高通量测序技术及应用

微生物所测试技术论坛第十七讲

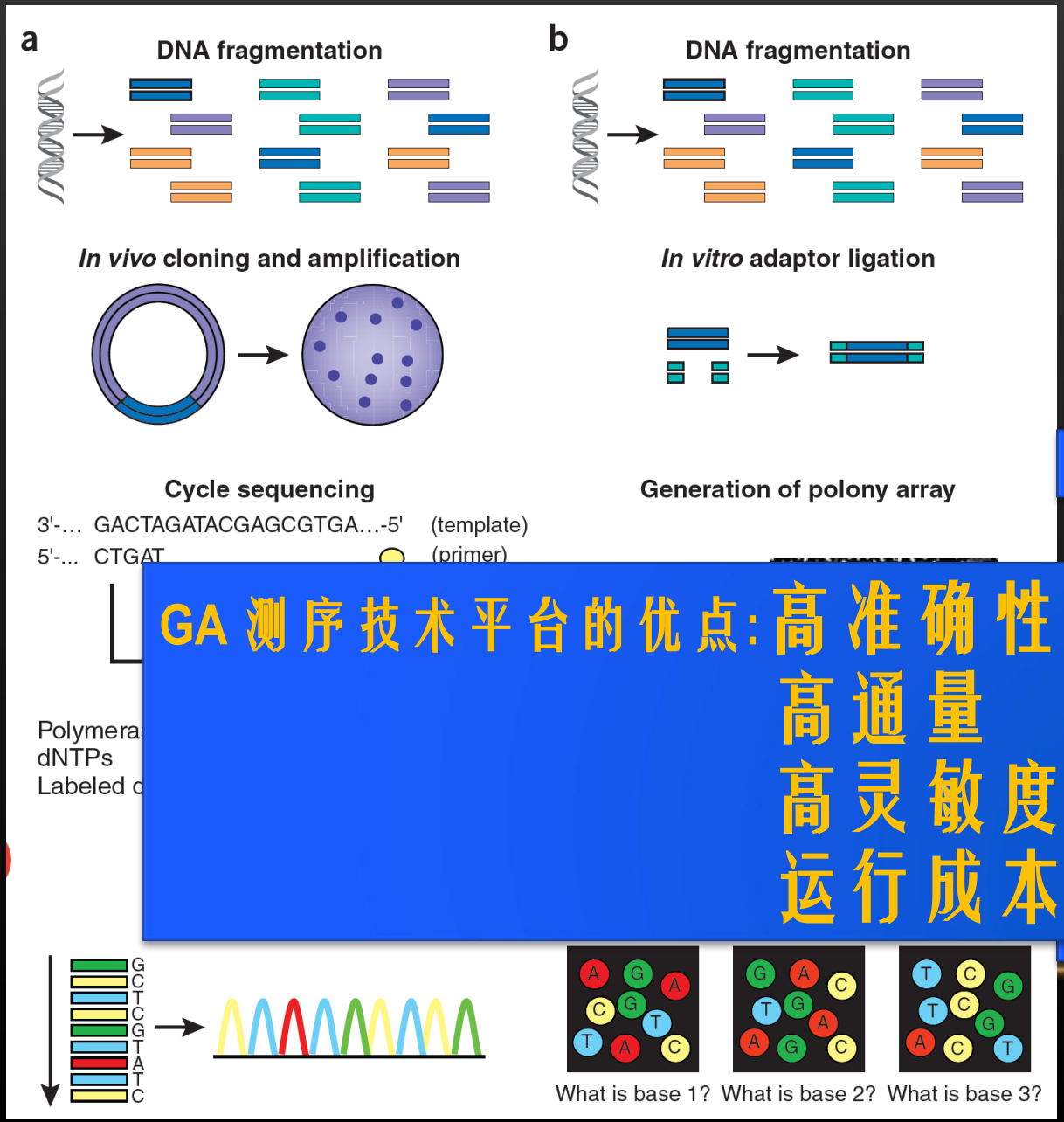
律 娜

第二代测序仪与第一代测序仪的不同

高通量测序可以做什么

高通量测序如何做

第二代测序仪与sanger法的比较



Roche: 454

ABI : SOLID

Illumina: GA IIx

Hiseq2000

无需再单独设计引物

的测序，
的生物
研究

力无限

GA测序技术平台的优点：高准确性
高通量
高灵敏度
运行成本低

Nature Biotechnology 2008

高通量测序可以做什么

基因组学研究:

DNA-seq

（全基因组测序、外显子测序）

功能基因组学:

RNA-seq

(mRNA, Non-Coding RNA, small RNA)

CHIP-seq

……

文章发表情况

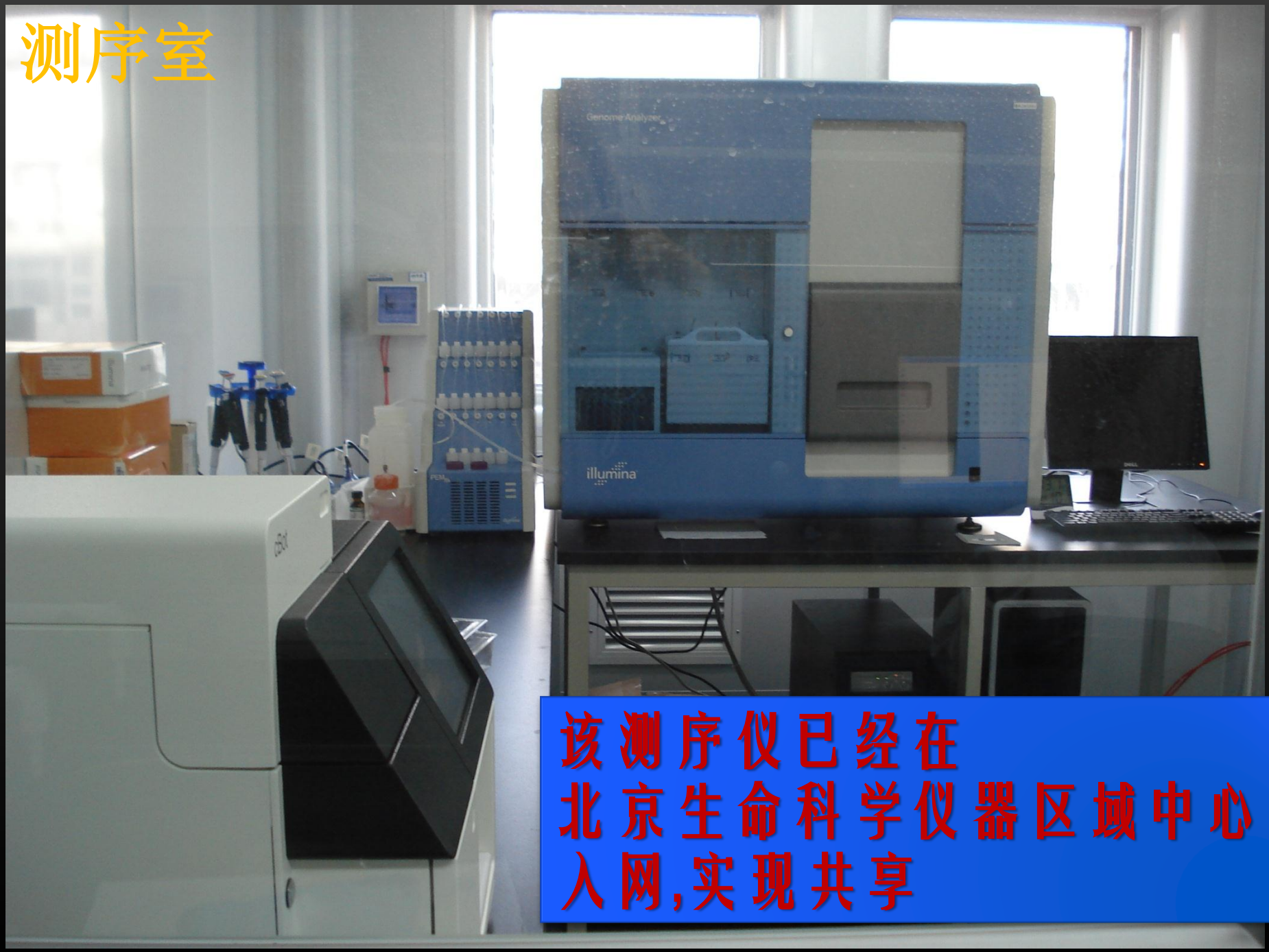
publications: sequencing applications	Sum	2009	2008	2007	2006	2005	2004
ChIP-Sequencing / Protein-Nucleic Acid Interactions	69	36	26	7	0	0	0
De Novo Sequencing	5	4	0	1	0	0	0
DNA Methylation Analysis	23	15	8	0	0	0	0
Histone Analysis	6	5	1	0	0	0	0
Metagenomics	3	2	1	0	0	0	0
Method	29	18	10	1	0	0	0
Targeted Resequencing	13	12	1	0	0	0	0
Transcriptomics - mRNA	57	35	22	0	0	0	0
Transcriptomics - Non-Coding RNA	80	22	51	6	1	0	0
Whole-Genome Resequencing	112	43	50	13	3	1	2

临床上的应用----产前检查等

illumina 官网	文章数
sequencing	1833
RNA sequencing	661
DNA sequencing	702
miRNA sequencing	123
NCBI	文章数
illumina sequencing	790
illumina DNA sequencing	676
illumina RNA sequencing	350
next generation sequencing	2595
illumina genome sequencing	580

