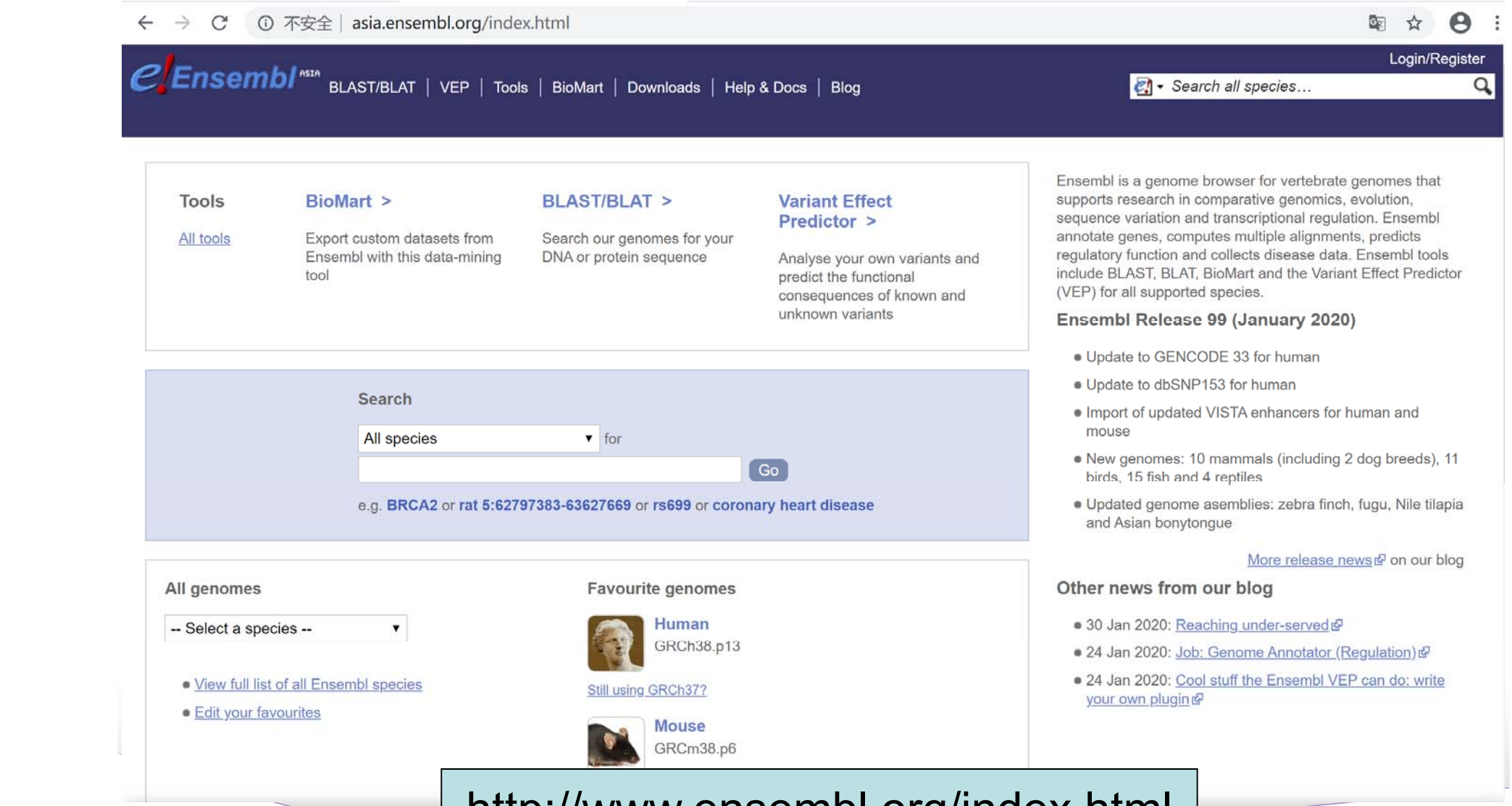
NGDC


不安全 | bigd.big.ac.cn
NGDC
Databases
Tools
Standards
Publications
About
English
简体中文
National Genomics Data Center
The National Genomics Data Center advances life & health sciences by providing open access to a suite of resources, with the aim to translate big data into big discoveries and support worldwide activities in both academia and industry.
Data Submission
All databases
Find a bioproject, biosample, gene, protein, tool, database...
Search
e.g., PRJCA000126; SAMC000385; tp53; EGFR; human; KaKs_Calculator; GenBank
http://bigd.big.ac.cn/
New
2019-nCoV Data Reso
Popular Resources
GSA
Genome Sequence Archive
BioProject
Biological Project Library
GVM
Genome Variation Map
BioSample
Biological Sample Library
GWH
Database Commons
Featured Resources
Human Genomics
EDK EWAS Data Hub PGG.SNV
PGG.Han
Biodiversity Databases
iDog iSheep IC4R eLMSG
PADS Arsenal LSD PED

Genome Sequence Archive

http://bigd.big.ac.cn/gsa/

Ensembl数据库



http://www.ensembl.org/index.html

Refseq数据库

- 提供高质量无冗余完整的序列信息
- 包括基因组的DNA，转录成的RNA以及蛋白质序列信息
- 序列文件的标识符：
  - DNA/RNA序列，NM_XXX, XM_XXX
  - 蛋白质序列：NP_XXX, XP_XXX
  - 染色体：NC_XXX

http://www.ncbi.nlm.nih.gov/RefSeq/

Announcements

January 10, 2020
RefSeq Release 98 is available for FTP

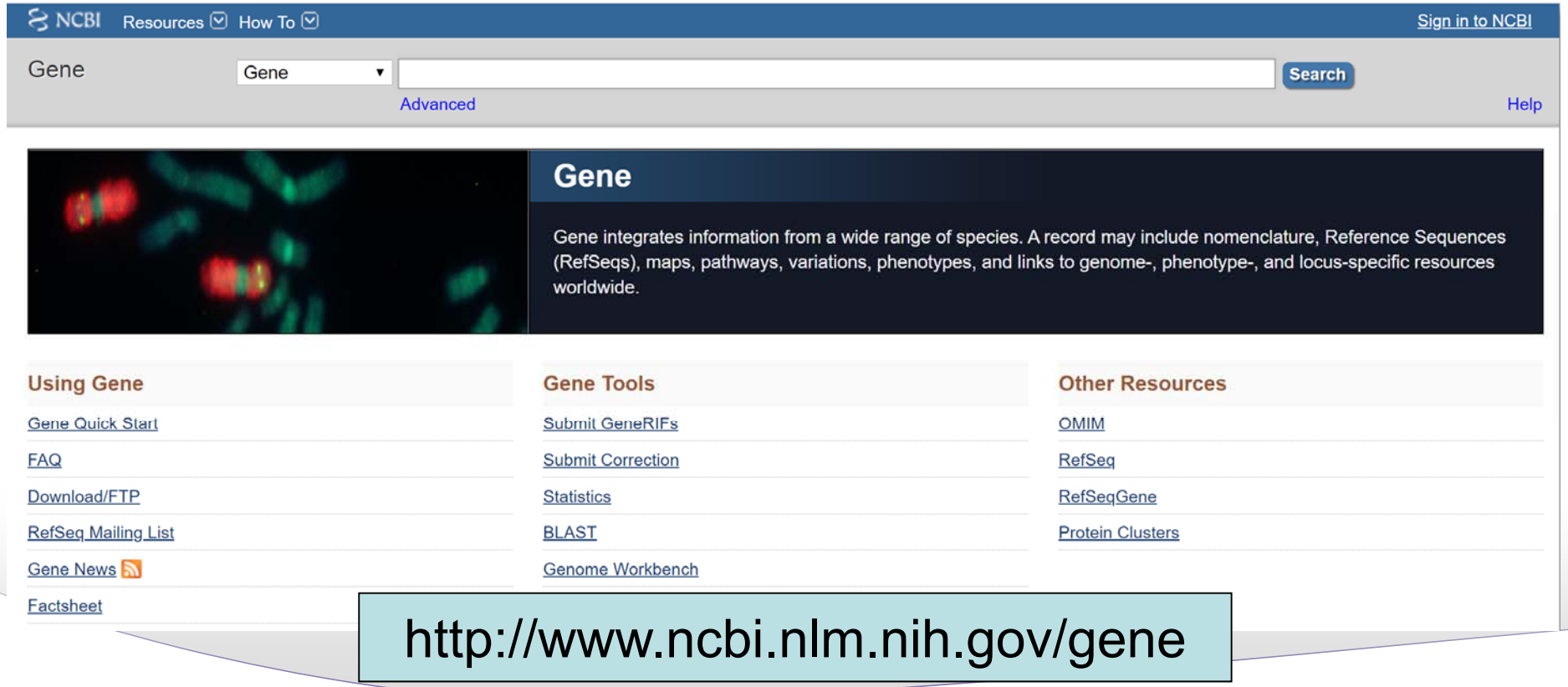
This release includes:

Proteins:	161,133,441
Transcripts:	29,134,515
Organisms:	98,406
Available at:	ftp://ftp.ncbi.nlm.nih.gov/refseq/release/
Documentation:	Release Notes

See previous announcements, follow NCBI on Twitter, or subscribe to NCBI's refseq-announce mail list to receive announcements.

NCBI Gene

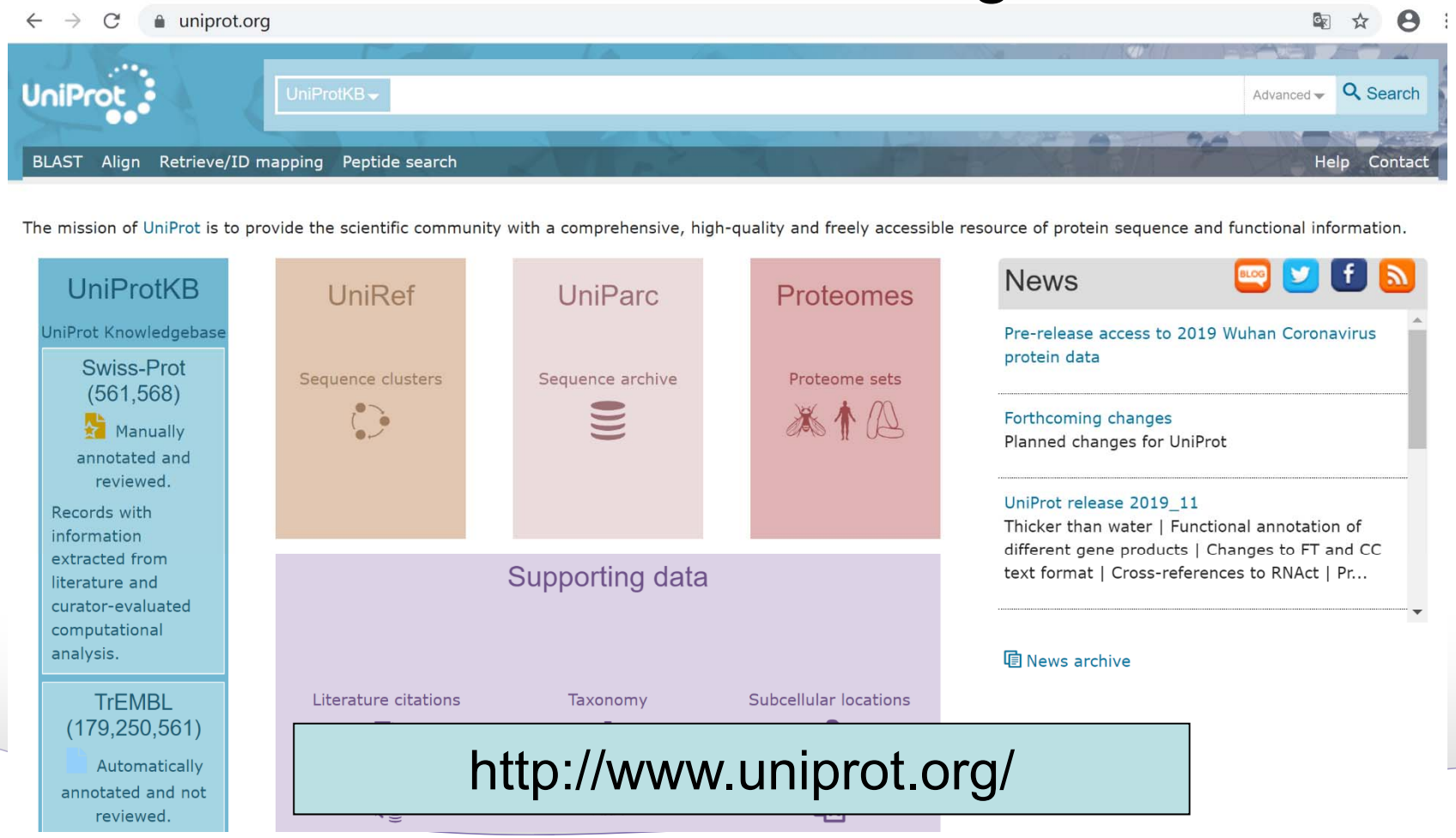
- 序列从Refseq数据库中得到
- 详尽的注释信息，包括基因在基因组的定位，基因名称、蛋白质名称，基因结构，等等



http://www.ncbi.nlm.nih.gov/gene

UniProt

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



http://www.uniprot.org/

序列数据的文件格式

- DNA/RNA/氨基酸代码的标识
- GenBank数据格式
- UniProt
- **FASTA**

DNA代码

Symbol	Meaning
G	G
A	A
T	T
C	C
R	A or G
Y	C or T
M	A or C
K	G or T
S	C or G
W	A or T
H	A,G or T not G
B	C, G or T not A
V	A,C or G not T/U
D	A,G or T not C
N	A,C,G,or T

氨基酸代码

1-letter code	3-letter code	Amin Acid
A	Ala	alanine
C	Cys	cysteine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
O	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
V	Val	valine
W	Trp	trypotophan
X	Xxx	Any
Y	Tyr	tyrosine
B	Asx	Asp or Asn
Z	Glx	Glu, or Gln

