

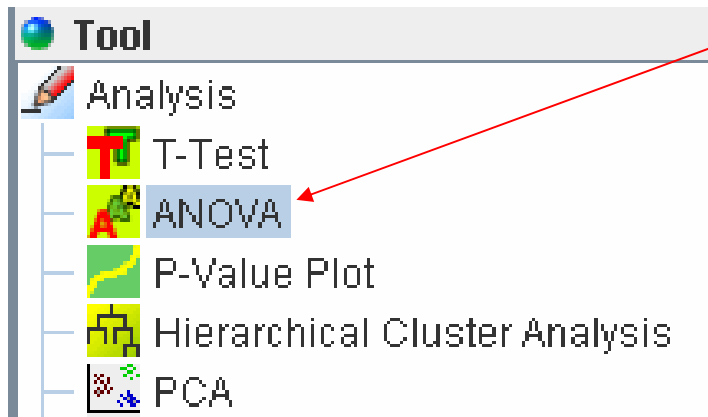
# Tutorial 2: Comparing multiple groups



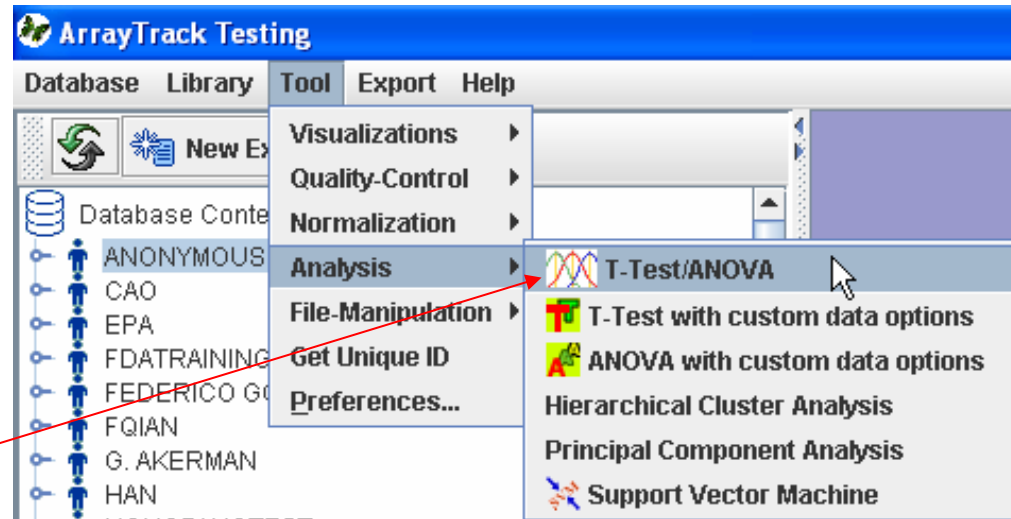
# Comparing Multiple Groups

User can use ANOVA to compare multiple groups. Running ANOVA is similar to running T-test (see tutorial 1 for detail about T-test).

The following are two ways to access ANOVA. But we recommend the third way to do ANOVA, see next slide.



Activate ANOVA from Tool panel



Activate ANOVA from pull-down menu

# Comparing Multiple Groups

Right-click the selected datasets, choose “Analysis” -> T-Test/ANOVA.

The screenshot displays a software interface with a 'Database Contents' tree on the left and a context menu on the right. The tree shows a hierarchy: ANONYMOUS > Affy\_Rat\_MAS5\_only > Gene Lists > D0\_T12\_B\_a D0\_T12\_B(Biotin) > MAS5 {D0\_T12\_B\_a} [file: Temp...]. Other similar entries are visible for B, C, and D groups. The context menu is open over the selected MAS5 files, listing various actions. The 'Analysis' option is highlighted, and its sub-menu is visible, with 'T-Test/ANOVA' selected. A red arrow points from the text above to this option. The 'Library' panel at the bottom left lists various biological databases like ID Converter, Gene Library, Pathway Library, Protein Library, IPI Library, and Orthologene Library.

