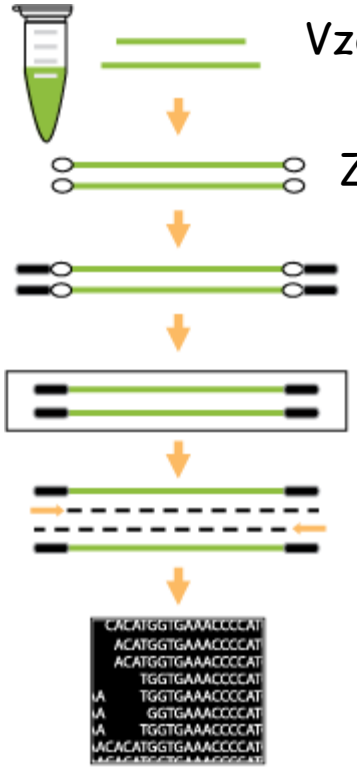
Sekvenátor II generace –MiniSeq, MiSeq, NextSeq, HiSeq, NovaSeq

Sekvenátor II generace

Příprava DNA knihovny



Vzorek DNA – fragmentace (Covaris, fragmentáza)

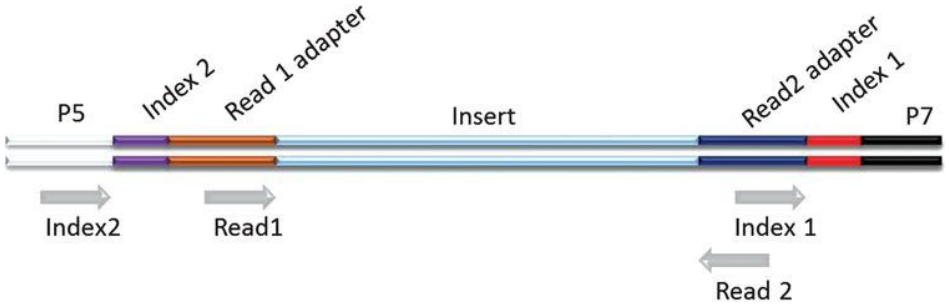
Začištění konců (DNA polymeráza)

Ligace adapterů (ligáza)

Výběr fragmentů dle velkosti (SPRI)

Amplifikace fragmentů

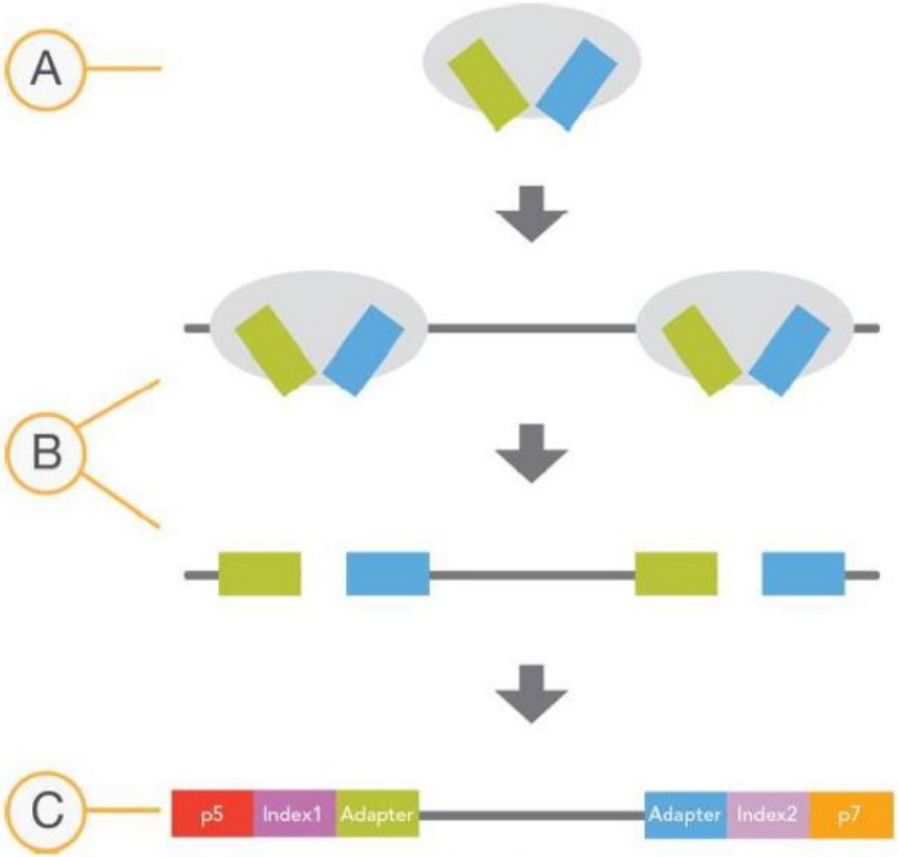
Sekvenace (Illumina, IonTorrent)



Sekvenátor II generace

Library Preparation

Illumina Nextera DNA Sample Preparation Kit



A Nextera Transposome with Adaptors
B Tagmentation to Fragment and Add Adaptors
C Limited Cycle PCR to Add Sequencing Primer Sequences and Indicies

