Reviewer Report

Title: Best genome sequencing strategies for annotation of complex immune gene families in wildlife

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Reviewer name: Michael Hiller

Reviewer Comments to Author:

Knowledge about immune genes is critical for species conservation programs. However, immune genes occur in large gene clusters that are difficult to assemble and annotate. This important and timely study uses a number of marsupial genomes and the platypus to assess which sequencing technologies enable complete reconstructions of immune gene clusters and which methods enable annotations of these immune genes.

I have the following comments.

Since Fgenesh++ and Maker produce automatic annotations, I wonder why not all 6 genomes were annotated with these two methods? This would allow a comparison between Fgenesh++ against Maker. Maybe it is possible to annotate at least a few genomes with both methods.

Direct assessments of assembly quality should ideally be done on different assemblies of the same species to rule out real differences between species.

Would it be possible to include previous koala or platypus genome that was much more fragmented?

Figure 1 shows a useful of all immune genes. However, some genes like TLRs are actually easy to annotate as they are have a standard gene structure.

Therefore, it would be informative to provide in this figure a breakdown of how well the different immune gene families are annotates, as the authors nicely did in table 2.

This would inform on which immune genes are particularly difficult to annotate.

Figure 3B is not colorblind friendly.

Line 275: The discussion makes it clear that this is a scaffolding error and not a real inversion. This should be clarified here as well.

I fully agree with the value of the manual annotations. Therefore, it would be helpful to provide the manual annotations also as a gff3 or gtf file that provide the full exon structure. Additional file 2 only lists the start and end coordinates of genes with multiple exons. The assembly accession should also be listed.

As a suggestion: A haplotype-resolved assembly of a marsupial is likely not yet available, but such an assembly would provide an opportunity to further investigate the influence of assembly quality and haplotype variation in immune genes.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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