

# Functional Iso-Transcriptomics with tappAS

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presented by Ana Conesa



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DE VALÈNCIA

Genomics   
 of Gene  
Expression Lab

# Reference and links



tappAS software: <https://github.com/ConesaLab/tappAS/releases>

Info: <https://app.tappas.org>

Fuente *et al.* *Genome Biology* (2020) 21:119  
<https://doi.org/10.1186/s13059-020-02028-w>

Genome Biology

**SOFTWARE** **Open Access**

**tappAS: a comprehensive computational framework for the analysis of the functional impact of differential splicing**

Lorena de la Fuente<sup>1,2†</sup>, Ángeles Arzalluz-Luque<sup>3</sup>, Manuel Tardáguila<sup>4,5†</sup>, Héctor del Risco<sup>4</sup>, Cristina Martí<sup>1</sup>, Sonia Tarazona<sup>3</sup>, Pedro Salguero<sup>1</sup>, Raymond Scott<sup>4</sup>, Alberto Lerma<sup>1</sup>, Ana Alastrue-Agudo<sup>6</sup>, Pablo Bonilla<sup>2</sup>, Jeremy R. B. Newman<sup>6,7</sup>, Shunichi Kosugi<sup>6,8</sup>, Lauren M. McIntyre<sup>6,9</sup>, Victoria Moreno-Manzano<sup>10†</sup> and Ana Conesa<sup>1,6†</sup> 



ConesaLab / tappAS

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Releases Tags Draft a new release

Latest release

v1.0.1  
8707749  
Verified

Compare

**tappAS v1.0.1** Edit

aarzalluz released this 27 days ago · 1 commit to master since this release

**PATCH** of version 1.0.0.

Changes:

- Fixed bug in DFI analysis for Multiple Time Course designs.
- Updated contact page.
- GO annotation has been updated (May 2, 2020).
- Miscellaneous changes.

Assets 3

 <b>tappas.jar</b>	17.4 MB
 <b>Source code (zip)</b>	
 <b>Source code (tar.gz)</b>	

Citation: de la Fuente, L., Arzalluz-Luque, Á., Tardáguila, M. et al. tappAS: a comprehensive computational framework for the analysis of the functional impact of differential splicing. *Genome Biol* 21, 119 (2020). <https://doi.org/10.1186/s13059-020-02028-w>

# Outline

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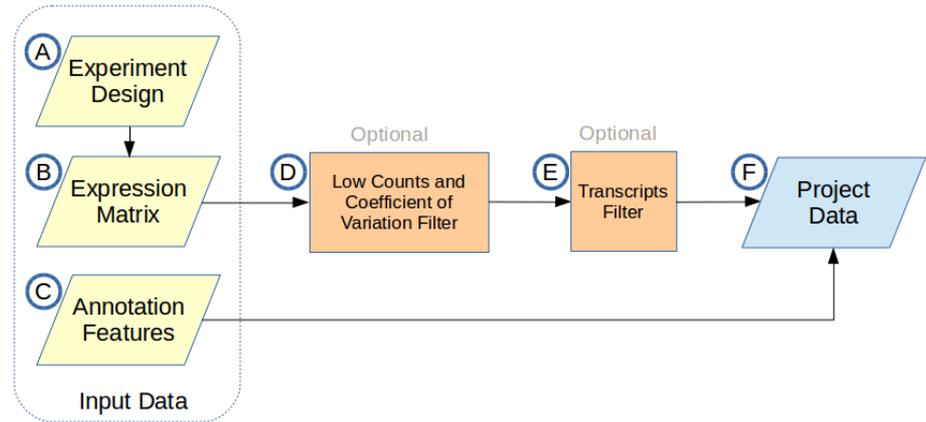
- Inputs and project creation.
- Brief guide to tappAS' interface.
- Analysis options.

How do I create a project in tappAS?

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# Inputs for project creation

- A. Design file.
- B. Expression matrix.
- C. Annotation file (gff3 format)\*



*\*Provided by tappAS for supported species/transcriptomes, can also be provided by user.*

# Inputs for project creation

A. Design file.

B. Expression matrix.

C. Annotation file (gff3 format)\*

sample	group
NSC1	NSC
NSC2	NSC
OLD1	OLD
OLD2	OLD

NSC1	NSC2	OLD1	OLD2	
PB.1668.1	13090.25	8635.61	5911.69	6028.91
PB.972.1	2900.91	2220.34	2890.57	2836.8
PB.5017.1	3833.06	2861.24	2889.58	2544.89
PB.5017.2	691.37	353.12	81.23	148.82
PB.5017.3	3755.89	755.2	1005.15	795.3
PB.5017.4	416.69	250.44	323.03	436.99
PB.1463.1	1549	1138	459	464

Two-Group Comparison:

- **Sample names** match column names in expression matrix.
- At least **two replicates** per group.

\*Provided by tappAS for supported species/transcriptomes, can also be provided by user.

# Inputs for project creation

A. Design files: other supported experimental designs.

B. Expression matrix.

C. Annotation file (gff3 format)\*

sample	time	group
OLD1-1	0	OLD
OLD1-2	0	OLD
OLD2-1	1	OLD
OLD2-2	1	OLD
OLD3-1	2	OLD
OLD3-2	2	OLD

Single Time Course:

- One group, two replicates.
- Three time points.

sample	time	group
OLD1-1	0	OLD
OLD1-2	0	OLD
OLD2-1	1	OLD
OLD2-2	1	OLD
OLD3-1	2	OLD
OLD3-2	2	OLD
NEUR1-1	0	NEUR
NEUR1-2	0	NEUR
NEUR2-1	1	NEUR
NEUR2-2	1	NEUR
NEUR3-1	2	NEUR
NEUR3-2	2	NEUR

Multiple Time Course:

- Two groups, two replicates.
- Three time points

\*Provided by tappAS for supported species/transcriptomes, can also be provided by user.

# Inputs for project creation

A.Design file.

B.Expression matrix.

