

Reading Genetic Material

Stanford BIOE 80

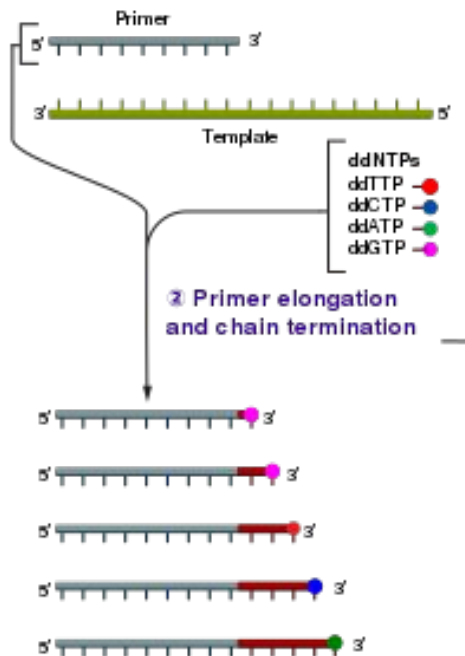
May 4, 2020

From an bioscience perspective, why is knowing the exact sequence of DNA or RNA in an organism interesting?

From an bioengineering perspective, why can knowing the exact sequence of DNA or RNA in an organism help us?

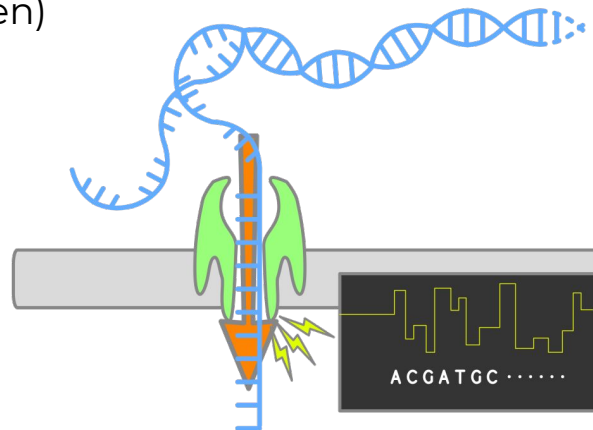
Sequencing by Synthesis

(1st gen)



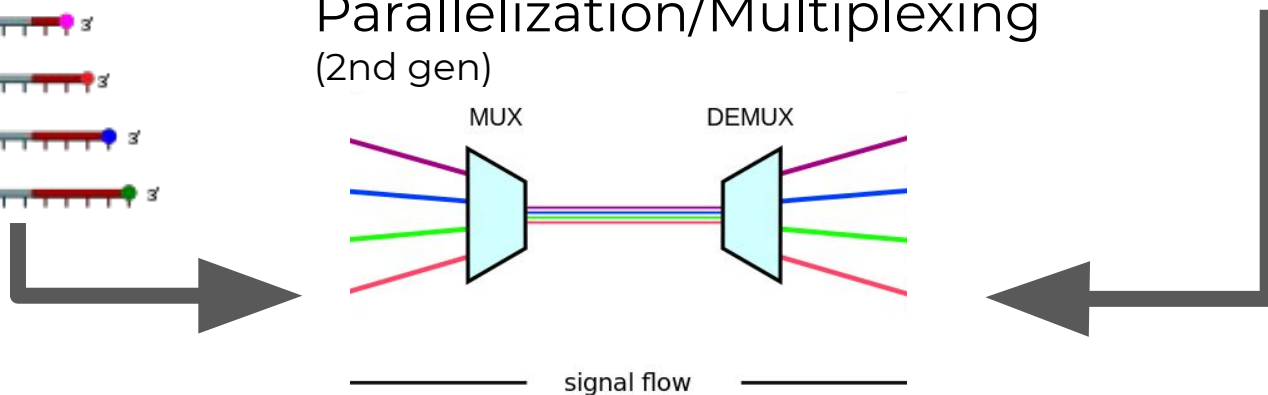
Nanopore-Based Sequencing

(3rd gen)

