REST-384 © :
an improved way of simple and precise qRT-PCR data evaluation

LightCycler
LightCycler
Page 1
Page 1

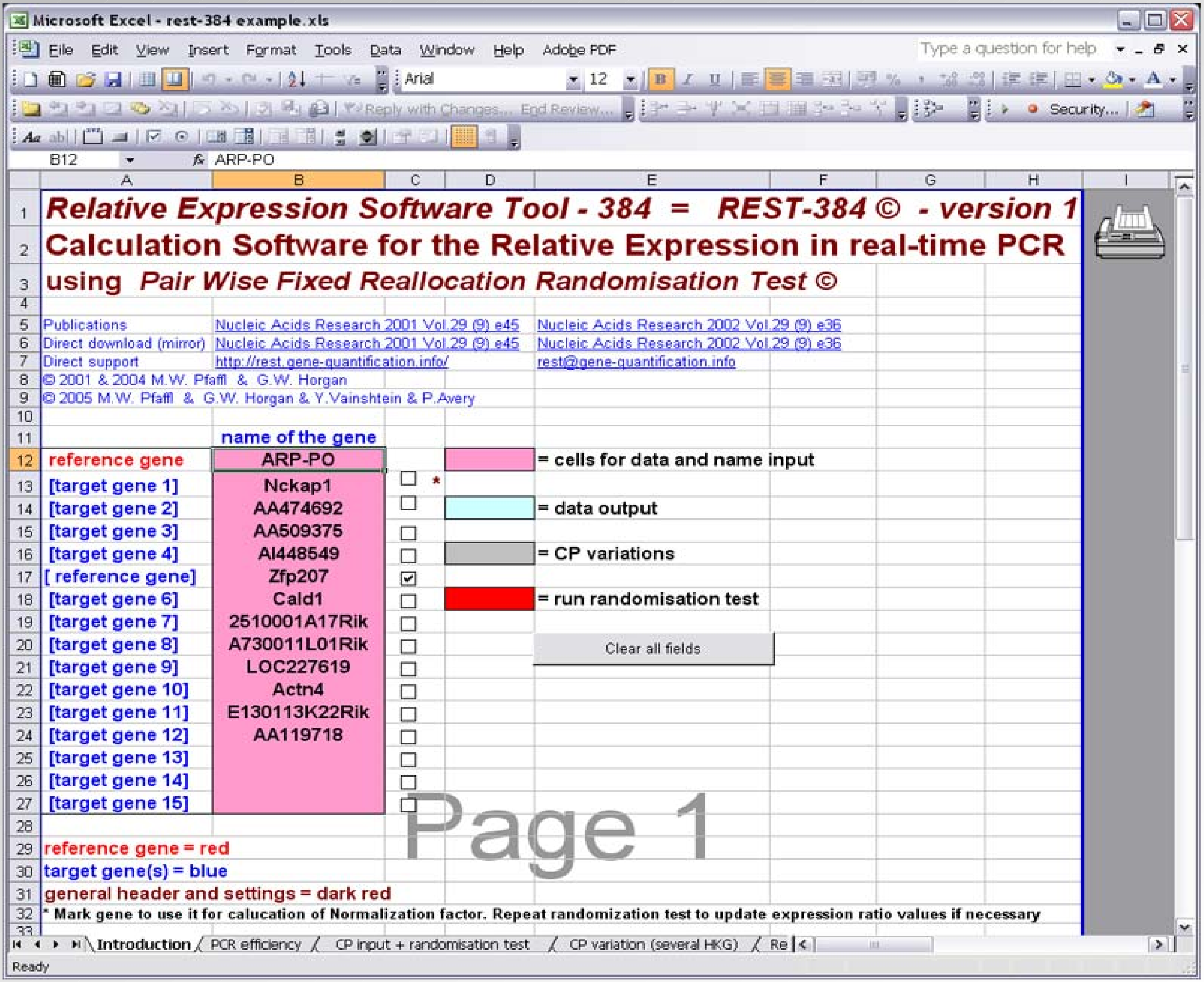
Relative Expression Software Tool

- Define target and control experiment
- Determine genes of interest
- Select one or more reference gene
- Determine the strategy for Efficiency correction.

