Reviewer’s report

Title: Characterizing Human Genomic Coevolution in Locus-Gene Regulatory Interactions

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Reviewer: Benjamin Schubert

Reviewer’s report:

The article by Savel and Koyutürk describes a coevolutionary analysis of eQTL SNPs and genes. The authors found strong correlations between the conservation status of the eQTL locus and the affected gene, and that pairs that strongly coevolved are mostly cis-eQTL locus. Consequently, Savel and Koyutürk propose to use coevolutionary analysis to priorities sites for cis-eQTL association testing.

The application of coevolutionary analysis to study eQTL interactions is interesting. However, the authors fail to recognize seven years of research and methods development for coevolutionary analysis in proteins, RNA, protein-protein and protein-RAN complex, as well as genome-wide analysis. Maximum Entropy models have widely replaced mutual information, pair-wise correlation, and co-frequencies as measures of coevolution. The overview section on Protein coevolution thus should be rewritten to reflect the state-of-the-art. See these papers for reference:


Especially the recent works by Skwark et al. (3) and Schubert et al. (4) on genome-wide coevolutionary analysis to identify coupled loci (Skwark) and its extension to prioritize pairs for epistatic GWAS testing (Schubert) should be mentioned here in this context, and their claim of novelty toned down accordingly.


I also do have major concerns regarding the rigor of the performed analyses, especially with regards to the statistical evaluation. The authors mostly rely on visual inspection of graphs to underpin their arguments. It would make the results more convincing if the authors would report a proper statistical evaluation as well. For example:
1) The observation that the conservation "at the transcription start site with upstream sequence undergoing accelerated evolution" (P8. l20ff) is elevated should be statistically compared to those pairs that do not undergo "accelerated evolution".

2) Similarly, at P8.l30ff "that the locus that harbors the SNP is undergoing accelerated evolution" should also be statistically underpinned.

3) To give the SNP- and gene-profile clustering results any merit, the authors should present a plot showing the within-cluster variance to the calculated centroids for each choice of k, to convince the reader that two was indeed the optimal choice or choose a clustering model that automatically selects the optimal choice of clusters.

4) The comparison of the cluster centroids and the first components of the SVD analysis is not surprising, as the first component will capture the mean tendencies of uncentered data. As the SVD analysis is not described in detail, the reader must assume that the data was not centered.

5) P10.l34ff "Fig 4a shows a comparison of the normalized histograms of eQTL interactions against the background. We see that the distribution of coevolution scores for eQTL interactions is shifted to the right as compared to the background distribution;" should be statistically underpinned and the figure only used as a visual aid, not as the main argument. The same should be done for the promoter analysis (P11.19ff).

6) The eQTL prioritization analysis is not convincing. It would be more important to show that the SNP-gene pairs with a high coevolutionary scores have low p-values (i.e., that ranks of the p-values and coevolution scores are anti-correlated); meaning that by focusing on highly coevolutionary coupled pairs one enriches for strongly associated eQTLs.

Minor Comments:

1) It was often not clear whether the authors presented results of an example or generalized results due to the confusing usage of singular and plural.

2) Descriptions of figures were often intermixed with the main text. Figure descriptions and the main text should be independent texts.

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