

Note 1. Concerning Figure 2A

The Red Line demarcated in Figures 2A1-2 represent a two-fold change in gene expression from moderate abundance onwards.

Hundreds of technical replicates were compared using the nCounter Analysis System.

In no instance has the code count between technical replicates, for any given gene, deviated beyond the demarcated threshold.

This serves to indicate the limit beneath which variation can be attributed to error in the instrument.

Such error increases when measuring exceedingly low abundance levels and the demarcated red line shown in Figure 2A1-2 reflects this phenomenon.

Note 2. Concerning the handling of Raw Code Counts (RCC)

Data manipulation outlined below is per Lane

1. $\sum$ ALL NEG controls (Barsi code v2 #188 through #197)

2. Calculate NEG NORM Factor as follows: 25 / (value obtained in step 1)

3. Multiply ALL the reads by the NEG NORM Factor obtained in step 2

4. Background correct by subtracting the Gene specific noise for each gene (only if the NO RNA runs suggest this is necessary)

5. If the counts for any given gene transcript turn out negative after Background correction, replace ALL negative values with 0.1 (if step 4 was not required, then step 5 may be ignored as well)

6. $\sum$ ALL counts w/o controls (Barsi code v2 #1 through #181)

7. Calculate POS NORM Factor as follows: 500,000 / (value obtained in step 6)

8. Multiply ALL the reads by the POS NORM Factor obtained in step 7

9. With the values obtained in step 8, calculate the following ratio: Experiment / Control

Note: In those instances where only one of the two values used in step 9 has been floored @ 0.1: replace the Ratio with a value of 0.5 if Experiment was floored OR replace with a value of 2 if Control has been floored

10. The values obtained in step 9 > 2 represent the Fold Difference UP

11. Furthermore, calculate the following: 1 / (value obtained in step 9)

12. The values obtained in step 11 > 2 represent the Fold Difference DOWN

13. Another useful way to assess fold difference is to calculate the following: Log_2 [value obtained in step 9]

14. Values obtained in step 13 > |1| represent Two Fold Difference, where (-) indicates DOWN & (+) indicates UP

15. Remember that the Ratio obtained in step 9 is meaningless for those instances where the Background corrected value has been floored @ 0.1 in step 5

16. Graphically represent data in the form of a labeled Scatter plot [i.e. x[Control] vs y[Experiment]]