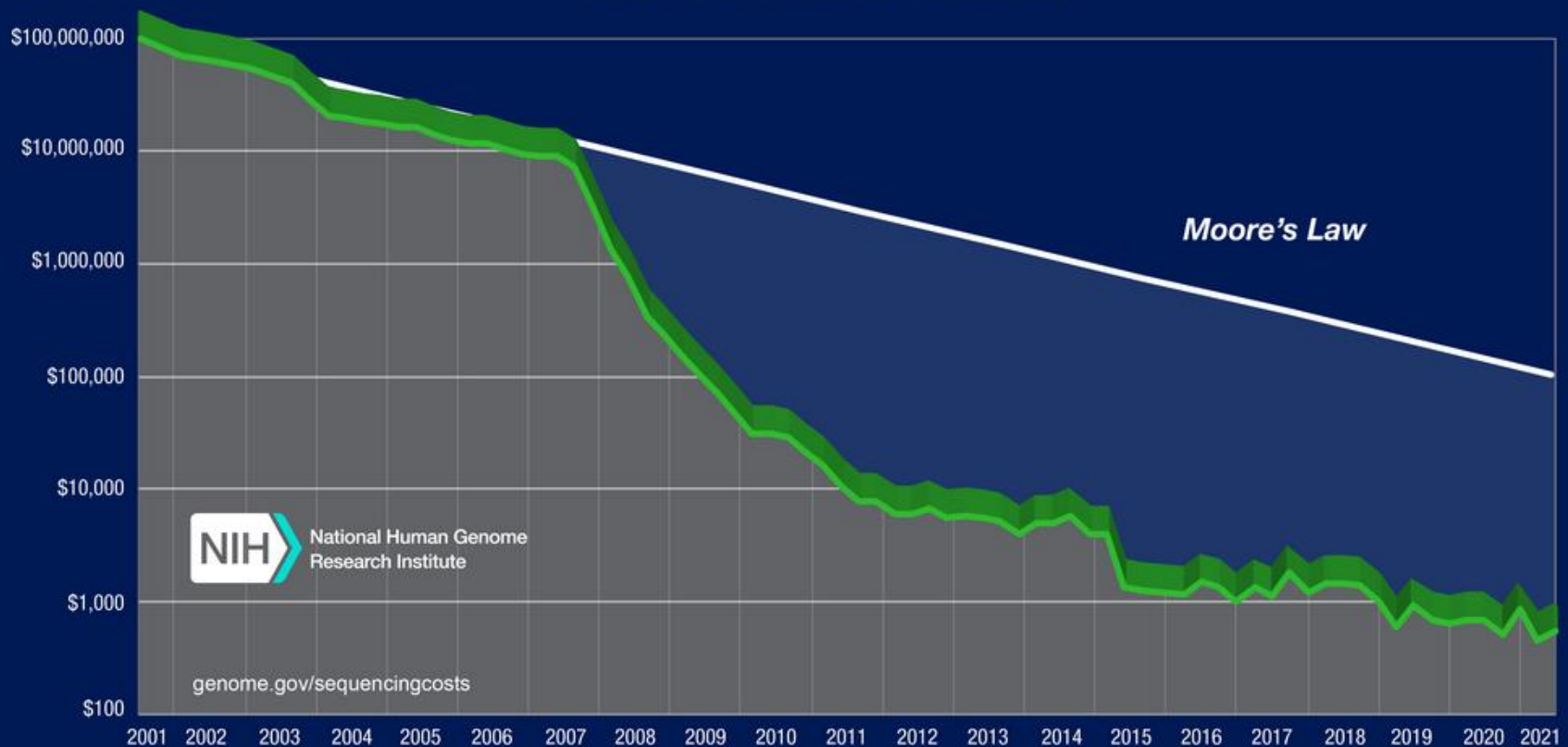


Tin Sinh học Bioinformatics

NGS - Next Generation Sequencing Giải trình tự gen thế hệ mới

Sequencing Human Genome Cost

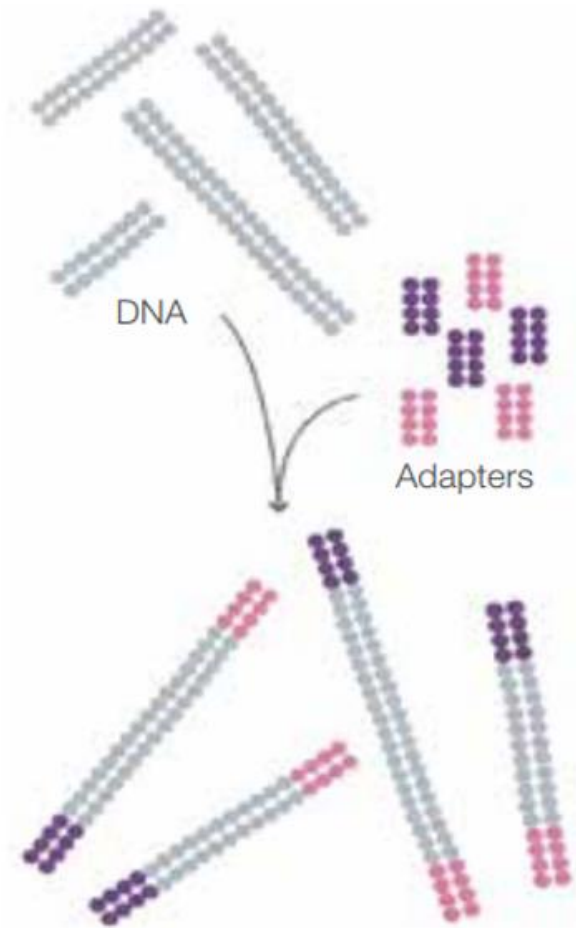
Cost per Human Genome



