SEKVENACE DNA – PŘEHLED METOD

- Sanger (dideoxy-NTP, DNA pol., gel) 600-900 bp

- “next-generation sequencing”
  - 454 (-> Roche) 120-150 bp
  - Illumina (Solexa->) 75-90 bp
  - SOLiD (-> ABI) 35-50 bp

- “single molecule sequencing”
  - Helicos tSMS
  - elektroporetická metoda (Oxford Nano)

- “SBH” (sekvenování hybridizací)

SEKVENACE DNA – PŘEHLED METOD

polymerizace:

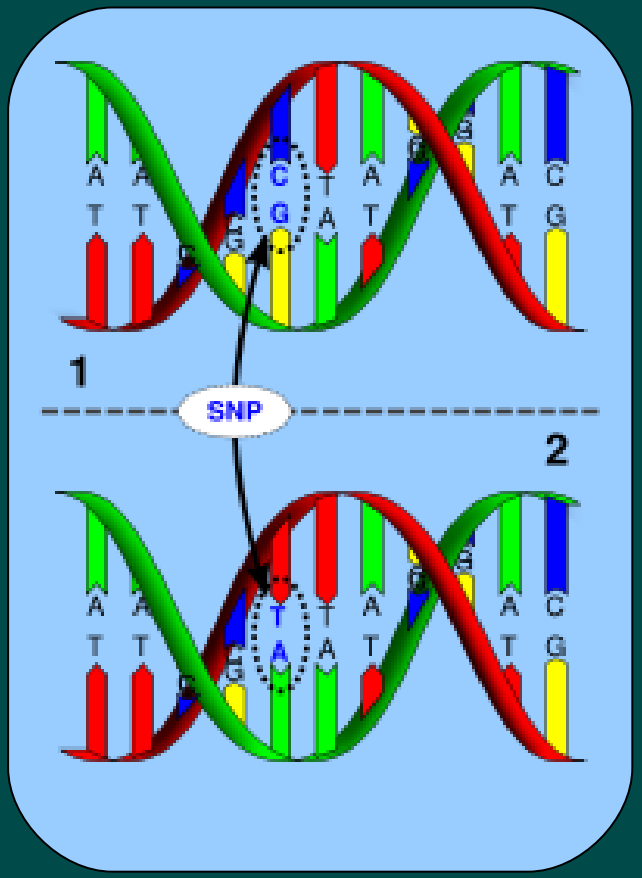
ATCAGTGC DNA pol A - (KOTVA)
TAGTCACG <-C*

hybridizace:

ATCAGTGCGATGCA - KOTVA
TAGTCACGCTACGT*

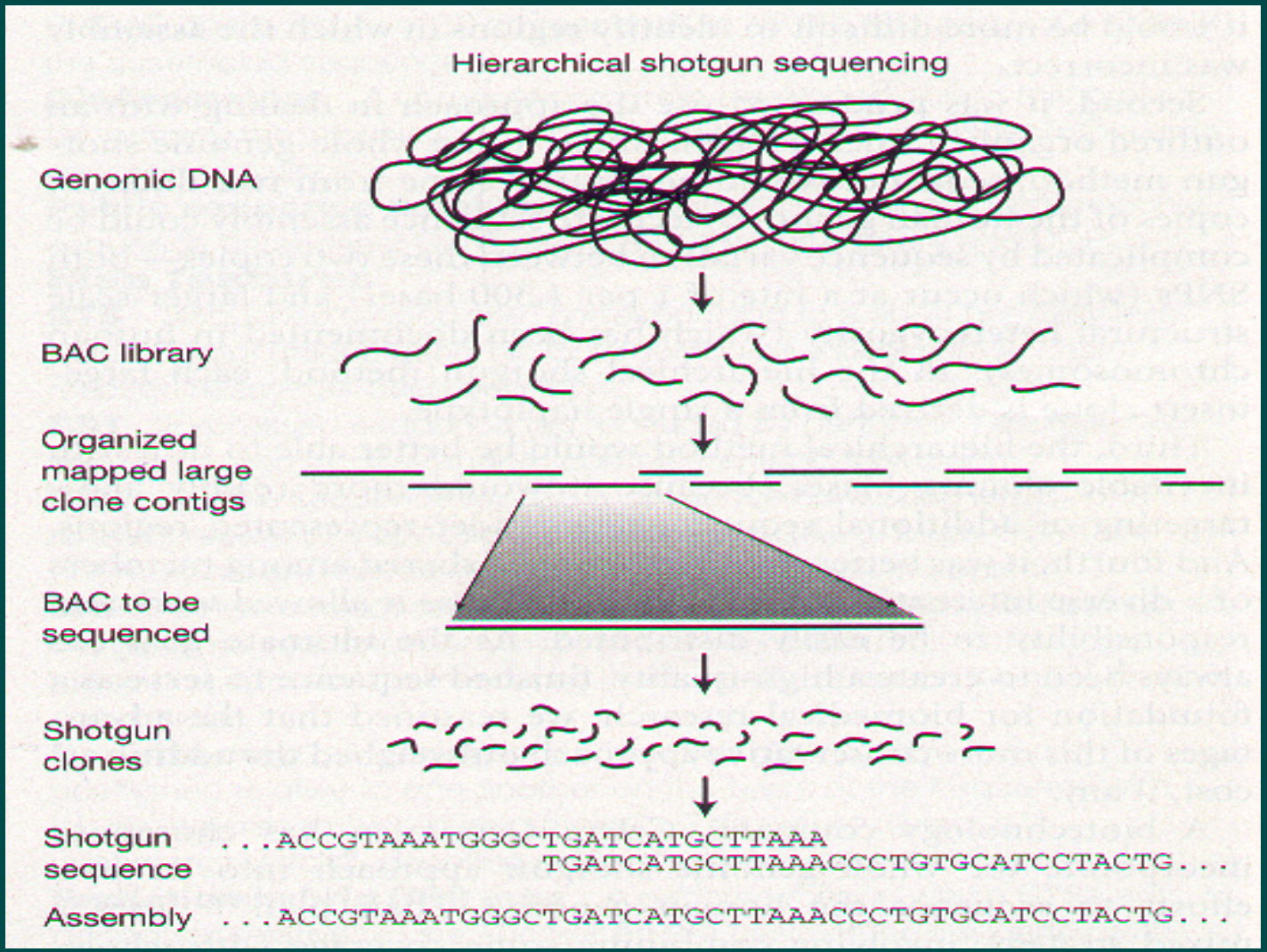
ligace:

ATCAGTGCGATGCA – KOTVA
TAGTCA DNA lig cgt*

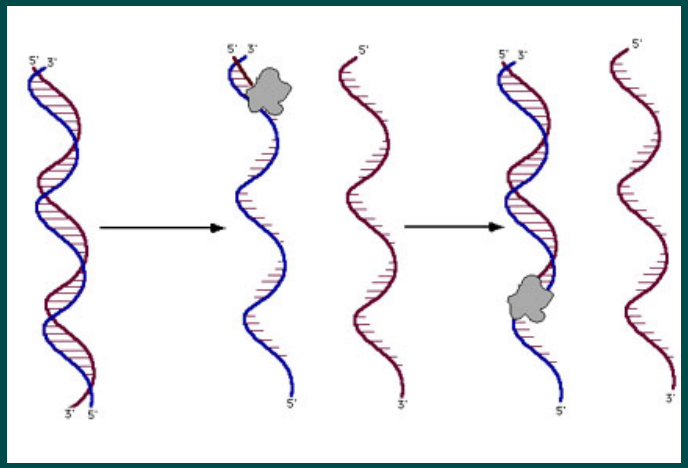


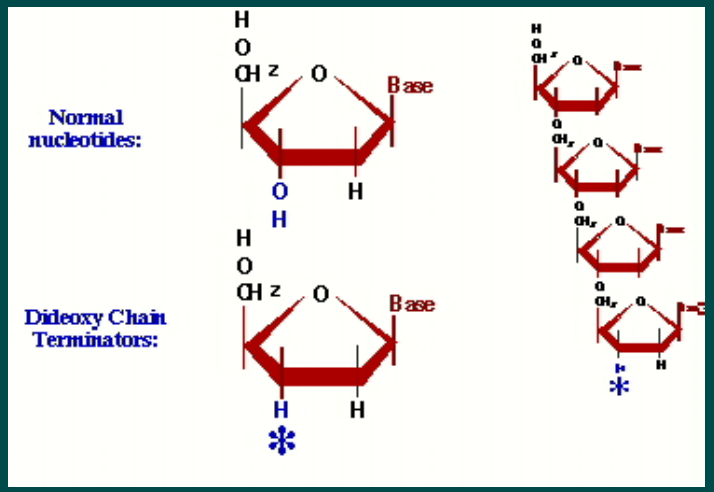
- de novo sekvenování
  - organizmy
  - populace
- resekvenování
- detekce polymorfizmu
  - SNP
  - přeskupení
- zjišťování metylace
- měření exprese (náhrada microarray)

