



TỔNG QUAN VỀ CÔNG NGHỆ VÀ HỆ THỐNG GIẢI TRÌNH TỰ ILLUMINA

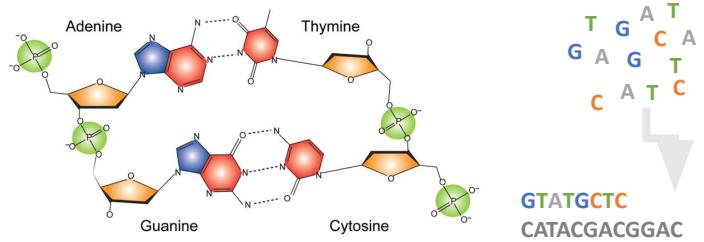
*Nguyễn Thị Hồng Nhung
MBA.Nguyễn Thuỳ Quốc Hương*

What is Next-Generation Sequencing?





The Ability to Read the Genetic Code

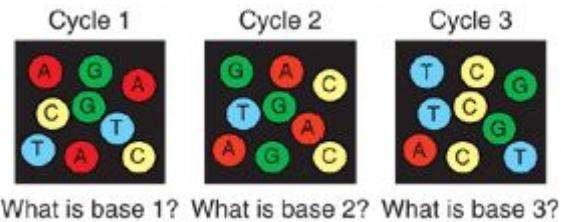
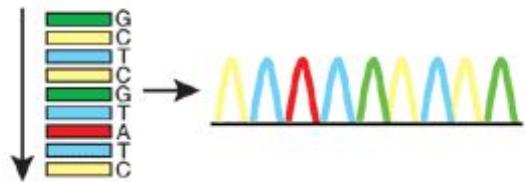


Basics of DNA sequencing

- To be able to read the sequence of DNA (or letters) requires the ability to distinguish between the different nucleotides
- Various methods are available:
 - Some recognize size and shape
 - The majority use pre-labelled fluorescent nucleotides that only bind to their complementary partner

Sanger-based electrophoresis (1977)

- 1 read / capillary
- 96 capillaries / run
- 1.2Mb / day



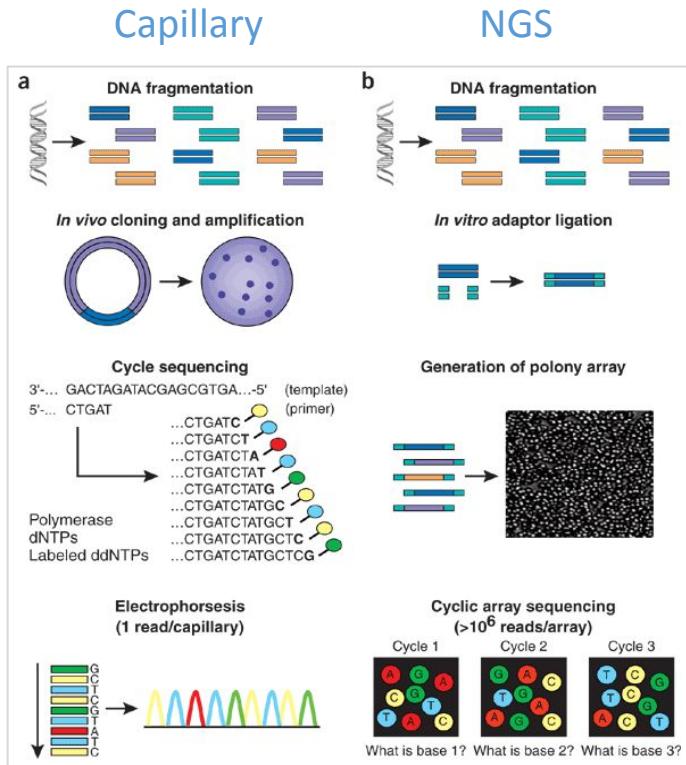
NGS Cyclic reads (2007)

- 4M-20B reads / flow cell
- ~1500Gb / day

Nucleic acids research 36, 3978–92 (2008)
Nature Biotechnology 26, 1135–1145 (2008), Published online: 9 October 2008



Next-Generation Sequencing



2016 capacity: $10^6 - 10^9$ reads/array

Shendure, J. et al. Next-generation DNA sequencing. *Nature Biotechnology* 26 1135 – 1145 (2008)
<http://www.illumina.com/systems/sequencing.html>



Next-Generation Sequencing



**Massively Parallel Sequencing
>100x-1000x**



```
AAAACCAGAGTCTAGCACCTTCTCATCAGGAGCA
AAACCGAGGTCTAGCACCTTCTCATCAGGAGCA
AACCGAGGTCTAGCACCTTCTCATCAGGAGCG
ACCGAGGTCTAGCACCTTCTCATCAGGAGGT
ACCGAGGTCTAGCACCTTCTCATCAGGAGCGT
CCAGAGTCTAGCACCTTCTCATCAGGAGGTC
GAGTCTAGCACCTTCTCATCAGGAGGTCTGC
CTAGCACCTTCTCATCAGGAGCTCTGCCTTC
TAGCACCTTCTCATCAGGAGCTCTGCCTTC
AGCACCTTCTCATCAGGAGCTCTGCCTTC
CCTTCTCATCAGGAGCTCTGCCTTC
ACCTTCTCATCAGGAGCTCTGCCTTC
CTTCTCATCAGGAGCTCTGCCTTC
ATCAGGAGCCGTCTGCCTTC
ATCAGGAGCCGTCTGCCTTC
TCAGGAGCCGTCTGCCTTC
GAGCACGTCTGCCTTC
GAGCACGTCTGCCTTC
AACGTCTGCCTTC
AACGTCTGCCTTC
AACGTCTGCCTTC
AACGTCTGCCTTC
```

AAAACCAGAGTCTAGCACCTTCTCATCAGGA**GCA****TCTGCCTTC****GCTAGGCTGACATCGCGGGACC**

NGS provides “digital” data, each read is analyzed independently and is quantitative

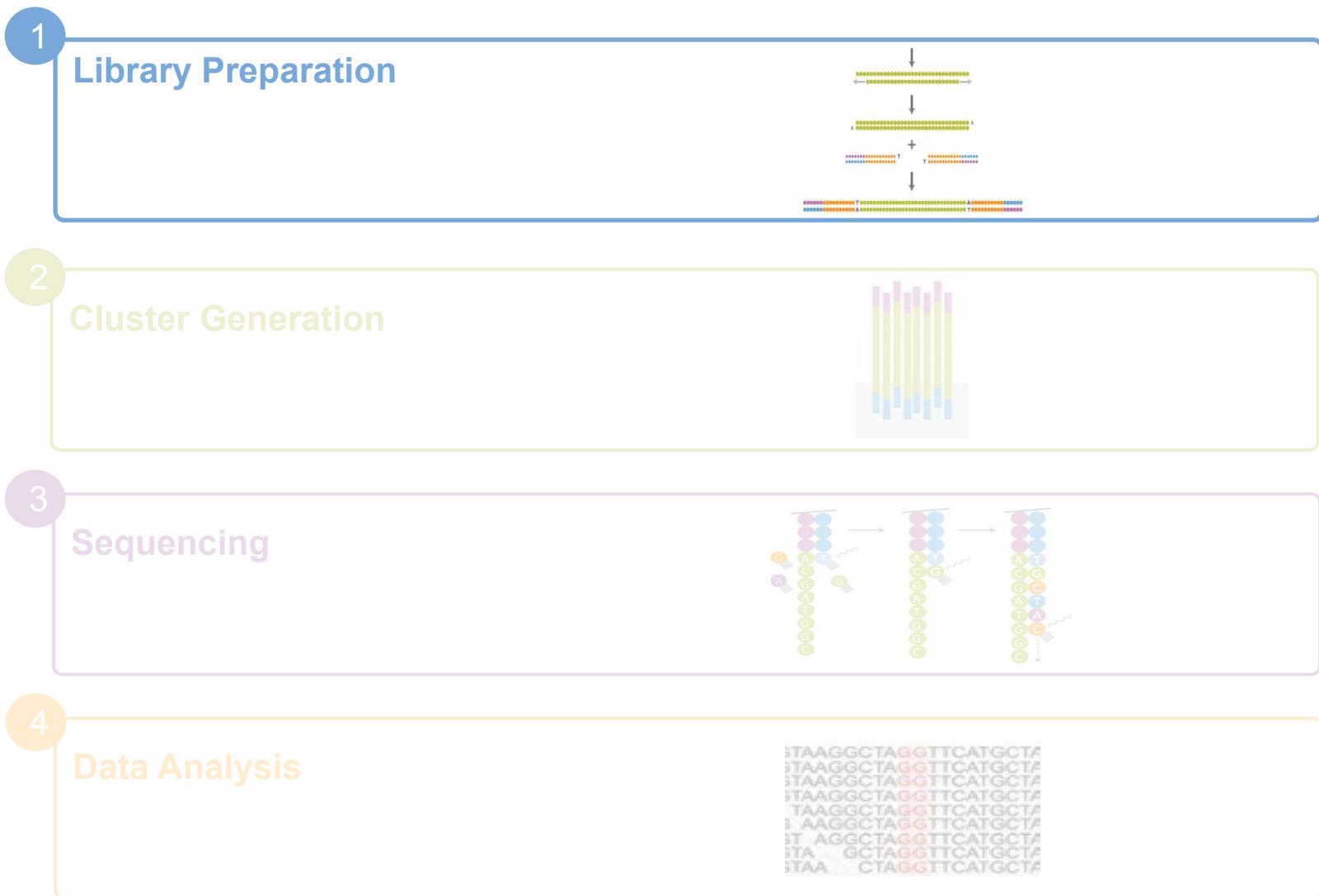
Overlapping reads are aligned together, resulting in quantitative and high confidence variant calling

Illumina Sequencing Workflow

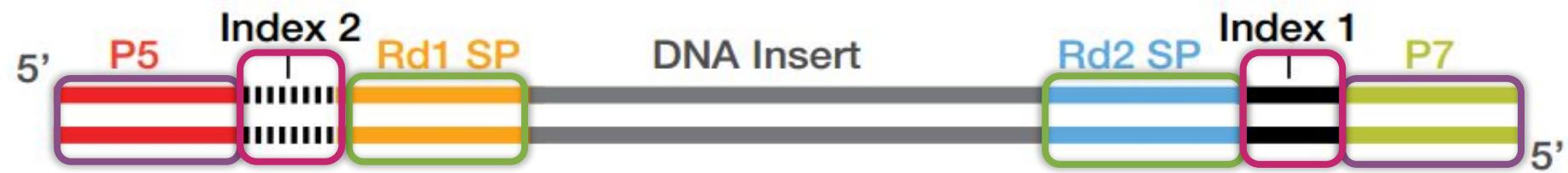
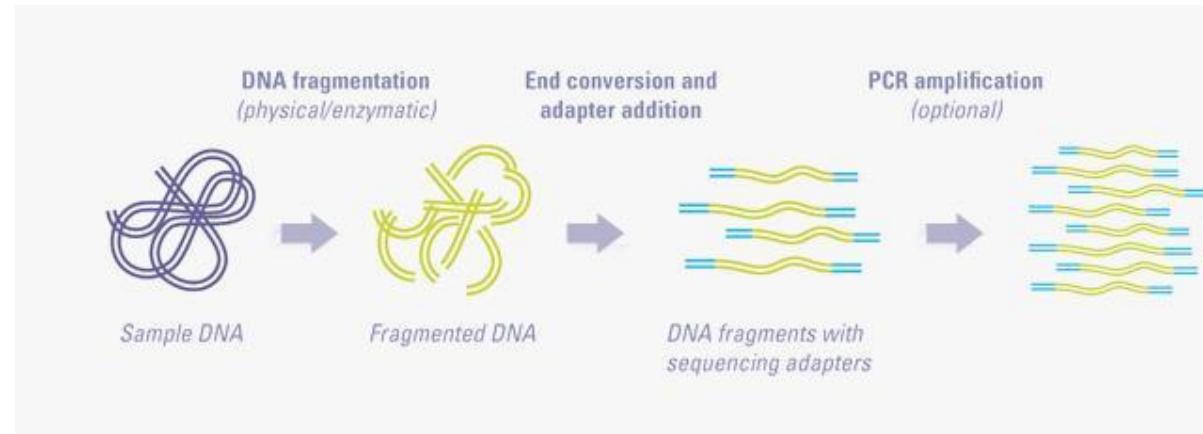
illumina®



