Introduction to Sequencing

CS 4390/5390
Fall 2019

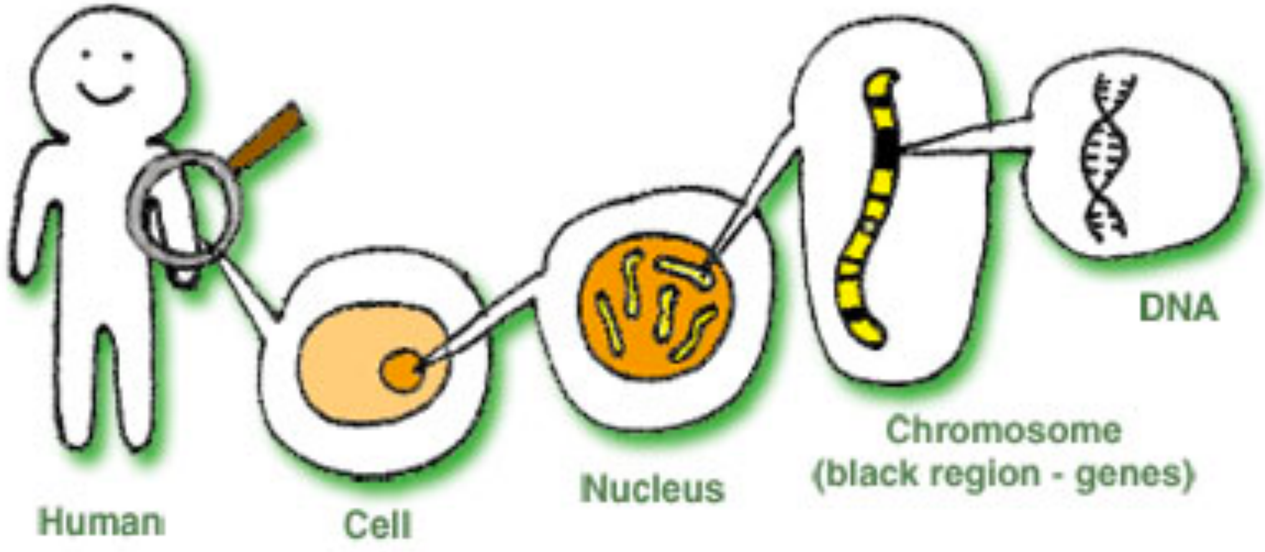
Associated Reading: Mäkinen, et al. Chapter 1
Gibson and Muse, Chapter 2

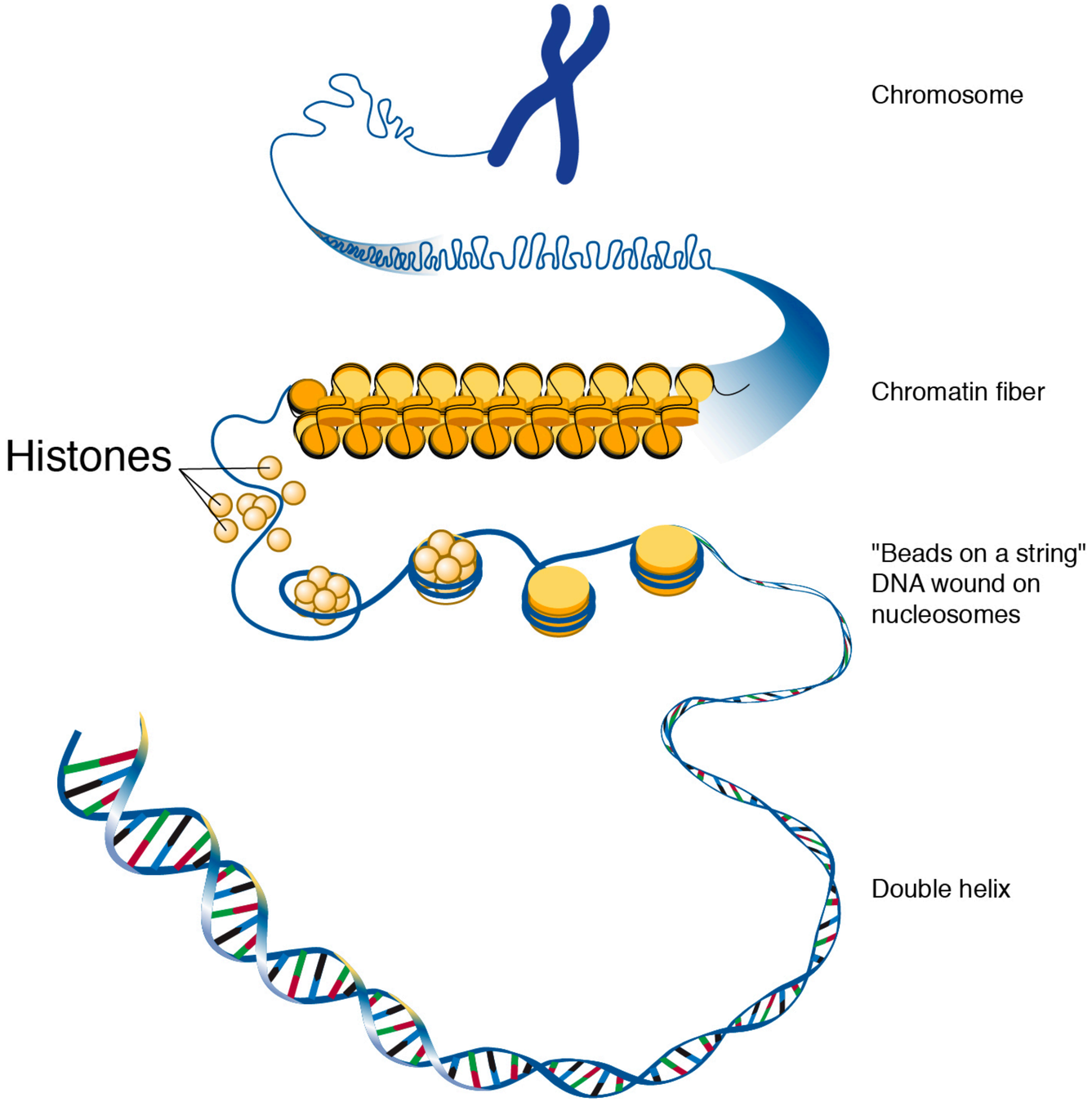
At the highest level

Organism are made up of one or multiple cells

inside the cell is the nucleus, which contains the DNA

humans are *diploid* meaning we have 2 copies of each chromosome (one from each parent)




Chromosome
Chromatin fiber
Histones
"Beads on a string"
DNA wound on
nucleosomes
Double helix

The Central Dogma

DNA

- double stranded
- contains all of the information for "you"
- only about 1.5% of the human genome encodes proteins