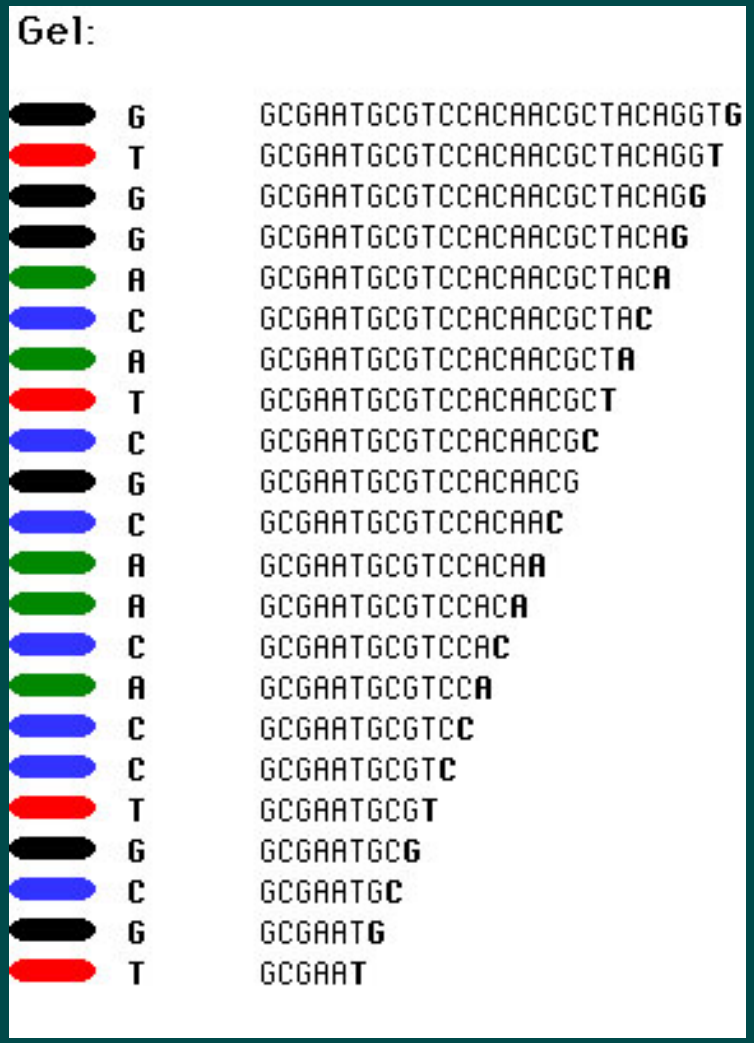
Tradiční způsoby sekvenace



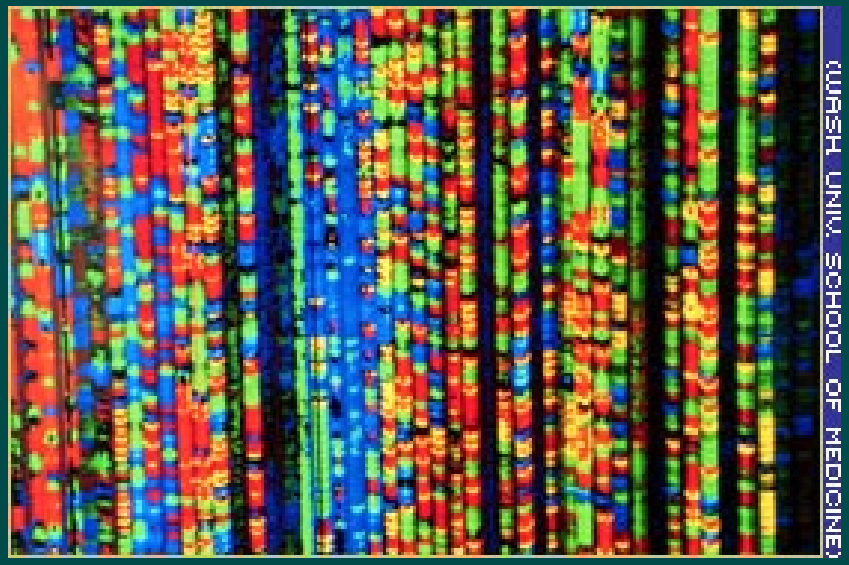
Sanger



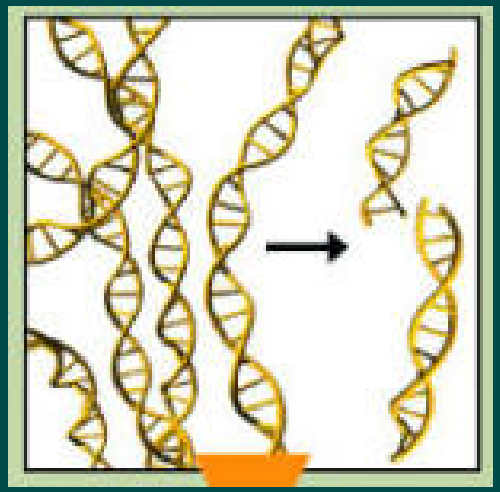

Normal nucleotides:
Base
Dideoxy Chain Terminators:
Base

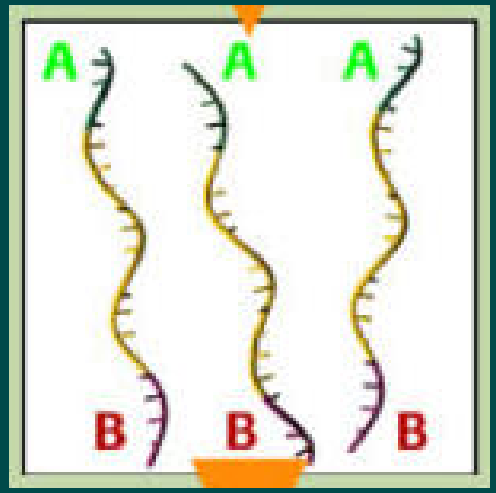

Gel:
G GCGAATGCGTCCACAACGCTACAGGTG
T GCGAATGCGTCCACAACGCTACAGGT
G GCGAATGCGTCCACAACGCTACAGG
G GCGAATGCGTCCACAACGCTACAG
A GCGAATGCGTCCACAACGCTACA
C GCGAATGCGTCCACAACGCTAC
A GCGAATGCGTCCACAACGCTA
T GCGAATGCGTCCACAACGCT
C GCGAATGCGTCCACAACGC
G GCGAATGCGTCCACAACG
C GCGAATGCGTCCACAAC
A GCGAATGCGTCCACAA
A GCGAATGCGTCCACA
C GCGAATGCGTCCAC
A GCGAATGCGTCCA
C GCGAATGCGTCC
C GCGAATGCGTC
T GCGAATGCGT
G GCGAATGCG
C GCGAATGC
G GCGAATG
T GCGAAT

