RNA-Seq Normalization in Babelomics 5

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GDA

International Course on
Genomic Data Analysis

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Outline

1 Introduction

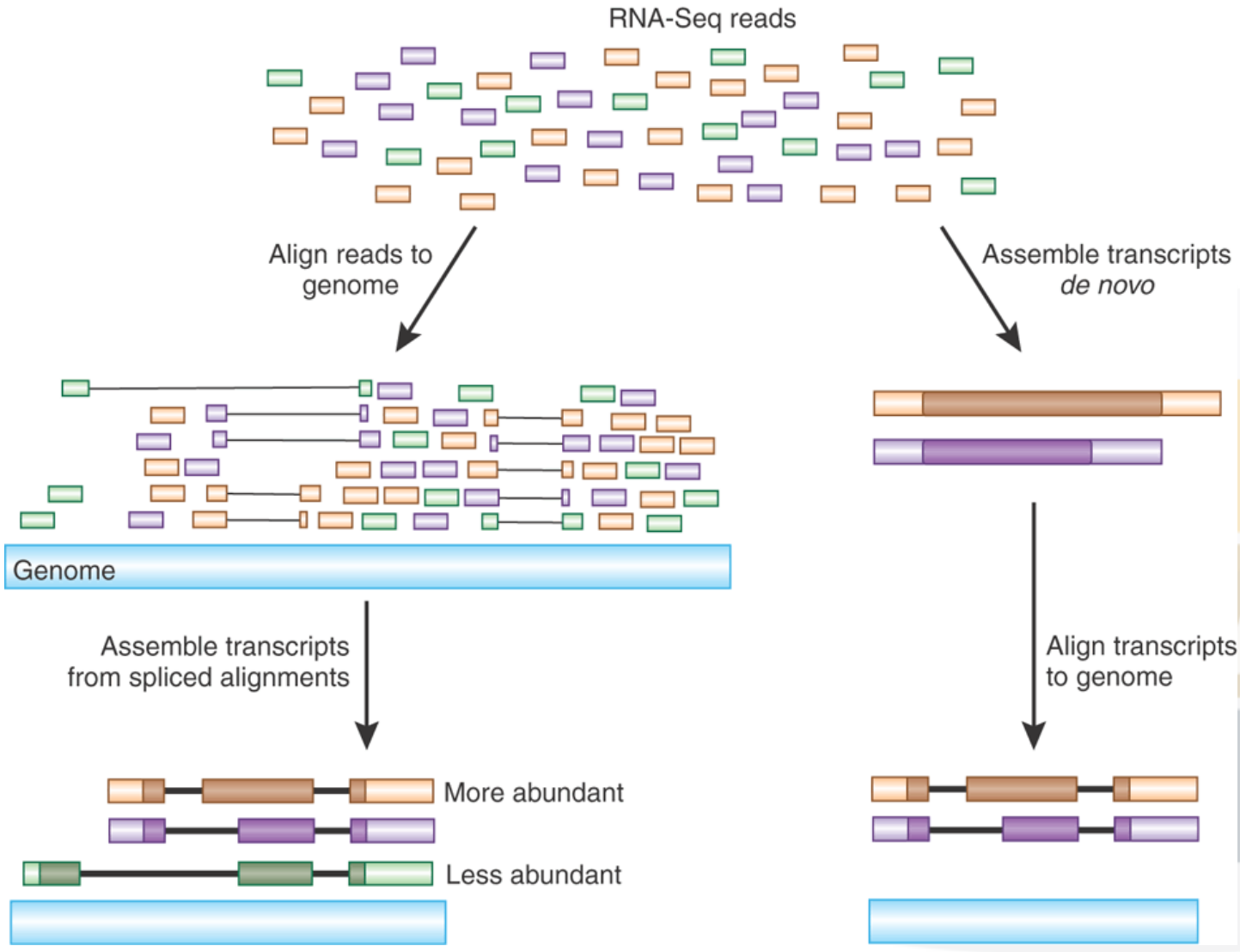
2 Biases

3 Normalization methods

4 Normalization in Babelomics 5

5 Exercises

Introduction



Introduction

What do we get? A counts matrix (integer data)

A1BG	203	698	643	176	177	247	100	125
A1CF	0	0	0	0	0	0	0	1
A2BP1	398	245	263	540	7	1	1	13
A2LD1	89	149	81	265	312	823	217	803
A2M	55336	76480	49882	16376	67193	21941	14414	10123
A2ML1	67	3	6	444	170	28	84	17
A4GALT	59	870	206	326	72	344	458	2109
A4GNT	2	1	0	1	0	2	0	0
AAA1	2	0	0	0	1	0	0	0
AAAS	759	1061	2607	2129	1151	8130	1649	3447
AACS	784	566	1168	639	643	4281	383	1756
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Introduction

Why normalizing?

- The technology introduces different biases
- We need to remove them to compare
  - Among genes in a sample
  - Among samples

Biases

Biases

1. Gene length
2. Library depth
3. RNA composition
4. Others

Gene length

Larger genes get more reads

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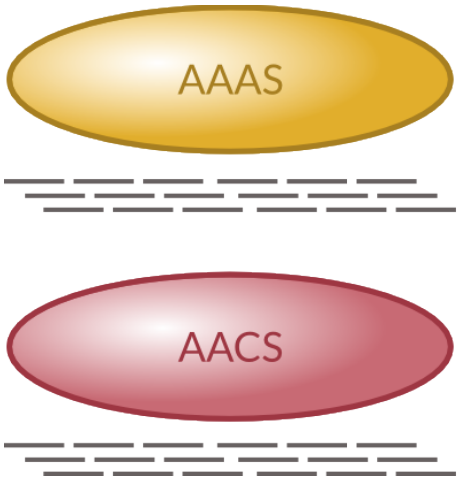

