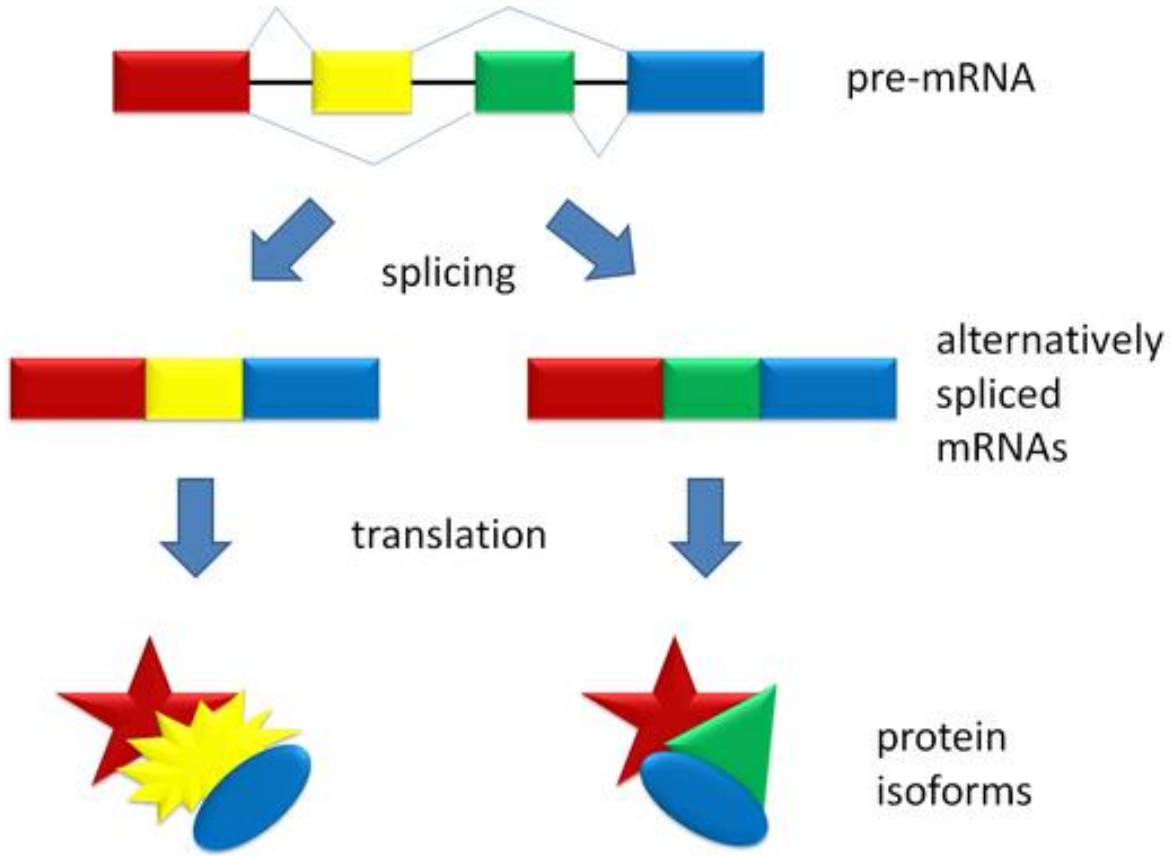
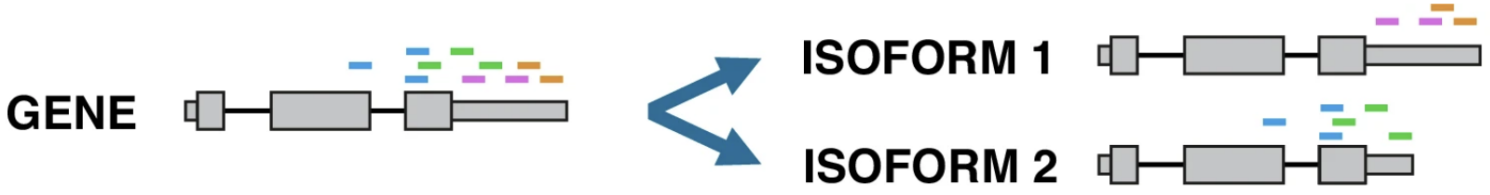
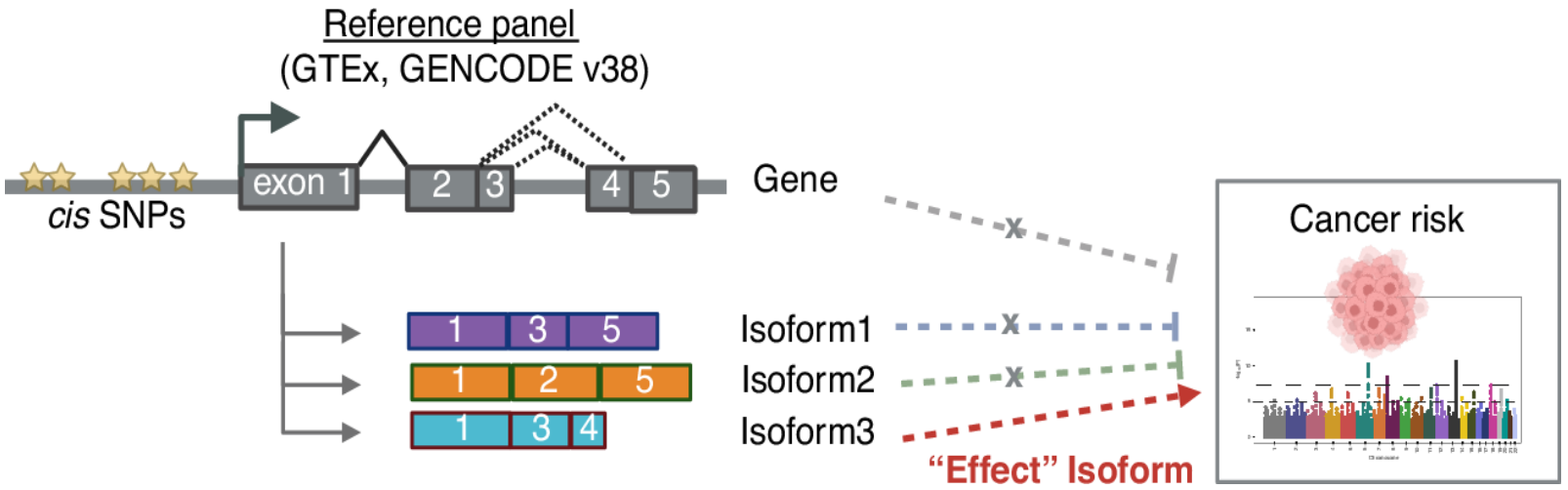
What is an isoform?



