A Computational Platform for Gene Expression Analysis

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Domain Problem

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.
- Two problems sparked the interest of biologists:
  - understanding differences in gene expression between individuals of the same species;
  - understanding interactions between genes and RNA binding proteins that bind with them.

Domain Problem

Introduction

Studying these problems involves:

- being able to take sequencing reads, align them against a reference genome and perform differential expression analysis;
- being able to take a list of gene identifiers, cross reference them between multiple online platforms and discovering their potential RBPs (as well as additional relevant information);
- being able to uncover implicit relationships in the produced information that might be useful to biologists, using data mining techniques.

Motivation and Objectives

Introduction

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.

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

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.

Overview

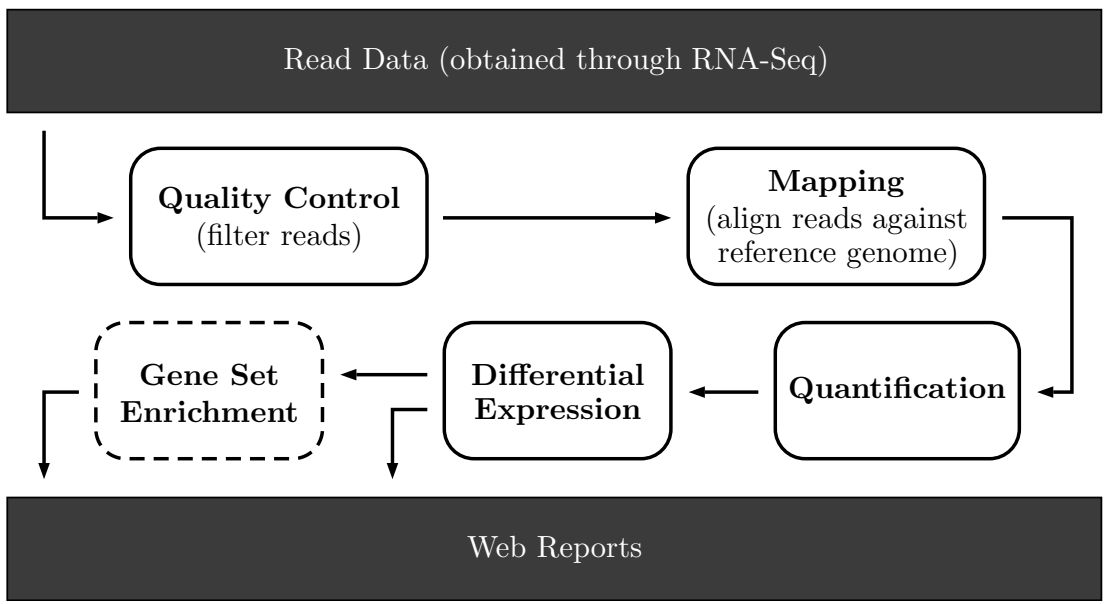
Developed Solution

- Develop two distinct pipelines: one for differential expression analysis and another for RBP discovery and analysis.
- Both pipelines should be available through web platforms. These platforms should be able to manage user accounts, user jobs (analysis tasks), view and export results, etc..
- These platforms should be able to overcome the three problems previously mentioned.

