Reviewer’s report

Title: Assessing the Contribution of Herpes Simplex Virus DNA Polymerase to Spontaneous Mutations.

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Reviewer: Dr Sandra Weller

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

The manuscript by Duffy et al has been significantly rewritten in response to the previous comments by reviewers. The rationale for experiments is now clear, and the control data for Table 2 is now addressed. New data has been presented on the conformation of HSV DNA in the tk gene region which may indicate that the tk gene from HSV-2 adopts an altered conformation. A couple of questions still remain, although the manuscript is much improved. Before publication, points 1-4 below should be addressed.

Major comments:
1. The control data for Table 2 has been addressed both in the M and Ms (pg 8 lines 14-Page 9, line 2) and also in Results (Page 12, first paragraph). In Table 2, the mutation frequency for test samples is given only as a percentage; this value reflects a normalization procedure involving the subtraction of the mutation frequency for the HP66 control in which the plasmid is assumed to be unable to replicate. However, it would be much more informative if the actual numbers of colonies in the test samples were shown. We are told that the mutation frequency for the HP66 control is 0.12% or 335 colonies in a total of 280,000. If the plasmid is not replicating in the HP66 infection control, how do the authors explain the seemingly high number of colonies. How does this number of colonies compare to the numbers obtained in the test samples? This data needs to be presented in order for the reader to evaluate the significance of the percentages shown in Table 2.
2. Page 13, line 8. The statement "However, our results clearly indicate that the HSV-2 6757 Pol is truly error-prone." This statement should be toned down somewhat since HSV-2 6757 Pol does not seem to be error prone in the recombinant bearing this gene in the HSV-1 background using the non-HSV-DNA assay (Table 2).
3. Page 14. The experiment showing S1 sensitivity of various plasmids is intriguing. One potential problem relates to reproducibility. Can the altered pattern observed with the tk gene from HSV-2 be related to some difference in the plasmid preparation itself; for instance, could there be more nicking in
one plasmid prep? How many separate preparations of this plasmid were analyzed?

4. The experiment shown in Fig. 2 implies that the HSV-1 and HSV-2 genes are different in terms of their ability to form an unusual conformation. The authors propose that the ability of the HSV-2 tk gene to form an anisomorphic DNA may contribute to the different mutation frequencies observed in the tk mutagenesis assay. It would be more convincing if they had actually transferred the HSV-2 tk gene into the HSV-1 genome and measured the frequency of ACV resistant virus. In the absence of this more convincing experiment, the authors should at least comment on whether the sequence of the HSV 1 and HSV-2 tk genes differ substantially from one another either in GC content or in homopolymer content.

Minor comments:
1. Pg 3, lines 5-7. This sentence should be reworded for clarity.
2. Page 8, Line 1. Should protease K be proteinase K?
3. Page 10, line 12. Typo - should read average
4. Page 11, last sentence of the first paragraph should be rewritten for clarity.
2. Page 11 second line from the bottom. Typo - should read "mutagenic".
3. Page 12, line 1. Bacterial should be bacteria.
4. Page 12, line 4. Clarity issue. Do the authors mean "Moreover, to ensure that"?
3. Page 12, line 6. Typo - should read "transfection".
5. Page12, line 14. The phrase "extrapolate from the tk mutagenesis assay..." is not entirely clear. This could be reworded.
6. Page. 13, lines 12-16. It should be made clear that this is a reference to an in vitro assay and has not been directly demonstrated in vivo. The statement could be toned down a bit to indicate that "nuclease-sensitve secondary structures within the HSV origin of replication are believed to be...."

Competing interests:

None declared.