**S5 Alignment. The X ORF has a potential AUG start codon in all erythro- and tetraparvoviruses**

Codon-based nucleotide alignment of the X ORFs of erythro- and tetraparvoviruses, derived from the reference alignment of the VP1 protein by using TranslatorX. Numbering corresponds to the VP1 CDS.

Putative AUG start codons of the X ORF are highlighted in yellow. For each species, the putative AUG start codon is conserved in all isolates (not shown in this alignment). Note how the AUGs tend to be in a similar location within erythroparvoviruses (except in chipmunk parvovirus and seal parvovirus), and within tetraparvoviruses.

**UAG** or **UGA**: stop codon

**AUG**: putative start codon of the X ORF

**Erythroparvoviruses**

Parvovirus B19 185 **UAG**UUGCUCGCAUUAAAAAUAACCUUAAAAAUUCUCCAGACUUAUAUAGUCAUCAUUUUCAAAGUC**AUG**GACAGUUAUCUGACCACCCCCAU---------GCCUUAUCAUCCAGUAGCAGUCAUG 301

Simian parvovirus 293 ACCUUAAA---CAACAUUUACAAGACUAUAUAGACAAUCCAGAUAAGUACACUU**UAG**ACUUGUCUC**AUG**GACCUCUCCCAGAUUUCAGAGAA---------ACUGAGGCAGAACACAAAUCCUUUA 406

Rhesus macaque 299 AUCUACAA---AAACAAAUUGAAAACUAUAAAAAUAAUCCAAACAAAUAUACAUUACAGUUGUCUC**AUG**GACCUCUCCCUGAUUUCAGAGAG---------UCUGAGGCAAAACAUGAAUCCUCUA 412

Pigtailed macaque 194 UGGUUAAU---CGCAUUAAAAAAAAUU**UAA**AAGACAAUCCUGAUAUUUACACUGACUCCUUAAGUC**AUG**GCGCUCUCCCAGAUUUCCGAGAA---------UCUAAGGCUGAGCAUGAGAAGUCUA 307

Chipmunk parvovirus 311 CCGUAGCU---AACACAGCCAAGCGGU**UAA**AAACUGACGAGGAUCCCUUA------UCCUUUGGGGCCCCCCCAC**UAA**CAGAAAACGCCCCGGUUCCCGUUGCGGAGCCAG**AUG**UGGCAAUUGUUU 427

Seal parvovirus 338 GGAGGCACUAUGGAUCCUCAAAGGAGCAGGGCGCAGCACCCCCCGCUGAUCCCAGACAUA**UAG**CAGGCCCUCCUCUCACAUCACAGGGCCCG---------GUGUUGGGGGAGC**AUG**UCCCUAUCA 454

**Tetraparvoviruses**

Human parvovirus 4 467 UUUUUGAA---GACUCGC**UAA**CGAACUUUGCCAAAGAAGACU**UAG**ACACCUGGCAACAACUCCACGAGCAGUUUAUCAAACUCUUUCACCCU---------CCAG**AUG**UCGGAGUCCACCUUGUUA 580

Porcine hokovirus 464 UCUUUGAG---GAGAGUGUAUCUCCUUGGUCUGAGGAAGACAAAAAAAUUU**UGA**AACAGAUUGAAGGGCAGUUCCAGGAAAUAUUCCAUCCACCCACAGAUACGGAGG**AUG**GAGCCGAUAGCUACG 586

Yak hokovirus 491 UUUUUCAG---GGCAUGUUAUCUCCCAUCAAACCUGAAGAUAGACCUAUAU**UAG**ACACCAUACAGAAGCAGUUUGAGGAGUUUUUUUAUCCUCCUA**UAG**UGGAAC**AUG**CUGGACCAGGCGACUACA 613

Deer tetraparvovirus 491 ACUUUGAU---GGCAUGUUGUCACCUAUUCCAGUGGAACAACAUCCAAUUG**UAG**AGCAGA**UAA**AGGCUCAGUUUU**UAA**AUAUUUUUCAUCCGCCACCUA**UAG**CUGGAG**AUG**GAGUCGGUGGUGACG 613

Ovine hokovirus 491 UUUUCAAU---GGUAUGUUGACGCCGUUUGAUUCCGACCAGCGACCGGUGGUCGAGCAGAUUCACCAGCAGUUCU**UAG**ACAUCUUUCACCCCCCACCAGUGCAUGGCC**AUG**GCGGAGGUGUCGACA 613

Rodent tetraparvo 467 ACUUUGAC---CAGUUCAUCACCCCGGUGGACCCCGGGGAGCCCGAGAUUC**UAA**AGCAGGCCUUCCAGCGCAUGG**UAG**AAUAUUACCACCCCGCCCCU---CAGG**AUG**GCGGACCUGCUCCUCCCC 586

Eidolon parvovirus 458 UUUUUGAU---GAGUCUGUGCUGGGCAGCGUUAGCGGAGACCCCGAAAACUUUGCUUUUGUGAAGCAGGCAG**UAG**UUAGUGCUUUUCAUCCUGCA------G**AUG**GAGGAGCCGCUACUGAACAGC 574

Oppossum tetraparvo 440 UUUUUACU---GACUCUAUUAGUGCGGGUG**UAG**CUGGAACA---CCACUCUGGGGAGAUACUGCAGAAGCUCUCAUGUCGGCCCUU---------------GCAGGGA**AUG**GAAGUAUCAGAGGGU 544