C.Annotation file (gff3 format)\*

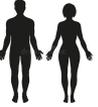
*Arabidopsis thaliana*  
Ensembl



*Drosophila melanogaster*  
Ensembl



*Homo sapiens*  
Ensembl, RefSeq



*Mus musculus*  
Demo, Ensembl, RefSeq



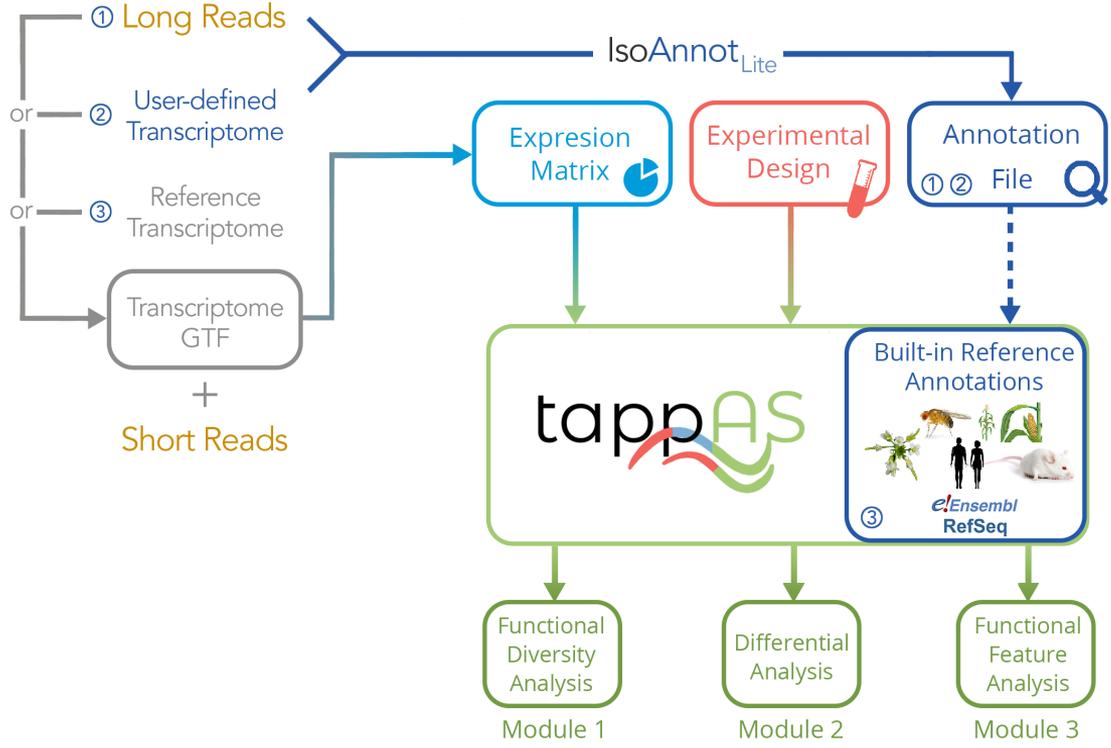
*Zea Mays*  
Ensembl



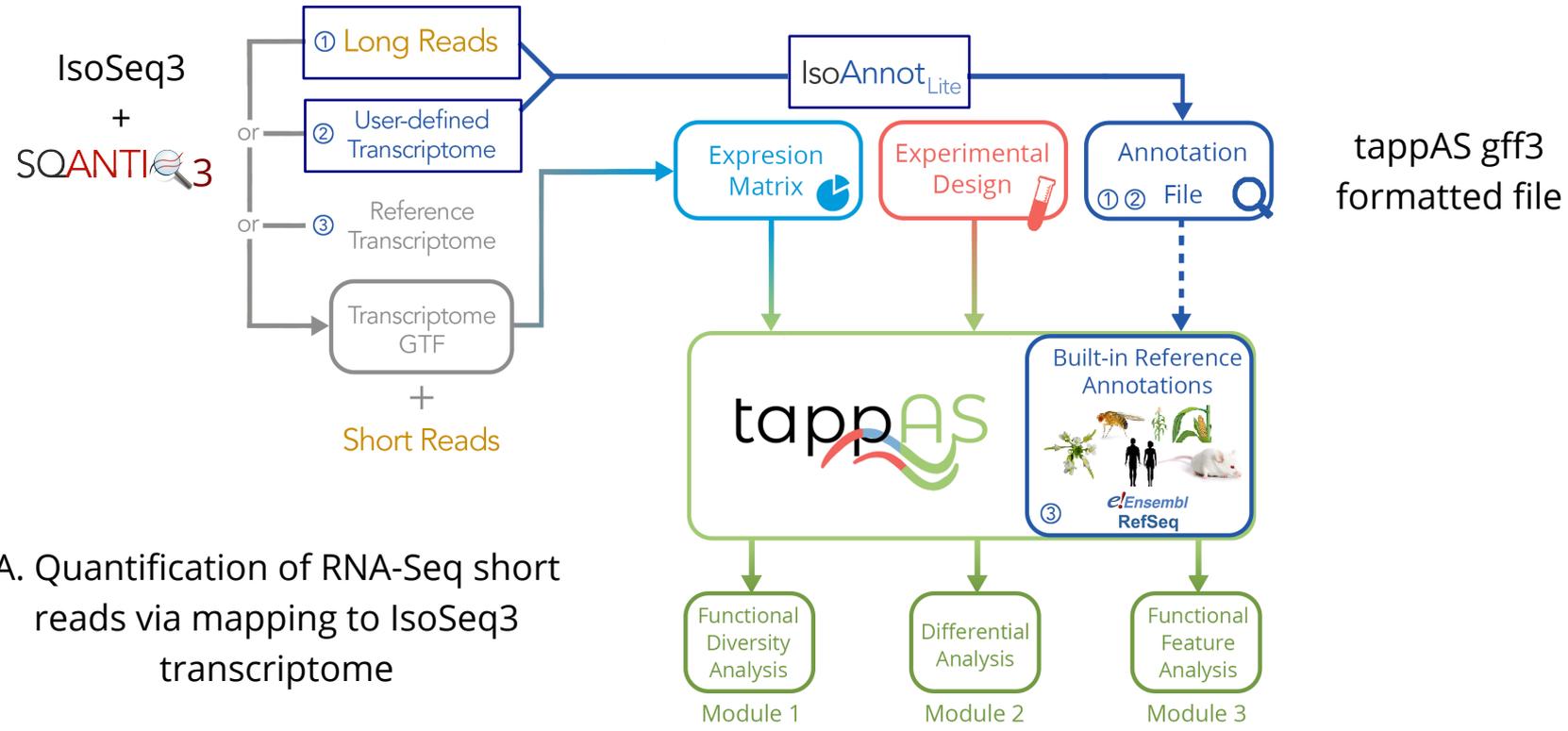
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PB.3189.4 tappAS transcript 1 1399 . + . ID=XM_006524897; primary_class=full-splice_match; PostType=T
PB.3189.4 tappAS gene 1 1399 . + . ID=Opct; Name=Opct; Desc=glutaminyl-peptide cyclotransferase (glutaminyl cyclase); PostType=T
PB.3189.4 tappAS CDS 10 948 . + . ID=NP_001303658.1; PostType=T
PB.3189.4 scanForMotifs PAS 1369 1398 . + . ID=MammalianPolyadenylationElement; Name=MammalianPolyadenylationElement; Desc=Mammalian Polyadenylation Element; PostType=T
PB.3189.4 scanForMotifs 3UTRmotif 1148 1222 . + . ID=tRNALikestructure; Name=tRNALikestructure; Desc=tRNA like structure; PostType=T
PB.3189.4 miRWalk miRNA Binding 1090 1097 . + . ID=mmu-miR-139-5p; Name=mmu-miR-139-5p; Desc=3UTR; PostType=T
PB.3189.4 miRWalk miRNA Binding 1100 1107 . + . ID=mmu-miR-376b-3p; Name=mmu-miR-376b-3p; Desc=3UTR; PostType=T
PB.3189.4 miRWalk miRNA Binding 1294 1301 . + . ID=mmu-miR-374b-5p; Name=mmu-miR-374b-5p; Desc=3UTR; PostType=T
PB.3189.4 tappAS genomic 1 1 . + . Chr=chr17; PostType=G
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PB.3189.4 tappAS protein 1 313 . + . ID=NP_001303658.1; PostType=P
PB.3189.4 Provean FunctionalImpact . . . . ID=lesser; Score=-111.14; PostType=N
PB.3189.4 GeneOntology C . . . . ID=G0:0070062; Name=extracellular exosome; PostType=N
PB.3189.4 GeneOntology F . . . . ID=G0:0008270; Name=zinc ion binding; PostType=N
PB.3189.4 GeneOntology F . . . . ID=G0:0016603; Name=glutaminyl-peptide cyclotransferase activity; PostType=N
PB.3189.4 GeneOntology P . . . . ID=G0:0017186; Name=peptidyl-pyroglutamic acid biosynthetic process, using glutaminyl-peptide cyclotransferase; PostType=N
```

\*Provided by tappAS for supported species/transcriptomes, can also be provided by user.

# How do I obtain an isoform-level expression matrix?

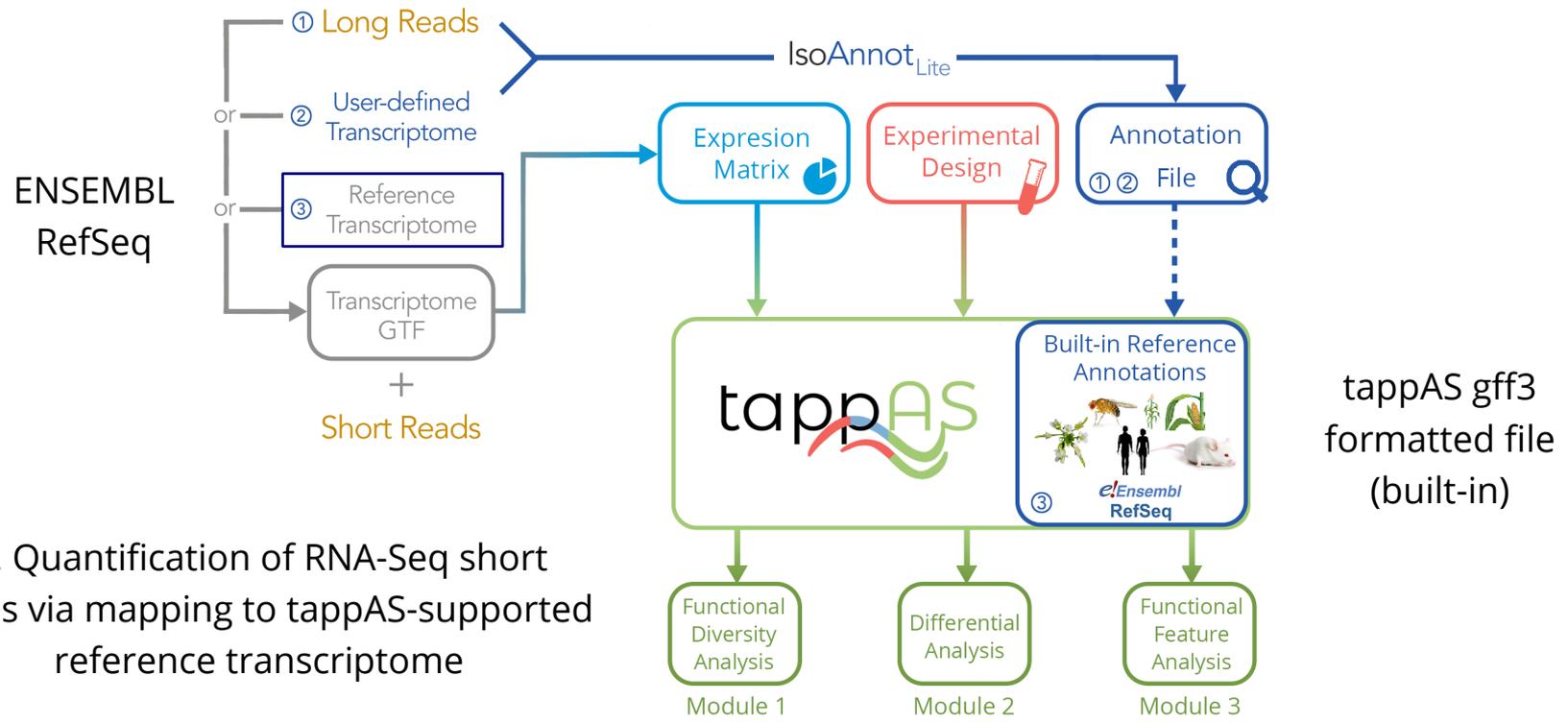


# How do I obtain an isoform-level expression matrix?



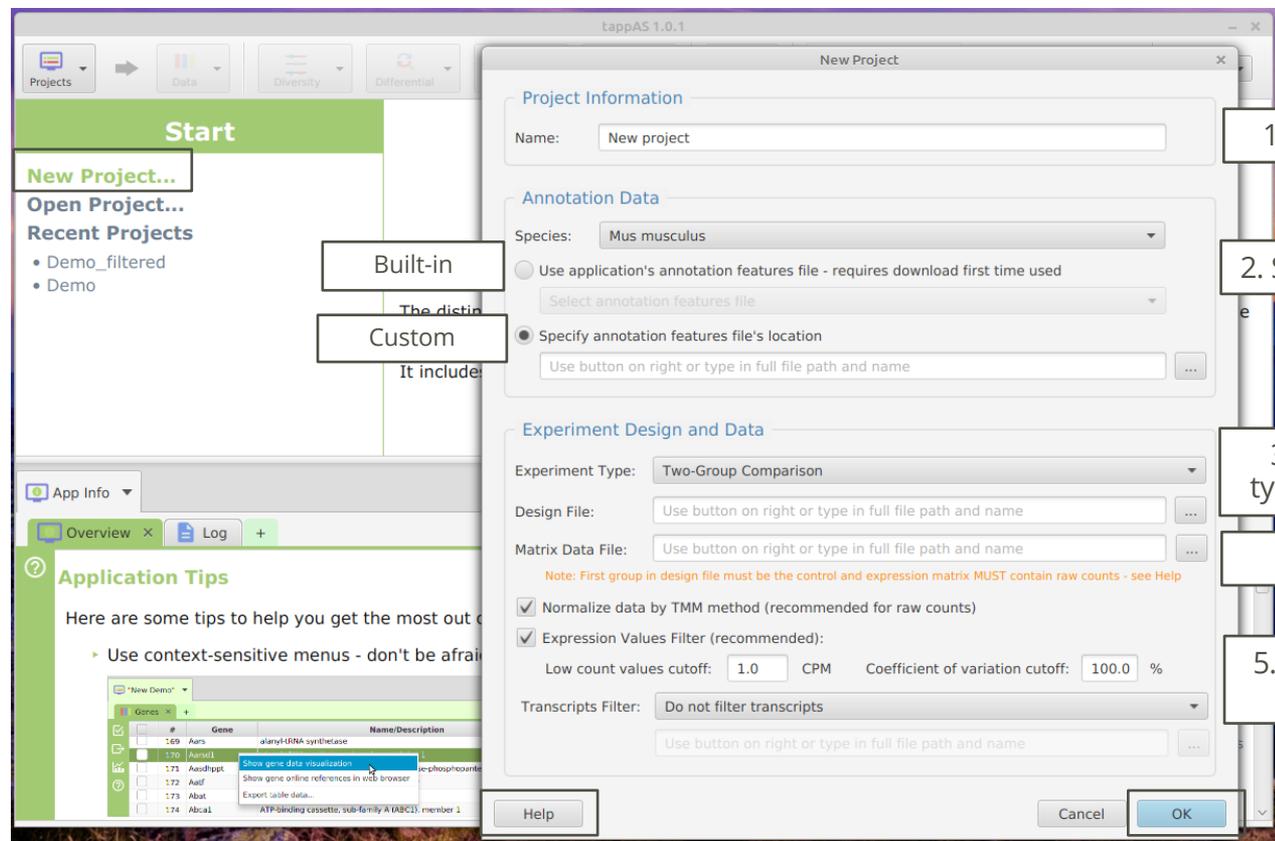
A. Quantification of RNA-Seq short reads via mapping to IsoSeq3 transcriptome

# How do I obtain an isoform-level expression matrix?



B. Quantification of RNA-Seq short reads via mapping to tappAS-supported reference transcriptome

# Project creation in tappAS



Built-in

Custom

1. Supply a name for the project

2. Select functional annotation

3. Design type and file

- Two-group comparison
- Single time course
- Multiple time course

4. Expression matrix file

5. Normalization and filtering options

App Info

Overview x Log +

Application Tips

Here are some tips to help you get the most out of...

Use context-sensitive menus - don't be afraid...

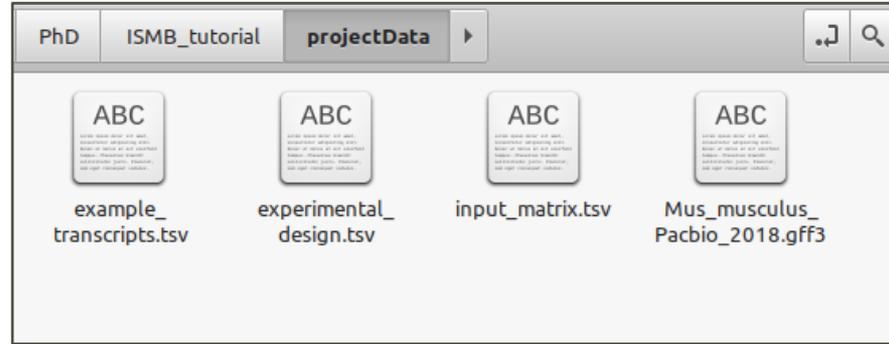
#	Gene	Name/Description
169	Aars	alanyl-tRNA synthetase
170	Aarsl	
171	Aasdrppt	show gene online references in web browser
172	Aaif	phosphoantigen
173	Abat	Export table data...
174	Abca1	ATP-binding cassette, sub-family A (ABC1), member 1

# Project creation in tappAS

- Execute the tappas.jar file with Java, or open a terminal window and run:

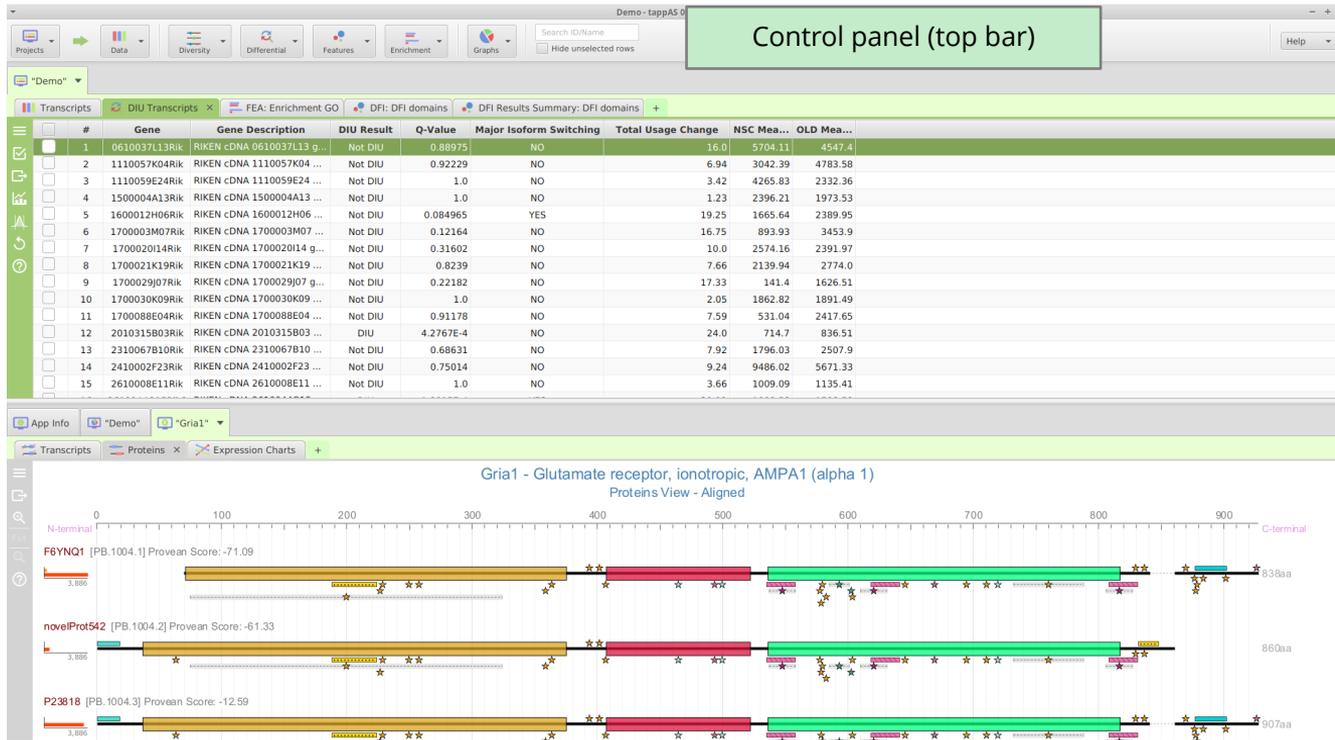
```
java -jar tappas.jar
```

- Locate the required input files:



# Guide to tappAS' interface

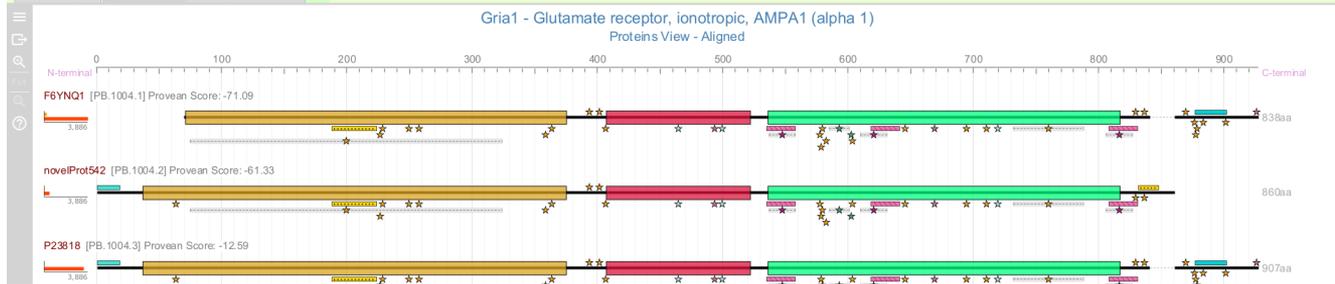
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Control panel (top bar)

Data and result tables panel (top panel)

Visualization panel (bottom panel)



# tappAS interface

Create new and view extant projects

Run analyses and view results

Search bar for data and results panel

Control panel (top bar)

The screenshot displays the tappAS interface with a top navigation bar and a main content area. The top bar includes a 'Projects' button, a search bar, and several analysis tool icons (Data, Diversity, Differential, Features, Enrichment, Graphs). The main area is divided into two panels. The upper panel shows a table of transcript data, and the lower panel shows a protein alignment view for Gria1.

#	Gene	Gene Description	DIU Result	Q-Value	Major Isoform Switching	Total Usage Change	NSC Mea...	OLD Mea...
1	0610037L13Rik	RIKEN cDNA 0610037L13 g...	Not DIU	0.88975	NO		16.0	5704.11
2	1110057K04Rik	RIKEN cDNA 1110057K04 ...	Not DIU	0.92229	NO		6.94	3042.39
3	1110059E24Rik	RIKEN cDNA 1110059E24 ...	Not DIU	1.0	NO		3.42	4265.83