Relative Expression Software Tool

- REST-384 ©
  - 2 groups of samples
  - up to 20 data points in each sample and control group
  - up to fifteen target genes
  - normalization to several housekeeping genes
  - calculation of standard error for the final ratio
  - Pair Wise Fixed Reallocation Randomisation Test ©

REST-384 ©



Relative Expression Software Tool - 384 = REST-384 © - version 1

Calculation Software for the Relative Expression in real-time PCR

using *Pair Wise Fixed Reallocation Randomisation Test ©*

Publications	Nucleic Acids Research 2001 Vol.29 (9) e45	Nucleic Acids Research 2002 Vol.29 (9) e36
Direct download (mirror)	Nucleic Acids Research 2001 Vol.29 (9) e45	Nucleic Acids Research 2002 Vol.29 (9) e36
Direct support	http://rest.gene-quantification.info/	rest@gene-quantification.info

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	name of the gene	
reference gene	ARP-PO	
[target gene 1]	Nckap1	☐ *
[target gene 2]	AA474692	☐
[target gene 3]	AA509375	☐
[target gene 4]	AI448549	☐
[reference gene]	Zfp207	☑
[target gene 6]	Cald1	☐
[target gene 7]	2510001A17Rik	☐
[target gene 8]	A730011L01Rik	☐
[target gene 9]	LOC227619	☐
[target gene 10]	Actn4	☐
[target gene 11]	E130113K22Rik	☐
[target gene 12]	AA119718	☐
[target gene 13]		☐
[target gene 14]		☐
[target gene 15]		☐

🟪 = cells for data and name input

🟦 = data output

⬜ = CP variations

🟥 = run randomisation test

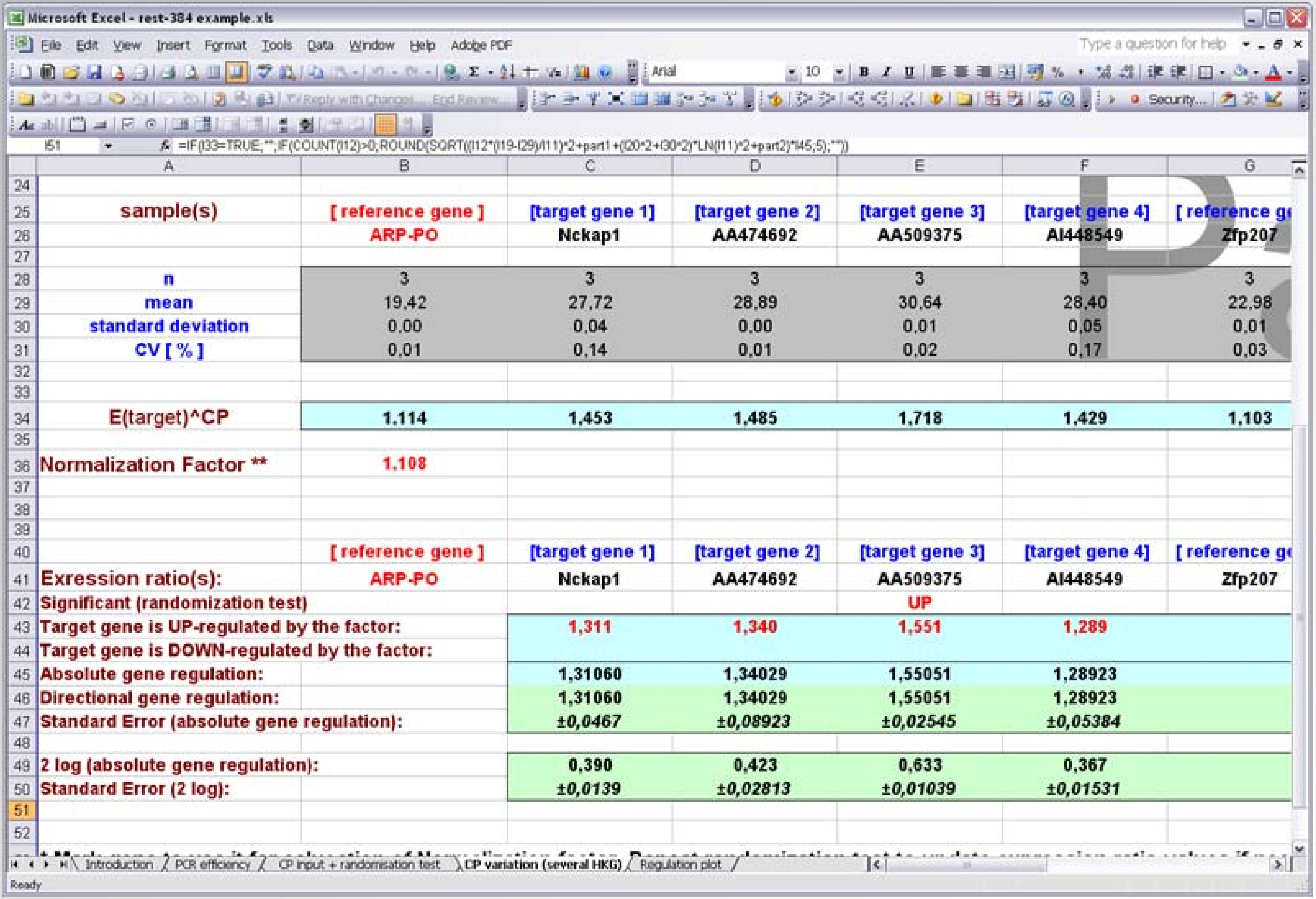
reference gene = red

target gene(s) = blue

general header and settings = dark red

* Mark gene to use it for calucation of Normalization factor. Repeat randomization test to update expression ratio values if necessary

