**LETTER TO THE EDITOR**

Dear Prof. Madan Babu,

Concerning our paper entitled “miR-Synth: a computational resource for the design of multi-site multi-target synthetic miRNAs” by Laganà et al. (NAR-03635-N-2013.R1), once again, we would like to thank you for giving us the possibility to address the reviewers’ observations, and we would also like to thank the reviewers for their hard work and insightful comments.

We did our best to address the concerns of the reviewer and implemented the filter that he suggested. We further elaborated on the off-target issue, in order to clearly state the advantages and limitations of the a-miR approach, which remains a better tool for multi-targeting, as it produces molecules with fewer off-targets than combinations of single-target molecules.

We have included a separate file with our responses to the points raised by the reviewers, a revised version of the manuscript and a revised version of the supplementary material.

Best personal regards,

Carlo M. Croce, M.D.

Distinguished University Professor

Chair, Department of Molecular Virology, Immunology and Medical Genetics

Alessandro Laganà, Ph.D.

**RESPONSE TO THE REVIEWERS**

**Reviewer’s comment:**

The authors ignored my comment and decided to stick with their initial estimate that effecient a-miRs can be designed to target 95% of all gene pairs. I provided argumentation that this number should be considerably lower (most likely <50%) because many a-miRs are extremely unspecific (matching thousands of mRNA 3'UTRs), meaning that their level of on-target repression will be weak and they will cause \*similar\* downregulation of hundreds of likely critical off-target mRNAs. It would have been very simple for the authors to include the suggested additional filter (max number of gene hits pr a-miR) in their new analysis, but for some reason they decided this was too much work.

**Answer:**

As suggested by the reviewer, we implemented a filter to discard all the a-miRs with a predicted number of off-target genes over a user-provided threshold. A variant of the filter allows the user to select the top 10 a-miRs with the smallest number of off-target hits. We also expanded on the off-target analysis in the manuscript, to include the percentages of gene pairs sharing a 7mer with no more than 2000 and 1000 off-target hits, yielding 43% and 5.6%, respectively. We further elaborated on the off-target issue and on the importance of such a filter. Changes in the manuscript are in red.