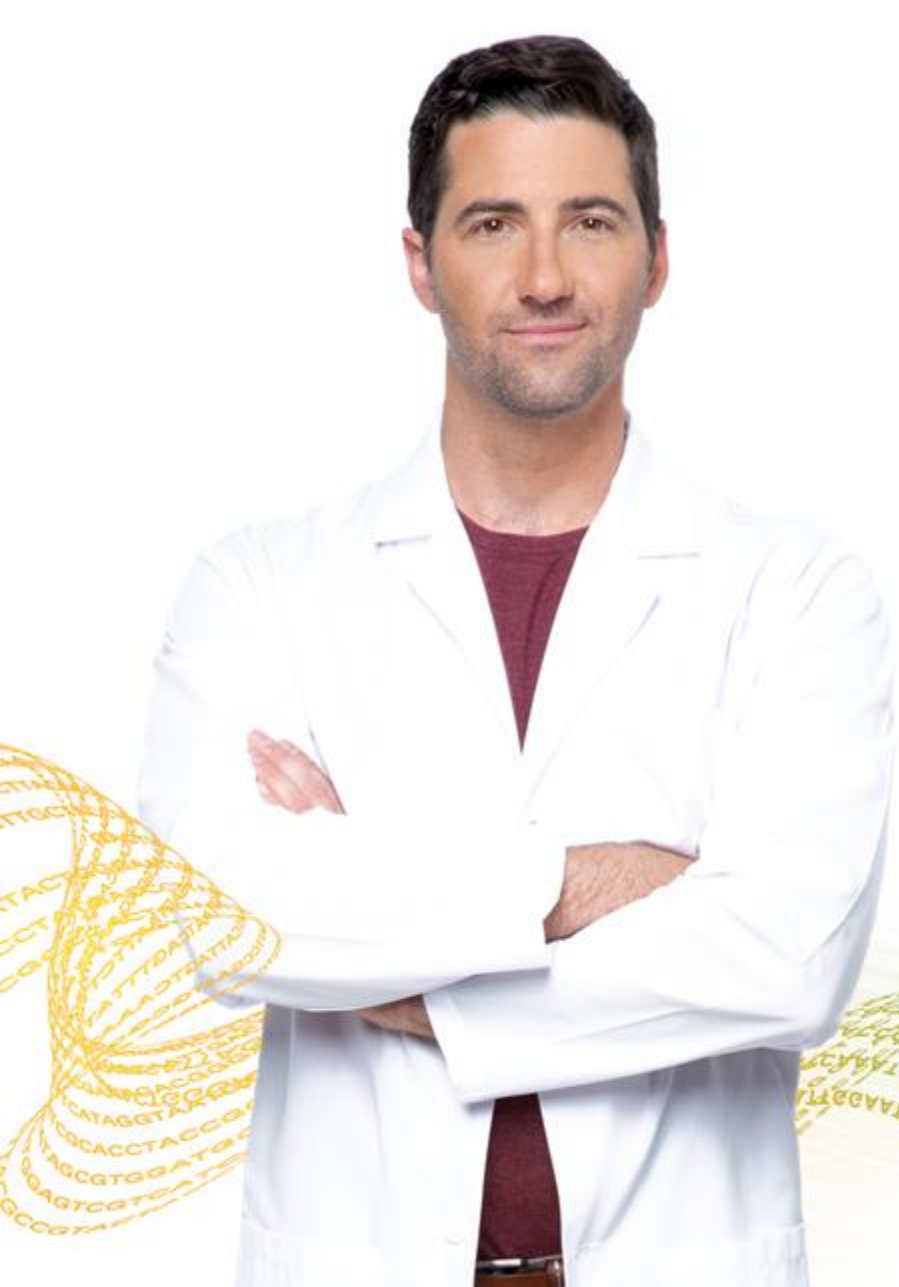


Illumina Sequencing Overview



Part # 15045845_Rev.C

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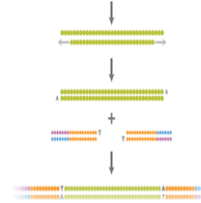
illumina[®]

Session Objectives

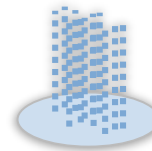
- ▶ By the end of this training, you will be able to:
 - List the major steps in the Illumina sequencing workflow
 - Describe cluster generation
 - Discuss the sequencing by synthesis process

Illumina Sequencing Workflow

1 Library Preparation



2 Cluster Generation



3 Sequencing



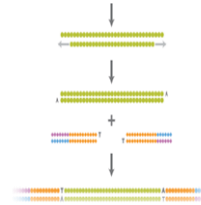
4 Data Analysis

```
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA
```

Illumina Sequencing Workflow

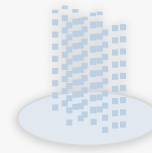
1

Library Preparation



2

Cluster Generation



3

Sequencing



4

Data Analysis

```
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
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3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA
```

Library Prep is Critical for Successful Sequencing



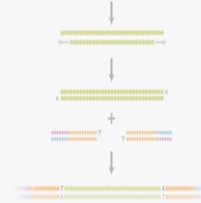
Dual Index Library shown

The aim of library prep is to obtain nucleic acid fragments with adapters attached on both ends

Illumina Sequencing Workflow

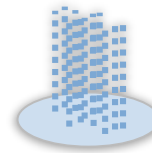
1

Library Preparation



2

Cluster Generation



3

Sequencing

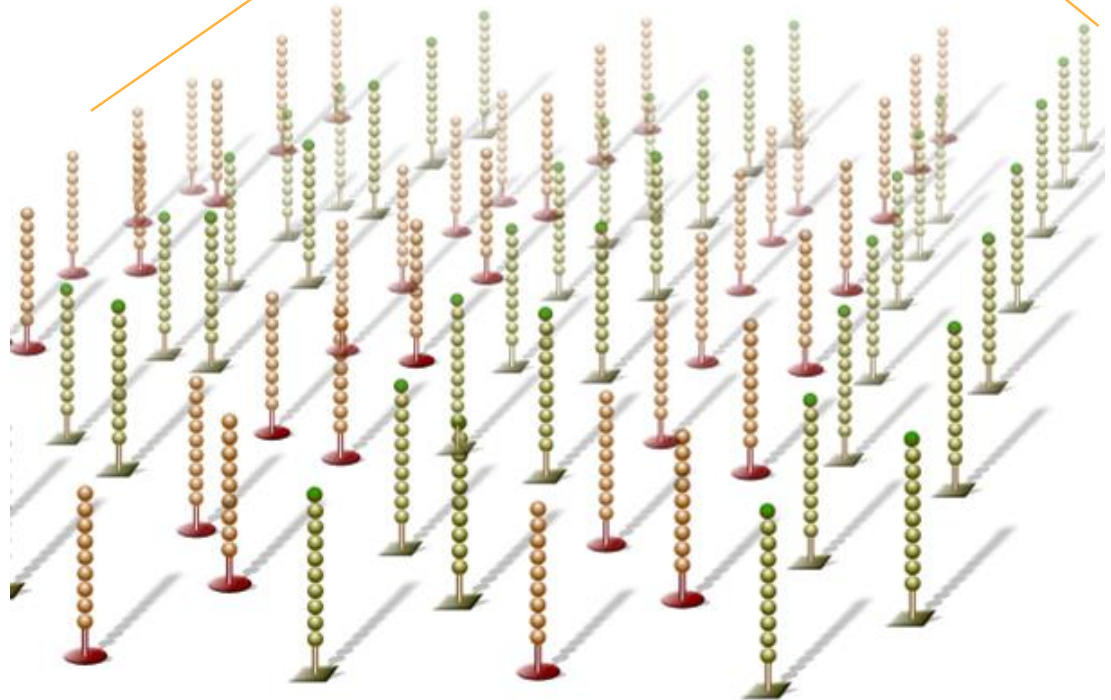
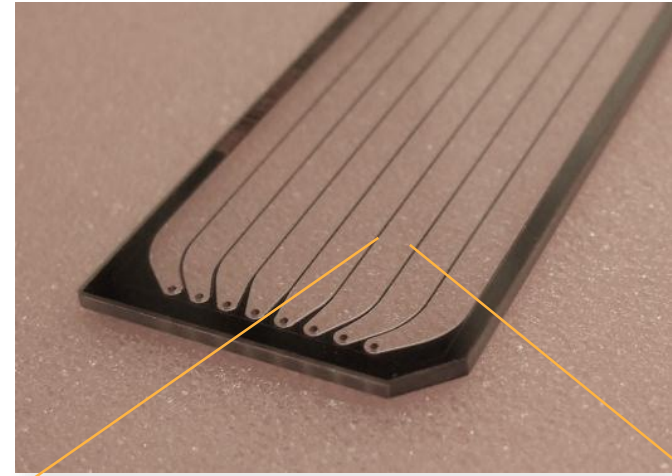


4

Data Analysis



What is a Flow Cell?



Cluster generation occurs on a flow cell

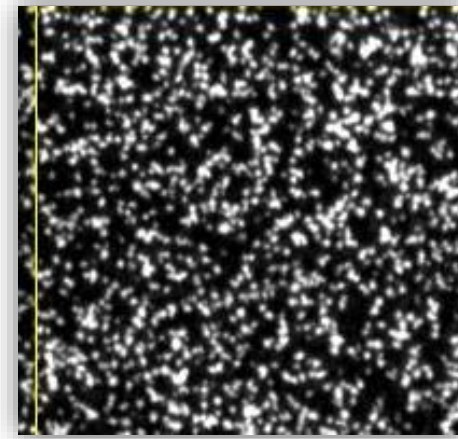
A flow cell is a thick glass slide with channels or lanes

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

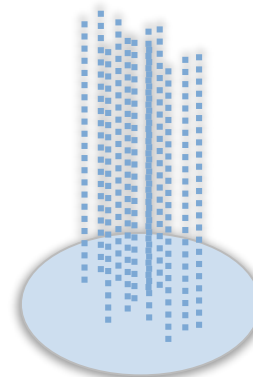
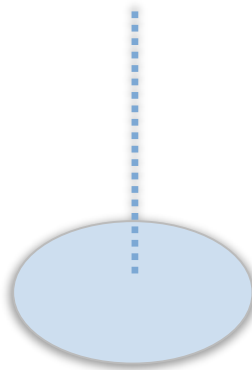
What is a Cluster?

