

Original Paper

## Genome-wide Search of DRE-Containing *Drosophila melanogaster* Genes

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**Abstract:** DNA replication-related element (DRE) which has the base sequence of 5'-TATCGATA-3' was found as a transcription activating element present in the upstream regions of *Drosophila melanogaster* genes encoding proteins required for DNA replication such as DNA polymerase  $\alpha$  and PCNA. A specific DRE-binding factor (DREF) is involved in this activation. In addition to DNA replication-related genes, a number of genes related to various reactions required for cell growth are now known regulated by the DRE/DREF system. Using the computer survey of the *Drosophila* whole genome permitted us to find out genes containing the DRE and DRE-like sequences with one base pair mismatches from DRE (DRE-1M) around the upstream regions of their transcription initiation sites. We found that 159 genes carry 200 copies of DRE sequences, and furthermore, 236 genes with 536 DRE-1M copies. Interestingly, many of these genes are related various reactions related to cell growth such as DNA metabolism, transcriptional regulation, protein metabolism, cell cycle, growth signal transduction etc. Immunological staining of the polytene chromosome from the salivary gland using anti-DREF antibody indicates that several hundreds of genes are under the regulation of DREF. These results suggest that the DRE/DREF system is one of important master key transcriptional regulatory systems of many genes required for cell growth.

**Key words:** *Drosophila* genome, cell proliferation, transcriptional regulation

### INTRODUCTION

Expression of genes encoding proteins involved in DNA replication is closely correlated with the proliferation state of cells, and is repressed in accordance with progression of differentiation in various tissues during development<sup>1)</sup>. The *Drosophila melanogaster* gene encoding PCNA and DNA polymerase  $\alpha$  (pol  $\alpha$ ) 180 kDa subunit<sup>2,3)</sup>, which are known as important factors in DNA replication, have been found to carry the common palindromic 8 base pair (bp) sequence 5'-TATCGATA-3' in these promoter regions<sup>3)</sup>. The sequence is required for the transcriptional activation, and termed the DNA

replication-related element (DRE). The requirement of DRE for promoter activities of these genes were confirmed in both cultured cells<sup>3-7)</sup> and transgenic flies<sup>8-10)</sup>. DRE can activate promoters through binding of DRE-binding factor (DREF), when it is placed within the 2 kb upstream region from the transcription initiation site<sup>3)</sup>.

DREF was purified from *Drosophila* Kc cells, and cDNA for DREF has been cloned<sup>11)</sup>. DREF consists of a polypeptide of 709 amino acid residues and is active in a form of homo-dimer of this polypeptide. Deletion experiments revealed that a part of the N-terminal region rich in basic amino acids (16-115 amino acids region) is responsible for the specific binding to DRE and the homo-dimer formation<sup>11)</sup>. Content of mRNA

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for DREF is high in embryos and ovaries of adult females, middle in larvae and low in pupae and adult males, which is similar to the expression pattern of mRNAs for PCNA and DNA polymerase  $\alpha$ <sup>11)</sup>.

The promoter activating function of the DRE/DREF system has been confirmed with genes for PCNA<sup>3)</sup>, Orc 2<sup>12)</sup>, Orc 5<sup>13)</sup>, DNA primase<sup>14)</sup>, DNA polymerase  $\alpha$  180 kDa<sup>15)</sup> and 73kDa subunits<sup>6)</sup>, RFC140<sup>16)</sup>, *raf*<sup>7)</sup>, *ras2/rop*<sup>4)</sup>, E2F<sup>10)</sup>, TBP<sup>17)</sup>, cyclin A<sup>5)</sup>, *skpA*<sup>18)</sup> and DREF itself. In addition, currently, regulation by DRE/DREF of genes for *caudal*<sup>19)</sup>, catalase<sup>20)</sup>, mitochondrial adenine nucleotide translocase<sup>21)</sup> and proteasome regulator REG gamma<sup>22)</sup> has also been reported.

Functions of DRE and DREF were examined using transgenic flies<sup>9, 23, 24)</sup> in addition to the experiments using cultured cells. Transgenic flies carrying fusion genes consisting of promoters from above mentioned genes with various mutations and the  $\beta$ -galactosidase gene were developed. By using these flies, the functions of regulatory elements in the PCNA gene were extensively studied. PCNA gene has at least three different elements: in order from the transcription initiation site to the distal, two E2F-binding sites (I and II), a DRE and a URE (upstream regulatory element)<sup>9)</sup>. Various deletion and point mutations revealed that DRE is essential for expression in embryos and larvae, and URE is necessary for expression in larval tissues<sup>9)</sup>. Moreover, it was found that expression of the PCNA gene is regulated by Zen (Zerknullt), which has a homeodomain-containing regulatory protein responsible for differentiation of the dorsal structure of *Drosophila* embryo, through the repression of expression level of DREF<sup>25-27)</sup>.

We established transgenic flies which express ectopically DREF in eye imaginal disc cells of larva by using the GAL4-UAS system<sup>24, 28)</sup>. The flies expressing the dominant negative N-terminal domain of DREF reduced DNA replication<sup>28)</sup>, and over-expression of the full-length of DREF in the eye-imaginal disc showed abnormal DNA synthesis, apoptosis, and differentiation failures of photo receptor cells after determination of differentiation<sup>24)</sup>.

Using the latter type of transgenic fly for searching mutant genes affecting DREF-induced phenotypes, a number of factors genetically interacting with DREF such as dE2F, *Dll* and *Polycomb/trithorax*-group genes (*brahma*, *moria* and *osa*) were identified<sup>24, 29)</sup>. Furthermore, dMLF, a *Drosophila* homologue of the human

myelodysplasia/myeloid leukemia factor 1 (hMLF1)<sup>30)</sup> was obtained using the yeast two-hybrid screening with DREF as a bait. Transgenic fly data also suggest the genetic interaction between DREF and dMLF *in vivo*<sup>30)</sup>. BEAF-32, which is involved in the boundary activity of *scs'* region of the *Drosophila hsp70* gene<sup>31)</sup>, can bind to the boundary element consisting of 5'-CGATA-3', a sequence present in DRE (5'-TATCGATA-3'), and antagonizes to DREF<sup>32, 33)</sup>. dMi-2, which is a member of DNA-stimulated ATPase of the chromatin remodeling complex, SWI/SNF2, is involved in repressing transcription of DRE-containing genes by inhibiting the binding of DREF to DRE<sup>34)</sup>. Dll, which has a homeodomain, also inhibits DREF by specific binding to the DNA binding domain of DREF<sup>29)</sup>. TRF2, a protein complex which is required for proper embryonic development and differentiation, contains DREF as a subunit functioning in recognition of the transcriptional elements in PCNA and DNA polymerase  $\alpha$  180 kDa promoter regions<sup>35)</sup>. These lines of evidences indicate that DRE cooperates with various factors to activate transcription, and its activity is repressed by factors related to differentiation induction (Zen, Dll) and gene silencing (BEAF, dMi-2, Polycomb).

In 1995, we tried to search DRE in about 3.5% of the *Drosophila* genome sequence which had been determined at that time, and found that 61 genes carried 73 copies of DRE sequences<sup>36)</sup>. These genes carry the DRE within 600 base pairs upstream regions of their transcription initiation sites, and interestingly many of them were related with reactions required for cell proliferation such as DNA replication, transcription, translation and growth signal. Simple extrapolation permitted us to estimate that more than one thousand *Drosophila* genes might carry DRE.

