Multi-omics data visualization
&
integration on dental pulp regeneration

Produced by
Victor DEGUISE, François VICTOR & Zakaria TOUGUI

under the supervision of
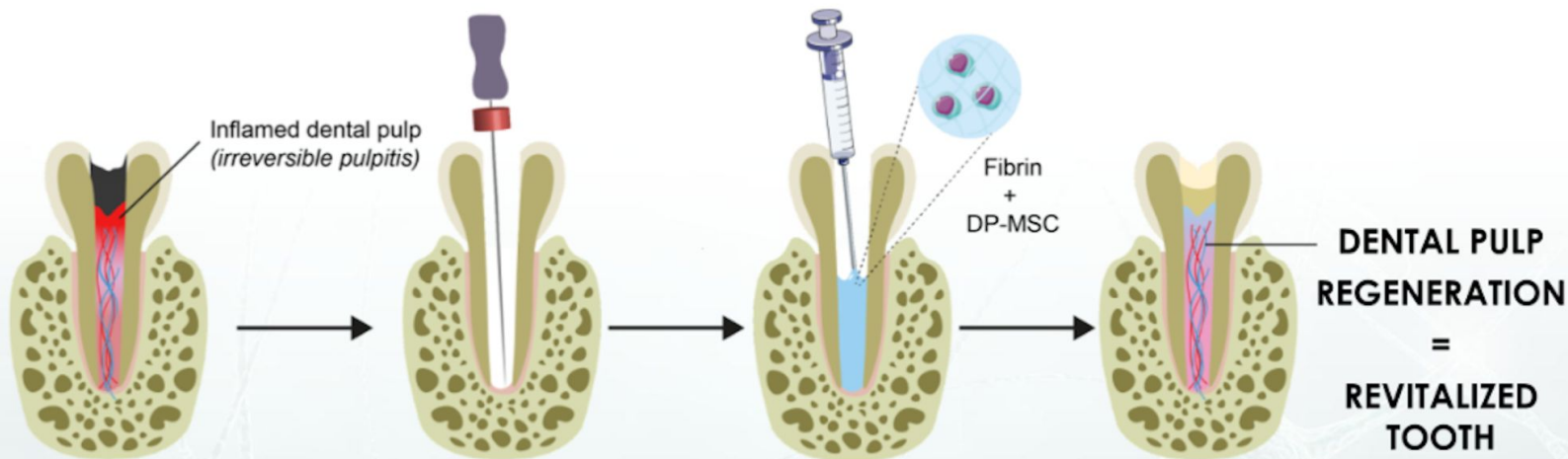Mourad **BEKHOUCHE** & Cécile **HILPERT**
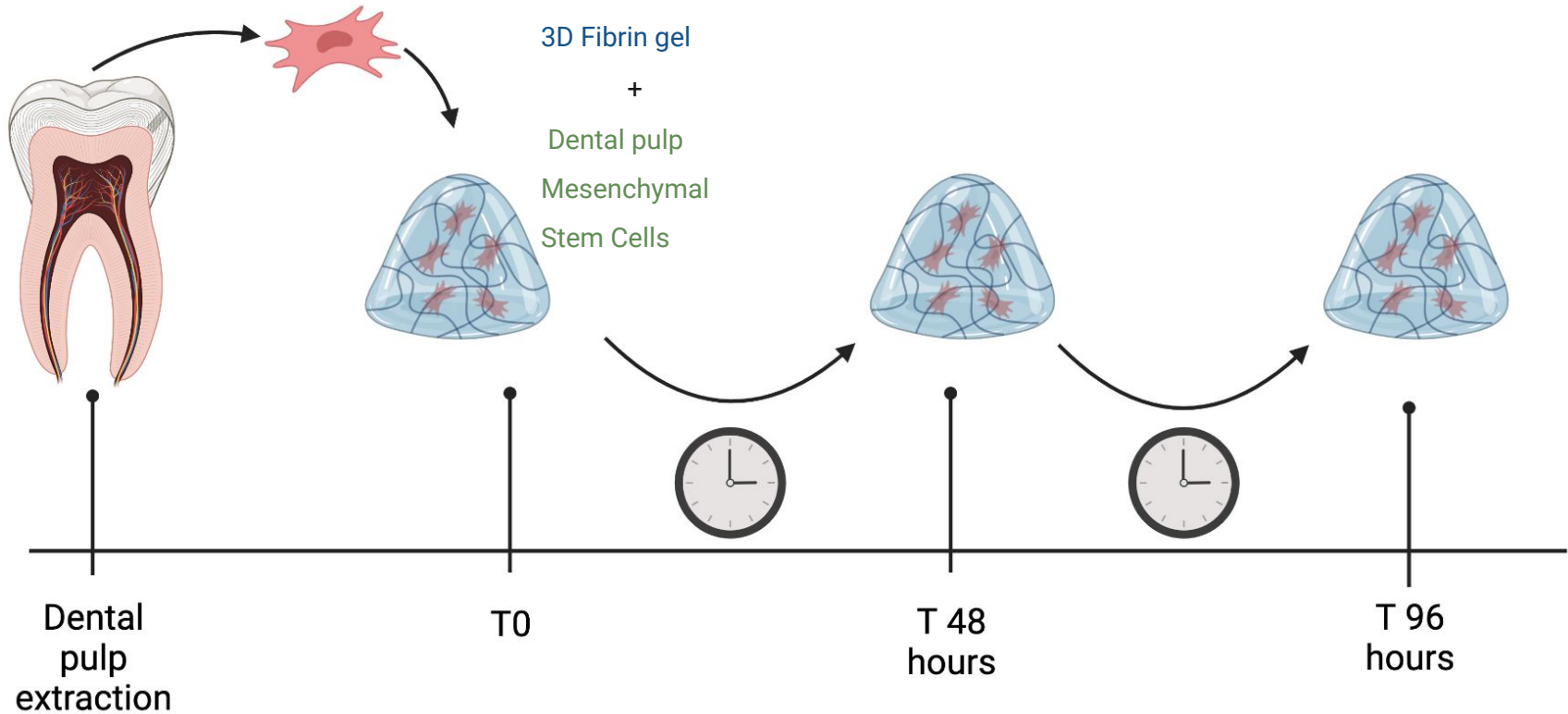
Université Claude Bernard Lyon 1

