Omics-based biomarkers detection

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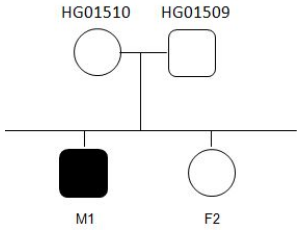
WODA

WEB-BASED OMICS DATA ANALYSIS

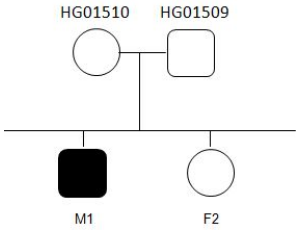
Unidad de Bioinformática y Bioestadística

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

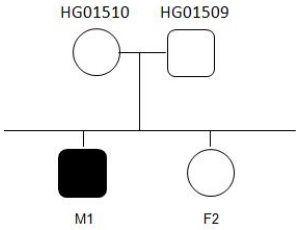
Detecting biomarkers

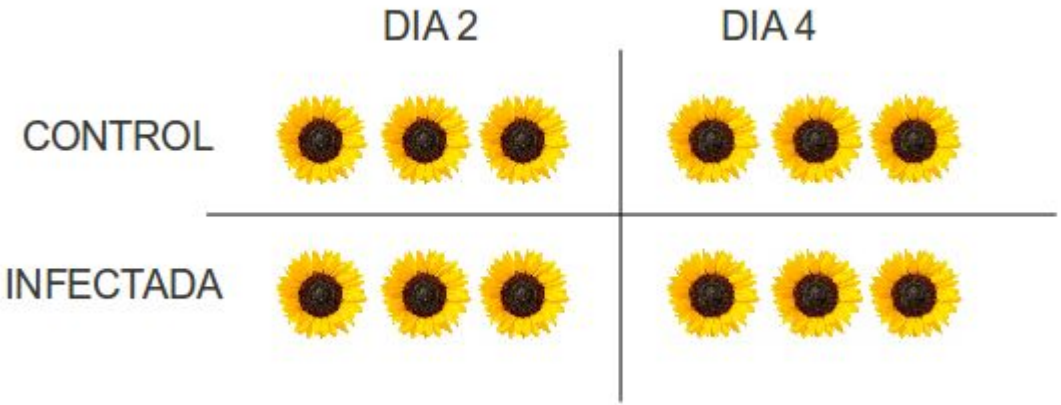


Detecting biomarkers

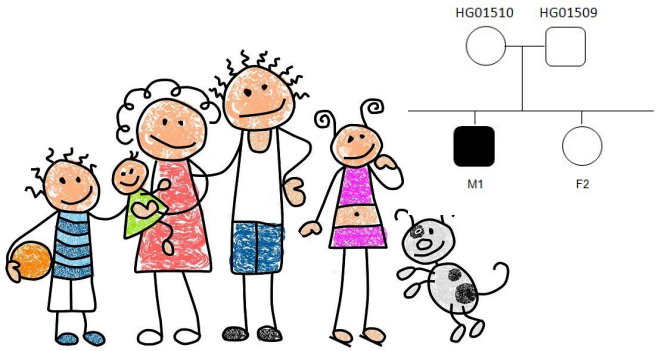


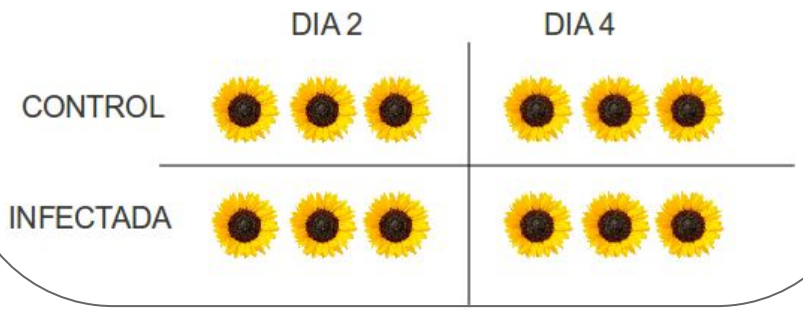
Detecting biomarkers