RNA-Seq Analysis Pipeline

Developed Solution

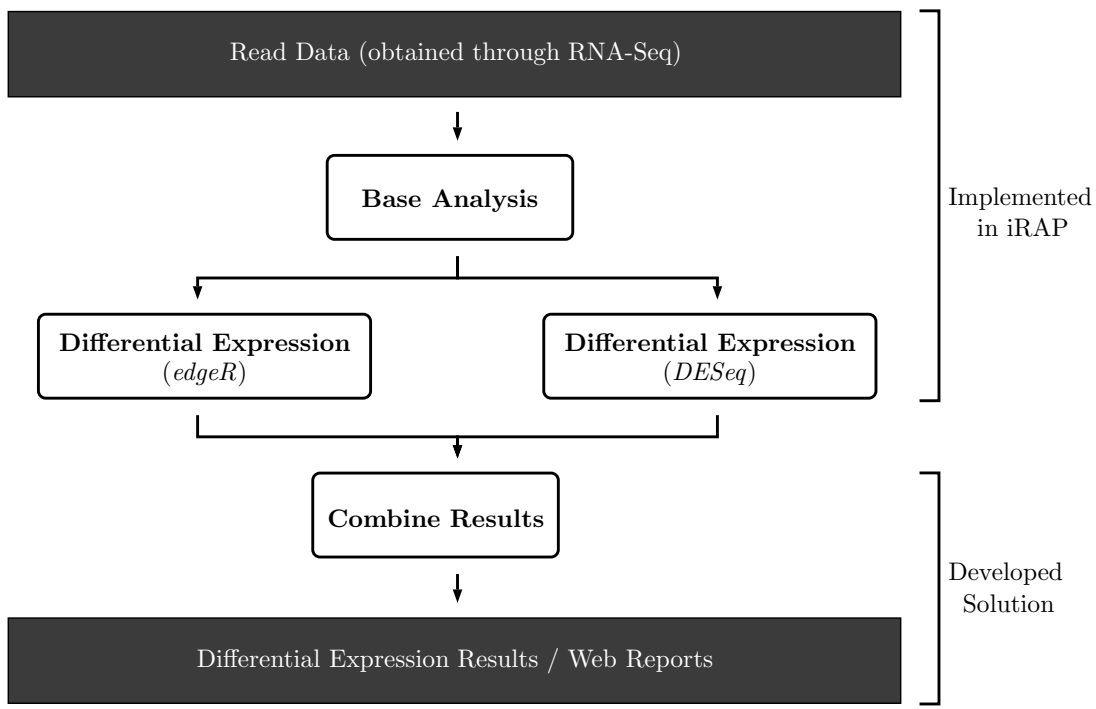
- Uses iRAP (an RNA-Seq analysis pipeline) as its base.
- iRAP is flexible and allows users to choose what tool should be used in each step of the process.
- But, if users have little experience with these tools, how can they choose the best one for the job?



RNA-Seq Analysis Pipeline

Developed Solution

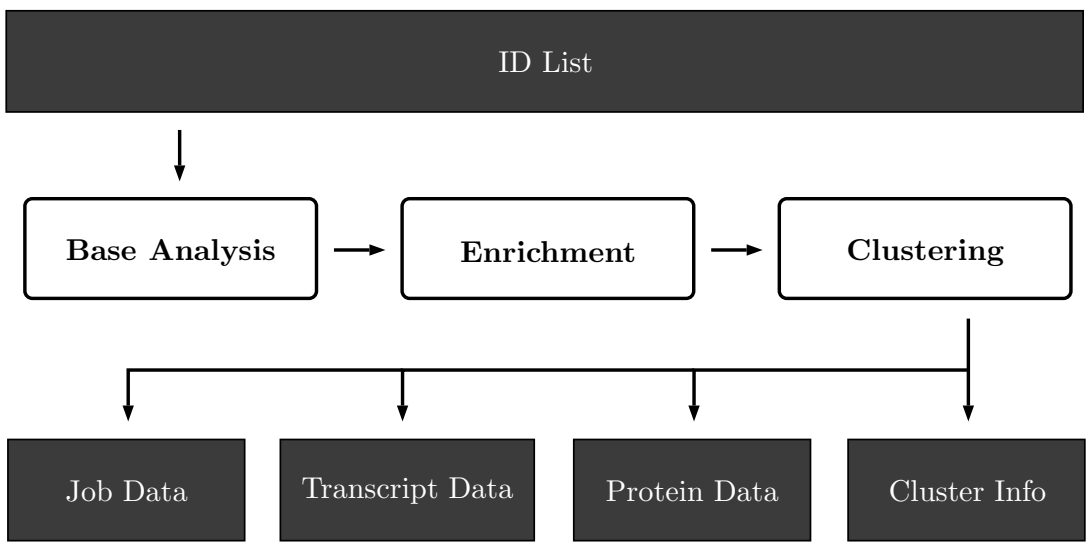
- Uses multiple differential expression analysis tools (edgeR e DESeq).
- Combines the results of those tools.



