DNA 测序技术的发展

三代测序的优势与应用

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Outline

一代: Sanger 法电泳测序

二代: (短读长的) 高通量测序

三代: (长读长的) 单分子测序

一代: Sanger 法电泳测序

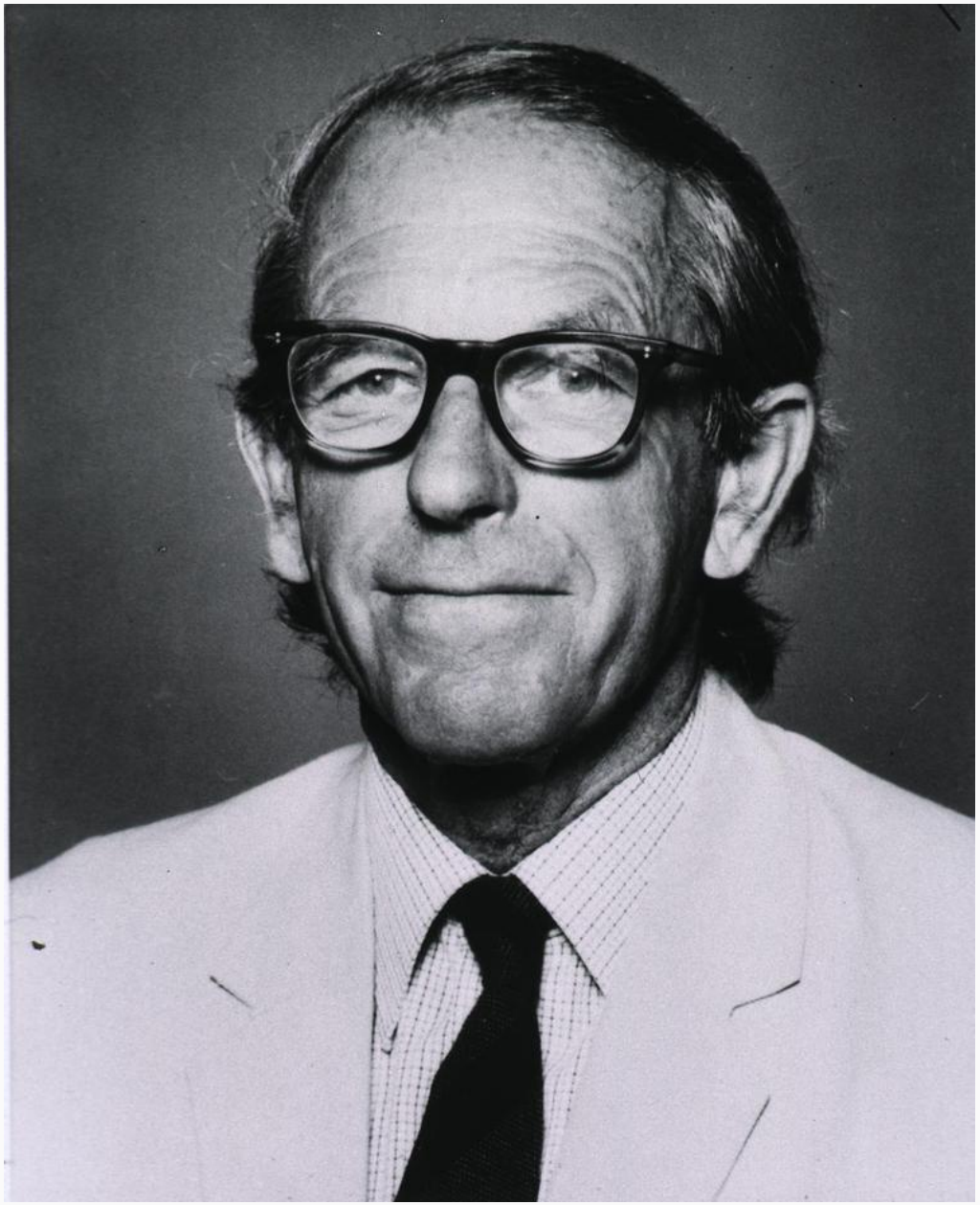


Figure 1. Frederick Sanger

- 1955 年, 第一个蛋白质测序, 胰岛素
- 1958 年, 诺贝尔化学奖
- 1975 年, 双脱氧法, ddNTP
- 1977 年, 第一个基因组, ϕ–X174 噬菌体
- 1980 年, 再度获得诺贝尔化学奖

人类基因组计划

- 1985 年, 美国能源部正式提出人类基因组测序.
- 1990 年, 正式启动人类基因组测序.

