

Course 2 How do these  
magic sequencers work?



## First generation



Sanger sequencing  
Maxam and Gilbert  
Sanger chain termination

Infer nucleotide identity using dNTPs,  
then visualize with electrophoresis

500–1,000 bp fragments

## Second generation (next generation sequencing)



+ BGI Nanoball sequencer  
454, Solexa,  
Ion Torrent,  
Illumina

High throughput from the  
parallelization of sequencing reactions

~50–500 bp fragments

## Third generation



PacBio  
Oxford Nanopore

Sequence native DNA in real time  
with single-molecule resolution

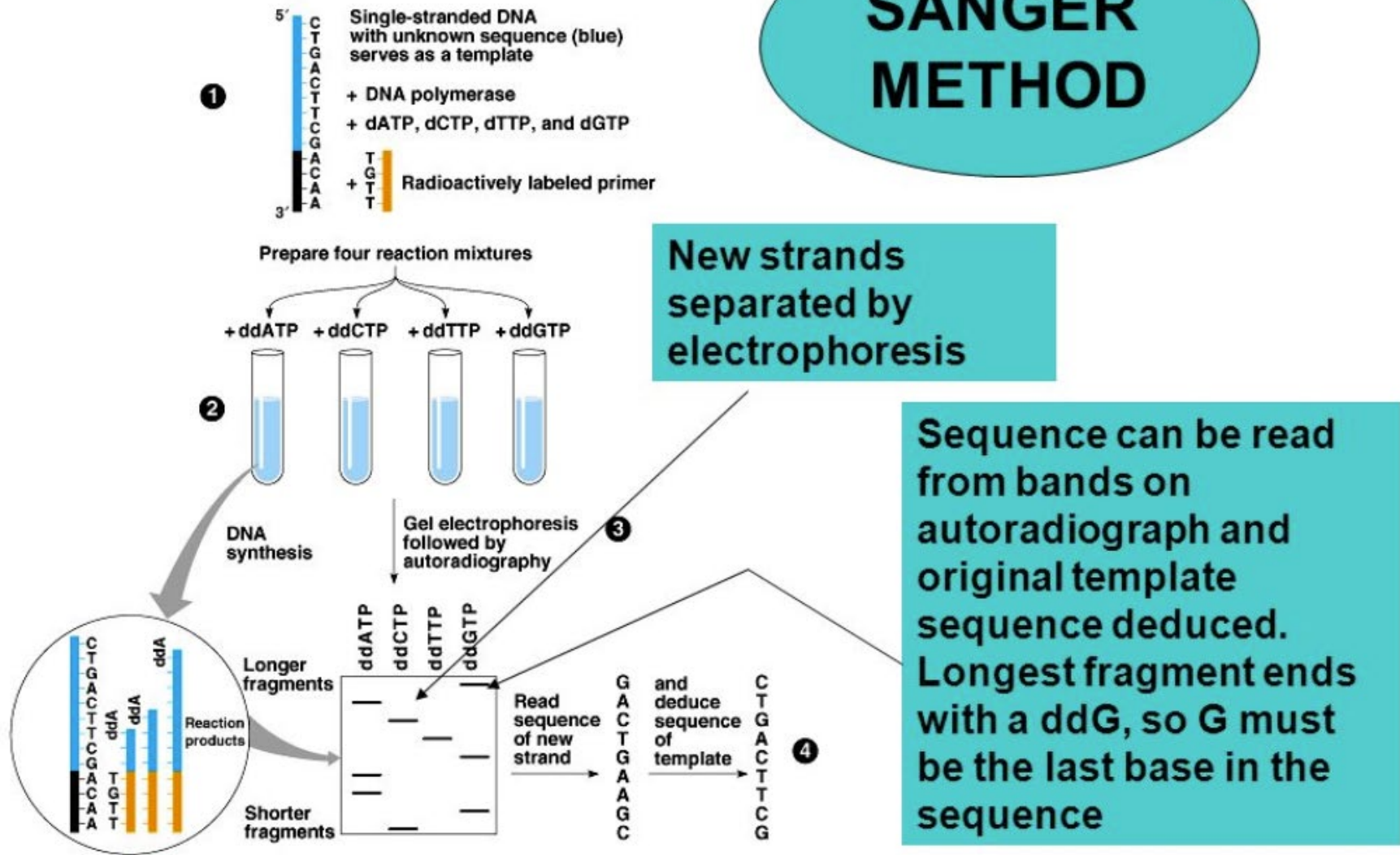
Tens of kb fragments, on average

Short-read sequencing

Long-read sequencing



# SANGER METHOD



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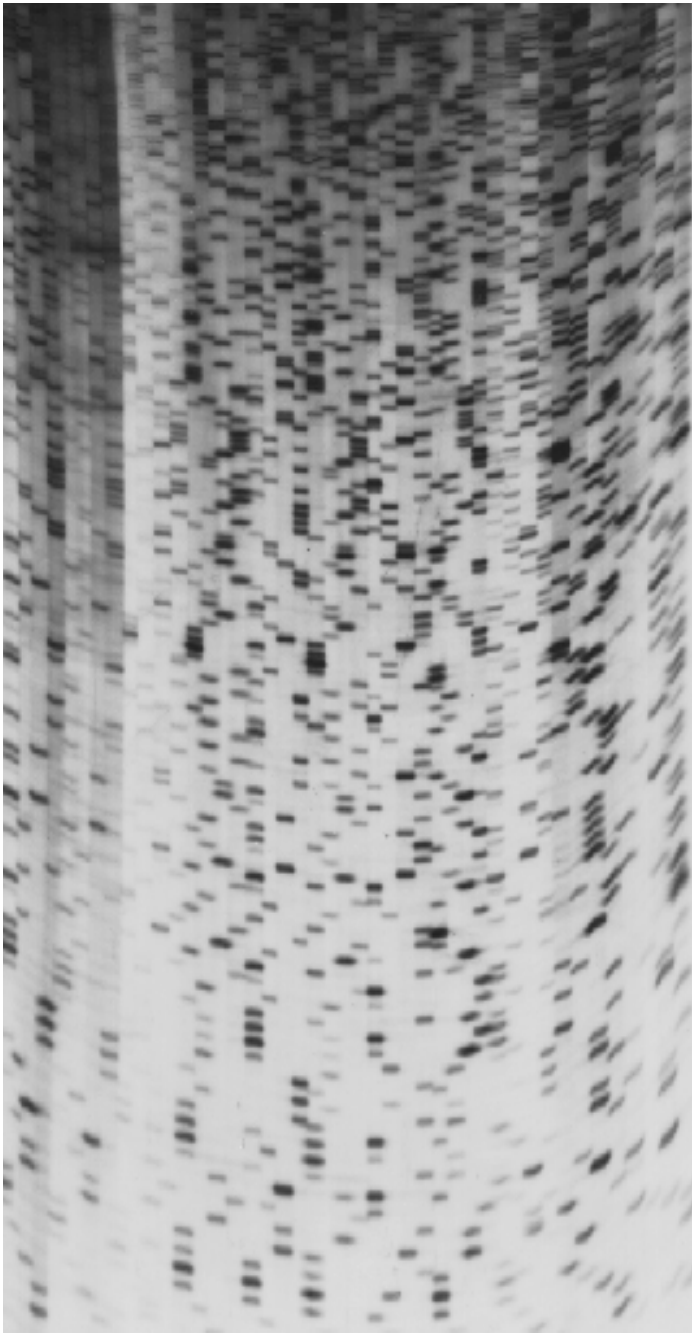
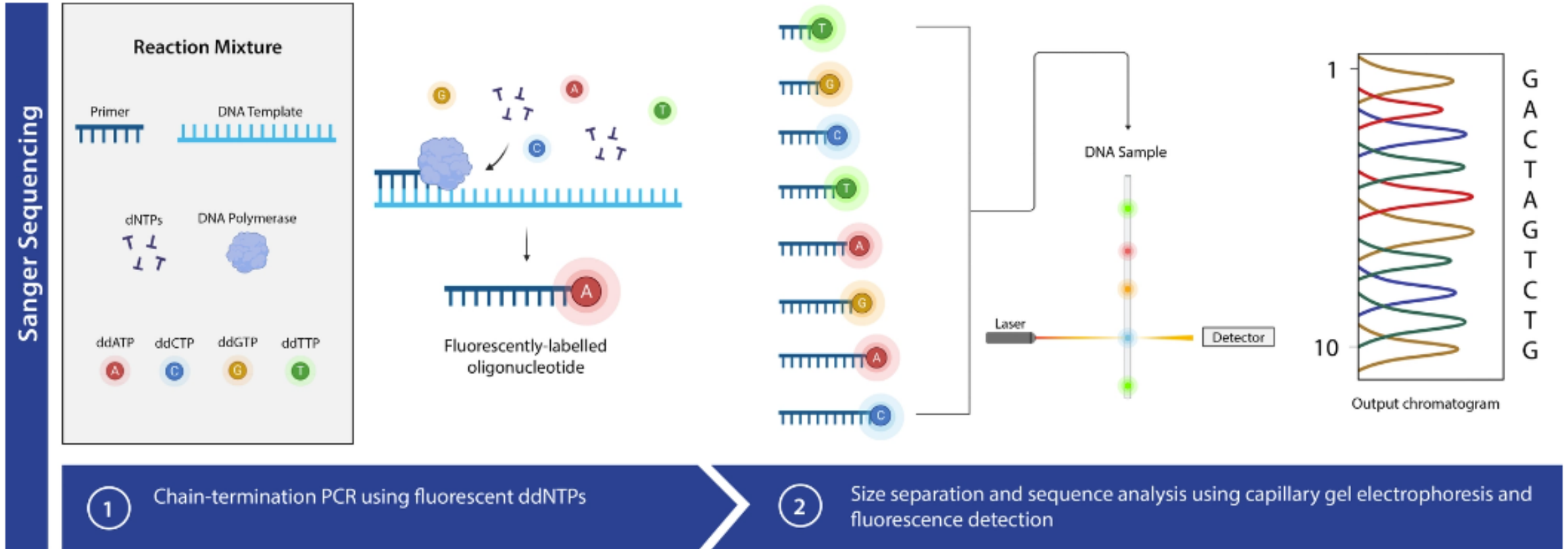