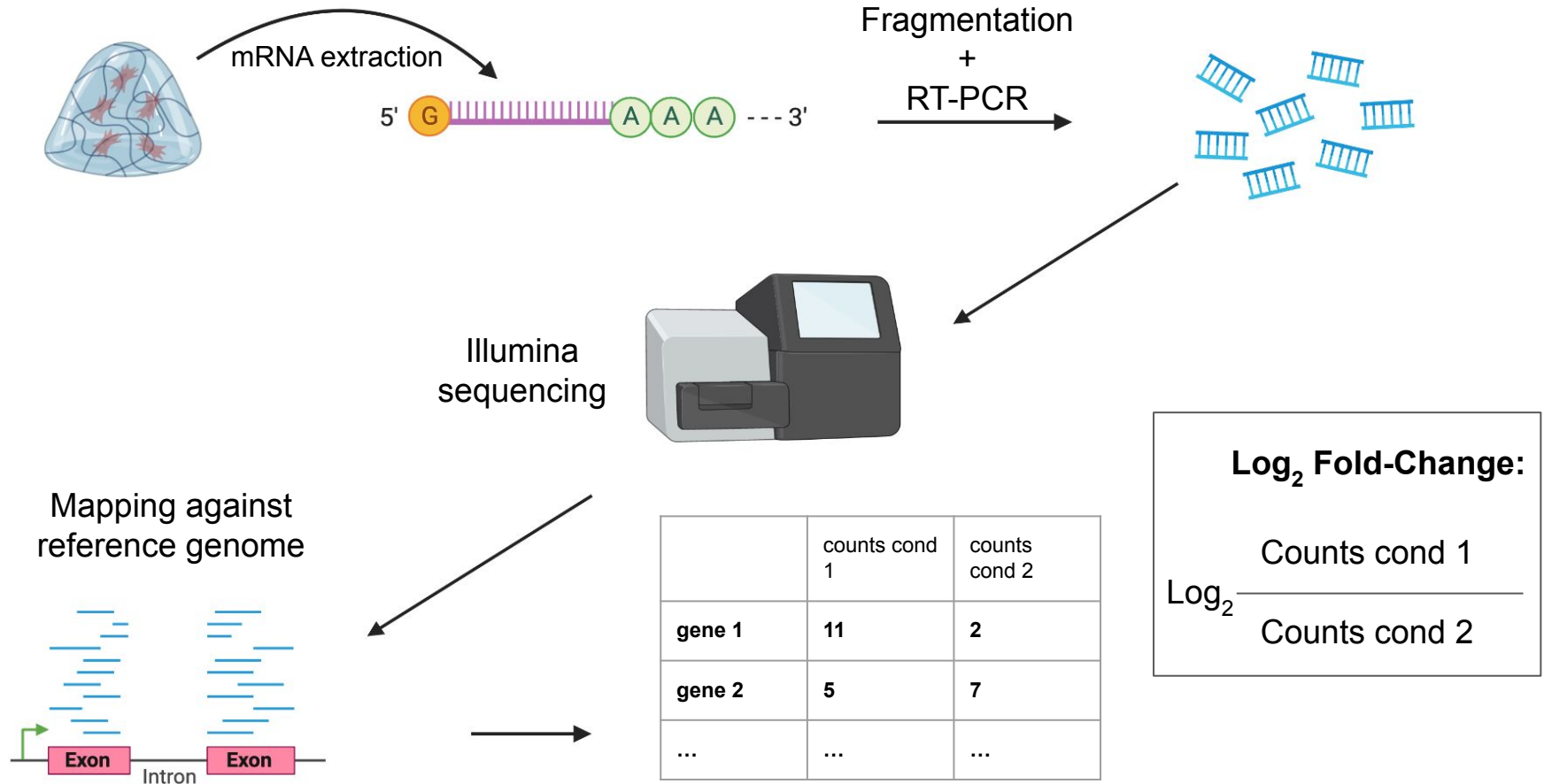# CONTEXT

REGENERATIVE ENDODONTIC PROCEDURES (REP)

Inflamed dental pulp (irreversible pulpitis)

Fibrin + DP-MSC

DENTAL PULP REGENERATION = REVITALIZED TOOTH

Ducret M, Costantini A, Gobert S, Farges J-C, Bekhouche M. Eur Cell Mater 2021;41:1–14.

