

Supporting Information

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SI Materials and Methods

Identification of an Su(H) in Vivo DNA Binding Site Consensus Sequence.

ChIP-seq to identify Su(H) protein in vivo within *Drosophila* early embryos was previously published (7). ChIP-Seq defined peaks were identified, and overrepresented motifs were identified by using Hypergeometric Optimization of Motif EnRichment (HOMER) (27). The second most common motif (second to a GAGA repeat sequence that likely represents an open chromatin signature) (21) was a sequence similar to Su(H) from JASPAR and was present in 24% of called peaks, which suggests this motif is the explanatory site for ChIP and represents an in vivo DNA binding site consensus sequence for *Drosophila* Su(H) in the early embryo.

Fly Crosses and Generation of Su(H) Germline Clones. Flies were reared under standard conditions at 23 °C. *run⁻* flies were rebalanced with *CyO fz-lacZ* marked balancer. For ectopic expression experiments, AP or DV enhancer reporter-line males were crossed to *yw*, *hs-run*, or *hs-Su(H)* females.

Su(H)Δ47 is a null allele and used previously for generation of females containing germline clones using the Flp-FRT system as described previously (5,7). *SuHΔ47 FRT40A P[*l*(2)35Bg +]/CyO hb-lacZ* males were crossed to females with germline clones of *Su(H)Δ47 FRT40A P[*l*(2)35Bg +]*.

Heat Shock-Mediated Ectopic Expression. For heat-shock experiments, 1–3-h embryos carrying one copy of *hs-Su(H)* (15) or *hs-run* (12) and one copy of a given reporter gene were collected and transferred into a 37 °C incubator for 20–25 min, allowed to recover at 25 °C for 35–40 min, and fixed immediately. Controls include comparisons of reporter expression associated with (i) embryos containing the *hs-Su(H)* or *hs-run* construct without the heat-shock treatment as well as (ii) heat-shocked *yw* embryos construct lacking either construct.

Candidate Enhancers and Mutagenesis. *hb_stripe*, *sog_Distal*, and *sog_Intronic* enhancer sequences were cloned into BglII/KpnI, BglII/NotI, or NaeI/NotI sites, respectively, associated with the *eve.promoter-lacZ-attB* vector (20), using standard techniques. Primers used to perform PCR of genomic fragments representing *hb_stripe*, *sog_Distal*, and *sog_Intronic* enhancers are listed as follows:

*hb_stripe*F5'-ATTAAGATCTTTTCATTGTCCGCCTTAATGG-3';

*hb_stripe*R5'-ATTAGGTACCCTTTCGGACGCTCAACAATG-3';

*sog_Distal*F5'-ATTAGCGCCGCGACAGATTCCCAGG-3';

*sog_Distal*R5'-ATTAAGATCTAACTGACAGGGGCAAGTCG-3';

*sog_Intronic*F5'-ATTAGCCGGCGTTGCCAATGCCA-3';
and

*sog_Intronic*R5'-ATTAGCGCCGCGCTTTATGGTCC-3'.

Site-directed transgenesis was carried out using a *Drosophila melanogaster* stock containing attP insertion site at position ZH-86Fb (Bloomington stock no. 23648).

The 14 AP reporter enhancer assayed include 13 provided by S. Small (i.e., HC_11, HC_34, gt1, HC_07, oc_EHE, HC_25, HC_35, HC_09, gt_23, hb_P2, tll_OE, HC_02, and eve2; Chen et al. (6)) as well as the *hb_stripe*, which was remade for the purpose of this study.

The *sog_Distal* and *sog_DistalΔrun* yellow reporters (*sog_Distal.y* and *sog_DistalΔrun.y*) were created by inserting gene-synthesized WT *sog_Distal* and Run-binding site-mutated *sog_Distal* sequences into the *eve2* promoter-MS2.yellow-attB vector (28). A *yellow* gene intronic probe was used to detect expression in fixed tissues. *sog_Distal.y* and *sog_DistalΔrun* reporter sequences are included in Table S1. The mutated Su(H) and Run binding sites are marked with red, compared with blue for the WT sites (Table S1).

In Situ Hybridizations, Immunohistochemistry, and Image Processing.

