WORKSHOP
Bioinformatics & Stitch in Biomolecular Sciences

Module III
Introduction to Differential Splicing Analysis

Board of European Students of Technology

BEST ALMADA

Paulo Caldas, PhD
p.caldas@fct.unl.pt
cOmics Lab

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

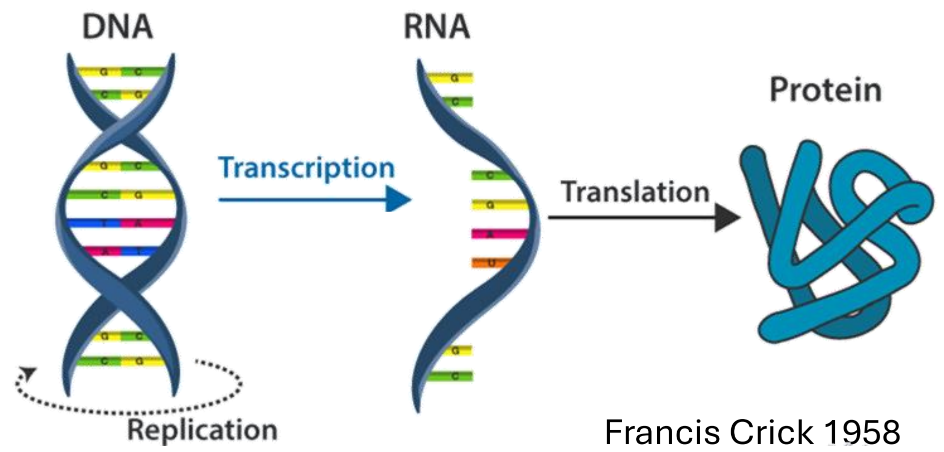
Computational Multi-Omics

EXPERIMENTAL VS. COMPUTATIONAL BIOLOGY

NOVA SCHOOL OF SCIENCE & TECHNOLOGY
Computational Multi-Omics
Paulo Caldas, PhD
p.caldas@fct.unl.pt
cOmics Lab

TRANSCRIPTION & TRANSLATION

Central Dogma of Biology

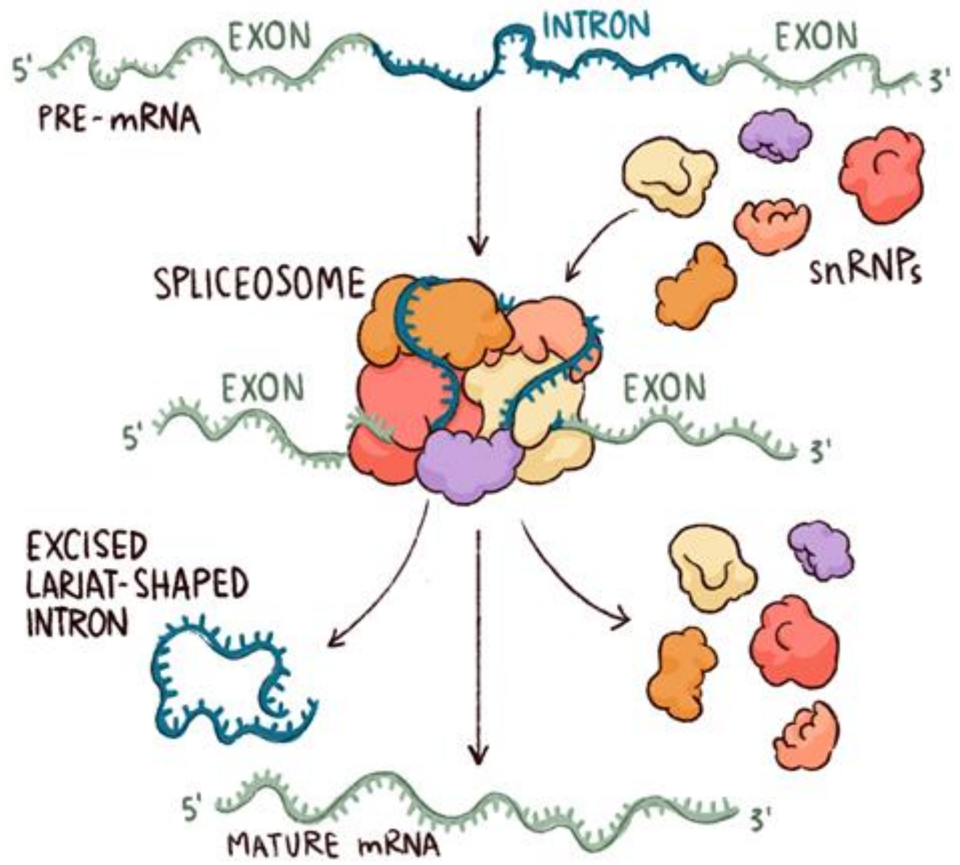


Francis Crick 1958

The flow of genetic information is more complex than initially thought ...

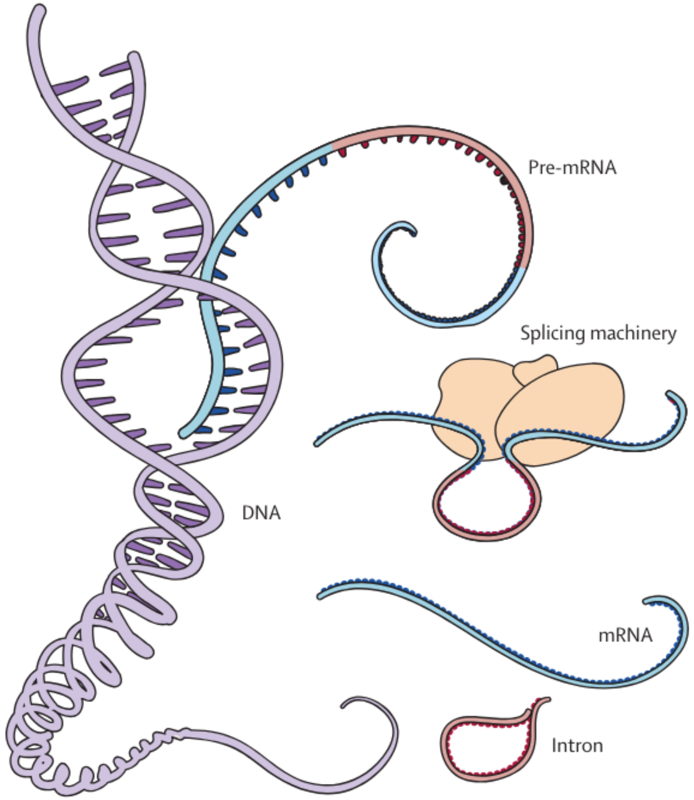
- Reverse Transcription (virus)
- Non-coding RNAs (regulatory functions)
- **Alternative Splicing**
- (...)

