Why are frameshift homologs widespread within and across species?

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Abstract

Frameshifted coding genes yield truncated and dysfunctional proteins, frameshift mutations have been therefore considered as utterly harmful and of little importance for the evolution of novel proteins. However, frameshifted yet functional proteins and coding genes have been frequently observed. Here we report that frameshift homologs are widespread within a genome and across species. We showed that protein coding genes have a *ca*-0.5 quasi-constant shiftability: given any protein coding sequence, at least 50% of the amino acids remain conserved in a frameshifted protein sequence. In the natural genetic code, amino acid pairs assigned to frameshift codon substitutions are more conservative than those to random codon substitutions, and the frameshift tolerability of the natural genetic code ranks among the best 6.3% of all compatible genetic codes. Hence, the shiftability of coding genes was predefined by the genetic code, while additional sequence-level shiftability was achieved through biased usages of codons and codon pairs. We concluded that during early evolution the genetic code was optimized to tolerate frameshifting.

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1. Introduction

The genetic code was discovered in the early 1960s [1]. It consists of 64 triplet codons: 61 sense codons for the twenty amino acids and the remaining three nonsense codons for stop signals. The natural genetic code has several important properties: (1) The genetic code is universal for all organisms, with only a few variations found in some organelles or organisms, such as mitochondrion, archaea and yeast; For details, see the webpage: https://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi. (2) The triplet codons are redundant, degenerative and wobble (the third base tends to be interchangeable); (3) In an open reading frame, an insertion/deletion (InDel) causes a frameshift unless the size of the InDel is a multiple of three.

The natural genetic code was optimized for translational error minimization [2], which is extremely efficient at minimizing the effects of mutation or mistranslation errors [3], and optimization for kinetic energy conservation in polypeptide chains [4]. Moreover, it was presumed that the natural genetic code resists frameshift errors by increasing the probability that a stop signal is encountered upon frameshifts, because frameshifted codons for abundant amino acids overlap with stop codons [5].

Presumably, most frameshifted coding DNA sequences (CDSs) yield truncated, non-functional, potentially cytotoxic products, lead to waste of cell energy, resources and the activity of the biosynthetic machinery [6, 7]. Therefore, frameshift mutations were considered as utterly harmful and of little importance for the evolution of novel proteins [8, 9]. However, frameshifted yet functional proteins and coding genes have been frequently observed [10-13]. For example, in yeast, a frameshifted coding gene for mitochondrial cytochrome c oxidase subunit II (COXII), the sequence is translated in an alternative frame [13]. Moreover, it was reported that frameshift mutations can be retained for millions of years and enable the acquisition of new gene functions [14], shed light into the role of frameshift mutation in molecular evolution.

A protein can be dysfunctioned even by changing a few residues, it is therefore a puzzle how the frameshift proteins kept their structures and functionalities while their amino acid sequences has been changed substantially. Here we report that frameshift homologs are widespread within a genome and across species, and this is because the natural genetic code was optimized to tolerate frameshifting in early evolution.

2. Materials and Methods

2.1 Protein and coding DNA sequences

All available protein sequences in all species (Release 2016_04 of 13-Apr-2016 of UniProtKB/TrEMBL, contains 63686057 sequence entries) were downloaded from the UniprotKB protein database. All available reference protein sequences and their coding DNA sequences (CDSs) in nine model organisms, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Xenopus tropicalis*, *Mus musculus* and *Homo sapiens*, were retrieved from *UCSC, Ensembl* and/or *NCBI* Genome Databases. Ten thousand CDSs each containing 500 random sense codons were simulated by *Recodon* 1.6.0 using default settings [15]. The human/simian immunodeficiency virus (HIV/SIV) strains were derived from the seed alignment in Pfam (pf00516). The CDSs of their envelop glycoprotein (GP120) were retrieved from the HIV sequence database [16].

2.2 Aligning and computing the similarity of the frameshifted protein sequences

A java program, *Frameshift-Align*, was written to translate CDSs in three reading frames, align the three translations and compute their similarities. Every CDS was translated into three protein sequences in its three reading frames in the same strand using the standard genetic code, while all internal nonsense codons were *readthrough* according to the above *readthrough rules* (Table 1). Each protein sequence and the two frameshifted amino acid sequences were aligned by ClustalW2 using default parameters. The pairwise similarity between a protein sequence and its frameshifted protein sequence is given by the percent of sites in which the matched amino acids are conserved (having a positive or zero amino acid substitution score in a scoring matrix, BLOSSUM62, PAM250 or GON250).

2.3 Blastp searching for frameshift homologs

A java program, *Frameshift-Translate*, was written and used to translate CDSs in the alternative reading frames, and the frameshift translations were used as queries to search against the UniprotKB protein database by local blastp, and the Blast hits were filtered with a stringent cutoff criterion (*E-value*≤1e-5, *identity*≥30%, and *alignment length*≥20 AAs).

Given a coding gene, its alternative reading frames often contain a certain number of off-frame stop codons. Therefore, frameshifted coding sequences are commonly translated into inconsecutive protein sequences interrupted by some stop signals (*). To find frameshift homologs by blastp, the query sequences is better to be consecutive sequences devoid of stop signals. Therefore, in *Frameshift-Translate*, when the CDSs were translated into protein sequences in the alternative reading frames, every internal nonsense codon was translated into an amino acid according to a set of *readthrough rules* (Table 1).

2.4 Computational analysis of frameshift codon substitutions

A protein sequence consisting of n amino acids is written as, $A_1 A_2 \ldots A_i A_{i+1} \ldots A_n$, where $A_i = \{ A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y \}$, $i = 1 \ldots n$; its coding DNA sequence consists of n triplet codons, which is written as,

$$\boldsymbol{B_1 B_2 B_3} \mid B_4 B_5 B_6 \mid \boldsymbol{B_7 B_8 B_9} | \ldots | \boldsymbol{B_{3i+1} B_{3i+2} B_{3i+3}} \mid B_{3i+4} B_{3i+5} B_{3i+6} | \ldots | B_{3n-2} B_{3n-1} B_{3n}$$

Where $B_k = \{ A, G, U, C \}$, $k = 1 \ldots 3n$. Without loss of generality, let a frameshift be caused by deleting or inserting one or two bases in the start codon:

(1) *Delete one:* $\boldsymbol{B_2 B_3} B_4 \mid B_5 B_6 \boldsymbol{B_7} | \ldots | \boldsymbol{B_{3i+2} B_{3i+3}} B_{3i+4} \mid B_{3i+5} B_{3i+6} \boldsymbol{B_{3i+7}} | \ldots$

(2) *Delete two:* $\boldsymbol{B_3} B_4 B_5 \mid B_6 \boldsymbol{B_7 B_8} | \ldots | \boldsymbol{B_{3i+3}} B_{3i+4} B_{3i+5} \mid B_{3i+6} \boldsymbol{B_{3i+7} B_{3i+8}} | \ldots$

(3) *Insert one:* $B_0 B_1 B_2 \mid \boldsymbol{B_3} B_4 B_5 \mid B_6 \boldsymbol{B_7 B_8} | \ldots | \boldsymbol{B_{3i+3}} B_{3i+4} B_{3i+5} \mid B_{3i+6} \boldsymbol{B_{3i+7} B_{3i+8}} | \ldots$

(4) *Insert two:* $B_{-1} B_0 \boldsymbol{B_1} \mid \boldsymbol{B_2 B_3} B_4 \mid B_5 B_6 \boldsymbol{B_7} | \ldots | \boldsymbol{B_{3i+2} B_{3i+3}} B_{3i+4} \mid B_{3i+5} B_{3i+6} \boldsymbol{B_{3i+7}} | \ldots$

So, if a frameshift mutation occurred in the first codon, the second codon $B_4 B_5 B_6$ and its encoded amino acid A_2 has two and only two possible changes:

(1) *Forward frameshifting* (*FF*): $\boldsymbol{B_3} B_4 B_5$ ($\rightarrow A_{21}$)

(2) *Backward frameshifting* (*BF*): $B_5 B_6 \boldsymbol{B_7}$ ($\rightarrow A_{22}$)

And so forth for each of the downstream codons. The results are two frameshifted protein sequences, which were denoted as *FF* and *BF*. In either case, in every codon all three bases are changed when compared base by base with the original codon. According to whether the encoded amino acid is changed or not, codon substitutions have been classified into two main types: (1) *Synonymous substitution* (SS); (2) *Nonsynonymous substitution* (NSS). Based on the above analysis, we classified codon substitutions into three subtypes: (1) *Random substitution*; (2) *Wobble substitution*; (3) *Frameshift substitution*.