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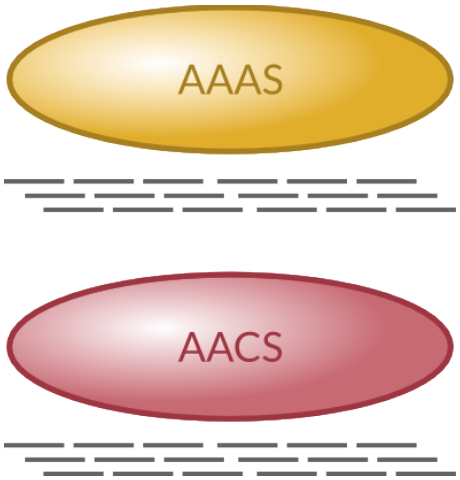

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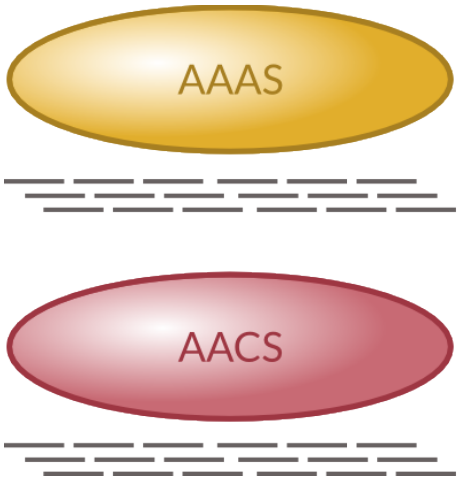

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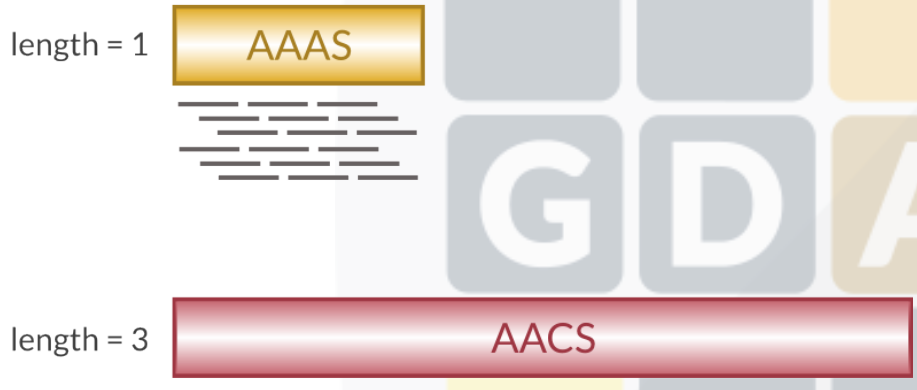

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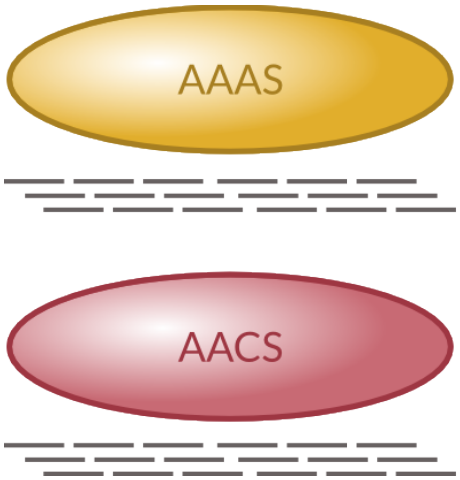

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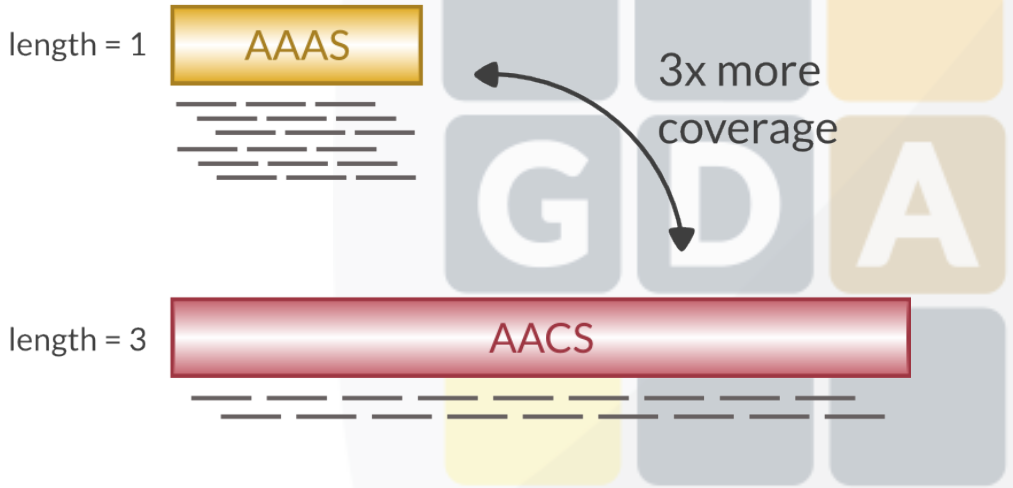