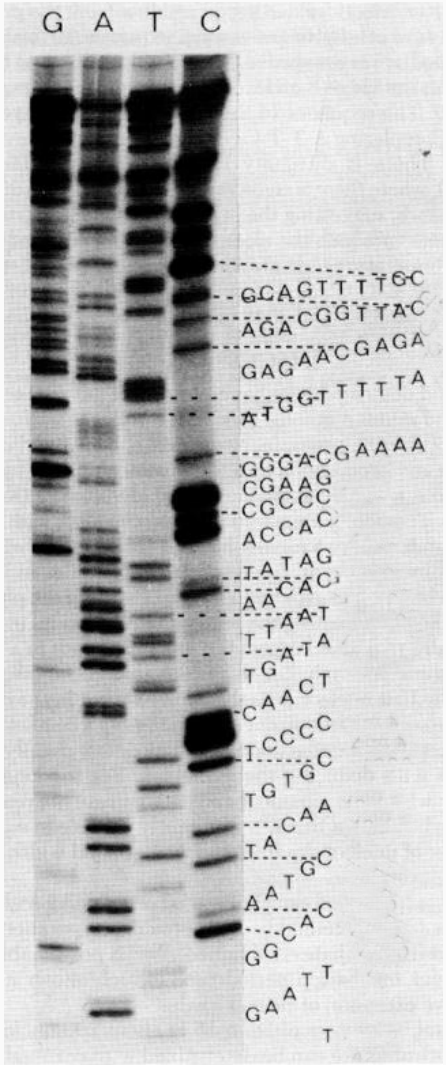


Figure 2. 聚丙烯酰胺凝胶电泳

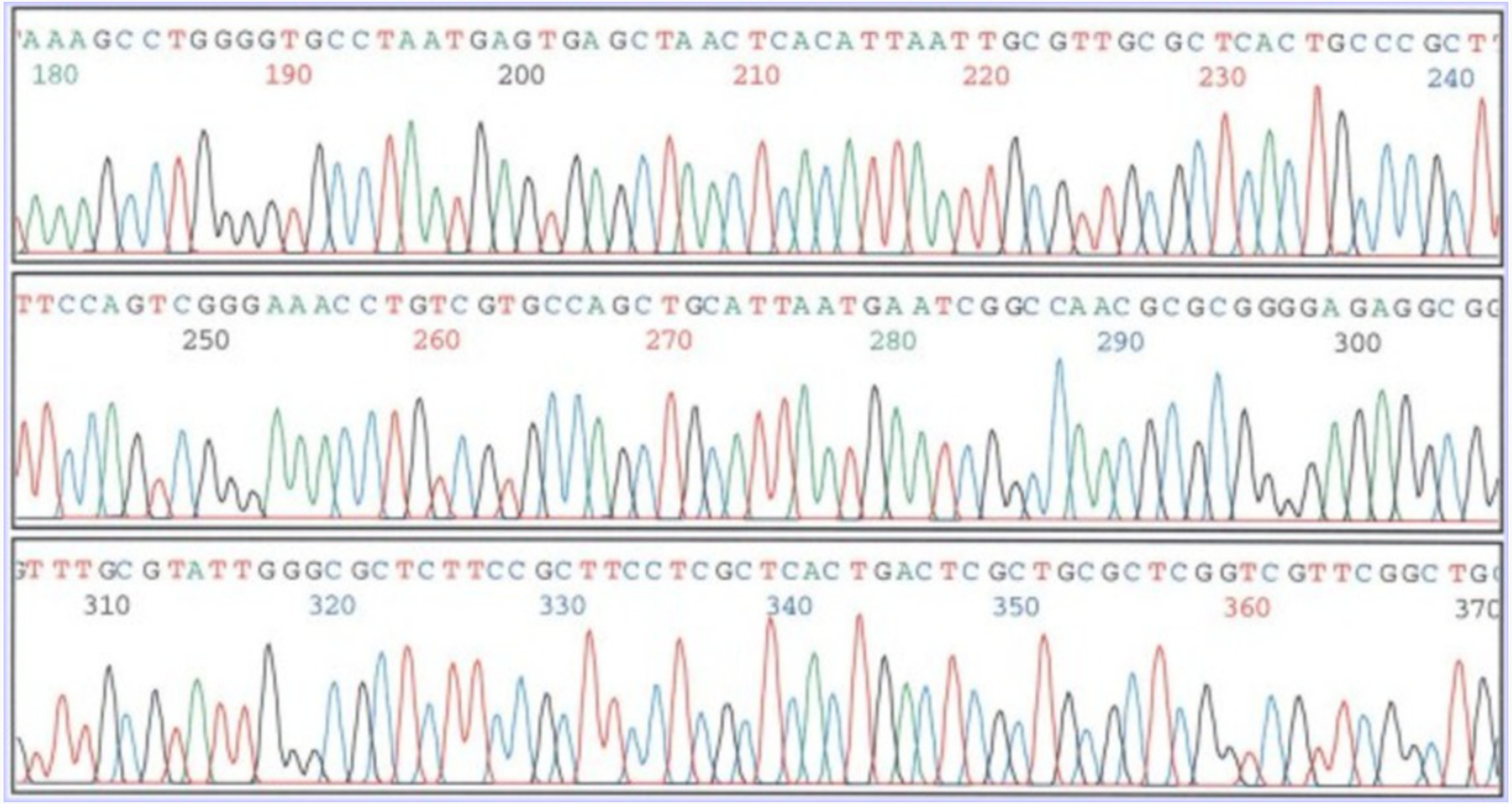


Figure 3. 毛细管电泳图谱

Figure 4. ABI 3730XL

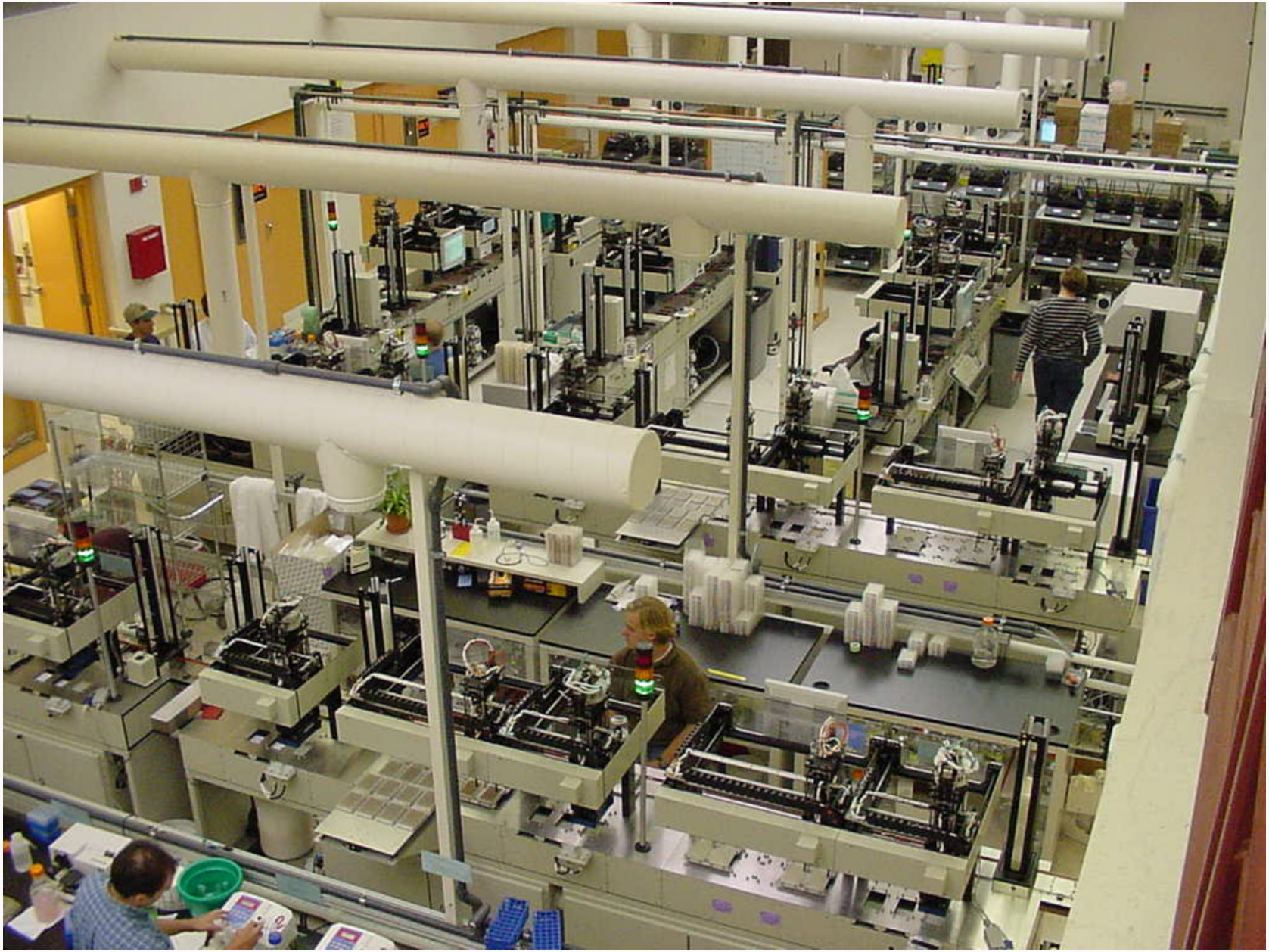


Figure 5. 模板与测序室

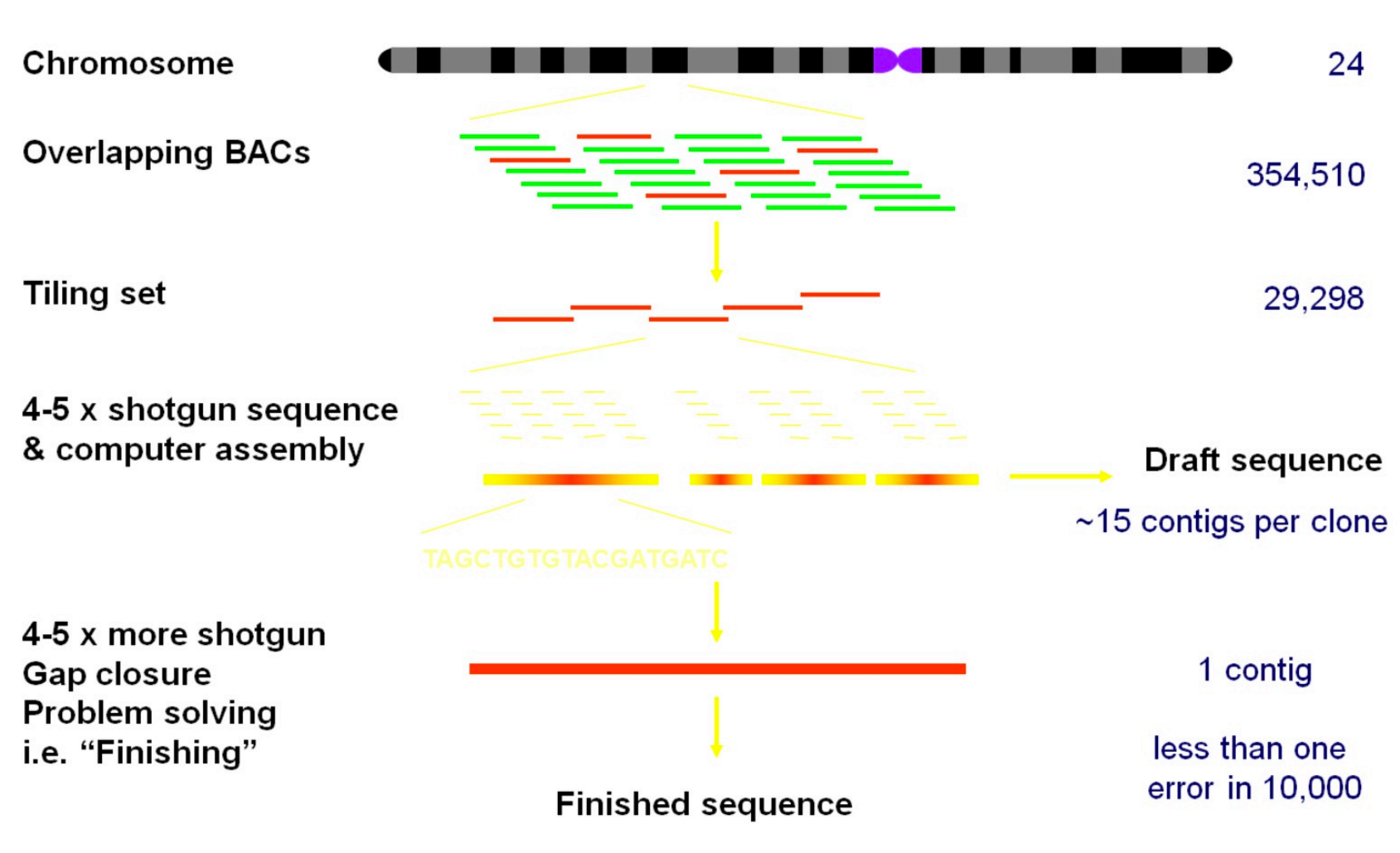


Figure 6. HGP sequencing strategy

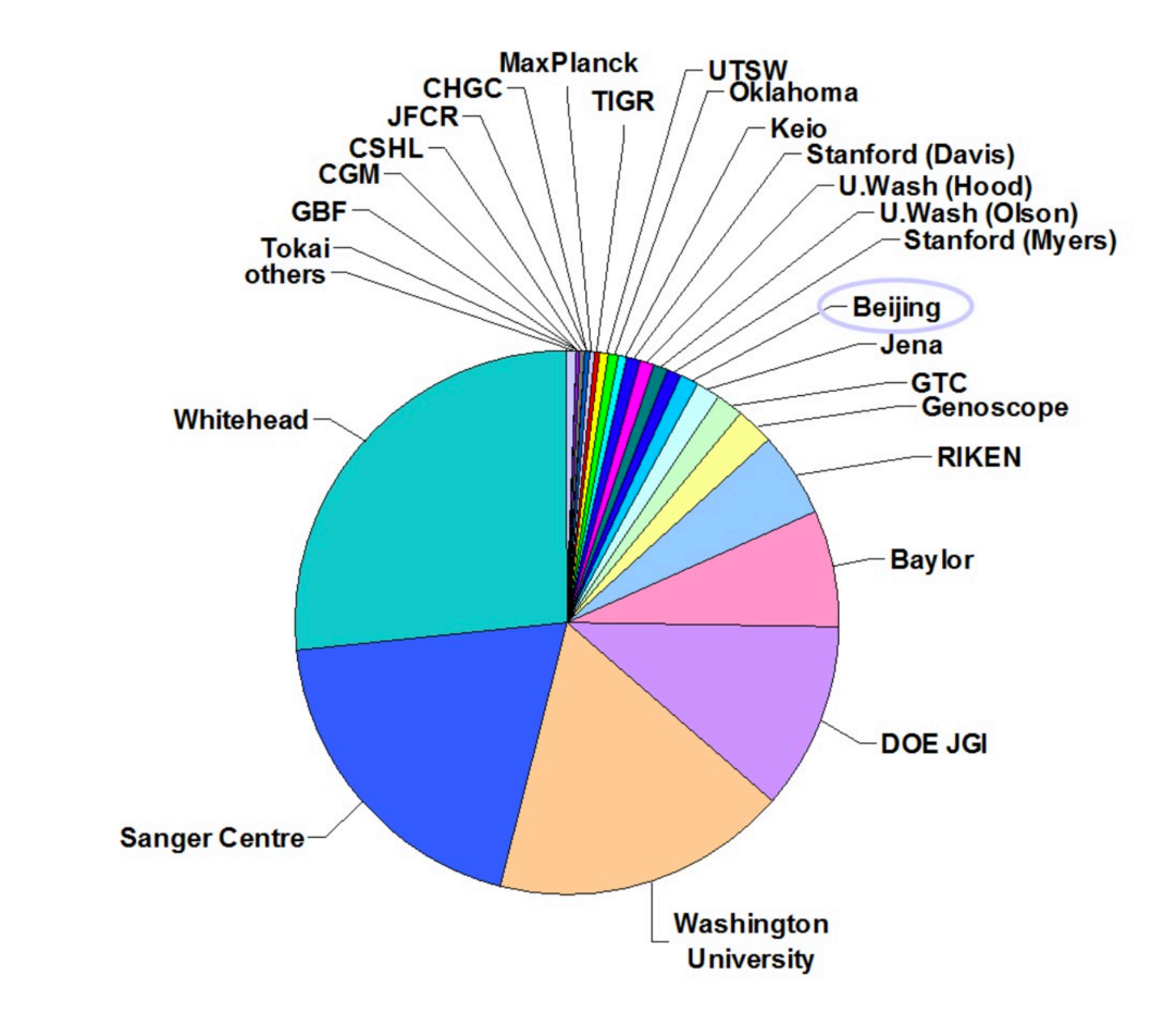