4	1500004A13Rik	RIKEN cDNA 1500004A13 ...	Not DIU	1.0	NO		1.23	2396.21
5	1600012H06Rik	RIKEN cDNA 1600012H06 ...	Not DIU	0.084965	YES		19.25	1665.64
6	1700003M07Rik	RIKEN cDNA 1700003M07 ...	Not DIU	0.12164	NO		16.75	893.93
7	1700020I14Rik	RIKEN cDNA 1700020I14 g...	Not DIU	0.31602	NO		10.0	2574.16
8	1700021K19Rik	RIKEN cDNA 1700021K19 ...	Not DIU	0.8239	NO		7.66	2139.94
9	1700029J07Rik	RIKEN cDNA 1700029J07 g...	Not DIU	0.22182	NO		17.33	141.4
10	1700030K09Rik	RIKEN cDNA 1700030K09 ...	Not DIU	1.0	NO		2.05	1862.82
11	1700088E04Rik	RIKEN cDNA 1700088E04 ...	Not DIU	0.91178	NO		7.59	531.04
12	2010315B03Rik	RIKEN cDNA 2010315B03 ...	DIU	4.2767E-4	NO		24.0	714.7
13	2310067B10Rik	RIKEN cDNA 2310067B10 ...	Not DIU	0.68631	NO		7.92	1796.03
14	2410002F23Rik	RIKEN cDNA 2410002F23 ...	Not DIU	0.75014	NO		9.24	9486.02
15	2610008E11Rik	RIKEN cDNA 2610008E11 ...	Not DIU	1.0	NO		3.66	1009.09

The lower panel shows a protein alignment view for Gria1 - Glutamate receptor, ionotropic, AMPA1 (alpha 1). It displays three protein isoforms: FB9YQ1 (838aa), novelProt542 (860aa), and P23818 (807aa). The alignment is shown against a scale from 0 to 900 amino acids, with N-terminal and C-terminal markers. Each isoform is represented by a horizontal bar with colored segments (yellow, red, green) and asterisks indicating conserved regions.

# tappAS interface

Run analyses and view results

Control panel (top bar)

The screenshot displays the tappAS interface with several key components:

- Control Panel (Top Bar):** Includes tabs for Data, Diversity, Differential, Features, Enrichment, and Graphs. A search bar and a 'Hide unselected rows' checkbox are also present.
- Data Table:** A table with columns: #, Gene, Gene Description, DIU Result, Q-Value, Major Isoform Switching, and Total Usage Change. It lists 15 genes with their respective DIU results and Q-values.
- Protein Alignment Chart:** Titled 'Gria1 - Glutamate receptor, ionotropic, Proteins View - Aligned'. It shows three protein isoforms: FBYNQ1 (Provean Score: -71.09), novelProt542 (Provean Score: -61.33), and P23818 (Provean Score: -12.59). Each isoform is represented by a horizontal bar with yellow and red segments and asterisks indicating alignment points.
- Differential Feature Inclusion Analysis Parameters Dialog:** A window for configuring analysis parameters. It includes:
  - Annotation Feature(s):** A list of features with checkboxes: SIGNALP\_EUK, PAR-CLIP, PFAM, and TMHMM.
  - Check Using:** A dropdown menu set to 'Feature genomic position'.
  - Analysis Method:** A dropdown menu set to 'DEXSeq'.
  - Analysis Parameters:** Fields for Name (placeholder: 'Enter Differential Feature Inclusion Analysis name'), Significance Level (0.05), and Filtering (checkbox for 'Filter minor isoforms', dropdown for 'Fold filtering').
  - Buttons:** 'Clear All', 'Check All', 'Help', 'Cancel', and 'OK'.

Analysis pre-run window to select parameters

Analysis info and help (built-in documentation)

# tappAS interface

Multi-option side bar

- Analysis log
- Filter table
- Export table
- Visualization options
- Change significance level
- Re-run analysis
- Help

Result subtabs for multiple analyses

The screenshot displays the tappAS interface with a table of analysis results and a protein alignment view below it.

#	Gene	Gene Description	DIU Result	Q-Value	Major Isoform Switching	Total Usage Change	NSC Mea...	OLD Mea...
1	0610037L13RIK	RIKEN cDNA 0610037L13 g...	Not DIU	0.88975	NO		16.0	5704.11
2	1110057K04RIK	RIKEN cDNA 1110057K04 ...	Not DIU	0.92229	NO		6.94	3042.39
3	1110059E24RIK	RIKEN cDNA 1110059E24 ...	Not DIU	1.0	NO		3.42	4265.83
4	1500004A13RIK	RIKEN cDNA 1500004A13 ...	Not DIU	1.0	NO		1.23	2396.21
5	1600012H06RIK	RIKEN cDNA 1600012H06 ...	Not DIU	0.084965	YES			1973.53
6	1700003M07RIK	RIKEN cDNA 1700003M07 ...	Not DIU	0.12164	NO			
7	1700020I14RIK	RIKEN cDNA 1700020I14 g...	Not DIU	0.31602	NO			
8	1700021K19RIK	RIKEN cDNA 1700021K19 ...	Not DIU	0.8239	NO		7.66	2139.94
9	1700029J07RIK	RIKEN cDNA 1700029J07 g...	Not DIU	0.22182	NO		17.33	141.4
10	1700030K09RIK	RIKEN cDNA 1700030K09 ...	Not DIU	1.0	NO		2.05	1862.82
11	1700088E04RIK	RIKEN cDNA 1700088E04 ...	Not DIU	0.91178	NO		7.59	531.04
12	2010315B03RIK	RIKEN cDNA 2010315B03 ...	DIU	4.2767E-4	NO		24.0	714.7
13	2310067B10RIK	RIKEN cDNA 2310067B10 ...	Not DIU	0.68631	NO		7.92	1796.03
14	2410002F23RIK	RIKEN cDNA 2410002F23 ...	Not DIU	0.75014	NO		9.24	9486.02
15	2610008E11RIK	RIKEN cDNA 2610008E11 ...	Not DIU	1.0	NO		3.66	1009.09

