

# Sekvenátor II generace -MiniSeq, MiSeq, NextSeq, HiSeq, NovaSeq

ially

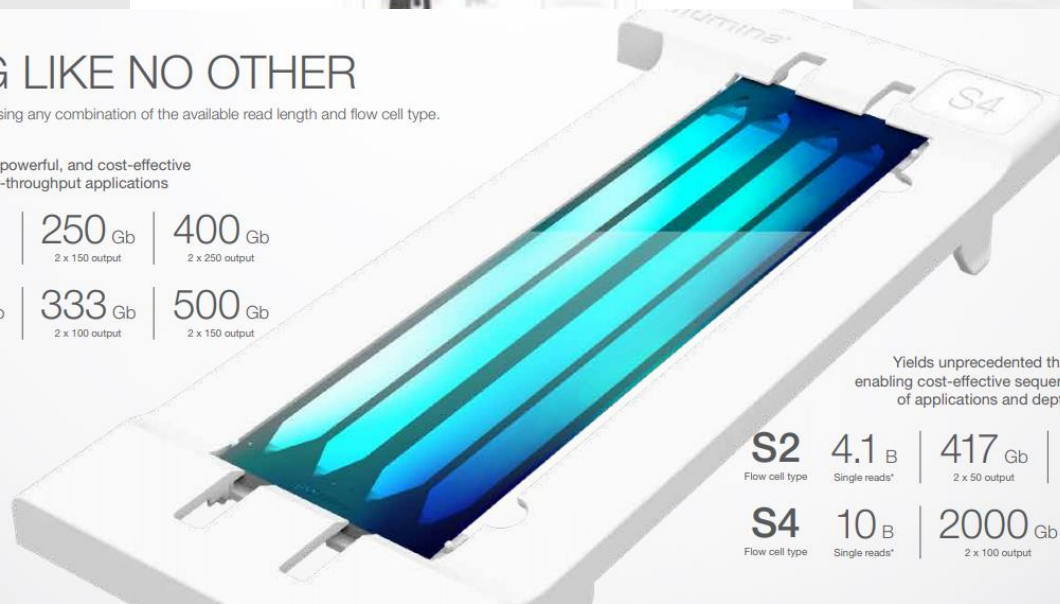
## SEQUENCING LIKE NO OTHER

Users can run 1 or 2 flow cells at a time, using any combination of the available read length and flow cell type.

Provides a quick, powerful, and cost-effective option for high-throughput applications

<b>SP</b> Flow cell type	0.8 <sub>B</sub> Single reads*	80 Gb 2 x 50 output	250 Gb 2 x 150 output	400 Gb 2 x 250 output
<b>S1</b> Flow cell type	1.6 <sub>B</sub> Single reads*	167 Gb 2 x 50 output	333 Gb 2 x 100 output	500 Gb 2 x 150 output

\*Clusters passing filter

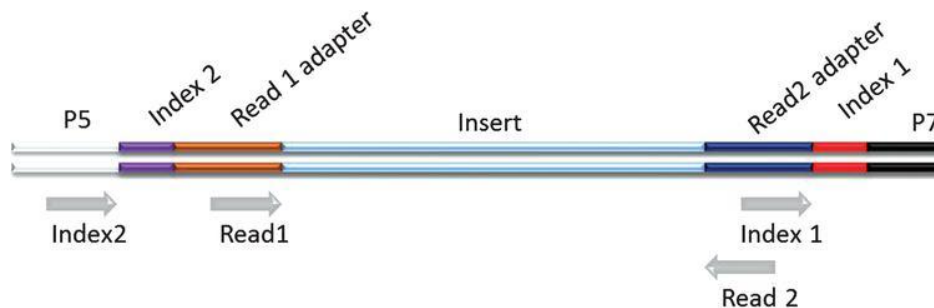
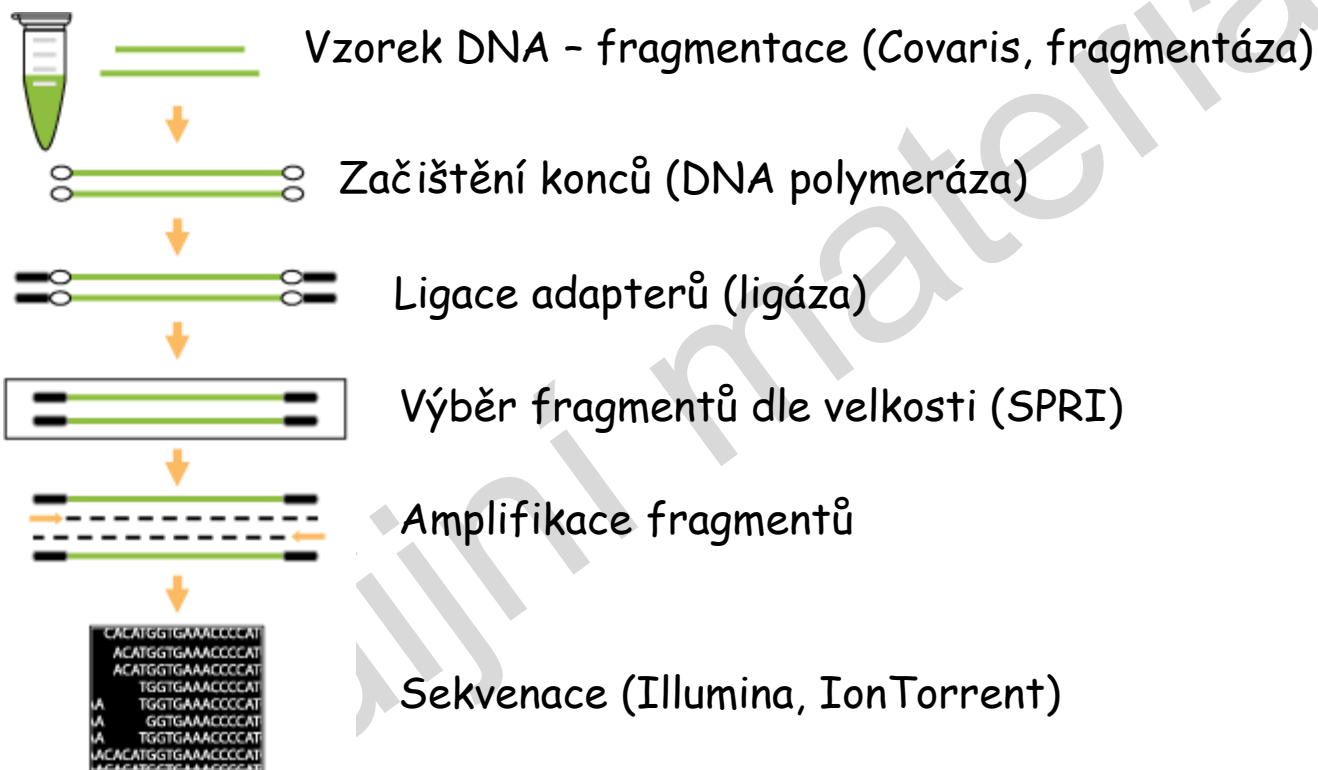


Yields unprecedented throughput while enabling cost-effective sequencing across a range of applications and depth of coverage

<b>S2</b> Flow cell type	4.1 <sub>B</sub> Single reads*	417 Gb 2 x 50 output	833 Gb 2 x 100 output	1250 Gb 2 x 150 output
<b>S4</b> Flow cell type	10 <sub>B</sub> Single reads*	2000 Gb 2 x 100 output	3000 Gb 2 x 150 output	

# Sekvenátor II generace

## Příprava DNA knihovny

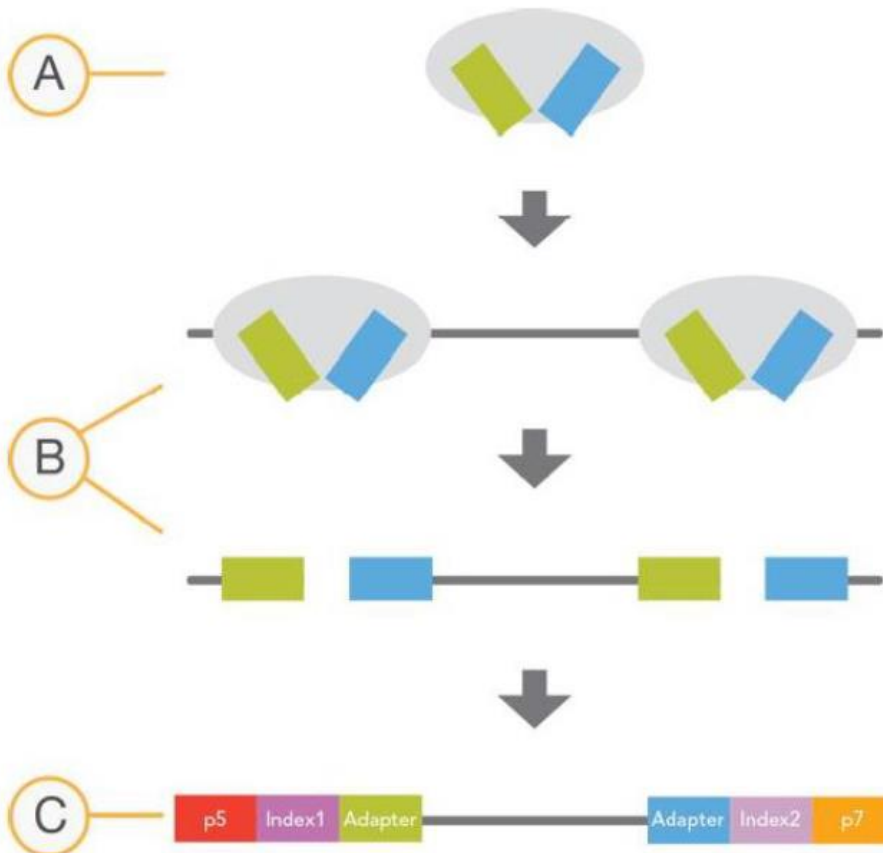




# Sekvenátor II generace

## Library Preparation

*Illumina Nextera DNA Sample Preparation Kit*



Separate fragmentation not required

Tag with enzyme mix

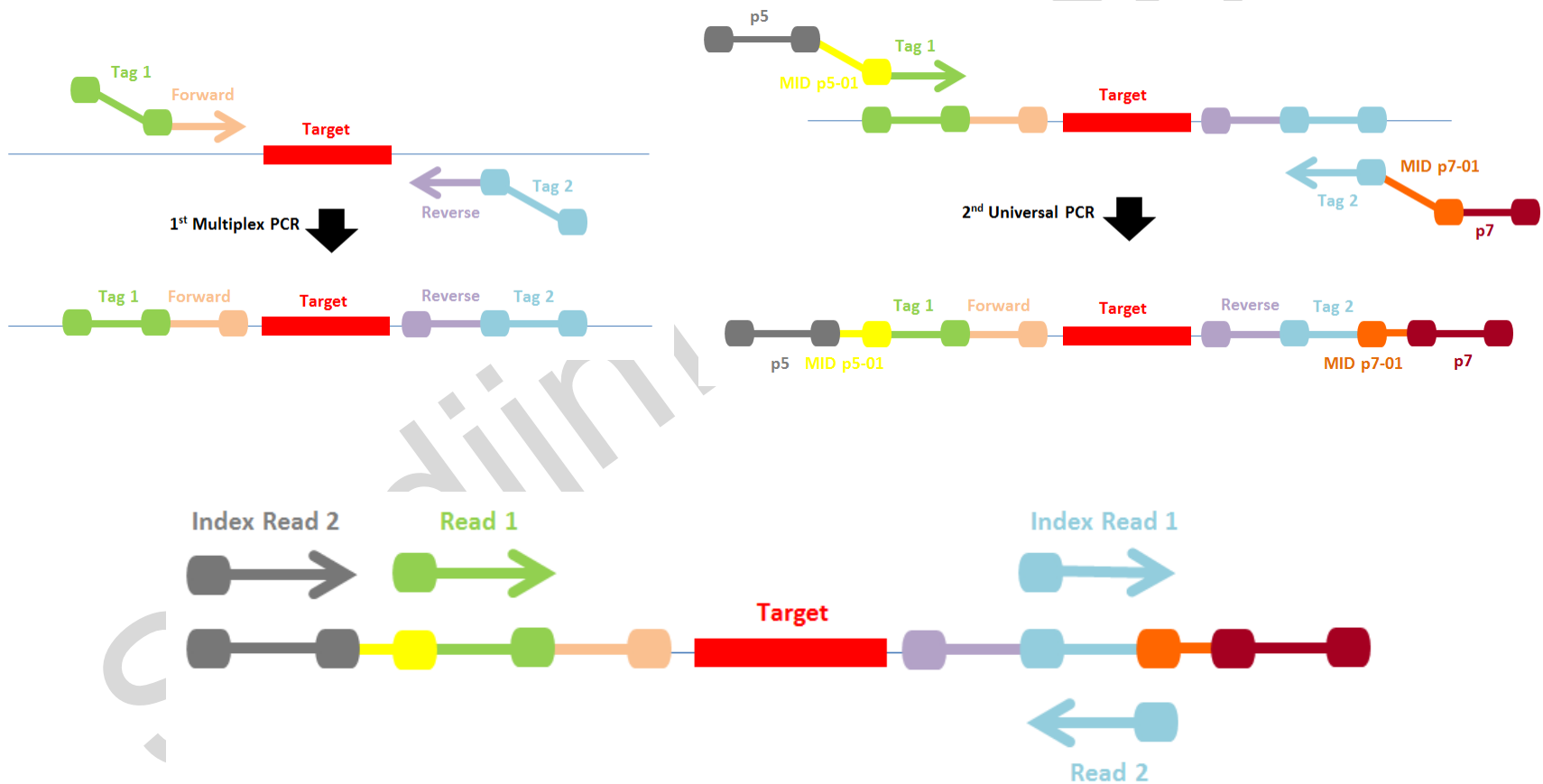
PCR  
Polishes fragment ends and incorporates optional indices