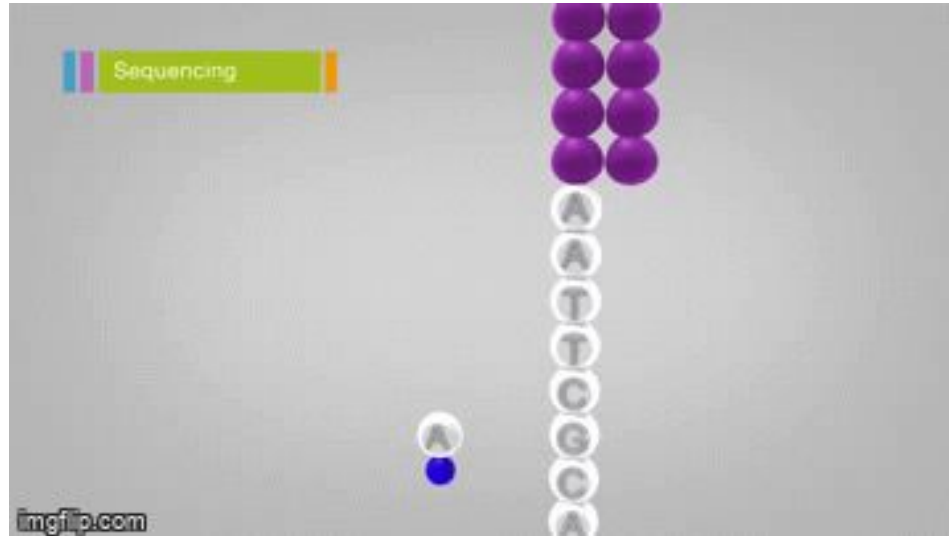


Parallelization/Multiplexing

(2nd gen)

