

Human Whole Genome OneArray™

Gene Expression Profiling Service Report

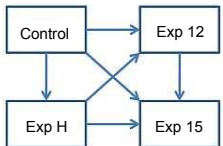
Customer Information

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Service Code	Sxx0xxx11x1
Date	200x/0x/xx

Service Laboratory Department
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Dear Customer

Following table is your final service content.

Service Content			
Item	Requisition	Alteration	Experimental Design 
RNA Sample (RNA QC)	4	None	
Target Preparation (aRNA)	4	None	
Single/Dual experiment	Single	None	
Array Type	Human	None	
Array Amount	12	None	

Phalanx OneArray™ service center finished your service and provided the service report containing two parts. One is the hard copy, and the other one is the CD.

	Content	Description	Note
Hard Copy	RNA Sample Information and QC Result		
	AminoAllyl aRNA preparation and labeling QC Result		
	Hybridization Result: Image and Signal segmented file		
	Data Reproducibility: R-Table		
	Box and Whiskers Plot		
	Scatter Plot		
	Description of the column in result file		
CD	Sxx0xxx11x1_Raw_Data.xls	Raw data of microarray experiment result	Data Folder
	Sxx0xxx11x1_Normalized_Data.xls	Normalized data of microarray experiment result	Data Folder
	HOA_V4.3.gal	Gal file used for GenePix pro software	
	HOA_ArrayLayout.xls	Array layout file	
	Annotation Release 2.0, 2009-04-30	Annotation based on: 1. Ensembl homo_sapiens_core_53_360, top-level sequences and core annotations; 2. NCBI RefSeq release 34	Annotation Folder
	Tiff file	Image files of microarray experiment	Image Folder
	GPR file	Signal segmented files of microarray experiment	Gpr Folder
	PDF file	Agilent Bioanalyzer result	RNAQC Folder
	Sxx0xxx11x1_Service Report	Service Report	

If you have any questions, please do not hesitate to contact us.

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RNA Sample Information and QC Result

Total RNA Sample No.	4					
Arrival Date	200x/0x/xx					
RNA Source	<input checked="" type="checkbox"/> Cell line <input type="checkbox"/> Tissue <input type="checkbox"/> Blood Other:					
RNA Status	<input checked="" type="checkbox"/> RNase-Free dH ₂ O / DEPC-dH ₂ O <input type="checkbox"/> EtOH / Isopropanol Precipitation Other:					
RNA Arrival Condition	<input checked="" type="checkbox"/> Frozen <input type="checkbox"/> De-frozen Other:					
Item	Sample Name	OD260/280 ≥ 1.8	Amount (µg) ≥ 6 µg	RIN >7	QC Result Pass or Fail	Note
1	Control	2.11	14.8	9.7	Pass	
2	Exp H	2.13	13.7	9.5	Pass	
3	Exp 12	2.09	16.5	9.5	Pass	
4	Exp 15	2.08	17.4	9.4	Pass	
Note For Agilent Bioanalyzer result, please see the electronic file (RNAQC Folder)						
Recommended processing for failed samples:						

- Optical density was measured by NanoDrop ND-1000. The ratio of absorbance at 260 nm and 280 nm provides an estimate of RNA purity. Ratios between 1.8 and 2.2 indicate a pure sample. The normal required amount of total RNA per sample is 12 µg for aRNA preparation method. Due to the different starting RNA amount, PhalanxBiotech accept at least 6 µg.
- RIN score was given by Agilent RNA 6000 Nano Assay. RNA Integrity Number (RIN) algorithm assigns a 1 to 10 RIN score, where level 10 RNA is completely intact. For the sake of good microarray result, RIN score should be above 7.
- Phalanx also proceed the agarose electrophoresis to check serious DNA contamination. Under this special circumstance, Phalanx will write down in the "Note" and recommend the customer to do DNase treatment.

