A Computational Platform for Gene Expression Analysis

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Outline

Domain Problem I

Introduction

- Molecular biology is a young field of study, with a lot of unknowns and partial knowledge.
- Studying gene expression is crucial to understand the mechanisms that control living organisms.
- We focused on two different areas:
  - differential expression analysis;
  - RNA-binding protein (RBP) discovery and analysis.

Domain Problem II

Introduction

Three distinct problems:

- Read alignment against a reference genome and differential expression analysis on the aligned data.
- RBP discovery, analysis and information enrichment.
- Further result analysis using data mining techniques.

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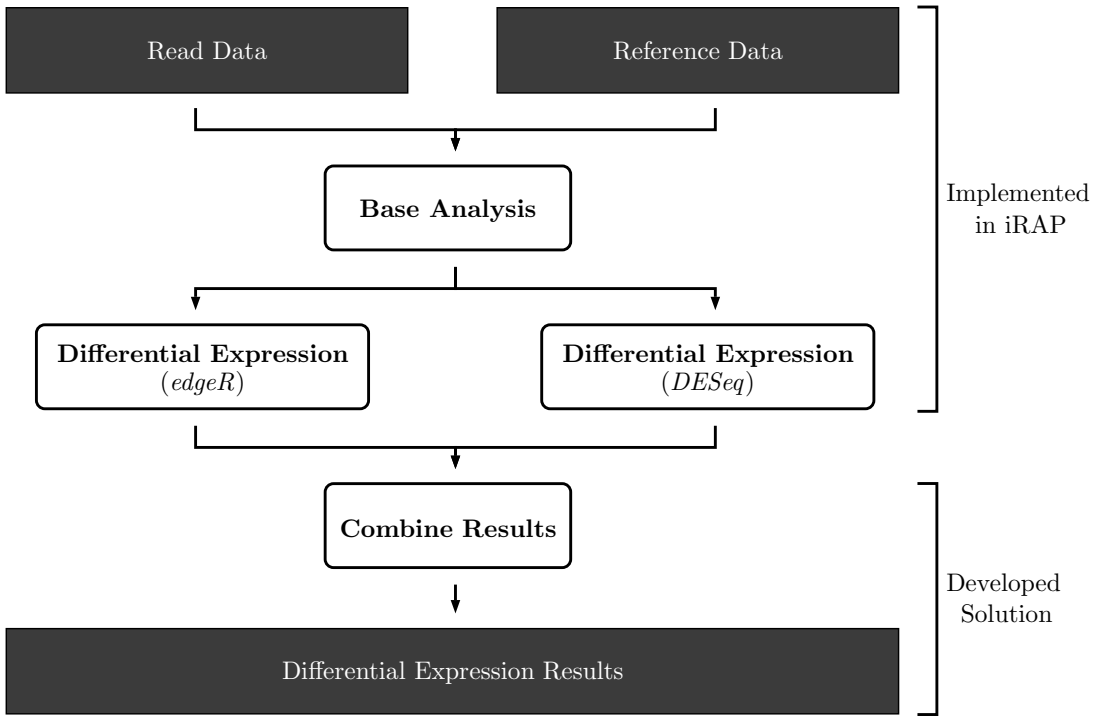
Gather information
Information should be contextually aggregated, allowing for quick access of relevant information.

- Two distinct problems warrant two different solutions.
- The developed system should be available anywhere, through the internet.
- The system should be as modular as possible, to allow future extensions.

RNA-Seq Analysis Pipeline I

Developed Solution

- Uses iRAP as the analysis pipeline.
- Conducts multiple differential expression analyses with different tools.
- Combines results from multiple tools.