From the above search, involvement of the DRE/DREF system in the regulation of a considerable variety of genes has been suggested. We are interested again DRE-containing genes in the whole *Drosophila* genome sequences to find out how many and what kinds of genes are regulated by the DRE/DREF system, and found that total 395 genes contain DRE and/or DRE-1M sequences in their regulatory regions.

## MATERIALS AND METHODS

### Data source

We have searched DRE (5'-TATCGATA-3') and also 24 kinds of DRE-1Ms using BDGP Pattern Search

([http://www.fruitfly.org/seq\\_tools/patscan.html](http://www.fruitfly.org/seq_tools/patscan.html)). DRE-1Ms are frequently observed in the upstream regions of genes, and some of them are active for DREF-binding and promoter activation like DRE<sup>10</sup>). We extracted these sequences that were located in upstream regions of the coding sequences (CDS) based on position information with NCBI accession numbers. Names of genes were confirmed using NCBI database and FlyBase data (Gad Fly Release 3 version 1 and version FB2006\_01). Then, we downloaded the sequences of genes carrying DRE and/or DRE-1M within 1 kilobase (1kb) upstream and 40 bp down stream from the transcription initiation sites using FlyBase GadFly Genome Annotation Database.

### Data analysis

#### Identification of sequences

Downloaded sequences were analyzed using the software GENETYX version 6.1. The programs were set as follows: TATCGATA (DRE) and DRE-1M sequences where mismatched bases are shown in [ ] :

[ACG]ATCGATA; T[CGT]TCGATA; TA[ACG]CGATA; TAT[AGT]GATA; TATC[ACT]ATA; TATCG[CGT]TA; TATCGA[ACG]A; TATCGAT[CGT]. Further analysis, scoring DRE and DRE-1Ms were used with Excel program.

#### Data classification

DRE and DRE-1M containing genes were then classified into 9 groups regarding to molecular functions and also biological processes according to FlyBase data; Group A: DNA metabolism (replication, repair, precursor synthesis), Group B: transcription and its regulation, Group C: chromosome structures, Group D: RNA processing and protein metabolism, Group E:

signal transduction and protein phosphorylation, Group F: germ line formation, Group G: cell cycle regulation, Group H: miscellaneous (metabolisms, cell structure, defense response etc.) and Group I: unknown functions. The data contain the positions of DRE and DRE-1M and the ID in FlyBase in addition to gene names.

### Immunological detection of DREF in the polytene chromosomes from salivary glands of the *Drosophila* larva

Salivary glands were excised from third-instar larvae and extended on a slide glass. The specimens were then fixed and immunologically stained using the mouse anti-DREF monoclonal antibody as the primary antibody and goat anti-mouse IgG antibody conjugated with Alexa 568. Photographs were taken with a fluorescent microscope.

## RESULTS

### Numbers of genes carrying DRE and/or DRE-1M sequences

456 DREs and 7323 of DRE-1Ms were found in the *Drosophila* genome data base. Genes carrying these sequences within the regions of  $-1$  kb and  $+40$  bp with respect to the transcription initiation sites were scored. 159 genes carried 200 copies of DREs and 236 genes of 536 copies of DRE-1Ms. Genes containing both DRE and DRE-1Ms were included in the former. DRE sequence functions as transcription activator of genes with a single-copy such as the PCNA gene or multiple-copies<sup>3)</sup> such as one for DNA polymerase  $\alpha$  180 kDa subunit. We found that 29 genes out of 159 had 2-4 copies of DREs. DRE-1M is also known to functions as an activator like DRE in some genes such

Table 1. Functions of genes and numbers of DRE and DRE-1M

Categories of gene functions	DRE		DRE-1M		Total numbers of	
	containing genes	numbers of sequences	containing genes	numbers of sequences	genes	sequences
A. DNA metabolism (replication, repair, precursor synthesis)	15	22	8	28	23	50
B. Transcription and its regulation	16	20	32	63	48	83
C. Chromosome structure	7	12	8	17	15	29
D. RNA processing and protein metabolism	43	53	37	98	80	151
E. Signal transduction and protein phosphorylation	21	24	40	98	61	122
F. Germ line formation	12	15	13	35	25	50
G. Cell cycle regulation	9	12	7	21	16	33
H. Miscellaneous (metabolism, cell structure, defence response etc.)	29	33	81	154	110	187
I. Unknown functions	7	9	10	22	17	31
Total	159	200	236	536	395	736

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Table 2. Detailed information of *Drosophila* genes containing DRE and/or DRE-1M

Category A				
	Name of genes	location	sequences	FlyBase ID
1	<i>DNA polymerase <math>\alpha</math> 180kD</i>	-10	TATCGATA	FBgn0004493
		-63	TATCGATA	
		-197	TATCGATA	
2	<i>DNA polymerase <math>\alpha</math> 73kD*</i>	+3	TATCGATA	FBgn0005696
		-44	TATCGATA	
		-77	TATCGATA	
3	<i>mutagen-sensitive 205</i>	-198	TATCGAT <sub>g</sub>	FBgn0002891
4	<i>lethal (1) 1Bi</i>	-1	TgTCGATA	FBgn0001341
		-66	TtTCGATA	
		-313	TATCGATA	
5	<i>Germ line transcription factor 1</i>	-70	TATCGATA	FBgn0004913
6	<i>mutagen-sensitive 209 (PCNA)</i>	-53	TATCGATA	FBgn0005655
		-758	TATtGATA	
7	<i>Topoisomerase 2</i>	-37	TATCGATA	FBgn0003732
8	<i>CG4049</i>	-356	TATCGATA	FBgn00034976
9	<i>RecQ4</i>	-258	TATCaATA	FBgn00040290
		-876	TATCGATt	
10	<i>homolog of RecQ (RecQ5)</i>	-35	TATCGAaA	FBgn0027375
		-58	cATCGATA	
		-456	TATCGAT <sub>g</sub>	
		-476	TATCGATt	
11	<i>okra</i>	-206	TATCGAT <sub>g</sub>	FBgn0002989
		-219	TATCaATA	
12	<i>Inverted repeat-binding protein</i>	+1	aATCGATA	FBgn0011774
		-536	aATCGATA	
13	<i>meiotic 41</i>	-82	TATCGATA	FBgn0004367
		-732	cATCGATA	
14	<i>mutagen-sensitive 210</i>	+3	TATCGATA	FBgn0004698
15	<i>DDB1</i>	-209	TATCGATA	FBgn0027049
16	<i>Eroc1</i>	-281	TtTCGATA	FBgn00028434
		-306	TATCGAaA	
17	<i>NTPase</i>	-62	TATCGATA	FBgn0024947
		-77	TATCGAT <sub>g</sub>	
		-83	TATCGtTA	
18	<i>nmdyn-D6</i>	-35	TATCGATA	FBgn0030573
		-660	TATCGAT <sub>g</sub>	
		-677	TATCGATA	
19	<i>belphegor</i>	-53	TATCGAT <sub>g</sub>	FBgn00040237
20	<i>rudimentary</i>	-299	TATCGATA	FBgn0003189
21	<i>Phosphoribosylamidotransferase</i>	-16	gATCGATA	FBgn0004901
		-33	TATCGATA	
		-57	TATCGATc	
22	<i>Adenine nucleotide translocase 2</i>	-87	TATCGATA	FBgn0025111
		-125	TATCGATA	
		-277	TATCGATA	
		-697	TtTCGATA	
23	<i>ATP synthase-<math>\beta</math></i>	-303	TATCaATA	FBgn0010217
		-650	TATCGAaA	
		-924	cATCGATA	
		-932	TATCGAT <sub>g</sub>	

