Reviewer #1 (Comments for the Authors (Required)):   
  
Despite addressing a number of outlined concerns, this manuscript still does not meet the bar necessary for publication in GENETICS. Three major issues that were considered critical for publication in the past review cycle remain unmet.   
  
First, the authors have not verified that their two non-sense alleles of dpy-22 produced truncated DPY-22 proteins (Major deficiency 1). This makes it problematic to interpret whether differing regions of the DPY-22 protein are involved in the arising phenotypes. The manuscript now addresses this deficiency by stressing that the authors are, instead, interested in the genetic regions missing in each allele, not in the missing part of the proteins. However, much greater insight would be learned if it was known whether their alleles resulted in stable mRNA transcripts and/or proteins. Circumstantial evidence already exists in other publications from this lab that supports the notion that both truncated proteins are produced, albeit sy622 is likely to be mis-localized.

*A prior publication (Moghal and Sternberg, 2003) fused GFP to the C-terminus of the protein products of both alleles, and showed stable expression. In addition, the* dpy-22(bx93)::gfp *and the* dpy-22(bx93) *alleles can complement* dpy-22(sy622)*.* *Finally, null alleles of dpy-22 are very likely lethal based on phenotypes observed from RNAi and from a strong loss-of-function allele,* dpy-22(e652)*. Taken together, these facts imply that there is functional product (protein) being generated from both alleles. In addition, the mRNA levels of* dpy-22 *transcript are not differentially expressed in either mutant homozygote.* dpy-22 *transcript levels are slightly down-regulated in the trans-heterozygote (FC ~ 0.75, q=0.010). These data show that the mRNA from either allele is stable.*

*We have added a sentence to the discussion regarding the stability of mRNA:*

***Line 377: In the case of our alleles, the relevant homozygotes had wild-type* dpy-22 *mRNA levels, suggesting that these mutations are affecting the biochemical properties of the protein product.***

Second, the authors did not logically describe how they arrived at their functional regions with sufficient clarity (Major deficiency 3). They re-wrote this section. However, my hunch is that there is now a labeling discrepancy between the text and Figure 6. That is, FR1 and FR2 seem switched in some descriptions. This makes it hard to follow the author's logic in this section.

*We have extensively detailed our logic regarding the inferences of functional regions in* dpy-22*. FR1 and FR2 are referred to correctly throughout the text.*

Third, the work does not measurably advance our understanding of dpy-22 biology to the standard required for publication in GENETICS. Though the authors have characterized the transcriptomic differences between two homozygous alleles, a trans-heterozygote, and the wild-type allele of dpy-22, the gene sets identified did not yield striking new insight into the biology of dpy-22. The authors added an analysis comparing dpy-22 mutant transcriptomes to the transcriptomes of worms impaired in two divergent downstream signaling pathways, Wnt or Ras, but failed to identify any separation of function or draw any memorable conclusions from these results.

*Our work revealed the existence of different functional units within the* dpy-22 *gene, some of which behave in a dosage-dependent manner. We associated these different units with independent genetic modules consisting of hundreds of genes. Finally, we asked whether these regions were interacting independently with Ras or Wnt. This test revealed a complex answer, which will require more research before we can determine how these units interact with the Ras and Wnt signaling pathways.*

*We submitted this paper as a proof-of-principle of a method we developed. Using a minimal set of alleles, we were able to successfully perform a complete genetic analysis. A major sign of success in our eyes is the ability to differentiate between the data that we understand and can explain in genetic terms and the data that we cannot explain and which challenges our models and biological intuition. Though our biological results may not be striking, we believe the methods we have developed show great potential.*

In addition to these major issues, the authors did not reference or mention the phenotype of the dpy-22 null or speculate how different the dpy-22(sy622) is likely to be from the null (this would probably need to be based off of dpy-22 RNAi assays). This seems like a crucial piece of information important for interpreting any study of an allelic series.

*The null phenotype is very likely to be lethal, based on available RNAi information and the increasingly severe phenotypes of loss-of-function alleles. Homozygotic hermaphrodites of a severe* dpy-22 *hypomorph (e652) are very sick (C. elegans II).*

The authors did go to great length to amend the paper by 1) clearly defining their meaning of gene activity, 2) removing unpublished data, 3) defining how they assigned putative functional regions, 4) addressing the direction of gene expression changes identified, 5) adding GO ontology analysis, 6) referencing only peer reviewed publications, and 7) testing whether dpy-22 mutants shared transcriptional phenotypes with strains impaired in two downstream signaling pathways.   
  
In addition, their original advances still remain in that the authors have taken several innovative approaches in tackling the problem of merging genomic RNA-seq technology to the genetic technique of studying allelic series. They developed an innovative false-hit analysis and an interesting dominance analysis metric. The python notebooks are quite well organized providing a useful online supplementary resource. And the concept of this work is very engaging, likely to stimulate a broad audience once key issues are addressed.   
  
*Thank you*  
  
  
  
  
  
CRITERIA FOR PUBLICATION IN GENETICS:   
• the study is of interest to a wide range of genetics and genomics investigators;   
• the results presented provide strong support for the conclusions reached;   
• the conclusions provide significant new insights into a biological process;   
or   
• the study demonstrates novel and creative approaches to an important biological problem,   
or   
• the manuscript describes development of new resources, methods, technologies or tools of interest to a wide range of geneticists.