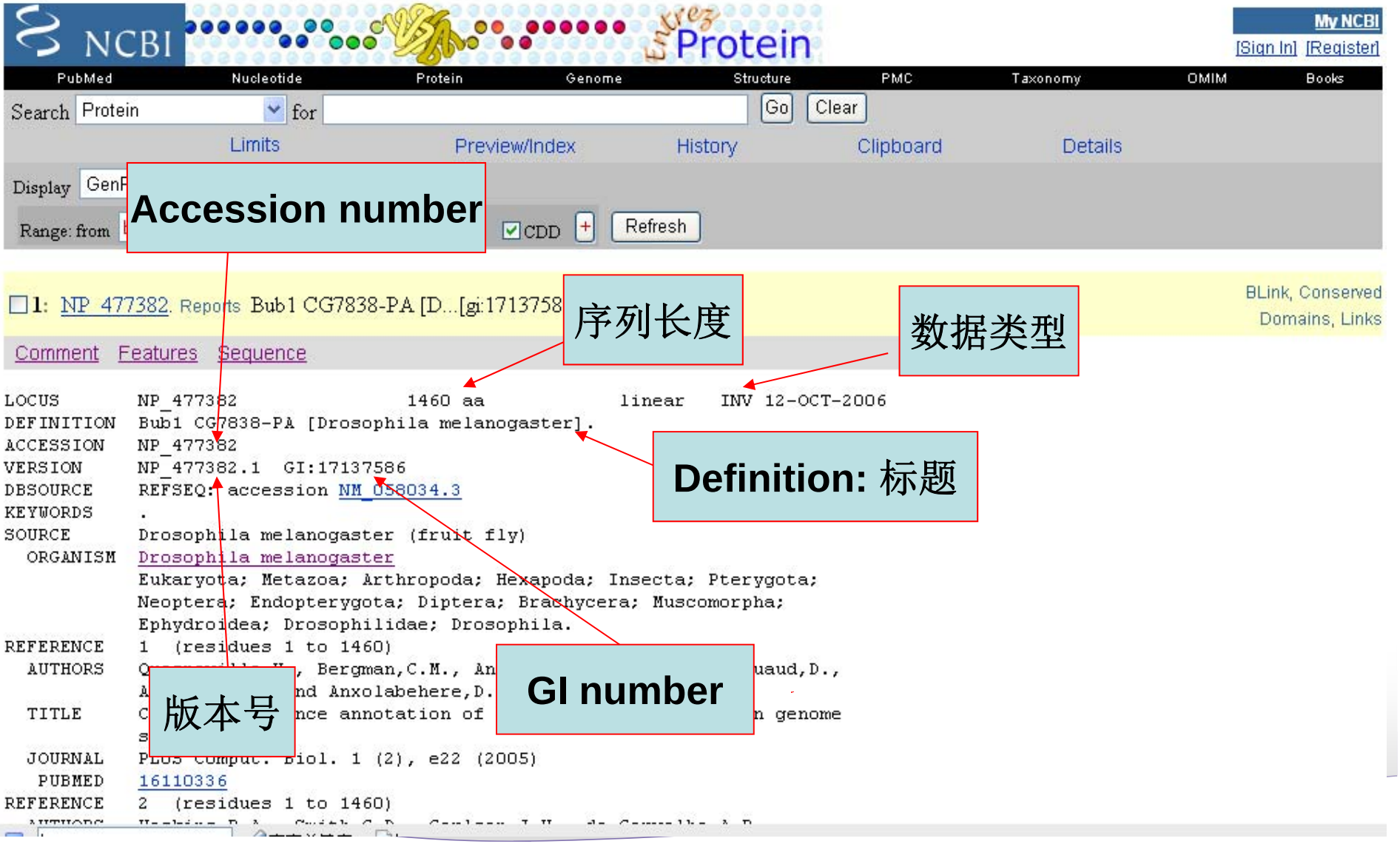
DNA代码

Symbol	Meaning
G	G
A	A
T	T
C	C
R	A or G
Y	C or T
M	A or C
K	G or T
S	C or G
W	A or T
H	A,G or T not G
B	C, G or T not A
V	A,C or G not T/U
D	A,G or T not C
N	A,C,G,or T

氨基酸代码

1-letter code	3-letter code	Amin Acid
A	Ala	alanine
C	Cys	cysteine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
O	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
V	Val	valine
W	Trp	trypotophan
X	Xxx	Any
Y	Tyr	tyrosine
B	Asx	Asp or Asn
Z	Glx	Glu, or Gln

GenBank数据格式

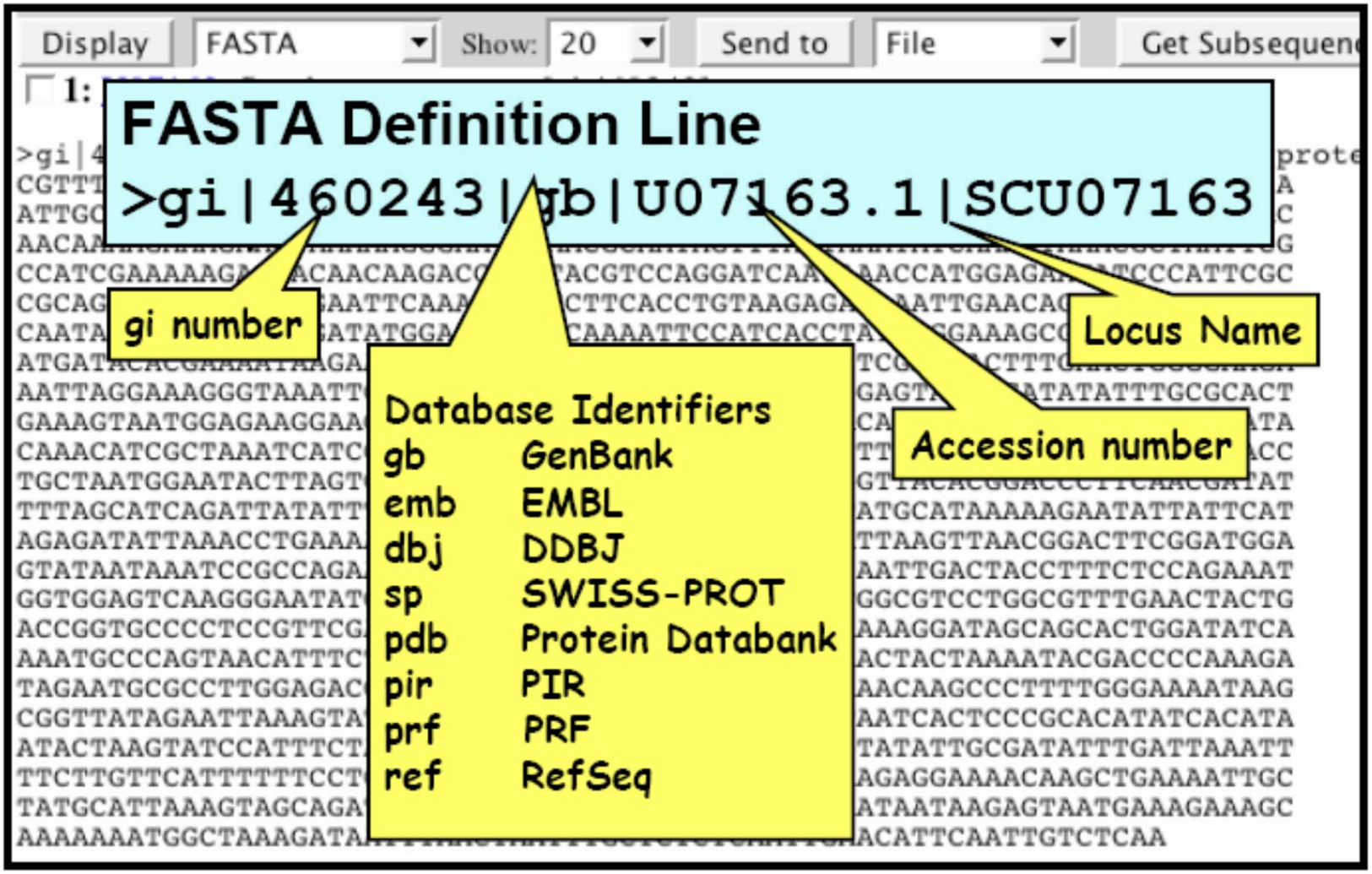


GenBank的数据类型

1. PRI - primate sequences
2. ROD - rodent sequences
3. MAM - other mammalian sequences
4. VRT - other vertebrate sequences
5. INV - invertebrate sequences
6. PLN - plant, fungal, and algal sequences
7. BCT - bacterial sequences
8. VRL - viral sequences
9. PHG - bacteriophage sequences
10. SYN - synthetic sequences
11. UNA - unannotated sequences
12. EST - EST sequences (expressed sequence tags)
13. PAT - patent sequences
14. STS - STS sequences (sequence tagged sites)
15. GSS - GSS sequences (genome survey sequences)
16. HTG - HTGS sequences (high throughput genomic sequences)
17. HTC - HTC sequences (high throughput cDNA sequences)
18. ENV - Environmental sampling sequences
19. CON - Constructed sequences

UniProt数据格式

FASTA格式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Locus Name
Accession number
Database Identifiers
gb GenBank
emb EMBL
dbj DDBJ
sp SWISS-PROT
pdb Protein Databank
pir PIR
prf PRF
ref RefSeq

序列数据的查询

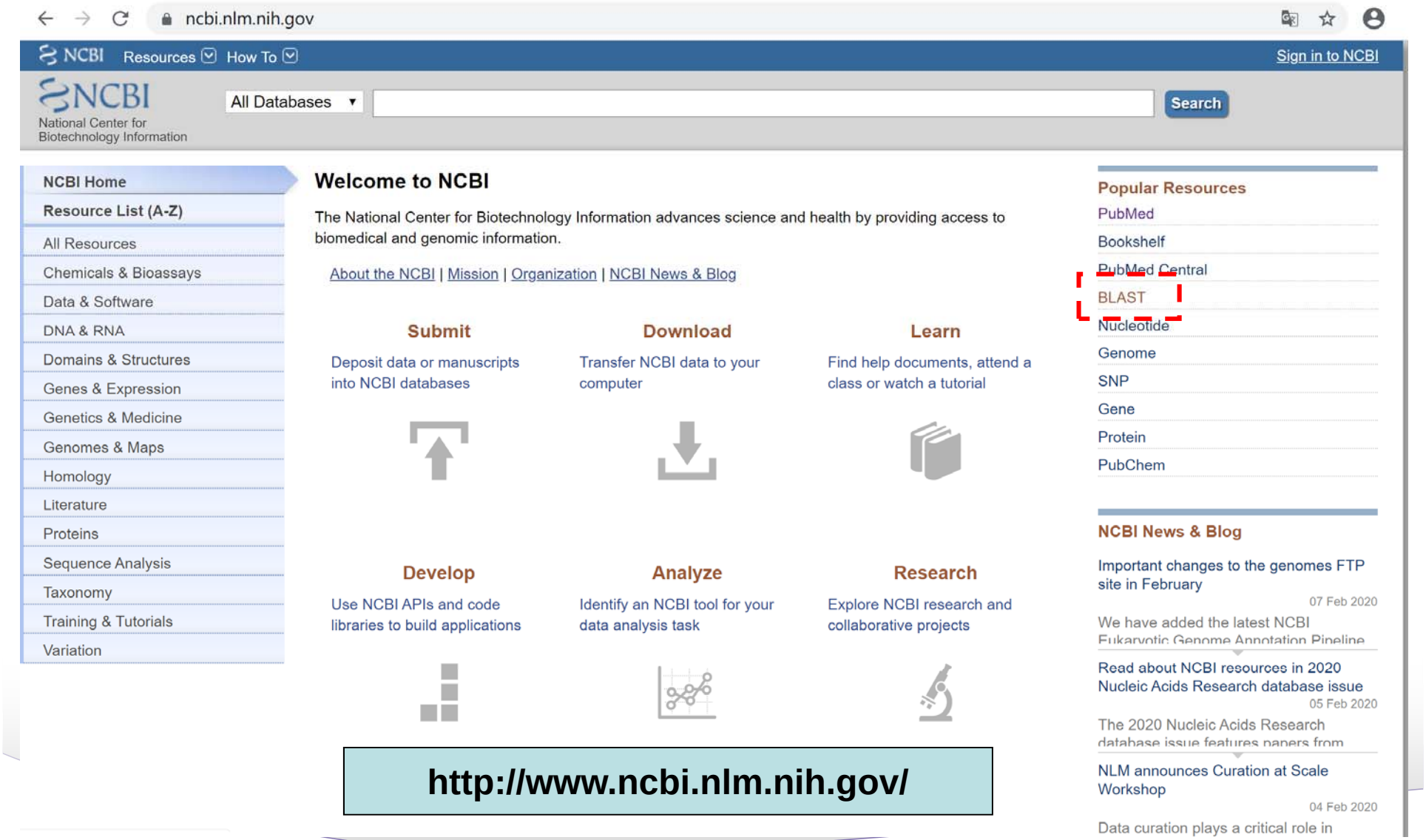
- 例：利用实验学方法（例如，酵母双杂交），发现了与有丝分裂期间某个蛋白可能相互作用的一个基因，测序结果如下（genotype）：

CCCCTGCCTGGCAGCCCTTTCTCAAGGACCACCGCATCTCTACATTCAAGA
ACTGGCCCTTCTTGGAGGGCTGCGCCTGCACCCCGGAGCGGATGGCCGA
GGCTGGCTTCATCCACTGCCCCACTGAGAACGAGCCAGACTTGGCCCAGT
GTTTCTTCTGCTTCAAGGAGCTGGAAGGCTGGGAGCCAGATGACGACCCC
ATAGAGGAACATAAAAAGCATTCGTCCGGTTGCGCTTTCCTTTCTGTCAAGA
AGCAGTTTGAAGAATTAACCCTTGGTGAATTTTTGAAACTGGACAGAGAAAG
AGCCAAGAACAAAATTGCAAAGGAAACCAACAATAAGAAGAAAGAATTTGAG
GAAACTGCGGAGAAAGTGCGCCGTGCCATCGAGCAGCTGGCTGCCATGGA
TTGAGGCCTCTGGC

问题：

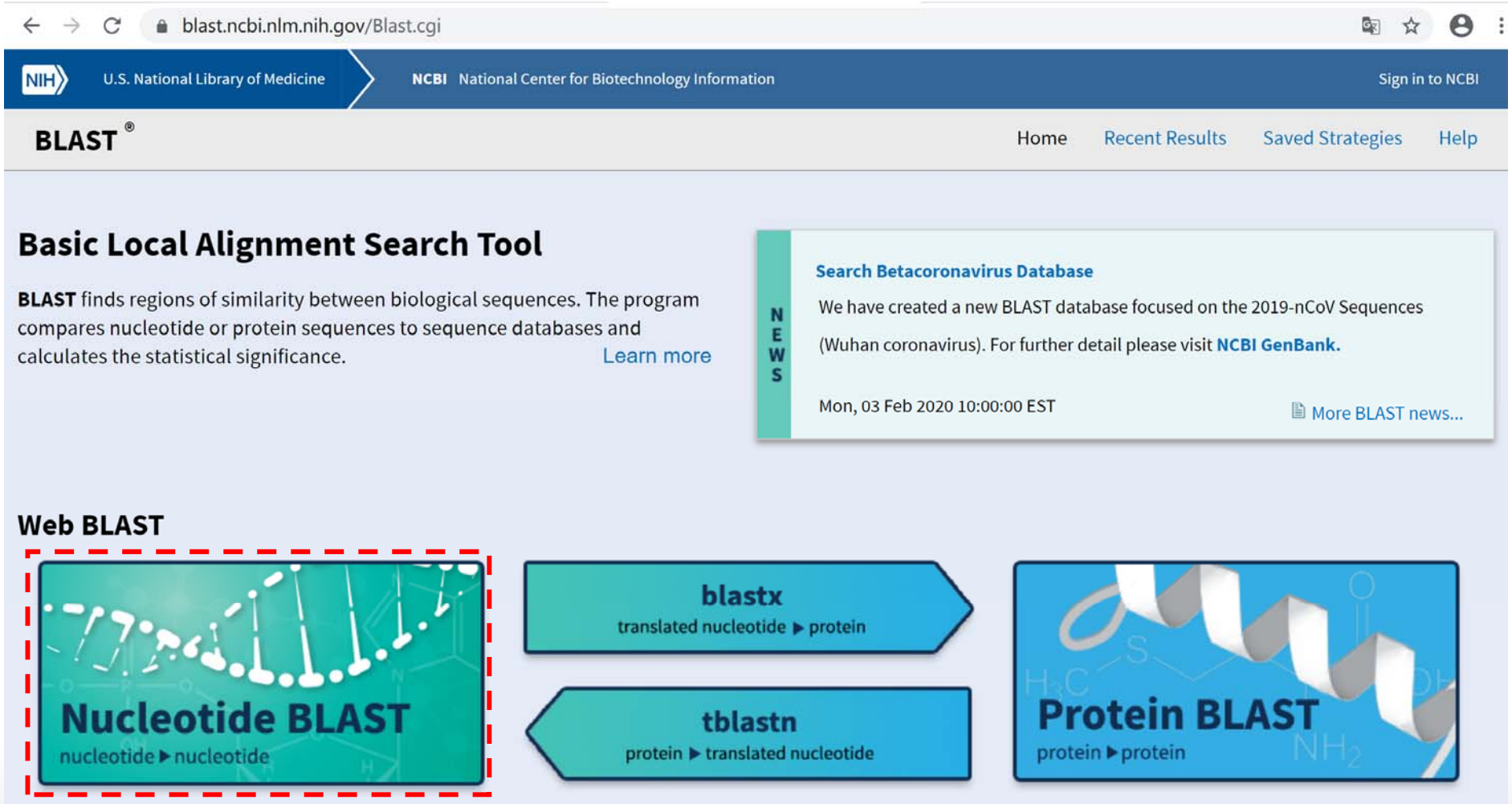
- 这是哪个基因？
- 编码的蛋白质序列是怎样的？
- 有没有保守的功能结构域（domain）？
- 它的功能是怎样的？
- 它在真核生物中保守吗？
- 有没有三级结构信息？