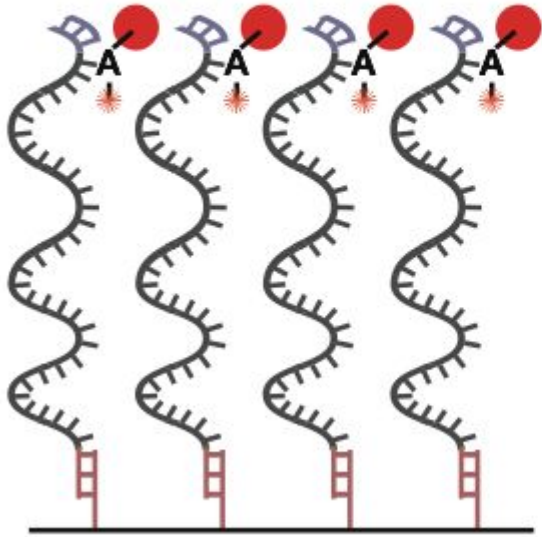


Sequencing By Synthesis

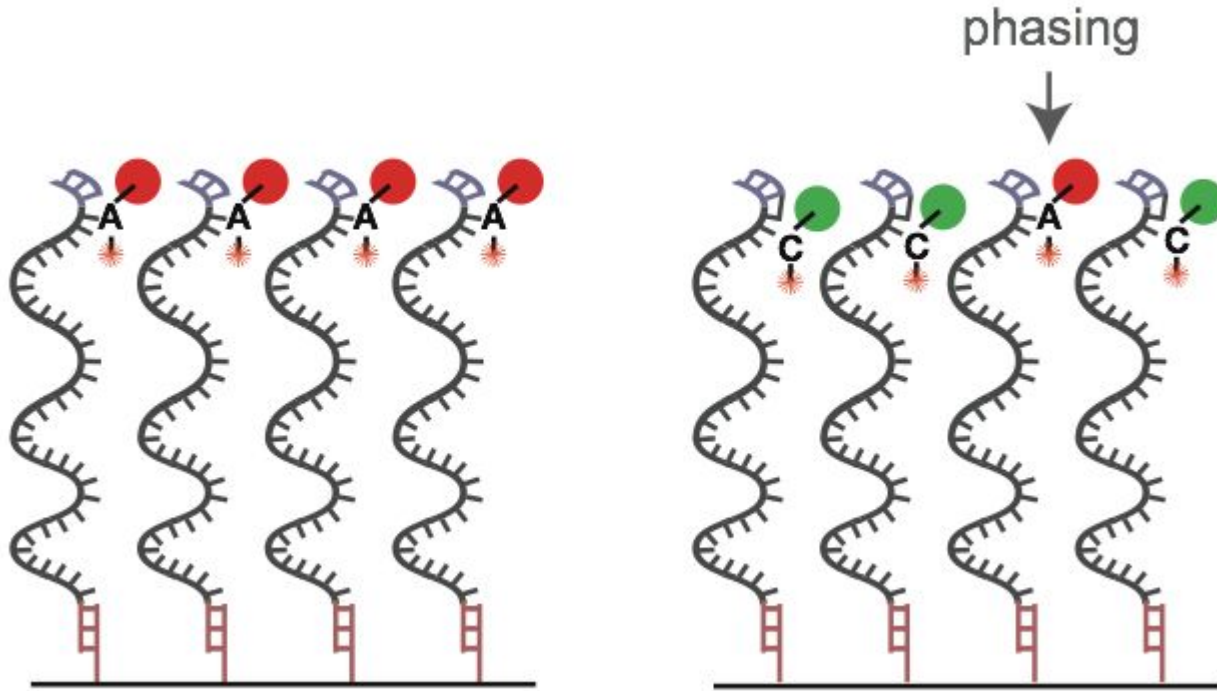


Illumina

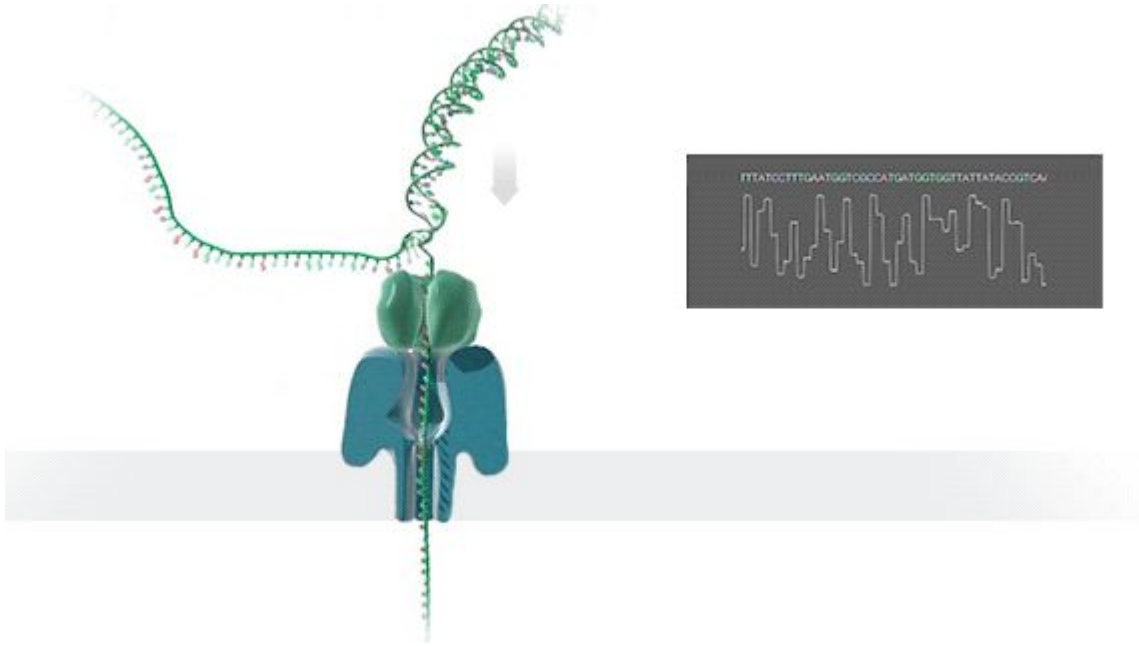
Seq By Synth. - Limited to short reads



Seq By Synth. - Limited to short reads



Nanopore-Based Sequencing

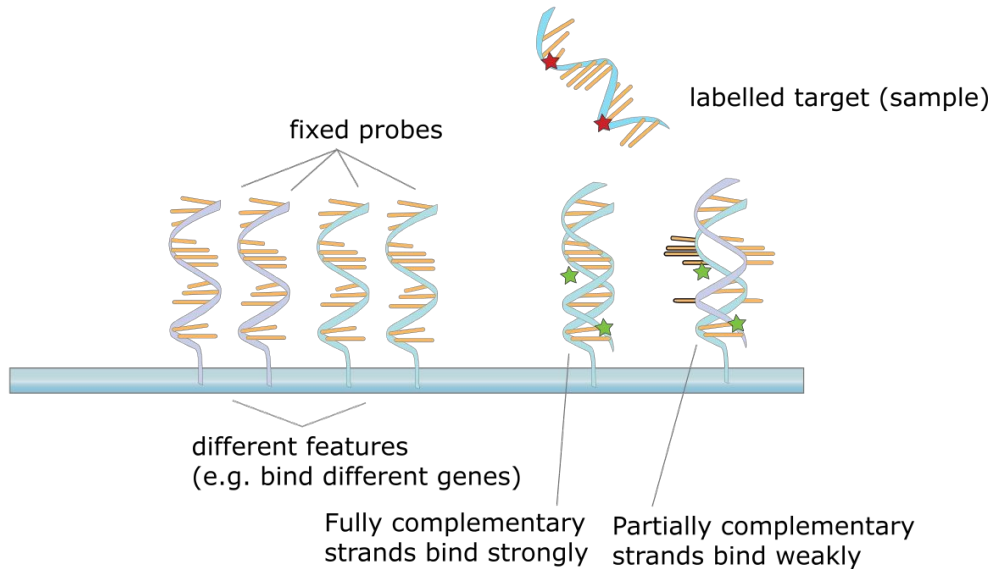


Pros and Cons of Sequencing Technologies

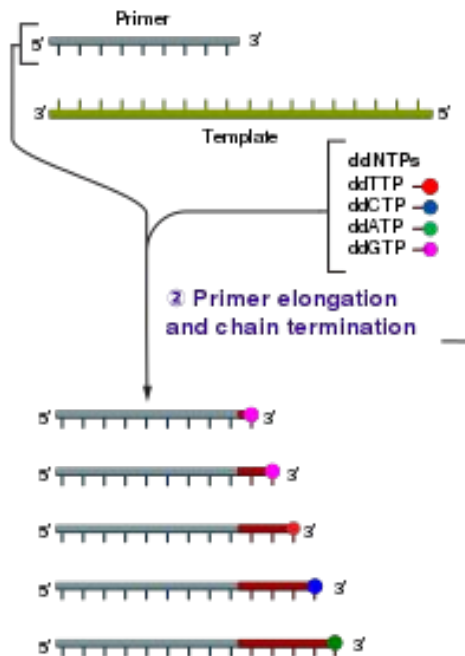
Platform	Pros	Cons
Sequencing by synthesis Illumina	Technology used widely by the WGS industry; lowest per-Gb sequencing cost range; highest confirmed output; wide range of Illumina machines suited for a wealth of applications and demands; lowest error rates	Rehybridization of template strands and low-copy-no. yields during bridge amplification; use of potentially biased DNA polymerases during bridge amplification; incomplete base extension (phasing, prephasing); shortest read lengths; long sequence runs; high instrument costs; no real-time data access
Oxford Nanopore Technologies	Fast sequencing; longest confirmed reads; smallest instrument footprint; lowest instrument and consumables costs; real-time measurement of base incorporation; real-time data output	Sensitivity of biological nanopores to changes in exptl environment; highest error rate of all platforms; the performance of the PromethION machine is not experimentally validated

