```
Original alignment block:
                  hg18.chrY CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
               panTro1.chrX CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
               rheMac2.chrX CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAGATCTCTTCTG
                  rn4.chr12 CGGGCTGCCGCTGCAAAATGCACACCAACACCGTGTGCAGAAAAATGTCTTCCG
           mm8.chrUn_random CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTA
     oryCun1.scaffold_202349 CTGGATGCCGTTGTAAAA---ACTCAAATACAGTTTGCTGCAAAGTTCCCACCG
        bosTau2.scaffold136 CAGGCTGCCGTTGTAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
               canFam2.chrX CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGGAAAATCTCTTCTG
      dasNov1.scaffold_74633 CAGGCTGCCGCTGTAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
       loxAfr1.scaffold_3802 CCGGATGCCGTTGTAAAA---ACTCAAACACAGTTTGCTGCAAAGTTCCCACTG
               monDom4.chrX CAGGCTGCCGATGCAAGAGTCACACCAACACCGTCTGCAGAAAGATCTCCTCTT
                galGal2.chr4 CCGGCTGCCGCTGTCAGACCCACACCAACACGGTGTGCAGAAAGATCTCCTCCT
       xenTro1.scaffold_211 CTGGCTGTCGATGTGAGAATCATACTAACACTGTCTGCAGGAAAATATCCTCAT
               tetNig1.chr20 CCGGCTGCCGCTGCAAGA---ACTCCAACACTGTGTACTGCA------------
                   fr1.chrUn CAGGCTGCAGGTGCCACA---ACACCAACACCGTGTGCCGCAAAATCTCCGCCT
                danRer3.chr21 CTGGTTGCCGATGCAGCA---ACACCAACACTGTGTGCCGCAAGATCTCTGCCA

      REFERENCE (CONSENSUS) CAGGCTGCCGCTGCAAGAGGCACACCAACACTGTGTGCAGAAAAATCTCTTCTG
         CONSERVATION CLASS 181131122181163261BBD12313112117 11C12161714594594554AA
       UNIQUE COLUMN NUMBER 12334536738539ABCDEFGD6H1IDDJD1K35L5M1N3ODPQRSTUVWXVYZ
```

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

```
Random alignment block produced by Multiperm:
                  hg18.chrY TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
               panTro1.chrX TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
               rheMac2.chrX TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAGATCTCTTCTG
                  rn4.chr12 TCACAGCCCAGACACCCATGCGCAACTGATGCGAGGGCATAGAAATGTCTTCCG
           mm8.chrUn_random TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTA
     oryCun1.scaffold_202349 TTACAGCCCATACTATTA---GCTAATGATGAGATGGCATCGAAGTTCCCACCG
        bosTau2.scaffold136 TTACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
               canFam2.chrX TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTGGAAATCTCTTCTG
      dasNov1.scaffold_74633 TCACAGCCCAAACACCTAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
       loxAfr1.scaffold_3802 TTACAGCCCACACTACTA---GCTAATGATGAGATGGCATCGAAGTTCCCACTG
               monDom4.chrX TAACAGCCCAAACACCCAGTCGCAACTGATGCGACGGCGTAGAGATCTCCTCTT
                galGal2.chr4 TCACCGCCCACACACCTACCCGCAACTGATGGGAGGGCGTAGAGATCTCCTCCT
       xenTro1.scaffold_211 TAACGGCCTATACATCTAATCGTAACTGATGTGACGGCGTGGAAATATCCTCAT
               tetNig1.chr20 TCACAGCCCACACTCCCA---GCTACTGATGTGAGGACGTCG------------
                   fr1.chrUn TGACCGCACAAACCCCCA---GCAACTGATGCGAGGGCCTCGAAATCTCCGCCT
                danRer3.chr21 TAACAGCCCATACCCCCA---GCAATTGGTGTGAGGGCCTCGAGATCTCTGCCA

      REFERENCE (CONSENSUS) TCACAGCCCAAACACCCAGGCGCAACTGATGTGAGGGCGTAGAAATCTCTTCTG
         CONSERVATION CLASS 181131122181163261BBD12313112117 11C12161714594594554AA
       UNIQUE COLUMN NUMBER 58D1A3176D2D1NIJ9DEFG36HD453B53K3DL3M1C5O3PQRSTUVWXVYZ
    DINUCL CONS VIOLATIONS  *        * *    ***          *  ** *  * * **^ *
```

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.