Al-Farabi Kazakh National University
Higher School of Medicine
Department of Fundamental Medicine

Genomic methods of research and diagnostics.

Lecturer and creator: PhD Pinsky Ilya Vladimirovich

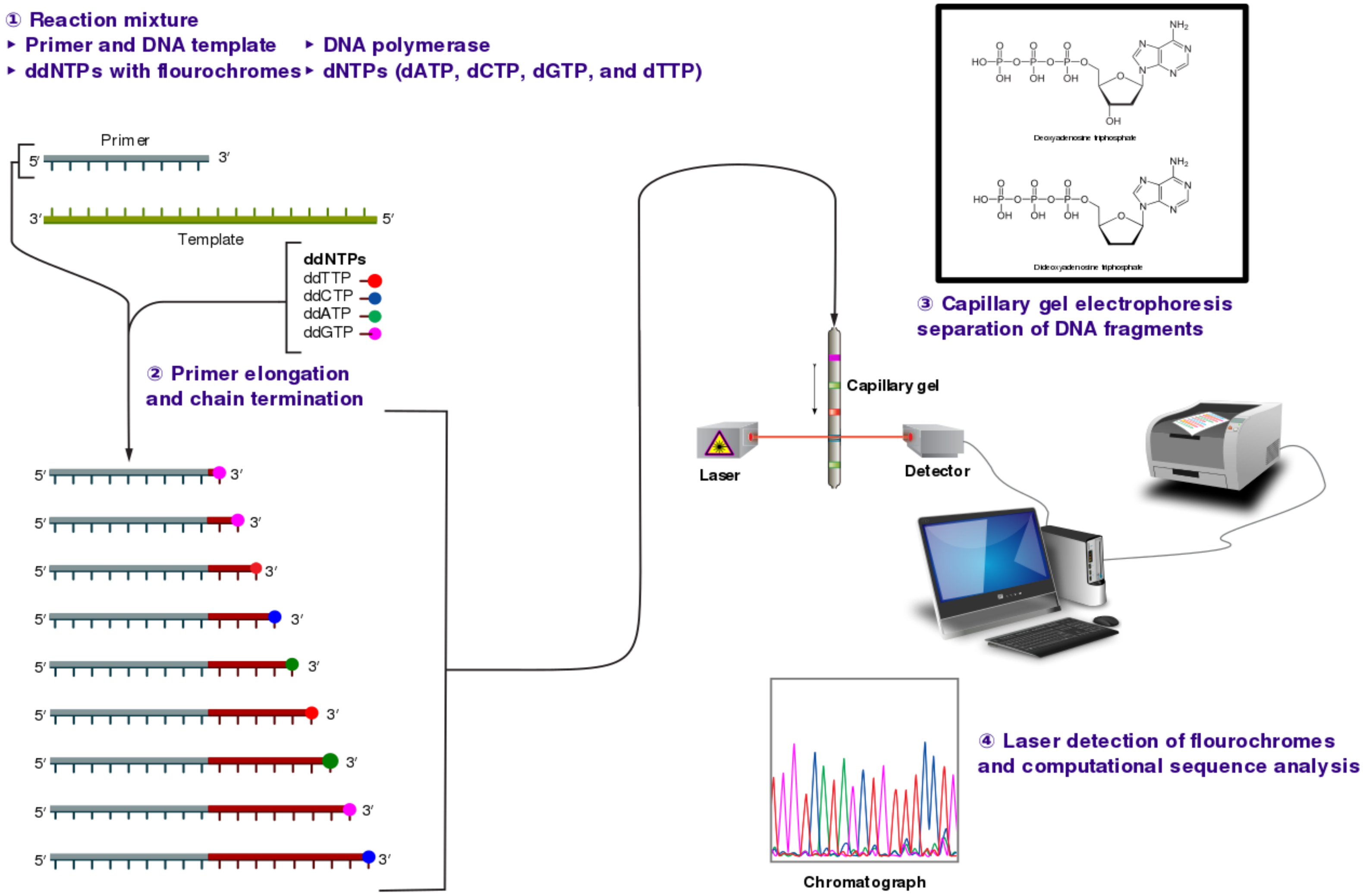
LEARNING OUTCOMES
As a result of the lesson you will be able to:

- 1. Analyze Sanger and Maxam-Gilbert methods of DNA sequencing.
- 2. Compare Sanger method with several methods of Next Generation Sequencing (NGS), analyze their advantages and disadvantages.
- 3. Describe "short gun-sequencing" and "chromosome walking" methods of genome sequencing.
- 4. Characterize bioinformatical methods of genome analysis (genome assembling, genome annotation, finding of open-reading frames (ORFs) and prediction of genes, alignment of nucleotide sequences, protein structure prediction and etc.). Give specific examples of used programs and bioinformatical databases.
- 5. Explain the main traditional methods of molecular biology that are used in genomic research: polymerase chain reaction (PCR), gel-electrophoresis, northern blotting, Southern blotting, restriction analysis and etc.
- 6. Describe the methods of DNA genotyping, DNA diagnostics, DNA fingerprinting and DNA microarray.

Methods of Genomics

- Genome sequencing
- Experimental genome analysis
- Bioinformatical (computational) genome analysis

The Sanger (chain-termination) method for DNA sequencing.



① **Reaction mixture**

- **Primer and DNA template**
- **ddNTPs with flourochromes**
- **DNA polymerase**
- **dNTPs (dATP, dCTP, dGTP, and dTTP)**

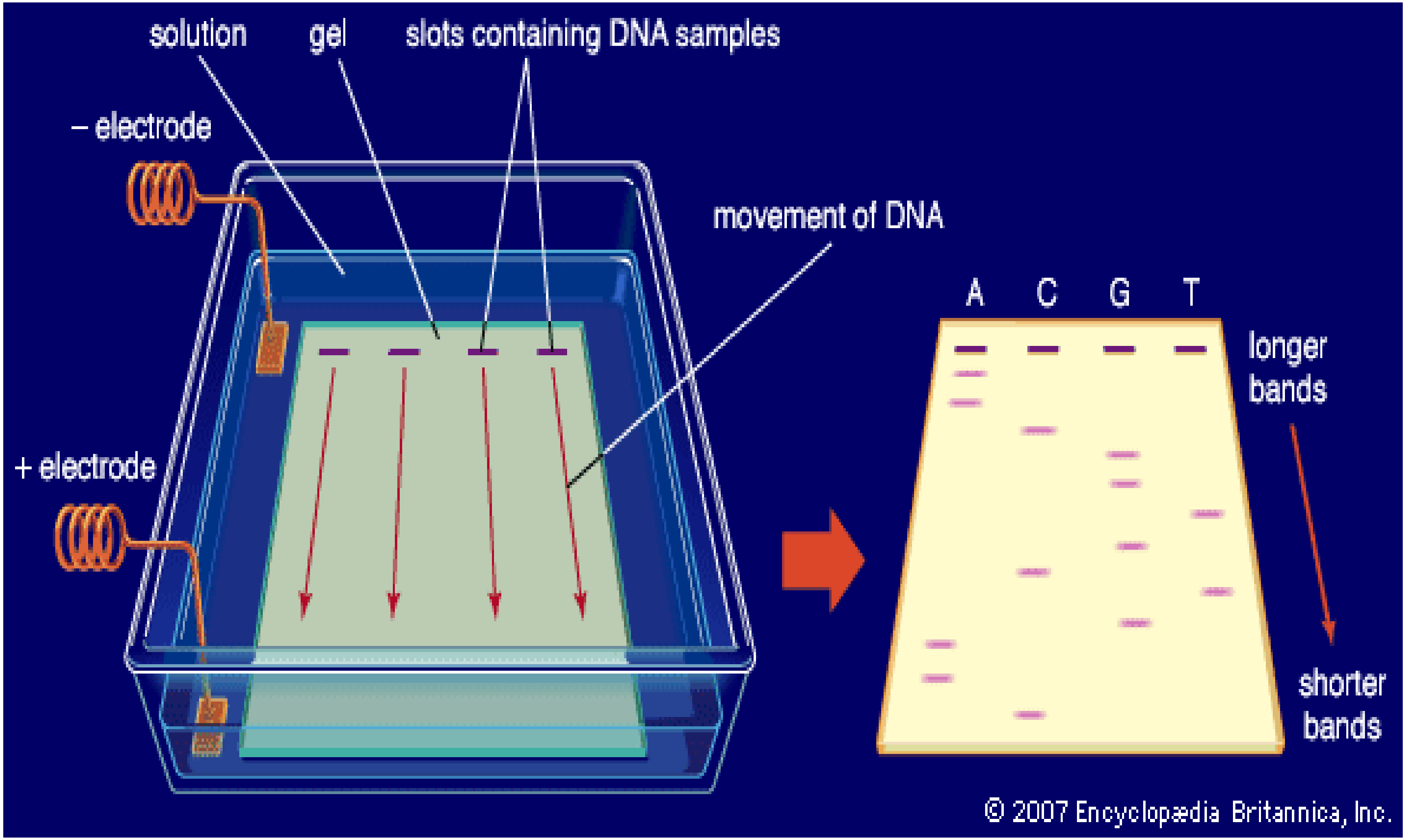
② **Primer elongation and chain termination**