REST-384 ©



Microsoft Excel - rest-384 example.xls

I51 =IF(I33=TRUE;"";IF(COUNT(I12)>0;ROUND(SQRT((I12*(I19-I29)/I11)^2+part1+(I20^2+I30^2)*LN(I11)^2+part2)*I45;5);""))

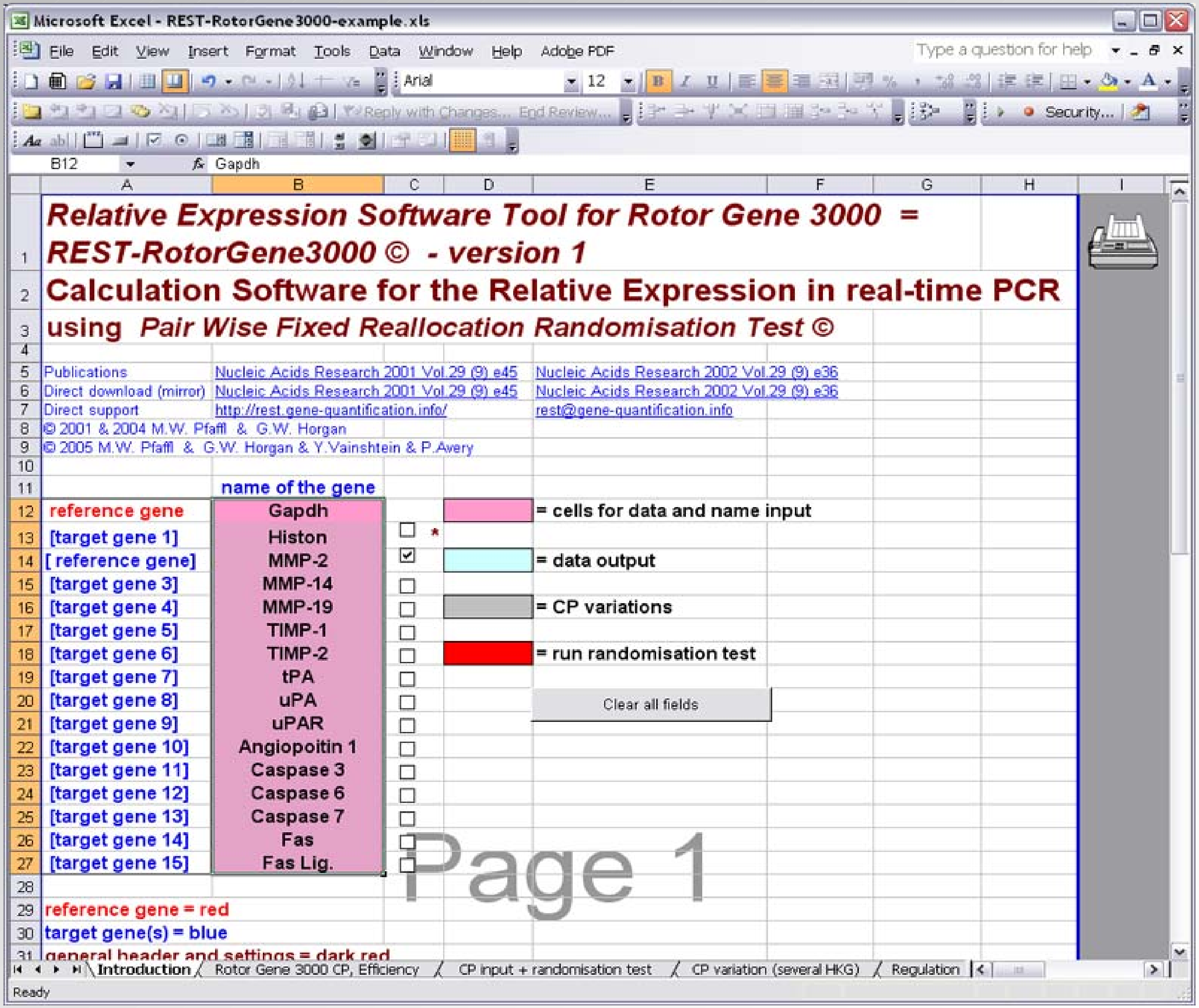
	A	B	C	D	E	F	G
24							
25	sample(s)	[reference gene]	[target gene 1]	[target gene 2]	[target gene 3]	[target gene 4]	[reference ge
26		ARP-PO	Nckap1	AA474692	AA509375	AI448549	Zfp207
27							
28	n	3	3	3	3	3	3
29	mean	19,42	27,72	28,89	30,64	28,40	22,98
30	standard deviation	0,00	0,04	0,00	0,01	0,05	0,01
31	CV [%]	0,01	0,14	0,01	0,02	0,17	0,03
32							
33							
34	E(target)^CP	1,114	1,453	1,485	1,718	1,429	1,103
35							
36	Normalization Factor **	1,108					
37							
38							
39							
40		[reference gene]	[target gene 1]	[target gene 2]	[target gene 3]	[target gene 4]	[reference ge
41	Expression ratio(s):	ARP-PO	Nckap1	AA474692	AA509375	AI448549	Zfp207
42	Significant (randomization test)				UP		
43	Target gene is UP-regulated by the factor:		1,311	1,340	1,551	1,289	
44	Target gene is DOWN-regulated by the factor:						
45	Absolute gene regulation:		1,31060	1,34029	1,55051	1,28923	
46	Directional gene regulation:		1,31060	1,34029	1,55051	1,28923	
47	Standard Error (absolute gene regulation):		±0,0467	±0,08923	±0,02545	±0,05384	
48							
49	2 log (absolute gene regulation):		0,390	0,423	0,633	0,367	
50	Standard Error (2 log):		±0,0139	±0,02813	±0,01039	±0,01531	
51							
52							