Database Contents

ANONYMOUS

Affy\_Rat\_MAS5\_only

Gene Lists

D0\_T12\_B\_a D0\_T12\_B(Biotin)

MAS5 {D0\_T12\_B\_a} [file: Temp...]

Mean/Median Scaling, ifs=N

D0\_T12\_B\_b D0\_T12\_B(Biotin)

MAS5 {D0\_T12\_B\_b} [file: Temp...]

Mean/Median Scaling, ifs=N

D0\_T12\_C\_a D0\_T12\_C(Biotin)

MAS5 {D0\_T12\_C\_a} [file: Temp...]

Mean/Median Scaling, ifs=N

D0\_T12\_C\_b D0\_T12\_C(Biotin)

MAS5 {D0\_T12\_C\_b} [file: Temp...]

Mean/Median Scaling, ifs=N

D0\_T12\_D\_a D0\_T12\_D(Biotin)

MAS5 {D0\_T12\_D\_a} [file: Temp...]

View data set(s) as wide spreadsheet - datasets side by side

Export

Convert affy cel files to probe sets

Mixed scatterplot

Virtual array images for data

Actual array images for data <<Dev. Only>>

Rank intensity plots for data

BarChart

Create gene list by data filtering...

**Analysis**

Quality Control

Normalize...

Duplicate data sets

Copy data sets for pasting elsewhere

Studies

Tree options...

T-Test/ANOVA

Correlation Matrix

T-Test with custom data options

ANOVA with custom data options

Hierarchical Cluster Analysis

Principal Component Analysis

Support Vector Machine <dev. only>

Do pairwise t-test combinations <<Dev. Only>>

Library

ID Converter

Gene Library

Pathway Library

Protein Library

IPI Library

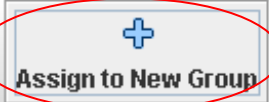


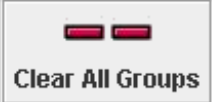

Orthologene Library

# Comparing multiple groups - continued

Assign data into 3 groups

Select Dataset Group Assignments for T-Test / ANOVA / Pairwise Tests

### Assign Data Sets Into Groups

Groups:     

	Hybridization	SAMPLE 1	VITRO DOSING 1	LABEL 1	ARRAYTYPENAME	SPECIES 1	ASSAY 1	CELLTYPE 1	SE	
1	1	D0_T12_B_a	D0_T12_B	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
2	1	D0_T12_B_b	D0_T12_B	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
3	2	D0_T12_C_a	D0_T12_C	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
4	2	D0_T12_C_b	D0_T12_C	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
5	3	D0_T12_D_a	D0_T12_D	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
6	3	D0_T12_D_b	D0_T12_D	Compound C 0 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
7	1	D2_T12_B_a	D2_T12_B	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
8	1	D2_T12_B_b	D2_T12_B	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
9	2	D2_T12_C_a	D2_T12_C	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
10	2	D2_T12_C_b	D2_T12_C	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
11	3	D2_T12_D_a	D2_T12_D	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male
12	3	D2_T12_D_b	D2_T12_D	Compound C 2 1	Biotin	Affy_RT-U34	Rat	In Vitro	Hepatocytes	Male

3 groups, sizes = [4, 4, 4]

T-Test fold changes are computed as  $\text{grp 1}/\text{grp 2}$ , so "up" regulation will mean  $\text{grp 1} > \text{grp 2}$  in any further analysis.

