



SeqDEAS

## Manual

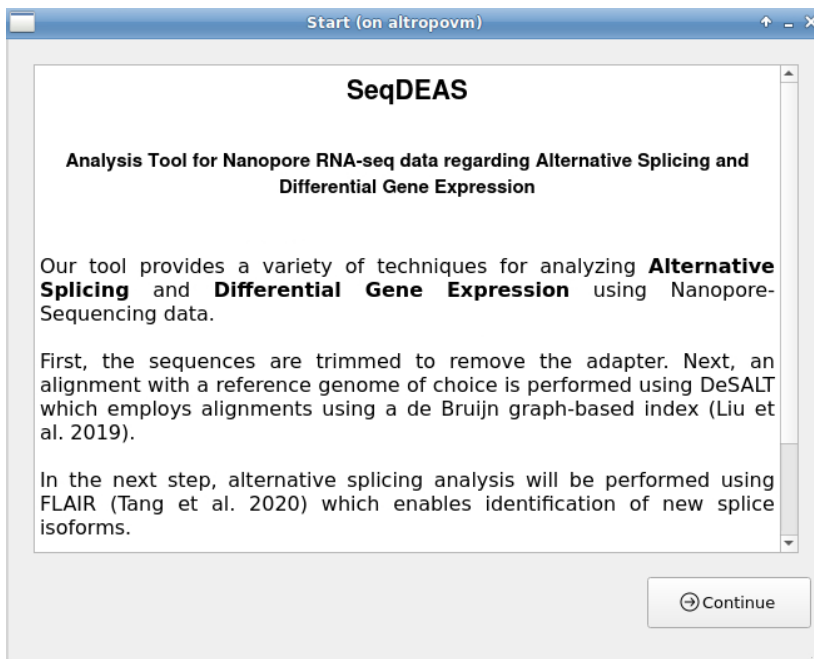
### 1. Installation

(Installer will be set up when software is done)

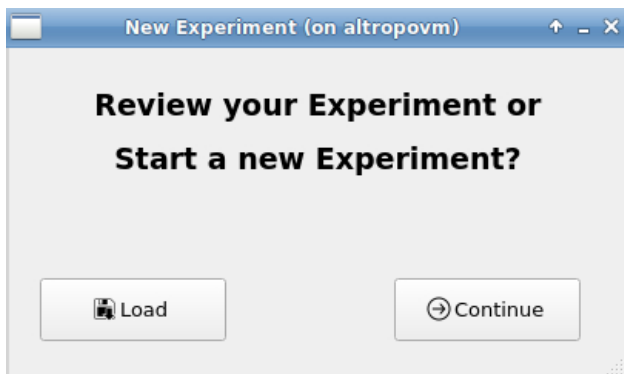
- 1.1. Git clone project: <https://github.com/anbergander/splivardet.git>
- 1.2. Install software packages from requirements.txt
- 1.3. Run project via terminal or pycharm

### 2. Software

- 2.1. Start Window will show up; Press Continue for the next window.



- 2.2. Decide whether you want to review an experiment or start a new one



- 2.3. In case: you decided to start a new experiment: you will be asked to create a manifest



- 2.4. In case: you decided to create a new manifest you need to enter the number of samples and click ADD. Then, you need to fill out the table and press CONTINUE. A manifest in TSV format will be generated

Create Manifest (on altropovm)

Group

Number of Samples: 2 Add

Continue

Group

Number of Samples: 2 Add

Sample	Group	Batch	Path
1			
2			

Continue

- 2.5. In case you decided to load a manifest, load your manifest by BROWSING

Load Manifest (on altropvm)

### Load Manifest for Grouping

Manifest

	Sample	Group	Batch	Path
1	barcode01	1	lv	C:...
2	barcode02	1	lv	C:...
3	barcode03	1	lv	C:...
4	barcode04	2	lv	C:...
5	barcode05	2	lv	C:...
6	barcode06	2	lv	C:...