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



# Sekvenátor II generace

## Sekvenace amplikonů



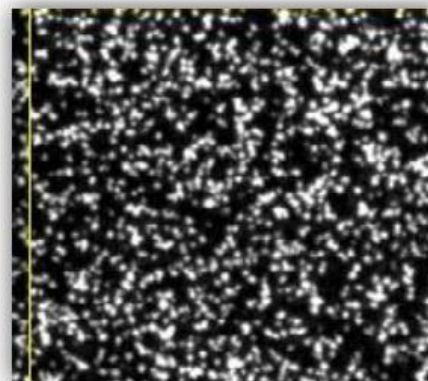


# Sekvenátor II generace

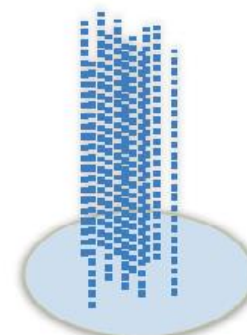
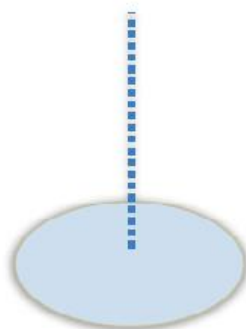
## Sekvenace probíhá v klastrech

Clusters are bright spots on an image

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



Single  
DNA  
Library



Amplified  
Clonal  
Cluster



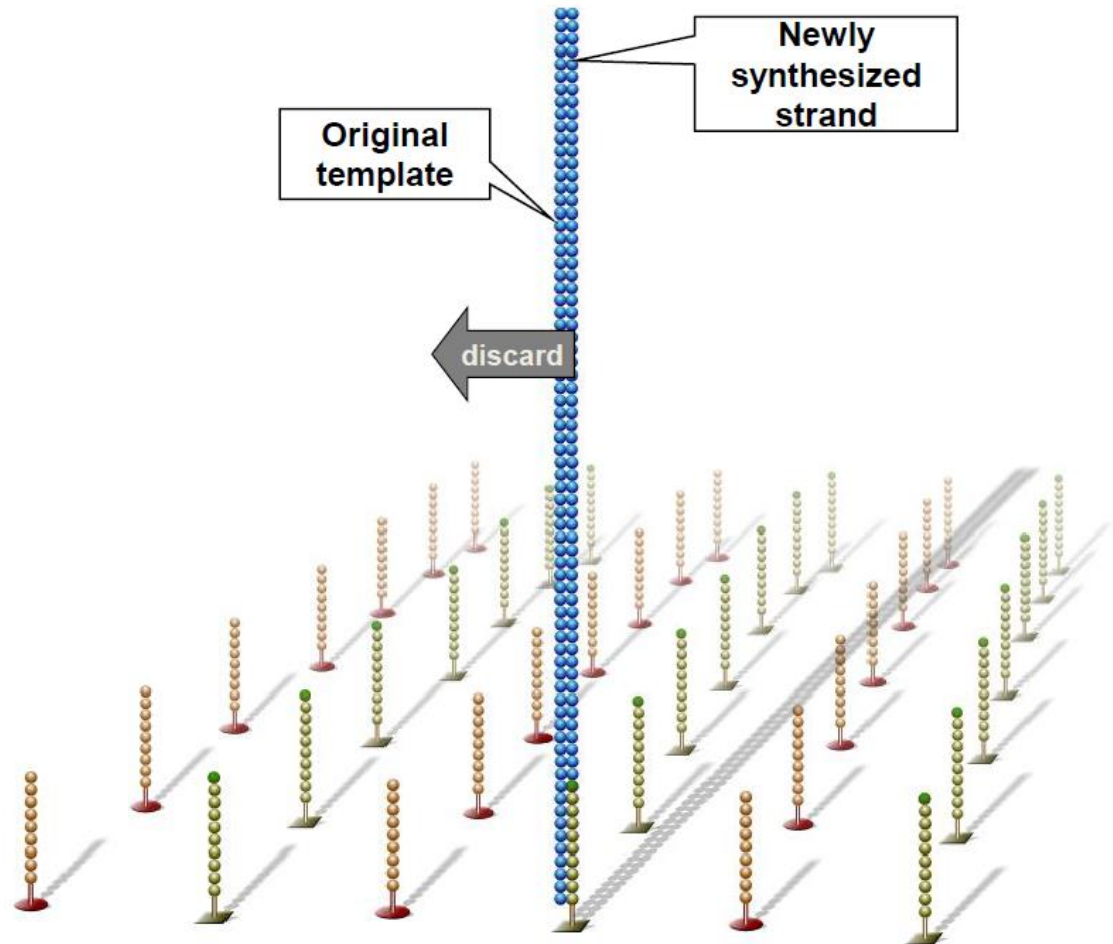
# Sekvenátor II generace

## 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





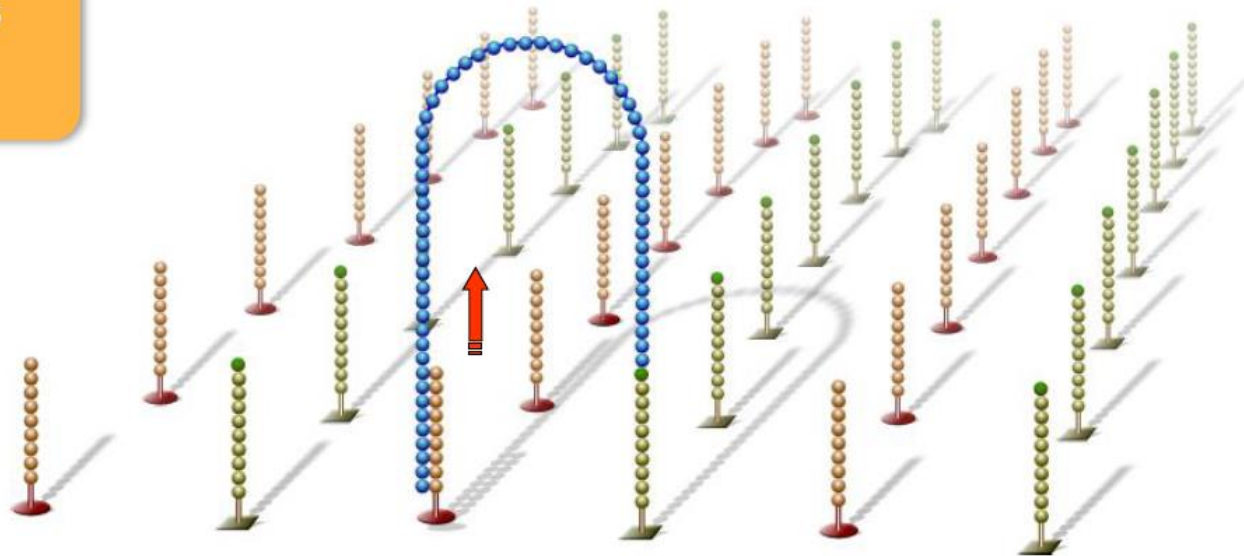