Next >

# Comparing Multiple Groups - continued

Select Dataset Group Assignments for T-Test / ANOVA / Pairwise Tests

Test Type ( Consistent with group selections )

ANOVA  Pairwise T-Tests

Filtering with a gene list

Only include genes from gene list

**Options for ANOVA**

Gene identifiers to include

Genbank Acc  Gene Mfr ID  LOCUSID  UNIGENEID  GENENAME

CLONEID  GEN\_DESCR\_MFR  REFSEQ  SPOTID

Data options

Subtract backgrounds when present (raw datasets only)

Apply logarithm to expression values

Exclude spots flagged as bad

< Back Do Tests

# Comparing Multiple Groups - continued

ANOVA result of 3 groups

ANOVA Results

File Selected-Spot All-Spots Advanced

	REFSEQ	SPOTID	P	F Ratio	Grp 1 Mean	Grp 1 Mean ...	Grp 1 Varia...	Grp 2 Mean	Grp 2 Mean ...	Grp 2 Varia...	(1) Grp 3 ...	Grp 3 Mean ...	Grp 3 Varia...
1		516642	0.1226	2.6747	0.0271	2.453	4.8052	1.7695	5.3662	3.9466	-1.3825	0.5308	2.4332
2	NM_017...	516728	0.1384	2.4837	0.0917	1.1206	0.314	0.1147	1.2876	1.0556	-0.9394	0.5596	0.3817
3		517045	0.0119	7.544	-0.798	0.6006	0.2479	0.3741	1.3606	0.272	-0.8101	0.5932	0.2161
4		516777	0.4129	0.9774	0.2041	1.2284	0.3384	0.3977	2.5057	3.567	-0.7313	0.6656	0.57
5	NM_016...	516803	0.4519	0.8687	0.5213	1.5517	0.4322	0.7535	2.1199	1.1335	0.017	1.0949	0.393
6		516594	0.8899	0.1182	0.0043	1.0365	0.1902	0.1046	1.1075	0.1547	0.1631	1.1807	0.3093
7		516809	0.052	4.1797	-0.0291	0.9916	0.0679	0.7364	1.6989	0.1142	0.2214	1.2195	0.2552
8		516802	0.8924	0.1153	0.1751	1.1358	0.033	0.2388	1.1842	0.0197	0.2833	1.2695	0.2549
9		516754	0.4983	0.7534	1.3581	2.6481	0.1847	1.6119	6.9747	4.2879	0.4272	1.7369	1.7249
10		516595	0.0441	4.5034	2.2098	5.7681	1.3627	1.949	4.2531	0.5743	0.431	1.4898	0.5218
11		516811	0.0407	4.6655	-0.1702	1.0047	0.6676	1.2518	2.6156	0.5054	0.4695	1.4183	0.1316
12		516851	0.631	0.4849	0.3829	1.3664	0.2403	0.1964	1.1651	0.095	0.5063	1.4892	0.267
13		516803	0.0705	3.6119	-0.292	0.8451	0.1886	0.0749	1.0952	0.1975	0.5402	1.5035	0.1918
14		516485	0.5241	0.6946	0.0503	1.058	0.1151	0.4892	1.7497	1.0519	0.5702	1.5302	0.1848
15	NM_009...	516838	0.9598	0.0412	0.8235	2.4535	1.7272	0.7298	2.6677	2.4203	0.5728	1.6113	0.5254
16		516901	0.037	4.8599	3.2954	10.6761	0.4793	1.3026	3.4833	2.2829	0.5806	2.2797	2.1198
17		516741	0.1353	2.5187	1.1233	2.2001	0.0546	0.9616	1.9534	0.0172	0.6151	1.5986	0.2494

1031 genes

Significance Filtering

P Values <  without adjustment

Target False Discovery Rate (FDR):

Select # genes  by lowest p-values

Mean Channel Intensities >  Bad Flags <=

Abs Fold Change >

# Comparing Multiple Groups - continued

Select Dataset Group Assignments for T-Test / ANOVA / Pairwise Tests

Test Type ( Consistent with group selections )

ANOVA  Pairwise T-Tests

**Options for Pairwise T-test**

T-Test Options

P values from dist.:  Welch t-test  Simple t-test  One class vs. mean:

P values from permutations:  All  Limit to:

Filtering with a gene list

Only include genes from gene list

Gene identifiers to include

Genbank Acc  Gene Mfr ID  LOCUSID  UNIGENEID  GENENAME

CLONEID  GEN\_DESCR\_MFR  REFSEQ  SPOTID

Dataset Naming

Hybridization names are always included.

add sample name(s) to hybridization names

add dye name(s) to hybridization names

Data options

Subtract backgrounds when present (raw datasets only)

Apply log (base 2) to expression values

Exclude spots flagged as bad

# Comparing Multiple Groups - continued

**Pairwise T-Tests Results**

Results Filtering

Filters: p <  Fold Change >  Mean Channel Intensities >

Apply

Group 1 vs group 2

Group 1 vs group 3

Group 2 vs group 3

Pairwise T-test result

Clicking one of the three group button will bring the detail table of the comparing result.

Gene List Creation

Place in experiment:  , within subfolder (optional):

Sort results by:   Ascending  Descending

Prefix gene list names with (optional):

Include gene list sizes in names

Create Gene Lists



# Comparing Multiple Groups - continued

**Group 1 vs Group 2**

File	Selected-Spot	All-Spots	Advanced										
	Genbank Acc	Gene Mfr ID	LOCUSID	GENENAME	REFSEQ	SPOTID	▲ (1) P	Abs Fold C...	Fold Chang...	Total Bad Fl...	Grp 1 Size	Grp 2 Size	Bad F
1	AA900476	rc_AA9004...	114490	Cited2		517317	0	1.8154	1.8154	0	4	4	0
2	AI007820	rc_AI00782...	301252	Hspcb		517371	0	1.6564	1.6564	0	4	4	0
3	X54686	X54686cds...	24517	Junb		517156	0.0001	2.2916	0.4364	0	4	4	0
4	U48592	U48592_at	25466	Il1rap	NM_012968	517061	0.0002	1.7567	0.5692	8	4	4	4
5	S45392	S45392_at	301252	Hspcb	NM_00100...	516965	0.0003	1.6925	1.6925	0	4	4	0
6	J03914	J03914cds...	24424	Gstm2	NM_177426	516763	0.0003	1.512	0.6614	0	4	4	0
7	Y07534	Y07534cds...	301517	Cyp27a1		517225	0.0004	1.7179	1.7179	0	4	4	0
8	AF081503	AF081503_...	60371	Birc2		516567	0.0004	5.9772	5.9772	8	4	4	4
9	S83436	S83436_i_at	297029	Gstk1	NM_181371	516977	0.0005	1.5451	1.5451	3	4	4	0
10	X70369	X70369_s_...	84032	Col3a1		517192	0.0005	3.6268	0.2757	8	4	4	4
11	M20629	M20629_s_...	24346	Es2		516864	0.0007	1.6922	0.591	0	4	4	0
12	U12187	U12187_at	83521	Rrad	NM_053338	517002	0.0008	2.6198	0.3817	8	4	4	4
13	U33314	U33314_at	29433	Pak3	NM_019210	517023	0.0009	3.0234	0.3308	8	4	4	4
14	AB008424	AB008424_...	24303	Cyp2d13	NM_173093	516517	0.001	1.59	0.6289	0	4	4	0
15	M31363	M31363m_...	24912	Sult2a1		516894	0.001	5.8238	0.1717	8	4	4	4
16	U04733	U04733_s_...	83790	Cyp2c23		516989	0.0012	1.8756	0.5332	0	4	4	0
17	AF013144	AF013144_...	171109	Dusp5	NM_133578	516533	0.0012	2.2075	0.453	0	4	4	0
18	U37462	U37462_s_...	29568	Map2k5		517036	0.0013	3.5246	3.5246	8	4	4	4
19	X57523	X57523_at	24811	Tap1		517164	0.0013	1.9986	1.9986	0	4	4	0

1031 genes

**Significance Filtering**

P Values <  without adjustment

Target False Discovery Rate (FDR):

Select # genes  by lowest p-values

Mean Channel Intensities >  Bad Flags <=

Abs Fold Change >  **Advanced>>**