RBP Analysis Pipeline (PBS Finder)

Developed Solution

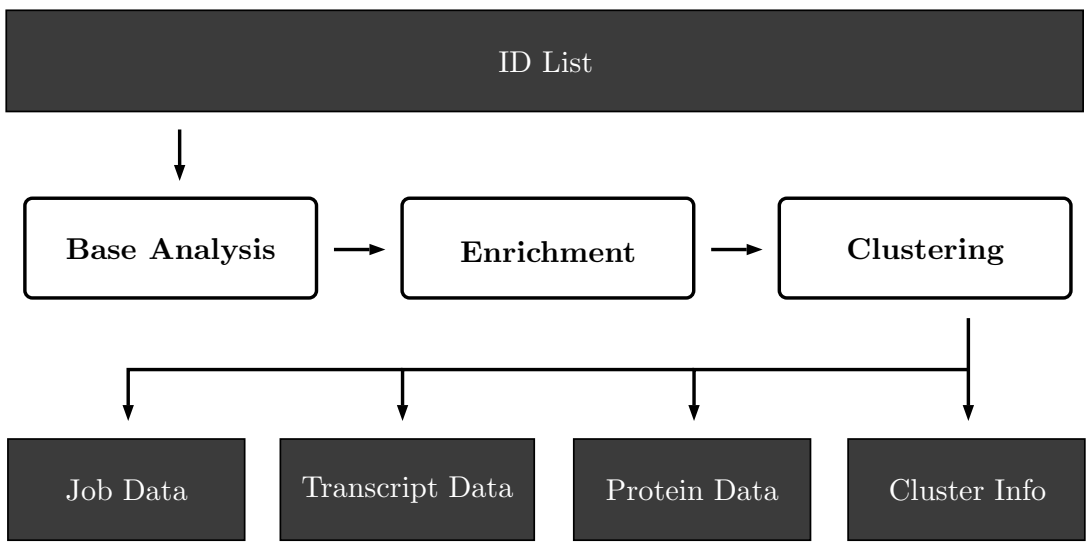
- Uses DB2DB, Ensembl and NCBI to convert identifiers, identify gene species, obtain basic information and extract genetic sequences (5' UTR, 3' UTR, 3' UTR downstream).



RBP Analysis Pipeline (PBS Finder)

Developed Solution

- Uses RBPDB to discover RNA binding proteins (based on the obtained genetic sequences).
- Uses UniProt and KEGG to enrich the obtained results and performs clustering analysis on those results.



RBP Analysis Pipeline (PBS Finder)