<https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost>

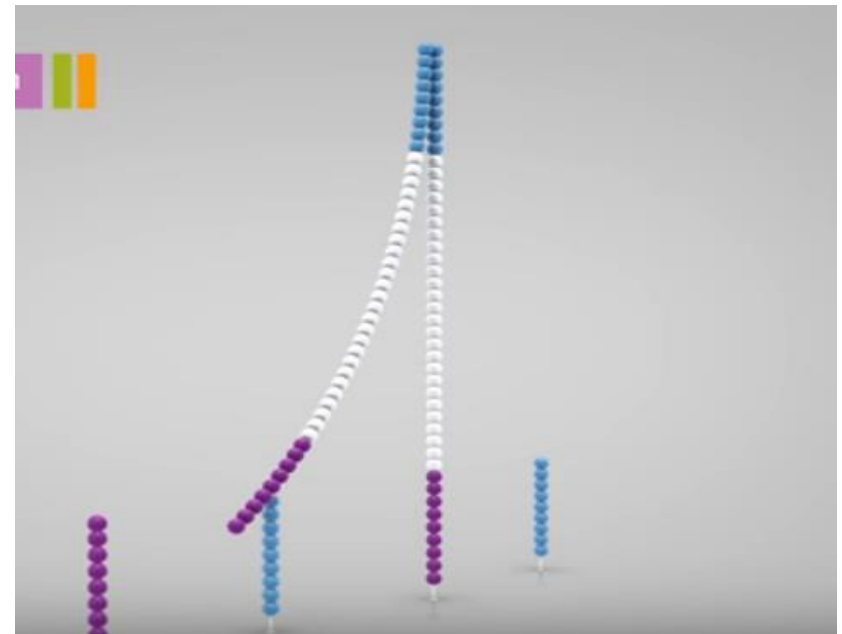
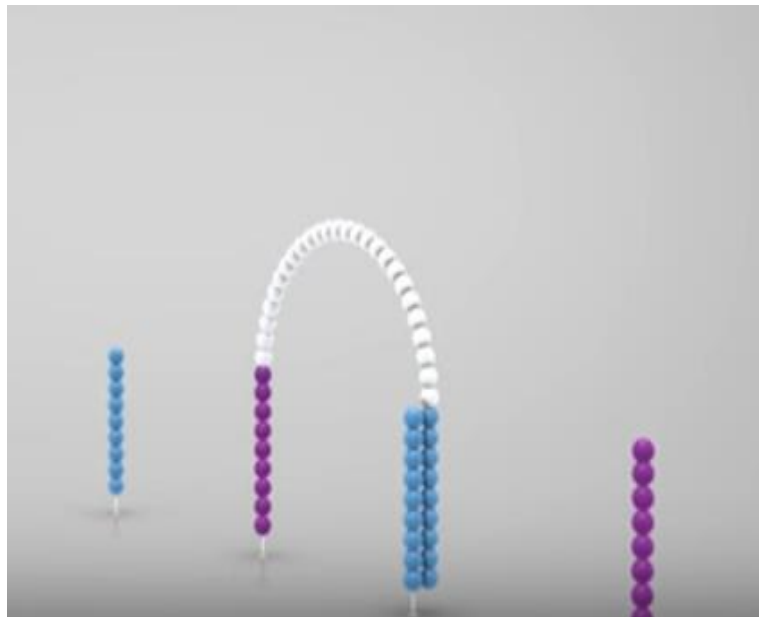
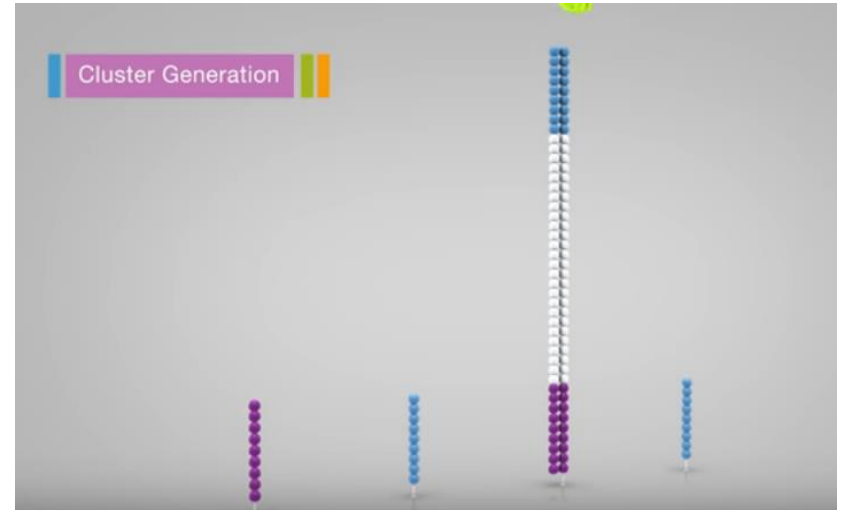
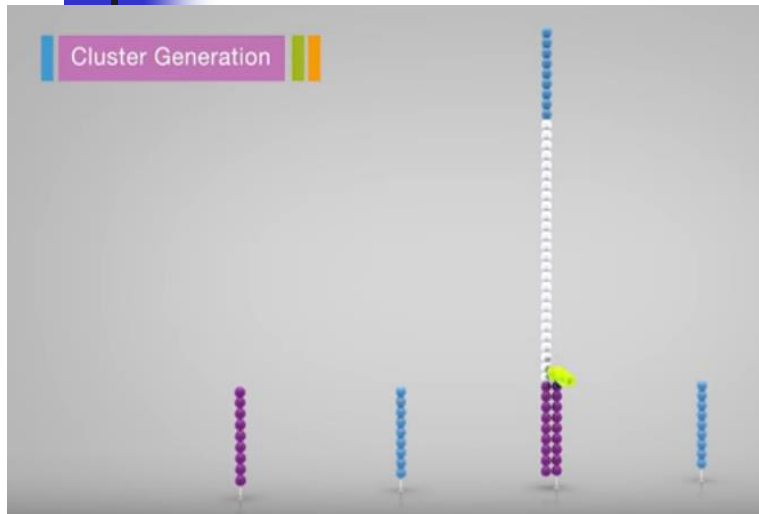
Illumina Sequencing

