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.

Tools are complex

Tools for biological data analysis often require a very technical set of skills.

Create simpler tools

Any user should be able to use the tools, with little to no training.

Motivation and Objectives

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Analysing high quantities of data can be repetitive, especially if executed manually.

Automate tasks

Automated systems should perform repetitive tasks, so that users can focus on their work.

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Information is scattered
Information is easy to acquire, but is often scattered through multiple platforms, services and institutions.

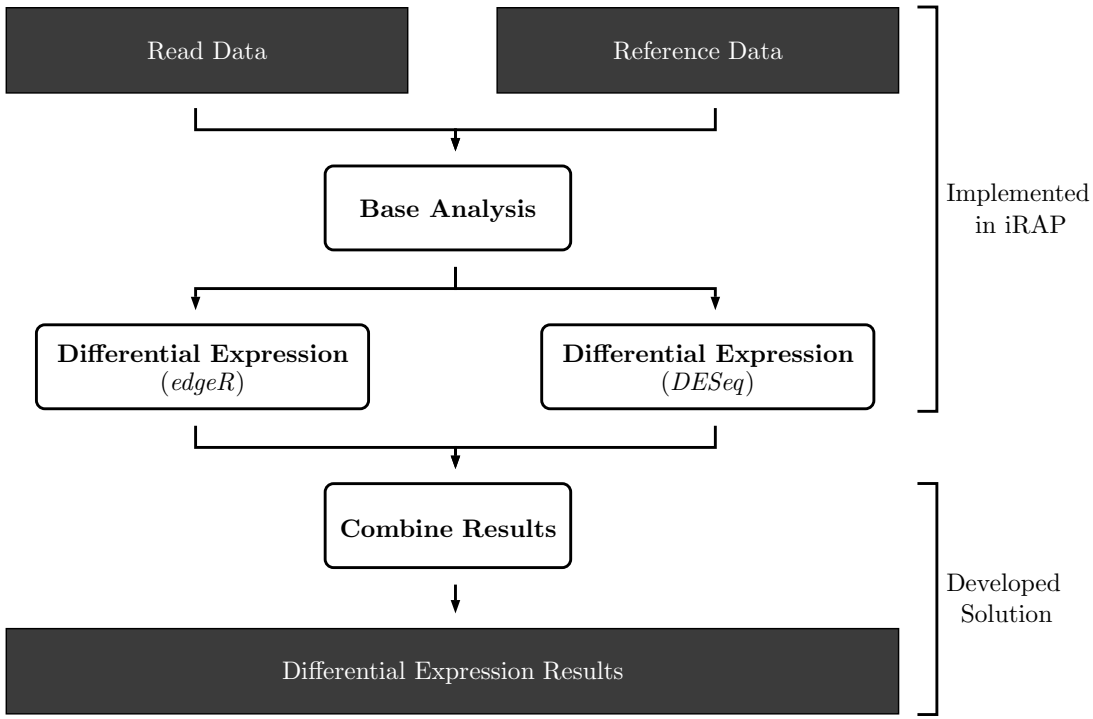
Gather information
Information should be contextually aggregated, allowing for quick access of relevant information.

- 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

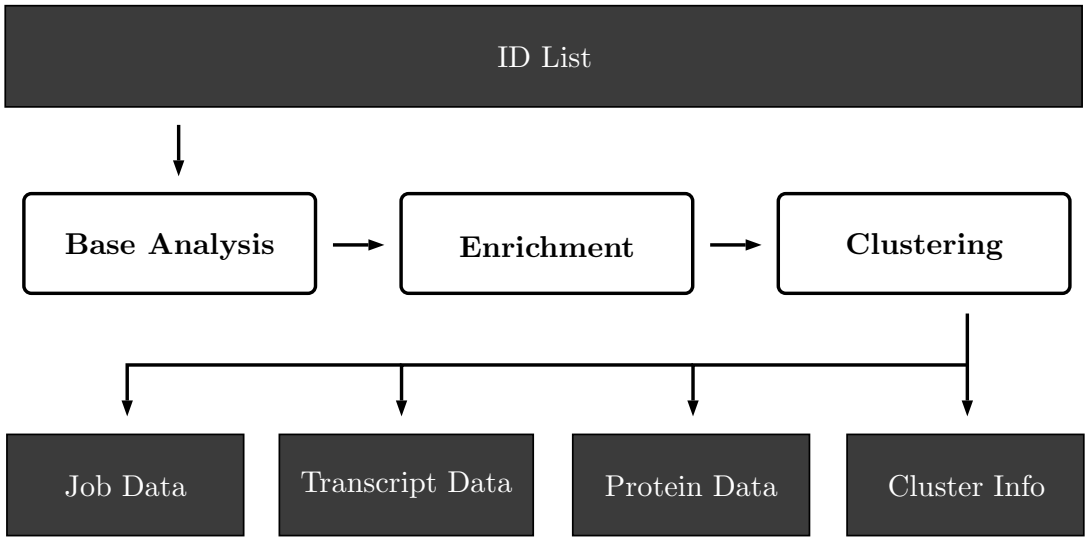
- Uses iRAP as the analysis pipeline.
- Conducts multiple differential expression analyses with different tools.
- Combines results from multiple tools.