Developed Solution

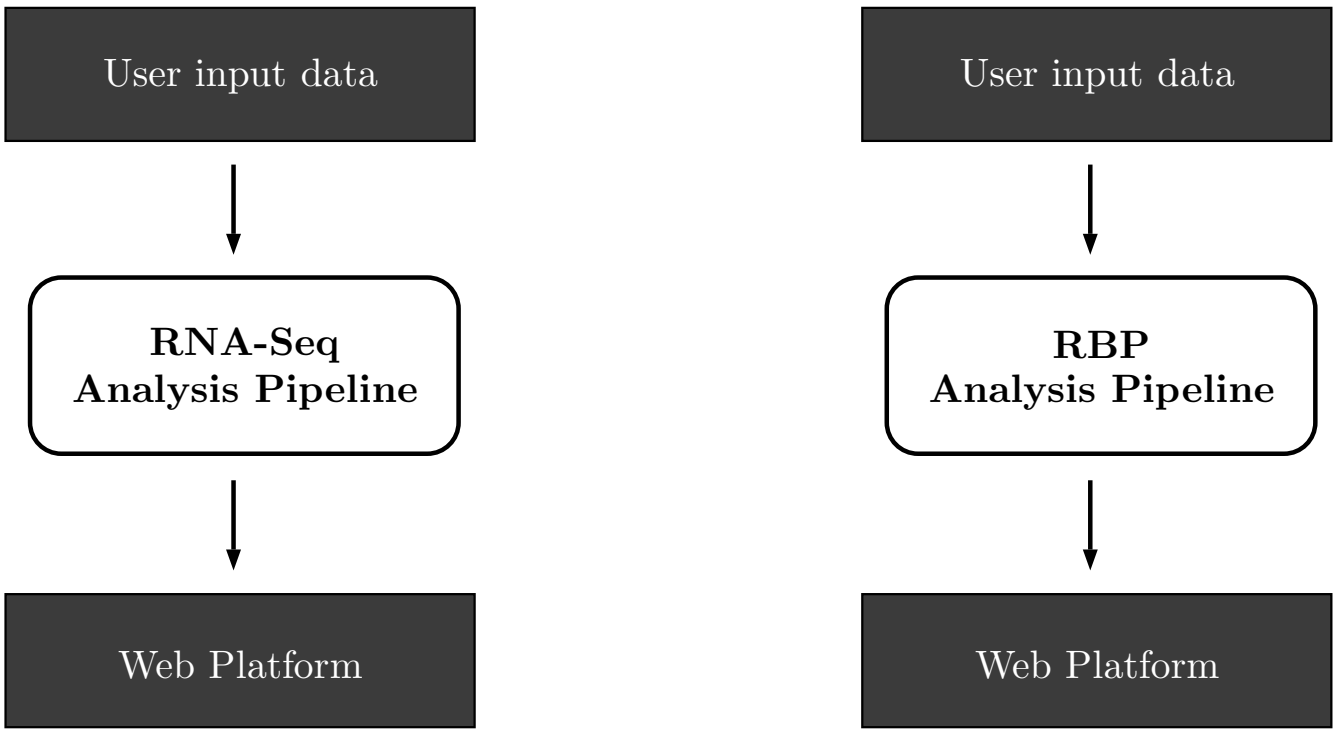
Clustering analysis:

- 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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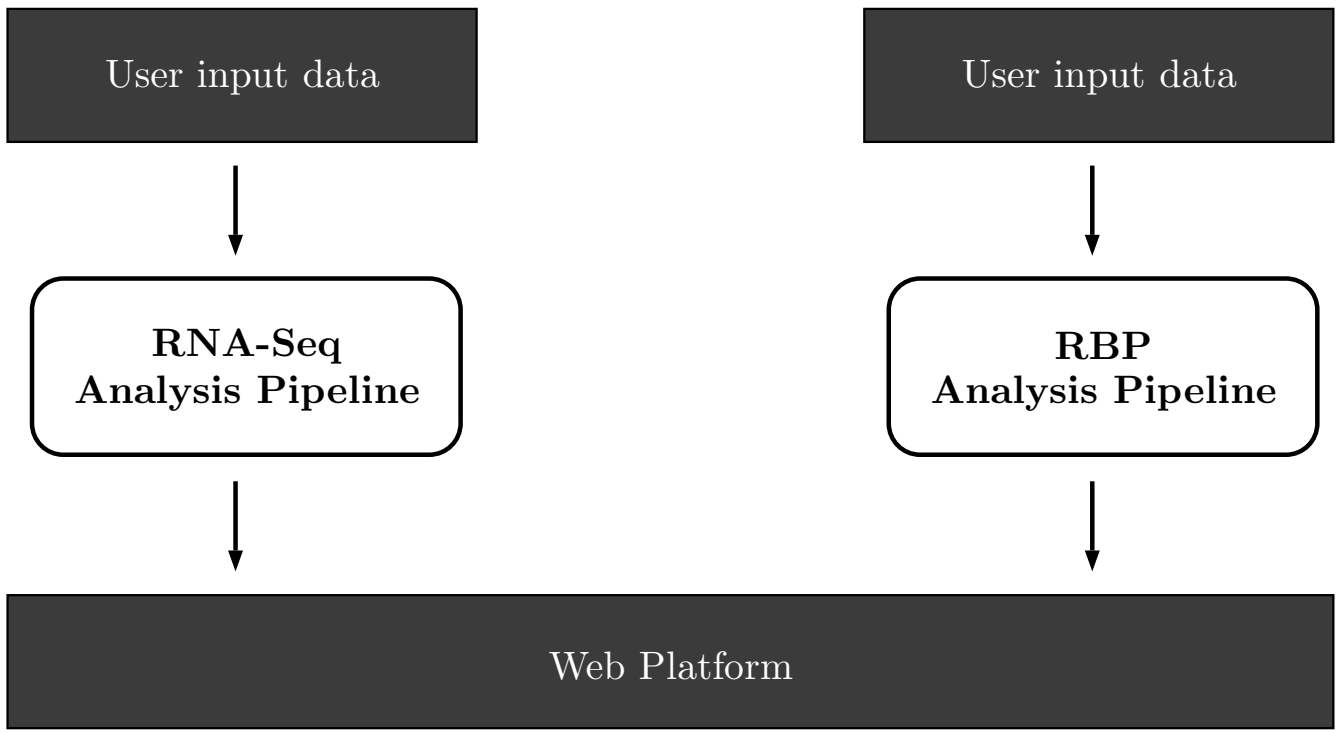
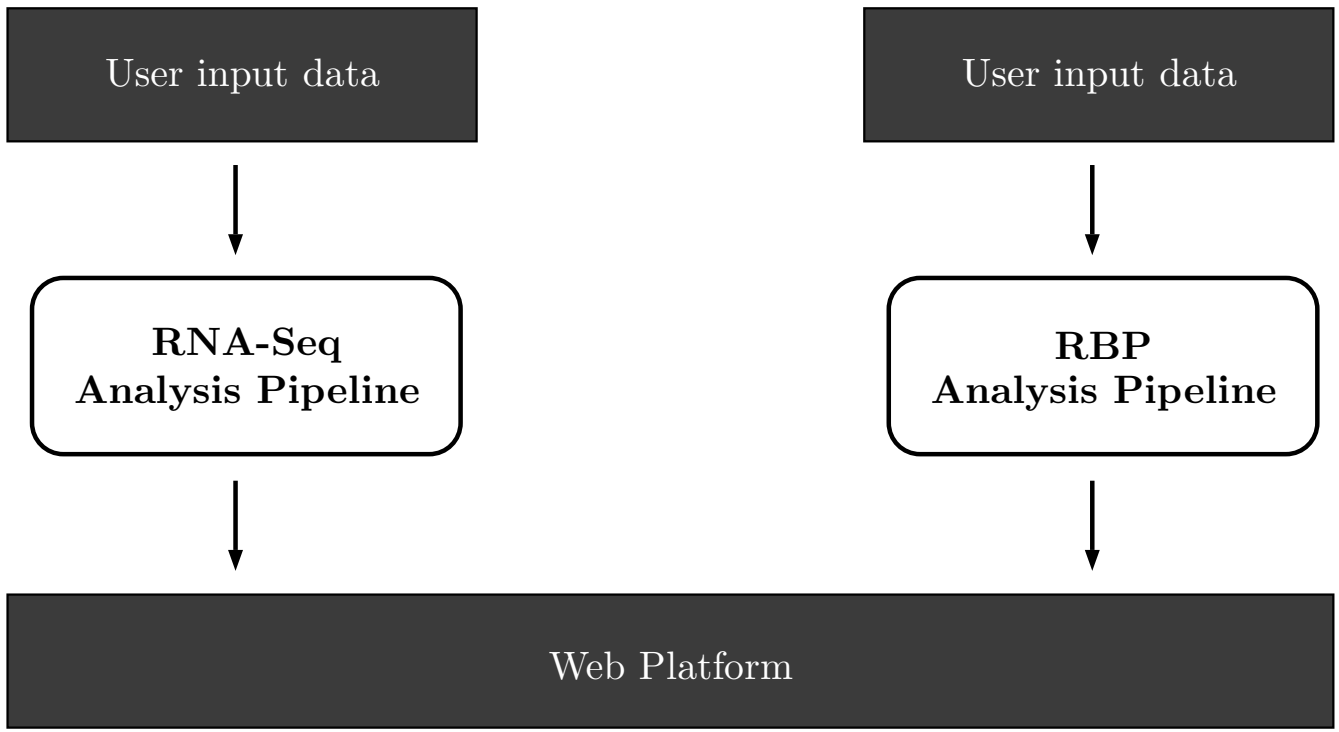