- Chuẩn bị thư viện
- Tạo cụm
- Giải trình tự Forward
- Giải trình tự Reverse

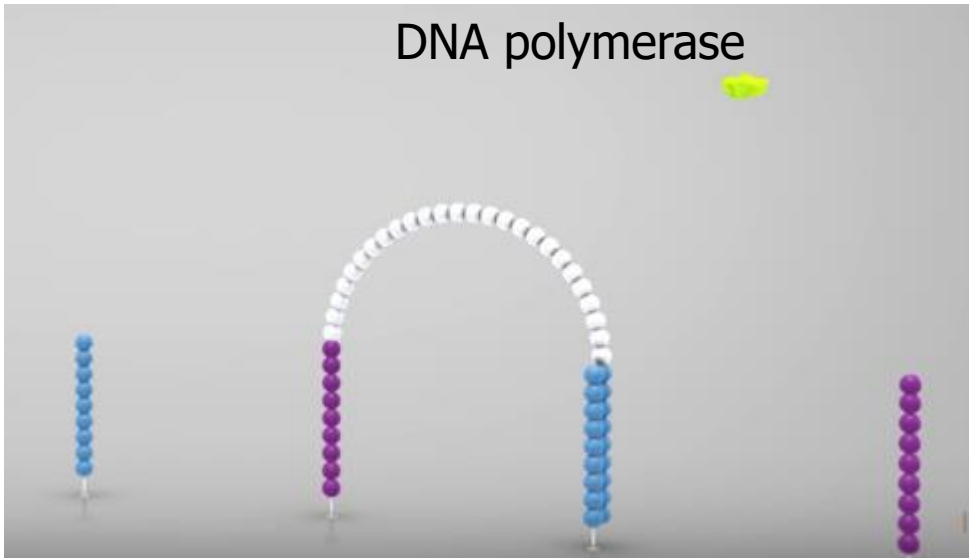


Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

Illumina Sequencing – Tạo cụm



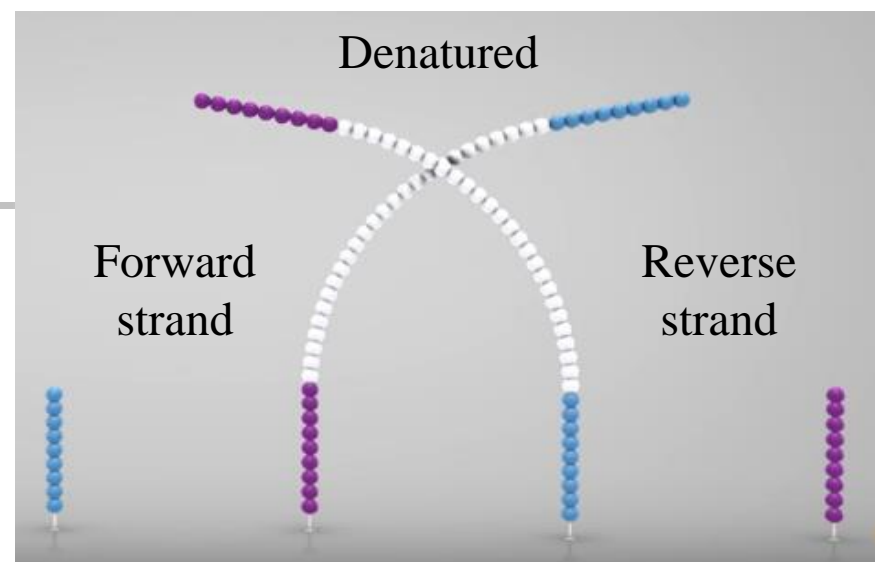
DNA polymerase



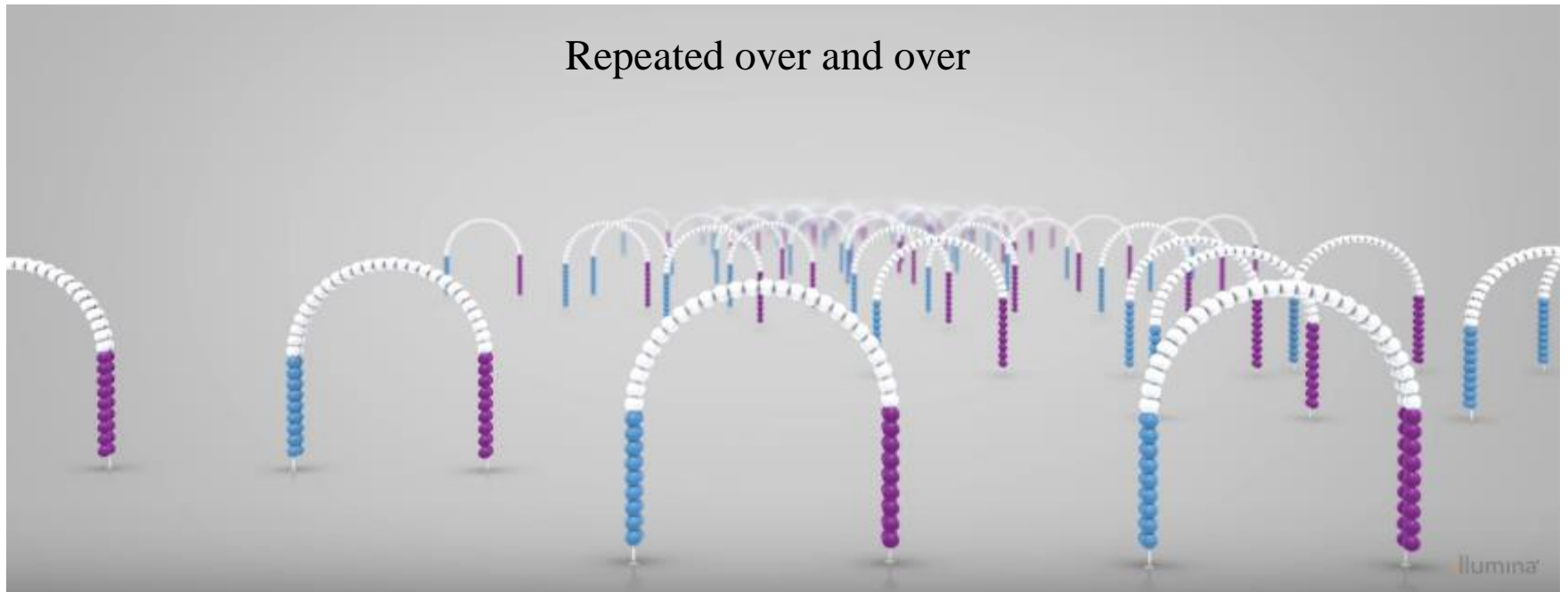
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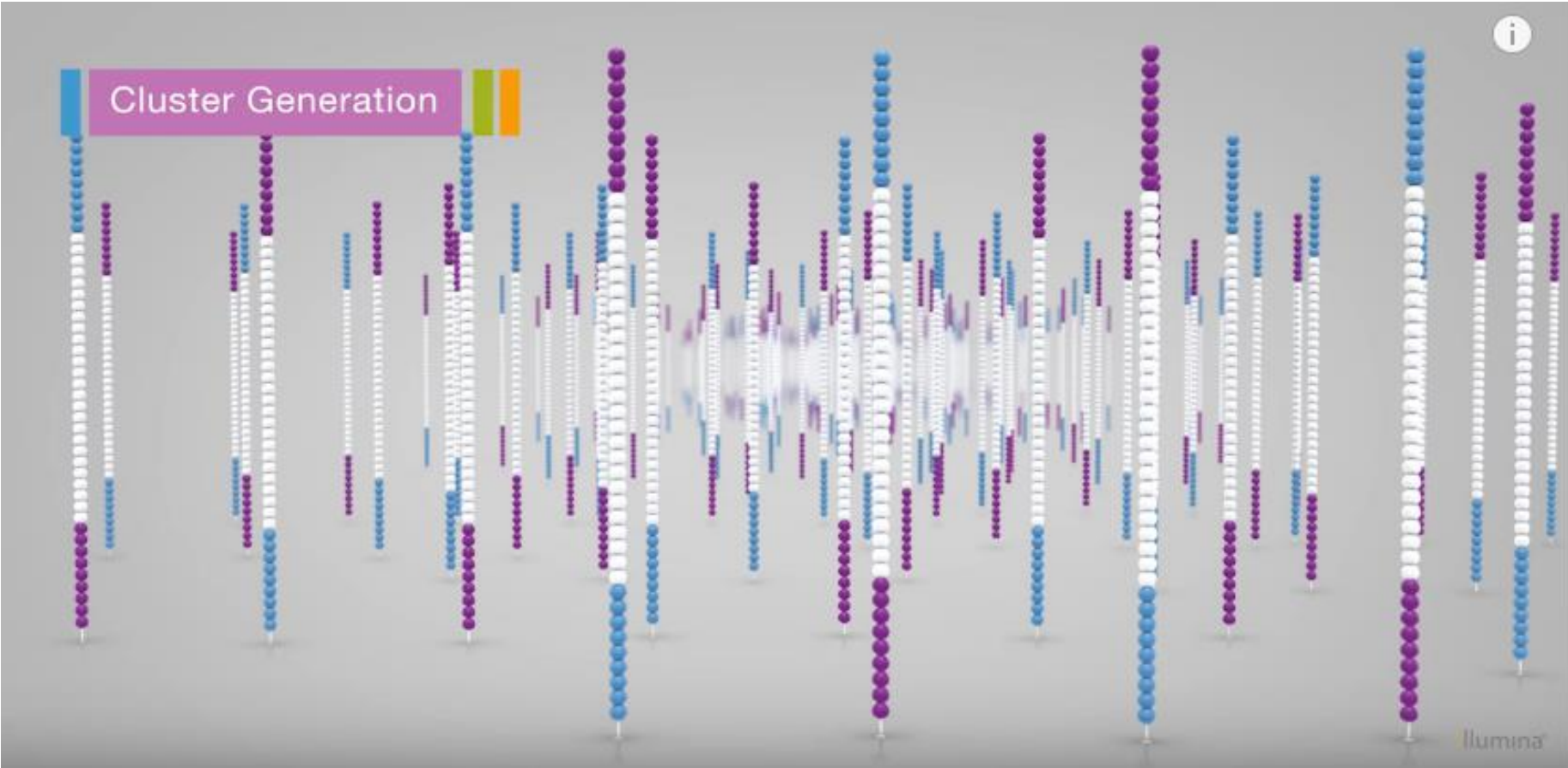
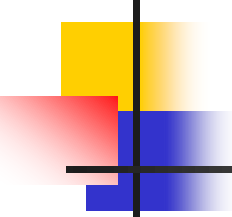
Forward
strand