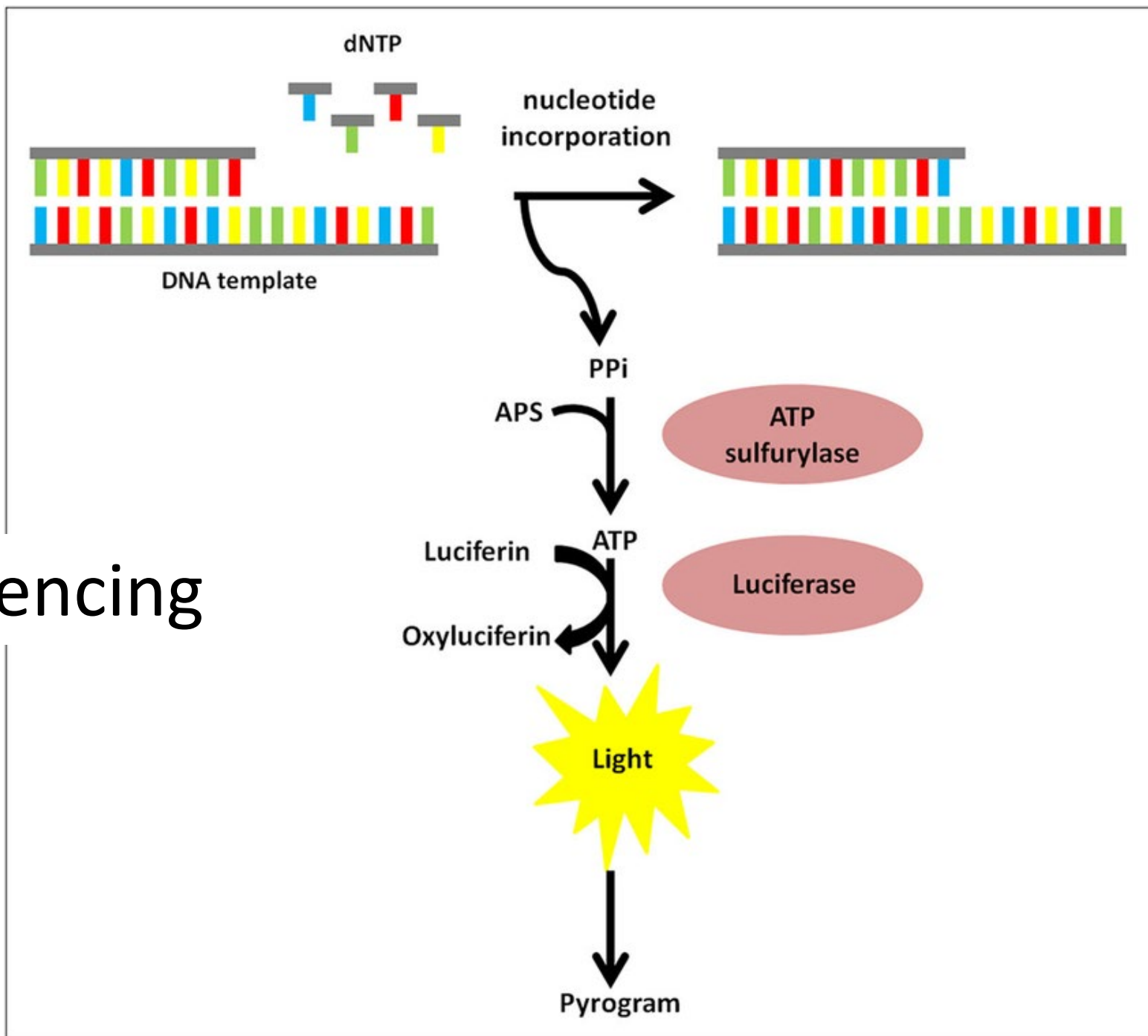
Figure: Diagrammatic representation of Sanger sequencing