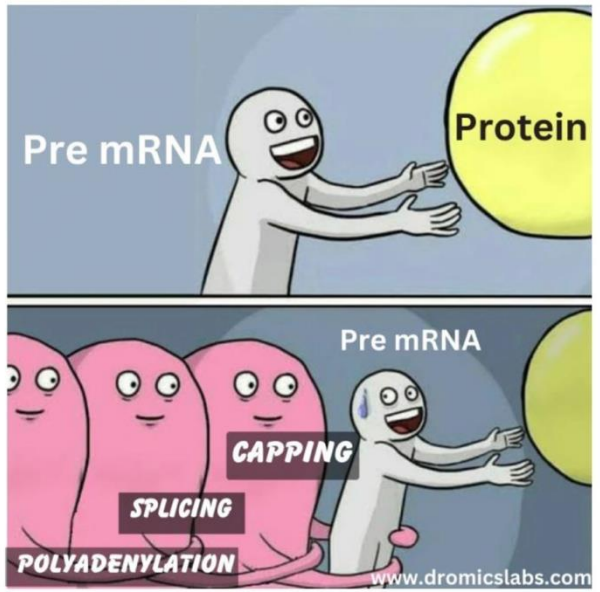


	Cell 1	Cell 2	...	Cell N
Gene A	8	4	...	6
Gene B	2	0	...	3
Gene C	0	2	...	1
...			...	
Gene X	5	7	...	6

		Cell 1	Cell 2	...	Cell N
Gene A	Iso 1	7	1	...	0
	Iso 2	1	3	...	6
Gene B	Iso 1	2	0	...	3
...					
Gene X	Iso 1	3	3	...	4
	Iso 2	2	4	...	2



Are isoforms good for us?