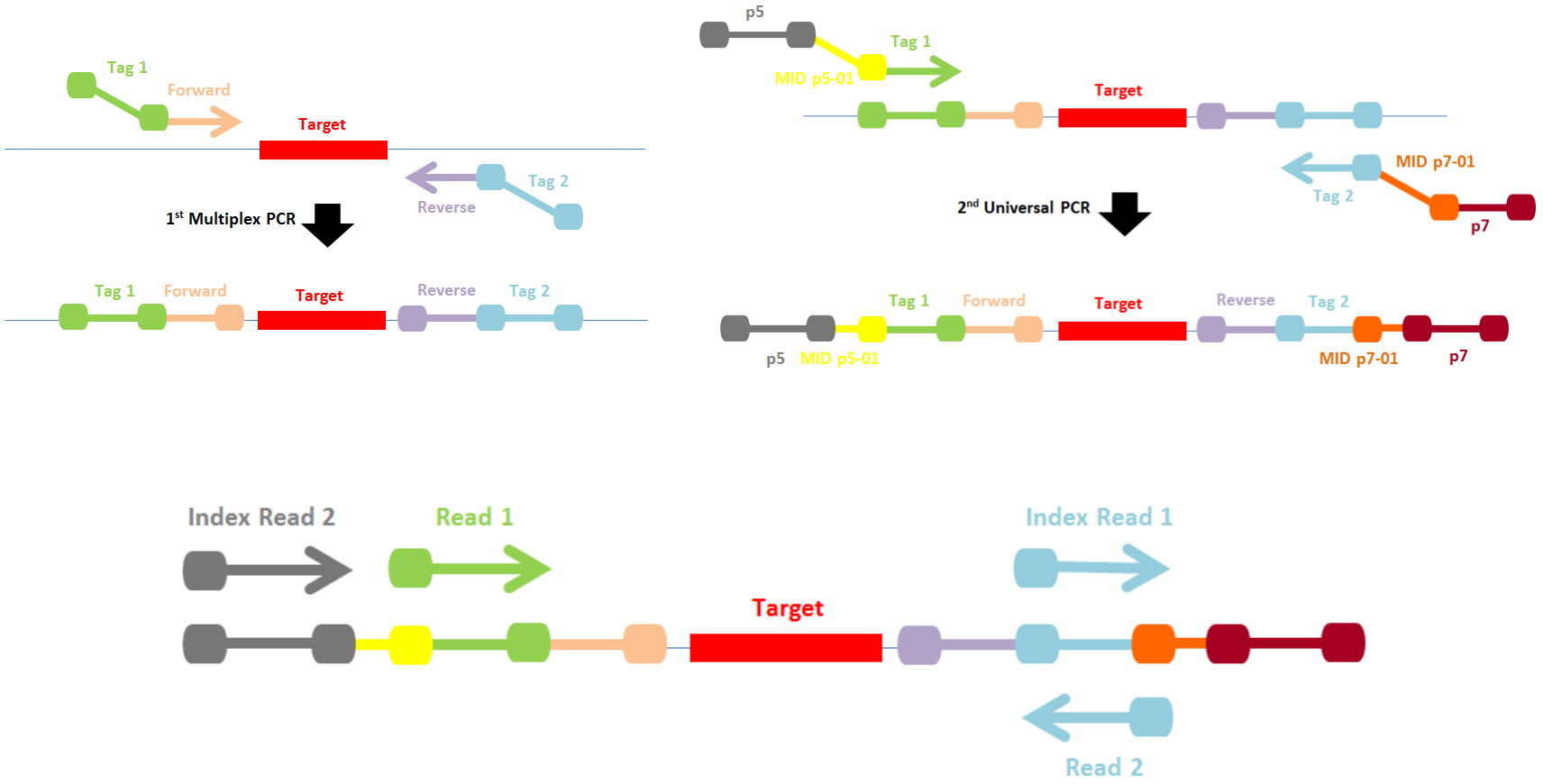
Separate fragmentation not required

Tag with enzyme mix

PCR
Polishes fragment ends and incorporates optional indices

Sekvenátor II generace

Sekvenace amplikonů

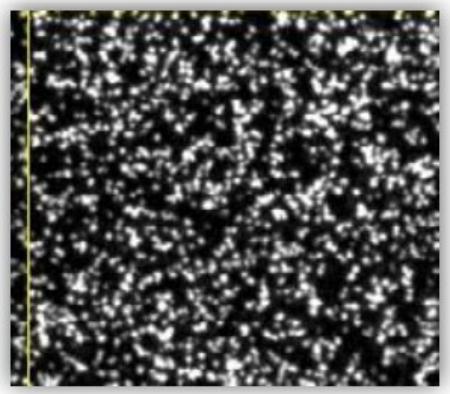


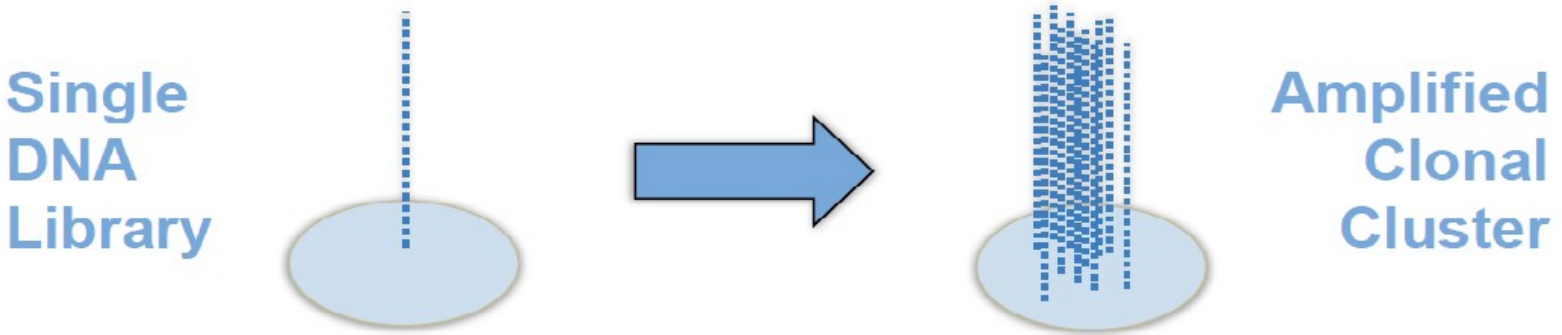
Sekvenátor II generace

Sekvenace probíhá v klastrech

Clusters are bright spots on an image

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





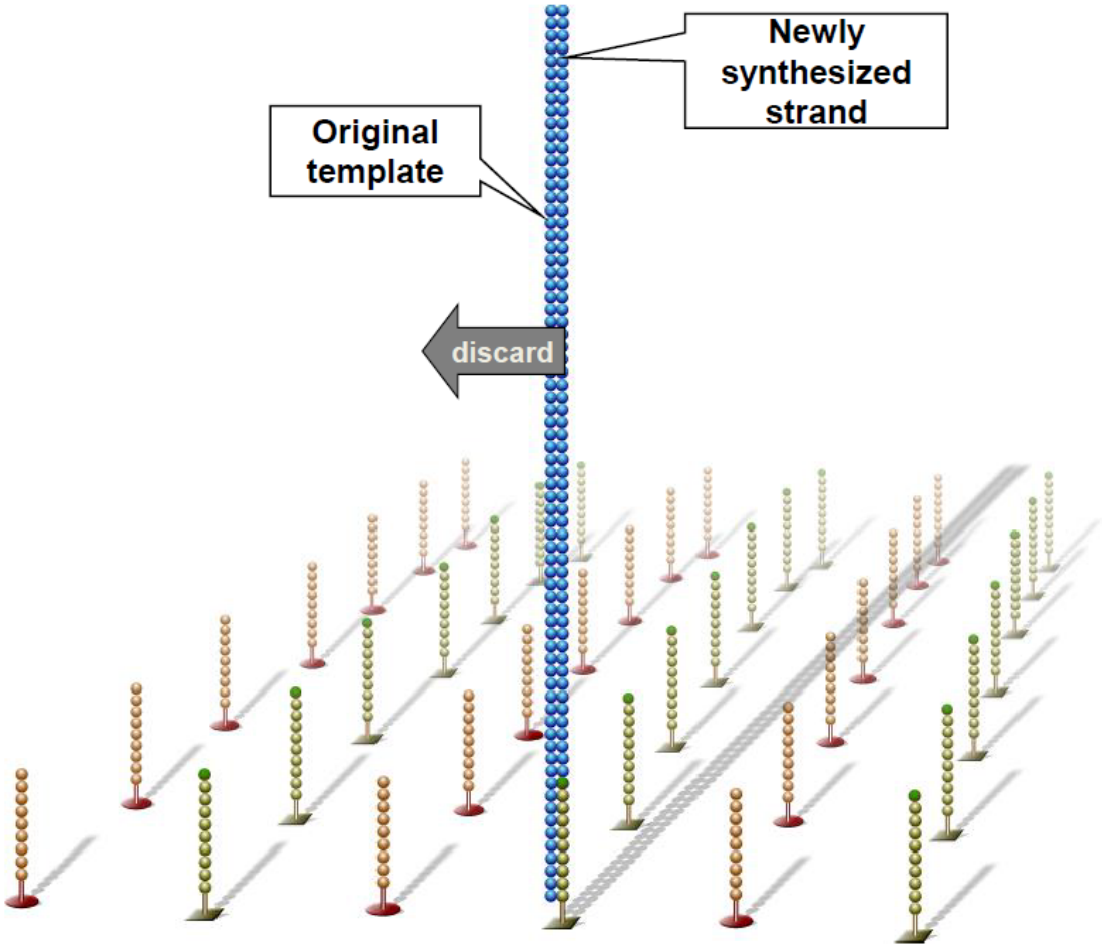
Sekvenátor II generace

1. Krok – hybridizace templátu na průtočnou celu

Double-stranded molecule is denatured

Original template washed away

Newly synthesized strand is covalently attached to flow cell surface

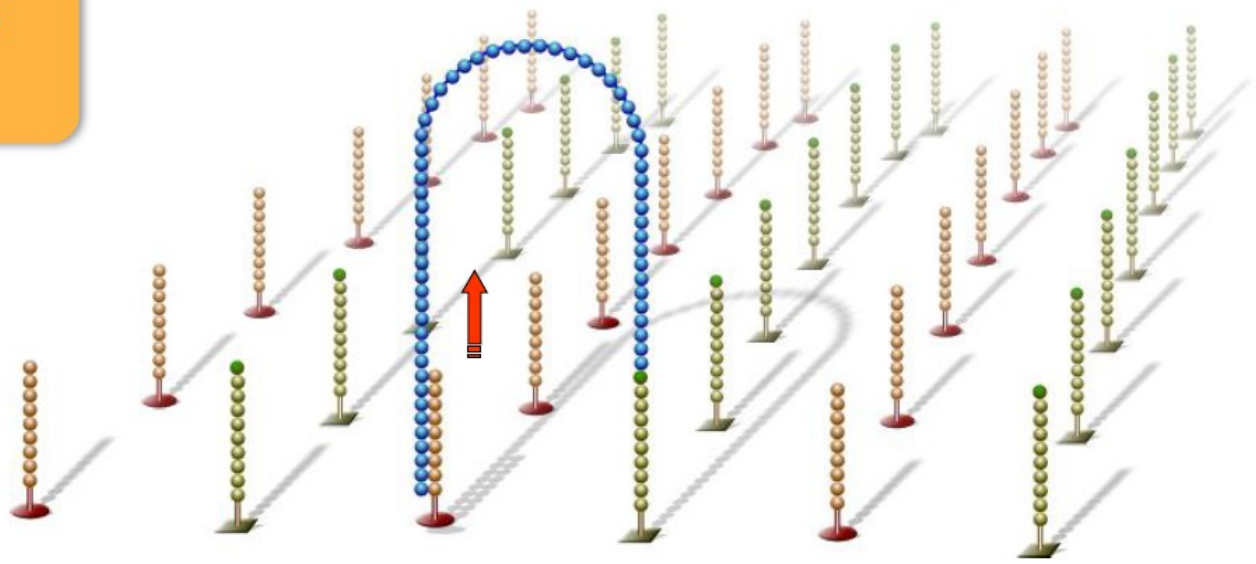


Sekvenátor II generace

2. Krok – Můstková PCR

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

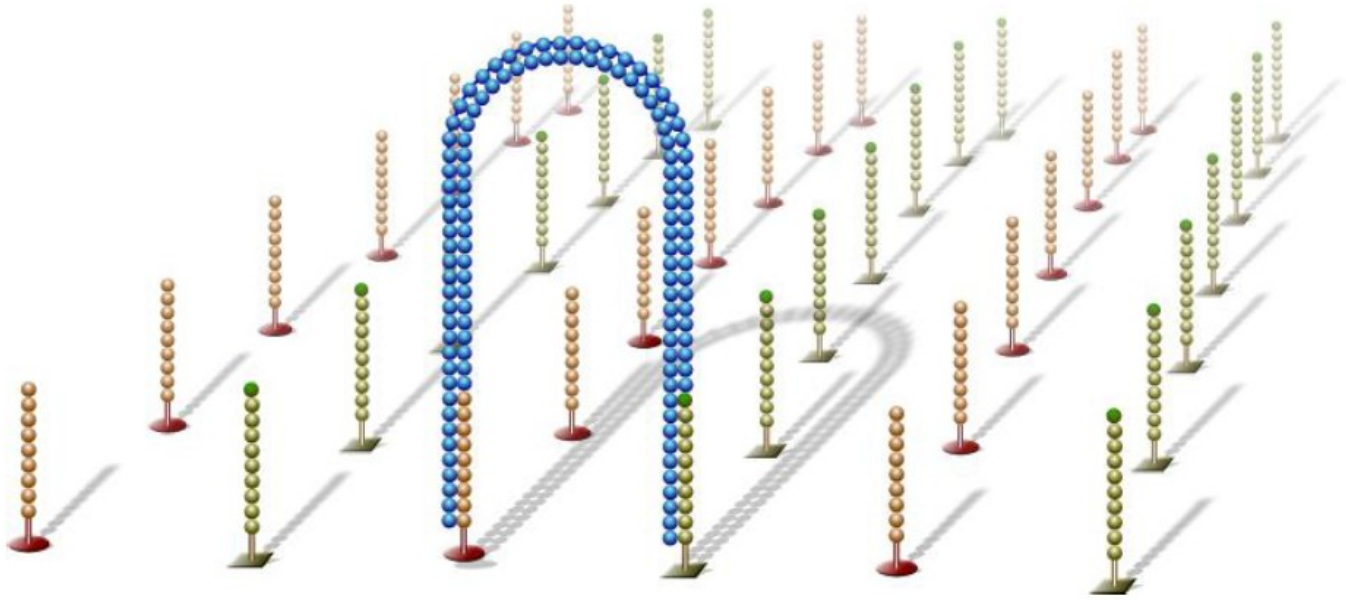
Hybridized primer is extended by polymerases