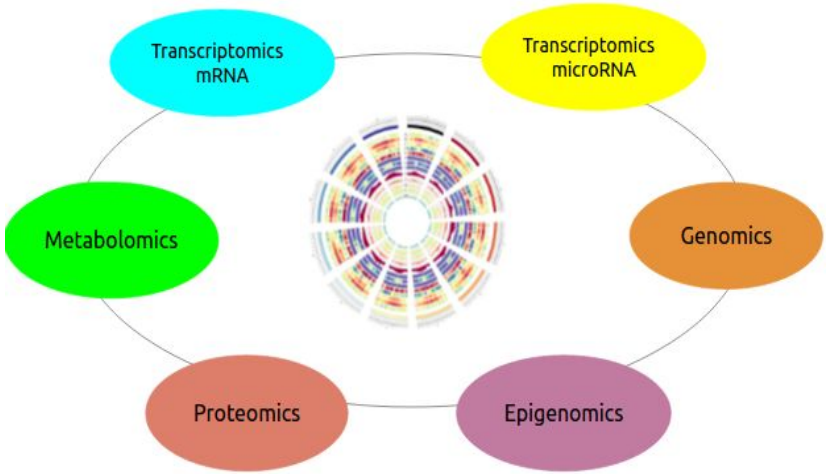




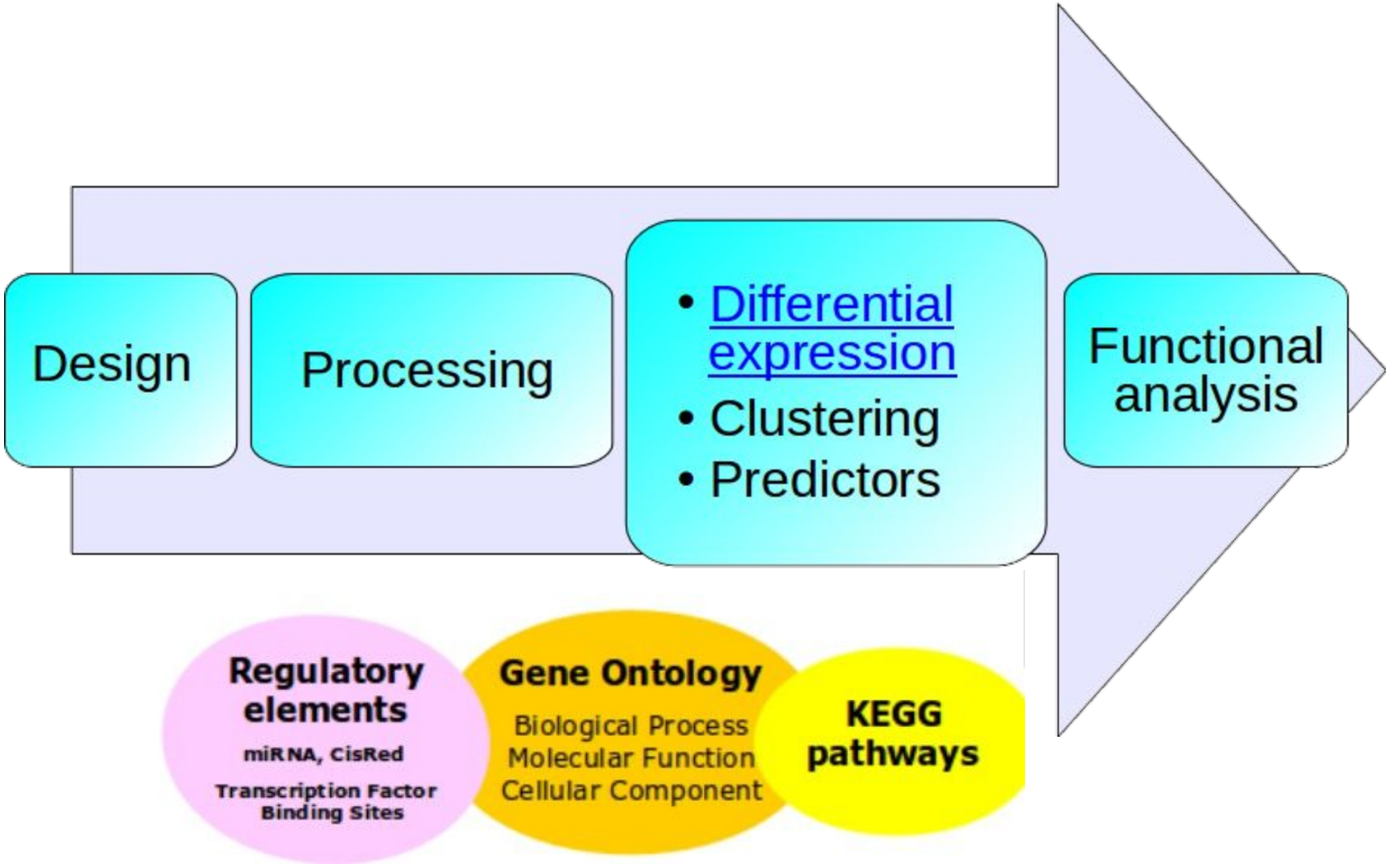
Detecting biomarkers



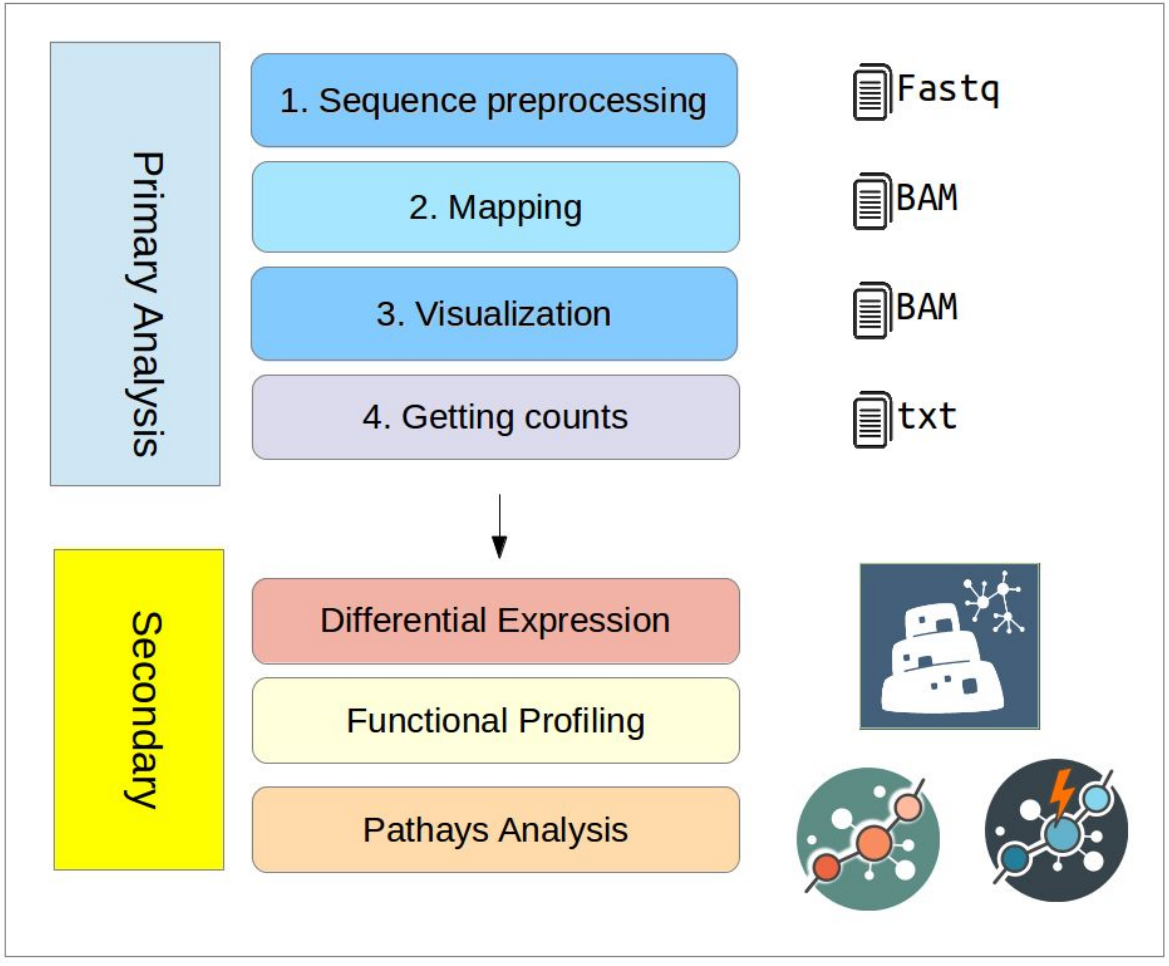




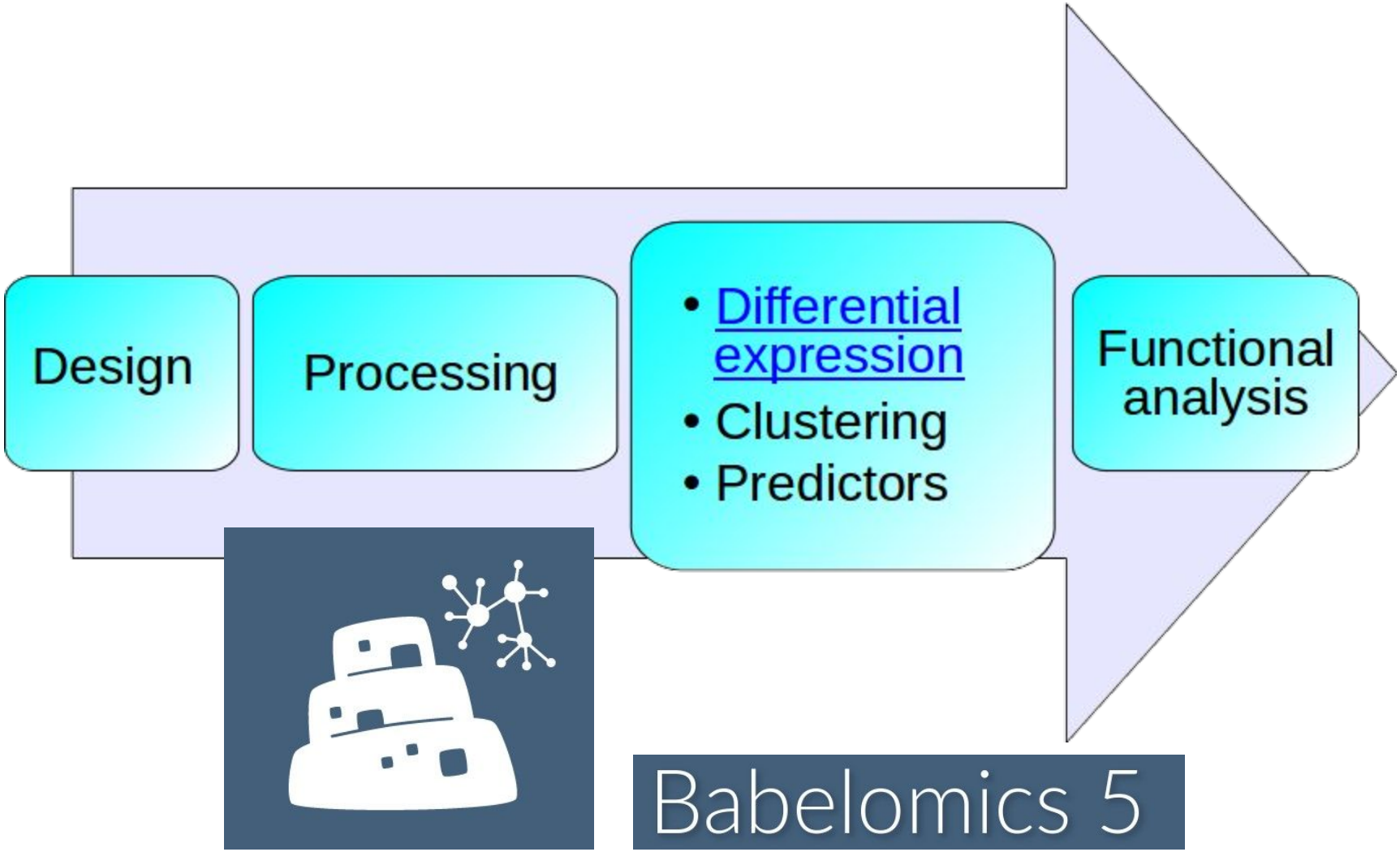
Data analysis workflow



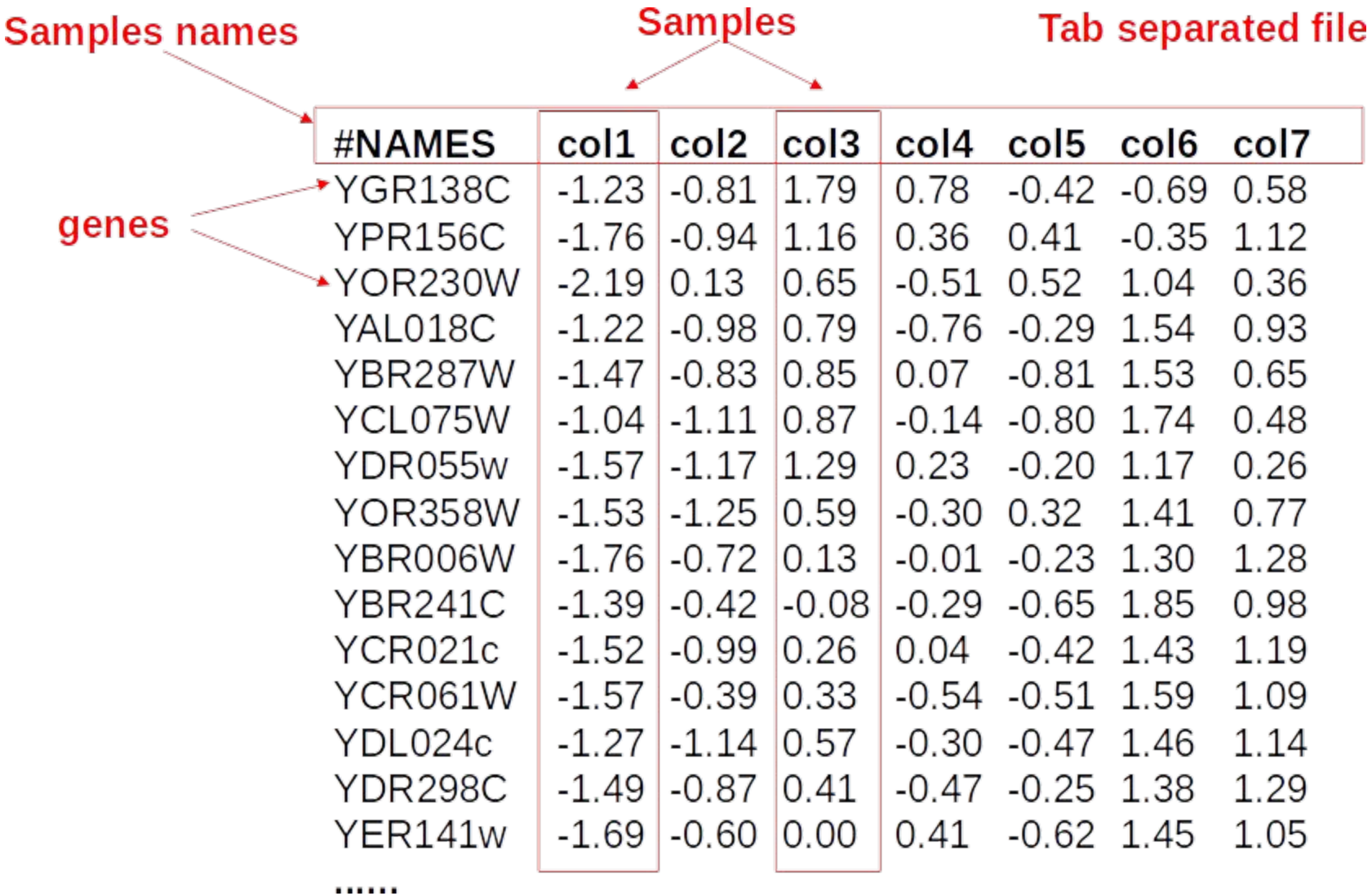
Data analysis workflow



Data analysis workflow



Input



#NAMES	col1	col2	col3	col4	col5	col6	col7
YGR138C	-1.23	-0.81	1.79	0.78	-0.42	-0.69	0.58
YPR156C	-1.76	-0.94	1.16	0.36	0.41	-0.35	1.12
YOR230W	-2.19	0.13	0.65	-0.51	0.52	1.04	0.36
YAL018C	-1.22	-0.98	0.79	-0.76	-0.29	1.54	0.93
YBR287W	-1.47	-0.83	0.85	0.07	-0.81	1.53	0.65
YCL075W	-1.04	-1.11	0.87	-0.14	-0.80	1.74	0.48
YDR055w	-1.57	-1.17	1.29	0.23	-0.20	1.17	0.26
YOR358W	-1.53	-1.25	0.59	-0.30	0.32	1.41	0.77
YBR006W	-1.76	-0.72	0.13	-0.01	-0.23	1.30	1.28
YBR241C	-1.39	-0.42	-0.08	-0.29	-0.65	1.85	0.98
YCR021c	-1.52	-0.99	0.26	0.04	-0.42	1.43	1.19
YCR061W	-1.57	-0.39	0.33	-0.54	-0.51	1.59	1.09
YDL024c	-1.27	-1.14	0.57	-0.30	-0.47	1.46	1.14
YDR298C	-1.49	-0.87	0.41	-0.47	-0.25	1.38	1.29
YER141w	-1.69	-0.60	0.00	0.41	-0.62	1.45	1.05
.....							

