

$$m\hat{N}_{cFDS}$$

In the genetic code table synonymous codons are arranged systematically as these are usually different from each other at the 3<sup>rd</sup> position. A set of four codons with degeneracy only at the 3<sup>rd</sup> position is grouped as a family box in this table. There are eight such family boxes in genetic code table: one for each amino acid having four or more synonymous codons. Third position in these codons is known as four-fold degenerate site (FDS). The FDS in coding sequences is important for studying the effect of any selection pressure on codon usage bias because nucleotide substitution *per se* is not under any such pressure at the site due to the unaltered amino acid sequence in a protein (Satapathy et al 2014).

$m\hat{N}_{cFDS}$  is a variation of  $m\hat{N}_c$  (Satapathy et al 2017) that calculate effective number of codons only for 32 family box codons.

## Reference

Satapathy SS, Sahoo AK, Ray SK and Ghosh TC (2017) Codon degeneracy and amino acid abundance influence the measures of codon usage bias: improved  $N_c$  ( $\hat{N}_c$ ) and ENCprime ( $\hat{N}'_c$ ) measures. *Genes to cells* 22:277–283.

Satapathy SS, Powdel BR, Dutta M, Buragohain AK and Ray SK (2014) Selection on GGU and CGU codons in the high expression genes in bacteria. *J Mol Evol* 78:13–23.