HELP to Use the macro FAMT

1 Download

Download the FAMT macro (Excel file) and open it.

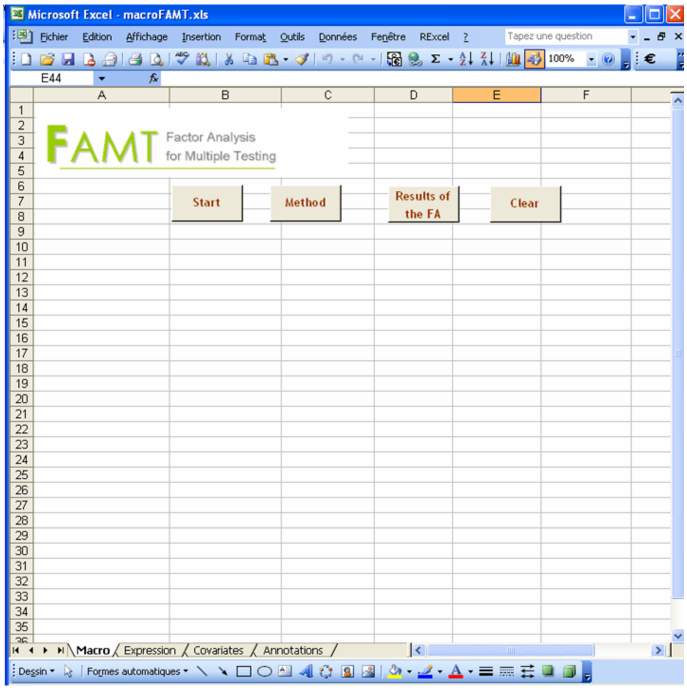


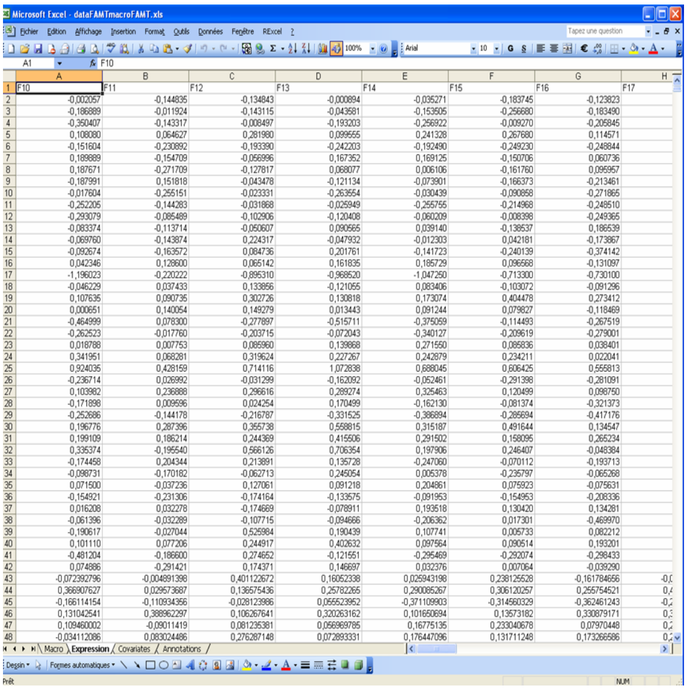
Figure 1: Illustration of the FAMT macro

The macro contains 4 sheets:

- The "Macro" sheet displays the results of the different steps of the Factor Analysis for multiple testing package.
- The "Expression" sheet contains the gene expressions data frame: genes are in rows without row names and arrays in columns (the column names are the identifier of arrays) (see Figure **??**).
- The "Covariates" sheet gives information about the experimental conditions: the identifier of each row (arrays), as used in the column names of Expression, is provided, with the value of the main explanatory variable in the testing issue and possibly other covariates (see Figure **??**).
- The "Annotations" sheet provides additional information about the response variables of the multiple testing procedure to be used to describe the results. One column must be named ID and gives the variable (gene) identifier (see Figure **??**).

The data must be stored in each sheet as above-mentioned. Note that Covariates and Annotations datasets are optional. The number of columns of expression must correspond to the number of rows of covariates and furthermore expression and annotations must have the same number of rows. If covariates dataset is not provided the procedure aims at testing the significance of the mean expression. If annotations dataset is not provided, a basic annotations dataset is created with row indices as variables identifiers.

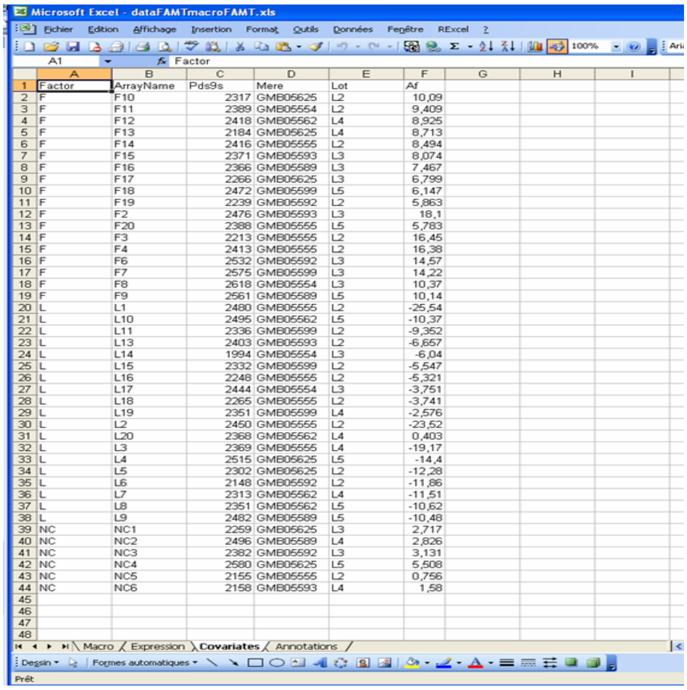
Import your data (copy and paste the data in each sheet or use the excel menu via import extern data). Be careful to the decimal mark (comma or point). The following figures present the different sheets with the data provided in the package. This dataset concerns hepatic transcriptome profiles for 9893 genes of 43 half sib male chickens selected for their variability on abdominal fatness.