The bottom panel shows a protein alignment view for 'Gria1 - Glutamate receptor, ionotropic, AMPA1 (alpha 1)'. It displays three protein isoforms: FB9YQ1 (838aa), novelProt542 (860aa), and P23818 (807aa). The alignment is shown against a protein structure with various domains and motifs highlighted.

Analysis result table

Data and result tables panel

Contains expression matrices for genes, transcripts and CDS, analysis result tables and result summary tables for up to 3 projects simultaneously.

# tappAS interface

Multi-option side bar

- Analysis log
- Filter table
- Export table
- Visualization options
- Change significance level
- Re-run analysis
- Help

Result subtabs for multiple analyses

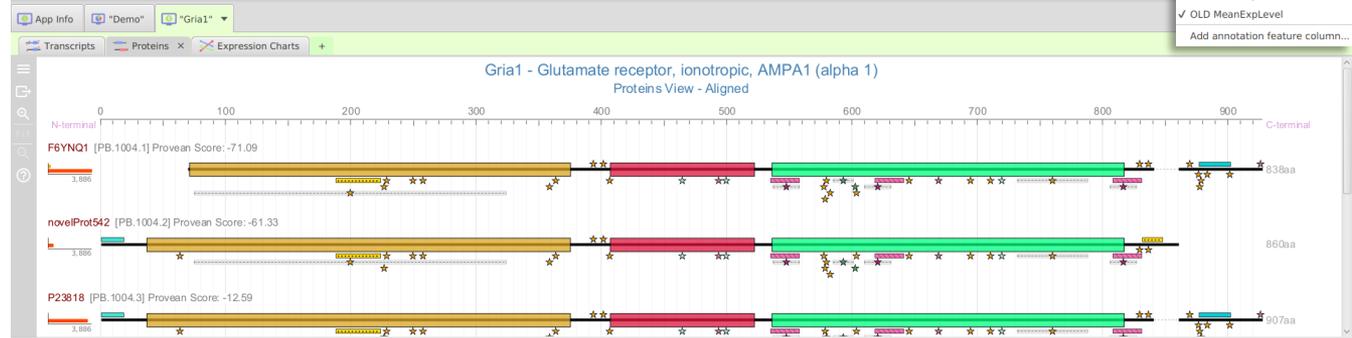
Table column customization

Analysis result table

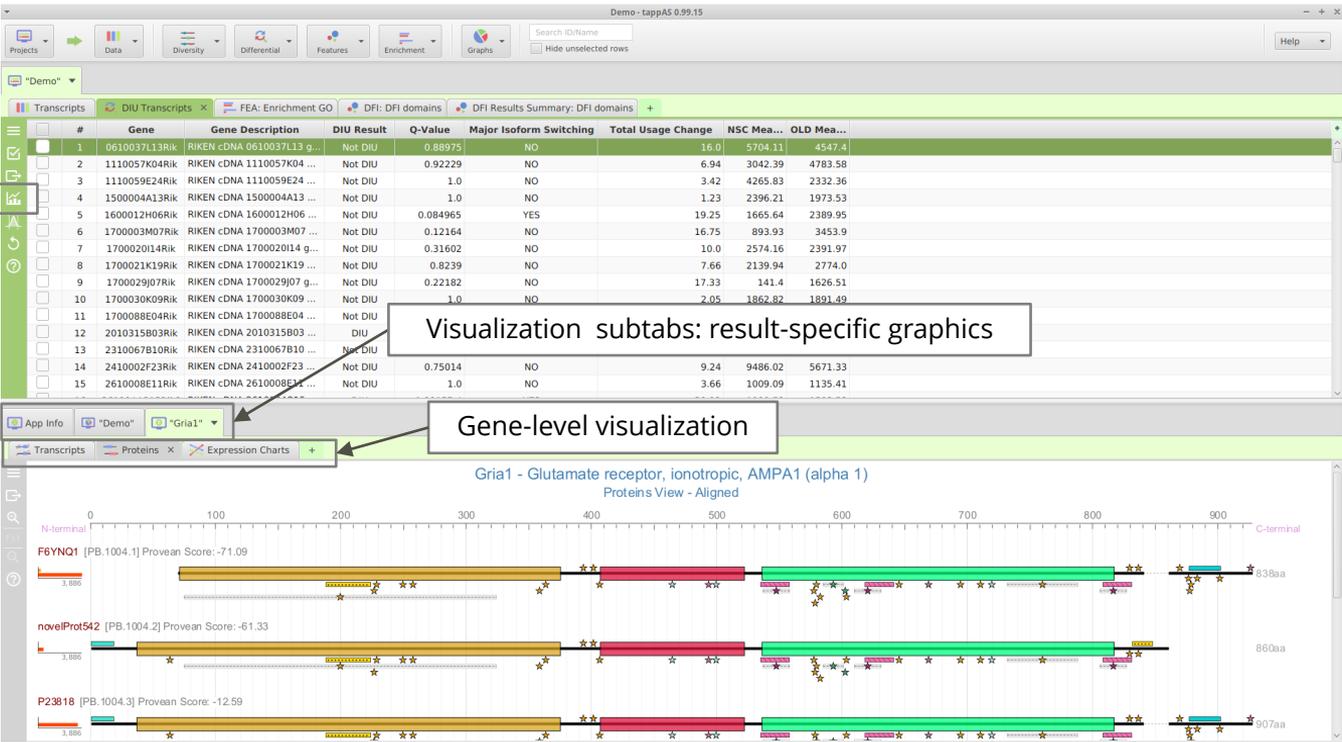
#	Gene	Gene Description	DIU Result	Q-Value	Major Isoform Switching	Total Usage Change	NSC Mean	OLD Mean
1	0610037L13RIK	RIKEN cDNA 0610037L13 g...	Not DIU	0.88975	NO		16.0	5704.11
2	1110057K04RIK	RIKEN cDNA 1110057K04 ...	Not DIU	0.92229	NO		6.94	3042.39
3	1110059E24RIK	RIKEN cDNA 1110059E24 ...	Not DIU	1.0	NO		3.42	4265.83
4	1500004A13RIK	RIKEN cDNA 1500004A13 ...	Not DIU	1.0	NO		1.23	2396.21
5	1600012H06RIK	RIKEN cDNA 1600012H06 ...	Not DIU	0.084965	YES			
6	1700003M07RIK	RIKEN cDNA 1700003M07 ...	Not DIU	0.12164	NO			
7	1700020I14RIK	RIKEN cDNA 1700020I14 g...	Not DIU	0.31602	NO			
8	1700021K19RIK	RIKEN cDNA 1700021K19 ...	Not DIU	0.8239	NO		7.66	2139.94
9	1700029J07RIK	RIKEN cDNA 1700029J07 g...	Not DIU	0.22182	NO		17.33	141.4
10	1700030K09RIK	RIKEN cDNA 1700030K09 ...	Not DIU	1.0	NO		2.05	1862.82
11	1700088E04RIK	RIKEN cDNA 1700088E04 ...	Not DIU	0.91178	NO		7.59	531.04
12	2010315B03RIK	RIKEN cDNA 2010315B03 ...	DIU	4.2767E-4	NO		24.0	714.7
13	2310067B10RIK	RIKEN cDNA 2310067B10 ...	Not DIU	0.68631	NO		7.92	1796.03
14	2410002F23RIK	RIKEN cDNA 2410002F23 ...	Not DIU	0.75014	NO		9.24	9486.02
15	2610008E11RIK	RIKEN cDNA 2610008E11 ...	Not DIU	1.0	NO		3.66	1009.09

Data and result tables panel

Contains expression matrices for genes, transcripts and CDS, analysis result tables and result summary tables for up to 3 projects simultaneously.



# tappAS interface



Analysis-specific visualization options

Visualization subtabs: result-specific graphics

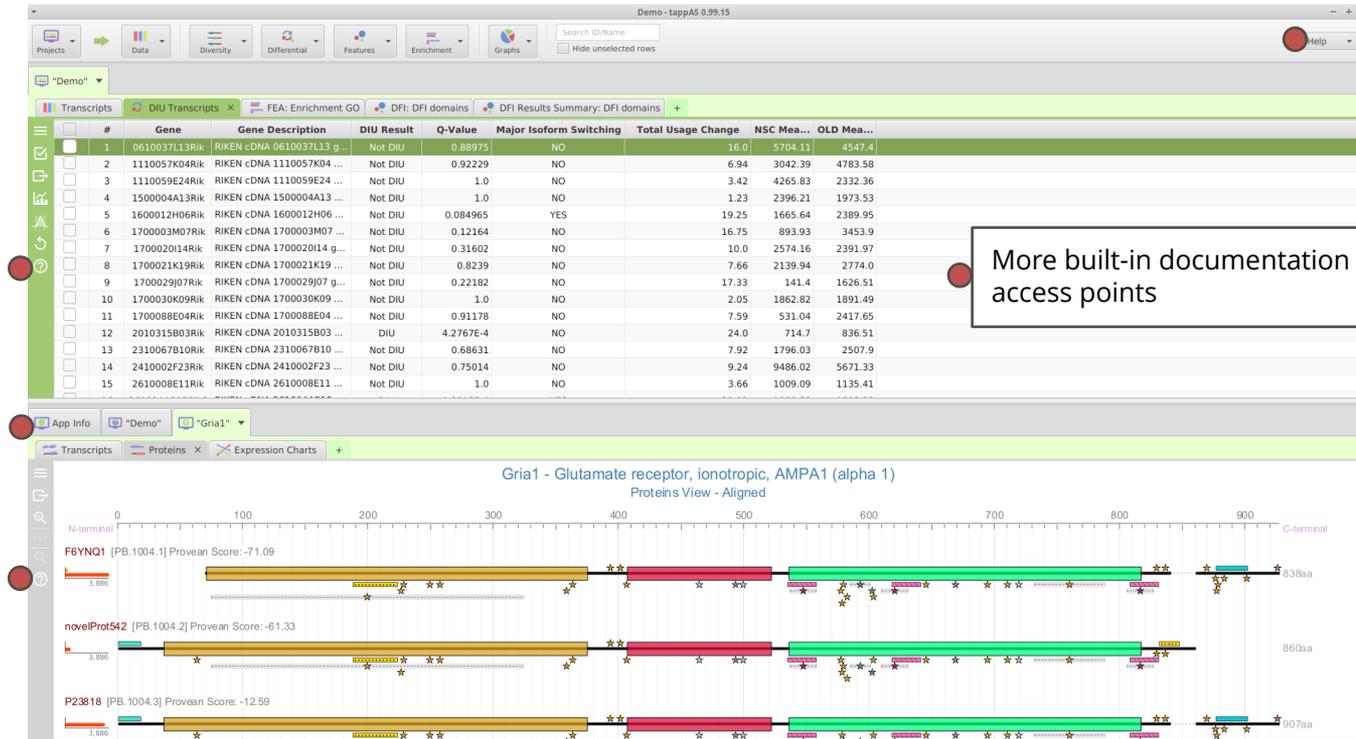
Multi-option side bar

Gene-level visualization

Visualization panel

- Analysis log
- Export image
- Zoom/adjust to screen.
- Help.

Contains gene-level visualization and result summary visualizations for the project under the project name subtab (e.g. Demo).



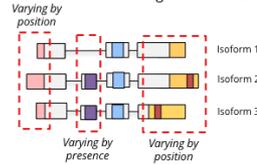
More built-in documentation access points

# tappAS analysis modules

### Module 1: Functional Diversity Analysis

Positional approach  
APA/UTR/CDS...

Presence/absence approach  
Signals/domains/NMD...



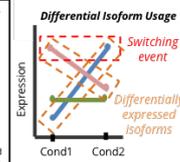
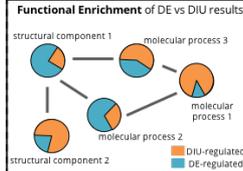
### Module 2: Differential Analysis

Global analysis : gene expression levels

Intra-gene analysis: AltTP

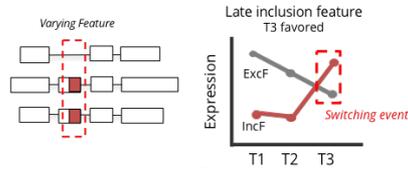
Differential Expression  
Transcript/Gene/CDS level

Differential Isoform Usage  
Transcript/CDS level



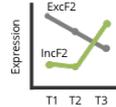
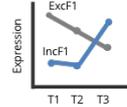
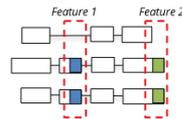
### Module 3: Feature Analysis

#### Differential Feature Inclusion

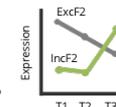
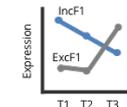
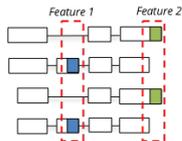


Co-DFI

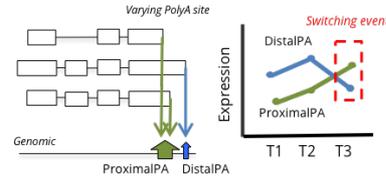
· Co-inclusion



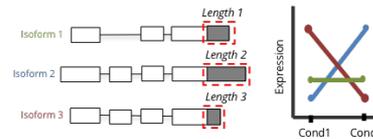
· Mutual exclusion



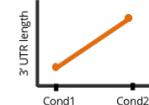
#### Differential Polydenylation



#### UTR shortening/lengthening



$$3'UTRLength_{Cond1} = Expr_1 \cdot Length_1 + Expr_2 \cdot Length_2 + Expr_3 \cdot Length_3$$

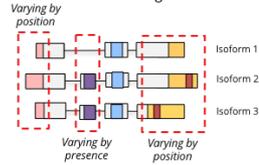


3' UTR lengthening  
in Cond2 vs Cond1

### Module 1: Functional Diversity Analysis

Positional approach  
APA/UTR/CDS...

Presence/absence approach  
Signals/domains/NMD...



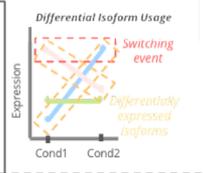
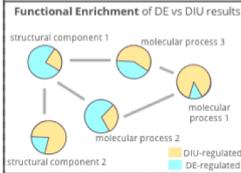
### Module 2: Differential Analysis

Global analysis : gene expression levels

Intra-gene analysis: AltTP

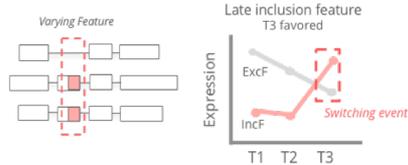
Differential Expression  
Transcript/Gene/CDS level

Differential Isoform Usage  
Transcript/CDS level

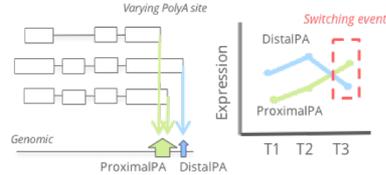


### Module 3: Feature Analysis

Differential Feature Inclusion

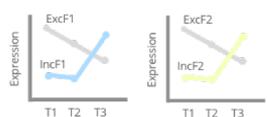
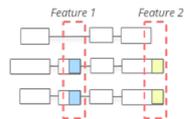


Differential Polydenylation

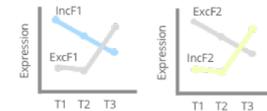
