

Figure S1 Schematic diagram of PCR characterization of the excision site on *MenEx1* alleles. Forward and reverse arrowheads represent forward and reverse primers, respectively, and are organized into pairs. Black blocks represent amplicons for their corresponding primer pairs. Primer pairs were designed to amplify partially overlapping ~1kb regions to ensure full amplification coverage. (A) A wild-type control showing amplification across the genomic region. (B) Hypothetical excision in which amplification within the excision site does not produce an amplicon through PCR amplification. Primers that flank the excision site (circled in red) were used to amplify around the excision and the product was sequenced.

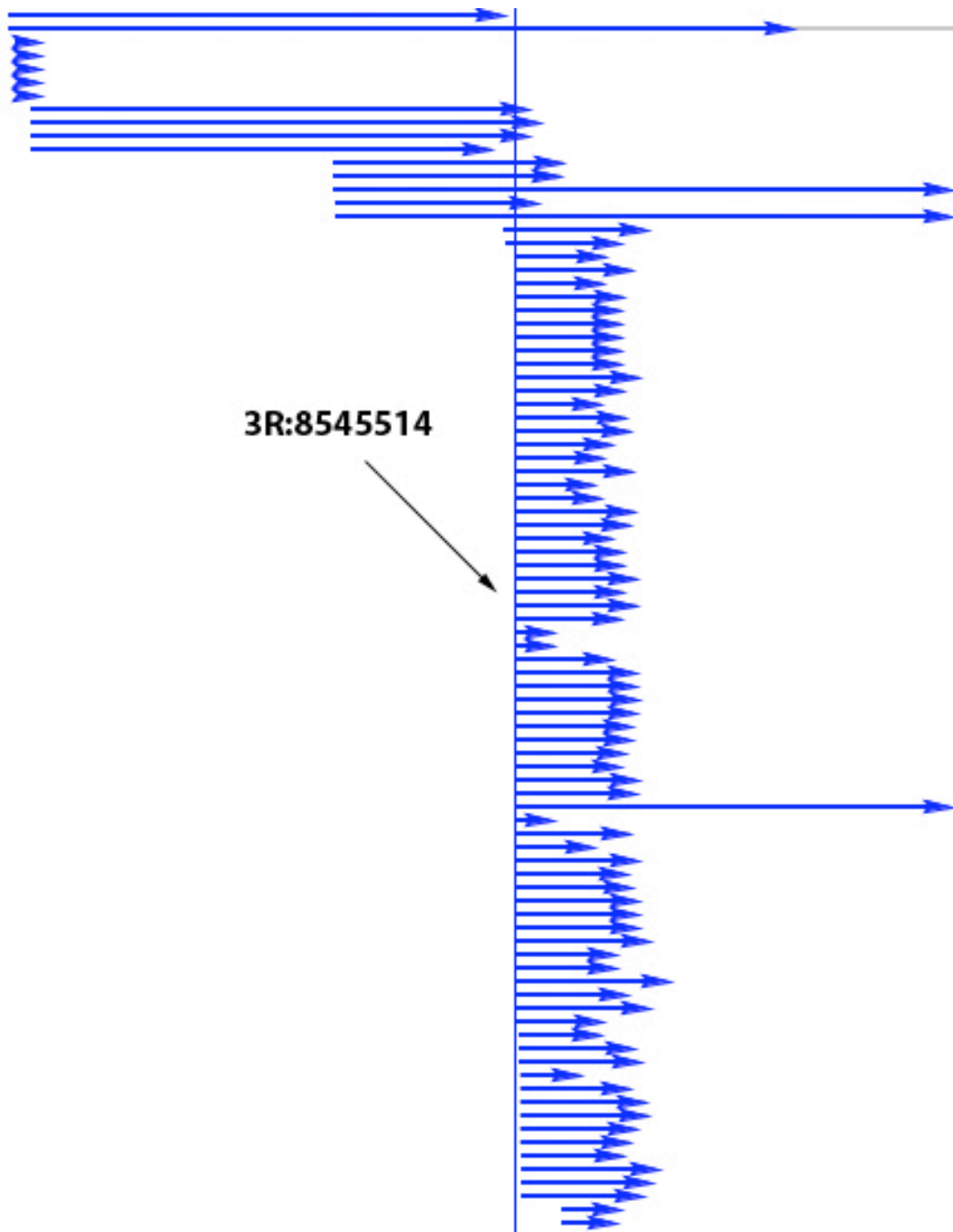


Figure S2 A visual alignment of ESTs of *D. melanogaster Malic enzyme*. The majority of ESTs begin at 3R:8545514 (indicated by the blue vertical line), the transcription start site (TSS) predicted through the PEAT project (Ni *et al.* 2010). All of our *MenEx*⁻ alleles are missing some portion of sequence flanking this TSS and are considered to be promoter deficient.

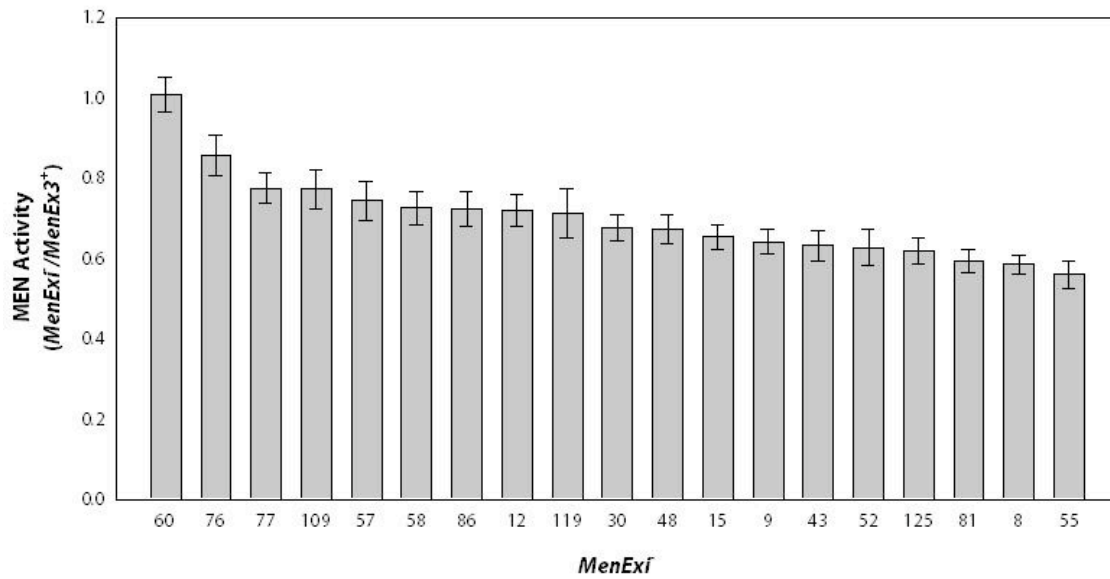


Figure S3 *MenEx1*/VT26 heterozygote MEN enzyme activity graphed as the ratio of wild-type activity (*MenEx3*/VT26). Error bars represent standard error and within each data point, n=6.

