MiSeq

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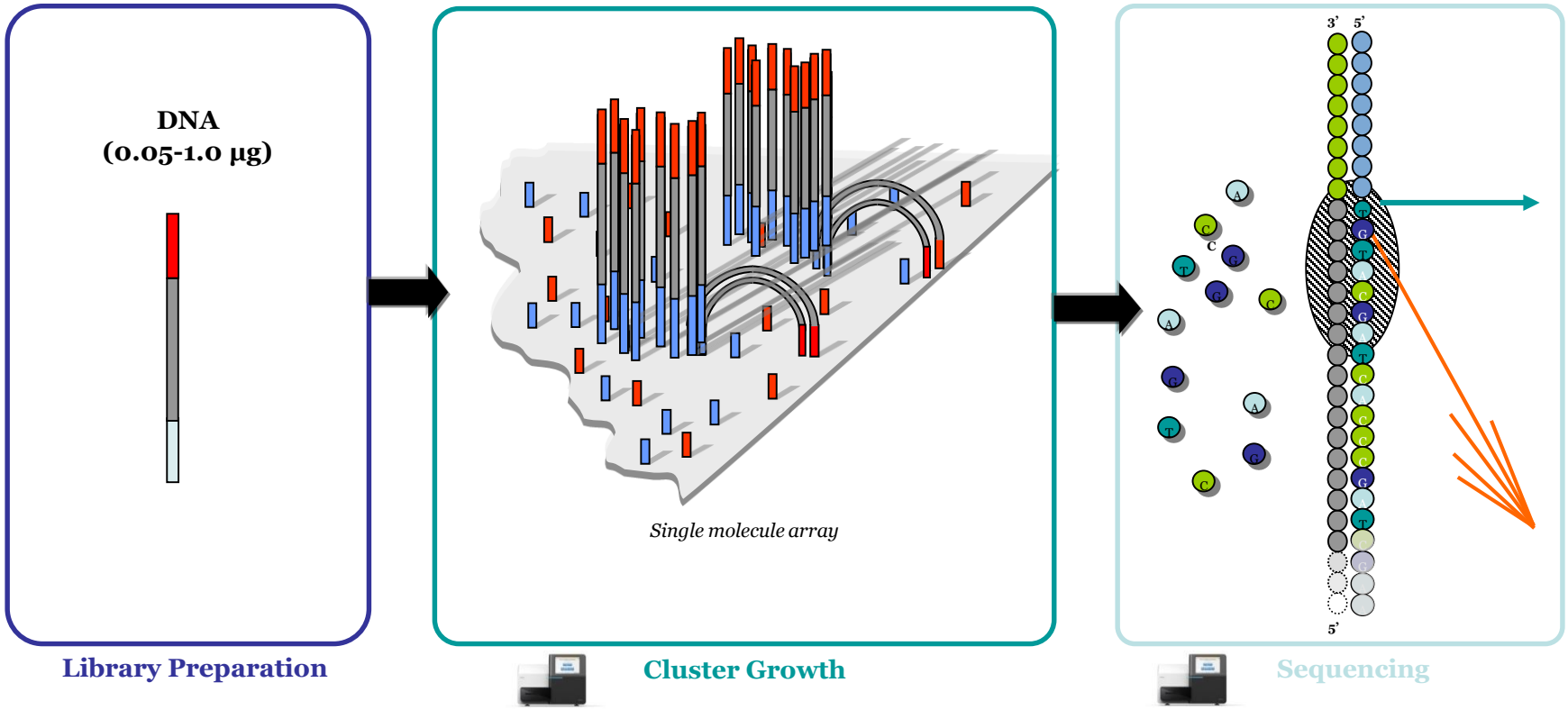
MiSeq

