**Assessment of robustness of MASS-PRF to demographic and recombination history using coalescent simulations**

To evaluate the impacts of demography and recombination on the estimation of selection intensity using MASS-PRF, we simulated six sets of neutrally evolved genes under three demographic events: bottleneck, constant and expansion scenarios with and without recombination. Firstly, we used Hudson’s ms (Hudson 2002) to simulate 20 coalescent graphs, each containing 101 samples, for six conditions incorporating three demographic events and recombination. Within each iteration of generating each coalescent graph, we specified a diploid population size of *N*0 = 104, a mutation rate of 10–8, and a recombination rate of *ρ* = 0.36 (as suggested in Hudson’s ms application) with a gene length of 900 bp. Within each iteration, the 101 samples include one divergent sequence and 100 polymorphic sequences, which diverged 6 mya (by eliminating migration from that time forward). We specified three demographic histories with and without recombination: an instantaneous fivefold bottleneck at 100 kya (thousand years ago), a constant population, and a 10-fold exponential growth beginning 100 kya. After generating 20 trees under each of the six scenarios, we used Fletcher and Yang’s INDELible (Fletcher and Yang 2009) to evolve sequences consistent with the coalescent trees under a neutral codon substitution model (the two-ratio model M1 with *ω*0 = 0, *ω*1 = 1, and *p*0 = 0.5) as demonstrated by Yang and Nielsen (Yang and Nielsen 2002). In the recombination scenarios, the trees for each sample that were output by ms were independently evolved in Indelible and then re-concatenated to produce full 900 bp sequences. MK tests were then applied to the simulated data to test the null hypothesis that genes were neutrally evolving. Finally, we estimated selection intensity for the six sets of simulated sequences using MASS-PRF with two divergence time strategies: (1) the site-specific divergence time calculated from clustering of silent sites, and (2) a fixed 6 million years ago divergence time corresponding to the human-chimpanzee species divergence time (of the human and chimpanzee lineages) specified in our ms coalescent simulations. False positive rates of selection intensities inferred by MASS-PRF on the simulated data were calculated.