Point-to-point response to the reviewers:

Reviewer 3

In this article, authors give a short review of 3 groups of reversible inducible gene expression systems, and an irreversible recombinase system. In the abstract, the authors also promise to provide basic standards and criteria for the selection of the most suitable mammalian inducible expression systems. The topic is very relevant and interesting, and there are not a lot of similar articles published. However, the review in its current form does not provide the best tool for researchers that wish to use the inducible system and need help with choosing the right one for their needs. There are couple of general and specific problems in this manuscript, which I list below.

Response: Thanks a lot for recognizing the necessity of our review manuscript and for your critical and constructive suggestions. Our initiative was to review the methodology development from a historical point of view. Meanwhile, as each improvement and invention was made to overcome the drawbacks of previous methods or obtain unique and novel features, we attempted to provide the reader with these rationales and include the analysis of cons and pros of each system. Following your valuable suggestion, to make the standards and criteria more clearly stated in our manuscript, we rewrote the last part, “Closing remarks and future perspectives”. Within this part, under the subtitle “7.1. How to choose the right system for the experiment”, we wrote 4 new paragraphs to provide standards and criteria in more detailed matter. In addition, we also summarized the cons and pros of each system in a new table 2 as a selection reference. To be noted, we emphasize that “No method is perfect in every aspect, the most suitable for each experiment is the one that comes with a maximum advantage for experimental purpose and with a minimum compromise for the other features”.

General problems:

The article does not provide the information promised in the abstract. “Basic standards and criteria for the selection of the most suitable mammalian inducible expression systems” are not given. The same goes also for the title.

Response: We thank the reviewer for this constructive suggestion. Following your valuable suggestion, to make the standards and criteria clearly stated in our manuscript. We rewrote the last part, “Closing remarks and future perspectives”. Within this part, under the subtitle “7.1. How to choose the right system for the experiment”, we wrote 4 paragraphs to provide standards and criteria. In addition, we also summarized the cons and pros of each system in a new table 2 to be used as a selection reference. It is to be noted, we have now emphasize that To be noted, we emphasize that “No method is perfect in every aspect, the most suitable for each experiment is the one that comes with a maximum advantage for experimental purpose and with a minimum compromise for the other features”.

Please see the table 2 in below.

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Reversability</th>
<th>Maximum induction</th>
<th>Overexpression</th>
<th>Knockdown Or knockout</th>
<th>Spatial regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>Yes</td>
<td>100-1000X</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Compound</td>
<td>Activity</td>
<td>Concentration</td>
<td>Induction</td>
<td>Expression</td>
<td>Advantages</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>---------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Cumate</td>
<td>Yes</td>
<td>100-1000X</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Yes</td>
<td>100-1000X</td>
<td>Yes</td>
<td>NT</td>
<td>No</td>
</tr>
<tr>
<td>FKCsA</td>
<td>Yes</td>
<td>100-1000X</td>
<td>Yes</td>
<td>NT</td>
<td>No</td>
</tr>
<tr>
<td>ABA</td>
<td>Yes</td>
<td>100-1000X</td>
<td>Yes</td>
<td>NT</td>
<td>No</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>No</td>
<td>30-50X</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Blue light</td>
<td>Yes</td>
<td>50-100X</td>
<td>Yes</td>
<td>NT</td>
<td>Yes</td>
</tr>
<tr>
<td>Riboswitch</td>
<td>Yes</td>
<td>5-9X</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

NT: not tested, ABA: abscisic acid

That manuscript could be structured more systematically: perhaps subtitles could be added and the text under each subtitle should follow the same scheme: starting with the description of the system, followed by examples of use, and finally the drawbacks and advantages of the system. I had a lot of problems in distinguishing what is a description of the system and what is an example of its use. I believe that better use of tenses (although I am not an expert in English language) and positioning of references could help with this.

Response: Thanks for this constructive suggestion. Following your suggestion, we have added several subtitles in order to make the structure clearer and more organized. Regarding the better use of tenses and positioning of references, we also made adjustments according to the attachment provided by the reviewer.

The third general problem is that too much attention is paid to details explaining the systems; for instance, the numbers of amino acids are really not important for understanding the paper. Instead, attention should be focused on giving some directions for users of the systems - like promised in the title and abstract.

Response: We thank the referee for raising this discussion. During reviewing of all the related literature, we realized how much differences these amino acids’ mutations could make for the function of the systems. Distinguishing these minor but sometimes critical changes in the process of choosing the right plasmid is quite confusing and complicated. That is why we decided to write as many details as possible. According to our practical experience, such information is very useful for choosing the right inducible expression system. Moreover, as the mutagenesis-driven optimization of each system is still going on, these details will also give a good reference for researchers to avoid re-establishing the same or similar mutations. We find this important and thus we hope that you will also find our explanation valid.

Specific problems:

The writing of “knock-out, knock-down” etc. should be unified: together or two separate words, with a dash?

Response: Thanks for pointing this out. Yes, we have corrected 3 places with this issue. We have now applied knockout, knockin, and knockdown.

Attention should be paid to all the abbreviations. Technical terms are not consistently used.

Response: Thanks for pointing this out. For each figure, we now have stated the full names of all the abbreviations. In the main text, we also have written full names of EGFP, BHK and so on. For technical terms, we are not quite sure what you mean.
When the authors are describing the composition of a system, they should replace the term “reporter gene” (which is just an example of its use) with “gene of interest.” Or, to include also the shRNAs, perhaps “expression cassette of interest” would be an even better option?

Response: Thanks for the advice. We have changed some of the “reporter” into “gene of interest”. But still in several cases, especially when we describe light switchable transgene system and ABA-inducible expression system, this is because these systems are so novel that all the assays done are still a proof of concept. So far, only a few reporter genes have been tested successfully.

In our figures, we now use the term “expression cassette of interest”. Thanks for this suggestion.

For additional recommendations, please see the attached file with annotations.

Response: Thanks for providing us with the attachment. It is most helpful. We have now corrected most of the parts. Only ones list below are not changed as we do not find them with confusions and mistakes:

1. Line 20, “temporarily and quantitively at will”, quantitively is spelled correctly.
2. Line 30, “lethality-causing genes or essential genes”, “lethal genes” are more oral ways to describe “lethality-causing genes”. So we still keep the original way.
3. Line 83, “newly engineered rtTAs with few mutations make them exponentially active and sensitive”, here “them” refers to rtTAs. From the context of the sentence, we feel there is no confusion.
4. Line 137 and 138, “Again, gene expression controlled by the minimal promoter is activated by removing cumate in the medium”, the reviewer suggested to remove “in” from the sentence, which makes the sentence grammarly problematic to our understanding. So we keep it as before.
5. Line 185, “Similarly, Gal4DBD has been fused with ABI1 and VP16 with PYL1”, “similarly” means “similarly” to the just mentioned rapamycin inducible expression system above the paragraph. Likewise, when people use “accordingly”, the word refers to the just mentioned logic or rule. People do not have to emphasize “accordingly to…”.
6. Line 189, “The first one got inspiration from the molecular basis of the circadian rhythm of fungi. Vivid (VVD)”, the reviewer suggested changing “get inspiration from” to “was inspired by”. We think the two ways hold similar meaning.
7. Line 212, 216, 224, regarding using “reporter” other than “gene of interest”, it is because they are in the description of the light-switchable transgene system and ABA-inducible expression system. As I explained above, these systems are so novel that all the assays done are still a proof of concept. So far, only a few reporter genes have been tested successfully. According to reviewer’s comments, we believe it is appropriate to use “reporter” (presents “example of its use”) here.