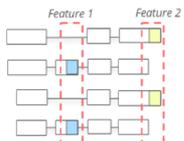


Co-DFI

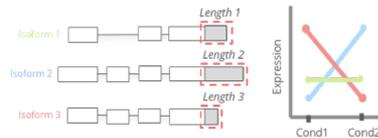
Co-inclusion



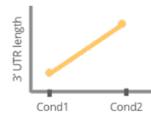
Mutual exclusion



UTR shortening/lengthening



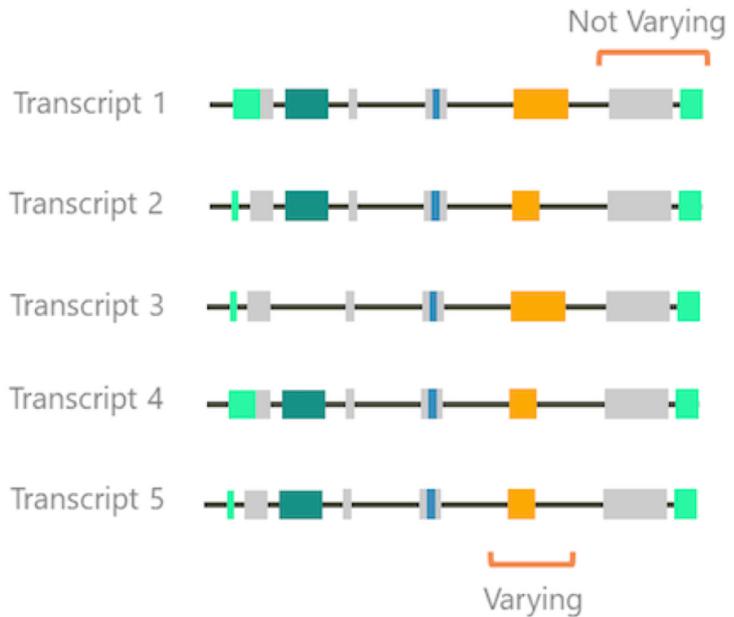
$$3'UTRLength_{Cond1} = Expr_1 \cdot Length_1 + Expr_2 \cdot Length_2 + Expr_3 \cdot Length_3$$



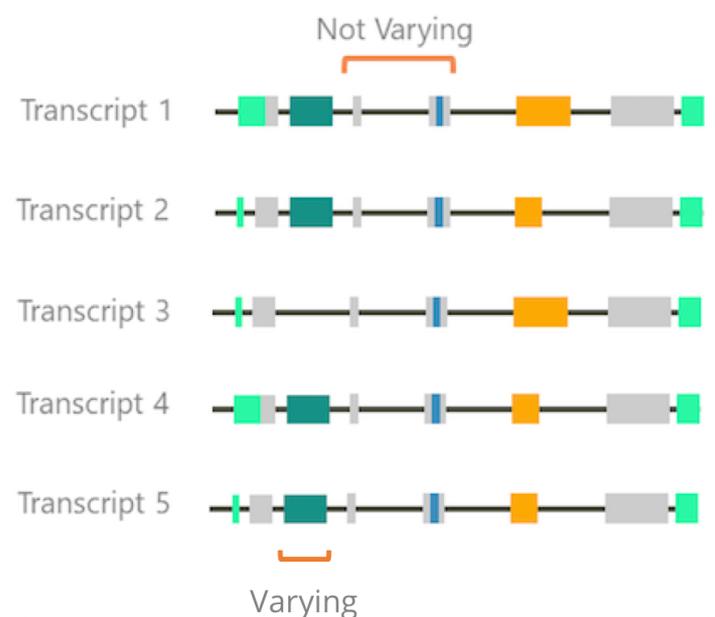
3' UTR lengthening in Cond2 vs Cond1

# Functional Diversity Analysis

### Genomic position variance



### Presence variance



# Functional Diversity Analysis

Functional Diversity Analysis Parameters

---

**List of genes**

Test list: All genes

List file: Use button on right or type in full file path and name ...

---

**Analysis Parameters**

Name: Enter Functional Diversity Analysis name

Analyze by:  Category  Id

Check Using: Feature genomic position

---

**Select Annotation Feature(s)**

- SIGNALP\_EUK
- PAR-CLIP
- PFAM
- TMHMM
- scanForMotifs
- RepeatMasker
- UniProtKB/Swiss-Prot\_Phosphosite
- COILS

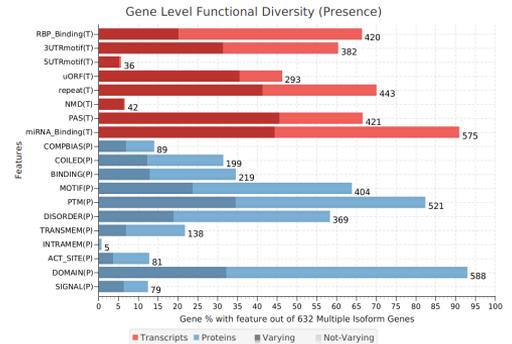
[Clear All](#) [Check All](#)

---

Help
Cancel
OK

Category result:

- Gene-level, for each feature.
- Varying/not varying.



		Transcript Annotation									
#	Gene	NMD	RBP_Binding	repeat	5UTRmotif	uORF	miRNA_Binding	3UTRmotif	PAS		
1	Cyth4						Not Varying	Not Varying	Not Varying		
2	Rnh1			Varying		Varying	Not Varying				
3	Pop4		Not Varying	Not Varying		Varying	Not Varying	Not Varying			
4	Smc6		Varying	Varying		Varying	Varying	Varying	Varying		
5	Ganc			Varying		Not Varying	Varying	Varying			
6	Smc2			Not Varying			Varying	Varying	Varying		
7	Ipo11		Varying	Not Varying			Not Varying	Not Varying	Varying		
8	Tex264		Not Varying	Not Varying			Not Varying				
9	Gm10825			Not Varying		Not Varying		Not Varying	Varying		
10	Scp2		Varying				Not Varying	Not Varying	Not Varying		
11	Myc		Not Varying	Varying			Not Varying	Not Varying	Not Varying		
12	Shmt1			Varying			Varying	Varying	Varying		
13	Akt2		Varying	Varying		Not Varying	Varying	Varying	Varying	Not Varying	
14	Cyb5d2			Not Varying		Varying	Not Varying			Not Varying	

# Functional Diversity Analysis (FDA)

Functional Diversity Analysis Parameters

List of genes

Test list: All genes

List file: Use button on right or type in full file path and name

Analysis Parameters

Name: Enter Functional Diversity Analysis name

Analyze by:  Category  Id

Check Using: Feature genomic position

Select Annotation Feature(s)

- SIGNALP\_EUK
- PAR-CLIP
- PFAM
- TMHMM
- scanForMotifs
- RepeatMasker
- UniProtKB/Swiss-Prot\_Phosphosite
- COILS

Clear All Check All

Help Cancel OK

ID result:

- Feature ID-level within a category.
- No. of varying/not varying occurrences across genes.

	#	Feature ID	Feature Description	P-Value	AdjP-Value	Protein Annotation	
						VARY...▼	NOT VARYING
<input type="checkbox"/>	1	PFAM/DOMAIN/PF00169	PH domain	0.005	1.0	8	3
<input type="checkbox"/>	2	PFAM/DOMAIN/PF00069	Protein kinase domain	0.4258	1.0	7	13
<input type="checkbox"/>	3	PFAM/DOMAIN/PF00096	Zinc finger, C2H2 type	0.241	1.0	6	8
<input type="checkbox"/>	4	PFAM/DOMAIN/PF00076	RNA recognition motif. (a.k.a. RRM,...	0.7906	1.0	5	15
<input type="checkbox"/>	5	PFAM/DOMAIN/PF00041	Fibronectin type III domain	0.0771	1.0	4	2
<input type="checkbox"/>	6	PFAM/DOMAIN/PF12796	Ankyrin repeats (3 copies)	0.2902	1.0	4	5
<input type="checkbox"/>	7	PFAM/DOMAIN/PF13927	Immunoglobulin domain	0.1745	1.0	3	2
<input type="checkbox"/>	8	PFAM/DOMAIN/PF01352	KRAB box	0.3729	1.0	3	4
<input type="checkbox"/>	9	PFAM/DOMAIN/PF13432	Tetratricopeptide repeat	0.0952	1.0	2	0
<input type="checkbox"/>	10	PFAM/DOMAIN/PF00307	Calponin homology (CH) domain	0.0952	1.0	2	0
<input type="checkbox"/>	11	PFAM/DOMAIN/PF01756	Acyl-CoA oxidase	0.0952	1.0	2	0
<input type="checkbox"/>	12	PFAM/DOMAIN/PF00433	Protein kinase C terminal domain	0.0952	1.0	2	0
<input type="checkbox"/>	13	PFAM/DOMAIN/PF09379	FERM N-terminal domain	0.0952	1.0	2	0
<input type="checkbox"/>	14	PFAM/DOMAIN/PF07719	Tetratricopeptide repeat	0.0952	1.0	2	0

### Module 1: Functional Diversity Analysis

**Positional approach**  
APA/UTR/CDS...

**Presence/absence approach**  
Signals/domains/NMD...

Varying by position

Varying by presence

Varying by position

Isoform 1

Isoform 2

Isoform 3

### Module 2: Differential Analysis

**Global analysis : gene expression levels**

**Intra-gene analysis: AltTP**

**Differential Expression**  
Transcript/Gene/CDS level

**Differential Isoform Usage**  
Transcript/CDS level

**Functional Enrichment of DE vs DIU results**

structural component 1

molecular process 3

molecular process 2

molecular process 1