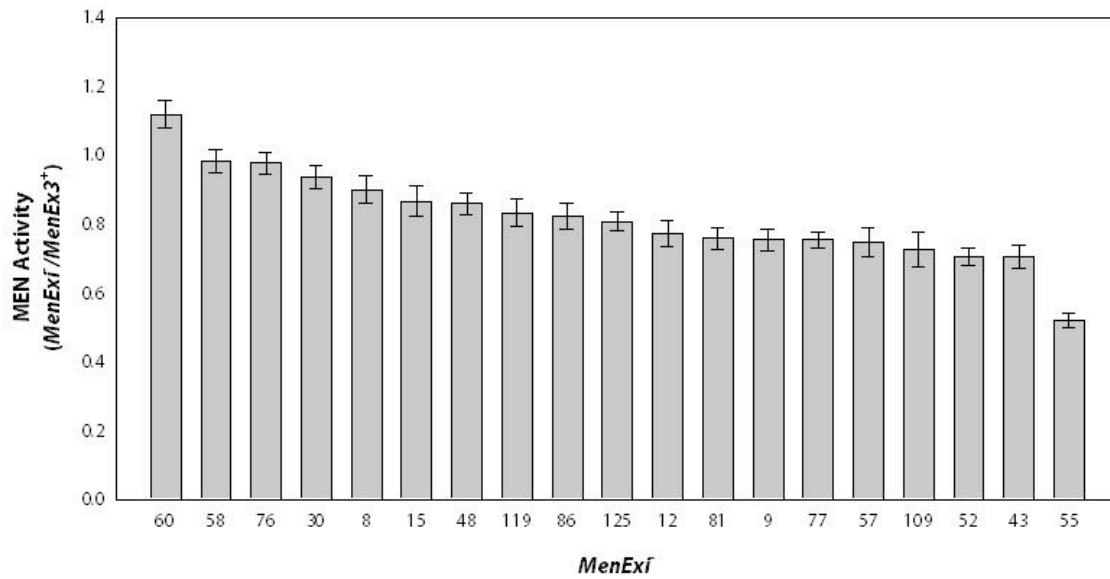


Figure S4 *MenEx1/HFL53* heterozygote MEN enzyme activity graphed as the ratio of wild-type activity (*MenEx3/VT26*). Error bars represent standard error and within each data point, n=6.