Results

name	statistic	p-value	adj. p-value
200067_x_at	5.5382	0.0000049746	0.00024376
200052_s_at	5.2111	0.00001452	0.00047431
200054_at	5.1028	0.000042635	0.0010445
200009_at	4.2093	0.00019599	0.0027557
200017_at	4.0805	0.00022496	0.0027557
1053_at	3.9461	0.00060822	0.0059605
200013_at	3.767	0.00070427	0.0062744
200071_at	3.518	0.0014872	0.012146
200076_s_at	3.1376	0.0039127	0.024703
177_at	3.0053	0.0061375	0.030074

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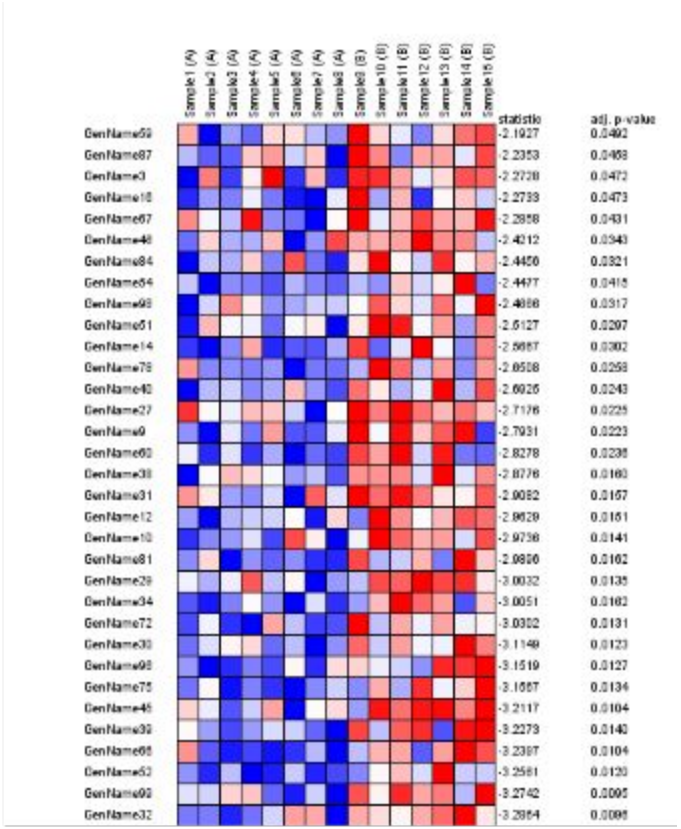
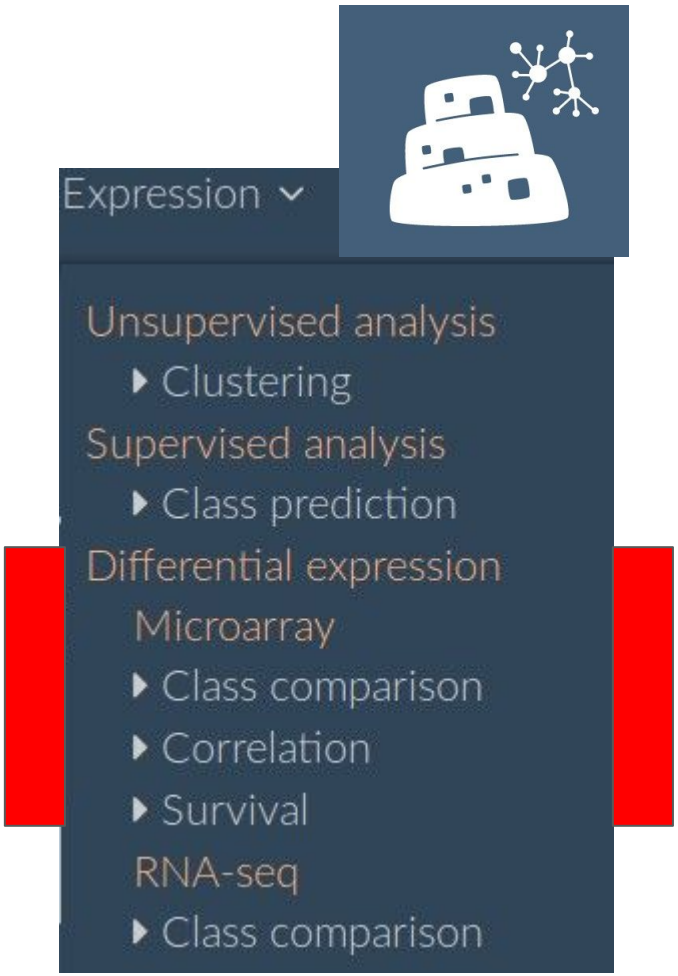
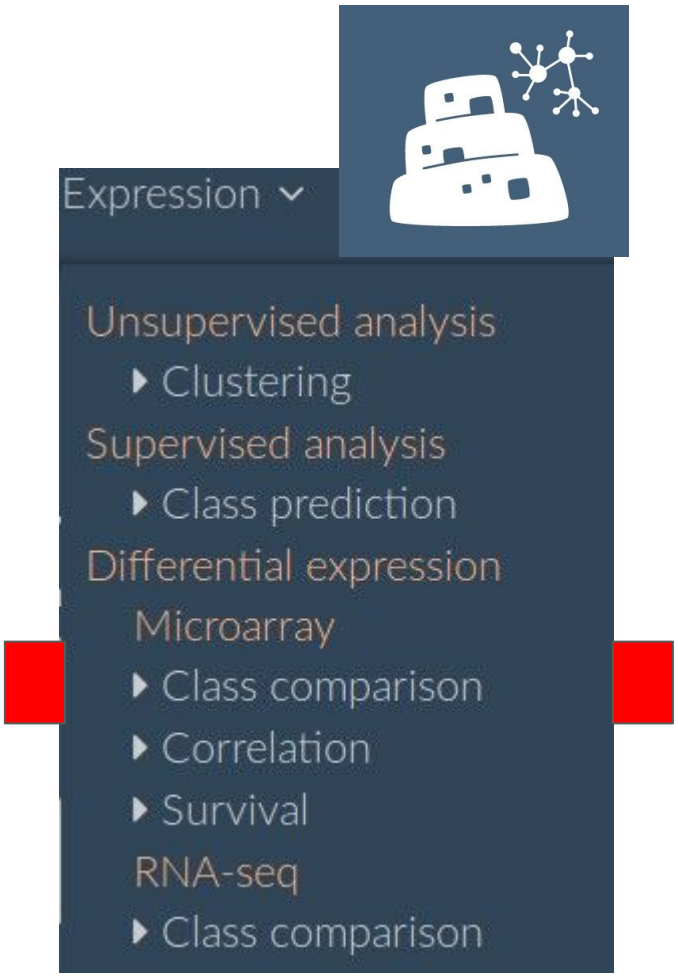


Detecting biomarkers



A. **Continuous** variables:
- Metabolomics
- Proteomics
- Transcriptomics arrays
- Experimental data

Different experimental designs



Class comparison

Methods:

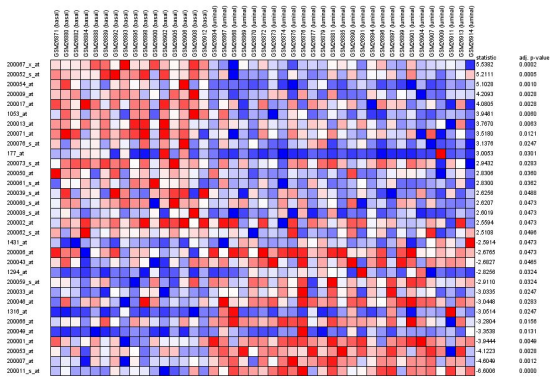
Limma, t-test:

$H_0: \mu_1 = \mu_2$
$H_a: \mu_1 \neq \mu_2$

Fold-change:

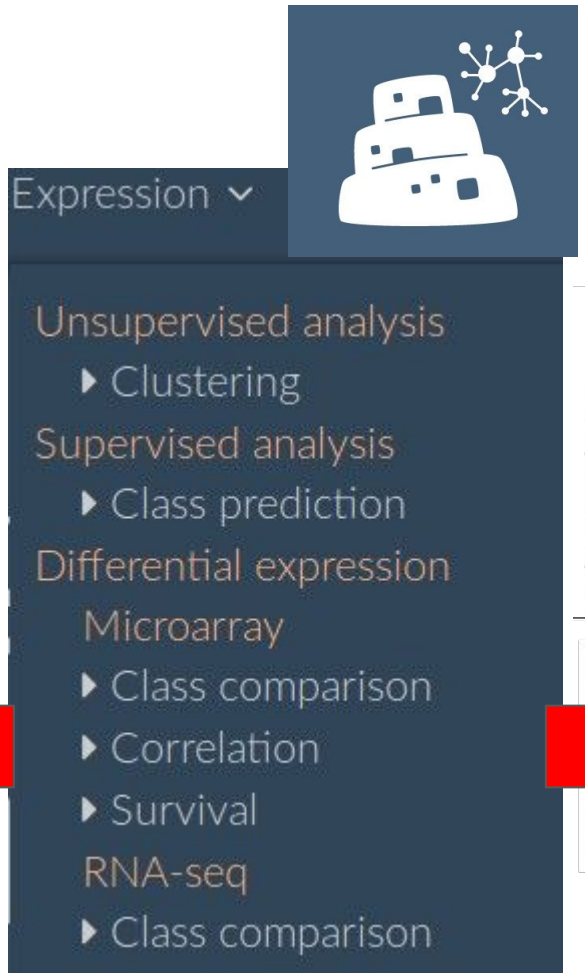
$\log_2 (\bar{y}_1 / \bar{y}_2)$

$\bar{y}_1 - \bar{y}_2$

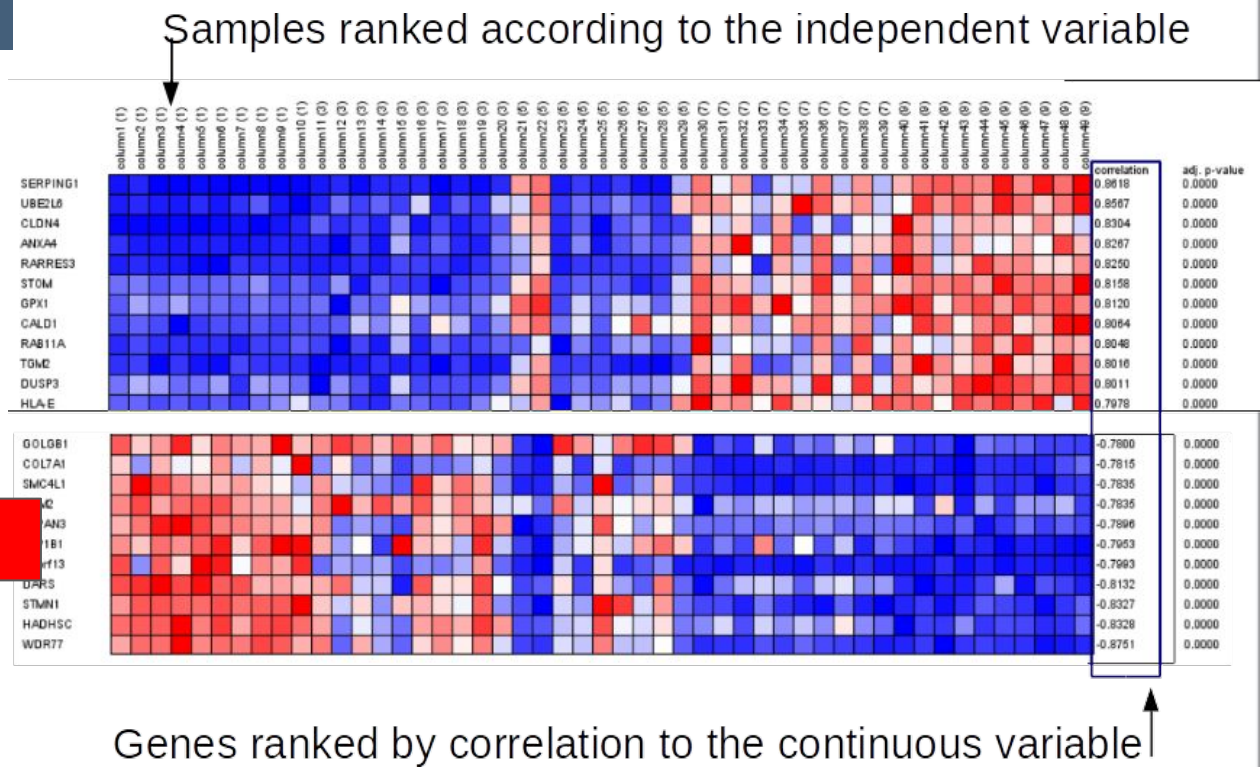


$H_0: \mu_1 = \mu_2 = \ldots = \mu_n$
$H_a:$ not H_0

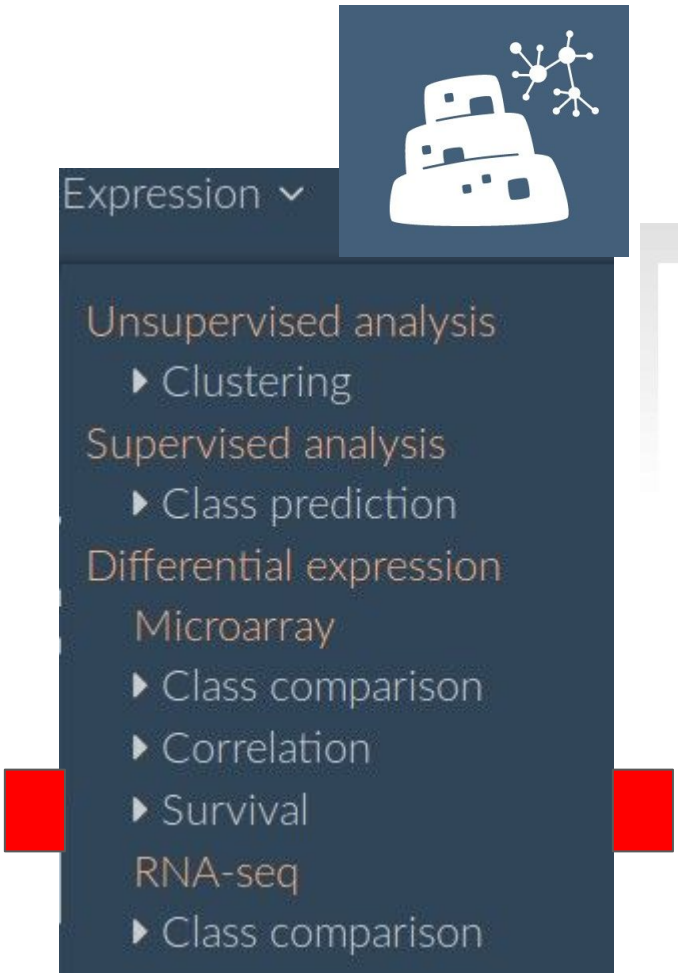
Different experimental designs



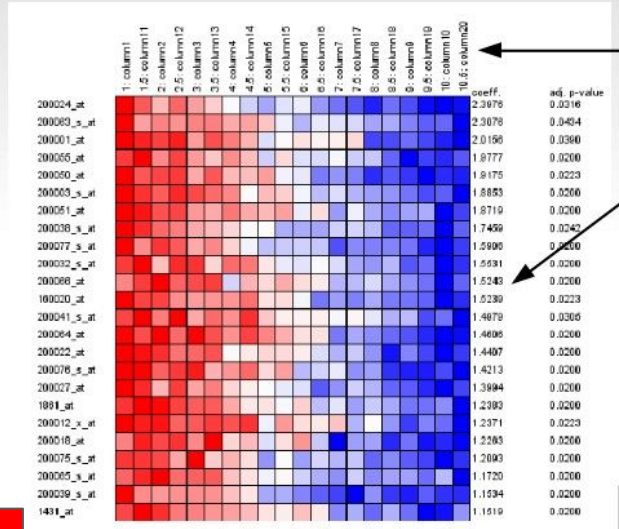
Correlation



Different experimental designs



Survival

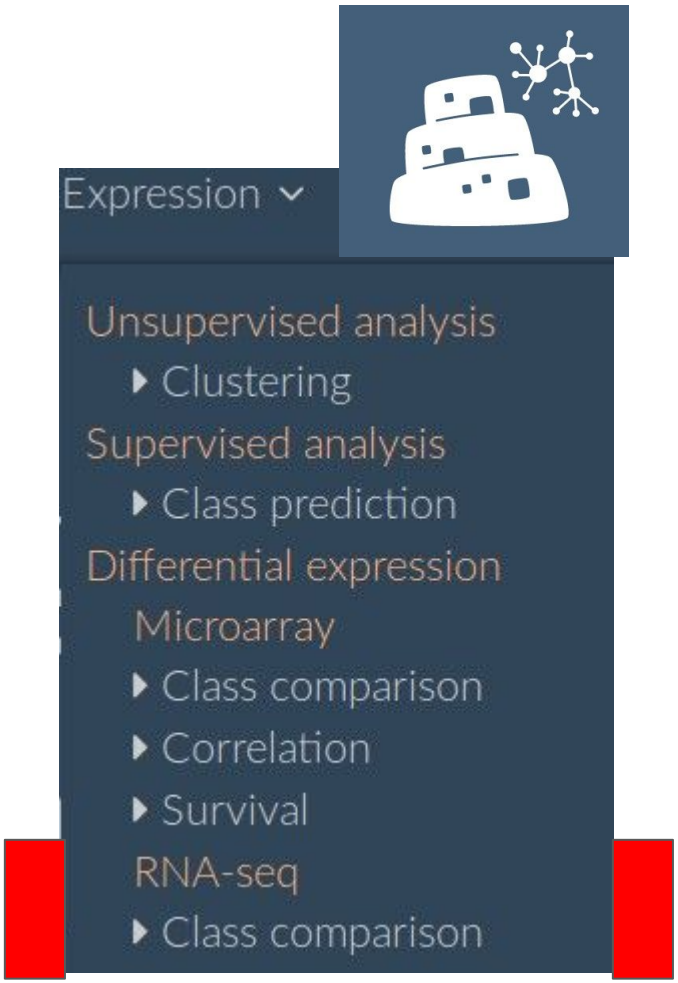


Samples ranked according to the survival time

Genes ranked by their relationship with survival time

- Cox model coefficients
- Estimate for the statistics
- p-values

Detecting biomarkers



B. **Discrete** variables:

- RNA-Seq
- Experimental data

RNA-Seq Data Analysis Pipeline

Primary

1. Sequence preprocessing
2. Mapping
3. Quantification

Secondary

4. Normalization
5. Differential expression
6. Functional Profiling

Fastq format

- We could say “it is a fasta with **qualities**”:
  - 1. Header (like the fasta but starting with “@”)
  - 2. Sequence (string of nt)
  - 3. “+” and sequence ID (optional)
  - 4. Encoded quality of the sequence

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

BAM/SAM format

```
@PG     ID:HPG-Aligner  VN:1.0
@SQ     SN:20   LN:63025520

HWI-ST700660_138:2:2105:7292:79900#2@0/1      16     20     76703  254    76=    *      0      0