Sekvenátor II generace

2. Krok – Můstková PCR

Double-stranded bridge is formed

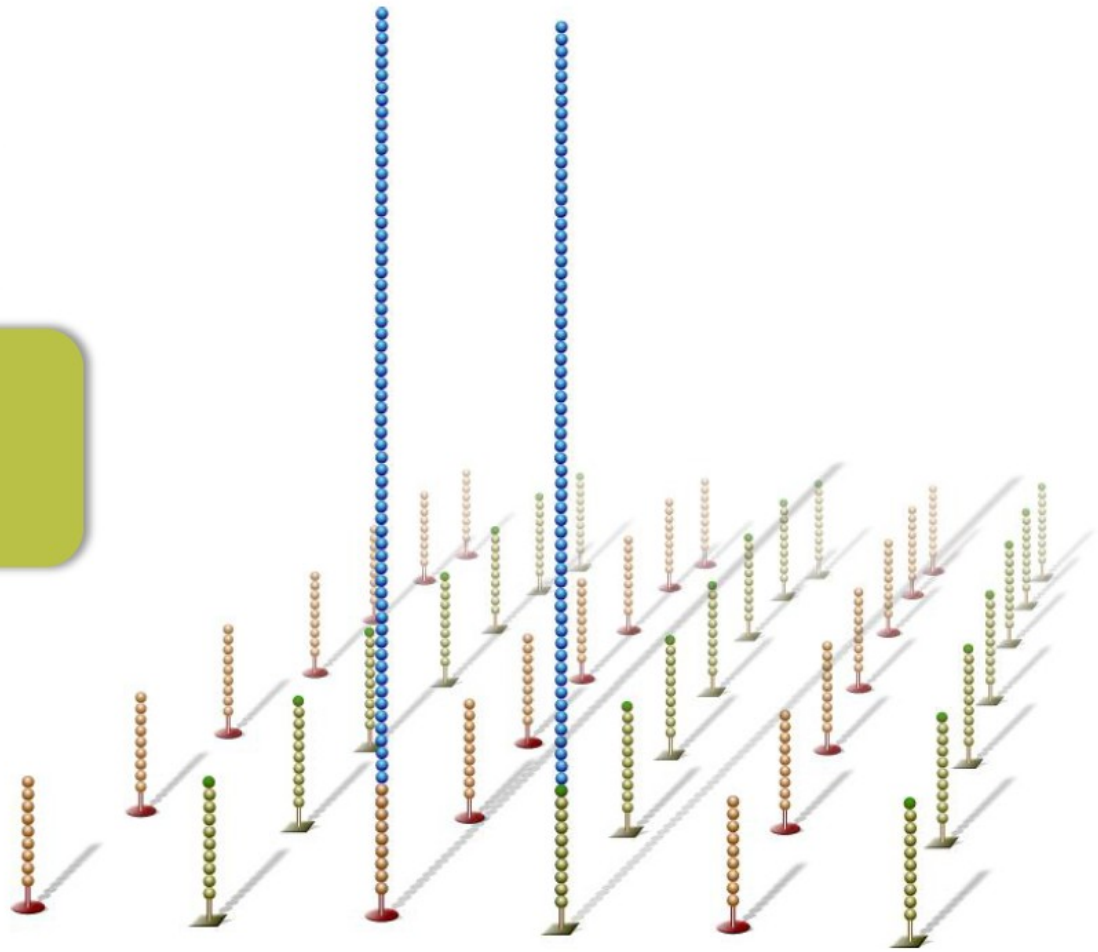


Sekvenátor II generace

2. Krok – Můstková PCR

Double-stranded bridge is denatured - 1st cycle denaturation

Result:
Two copies of covalently bound single-stranded templates

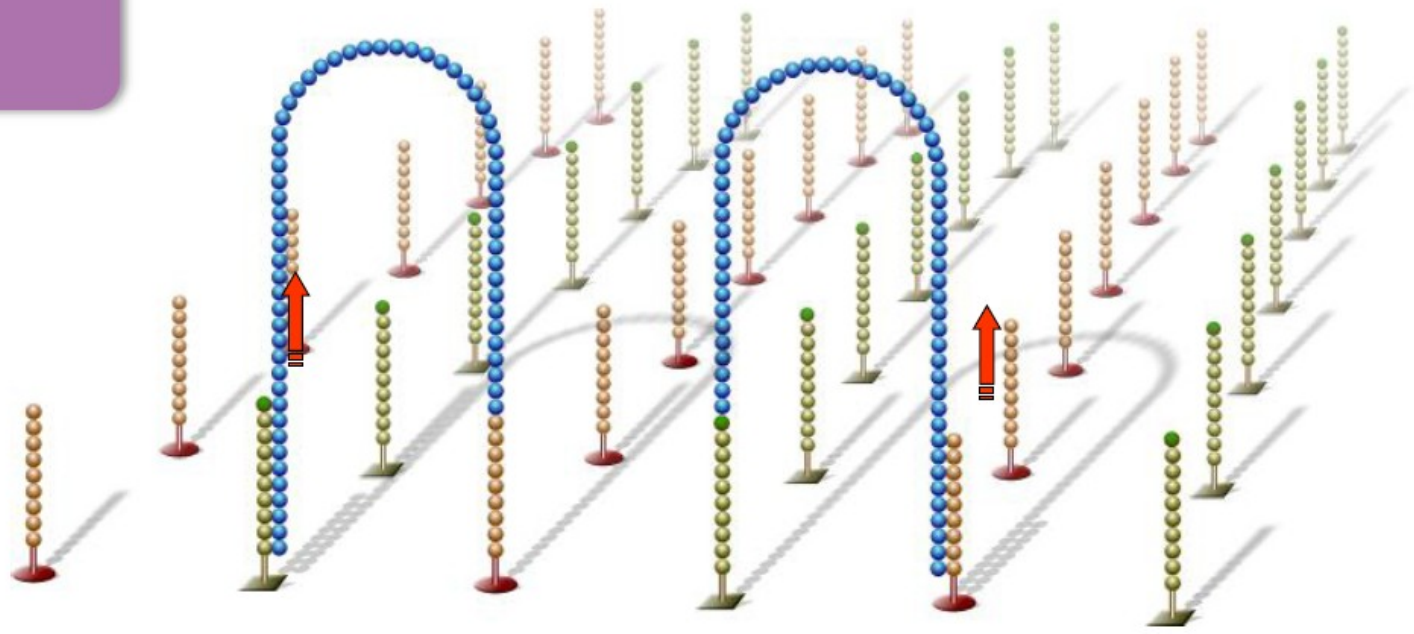


Sekvenátor II generace

2. Krok – Můstková PCR

Single-stranded molecules flip over to hybridize to adjacent primers

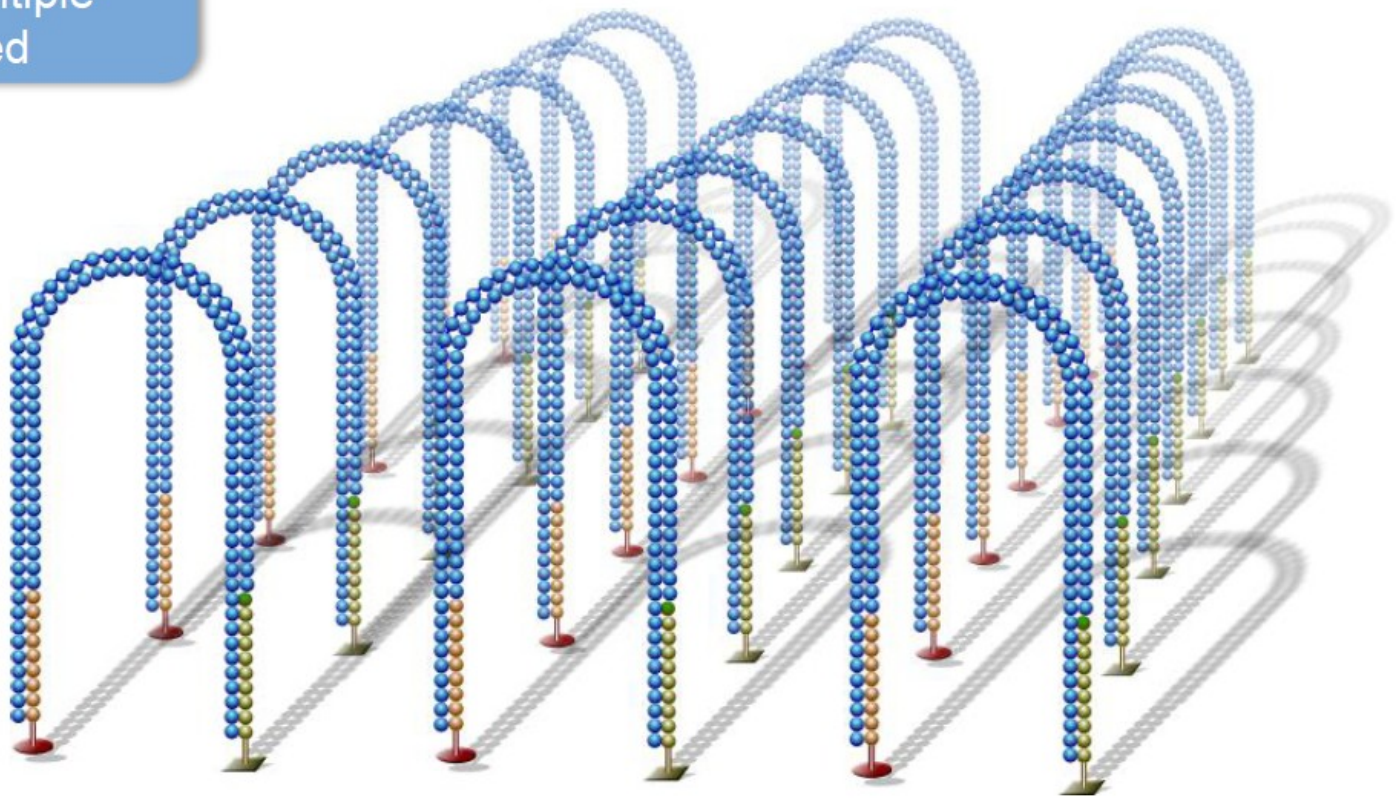
Hybridized primer is extended by polymerase



Sekvenátor II generace

2. Krok – Můstková PCR

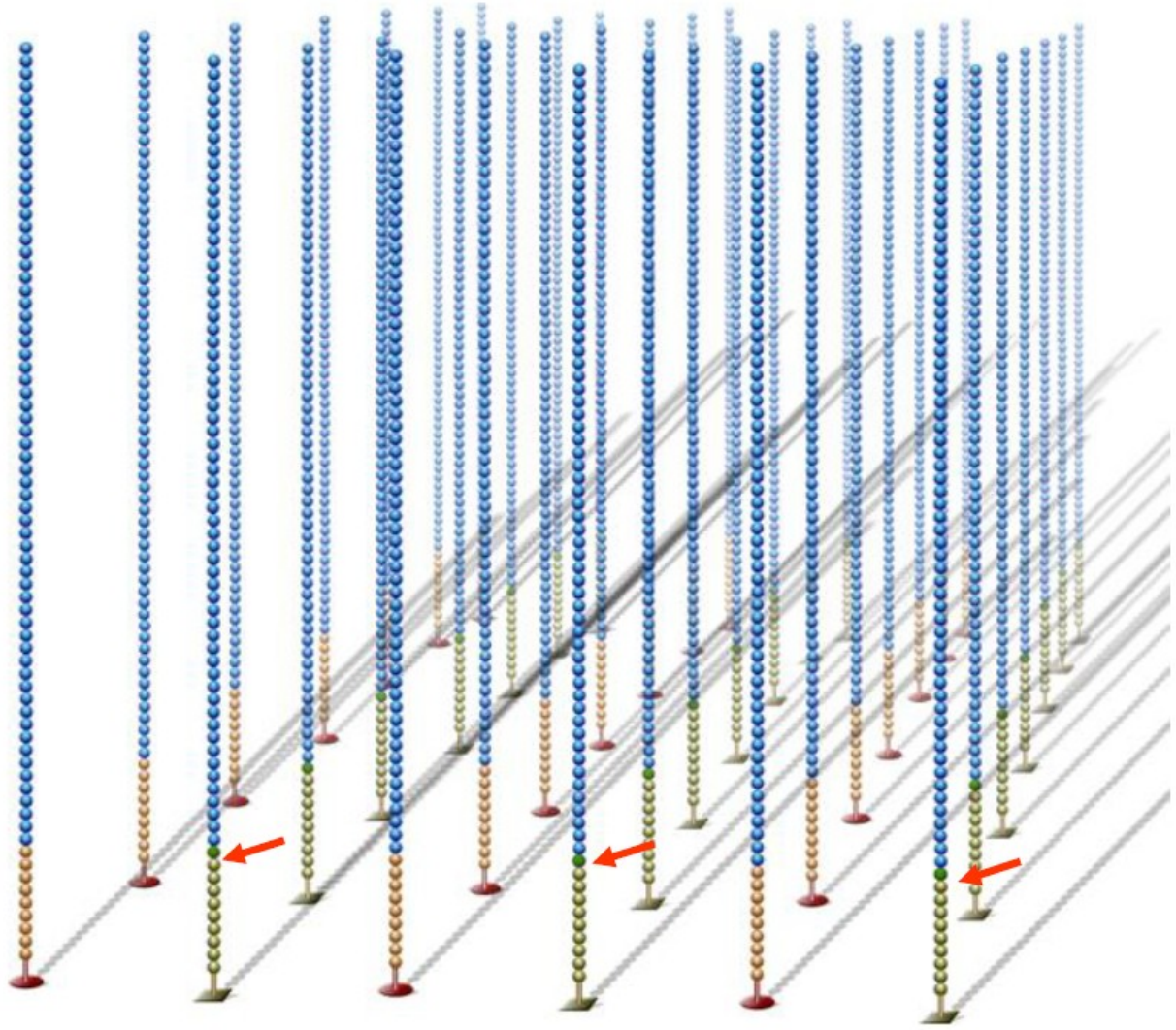
Bridge amplification cycle repeated until multiple bridges are formed