AminoAllyl aRNA and labeling QC Result

Item	Sample Name	OD260/280 > 1.8	Labeling Efficiency Cy5 >10	Quality Pass or Fail	Note
1	Control	1.94	27.0	Pass	
2	Exp H	1.93	30.3	Pass	
3	Exp 12	1.94	29.4	Pass	
4	Exp 15	1.93	27.2	Pass	
Note					
1. Labeling Efficiency: # dye molecules/per 1000 nucleotides					
Recommended processing for failed samples:					

- 1 The sample preparation reagent used for this service is Ambion MessageAmp aRNA kit (Cat. No. 1753) or Ambion amino allyl cDNA kit (Cat. No. 1705).
- 2 Optical density was measured by NanoDrop ND-1000. The ratio of absorbance at 260 nm and 280 nm provides a estimate of RNA purity. Ratios between 1.8 and 2.2 indicate a pure sample.
- 3 The reactive amino group of 5-(3-aminoallyl)-UTP/5-(3-aminoallyl)-dUTP was used to conjugate the purified aRNA/cDNA with the NHS-CyDye. Labeling efficiency can be calculated by the concentration of CyDye and aRNA/cDNA measured by NanoDrop ND-1000. For the sake of good microarray result, labeling efficiency should be above 10.

Example

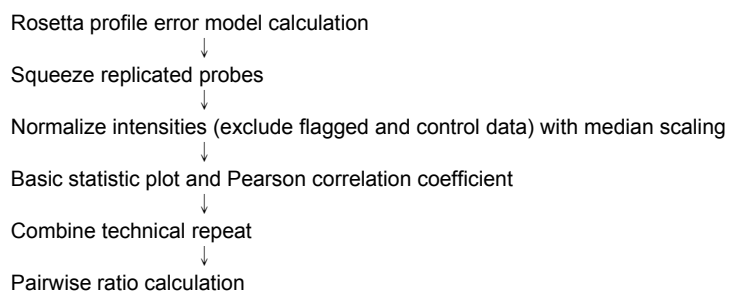
Hybridization Result: Image (Tiff file) and Signal segmented file (GPR file)

Basic information of microarray experiment

Hybridization Amount 5 µg Cy5-labeled aRNA
 Hybridization Protocol Phalanx Hybridization Protocol
 Array Version HOA 4.3
 Total array amount 12 slides

Item	Sample Name	Cy5/ Cy3	Tiff File Name	GPR File Name	Label
1	Control	Cy5 Cy3	H01-0x070082xx_0635.tif H01-0x070082xx_0532.tif	H01-0x070082xx.gpr	T_H01
2	Control	Cy5 Cy3	H02-0x070082xx_0635.tif H02-0x070082xx_0532.tif	H02-0x070082xx.gpr	T_H02
3	Control	Cy5 Cy3	H03-0x070082x9_0635.tif H03-0x070082x9_0532.tif	H03-0x070082x9.gpr	T_H03
4	Exp H	Cy5 Cy3	H04-0x07008xx7_0635.tif H04-0x07008xx7_0532.tif	H04-0x07008xx7.gpr	TH_H04
5	Exp H	Cy5 Cy3	H05-0x07008xx8_0635.tif H05-0x07008xx8_0532.tif	H05-0x07008xx8.gpr	TH_H05
6	Exp H	Cy5 Cy3	H06-0x07008xx9_0635.tif H06-0x07008xx9_0532.tif	H06-0x07008xx9.gpr	TH_H06
7	Exp 12	Cy5 Cy3	H07-0x070082x0_0635.tif H07-0x070082x0_0532.tif	H07-0x070082x0.gpr	C12_H07
8	Exp 12	Cy5 Cy3	H08-0x070082x1_0635.tif H08-0x070082x1_0532.tif	H08-0x070082x1.gpr	C12_H08
9	Exp 12	Cy5 Cy3	H09-0x070082x3_0635.tif H09-0x070082x3_0532.tif	H09-0x070082x3.gpr	C12_H09
10	Exp 15	Cy5 Cy3	H10-0x07008x7x_0635.tif H10-0x07008x7x_0532.tif	H10-0x07008x7x.gpr	C15_H10
11	Exp 15	Cy5 Cy3	H11-0x07008xx2_0635.tif H11-0x07008xx2_0532.tif	H11-0x07008xx2.gpr	C15_H11
12	Exp 15	Cy5 Cy3	H12-0x07008x7x_0635.tif H12-0x07008x7x_0532.tif	H12-0x07008x7x.gpr	C15_H12
Note					
Recommended processing for failed samples:					

Analysis Overview



R table

Pearson correlation tables (R values) for each technical repeats. R values were calculated from raw \log_2 intensity (R) and normalized \log_2 intensity (N) values for each dataset and compared to each other. Only probes with P value (detected) less than 0.01 were included in the calculation.

