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anses

Comparative study of the pulmonary toxicity of actinolite cleavage fragments and asbestos fibers

(ToxiPulFraCam)

Bara LO

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Introduction

Mineral particles

Mineral elongated particles or fibers

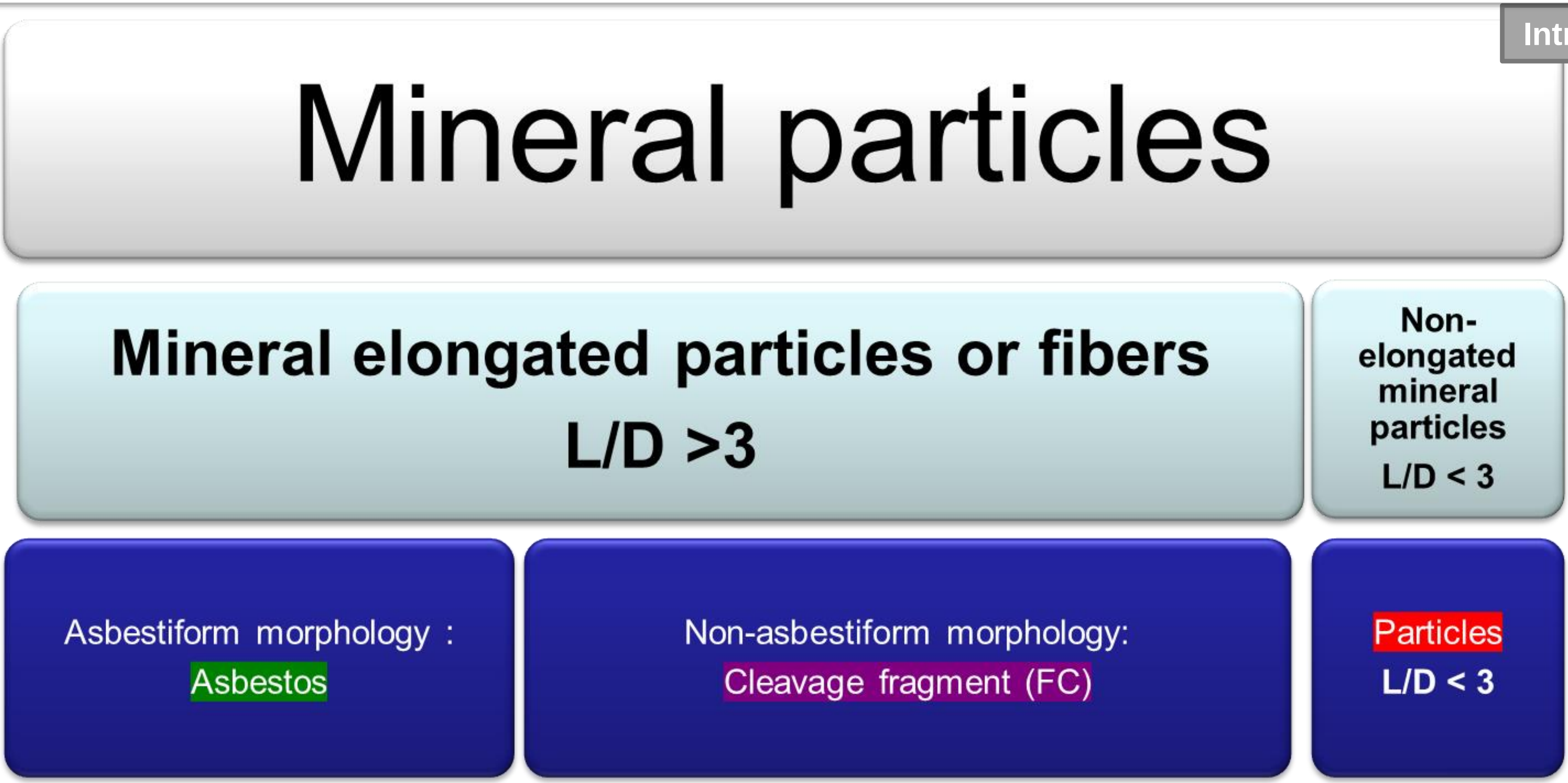
L/D >3

Non-elongated mineral particles

L/D < 3

NF X 43-050

Introduction



Mineral particles

Mineral elongated particles or fibers

L/D >3

Non-elongated mineral particles

L/D < 3

NF X 43-050

Asbestiform morphology :
Asbestos

Non-asbestiform morphology:
Cleavage fragment (FC)

Particles
L/D < 3

Introduction

Mineral particles

Mineral elongated particles or fibers
L/D >3

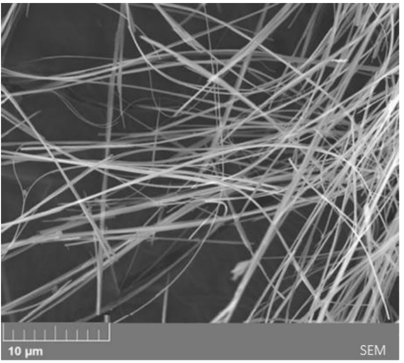
Non-elongated mineral particles
L/D < 3

NF X 43-050

Asbestiform morphology :
Asbestos

Non-asbestiform morphology:
Cleavage fragment (FC)

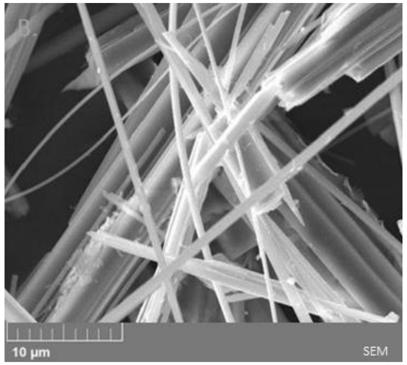
Particles
L/D < 3



Asbestos

- Hydrated silicates
- Unidimensional growth
  - ➢ Long and filamentous fibers
- Regulated (WHO) :
  - ➢ L > 5 µm - D < 3 µm - L/D > 3

Fibres d'amiante d'actinolite
(Lahondère et al., 2018)



Cleavage fragment

- Same chemical composition as asbestos
- Bi/tridimensional growth
  - ➢ Shorter and wider fibers
- No regulation

Fragments de clivage d'actinolite
(Lahondère et al., 2018)

Context

- Fragmentation generated by mechanical stress applied to amphibole-type rocks
  - ❑ Dispersion of asbestos fibers (FA) and actinolite cleavage fragments (FC) in the environment

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- Well-known toxicological effects of "asbestiform" fibers defined by the WHO
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  - Lung cancers
  - Fibrosis, …

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  - ❑ Lung cancers
  - ❑ Fibrosis, …
- Limited data on cleavage fragments (FC)
  - ❑ Epidemiological studies do not exclude health risk : non-asbestiform amphibole fragments
  - ❑ Amphiboles linked to cancer : not avaible on rigorously characterized cleavage fragments
  - ❑ No evidence : "WHO" fragments less toxic than their asbestiform counterparts
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Context

- Fragmentation generated by mechanical stress applied to amphibole-type rocks
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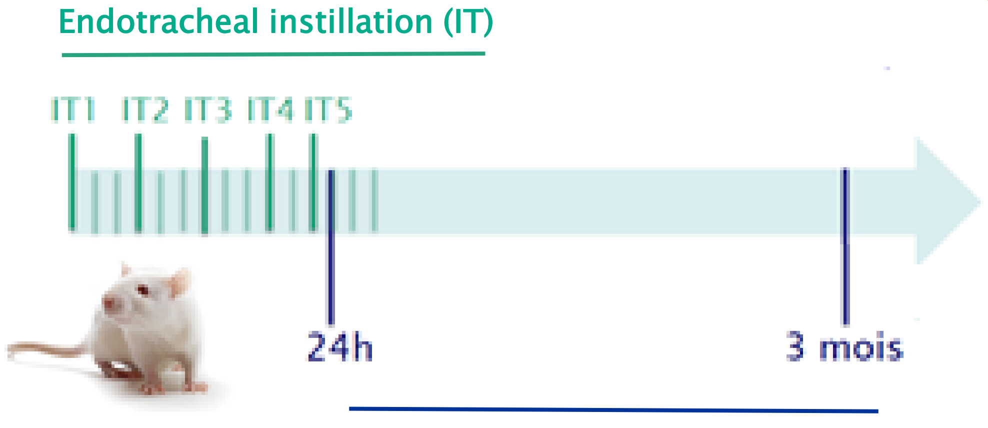
- Recommendation from ANSES to "conduct toxicology studies using rigorously characterized samples of elongated particles "
  - ❑ Preparation of Actinolite FC (with less than 1.2% FA content): BRGM (Bureau of Geological and Mining Research)
  - ❑ Preparation of Actinolite FA: AD-LAB Company (a group of asbestos experts and professional analysts)

Objectives

- Assessment of the pulmonary toxicity of actinolite cleavage fragments [FC] compared to asbestiform actinolite (actinolite asbestos fibers [FA]) in vivo in rats
  - Pulmonary toxicological effects of FC after short- and medium-term exposure
  - Biopersistence of these fibers through biometrology studies

Experimental plan



Sacrifice of rats

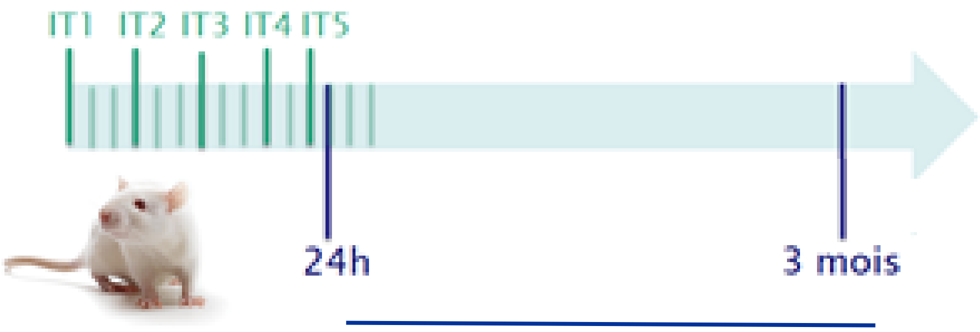
Groupes de rats

① Control	➡	véhicule (NaCl 0,9%)
② Asbestos Fibers (FA)	➡	0,6 mg/rat/IT : **3 mg/rat**
③ Cleavage fragment (FgtC)	➡	0,6 mg/rat/IT : **3 mg/rat**
④ Cleavage fragment (FgtC)	➡	1,4 mg/rat/IT : **7 mg/rat**

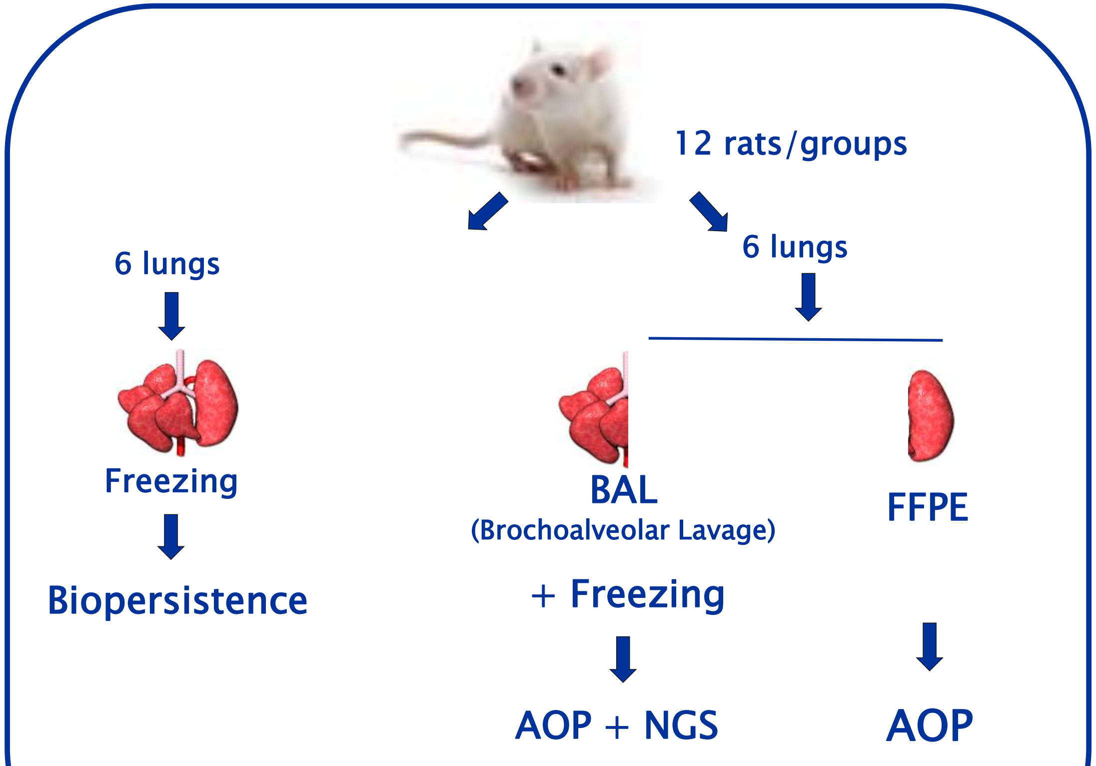
FgtC/FA : weight dose ➡ number dose ➡

Experimental plan

Endotracheal instillation (IT)



Sacrifice of rats



12 rats/groups

6 lungs

Freezing

Biopersistence

6 lungs

BAL
(Brochoalveolar Lavage)

+ Freezing

AOP + NGS

FFPE

AOP

Groupes de rats

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Experimental plan

- Assessment of the pulmonary toxicity of actinolite cleavage fragments [FC] compared to asbestiform actinolite (actinolite asbestos fibers [FA]) in vivo in rats

Axe 1 : Biopersistence analysis

Axe 2 :
Effects on Adverse Outcome Pathways (AOP) : precancerous and fibrosis marker assessments and Histopathological analyses

Conclusion : Axe 1 and 2

- Asbestos instillation: 50% fibers (25% WHO fibers)
- Cleavage fragment instillation: 25% fibers (5% WHO fibers)
- Short fibers ($L < 5\mu m$) $> 3/4$ of total fibers in the lungs
- Between J1 and J90, on average, the lung eliminates approximately 70% of the WHO fibers
- Histopathologicals analyses and Precancerous and fibrosis markers
  - Cleavage fragments display:
    - Temporary effects: lesions, hemorrhage, inflammation in the BAL
    - Persistent effects: inflammation, collagen deposits, apoptosis
  - Asbestos shows effects only persisting at D90

Experimental plan

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Axe 1 : Biopersistence analysis

Axe 2 :
Effects on Adverse Outcome Pathways (AOP) : precancerous and fibrosis marker assessments and Histopathological analyses

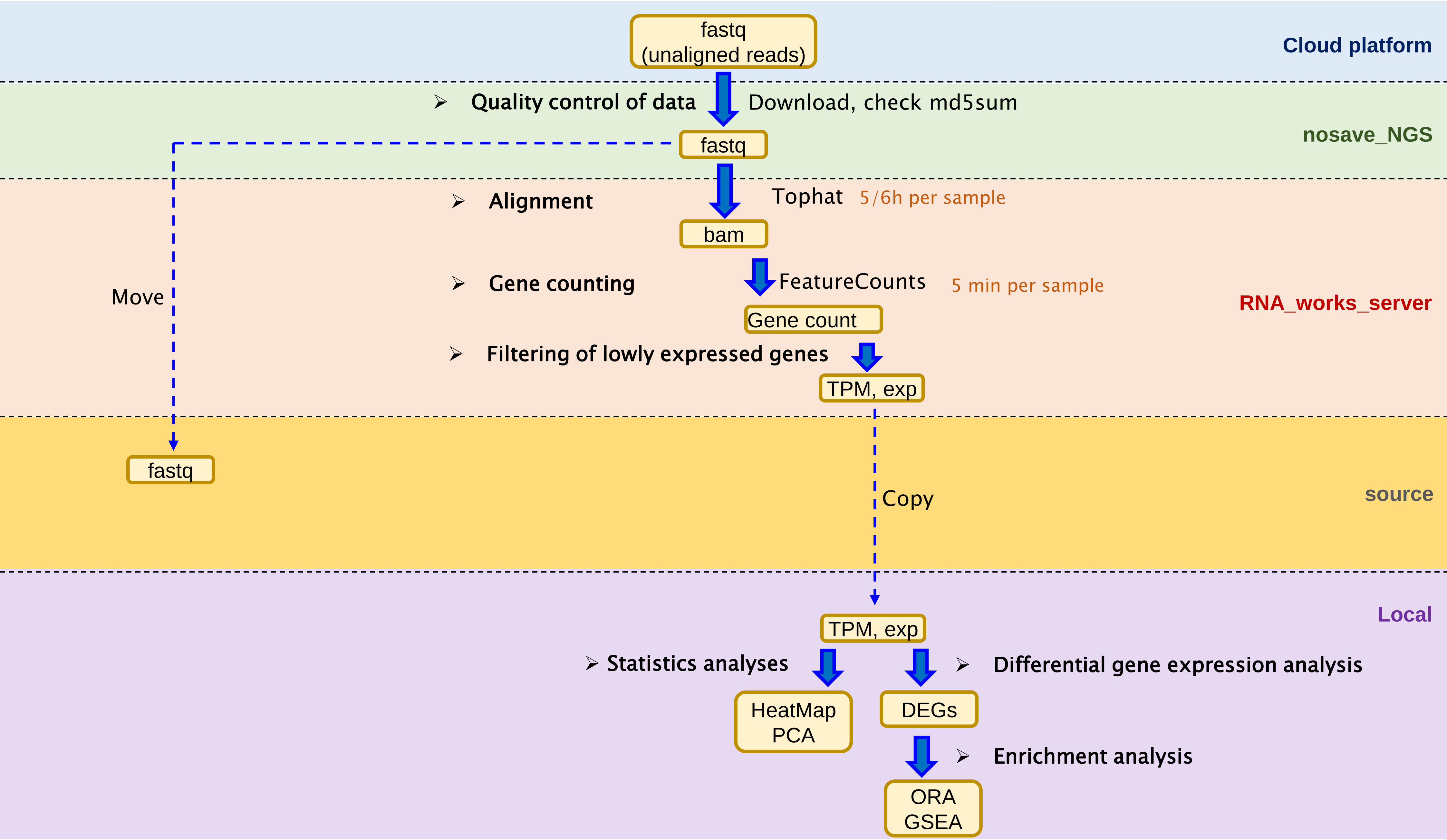
Axe 3 : Transcriptome analysis by RNA-Seq

Methylome and miRNome analysis by Methyl-Seq and miRNA-Seq

Transcriptome analysis by RNA-Seq

RNA-Seq workflow

Transcriptome analysis by RNA-Seq

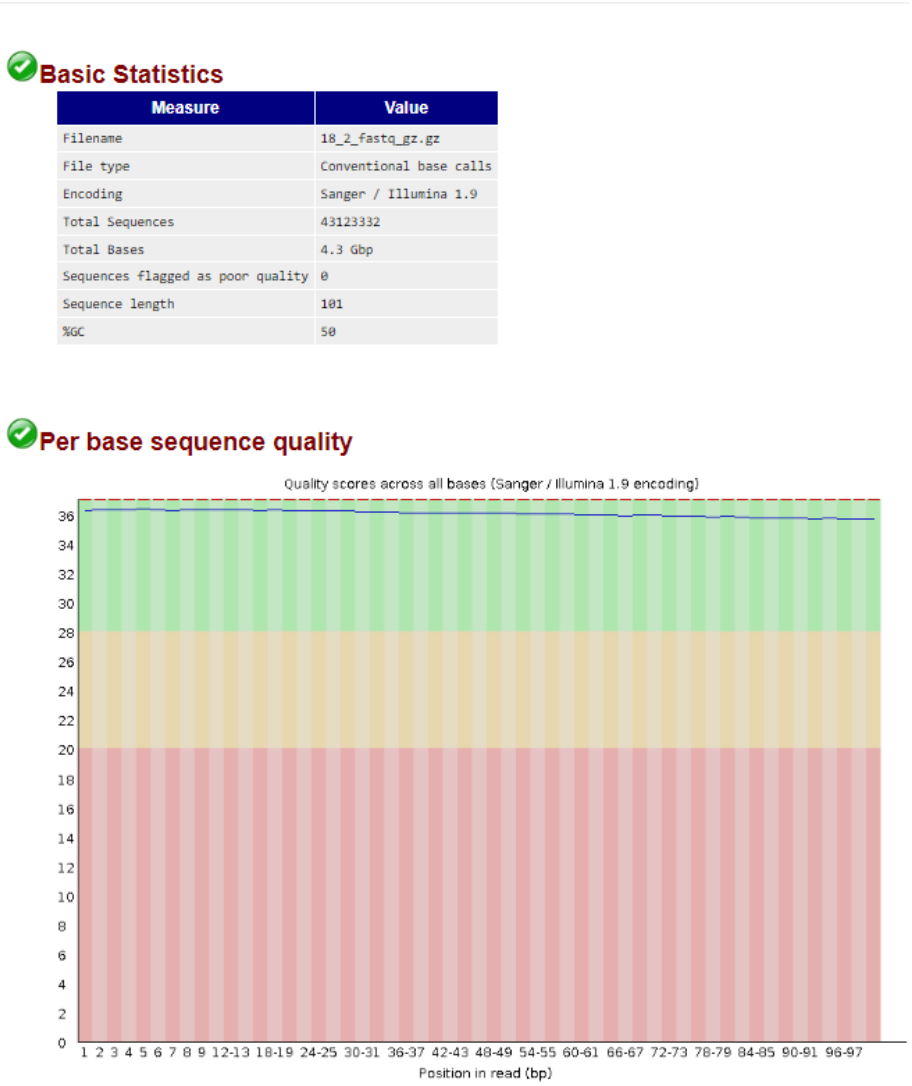


- ➢ **Quality control of data**
- ➢ **Alignment**
- ➢ **Gene counting**
- ➢ **Filtering of lowly expressed genes**
- ➢ Statistics analyses
- ➢ Differential gene expression analysis
- ➢ Enrichment analysis

RNA-Seq workflow

Transcriptome analysis by RNA-Seq

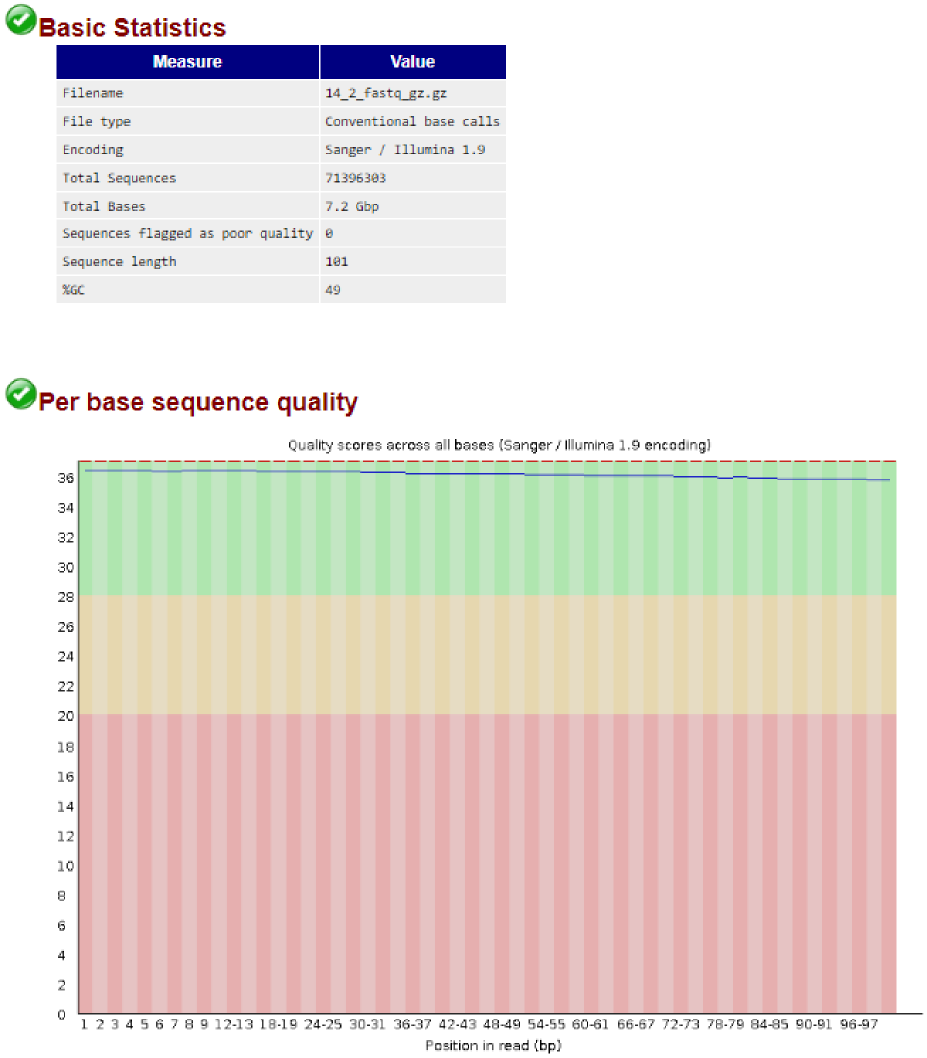
- **Quality control of data :** FastQC to assess the quality of raw data (fastq)



