



SDI FINAL EVALUATION FORM 1.1

The aPART 1:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_14058
Title of the Manuscript:	Phosphatidylcholine-mediated aqueous diffusion of cellular cholesterol down-regulates the ABCA1 transporter in human skin fibroblasts

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
The authors solved all comments, except the compulsory revision comments. The authors should have done a BLAST with the sequence of the primers employees, regardless of grants and articles that have been employed. If the authors do not know the BLAST, need study for do it. Probably there are 5 articles published with erroneous results for a primer design problem. Unfortunately there are things that are assumed and not verified by reviewers, such as the design of the primers, especially when the sequence is a picture (usually is text) and the reviewer can't copy the sequence for test it. I add a PDF with the BLAST.	In the compulsory revision comment, the reviewer made two points regarding the design of the actin primers: (1). That there are 'gaps' on the 3' and 5' side and that the gaps can be a limitation for the Taq Polymerase. (2) That the primers are not specific for β-actin. In this final evaluation, the reviewer states that we should have done a blast with the primers. Response: We would like to point out the purpose of amplifying the actin transcript. It is used as a control housekeeping gene to show that any change in the level of ABCA1 transcript is not due to artifacts such as cell death or error in RNA quantification. As such, one can also use a combination of primers that are all for housekeeping genes (genes that are not regulatable but they code for structural proteins). The purpose is to just compare the relative expression between two samples and show that while the expression level of the housekeeping genes is not altered upon treatment, the gene of interest is induced/repressed. Thus, we are only interested in relative intensity rather than absolute quantification of the PCR products. Thus the reviewer's comment(1) that the Taq Polymerase may be limited, is not apparent from the gel images. And even if there is a reduced efficiency of amplification, it would be so in both the control and test samples, so there would be no impact on our interpretation. (2) The reviewer is concerned about specificity of our actin primer pair. As suggested by the reviewer, we have run a Blast using the actin primer pairs and have obtained a list of genes in the human genome that would be amplified. As shown in the attached file (Actin Primer-Blast Results.pdf), all of the templates that the primer pairs anneal to are some variant of the actin gene (gamma, beta or alpha). Since alpha-actin is only expressed in muscle cells, the fibroblast cells used in our study may express both beta- and gamma-actin; isoforms that differ in only 4 amino acids at the amino-terminus. The Blast results show that some isoforms of the POTE ankyrin domain family are also targets of the primer pair used. This family is classified as being 'actin-like' with the C-terminal region of the gene product belonging to the actin family. This gene is usually expressed in reproductive tissues such as prostate, testes, ovary and placenta, but not expressed in fibroblasts. As seen from the Blast results, all of the putative amplicon products are of 285 bp in size. Amplification of multiple isoforms in our experiments does not in any way negate or invalidate our data or interpretation.