Biases

1. Gene length
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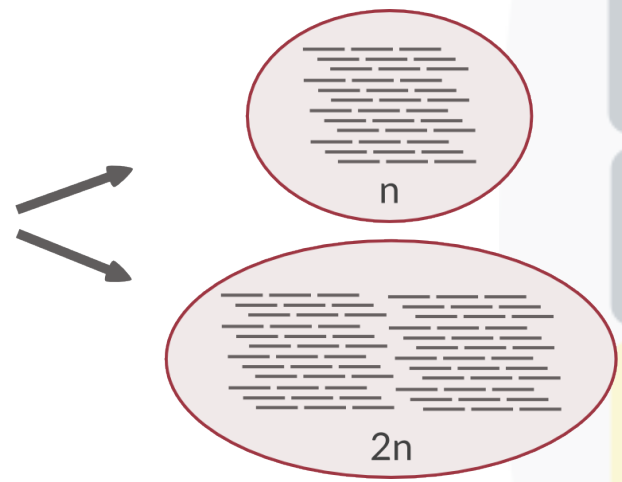
4. Others

Library depth

Deeper libraries give more reads



Biases

1. Gene length
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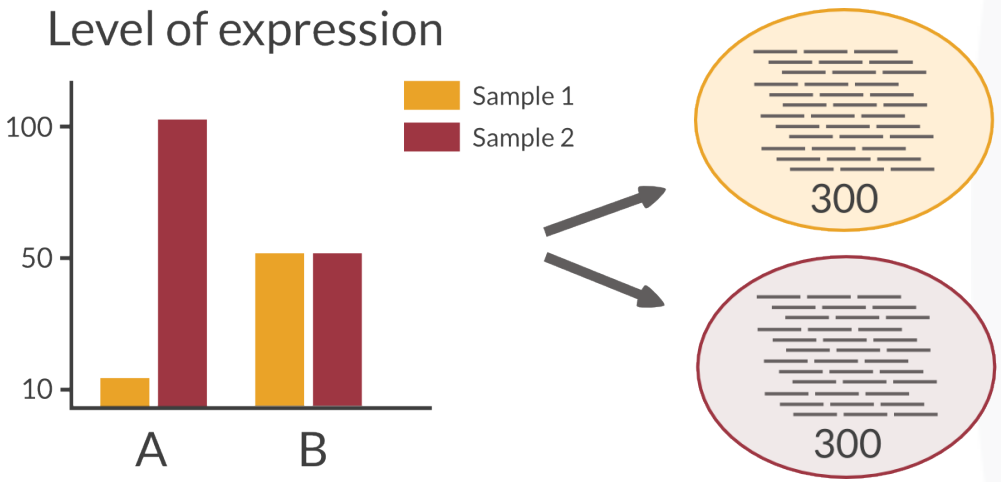
3. RNA composition
4. Others

RNA composition

A greedy gene steals reads from the others



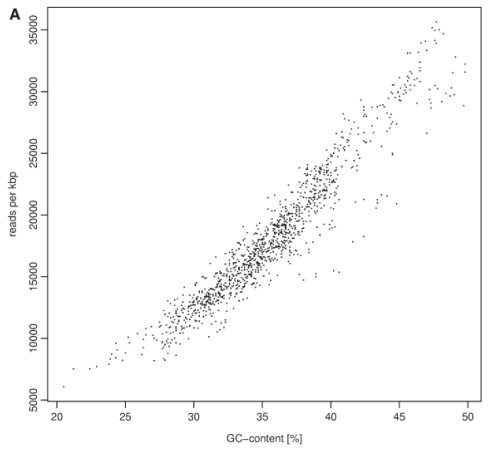
	Sample 1	Sample 2
A	50 (17%)	200 (66%)
B	250 (83%)	100 (33%)

Biases

1. Gene length
2. Library depth
3. RNA composition
4. Others

Others

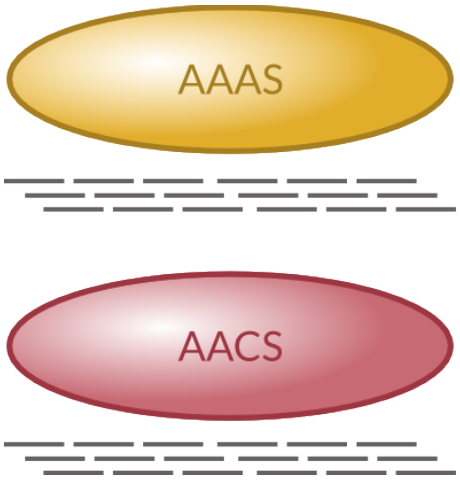
- GC-content
- Dinucleotide frequencies



Normalization methods

Normalization methods

1. Gene length
2. Library depth
3. RPKM
4. TMM
5. Quantiles



Gene length

Divide by gene length

Normalization methods

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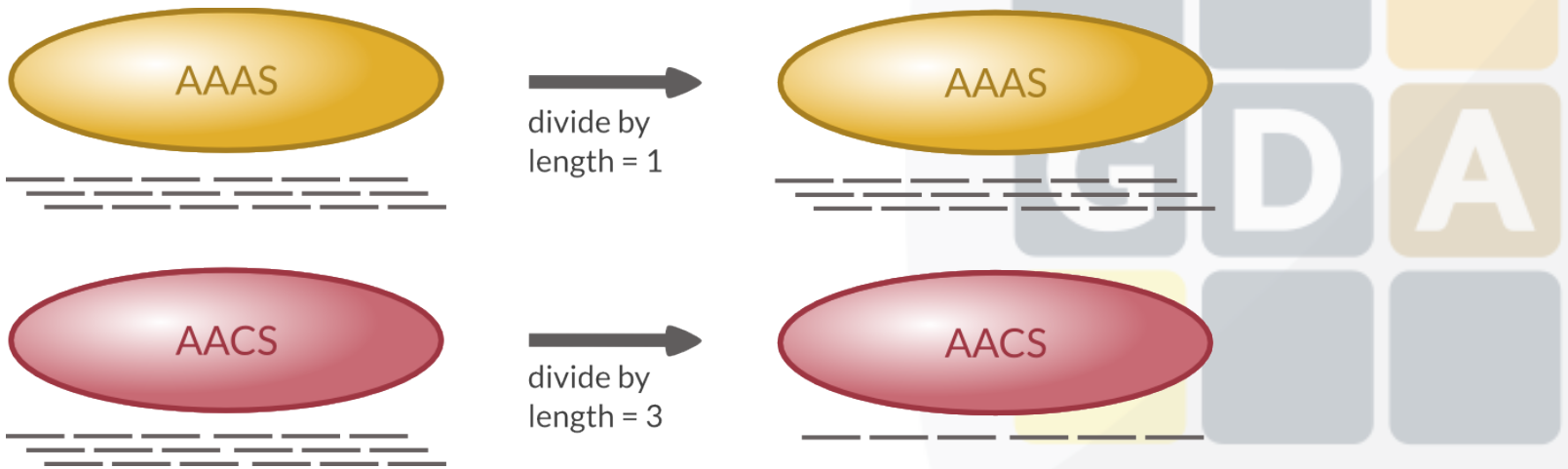
Gene length