# Capillary sequencers

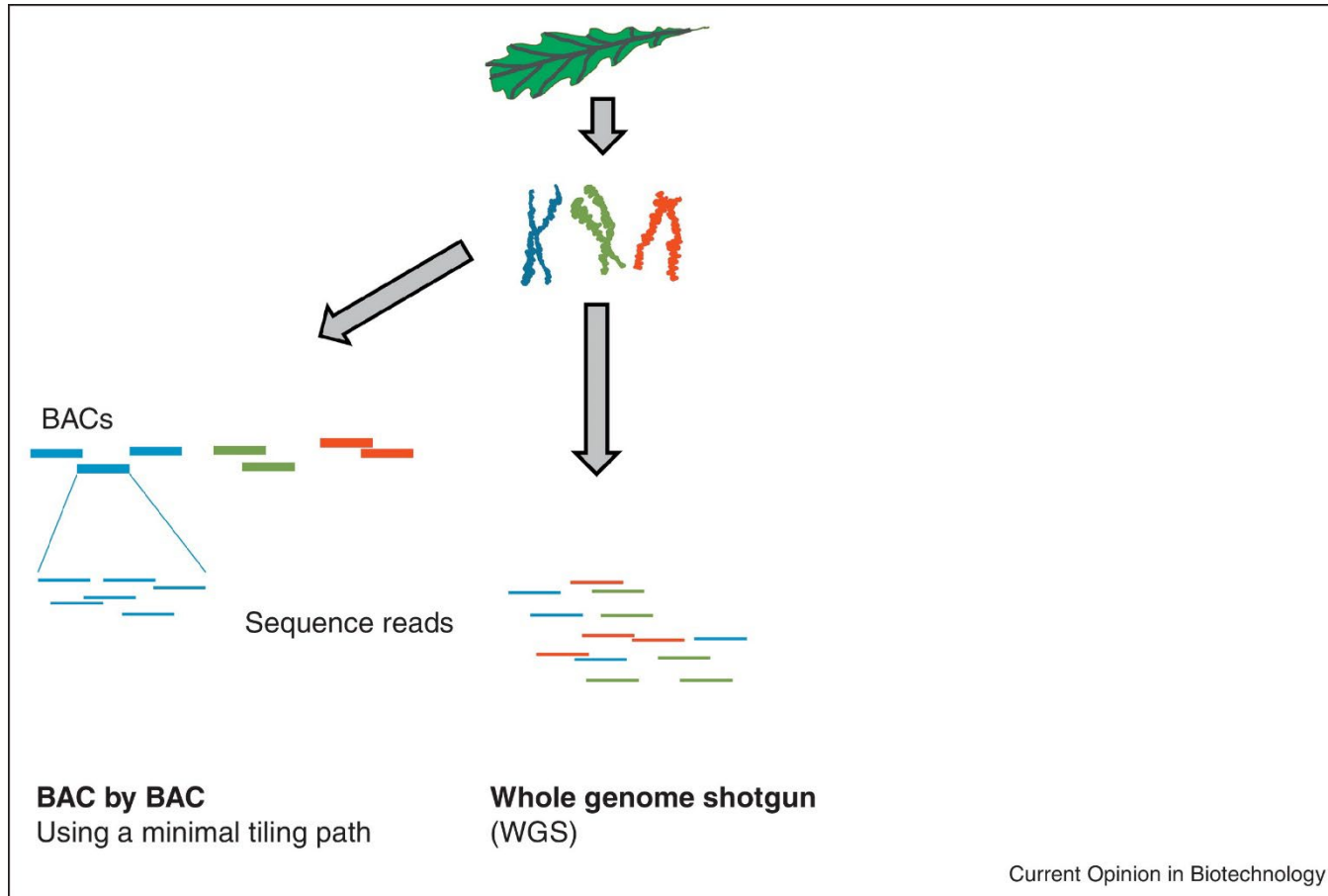


An illustration of Sanger sequencing by capillary electrophoresis (figure made in BioRender).

# Pyrosequencing



# The „shotgun“ idea



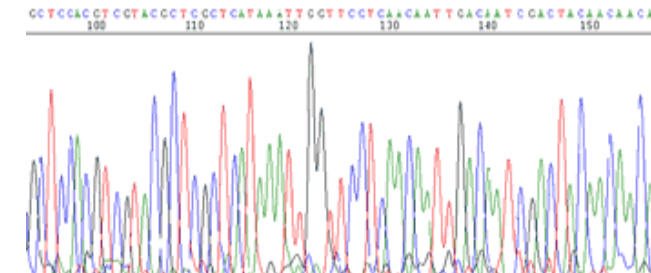
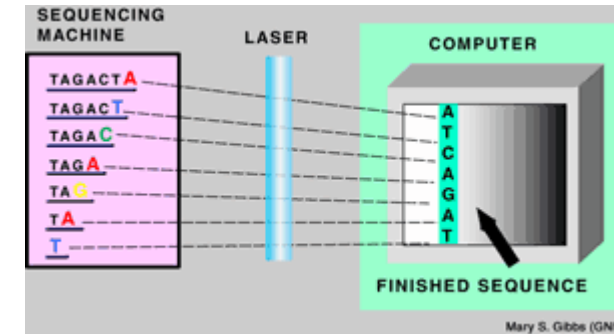
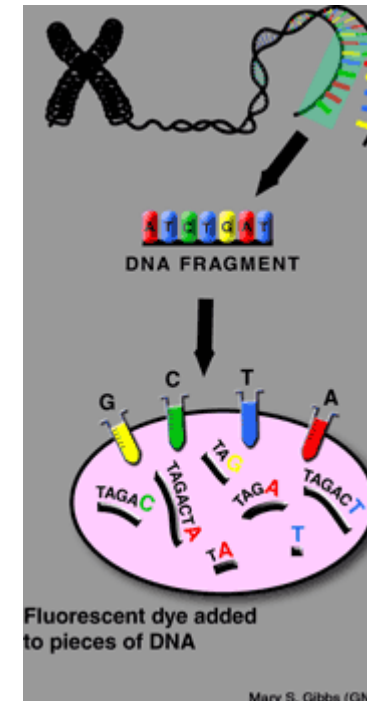
Instead of sequencing BAC libraries that maintain the genome structure, sequence **many** random fragments and reconstruct structure by assembly of aligning fragments