Category B				
	Name of genes	location	sequences	FlyBase ID
1	<i>TATA binding protein</i>	-35	TATCGATA	FBgn0003687
		-62	TAaCGATA	
		-222	TATCGATt	
2	<i>Transcription-factor-2A-S</i>	-257	TATCGATA	FBgn0013347
3	<i>TBP-associated factor 4</i>	-568	TATCGATt	FBgn0010280
4	<i>bicaudal</i>	-69	TATCGATA	FBgn0000181
		-499	aATCGATA	
		-616	TATaGATA	
		-755	aATCGATA	
5	<i>caudal</i>	-538	TATgGATA	FBgn0000251
		-949	aATCGATA	
6	<i>Jun-related antigen</i>	-922	TATaGATA	FBgn0001291
7	<i>engrailed</i>	-349	TgTCGATA	FBgn0000577
		-624	aATCGATA	
8	<i>Relish</i>	-937	TATCaATA	FBgn0014018
9	<i>scute</i>	-276	TtTCGATA	FBgn0004170
10	<i>Ultrabithorax</i>	-600	TATCaATA	FBgn0003944
11	<i>zerknüllt</i>	-1000	TATtGATA	FBgn0004053
12	<i>NFAT</i>	-38	TgTCGATA	FBgn00030505
		-59	TATCGATA	
13	<i>Chorion factor 2</i>	+35	aATCGATA	FBgn0000286
		-36	TATCGtTA	
		-151	TATCGAaA	
14	<i>Clock</i>	-450	TATaGATA	FBgn0023076
15	<i>nubbin</i>	-805	TATCGAaA	FBgn0002970
16	<i>senseless</i>	-362	TATCGAgA	FBgn0002573
17	<i>Sp1</i>	+5	TATtGATA	FBgn00020378
18	<i>vvl</i>	-781	TATtGATA	FBgn0008680
19	<i>Zn finger homeodomain 1</i>	-514	TATCaATA	FBgn0004606
		-609	TATtGATA	
		-847	TAaCGATA	

Category B				
	Name of genes	location	sequences	FlyBase ID
20	<i>Zn finger homeodomain 2</i>	-913	TATCGATA	FBgn0004607
21	<i>lilliputian</i>	-26	TAaCGATA	FBgn0041111
		-32	cATCGATA	
		-47	TATCGATA	
22	<i>Mix interactor</i>	-501	TATCGATA	FBgn0032940
23	<i>Myb oncogene-like</i>	-54	TATCGATA	FBgn0002914
		-128	TATCGATA	
		-554	TgTCGATA	
24	<i>pannier</i>	-85	TATCGATA	FBgn0003117
25	<i>pipsqueak</i>	-720	TATCGATA	FBgn0004399
26	<i>binou</i>	-668	TAaCGATA	FBgn0045759
27	<i>Eip93F</i>	-647	TATCGAaA	FBgn0013948
28	<i>eyegone</i>	-552	TAaCGATA	FBgn0000625
		-623	gATCGATA	
		-794	TATtGATA	
29	<i>hermaphrodite</i>	-8	aATCGATA	FBgn0001185
		-28	aATCGATA	
30	<i>rough</i>	-701	cATCGATA	FBgn0003267
31	<i>Sox21b</i>	-805	TtTCGATA	FBgn0042630
32	<i>split ends</i>	-376	TAaCGATA	FBgn0016977
33	<i>cap-n-collar</i>	-539	TATCaATA	FBgn0000338
		-545	TATCaATA	
		-603	TATCaATA	
		-759	TATCaATA	
34	<i>E2F transcription factor</i>	-793	TATCaATA	FBgn0011766
35	<i>similar</i>	-295	TATCGATc	FBgn0015542
36	<i>multi-protein bridging factor 1</i>	-354	TATCGATt	FBgn0026208
		-552	TtTCGATA	
37	<i>suppressor of sable</i>	-31	TATCGAT <sub>g</sub>	FBgn0003575
		-301	TATCGATA	
		-307	gATCGATA	
38	<i>E(spl) region transcript m3 (HLHm3)</i>	-230	TATtGATA	FBgn0002609
39	<i>Bicoid interacting protein 1</i>	+16	TATCGAaA	FBgn0024491
40	<i>Hairless</i>	-279	TATCGcTA	FBgn0001169
41	<i>TH1</i>	-276	TATCGATA	FBgn0010416
42	<i>lethal(1)10Bb</i>	-52	TATCGATA	FBgn0001491
		-955	TATCGATA	
43	<i>Retinoblastoma-family protein 2</i>	-4	TtTCGATA	FBgn0038390
		-46	TATCGATA	
44	<i>refractory to sigma P</i>	+3	TATCGATA	FBgn0003231
		-94	TATCGATA	
		-144	TATCGATA	
		-150	TtTCGATA	
		-367	TATCaATA	
45	<i>Ragena</i>	-26	TcTCGATA	FBgn0017550
		-43	TATCGATA	
		-179	TATCGATt	
		-252	aATCGATA	
46	<i>males absent on the first</i>	-37	cATCGATA	FBgn0014340
		-462	TATaGATA	
47	<i>zeste</i>	+32	TATaGATA	FBgn0004050
48	<i>Ecdysone receptor</i>	-453	TATCGAcA	FBgn0000546

Category C				
	Name of genes	location	sequences	FlyBase ID
1	<i>JIL-1</i>	-44	TAaCGATA	FBgn0020412
		-50	TATCGATA	
		-214	TATtGATA	
2	<i>Suppressor of variegation 3-9</i>	+8	TATCGATA	FBgn0003600
		-61	TATCGATA	
		-80	TATCGATA	
		-701	TATCGATA	
3	<i>Imitation SWI</i>	-7	TATCGATA	FBgn0011604
4	<i>Nucleosome remodeling factor - 38kD</i>	-4	TATCGATA	FBgn0016687
		-519	TATCGAT <sub>g</sub>	
5	<i>Ip1l-aurora-like kinase</i>	-323	TATtGATA	FBgn0024227
		-816	TAaCGATA	
6	<i>Origin recognition complex subunit 2</i>	-15	TATCGATA	FBgn0015270
		-233	TATCGATA	
		-737	TATCGATA	
7	<i>Origin recognition complex subunit 5</i>	-89	TATCGATA	FBgn0015271
		-476	TATCGtTA	
8	<i>fruitless</i>	-467	TATCGAgA	FBgn0004652
9	<i>spt4</i>	-55	cATCGATA	FBgn0028683
10	<i>Spt6</i>	-243	TATCaATA	FBgn0028982
11	<i>Polycomb</i>	-57	TATCGATt	FBgn0003042
12	<i>kismet</i>	-126	TATCGAT <sub>g</sub>	FBgn0001309
		-146	TAaCGATA	
		-217	TATCGtTA	
13	<i>female sterile (1) Young arrest</i>	-72	aATCGATA	FBgn0000927
14	<i>Histone H2A variant</i>	-63	cATCGATA	FBgn0001197
		-78	TATCGATt	
15	<i>taranis</i>	-122	TATCGATA	FBgn0040071
		-156	TATCGATc	

