In this white paper, we provide quality assessment results of 180 microarray data sets for HuBase® xenograft models. We show that the data is of high quality and expression profiles are well correlated in different passages from the same primary tumor tissue.

QA of mRNA arrays

We performed comprehensive quality assessment for 180 Robust Multi-array Average (RMA) normalized Affymetrix HG-U219 arrays. We found that only 9 models failed 1 testing criterion, and 2 models failed 2 testing criteria (Table 1). However, none of these violations is exceedingly large from normal values (details in text). We conclude that our microarray data is of high quality and is suitable for downstream biological and quantitative analysis, albeit some caution should be exercised when models in Table 1 are used. A detailed report generated by the Bioconductor package arrayQualityMetrics is provided as supplemental data [1].

Table 1. Arrays failed at least 1 outlier detection procedure*

Array No.	Sample Names	RLE	NUSE	MA plot	Intensity spatial distribution	# Outlier criteria
2	BL0597-P1.cel		x			1
11	CR0012-P8.cel				x	1
13	CR0028-P7.cel	x				1
35	CR1520-P2.cel			x		1
40	CR1634-P2.cel				x	1
43	CR2501-P2.cel		x			1
67	ES0215-P4.cel				x	1
72	GA0006-P7.cel	x				1
127	LI1078-P2.cel	x	x			2
129	LI1088-P2.cel		x			1
178	SA0665-P4.cel	x	x			2

RLE: relative log expression, NUSE: normalized unscaled standard error.

Between array comparison

Between array distance measures the similarity between arrays. An array with exceptionally large average distance to all other arrays is potentially problematic, for example, contamination introduced by sample mishandling. In our samples, 7 arrays have slightly large values for this metric (Figure 1, also S1-Figure 2): ES0026-P3, GA0046-P4, GAL0608-P2, LI0801-P4, LU0741-P1, LU1143-P3, SA0665-P1. However, none of them is visibly an outlier in principal component analysis (PCA), a dimensionality reduction method to reveal the relative distance between arrays (Figure 2, also S1-Figure 3). It is not unlikely that these 7 arrays have their tumor specific expression profile. The PCA graph also shows separation of different cancer types (Figure 3).

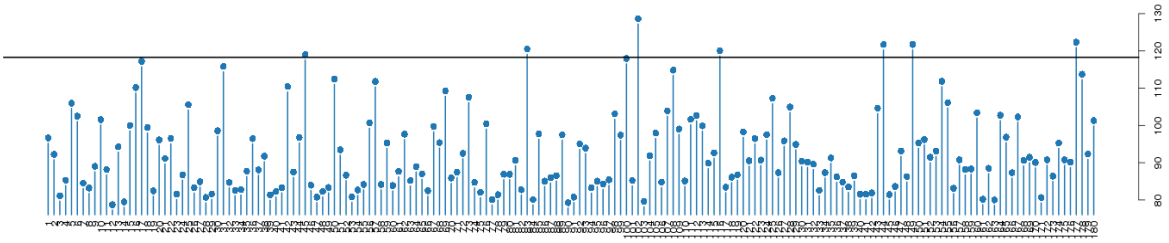

Figure 1. Each array's average distance to other arrays, the horizontal line is a threshold computed from the distribution of the average distances. See S1-Table 1 for ordering of the arrays.

