

AS-Quant User Manual

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1. Download

AS-Quant tool can be downloaded directly from github [AS-Quant](#). User need to have python installed in their machine. It can work on Windows, Linux and Mac platform.

2. Required software

- a. [Python](#) (version 3.0 or higher)
- b. [Samtools 0.1.8*](#) [This specific version]

3. Required python packages

- a. [matplotlib](#)
- b. [scipy](#)
- c. [pandas](#)

4. Run AS-Quant

AS-Quant is designed for handling both human and mouse Alternative Splicing events. The supplementary data (the five types of Alternative Splicing target dataset and the annotation) is provided in the project directory.

Users have to run the following two python files in order to run AS-Quant:

- i) `as_quant.py`: the main function which the user need to run
- ii) `make_plots.py`: generates figures for visual representation of data

Run `as_quant.py`:

```
$python3 as_quant.py -s species -o output_directory input1_directory input2_directory
```

Example:

```
$python3 as_quant.py -s human -o results home/Naima/Desktop/input1.bam home/Naima/Desktop/input2.bam
```

Options: (* refers to mandatory field)

<code>-s/-S*</code>	: Species name. AS-Quant can handle both human and mouse.
<code>-o/-O</code>	: Output directory. User can specify desired output directory for writing the results. [Optional] Output directory must be a folder name/directory without a '/' at the end.
<code>input1*</code>	: Specifies directory for the first sample. Input1 is the name of a bam file aligned to reference genome.
<code>input2*</code>	: Specifies directory for the second sample. Input2 is the name of a bam file aligned to reference genome.

as_quant.py will generate a file in the output directory after computing the significance of the association between the two samples. The following image is showing some of the generated fields:

	A	B	C	D	E	F	G	H
1	Chrom	Gene Name	Exon Start	Exon End	p-value	Ratio difference	Absolute Ratio difference	Chrom region
2	chr1	DPH5	101467022	101467100	0.9121302401	0.1000592552	0.1000592552	chr1:DPH5:101467022-101467100
3	chr1	DPH5	101479265	101479374	0.5834973654	0.1790025326	0.1790025326	chr1:DPH5:101479265-101479374
4	chr1	APITD1-CORT	10494713	10494747	0.3359058847	-0.972067033	0.972067033	chr1:APITD1-CORT:10494713-10494747
5	chr1	PEX14	10596269	10596354	0.9023390528	0.137534426	0.137534426	chr1:PEX14:10596269-10596354
6	chr1	PEX14	10659294	10659423	0.6931817062	0.2498379422	0.2498379422	chr1:PEX14:10659294-10659423
7	chr1	AMPD2	110167924	110168055	0.563988963	0.1287839664	0.1287839664	chr1:AMPD2:110167924-110168055
8	chr1	SLC16A4	110924273	110924417	0.5841909446	-0.8237182045	0.8237182045	chr1:SLC16A4:110924273-110924417
9	chr1	SLC16A4	110925455	110925588	0.6872561265	-0.5067973124	0.5067973124	chr1:SLC16A4:110925455-110925588
10	chr1	ST7L	113098489	113098640	0.9287630199	0.196174765	0.196174765	chr1:ST7L:113098489-113098640
11	chr1	ST7L	113140592	113140708	0.7976173158	0.366711548	0.366711548	chr1:ST7L:113140592-113140708
12	chr1	ST7L	113143415	113143470	0.9416810826	-0.1870431467	0.1870431467	chr1:ST7L:113143415-113143470

Run **make_plots.py**:

```
$python3 make_plots.py -s species -o output_directory input1 input2
```

Example:

```
$python3 make_plots.py -s human -o /home/Naima/Desktop/Annotation_plot inputs/sample1 inputs/sample2
```

Options: (* refers to mandatory field)

-s/-S* : Species name. AS-Quant can handle both human and mouse. [Mandatory]

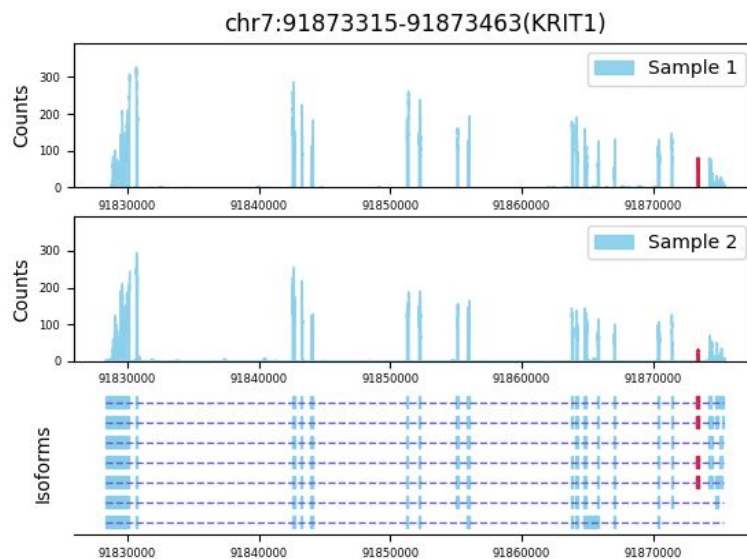
-o/-O : output directory for the generated plots. [Optional]

Input1* : The aligned read files directory for which you want to generate the plot, without '/' at the end.

Input2* : The aligned read files directory for which you want to generate the plot, without '/' at the end.

make_plots.py will generate the read coverage plot for the given gene along with the whole annotation plot with all exons information of that gene.

The output will produce a figure like the following:



The first two subplots of the figure represents the read coverage of the two biological conditions. The bottom subplot shows the gene annotation along with all the exons information of that gene.

5. Run AS-Quant with provided sample input

```
$ python3 as_quant.py -s mouse sample_input_mouse/s1/accepted_hits.bam  
sample_input_mouse/s2/accepted_hits.bam
```

It will generate the output tables inside of folder 'Output' in the same directory. Or you can generate output in your desired directory, such as 'Results':

```
$ python3 as_quant.py -s mouse -o Results sample_input_mouse/s1/accepted_hits.bam  
sample_input_mouse/s2/accepted_hits.bam
```