RNA-Seq Analysis Pipeline II

Developed Solution

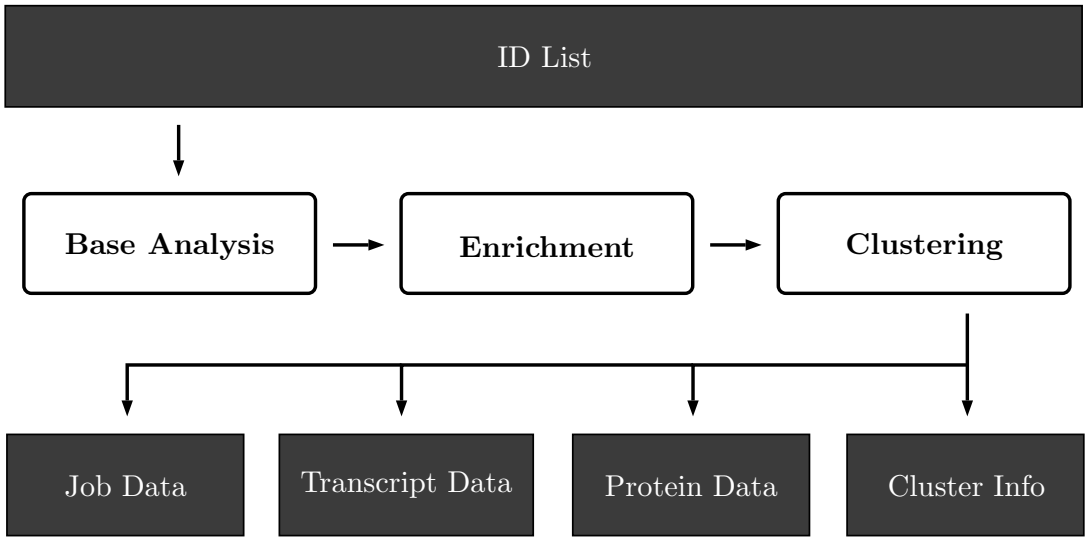
Additional features:

- Access to iRAP's web reports and gene browser, along with result visualization in the web interface.
- Synchronization with Ensembl's reference genome repositories.
- Graphical job configuration.
- Possibility to easily include other differential expression tools.

RBP Analysis Pipeline (PBS Finder) I

Developed Solution

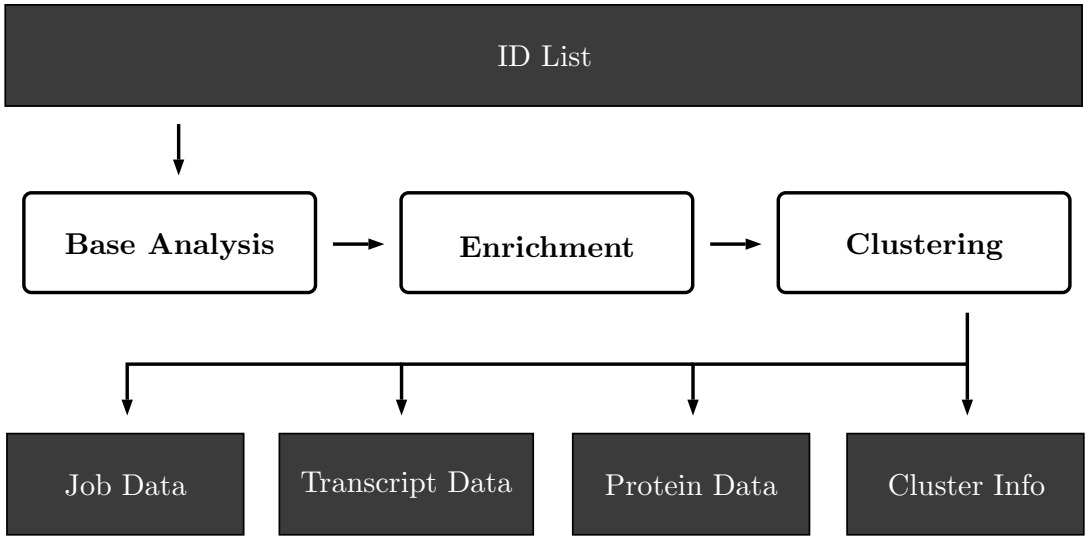
- Uses Ensembl and NCBI to identify gene species, obtain basic information and extract genetic sequences (5' UTR, 3' UTR, 3' UTR downstream).