Basic Statistics

Measure	Value
Filename	18_2_fastq_gz.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	43123332
Total Bases	4.3 Gbp
Sequences flagged as poor quality	0
Sequence length	101
%GC	50

Per base sequence quality



Basic Statistics

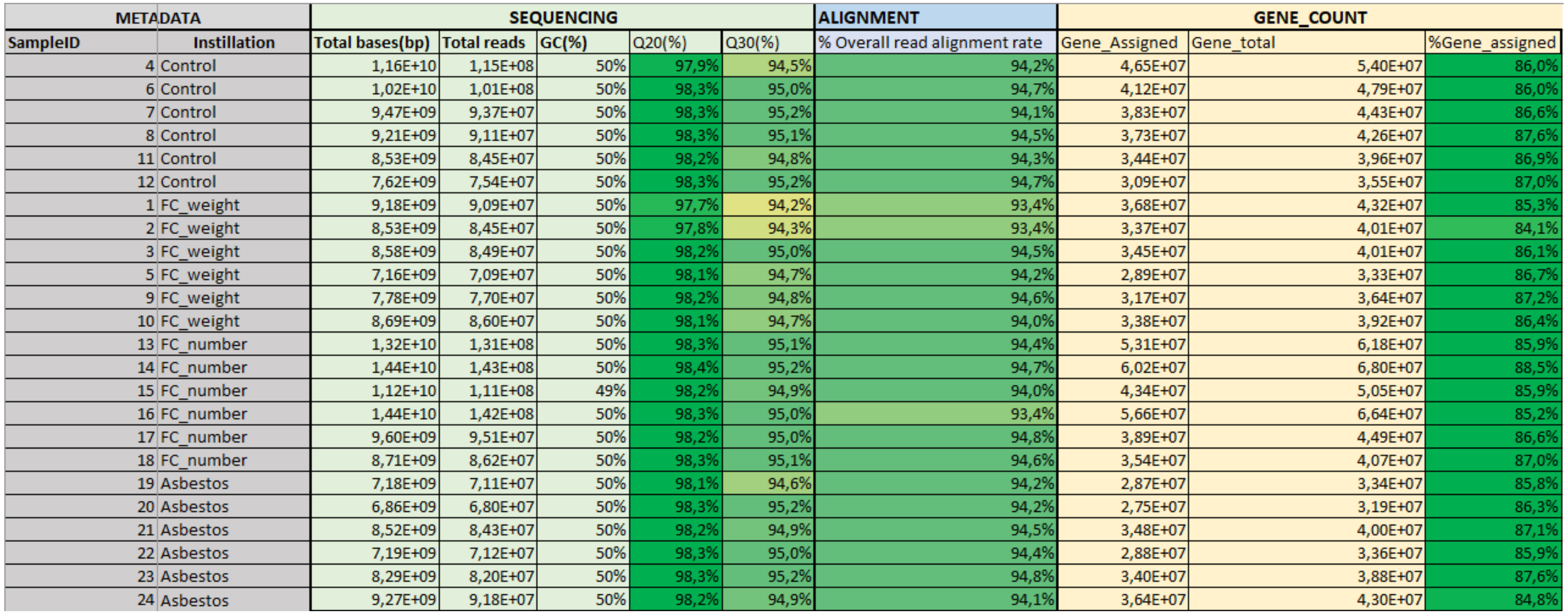
Measure	Value
Filename	14_2_fastq_gz.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	71396303
Total Bases	7.2 Gbp
Sequences flagged as poor quality	0
Sequence length	101
%GC	49

Per base sequence quality

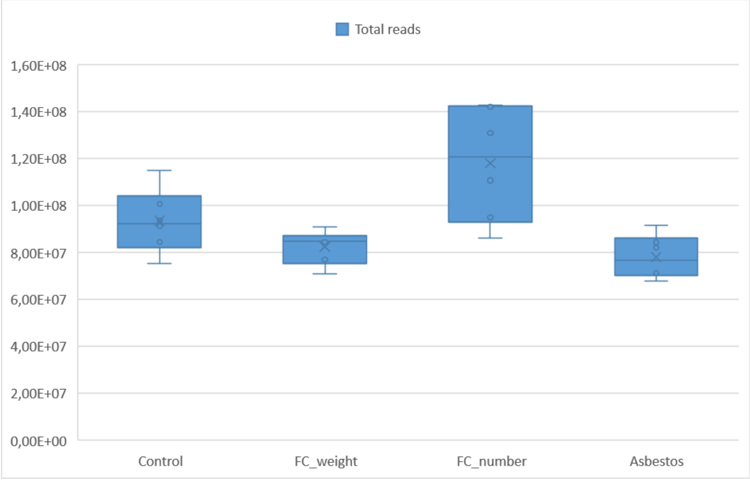
RNA-Seq workflow

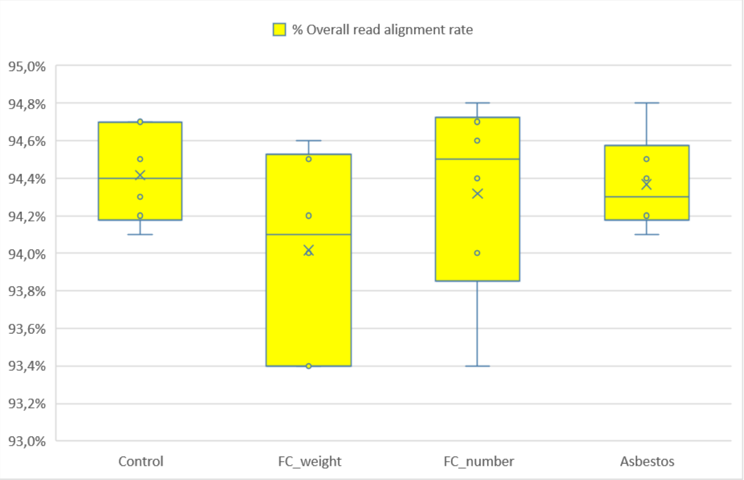
Transcriptome analysis by RNA-Seq

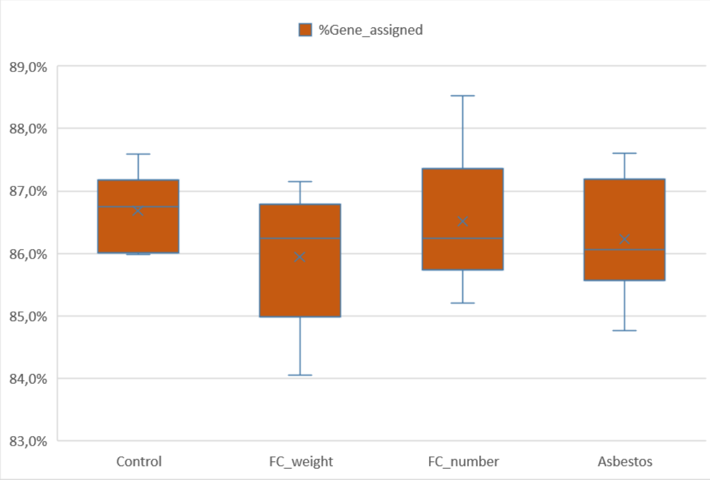
- **Alignment** : Aligning reads to the rat genome rnor7 (**TopHat2**)
- **Gene counting** : Counting the number of reads aligned to each gene (**FeatureCounts**)



METADATA		SEQUENCING					ALIGNMENT	GENE_COUNT		
SampleID	**Instillation**	**Total bases(bp)**	**Total reads**	**GC(%)**	Q20(%)	Q30(%)	% Overall read alignment rate	Gene_Assigned	Gene_total	%Gene_assigned
4	Control	1,16E+10	1,15E+08	50%	97,9%	94,5%	94,2%	4,65E+07	5,40E+07	86,0%
6	Control	1,02E+10	1,01E+08	50%	98,3%	95,0%	94,7%	4,12E+07	4,79E+07	86,0%
7	Control	9,47E+09	9,37E+07	50%	98,3%	95,2%	94,1%	3,83E+07	4,43E+07	86,6%
8	Control	9,21E+09	9,11E+07	50%	98,3%	95,1%	94,5%	3,73E+07	4,26E+07	87,6%
11	Control	8,53E+09	8,45E+07	50%	98,2%	94,8%	94,3%	3,44E+07	3,96E+07	86,9%
12	Control	7,62E+09	7,54E+07	50%	98,3%	95,2%	94,7%	3,09E+07	3,55E+07	87,0%
1	FC_weight	9,18E+09	9,09E+07	50%	97,7%	94,2%	93,4%	3,68E+07	4,32E+07	85,3%
2	FC_weight	8,53E+09	8,45E+07	50%	97,8%	94,3%	93,4%	3,37E+07	4,01E+07	84,1%
3	FC_weight	8,58E+09	8,49E+07	50%	98,2%	95,0%	94,5%	3,45E+07	4,01E+07	86,1%
5	FC_weight	7,16E+09	7,09E+07	50%	98,1%	94,7%	94,2%	2,89E+07	3,33E+07	86,7%
9	FC_weight	7,78E+09	7,70E+07	50%	98,2%	94,8%	94,6%	3,17E+07	3,64E+07	87,2%
10	FC_weight	8,69E+09	8,60E+07	50%	98,1%	94,7%	94,0%	3,38E+07	3,92E+07	86,4%
13	FC_number	1,32E+10	1,31E+08	50%	98,3%	95,1%	94,4%	5,31E+07	6,18E+07	85,9%
14	FC_number	1,44E+10	1,43E+08	50%	98,4%	95,2%	94,7%	6,02E+07	6,80E+07	88,5%
15	FC_number	1,12E+10	1,11E+08	49%	98,2%	94,9%	94,0%	4,34E+07	5,05E+07	85,9%
16	FC_number	1,44E+10	1,42E+08	50%	98,3%	95,0%	93,4%	5,66E+07	6,64E+07	85,2%
17	FC_number	9,60E+09	9,51E+07	50%	98,2%	95,0%	94,8%	3,89E+07	4,49E+07	86,6%
18	FC_number	8,71E+09	8,62E+07	50%	98,3%	95,1%	94,6%	3,54E+07	4,07E+07	87,0%
19	Asbestos	7,18E+09	7,11E+07	50%	98,1%	94,6%	94,2%	2,87E+07	3,34E+07	85,8%
20	Asbestos	6,86E+09	6,80E+07	50%	98,3%	95,2%	94,2%	2,75E+07	3,19E+07	86,3%
21	Asbestos	8,52E+09	8,43E+07	50%	98,2%	94,9%	94,5%	3,48E+07	4,00E+07	87,1%
22	Asbestos	7,19E+09	7,12E+07	50%	98,3%	95,0%	94,4%	2,88E+07	3,36E+07	85,9%
23	Asbestos	8,29E+09	8,20E+07	50%	98,3%	95,2%	94,8%	3,40E+07	3,88E+07	87,6%
24	Asbestos	9,27E+09	9,18E+07	50%	98,2%	94,9%	94,1%	3,64E+07	4,30E+07	84,8%







RNA-Seq workflow

Transcriptome analysis by RNA-Seq

- **Filtering of lowly expressed genes :** TPM > 1 in at least 1 of the groups

```
[1] "Generating fpkm, tpm protein coding tables : DONE"
[1] "table dimension:"
[1] 23096    24

[1] "Exp table filtered low expressed : DONE"
[1] 7274    24
```

$$\text{TPM} = \frac{(C_g * 1e^3 / L_g) * 1e^6}{\sum_{g=1}^{N} (C_g * 1e^3 / L_g)}$$

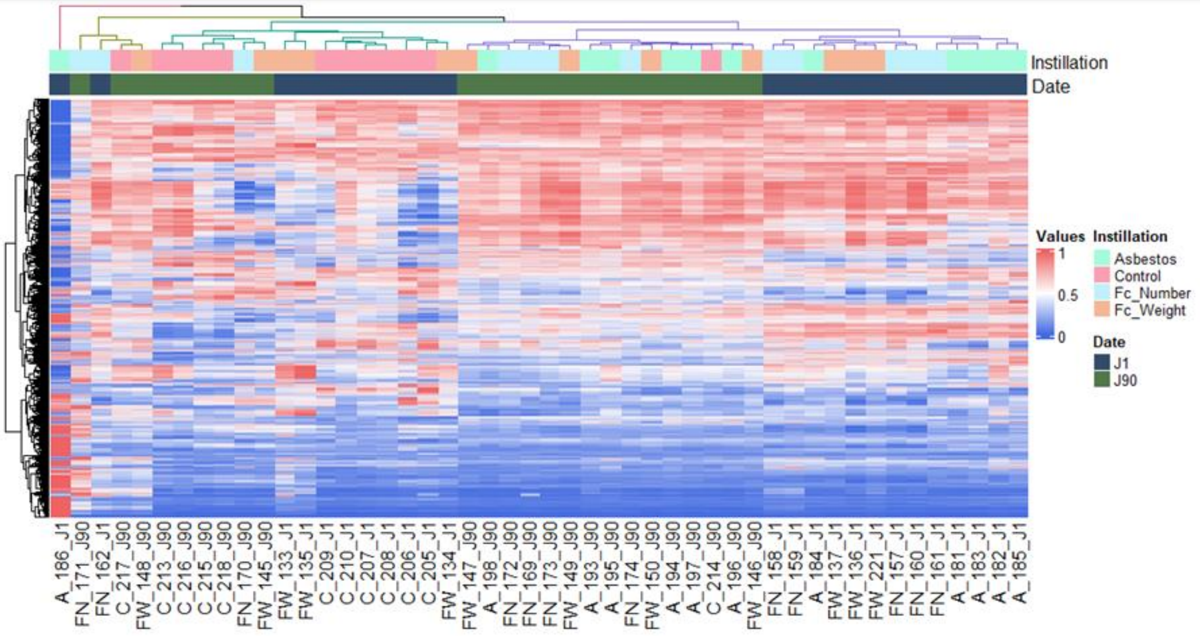
C_g = count of reads aligned to gene g

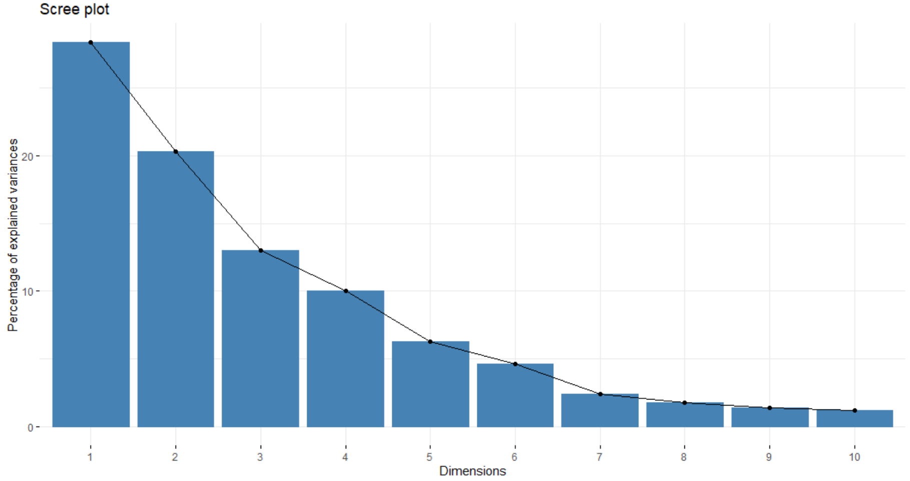
Lg = union length of exons of gene g

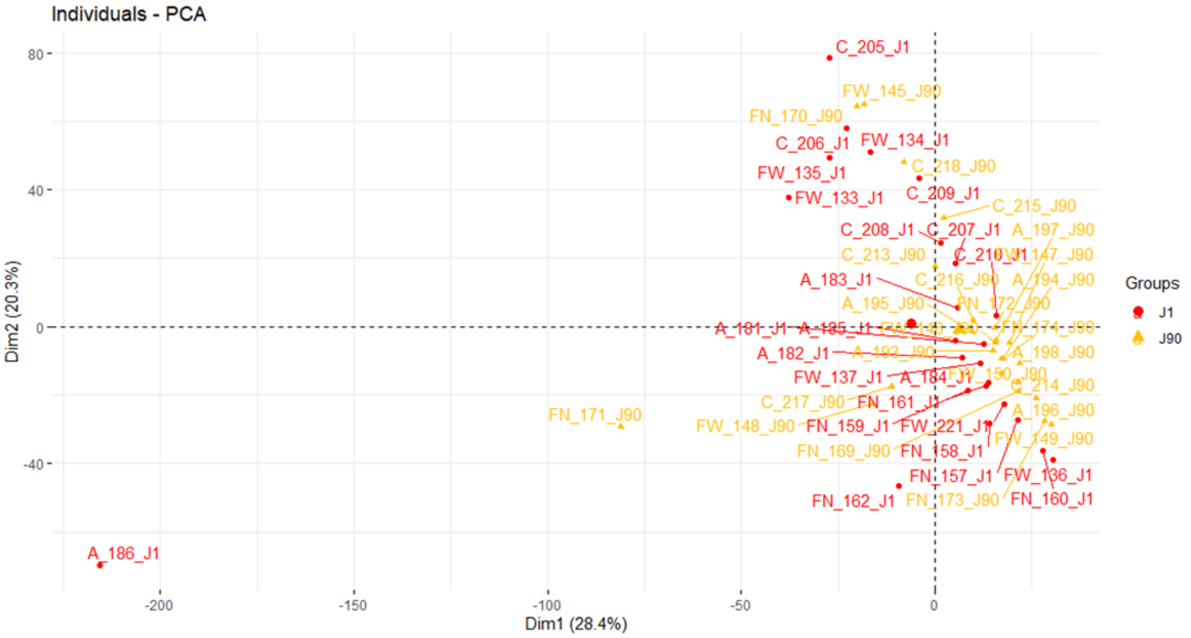
- **Analysis without prior knowledge:** Analysis on the 5000 most variable genes:
  - **Heatmap to visualize gene expression between samples**
  - **PCA to visualize variance and detect potential biological or technical groups**

Elimination of outlier samples

Transcriptome analysis by RNA-Seq







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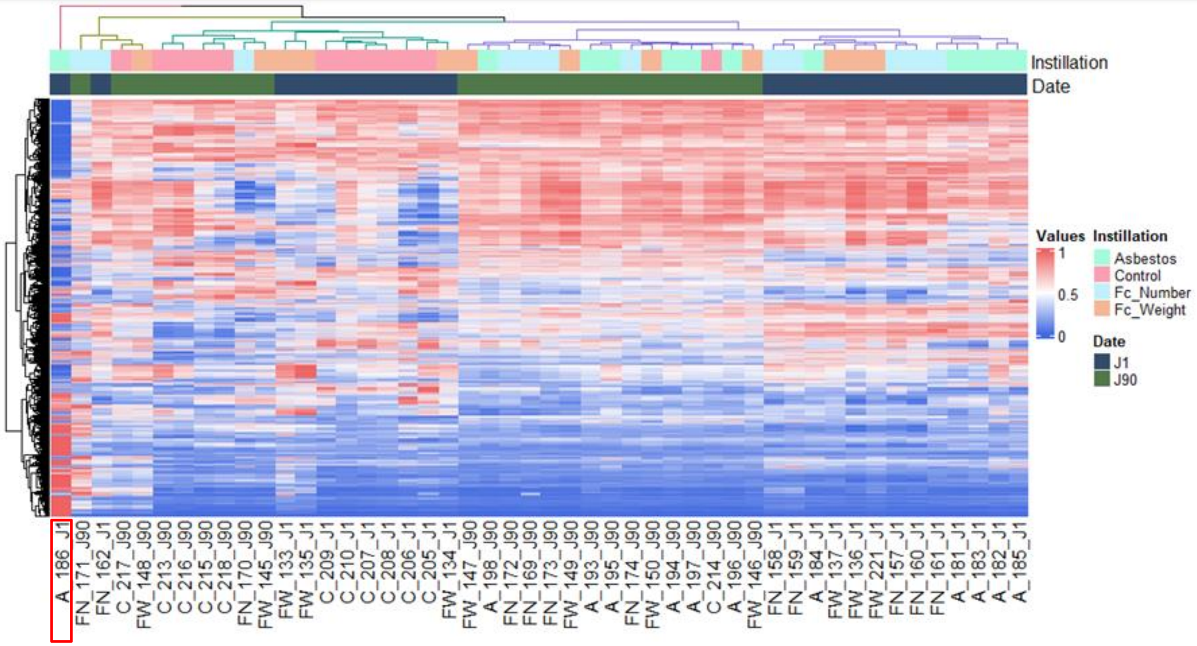