MiSeq

Illumina Sequencing Workflow



Illumina sequencing read strategy

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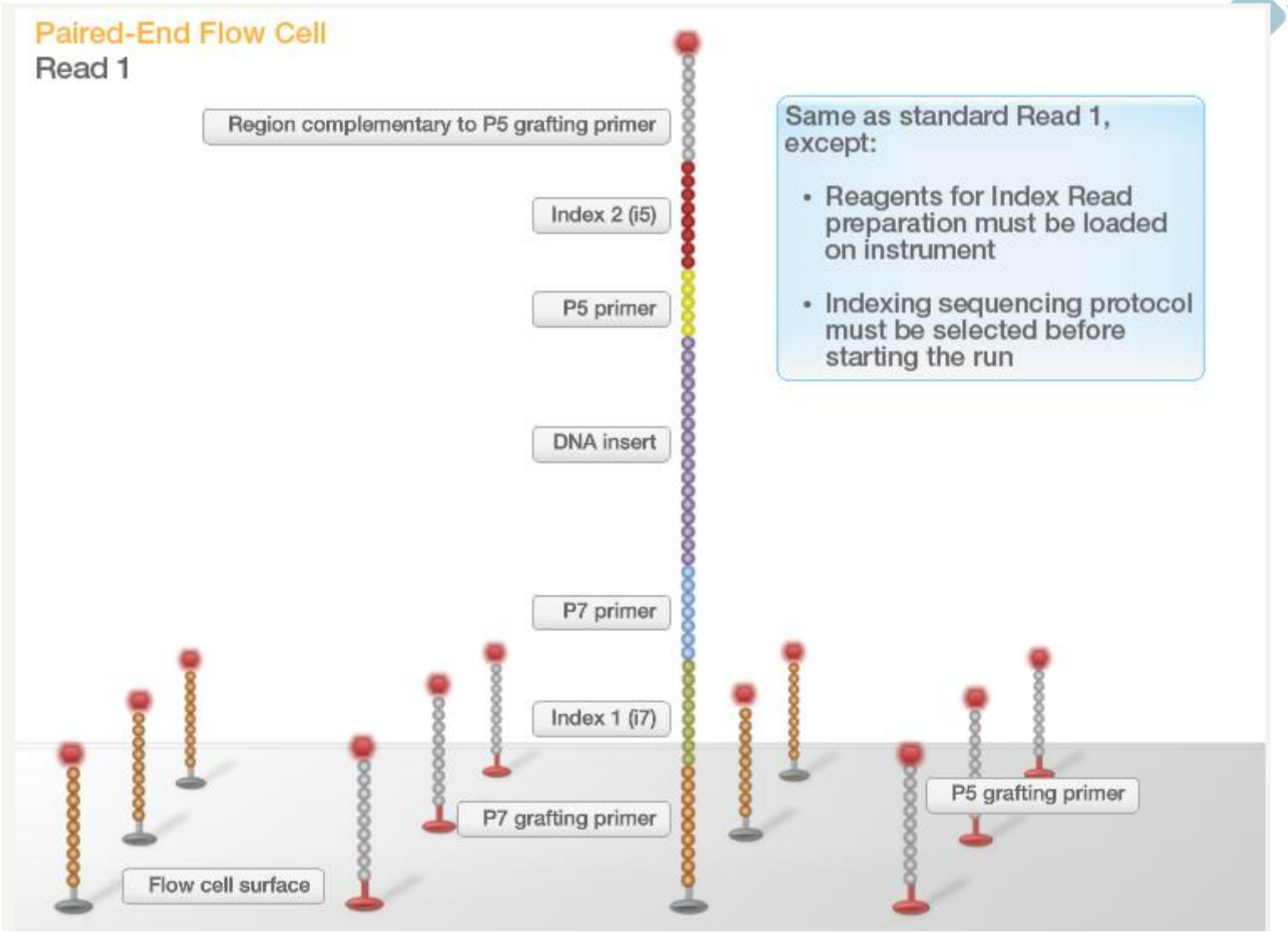
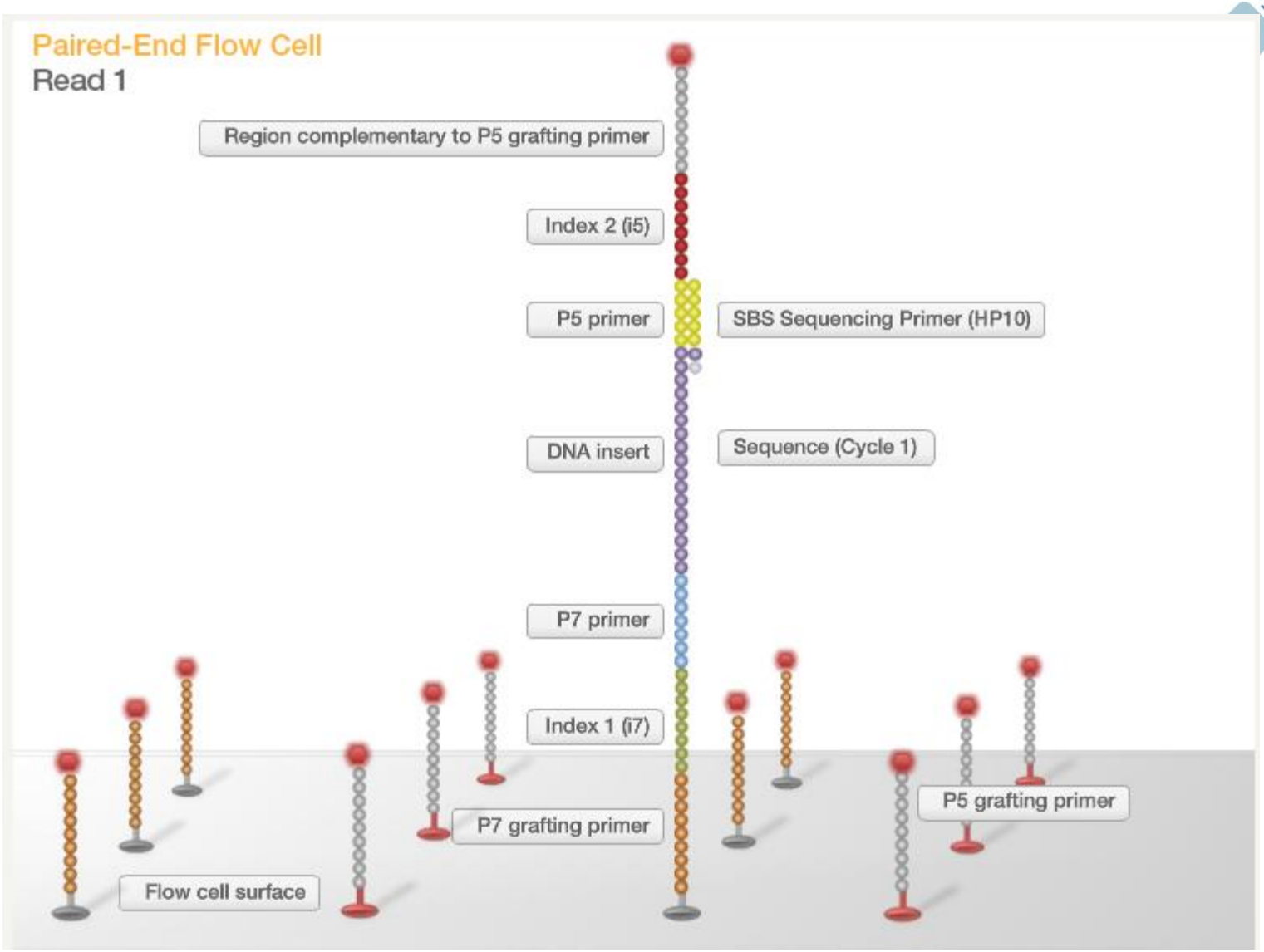
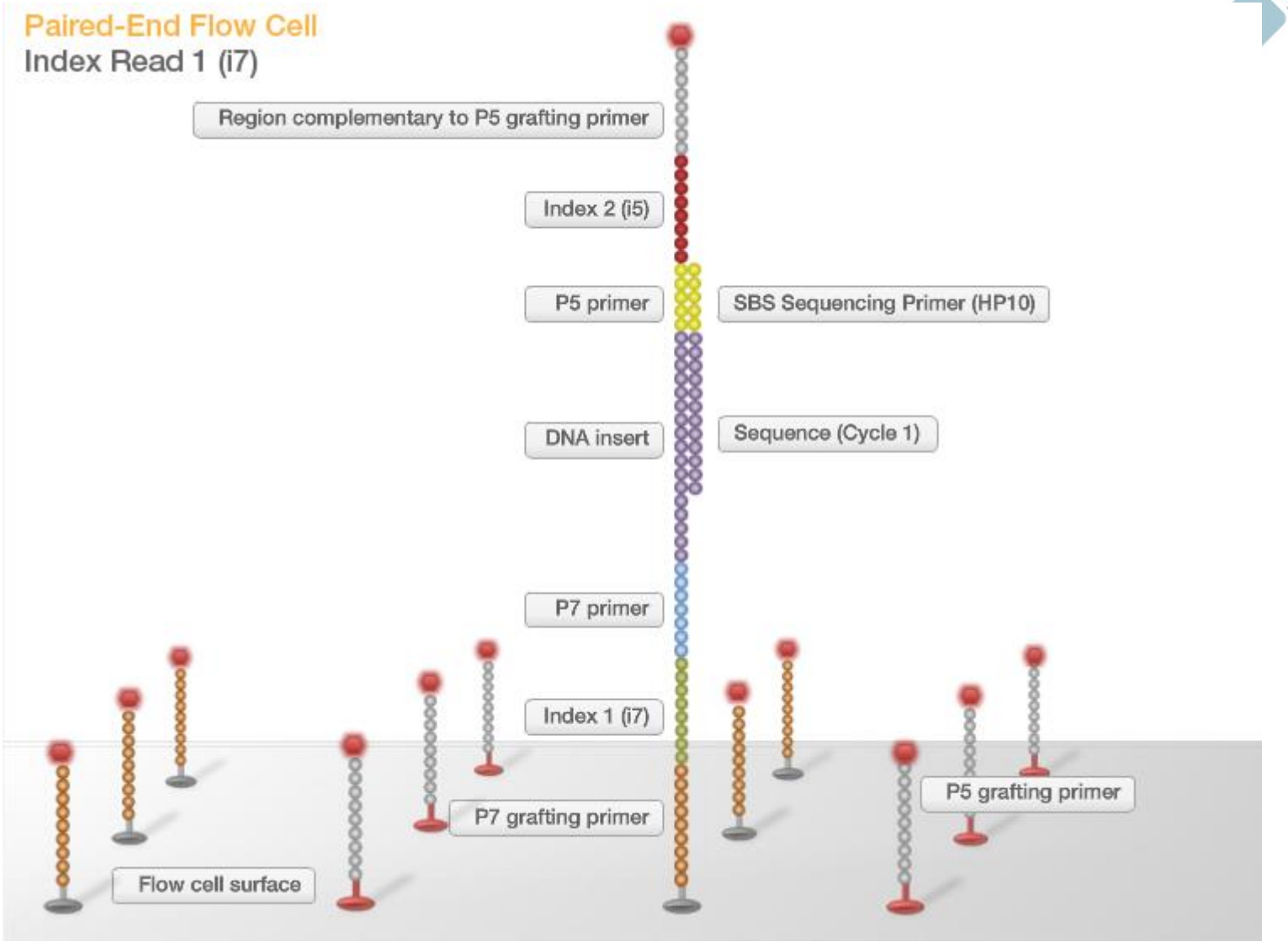
