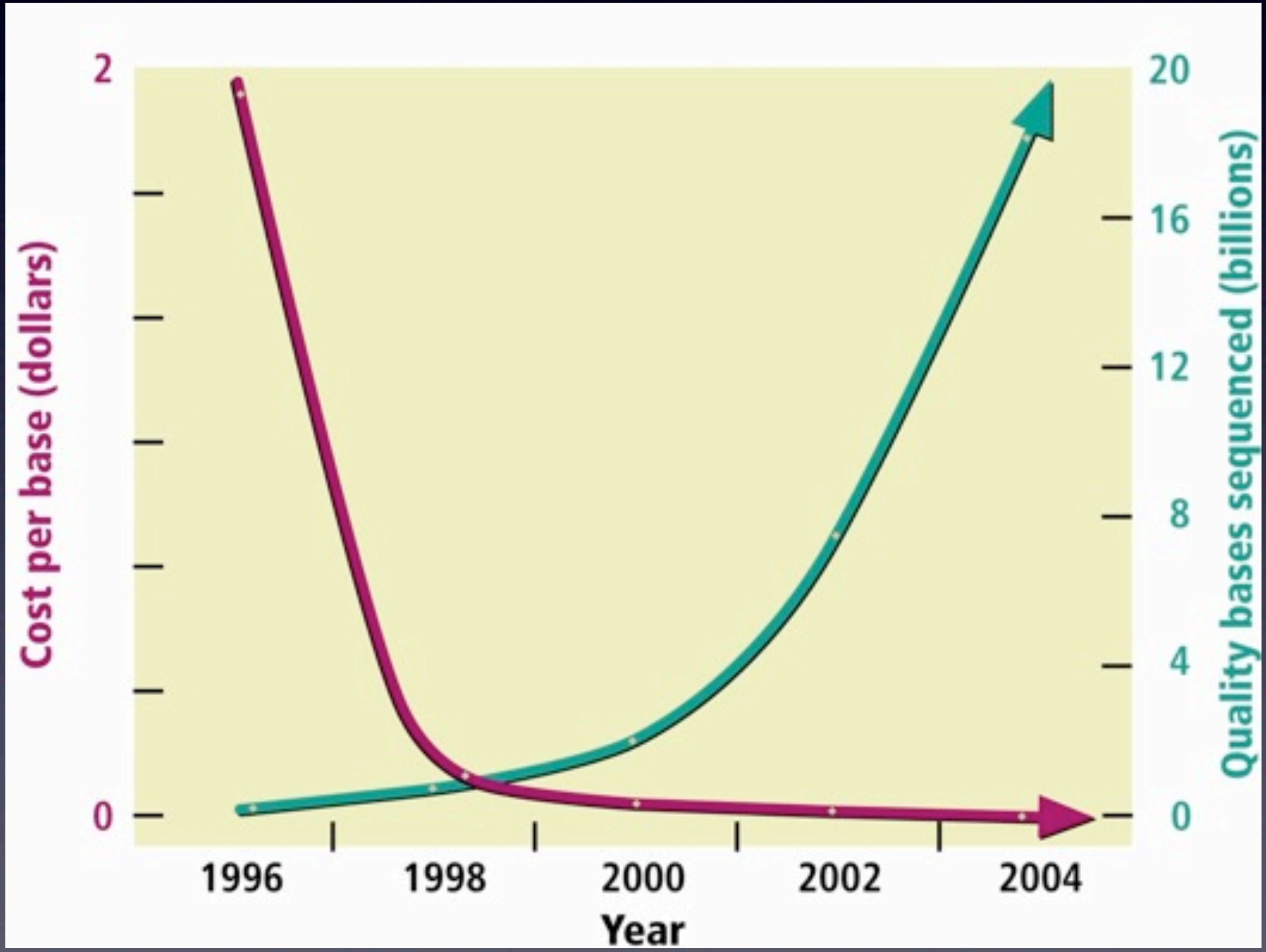
Sequencing Technology

MCDB 187

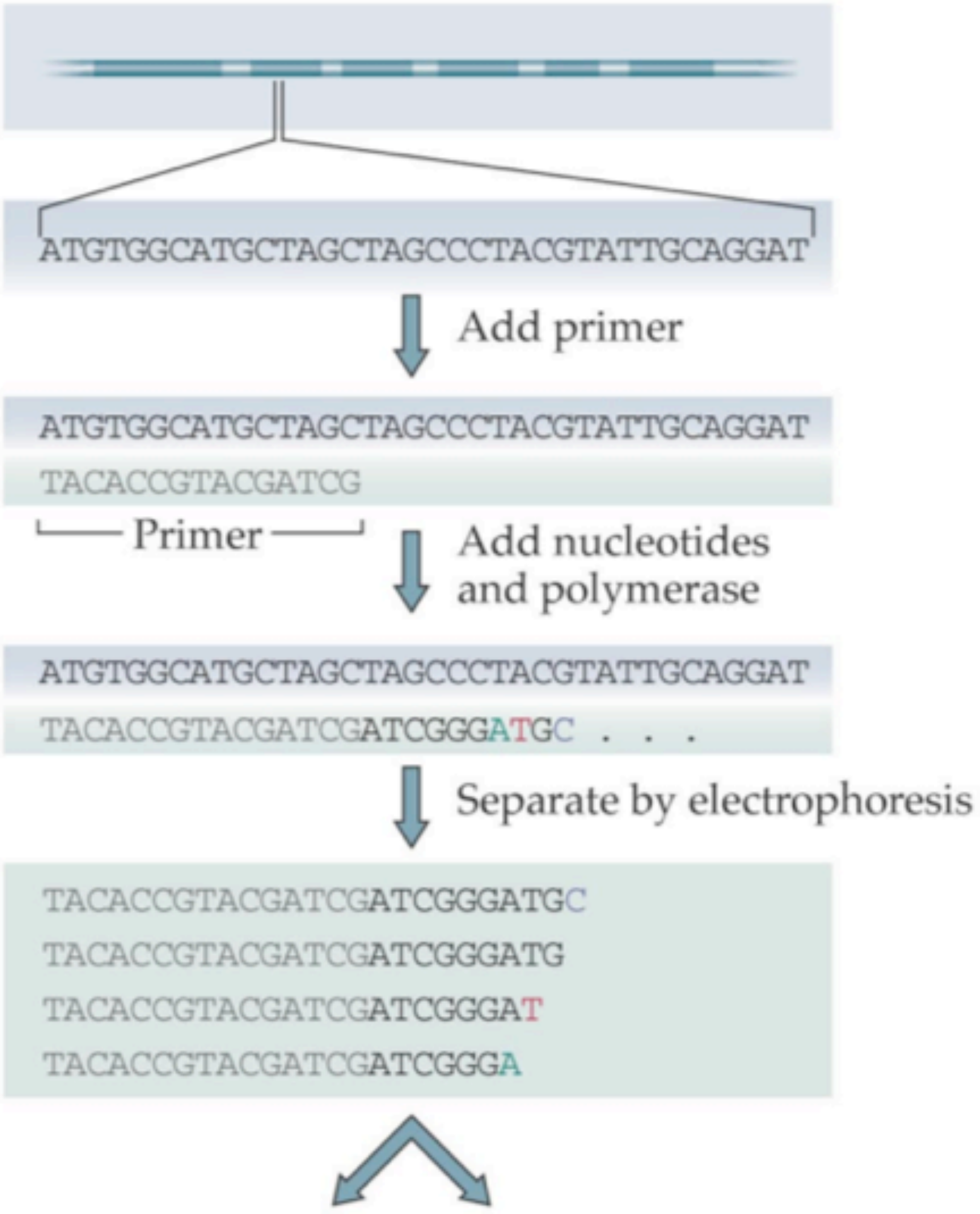
Matteo Pellegrini

Sequencing Technology

- Sanger Sequencers
  - Applied Biosystems
- New technologies:
  - 454, SOLiD, Solexa



Sanger sequencing



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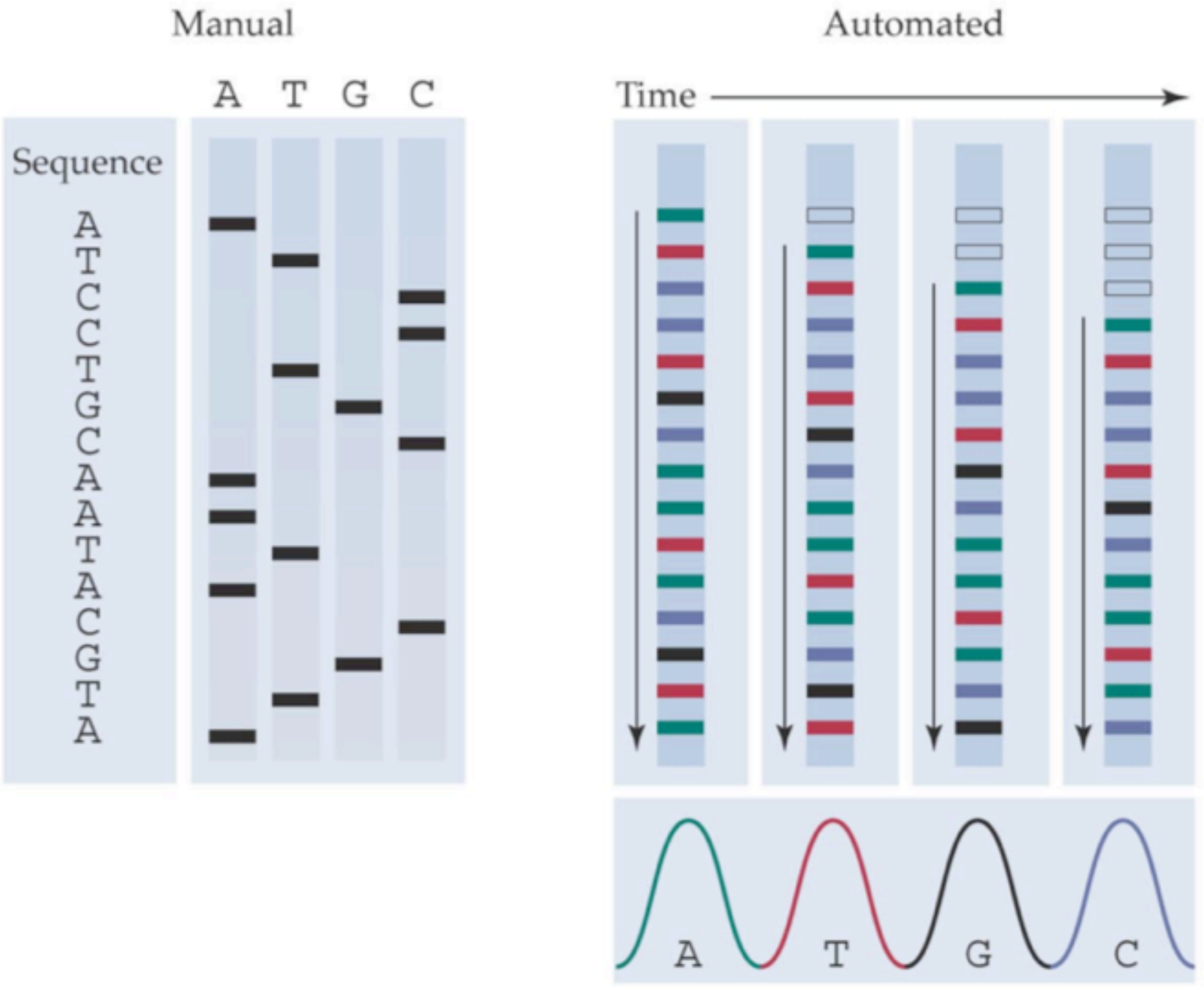
Sanger Sequencing



