

Ancestral Resurrection of the *Drosophila* S2E enhancer

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We have developed a novel computational approach for the custom design of complex cis-regulatory regions expressing in arbitrary patterns, as well as methods for predicting the evolution and putative ancestral sequences of extant enhancers in *Drosophila*. Our methodology involves the use of a feed-forward transcriptional model, capable of predicting gene expression patterns directly from enhancer sequence, as a tool for enhancer design and to provide a functional constraint for predicting enhancer evolution. In the former case, enhancer design was achieved through the use of simulated annealing in conjunction with a transcriptional model in order to efficiently search the sequence space for novel enhancers having the desired expression pattern. For the latter, Bayesian inference was used to generate a set of possible ancestral eve stripe 2 enhancer (S2E) sequences for the sim-sec, mel-sim-sec, and ere-yak internal nodes of the *Drosophila* phylogenetic tree. Candidate ancestral sequences were selected for synthesis and experimental validation by checking the model predicted expression patterns of each sequence against a reference eve stripe 2 expression pattern. By combining an phylogenomics with transcriptional modeling, we show that compensatory evolution between Bcd and Gt transcription binding sites have occurred during the course of stripe 2 evolution.