IMIM Parc de Salut MAR Barcelona

Institut Hospital del Mar d'Investigacions Mèdiques

Gene Set Enrichment Analysis

february 2020

MARGenomics

Gene Set Enrichment Analysis (GSEA)

List of genes with statistics (p.Value and logFC)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Symbol	FC.Tumoral.vs.Ctrl	logFC.Tumoral.vs.Ctrl	P.Value.Tumoral.vs.Ctrl	adj.P.Val.Tumoral.vs.Ctrl	mean.Ctrl	mean.LINC	CONTROL1.RMA	CONTROL3.RMA	CONTROL4.RMA	CONTROL5.RMA	CONTROL6.RMA	Tumoral1.RMA	Tumoral2.RMA	Tumoral3.RMA	Tumoral4.RMA	Tumoral5.RMA
2	FAM231D	-2.02	-1.02	0.00000	0.01856	7.25	6.23	7.11	7.46	7.37	7.38	6.91	6.39	6.31	6.10	6.00	6.35
3	CHD1	-1.97	-0.98	0.00000	0.02406	7.16	6.18	6.97	7.42	7.34	7.01	7.06	6.25	6.36	5.90	6.00	6.37
4	DOCK5	-1.66	-0.73	0.00012	0.41294	5.04	4.31	5.25	5.11	5.23	4.95	4.69	4.46	4.18	4.51	4.45	3.97
5	XG	-1.58	-0.66	0.00056	0.58249	7.10	6.45	7.09	7.25	7.09	6.88	7.19	6.58	6.58	6.81	6.29	5.96
6	DST	-1.57	-0.65	0.00140	0.81165	5.68	5.03	5.71	5.98	5.48	5.80	5.41	4.81	4.62	5.07	5.16	5.48
7	MPC1L	-1.44	-0.53	0.00008	0.34905	4.58	4.05	4.39	4.64	4.71	4.43	4.71	4.03	4.04	3.86	4.10	4.20
8	BLOC1S1-F	-1.43	-0.51	0.00766	0.98116	6.24	5.73	6.02	6.41	6.42	5.96	6.37	6.14	5.82	5.26	5.51	5.91
9	MKRN1	-1.41	-0.49	0.03340	0.98116	6.42	5.92	6.38	6.61	6.26	6.61	6.22	6.18	5.63	5.73	5.40	6.67
10	SNUPN	-1.41	-0.49	0.00041	0.53985	5.59	5.10	5.71	5.63	5.69	5.34	5.57	4.87	4.97	5.23	5.31	5.11
11	RPL36A	-1.40	-0.49	0.00002	0.11241	7.25	6.76	7.29	7.22	7.22	7.20	7.30	6.67	6.80	6.63	6.94	6.75
12	SERF2	-1.40	-0.48	0.00538	0.98116	5.22	4.74	5.59	5.09	5.39	5.20	4.86	4.80	4.56	4.69	4.55	5.11
13	DHRS4L2	-1.39	-0.48	0.00133	0.81165	4.93	4.45	4.79	5.03	5.16	4.69	4.99	4.51	4.61	4.18	4.33	4.65
14	LGALS14	-1.39	-0.48	0.00251	0.86826	4.20	3.72	4.05	4.16	4.05	4.26	4.47	3.67	3.86	3.81	3.30	3.96

Pre-ranked analysis

Gene Expression matrix (microarrays)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Symbol	FC.Tumoral.vs.Ctrl	logFC.Tumoral.vs.Ctrl	P.Value.Tumoral.vs.Ctrl	adj.P.Val.Tumoral.vs.Ctrl	mean.Ctrl	mean.LINC	CONTROL1.RMA	CONTROL3.RMA	CONTROL4.RMA	CONTROL5.RMA	CONTROL6.RMA	Tumoral1.RMA	Tumoral2.RMA	Tumoral3.RMA	Tumoral4.RMA	Tumoral5.RMA
2	FAM231D	-2.02	-1.02	0.00000	0.01856	7.25	6.23	7.11	7.46	7.37	7.38	6.91	6.39	6.31	6.10	6.00	6.35
3	CHD1	-1.97	-0.98	0.00000	0.02406	7.16	6.18	6.97	7.42	7.34	7.01	7.06	6.25	6.36	5.90	6.00	6.37
4	DOCK5	-1.66	-0.73	0.00012	0.41294	5.04	4.31	5.25	5.11	5.23	4.95	4.69	4.46	4.18	4.51	4.45	3.97
5	XG	-1.58	-0.66	0.00056	0.58249	7.10	6.45	7.09	7.25	7.09	6.88	7.19	6.58	6.58	6.81	6.29	5.96
6	DST	-1.57	-0.65	0.00140	0.81165	5.68	5.03	5.71	5.98	5.48	5.80	5.41	4.81	4.62	5.07	5.16	5.48
7	MPC1L	-1.44	-0.53	0.00008	0.34905	4.58	4.05	4.39	4.64	4.71	4.43	4.71	4.03	4.04	3.86	4.10	4.20
8	BLOC1S1-F	-1.43	-0.51	0.00766	0.98116	6.24	5.73	6.02	6.41	6.42	5.96	6.37	6.14	5.82	5.26	5.51	5.91
9	MKRN1	-1.41	-0.49	0.03340	0.98116	6.42	5.92	6.38	6.61	6.26	6.61	6.22	6.18	5.63	5.73	5.40	6.67
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13	DHRS4L2	-1.39	-0.48	0.00133	0.81165	4.93	4.45	4.79	5.03	5.16	4.69	4.99	4.51	4.61	4.18	4.33	4.65
14	LGALS14	-1.39	-0.48	0.00251	0.86826	4.20	3.72	4.05	4.16	4.05	4.26	4.47	3.67	3.86	3.81	3.30	3.96

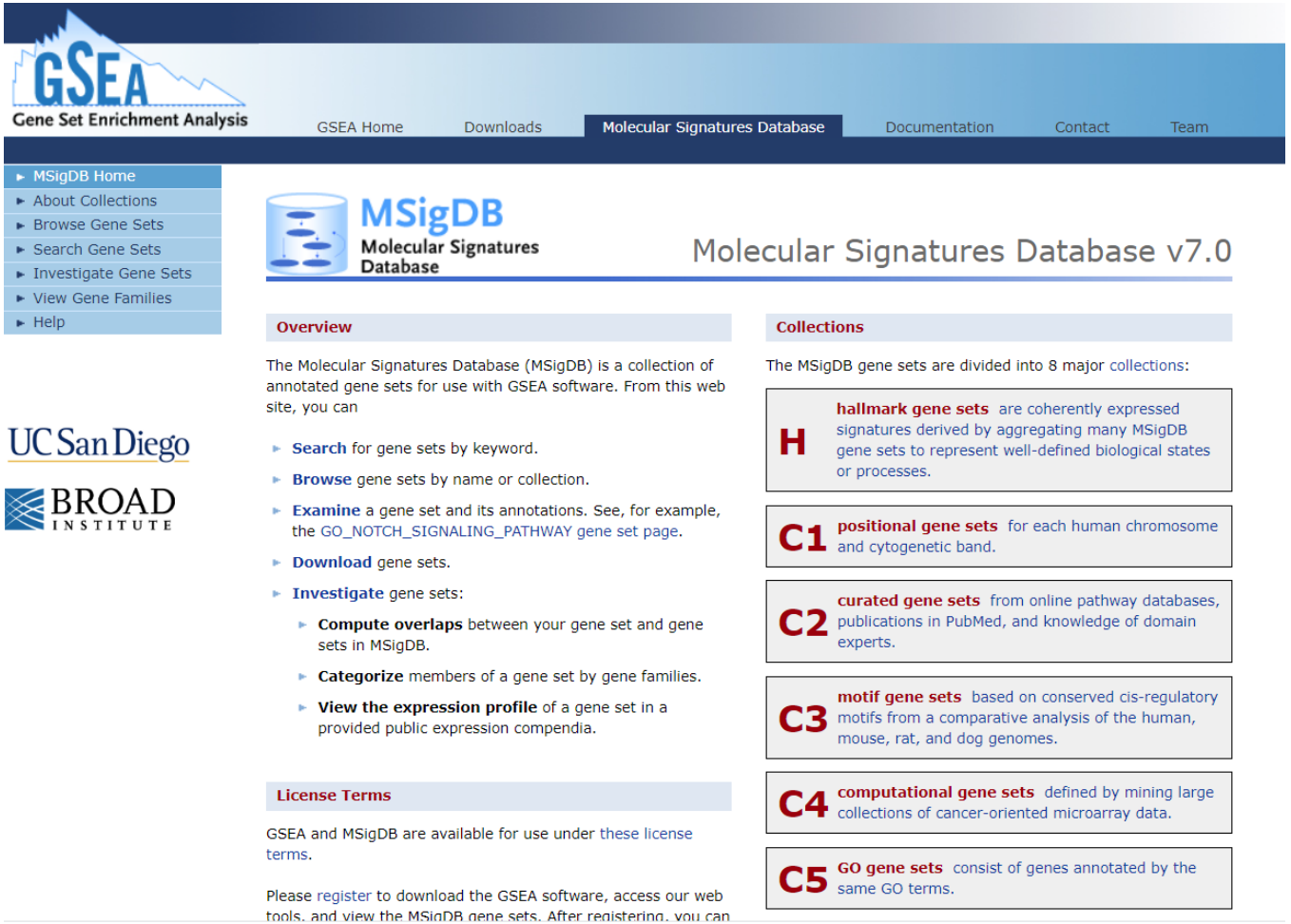
Classic analysis

List of over represented pathways

GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
NADLER_OBESITY_DN	Details ...	46	0.80	2.65	0.000	0.000	0.000
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	Details ...	42	0.73	2.48	0.000	0.000	0.000
HSIAO_LIVER_SPECIFIC_GENES	Details ...	218	0.59	2.47	0.000	0.000	0.000
KIM_LIVER_CANCER_POOR_SURVIVAL_DN	Details ...	38	0.75	2.42	0.000	0.000	0.000
WOO_LIVER_CANCER_RECURRENCE_DN	Details ...	70	0.66	2.42	0.000	0.000	0.000
LEE_LIVER_CANCER_CIPROFIBRATE_DN	Details ...	61	0.65	2.33	0.000	0.000	0.000
CHIANG_LIVER_CANCER_SUBCLASS_PROLIFERATION_DN	Details ...	161	0.56	2.31	0.000	0.000	0.000
LEE_LIVER_CANCER_SURVIVAL_UP	Details ...	159	0.55	2.27	0.000	0.000	0.000
KEGG_PROPANOATE_METABOLISM	Details ...	31	0.73	2.26	0.000	0.000	0.000
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	Details ...	31	0.73	2.26	0.000	0.000	0.000
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	Details ...	48	0.67	2.25	0.000	0.000	0.000
LEE_LIVER_CANCER_DENA_DN	Details ...	73	0.62	2.21	0.000	0.000	0.003

Molecular Signatures Database (MSigDB)

https://www.gsea-msigdb.org/gsea/msigdb/index.jsp



Molecular Signatures Database (MSigDB)