RNA Splicing

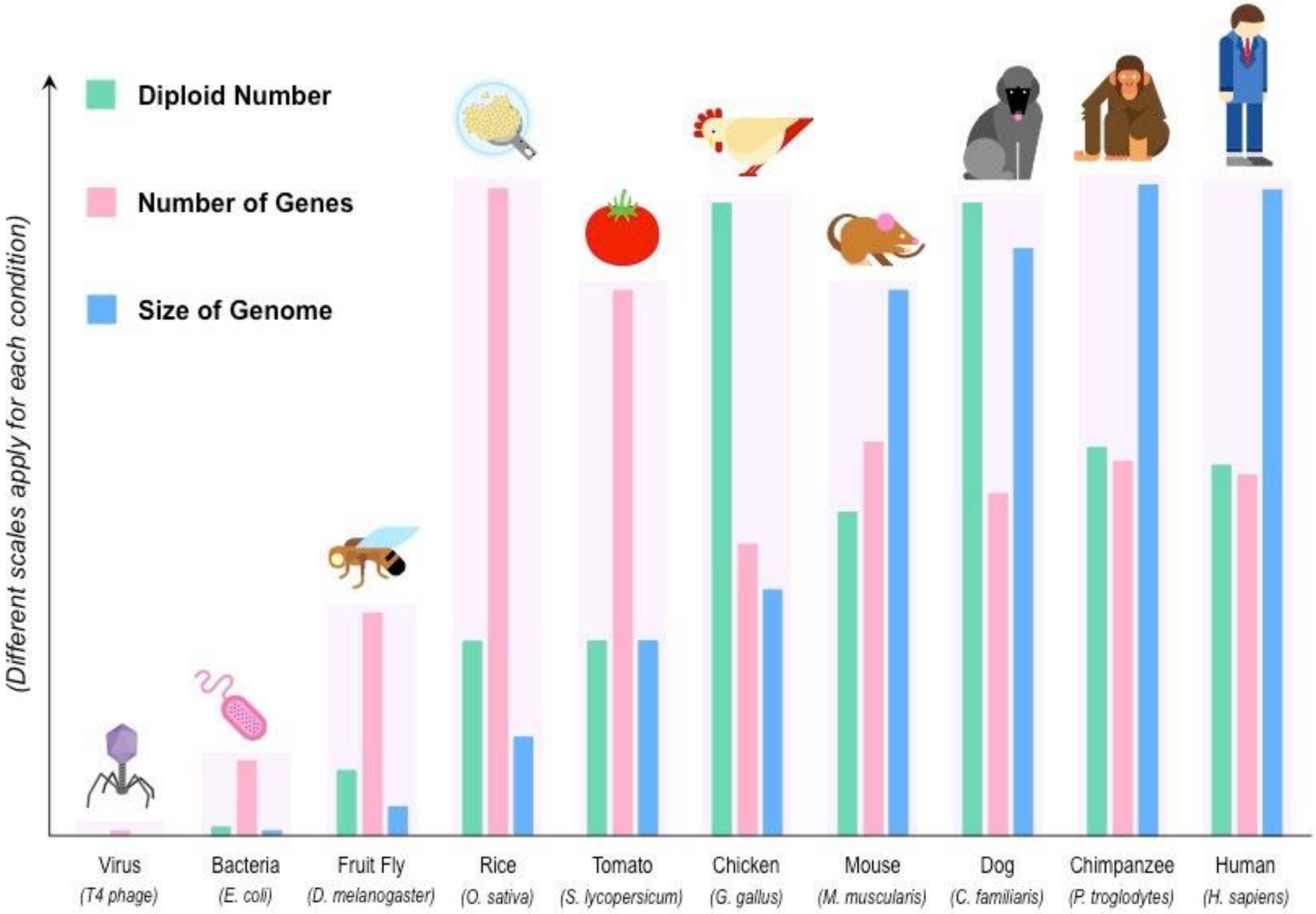


NOVA SCHOOL OF SCIENCE & TECHNOLOGY

TRANSCRIPTION & SPLICING



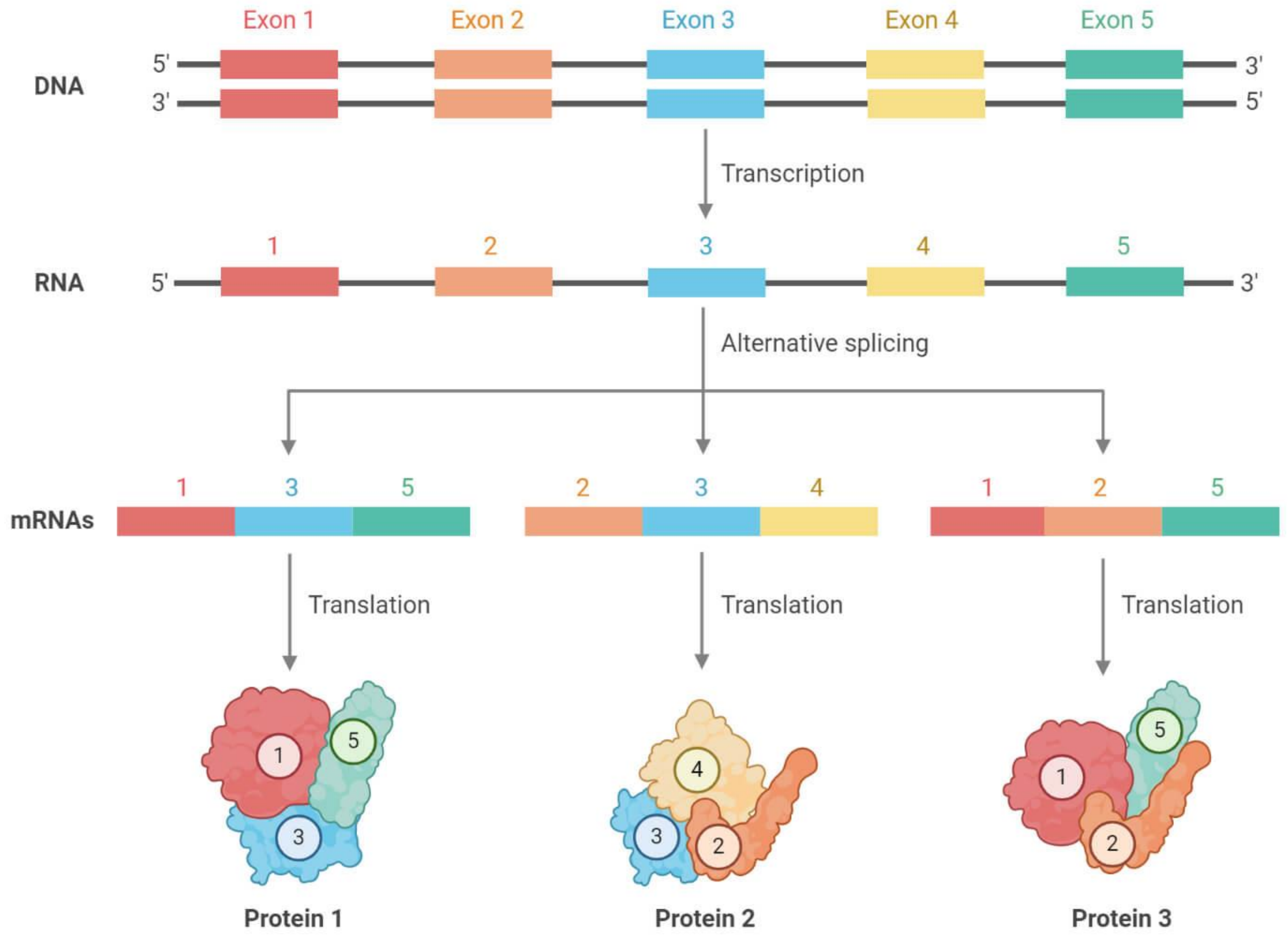
GENE NUMBER VS. COMPLEXITY



Alternative Splicing
Gilbert, 1978

ALTERNATIVE SPLICING

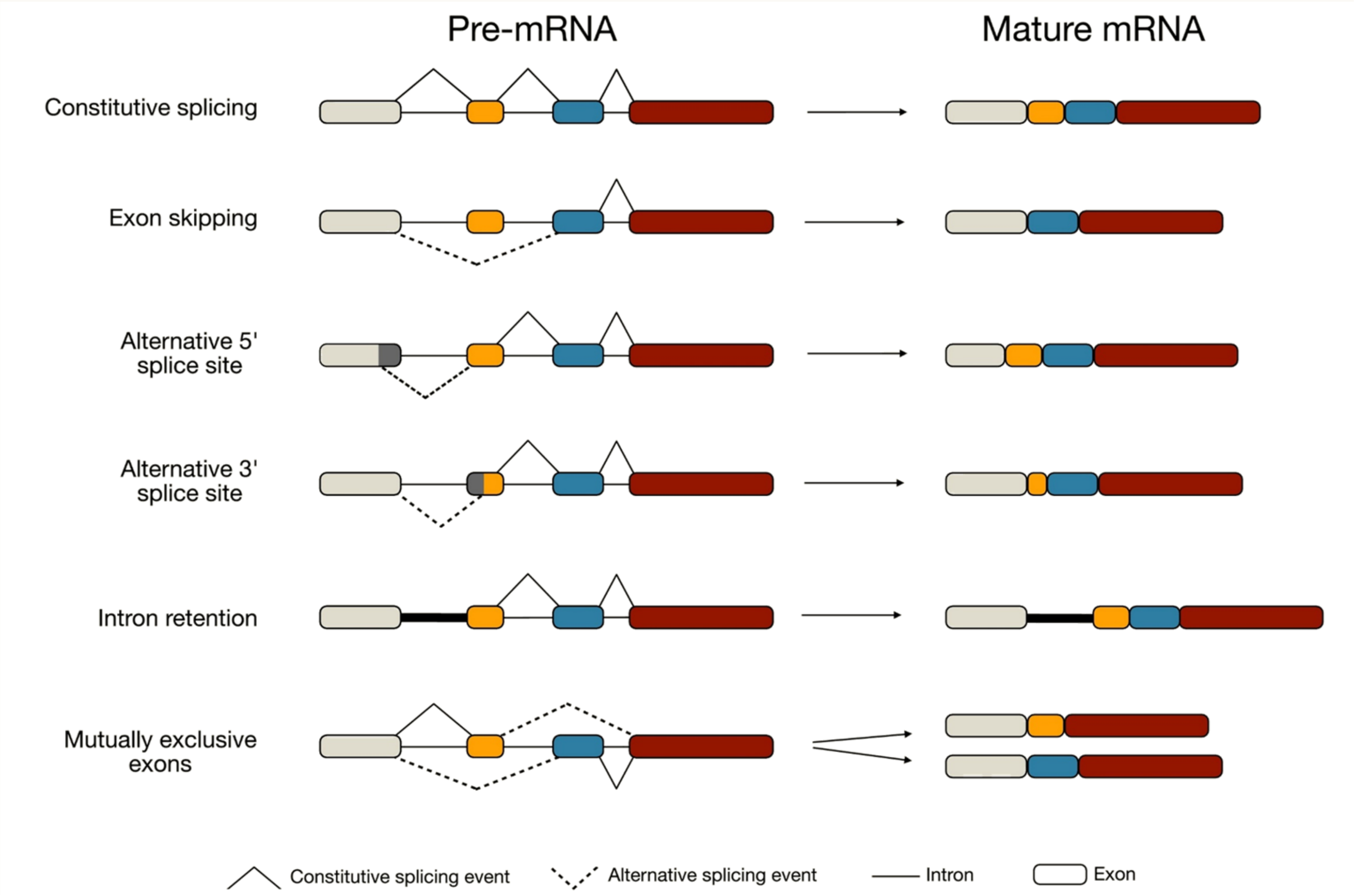
The human genome has aprox. 20.000 genes
But generates more than 100.000 different proteins ...



Increased diversify and functionality!

Alternative Splicing
Gilbert, 1978