MaxPlanck
UTSW
CHGC
Oklahoma
TIGR
JFCR
Keio
CSHL
Stanford (Davis)
CGM
U.Wash (Hood)
GBF
U.Wash (Olson)
Tokai
Stanford (Myers)
others
Beijing
Jena
GTC
Genoscope
Whitehead
RIKEN
Baylor
DOE JGI
Sanger Centre
Washington University

Figure 7. 贡献

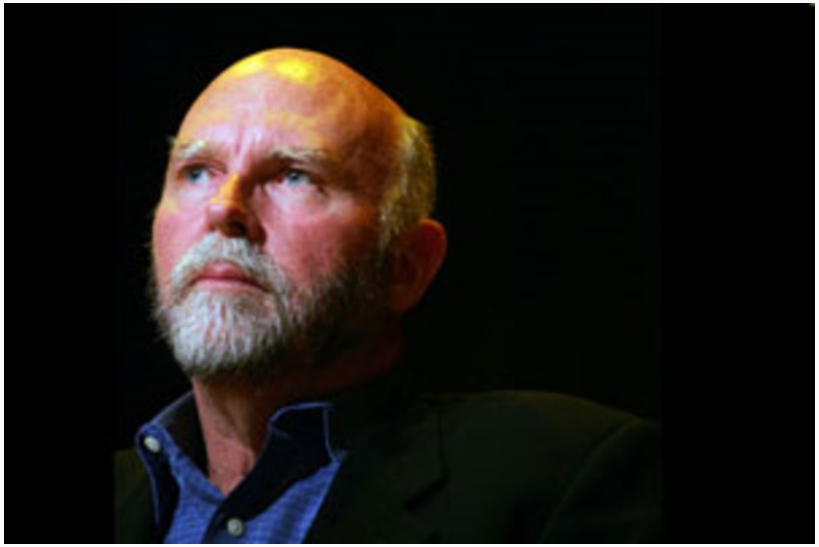


Figure 8. J. Craig Venter (文特尔)

