BGI华大

文献阅读与科研展示

第三组-王硕 吕志远 赵万东 孙士瑶 吕丰源 苏光烨

2021.10.09

华大生命科学研究院
BGI · research

1.背景介绍
2.文献讲解-Roche公司的454技术（合成法）
3.文献讲解-ABI公司的SOLiD技术（连接法）

一、背景介绍


腾讯会议
vuan的快速会议
会议号：520 375 152
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创建者：vuan

二、罗氏454技术

nature Vol 437|15 September 2005|doi:10.1038/nature03959

ARTICLES

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies[1]*, Michael Egholm[1]*, William E. Altman[1], Said Attiya[1], Joel S. Bader[1], Lisa A. Bemben[1], Jan Berka[1], Michael S. Braverman[1], Yi-Ju Chen[1], Zhoutao Chen[1], Scott B. Dewell[1], Lei Du[1], Joseph M. Fierro[1], Xavier V. Gomes[1], Brian C. Godwin[1], Wen He[1], Scott Helgesen[1], Chun He Ho[1], Gerard P. Irzyk[1], Szilveszter C. Jando[1], Maria L. I. Alenquer[1], Thomas P. Jarvie[1], Kshama B. Jirage[1], Jong-Bum Kim[1], James R. Knight[1], Janna R. Lanza[1], John H. Leamon[1], Steven M. Lefkowitz[1], Ming Lei[1], Jing Li[1], Kenton L. Lohman[1], Hong Lu[1], Vinod B. Makhijani[1], Keith E. McDade[1], Michael P. McKenna[1], Eugene W. Myers[2], Elizabeth Nickerson[1], John R. Nobile[1], Ramona Plant[1], Bernard P. Puc[1], Michael T. Ronan[1], George T. Roth[1], Gary J. Sarkis[1], Jan Fredrik Simons[1], John W. Simpson[1], Maithreyan Srinivasan[1], Karrie R. Tartaro[1], Alexander Tomasz[3], Kari A. Vogt[1], Greg A. Volkmer[1], Shally H. Wang[1], Yong Wang[1], Michael P. Weiner[4], Pengguang Yu[1], Richard F. Begley[1] & Jonathan M. Rothberg[1]

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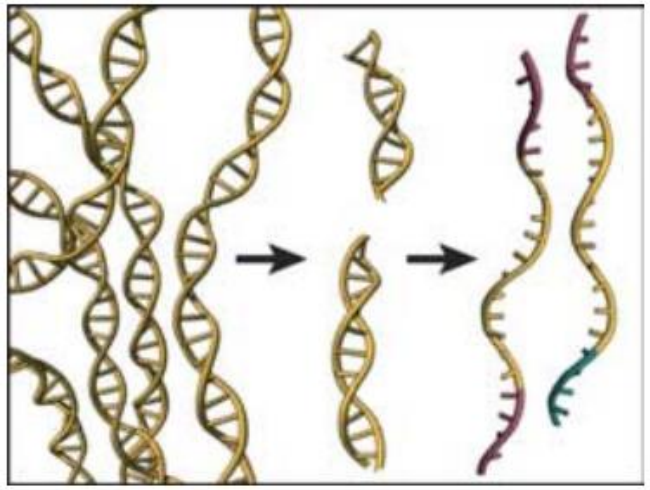
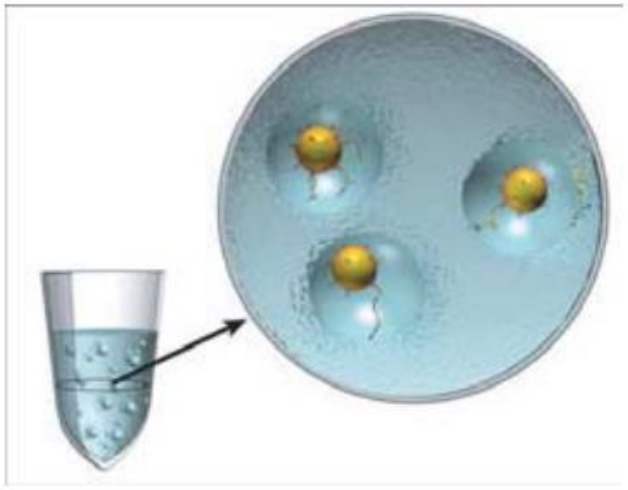
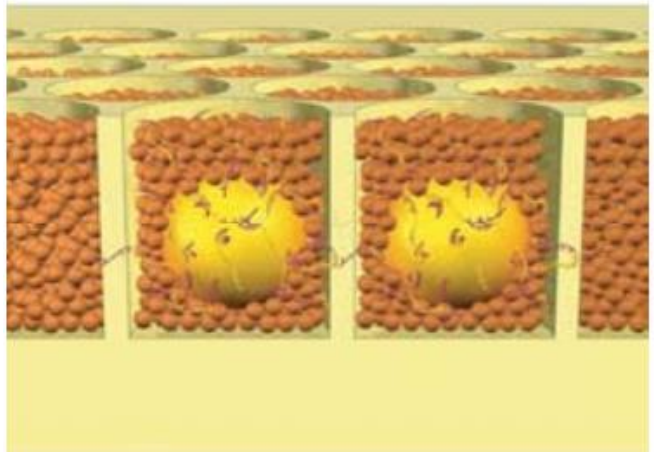
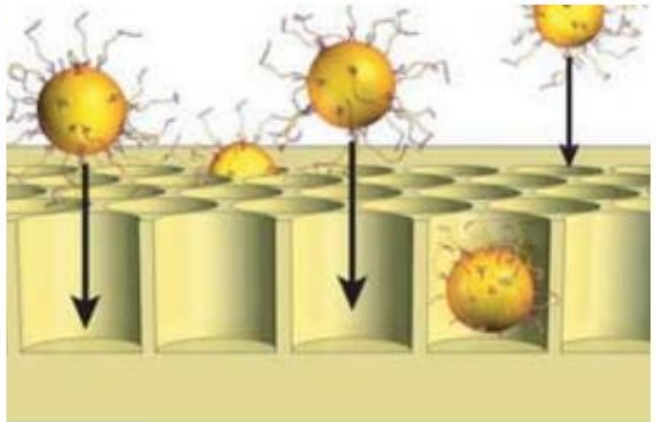
*These authors contributed equally to this work.









Sample preparation

top left

Genomic DNA is isolated, fragmented,
ligated to adapters and separated into single strands

top right

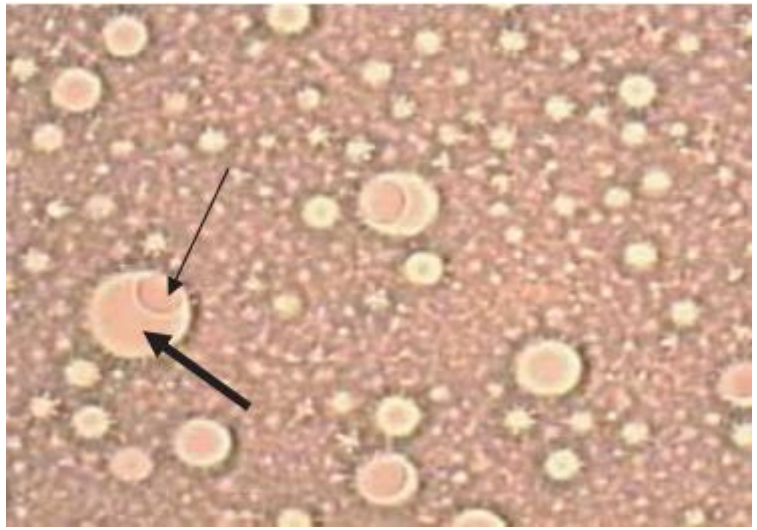
Emulsion-based PCR

bottom right

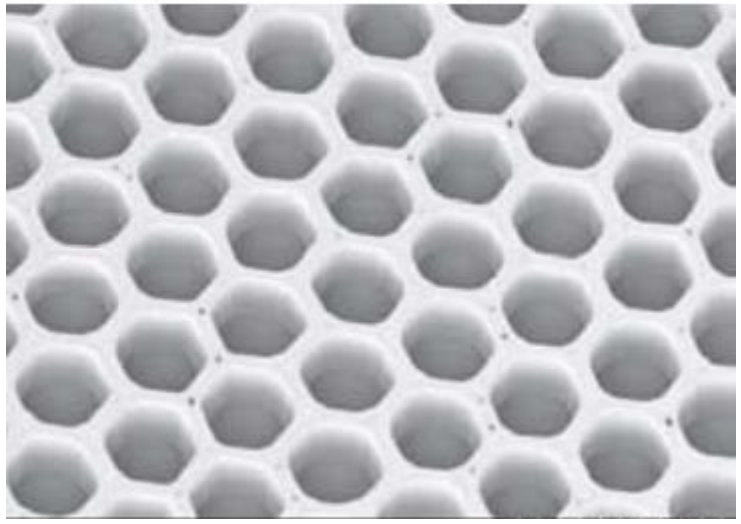
The emulsion is broken, the DNA strands are denatured, and beads carrying
single-stranded DNA clones are deposited into wells of a fifibre-optic slide