RBP Analysis Pipeline (PBS Finder) I

Developed Solution

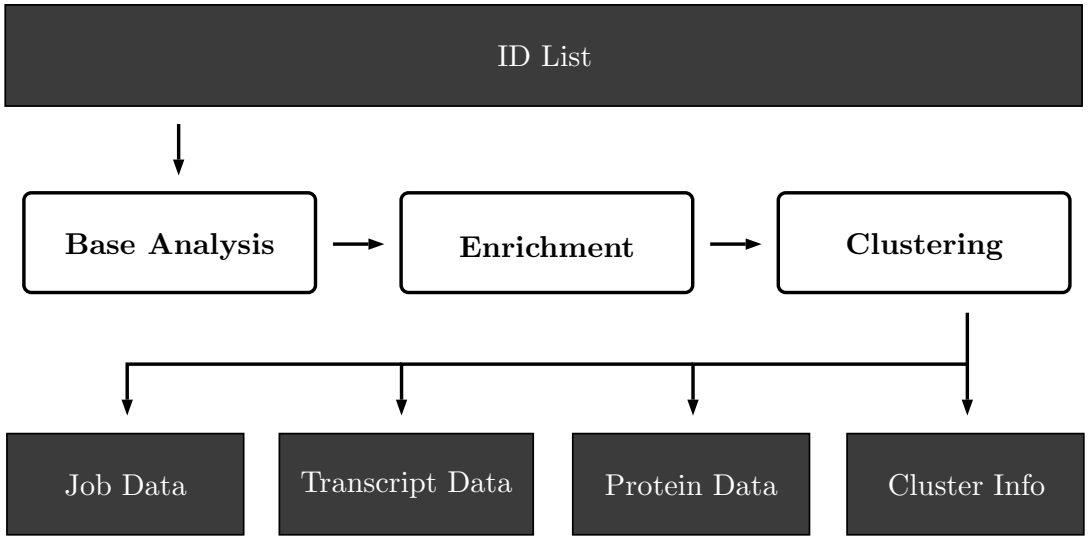
- Uses Ensembl and NCBI to identify gene species, obtain basic information and extract genetic sequences (5' UTR, 3' UTR, 3' UTR downstream).