# Sekvenátor II generace

## 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

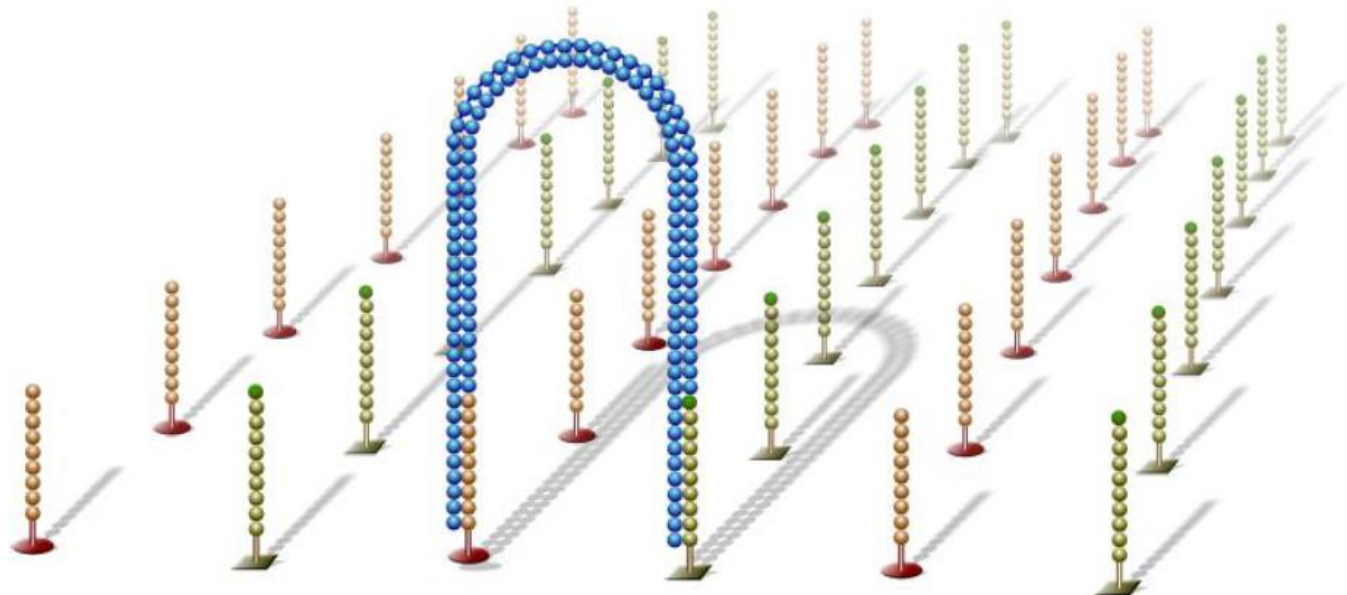




# Sekvenátor II generace

## 2. Krok - Můstková PCR

Double-stranded bridge is formed





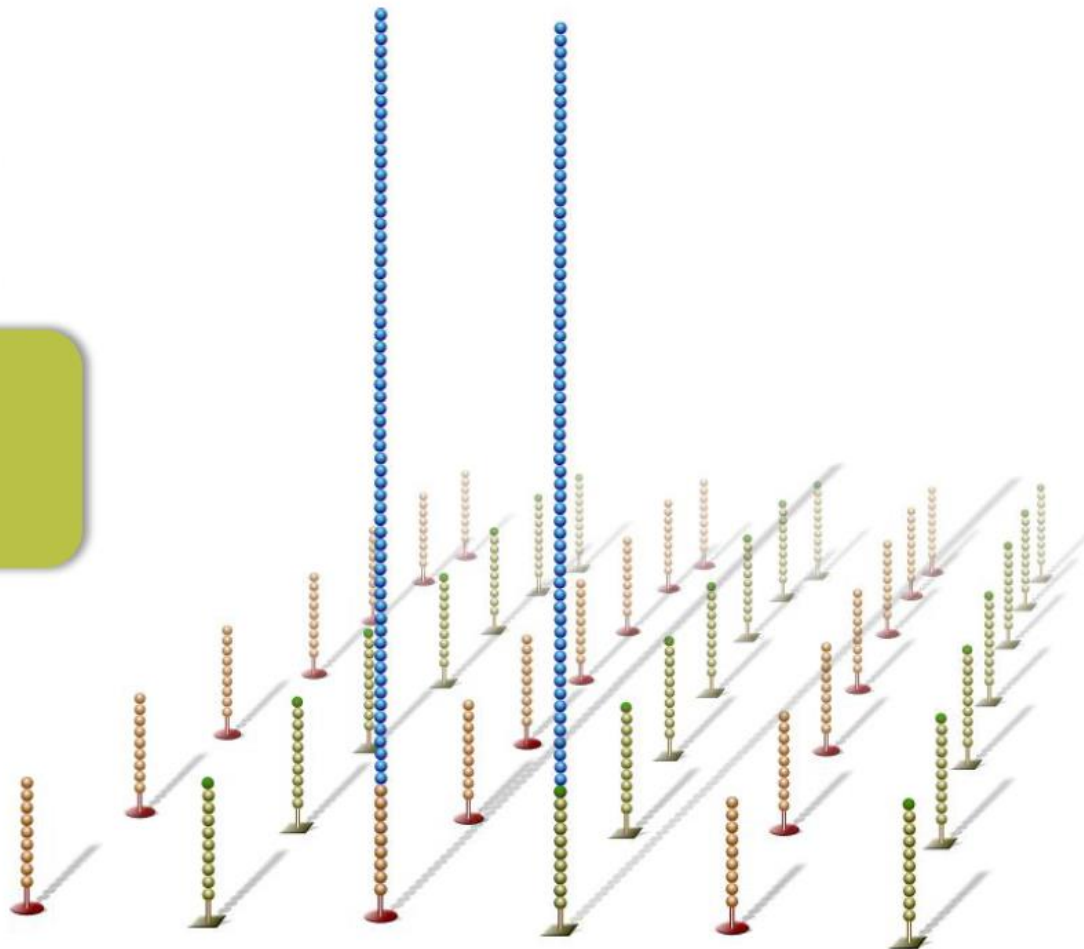


# Sekvenátor II generace

## 2. Krok - Můstková PCR

Double-stranded bridge is denatured - 1<sup>st</sup> cycle denaturation

Result:  
Two copies of covalently bound single-stranded templates



riály

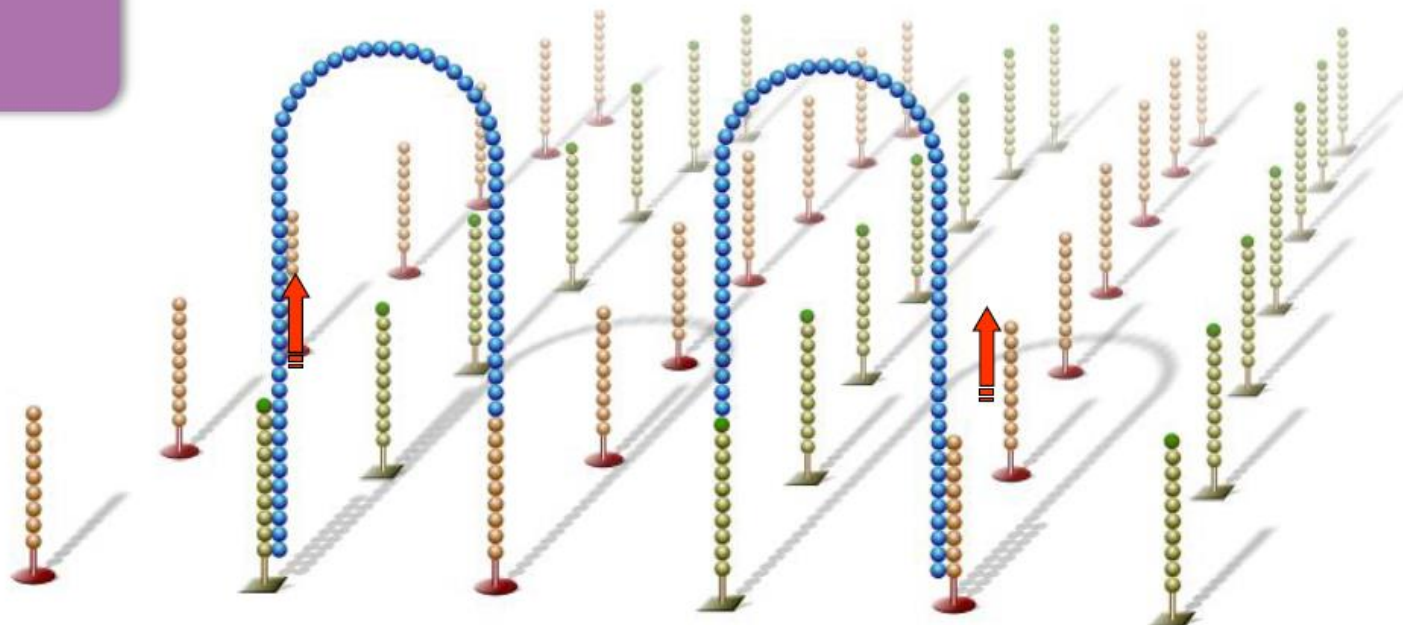


# Sekvenátor II generace

## 2. Krok - Můstková PCR

Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase



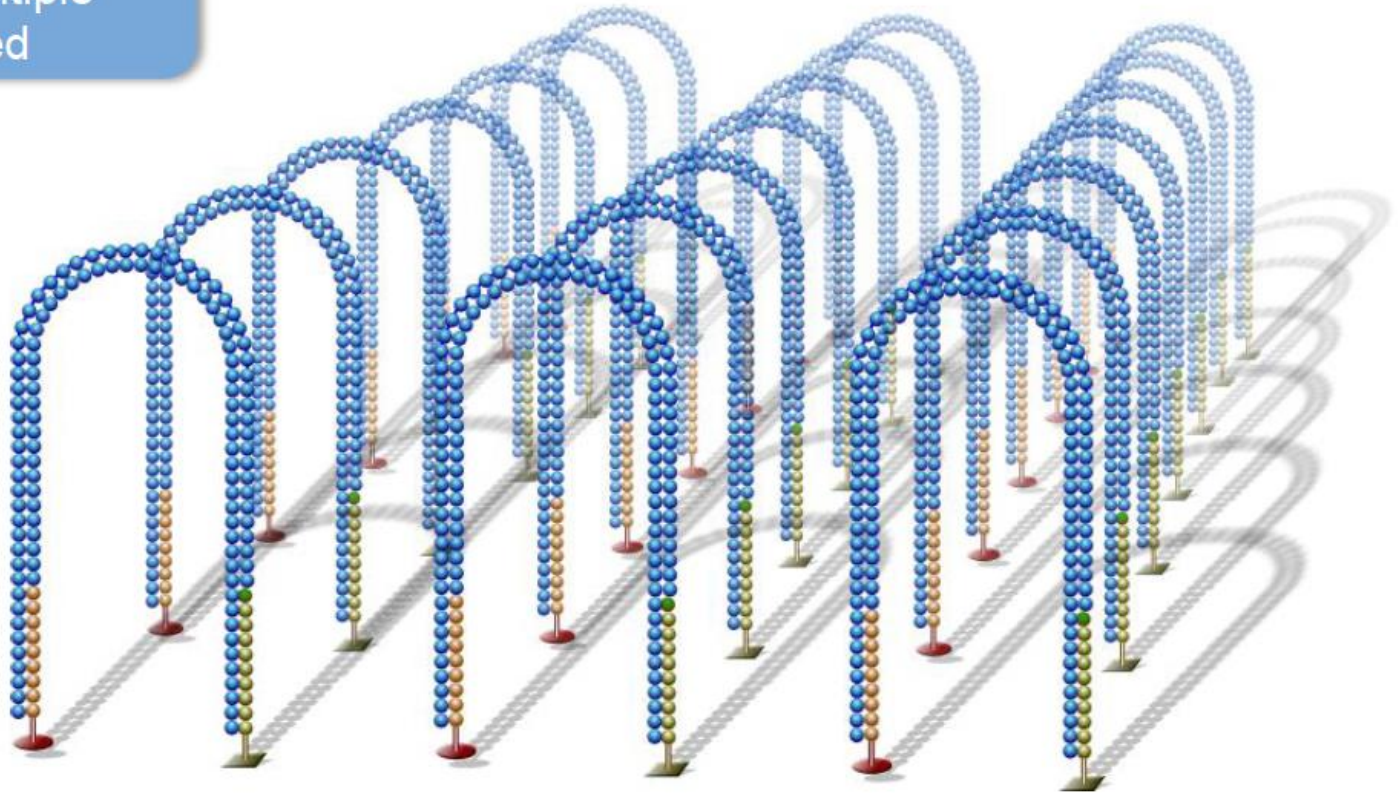