Experimental design

3D Fibrin gel

+

Dental pulp
Mesenchymal
Stem Cells

Dental
pulp
extraction

T0

T 48
hours

T 96
hours

Transcriptomic data

mRNA extraction

5' G AAA - - - 3'

Fragmentation
+
RT-PCR

Illumina
sequencing

Mapping against
reference genome

Exon   Exon
Intron

	counts cond 1	counts cond 2
gene 1	11	2
gene 2	5	7
...	...	...

Log$_2$ Fold-Change:

$$\text{Log}_2 \frac{\text{Counts cond 1}}{\text{Counts cond 2}}$$

Proteomic data

TMT 9-Plex

D0   D2   D4

Donor 1

Donor 2

Donor 3

Selection

Quantification
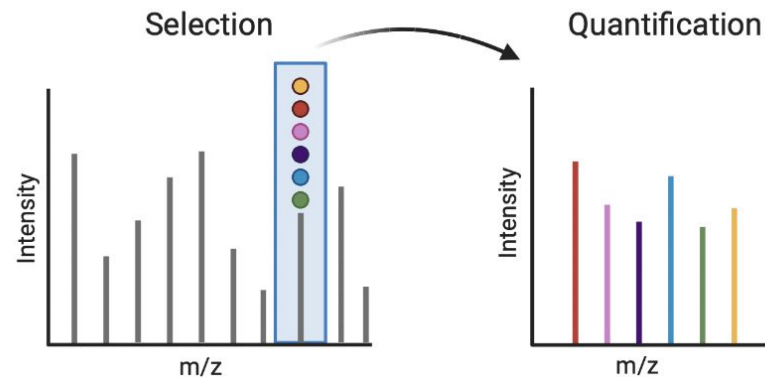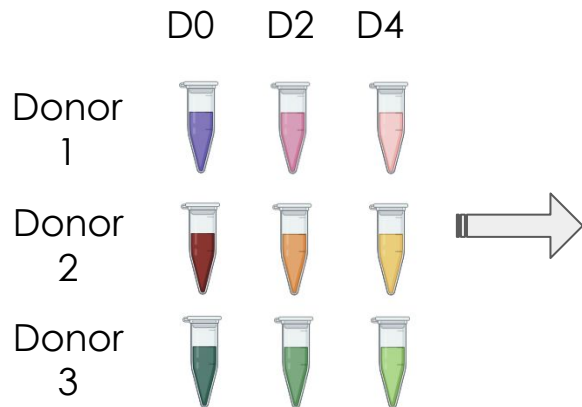
LC-MS/MS analysis

