Supplemental Material

CBE—Life Sciences Education

Stefanski *et al.*

Reviewer #2 (Remarks to the Author):

A few specific suggestions:
1. You state that the complete LOCI can be provided separately to readers, on the assumption that to publish it with the article would invite student access. On the one hand, I doubt that undergraduates would encounter LSE accidentally in the course of their studies. On the other hand, wouldn't that be an acceptable way for them to correct their misconceptions? Besides, your detailed discussion of the items indicates quite clearly what the expected answers are. I encourage you to provide the complete LOCI questions as a supplement to the paper.

The authors have chosen leave the complete LOCI out of the manuscript to maintain the integrity of the instrument. We will gladly and freely share it with instructors or researchers who may wish to use it in the future.We simply wish to communicate with those who wish to use it in order to ensure that the LOCI is not widely disseminated or used inappropriately. Not only are we concerned that students may happen upon it, but the more likely event is thatsome instructors may use the items as test questions leading to more broad dissemination. We feel it is appropriate and in keeping with precedent to ask instructors to request the LOCI (Smith *et al.,* 2008; Kalas*et al*., 2013; Deane *et al*., 2014).

2. Some grammatical and punctuation suggestions:
Lines 33-35 and 36-37 both have dangling introductory participial phrases. Recast to provide an answer to the question, "WHO is using?" Here is where the active voice can be a big help.
Line 39: "The data...were.." Make the verb plural to match the subject.
Line 48: This sentence seems incomplete...
Lines 71-79: This seems unnecessarily general and does not advance your argument.
Line 76: "...benefit from the gaining knowledge of..." What does this mean?
Lines 101-102: I'd omit the sentence about the Nobel Prize.
Line 130: Here might be a good place to point out that CI's directed at undergraduates are even rarer than those directed at high-school students. Suggest: "... of developing undergraduate learning tools..."
Line 147 and 150 are repetitious of each other.
Line 172: "...process, the sample sizes for each stage..." Add a comma and remove the "s" from sample.
Line 189: remove the comma after "...role of,..."
Line 208: remove "that" to make parallel construction of the things being ensured.
Line 223: remove "enough;" "not sufficient" is sufficient!
Line 225: Your refer to Item 5 but only later do you say what it is.
Lines 235-239: You use the word "thoroughly" three times in this short space; reword?
Line 246: put an apostrophe in "student's"
Line 279: "...data were limited...but are large..." (or for the second one you could say "but the set is large"
Lines 296-301: I recommend moving the sentence that begins "For this analysis..." to the end of that paragraph for logical flow.
Line 353: put a comma after "mutations"
Line 417: say "proportion" instead of "portion"
Line 454: Shouldn't this reference be to Figure 2, now that you have a Figure 1?
Line 474: "...showed that many students..." Add a "that"
Lines 496-498: This is an incomplete sentence

Line 552: "..within in..." Remove the "in"

All of the above line by line grammatical corrections have been made. Edits are indicated in green or by strikethrough.

Reviewer #3 (Remarks to the Author):

I agree with the authors' claim that the field could use more CIs that cover a broader range of core biology concepts. However, I am not sure the lac operon, as a specific example, qualifies as a core idea. The regulation of gene expression is absolutely a central concept, but knowing the details of this specific operon (as opposed to other operons, like the trp operon), I would argue, is not important. In fact, it is often the case that students get confused about details and do not see the big picture of gene regulation (and data reported herein support this assertion given the kinds of misconceptions students exhibit). That students think the enzyme genes are not transcribed when lactose is present is a major issue that suggests they are missing the entire point of the operon mechanism. The learning goals and instrument also do not address transfer; can students use the mechanism of this specific operon to speculate about other phenomena that are novel to them? (some examples of other phenomena were discussed in the manuscript). Again, this is due to the overly narrow focus of the goals; it is unclear that even students who know the lac operon well understand gene regulation more broadly. I suggest that the authors justify their choice of such a narrow focus in their initial argument and better motivate the need for such an instrument.

The following text has been added to the manuscript to better defend the utility of learning the lac operon in the larger scheme of learning gene regulation and that we anticipate that this CI will be used in the context of a course and be only one of many examples of gene regulation that a genetics or microbiology student would learn.

"Two well understood model transcriptional regulatory systems for learning the basic principles of gene regulation are the *trp* and *lac* operons, one of which is inducible and the other is repressible. Having a firm understanding of one of these operons makes understanding the other simpler as their mechanisms of action only vary in how binding at the allosteric site affects the repressor. Once students have a grasp of these examples of prokaryotic gene regulation that knowledge serves as a base for the scaffolding of other bacterial operons and also more complicated systems of gene regulation, such as complex eukaryotic transcriptional regulation, epigenetics, negative feedback loops, molecular cloning, and systems biology (e.g., Olaharski et al 2005; Cronan, 1988; Zoller et al, 2015). While the focus of this particular CI is a single operon, instruction within the context of the entire course would provide opportunities for students to observe and evaluate the similarities and differences in other gene regulation systems."

Other issues:

• On page 9 the authors note that the existence of a CI can encourage others to develop new instructional methods for these concepts. I don't see why this is the case. Moreover, in relation to my major concern above, I do not think that additional focus on this specific operon is really beneficial if what we want is for students to understand gene expression and regulation thereof.

