Reviewer’s report

Title: Small nucleolar RNA U91 is a new internal control for accurate microRNAs quantification in pancreatic cancer.

Version: 2 Date: 8 September 2015

Reviewer: Emma Dorris

Reviewer’s report:

- Major Compulsory Revisions

1. The authors use ANOVA on RQ data. Can they confirm that tests for normality were performed and clarify if any transformation of data was required, and if so, what transformations were used.

2. The authors conclude that U91 is a stable internal control for miRNA expression in pancreatic cancer. Were calibration curves performed on each assay to ensure that all endogenous control assays were equivalent and that this conclusion is not based on differences in PCR efficiency between assays?

- Minor Essential Revisions

1. Line 70: remove the word ‘of’
2. Line 203: states “miR-2” which should read “miR-21”
3. Line 250: use of comma instead of period (reads “value=0.056”)
4. Line 275: reads “a least” rather than “at least”
5. Line 624: should “relatively” read “relative to”?
6. Line 639: Should read “relative to” rather than “relatively to”
7. Consistency of terminology: microRNAs, miRNAs and miRs are used interchangeably. One abbreviation should be used consistently throughout.
8. During the discussion the authors discuss differences in the mean expression of miRs between studies. The authors state that “the differences in the mean expression of miRs may be a least partially explained by the choice of controls for normalization.” It should be noted that many factors including reagents/materials and data-processing algorithms can also contribute to the variation, rather than solely focusing on the reference gene used.
9. Figure 1: The X-Axis label is awkwardly placed. It should be rotated and at the axis.

- Discretionary Revisions

1. Line 221: consider rephrasing “not statistically significant almost for all miRNAs”
2. The MIQE guidelines by Bustin et al, 2009 should be referenced.

3. Table 4 and 6: Consider using bold to highlight the statistically significant rather than statistically insignificant results.

4. Table 5: Is it possible to relabel the group identifiers on the Tables? The legend is outlined in the Table heading but the tables would read easier if directly labeled Normal and PDACs rather than 1 and 2.

5. Table 7: Phrasing is ambiguous “vary up to several times”, consider using an actual figure (for example: vary up to 8-fold) or else rephrase the heading (for example: Expression values of candidate endogenous control genes are highly variable in PDACs compared with normal control tissues).

6. Figure 1: Indicators of statistical significance (often denoted by *) would be useful on the graph.

7. Figure 1: Patterned bars would be easier for those who print in black and white.

8. Figure 2: This figure would be more informative if converted to a table with the mean difference and range (min/max) between matched samples.

9. Figure 3: Table 7 is more informative than the single representative sample shown in figure 3. This figure seems redundant.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.