Table 1: Sample T

	R_T_H01	R_T_H02	R_T_H03	N_T_H01	N_T_H02	N_T_H03
R_T_H01	1.000	0.983	0.989	1.000	0.983	0.989
R_T_H02	0.983	1.000	0.985	0.983	1.000	0.985
R_T_H03	0.989	0.985	1.000	0.989	0.985	1.000
N_T_H01	1.000	0.983	0.989	1.000	0.983	0.989
N_T_H02	0.983	1.000	0.985	0.983	1.000	0.985
N_T_H03	0.989	0.985	1.000	0.989	0.985	1.000

Table 2: Sample TH

	R_TH_H04	R_TH_H05	R_TH_H06	N_TH_H04	N_TH_H05	N_TH_H06
R_TH_H04	1.000	0.979	0.980	1.000	0.979	0.980
R_TH_H05	0.979	1.000	0.977	0.979	1.000	0.977
R_TH_H06	0.980	0.977	1.000	0.980	0.977	1.000
N_TH_H04	1.000	0.979	0.980	1.000	0.979	0.980
N_TH_H05	0.979	1.000	0.977	0.979	1.000	0.977
N_TH_H06	0.980	0.977	1.000	0.980	0.977	1.000

Table 3: Sample C12

	R_C12_H07	R_C12_H08	R_C12_H09	N_C12_H07	N_C12_H08	N_C12_H09
R_C12_H07	1.000	0.987	0.987	1.000	0.987	0.987
R_C12_H08	0.987	1.000	0.987	0.987	1.000	0.987
R_C12_H09	0.987	0.987	1.000	0.987	0.987	1.000
N_C12_H07	1.000	0.987	0.987	1.000	0.987	0.987
N_C12_H08	0.987	1.000	0.987	0.987	1.000	0.987
N_C12_H09	0.987	0.987	1.000	0.987	0.987	1.000

Table 4: Sample C15

	R_C15_H10	R_C15_H11	R_C15_H12	N_C15_H10	N_C15_H11	N_C15_H12
R_C15_H10	1.000	0.983	0.976	1.000	0.983	0.976
R_C15_H11	0.983	1.000	0.972	0.983	1.000	0.972
R_C15_H12	0.976	0.972	1.000	0.976	0.972	1.000
N_C15_H10	1.000	0.983	0.976	1.000	0.983	0.976
N_C15_H11	0.983	1.000	0.972	0.983	1.000	0.972
N_C15_H12	0.976	0.972	1.000	0.976	0.972	1.000

Box and Whiskers Plot

Distribution of expression signal before and after normalization between technical repeats. The \log_2 scale of the expression signal values were plotted for all probes, excluding control and flagged probes.

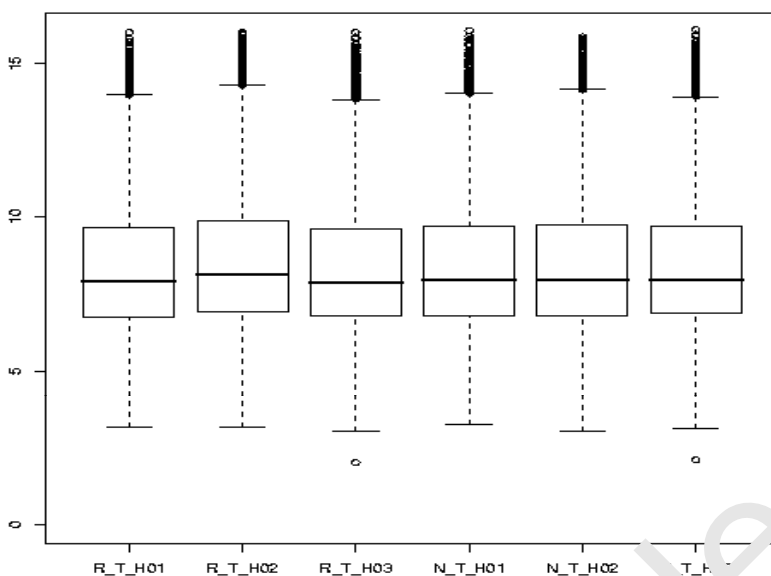


Figure 1.