	A	B	C	D	E	F	G	H
1	F10	F11	F12	F13	F14	F15	F16	F17
2	-0,002057	-0,144835	-0,134843	-0,000894	-0,035271	-0,183745	-0,123823	
3	-0,186889	-0,011924	-0,143115	-0,043581	-0,153505	-0,256680	-0,183490	
4	-0,350407	-0,143317	-0,008497	-0,193203	-0,256922	-0,009270	-0,205845	
5	0,108080	0,064627	0,281980	0,099555	0,241328	0,267680	0,114571	
6	-0,151604	-0,230892	-0,193390	-0,242203	-0,192490	-0,249230	-0,248844	
7	0,189889	-0,154709	-0,056996	0,167352	0,169125	-0,150706	0,060736	
8	0,187671	-0,271709	-0,127817	0,068077	0,006106	-0,161760	0,095957	
9	-0,187991	0,151818	-0,043478	-0,121134	-0,073901	-0,166373	-0,213461	
10	-0,017604	-0,255151	-0,023331	-0,263554	-0,030439	-0,090858	-0,271865	
11	-0,252205	-0,144283	-0,031868	-0,025949	-0,255755	-0,214968	-0,248510	
12	-0,293079	-0,085489	-0,102906	-0,120408	-0,060209	-0,008398	-0,249365	
13	-0,083374	-0,113714	-0,050607	0,090565	0,039140	-0,138537	0,186539	
14	-0,069760	-0,143874	0,224317	-0,047932	-0,012303	0,042181	-0,173867	
15	-0,092674	-0,163572	0,084736	0,201761	-0,141723	-0,240139	-0,374142	
16	0,042346	0,128600	0,065142	0,161835	0,185729	0,096568	-0,131097	
17	-1,196023	-0,220222	-0,895310	-0,968520	-1,047250	-0,713300	-0,730100	
18	-0,046229	0,037433	0,133856	-0,121055	0,083406	-0,103072	-0,091296	
19	0,107635	0,090735	0,302726	0,130818	0,173074	0,404478	0,273412	
20	0,000651	0,140054	0,149279	0,013443	0,091244	0,079827	-0,118469	
21	-0,464999	0,078300	-0,277897	-0,515711	-0,375059	-0,114493	-0,267519	
22	-0,262523	-0,017760	-0,203715	-0,072043	-0,340127	-0,209619	-0,279001	
23	0,018788	0,007753	0,085960	0,139868	0,271550	0,085836	0,038401	
24	0,341951	0,068281	0,319624	0,227267	0,242879	0,234211	0,022041	
25	0,924035	0,428159	0,714116	1,072838	0,688045	0,606425	0,555813	
26	-0,236714	0,026992	-0,031299	-0,162092	-0,052461	-0,291398	-0,281091	
27	0,103982	0,236888	0,296616	0,289274	0,325463	0,120499	0,098750	
28	-0,171898	0,009596	0,024254	0,170499	-0,162130	-0,081374	-0,321373	
29	-0,252686	-0,144178	-0,216787	-0,331525	-0,386894	-0,285694	-0,417176	
30	0,196776	0,287396	0,355738	0,558815	0,315187	0,491644	0,134547	
31	0,199109	0,186214	0,244369	0,415506	0,291502	0,158095	0,265234	
32	0,335374	-0,195540	0,566126	0,706354	0,197906	0,246407	-0,048384	
33	-0,174458	0,204344	0,213891	0,135728	-0,247060	-0,070112	-0,193713	
34	-0,098731	-0,170182	-0,062713	0,245054	0,005378	-0,235797	-0,065268	
35	0,071500	-0,037236	0,127061	0,091218	0,204861	0,075923	-0,075631	
36	-0,154921	-0,231306	-0,174164	-0,133575	-0,091953	-0,154953	-0,208336	
37	0,016208	0,032278	-0,174669	-0,078911	0,193518	0,130420	0,134281	
38	-0,061396	-0,032289	-0,107715	-0,094666	-0,206362	0,017301	-0,469970	
39	-0,190617	-0,027044	0,525984	0,190439	0,107741	0,005733	0,082212	
40	0,101110	0,077206	0,244917	0,402632	0,097564	0,090514	0,193201	
41	-0,481204	-0,186600	0,274652	-0,121551	-0,295469	-0,292074	-0,298433	
42	0,074886	-0,291421	0,174371	0,146697	0,032376	0,007064	-0,039290	
43	-0,072392796	-0,004891398	0,401122672	0,16052338	0,025943198	0,238125528	-0,161784656	-0,0
44	0,366907627	0,029573687	0,136575436	0,25782265	0,290085267	0,306120257	0,255754521	0,
45	-0,166114154	-0,110934356	-0,028123986	0,055523952	-0,371109903	-0,314560329	-0,362461243	-0,
46	0,131042541	0,388962297	0,106267641	0,320263162	0,101650694	0,13573182	0,330879171	0,
47	0,109460002	-0,09011419	0,081235381	0,056969785	0,16775135	0,233040678	0,07970448	0,
48	-0,034112086	0,083024486	0,276287148	0,072893331	0,176447096	0,131711248	0,173266586	0,

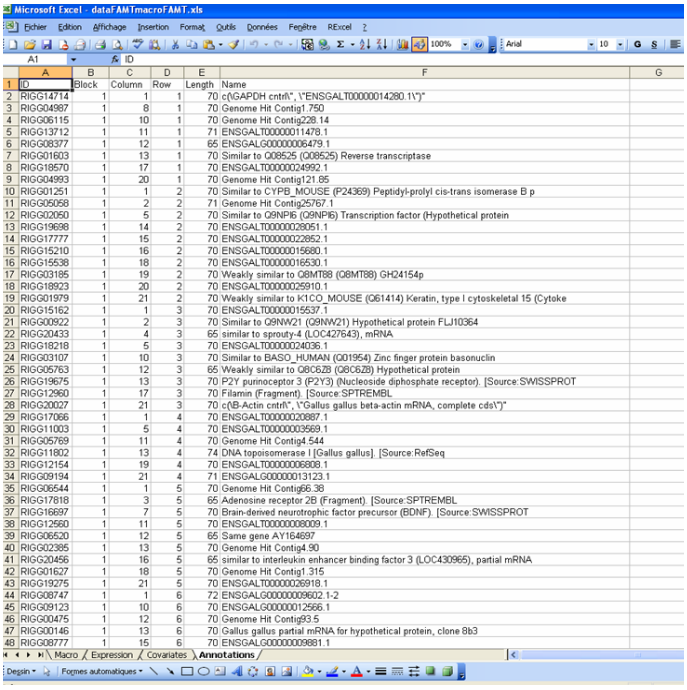
Figure 2: An example of the Expression sheet

Another sheet, called "graph", is automatically created to store all the graphs provided by the functions of the package.
The buttons in the "Macro" sheet allow to run the package by step. The CLEAR button clears the "Macro" sheet and the "graph" sheet.



	A	B	C	D	E	F
1	Factor	ArrayName	Pds9s	Mere	Lot	Af
2	F	F10	2317	GMB05625	L2	10,09
3	F	F11	2389	GMB05554	L2	9,409
4	F	F12	2418	GMB05562	L4	8,925
5	F	F13	2184	GMB05625	L4	8,713
6	F	F14	2416	GMB05555	L2	8,494
7	F	F15	2371	GMB05593	L3	8,074
8	F	F16	2366	GMB05589	L3	7,467
9	F	F17	2266	GMB05625	L3	6,799
10	F	F18	2472	GMB05599	L5	6,147
11	F	F19	2239	GMB05592	L2	5,863
12	F	F2	2476	GMB05593	L3	18,1
13	F	F20	2388	GMB05555	L5	5,783
14	F	F3	2213	GMB05555	L2	16,45
15	F	F4	2413	GMB05555	L2	16,38
16	F	F6	2532	GMB05592	L3	14,57
17	F	F7	2575	GMB05599	L3	14,22
18	F	F8	2618	GMB05554	L3	10,37
19	F	F9	2561	GMB05589	L5	10,14
20	L	L1	2480	GMB05555	L2	-25,54
21	L	L10	2495	GMB05562	L5	-10,37
22	L	L11	2336	GMB05599	L2	-9,352
23	L	L13	2403	GMB05593	L2	-6,657
24	L	L14	1994	GMB05554	L3	-6,04
25	L	L15	2332	GMB05599	L2	-5,547
26	L	L16	2248	GMB05555	L2	-5,321
27	L	L17	2444	GMB05554	L3	-3,751
28	L	L18	2265	GMB05555	L2	-3,741
29	L	L19	2351	GMB05599	L4	-2,576
30	L	L2	2450	GMB05555	L2	-23,52
31	L	L20	2368	GMB05562	L4	0,403
32	L	L3	2369	GMB05555	L4	-19,17
33	L	L4	2515	GMB05625	L5	-14,4
34	L	L5	2302	GMB05625	L2	-12,28
35	L	L6	2148	GMB05592	L2	-11,86
36	L	L7	2313	GMB05562	L4	-11,51
37	L	L8	2351	GMB05562	L5	-10,62
38	L	L9	2482	GMB05589	L5	-10,48
39	NC	NC1	2259	GMB05625	L3	2,717
40	NC	NC2	2496	GMB05589	L4	2,826
41	NC	NC3	2382	GMB05592	L3	3,131
42	NC	NC4	2580	GMB05625	L5	5,508
43	NC	NC5	2155	GMB05555	L2	0,756
44	NC	NC6	2158	GMB05593	L4	1,58
45						
46						
47						
48						