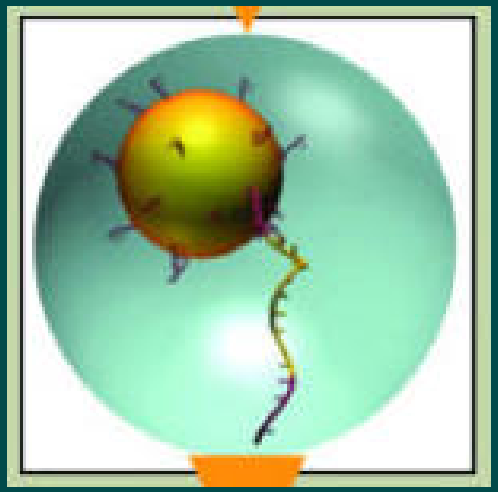
Sanger



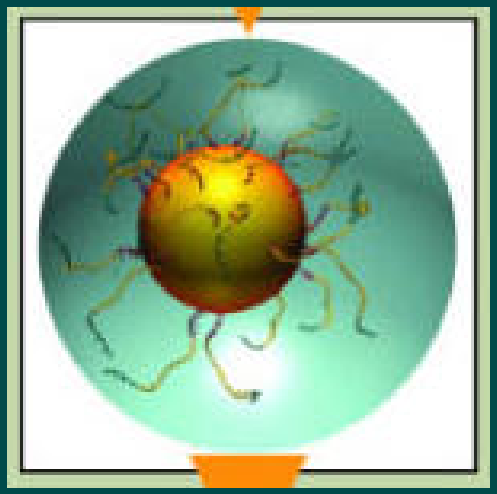
454 sequencing (pyrosequencing)

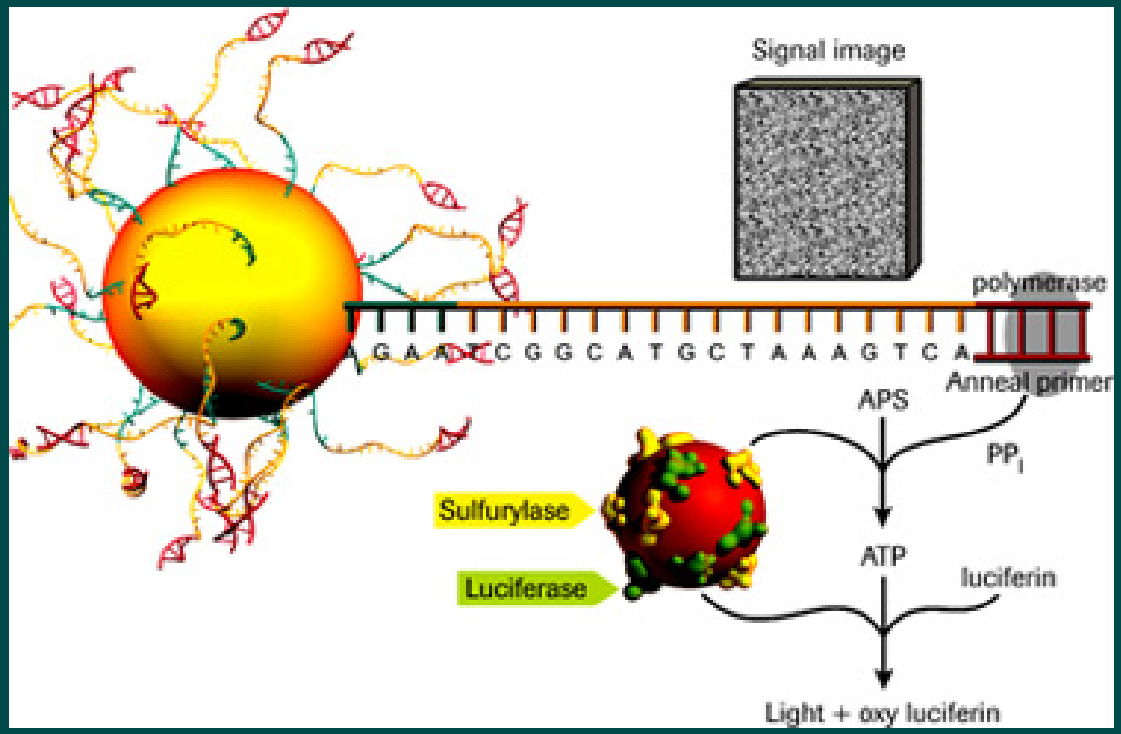




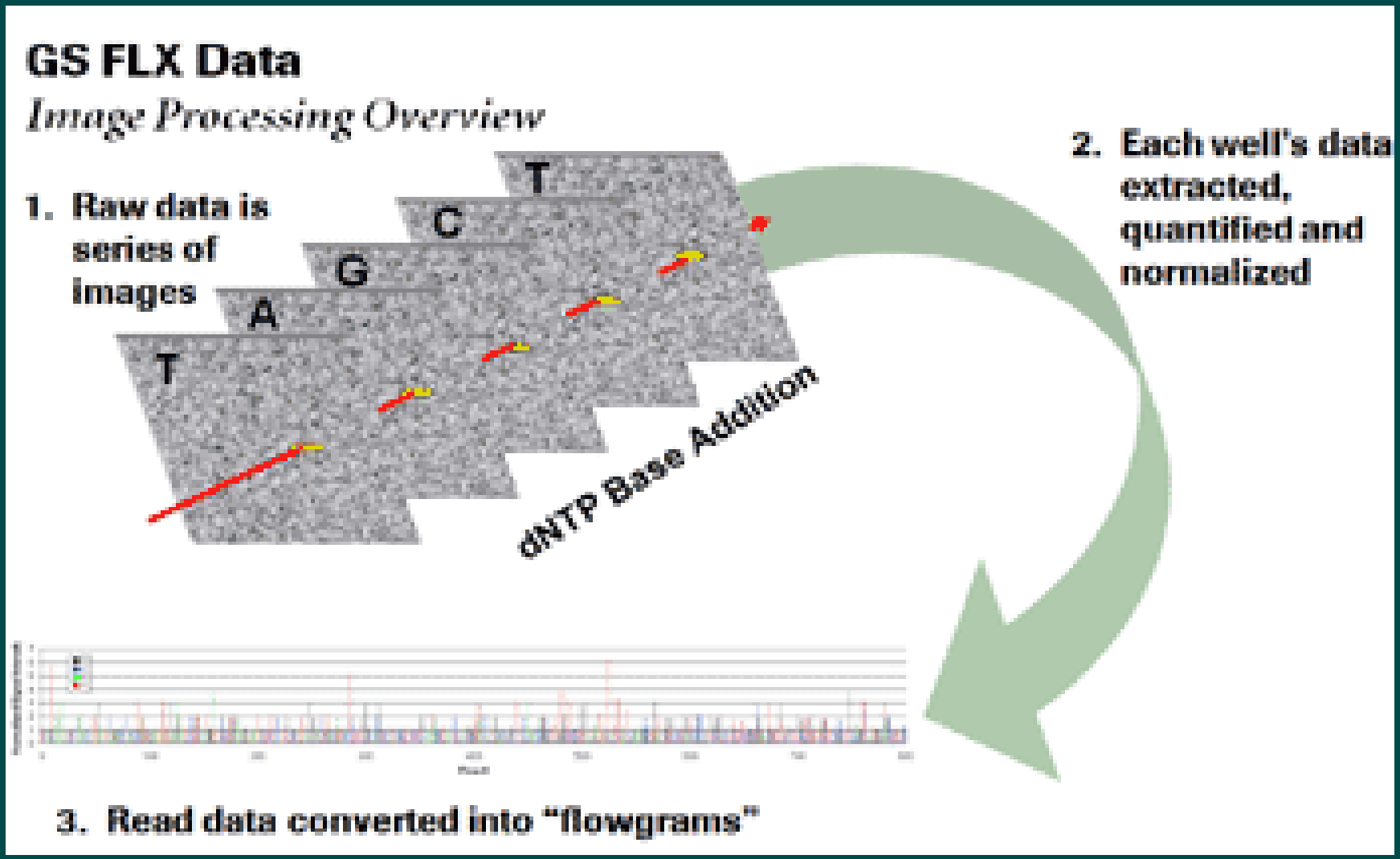


454 sequencing (pyrosequencing)