Clusters are bright spots on an image

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



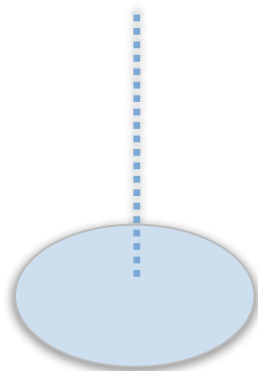
**Single
DNA
Library**



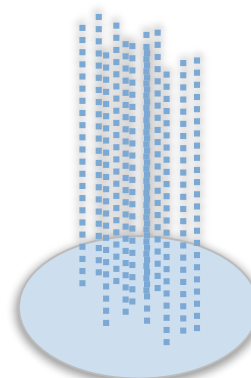
**Amplified
Clonal
Cluster**

Instrumentation

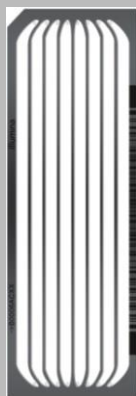
Single
DNA
Library



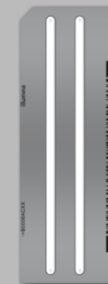
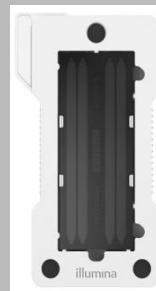
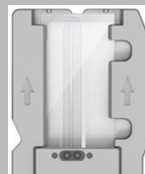
Amplified
Clonal
Cluster



cBot



Sequencer



Hybridize Fragment & Extend

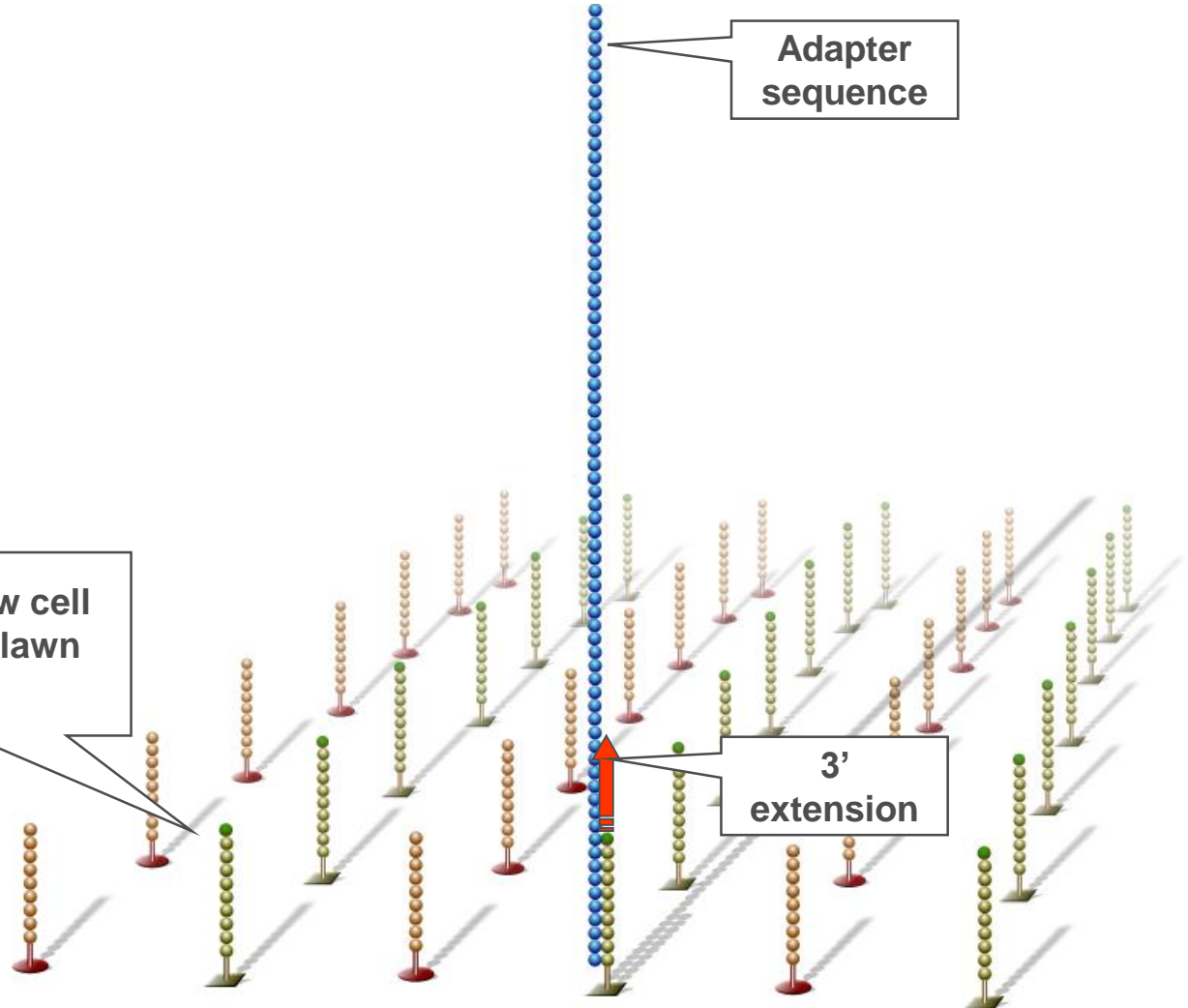
Single DNA libraries are hybridized to primer lawn

Bound libraries are then extended by polymerases

Surface of flow cell coated with a lawn of oligo pairs

Adapter sequence

3' extension

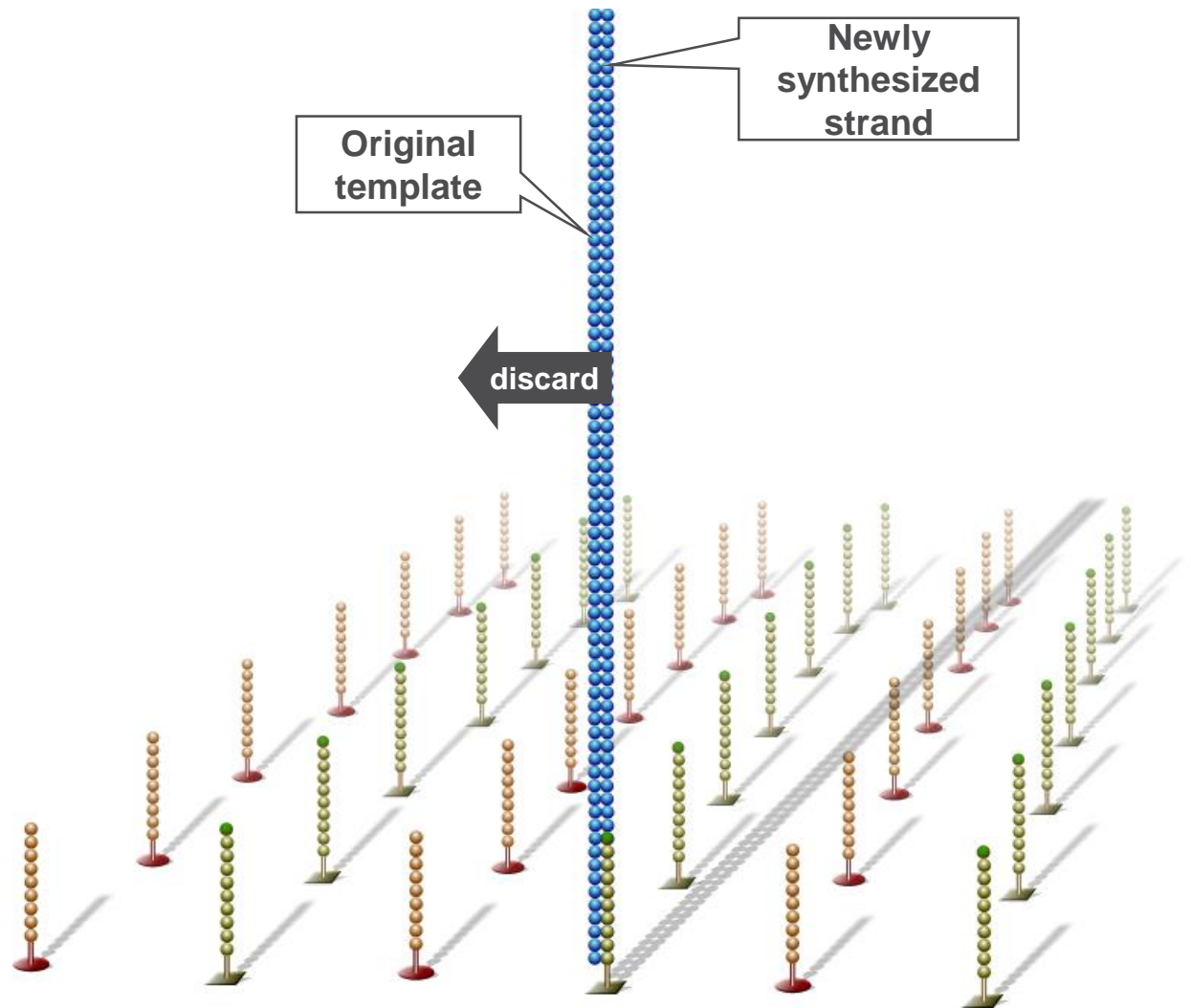


Denature Double-Stranded DNA

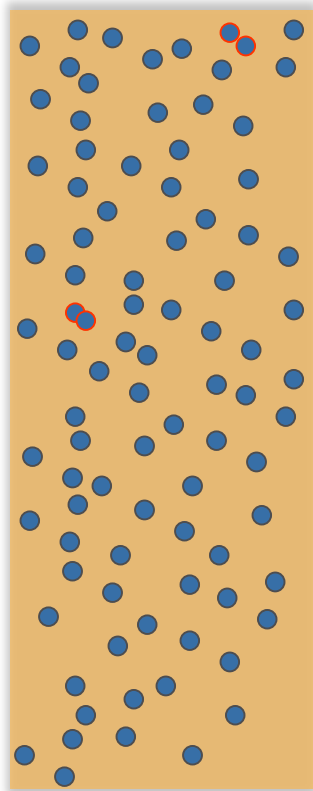
Double-stranded molecule is denatured

Original template washed away

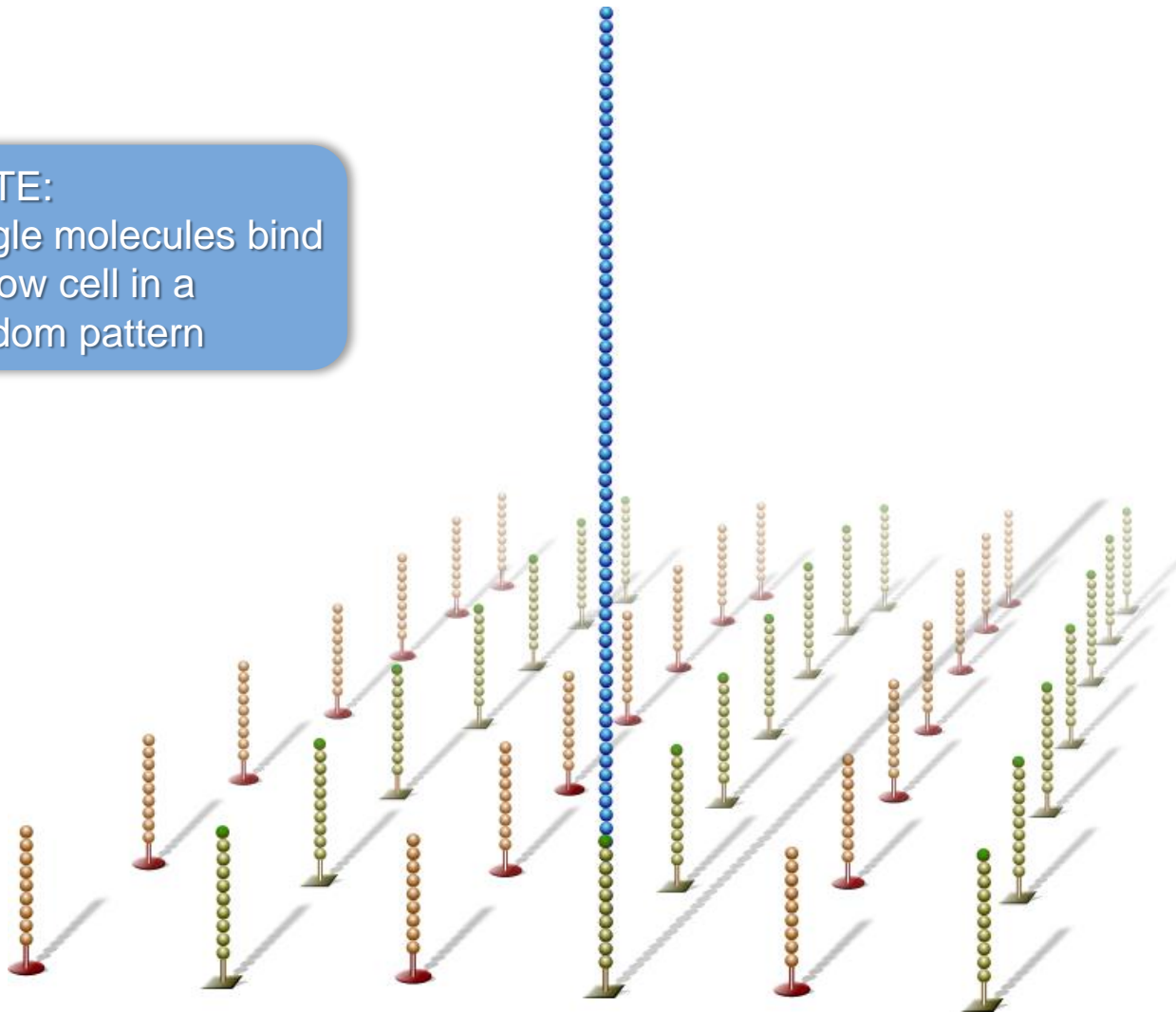
Newly synthesized strand is covalently attached to flow cell surface