C5: GO gene sets (browse 9996 gene sets)	Gene sets that contain genes annotated by the same GO term. The C5 collection is divided into three sub-collections based on GO ontologies: BP, CC, and MF. details	Download GMT Files gene symbols NCBI (entrez) gene ids
BP: GO biological process (browse 1350 gene sets)	Gene sets derived from the GO Biological Process Ontology.	Download GMT Files gene symbols NCBI (entrez) gene ids
CC: GO cellular component (browse 1001 gene sets)	Gene sets derived from the GO Cellular Component Ontology.	Download GMT Files gene symbols NCBI (entrez) gene ids
MF: GO molecular function (browse 1645 gene sets)	Gene sets derived from the GO Molecular Function Ontology.	Download GMT Files gene symbols NCBI (entrez) gene ids

Gene Sets: GMT format

- Genes are grouped in families describing biological pathways, molecular functions, disease mechanisms etc..
- Example: Collection 5 from MSigDB Biological processes (c5.bp.v7.0.symbols.gmt)

GO Biological Process (Column A)

Extra information (Column B)

Genes in the GO BP

	A	B	C	D	E	F	G	H	I	J	K
2	GO_CARDIAC_CHAMBER_DEVELOPMENT	http://www.gsea-msi	ZMPSTE24	UBE4B	CITED2	TAB1	SEMA3C	FRS2	PPP1R13L	ADAMTS6	XIRP2
3	GO_DNA_DEPENDENT_DNA_REPLICATION_MAINTENANCE_OF_FIDELITY	http://www.gsea-msi	ALYREF	CDK9	EME1	DDX11	DNA2	EME2	CENPX	PRIMPOL	ZNF365
4	GO_CIRCADIAN_RHYTHM	http://www.gsea-msi	ADA	NR1H3	NAMPT	CDK4	HNRNPR	PRMT5	NCOA2	MYBBP1A	ATG7
5	GO_PHOSPHATIDYLSERINE_ACYL_CHAIN_REMODELING	http://www.gsea-msi	PLA2G4B	LPCAT3	PLAAT3	OSBPL5	OSBPL8	OSBPL10	PLA2G4E	MBOAT1	LPCAT4
6	GO_SPINAL_CORD_DEVELOPMENT	http://www.gsea-msi	OLIG2	GDF11	ADARB1	LBX1	DBX1	FOXN4	GDF7	DAB1	MDGA2
7	GO_PLATELET_DERIVED_GROWTH_FACTOR_RECEPTOR_SIGNALING_PATHWAY	http://www.gsea-msi	TXNIP	RGS14	F3	F7	FER	ABL1	TIPARP	RAPGEF1	HIP1
8	GO_CELLULAR_RESPONSE_TO_LIPOPROTEIN_PARTICLE_STIMULUS	http://www.gsea-msi	CDH13	TLR6	TICAM1	ABCA1	AKT1	FCER1G	FGF21	HMGCS1	APOE
9	GO_POSITIVE_REGULATION_OF_EPITHELIAL_CELL_DIFFERENTIATION	http://www.gsea-msi	CD24	CDH5	TRIM16	ADD1	KDF1	CTNNB1	CYP27B1	ETV2	FOXC1
10	GO_POSITIVE_REGULATION_OF_KINASE_ACTIVITY	http://www.gsea-msi	ABI1	CD24	TOM1L1	ADAM8	RAD50	RASGRP1	TENM1	CDK5	SPRY2
11	GO_POTASSIUM_ION_TRANSPORT	http://www.gsea-msi	KCNE3	KCNJ18	HCN4	ABCC9	KCNK7	AKAP9	CDK2	KCNMB2	CDKN1B
12	GO_REGULATION_OF_T_CELL_RECEPTOR_SIGNALING_PATHWAY	http://www.gsea-msi	ADA	BTN2A2	CD226	MALT1	LILRB4	CD160	CD300A	CCR7	RC3H1
13	GO_NEGATIVE_REGULATION_OF_EPITHELIAL_CELL_PROLIFERATION	http://www.gsea-msi	MIR2355	CDK6	RIDA	CDKN1B	CDKN1C	CDKN2B	CNMD	STRAP	DUSP10
14	GO_MOVEMENT_IN_ENVIRONMENT_OF_OTHER_ORGANISM_INVOLVED_IN_SYMBIOTIC_INTERACTION	http://www.gsea-msi	TRIM28	TRIM13	MID2	TRIM31	CHMP4B	DDB1	TRIM32	TRIM35	CHMP2B
15	GO_REGULATION_OF_PROTEIN_TARGETING_TO_MITOCHONDRION	http://www.gsea-msi	SAE1	HUWE1	ARIH2	HAX1	UBE2J2	LRRK2	CSNK2A2	UBL4B	ABLIM3
16	GO_APICAL_PROTEIN_LOCALIZATION	http://www.gsea-msi	HCN1	SHROOM2	ARF4	MAL	GOPC	VANGL2	SHROOM3	RDX	NAPA
17	GO_REGULATION_OF_ESTABLISHMENT_OF_PLANAR_POLARITY	http://www.gsea-msi	GPC6	PSME3	PSMD14	SEC24B	CTHRC1	AP2M1	AP2S1	CLTC	PSMB11
18	GO_FOREBRAIN_NEURON_DEVELOPMENT	http://www.gsea-msi	SEMA3A	DCLK2	ARX	DRD1	DRD2	FGF8	FGFR2	ATF5	FOXG1
19	GO_POSITIVE_REGULATION_OF_PROTEIN_MATURATION	http://www.gsea-msi	ADAM8	SPON1	TIMM17A	CCBE1	ENO1	F12	GSN	HPN	KLKB1
20	GO_NEUROMUSCULAR_JUNCTION_DEVELOPMENT	http://www.gsea-msi	GPHN	CACNG2	UNC13B	AFG3L2	RER1	CHRNA1	LRRK2	COL4A1	COL4A5
21	GO_MITOTIC_CYTOKINESIS	http://www.gsea-msi	PDCD6IP	KIF20A	CENPA	STAMBP	CFL1	JTB	RAB35	CIT	SNX18
22	GO_NEGATIVE_REGULATION_OF_RESPONSE_TO_ENDOPLASMIC_RETICULUM_STRESS	http://www.gsea-msi	RACK1	CREB3	HYOU1	OS9	PARK7	CLU	LRRK2	ATF6B	PPP1R15A
23	GO_SMAD_PROTEIN_SIGNAL_TRANSDUCTION	http://www.gsea-msi	TOB1	GDF11	RBM14	LEFTY1	SUB1	RBPMS	BTBD11	PARP1	GDF7
24	GO_CYTOPLASMIC_TRANSLATION	http://www.gsea-msi	DPH3P1	EIF3M	DNAJC24	CPEB2	JAKMIP1	AARS	DHX9	DHX36	DPH1
25	GO_MEIOTIC_CHROMOSOME_SEGREGATION	http://www.gsea-msi	SYCE1L	RNF212B	SMC4	ACTR3	ACTR2	MEI4	SYCP2	SMC2	CENPC
26	GO_POSITIVE_REGULATION_OF_CALCIUM_ION_TRANSPORT	http://www.gsea-msi	KCNE3	RAMP3	TRDN	LILRA2	ADCYAP1R1	CCR1	CRH	TRPV3	DRD1
27	GO_REGULATION_OF_DOUBLE_STRAND_BREAK_REPAIR	http://www.gsea-msi	PARP3	ACTR2	MAD2L2	RAD51AP1	POLQ	CHEK1	SHLD3	CGAS	RMI2
28	GO_RNA_DEPENDENT_DNA_BIOSYNTHETIC_PROCESS	http://www.gsea-msi	TEN1	RAD50	CCT7	CCT4	CCT2	CCT8	PTGES3	PNKP	NEK7
29	GO_REGULATION_OF_B_CELL_RECEPTOR_SIGNALING_PATHWAY	http://www.gsea-msi	CD300A	FCRL3	CMTM3	GCSAML	NFAM1	ELF1	ELF2	FCGR2B	PLCL2
30	GO_DENDRITE_DEVELOPMENT	http://www.gsea-msi	ABI1	HDAC6	FOXO6	ARMCX5-GPI	DNM1L	PQBP1	ACTR2	ABI2	FARP1

GSEA statistics

Enrichment Score (ES): Degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes (the default ranking metric is signal-to-noise ratio)

Examples for positive enriched gene sets in the contrast LINC vs CTRL (enriched in LINC)

Table: GSEA Results Summary

Dataset	LINC.vs.Ctrl
Phenotype	NoPhenotypeAvailable
Upregulated in class	na_pos
GeneSet	GO_CELLULAR_RESPONSE_TO_ZINC_ION
Enrichment Score (ES)	0.8632142
Normalized Enrichment Score (NES)	2.4628952
Nominal p-value	0.0
FDR q-value	0.0
FWER p-Value	0.0

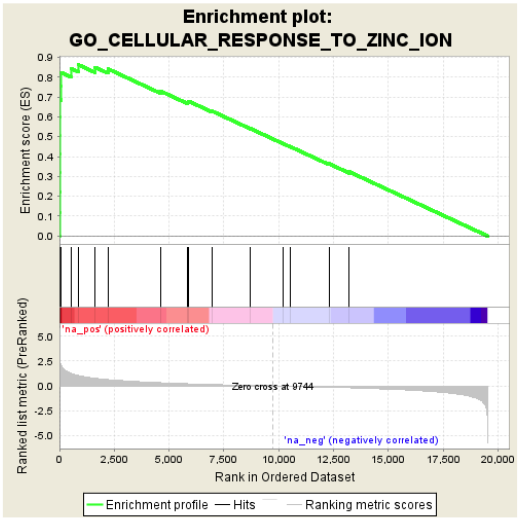


Fig 1: Enrichment plot: GO_CELLULAR_RESPONSE_TO_ZINC_ION Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

Table: GSEA Results Summary

Dataset	LINC.vs.Ctrl
Phenotype	NoPhenotypeAvailable
Upregulated in class	na_pos
GeneSet	GO_ACTIN_MEDIATED_CELL_CONTRACTION
Enrichment Score (ES)	0.38933018
Normalized Enrichment Score (NES)	1.5310684
Nominal p-value	0.0055452865
FDR q-value	0.5675065
FWER p-Value	1.0

Enrichment score

Leading edge subset

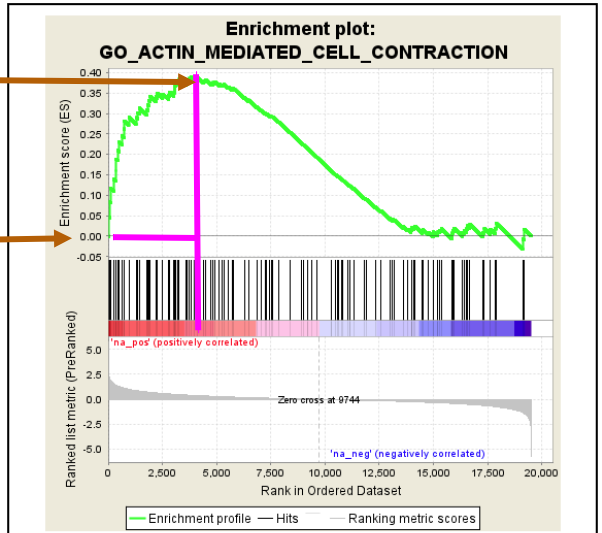


Fig 1: Enrichment plot: GO_ACTIN_MEDIATED_CELL_CONTRACTION Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