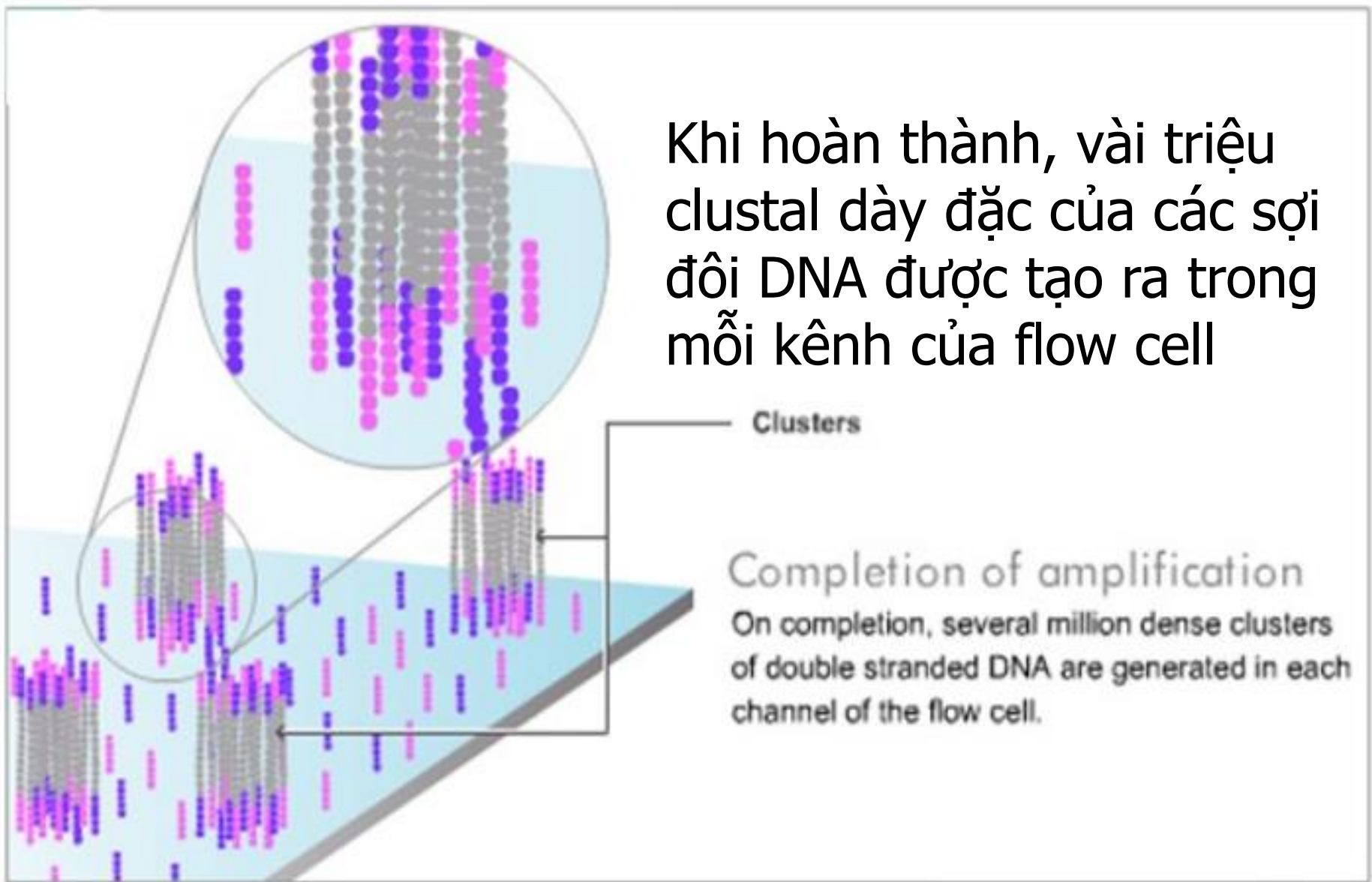
Reverse
strand

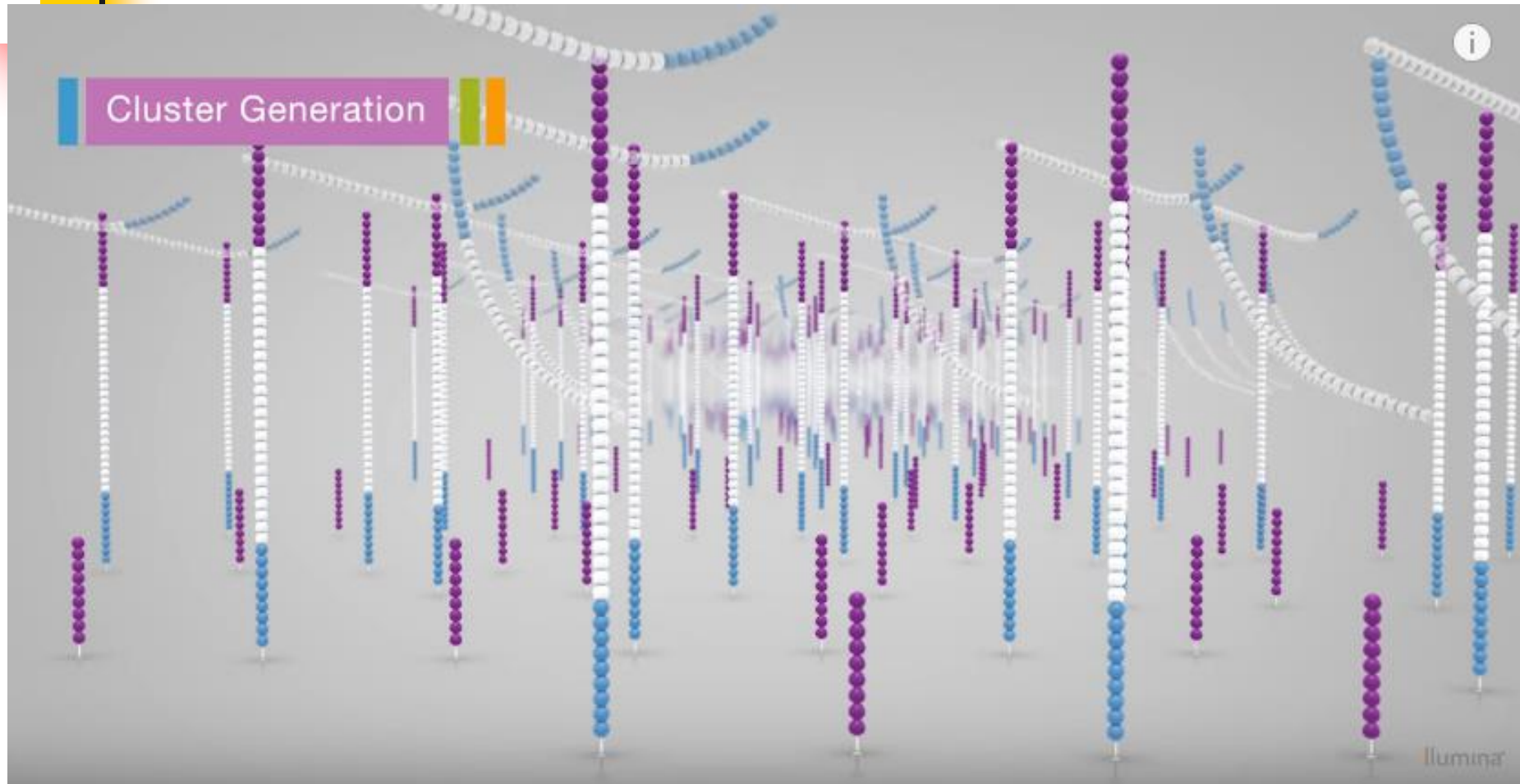


Repeated over and over



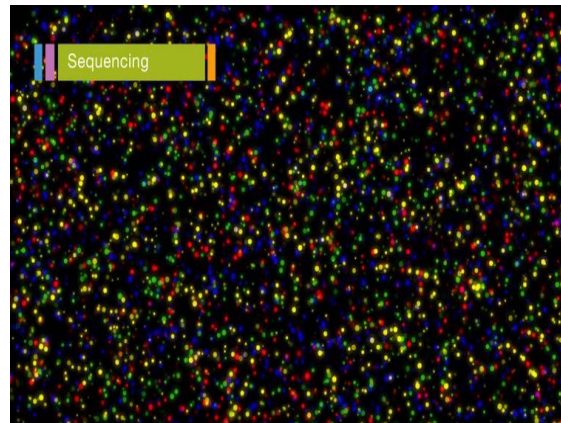