RBP Analysis Pipeline (PBS Finder) II

Developed Solution

- Uses RBPDB to discovery RNA binding proteins based on the obtained sequences.
- Uses UniProt to enrich the obtained results and performs clustering analysis on those results.



RBP Analysis Pipeline (PBS Finder) III

Developed Solution

Clustering analysis:

- Uses k-medoids and hierarchical clustering, both with Jaccard and binary distance matrices.
- Executes every possible combination of clustering setups (alternates algorithms, distance matrices, used features, etc.).
- Results are filtered (acceptable solutions must have a minimum percentage of entries per cluster, clusters must have defining features, etc.).
- Solution quality internally determined based on the average silhouette.

RBP Analysis Pipeline (PBS Finder) IV

Developed Solution

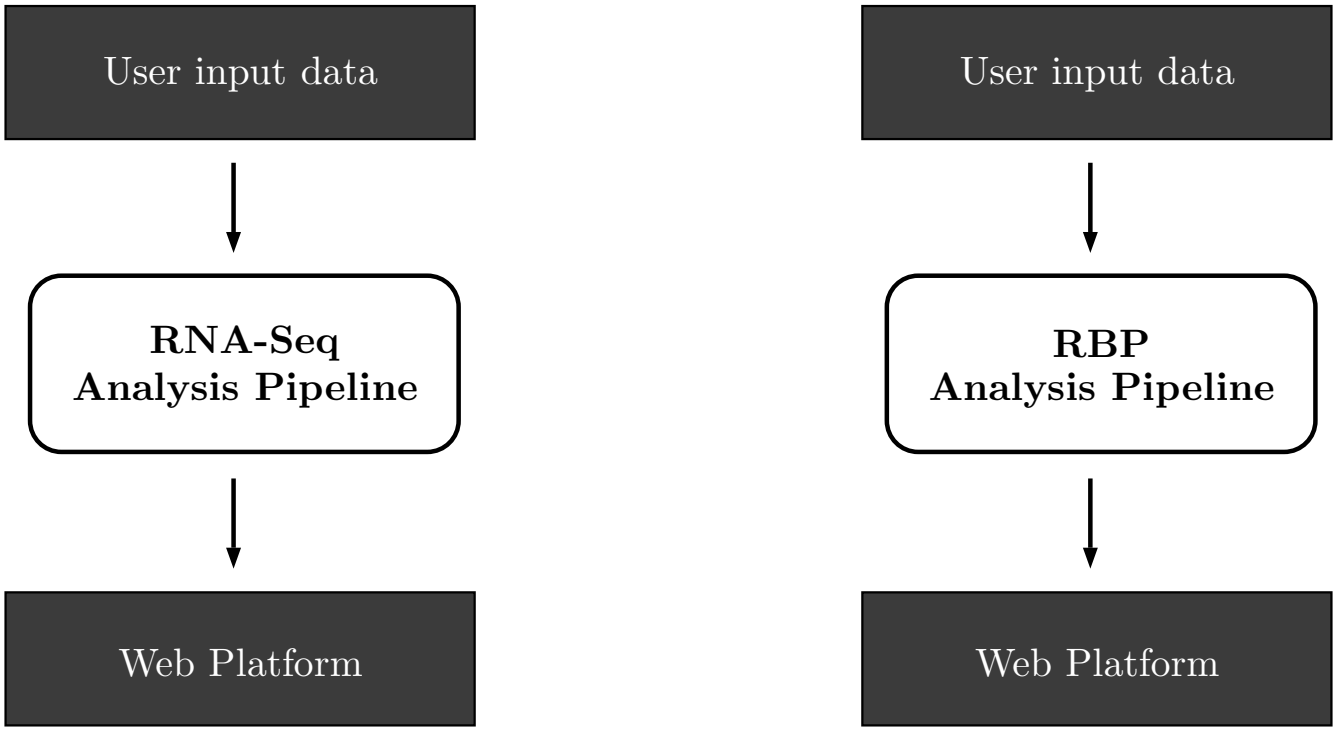
Additional features:

- User account and job management system.
- References to external platforms with relevant information based on context.
- Support for multiple identifier notations (Ensembl, Entrez, RefSeq and GenBank).
- Visualization of defining features for each cluster.
- Job completed notification system.