GSEA statistics

Enrichment Score (ES): Degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes (the default ranking metric is signal-to-noise ratio)

Examples for negative enriched gene sets in the contrast LINC vs CTRL (enriched in CTRL)

Table: GSEA Results Summary

Dataset	LINC.vs.Ctrl
Phenotype	NoPhenotypeAvailable
Upregulated in class	na_neg
GeneSet	GO_LONG_CHAIN_FATTY_ACID_IMPORT_INTO_CELL
Enrichment Score (ES)	-0.8595188
Normalized Enrichment Score (NES)	-2.2617462
Nominal p-value	0.0
FDR q-value	0.0023107266
FWER p-Value	0.002

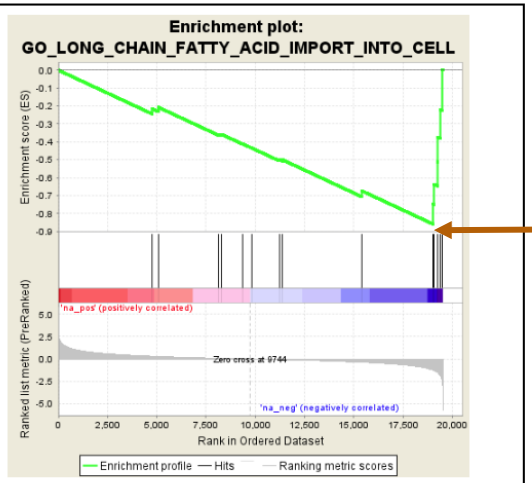


Fig 1: Enrichment plot: GO_LONG_CHAIN_FATTY_ACID_IMPORT_INTO_CELL

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

Enrichment score

Table: GSEA Results Summary

Dataset	LINC.vs.Ctrl
Phenotype	NoPhenotypeAvailable
Upregulated in class	na_neg
GeneSet	GO_CELLULAR_RESPONSE_TO_IONIZING_RADIATION
Enrichment Score (ES)	-0.45991984
Normalized Enrichment Score (NES)	-1.650384
Nominal p-value	0.006289308
FDR q-value	0.49168018
FWER p-Value	1.0

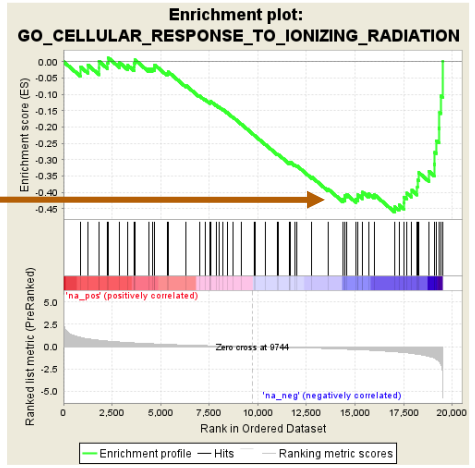


Fig 1: Enrichment plot: GO_CELLULAR_RESPONSE_TO_IONIZING_RADIATION

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

GSEA statistics

Normalized enrichment score (NES)

- Obtained by normalizing the enrichment score
- Accounts for differences in gene set size and in correlations between gene sets and the expression dataset
- Used to compare analysis results across gene sets

$$NES = \frac{\text{actual ES}}{\text{mean(ESs against all permutations of the datset)}}$$

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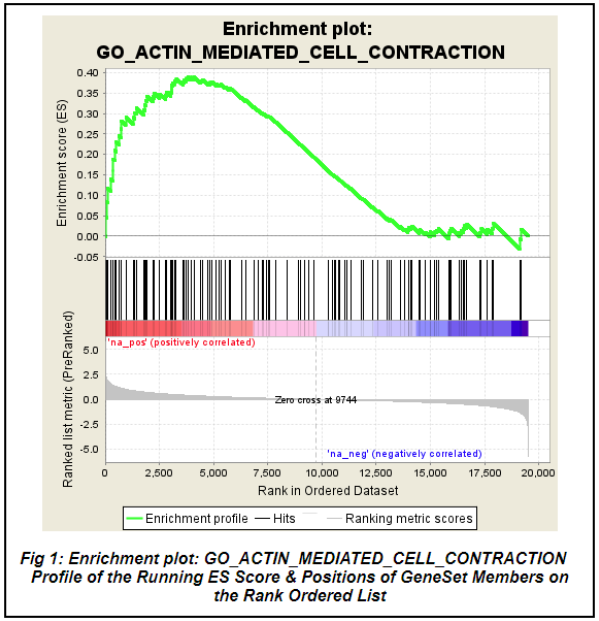

Fig 1: Enrichment plot: GO_ACTIN_MEDIATED_CELL_CONTRACTION Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

GSEA statistics

Nominal p-value: Estimates the statistical significance of the enrichment score for a single gene set

FDR q-value: The false discovery rate (FDR) is the estimated probability that a gene set with a given NES represents a false positive finding (Ex: an FDR of 25% indicates that the result is likely to be valid 3 out of 4 times)

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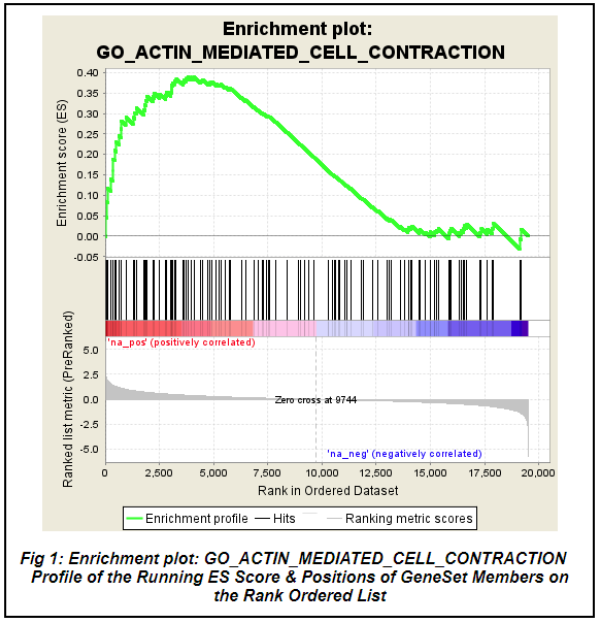


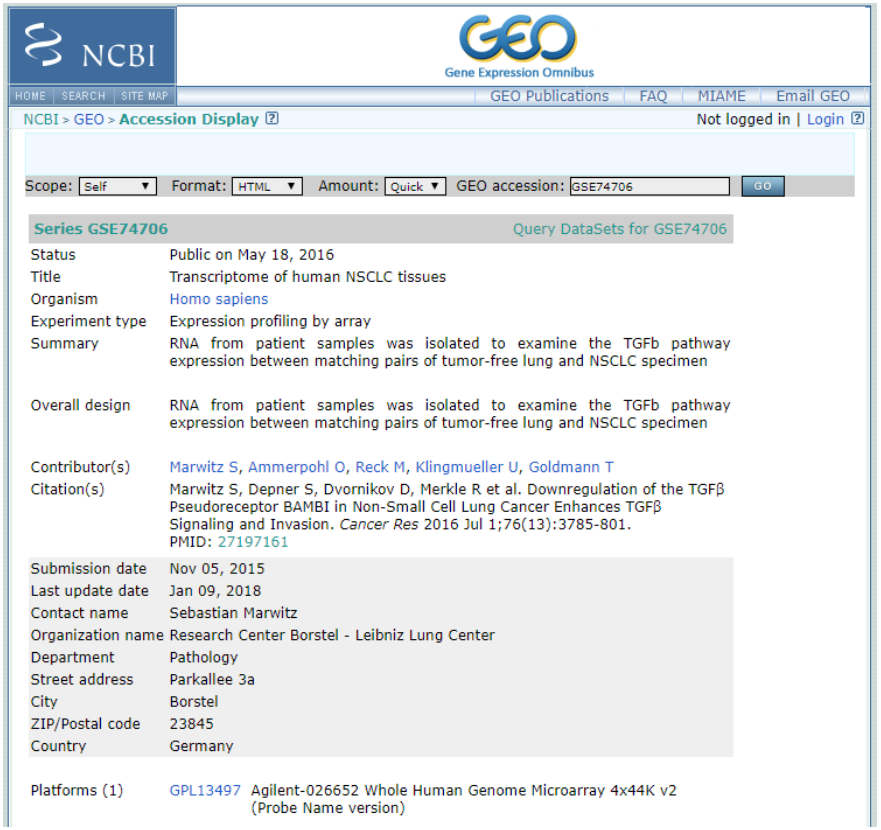
Fig 1: Enrichment plot: GO_ACTIN_MEDIATED_CELL_CONTRACTION Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

GSEA tool practical

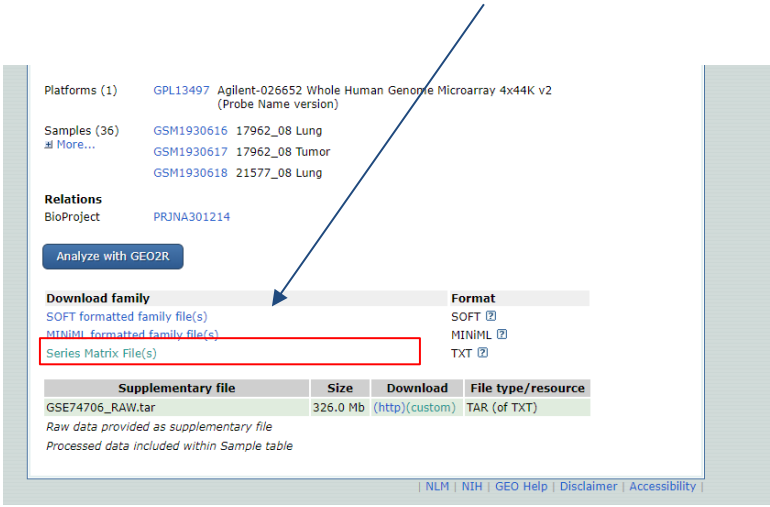
Input data preparation

Input Data: Lung Cancer dataset example

- Transcriptome of Human NSCLC tissues (GEO accession: GSE74706)
  - NSCLC: n=18
  - TumorFree: n=18



Series matrix



Input data: GCT format

GCT format: For microarrays, contains the expression data. Tabulator separated.

