Illumina Sequencing Overview

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Session Objectives

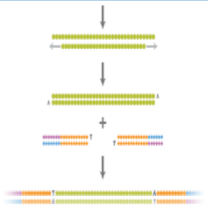
- By the end of this training, you will be able to:
  - List the major steps in the Illumina sequencing workflow
  - Describe cluster generation
  - Discuss the Sequencing By Synthesis process

Sequencing Workflow Review

Illumina Sequencing Workflow

1

Library Preparation



2

Cluster Generation

cBot
MiSeq

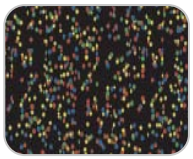
3

Sequencing

HiSeq
HiScan SQ
GA IIx
MiSeq

4

Data Analysis



ICS/RTA
CASAVA
MSR
BaseSpace

Illumina Sequencing Workflow

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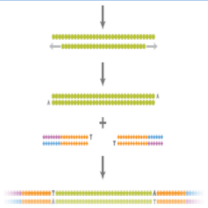

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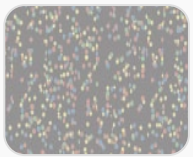

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Sample Prep is Critical for Successful sequencing

Dual Index Library shown

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

Illumina Sequencing Workflow

1

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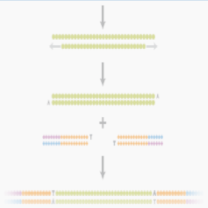

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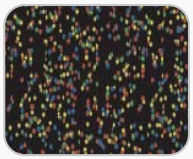

ICS/RTA
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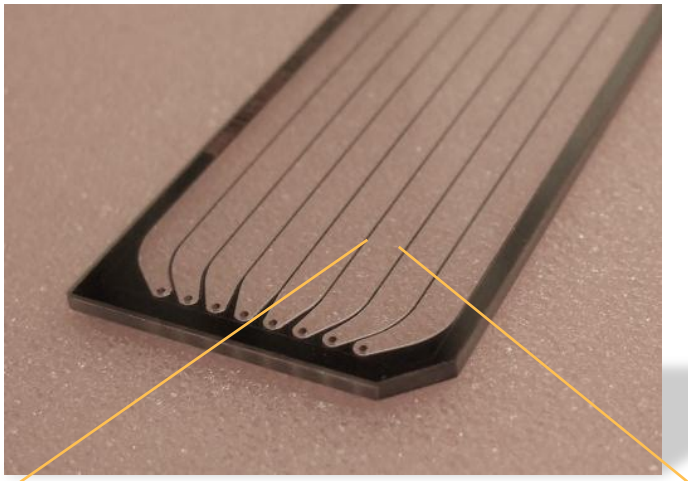
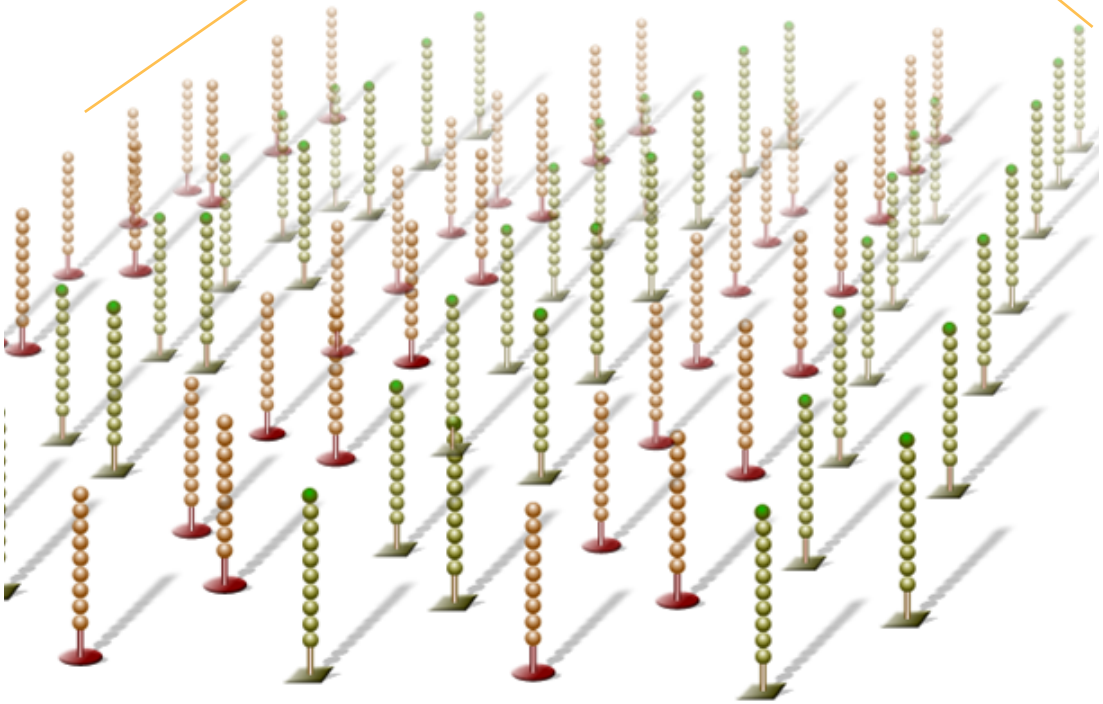
What is a Flow Cell?

Cluster generation occurs on a flow cell

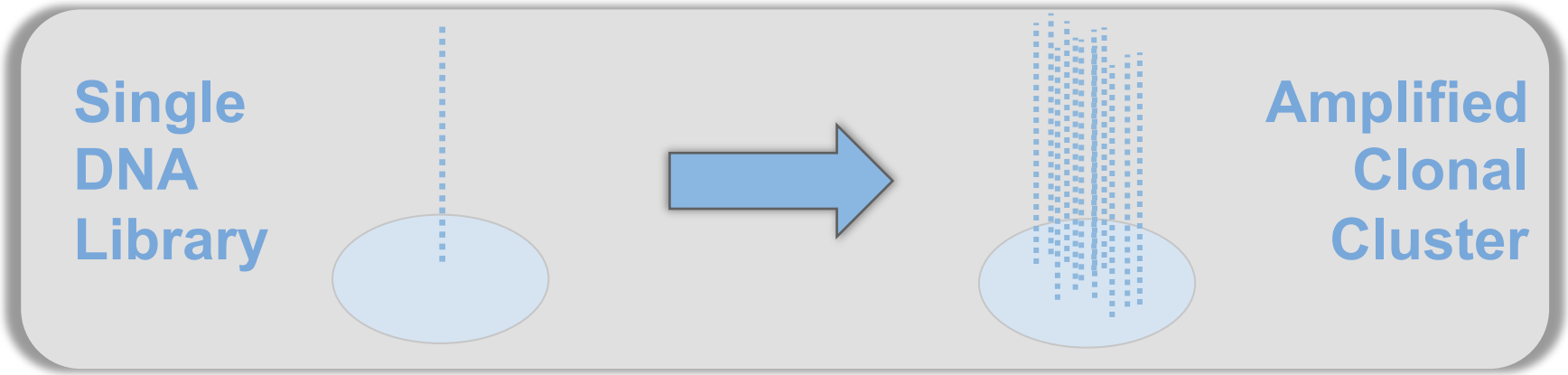
A flow cell is a thick glass slide with channels or lanes