Relative Expression Software Tool

- REST-384 © for RotorGene 3000
  - 2 groups of samples
  - up to 20 data points in each sample and control group
  - up to fifteen target genes
  - normalization to several housekeeping genes
  - calculation of standard error for the final ratio
  - Pair Wise Fixed Reallocation Randomisation Test ©

  - Direct import of RotorGene Efficiencies and Ct values

REST-384 © for RotorGene 3000



Relative Expression Software Tool for Rotor Gene 3000 = REST-RotorGene3000 © - version 1

Calculation Software for the Relative Expression in real-time PCR

using *Pair Wise Fixed Reallocation Randomisation Test ©*

Publications	Nucleic Acids Research 2001 Vol.29 (9) e45	Nucleic Acids Research 2002 Vol.29 (9) e36
Direct download (mirror)	Nucleic Acids Research 2001 Vol.29 (9) e45	Nucleic Acids Research 2002 Vol.29 (9) e36
Direct support	http://rest.gene-quantification.info/	rest@gene-quantification.info

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	name of the gene	
reference gene	**Gapdh**	
[target gene 1]	**Histon**	☐ *
[reference gene]	**MMP-2**	☑
[target gene 3]	**MMP-14**	☐
[target gene 4]	**MMP-19**	☐
[target gene 5]	**TIMP-1**	☐
[target gene 6]	**TIMP-2**	☐
[target gene 7]	**tPA**	☐
[target gene 8]	**uPA**	☐
[target gene 9]	**uPAR**	☐
[target gene 10]	**Angiopoitin 1**	☐
[target gene 11]	**Caspase 3**	☐
[target gene 12]	**Caspase 6**	☐
[target gene 13]	**Caspase 7**	☐
[target gene 14]	**Fas**	☐
[target gene 15]	**Fas Lig.**	☐