The box plots from left to right are as follows: raw data of T_H01 ~ H03 normalized data of T_H01 ~ H03.

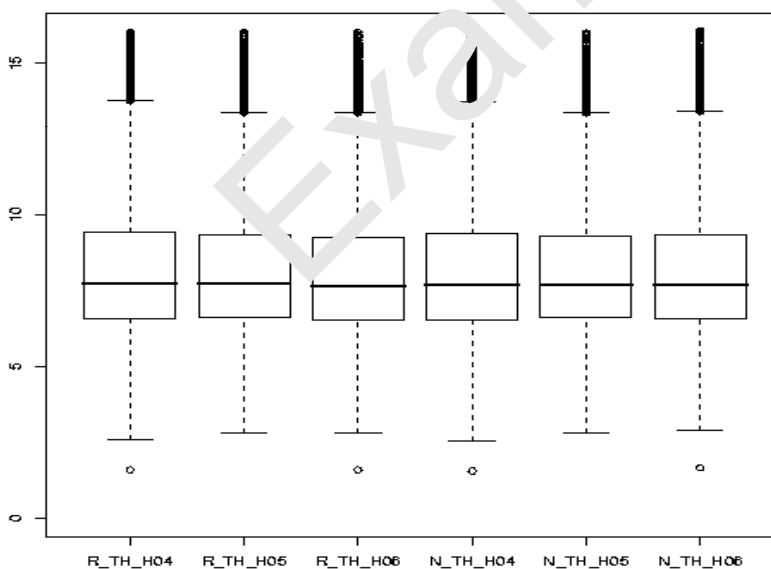


Figure 2.

The box plots from left to right are as follows: raw data of TH_H04 ~ H06, normalized data of TH_H04 ~ H06.

Box and Whiskers Plot

Distribution of expression signal before and after normalization between technical repeats. The \log_2 scale of the expression signal values were plotted for all probes, excluding control and flagged probes.

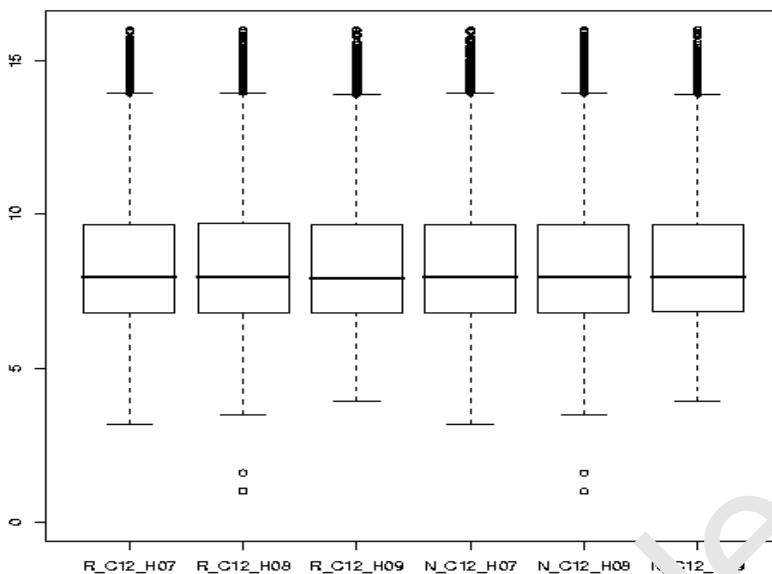


Figure 3.

The box plots from left to right are as follows: raw data of C12_H07 ~ H09, normalized data of C12_H07 ~ H09.