Experiment_1.xlsx

Experiment_2.xlsx

Two replicates with 3 timestamps each time

Proteomic data

Protein FDR Confidence	Master	Accession	Description	Contaminant	Sum PEP	Coverage	# Peptides	# PSMs	# Unique	# AAs	MW [kDa]	calc. pI	Score Sequest	# Peptides
High	Master Protein	P02751	Fibronectin OS=Homo sapiens OX=9606 GN=FN1 PE=1 SV=5	0	1079,65	55	92	607	92	2477	272,2	5,5	2264,21	92
High	Master Protein	sp	ALBU_BOVIN	1	573,971	77	46	652	42	607	69,2	6,18	1876,85	46
High	Master Protein	sp	ALBU_HUMAN	1	516,103	75	42	396	38	609	69,3	6,28	1273	42
High	Master Protein	sp	GELS_HUMAN	1	149,977	39	24	80	24	782	85,6	6,28	236,71	24
High	Master Protein	Q9P2E9	Ribosome-binding protein 1 OS=Homo sapiens OX=9606 GN=RRBP	0	144,903	41	35	82	35	1410	152,4	8,6	219,94	35
High	Master Protein	sp	PPIA_HUMAN	1	91,763	72	10	75	1	164	17,9	7,81	218,43	10
High	Master Protein	sp	GSTP1_HUMAN	1	91,288	61	10	40	10	209	23,2	5,64	180,46	10
High	Master Protein	sp	K1M1_SHEEP	1	88,686	47	19	63	10	412	46,6	4,81	182,94	19
High	Master Protein	P0DOX6	Immunoglobulin mu heavy chain OS=Homo sapiens OX=9606 PE	0	78,102	34	15	64	1	576	63,4	7,87	173,33	15
High	Master Protein	sp	PRDX1_HUMAN	1	76,513	65	12	58	9	199	22,1	8,13	168,52	12
High	Master Protein	sp	SYH_HUMAN	1	67,007	30	13	34	13	509	57,4	5,88	101,76	13
High	Master Protein	sp	RS27A_HUMAN	1	59,727	51	8	76	1	156	18	9,64	208,18	8
High	Master Protein	P04179	Superoxide dismutase [Mn], mitochondrial OS=Homo sapiens O	0	58,392	54	11	36	11	222	24,7	8,25	121,19	11
High	Master Protein	sp	TRYP_PIG	1	53,07	25	4	38	4	231	24,4	7,18	136,37	4
High	Master Protein	sp	NQO1_HUMAN	1	52,919	36	10	35	10	274	30,8	8,88	110,05	10
High	Master Protein	sp	K2M2_SHEEP	1	47,472	25	14	50	7	491	53,6	5,57	114,28	14
High	Master Protein	Q99873	Protein arginine N-methyltransferase 1 OS=Homo sapiens OX=9	0	35,395	32	10	28	10	371	42,4	5,35	71,52	10
High	Master Protein	sp	B2MG_HUMAN	1	32,511	55	5	15	5	119	13,7	6,52	54,48	5
High	Master Protein	sp	UBE2I_HUMAN	1	32,062	37	4	14	4	158	18	8,66	38,92	4
High	Master Protein	sp	K1HB_HUMAN	1	31,56	16	7	28	1	404	46,2	4,84	71,92	7
High	Master Protein	sp	K2M1_SHEEP	1	26,617	59	5	13	3	109	12,7	4,59	46,82	5
High	Master Protein	sp	KRHB5_HUMAN	1	25,06	16	9	31	2	507	55,8	6,55	61,25	9
High	Master Protein	sp	CATD_HUMAN	1	20,242	14	6	16	6	412	44,5	6,54	35,42	6
High	Master Protein	sp	ANT3_HUMAN	1	14,791	13	5	11	5	464	52,6	6,71	27,63	5
High	Master Protein	sp	K1H6_HUMAN	1	13,875	10	5	10	1	467	52,2	4,94	24,3	5
High	Master Protein	P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens OX=9606 GN=AHSG	0	13,249	20	5	9	5	367	39,3	5,72	14,91	5
High	Master Protein	Q9H3H3	UPF0696 protein C11orf68 OS=Homo sapiens OX=9606 GN=C11	0	12,236	16	4	7	4	292	31,4	6,32	17,31	4
High	Master Protein	O00468	Agrin OS=Homo sapiens OX=9606 GN=AGRN PE=1 SV=6	0	9,413	2	3	5	3	2068	217,2	6,39	10,48	3
High	Master Protein	sp	IGF2_HUMAN	VRAI	8,892	14	2	5	2	180	20,1	9,32	15,3	2
High	Master Protein	O43847	Nardilysin OS=Homo sapiens OX=9606 GN=NRDC PE=1 SV=3	FAUX	5,947	1	2	3	2	1151	131,6	4,98	7,08	2
High	Master Protein	sp	BID_HUMAN	VRAI	5,852	8	1	1	1	195	22	5,44	3,32	1
High	Master Protein	Q9NZL4	Hsp70-binding protein 1 OS=Homo sapiens OX=9606 GN=HSPBP	FAUX	5,39	4	1	2	1	359	39,3	5,21	6,1	1
High	Master Protein	sp	RETBP_HUMAN	VRAI	5,272	9	1	1	1	201	23	6,07	4,56	1
High	Master Protein	Q52LJ0	Protein FAM98B OS=Homo sapiens OX=9606 GN=FAM98B PE=1	FAUX	5,125	4	2	4	2	433	45,5	8,69	8,37	2

Functional requirements

1 : Identify significantly differentially expressed genes and differentially abundant proteins

2 : Identify the functions and associated biological processes by querying a database of annotations (GO database) and performing enrichment tests

