

Tutorial 2: Comparing multiple groups

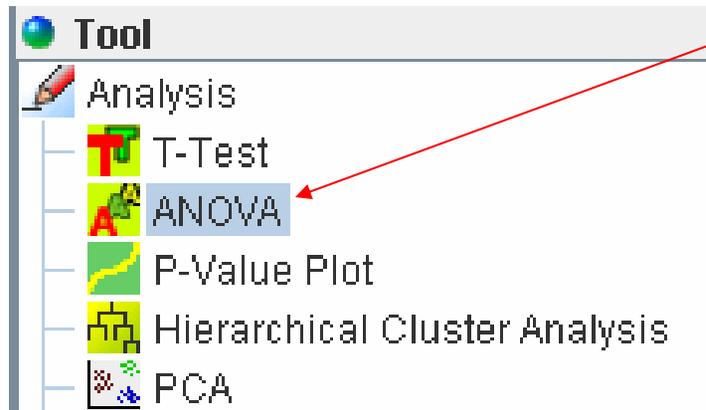


Comparing Multiple Groups

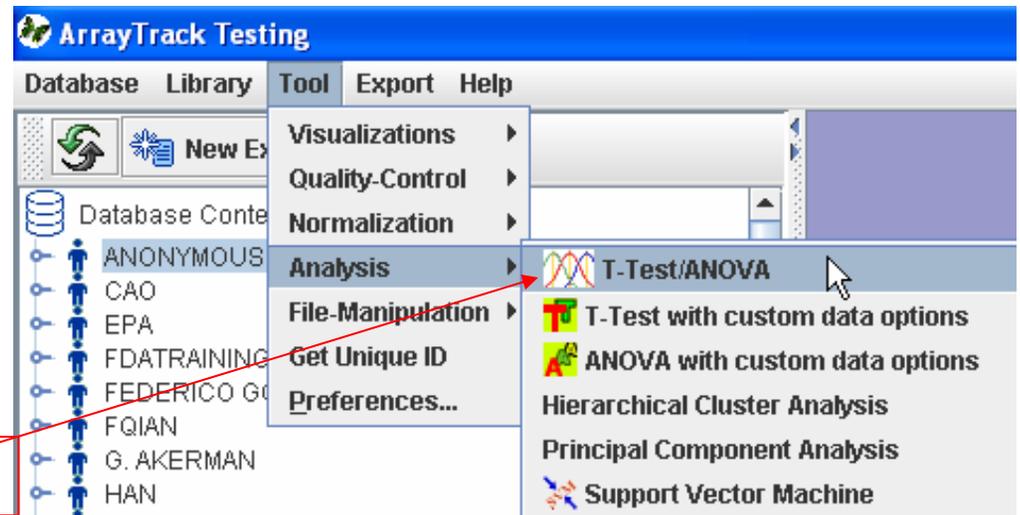
- User can choose ANOVA tool to compare multiple groups of data.
- There are three ways to activate ANOVA
 1. from TOOL panel.
 2. from pull-down menu.
 3. from selected dataset (database panel).We recommend the third way.

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).



1. Activate ANOVA from Tool panel



2. Activate ANOVA from pull-down menu

Comparing Multiple Groups – continued.

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

3. Activate ANOVA from database panel

The screenshot shows a software interface with a 'Database Contents' panel on the left. The panel is expanded to show a folder named 'Affy_Rat_MAS5_only', which contains several sub-folders: 'Gene Lists', 'D0_T12_B_a D0_T12_B(Biotin)', 'D0_T12_B_b D0_T12_B(Biotin)', 'D0_T12_C_a D0_T12_C(Biotin)', 'D0_T12_C_b D0_T12_C(Biotin)', and 'D0_T12_D_a D0_T12_D(Biotin)'. Each sub-folder contains a 'MAS5' dataset and a 'Mean/Median Scaling' option. A right-click context menu is open over the 'MAS5 {D0_T12_C_b}' dataset. The menu items are: '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', and 'Tree options...'. The 'Analysis' option is selected, and a sub-menu is open showing: '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>', and 'Do pairwise t-test combinations <<Dev. Only>>'. A red box highlights the 'T-Test/ANOVA' option, and a red arrow points from the box to the option. A mouse cursor is hovering over the 'T-Test/ANOVA' option.