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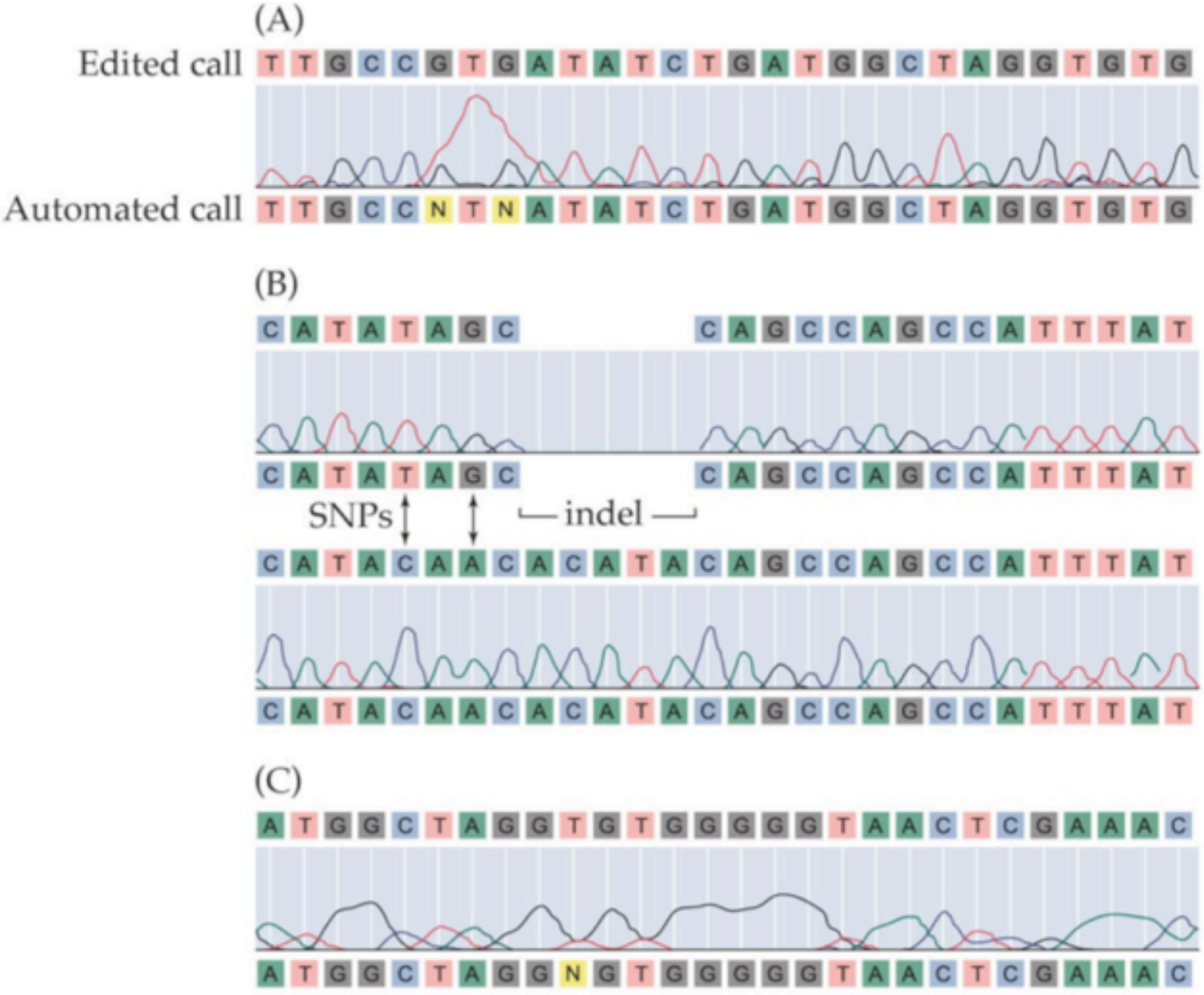
Sanger Sequencing



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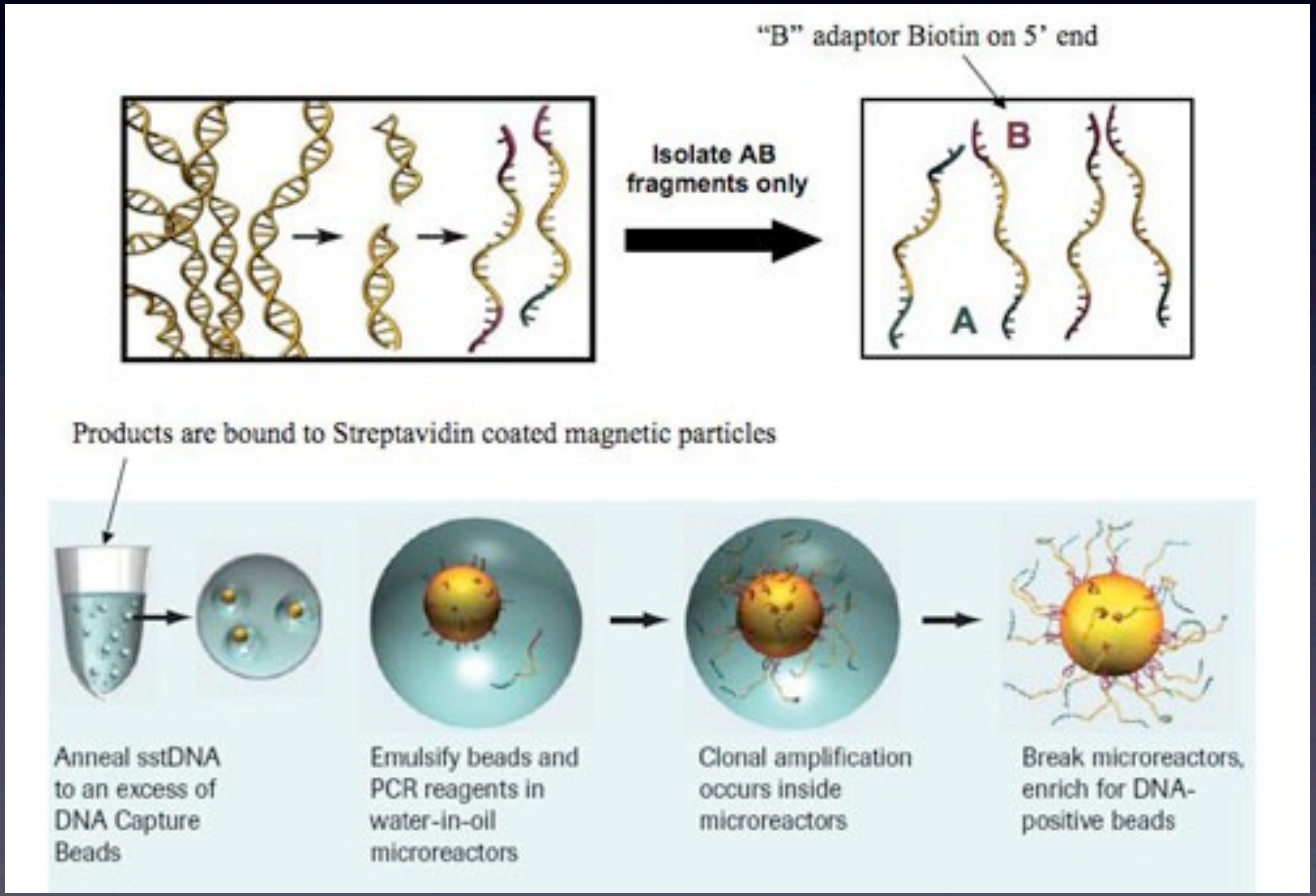
Sanger Sequencing Throughput