# Sekvenátor II generace



## 2. Krok - Můstková PCR

Bridge amplification cycle repeated until multiple bridges are formed



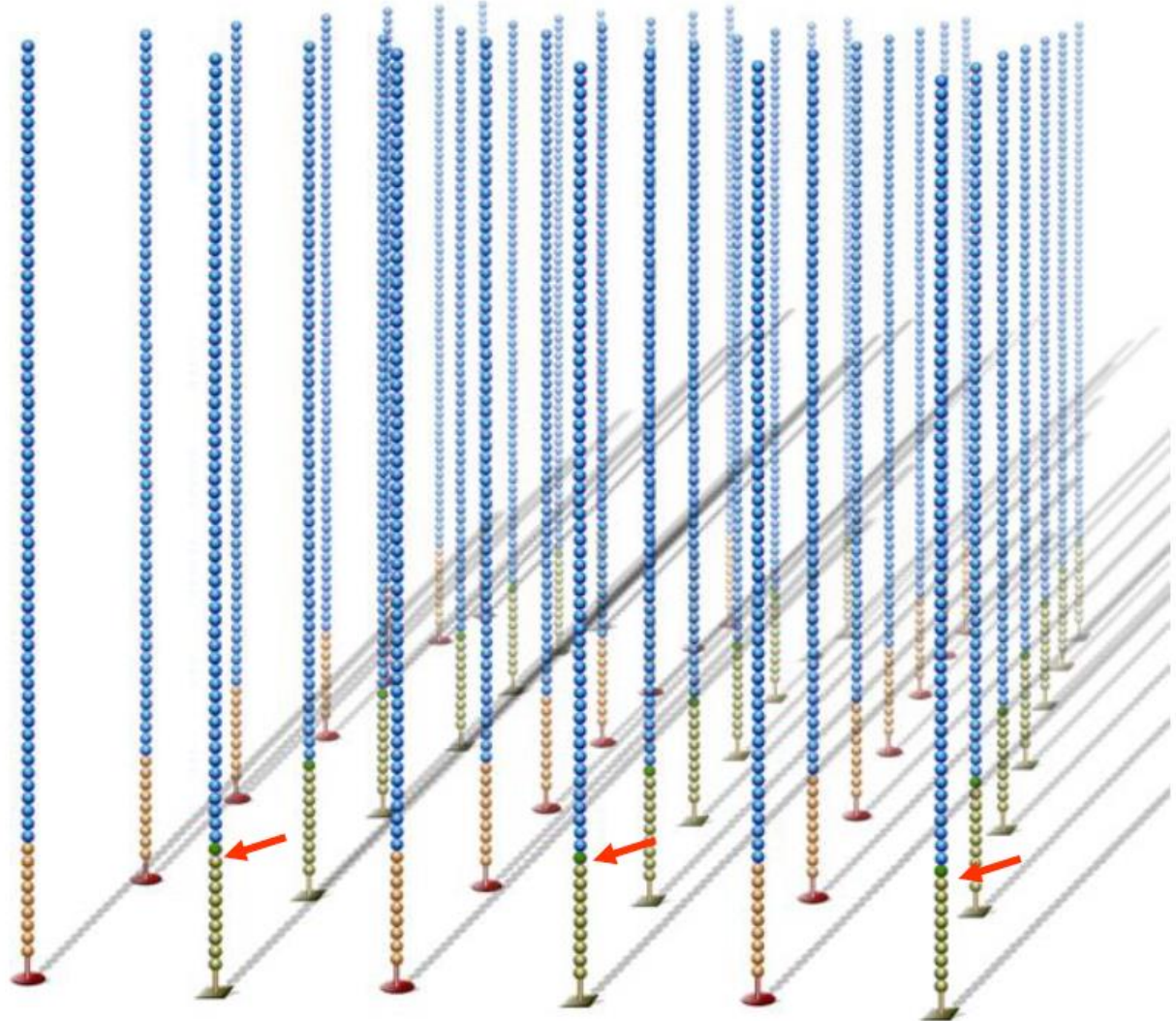




# Sekvenátor II generace

## 3. Krok - Linearizace

dsDNA bridges are denatured

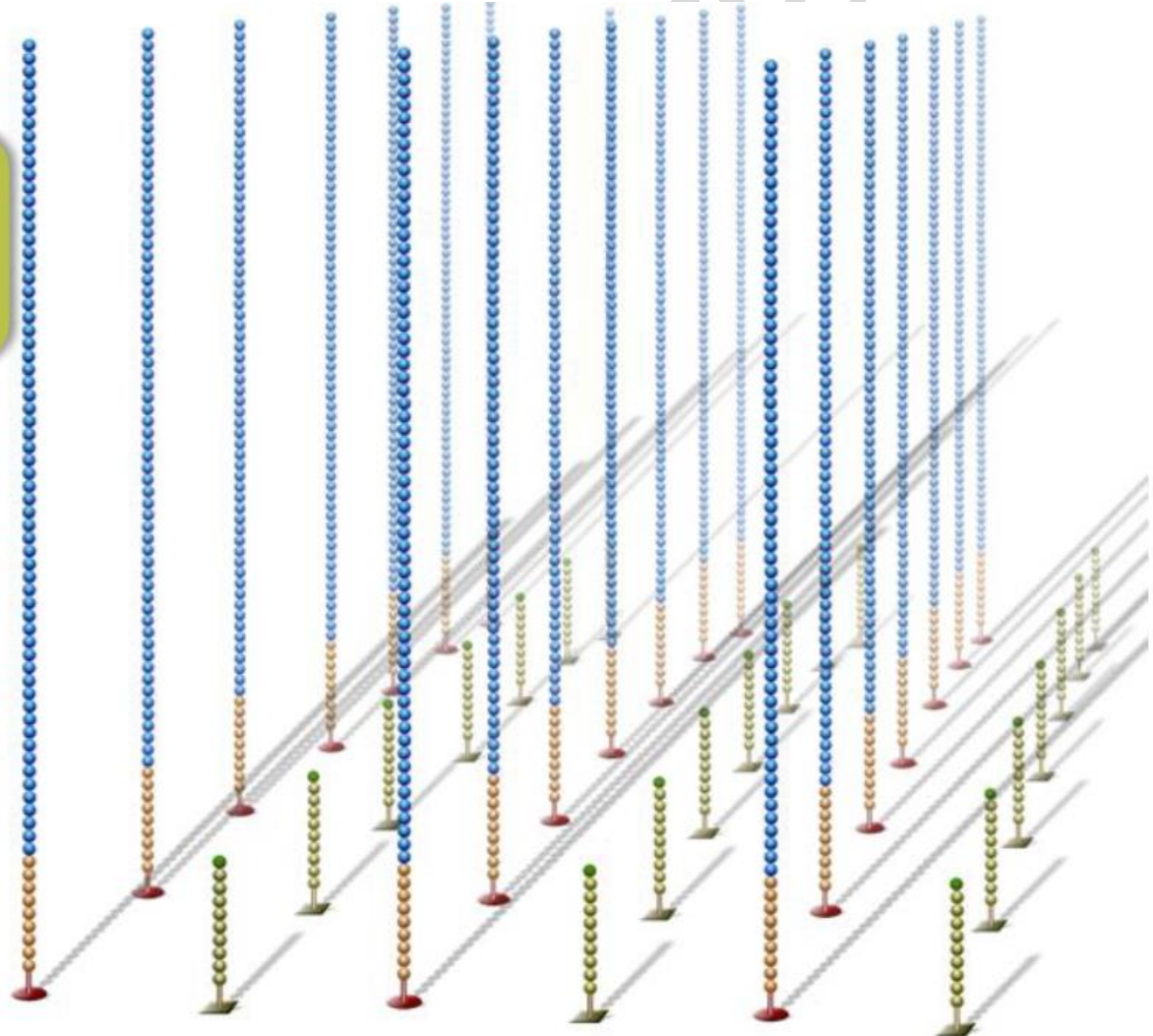




# Sekvenátor II generace

## 4. Krok - Odštěpení reverzního vlákna

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

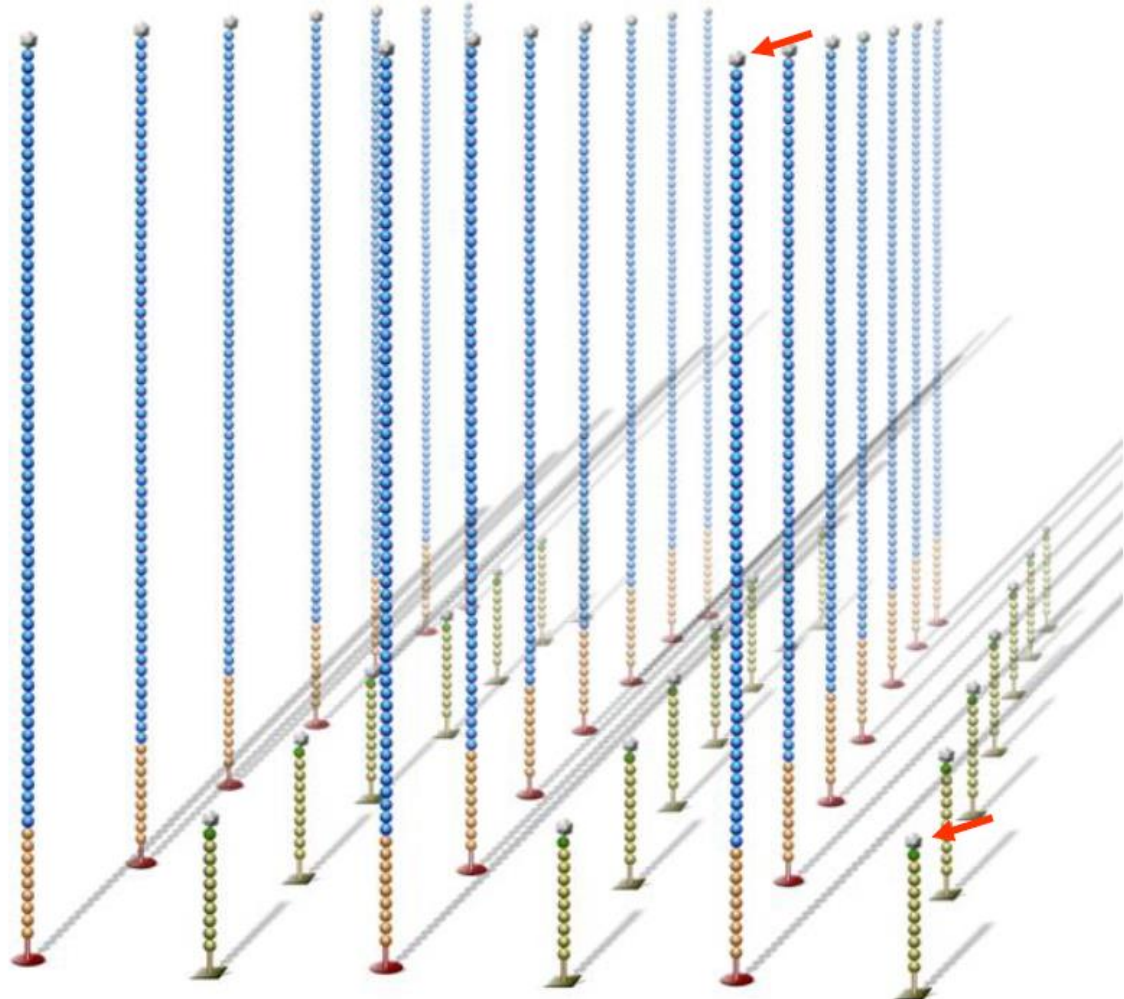




# Sekvenátor II generace

## 5. Krok - Blokace volného 5' konce

Free 3' ends are blocked to prevent unwanted DNA priming





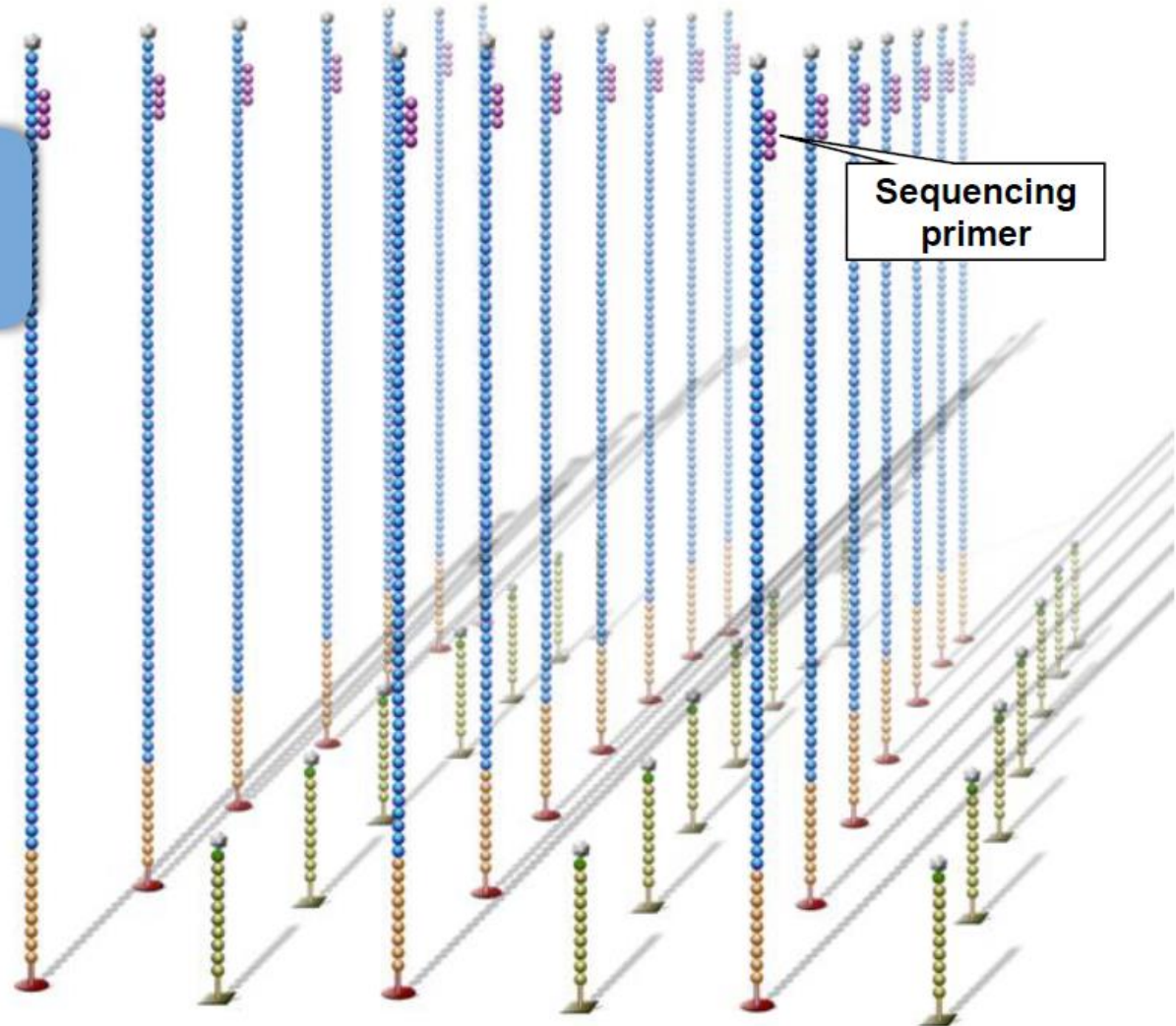