Integration

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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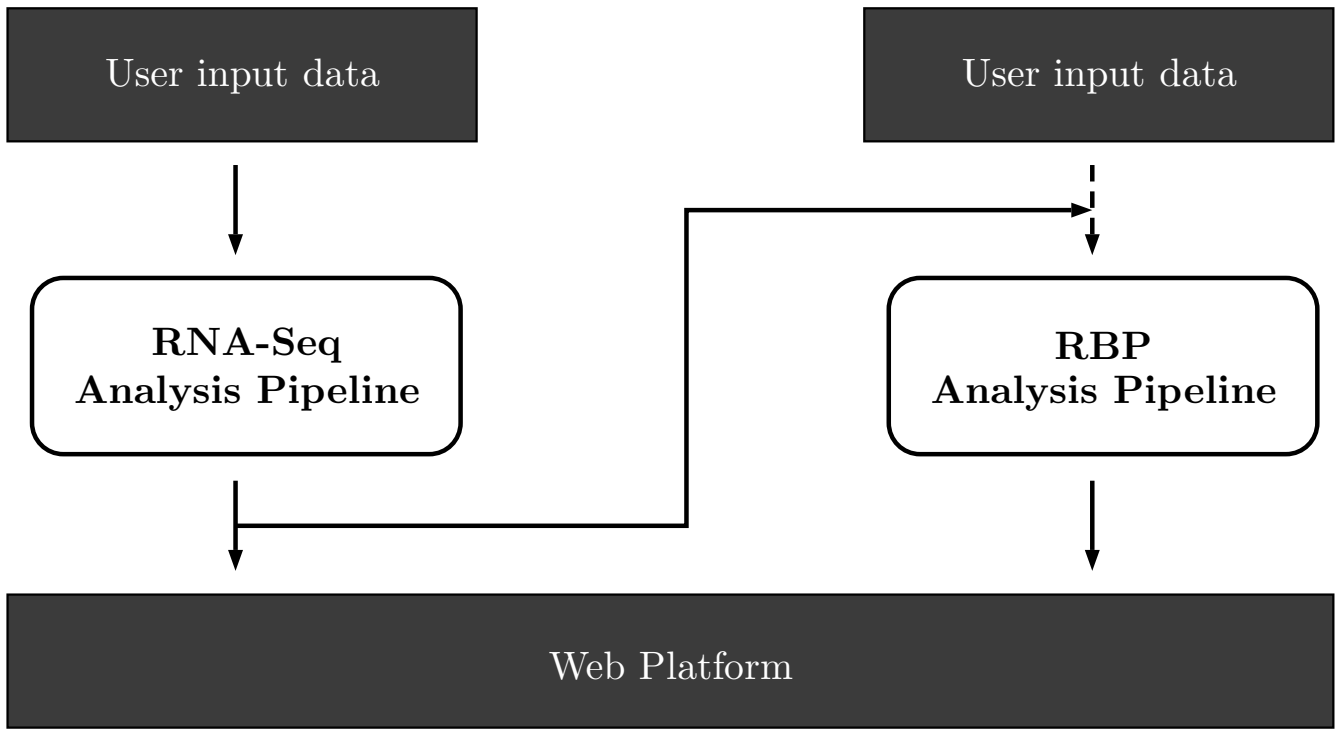
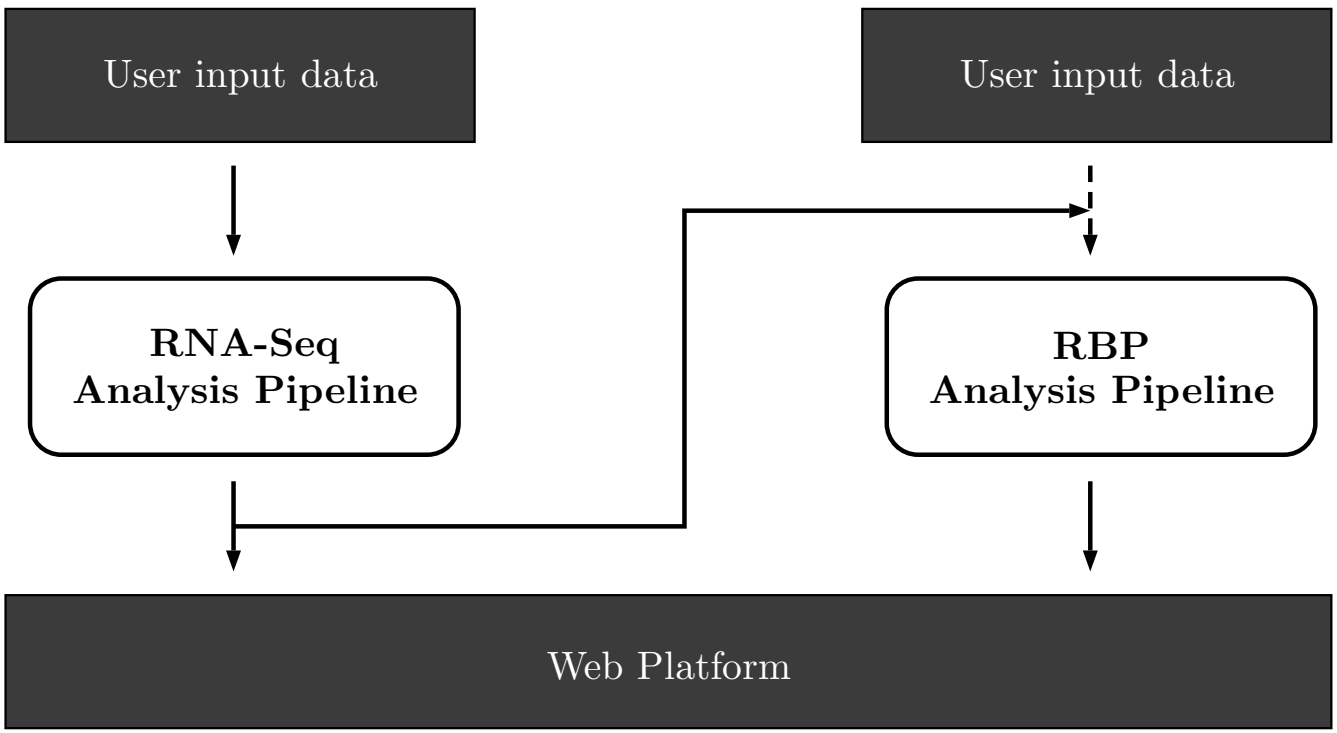


Integration

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.



RNA-Seq Analysis Pipeline

Case Studies

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.

RNA-Seq Analysis Pipeline

Case Studies

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)

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)

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.

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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