DNA {A,C,T,G}

↓ Transcription

RNA {A,C,U,G}

↓ Translation

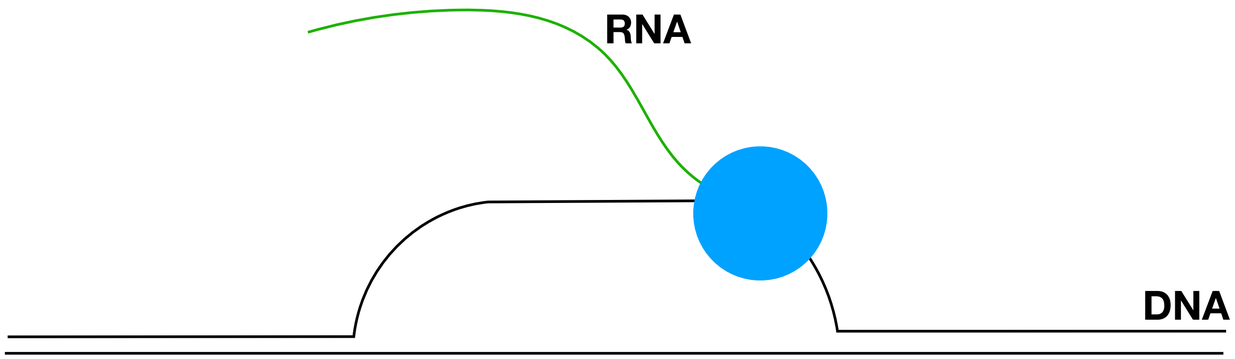
Proteins {A,C,D,E,F,G,H,I,K,L,M, N,P,Q,R,S,T,V,W,Y}

DNA

The Central Dogma

Transcription

- process of uncoiling, seperating, and copying DNA into RNA
- first stage is called "pre-mRNA" in the case of protein coding genes



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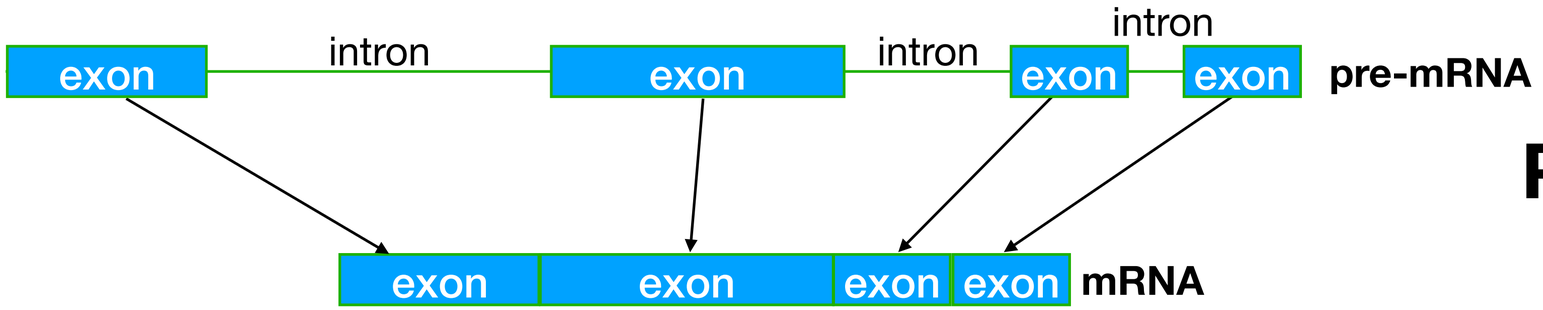
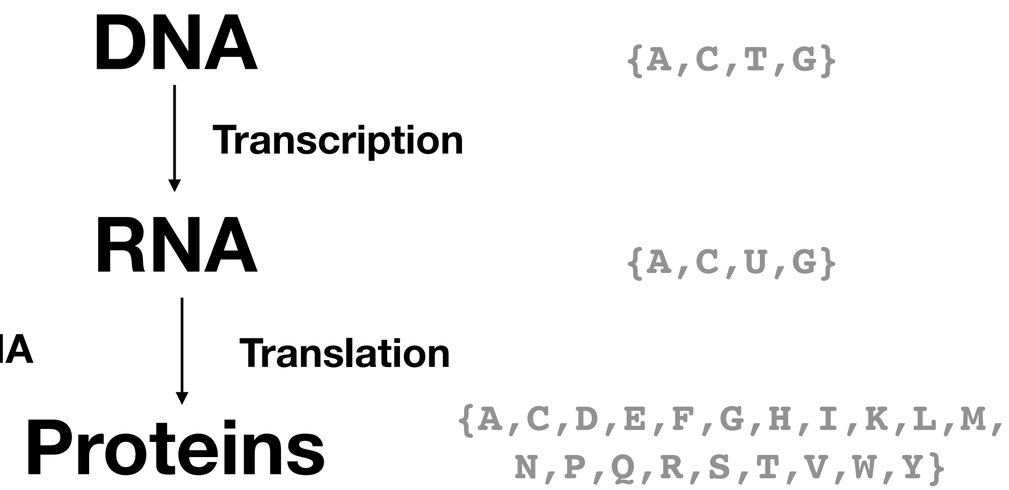
RNA

- pre-mRNA undergo splicing to remove the *introns* and leave only (some) *exons*
- some RNA perform functions on their own and are not spliced, called ncRNA (non-coding RNA)





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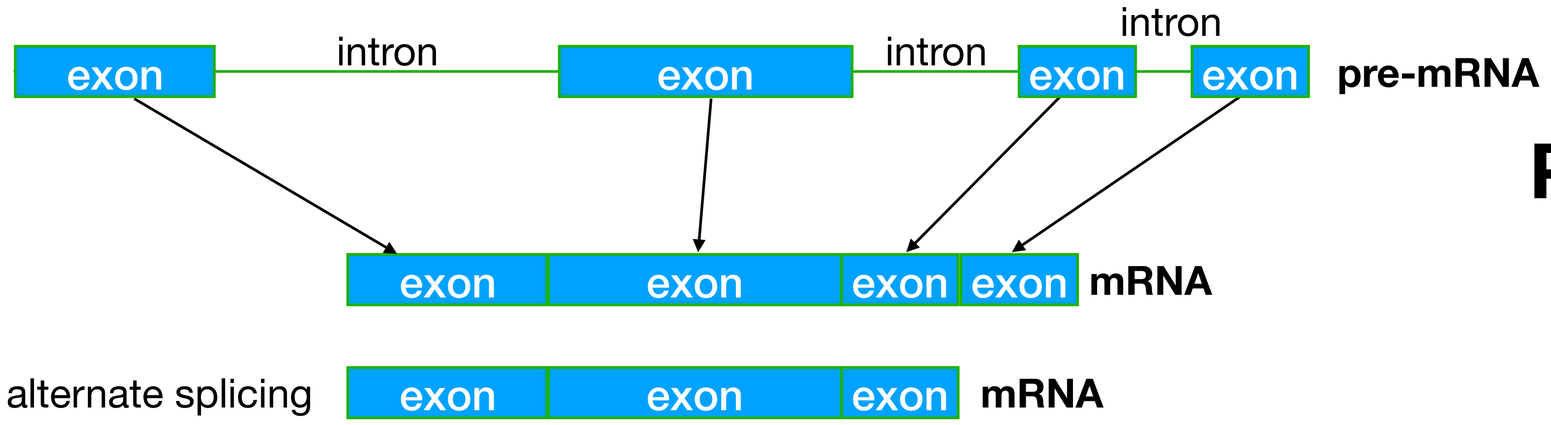