TYPES OF ALTERNATIVE SPLICING

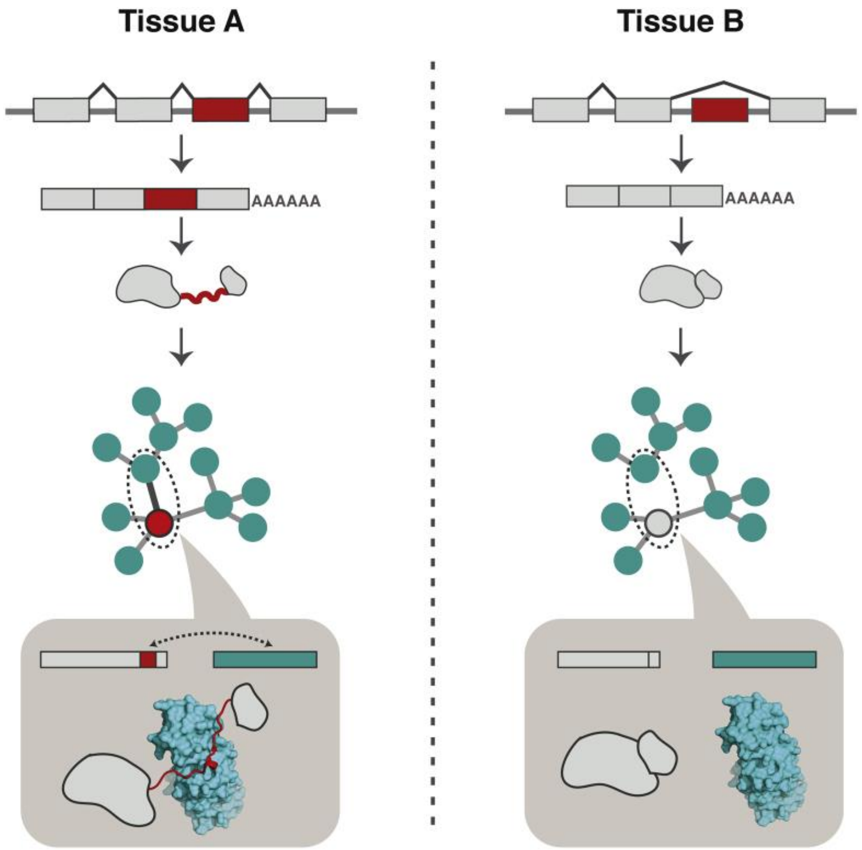


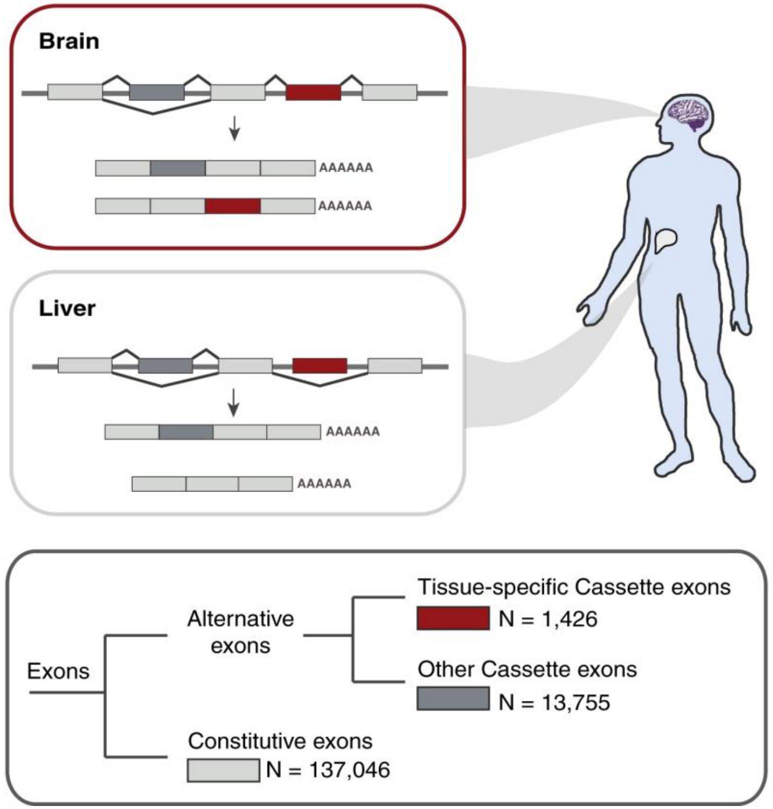
Jukka-Pekka Verta & Arne Jacobs 2022 Trends in Ecology & Evolution

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

TISSUE SPECIFIC ALTERNATIVE SPLICING

Alternative splicing can result in proteins with tissue-specific functions in muscles, neurons, or liver cells, contributing to the complexity and adaptability of biological processes in multicellular organisms.



