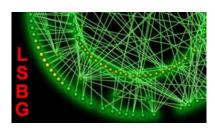


Laboratory of Systems Biology and Genetics

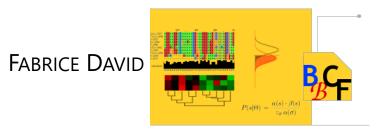
ASAPAutomated Single-cell Analysis Pipeline

WEB-BASED PIPELINE FOR THE ANALYSIS AND INTERACTIVE VISUALIZATION OF SINGLE-CELL RNA-SEQ DATA

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Few words about Deplancke's lab



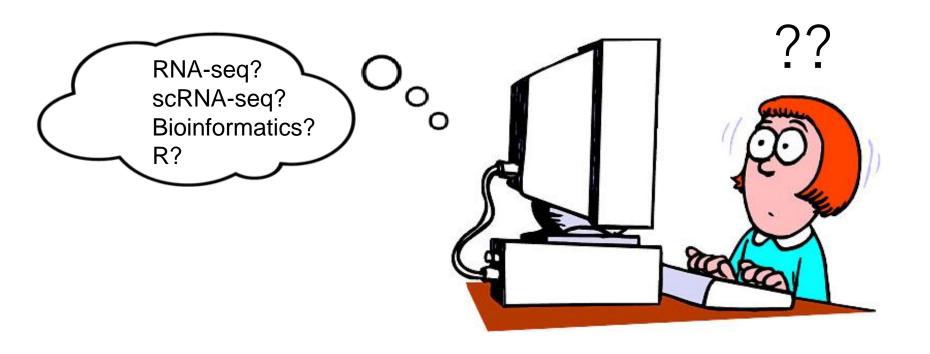
- Located at EPFL (Ecole Polytechnique Fédérale de Lausanne), Lausanne, Switzerland
- 1 Bart, 5 Postdocs, 11 (+ 2 shared) PhD students, 2 Master students, 2 lab tech
- Working in Fly genomics, Aging, Obesity, and Microfluidics tech
- ⇒RNAseq, ATACseq, ChIPseq, WES, Proteomics, ...
- Also, more and more datasets generated from singlecell technologies: Dropseq & Smartseq2
- ⇒And only 2 bioinformaticians!

Community needs: « I want to do scRNA-seq! »





Community needs: « But how do I analyze scRNA-seq data? »



⇒ This is a typical bottleneck, and was routinely experienced in our own lab

scRNA-seq Computational Workflow



Quality control & filtering (I)

Mapping to reference genome

Read/transcript matrix (raw counts/UMI or normalized data)



Quality control & filtering (II)

Normalization, noise removal

Study-specific downstream analyses to generate new biological insight

Cell type identification
Cell type characterization
Gene network analysis
Kinetics of transcription

scRNA-seq Computational Workflow



Quality control & filtering (I)

Mapping to reference genome

Read/transcript matrix (raw counts/UMI or normalized data)



"Black box" fixed pipeline?

scRNA-seq pipeline may not be applicable to all datasets

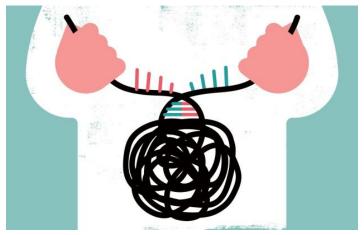


Single-cell sequencing made simple

Data from thousands of single cells can be tricky to analyse, but software advances are making it easier.

Jeffrey M. Perkel

03 July 2017 | Corrected: 05 July 2017, 06 July 2017



"The tools aren't perfect for every situation"

⇒ "A pipeline that excels at identifying cell types, for instance, might stumble with pseudotime analysis"

"Appropriate methods are 'very data-set dependent", says Sandrine Dudoit, (biostatistician at the University of California, Berkeley).

⇒ "The methods and tuning parameters may need to be adjusted to account for variables such as sequencing length"

ASAP: Automated Single-cell Analysis Pipeline

ASAP

Platform

Dimensionality

reduction

Normalization _



Normalization:

- Scaling, Log2, RPKM
- Voom, TMM, DESeq2
- scLVM (can use spike ins)
- Batch effect correction (ComBat)

Filtering

2D and 3D interactive visualization for :

- PCA, tSNE, MDS, ZIFA

Clustering

Differential

expression

Functional

enrichment

- Cell colouring according to expression or clustering
- Manual selection of cells from the plots

Filtering:

- Expression, Coeff. of Var.. CPM
- PAGODA
- SCAN/UPC

Input file:

- scRNA-seg read count data

Input Data

- already normalized matrix

Processing:

- duplicates handling
- ERCC spike-ins detection
- ENSEMBL automatic mapping

Clustering algorithms:

- K-Means, PAM, Hierarchical Clustering, SC3

DE algorithms :

- Marker genes or 2 groups comparison - limma-Voom, edgeR, DESeq2, SCDE

Functional annotation databases :

 Gene atlas, Gene ontology (GO), KEGG Pathways

Technically, what is ASAP?

