Comments to manuscript #62918

In the manuscript #62918, entitled "A chimeric vector for dual use in cyanobacteria and *Escherichia coli*, tested with cystatin, a non fluorescent reporter protein**"** The article describes the development of an improved version of RSF1010 derived shuttle vector that is smaller in size, and would have a higher copy number and better biosafety that can replicate in *E.coli* and cyanobacteria. The functionality of the new vector was confirmed through GFP expression and cystatin activity.
The authors concluded that the novel that the newly constructed vector has higher copy number, better biosafety and stability which will serve greatly in expanding the genetic toolbox of cyanobacteria.

The experimental design and the data obtained seem to be solid and representative. The article contains important novel data, which might be of interest to academic researcher and biotechnologists.

I have few comments for improving the manuscript,

1- In page 25, lines 420 and 421, the authors addressed three drawbacks, one of them, the authors stated that the currently used episomal vectors and reporters have high reporter signal backgrounds resulting from cellular components.

But in matter of fact, it has already been reported that a tight genetic regulation can be achieved through the use of orthogonal transcriptional systems as no cross talks happening with the host organism. This work has been clearly demonstrated by Badary et al., 2015 when evaluating an introduced two-component derived from *Synechocystis* sp. PCC 6803 into the marine cyanobacterial strain *Synechococcus* using gfp, reporter gene compared to the regulation of the same two-component system in its native host.
Moreover, some of the backgrounds that have been observed in other transcriptional systems using fluorescent reporters have been solved through many approaches.

Throughout the manuscript, please state clearly the importance of using cystatin reporter as new tool for characterization and also addressing the tight genetic regulation systems that have been established in cyanobacteria that are based on fluorescent reporters.

2- In page 9, lines 67-76, the authors claimed that episomal expression is preferred over genomic insertion through only addressing the pros of episomal expression over genomic insertion, although genomic insertion has a lot of advantages not found in replicative vectors as long-term inheritance, high stability of host genome and reducing gene dosage variation caused by copy number variation of replicative plasmids. Roubstness of replicative vectors can be claimed as it is not needed to make sure that construct is present in all copies through the seggregation procedure.

Please consider re-writing that paragraph with references.

3- In pages 28 and 29, lines 492-517, the authors only mentioned chemical gene induction systems that carried on RSF1010-based shuttle backbones that have been tested in cyanobacteria, please discuss other gene induction systems based on physical stimuli such as light that are carried on RSF1010 derived vectors.

Also, please consider giving a more clear description of the L21 promoter when implemented in other systems.

4- In page 8, lines 47 and 48, regarding the citation of book chapters and review articles, there are more recent publications than the years 2012 and 2017 discussing in details the applications of synthetic biology in cyanobacteria which can be added such as:

Badaryet al., 2021, Marine cyanobacteria (book chapter)

Ashley et al., 2021, Recent advancements of the genetic engineering of microalgae (Review article)

Please consider adding these most recent reviews and book chapters especially they have some content not included in the already cited articles to ensure full understanding of the current status of synthetic biology in cyanobacteria.

5- In page 25, lines 432 and 433, please cite the references showing the successful genetic transformation by electroporation that have been achieved in many cyanobacteria. There have been successes in transformation by electroporation in marine *Synechococcus,* filamentous and heterocyst in the following articles:

- Transformation of the Cyanobacterium Leptolyngbyaboryana by Electroporation, 2015
- The development and characterization of an exogenous green-light-regulated gene expression system in marine cyanobacteria, 2015
- Characterization of the hupSL promoter activity in *NostocpunctiformeATCC* 29133, 2009