Integration

Developed Solution

While focusing on aggregation and quick access to information, does it make sense to separate the results into two different platforms?



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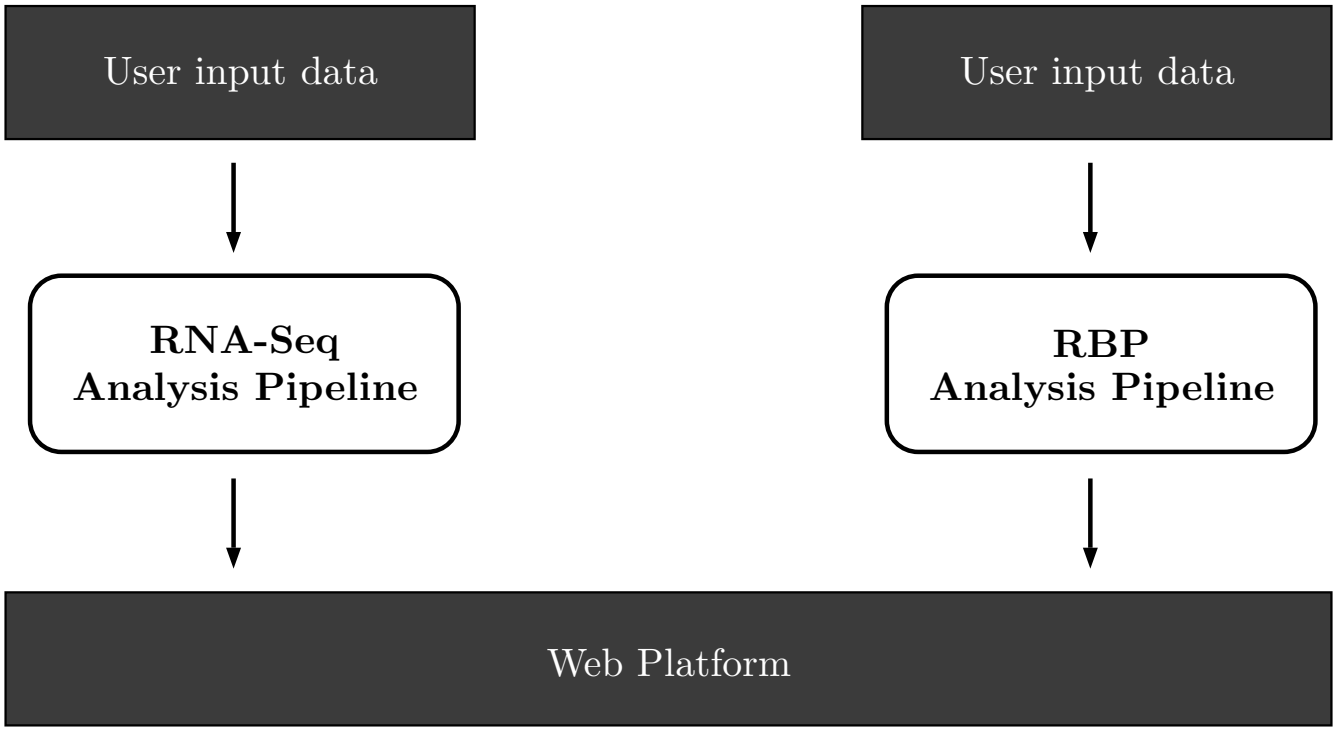