第二代测序仪中，illumina公司的测序仪应用的最为广泛。

第二代高通量测序仪的实验流程


测序室
illumina
该测序仪已经在
北京生命科学仪器区域中心
入网,实现共享

1

样品制备

2

生成
分子簇

3

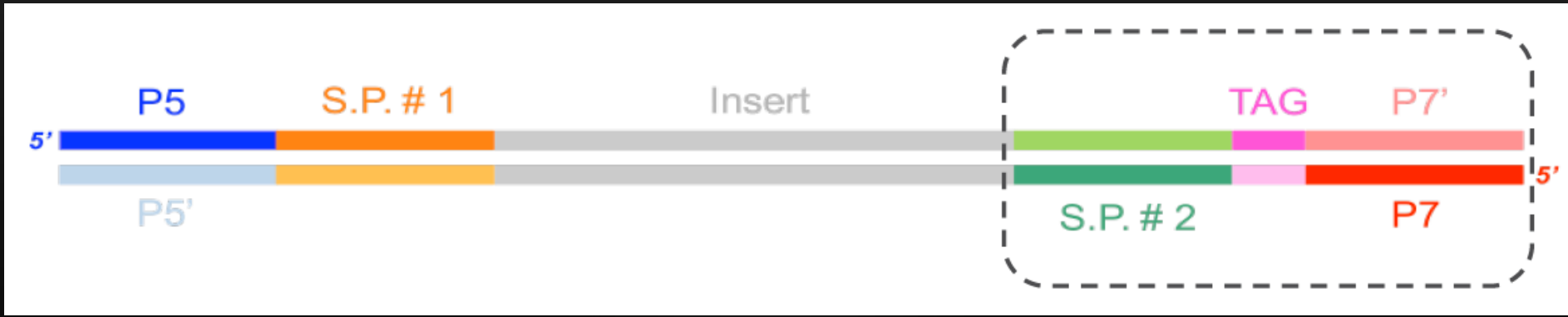
测序

4

数据分析

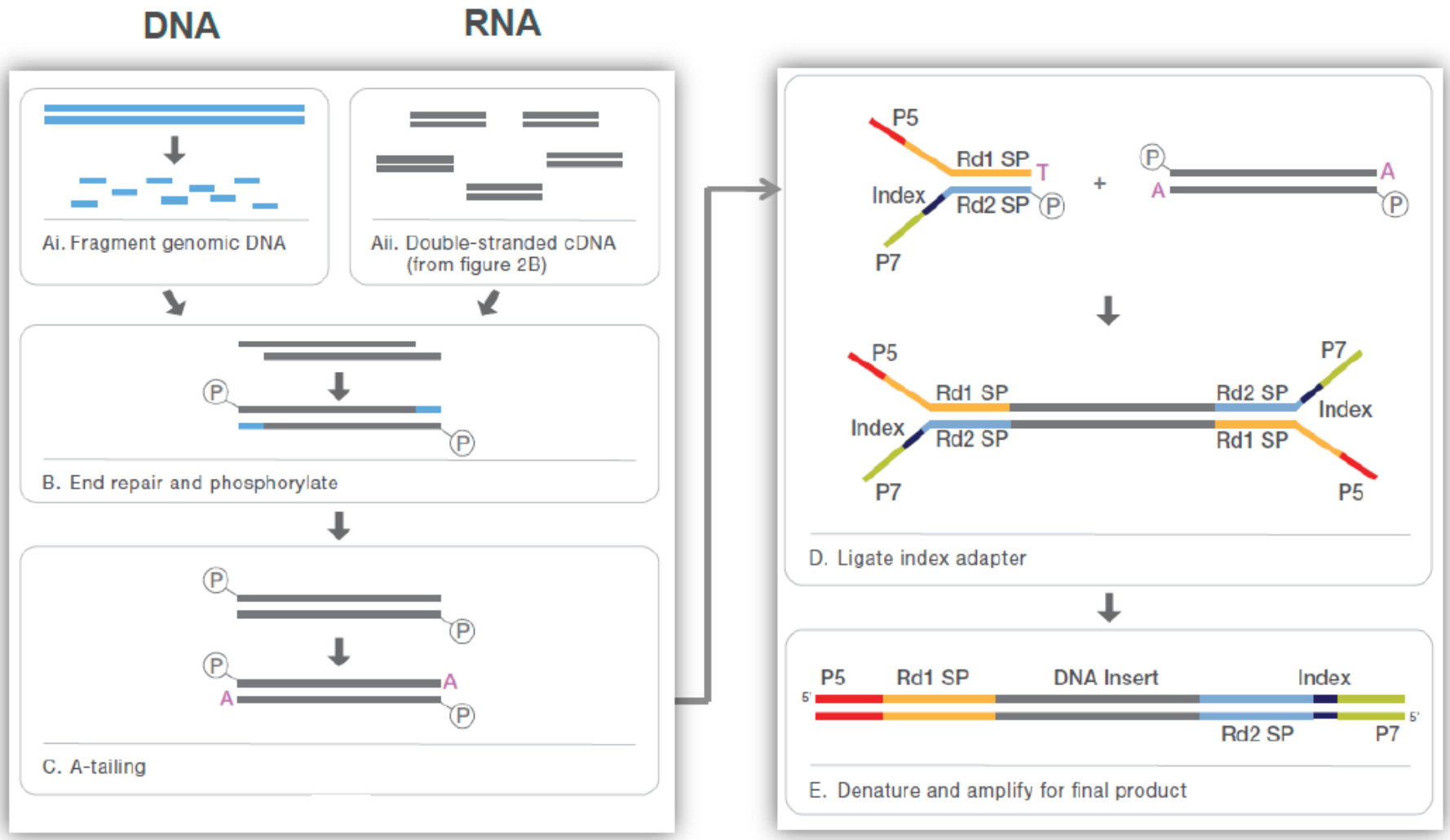
样品制备

- DNA 样品制备
- mRNA 样品制备
- Small RNA样品制备
- CHIP-seq 样品制备
- Mate Pair 样品制备
- ⋮

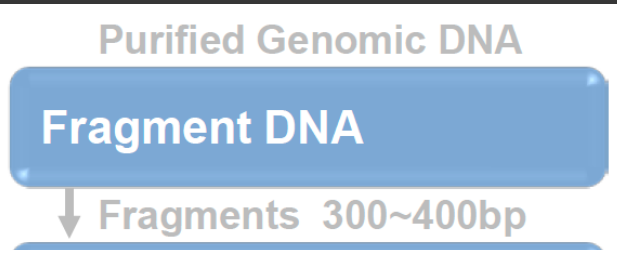


分子库构建关键步骤

Truseq adapter



DNA样本制备



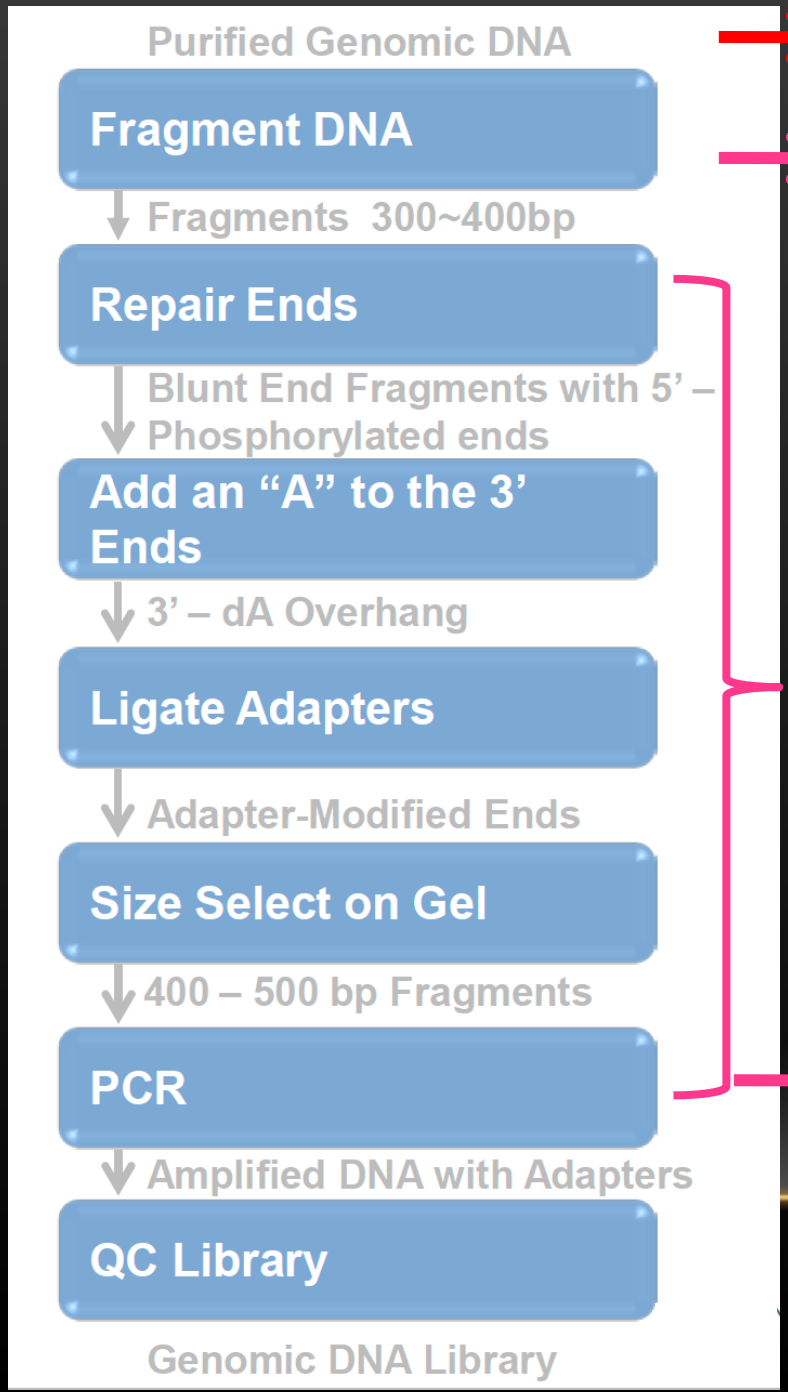
非常重要,实验成败的关键

样品总量>10μg

260/280值：1.8~2.0之间

	Sample Submission		
Application	**Absolute Minimum Concentration**	**Input Requirements**	**Buffer**
DGE (Digital Gene Expression): *Nla*III or *Dpn*II Tag Profiling	RNA: 500ng/ul	Minimum of 10 uL at 500ng/ul (5ug minimum)	DEPC water or appropriate kit buffer
DGE: smallRNA	RNA: 500ng/ul	Minimum of 40uL at 500ng/ul (20ug min)	DEPC water or appropriate kit buffer
mRNA-Seq (aka Full Length cDNA Sequencing)	RNA: 500ng/ul	Minimum of 40 uL at 500ng/ul (20ug min)	DEPC water or appropriate kit buffer
ChIP-Seq	DNA: 2ng/ul quanted using PicoGreen®	Minimum of 10 uL at 2ng/ul (20ng min)	Buffer (TE)
Genomic Resequencing	DNA: 500ng/ul	Minimum of 10 uL at 500ng/ul (5ug minimum)	Buffer (TE)
Paired End Sequencing	DNA: 500ng/ul	Minimum of 10 uL at 500ng/ul (5ug minimum)	Buffer (TE)

100ng/ μl



非常重要,实验成败的关键

超声破碎仪

Illumina :

TruSeq DNA Sample Preparation Kit 试剂，Adapter (含有index)

NEB : NEBNext™ DNA 样品准备试剂 试剂

……

Cycle数：10~12　最大不能超过15个

RNA样本制备

Total RNA 的质量：260/280 1.8-2.2
28S:18S ≥ 1
RIN >8

纯化mRNA
- 选择mRNA
- 去除核糖体RNA（原核生物）

mRNA片段化

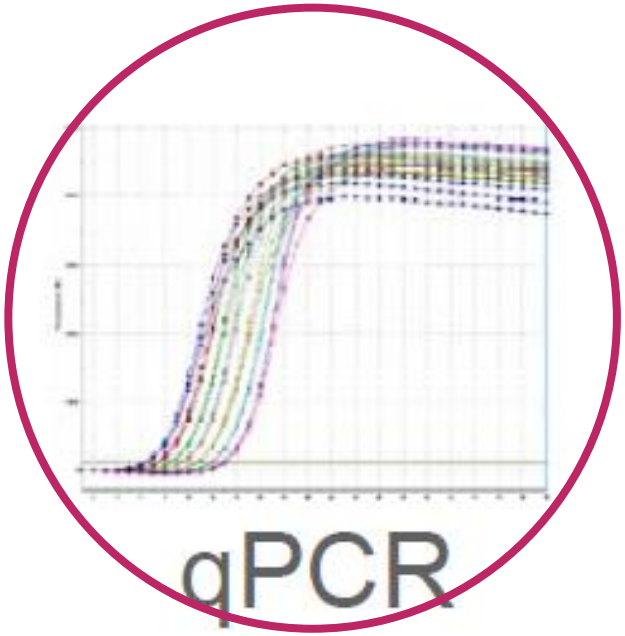
反转录
- Random primer → 全转录组测序（EP100bp）
- Oligo dT → 检测表达量（SR36bp）

与DNA建库方法相同

分子库的质控

- Quantitate by NanoDrop or Qubit
  - NanoDrop can overestimate concentration
  - Qubit or PicoGreen gives more accurate measurement