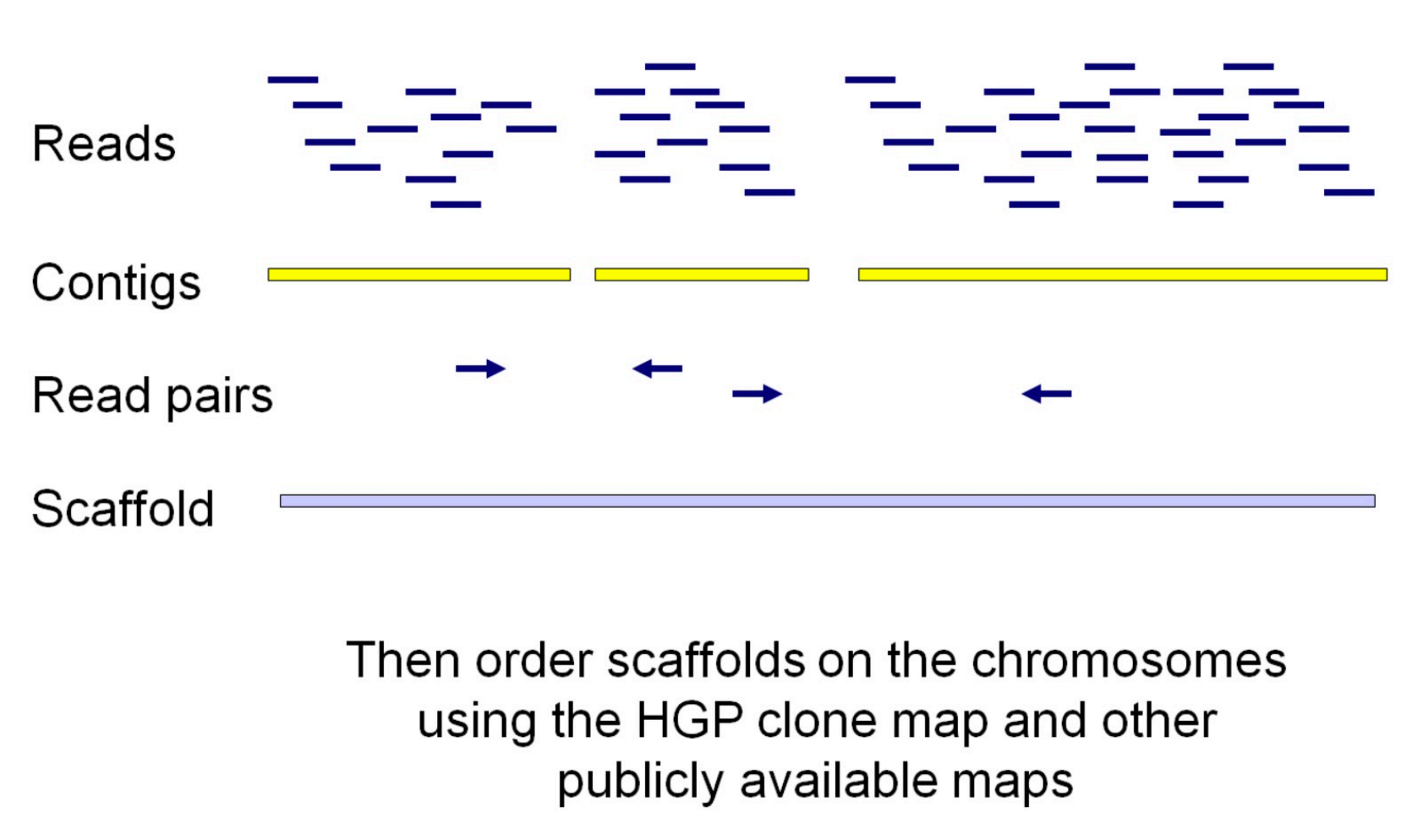
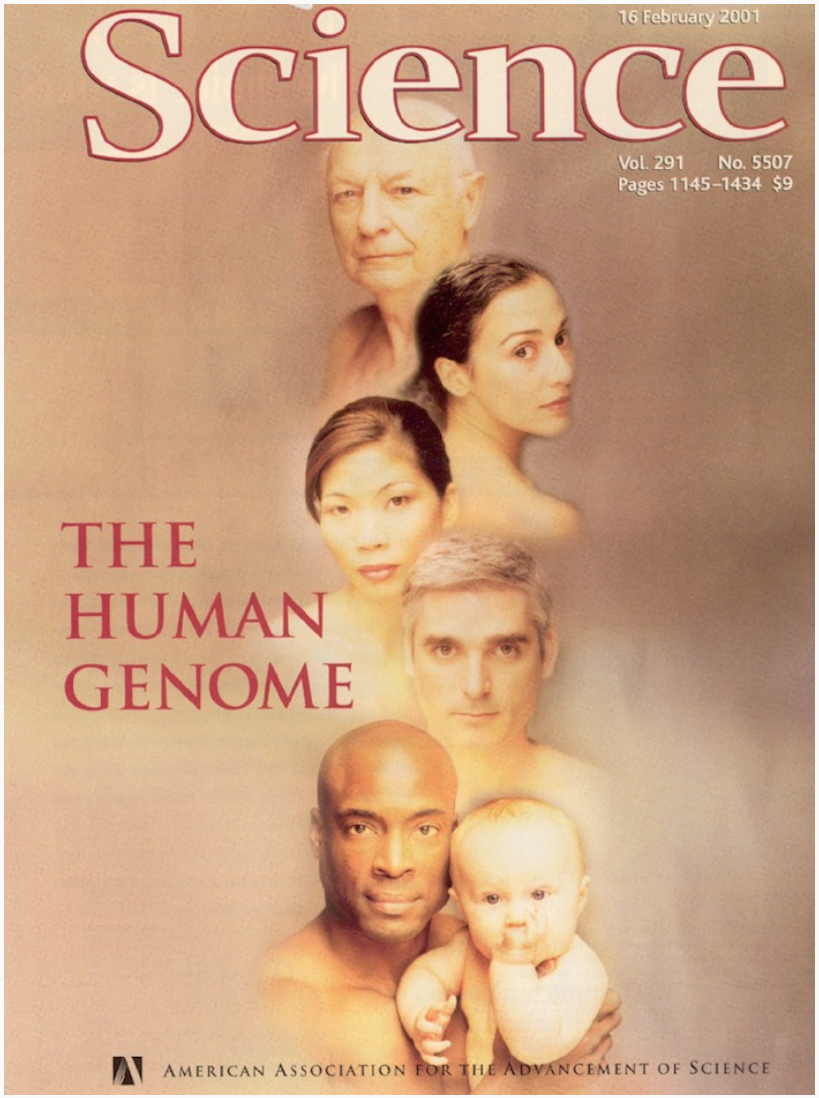


Figure 9. Celera assembly strategy

Figure 10. Celera stocks

(a) Nature



(b) Science

Figure 11. 基因组草图, 2001

二代: (短读长的) 高通量测序

Personal Genomes

- Craig Venter
- James Watson
- Stephen Quake
- George Church
- Marjolein Kriek
- Hermann Hauser
- Han Chinese
- Seong-Jin Kim
- Korean AK1
- Yoruban African NA18507
- 14 others sequenced by Complete Genomics
- Unknown number sequenced by Knome
- 6 genomes sequenced at high depth by the 1000 Genomes Project
- 180 genomes sequenced at low coverage by the 1000 Genomes Project
- Two acute myeloid leukemia patients