🟪 **= cells for data and name input**

🟦 **= data output**

⬜ **= CP variations**

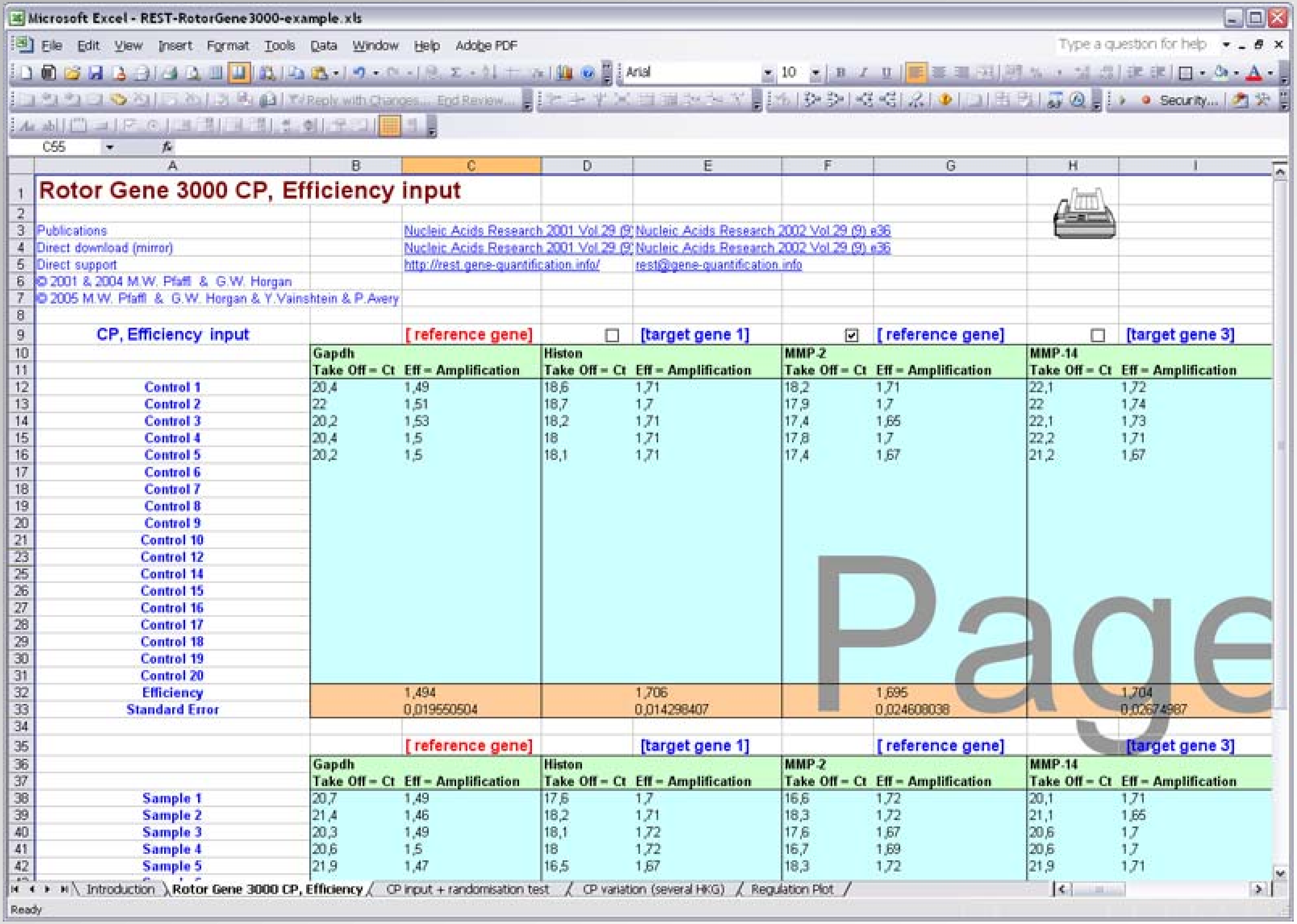
🟥 **= run randomisation test**

reference gene = red

target gene(s) = blue

general header and settings = dark red

REST-384 © for RotorGene 3000



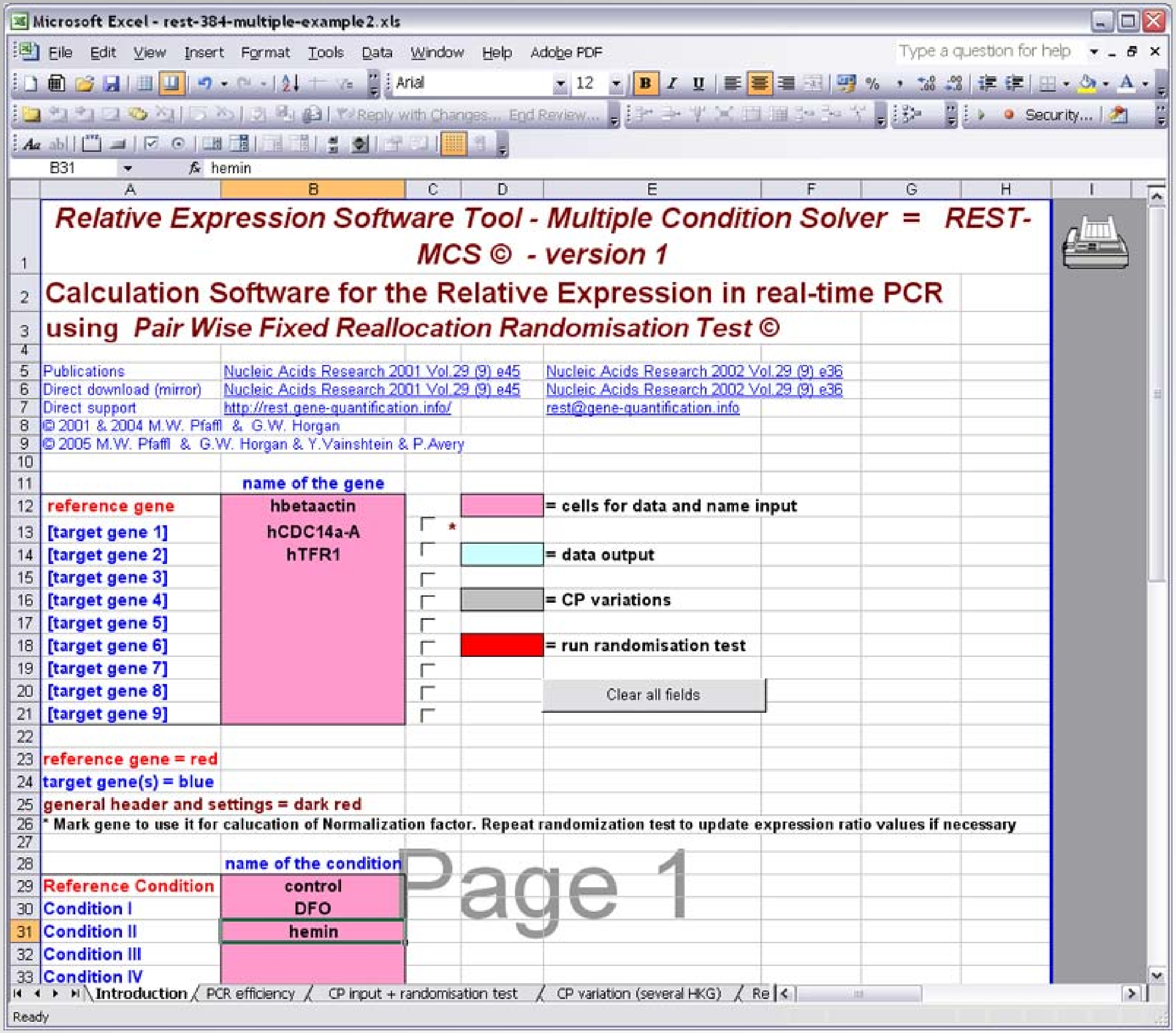
	A	B	C	D	E	F	G	H	I
1	Rotor Gene 3000 CP, Efficiency input								
2									
3	Publications		Nucleic Acids Research 2001 Vol.29 (9	Nucleic Acids Research 2002 Vol.29 (9) e36					
4	Direct download (mirror)		Nucleic Acids Research 2001 Vol.29 (9	Nucleic Acids Research 2002 Vol.29 (9) e36					
5	Direct support		http://rest.gene-quantification.info/	rest@gene-quantification.info					
6	© 2001 & 2004 M.W. Pfaffl & G.W. Horgan								
7	© 2005 M.W. Pfaffl & G.W. Horgan & Y.Vainshtein & P.Avery								
8									
9	CP, Efficiency input		[reference gene]	☐	[target gene 1]	☑	[reference gene]	☐	[target gene 3]
10		Gapdh		Histon		MMP-2		MMP-14	
11		Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification
12	Control 1	20,4	1,49	18,6	1,71	18,2	1,71	22,1	1,72