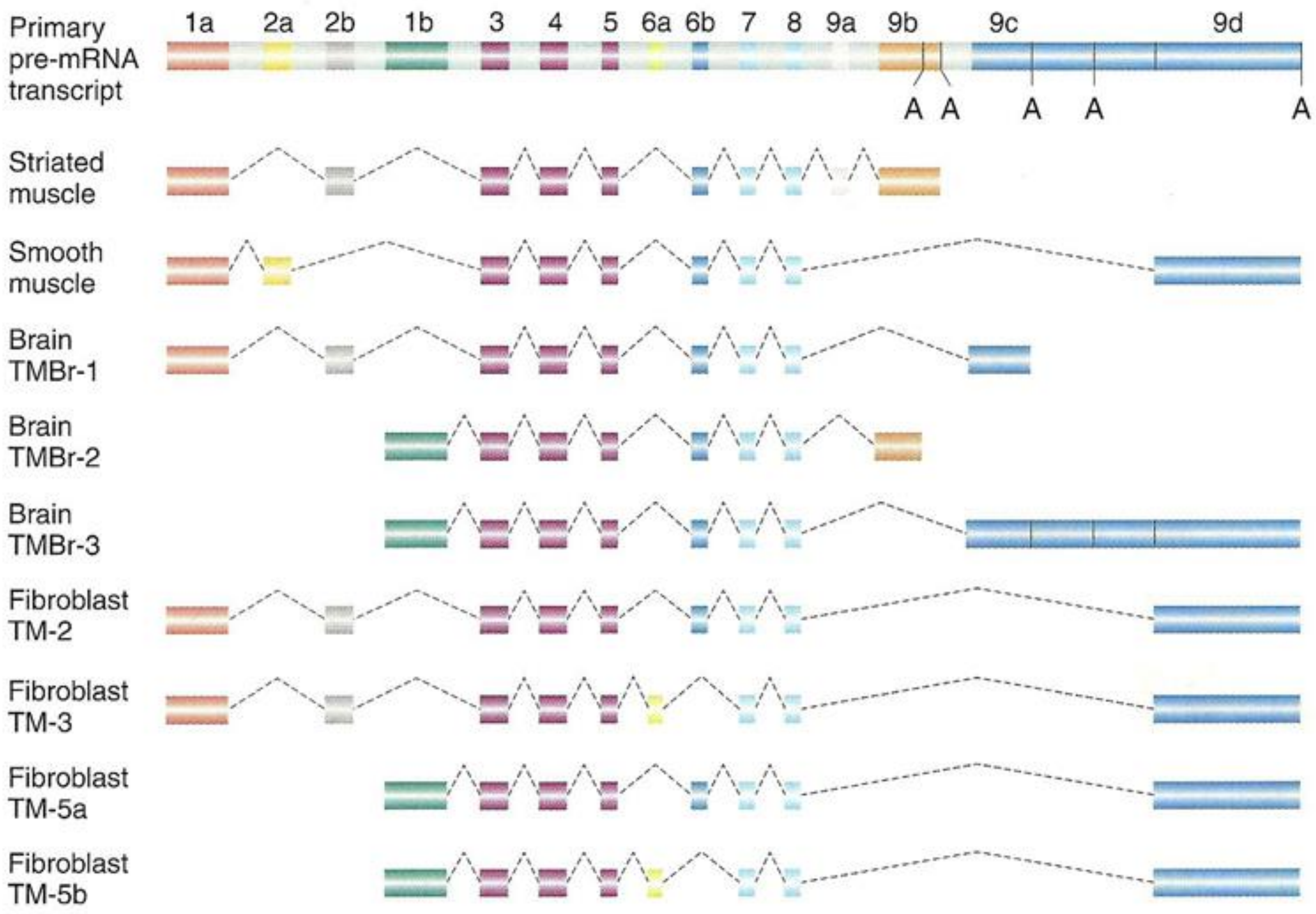


Buljan M, et al. Mol Cell. 2012.

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

TISSUE SPECIFIC ALTERNATIVE SPLICING

Tropomyosin Alternative Splicing



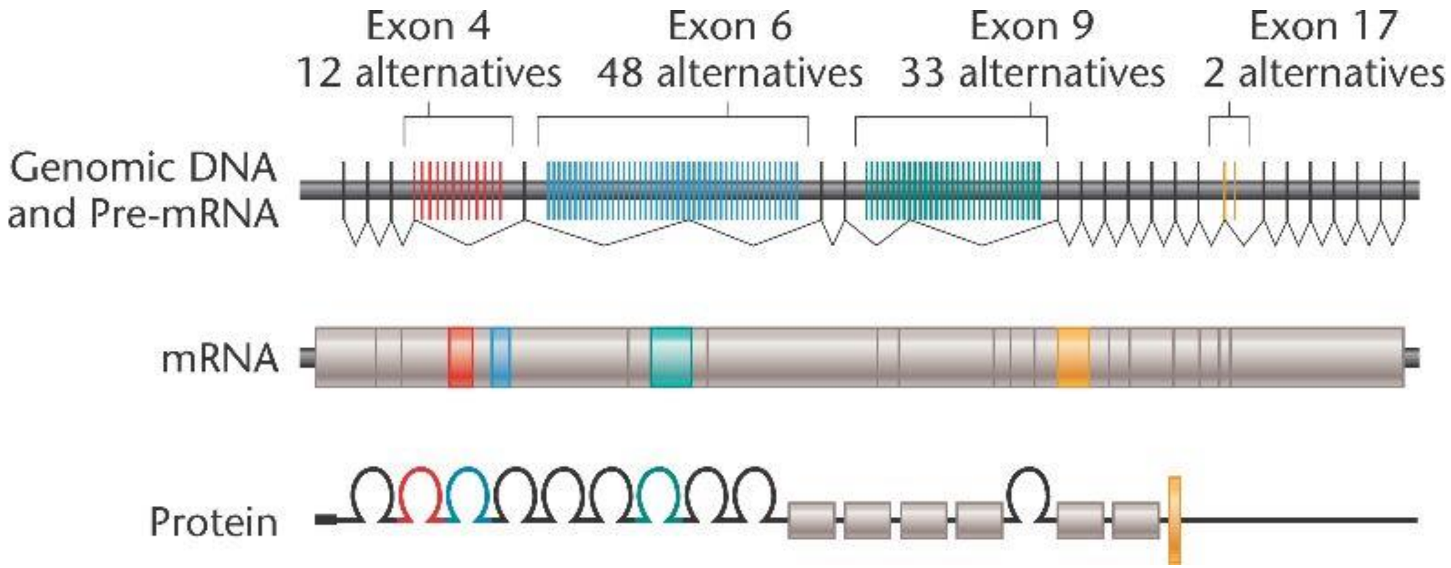
In skeletal muscles: isoforms that regulate actin-myosin interactions for muscle contraction.
In smooth muscles and non-muscle cells: isoforms that contribute to cytoskeletal stability and cell motility.

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

ALTERNATIVE SPLICING: RECORD HOLDER!