Figure 3: An example of the Covariates sheet



	A	B	C	D	E	F
1	ID	Block	Column	Row	Length	Name
2	RIGG14714	1	1	1	70	c(\GAPDH cntrl\", \"ENSGALT00000014280.1\")"
3	RIGG04987	1	8	1	70	Genome Hit Contig1.750
4	RIGG06115	1	10	1	70	Genome Hit Contig228.14
5	RIGG13712	1	11	1	71	ENSGALT00000011478.1
6	RIGG08377	1	12	1	65	ENSGALG00000006479.1
7	RIGG01603	1	13	1	70	Similar to Q08525 (Q08525) Reverse transcriptase
8	RIGG18570	1	17	1	70	ENSGALT00000024992.1
9	RIGG04993	1	20	1	70	Genome Hit Contig121.85
10	RIGG01251	1	1	2	70	Similar to CYPB_MOUSE (P24369) Peptidyl-prolyl cis-trans isomerase B p
11	RIGG05058	1	2	2	71	Genome Hit Contig25767.1
12	RIGG02050	1	5	2	70	Similar to Q9NPI6 (Q9NPI6) Transcription factor (Hypothetical protein
13	RIGG19698	1	14	2	70	ENSGALT00000028051.1
14	RIGG17777	1	15	2	70	ENSGALT00000022852.1
15	RIGG15210	1	16	2	70	ENSGALT00000015680.1
16	RIGG15538	1	18	2	70	ENSGALT00000016530.1
17	RIGG03185	1	19	2	70	Weakly similar to Q8MT88 (Q8MT88) GH24154p
18	RIGG18923	1	20	2	70	ENSGALT00000025910.1
19	RIGG01979	1	21	2	70	Weakly similar to K1CO_MOUSE (Q61414) Keratin, type I cytoskeletal 15 (Cytoke
20	RIGG15162	1	1	3	70	ENSGALT00000015537.1
21	RIGG00922	1	2	3	70	Similar to Q9NW21 (Q9NW21) Hypothetical protein FLJ10364
22	RIGG20433	1	4	3	65	similar to sprouty-4 (LOC427643), mRNA
23	RIGG18218	1	5	3	70	ENSGALT00000024036.1
24	RIGG03107	1	10	3	70	Similar to BASO_HUMAN (Q01954) Zinc finger protein basonuclin
25	RIGG05763	1	12	3	65	Weakly similar to Q8C6Z8 (Q8C6Z8) Hypothetical protein
26	RIGG19675	1	13	3	70	P2Y purinoceptor 3 (P2Y3) (Nucleoside diphosphate receptor). [Source:SWISSPROT
27	RIGG12960	1	17	3	70	Filamin (Fragment). [Source:SPTREMBL
28	RIGG20027	1	21	3	70	c(\B-Actin cntrl\", \"Gallus gallus beta-actin mRNA, complete cds\")"
29	RIGG17066	1	1	4	70	ENSGALT00000020887.1
30	RIGG11003	1	5	4	70	ENSGALT00000003569.1
31	RIGG05769	1	11	4	70	Genome Hit Contig4.544
32	RIGG11802	1	13	4	74	DNA topoisomerase I [Gallus gallus]. [Source:RefSeq
33	RIGG12154	1	19	4	70	ENSGALT00000006808.1
34	RIGG09194	1	21	4	71	ENSGALG00000013123.1
35	RIGG06544	1	1	5	70	Genome Hit Contig66.38
36	RIGG17818	1	3	5	65	Adenosine receptor 2B (Fragment). [Source:SPTREMBL
37	RIGG16697	1	7	5	70	Brain-derived neurotrophic factor precursor (BDNF). [Source:SWISSPROT
38	RIGG12560	1	11	5	70	ENSGALT00000008009.1
39	RIGG06520	1	12	5	65	Same gene AY164697
40	RIGG02385	1	13	5	70	Genome Hit Contig4.90
41	RIGG20456	1	16	5	65	similar to interleukin enhancer binding factor 3 (LOC430965), partial mRNA
42	RIGG01627	1	18	5	70	Genome Hit Contig1.315
43	RIGG19275	1	21	5	70	ENSGALT00000026918.1
44	RIGG08747	1	1	6	72	ENSGALG00000009602.1-2
45	RIGG09123	1	10	6	70	ENSGALG00000012566.1
46	RIGG00475	1	12	6	70	Genome Hit Contig93.5
47	RIGG00146	1	13	6	70	Gallus gallus partial mRNA for hypothetical protein, clone 8b3
48	RIGG08777	1	15	6	70	ENSGALG00000009881.1

Figure 4: An example of the Annotations sheet

2 The DATA button

The DATA button enables you to define the datasets, to create the FAMT data and to summarize the FAMT data. You have to select the available datasets (expression, covariates and/or

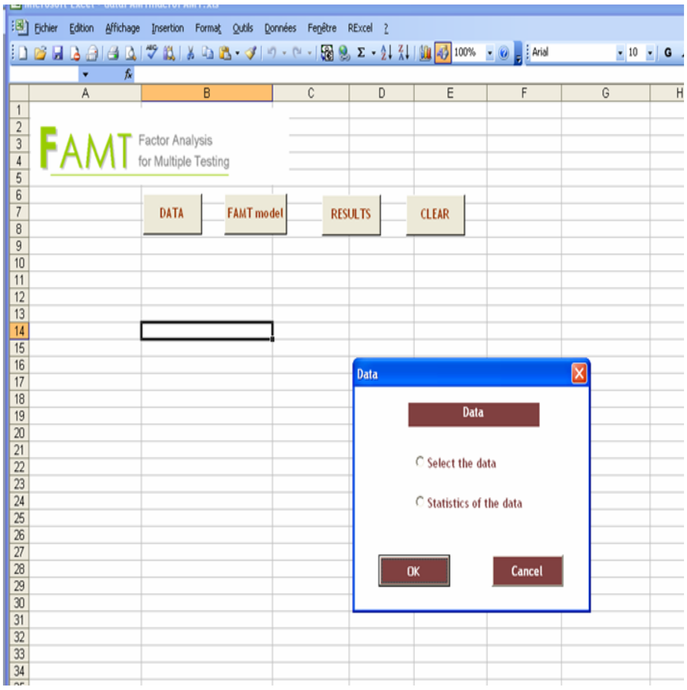


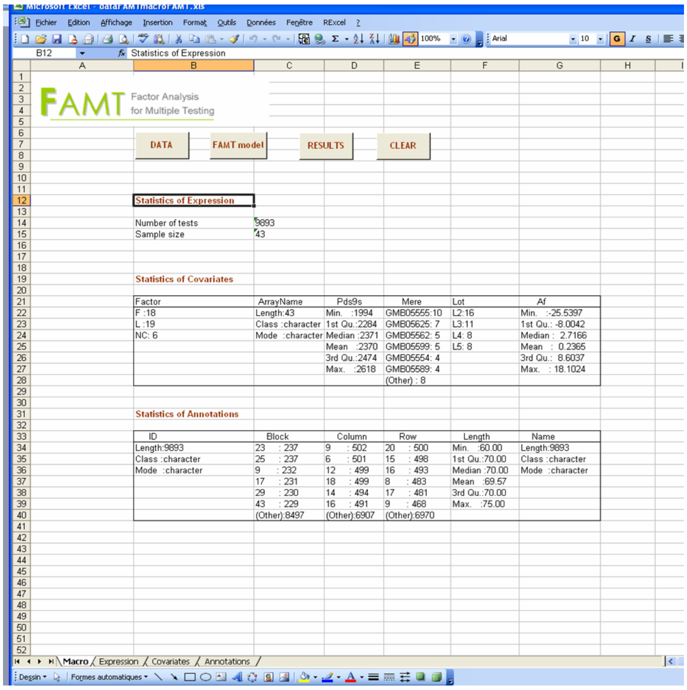
Figure 5: The DATA button

annotations), then some other dialog box allow to precise the class of variables.