structural component 2

DIU-regulated

DE-regulated

**Differential Isoform Usage**

Expression

Cond1

Cond2

Switching event

Differentially expressed isoforms

### Module 3: Feature Analysis

**Differential Feature Inclusion**

Varying Feature

Late inclusion feature  
T3 favored

Expression

ExcF

IncF

T1

T2

T3

Switching event

Co-DFI

**Differential Polydenylation**

Varying PolyA site

Genomic

ProximalPA

DistalPA

Switching event

Expression

DistalPA

ProximalPA

T1

T2

T3

**UTR shortening/lengthening**

Co-inclusion

Feature 1

Feature 2

ExcF1

ExcF2

IncF1

IncF2

Expression

T1

T2

T3

Length 1

Length 2

Length 3

Length 1

Length 2

Length 3

Expression

Cond1

Cond2

Mutual exclusion

Feature 1

Feature 2

ExcF1

ExcF2

IncF1

IncF2

Expression

T1

T2

T3

3' UTR length

Cond1

Cond2

3' UTR lengthening in Cond2 vs Cond1

$$3'UTRLength_{Cond1} = Expr_1 \cdot Length_1 + Expr_2 \cdot Length_2 + Expr_3 \cdot Length_3$$

# Differential Isoform Usage Analysis (DIU)

Differential Isoform Usage Parameters

Project "Demo\_filtered"

**Data Type**

Transcripts     CDS

**Analysis Method**

DIU Method: DEXSeq

**Analysis Parameters**

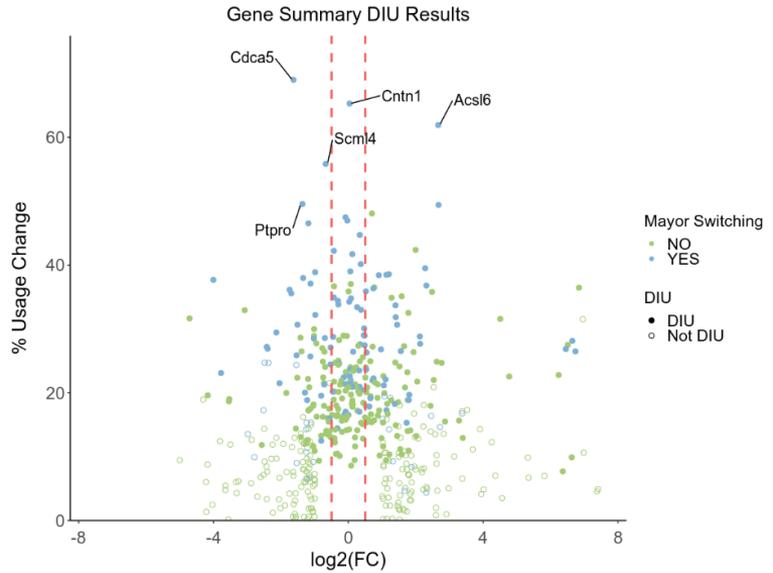
Significance Level: 0.05

Filtering:  Filter minor isoforms    Proportion filtering

Prop. expression difference: 0.2 (default: 0.2)

Help
Cancel
OK

- Result:
- Isoform switching.
  - DIU/not DIU.
  - Total Usage Change.



# Enrichment analyses (FEA and GSEA)

Functional Enrichment Analysis Parameters

Project "Demo\_filtered"

**Analysis Information**

Name:

**Data Type**

Genes
  Transcripts
  CDS

**Genes Lists**

Test List:

List file:

Background List:

List file:

**Annotation Feature(s)**

- REACTOME
- UTRsite
- GeneOntology
- miRWalk

You may select one or more features from a single source

**Analysis Parameters**

Statistical Test:

Number of Samples:

Use w/o Category:

Significance Level:

Result:

- Significantly enriched feature categories.
- No. of genes with feature in test list/background list.



### Module 1: Functional Diversity Analysis

**Positional approach**  
APA/UTR/CDS...

**Presence/absence approach**  
Signals/domains/NMD...

### Module 2: Differential Analysis

Global analysis : gene expression levels  
Intra-gene analysis: AltTP

**Differential Expression**  
Transcript/Gene/CDS level

**Differential Isoform Usage**  
Transcript/CDS level

**Functional Enrichment of DE vs DIU results**

**Differential Isoform Usage**

### Module 3: Feature Analysis

**Differential Feature Inclusion**

Late inclusion feature  
T3 favored

ExcF  
IncF

Switching event

Expression

T1 T2 T3

**Differential Polydenylation**

Varying PolyA site

DistalPA  
ProximalPA

Switching event

Expression

T1 T2 T3

Genomic

ProximalPA DistalPA

**Co-inclusion**

Feature 1 Feature 2

ExcF1  
IncF1

ExcF2  
IncF2

Expression

T1 T2 T3

**Mutual exclusion**

Feature 1 Feature 2

IncF1  
ExcF1

ExcF2  
IncF2

Expression

T1 T2 T3

**UTR shortening/lengthening**

Length 1  
Length 2  
Length 3

Expression

Cond1 Cond2

**3' UTR lengthening in Cond2 vs Cond1**

$$3UTRLength_{Cond_i} = Expr_i \cdot Length_1 + Expr_i \cdot Length_2 + Expr_i \cdot Length_3$$

3' UTR length

Cond1 Cond2

# Differential Feature Inclusion Analysis (DFI)

Differential Feature Inclusion Analysis Parameters

Project "Demo\_filtered"

**Annotation Feature(s)**

- SIGNALP\_EUK
- NMD
- PAR-CLIP
- PFAM

You may select annotation features from multiple sources. Clear All Check All

Check Using: Feature presence

**Analysis Method**

Method: DEXSeq

**Analysis Parameters**

Name:

Significance Level:

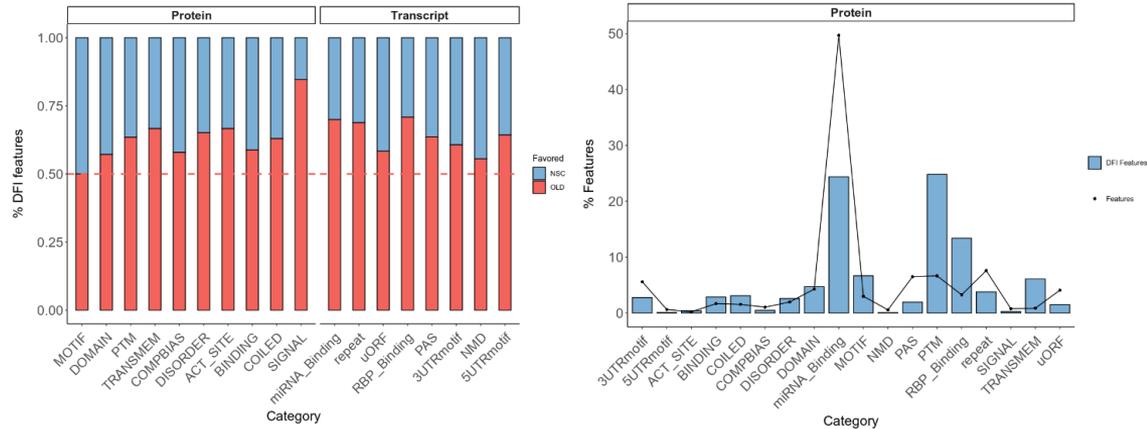
Filtering:  Filter minor isoforms Proportion filtering

Prop. expression difference:  (default: 0.2)

Help
Cancel
OK

Result:

- DFI status and significance.
- Global statistical testing of each functional category.
- Favored condition/time point.
- Switching for feature usage and  $\Delta FI$ .



# Differential Polyadenylation Analysis (DPA)

Project "Demo\_filtered"

**PolyAdenylation Range**

Minimum value for differentiation:  (default: 60)

**Analysis Method**

Method: DEXSeq

**Analysis Parameters**

Significance Level:

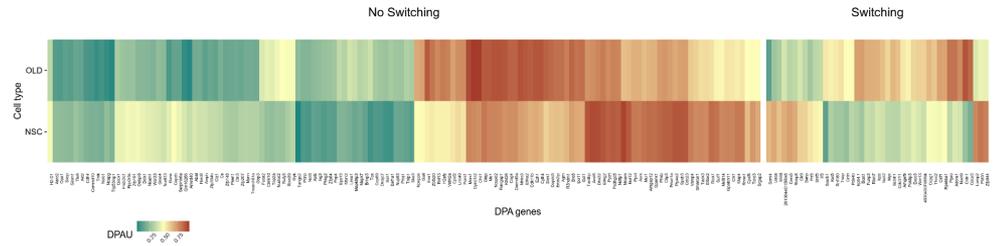
Filtering:  Filter minor isoforms    Proportion filtering

Prop. expression differen...  (default: 2)

Help
Cancel
OK

Result:

- DPA status and significance.
- Proximal and distal polyA site estimated expression.
- Distal condition.
- Switching for polyA site usage and  $\Delta$ DPAU.



# 3' and 5' UTR Lengthening (UL)

UTR Lengthening Analysis Parameters

