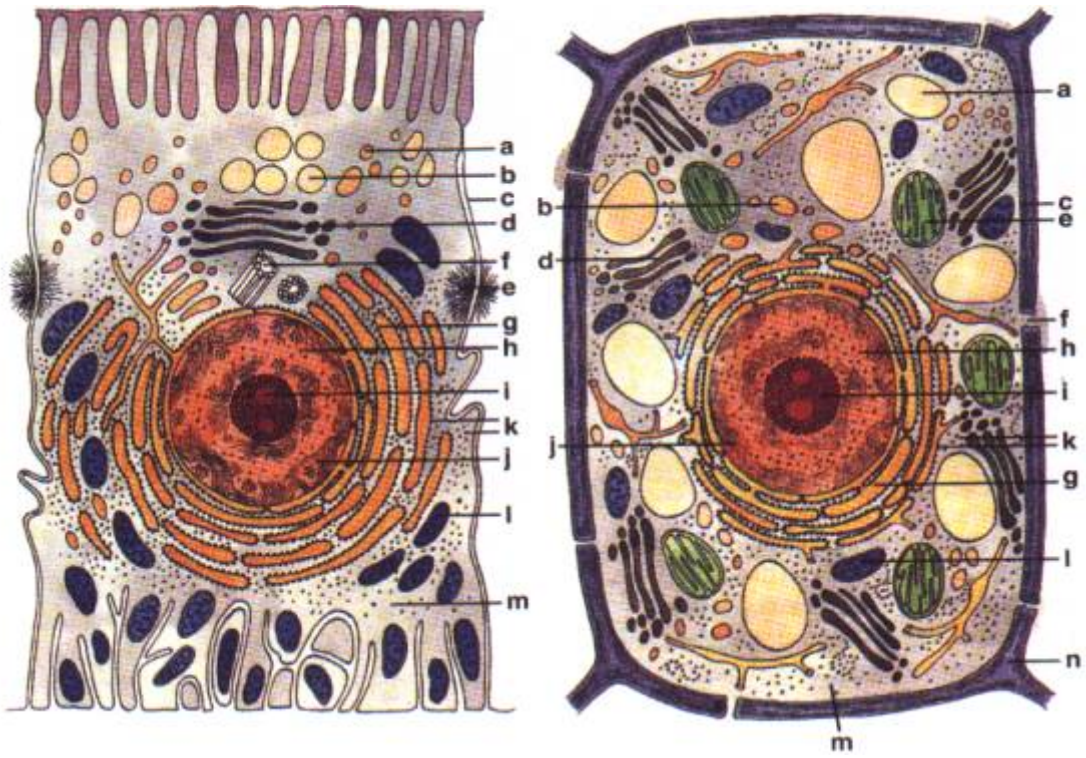
CO JE TO SEKVENACE A CO SE BUDE SEKVENOVAT?