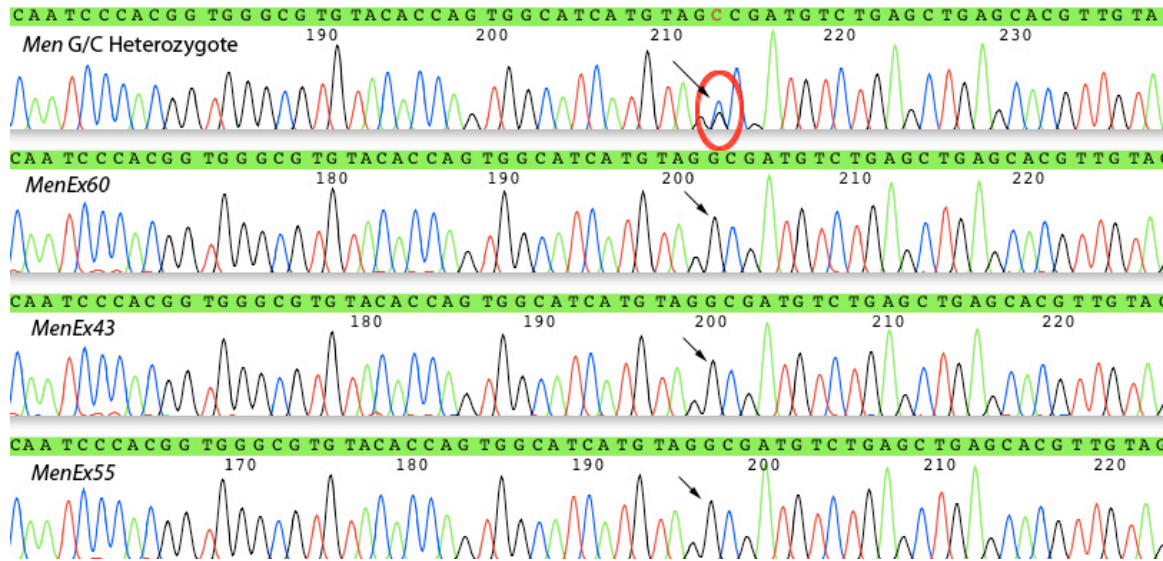


Figure S5 Chromatograms from the sequencing of *Men* cDNA from *MenEx60*⁻, *MenEx43*⁻, and *MenEx55*⁻. The *Men* gene has a G/C polymorphism at position 338 (indicated by the black arrow). *Men*^{G_{Ex}} lines were crossed to a *Men*^C line, *MD5*. In cases where both alleles are being expressed, a G/C double peak is observed (highlighted by the red circle). In the *Men*^{G_{Ex}}/*Men*^C heterozygotes, only a single peak is observed indicating that only the wild-type allele is being expressed. Note that because sequencing was done with the reverse primer, nucleotides need to be reversed (e.g. G represents a C).

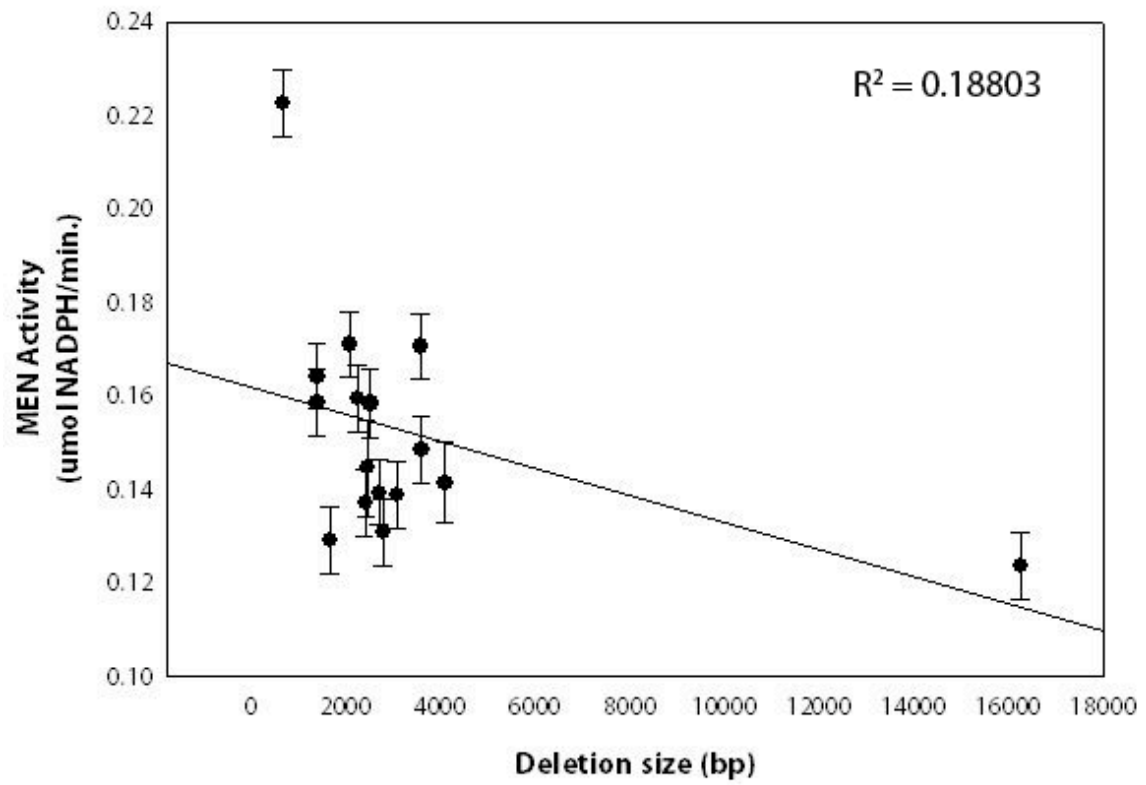


Figure S6 MEN enzyme activity plotted against deletion size. Note, the low correlation between *MenExi* deletion size and MEN enzyme activity suggests that another characteristic of each excision, not simply the size of the deletion, is impacting *trans* interactions with the wild-type allele.

