UNIVERSITY OF GOTHENBURG

Introduction to scientific writing

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Introduction to scientific writing

- Reading a research article - T1
- Summarize, report and discuss scientific results – T1 & T3

- Searching scientific literature– T3
- Writing a research article – T3

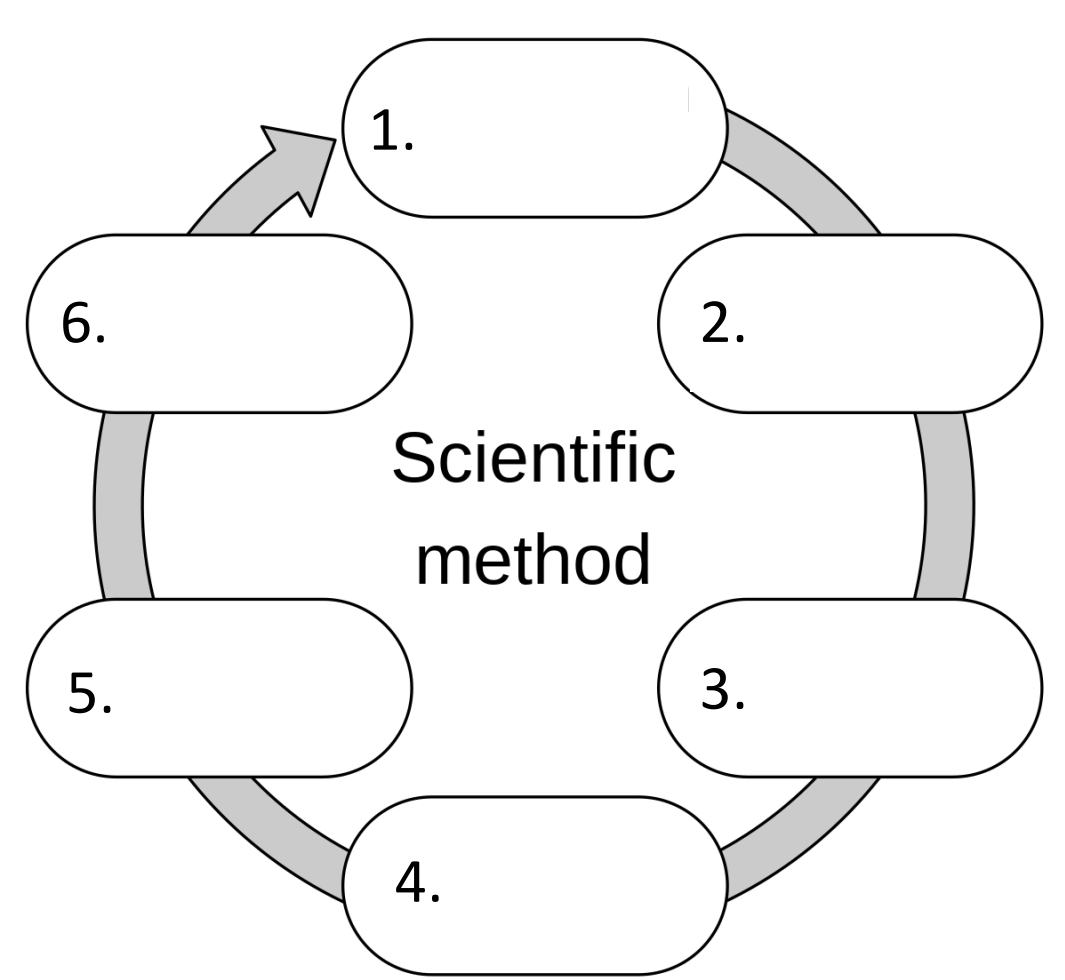
Introduction to scientific writing

- Lectures:
  - Structure of a scientific article
  - Methods and models in biomedical research

- Own work
  - Read an article, understand the methods and results, prepare a short presentation to discuss in small groups
- Group work
  - Discuss article with classmates and prepare a group presentation

Background: The Scientific Method

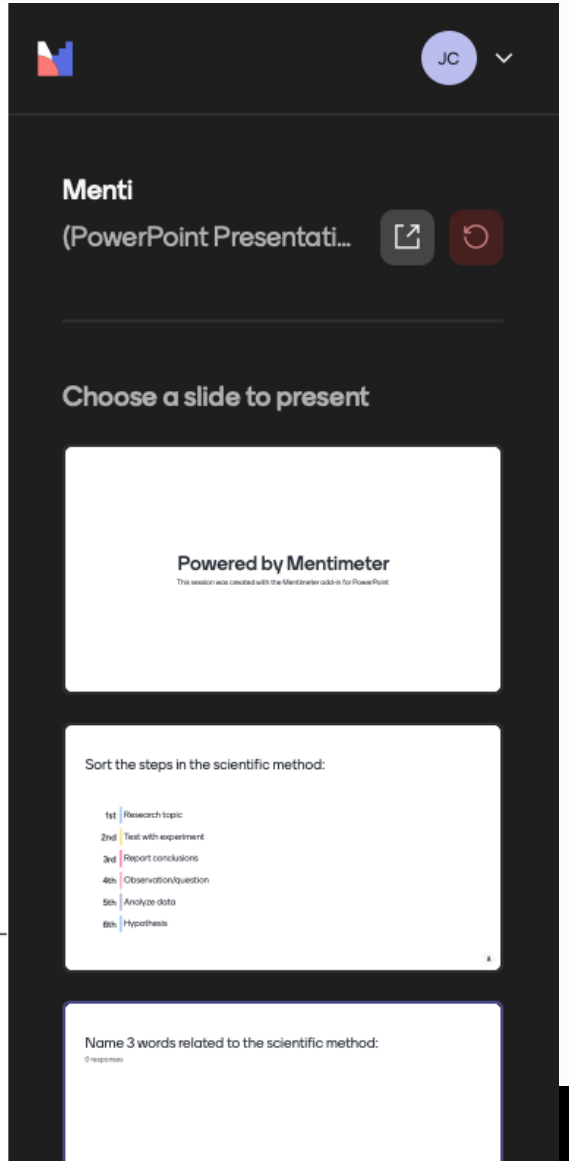
- The process by which science is carried out.

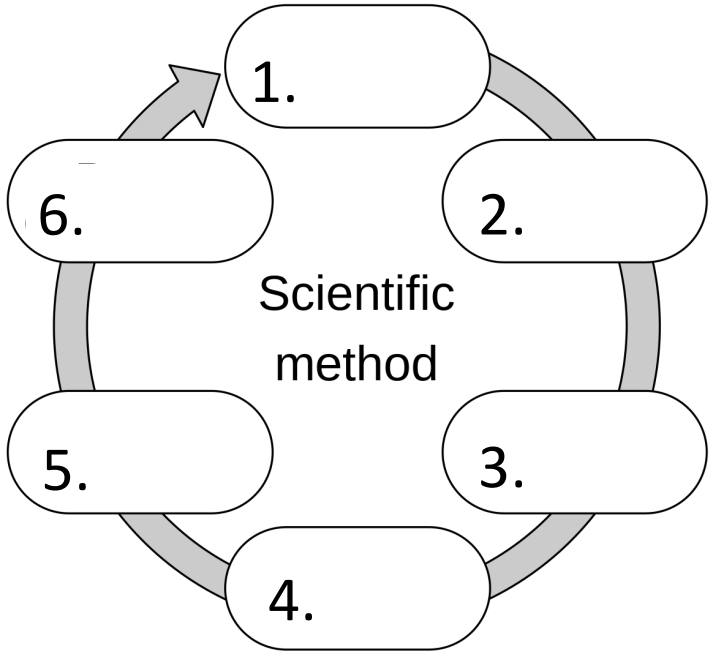


Background: The Scientific Method

Join at menti.com | use code 7692 2773

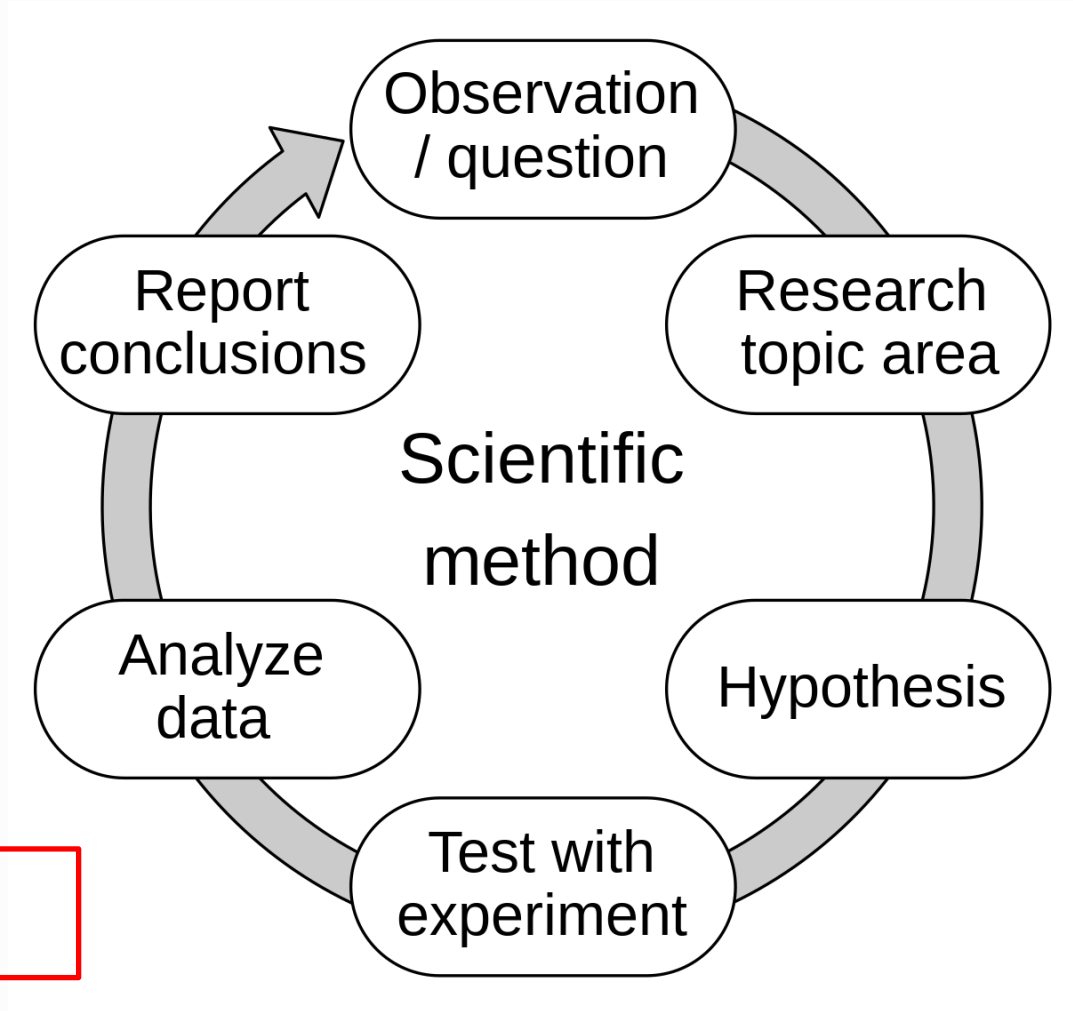
Name 3 words related to the scientific method:





Background: The Scientific Method

- The process by which science is carried out.
- An example: The structure of DNA
  - *Question:* How does DNA store genetic information?
  - *Hypothesis*: Crick & Watson propose helical structure.
  - *Prediction:* X-rays image of DNA should show an X-shape.
  - *Experiment:* Franklin obtains 1st X-ray image of DNA: It has an X-shape!
  - *Analysis:* Model of DNA helix structure to store genetic information.
  - *Report conclusions:* Seminal paper 'Molecular Structure of Nucleic Acids'.



Suggested Readings: Thomas S. Kuhn – The Structure of Scientific Revolutions (1962)

Types of research articles

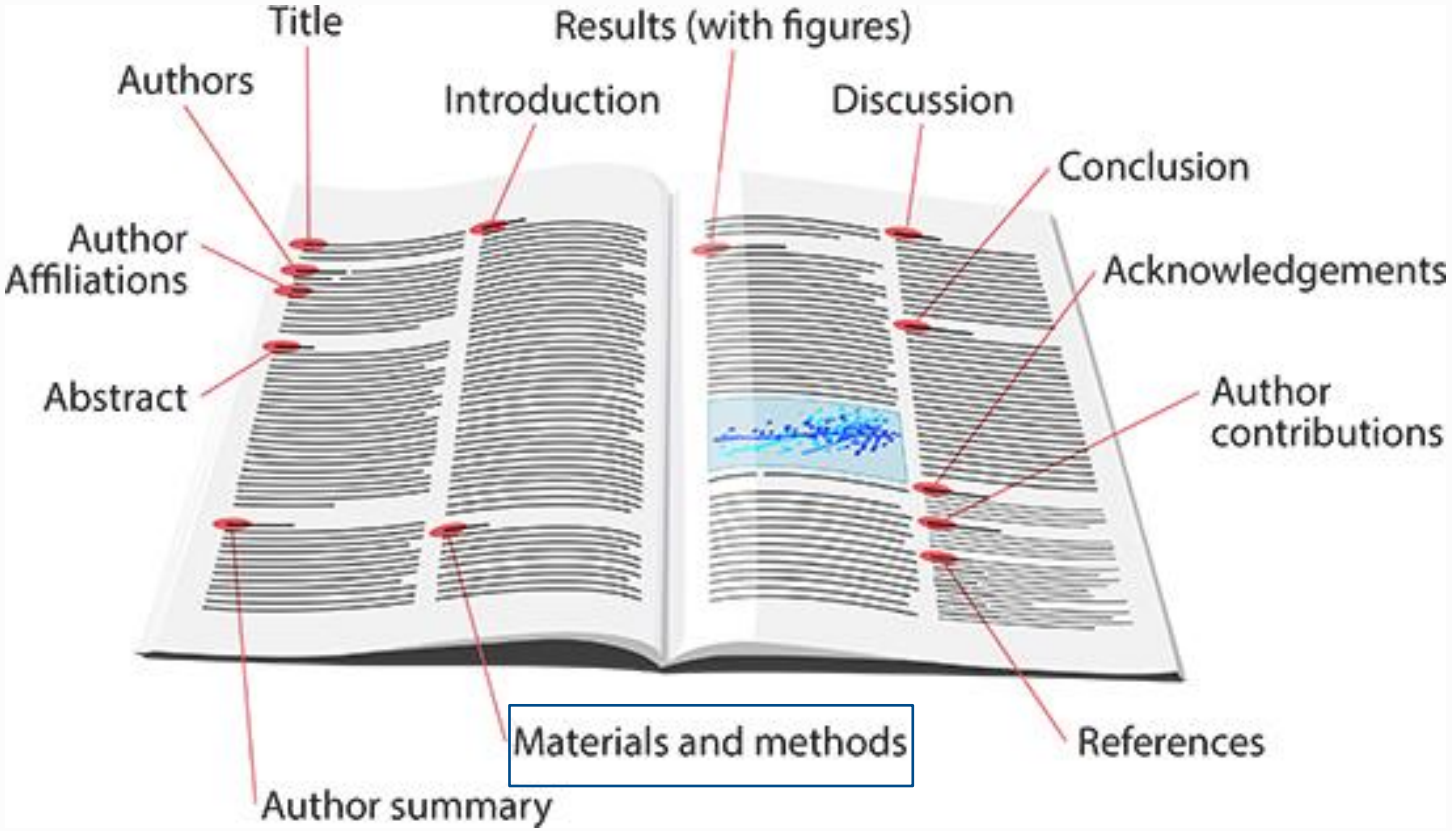
- Abstracts - *Kongressrapporter*
- Original articles- *Originalartiklar* *<- Term 1*
- Review articles – *Översiktsartiklar* *<- Term 3*
- Dissertations - *Avhandlingar*
- Text books - *Textböcker*
- Guidelines - *Vårdprogram*

How to distinguish an original research article from reviews?