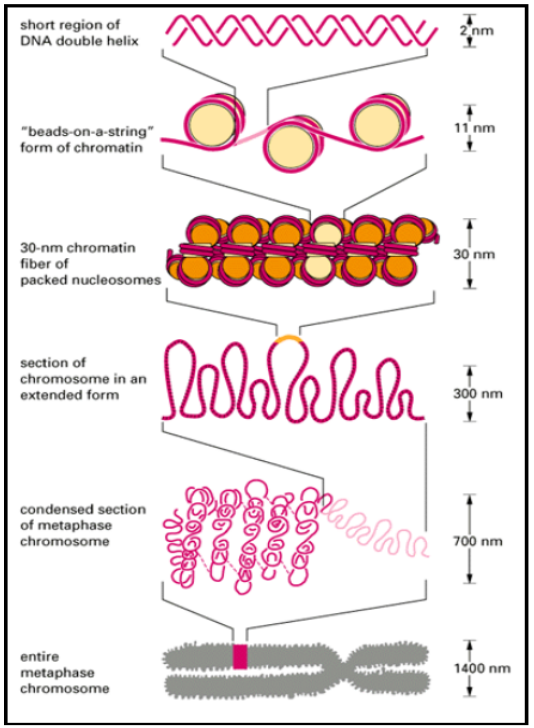
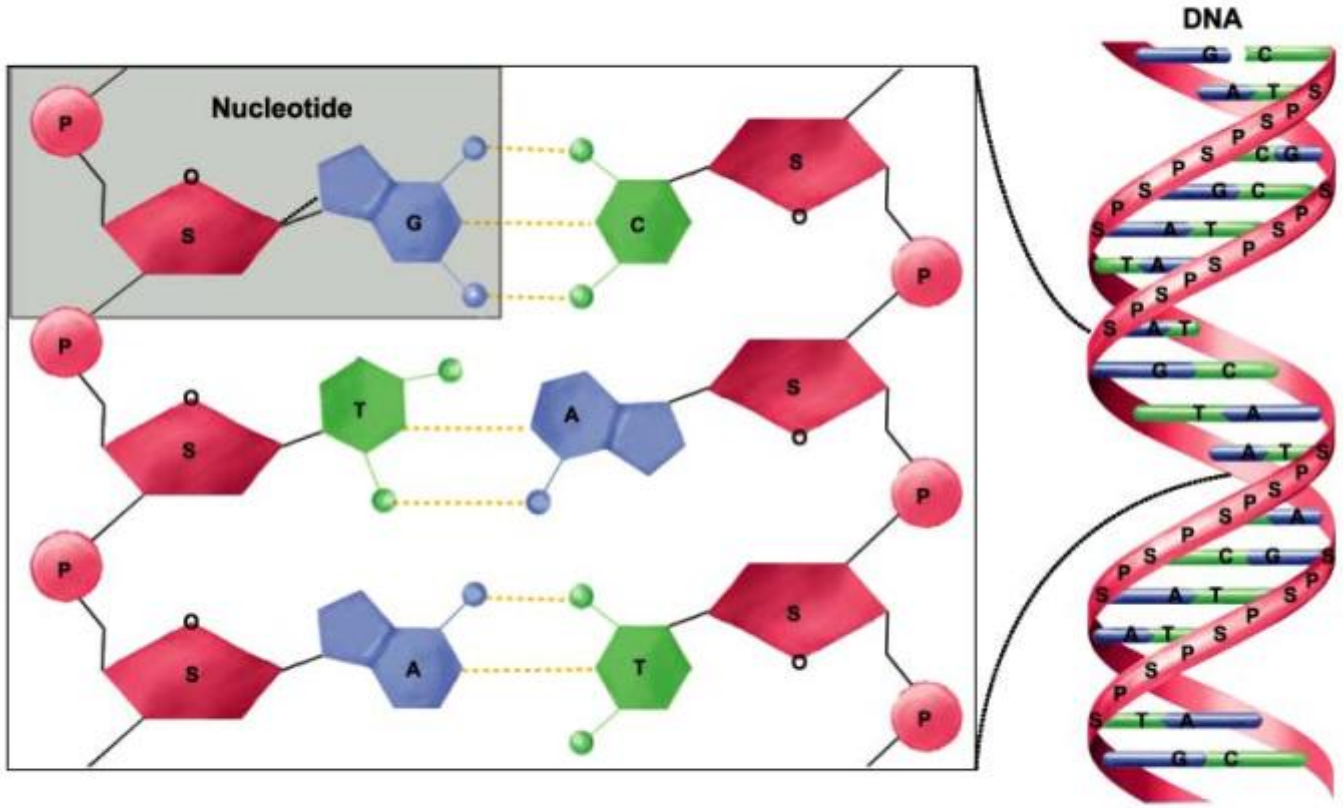


Figure 8-30. Model of chromatin packing. Alberts ,,Molecular Biology of the Cell"

POŘADÍ NUKLEOTIDU V DNA



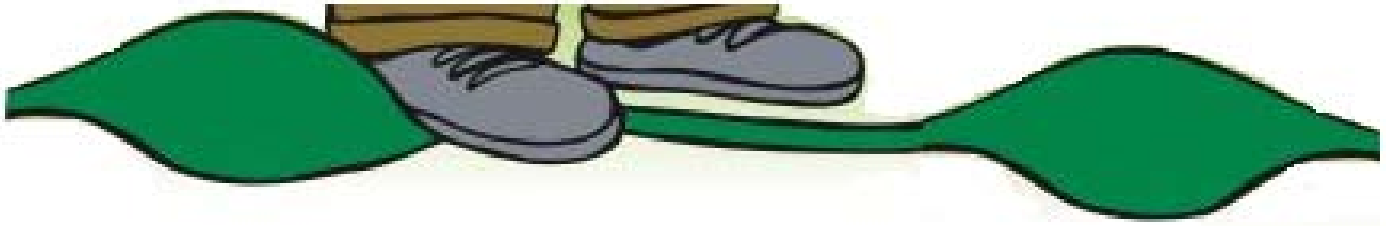
SEKVENOVÁNÍ DNA
od manuálních metod po plně automatizované

1. generace (od cca 1976):
   Maxam-Gilbert (čtyři štěpící reakce – G, AG, C, TC)
   Sangerova metoda (terminace syntezy druhého vlákna)

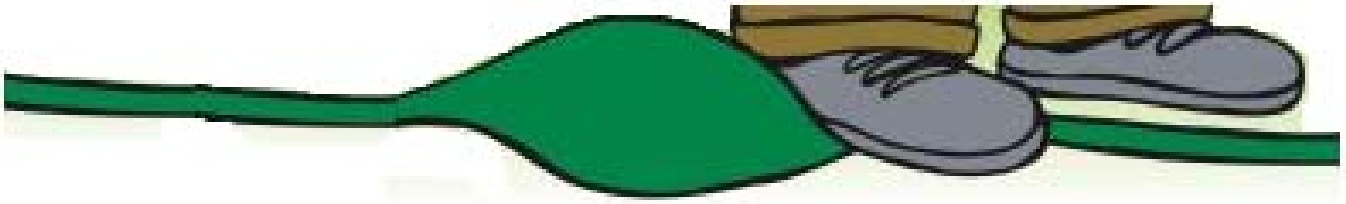
2. generace (next-gen; NGS; od cca 2000-2005):
   Solexa/Illumina (polymerace fluorescenčně značených oligonukleotidů)
   454/Roche (pyrosekvenace, PP enzymaticky vázán na emisi světla)
   SOLiD/LifeTechnologies (ligace fluorescenčně znač. oligonukleotidů)
   Polonator (ligace fluorescenčně znač. oligonukleotidů)
   Complete Genomics (nepoužívá PCR, ligace na shluky ssDNA)