③ **Capillary gel electrophoresis separation of DNA fragments**

④ **Laser detection of flourochromes and computational sequence analysis**

https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Gel electrophoresis



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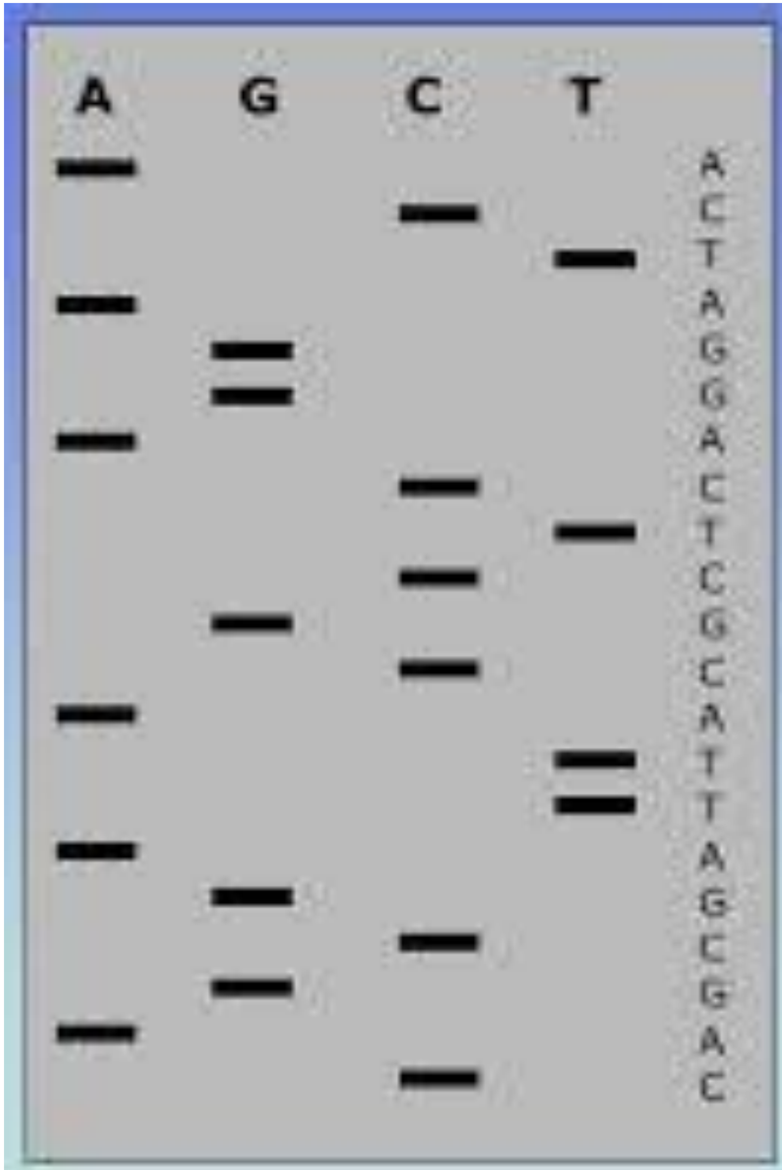
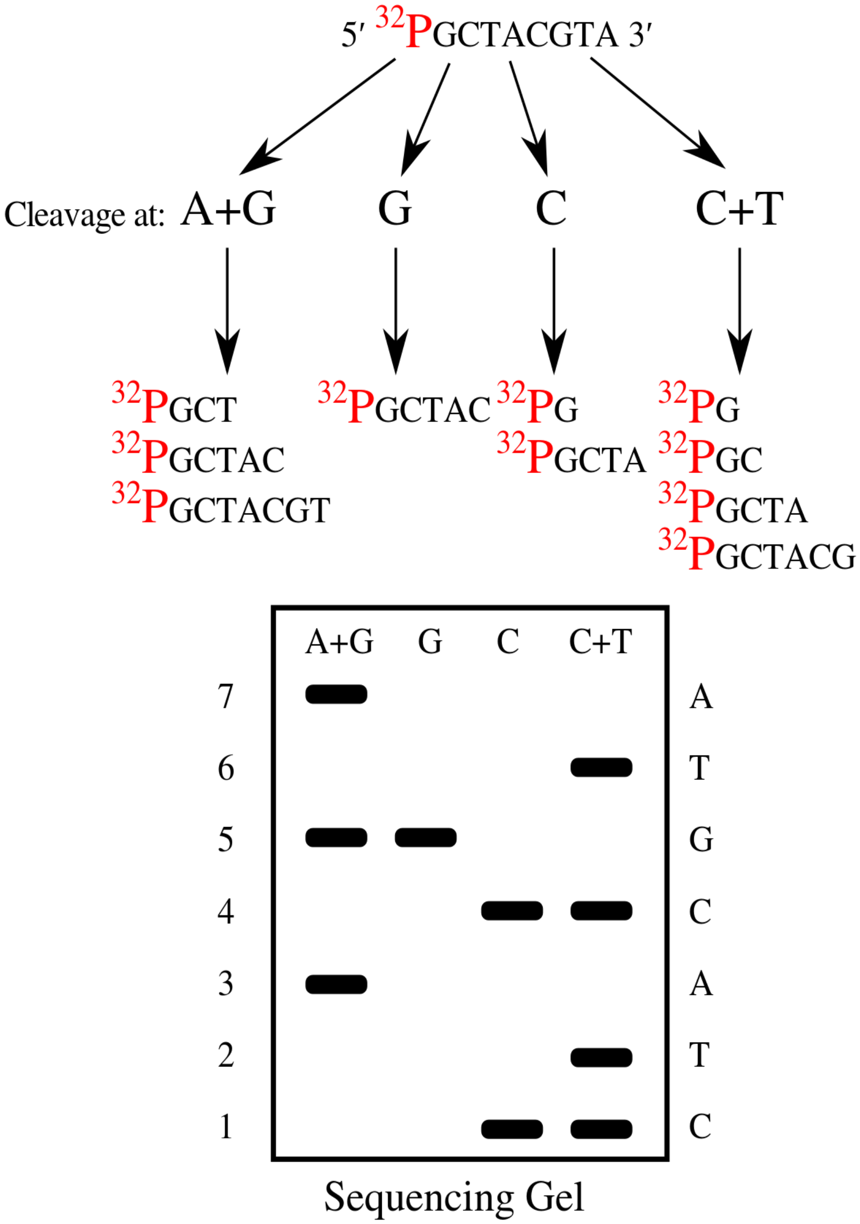


Figure 1: Manual Sequencing Using Radiolabeled ddNTPs

Manual sequencing using the Sanger method originally used radioactively labeled ddNTPs. Because there was no method to detect the difference between the A,G,C, and T bases, the reaction for each bases was done separately and loaded into separate lanes on a polyacrylamide gel as shown.

https://agctsequencing.wordpress.com/2012/08/01/sanger-sequencing-historical-development-of-automated-dna-sequencing/

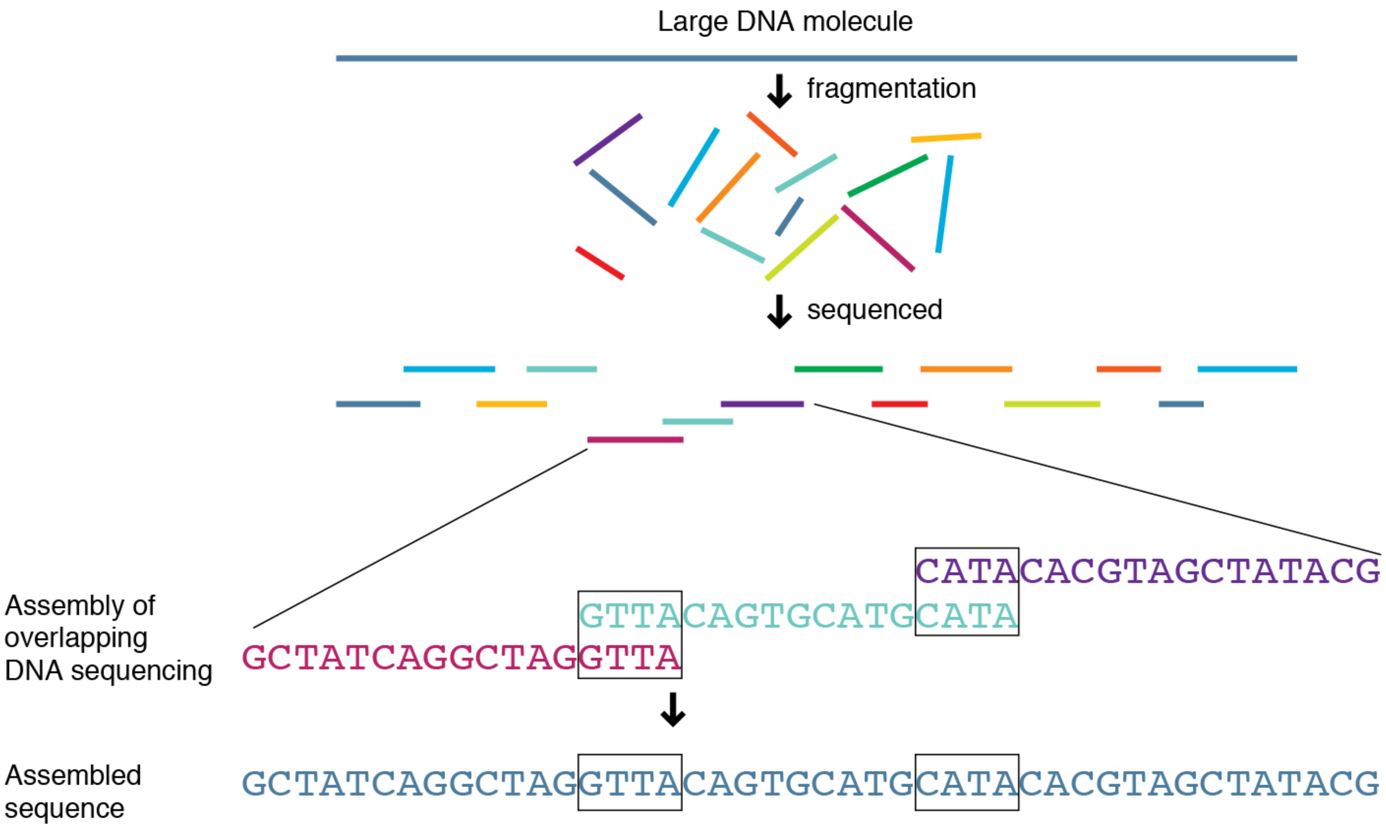
Maxam-Gilbert DNA sequencing



Sequencing Gel

An example of Maxam–Gilbert sequencing reaction. Cleaving the same tagged segment of DNA at different points yields tagged fragments of different sizes. The fragments may then be separated by gel electrophoresis. https://en.wikipedia.org/wiki/Maxam%E2%80%93Gilbert_sequencing