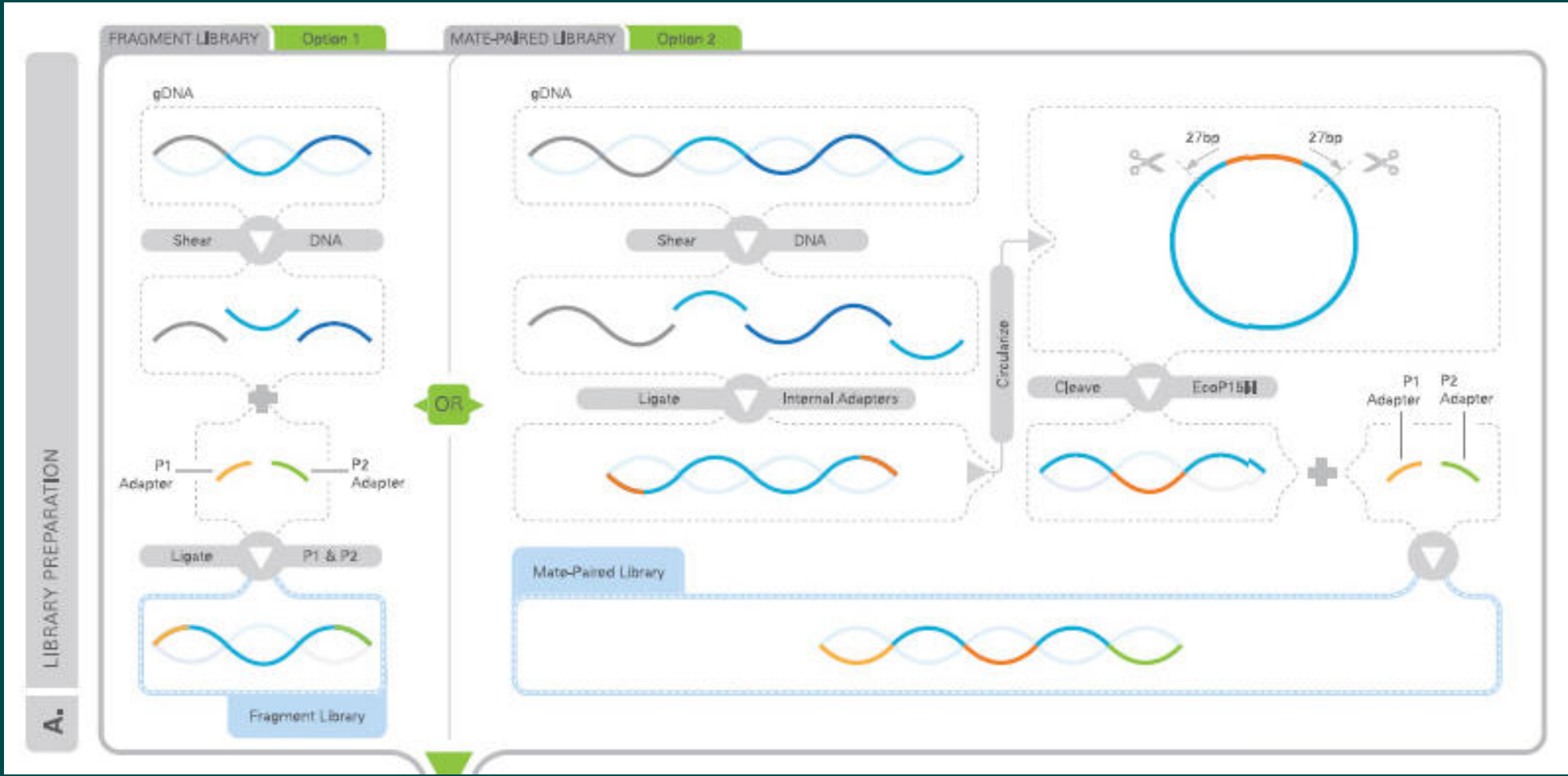




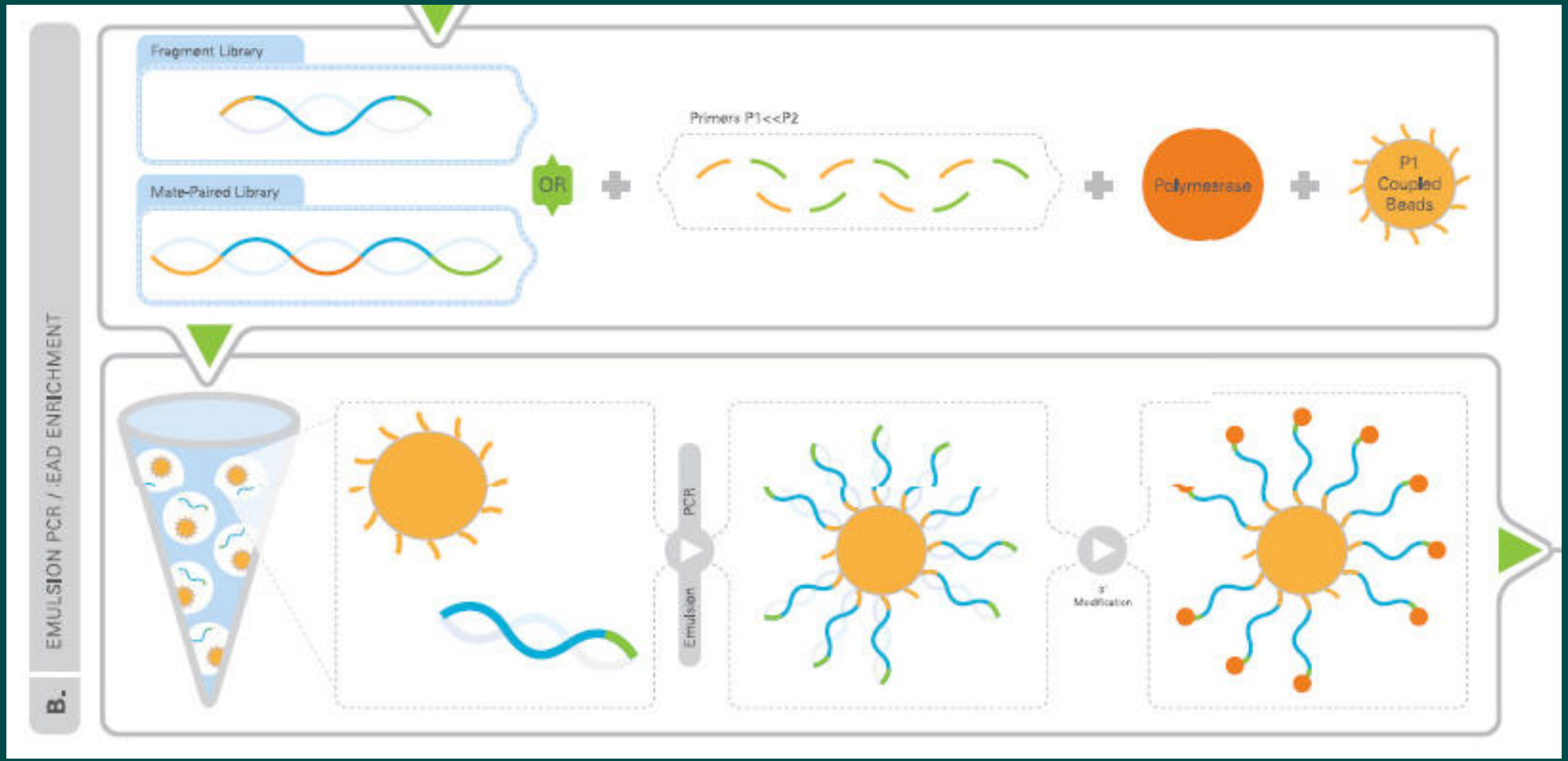
454 sequencing (pyrosequencing)