Sekvenátor II generace

3. Krok – Linearizace

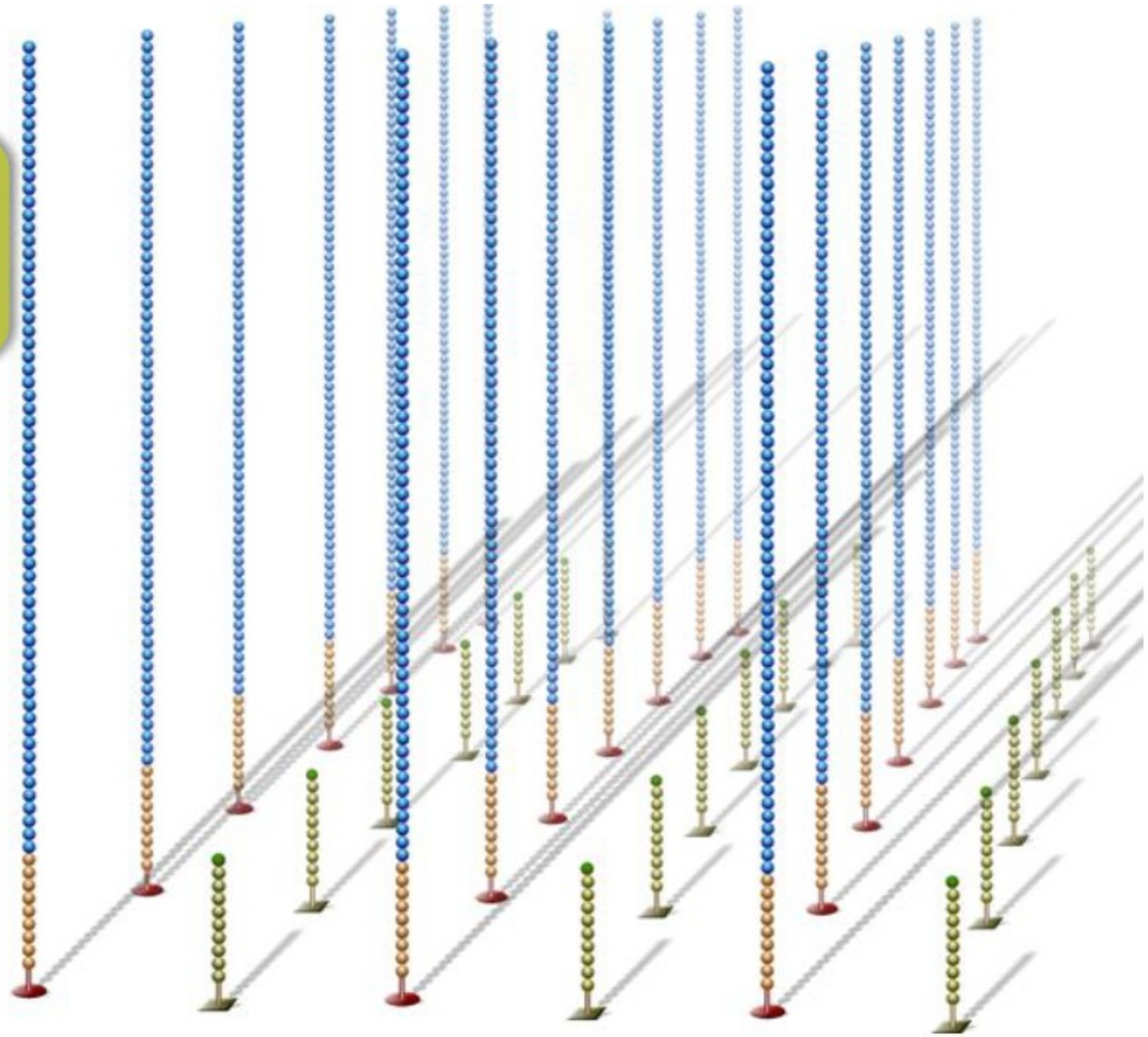
dsDNA bridges are denatured



Sekvenátor II generace

4. Krok – Odštěpení reverzního vlákna

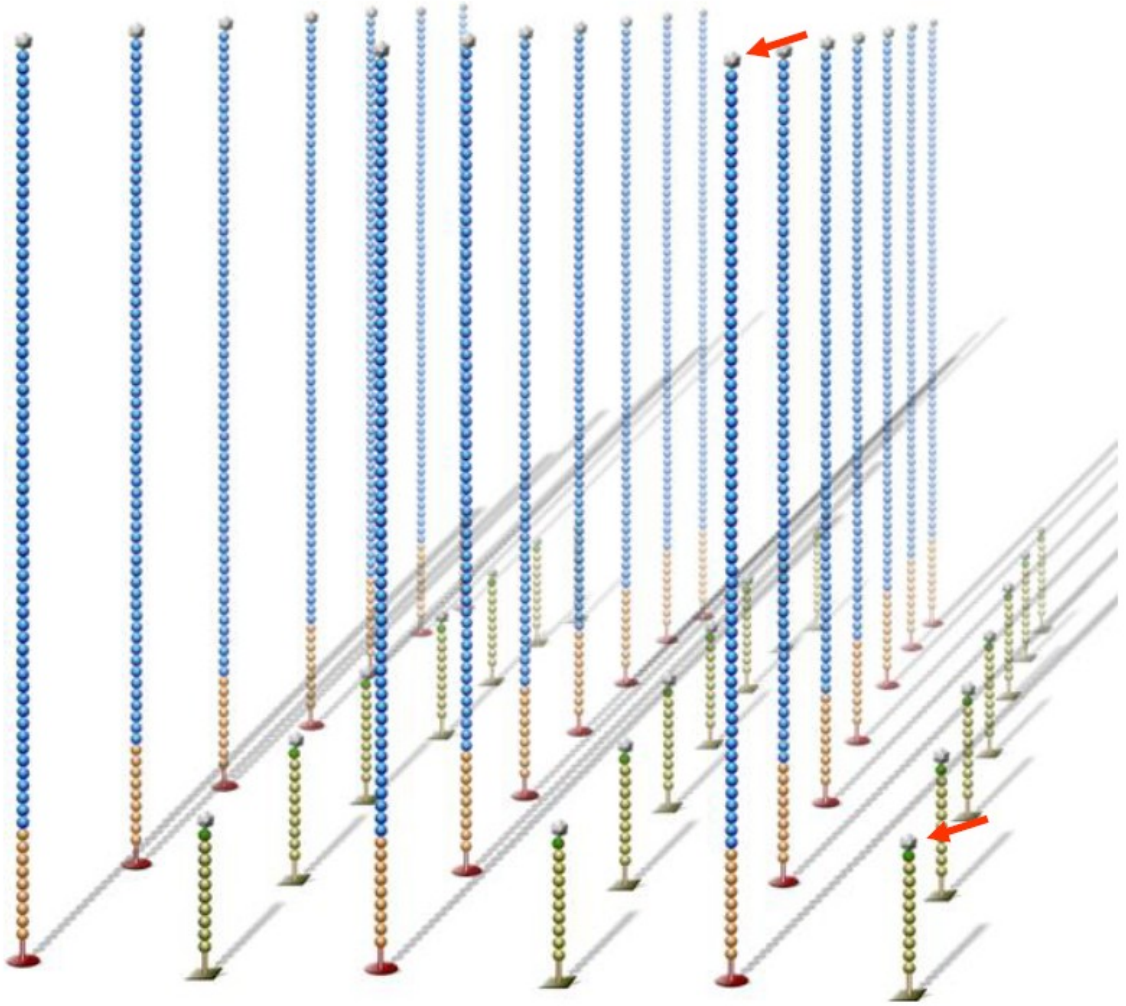
Reverse strands cleaved and washed away, leaving a cluster with forward strands only



Sekvenátor II generace

5. Krok – Blokace volného 5' konce

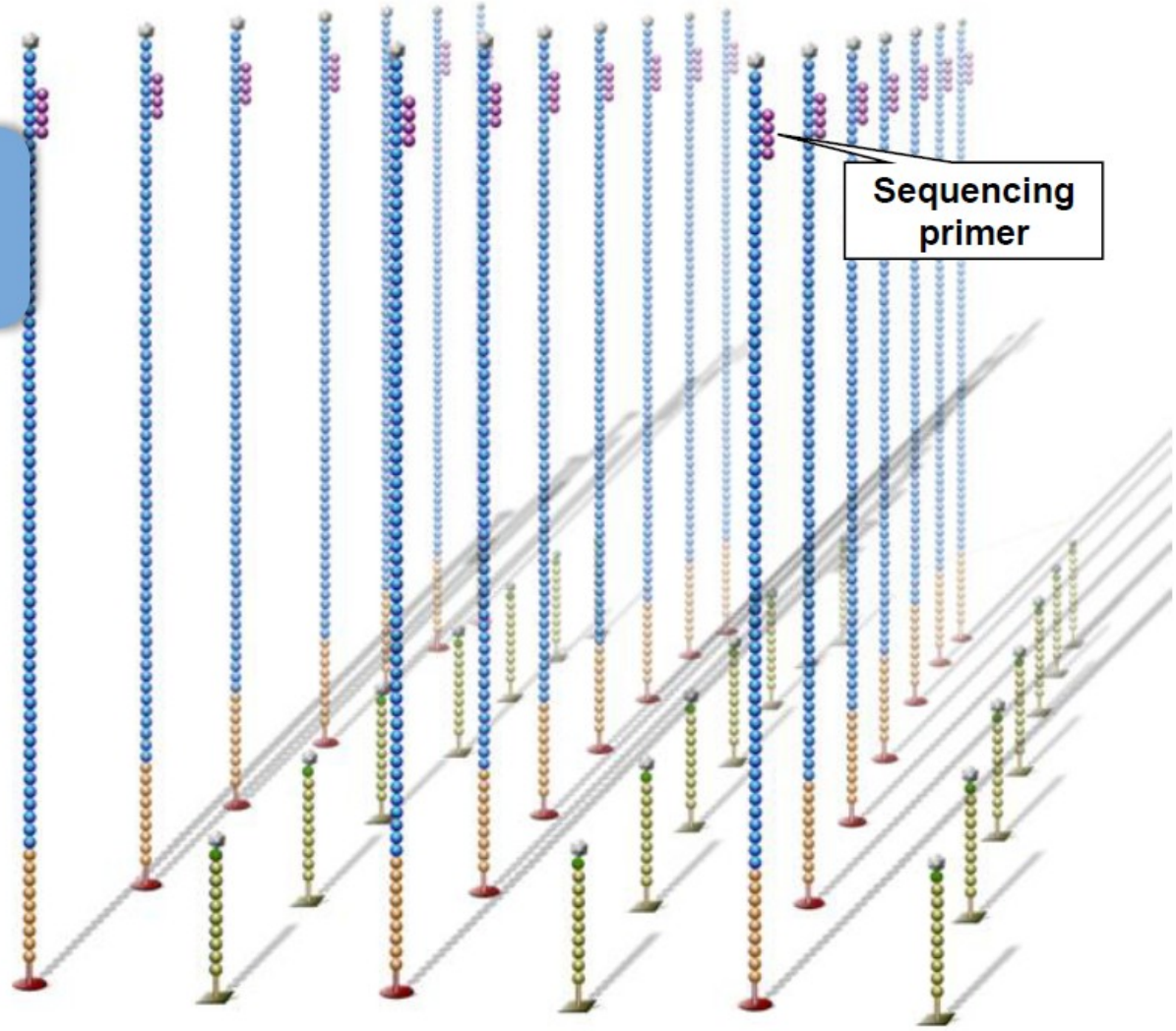
Free 3' ends are blocked to prevent unwanted DNA priming