The amino acid substitution score of a frameshift codon substitution is defined as frameshift substitution score (FSS). A java program, *Frameshift-CODON,* was written to compute the average substitution scores for distinct kinds of codon substitutions by using a scoring matrix (BLOSSUM62, PAM250 or GON250).

2.5 Computational analysis of alternative codon tables

A java program, *Frameshift-GC*, was written to produce “compatible” alternative codon tables according to the method used in reference [3], by changing amino acids assigned to sense codons and keeping degenerative codons synonymous. One million alternative genetic codes were randomly selected from all ($20! = 2.43290201\times10^{18}$) “compatible” genetic codes. The sum and average FSSs for each genetic code were computed and sorted, and compared with that of the natural genetic code.

2.6 Analysis of codon pairs and their frameshift substitution scores

For a given pair of amino acids, written as, $A_1 A_2$, where $A_i = \{ A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y \}$, $i = 1, 2$; its encoding codon pair is written as, $\boldsymbol{B_1} \boldsymbol{B_2} \boldsymbol{B_3} \mid B_4 B_5 B_6$, where $B_k = \{ A, G, U, C \}$, $k = 1…6$. There are 400 different amino acid pairs and 4096 different codon pairs.

Without loss of generality, let a frameshift be caused by inserting or deleting one base in the first codon, the codon pair and its encoded amino acids has two and only two types of changes:

(1) *Forward frameshifting:* $B_0 \boldsymbol{B_1} \boldsymbol{B_2} \mid \boldsymbol{B_3} B_4 B_5$ ($\rightarrow A_{11}A_{21}$)

(2) *Backward frameshifting:* $\boldsymbol{B_2} \boldsymbol{B_3} B_4 \mid B_5 B_6 \boldsymbol{B_7}$ ($\rightarrow A_{12}A_{22}$)

A java program, *Frameshift-CODONPAIR*, was written to compute the average amino acid substitution scores for each codon pair. The result of these calculations is a list of 4096 codon pairs with their corresponding FSSs.

2.7 Computational analysis of the usage of codon and codon pairs

The usage of codons and codon pairs was analyzed on the above dataset using the same method used in reference [17]. The program *CODPAIR* was rewritten in java as the original program is not available. For each sequence, it enumerates the total number of codons, and the number of occurrences for each codon and codon pair. The observed and expected frequencies were then calculated for each codon and codon pair. The result of these calculations is a list of 64 codons and 4096 codon pairs, each with an expected (E) and observed (O) number of occurrences, usage frequency, together with a value for $\chi_1^2 = (O \square E)^2/E$. The codons and dicodons whose *O-value* is greater/smaller than their *E-value* were identified as *over-/under-represented*, their average FSSs and the total weighted average FSSs were computed and compared.

3. Results and Analysis

3.1 Frameshift homologs are widespread within and across species

Frameshift mutations disrupt the function of proteins, as every codon is changed, and often many nonsense codons emerge in a frameshifted CDS. However, in the development of codon and amino acid unified sequence alignment (CAUSA) [18, 19], we noticed that protein sequences encoded by frameshifted CDSs are highly similar to the wild-type protein sequences when they were aligned with each other. For example, in different HIV/SIV strains, including HIV, SIVCZ and SIVGB, HIV was originated from SIVCZ, and SIVCZ was from SIVGB [20-22]. As shown in Fig 1A, the envelop glycoprotein gene (*gp120*) underwent a series of evolutionary events, including base substitution, insertion, deletion, frameshifting and recombination. Especially, several whole or partial, forward or backward frameshifting events occurred in *gp120*, but their encoded protein sequences remain highly similar to each other (Fig 1B), and these frameshifted proteins (GP120) are surely all functional, as the infection of these virus into their host cells relies on these proteins.

As we know, a frameshift mutation is caused by one or more InDels in a protein coding gene whose length is not a multiple of three. Consequently, the reading frame is altered, either fully or partially. In this study, a *frameshift homolog* is defined as a blastp hit using an artificially frameshifted protein sequence as a query. A frameshift homolog is not a frameshift pseudogene, which often contains a certain number of internal nonsense codons and is usually considered as dysfunctional. A frameshift homolog, however, does not necessarily contain internal stop codons, and is usually a frameshifted coding gene that encodes a functional protein.

By searching Uniprot database using blastp with artificially frameshifted protein sequences as queries, we found that frameshift homologs are widespread within a genome and across species. These frameshift homologs were classified into two types:

(1) ***Frameshift ortholog****:* given a coding gene *A* in a species (*sp*. 1), a frameshift homolog (gene *a*) exists in another species (*sp*. 2), which was evolved from a common ancestral gene via speciation and frameshifting (Fig 2A).

(2) ***Frameshift paralog***: given a coding gene *A* in a species, a frameshift homolog (gene *B*) exists in the same species, which was evolved from a common ancestral gene via duplication and frameshifting (Fig 2B).

As shown in Supplementary Dataset 1(Frameshift homologs.xlsx), large numbers of frameshift paralogs and orthologs were found in the genomes of all species tested. For example, in *Homo sapiens*, using frameshifted protein sequences translated from the alternative reading frames of human reference CDSs (hg38, GRCh38) as queries, blastp detected 3974 frameshift paralogs in the human genome and 23224 frameshift homologs (including frameshift orthologs and frameshift paralogs) in all species. These frameshift homologs were mapped onto the human genome and displayed in the UCSC genome browser in two custom tracks, *frameshift homologs* and *frameshift paralogs* (Fig 1C), respectively. The supplementary dataset, the source code of the programs, and the custom track files for the UCSC genome browser are available in the supporting information listed in the end of this article.

A modified blastp method for searching frameshift homologs was first established by Claverie in 1993 [8] and then by Pellegrini and Yeates in 1999 [9]. Both studies relied on more sophisticated use of amino acid scoring tables as a way to account for models of protein sequence divergence, and both provided more robust statistical treatments. Claverie suggested that, setting aside cases of accepted overlapping genes in certain microbes and viruses, and cases of likely sequencing errors, there were only a very small number of detectable cases of frameshift homologs. Pellegrini and Yeates performed more careful sequence shuffling experiments to establish a baseline for random expectations, and concluded that some weak signal existed in the databases to suggest frameshifting as an evolutionary mechanism, concluded that strong inferences about frameshift relationships between specific modern sequences was not possible.

Their method is more sophisticated and could be better than ours in the matter of specificity and accuracy. However, their results published in the 1990s were based on small datasets: Claverie used only 28,154 protein sequences from UniProt in 1993. The method of Pellegrini and Yeates requires consensus sequences and there were only 8,823 entries of consensus sequences available in Prodom in 1999.

The size of the UniProtKB database has been growing exponentially. The UniProt data we used (Release 2016_04) contains 63,686,057 entries, which is 2262-fold greater than the size of database used by Claverie. The blastp hits were filtered with a very rigorous cutoff criteria (E-value≤1e-5, identity≥30% and alignment length≥20 AAs), but it might be not sufficient to filter out all false positives. Although the method we used is rudimentary, it is based on the well-established blastp program and we can adjust the cutoff criterion to raise the specificity and ensure that most of the hits are true frameshift homologs. In human, when the cutoff criteria was raise to E-value≤1e-5, identity≥50% and alignment length≥20 AAs, there were still 1120 frameshift paralogs in human genome and 6371 frameshift homologs in all species.

Moreover, hundreds of frameshifted queries are ≥95% identical to other known protein sequences, and hundreds of them have a match length ≥100 AAs. Clearly, they are recently derived from frameshifting of other coding genes. Despite there may still be some false positives (e.g., frameshifts caused by sequencing errors), most of them were considered as true frameshift homologs evolved from a common ancestral gene via frameshifting rather than random similarities or artifacts. Finally, in the last decade, a few studies have already been reported that frameshift homologs are widely exist in many species [14, 23]. Therefore, we concluded that: *frameshift homologs are widespread within and across species*.