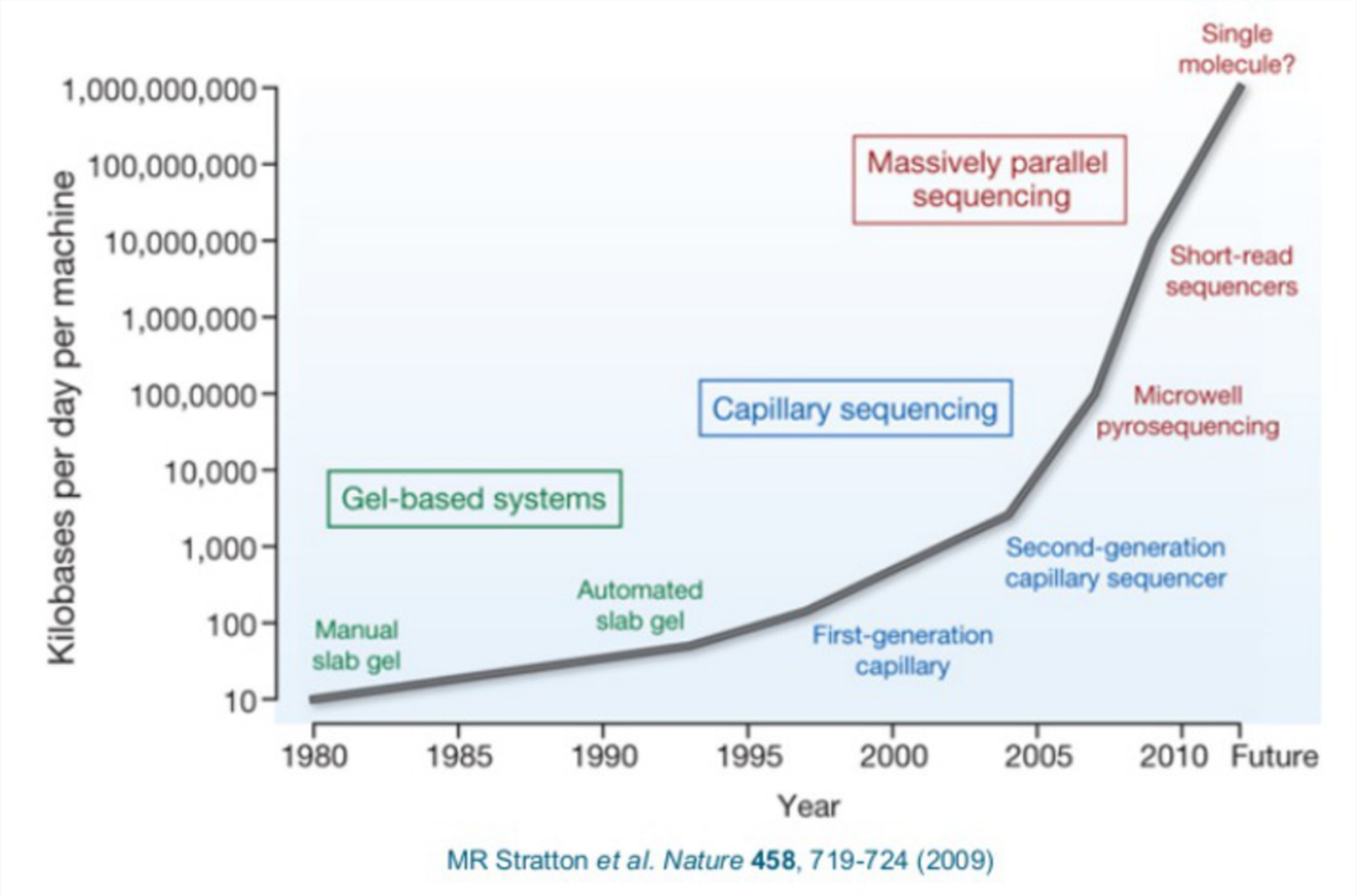


Figure 12. 测序能力的增长

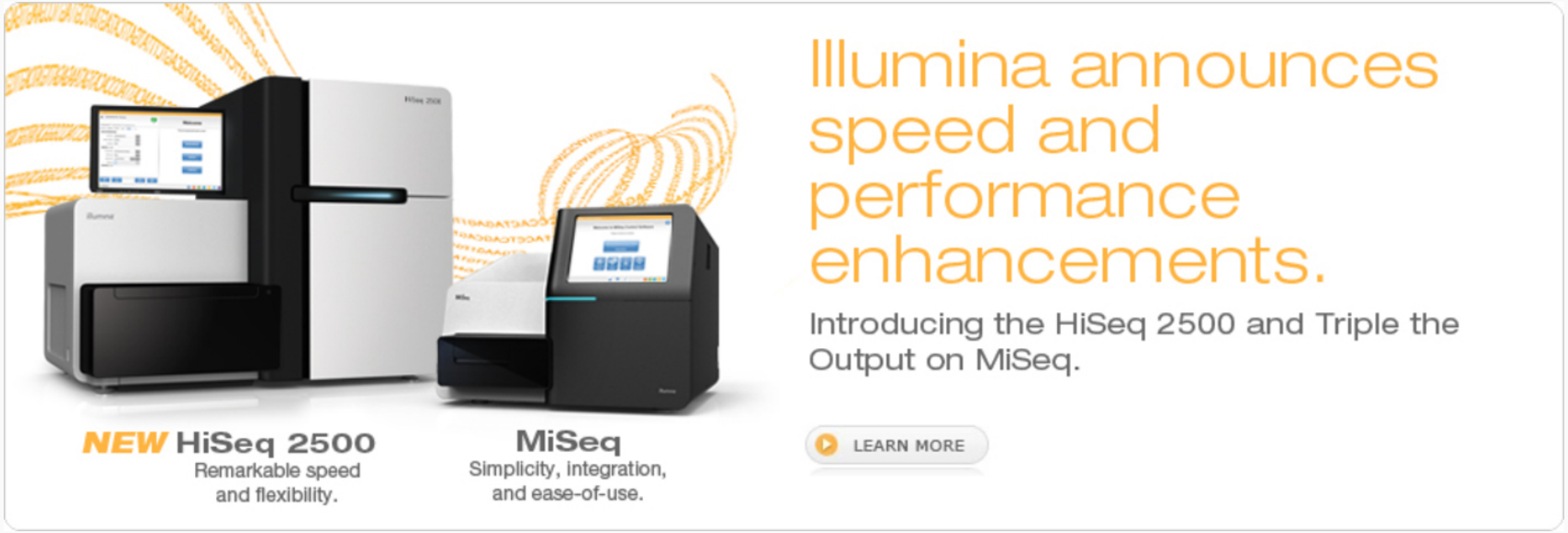


Figure 13. Illumina HiSeq

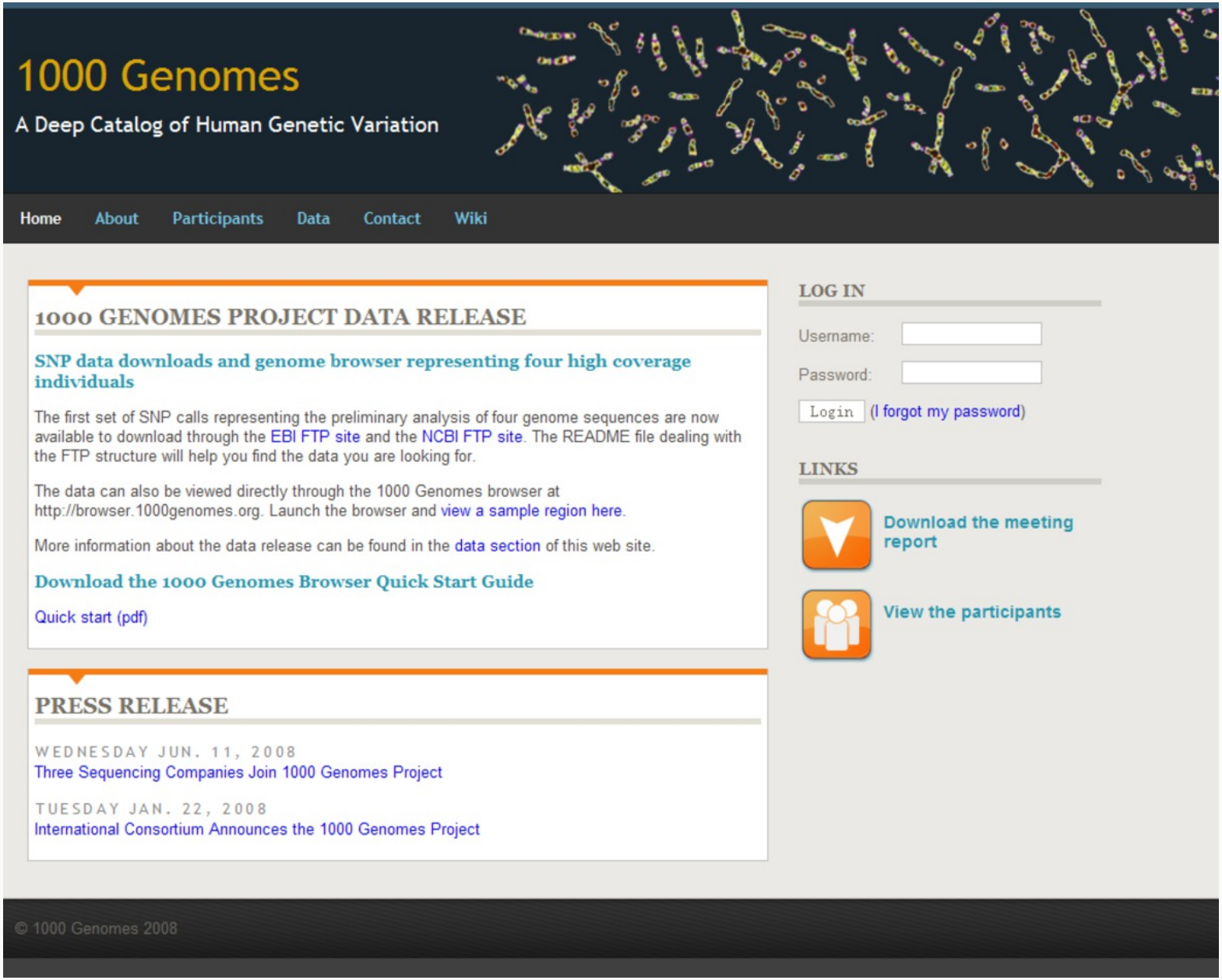


Figure 14. 千个基因组计划

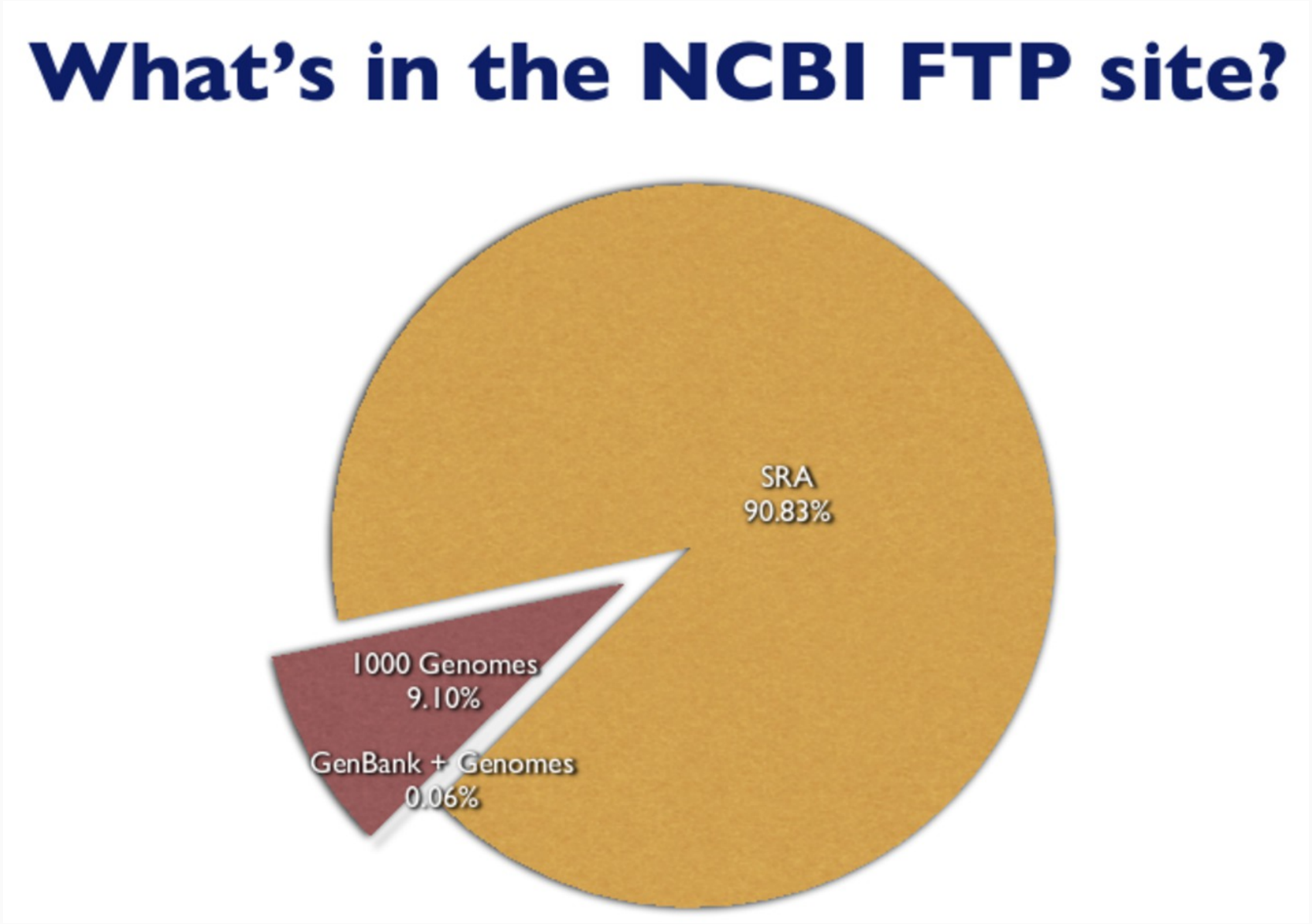


Figure 15. 爆发性增长的数据量

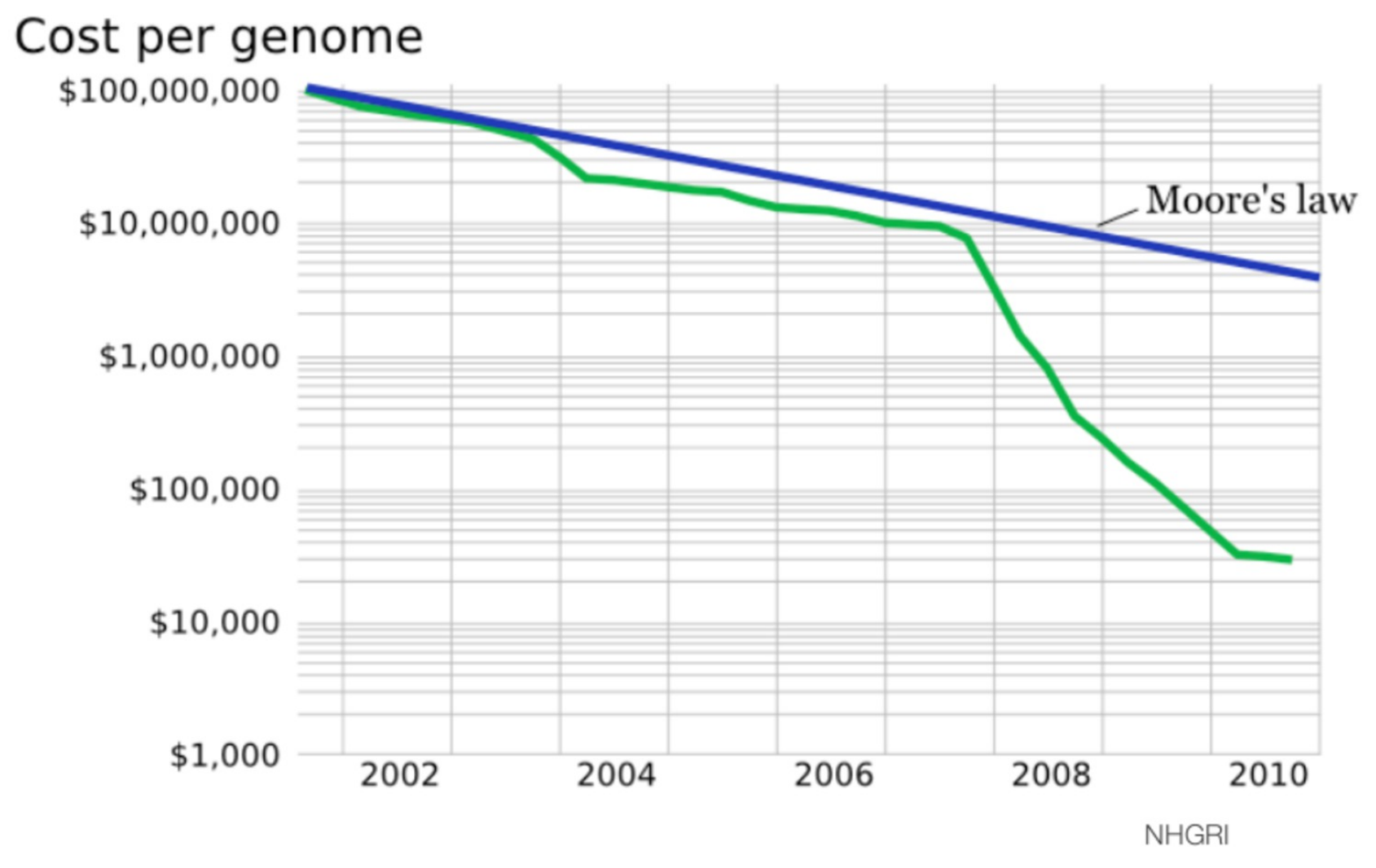


Figure 16. 比摩尔定律更快

Figure 17. HiSeq X

100 GB ≈ 33X Human genome ≈ 10,000 CNY

别再升级通量了，测序仪卖不出去了.

CEO，侬脑子哇特了？

其它二代方法

- Ion Torrent
- 454
- SOLiD

在最近与彭博社的一次采访中, 奥巴马透露了自己想要进入科技风投界的想法.

> 我与硅谷以及风投的交流, 极大地满足了我对科学和组织的兴趣... 你可以只花几千美元, 而不是十万美元, 就可以把个人的基因绘制出来. 你可以有能力辨识自己的(基因)倾向, 去生产对你这个个体而言最有效的药物. 这只是可以让我坐下来, 与别人谈几个小时的科技创业的例子之一.

三代: (长读长的) 单分子测序

二代的缺点

- 建库过程中扩增带来的偏性
- 高 GC 区域的覆盖度与准确性
- 读长短
  - 转座子 (transposons and retrotransposons)
  - 片段重复 (tandom or segment duplications)
  - 一般不超过 5 kbp

商业化服务

- http://allseq.com/providers/
- http://www.pacb.com/products-and-services/service-providers/

