Reviewer #1 (Comments for the Authors (Required)):   
  
This manuscript, "Genome Assembly of Synthetic Allotetraploid Brassica napus Reveals Homoeologous Exchanges between Subgenomes," provides yet another genome of Brassica napus, in this case the synthetic Brassica napus cultivar, Da-Ae. The manuscript mainly compared the genome assembly results of different methods, and to a lesser extent with previously published genomes.   
  
The manuscript has numerous flaws.   
  
1. The title of the manuscript is misleading with respect to revealing any insight about homoeologous exchanges (HE). The authors fail to cite the extensive literature on this top (original classical papers and many reviews, on both HE in general and specifically in Brassica allopolyploids. Even the literature on HE in resynthesized Brassica napus allopolyploids is extensive and ignored. Nothing new is revealed about HE in this manuscript not already described in the published literature.

*Thank you for pointing out this omission. We now include citations to Higgins, 2018 and 2021; Xiong 2021; Gaeta, 2007; Stein, 2017, Lloyd, 2018, and Raman 2022 when discussing HE in the introduction (lines 68-72).We have also increased our comparison of HE regions between varieties (lines 441 – 512).*  
  
2. The genome comparisons are inadequate. As the author mentioned, there are many new and high quality Brassica napus genomes available now, specifically a updated Darmor-bzh genome (v10, <https://doi.org/10.1093/gigascience/giaa137>). Why are the authors using older versions in their comparisons?

*We now compare with Darmor-BZH\_V10, GanganF73, No2127, QuintaA, Shengli3, Tapidor, Westar, Zheyou73, and ZS11*  
  
3. The genome assembly methods are inadequate in numerous ways.   
Fist, based on the sequencing technology they used in this study, why is it that other published studies can get a chromosome level assembly? Why can this study not add Pacbio and HiC sequencing? What does the HiC result look like?

*I am not sure what is being asked here. We did use PacBio and HiC sequencing and we did achieve chromosome-level assemblies. In this new version of the manuscript we have removed description of our intermediate assemblies, perhaps that was the source of confusion.*

Second, the authors try to verify the discrepancy between Da-Ae and Damor-bzh assembly, and the homoeologous exchanges based on mapping results, but what does the HiC matrix looks like in these regions?

*We have added the Hi-C matrix plot; it supports our assembly. Also, while we used Darmor\_4.1 for this comparison (V 10 was not available at that time), Darmor 4 and Darmor 10 are co-linear so the results are the same as if we had used the newer assembly.*

Third, the authors claim "assembly had significant support from the mapped reads and scaffolds" in line 207, but what is the standard for significant support?

*Document how? Change “significant” to “substantial” and define what we mean / what our threshold is.*

Fourth, is GO annotation a better method to evaluate genome completeness than BUSCO or LAI?

*There must be a misunderstanding here. We did not use GO annotation to evaluate genome completeness, we used BUSCO both in the original submission and in the current submission. We did use GO to evaluate presence-absence variation between genomes. For the purpose of asking there is enrichment for particular categories of genes affected by presence-absence variation, GO is indeed the better choice*

Fifth, with respect to homoeologous exchanges (HEs) identified a the "gene-level" and "sequence-level" - how consistent are these two methods? How many HE genes are in the HE sequences? 

*We have changed our HE analysis methods and now use a synteny/homology based approach. We discuss and illustrate when HE detected by synteny will and will not lead to a change in coverage.*

Minor comment: Figure 7 should have a higher resolution.   
  
  
  
  
Reviewer #3 (Comments for the Authors (Required)):   
  
This paper presents a reference-quality genome assembly of a resynthesized Brassica napus line produced by third-generation genome technology. By my assessment the genome assembly is of very high quality. However I have some substantial concerns about other aspects of the manuscript including its framing and claimed significance and potential confounders. While the genome assembly is commendable I believe the authors need to seriously reconsider the framing and potentially include analyses that truly highlight the utility of such a resource.   
  
Major comments:   
  
1) There is a major conceptual confusions I believe the authors possess that flows through the manuscript. Above all, the authors treat the assembly as truly Brassica napus, which I do not believe is correct.   
  
For example, lines 98-100 read: Concurrently we have generated a new genomic reference for a synthetic B. napus that includes a significant number of previously unscaffolded sequences. Additionally, this new assembly reveals shared and unique homoeologous exchange events in different B. napus lines.   
  
However, while this synthetic B. napus assembly is more contiguous than many previous natural B. napus assemblies, it is uncalled for to claim they were "previously unscaffolded". The subgenomes and chromosomes of the synthetic B. napus are extant Brassica rapa and Brassica oleracea genomes. They are evolutionary distinct from the ancestral populations that served as direct progenitors for natural B. napus ~12,000 years ago. As such intraspecific genomic variation in gene and genome content will introduce true biological differences between the extant Brassica progenitors and the natural B. napus subgenomes. Additionally, the process of fractionation and diploidization which has begun in natural B. napus but not in a substantial way in the synthetic B. napus will introduce true biological differences in genome structure between synthetic and natural B. napus. Indeed, analysis from the B. napus genome paper (Chalhoub et al. 2014) highlight the sequence and structural divergence of B. napus subgenomes and extant progenitors.   
  