Enzymatic in situ hybridizations were performed with antisense RNA probes labeled with digoxigenin, biotin, or FITC-UTP to detect reporter or in vivo gene expression (7, 20). *Kr*, *tll*, *oc*, *hb*, *en*, *sna*, *sog*, and *eve* riboprobes were used for multiplex FISH, as well as *hb* and *yellow* intronic riboprobes. Images were taken under the same settings, 15 z-sections at 0.5-μm intervals, on a Zeiss Pascal confocal. *P* values across the study were calculated by using a one-tailed *t* test.

Antibody staining was performed by incubating fixed embryos with mouse anti-Nrt (BP 106 Developmental Studies Hybridoma Bank) at 1:50 dilutions; rat anti-Bcd (no. 807) (29), goat anti-Su(H) (sc-15813; Santa Cruz Biotechnology), and guinea pig anti-Runt antibodies (no. 638) (29) at 1:200 dilutions followed by incubation with Alexa Fluor 488 donkey anti-rat IgG secondary antibodies (1:500 dilutions; Invitrogen). Heat/methanol-fixed embryos were used for the anti-Nrt staining.

Measurement of Gene Expression Boundary Positions. To measure boundary positions of *Kr* central and *tll* anterior expression domain, lateral images of alkaline phosphatase-stained embryos were taken by using a 20× objective on an Axioplan microscope. Eight to 15 embryos of each genotype [WT, *Su(H)*⁻ mutant, *hs-Su(H)*, *hs-run*] of appropriate ages and orientation were then analyzed for expression patterns. A line was drawn at the center of each embryo from the anterior edge to the posterior edge to measure EL. AP positions were displayed as a percentage of EL with the anterior pole as 100%. Following the same logic, a line was drawn from the anterior pole to the anterior boundary of the central *Kr* domain (marked as “ab”), whereas the “bc” line demarcates the central *Kr* domain width. Similar to *Kr*, “ab” is the length of the anterior boundary of the *tll* anterior domain and “bc” is the anterior *tll* domain length. Each of the “ab” and “bc” measurements was then divided by EL, resulting in the distance from the anterior pole or the width of the expression domain as a percentage. Significance was tested by Student’s two-tailed *t* test and was designated by a *P* value of <0.05. For the *tll* posterior domain, all expressed cells of the blastoderm periphery were counted in WT, *hs-Su(H)*, and *hs-run* embryos. All comparisons were made by number of cells counted, and the analysis revealed an average of seven fewer cells expressed per embryo upon ectopic expression of either *Su(H)* or *run*.

Viability Experiments. For each of the genotypes WT, *hs-run*, and *hs-Su(H)*, 10 male and 10 virgin female *D. melanogaster* flies were crossed, and the number of progeny counted to establish a measure of viability resulting after heat shock and ectopic expression of *run* or *Su(H)*. After a 24-h egg-laying period, the adult flies were transferred away, and the eggs were then allowed to develop at 23 °C. This procedure was performed in triplicates. The number of adult flies that emerged from each genotype was counted, and an average of three experiments is presented in Fig. S6.



Fig. S1. Ectopic expression of Runt and Su(H) impacts *En* expression in germband-elongated embryos. Germband-elongated embryos are oriented with anterior aspect to the left. In situ hybridization using a riboprobe to *en* shows expression within 14 stripes in WT embryos undergoing germband elongation (A). Upon ectopic expression of Runt, *en* is down-regulated in odd-numbered stripes (B; even stripes marked by arrowhead), whereas ectopic expression of Su(H) has a distinct phenotype resulting in an increase in interstripe distance (C, red box). *hs-Su(H)* and *Su(H)* mutant embryos exhibit opposite phenotypes: compare red boxes in C and in Fig. 1L.