3.2 Frameshift proteins are always highly similar to their wild-types

As mentioned above, we noticed that frameshifted protein sequences are always highly similar to their wild-types. To further validate this, the coding sequences were translated each into three protein sequences in their three reading frames, the three translations were aligned by ClustalW, and their pairwise similarities were computed. For a given CDS, let $\delta_{ij} = \delta_{ji}$ be the similarity between a pair of protein sequences encoded in reading frame i and j (i, $j=1,2,3$, $i \neq j$), the average pairwise similarity among the three protein sequences translated from the three different reading frames on the same strand is defined as *the shiftability of the protein coding gene* (δ),

$$\delta = \frac{1}{3}(\delta_{12} + \delta_{13} + \delta_{23})$$

By analyzing all available reference CDSs in nine major model organisms, we show that δ was centered approximately at 0.5 in all CDSs, in all species, as well as in the simulated CDSs (Table 2 and Supplementary Dataset 2). In other words, *in most coding genes*, the three protein sequences encoded in their three reading frames are always highly similar to each other, with an average similarity of ~50%. Therefore, we proposed that *protein coding genes have ca-0.5 quasi-constant shiftability, i.e., in a protein coding gene, approximately 50% of its amino acids remain conserved in a completely frameshifted protein sequence*.

For partially frameshifted coding genes, obviously, site conservation is inversely proportional to the numbers of frameshifted sites, therefore, partial frameshifts are all highly similar to the wild-type. Hence, it is guaranteed that in a frameshifted protein at least half of its aa sites are conserved when compared to the wide-type. This does not mean that frameshifted variants are all functional, however, quite many of them could maintain their structure and function, forming the basis of frameshift tolerating.

In addition, the wild type is not necessarily the "best" form. In a frameshifted protein, the other half of sites change into dissimilar amino acids, provides a fast and effective means of molecular evolution for improving or altering the structure and function of proteins, or developing the overlapping genes.

2.8 Explanation of the readthrough rules and their impact on computation

The *readthrough rules* were summarized from nonsense suppression tRNAs reported in *E. coli*. The suppressor tRNAs are expressed *in vivo* to correct nonsense mutations, including *amber suppressors* (*supD* [24], *supE* [25], *supF* [26]), *ochre suppressor*s (*supG* [27]) and *opal suppressors* (*supU* [26], *su9* [28]). These suppressor tRNAs are taken as *readthrough rules*, because *translational readthrough* occurs upon activity of a suppressor tRNA with an anticodon matching a stop codon. The suppressor tRNAs frequently occur in the negative strand of a regular tRNA [29-31]. It was found that translational readthrough occurred by using these suppressor tRNAs allows the translation of off-frame peptides [32-35]. There have been a lot of reports that translational readthrough functions not only in *E. coli*, but also in yeast and many eukaryotes species (including human), while the readthrough rules may vary [36, 37]. In addition, there have been increasing evidences show that translational readthrough is related to frameshift tolerating, ribosomal frameshifting or frameshift repair. For example, interaction of eRF3 with RNase L leads to increased readthrough efficiency at premature termination codons and +1 frameshift efficiency [38].

However, in this study, the readthrough-rules are taken simply as 'computational rules' borrowed from biology to obtain consecutive frameshifted protein sequences, without the interruption of stop signals. Therefore, the artificial frameshifting and *in silicon* readthrough operations performed on the coding sequences are distinct from *in vivo* translational readthrough, since the frameshifted amino acid sequences translated from the artificially frameshifted CDSs were used as inputs to ClustalW for multiple sequence alignment (MSA). The purpose of MSA is only to compute the similarities of the protein sequences encoded in the three reading frames.

The artificially frameshifted protein sequences were also used as query for blastp to search for frameshift homologs in the Uniprot database. Although the frameshifts themselves are not really exist in biology, a blastp hit found in the Uniprot database is a true biological protein sequences in most cases (unless the hit itself contains an artificial sequencing error), and the hits are the homologs (ancestors or descendants) of the corresponding frameshifted query, called *frameshift homologs*.

We performed ClustalW aligning and blastp searching by using both readthrough and non-readthrough frameshifted protein sequences. For example, as shown in Fig 2D, in the MSA of wild-type zebrafish VEGFAA with their frameshifted translations, the alignment for readthrough and non-readthrough frameshifted protein sequences are same to each other, except for the stop signals presented in the alignments. As shown in Fig 2D, 62.2% (117/188) of their sites are kept conserved in physiochemical properties. The shiftability of vegfaa computed by readthrough and non-readthrough is 0.5354 and 0.5573, respectively. So, *in silicon* readthrough has a negligible impact on the computation of the shiftability.

The blastp results, however, were slightly better (higher score, more positives and better E-value) in readthrough than in non-readthrough queries. As shown in Fig 2E, in the blastp result of a frameshifted query, the stop signals of the query match each with an amino acid in the subject, suggesting that the corresponding stop codons were substituted each by a sense codon in evolution. So, we translated the frameshifted coding sequences by using the readthrough rules, but, it does not require or imply that these *in-silicon* readthrough rules must function in *E. coli* or any other species, but simply a computational method to obtain consecutive frameshifted protein sequences.

3.3 The genetic code was optimized for frameshift tolerating

In Table 2, the shiftability of the protein coding genes is similar in all species, and all genes, and the standard deviation is very small, suggesting that the shiftability is largely species- and sequence-independent. This implies that the shiftability is defined mainly by the genetic code rather than by the coding sequences themselves. This is also suggested by the simulated coding sequences, whose shiftability is comparable with those of the real coding genes.

As described above in the method section, we computed the average amino acid substitution scores respectively for random, wobble and forward/backward frameshift codon substitutions. As shown in Table 3 and Supplementary Dataset 3, in all 4096 possible codon substitutions, most (192/230=83%) of the synonymous substitutions are wobble, and most (192/256=75%) wobble substitutions are synonymous, thus the average substitution score of the wobble substitutions is the highest. For frameshift codon substitutions, including the four triplet codons (TTT, AAA, GGG, CCC) which are kept unchanged in frameshifting, only a small proportion (28/512=5.5%) of the frameshift codon substitutions are synonymous (Table 4), and the others (95.9%) are all nonsynonymous. However, a substantial proportion (35.9%) of them are positive (including SSs and positive NSSs), which is significantly higher than the proportion of positive substitutions in random codon substitutions (25.7%). In summary, in the natural genetic code, SSs are assigned mainly to wobble codon substitutions, while positive NSSs are assigned mainly to frameshift substitutions.

In addition, no matter which substitution scoring matrix (BLOSSUM62, PAM250 or GON250) was used for computation, the average FSSs are significantly higher than the substitution scores of the random codon substitutions (t-test P << 0.01), suggesting that the amino acid substitutions assigned to the frameshift substitutions are more conservative than those to the random substitutions.

The amino acid substitution scoring matrix is widely used to determine similarity and conservation in sequence alignment and blast searching, which forms the basis of most bioinformatics analysis. In commonly used scoring matrix, either BLOSSUM62, PAM250 or GON250, most of the amino acid substitution scores are negative and the percent of positive scores is less than 30%. So, percent of positive scores for random codon substitutions is ~30%. However, as shown in Table 2, the frameshifted protein sequences are always ~50% similar to the wild types: the ~35% similarity derived from the frameshift codon substitutions, combined with the ~25% similarity from the random codon substitutions, deduct their ~10% intersection, well explains the ~50% similarities observed among the frameshifted protein sequences and the wild types. Therefore, it is suggested that the shiftability of coding genes was predefined mainly by the genetic code, and is largely independent on the coding sequences themselves.

To further investigate optimization for frameshift tolerance of the natural genetic code, one million alternative genetic codes were randomly selected from all (20! = 2.43290201×10^{18}) "compatible" genetic codes by changing the amino acids assigned to the sense codons randomly, while keeping all degenerative codons synonymous. By computing and sorting the average FSSs for these alternative genetic codes (Table 5), the FSSs of the natural genetic code ranks in the best 6.3% of all compatible genetic codes. Hence the genetic code was indeed optimized for tolerating frameshifts, clearly demonstrating that the shiftability of coding genes is defined by the genetic code.

3.4 The genetic code is symmetric in frameshift tolerating

The genetic code shows the characteristics of symmetry in many aspects [39-41], and it evolved probably through progressive symmetry breaking [42-44]. Here in all CDSs both forward and backward frameshift proteins have comparable similarities with the wild-type (Table 2). In addition, in the natural genetic code both forward and backward frameshift substitutions have the same number of SSs/NSSs and roughly equal FSSs (Table 3). These data suggested that the genetic code is also symmetric in terms of shiftability and frameshift tolerating, so that a coding gene has an ability to tolerate frameshifting in both forward and backward directions at the same time (Fig 2). This could also explain why the codons in the natural genetic code are not tetrad but triplet: triplet codon could be kept symmetric for both forward and backward frameshifting, while for tetrad codons the situation will be much more complicated in frameshifting.