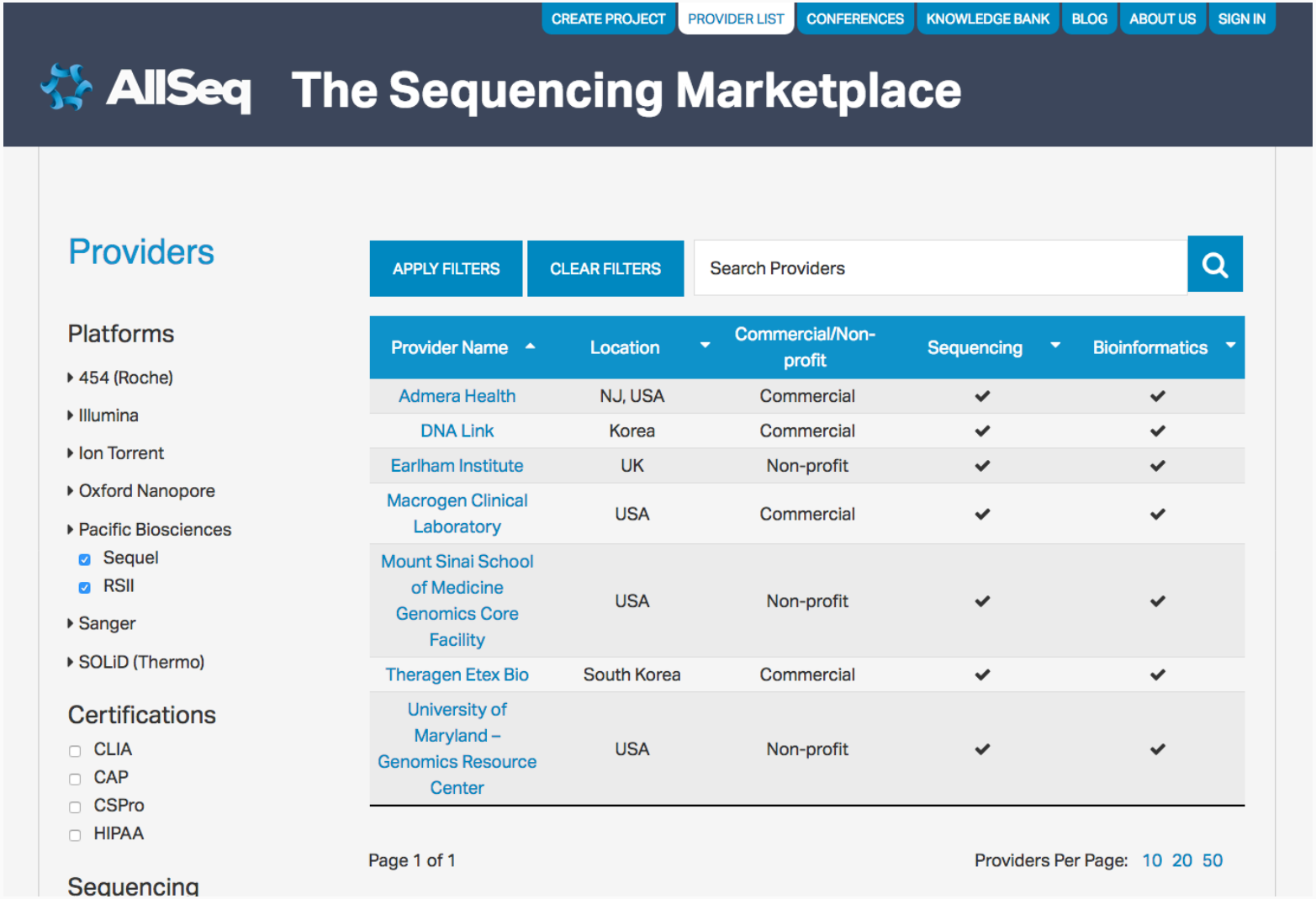


Figure 18. 商业化服务列表

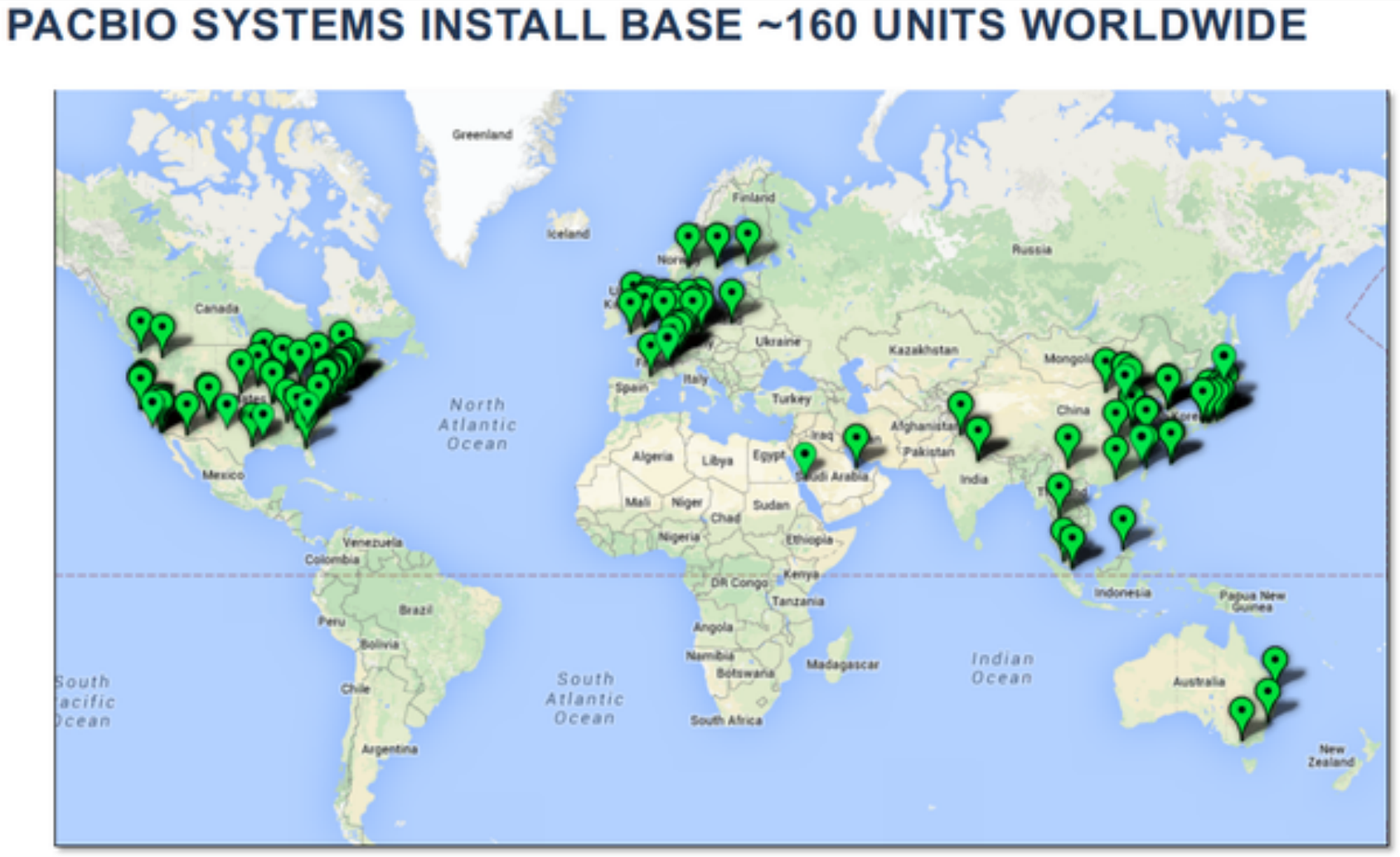


Figure 19. 2015 年全球装机约 160 台

武汉菲沙基因信息有限公司联合美国 Pacific Biosciences 公司在武汉东湖综合保税区共建高通量测序中心, 引进了不少于 6 台 Sequel 三代测序系统, 将建成大规模的三代测序中心.

RS II 与 Sequel 对比

	RS II (P6-C4)	Sequel
运行时间	240 min	240 min
输出量	0.5-1 Gb	5-10 Gb
每日输出量	2 Gb	20 Gb
平均读长	10-15 kb	10-15 kb
单程准确率	~86%	~86%
30X 准确率	>99.999%	>99.999%
Reads 数	50k	500k
平台价格	$700k	$350k
运行成本	$400	$850

适用范围

	Sequel	原因
人类全基因组	Ok/**Good**	贵; 利于鉴定结构变异及组装