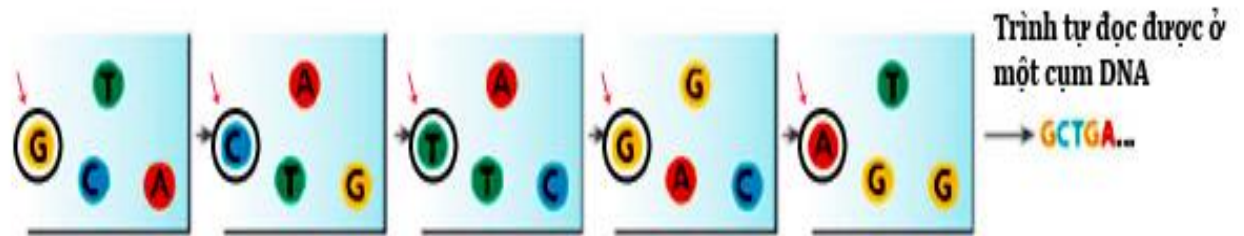
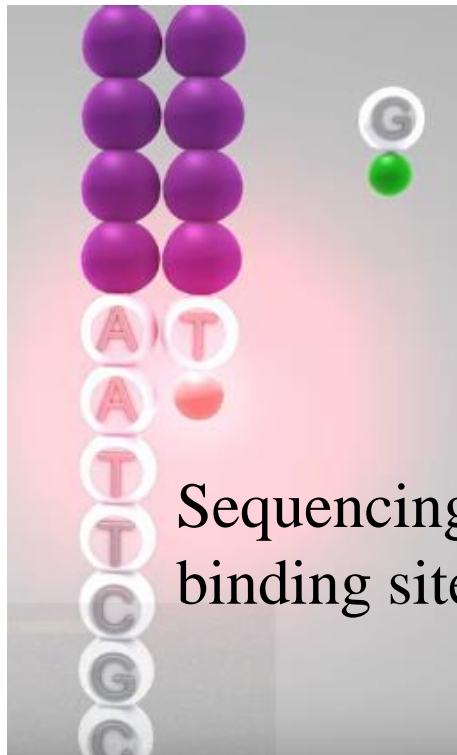
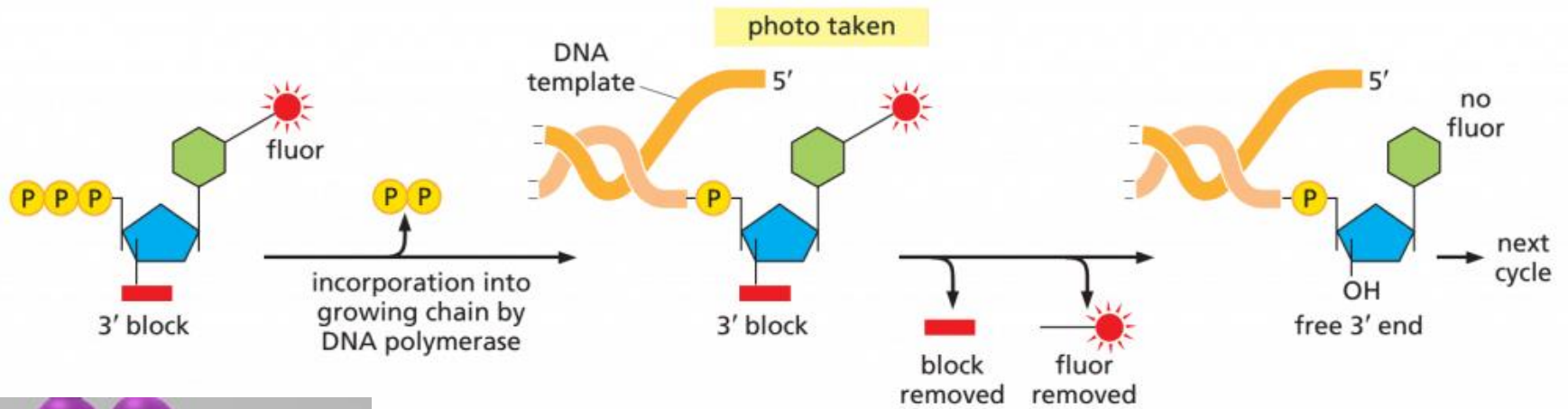