Single-Stranded DNA



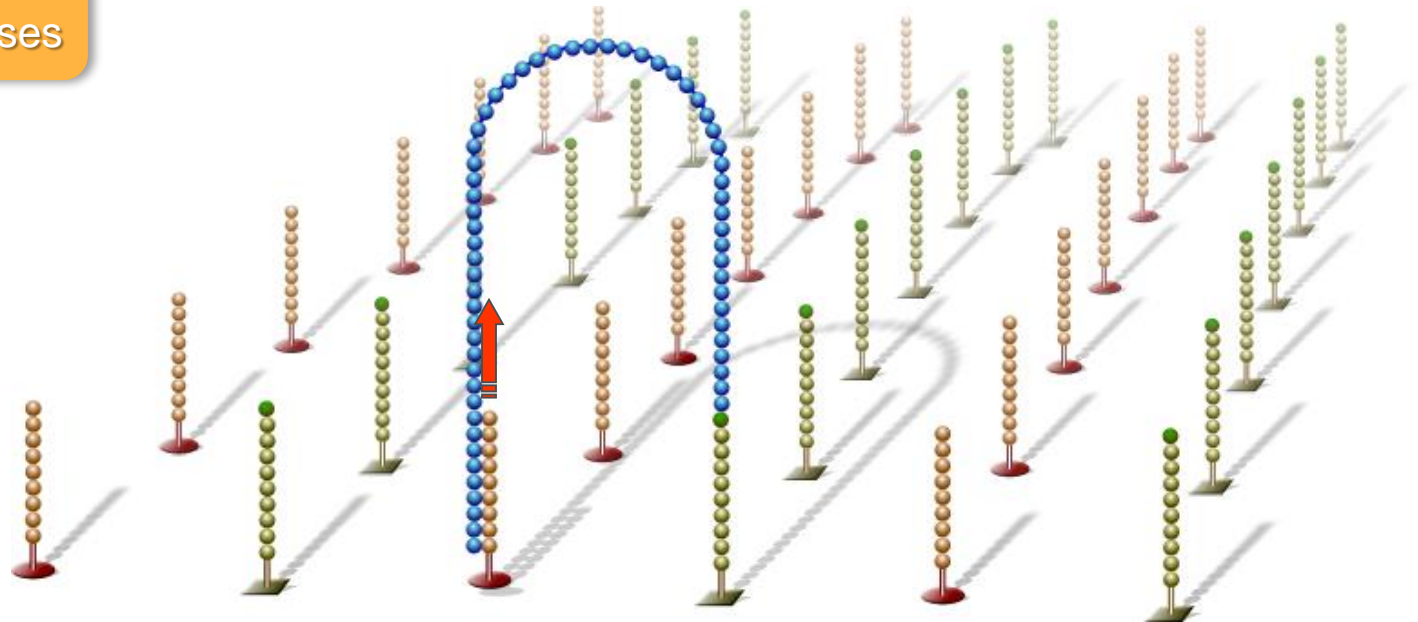
NOTE:
Single molecules bind
to flow cell in a
random pattern



Bridge Amplification

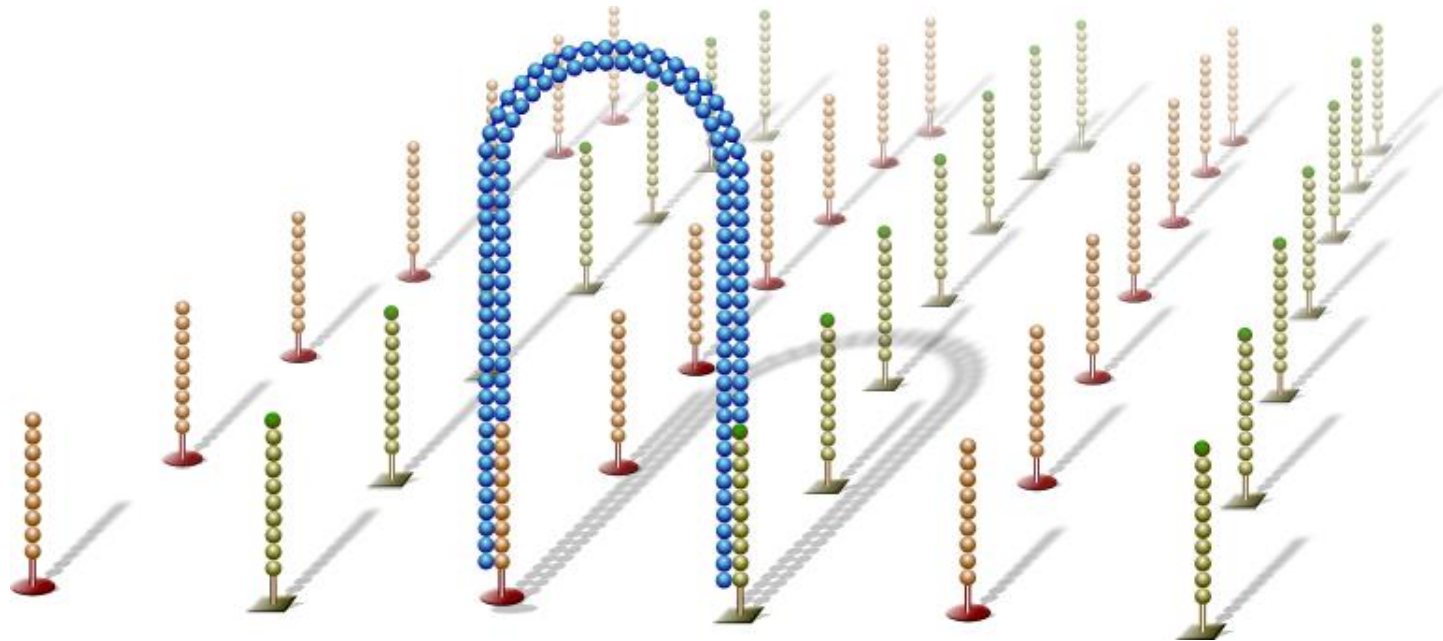
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer

Hybridized primer is extended by polymerases



Bridge Amplification

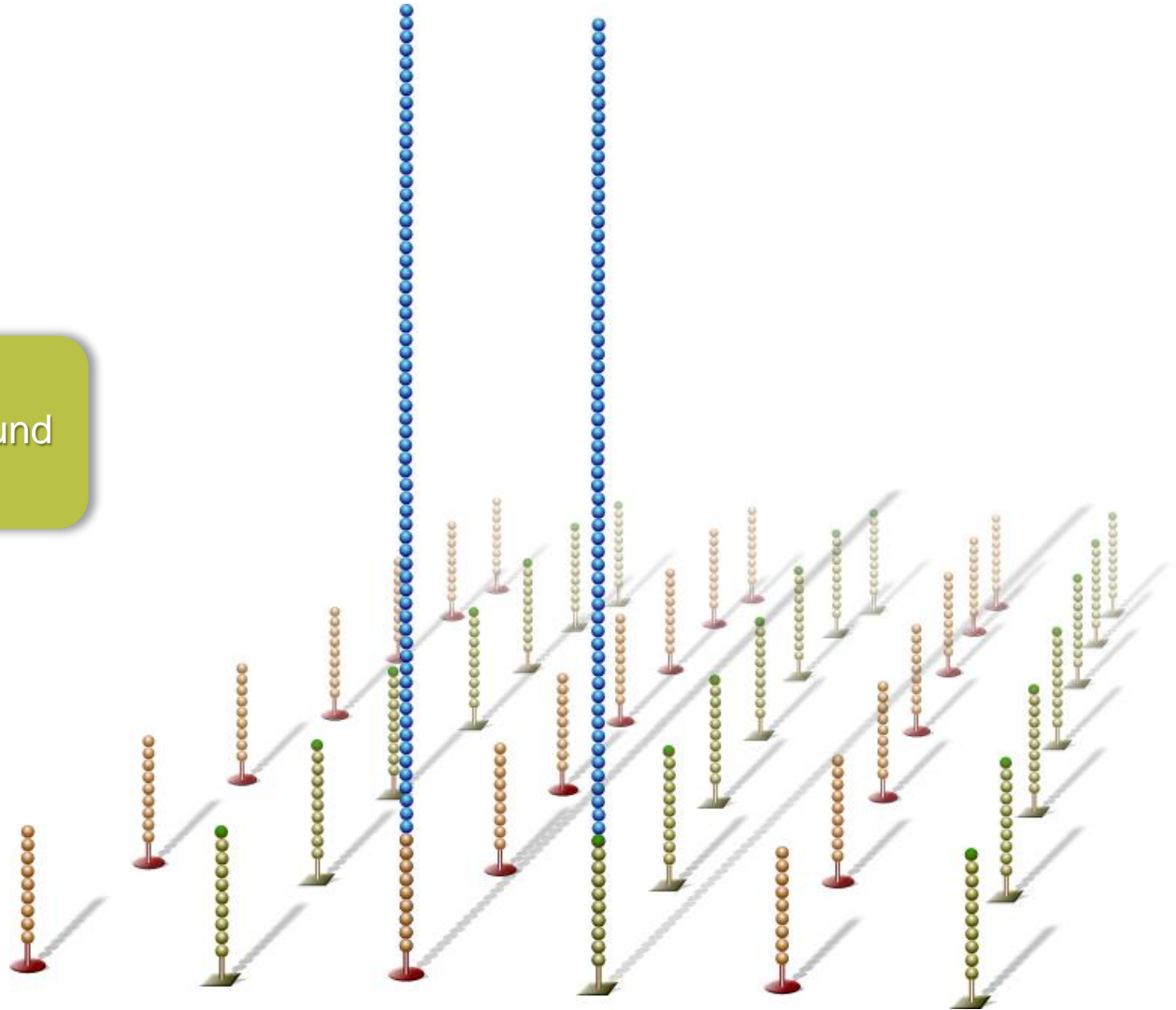
Double-stranded bridge is formed



Denature Double-Stranded Bridge

Double-stranded bridge is denatured

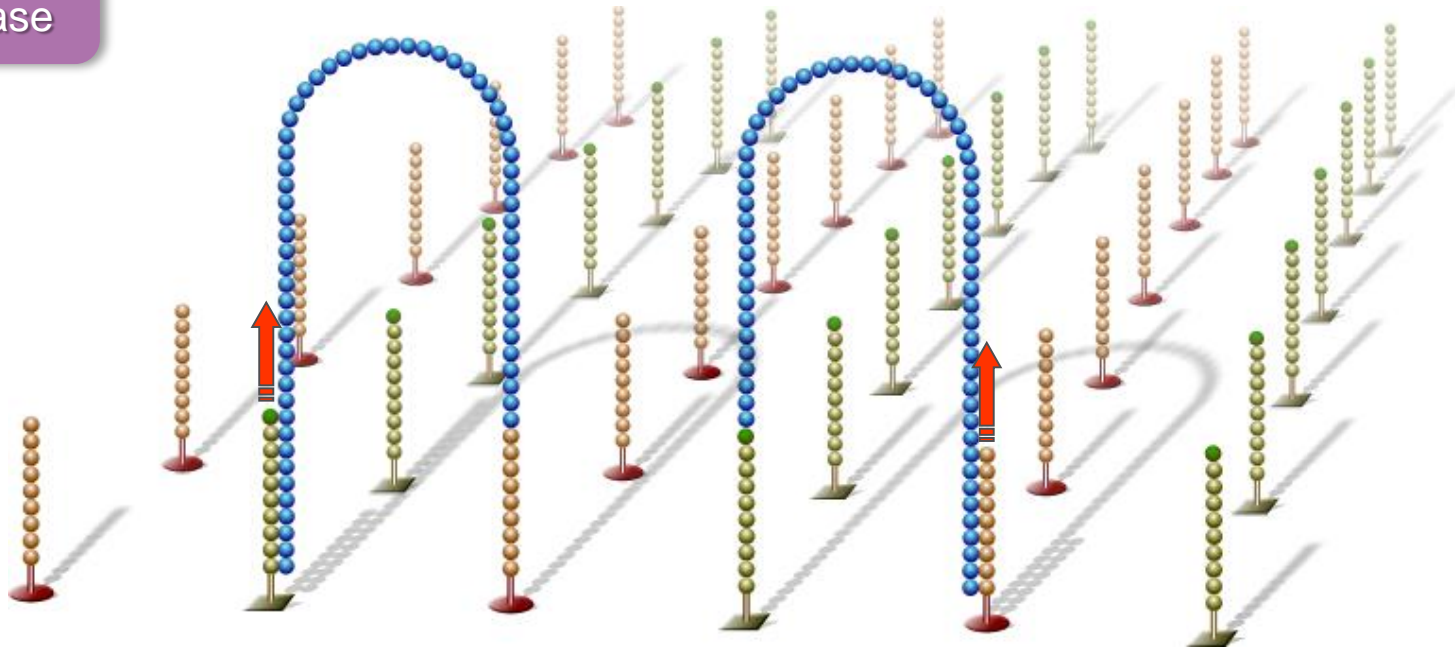
Result:
Two copies of covalently bound single-stranded templates