   Sekvenování jednotlivých molekul:
      Helicos (optika)
      SMRT/Pacific Biosciences (nanopovrch s ukotvenou polymerázou)
      IonTorrent (polovodičové sekvenování, místo světla protony)
   Sekvenování na membránách:
      Oxford Nanopore
      NABSys
      NobleGen

KRITICKÁ FÁZE ANALÝZ
1. a 2. GENERACE



Příprava knihovny → Sekvenace → Analýza dat



PRINCIPY SEKVENACE DNA

sekvenčně-specifické štěpení DNA

polymerace:

ATCAGTGCGATGCA - (ANCHOR)
TAGTCACG DNA pol <-C*

hybridizace:

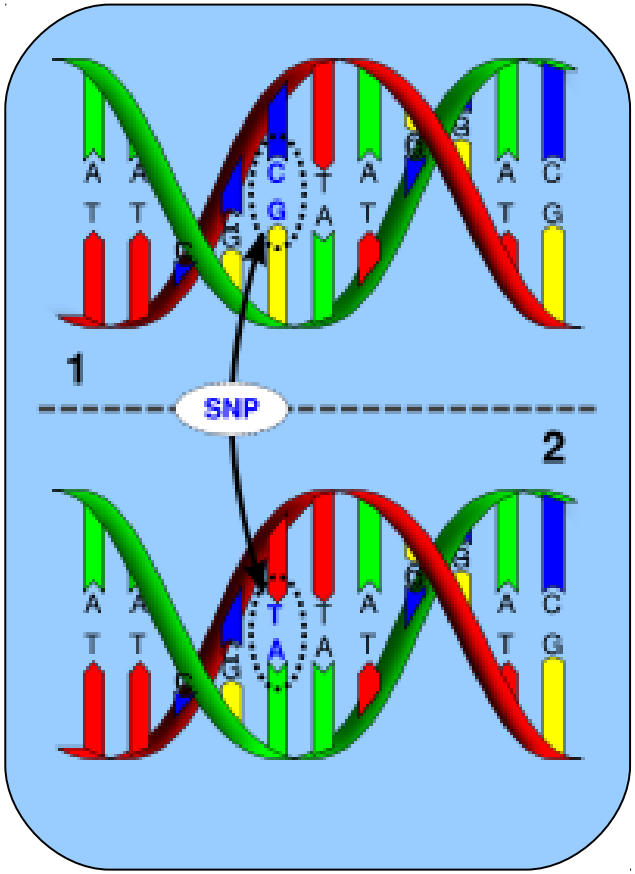
ATCAGTGCGATGCA - ANCHOR
TAGTCACGCTACGT*

ligace:

ATCAGTGCGATGCA – ANCHOR
TAGTCACG----CTacgt*
DNA lig

nanopory a průchod membránou

DUVODY SEKVENACE



- de novo sekvenace
  - organismy
  - populace (metagenomika)

- resekvenování
- detekce polymorfismů
  - SNP
  - rekombinace

- zjištění stavu metylace

- měření exprese
(jako náhrada technik založených na hybridizaci)

DRUHY SEKVENACE

dle způsobu přípravy vzorků a použitých reaktantů

DNA-seq

ChIP-seq (imunoprecipitace chromatinu)

RNA-seq

sRNA-seq

Bis-seq, BisChIP-seq (metylační analýza)

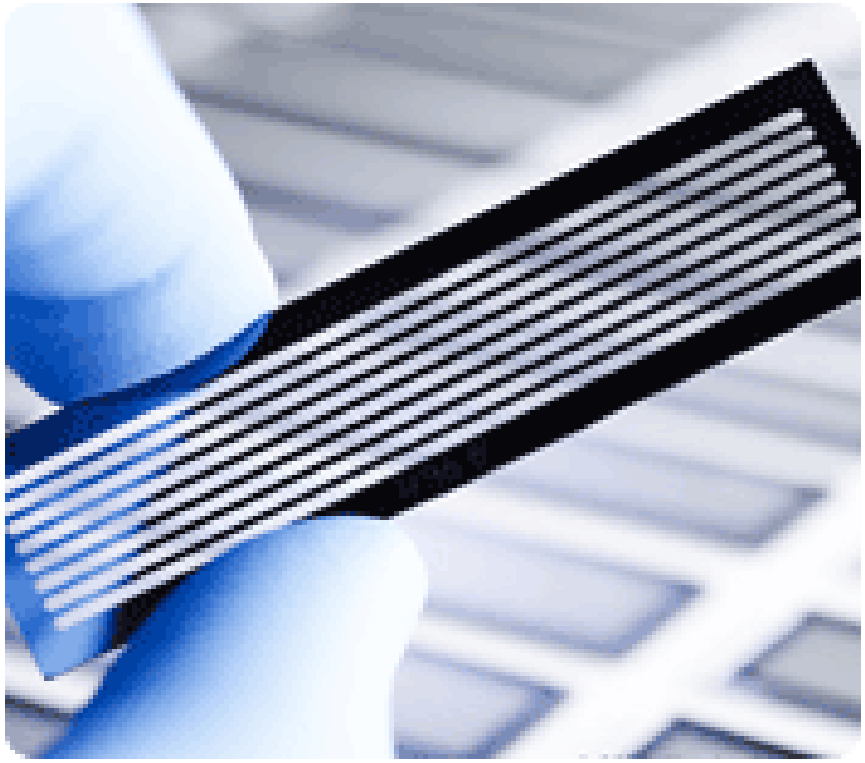
CLIP-seq (“cross-linking immunopreciputation”; RNA-prot)