Sekvenátor II generace

6. Krok: Čtení – hybridizace sek. primeru

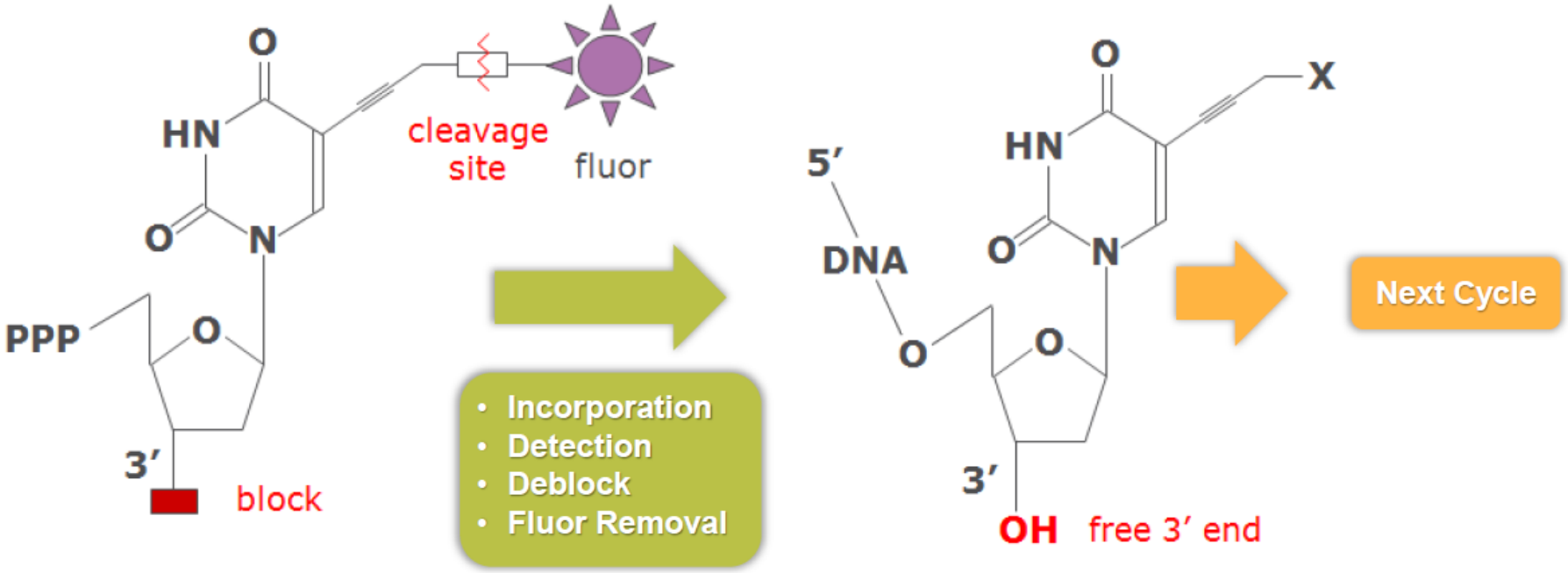
Sequencing primer is hybridized to adapter sequence



Sekvenátor II generace

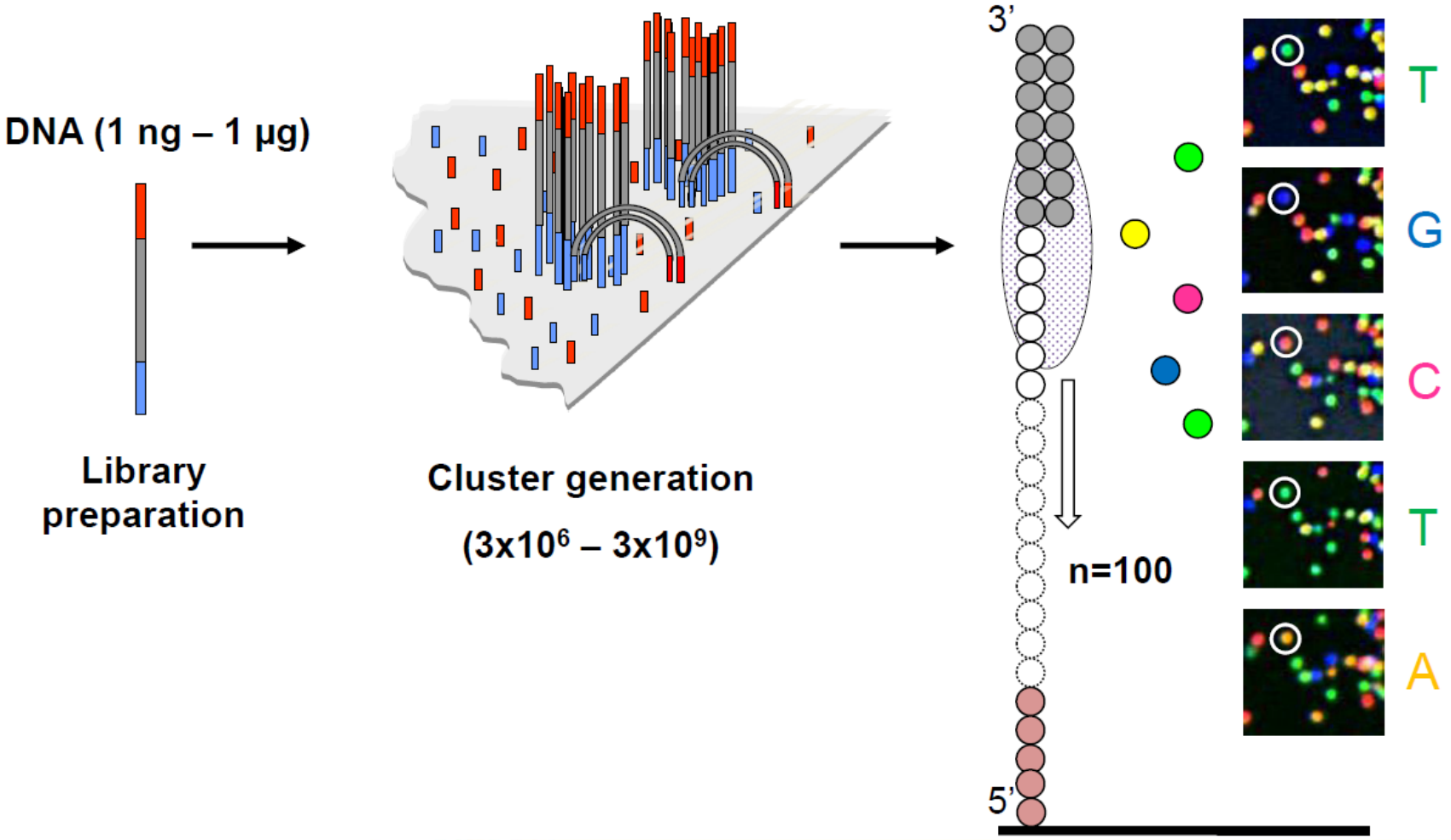
Chemie reverzibilních terminátorů

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



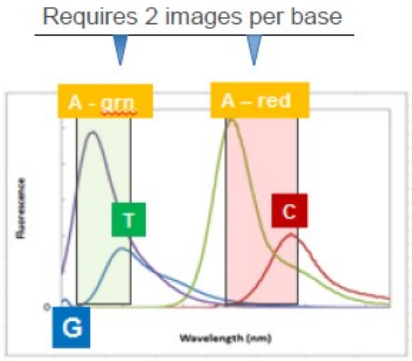
Sekvenátor II generace

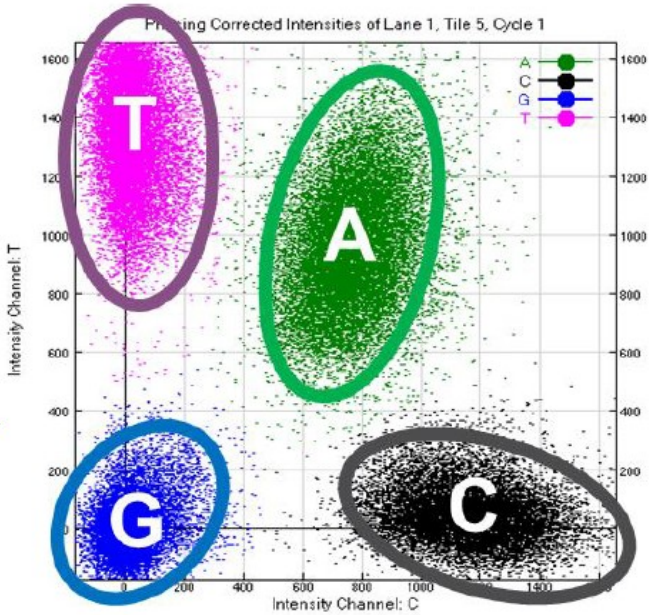
Sekvenace pomocí syntézy (SBS)