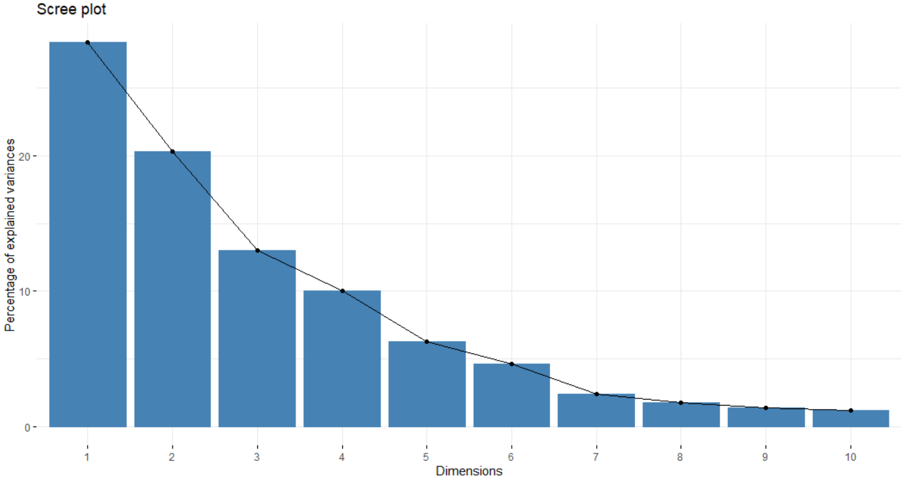

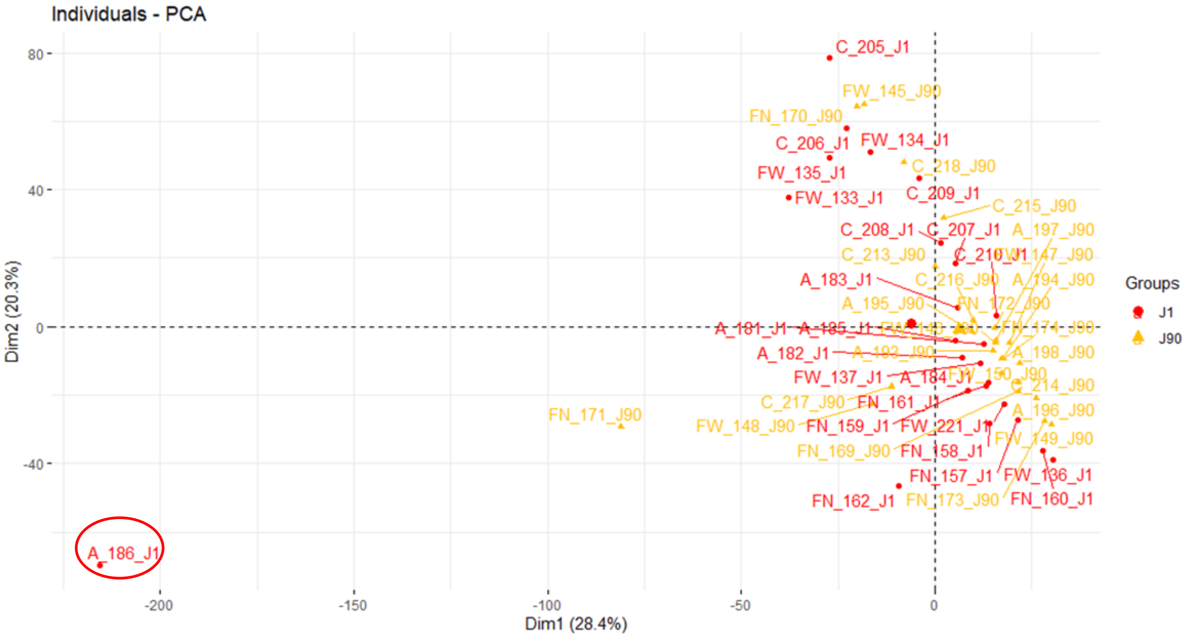

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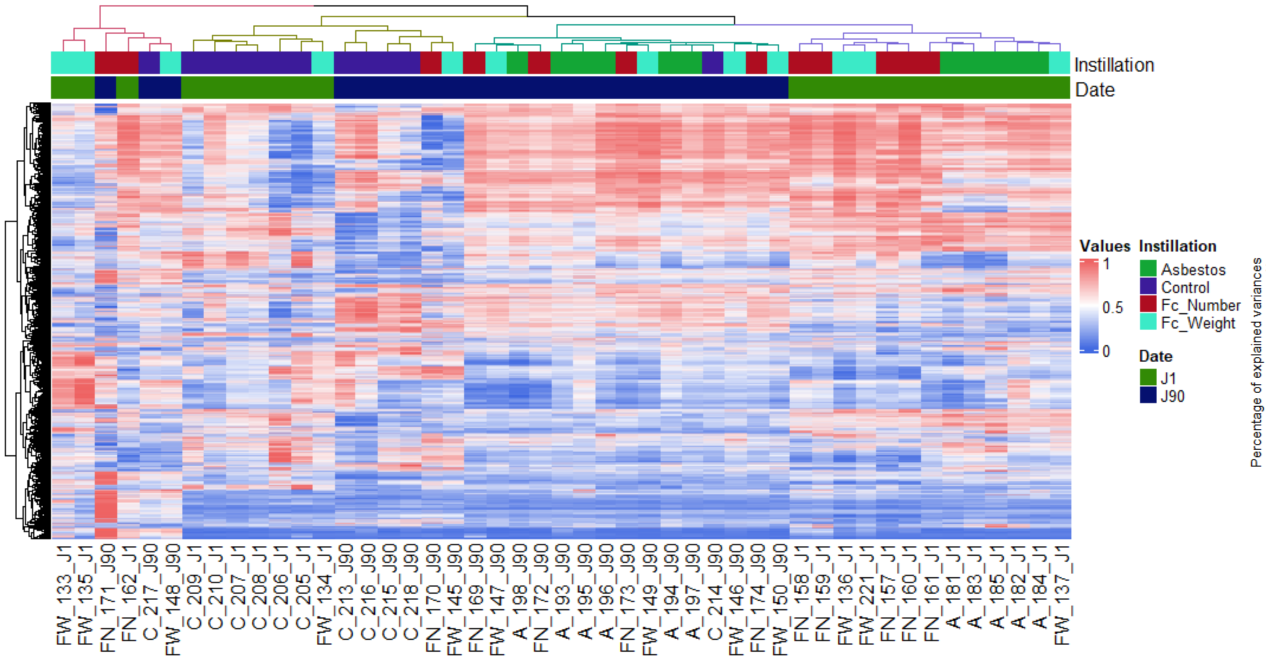

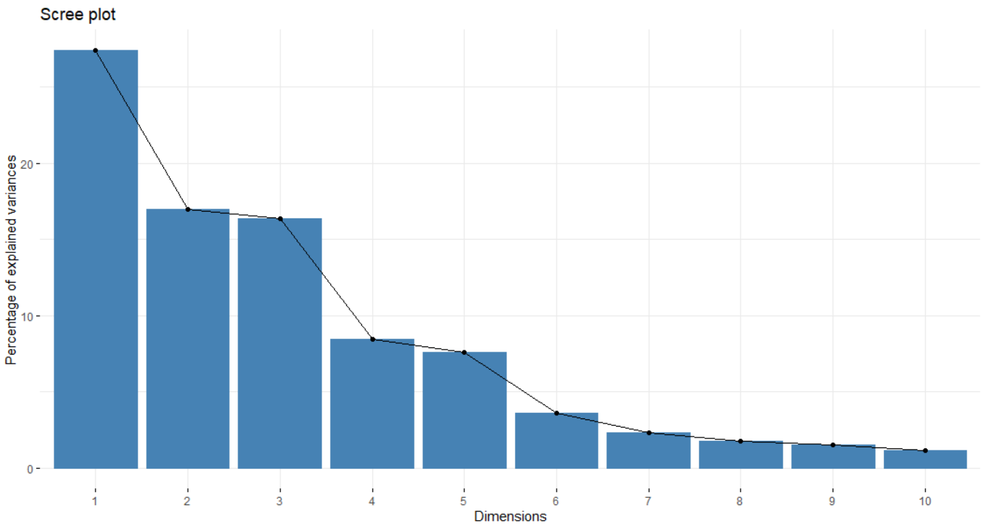

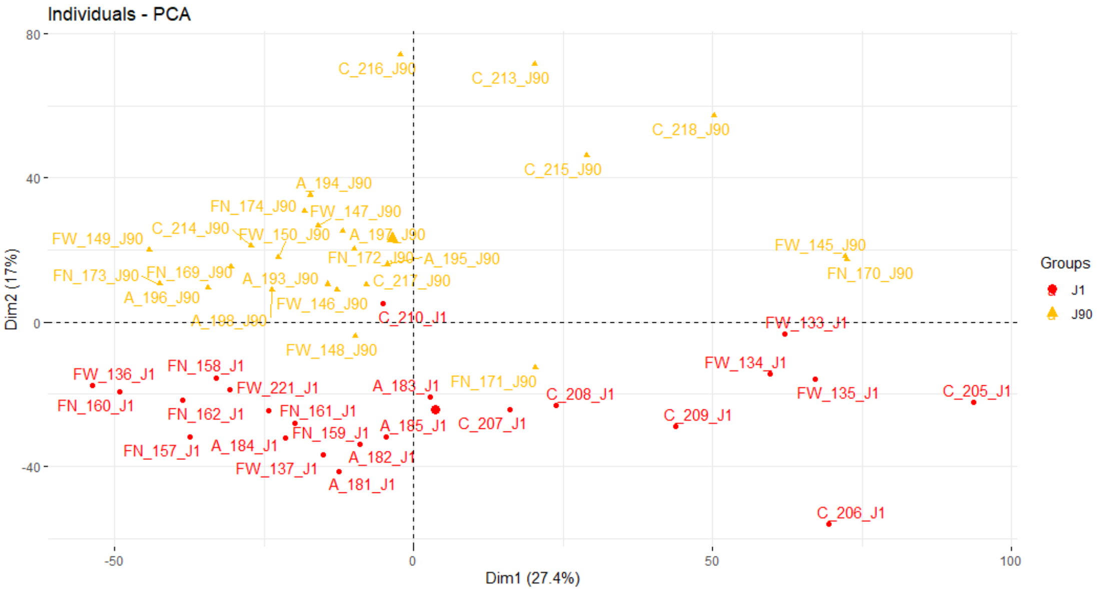

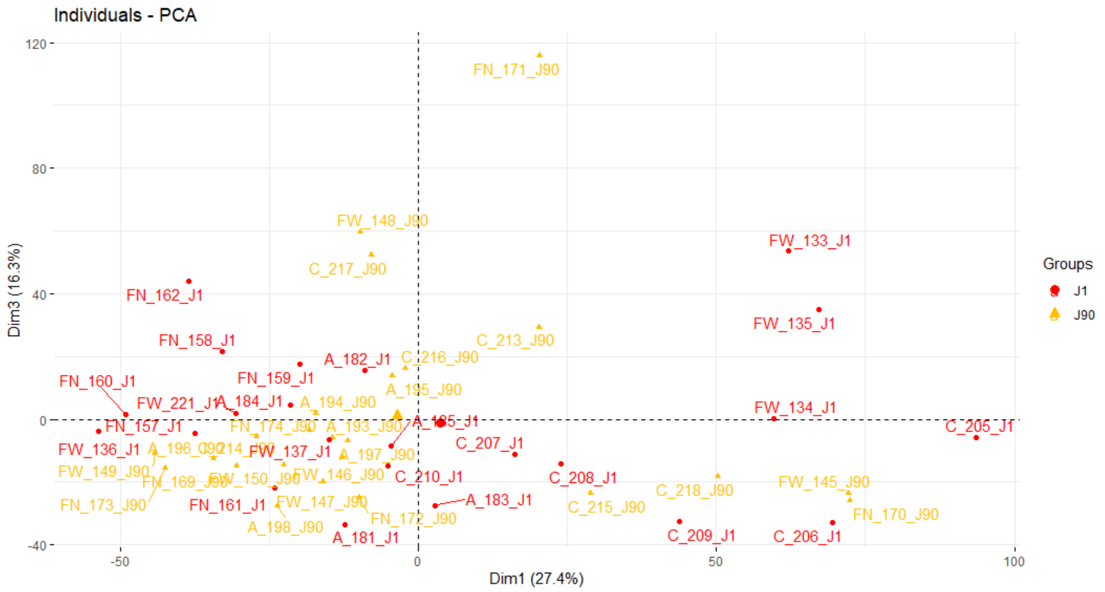

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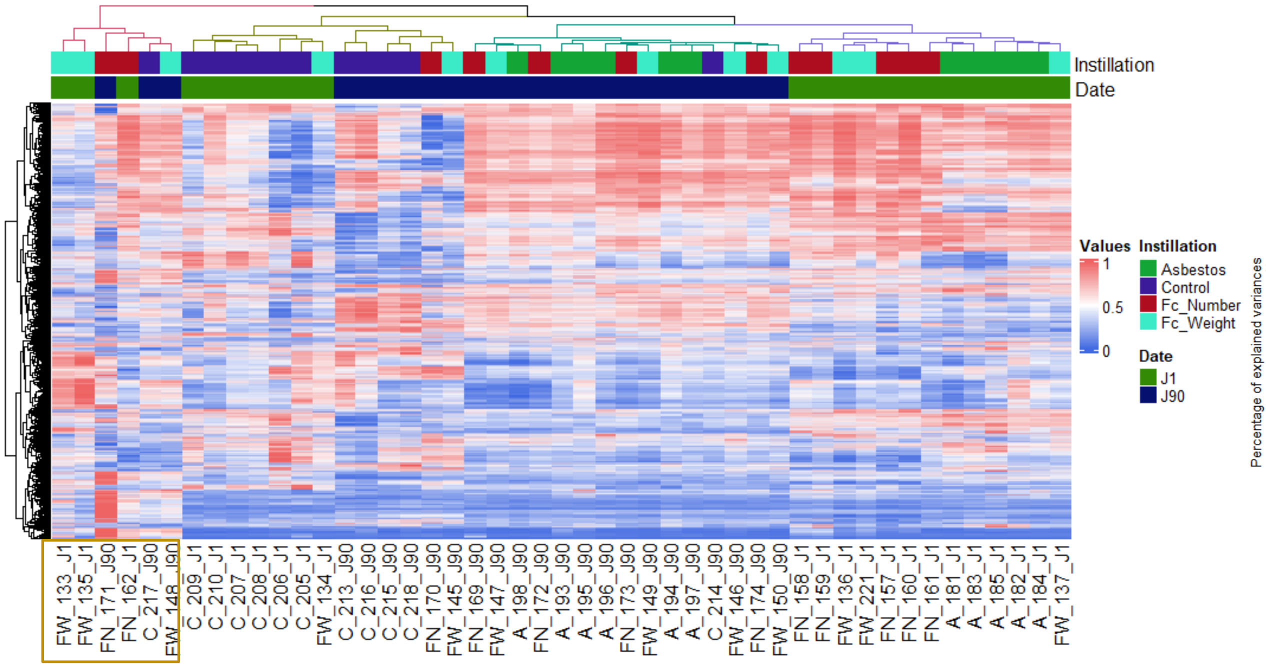

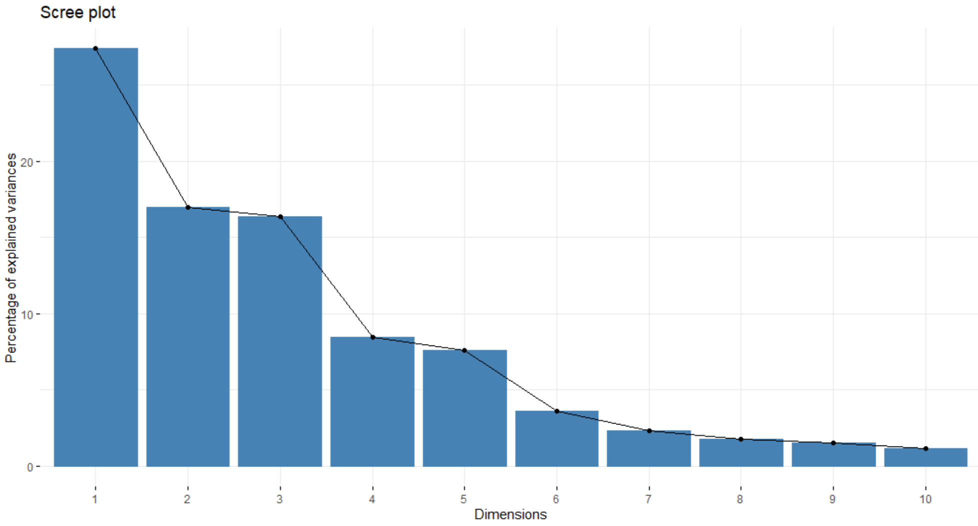

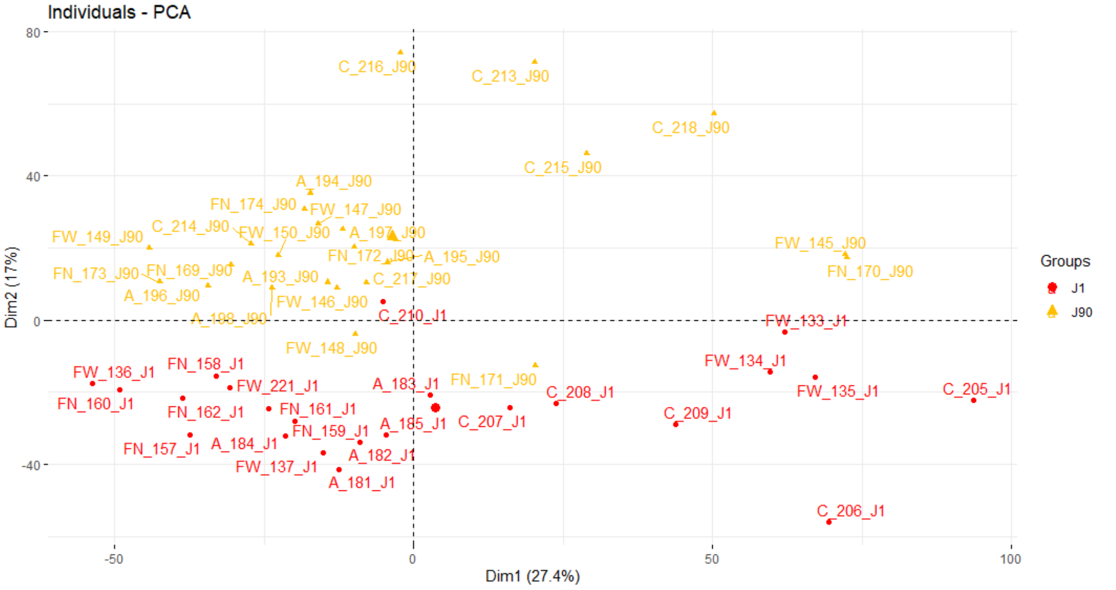

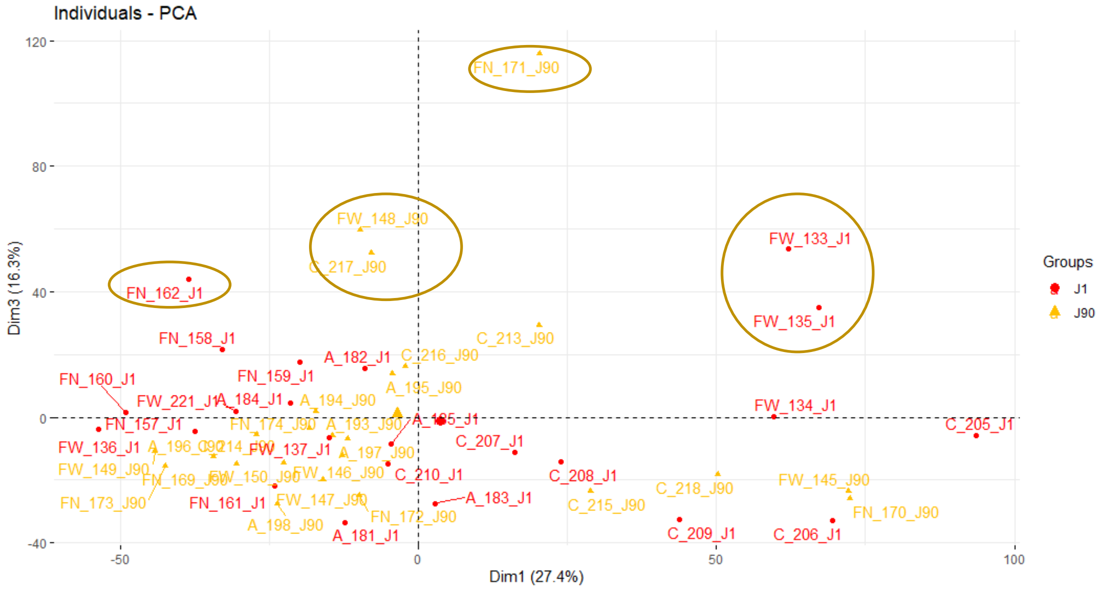

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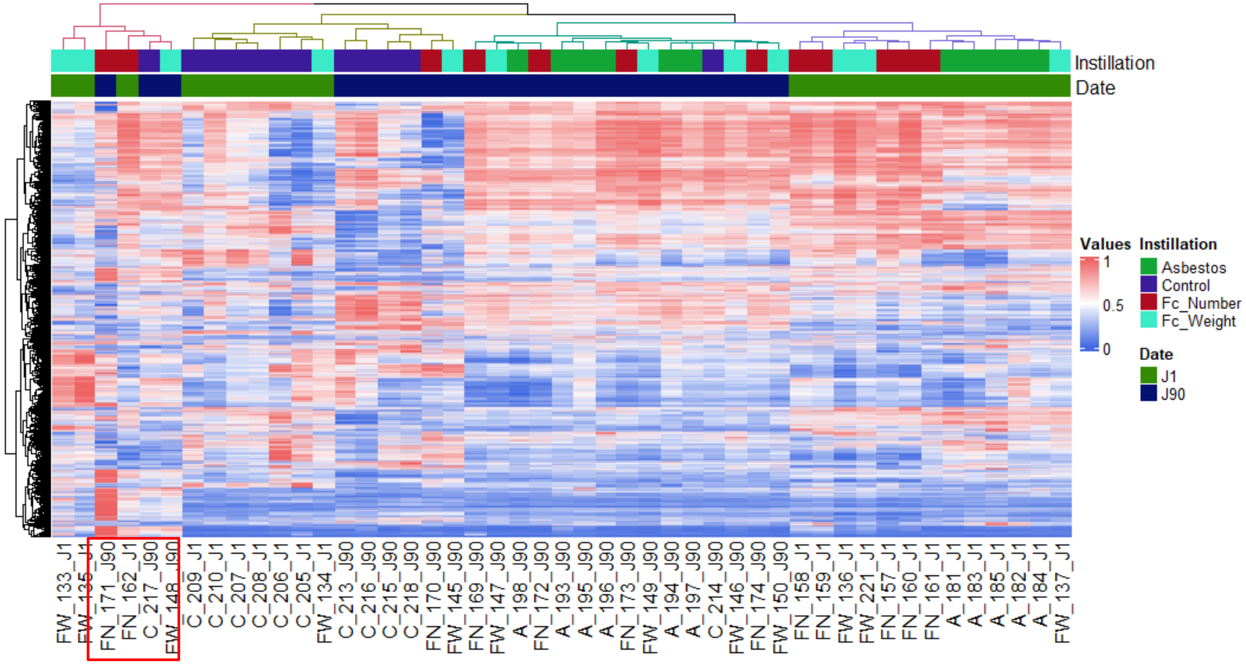
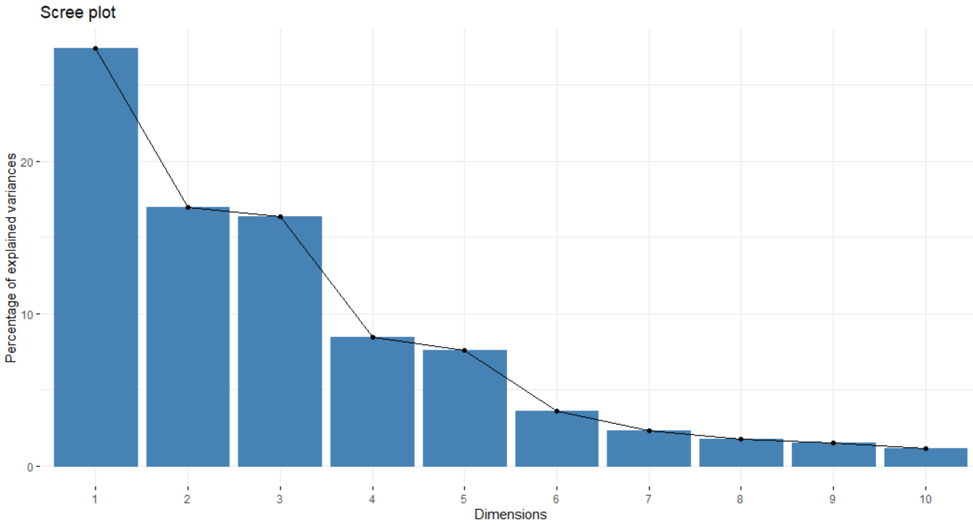
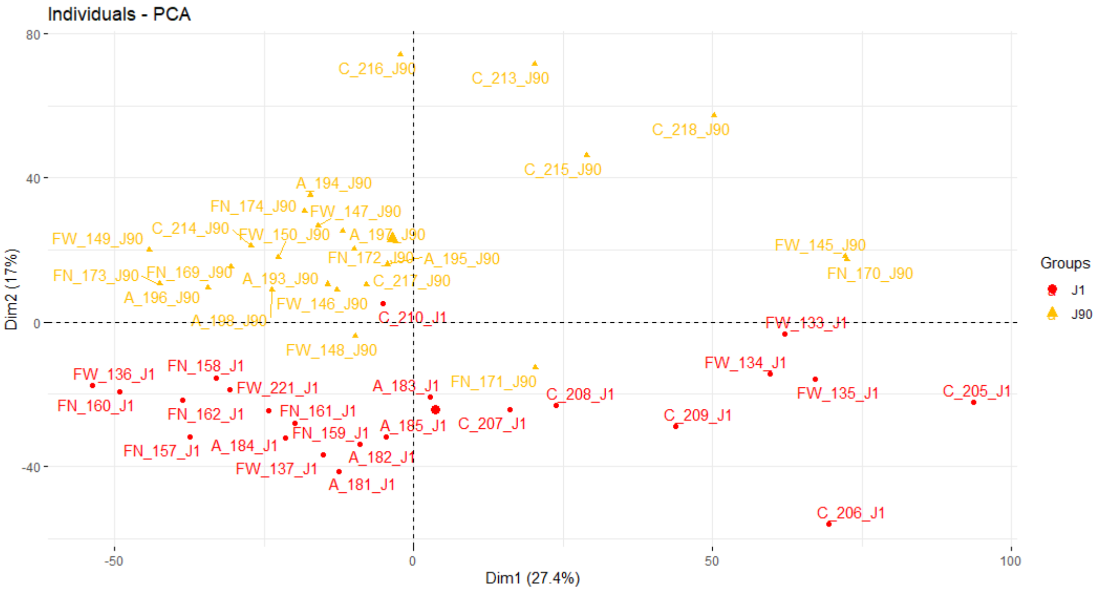
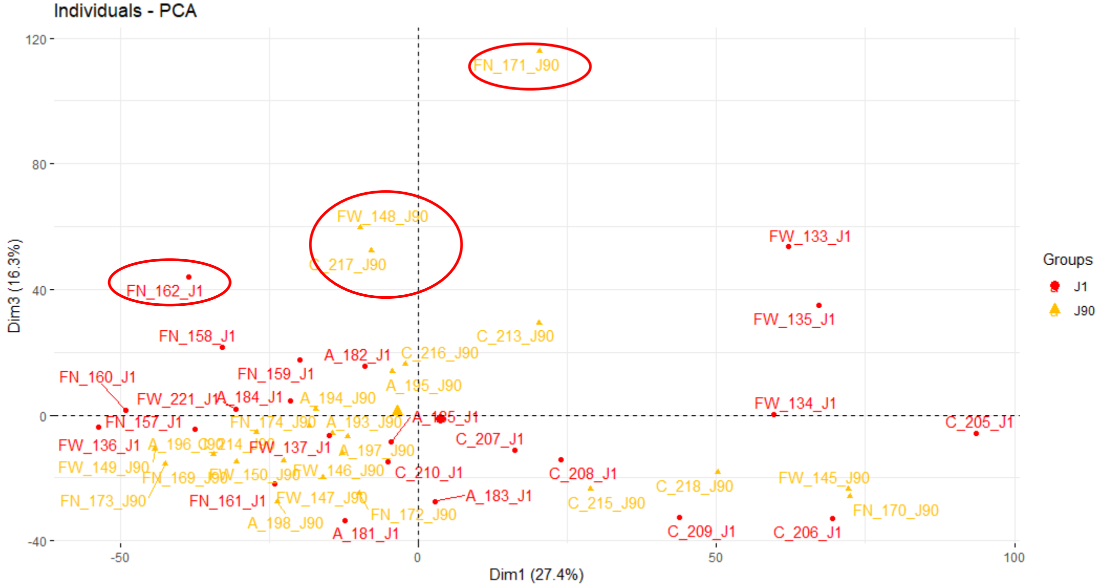