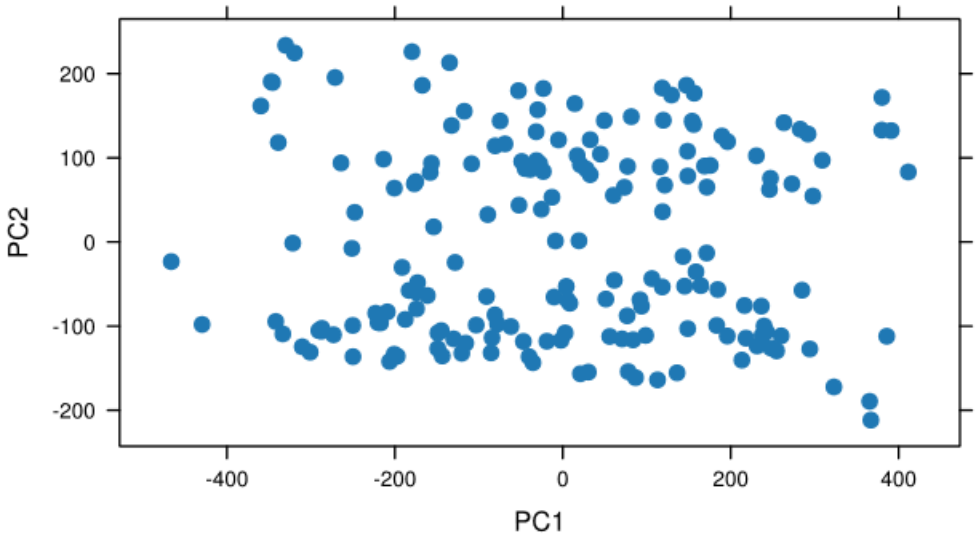

Figure 2. Relative array positions from the first two axes of principal component (PC) analysis.

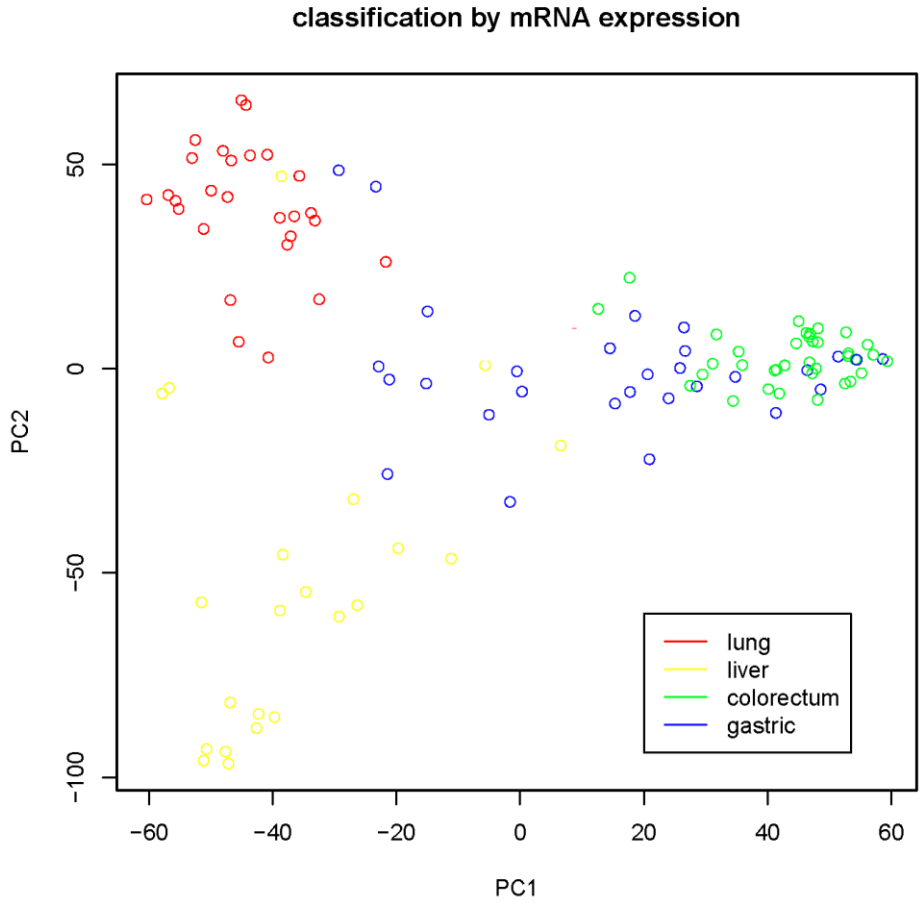

Figure 3. Separation of samples for 4 major cancer types by the first two axes of principal component (PC) analysis.

Array intensity distributions

Array intensity analysis checks arrays with very different distribution for probe set intensities (Figure 4, also S1-Figure 4), and we found no outlier (S1-Figure 5).