- **Original Article:** Reports **new findings** from a study or experiment.
- **Review Article**: Summarize and critically analyze **existing research** on a topic.

	Original article	Review article
Based on?	Unpublised data (analysis)	Published data (analysis)
Whose work?	Your own work	Mostly work by others
Focus?	Narrow: Your research hypothesis	Broad: Topic or field
Other characteristics	-Active voice (We found…) -Shorter bibliography -Detailed Methods section	-Passive voice (Studies suggest…) -Long bibliography -Often No Methods section

Anatomy of an original research article



Other sections in original articles

- Abbreviations - Used frequently & defined the first time that are mentioned

- Supplementary Information:
  - Additional methods
  - Additional Figures and Tables
  - Additional Movies

- Data Availability - Most journals now require data to be available
  - Zenodo, Figshare (research data, figures, supplemenets)
  - Github (code, software)
  - SRA, GEO (genomic data)

Anatomy of a Scientific Paper

Are All Apples Red?

by

Ida Cortland

Abstract:

We examined several apples' color. Although most are red, some are not.

Introduction:

An age-old question is: are all apples red? MacIntosh (1993) thought so. G. Smith (1999) begs to differ. We hope to resolve this issue once and for all.

Methods:

We went to the local grocery store and bought one of every apple they had. We took them home and looked at them.

Results:

We found four red apples, one green apple, and two yellow apples.

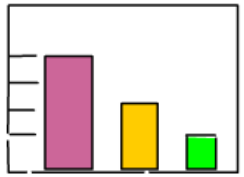


Figure 1

Discussion:

Since we found one yellow apple and two green apples, it must be true that all apples are not red. We concur with G. Smith's findings.

References:

MacIntosh (1993) *Journal of Fruit Science*. 4(3): 121-135.

Smith, G. (1999) *Apple Technology Today*. 7(3):4-8.

Review article

- Evaluate and summarize previous work in a research topic
- Identify unsolved questions and future directions

Biophysical Reviews (2024) 16:89–107
https://doi.org/10.1007/s12551-023-01174-2

REVIEW

Integrating single-cell transcriptomics with cellular phenotypes: cell morphology, Ca^{2+} imaging and electrophysiology

Joan Camunas-Soler[1,2]

Received: 18 May 2023 / Accepted: 29 November 2023 / Published online: 18 December 2023
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Abstract

I review recent technological advancements in coupling single-cell transcriptomics with cellular phenotypes including morphology, calcium signaling, and electrophysiology. Single-cell RNA sequencing (scRNAseq) has revolutionized cell type classifications by capturing the transcriptional diversity of cells. A new wave of methods to integrate scRNAseq and biophysical measurements is facilitating the linkage of transcriptomic data to cellular function, which provides physiological insight into cellular states. I briefly discuss critical factors of these phenotypical characterizations such as timescales, information content, and analytical tools. Dedicated sections focus on the integration with cell morphology, calcium imaging, and electrophysiology (patch-seq), emphasizing their complementary roles. I discuss their application in elucidating cellular states, refining cell type classifications, and uncovering functional differences in cell subtypes. To illustrate the practical applications and benefits of these methods, I highlight their use in tissues with excitable cell-types such as the brain, pancreatic islets, and the retina. The potential of combining functional phenotyping with spatial transcriptomics for a detailed mapping of cell phenotypes in situ is explored. Finally, I discuss open questions and future perspectives, emphasizing the need for a shift towards broader accessibility through increased throughput.

Keywords Single-cell · Morphology · Phenotypes · Patch-seq · Imaging · Calcium · Transcriptomics · Cell-type · Excitability · Function

THE

NEW ENGLAND JOURNAL

OF

MEDICINE AND SURGERY.

VOL. I.] JANUARY, 1812. [No. I.

REMARKS ON ANGINA PECTORIS.

BY JOHN WARREN, M. D.

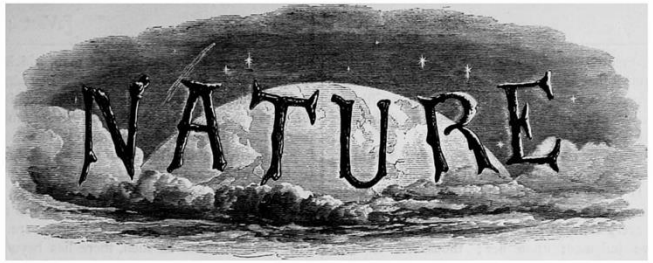
In our inquiries into any particular subject of Medicine, our labours will generally be shortened and directed to their proper objects, by a knowledge of preceding discoveries.

When Dr. Heberden, in the London Medical Transactions, first described a disease under the name of Angina Pectoris, so little had it attracted the attention of physicians, that much surprise was excited by the communication. From the most striking and distressing symptoms, with which it was attended, pain and stricture about the breast, it received from him its denomination; and he soon after published farther remarks on this subject, with the history of a case and appearances on dissection.

That all the cases which this author had noticed as accompanied with affections of *a somewhat similar nature*, were instances of true Angina Pectoris, is by no means probable; for not less than one hundred of those were supposed by him to have fallen under his observation. Of those, three only were women, one a boy; all the rest were men, and about the age of fifty.

In the same work were communicated some observations on this disease made by Dr. Wall, who likewise added a case of dissection.

Dr. Fothergill, in the fifth volume of the London Medical Observations and Inquiries, 1774, published his remarks upon An-



A WEEKLY ILLUSTRATED JOURNAL OF SCIENCE

"To the solid ground
Of Nature trusts the mind which builds for aye."—WORDSWORTH

THURSDAY, NOVEMBER 4, 1869

NATURE: APHORISMS BY GOETHE

NATURE! We are surrounded and embraced by her: powerless to separate ourselves from her, and powerless to penetrate beyond her.

Without asking, or warning, she snatches us up into her circling dance, and whirls us on until we are tired, and drop from her arms.

She is ever shaping new forms: what is, has never yet been; what has been, comes not again. Everything is new, and yet nought but the old.

We live in her midst and know her not. She is incessantly speaking to us, but betrays not her secret. We constantly act upon her, and yet have no power over her.

The one thing she seems to aim at is Individuality; yet she cares nothing for individuals. She is always building up and destroying; but her workshop is inaccessible.

Her life is in her children; but where is the mother? She is the only artist; working-up the most uniform material into utter opposites; arriving, without a trace of effort, at perfection, at the most exact precision, though always veiled under a certain softness.

Each of her works has an essence of its own; each of her phenomena a special characterisation: and yet their diversity is in unity.

She performs a play; we know not whether she sees it herself, and yet she acts for us, the lookers-on.

Incessant life, development, and movement are in her, but she advances not. She changes for ever and ever, and rests not a moment. Quietude is inconceivable to her, and she has laid her curse upon rest. She is firm. Her steps are measured, her exceptions rare, her laws unchangeable.

She has always thought and always thinks; though not as a man, but as Nature. She broods over an all-comprehending idea, which no searching can find out.

Mankind dwell in her and she in them. With all men she plays a game for love, and rejoices the more they win. With many, her moves are so hidden, that the game is over before they know it.

That which is most unnatural is still Nature; the stupidest philistinism has a touch of her genius. Whoso cannot see her everywhere, sees her nowhere rightly.

She loves herself, and her innumerable eyes and affections are fixed upon herself. She has divided herself that she may be her own delight. She causes an endless succession of new capacities for enjoyment to spring up, that her insatiable sympathy may be assuaged.

She rejoices in illusion. Whoso destroys it in himself and others, him she punishes with the sternest tyranny. Whoso follows her in faith, him she takes as a child to her bosom.

Her children are numberless. To none is she altogether miserly; but she has her favourites, on whom she squanders much, and for whom she makes great sacrifices. Over greatness she spreads her shield.

She tosses her creatures out of nothingness, and tells them not whence they came, nor whither they go. It is their business to run, she knows the road. Her mechanism has few springs—but they never wear out, are always active and manifold.

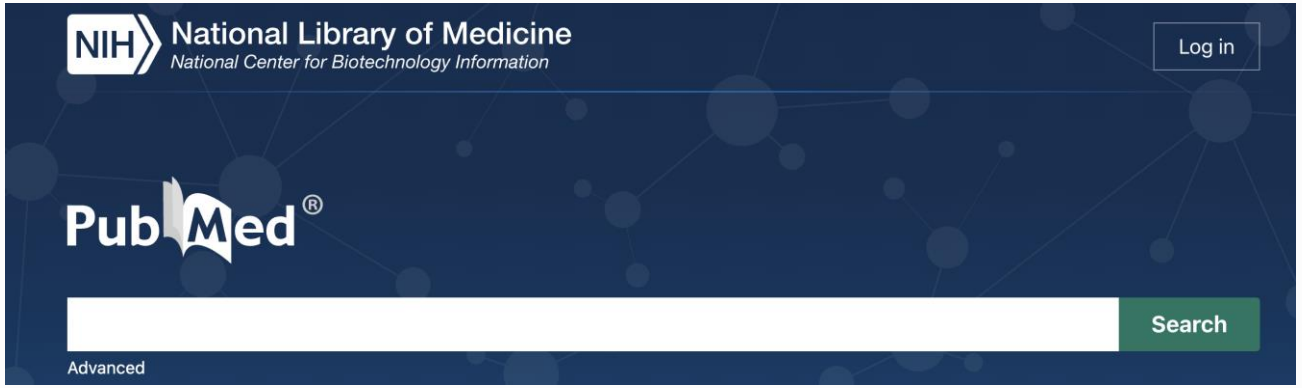
The spectacle of Nature is always new, for she is always renewing the spectators. Life is her most exquisite invention; and death is her expert contrivance to get plenty of life.

She wraps man in darkness, and makes him for ever long for light. She creates him dependent upon the earth, dull and heavy; and yet is always shaking him until he attempts to soar above it.

Scientific journals

Interdisciplinary and specialized journals

Research articles and where to find them:



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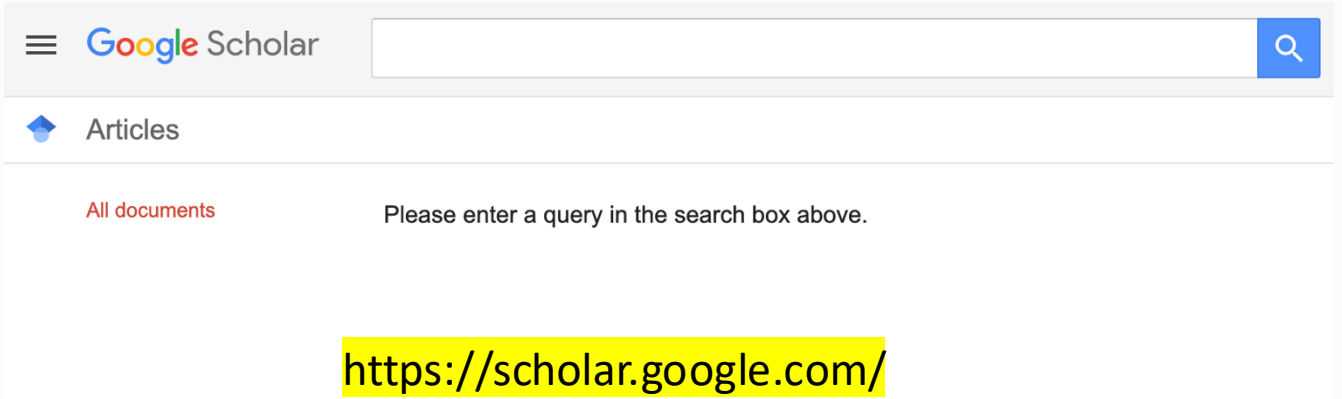

https://scholar.google.com/

PubMed Search results: *Cell*

PubMed®

"Cell" Search

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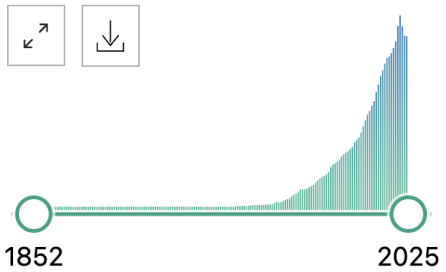
5,827,984 results Page 1 of 582,799

RESULTS BY YEAR



PUBLICATION DATE

- 1 year
- 5 years
- 10 years

1 Cite Share

Harnessing the biology of regulatory T cells to treat disease.

Wardell CM, Boardman DA, Levings MK.

Nat Rev Drug Discov. 2024 Dec 16. doi: 10.1038/s41573-024-01089-x. Online ahead of print.

PMID: 39681737 Review.

Furthermore, multi-dimensional methods to interrogate the biology of T(reg) cells are leading to a refined understanding of T(reg) **cell** biology and new approaches to harness tissue-specific functions for therapy. A new generation of T(reg) **cell** clinical trials is no ...

2 Cite Share

qPCR-based quantification reveals high plant host-specificity of endophytic colonization levels in leaves.

de Paula CCP, Bárta J, Borovec J, Frouz J, Rychtecký P, Sirová D.

Am J Bot. 2024 Dec 16:e16448. doi: 10.1002/ajb2.16448. Online ahead of print.

PMID: 39682006

PubMed Search results: *Cell (The Journal)*

PubMed®

"Cell"[Journal] Search

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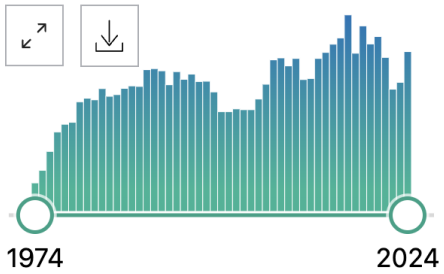
22,817 results Page 1 of 2,282

RESULTS BY YEAR



PUBLICATION DATE

- 1 year
- 5 years

1 Cite Share

Intermittent fasting triggers interorgan communication to suppress hair follicle regeneration.

Chen H, Liu C, Cui S, Xia Y, Zhang K, Cheng H, Peng J, Yu X, Li L, Yu H, Zhang J, Zheng JS, Zhang B.

Cell. 2024 Nov 26:S0092-8674(24)01311-4. doi: 10.1016/j.cell.2024.11.004. Online ahead of print.

PMID: 39674178

2 Cite Share

Engineering source-sink relations by prime editing confers heat-stress resilience in tomato and rice.

Lou H, Li S, Shi Z, Zou Y, Zhang Y, Huang X, Yang D, Yang Y, Li Z, Xu C.

Cell. 2024 Dec 9:S0092-8674(24)01321-7. doi: 10.1016/j.cell.2024.11.005. Online ahead of print.