2.1 Statistics of the FAMT data

When you have defined the data frame, you can ask for summary of the FAMT data by clicking on Statistics of data.
The results are displayed in the "macro" sheet. The function provides:

- For Expression: the number of tests which corresponds to the number of rows, the sample size which is the number of columns.
- For Covariates and Annotations: classical summaries.



Statistics of Expression

Number of tests	9893
Sample size	43

Statistics of Covariates

Factor	ArrayName	Pds9s	Mere	Lot	Af
F :18	Length:43	Min. :1994	GMB05555:10	L2:16	Min. :-25.5397
L :19	Class :character	1st Qu.:2284	GMB05625: 7	L3:11	1st Qu.: -8.0042
NC: 6	Mode :character	Median :2371	GMB05562: 5	L4: 8	Median : 2.7166
		Mean :2370	GMB05599: 5	L5: 8	Mean : 0.2365
		3rd Qu.:2474	GMB05554: 4		3rd Qu.: 8.6037
		Max. :2618	GMB05589: 4		Max. : 18.1024
			(Other) : 8		

Statistics of Annotations

ID	Block	Column	Row	Length	Name
Length:9893	23 : 237	9 : 502	20 : 500	Min. :60.00	Length:9893
Class :character	25 : 237	6 : 501	15 : 498	1st Qu.:70.00	Class :character
Mode :character	9 : 232	12 : 499	16 : 493	Median :70.00	Mode :character
	17 : 231	18 : 499	8 : 483	Mean :69.57	
	29 : 230	14 : 494	17 : 481	3rd Qu.:70.00	
	43 : 229	16 : 491	9 : 468	Max. :75.00	
	(Other):8497	(Other):6907	(Other):6970		

Figure 6: Display of the results of statistics of the FAMT data

3 The FAMT MODEL button

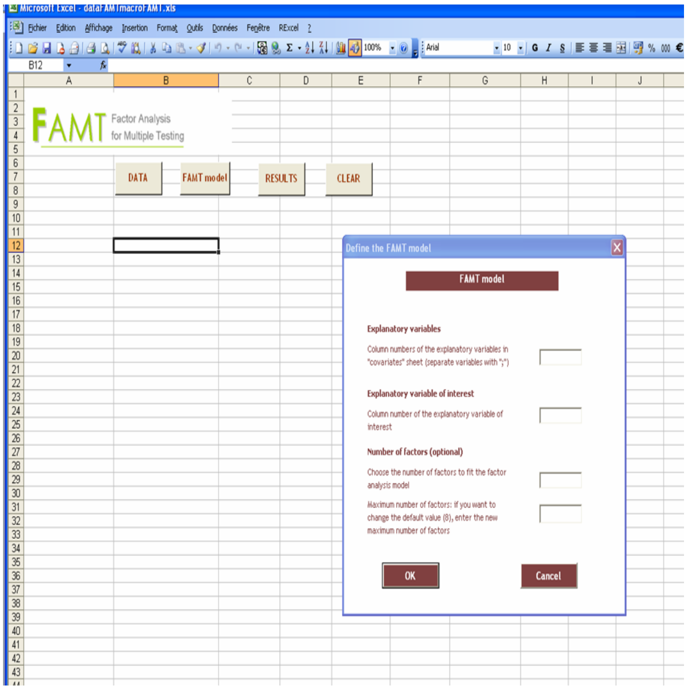
The FAMT model button enables to implement the FAMT complete multiple testing procedure. When you click on the FAMT model, a form with 4 text box is opened (see Figure **??**):

- The first box determines the experimental condition and the optional covariates.
- The second box corresponds to the experimental condition on which the test is done.
- The third and fourth box are optional and they refer to the number of factors. You can select a number of factors to fit the FA model (in the third box) or this number is estimated. The last box allows to change the default value of the maximum number of factors tested to estimate the optimal number of factors.

If you don't fill the box, the **default values** are kept for the fitting of the FA model:

- The experimental condition is the column 1 in the "Covariates" sheet (x=1)
- The test is done on the 1st column of the previous vector (x[1]). If x=1, test = 1 too.
- The function estimates the optimal number of factors
- The maximum number of factors tested to estimate the optimal number of factors is 8.

In our illustrative example (data provided with the FAMT package), we test the significance of the relationship between each gene expression and the abdominal fatness (6th column of covariates), taking into account the effect of the dam (4th column of covariates). So, in the first box, we write 4 and 6, column numbers corresponding to the experimental condition and covariates (column numbers are separated by semicolon), and in the second box, we type 6, column number of the explanatory variable of interest (see Figure **??**).



Define the FAMT model

FAMT model

Explanatory variables

Column numbers of the explanatory variables in "covariates" sheet (separate variables with ";")

Explanatory variable of interest

Column number of the explanatory variable of interest

Number of factors (optional)

Choose the number of factors to fit the factor analysis model

Maximum number of factors: if you want to change the default value (8), enter the new maximum number of factors

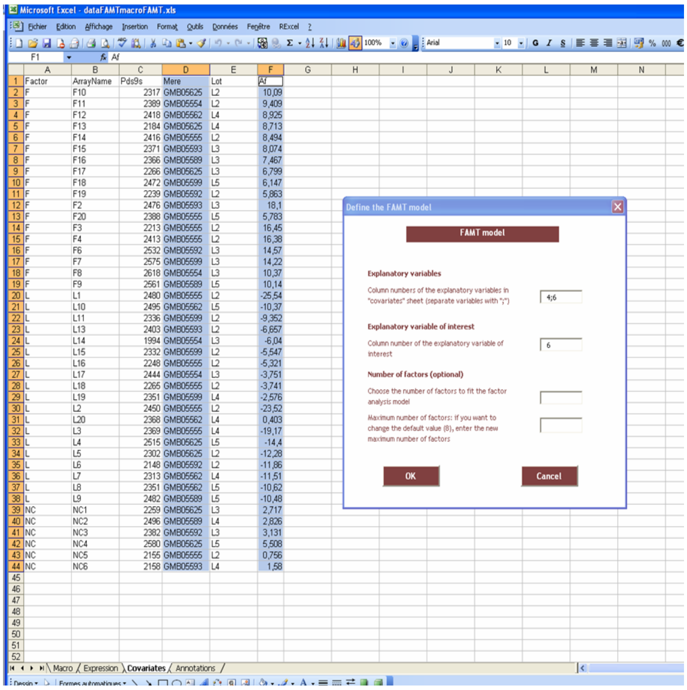
Figure 7: Dialog box of the FAMT model button

The optimal number of factors used to fit the model is given in the ”Macro” sheet. The ”graph” sheet, automatically added in the excel file, contains three graphs (see Figure **??**):

- The values of the variance inflation criteria for each number of factors are plotted
- The histograms of p-values and adjusted p-values.

If you want to use the FAMT method as a classical multiple testing procedure without any modeling for the dependence structure across the variables, choose 0 for the number of factors to adjust the FA model.