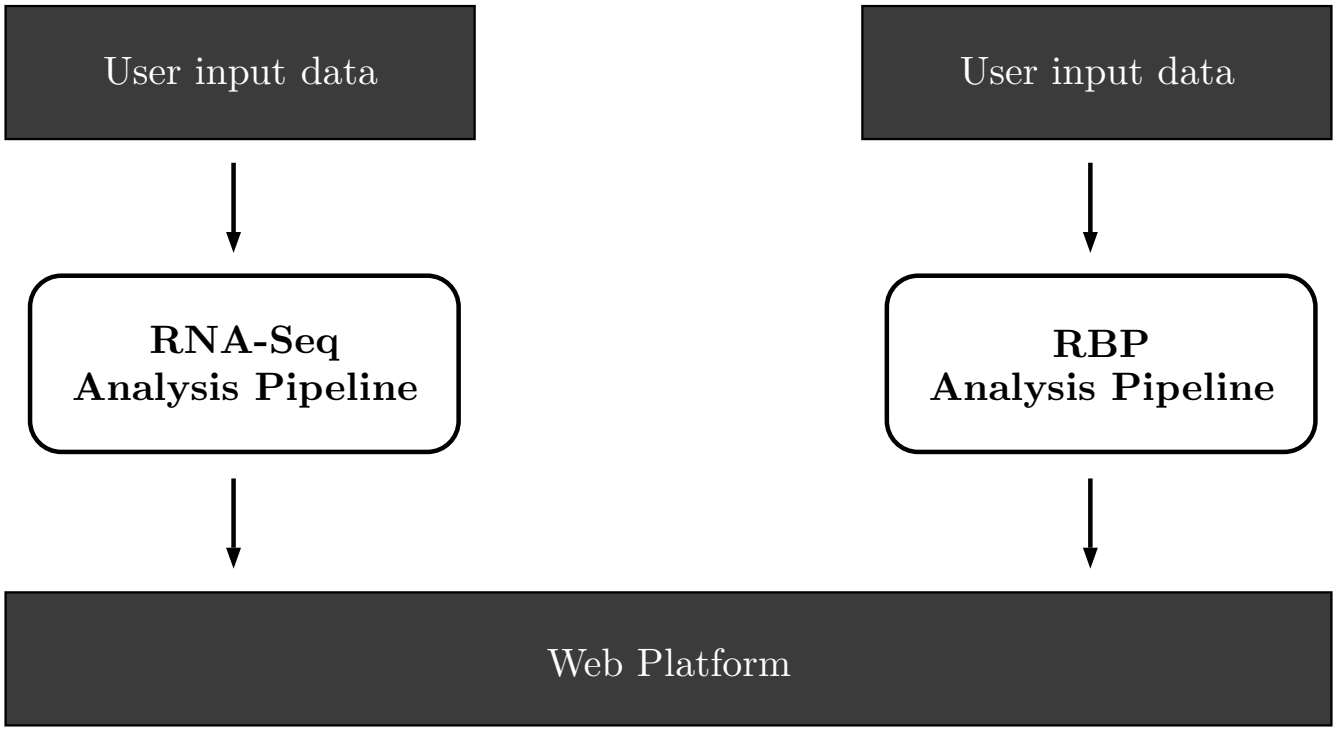


Integration

Developed Solution

A list of differentially expressed genes is not very useful without further information about those genes. Does it make sense for a user to launch a new gene enrichment task by hand?



