

## Reviewer Report

**Title: PseudoFuN: Deriving functional potentials of pseudogenes from integrative relationships with genes and miRNAs across 32 cancers**

**Version: Original Submission**    **Date: 10/21/2018**

**Reviewer name: Cameron Bracken**

### Reviewer Comments to Author:

The role of pseudogenes in the modulation of gene regulation is a burgeoning field that is ideally placed to benefit from integrative approaches that utilise "big data" that is currently available. A user friendly tool such as PseudoFun is therefore of use as a possible discovery mechanism for new relationships. Not having used PseudoFun at this stage, it is difficult to fully evaluate its performance, though the approach described appears useful and the presentation of new relationships such as that suggested between PPP4R1L, SOX15 and miR-375 highlight a potential to identify new avenues for further investigation. I have only minor suggestions for improvement in presentation.

- 1) In Figure 5 (and much of the supplementary figures presented in a similar fashion), is the miRNA associated directly targeting the gene/pseudogene. Visually, only a correlative expression relationship is indicated.
- 2) Figure 4 does little to add clarity. If the goal is to highlight regulatory relationships, the ENSTxxx labelling does not lend for easy interpretation and the miRNAs are not shown. If the intended purpose is to illustrate a style at which data is outputted, perhaps this is better served by a user friendly series of screenshots illustrating a beginning to end data query - result flow?
- 3) In Figure 5 and some supplementary figures, co-expression visually is not well represented by the colour scheme. ie: the tumour relationship between PPP4R1L and SOX15. The stats support this, the visual representation less so. Perhaps blanking out the 1:1 same gene : same gene diagonal would allow re-setting of the colour scheme to better represent co-expression?
- 4) In paragraph 2 of the results, I was unclear what the "alignment score above 54" means... What degree of alignment is this? I found understanding this to be hard to gauge. Relating to this, could the authors comment more extensively on their findings of tremendous levels of alignment for some pseudogenes?

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