This step builds the ”FAMT model”, and enables you to analyse results (with the Results button). You can fit different models but the last one is used for the analysis of the results.



	A	B	C	D	E	F
1	Factor	ArrayName	Pds9s	Mere	Lot	Af
2	F	F10	2317	GMB05625	L2	10,09
3	F	F11	2389	GMB05554	L2	9,409
4	F	F12	2418	GMB05562	L4	8,925
5	F	F13	2184	GMB05625	L4	8,713
6	F	F14	2416	GMB05555	L2	8,494
7	F	F15	2371	GMB05593	L3	8,074
8	F	F16	2366	GMB05589	L3	7,467
9	F	F17	2266	GMB05625	L3	6,799
10	F	F18	2472	GMB05599	L5	6,147
11	F	F19	2239	GMB05592	L2	5,863
12	F	F2	2476	GMB05593	L3	18,1
13	F	F20	2388	GMB05555	L5	5,783
14	F	F3	2213	GMB05555	L2	16,45
15	F	F4	2413	GMB05555	L2	16,38
16	F	F6	2532	GMB05592	L3	14,57
17	F	F7	2575	GMB05599	L3	14,22
18	F	F8	2618	GMB05554	L3	10,37
19	F	F9	2561	GMB05589	L5	10,14
20	L	L1	2480	GMB05555	L2	-25,54
21	L	L10	2495	GMB05562	L5	-10,37
22	L	L11	2336	GMB05599	L2	-9,352
23	L	L13	2403	GMB05593	L2	-6,657
24	L	L14	1994	GMB05554	L3	-6,04
25	L	L15	2332	GMB05599	L2	-5,547
26	L	L16	2248	GMB05555	L2	-5,321
27	L	L17	2444	GMB05554	L3	-3,751
28	L	L18	2265	GMB05555	L2	-3,741
29	L	L19	2351	GMB05599	L4	-2,576
30	L	L2	2450	GMB05555	L2	-23,52
31	L	L20	2368	GMB05562	L4	0,403
32	L	L3	2369	GMB05555	L4	-19,17
33	L	L4	2515	GMB05625	L5	-14,4
34	L	L5	2302	GMB05625	L2	-12,28
35	L	L6	2148	GMB05592	L2	-11,86
36	L	L7	2313	GMB05562	L4	-11,51
37	L	L8	2351	GMB05562	L5	-10,62
38	L	L9	2482	GMB05589	L5	-10,48
39	NC	NC1	2259	GMB05625	L3	2,717
40	NC	NC2	2496	GMB05589	L4	2,826
41	NC	NC3	2382	GMB05592	L3	3,131
42	NC	NC4	2580	GMB05625	L5	5,508
43	NC	NC5	2155	GMB05555	L2	0,756
44	NC	NC6	2158	GMB05593	L4	1,58

Define the FAMT model

FAMT model

Explanatory variables

Column numbers of the explanatory variables in "covariates" sheet (separate variables with ";") 4;6

Explanatory variable of interest

Column number of the explanatory variable of interest 6

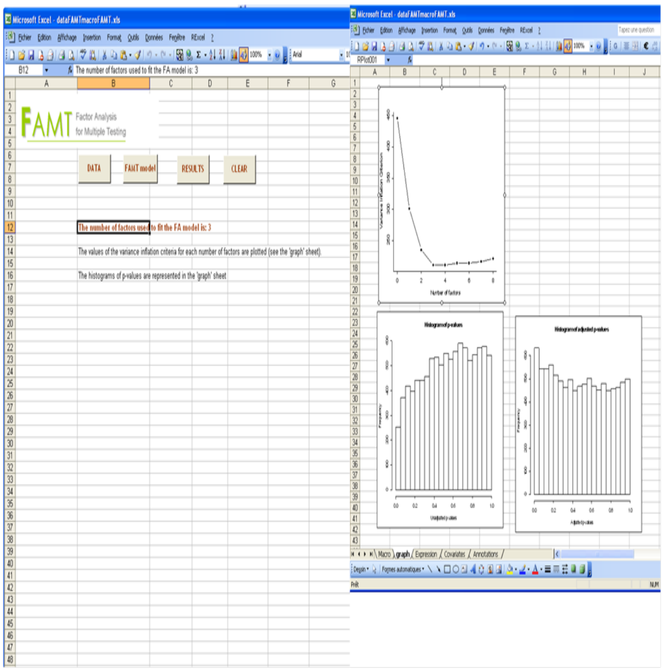
Number of factors (optional)

Choose the number of factors to fit the factor analysis model

Maximum number of factors: if you want to change the default value (8), enter the new maximum number of factors

OK Cancel

Figure 8: Example of a FAMT model



The number of factors used to fit the FA model is: 3

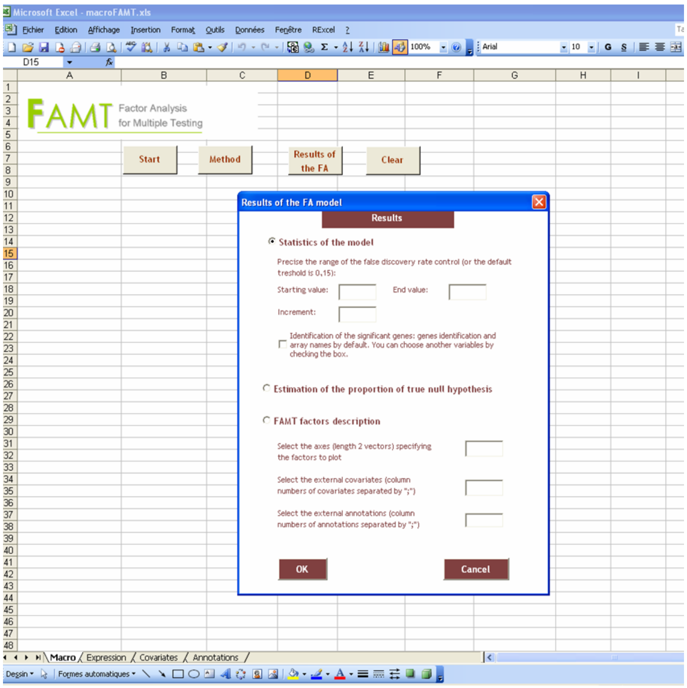
The values of the variance inflation criteria for each number of factors are plotted (see the 'graph' sheet).

The histograms of p-values are represented in the 'graph' sheet

Figure 9: Display of the results of a FAMT model

4 The RESULTS button

The RESULTS button proposes three functions to analyse and display the results (see Figure **??**). The first function provides information about the rejected genes, the second one gives an estimation of the proportion of true null hypotheses, and the last one helps the user to describe and interpret the factors.



● Statistics of the model

Precise the range of the false discovery rate control (or the default treshold is 0,15):

Starting value: End value:

Increment:

☐ Identification of the significant genes: genes identification and array names by default. You can choose another variables by checking the box.