PMID: 39674177

Publishing an article - Peer Review

- **Peer Review** - All manuscripts are reviewed by at least 2 scientists before publishing
- Reviewers can ask for new experiments and analysis
- Many times manuscripts are rejected or require extensive review
- Can be a long process (3 months – 2 years)

Impact factor (IF)

- A measure often associated to journal ‘fame’ or ‘reputation’

- IF = (Number of citations received) / (Number of papers published)

- Interdisciplinary Journals: Nature 50.5, Science 47.7, Cell 66.9 , NEJM 92,
- Specialized Journals: Nature Immunology 27.8, Immunology 7.5, Biochemistry 2.9

- IF is often an important consideration, but can sometimes be misleading

Predatory Journals

- **Journals that publish papers with little or no peer-review for exorbitant fees.**

- How to identify them:
  - False or misleading information (IF, address, editors)
  - Lack of transparency
  - Agressive solicitation of papers
  - Other: spamming, poor English

https://beallslist.net/
https://www.nature.com/articles/d41586-019-03759-y

Choosing a Journal to publish your work

- Explore Journal content:
  - Does it publish work similar to your work?
  - Do you read it and cite it?

- Other important factors:
  - Impact factor (IF)
  - Avoid Predatory Journals

"Predatory journals have rapidly increased their publication volumes from 53,000 in 2010 to an estimated 420,000 articles in 2014, published by around 8,000 active journals." (Wikipedia)

Instructions for the Research Article Assignment

Objectives

- Learning objectives:

  To understand the structure of a research paper.

  To summarize the main results of a research paper.

  To learn how to present the results of a research paper to an audience.

  To critically assess and discuss the methods used in a research paper and prepare questions about it.

Organizing subgroups and assigning articles

Today:

- Organize groups (A1-10 and B1-10)
- Divide each group in half (3/4 students), with each half preparing **one of the two articles**.

Group A:

Article A1: Fey, M. F., et al. "Clonal analysis of human tumors with M27 beta, a highly informative polymorphic X chromosomal probe." *The Journal of clinical investigation* 89.5 (1992): 1438-1444.

Article A2: Gale, RE., et al. "Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age." *British journal of haematology* 98.3 (1997): 512-519

Group B:

Article B1: Salomon, D., & Meda, P. (1986). Heterogeneity and contact-dependent regulation of hormone secretion by individual B cells. *Experimental cell research*, *162*(2), 507-520.

Article B2: Dorrell, C, et al. "Human islets contain four distinct subtypes of β cells." *Nature communications* 7.1 (2016): 11756.

Preparing your subgroup presentation

Between Today and January 16th:

- **Each subgroup prepares a 10 minute presentation of their article.**

- The presentation **MUST** include these sections:
  - Introduction and scientific questions addressed in the article
  - Summary of main methods and results
  - Select 1-2 figures and present them
  - Discussion and conclusion

- **Every student must present at least one section**

Class Activity and Presentation

Activity on January 16th (Room T Bjurström, Group B: Morning, Group A: Afternoon)

- **Within group presentation:**
  - Each subgroup presents their article to the other subgroup (10 minutes).
  - Questions and in-depth discussion of the two articles in each group

- **Prepare for class presentation:**
  - Instructors will assign one section (introduction, figure, methods) of the articles to each group
  - The group will prepare to present it to the class.

- **Class presentation**
  - Short presentation of the assigned section to the class (10 minutes)
  - Figures can be displayed
  - Paper notes and blackboard allowed, powerpoint not required

Questions?

UNIVERSITY OF GOTHENBURG

Research methods in life sciences

Joan Camuñas-Soler
Department of Medical Biochemistry and Cell Biology
Wallenberg Center For Molecular and Translational Medicine
Institute of Biomedicine
Sahlgrenska Academy
University of Gothenburg

Biomedical research

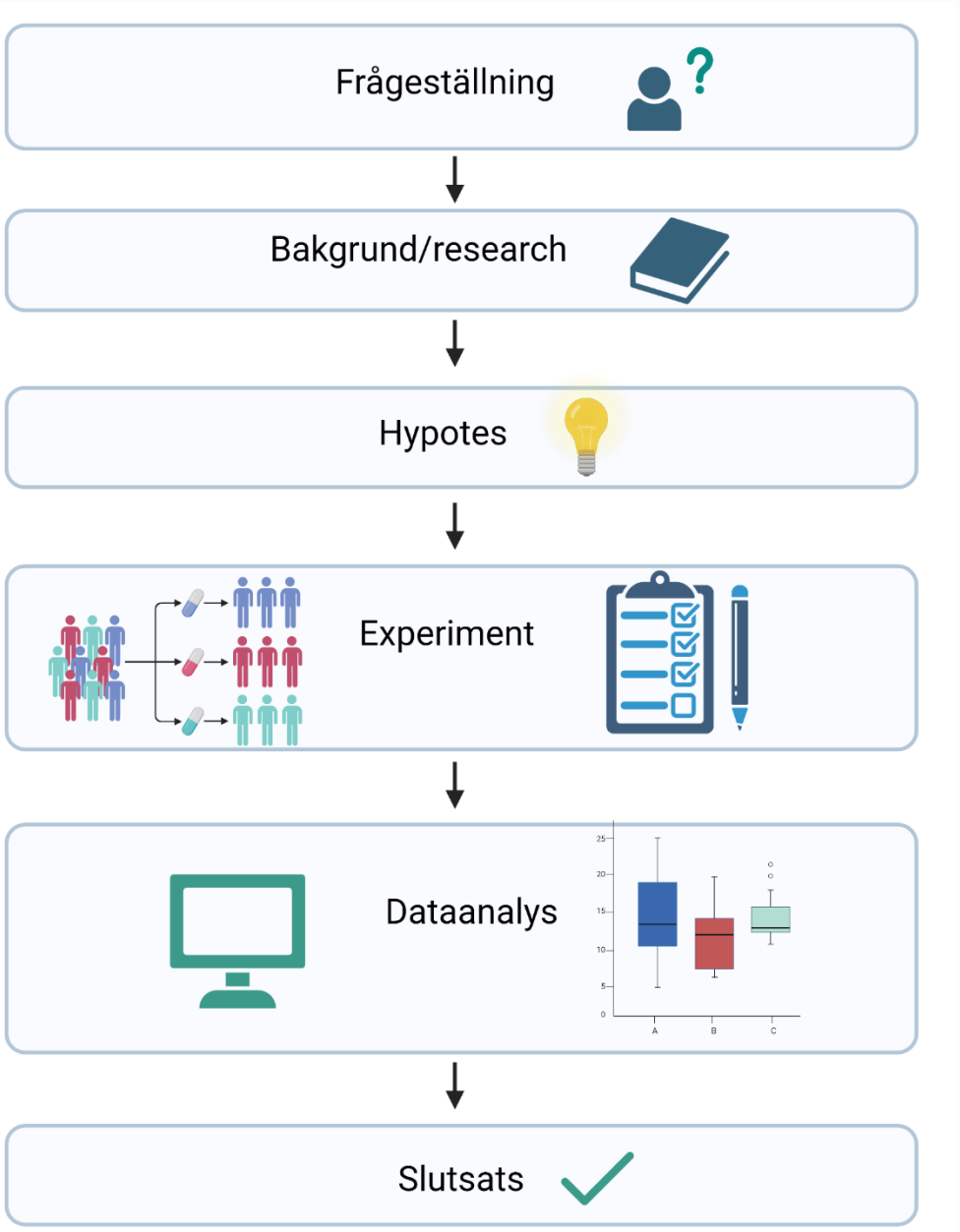
- Study biological processes and systems to understand health, disease and treatments.

- How do they work?
  - Organisms
  - Cells
  - Molecules

- Understand disease process, test how treatments affect biology -> implement in clinical practice

Types of research

Scientific Method



Created with BioRender.com

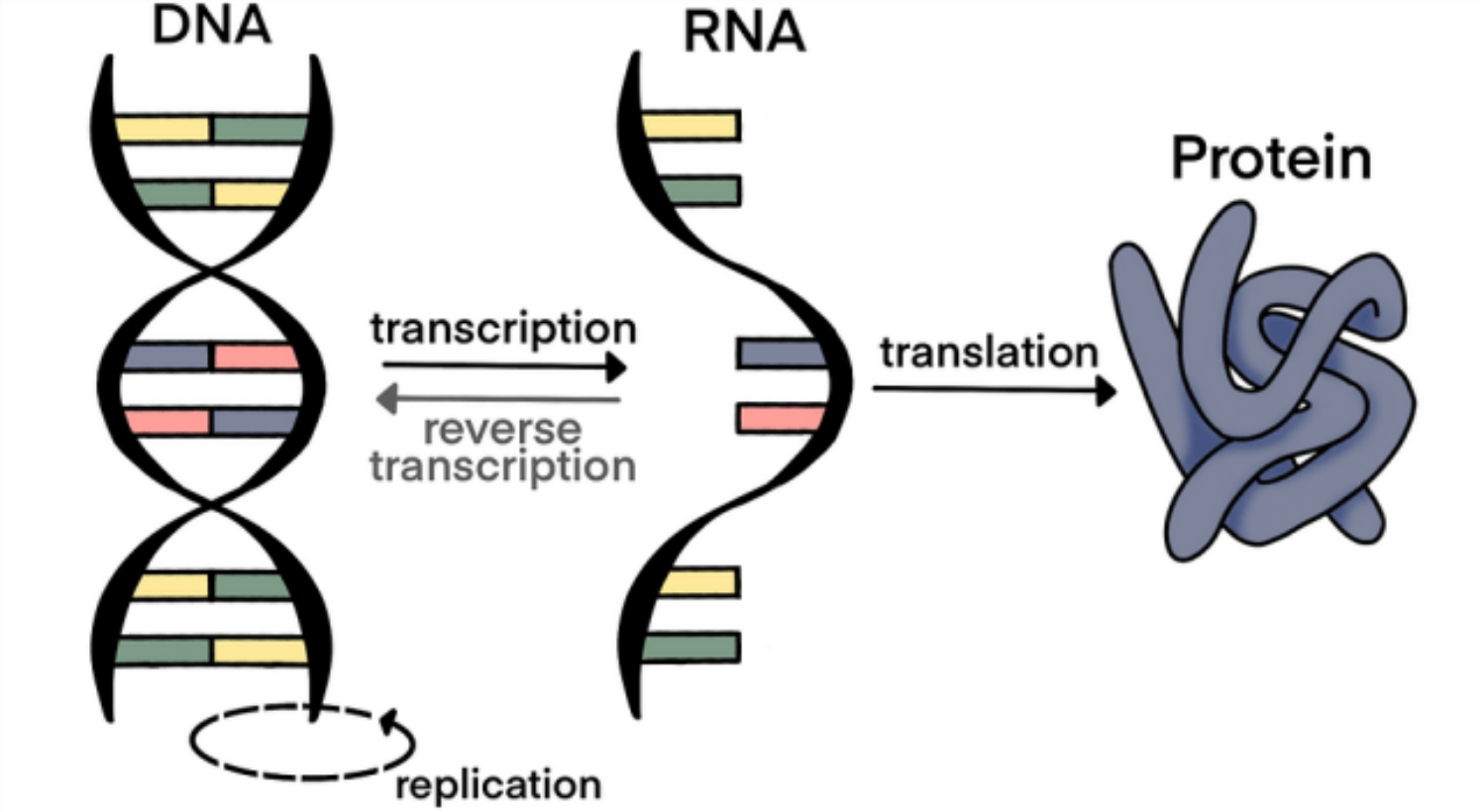
Methods in biomedical research

Central Dogma of Molecular Biology

•All human cells contain the **same DNA**.

•Different genes are expressed (mRNA) in each cell to produce **proteins to perform specific functions** and respond to their **environment.**

•**Example**: Beta cells in the pancreas express the **INS** gene to produce insulin, which regulates glucose levels.

