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Reframing the situation:

In translation, mRNA is ‘read’ three nucleotides at a time, with these three letters coding for a specific protein. Consequently, mutations in mRNA that do not shift the ‘frame’ of translation – substitutions, or indels that occur in multiples of 3 – should be observed more often in conserved sequences than frameshifting mutations.

To account for this, I adapted the Global Sequenqueor te

For the experimental portion of my project, I compared alignments of mRNA sequences made with Global, Local, and Frame Align functions. I chose to align mRNA of the NMDA-type glutamate receptor (subunit 1; NMDAR1) from , , and . NMDAR subunit1 is a useful sequence to compare these alignment functions because splice variants of this receptor have been sequenced in humans. I compared alignments of the GluN1-3b splice variant to the GluN1-5b splice variant as a proof of concept. Frame Align was able to successfully identify the location of alternative splicing between GluN1-3b and -5b, where the Global Align and Local Align (not shown) functions were not.

Figure 2. Section of alignments of GluN1-3b (bottom two rows) to GluN1-5b (top) produced with Frame and Global Align functions.

I then aligned NMDAR 1 mRNA from Xenopus tropicalis, a vertebrate, and Apis mellifera, the western honey bee, with GluN1-5b using the three alignment functions. These sequences were obtained in FASTA format from Nucleotide (NCBI). Because Frame Align adds bonuses for continuous diagonal movement, the raw scores of the three alignment functions are not directly comparable (figure 3, left); however, looking at the score of the and sequences as a percentage of the score of the alignment of GluN1-5b with itself using each function (figure 3, right).

Figure 2]TJE Q0.5(1242130 5 36B 41 0.41 0 TmT Tf()TjE Q24 0.242130 5 36B 41 0.41 0 TmT1 1 Tf()TjE Q24 0.6124213

from or ; However, Frame Align was able to identify what appears to be a large indel or alternative splicing site that in the NMDAR1 mRNA that did not appear in the global or local alignments (Figure 4).

Figure 4. Frame alignment of NMDAR1 with GluN1-5b.

Surprisingly, frame align works slightly better than our previously built Global Align function at detecting large indels; however, it remains to be seen how this function would compare to a function built specifically to account for large indels (such as the affine gap function). On the other hand, accounting for frame in the alignment of mRNA sequences did not substantially improve the scores of these alignments, and actually produced an alignment of GluN1-5b to