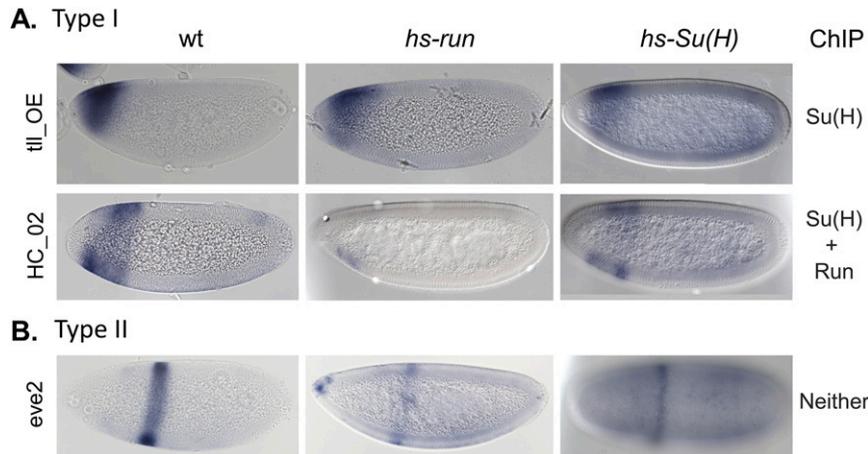


Fig. S2. Runt and Su(H) ChIP occupancy of genomic enhancer sequences does not always correlate with the ability of these factors to repress reporter constructs. Expression patterns of some reporter genes from type I (A) and type II (B) enhancers (Left) in embryos collected from three different genetic backgrounds: WT, *hs-run*, or *hs-Su(H)*. All embryos were equivalently processed, including heat shock of WT, which serves as control. Runt and Su(H) ectopic expression does not completely silence the expression of *tll_OE*, *HC_02* (*slp2* enhancer), and *eve2* reporters, despite detection of ChIP binding to these sequences at their endogenous locations. No binding was detected to the *eve2* locus.

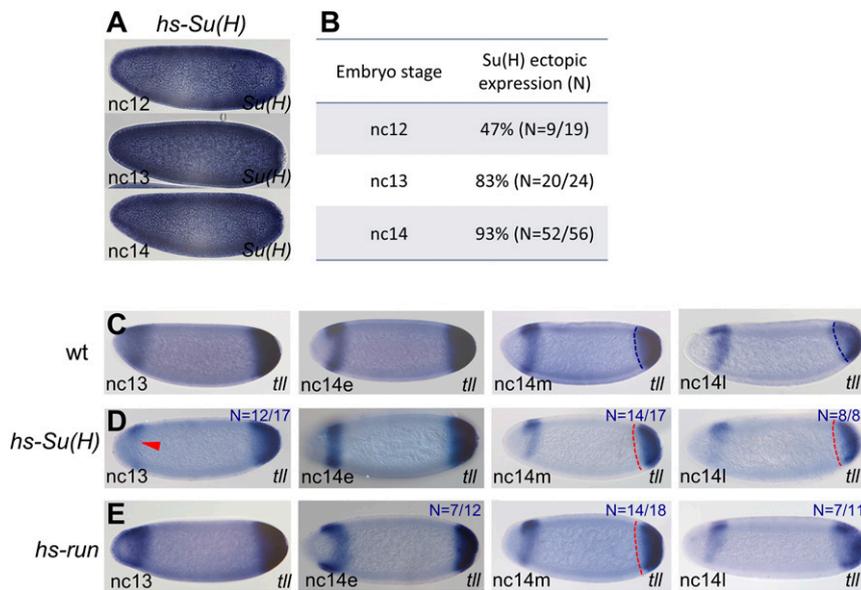


Fig. S3. *tll* expression at the anterior and posterior poles is dynamic and differentially impacted by *hs-Su(H)* and *hs-run*. (A) *hs-Su(H)* embryos overexpressing Su(H) gene at different stages of early development. (B) Quantification of ectopic expression assay efficiency at different ncs (nc 12–14). (C–E) Developmental time-course of *tll* expression within early embryos of indicated stages detected by in situ hybridization using *tll* riboprobes. All embryos were heat-shocked, including WT for comparative purposes. *tll* expression is shown for embryos of WT (C), *hs-Su(H)* (D), or *hs-run* (E) background at nc 13 until late nc 14. *tll* expression at the anterior and posterior poles is dynamic. Quantification of embryos that exhibit similar to the depicted phenotypes are present on the right corner of each image. Fifteen embryos were examined and the ratio was 14/15 embryos or greater if it is not presented. Red arrowhead and dashed lines indicate domains of reduced expression. *SI Materials and Methods* includes details about how *tll* posterior expression measurements were made.