Ig-seq,…(viz *Seq @ http://liorpachter.wordpress.com/seq/

Klíčové kroky a parametry sekvenování DNA

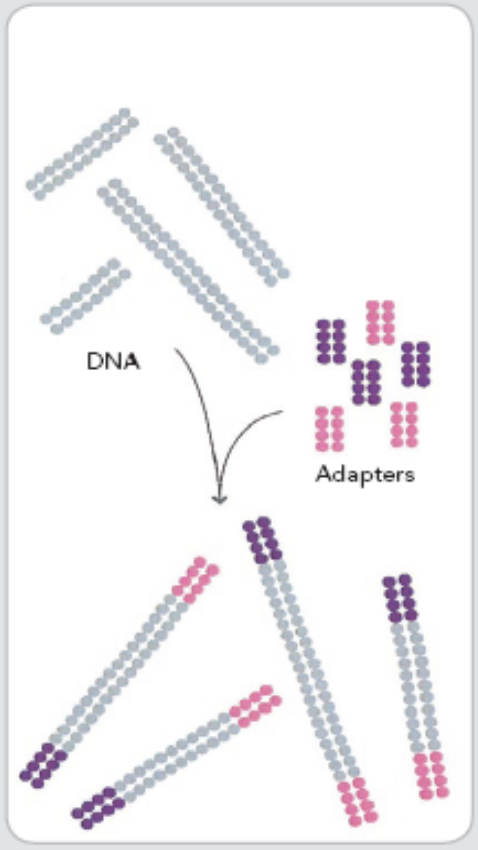
- izolace DNA
- fragmentace DNA
- ligace adaptérů
- namnožení sekvencí
- typ podložky nebo média
- typ enzymů (pol, lig)
- typ detekce (světlo, proud)

Illumina



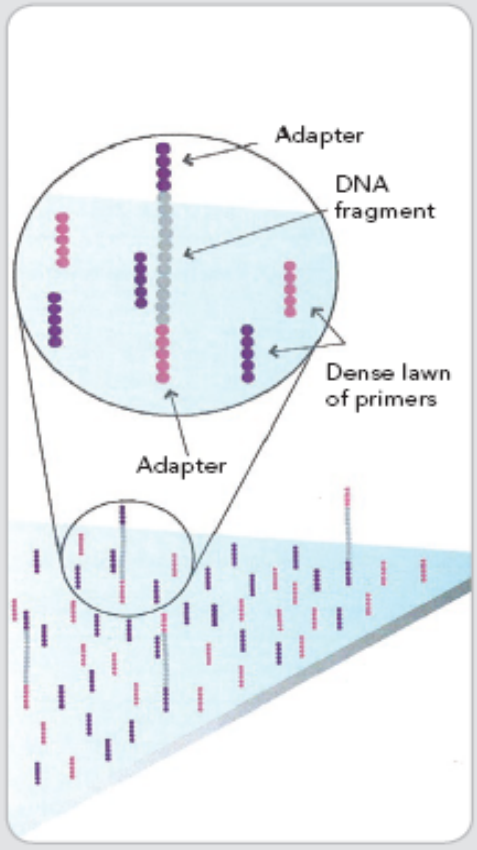
Illumina (“bridge amplification”)

1. PREPARE GENOMIC DNA SAMPLE



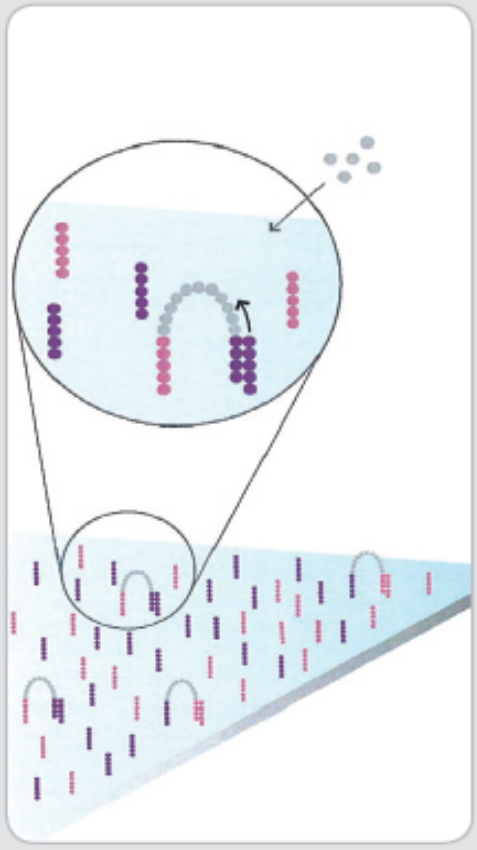
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