bottom left

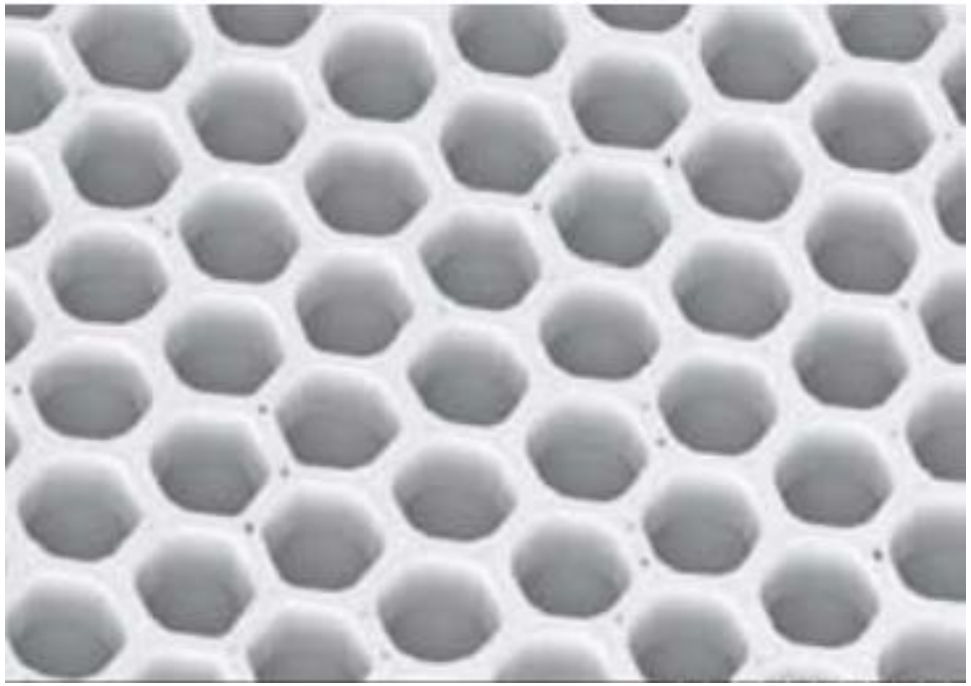
Smaller beads carrying immobilized enzymes required for pyrophosphate sequencing are deposited into each well



Microscope photograph of emulsion showing droplets containing a bead and empty droplets. The thin arrow points to a 28-mm bead; the thick arrow points to an approximately 100-mm droplet.



Scanning electron
micrograph of a portion of a fibre-optic slide, showing fibre-optic cladding and wells before bead deposition.



Fibre-optic core diameter : 44 um

Fibre-optic core cladding : 2–3

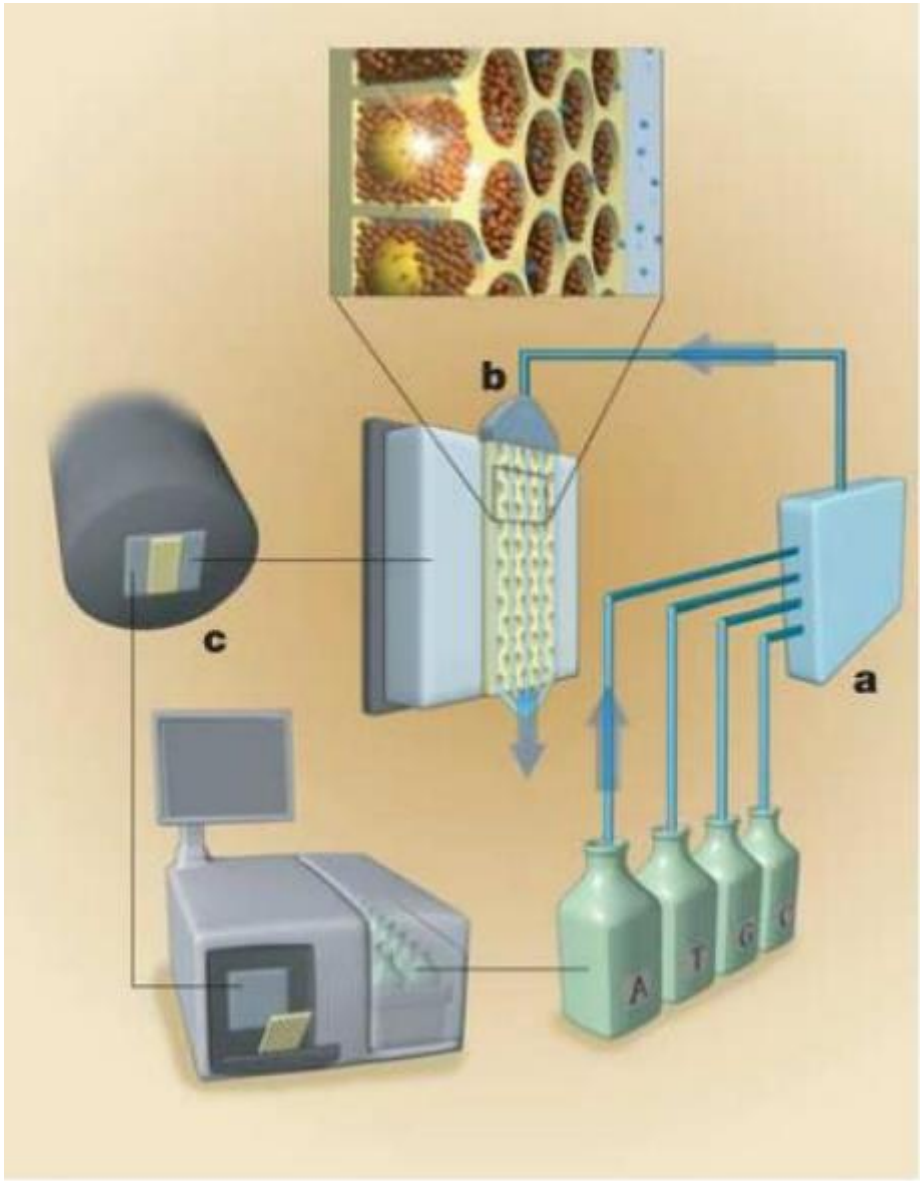
Fibre-optic core depth : 55 um

Fibre-optic core centre-to-centre distance : 50 um

The well density 480 wells um^{-2}

The slide contain 1.6 million wells

The flow chamber designed to create a 300-um high channel



Sequencing instrument.
(a)流体组件
(b)一个流动室，包括含有孔的光纤载玻片
(c)A CCD 电荷耦合器件传感器 camera-based imaging assembly

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三、SOLiD技术

Science

AAAS

Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome

Jay Shendure *et al.*

Science **309**, 1728 (2005);
DOI: 10.1126/science.1117389

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Genetics, [3]Howard Hughes Medical Institute, Washington University, St. Louis, MO 63110, USA.