3 : Comparative analysis between transcripts and proteins

4 : Deploying interactive notebook online without visible code
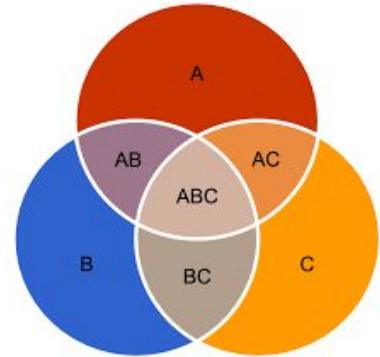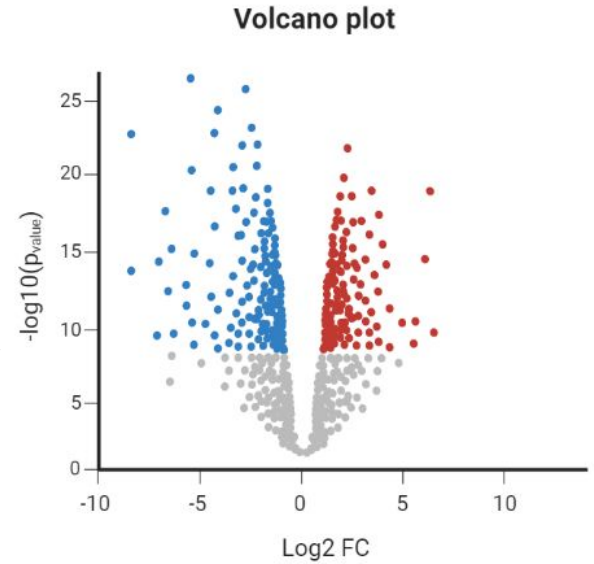
Volcano plot
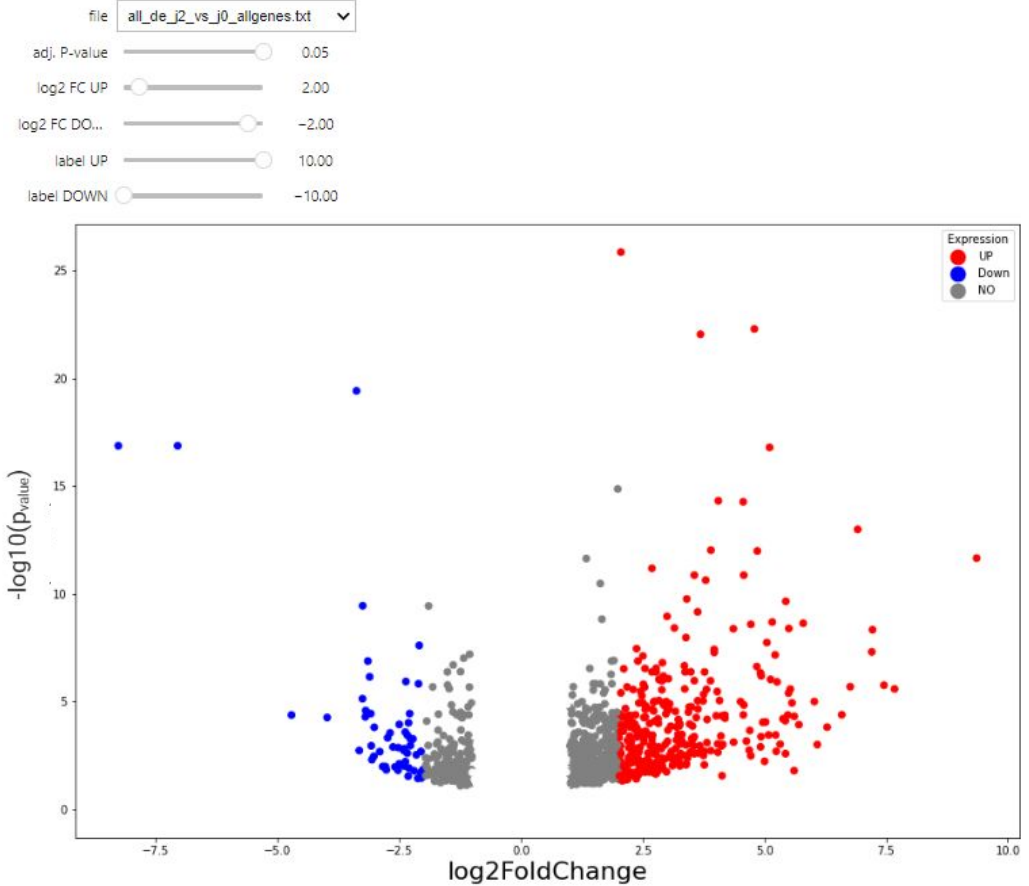
Volcano plots


Volcano plot of the transcripts
fold change between D0 and D2

8

Table to show the results viewed on the volcano plot

file J2/J0 ▾

Show 10 ▾ entries

Search: [   ]

symbol	baseMean	log2FoldChange	lfcSE	pvalue	padj
5_8S_rRNA	0	NaN	NaN	NaN	NaN
5S_rRNA	0.038653	-0.001017	0.274193	0.9825698	NaN
7SK	0.517037	-0.019322	0.273432	0.6740422	NaN
A1BG	595.000641	-0.027497	0.221786	0.831401	0.9279159
A1BG-AS1	391.792709	-0.199175	0.182067	0.1597196	0.4158482
A1CF	0.084189	0.011649	0.274288	0.8800164	NaN
A2M	281.240669	6.91734	0.919416	6.1939e-17	1.01879e-13
A2M-AS1	7.184134	-0.163297	0.312138	0.1476119	0.3982945
A2ML1	0	NaN	NaN	NaN	NaN
A2ML1-AS1	0	NaN	NaN	NaN	NaN

