

Matrix with all regression coefficients

	cultivation parameters					
	a	b	c	a&b	a&c	...
g1	0	1.4	2	0	0	
g2	0	-2.7	0	0	0	
g3	0.6	0	0	0	0	
g4	0	0	0	0	-0.6	
g5	-1.7	0	0	0	0	
g6	2.1	-0.5	0	0	0	
g7	0.9	0	0	0	0	
g8	0	-2.2	0	0.6	0	
g9	-3.2	0	0	0	0	
g10	0	0	0	2.1	0	
g11	0	0	0	0.9	0	
...						

1

Gene groups derived from regression coefficients

	a	b	...
up	g3 g6 g7 ...	g1 ...	
down	g5 g9 ...	g2 g6 g8 ...	
both	g3 g5 g6 g7 g9 ...	g1 g2 g6 g8 ...	

Gene groups derived from GO, MIPS, KEGG, TF functional categories

funcat1	g1 g10 g11...
funcat2	g3 g6 ...
...	

2

Hypergeometric test

	a	b	...			
funcat1	.9	1	1	1	1	.85
funcat2	.003	1	.054	.12	.67	.05
...						

Matrix with enrichment P-values of overlap between gene groups

Flowchart of Methods - Enrichment analysis

All significant regression weights are stored in a large matrix. This matrix has 6005 rows, which represent all differentially expressed genes for which the regression analysis was performed, and 227 columns, which represent the (combinatorial) cultivation parameters, which were used as predictors in the regression model. An element of this matrix indicates the assigned regression weight of a cultivation parameter to explain the expression pattern of a gene. A zero indicates that the cultivation parameter was not selected as a significant predictor for a gene. Step 1: For each column of this matrix, we group the genes that were upregulated (positive weights), downregulated (negative weights) or either up- or downregulated (non-zero weights) by the cultivation parameter related to that column. Step 2: These groups are analyzed for significant overlap with gene groups derived from functional annotation databases and TF binding information using the hypergeometric test. The enrichment results are visually represented in Additional File 2.

Matrix with all regression coefficients

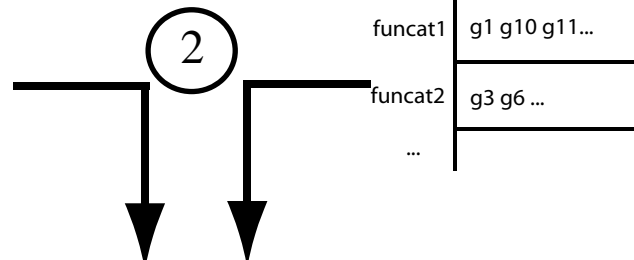
		cultivation parameters					
		a	b	c	a&b	a&c	...
genes	g1	0	1.4	2	0	0	
	g2	0	-2.7	0	0	0	
	g3	0.6	0	0	0	0	
	g4	0	0	0	0	-0.6	
	g5	-1.7	0	0	0	0	
	g6	2.1	-0.5	0	0	0	
	g7	0.9	0	0	0	0	
	g8	0	-2.2	0	0.6	0	
	g9	-3.2	0	0	0	0	
	g10	0	0	0	2.1	0	
	g11	0	0	0	0.9	0	
...							

Gene groups that respond to a combinatorial effect or one or both of the single effects that constitute the comb. effect



Gene groups derived from GO, MIPS, KEGG, TF functional categories

	combinatorial effects	
	a&b	a&c
first single effect	g3 g5 g6 g7 g9	g3 g5 g6 g7 g9 ...
second single effect	g1 g2 g6 g8 ...	g1 ...
both single effects	g6
comb effect	g8 g10 g11 ...	g4 ...



funcat1	g1 g10 g11...
funcat2	g3 g6 ...
...	