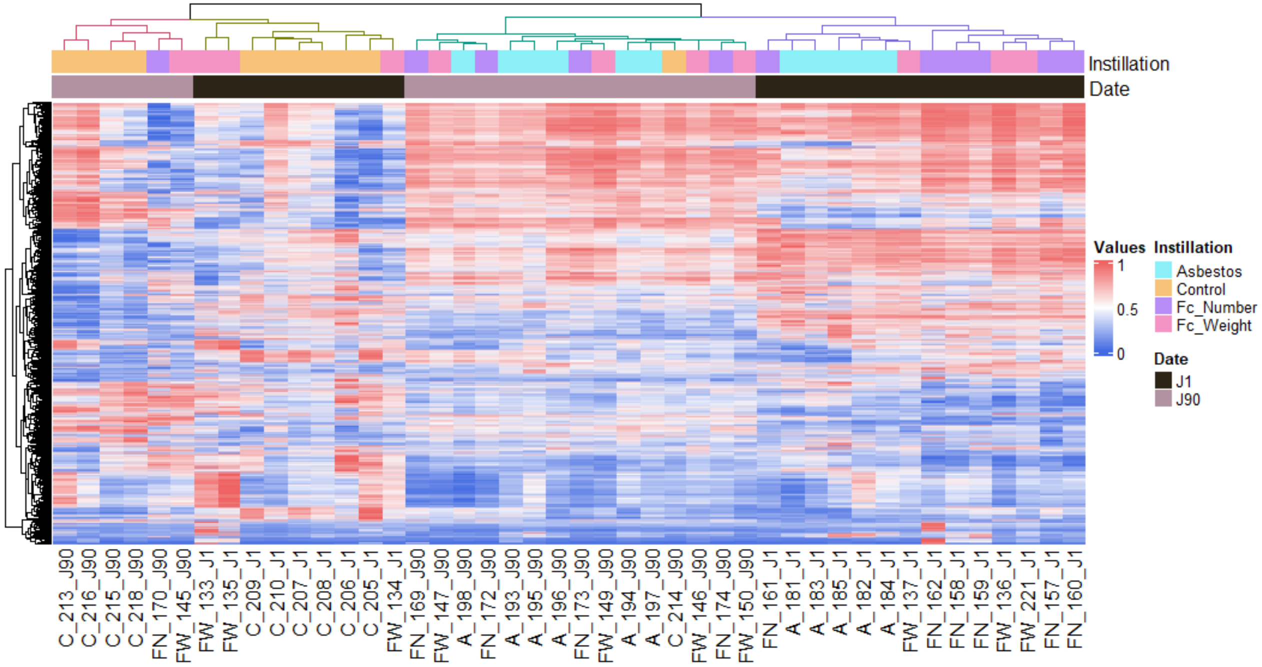


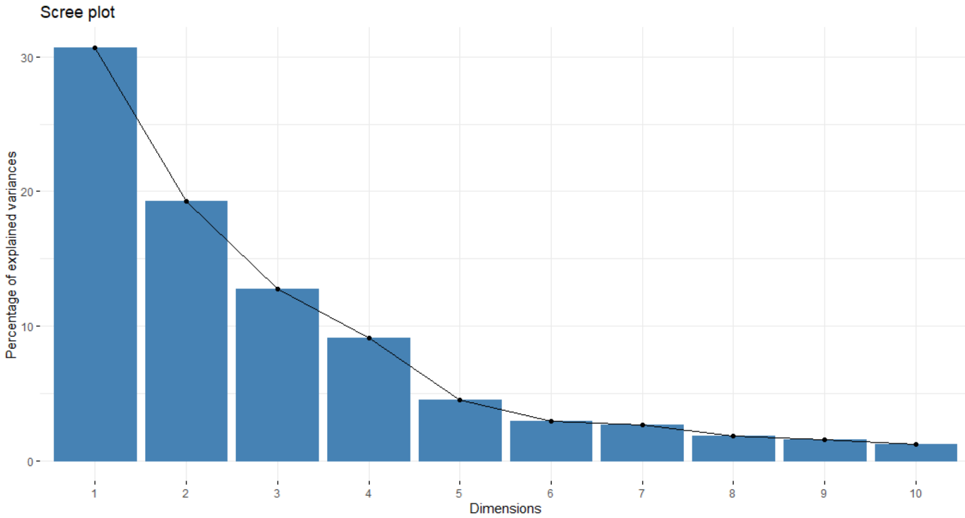


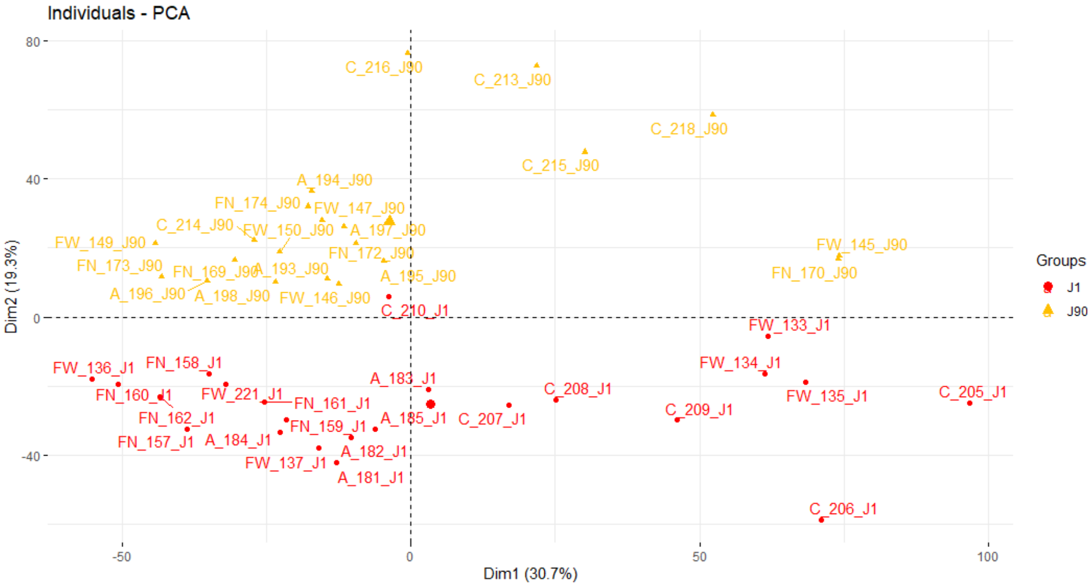


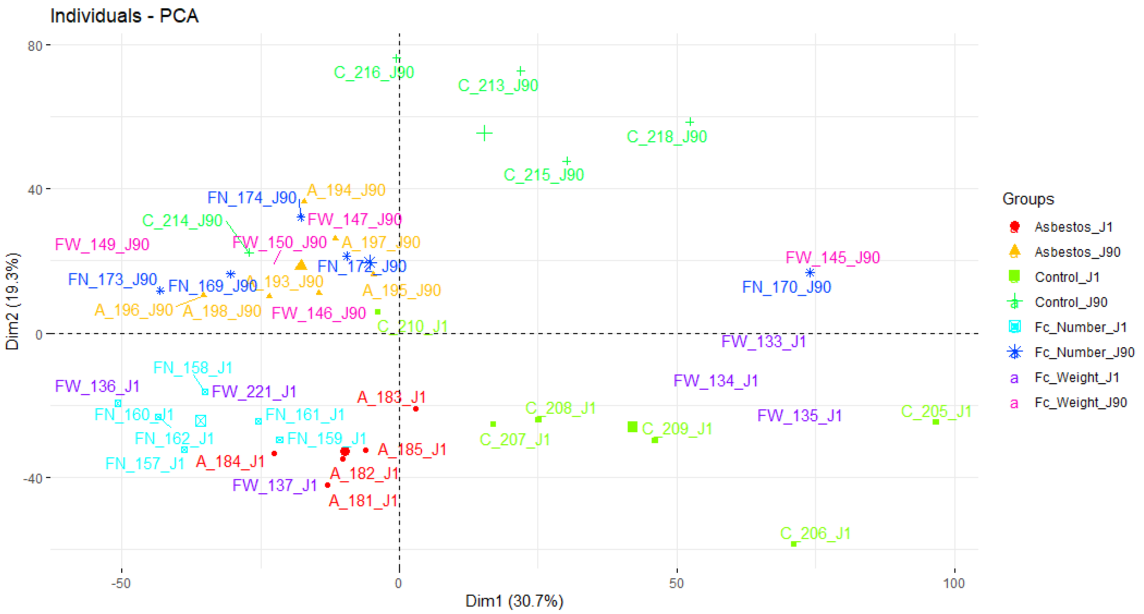
Statistical analyses for all samples

Transcriptome analysis by RNA-Seq









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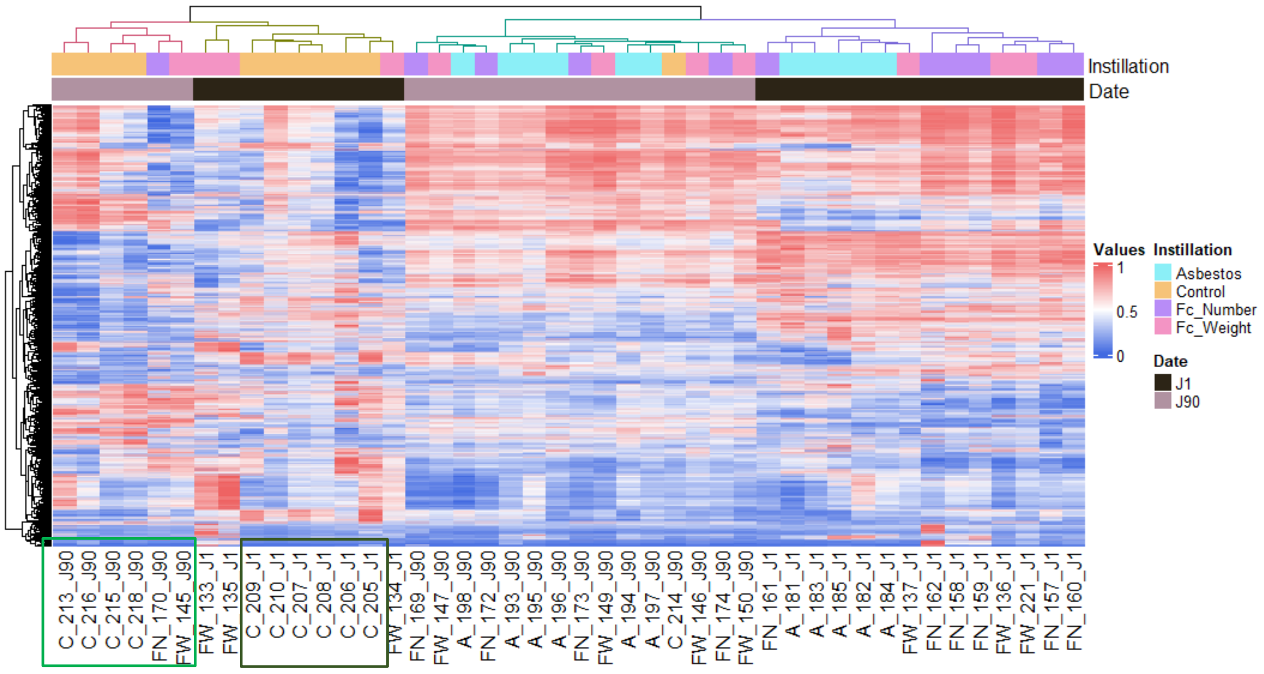
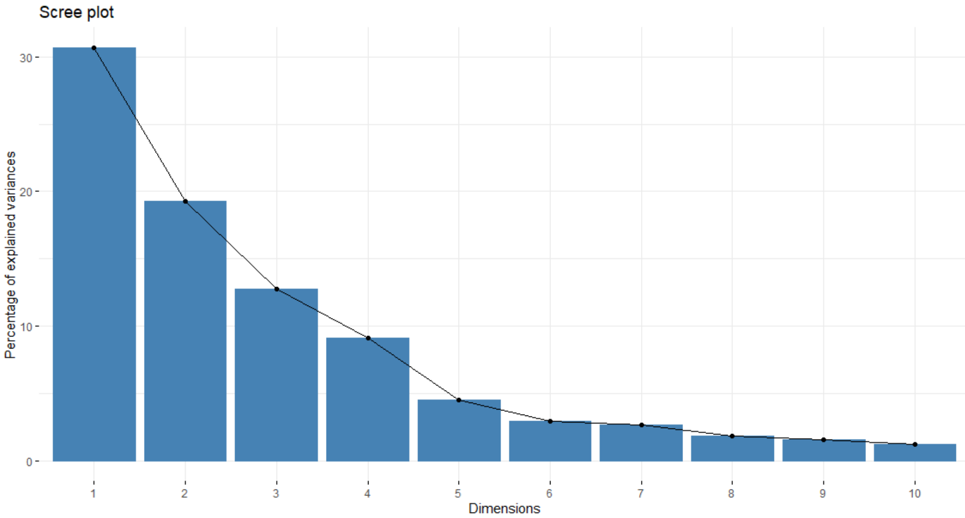
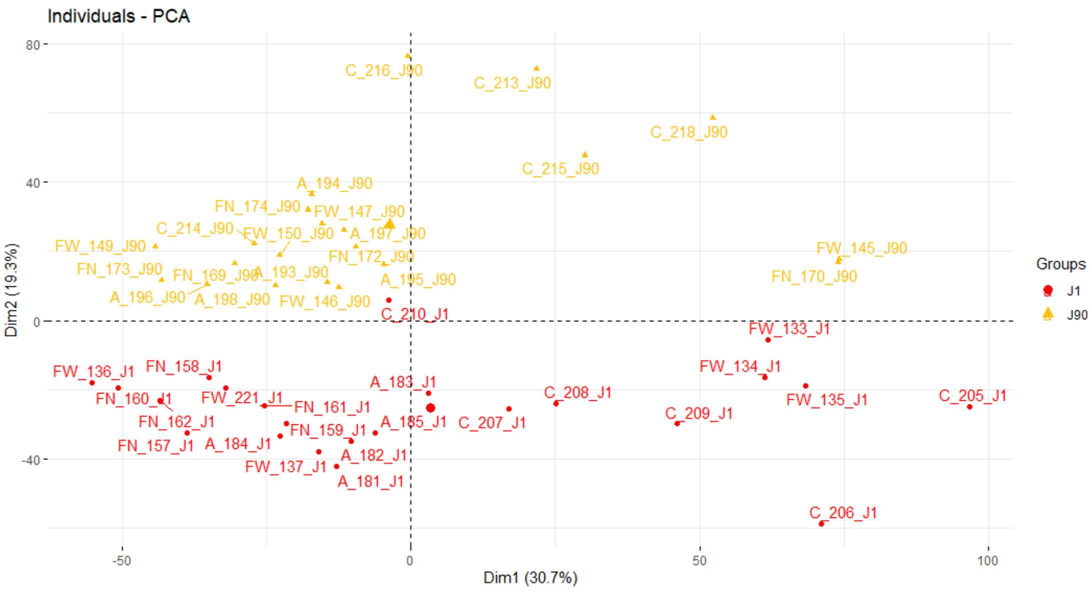
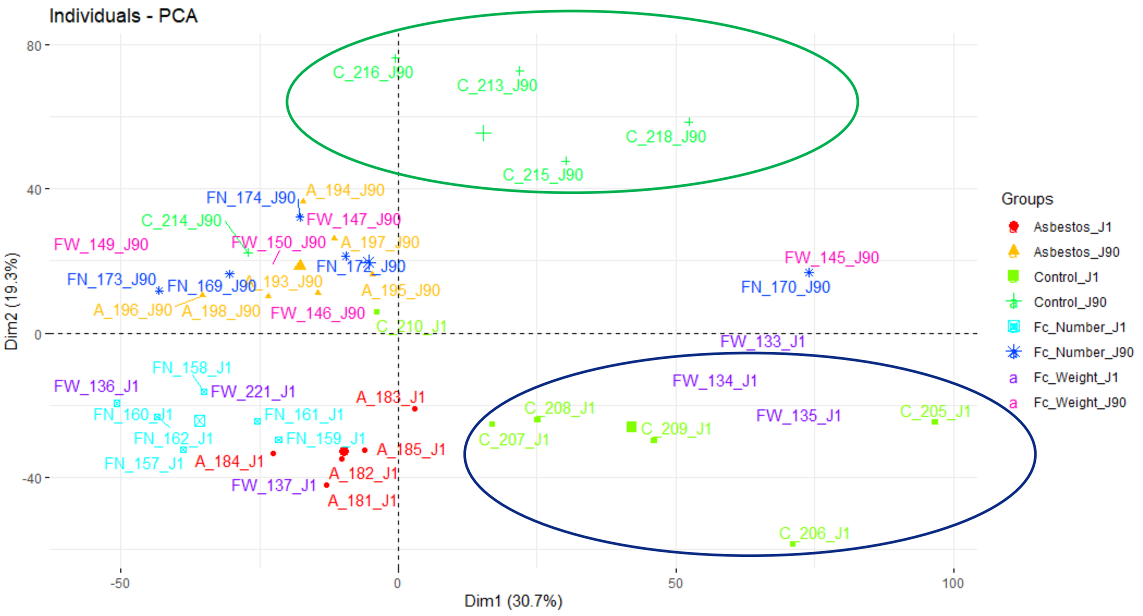
Transcriptome analysis by RNA-Seq