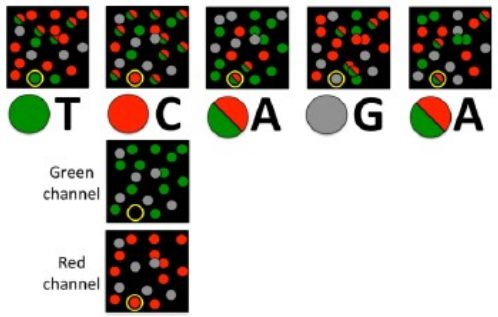


Sekvenátor II generace

2-Channel SBS Chemistry: MiniSeq, NextSeq





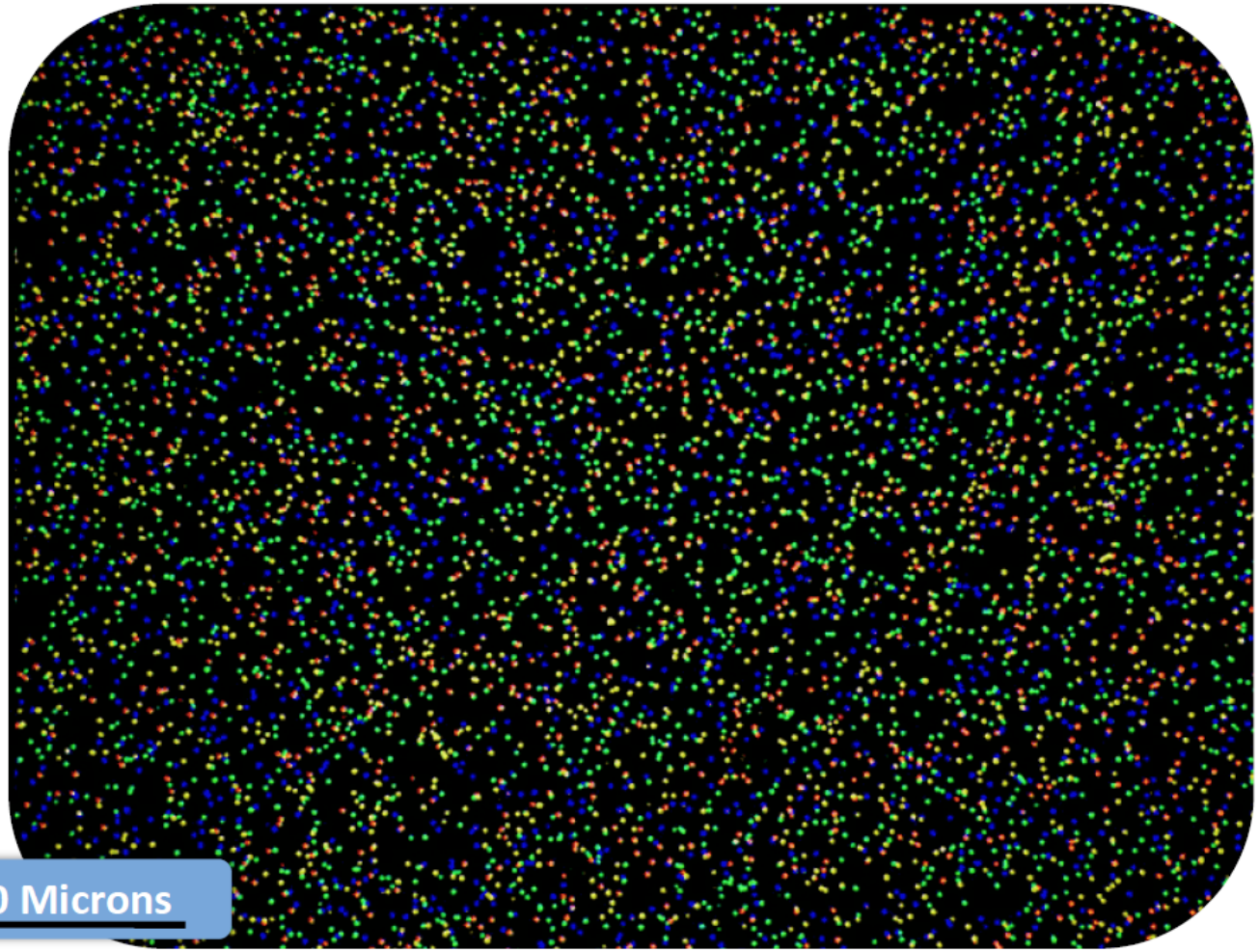


High performance with half the pictures

50% fewer images

Sekvenátor II generace

Clusters

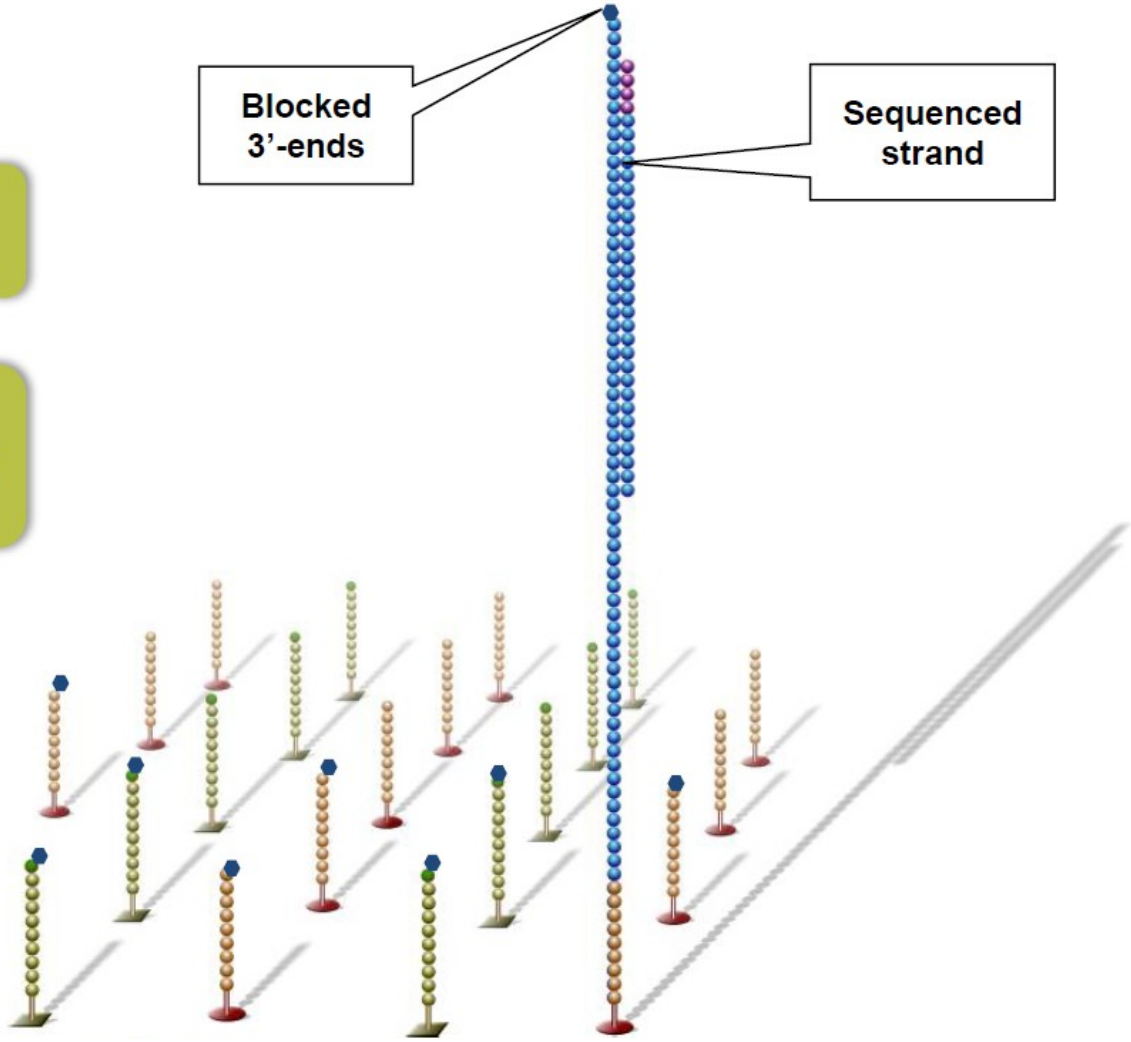


Sekvenátor II generace

7. Krok – Pair-End sekvenace

Sequenced strand is stripped off

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

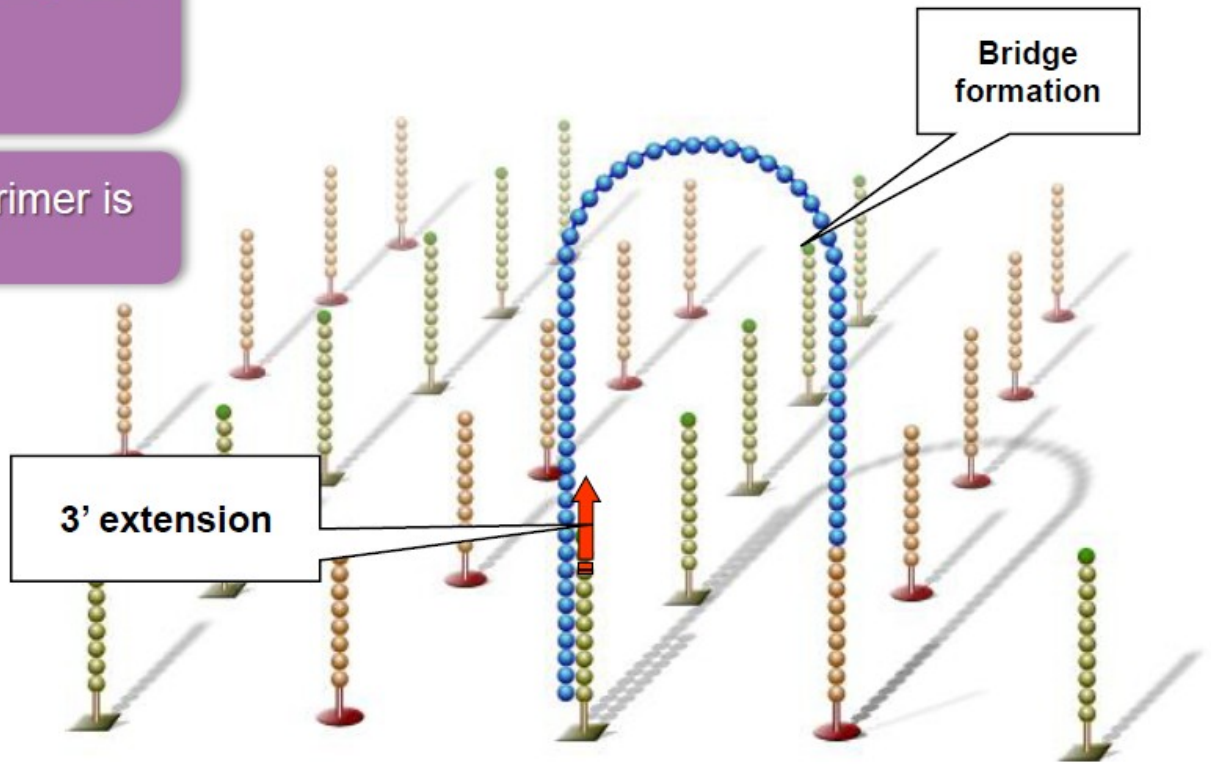


Sekvenátor II generace