We feel that faculty may be more willing to assess and adjust their current teaching methodologies if an assessment method with results that were shown to be valid was already in existence and freely available.

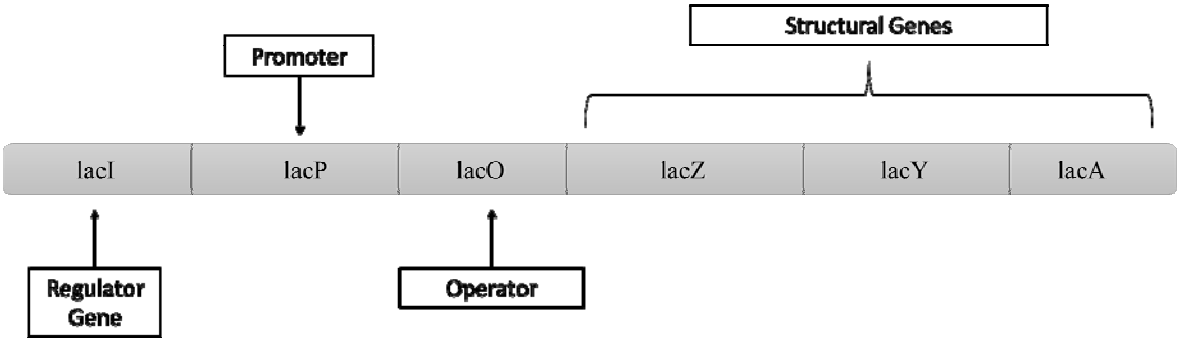
We have addressed this in part, on page 9 with the addition of the following text:
"It is hoped that the availability of this concept inventory will remove hurdles for faculty who wish assess and perhaps publish their own teaching methods and activities regarding gene regulation, but do not have the time to develop such assessments of their own. Well-designed concept inventories allow for valid and reliable means to assess student knowledge of particular concepts that may not be capture in other more traditional assessments. As such, the fine-grain analysis of particular items allows educators to adjust instruction tied to particular learning objectives and enhance the overall learning experience for students."

Also, as noted above, we anticipate that this CI will be used within context of a genetics or microbiology course and be one of many examples of gene regulation that a genetics or microbiology student would learn.

• Description of statistical indicators. I agree with prior reviewers that there is no need to provide the actual formulas (e.g. page 17), however, I think that in removing these some more important text was also removed (base don't track changes). I think that it would be helpful to keep in the more lay explanation of what these parameters tell you about the test and what are acceptable ranges for values.

Explanations of each statistic calculated has been put back into the manuscript along with the possible range for each.

Lac Operon Assessment Name _____



I. Using the above diagram and your knowledge of the lac operon answer the following questions.

Lac Operon Structures/Components

1. The lac repressor binds to what site within the lac operon?
    a. Promoter
    b. Operator
    c. Regulator
    d. Structural Genes

2. Which of the following does NOT encode for a protein that is directly involved in metabolizing lactose?
    a. lacZ
    b. lacY
    c. lacI
    d. lacA

3. The lac repressor is inactivated by binding to _____ whichis derived from _____?
    a. Allolactose, lactose
    b. β-galactosidase, galactose
    c. Transcription factors, glucose
    d. RNA Polymerase, lactose

4. Which of the following encode a protein that plays a direct role inregulating the expression of the Lac operon?
    a. lacI
    b. lacP
    c. lacO
    d. lacZ

Predicting Outcomes

5. Is mRNA transcribed from the lac operon when lactose is present AND glucose is not present in the cell? Why or why not?
    a. No, because the inducer is not present
    b. Yes, because the inducer is present
    c. No, because the inducer is present
    d. Yes, because the inducer is not present

6. Is the lac repressor active in the absence of lactose within the cell? Why or why not?
    a. Yes, the repressor is active because the inducer is not present.
    b. Yes, the repressor is only active in the presence of lactose
    c. No, the repressor is not active because the inducer is not present.
    d. None of the above

II. For the following questions use the information provided in each question, the diagram, and your knowledge of the lac operon to give your best answer.

Mutations

7. Predict the phenotype of an *E. coli* with a *lacI*$^-$ mutation in an otherwise normal genome.
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.

8. If a second, normal (wild-type), copy of the *lacI* gene is inserted into a cell with a *lacI*$^-$ mutation what will the phenotype of this partial diploid be?
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.

9. Predict the phenotype of an *E. coli* cell with a *lacI*^{S}, "super repressor," mutation in an otherwise normal genome.
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.

10. If a normal copy of the *lacI*gene were inserted into a cell with the *lacI*^{S} mutation what would the phenotype of this partial diploid be?
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.

11. An operator mutation, $lacO^C$, prevents the operator from being bound by the repressor. Predict the phenotype of an *E. coli* cell with this mutation.
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.

12. Using your knowledge of the lac operon, predict the phenotype of a cell that exhibits a loss of function mutation in the promoter.
    a. The lac genes would only be transcribed in the absence of lactose.
    b. The lac genes would only be transcribed in the presence of lactose.
    c. The lac genes would be transcribed continuously.
    d. The lac genes would never be transcribed efficiently.