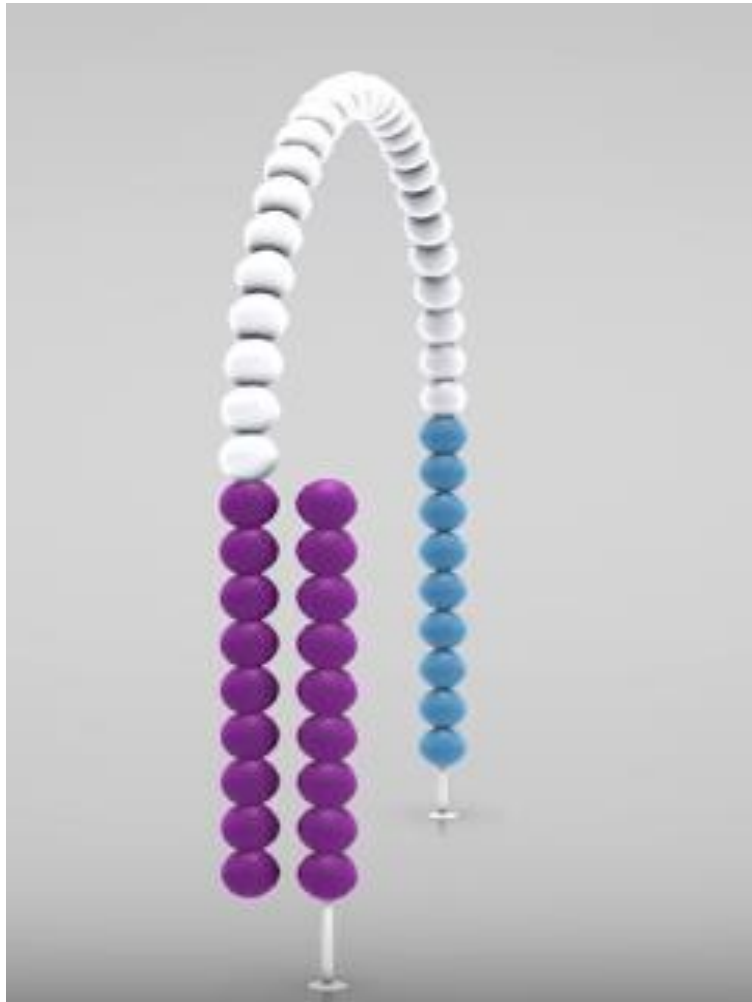


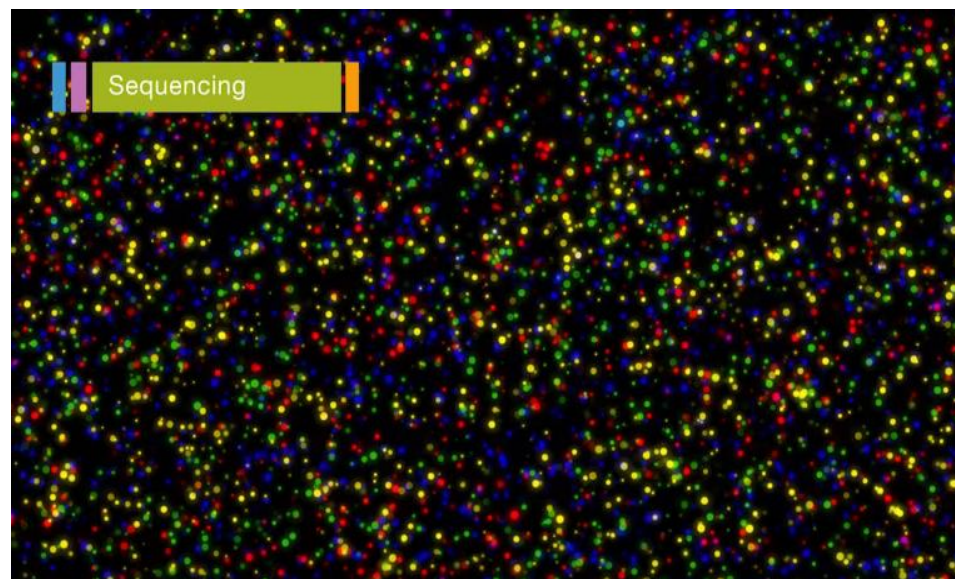
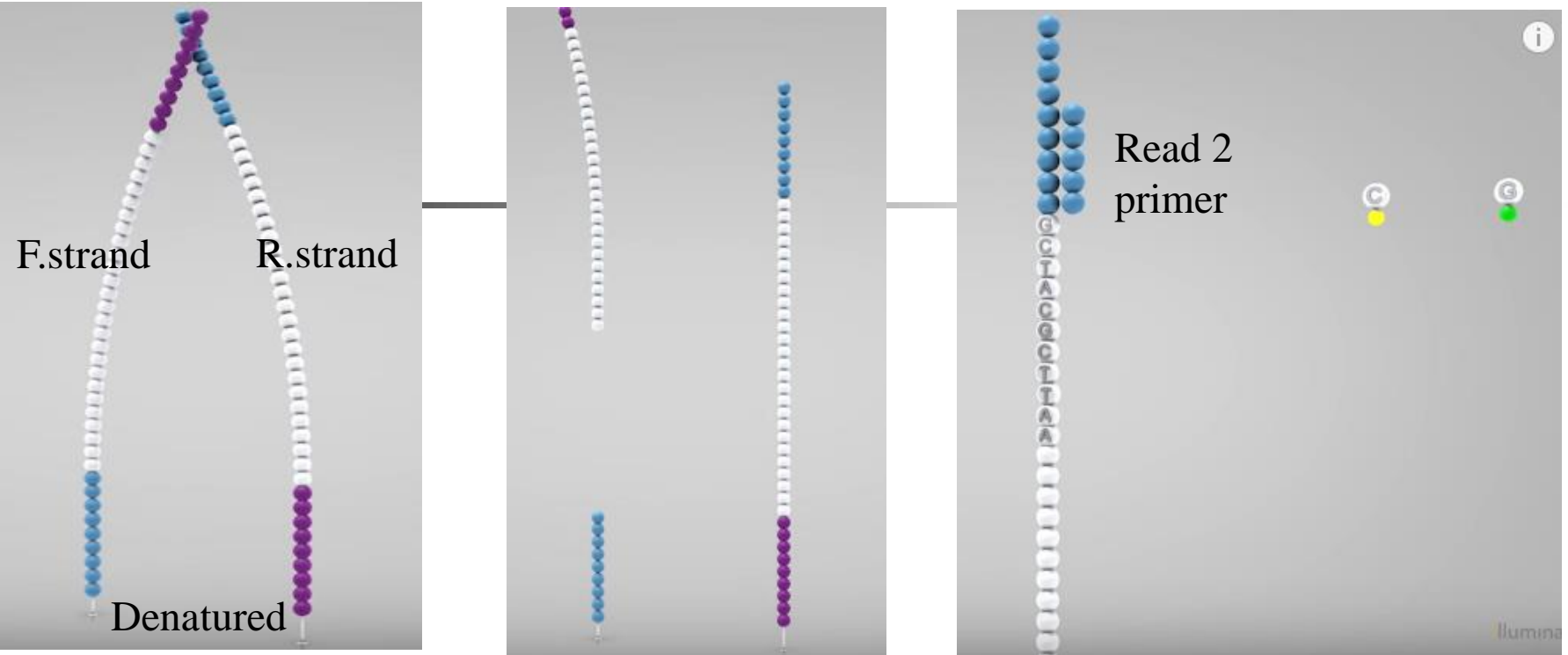
Reverse strands are cleaved and washed out, leaving only the forward strands

Illumina Sequencing



Giải trình tự Reverse





Local sequencing clustering

```
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
```

```
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
```

```
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
```

Similar sequences

```
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
```

```
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
```

Creat contiguous sequencing (Tạo ra các tập con trình tự)

Forward read

```
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
CTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGAAACAA
```

Reverse read

```
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
ACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCTTATTCTTAGA
```

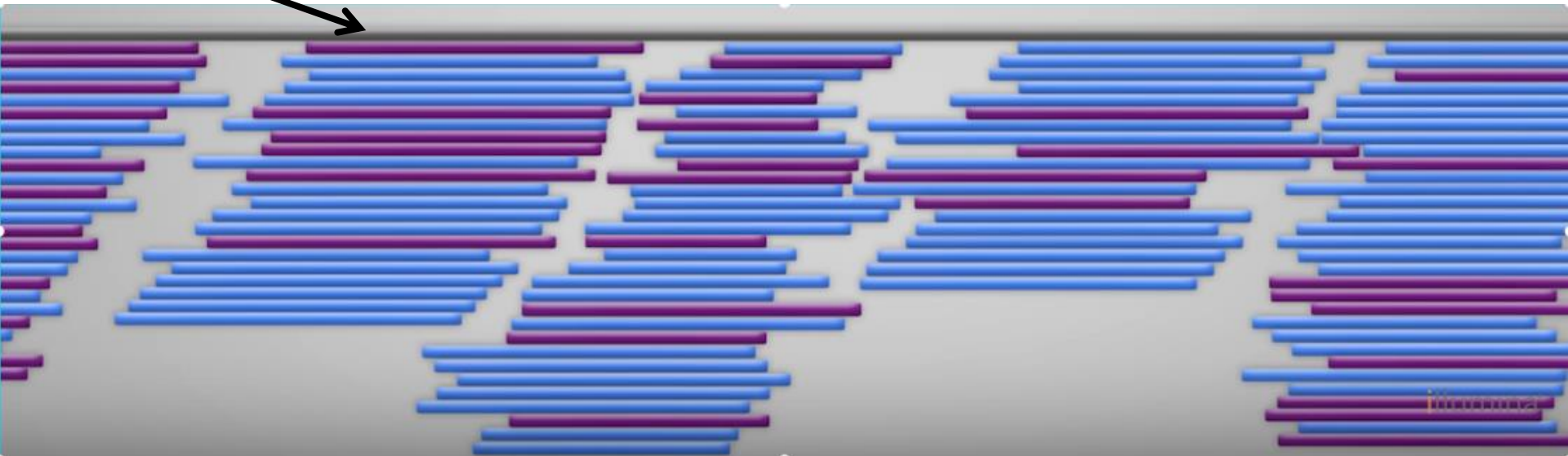
```
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
CTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGACAAACCTCCTTCT
```

```
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
AATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATTGAC
```

```
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
CTCAATGGCTAAGGCTTACGCCGTACTACCTCAGCAGTAGTAAGAAACAAAAGCAATT
```