13	Control 2	22	1,51	18,7	1,7	17,9	1,7	22	1,74
14	Control 3	20,2	1,53	18,2	1,71	17,4	1,65	22,1	1,73
15	Control 4	20,4	1,5	18	1,71	17,8	1,7	22,2	1,71
16	Control 5	20,2	1,5	18,1	1,71	17,4	1,67	21,2	1,67
17	Control 6								
18	Control 7								
19	Control 8								
20	Control 9								
21	Control 10								
23	Control 12								
25	Control 14								
26	Control 15								
27	Control 16								
28	Control 17								
29	Control 18								
30	Control 19								
31	Control 20								
32	Efficiency		1,494		1,706		1,695		1,704
33	Standard Error		0,019550504		0,014298407		0,024608038		0,02674987
34									
35			[reference gene]		[target gene 1]		[reference gene]		[target gene 3]
36		Gapdh		Histon		MMP-2		MMP-14	
37		Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification	Take Off = Ct	Eff = Amplification
38	Sample 1	20,7	1,49	17,6	1,7	16,6	1,72	20,1	1,71
39	Sample 2	21,4	1,46	18,2	1,71	18,3	1,72	21,1	1,65
40	Sample 3	20,3	1,49	18,1	1,72	17,6	1,67	20,6	1,7
41	Sample 4	20,6	1,5	18	1,72	16,7	1,69	20,6	1,7
42	Sample 5	21,9	1,47	16,5	1,67	18,3	1,72	21,9	1,71