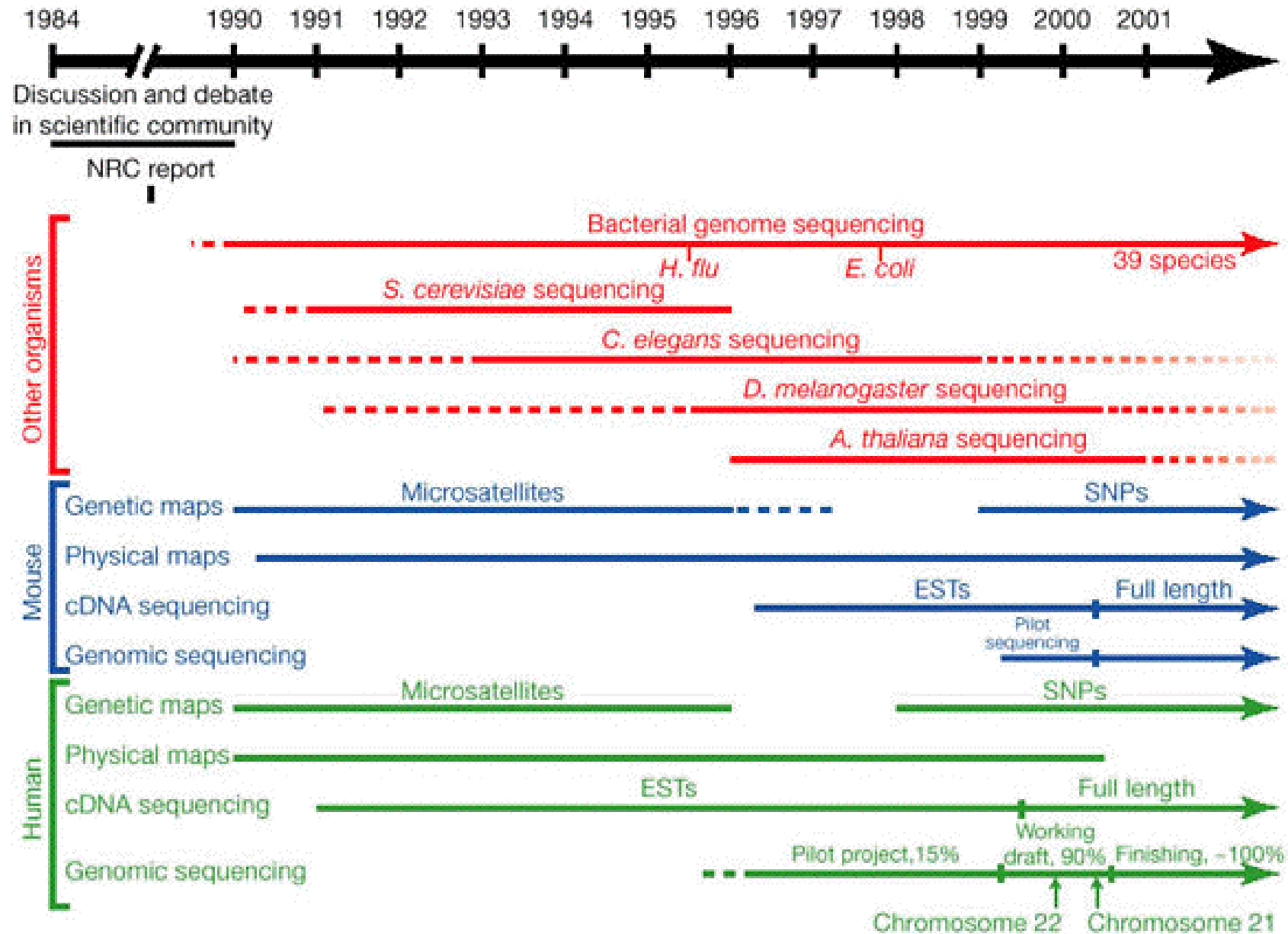


# Parallel sequencing – more, faster, cheaper

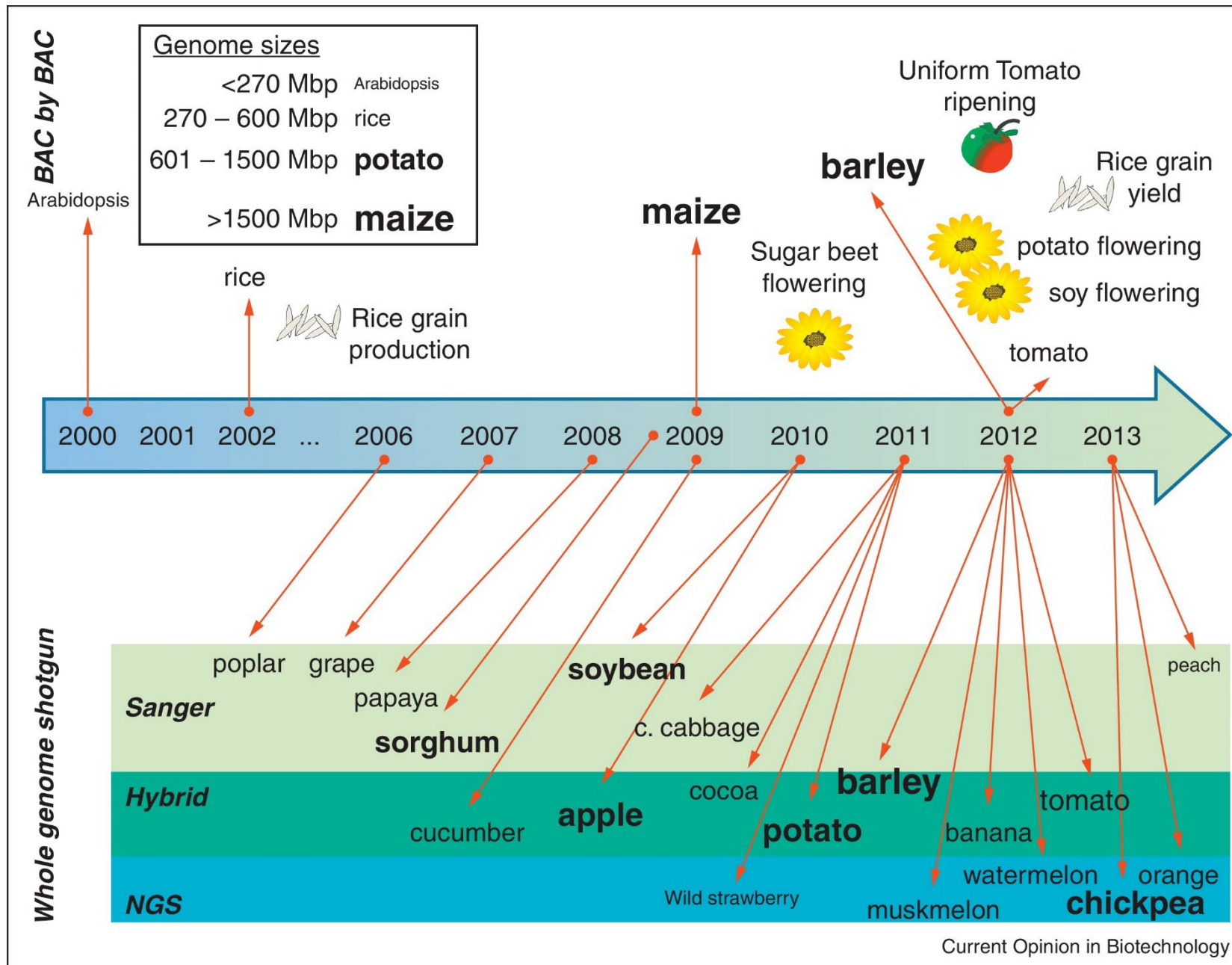