- 35kbases per day
- 1000 base read length

New Sequencing Technology: 454

- 200-400 base reads
- 200,000 reads per run
- 100 million bases per run
- 1 day



Solexa Sequencing–by–Synthesis

animation adapted from *www.illumina.com*

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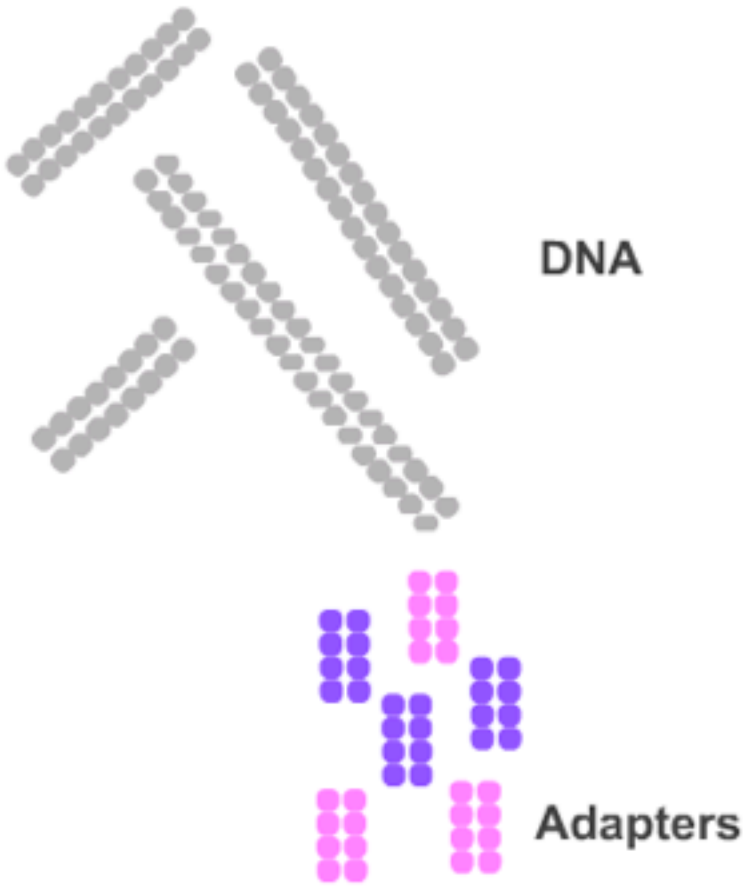

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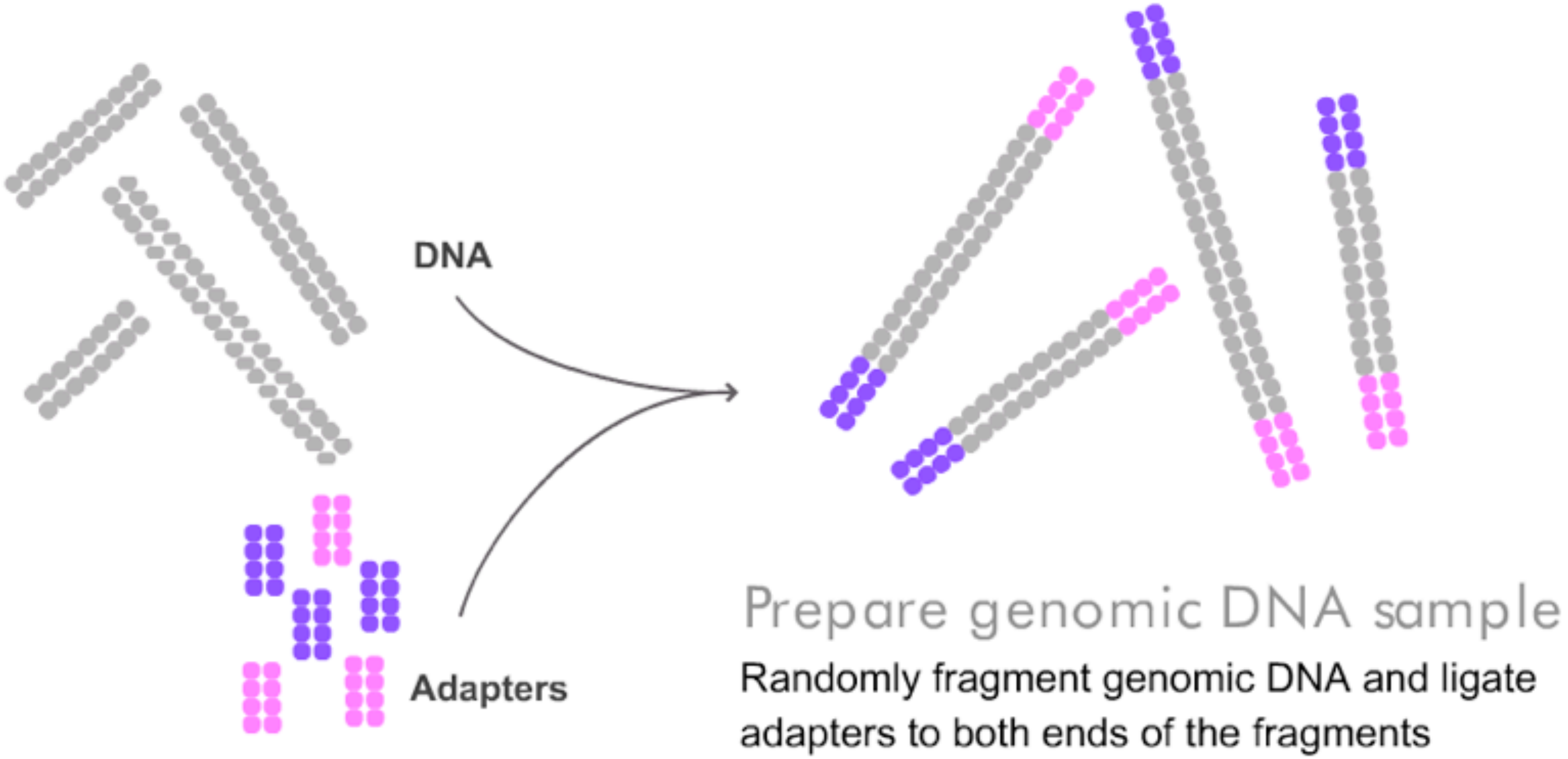

Prepare genomic DNA sample

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

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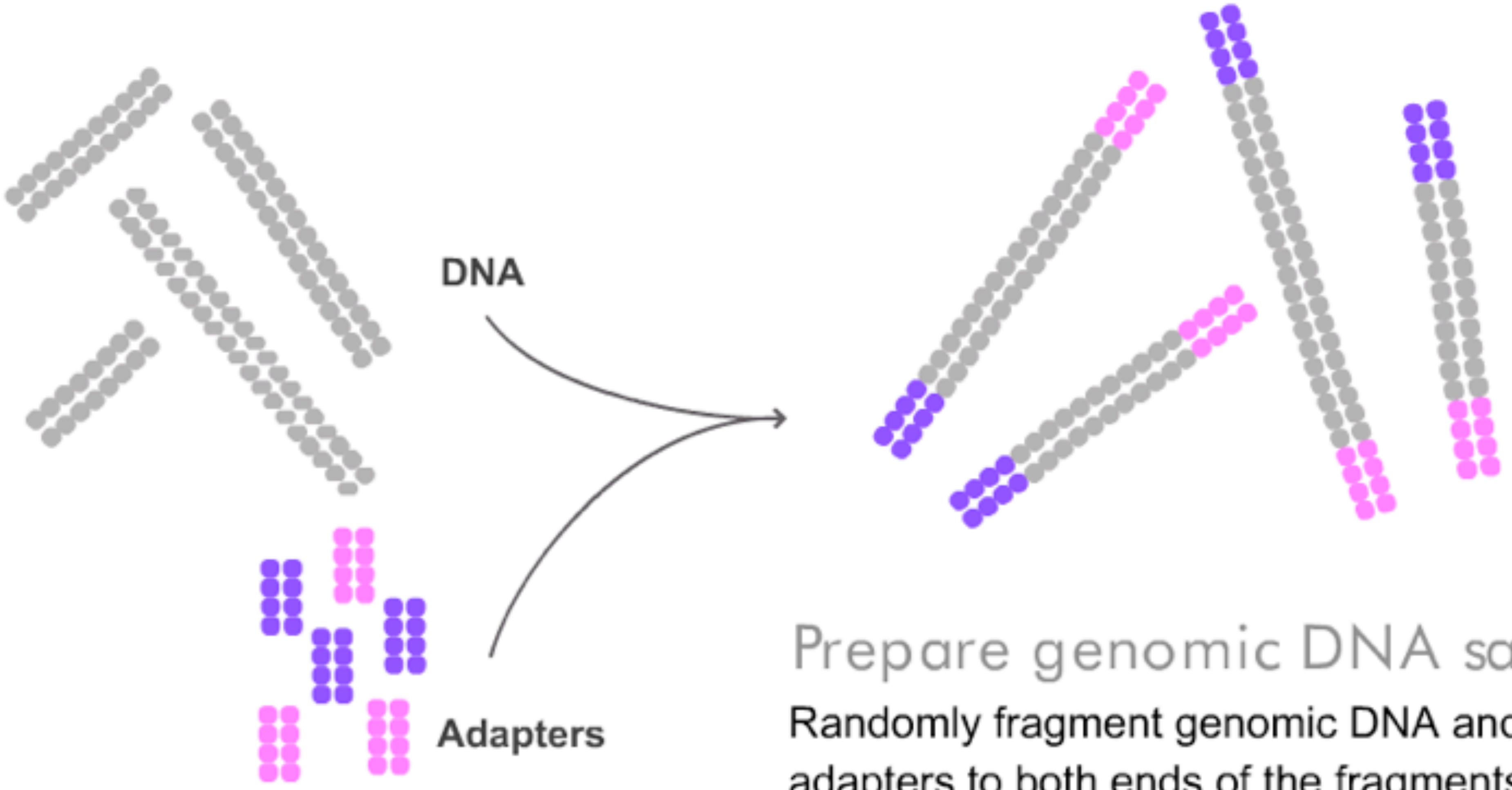