小基因组	**Good**	长读长, 只需要较低的通量
靶向测序	**Good**	长读长, 只需要较低的通量
转录组	Poor/**Good**	贵; 可得到全长的转录本
宏基因组	Poor/Ok	贵; 利于 de novo 组装
外显子组	Poor	贵; 长读长对外显子没有用处
表达谱	Poor	贵
ChIP-Seq	Poor	贵

信息学的问题

SMRT Analysis Software 包括了一些第三方程序:

- 编程语言
  - Java 7
  - Mono 3
  - Perl 5.8
  - Python 2.7
  - Scala 2.9
- 平台
  - Tomcat 7.0.23
  - MySQL 5.1.73
  - Docutils
- 生物信息学工具
  - Celera Assembler 8.1
  - GMAP

其它三代方法

- Helicos, 读长过短, 已经破产
- Oxford Nanopore

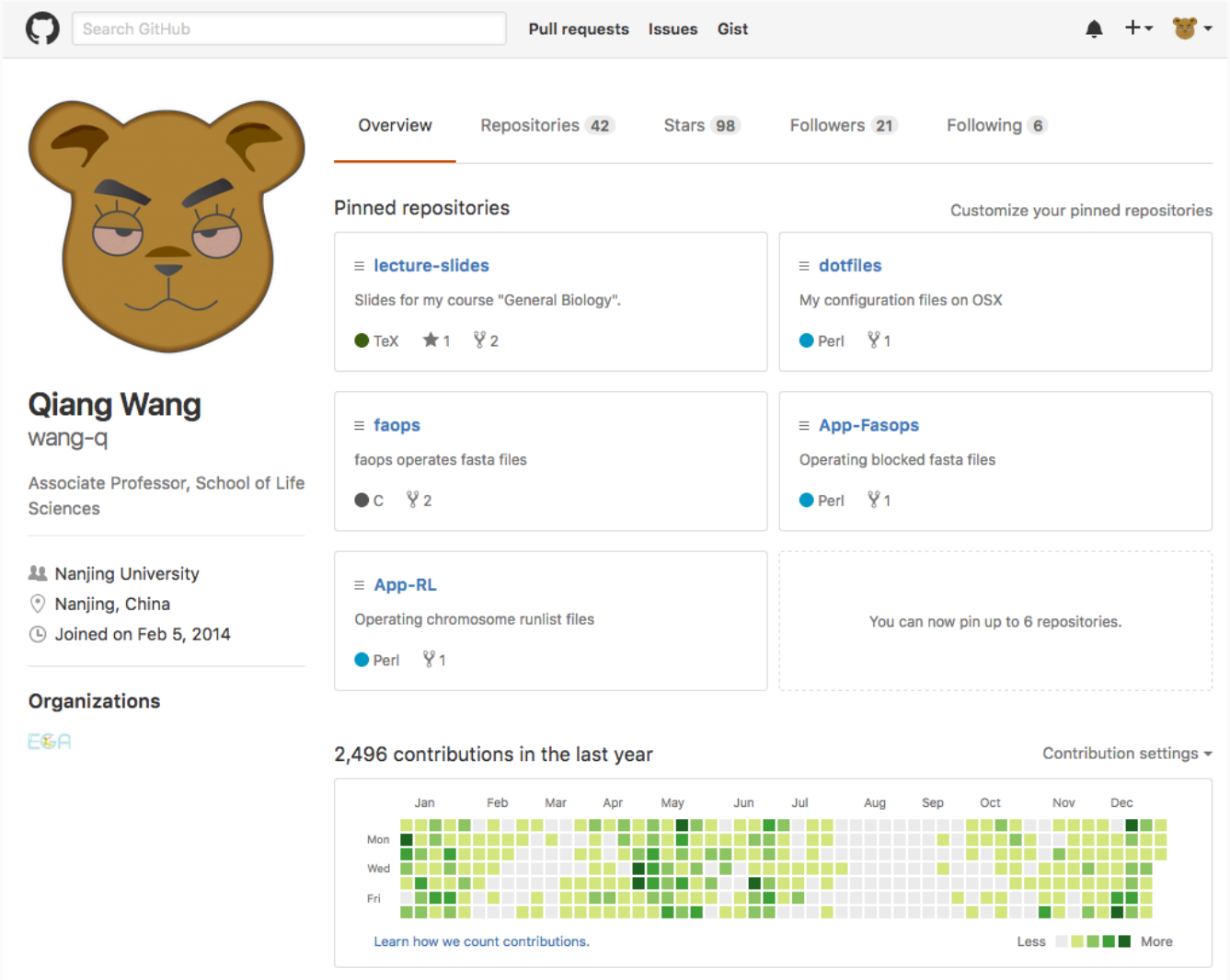


Figure 20. https://github.com/wang-q