Reviewer Report

Title: RepeatFiller newly identifies megabases of aligning repetitive sequences and improves annotations of conserved non-exonic elements

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Reviewer Comments to Author:

The authors presented a neat way of rescuing the potentially missing whole genome alignments for the repeat masked regions. I have a few questions and concerns regarding this manuscript.
1. The authors demonstrated the application of RepeatFiller on human hg38 against other 20 mammals. I am curious to see how well RepeatFiller can be applied to other vertebrates or even invertebrates? How it copes with highly fragmented assembly? The guidelines of how to choose the options on other cases are highly desired from the authors.
2. In "Generating pairwise genome alignments", the authors used the same lastz alignment parameters and default scoring matrix for genome alignment against hg38. I wonder why this is case? For instance, in UCSC, Human vs. Rhesus uses the human_chimp.v2.q scoring matrix for closer species. I expect to get many spurious alignments from Human vs. Rhesus alignment, hence much lower "added aligning sequence" for Rhesus in Figure 2.
3. Can authors explain a bit on what factors might be related to amount of "added aligning sequence" in Figure 2? I would expect to see a higher recovery rate for the species that are more evolutionary distant and have better assembly quality, because the co-linear alignments should be anchored better in the first round of alignment. However, it doesn't seem to be the case.
4. In terms of the novel repeat-derived conserved non-exonic elements, further details of those CNEs is needed. I have concerns about how genuine those CNEs are. Do they similar characteristics, compared to other normal CNEs? Are these repeat-derived CNEs also AT-rich? Do the widths of CNEs follow a power-law distribution? Is there any locus where only repeat-derived CNEs exist?

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