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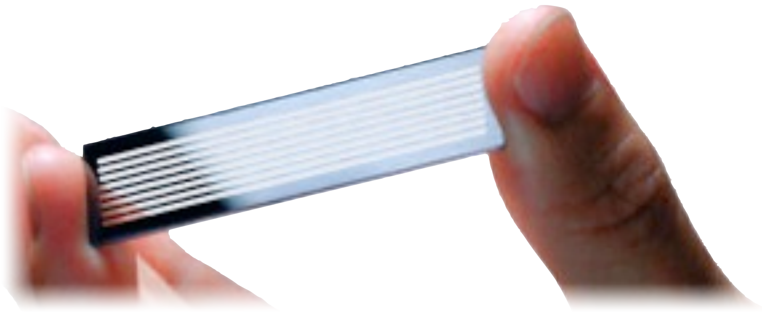

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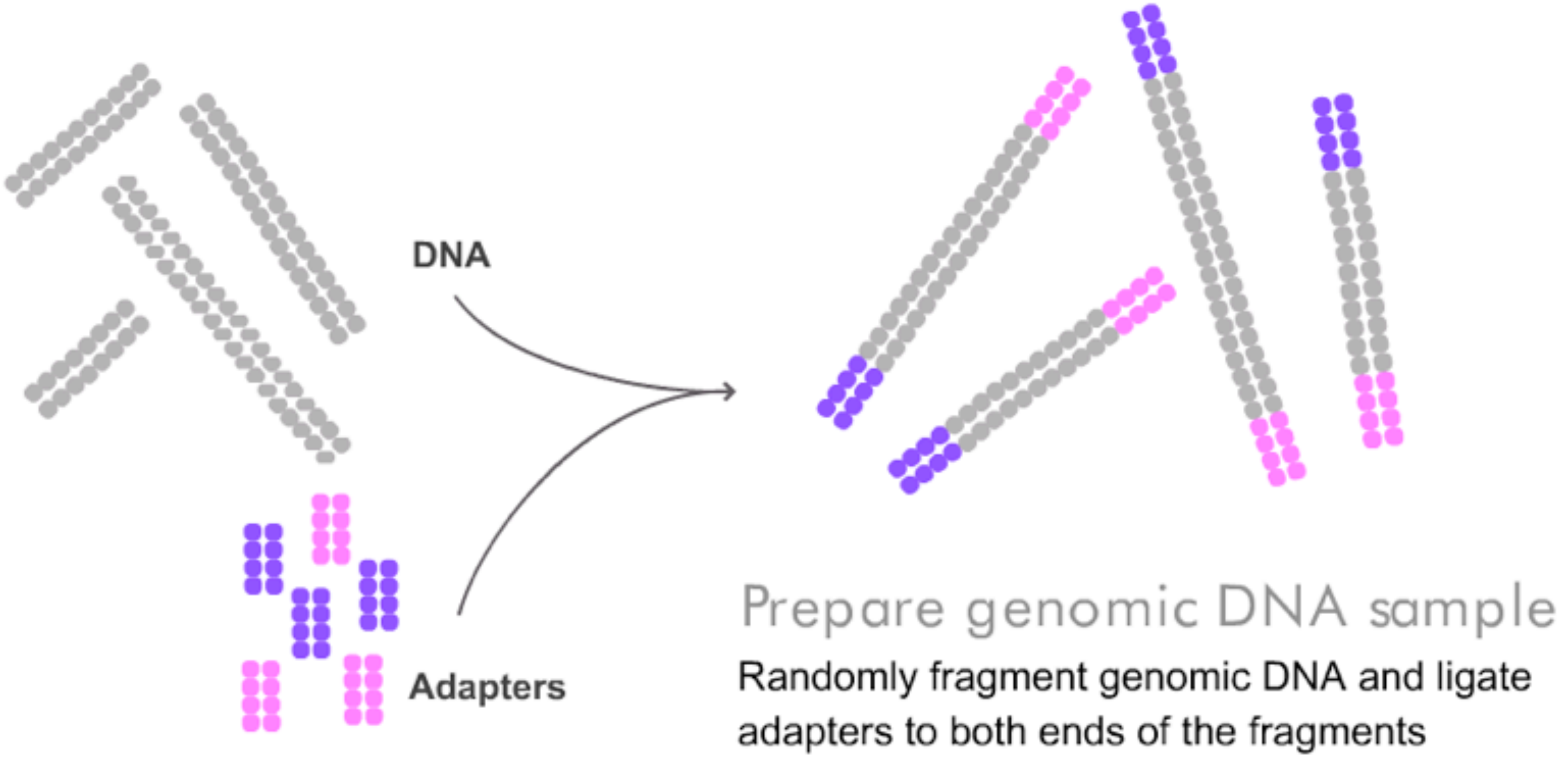

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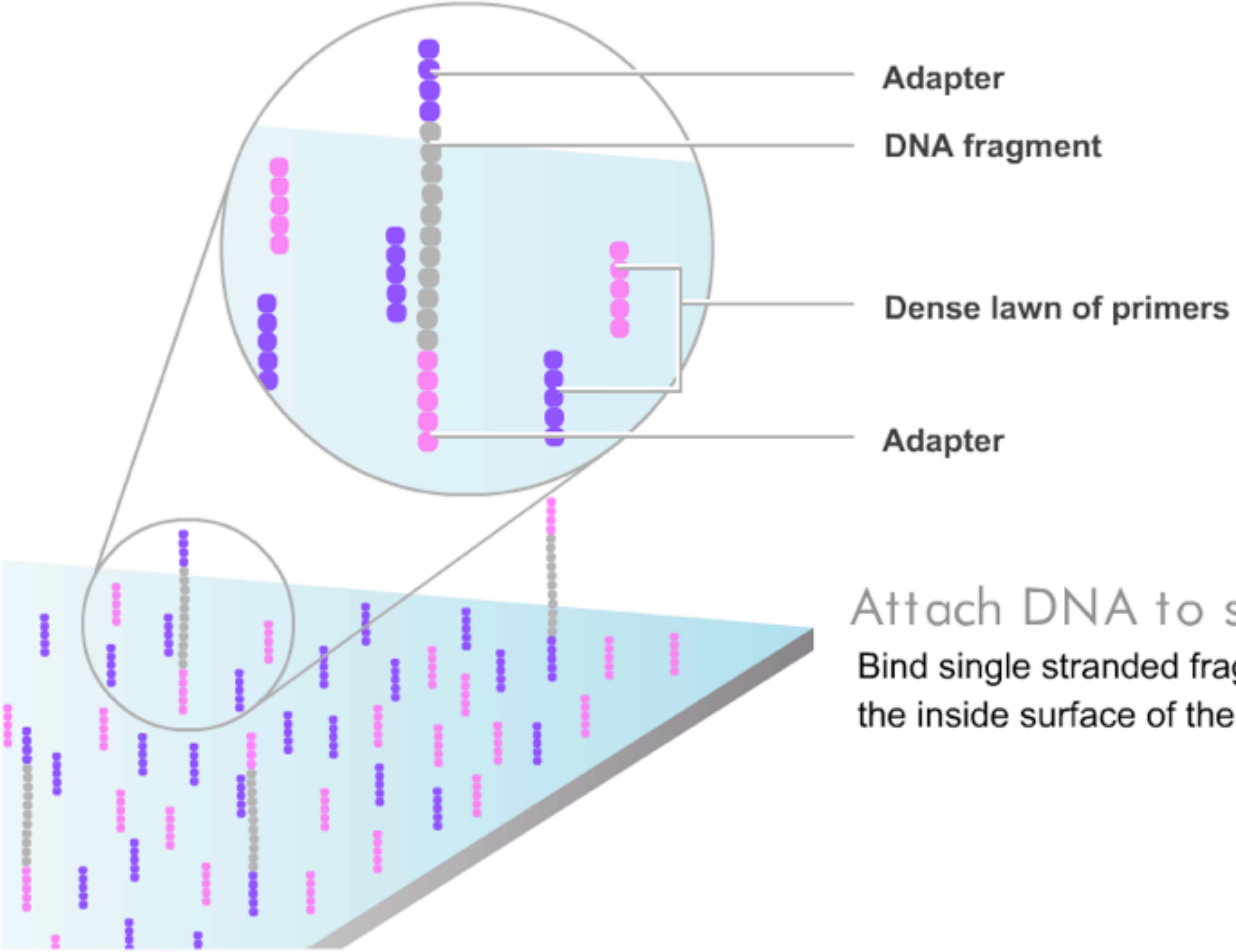
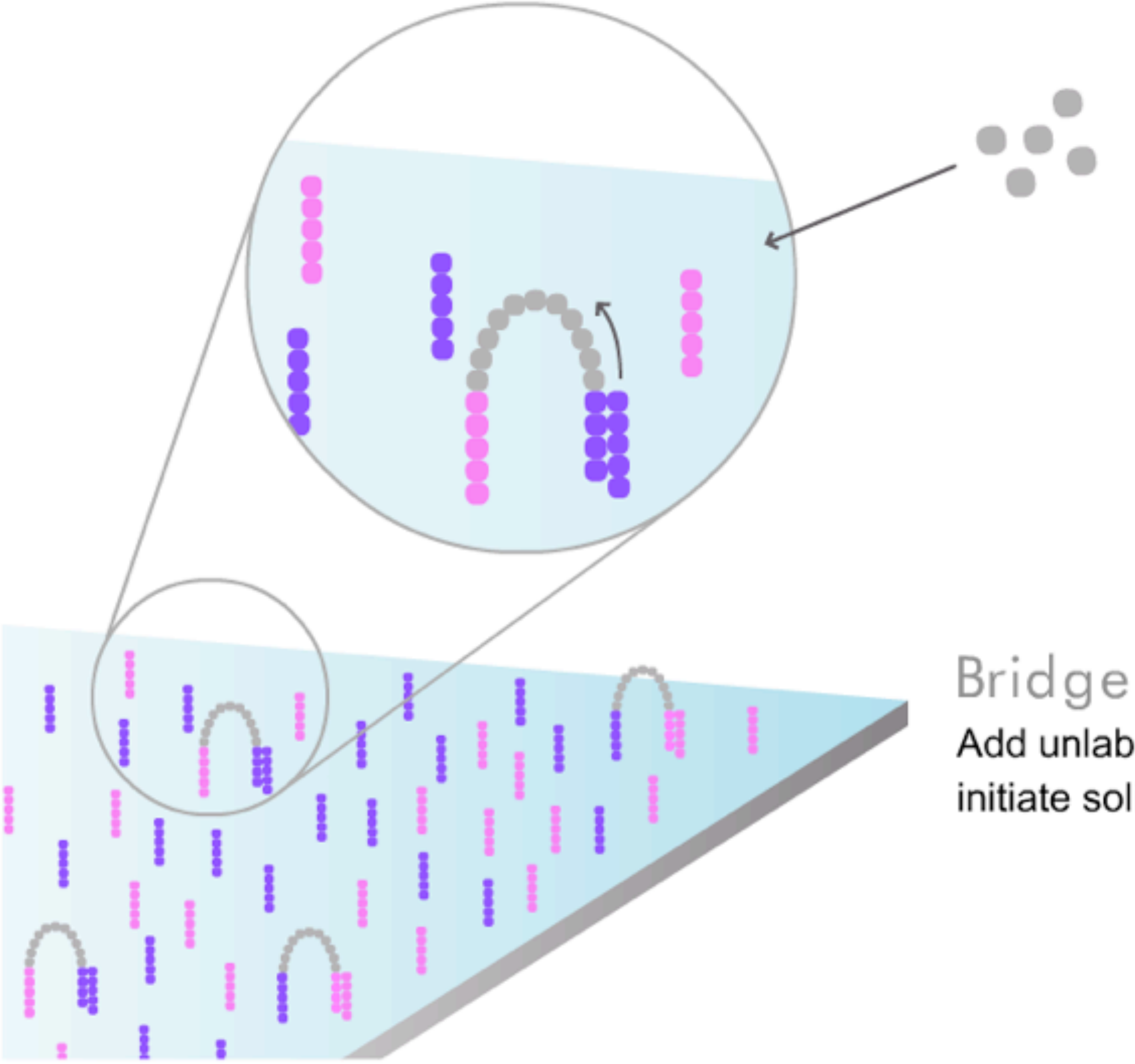