○ Estimation of the proportion of true null hypothesis

○ FAMT factors description

Select the axes (length 2 vectors) specifying the factors to plot

Select the external covariates (column numbers of covariates separated by ";")

Select the external annotations (column numbers of annotations separated by ";")

Figure 10: Dialog box of the RESULTS button

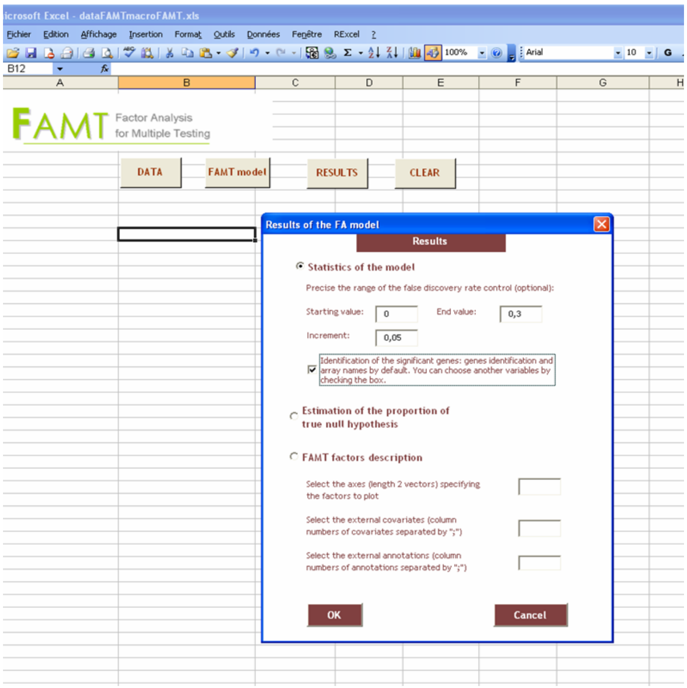
4.1 Statistics of the FAMT model

The selection of ”Statistics of the FAMT model” gives the number of rejected genes according to raw analysis and FAMT analysis, the annotations characteristics of significant genes, and the estimated proportion of true null hypotheses.

The number of positive tests is provided for each level of False Discovery Rate (FDR) control chosen by the user (the default value is 0.15). If you want to change the level of FDR control, you have to define the range of the FDR control. In our illustrative example, we select a range from 0 to 0.3 with increment of 0.05 (see Figure **??**).

The significant genes are listed with the genes identification and array names in the original data frames. You can change the identifiers (add some characteristics for example) by clicking in the check box ”identification of the significant genes” (see Figure **??**).
A new dialog box is displayed and you can select the identifiers among the annotations variables. Results are shown in the ”Macro” sheet (see Figure **??**). The list of positive genes is given for the highest level of FDR.

If you don’t fill the box of the ”Statistics of the model”, the default values are selected : the FDR control is 0.15 by default, the significant genes are characterised by the genes identification and their name in the Annotations file.



Results of the FA model

Results

● Statistics of the model

Precise the range of the false discovery rate control (optional):

Starting value: 0 End value: 0,3

Increment: 0,05

☑ Identification of the significant genes: genes identification and array names by default. You can choose another variables by checking the box.

○ Estimation of the proportion of true null hypothesis

○ FAMT factors description

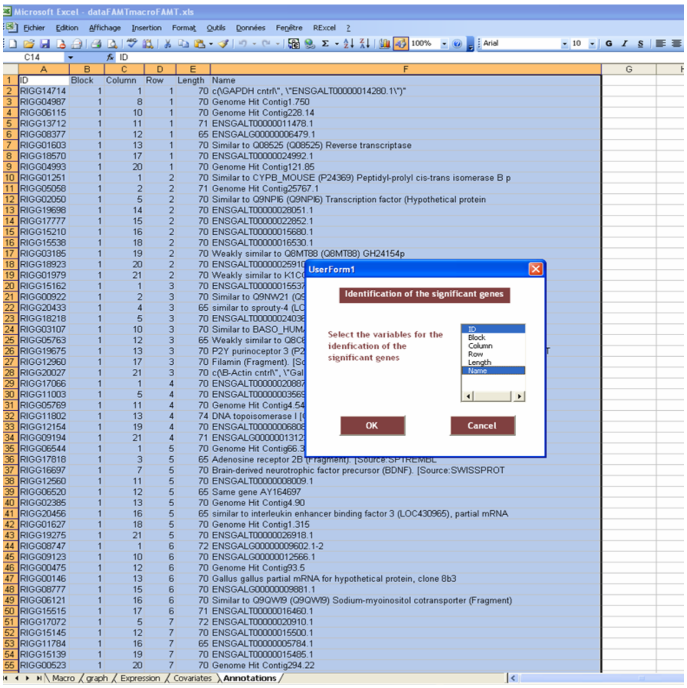
Select the axes (length 2 vectors) specifying the factors to plot

Select the external covariates (column numbers of covariates separated by ";")

Select the external annotations (column numbers of annotations separated by ";")

OK Cancel

Figure 11: Statistics of the model and choice of FDR control



	A	B	C	D	E	F
1	ID	Block	Column	Row	Length	Name
2	RIGG14714	1	1	1	70	c(\GAPDH cntrl\", \"ENSGALT00000014280.1\")"
3	RIGG04987	1	8	1	70	Genome Hit Contig1.750
4	RIGG06115	1	10	1	70	Genome Hit Contig228.14
5	RIGG13712	1	11	1	71	ENSGALT00000011478.1
6	RIGG08377	1	12	1	65	ENSGALG00000006479.1
7	RIGG01603	1	13	1	70	Similar to Q08525 (Q08525) Reverse transcriptase
8	RIGG18570	1	17	1	70	ENSGALT00000024992.1
9	RIGG04993	1	20	1	70	Genome Hit Contig121.85
10	RIGG01251	1	1	2	70	Similar to CYPB_MOUSE (P24369) Peptidyl-prolyl cis-trans isomerase B p
11	RIGG05058	1	2	2	71	Genome Hit Contig25767.1
12	RIGG02050	1	5	2	70	Similar to Q9NPI6 (Q9NPI6) Transcription factor (Hypothetical protein
13	RIGG19698	1	14	2	70	ENSGALT00000028051.1
14	RIGG17777	1	15	2	70	ENSGALT00000022852.1
15	RIGG15210	1	16	2	70	ENSGALT00000015680.1
16	RIGG15538	1	18	2	70	ENSGALT00000016530.1
17	RIGG03185	1	19	2	70	Weakly similar to Q8MT88 (Q8MT88) GH24154p
18	RIGG18923	1	20	2	70	ENSGALT00000025910
19	RIGG01979	1	21	2	70	Weakly similar to K1C
20	RIGG15162	1	1	3	70	ENSGALT00000015537
21	RIGG00922	1	2	3	70	Similar to Q9NW21 (Q
22	RIGG20433	1	4	3	65	similar to sprouty-4 (LC
23	RIGG18218	1	5	3	70	ENSGALT00000024036
24	RIGG03107	1	10	3	70	Similar to BASO_HUM
25	RIGG05763	1	12	3	65	Weakly similar to Q8C
26	RIGG19675	1	13	3	70	P2Y purinoceptor 3 (P2
27	RIGG12960	1	17	3	70	Filamin (Fragment). [S
28	RIGG20027	1	21	3	70	c(\B-Actin cntrl\", \"Gal
29	RIGG17066	1	1	4	70	ENSGALT00000020887
30	RIGG11003	1	5	4	70	ENSGALT00000003569
31	RIGG05769	1	11	4	70	Genome Hit Contig4.54
32	RIGG11802	1	13	4	74	DNA topoisomerase I [
33	RIGG12154	1	19	4	70	ENSGALT00000006808
34	RIGG09194	1	21	4	71	ENSGALG00000013121
35	RIGG06544	1	1	5	70	Genome Hit Contig66.3
36	RIGG17818	1	3	5	65	Adenosine receptor 2B (Fragment). [Source:SPTREMBL
37	RIGG16697	1	7	5	70	Brain-derived neurotrophic factor precursor (BDNF). [Source:SWISSPROT
38	RIGG12560	1	11	5	70	ENSGALT00000008009.1
39	RIGG06520	1	12	5	65	Same gene AY164697
40	RIGG02385	1	13	5	70	Genome Hit Contig4.90
41	RIGG20456	1	16	5	65	similar to interleukin enhancer binding factor 3 (LOC430965), partial mRNA
42	RIGG01627	1	18	5	70	Genome Hit Contig1.315
43	RIGG19275	1	21	5	70	ENSGALT00000026918.1
44	RIGG08747	1	1	6	72	ENSGALG00000009602.1-2
45	RIGG09123	1	10	6	70	ENSGALG00000012566.1
46	RIGG00475	1	12	6	70	Genome Hit Contig93.5
47	RIGG00146	1	13	6	70	Gallus gallus partial mRNA for hypothetical protein, clone 8b3
48	RIGG08777	1	15	6	70	ENSGALG00000009881.1
49	RIGG06121	1	16	6	70	Similar to Q9QWI9 (Q9QWI9) Sodium-myoinositol cotransporter (Fragment)
50	RIGG15515	1	17	6	71	ENSGALT00000016460.1
51	RIGG17072	1	5	7	72	ENSGALT00000020910.1
52	RIGG15145	1	12	7	70	ENSGALT00000015500.1
53	RIGG11784	1	16	7	65	ENSGALT00000005784.1
54	RIGG15139	1	19	7	70	ENSGALT00000015485.1
55	RIGG00523	1	20	7	70	Genome Hit Contig294.22