Project "Demo\_filtered"

**Lengthening Range**

Minimum value for differentiation:  (default: 100)

**Analysis Method**

Method:

**Analysis Parameters**

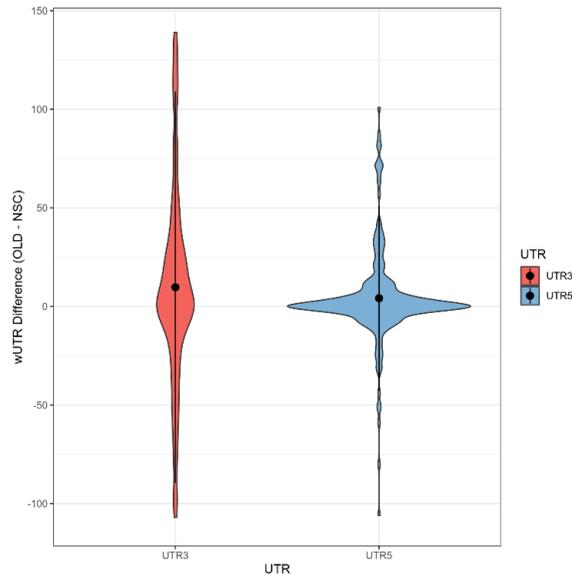
Significance Level:

Filtering:  Filter minor isoforms

Prop. expression differen...  (default: 0.2)

Result:

- Expression-weighted UTR length (wUTR) per gene.
- Significance of change in UTR length across conditions for 3' and 5' UTRs.



---

# Hands on time!

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tappAS

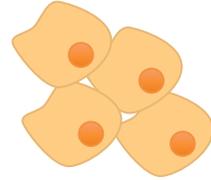
The logo for tappAS features the text 'tappAS' in a sans-serif font. The 't' and 'a' are black, 'p' is red, and 'AS' is green. Below the text are three overlapping, wavy lines: a red one on the left, a blue one in the middle, and a green one on the right, all curving upwards.

# Demo data: OPC differentiation from NPC

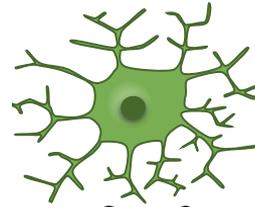
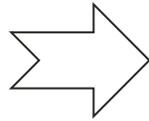


Neural Precursor Cells

Oligodendrocytes



NPC



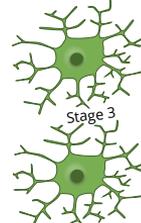
Stage 3



NPC

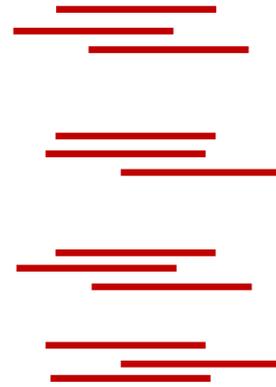


NPC

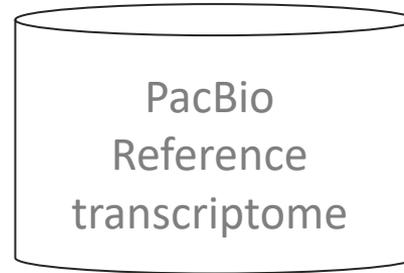
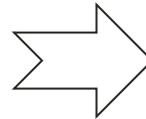


Stage 3

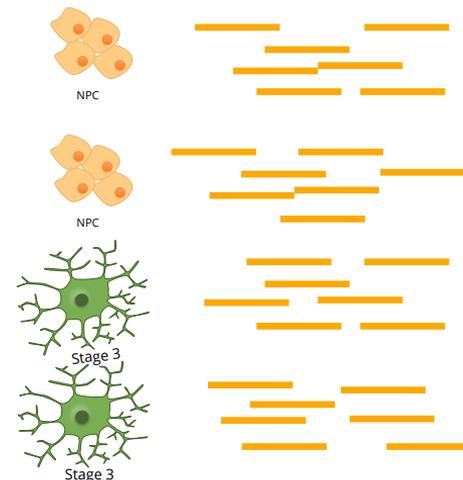
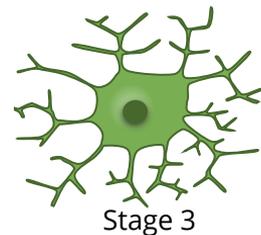
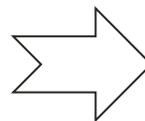
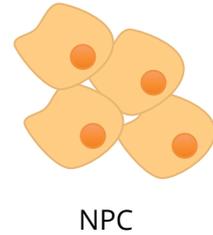
Stage 3



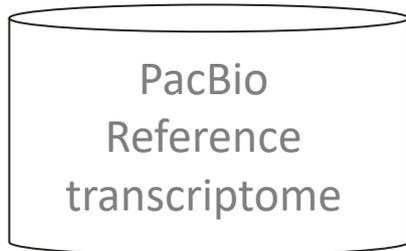
Iso-seq3  
SQANTI



# Demo data: OPC differentiation from NPC

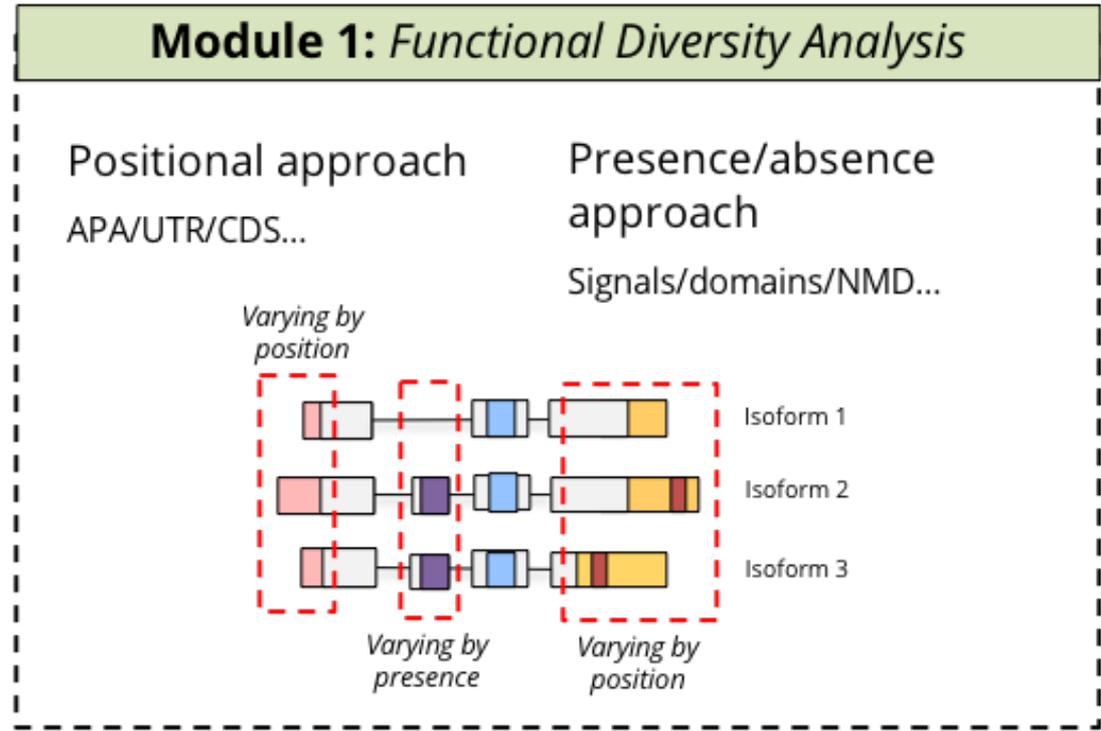


Mapping  
STAR



	NPC1	NPC2	OLD1	OLD2
PB1	-	-	-	-
PB2	-	-	-	-
PB3	-	-	-	-
...				
...				

## Exercise 1: Functional Diversity Analysis (FDA)



## Exercise 1: Functional Diversity Analysis (FDA)

1. Run a category FDA analysis using **genomic position** as the *varying* criteria.
2. Run a category FDA analysis using **presence** as the *varying* criteria.
3. **Visualization:** [Tip: use the left-hand side bar, and look for the graphics icon. Results will show in bottom panel.]
4. Look up gene **Rnh1** gene and find the differences in varying status for the Domain category between the presence and genomic position options
5. Run two ID-level FDA for PFAM domains, one using each *varying* criteria. Select the most significant varying ID and find the gene differences in the annotation.
6. **Visualization:** open the combined ID results tab and its results summary graphics. How can the results be interpreted?

## Exercise 4: Differential Feature Inclusion (DFI) analysis

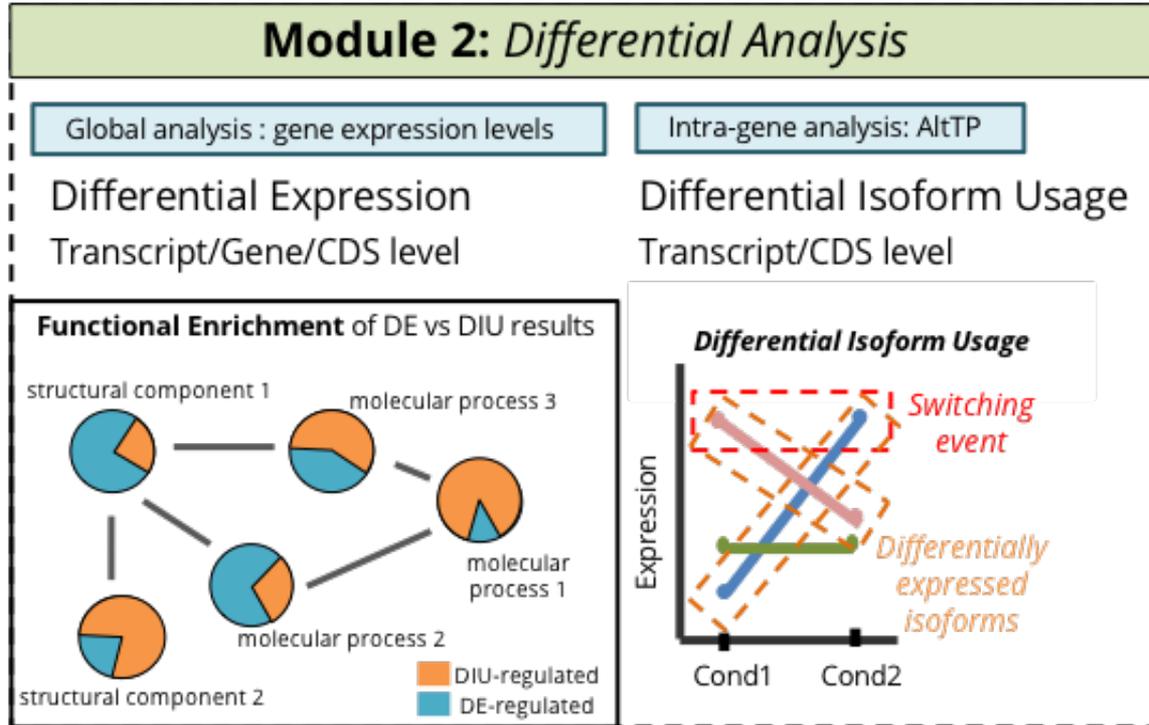
1. Run a **DFI analysis** for selected annotation categories (PAR-CLIP, NLS mapper, mirWalk, PFAM, UTRsite). You may use **genomic position** or **presence** as the differential criteria.

**NOTE:** this analysis will take ~10 min, so we'll leave this running and continue with Exercise 2.  
We'll come back to Exercise 4 and the DFI results later!

## Exercise 2: Differential Expression (DE) and Differential Isoform Usage (DIU)

1. Run a **DE analysis for genes**. You may use any of the available analysis methods.
  2. **Filtering**: filter de DEA genes tab by DE status. How many genes are DE? Export the list of DE genes (gene IDs only).  
[Tip: use the left hand-side bar, and look for the filtering and export icons]
  3. **Filtering**: try adding the «Isoform» column and filter again by isoform no. to detect how many DE genes have more than one isoform.  
[Tip: use the [+] button on the right side of the top panel]
1. **Visualization**: open the Results Summary graphics and try to find the no. of DE genes and the no. of multi-isoform DE genes in this other view.

## Exercise 2: Differential Expression (DE) and Differential Isoform Usage (DIU)



## Exercise 2: Differential Expression (DE) and Differential Isoform Usage (DIU)

5. Run a **DIU analysis for transcripts**. Run a DIU analysis for CDS, i.e. a DCU (Differential Coding region Usage) analysis.

[Tip: do not forget to apply the minor isoform filtering option at the bottom of the window!]

6. **Filtering:** filter the DIU results table by DIU status. Export the DIU gene IDs list. Do the same for the DCU results table (DIU protein).

7. Combine results and search for the ***Ctnnd1* gene**. Obtain gene visualization. Open the Expression Charts, and notice that there is isoform switching for two of its isoforms. Is the gene DIU? And DE?

[Tip: use the «Search» bar at the upper bar of the application, and then right-click on the gene.]

8. **Visualization:** open the DIU results summary. What is the relationship between isoform switching and % Usage Change?