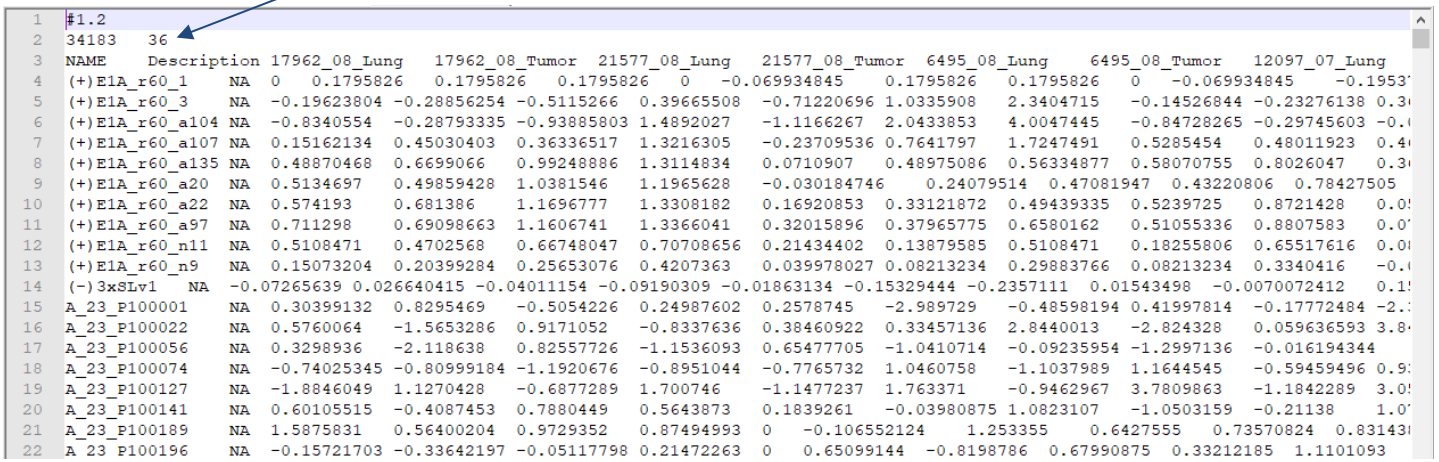
First row is always #1.2

Second row has two tab separated values:

34183 = Number of geneID

36 = Number of samples

From the **third row** on starts the expression matrix



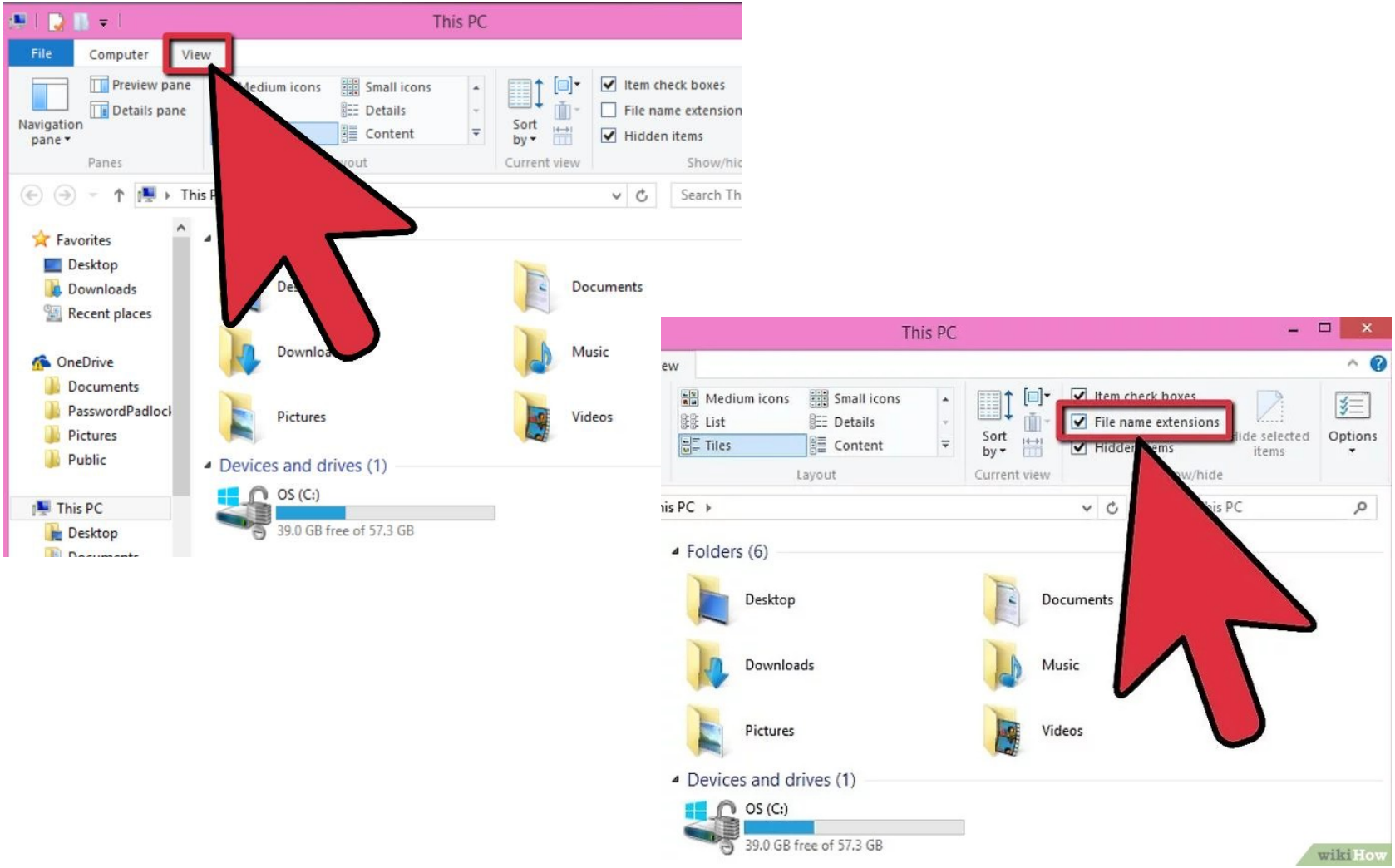
```
#1.2
34183   36
NAME    Description 17962_08_Lung   17962_08_Tumor  21577_08_Lung   21577_08_Tumor  6495_08_Lung    6495_08_Tumor   12097_07_Lung
(+)E1A_r60_1    NA  0   0.1795826   0.1795826   0.1795826   0   -0.069934845    0.1795826   0.1795826   0   -0.069934845    -0.1953
(+)E1A_r60_3    NA  -0.19623804 -0.28856254 -0.5115266  0.39665508  -0.71220696 1.0335908   2.3404715   -0.14526844 -0.23276138 0.3
(+)E1A_r60_a104 NA  -0.8340554  -0.28793335 -0.93885803 1.4892027   -1.1166267  2.0433853   4.0047445   -0.84728265 -0.29745603 -0.
(+)E1A_r60_a107 NA  0.15162134  0.45030403  0.36336517  1.3216305   -0.23709536 0.7641797   1.7247491   0.5285454   0.48011923  0.4
(+)E1A_r60_a135 NA  0.48870468  0.6699066   0.99248886  1.3114834   0.0710907   0.48975086  0.56334877  0.58070755  0.8026047   0.3
(+)E1A_r60_a20  NA  0.5134697   0.49859428  1.0381546   1.1965628   -0.030184746    0.24079514  0.47081947  0.43220806  0.78427505
(+)E1A_r60_a22  NA  0.574193    0.681386    1.1696777   1.3308182   0.16920853  0.33121872  0.49439335  0.5239725   0.8721428   0.0
(+)E1A_r60_a97  NA  0.711298    0.69098663  1.1606741   1.3366041   0.32015896  0.37965775  0.6580162   0.51055336  0.8807583   0.0
(+)E1A_r60_n11  NA  0.5108471   0.4702568   0.66748047  0.70708656  0.21434402  0.13879585  0.5108471   0.18255806  0.65517616  0.0
(+)E1A_r60_n9   NA  0.15073204  0.20399284  0.25653076  0.4207363   0.039978027 0.08213234  0.29883766  0.08213234  0.3340416   -0.
(-)3xSLv1   NA  -0.07265639 0.026640415 -0.04011154 -0.09190309 -0.01863134 -0.15329444 -0.2357111  0.01543498  -0.0070072412   0.1
A_23_P100001    NA  0.30399132  0.8295469   -0.5054226  0.24987602  0.2578745   -2.989729   -0.48598194 0.41997814  -0.17772484 -2.
A_23_P100022    NA  0.5760064   -1.5653286  0.9171052   -0.8337636  0.38460922  0.33457136  2.8440013   -2.824328   0.059636593 3.8
A_23_P100056    NA  0.3298936   -2.118638   0.82557726  -1.1536093  0.65477705  -1.0410714  -0.09235954 -1.2997136  -0.016194344
A_23_P100074    NA  -0.74025345 -0.80999184 -1.1920676  -0.8951044  -0.7765732  1.0460758   -1.1037989  1.1644545   -0.59459496 0.9
A_23_P100127    NA  -1.8846049  1.1270428   -0.6877289  1.700746    -1.1477237  1.763371    -0.9462967  3.7809863   -1.1842289  3.0
A_23_P100141    NA  0.60105515  -0.4087453  0.7880449   0.5643873   0.1839261   -0.03980875 1.0823107   -1.0503159  -0.21138    1.0
A_23_P100189    NA  1.5875831   0.56400204  0.9729352   0.87494993  0   -0.106552124    1.253355    0.6427555   0.73570824  0.8314
A_23_P100196    NA  -0.15721703 -0.33642197 -0.05117798 0.21472263  0   0.65099144  -0.8198786  0.67990875  0.33212185  1.1101093
```

First column in the expression matrix contains gene IDs

Second column in the expression matrix contains description but is ignored by the program (NA in this case)

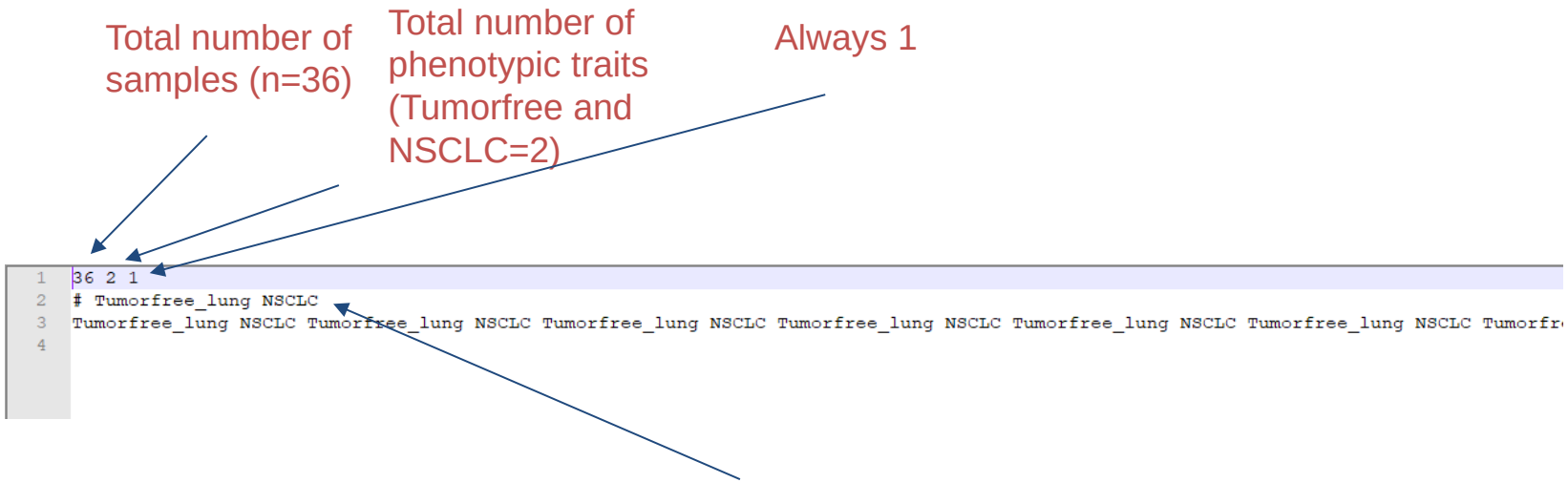
The rest of the columns in the expression matrix contain expression values for each sample

Change file extension in Windows



Input data: CLS format

CLS format: For microarrays, contains the phenotypic data. Blank space separated.