Genotyping vs. Sequencing

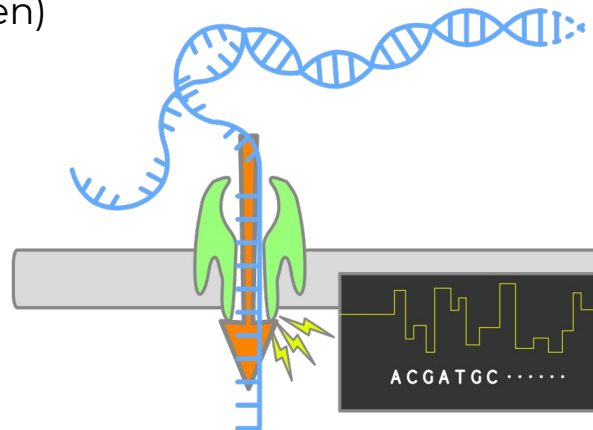
“What about 23andMe?”



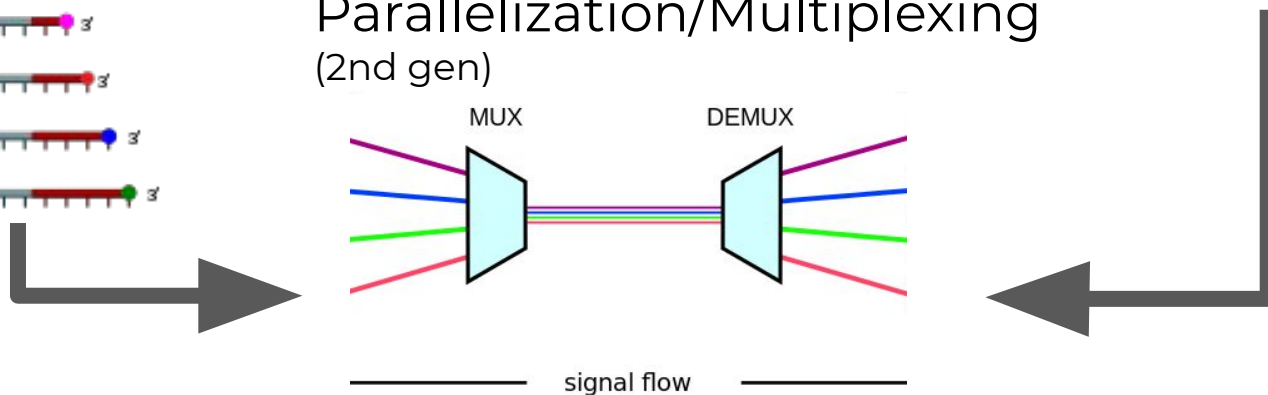
Sequencing by Synthesis (1st gen)



Nanopore-Based Sequencing (3rd gen)



Parallelization/Multiplexing (2nd gen)



Parallelization/Multiplexing

To the invisible kindergartner next to you:

Explain what it means to **parallelize** something.

Parallelization/Multiplexing

Parallelize: *To modify a system so that computations or [processes](#) can be carried out simultaneously.*

Multiplex: *Method by which multiple signals are combined into one signal over a shared medium, in order to share a scarce resource.*

Multiplexing and parallelization often go hand-in-hand in bioengineering.

