Reviewer Report

Title: Sequencing smart: De novo sequencing and assembly approaches for a non-model mammal.

Version: Revision 1 **Date:** 3/19/2020

Reviewer name: Ellie Armstrong

Reviewer Comments to Author:

Etherington et al. have responded well to all reviewer comments and have produced a very nice and thorough paper investigating various genome assembly technologies and their impact on degraded samples. The paper reads extremely well and I think the authors have done a particularly good job explaining some more advanced genome assembly concepts that I think will be very beneficial to those in the field dealing with non model organisms. I have included just some brief comments below that address some minor grammatical/sentence errors. I also hope the authors are doing well in this very uncertain time and wish them all the best with their future research. Great work to all!

Abstract, Background: "little is known about the correlation between genome sequencing..." I would just write relationship instead of correlation here. Correlation is a bit of a loaded word.

Introduction, top of pg 4: "the cost of generating this amount of data and assemble..." assemble should be "assembling"

Introduction, pg 5, paragraph 2: First sentence reads a bit funny saying that organisms are species from populations, maybe rephrase to: Many samples from non-model organisms originate from wild populations that are highly heterozygous"

Page 6 Sequencing technologies: "Recent machines" to just "Machines"

Page 7, Long Mate Pair sequencing: Clarification on this, is 4ug of DNA required to generate all 12 LMP libraries or is this per LMP library?

Materials and methods, page 9: "Using the same sample of a roadkill European Polecat sample"--delete second "sample", redundant

Materials and methods, page 9: You list the coverage for all the libraries except the four lanes of 150bp PE 10x, so maybe just add that in.

Page 15, Ranking assemblies: The first two sentences of this paragraph can probably be combined, seems repetative?

General comment: It might be useful in your tables and the text to add in ',' to your numbers. For example, 300,334 instead of 300334. I find that with a lot of big numbers it is easier to read this way and quickly get a sense for the magnitude of the difference, i.e. if its 1,000,000 vs 100,000 makes it easier to see that is larger than if you just have 1000000 vs 100000.

Materials and methods, page 9: "We generated 664 Gb of Bionano molecules" --just checking on how this is supposed to be referred to. Do you generate "Bionano molecules" or "Bionano data". Just wondering if this would make more sense to refer to this as data rather than molecules here and later in the paragraph.

Irrelevant, but interesting: In table 4 for the reapr breaks for 10x, i wonder if these would vary much between the two pseudohap outputs...

Discussion pg 28: You mention that you address heterozygosity, but that wasn't explicitly addressed in your analyses, so may just want to reword slightly or mention something about how much more (or not) the polecat is from the ferret or from the rat?
Irrelevant also: I really am bummed that 10x assemblies wont be able to be generated for much longer because of the lawsuit. Idk if it would be worth mentioning that here or towards the end of the discussion, but it certainly makes your call to com up with new ways to do linked read assemblies more relevant...
General, supplement: Do you happen to have the pre-bionano tape station or agilent traces from the ferret? It might be interesting to show the difference of molecule dist sizes between that and the roadkill ferret sample.

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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