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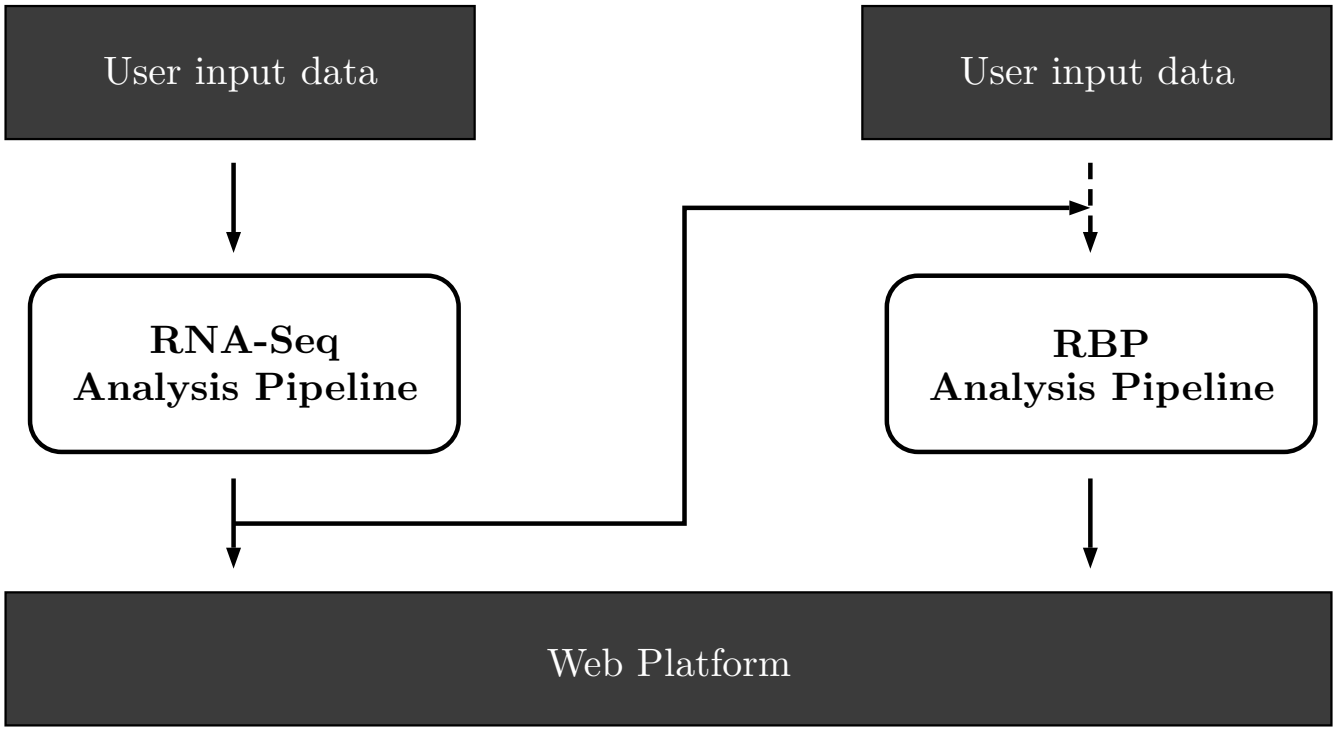
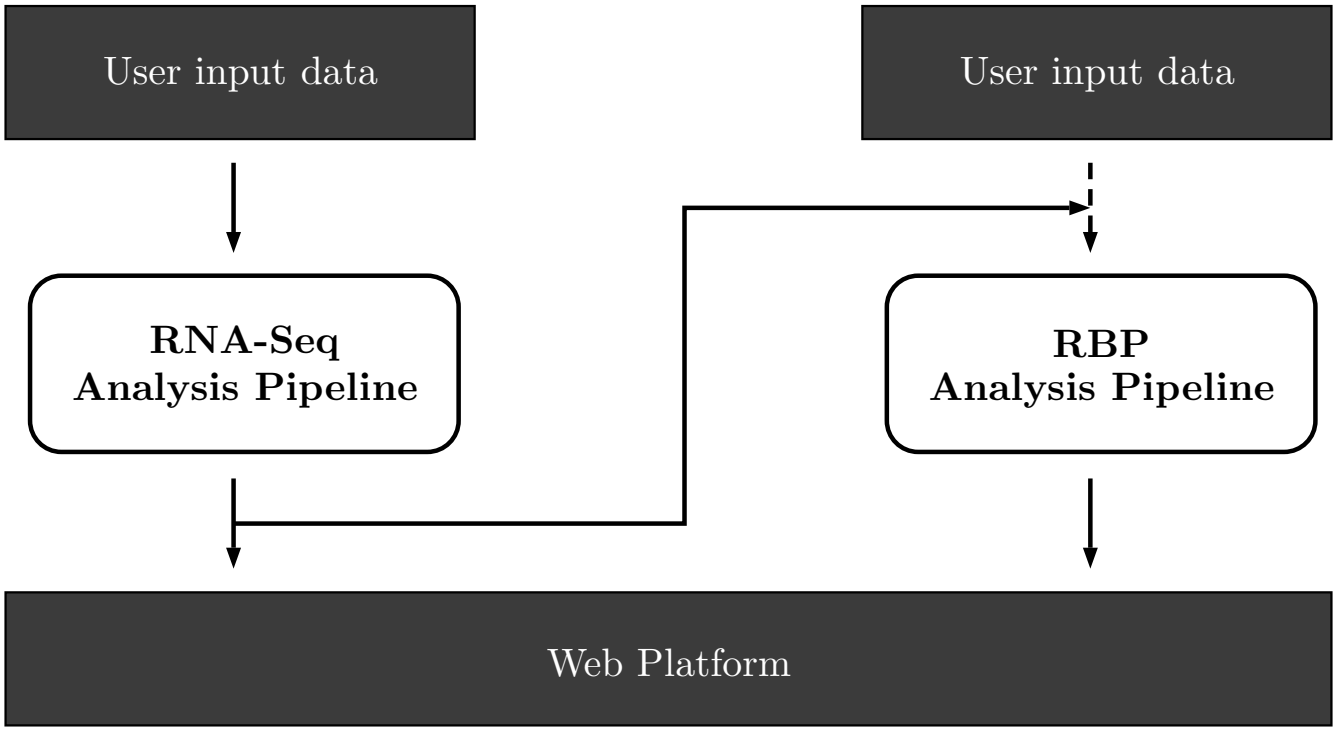