Previous 1 2 3 4 5 … 137 Next

Venn diagrams
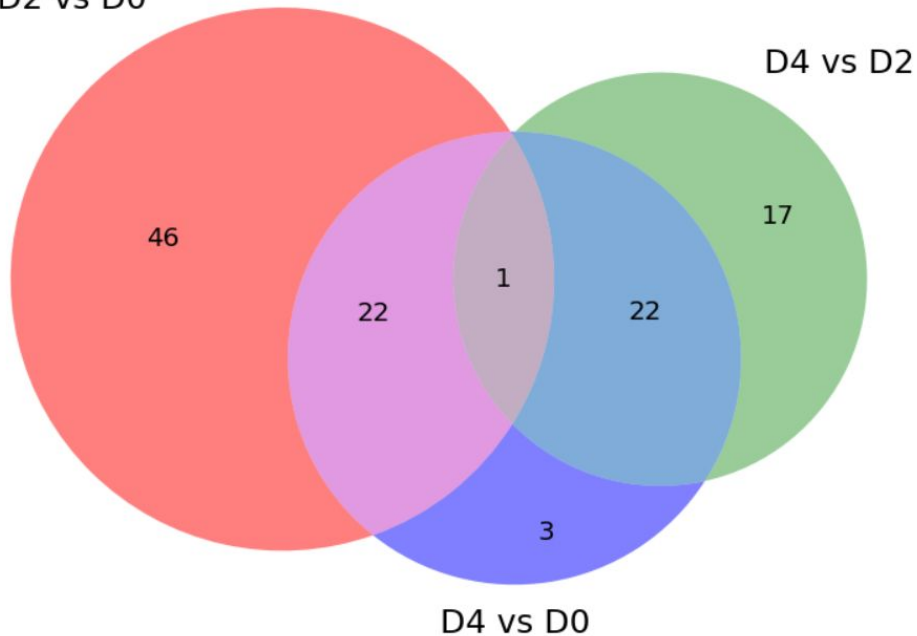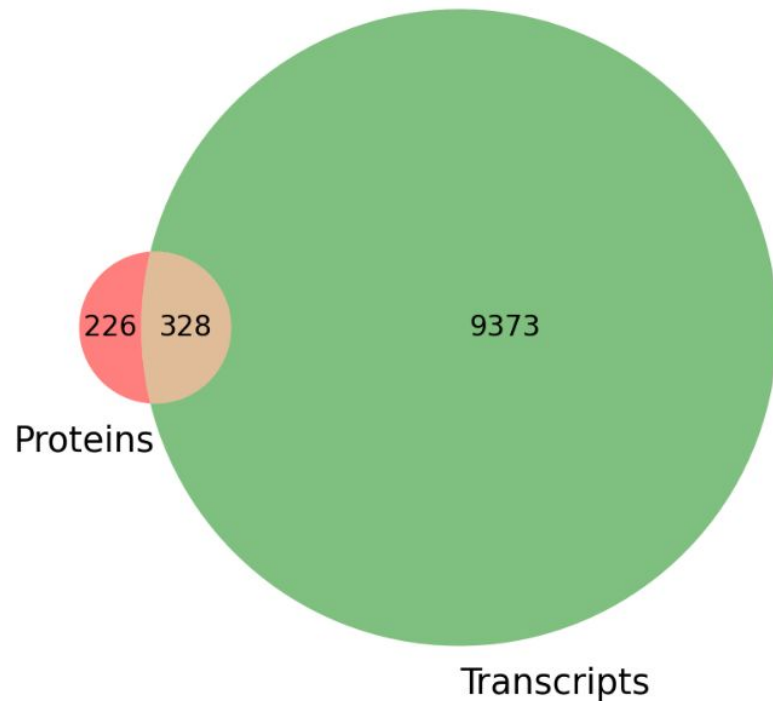
Gene Set Enrichment Analysis

- Competitive null hypothesis against Gene Ontology terms
- J2/J0 differentially abundant transcripts enrichment
  - → **Glycolysis, signal transduction, perception of EC environment**

	Gene_set	Term	Overlap	Adjusted P-value	Genes
0	GO_Biological_Process_2021	glycolytic process (GO:0006096)	10/29	0.001133	LDHA;TPI1;PKLR;HKDC1;PGK1;ALDOC;ENO2;ALDOA;GAP...
1	GO_Biological_Process_2021	canonical glycolysis (GO:0061621)	9/24	0.001133	TPI1;PKLR;PGK1;ALDOC;ENO2;ALDOA;GAPDH;HK2;PFKP
2	GO_Biological_Process_2021	glucose catabolic process to pyruvate (GO:0061...	9/24	0.001133	TPI1;PKLR;PGK1;ALDOC;ENO2;ALDOA;GAPDH;HK2;PFKP
3	GO_Biological_Process_2021	glycolytic process through glucose-6-phosphate...	9/25	0.001270	TPI1;PKLR;PGK1;ALDOC;ENO2;ALDOA;GAPDH;HK2;PFKP
4	GO_Biological_Process_2021	cellular response to cytokine stimulus (GO:007...	47/482	0.003846	RIPOR2;IL1RN;CCL11;SELPLG;IL24;RORA;AKAP6;CXCL...