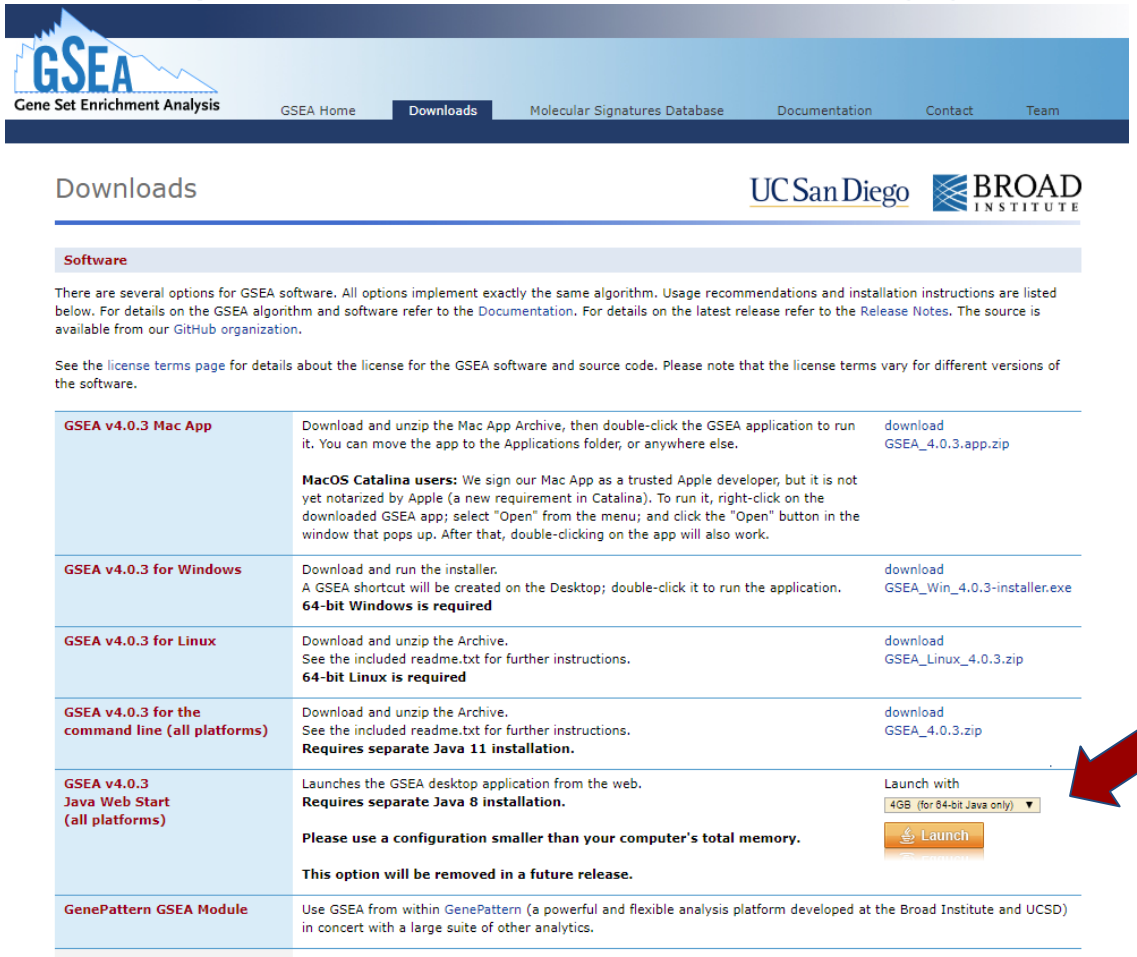


Third row: Names of the phenotypic traits for each sample/column in the expression matrix (Important: the order has to be the same)

Second row: Names of the phenotypic traits. IMPORTANT: First condition is Tumorfree_lung, like in the third row.