NCBI: BLAST



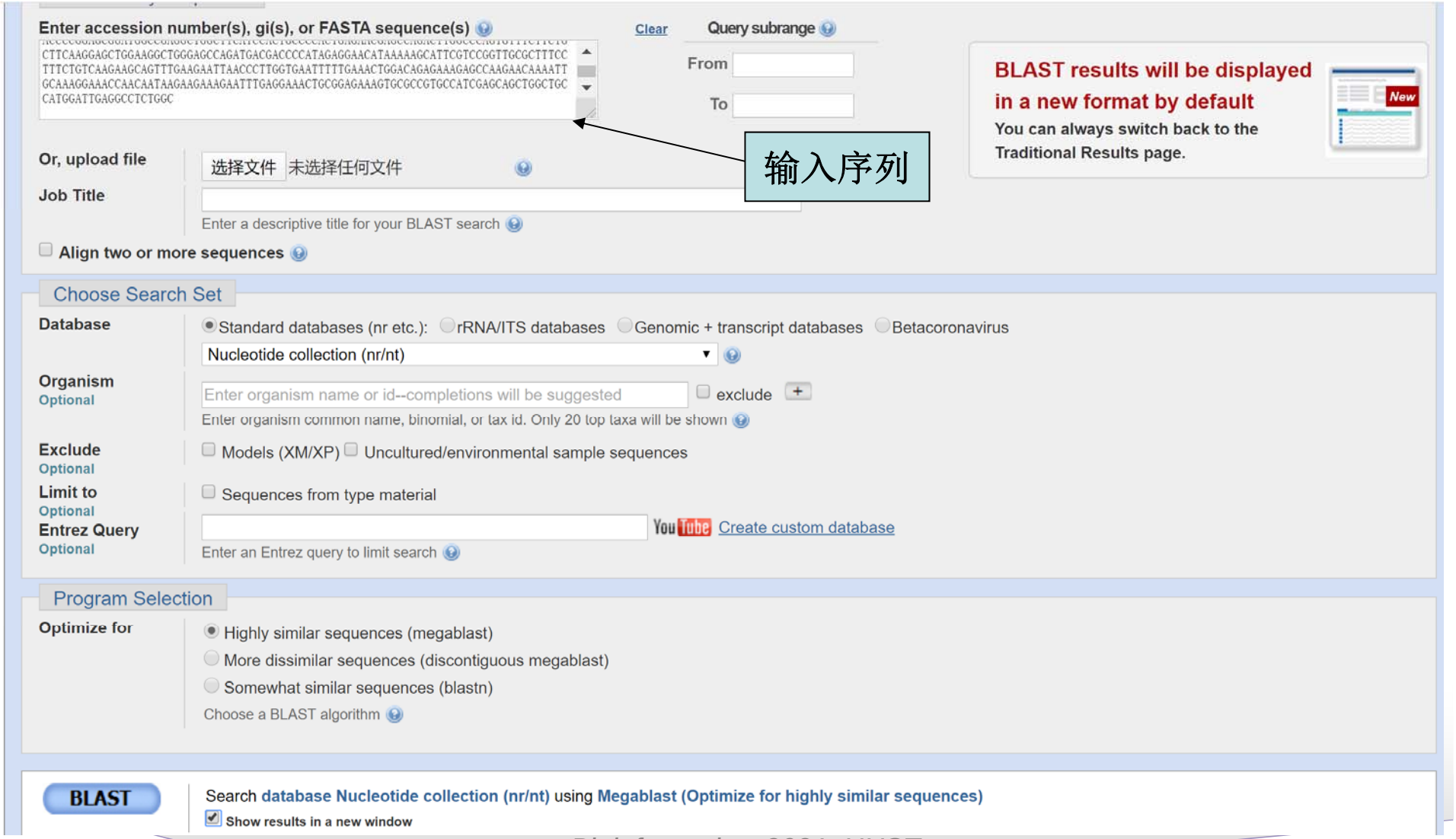
http://www.ncbi.nlm.nih.gov/

Nucleotide blast

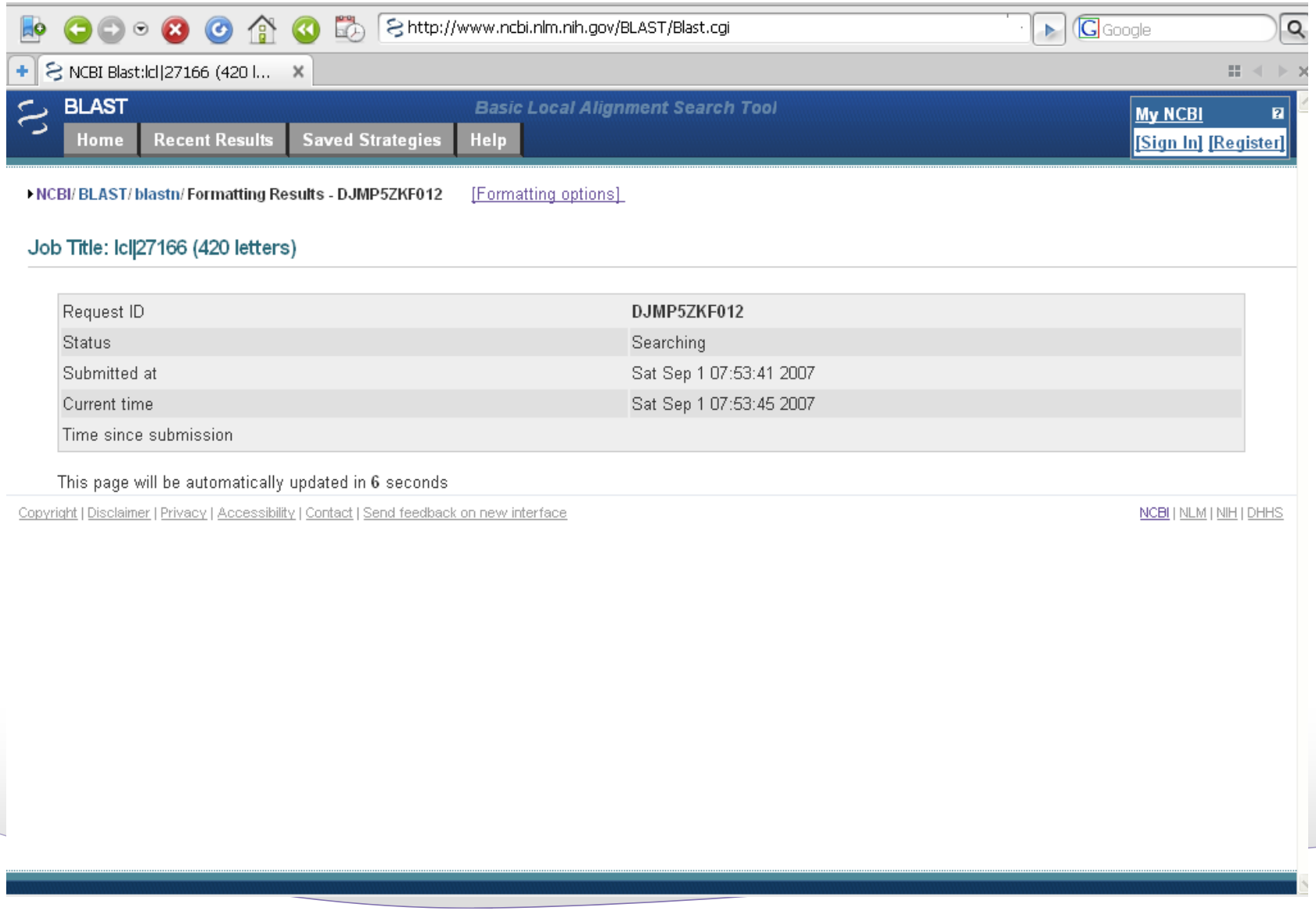


https://blast.ncbi.nlm.nih.gov/Blast.cgi

Megablast: 找基因序列



提交序列


http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi
Google
NCBI Blast:lcl|27166 (420 l...
BLAST
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Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
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[Formatting options]
Job Title: lcl|27166 (420 letters)
Request ID
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Status
Searching
Submitted at
Sat Sep 1 07:53:41 2007
Current time
Sat Sep 1 07:53:45 2007
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NM_001168.3：BIRC5 (Survivin)

Distance tree of results MSA viewer

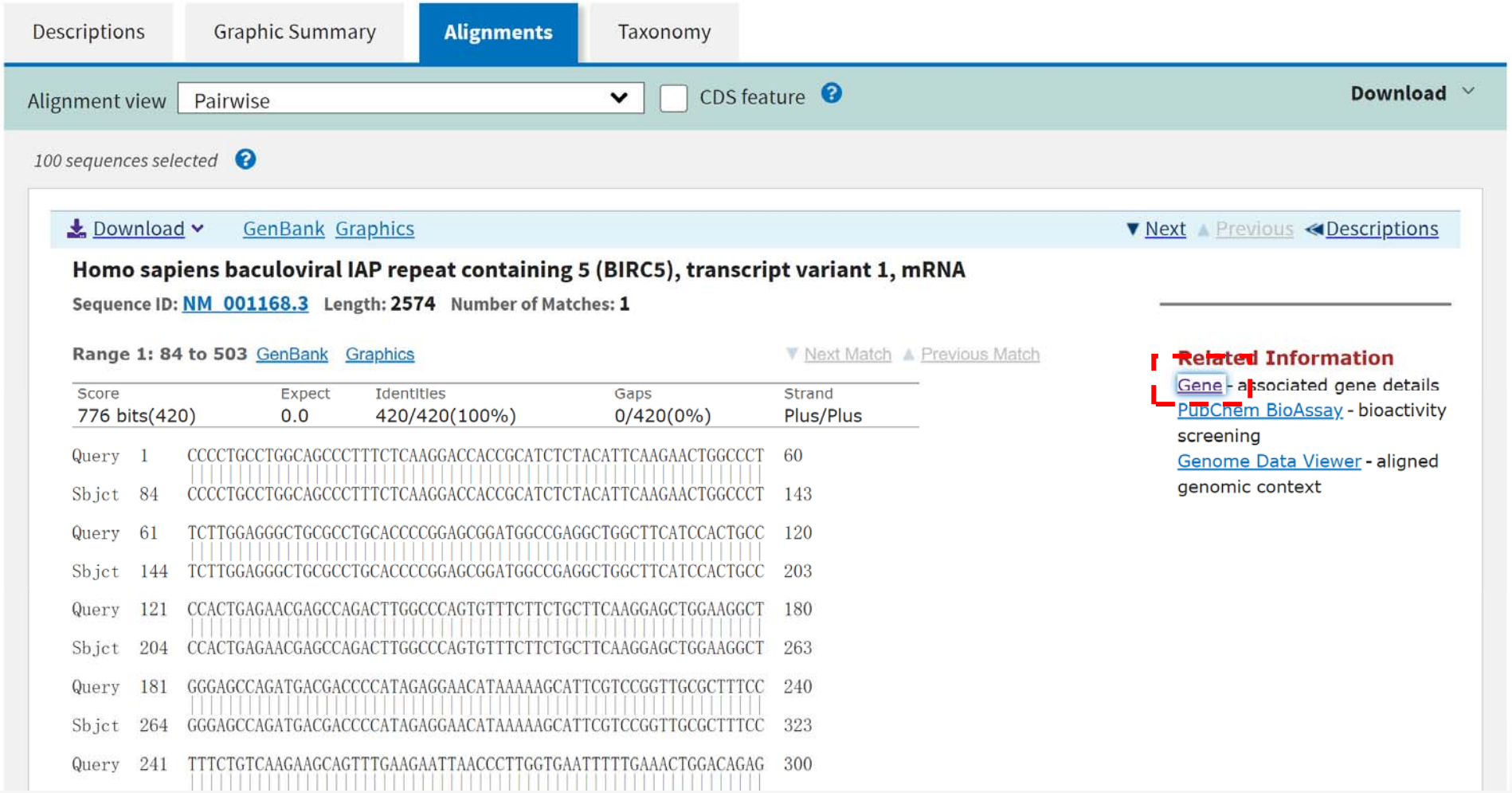
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Homo sapiens baculoviral IAP repeat containing 5 (BIRC5), transcript variant 1, mRNA	776	776	100%	0.0	100.00%	NM_001168.3
Homo sapiens baculoviral IAP repeat-containing 5 (BIRC5) mRNA, complete cds	771	771	100%	0.0	99.76%	HM625836.1
Synthetic construct Homo sapiens baculoviral IAP repeat-containing 5 (survivin), mRNA (cDNA clone IMAGE:2964713), **** W	771	771	100%	0.0	99.76%	BC002388.1
Homo sapiens baculoviral IAP repeat-containing 5, mRNA (cDNA clone MGC:75168 IMAGE:5394399), complete cds	771	771	100%	0.0	99.76%	BC065497.1
Pongo abelii baculoviral IAP repeat containing 5 (BIRC5), mRNA	771	771	100%	0.0	99.76%	NM_001132255.1
Homo sapiens inhibitor of apoptosis homolog mRNA, complete cds	771	771	100%	0.0	99.76%	AF077350.1
Homo sapiens baculoviral IAP repeat-containing 5, mRNA (cDNA clone MGC:8592 IMAGE:2961114), complete cds	771	771	100%	0.0	99.76%	BC008718.2
Homo sapiens baculoviral IAP repeat-containing 5, mRNA (cDNA clone MGC:32768 IMAGE:4656567), complete cds	771	771	100%	0.0	99.76%	BC034148.1
PREDICTED: Gorilla gorilla gorilla baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_019028170.2
PREDICTED: Pan troglodytes baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_009433243.3
PREDICTED: Pan paniscus baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_003818274.2