Bridge Amplification

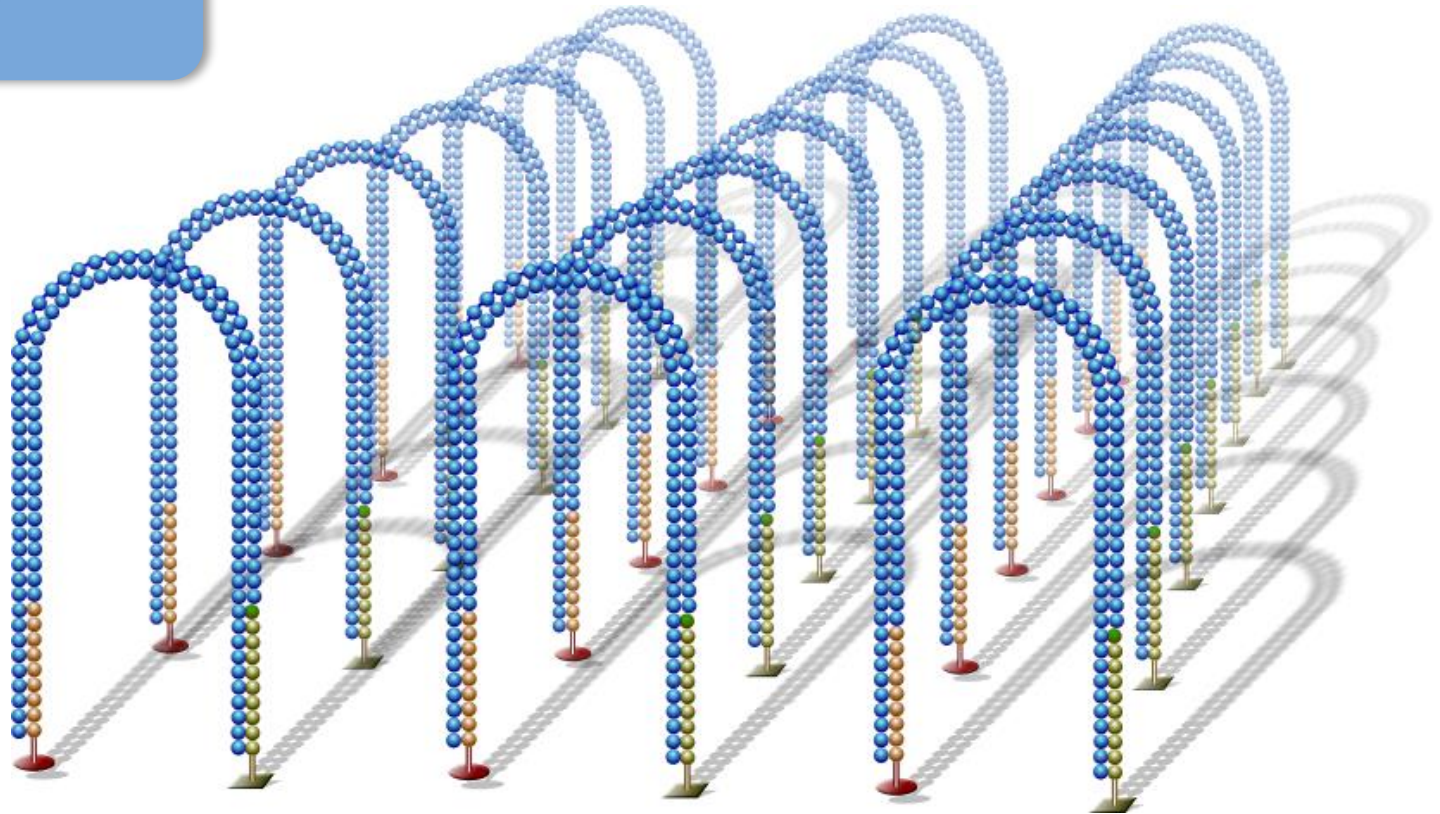
Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase



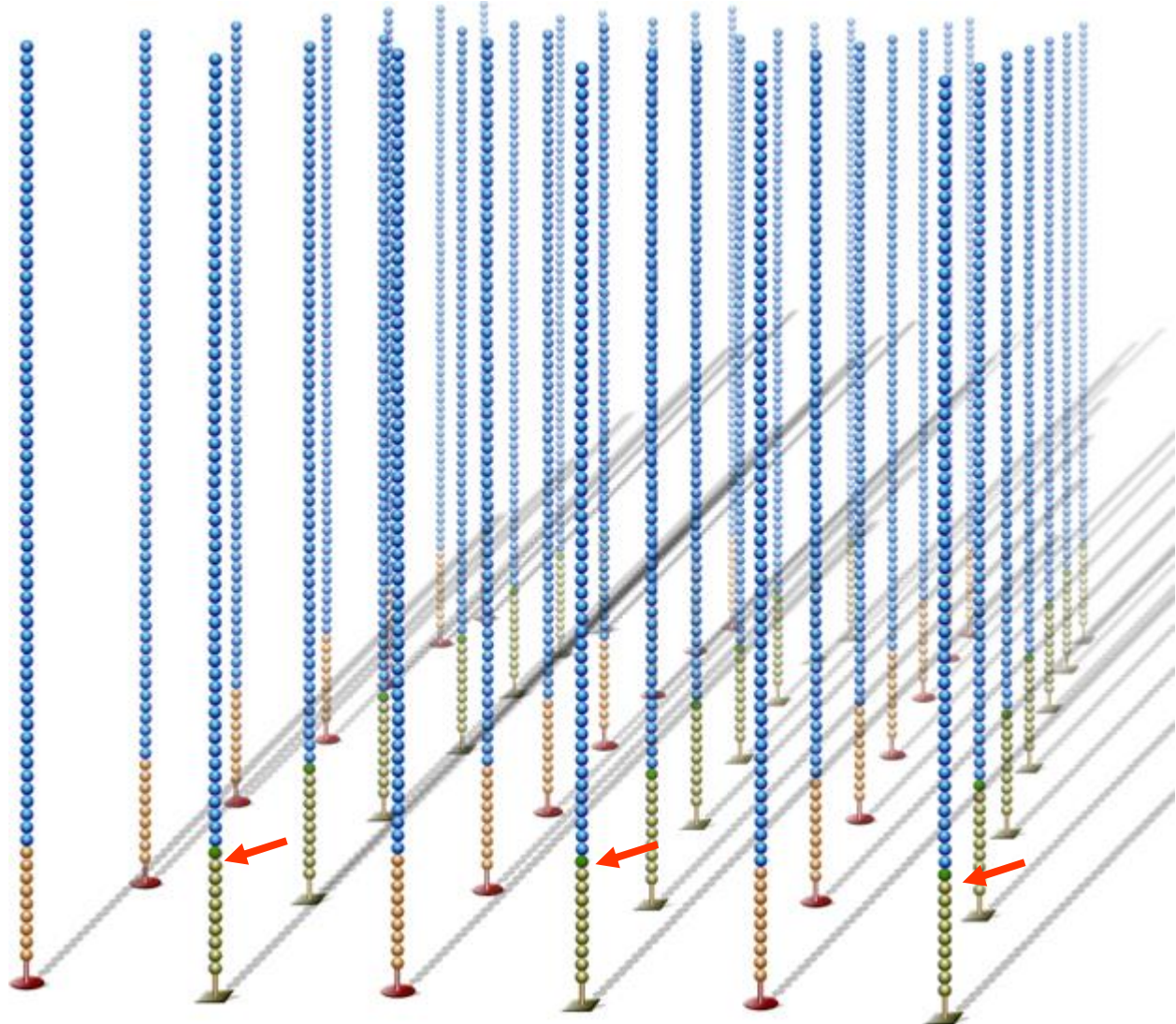
Bridge Amplification

Bridge amplification cycle is repeated until multiple bridges are formed



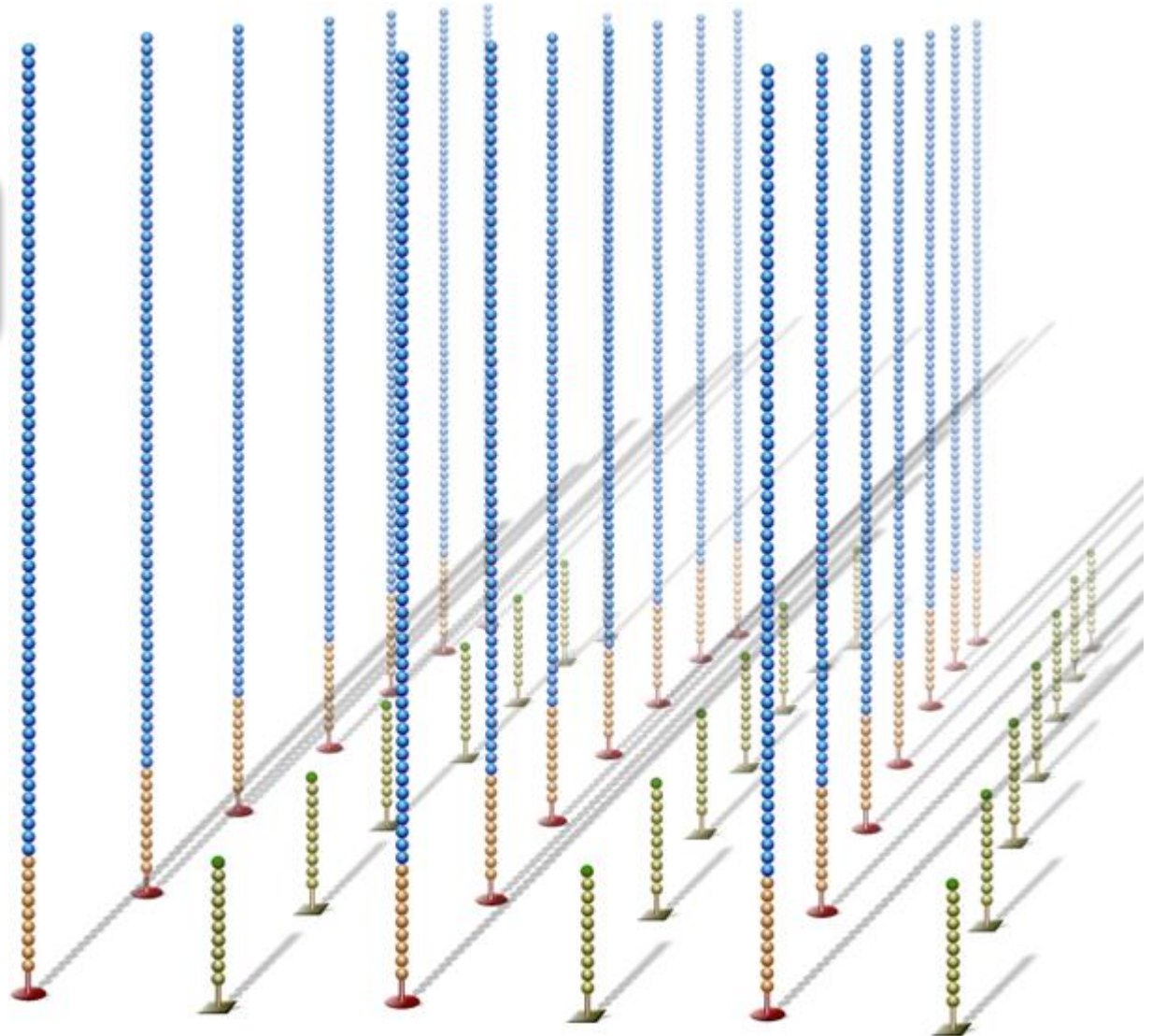
Linearization

dsDNA bridges are denatured



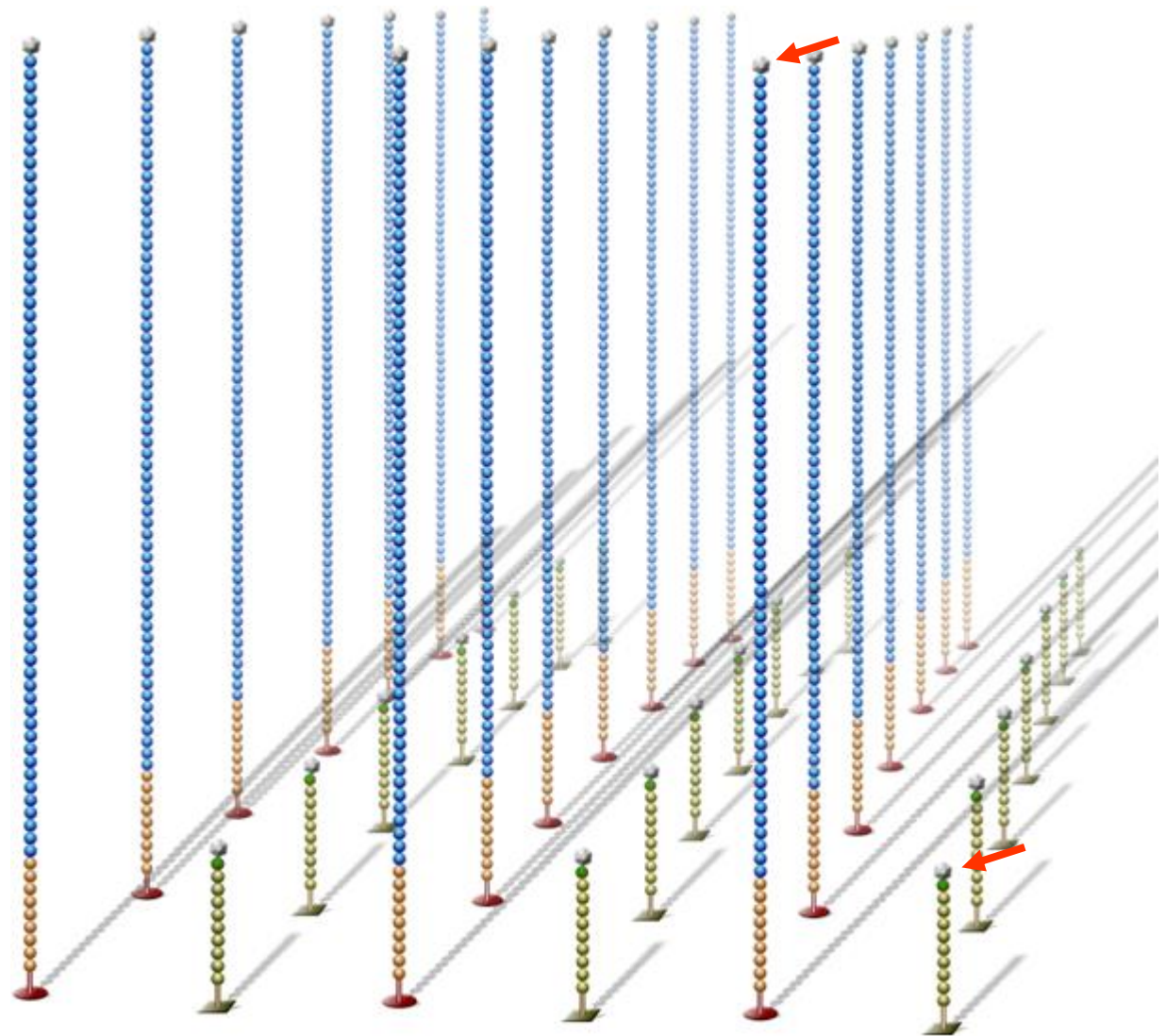
Reverse Strand Cleavage

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



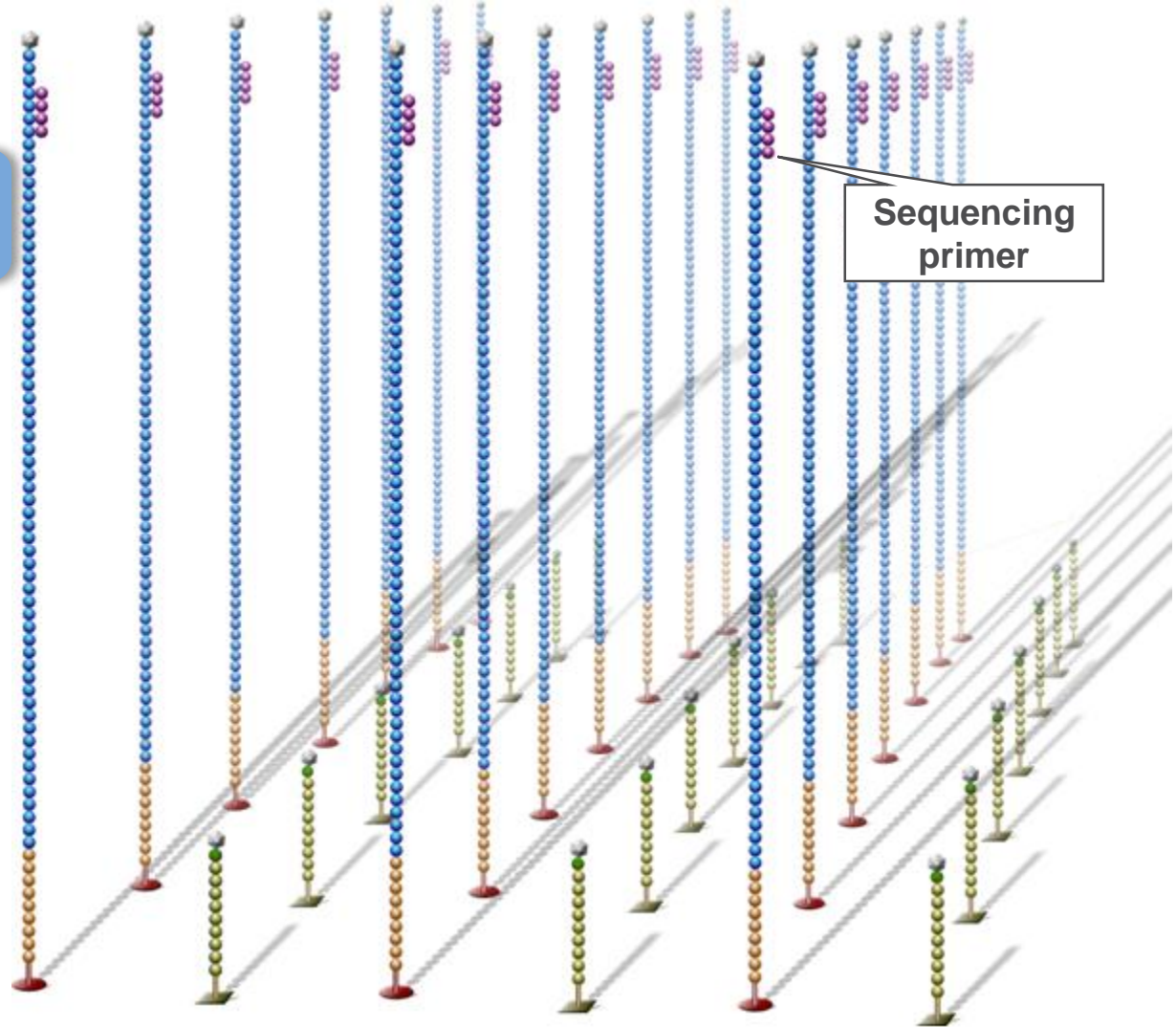
Blocking

Free 3' ends are blocked to prevent unwanted DNA priming



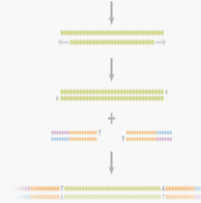
Read 1 Primer Hybridization

Sequencing primer is hybridized to adapter sequence

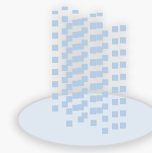


Illumina Sequencing Workflow

1 Library Preparation



2 Cluster Generation



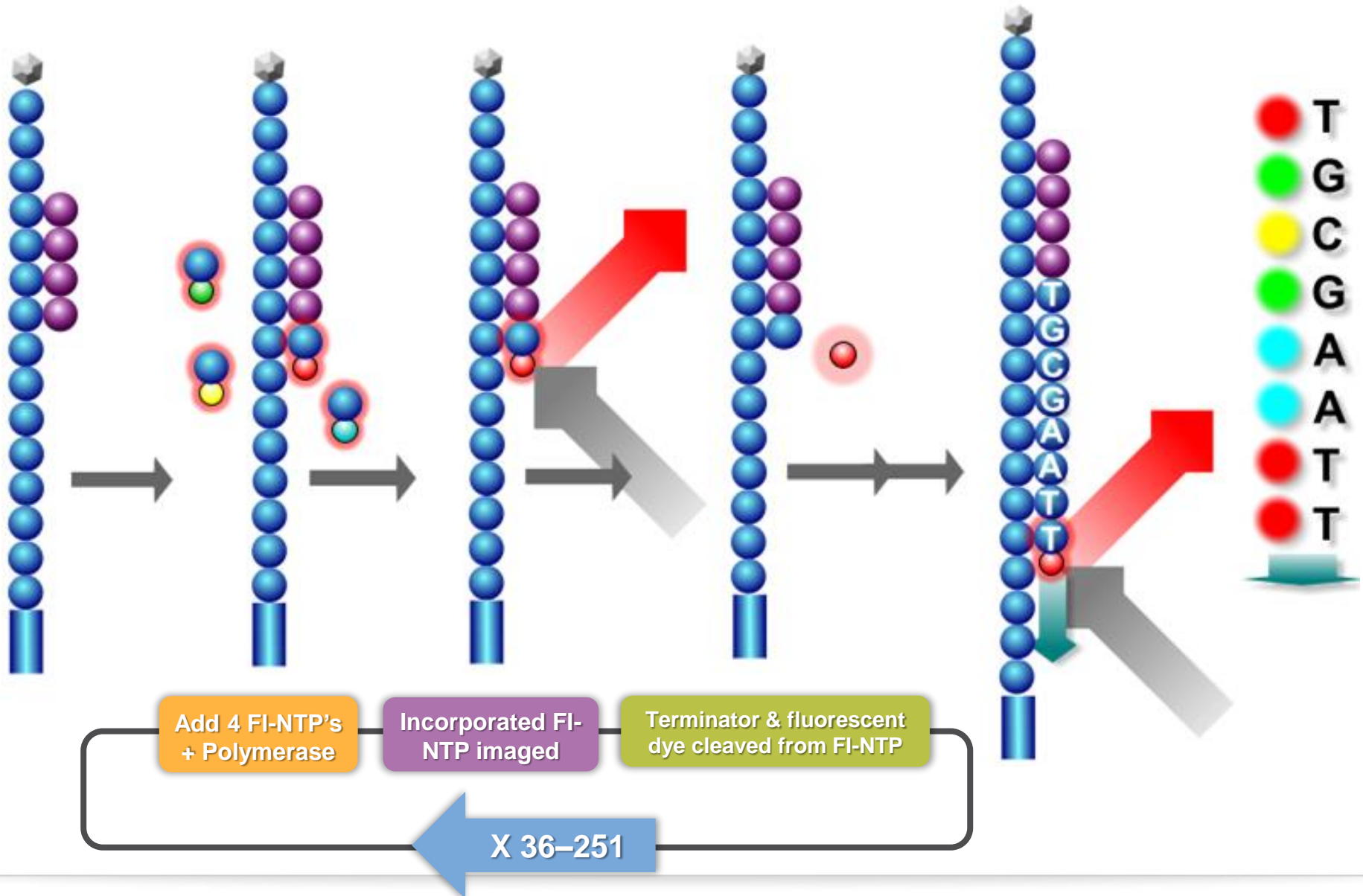
3 Sequencing



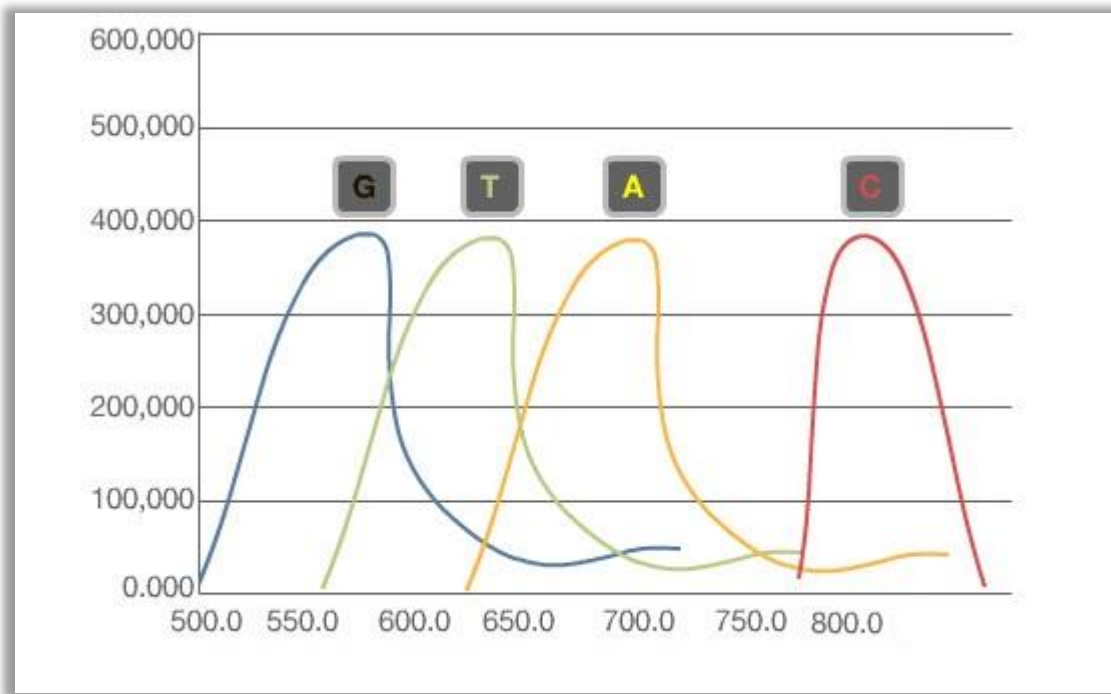