Qubit™



qPCR

分子簇(Cluster)生成

样品制备

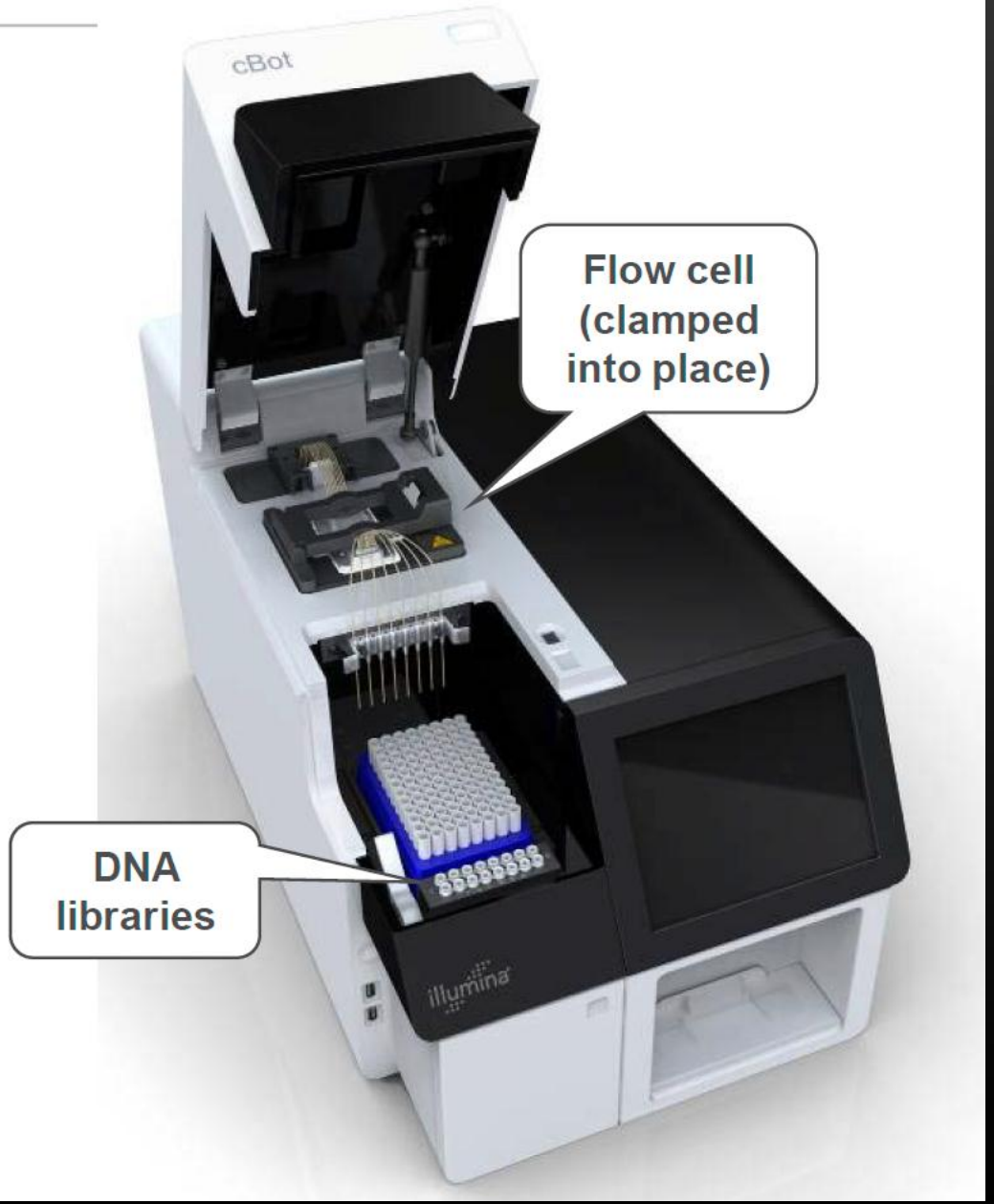
生成
分子簇

测序

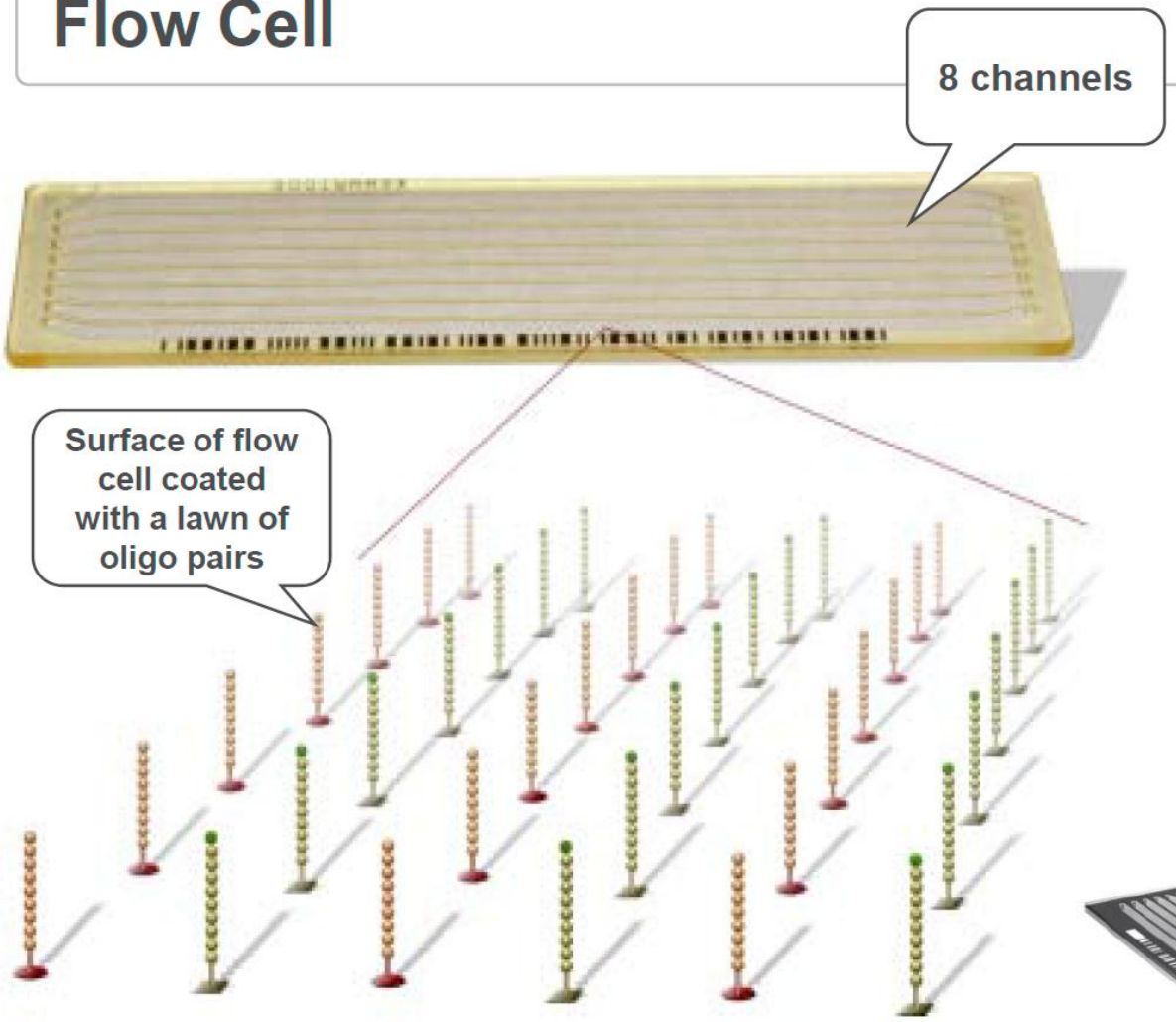
数据分析

cBot

- Aspirates DNA samples into flow cell
- Automates the formation of amplified clonal clusters from the DNA single molecules



Flow Cell



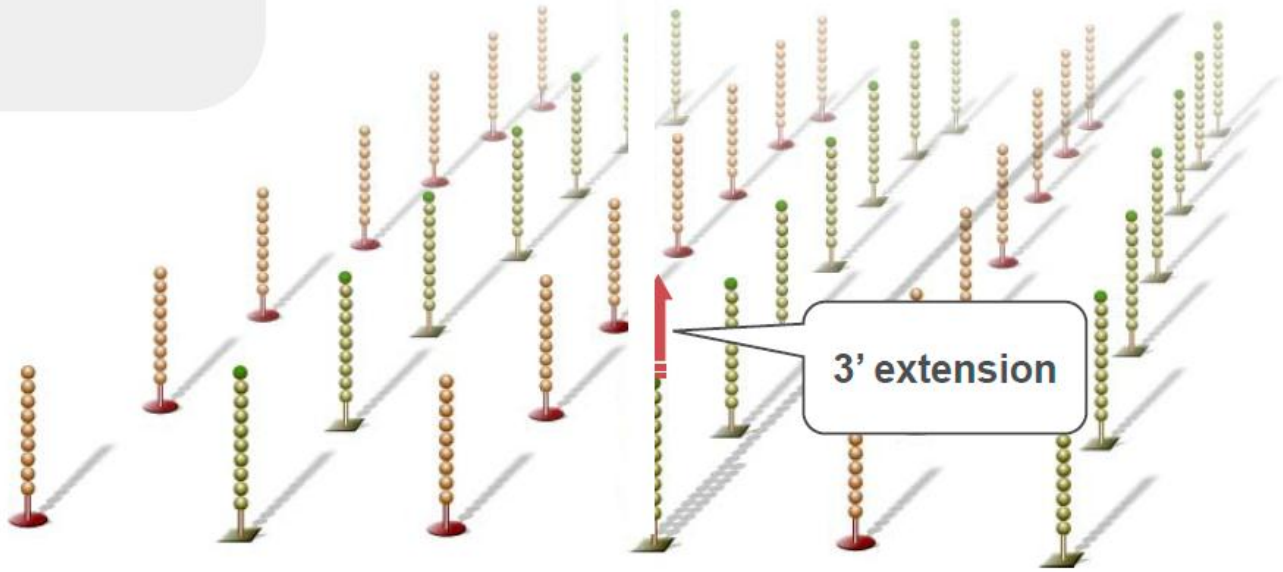
Key to the simplified workflow

- Clonal clusters are generated in a contained environment (need no clean rooms)
- Sequencing also performed in the flow cell on the generated clusters