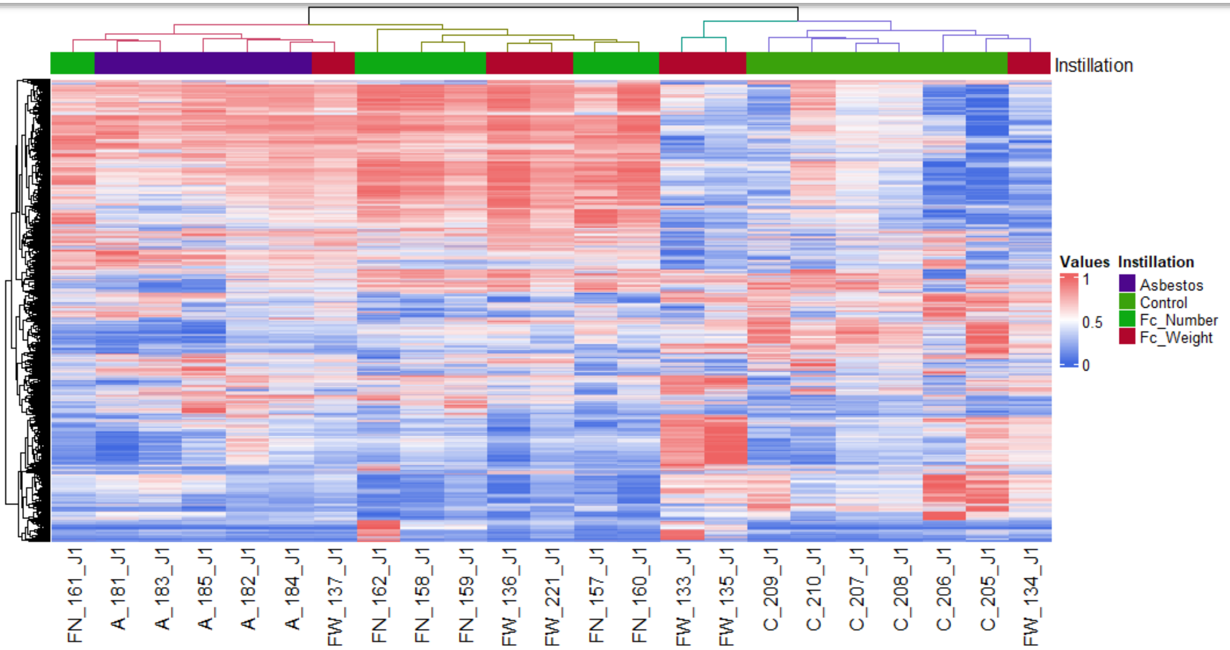


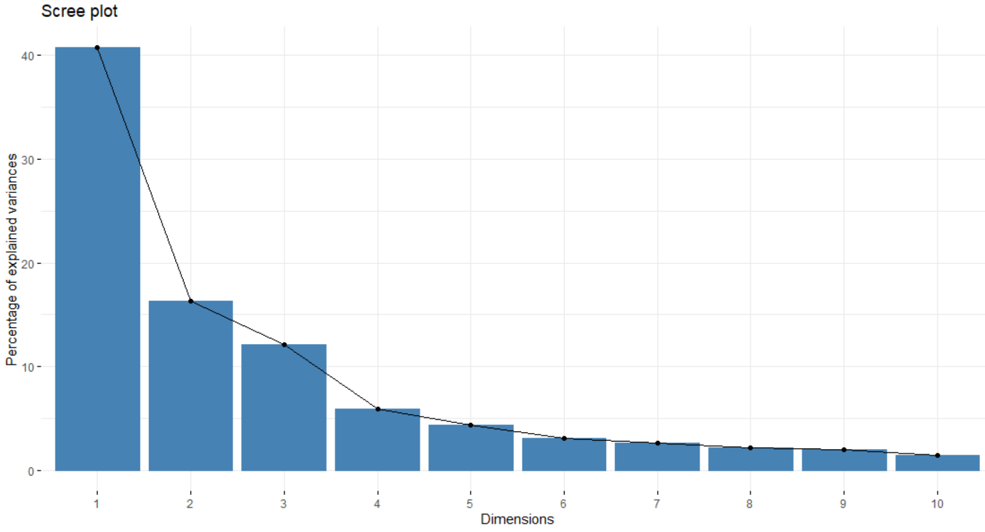


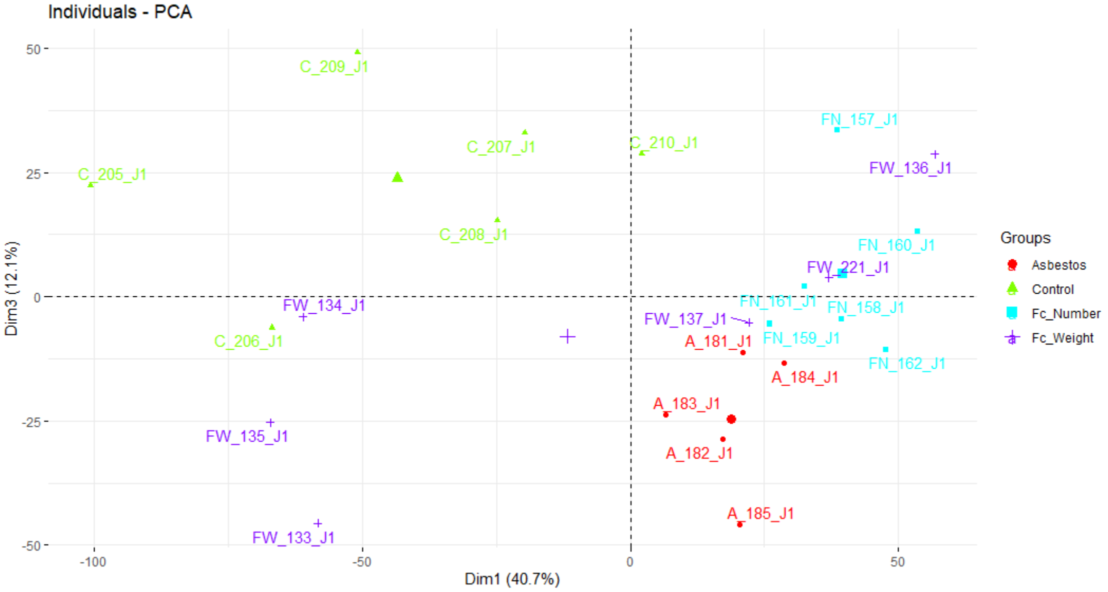


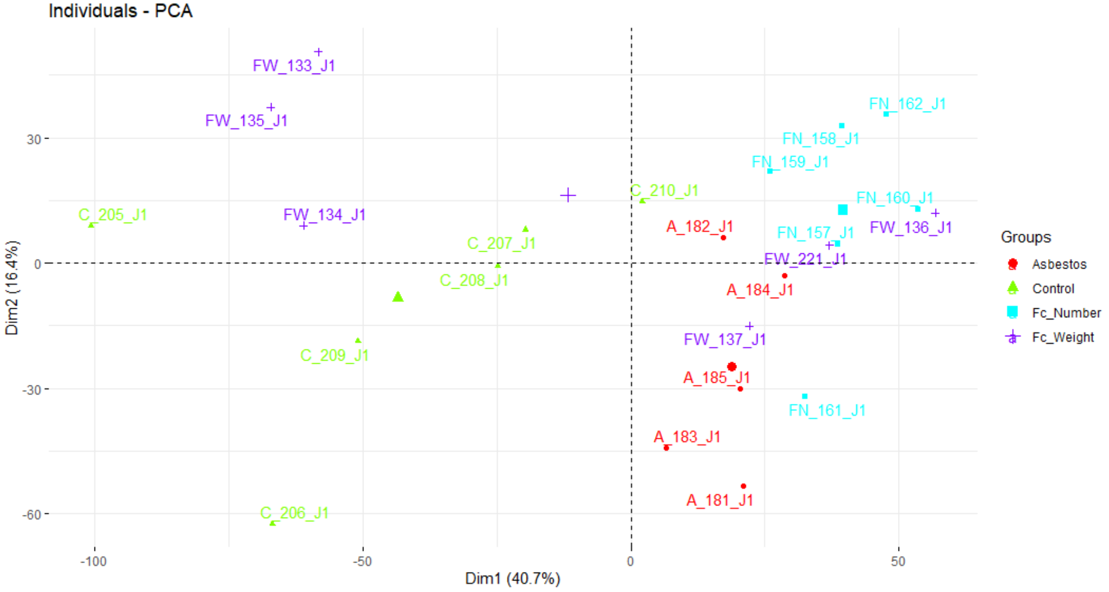
Statistical analyses for D1

Transcriptome analysis by RNA-Seq



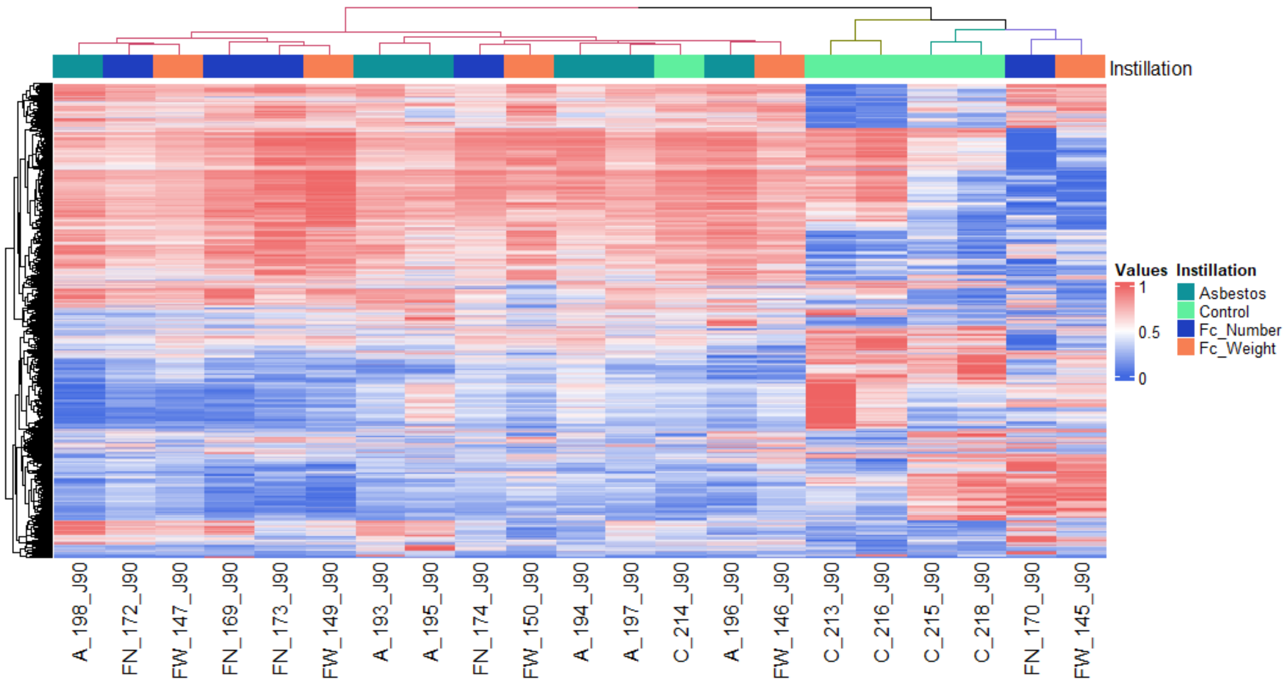


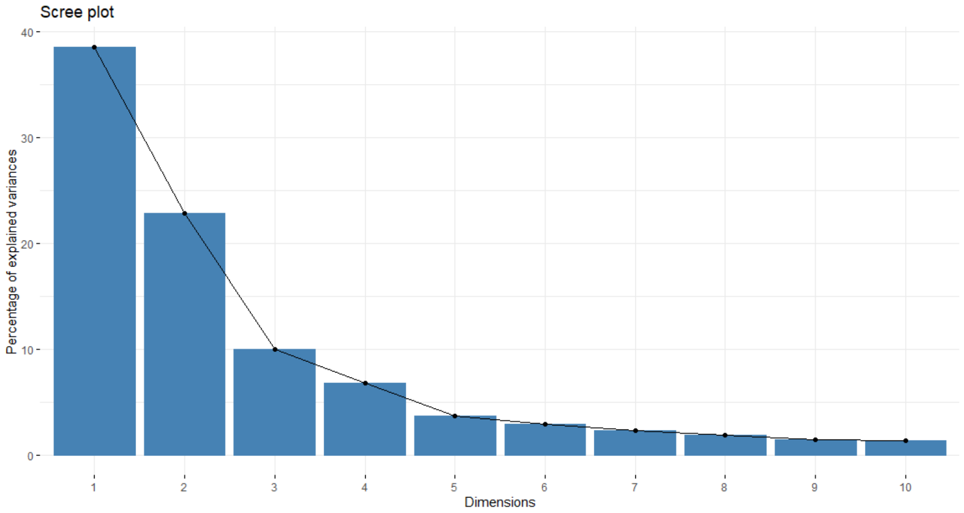


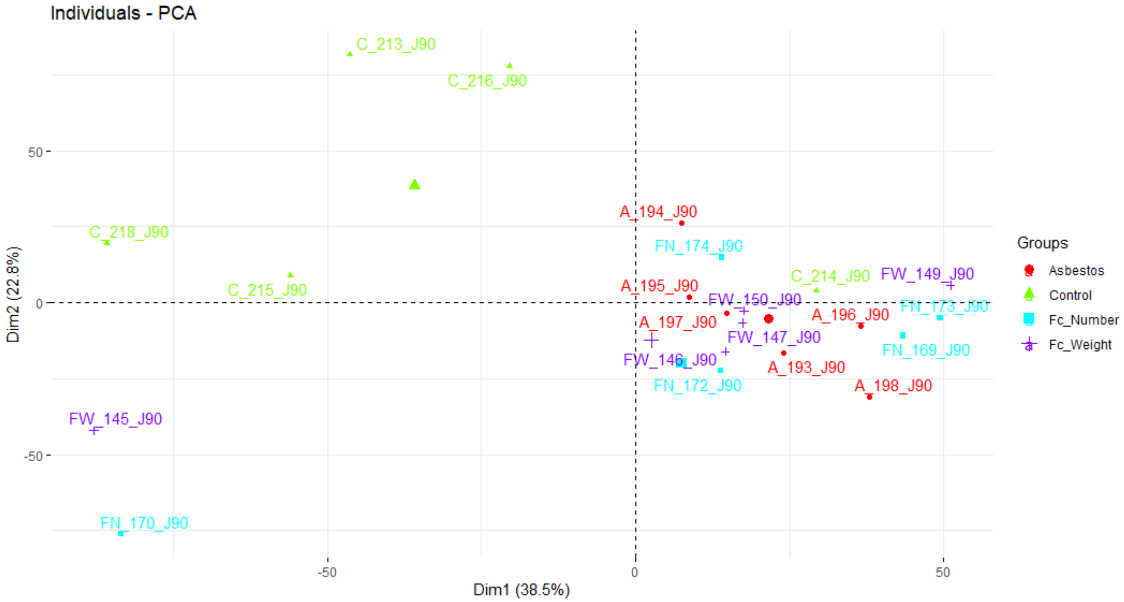


Statistical analyses for D90

Transcriptome analysis by RNA-Seq







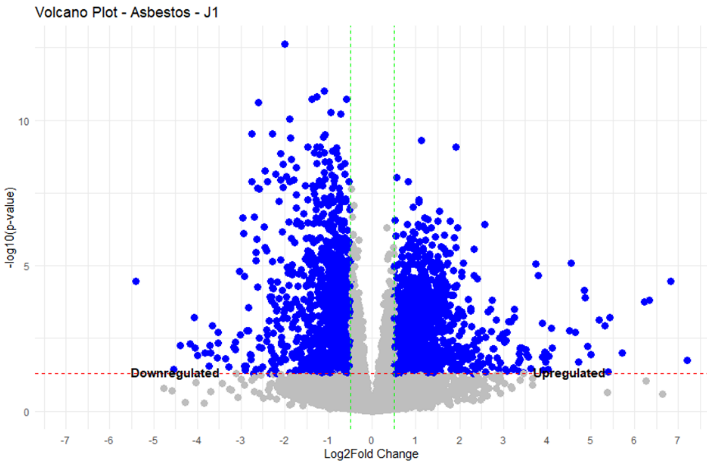
Analysis of differentially expressed genes

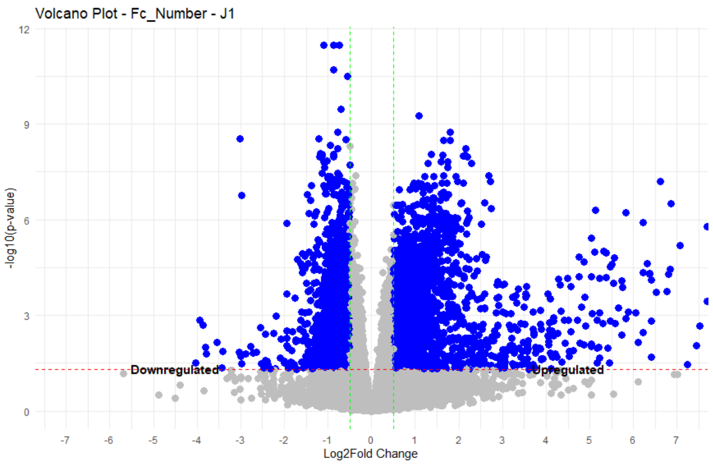
Transcriptome analysis by RNA-Seq

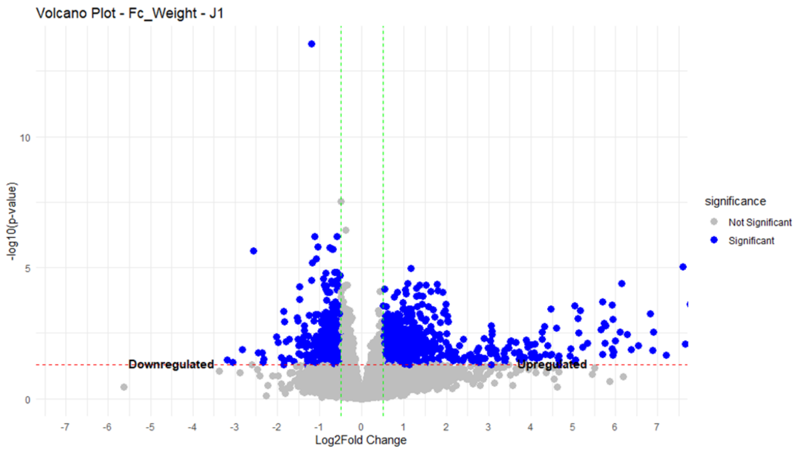
- **Differential gene expression analysis :** DESeq2 to detect DEGs between sample groups

D1

	Overexpression	**Underexpression**
Asbestos	**1888**	**2036**
FC_number	**2617**	**2546**
FC_weight	**481**	**498**







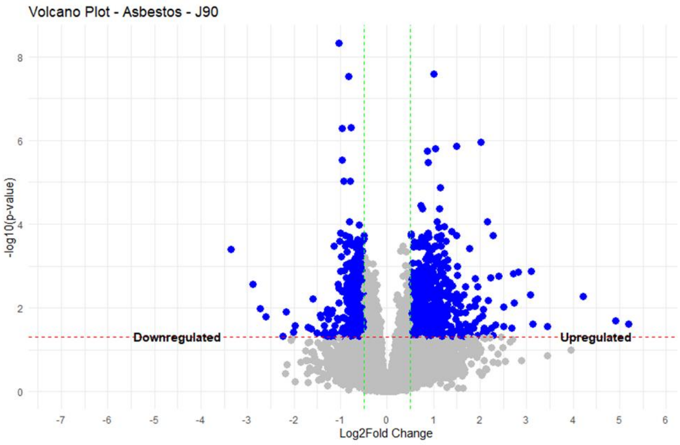
Analysis of differentially expressed genes

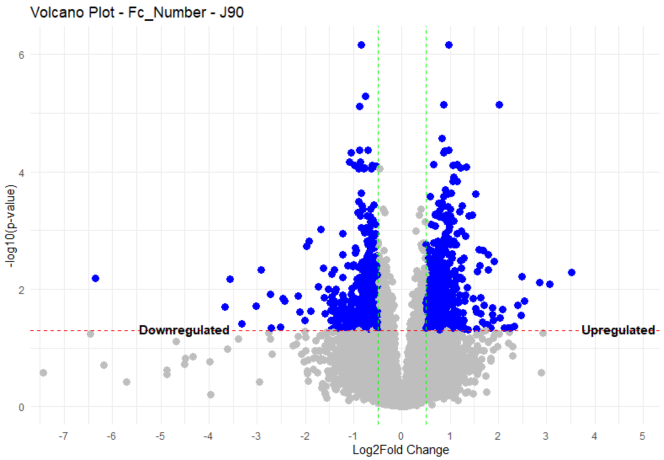
Transcriptome analysis by RNA-Seq

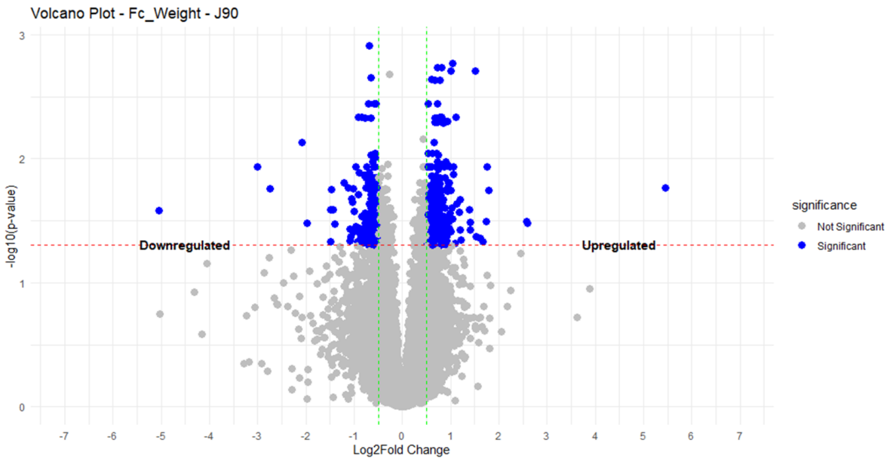
- **Differential gene expression analysis :** DESeq2 to detect DEGs between sample groups

D90

	Overexpression	**Underexpression**
Asbestos	**909**	**848**
FC_number	**725**	**688**
FC_weight	**399**	**279**







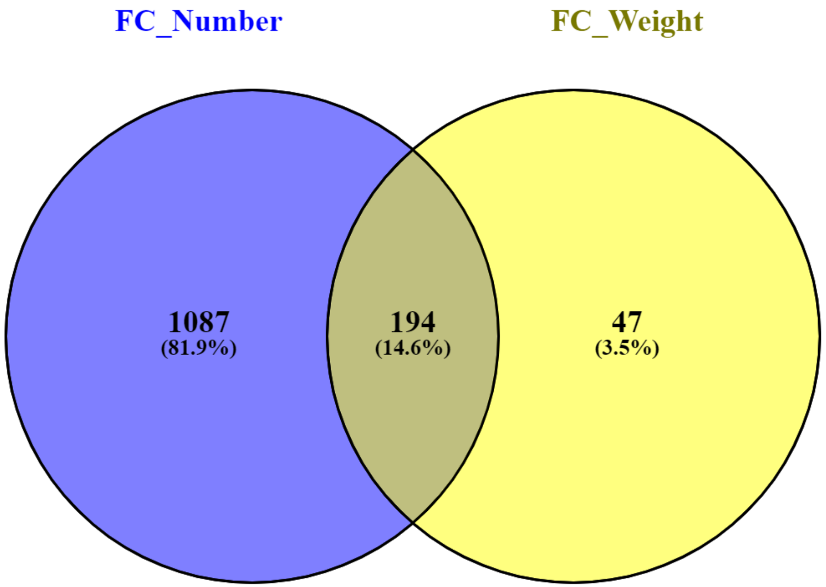
Analysis of differentially expressed genes