GTTTAGATACTGAAAGGTACATACTTCTTTGTAGGAACAAGCTATCATGCTGCATTTCTATAATATCACATGAATA
GIJGJLGGFLILGGIEIFEKEDELIGLJIHJFIKKFELFIKLFFGLGHKKGJLFIIGKFFEFFEFGKCKFHHCCCF    AS:i:254      NH:i:1  NM:i:0

HWI-ST700660_138:2:2208:6911:12246#2@0/1      16     20     76703  254    76=    *      0      0
GTTTAGATACTGAAAGGTACATACTTCTTTGTAGGAACAAGCTATCATGCTGCATTTCTATAATATCACATGAATA
HHJFHLGFFLILEGIKIEEMGEDLIGLHIHJFIKKFELFIKLEFGKGHEKHJLFHIGKFFDFFEFGKDKFHHCCCF    AS:i:254      NH:i:1  NM:i:0

HWI-ST700660_138:2:1201:2973:62218#2@0/1      0      20     76655  254    76M    *      0      0
AACCCCAAAAATGTTGGAAGAATAATGTAGGACATTGCAGAAGACGATGTTTAGATACTGAAAGGGACATACTTCT
FEFFGHHHGGHFKCCJKFHIGIFFIFLDEJKGJGGFKIHLFIJGIEGFLDEDFLFGEIIMHHIKL$BBGFFJIEHE    AS:i:254      NH:i:1  NM:i:1

HWI-ST700660_138:2:1203:21395:164917#2@0/1    256    20     68253  254    4M1D72M *      0      0
NCACCCATGATAGACCAGTAAAGGTGACCACTTAAATTCCTTGCTGTGCAGTGTTCTGTATTCCTCAGGACACAGA
#4@ADEHFJFFEJDHJGKEFIHGHBGFHHFIICEIIFFKKIFHEGJEHHGLELEGKJMFGGGLEIKHLFGKIKHDG    AS:i:254      NH:i:3  NM:i:1

HWI-ST700660_138:2:1105:16101:50526#6@0/1      16     20     126103 246    53M4D23M       *      0      0
AAGAAGTGCAAACCTGAAGAGATGCATGTAAAGAATGGTTGGGCAATGTGCGGCAAAGGGACTGCTGTGTTCCAGC
  FEHIGGHIGIGJI6FCFHJIFFLJJCJGJHGFKKKKGIJKHFFKIFFFKHFLKHGKJLJGKILLEFFLIHJIEIIB    AS:i:368      NH:i:1  NM:i:4
```