Cluster generation: Hybridize fragment & extend

Single molecules hybridize to the lawn of primers

Bound molecules are then extended by polymerases

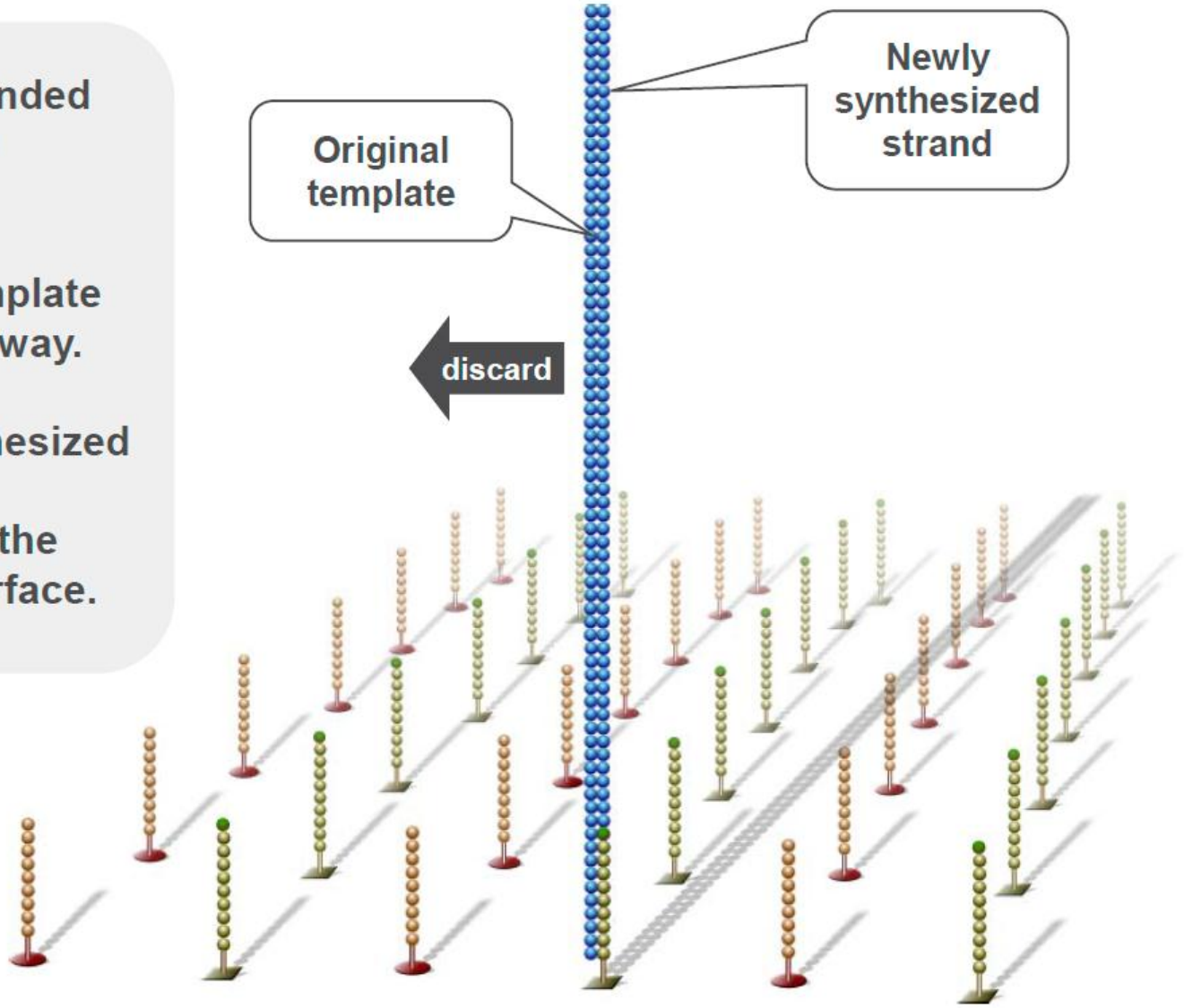


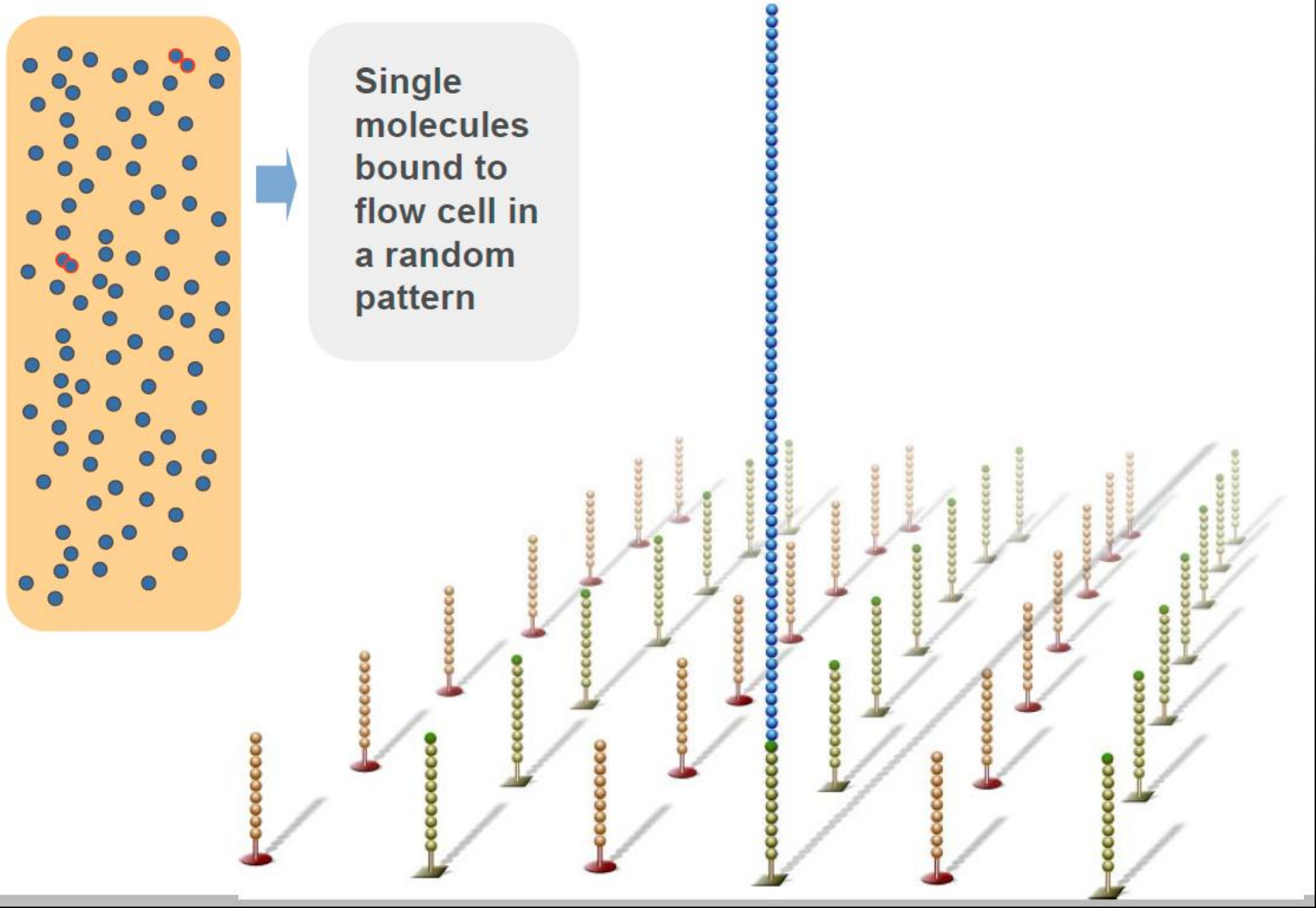
Cluster generation:
Denature double-stranded DNA

Double-stranded molecule is denatured.

Original template is washed away.

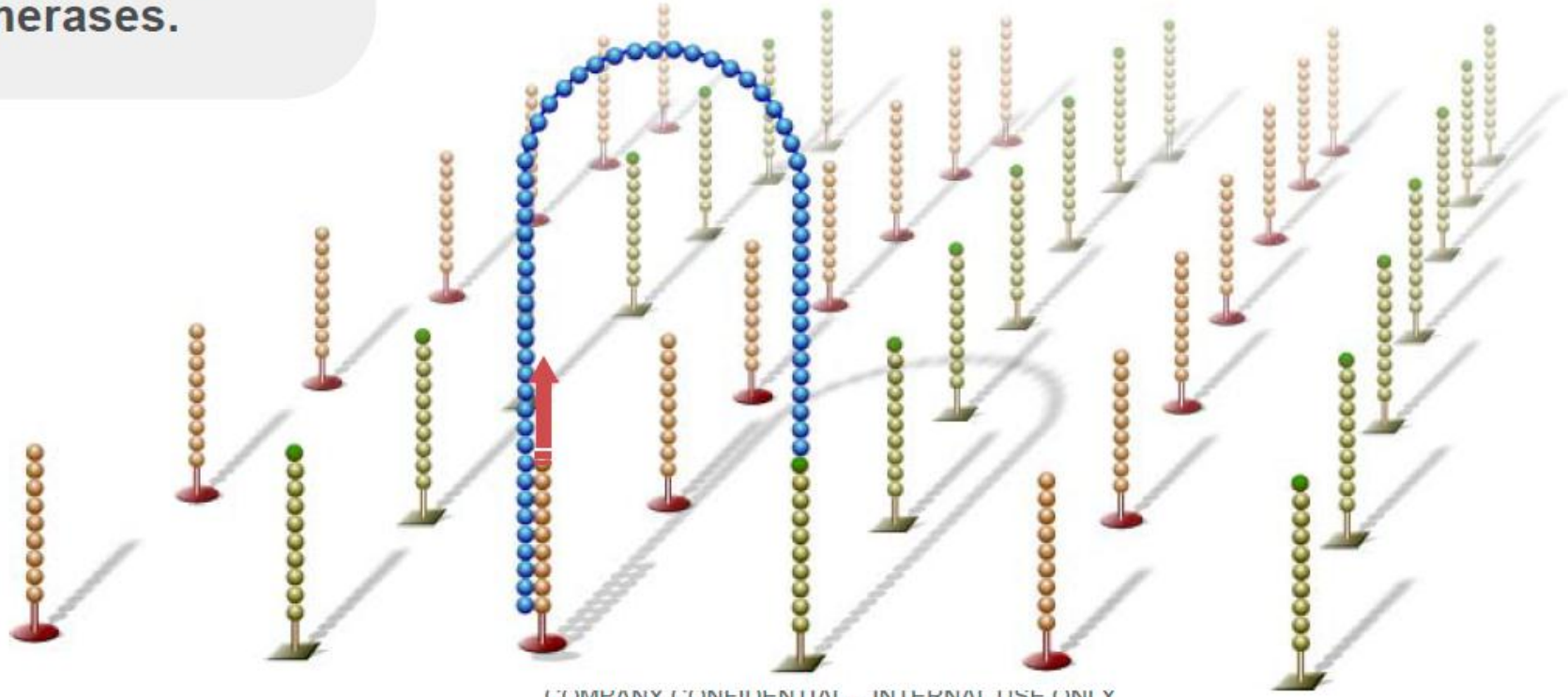
Newly synthesized covalently attached to the flow cell surface.



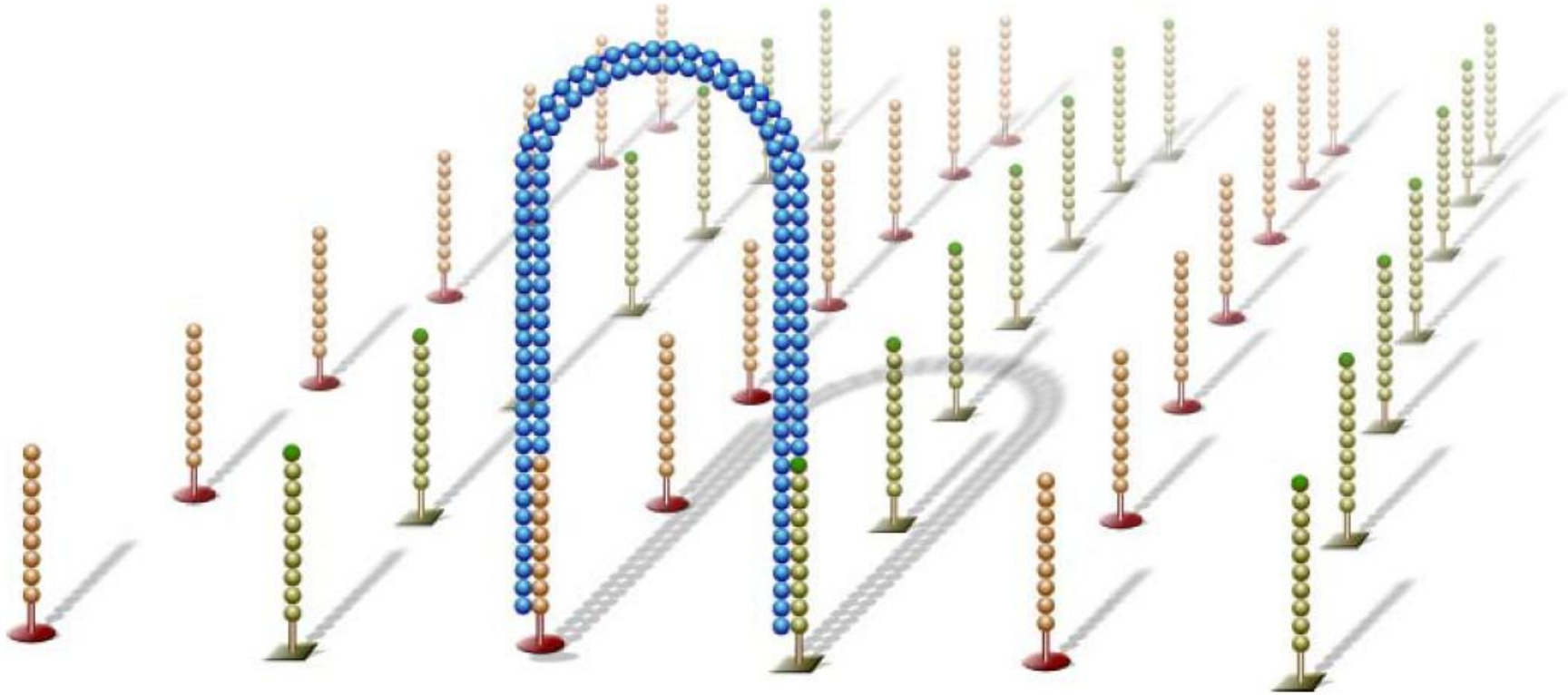

Single molecules bound to flow cell in a random pattern

Single-strand flips over to hybridize to adjacent primers to form a bridge.