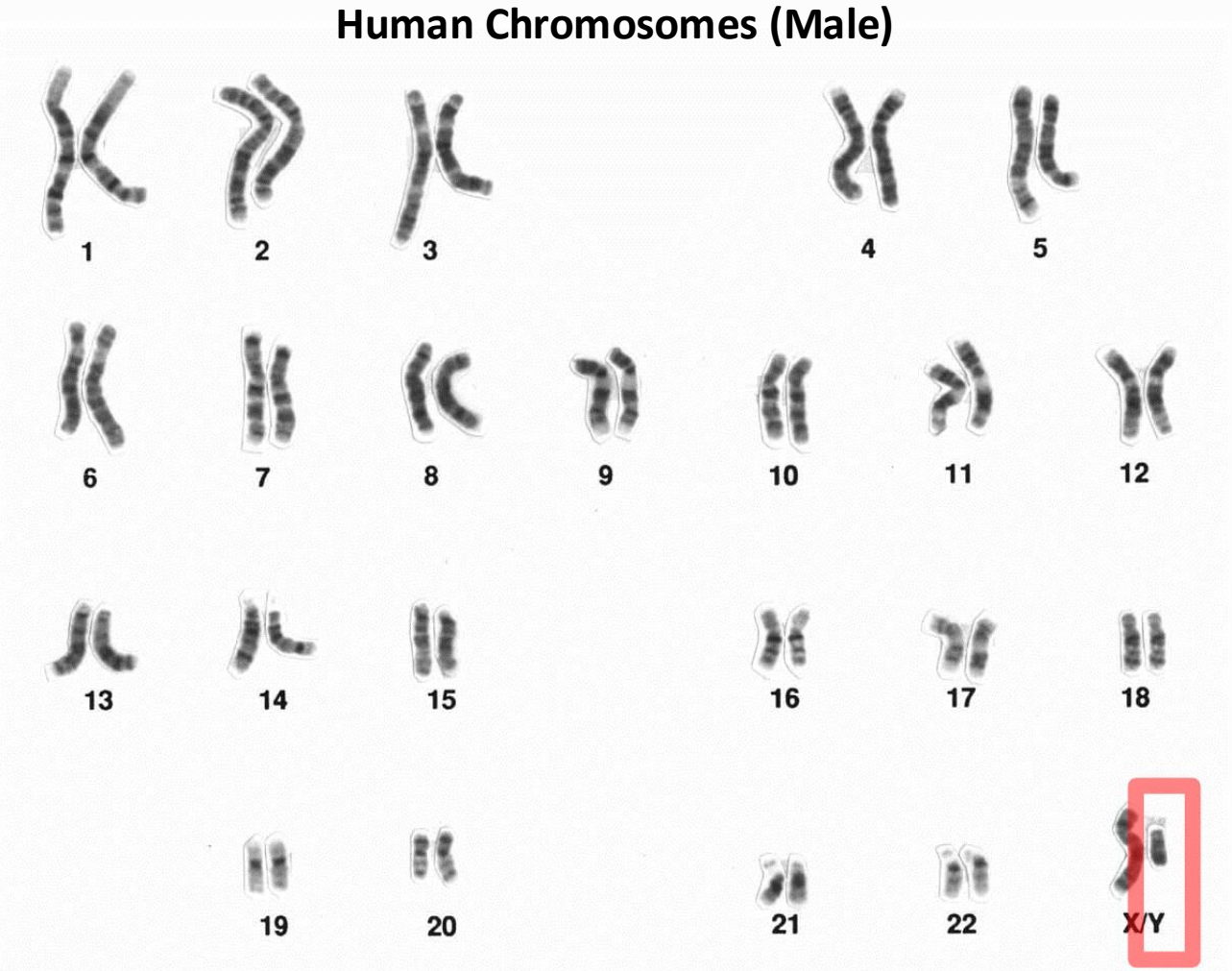


Store information

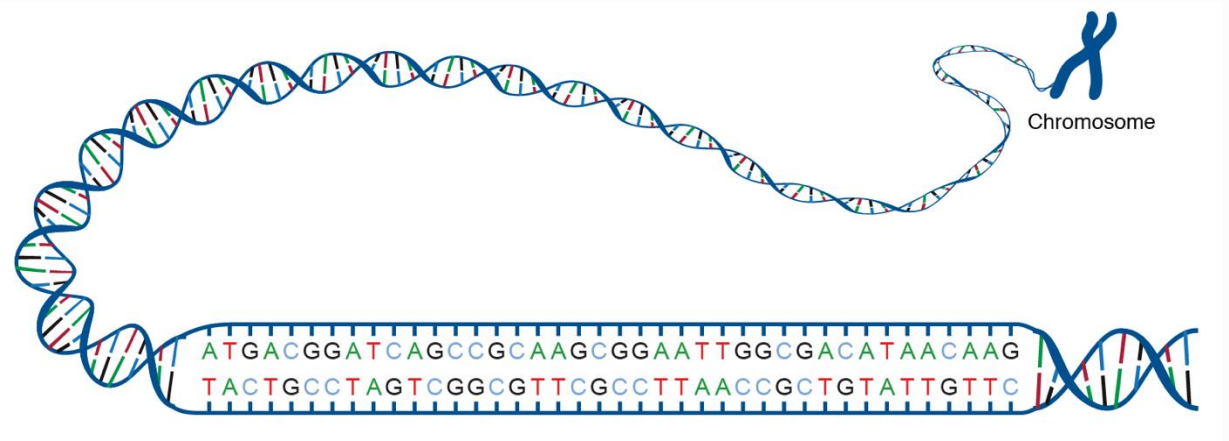
Gene expression to make proteins

Function in cells

The human genome

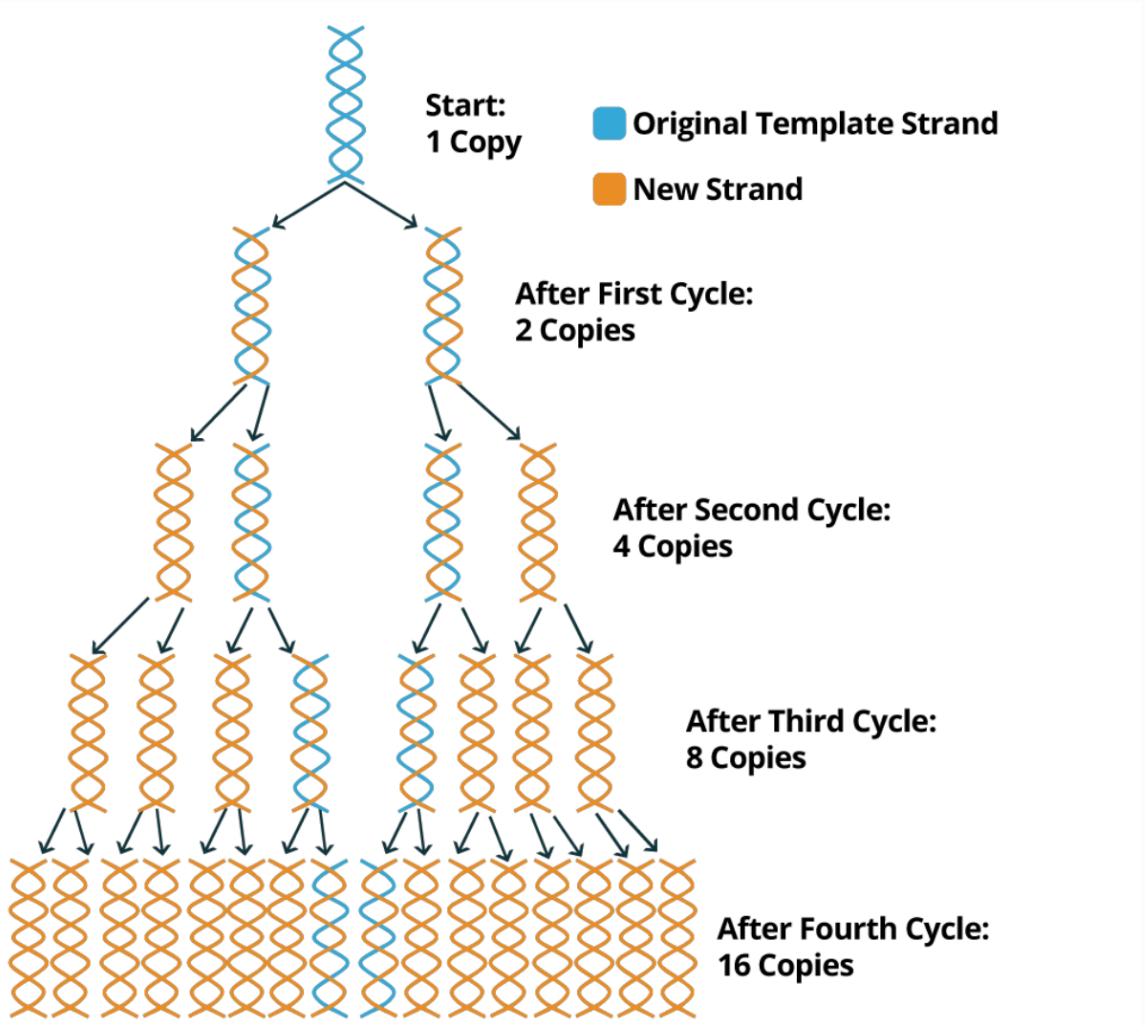


- The complete set of DNA in humans.
- Size: 3 x 10^7 base pairs.
- 23 chromosome pairs (22 autosomes + X/Y)
- ~20,000 protein-coding genes.
- Only 1.5% of genome is protein-coding
- Human Genome Project (1990-2003): First full sequence of the human genome.



Polymerase Chain Reaction (PCR)

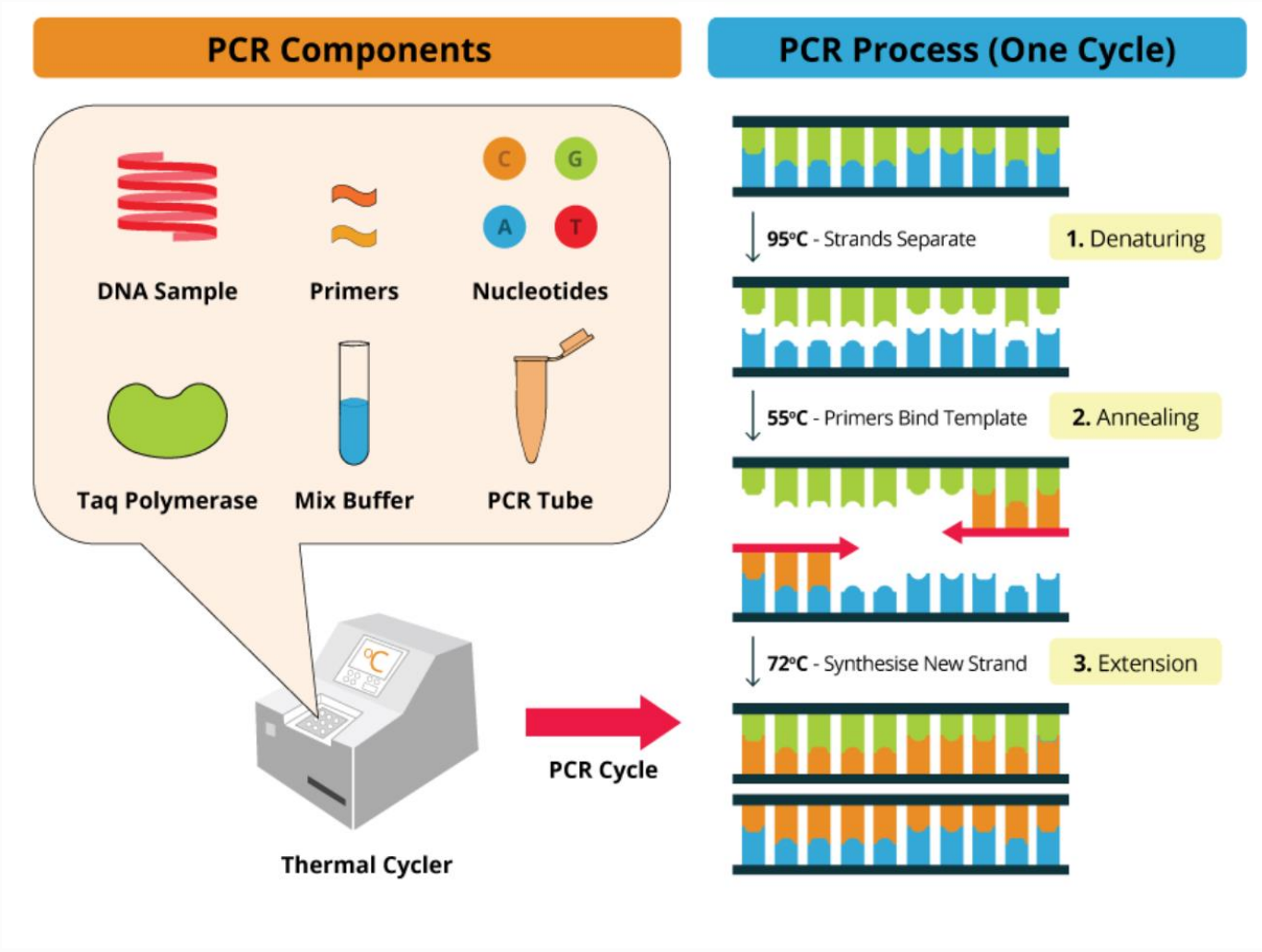
- **Foundation of modern molecular biology**
- **Rapid DNA amplification**: Millions of copies
- Each cycle doubles number of molecules:
  - **2^N amplification** (N: number of cycles)
  - 20 cycles: 1 million copies
- Key applications:
  - **Diagnostics**: viral detection, mutations
  - **Forensics**: suspect identification
  - **Research**: clone genes, sequencing



How to become a molecular biologist in 4 days. DNA & RNA. BosterBio.

Polymerase Chain Reaction (PCR)

- **Foundation of modern molecular biology**
- **Rapid DNA amplification**: Millions of copies
- Each cycle doubles number of molecules:
  - **2^N amplification** (N: number of cycles)
  - 20 cycles: 1 million copies
- Key applications:
  - **Diagnostics**: viral detection, mutations
  - **Forensics**: suspect identification
  - **Research**: clone genes, sequencing
- Basic requirements:
  - DNA/RNA template, DNA polymerase (Taq), primers, nucleotides, thermal cycler.

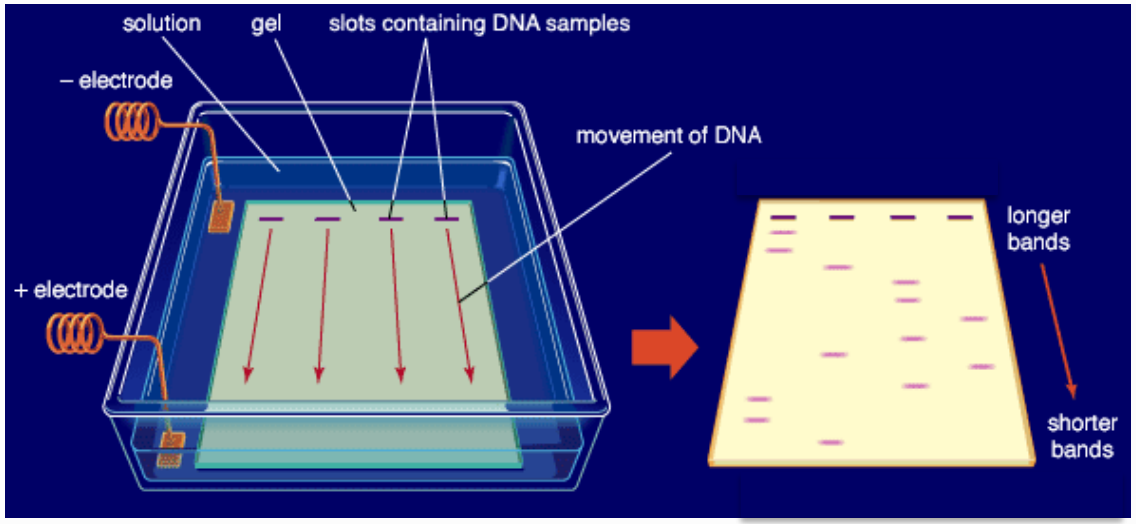


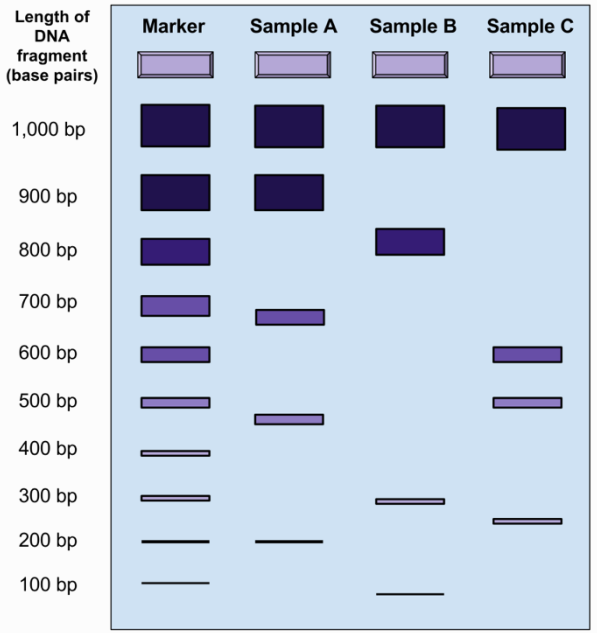
How to become a molecular biologist in 4 days. DNA & RNA. BosterBio.

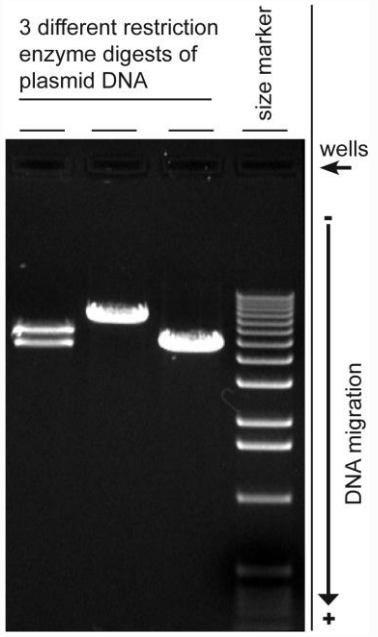
DNA gel electrophoresis

Simplest method to detect and quantify DNA:

1. Load DNA samples in wells of a gel matrix.
2. Electrophoresis – Short DNA fragments move faster in an electric field.
3. Fragments separate by their size.
4. Add fluorescent dye to visualize DNA.
5. Use a *DNA marker* with fragments of known size as a reference.







Encyclopedia Britannica &Wikicommons

Quantitative PCR (qPCR)

•Measure **real-time DNA or RNA amplification** to quantify genetic material.

•During amplification, **fluorescent dyes or probes** emit a signal that increases as the target DNA is amplified.