## Category D

1	<i>RNA polymerase III 128kD subunit</i>	-63	TATCGATA	FBgn0004463
2	<i>abstrakt</i>	-42	aATCGATA	FBgn0015331
		-595	TATCtATA	
		-647	aATCGATA	
		-692	TATCGATA	
3	<i>DEAD box protein 45A</i>	-49	TATCGATA	FBgn0010220
		-35	TAcCGATA	
4	<i>Dead box protein 73D</i>	-18	TATCGATA	FBgn0004556
5	<i>Helicase at 25E</i>	-26	TAcCGATA	FBgn0014189
		-37	TATCGATA	
		-233	aATCGATA	
6	<i>kurz</i>	+28	TATCGATA	FBgn0001330
		-111	TtTCGATA	
7	<i>Ribonuclease X25</i>	+18	TATCGATA	FBgn0010406
8	<i>ribonuclease H1</i>	+13	TtTCGATA	FBgn0023171
9	<i>no on or off transient A</i>	-25	TAcCGATA	FBgn0004227
		-180	TATCGATA	
		-208	TATCaATA	
10	<i>SF2</i>	-52	TATCGATA	FBgn0040284
		-105	TATCGATA	
		-264	TATCGtTA	
11	<i>Spliceosomal protein on the X</i>	-13	TtTCGATA	FBgn0015818
		-26	TATCGATA	
		-39	TATCGATt	
		-87	TtTCGATA	
		-323	TATCGATA	
12	<i>suppressor of white-apricot</i>	-14	cATCGATA	FBgn0003638
		-61	TcTCGATA	
		-90	TATCGATA	
13	<i>transformer 2</i>	+1	aATCGATA	FBgn0003742
		-292	TATCGcTA	
14	<i>nanos</i>	-60	TATCGATA	FBgn0002962
15	<i>Pabp2</i>	-32	TATCGATA	FBgn0005648
		-258	TATCGtTA	
16	<i>Adar</i>	-38	TATCGATA	FBgn0026086
		-84	TATCGATA	
		-800	TATCGATg	
17	<i>Heterogeneous nuclear</i>	-734	TATCGATA	FBgn0004237
18	<i>smooth</i>	-857	TATCGAcA	FBgn0003435
19	<i>small bristles</i>	-338	TATCGATt	FBgn0003321
20	<i>exuperantia</i>	+3	TATCGATA	FBgn0000615
		-10	TATCGATA	
21	<i>Phenylalanyl-tRNA synthetase</i>	-39	TATCGATA	FBgn0020766
22	<i>pygopus</i>	-218	TATCGATA	FBgn0043900
23	<i>dribble</i>	+6	TATCaATA	FBgn0020305
		-289	TATaGATA	
24	<i>aubergine</i>	-96	TATaGATA	FBgn0000146
25	<i>Nucleolar protein at 60B</i>	-254	TtTCGATA	FBgn0023184
		-262	TATCaATA	
		-281	TATCGATt	
26	<i>Eukaryotic initiation factor 4a</i>	-47	TATCGATA	FBgn0001942
		-274	TATCGATA	
27	<i>Eukaryotic initiation factor 4E</i>	-21	aATCGATA	FBgn0015218
		-831	TATCGtTA	
28	<i>Eukaryotic-initiation-factor-4G</i>	-13	TATCGATg	FBgn0023213
		-480	TATCGATA	
29	<i>Ribosomal protein S6</i>	-57	TATCGATA	FBgn0004922
		-396	TAcCGATA	
		-503	TtTCGATA	
30	<i>Ribosomal protein S13</i>	-50	TATCGATA	FBgn0010265
		-68	TATCGcTA	
		-193	TATCGATA	
31	<i>Ribosomal protein L14</i>	-172	TATCGAaA	FBgn0017579
32	<i>Ribosomal protein S14a</i>	-68	TATCGATA	FBgn0004403
		-295	cATCGATA	
		-439	TATCGATA	
33	<i>Ribosomal protein S19a</i>	-45	aATCGATA	FBgn0010412
34	<i>Ribosomal protein L36</i>	-15	TATCGATA	FBgn0002579
		-262	TATCGAaA	
		-327	TATCGAcA	
35	<i>Ribosomal protein L40</i>	-611	TATCGATt	FBgn0003941
		-637	TATCGAaA	
		-684	cATCGATA	
36	<i>overgrown hematopoietic organs at 23B</i>	-253	TATtGATA	FBgn0015521
37	<i>string of pearls</i>	-34	TATCGATg	FBgn0004867
		-61	TATCGATt	
38	<i>technical knockout</i>	-7	TATCGAcA	FBgn0003714
		-490	TATtGATA	
39	<i>Signal recognition particle protein 19</i>	-40	TATCGATt	FBgn0015298
		-240	cATCGATA	
		-276	TATCGATA	
40	<i>spaghetti</i>	+10	TATCGAcA	FBgn0015544
41	<i>karyopherin <math>\alpha 1</math></i>	-49	TATCGATA	FBgn0024889
		-55	TATCGcTA	
42	<i>karyopherin <math>\alpha 3</math></i>	-215	TATCGATA	FBgn0027338