Hybridized primer is extended by polymerases.

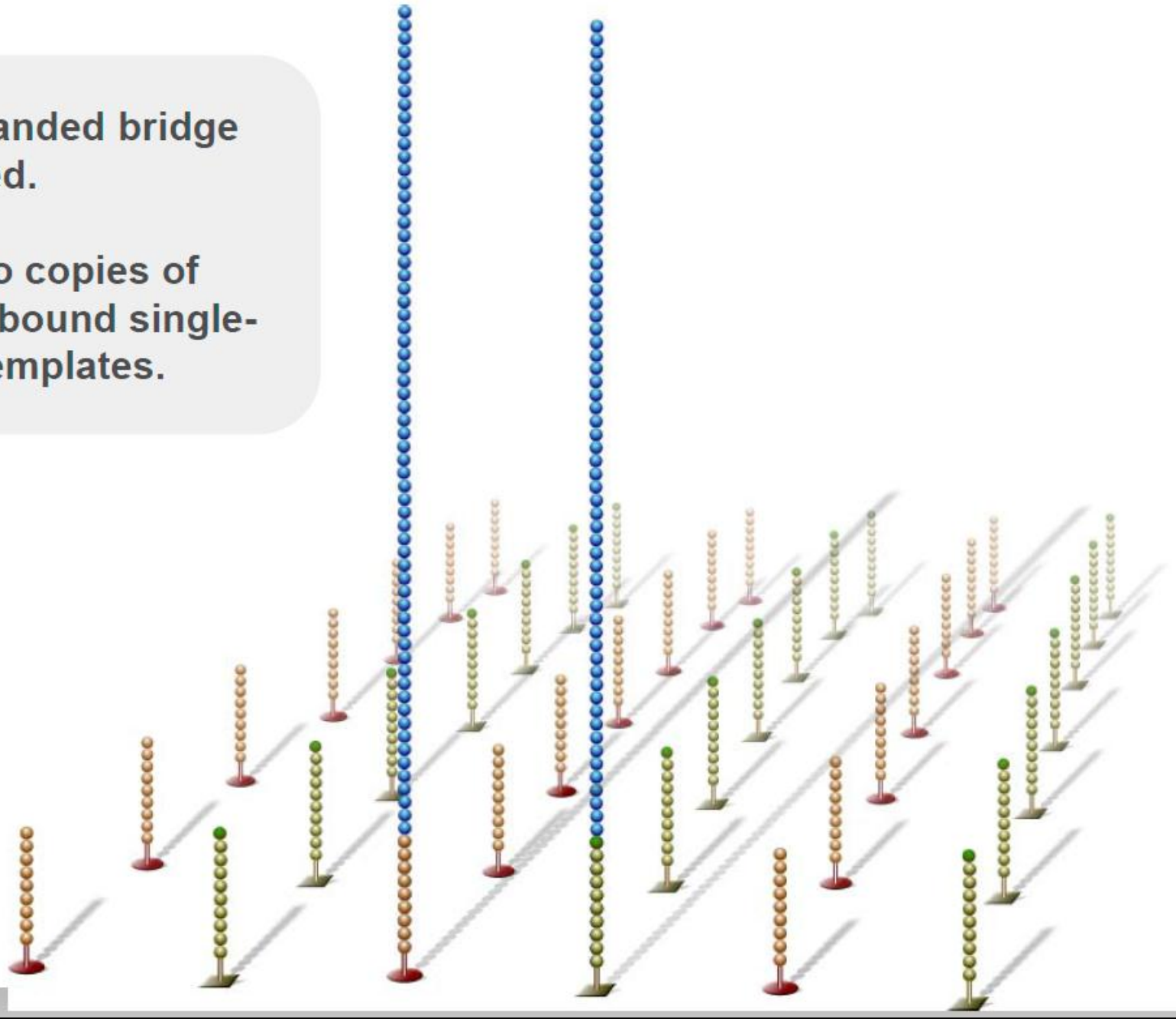


→ double-stranded bridge is formed.



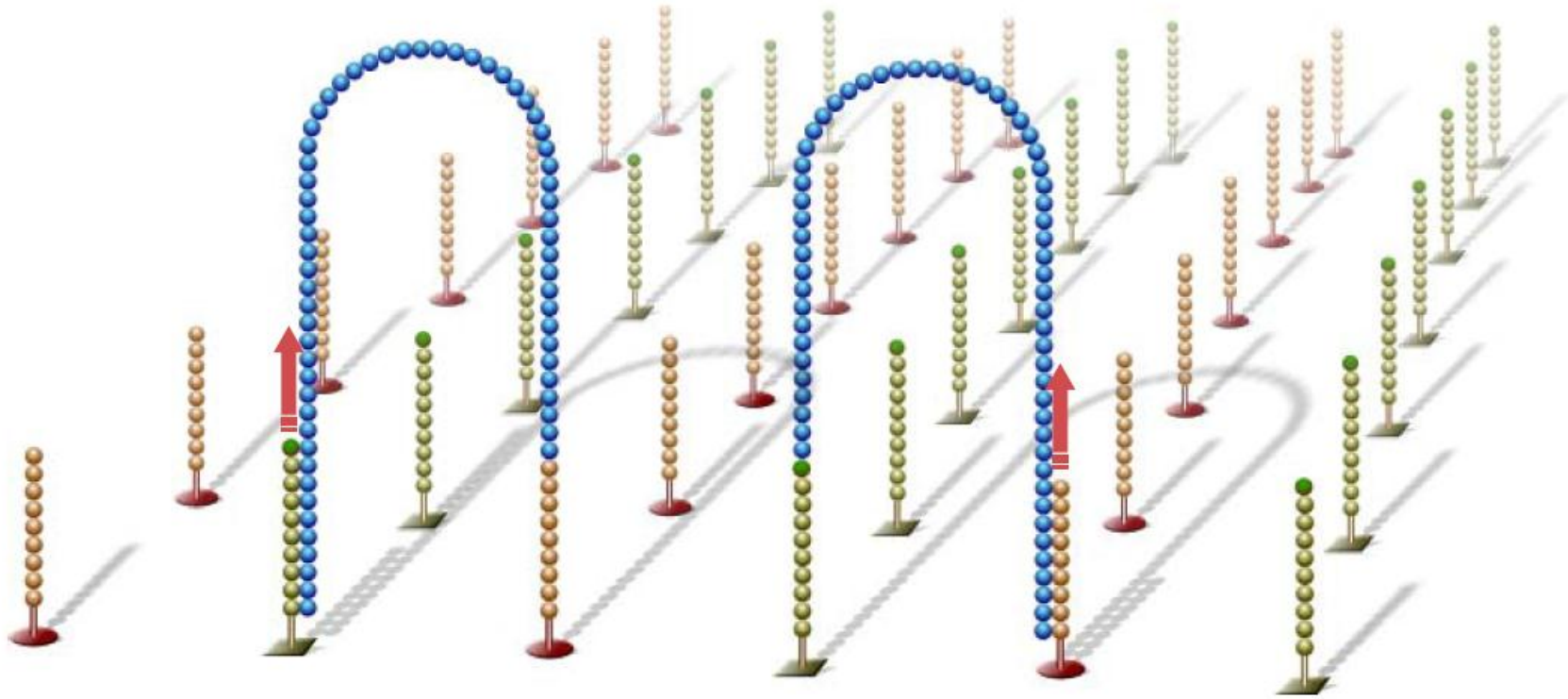
Double-stranded bridge is denatured.

Result: Two copies of covalently bound single-stranded templates.

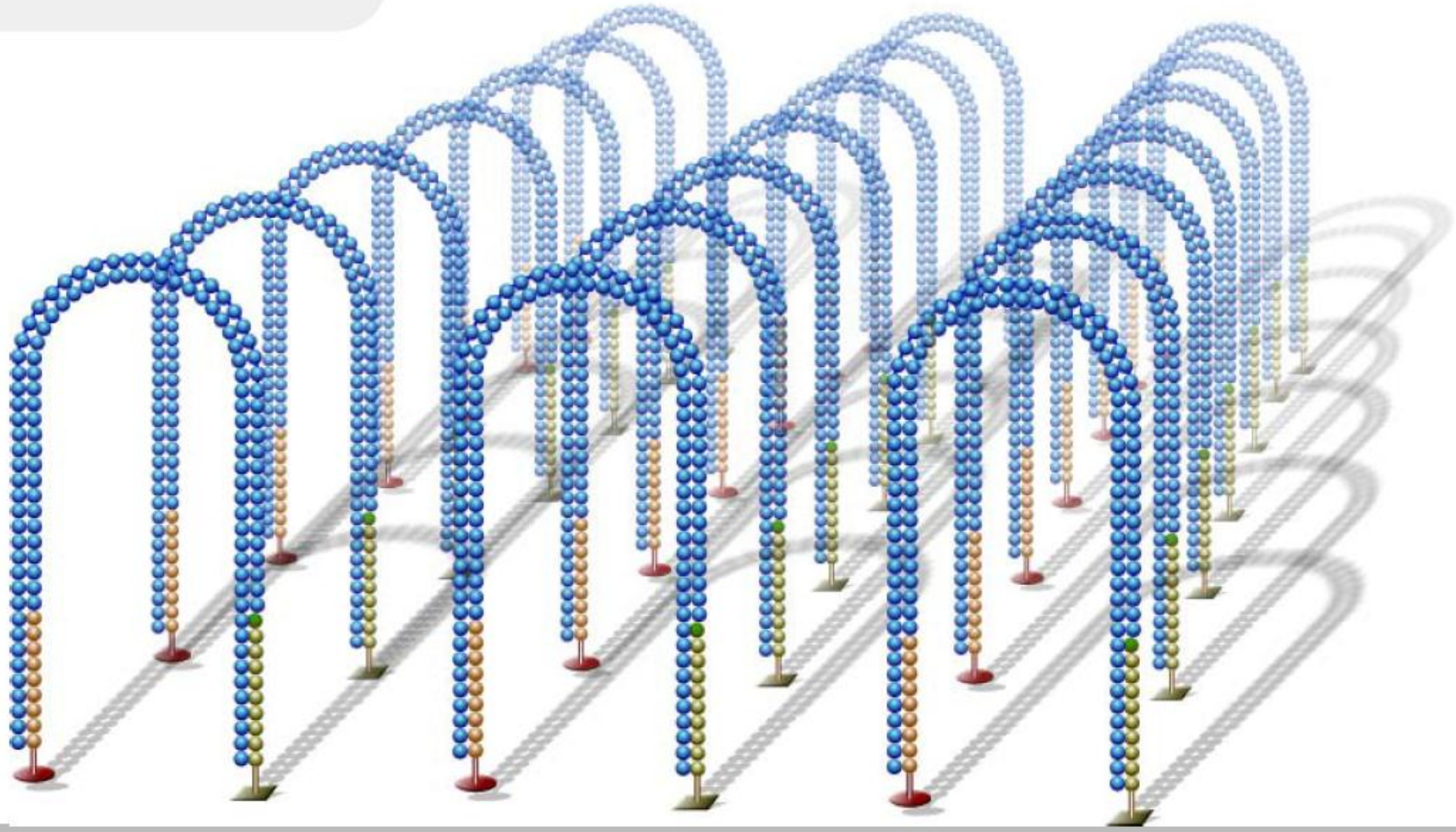


Single-strands flip over to hybridize to adjacent primers to form bridges.