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

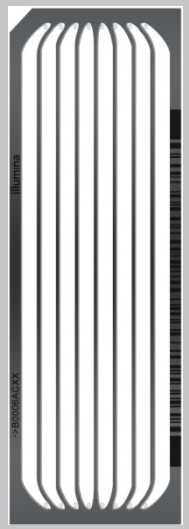




Instrumentation



cBot

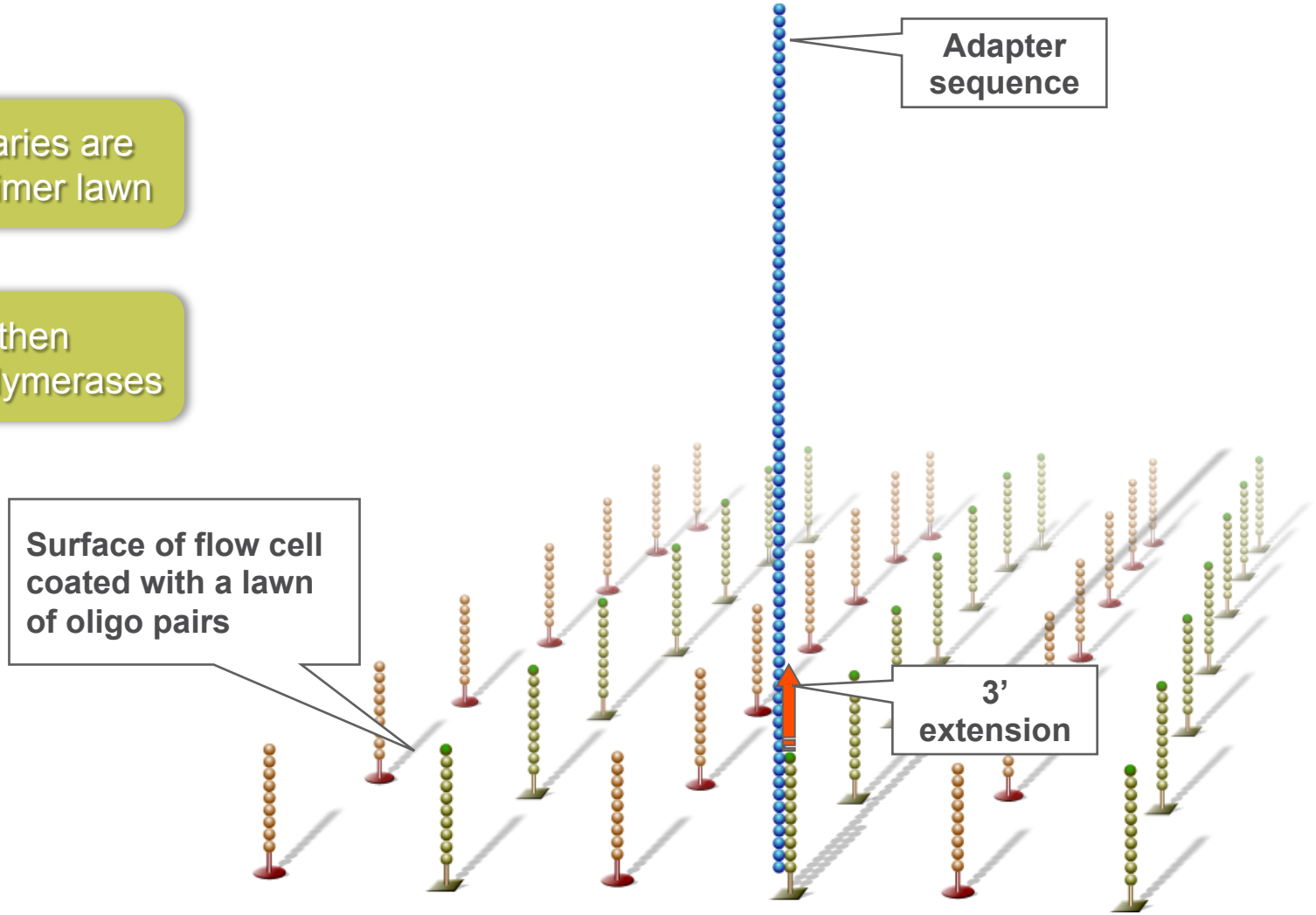


Sequencer

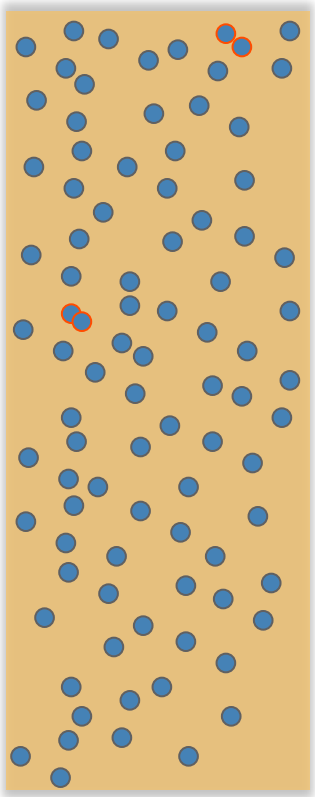
Hybridize Fragment & Extend

Single DNA libraries are hybridized to primer lawn

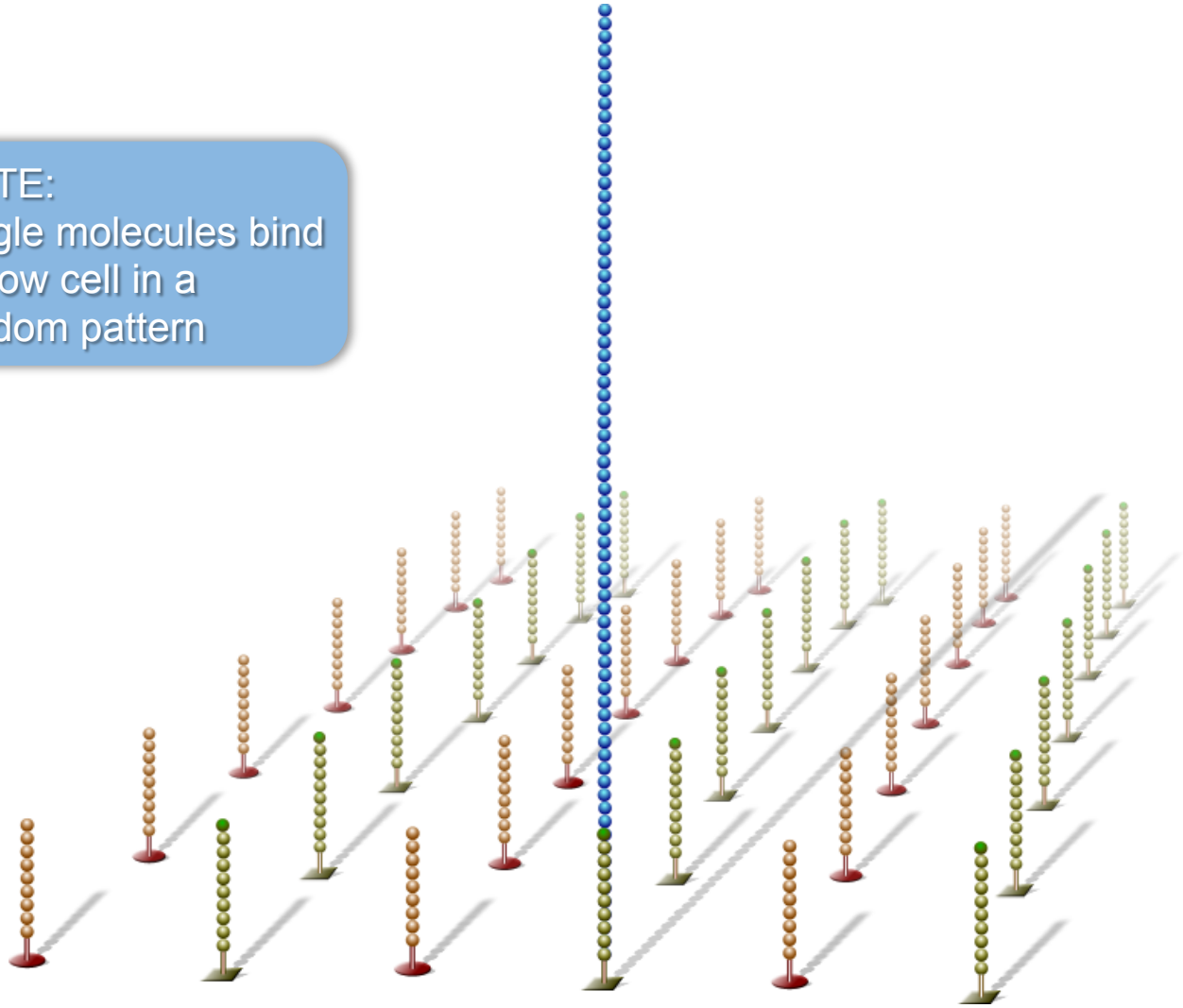
Bound libraries then extended by polymerases



Hybridize Fragment & Extend

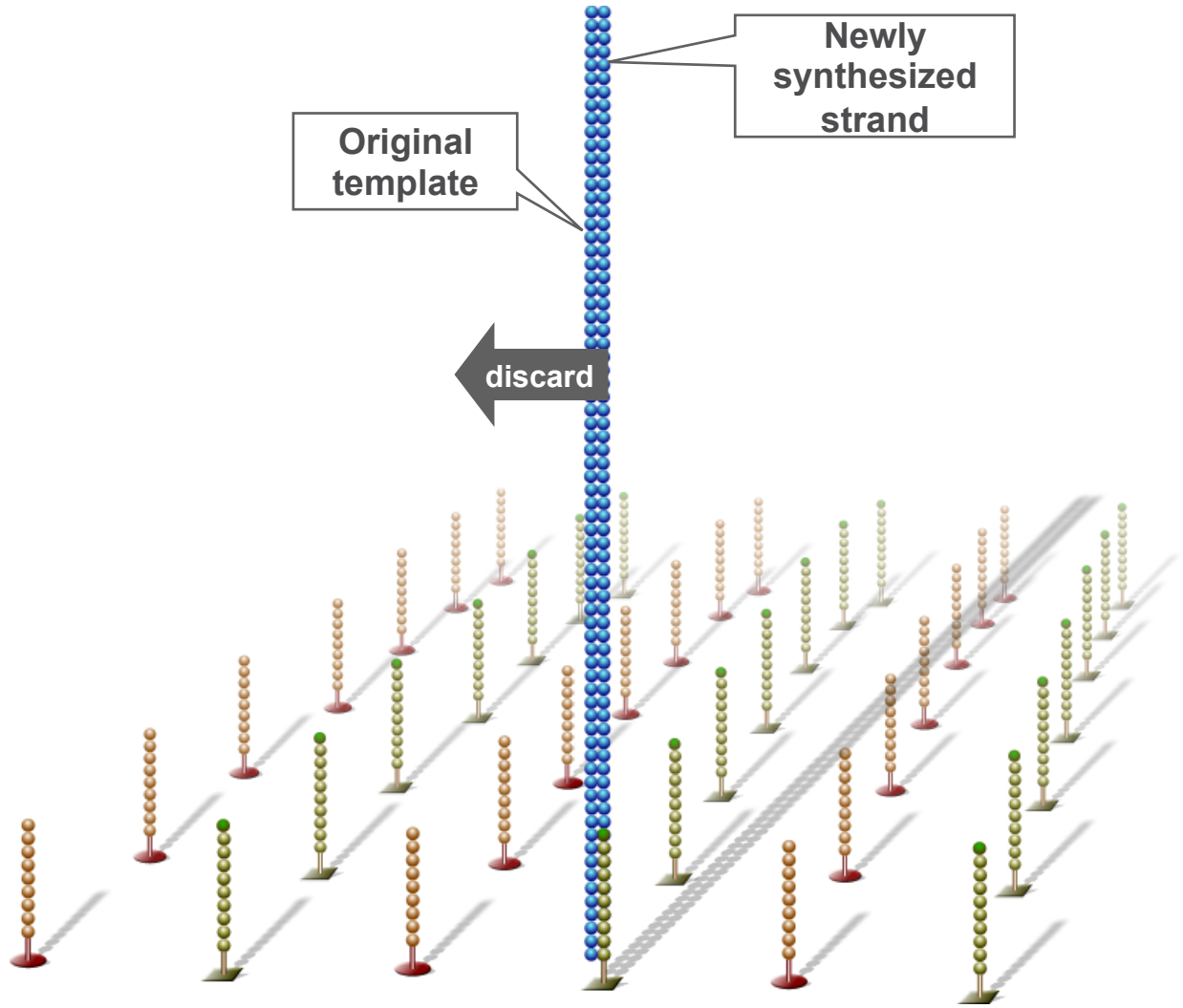


NOTE:
Single molecules bind to flow cell in a random pattern



Denature Double-stranded DNA

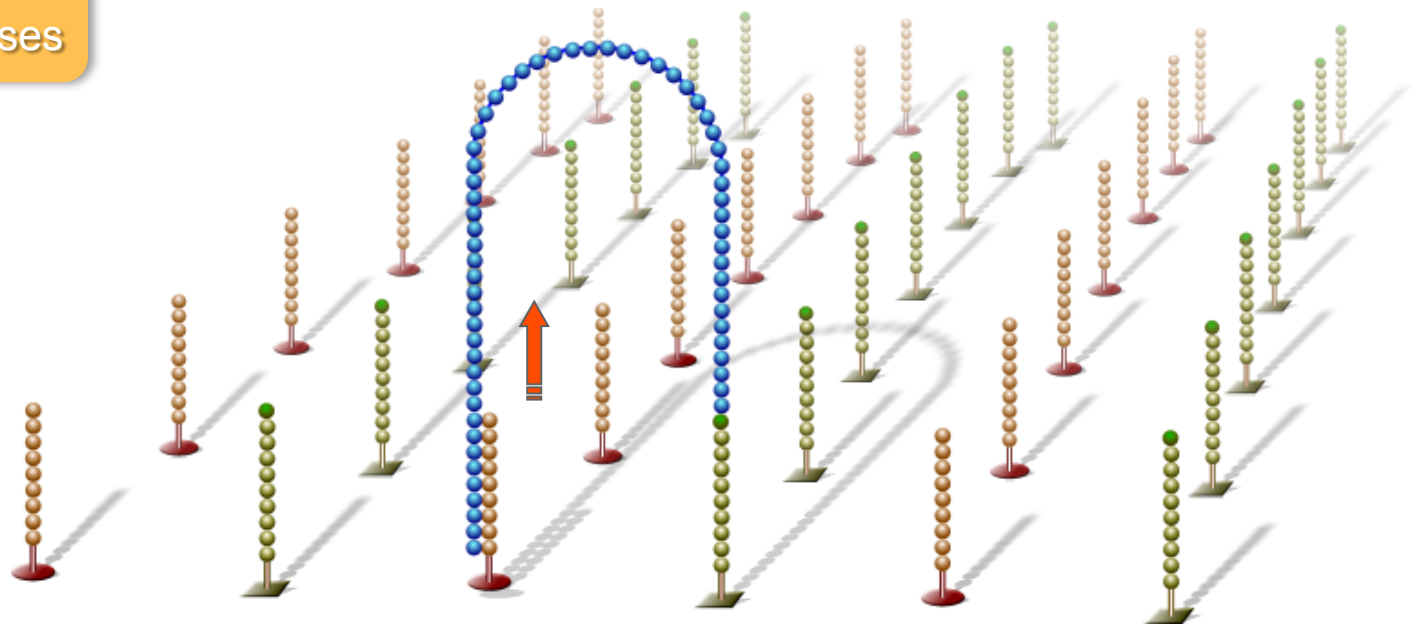
- Double-stranded molecule is denatured
- Original template washed away
- Newly synthesized strand is covalently attached to flow cell surface