ALGORITHM ;)

assign random values to all $f(i)$
while not converged **do**
E-step:
initialize all $n(j)$ to 0
for each read r **do**
sum $= \sum_{j:w_{r,j}>0} w_{r,j} f(j)$
for each isoform j with $w_{r,j} > 0$ **do**
$n(j) += w_{r,j} f(j)/\text{sum}$
end for
end for
M-step:
$s = \sum_j n(j)/(l(j) - \mu + 1)$
for each isoform j **do**
$f(j) = \frac{n(j)/(l(j)-\mu+1)}{s}$
end for
end while

DPI calculation (Delta Percent Isoform)

$$DPI = \frac{\sum |\Delta PI_i|}{2}$$

2 DPI= all of the isoforms pi
Delts PI = absolute value of pi's

pos → acceptor (3′ splice site)

neg → donor (5' splice site)

2 most found isoforms are shown, where are the others??

gid	p_val	dpi	pos_iso_1	pos_iso_2	pos_iso_1_dpi	pos_iso_2_dpi	neg_iso_1	neg_iso_2	neg_iso_1_dpi	neg_iso_2_dpi	adj_p_val	gname
ENSG00000079150	0.001453499946493 2115	28.125	ENST00000419184.1	ENST00000470945.1	14.543270111083984	6.911057949066162	ENST00000424785.6	ENST00000064248.1	-23.4375	-4.6875	0.011643364380367663	FKBP7

$$\sum |\Delta PI| = 14.54327 + 6.91106 + 23.4375 + 4.6875$$

$$= 49.57933$$

Now divide by 2:

$$DPI = \frac{49.57933}{2} = 24.79$$

$$\sum |\Delta PI_{all}| = 2 \times DPI = 2 \times 28.125 = 56.25$$

$$\sum |\Delta PI| = 49.57933 + 6.67067 = 56.25$$

$$DPI = \frac{56.25}{2} = 28.125$$

Motivation

Standard DPI / isoform analyses:
•Typically report only the **top 2 positive and top 2 negative isoforms**
What if I am interested in other isoforms of genes??
•Remaining isoforms are often treated as **noise or biologically negligible**

This study introduces **$\mathbf{Dpi_3}$**, a quantitative metric defined as:

$$\mathbf{Dpi_3} = (2 \times \mathtt{dpi}) - \sum |\text{dominant isoform DPI values}|$$

Gen	Dpi_3	Significance
CFLAR	~30.9	Very powerful alternative splicing/apoptotic switch
CALD1	~16.5	Cytoskeletal reorganization
SEC31A	~16.0	ER–Golgi transport regulation
TPM1	~10.2	Actin filament dynamics
RTKN	~11.2	Rho-GTPase signal

Ouput file called “third_iso.csv”

gid	p_val	Dpi_3	gname
ENSG00000079150	0.0014534999464932115	21.35	**FKBP7**

>=10

- Write this gene to the visuzaltion code
- Go to this web page: https://freese.gitbook.io/swan/tutorials/visualization

mRNA and Protein(s)

1. NM_001135212.2 → NP_001128684.1 **peptidyl-prolyl cis-trans isomerase FKBP7 isoform b precursor**

   See identical proteins and their annotated locations for NP_001128684.1

   Status: REVIEWED

Description	Transcript Variant: This variant (2) uses an alternate in-frame splice site in the coding region, compared to variant 1. The resulting isoform (b) is 1 amino acid shorter than isoform a.
Source sequence(s)	AA447628, AC009948, AY353086, BC009711, DA020387
Consensus CDS	CCDS46462.1
UniProtKB/Swiss-Prot	Q9Y680
Related	ENSP00000415486.2, ENST00000434643.6

Conserved Domains (3) summary

pfam00254 Location:46 → 140	FKBP_C; FKBP-type peptidyl-prolyl cis-trans isomerase
pfam13499 Location:151 → 213	EF-hand_7; EF-hand domain pair
cl08302 Location:151 → 212	EFh; EF-hand, calcium binding motif; A diverse superfamily of calcium sensors and calcium signal modulators; most examples in this alignment model have 2 active canonical EF hands. Ca2+ binding induces a conformational change in the EF-hand motif, leading to ...