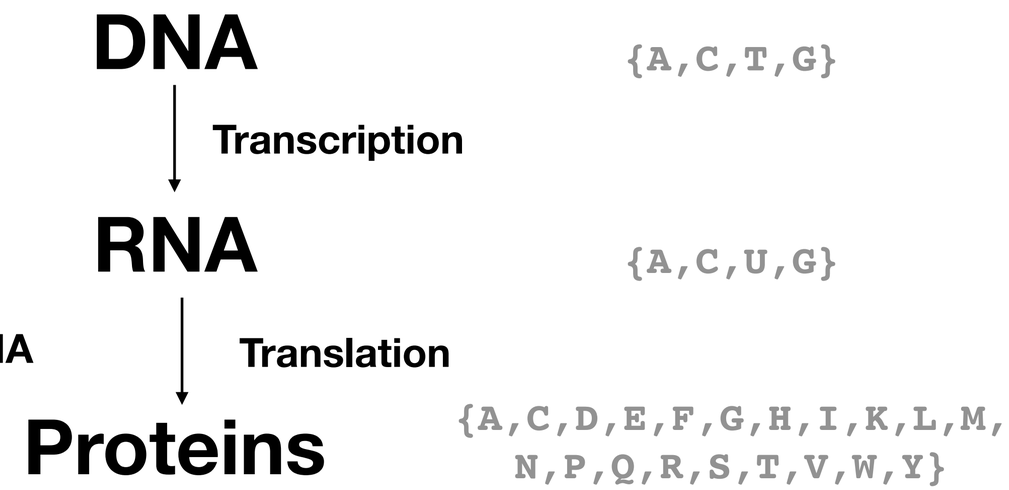

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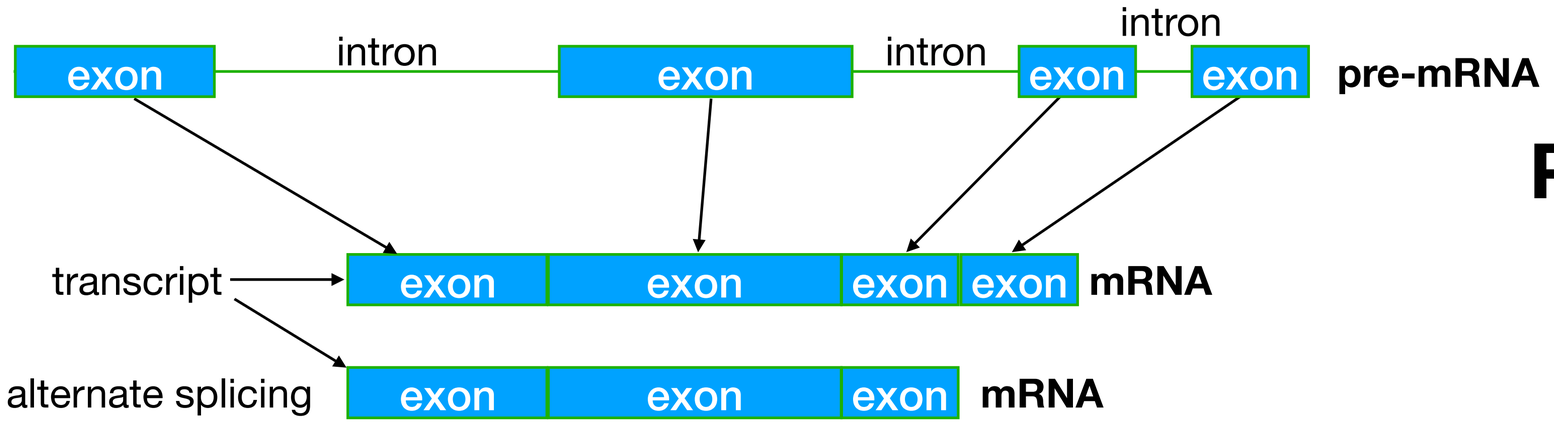

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↓ **Translation**

Proteins {A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y}

The Central Dogma

Translation

- 3-letter groups of RNA characters, *codons,* are converted to amino acids, the building blocks for proteins

First Char.	Second Character: A		C		U		G		Third Char.
A	AAC	N	ACC	T	AUC	I	AGC	S	C
	AAU		ACU		AUU		AGU		U
	AAA	K	ACA		AUA		AGA	R	A
	AAG		ACG		AUG	M/start	AGG		G
C	CAC	H	CCC	P	CUC	L	CGC	R	C
	CAU		CCU		CUU		CGU		U
	CAA	Q	CCA		CUA		CGA		A
	CAG		CCG		CUG		CGG		G
U	UAC	Y	UCC	S	UUC	F	UGC	C	C
	UAU		UCU		UUU		UGU		U
	UAA	stop	UCA		UUA	L	UGA	stop	A
	UAG		UCG		UUG		UGG	W	G
G	GAC	D	GCC	A	GUC	V	GGC	G	C
	GAU		GCU		GUU		GGU		U
	GAA	E	GCA		GUA		GGA		A
	GAG		GCG		GUG		GGG		G

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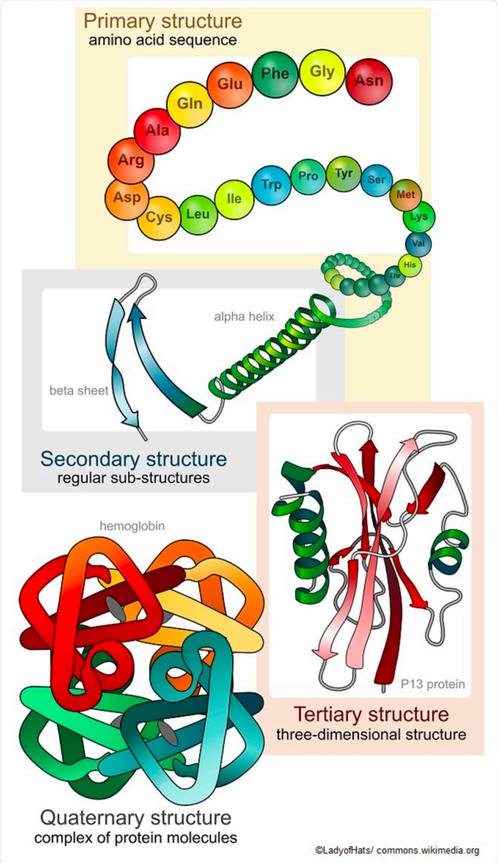
Proteins {A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y}

The Central Dogma

Proteins

- Do stuff in the cell, including help with translation and transcription



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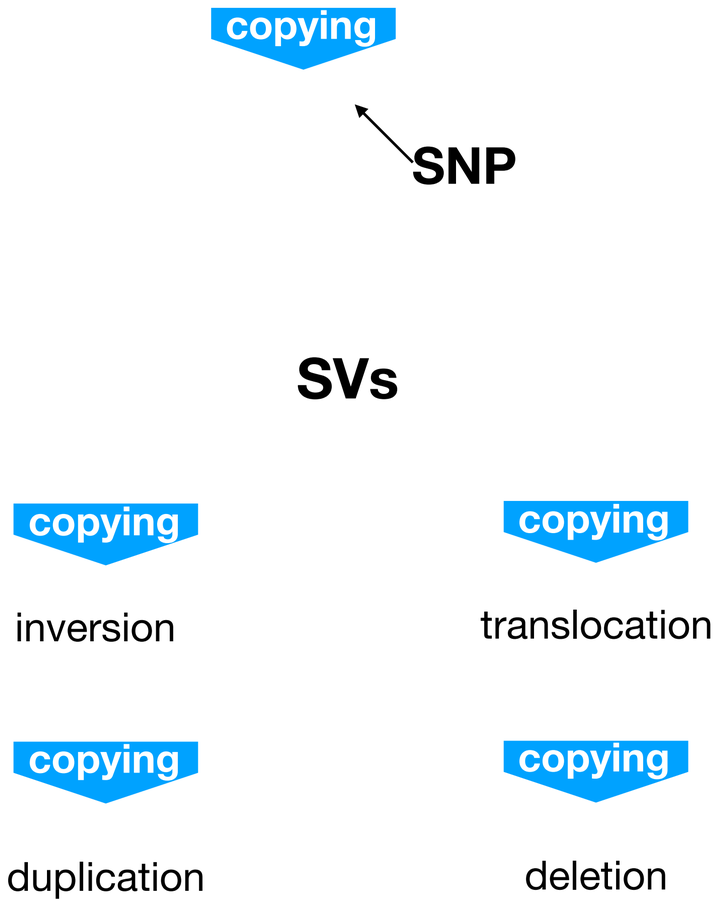
Genetic Variants

