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Original alignment block:
  hg18.chrY CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
  panTrol.chrX CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
  rheMac2.chrX CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
  rn4.chr12 CGGGCTGCCGCTGCAAAATGCACACCAACACCGTGTGCAGAAAAATGTCTTCCG
  mm8.chrUn_random CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTA
oryCun1.scaffold_202349 CTGGATGCCGTTGTAAAA---ACTCAAATACAGTTTGTCTGCAAAGTCCCACCG
  bosTau2.scaffold136 CAGGCTGCCGTTGTAAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
  canFam2.chrX CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGGAAAAATCTCTTCTG
dasNov1.scaffold_74633 CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
  loxAfr1.scaffold_3802 CCGGATGCCGTTGTAAAA---ACTCAAACACAGTTTGTCTGCAAAGTCCCACCTG
  monDom4.chrX CAGGCTGCCGATGCAAGAGTACACCAACACCGTCTGCAGAAAGATCTCCTCTT
  galGal2.chr4 CCGGCTGCCGCTGTGACACCAACACCGTGTGCAGAAAGATCTCCTCCT
  xenTrol.scaffold_211 CTGGCTGTCGATGTGAGAATCATACTAACACTGTCTGCAGAAAAATATCCTCAT
  tetNig1.chr20 CCGGCTGCCGCTGCAAGA---ACTCCAACACTGTGTACTGCA-----
  fr1.chrUn CAGGCTGCAGGTGCCACA---ACACCAACACCGTGTGCCGAAAAATCTCCGCCT
  danRer3.chr21 CTGGTTGCCGATGCAGCA---ACACCAACACTGTGTGCCGCAAGATCTCTGCCA

REFERENCE (CONSENSUS) CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
  CONSERVATION CLASS 181131122181163261BBD1231311211711C12161714594594554AA
  UNIQUE COLUMN NUMBER 12334536738539ABCEFGD6H1IDDJD1K35L5M1N3ODPQRSTUVWXYZ

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Original edge list:
A: AGCACG ACC AT A G A G
  111111 222 44 5 6 9 B
C: TGTAAG C AAC T A T A T T
  111111 2 333 5 6 7 8 9 A
G: CGATTC C C C C G C
  111111 2 3 6 8 B D
T: GGG G CC CT G G
  111 2 44 55 A C

Shuffled edge list:
A: GCGACA CCA AT A G A G
  111111 222 44 5 6 9 B
C: AAAGTT C ACA T A T A T T
  111111 2 333 5 6 7 8 9 A
G: TCTAGC C C C C G C
  111111 2 3 6 8 B D
T: GGG G CC CT G G
  111 2 44 55 A C

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Random alignment block produced by Multiperm:
  hg18.chrY TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
  panTrol.chrX TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
  rheMac2.chrX TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
  rn4.chr12 TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAAATGTCTTCCG
  mm8.chrUn_random TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTA
oryCun1.scaffold_202349 TTACAGCCCATACTATTA---GCTAATGATGAGATGGCATCGAAGTCCCACCG
  bosTau2.scaffold136 TTACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
  canFam2.chrX TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTGAAATCTCTTCTG
dasNov1.scaffold_74633 TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
  loxAfr1.scaffold_3802 TTACAGCCCACTACTA---GCTAATGATGAGATGGCATCGAAGTCCCACCTG
  monDom4.chrX TAACAGCCCAAACACCCAGTGCCTGCAACTGATGCGACGGCGTAGAGATCTCCTCTT
  galGal2.chr4 TCACCGCCCAACACCTACCCGCAACTGATGGGAGGGCGTAGAGATCTCCTCCT
  xenTrol.scaffold_211 TAACGGCTATACATCTAATCGTAACTGATGTGACGGCGTGAAATATCCTCAT
  tetNig1.chr20 TCACAGCCCACTCCCA---GCTACTGATGTGAGGACGTCG-----
  fr1.chrUn TGACCGCACAACCCCA---GCAACTGATGCGAGGGCCTCGAAATCTCCGCCT
  danRer3.chr21 TAACAGCCCATACCCCA---GCAATTGGTGTGAGGGCCTCGAGATCTCTGCCA

REFERENCE (CONSENSUS) TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
  CONSERVATION CLASS 181131122181163261BBD1231311211711C12161714594594554AA
  UNIQUE COLUMN NUMBER 58D1A3176D2D1NIJ9DEFG36HD453B53K3DL3M1C5O3PQRSTUVWXYZ
  DINUCL CONS VIOLATIONS * * * * * * * * * * * * * * * *

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**Fig. S1.** Sample shuffle of a multiple sequence alignment using Multiperm at level 1. The shufflability (with 1000 shuffles) of this alignment is 0.524. The Multiperm algorithm a) computes the conservation class for each column using mean pairwise identity, b) constructs the original edge list from start nucleotide to end nucleotide and end conservation class, c) shuffles the edge list and d) randomly walks the shuffled edge list starting from a randomly chosen start column. The start column is indicated by the caret on the last line. All the columns in the same conservation class are labeled with the same symbol. The conservation class of a column is preserved since the composition of the column is not changed.