Shortgun sequencing



Large DNA molecule

fragmentation

sequenced

Assembly of overlapping DNA sequencing

CATACACGTAGCTATACG

GTTACAGTGCATGCATA

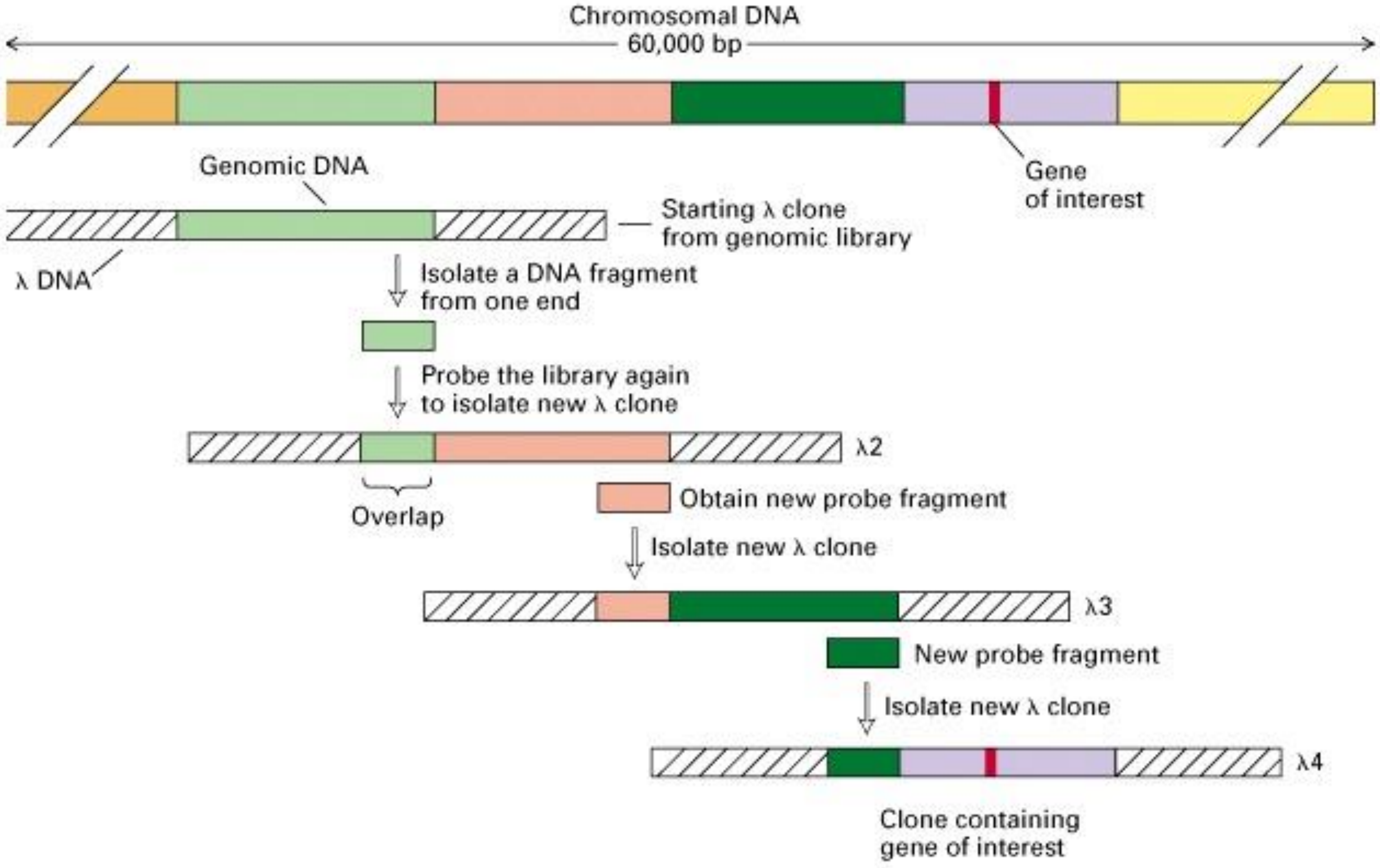
GCTATCAGGCTAGGTTA

Assembled sequence

GCTATCAGGCTAGGTTACAGTGCATGCATACACGTAGCTATACG

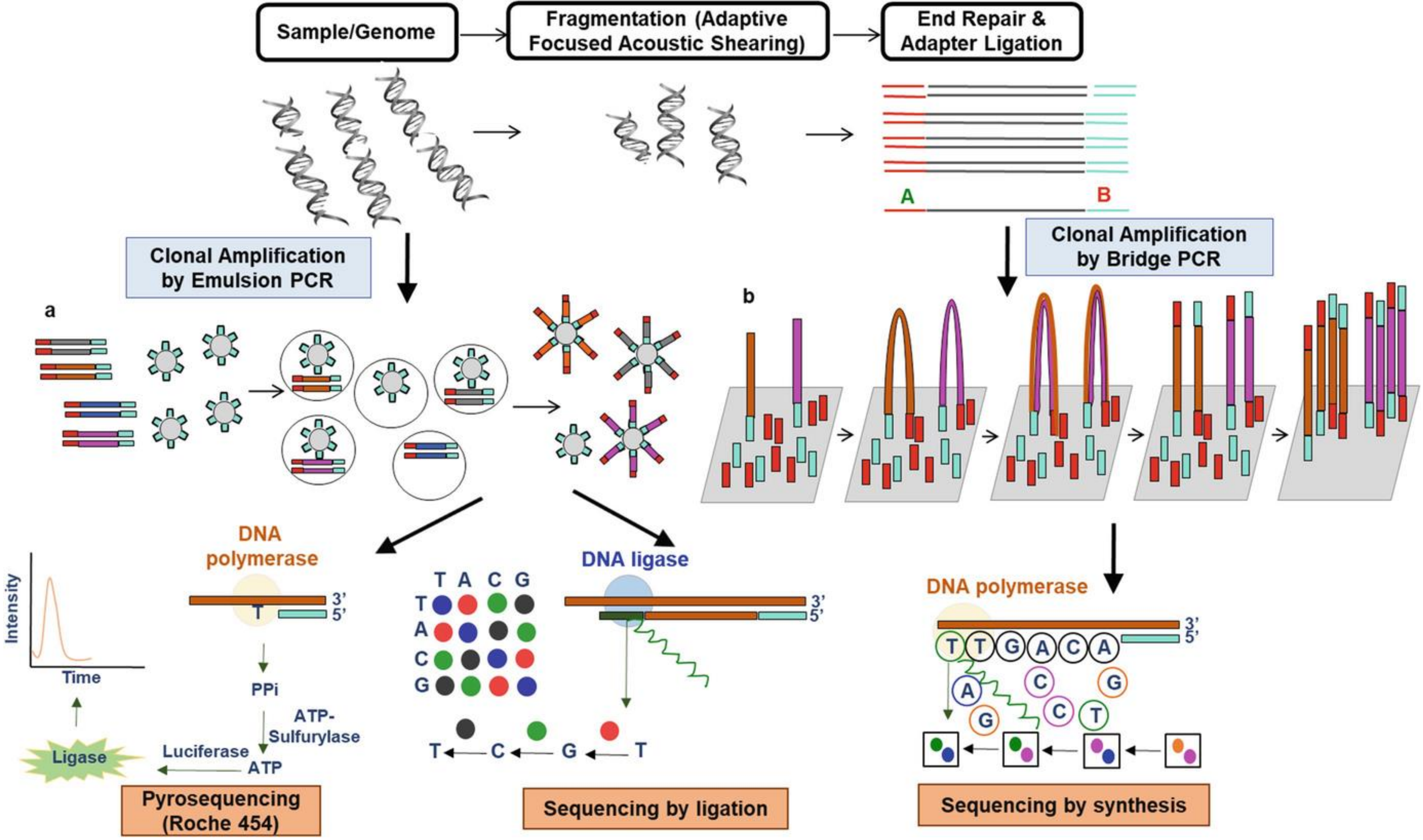
https://www.genome.gov/genetics-glossary/Shotgun-Sequencing