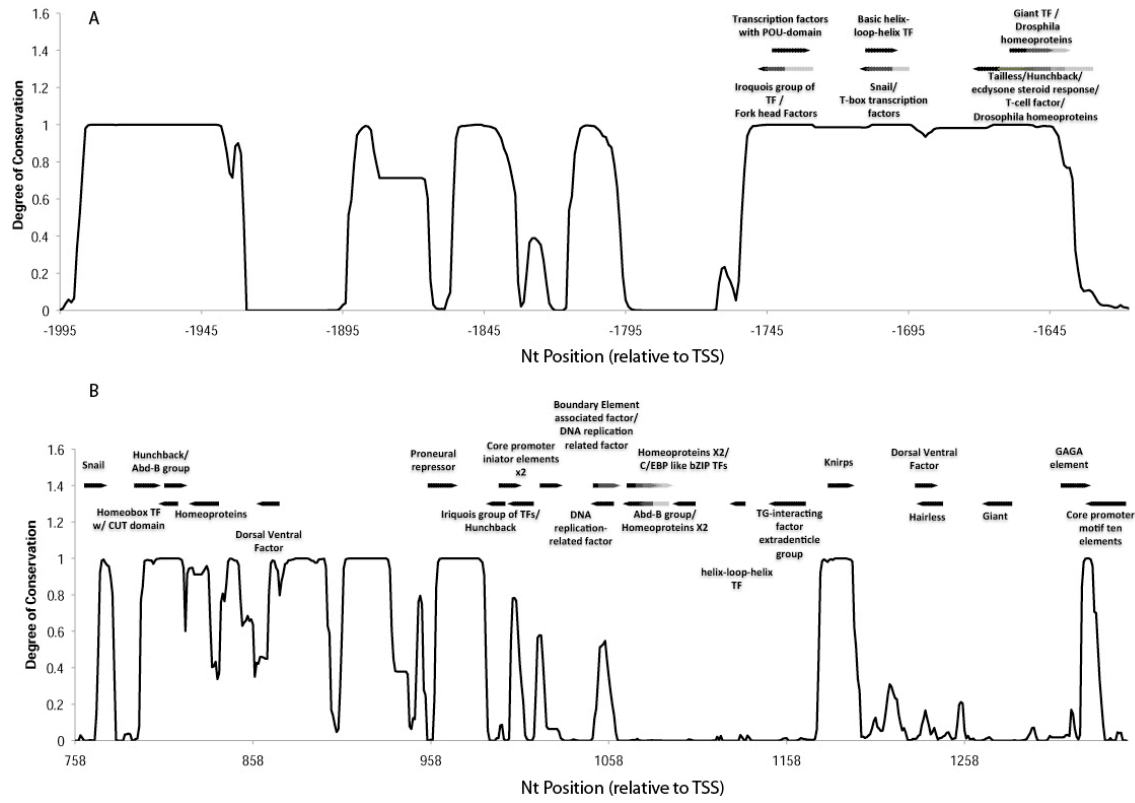


Figure S7 The degree of conservation, measured between 0 and 1, in regions ii and iii (Figure 4) and regulatory element prediction. Regions closer to 1 represent high conservation whereas 0 represents no conservation. Overlapping predicted regulatory elements are shown in different shades. Regulatory elements in the top row are in the forward direction whereas regulatory elements in the bottom row are in the reverse direction.