Illumina Sequencing Workflow



Library Prep is Critical for Successful Sequencing

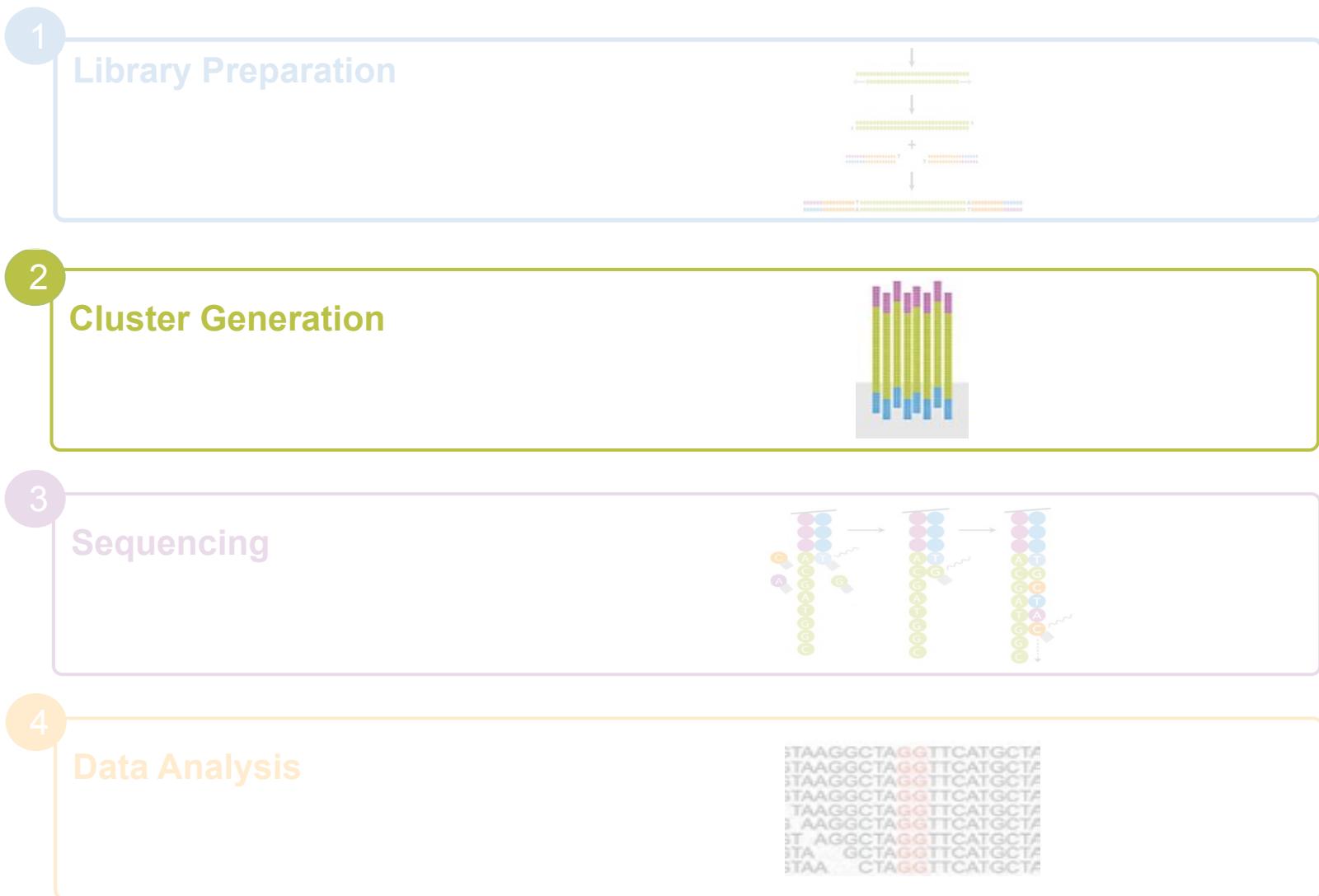


For clustering:
Libraries must have P5 and P7 binding regions on either end of a library

For sequencing:
Libraries must have sequencing primer binding regions

For mixing samples:
Libraries must have a unique index or barcodes sequence

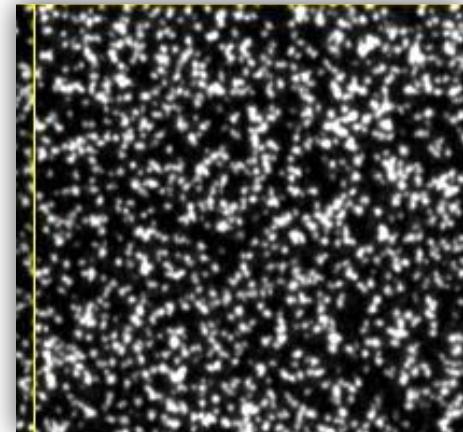
Illumina Sequencing Workflow



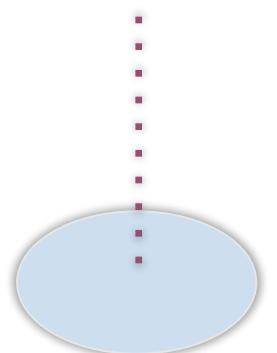
What is a Cluster?

Clusters are a group of DNA strands positioned closely together

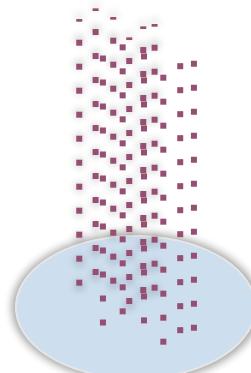
Each cluster represents thousands of copies of the same DNA strand in a 1–2 micron spot



**Single
DNA
Library
Molecule**



Amplification



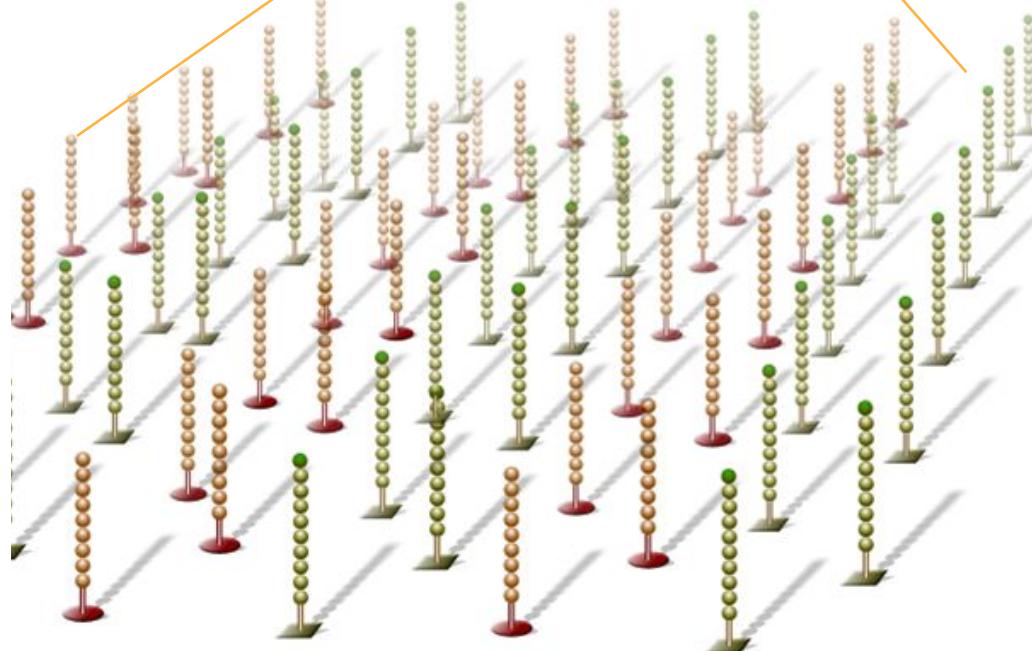
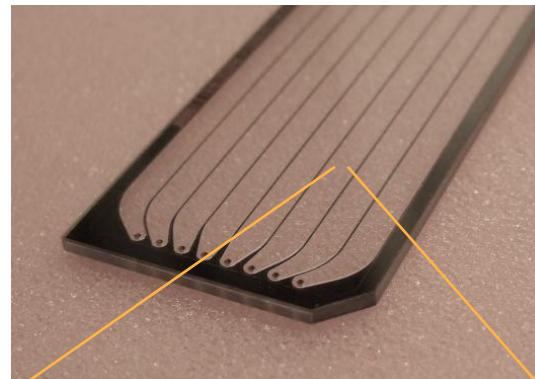
**Amplified
Clonal
Cluster**

What is a Flow Cell?

Cluster generation occurs on a flow cell

A flow cell is a thick glass slide with channels or lanes

Each lane is coated with a lawn of oligos complementary to library adapters



Flow Cell Architecture

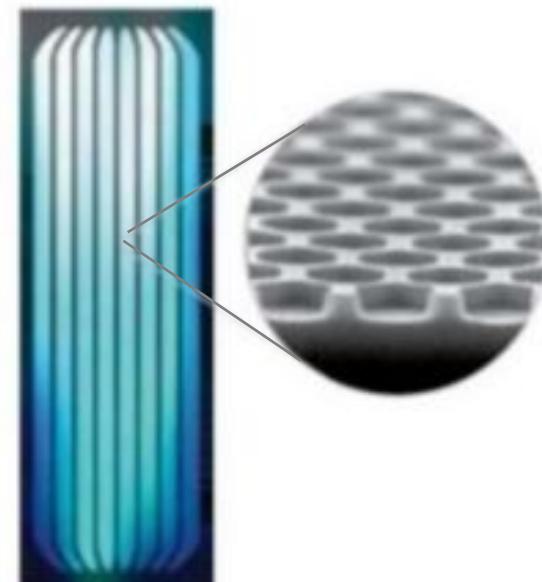
Random vs Patterned

Random Flow Cell

- HiSeq 2500, MiSeq, NextSeq, MiniSeq
- Randomly spaced clusters
- Variable Insert Sizes
- Lower Duplication Rates

Patterned Flow Cell

- HiSeq 3K/4K/X, NovaSeq, iSeq 100
- Defined size and spacing
- Increased Cluster density
- Simplified imaging