2. ATTACH DNA TO SURFACE



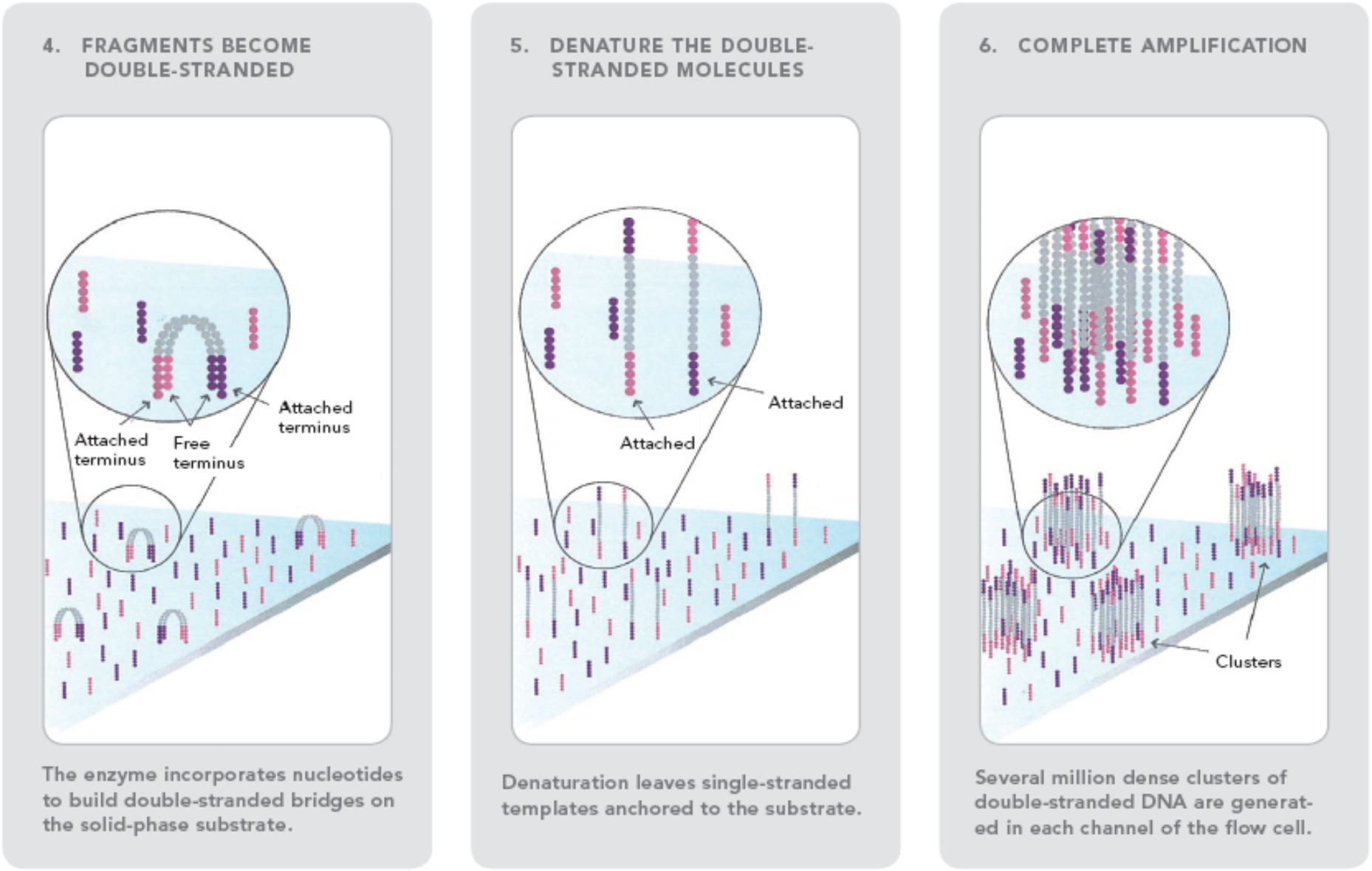
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

3. BRIDGE AMPLIFICATION



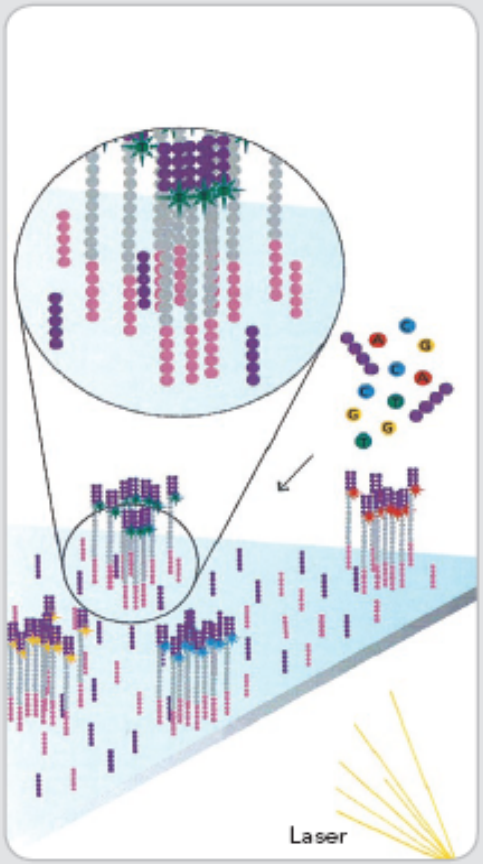
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Illumina (“bridge amplification”)