When copying a genome "errors" may occur, these changes are what make people different

- 99.99% of our genomes are identical
- **Single Nucleotide Polymorphism (SNP)** -- a change at a single base
- **Structural Variants (SV)** -- large scale changes



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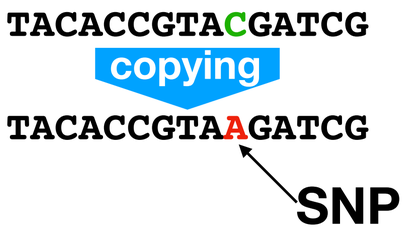
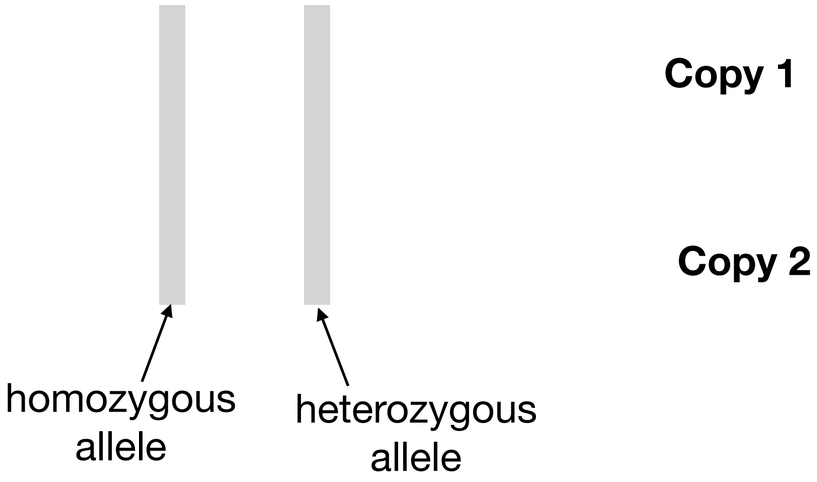


SVs

Genetic Variants

- **Deleterious Mutations** -- changes that are harmful (lethal) to a cell
- **Germline Mutations** -- changes passed to offspring
- **Somatic Mutations** -- those not passed down
- **Heterozygous** -- different beween copies
- **Homozygous** -- same on both copies
- **Allele** -- specific position on a chromosome



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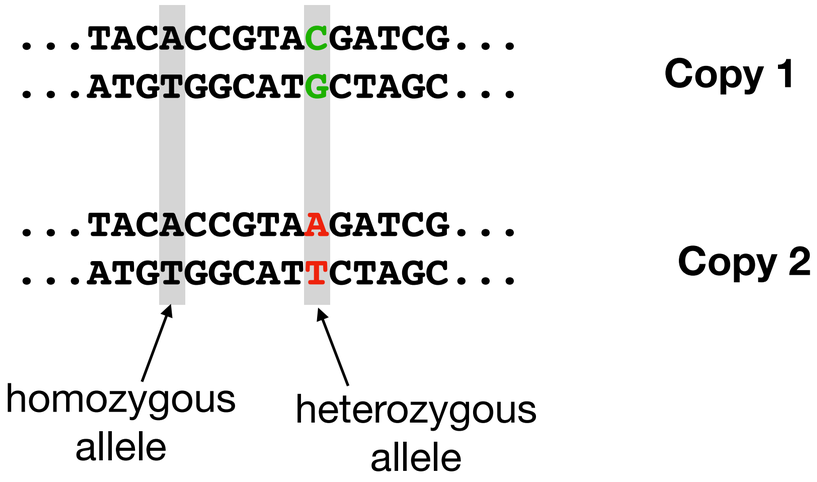
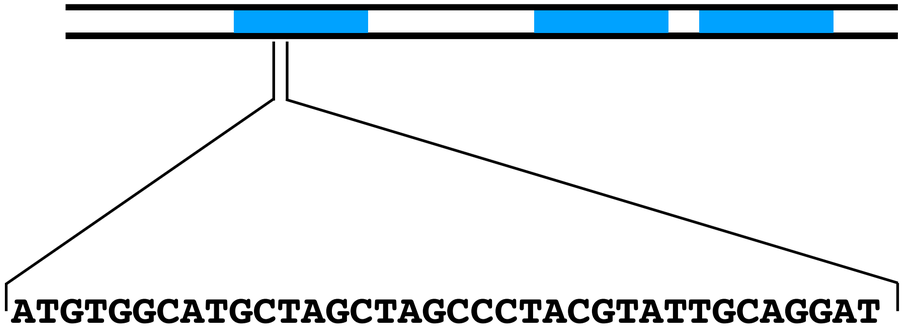


Sanger Sequencing



The basis of all modern sequencing.

figure adapted from Gibson and Muse, 3rd Edition (2009)

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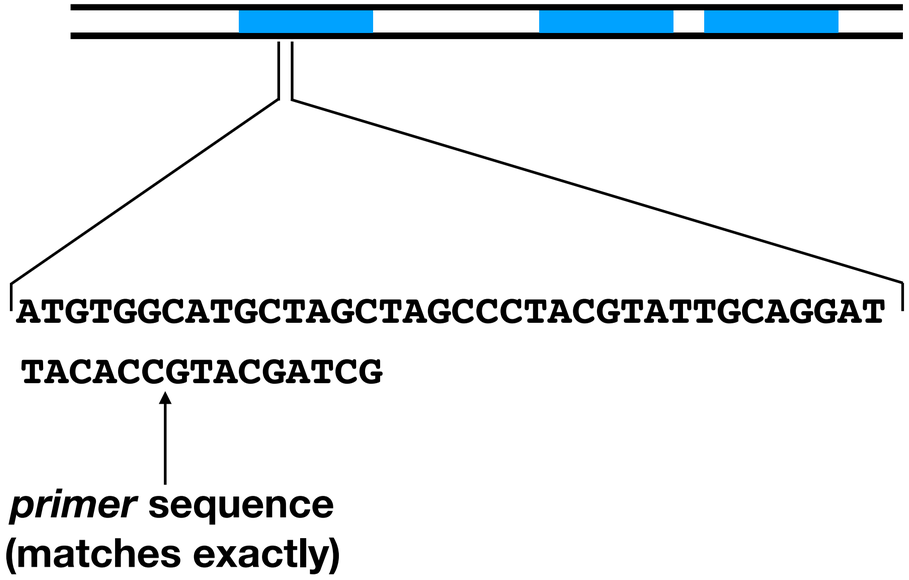