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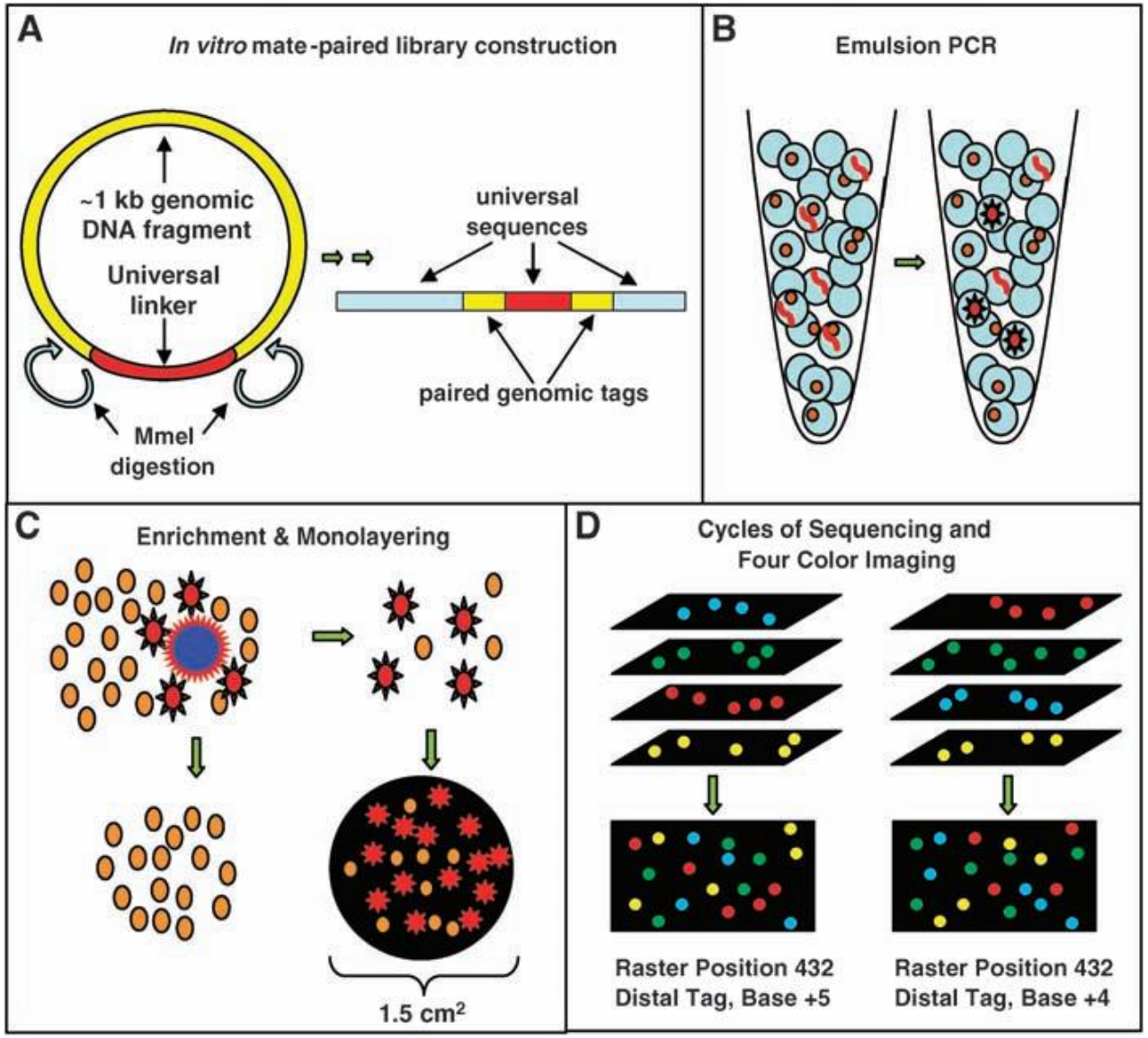
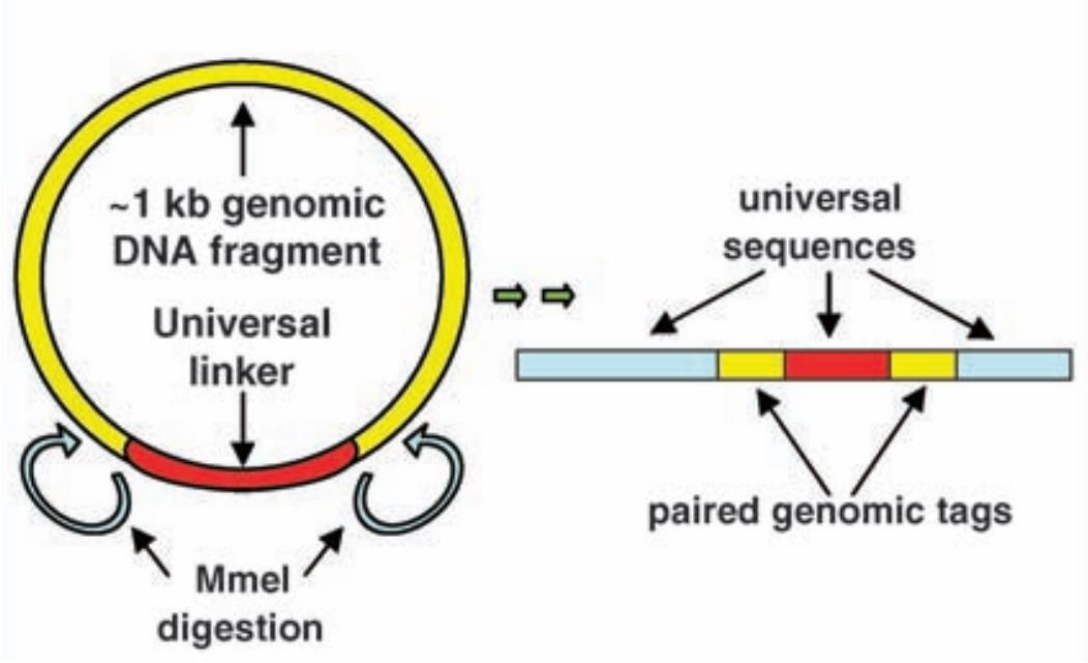
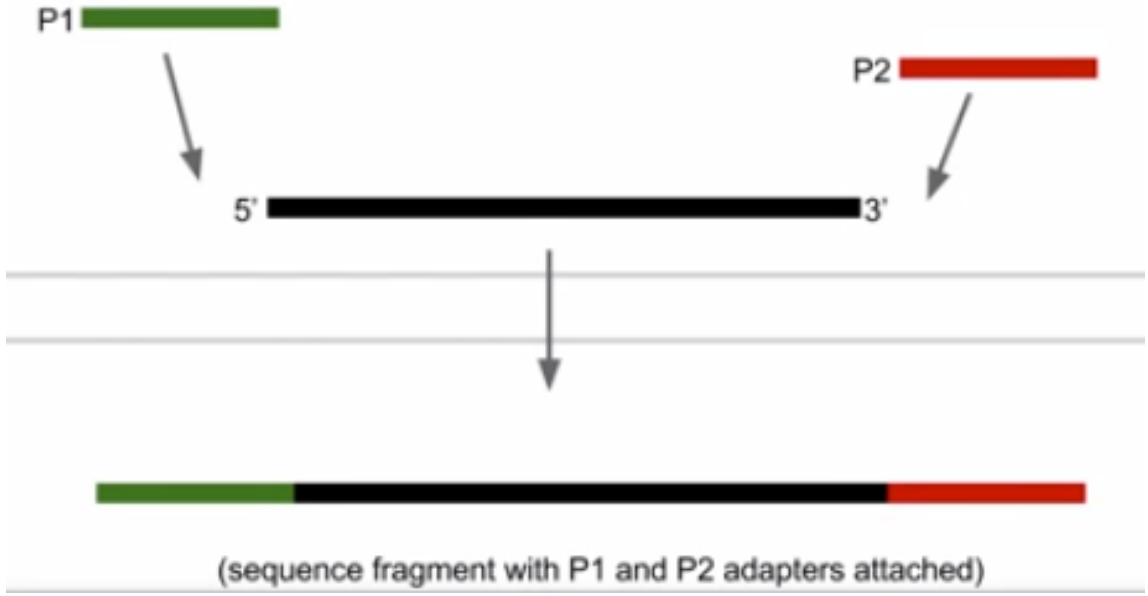
波士顿哈佛医学院遗传学系

