Dear Editors and Reviewers,

We thank you very much for the insightful feedback and have addressed each point below. We believe that after revision we have greatly improved this manuscript and appreciate the consideration for publication. Our responses are below each concern outlined below.

Editor's comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

We have taken the time to proofread the manuscript to remove spelling errors and standardize abbreviations.

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

We believe we have corrected this throughout the manuscript.

3. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We have added more detail to specific sections such as both PCR protocols and added references for more information on PCR. We have also added more details to the nucleosome assembly section to clarify the use of the peristaltic pump.

4. Section 1: Besides the general instructions, please include some specific example that can be included in the video and that will help the readers/viewers see the implementation of your protocol. For details that will not be included in the video, as stated above, please cite references.

5. Section 4: what are the PCR conditions?

As noted above, we have added details on procedure, conditions, and a few references for both sections involving PCR.

6. Section 5: lines 131-132: what do you mean by "dissolve and combine like aliquots of histone protein pellets …"?

This was meant to emphasize that all aliquots of H4 were combined, all aliquots of H3 were combined etc. We have rewritten this sentence to be more clear.

7. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.

We have included the space and highlighted sections for the video.

8. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

In our first paragraph, we have added some details on the critical steps within the protocol including some modifications and troubleshooting of our technique as well as the major limitations. The next several paragraphs then discuss the significance with respect to existing methods and the extensive future applications of the technique. We have used some of our previous work as examples.

Reviewers' comments:

Reviewer #1:

Major Concerns:

None

Minor Concerns:

1. Line 55, describe key features of the plasmid used to express histone, such as origin, copy number and promoter.
2. Line 68, briefly describe the acetyllysine incorporation system. Origin and copy number of the plasmid? Which AckRS and tRNA mutants are used? How is the expression of AckRS and tRNA controlled, i.e. promoters? A reference is also needed here.
3. Line 106, Can the authors comment on the yield of the modified protein? How many mg of protein is obtained from 1 L culture?

This reviewer has brought up an excellent point brought up and we have added this information for all the plasmids. We have added several sentences describing each of the plasmids used as well as their origin, copy number and promoter of each. We have also added estimates of protein yield of wild type versus acetylated proteins.

Reviewer #2:

Major Concerns:

None

Minor Concerns:

For the most part, the protocol is easy to follow. I have just a few suggestions:

1. I know that there will be a table of abbreviations, but I urge the authors to define non-common abbreviations in the protocol. And, do so for relatively naïve readers. For example, pEVOL-AckRS, GuHCl and 601 DNA.

This issue was brought up by the editors and our first reviewer as well. We have gone through and defined and standardized all abbreviations as well as included a short introduction to 601 DNA and its origins.

2. Be a little more explicit. Don't make your readers assume or infer your meaning. In plasmid construction 1.1, the authors mention 4 histone protein plasmids, but these plasmids were never introduced. Also, the authors never explain how to do whole-plasmid PCR (1.2).

See our responses above. We believe we have successfully addressed these issues.

3. When the authors introduce CobB, they should mention that it is the only KDAC in E. coli. Since CobB is a sirtuin, then it makes sense that the sirtuin inhibitor nicotinamide can be used instead of a cobB deletion mutant.

This is excellent information that should be included. We have pulled a some references and included the background information on CobB and the rationale behind using nicotinamide as an alternative.

4. Please refrain from jargon. I know that "knock-out" is in common usage, but the formal term is "deletion," which is more explicit.

This terminology has been updated.

5. Under plasmid construction 3, I think the authors have made a typo - shouldn't it be 3.1 and not 1.1?

These typos have been fixed.

6. The authors mention the use of nicotinamide to inhibit the sirtuin CobB, but they never say when to add the nicotinamide nor the appropriate concentration.

We have added the appropriate concentration and note to add it with inducers.

7. On line 206, please define "extended periods."

In this case, we have added that it means more than 2 weeks.

Reviewer #3:

Major Concerns:

1. Line 61: Is it PFU DNA polymerase , PFU turbo DNA polymerase? The authors should clarify with the vendor name.

We express an in-house construct of PFU polymerase that we do not have complete sequence information for. Really any polymerase is acceptable for PCR. We have added that information to our manuscript, but is secondary to the procedure.

2. Line 160: liquid transfer pump should be changed to peristaltic pump. And it is also better to put the speed (and/or length) of no salt TE buffer.

We have added more details on how this is done. Our pump does not have a speed setting beyond the vague options of slow, fast, and purge, but we have included our specific method in the protocol with this pump.

3. Figure 1, the authors should label the lanes. I cannot see the bands of tetramers in H3K14Ac, H3K27Ac, H3K37Ac, and H3K64Ac.

We have added markers for each histone protein which should make this more clear. Some of the bands are very faint because we work at low protein concentrations once we are at this point in the protocol.

4. Figure 2, what are upper bands (~30 kDa and ~25kDa) in the H3K4Ac?

We have added protein markers. We believe the ~25kDa band is left over His6x-SUMO but it is difficult to be sure.

5. Figure 3, the authors should remove the gel well part. And it is also good to load at least one of acetylated nucleosomes as well.

This is an excellent point and we have replaced Figure 3 with an example of H3K64Ac assembled nucleosome.

6. Figure 4, there are three panels, the authors labelled after nucleosome thermal positioning in the center. What are left and right panel? The authors should describe in the legends. And it is also nicer with at least one of acetylated nucleosomes. Should we apply thermal positioning even in the destabilized acetylated nucleosome?

We have updated this figure to be more clear and removed one of the panels since it's not explicitly necessary. It's certainly possible that thermal positioning could further destabilize the nucleosome and have included an example of this in figure 3. It may not always be advised especially for acetyl lysine sites that are known to be more unstable. We have updated our protocol to include this.

We deeply appreciate the constructive feedback and opportunity to submit this manuscript for your reconsideration.

Best,

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