BIRC5 Gene


Descriptions
Graphic Summary
Alignments
Taxonomy
Alignment view
Pairwise
CDS feature
Download
100 sequences selected
Download
GenBank
Graphics
Next
Previous
Descriptions
Homo sapiens baculoviral IAP repeat containing 5 (BIRC5), transcript variant 1, mRNA
Sequence ID: NM_001168.3 Length: 2574 Number of Matches: 1
Range 1: 84 to 503 GenBank Graphics
Next Match
Previous Match
Score
776 bits(420)
Expect
0.0
Identities
420/420(100%)
Gaps
0/420(0%)
Strand
Plus/Plus
Query 1 CCCCTGCCTGGCAGCCCTTTCTCAAGGACCACCGCATCTCTACATTCAAGAACTGGCCCT 60
Sbjct 84 CCCCTGCCTGGCAGCCCTTTCTCAAGGACCACCGCATCTCTACATTCAAGAACTGGCCCT 143
Query 61 TCTTGGAGGGCTGCGCCTGCACCCCGGAGCGGATGGCCGAGGCTGGCTTCATCCACTGCC 120
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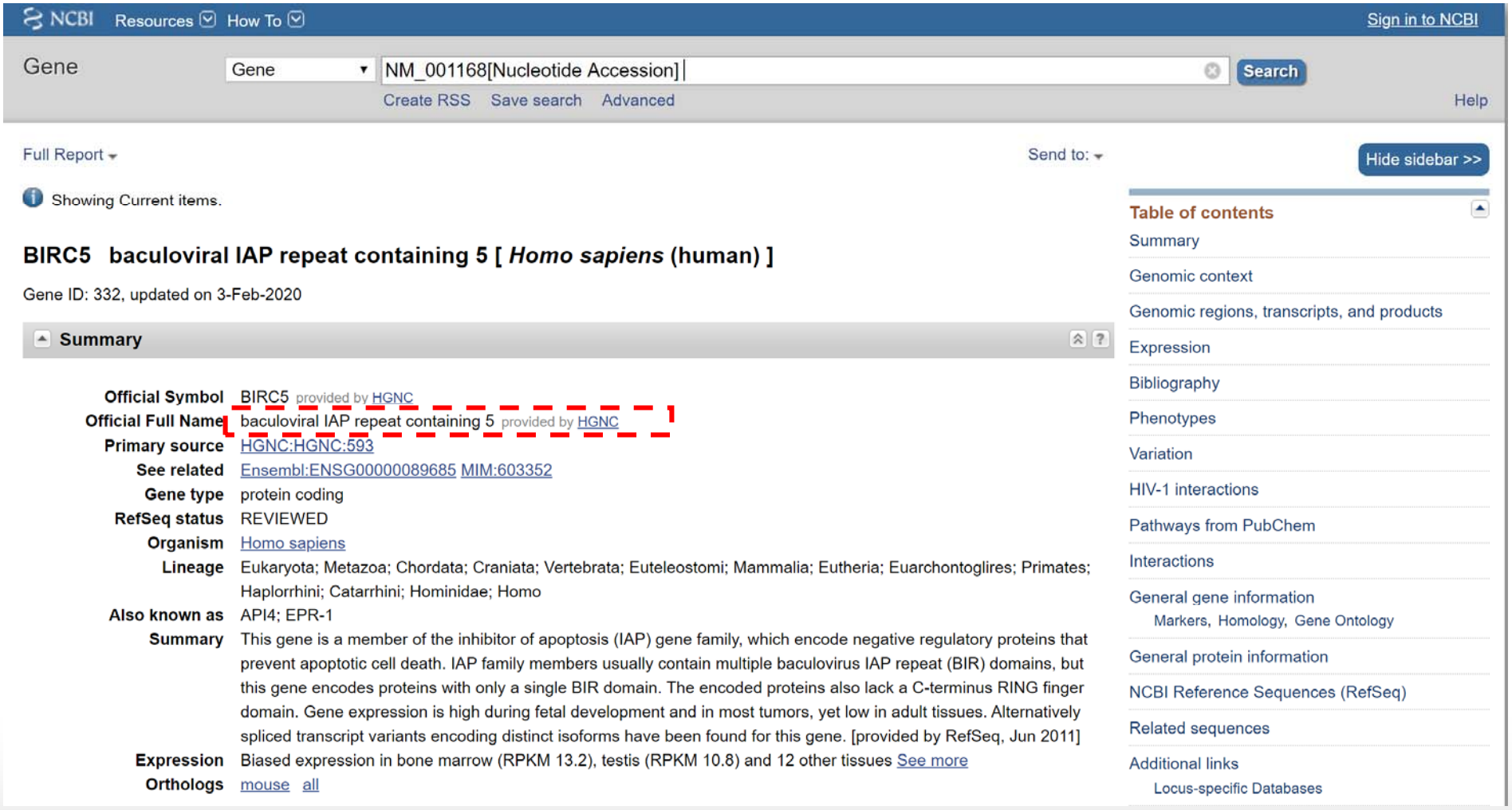
Sbjct 204 CCACTGAGAACGAGCCAGACTTGGCCCAGTGTTTCTTCTGCTTCAAGGAGCTGGAAGGCT 263
Query 181 GGGAGCCAGATGACGACCCCATAGAGGAACATAAAAAGCATTCGTCCGGTTGCGCTTTCC 240
Sbjct 264 GGGAGCCAGATGACGACCCCATAGAGGAACATAAAAAGCATTCGTCCGGTTGCGCTTTCC 323
Query 241 TTTCTGTCAAGAAGCAGTTTGAAGAATTAACCCTTGGTGAATTTTTGAAACTGGACAGAG 300
Related Information
Gene - associated gene details
PubChem BioAssay - bioactivity screening
Genome Data Viewer - aligned genomic context

BIRC5 gene information



Gene info: 17号染色体

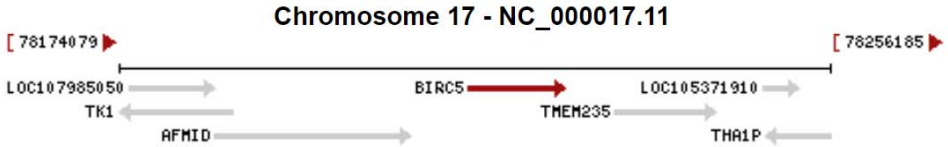
Genomic context

Location: 17q25.3

See BIRC5 in Genome Data Viewer

Exon count: 6

Annotation release	Status	Assembly	Chr	Location
109.20191205	current	GRCh38.p13 (GCF_000001405.39)	17	NC_000017.11 (78214253..78225635)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	17	NC_000017.10 (76210277..76221716)

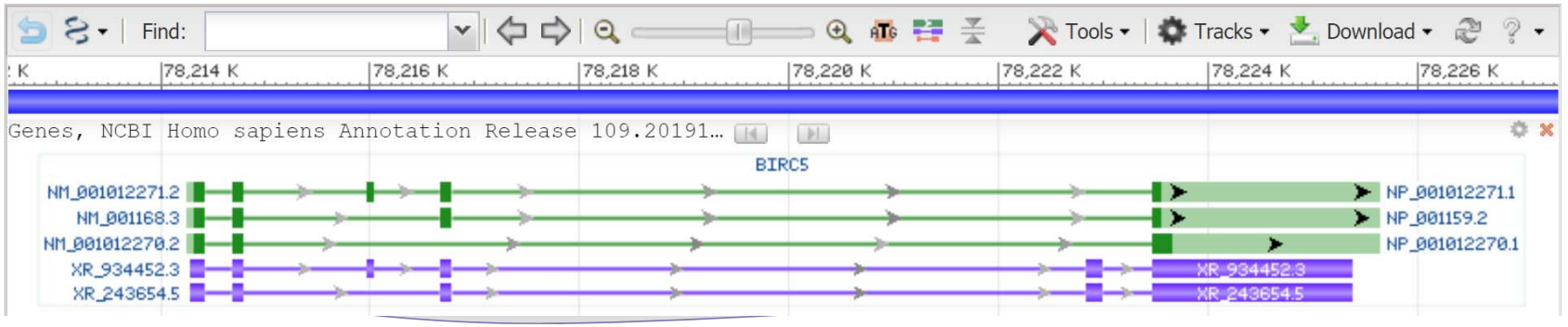


Genomic regions, transcripts, and products

Go to reference sequence details

Genomic Sequence: NC_000017.11 Chromosome 17 Reference GRCh38.p13 Primary Assembly

Go to nucleotide: Graphics FASTA GenBank



功能注释：Gene Ontology

Process	Evidence Code	Pubs
apoptotic process	IEA	
cell division	IEA	
chromosome segregation	IEA	
cytokine-mediated signaling pathway	TAS	
mitotic spindle assembly	IBA	PubMed
negative regulation of apoptotic process	IDA	PubMed
negative regulation of apoptotic process	IMP	PubMed
negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	IBA	PubMed
negative regulation of transcription, DNA-templated	IMP	PubMed
positive regulation of cell proliferation	TAS	PubMed
protein phosphorylation	IDA	PubMed
protein-containing complex localization	IMP	PubMed
regulation of apoptotic process	TAS	
sensory perception of sound	IEP	PubMed

结论1

- 核酸序列标识符：NM_001168.3
- 该基因为人的BIRC5基因
- 染色体定位：17q25.3
- 基因组坐标：78214253-78225635
- 初步的功能分析：凋亡，细胞分裂，染色体分离，等等

NM_001168.3：BIRC5

Distance tree of results MSA viewer

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
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Homo sapiens baculoviral IAP repeat-containing 5 (BIRC5) mRNA, complete cds	771	771	100%	0.0	99.76%	HM625836.1
Synthetic construct Homo sapiens baculoviral IAP repeat-containing 5 (survivin), mRNA (cDNA clone IMAGE:2964713), **** W	771	771	100%	0.0	99.76%	BC002388.1
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Pongo abelii baculoviral IAP repeat containing 5 (BIRC5), mRNA	771	771	100%	0.0	99.76%	NM_001132255.1
Homo sapiens inhibitor of apoptosis homolog mRNA, complete cds	771	771	100%	0.0	99.76%	AF077350.1
Homo sapiens baculoviral IAP repeat-containing 5, mRNA (cDNA clone MGC:8592 IMAGE:2961114), complete cds	771	771	100%	0.0	99.76%	BC008718.2
Homo sapiens baculoviral IAP repeat-containing 5, mRNA (cDNA clone MGC:32768 IMAGE:4656567), complete cds	771	771	100%	0.0	99.76%	BC034148.1
PREDICTED: Gorilla gorilla gorilla baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_019028170.2
PREDICTED: Pan troglodytes baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_009433243.3
PREDICTED: Pan paniscus baculoviral IAP repeat containing 5 (BIRC5), transcript variant X1, mRNA	765	765	100%	0.0	99.52%	XM_003818274.2

Human BIRC5 !

NCBI Resources How To

Nucleotide | Nucleotide | Advanced

GenBank | Send to:

Homo sapiens baculoviral IAP repeat containing 5 (BIRC5), transcript variant 1, mRNA

NCBI Reference Sequence: NM_001168.3

FASTA Graphics

Go to:

```
LOCUS       NM_001168              2574 bp    mRNA    linear   PRI 13-JAN-2020
DEFINITION  Homo sapiens baculoviral IAP repeat containing 5 (BIRC5),
            transcript variant 1, mRNA.
ACCESSION   NM_001168
VERSION     NM_001168.3
KEYWORDS    RefSeq; MANE Select.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 2574)
  AUTHORS   Zou J, Liao X, Zhang J and Wang L.
  TITLE     Dysregulation of miR-195-5p/-218-5p/BIRC5 axis predicts a poor
            prognosis in patients with gastric cancer
  JOURNAL   J. Biol. Regul. Homeost. Agents 33 (5), 1377-1385 (2019)
   PUBMED   31663299
  REMARK    GeneRIF: dysregulation of miR-195-5p/-218-5p/BIRC5 axis predicts a
```

获取蛋白质的序列信息

CDS 65..493
/gene="BIRC5"
/gene_synonym="API4; EPR-1"
/note="isoform 1 is encoded by transcript variant 1; survivin variant 3 alpha; apoptosis inhibitor 4; baculoviral IAP repeat-containing protein 5; apoptosis inhibitor survivin"
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/protein_id="NP_001159.2"
/db_xref="CCDS:CCDS11755.1"
/db_xref="GeneID:332"
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/db_xref="MIM:603352"
/translation="MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFI
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KLDRERAKNKIAKETNNKKKEFEETAEKVRRAIEQLAAMD"

BIRC5：142aa

NCBI Resources How To

Protein Protein Advanced

GenPept Send to:

baculoviral IAP repeat-containing protein 5 isoform 1 [Homo sapiens]

NCBI Reference Sequence: NP_001159.2

Identical Proteins FASTA Graphics

Go to:

```
LOCUS       NP_001159                142 aa            linear   PRI 13-JAN-2020
DEFINITION  baculoviral IAP repeat-containing protein 5 isoform 1 [Homo
            sapiens].
ACCESSION   NP_001159
VERSION     NP_001159.2
DBSOURCE    REFSEQ: accession NM_001168.3
KEYWORDS    RefSeq; MANE Select.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 142)
  AUTHORS   Zou J, Liao X, Zhang J and Wang L.
  TITLE     Dysregulation of miR-195-5p/-218-5p/BIRC5 axis predicts a poor
            prognosis in patients with gastric cancer
  JOURNAL   J. Biol. Regul. Homeost. Agents 33 (5), 1377-1385 (2019)
```

结论2

- 人的BIRC5蛋白质包含142个氨基酸，序列标识符为：NP_001159.2

获取FASTA序列

NCBI Resources How To

Protein Protein Advanced

GenPept Send to:

baculoviral IAP repeat-containing protein 5 isoform 1 [Homo sapiens]

NCBI Reference Sequence: NP_001159.2

Identical Proteins FASTA Graphics

Go to:

```
LOCUS       NP_001159               142 aa            linear   PRI 13-JAN-2020
DEFINITION  baculoviral IAP repeat-containing protein 5 isoform 1 [Homo
            sapiens].
ACCESSION   NP_001159
VERSION     NP_001159.2
DBSOURCE    REFSEQ: accession NM_001168.3
KEYWORDS    RefSeq; MANE Select.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 142)
  AUTHORS   Zou J, Liao X, Zhang J and Wang L.
  TITLE     Dysregulation of miR-195-5p/-218-5p/BIRC5 axis predicts a poor
            prognosis in patients with gastric cancer
  JOURNAL   J. Biol. Regul. Homeost. Agents 33 (5), 1377-1385 (2019)
```

FASTA格式的序列

FASTA

baculoviral IAP repeat-containing protein 5 isoform 1 [Homo sapiens]