Chromosome walking



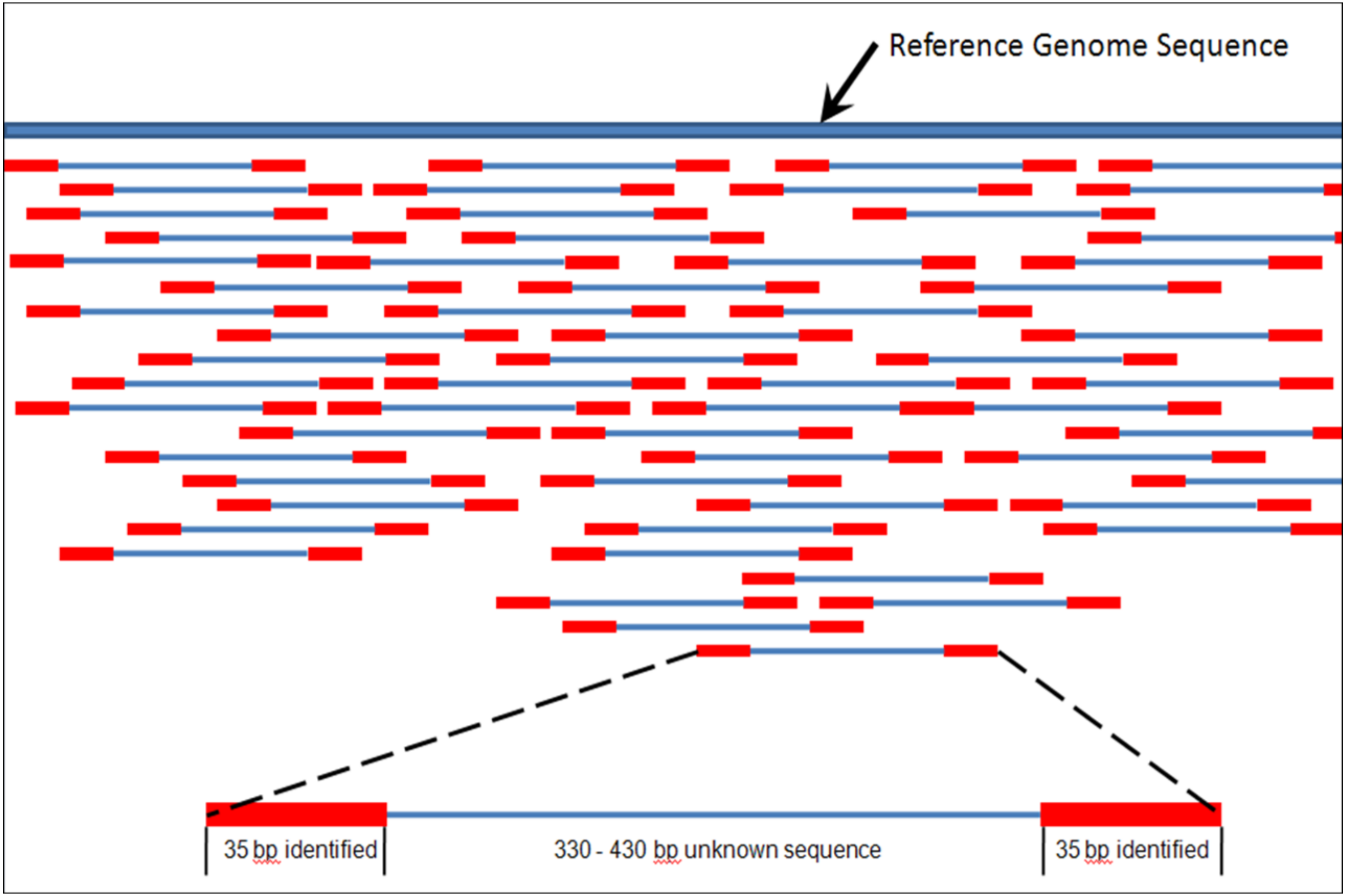
https://www.google.com/url?sa=i&url=http%3A%2F%2Fgvbioteck.blogspot.com%2F2012%2F09%2Fchromosome-walking.html&psig=AOvVaw1Efr8a66zZIvm2f80hb2UM&ust=1614627414190000&source=images&cd=vfe&ved=2ahUKEwjbkIH9qY3vAhWYuyoKHan9AbIQr4kDegUIARC2AQ

Next generation sequencing

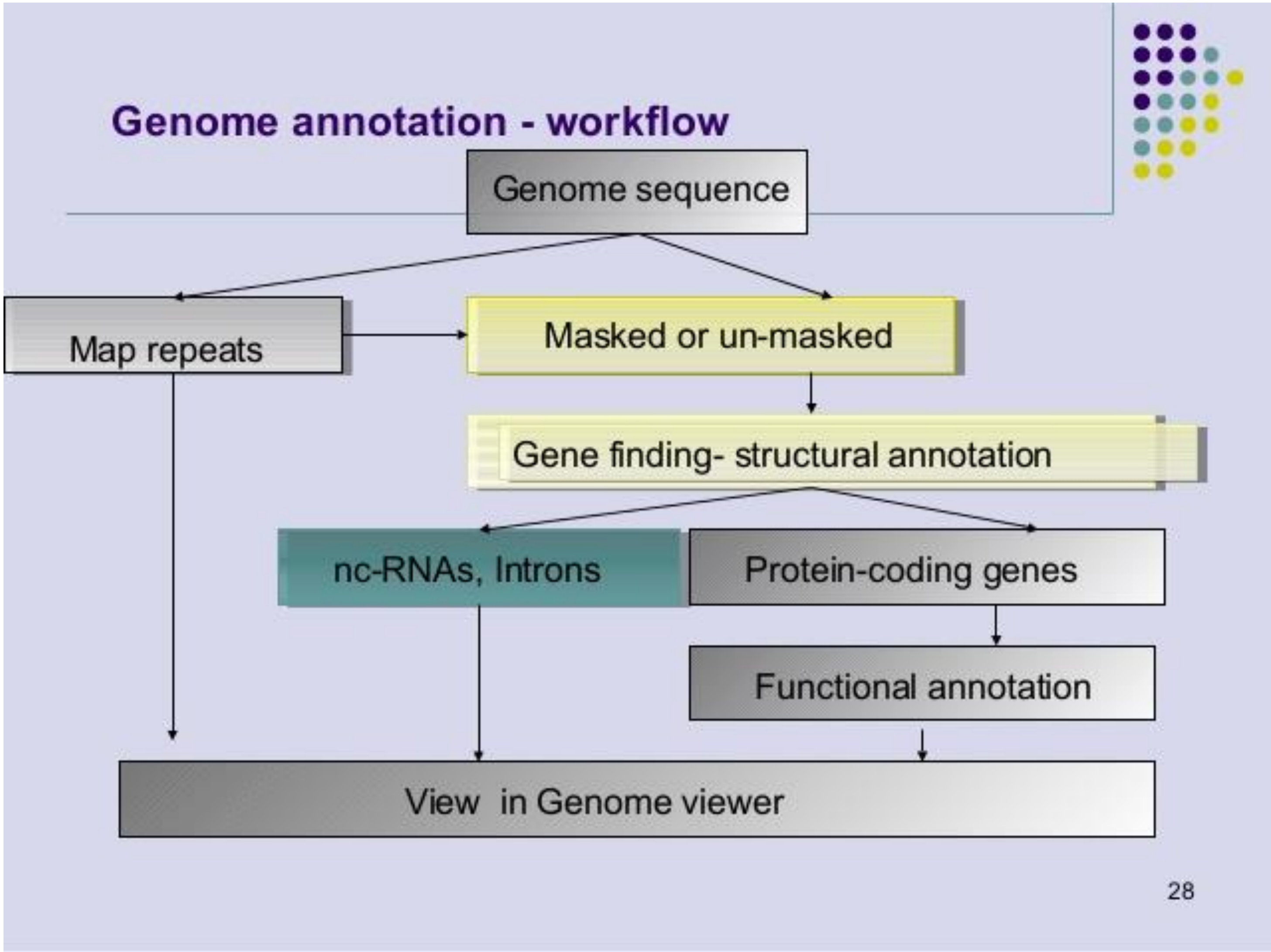


link.springer.com

Genome assembling



https://en.wikipedia.org/wiki/Genomics#/media/File:Mapping_Reads.png



Genome annotation - workflow

Genome sequence

Map repeats

Masked or un-masked

Gene finding- structural annotation

nc-RNAs, Introns

Protein-coding genes

Functional annotation

View in Genome viewer

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.slideshare.net%2Fkaranppt%2Fgenome-annotation-2013&psig=AOvVaw3W0yVtMND0yOoaRAbiACi3&ust=1614628815714000&source=images&cd=vfe&ved=2ahUKEwjtt6eZr43vAhXaBncKHVEDCNQQr4kDegUIARDIAQ