Hybridized primer is extended by polymerase.

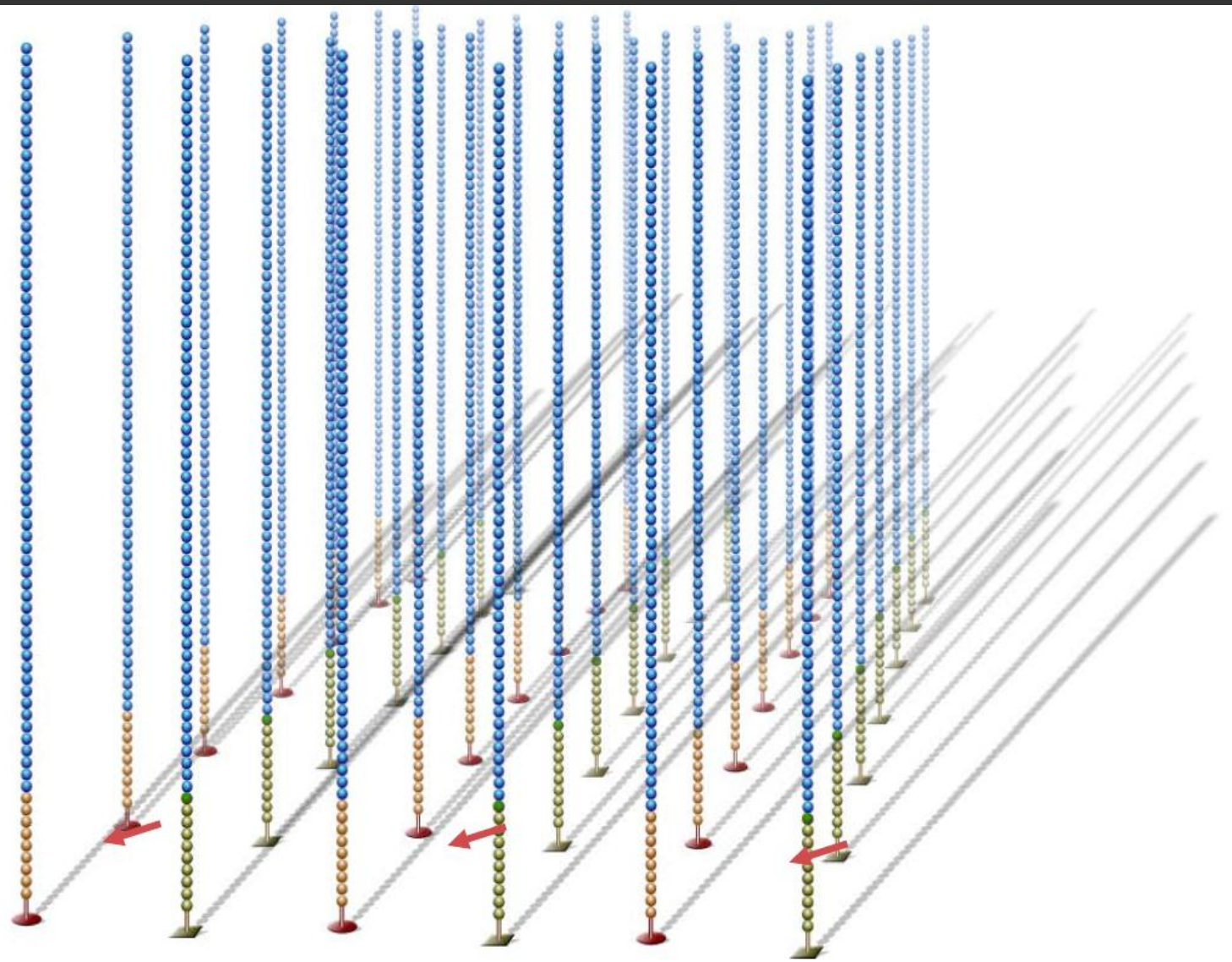


Bridge amplification cycle repeated till multiple bridges are formed

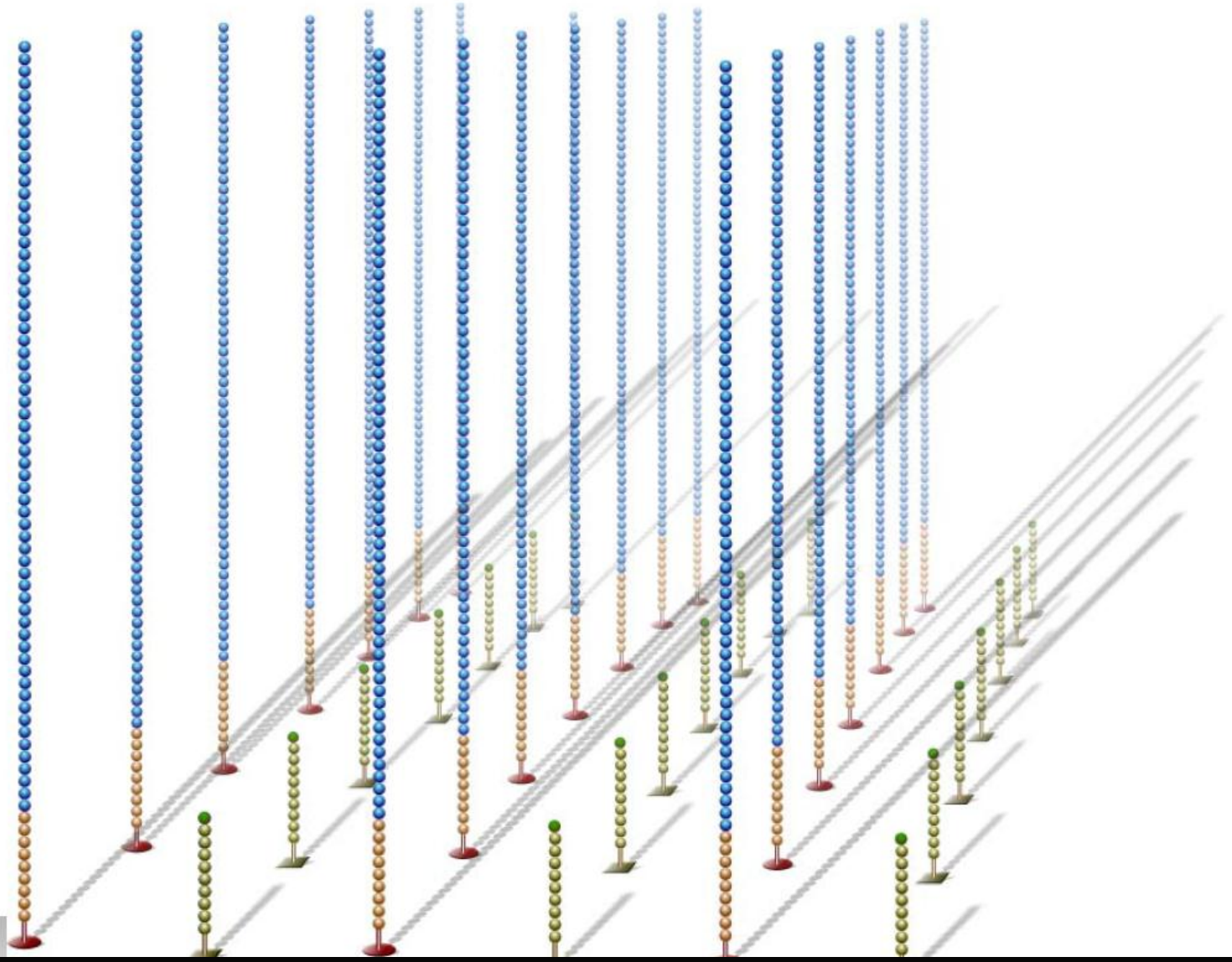


dsDNA bridges denatured.

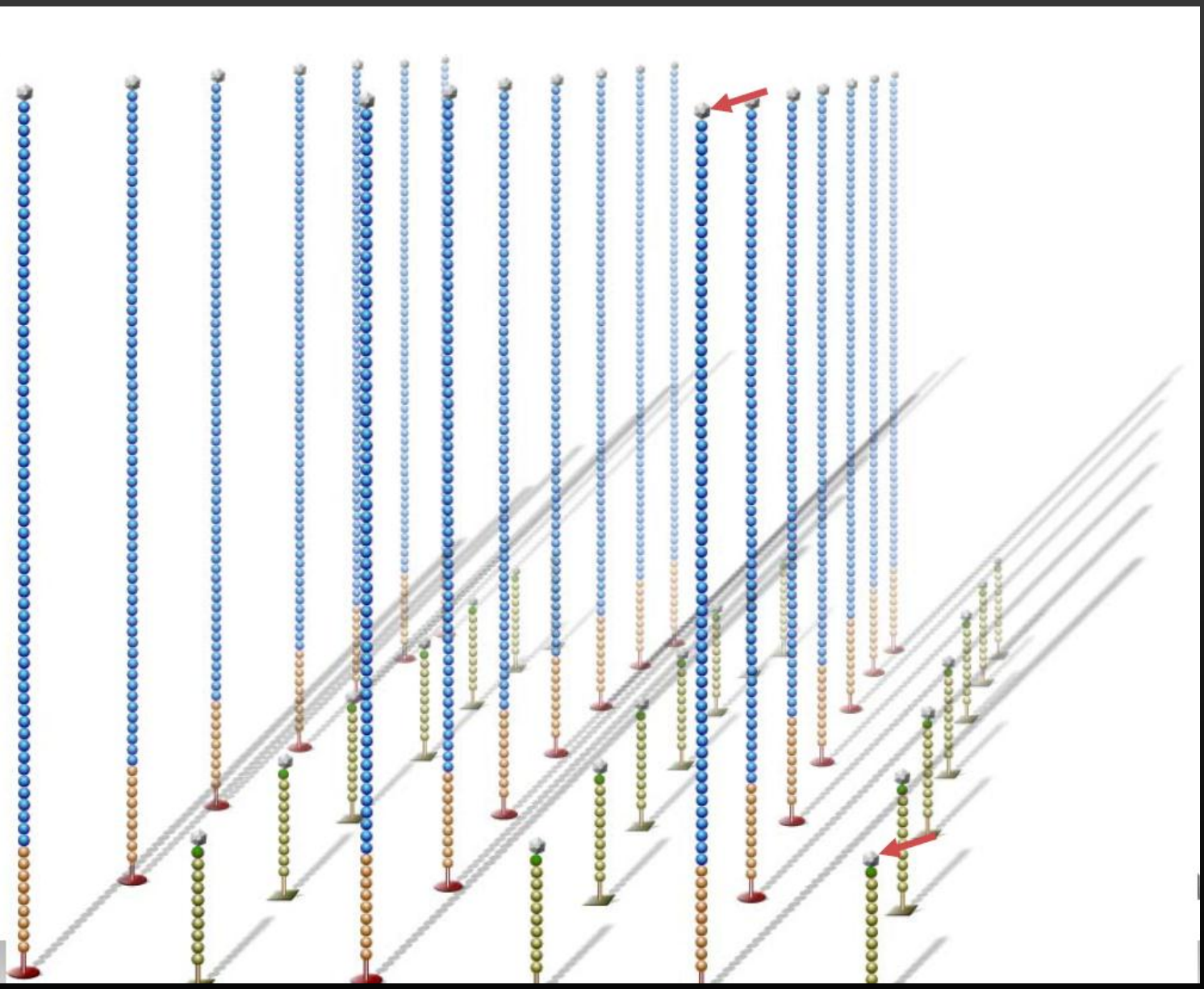
Reverse strands cleaved and washed away.



