Supplemental Figure 1. Identification of Binding Sites for the Transcription Factors TEF and SRF in the Random DNA Library.

(A) Competition of random DNA plasmid pools for binding to recombinant TEF in an EMSA experiment with a probe containing a PAR bZIP consensus site. Different amounts of non-radioactive probe (5x, 25x, and 125x the amount of the radioactive probe) were included in three reactions as a positive control for specific competition to the radioactive probe. (B) The individual plasmids of the competing DNA-pool 6 were used as competitor DNA in an EMSA experiment identifying a PAR bZIP binding site in plasmid 98. (C) Competition of random DNA plasmid pools for binding to serum response factor (SRF) in mouse liver nuclear extract with a probe containing an SRF consensus site. Different amounts of non-radioactive probe (10x and 100x of non-radioactive probe, and 100x of an oligonucleotide with an unrelated sequence) were included in three reactions as a positive control for specific competition to the radioactive probe. Asterisks mark unspecific protein-DNA complexes, whose formation seems to be triggered by high amounts of competitor DNA in the EMSA reactions, perhaps by titration of inhibitory activities of the corresponding proteins. (D) Competition of the individual plasmids of the competing pools 1 and 4 for binding to SRF. (E) EMSA with probes originating from plasmids 61 and 94 that competed for binding to SRF.

Supplemental Figure 2. Strategy for the Identification of an Unknown Binding Site in Random DNA Probes.

(A) The two 50 bp random DNA sequences of a random DNA insert are separately amplified by PCR and used as competitor DNA in EMSA experiments. Six oligonucleotides (arrows) are then designed for the competing fragment and are used in competition experiments individually or in combination with each other, annealed or filled-in, according to the scheme shown in (B). (C) Circadian bandshift of probe 50 containing a tripartite heat shock element. (D) Narrowing down the binding site of probe 50 to 20 bp using the procedure described
above. The various combinations of annealed and filled-in oligonucleotides that were used as competitor are indicated on top of the autoradiograph. The minimal sequence required for competition was determined as TGGCGTTCTAGAACTTGCCG (HSE underlined).