4 Data Analysis

```
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA
```


Sequencing By Synthesis (SBS)



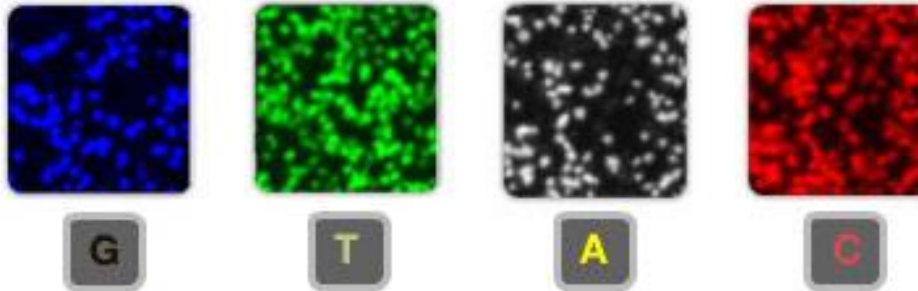
Four Channel SBS Chemistry: GA, HiSeq, MiSeq



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

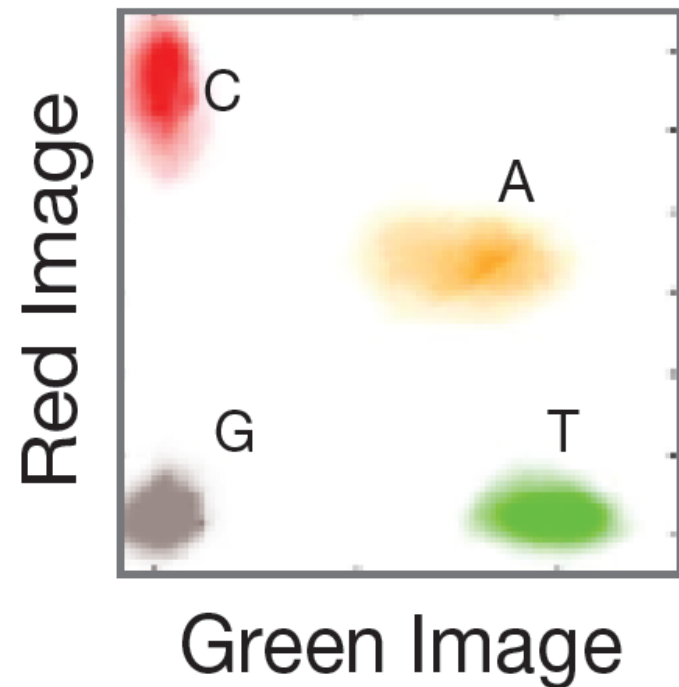
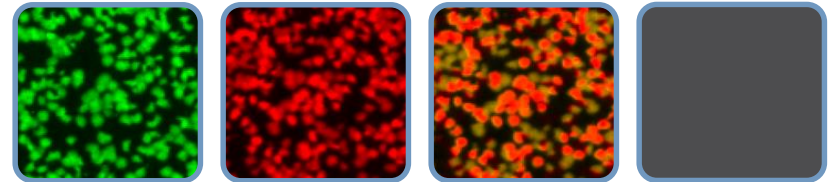
Collects four images:

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

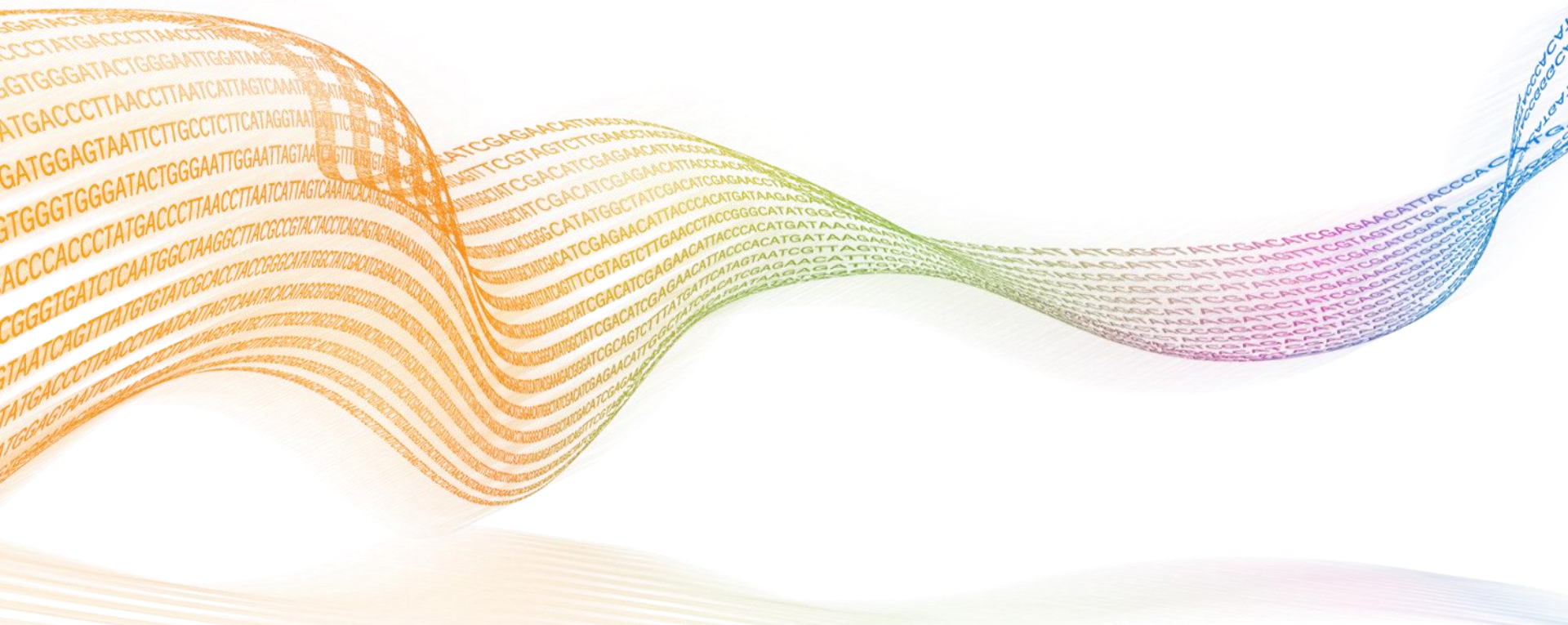


Two Channel SBS – NextSeq 500

- ▶ Two channel SBS uses two images
- ▶ Clusters appearing in green only are **T**
- ▶ Clusters appearing in red only are **C**
- ▶ Clusters appearing in both images are **A**
- ▶ Clusters not present in either green nor red are **G**
- ▶ Cluster intensities are plotted and bases are called accordingly