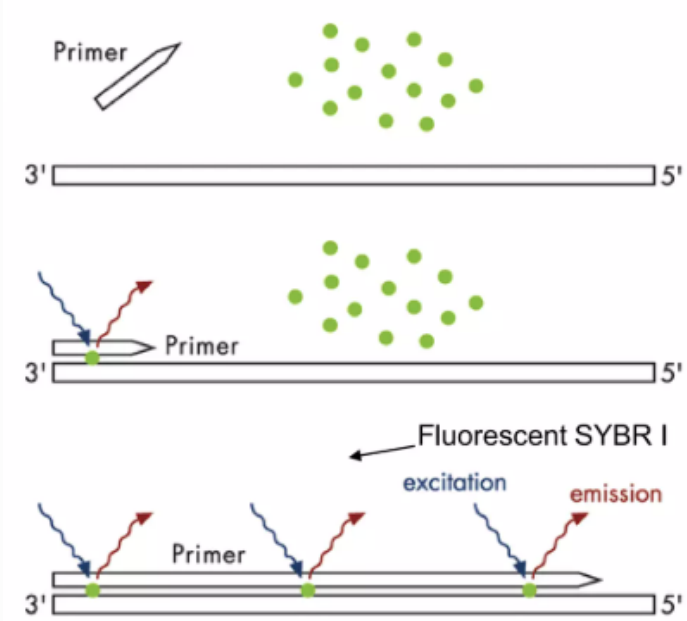
•Essentially, it is a PCR where DNA fluorescence is measured at every PCR cycle.

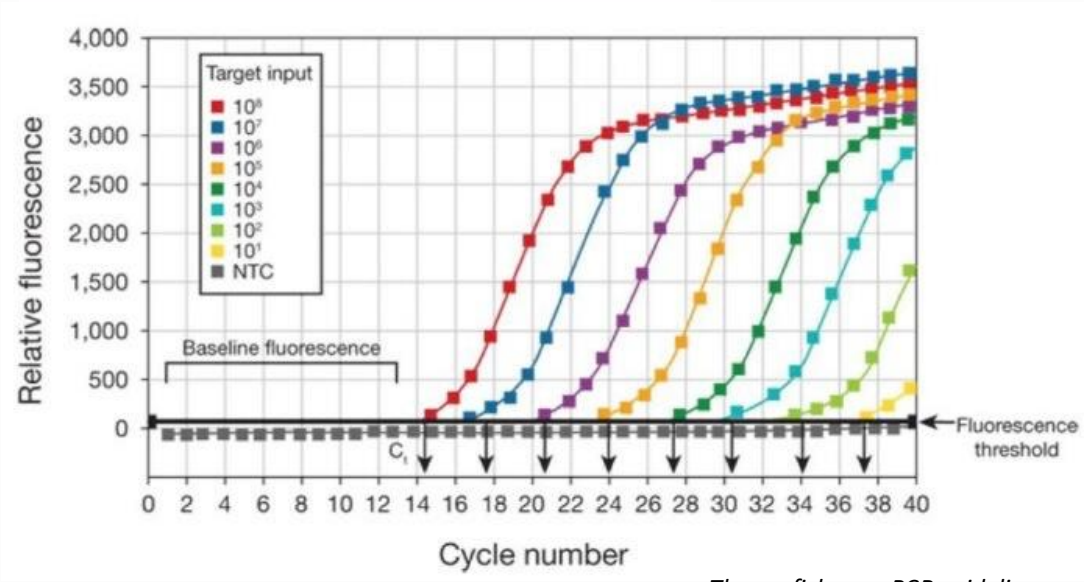
Key Components

- PCR components (DNA, primers, polymerase, nucleotides)
- Fluorophore (e.g., DNA dye, TaqMan probe)
- Thermal cycler with fluorescence detection

Applications

- Gene expression analysis
- Viral load quantification
- Mutation detection





Thermofisher – qPCR guidelines
Qiagen - Introduction to real-time PCR.

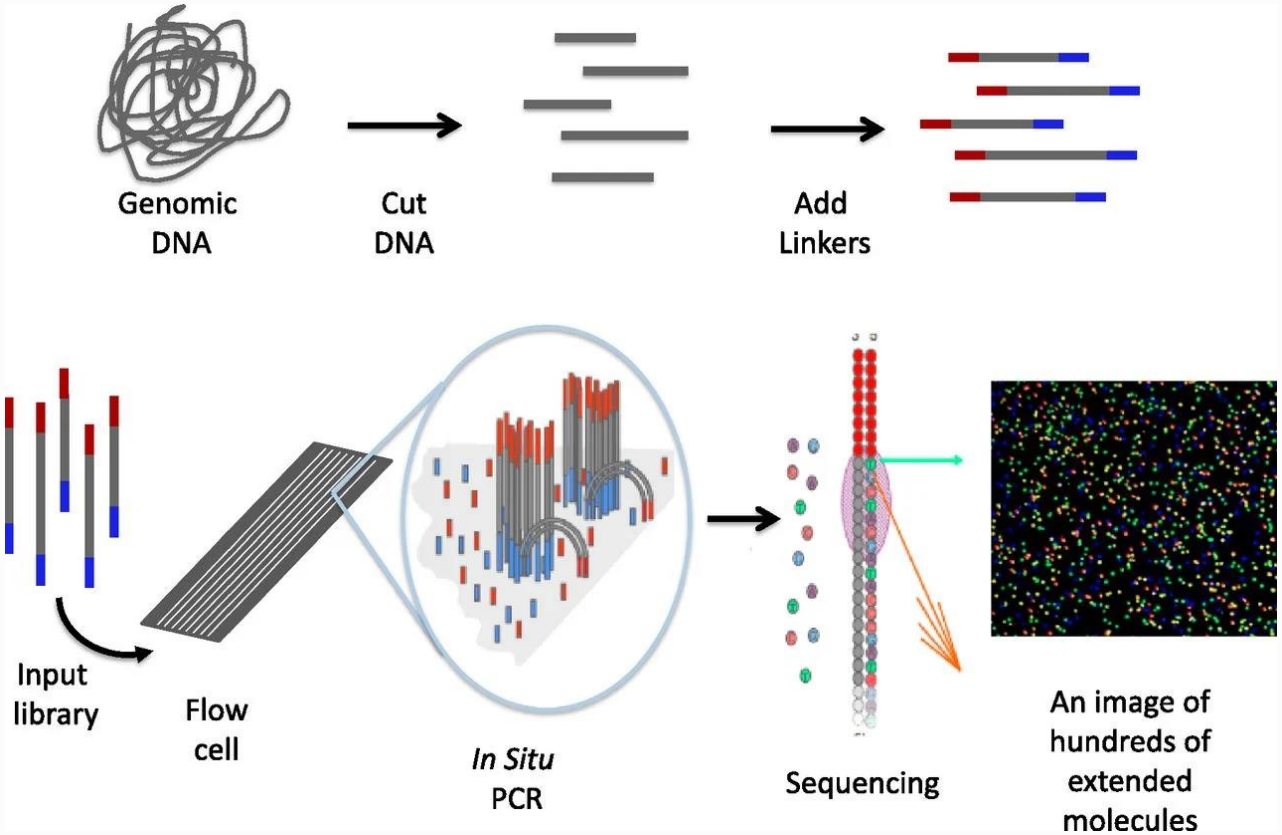
Next Generation Sequencing

- High-throughput method for sequencing **DNA** and **RNA**.
- Enables rapid, large scale **genomic analysis.**

- **How it works:**
  - Fragment DNA/RNA, attach adapters, bind to flow cell.
  - In the flow cell, identical clusters are generated and bases read with **fluorescence microscopy**.
  - Bioinformatic analysis to map sequences to the human genome

- **Applications**
  - Whole genome sequencing, RNA-seq, variant detection (e.g. mutations, deletions)

10^{13} bp per run (1 day)
100 Human genome (30x)



Microbe Notes

Next Generation Sequencing

- High-throughput method for sequencing **DNA** and **RNA**.
- Enables rapid, large scale **genomic analysis.**

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  - Fragment DNA/RNA, attach adapters, bind to flow cell.
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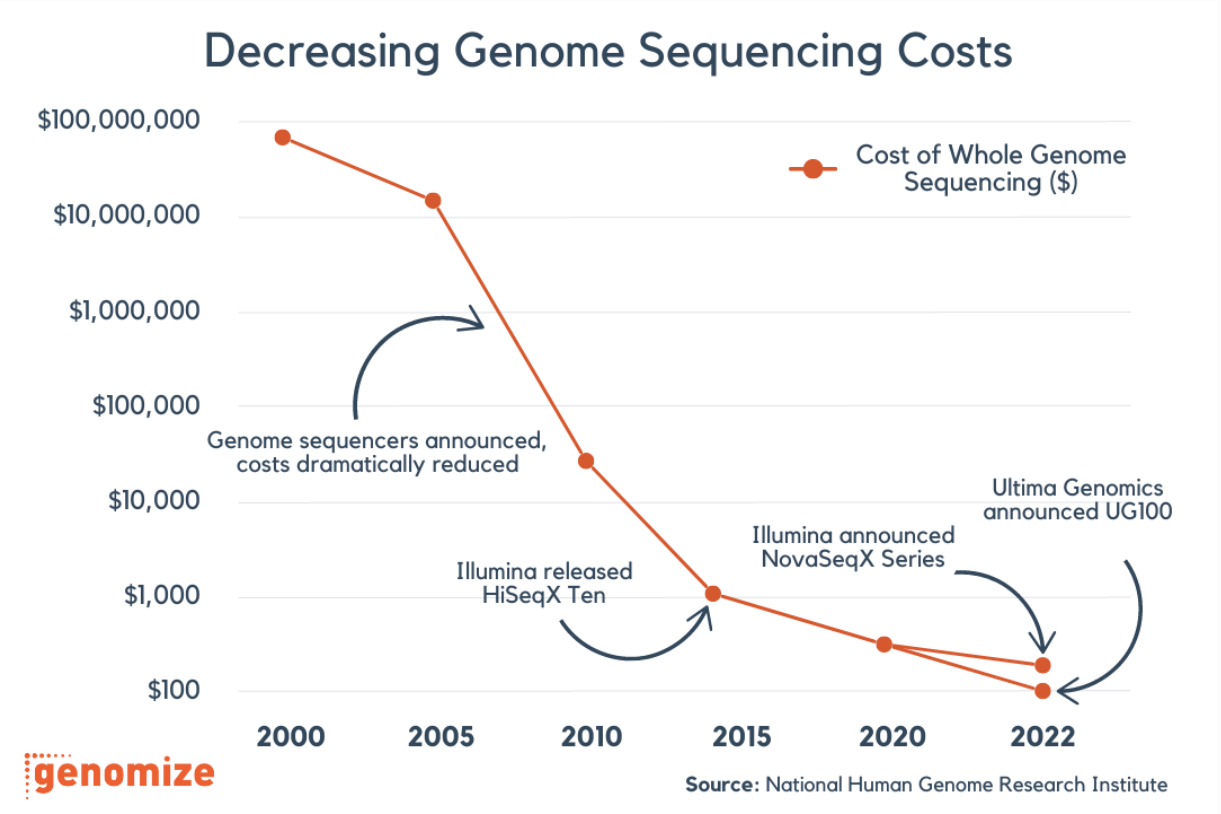
- **Applications**
  - Whole genome sequencing, RNA-seq, variant detection (e.g. mutations, deletions)

- **Key Technologies**
  - Illumina , PacBio, Oxford Nanopore

- **Advantages**
  - High throughput, deep coverage, and broad applications in **diagnostics** and **research**.



"Progress in science depends on new techniques, new discoveries and new ideas, probably in that order."
Sydney Brenner

Gene editing

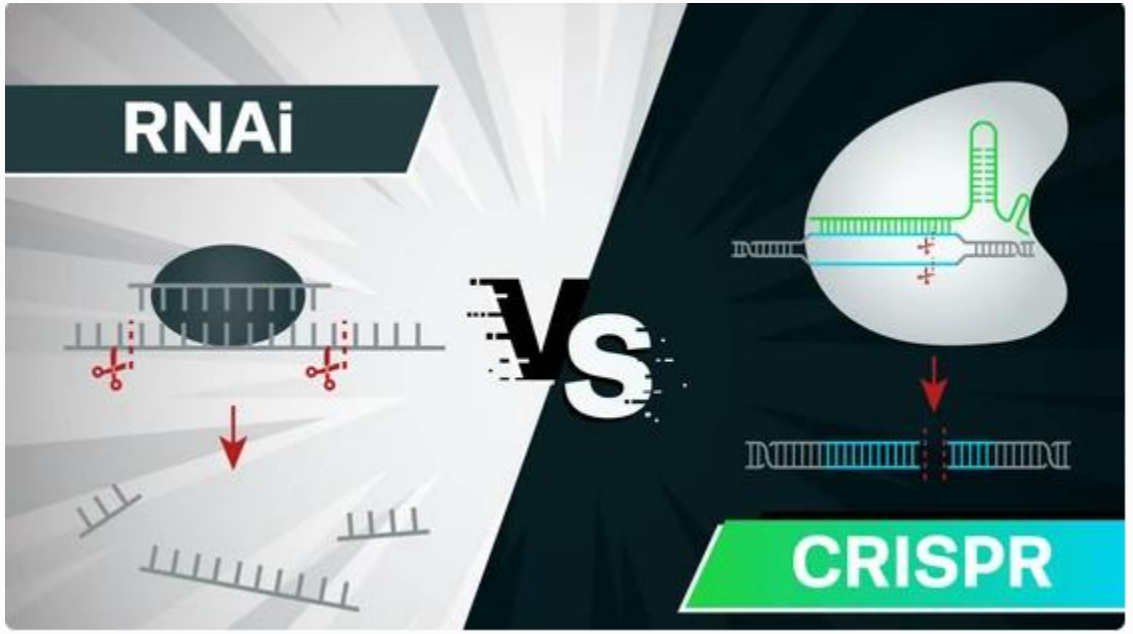
- **Research**: Study underlying mechanisms.
- **Disease Treatment**: Modify genes in somatic cells or germ cells/embryos.