3.5 The shiftability at sequence level

Although the shiftability of a coding sequence is predefined mainly by the genetic code, shiftability may also exist at the sequence level. Functionally important coding genes, such as housekeeping genes, which are more conserved, may also have greater shiftability when compared with other genes. At first, we thought that a biased usage of codons may contribute to the sequence-level shiftability. However, as shown in Table 6 and Supplementary Dataset 4, it is somewhat surprising that in *E. coli* and *C. elegans* the average FSSs weighted by their codon usages are even lower than for unweighted calculations (equal usage of codons). In the other species tested, although the weighted average FSSs are higher than for unweighted analyses, the difference is not statistically significant in all species tested (P>0.05), suggesting that the usage of codons has little or no direct impact on the shiftability. However, the usage of codons may influence the shiftability indirectly, *e.g.*, by shaping the pattern of codon pairs.

Given a pair of amino acids, $A_1 A_2$, if A_1 and A_2 have m_1 and m_2 degenerative codons, respectively, their encoding dicodons, $B_1 B_2 B_3 \mid B_4 B_5 B_6$, has $m_1 \times m_2$ possible combinations, called *degenerative codon pairs* (DCPs). It has been reported that codon pair usages are highly biased in various species, such as bacteria, human and animals [17, 45-50]. As shown in Table 7, and Supplementary Dataset 5, in all species tested, the average FSSs of the over-represented codon pairs are all positive, while those of the under-represented codon pairs are all negative; in addition, the weighted average FSSs of codon pairs are all positive, while that of the equal usage of codon pairs is negative, suggesting that a selective pressure was working on the codon pairs, so that frameshift-tolerable DCPs are present more frequently in these genomes than non-frameshift-tolerable DCPs. Therefore, sequence-level shiftability does exist, and was achieved through a biased usage of codons and codon pairs. There have been many studies on the causes and consequences of the usage of codons, such as gene expression level [51-56], mRNA structure [57-64], protein abundance [61, 65-67], and stability [68-70]. The above analysis suggested that the usages of codon pairs is either a cause or a consequence of the shiftability of the protein-coding genes.

4. Discussion

4.1 The genetic code was optimized for frameshift tolerating

The natural genetic code results from selection during early evolution, and it was optimized along several properties when compared with other possible genetic codes [71-82]. It was reported that the natural genetic code was optimized for translational error minimization, because the amino acids whose codons differed by a single base in the first and third positions were similar with respect to polarity and hydropathy, and the differences between amino acids were specified by the second position is explained by selection to minimize the deleterious effects of translation errors during the early evolution of the genetic code [2]. In addition, it was reported that only one in every million alternative genetic codes is more efficient than the natural genetic code, which is extremely efficient at minimizing the effects of point mutation or translation errors [3]. It was demonstrated that the natural genetic code is nearly optimal for allowing additional information within coding sequences, such as out-of-frame hidden stop codons (HSCs) and secondary structure formation (self-hybridization) [5].

In the above, we showed that the code- and sequence-level shiftability of coding genes guaranteed at least half of the sites are kept conserved in a frameshifted protein when compared with the wild-type protein. This is the basis for frameshift tolerating, and explains why the usage of codons and codon pairs are biased and why frameshift homologs are widespread within and across species.

The sequence-level shiftability caused by the biased usages of codon pairs are probably relevant to the circular code. The circular code is a set of 20 codons that are overrepresented in the regular coding frame of genes as compared to frameshifted frames [83-85]. The mechanism by which the circular code maintains the translation frame is unknown [85-89], but *in silico* frame detection was made possible by using the empirical circular code [88-93]. However, the relationship among the shiftability, the biased usages of codons and codon pairs, and the circular code is unknown.

4.2 The universality of the shiftability

Here we analyzed the shiftability of protein-coding genes only in some model organisms, thus it is interesting to further validate this mechanism in other species. It has been reported that in some animal species frameshift mutations are tolerated by the translation systems in mitochondrial genes [94-96]. For example, a (+1) frameshift insertion is tolerated in the *nad3* in birds and reptiles [94]. Moreover, frameshifted overlapping coding genes have been found in mitochondria genes in fruit fly and turtles [97, 98]. It was reported that the levels of translational readthrough and frameshifting in *E. coli* are both high and growth phase dependent [99]. Meanwhile, translational readthrough has been widely observed in various species [100-107]. Frameshift tolerating has also been explained by *ribosomal frameshifting* [108-111].

However, the shiftability of protein coding genes may also contribute, at least partially, to the functioning, repairing and evolution of the frameshifted protein coding genes.

5. Conclusion

The above analysis conclude that frameshift homologs are widespread within and across species, and this is because the genetic code was optimized for frameshift tolerating. The shiftability of coding genes guarantees a near-half conservation after a frameshifting event, endows coding genes an inherent ability to tolerate frameshifting. The natural genetic code, which exists since the origin of life, was optimized by competition with other alternative genetic codes during early evolution [112-115]. As the *bottom design* for all genes and genomes for all species, the natural genetic code allows coding genes to tolerate both forward and backward frameshifting, could have a better fitness in the early evolution. Thanks to this ingenious property of the genetic code, the shiftability serves an innate mechanism for protein-coding genes to deal with frameshift mutations, by which the disastrous frameshifting events were utilized, becoming a driving force for molecular evolution.

Author Contributions

Xiaolong Wang conceived the study, coded the programs, analyzed the data, prepared the figures, tables and wrote the paper; Quanjiang Dong proofread the paper and gave conceptual advices. Gang Chen and Jianye Zhang provided materials and supports. Yujia Cai, Yongqiang Liu and Jinqiao Zhao analyzed the data for alternative genetic codes.

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Figure Legends

Fig 1. The alignment of the coding and the protein sequences of HIV/SIV GP120. (A) The alignment of coding sequences, with highlights showing that the coding genes contain several frameshifting events. In other words, the coding gene is expressed in different reading frames in

different virus strains. (B) The alignment of protein sequences, showing that the GP120 sequences for different virus, which are encoded in different reading frames of *gp120*, are highly similar.

Fig 2. Diagram of different frameshift homologs. (A) Frameshift orthologs; (B) Frameshift paralog; (C) Custom tracks for the frameshift homologs displayed in the UCSC genome browser; (D) the ClustalW alignment of the wild-type VEGFAA and its readthrough or non-readthrough frameshifts; (E) The outputs of blastp searching for frameshift homologs, the query is an artificial protein sequence translated from a frameshifted CDS by readthrough or non-readthrough.

Additional Information

We declare that the authors have no competing interests.

Table 1. The natural suppressor tRNAs (*readthrough rules*) for nonsense mutations.

Site	tRNA	Wild type		Correction	
	(AA)	**Code**	**Anti-code**	**Code**	**Anti-code**
supD	Ser (S)	→ UCG	CGA←	→ UAG	CUA←
supE	Gln (Q)	→ CAG	CUG←	→ UAG	CUA←
supF	Tyr (Y)	→ UAC	GUA←	→ UAG	CUA←
supG	Lys (K)	→ AAA	UUU←	→ UAA	UUA←
supU	Trp (W)	→ UGG	CCA←	→ UGA	UCA←

Table 2. The similarities of natural and simulated proteins and their frameshift forms.

No.	***Species***	***Number of CDSs***	***Average Similarity***					
			δ_{12}	δ_{13}	δ_{23}	δ	***MAX***	***MIN***
1	*H. sapiens*	71853	0.5217±0.0114	0.5044±0.0122	0.4825±0.0147	0.5028±0.0128	0.5948	0.4357
2	*M. musculus*	27208	0.5292±0.042	0.5058±0.0437	0.4869±0.0418	0.5073±0.0425	0.8523	0.1000*
3	*X. tropicalis*	7706	0.5190±0.0013	0.4987±0.0013	0.4855±0.0008	0.5010±0.0008	0.5962	0.4790
4	*D. rerio*	14151	0.5234±0.0007	0.5022±0.0008	0.4921±0.0005	0.5059±0.0004	0.5240	0.4784
5	*D. melanogaster*	23936	0.5162±0.0015	0.4921±0.001	0.4901±0.0013	0.4995±0.0008	0.6444	0.4667
6	*C. elegans*	29227	0.5306±0.0007	0.5035±0.0008	0.5002±0.001	0.5115±0.0006	0.6044	0.4864
7	*A. thaliana*	35378	0.5389±0.0508	0.5078±0.0481	0.5062±0.048	0.5176±0.0388	0.9540	0.2162*
8	*S. cerevisiae*	5889	0.5174±0.0011	0.4811±0.001	0.5072±0.0006	0.502±0.0007	0.5246	0.4577
9	*E. coli*	4140	0.5138±0.0019	0.4871±0.0046	0.481±0.0015	0.494±0.0012	0.7778	0.4074
10	*Simulated*	10000	0.5165±0.0282	0.4745±0.0272	0.4773±0.0263	0.4894±0.0013	0.6489	0.3539

* Very large and small similarity values were observed in a few very short or repetitive peptides.

