

| Name                              | Implementation   | FASTQ<br>Processing | Gene<br>Filtering | Dimensionality<br>Reduction | Clustering | Interactive<br>Clustering | Differential<br>Expression | Functional<br>Analysis | Cell<br>populations<br>/ pseudotime | Comprehensive<br>Reports | Setting of<br>Parameters | Spatial<br>Plots | PMID                     |
|-----------------------------------|------------------|---------------------|-------------------|-----------------------------|------------|---------------------------|----------------------------|------------------------|-------------------------------------|--------------------------|--------------------------|------------------|--------------------------|
| <a href="#">SC3</a>               | R                | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | No                     | Yes                                 | No                       | Yes                      | No               | <a href="#">28346451</a> |
| <a href="#">ASAP</a>              | Web/Cloud        | No                  | Yes               | Yes                         | Yes        | Yes                       | Yes                        | Yes                    | Yes                                 | No                       | No                       | No               | <a href="#">32449934</a> |
| <a href="#">Granatum</a>          | Shiny web server | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | Yes                    | Yes                                 | No                       | No                       | No               | <a href="#">29202807</a> |
| <a href="#">SEURAT</a>            | R                | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | No                     | Yes                                 | No                       | Yes                      | Yes              | <a href="#">31178118</a> |
| <a href="#">MOANA</a>             | Python           | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | No                     | Yes                                 | No                       | No                       | No               | NA                       |
| <a href="#">STREAM</a>            | Web/Cloud        | No                  | Yes               | Yes                         | Yes        | No                        | No                         | No                     | No                                  | No                       | No                       | No               | <a href="#">31015418</a> |
| <a href="#">SINCERA</a>           | R                | No                  | Yes               | No                          | Yes        | No                        | Yes                        | Yes                    | Yes                                 | No                       | Yes                      | No               | <a href="#">26600239</a> |
| <a href="#">CIDR</a>              | R                | No                  | Yes               | Yes                         | Yes        | No                        | No                         | No                     | No                                  | No                       | Yes                      | No               | <a href="#">28351406</a> |
| <a href="#">iCellR</a>            | R                | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | Yes                    | Yes                                 | No                       | Yes                      | Yes              | NA                       |
| <a href="#">IRIS - EDA</a>        | Shiny web server | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | Yes                    | No                                  | No                       | No                       | No               | <a href="#">30763315</a> |
| <a href="#">Biocond. workflow</a> | R                | No                  | Yes               | Yes                         | Yes        | No                        | Yes                        | No                     | No                                  | No                       | Yes                      | No               | <a href="#">28868140</a> |
| <a href="#">FASTGenomics</a>      | Web/Cloud        | Yes                 | Yes               | Yes                         | Yes        | No                        | Yes                        | No                     | No                                  | No                       | No                       | No               | NA                       |
| <a href="#">SingleCAnalyzer</a>   | Web/Cloud        | Yes                 | Yes               | Yes                         | Yes        | Yes                       | Yes                        | Yes                    | Yes                                 | Yes                      | No                       | No               | NA                       |

**Supplementary table S1.** Comparison of pipelines, web servers and cloud platforms for the analysis of scRNA-Seq data.

| Project Identifier | Organism     | N° Samples | N° Fragments | Bases x Frag. | Uploading   | Quantification | Counts      | Clustering  | DE          | GSEA        | GOSEQ       | Total       |
|--------------------|--------------|------------|--------------|---------------|-------------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| PRJNA229411        | Mus musculus | 201        | 484268670    | 2x101         | 00h 24m 19s | 00h 26m 41s    | 00h 00m 06s | 00h 00m 12s | 00h 06m 00s | 00h 00m 38s | 00h 00m 24s | 00h 33m 37s |
| PRJNA481319        | Homo sapiens | 135        | 3193027317   | 66            | 00h 39m 39s | 00h 43m 37s    | 00h 00m 06s | 00h 00m 05s | 00h 01m 32s | 00h 00m 47s | 00h 00m 27s | 00h 46m 07s |
| PRJNA314949        | Homo sapiens | 384        | 837257090    | 2x125         | 00h 37m 31s | 01h 27m 34s    | 00h 00m 11s | 00h 00m 25s | 00h 28m 17s | 00h 00m 38s | 00h 00m 34s | 01h 57m 05s |
| PRJNA296119        | Homo sapiens | 121        | 502602411    | 2x100         | 00h 20m 22s | 00h 26m 13s    | 00h 00m 06s | 00h 00m 07s | 00h 01m 44s | 00h 00m 30s | 00h 00m 17s | 00h 28m 40s |
| PRJNA286698        | Mus musculus | 148        | 382295820    | 90            | 00h 04m 56s | 00h 10m 15s    | 00h 00m 05s | 00h 00m 07s | 00h 05m 06s | 00h 00m 35s | 00h 00m 15s | 00h 16m 08s |
| PRJNA573109        | Homo sapiens | 14         | 1176159212   | 58            | 00h 09m 23s | 00h 11m 54s    | 00h 00m 02s | 00h 00m 03s | 00h 00m 21s | 00h 00m 46s | 00h 00m 39s | 00h 13m 06s |
| PRJNA503340        | Homo sapiens | 72         | 543308382    | 50            | 00h 06m 36s | 00h 08m 02s    | 00h 00m 04s | 00h 00m 02s | 00h 00m 28s | 00h 00m 34s | 00h 00m 25s | 00h 09m 10s |
| PRJNA558893        | Homo sapiens | 66         | 1825947716   | 30+70         | 00h 27m 35s | 00h 38m 07s    | 00h 00m 05s | 00h 00m 03s | 00h 00m 49s | 00h 00m 40s | 00h 00m 30s | 00h 39m 44s |
| PRJNA393431        | Danio rerio  | 246        | 272727507    | 2x38          | 00h 02m 42s | 00h 08m 43s    | 00h 00m 06s | 00h 00m 10s | 00h 12m 04s | 00h 00m 32s | 00h 00m 16s | 00h 21m 35s |

**Supplementary Table S2.** Execution time of each process in the pipeline of SingleCAnalyzer. This test was executed on an Intel Xeon Gold 6146 processor (3.2 Ghz) with 256GB of RAM.