## Exercise 3: Coupling the Differential and Enrichment Modules.

1. Run a **Functional Enrichment Analysis** using **DE genes as test list** and all genes as background list. Select GeneOntology as the functional database for analysis.
2. Run a **Functional Enrichment Analysis for DIU genes**. To study the functional categories that are regulated by splicing, select all functional annotation databases.
3. Run the same analysis for **DCU genes**. How do results change when coding potential is considered?
4. **Visualization:** for the DIU and DCU gene enrichments, open the Enriched Features Chart. Customize the no. of features shown in the chart. Export the images.

## Exercise 3: coupling the Differential and Enrichment Modules.

5. Run a **Functional Enrichment Analysis**, using DIU genes as test list and DE genes as background. Select «Use input file...» and use the exported files in the previous exercise.

[Tip: use the left hand-side bar, and look for the graphics and export icons]

6. For the NLS category, find the list of genes that contains the feature in the test list. How many are there?

[Tip: you can right click on any functional category, and select «Drill down data» to view the genes that contain a given feature within the test list.]

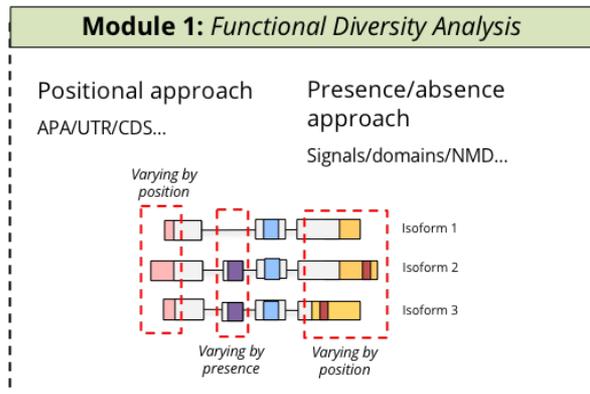
1. **Extra exercise:** go back to the DE and DIU results, filter and export them as **gene ranked lists** (i.e. gene ID and p-value). Perform **unidimensional and multidimensional Gene Set Enrichment Analysis (GSE)**.

# tappAS hands-on

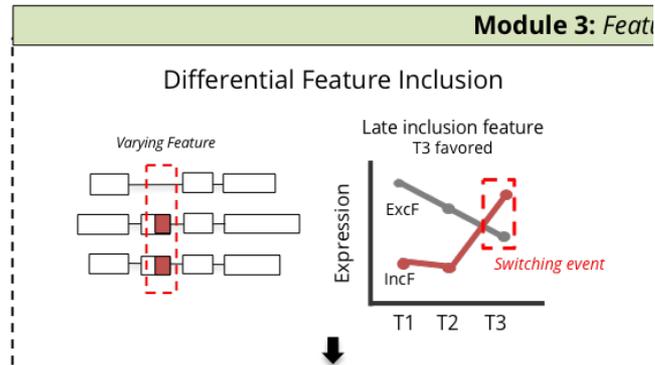
## Exercise 4: Differential Feature Inclusion (DFI) analysis

1. Run a **DFI analysis** for all annotation categories. You may use **genomic position** or **presence** as the differential criteria.

### FDA only uses annotation



### DFI uses gene expression



## Exercise 4: Differential Feature Inclusion (DFI) analysis

1. Run a **DFI analysis** for all annotation categories. You may use **genomic position** or **presence** as the differential criteria.  
[!] The analysis should have finished by now -if not, it may be due to low RAM. tappAS can be run with more RAM by using the `java -XmsAM -XmxBM -jar tappas.jar` command
2. **Visualization**: open the Results Summary graphics. What categories are globally significantly DFI in the transcriptome?
3. Select a DFI gene and open the **feature ID data visualization**. Open the Expression Charts in the gene data visualization for that gene, and compare them. Is there switching for the feature? Is there switching for the isoforms?

## Exercise 4: Differential Feature Inclusion (DFI) analysis

4. For this gene, open the **transcript and protein annotations**. Can you locate the DFI feature in the transcript and/or protein models? Which are the isoforms that include them, and how does their expression change?
2. Open the **DFI Results Summary** tab. What is the most frequently DFI functional annotation category? In which condition is its inclusion favored?
1. Open the **coDFI Results** tab. Which pair of functional features are more frequently co-DFI?

## Exercise 5: other analyses in the Features module.

1. Run a **Differential PolyAdenylation (DPA)** analysis and a  
[Tip: notice the minimum differentiation value threshold. The higher, the more stringent the analysis, i.e. higher bp difference between the polyA sites of all isoforms will be required to consider a gene with two polyA sites, distal and proximal.]
2. **Visualization:** open the Results Summary graphics, and look for the total no. of DPA genes.
3. Select a DPA gene, and open the **feature ID data visualization**. Is there polyA site switching? How does this compare to the gene's isoforms expression? Can you locate the different polyA sites in the transcript model annotations?
4. Run a **UTR Lengthening (UL)** analysis.

## Exercise 5: other analyses in the Features module.

### 4. Run a UTR Lengthening (UL) analysis.

[Tip: notice the minimum differentiation value threshold. The higher, the more stringent the analysis, i.e. higher bp difference between the polyA sites of all isoforms will be required to consider a gene with two UTRs, a shorter and a longer.]

1. **Visualization:** open the Results Summary graphics. Is there significant 3' and/or 5' UTR lengthening between the conditions?

2. Add the «Isoforms» column to the UL results table. Find a gene with many isoforms, and open the **gene data visualization**. How many different UTR lengths do you see, and how do you think they were collapsed?