**Follow-up Clarifying Question about tRNA and protein genes not overlapping.**

In my previous post entitled “**Is there any recent evidence of a tRNA overlapping a protein gene, even by a few bp?**” (https://seaphages.org/forums/topic/5365/) I thought this question was settled. I want to delete the gene in phage Glaske16 at position 60940-61320 bp, but because several recent annotations have kept it, and it has more than 70 hits to the HNH endonuclease in phagesDB, I am seeking a second opinion on this. Its sequence is MQREYMRRWVANRRSAFFASKQCAMCGAGEELELDHIDPTKKVDHRIWSWTDARRSEELAKCQVLCASCHKKKTGEQWYANRSVSENAHHGTSRRYRKMKCRCGLCRLGNTNRSRALRQRHRVPVE

Despite more than 70 hits to HNH endonuclease in phagesDB, this gene has low (<50%) coding potential in Genemark\_S, and entirely no CP in Genemark\_smeg, and TB. THIS GENE OVERLAPS 15bp WITH A tRNA CALLED BY ARAGORN v1.2.41 AND tRNA-SE v. 2.0. WITH AN INFERNAL SCORE OF 55.5.

I am asking this question because of the statement from the Resource Guide entitled, “Predicting tRNA and tmRNA genes” (https://seaphagesbioinformatics.helpdocsonline.com/article-40):

“It is highly unusual that a phage tRNA will:(i) Be encoded within an ORF called by Glimmer and GeneMark that has high coding potential, (ii) Be encoded on the opposite strand as a number of other phage tRNAs found in the same genome, (iii) Be encoded at a genomically distant location from the other tRNA genes in a genome. In general, violation of any of the three preceding conditions is sufficient for exclusion of a potential tRNA from an annotation (we have found a single high scoring tRNA that is not part of the rest of the large cluster, however this situation is very rare).”

Whereas I am inclined to delete the protein coding gene and keep the tRNA since it is called by ARAGORN and tRNAscan-SE with a high infernal score (55.5), I also note that this tRNA is distant from other tRNAs, being 387 bp apart, which seems to violate caveat iii above.

According to [the forum post](https://seaphages.org/forums/topic/88/), “How close can one pack protein and tRNA's genes” of Feb 24, 2016, Dr Pope stated that, “*We tend to steer clear of a tRNA and a protein occupying the same space, but there are definitely genomes where they get pretty close*.”

I realize that there may be exceptions though but wanted to be sure. Some M1 phages such as Reindeer & Iphrane7 do not have this gene but have the “Glu” tRNA (61306-61380 bp in Glaske16; see fig below), and we know that tRNAs tend to be conserved.

Timeline

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**Phamerator map**

Timeline

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**Sample phagesDB hits**

Graphical user interface, text

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