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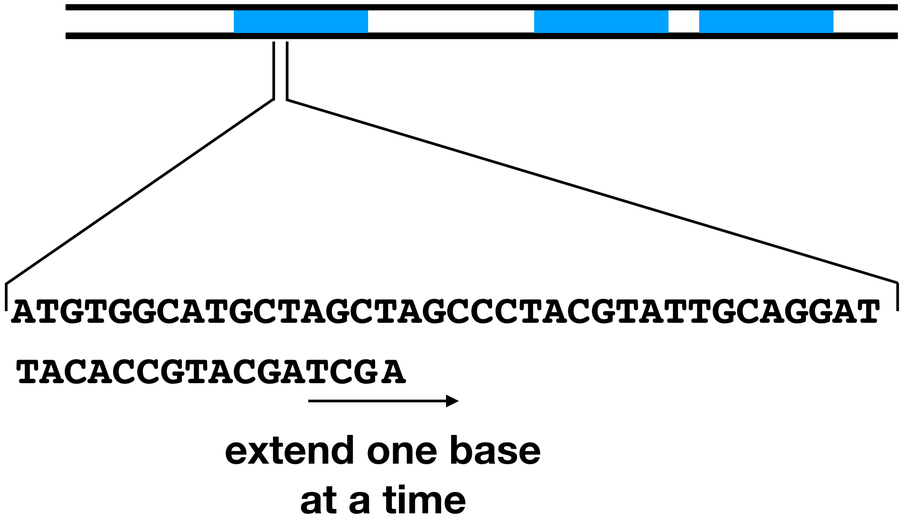

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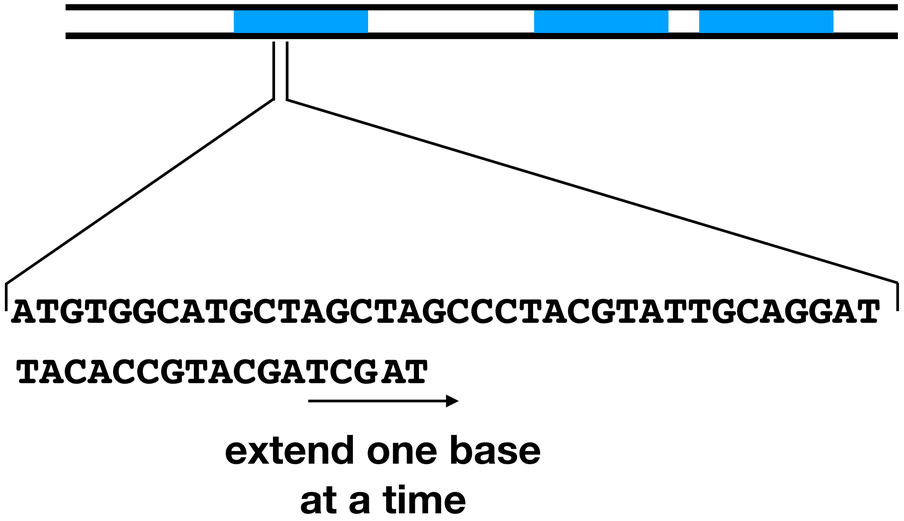

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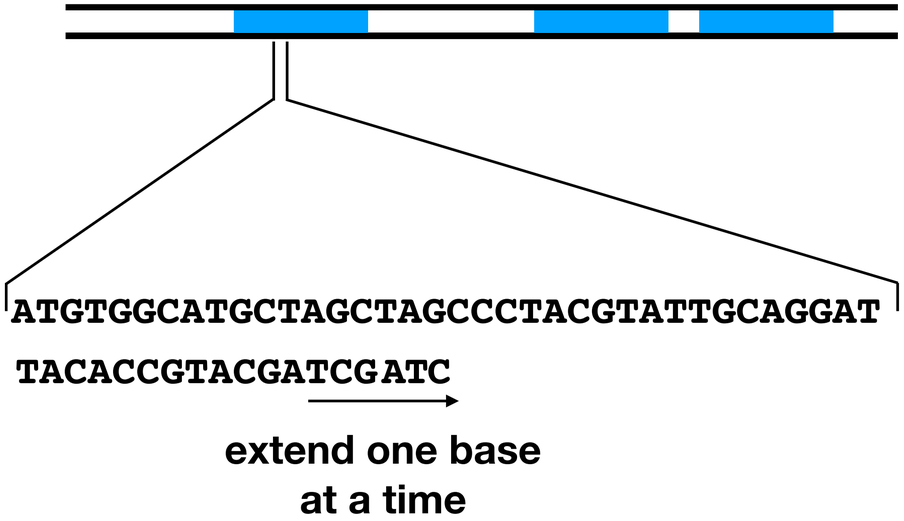

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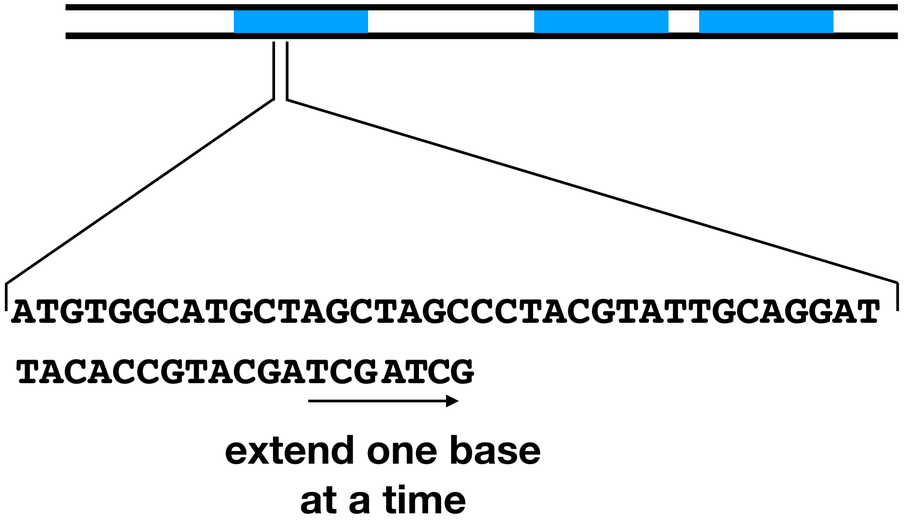

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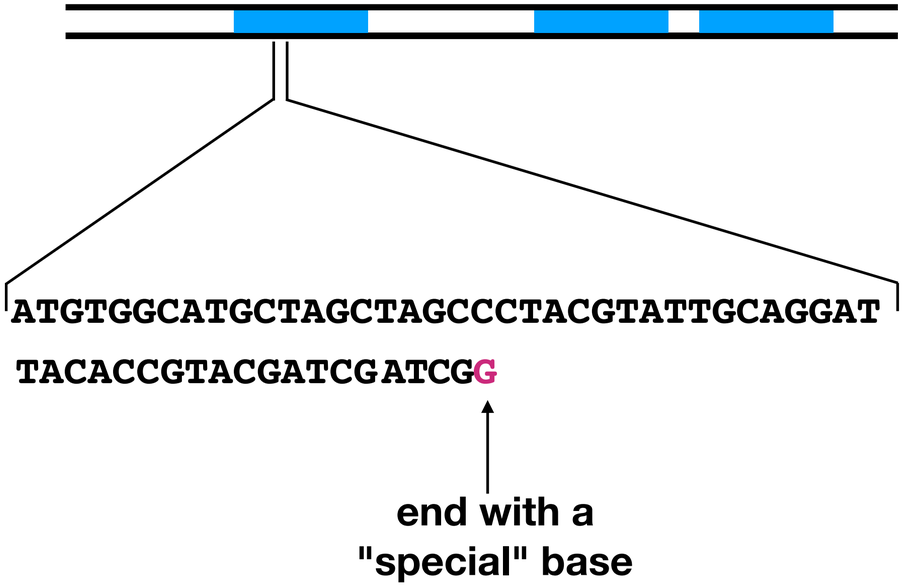