Variant identification

Reference



Cấu tạo chi tiết Adapter

Library Prep is Critical for Successful Sequencing

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



Dual Index Library shown

P5 and P7 sequences are complimentary to oligos bound to flow cell surface and *required* for any library

Indexes are used to tag individual samples to allow pooling of multiple libraries

Rd1 and Rd2 sequencing primers regions are used to initiate sequencing

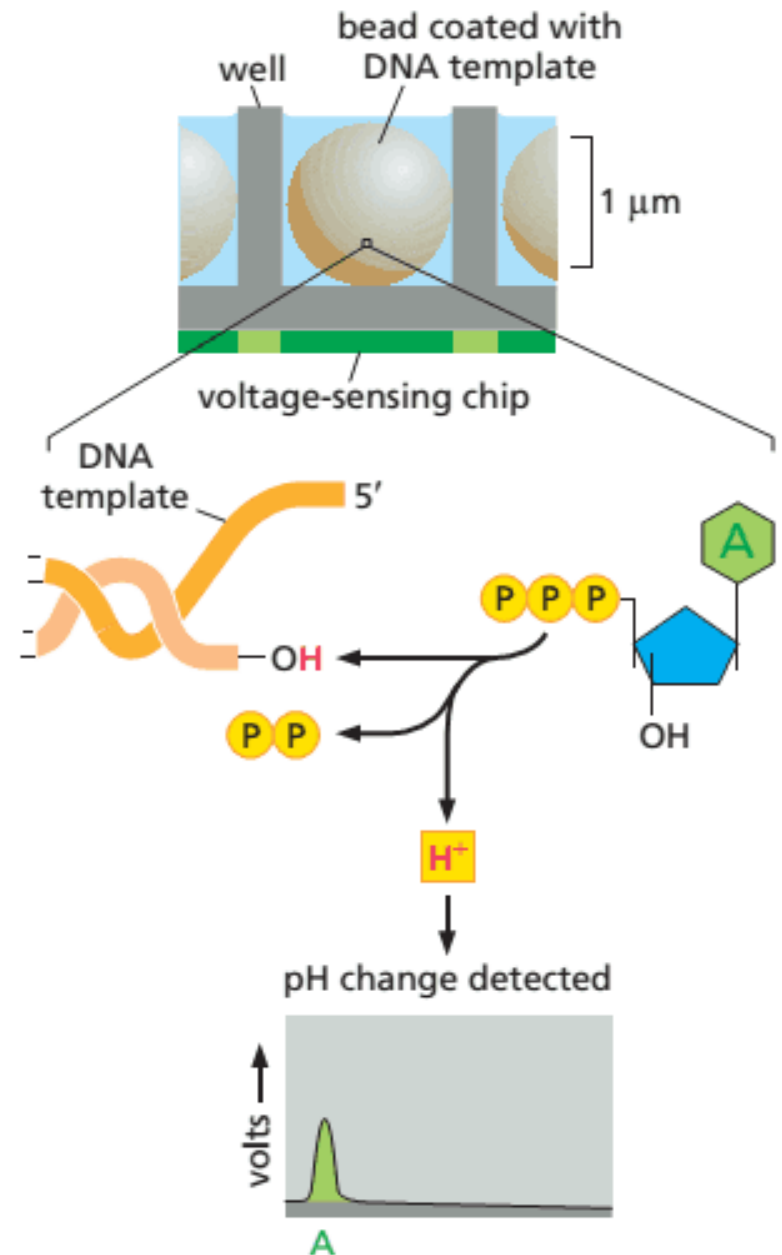


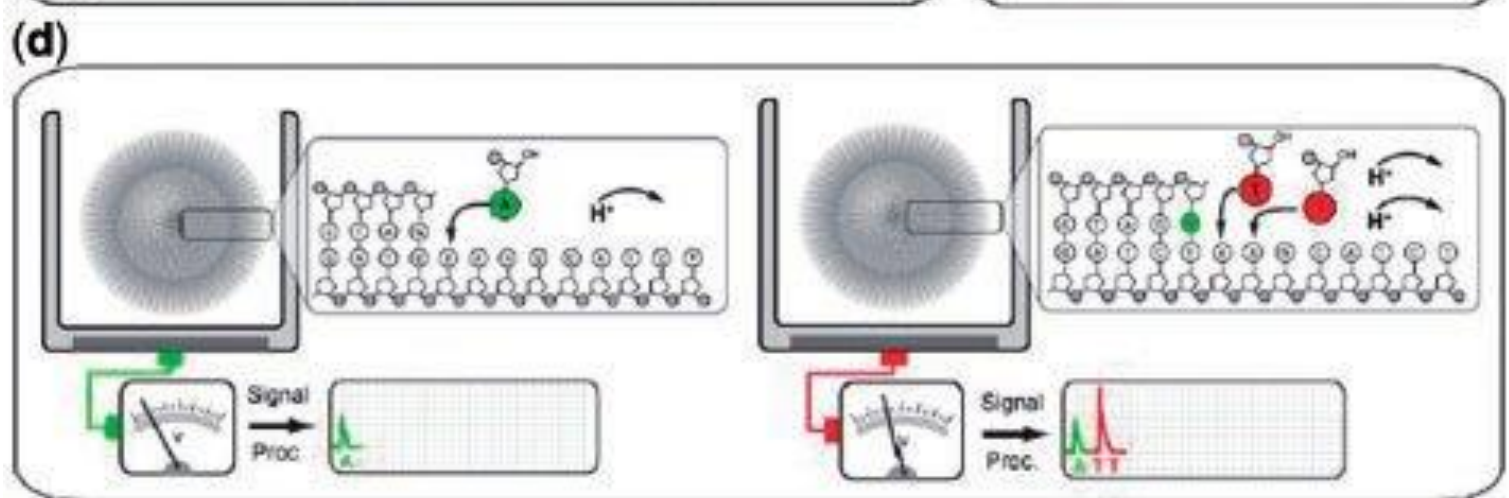
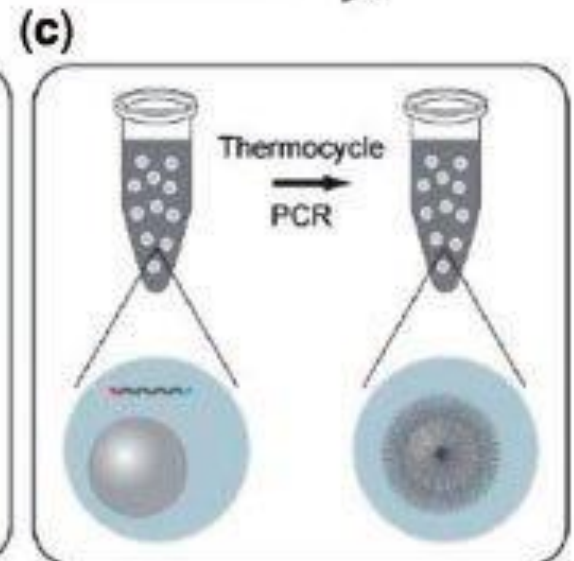
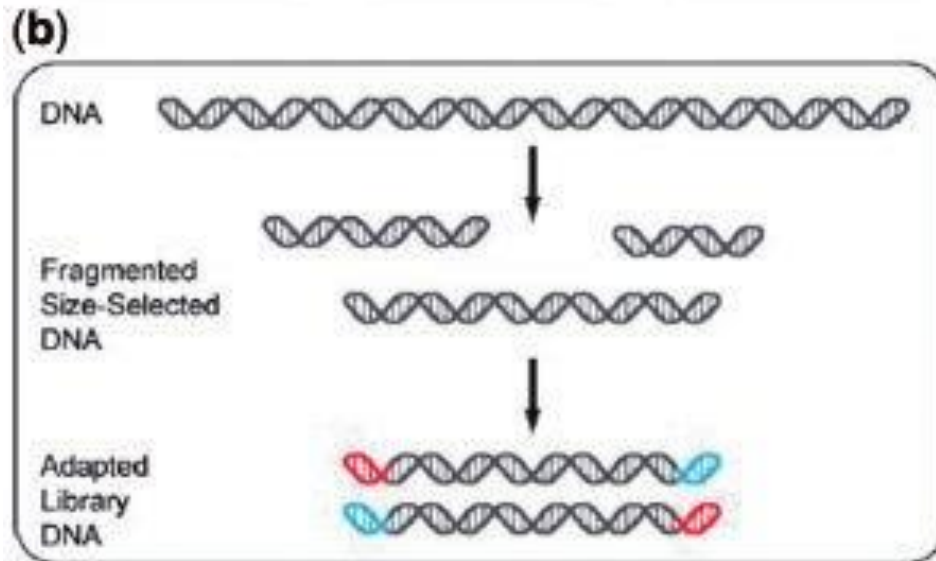
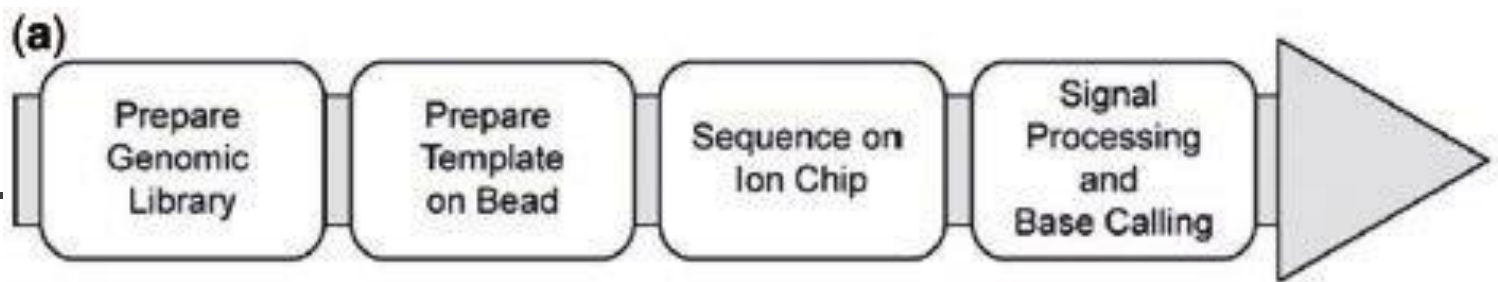
Bài tập