SOLiD流程

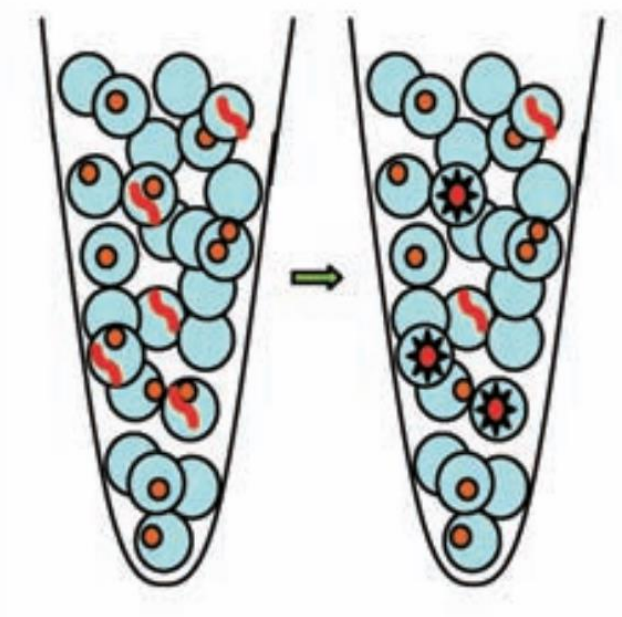

A
In vitro mate-paired library construction
~1 kb genomic DNA fragment
Universal linker
MmeI digestion
universal sequences
paired genomic tags
B
Emulsion PCR
C
Enrichment & Monolayering
1.5 cm²
D
Cycles of Sequencing and Four Color Imaging
Raster Position 432
Distal Tag, Base +5
Raster Position 432
Distal Tag, Base +4

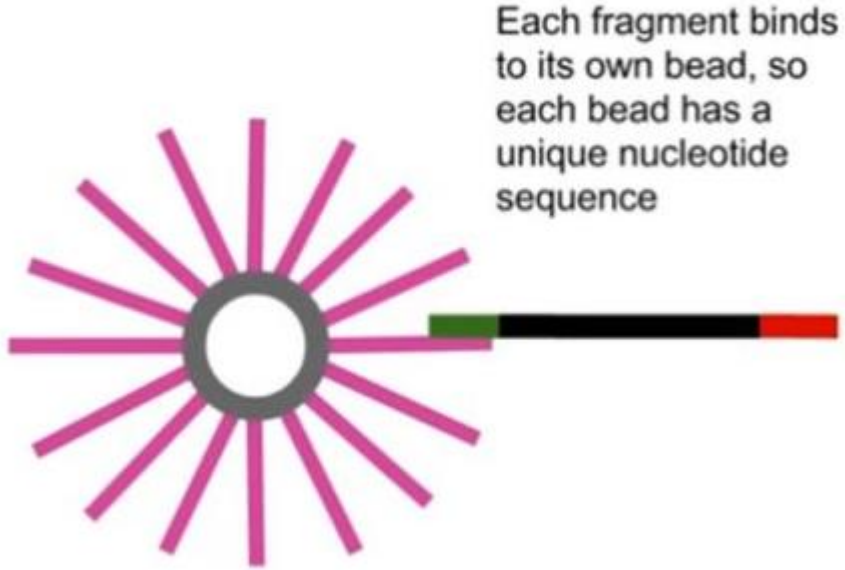
SOLiD流程1 打断，构建文库





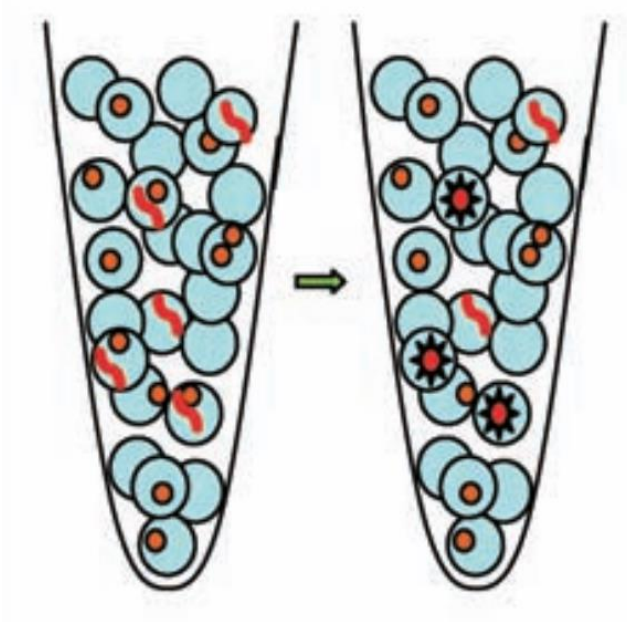
SOLiD流程

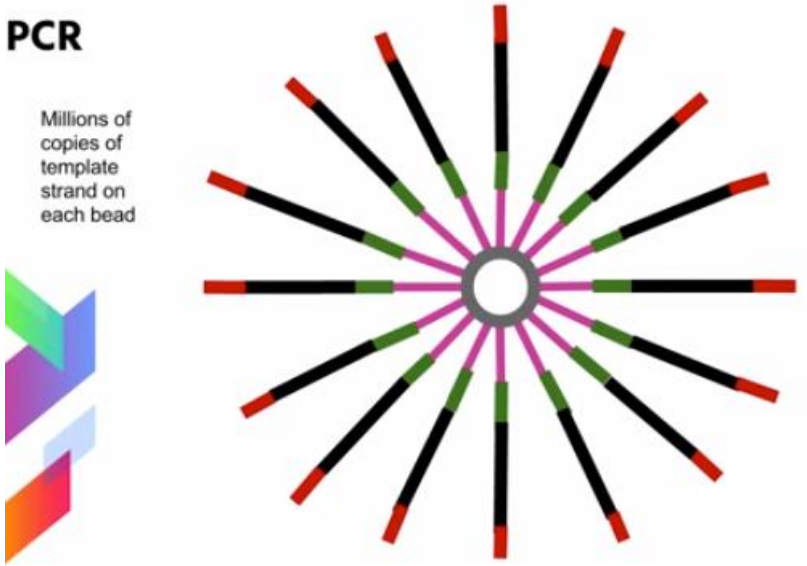


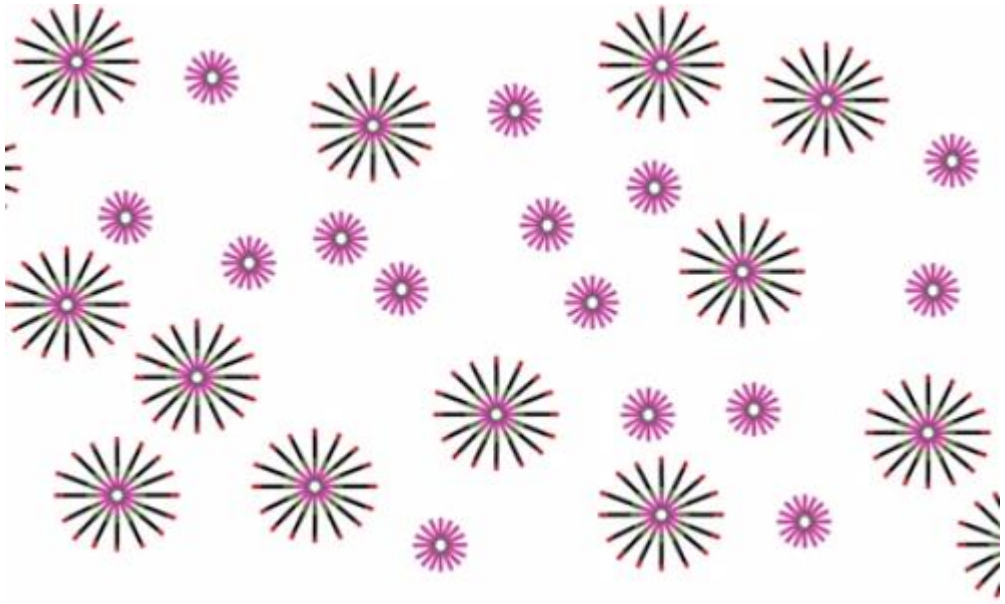


•fragment可以结合特定bead，不是所有bead都有结合的fragment
•结合有fragment的bead，通过离心筛选出来，然后结合到glass slide