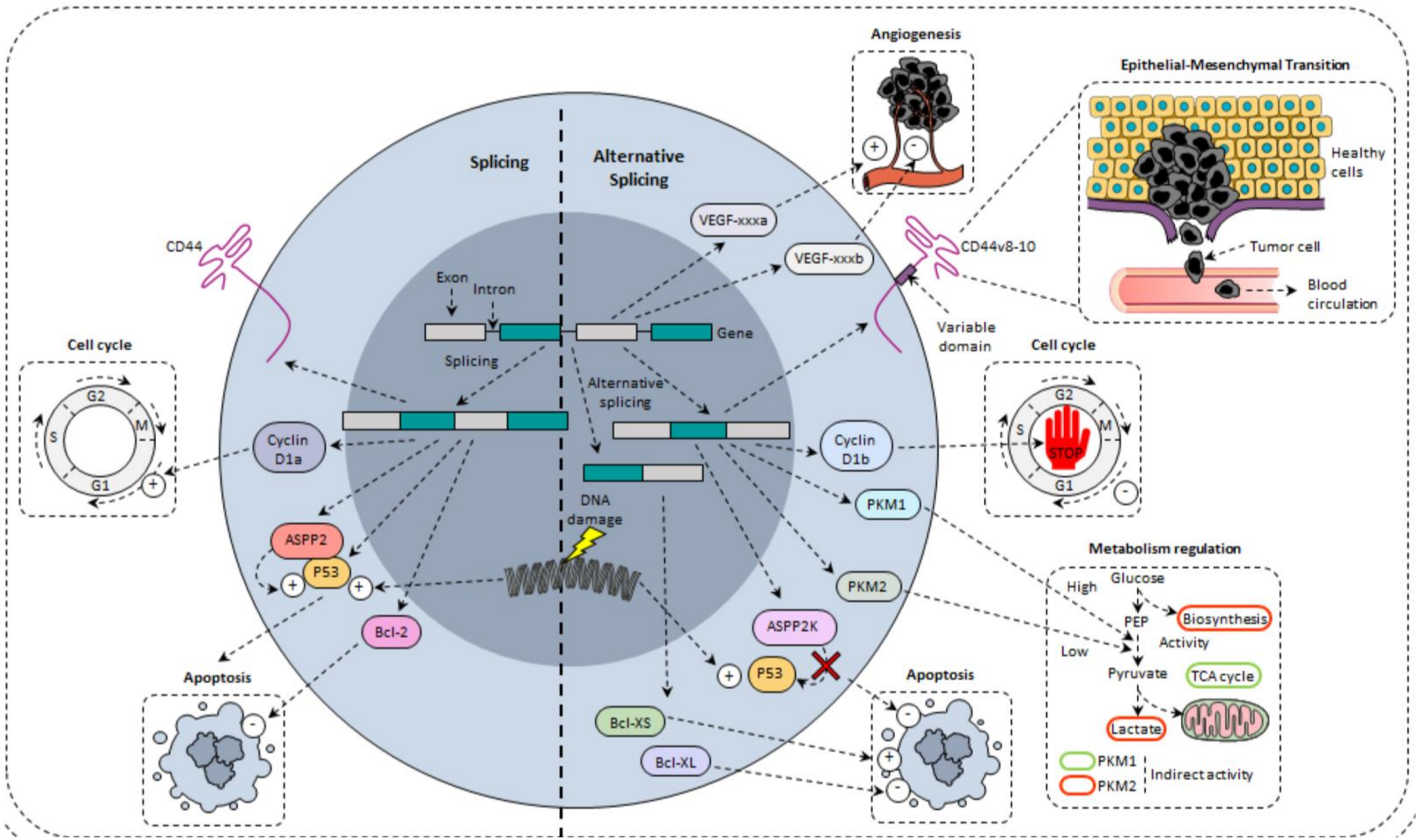
Dscam Gene in Drosophila

Involved in adhesion between neurons
Contains 4 clusters of exons that are spliced a mutually exclusive fashion



If all combinations of these exons are used in alternative splicing, the Dscam gene can produce **38,016** different proteins !

ALTERNATIVE SPLICING IN CANCER



Bernard, A. et. al., Cancers. 2022

Resistance to Apoptosis
Caspase-3 : Alternative splicing generates caspase-3s, an anti-apoptotic isoform lacking a catalytic site, inhibiting apoptosis.

Bcl-2: Splicing produces anti-apoptotic Bcl-XL, common in cancers, contributing to chemotherapy resistance.

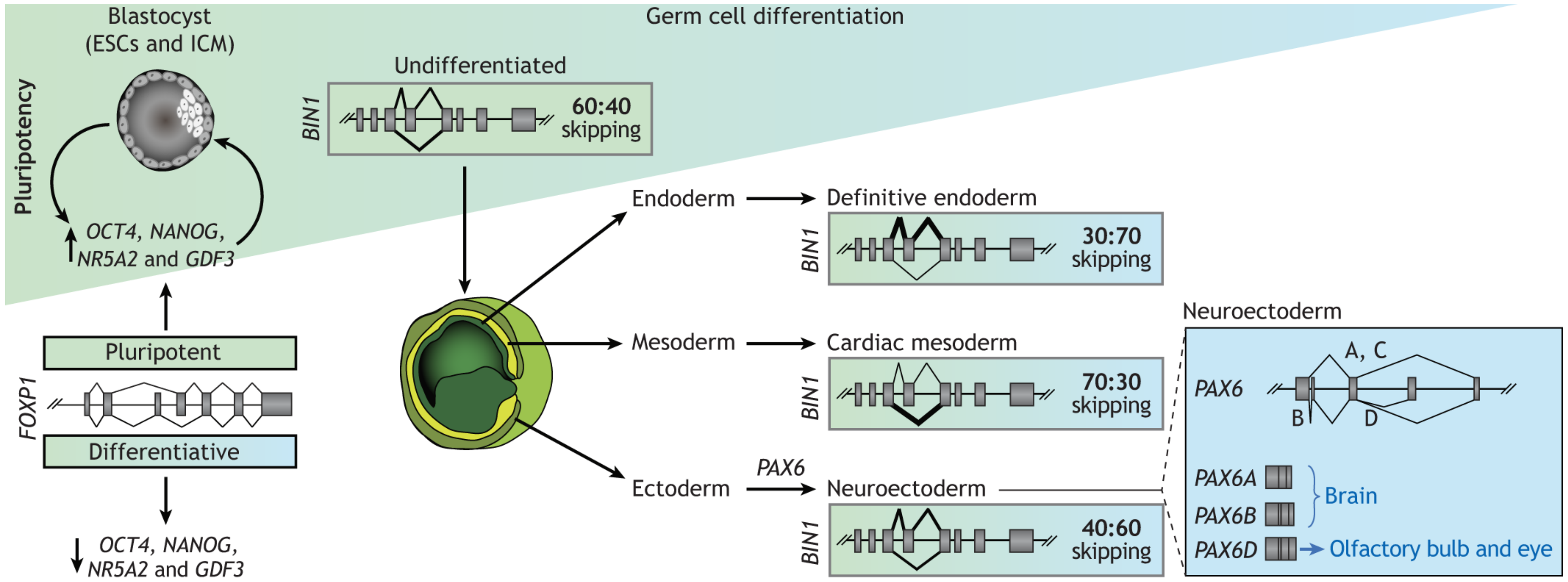
Enhanced Angiogenesis
VEGF-A: Alternative splicing increases pro-angiogenic isoforms leading to increased tumor growth.

Metabolism Regulation:
Cancer cells often shift to aerobic glycolysis facilitated by AS of metabolic genes.