Table 3. The amino acid substitution scores for different kinds of codon substitutions.

Codon Substitution		*ALL (Random)*	*Frameshift*		*Wobble*
			FF	***BF***	
Type of Codon Substitution	*All*	4096	256	256	256
	Unchanged (%)	64 (1.6%)	4 (1.6%)	4 (1.6%)	64 (25%)
	Changed (%)	4032 (98.4%)	252 (98.4%)	252 (98.4%)	192 (75%)
	SS (%)	230 (5.6%)	14 (5.5%)	14 (5.5%)	192 (75%)
	NSS-Positive (%)	859 (20.1%)	76 (29.7%)	76 (29.7%)	40 (15.6%)
	NSS-Negative (%)	3007 (73.4%)	166 (64.8%)	166 (64.8%)	24 (9.4%)
Average Substitution Score	*BLOSSUM62*	-1.29	-0.61	-0.65	3.77
	PAM250	-4.26	-0.84	-0.84	3.68
	GON250	-10.81	-1.78	-1.78	35.60

SS/NSS: synonymous/nonsynonymous substitution; FF/BF: forward/backward frameshift codon substitution.

Table 4. The synonymous frameshift substitutions

Forward Frameshifting					*Backward Frameshifting*				
From			*To*		*From*			*To*	
1	AAA	K	AAA	K	1	AAA	K	AAA	K
2	AAA	K	AAG	K	2	AAG	K	AAA	K
3	GGG	G	GGA	G	3	GGA	G	GGG	G
4	GGG	G	GGG	G	4	GGG	G	GGG	G
5	GGG	G	GGC	G	5	GGC	G	GGG	G
6	GGG	G	GGT	G	6	GGT	G	GGG	G
7	CCC	P	CCA	P	7	CCA	P	CCC	P
8	CCC	P	CCG	P	8	CCG	P	CCC	P
9	CCC	P	CCC	P	9	CCC	P	CCC	P
10	CCC	P	CCT	P	10	CCT	P	CCC	P
11	CTT	L	TTA	L	11	TTA	L	CTT	L
12	CTT	L	TTG	L	12	TTG	L	CTT	L
13	TTT	F	TTC	F	13	TTC	F	TTT	F
14	TTT	F	TTT	F	14	TTT	F	TTT	F

Table 5. The frameshift substitution score of the natural and alternative genetic codes (computed by using the amino acid scoring matrix BLOSSUM62).

Number of alternative	The natural genetic code		FSS of the alternative genetic codes				
genetic codes Sampled	FSS Score	Rank	MAX	MIN	Average A*	Average B**	Average
1,000,000	-294	62007	-43	-814	-256.842	-438.930	-427.375

* Average A: the average FSS of the genetic codes ranks above (better than) the natural genetic code;

** Average B: the average FSS of the genetic codes ranks below (worse than) the natural genetic code;

Table 6. The usage of codons and their weighed average FSSs (Gon250)

NO	***Species (Codon Usage)***	***Weighted Average FSS***
1	*H. sapiens*	-9.82
2	*M. musculus*	-13.47
3	*X. tropicalis*	-12.75
4	*D. rerio*	-20.58
5	*D. melanogaster*	-19.43
6	*C. elegans*	-23.38
7	*A. thaliana*	-22.52
8	*S. cerevisiae*	-14.08
9	*E. coli*	-28.59
10	*Equal usage*	-22.27

Table 7. The usage of codon pairs and their weighed average FSSs (Gon250)

NO	***Species (Codon Usage)***	***Average FSS of over-represented Codon pairs***	***Average FSS of under-represented Codon pairs***	***Weighted Average FSS of All Codon pairs***
1	*H. sapiens*	41.30	-25.94	102.41
2	*M. musculus*	41.09	-26.09	98.55
3	*X. tropicalis*	42.20	-25.81	98.24
4	*D. rerio*	40.91	-26.17	87.38
5	*D. melanogaster*	39.77	-25.95	79.51
6	*C. elegans*	40.85	-26.18	81.48
7	*A. thaliana*	40.54	-26.09	90.64
8	*S. cerevisiae*	40.85	-26.18	99.21
9	*E. coli*	39.27	-30.75	77.03
10	*Equal Usage*	N/A	N/A	-28.50

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```
                 *        20         *        40
HV1J3 : -----------ATGAGAGTGAAGGGGATCAGGAAGAA--TTA :   29
SIVCZ : -----------ATGAAAGTAATGGAGAAGAAGAAGAG--AGA :   29
SIVGB : ATGTCTACAGGAAACGTGTACCAGGAACTAATAAGAAGATAC :   42

               *        60         *        80
HV1J3 : TCAGCACTTGTGGAGATGGGGCACGATGCTCCTTGGGATATT :   71
SIVCZ : CTGGAACAGCTTATCCATAATTACAATCATAACAATCATTTT :   71
SIVGB : CTGGTAGTGGTGAAGAAGCTATACGAAGGTAAGTATGAAGTG :   84

             *       100         *       120
HV1J3 : GATGATCTGTAGTGCTGCAGAACAATTGTGGGTCACAGTC-- :  111
SIVCZ : GCTAACCCCATGTTTGACCTCTGAGTTATGGGTAACAGTA-- :  111
SIVGB : TCCAGGTCTTTTTCTTATACTATGTTTA-GCCTACTAGTAGG :  125

           *       140         *       160
HV1J3 : TATTATGGGGTACCTGTGTGGAAAGAAGCAGCCACCACTCTA :  153
SIVCZ : TATTATGGAGTACCTGTTTGGCATGATGCTGACCCGGTACTC :  153
SIVGB : TATTATAGGAAAACAATATGTGACAGT-CTTCTATGGAGTAC :  166

         *       180         *       200         *
HV1J3 : TTTTGTGCATCAGATGCTAAAGCATAT---------GATACA :  186
SIVCZ : TTTTGTGCCTCAGACGCTAAGGCACAT---------AGTACA :  186
SIVGB : CAGTATGGAA-GGAAGCTAAAACACATTTGATTTGTGCTACA :  207

               220         *       240         *
HV1J3 : GAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACA :  228
SIVCZ : GAGGCTCATAATATTTGGGCCACACAGGCATGTGTACCTACA :  228
SIVGB : GATAATTCAAGTCTCTGGGTAACCACTAATTGCATACCTTCA :  249

             260         *       280         *
HV1J3 : GACCCCAACCCACAAGAAGTAGTATTGGAAAATGTGACAGAA :  270
SIVCZ : GATCCCAGTCCTCAGGAAGTATTTCTTCCAAATGTAATAGAA :  270
SIVGB : TTGCCAGATTATGATGAGGTAGAAATTCCTGATATAAAGGAA :  291

           300         *       320         *
HV1J3 : AAATTTAA------CATGTGGAAAAATAACATGGTAGAACAG :  306
SIVCZ : TCATTTAA------CATGTGGAAAAATAATATGGTGGACCAA :  306
SIVGB : AATTTTACAGGACTTATAAGGGAAAATCAGATAGTTTATCAA :  333
```

Fig 1 (A). Alignment of coding sequences of HIV/SIV GP120

```
                 *        20         *        40
HV1J3 : --------------MRVKGIRKNYQHLWRWGTMLLGILMICSA :  29
SIVCZ : --------------MKVMEKKKRDWNSLSIITIITIILLTPCL :  29
SIVGB : MSTGNVYQELIRRYLVVVKKLYEGKYEVSRSFSYTMFSLLVGI :  43
                      6 V  k k      s  t  t il6

              *        60         *        80
HV1J3 : AEQLWVTVYYGVPVWKEAATTLFCASDAKAYDTEVHNVWATHA :  72
SIVCZ : TSELWVTVYYGVPVWHDADPVLFCASDAKAHSTEAHNIWATQA :  72
SIVGB : IGKQYVTVFYGVPVWKEAKTHLICATDNSS-------LWVTTN :  79
           l5VTV5YGVPVWkeA t LfCA3Daka  te hn6WaT a