Relative Expression Software Tool

- REST-384 © for multiple conditions
  - – up to fifteen target genes
  - – normalization to several housekeeping genes
  - – calculation of standard error for the final ratio

  - – 7 groups of samples (1 reference condition and 6 different test conditions)
  - – up to 10 data points in each sample and control group
  - – Pair Wise Fixed Reallocation Randomisation Test © for each combination of reference and test condition

REST-384 © for multiple conditions



Relative Expression Software Tool - Multiple Condition Solver = REST-MCS © - version 1

Calculation Software for the Relative Expression in real-time PCR

using *Pair Wise Fixed Reallocation Randomisation Test ©*

Publications: Nucleic Acids Research 2001 Vol.29 (9) e45 | Nucleic Acids Research 2002 Vol.29 (9) e36

Direct download (mirror): Nucleic Acids Research 2001 Vol.29 (9) e45 | Nucleic Acids Research 2002 Vol.29 (9) e36

Direct support: http://rest.gene-quantification.info/ | rest@gene-quantification.info

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	name of the gene	
reference gene	hbetaactin	
[target gene 1]	hCDC14a-A	☐ *
[target gene 2]	hTFR1	☐
[target gene 3]		☐
[target gene 4]		☐
[target gene 5]		☐
[target gene 6]		☐
[target gene 7]		☐
[target gene 8]		☐
[target gene 9]		☐