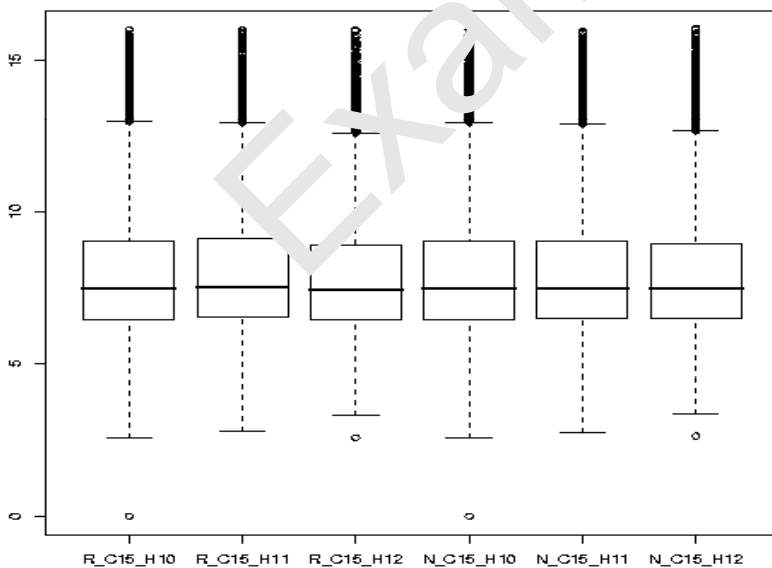


Figure 4.

The box plots from left to right are as follows: raw data of C15_H10 ~ H12, normalized data of C15_H10 ~ H12.

Scatter Plot

Repeatability of expression signal between technical repeats. Figures below contain pairwise scatter plots for each sample, including both the raw \log_2 intensity (R) and normalized \log_2 intensity (N) data. Only probes with P value (detected) less than 0.01 were included.

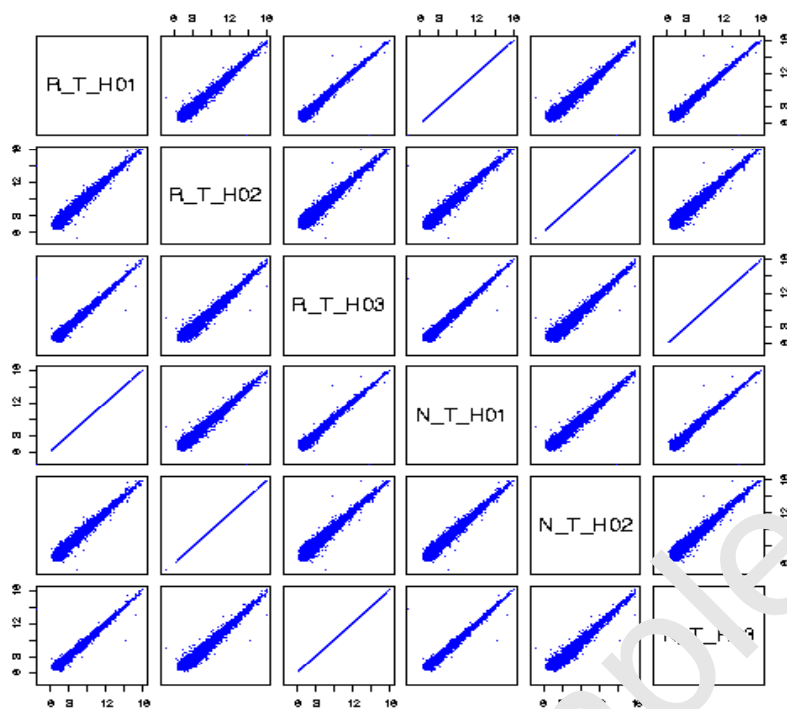


Figure 5.

The figures from left to right were labeled as follows : raw data of T_H01 ~ H03, normalized data of T_H01 ~ H03. All data were plotted in the same scale: both the X-axis and the Y-axis represent \log_2 intensity.