Bridge Amplification

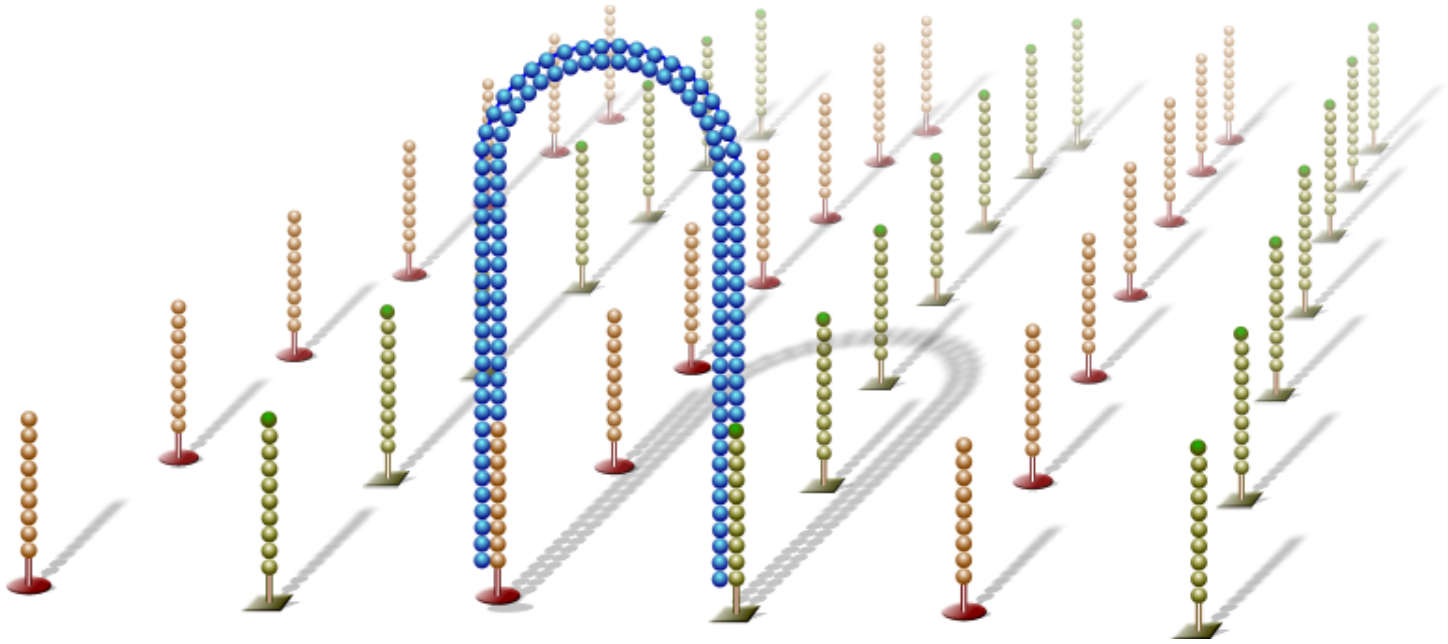
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer

Hybridized primer is extended by polymerases



Bridge Amplification

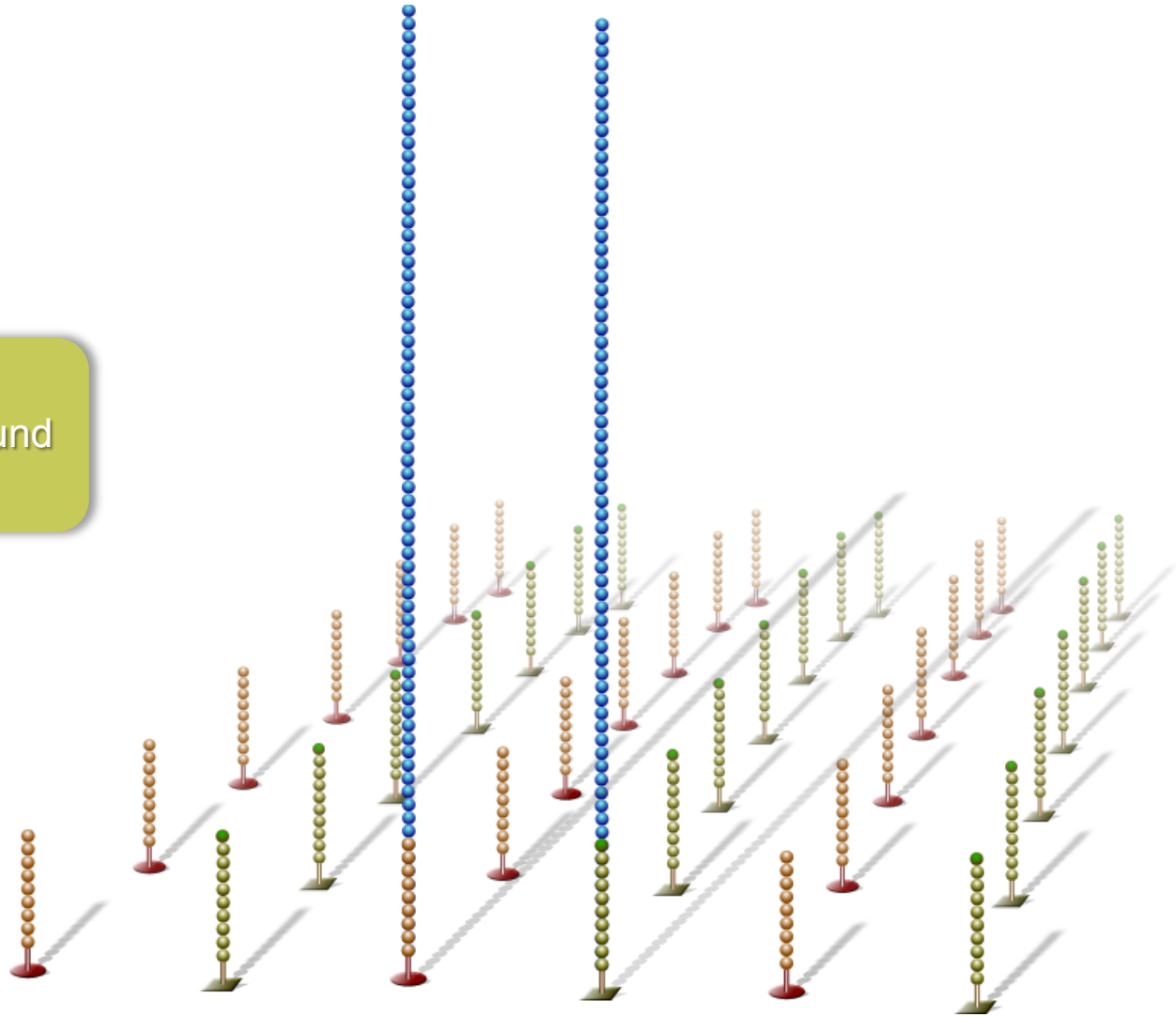
Double-stranded bridge is formed



Denature Double-stranded Bridge

Double-stranded bridge is denatured

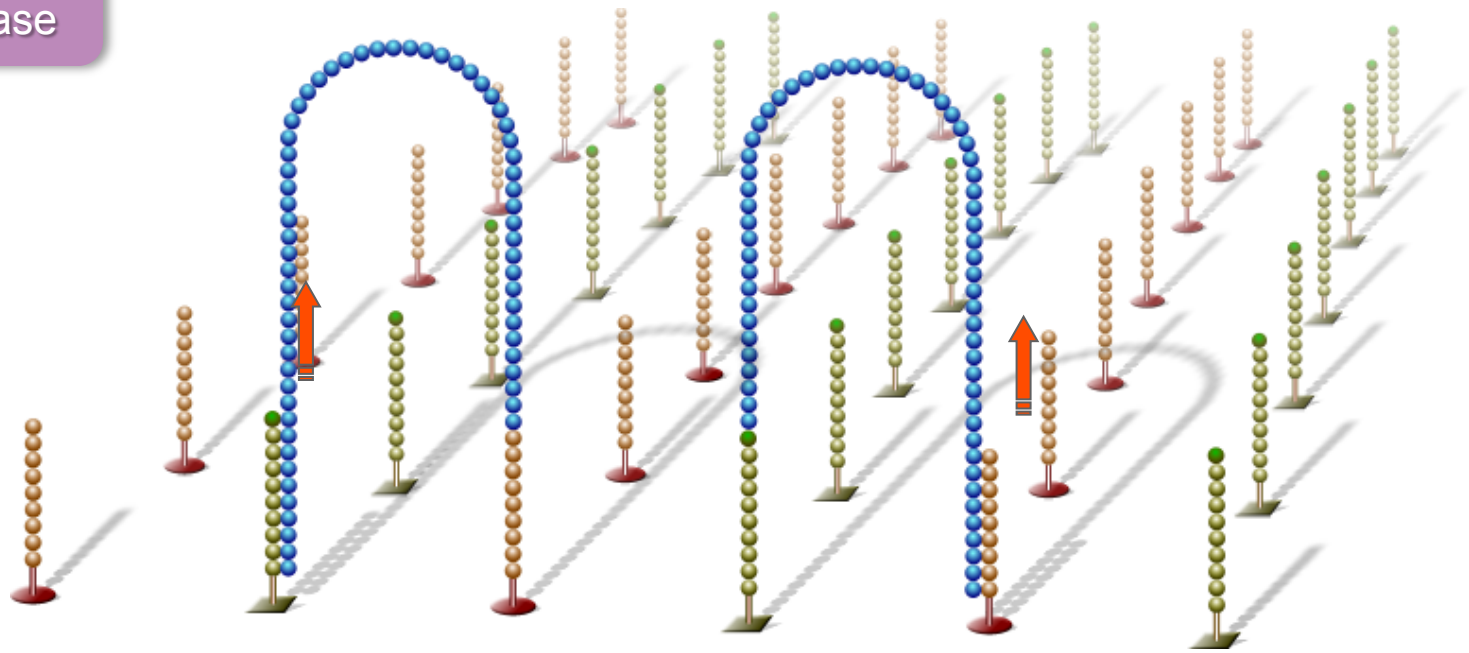
Result:
Two copies of covalently bound single-stranded templates



Bridge Amplification

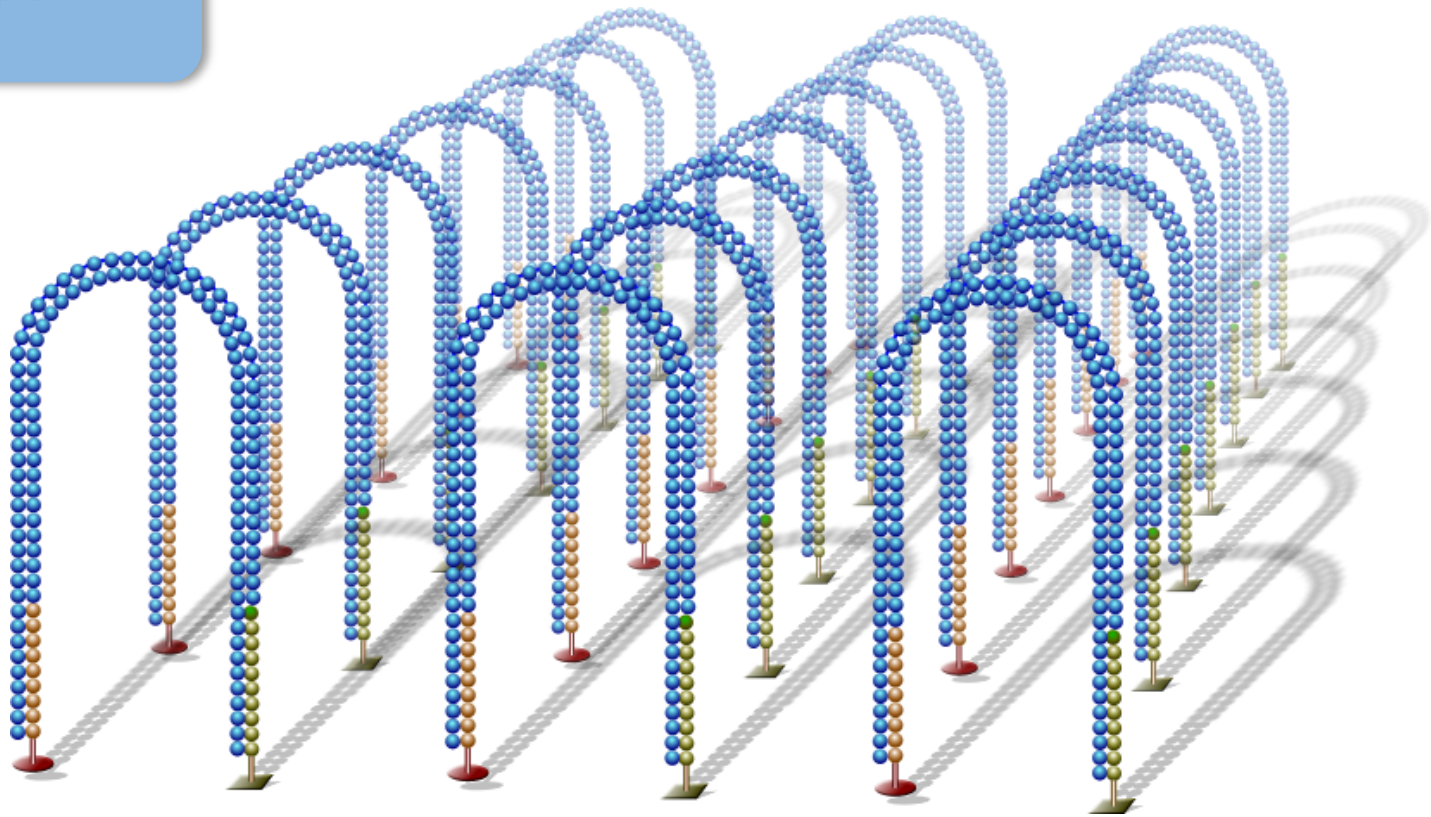
Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase



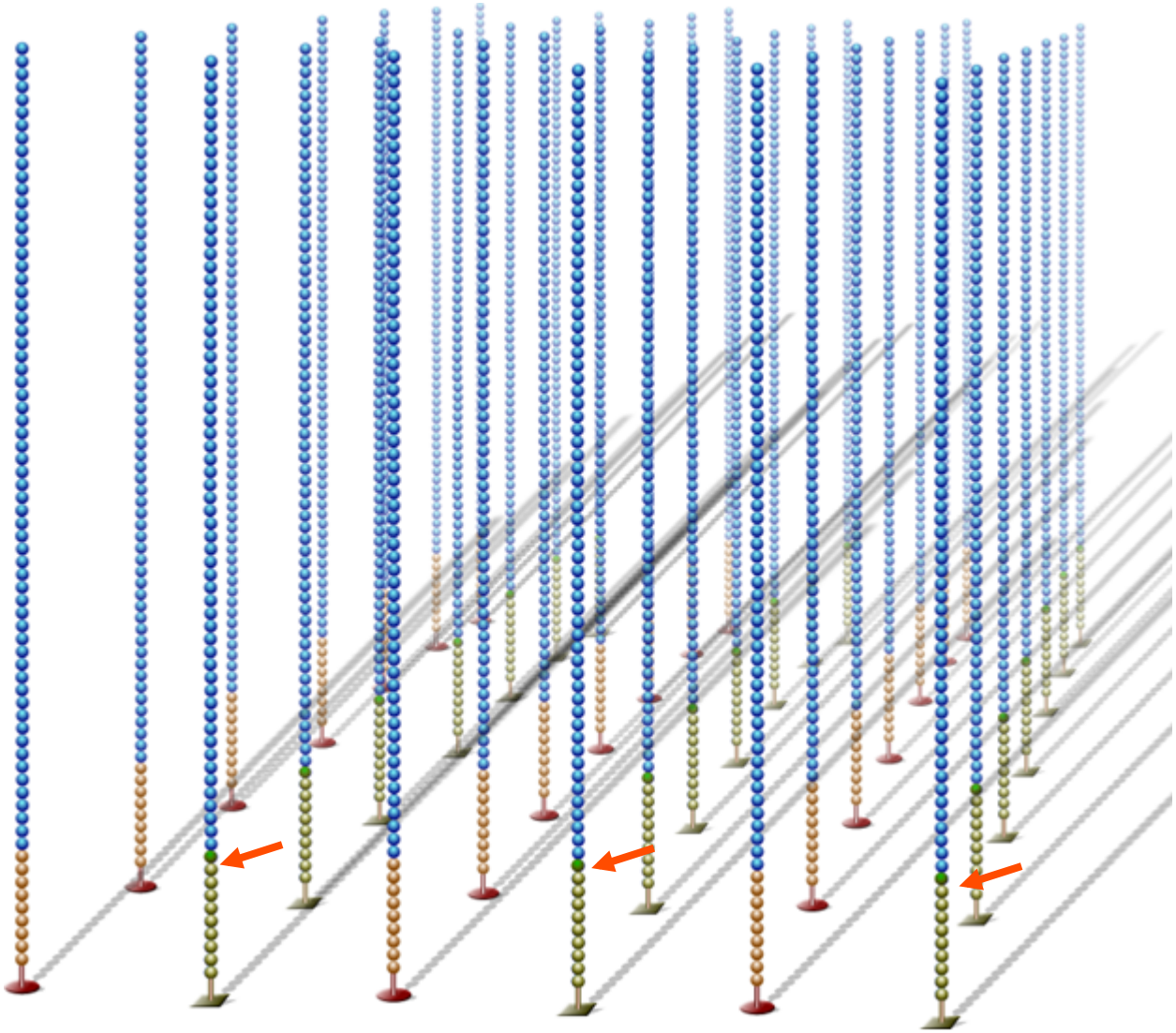
Bridge Amplification

Bridge amplification cycle repeated until multiple bridges are formed



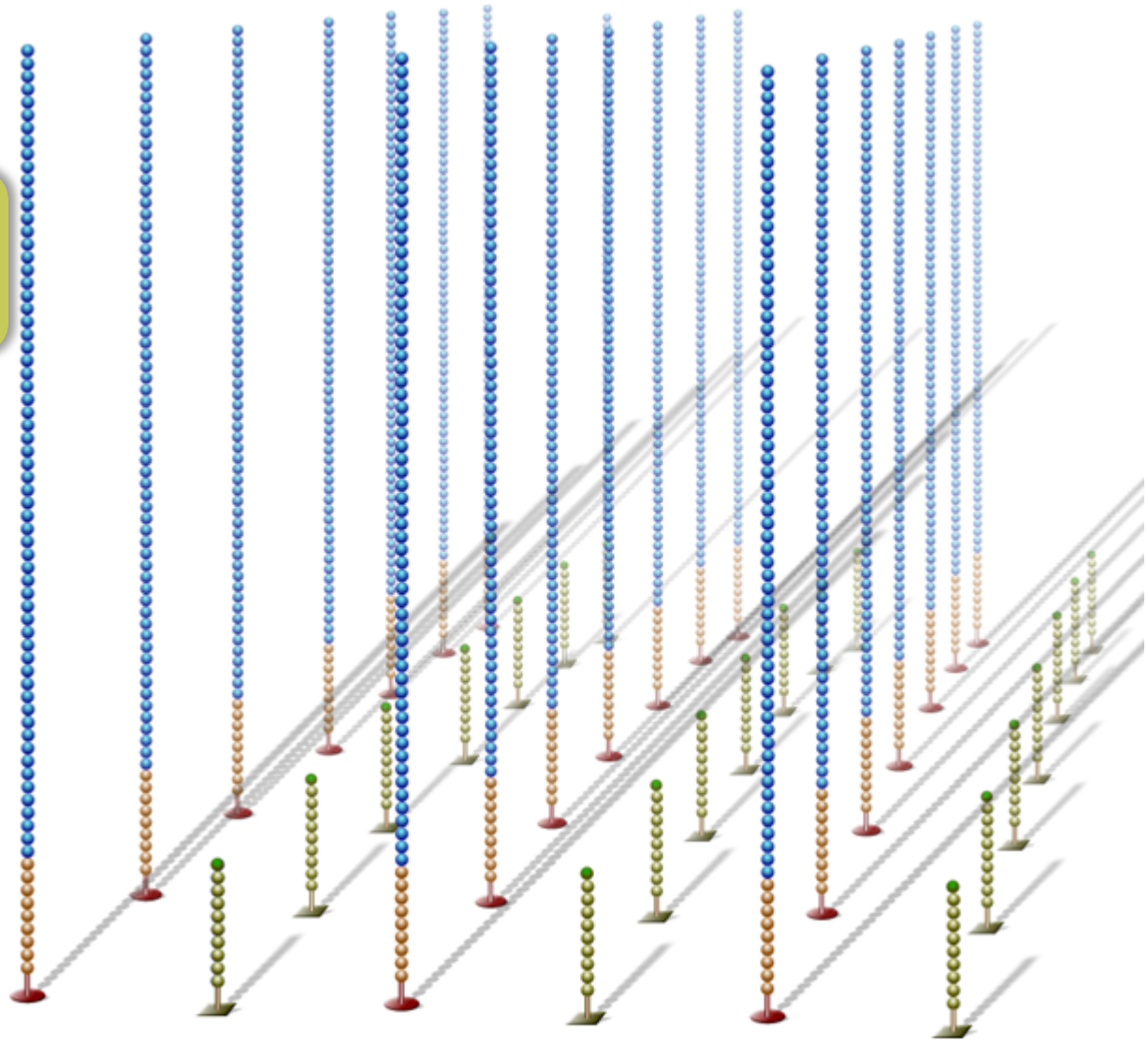
Linearization

dsDNA bridges are denatured



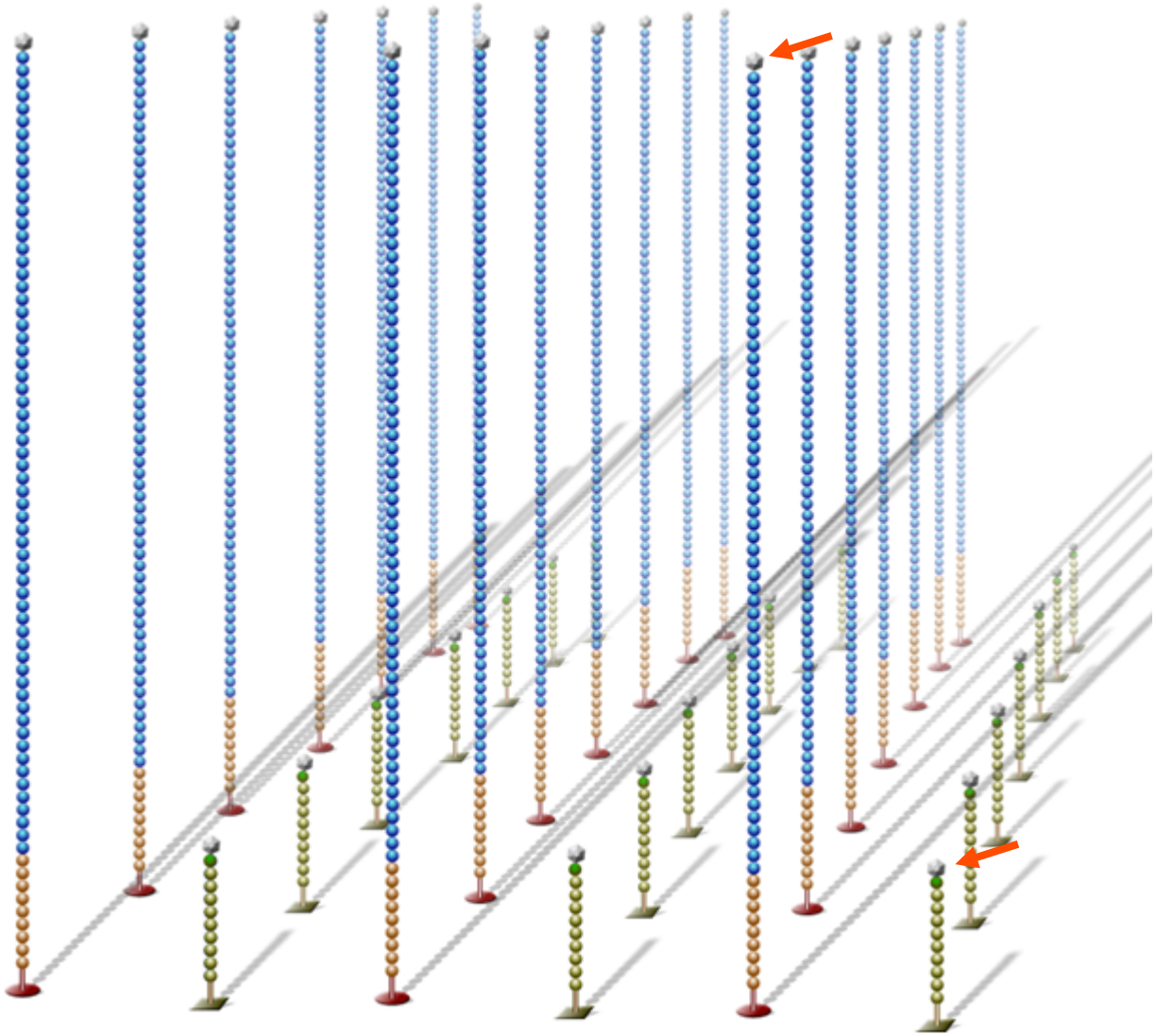
Reverse Strand Cleavage

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



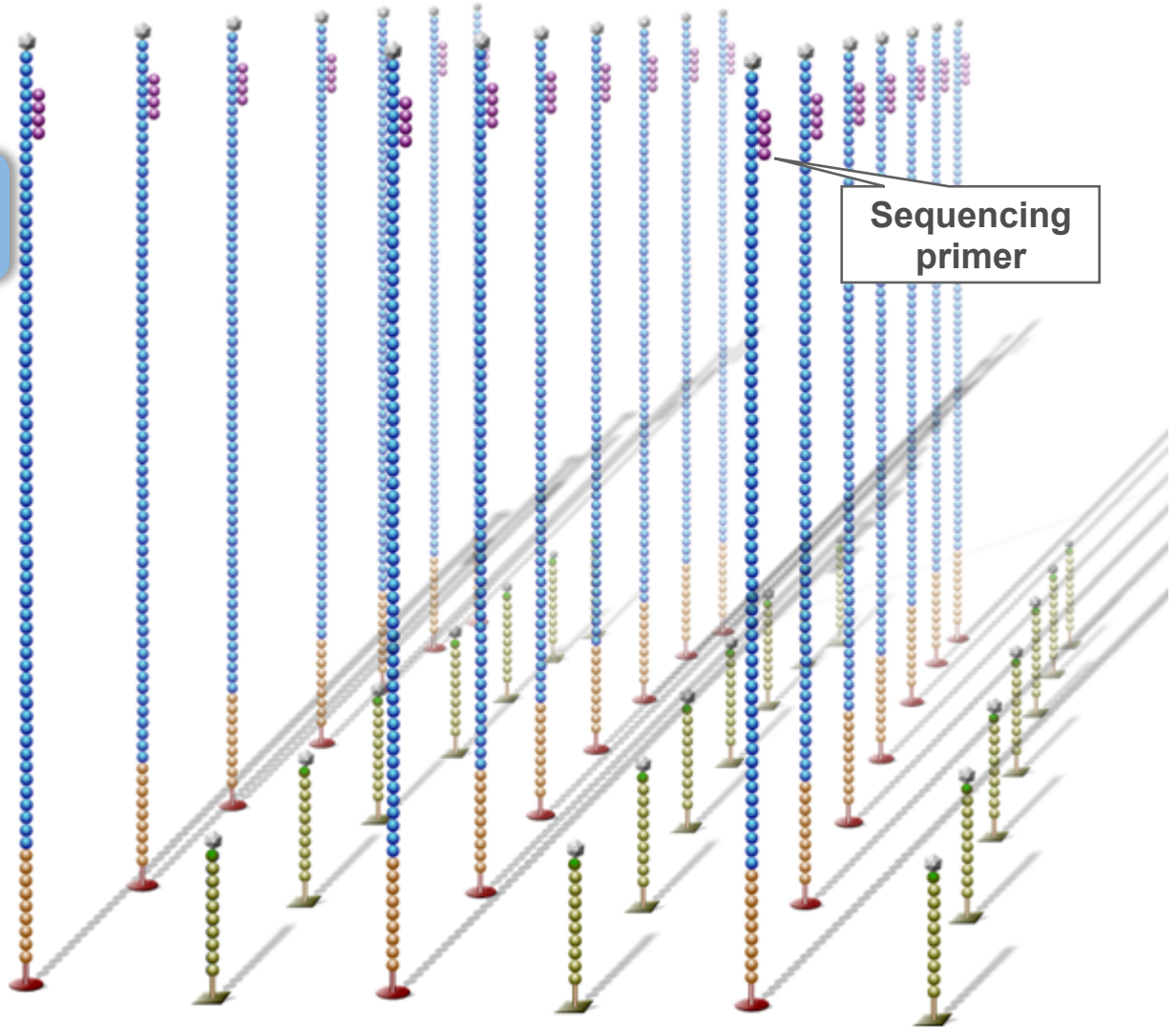
Blocking