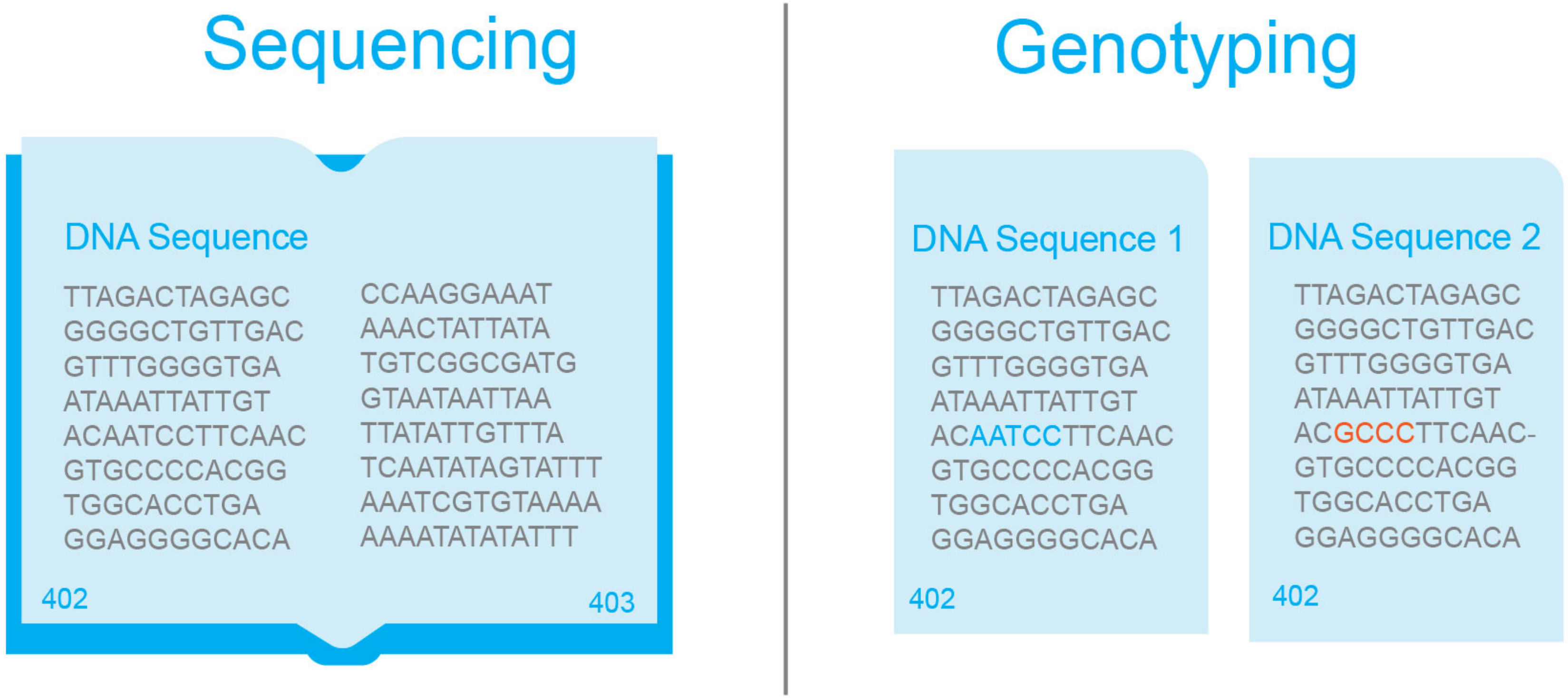
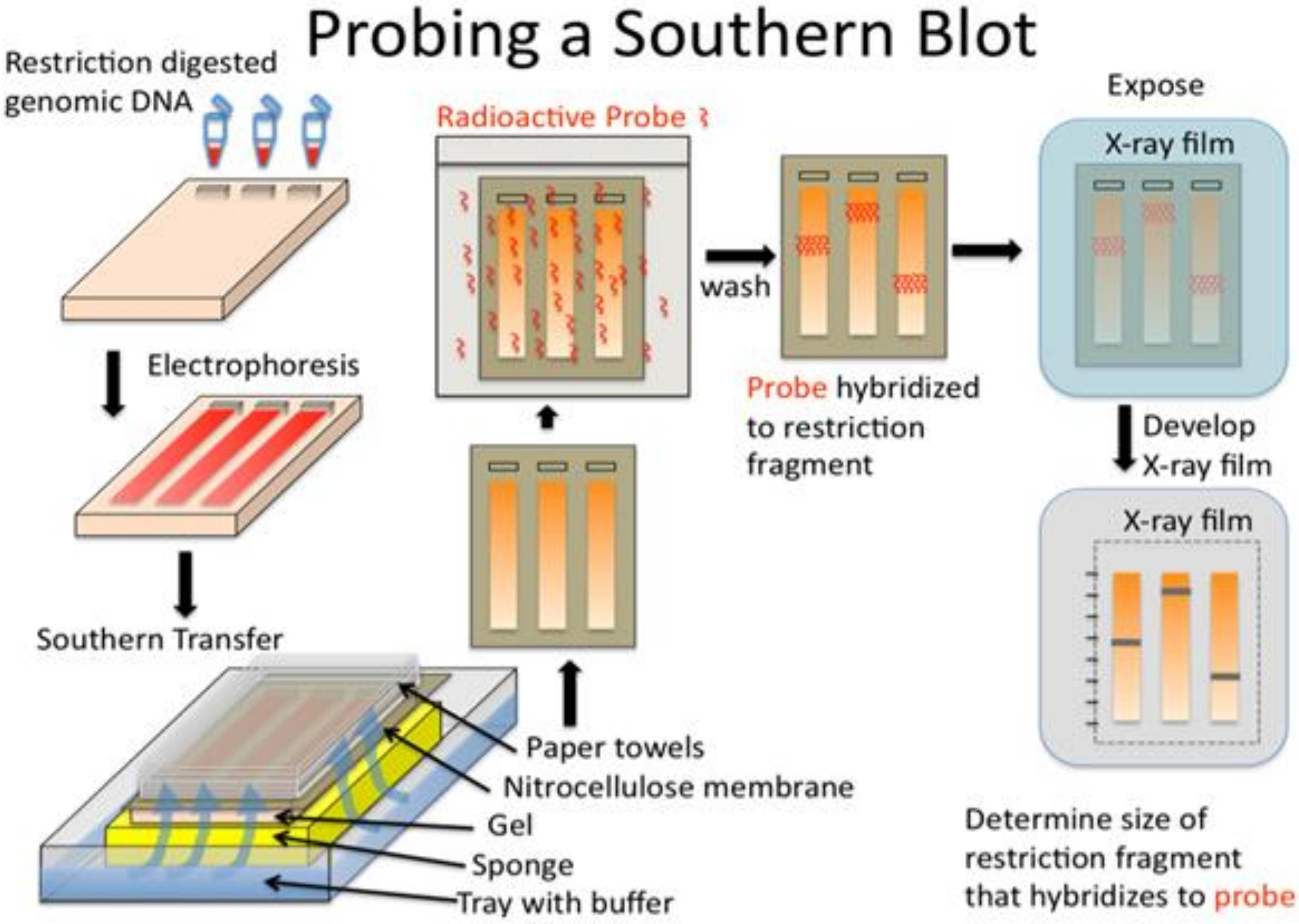


Figure 1. *Illustrates the analogy of a book being used to compare sequencing to genotyping. Sequencing (left) is like reading out all of the letters in a book. Genotyping (right) is like looking at a specific section of a specific page, and comparing to others of the same page.*