- **RNA Interference (RNAi):**
  - Double-stranded RNA (dsRNA)
  - MicroRNA (miRNA)
  - Small interfering RNAs (siRNA)
  - Short hairpin RNAs (shRNA)

- **Gene Editing Technologies:**
  - Zinc Finger Nucleases (ZFNs)
  - Transcription Activator-Like Effector Nucleases (TALENs)
  - CRISPR/Cas9

RNA interference and Gene editing

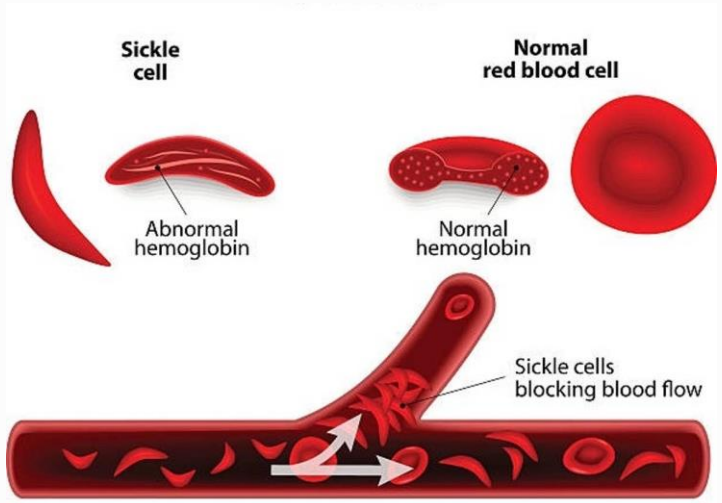
- RNA interference
  - Nobel Prize in Physiology, 2006, Fire & Mello, *"For their discovery of RNA interference - gene silencing by double-stranded RNA"*
  - Silence a gene by reducing mRNA expression - knockdown
- Crispr-Cas9 gene editing
  - Nobel Prize in Chemistry 2020, Charpentier & Doudna, *"For the development of a method for genome editing"*
  - Origin: Bacterial defense mechanism against viruses
  - Silence a gene by editing DNA – knockout
  - Can also be used to modify a gene (e.g. introduce or correct a mutation)

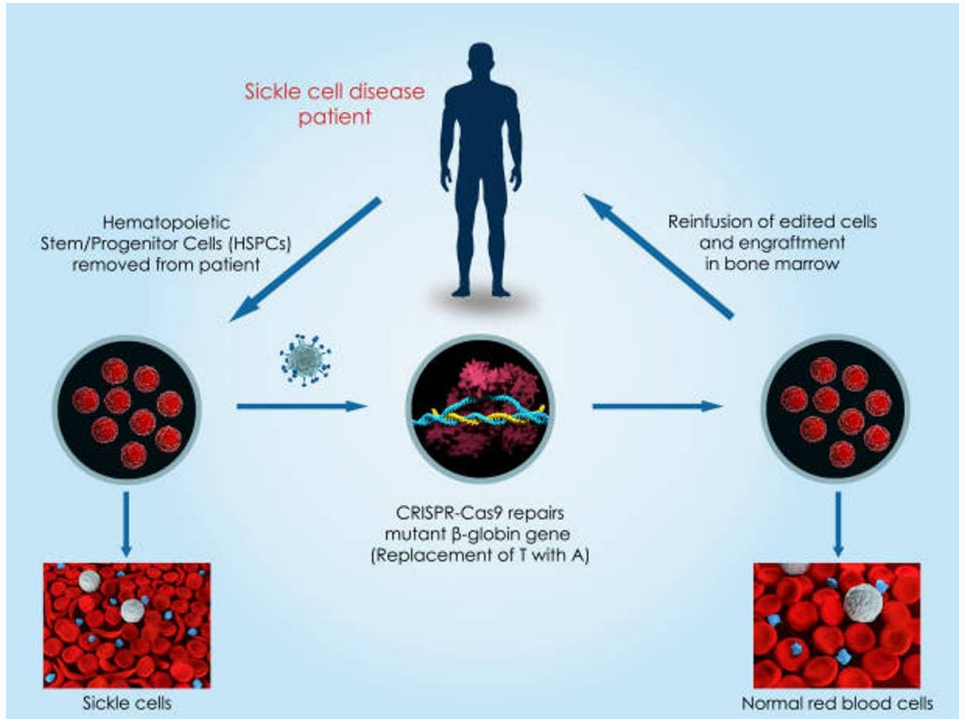


https://www.synthego.com

CRISPR as a therapy

- Sickle-cell Disease: Mutation i β-globin gene



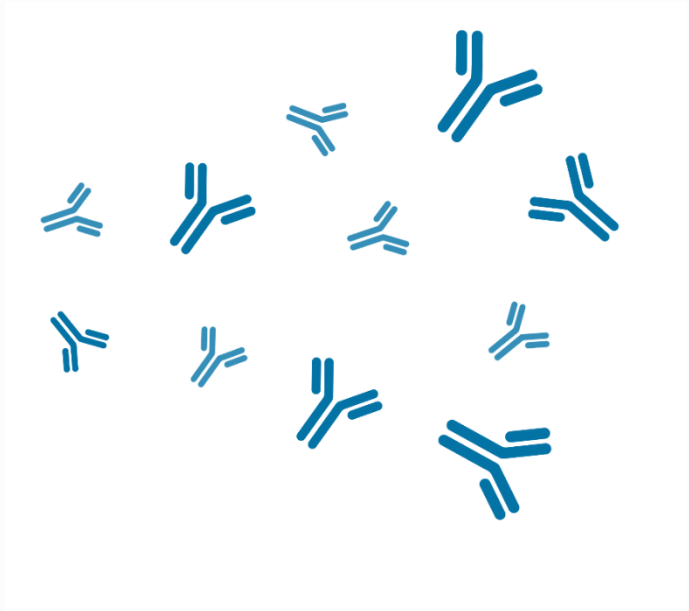


- *Approved in the UK (nov 2023)*

https://www.synthego.com/crispr-sickle-cell-disease

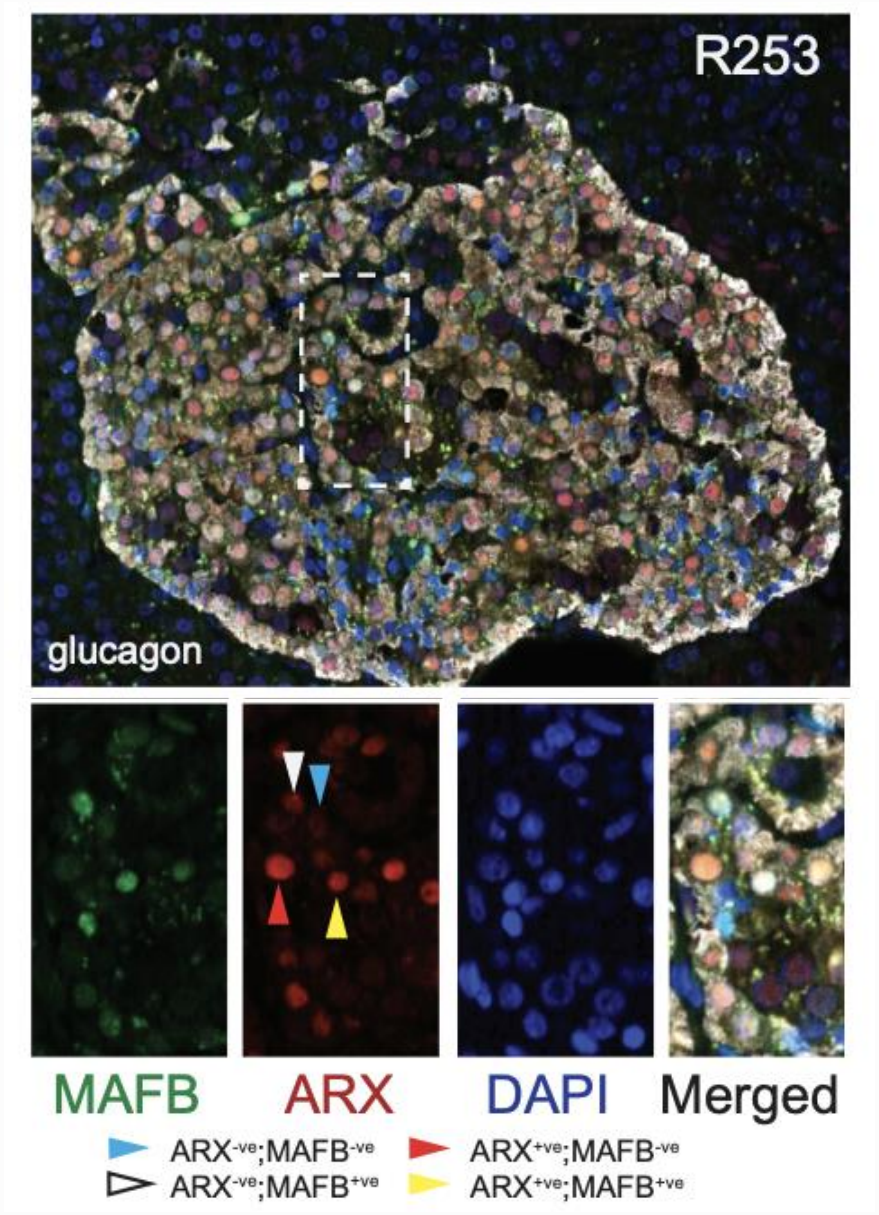
Studying proteins - Methods based on antibodies

- Immunostaining (ICC and IHC)
- Enzyme-linked immunosorbent assay (ELISA)
- Western blot (WB)
- Flow cytometry and FACS



Immunostaining

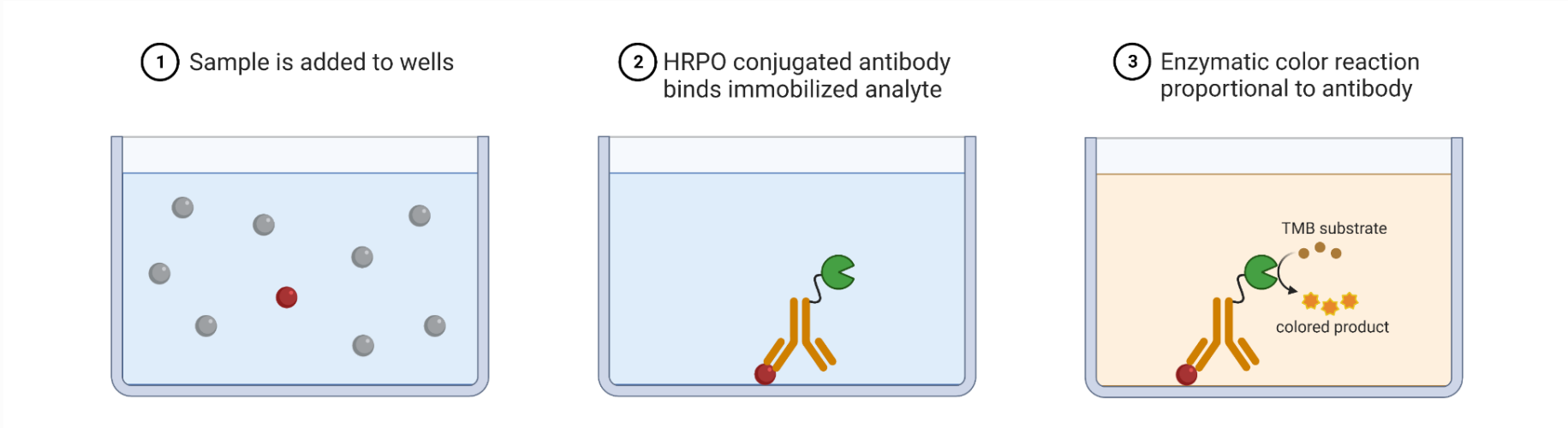
- Label-based method to detect proteins in cells using antibodies with a fluorophore
- Use of antibodies that specifically bind to proteins of interest (antigen)
- Antibody is conjugated with a fluorescent die – can combine multiple colors (3/4 usually)
- Immunohistochemistry: In tissue slices



Dai X, Camunas-Soler, et al. Cell Metabolism 2022

ELISA (Enzyme-Linked ImmunoSorbent Assay)

- Detect an antigen in a biological sample
- The antibody gives a signal that is proportional to the concentration of the antigen
- It is a bulk measurement, usually performed in plates (96- or 384-well plates)

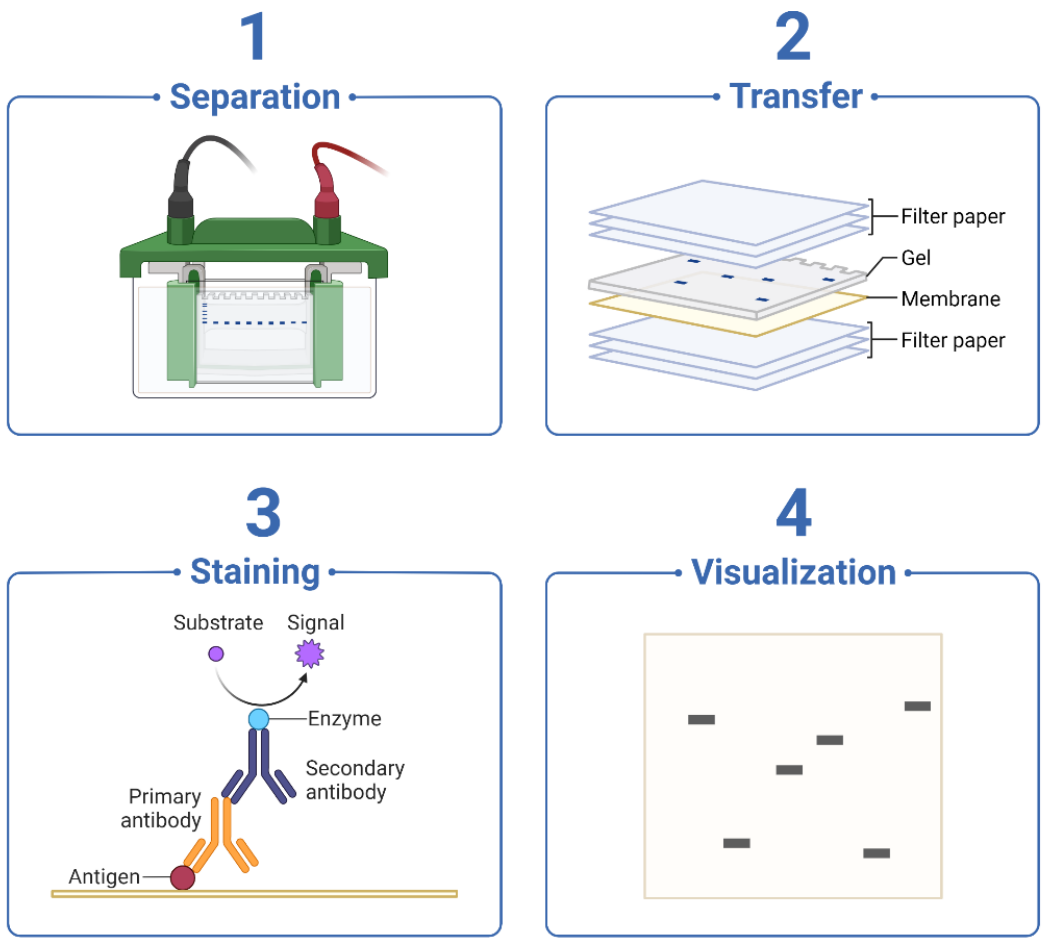


Created with BioRender.com

Western blot

Detect and quantify protein:

1. Size separation of the protein usin electrophoresis
2. Transfer proteins to a membrane
3. Bind primary and secondary antibodies to the protein
4. Visualize the proteins



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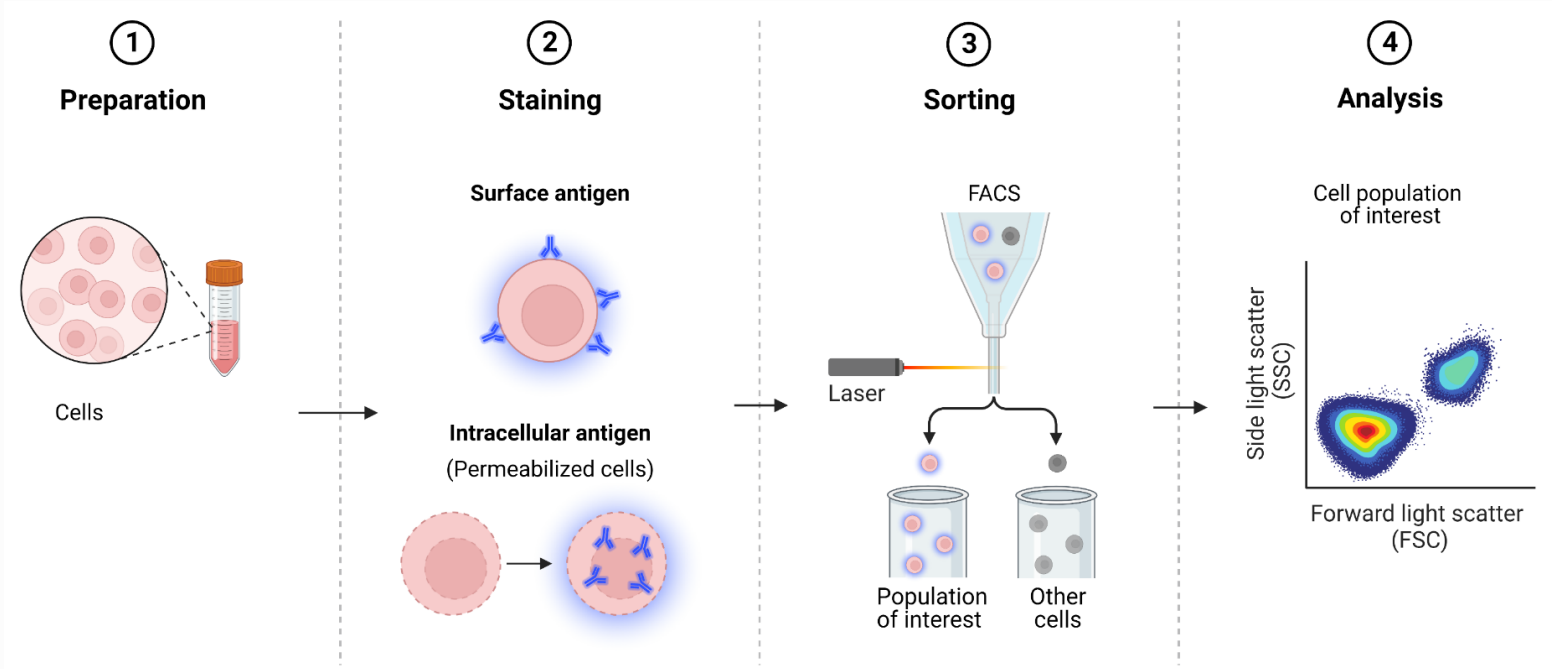
Flow Cytometry and Fluorescence-activated cell sorting (FACS)

- **Laser-based analysis:** Flow of cells is illuminated with laser beam.
- **Fluorescence Detection:** Dyes or antibody markers emit signals to identify cell features.
- **Cell Identification**: Differentiates cell types in mixed populations using surface markers.
- **FACS**: Sorts and isolates into wells

cells based on fluorescence signals.



