

Table S10: Summary of Twist Binding divergence estimated using different methods

	Pvalue 10-5	Pvalue 10-10	Pvalue 10-15	All	TWOB	FWOB
<i>D.mel</i> rep1	0	0	0	0	0	0
<i>D.mel</i> rep2	2%	6%	13%			
<i>D.sim</i> rep1	18%	29%	41%	39.7%	37.8%	36.9%
<i>D.sim</i> rep2	3%	52%	68%			
<i>D.yak</i> rep1	19%	28%	36%	37.2%	36.8%	36.4%
<i>D.yak</i> rep2	22%	34%	43%			
<i>D.pse</i> rep1	40%	48%	53%	59.2%	53.3%	53.2%
<i>D.pse</i> rep2	42%	53%	59%			
offset 20kb	87%	93%	95%	NA	NA	NA

Note: the divergence rate in column 2,3,4 are calculated from the conservation rate obtained from He *et al.* paper. These conservation rates were estimated by comparing the binding site sets identified using different P value cutoffs in other species to the high stringent binding site set identified in the *D. melanogaster* reference replicate (here *D. mel* rep1). Column 4,5,6 are the conservation rate estimated by applying our methods to the Twist data. We found that our estimates of conservation rate is generally higher than He *et al.* method, and consistent with the estimate variations as well as False Negative Rate (1- offset estimate) observed using their method between replicates for each species.