## Category D

43	<i>Female sterile (2) Ketel</i>	-75	TATCGATA	FBgn0000986
		-255	TtTCGATA	
44	<i>Synaptobrevin</i>	-36	TATCGATA	FBgn0003660
		-45	cATCGATA	
45	<i>Ras opposite</i>	-46	TATCGtTA	FBgn0004574
46	<i>Cyclophilin 1</i>	-552	TATCGAaA	FBgn0004432
47	<i>Heat shock protein 23</i>	-218	TAgCGATA	FBgn0001224
48	<i>Heat shock gene 67Ba</i>	-559	TATgGATA	FBgn0001227
49	<i>Heat shock protein 68</i>	-512	TATCtATA	FBgn0001230
		-566	TATCGATc	
		-667	TATCtATA	
		-899	TATCGATt	
		-950	TATCGATg	
50	<i>Heat shock protein 83</i>	-240	TATCGATA	FBgn0001233
		-488	gATCGATA	
		-518	cATCGATA	
		-531	TATCGATg	
51	<i>Hsp70/Hsp90 organizing protein homolog</i>	+24	TATCGATt	FBgn0024352
		-375	TtTCGATA	
		-407	TATCGATA	
52	<i>Ubiquitin activating enzyme 1</i>	-78	TATCGATA	FBgn0023143
53	<i>effete</i>	-119	TATCGAcA	FBgn00011217
		-140	TATCGATA	
		-373	TATCGATg	
54	<i>lesswright</i>	-30	TATCGATA	FBgn0010602
		-586	TtTCGATA	
55	<i>deep orange</i>	-16	TATCGATA	FBgn0000482
		-69	TAcCGATA	
		-845	TATaGATA	
56	<i>unkempt</i>	-43	TATCGATA	FBgn0004395
		-71	aATCGATA	
57	<i>highwire</i>	-318	gATCGATA	FBgn0030600
58	<i>lethal with a checkpoint kinase</i>	-73	aATCGATA	FBgn0029006
59	<i>Ubiquitin carboxy-terminal hydrolase</i>	-39	TATCGATA	FBgn0010288
60	<i>non-stop</i>	-273	TgTCGATA	FBgn0013717
		-625	TATCGATt	
61	<i>Proteasome 35kD subunit</i>	-18	TATCGATA	FBgn0003151
62	<i>Proteasome <math>\alpha 7</math> subunit</i>	-575	cATCGATA	FBgn0023175
63	<i>Proteasome <math>\beta 2</math> subunit</i>	-4	TATCGATt	FBgn0023174
		-15	TATCGATA	
		-265	TATCGATA	
		-766	TATCGATt	
64	<i>Cysteine proteinase-1</i>	-45	TATCGATA	FBgn0013770
65	<i>Diphenol oxidase A2</i>	+37	TATCGATA	FBgn0000486
		-231	TATCGAaA	
		-237	TATCGATA	
66	<i>infertile crescent</i>	-4	TATCGATt	FBgn0001941
		-15	TATCGATA	
		-596	TATtGATA	
67	<i>cinnamon</i>	-71	TATCGAaA	FBgn0000316
		-552	TATCcATA	
68	<i>Disabled</i>	-567	aATCGATA	FBgn0000414
69	<i>Furin 1</i>	-701	TATCcATA	FBgn0004509
70	<i>huntingtin</i>	-82	TAcCGATA	FBgn0027655
71	<i>kuzbanian</i>	-242	TATCaATA	FBgn0015954
72	<i><math>\beta</math> Trypsin</i>	-175	cATCGATA	FBgn0010357
73	<i><math>\epsilon</math> Trypsin</i>	-109	TATCGATg	FBgn0010425
74	<i>Presenilin</i>	-32	TATCGcTA	FBgn0019947
75	<i>Cystatin-like</i>	-126	TATtGATA	FBgn0004629
76	<i>E(spl) region transcript m1</i>	+11	aATCGATA	FBgn0002578
77	<i>pugilist</i>	-16	TATCGATA	FBgn0020385
		-51	TATCGATA	
78	<i>Dihydropteridine reductase</i>	-398	TATCtATA	FBgn0035964
79	<i>Eip55E</i>	-95	TATCGtTA	FBgn0000566
80	<i>Ecdysone-induced protein 28/29kD</i>	-82	TAcCGATA	FBgn0000565
		-843	TATCGAaA	

## Category E

1	<i>G protein <math>\alpha</math> 60A</i>	-5	TATCGATA	FBgn0001123
		-344	TATgGATA	
2	<i>G protein <math>\beta</math>-subunit 76C</i>	-158	TtTCGATA	FBgn0004623
3	<i>Ras oncogene at 64B</i>	-44	TATCGATA	FBgn0003206
		-57	TAgCGATA	
		-598	TAgCGATA	
4	<i>Ras oncogene at 85D</i>	-24	TATCGATA	FBgn0003205
		-563	TATCcATA	
5	<i>G protein <math>\alpha</math> 47A</i>	-565	aATCGATA	FBgn0001122
6	<i>Rho1</i>	-10	TATCGATt	FBgn0014020
7	<i>crowded</i>	-358	TATCtATA	FBgn0015372
		-444	TATCGcTA	
8	<i>pebble</i>	-197	TATCGAcA	FBgn0003041
		-412	TATCGATA	
9	<i>Ral guanine nucleotide exchange factor 2</i>	+23	TATCGcTA	FBgn0026376
		+3	TATaGATA	



Genome-wide Search of DRE-Containing *Drosophila melanogaster* Genes

Category E

10	<i>C3G</i>	-89	TATCGATt	FBgn0026145
		-101	TATCGAcA	
		-158	TATCGATA	
11	<i>Neurofibromin 1</i>	-62	TATCGATA	FBgn0015269
		-336	TATtGATA	
		-934	TATCaATA	
12	<i>locomotion defects</i>	-38	TATCGATA	FBgn0020278
13	<i>GTPase-activating protein 69C</i>	-11	TATCGATA	FBgn0020655
14	<i>SNF1A/AMP-activated protein kinase</i>	-82	TATCGATA	FBgn0023169
		-952	TATtGATA	
15	<i>licorne</i>	-7	TATCGATA	FBgn0015763
		-117	aATCGATA	
		-126	TATaGATA	
		-338	TATCGAaA	
		-643	TATCGATg	
16	<i>nemo</i>	-55	TATCGATg	FBgn0011817
		-104	cATCGATA	
		-382	TATCGATg	
		-402	TATCGATA	
17	<i>pole hole (D-raf)*</i>	-370	TATCGtTA	FBgn0003079
		-364	TATCGATt	
18	<i>Downstream of raf1</i>	-26	TATCGAGA	FBgn0010269
		-45	TATCGtTA	
		-577	TATaGATA	
19	<i>slingshot</i>	-19	TATCGATt	FBgn0029157
		-565	TATCGATt	
		-830	TATtGATA	
20	<i>MAP kinase kinase 4</i>	-22	TATCGATt	FBgn0024326
		-32	TATCGATg	
21	<i>PAK-kinase</i>	-1	TATtGATA	FBgn0014001
		-9	TATCGATA	
		-60	cATCGATA	
		-619	TATCGATt	
22	<i>Src oncogene at 64B</i>	-59	aATCGATA	FBgn0003501
23	<i>Rho-kinase</i>	-422	TATCGATt	FBgn0026181
24	<i>minibrain</i>	-7	cATCGATA	FBgn0002777
		-238	TtTCGATA	
		-313	TATCGATt	
25	<i>Focal Adhesion Kinase</i>	-238	TATCGATt	FBgn0020440
		-355	TaACGATA	
26	<i>Abl tyrosine kinase</i>	-958	TtTCGATA	FBgn0000017
27	<i>bent</i>	-347	TATCaATA	FBgn0005666
		-857	TATCGtTA	
28	<i>salimus</i>	-839	TATCtATA	FBgn0003432
29	<i>Casein kinase II <math>\beta</math> subunit</i>	+38	aATCGATA	FBgn0000259
		-877	TATCaATA	
30	<i>Protein phosphatase 1 at 87B</i>	-49	TATCGATt	FBgn0004103
		-76	TATCGATt	
		-547	TATCaATA	
		-612	TATCGATA	
31	<i>Pten</i>	-104	cATCGATA	FBgn0026379
		-115	TATCGATg	
		-468	TATCGATt	
32	<i>domeless</i>	-10	TtTCGATA	FBgn0043903
		-234	aATCGATA	
33	<i>Protein phosphatase 2B at 14D</i>	-450	TATtGATA	FBgn0011826
34	<i>retinal degeneration C</i>	-355	TtTCGATA	FBgn0004366
		-670	TATCGtTA	
35	<i>Calcineurin B</i>	-378	TATtGATA	FBgn0010014
36	<i>14-3-3 <math>\epsilon</math></i>	-178	TATCGATA	FBgn0020238
37	<i>Nuclear inhibitor of Protein phosphatase 1</i>	-313	TcTCGATA	FBgn0026402
38	<i>Inhibitor-2</i>	-36	TATCGATt	FBgn0028429
39	<i>PP2A-B'</i>	-268	TATCGAaA	FBgn0042693
40	<i>cAMP-dependent protein kinase R1</i>	-21	TATCGATA	FBgn0000275
		-53	TATCGATA	
41	<i>Signal transducing adaptor molecule</i>	-130	TATCaATA	FBgn0027363
42	<i>Epidermal growth factor receptor</i>	-924	TATCGATA	FBgn0003731
		-946	TATCGtTA	
43	<i>Eph receptor tyrosine kinase</i>	+18	TATCGATA	FBgn0025936
		-876	TATCaATA	
44	<i>plexin A</i>	+1	TATCGATA	FBgn0025741
		-73	TATCGATA	
		-284	TATCGATA	
		-661	TATCtATA	
		-804	TATgGATA	
45	<i>saxophone</i>	+17	TATCGATA	FBgn0003317
		-440	gATCGATA	
46	<i>PDGF- and VEGF-receptor related</i>	-83	cATCGATA	FBgn0032006
		-591	TATCaATA	
		-955	TATaGATA	
47	<i>sevenless</i>	-398	TATCtATA	FBgn0003366
48	<i>neither inactivation nor afterpotential E</i>	-146	TATgGATA	FBgn0002940
49	<i>Trehalose-sensitivity</i>	-619	TATCGATt	FBgn0003747
		-646	TATCGATt	
		-875	TATCGAaA	