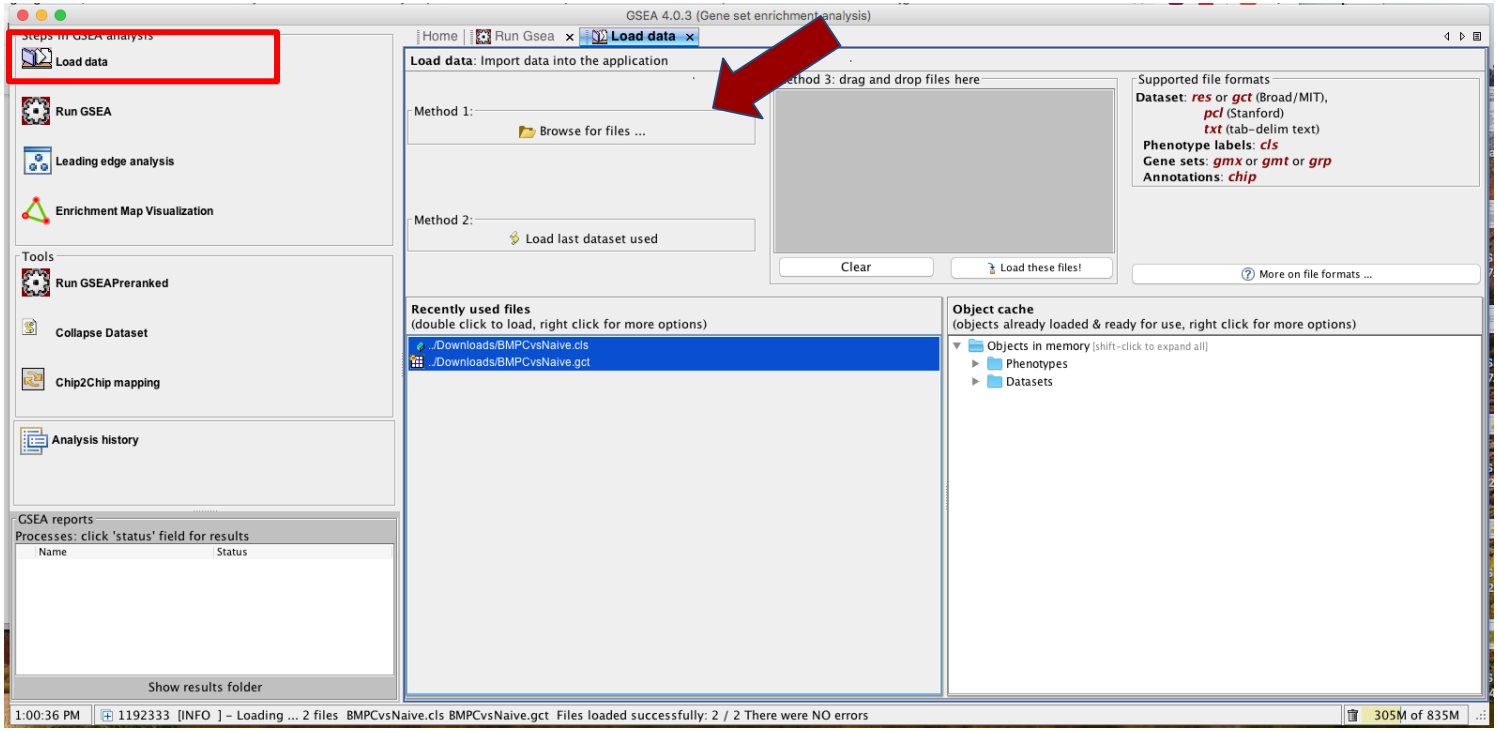
GSEA tool

Available at: https://software.broadinstitute.org/gsea/index.jsp



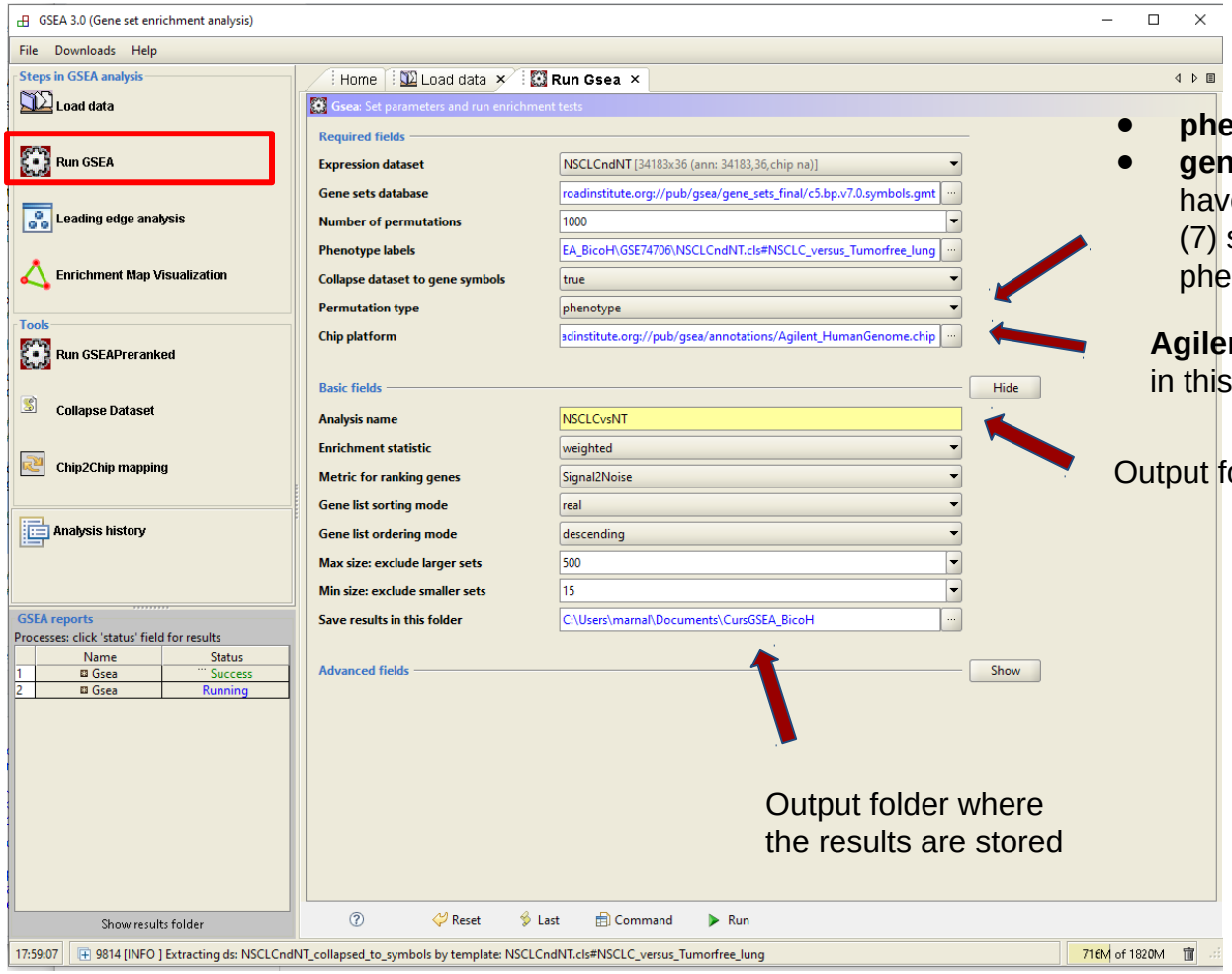
GSEA Classic Analysis

1. Download GSEA: GSEA v4.0.3 Java Web Start (all platforms)
2. Download gct and cls files in the folder “MaterialsCursGSEA”
3. Open the app
4. “Load data” - “Browse for files” - load the .cls and .gct files



GSEA Classic Analysis

1. Go to “Run GSEA” - “Required fields” and “Basic fields”



- **phenotype**
- **gene_set** = When you have fewer than seven (7) samples in any phenotype

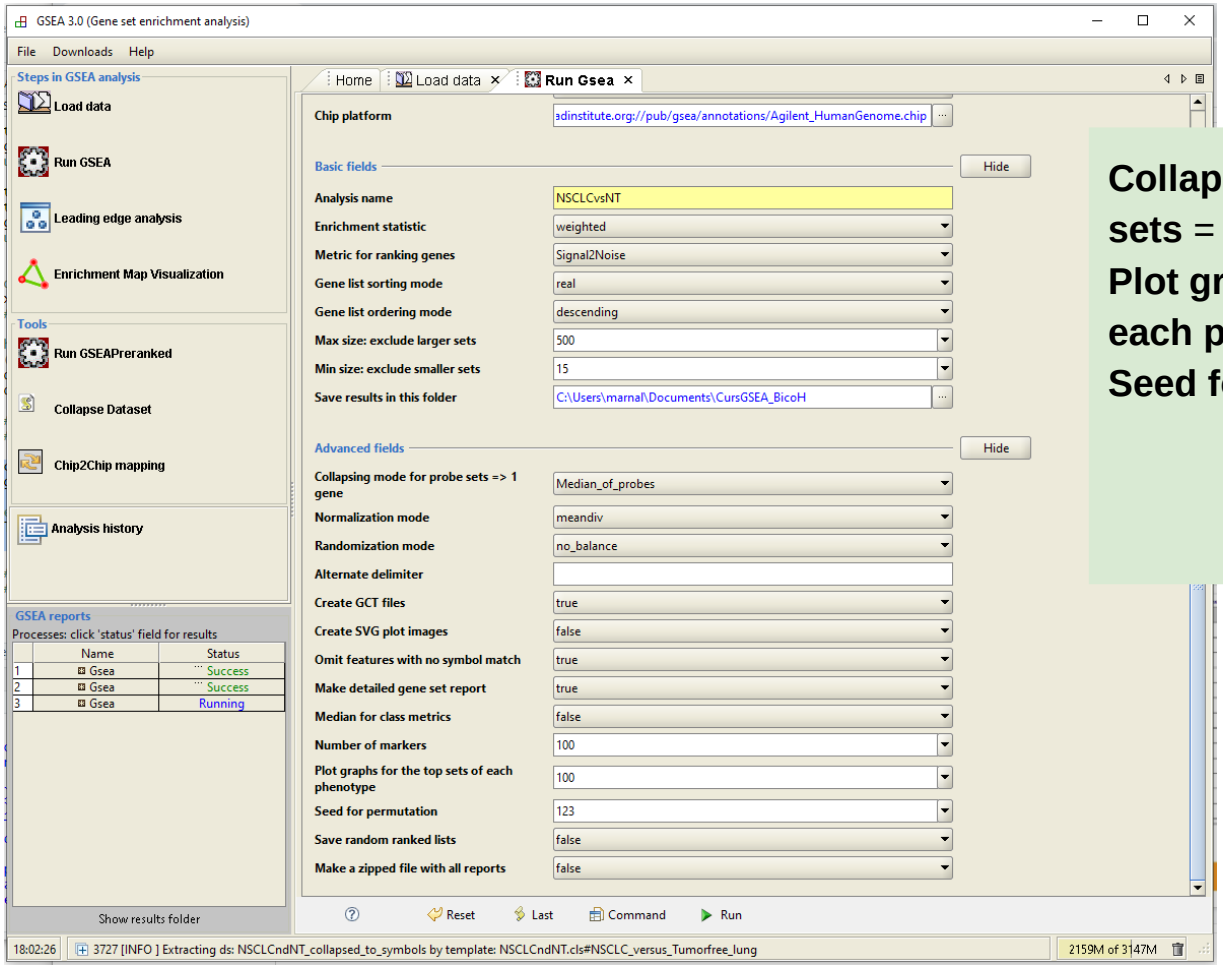
Agilent_HumanGenome in this case

Output folder name

Output folder where the results are stored

GSEA Classic Analysis

1. Go to “Run GSEA” - “Advanced fields”



Collapsing mode for probe sets = Median_of_probes
Plot graphs for the top sets of each phenotype = 100
Seed for permutation=123

GSEA Classic results

GSEA Report for Dataset NSCLCndNT

Enrichment in phenotype: NSCLC (18 samples)

- 958 / 3305 gene sets are upregulated in phenotype **NSCLC**
- 384 gene sets are significant at FDR < 25%
- 189 gene sets are significantly enriched at nominal pvalue < 1%
- 288 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Enriched in NSCLC

Enrichment in phenotype: Tumorfree_lung (18 samples)

- 2347 / 3305 gene sets are upregulated in phenotype **Tumorfree_lung**
- 1291 gene sets are significantly enriched at FDR < 25%
- 490 gene sets are significantly enriched at nominal pvalue < 1%
- 868 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Enriched in TumorFree

Dataset details

- The dataset has 34183 native features
- After collapsing features into gene symbols, there are: 14110 genes

Gene set details

- Gene set size filters (min=15, max=500) resulted in filtering out 4045 / 7350 gene sets
- The remaining 3305 gene sets were used in the analysis
- List of gene sets used and their sizes (restricted to features in the specified dataset)

index.html in the folder contains the results summary

GSEA_Plots (Details)

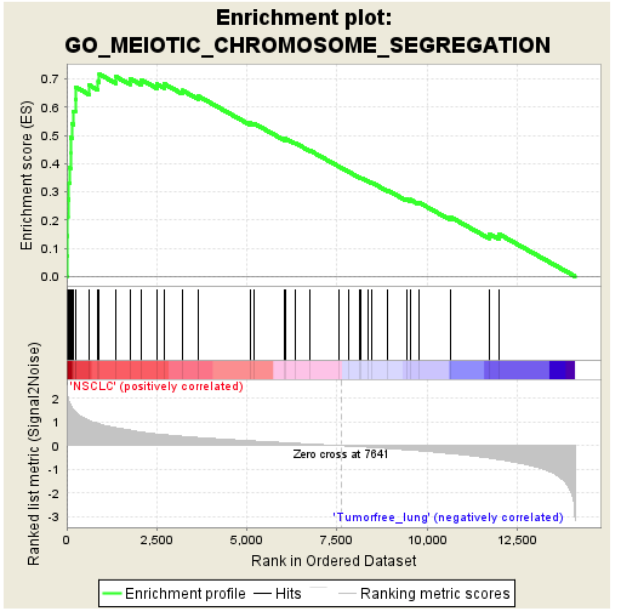
Gene set information

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	GO_MEIOTIC_CHROMOSOME_SEGREGATION	Details ...	41	0.72	2.09	0.000	0.041	0.043	904
2	GO_REGULATION_OF_CHROMOSOME_SEGREGATION	Details ...	66	0.67	2.05	0.000	0.042	0.088	1023
3	GO_MEIOTIC_CELL_CYCLE_PROCESS	Details ...	98	0.59	2.05	0.000	0.030	0.092	1401
4	GO_MEIOSIS_I_CELL_CYCLE_PROCESS	Details ...	65	0.61	2.04	0.000	0.025	0.099	904
5	GO_SIGNAL_TRANSDUCTION_INVOLVED_IN_CELL_CYCLE_CHECKPOINT	Details ...	44	0.61	2.03	0.000	0.024	0.118	1162
6	GO_DNA_PACKAGING	Details ...	125	0.67	2.03	0.000	0.020	0.119	1156
7	GO_NEGATIVE_REGULATION_OF_DNA_REPLICATION	Details ...	22	0.75	2.02	0.000	0.020	0.131	1272
8	GO_REGULATION_OF_NUCLEAR_DIVISION	Details ...	132	0.51	2.01	0.000	0.022	0.154	1082
9	GO_MITOTIC_CELL_CYCLE_CHECKPOINT	Details ...	105	0.58	2.00	0.000	0.021	0.164	1162
10	GO_NEGATIVE_REGULATION_OF_CHROMOSOME_SEGREGATION	Details ...	29	0.72	2.00	0.000	0.019	0.164	876
11	GO_REGULATION_OF_SISTER_CHROMATID_SEGREGATION	Details ...	52	0.64	2.00	0.000	0.017	0.164	972
12	GO_MEIOTIC_CELL_CYCLE	Details ...	137	0.54	2.00	0.000	0.017	0.169	1401

GSEA classic results

GSEA plots in “Details…”

- Enrichment plot
- Table with genes in the gene set
- Heatmap
- Random ES distribution