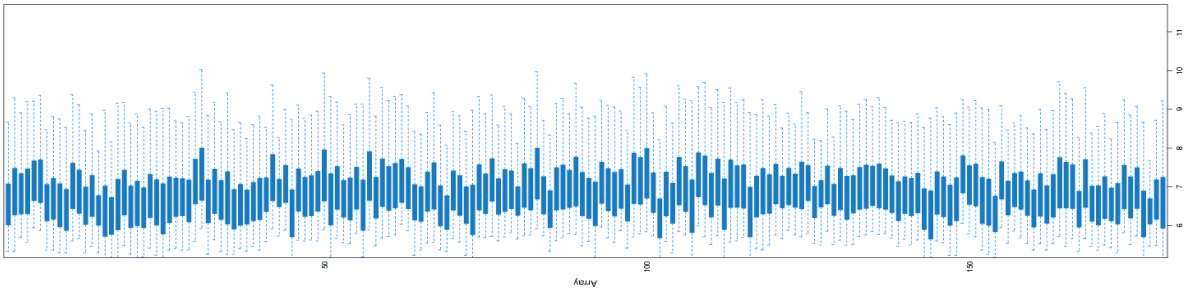

Figure 4. Boxplot of array signal intensity distributions.

Variance mean dependence

Due to the large dynamic range of mRNA expression levels, it is frequent to have signal saturation at high intensities. We observe mild such trend in our data. Out analyses confirmed that little impact is brought into downstream analysis (Figure 5, also S1-Figure 7).

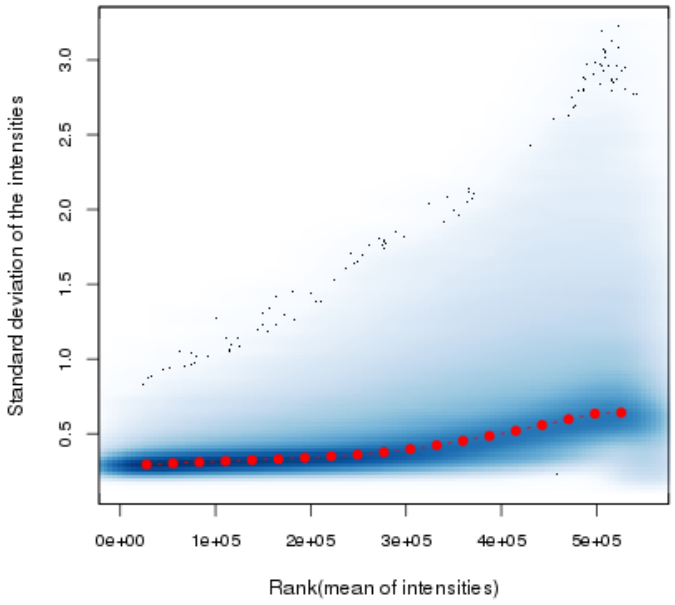

Figure 5. Density plot of the standard deviation of the intensities across arrays versus the rank of intensity mean. The red dots are the running median of the stand deviations.

RNA degradation assessment

The RNA degradation plot shows that all arrays have very close degradation levels, indicating consistent sample preparation and handling practice and arrays are suitable for comparative studies (Figure 6).

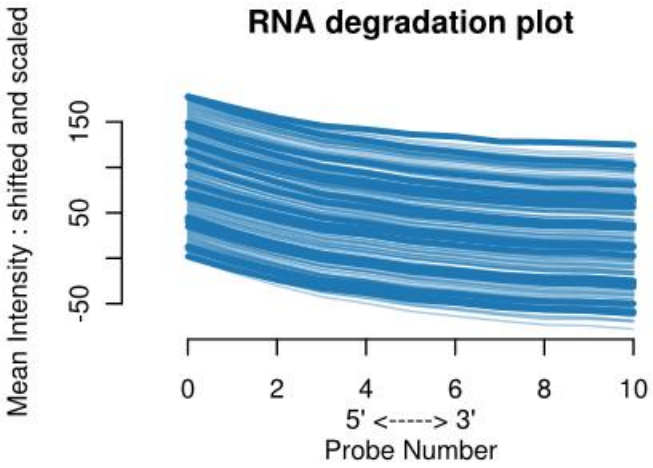

Figure 6. Each line represent an array, intensities are computed from the preprocessed data after background correction and normalization.

Individual array quality

The individual array quality is assessed by MA plots (see S1-Figure 13) and intensity spatial distribution plots (see S1-Figure 15). The MA plots evaluate if arrays have different background intensities and signal saturation, and only 1 array mildly crosses the threshold (Figure 7, also S1-Figure 14). The intensity spatial distribution plot checks if the signal intensities are randomly distributed in the array. Any violation, such as a pattern of intensity distribution, indicates possible systematic error introduced in array experiment, for example, unintended smear. Three array mildly surpass the threshold (Figure 8).

