Gillingham and Co-workers have developed a workflow to improve the genotyping of MHC class I and IIB genes in non-model organisms. The MHC genes are often highly duplicated and although the different gene copies are extremely polymorphic different alleles can be highly similar. High-throughput amplicon sequencing enables the characterization of the MHC genotype in individuals/species with extreme MHC gene copy numbers.

The first main step in the laboratory is a PCR that amplifies over the entire multi-locus of MHC I or MHC IIB (all gene copies are amplified at the same time) and then follows high-throughput amplicon sequencing, here using Illumina. Both these steps in the laboratory generate artefacts, hence MHC alleles that do not really exist. These artefacts need to be filtered away and this is the main topic of the present manuscript. How can we filter away artefactual MHC alleles while keeping all the true alleles?

Several different research groups have been engaged in this filtering process and different laboratories use different programs / workflows. The present study provides a partly novel pipeline how to filter MHC data, ACACIA, it is user friendly and flexible according to the authors. ACACIA might be advantageous to the existing programs / workflows, this is not really fully tested in the manuscript but I think the ACACIA workflow seems excellent and I am very positive to this part of the manuscript!

Gillingham and Co-workers use three different data sets that they filter using their workflow ACACIA:

- 241bp MHC class IIB that has been amplified using perfect chicken primers, in a PCR with 30 plus 10 cycles with 10 minutes of extension.
- 151bp MHC class IIB that has been amplified using poor chicken primers, in a PCR with 30 plus 10 cycles with 10 minutes of extension.
- 236bp MHC class I that has been amplified using primate primers, in a PCR with 25 plus 7 cycles without any 10 minute extension.

These three data sets differ in amplicon length, number of PCR cycles, PCR settings, target genes, amplicon read depths etc.. These differences are major and all have considerable effects on the outcome of the filtering. It is impossible, or at least very difficult, to draw any conclusions on findings considering artefacts / chimeras etc. between these data sets since a large number of the parameters differ between the data sets. The authors should either have run all settings in one study data-set or one setting in all data sets (or all combinations for all data sets).

However, in my opinion the authors have an excellent set-up with the chicken and the artificial MHC genotypes, very clever design, and my advice would be to focus your ACACIA manuscript on the chicken only and present your nice ACACIA workflow based on the chicken data with the perfect chicken primers. The MS would also improve if you compared the output and repeatability of the ACACIA with several different MHC filtering pipe-lines. At present you only test amplisas.

I think you should compare your ACACIA pipeline with that of other filtering programs / workflows and explain how they differ and also what similarities they have, and why you choose the setting that you did.

Finally, figure 2 reuses data, 2b plus 2c is equal to 2a, not so, moreover it is tricky to interpret and understand figure 2, think about rearranging this figure.