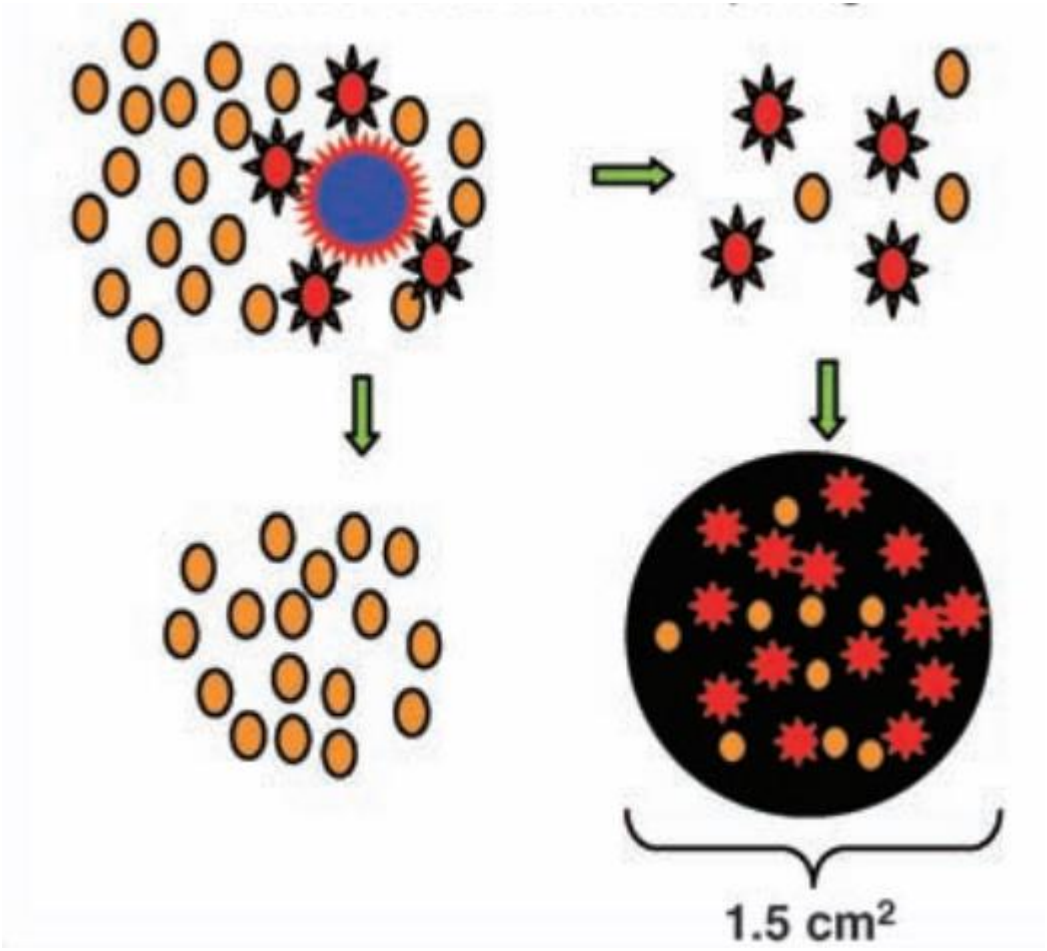
SOLiD流程

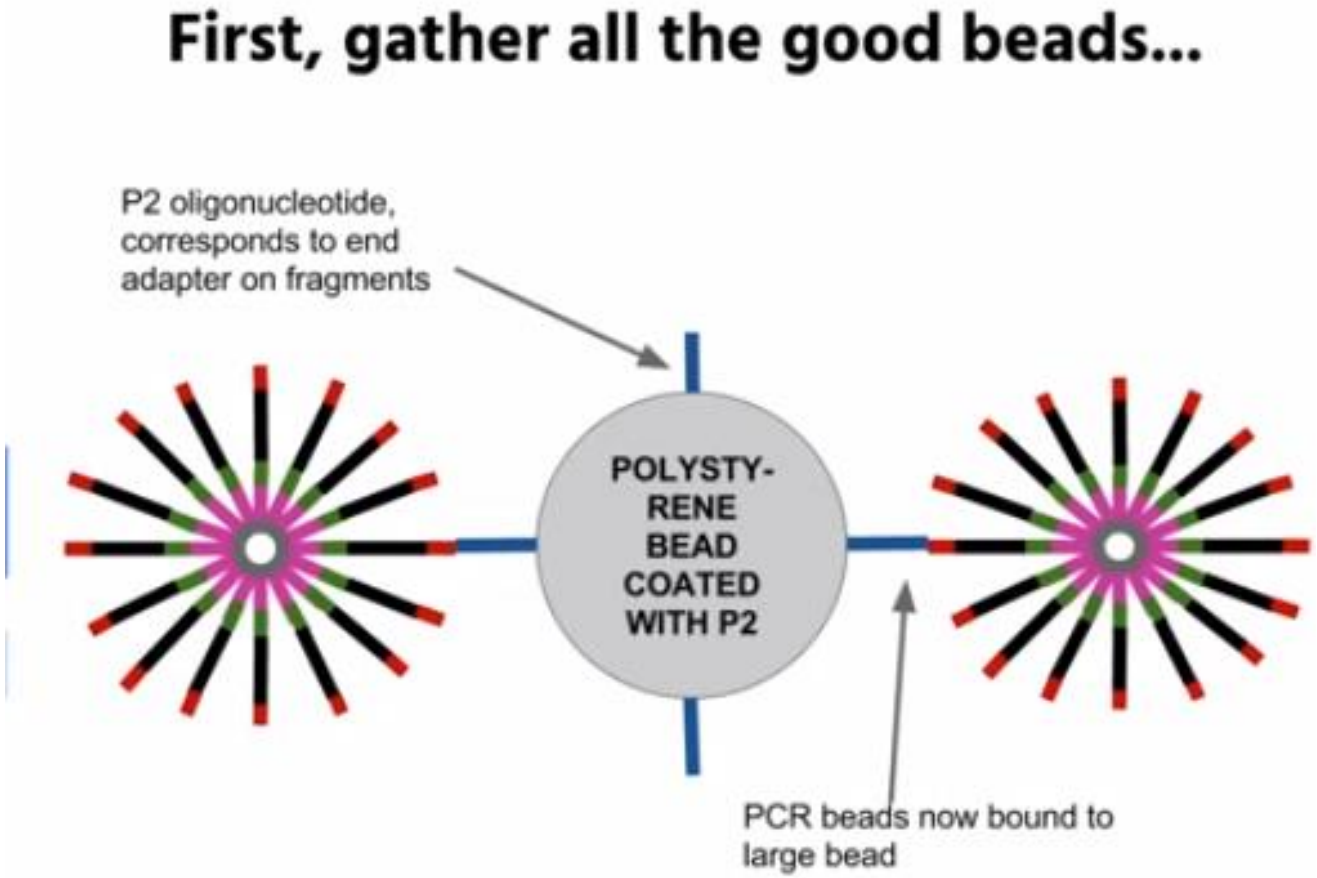




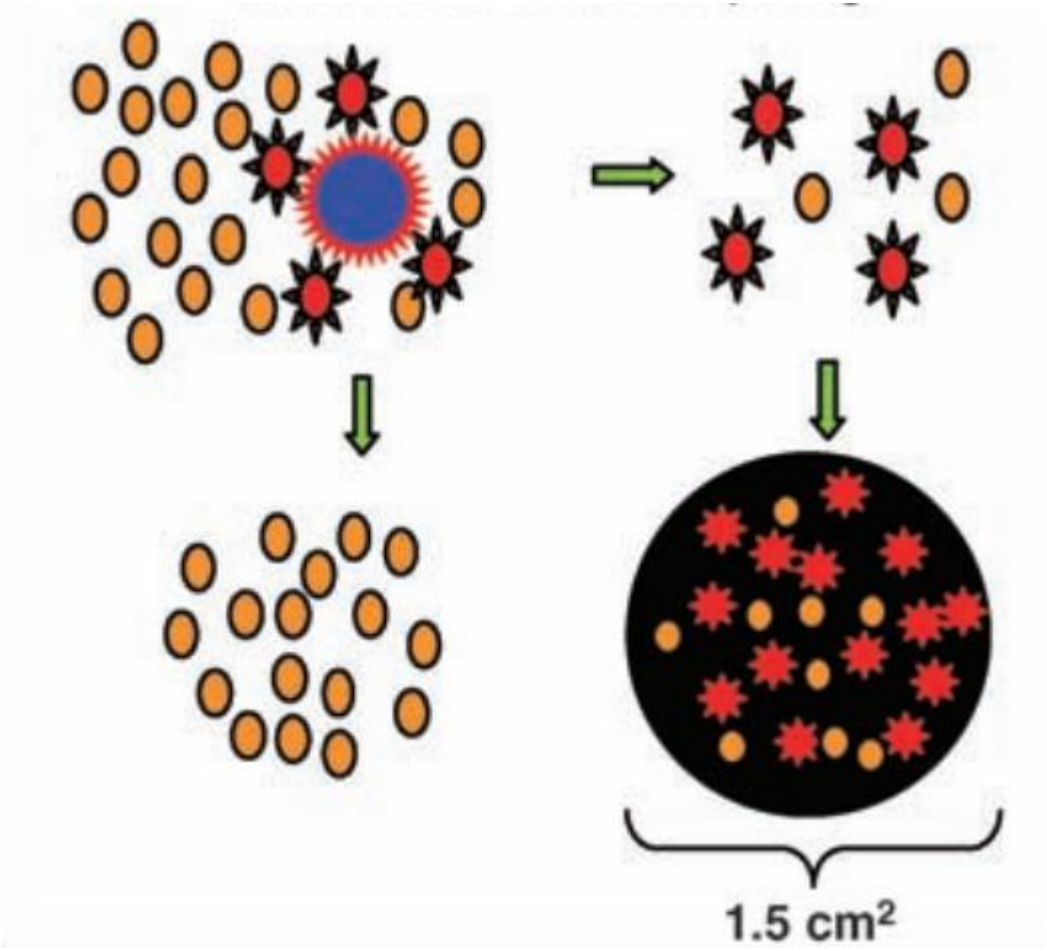


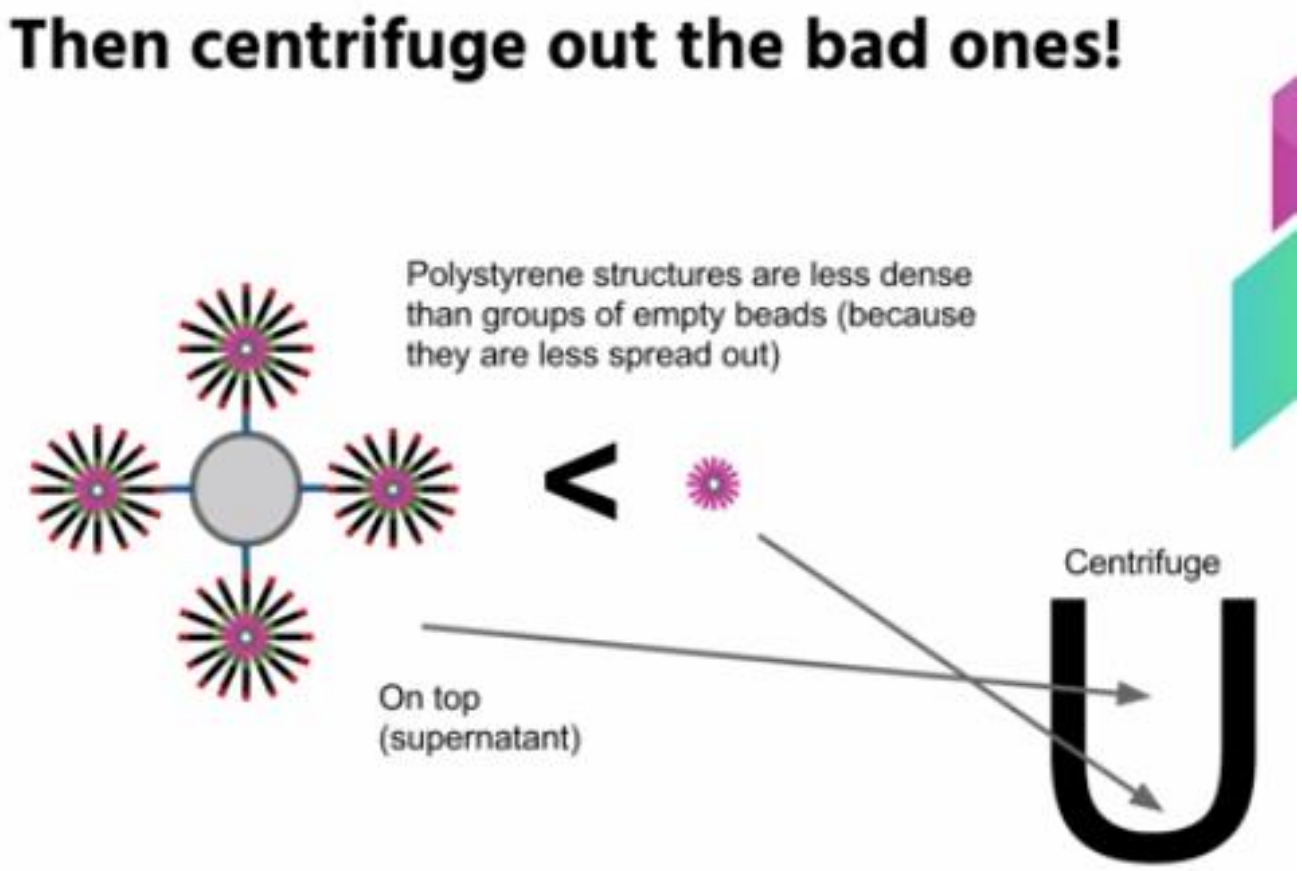
SOLiD流程


1.5 cm²

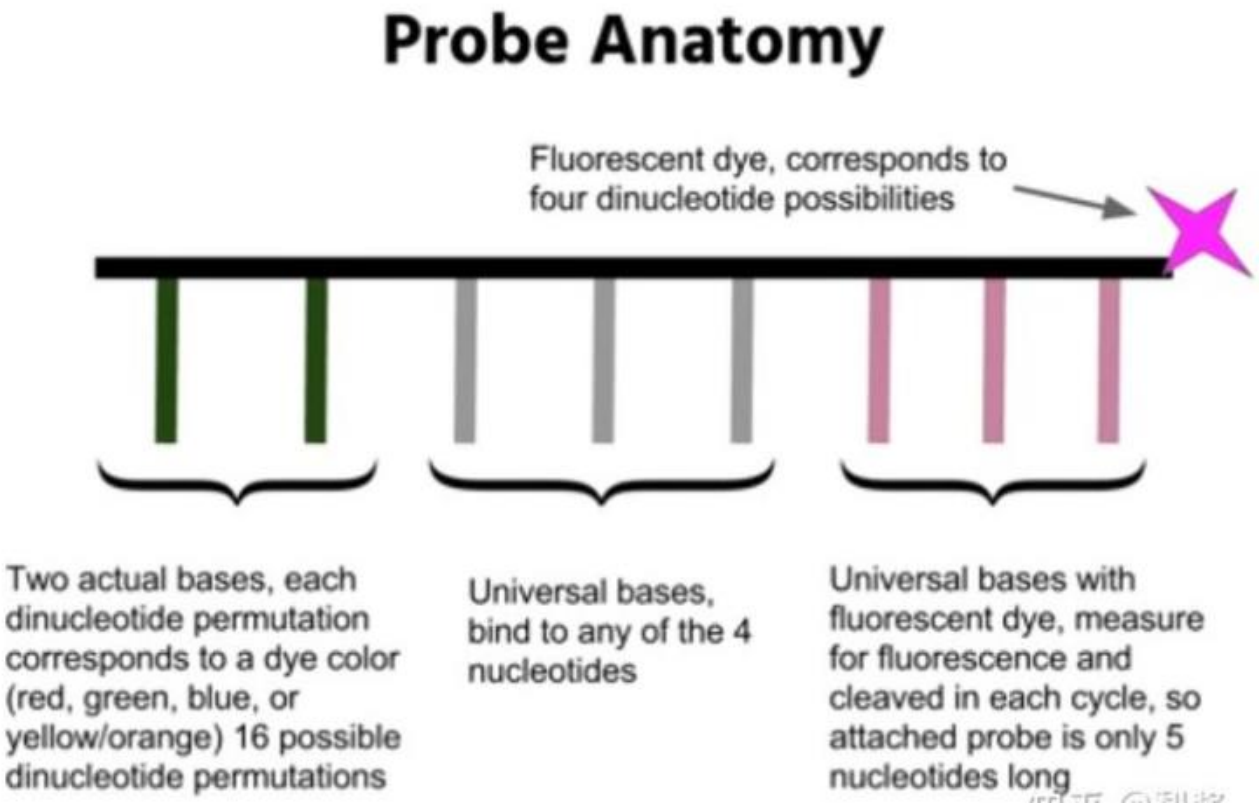

First, gather all the good beads...