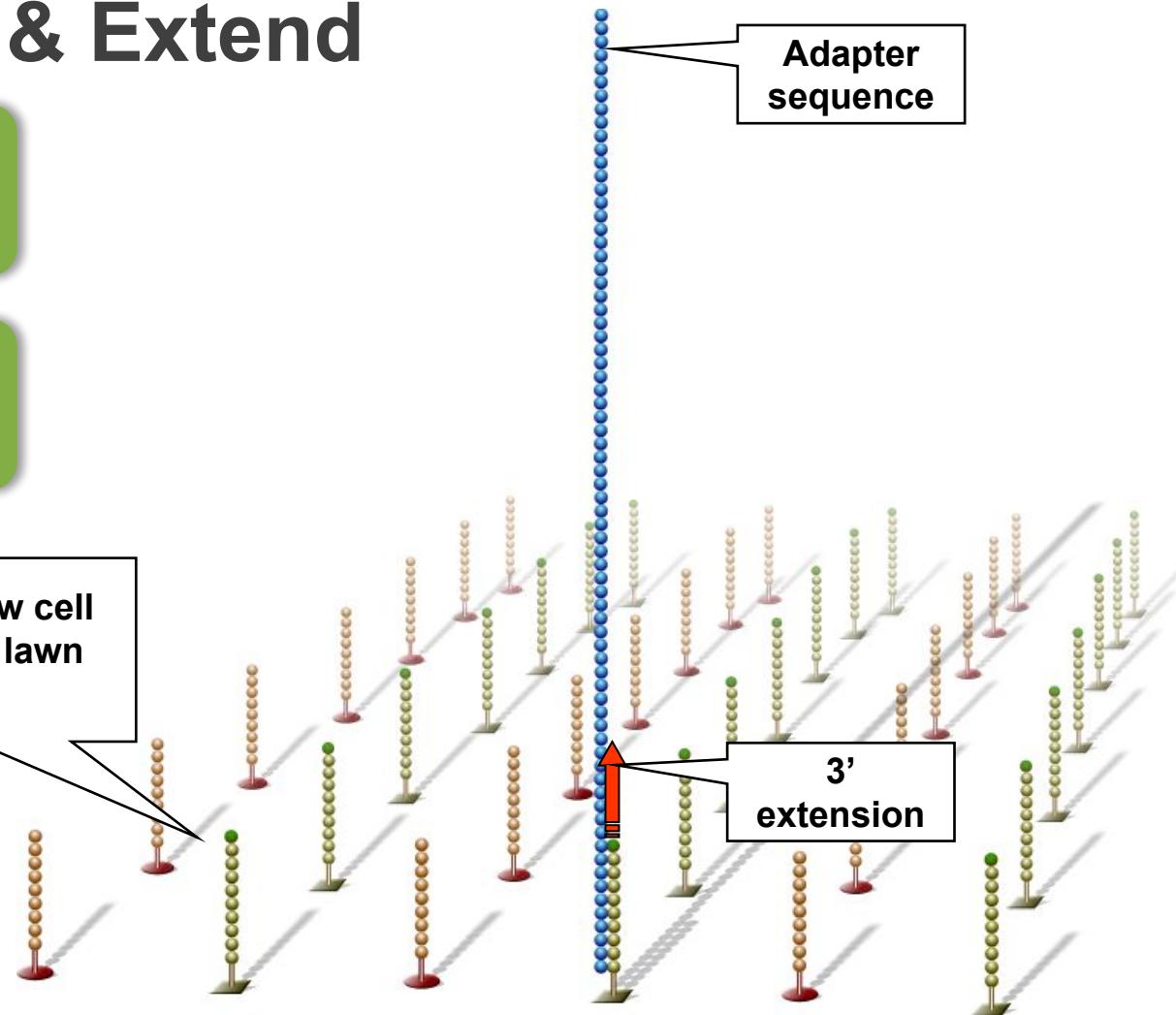


Hybridize Fragment & Extend

Single DNA libraries are hybridized to primer lawn

Bound libraries are then extended by polymerases

Surface of flow cell
coated with a lawn
of oligo pairs

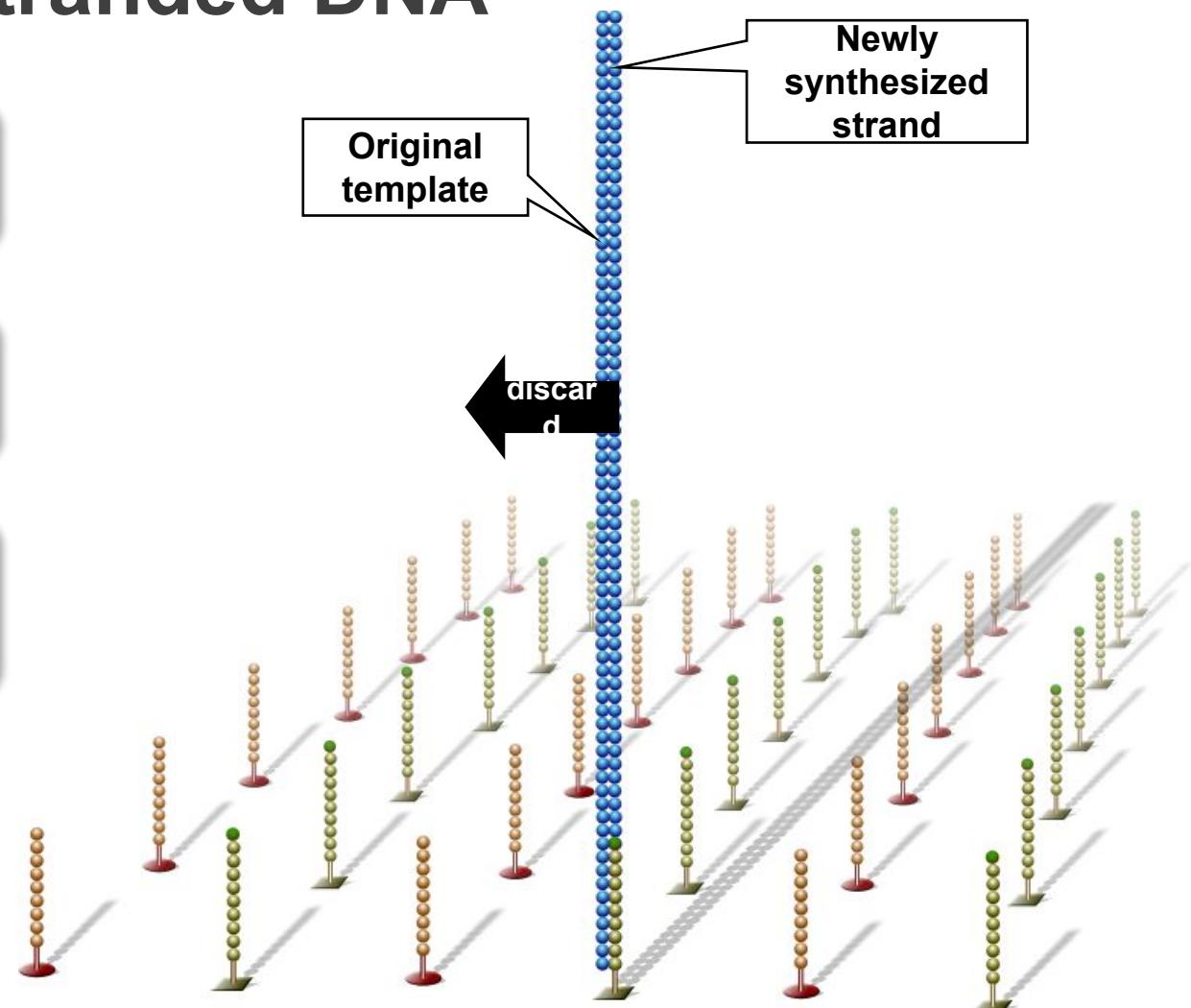


Denatured Double-Stranded DNA

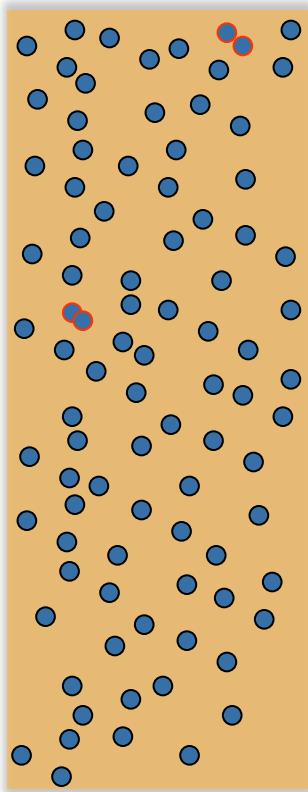
Double-stranded
molecule is denatured

Original template
washed away

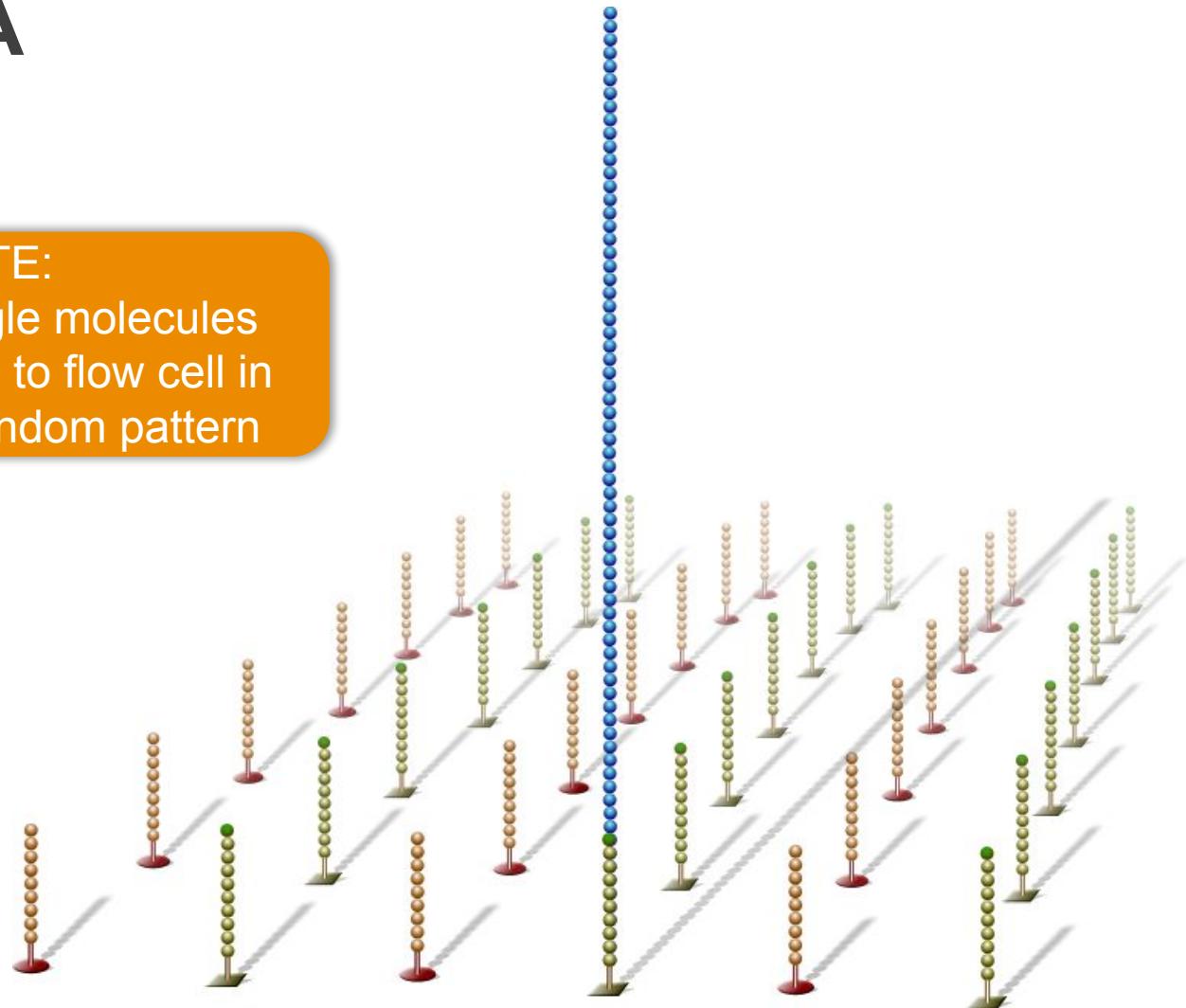
Newly synthesized
strand is covalently
attached to flow cell
surface



Single-Stranded DNA



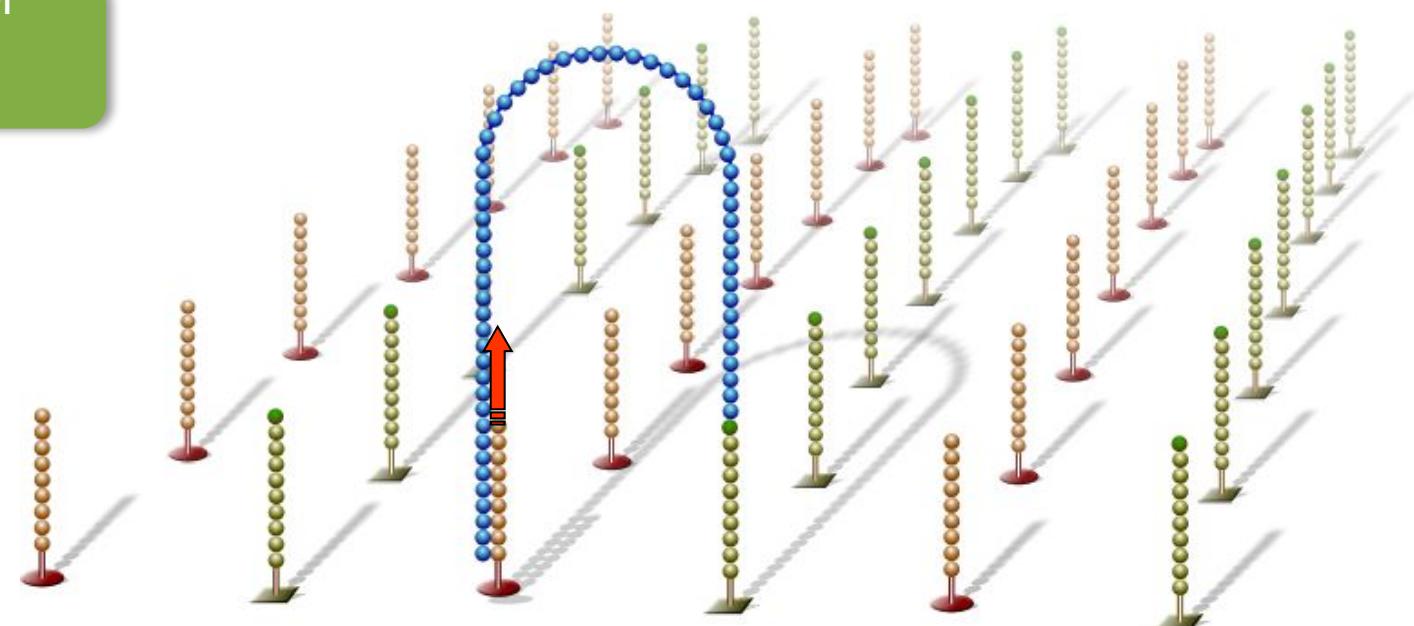
NOTE:
Single molecules
bind to flow cell in
a random pattern



Bridge Amplification

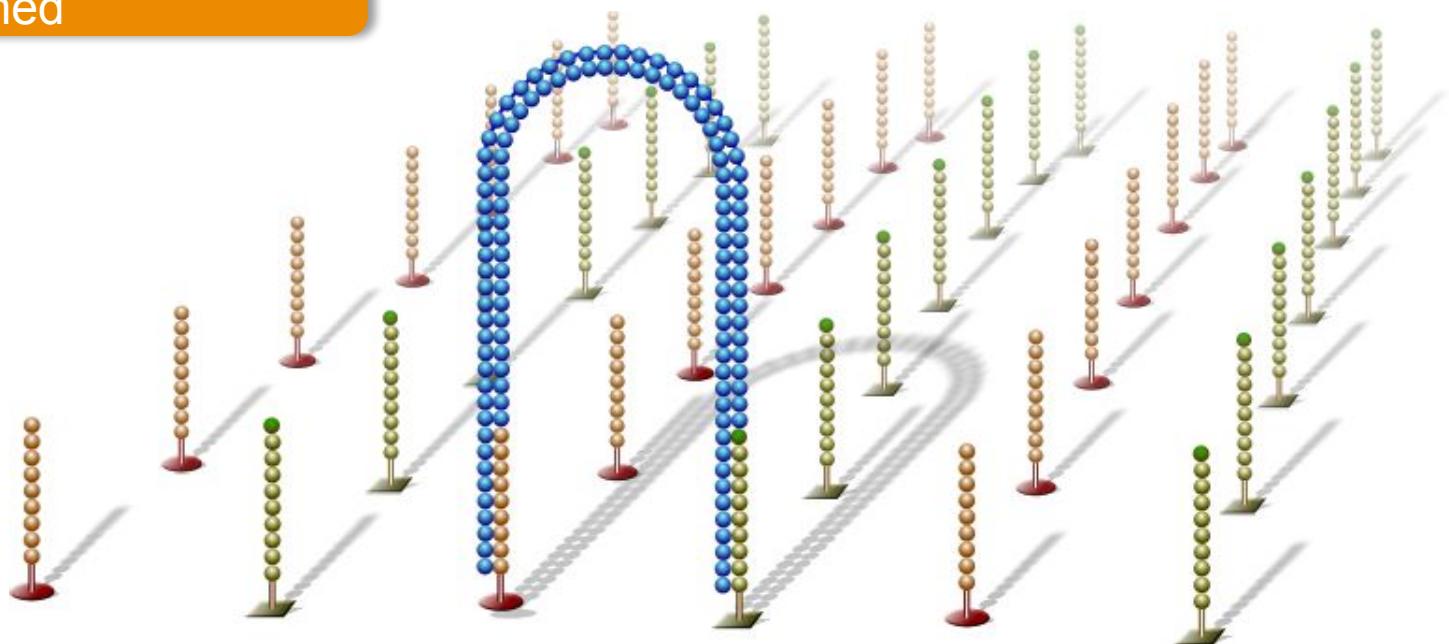
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer

Hybridized primer extends by polymerases



Bridge Amplification

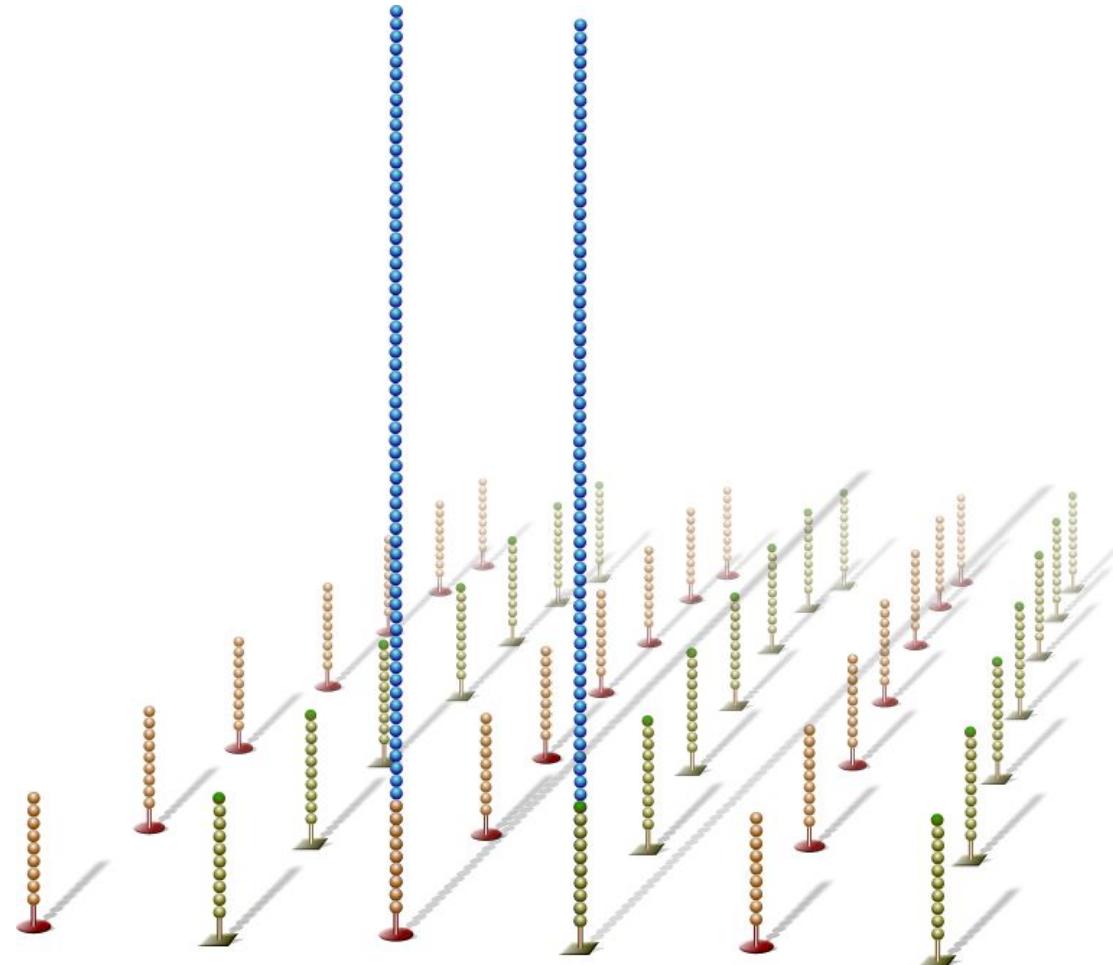
Double-stranded bridge is formed



Denature Double-Stranded Bridge

Double-stranded bridge is denatured

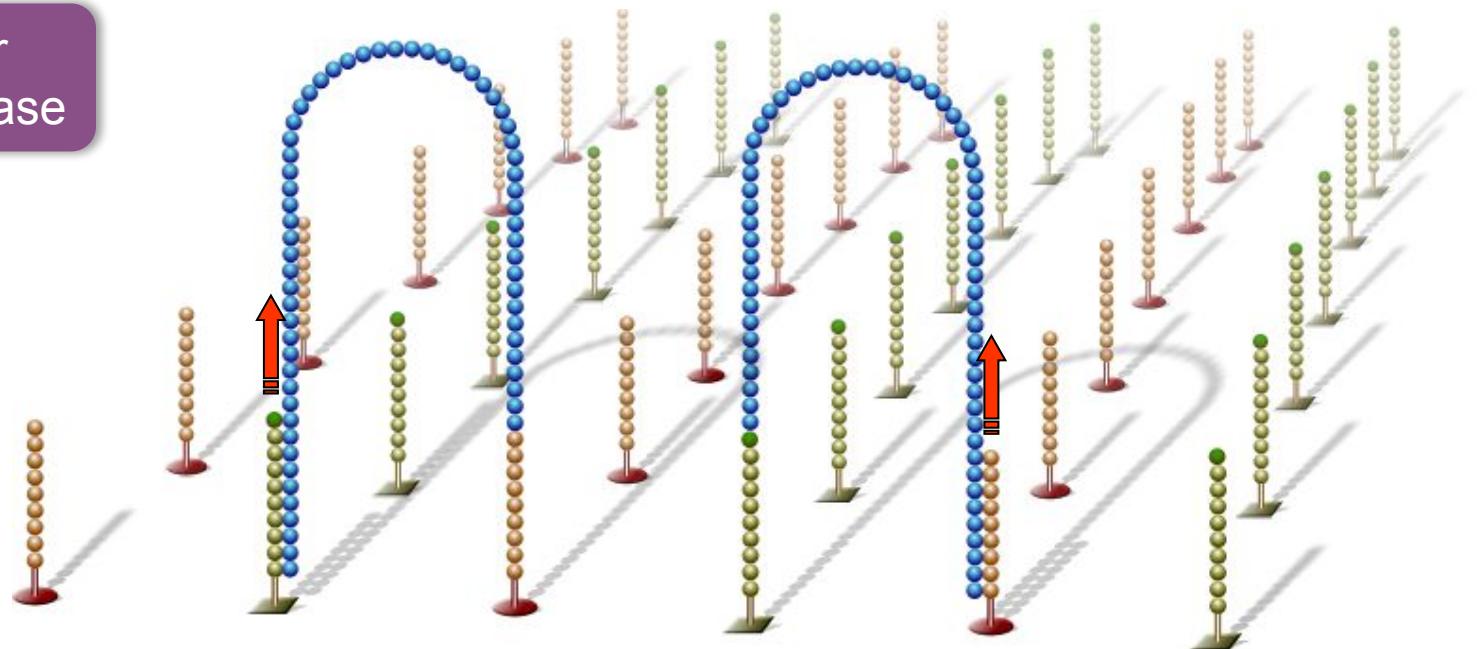
Result:
Two copies of covalently bound single-stranded templates



Bridge Amplification

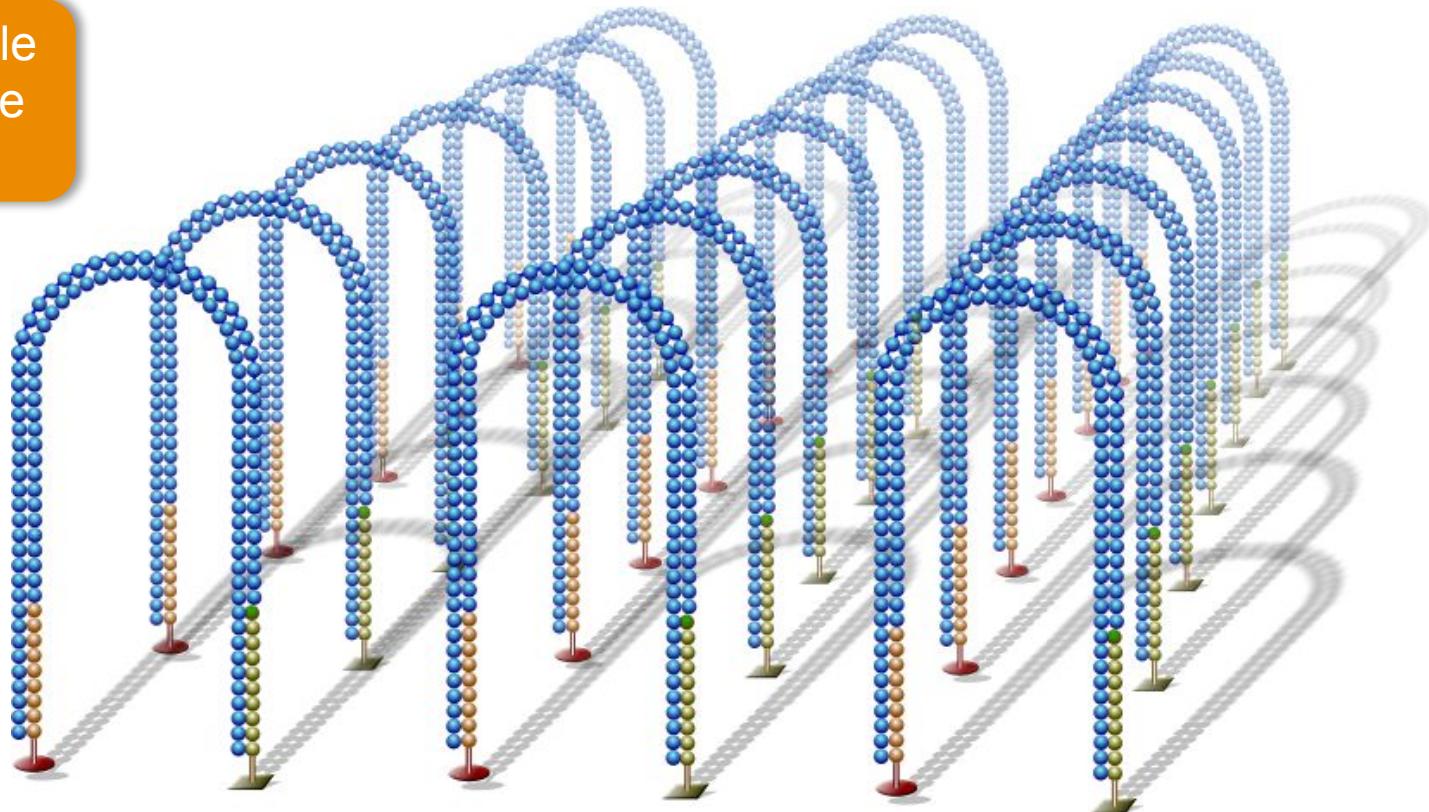
Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer extends by polymerase