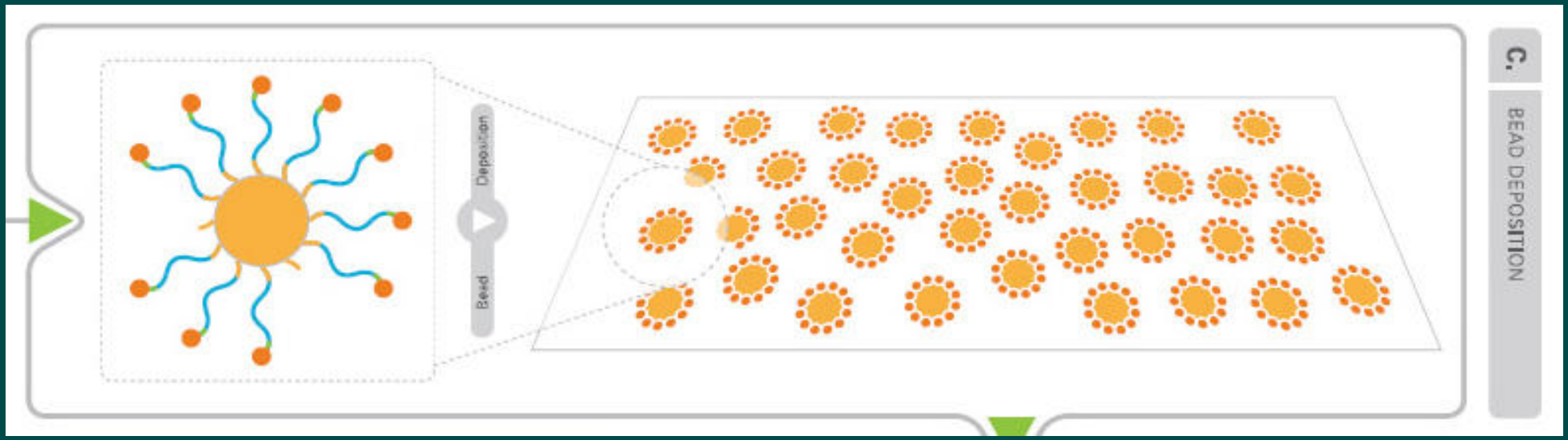
SOLiD (seq by ligation)



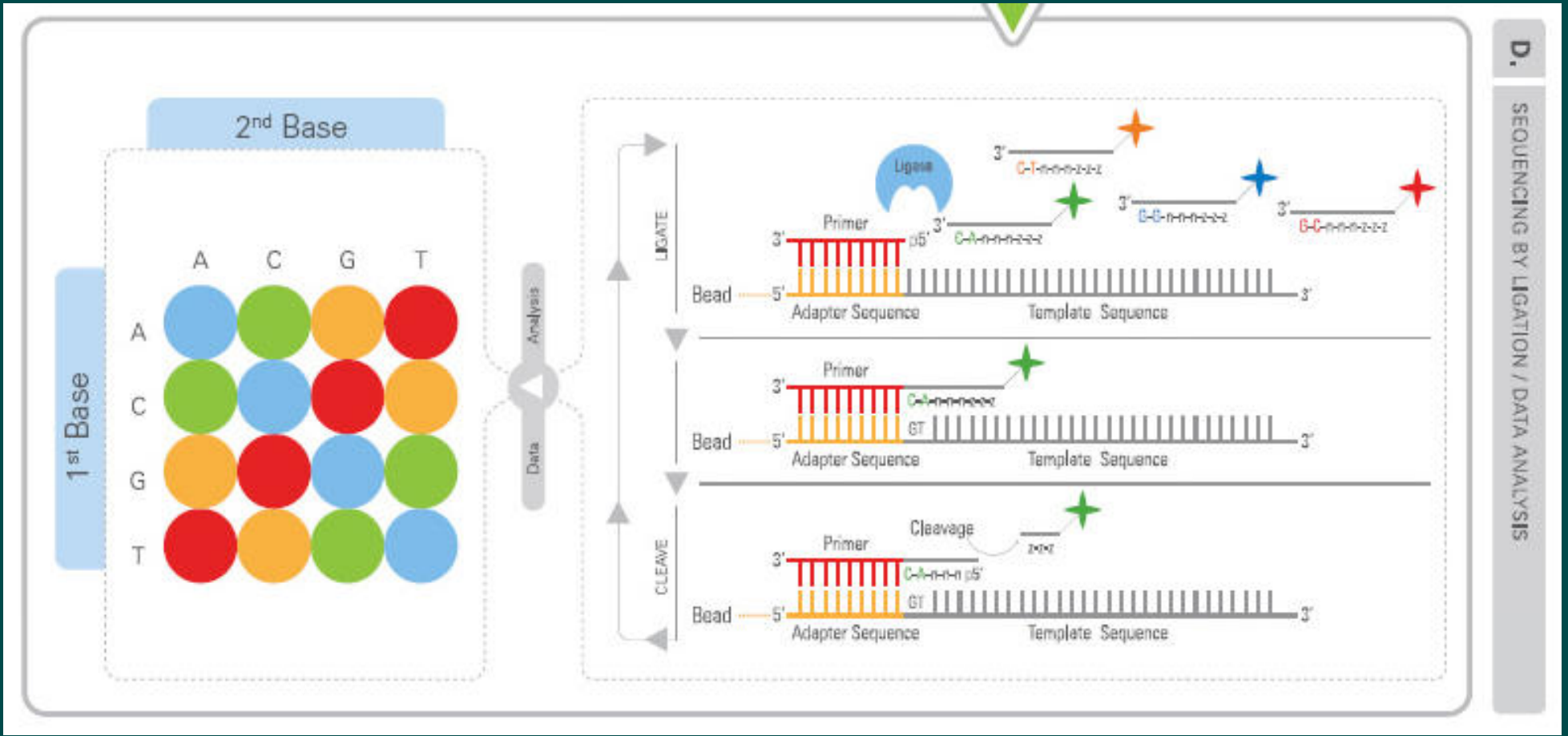
SOLiD (seq by ligation)



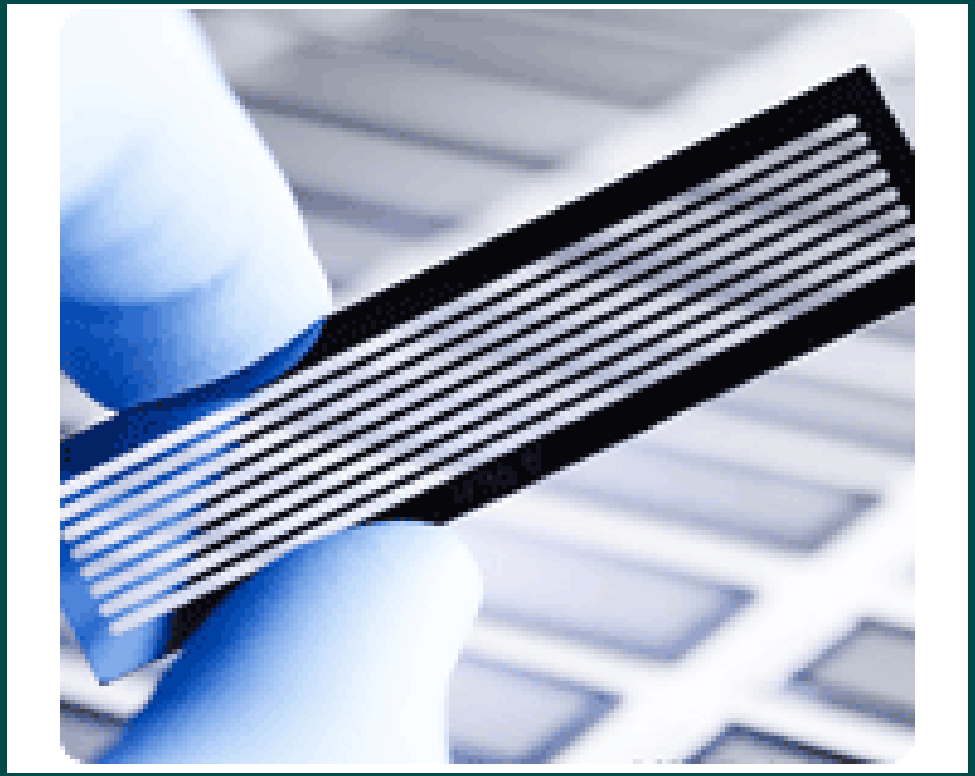
SOLiD (seq by ligation)