Category E

50	<i>trapped in endoderm-1</i>	-113	aATCGATA	FBgn0046687
		-140	aATCGATA	
51	<i>myoglianin</i>	-505	TATCaATA	FBgn0026199
		-830	TATCGAcA	
		-893	TATCGATA	
52	<i>maverick</i>	+40	TATCGATg	FBgn0039914
		-766	TATCaATA	
53	<i>gurken</i>	-159	TATCaATA	FBgn0001137
		-343	TATCaATA	
54	<i>wingless</i>	-639	TATCGATt	FBgn0004009
55	<i>Axin</i>	-525	aATCGATA	FBgn0026597
		-536	TAgCGATA	
		-547	aATCGATA	
56	<i>Arrestin 2</i>	-765	TATCGAaA	FBgn0000121
57	<i>SHC-adaptor protein</i>	-40	TATCGATA	FBgn0015296
		-163	TATCGAaA	
58	<i>E(spl) region transcript m2</i>	-605	TATgGATA	FBgn0002592
59	<i>E(spl) region transcript m <math>\alpha</math></i>	-959	TATCtATA	FBgn0002732
60	<i>Twin of m4</i>	-456	TATtGATA	FBgn0026320
61	<i>Star</i>	-114	TATCGATt	FBgn0003310
		-126	TATCGATg	

Category F

1	<i>female sterile (1) K10</i>	+39	TATCGAaA	FBgn0000810
		-100	TATCGATA	
2	<i>sans fille</i>	+20	TATCGATg	FBgn0003449
		-14	TATCGATA	
		-272	TATCGATg	
3	<i>vasa</i>	-50	TATCGAaA	FBgn0003970
4	<i>Protein tyrosine phosphatase 61F</i>	-360	TcTCGATA	FBgn0003138
5	<i>mago nashi</i>	-21	TATCGATA	FBgn0002736
		-84	TATCGATA	
6	<i>mutagen-sensitive 101</i>	-257	TATCGATA	FBgn0002878
		-282	TATCGATA	
7	<i>Yolk protein 2</i>	-511	TtTCGATA	FBgn0005391
		-562	TATCGATg	
8	<i>Accessory gland peptide 70A</i>	-473	TATCGAaA	FBgn0003034
9	<i>Bicaudal D</i>	+30	TATCGAaA	FBgn0000183
		-237	TATCGATg	
10	<i>Cortactin</i>	+4	TATCGATg	FBgn0025865
		-316	TATCGATg	
		-324	TATCGATA	
11	<i>kelch</i>	-262	TATaGATA	FBgn0001301
		-266	TATCtATA	
		-277	TATCtATA	
		-527	TATCaATA	
12	<i>discontinuous actin hexagon</i>	-56	TATCGATA	FBgn0015926
		-473	TATtGATA	
13	<i>Ribonuclear protein at 97D</i>	-24	TATCGATA	FBgn0004903
		-846	TATCGATA	
14	<i>always early</i>	-155	TATtGATA	FBgn0004372
		-898	aATCGATA	
15	<i>boule</i>	-221	TATCGcTA	FBgn0011206
16	<i>bag of marbles</i>	+20	TATCGATA	FBgn0000158
17	<i>xmas-1</i>	-184	TATCGtTA	FBgn0016080
18	<i>xmas-2</i>	-14	TATCGATt	FBgn0028974
		-382	TtTCGATA	
		-674	cATCGATA	
19	<i>hu li tai shao</i>	-9	cATCGATA	FBgn0004873
		-21	TtTCGATA	
		-53	TATCGATA	
		-607	TATCaATA	
20	<i>piwi</i>	-600	TATCGgTA	FBgn0004872
21	<i>outstretched</i>	-54	TcTCGATA	FBgn0004956
		-480	gATCGATA	
22	<i>janus A</i>	-19	TATCGATA	FBgn0001280
		-155	aATCGATA	
23	<i>janus B</i>	-818	TATCGATA	FBgn0001281
		-954	aATCGATA	
24	<i>Esterase 6</i>	-467	TATCtATA	FBgn0000592
		-605	TtTCGATA	
25	<i>Serendipity <math>\beta</math></i>	-47	TATCGATt	FBgn0003511
		-183	TATCGATA	

Category G

1	<i>Cyclin A</i>	-12	TATCGATA	FBgn0000404
		-32	TgTCGATA	
		-318	TATgGATA	
2	<i>Cyclin B</i>	-12	aATCGATA	FBgn0000405
		-117	TATCGAaA	
		-156	cATCGATA	
3	<i>Cyclin-dependent kinase 8</i>	-31	TATCGtTA	FBgn0015618
		-415	TATtGATA	
4	<i>Cdc37</i>	-63	TATCGATg	FBgn0011573

## Category G

5	<i>α-Tubulin at 87C</i>	-117	TATCGATA	FBgn0004236
		-742	cATCGATA	
6	<i>α-Tubulin at 84D</i>	-306	gATCGATA	FBgn0003885
		-327	aATCGATA	
		-873	TATCGtA	
7	<i>dodo</i>	-36	TATCGAt	FBgn0015379
		-60	TATCGATA	
8	<i>tricornered</i>	-56	TATCGATA	FBgn0003744
9	<i>skpA</i>	-21	TATCGATA	FBgn0025637
10	<i>twinstar</i>	-406	TATCGtA	FBgn0011726
		-570	TgTCGATA	
		-599	TATCGATA	
11	<i>warts</i>	+18	TATCGATA	FBgn0011739
		+4	TATCGATA	
		-56	TATCGATA	
		-86	TATCGAtg	
		-509	TATCGATA	
12	<i>Bub3</i>	-13	TATCGAA	FBgn0025457
		-25	TATCGATA	
13	<i>Protein phosphatase 19C</i>	-24	TATCGATA	FBgn0023177
14	<i>meiotic P22</i>	-128	TgTCGATA	FBgn0016036
15	<i>Kinesin-like protein at 61F</i>	-770	TAcCGATA	FBgn0004378
16	<i>lamming</i>	-100	aATCGATA	FBgn0029004
		-139	gATCGATA	

