Mapping active regulatory elements genome-wide in cultured primary neurons using ATAC-seq. Response to reviewer comments

Reviewer 1:

> Reviewer #1: The protocol submitted by Maor-Nof et al., describes the application of optimized ATAC-seq to live plated primary neurons. It is well written, clear and concise. Some steps and quality controls would require a bit of clarification and some additional explanation as indicated in my comments.
>
> Overall, I found this protocol quite straightforward (for people used to perform ATAC-seq) and clear and therefore recommend it for publication in STAR Protocols, pending the required revisions are made.

We thank the reviewer for the useful comments and suggestions and have made the recommended changes to the manuscript.

Reviewer 2:

> Reviewer #2: This protocol from Maor-Nof et al. provides an updated ATAC-seq procedures specific for cultured primary neurons. Overall, the protocol is well-written and will be ready for publication once the details mentioned in the review are clarified.

We thank the reviewer for the provided comments and suggestions. We have changed the manuscript accordingly.

Reviewer 3:

> Reviewer #3: In this study, Maya Maor-Nof and colleagues introduce a new protocol assay aimed at identifying robust and seamless footprints of Transposase Accessible Chromatin sequencing and associated mapping of active regulatory elements genome-wide in cultured primary neurons. Overall, a comprehensive and detailed protocol by Maor-Nof et al that will be very useful to the primary culture research community studying gene regulation. I recommend acceptance with minor revisions as indicated in the comments below.

We thank the reviewer for the suggestions.

> 1. A small introduction describing what each major step sets out to achieve at the start would be useful.

We have added such introductions to most sections.

> 2. Analysis workflow for ATAC-seq data and profiling is missing and need to be included.

We agree with the reviewer that ATAC-seq data processing and analysis is an important subject, but this goes beyond the scope of the current manuscript, the focus of which is the experimental protocol for carrying out ATAC-seq on plated neurons.

We have added an explicit referral to our recent detailed description of ATAC-seq data and processing (Marinov & Shipony 2021) regarding processing and analysis.

> 3. More information regarding index hoping would be useful, or reference to the literature.

The protocol described here is largely agnostic regarding the particulars of the sequencing downstream, aside from that sequencing having to be done on a Illumina instrument. Index hopping is an esoteric subject that is beyond the scope of the current protocol; it has also never been shown to be a significant issue for typical bulk ATAC-seq experiments. We have added a note to the text advising users to pick optimally compatible sequencing index pairs prior to pooling of libraries for sequencing.

4. Heat maps depicting normalized ATAC-seq signal at all TSS, sorted by signal intensity should be part of the manuscript and missing here.

We have added such a heat map.

5. Real-time qPCR for the assessment of the quality of ATAC-Seq libraries is missing.

We use qPCR to evaluate the optimal number of amplification cycles for final library generation and this is already in the manuscript.

We are assuming that the reviewer refers instead to running qPCR against a panel of positive known open chromatin sites and a set of negatives and verifying that there is enrichment in the former relative to the latter. We have never used qPCR to evaluate quality in such away for ATAC-seq. In our opinion, recommending to do that would not be of benefit for readers of the protocol, and it would actually be a net negative for their workflows. The TSS score metric that we use is the gold standard for ATAC-seq QC, and can be applied on a small QC run (i.e. just a few hundred thousand reads for each library), before committing to a full production run (this is something we do routinely). We have added that advice to the text. Evaluating enrichment with qPCR against open known chromatin targets is redundant and only adds an unnecessary extra expense in terms of reagents and time.

6. Major step of ATAC-seq data analysis is to identify accessible regions (also referred to as peaks) and is the basis for advanced analysis which is again lacking in this article. Although Peak tracks generated by these tools analyzed and visualized in this manuscript, count-based analysis behaves similarly but are quite different from shape-based tools.

As we noted above, the processing and analysis of ATAC-seq data is beyond the scope of this manuscript (if we were to provide a proper treatment of that subject in this manuscript, its length would likely have to quadruple) and we have already described all our procedures in complete detail previously.

7. By its nature ATAC-seq reveals multiple aspects of transcriptional regulation, the major step involving a protocol paper interpretation at four different levels: peak, motif, nucleosome, and TF footprint. Accordingly, authors need to provide convincing data that their experiments achieve motif and TF footprints in these parameters.

We are not sure what exactly the reviewer is requesting here, as the quality of the neuronal ATAC-seq libraries is very high, as shown in Figure 2C. TSS scores between 20 and 30 are as good as have ever been seen in ATAC-seq libraries, generated by us or anyone else. There has never been a question in the literature whether ATAC-seq datasets of such high-quality could somehow be inferior in terms of footprint detection, and therefore there is no basis for comparison and evaluation. It is also not the case that a pure protocol manuscript is not the place for such analytical comparisons. We have added an example of TF footprints based on neuronal ATAC-seq data for illustrative purposes.

8. I also suggest adding a section on ATAC-seq QC of the NGS data. A brief explanation about Diffbind and what to look for would be helpful prior to moving forward to Diffbind.

We are not sure why the reviewer suggests that we should talk about Diffbind. We have never used it, not are we aware of it being widely used for ATAC-seq analysis. There is a vast array of tools for analysis of NGS data, and their comprehensive description is beyond the scope of a manuscript dedicated to a experimental protocol.

9. In some cases critical steps and alternatives are missing for equipment or reagents.

There is indeed a wide variety of reagents that could probably be used in several steps in the protocol. However, we can only describe the reagents that we ourselves have used and can verify that they work. We have included alternatives in the cases where we have used more than one reagent, but we cannot go beyond that as we cannot verify that those further alternatives in fact work as well as what we use.