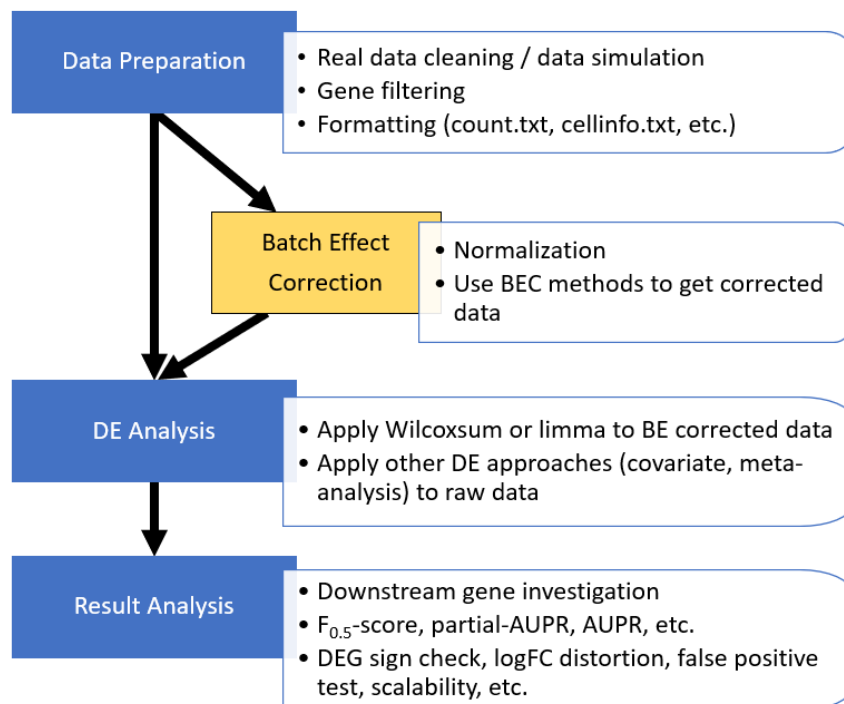
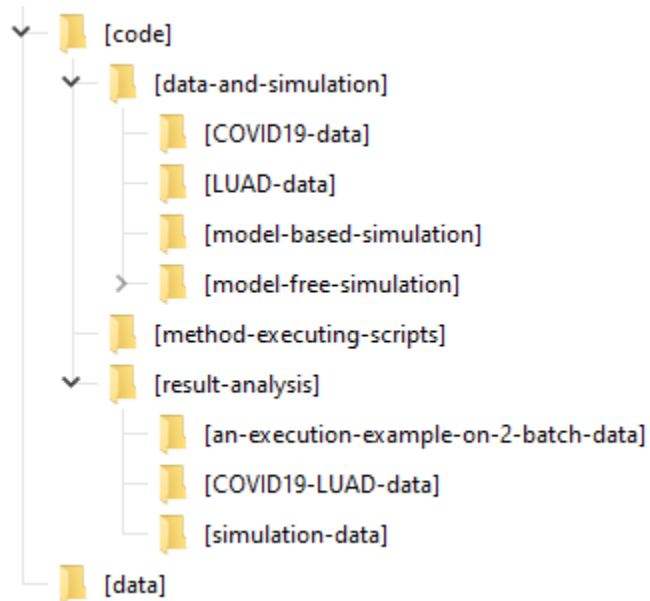


# Benchmarking integration of single-cell differential expression

## How-to-use-the-code



## 1. GitHub folder structure



- **'code'** contains the core analysis Python & R scripts for this study
  - **'data-and-simulation'** contains sample data and scripts for data preparation step
    - **'COVID19-data'** gives scripts for data preparation using COVID-19 data
    - **'LUAD-data'** gives scripts for data preparation using LUAD data
    - **'model-based-simulation'** gives scripts for simulating data using MCA and Pancreas data
    - **'model-free-simulation'** gives scripts for simulating data using Splatter
  - **'method-executing-scripts'** contains implementation for each considered method
  - **'result-analysis'** contains **<ANA-function>** scripts for analyzing
- **'data'** contains figures and tables of the experimental results for illustration

## 2. Analysis code input-output

- R BEC-limma
- R BEC-pseudobulk\_edger
- R BEC-seurat3
- R COV-deseq2
- R COV-edger
- R COV-edger\_DetRate
- R COV-limma\_trend
- R COV-limma\_trend\_Combat\_false
- R COV-limma\_trend\_False
- R COV-limma\_trend\_mnnCorrect
- R COV-limma\_trend\_scMerge
- R COV-limma\_voom
- R COV-mast
- R COV-zinbwave\_deseq2
- R DE-DEGs\_from\_Seurat\_auc
- R DE-Seurat\_DEG\_analysis\_auc

- ‘<method-category>\_<method-name>’: script to specifically test a particular method

○ < method-category >: includes ‘BEC’, ‘COV’, ‘META’, ‘DE’, ‘ANA’ indicating the characteristic of a method.