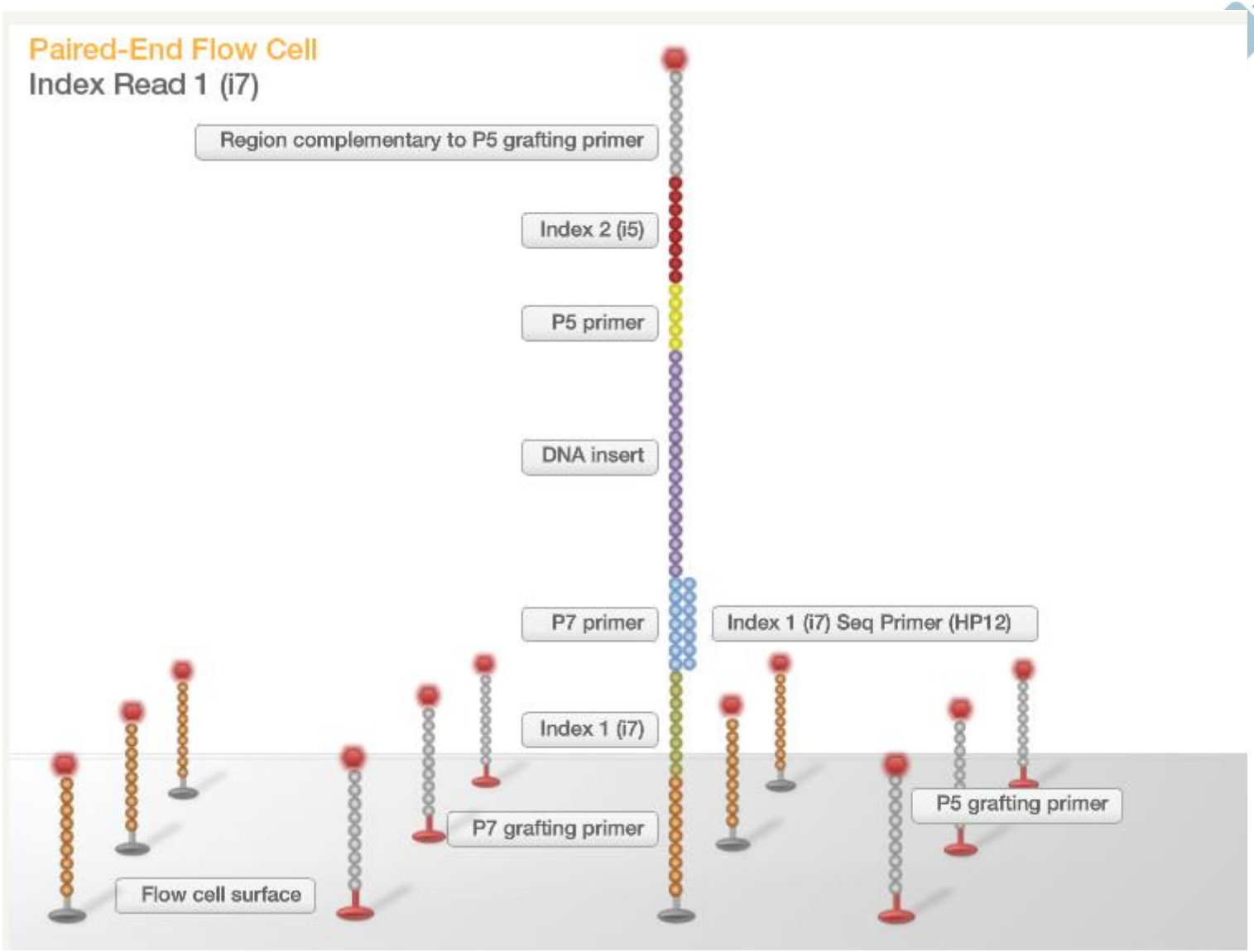
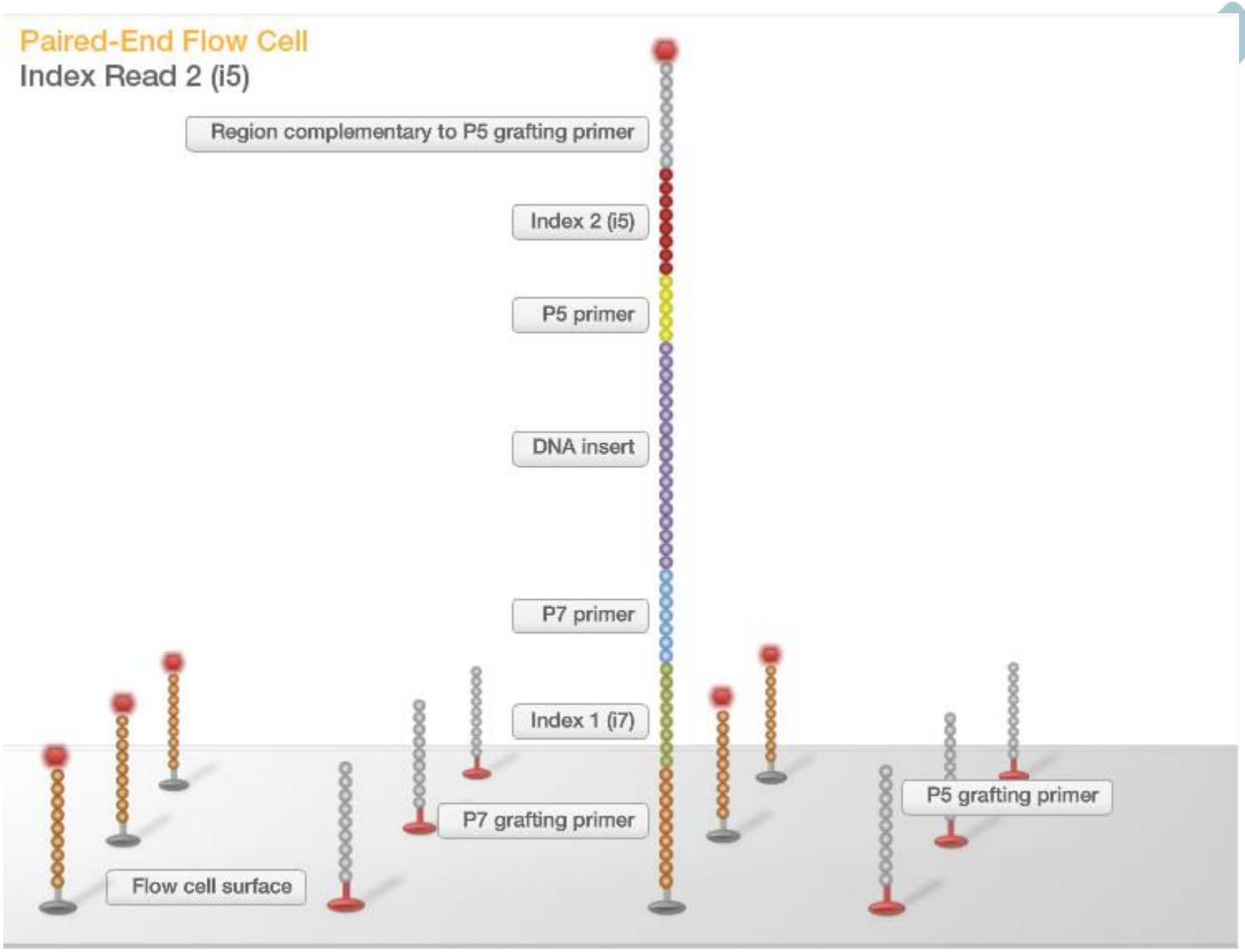
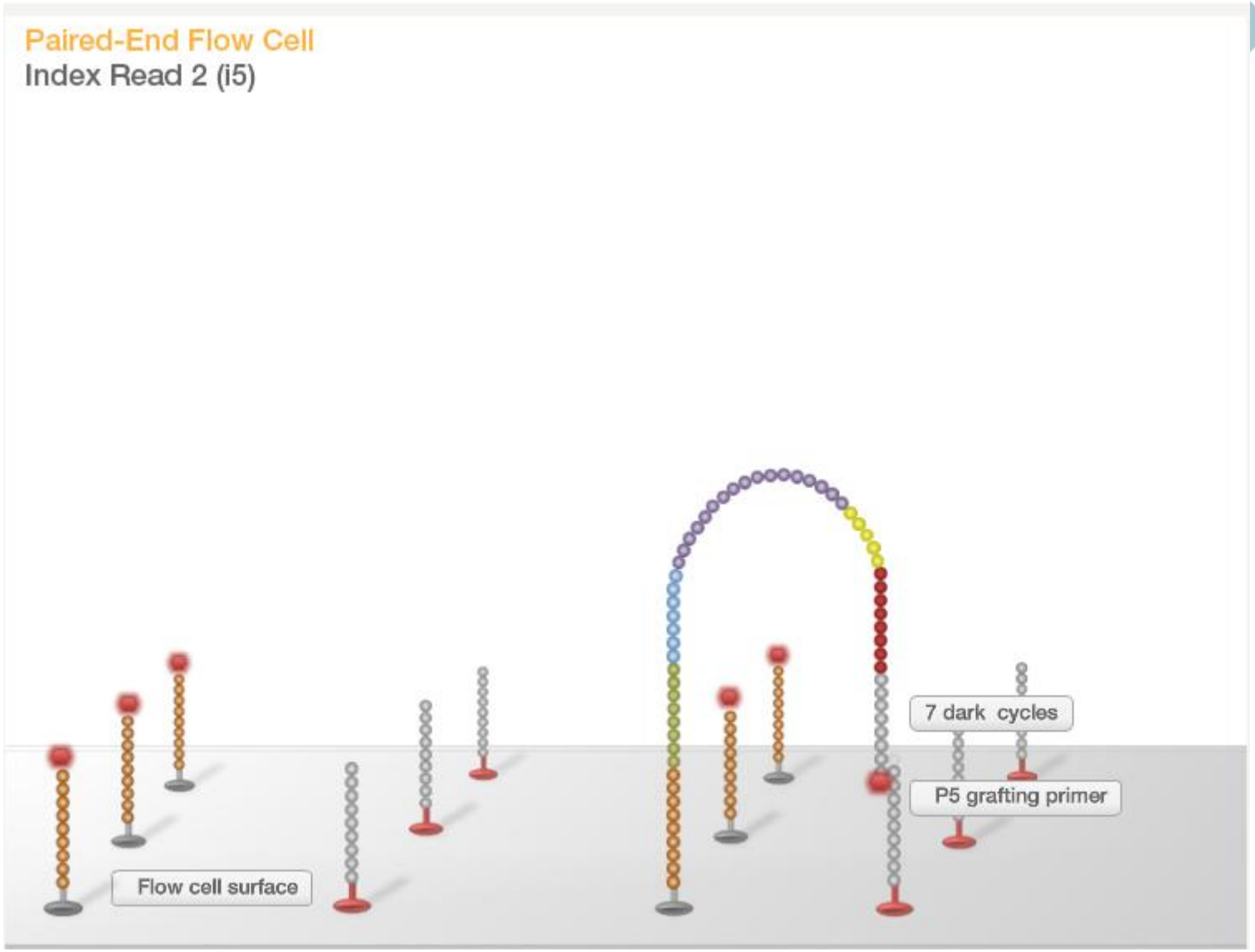