	Gene_set	Term	Overlap	Adjusted P-value	Genes
0	GO_Molecular_Function_2021	receptor ligand activity (GO:0048018)	32/307	0.028938	SEMA7A;COLEC10;CCL11;TNFRSF11B;FGF1;AREG;PTHLH...

	Gene_set	Term	Overlap	Adjusted P-value	Genes
0	GO_Cellular_Component_2021	ionotropic glutamate receptor complex (GO:0008...	8/32	0.016052	GRIA1;OLFM2;DLG4;GRIK4;GRIK2;GRIN2C;CNIH3;GRIN1
1	GO_Cellular_Component_2021	collagen-containing extracellular matrix (GO:0...	36/380	0.016052	COL17A1;LRRC15;SEMA7A;SERPINA1;COL13A1;LAMC3;L...
2	GO_Cellular_Component_2021	integral component of plasma membrane (GO:0005...	101/1454	0.018797	ICAM5;ICAM1;SLC8A2;AQP1;MILR1;EDNRA;HTR7;SLC16...
3	GO_Cellular_Component_2021	potassium channel complex (GO:0034705)	12/80	0.031223	KCNF1;KCNJ8;KCNIP2;KCNC3;KCNIP3;GRIK4;ABCC9;GR...

Gene Set Enrichment Analysis

- Competitive null hypothesis against Gene Ontology terms
- J2/J0 differentially abundant proteins enrichment
  - → **ECM organization, exocytosis, regulation of fibrinolysis**

	Gene_set	Term	Overlap	Adjusted P-value	Genes
0	GO_Biological_Process_2021	extracellular matrix organization (GO:0030198)	16/300	7.015200e-20	APP;VWF;MMP2;SERPINF2;MMP3;SERPINE1;PLG;THBS1;...
1	GO_Biological_Process_2021	extracellular structure organization (GO:0043062)	13/216	1.340338e-16	APP;VWF;MMP2;MMP3;SERPINE1;THBS1;DCN;COL3A1;CO...
2	GO_Biological_Process_2021	external encapsulating structure organization ...	13/217	1.340338e-16	APP;VWF;MMP2;MMP3;SERPINE1;THBS1;DCN;COL3A1;CO...
3	GO_Biological_Process_2021	platelet degranulation (GO:0002576)	9/125	1.046657e-11	APP;VWF;SERPINF2;SERPINE1;APOA1;PLG;TIMP1;THBS...
4	GO_Biological_Process_2021	regulated exocytosis (GO:0045055)	9/180	2.330239e-10	APP;VWF;SERPINF2;SERPINE1;APOA1;PLG;TIMP1;THBS...
5	GO_Biological_Process_2021	collagen fibril organization (GO:0030199)	7/89	3.065533e-09	COL3A1;COL1A2;COL5A1;COL4A2;SERPINF2;COL6A1;CO...
6	GO_Biological_Process_2021	regulation of complement activation (GO:0030449)	6/50	5.697646e-09	C1QB;C3;C5;C1S;CFP;C1QC
7	GO_Biological_Process_2021	regulation of immune effector process (GO:0002...	6/53	7.175437e-09	C1QB;C3;C5;C1S;CFP;C1QC
8	GO_Biological_Process_2021	regulation of humoral immune response (GO:0002...	6/54	7.175437e-09	C1QB;C3;C5;C1S;CFP;C1QC
9	GO_Biological_Process_2021	negative regulation of fibrinolysis (GO:0051918)	4/9	2.376390e-08	SERPINE1;SERPINF2;PLG;THBS1
10	GO_Biological_Process_2021	supramolecular fiber organization (GO:0097435)	9/351	4.116684e-08	APP;COL3A1;COL1A2;COL5A1;COL4A2;SERPINF2;COL6A...
11	GO_Biological_Process_2021	regulation of fibrinolysis (GO:0051917)	4/13	1.119484e-07	SERPINE1;SERPINF2;PLG;THBS1
12	GO_Biological_Process_2021	positive regulation of blood coagulation (GO:0...	4/17	3.426633e-07	SERPINE1;SERPINF2;PLG;THBS1

Gene Set Enrichment Analysis

- Competitive null hypothesis against Gene Ontology terms
- J4/J2 differentially abundant transcripts enrichment → **mitotic processes**