- Mô tả chi tiết các bước hoạt động trong quá trình “Tạo cụm”, “Giải trình tự Forward”, “Giải trình tự Reverse”

Ion Torrent sequencing

Ion semiconductor sequencing





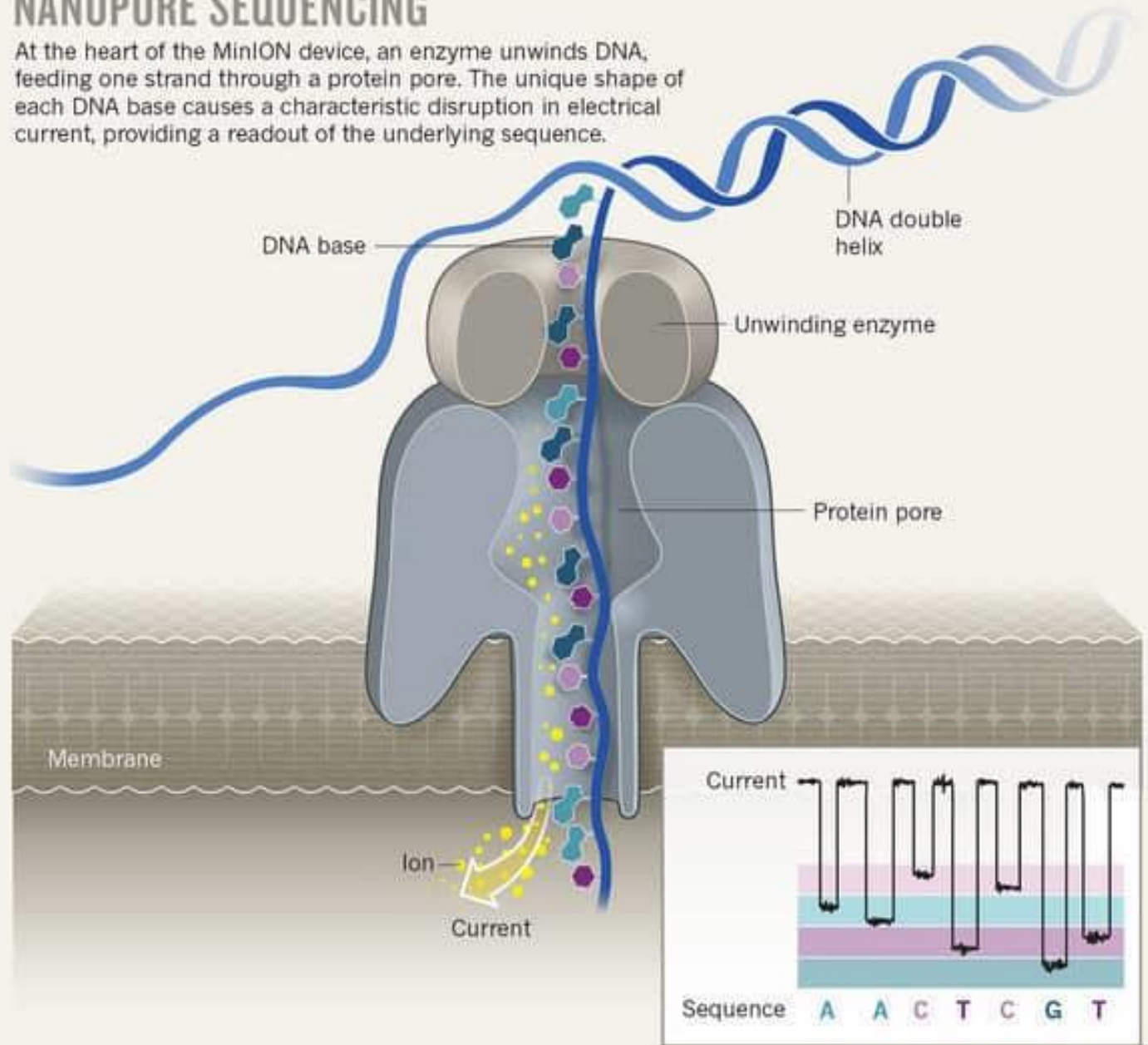
Giải trình tự thể hệ thứ 4 – Oxford Nanopore DNA Sequencing Technology

- Giải trình tự tức thời dựa trên tín hiệu điện phân tử



NANOPORE SEQUENCING

At the heart of the MinION device, an enzyme unwinds DNA, feeding one strand through a protein pore. The unique shape of each DNA base causes a characteristic disruption in electrical current, providing a readout of the underlying sequence.



Nanopore:
Alpha hemolysin (α HL) và
Mycobacterium
smegmatis porin A
(MspA)