# Sekvenátor II generace

## 6. Krok: Čtení - hybridizace sek. primeru

Sequencing primer is hybridized to adapter sequence

Sequencing primer

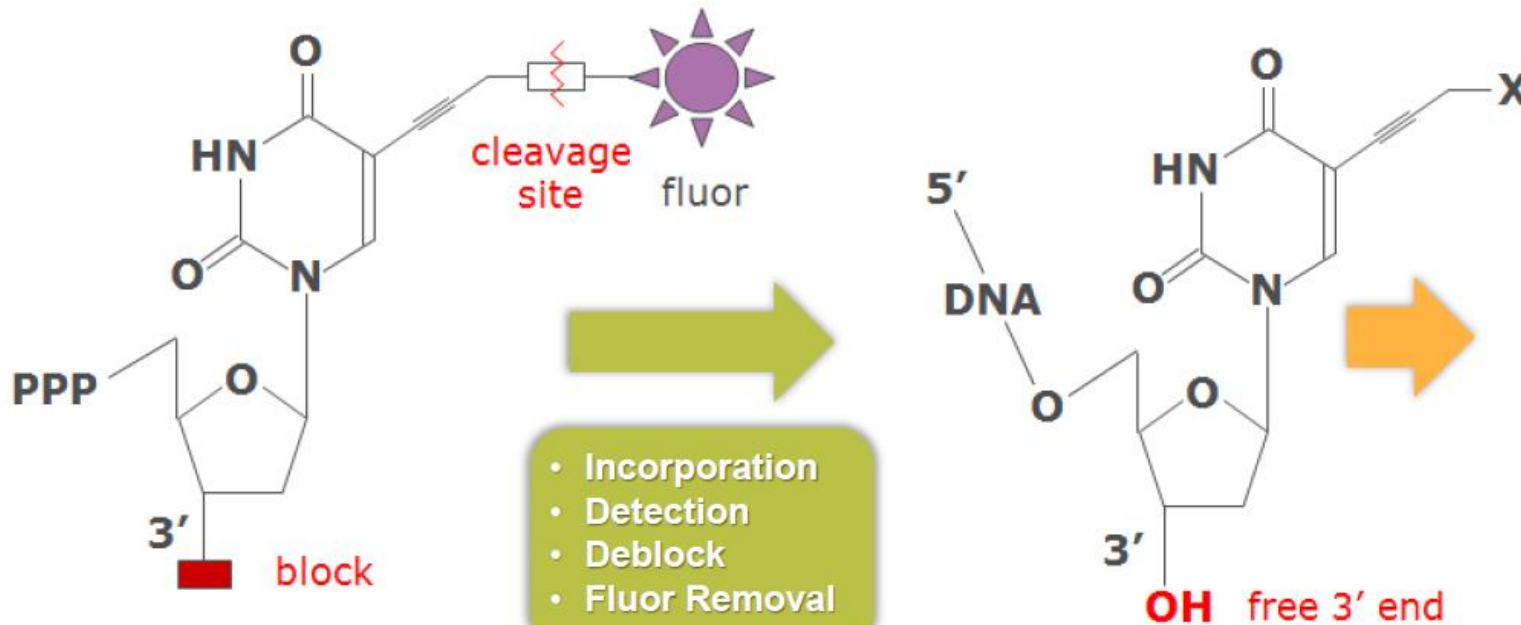




# Sekvenátor II generace

## Chemie reverzibilních terminátorů

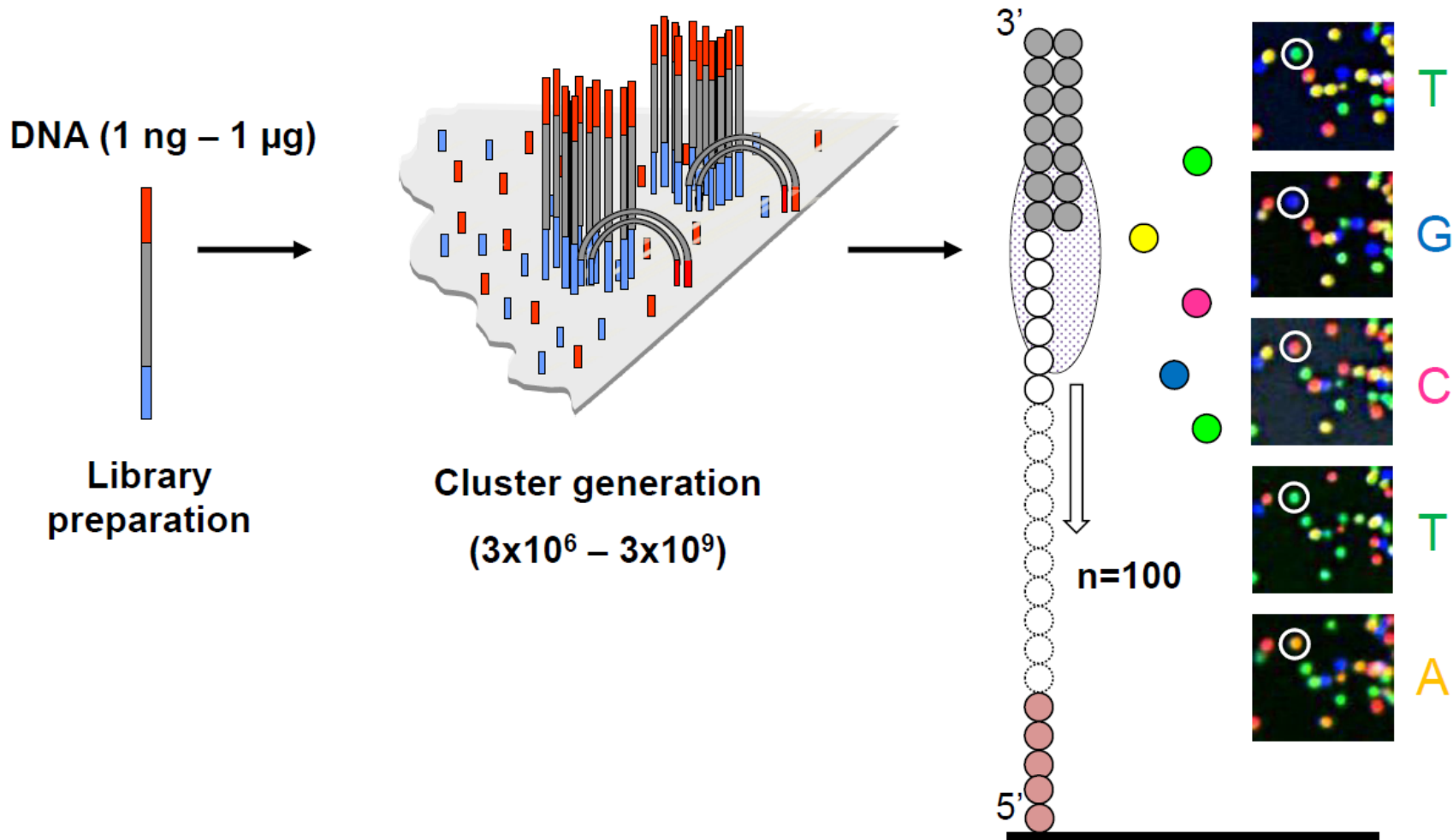
- All 4 nucleotides in 1 reaction
- Higher accuracy
- No problems with homopolymer repeats





# Sekvenátor II generace

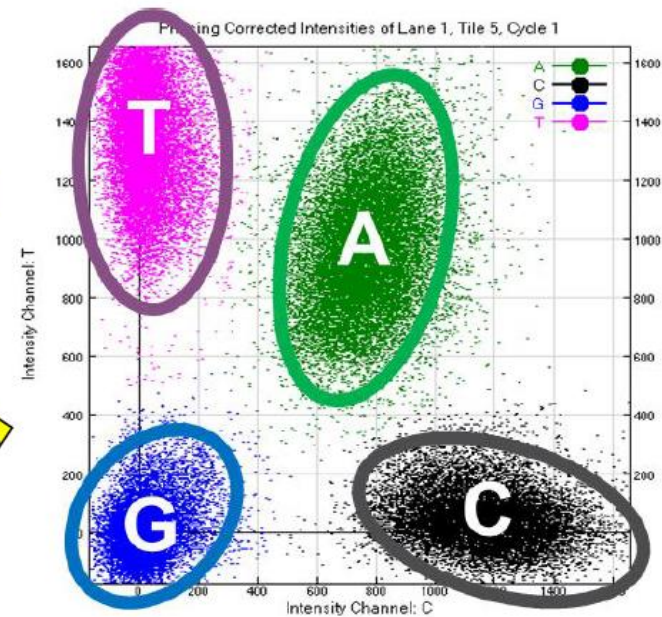
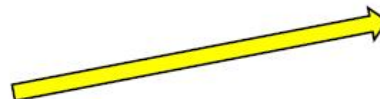
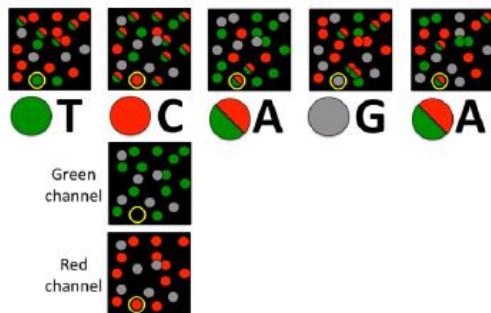
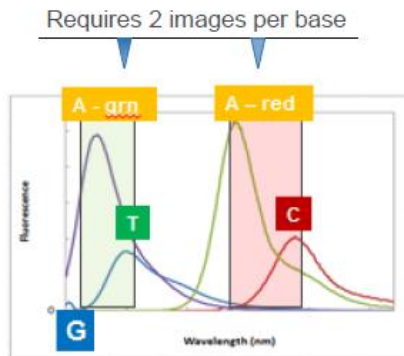
## Sekvenace pomocí syntézy (SBS)





