

Editorial comments

- 1) The manuscript was proofread.
- 2) Additional keywords/ phrase added to bring the total to six.
- 3) Summary shortened to less than 50 words. The protocol and applications are described in the protocol.
- 4) The abstract was revised to describe the method in more general terms. Also, the purpose of the method, advantages and limitations are stated in the abstract and discussion.
- 5) All commercial language has been removed.
- 6) Personal pronouns have been removed from the protocol.
- 7) Protocol has been revised to contain action steps only. Text that could not be in imperative tense was added as note.
- 8) Protocol has been revised to contain no more than 3 action steps and no more than 4 sentences.
- 9) Weblink has been removed and reference has been inserted.
- 10) Limitations of technique has been added.
- 11) Figure 2 has been combined into one image file. Figure 2 contains two histograms and a cartoon.
- 12) Trademarks have been removed. (Needs to be removed from table of authors).

Reviewer 1 comments.

- 1) Personal pronouns such as we have been removed from the text.
- 2) *Dm ime4* full name and function has been added to the text.
- 3) Homozygotes have been substituted for homozygous line 67.
- 4) Commercial names such as Genetivision has been removed.
- 5) Described has been removed line 86.
- 6) The p-values have been added to the figure legends. Original excel files will be sent along as Supplemental Materials.
- 7) The percentage of embryos that developed to adulthood along with standard deviation have been added to the legend for Figure 2. Also appearing in the excel sheet.

Reviewer 2 comments

- 1) Each experiment was performed a minimum of 3 times, independent biological replicates were used to ensure reproducibility. Each experimental cross included two virgin wildtype females and 1 wildtype male.
- 2) A Chi square test was used to determine if there was a significant difference between the two different grape agar positions. The p-values have been added to the legend of Figure 2. Also highlighted in yellow in the excel sheet used in all calculations and bar graphs.
- 3) A Chi square test was used to determine the viability phenotype of *Dm ime4* mutants, the p-values have been added to the figure legend.
- 4) The error bar represents the actual numbers of the counts at individual stages for each biological replicate. The variance in the error bars are a result of different starting numbers of progeny per trial, not the percentages of progeny lost. However, the statistical analysis does suggest a significant difference between the agar positions. Please refer to the excel sheet sent as Supplemental Materials.
- 5) Although we did not examine L3, L3 larvae can be quantified as they crawl up the side of the vial. The L3 quantification statement has been added to the discussion (Line 177).

- 6) This is a valid point that it would be beneficial to look at viability of different genotypes and phenotypes; however, we used wildtype OreR to develop the reported assay and for proof of principle and troubleshooting. Thus wildtype Ore-R is used to provide a baseline for future experiments