Note that all results of ‘BEC’, ‘COV’, ‘META’, ‘DE’ functions are **required** before running ‘ANA’ functions or else **code modification** is essential.

○ < method-name >: indicate the specific method

- input:
  - a count matrix (genes  $\times$  cells)

| ^             | X1_A | X1_A.1 | X1_A.2 | X1_A.3 | X1_A.4 | X1_A.5 | X1_A.6 | X1_A.7 | X1_A.8 | X1_A.9 | X1_A.10 |
|---------------|------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|
| 0610007P14Rik | 0    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0       |
| 0610012G03Rik | 0    | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |
| 1110004E09Rik | 0    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 2      | 0      | 0       |
| 1110004F10Rik | 0    | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0       |
| 1110008F13Rik | 0    | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 2      | 0      | 0       |
| 1110008P14Rik | 0    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1       |
| 1110038B12Rik | 0    | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0       |
| 1110038F14Rik | 0    | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |
| 1110059E24Rik | 0    | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0       |
| 1110059G10Rik | 0    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1       |
| 1300002E11Rik | 0    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |
| 1600020E01Rik | 0    | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0       |
| 1700037H04Rik | 1    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |
| 1700097N02Rik | 0    | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0       |

- a data frame of cell descriptions (group, batch, ... information)

|    | Group | Batch |
|----|-------|-------|
| 1  | A     | 1     |
| 2  | A     | 1     |
| 3  | A     | 1     |
| 4  | A     | 1     |
| 5  | A     | 1     |
| 6  | A     | 1     |
| 7  | A     | 1     |
| 8  | A     | 1     |
| 9  | A     | 1     |
| 10 | A     | 1     |

- a data frame of gene descriptions (id, name, code, ...information)

|    | x             |
|----|---------------|
| 1  | 0610007P14Rik |
| 2  | 0610012G03Rik |
| 3  | 1110004E09Rik |
| 4  | 1110004F10Rik |
| 5  | 1110008F13Rik |
| 6  | 1110008P14Rik |
| 7  | 1110038B12Rik |
| 8  | 1110038F14Rik |
| 9  | 1110059E24Rik |
| 10 | 1110059G10Rik |

○ **output:**

- batch effect correction methods: a matrix of corrected values (genes  $\times$  cells)

|               | X1_A       | X1_A.1     | X1_A.2     | X1_A.3     | X1_A.4     | X1_A.5     | X1_A.6     | X1_A.7     |
|---------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 0610007P14Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 0610012G03Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.03600818 | 0.00000000 |
| 1110004E09Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110004F10Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.04656252 | 0.00000000 | 0.04371717 | 0.03600818 | 0.00000000 |
| 1110008F13Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110008P14Rik | 0.00000000 | 0.00000000 | 0.04795441 | 0.04656252 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110038B12Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110038F14Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110059E24Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1110059G10Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 |
| 1300002E11Rik | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.00000000 | 0.04371717 | 0.00000000 | 0.00000000 |

- Wilcoxon rank sum test: a data frame of gene ranking analysis

|    | X      | p_val        | avg_log2FC | pct.1 | pct.2 | p_val_adj    |
|----|--------|--------------|------------|-------|-------|--------------|
| 1  | Rpl41  | 3.361860e-32 | 0.8112850  | 0.989 | 0.934 | 1.028393e-28 |
| 2  | Gzma   | 7.953368e-01 | 0.7430536  | 0.093 | 0.079 | 1.000000e+00 |
| 3  | H3f3b  | 1.874244e-14 | 0.7032320  | 0.956 | 0.968 | 5.733313e-11 |
| 4  | Rpl4   | 1.286567e-18 | 0.6976022  | 0.995 | 0.984 | 3.935608e-15 |
| 5  | Rps29  | 4.303247e-23 | 0.6938563  | 0.989 | 0.950 | 1.316363e-19 |
| 6  | Rpl35a | 4.384710e-18 | 0.6661287  | 0.913 | 0.807 | 1.341283e-14 |
| 7  | Rps15  | 6.610199e-16 | 0.6603338  | 0.934 | 0.882 | 2.022060e-12 |
| 8  | Rpl27a | 2.439341e-23 | 0.6384096  | 0.973 | 0.825 | 7.461943e-20 |
| 9  | Rpl10a | 1.966441e-16 | 0.6215998  | 0.896 | 0.744 | 6.015343e-13 |
| 10 | Nkg7   | 4.653614e-02 | 0.6019261  | 0.765 | 0.794 | 1.000000e+00 |

