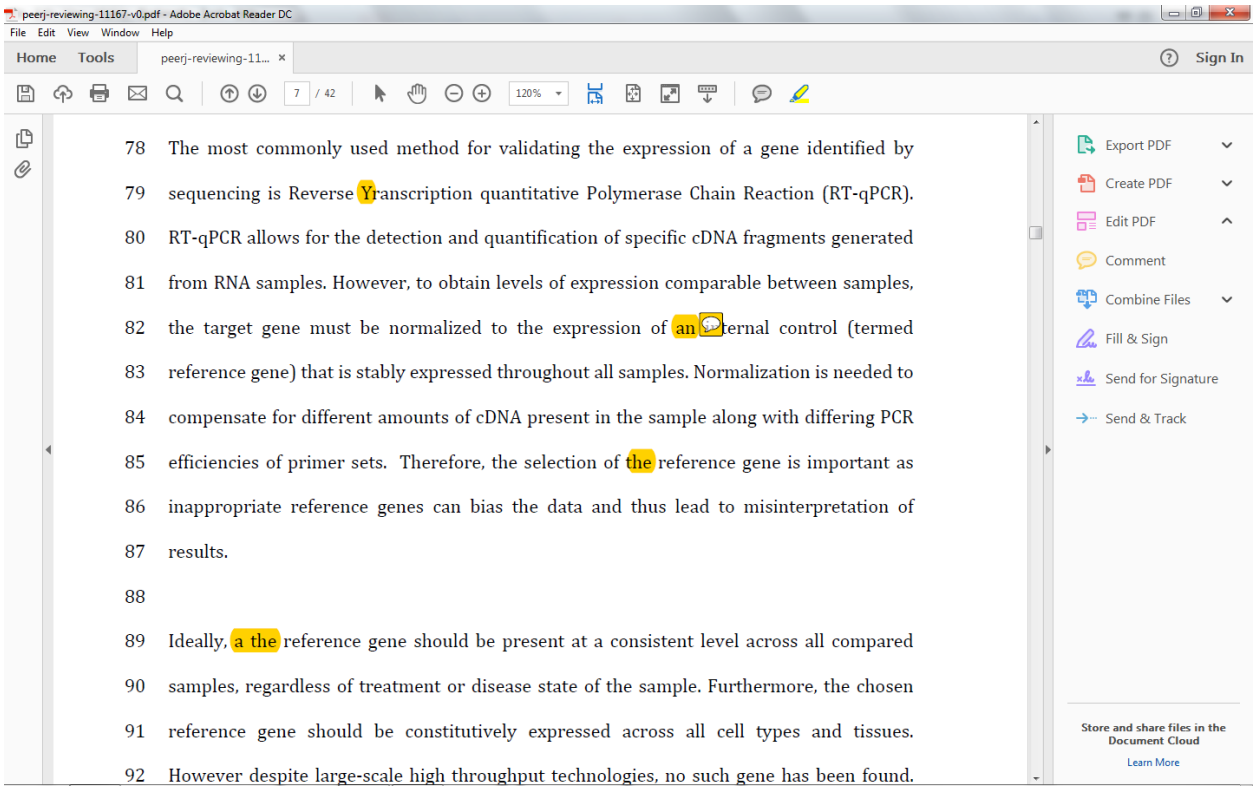
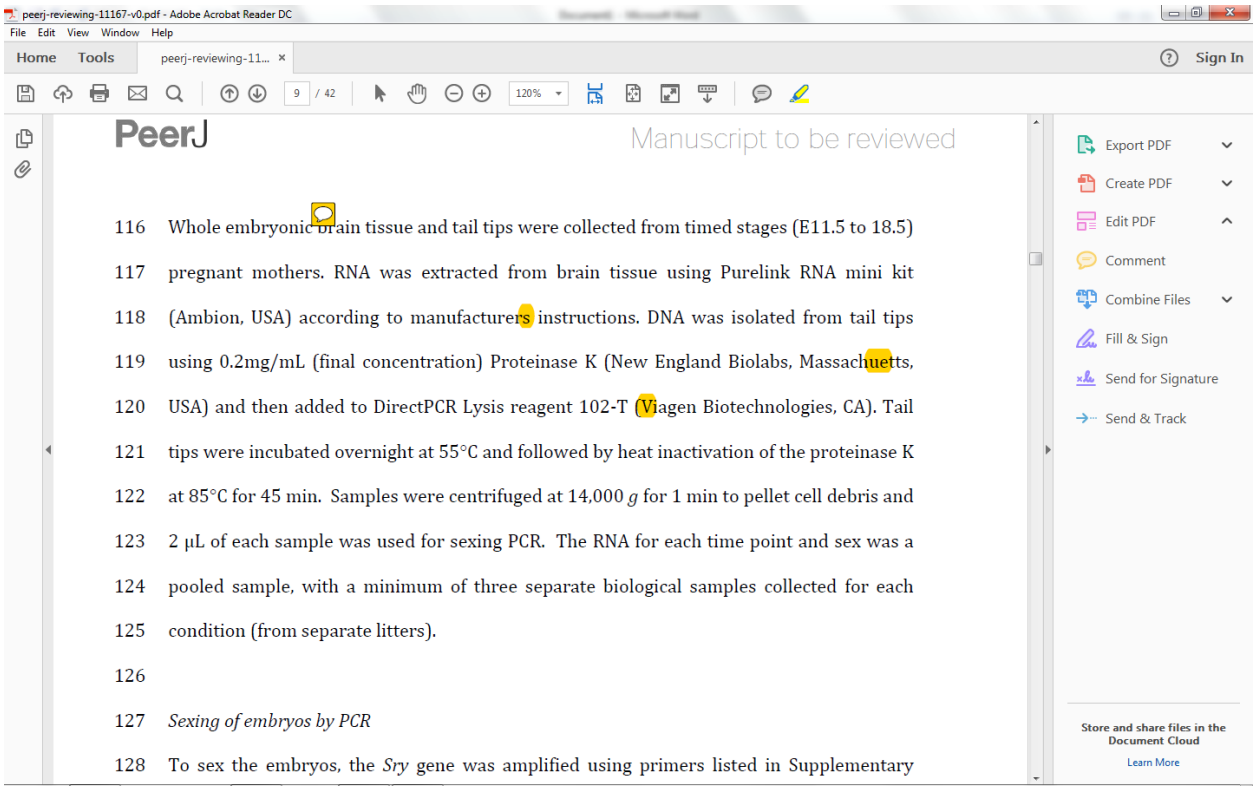