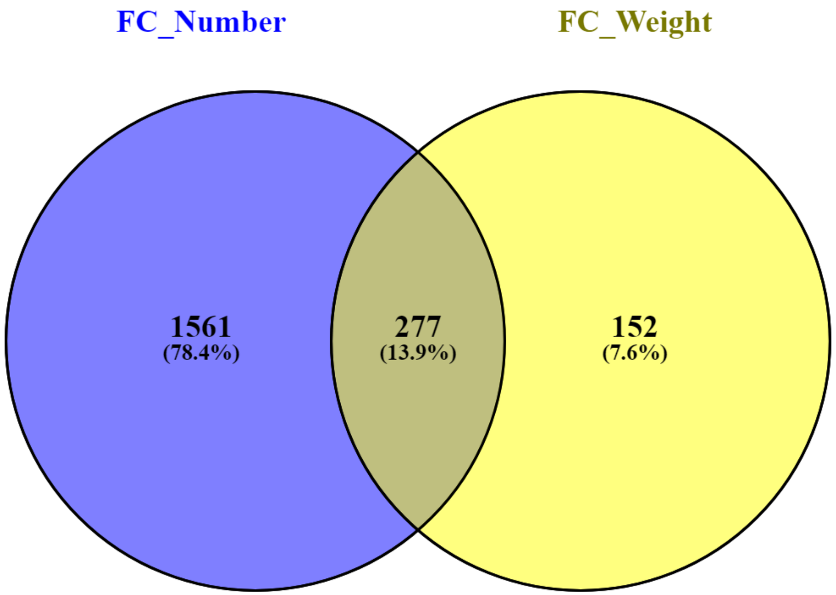
Transcriptome analysis by RNA-Seq

UNDEREXPRESSION

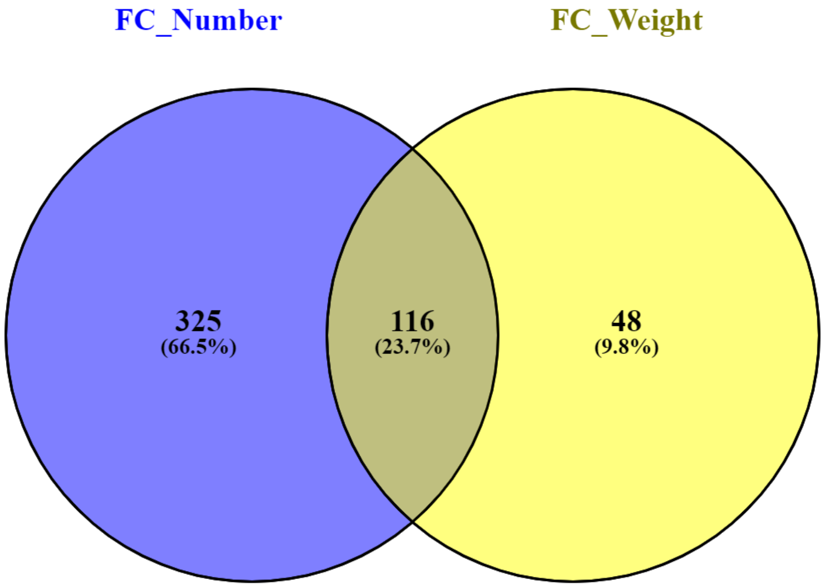
J1

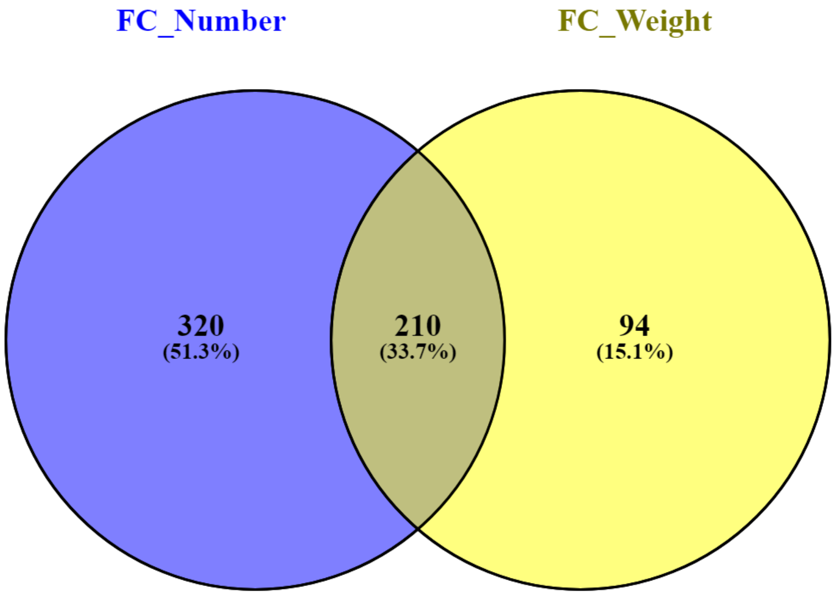
OVEREXPRESSION





J90





Enrichment analysis

– Pathway analysis

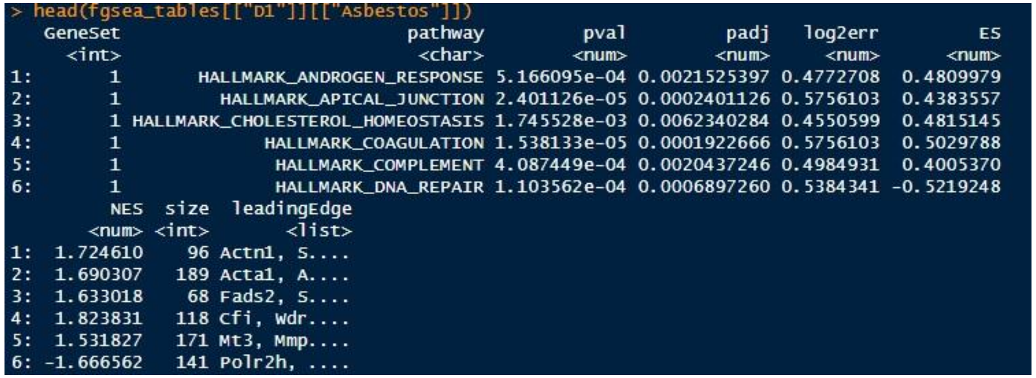
Transcriptome analysis by RNA-Seq

- **Pathway database as reference** : GO:BP, Reactome, KEGG, and Hallmark

Enrichment analysis

– Pathway analysis

- **Pathway database as reference :** GO:BP, Reactome, KEGG, and Hallmark
- GSEA on all genes R RStudio (fgsea)



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> head(fgsea_tables[["D1"]][["Asbestos"]])
   GeneSet                         pathway         pval         padj   log2err         ES
     <int>                          <char>        <num>        <num>     <num>      <num>
1:       1        HALLMARK_ANDROGEN_RESPONSE 5.166095e-04 0.0021525397 0.4772708  0.4809979
2:       1          HALLMARK_APICAL_JUNCTION 2.401126e-05 0.0002401126 0.5756103  0.4383557
3:       1 HALLMARK_CHOLESTEROL_HOMEOSTASIS 1.745528e-03 0.0062340284 0.4550599  0.4815145
4:       1             HALLMARK_COAGULATION 1.538133e-05 0.0001922666 0.5756103  0.5029788
5:       1              HALLMARK_COMPLEMENT 4.087449e-04 0.0020437246 0.4984931  0.4005370
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         NES  size  leadingEdge
       <num> <int>       <list>
1:  1.724610    96 Actn1, S....
2:  1.690307   189 Acta1, A....
3:  1.633018    68 Fads2, S....
4:  1.823831   118 Cfi, Wdr....
5:  1.531827   171 Mt3, Mmp....
6: -1.666562   141 Polr2h, ....
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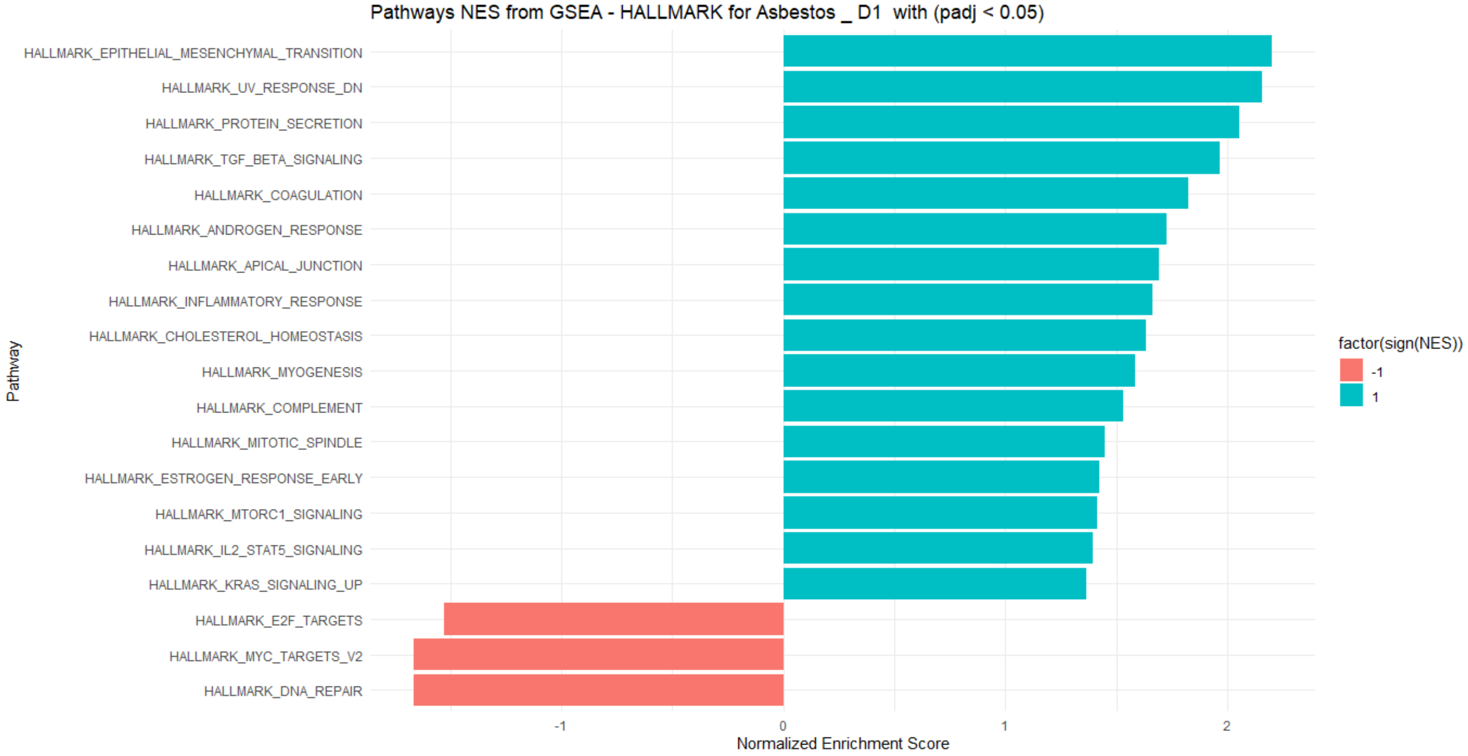
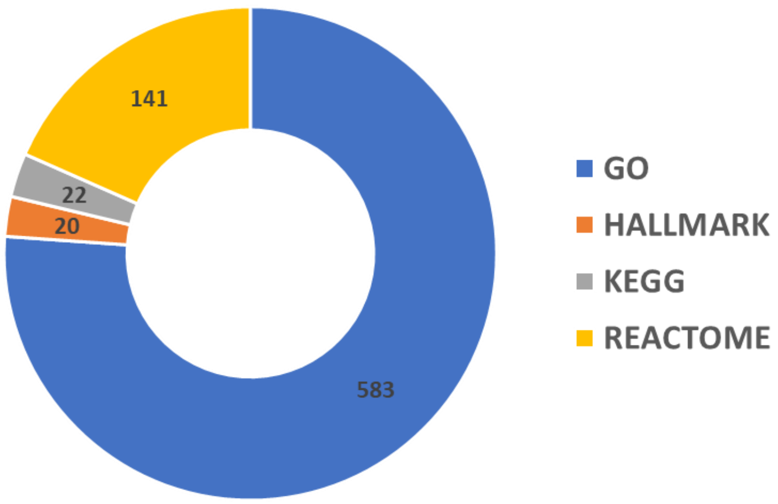


Gene Set Enrichment Analysis

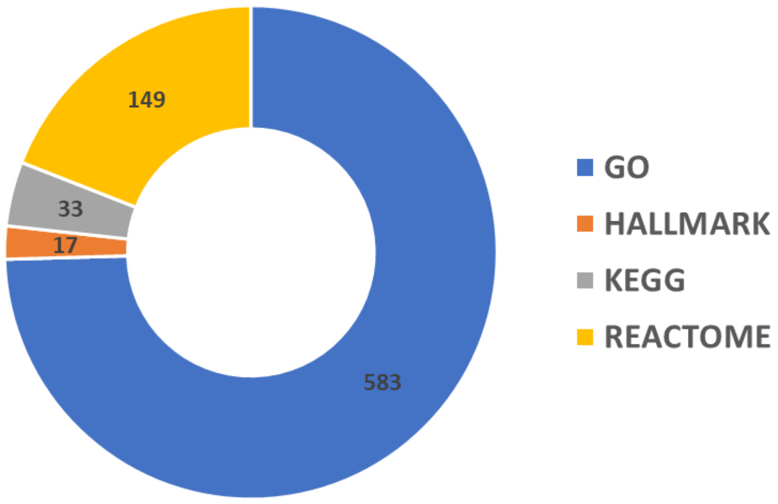
Transcriptome analysis by RNA-Seq

J1

Asbestos

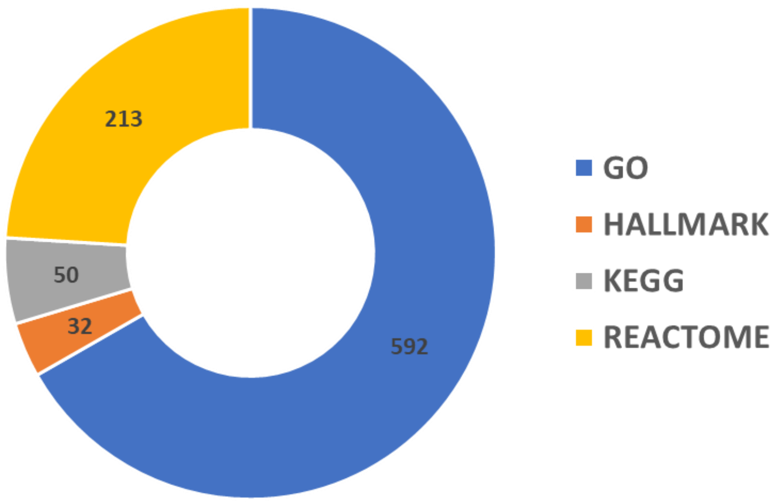


FC_Number



J90

Asbestos



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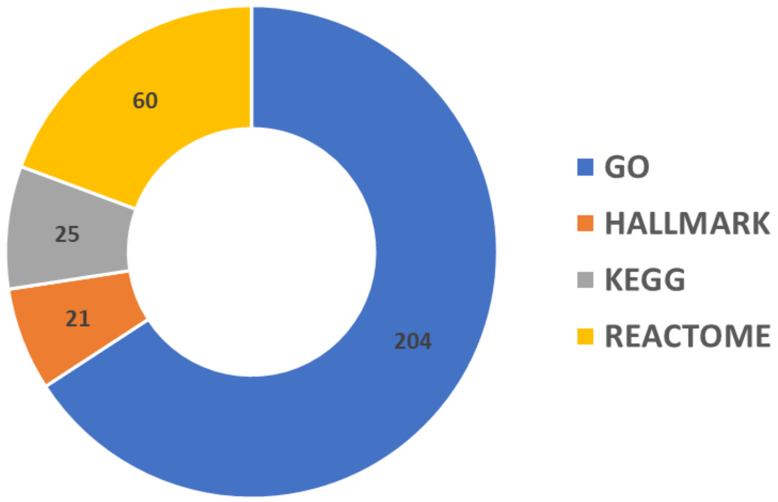
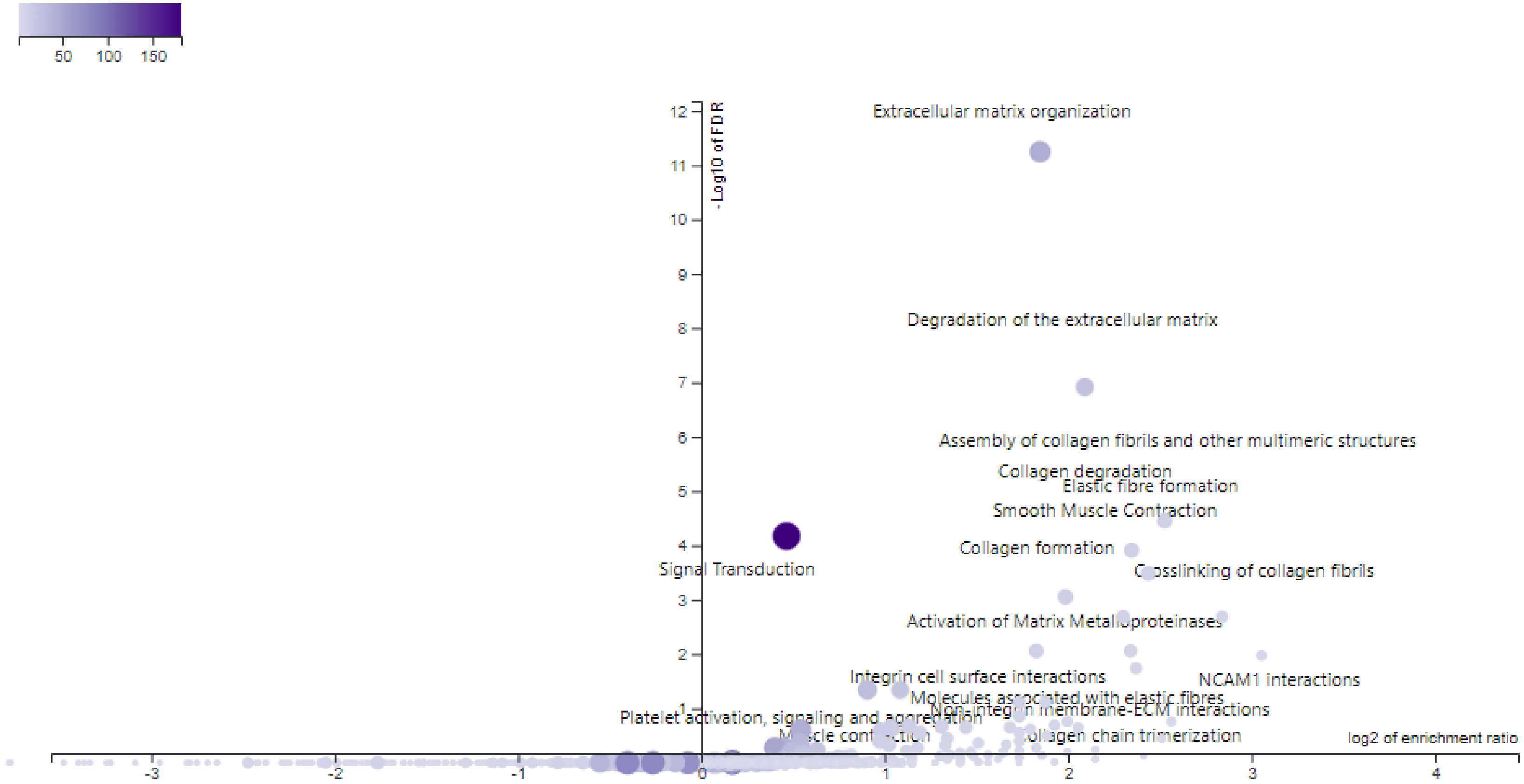


Enrichment analysis

– Pathway analysis

- **Pathway database as reference** : GO:BP, Reactome, KEGG, and Hallmark
- **GSEA on all genes** **(fgsea)**
- **Over-Representation Analysis on DE gene lists**

https://www.webgestalt.org/



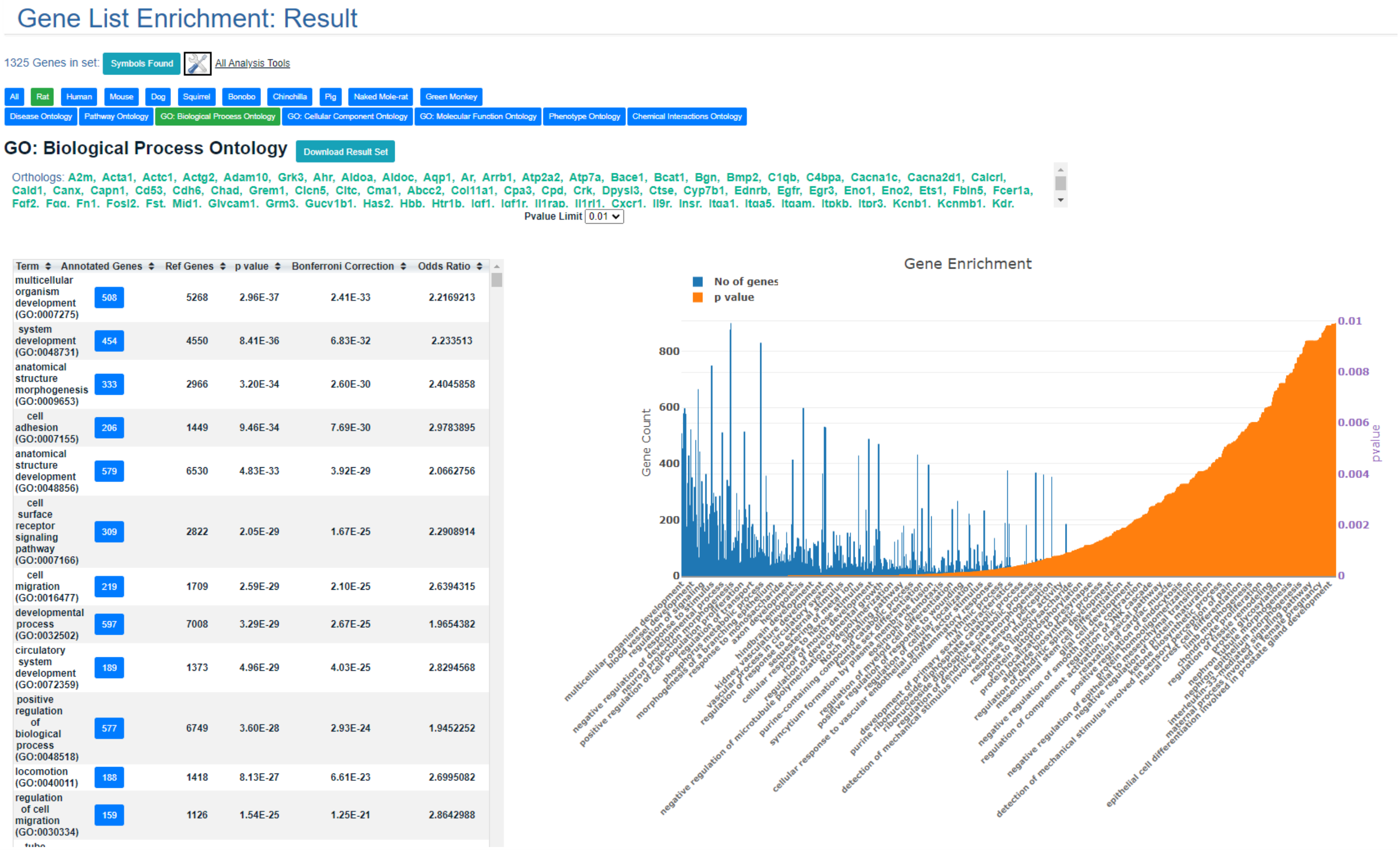
Enrichment analysis

– Pathway analysis

Transcriptome analysis by RNA-Seq

- **Pathway database as reference :** GO:BP, Reactome, KEGG, and Hallmark
- **GSEA on all genes** (fgsea)
- **Over-Representation Analysis on DE gene lists**

https://rgd.mcw.edu/



Gene List Enrichment: Result

1325 Genes in set: Symbols Found All Analysis Tools

GO: Biological Process Ontology

Orthologs: A2m, Acta1, Actc1, Actg2, Adam10, Grk3, Ahr, Aldoa, Aldoc, Aqp1, Ar, Arrb1, Atp2a2, Atp7a, Bace1, Bcat1, Bgn, Bmp2, C1qb, C4bpa, Cacna1c, Cacna2d1, Calcrl, Cald1, Canx, Capn1, Cd53, Cdh6, Chad, Grem1, Clcn5, Cltc, Cma1, Abcc2, Col11a1, Cpa3, Cpd, Crk, Dpysl3, Ctse, Cyp7b1, Ednrb, Egfr, Egr3, Eno1, Eno2, Ets1, Fbln5, Fcer1a, Fgf2, Fga, Fn1, Fosl2, Fst, Mid1, Glycam1, Grm3, Gucy1b1, Has2, Hbb, Htr1b, Igf1, Igf1r, Il1rap, Il1rl1, Cxcr1, Il9r, Insr, Itga1, Itga5, Itgam, Itpkb, Itpr3, Kcnb1, Kcnmb1, Kdr,