# Sekvenátor II generace

## 2-Channel SBS Chemistry: MiniSeq, NextSeq



High performance with  
half the pictures

50%  
fewer images

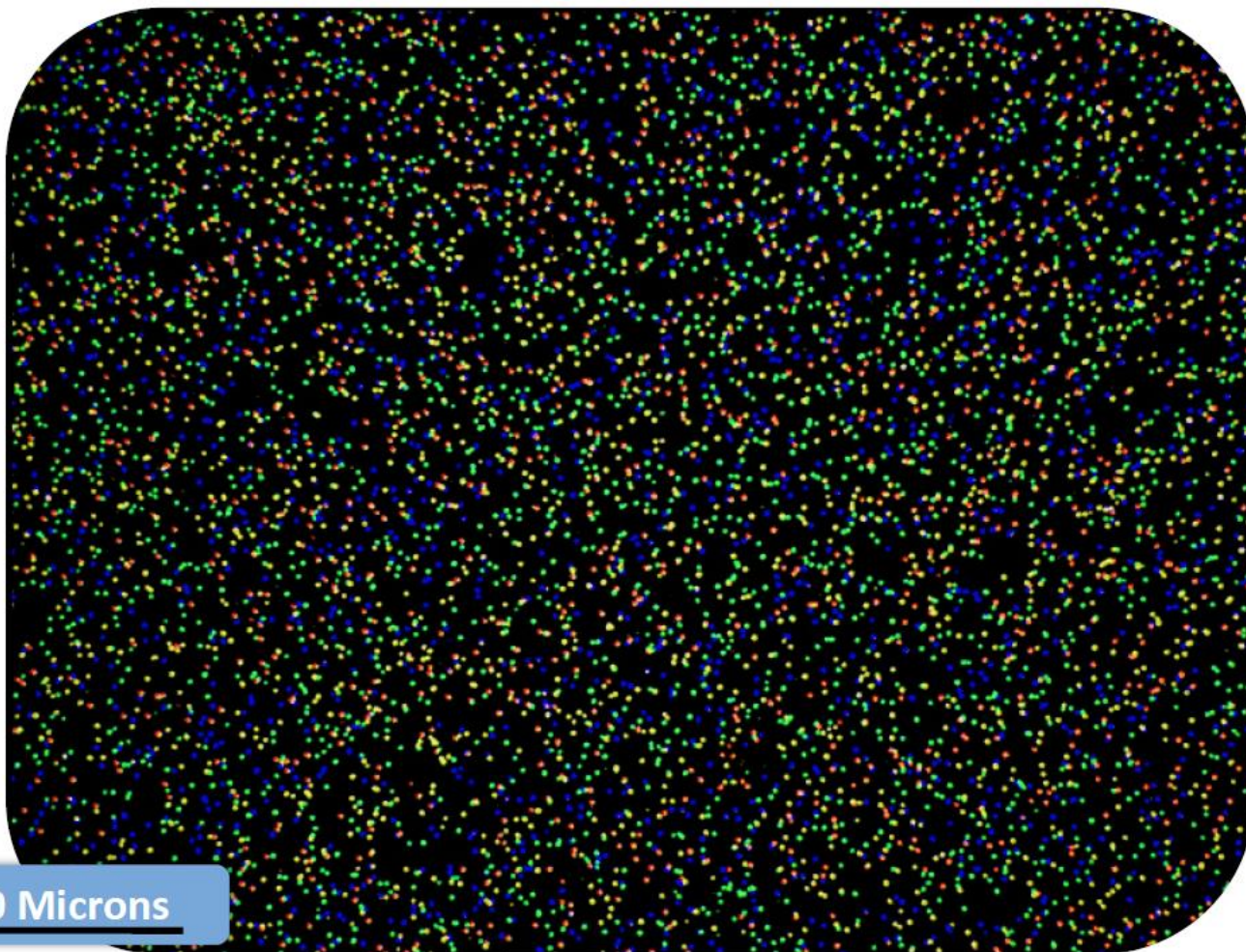




# Sekvenátor II generace



## Clusters



100 Microns

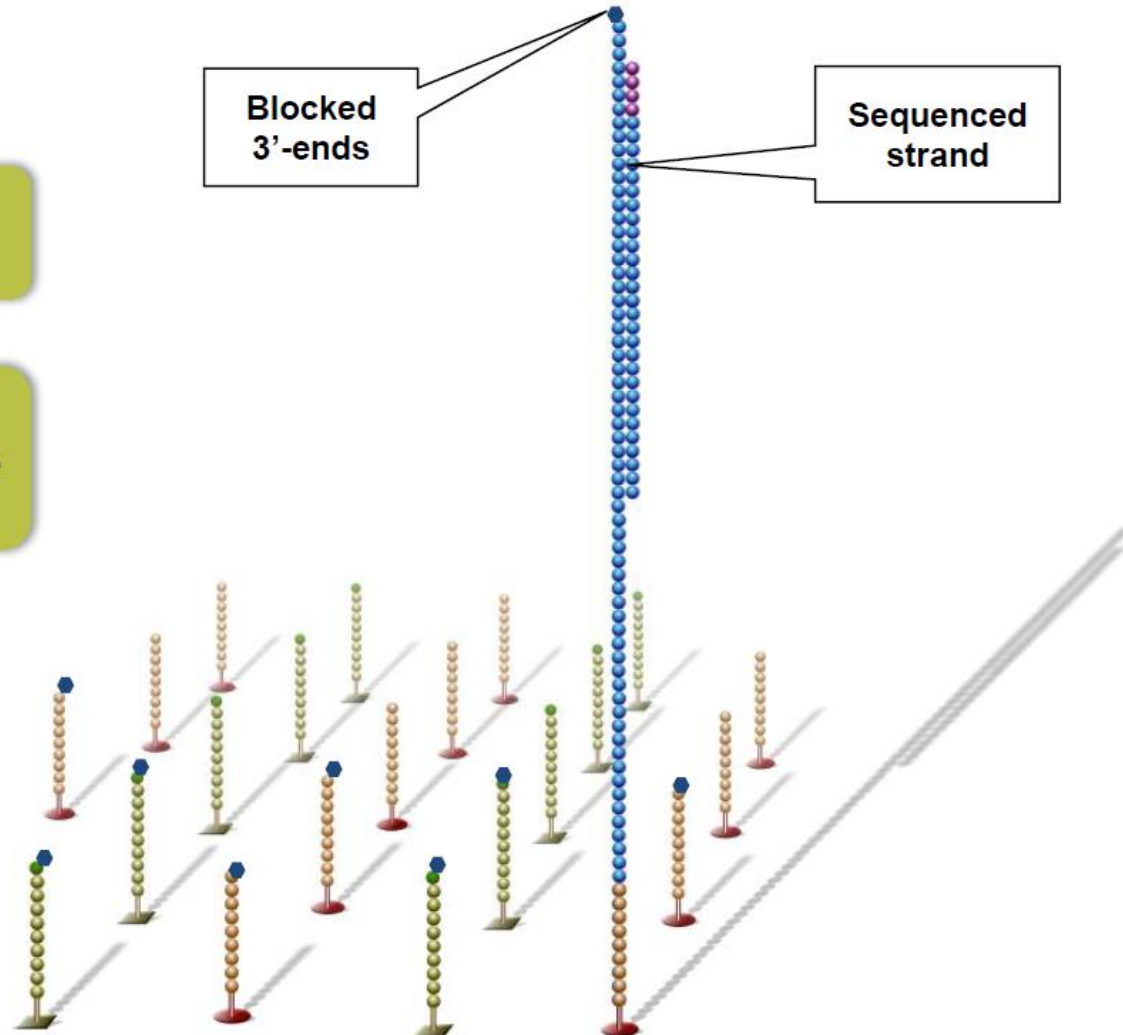


# Sekvenátor II generace

## 7. Krok - Pair-End sekvenace

Sequenced strand is stripped off

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





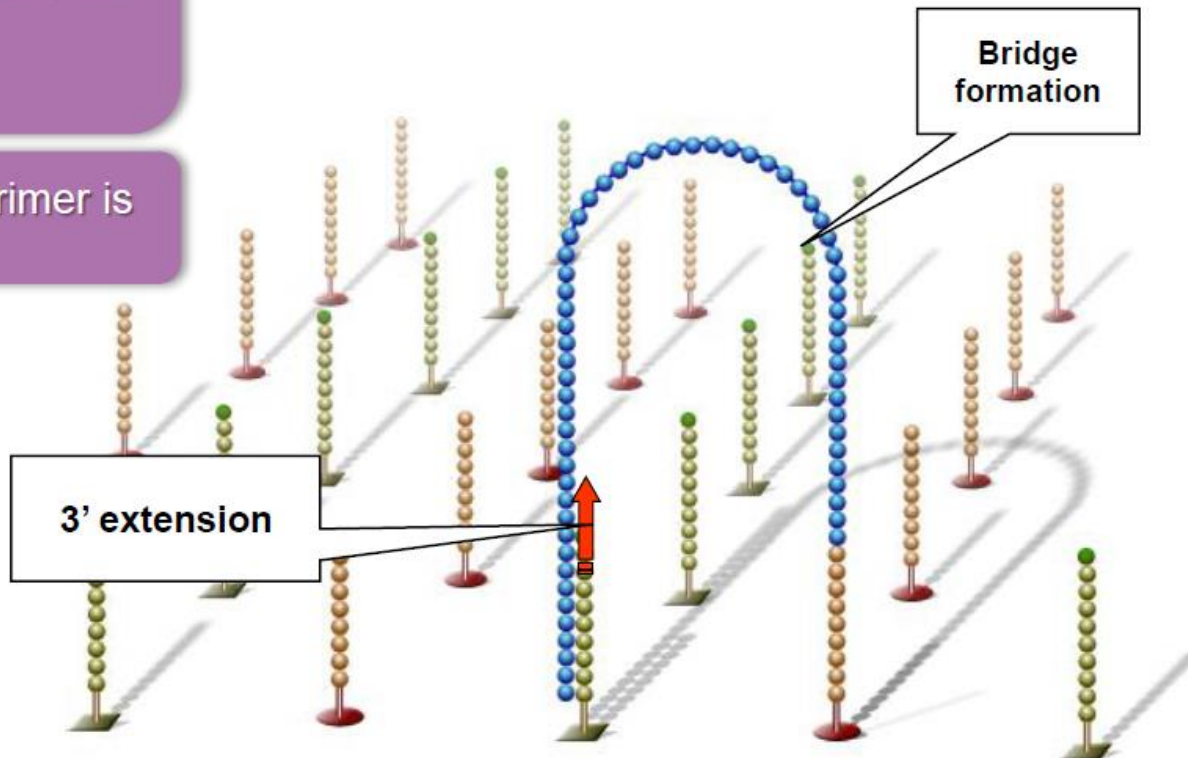


# Sekvenátor II generace

## 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

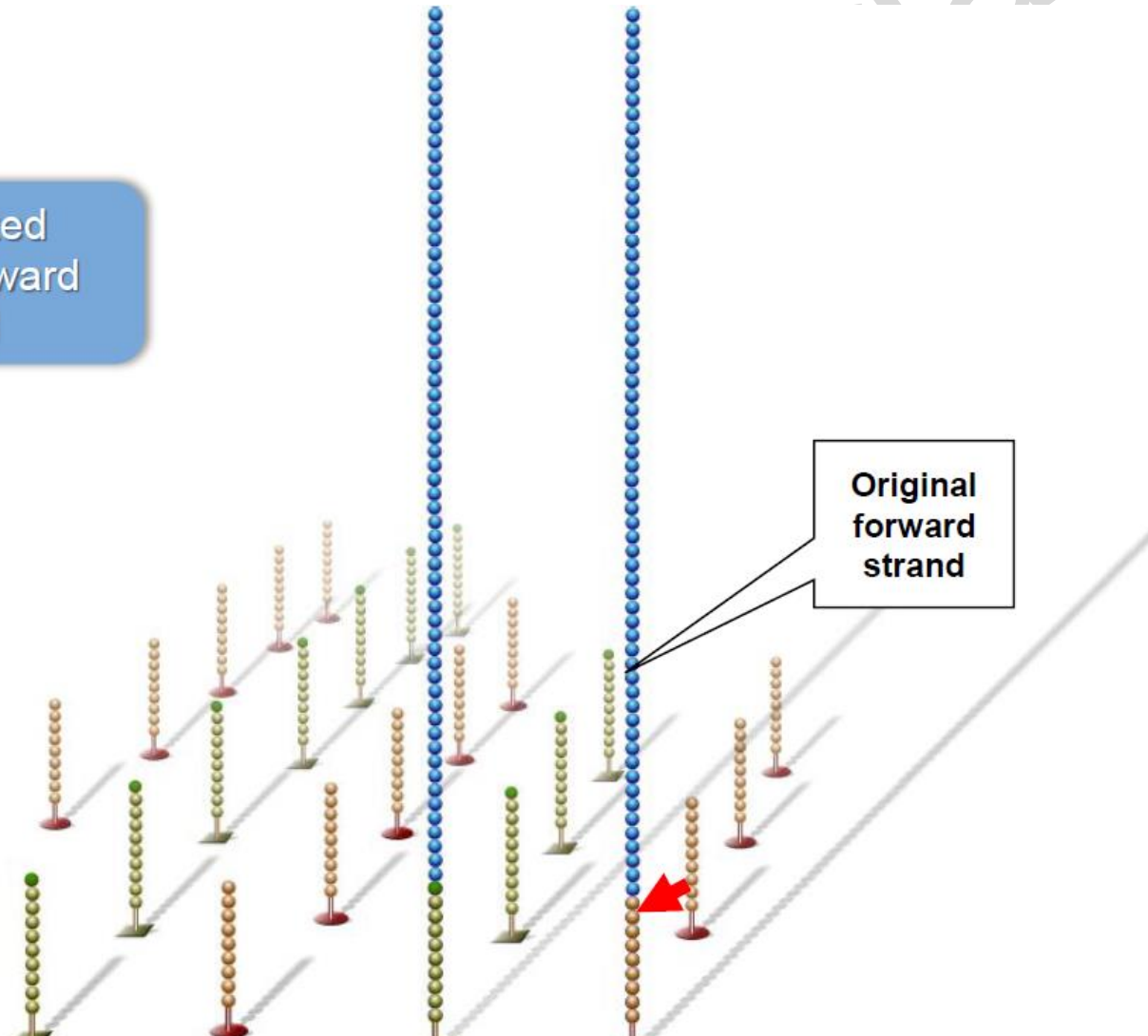




# Sekvenátor II generace

## 7. Krok - Pair-End sekvenace

Bridges are linearized and the original forward template is cleaved



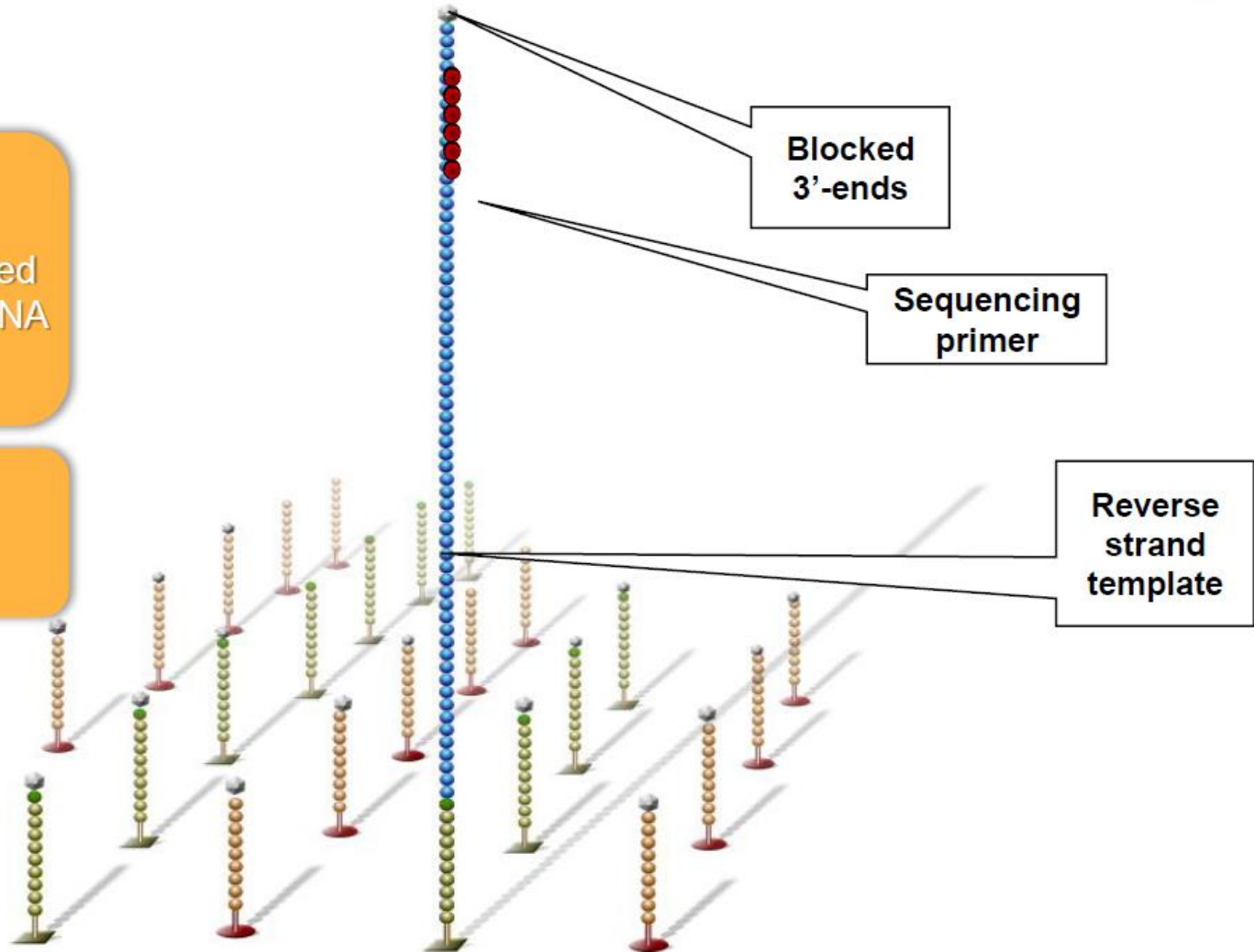


# Sekvenátor II generace

## 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



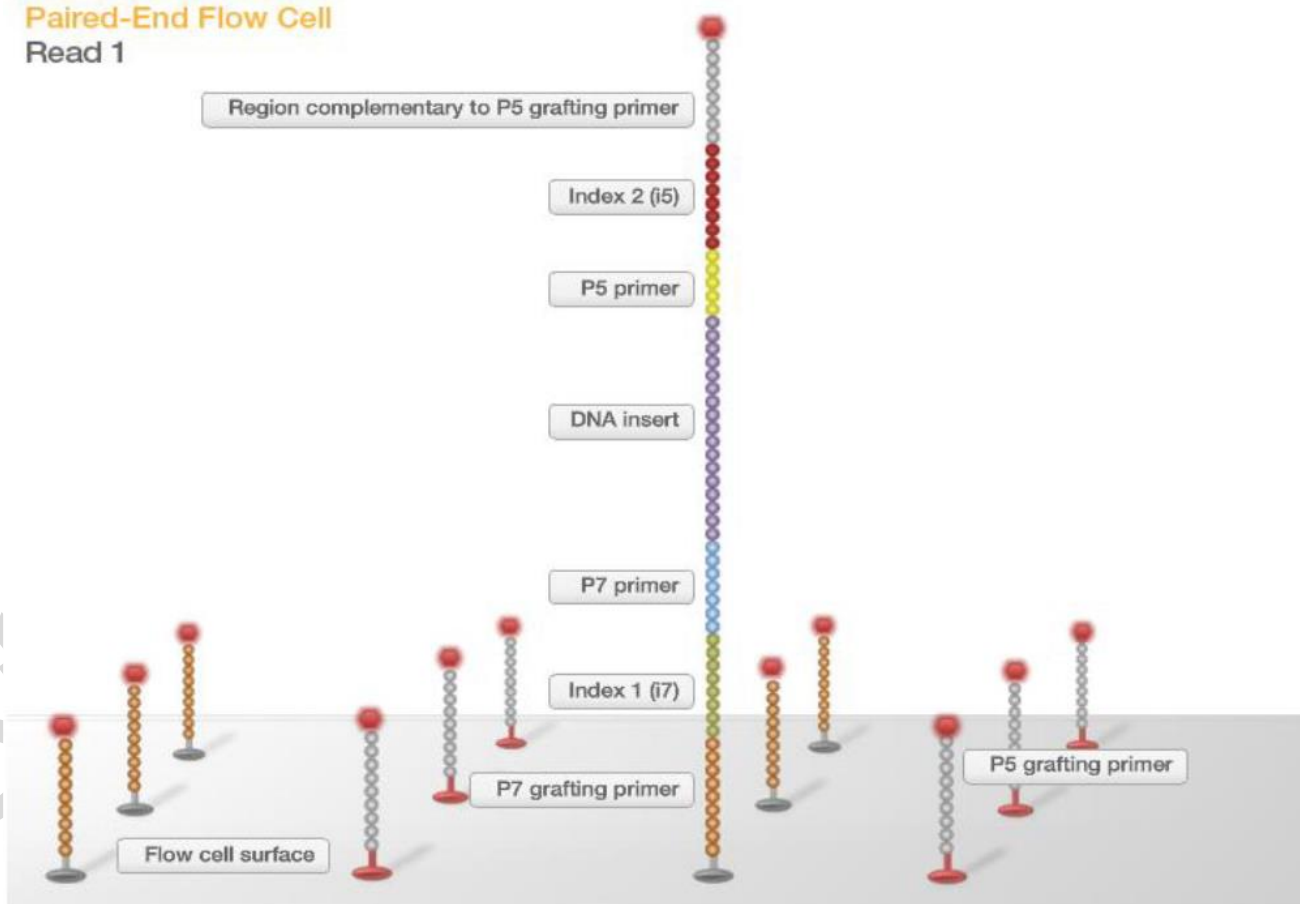