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

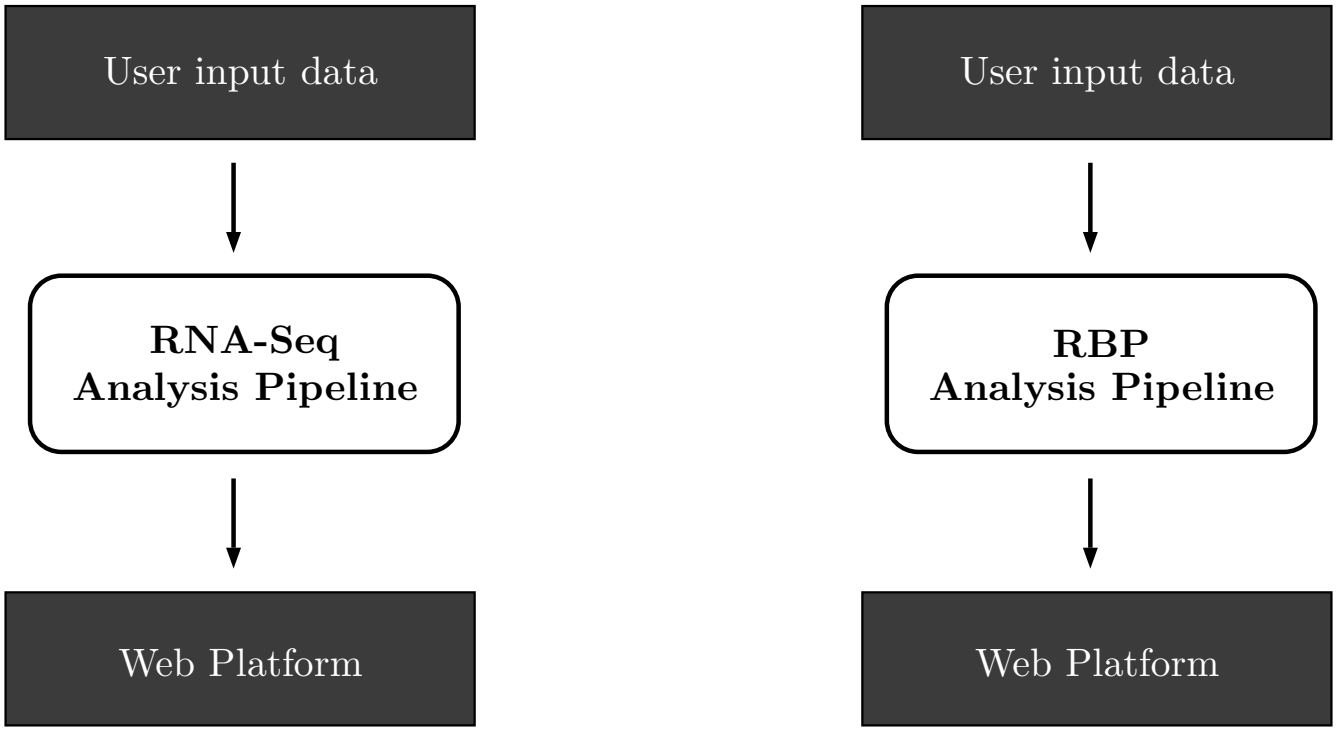
Clustering analysis:

- 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.