Probing a Southern Blot



bio.libretexts.org

DNA microarray

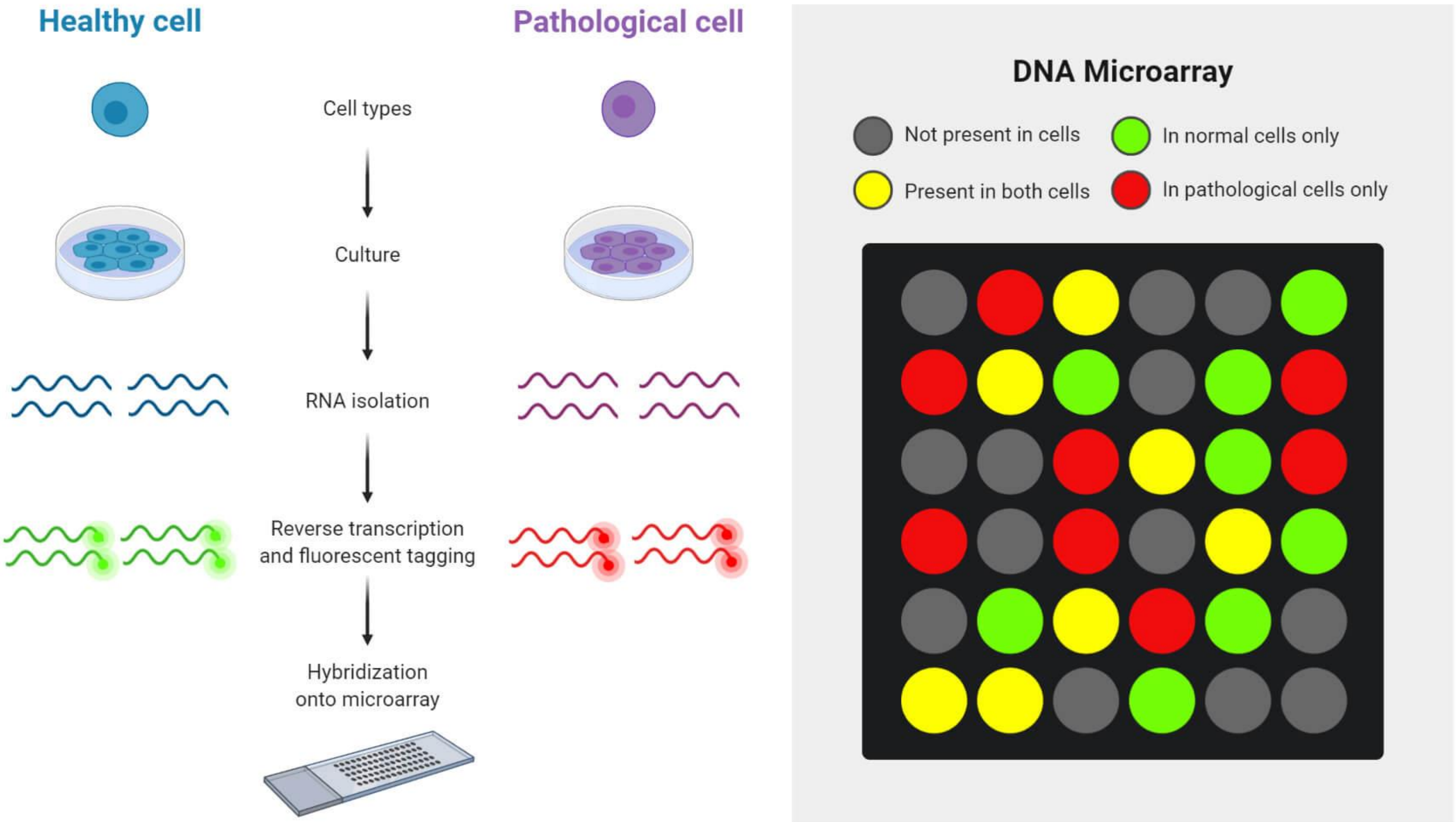
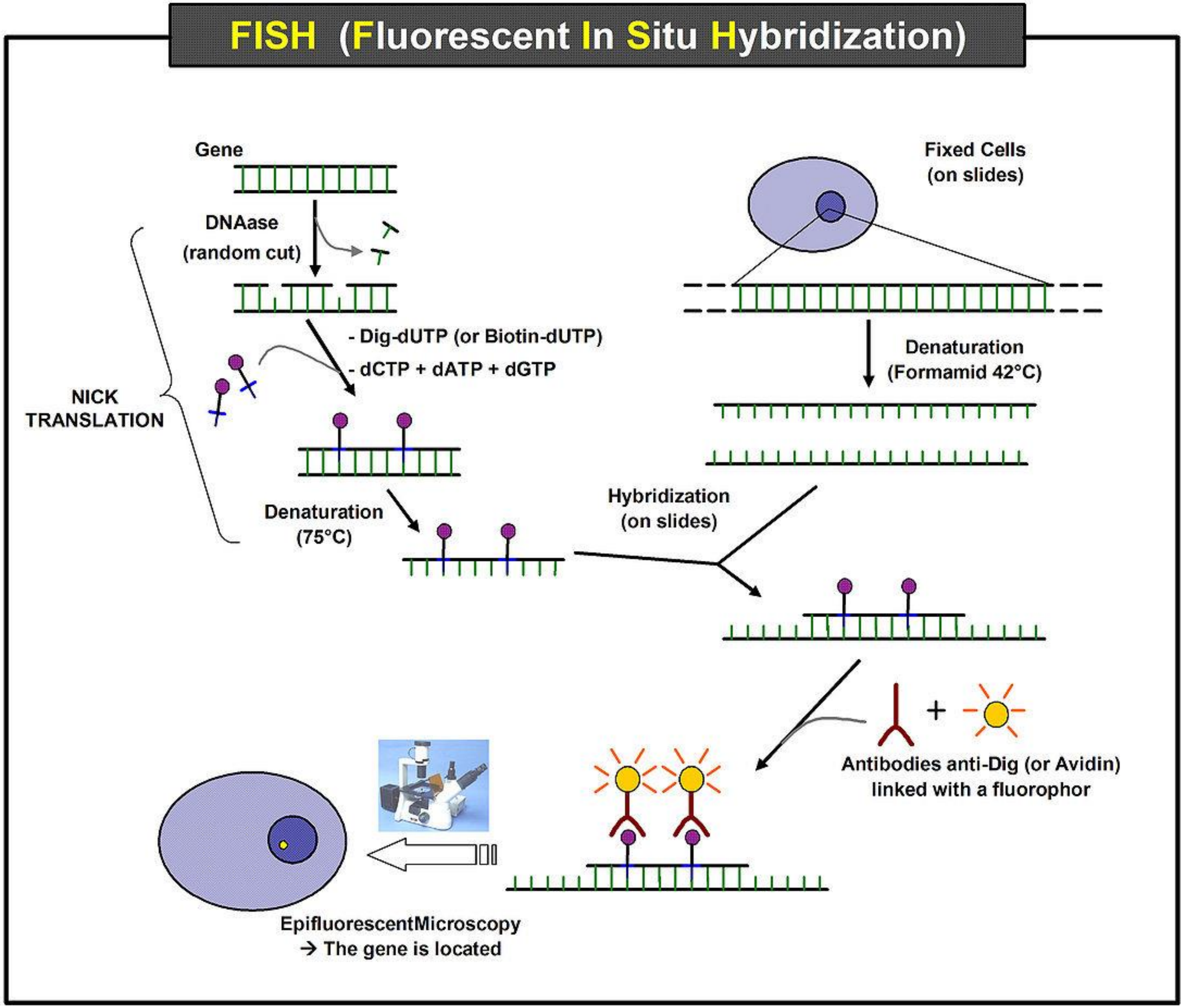
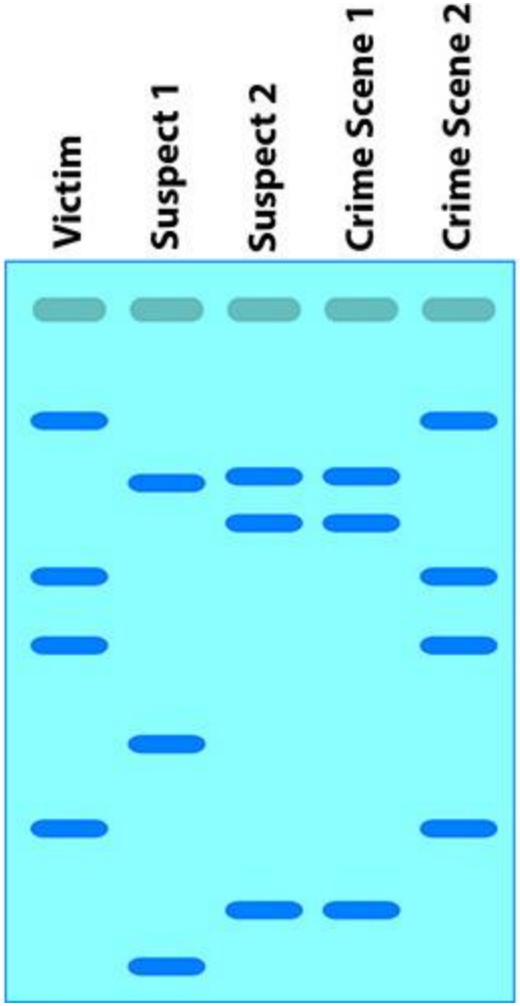


Image By Sagar Aryal, created using biorender.com



https://en.wikipedia.org/wiki/Fluorescence_in_situ_hybridization#/media/File:FISH_(Fluorescent_In_Situ_Hybridization).jpg

DNA fingerprinting



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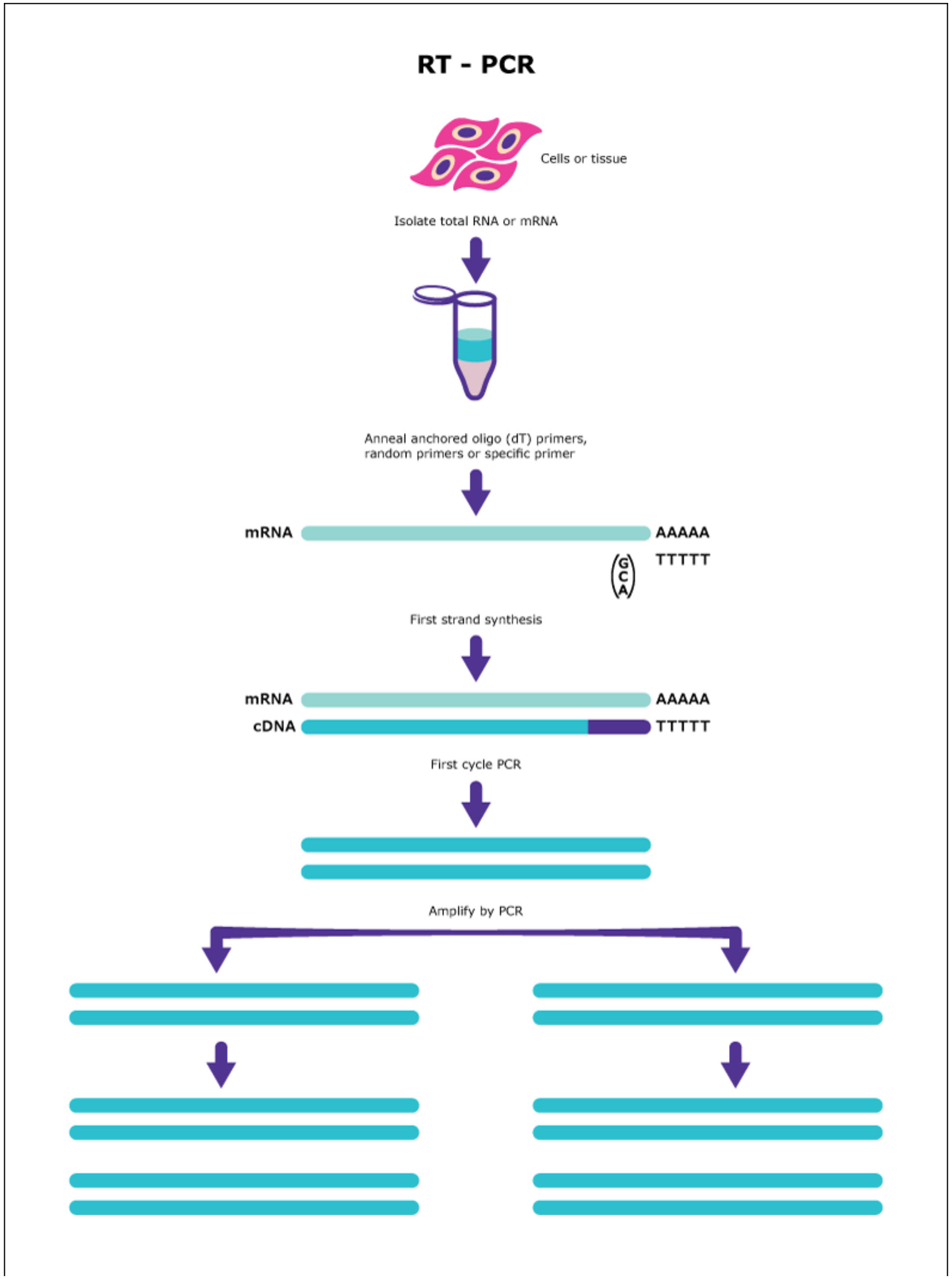
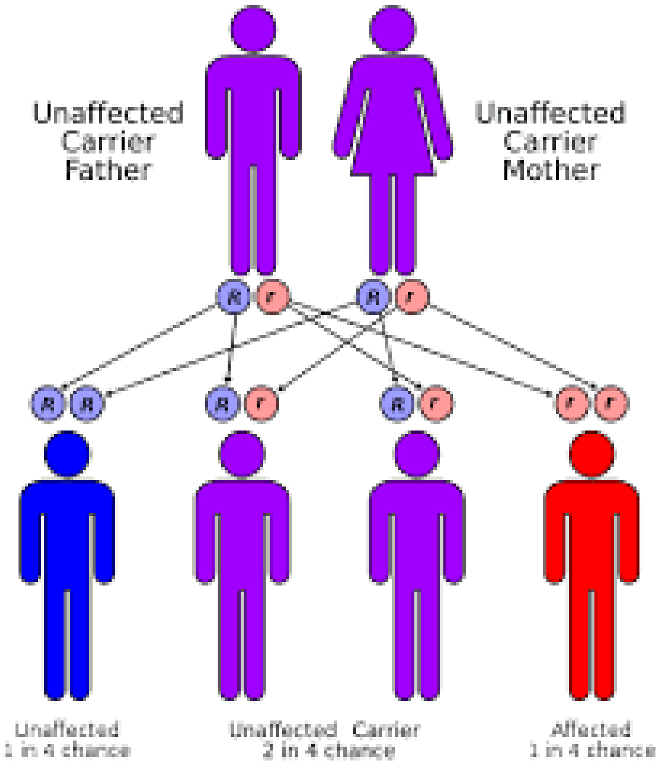