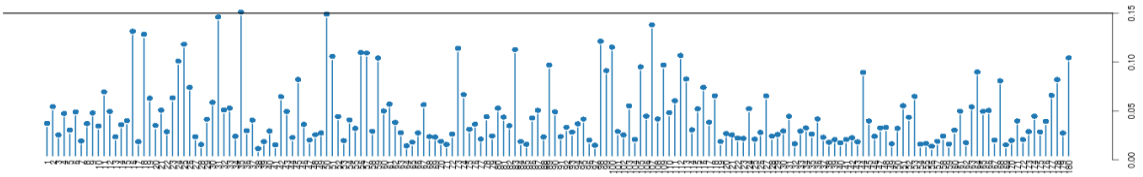

Figure 7. A bar chart showing values of a statistic used for outlier detection for MA plots. The top horizontal line represents the threshold for detecting outliers.

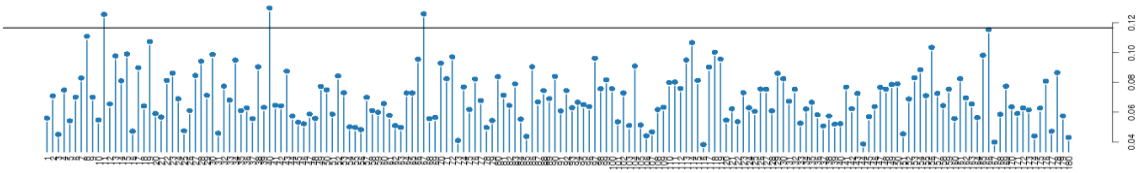

Figure 8. A bar chart showing values of a statistic used for outlier detection for intensity spatial distribution plots. The top horizontal line represents the threshold for detecting outliers.

RLE and NUSE assessment

Both the relative log expression (RLE) analysis and the normalized unscaled standard error (NUSE) analysis assume that each array has only a small number of differentially expressed genes and hence probe sets. The RLE plot measures the variation of probe set level expression across arrays. It computes the log ratio (i.e., relative log expression) between the expression of a probe set in an array and the median expression of that probe set across all arrays. By assumption, the box plots of the relative log expression of all probe sets in any array should be centered around 0 and similar in range (S1-Figure 8). We observed that only 4 arrays exceed the threshold in a statistical test of their RLE values (Figure 9, also S1-Figure 9).

In the normalized unscaled standard error (NUSE) analysis, the normalized standard error of a probe set is calculated from its expression across all arrays. Similar to RLE, the box plot of the NUSE for all arrays should be centered around 1 and similar in range (S1-Figure 10), and there are only 3 outliers in statistical test of NUSE values (Figure 10, also S1-Figure 11).

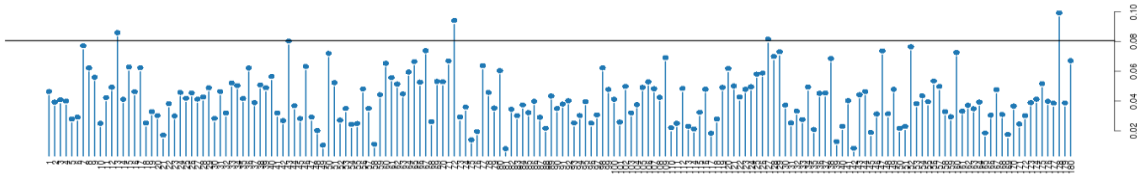

Figure 9. A bar chart showing values of a statistic used for outlier detection for relative log expression (RLE) of arrays. The top horizontal line represents the threshold for detecting outliers.

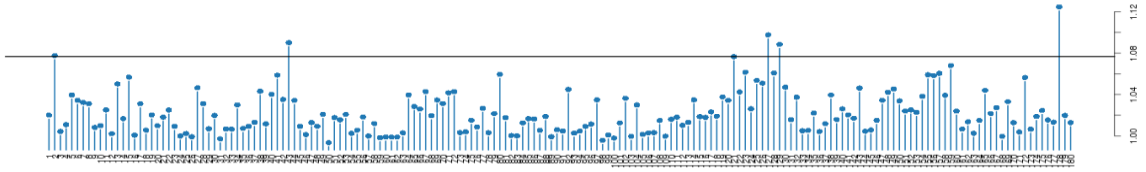

Figure 10. A bar chart showing values of a statistic used for outlier detection for normalized unscaled standard error (NUSE) of arrays. The top horizontal line represents the threshold for detecting outliers.