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

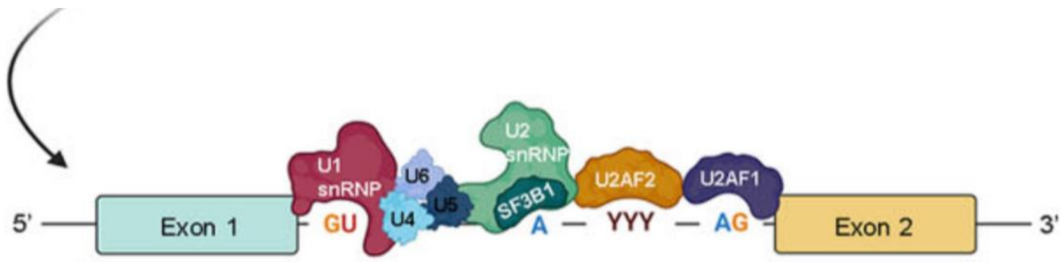
ALTERNATIVE SPLICING IN VERTEBRATE DEVELOPMENT

AS is essential for tissue-specific functions and complex developmental processes
leads to diverse protein isoforms essential for different developmental stages and tissues



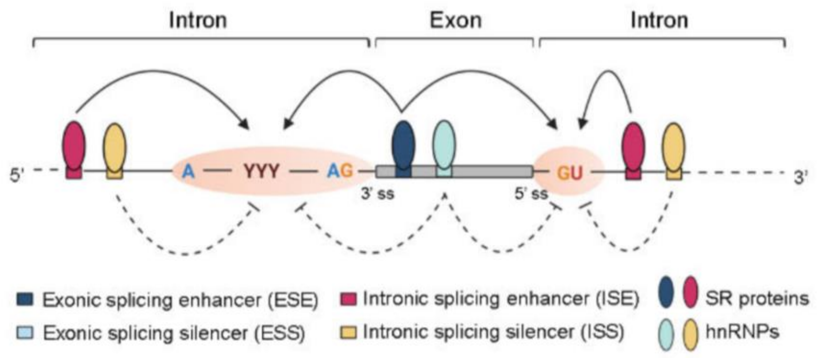
Anouk M., et al,. Development, 2022.

REGULATION OF ALTERNATIVE SPLICING



Several RNA elements recognize splice sites in a cell-type, condition or species- dependent manner

The combination/presence/absence of these elements in each context modulates the final mRNA product.



<u>**Key Points About Alternative Splicing**</u>

Increases Protein Diversity
A single gene produces multiple protein isoforms (functional versatility)

Regulates Gene Expression
Influences mRNA stability, export, and translation efficiency

Crucial for Development and Adaptation
Correct cell differentiation and response to environmental changes.

Linked to Diseases
AS dysregulation can result in disease-associated isoforms, making it a potential therapeutic target.

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DIFFERENTIAL SPLICING ANALYSIS

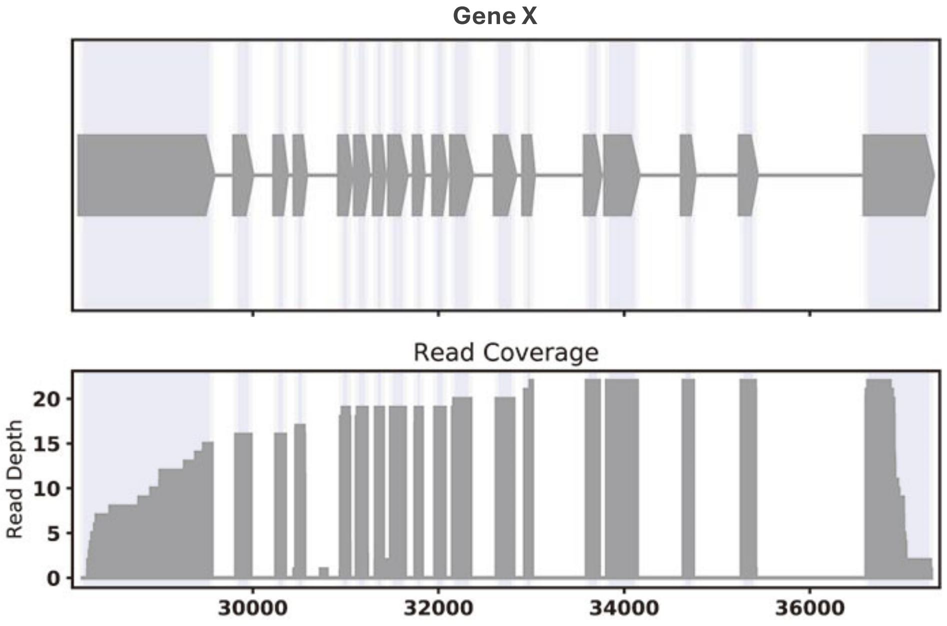
Differential Gene Expression

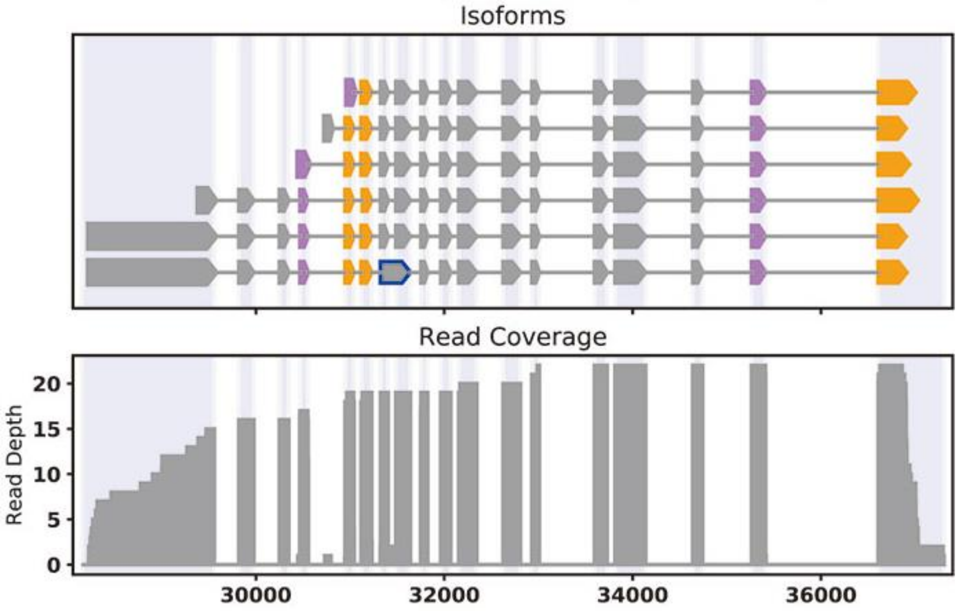
Significant change in gene expression between two conditions (i.e. quantity of mRNA transcribed from a gene)

VS.

Differential Splicing

Significant change in the expression of all isoforms relative to the total gene expression between two conditions