Paired-End Flow Cell
Read 1
Region complementary to P5 grafting primer
Index 2 (i5)
P5 primer
DNA insert
P7 primer
Index 1 (i7)
P7 grafting primer
Flow cell surface
P5 grafting primer
Same as standard Read 1, except:
• Reagents for Index Read preparation must be loaded on instrument
• Indexing sequencing protocol must be selected before starting the run


Paired-End Flow Cell
Read 1
Region complementary to P5 grafting primer
Index 2 (i5)
P5 primer
SBS Sequencing Primer (HP10)
DNA insert
Sequence (Cycle 1)
P7 primer
Index 1 (i7)
P7 grafting primer
P5 grafting primer
Flow cell surface


Paired-End Flow Cell
Index Read 1 (i7)
Region complementary to P5 grafting primer
Index 2 (i5)
P5 primer
SBS Sequencing Primer (HP10)
DNA insert
Sequence (Cycle 1)
P7 primer
Index 1 (i7)
P7 grafting primer
P5 grafting primer
Flow cell surface

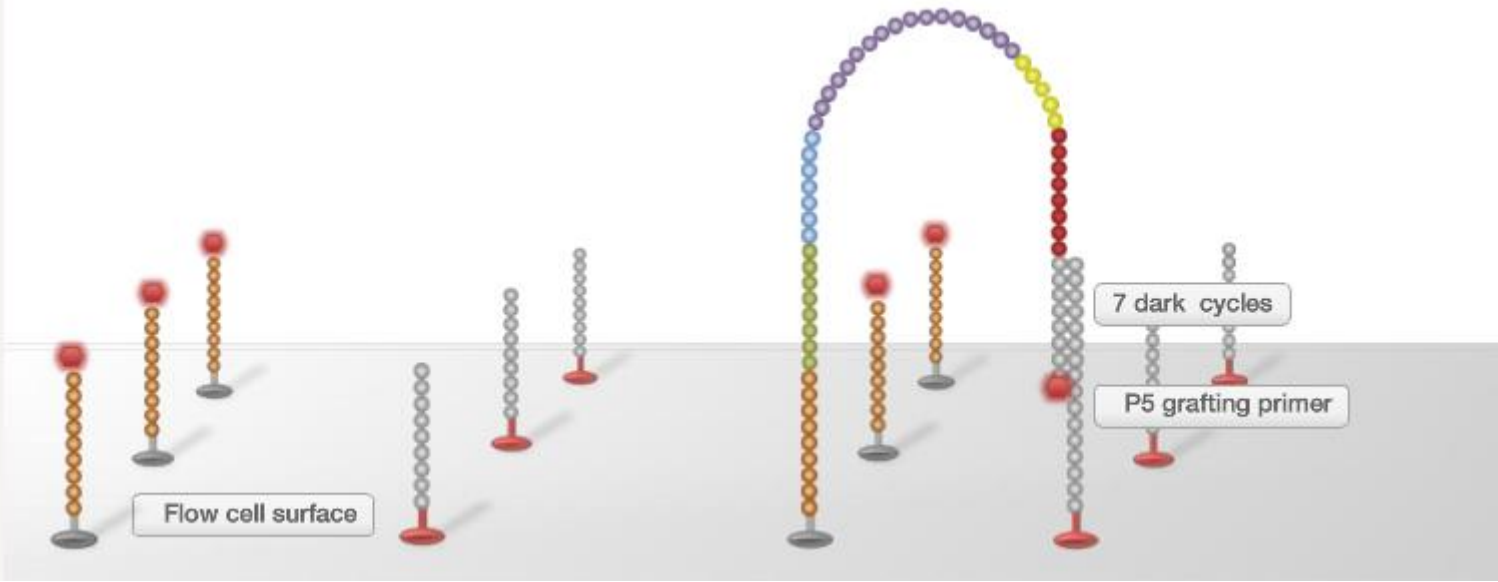

Paired-End Flow Cell
Index Read 1 (i7)
Region complementary to P5 grafting primer
Index 2 (i5)
P5 primer
DNA insert
P7 primer
Index 1 (i7) Seq Primer (HP12)
Index 1 (i7)
P7 grafting primer
P5 grafting primer
Flow cell surface


Paired-End Flow Cell
Index Read 2 (i5)
Region complementary to P5 grafting primer
Index 2 (i5)
P5 primer
DNA insert
P7 primer
Index 1 (i7)
P7 grafting primer
P5 grafting primer
Flow cell surface


Paired-End Flow Cell
Index Read 2 (i5)
7 dark cycles
P5 grafting primer
Flow cell surface

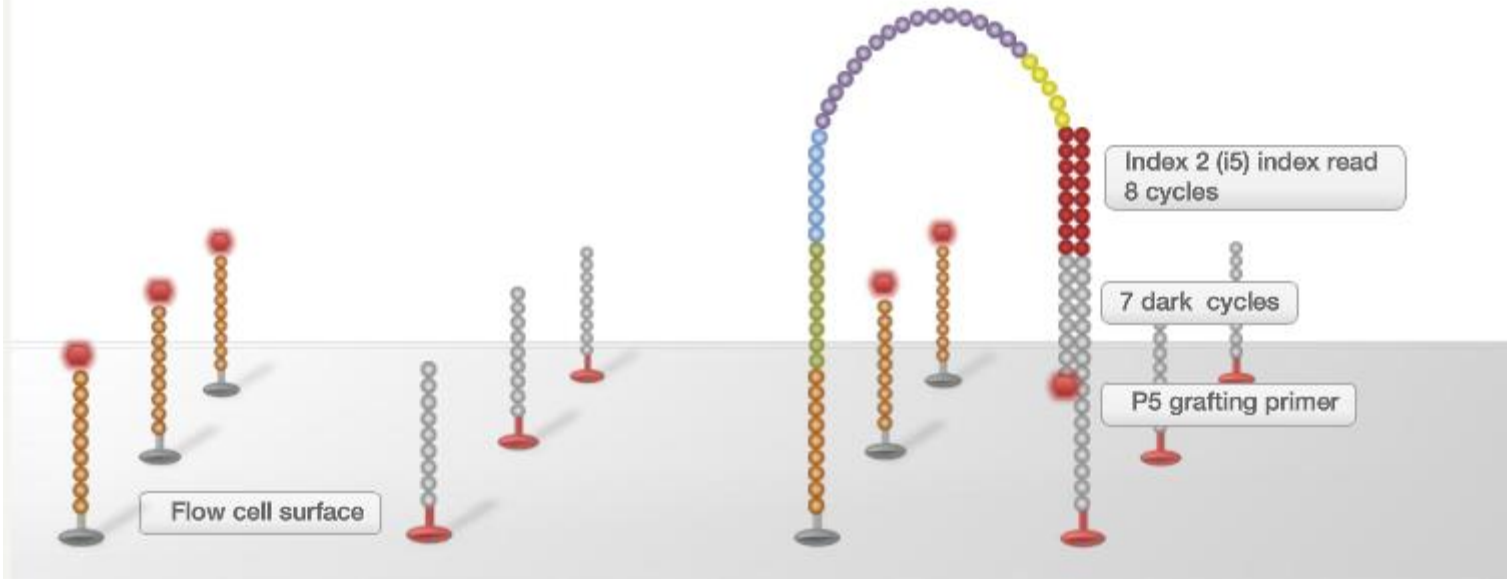
Paired-End Flow Cell

Index Read 2 (i5)