## Category H

1	<i>roadblock</i>	-69	TATCGATA	FBgn0024196
2	<i>Dynein light chain 90F</i>	-259	TATCGAt	FBgn0024432
		-462	TAgCGATA	
3	<i>Myosin light chain cytoplasmic</i>	-4	TATCGAt	FBgn0004687
4	<i>Myosin light chain 2</i>	-216	TATCtATA	FBgn0002773
		-836	TATaGATA	
5	<i>Kinesin-like protein at 67A</i>	-113	TATCGATA	FBgn0004379
		-281	TATCGATA	
		-410	TATCGATA	
		-430	cATCGATA	
6	<i>coosta</i>	+16	TATCGAt	FBgn0000352
		-169	TATCtATA	
7	<i>Glued</i>	-44	TAgCGATA	FBgn0001108
8	<i>Actin 5C</i>	-944	TATCGtA	FBgn0000042
9	<i>Paramyosin</i>	-108	TATaGATA	FBgn0003149
10	<i>capping protein beta</i>	-59	aATCGATA	FBgn0011570
11	<i>Tropomyosin 2</i>	-600	TATCGtA	FBgn0004117
		-646	TATCtATA	
12	<i>lethal (2) giant larvae</i>	-23	TtTCGATA	FBgn0002121
13	<i>hook</i>	-16	TtTCGATA	FBgn0001202
		-45	TATCGATA	
		-58	TAaCGATA	
14	<i>Lamin</i>	+10	TATCGAt	FBgn0002525
		-187	TATCGATA	
		-198	TATCGtA	
15	<i>prickle</i>	-612	TATCcATA	FBgn0003090
16	<i>Centrosomal protein 190kD</i>	-40	TATCGATA	FBgn0000283
17	<i>pickel</i>	+29	TATCGAt	FBgn0013720
18	<i>vestigial</i>	-171	TATCaATA	FBgn0003975
		-516	TtTCGATA	
		-987	TATCaATA	
19	<i>misato</i>	-55	aATCGATA	FBgn0020272
		-73	TATCGATA	
		-244	TATCGATA	
		-844	TATCaATA	
20	<i>Amylase distal</i>	-400	TATCGATA	FBgn0000078
21	<i>Formaldehyde dehydrogenase</i>	-36	TATCGATc	FBgn0011768
22	<i>α-Mannosidase II</i>	+1	TATCGAt	FBgn0011740
		-437	aATCGATA	
23	<i>Phosphoglyceromutase</i>	-616	TAgCGATA	FBgn0014869
24	<i>Glycerol 3 phosphate dehydrogenase</i>	-129	TAaCGATA	FBgn0001128
25	<i>quemao</i>	-32	TATCGATA	FBgn0019662
		-68	TATCGAtg	
		-268	aATCGATA	
26	<i>desat2</i>	-954	TgTCGATA	FBgn0043043
27	<i>HMG Coenzyme A synthase</i>	-8	gATCGATA	FBgn0010611
		-950	TATGgATA	
28	<i>Thiolase</i>	-21	TtTCGATA	FBgn0025352
29	<i>α-Esterase-4</i>	-37	TATCGATA	FBgn0015572
		-826	TATCGAt	
30	<i>α-Esterase-5</i>	-58	TATCGAA	FBgn0015573
		-130	TATCtATA	
		-601	TATCGAt	
31	<i>Esterase P</i>	-347	TATCaATA	FBgn0000594
		-809	TATCGAtg	
32	<i>prune</i>	-262	TATCGATA	FBgn0003116
		-268	TATCGcTA	
33	<i>Na pump α subunit</i>	+26	TATCGATA	FBgn0002921
		-953	TATCaATA	
34	<i>Calcium ATPase at 60A</i>	-516	TATgGATA	FBgn0004551

## Category H

35	<i>nervana 2</i>	-328	TATaGATA	FBgn0015777
36	<i>Dopa decarboxylase</i>	-824	TATtGATA	FBgn0000422
		-882	TeTCGATA	
37	<i>S-adenosylmethionine decarboxylase</i>	-30	cATCGATA	FBgn0019932
		-56	TATCGAtg	
38	<i>CG3253</i>	-48	TATCGATA	FBgn0041706
		-583	TATgGATA	
39	<i>l(1)G0020</i>	-47	TATCGAcA	FBgn0027330
40	<i>selenide,water dikinase</i>	-89	aATCGATA	FBgn0020615
41	<i>FK506-binding protein FKBP59</i>	-169	TATCGtA	FBgn0029174
42	<i>Catalase</i>	-41	TATCGATA	FBgn0000261
		-198	TATCaATA	
43	<i>Superoxide dismutase</i>	-636	TATCtATA	FBgn0003462
		-685	TATCGAt	
44	<i>Superoxide dismutase 2 (Mn)</i>	-26	TtTCGATA	FBgn0010213
		-393	TATCGAt	
45	<i>Microsomal glutathione S-transferase-like</i>	-70	aATCGATA	FBgn0025814
46	<i>maroon-like</i>	-18	TATCGAtg	FBgn0002641
47	<i>prolyl-4-hydroxylase-α EFB</i>	-33	TATCtATA	FBgn0039776
48	<i>Ferredoxin</i>	-237	TATCGATA	FBgn0011769
49	<i>Cytochrome P450-4e2</i>	-686	TATCGATA	FBgn0014469
50	<i>Cytochrome P450-6a2</i>	-405	TATCGAtg	FBgn0000473
51	<i>bon92</i>	-529	TATgGATA	FBgn0013432
52	<i>fa1en</i>	-343	aATCGATA	FBgn0028380
		-686	aATCGATA	
		-777	TAcCGATA	
53	<i>Cysteine string protein</i>	-423	TATCGAA	FBgn0004179
		-448	TAcCGATA	
		-878	TeTCGATA	
54	<i>Male-specific RNA 84Db</i>	-82	TATgGATA	FBgn0004173
55	<i>CG3321</i>	+27	TATCGATA	FBgn0038224
		-257	TATCcATA	
56	<i>white</i>	-795	TATtGATA	FBgn0003996
57	<i>Early gene at 23</i>	-95	TtTCGATA	FBgn0020445
		-398	TATgGATA	
		-646	TATCGAA	
58	<i>nicotinic Acetylcholine Receptor alpha 7E</i>	-159	TATaGATA	FBgn0015519
		-448	TATCtATA	
59	<i>Shaker</i>	-280	TATCGAA	FBgn0003380
		-488	TATCaATA	
60	<i>Catecholamines up</i>	-321	TAaCGATA	FBgn0002022
61	<i>lethal-(2)-denticleless</i>	-960	TATCtATA	FBgn0013548
62	<i>drongo</i>	-152	TtTCGATA	FBgn0020304
63	<i>Allatostatin C</i>	+33	cATCGATA	FBgn0032336
64	<i>neuropeptide F</i>	-488	TATCGAA	FBgn0027109
65	<i>Peptidoglycan recognition protein LE</i>	-80	TATCGATA	FBgn0030695
		-93	cATCGATA	
		-189	TATCGAA	
66	<i>Scavenger receptor class C, type I</i>	+6	TATCtATA	FBgn0014033
67	<i>BQ4</i>	-41	gATCGATA	FBgn0038928
		-197	TATCGAtg	
68	<i>inflated</i>	-433	TATCGATA	FBgn0001250
69	<i>mysospheroid</i>	-12	TATCGAA	FBgn0004657
		-232	cATCGATA	
		-558	TATtGATA	
70	<i>Tetraspanin 3A</i>	-49	aATCGATA	FBgn0040334
		-56	TATCGtA	
71	<i>Tetraspanin 97E</i>	+17	TATCtATA	FBgn0039465
		+11	aATCGATA	
		+5	TATCGAA	
		-2	TATCGATA	
		-208	TATCGATA	
		-791	TATCGAtg	
72	<i>Surfeit 4</i>	+25	TAaCGATA	FBgn0019925
		+8	TATCGATA	
73	<i>inactivation no afterpotential D</i>	-80	aATCGATA	FBgn0001263
		-271	aATCGATA	
		-780	TATtGATA	
74	<i>shaking B</i>	-467	gATCGATA	FBgn0003037
75	<i>muscleblind</i>	-704	TATCtATA	FBgn0053197
76	<i>CG11418</i>	-217	aATCGATA	FBgn0024360
77	<i>Misexpression suppressor of ras 4</i>	+19	TATCGAA	FBgn0034240
78	<i>terminus</i>	-779	TATCGAt	FBgn0003683
79	<i>couch potato</i>	-680	TATCGATA	FBgn0000363
80	<i>DISCO Interacting Protein 1</i>	-33	TATCGATA	FBgn0024807
		-701	TATaGATA	
81	<i>yantra</i>	-36	aATCGATA	FBgn0021895
82	<i>Calphotin</i>	-288	aATCGATA	FBgn0010218
83	<i>Calreticulin</i>	-125	TATCGAA	FBgn0005585
		-157	aATCGATA	
		-570	TATaGATA	
84	<i>Yippee</i>	-23	cATCGATA	FBgn0026749
		-326	TATCGATA	
		-543	TATCtATA	
85	<i>Chorion protein 19</i>	-444	TATaGATA	FBgn0000358
		-481	TATCGtA	