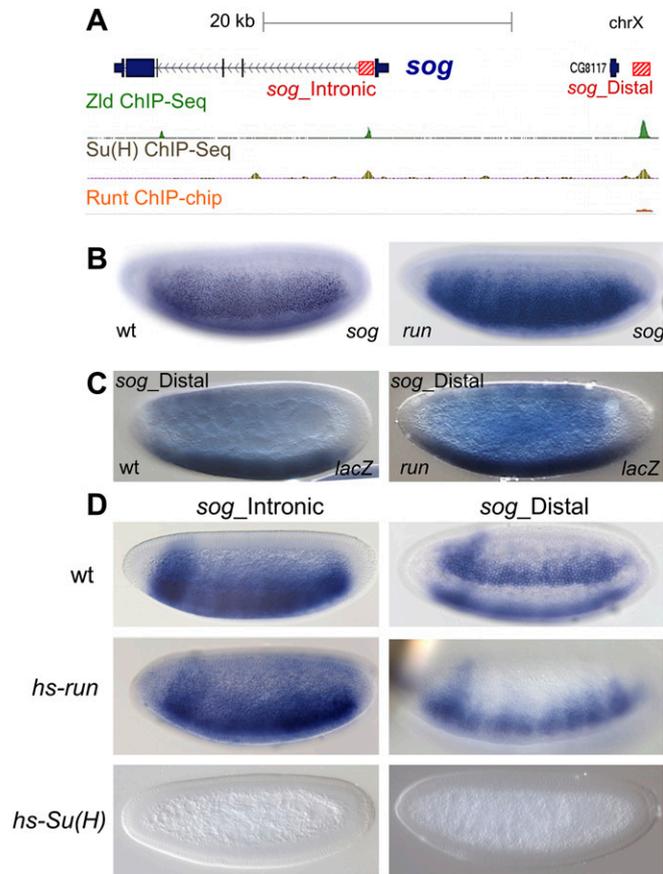


Fig. S5. Assays to test the role for Runt, in addition to Su(H), in the regulation of the *sog* gene. (A) ChIP data for Zld, Su(H), and Runt showing occupancy at the *sog* gene locus. Position of two enhancers controlling *sog* early embryonic expression, *sog_Intronic* and *sog_Distal*, are indicated by red boxes. All three factors exhibit binding to *sog_Distal*, but only two of the three factors [Zld and Su(H)] exhibit binding to *sog_Intronic*. (B–D) Expression of transcripts in embryos was obtained using in situ hybridization and specific riboprobes. (B) Expression of *sog* in WT or *run* mutant embryos at late nc 14. *sog* is expanded in *run* mutants. (C) Expression of *lacZ* in embryos, either WT or *run* mutants, containing the *sog_Distal* reporter at late nc 13. The reporter is more broadly expressed in the *run* mutant compared with WT embryos. (D) Expression of *sog_Intronic* or *sog_Distal* in WT, *hs-run*, and *hs-Su(H)* embryos that have been heat-shocked. Ectopic Su(H) represses expression of both reporters, suggesting a role for Su(H) in regulation of both *sog* enhancers. Alternatively, ectopic Runt was not able to repress expression of either reporter.