Parallelization vs Multiplexing

E.g., in a department-wide brownie-baking effort, each student in the BIOE department spoons batter into a pan simultaneously. We then put all the pans in a giant oven so that they can bake at the same time. These brownie batches have been poured and cooked ***in parallel.***

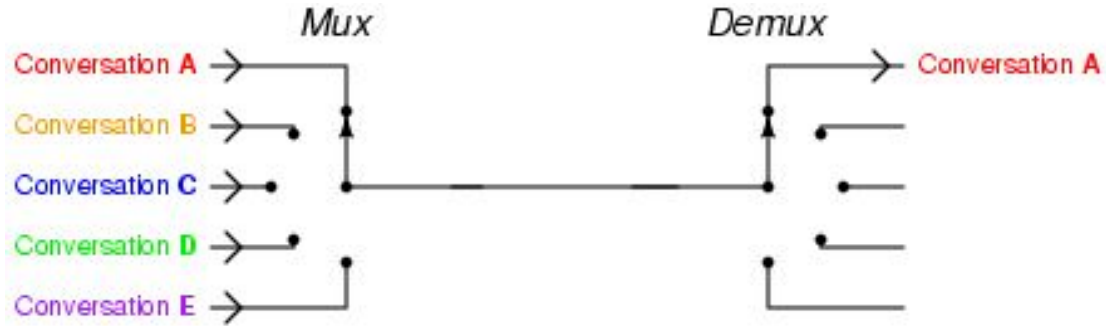
Parallelization vs **Multiplexing**

We want to be able to identify which brownies belong to whom when they come out of the oven. So, each person puts a dye into their brownies, and when the brownies come out we separate them by color. The process of putting dye into the brownies is ***multiplexing***, and the process of separating them by color ***demultiplexing***.

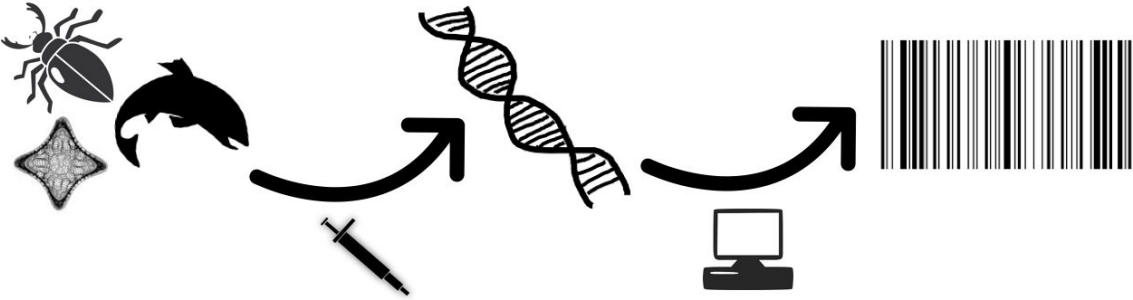
Parallelization/Multiplexing

Come up with an example:

1. From day-to-day life?
2. From engineering?
3. From bioengineering?

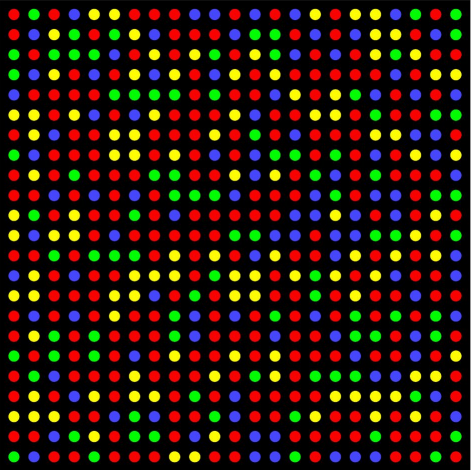


Parallelization/Multiplexing for Sequencing



DNA barcoding

Sequencing flow cell



“Massively Parallel”

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

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