Divide by gene length



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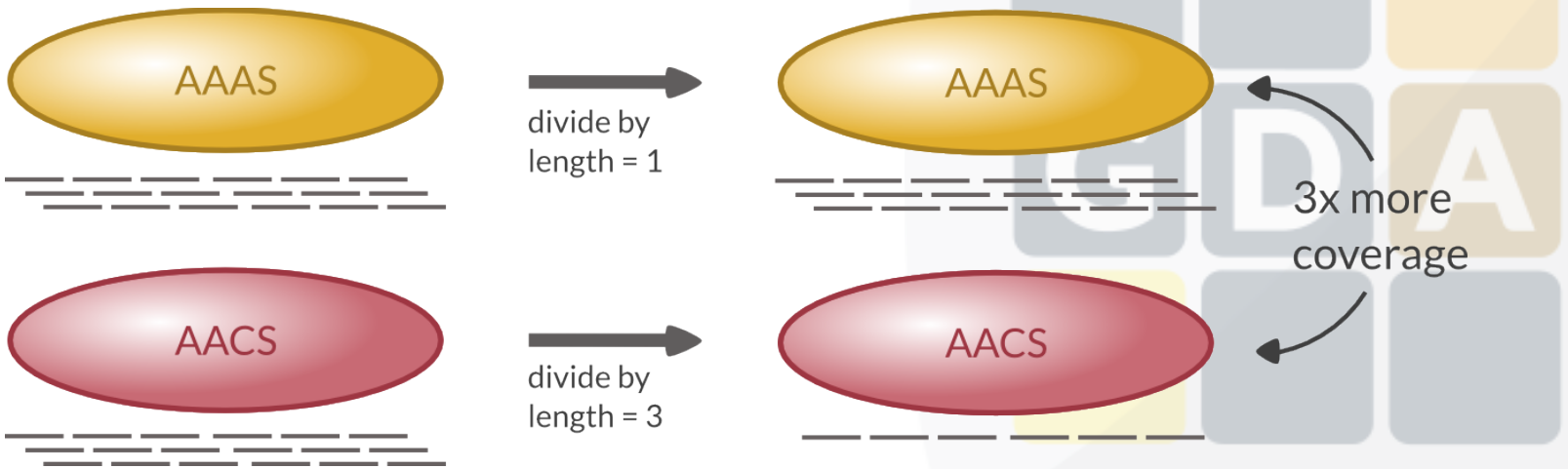

Normalization methods

1. Gene length
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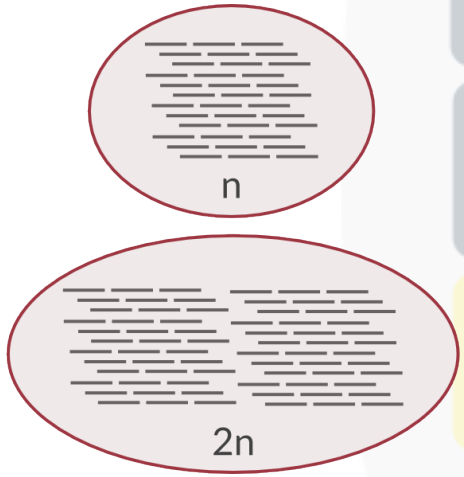
Library depth

Divide by library depth



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Library depth

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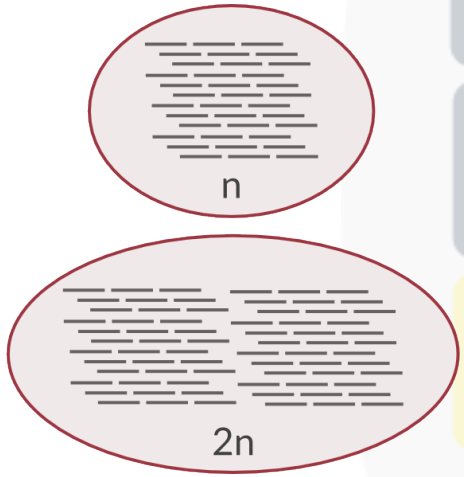


Normalization methods

1. Gene length
2. Library depth
3. RPKM
4. TMM
5. Quantiles

RPKM

- Reads per Kilobase per Million
- Remove gene length and library depth biases

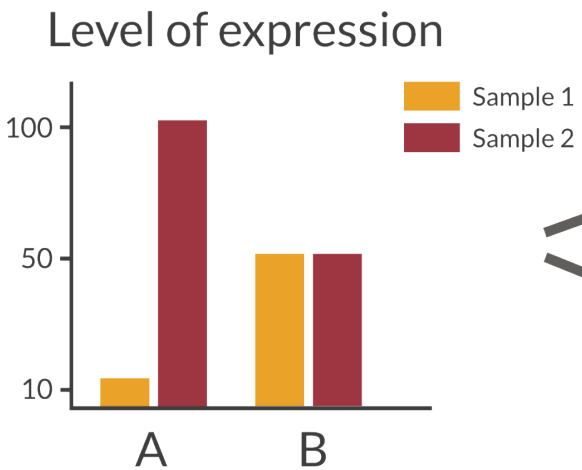
$$\text{RPKM} = \frac{total\ exon\ reads}{mapped\ reads\ (millions) * exon\ length\ (KB)}$$

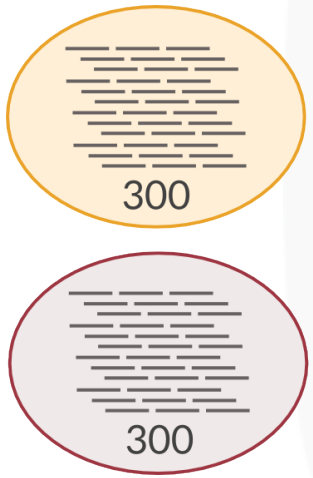
Normalization methods

1. Gene length
2. Library depth
3. RPKM
4. TMM
5. Quantiles

TMM

- Trimmed Means of M-values
- Assumes only a few genes are DE
- Changes library depth