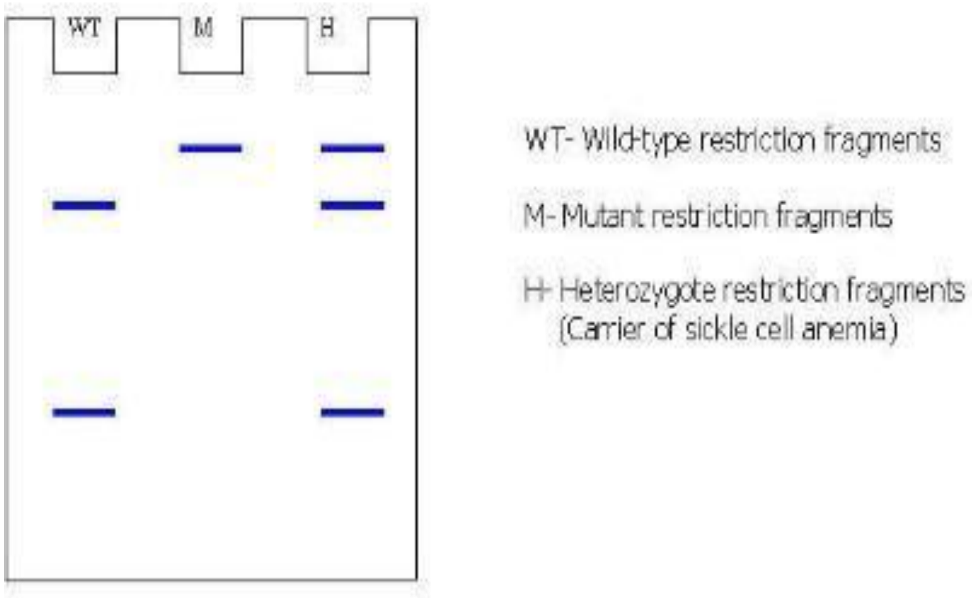
PCR diagnostics.

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.reddit.com%2Fr%2FCOVID19%2Fcomments%2Ffb859l%2Frtpcr_diagnostics_what_are_they_and_how_do_they%2F&psig=AOvVaw2xxYqnR3Tv9-QQkfRIi37h&ust=1614630255452000&source=images&cd=vfe&ved=2ahUKEwi4g-rHtI3vAhUGwSoKHSIoDzgQr4kDegUIARC7AQ

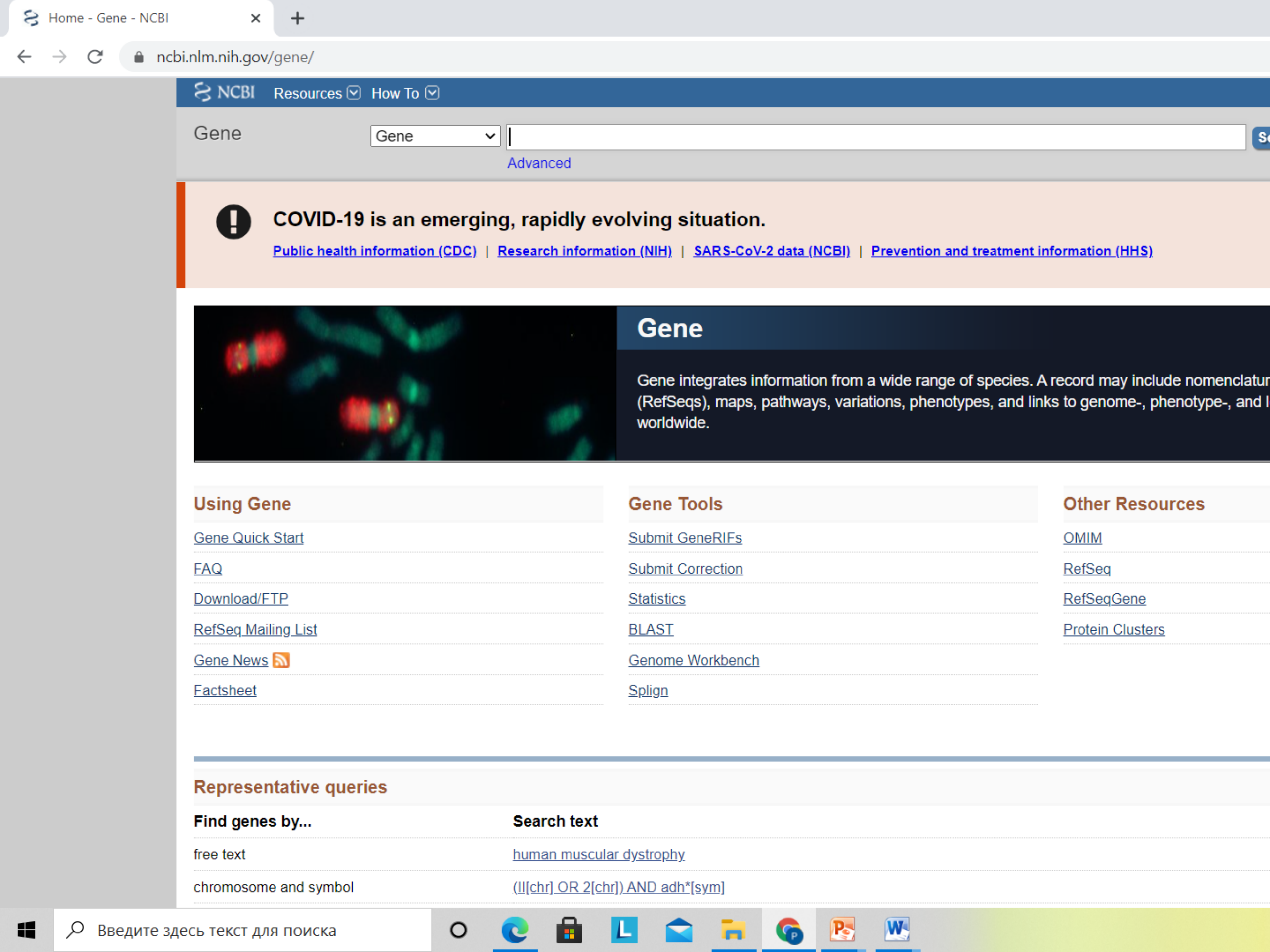
Restriction analysis of sickle-cell anemia



Sickle cell disease - Wikiwand
wikiwand.com



Sickle Cell Anemia | Muhlenberg College
muhlenberg.edu



COVID-19 is an emerging, rapidly evolving situation.