Free 3’ ends are blocked to prevent unwanted DNA priming



Read 1 Primer Hybridization

Sequencing primer is hybridized to adapter sequence



Illumina Sequencing Workflow

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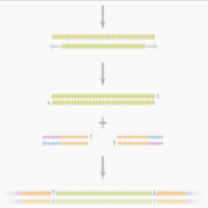

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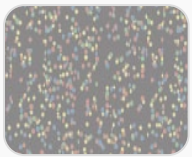
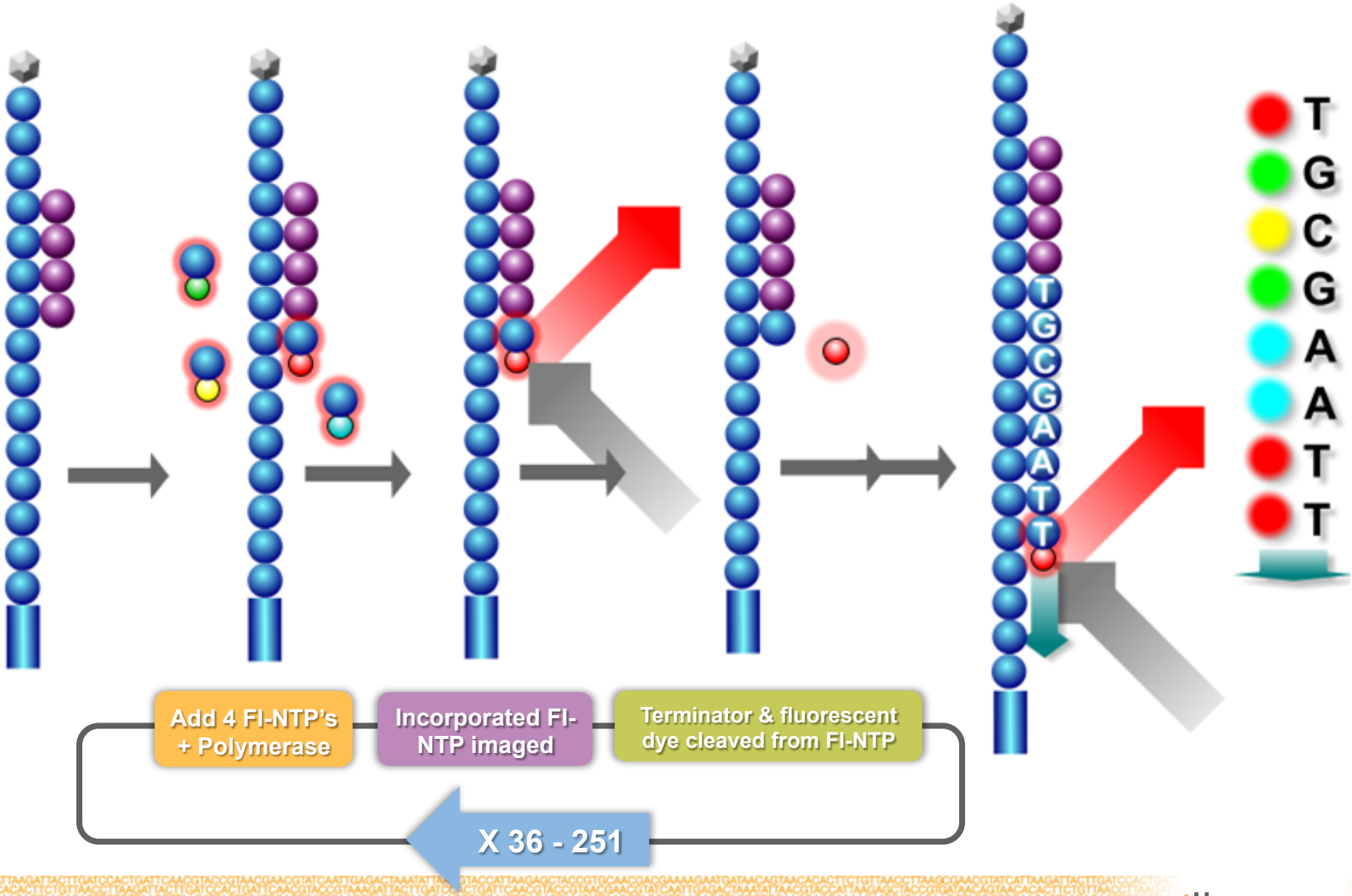
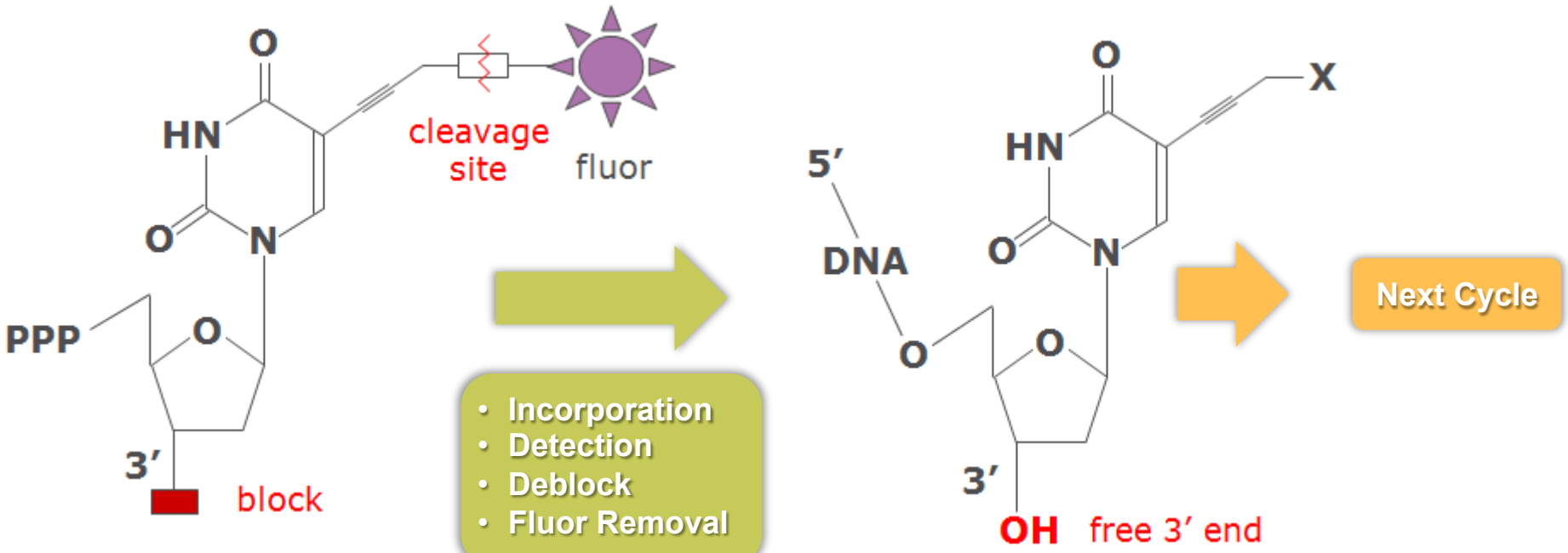