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Normalization methods

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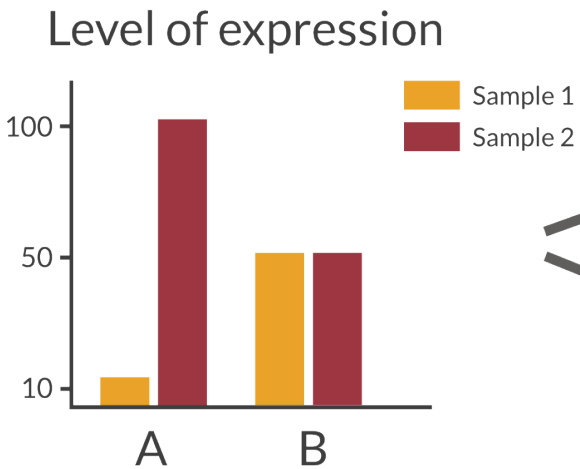
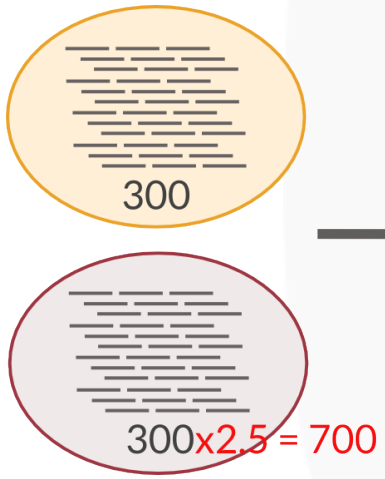




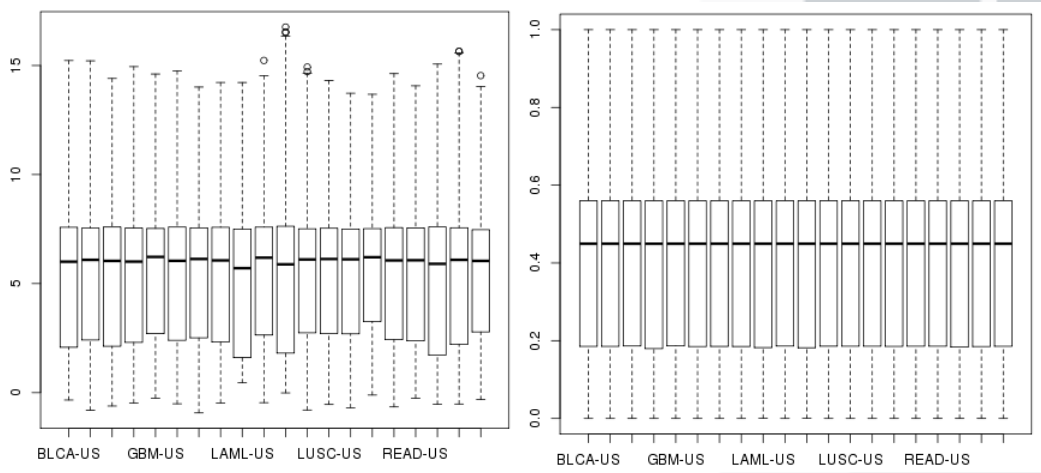
	Sample 1	Sample 2
A	50 (17%)	200x2.5= 500
B	250 (83%)	100x2.5= 250