SAM Specification:
http://samtools.sourceforge.net/SAM1.pdf

counts file

Gene

Sample

Ensembl	Gene.Name	T1	T2	T3	T4	T5	WT1	WT2	WT3	WT4	WT5	WT6
ENSMUSG00000000134	Tfe3	312	295	333	258	392	257	344	223	423	277	389
ENSMUSG00000000142	Axin2	165	171	138	166	203	170	172	119	203	147	178
ENSMUSG00000000148	Brat1	213	196	207	224	350	204	268	143	300	177	288
ENSMUSG00000000149	Gna12	684	684	613	545	900	496	672	426	1023	583	797
ENSMUSG00000000154	Slc22a18	3	2	3	2	2	3	3	2	1	1	3
ENSMUSG00000000157	Itgb2l	0	0	0	0	0	0	0	0	0	0	0
ENSMUSG00000000159	Igsf5	0	0	0	0	0	0	0	0	0	0	0
ENSMUSG00000000167	Pih1d2	15	19	6	10	9	5	5	5	7	6	6
ENSMUSG00000000168	Dlat	899	777	967	756	1116	777	1047	614	1155	894	1126
ENSMUSG00000000171	Sdhd	1055	1003	1047	914	1430	939	1192	766	1390	916	1412
ENSMUSG00000000182	Fgf23	1	0	3	1	0	2	0	2	2	0	0
ENSMUSG00000000183	Fgf6	0	0	0	0	0	0	0	1	0	0	0
ENSMUSG00000000184	Ccnd2	1961	1978	1804	1779	2090	1655	2148	1585	2504	1895	2274
ENSMUSG00000000194	Gpr107	784	733	667	615	889	654	818	483	1034	627	1015
ENSMUSG00000000197	Nalcn	1120	1009	1047	917	1356	1129	1202	758	1625	1127	1044

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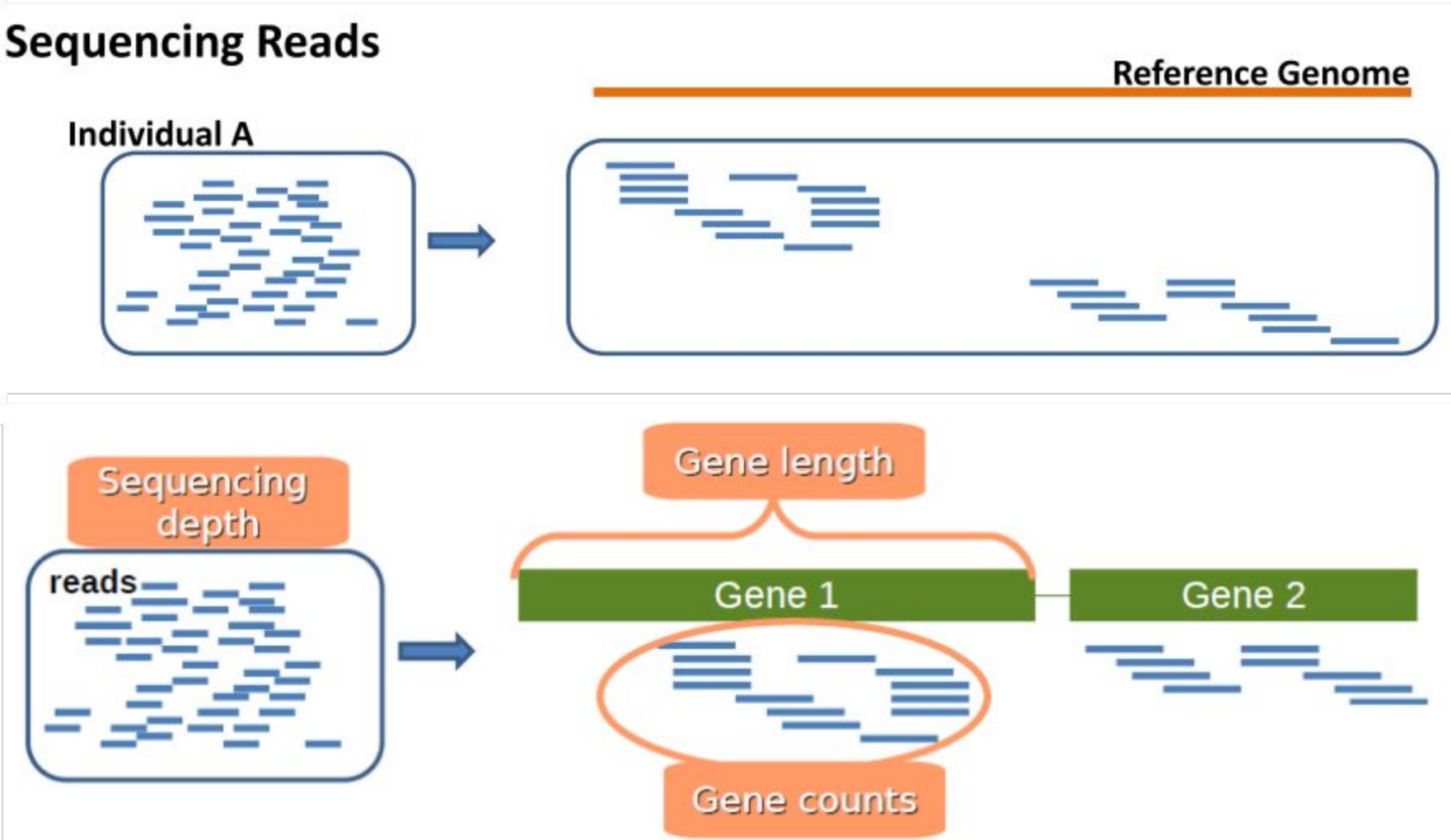
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

General context



Count Normalization

- **Transcript length**: *within* library
- **Library size**: *between* libraries
- Many **other biases** ...
  - Differences on the read count distribution among samples.
  - GC content of the gene affects the detection of that gene (Illumina)
  - sequence-specific bias is introduced during the library preparation