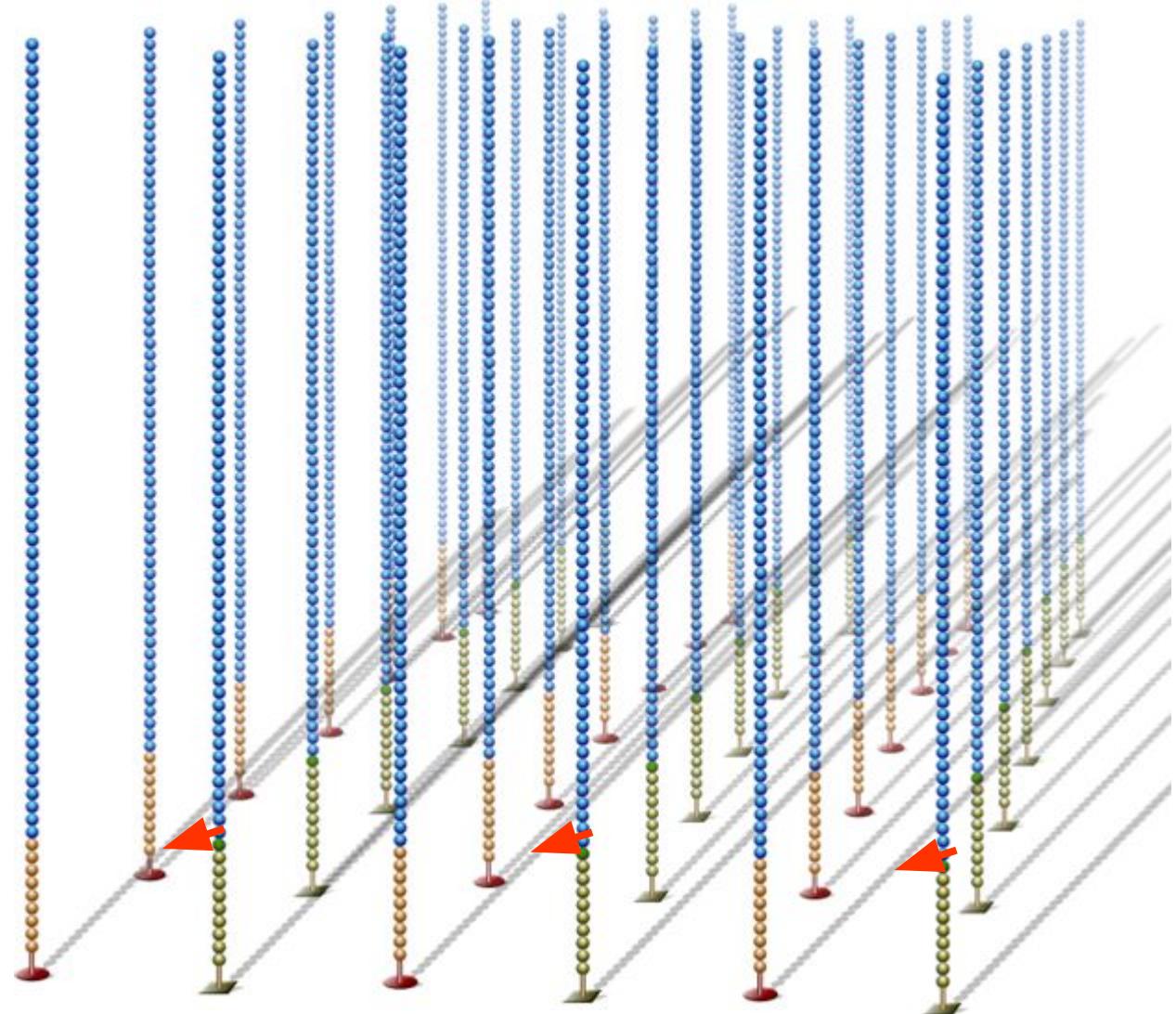
Bridge Amplification

Bridge amplification cycle
is repeated until multiple
bridges are formed



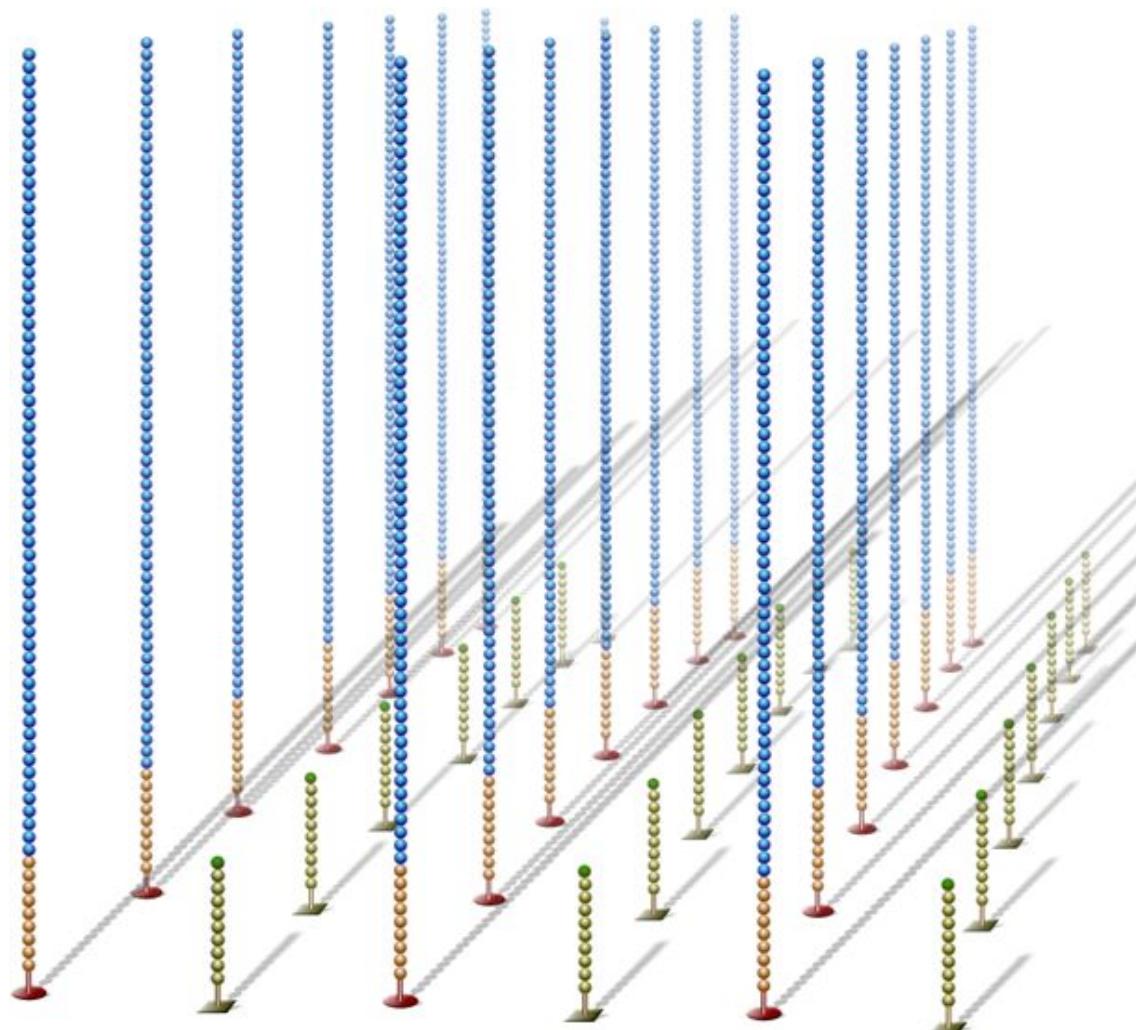
Linearization

dsDNA bridges are denatured



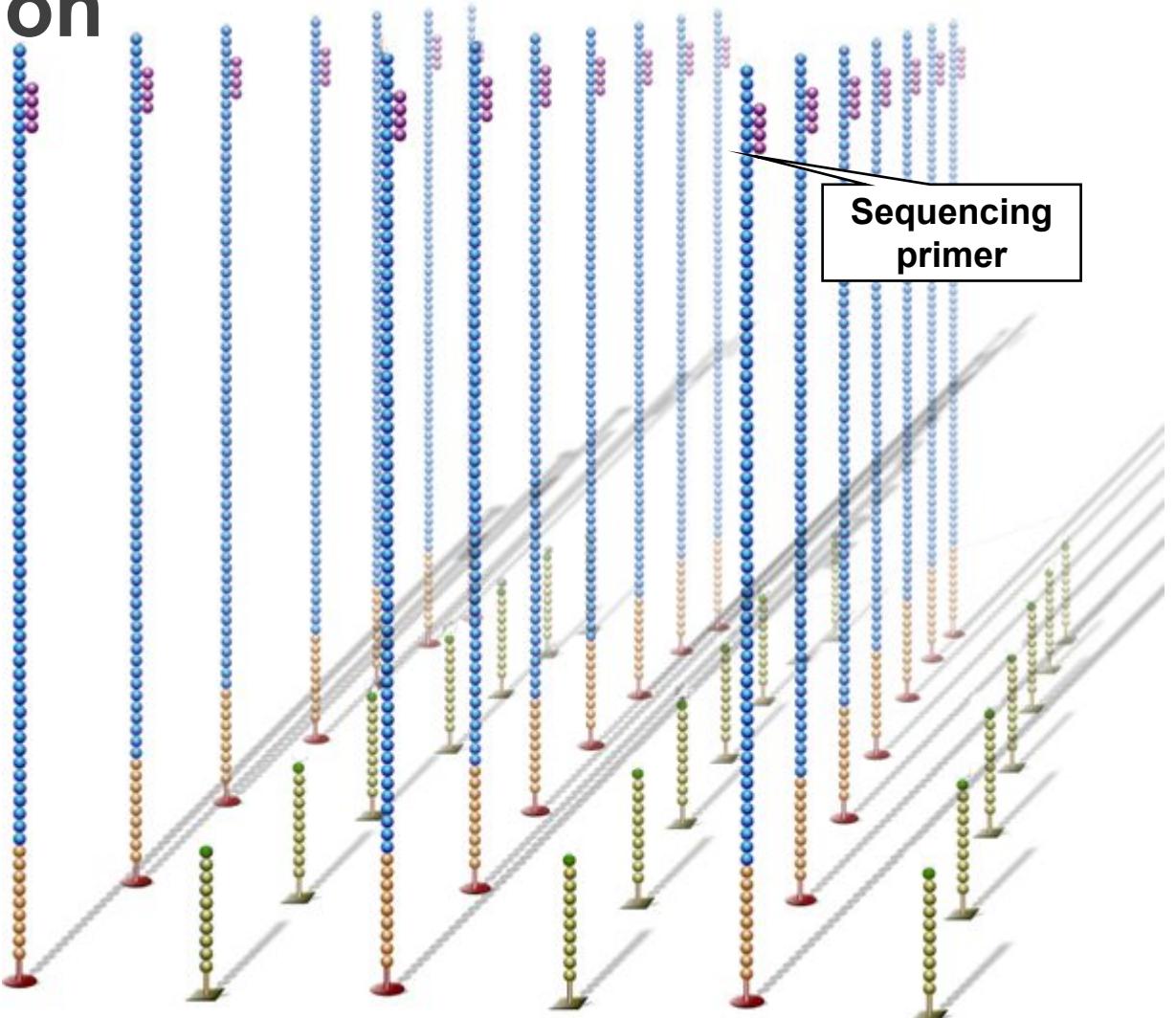
Reverse Strand Cleavage

Reverse strands are cleaved and washed away, leaving a cluster with forward strands only