Sequencing By Synthesis

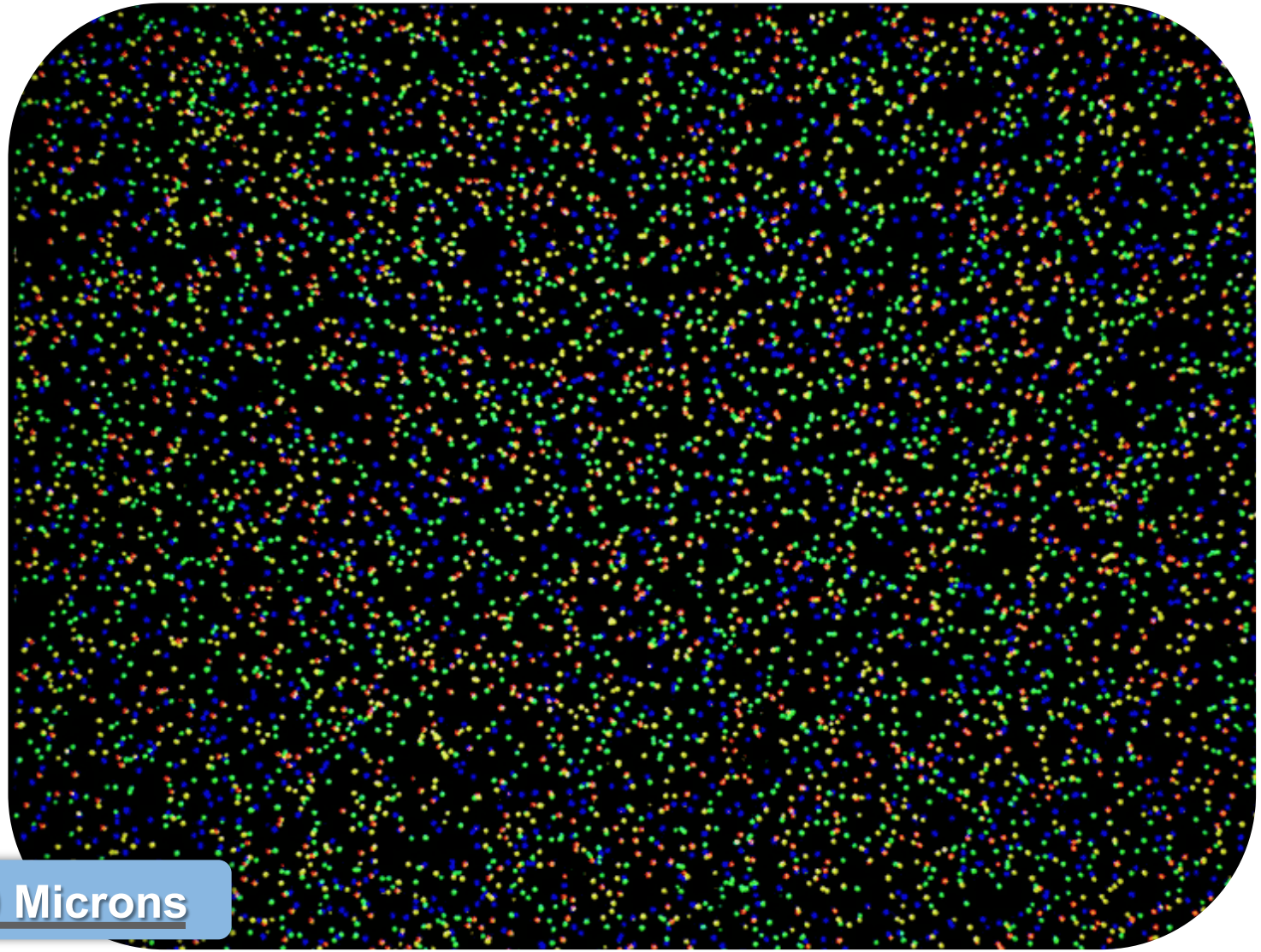


Reversible Terminator Chemistry

- All 4 labeled nucleotides in 1 reaction
- Higher accuracy
- No problems with homopolymer repeats



Clusters



Illumina Sequencing Workflow

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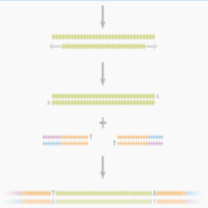


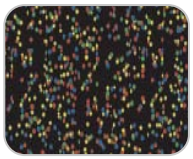
2 **Cluster Generation**

cBot
MiSeq

3 **Sequencing**

HiSeq
HiScan SQ
GA IIx
MiSeq

4 **Data Analysis**



ICS/RTA
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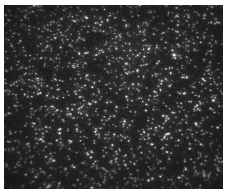
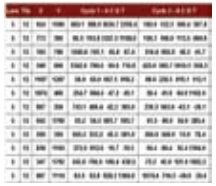
Data Analysis Overview

Primary and Secondary Analysis Overview

Analysis Type	Software	Outputs
Sequencing	 ICS/RTA	 Images/TIFF files
Primary Analysis	 ICS/RTA	 Intensities  Base Calling
Secondary Analysis	BaseSpace MiSeq Reporter CASAVA	 Alignments and Variant Detection

Paired End Sequencing

Sequencing with Paired Ends

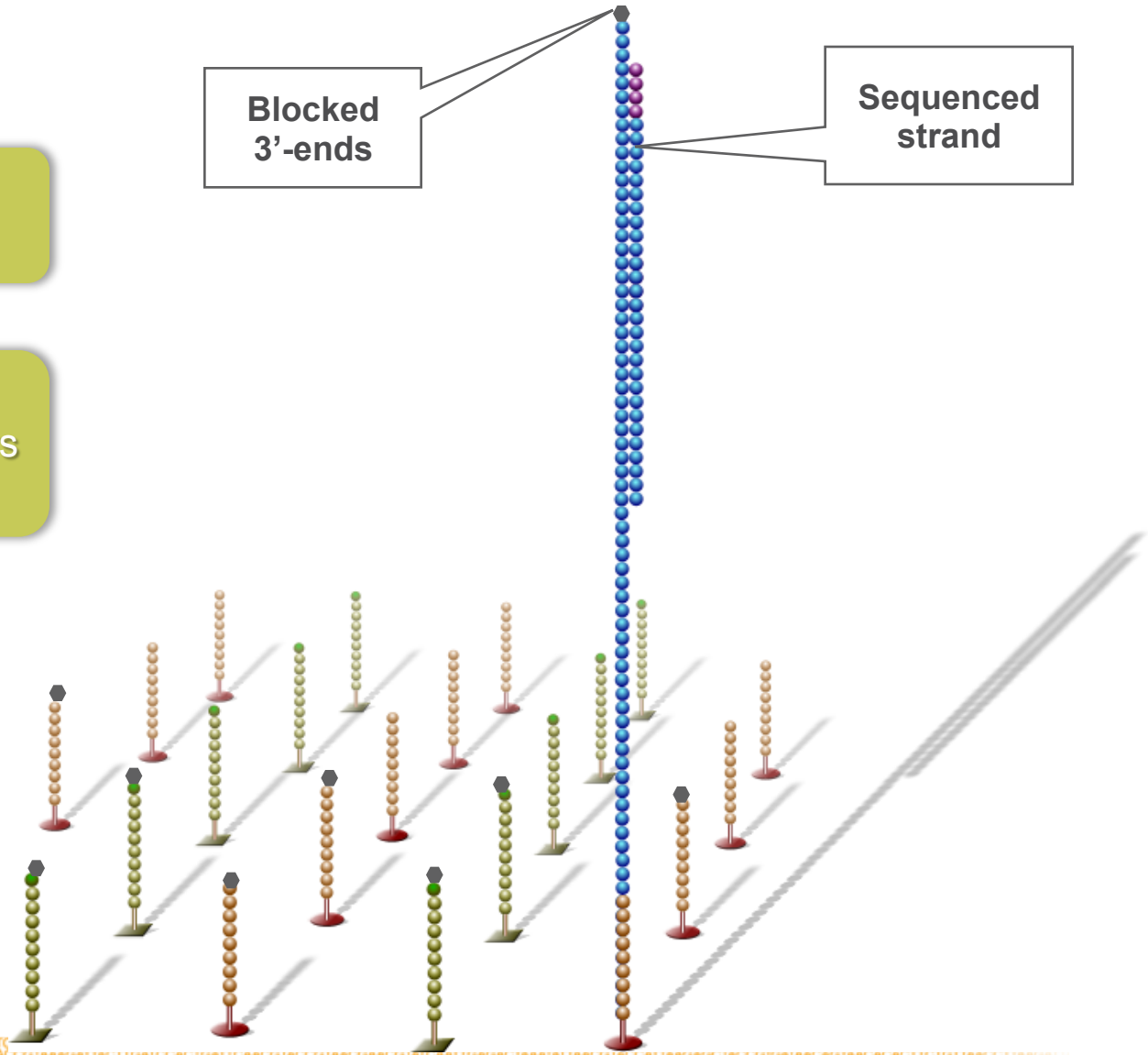
Reference	This is really the best way to do sequencing
Single-reads	This is
…	is really
…	really the
…	the best
…	sequencing
Paired-reads	This is (----100 characters-------) sequencing

Assembly becomes easier!!

Paired End Sequencing

Sequenced strand is stripped off

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



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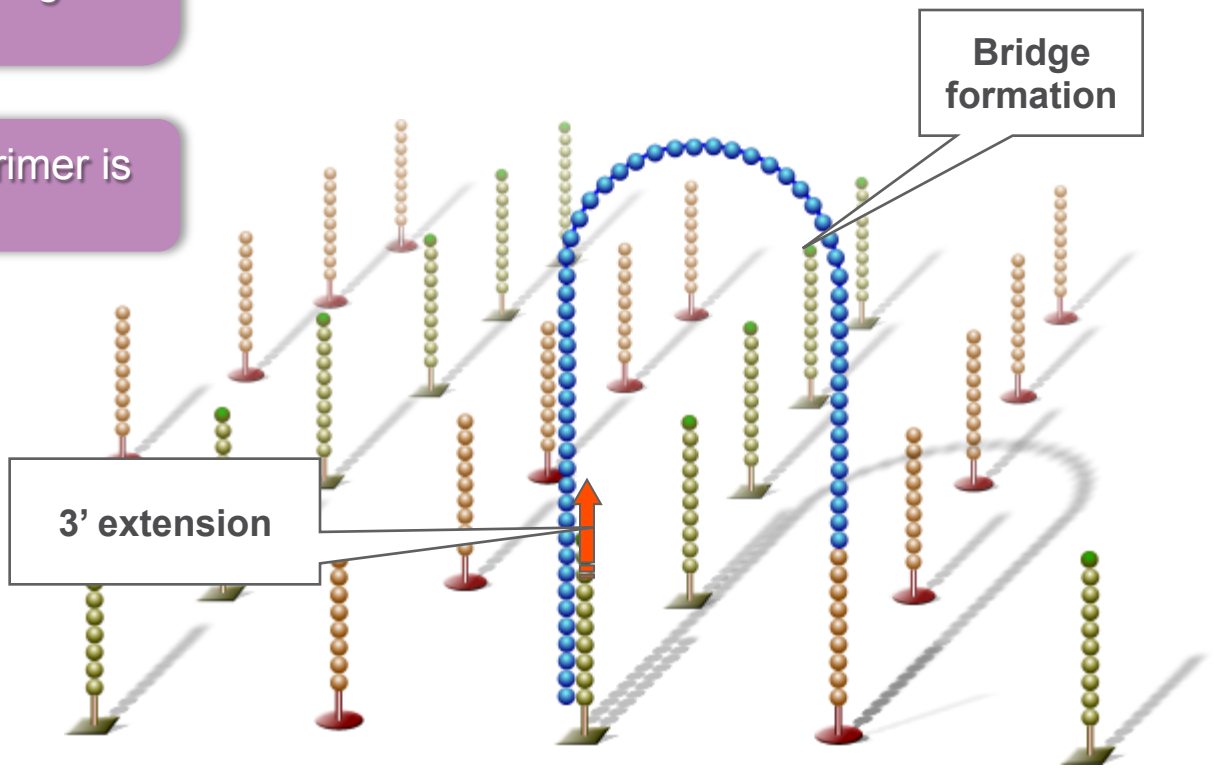
Paired End Sequencing