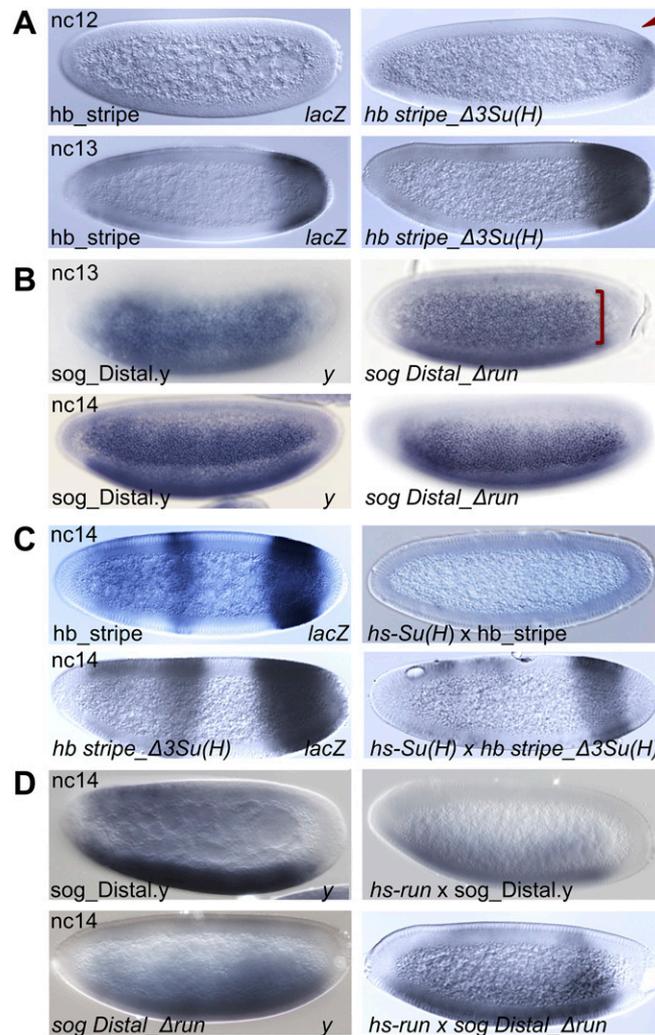


Fig. S6. Mutagenesis of Su(H) and Run binding sites within enhancers demonstrates a direct role for these factors in the regulation of *hb* and *sog* gene expression. (A and B) Reporter gene expression in embryos of indicated stages driven by *hb_stripe* (A) and *sog_Distal.y* (B) WT or mutated enhancers assayed using riboprobes to reporters *lacZ* (A) or *yellow* (B). Three Su(H) sites (matches to the binding site consensus) were mutated in *hb_stripe* enhancer sequence [*hb_stripe_Δ3Su(H)*], and one Run site was mutated in *sog_Distal* enhancer sequence [*sogDistal_Δrun*]. Mutation of Su(H) sites results in the *hb_stripe* enhancer reporter supporting earlier expression relative to WT, whereas mutation of the Run site in the *sog_Distal* enhancer reporter results in expansion of expression at nc 13 but has little effect at nc 14. Red arrowhead and bracket indicate domains where patterns exhibit alterations. (C and D) Heat shock-mediated ectopic expression of Su(H) or Run on WT or mutated *hb_stripe* and *sog_Distal* enhancer sequences. Whereas ectopic expression of Su(H) is able to silence expression of the *hb_stripe* reporter, the *hb_stripe_Δ3Su(H)* reporter was refractory to repression (C). Similarly, whereas ectopic expression of Run down-regulates expression of the *sog_Distal.y* reporter, the *sogDistal_Δrun* reporter was refractory to repression (D). The control enhancers [*hb_stripe*, *hb_stripe_Δ3Su(H)*, *sog_Distal.y*, *sogDistal_Δrun*] were similarly treated as the *hs-Su(H)* samples (i.e., heat-shocked).

Table S1. Mutagenesis of enhancer sequence variants (Fig. S6)