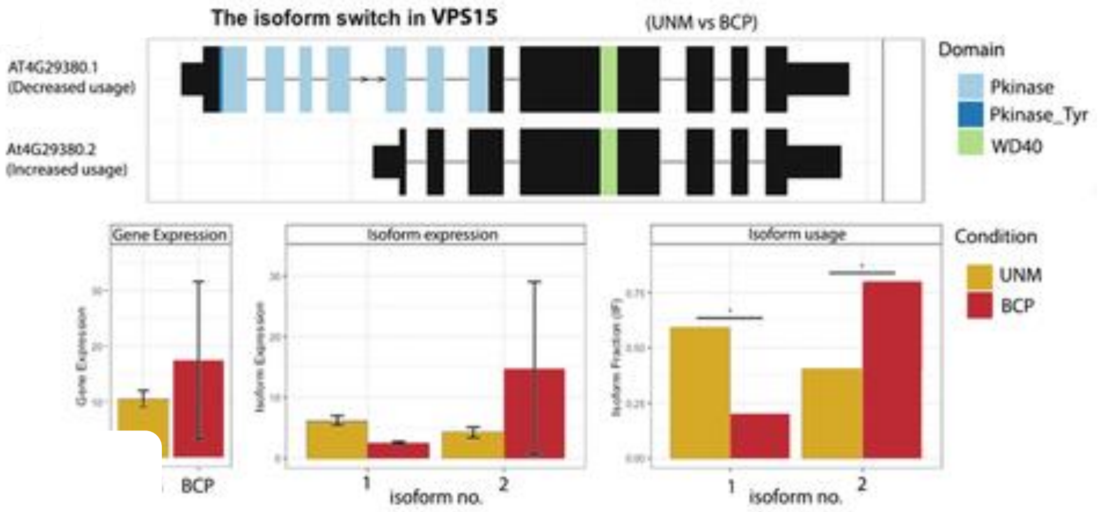


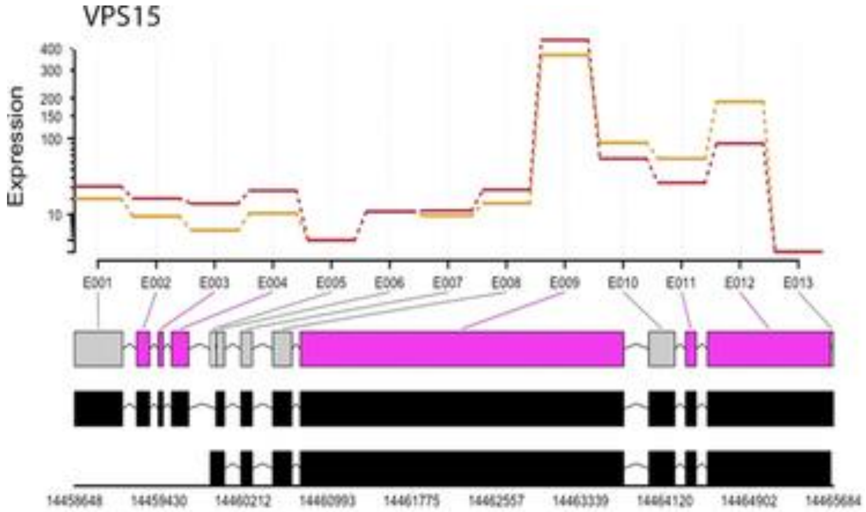
DIFFERENTIAL SPLICING ANALYSIS

<u>Differential Gene Expression</u>
Significant change in gene expression between two conditions (i.e. quantity of mRNA transcribed from a gene)

VS.

<u>Differential Splicing</u>
Significant change in the expression of all isoforms relative to the total gene expression between two conditions

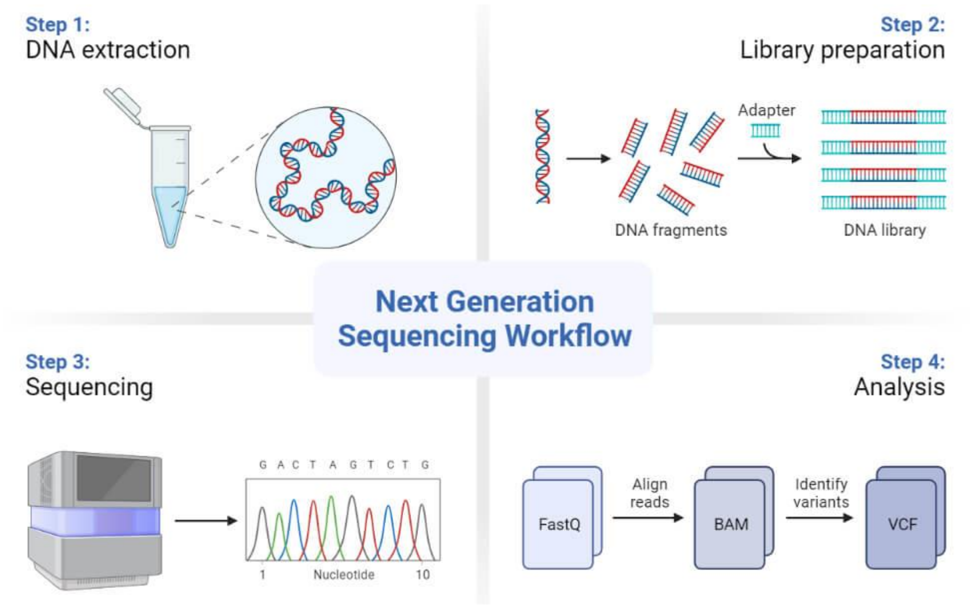




Which isoforms are more/less expressed in two different conditions

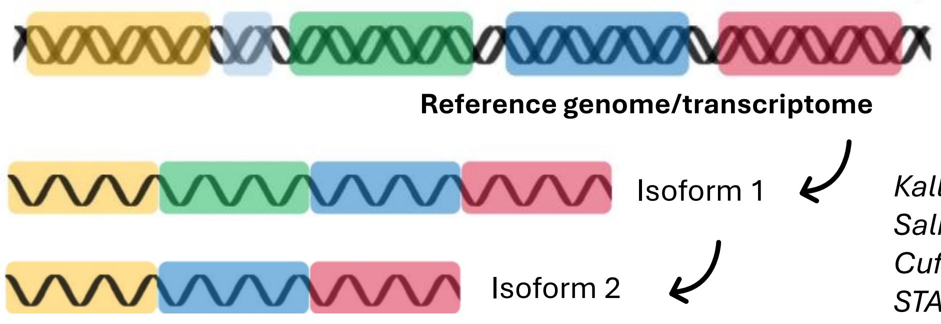
Which exons are being in/excluded from the final product ?

DIFFERENTIAL SPLICING ANALYSIS



Isoform-Centred Approach

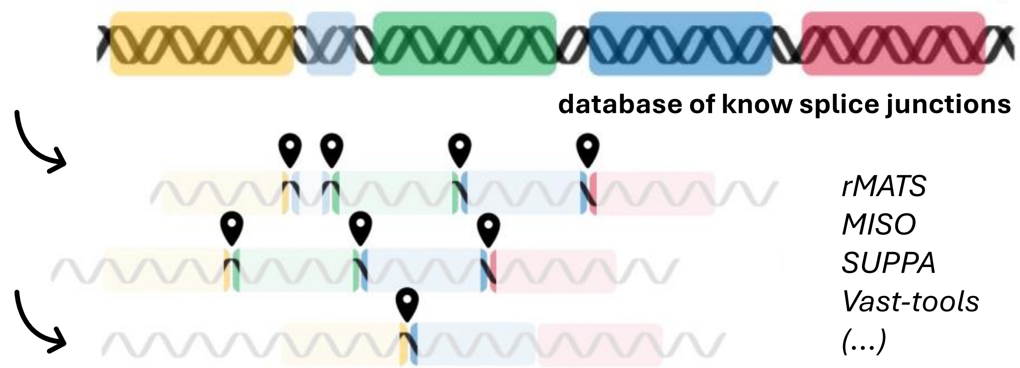
How much of the gene's expression if from each transcript?



Kallisto
Salmon
Cufflinks
STAR
(...)

Event-Centred Approach

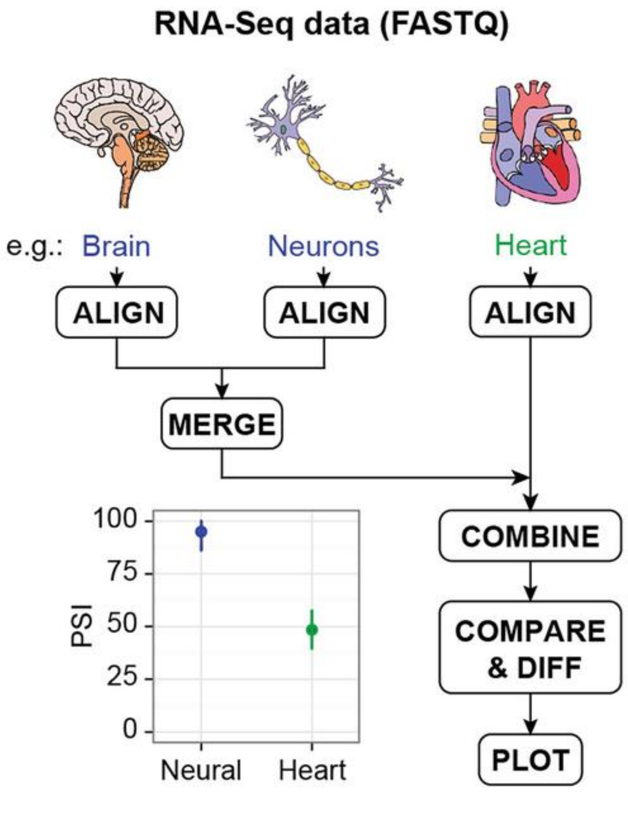
What is the proportion of transcripts that include a given exon?