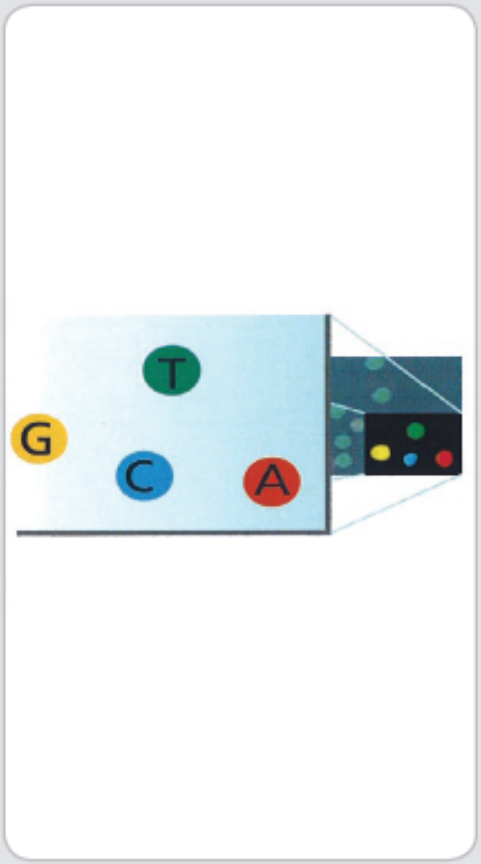
Illumina

7. DETERMINE FIRST BASE



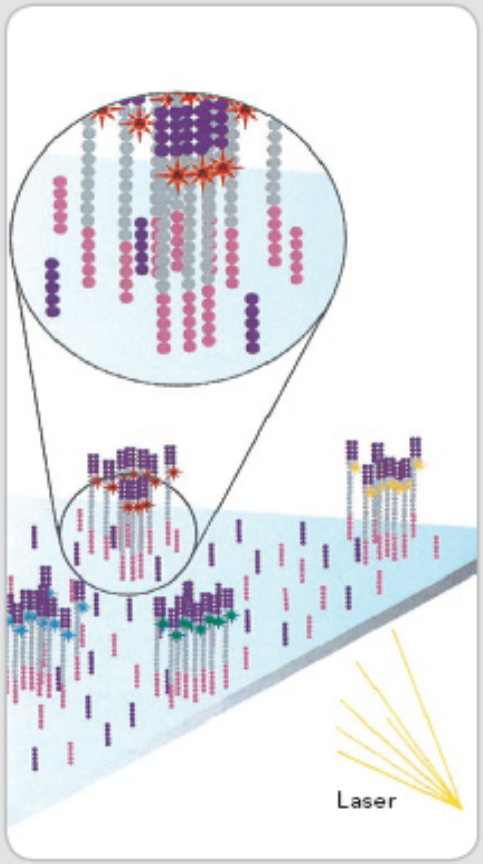
The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

8. IMAGE FIRST BASE



After laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified.

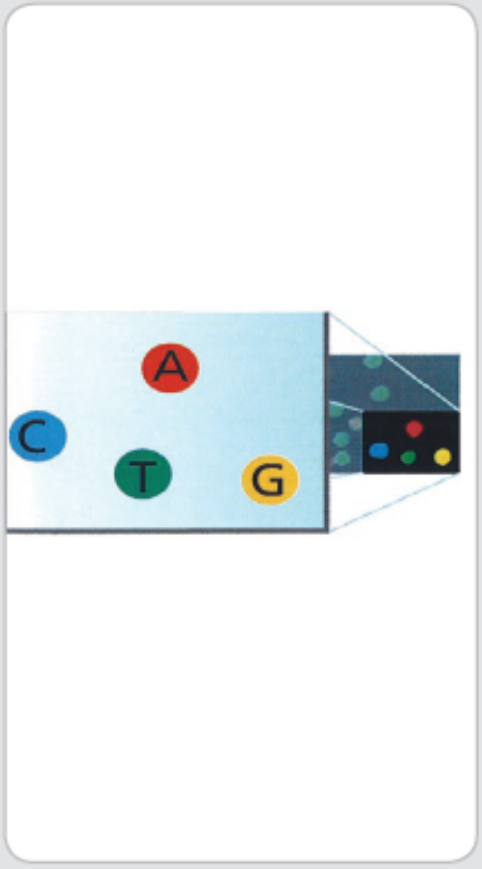
9. DETERMINE SECOND BASE



The next cycle repeats the incorporation of four labeled reversible terminators, primers, and DNA polymerase.