Construct name	Sequence
hb_stripe and hb_stripe with three Su(H) sites mutated	
hb_stripe	AGATCTTTTCATTGTCCGCCTTAATGGTTACGCCGTAAAAATTGGCTATGC 50
hb_stripe_Δ3Su(H)_sites	AGATCTTTTCATTGTCCGCCTTAATGGTTACGCCGTAAAAATTGGCTATGC 50
hb_stripe	GGCCAAACAATAGTGCAGGACGACGGCAGGACGCGCAGGACAATCGTC 100
hb_stripe_Δ3Su(H)_sites	GGCCAAACAATAGTGCAGGACGACGGCAGGACGCGCAGGACAATCGTC 100
hb_stripe	TGGTGGATTTCCAGTCGACACGCCACGAGATTTTATGAAGGCAACTCGCT 150
hb_stripe_Δ3Su(H)_sites	TGGTGGATTTCCAGTCGACACGCCACGAGATTTTATGAAGGCAACTCGCT 150
hb_stripe	TTGCATGTTATTCCATAGATTTTCGCTTCGGTCCCGGTTTGGTTGGTCAG 200
hb_stripe_Δ3Su(H)_sites	TTGCATGTTATTCCATAGATTTTCGCTTCGGTCCCGGTTTGGTTGGTCAG 200
hb_stripe	GTAAGACCTTCGATTAACAATGAAAGTAGCTGGAAAAATCGCGAGAACTT 250
hb_stripe_Δ3Su(H)_sites	GTAAGACCTTCGATTAACAATGAAAGTAGCTGGAAAAATCGCGAGAACTT 250
hb_stripe	CGAAAAGACACAAAGATACAATATCTATGAGTCTAATGGTCATTAGAGC 300
hb_stripe_Δ3Su(H)_sites	CGAAAAGACACAAAGATACAATATCTATGAGTCTAATGGTCATTAGAGC 300
hb_stripe	GGTGCGCTCTACATAACAATTGTACCAGCCGCTTGTGTTGAAGCCTAAAA 350
hb_stripe_Δ3Su(H)_sites	GGTGCGCTCTACATAACAATTGTACCAGCCGCTTGTGTTGAAGCCTAAAA 350
hb_stripe	ACGTCGCAAAAAACACACTTCCGCGTAAGACATCCCATTCTGTGGTCCG 400
hb_stripe_Δ3Su(H)_sites	ACGTCGCAAAAAACACACTTCCGCGTAAGACATCCCATTCTGTGGTCCG 400
hb_stripe	ATCGTAAAAATATTTAGTTTTTTATGACCAACGGTCCGGCAGGTAGCTGG 450
hb_stripe_Δ3Su(H)_sites	ATCGTAAAAATATTTAGTTTTTTATGACCAACGGTCCGGCAGGTAGCTGG 450
hb_stripe	CTGCCGTTTTTTGTGCGCGACCTCAACCCTTTCACCCATTAAGAAAAAAT 500
hb_stripe_Δ3Su(H)_sites	CTGCCGTTTTTTGTGCGCGACCTCAACCCTTTCACCCATTAAGAAAAAAT 500
hb_stripe	CGCATCC TGTG AGTGTCTTGCCTTCCCTCGAAACGG CCACACA ATTT 550
hb_stripe_Δ3Su(H)_sites	CGCATCC TCTGT GTGTCTTGCCTTCCCTCGAAACGG CCGTCA ATTT 550
hb_stripe	GTGTGCTTTGCGTTTTCTCCTCTCTTTTTGTTTTCCACCTAATGTCGGCGT 600
hb_stripe_Δ3Su(H)_sites	GTGTGCTTTGCGTTTTCTCCTCTCTTTTTGTTTTCCACCTAATGTCGGCGT 600
hb_stripe	CATTGCTTCTTTATGACGCCCTCGGTTGTTCTTTTTTATGGTGCCTTT 650
hb_stripe_Δ3Su(H)_sites	CATTGCTTCTTTATGACGCCCTCGGTTGTTCTTTTTTATGGTGCCTTT 650
hb_stripe	GTCCTTTGAGCCTCGTTCACGGCCAAATCCCTACTTCCCTCAACCCTTTG 700
hb_stripe_Δ3Su(H)_sites	GTCCTTTGAGCCTCGTTCACGGCCAAATCCCTACTTCCCTCAACCCTTTG 700
hb_stripe	GCGGACGAGAAAGTTGCTAGGAGGAGAACGGGTTAAGCGAAAACTCCATT 750
hb_stripe_Δ3Su(H)_sites	GCGGACGAGAAAGTTGCTAGGAGGAGAACGGGTTAAGCGAAAACTCCATT 750