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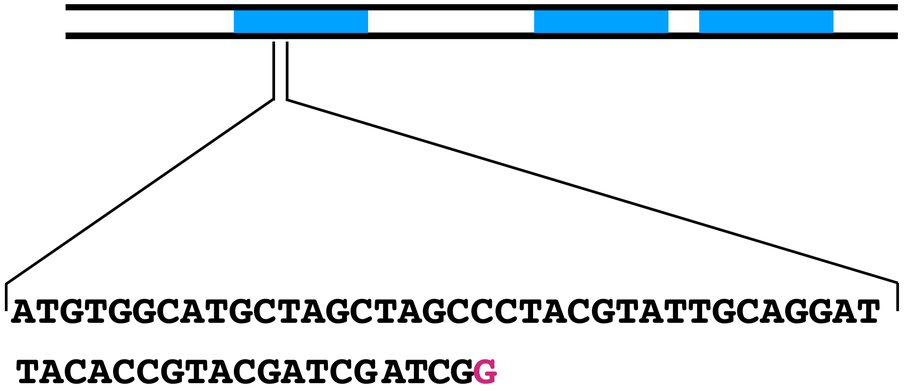

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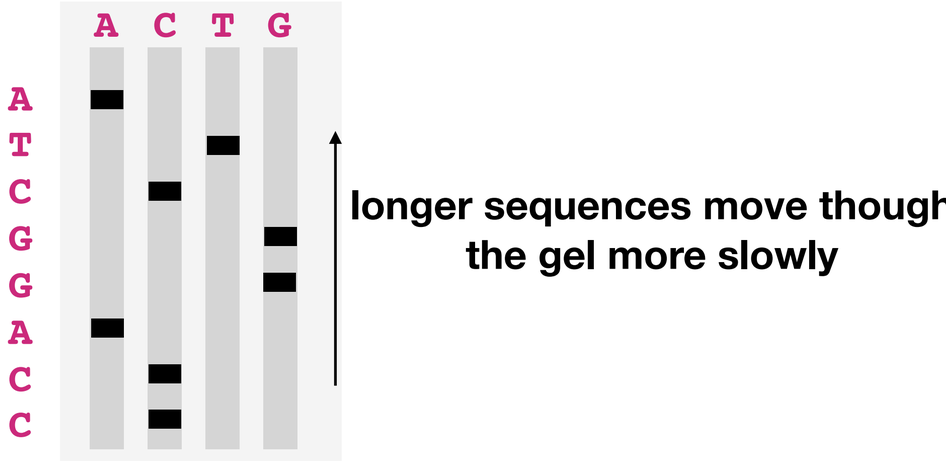
Sanger Sequencing

The basis of all modern sequencing.

```
...
TACACCGTACGATCGATCGG
TACACCGTACGATCGATCG
TACACCGTACGATCGATC
TACACCGTACGATCGAT
TACACCGTACGATCGA
```



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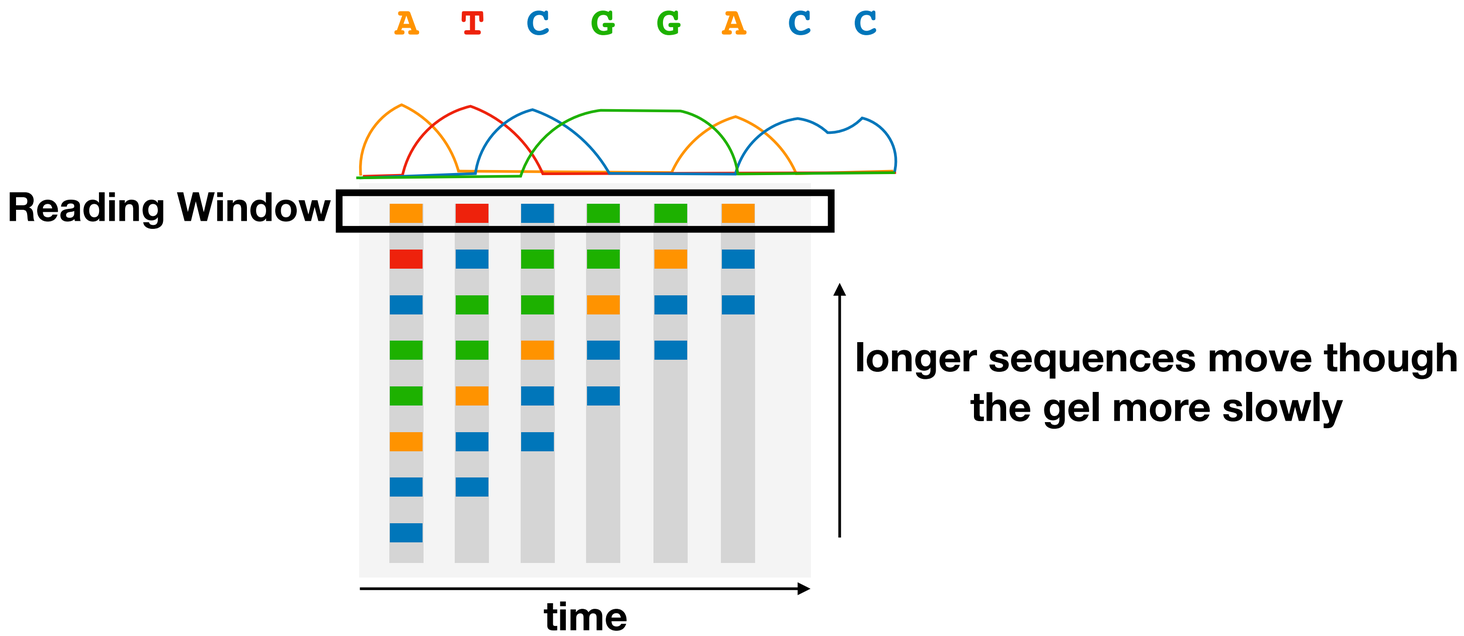


figure adapted from Gibson and Muse, 3rd Edition (2009)