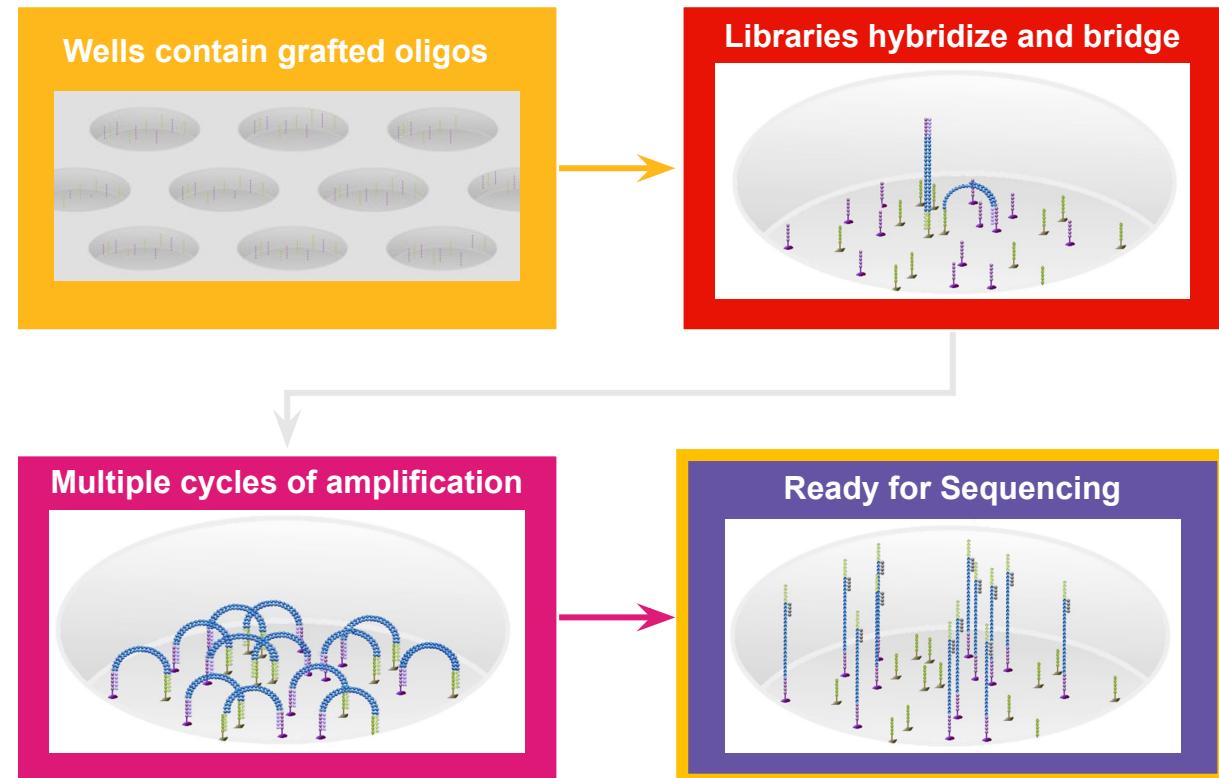
Read 1 Primer Hybridization

Sequencing primer is hybridized to adapter sequence

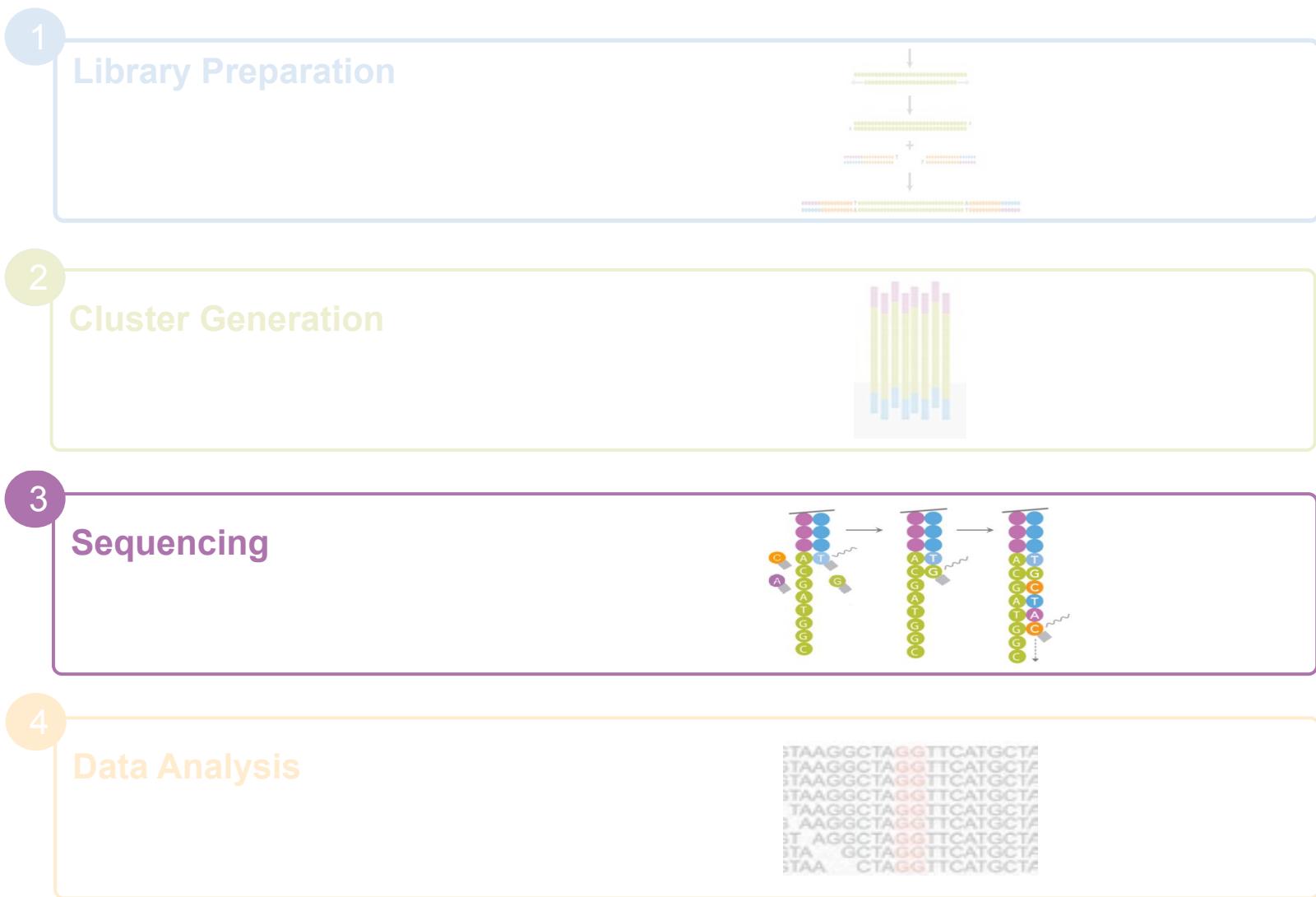


Sequencing
primer

Pattern Flow Cell and ExAmp Technology

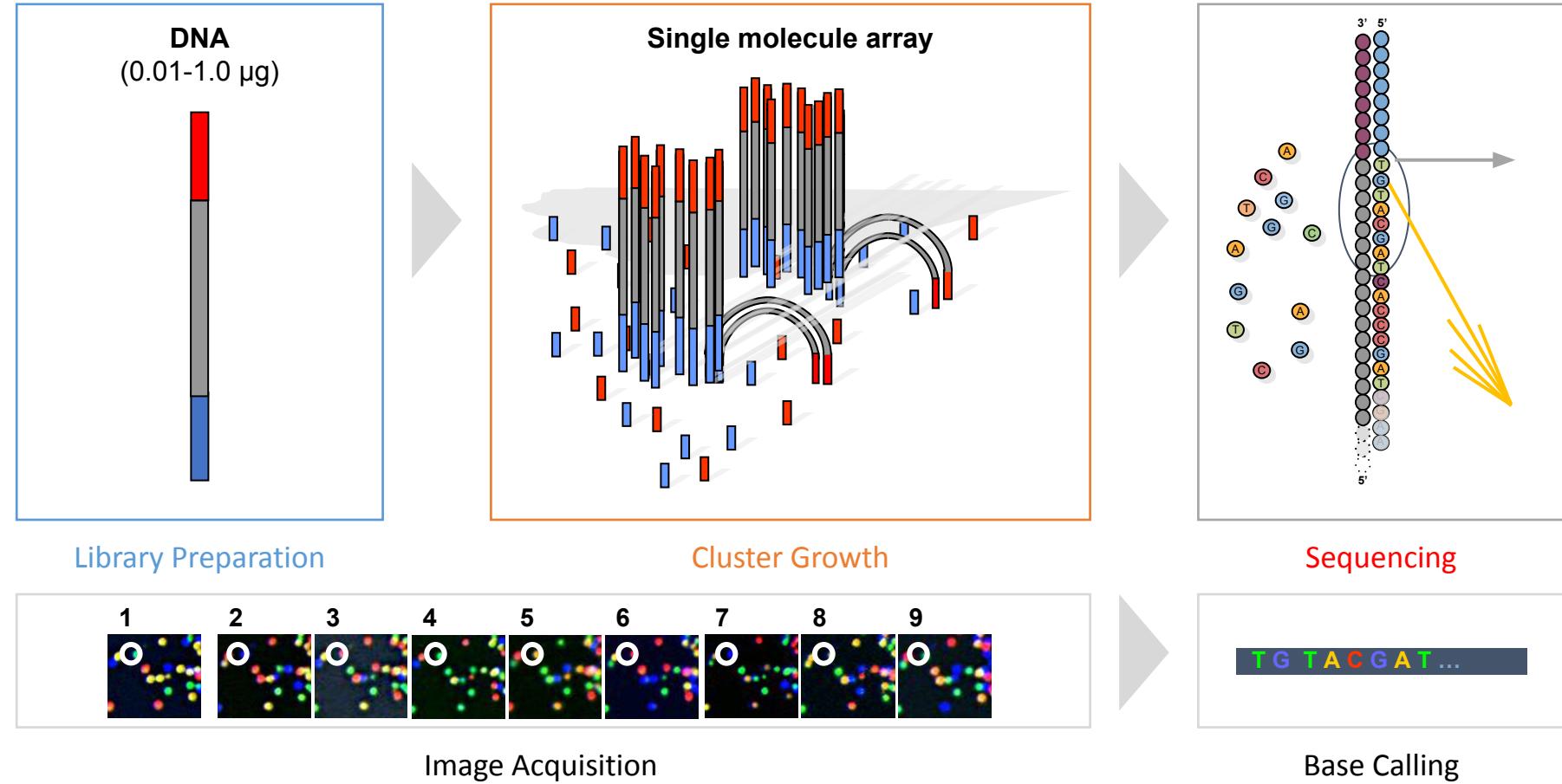


Illumina Sequencing Workflow



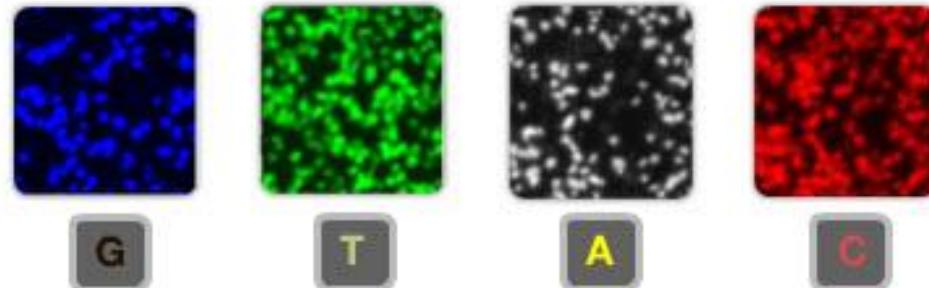
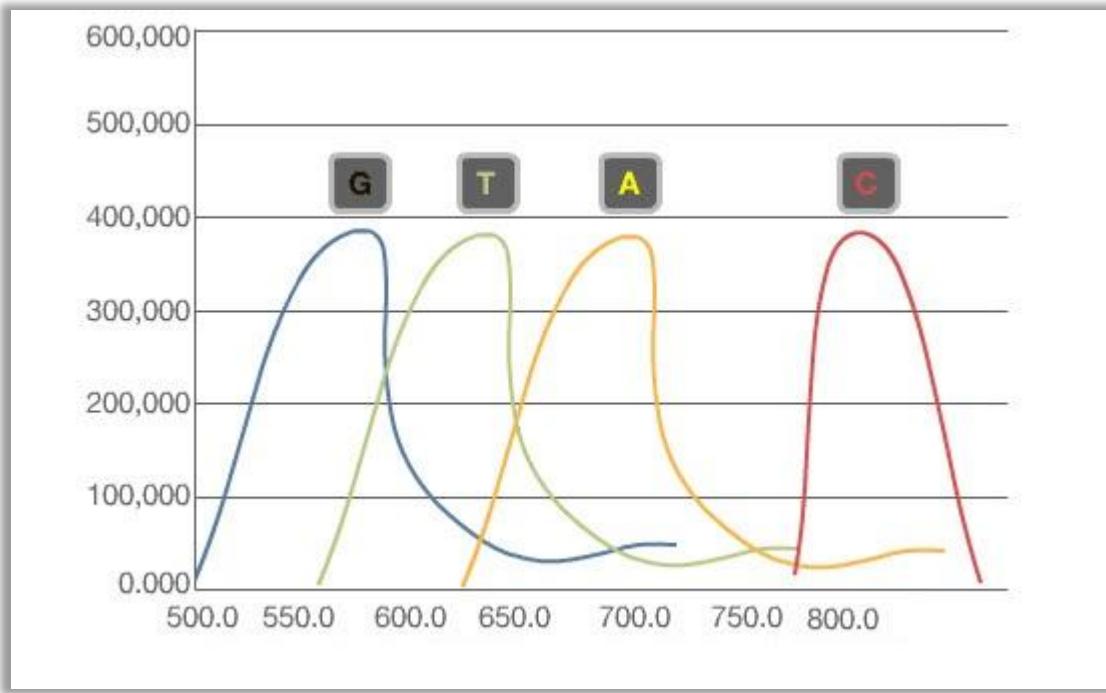
Sequencing

Sequencing-by-Synthesis (SBS)



Four Channel SBS Chemistry:

HiSeq, MiSeq



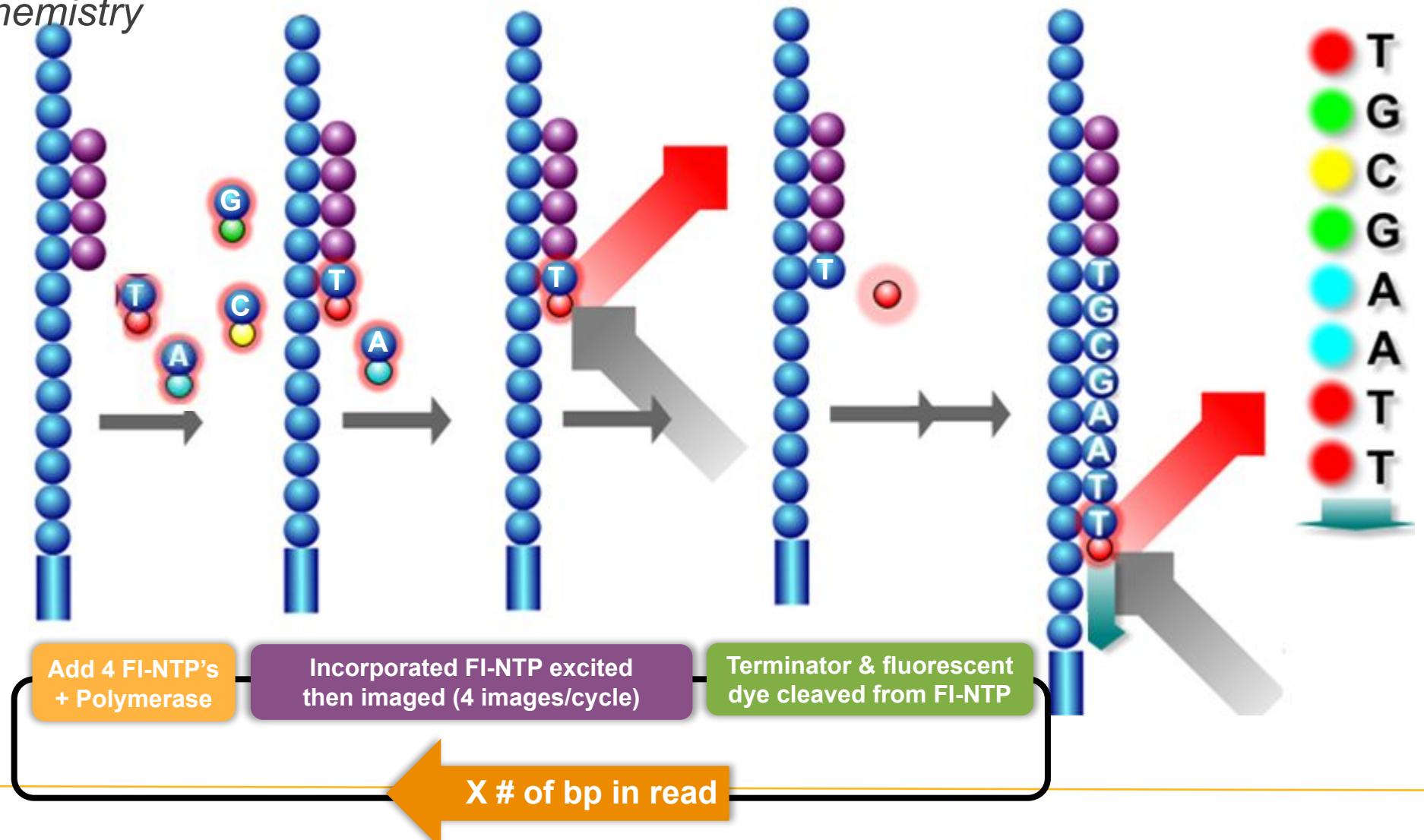
Each of the four DNA bases emits an intensity of a unique wavelength

Collects four images:

- During each cycle, each cluster appears in only one of four images

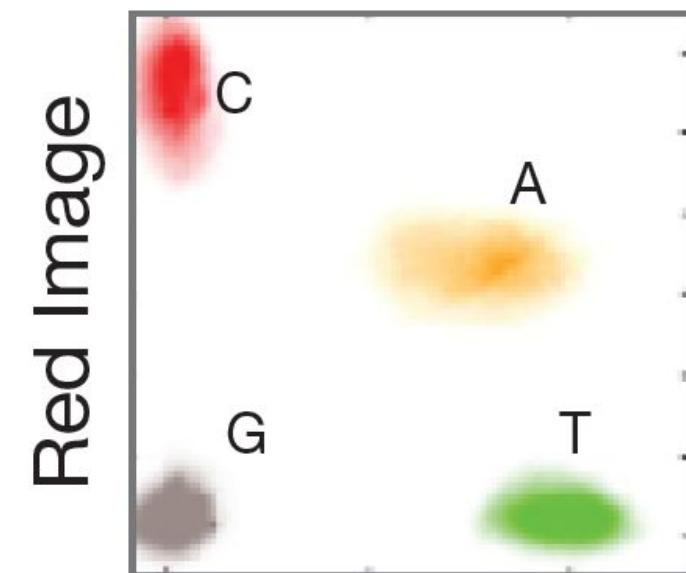
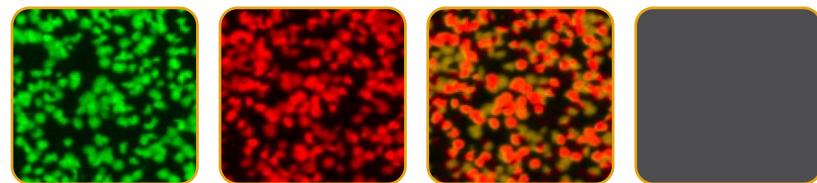
A Closer Look At 4-Dye Chemistry

Four channel chemistry



Two Channel SBS Chemistry:

NextSeq 550, MiniSeq, NovaSeq

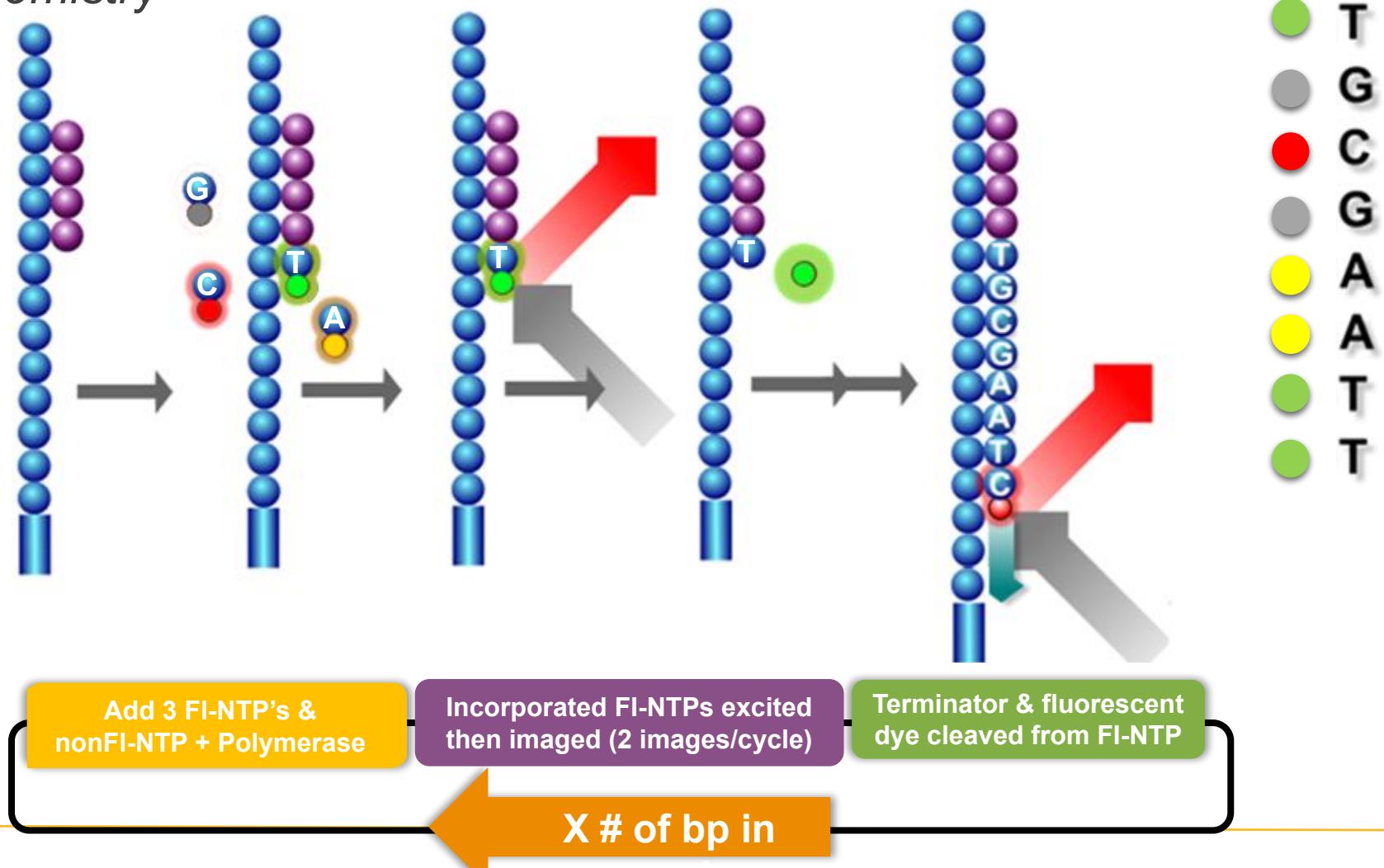


Red Image

Green Image

A Closer Look At 2-Dye Chemistry

Two channel chemistry



One-Channel SBS Chemistry

iSeq 100

SBS Chemistry combined with Complementary Metal-oxide-semiconductor (CMOS) technology