hb_stripe	GCACTTTTTACAAGCCCGATCTTCTTGAATTAGTTTTGGTCATTAGGC 800
hb_stripe_Δ3Su(H)_sites	GCACTTTTTACAAGCCCGATCTTCTTGAATTAGTTTTGGTCATTAGGC 800
hb_stripe	GAAAGGGTTAATTTTCGATTTTGGCTCTCGGTGGGTTACTGAGTGAATTC 850
hb_stripe_Δ3Su(H)_sites	GAAAGGGTTAATTTTCGATTTTGGCTCTCGGTGGGTTACTGAGTGAATTC 850
hb_stripe	AATGGGCTAAGGCGAGTAAAGGGTTATACTGTTTTTACATTTTACTACTT 900
hb_stripe_Δ3Su(H)_sites	AATGGGCTAAGGCGAGTAAAGGGTTATACTGTTTTTACATTTTACTACTT 900
hb_stripe	GGAAAACTGAAGAACTTGTAGGAAAAATTTCCAGCACTTTTAAAAAGCC 950
hb_stripe_Δ3Su(H)_sites	GGAAAACTGAAGAACTTGTAGGAAAAATTTCCAGCACTTTTAAAAAGCC 950
hb_stripe	ATATATAACTTTATGAATATGAACCTTCAAATGTA AAAACCTGAAAGTGAC 1000
hb_stripe_Δ3Su(H)_sites	ATATATAACTTTATGAATATGAACCTTCAAATGTA AAAACCTGAAAGTGAC 1000
hb_stripe	ATGTAGTTATTTTAAAGTCTTGA AAAATGATCATCGTCTAAAATTTCTTT 1050
hb_stripe_Δ3Su(H)_sites	ATGTAGTTATTTTAAAGTCTTGA AAAATGATCATCGTCTAAAATTTCTTT 1050
hb_stripe	TTTTTAAATAATTTTAAAAATATTTTTTGATAGCATACGAAGTATTTAA 1100
hb_stripe_Δ3Su(H)_sites	TTTTTAAATAATTTTAAAAATATTTTTTGATAGCATACGAAGTATTTAA 1100
hb_stripe	AAATGTGAACAGATTA AACACATTAATTTATAAAAAGTAAATACAACAGA 1150
hb_stripe_Δ3Su(H)_sites	AAATGTGAACAGATTA AACACATTAATTTATAAAAAGTAAATACAACAGA 1150
hb_stripe	TTTAGCATAGAAATAAAAAATCATTTTAAATGTTCCGTCCTAAGTAACGGT 1200
hb_stripe_Δ3Su(H)_sites	TTTAGCATAGAAATAAAAAATCATTTTAAATGTTCCGTCCTAAGTAACGGT 1200
hb_stripe	CGTGGAAAATCTTGA AAAAT CCCA CAATTTATATTCGATCCCTTTGGCCG 1250
hb_stripe_Δ3Su(H)_sites	CGTGGAAAATCTTGA AAAAT CGGACA AATTTATATTCGATCCCTTTGGCCG 1250
hb_stripe	AACATTTGGTGCATACATTCGTAATTCGCTGGA AAAATTAAGGCCACTAA 1300
hb_stripe_Δ3Su(H)_sites	AACATTTGGTGCATACATTCGTAATTCGCTGGA AAAATTAAGGCCACTAA 1300
hb_stripe	GTCGCCAGCGAAATGAATTCGACATTTGGGCAATGGACAAAATGTA AAAAG 1350
hb_stripe_Δ3Su(H)_sites	GTCGCCAGCGAAATGAATTCGACATTTGGGCAATGGACAAAATGTA AAAAG 1350
hb_stripe	GACTCTAGAGCCCGACCATTTGCAATGGTCCATTGTTGAGCGTCCGAAAG 1400
hb_stripe_Δ3Su(H)_sites	GACTCTAGAGCCCGACCATTTGCAATGGTCCATTGTTGAGCGTCCGAAAG 1400
hb_stripe	GGTACC 1406
hb_stripe_Δ3Su(H)_sites	GGTACC 1406
sog_Distal.y and sog Distal with one Run sites mutated	
sog_Distal.y	GCGGCCGCGACAGATTC CCGGGTTTCAGCGGAACAGGTAGGCTGGTTCGAT 50
sog_Distal_Δrun_site	GCGGCCGCGACAGATTC CCGGGTTTCAGCGGAACAGGTAGGCTGGTTCGAT 50