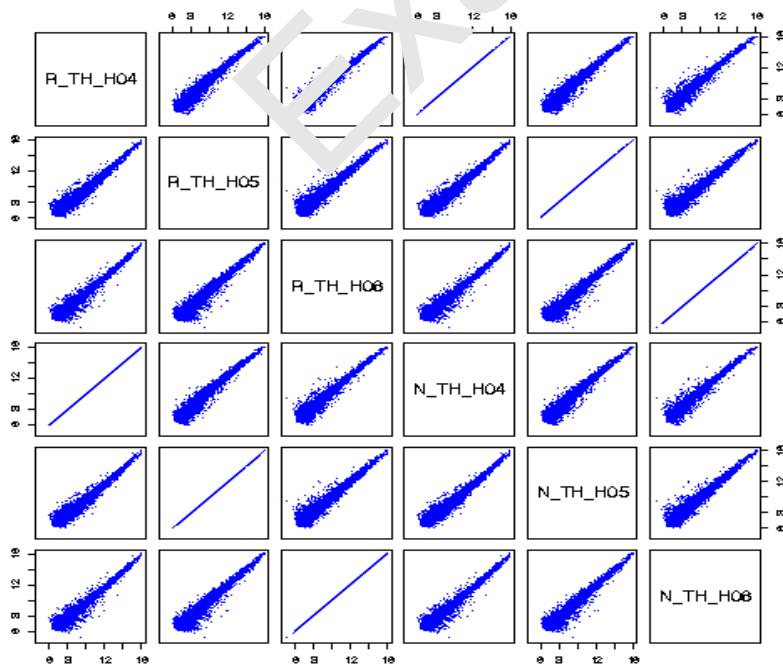


Figure 6.

The figures from left to right were labeled as follows : raw data of TH_H04 ~ H06, normalized data of TH_H04 ~ H06. All data were plotted in the same scale: both the X-axis and the Y-axis represent \log_2 intensity.

Scatter Plot

Repeatability of expression signal between technical repeats. Figures below contain pairwise scatter plots for each sample, including both the raw \log_2 intensity (R) and normalized \log_2 intensity (N) data. Only probes with P value (detected) less than 0.01 were included.

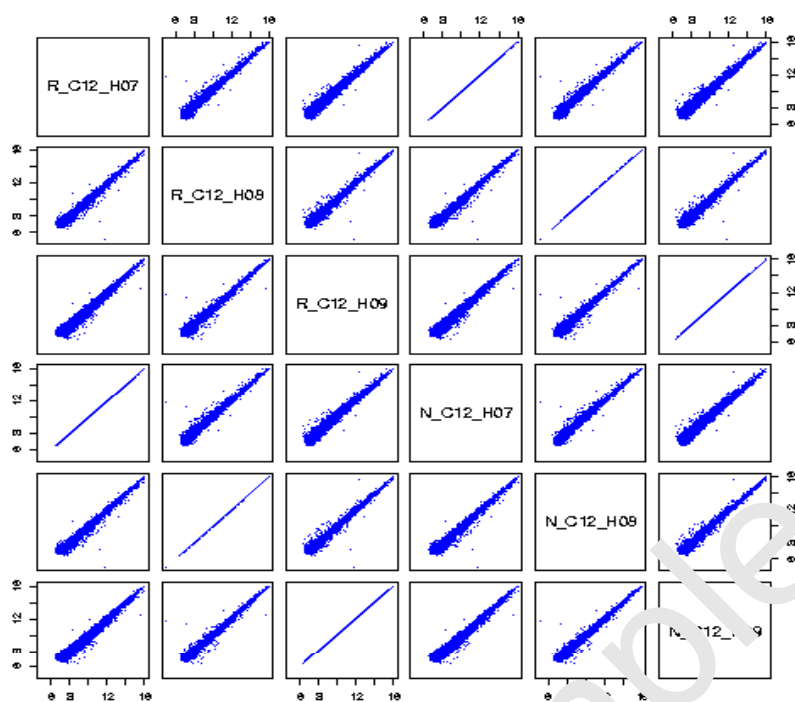


Figure 7.

The figures from left to right were labeled as follows : raw data of C12_H07 ~ H09, normalized data of C12_H07 ~ H09. All data were plotted in the same scale: both the X-axis and the Y-axis represent \log_2 intensity.

