

**Table S2:** Summary of promoter positions with significant mutation rates following both selection screens<sup>a</sup>

Position	Site	WT Base	# Mutations (Selected library)		Expected # Mutations <sup>b</sup> (Unselected library)		Log <sub>10</sub> (p-value) (pairwise) <sup>c</sup>		Log <sub>10</sub> (Min p-value <sup>2</sup> ) <sup>d</sup>
			Activate	Deactivate	Activate	Deactivate	Activate	Deactivate	Combined
380	Sp1 III, p4	G	15	10	0.225	0.235	<-25	-12.7	-25.5
426	TATA, p2	A	9	6	0.917	0.958	-6.29	-3.28	-6.56
412	Core (n.i. <sup>e</sup> )	C	3	19	0.173	0.179	-3.10	<-25	-6.20
382	Sp1 III, p6	G	10	3	0.218	0.235	-13.1	-2.72	-5.43
391	Sp1 II, p4	G	3	3	0.225	0.235	-2.77	-2.72	-5.43
192	U3 (n.i.)	A	5	17	0.917	0.958	-2.54	-15.5	-5.07
431	TATA, p7	A	5	5	0.917	0.958	-2.54	-2.45	-4.90

- Mutated positions were considered significant if both pairwise p-values < -2 ( $p < 0.01$ ) and the  $\log_{10}(\min p\text{-value}^2) < -4$ .
- Expected mutation rate is base-type specific but not position-specific in the initial library.
- Pairwise comparison of mutation rates between an individual screen and the unselected library
- Log<sub>10</sub> of the square of the minimum of the pairwise p-values, which is a measure of the probability of the weaker screen giving that p-value or better, under the null hypothesis that the all 3 rates are the same.
- n.i. = Not identified