Normalization methods

1. Gene length
2. Library depth
3. RPKM
4. TMM
5. Quantiles

Quantiles

Makes all sample distributions the same



Normalization in Babelomics 5

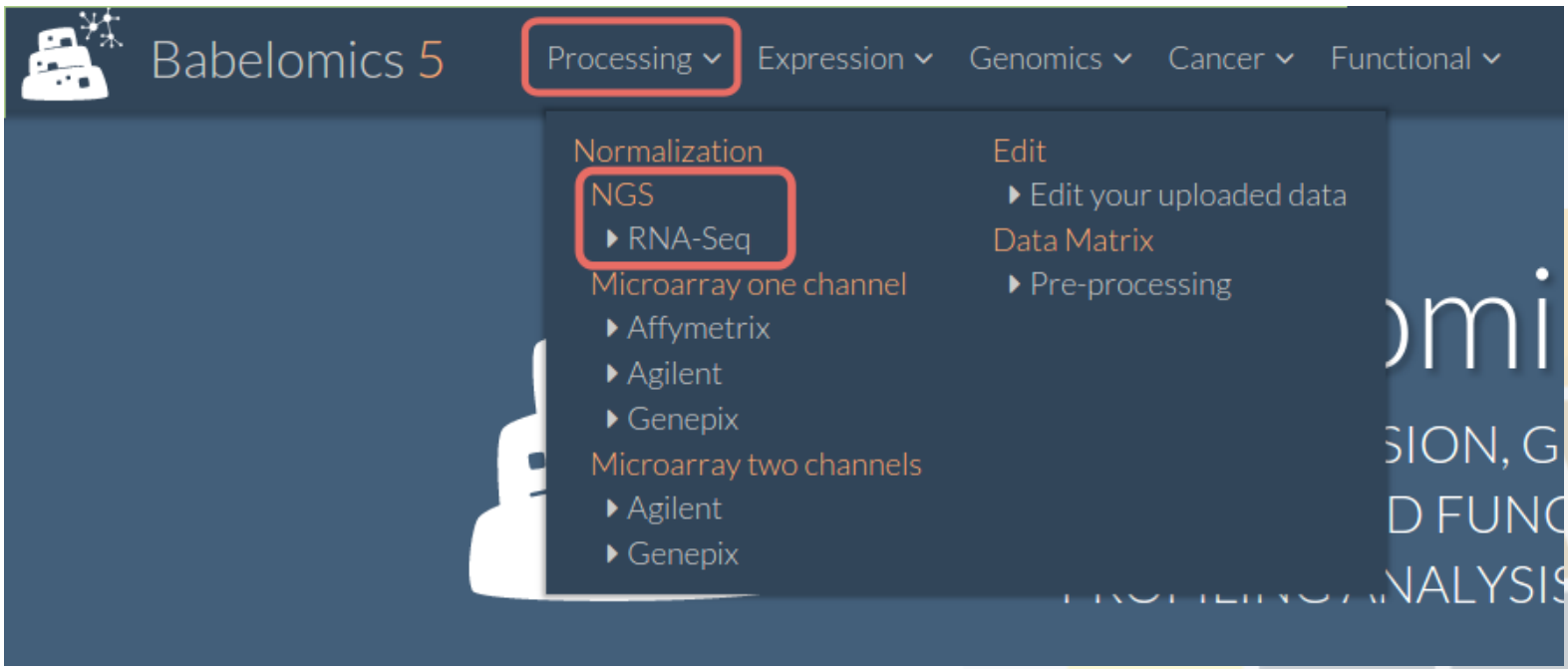
Normalization in Babelomics 5

Available normalization methods in Babelomics 5

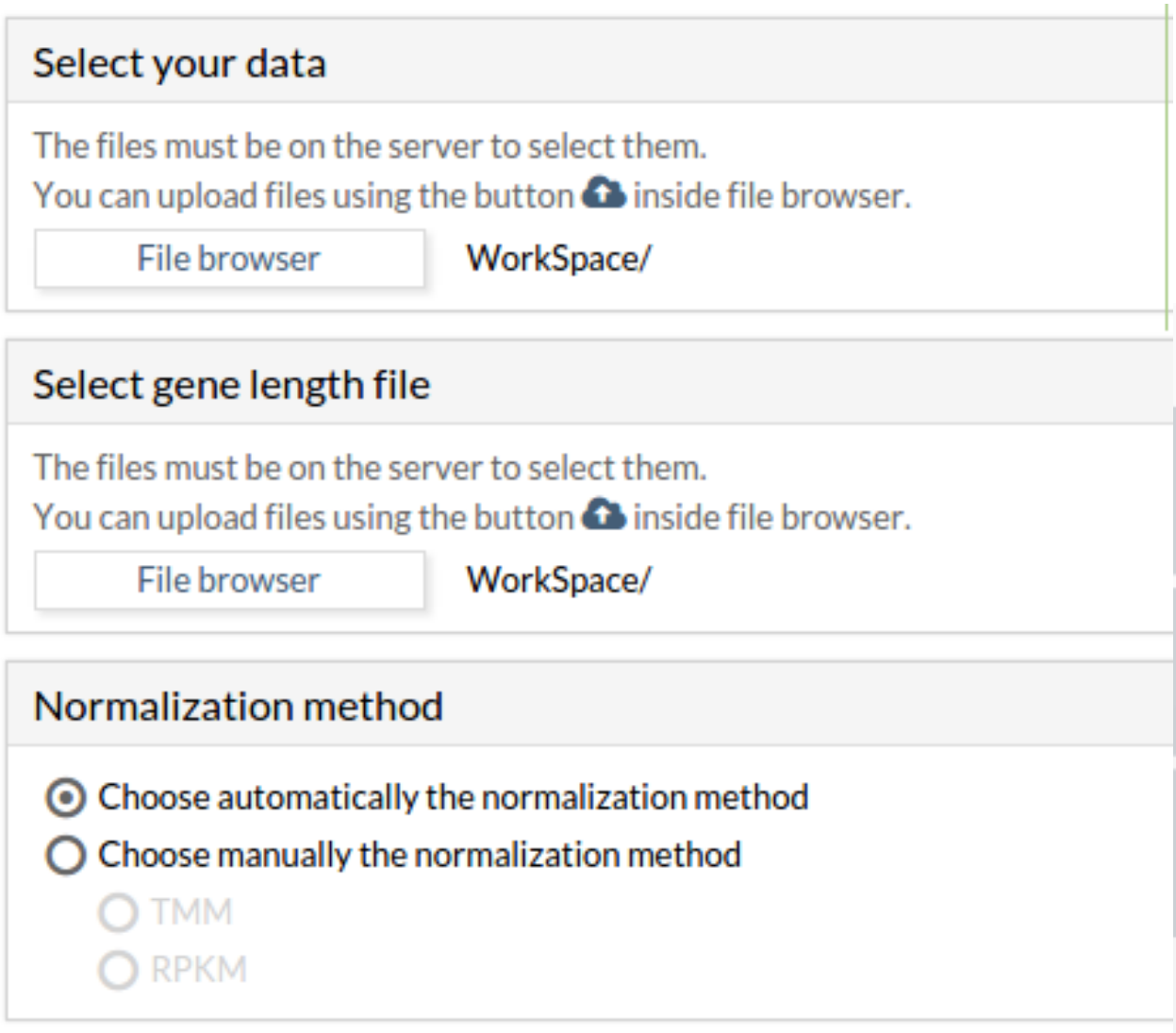
1. RPKM (gene length required)
2. TMM
3. TMM with gene length correction (gene length required)
4. Automatic selection of the method based on the diagnostic test for differences in RNA composition from NOISeq

Normalization in Babelomics 5

Where can we find RNA-Seq normalization in Babelomics 5?