Attach DNA to surface

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

Solexa Sequencing–by–Synthesis

animation adapted from *www.illumina.com*

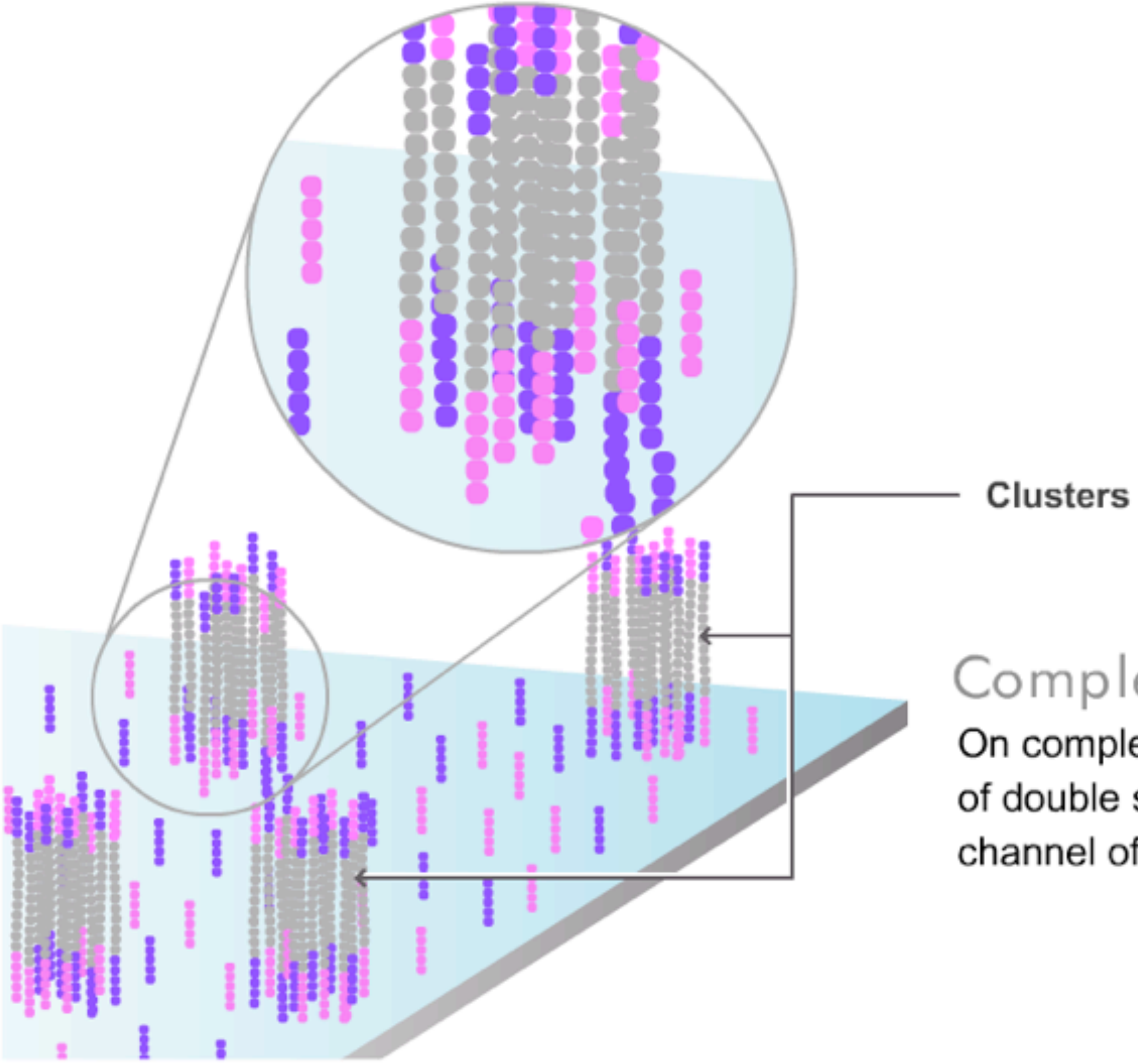


Bridge amplification

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

Solexa Sequencing–by–Synthesis

animation adapted from *www.illumina.com*



Completion of amplification

On completion, several million dense clusters of double stranded DNA are generated in each channel of the flow cell.

Solexa Sequencing–by–Synthesis

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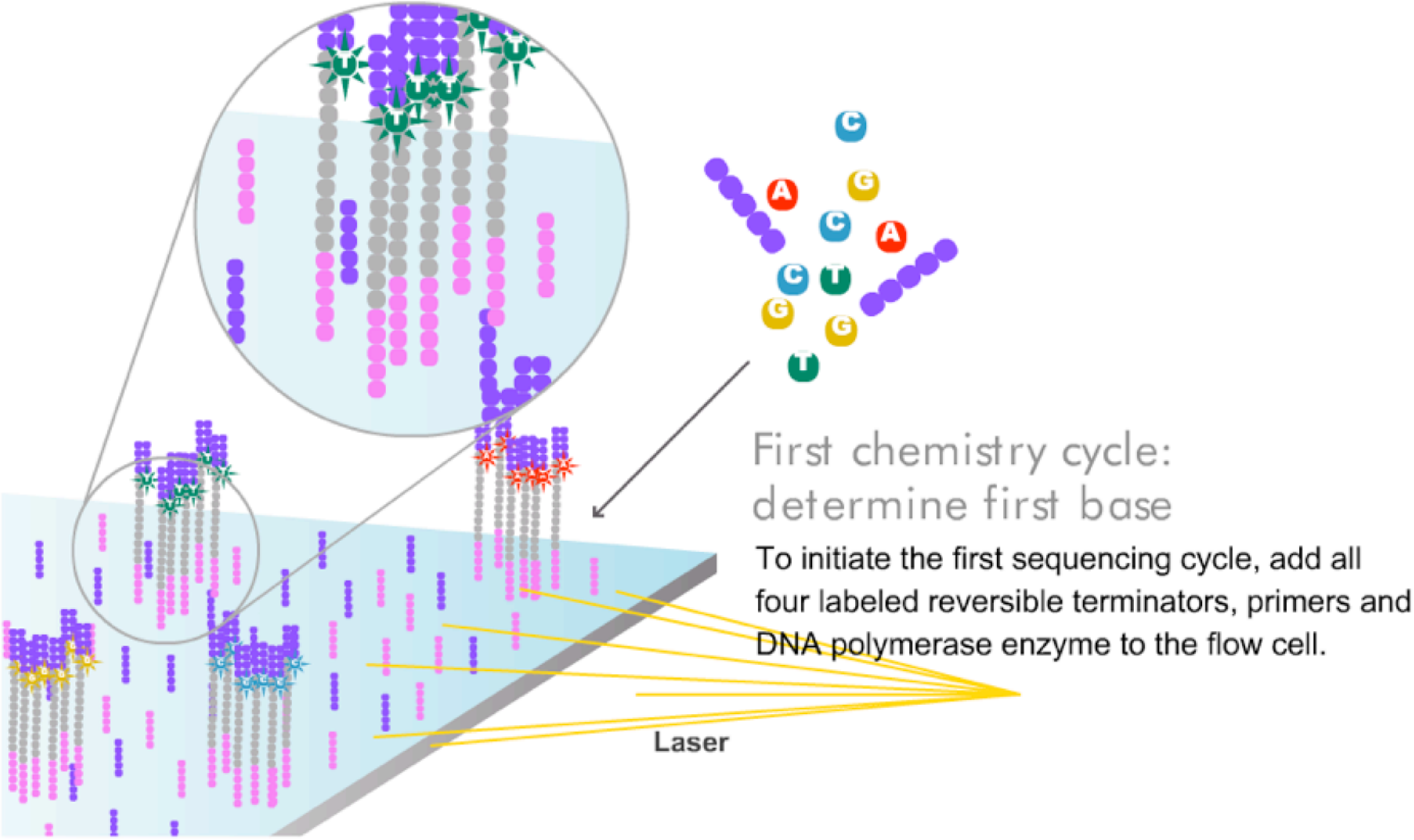