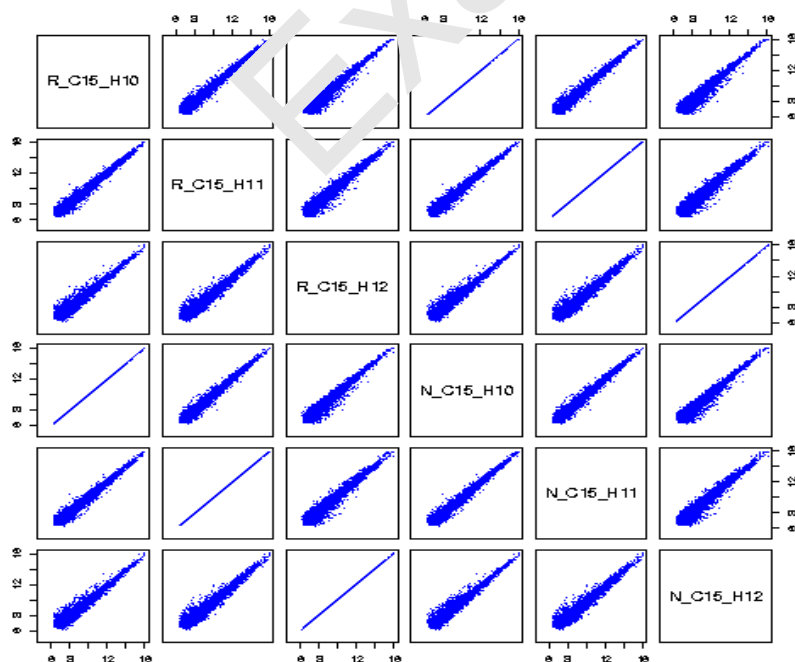


Figure 8.

The figures from left to right were labeled as follows : raw data of C15_H10 ~ H12, normalized data of C15_H10 ~ H12. All data were plotted in the same scale: both the X-axis and the Y-axis represent \log_2 intensity.

Description of column in result file

Item	File Name	Data Sheet Name	Content	Note
1	Sxx0xxx11x1_Raw_Data.xls	Slide lists	Slide information (gpr file) and corresponding sample name and labelling name	
		Raw data	<p>Column name : T_H01 ~ T_H03 : "Control" 3 replicate samples TH_H04 ~ TH_H06 : "Exp H" 3 replicate samples C12_H07 ~ C12_H09 : "Exp 12" 3 replicate samples C15_H10 ~ C15_H12 : "Exp 15" 3 replicate samples</p> <p>Description : Column A : Gene symbol Column B : Phalanx probe ID Column C ~ N : Raw data of T_H01 ~ C15_H12 samples Column O ~ Z : P-value (Detected) of T_H01 ~ C15_H12 samples Column AA ~ AL : Flags of T_H01 ~ C15_H12 samples from GPR files</p> <p>Note : 1. If P-value (Detected) is greater than the threshold it means the probe is "absent", otherwise the probe is "present". 2. Flags are determined by Genepix Pro software. When GPR files are saved, flags are given the following numerical values. 100: Good, -100: Bad, -50: Not Found, -75: Absent, 0: Found (Unflagged)</p>	
2	Sxx0xxx11x1_Normalized_Data.xls	Slide lists	Slide information (gpr file) and corresponding sample name and labelling name	
		Normalized data	<p>Column name : Column A : Phalanx probe ID Column B ~ E : P-value (Detected) of T · TH · C12 and C15 samples Column F ~ I : Normalized data of T · TH · C12 and C15 samples Column J ~ M : Error of T · TH · C12 and C15 samples Column N ~ S : log2 ratio of each compared dataset (TH/T · C12/T · C15/T · C12/TH · C15/TH and C15/C12) Column T ~ Y : P-value (Differentially expressed) of each compared dataset (TH/T · C12/T · C15/T · C12/TH · C15/TH and C15/C12) Column Z ~ AI : Gene information</p> <p>Note : 1. If P-value (Detected) is greater than the threshold it means the probe is "absent", otherwise the probe is "present". 2. Error means "measurement error of intensity" from the error model used by Rosetta Resolver®. 3. If P-value (Differentially expressed) is greater than the threshold it means no differential expression was observed.</p>	

Data comparison is presented in its entirety. Phalanx respects the scientific judgment from each customer so we will not arbitrarily select for a list of differentially expressed genes based on prior set criteria. However, Phalanx provides the fold change (log2 ratio) and p-value (if the customer chooses technical repeats) for each probe that passed the intensity stringency filter. Based on the MAQC Phase I results, differential expression data show good correlation between platforms when filtered stringently for fold change and loosely for significance (p-value). (Reference: <http://www.nature.com/nbt/journal/v24/n10s/index.html>). Thus, using the "sort" function in the Excel program, each customer can view the data under different combination of selection criteria for fold change and p-value in order to test his/her biological hypothesis.