Reviewer's report

Title: Application of Affymetrix Array and Massively Parallel Signature Sequencing for Identification of Genes Involved in Prostate Cancer Progression

Version: 1 Date: 25 May 2005

Reviewer: William Gerald

Reviewer's report:

Comments to authors

General

This manuscript describes a comparison of two different transcript profiling technologies using prostate cancer cell lines. The study demonstrates that the different methods provide somewhat different results and conclude that a combination of the two is a more robust procedure for profiling. Several findings were evaluated using human tissue samples. This is a thoughtful study addressing a timely topic. Given the complexity of performing such a study, the authors have applied a reasonable approach, although I am sure that every statistician asked would suggest a different one. The conclusion that the two methods are better than either alone, although generally true, is not well founded since the accuracy of either was not fully explored. A disappointment was the lack of an effort regarding comparison of accuracy of the two methods. In particular the followup RTPCR studies would have been much more informative if they had been more quantitative. I would recommend the authors address the following specific issues:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Although the analysis using Affymetrix default calls is reasonable, I would have preferred a more quantitative approach using the signal intensities versus TPM directly. At the very least the authors should defend the final methods of measurement as defined, or discuss the various options.

2. The discussion of the reasons for discrepancies seems to be a bit one sided focused on the faults in the Affymetrix methodology. A more thorough critique of the two methods would be very informative to readers. For example how much is the accuracy of the MPS method affected by the location of the first DpnII site or cloning efficiency, or microbead attachment or accuracy of sequencing or accurate matching of the sequence to gene, etc.

3. It is a little hard to follow the trimming method defining the unique genes and there is no real discussion of the reasons for the methods of trimming finally chosen. Some discussion or diagrams or simplification would be helpful.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The assays of human tissues are not very helpful. Are these single examples of each type or pools. If single examples I would suggest that no extrapolations as to the significance be made.
Discretionary Revisions (which the author can choose to ignore)

1. Quantitative RTPCR would be very helpful for determining accuracy.

**What next?:** Accept after minor essential revisions

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

**Quality of written English:** Acceptable

**Statistical review:** Yes