Second Generation Sequencing

Also called next generation sequencing

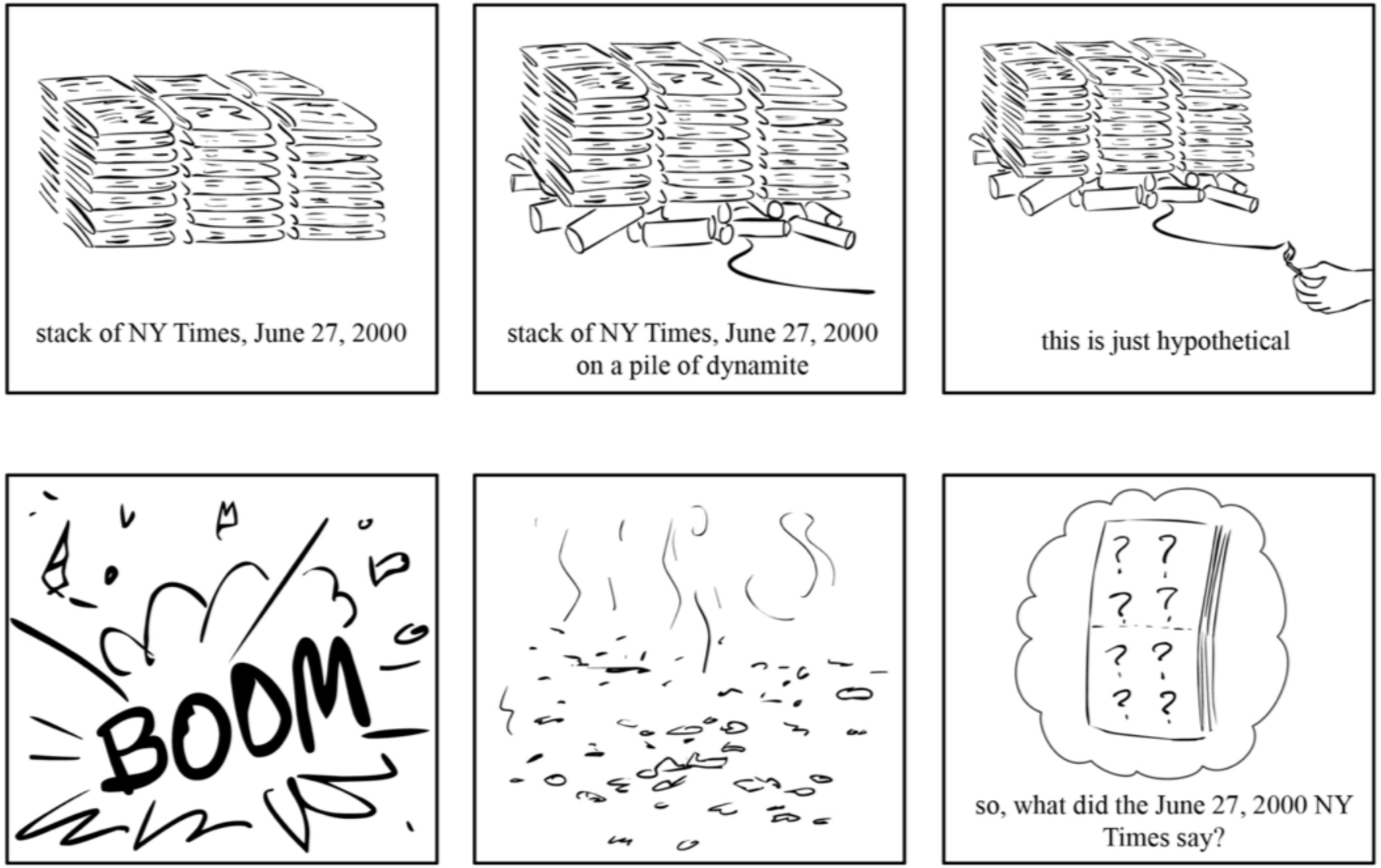
Based on the same principles, but at a much larger scale

Improvements were made in the amplification and reading with better microscopes

With this came shorter sequences

- Sanger could do >1,000 bases (characters) at once but all done by hand, so 10s of sequences, very accurate
- Illumina (current standard) ~250 base reads, 1,000,000s of sequences, some errors

Second Generation Sequencing

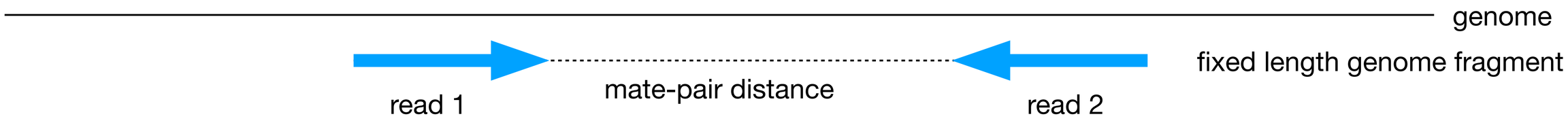


from Compeau and Pevzner 2018

Second Generation Sequencing

NextGen sequencing also introduced paired-end reads

- take a long piece of sequence (much longer than the read size, but predictable size)
- sequence both ends but keep them together
- gives two reads that you know are a certain distance from each other



Third Generation Sequencing

Recently Pacific Biosciences and Oxford Nanopore have introduced new technologies that:

- have long reads
- with high(er) error rates

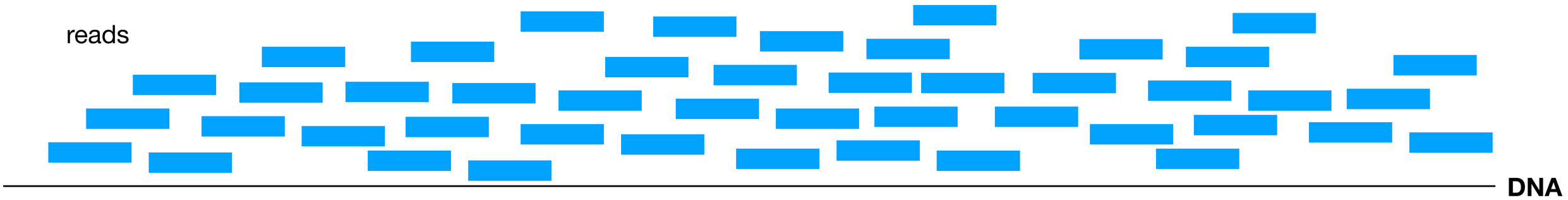
	Sanger	Next-Generation	Third-Generation
Launched	1977 Basic chemistry 1998 Modern form	2005 with significant improvements since	2010 with significant improvements since
Estimated Error Rate	0.001% - 1%	0.46% - 2.4%	11% - 14% (but decreasing)
Cost			
Throughput			
Currently Available Platforms	Applied Biosystems*	Illumina Ion Torrent* Qiagen (Europe) Complete Genomics (China)**	Pacific Biosciences Oxford Nanopore
Clinical Uses	Many (but dwindling)	Many (and growing)	Niche uses (today)

*Part of Thermo Fisher **Part of BGI

Error rate figures courtesy of G. Corey Shan from https://shanguangyu.com/articles/comparison-of-sanger-NGS-and-SMS/

Adapted from slide by Erica Ramos via SlideShare https://www.slideshare.net/EricaRamos14/acmg-2017-the-data-behind-the-results-bioinformatics-for-clinicians/7

Sequencing Applications



whole genome sequencing

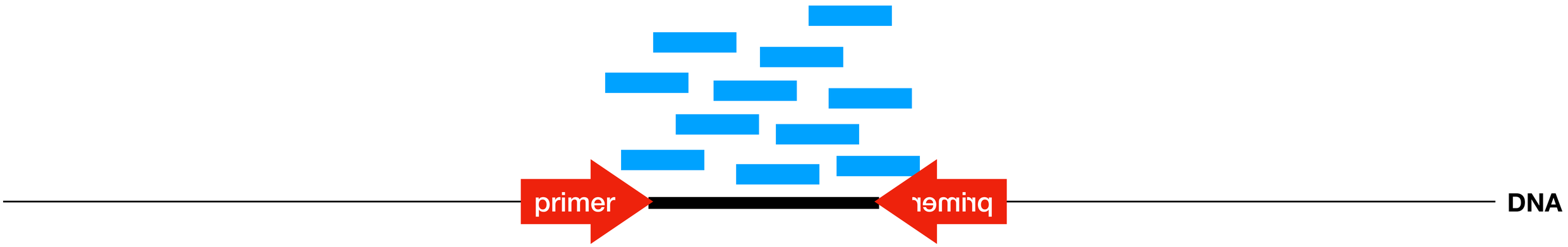
adapted from figure 1.2 in Mäkinen, *et al.* 2015