Integration

Developed Solution

A fully integrated solution: the analysis pipelines can be used separately or automatically executed in sequence; result visualization for both pipelines is isolated.



Objective

- Ascertain if combining the results of multiple tools has impact on the set of differentially expressed genes.

Data set

- Reproduction of ArrayExpress experiment E-GEOD-48829 (*Escherichia coli*).
- Reference genome obtained from Ensembl Genomes and read data obtained from ENA Sequence Read Archive.

Results (number of differentially expressed genes)

	Raw results	***Filtered results***	***Combined results***
edgeR	4494	386	191
DESeq	4494	204	

Conclusions

- Combining results impacts the final differentially expressed gene list by reducing its size.
- The combined results will hopefully give researchers an higher confidence in the experimental results.

RBP Analysis Pipeline (PBS Finder) I

Case Studies

Objectives

- Assess the general usefulness of PBS Finder.
- Compare PBS Finder with the existing techniques of manual analysis.
- Assess the impact of differences in hardware performance in the overall performance of the platform.

Data set

- 23 genes from the *RhoGTPase* family (*Rattus norvegicus*) provided by IBMC.

RBP Analysis Pipeline (PBS Finder) II

Case Studies

Results (expert estimation of 30 minutes per gene analysed)

Number of IDs	***Machine1***	***Machine2***	***Manual method***
100	$9m\ 56s$	$11m\ 1s$	$\approx 50h$
500	$41m\ 47s$	$55m\ 51s$	$\approx 250h$
900	$1h\ 33m\ 32s$	$2h\ 7m\ 4s$	$\approx 450h$