SOLiD (seq by ligation)

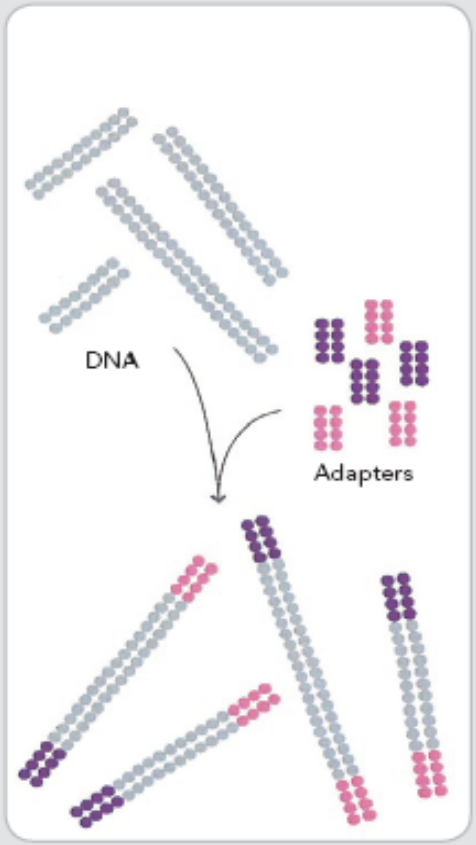


Illumina



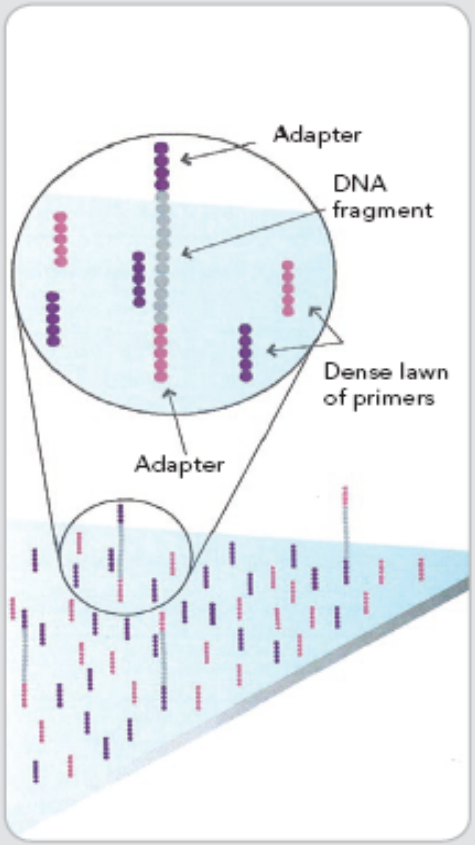
Illumina

1. PREPARE GENOMIC DNA SAMPLE



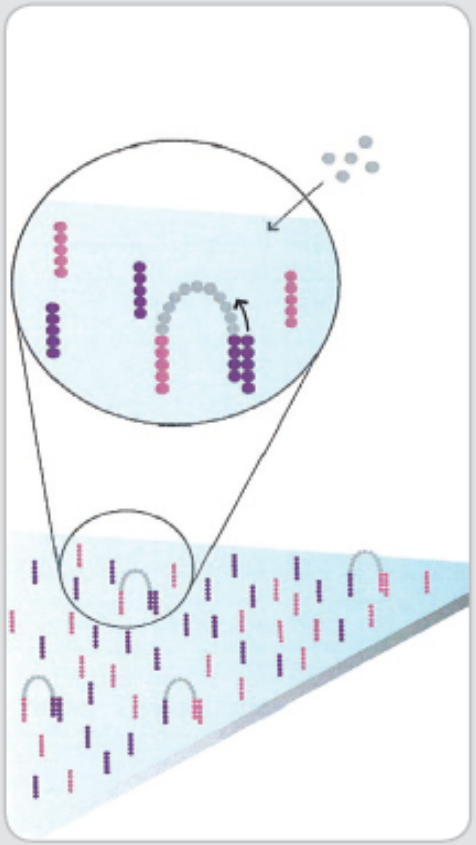
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

2. ATTACH DNA TO SURFACE



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

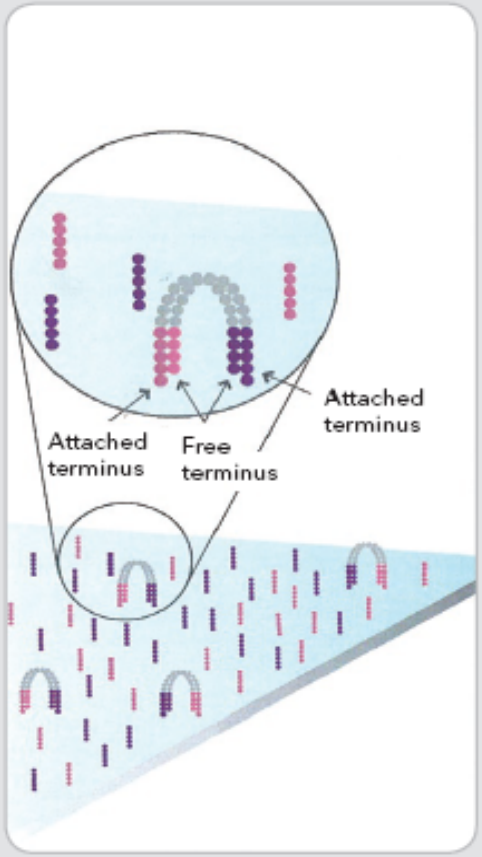
3. BRIDGE AMPLIFICATION



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

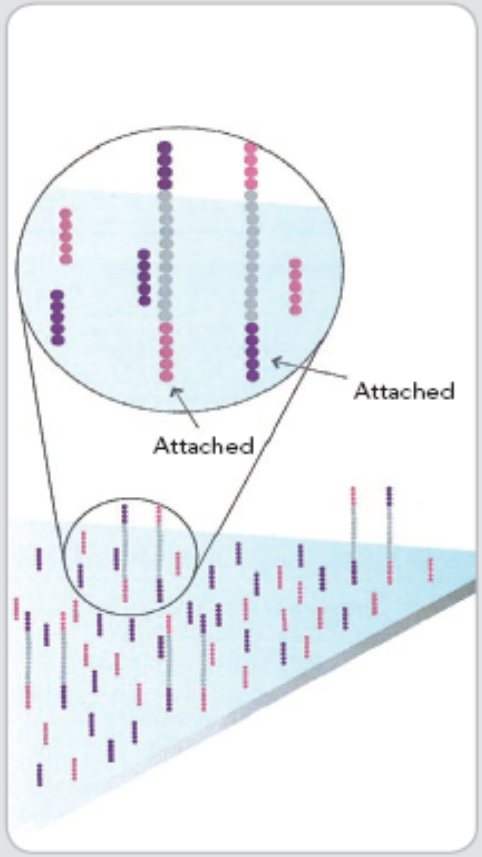
Illumina

4. FRAGMENTS BECOME DOUBLE-STRANDED



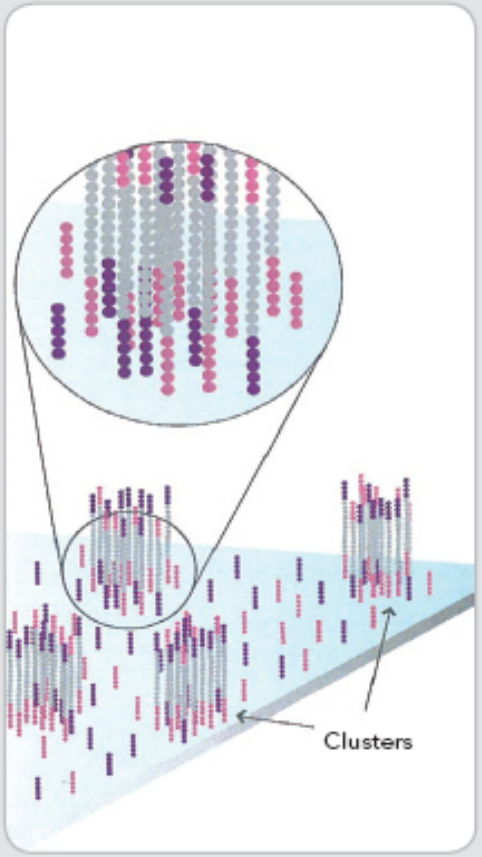
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



Denaturation leaves single-stranded templates anchored to the substrate.