7. Krok – Pair-End sekvenace

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

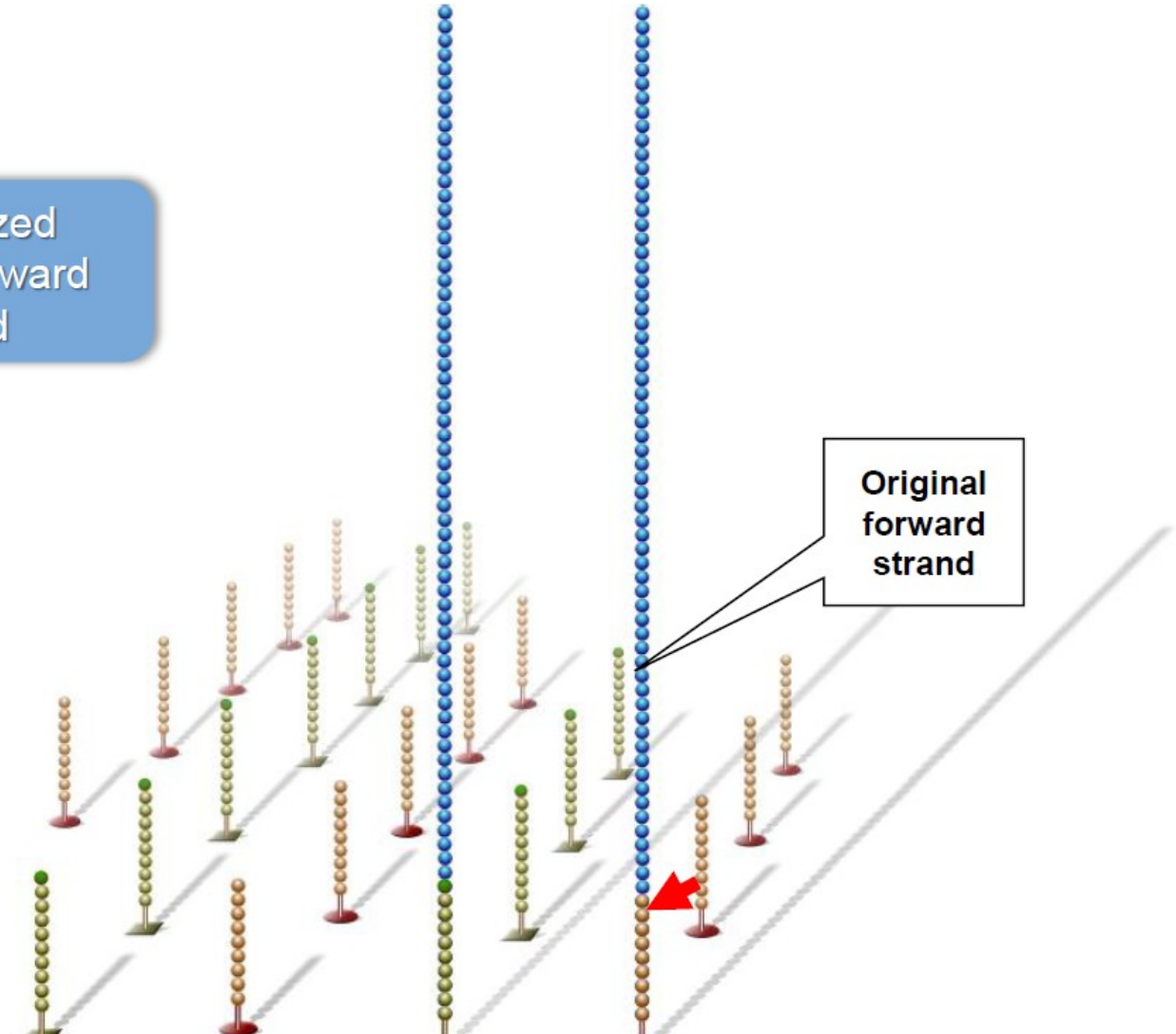
3'-ends of lawn primer is extended



Sekvenátor II generace

7. Krok – Pair-End sekvenace

Bridges are linearized and the original forward template is cleaved

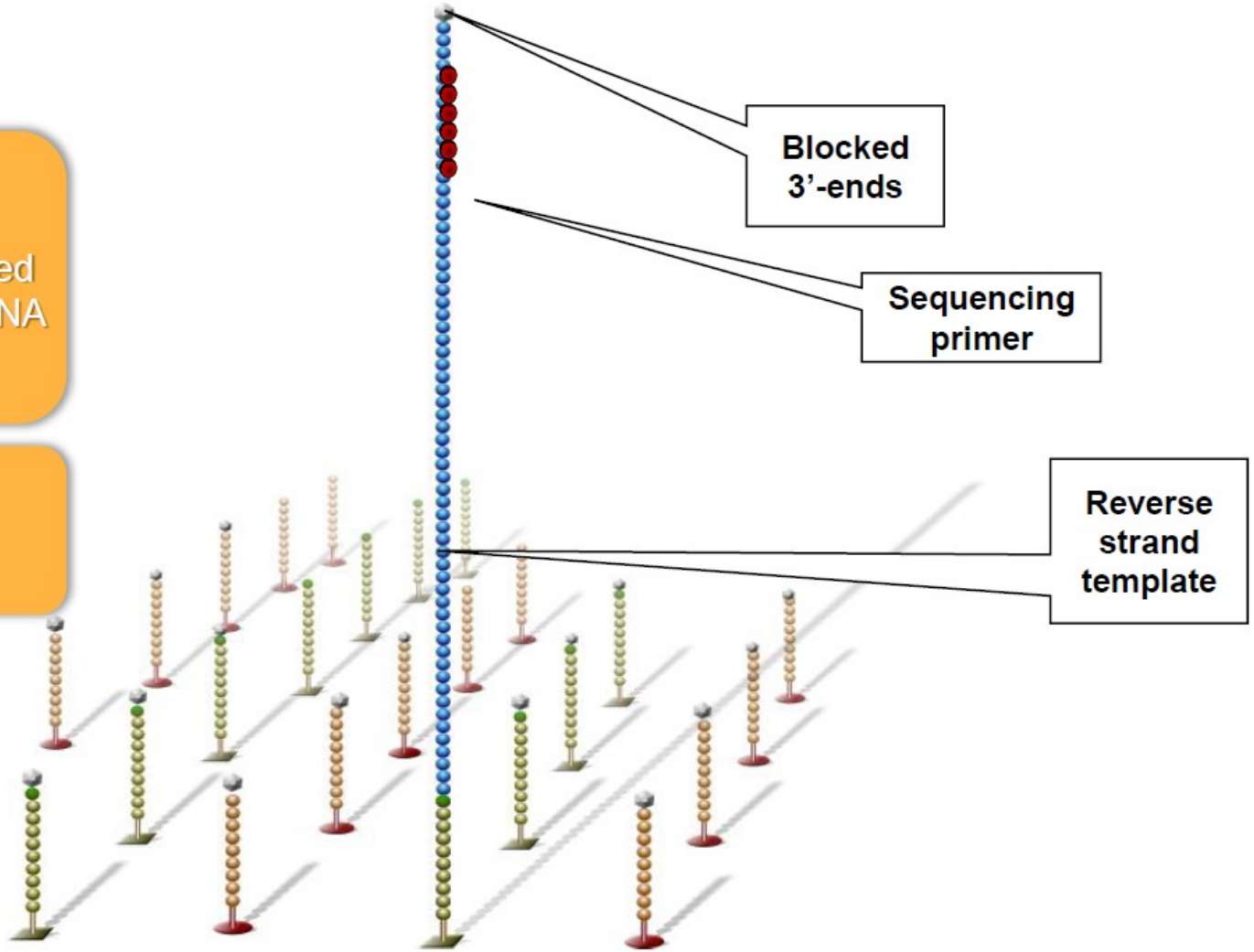


Sekvenátor II generace

7. Krok – Pair-End sekvenace

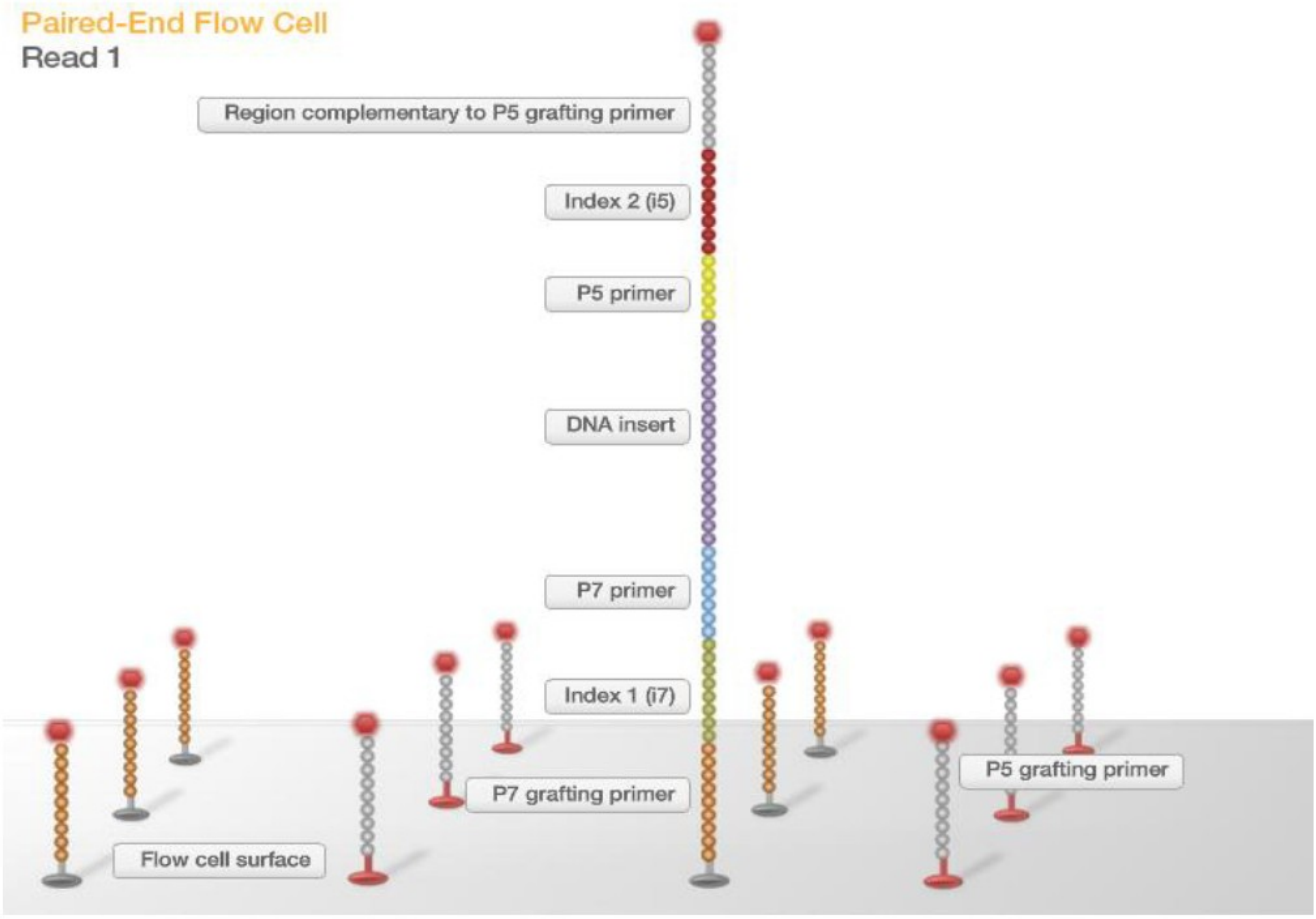
Free 3’ ends of the reverse template and lawn primers are blocked to prevent unwanted DNA priming