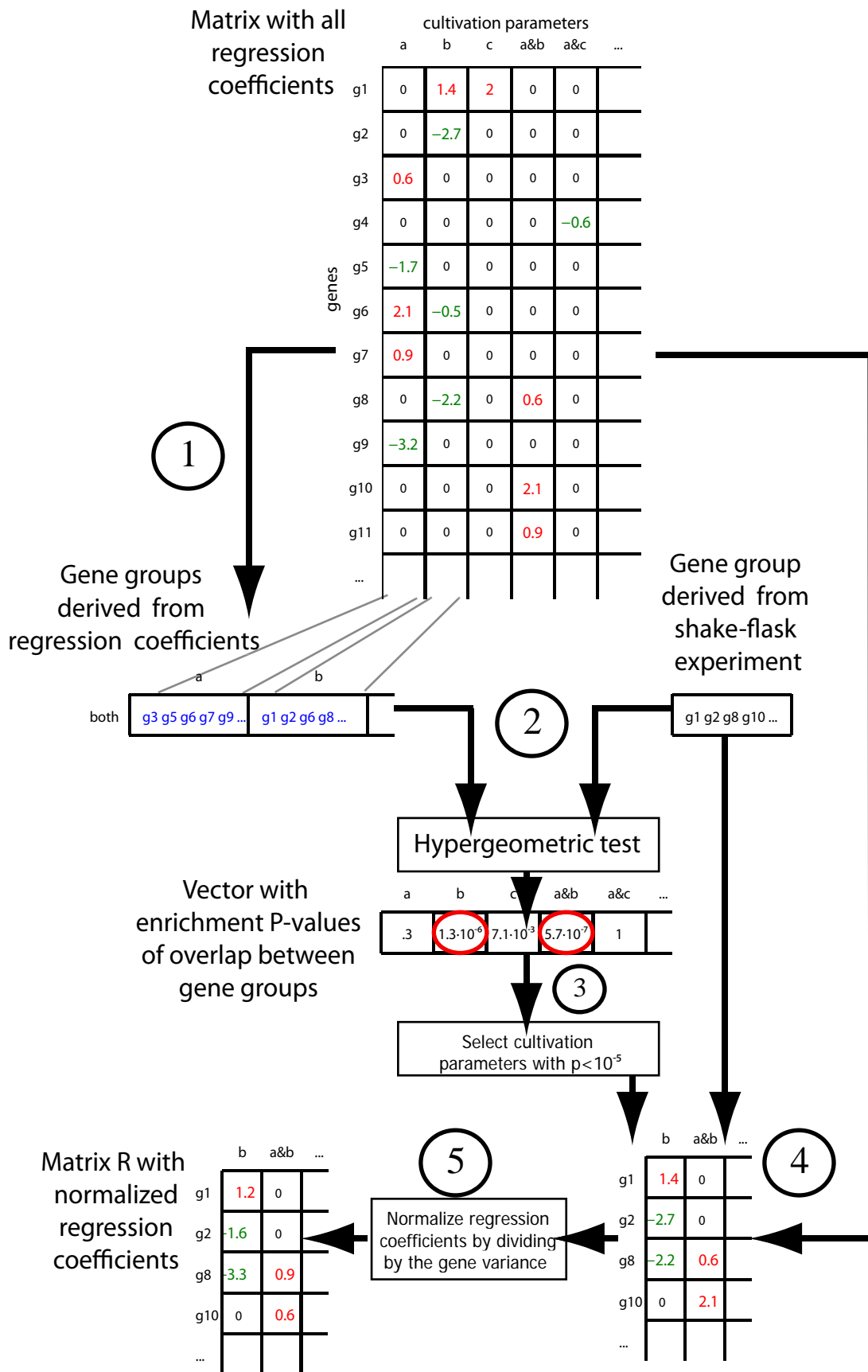
Hypergeometric test

	a&b		a&c		...			
	1	0	1	0	1	0		
funcat1	1	.85	1	.01	1	.2	1	1
funcat2	.054	.05	0.4	1	.054	1	1	1
...								

Matrix with enrichment P-values of overlap between gene groups

Flowchart of Methods - Functional categories specifically influenced by a combinatorial effect

Step 1: Gene groups are derived based on the large matrix with regression weights. In this case, for each combinatorial effect we create four gene groups: 1) We group the genes that respond to the combinatorial effect (non-zero weights). 2) We group the genes that respond to one of the single effects that constitute the combinatorial effect. 3) We group the genes that respond to the other single effect. 4) We group genes that respond to both single effects. Step 2: Again, the hypergeometric test is employed to analyze these gene groups for significant overlap with gene groups derived from functional categories.



Flowchart of Methods - Clustering of genes based on regression coefficients

Step 1: Firstly, genes that respond (non-zero weights) to a particular (combinatorial) cultivation parameter are grouped. (These groups are identical to the blue groups generated in the enrichment analysis flowchart on Page 1 of this document.)

Step 2: These groups are analyzed for significant overlap with the gene group derived from the shake-flask experiment.

Step 3: The significant cultivation parameters are selected. (These are the cultivation parameters to which significantly many of the genes in the shake-flask gene group respond.)

Step 4: A new matrix is derived. This matrix contains the regression weights of the genes of the shake-flask gene group for the significant cultivation parameters. Basically, this matrix is a submatrix of the original regression weight matrix.

Step 5: By normalizing these regression weights, we arrive at matrix **R**, which is used in the subsequent cluster analysis.