UserForm1

Identification of the significant genes

Select the variables for the idenfication of the significant genes

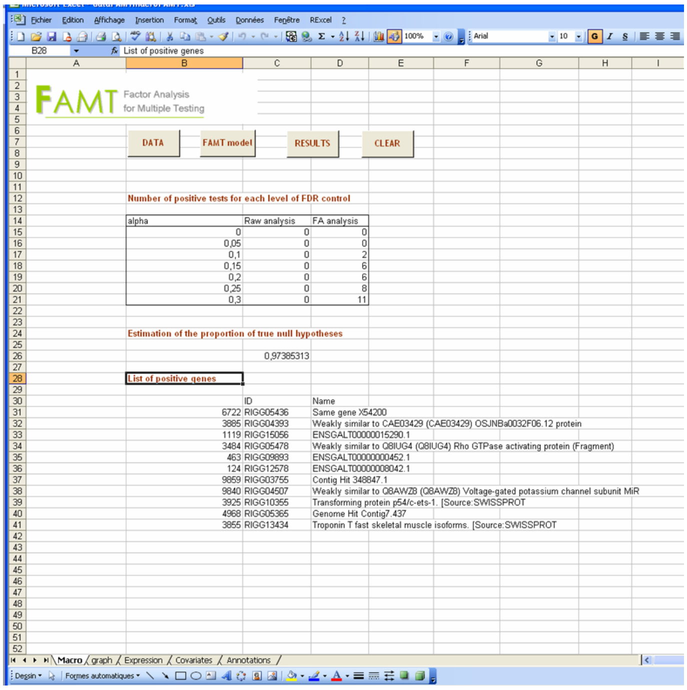
ID (selected)
Block
Column
Row
Length
Name (selected)

OK Cancel

Figure 12: Choice of the identifiers of the significance genes

4.2 Estimation of the proportion of true null hypotheses

The function estimates the proportion of true null hypotheses (pi0). The histogram of the p-values with the estimate of pi0 null line is plotted in the ”graph” sheet. An additional graph is displayed



Factor Analysis for Multiple Testing

Number of positive tests for each level of FDR control

alpha	Raw analysis	FA analysis
0	0	0
0,05	0	0
0,1	0	2
0,15	0	6
0,2	0	6
0,25	0	8
0,3	0	11

Estimation of the proportion of true null hypotheses

0,97385313

List of positive genes

	ID	Name
6722	RIGG05436	Same gene X54200
3885	RIGG04393	Weakly similar to CAE03429 (CAE03429) OSJNBa0032F06.12 protein
1119	RIGG15056	ENSGALT00000015290.1
3484	RIGG05478	Weakly similar to Q8IUG4 (Q8IUG4) Rho GTPase activating protein (Fragment)
463	RIGG09893	ENSGALT00000000452.1
124	RIGG12578	ENSGALT00000008042.1
9859	RIGG03755	Contig Hit 348847.1
9840	RIGG04507	Weakly similar to Q8AWZ8 (Q8AWZ8) Voltage-gated potassium channel subunit MiR
3925	RIGG10355	Transforming protein p54/c-ets-1. [Source:SWISSPROT
4968	RIGG05365	Genome Hit Contig7.437
3855	RIGG13434	Troponin T fast skeletal muscle isoforms. [Source:SWISSPROT

Figure 13: Display of the results of the Statistics of the model

showing the spline curve used to estimate pi0 (see Figure ??).

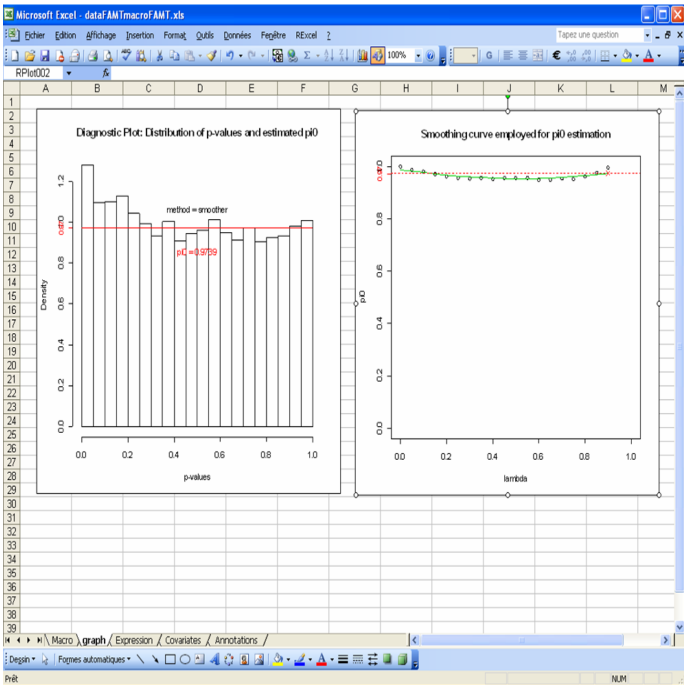


Figure 14: Display of the results of the pi0

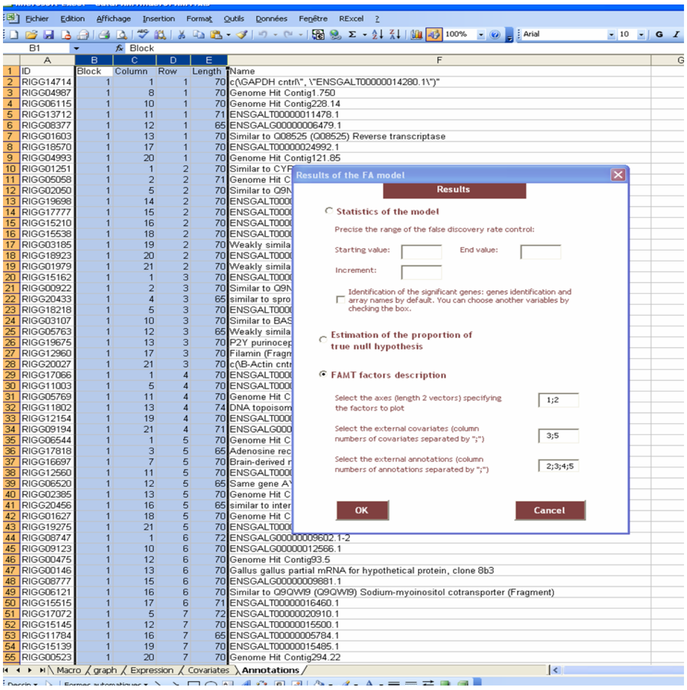
The algorithm used to estimate the proportion of true null p-values is the ”smoother” method (this method uses the smoothing spline approach proposed by Storey and Tibshirani (2003)).