           *       100         *       120
HV1J3 : CVPTDPNPQEVVLENVTEKFN--MWKNNMVEQMHEDIISLWDQ : 113
SIVCZ : CVPTDPSPQEVFLPNVIESFN--MWKNNMVDQMHEDIISLWDQ : 113
SIVGB : CIPSLPDYDEVEIPDIKENFTGLIRENQIVYQAWHAMGSMLDT : 122
        C6P3dP pqEV 6p16 E Fn  6wkNn6V Qmhed6iS6wDq

        *       140         *       160         *
HV1J3 : SLKPCVKLTPLCVTLNCIDWGNDTSPNATNTTSSGGEKMEKGE : 156
SIVCZ : SLKPCVKLTPLCVTLQCSKANFSQAKNLTNQTSS-----PPLE : 151
SIVGB : ILKPCVKINPYCVKMQCQETENVSATTAKPITTPTTTSTVASS : 165
        sLKPCVK6tPlCVt6qC    n  a natn T3s        e

             180         *       200         *
HV1J3 : MKNCSFNITTSIRDKVQKEHALFY------KHDVVPINNSTKD : 193
SIVCZ : MKNCSFNVTTELRDKKKQVYSLFY------VEDVVNLG----- : 183
SIVGB : TEIYLDVDKNNTEEKVERNHVCRYNITGLCRDSKEEIVTNFRG : 208
        mkncsfn tt  rdKv   h lfY        dvv 6

          220         *       240         *       2
HV1J3 : NIKNDNSTRYRLISCNTSVITQACPKISFEPIPIHYCAPAGFA : 236
SIVCZ : ---NENNT-YRIINCNTTAITQACPKTSFEPIPIHYCAPAGFA : 222
SIVGB : DDVKCENNTCYMNHCNESVNTEDCQKG-LLIRCILGCVPPGYV : 250
           n nnt yr6i CNt3viT2aCpK sfepipIhyCaPaG5a

        60         *       280         *       300
HV1J3 : IIKCNDKKFNGTGPCTNVSTVQCTHGIKPVVSTQLLLNGSLAE : 279
SIVCZ : ILKCNDKDFSGKGKCTNVSTVHCTHGIKPVVTTQLLINGSLAE : 265
SIVGB : MLRYN-EKLNNNKLCSNISAVQCTQHLVATVSSFFGFNGTMHK : 292
        664cNdkkfng g C3N6StVqCThg6kpvV33qll NG36ae