In this way it is not possible to know what differences are due to genome assembly differences and which are due to true differences between extant Brassica rapa and Brassica oleracea and the respective subgenomes in B. napus.   
  
This same concern applies to comments about relative genome size and gene composition made throughout the paper and treated as a major selling point of the genome. The hypothesized genome size of B. napus from combined Brassica progenitors is not a proper reference. Differences in genome size of different Brasssica rapa and Brassica oleracea cultivars can induce genome size differencs as well as the natural downsizing of subgenomes in polyploids and lineage specific duplications and deletions of genes.   
  
That said, there are purposes where a resynthesized polypliod does provide important insights about a natural polyploid. They are often related to early processes of response to polyploidy and subsequent genome evolution. In that sense the results about homoeologous exchanges being shared by natural and resynthesized polyploids is exciting and builds upon some early findings in the original B. napus genome paper (e.g. Figure 3 in Chaloub et al. 2014).

*We thank the reviewer for these important points. When we said “previously unscaffolded” content we were referring to sequence that was present in Darmor-bzh 4.1, but only in contig form (not in the chromosome-level assemblies), so in this particular case the reviewers concern does not apply. However, we do agree in general with the reviewer that many differences in these assemblies could arise from the different biological histories of the synthetic and historic B. napus lines and we have modified the manuscript accordingly. In addition, there are now many B. napus assemblies that are roughly comparable to ours in terms of quality so we have generally removed discussion of the our Da-Ae assembly being an improvement.*   
  
  
2) One other potential issue I believe the authors should address. Past work on resynthesized B. napus has shown they are afflicted by regular aneuploidy, (see Xiong, Z., Gaeta, R. T., & Pires, J. C. (2011). Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid Brassica napus. Proceedings of the National Academy of Sciences, 108(19), 7908-7913.)   
  
I believe the authors should verify that this resynthesized line retains a balanced karyotype after 7 generations of selfing. It is likely this is not the case. In fact, Supp figure 1. seems to show that the read-mapping coverage is not at 0, but slightly below for virtually the length of the whole chromosome, potentially reflecting aneuploidy that alters the proportion of read mapping to be unbalanced for the entire length of the chromosome.   
  
If in fact the genome is stable and does not show aneuploidy that is incredibly interesting and the authors may want to consider including an analysis that genotypes the parental lines and the resynthesized polyploid for loci and features that have been associated with homoeologous exchange and genome instability, e.g.   
  
Gonzalo, A., Lucas, MO., Charpentier, C. et al. Reducing MSH4 copy number prevents meiotic crossovers between non-homologous chromosomes in Brassica napus. Nat Commun 10, 2354 (2019). <https://doi.org/10.1038/s41467-019-10010-9>   
  
and   
  
Ferreira de Carvalho, J., Stoeckel, S., Eber, F., Lodé‐Taburel, M., Gilet, M. M., Trotoux, G., ... & Rousseau‐Gueutin, M. (2021). Untangling structural factors driving genome stabilization in nascent Brassica napus allopolyploids. New Phytologist, 230(5), 2072-2084.   
  
*We thank the reviewer for these suggestions. The apparent below average coverage in the previous version of the manuscript was an artifact of how the normalized coverage was being generated. Previously we took the mean coverage per chromosome and normalized to that on a chromosome-by-chromosome basis. Because most assemblies have collapsed sequences at the centromeric repeats, the apparent coverage in these regions can be very high, thereby skewing the chromosome-wide average and causing “normal” regions to appear to map low. We have rectified this by using median instead of mean coverage to normalize, and by using the genome-wide instead of chromosome-wide median for all calculations (indeed, chromosome-wide analysis could have made aneuploidy hard to detect). Looking at the new coverage plots, we see that the normal regions are now at 1X coverage (we have shifted our scale so that “1” represents normal coverage, not 0). There are regions with both increased and decreased coverage, some of these are due to homoeologous exchange and others could be due to aneuploidy and we note this in the manuscript.*

3) Additionally, one of the rationales for the paper given by the authors at page 88-89 is: Consequently, it has been challenging to generate a standard public consensus genome assembly for B. napus.   
  
But this doesn't make sense. There will not be a "consensus" genome because no single genome can capture the genomic diversity of a species, hence the growing interest in pan-genomes. While this assembly is of higher quality it does not get us closer to a "standard public consensus genome assembly", even if it was a natural B. napus cultivar. 

*We agree. This section has been changed to read “*Consequently, it is important to have genome assemblies from multiple different *B. napus* varieties as an aid to building a pan genome for this species.”