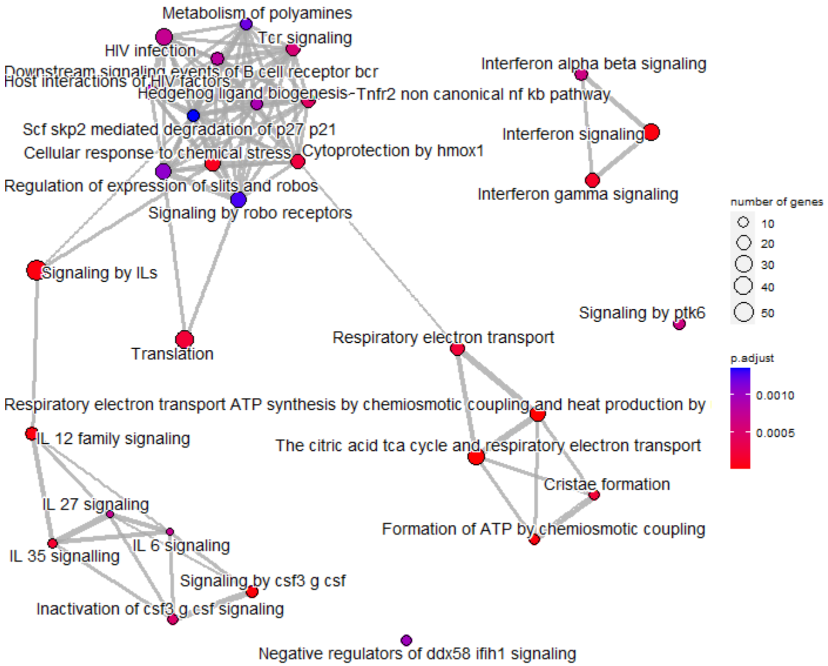
Pvalue Limit 0.01

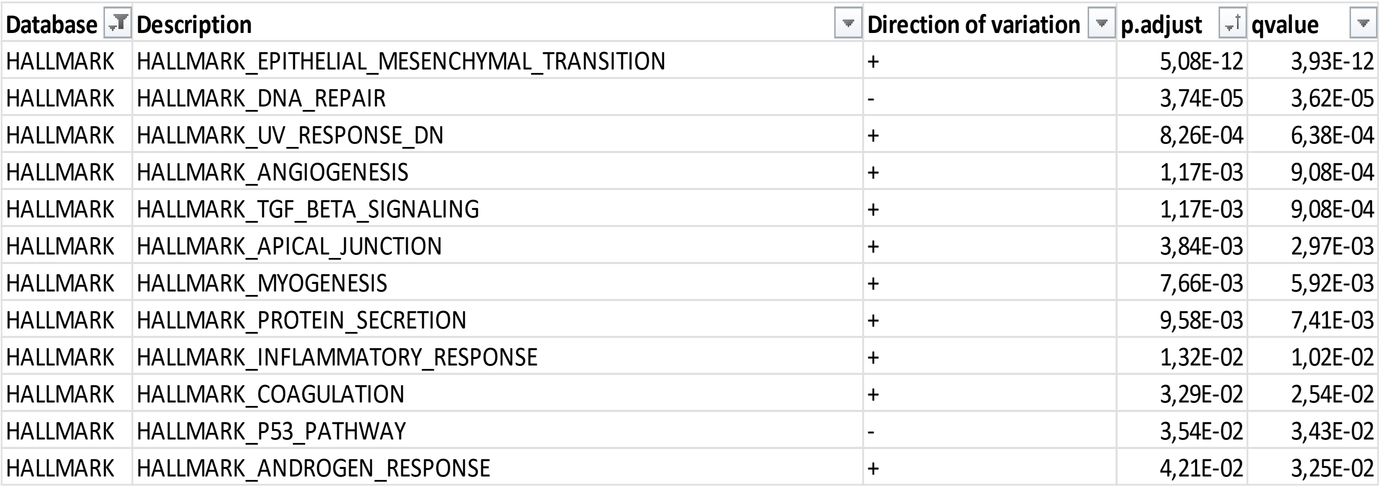
Term	Annotated Genes	Ref Genes	p value	Bonferroni Correction	Odds Ratio
multicellular organism development (GO:0007275)	508	5268	2.96E-37	2.41E-33	2.2169213
system development (GO:0048731)	454	4550	8.41E-36	6.83E-32	2.233513
anatomical structure morphogenesis (GO:0009653)	333	2966	3.20E-34	2.60E-30	2.4045858
cell adhesion (GO:0007155)	206	1449	9.46E-34	7.69E-30	2.9783895
anatomical structure development (GO:0048856)	579	6530	4.83E-33	3.92E-29	2.0662756
cell surface receptor signaling pathway (GO:0007166)	309	2822	2.05E-29	1.67E-25	2.2908914
cell migration (GO:0016477)	219	1709	2.59E-29	2.10E-25	2.6394315
developmental process (GO:0032502)	597	7008	3.29E-29	2.67E-25	1.9654382
circulatory system development (GO:0072359)	189	1373	4.96E-29	4.03E-25	2.8294568
positive regulation of biological process (GO:0048518)	577	6749	3.60E-28	2.93E-24	1.9452252
locomotion (GO:0040011)	188	1418	8.13E-27	6.61E-23	2.6995082
regulation of cell migration (GO:0030334)	159	1126	1.54E-25	1.25E-21	2.8642988

Enrichment analysis

– Pathway analysis

- **Pathway database as reference** : GO:BP, Reactome, KEGG, and Hallmark
- **GSEA on all genes** (fgsea)
- **Over-Representation Analysis on DE gene lists** (ClusterProfiler)





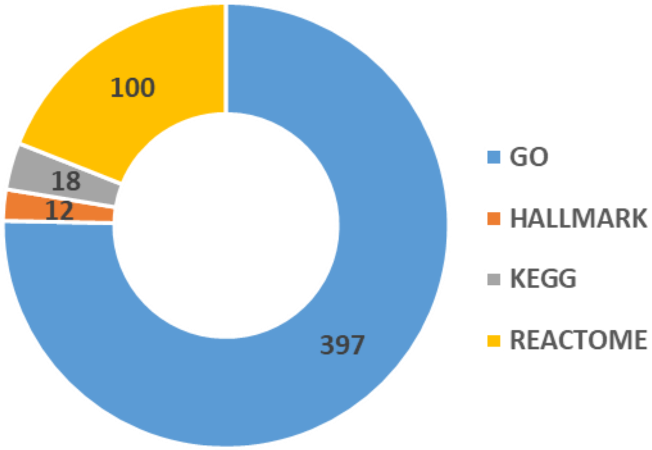
Database	Description	Direction of variation	p.adjust	qvalue
HALLMARK	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	+	5,08E-12	3,93E-12
HALLMARK	HALLMARK_DNA_REPAIR	-	3,74E-05	3,62E-05
HALLMARK	HALLMARK_UV_RESPONSE_DN	+	8,26E-04	6,38E-04
HALLMARK	HALLMARK_ANGIOGENESIS	+	1,17E-03	9,08E-04
HALLMARK	HALLMARK_TGF_BETA_SIGNALING	+	1,17E-03	9,08E-04
HALLMARK	HALLMARK_APICAL_JUNCTION	+	3,84E-03	2,97E-03
HALLMARK	HALLMARK_MYOGENESIS	+	7,66E-03	5,92E-03
HALLMARK	HALLMARK_PROTEIN_SECRETION	+	9,58E-03	7,41E-03
HALLMARK	HALLMARK_INFLAMMATORY_RESPONSE	+	1,32E-02	1,02E-02
HALLMARK	HALLMARK_COAGULATION	+	3,29E-02	2,54E-02
HALLMARK	HALLMARK_P53_PATHWAY	-	3,54E-02	3,43E-02
HALLMARK	HALLMARK_ANDROGEN_RESPONSE	+	4,21E-02	3,25E-02

Over-Representation Analysis

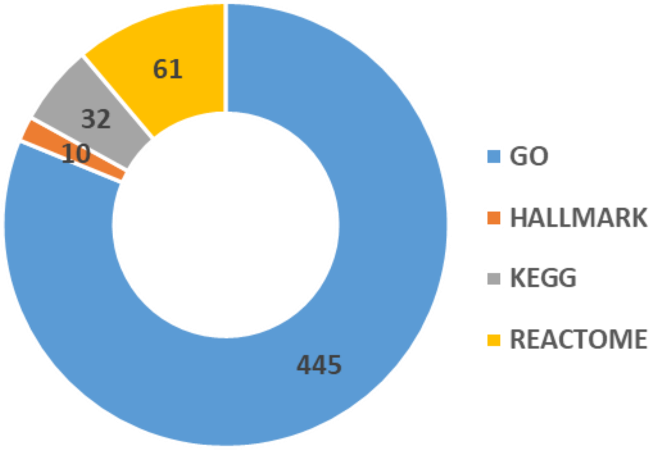
Transcriptome analysis by RNA-Seq

J1

Asbestos

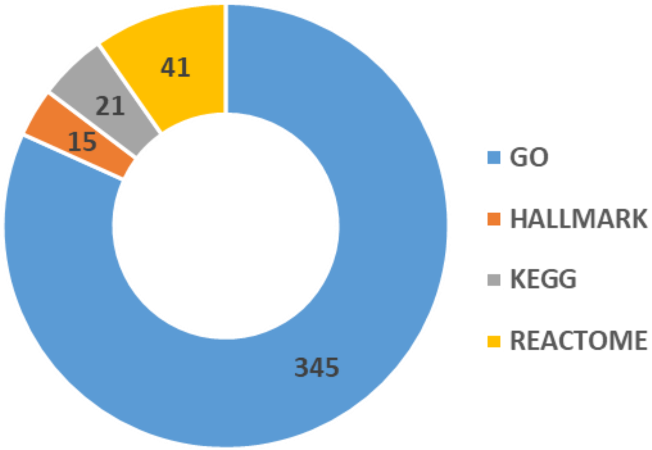


FC_Number



J90

Asbestos



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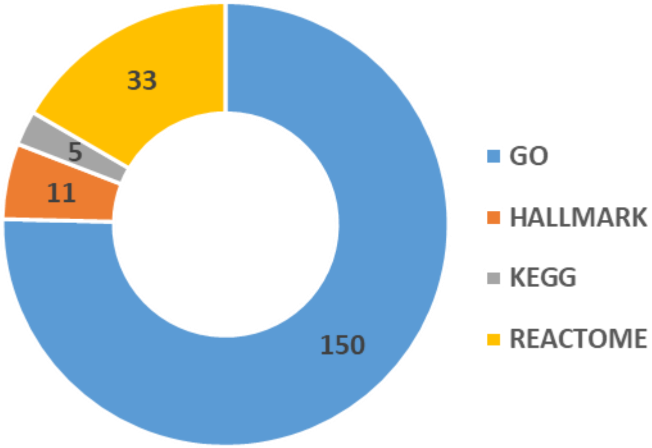
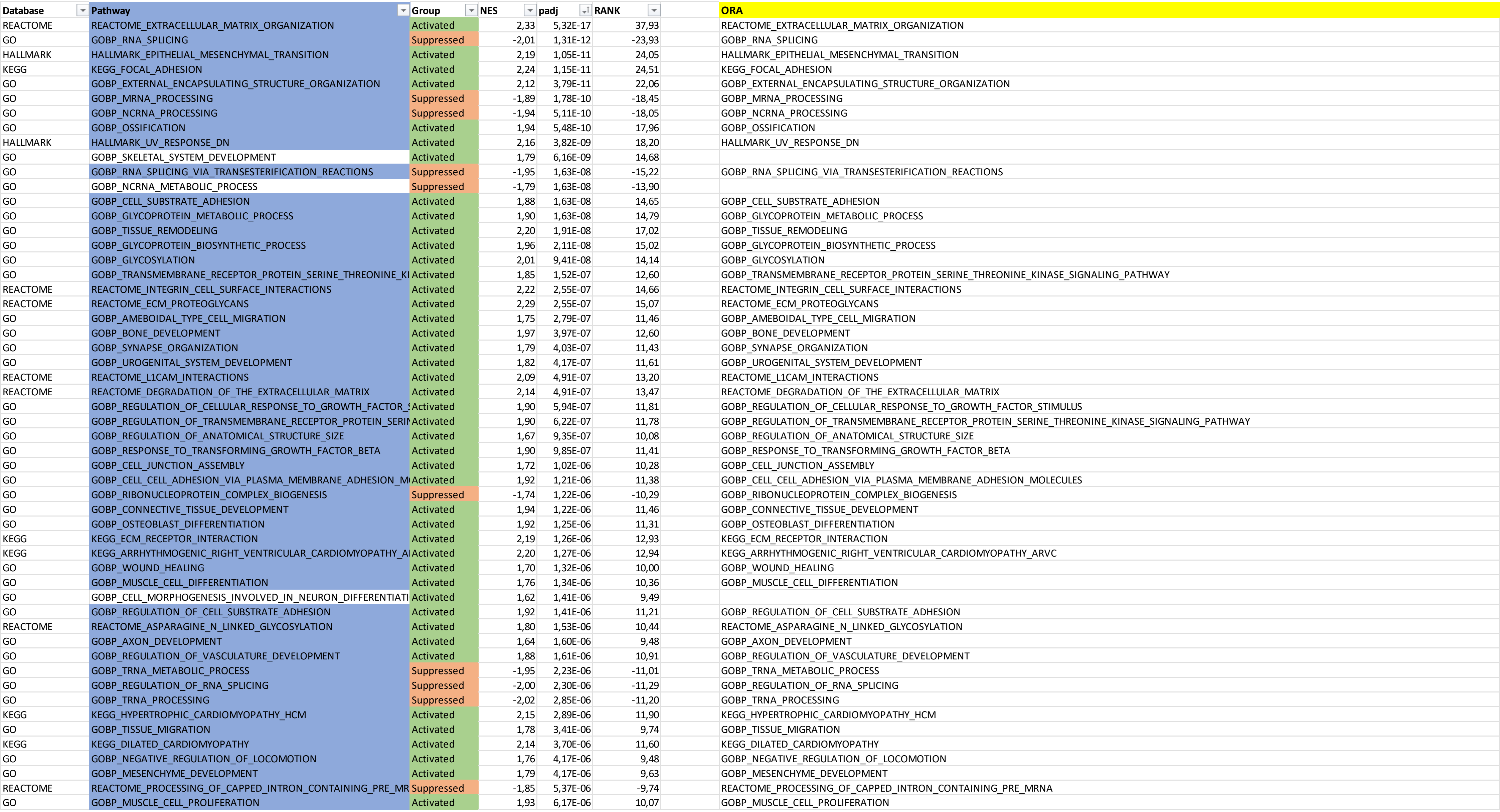


GSEA vs ORA

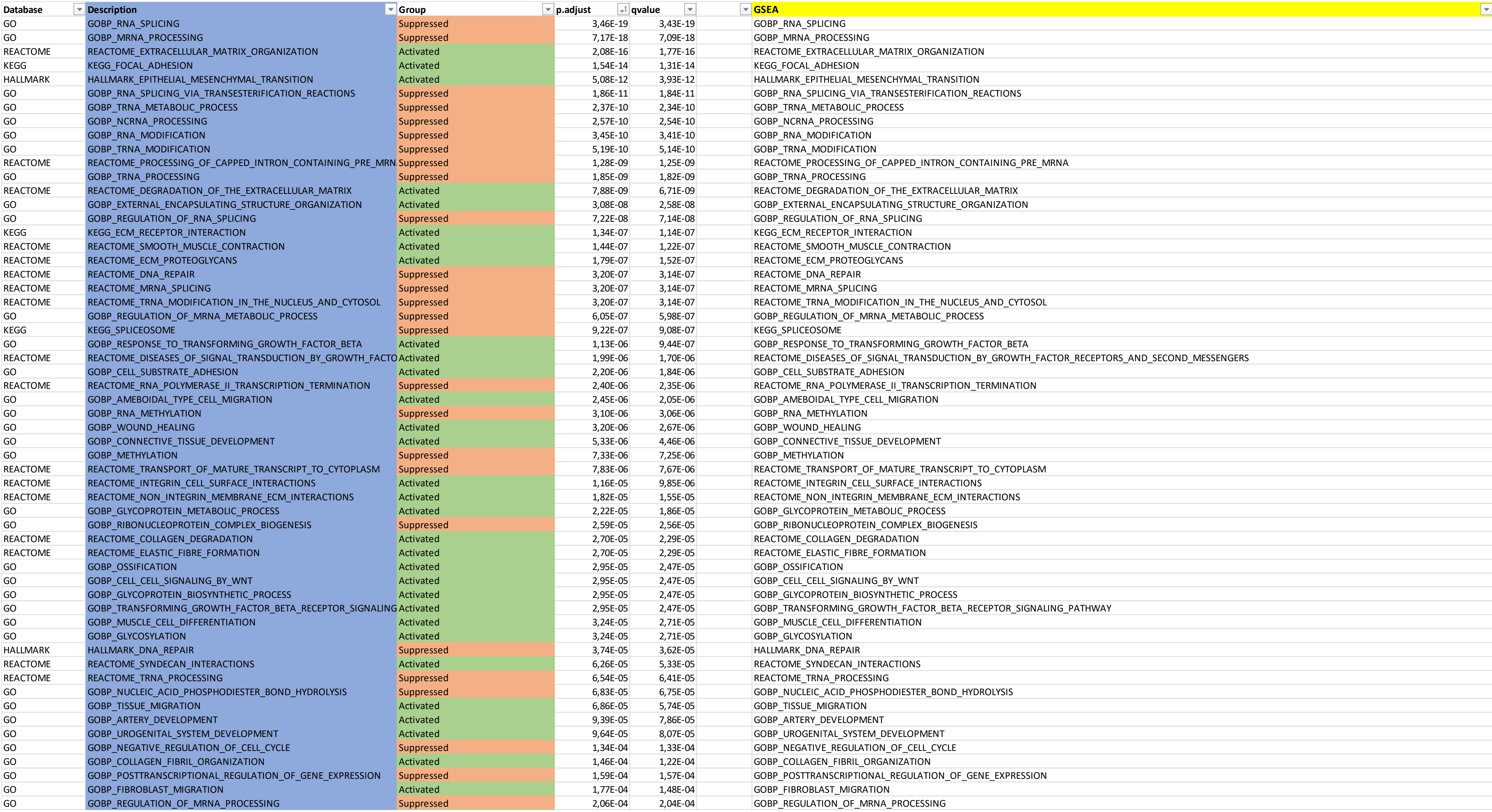
Transcriptome analysis by RNA-Seq



Database	Pathway	Group	NES	padj	RANK	ORA
REACTOME	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION	Activated	2,33	5,32E-17	37,93	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
GO	GOBP_RNA_SPLICING	Suppressed	-2,01	1,31E-12	-23,93	GOBP_RNA_SPLICING
HALLMARK	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	Activated	2,19	1,05E-11	24,05	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
KEGG	KEGG_FOCAL_ADHESION	Activated	2,24	1,15E-11	24,51	KEGG_FOCAL_ADHESION
GO	GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION	Activated	2,12	3,79E-11	22,06	GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION
GO	GOBP_MRNA_PROCESSING	Suppressed	-1,89	1,78E-10	-18,45	GOBP_MRNA_PROCESSING
GO	GOBP_NCRNA_PROCESSING	Suppressed	-1,94	5,11E-10	-18,05	GOBP_NCRNA_PROCESSING
GO	GOBP_OSSIFICATION	Activated	1,94	5,48E-10	17,96	GOBP_OSSIFICATION
HALLMARK	HALLMARK_UV_RESPONSE_DN	Activated	2,16	3,82E-09	18,20	HALLMARK_UV_RESPONSE_DN
GO	GOBP_SKELETAL_SYSTEM_DEVELOPMENT	Activated	1,79	6,16E-09	14,68	
GO	GOBP_RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS	Suppressed	-1,95	1,63E-08	-15,22	GOBP_RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS
GO	GOBP_NCRNA_METABOLIC_PROCESS	Suppressed	-1,79	1,63E-08	-13,90	
GO	GOBP_CELL_SUBSTRATE_ADHESION	Activated	1,88	1,63E-08	14,65	GOBP_CELL_SUBSTRATE_ADHESION
GO	GOBP_GLYCOPROTEIN_METABOLIC_PROCESS	Activated	1,90	1,63E-08	14,79	GOBP_GLYCOPROTEIN_METABOLIC_PROCESS
GO	GOBP_TISSUE_REMODELING	Activated	2,20	1,91E-08	17,02	GOBP_TISSUE_REMODELING
GO	GOBP_GLYCOPROTEIN_BIOSYNTHETIC_PROCESS	Activated	1,96	2,11E-08	15,02	GOBP_GLYCOPROTEIN_BIOSYNTHETIC_PROCESS
GO	GOBP_GLYCOSYLATION	Activated	2,01	9,41E-08	14,14	GOBP_GLYCOSYLATION
GO	GOBP_TRANSMEMBRANE_RECEPTOR_PROTEIN_SERINE_THREONINE_KI	Activated	1,85	1,52E-07	12,60	GOBP_TRANSMEMBRANE_RECEPTOR_PROTEIN_SERINE_THREONINE_KINASE_SIGNALING_PATHWAY
REACTOME	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	Activated	2,22	2,55E-07	14,66	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
REACTOME	REACTOME_ECM_PROTEOGLYCANS	Activated	2,29	2,55E-07	15,07	REACTOME_ECM_PROTEOGLYCANS
GO	GOBP_AMEBOIDAL_TYPE_CELL_MIGRATION	Activated	1,75	2,79E-07	11,46	GOBP_AMEBOIDAL_TYPE_CELL_MIGRATION
GO	GOBP_BONE_DEVELOPMENT	Activated	1,97	3,97E-07	12,60	GOBP_BONE_DEVELOPMENT
GO	GOBP_SYNAPSE_ORGANIZATION	Activated	1,79	4,03E-07	11,43	GOBP_SYNAPSE_ORGANIZATION
GO	GOBP_UROGENITAL_SYSTEM_DEVELOPMENT	Activated	1,82	4,17E-07	11,61	GOBP_UROGENITAL_SYSTEM_DEVELOPMENT
REACTOME	REACTOME_L1CAM_INTERACTIONS	Activated	2,09	4,91E-07	13,20	REACTOME_L1CAM_INTERACTIONS
REACTOME	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	Activated	2,14	4,91E-07	13,47	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX
GO	GOBP_REGULATION_OF_CELLULAR_RESPONSE_TO_GROWTH_FACTOR_	Activated	1,90	5,94E-07	11,81	GOBP_REGULATION_OF_CELLULAR_RESPONSE_TO_GROWTH_FACTOR_STIMULUS
GO	GOBP_REGULATION_OF_TRANSMEMBRANE_RECEPTOR_PROTEIN_SERIN	Activated	1,90	6,22E-07	11,78	GOBP_REGULATION_OF_TRANSMEMBRANE_RECEPTOR_PROTEIN_SERINE_THREONINE_KINASE_SIGNALING_PATHWAY
GO	GOBP_REGULATION_OF_ANATOMICAL_STRUCTURE_SIZE	Activated	1,67	9,35E-07	10,08	GOBP_REGULATION_OF_ANATOMICAL_STRUCTURE_SIZE
GO	GOBP_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA	Activated	1,90	9,85E-07	11,41	GOBP_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA
GO	GOBP_CELL_JUNCTION_ASSEMBLY	Activated	1,72	1,02E-06	10,28	GOBP_CELL_JUNCTION_ASSEMBLY
GO	GOBP_CELL_CELL_ADHESION_VIA_PLASMA_MEMBRANE_ADHESION_M	Activated	1,92	1,21E-06	11,38	GOBP_CELL_CELL_ADHESION_VIA_PLASMA_MEMBRANE_ADHESION_MOLECULES
GO	GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS	Suppressed	-1,74	1,22E-06	-10,29	GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS
GO	GOBP_CONNECTIVE_TISSUE_DEVELOPMENT	Activated	1,94	1,22E-06	11,46	GOBP_CONNECTIVE_TISSUE_DEVELOPMENT
GO	GOBP_OSTEOBLAST_DIFFERENTIATION	Activated	1,92	1,25E-06	11,31	GOBP_OSTEOBLAST_DIFFERENTIATION
KEGG	KEGG_ECM_RECEPTOR_INTERACTION	Activated	2,19	1,26E-06	12,93	KEGG_ECM_RECEPTOR_INTERACTION
KEGG	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_A	Activated	2,20	1,27E-06	12,94	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC
GO	GOBP_WOUND_HEALING	Activated	1,70	1,32E-06	10,00	GOBP_WOUND_HEALING
GO	GOBP_MUSCLE_CELL_DIFFERENTIATION	Activated	1,76	1,34E-06	10,36	GOBP_MUSCLE_CELL_DIFFERENTIATION
GO	GOBP_CELL_MORPHOGENESIS_INVOLVED_IN_NEURON_DIFFERENTIATI	Activated	1,62	1,41E-06	9,49	
GO	GOBP_REGULATION_OF_CELL_SUBSTRATE_ADHESION	Activated	1,92	1,41E-06	11,21	GOBP_REGULATION_OF_CELL_SUBSTRATE_ADHESION
REACTOME	REACTOME_ASPARAGINE_N_LINKED_GLYCOSYLATION	Activated	1,80	1,53E-06	10,44	REACTOME_ASPARAGINE_N_LINKED_GLYCOSYLATION
GO	GOBP_AXON_DEVELOPMENT	Activated	1,64	1,60E-06	9,48	GOBP_AXON_DEVELOPMENT
GO	GOBP_REGULATION_OF_VASCULATURE_DEVELOPMENT	Activated	1,88	1,61E-06	10,91	GOBP_REGULATION_OF_VASCULATURE_DEVELOPMENT
GO	GOBP_TRNA_METABOLIC_PROCESS	Suppressed	-1,95	2,23E-06	-11,01	GOBP_TRNA_METABOLIC_PROCESS
GO	GOBP_REGULATION_OF_RNA_SPLICING	Suppressed	-2,00	2,30E-06	-11,29	GOBP_REGULATION_OF_RNA_SPLICING
GO	GOBP_TRNA_PROCESSING	Suppressed	-2,02	2,85E-06	-11,20	GOBP_TRNA_PROCESSING
KEGG	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	Activated	2,15	2,89E-06	11,90	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM
GO	GOBP_TISSUE_MIGRATION	Activated	1,78	3,41E-06	9,74	GOBP_TISSUE_MIGRATION
KEGG	KEGG_DILATED_CARDIOMYOPATHY	Activated	2,14	3,70E-06	11,60	KEGG_DILATED_CARDIOMYOPATHY
GO	GOBP_NEGATIVE_REGULATION_OF_LOCOMOTION	Activated	1,76	4,17E-06	9,48	GOBP_NEGATIVE_REGULATION_OF_LOCOMOTION
GO	GOBP_MESENCHYME_DEVELOPMENT	Activated	1,79	4,17E-06	9,63	GOBP_MESENCHYME_DEVELOPMENT
REACTOME	REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MR	Suppressed	-1,85	5,37E-06	-9,74	REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA
GO	GOBP_MUSCLE_CELL_PROLIFERATION	Activated	1,93	6,17E-06	10,07	GOBP_MUSCLE_CELL_PROLIFERATION