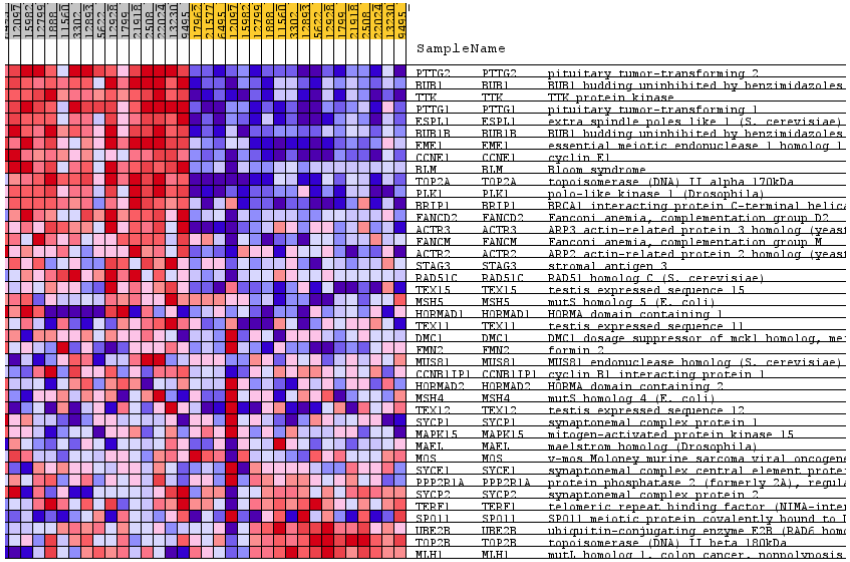
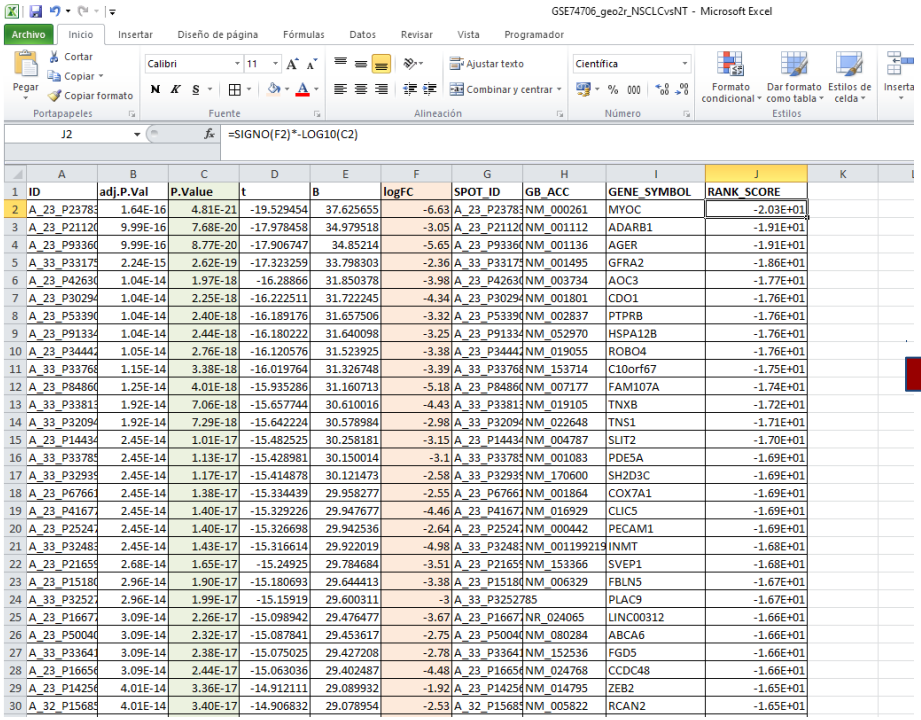


Fig 2: GO_MEIOTIC_CHROMOSOME_SEGREGATION
Blue-Pink O' Gram in the Space of the Analyzed GeneSet

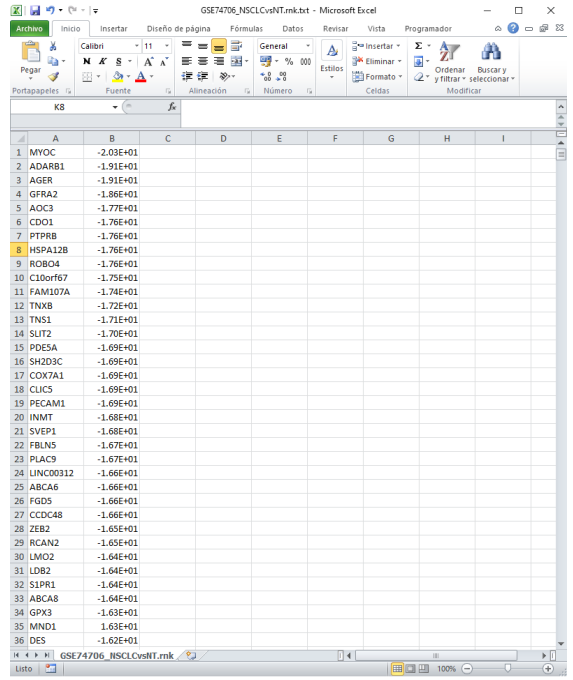
Input data: RNK format

RNK file:

- Valid for microarrays and RNAseq
- A rank score is given for each gene: Rank Score = -log10(pvalue) * sign(logFC)
- A gene differentially expressed at a significant level (low pvalue close to 0) will be assigned with a high score