NCBI Reference Sequence: NP_001159.2

GenPept Identical Proteins Graphics

```
>NP_001159.2 baculoviral IAP repeat-containing protein 5 isoform 1 [Homo sapiens]
MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDLAQCFFCFKELEGWEPD
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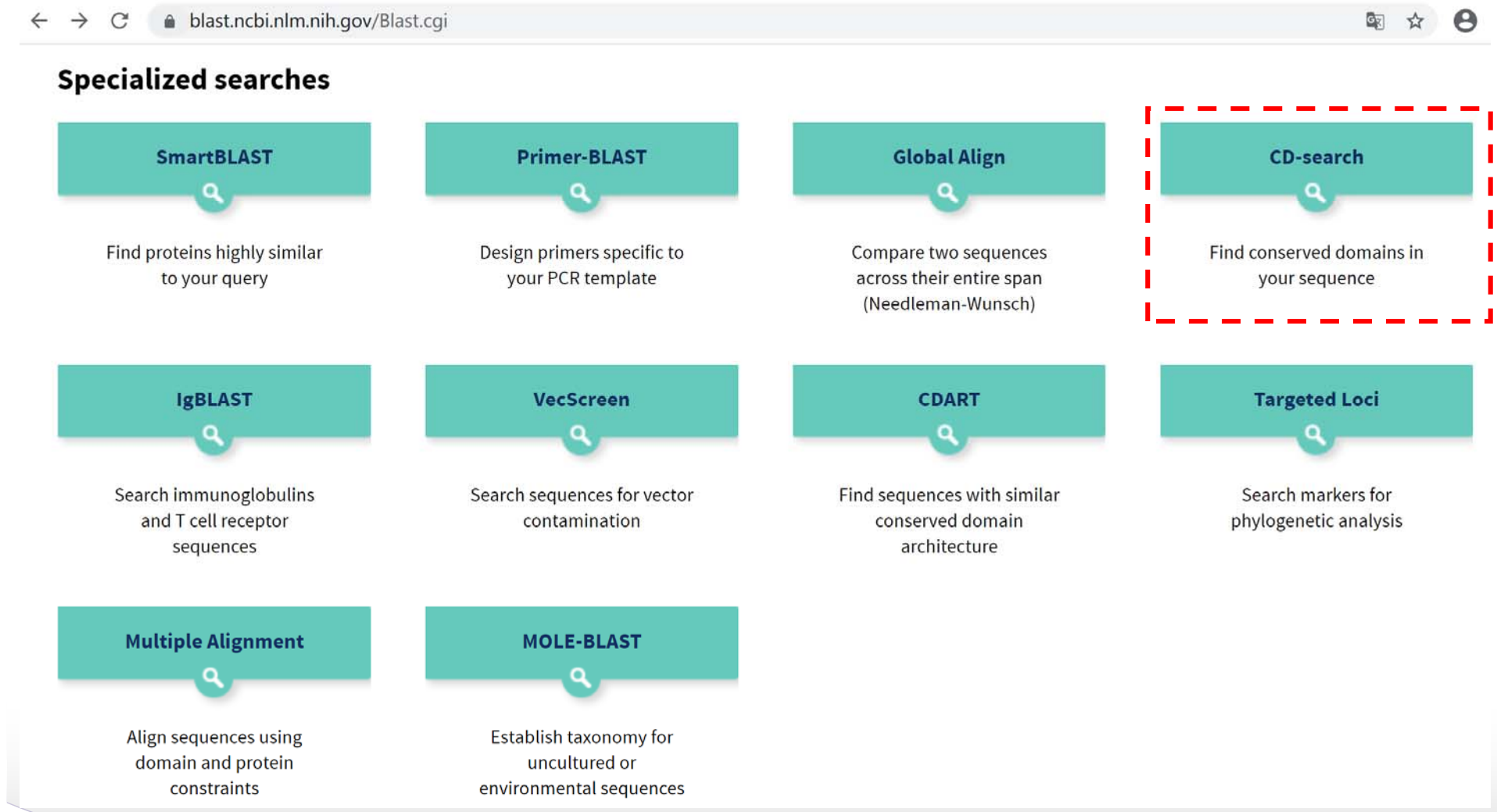
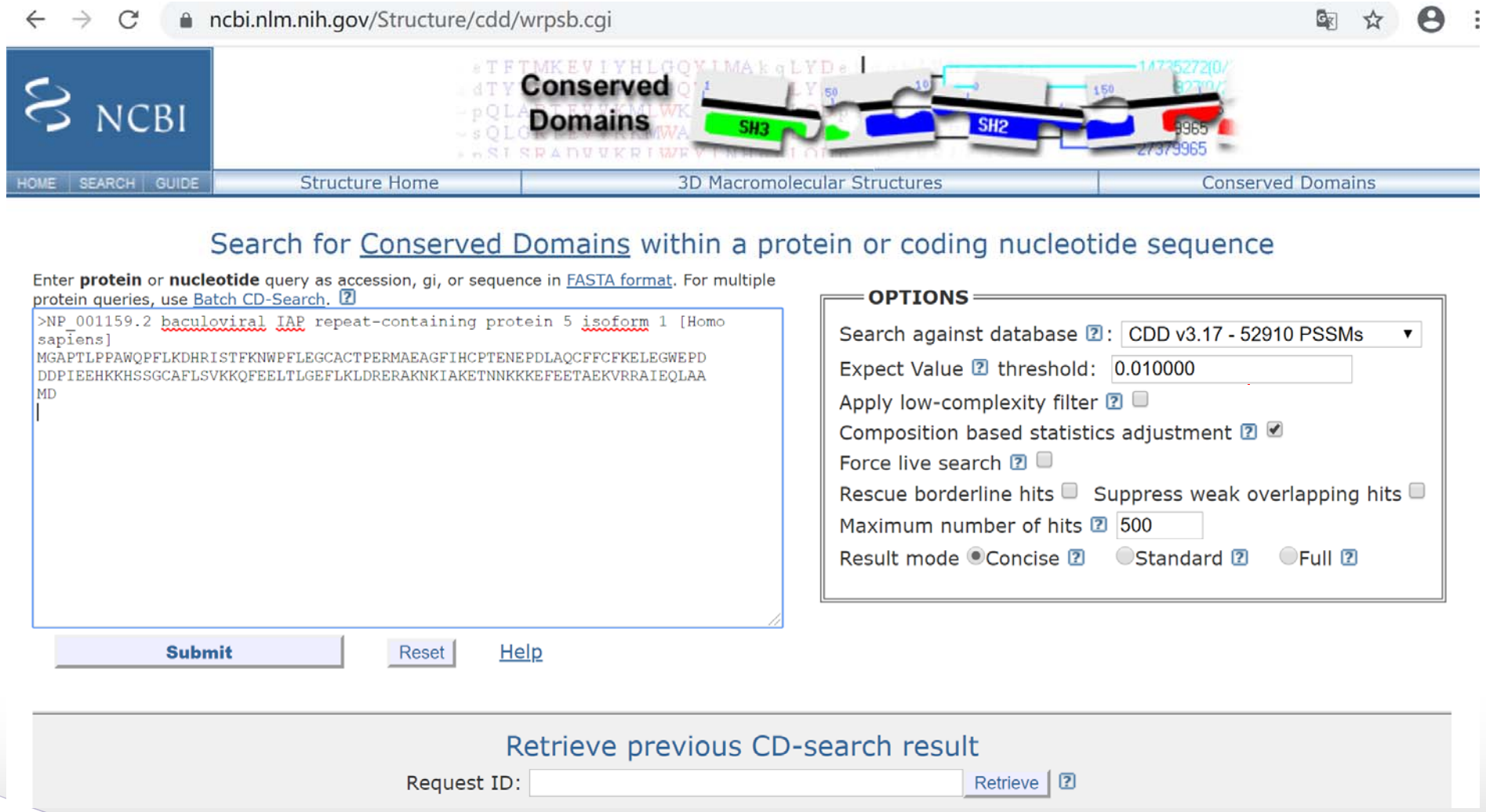
MD
```

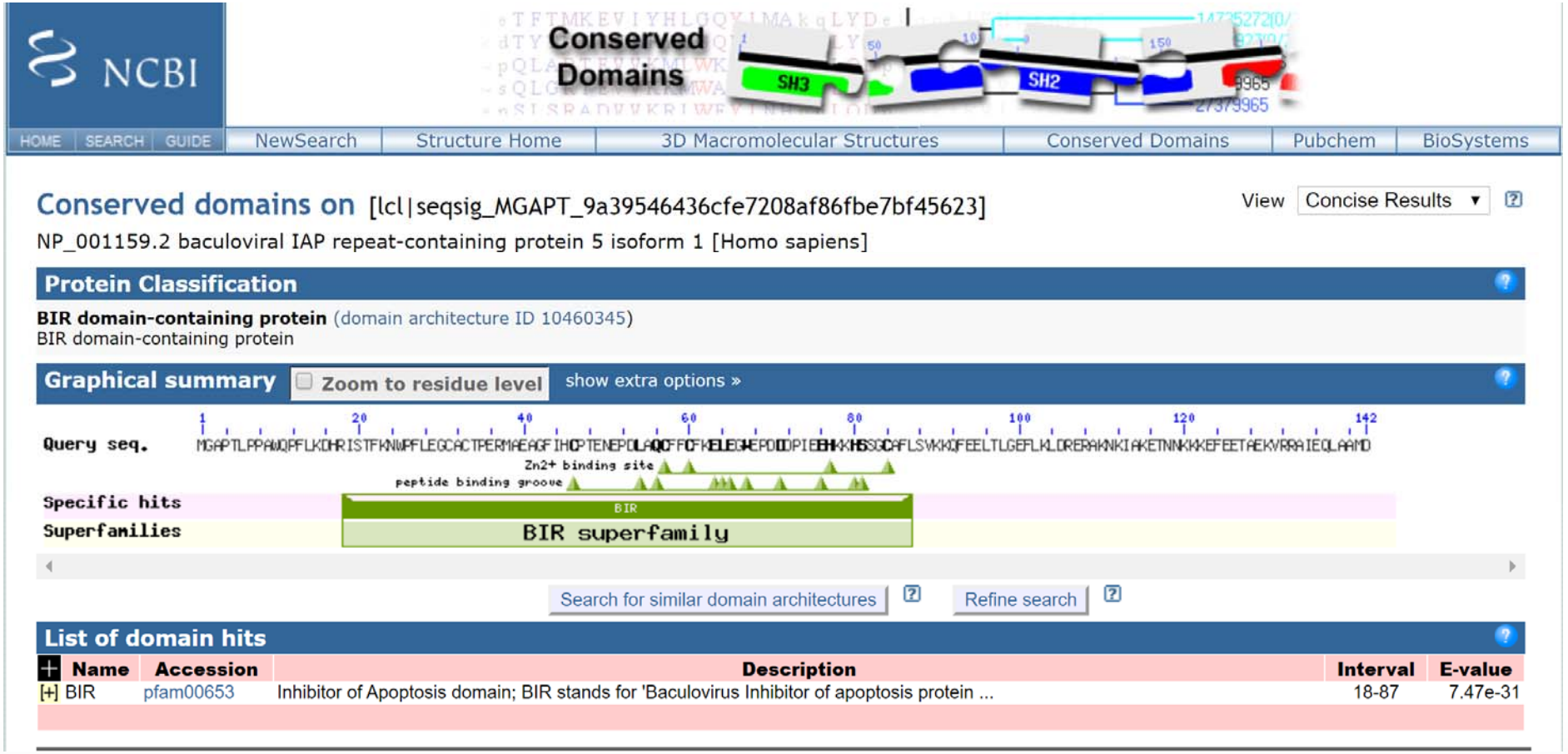
PHI-BLAST: find domain



填入蛋白质的FASTA序列



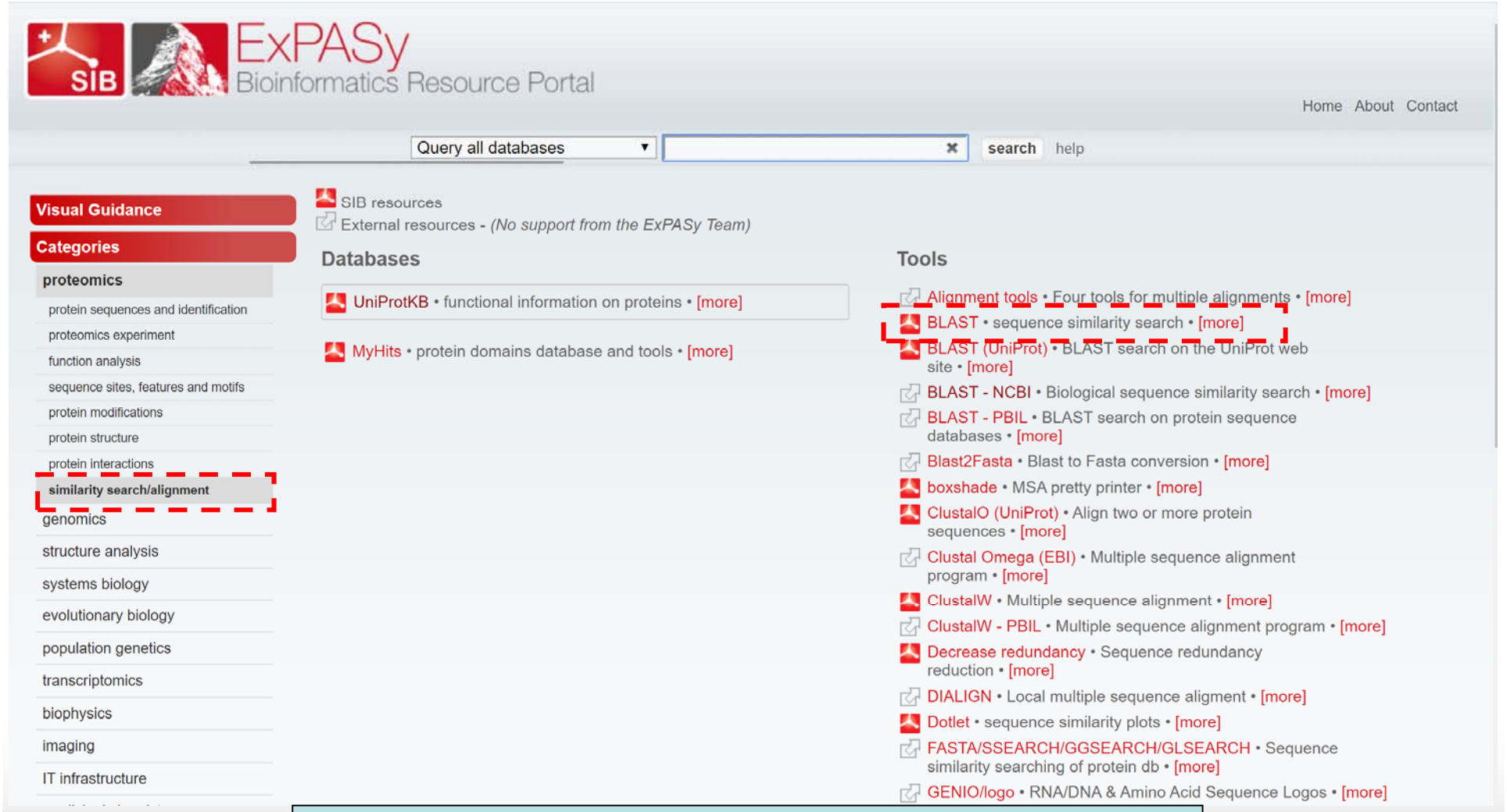
BIR domain


NCBI
Conserved Domains
SH3
SH2
HOME
SEARCH
GUIDE
NewSearch
Structure Home
3D Macromolecular Structures
Conserved Domains
Pubchem
BioSystems
Conserved domains on [lcl|seqsig_MGAPT_9a39546436cfe7208af86fbe7bf45623]
View
Concise Results
NP_001159.2 baculoviral IAP repeat-containing protein 5 isoform 1 [Homo sapiens]
Protein Classification
BIR domain-containing protein (domain architecture ID 10460345)
BIR domain-containing protein
Graphical summary
Zoom to residue level
show extra options »
Query seq.
Zn2+ binding site
peptide binding groove
Specific hits
BIR
Superfamilies
BIR superfamily
Search for similar domain architectures
Refine search
List of domain hits
Name
Accession
Description
Interval
E-value
[+] BIR
pfam00653
Inhibitor of Apoptosis domain; BIR stands for 'Baculovirus Inhibitor of apoptosis protein ...
18-87
7.47e-31