Passage effect

There are 12 pairs of samples in our data, each pair from the same patient but in different xenograft passages. The mRNA expression levels in all pairs are highly correlated, indicating not only high quality of sample handling and RNA profiling, but also faithful preservation of the tumor genetics in our xenograft models (Figure 11, see also S2).

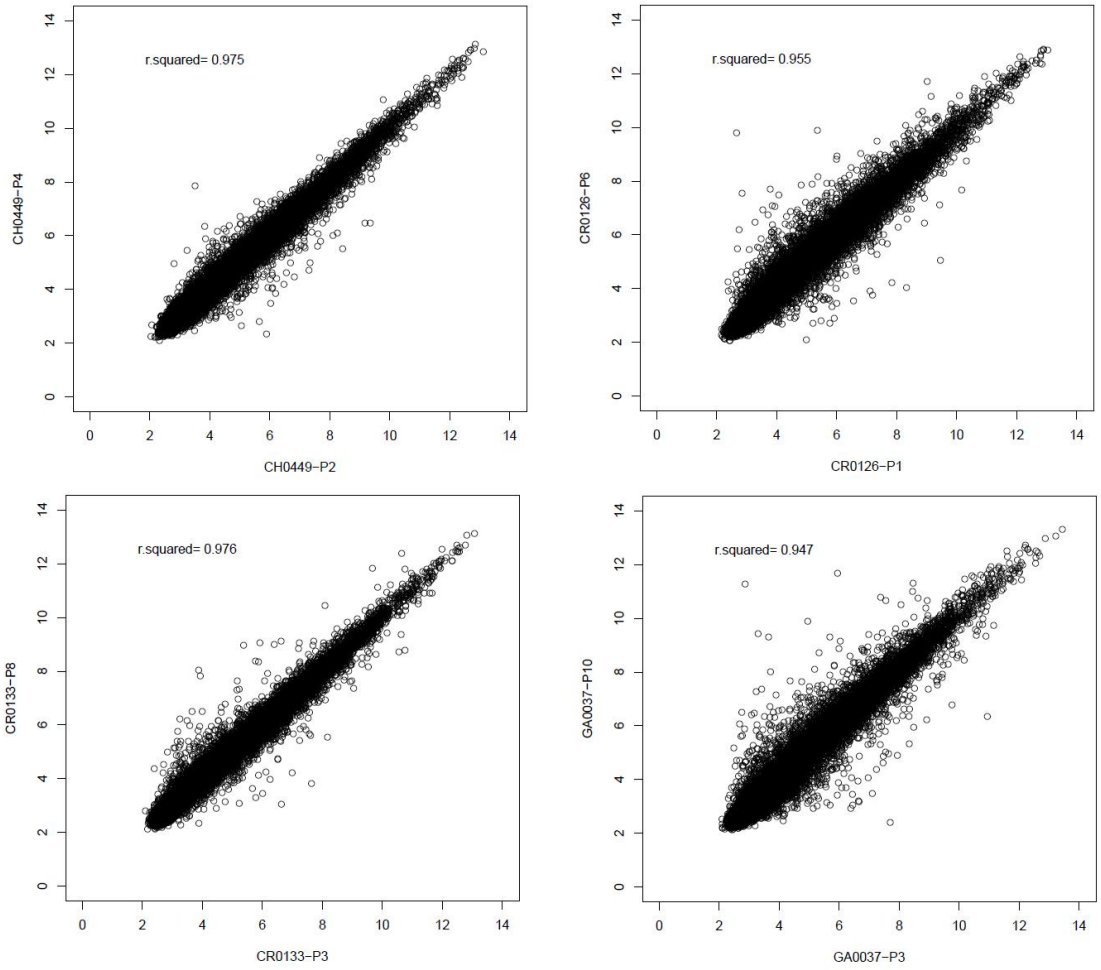

Figure 11. mRNA expression levels are highly correlated in different xenograft passages from the same patient.

Supplemental Data

[1] S1: see folder mRNAarrayqc, and click mRNAindex.html to view the detailed report.

[2] S2: see mRNA_passage_effect.pdf