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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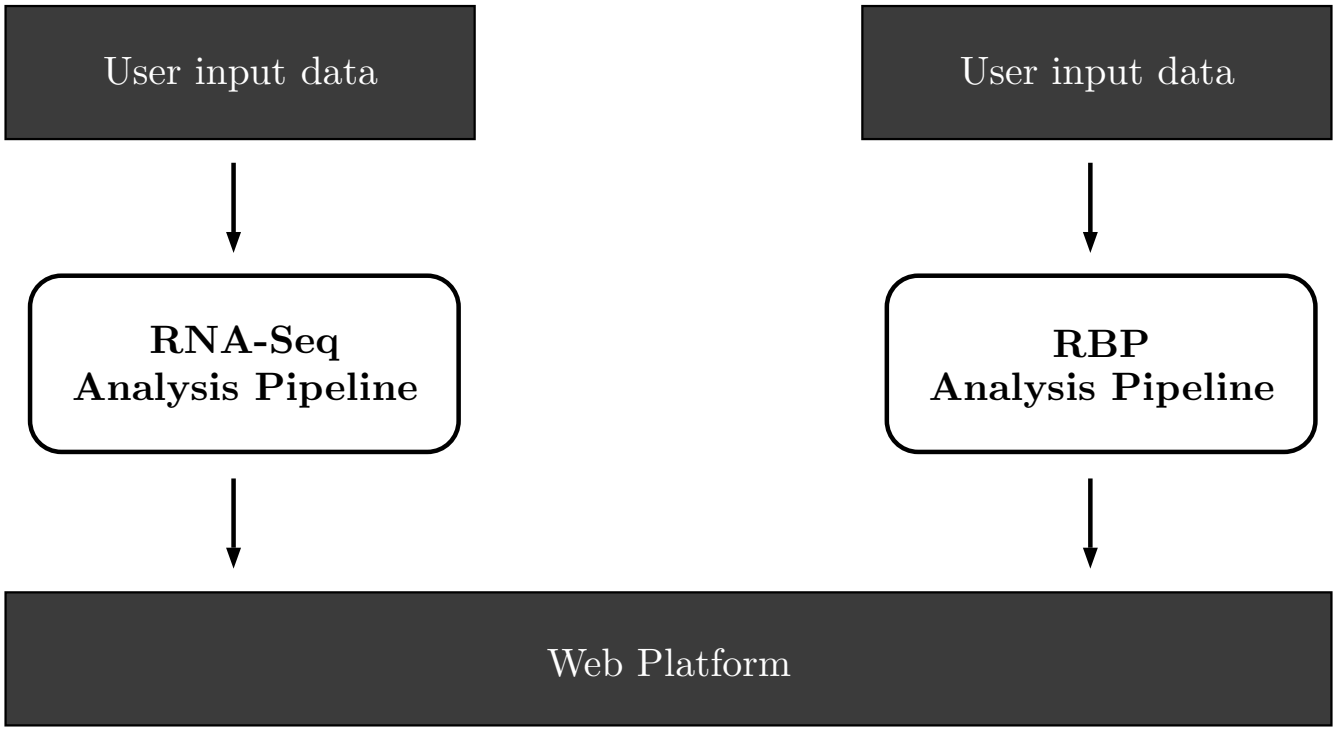
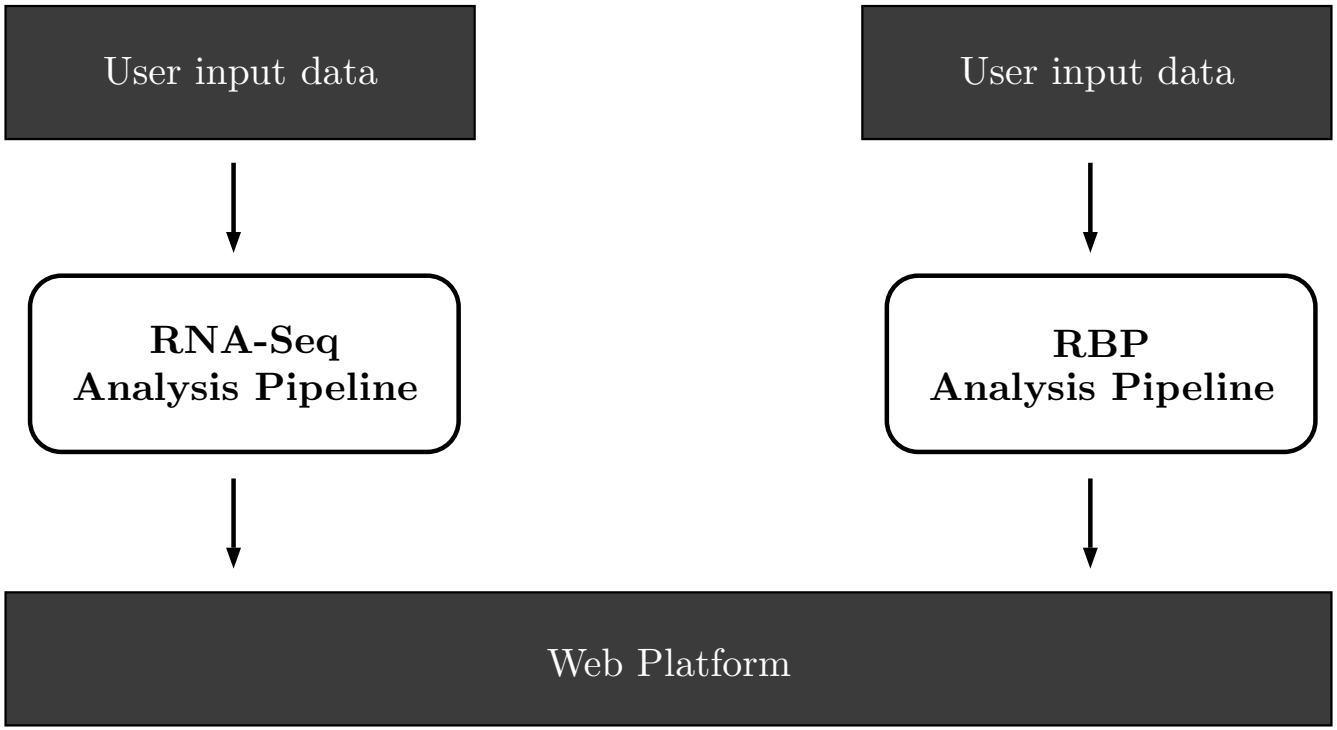