Sequencing primer is hybridized to adapter sequence



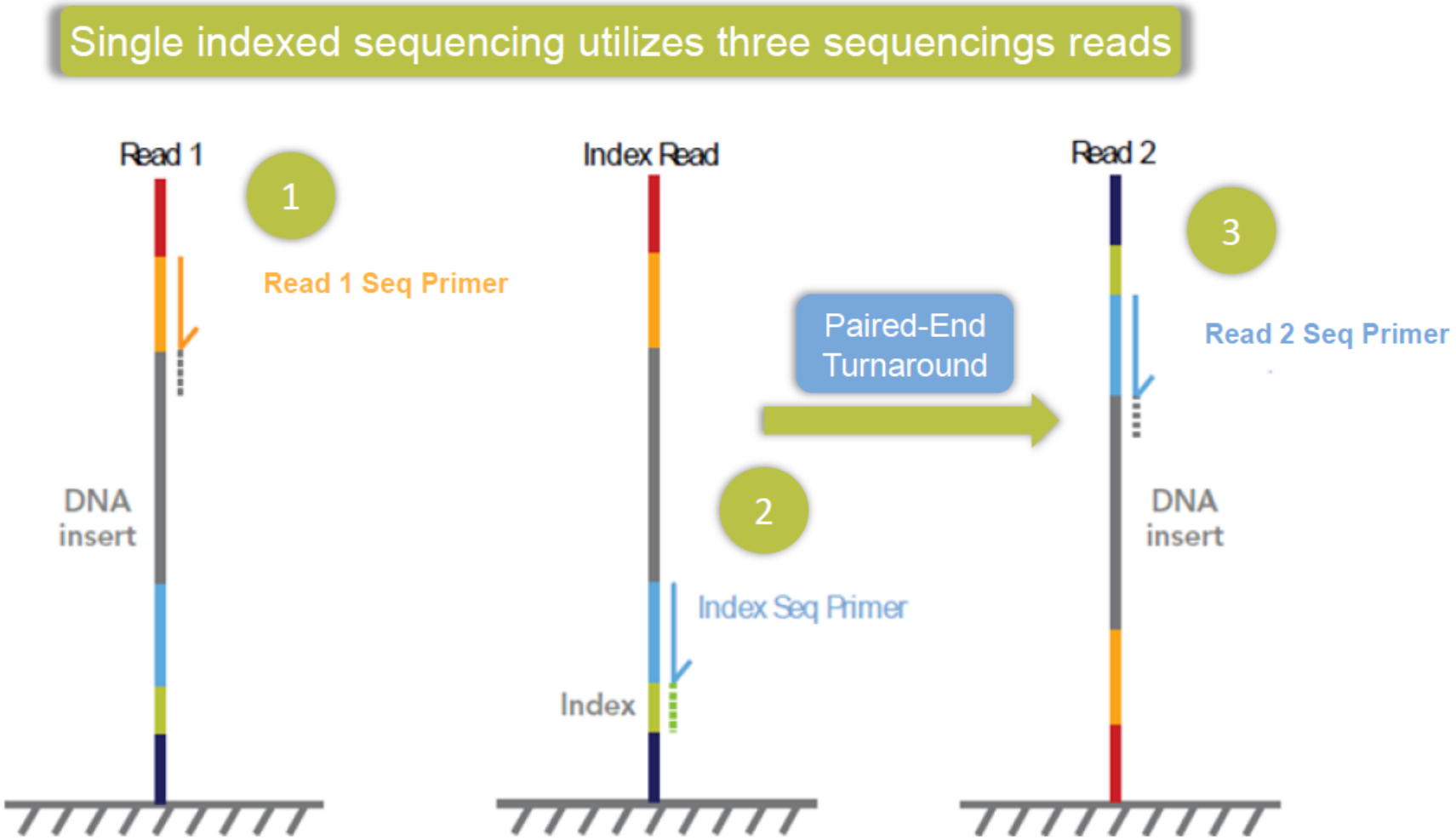
Sekvenátor II generace

Čtení indexů



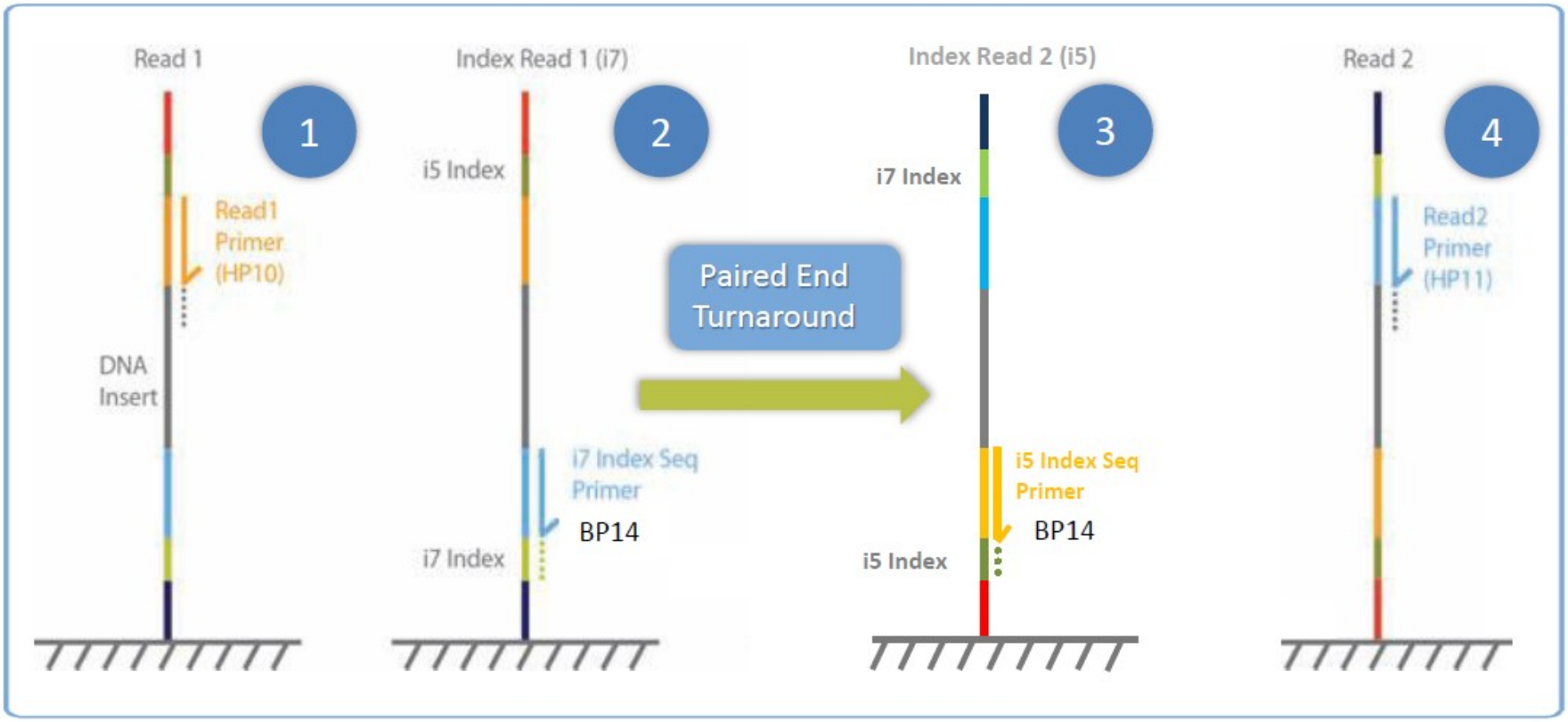
Sekvenátor II generace

Single index čtení



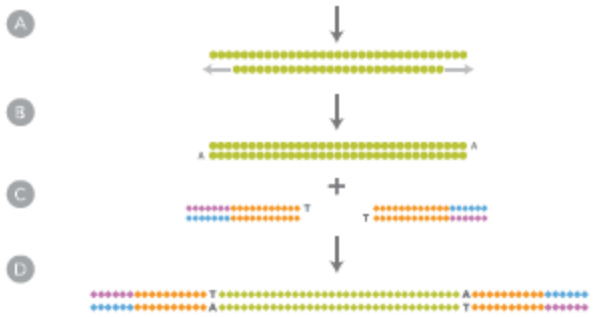
Sekvenátor II generace

Dual index čtení
(iSeq, MiniSeq, NextSeq,HiSeq)



1 LIBRARY PREPARATION

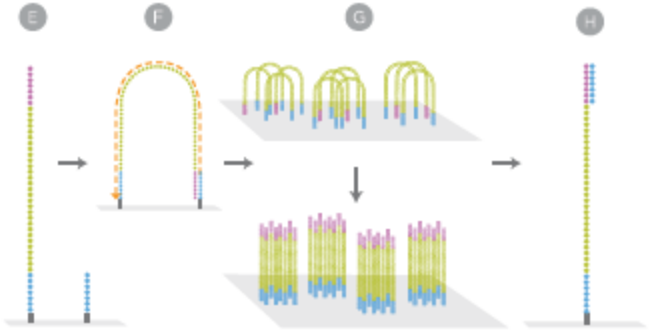
6 hours
3 hours hands-on time



- A Fragment DNA
- B Repair ends
  Add A overhang
- C Ligate adapters
- D Select ligated DNA

2 CLUSTER GENERATION

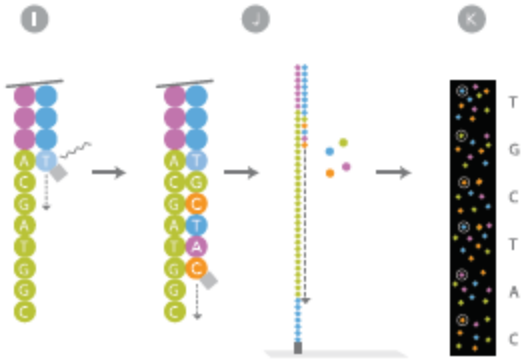
4 hours
< 10 minutes hands-on time
1–96 samples



- E Attach DNA to
  flow cell
- F Perform bridge
  amplification
- G Generate clusters
- H Anneal sequencing
  primer

3 SEQUENCING

1–3 days single-read run
3–9 days paired-end run
30 minutes hands-on time
8 lanes, up to 96 samples
per flow cell (run)



- I Extend first base,
  read, and deblock
- J Repeat step above
  to extend strand
- K Generate base calls

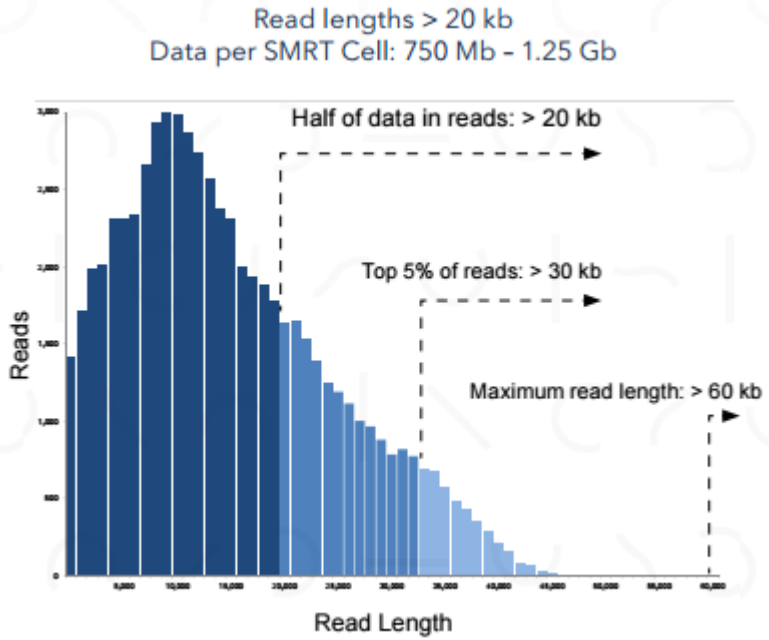
Sekvenátor III generace – PacBio

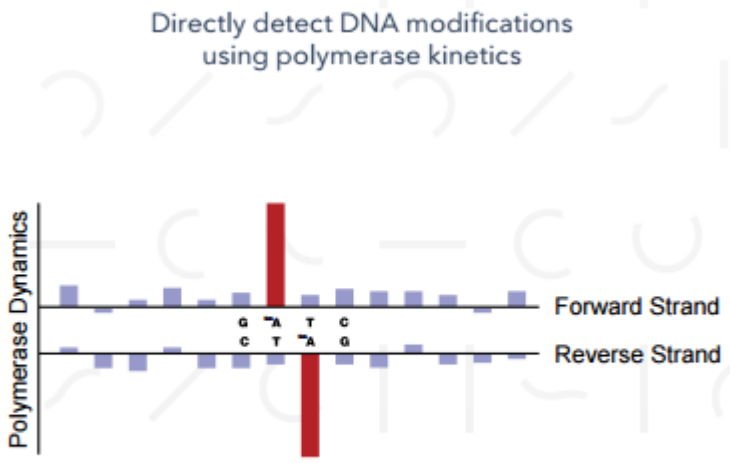
Sequel System

PacBio RS II

Sekvenátor III generace – PacBio

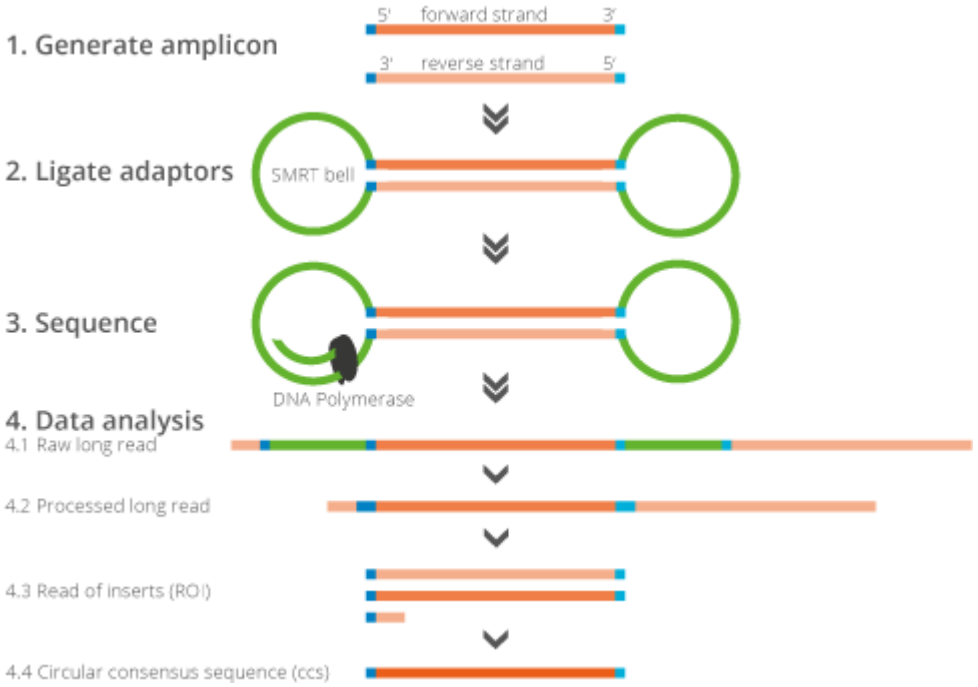
- Sekvenace založena na Single Molecule, Real-Time (SMRT®) technologii
- Vyžívá tzv. Zero-Mode Waveguides (ZMWs) umožňujcící osvícení pouze spodní části jamky, ve které je dole imobilizována DNA polymeráza
- Hlavní výhoda je možnost dlouhého čtění (až 20 kb)
- Další výhoda je možnost přímé detekce methylovaných bazí (epigenom)





Sekvenátor III generace – PacBio

Příprava knihovny



https://www.youtube.com/watch?v=v8p4ph2MAvI

Sekvenátor III generace
Oxford Nanopores

- Základem technologie jsou nanopóry (nanodíry)
- Na začátku sekvenace je NK navázána na nanopór tvořený proteinem
- Poté je rozpletena a prochází přes nanopór, což generuje změnu proudu
- Na základě pozorovanéí zmšny jsou odečítání v reálním čase jednotlivé báze
- Umožňuje sekvenaci velmi dlouhých úšeku (desítky až stovky kilobází)
- Nevýhodou je vyšší chybovost, správnost >90%