- parametric and integration methods: a data frame of gene ranking analysis

|               | pvalue       | adjpvalue    | logFC        |
|---------------|--------------|--------------|--------------|
| 0610007P14Rik | 2.152735e-03 | 2.374718e-02 | 1.223651041  |
| 0610012G03Rik | 4.866872e-01 | 8.331147e-01 | -0.248451779 |
| 1110004E09Rik | 8.736544e-01 | 9.750514e-01 | -0.060952425 |
| 1110004F10Rik | 6.314026e-01 | 8.961051e-01 | 0.166795670  |
| 1110008F13Rik | 5.896157e-01 | 8.806809e-01 | 0.185851914  |
| 1110008P14Rik | 1.379971e-01 | 4.504801e-01 | 0.575286590  |
| 1110038B12Rik | 5.304236e-04 | 7.953754e-03 | 1.536010377  |
| 1110038F14Rik | 4.149591e-02 | 2.073660e-01 | -0.725644048 |
| 1110059E24Rik | 2.805607e-01 | 6.566887e-01 | -0.412511845 |
| 1110059G10Rik | 4.577728e-02 | 2.218105e-01 | -0.708648964 |

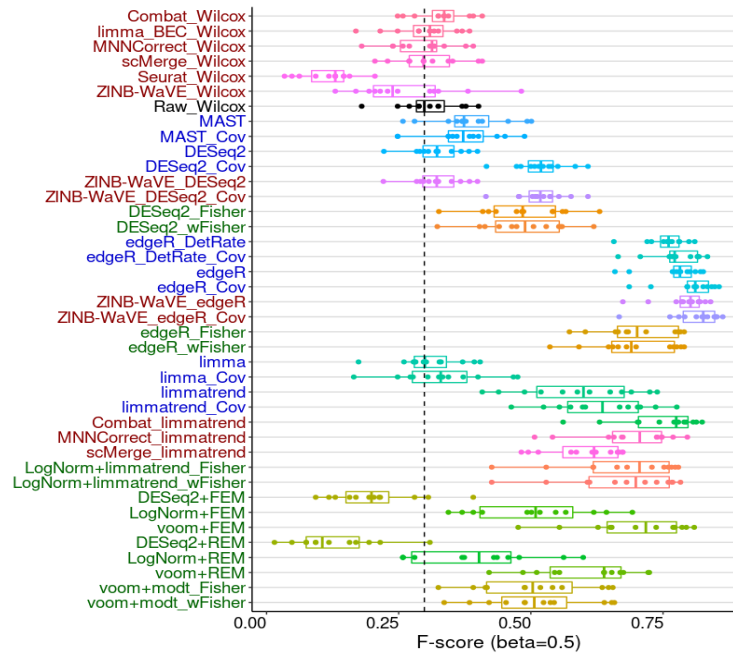
- meta-analysis methods: a data frame of gene ranking analysis

| Name                   | Type                     | Value   |
|------------------------|--------------------------|---|
| MetaDE.Res\$`voom+FEM` | list [5] (S3: MetaDE.ES) | List of length 5                                    |
| mu.hat                 | double [2609]            | 0.1070 -0.0710 -0.1316 -0.1213 0.0643 0.0697 ...    |
| mu.var                 | double [2609]            | 0.00920 0.00920 0.00919 0.00919 0.00918 0.00919 ... |
| zval                   | double [2609]            | 1.116 -0.741 -1.373 -1.266 0.671 0.727 ...          |
| pval                   | double [2609]            | 0.8677 0.2294 0.0849 0.1028 0.7489 0.7664 ...       |
| FDR                    | double [2609 x 1]        | 0.939 0.592 0.416 0.433 0.881 0.893 ...             |

### 3. Visualization

#### ○ ‘GBC-meta’:

- Aggerate all output results and visualize F-beta performance



#### ○ ‘GBC-meta\_PR’:

- Aggerate all output results and illustrate the AUPR curve