First chemistry cycle: determine first base

To initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

Solexa Sequencing–by–Synthesis

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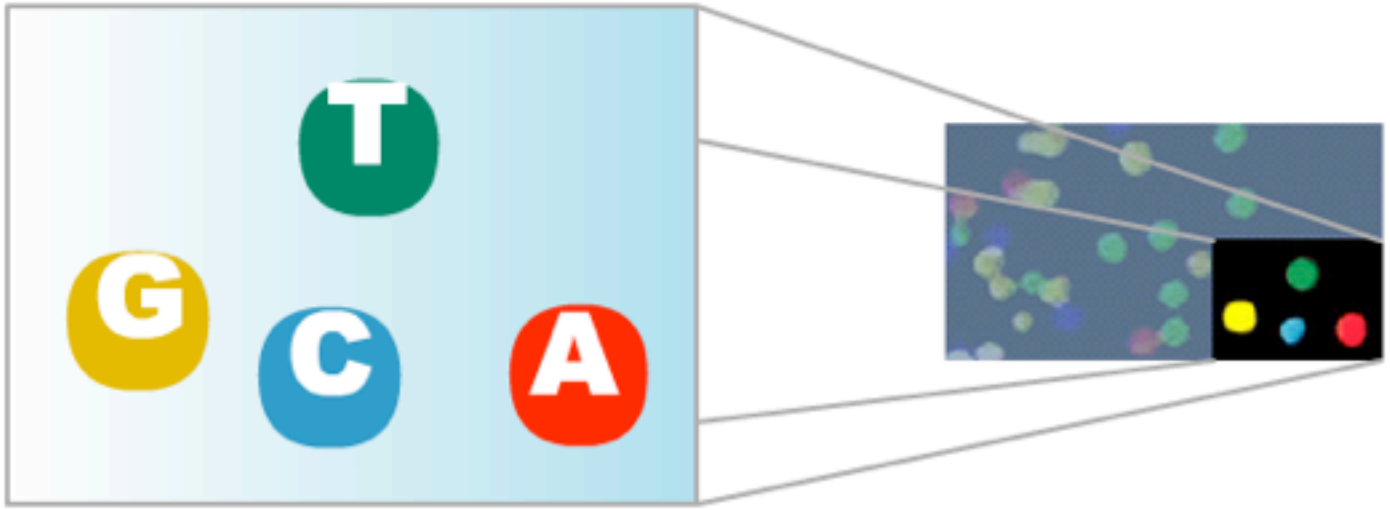


Image of first chemistry cycle

After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

Before initiating the next chemistry cycle

The blocked 3' terminus and the fluorophore from each incorporated base are removed.

Solexa Sequencing–by–Synthesis

animation adapted from *www.illumina.com*

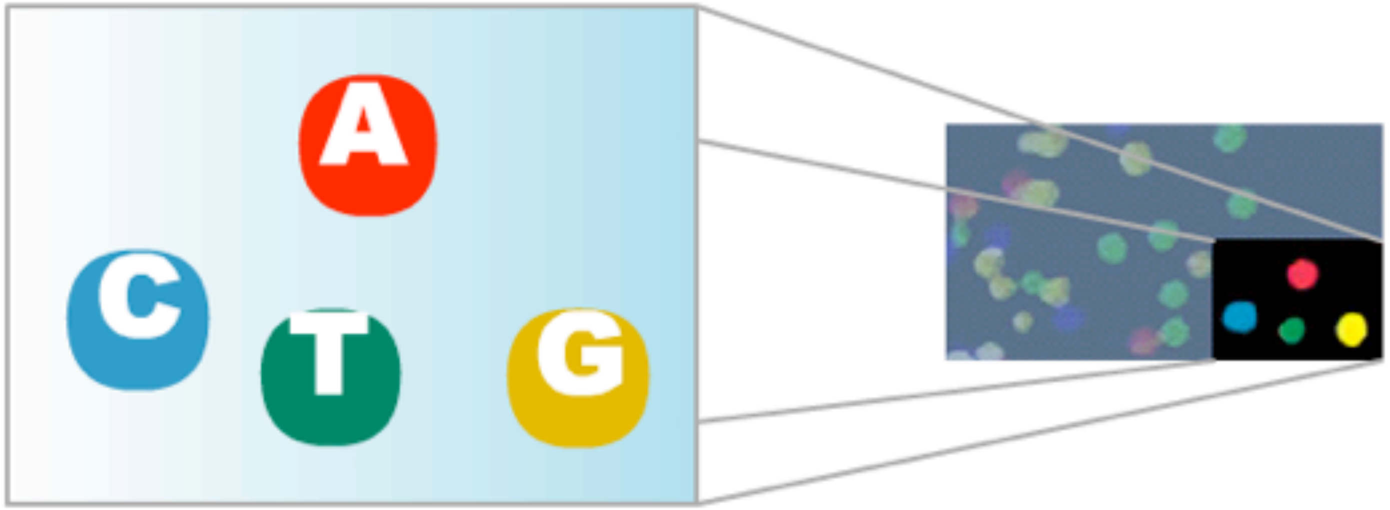
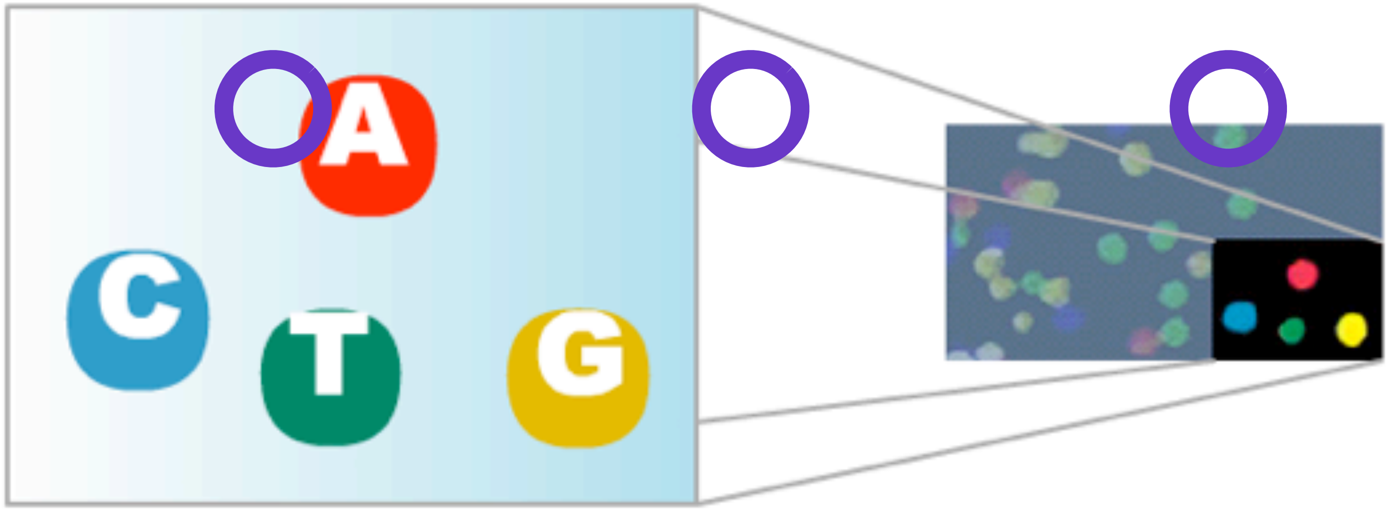


Image of second chemistry cycle is captured by the instrument

After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

Solexa Sequencing–by–Synthesis

animation adapted from *www.illumina.com*



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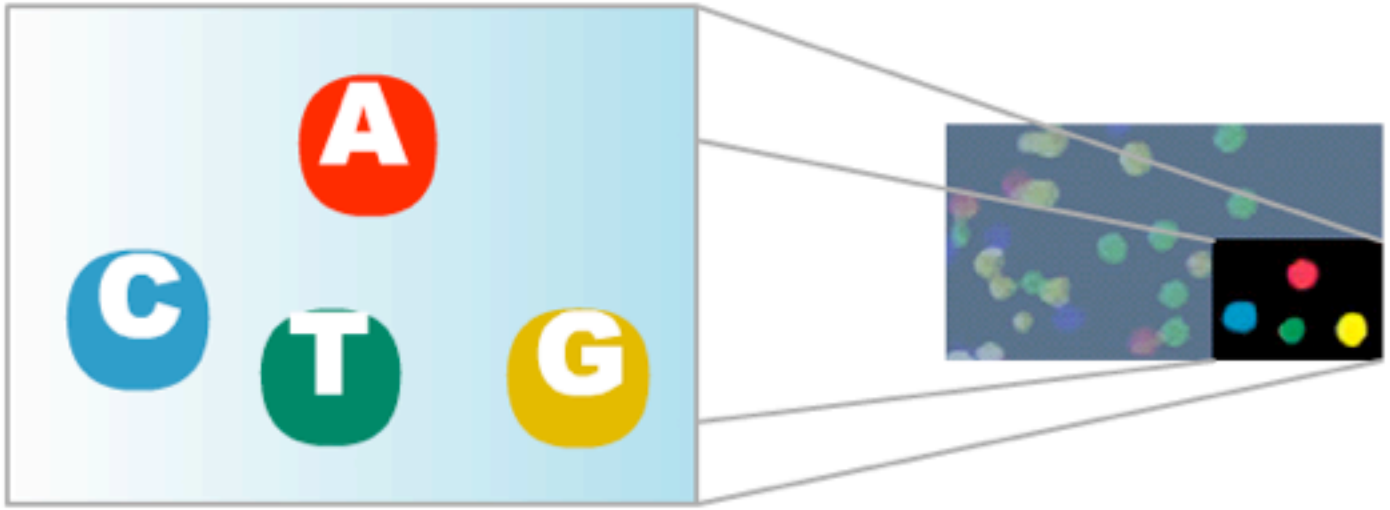