Line 97 – missing adjective

Line 112: This is a weak point, as C57BL/6 mice have a number of specificities about their brains relative to other strains. How about a more robust sample of standard variation in the mouse population, using pregnant Swiss or other outbred mice?

Page 9/4; line 116: Meaning, the top of the head? Where does "the brain" stop in this definition? Of course, there is a great deal of mesenchyme, oral endoderm, and ectoderm in these samples.



Line 136: with how much total RNA per reaction? How was that quantified? If not, how can you assert that there are sex-specific differences when there may have just been quality and quantity differences between individual samples? Related to line 142: what is 2 µL of cDNA? Ct values 15-28 implies that all 2 uL are far from equivalent and perhaps not comparable.

Line 163-164 – grammar

What about Tbp, for example? Why only five genes, and after the more common top three, why not others?

Line 221: Please respond to this assertion:

"In general, it is recommended to use between two and five validated stably expressed reference genes for normalization. Unfortunately, single reference genes continue to being used, assuming they are stably expressed. A geNorm pilot study in which the stability of a panel of (eight) candidate reference genes is evaluated in a representative set of (ten) samples is the preferred way to determine the best set and required number of reference genes to be used. As long as the experimental conditions do not change, one can use the results of such a pilot study to achieve the optimal normalization for all future studies. Normalization using multiple stably expressed reference genes will provide statistically more significant results and will enable detection of small expression differences."

Line 268: "Anterior folded neural tube" is not a term in embryology. Anterior fold of the neural tube, perhaps, but I suggest just removing this. Forebrain is fine, too.

Line 285: At “higher expression”, bring -fold increases into the text here as in previous paragraph.

Line 372: typo

The authors should read and respond to the suggestion in this article that three reference genes should be used, rather than two. Arguments based on practicality and cost are admitted, but should be mentioned if relevant. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3825189/pdf/13353_2013_Article_173.pdf