结论3

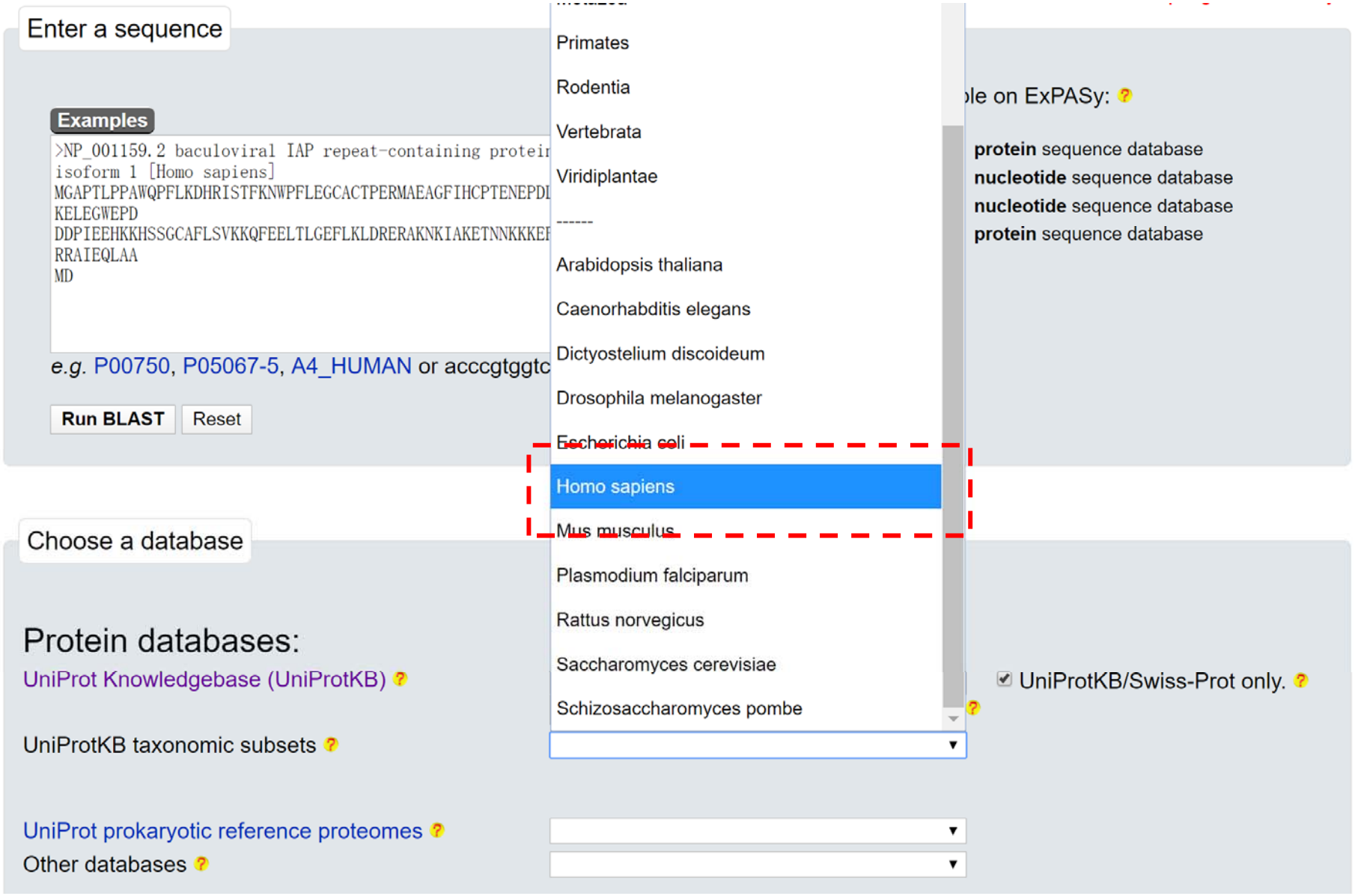
- BIRC5具有保守的功能结构域BIR

ExPASy: 生物信息资源



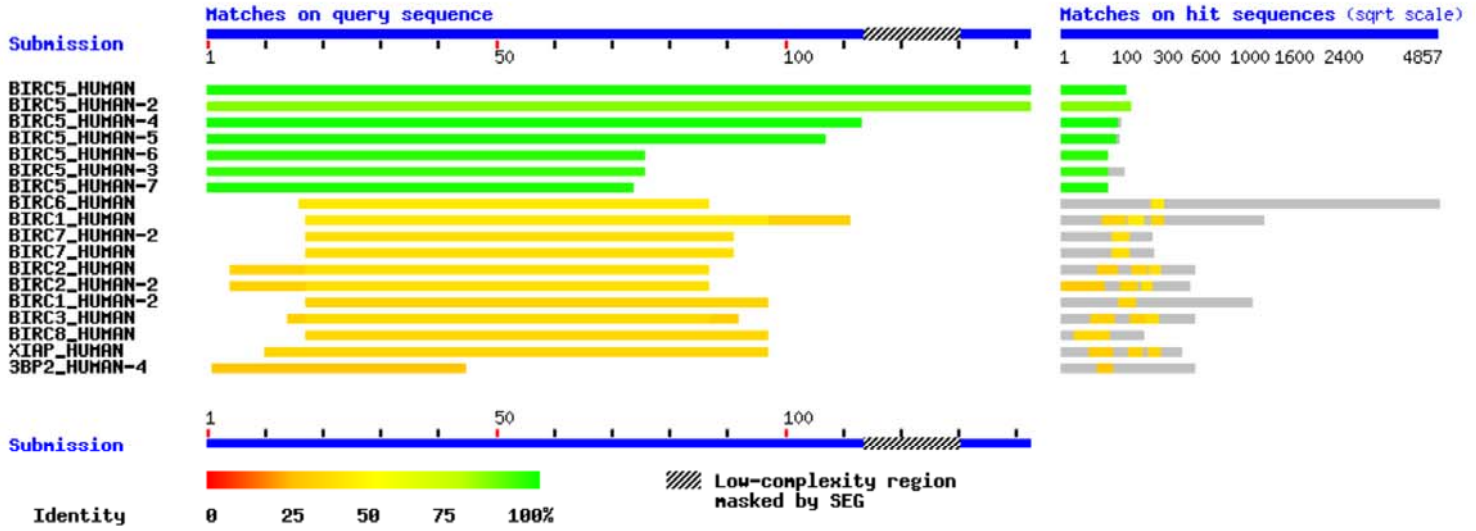
https://www.expasy.org/

http://web.expasy.org/blast/



BIRC5：O15392

Graphical overview of the alignments



List of the matches

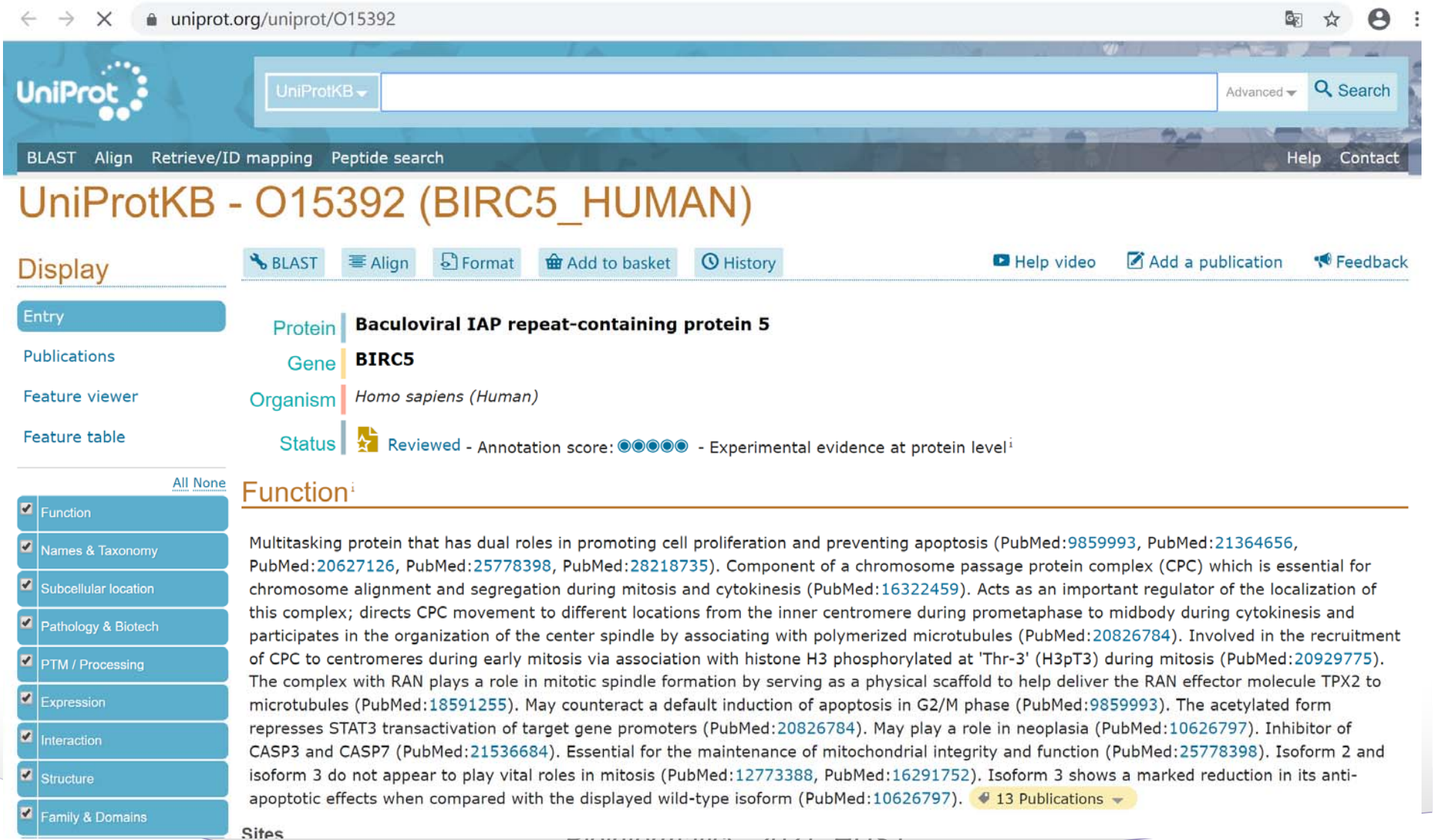
Clustal W (multiple alignment) ▾ 提交

Select up to...

Include query sequence

		Accession	Length	Db	Description	Score	E-value
☐	1	O15392 (BIRC5_HUMAN)	142		Baculoviral IAP repeat-containing protein ...	256 bits (654)	1e-87

BIRC5的蛋白质信息



翻译后修饰

Amino acid modifications

Feature key	Position(s)	Description Actions	Graphical view	Length
Modified residue	20	Phosphoserine; by AURKC 1 Publication		1
Modified residue	23	N6-acetyllysine 1 Publication		1
Modified residue	34	Phosphothreonine; by CDK1 and CDK15 Combined sources 2 Publications		1
Modified residue	48	Phosphothreonine; by CK2; in vitro 1 Publication		1
Modified residue	90	N6-acetyllysine 1 Publication		1
Modified residue	110	N6-acetyllysine 1 Publication		1
Modified residue	112	N6-acetyllysine 1 Publication		1
Modified residue	115	N6-acetyllysine 1 Publication		1
Modified residue	117	Phosphothreonine; by AURKB 1 Publication		1
Modified residue	121	N6-acetyllysine 1 Publication		1
Modified residue	129	N6-acetyllysine 1 Publication		1

Post-translational modification

Ubiquitinated by the Cul9-RING ubiquitin-protein ligase complex, leading to its degradation. Ubiquitination is required for centrosomal targeting. 2 Publications

In vitro phosphorylation at Thr-117 by AURKB prevents interaction with INCENP and localization to mitotic chromosomes (PubMed:14610074). Phosphorylation at Thr-48 by CK2 is critical for its mitotic and anti-apoptotic activities (PubMed:21252625). Phosphorylation at Thr-34 by CDK15 is critical for its anti-apoptotic activity (PubMed:24866247). Phosphorylation at Ser-20 by AURKC is critical for regulation of proper chromosome alignment and segregation, and possibly cytokinesis. 5 Publications

Acetylation at Lys-129 by CBP results in its homodimerization, while deacetylation promotes the formation of monomers which heterodimerize with XPO1/CRM1 which facilitates its nuclear export. The acetylated form represses STAT3 transactivation. The dynamic equilibrium between its acetylation and deacetylation at Lys-129 determines its interaction with XPO1/CRM1, its subsequent subcellular localization, and its ability to inhibit STAT3 transactivation. 1 Publication

KEGG: Pathway

- http://www.genome.jp/kegg/kegg2.html

KEGG - Table of Contents

Menu PATHWAY BRITE MODULE KO GENES LIGAND NETWORK DISEASE DRUG DBGET

Search KEGG for BIRC5 Go

Data-oriented entry points

Category	Entry Point	Content	DBGET Search
Systems information	**KEGG PATHWAY**	KEGG pathway maps	PATHWAY
	KEGG BRITE	BRITE hierarchies and tables	BRITE
	KEGG MODULE	KEGG modules	MODULE
Genomic information	**KO** (KEGG Orthology)	Functional orthologs	ORTHOLOGY
	KEGG GENOME	KEGG organisms (complete genomes)	GENOME
	KEGG GENES	Genes and proteins	GENES
	KEGG SSDB	GENES sequence similarity	