🟪 = cells for data and name input

🟦 = data output

⬜ = CP variations

🟥 = run randomisation test

Clear all fields

reference gene = red

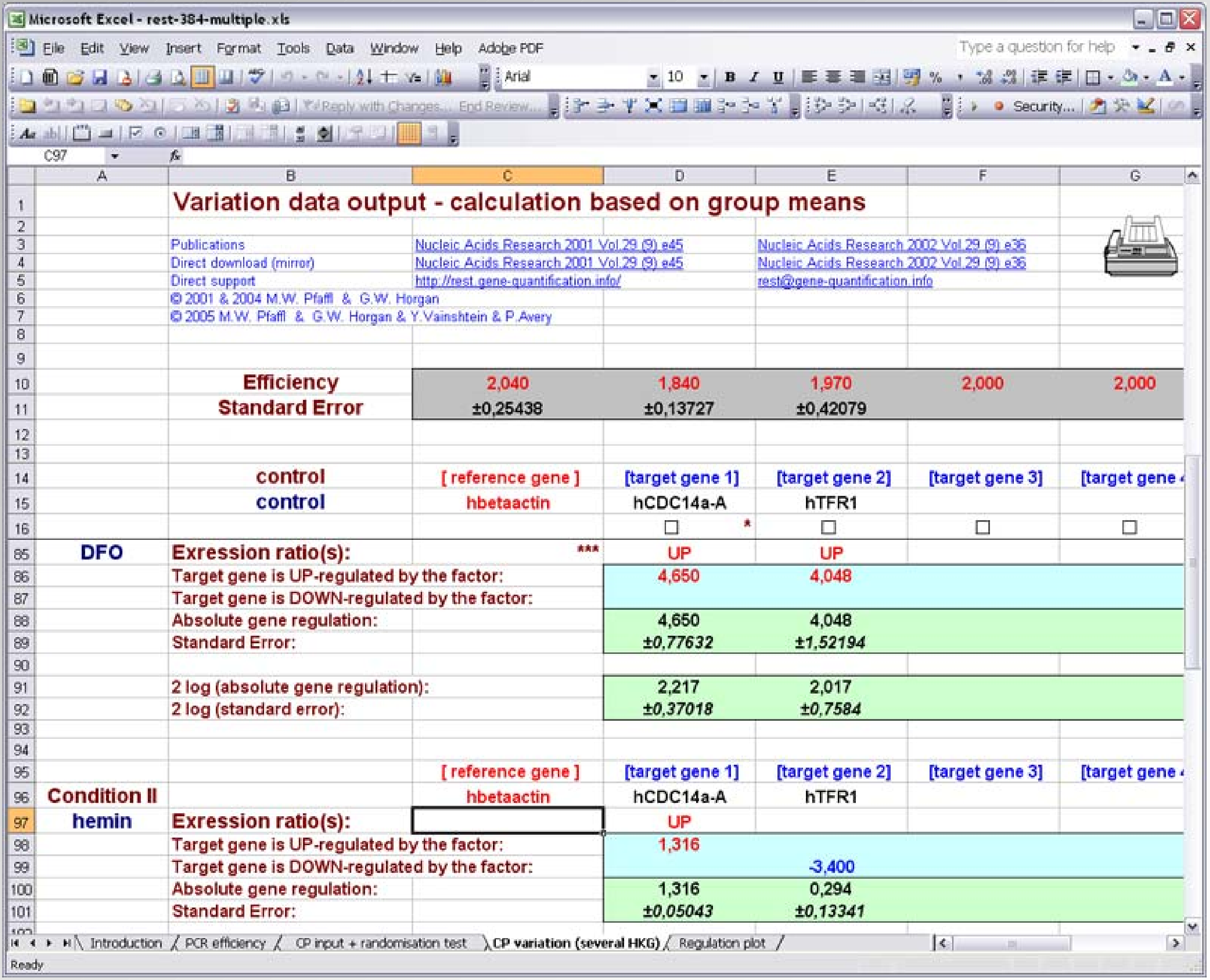
target gene(s) = blue

general header and settings = dark red

* Mark gene to use it for calucation of Normalization factor. Repeat randomization test to update expression ratio values if necessary

	name of the condition
Reference Condition	control
Condition I	DFO
Condition II	hemin
Condition III	
Condition IV	

REST-384 © for multiple conditions



	A	B	C	D	E	F	G
1		Variation data output - calculation based on group means					
2							
3		Publications	Nucleic Acids Research 2001 Vol.29 (9) e45		Nucleic Acids Research 2002 Vol.29 (9) e36		
4		Direct download (mirror)	Nucleic Acids Research 2001 Vol.29 (9) e45		Nucleic Acids Research 2002 Vol.29 (9) e36		
5		Direct support	http://rest.gene-quantification.info/		rest@gene-quantification.info		
6		© 2001 & 2004 M.W. Pfaffl & G.W. Horgan					
7		© 2005 M.W. Pfaffl & G.W. Horgan & Y.Vainshtein & P.Avery					
8							
9							
10		**Efficiency**	2,040	1,840	1,970	2,000	2,000
11		**Standard Error**	±0,25438	±0,13727	±0,42079		
12							
13							
14		**control**	[reference gene]	[target gene 1]	[target gene 2]	[target gene 3]	[target gene
15		**control**	hbetaactin	hCDC14a-A	hTFR1		
16				☐ *	☐	☐	☐
85	**DFO**	**Exression ratio(s):**	***	UP	UP		
86		Target gene is UP-regulated by the factor:		4,650	4,048		
87		Target gene is DOWN-regulated by the factor:					
88		Absolute gene regulation:		4,650	4,048		
89		Standard Error:		*±0,77632*	*±1,52194*		
90							
91		2 log (absolute gene regulation):		2,217	2,017		
92		2 log (standard error):		*±0,37018*	*±0,7584*		
93							
94							
95			[reference gene]	[target gene 1]	[target gene 2]	[target gene 3]	[target gene
96	**Condition II**		hbetaactin	hCDC14a-A	hTFR1		
97	**hemin**	**Exression ratio(s):**		UP			
98		Target gene is UP-regulated by the factor:		1,316			
99		Target gene is DOWN-regulated by the factor:			-3,400		
100		Absolute gene regulation:		1,316	0,294		
101		Standard Error:		*±0,05043*	*±0,13341*		

Thanks!

EMBL, Heidelberg

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Martina Muckenthaler

Mayka Sanchez

Vladimir Benes

REST Team

Michael Pfaffl

Graham Horgan

Peter Avery

REST error calculating using Taylor’s series

by Peter J. Avery, University of Newcastle, Mathematics and Statistics

calculated expression ratio = φ

calculated variation is dependent of 6 different SEs *[2 x SE(Eff.); 4 x SE(mean CP)]*

SE (Ratio by REST) = around 20-30%

$$
\begin{aligned}
S.E.(\hat{\varphi}) = \varphi\{ & \frac{(CP_{targ,cont} - CP_{targ,test})^2}{\hat{E}^2_{targ}} SE^2(\hat{E}_{targ}) \\
& + \frac{(CP_{ref,cont} - CP_{ref,test})^2}{\hat{E}^2_{ref}} SE^2(\hat{E}_{ref}) \\
& + (\log_e E_{targ})^2 (SE^2(CP_{targ,cont}) + SE^2(CP_{targ,test})) \\
& + (\log_e E_{ref})^2 (SE^2(CP_{ref,cont}) + SE^2(CP_{ref,test}))\}^{0.5}
\end{aligned}
$$