Public health information (CDC) | Research information (NIH) | SARS-CoV-2 data (NCBI) | Prevention and treatment information (HHS)

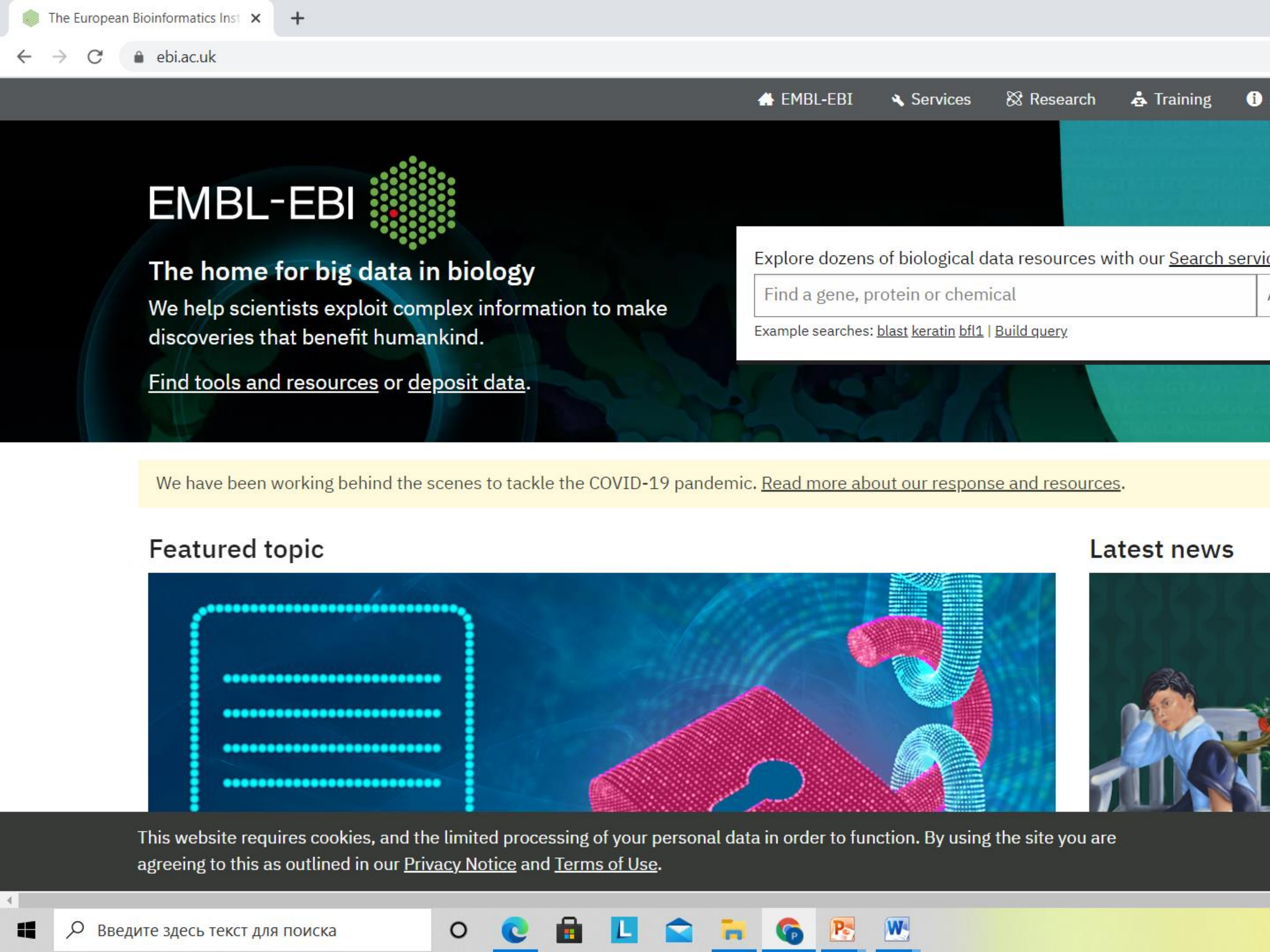
Gene

Gene integrates information from a wide range of species. A record may include nomenclatur
(RefSeqs), maps, pathways, variations, phenotypes, and links to genome-, phenotype-, and l
worldwide.

Using Gene	Gene Tools	Other Resources
Gene Quick Start	Submit GeneRIFs	OMIM
FAQ	Submit Correction	RefSeq
Download/FTP	Statistics	RefSeqGene
RefSeq Mailing List	BLAST	Protein Clusters
Gene News	Genome Workbench	
Factsheet	Splign	

Representative queries

Find genes by...	Search text
free text	human muscular dystrophy
chromosome and symbol	(II[chr] OR 2[chr]) AND adh*[sym]



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We help scientists exploit complex information to make discoveries that benefit humankind.

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Example searches: blast keratin bfl1 | Build query

We have been working behind the scenes to tackle the COVID-19 pandemic. Read more about our response and resources.

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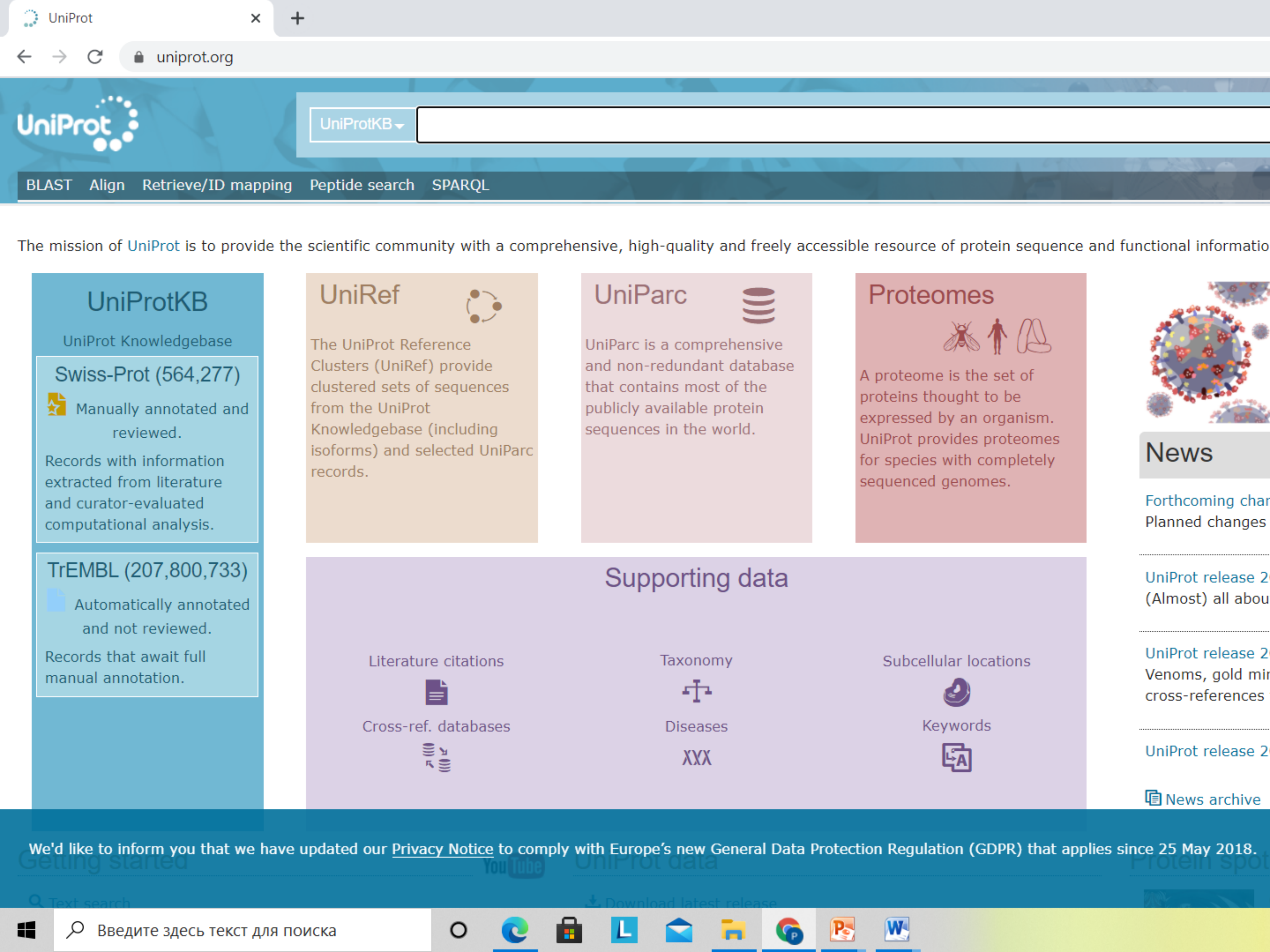

UniProt

uniprot.org

UniProtKB

BLAST Align Retrieve/ID mapping Peptide search SPARQL

The mission of UniProt is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional informatio

UniProtKB

UniProt Knowledgebase

Swiss-Prot (564,277)

Manually annotated and reviewed.

Records with information extracted from literature and curator-evaluated computational analysis.

TrEMBL (207,800,733)

Automatically annotated and not reviewed.

Records that await full manual annotation.

UniRef

The UniProt Reference Clusters (UniRef) provide clustered sets of sequences from the UniProt Knowledgebase (including isoforms) and selected UniParc records.

UniParc

UniParc is a comprehensive and non-redundant database that contains most of the publicly available protein sequences in the world.

Proteomes

A proteome is the set of proteins thought to be expressed by an organism. UniProt provides proteomes for species with completely sequenced genomes.

Supporting data

Literature citations

Taxonomy

Subcellular locations

Cross-ref. databases

Diseases

Keywords

News

Forthcoming char

Planned changes

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(Almost) all abou

UniProt release 2

Venoms, gold mi

cross-references

UniProt release 2

News archive

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