2. NM_001410972.1 → NP_001397901.1 **peptidyl-prolyl cis-trans isomerase FKBP7 isoform c precursor**

   Status: REVIEWED

Source sequence(s)	AC009948, AK299215
Consensus CDS	CCDS92911.1
UniProtKB/TrEMBL	B4DRE2
Related	ENSP00000509396.1, ENST00000464248.1

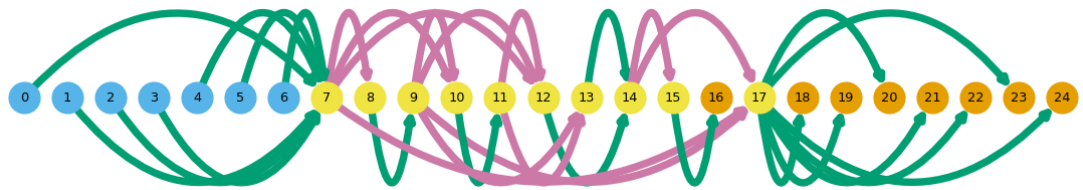
3. NM_181342.3 → NP_851939.1 **peptidyl-prolyl cis-trans isomerase FKBP7 isoform a precursor**

   See identical proteins and their annotated locations for NP_851939.1

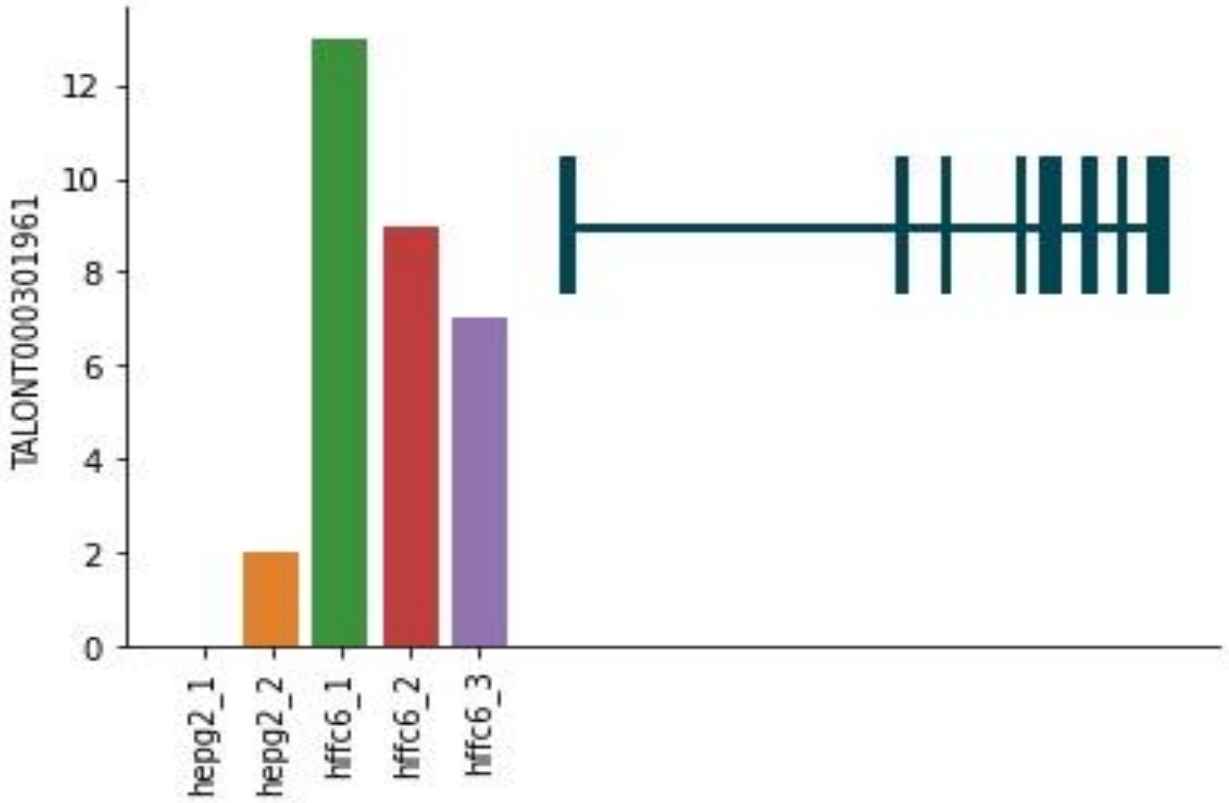
   Status: REVIEWED

