Author's response to reviews

Title: Evaluation of a New High-Dimensional miRNA Profiling Platform

Authors:

Julie M Cunningham (cunningham.julie@mayo.edu)
Ann L Oberg (oberg.ann@mayo.edu)
Pedro M Borralho (borralho@ff.ul.pt)
Betsy T Kren (krenx001@umn.edu)
Amy J French (french.amy@mayo.edu)
Liang Wang (wang.liang@mayo.edu)
Brian M Bot (bot.brian@mayo.edu)
Bruce W Morlan (morlan.bruc@mayo.edu)
Kevin AT Silverstein (silve023@umn.edu)
Rod Staggs (stag004@umn.edu)
Yan Zeng (zengx003@umn.edu)
Anne-Francoise Lamblin (lambl001@umn.edu)
Christopher A Hilker (hilker.christopher@mayo.edu)
Jian-Bing Fan (jfan@illumina.com)
Clifford J Steer (steer001@umn.edu)
Stephen N Thibodeau (sthibodeau@mayo.edu)

Version: 2  Date: 18 May 2009

Author's response to reviews: see over
May 18, 2009

Scott Edmunds PhD
Senior Editor
BioMed Central Editorial Team

To Whom It May Concern:

We sincerely thank the *BMC Medical Genomics* reviewers for their thoughtful comments on our manuscript entitled, “Evaluation of a New High-Dimensional miRNA Profiling Platform”. We are submitting a revised version addressing the reviewers points as follows:

Reviewer 1 Robert Blelloch

“This study is very similar to a study published by Chen et al in NAR (NAR36:e87) an independent verification of the reproducibility of this platform will be valuable to some readers. Unlike the previous publication, this paper does very little to evaluate the accurateness of this platform. It would be nice if a similar analysis for at least a couple of samples were done for this paper”

We thank reviewer 1 for this comment. The primary purpose of the present paper is to address the performance and reproducibility of the platform, rather than the accuracy per se. No other paper has addressed this for the Illumina miRNA platform to date, and the authors feel it singularly important in light of the issues encountered in mRNA profiling (MAQC consortium. The microarray quality control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nature Biotechnology 24:1151-1161, 2006; A. Kohlman et al Intraplatform reproducibility and technical precision of gene expression profiling in 4 laboratories investigating 160 leukemia samples: the DACH study, Clin Chem 54:1-11, 2008; L. Shi et al Reproducible and reliable microarray results through quality control: good laboratory proficiency and appropriate data analysis practices are essential. Current Opinion in Biotechnology 19:10-18. 2008). The paper by Chen et al used rtPCR (NAR36:e87) and digital gene expression profiling to assess the accuracy of the platform; we have included rtPCR data in the present manuscript. While we do agree that inclusion of digital gene expression profiling for some of the samples would have been nice, this was not possible due to the current prohibitively high cost.

Minor essential changes

1) “Authors claim that in their study they evaluated reproducibility, dynamic range and sensitivity, but the really only strong conclusion is the reproducibility……..”

The authors agree with this statement and have amended the text (page 5, paragraph 2, line 8 and page 19, paragraph 3, line 3) to reflect that the focus of this paper is reproducibility.

2) “I would like much more of a discussion of the their method of normalization (i.e. fastlo) including its value, weaknesses and strengths”
The authors have addressed this by adding a paragraph describing the algorithm, its assumptions, strengths and weaknesses and how it was applied to this study (please see pages 10-11).

3) “Figures need to better labeled and figure legends more detailed”
   For Figure 1, a new figure has replaced the original, which the authors feel better depicts the study design. Plate 4, from which data for only the cell lines, has been included to allow a balanced figure design. Figure captions have been added to all figures. An additional axis label has been added to Figure 5b. We agree that additional axis labels are desirable for figures 2, 3 and 4. However, we were unable to add clear labels without making the plots more ‘busy’ and even more difficult to read. Therefore, the remaining figures are unchanged. We have reviewed all figure legends, and clarified them where possible.

4) “There are some minor spelling and grammar errors in the text”
   We thank the reviewer for pointing these out. The manuscript has been carefully reviewed for grammatical and spelling errors. Changes have been to correct tense and spelling and improve sentence structure.

Reviewer 2 Rickard Sandberg

Major compulsory Revisions

1) “Overall, the authors must clarify what is new in the study with comparison to existing literature on using Illumina beads and how do the observed reproducibility relate the other published methods, e.g. digital gene expression and qRT-PCR”
   The authors have added two sentences to page 5, lines 11-16 of paragraph 2
   “Other than the original paper by Chen et al (NAR36:e87), describing the platform, there is no other publication addressing reproducibility and performance of the Illumina miRNA profiling, so it is highly relevant to evaluate reproducibility and this forms the primary focus of this report. More specifically, the goals of this study were to understand variability due to plate, extraction, dilution and technical replication using clinical samples”

2) The presentation on terms of figures and writing needs further work to enhance clarity, below follows a few examples”

   Figure 1 in B there is no clear indication of what the arrows signify given that all starting amounts total RNA amounts are different. Add units to all numbers and avoid unnecessary acronyms....Altogether figure 1 looks more like a sketch than a figure”

As noted above, a new figure 1 has replaced the original, which the authors feel more clearly depicts the study design. Plate 4, from which data for only the cell lines, has been included to allow a balanced figure design. Figure captions and labels have been improved.
“Page 13, the text is broken up into many short sentences indicates ad draft rather than finished manuscript. I would also advice that the paragraph “Please note that figure 3…:” would be mover earlier in the text where is would help clarify the presentation of figures. In its current place it, the readers would have already had to deduce the figure layout himself/herself.

The authors have revised this section to be more polished, keeping each concept together in only one or two sentences. In addition, a portion of the conclusions were moved to the discussion (page 20, last paragraph, line 4).

In addition, “Please note that figure 3…:” The authors have moved the paragraph to a place earlier in the text, from the bottom of page 13 to the bottom of page 12.

Minor essential revisions

Figure 4 and 5: the usage if letter notation for denoting different comparisons is confusing/unclear. Authors might prefer to instead label the different comparisons made” As discussed above, we could not add labels that were both meaningful and not distracting to figure 4. A caption has been added to figure 5 indicating that the numbers correspond to Input RNA.

We believe these revisions have improved the manuscript and hope that you now find it acceptable. Thank you very much for your consideration.

Sincerely,

Julie M. Cunningham, Ph.D.
Director, Genotyping Shared Resource
Advanced Genomic Technology Center
Department of Laboratory Medicine & Pathology
Mayo Clinic College of Medicine
200 1st St. SW
Rochester, MN55902CA
Phone: 507-538-6863
Fax: 507-266-0340
cunningham.julie@mayo.edu