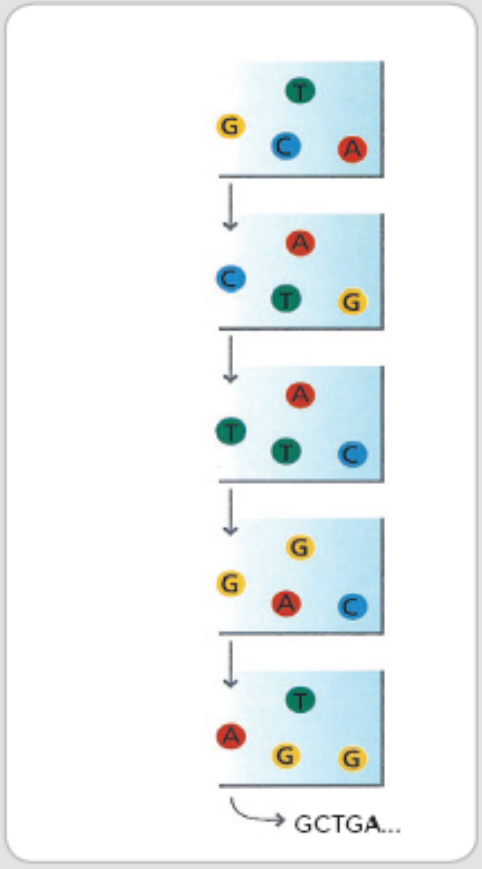
Illumina

10. IMAGE SECOND CHEMISTRY CYCLE



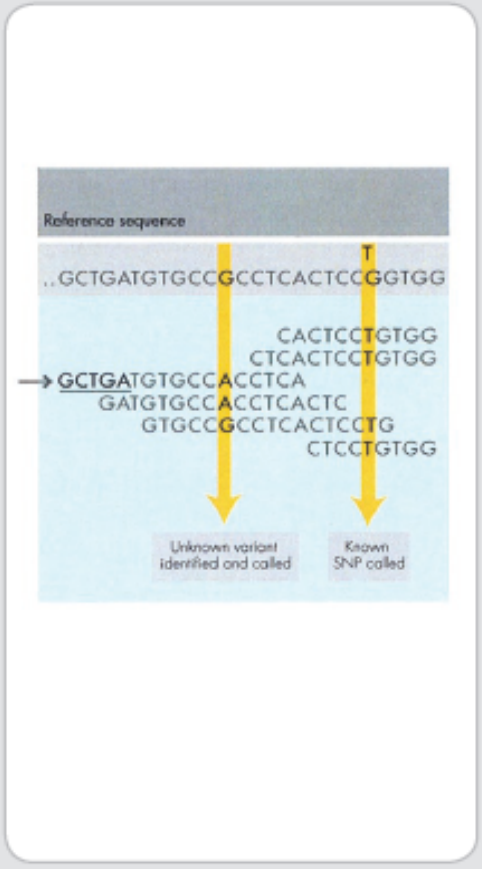
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

11. SEQUENCING OVER MULTIPLE CHEMISTRY CYCLES



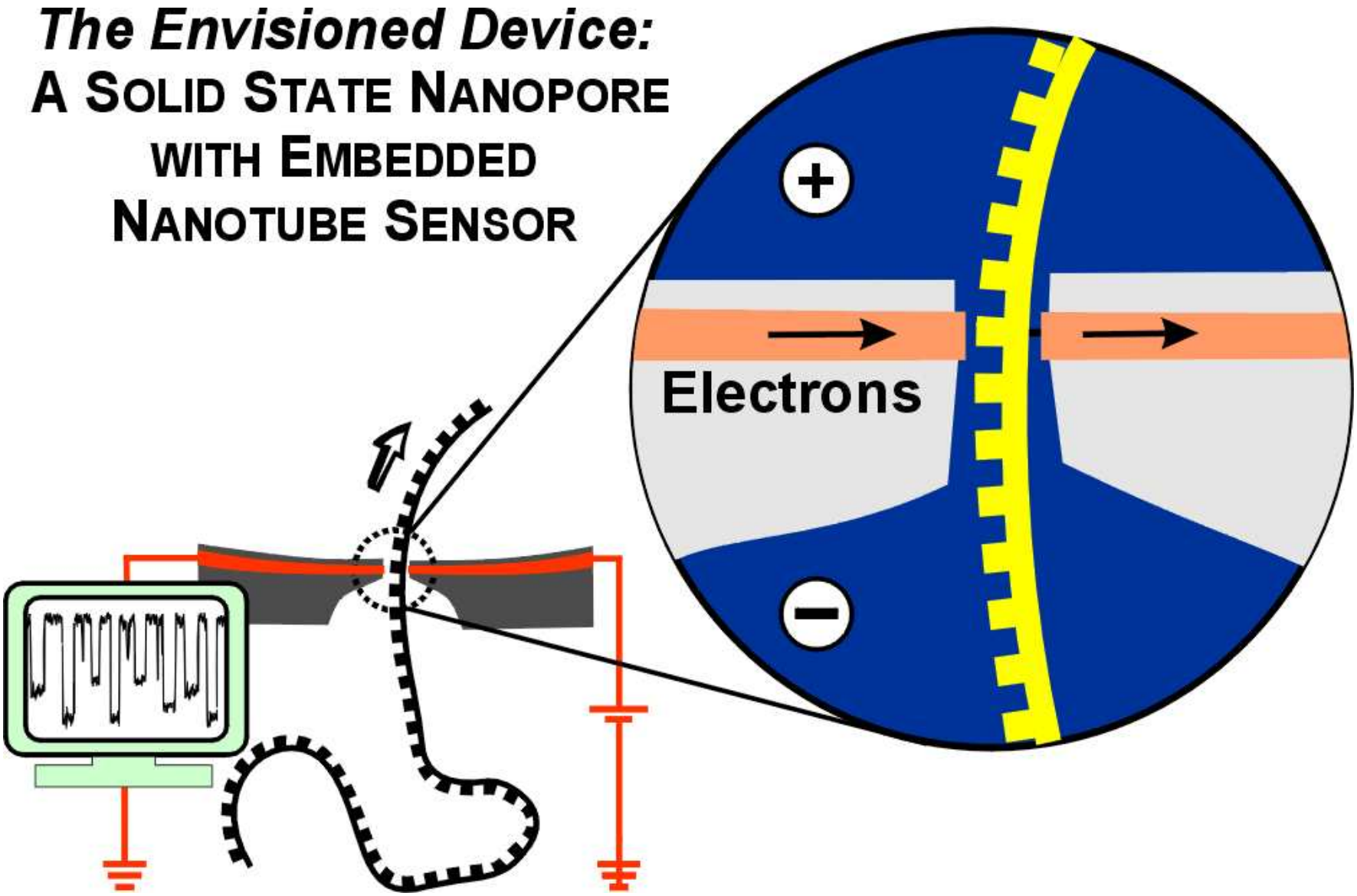
The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