P2 oligonucleotide, corresponds to end adapter on fragments
POLYSTY-RENE BEAD COATED WITH P2
PCR beads now bound to large bead

SOLiD流程


1.5 cm²


Then centrifuge out the bad ones!
Polystyrene structures are less dense than groups of empty beads (because they are less spread out)
<
Centrifuge
On top (supernatant)

SOLiD流程



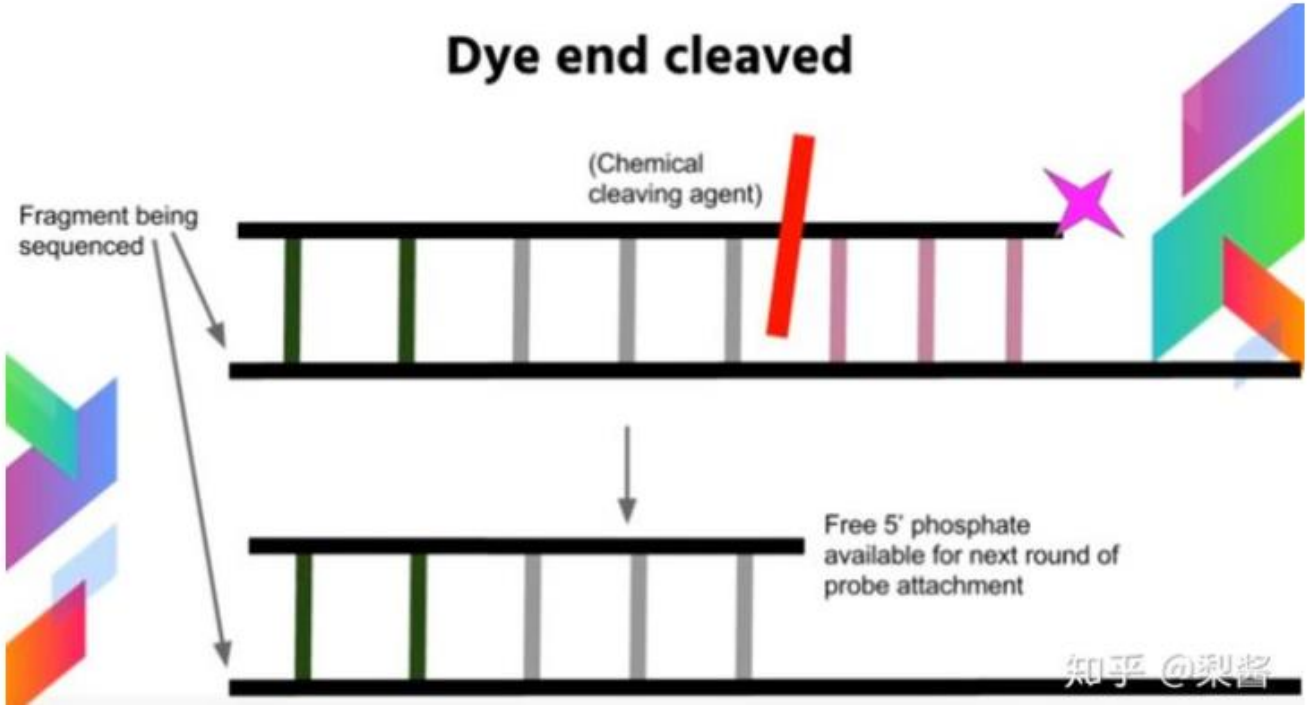
带有不同荧光标记的8碱基探针（different fluorescently dyed oligonucleotide 8-mer bases）

•前2个碱基是真实的碱基。组合起来可以有16种可能，AA\AT\AG\AC, GG\GA\GT\GC……每种排列对应一种荧光颜色（红、绿、蓝、黄）。**注意！**也就是说每种颜色可以对应4种排列。