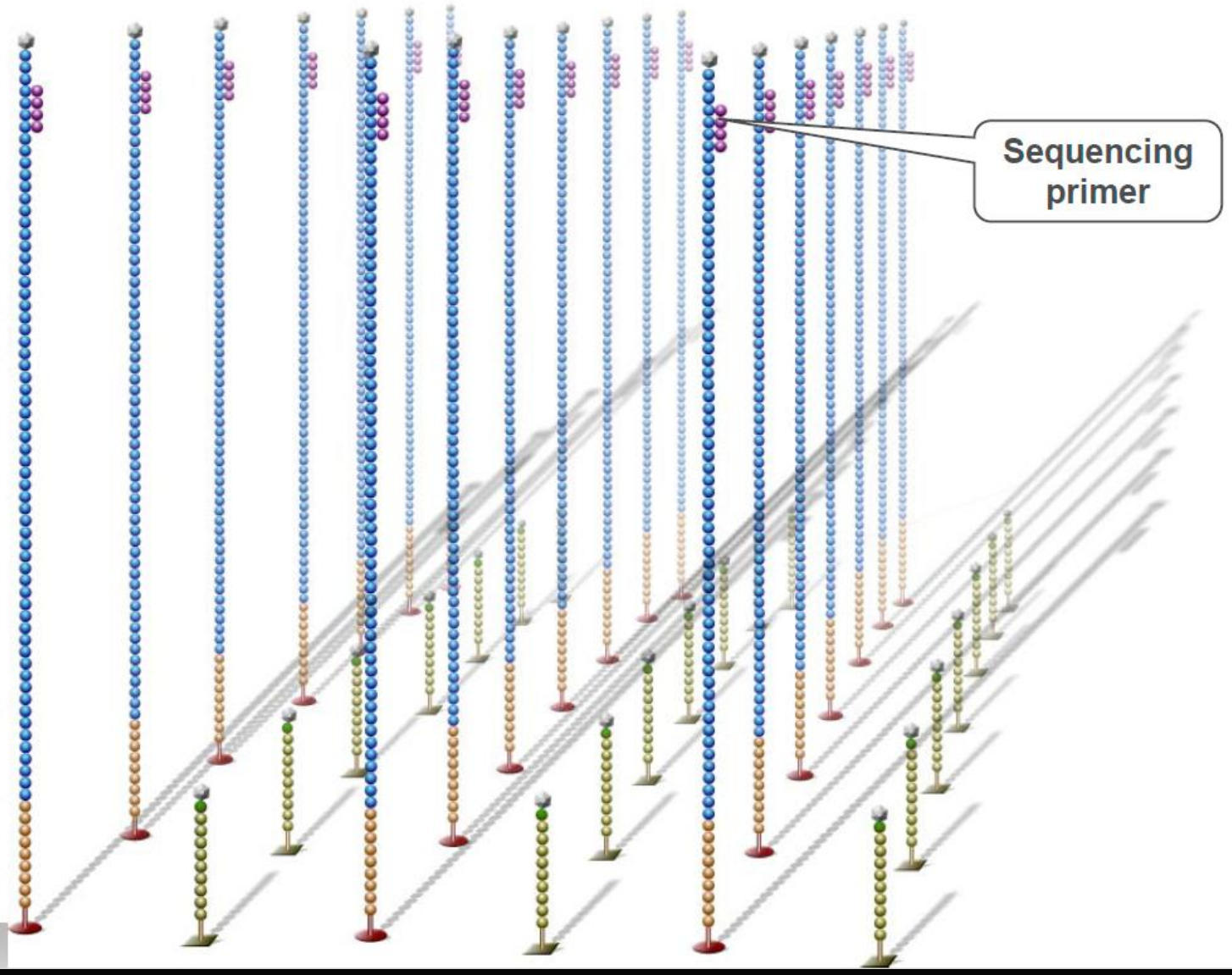
… leaving a cluster with forward strands only.



Free 3’ ends are blocked to prevent unwanted DNA priming.



Sequencing primer is hybridized to adapter sequence.



测 序

1

样品制备

2

生成
分子簇

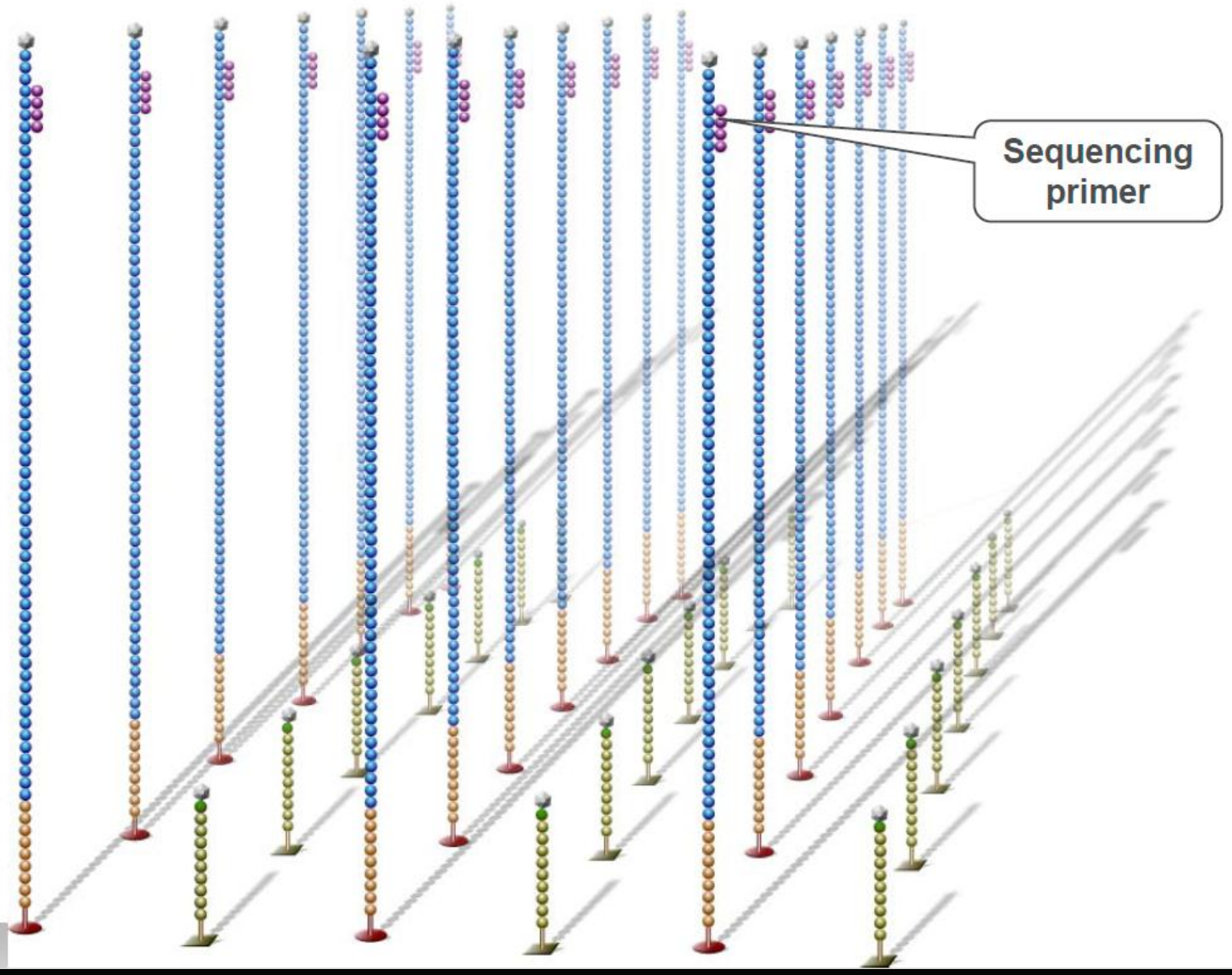
3

测序

4

数据分析

Sequencing primer is hybridized to adapter sequence.