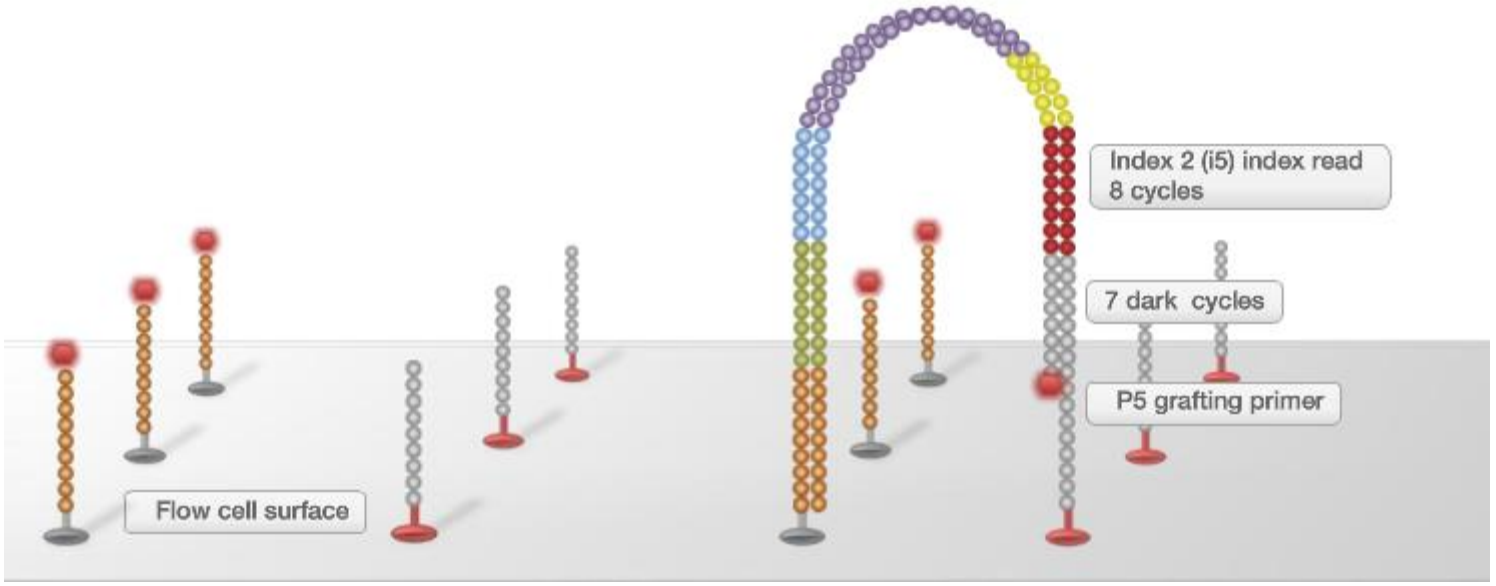
Paired-End Flow Cell

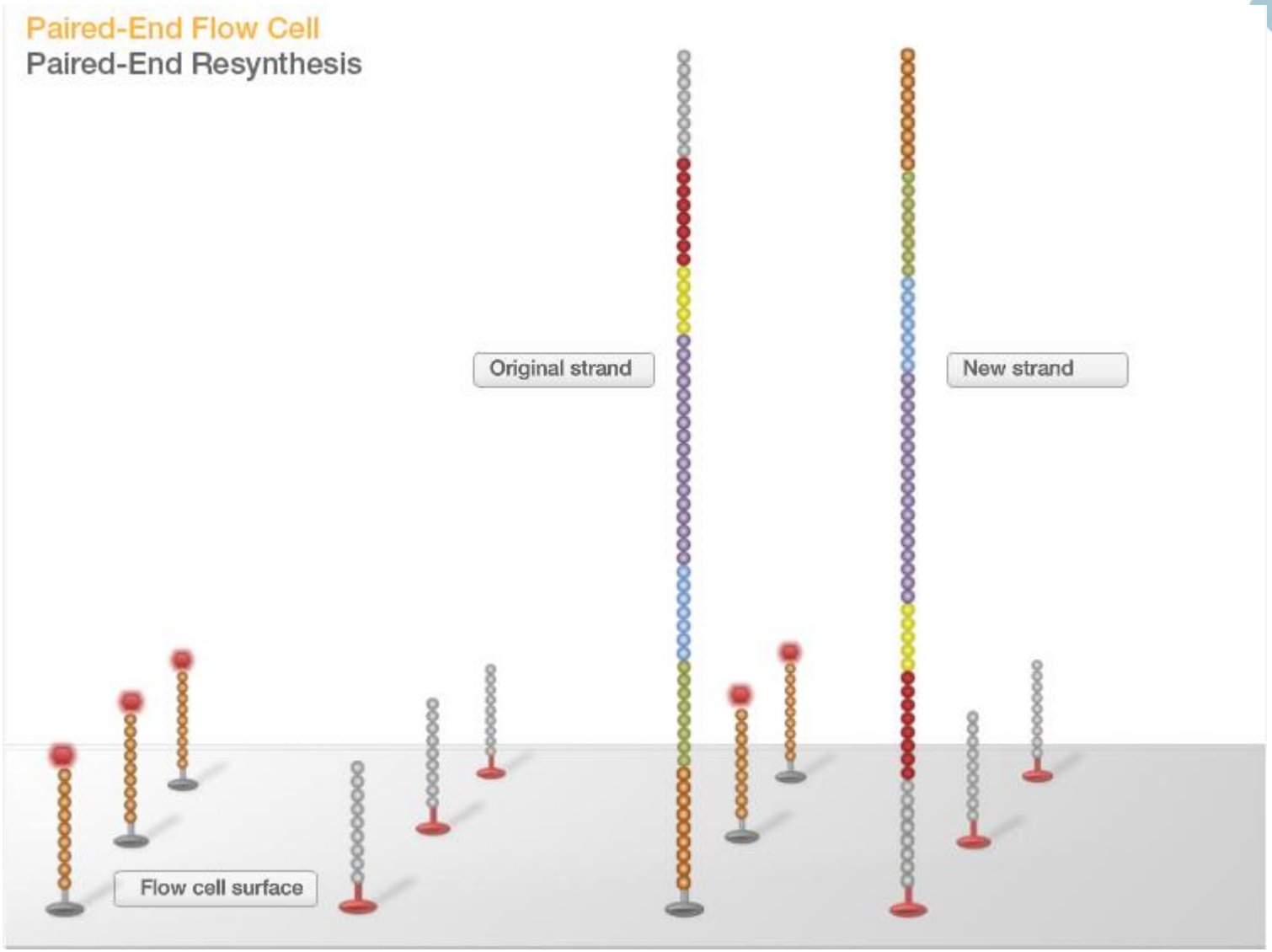
Index Read 2 (i5)