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

3’-ends of lawn primer is extended



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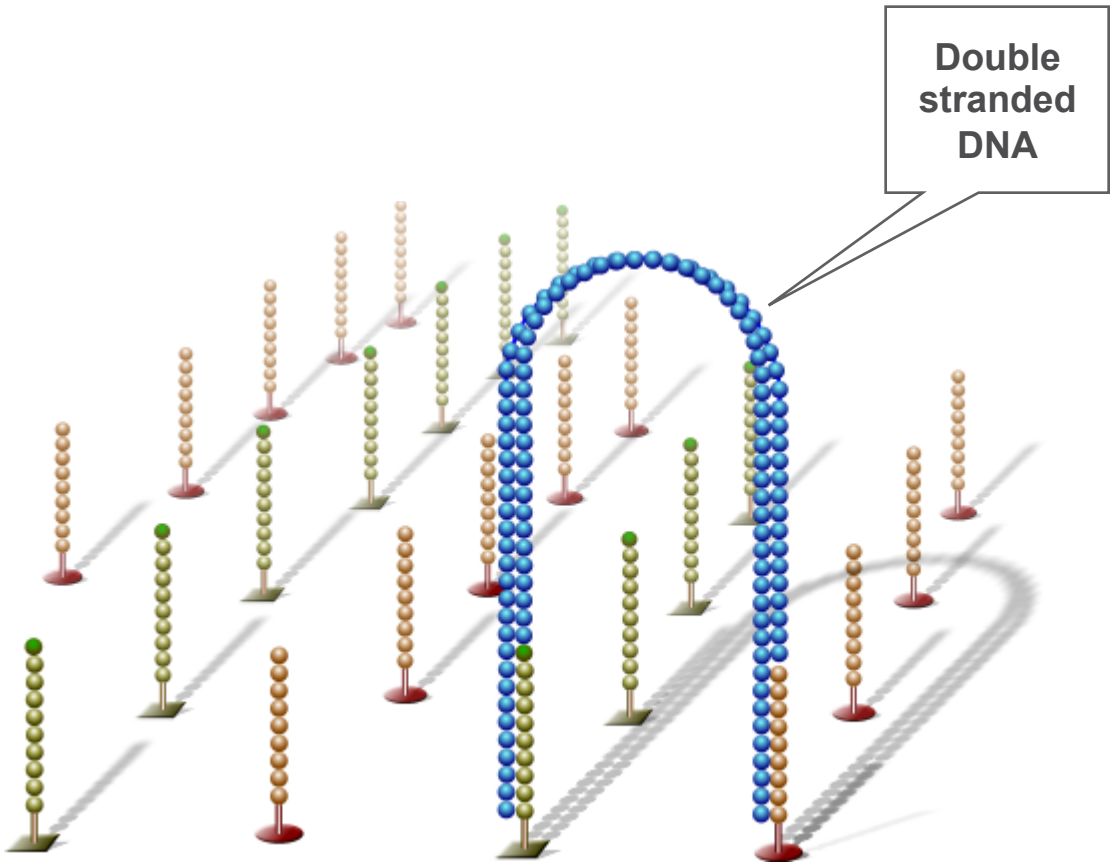
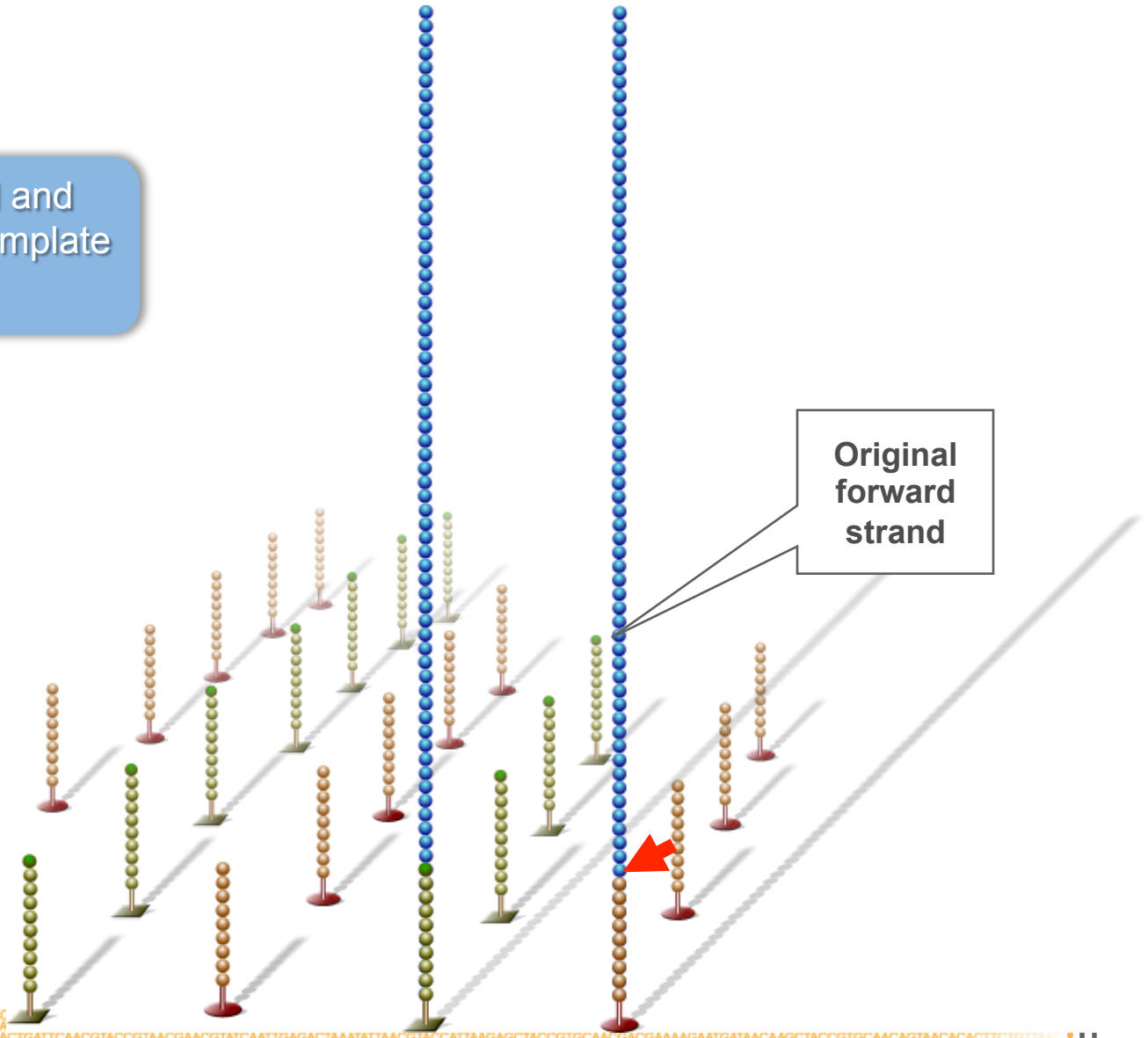
Paired End Sequencing



Paired End Sequencing

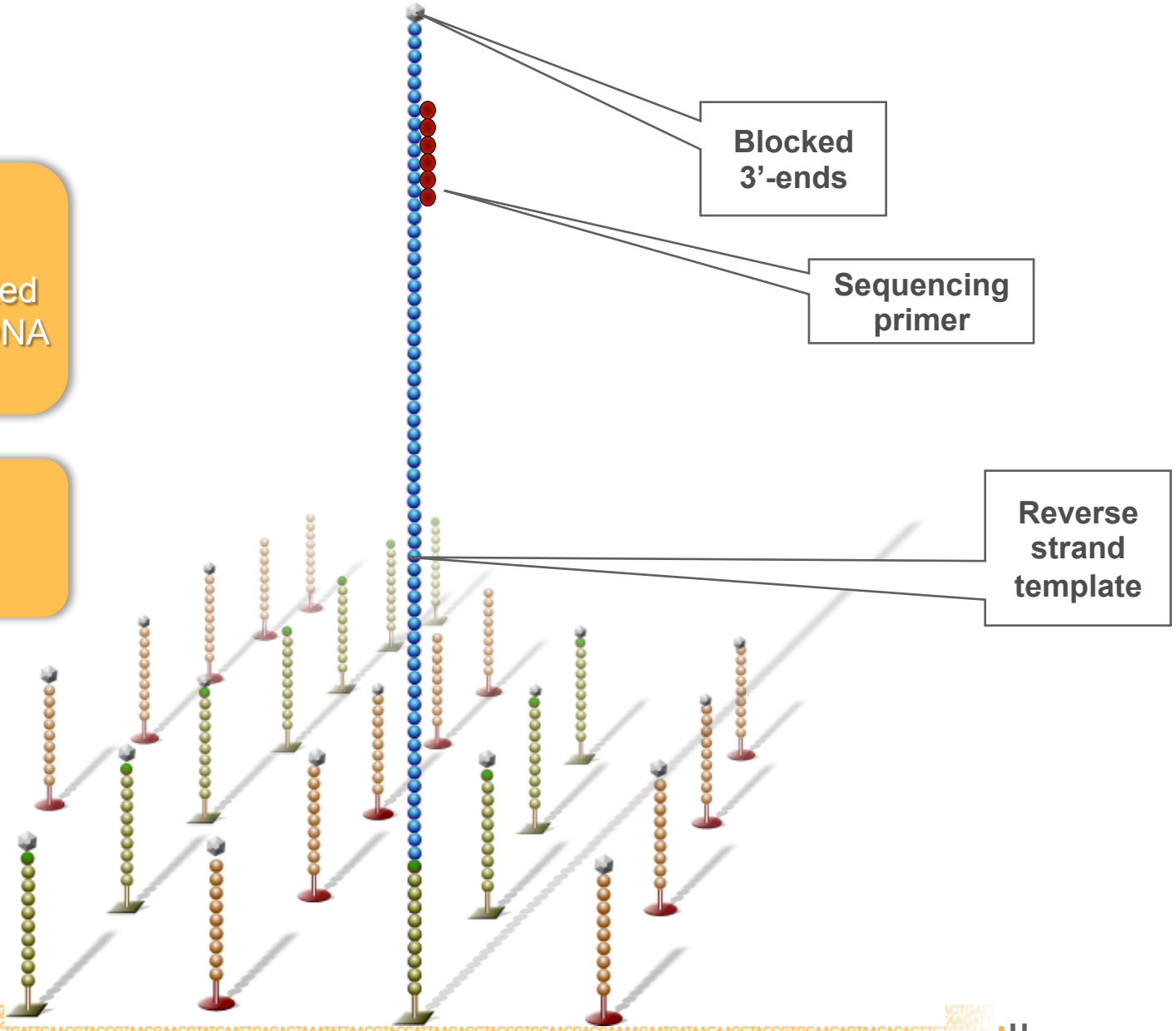
Bridges are linearized and the original forward template is cleaved