•中间3个通用碱基，

•后3个有荧光标记的碱基

SOLiD流程



1.加上8碱基探针后:
2.用激光激发荧光。
3.用化学方法剪切掉后面3个荧光标记的碱基，只留下前5个碱基和游离的磷酸基团，以便加上第二个探针。

SOLiD流程

1.加上8碱基探针后:
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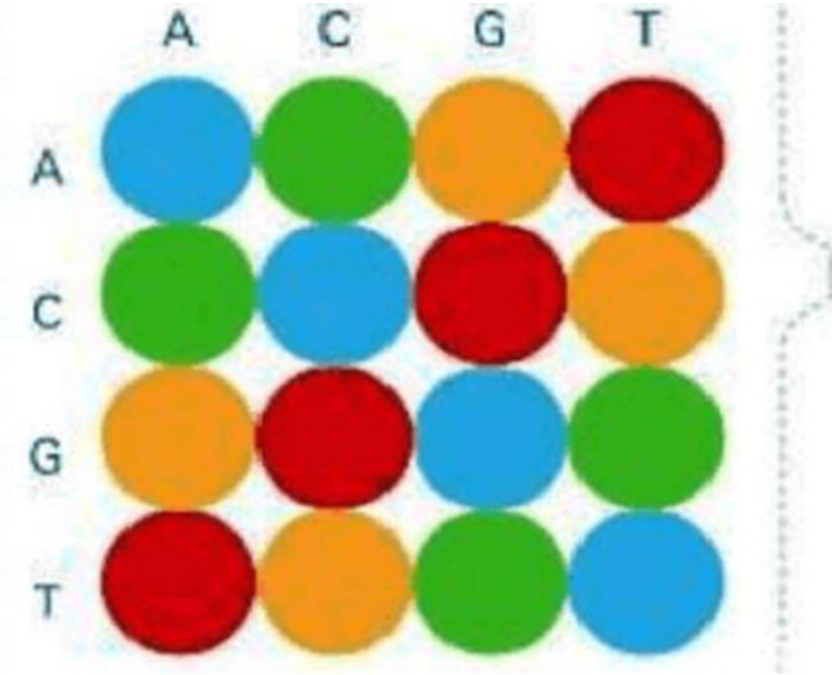
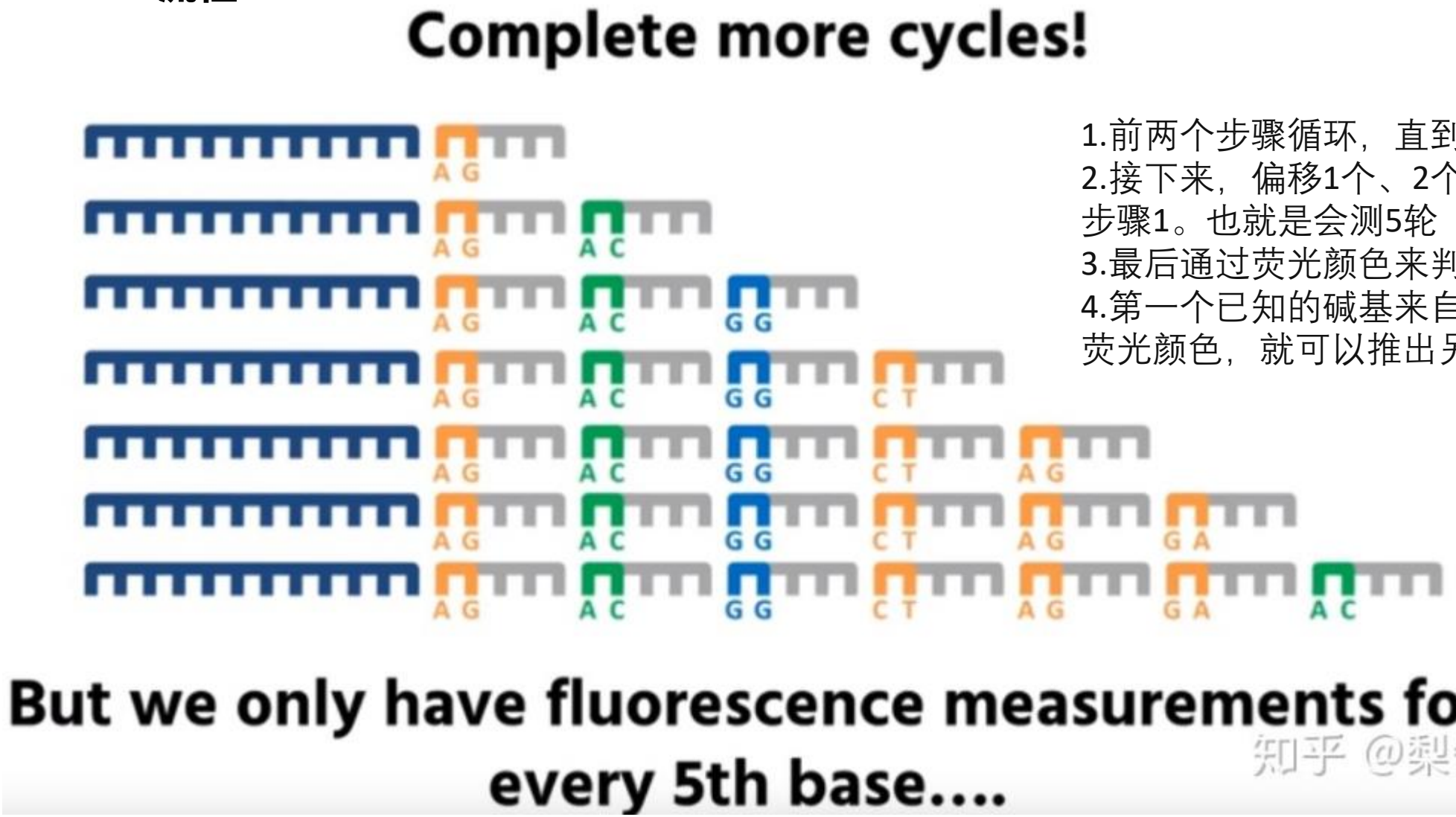
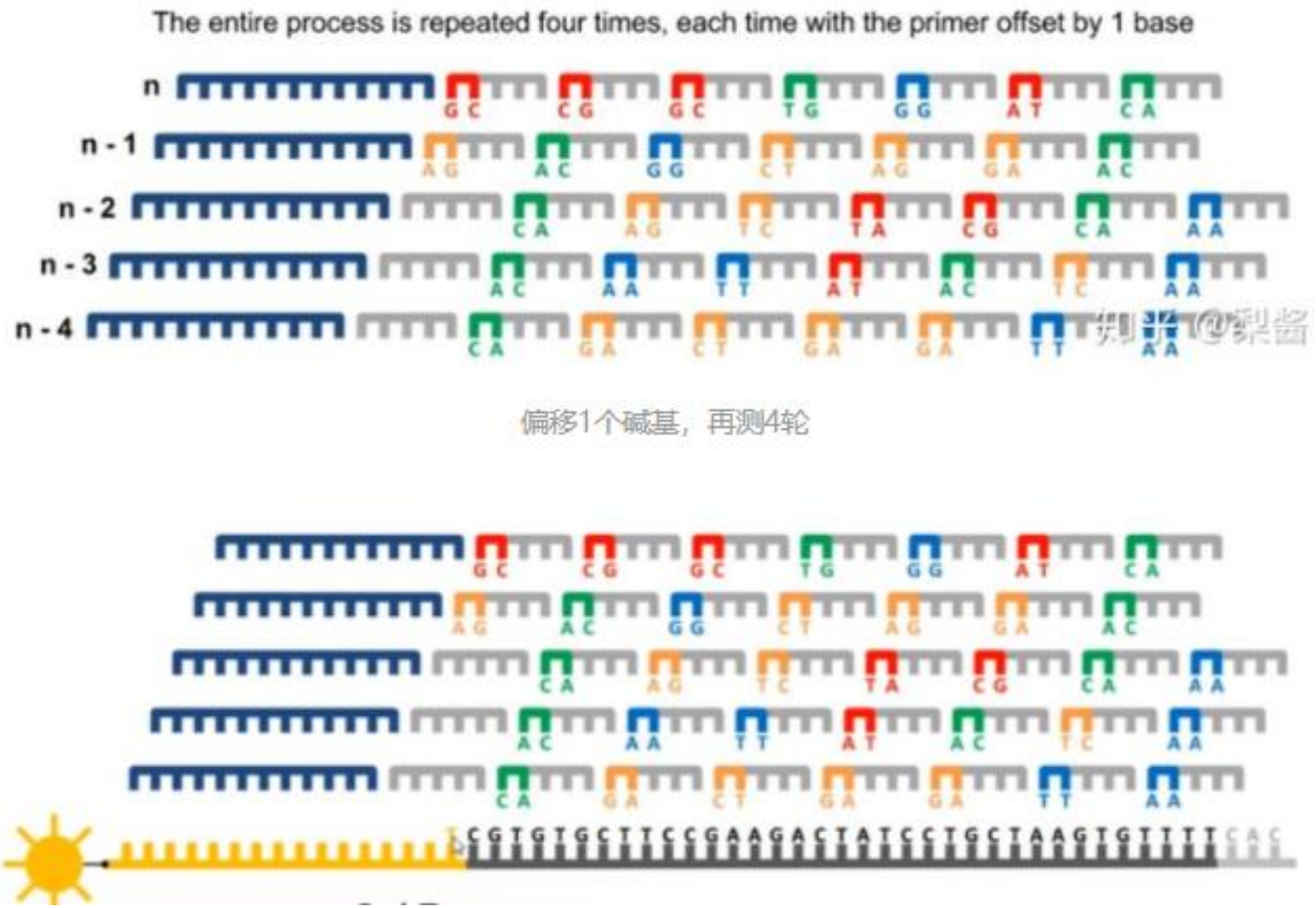


SOLiD流程



1.前两个步骤循环，直到合成整条链。
2.接下来，偏移1个、2个、3个、4个碱基，重复步骤1。也就是会测5轮
3.最后通过荧光颜色来判断
4.第一个已知的碱基来自5’或3’的adapter，又已知荧光颜色，就可以推出另外一个碱基

SOLiD流程



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2.最后通过荧光颜色来判断
3.第一个已知的碱基来自5’或3’的adapter，又已知荧光颜色，就可以推出另外一个碱基

华大BGI

优缺点

a) 优点：

•目前二代测序技术中准确度最高

•除了测序和重测序之外，还能进行全基因组表达图谱分析、SNP、microRNA、甲基化等分析。

b) 缺点：

•读长短，拼接复杂

•双碱基对应一个荧光信号，如果发生读码错误，将发生连锁读码反应。

二者区别

- ePCR的过程中，SOLiD采用的磁珠直径更小，仅有1微米；而454使用两种磁珠，小磁珠上有固定酶，大磁珠上有reads
- SOLiD的独特之处在于没有使用常用的DNA聚合酶，而是使用DNA连接酶

相同之处

- 均使用ePCR技术
- 均使用磁珠进行扩增

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THANKS

OMICS FOR ALL

基 因 科 技 造 福 人 类