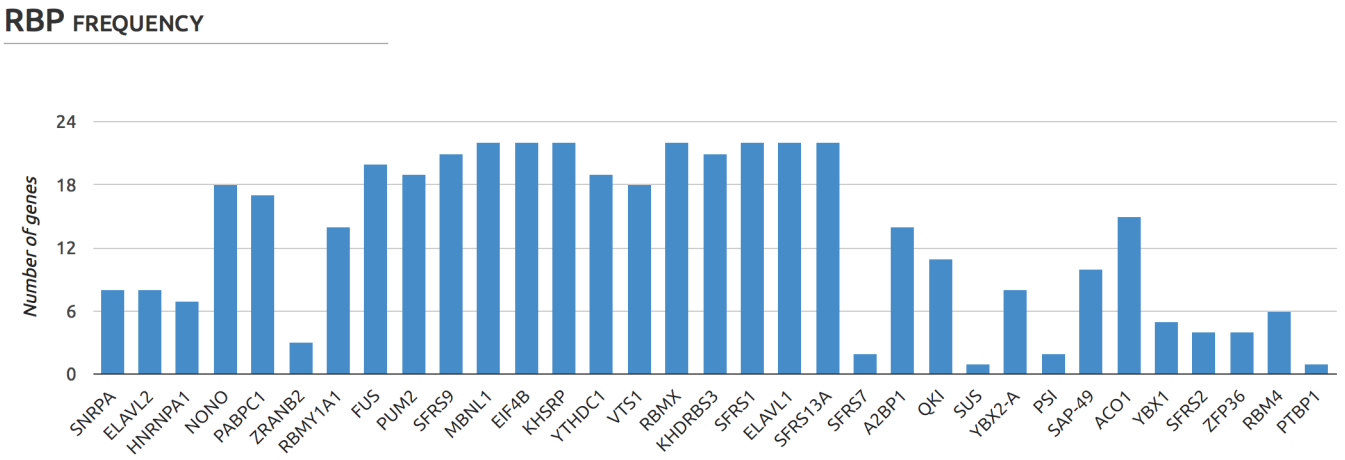
Conclusions

- PBS Finder can reproduce the results an expert would get.
- Months worth of an expert's manual work can be accomplished in a few hours.
- While hardware performance has a significant impact on analysis time, the platform achieves satisfactory performance on personal computer-level hardware.

RBP Analysis Pipeline (PBS Finder) III

Case Studies

Results (viewed in PBS Finder)



Function	Proteins	Species	Gene (view original)	Transcript	Protein
		Rattus norvegicus (Rat)	ENSRNOG00000013536 (Cdc42)	ENSRNOT00000029025 (Cdc42-201)	Q8CFN2 (CDC42)
		Rattus norvegicus (Rat)	ENSRNOG00000013536 (Cdc42)	ENSRNOT00000018118 (Cdc42-202)	Q8CFN2 (CDC42)
		Rattus norvegicus (Rat)	ENSRNOG00000021919 (Rhoj)	ENSRNOT00000031979 (Rhoj-201)	Q5RJS2 (RHOJ)
		Rattus norvegicus (Rat)	ENSRNOG00000015415 (Rhoq)	ENSRNOT00000020822 (Rhoq-201)	Q9JJL4 (RHOQ)
		Rattus norvegicus (Rat)	ENSRNOG00000020393 (Rhog)	ENSRNOT00000027641 (Rhog-201)	Q32PX6 (RHOG)

Objective Fulfilment

Conclusions

- RBP analysis pipeline and web platform (PBS Finder) implemented and tested. PBS Finder has been in production for several months; during this time it was thoroughly tested by IBMC experts.
- RNA-Seq analysis pipeline implemented and tested (iRAP deployed and result consolidation tool implemented).
- Integration of both tools could not be accomplished.

Future Work

Conclusions

- Fully integrate the RNA-Seq analysis pipeline with the web platform (automatic job configuration, result visualization, etc.).

- Study the requirements for deploying the platform in large scale, and assess the feasibility of making it available internet-wide.

Review

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   - RNA-Seq Analysis Pipeline
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4. Conclusions
   - Objective Fulfilment
   - Future Work

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