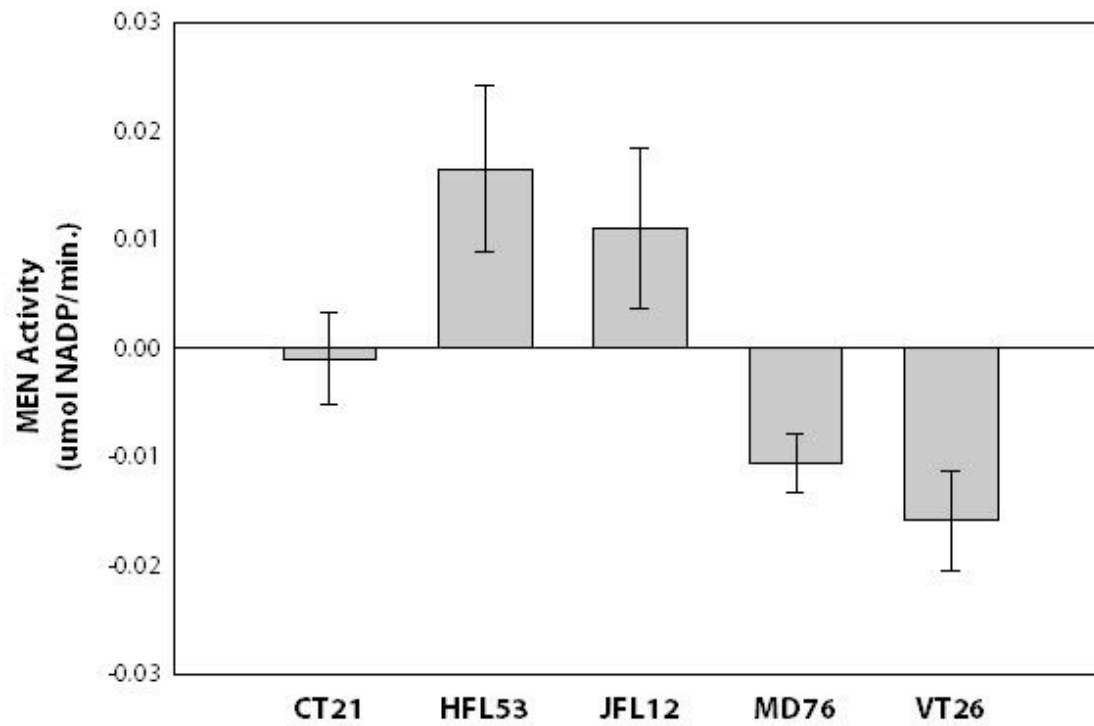


Figure S8 To determine the differences in background specific MEN activity across all genetic backgrounds, the average activity was taken across all standardized excision groups for each genetic background.

Table S1 Summary of regulatory elements predicted by MatInspector

Detailed Family Information	Start pos	End pos	Strand	Core		Sequence
				sim.	Matrix sim.	
Iroquois group of transcription factors	-1944	-1936	-	1	0.974	atattACA acctcaTAAAt
Drosophila fork head factors	-1942	-1926	-	1	0.981	attaa
Transcription factors with POU-domain - N-terminal to homeobox domain	-1940	-1928	+	0.889	0.931	aatatttATGAa g
Drosophila snail protein	-1908	-1898	-	0.868	0.931	cacACATgttt
Drosophila basic helix-loop-helix transcription factors	-1907	-1897	+	1	0.991	aacaTGTGtga
Drosophila T-box transcription factors	-1906	-1892	-	0.893	0.905	atatatCACa tgt
Drosophila segmentation gene tailless	-1868	-1860	-	0.897	0.938	gaaagTAAA
Drosophila gap gene hunchback	-1860	-1848	-	1	0.984	tacacAAAAatt g
Drosophila broad-complex for ecdysone steroid response	-1860	-1842	-	1	0.905	aattattaca Aaaattg
Drosophila giant transcription factor	-1856	-1842	+	1	0.981	ttttgtGTAAtaa tt
Drosophila homeoproteins	-1851	-1837	-	1	0.978	tattcaATTAtta ca
Drosophila homeoproteins	-1850	-1836	+	1	0.96	gtaaTAATgaa tat
Drosophila T-cell factor	-1839	-1827	-	1	0.897	ctgtTTGatatat
Drosophila OVO transcription factor	-162	-146	+	1	0.917	cggctGTTAcac gaaga
GAGA element, binding sites for proteins of the trithorax group (trxG)	-114	-100	+	1	0.978	tgagagAGAGa gtca
Zeste transvection gene product	-48	-38	-	1	0.948	attcGAGTgtg
Drosophila OVO transcription factor	121	137	+	1	0.923	ctgccGTTAtcgt tatc
DNA replication-related	125	135	-	1	0.92	taaCGATaacg