ABI sequencer machine park (from Wikipedia)

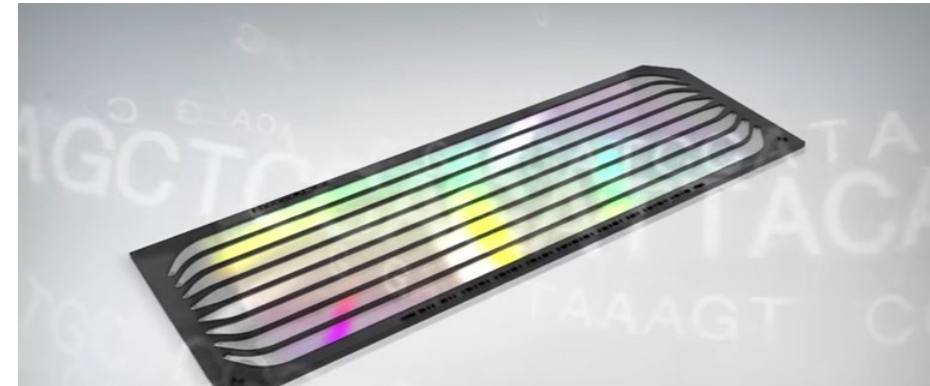
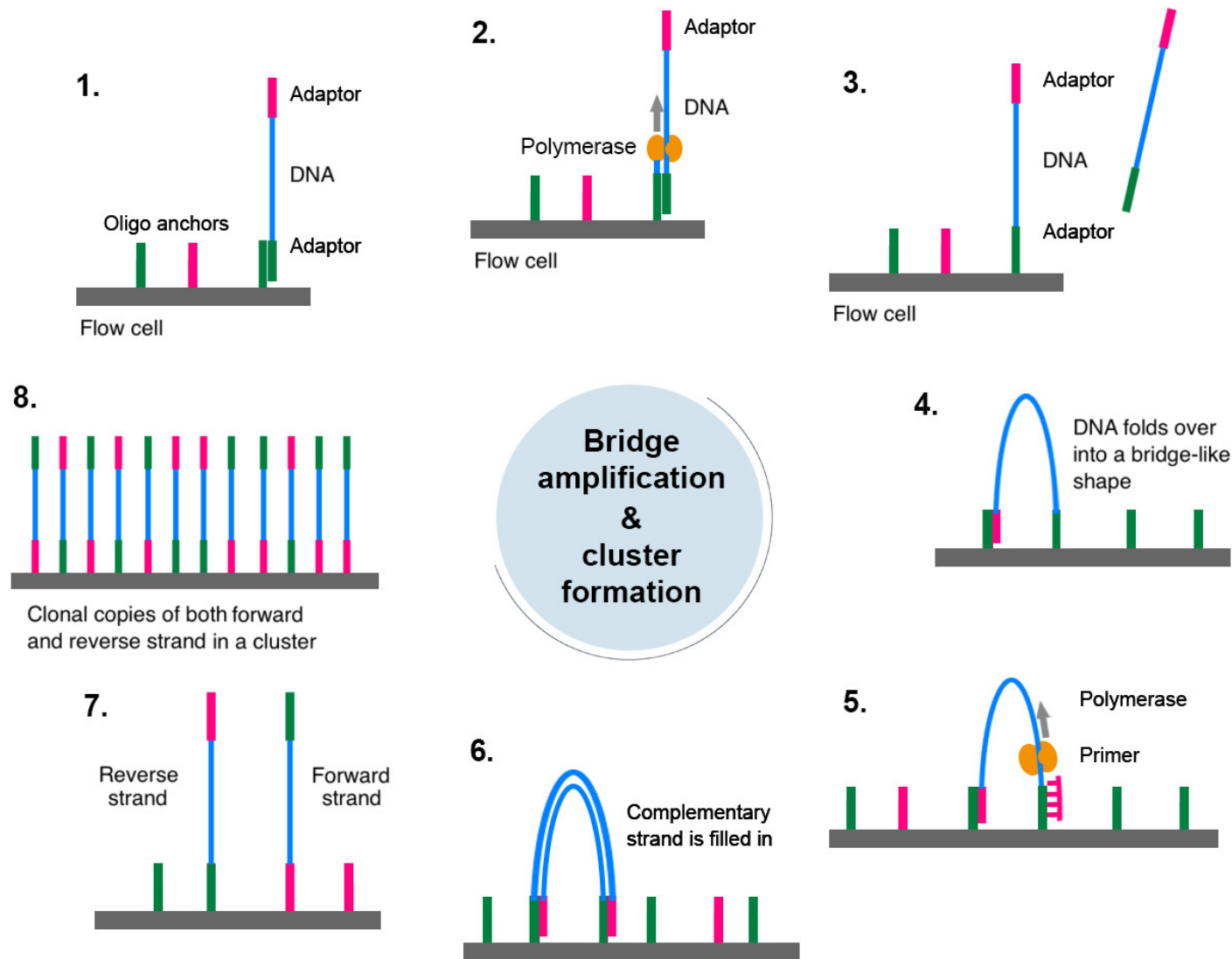