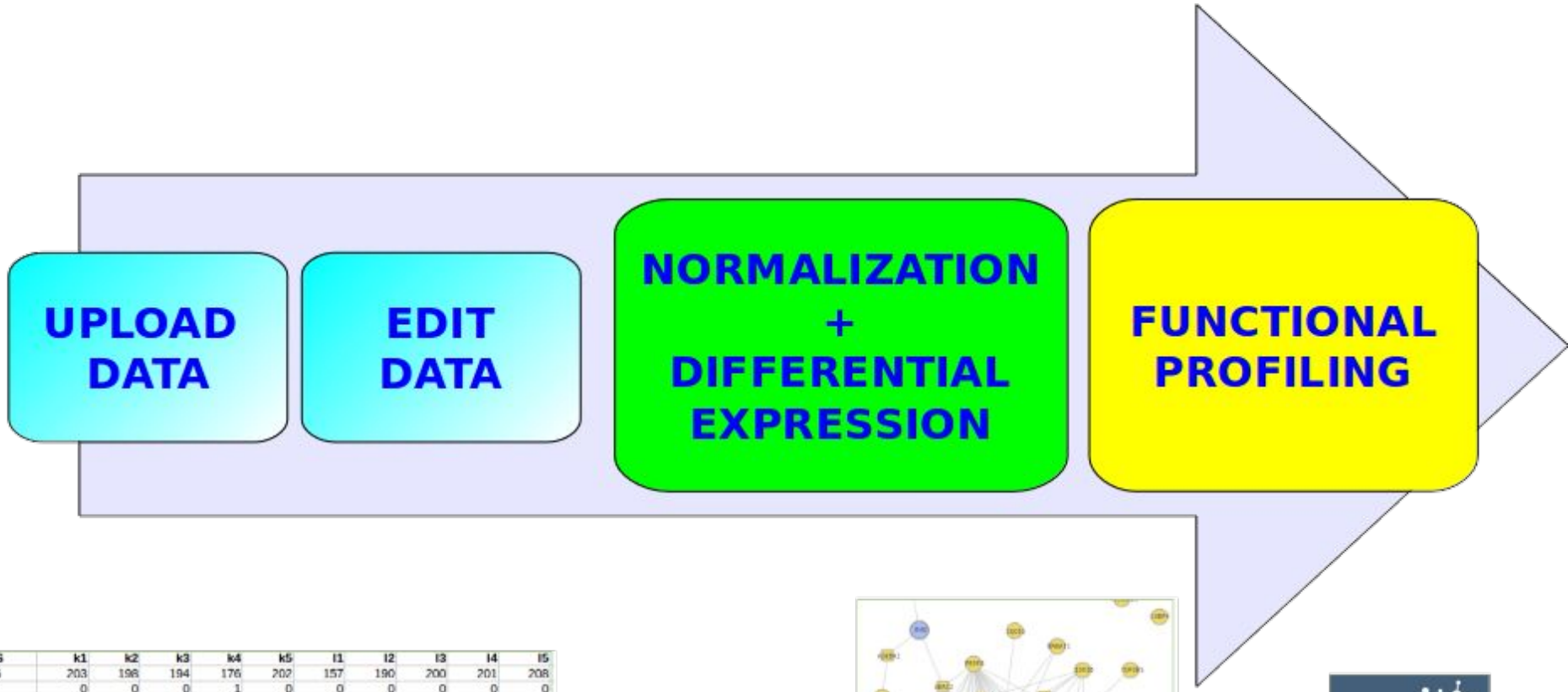
Count Normalization

- **RPKM**: Reads Per Kilobase of the transcript per Million mapped reads

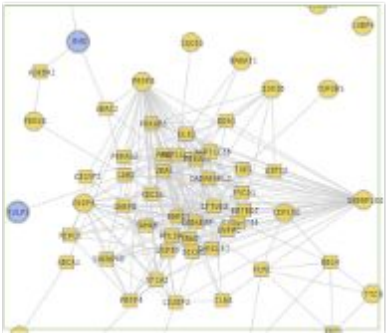
$$RPKM = 10^9 \times \frac{C}{N*L}$$

- **C** is the number of mappable reads mapped onto the gene's exons.
- **N** is the total number of mappable reads in the experiment.
- **L** is the total length of the exons in base pairs.
- Fragments Per Kilobase of exon per Million fragments mapped (FPKM),

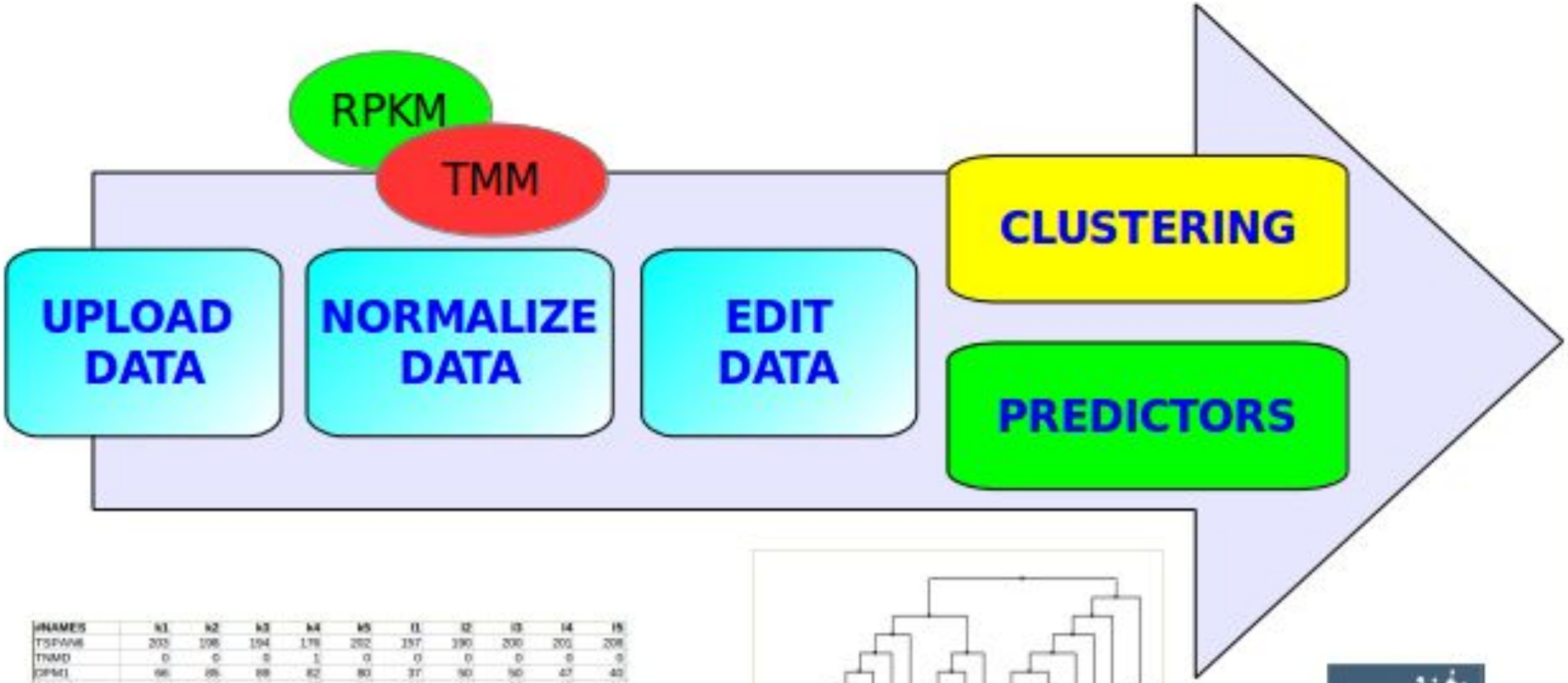
Working in Babelomics



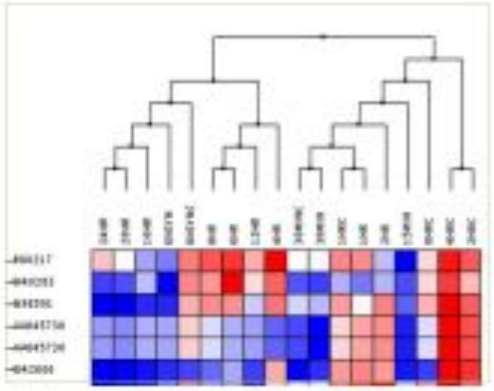
#NAMES	k1	k2	k3	k4	k5	l1	l2	l3	l4	l5
TSPAN6	203	198	194	176	202	157	190	200	201	208
TNMD	0	0	0	1	0	0	0	0	0	0
DPM1	66	85	89	82	80	37	50	50	47	40
SCYL3	21	30	31	27	31	28	31	37	15	21
C1orf112	10	12	8	11	18	17	22	12	12	19
FGR	19	28	18	20	10	47	50	43	49	48
FUCA2	240	272	261	256	211	76	82	85	68	83
GCLC	98	100	84	94	86	354	362	373	369	326
NFYA	59	61	53	56	59	59	66	63	66	62
STPG1	34	43	41	31	48	6	7	7	8	7



Working in Babelomics



#NAME/S	k1	k2	k3	k4	k5	l1	l2	l3	l4	l5
TSPAN8	203	198	194	178	202	197	190	200	201	208
TNMD	0	0	0	1	0	0	0	0	0	0
DPM1	86	89	88	82	80	37	50	50	47	40
SCYL3	21	30	31	27	31	28	31	37	15	21
C1orf112	10	12	8	11	18	17	22	12	12	19
FGR	19	28	18	20	10	47	50	43	49	48
FUCA2	340	272	261	256	211	76	82	85	68	63
GCLC	98	100	94	94	86	354	362	273	369	326
NFYA	59	61	53	58	59	59	66	63	66	62
STPG1	34	43	41	31	46	6	7	7	8	7



Any question?