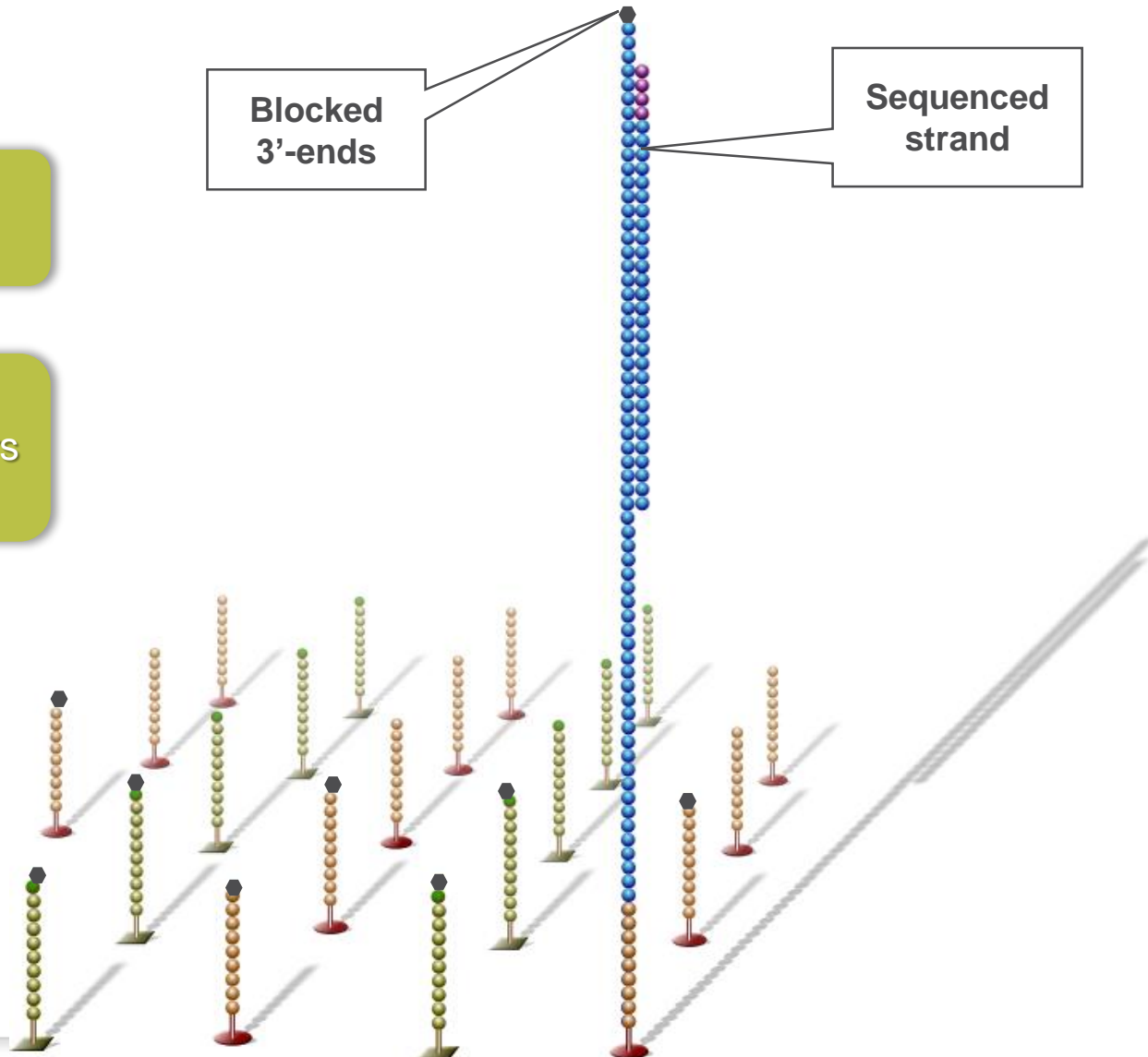
Paired-End Sequencing



Paired-End Sequencing

Sequenced strand is stripped off

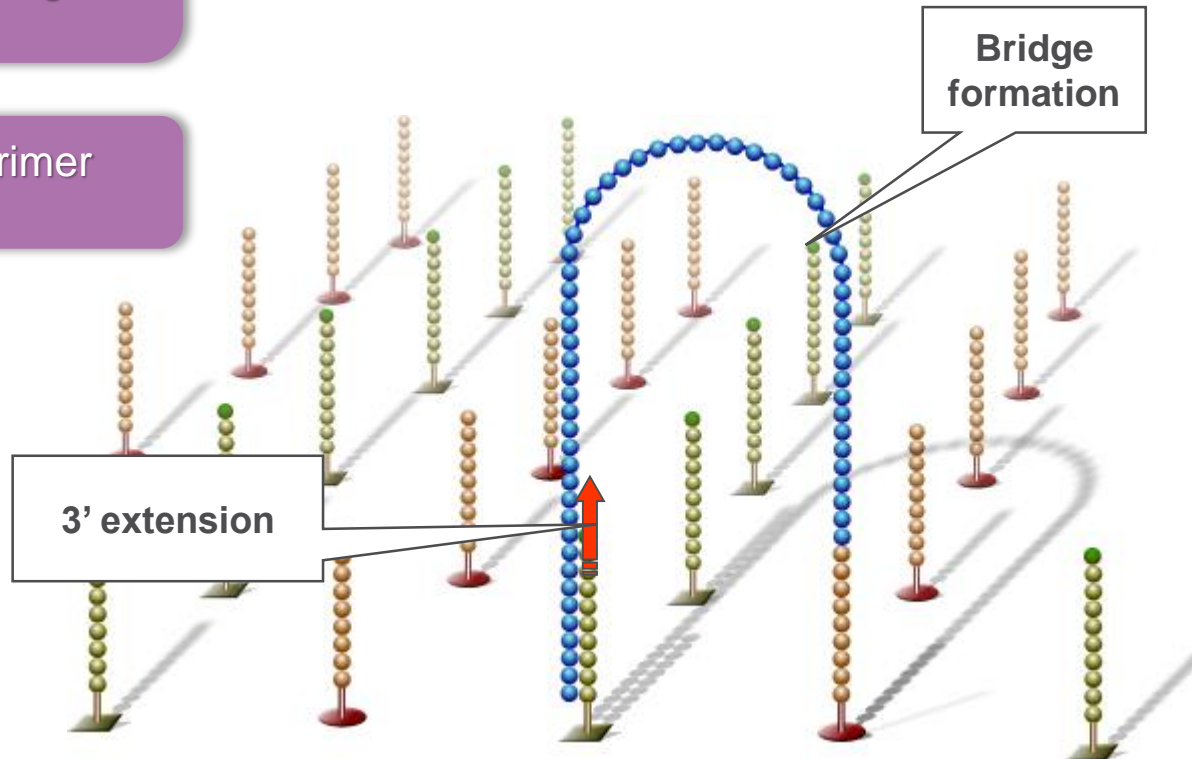
3'-ends of template strands and lawn primers are unblocked



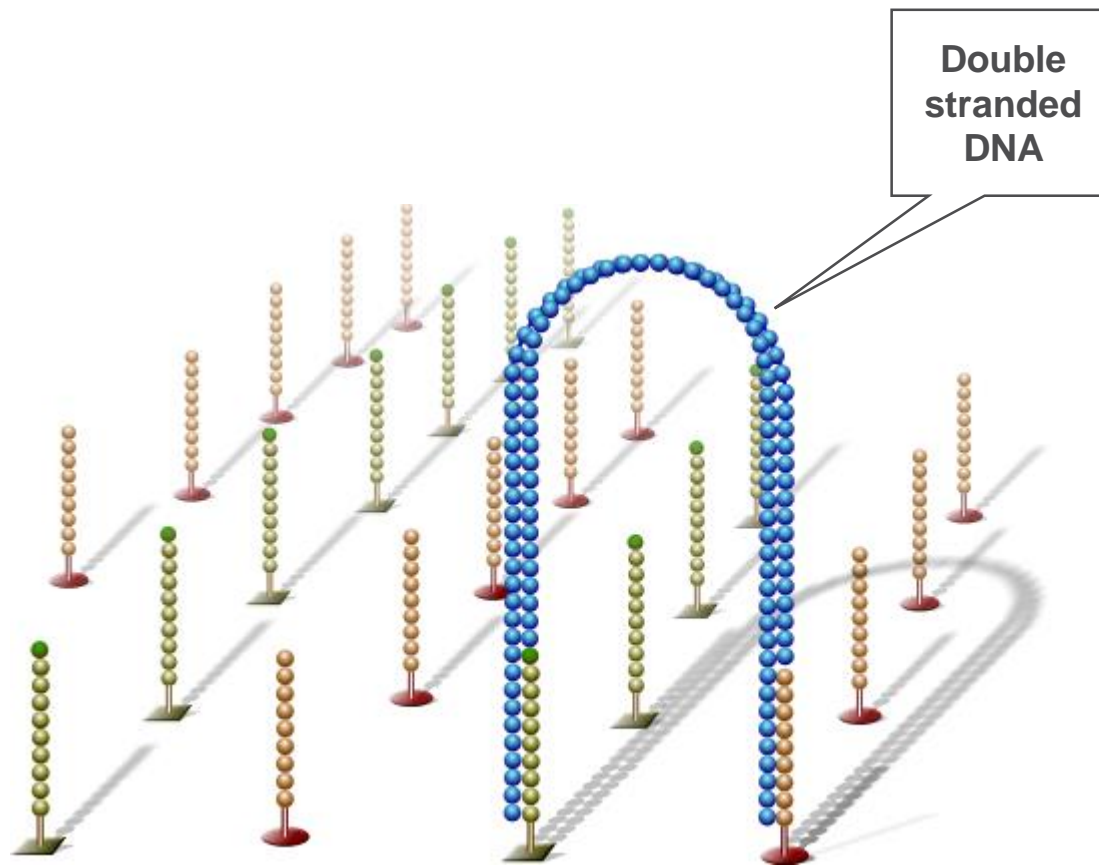
Paired-End Sequencing

Single-stranded template loops over to form a bridge by hybridizing with a lawn primer