2.6. Next you need to fill out the parameter form. All fields need to be filled except for the output path. Then press CONTINUE

Parameter for Config (on altropvm)

### Select Parameters

Reference Genome

☐ Build DeSALT index

Threads

FastQ Files

gtf File

Flair Manifest

Output

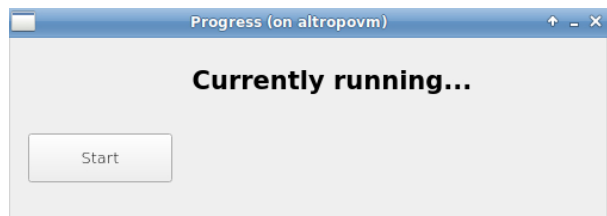
Genome Fasta

Index Directory

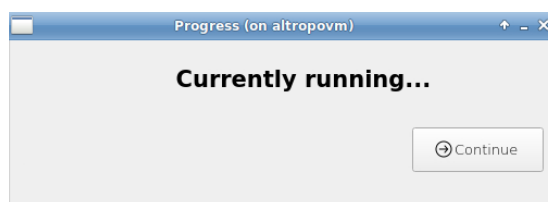
2.7. You will be asked to start the run



2.8. Press the START Button to start.



2.9. After the run is done: a CONTINUE button will be disabled.



2.10. Next, you can generate a report for the run with the important steps in PDF format. You can fill out the experiment name, the reference genome and the annotation

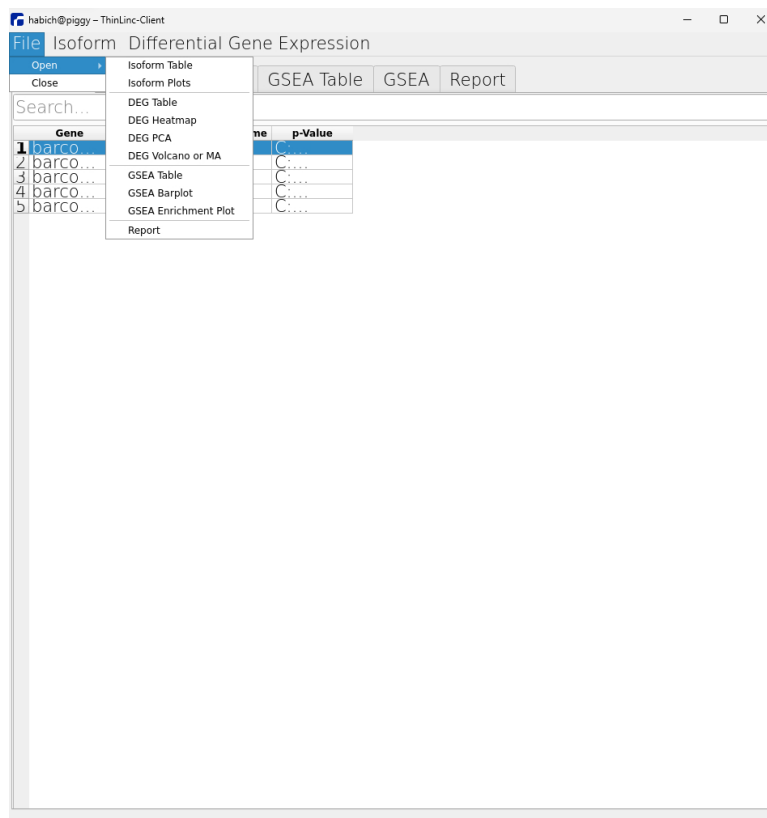
A dialog box titled "Report (on altropovm)" with a blue header bar. The main text says "Report". Below the text is a form with several sections, each with a label on the left and input fields on the right:

- Experiment**: A single-line text input field.
- Reference Genome**: A single-line text input field.
- gtf Annotation**: A single-line text input field.
- Trimming**: Two input fields. The first is labeled "Method/ Tool" and contains "PoreChop". The second is labeled "Parameter/Values" and contains "none".
- Data Processing**: Two input fields. The first is labeled "Method/ Tool" and contains "Seqkit". The second is labeled "Parameter/ Values" and contains "-m 100 (Minimum length)".
- Alignment**: Two input fields. The first is labeled "Method/ Tool" and contains "DeSALT". The second is labeled "Parameter/ Values" and contains "-s 2 (Seed step), -l 14 (Seeding l-mer), -x ont1d (Read type)".
- Post-Processing**: Two input fields. The first is labeled "Method/ Tool" and contains "Samtools". The second is labeled "Parameter/ Values" and contains "none".
- Isoform Detection**: Two input fields. The first is labeled "Method/ Tool" and contains "FLAIR". The second is labeled "Parameter/ Values" and contains "FLAIR-correct, FLAIR-quantify -quality 4".
- Statistics**: Two input fields. The first is labeled "Method/ Tool" and contains "ZO-transformed, Bonferroni-corrected Beta Regression". The second is labeled "Parameter/ Values" and contains "Significance niveau: 0.05".