4.3 FAMT factors description

This function provides diagnostic plots to interpret and describe the factors using external information either on genes or arrays. To use this option, the FAMT data must contain ”Annotations” dataset. You have to fill three items:

- the axes: a length 2 vector specifying the factors to plot
- the covariates
- the factors of annotations.

The default value of the axes is the factors 1 and 2.
The function takes all covariates except those used in the model and the array name.
The function takes all variables of annotations of factor type.
In our illustrative example (see Figure **??**), the axes are the two first factors, the external covariates are the column 3 (Pds9s: the body weight) and the column 5 (Lot: the hatch), the external annotations are the columns 2 (Block) 3 (Column) 4 (Row) which correspond to the location on the microarray and the column 5(Length: oligonucleotide size).



	A	B	C	D	E	F
1	ID	Block	Column	Row	Length	Name
2	RIGG14714	1	1	1	70	c(\GAPDH cntrl\", \"ENSGALT00000014280.1\")"
3	RIGG04987	1	8	1	70	Genome Hit Contig1.750
4	RIGG06115	1	10	1	70	Genome Hit Contig228.14
5	RIGG13712	1	11	1	71	ENSGALT00000011478.1
6	RIGG08377	1	12	1	65	ENSGALG00000006479.1
7	RIGG01603	1	13	1	70	Similar to Q08525 (Q08525) Reverse transcriptase
8	RIGG18570	1	17	1	70	ENSGALT00000024992.1
9	RIGG04993	1	20	1	70	Genome Hit Contig121.85
10	RIGG01251	1	1	2	70	Similar to CYF
11	RIGG05058	1	2	2	71	Genome Hit C
12	RIGG02050	1	5	2	70	Similar to Q9N
13	RIGG19698	1	14	2	70	ENSGALT000
14	RIGG17777	1	15	2	70	ENSGALT000
15	RIGG15210	1	16	2	70	ENSGALT000
16	RIGG15538	1	18	2	70	ENSGALT000
17	RIGG03185	1	19	2	70	Weakly simila
18	RIGG18923	1	20	2	70	ENSGALT000
19	RIGG01979	1	21	2	70	Weakly simila
20	RIGG15162	1	1	3	70	ENSGALT000
21	RIGG00922	1	2	3	70	Similar to Q9N
22	RIGG20433	1	4	3	65	similar to spro
23	RIGG18218	1	5	3	70	ENSGALT000
24	RIGG03107	1	10	3	70	Similar to BAS
25	RIGG05763	1	12	3	65	Weakly simila
26	RIGG19675	1	13	3	70	P2Y purinocep
27	RIGG12960	1	17	3	70	Filamin (Fragm
28	RIGG20027	1	21	3	70	c(\B-Actin cntr
29	RIGG17066	1	1	4	70	ENSGALT000
30	RIGG11003	1	5	4	70	ENSGALT000
31	RIGG05769	1	11	4	70	Genome Hit C
32	RIGG11802	1	13	4	74	DNA topoisom
33	RIGG12154	1	19	4	70	ENSGALT000
34	RIGG09194	1	21	4	71	ENSGALG000
35	RIGG06544	1	1	5	70	Genome Hit C
36	RIGG17818	1	3	5	65	Adenosine rec
37	RIGG16697	1	7	5	70	Brain-derived r
38	RIGG12560	1	11	5	70	ENSGALT000
39	RIGG06520	1	12	5	65	Same gene AY
40	RIGG02385	1	13	5	70	Genome Hit C
41	RIGG20456	1	16	5	65	similar to inter
42	RIGG01627	1	18	5	70	Genome Hit C
43	RIGG19275	1	21	5	70	ENSGALT000
44	RIGG08747	1	1	6	72	ENSGALG00000009602.1-2
45	RIGG09123	1	10	6	70	ENSGALG00000012566.1
46	RIGG00475	1	12	6	70	Genome Hit Contig93.5
47	RIGG00146	1	13	6	70	Gallus gallus partial mRNA for hypothetical protein, clone 8b3
48	RIGG08777	1	15	6	70	ENSGALG00000009881.1
49	RIGG06121	1	16	6	70	Similar to Q9QWI9 (Q9QWI9) Sodium-myoinositol cotransporter (Fragment)
50	RIGG15515	1	17	6	71	ENSGALT00000016460.1
51	RIGG17072	1	5	7	72	ENSGALT00000020910.1
52	RIGG15145	1	12	7	70	ENSGALT00000015500.1
53	RIGG11784	1	16	7	65	ENSGALT00000005784.1
54	RIGG15139	1	19	7	70	ENSGALT00000015485.1
55	RIGG00523	1	20	7	70	Genome Hit Contig294.22

Results of the FA model

Results

○ **Statistics of the model**

Precise the range of the false discovery rate control:

Starting value: ☐ End value: ☐

Increment: ☐

☐ Identification of the significant genes: genes identification and array names by default. You can choose another variables by checking the box.

○ **Estimation of the proportion of true null hypothesis**

● **FAMT factors description**

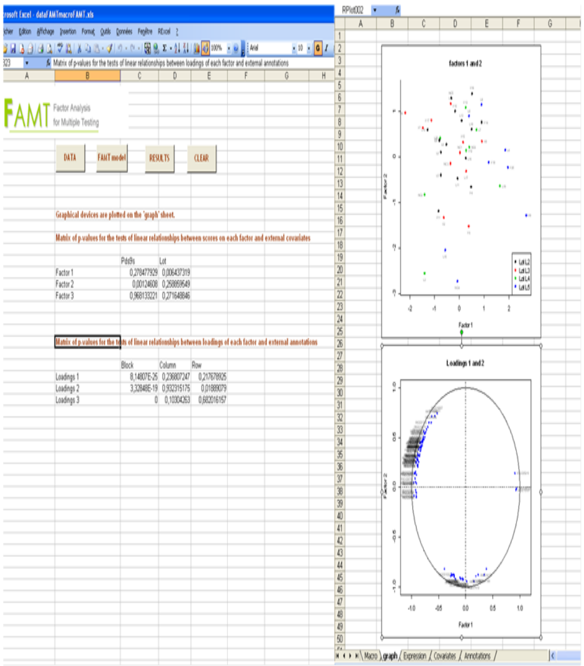
Select the axes (length 2 vectors) specifying the factors to plot: 1;2

Select the external covariates (column numbers of covariates separated by ";"): 3;5

Select the external annotations (column numbers of annotations separated by ";"): 2;3;4;5

Figure 15: FAMT factors description

Graphical devices are plotted in the ”graph” sheet if the FAMT model has more than one factor (see Figure **??**).
The tables of p-values are displayed in the ”Macro” sheet: p-values of the test whereas the score of each factor are affected by the selected covariates, and p-values of the test whereas the score of each factor are affected by the selected annotations.



Graphical devices are plotted on the 'graph' sheet.

Matrix of p-values for the tests of linear relationships between scores on each factor and external covariates

	Pds9s	Lot
Factor 1	0,278477929	0,006437319
Factor 2	0,00124608	0,258859549
Factor 3	0,968133221	0,271648846

Matrix of p-values for the tests of linear relationships between loadings of each factor and external annotations

	Block	Column	Row
Loadings 1	8,14807E-25	0,236807247	0,217678925
Loadings 2	3,32848E-19	0,932315175	0,01889079
Loadings 3	0	0,10304263	0,682016157

Figure 16: Display of the results of the FAMT factors description