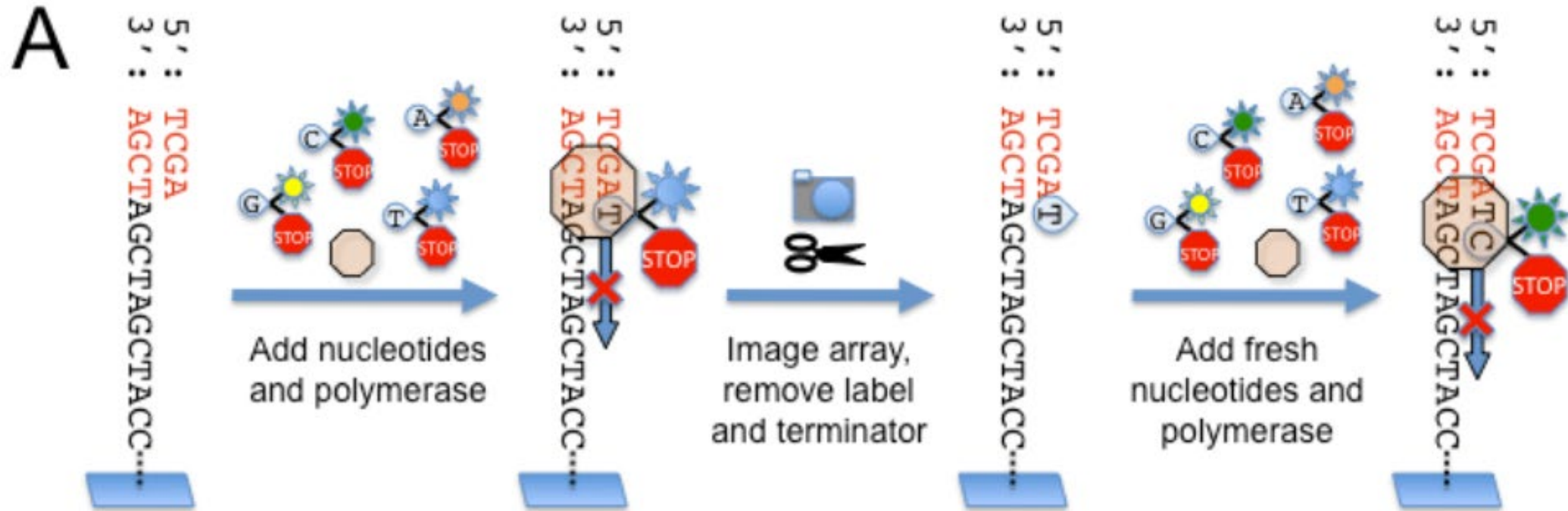
# Principle of Illumina-type sequencers



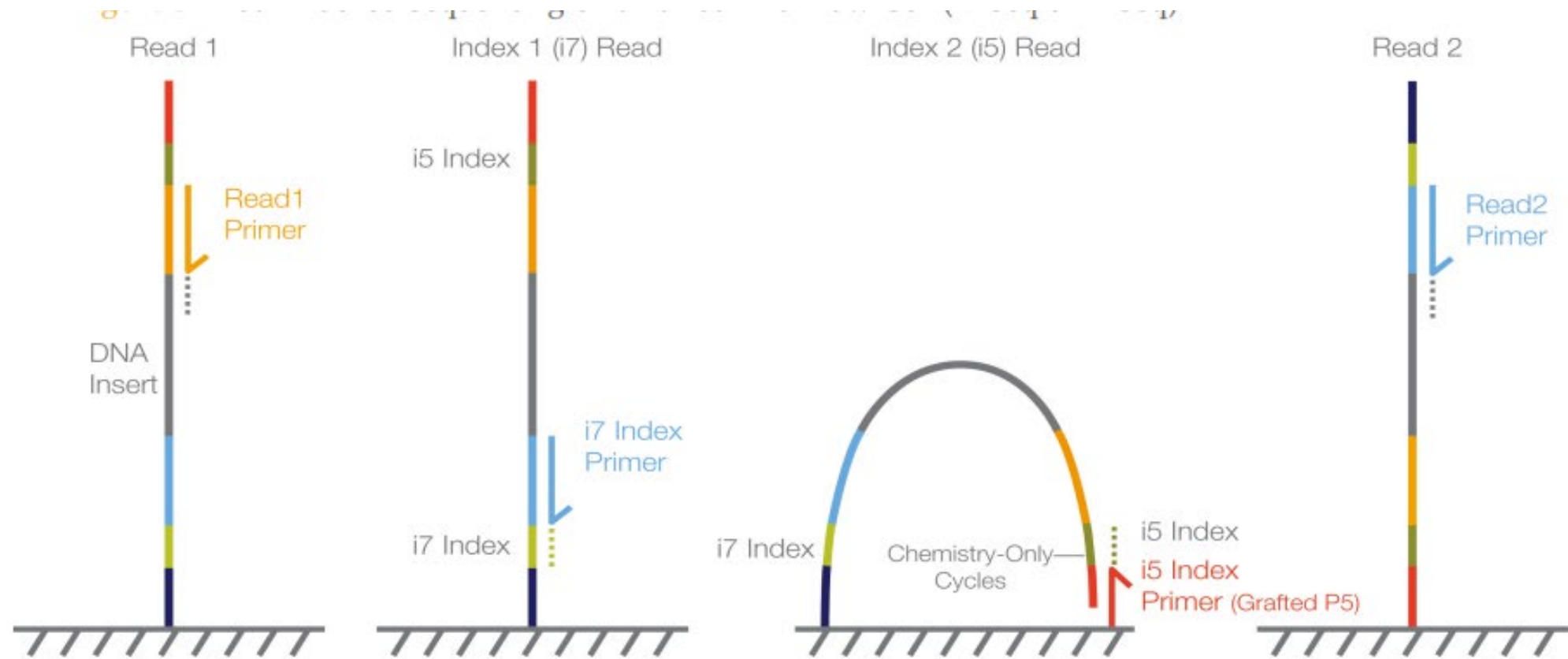
Flowcell: 1000-1500 cluster per mm<sup>2</sup>  
Currently ~  $5 \times 10^8$  clusters per flowcell

Newest: HiSeq3000 and Novaseq

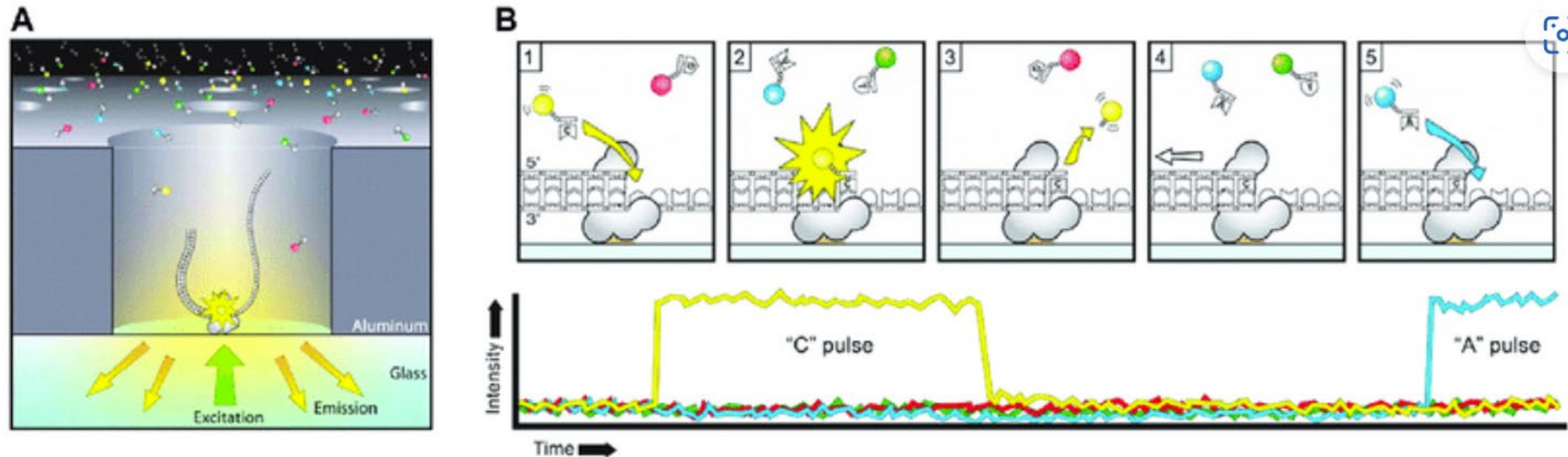
Sequencing of the Illumina cluster is like a miniature scale sanger sequencing



# Illumina primers and adaptors



# Principle of long-read sequencing by PacBio



Polymerase fixed in microwell

[https://youtu.be/\\_ID8JyAbwEo](https://youtu.be/_ID8JyAbwEo)

# Oxford Nanopore sequencing

<https://youtu.be/RcP85JHLmnl>

<https://youtu.be/e9wtAlvPPxY>



# Single cell sequencing and 1001 genomes

An overview of the single-cell RNA-sequencing procedures