GSEA vs ORA

Transcriptome analysis by RNA-Seq



Database	Description	Group	p.adjust	qvalue		GSEA
GO	GOBP_RNA_SPLICING	Suppressed	3,46E-19	3,43E-19		GOBP_RNA_SPLICING
GO	GOBP_MRNA_PROCESSING	Suppressed	7,17E-18	7,09E-18		GOBP_MRNA_PROCESSING
REACTOME	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION	Activated	2,08E-16	1,77E-16		REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
KEGG	KEGG_FOCAL_ADHESION	Activated	1,54E-14	1,31E-14		KEGG_FOCAL_ADHESION
HALLMARK	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	Activated	5,08E-12	3,93E-12		HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
GO	GOBP_RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS	Suppressed	1,86E-11	1,84E-11		GOBP_RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS
GO	GOBP_TRNA_METABOLIC_PROCESS	Suppressed	2,37E-10	2,34E-10		GOBP_TRNA_METABOLIC_PROCESS
GO	GOBP_NCRNA_PROCESSING	Suppressed	2,57E-10	2,54E-10		GOBP_NCRNA_PROCESSING
GO	GOBP_RNA_MODIFICATION	Suppressed	3,45E-10	3,41E-10		GOBP_RNA_MODIFICATION
GO	GOBP_TRNA_MODIFICATION	Suppressed	5,19E-10	5,14E-10		GOBP_TRNA_MODIFICATION
REACTOME	REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRN	Suppressed	1,28E-09	1,25E-09		REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA
GO	GOBP_TRNA_PROCESSING	Suppressed	1,85E-09	1,82E-09		GOBP_TRNA_PROCESSING
REACTOME	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	Activated	7,88E-09	6,71E-09		REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX
GO	GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION	Activated	3,08E-08	2,58E-08		GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION
GO	GOBP_REGULATION_OF_RNA_SPLICING	Suppressed	7,22E-08	7,14E-08		GOBP_REGULATION_OF_RNA_SPLICING
KEGG	KEGG_ECM_RECEPTOR_INTERACTION	Activated	1,34E-07	1,14E-07		KEGG_ECM_RECEPTOR_INTERACTION
REACTOME	REACTOME_SMOOTH_MUSCLE_CONTRACTION	Activated	1,44E-07	1,22E-07		REACTOME_SMOOTH_MUSCLE_CONTRACTION
REACTOME	REACTOME_ECM_PROTEOGLYCANS	Activated	1,79E-07	1,52E-07		REACTOME_ECM_PROTEOGLYCANS
REACTOME	REACTOME_DNA_REPAIR	Suppressed	3,20E-07	3,14E-07		REACTOME_DNA_REPAIR
REACTOME	REACTOME_MRNA_SPLICING	Suppressed	3,20E-07	3,14E-07		REACTOME_MRNA_SPLICING
REACTOME	REACTOME_TRNA_MODIFICATION_IN_THE_NUCLEUS_AND_CYTOSOL	Suppressed	3,20E-07	3,14E-07		REACTOME_TRNA_MODIFICATION_IN_THE_NUCLEUS_AND_CYTOSOL
GO	GOBP_REGULATION_OF_MRNA_METABOLIC_PROCESS	Suppressed	6,05E-07	5,98E-07		GOBP_REGULATION_OF_MRNA_METABOLIC_PROCESS
KEGG	KEGG_SPLICEOSOME	Suppressed	9,22E-07	9,08E-07		KEGG_SPLICEOSOME
GO	GOBP_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA	Activated	1,13E-06	9,44E-07		GOBP_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA
REACTOME	REACTOME_DISEASES_OF_SIGNAL_TRANSDUCTION_BY_GROWTH_FACTO	Activated	1,99E-06	1,70E-06		REACTOME_DISEASES_OF_SIGNAL_TRANSDUCTION_BY_GROWTH_FACTOR_RECEPTORS_AND_SECOND_MESSENGERS
GO	GOBP_CELL_SUBSTRATE_ADHESION	Activated	2,20E-06	1,84E-06		GOBP_CELL_SUBSTRATE_ADHESION
REACTOME	REACTOME_RNA_POLYMERASE_II_TRANSCRIPTION_TERMINATION	Suppressed	2,40E-06	2,35E-06		REACTOME_RNA_POLYMERASE_II_TRANSCRIPTION_TERMINATION
GO	GOBP_AMEBOIDAL_TYPE_CELL_MIGRATION	Activated	2,45E-06	2,05E-06		GOBP_AMEBOIDAL_TYPE_CELL_MIGRATION
GO	GOBP_RNA_METHYLATION	Suppressed	3,10E-06	3,06E-06		GOBP_RNA_METHYLATION
GO	GOBP_WOUND_HEALING	Activated	3,20E-06	2,67E-06		GOBP_WOUND_HEALING
GO	GOBP_CONNECTIVE_TISSUE_DEVELOPMENT	Activated	5,33E-06	4,46E-06		GOBP_CONNECTIVE_TISSUE_DEVELOPMENT
GO	GOBP_METHYLATION	Suppressed	7,33E-06	7,25E-06		GOBP_METHYLATION
REACTOME	REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM	Suppressed	7,83E-06	7,67E-06		REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM
REACTOME	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	Activated	1,16E-05	9,85E-06		REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
REACTOME	REACTOME_NON_INTEGRIN_MEMBRANE_ECM_INTERACTIONS	Activated	1,82E-05	1,55E-05		REACTOME_NON_INTEGRIN_MEMBRANE_ECM_INTERACTIONS
GO	GOBP_GLYCOPROTEIN_METABOLIC_PROCESS	Activated	2,22E-05	1,86E-05		GOBP_GLYCOPROTEIN_METABOLIC_PROCESS
GO	GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS	Suppressed	2,59E-05	2,56E-05		GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS
REACTOME	REACTOME_COLLAGEN_DEGRADATION	Activated	2,70E-05	2,29E-05		REACTOME_COLLAGEN_DEGRADATION
REACTOME	REACTOME_ELASTIC_FIBRE_FORMATION	Activated	2,70E-05	2,29E-05		REACTOME_ELASTIC_FIBRE_FORMATION
GO	GOBP_OSSIFICATION	Activated	2,95E-05	2,47E-05		GOBP_OSSIFICATION
GO	GOBP_CELL_CELL_SIGNALING_BY_WNT	Activated	2,95E-05	2,47E-05		GOBP_CELL_CELL_SIGNALING_BY_WNT
GO	GOBP_GLYCOPROTEIN_BIOSYNTHETIC_PROCESS	Activated	2,95E-05	2,47E-05		GOBP_GLYCOPROTEIN_BIOSYNTHETIC_PROCESS
GO	GOBP_TRANSFORMING_GROWTH_FACTOR_BETA_RECEPTOR_SIGNALING	Activated	2,95E-05	2,47E-05		GOBP_TRANSFORMING_GROWTH_FACTOR_BETA_RECEPTOR_SIGNALING_PATHWAY
GO	GOBP_MUSCLE_CELL_DIFFERENTIATION	Activated	3,24E-05	2,71E-05		GOBP_MUSCLE_CELL_DIFFERENTIATION
GO	GOBP_GLYCOSYLATION	Activated	3,24E-05	2,71E-05		GOBP_GLYCOSYLATION
HALLMARK	HALLMARK_DNA_REPAIR	Suppressed	3,74E-05	3,62E-05		HALLMARK_DNA_REPAIR
REACTOME	REACTOME_SYNDECAN_INTERACTIONS	Activated	6,26E-05	5,33E-05		REACTOME_SYNDECAN_INTERACTIONS
REACTOME	REACTOME_TRNA_PROCESSING	Suppressed	6,54E-05	6,41E-05		REACTOME_TRNA_PROCESSING
GO	GOBP_NUCLEIC_ACID_PHOSPHODIESTER_BOND_HYDROLYSIS	Suppressed	6,83E-05	6,75E-05		GOBP_NUCLEIC_ACID_PHOSPHODIESTER_BOND_HYDROLYSIS
GO	GOBP_TISSUE_MIGRATION	Activated	6,86E-05	5,74E-05		GOBP_TISSUE_MIGRATION
GO	GOBP_ARTERY_DEVELOPMENT	Activated	9,39E-05	7,86E-05		GOBP_ARTERY_DEVELOPMENT
GO	GOBP_UROGENITAL_SYSTEM_DEVELOPMENT	Activated	9,64E-05	8,07E-05		GOBP_UROGENITAL_SYSTEM_DEVELOPMENT
GO	GOBP_NEGATIVE_REGULATION_OF_CELL_CYCLE	Suppressed	1,34E-04	1,33E-04		GOBP_NEGATIVE_REGULATION_OF_CELL_CYCLE
GO	GOBP_COLLAGEN_FIBRIL_ORGANIZATION	Activated	1,46E-04	1,22E-04		GOBP_COLLAGEN_FIBRIL_ORGANIZATION
GO	GOBP_POSTTRANSCRIPTIONAL_REGULATION_OF_GENE_EXPRESSION	Suppressed	1,59E-04	1,57E-04		GOBP_POSTTRANSCRIPTIONAL_REGULATION_OF_GENE_EXPRESSION
GO	GOBP_FIBROBLAST_MIGRATION	Activated	1,77E-04	1,48E-04		GOBP_FIBROBLAST_MIGRATION
GO	GOBP_REGULATION_OF_MRNA_PROCESSING	Suppressed	2,06E-04	2,04E-04		GOBP_REGULATION_OF_MRNA_PROCESSING

Enrichment analysis

– Pathway analysis

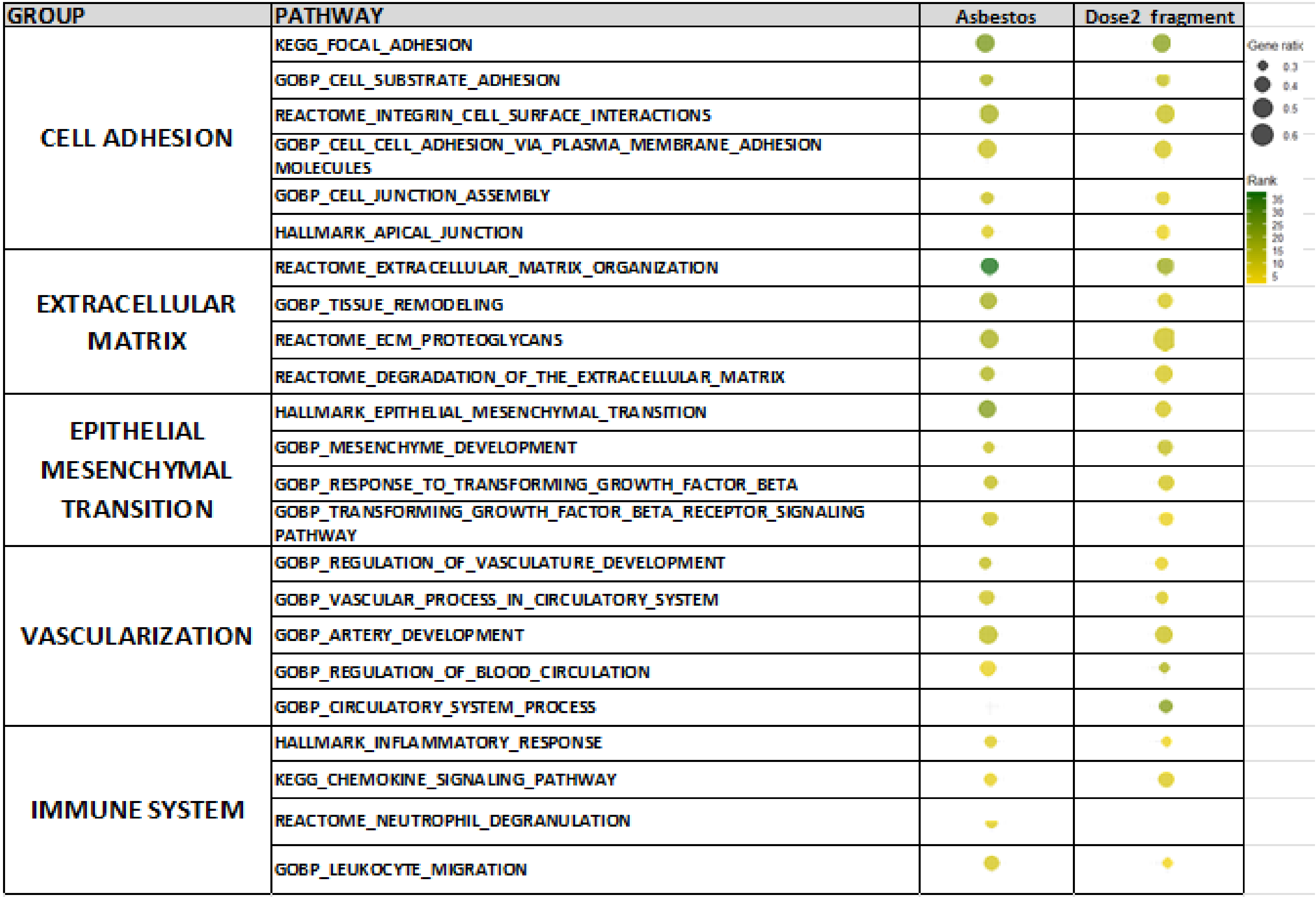
- **Pathway database as reference** : GO:BP, Reactome, KEGG, and Hallmark
- GSEA on all genes (fgsea)
- Over-Representation Analysis on DE gene lists (ClusterProfiler)

– Significant enrichments

- biological pathways with significant enrichments (p-value adjusted < 0.05)
- Focused on presenting GSEA results, as both analyses gave consistent findings
- Classified and grouped these pathways into main categories for in-depth analysis

Gene Set Enrichment Analysis (GSEA) : J1

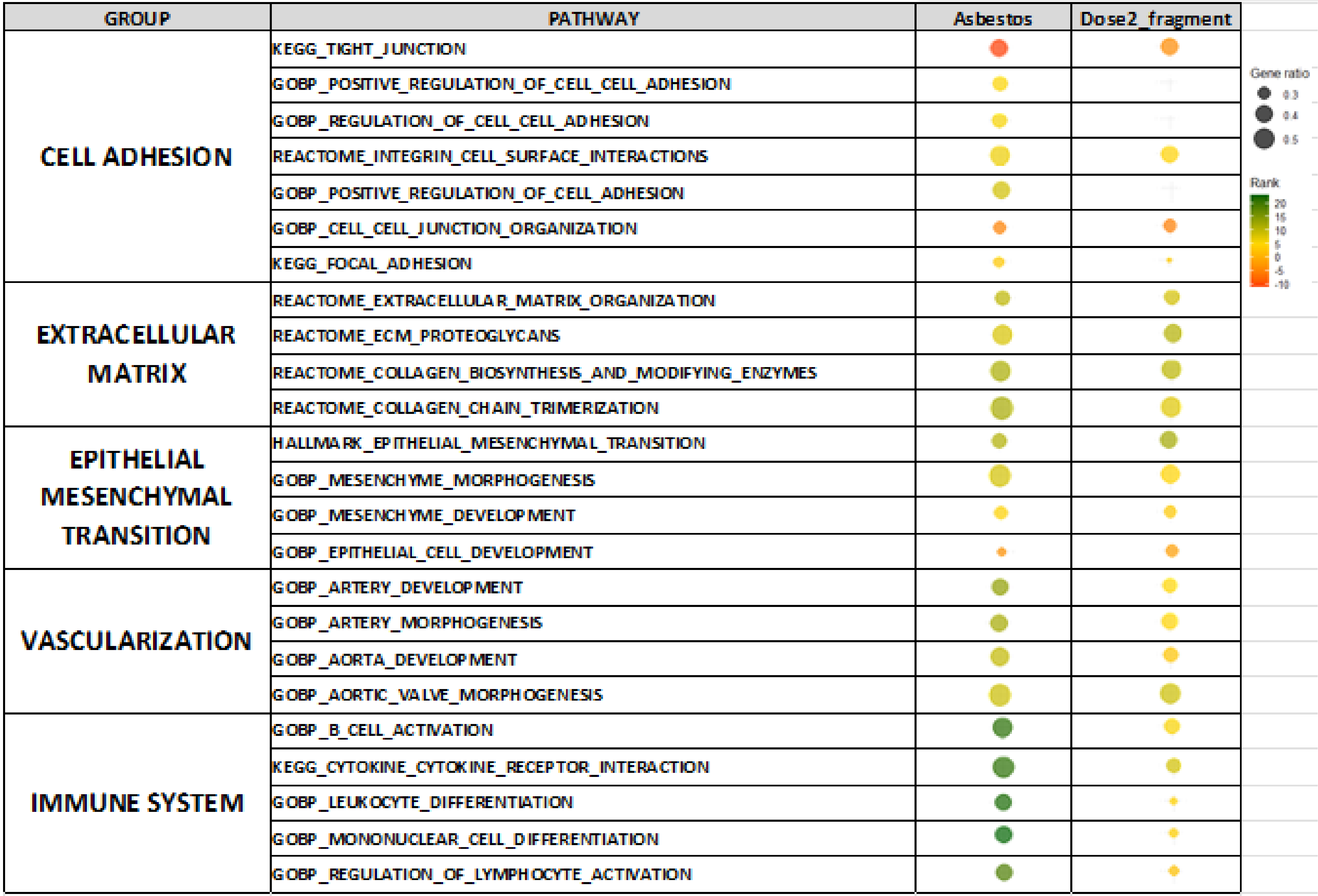
Transcriptome analysis by RNA-Seq



GROUP	PATHWAY	Asbestos	Dose2 fragment
CELL ADHESION	KEGG_FOCAL_ADHESION	●	●
	GOBP_CELL_SUBSTRATE_ADHESION	●	●
	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	●	●
	GOBP_CELL_CELL_ADHESION_VIA_PLASMA_MEMBRANE_ADHESION MOLECULES	●	●
	GOBP_CELL_JUNCTION_ASSEMBLY	●	●
	HALLMARK_APICAL_JUNCTION	●	●
EXTRACELLULAR MATRIX	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION	●	●
	GOBP_TISSUE_REMODELING	●	●
	REACTOME_ECM_PROTEOGLYCANS	●	●
	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	●	●
EPITHELIAL MESENCHYMAL TRANSITION	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	●	●
	GOBP_MESENCHYME_DEVELOPMENT	●	●
	GOBP_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA	●	●
	GOBP_TRANSFORMING_GROWTH_FACTOR_BETA_RECEPTOR_SIGNALING PATHWAY	●	●
VASCULARIZATION	GOBP_REGULATION_OF_VASCULATURE_DEVELOPMENT	●	●
	GOBP_VASCULAR_PROCESS_IN_CIRCULATORY_SYSTEM	●	●
	GOBP_ARTERY_DEVELOPMENT	●	●
	GOBP_REGULATION_OF_BLOOD_CIRCULATION	●	●
	GOBP_CIRCULATORY_SYSTEM_PROCESS		●
IMMUNE SYSTEM	HALLMARK_INFLAMMATORY_RESPONSE	●	●
	KEGG_CHEMOKINE_SIGNALING_PATHWAY	●	●
	REACTOME_NEUTROPHIL_DEGRANULATION	●	
	GOBP_LEUKOCYTE_MIGRATION	●	●

Gene Set Enrichment Analysis (GSEA) : J90

Transcriptome analysis by RNA-Seq



GROUP	PATHWAY	Asbestos	Dose2_fragment
CELL ADHESION	KEGG_TIGHT_JUNCTION	●	●
	GOBP_POSITIVE_REGULATION_OF_CELL_CELL_ADHESION	●	
	GOBP_REGULATION_OF_CELL_CELL_ADHESION	●	
	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	●	●
	GOBP_POSITIVE_REGULATION_OF_CELL_ADHESION	●	
	GOBP_CELL_CELL_JUNCTION_ORGANIZATION	●	●
	KEGG_FOCAL_ADHESION	●	●
EXTRACELLULAR MATRIX	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION	●	●
	REACTOME_ECM_PROTEOGLYCANS	●	●
	REACTOME_COLLAGEN_BIOSYNTHESIS_AND_MODIFYING_ENZYMES	●	●
	REACTOME_COLLAGEN_CHAIN_TRIMERIZATION	●	●
EPITHELIAL MESENCHYMAL TRANSITION	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	●	●
	GOBP_MESENCHYME_MORPHOGENESIS	●	●
	GOBP_MESENCHYME_DEVELOPMENT	●	●
	GOBP_EPITHELIAL_CELL_DEVELOPMENT	●	●
VASCULARIZATION	GOBP_ARTERY_DEVELOPMENT	●	●
	GOBP_ARTERY_MORPHOGENESIS	●	●
	GOBP_AORTA_DEVELOPMENT	●	●
	GOBP_AORTIC_VALVE_MORPHOGENESIS	●	●
IMMUNE SYSTEM	GOBP_B_CELL_ACTIVATION	●	●
	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	●	●
	GOBP_LEUKOCYTE_DIFFERENTIATION	●	●
	GOBP_MONONUCLEAR_CELL_DIFFERENTIATION	●	●
	GOBP_REGULATION_OF_LYMPHOCYTE_ACTIVATION	●	●

Conclusion and Perspectives

- Transcriptomic analyses
- Observed notable results in various biological processes:
  - Cell adhesion
  - Extracellular matrix
  - Epithelial-mesenchymal transition (EMT)
  - Vascularization
  - Immune system

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  - Conducted methylation analysis via Methyl-Seq using RREM-Seq
  - Data delivered in mid-July and is currently being processed and analyzed in the INSERM laboratory

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- Insight into the carcinogenic potential of cleavage fragments ? Short fibers ?
- Potential implications for occupational health and environmental health

Thank you for your attention

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