Filling in the formular



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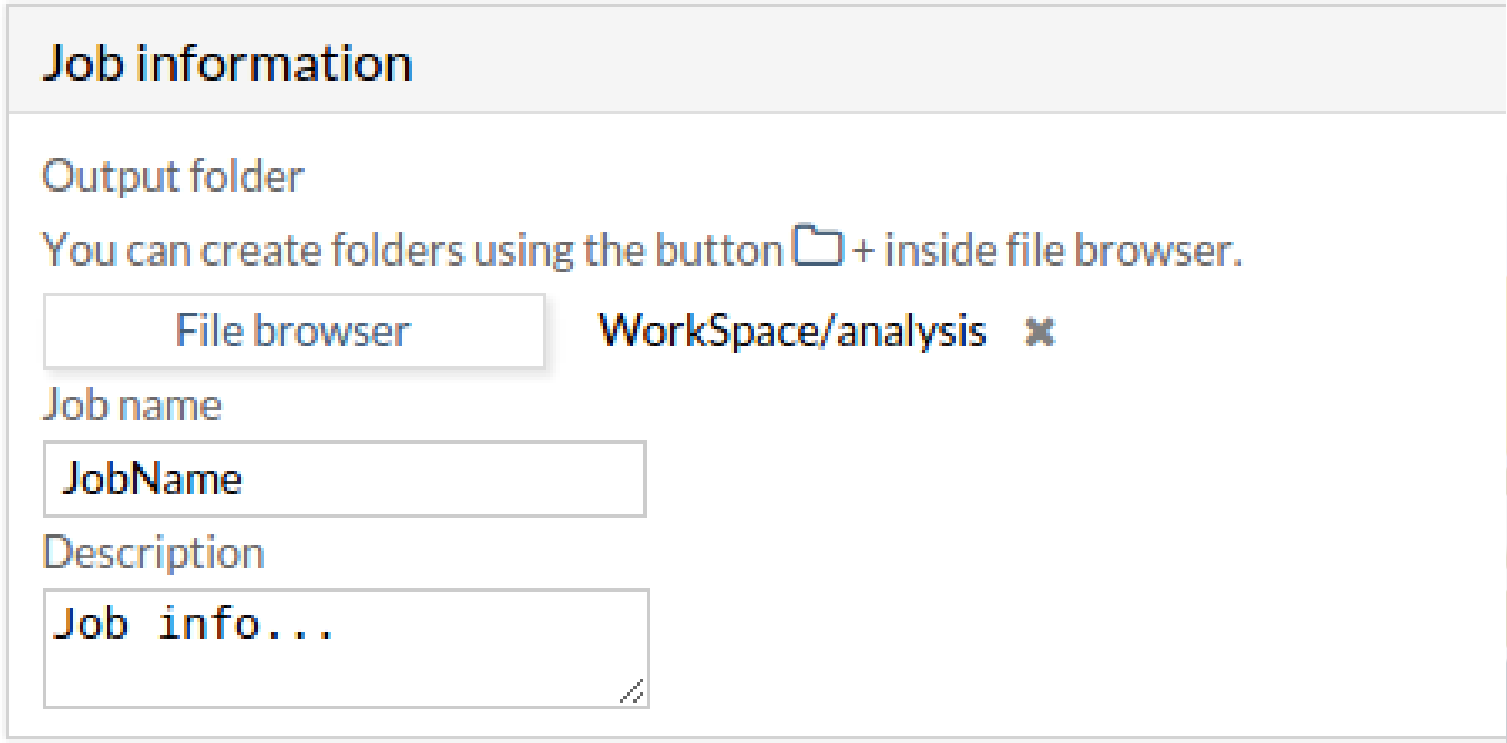
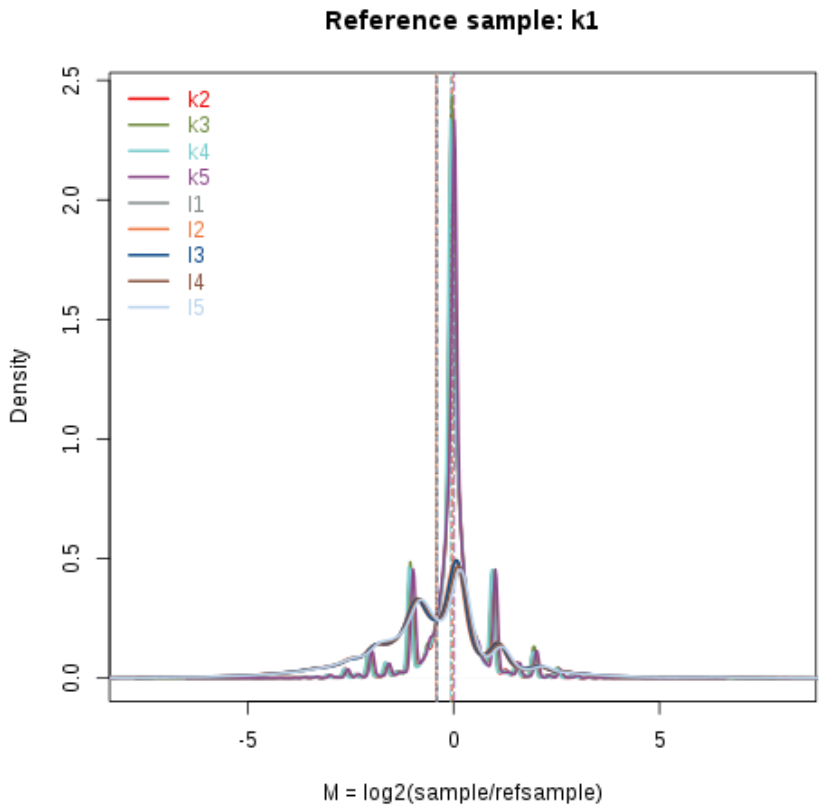