Used to analyze immune cell populations or sort stem cells

Model organisms in research

Disease models

- Most diseases cannot be exclusively studied in humans - practical, ethical reasons

- Reduced complexity in model organisms:
  - Controlled environment
  - Study one aspect at a time
  - Large control: inbreed animals, same sex, genetic background

- Model organisms can be edited and manipulated:
  - Gene edits, drug treatment, stress, toxins, infections, microbiota.

Considerations

- **Monogenic disease**:
  - Diseases caused by defects in a single gene.

    Cystic Fibrosis: mutations in the CFTR gene

- **Complex diseases**:
  - Many genes involved, sometimes in combination with environment

    Type 2 Diabetes, Cancer, Heart Disease
  - Patient Variability

Genetics and environment are many times associated to disease development

The immune system can affect many diseases as well

Model systems

- Cells
  - Human cell lines
  - Bacteria
  - Yeast
- Simple Organisms
  - Caenorhabditis elegans (worm)
  - Drosophila melanogaster (fruit fly)
  - Danio rerio (zebra fish)
- Animal Models
  - Mouse
  - Rat
  - Primates

Why using model systems?

- Ethics
- Cost
- Easier to understand how a system works when you can modify it and break it down

Choosing a model system

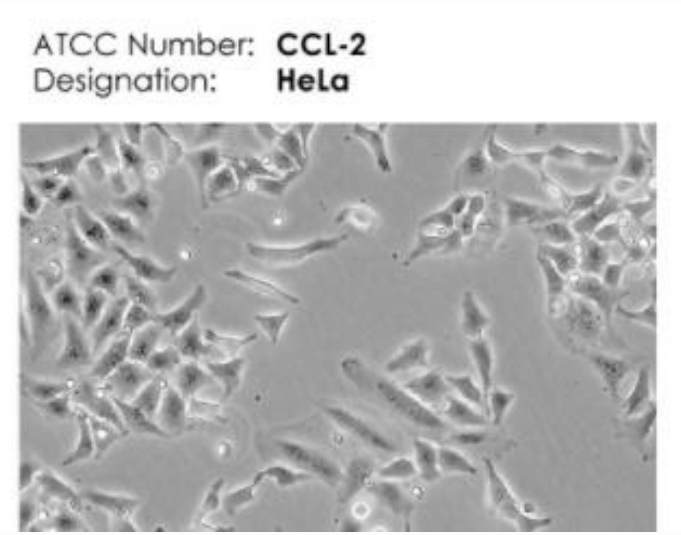
- Cells:
  - Pros: Practical, cost-effective, many replicates - easy to modify, test and repeat
  - Cons: Low complexity – may not fully replicate disease biology

- Animals:
  - Pros: high complexity – allows studying disease in a whole organism (closer to humans)
  - Cons: Expensive, time-consuming, ethical concerns, complex handling

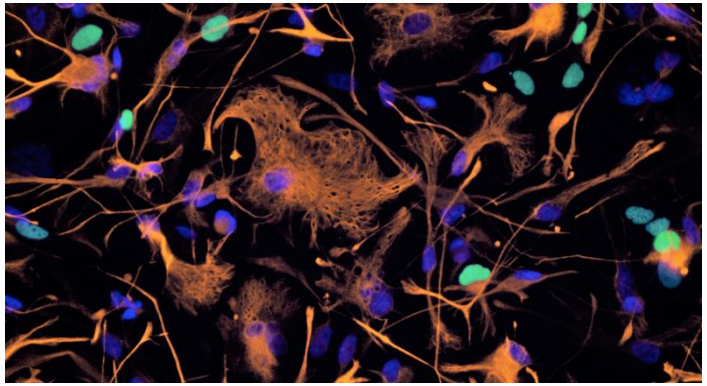
- Combine models (cells, animals and human tissues)

Human cells

- Cell lines:
  - **Immortalized**: Edited to resist an unlimited number of cell divisions
  - Can be grown ‘forever’ on a dish
  - Easily available
  - Well studied (HeLa cells: 115,757 publications)
  - Easy to add genetic modifications (e.g. CRISPR)
  - Cells in culture acquire mutations not present originally

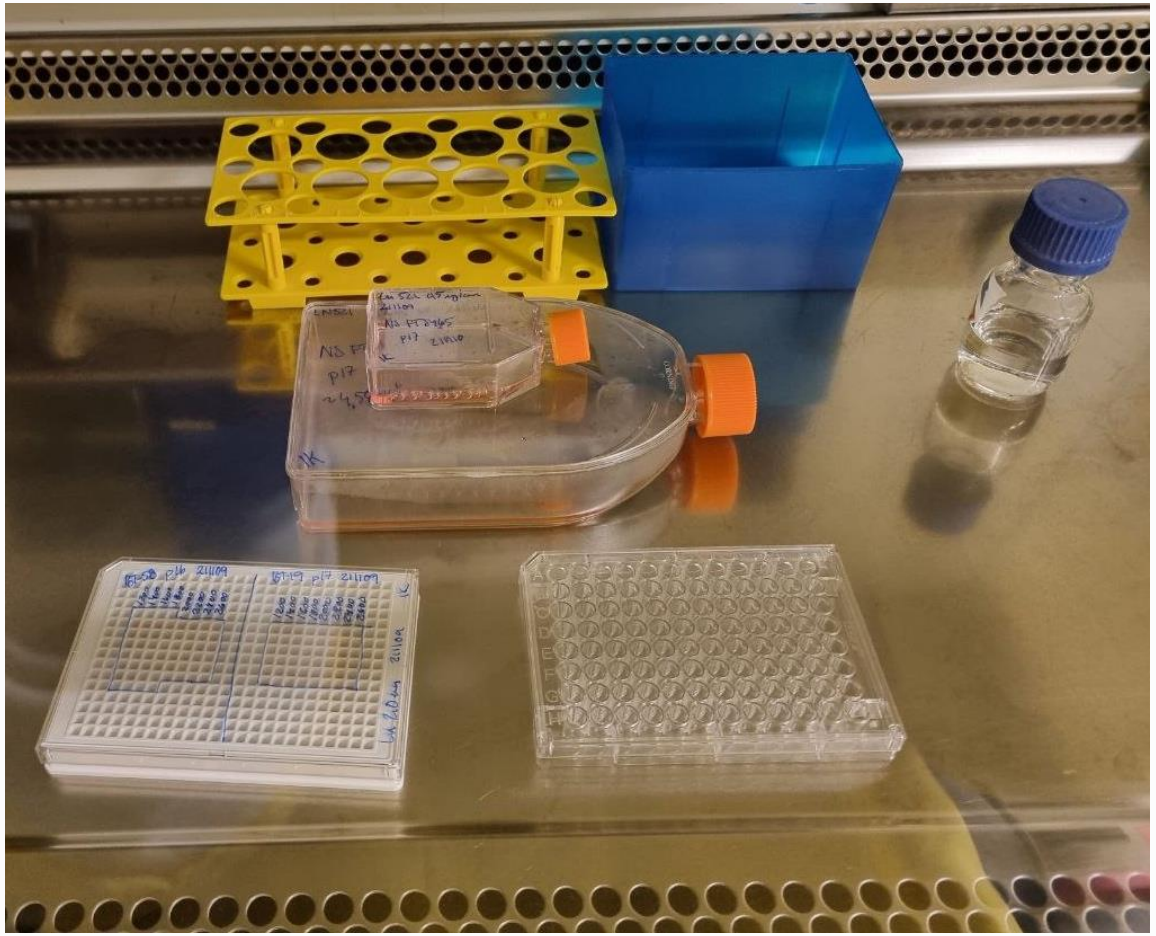


- Primary culture:
  - Cells directly obtained from animals or humans
  - Grown on a dish for a limited number of day (stop dividing / die)
  - Closer to disease physiology
  - Genetic background changes from patient to patient



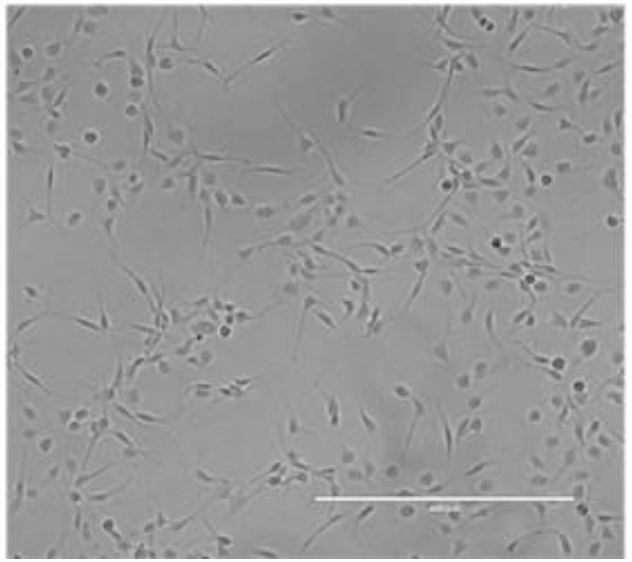
Neurala cancer stem cells, Carén lab

Human cells – culture methods (I)

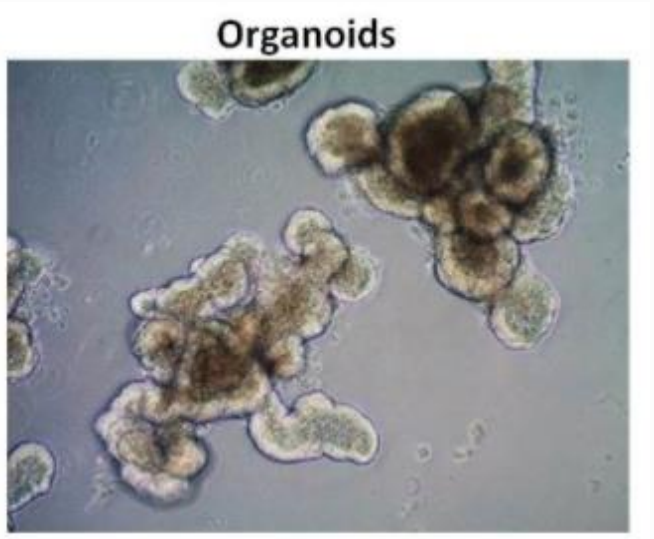


Human cells – culture methods (II)

- 2D culture
  - Adherent cells: Grow on plastic surface (e.g., fibroblasts)
  - Suspension cells: Grow freely in solution (e.g., T-cells, B-cells)
  - Simple, cost-effective, and useful to study isolated cells or monolayers.
  - Lacks complexity of 3D tissue microenvironment

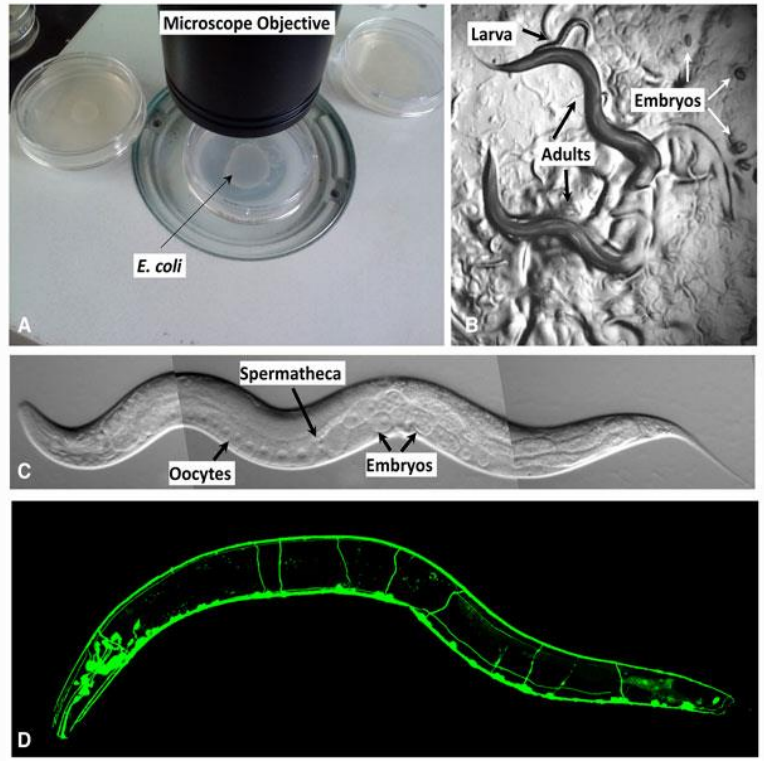


- 3D culture
  - Organoids: Aggregate of primary or stem cells that self-organize and mimic organ-like features
  - Closer to in vivo physiology
  - Limitations:
    - Short cultivation periods
    - Size limited due to lack of vascularization, affecting nutrient and oxygen supply.