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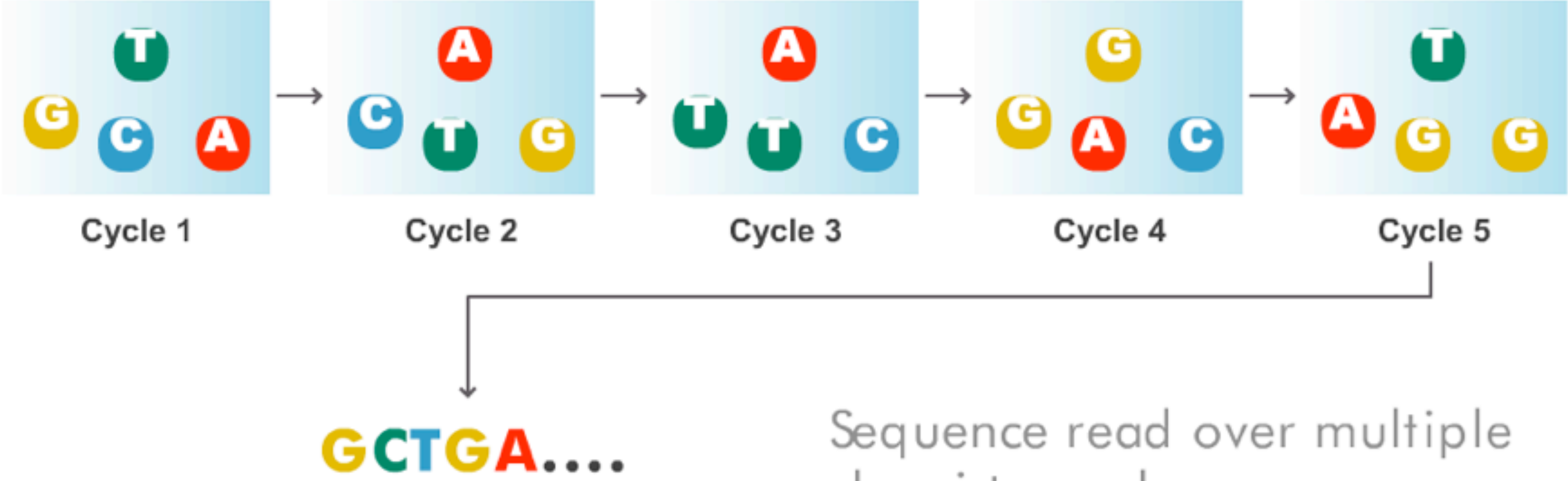


Sequence read over multiple chemistry cycles

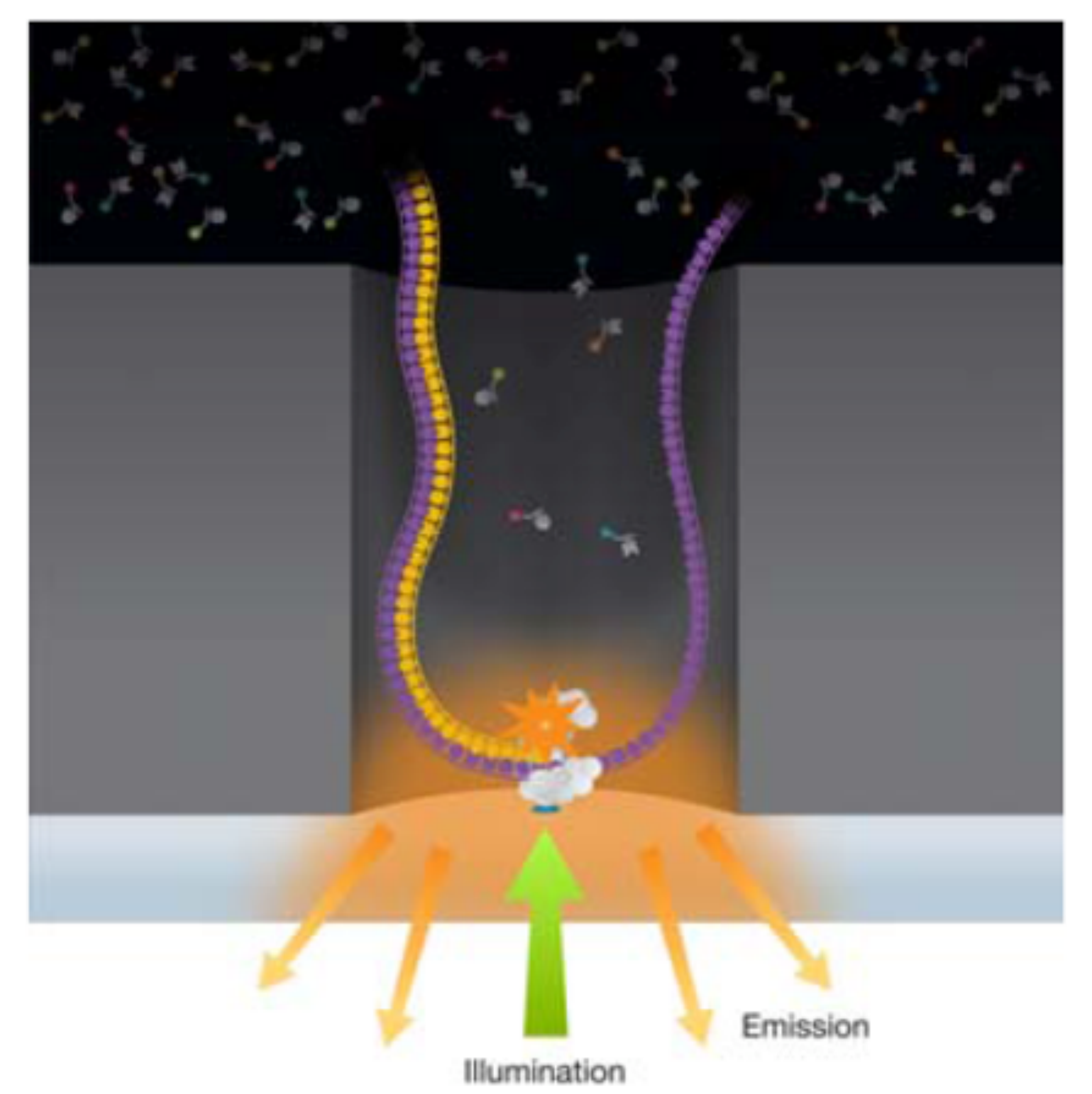
Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at a time.

Solexa Sequencer

- One run generates one hundred billion bases
- The first 76 to 100 bases of a read are usually sequenced
- Paired-end mode permits sequencing of both pairs of a read
- Cost of a run is about $4K

Future Sequencers

- Pacific Biosciences
- Individual Polymerases anchored to bottom of wells
- DNA sequenced in real time (1 base/ second)



Labeled Nucleotides

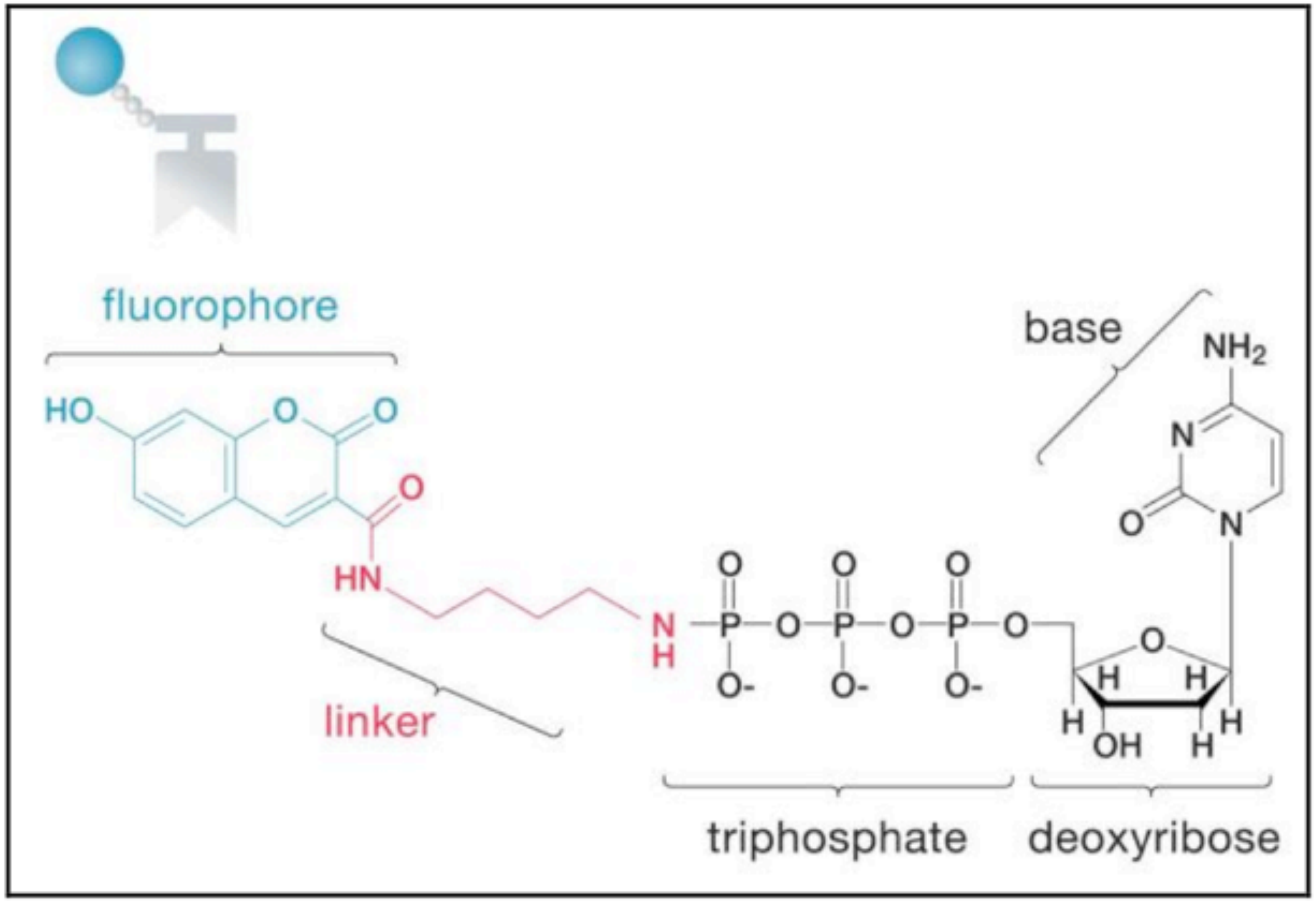


Figure 1. Phospholinked nucleotides

Sequencing Steps

Figure 2. Processive Synthesis with Phospholinked Nucleotides.