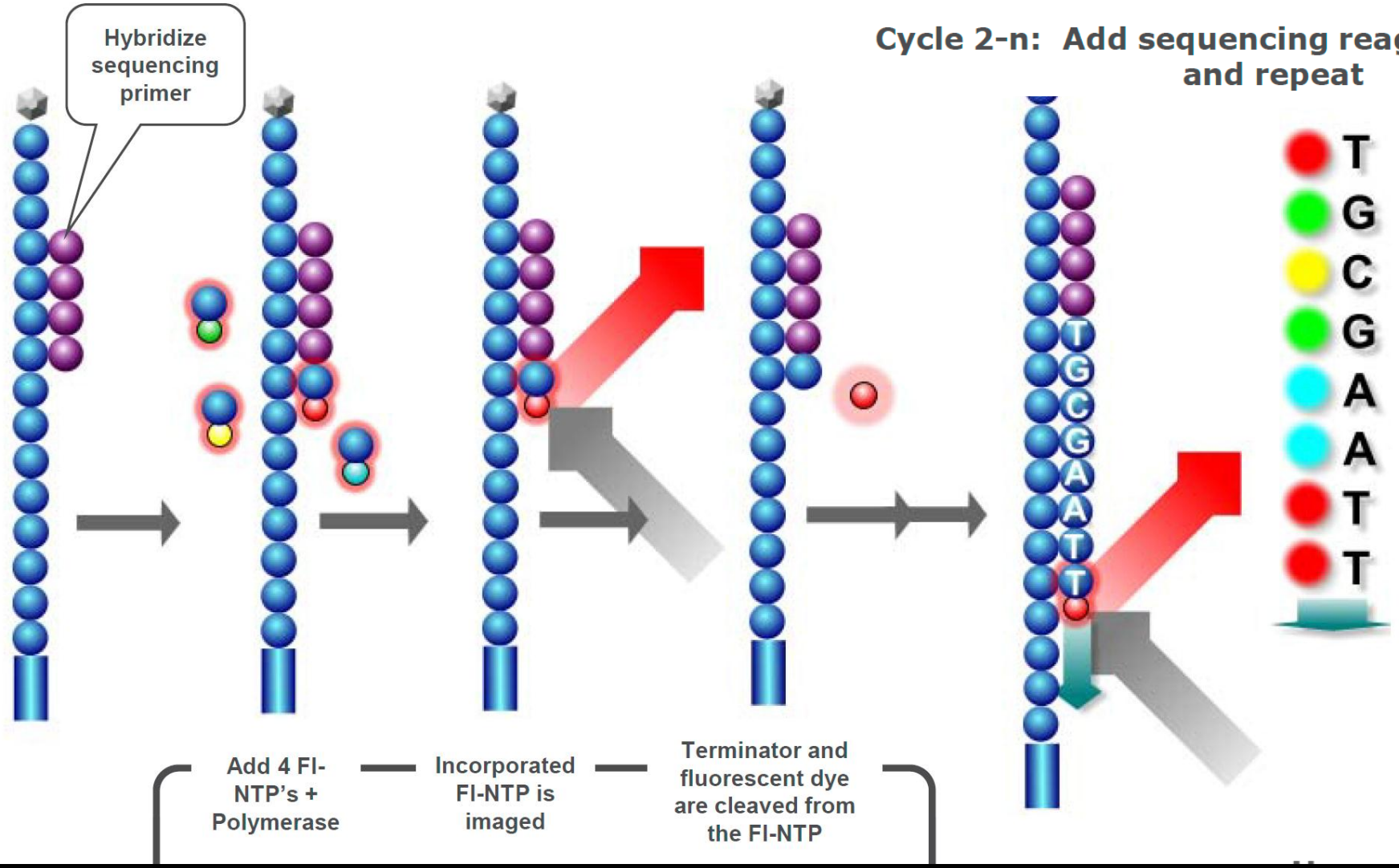
Cycle 1: Add sequencing reagents

First base incorporated

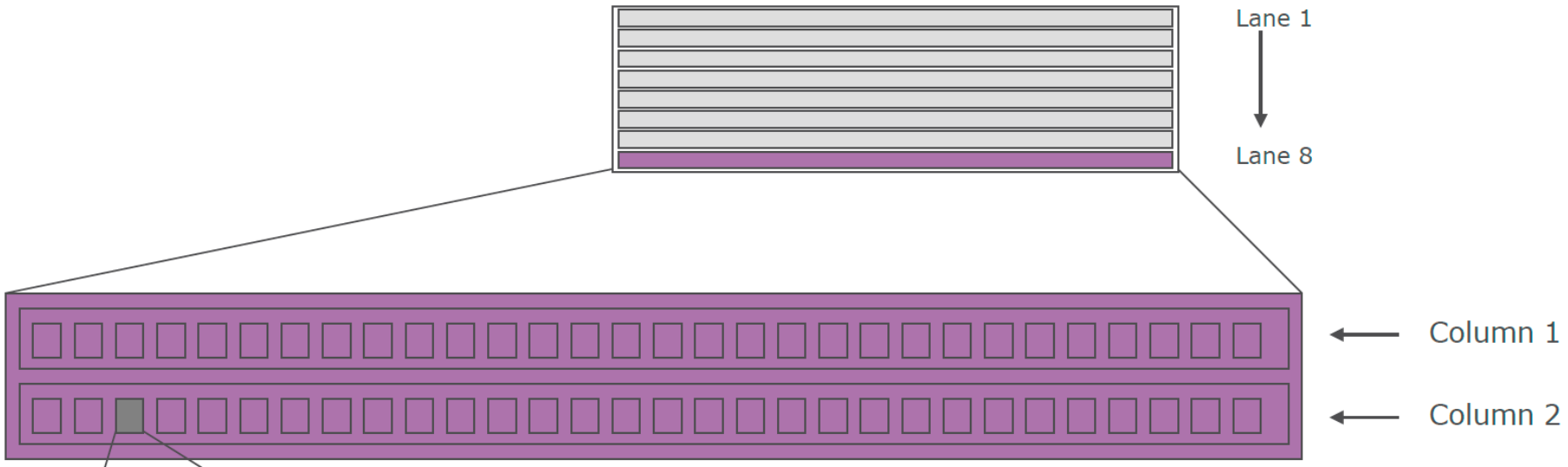
Detect Signal

Cleave Terminator and Dye

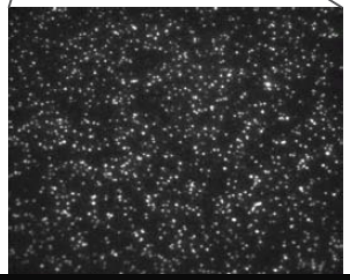
Cycle 2-n: Add sequencing reagents and repeat



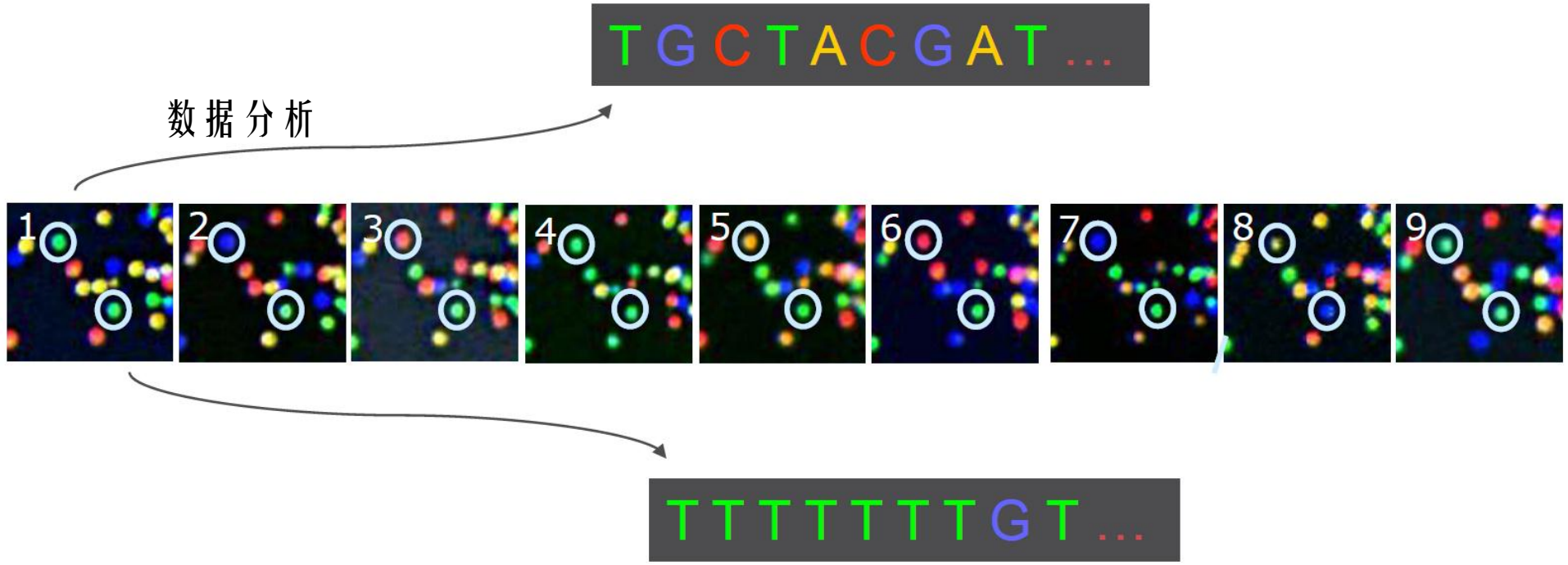
A **flowcell** contains eight lanes



Each **column** contains **multiple tiles** – total 120



- There are 8 lanes per flow cell
- One lane contains 2 columns
- One column contains 120 tiles
- Each tile is imaged four times per cycle – one image per base.


TGCTACGAT...
数据分析
1
2
3
4
5
6
7
8
9
TTTTTTTGT...

自己测序的好处

- 价格优势

	测序公司	研究所
测序仪	√	✗
超算（大型计算机）	√	✗
试剂费用	√	○
人员费用	√	○
税费	√	✗

6000元/Gb

- 人员优势
  （可以共同讨论实验结果及分析方法）
- 时间优势

谢 谢！

优点

1. 可扩展的超高通量

量还有望上升到95 GB，相当于人类基因组的30 倍覆盖度。

2. 需要样品量少

Genome Analyzer 系统需要的样品量低至100ng，能应用在很多样品有限的实验

3. 简单、快速、自动化

4. 新颖的测序化学技术

Genome Analyzer 通过合成测序来支持大规模并行测序。

利用新颖的可逆荧光标记终止子,可以在DNA 链延伸的过程中检测单个碱基掺入。

由于四个可逆终止子dNTP 在每个测序循环都存在，自然的竞争减少了掺入的误差。

5. 单个或配对末端支持

6. 相对于其它高通量测序仪它的成本更低

不足

测序片段长度有待增加

前期对样本的要求比较高，分子库构建程序比较复杂

KAPA Library Quant Kits

不用自备标准品

适用平台:

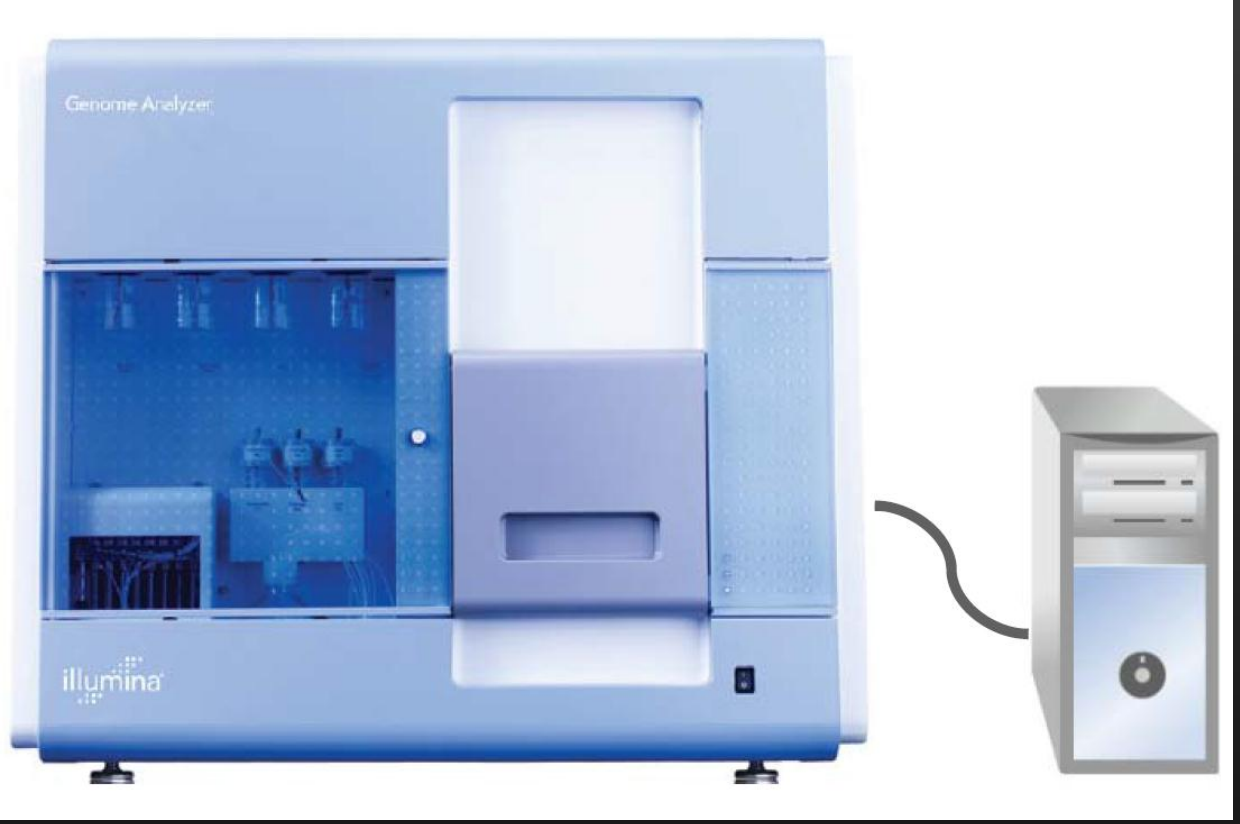
1、Illumina Genome Analyzer

2、Roche 454 GS Titanium and FLX

3、ABI SOLiD

KAPA SYBR FAST qPCR试剂盒优点:

1、反应效率高;
2、可扩增AT/GC含量高的模板;
3、高灵敏度和线性特征;
4、动态范围宽
5、重复性强;
6、速度快，且灵敏度与效率高。


Genome Analyzer
illumina