# Sekvenátor II generace

## Čtení indexů

riály

Paired-End Flow Cell  
Read 1

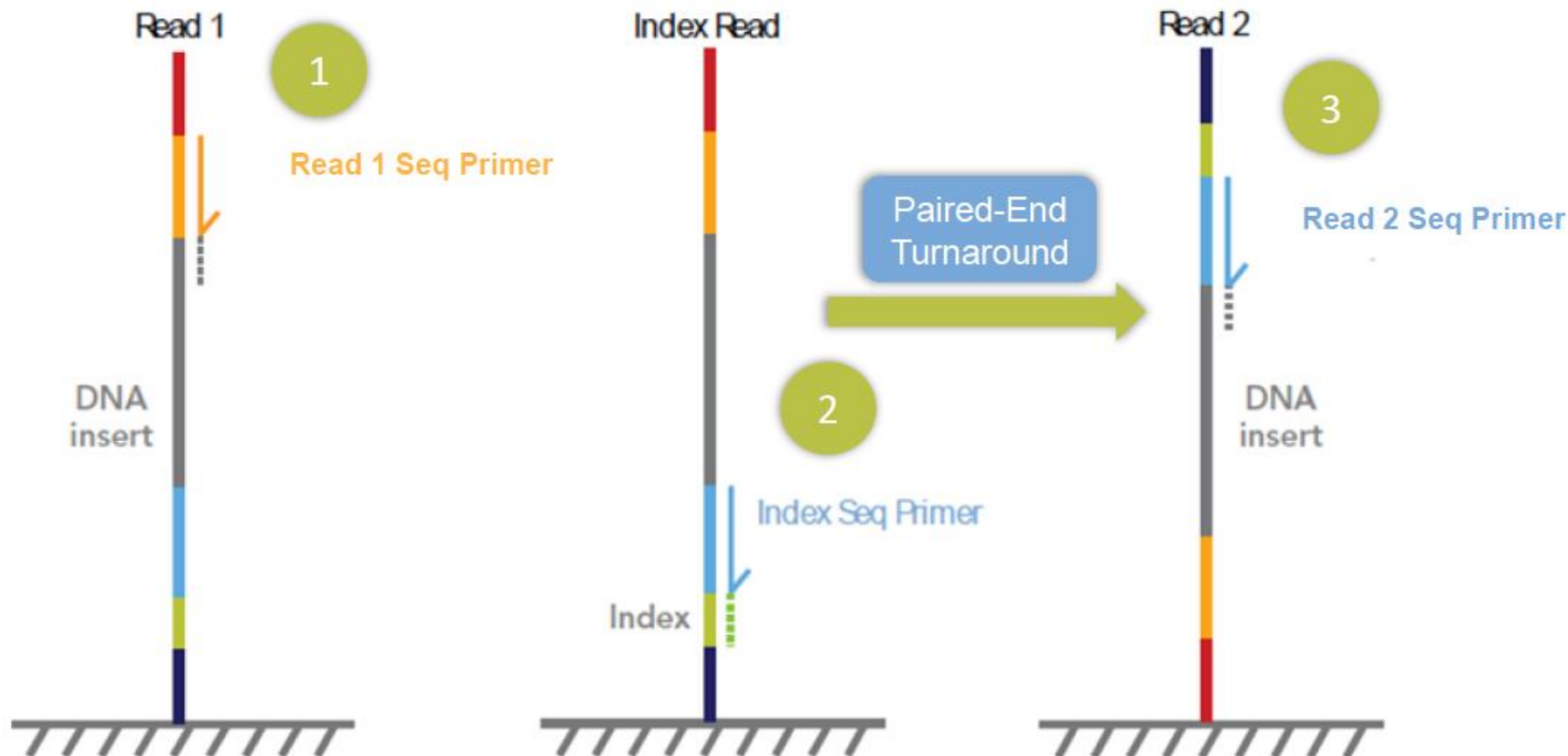




# Sekvenátor II generace

## Single index čtení

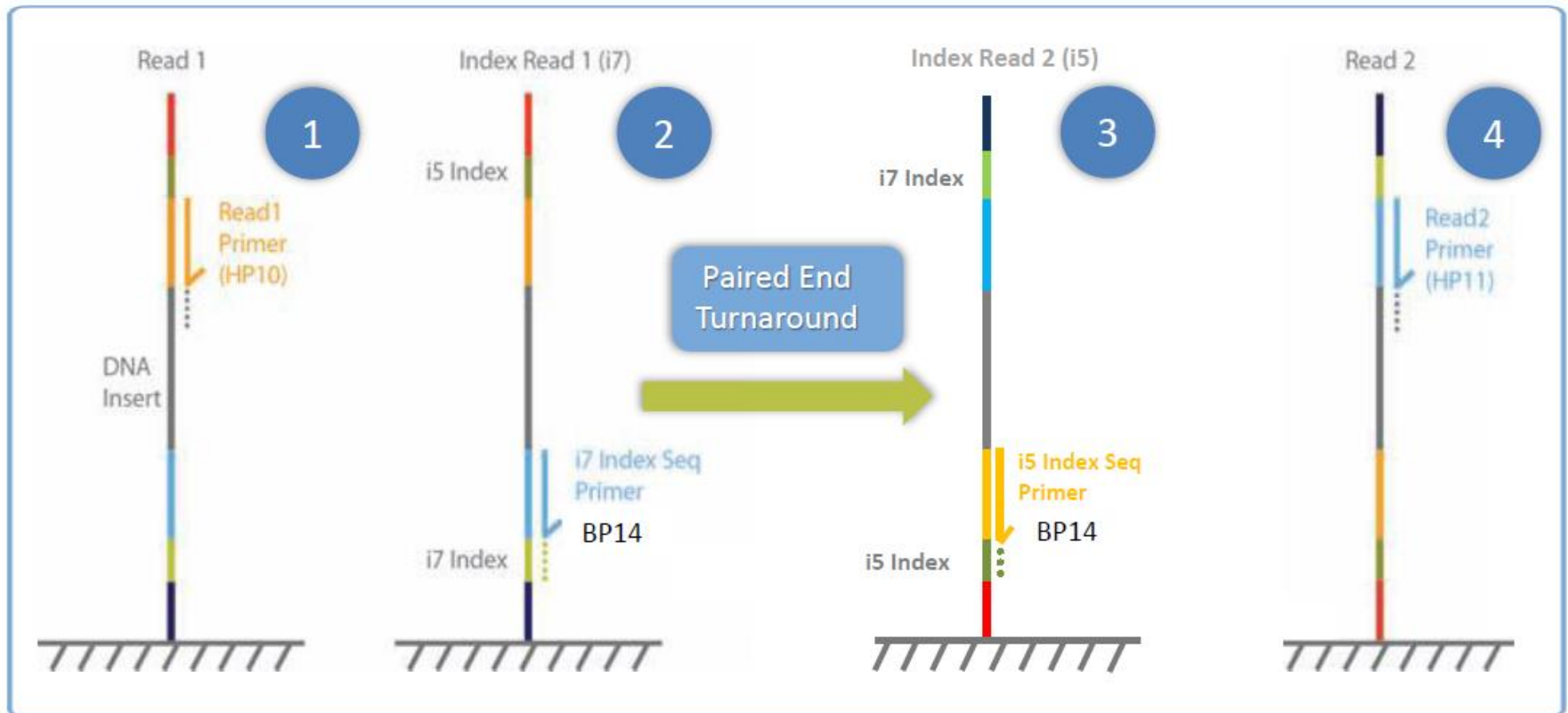
Single indexed sequencing utilizes three sequencing reads





# Sekvenátor II generace

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

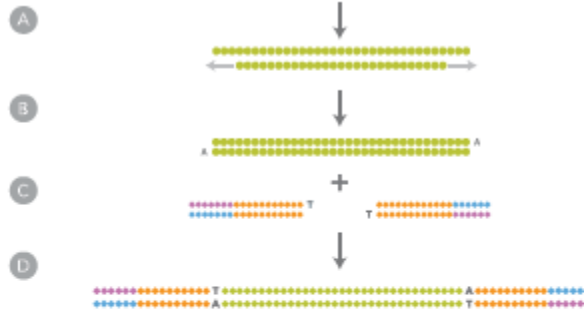




1

**LIBRARY PREPARATION**

6 hours  
3 hours hands-on time

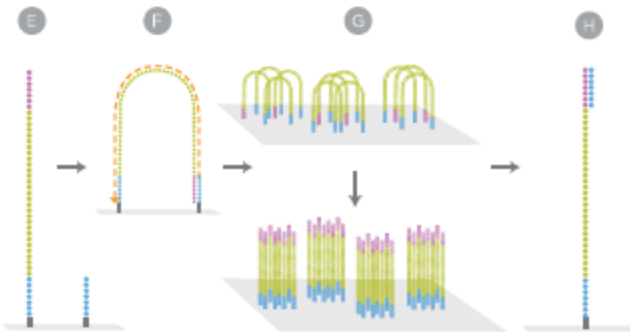


- A Fragment DNA
- ↓
- B Repair ends  
Add A overhang
- ↓
- C Ligate adapters
- ↓
- D Select ligated DNA

2

**CLUSTER GENERATION**

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

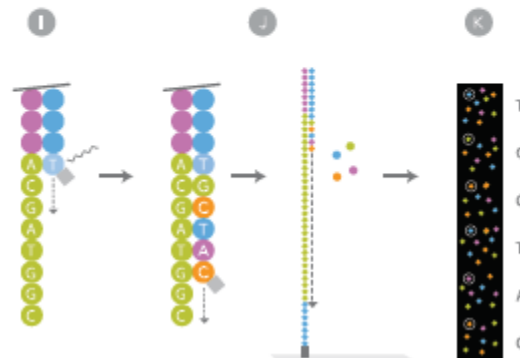


- E Attach DNA to  
flow cell
- ↓
- F Perform bridge  
amplification
- ↓
- G Generate clusters
- ↓
- H Anneal sequencing  
primer

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