- The system uses a patterned flow cell with nanowells fabricated over a CMOS chip
- Each sequencing cycle has two chemistry steps in order to determine bases
- Two images are captured within one cycle of sequencing run

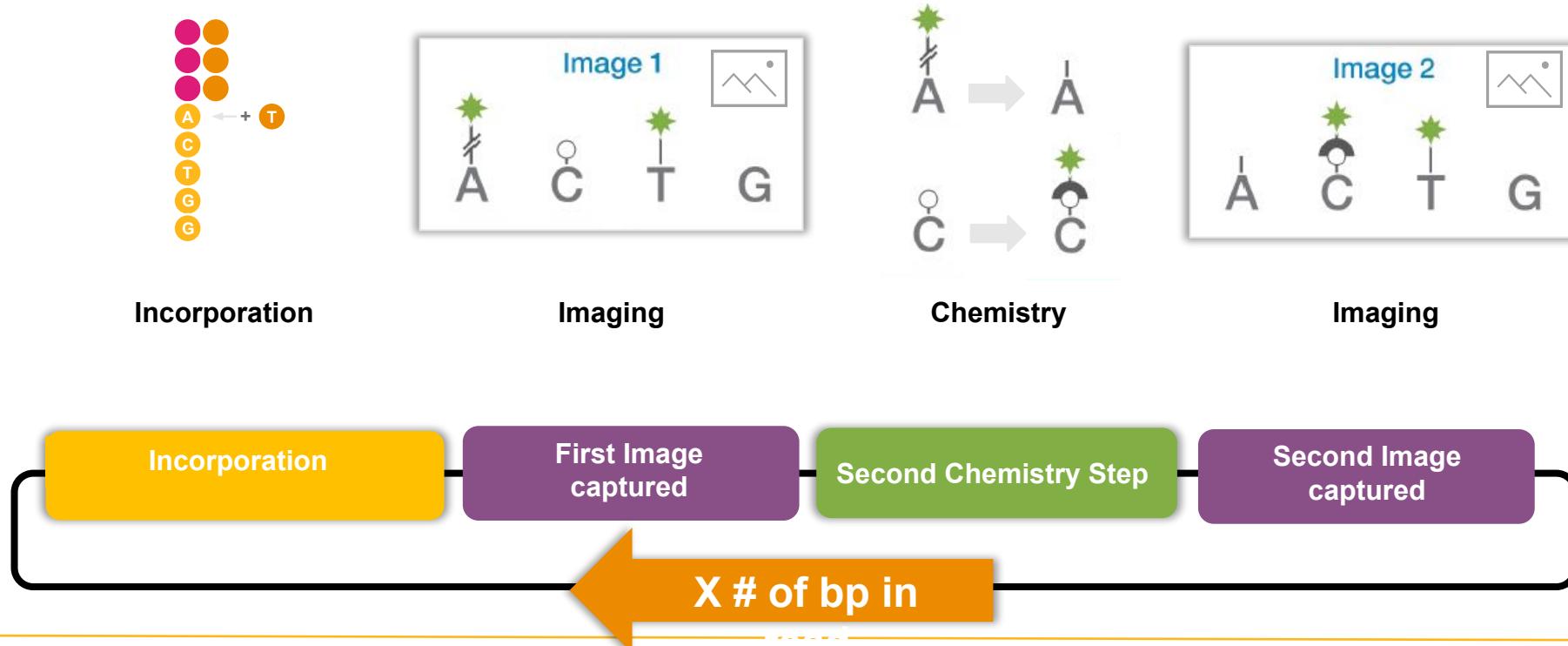
Based on the signal pattern across two images, base calls can be determined

- Intensities extracted from one image and compared to a second image result in four distinct populations, each corresponding to a nucleotide.

A Closer Look At 1-Dye Chemistry

One channel chemistry

One Sequencing Cycle

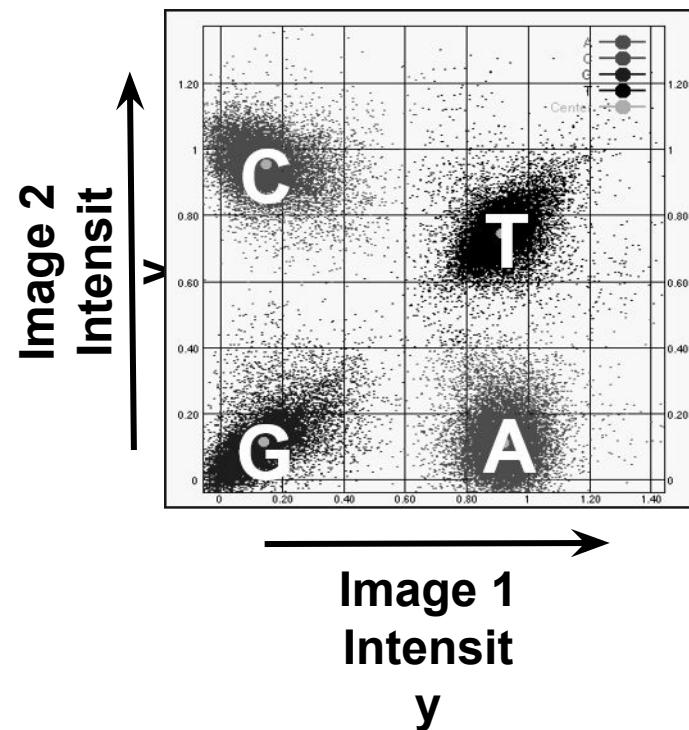


Sequencing by Synthesis with CMOS Detection

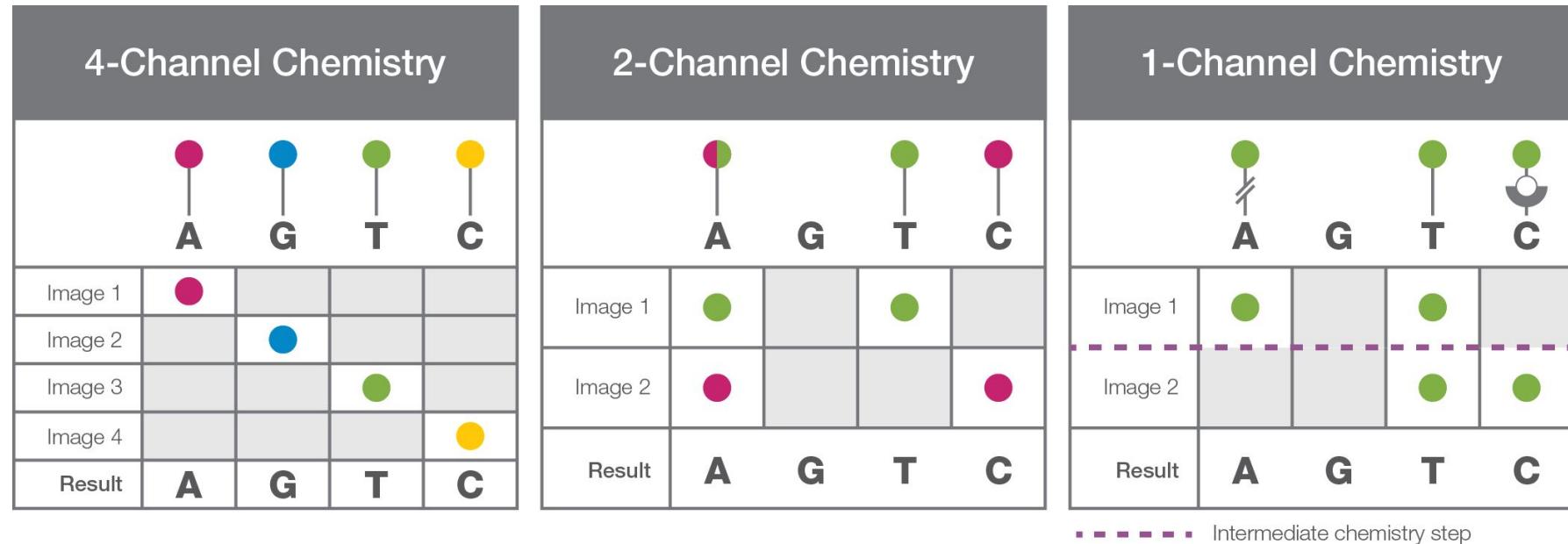
One channel chemistry

- The iSeq 100 System uses one-dye sequencing, which requires one dye and two images to encode data for the four bases

Base	Image 1	Image 2
T	ON	ON
A	ON	OFF
C	OFF	ON
G	OFF	OFF



Illumina Chemistry Comparison



Four-channel SBS

- Bases are identified using four different fluorescent dyes, one for each base and four images per sequencing cycle

Two-channel SBS

- Simplified nucleotide detection by using two fluorescent dyes and two images to determine all four base calls

One-channel SBS

- System uses a patterned flow cell with nanowells fabricated over a CMOS chip to determine base calls using only two images



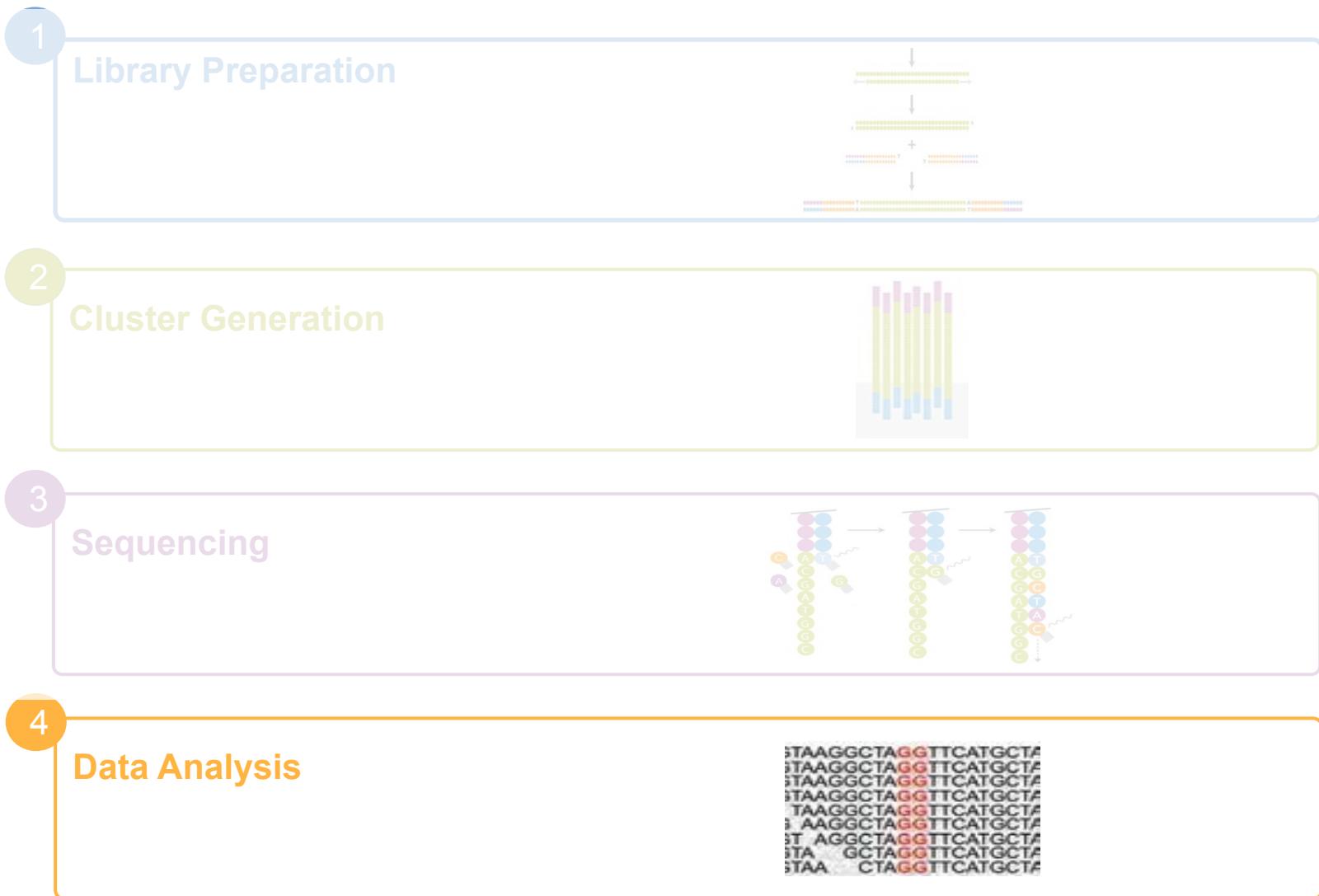
Benefits of SBS Technology

- Robust Performance and Data Quality
 - SBS chemistry delivers the highest yield of error-free reads¹, enabling robust base calling across the genome.
- Proven Base Calling Accuracy
 - SBS produces the highest percentage of sequenced bases over **Q30** - a quality score indicating a 0.1% probability that a base was called incorrectly.
- Unbiased Coverage Across Genome
 - SBS uses base-by-base, reversible-terminator chemistry, which virtually eliminates the homopolymer errors seen in ion-semiconductor or pyrosequencing technologies²
- High-Quality Alignment with Paired-End Sequencing
 - SBS allows for paired-end sequencing —sequencing DNA library fragments from both ends, which generates high-quality sequence data.
- Cost-Effective for Any Throughput or Scale

1.Based on a comparison of the top 3 industry-leading NGS platforms. Data calculations on file. Illumina, Inc.
2016.

