

Table S2. Orthologous TSS conservation. For each pair of TSS-classes we compare the ratio of orthologous TSSs to the total frequency of TSSs (using Fisher exact test). The class with a larger ratio includes more positional orthologs and is therefore more conserved. TSSs that fit into more than one class (i.e., gaTSS, giTSS, aiTSS and gaiTSS) were excluded from the analysis.

A) Comparison of *F. muscicola* and *F. thermalis*

TSS-Type	# gTSS	# aTSS	# iTSS		H1: gTSS > aTSS	H1: gTSS < aTSS	H1: gTSS > iTSS	H1: gTSS < iTSS	H1: aTSS > iTSS	H1: aTSS < iTSS
Ortholog	796	994	513	p-value:	0.991	0.011	0.332	0.693	0.006	0.995
Singleton	1,639	1,786	1,091	FDR corrected:	0.991	0.032	0.498	0.995	0.018	0.995
Total	2,435	2,780	1,604							

Conclusion: aTSS positional orthologs are enriched (i.e., more conserved) in comparison to gTSS and iTSS. No difference between gTSS and iTSS

B) Comparison of *F. muscicola* and *C. fritschii*

TSS-Type	# gTSS	# aTSS	# iTSS		H1: gTSS > aTSS	H1: gTSS < aTSS	H1: gTSS > iTSS	H1: gTSS < iTSS	H1: aTSS > iTSS	H1: aTSS < iTSS
Ortholog	449	314	147	p-value:	2.200E-16	1.000	2.200E-16	1.000	0.001	0.999
Singleton	2,397	3,422	2,195	FDR corrected:	3.300E-16	1.000	3.300E-16	1.000	0.001	1.000
Total	2,846	3,736	2,342							

Conclusion: gTSS positional orthologs are enriched (i.e., more conserved) in comparison to aTSS and iTSS. aTSS positional orthologs are enriched in comparison to iTSS.