	Gene_set	Term	Overlap	Adjusted P-value	Genes
0	GO_Biological_Process_2021	microtubule cytoskeleton organization involved...	40/128	4.757960e-16	ERCC6L;BUB1B;CDCA8;TTK;KIF11;CENPA;SKA1;AURKB;...
1	GO_Biological_Process_2021	mitotic spindle organization (GO:0007052)	43/157	2.806952e-15	ERCC6L;BUB1B;CDCA8;TTK;KIF11;CENPA;SKA1;AURKB;...
2	GO_Biological_Process_2021	mitotic sister chromatid segregation (GO:0000070)	27/102	1.061352e-08	CDCA5;NCAPG2;KIF14;NCAPG;CDCA8;NCAPH;CCNB1;NUS...
3	GO_Biological_Process_2021	kinetochore organization (GO:0051383)	9/13	2.860446e-06	CDT1;CENPE;CENPF;CENPW;CENPH;NUF2;CENPA;NDC80;...
4	GO_Biological_Process_2021	centromere complex assembly (GO:0034508)	14/37	3.911693e-06	CENPU;CENPV;CENPW;HJURP;KNL1;CENPA;CENPE;CENPF...
5	GO_Biological_Process_2021	sister chromatid segregation (GO:0000819)	13/34	9.888964e-06	TOP2A;SPAG5;NCAPG2;PLK1;CDCA8;KNSTRN;ZWINT;NDC...
6	GO_Biological_Process_2021	mitotic nuclear division (GO:0140014)	18/74	4.841909e-05	SPAG5;UBE2C;NDE1;NCAPG2;PLK1;CDCA8;KIF11;KNSTR...
7	GO_Biological_Process_2021	regulation of cyclin-dependent protein serine/...	19/82	4.841909e-05	BLM;CDKN2B;CCNF;PLK1;PROX1;PKD2;CDC25C;PKMYT1;...
8	GO_Biological_Process_2021	glycolytic process (GO:0006096)	11/29	9.267617e-05	PFKFB2;GPI;LDHA;TPI1;PGAM1;PGK1;ALDOC;ENO1;ENO...
9	GO_Biological_Process_2021	canonical glycolysis (GO:0061621)	10/24	9.497093e-05	GPI;TPI1;PGAM1;PGK1;ALDOC;ENO1;ENO2;ALDOA;HK2;...

Gene Set Enrichment Analysis

- Competitive null hypothesis against Gene Ontology terms
- J4/J2 overabundant proteins enrichment → **Metabolism, cell morphogenesis**

	Gene_set	Term	Overlap	Adjusted P-value	Genes
403	GO_Cellular_Component_2021	intracellular organelle lumen (GO:0070013)	11/848	1.141924e-08	GLUD1;HMGCL;FH;ECHS1;HSPA5;MDH2;C1QBP;FGG;SERP...
404	GO_Cellular_Component_2021	mitochondrial matrix (GO:0005759)	8/348	4.144643e-08	GLUD1;HMGCL;FH;ECHS1;MDH2;C1QBP;DLD;ACAT1
0	GO_Biological_Process_2021	cellular amino acid catabolic process (GO:0009...	5/90	1.084072e-05	GLUD1;HMGCL;ECHS1;DLD;ACAT1
1	GO_Biological_Process_2021	branched-chain amino acid catabolic process (G...	3/20	1.281631e-04	ECHS1;DLD;ACAT1
2	GO_Biological_Process_2021	branched-chain amino acid metabolic process (G...	3/20	1.281631e-04	ECHS1;DLD;ACAT1
3	GO_Biological_Process_2021	positive regulation of substrate adhesion-depe...	3/36	5.479377e-04	FGB;C1QBP;FGG
4	GO_Biological_Process_2021	platelet degranulation (GO:0002576)	4/125	5.479377e-04	FGB;FGG;LAMP2;SERPING1
5	GO_Biological_Process_2021	negative regulation of blood coagulation (GO:0...	3/40	5.479377e-04	FGB;FGG;SERPING1
6	GO_Biological_Process_2021	positive regulation of peptide hormone secreti...	3/43	5.854625e-04	FGB;GLUD1;FGG
7	GO_Biological_Process_2021	positive regulation of cell morphogenesis invo...	3/50	6.833829e-04	FGB;C1QBP;FGG
8	GO_Biological_Process_2021	regulation of substrate adhesion-dependent cel...	3/51	6.833829e-04	FGB;C1QBP;FGG
9	GO_Biological_Process_2021	fatty acid beta-oxidation (GO:0006635)	3/52	6.833829e-04	HADHB;ECHS1;ACAT1
10	GO_Biological_Process_2021	malate metabolic process (GO:0006108)	2/7	6.833829e-04	FH;MDH2

Displaying interactive results online

Transcripto

file	all_de_j2_vs_j0_allgenes.txt
adj. P-value	0.05
log2 FC UP	2.00
log2 FC DO...	−2.00
label UP	10.00
label DOWN	−10.00


```
<function __main__.volcano_plot(file, pval, l2FC_up, l2FC_down, label_up, label_down)>
```

file	J2/J0

Show 10 entries

Search:

symbol   baseMean   log2FoldChange   lfcSE   pvalue   padj

Conclusion

To sum up:

- Interactive visualisations accessible for users without programming background
- Functional annotations and geneset comparison between datasets

Limitations:

- Few options for displaying a Jupyter notebook into a webpage
- Data integration is specific to the formats used → limited reusability
- Experimental design was not intended for multi-omic integration → no standard method to combine the two types of data

Further improvements

- Deploying the html page on university's servers with the help of iCap

  → **URL**: https://momics.univ-lyon1.fr

- Adding more options for the input format

Thank you for your attention !

Any questions ?