                *       320         *       340
HV1J3 : EEVVIRSENFTDNAK-------TIIVQLKEPVVINCTRPSKTT : 315
SIVCZ : GNITVRVENKSKNTD-------VWIVQLVEAVSLNCHRPGNNT : 301
SIVGB : EGELIPIDDKYRGPEEFHQRKFVYKVPGKYGLKIECHRKGNRS : 335
        e   6r e1k  n         v iVqlke 6 6nChRpgn 3
```

Fig 1 (B). Alignment of protein sequences of HIV/SIV GP120

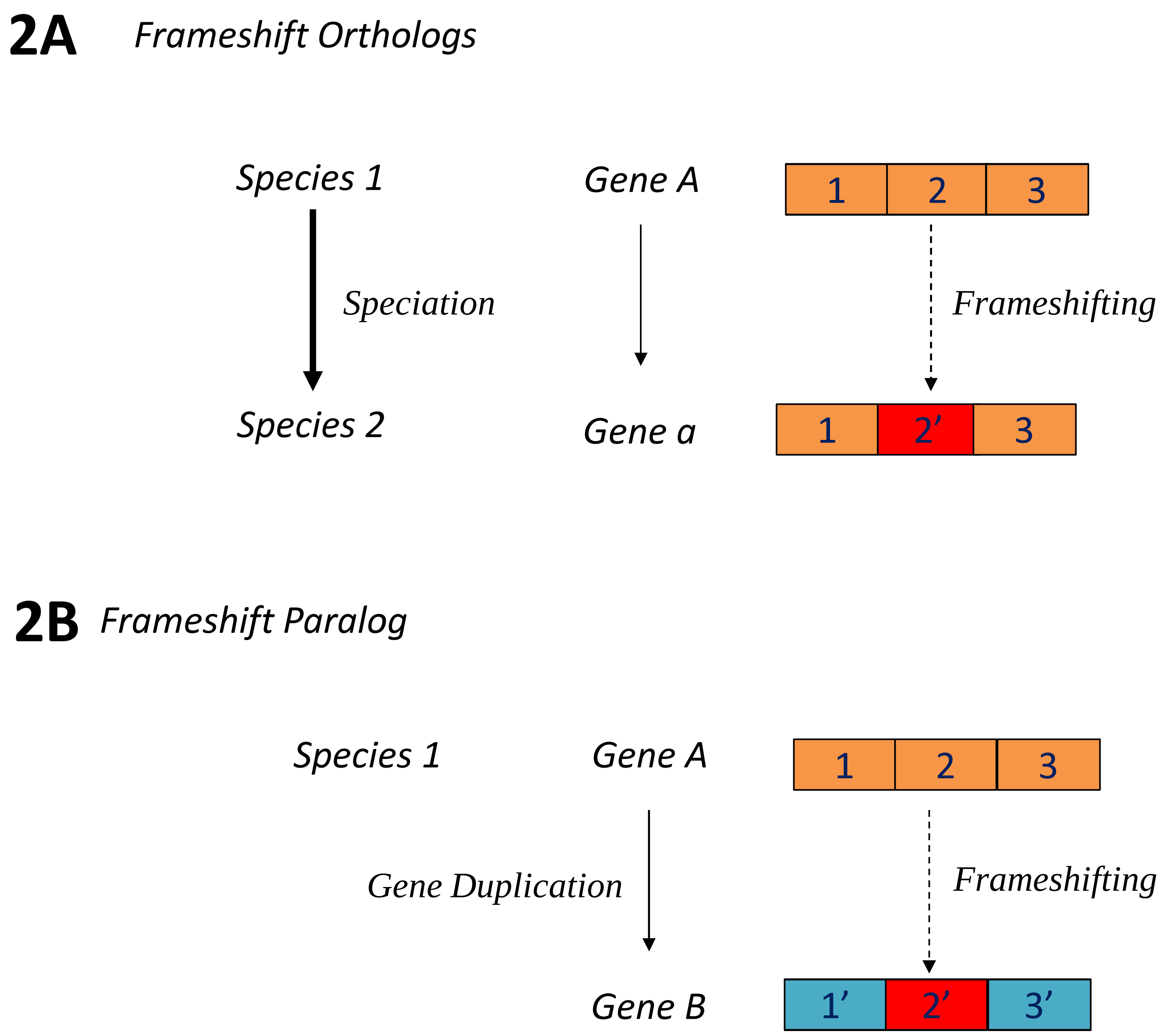
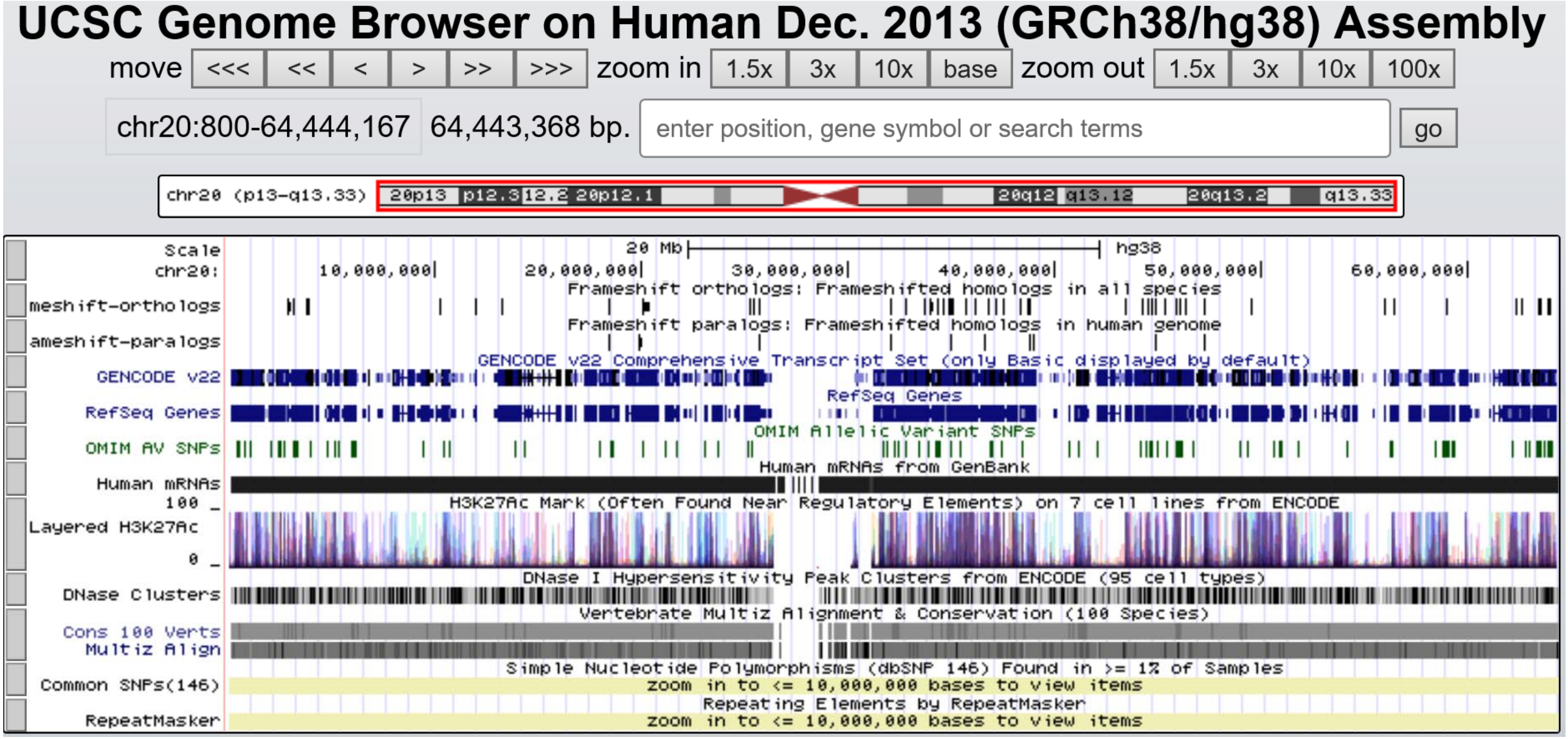

2A
Frameshift Orthologs
Species 1
Gene A
1
2
3
Speciation
Frameshifting
Species 2
Gene a
1
2'
3
2B
Frameshift Paralog
Species 1
Gene A
1
2
3
Gene Duplication
Frameshifting
Gene B
1'
2'
3'

Fig 2

2C


UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

chr20:800-64,444,167 64,443,368 bp.

Frameshift orthologs: Frameshifted homologs in all species

Frameshift paralogs: Frameshifted homologs in human genome

GENCODE v22 Comprehensive Transcript Set (only Basic displayed by default)

RefSeq Genes

OMIM Allelic Variant SNPs

Human mRNAs from GenBank

H3K27Ac Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE

DNase I Hypersensitivity Peak Clusters from ENCODE (95 cell types)

Vertebrate Multiz Alignment & Conservation (100 Species)

Simple Nucleotide Polymorphisms (dbSNP 146) Found in >= 1% of Samples

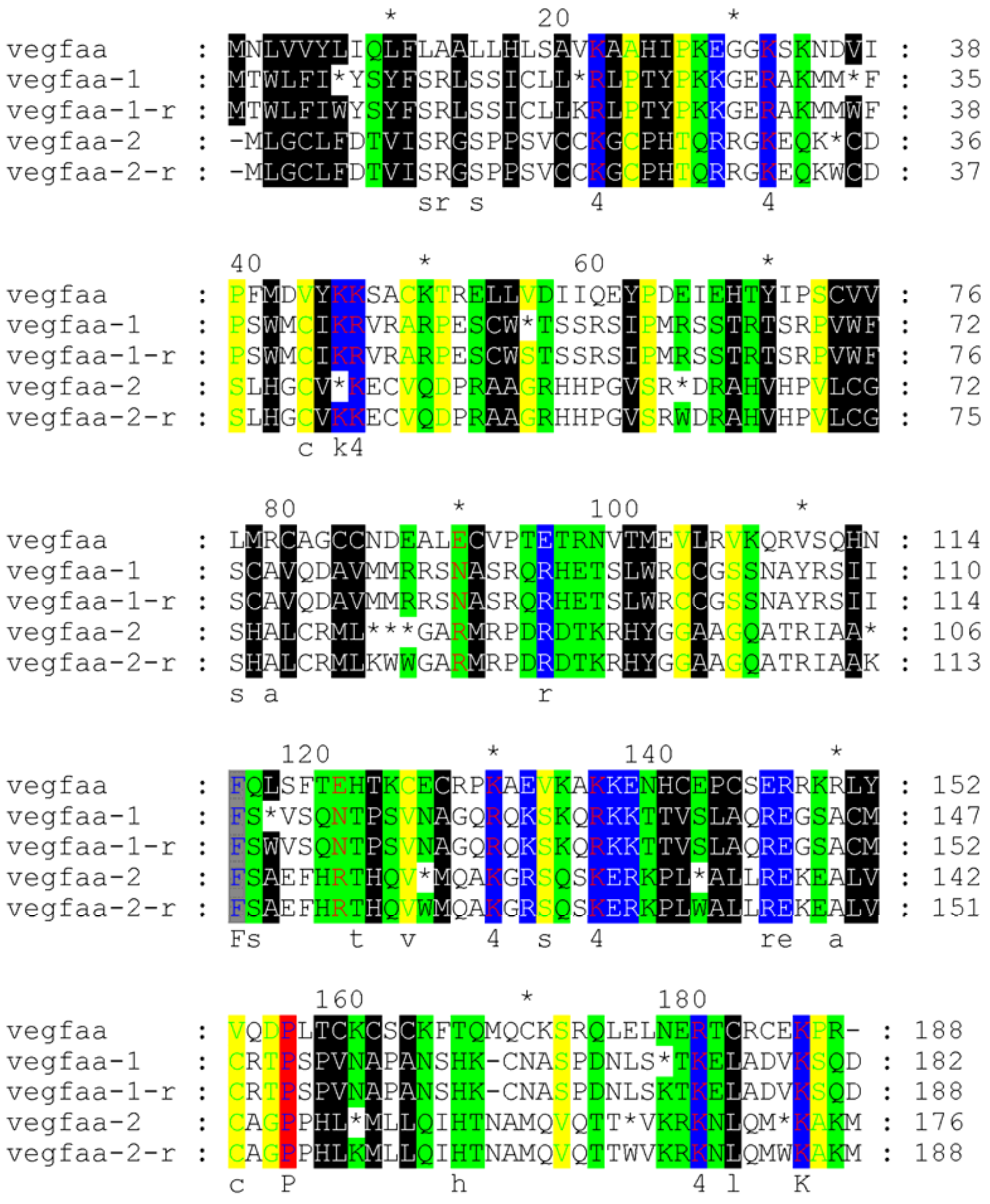
zoom in to <= 10,000,000 bases to view items

Repeating Elements by RepeatMasker

zoom in to <= 10,000,000 bases to view items

Fig 2

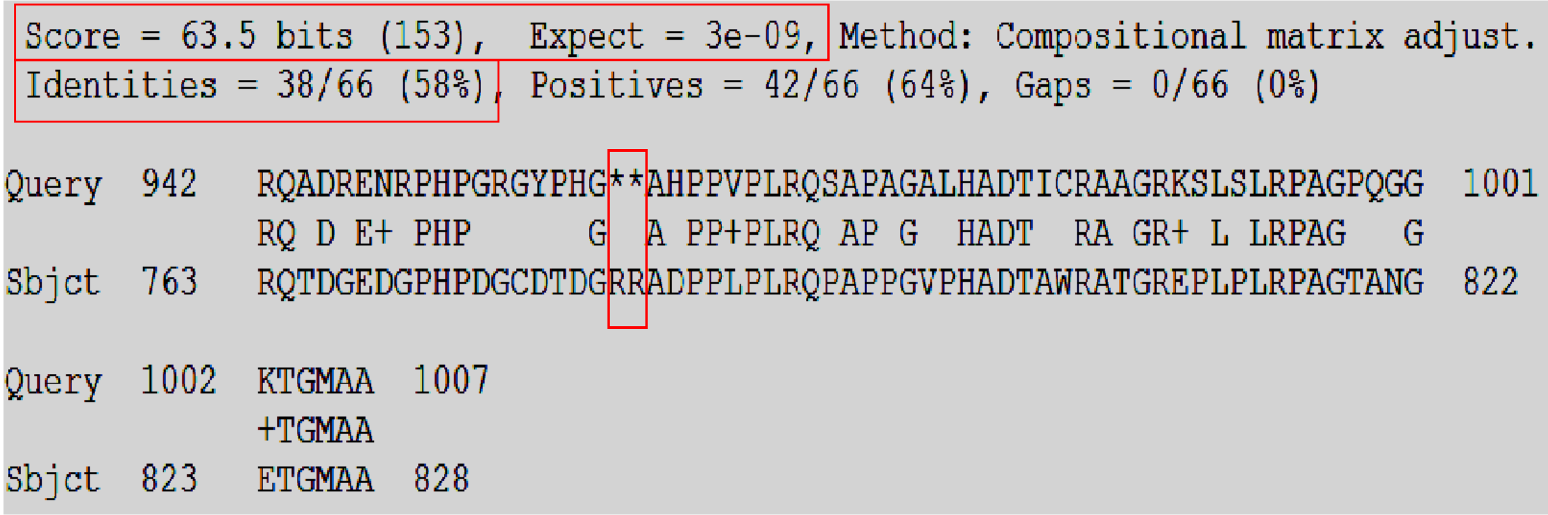
2D VEGFAA and its frameshifts