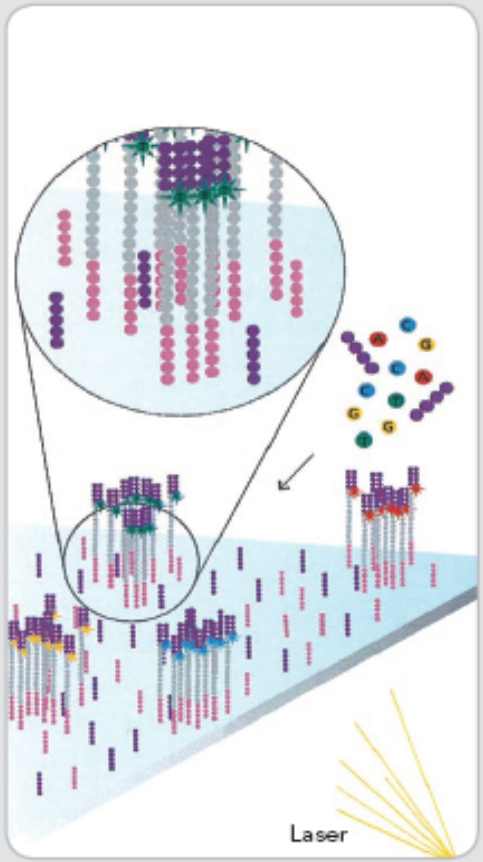
6. COMPLETE AMPLIFICATION



Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Illumina

7. DETERMINE FIRST BASE

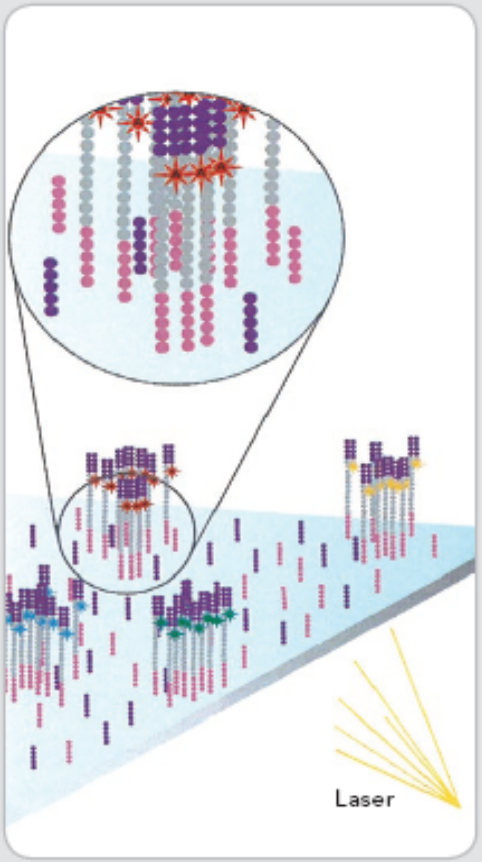


The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

8. IMAGE FIRST BASE

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

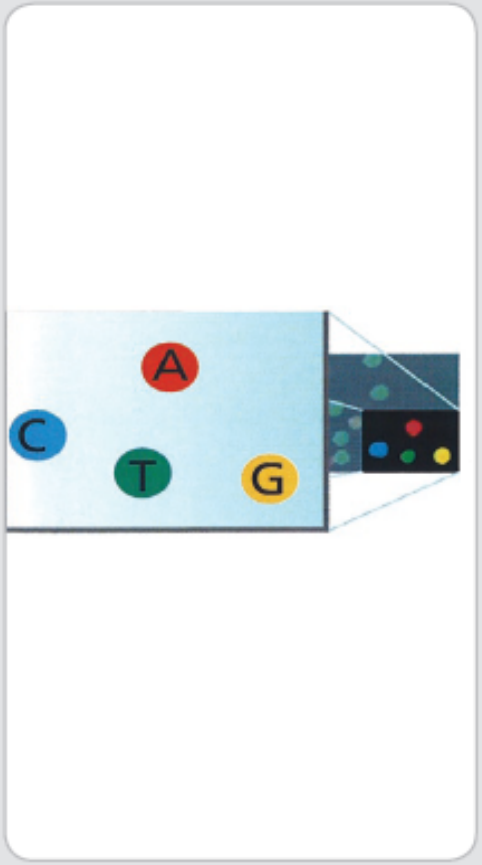
9. DETERMINE SECOND BASE



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

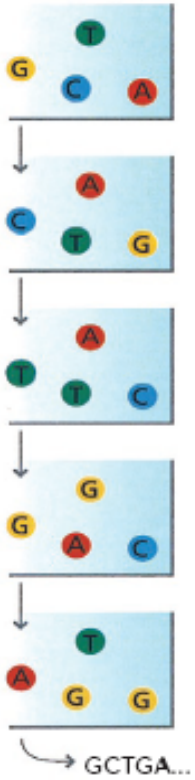
Illumina

10. IMAGE SECOND CHEMISTRY CYCLE



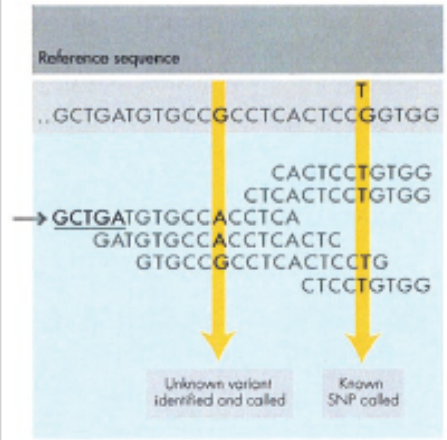
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