Paired-End Flow Cell

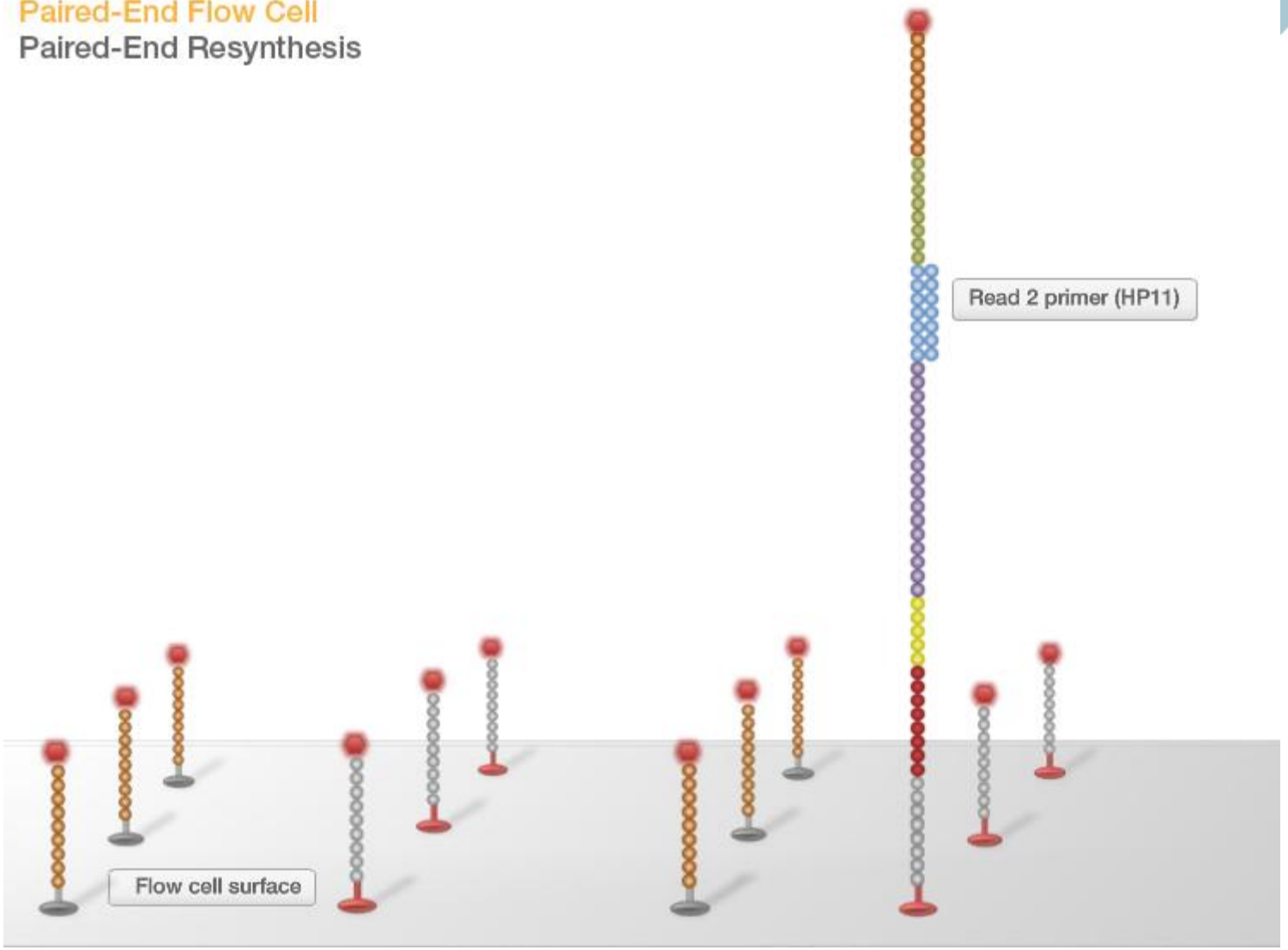
Paired-End Resynthesis




Paired-End Flow Cell
Paired-End Resynthesis
Original strand
New strand
Flow cell surface