12. ALIGN DATA

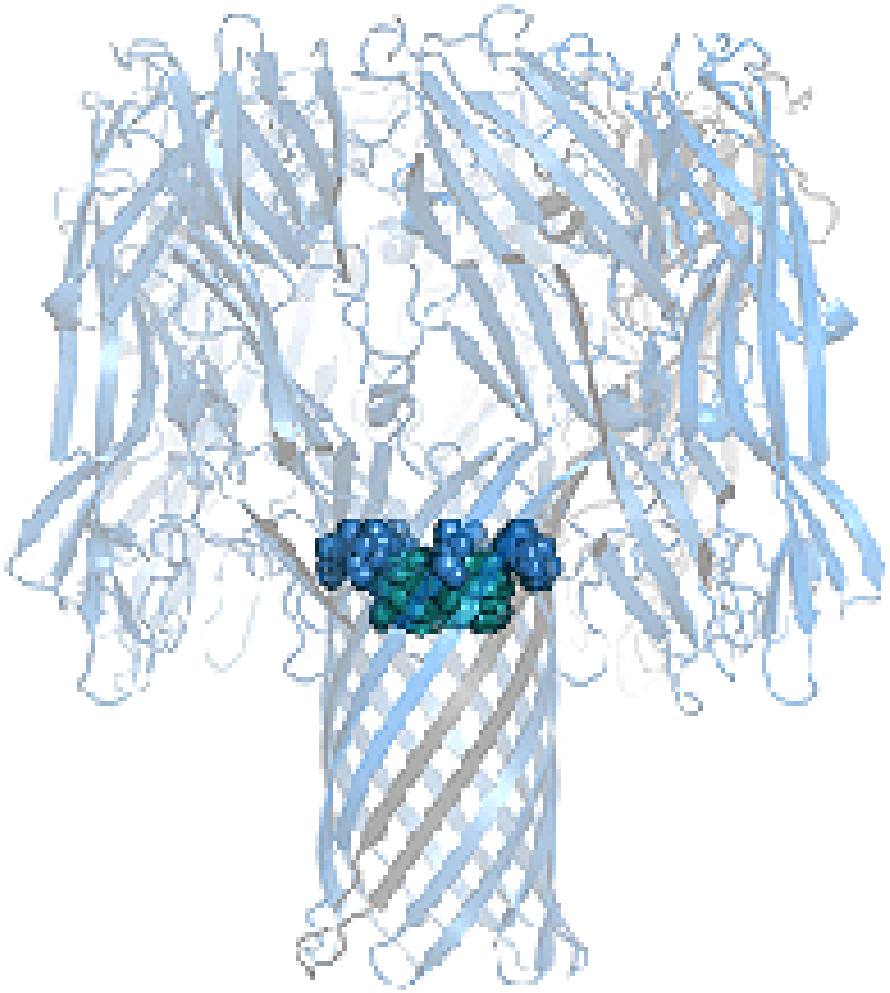


The data are aligned and compared to a reference, and sequencing differences are identified.

Sekvenování nanopory



Oxford Nanopore Technologies



Nanopor tvořen proteinem alpha-hemolysin s cyclodextrinovým adapterem (místo detekce nukleotidů).