Caenorhabditis elegans (worm)

- Multicellular eukaryote
- Live cycle ~2 weeks -> quick!
- Hermaphrodyte, 1 mm long, ~1000 eggs/day
- Transparent – Follow individual cells in organism.
- Sequenced genome, 60-80% of human genes have homolog in C Elegans, 40% of genes related to diseases
- Easy to screen new drugs
- Easy to mutate genes to study their function
- Applications:
  - Studies of aging
  - Development of nervous system



Corsi et al, Wormbook

Drosophila melanogaster (fruit fly)

- Life cycle of 2 weeks -> quick
- Sequenced genome, 60-75% of human disease genes
- Genetic studies of inheritance, but also cognitive studies, infections, etc
- Does not require ethical permission

Groundbreaking discoveries with Drosophila

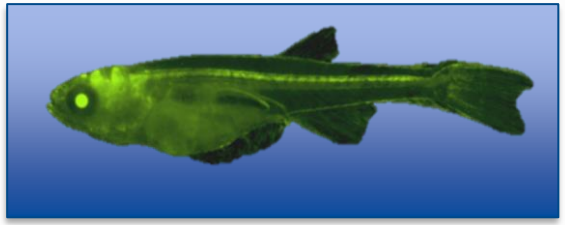
- Genes are organized in chromosomes
- Gender is controlled by chromosomal inheritance & offspring become a mixture of parents by combining genetic material – Nobel prize (Morgan, 1933)
- Radiation leads to mutations – Nobel prize (Müller, 1946)
- Isolation of mutation in the tumor suppressor genees
- Identification of genes regulating embryonic development– Nobel prize (Lewis & Nüsslein-Volhard & Eric F. Wieschaus, 1995)

Danio rerio (Zebra Fish)

- Vertebrate – organs and tissues like humans
- 3 months for an adult fish
- 200-300 eggs/week – external embryonic development
- Fully sequenced genome
- 70% of human genes have a homolog
- 84% of human genes associated with disease present
- Transparent - life-imaging of inner organs

AB wild-type

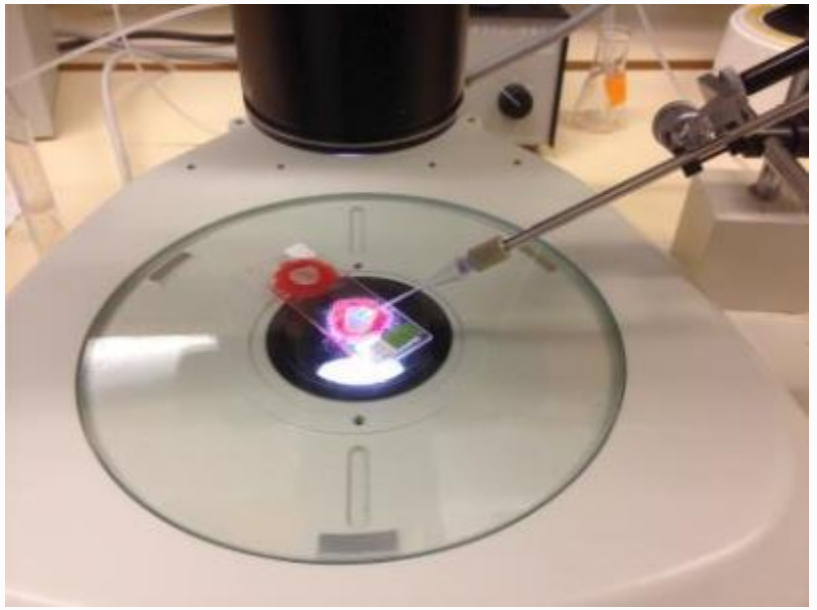
Casper

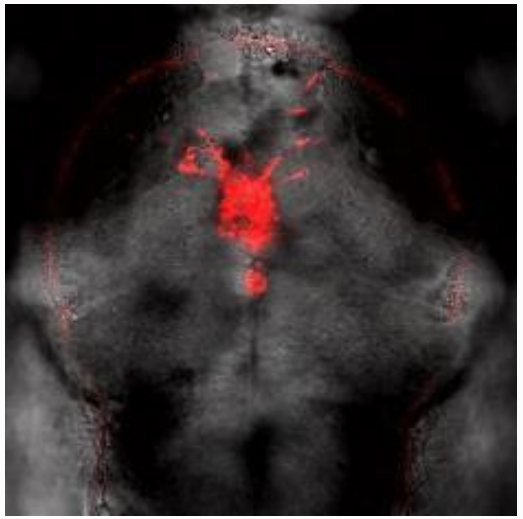


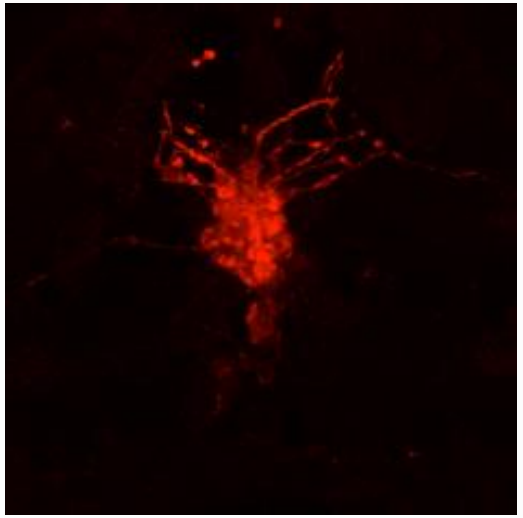
Transgenic GFP

In vivo imaging in zebrafish

- Developmental biology, regenerative medicine, diabetes, immune research, cancer
- Drug screening







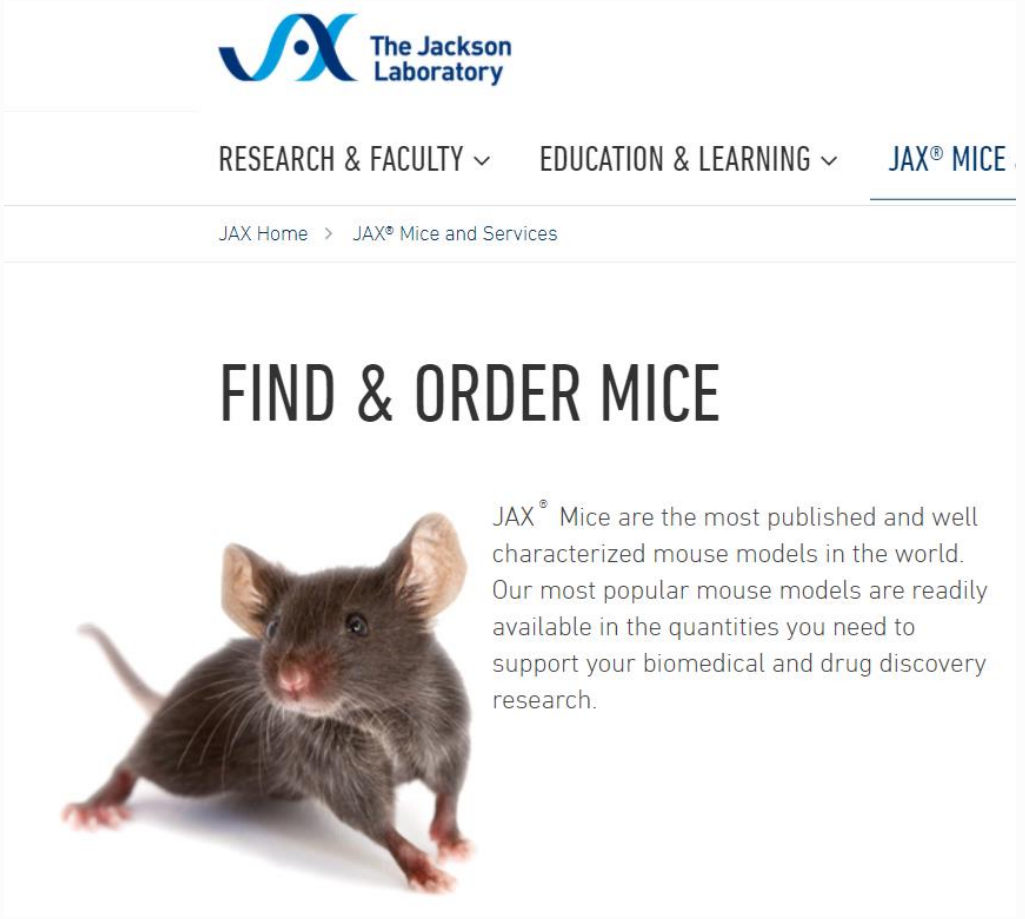
Mus musculus (I)

- Most common mammal model
- Genome very similar to human, share ~90%
- Shares many physiological and anatomical functions with humans
- Longer lifespan(2-3 years)
- Complex diseases– aging, neurodegenerative disorders, heart disease, obesity, cancer

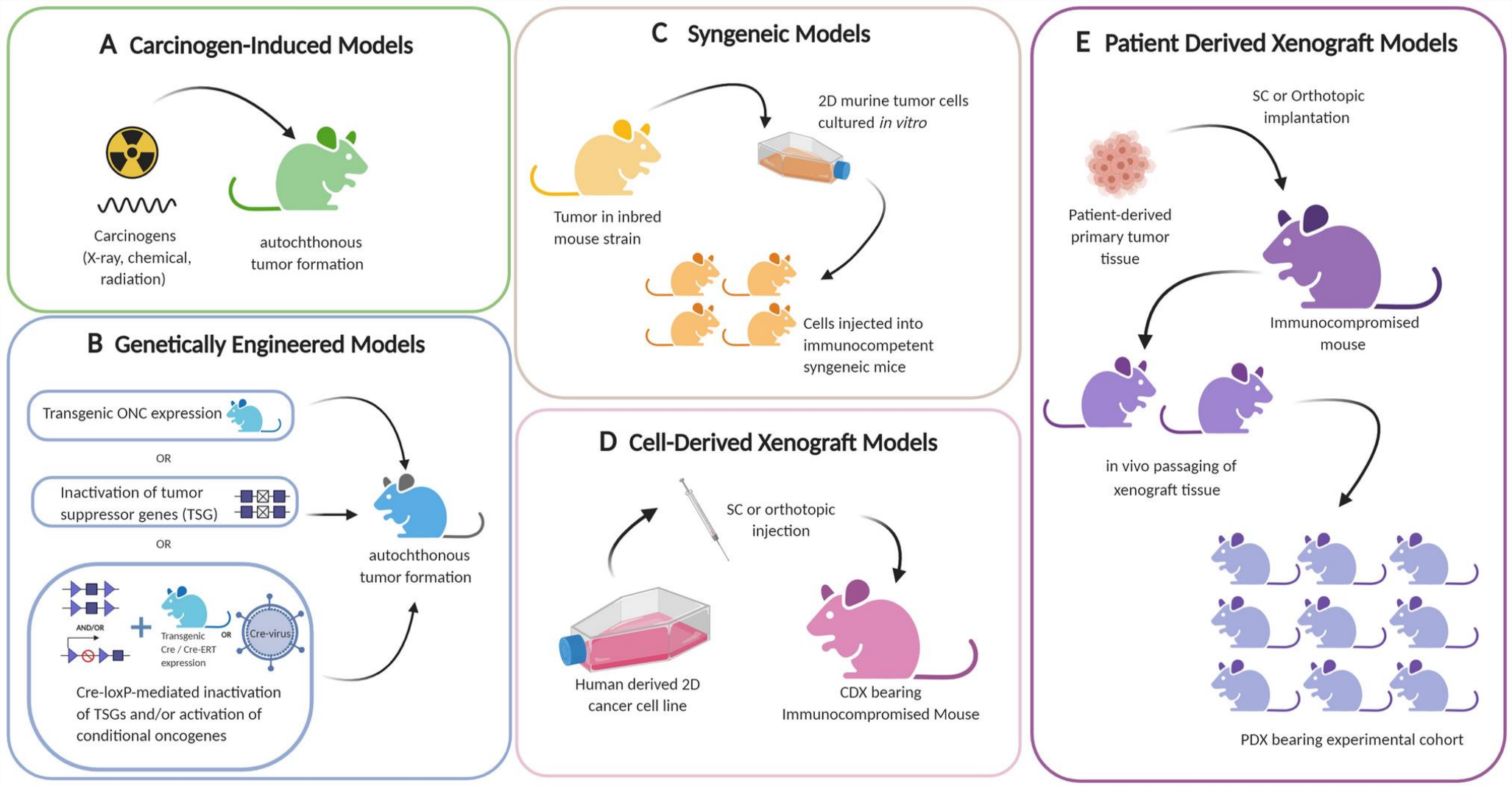
- Essential in drug development
  - Used to study metabolism, safety and efficacy

Mus musculus (II)

- Genetically inbreed strains available
- Strains with different characteristics. e.g., db mouse model develops obesity and glucose intolerance
- Possible to cross mice with different mutations
- Types:
  - Wild-type
  - Knock-out:
    - Homozygot
    - Heterozygot



Other mouse models(I)



Bareham et al, Cancer Immunology, Immunotherapy, 2021

Other animals in research

- Rat, pig, dog, horse, apes.
- Have greater similarities to humans
- Have longer lifespan:
  - Long term effects on treatments
  - Chronic disease
- Higher ethical bar

- Atypical animals can be of high interest:
  - Axolotl – Regrows body parts
  - Blind Mole Rat – Longevity (40 yrs vs 2 yrs most rodents)

Axolotl

Blind Mole Rat

Animal experimentation

- Animal experimentation is when you use animals in:
  - Basic and applied research
  - Diagnosis of multiple diseases
  - Development and manufacture of pharmaceuticals or other chemical products
  - Perform surgical procedures in animals, inject or draw blood, or if you cause animal suffering in any other way
  - or for other equivalent purposes

Research in animals – Ethics (I)

- Various permits and approvals are required to perfom research in animals.
- Personnel taking care of the animals must have the right training and competence.
- Experiments involving animals are approved by a regional ethical board.
- The benefit of the experiment must be greater than potential animal suffering, and there must not be an equally good alternative.
- The requirement for ethical review applies generally to vertebrates (e.g. fish, mammals birds). Embryos are also covered.

Animal Research – Ethics (II)

- Strict rules
  - In general stricter than in other environments (food production, tick control)
- 3 R – replace, reduce, refine
- Replace:
  - Alternative methods
- Reduce:
  - Use fewer animals through statistical planning
  - Minimize variation using animals with same genetics background
  - Controlled environment
- Refine:
  - Plan research to minimize pain and distress to animals
  - Improve living conditions with enrichment and group housing when possible.

Animal use in research 2018

- Basic research:
  - 212,500 animals
  - Cardiovascular, blood and lymphatic system (22 %)
  - Nervous system (17 %)
  - Immune system (15%)

- Translation and applied research:
  - 46,000 animals
  - Nervous and mental disorders in humans(24 %)
  - Cancer in humans(17 %)
  - Endocrine disorders and toxicology (14 %)

Djurgrupp	Djurslag	Totalt	
		Antal	%
Gnagare	Husmus	173 998	63
	Brunråtta	15 438	6
	Marsvin	623	<1
	Guldhamster	39	<1
	Kinesisk dvärghamster	0	0
	Mongolisk ökenråtta	0	0
	Övriga gnagare	181	<1
Hardjur	Kanin	1 738	<1
Rovdjur	Katt	4	<1
	Hund	531	<1
	Tamiller	0	0
	Övriga rovdjur	237	<1
Hovdjur	Hästar, åsnor	146	<1
	Svin	1 579	<1
	Getter	261	<1
	Får	46	<1
	Nötkreatur	2 394	<1
Primater	Strepsirrhini (Halvapor)	0	0
	Silkesapor och tamariner	0	0
	Krabbmakak	10	<1
	Rhesusmakak	10	<1
	Gröna markattor	0	0
	Babianer	0	0
	Dödskalleapor	0	0
	Övriga arter av gamla världens apor (Cercopithecoidea)	0	0
	Övriga arter av nya världens apor (Ceboidea)	0	0
	Människoartade apor (Hominoidea)	0	0
Övriga däggdjur	Övriga däggdjur	260	<1
Fåglar	Tamhöns	1 153	<1
	Övriga fåglar	10 625	4
Kräldjur	Kräldjur	529	<1
Groddjur	Grodor (om Rana spp.*)	2	<1
	Klogrodor	298	<1
	Övriga groddjur	2 538	<1
Fiskar	Zebrafisk	36 476	13
	Övriga fiskar	25 539	9
Bläckfiskar	Bläckfiskar	0	0
	Totalt	274 655	**100**

Statistik, Jordbruks verket

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https://www.camunaslab.org/