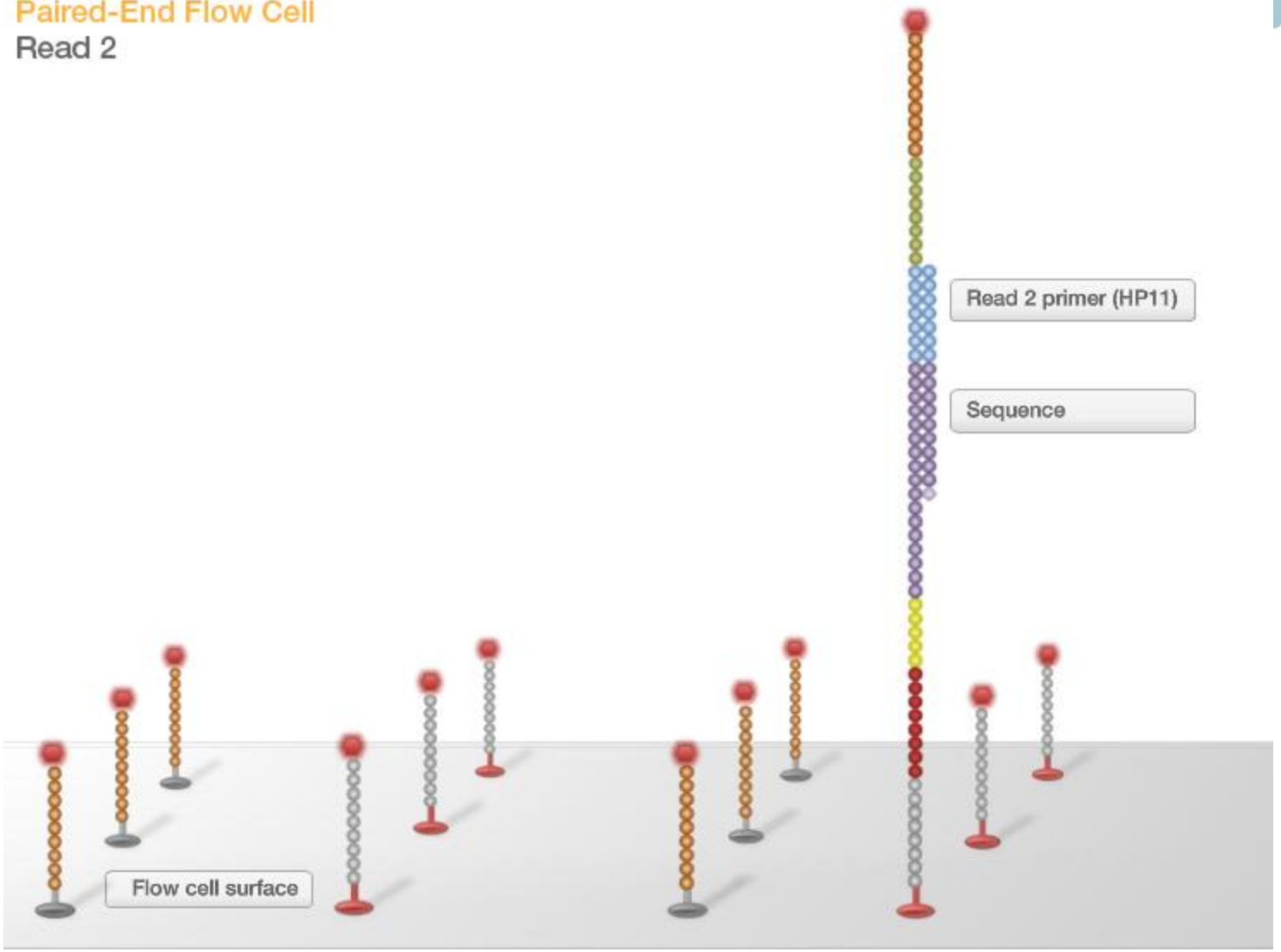
Paired-End Flow Cell

Paired-End Resynthesis



Paired-End Flow Cell

Read 2



MiSeq features

- Fast
- On board clustering
- High accuracy……. Long read lengths
  Easy to use
- In built analysis modules

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MiSeq requirements

- Library
  - Same libraries as for GAII and HiSeq
  - Can be multiplexed
  - 10µl of 2nM
  - Denatured with 0.1N NaOH prior to loading

MiSeq requirements

» Library

» Reagents

MiSeq Reagent Kits

Description	Kit Size	# Reads	Max. Output	# Tiles Imaged	Cap Color	Price
MiSeq Reagent Nano Kit v2	300-cycle	up to 1M	300 Mb	2	Yellow	$405
MiSeq Reagent Micro Kitv2	300-cycle	up to 4M	1.2 Gb	4 (dual)	Green	$609
MiSeq Reagent Nano Kit v2	500-cycle	up to 1M	500 Mb	2	Yellow	$486
MiSeq® Reagent Kit v2	50 cycle	Up to 14M	0.7Gb			$568
MiSeq® Reagent Kit v2	300 cycle	Up to 14M	4 Gb			$732
MiSeq® Reagent Kit v2	500 cycle	Up to 14M	8 Gb			$812

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MiSeq v3 Kits

V3 - 150 cycle	1x150bp 2x75bp	25M	Counting Apps: • RNA seq (25M PE) • Exome (low cost)
V3 – 600 cycle	Up to 2x300bp	25M	• Long-read Amplicon • Small Genome de novo • HLA • Exome

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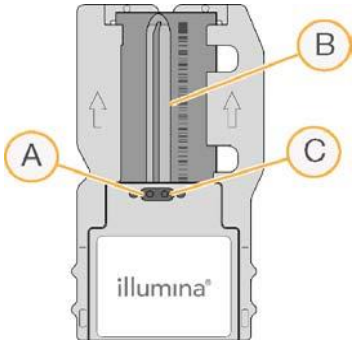
MiSeq requirements

» Library

» Reagents

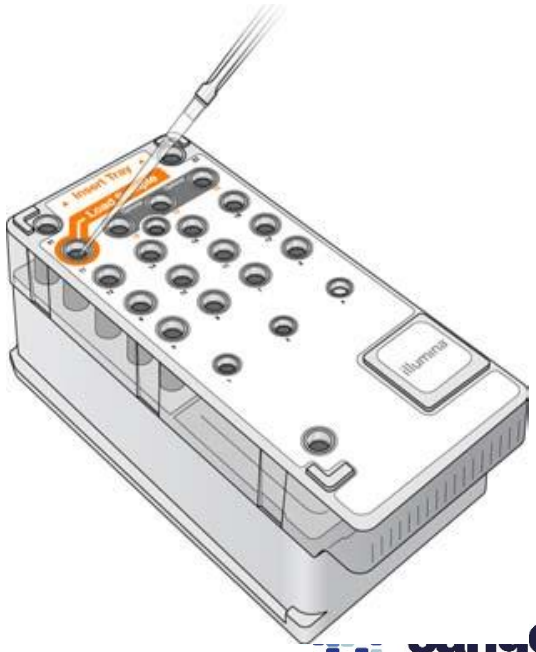
» Flowcell



A- Outlet Port
B- Imaging Area
C- Inlet Port

MiSeq requirements

- Library
- Reagents
  - Reagent cartridge (-20C)



MiSeq requirements

» Library
» Reagents
» Sample sheet

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MiSeq requirements

» Library
» Reagents
» Sample sheet

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NGS course samplesheet

[Header]				
Investigato	mq1			
Project Nar	WTAC test			
Experiment	test genomes			
Date	24/03/2025			
Workflow	GenerateFASTQ			
Chemistry	Default			
[Reads]				
151				
151				
[Settings]				
[Manifests]				
[Data]				
Sample_ID	Sample_Nan	GenomeFolder	Index	Index2
Library1	Library1		TTACCGAC	CGAATACG
Library2	Library2		TCGTCTGA	GTCCTTGA
Library3	Library3		TTCCAGGT	CAGTGCTT
Library4	Library4		TACGGTCT	TCCATTGC
Library5	Library5		AAGACCGT	GTCGATTG
Library6	Library6		CAGGTTCA	ATAACGCC
Library7	Library7		TAGGAGCT	GCCTTAAC
Library8	Library8		TACTCCAG	GGTATAGG
Library9	Library9		AGTGACCT	TCTAGGAG

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Any Questions ?

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