element factor						
Boundary element associated						ggataaCGATa
factor	126	138	-	1	0.94	ac
DNA replication-related						
element factor	128	138	+	0.75	0.802	tatCGTTatcc
Drosophila neuronal cis						gaatcgGGTTtg
element binding factor	259	275	-	1	0.879	ctcat
Core promoter motif ten						cagcgcgATCGc
elements	295	315	+	0.875	0.773	ctgggcctt
Drosophila C/EBP like bZIP						cATTGtcaccag
transcription factors	322	334	-	1	0.911	t
Drosophila snail protein	765	775	+	1	1	gccACCTgcta
Drosophila gap gene						agaatAAAAaa
hunchback	793	805	+	1	1	at
Drosophila homeobox						
transcription factor with CUT						
domain	808	816	-	1	0.935	tatGATTtg
Drosophila Abd-B group	810	820	+	1	0.96	aatcATAAata
						cgccTAATagtt
Drosophila homeoproteins	825	839	-	1	0.901	att
Drosophila Dorsal Ventral						
Factor	863	873	-	1	0.93	ttttTTCgct
Drosophila proneural						ggcaCACGcgcc
repressor	958	972	+	1	0.945	act
Iroquois group of						
transcription factors	992	1000	-	1	0.997	acaaaAACA
Core promoter initiator						
elements	998	1008	+	1	0.952	tgTCAGttttt
Drosophila gap gene						gcaacAAAAaa
hunchback	1004	1016	-	1	0.986	aa
Core promoter initiator						
elements	1021	1031	+	0.969	0.949	ttTCATtttt
DNA replication-related						
element factor	1051	1061	-	1	0.801	tatCGATtttc
Boundary element associated						gaaaatCGATa
factor	1051	1063	+	1	0.88	ga
DNA replication-related						
element factor	1054	1064	+	1	0.936	aatCGATagac
Drosophila Abd-B group	1068	1078	-	1	0.947	aattATAAaag

Drosophila homeoproteins	1069	1083	-	1	0.978	gcctcaATTAta aaa ttaaTAATtgagg
Drosophila homeoproteins	1070	1084	+	1	0.976	ca
Drosophila C/EBP like bZIP transcription factors	1075	1087	+	1	0.914	aATTGaggcaat t cagcTAATtgcc
Drosophila homeoproteins	1078	1092	-	1	0.974	tca gaggcaATTAgc
Drosophila homeoproteins	1079	1093	+	1	0.974	tgt
Drosophila basic helix-loop- helix transcription factors	1097	1107	-	1	0.957	ggcaTGTGcca
TGIF (TG-interacting factor)- Exd (extradenticle) group	1129	1135	-	1	1	TGTCaac
Drosophila broad-complex for ecdysone steroid response	1151	1169	-	1	0.94	taaatttgTAAA cgaatc
Drosophila segmentation gene knirps	1183	1195	+	1	0.917	tttcaaGTTCaat
Drosophila Dorsal Ventral Factor	1232	1242	+	1	0.944	ctttTTTCcca tgcGTGGgaaa
Drosophila supressor of Hairless	1234	1246	-	1	0.923	aa
Drosophila giant transcription factor	1271	1285	-	0.88	0.887	tatdddGTGAaac aa
GAGA element, binding sites for proteins of the trithorax group (trxG)	1314	1328	+	1	0.988	gaagagAGAGc gcgc
Core promoter motif ten elements	1329	1349	-	0.938	0.771	ataaacaAACG caaagcccat

Start and End position are relative to the TSS.