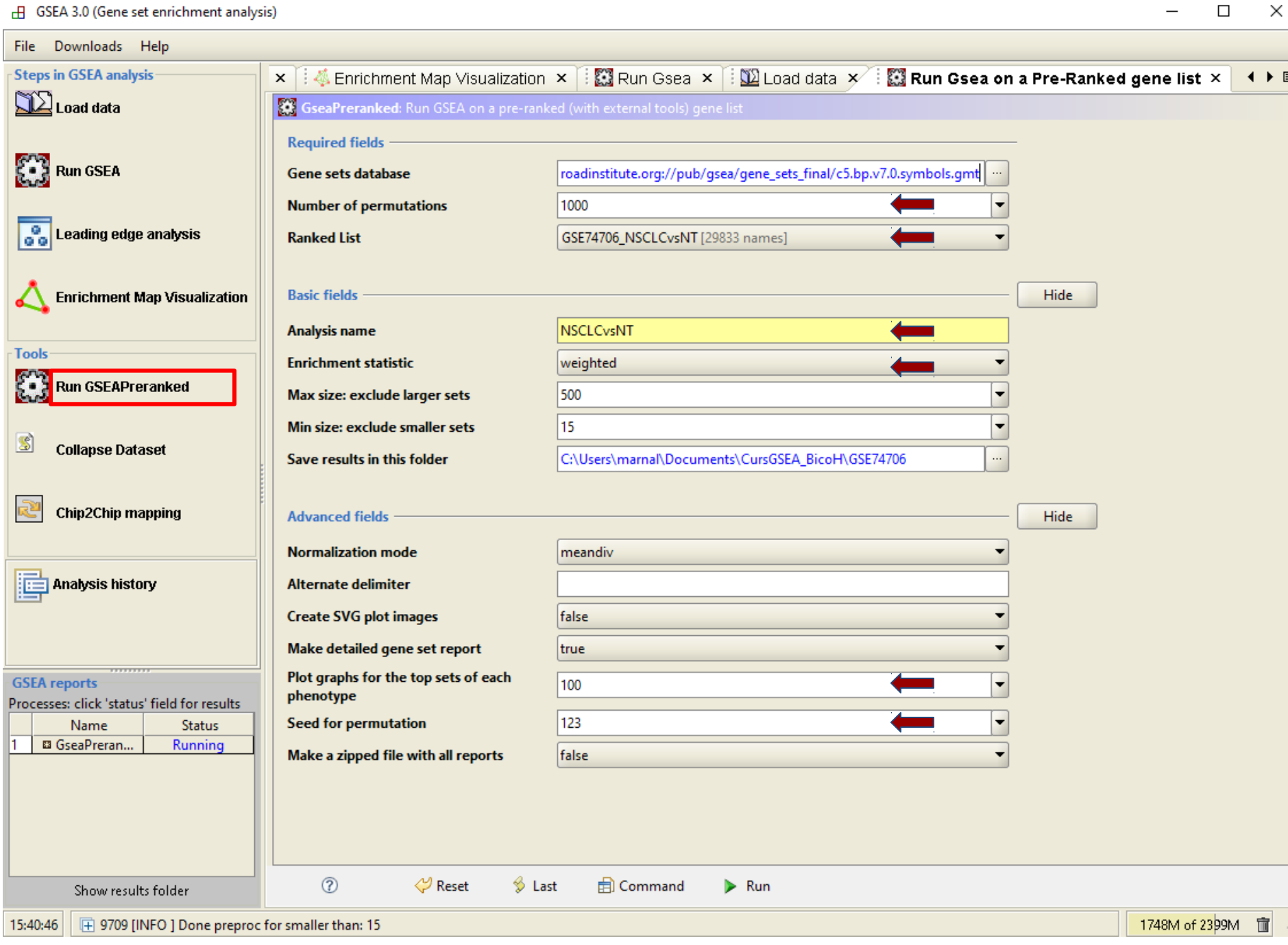


	A	B	C	D	E	F	G	H	I	J
1	ID	adj.P.Val	P.Value	t	B	logFC	SPOT_ID	GB_ACC	GENE_SYMBOL	RANK_SCORE
2	A_23_P23783	1.64E-16	4.81E-21	-19.529454	37.625655	-6.63	A_23_P23783	NM_000261	MYOC	-2.03E+01
3	A_23_P21120	9.99E-16	7.68E-20	-17.978458	34.979518	-3.05	A_23_P21120	NM_001112	ADARB1	-1.91E+01
4	A_23_P93360	9.99E-16	8.77E-20	-17.906747	34.85214	-5.65	A_23_P93360	NM_001136	AGER	-1.91E+01
5	A_33_P33175	2.24E-15	2.62E-19	-17.323259	33.798303	-2.36	A_33_P33175	NM_001495	GFRA2	-1.86E+01
6	A_23_P42630	1.04E-14	1.97E-18	-16.28866	31.850378	-3.98	A_23_P42630	NM_003734	AOC3	-1.77E+01
7	A_23_P30294	1.04E-14	2.25E-18	-16.222511	31.722245	-4.34	A_23_P30294	NM_001801	CDO1	-1.76E+01
8	A_23_P53390	1.04E-14	2.40E-18	-16.189176	31.657506	-3.32	A_23_P53390	NM_002837	PTPRB	-1.76E+01
9	A_23_P91334	1.04E-14	2.44E-18	-16.180222	31.640098	-3.25	A_23_P91334	NM_052970	HSPA12B	-1.76E+01
10	A_23_P34442	1.05E-14	2.76E-18	-16.120576	31.523925	-3.38	A_23_P34442	NM_019055	ROBO4	-1.76E+01
11	A_33_P33768	1.15E-14	3.38E-18	-16.019764	31.326748	-3.39	A_33_P33768	NM_153714	C10orf67	-1.75E+01
12	A_23_P84860	1.25E-14	4.01E-18	-15.935286	31.160713	-5.18	A_23_P84860	NM_007177	FAM107A	-1.74E+01
13	A_33_P33813	1.92E-14	7.06E-18	-15.657744	30.610016	-4.43	A_33_P33813	NM_019105	TNXB	-1.72E+01
14	A_33_P32094	1.92E-14	7.29E-18	-15.642224	30.578984	-2.98	A_33_P32094	NM_022648	TNS1	-1.71E+01
15	A_23_P14434	2.45E-14	1.01E-17	-15.482525	30.258181	-3.15	A_23_P14434	NM_004787	SLIT2	-1.70E+01
16	A_33_P33785	2.45E-14	1.13E-17	-15.428981	30.150014	-3.1	A_33_P33785	NM_001083	PDE5A	-1.69E+01
17	A_33_P32935	2.45E-14	1.17E-17	-15.414878	30.121473	-2.58	A_33_P32935	NM_170600	SH2D3C	-1.69E+01
18	A_23_P67661	2.45E-14	1.38E-17	-15.334439	29.958277	-2.55	A_23_P67661	NM_001864	COX7A1	-1.69E+01
19	A_23_P41677	2.45E-14	1.40E-17	-15.329226	29.947677	-4.46	A_23_P41677	NM_016929	CLIC5	-1.69E+01
20	A_23_P25247	2.45E-14	1.40E-17	-15.326698	29.942536	-2.64	A_23_P25247	NM_000442	PECAM1	-1.69E+01
21	A_33_P32483	2.45E-14	1.43E-17	-15.316614	29.922019	-4.98	A_33_P32483	NM_001199219	INMT	-1.68E+01
22	A_23_P21655	2.68E-14	1.65E-17	-15.24925	29.784684	-3.51	A_23_P21655	NM_153366	SVEP1	-1.68E+01
23	A_23_P15180	2.96E-14	1.90E-17	-15.180693	29.644413	-3.38	A_23_P15180	NM_006329	FBLN5	-1.67E+01
24	A_33_P32527	2.96E-14	1.99E-17	-15.15919	29.600311	-3	A_33_P3252785		PLAC9	-1.67E+01
25	A_23_P16671	3.09E-14	2.26E-17	-15.098942	29.476477	-3.67	A_23_P16671	NR_024065	LINC00312	-1.66E+01
26	A_23_P50040	3.09E-14	2.32E-17	-15.087841	29.453617	-2.75	A_23_P50040	NM_080284	ABCA6	-1.66E+01
27	A_33_P33641	3.09E-14	2.38E-17	-15.075025	29.427208	-2.78	A_33_P33641	NM_152536	FGD5	-1.66E+01
28	A_23_P16656	3.09E-14	2.44E-17	-15.063036	29.402487	-4.48	A_23_P16656	NM_024768	CCDC48	-1.66E+01
29	A_23_P14256	4.01E-14	3.36E-17	-14.912111	29.089932	-1.92	A_23_P14256	NM_014795	ZEB2	-1.65E+01
30	A_32_P15685	4.01E-14	3.40E-17	-14.906832	29.078954	-2.53	A_32_P15685	NM_005822	RCAN2	-1.65E+01



	A	B
1	MYOC	-2.03E+01
2	ADARB1	-1.91E+01
3	AGER	-1.91E+01
4	GFRA2	-1.86E+01
5	AOC3	-1.77E+01
6	CDO1	-1.76E+01
7	PTPRB	-1.76E+01
8	HSPA12B	-1.76E+01
9	ROBO4	-1.76E+01
10	C10orf67	-1.75E+01
11	FAM107A	-1.74E+01
12	TNXB	-1.72E+01
13	TNS1	-1.71E+01
14	SLIT2	-1.70E+01
15	PDE5A	-1.69E+01
16	SH2D3C	-1.69E+01
17	COX7A1	-1.69E+01
18	CLIC5	-1.69E+01
19	PECAM1	-1.69E+01
20	INMT	-1.68E+01
21	SVEP1	-1.68E+01
22	FBLN5	-1.67E+01
23	PLAC9	-1.67E+01
24	LINC00312	-1.66E+01
25	ABCA6	-1.66E+01
26	FGD5	-1.66E+01
27	CCDC48	-1.66E+01
28	ZEB2	-1.65E+01
29	RCAN2	-1.65E+01
30	LMO2	-1.64E+01
31	LDB2	-1.64E+01
32	S1PR1	-1.64E+01
33	ABCA8	-1.64E+01
34	GPX3	-1.63E+01
35	MND1	1.63E+01
36	DES	-1.62E+01

IMPORTANT: Delete missings and dates in gene symbol column


GSEA 3.0 (Gene set enrichment analysis)
File
Downloads
Help
Steps in GSEA analysis
Load data
Run GSEA
Leading edge analysis
Enrichment Map Visualization
Tools
Run GSEAPreranked
Collapse Dataset
Chip2Chip mapping
Analysis history
GSEA reports
Processes: click 'status' field for results
Name
Status
1
GseaPreran...
Running
Show results folder
Enrichment Map Visualization
Run Gsea
Load data
Run Gsea on a Pre-Ranked gene list
GseaPreranked: Run GSEA on a pre-ranked (with external tools) gene list
Required fields
Gene sets database
roadinstitute.org://pub/gsea/gene_sets_final/c5.bp.v7.0.symbols.gmt
Number of permutations
1000
Ranked List
GSE74706_NSCLCvsNT [29833 names]
Basic fields
Hide
Analysis name
NSCLCvsNT
Enrichment statistic
weighted
Max size: exclude larger sets
500
Min size: exclude smaller sets
15
Save results in this folder
C:\Users\marnal\Documents\CursGSEA_BicoH\GSE74706
Advanced fields
Hide
Normalization mode
meandiv
Alternate delimiter
Create SVG plot images
false
Make detailed gene set report
true
Plot graphs for the top sets of each phenotype
100
Seed for permutation
123
Make a zipped file with all reports
false
Reset
Last
Command
Run
15:40:46
9709 [INFO] Done preproc for smaller than: 15
1748M of 2399M

GSEA Preranked results

GSEA Report for Dataset GSE74706_NSCLCvsNT

Enrichment in phenotype: na

- 970 / 3897 gene sets are upregulated in phenotype **na_pos**
- 569 gene sets are significant at FDR < 25%
- 368 gene sets are significantly enriched at nominal pvalue < 1%
- 469 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

na_pos=NSCLC

Enrichment in phenotype: na

- 2927 / 3897 gene sets are upregulated in phenotype **na_neg**
- 1757 gene sets are significantly enriched at FDR < 25%
- 794 gene sets are significantly enriched at nominal pvalue < 1%
- 1272 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

na_neg=NT

Dataset details

- The dataset has 21754 features (genes)
- No probe set => gene symbol collapsing was requested, so all 21754 features were used

Gene set details

- Gene set size filters (min=15, max=500) resulted in filtering out 3453 / 7350 gene sets
- The remaining 3897 gene sets were used in the analysis
- List of gene sets used and their sizes (restricted to features in the specified dataset)

GSEA plots in “Details…”

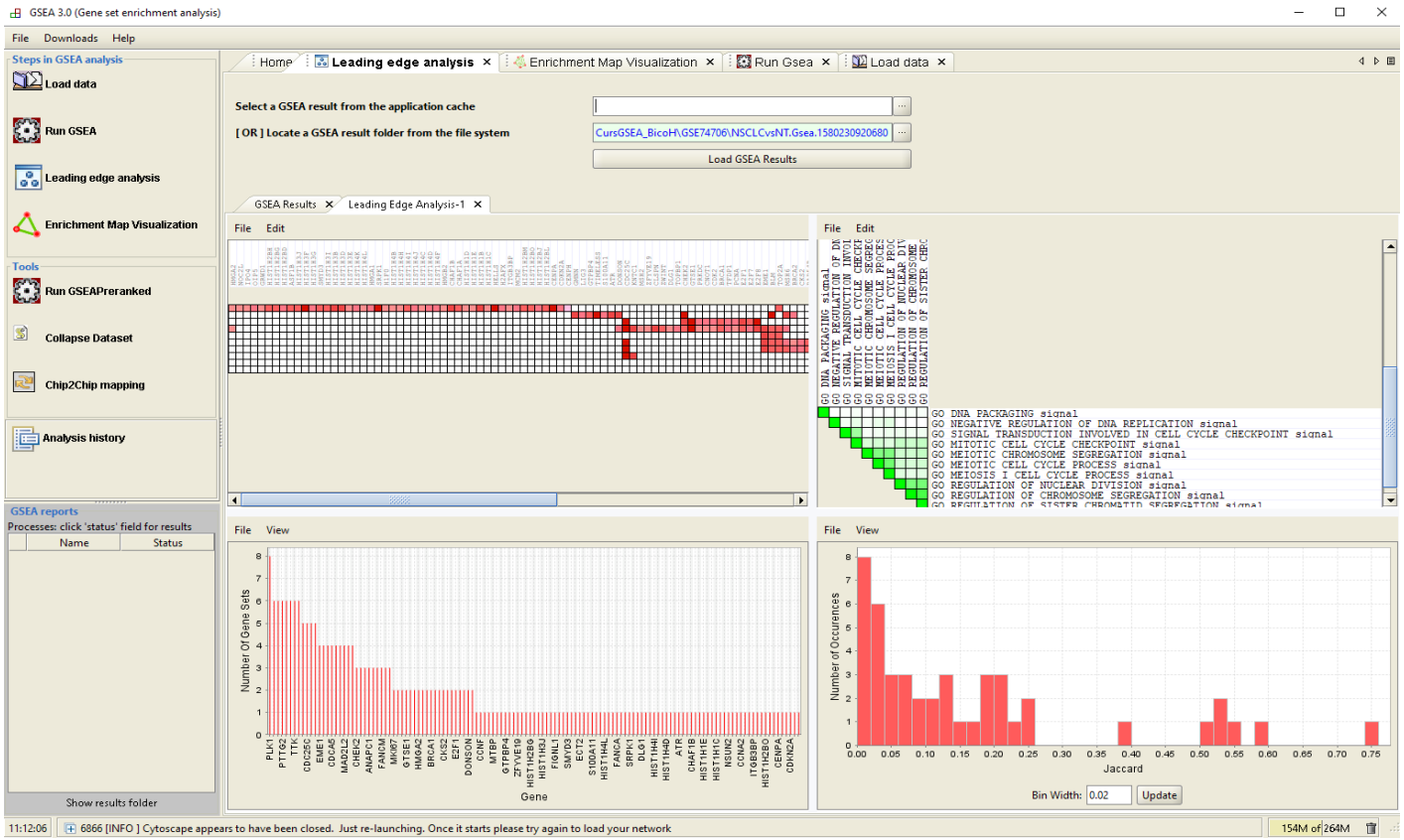
- Enrichment plot
- Table with genes in the gene set
- Random ES distribution

(no heatmap)

GSEA Leading edge analysis

Leading edge analysis to compare two gene sets:

- Load a folder with GSEA results
- Select the gene sets to compare



Practical Exercise

Dataset GSE5600 to perform GSEA analysis:

Classic analysis

1. Download the series matrix from GEO
2. Use Sublime Text to generate the GCT and CLS files
3. Load files in GSEA tool - Load data
4. Perform a GSEA - Run GSEA with the parameters previously described

Pre-Ranked analysis

5. Use the differential expression analysis results from GEOtoR
6. Calculate the RNK score in excel (=SIGNO(F2)*-LOG10(C2))
7. Change the extension of the file to .rnk
8. Load .rnk file in GSEA tool - Load data
9. Perform a GSEA - Run GSEAPreranked