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: **Assign to New Group** Unassign Assign to... Clear All Groups Swap Dyes

| | 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 grp 1/grp 2, so "up" regulation will mean grp 1 > 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

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

Comparing Multiple Groups - continued

ANOVA result of 3 groups

| File | Selected-Spot | All-Spots | Advanced | Genbank Acc | Gene Mfr ID | LOCUSID | GENENAME | REFSEQ | SPOTID | P | F Ratio | Num Gr... | Total Bad Fl... | Abs Fold C... | Fold Chang... | Diff Means ... | Grp 1 Size | Grp 1 Bad F... |
|------|---------------|-----------|----------|-------------|--------------|---------|----------|-----------|--------|--------|---------|-----------|-----------------|---------------|---------------|----------------|------------|----------------|
| 1 | | | | U70210 | U70210_at | 11787 | Apbb2 | NM_009686 | 517087 | 0.6343 | 0.4791 | 3 | 12 | 1.491 | 1.491 | 0.5763 | 4 | 4 |
| 2 | | | | M83649 | AFFX-MurF... | 14102 | Fas | NM_007987 | 516597 | 0.1063 | 2.9053 | 3 | 12 | 3.4635 | 3.4635 | 1.7922 | 4 | 4 |
| 3 | | | | M37897 | AFFX-MurL... | 16153 | Il10 | NM_010548 | 516598 | 0.1621 | 2.2421 | 3 | 12 | 2.5044 | 0.3993 | -1.3244 | 4 | 4 |
| 4 | | | | M16762 | AFFX-MurL... | 16183 | Il2 | | 516599 | 0.3922 | 1.0405 | 3 | 12 | 1.3329 | 1.3329 | 0.4145 | 4 | 4 |
| 5 | | | | M25892 | AFFX-MurL... | 16189 | Il4 | NM_021283 | 516600 | 0.4113 | 0.9821 | 3 | 12 | 2.2239 | 2.2239 | 1.1531 | 4 | 4 |
| 6 | | | | L22190 | L22190mR... | 20209 | Saa2 | | 516814 | 0.1353 | 2.5191 | 3 | 9 | 2.0445 | 2.0445 | 1.0318 | 4 | 4 |
| 7 | | | | L42293 | L42293mR... | 20652 | Soat1 | NM_009230 | 516838 | 0.9597 | 0.0413 | 3 | 12 | 1.1901 | 1.1901 | 0.251 | 4 | 4 |
| 8 | | | | J02791 | J02791_at | 24158 | Acadm | NM_016986 | 516751 | 0.0011 | 15.9787 | 3 | 0 | 1.6787 | 0.5957 | -0.7474 | 4 | 0 |
| 9 | | | | U20643 | U20643m... | 24189 | Aldoa | | 517015 | 0.034 | 5.0392 | 3 | 0 | 1.4411 | 1.4411 | 0.5271 | 4 | 0 |
| 10 | | | | M60322 | M60322_at | 24192 | Akr1b4 | NM_012498 | 516918 | 0.0036 | 11.1745 | 3 | 8 | 2.0299 | 0.4926 | -1.0214 | 4 | 4 |
| 11 | | | | M60322 | M60322_g... | 24192 | Akr1b4 | NM_012498 | 516919 | 0.0539 | 4.1123 | 3 | 12 | 4.0823 | 0.245 | -2.0294 | 4 | 4 |
| 12 | | | | M28647 | M28647_at | 24211 | Atp1a1 | | 516884 | 0.2325 | 1.7231 | 3 | 12 | 1.621 | 0.6169 | -0.6969 | 4 | 4 |
| 13 | | | | M28647 | M28647_g... | 24211 | Atp1a1 | | 516885 | 0 | 72.5941 | 3 | 0 | 1.7933 | 0.5576 | -0.8426 | 4 | 0 |
| 14 | | | | D90049 | D90049exo... | 24212 | Atp1a2 | | 516727 | 0.0842 | 3.2988 | 3 | 12 | 1.4824 | 0.6746 | -0.568 | 4 | 4 |
| 15 | | | | M28648 | M28648_s... | 24213 | Atp1a3 | | 516886 | 0.2389 | 1.6855 | 3 | 12 | 2.3333 | 0.4286 | -1.2224 | 4 | 4 |
| 16 | | | | D90048 | D90048exo... | 24214 | Atp1b2 | | 516725 | 0.062 | 3.8483 | 3 | 12 | 2.4469 | 0.4087 | -1.291 | 4 | 4 |
| 17 | | | | D90048 | D90048exo... | 24214 | Atp1b2 | | 516726 | 0.0323 | 5.1517 | 3 | 12 | 3.1855 | 0.3139 | -1.6715 | 4 | 4 |
| 18 | | | | L14680 | L14680_at | 24224 | Bcl2 | NM_016993 | 516803 | 0.4519 | 0.8686 | 3 | 12 | 1.6661 | 1.6661 | 0.7365 | 4 | 4 |
| 19 | | | | L14680 | L14680_g... | 24224 | Bcl2 | NM_016993 | 516804 | 0.5709 | 0.5969 | 3 | 12 | 1.2519 | 0.7988 | -0.3242 | 4 | 4 |
| 20 | | | | Z50051 | Z50051_at | 24235 | C4bpa | NM_012516 | 517239 | 0.0019 | 13.5576 | 3 | 0 | 1.8727 | 0.534 | -0.9052 | 4 | 0 |
| 21 | | | | X13933 | X13933_s... | 24242 | Calm1 | | 517136 | 0.1177 | 2.7397 | 3 | 0 | 1.3836 | 0.7227 | -0.4684 | 4 | 0 |
| 22 | | | | M11670 | M11670_at | 24248 | Cat | NM_012520 | 516842 | 0.297 | 1.3936 | 3 | 0 | 1.3653 | 0.7324 | -0.4492 | 4 | 0 |
| 23 | | | | AA926149 | rc_AA9261... | 24248 | Cat | | 517330 | 0.73 | 0.326 | 3 | 12 | 1.6428 | 1.6428 | 0.7162 | 4 | 4 |
| 24 | | | | AA926149 | rc_AA9261... | 24248 | Cat | | 517331 | 0.3518 | 1.1758 | 3 | 0 | 1.1945 | 0.8372 | -0.2564 | 4 | 0 |
| 25 | | | | M60753 | M60753_s... | 24267 | Comt | | 516920 | 0.0014 | 14.9522 | 3 | 0 | 2.2705 | 2.2705 | 1.183 | 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>>