Human BIRC5

- 参与通路：癌症、直肠癌、乙肝

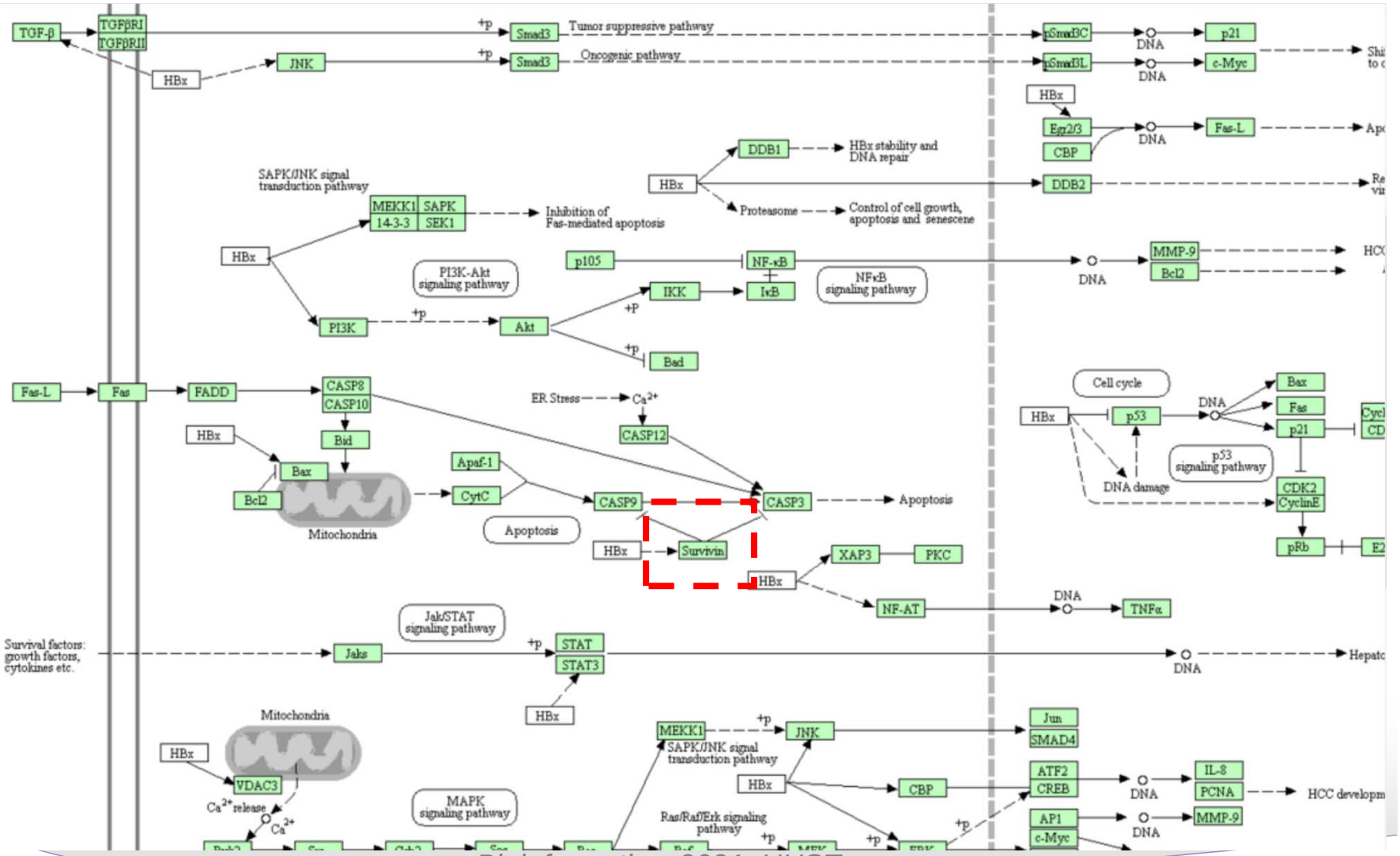
KEGG

Homo sapiens (human): 332

Help

Entry	332 CDS T01001
Gene name	BIRC5, API4, EPR-1
Definition	(RefSeq) baculoviral IAP repeat containing 5
KO	K08731 baculoviral IAP repeat-containing protein 5
Organism	hsa Homo sapiens (human)
Pathway	hsa01524 Platinum drug resistance hsa04210 Apoptosis hsa04215 Apoptosis - multiple species hsa04390 Hippo signaling pathway hsa05161 Hepatitis B hsa05200 Pathways in cancer hsa05210 Colorectal cancer
Network	nt06131 Apoptosis (viruses and bacteria) nt06162 Hepatitis B virus (HBV) nt06215 WNT signaling nt06260 Colorectal cancer nt06261 Gastric cancer nt06263 Hepatocellular carcinoma nt06271 Endometrial cancer
Element	N00057 Mutation-inactivated APC to Wnt signaling pathway N00058 Mutation-activated CTNNB1 to Wnt signaling pathway N00533 HBV HBx to Crosstalk between extrinsic and intrinsic apoptotic pathways

Hepatitis B (hsa05161)



结论4：功能分析

- 在肿瘤形成过程中可能起一定作用
- 阻碍G2/M期的细胞编程性凋亡
- Chromosomal passenger complex (CPC) 的成员之一
- 细胞亚定位：中心粒、染色体、胞质
- 参与的生物学通路：癌症、直肠癌、乙肝

