Reviewer's report

Title: Impact of RNA degradation on gene expression profiling

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Reviewer: Giacomo Spinsanti

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Unfortunately, I'm not able to change my overall opinion on this work. Up to my personal experience gene expression analysis is a really complex procedure that can easily lead to misleading results for a number of "operating errors" that too often are not considered by researchers. A key point of this procedure is the integrity of RNA and, as I mentioned in the previous review, I do not agree with the fact that RNAs with different levels of degradation can be easily compared.

Moreover, I think that this message could cause problems with future publications and the "optimal standards" defined to publish reliable gene expression data. If you give a look to publications of Bustin, Vandesompele and Pfaffl you can easily understand the importance of RNA degradation in gene expression protocols. The authors conclusion that "...RNA of different quality can be used to perform gene expression analysis due to a much higher biological variance, like in our case compared to the effect that is imposed by degradation of RNA." is not acceptable up to my knowledge in this field. What I mean is that the biological variance between samples is not a reason to accept a comparison of gene expression between samples at different degradation stages.