Integration

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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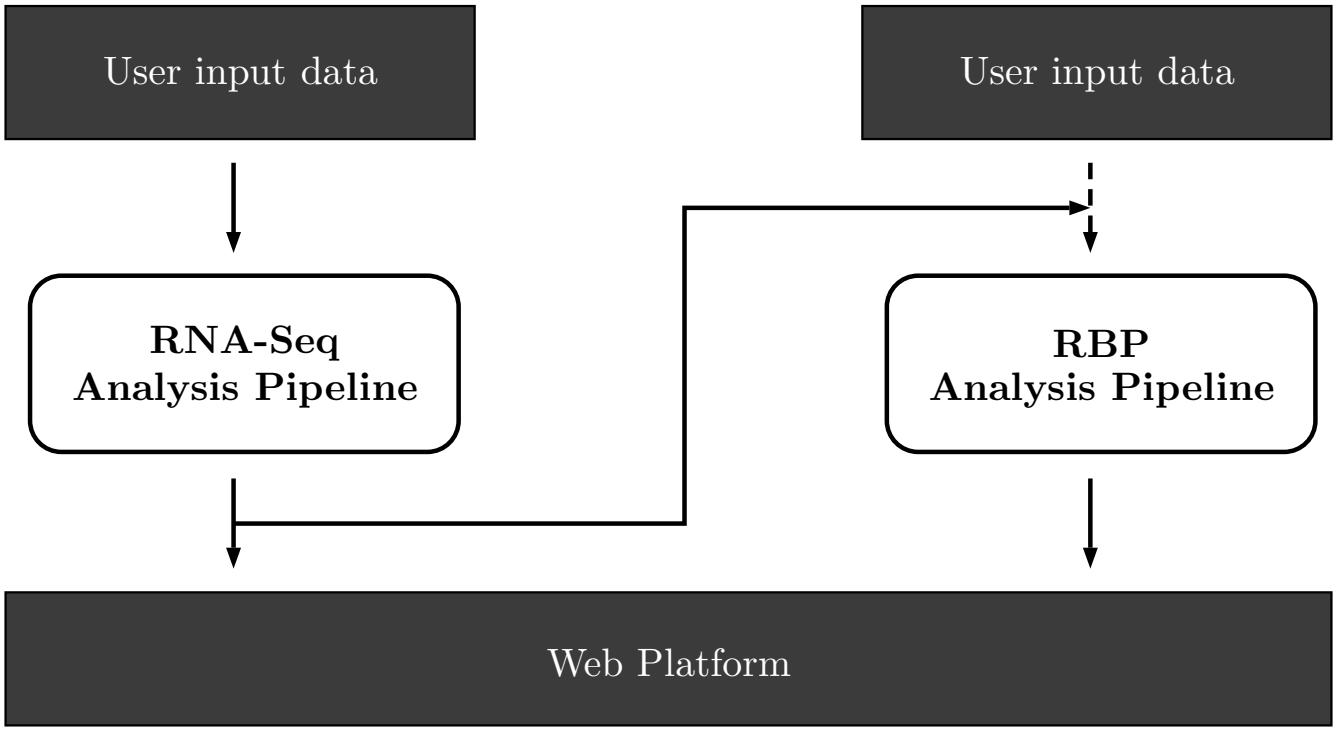
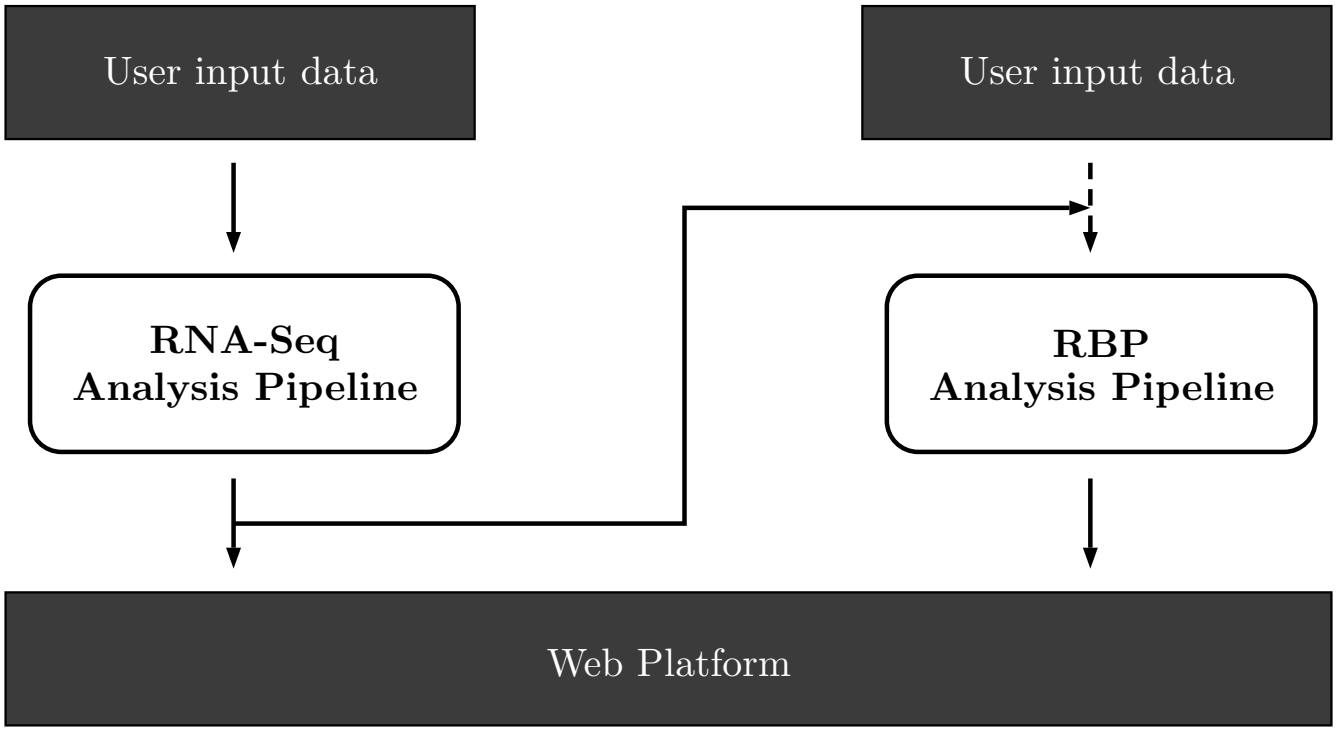