人类BIRC5在酵母中有同源序列吗？


```
>sp|O15392|BIRC5_HUMAN Baculoviral IAP repeat-containing protein 5 OS=Homo sapiens OX=9606 GN=BIRC5 PE=1 SV=3
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FKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDRERAKNKIAKETNNK
KKEFEETAKKVRRAIEQLAAMD
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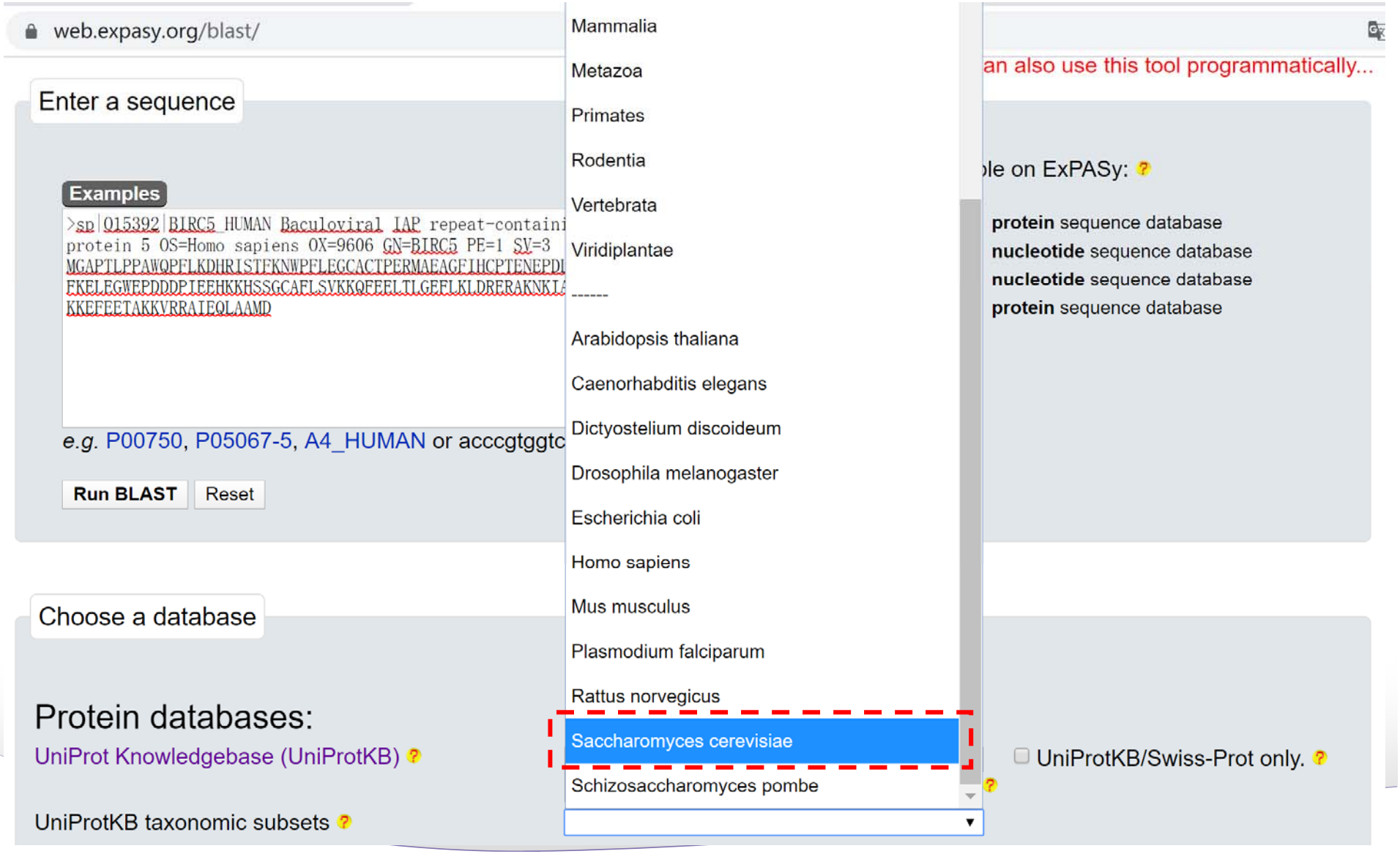
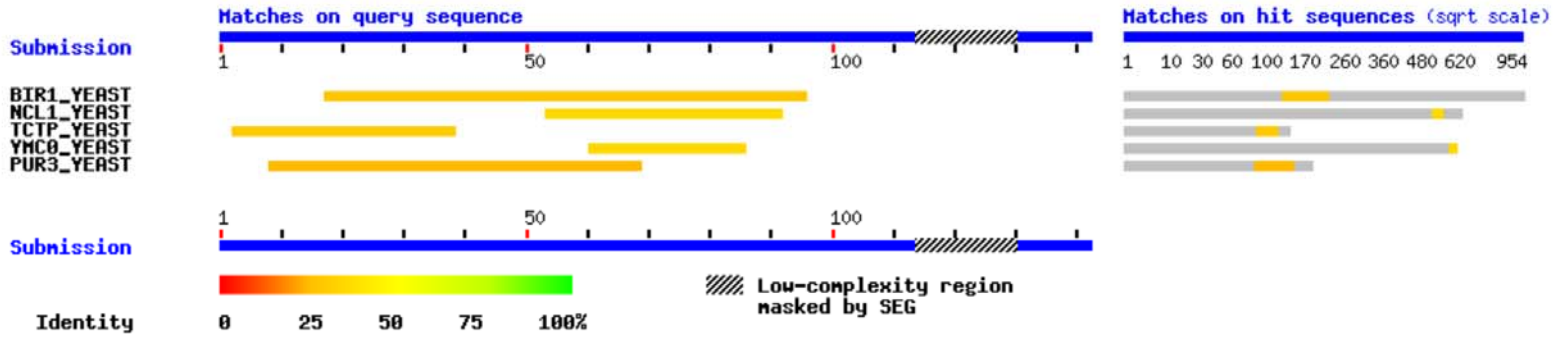
在酵母中进行序列比对


web.expasy.org/blast/
Enter a sequence
Examples
>sp|O15392|BIRC5_HUMAN Baculoviral IAP repeat-containi
protein 5 OS=Homo sapiens OX=9606 GN=BIRC5 PE=1 SV=3
MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDL
FKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDRERAKNKIA
KKEFEETAKKVRRAIEQLAAMD
e.g. P00750, P05067-5, A4_HUMAN or acccgtggtc
Run BLAST
Reset
Mammalia
Metazoa
Primates
Rodentia
Vertebrata
Viridiplantae

Arabidopsis thaliana
Caenorhabditis elegans
Dictyostelium discoideum
Drosophila melanogaster
Escherichia coli
Homo sapiens
Mus musculus
Plasmodium falciparum
Rattus norvegicus
Saccharomyces cerevisiae
Schizosaccharomyces pombe
an also use this tool programmatically...
ble on ExPASy:
protein sequence database
nucleotide sequence database
nucleotide sequence database
protein sequence database
Choose a database
Protein databases:
UniProt Knowledgebase (UniProtKB)
UniProtKB/Swiss-Prot only.
UniProtKB taxonomic subsets

酵母BIR1: P47134

Graphical overview of the alignments



List of the matches

Clustal W (multiple alignment) ▾ 提交

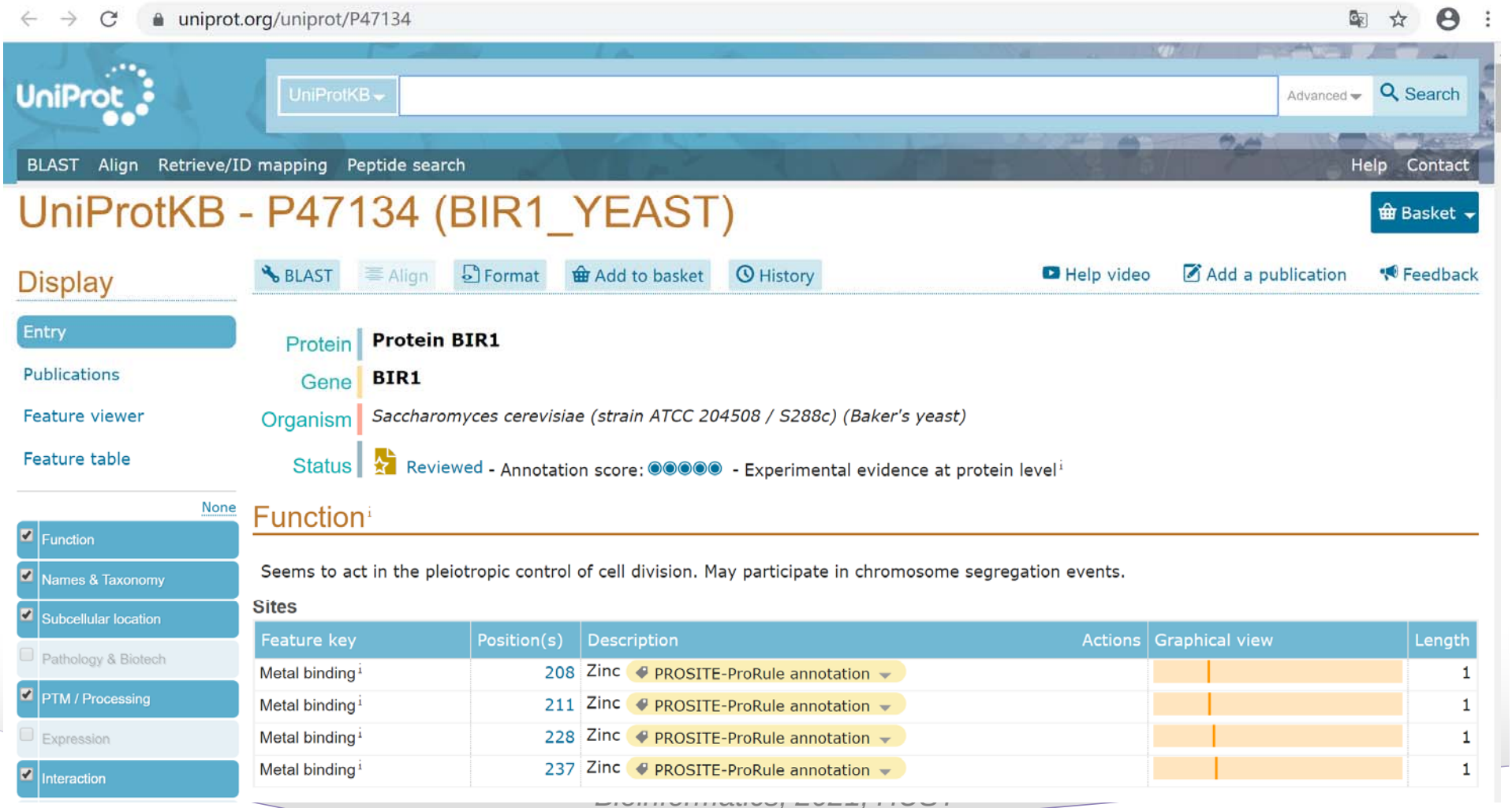
Select up to...

Include query sequence

	Accession	Length	Db	Description	Score	E-value
1	P47134 (BIR1_YEAST)	954		Protein BIR1 OS=Saccharomyces cerevisiae (s...	39.7 bits (91)	7e-05
2	P38205 (NCL1_YEAST)	684		Multisite-specific tRNA:(cytosine-C(5))-met...	30.8 bits (68)	0.070
3	P35691 (TCTP_YEAST)	167		Translationally-controlled tumor protein ho...	26.2 bits (56)	1.7
4	Q03722 (YMC0_YEAST)	664		Uncharacterized protein YML020W OS=Saccharo...	24.6 bits (52)	7.7
5	P04161 (PUR3_YEAST)	214		Phosphoribosylglycinamide formyltransferase...	24.3 bits (51)	8.4

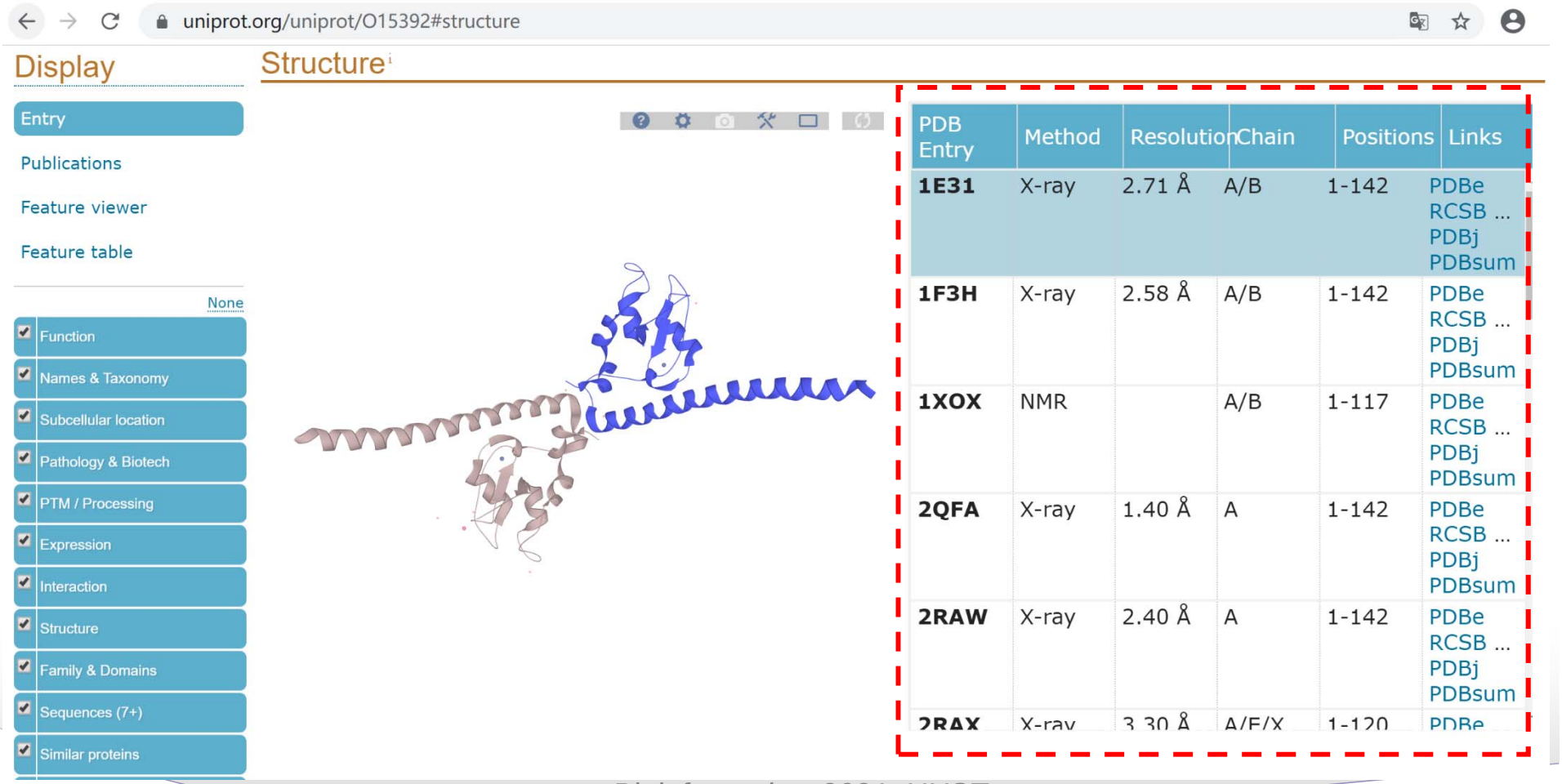
酵母BIR1的信息

- 人类BIRC5在酵母中的同源序列可能是BIR1



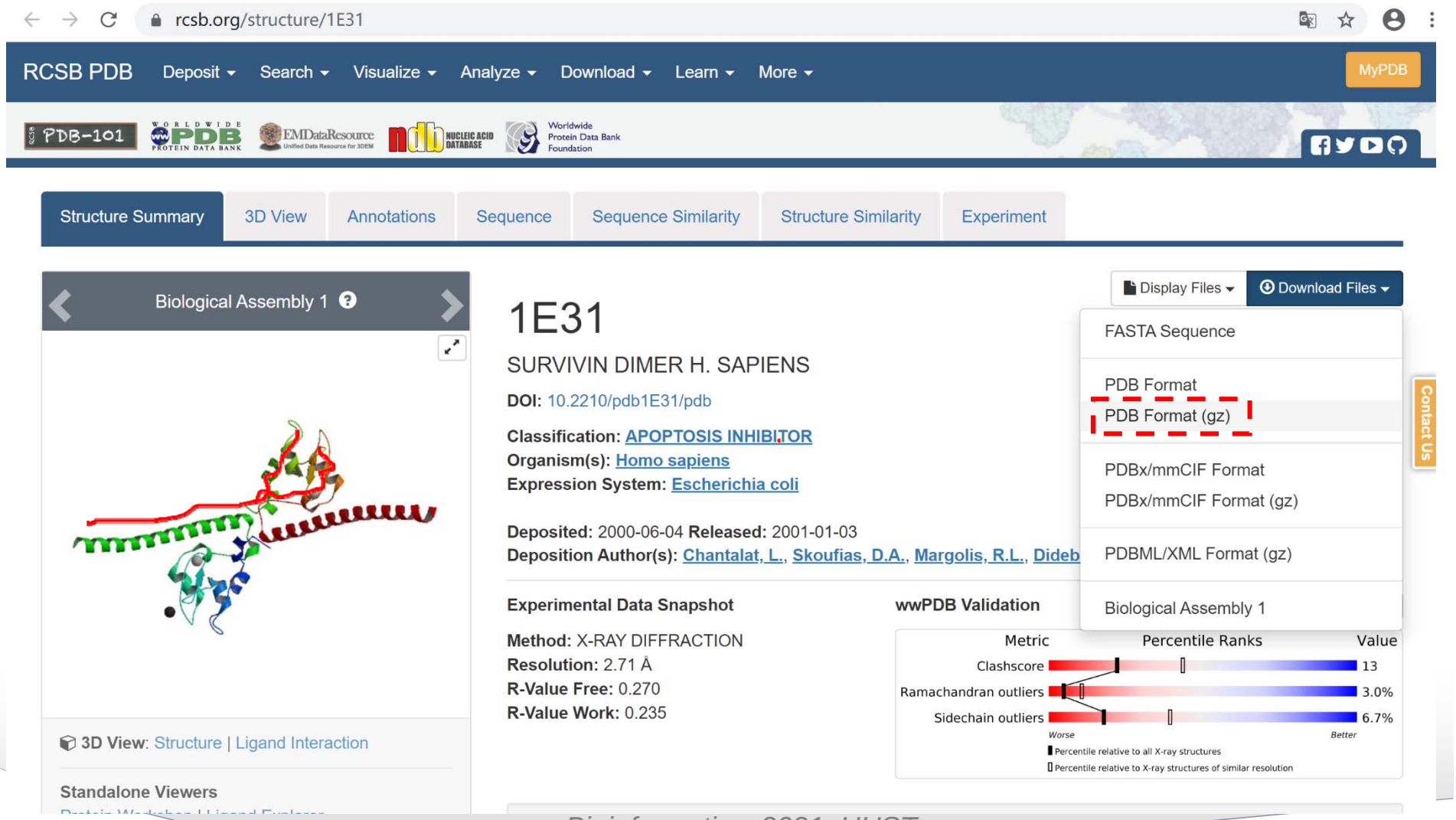
PDB：三级结构数据库

http://www.uniprot.org/uniprot/O15392

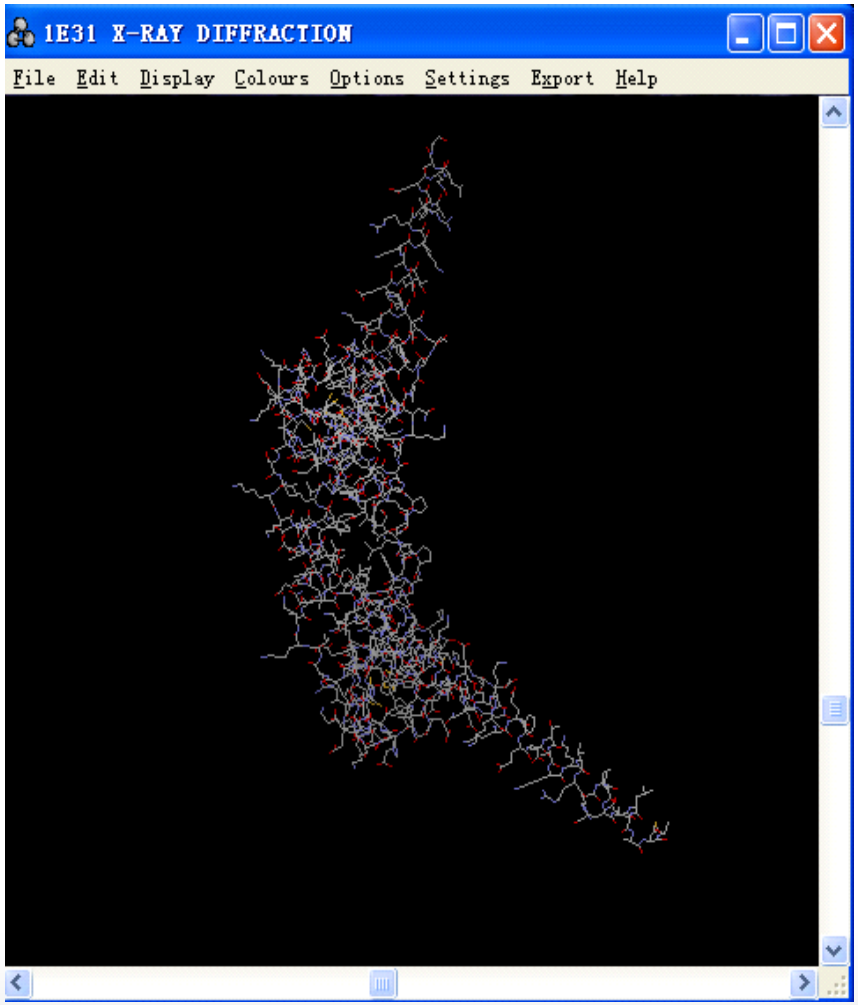


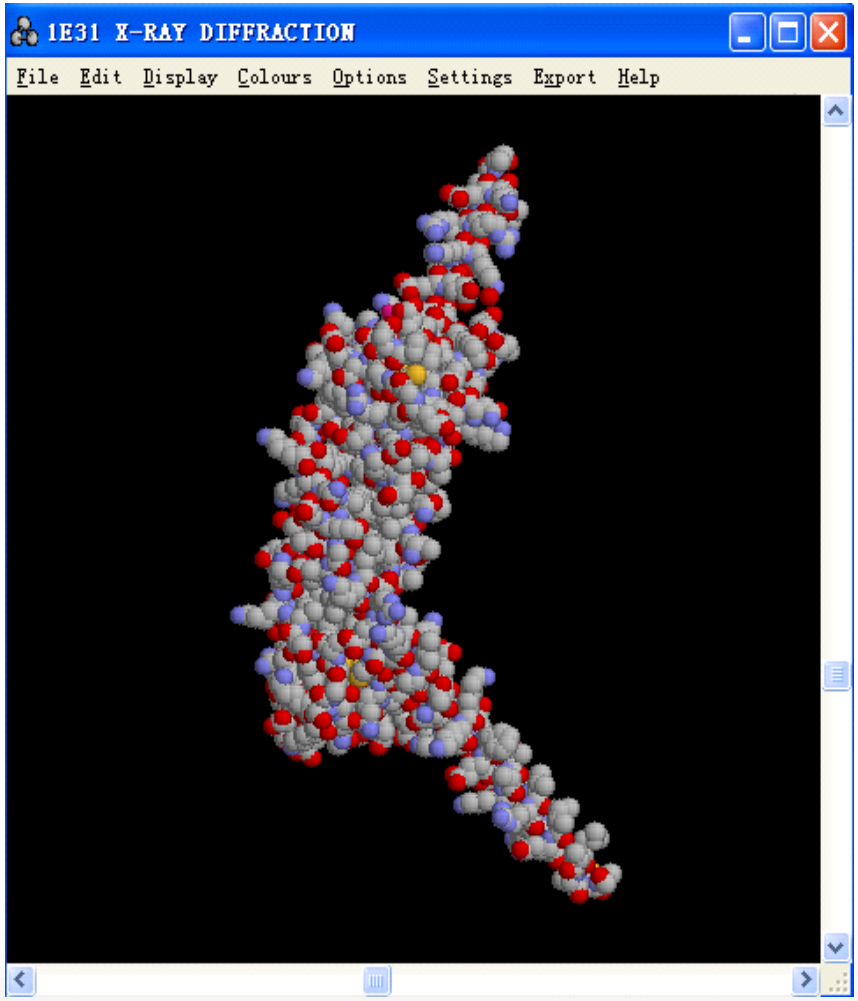
PDB Entry	Method	Resolution	Chain	Positions	Links
1E31	X-ray	2.71 Å	A/B	1-142	PDBe RCSB ... PDBj PDBsum
1F3H	X-ray	2.58 Å	A/B	1-142	PDBe RCSB ... PDBj PDBsum
1XOX	NMR		A/B	1-117	PDBe RCSB ... PDBj PDBsum
2QFA	X-ray	1.40 Å	A	1-142	PDBe RCSB ... PDBj PDBsum
2RAW	X-ray	2.40 Å	A	1-142	PDBe RCSB ... PDBj PDBsum
2RAX	X-ray	3.30 Å	A/E/X	1-120	PDBe

BIRC5的三级结构信息



Raswin: 三级结构显示





总结

- 1. 该基因为人类BIRC5基因，染色体定位：17q25.3，78214253-78225635；基因标识符：NM_001168.3
- 2. 人的BIRC5蛋白质包含142个氨基酸，序列标识符为：NP_001159.2
- 3. BIRC5具有保守的功能结构域BIR
- 4. BIRC5的细胞亚定位：中心粒、染色体等，其功能有：
  - (1) 在肿瘤形成过程中可能起一定作用
  - (2) 阻碍G2/M期的细胞编程性凋亡
  - (3) Chromosomal passenger complex (CPC) 的成员之一
  - (4) 参与的生物学通路：癌症、直肠癌、乙肝
- 5. 人的BIRC5在酵母中的同源序列可能是BIR1
- 6. BIRC5的三级结构已知，在PDB中的标识符为1E31

Homework 1#

- **A mitosis-associated gene *X* was experimentally identified in *Mus musculus*. By DNA sequencing, a partial sequence was obtained as below:**

GATGAGCTGCTTATCCTACAACGAGAAGTCGGACATCTGGTCCTTG
GGCTGCCTGCTGTATGAGCTGTGTGCACTAATGCCTCCCTTTACAG
CTTTCAACCAAAAAGAGCTAGCTGGGAAAATCAGGGAAGGGAGGT
TCAGGCGCATCCCCTACCGCTACTCTGATGGCTTGAATGACCTCAT
CACTCGGATGCTGAATTTAAAGGACTACCATCGACCTTCAGTGGAA
GAAATTCTGGAGAGCCCTTTGATAGCAGACTTGGTTGCAGAAGAGC
AAAGGAGAAATCTGGAGAGGAGAGGACGGCGCTCAGGCGAGCCT
TCGAAGCTGCCGGACTCCAGCCCTGTGCTGAGCGAGCTCAAGTTG
AAGGAAAGGCAACTGCAGGATCGAGAGCAAGCACTCAGAGCTCGG
GAGGACATCCT

Questions:

- **1. What's the name of gene *X* in *Mus musculus?* Its accession number in GenBank database and coordinates of mouse genome.**
- **2. The homolog of gene *X* in human, and its accession number.**
- **3. In human, the protein product of this gene. Its functions, sub-cellular localizations. Whether it's a enzyme? If so, does it have a conserved functional domain?**
- **4. Whether this gene is conserved in yeast? If so, identify its potential homolog.**
- **5. The 3D structural information of gene *X* in human, but not mouse. It's accession number in PDB.**