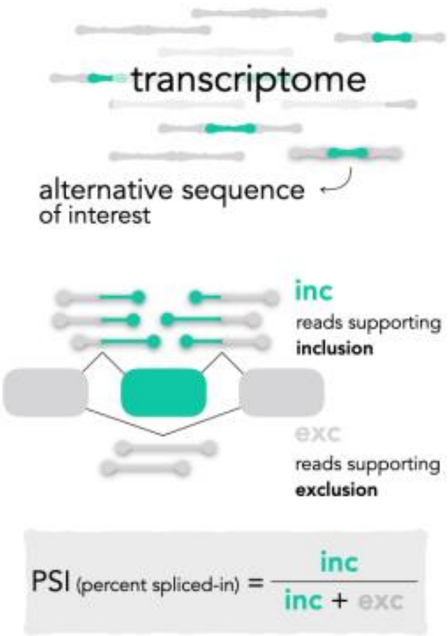
rMATS
MISO
SUPPA
Vast-tools
(...)

DIFFERENTIAL SPLICING ANALYSIS WORKFLOW

VAST-TOOLS WORKFLOW



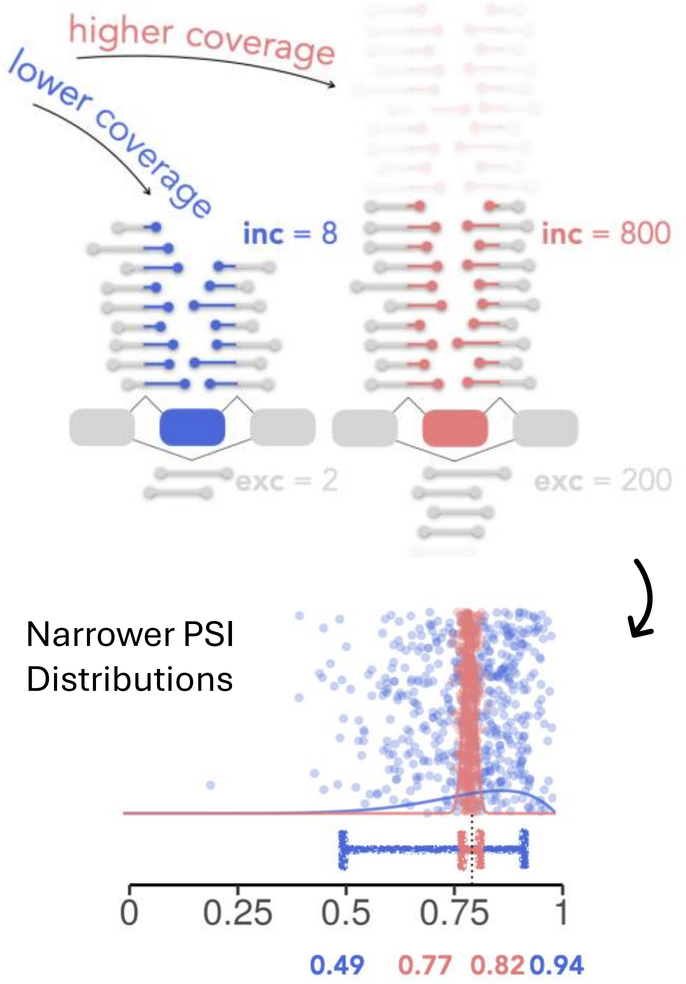
<u>OUTPUT</u>
Table with PSI values for each exon/intron in the DB



ratio of normalized read counts of exon/intron inclusion relative to the total reads for that event

-

Varies between 0 and 1

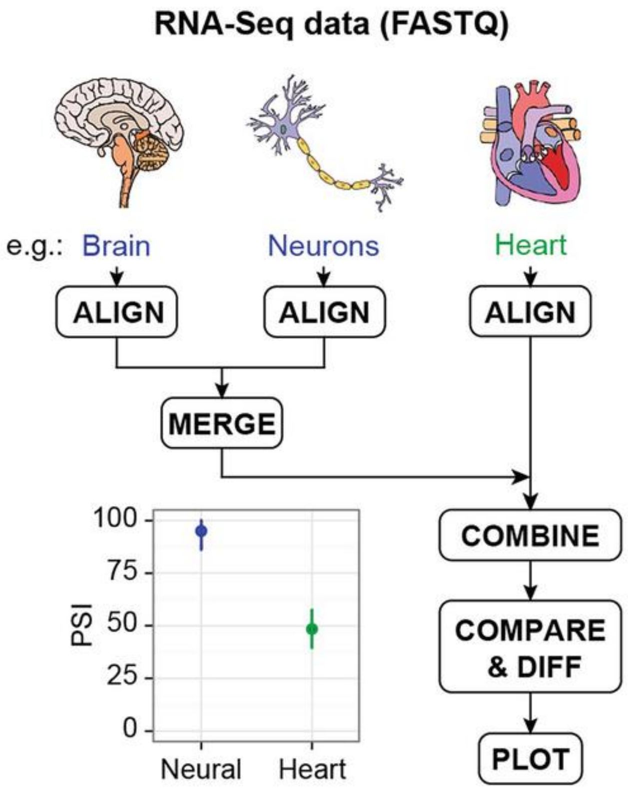


Ascensão-Ferreira, M. et al,. RNA 2024

HANDS-ON: DIFFERENTIAL SPLICING ANALYSIS

https://github.com/vastgroup/vast-tools

VAST-TOOLS WORKFLOW



vast-tools align output

GENE	EVENT	COORD	LENGTH	SAMPLE	SAMPLE	SAMPLE	SAMPLE	SAMPLE	SAMPLE	...
EPN1	HsaEX0022820	chr19:56200663-56200737	75	S	11.33	11.33	35.89	12.66	87.17	...
SUGP2	HsaEX0062842	chr19:19104457-19104549	93	S	19.54	18.02	11.93	7.85	22.22	...
APLP2	HsaEX0005151	chr11:130007151-130007186	36	S	88.68	94.33	69.53	87.48	11.50	...
PSAP	HsaEX0050522	chr10:73583645-73583653	9	MIC	94.02	96.51	58.55	93.72	91.46	...
INTS10	HsaEX0031861	chr8:19706673-19706750	78	S	84.27	75.44	50.91	77.14	18.92	...
...	...	...	...	...	...	...	...	...	...	...

vast-tools diff output

GENE	EVENT	BRAIN	LIVER	dPSI	MV[dPsi]
LAS1L	HsaEX0035417	0.970019	0.987019	-0.017000	0.00
KRIT1	HsaEX0035028	0.988664	0.983987	0.004676	0.03
LAP3	HsaEX0035326	0.926628	0.985192	-0.058563	0.00
CD99	HsaEX0013932	0.736958	0.962170	-0.225211	0.07
SPATA20	HsaEX0061239	0.988164	0.982821	0.005343	0.04
...	...	...	...	...	...

HANDS-ON: DIFFERENTIAL SPLICING & PYTHON

You should have Anaconda Installed (!)
Download Files & Open the Jupyter Notebook

BUT FIRST … COFFEE …