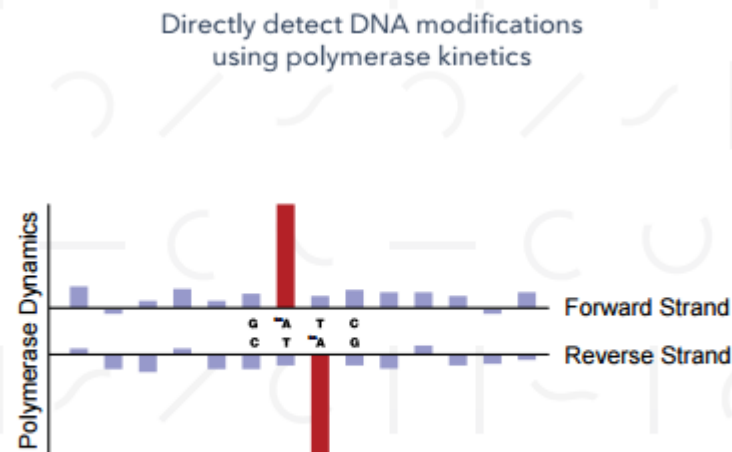
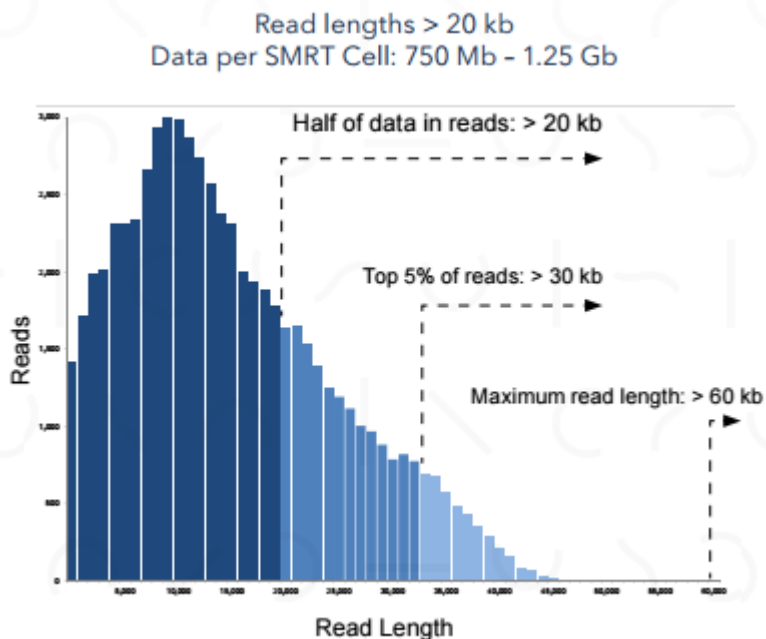




# Sekvenátor III generace - PacBio

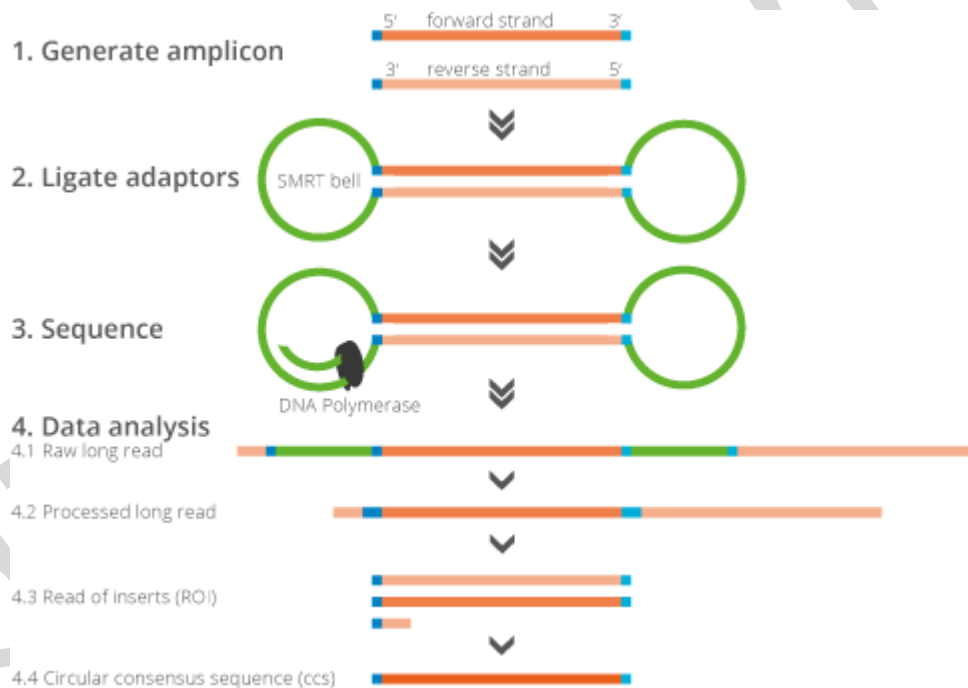


- Sekvence založena na Single Molecule, Real-Time (SMRT®) technologii
- Vyžívá tzv. Zero-Mode Waveguides (ZMWs) umožňující osvětlení pouze spodní části jamky, ve které je dole imobilizována DNA polymeráza
- Hlavní výhoda je možnost dlouhého čtení (až 20 kb)
- Další výhoda je možnost přímé detekce methylovaných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





## Příprava knihovny



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



# Sekvenátor III generace Oxford Nanopores



Minion

- 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 pozorování změny jsou odečítány v reálném čase jednotlivé báze
- Umožňuje sekvenaci velmi dlouhých úseků (desítky až stovky kilobází)
- Nevýhodou je vyšší chybovost, správnost >90%