At the bottom of the form is a large button labeled "Generate".

2.11. Click GENERATE to go to the main window

2.12. Main Window will appear



- 2.13. Here you have a various choices to display data from your experiment or start a DEG or GSEA in case you have count data generated
- 2.14. For the DEG, click Differential Gene Expression; then perform DEG Analysis



- 2.15. In order to perform a DEG, you need to enter your count table, an output path, the factors you are interested in and optionally a list of genes you want to see in the volcano plot. The list for the genes and factors you should be separated by “,”

- 2.16. Enter the number of samples and factors and click CREATE METADATA TABLE for creating your own metadata table. Fill out the table before you click START in order to perform the DEG

	Sample	Condition
1		
2		
3		
4		
5		
6		
7		
8		
9		

- 2.17. After finishing a message will show up. Close all windows  
2.18. For the GSEA, click Differential Gene Expression; then perform GSEA

Start GSEA (on altropovm)

GSEA

DEG Table  Browse

Output  Browse

Start

- 2.19. For the GSEA, enter your DEG data and the output path before clicking START.
- 2.20. After finishing a message will show up
- 2.21. In order to create a report for the DEG click Differential Gene Expression > Create Report for DEG
- 2.22. Enter your Experiment name, Annotation and Reference Genome and click GENERATE. You will obtain a report in PDF formate

**Report (on altropovm)**

## Report: DEG and Pathway Enrichment

Experiment	<input type="text"/>		
Reference genome	<input type="text"/>	Annotation	<input type="text"/>
<b>DEG</b>		<b>Pathway Enrichment</b>	
Method	<input type="text" value="PyDESeq2"/>	Method	<input type="text" value="GSEAPy"/>
Output	Metadata (metadata.tsv) DE Table (result_table.tsv) Significant Genes (significant_de.tsv) Volcano-Plot (volcano.svg; volcano.png) Heatmap (heatmap_de.svg) PCA (pca_normalized.svg)	Output	GSEA Table (gsea.tsv) Enrichment Plots (gsea_enrichment_plot.svg) Enriched Pathways (gsea_terms_enriched.svg) Reduced Pathways (gsea_terms_decreased.svg)
<input type="button" value="Generate"/>			


2.23. In case you decided to load your data in step 2.2, you will be seeing this window

**Load Previous Experiment (on altropovm)**

## Load Data

Report	Optional: Path to Report	<input type="button" value="Browse"/>
Gene Expression Table	Optional Path to Table for Gene Expression Data	<input type="button" value="Browse"/>
DE Heatmap	Optional: Path to Heatmap	<input type="button" value="Browse"/>
DE Volcano	Optional: Path to Volcano Plot	<input type="button" value="Browse"/>
DE PCA	Optional: Path to PCA	<input type="button" value="Browse"/>
GO Term Table	Optional: GO Term Table	<input type="button" value="Browse"/>
GO Term Enrichment Plot	Optional: Path to Enrichment Plot	<input type="button" value="Browse"/>
GO Term Barplot	Optional: Path to Barplot	<input type="button" value="Browse"/>
Isoform Expression Table	Optional: Path to Isoform Expression Table	<input type="button" value="Browse"/>
Isoform Heatmap	Optional: Path to Isoform Heatmap	<input type="button" value="Browse"/>
<input type="button" value="Load"/>		

2.24. Fill out the data you want to load and click LOAD. Then you will see your data in the main menu having the same functions as before.


habich@piggy - ThinLinc-Client

File
Isoform
Differential Gene Expression

Isoform

DEG Table

DEG

GSEA Table

GSEA

Report

Search...

	Gene	ase Mea	Fold Ch	lfcSE	Stat	P-value	
1	ENSG0...	3077....	-1.038...	0....	-6.771...	1.278777569261189e-11	4.840173

2.25. Close the window either by clicking X or File > Close