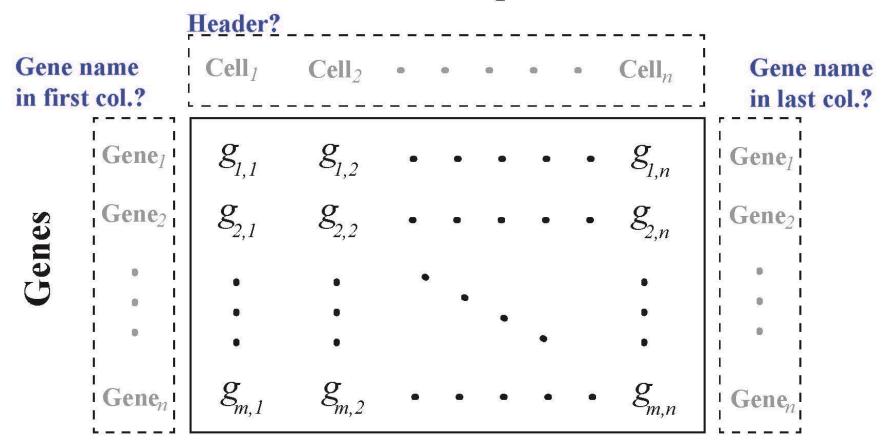
- Centralized computational resources: Ruby-on-rails server currently hosted at the BBCF
- Implementation of the "delayed-jobs" gem that allows job queuing management
- Single-cell analysis scripts are written in R (mostly), Python (dimension reduction) and Java (parsing, functional enrichment)
- ⇒Generates JSON files that are interpreted by the browser
- Interactive and user-friendly web interface with 2D/3D visualization (using plotly JS) [currently moving to plotly webGL]

Dataset formatting



ASAP input file: count matrix or already normalized gene expression matrix

Cells / Samples



Demonstration



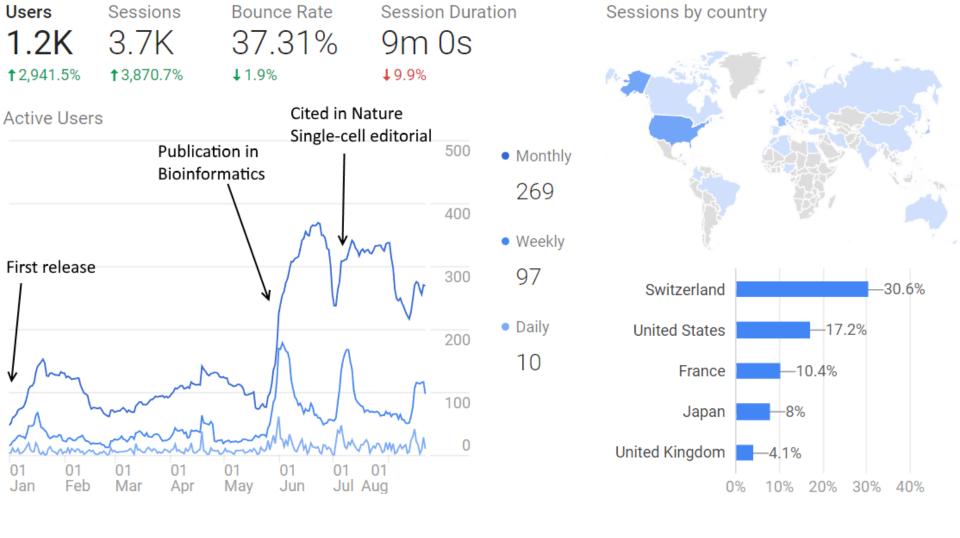
https://asap.epfl.ch

Conclusions



- To open state-of-the-art analysis algorithms to biology laboratories and non-bioinformaticians experts.
- Fast platform for checking and having an overview of everyday data output
- Still in development, many things can be done/added

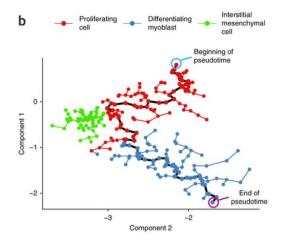
ASAP usage since first release



Future developments



- Improve visualization/responsiveness (front-end developer)
- ⇒ Many plots are in creation on the dev server
- Improve reproducibility (Docker, back-end dev)
- Allow projects to be collaboratively accessed/shared
- Add new tools / algorithms as they are now published (M3DROP, BPSC, ...)
- Mapping dynamic processes / transcriptional trajectory (Monocle, ...)



- Add other databases for functional enrichment (pharmgkb, oncogenes, ...)
- Handle bigger datasets (>50k cells) for e.g. implementing HDF5 matrices