Description	Transcript Variant: This variant (1) represents the longer transcript and encodes the longer isoform (a).
Source sequence(s)	AA447628, AC009948, BC009711, DA020387
Consensus CDS	CCDS2280.1
UniProtKB/Swiss-Prot	Q4ZG70, Q6V3B2, Q86U65, Q96DA4, Q9Y680, Q9Y6B0
Related	ENSP00000413152.2, ENST00000424785.7

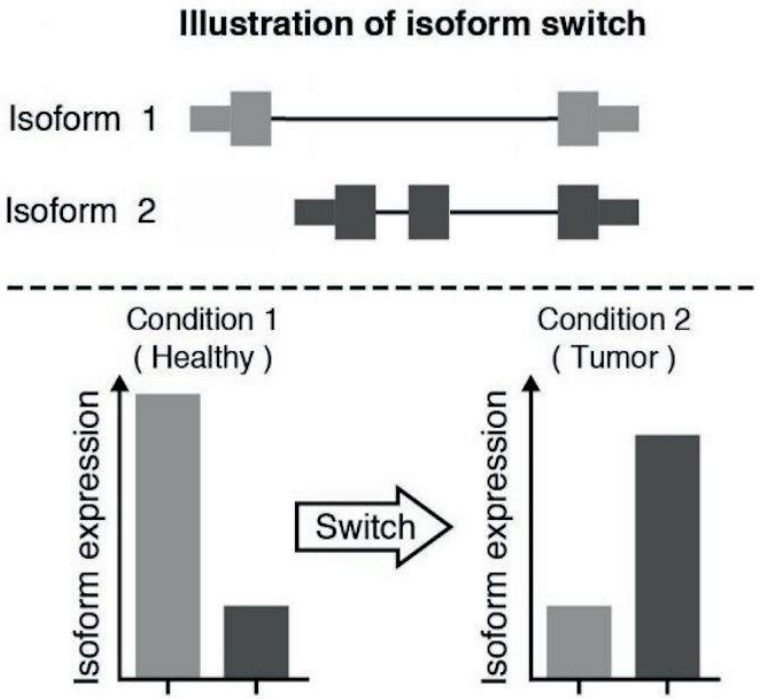
Conserved Domains (2) summary



Swan assigns a node to each unique splice site.



Gene is the same
Isoform is the same
Isoform function is different! Why?



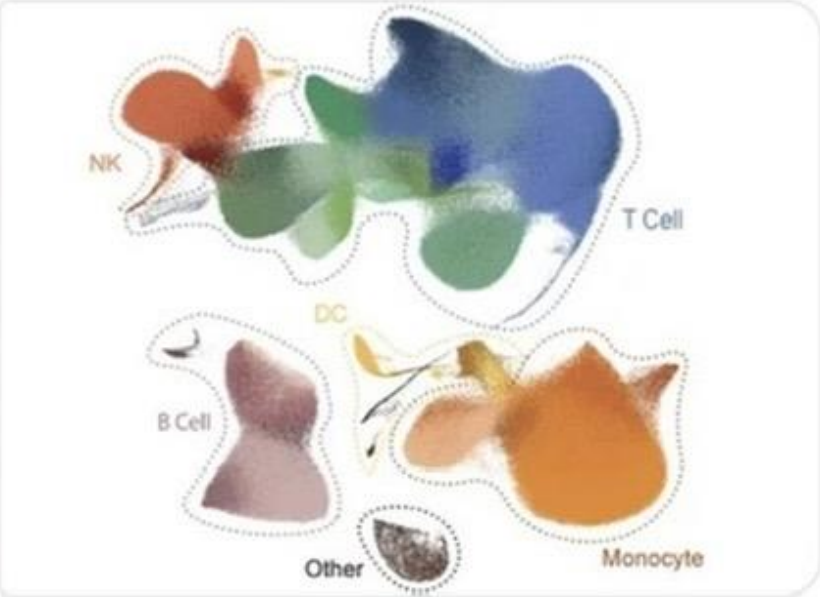
TALONT00311961L isoform shows:

Very low expression in hepg2_1 and hepg2_2 cell lines
High expression in hffc6_2 and hffc6_3 cell lines
This shows the **change** in usage across different cell lines→ **isoform switch potential**

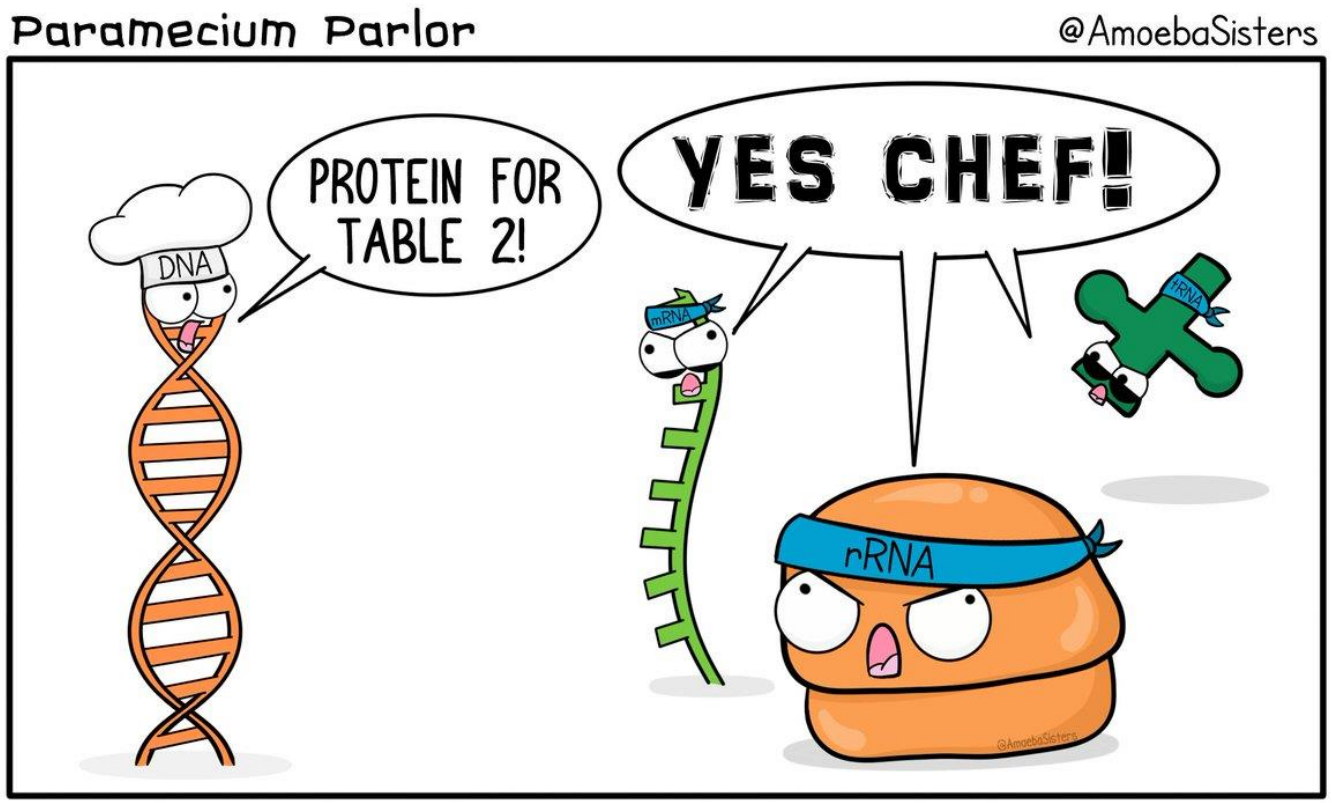
- Bioinformatics Tutorials -> https://ngs101.com/tutorials/

  For advanced isoform analysis -> https://bioconductor.org/packages/release/bioc/vignettes/IsoformSwitchAnalyzeR/inst/doc/IsoformSwitchAnalyzeR.html#importing-data-from-salmon-via-tximeta

  Github101 -> https://github.com/nuryagli/git-github-101-networking



Thanks for attending!!
Any questions???



RNA: If you can't help make proteins, get out of the kitchen.