Author's response to reviews

Title: An iteration normalization and test method for differential expression analysis of RNA-seq data

Authors:

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Author's response to reviews: see over
Dear Editor,

*An iteration normalization and test method for differential expression analysis of RNA-seq data*

(ID 3968892491123558)

Thank you for your letter regarding our paper. We greatly appreciate you, the associate editor and reviewers for great effort deployed on this paper. We have carefully considered the comments from the reviewer and finished the revision of our manuscript.

According to reviewer’s comments, we have now done some analysis from RNA-seq dataset. We also found something new in the real data we analyzed. We have added a paragraph to section 4 and perhaps a paragraph to the conclusions. We have put some of figures in supplementary material.

At below, we provide detailed, point-by point responses to the associate editor and reviewers’ comments.

We shall look forward to your evaluation on this revision.

Yours sincerely,

Yan Zhou, Nan Lin, Yang Zhang and Baoxue Zhang
This is a well-written paper that provides some new methods for normalizing RNA-seq data. The following are some suggestions for improving the paper.

We sincerely thank you for Professor Jason Moore’s comments. We have revised the paper as the suggestions of Professor Jason Moore.

Major Compulsory Revisions

1) The paper could be improved by identifying at least one biological insight that can be highlighted and discusses. The results are interesting from an RNA-seq analysis point of view. However, it would be nice to know if you found something new in the real data you analyzed. How will this help biologists doing RNA-seq analysis? You might consider adding a paragraph to section 4 and perhaps a paragraph to the conclusions.

We sincerely thank you for the comments. We have now improved the paper by identifying at least one biological insight that can be highlighted and discusses. And we also found some interesting results from an RNA-seq analysis point of view. We added the biological analysis for liver versus kidney data set in paragraph three and four of section 4.1. We downloaded lung and kidney RNA-seq data form bodymap project[1]. DE genes (only protein coding genes were considered) detected by IMM were intersected with DE genes detected by TMM and DE genes only detected by one method were retrieved. Then, we compared the gene expression of these genes in liver and kidney based on bodymap data. And we found that DE genes detected by IMM are more consistent with gene expression level reported by bodymap data than DE genes detected by IMM. In addition, we found some DE genes which detected by IMM but not by TMM are associated with the liver or kidney diseases. For example, UNC5C [2] has a direct association with kidney cancer.

We found that DE genes detected by IMM are more accurate than DE genes detected by TMM. What’s more, we found DE genes identified only by IMM are more likely related to liver or kidney tissue than DE genes detected by TMM. Therefore, IMM normalization method is a
useful method in RNA-seq data analysis for biologists. And we also added these contents to the end of Conclusions (section 5).

Minor Essential Revisions

1) There are a lot of figures. Can some of these be included as supplementary material?

We have now put some tables and all figures to supplementary.

2) Please provide additional details about your software and how it can be accessed.

Thanks for the comments! Unfortunately, we have no package for our method. But we can put the codes to web.

References