2.Ross MG, Russ C, Costello M, et al. Characterizing and measuring bias in sequence data. Gen Biol 2013;14:R51

Illumina Sequencing Workflow



Data Analysis



Sequencing reads aligned to a reference genome

CGATTAGTAC

ACTCGATTAG

ACGGGCTCGA

ACGACGGACT

Reference Sequence

CATACGACGGACTCGATTAGTACTCGTA

Variant/genotype calling

Annotation & filtering

Functional effect

20bp region

6bp @ 1X

8bp @ 2X

6bp @ 3X

Average = 2X

Analysis Overview

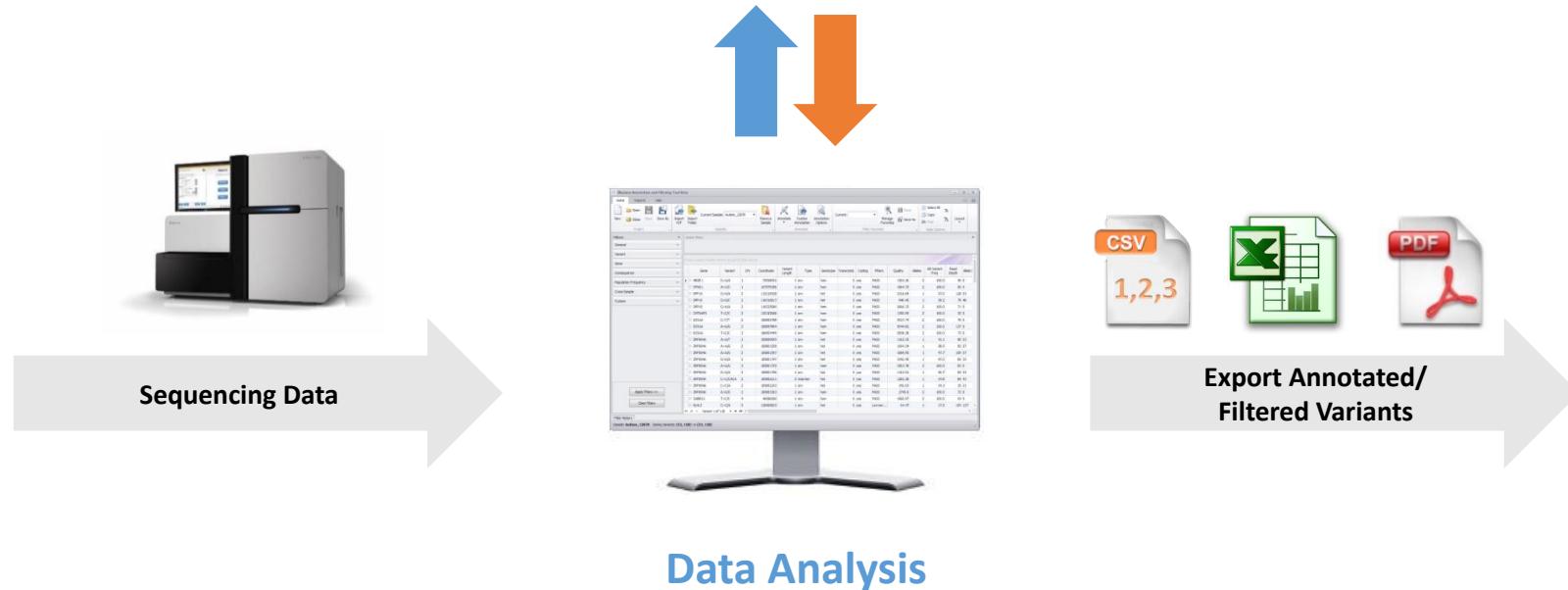
Analysis Type	Software	Outputs
Control Software		 Images, Intensities and Base Calls 
Analysis Software	  	 Alignments, Variant Detection
Visualization Software	 	  Annotation, Filtering, Reports

Analysis and Interpretation of Sequencing Data

Data in, biological knowledge out



Public Databases & Private Data Centres



For Research Use Only. Not for use in diagnostic procedures.

Illumina Systems

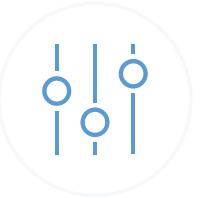
illumina®



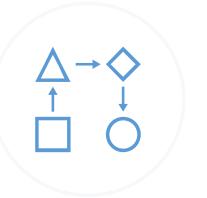
Illumina System Benefits



Fast turnaround
for time-critical projects



Integrates cluster generation
and sequencing **in ONE system**



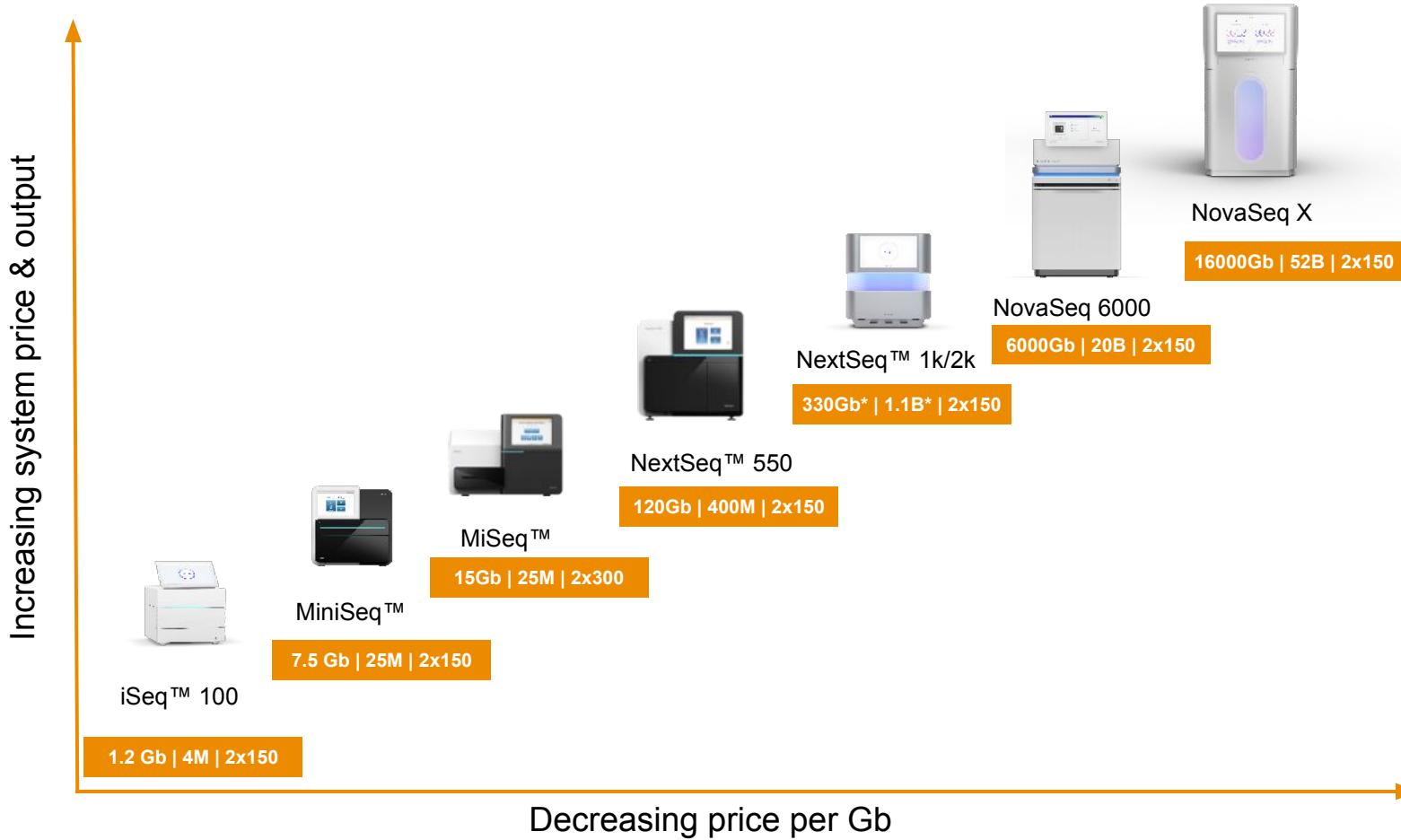
Simple workflow for limited
impact to **lab overhead**



Integrates with Illumina library
prep and **informatics**



Sequencing Power for Every Scale



Key Takeaway:

- ✓ Greater than 20,000 sequencers shipped globally
- ✓ 300,000 peer-reviewed publications, 5x vs. all other participants*

*Data on file

FDA-regulated, CE-IVD marked Sequencer



- Illumina is the leading provider of NGS IVD solutions, with three Dx platforms currently on the market and a growing menu of IVD tests
- NovaSeqDx will be a fit-for-purpose clinical instrument that is designed to run IVD assays, developed either by Illumina or our partners.
- It will also function as an open platform system, which allows development of IVD assays for site specific use or for distribution.

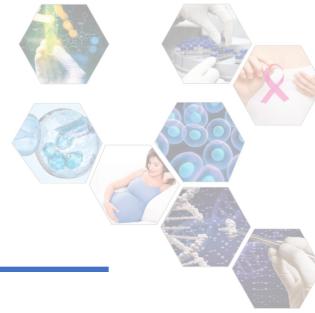
From left to right:
MiSeqDx, NovaSeqDx, NextSeq 550Dx

Application By Sequencer



Amount of Data	Sequencer	Applications
Most	NovaSeq™ 6000 NovaSeq X series	Whole Genome, Transcriptome, Methylome, Liquid Biopsy
	NextSeq™ 2000	Single cells, Trios, Exomes, Enrichment
	NextSeq™ 550	NIPT, Oncology Panels, Small Genomes, Enrichment
	MiSeq™	
	MiniSeq™	Oncology Panels, PGT-A (For MiSeq only), Small RNA, Targeted Panels , Metagenomics
	iSeq™ 100	Amplicon, Bacterial WGS

ĐỐI TÁC CHIẾN LƯỢC



- Biomedic JSC., đã và đang là **nhà phân phối độc quyền** của Illumina Inc., tại thị trường Việt Nam kể từ năm 2011
- Năm 2018 – 2021, Biomedic JSC., tự hào được vinh danh là **Global Elite Channel Partner** của Illumina Inc.,





Số đầu máy NGS/ Scan Biomedic phục vụ



Array Scanners



> 70 hệ thống máy NGS, server phân tích đã được cài đặt trên toàn quốc



CUNG CẤP GIẢI PHÁP TOÀN DIỆN



Prepare library



Sequence



Analyze data

Simplified
library prep

Custom
content

Flexible, economical
sequencing

Integrated
analysis

KHÁCH HÀNG TOÀN QUỐC

Our customers

SẢN KHOA

OBSTETRICS & GYNECOLOGY



UNG THƯ

ONCOLOGY



TRUYỀN NHIỄM

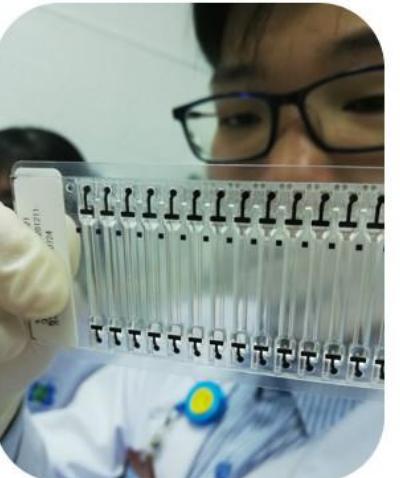
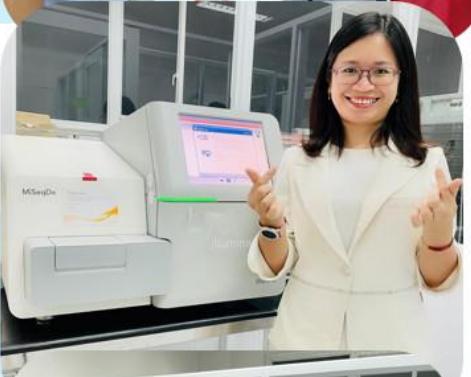
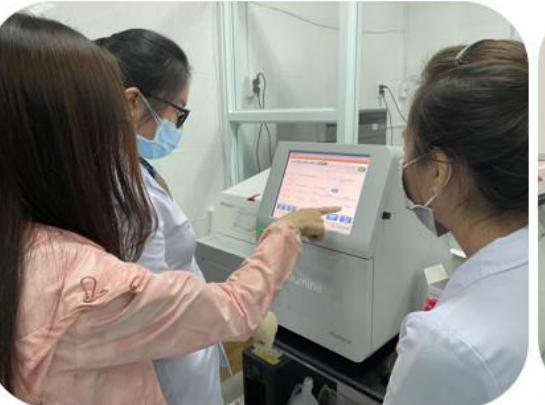
INFECTIOUS DISEASES



HÌNH SỰ

FORENSICS





Meaning
time





TRÂN TRỌNG CẢM ƠN!

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