Sequencing Applications

bisulphite sequencing

adapted from figure 1.2 in Mäkinen, *et al.* 2015

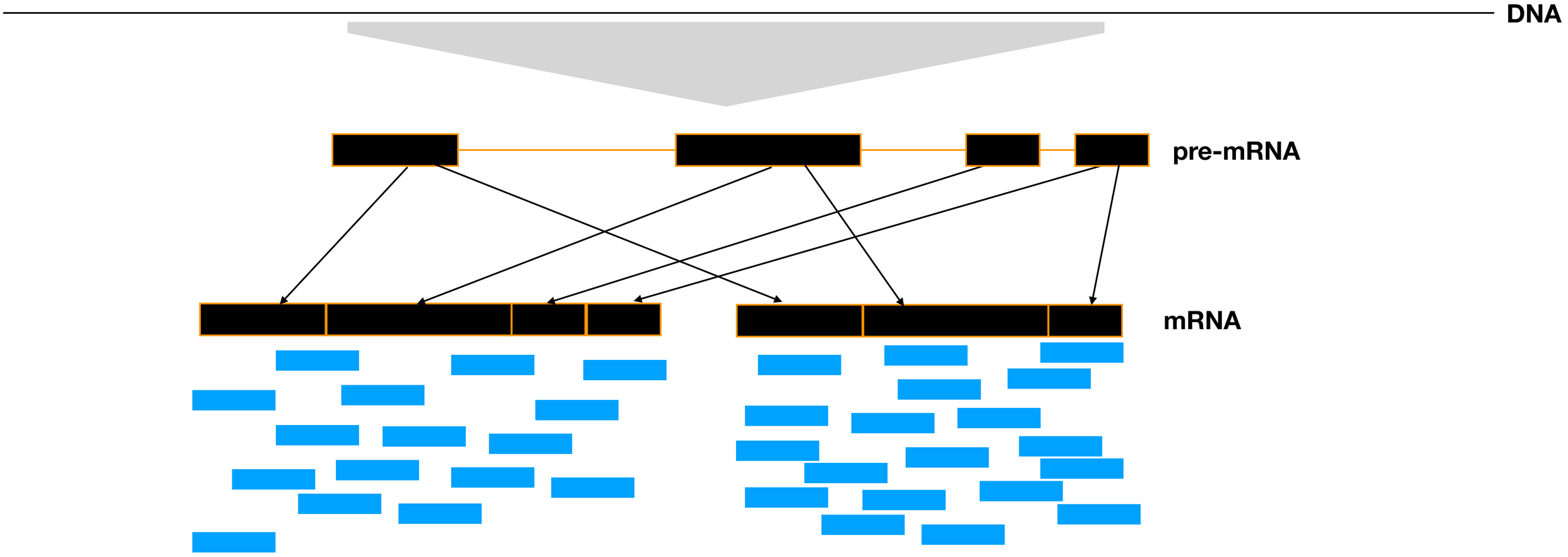
Sequencing Applications



targeted sequencing

adapted from figure 1.2 in Mäkinen, *et al.* 2015

Sequencing Applications



RNA sequencing

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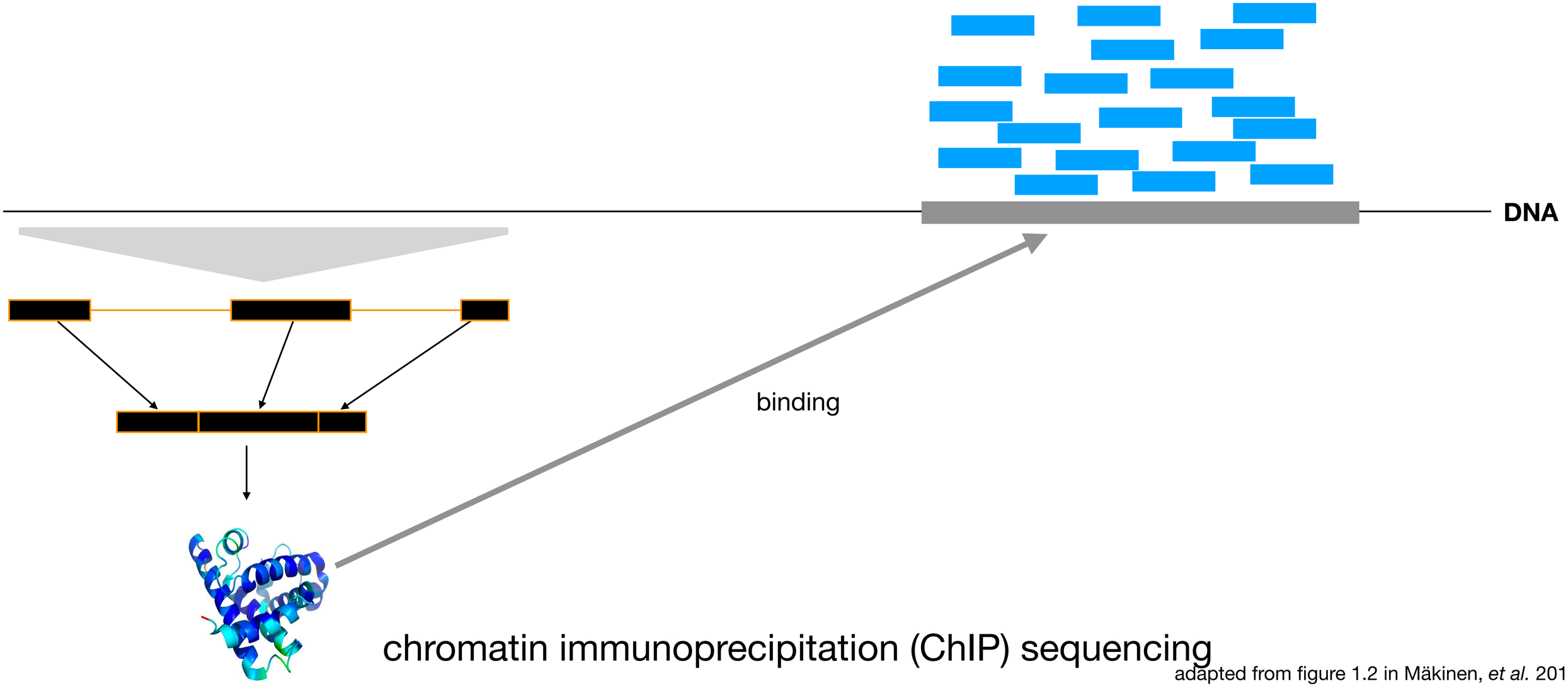
Sequencing Applications



chromatin immunoprecipitation (ChIP) sequencing

adapted from figure 1.2 in Mäkinen, *et al.* 2015