3'-ends of lawn primer are extended

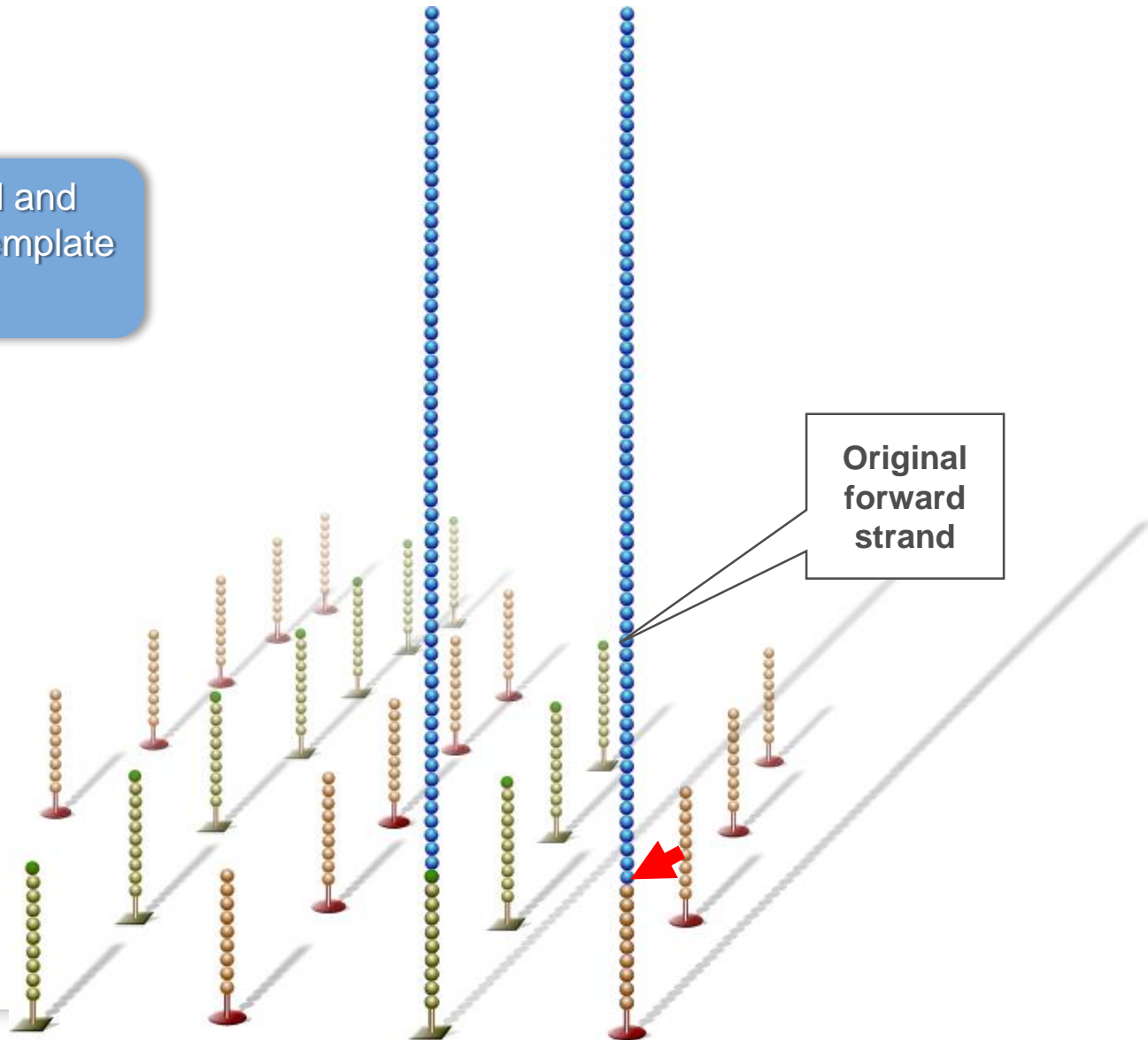


Paired-End Sequencing



Paired-End Sequencing

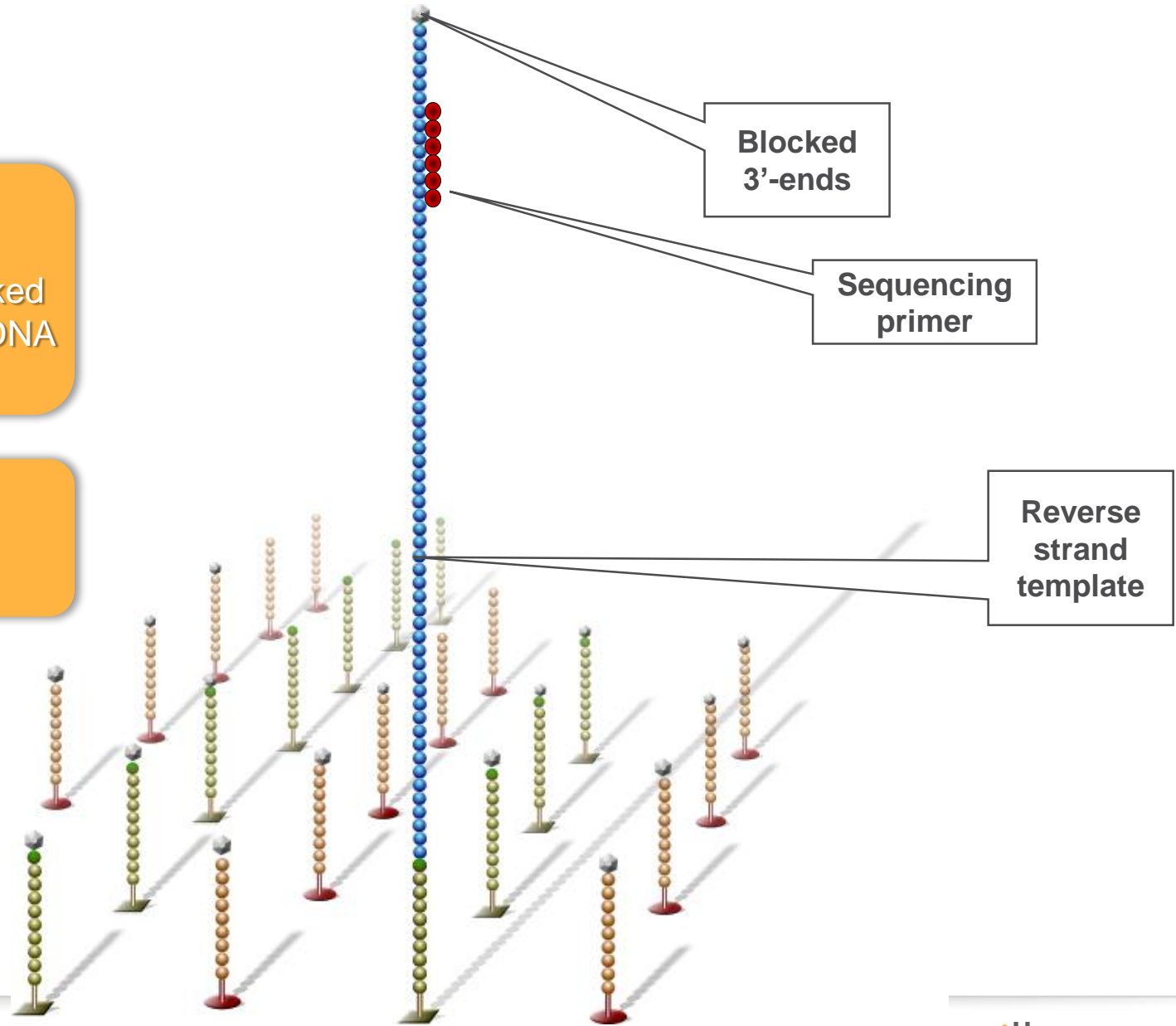
Bridges are linearized and the original forward template is cleaved



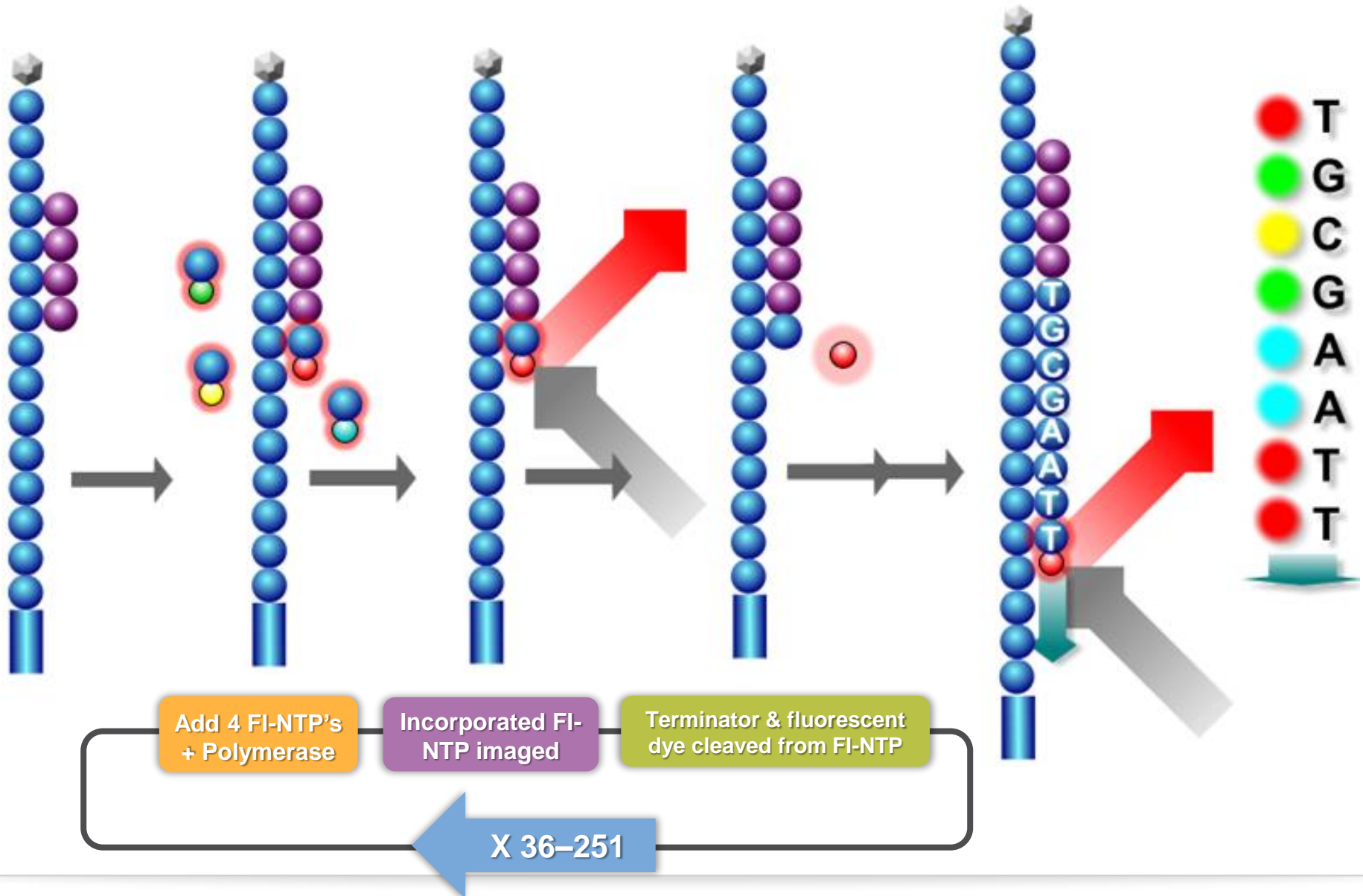
Paired-End Sequencing

Free 3' ends of the reverse template and lawn primers are blocked to prevent unwanted DNA priming

Sequencing primer is hybridized to adapter sequence




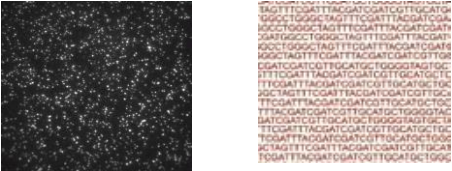




Sequencing By Synthesis 2nd Read



Data Analysis Overview



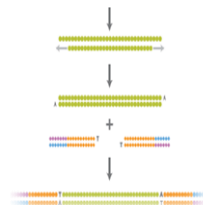
Analysis Overview

Analysis Type	Software	Outputs
Control Software	 <p>ICS/MCS/RTA</p>	 <p>Images, Intensities and Base Calls</p>
Analysis Software	 <p>CASAVA HiSeq Analysis Software MiSeq Reporter</p>	 <p>Alignments, Variant Detection</p>
Visualization Software		 <p>Annotation, Filtering, Reports</p>

Summary

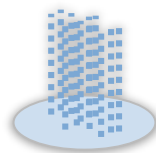
1

Library Preparation



2

Cluster Generation



3

Sequencing

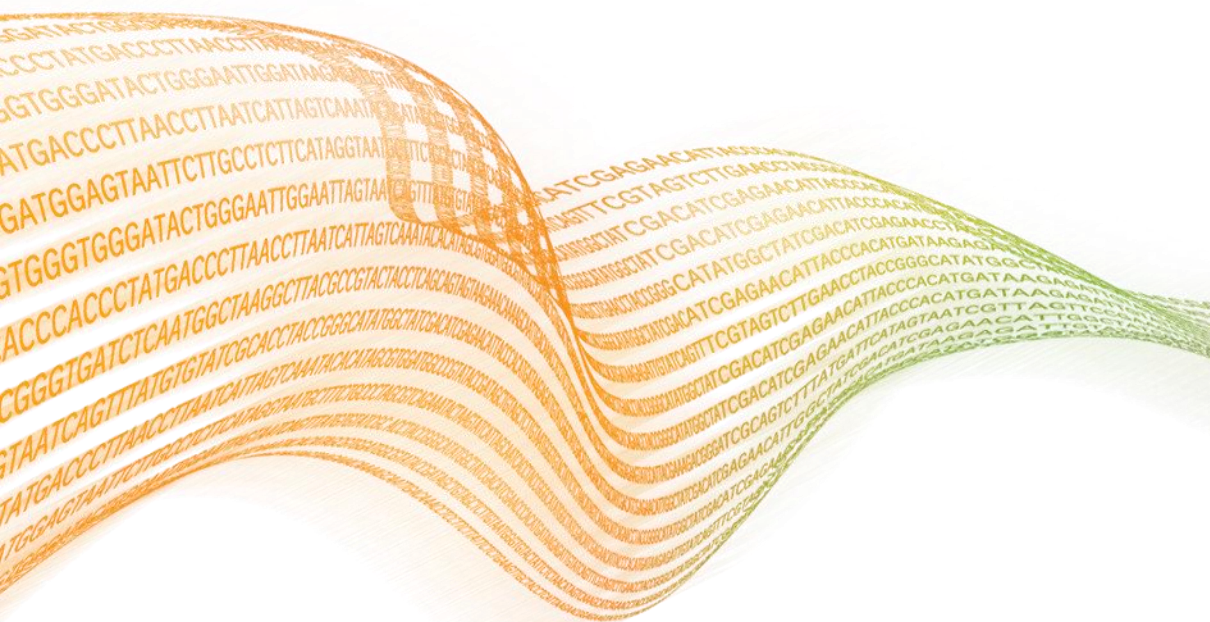


4

Data Analysis

```
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
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3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA  
3TAAGGCTAGGTTTCATGCTA
```

Questions?



AGTCAGGTCAGTCAAGCTTACTG
GGTCAGTCAAGCTTACTGCATCG
TCAGGTCAGTCAAGCTTACTGAA
GAGTCAGTCAAGCTTACTG
AAGTTAGTCAAGCTTACTG
CTTAAGTCAAGCTTACTG
GGTCAGTCAAGCTTACTG
AGTCAGGTCAGTCAAGCTTACTG
GGTCAGTCAAGCTTACTG
TCAGGTCAGTCAAGCTTACTGAA
GAGTCAGTCAAGCTTACTG
AAGTTAGTCAAGCTTACTG
CTTAAGTCAAGCTTACTG
AGTCAGGTCAGTCAAGCTTACTG
CTTAAGTCAAGCTTACTG
GGTCAGTCAAGCTTACTG
AGTCAGGTCAGTCAAGCTTACTG