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



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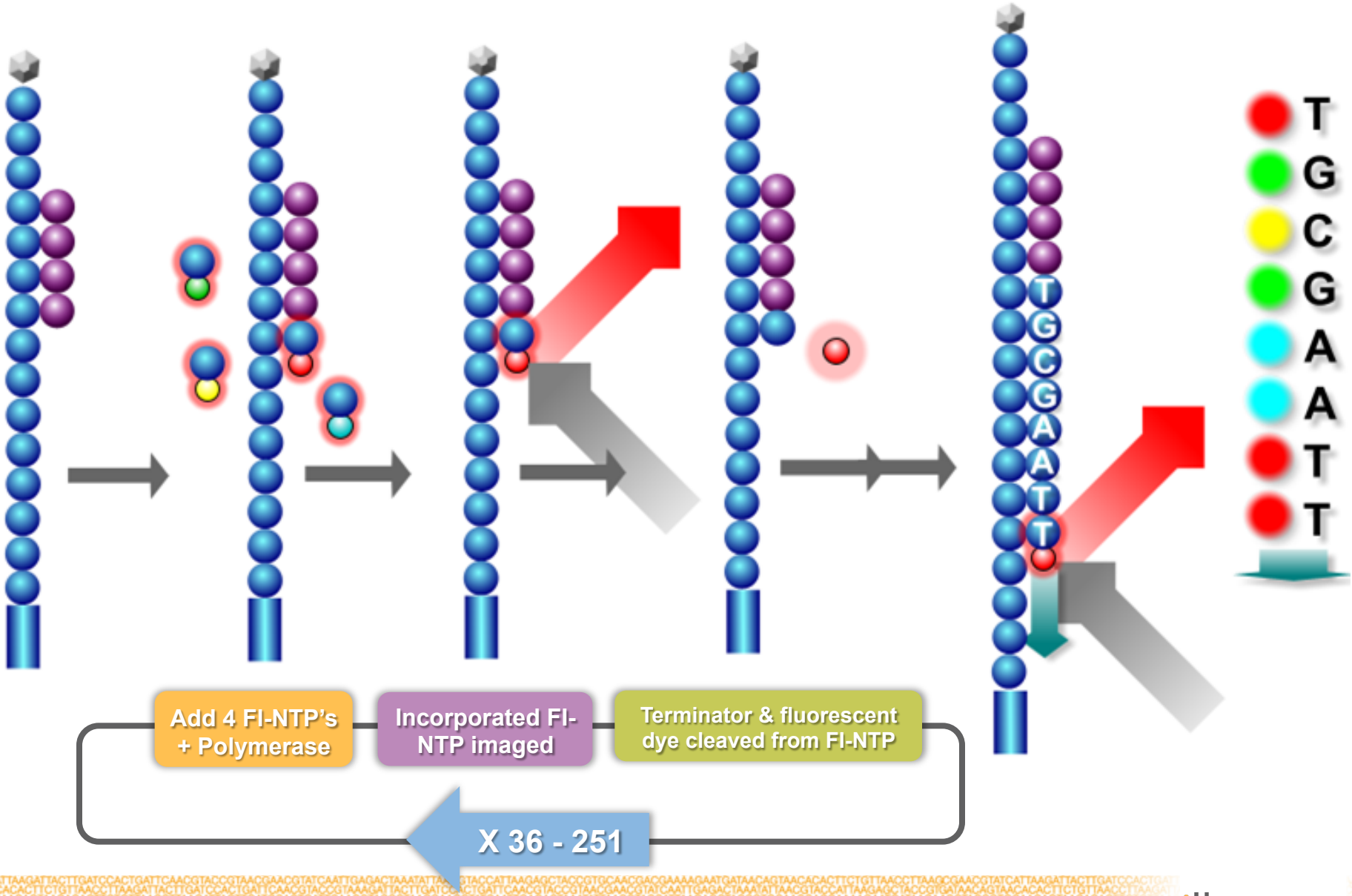
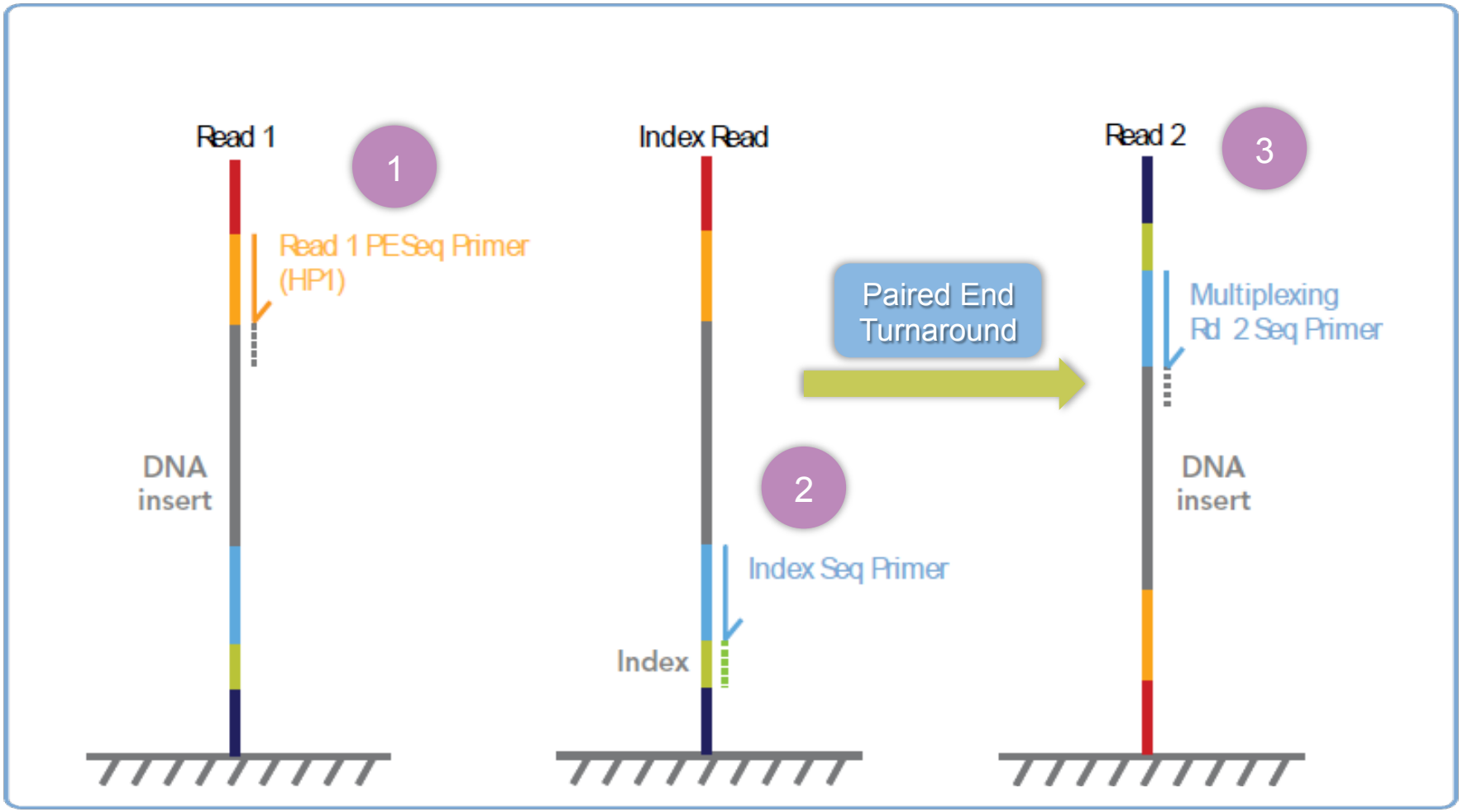
Sequencing By Synthesis 2nd Read



Sequencing Paired End Libraries with Single Index Read

Sequencing Paired End Libraries with Single Index Read



Single Index Sequencing Utilizes 3 Sequencing Reads

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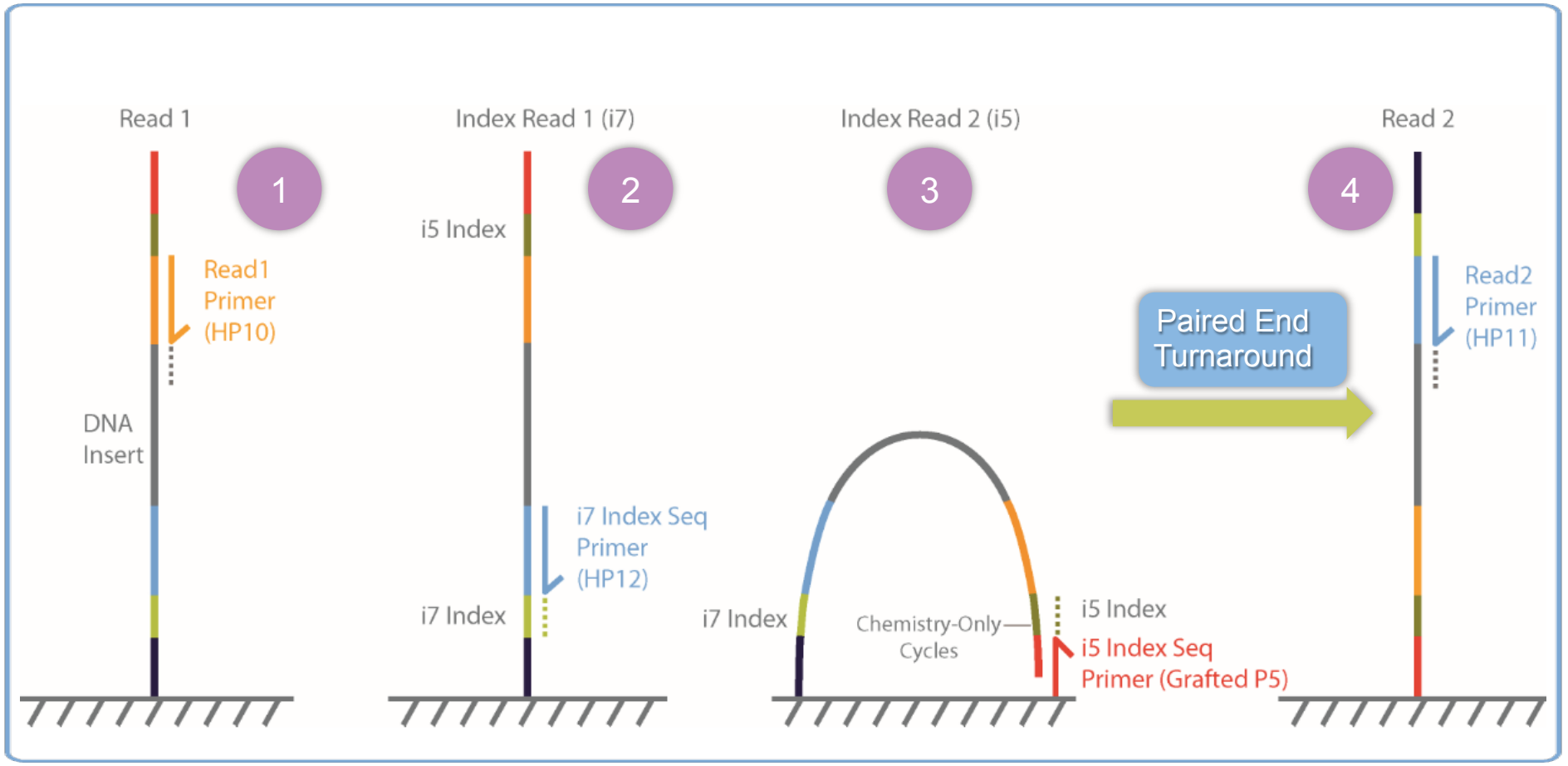
Sequencing Paired End Libraries with Dual Index Read

Sequencing Paired End Libraries with Dual Index Read



Dual Index Sequencing Utilizes 4 Sequencing Reads

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Summary

1

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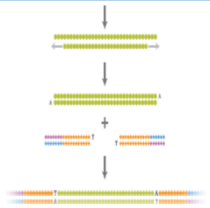

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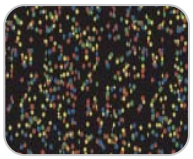

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Questions?

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