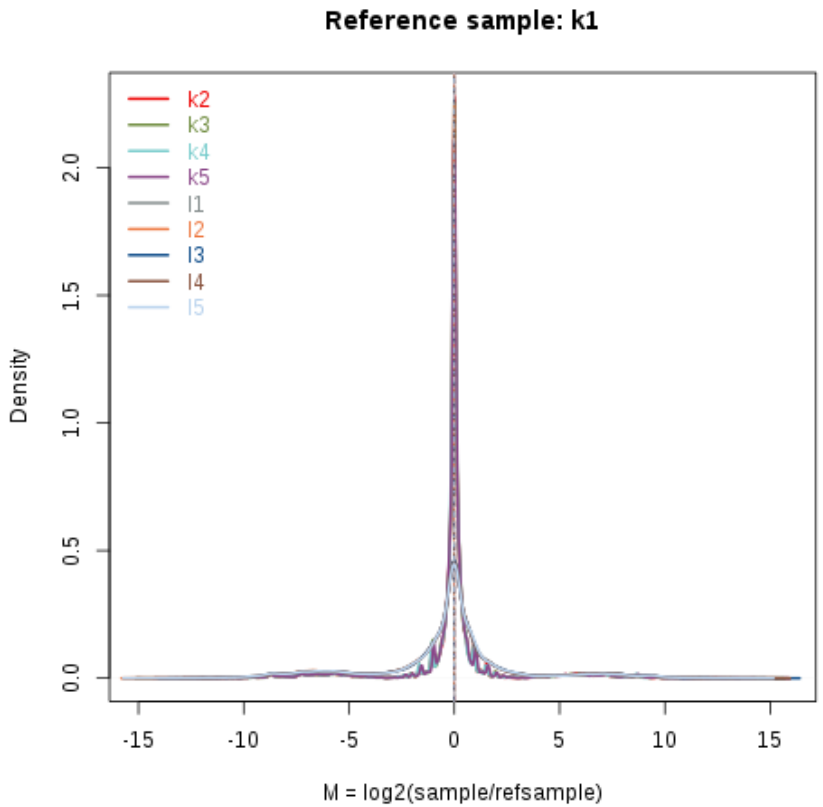
The results

RNA composition

RNA composition before normalization



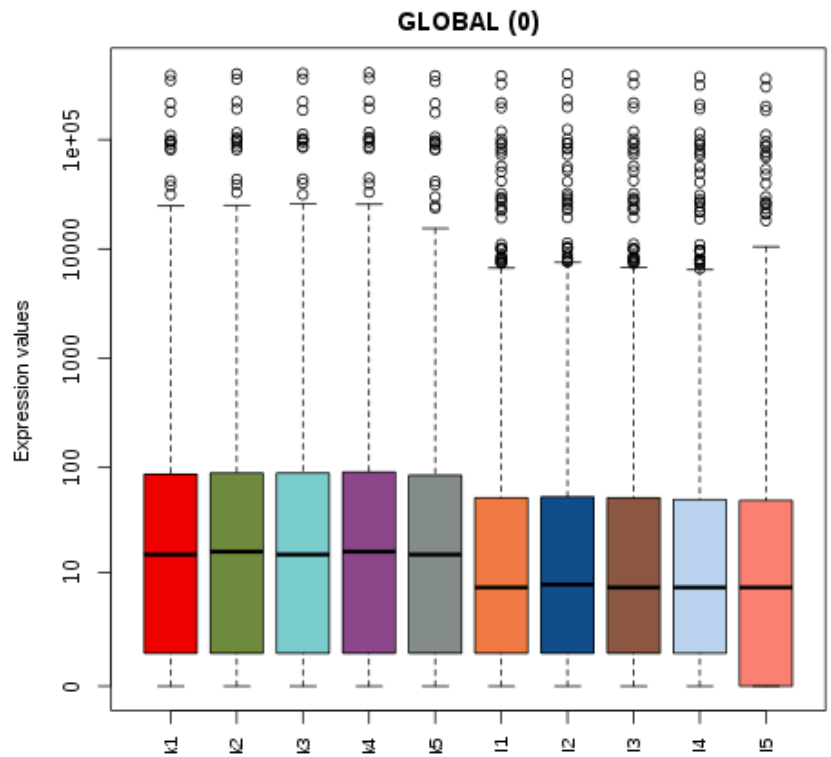
RNA composition after normalization



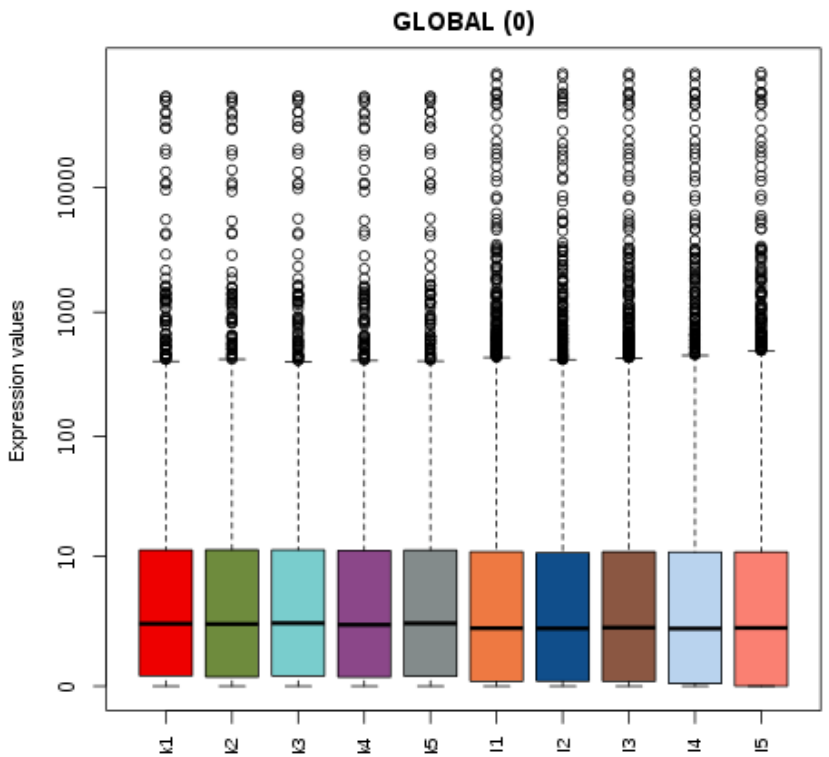
The results

Distribution of Expression values

Boxplot expression values before normalization



Boxplot expression values after normalization



The results

Table of Normalized values

File *normalized_results.txt*

#NAMES	k1	k2	k3	k4	k5
TSPAN6	42.11	39.49	39.02	34.59	42.55
TNMD	0	0	0	0.22	0
DPM1	13.17	16.31	17.22	15.5	16.21
SCYL3	1.54	2.12	2.21	1.88	2.31
C1orf112	1	1.15	0.77	1.04	1.82
FGR	2.25	3.19	2.07	2.24	1.2
FUCA2	37.84	41.24	39.91	38.24	33.78
GCLC	25.88	25.39	21.51	23.51	23.06
NFYA	4.62	4.59	4.03	4.16	4.69
STPG1	5.82	7.08	6.81	5.03	8

29405 Results

Send to edit

Exercises

Normalization exercises

Exercise 1

Perform a normalization of the breast cancer data in the file *brca_demo_counts_4babelomics.txt*

Exercise 2

We will use a Kidney Renal Clear Cell carcinoma (KIRC) dataset from the TCGA

1. Go to the GDA 2016 wiki
2. Download the *kirc_demo_counts_4babelomics.txt*
3. Upload this file to Babelomics 5
4. Normalize the data

For help, ask or visit the normalization tutorial