```
                      *        20         *
vegfaa     : MNLVVYLIQLFLAALLHLSAVKAAHIPKEGGKSKNDVI :  38
vegfaa-1   : MTWLFI*YSYFSRLSSICLL*RLPTYPKKGERAKMM*F :  35
vegfaa-1-r : MTWLFIWYSYFSRLSSICLLKRLPTYPKKGERAKMMWF :  38
vegfaa-2   : -MLGCLFDTVISRGSPPSVCCKGCPHTQRRGKEQK*CD :  36
vegfaa-2-r : -MLGCLFDTVISRGSPPSVCCKGCPHTQRRGKEQKWCD :  37
                        sr s      4         4

             40         *        60         *
vegfaa     : PFMDVYKKSACKTRELLVDIIQEYPDEIEHTYIPSCVV :  76
vegfaa-1   : PSWMCIKRVRARPESCW*TSSRSIPMRSSTRTSRPVWF :  72
vegfaa-1-r : PSWMCIKRVRARPESCWSTSSRSIPMRSSTRTSRPVWF :  76
vegfaa-2   : SLHGCV*KECVQDPRAAGRHHPGVSR*DRAHVHPVLCG :  72
vegfaa-2-r : SLHGCVKKECVQDPRAAGRHHPGVSRWDRAHVHPVLCG :  75
                 c k4

               80         *       100         *
vegfaa     : LMRCAGCCNDEALECVPTETRNVTMEVLRVKQRVSQHN : 114
vegfaa-1   : SCAVQDAVMMRRSNASRQRHETSLWRCCGSSNAYRSII : 110
vegfaa-1-r : SCAVQDAVMMRRSNASRQRHETSLWRCCGSSNAYRSII : 114
vegfaa-2   : SHALCRML***GARMRPDRDTKRHYGGAAGQATRIAA* : 106
vegfaa-2-r : SHALCRMLKWWGARMRPDRDTKRHYGGAAGQATRIAAK : 113
             s a               r

                120         *       140         *
vegfaa     : FQLSFTEHTKCECRPKAEVKAKKENHCEPCSERRKRLY : 152
vegfaa-1   : FS*VSQNTPSVNAGQRQKSKQRKKTTVSLAQREGSACM : 147
vegfaa-1-r : FSWVSQNTPSVNAGQRQKSKQRKKTTVSLAQREGSACM : 152
vegfaa-2   : FSAEFHRTHQV*MQAKGRSQSKERKPL*ALLREKEALV : 142
vegfaa-2-r : FSAEFHRTHQVWMQAKGRSQSKERKPLWALLREKEALV : 151
             Fs     t  v    4  s  4         re  a

                  160         *       180
vegfaa     : VQDPLTCKCSCKFTQMQCKSRQLELNERTCRCEKPR- : 188
vegfaa-1   : CRTPSPVNAPANSHK-CNASPDNLS*TKELADVKSQD : 182
vegfaa-1-r : CRTPSPVNAPANSHK-CNASPDNLSKTKELADVKSQD : 188
vegfaa-2   : CAGPPHL*MLLQIHTNAMQVQTT*VKRKNLQM*KAKM : 176
vegfaa-2-r : CAGPPHLKMLLQIHTNAMQVQTTWVKRKNLQMWKAKM : 188
             c  P         h             4 l   K
```

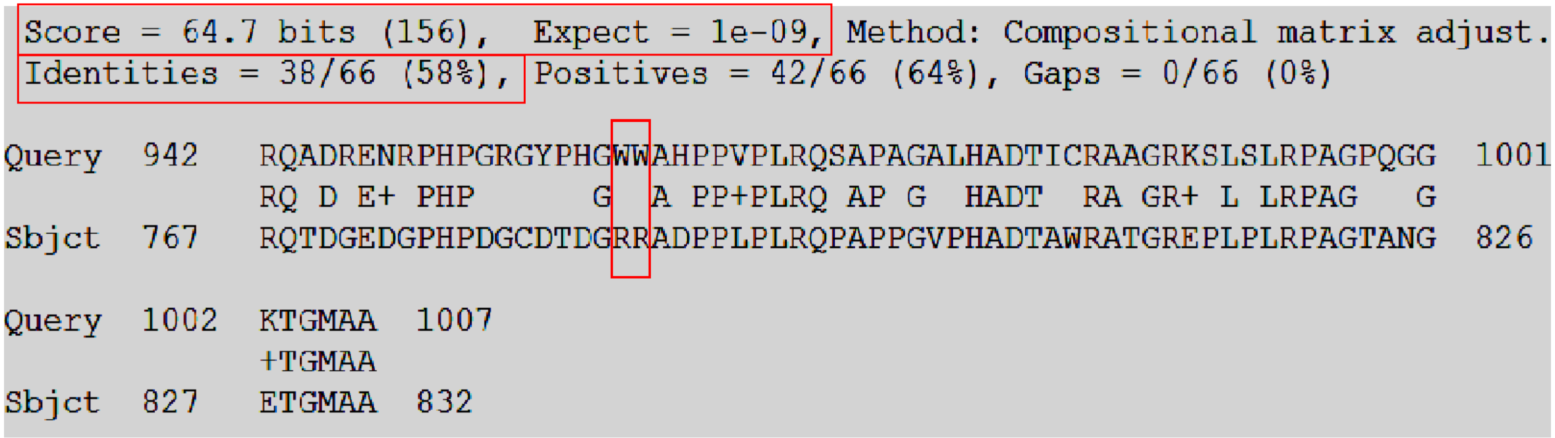
Fig 2

2E



```
Score = 63.5 bits (153),  Expect = 3e-09, Method: Compositional matrix adjust.
Identities = 38/66 (58%), Positives = 42/66 (64%), Gaps = 0/66 (0%)

Query  942   RQADRENRPHPGRGYPHG**AHPPVPLRQSAPAGALHADTICRAAGRKSLSLRPAGPQGG  1001
             RQ D E+ PHP      G  A PP+PLRQ AP G  HADT  RA GR+ L LRPAG   G
Sbjct  763   RQTDGEDGPHPDGCDTDGRRADPPLPLRQPAPPGVPHADTAWRATGREPLPLRPAGTANG  822

Query  1002  KTGMAA  1007
             +TGMAA
Sbjct  823   ETGMAA  828
```

Query translation is non-readthrough



```
Score = 64.7 bits (156),  Expect = 1e-09, Method: Compositional matrix adjust.
Identities = 38/66 (58%), Positives = 42/66 (64%), Gaps = 0/66 (0%)

Query  942   RQADRENRPHPGRGYPHGWWAHPPVPLRQSAPAGALHADTICRAAGRKSLSLRPAGPQGG  1001
             RQ D E+ PHP      G  A PP+PLRQ AP G  HADT  RA GR+ L LRPAG   G
Sbjct  767   RQTDGEDGPHPDGCDTDGRRADPPLPLRQPAPPGVPHADTAWRATGREPLPLRPAGTANG  826

Query  1002  KTGMAA  1007
             +TGMAA
Sbjct  827   ETGMAA  832
```

Query translation is readthrough

Fig 2