Apply Filters Clear Filters

P-Value Plot Create Sig. Gene List HCA PCA K-Means Volcano Plot

User can further filter the results by setting criteria like P value, fold change, etc.

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. See Next slide.

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 Flags ... | Bad Flags ... | |
| 1 | AA900476 | rc_AA9004... | 114490 | Cited2 | | 517317 | 0 | 1.8154 | 1.8154 | 0 | 4 | 4 | 0 | 0 | 0 ▲ |
| 2 | AI007820 | rc_AI00782... | 301252 | Hspcb | | 517371 | 0 | 1.6564 | 1.6564 | 0 | 4 | 4 | 0 | 0 | 0 |
| 3 | X54686 | X54686cds... | 24517 | Junb | | 517156 | 0.0001 | 2.2916 | 0.4364 | 0 | 4 | 4 | 0 | 0 | - |
| 4 | U48592 | U48592_at | 25466 | Il1rap | NM_012968 | 517061 | 0.0002 | 1.7567 | 0.5692 | 8 | 4 | 4 | 4 | 4 | - |
| 5 | S45392 | S45392_at | 301252 | Hspcb | NM_00100... | 516965 | 0.0003 | 1.6925 | 1.6925 | 0 | 4 | 4 | 0 | 0 | 0 |
| 6 | J03914 | J03914cds... | 24424 | Gstm2 | NM_177426 | 516763 | 0.0003 | 1.512 | 0.6614 | 0 | 4 | 4 | 0 | 0 | - |
| 7 | Y07534 | Y07534cds... | 301517 | Cyp27a1 | | 517225 | 0.0004 | 1.7179 | 1.7179 | 0 | 4 | 4 | 0 | 0 | 0 |
| 8 | AF081503 | AF081503_... | 60371 | Birc2 | | 516567 | 0.0004 | 5.9772 | 5.9772 | 8 | 4 | 4 | 4 | 4 | 2 |
| 9 | S83436 | S83436_i_at | 297029 | Gstk1 | NM_181371 | 516977 | 0.0005 | 1.5451 | 1.5451 | 3 | 4 | 4 | 0 | 3 | 0 |
| 10 | X70369 | X70369_s_... | 84032 | Col3a1 | | 517192 | 0.0005 | 3.6268 | 0.2757 | 8 | 4 | 4 | 4 | 4 | - |
| 11 | M20629 | M20629_s_... | 24346 | Es2 | | 516864 | 0.0007 | 1.6922 | 0.591 | 0 | 4 | 4 | 0 | 0 | - |
| 12 | U12187 | U12187_at | 83521 | Rrad | NM_053338 | 517002 | 0.0008 | 2.6198 | 0.3817 | 8 | 4 | 4 | 4 | 4 | - |
| 13 | U33314 | U33314_at | 29433 | Pak3 | NM_019210 | 517023 | 0.0009 | 3.0234 | 0.3308 | 8 | 4 | 4 | 4 | 4 | - |
| 14 | AB008424 | AB008424_... | 24303 | Cyp2d13 | NM_173093 | 516517 | 0.001 | 1.59 | 0.6289 | 0 | 4 | 4 | 0 | 0 | - |
| 15 | M31363 | M31363m... | 24912 | Sult2a1 | | 516894 | 0.001 | 5.8238 | 0.1717 | 8 | 4 | 4 | 4 | 4 | - |
| 16 | U04733 | U04733_s_... | 83790 | Cyp2c23 | | 516989 | 0.0012 | 1.8756 | 0.5332 | 0 | 4 | 4 | 0 | 0 | - |
| 17 | AF013144 | AF013144 | 171109 | Dusp5 | NM_133578 | 516533 | 0.0012 | 2.2075 | 0.453 | 0 | 4 | 4 | 0 | 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 >