Integration

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	$1h\ 33m\ 32s$	$2h\ 7m\ 4s$	$\approx 450h$

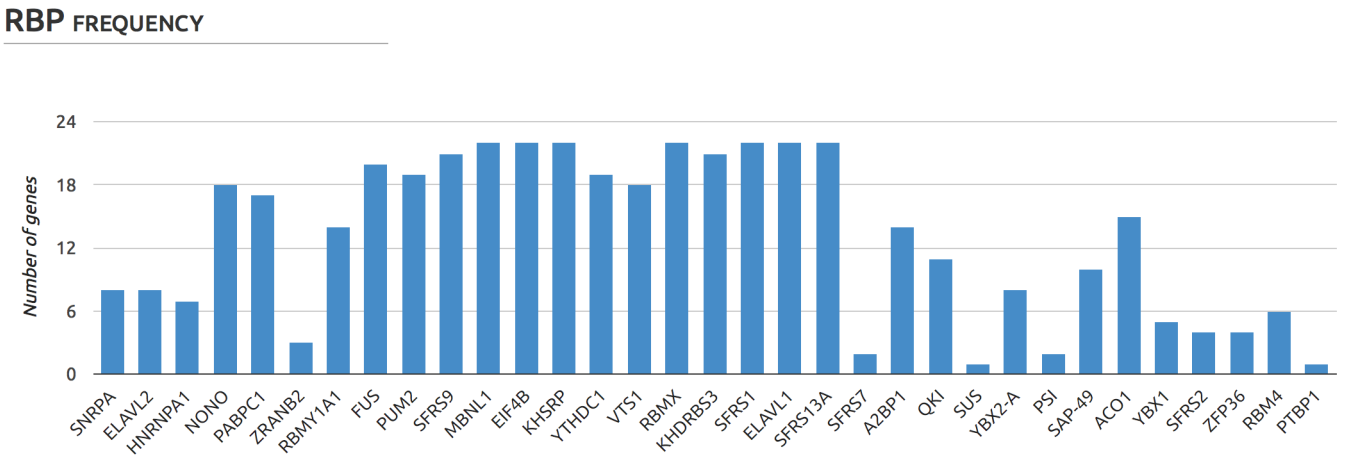
Conclusions

- 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)

SNRPA ELAVL2 HNRNPA1 NONO PABPC1 ZRANB2 RBMY1A1 FUS PUM2 SFRS9 MBNL1 EIF4B

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

1. Introduction
   - Domain Problem
   - Motivation and Objectives
2. Developed Solution
   - Overview
   - RNA-Seq Analysis Pipeline
   - RBP Analysis Pipeline (PBS Finder)
   - Integration
3. Case Studies
   - RNA-Seq Analysis Pipeline
   - RBP Analysis Pipeline (PBS Finder)
4. Conclusions
   - Objective Fulfilment
   - Future Work

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