11. SEQUENCING OVER MULTIPLE CHEMISTRY CYCLES



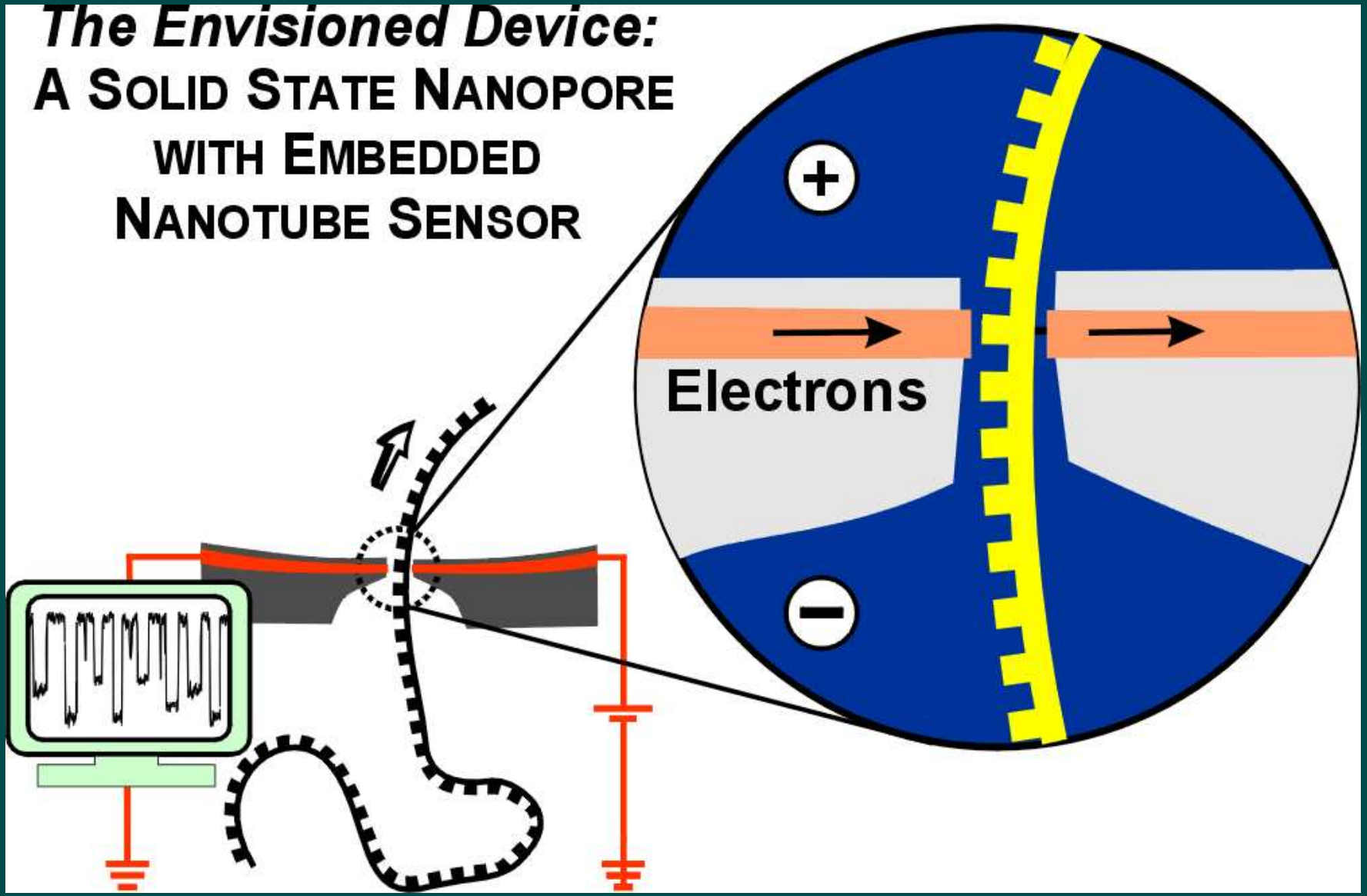
The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

12. ALIGN DATA

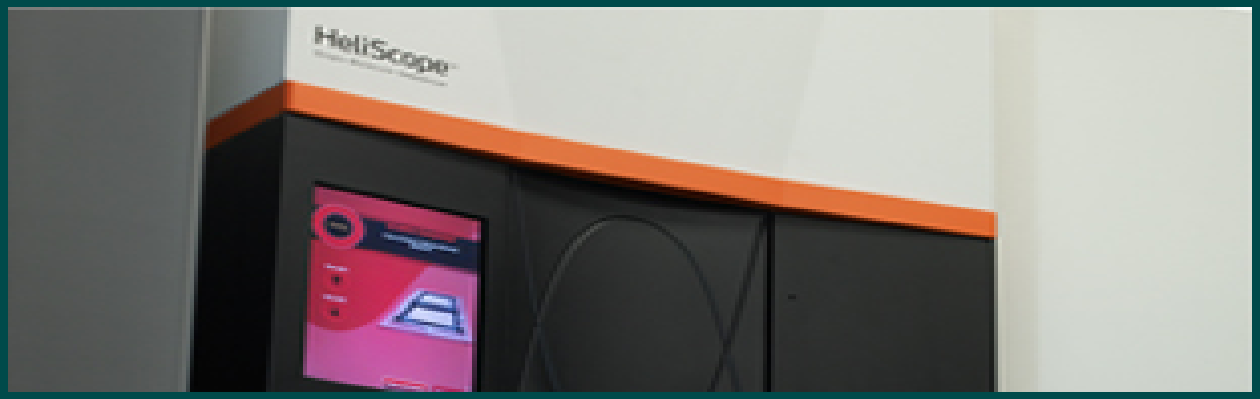


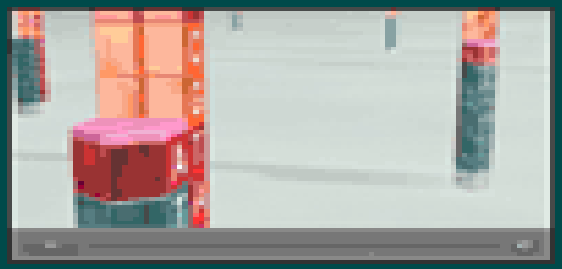
The data are aligned and compared to a reference, and sequencing differences are identified.

Sekvenování elektropórem



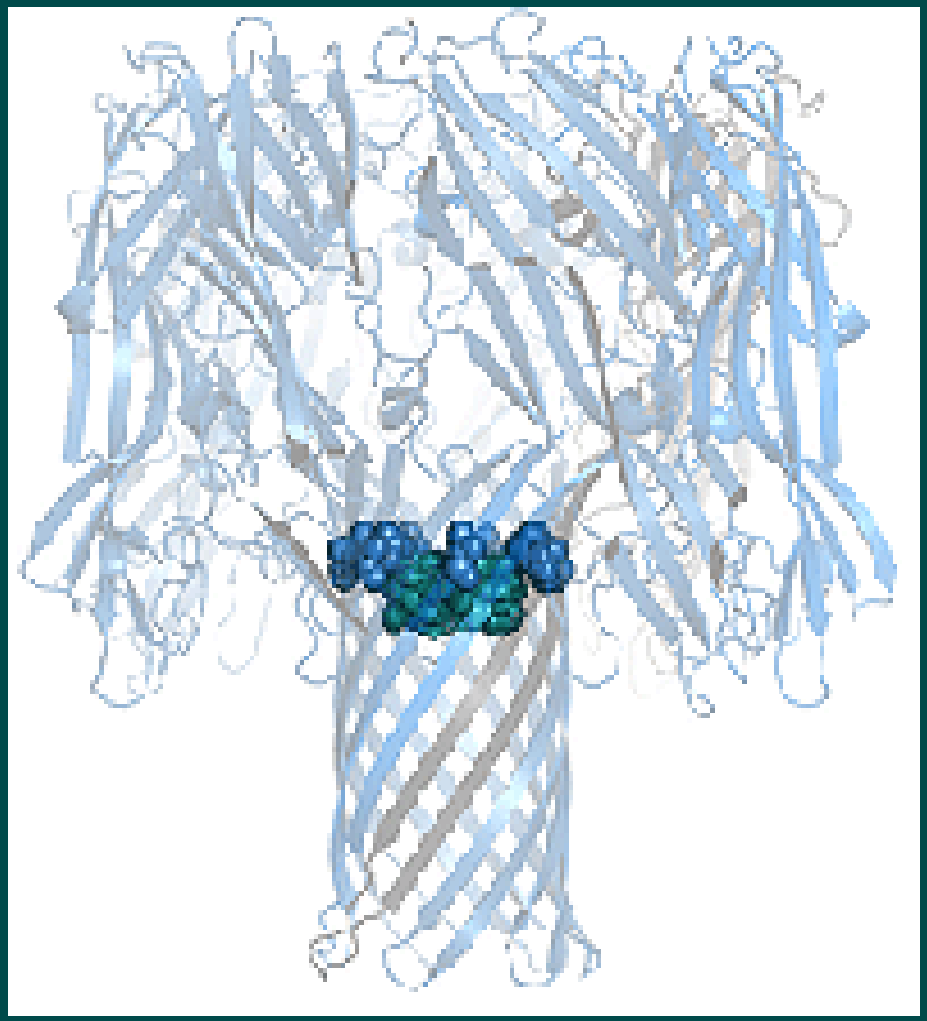
tSMS – single molecule sequencing





- 10^9 bp / day
- >35bp readlength

Oxford Nanopore Technologies



Alpha haemolysin nanopore showing cyclodextrin adapter molecule (the DNA binding site).