Step 1: Fluorescent phospholinked labeled nucleotides are introduced into the ZMW.
Step 2: The base being incorporated is held in the detection volume for tens of milliseconds, producing a bright flash of light.
Step 3: The phosphate chain is cleaved, releasing the attached dye molecule.
Step 4-5: The process repeats.

Zero Mode Waveguides

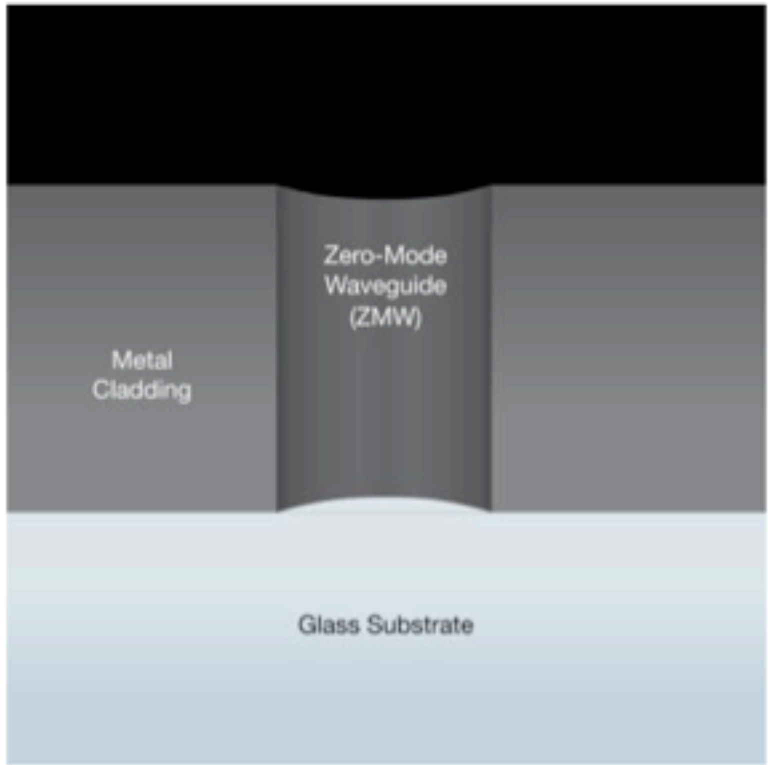


Figure 3. Individual ZMW

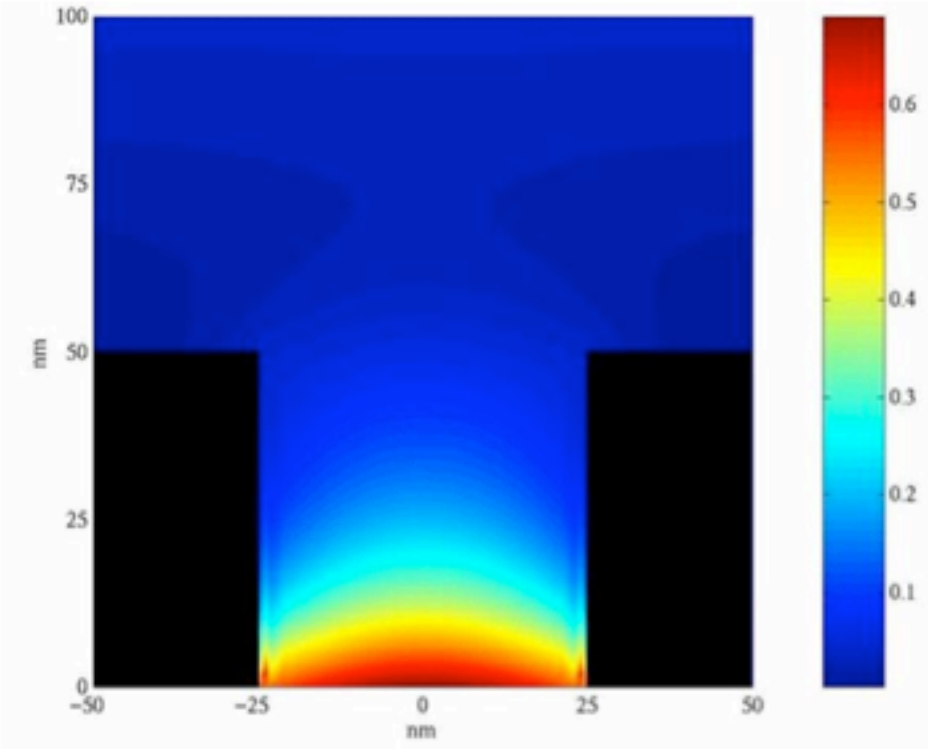


Figure 4. Detection volume

Attenuated light from the excitation beam penetrates the lower 20-30nm of each waveguide, creating a detection volume of only 20 zeptoliters (10-21 liters)

Architecture of Sequencer

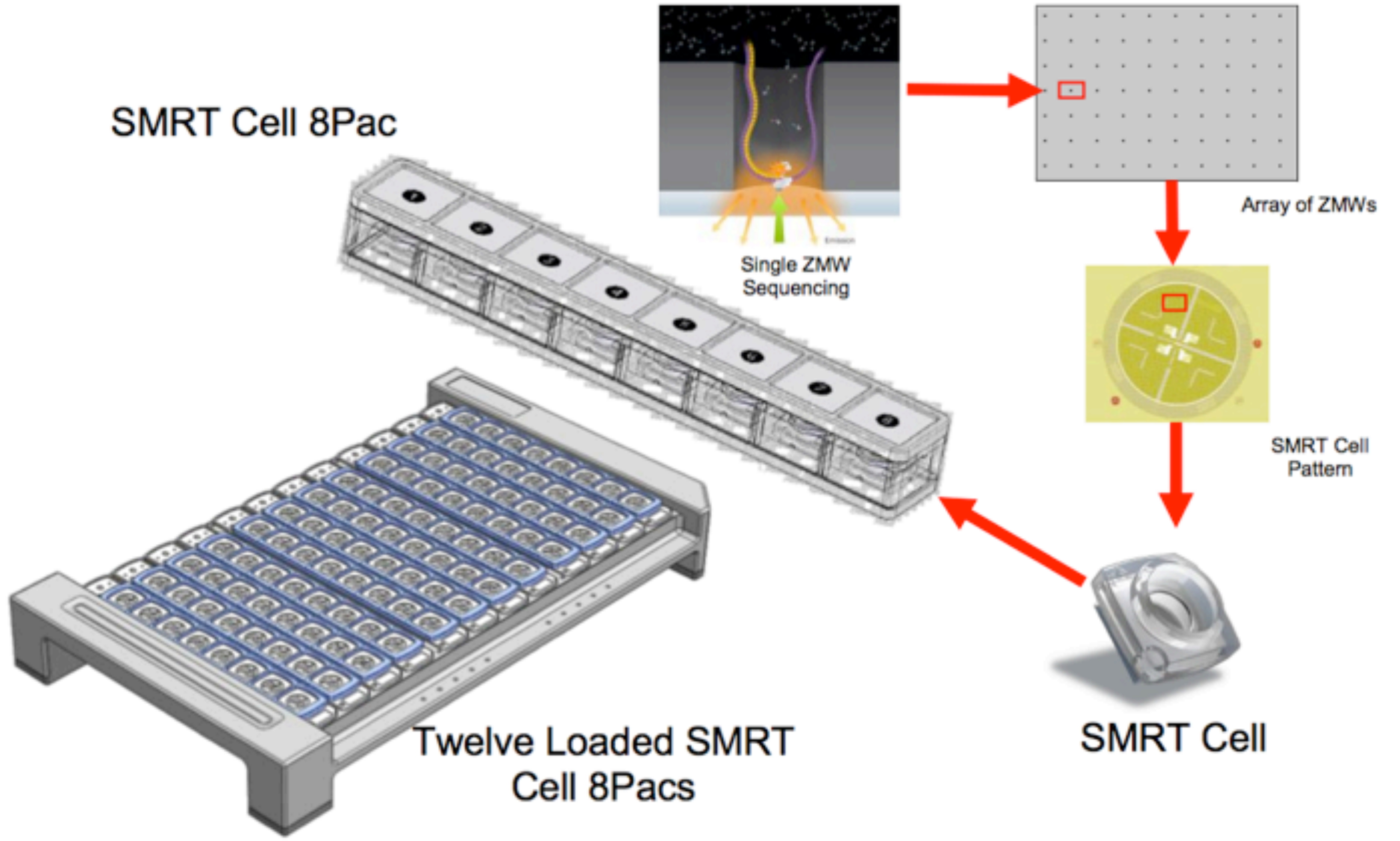


Figure 6. SMRT Cell consumable architecture

Pacific biosciences

- Long Reads
- Thousands of wells
- Human genome in minutes for $100?