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Category H

86	<i>Chorion protein 36</i>	-500	TATaGATA	FBgn0000359
87	<i>Lop65Aa</i>	-619	TATCGATt	FBgn0020645
88	<i>Lop65Af</i>	-25	aATCGATA	FBgn0020639
		-290	TATCGtTA	
89	<i>Lop65Ag1</i>	-263	TATtGATA	FBgn0020638
90	<i>Lop65Ag2</i>	-455	aATCGATA	FBgn0020637
		-544	TATCGAaA	
		-702	TAcCGATA	
91	<i>Larval outcicle protein 1</i>	-753	TATCtATA	FBgn0002531
92	<i>new glue 2</i>	-929	TtTCGATA	FBgn0010294
93	<i>Salivary gland secretion 4</i>	-426	TATCGATc	FBgn0003374
94	<i>M6</i>	-34	cATCGATA	FBgn0037092
		-295	TATCGATA	
95	<i>Laminin B1</i>	-67	TATCGATc	FBgn0002527
		-681	TATCGATt	
96	<i>out at first</i>	-175	TATaGATA	FBgn0011818
		-181	TATaGATA	
97	<i>expanded</i>	-22	TATCGcTA	FBgn0004583
98	<i>Fascioline 1</i>	+4	TATCGATA	FBgn0000634
99	<i>unzipped</i>	-470	cATCGATA	FBgn0004055
		-526	cATCGATA	
		-534	TATCGATg	
100	<i>caectin</i>	-85	cATCGATA	FBgn0031114
		-146	TtTCGATA	
		-195	TATCGATA	
		-719	TcTCGATA	
101	<i>melted</i>	-209	TtTCGATA	FBgn0023001
102	<i>mushroom body defect</i>	-189	TATCGATc	FBgn0002873
		-242	TATCGATA	
103	<i>vegetable</i>	-7	TATCGAaA	FBgn0015562
		-18	TATCGATA	
		-165	TATCGATt	
		-207	TATCGATt	
104	<i>dalmatian</i>	-129	TATCGAcA	FBgn0016792
105	<i>von Hippel-Lindau</i>	+30	TATcTATA	FBgn0041174
		-58	TATCGATA	
106	<i>Ecdysone-induced gene 71Ed</i>	-694	aATCGATA	FBgn0004591

Category H

107	<i>Attacin-A</i>	-393	TATtGATA	FBgn0012042
108	<i>Defensin</i>	-849	TATaGATA	FBgn0010385
109	<i>Diptericin</i>	-589	TATCGATt	FBgn0004240
110	<i>Drosocin</i>	-682	aATCGATA	FBgn0010388

Category I

1	<i>angel</i>	-63	TATCGATA	FBgn0016762
		-89	TtTCGATA	
		-218	TAcCGATA	
2	<i>Bem46</i>	-41	TATCGATA	FBgn0025109
		-273	TATtGATA	
3	<i>CG3224</i>	-8	TATCGATA	FBgn0029885
		-981	TATCGATA	
4	<i>CG3760</i>	+35	TATCGATA	FBgn0022343
		-148	TATgGATA	
		-170	TATCGATA	
5	<i>CG3839</i>	-6	TtTCGATA	FBgn0040396
		-133	TATCGATA	
6	<i>Rib1</i>	-265	TATCGATA	FBgn0014022
		-500	TATCaATA	
7	<i>Suppressor of ref(2)P sterility</i>	-252	TATCGATA	FBgn0004465
8	<i>asteroid</i>	-62	aATCGATA	FBgn0015905
9	<i>bicoid-interacting protein 3</i>	-252	aATCGATA	FBgn0033073
		-426	TATCGgTA	
		-442	TATCGAcA	
10	<i>Caldesmon-related protein</i>	-63	aATCGATA	FBgn0029501
		-201	TATCGAaA	
		-439	TATCGATt	
11	<i>Ecdysone-induced protein 63F 2</i>	-20	TATCGATc	FBgn0004911
12	<i>Ecdysone-induced gene 71Ea</i>	-960	TATCGATg	FBgn0004588
		-966	TATcTATA	
13	<i>GCR(1ch)</i>	-922	TATcTATA	FBgn0029170
		-928	TATCGtTA	
14	<i>K(3)05822</i>	-69	aATCGATA	FBgn0010877
15	<i>lamina ancestor</i>	-179	TtTCGATA	FBgn0016031
16	<i>Male-specific RNA 84Dc</i>	-435	TATgGATA	FBgn0004174
17	<i>SPT173</i>	-862	TATCGAaA	FBgn0035710

\* Experimentally confirmed

as that for the transcription factor dE2F<sup>10</sup>).

The classification analysis showed that 268 out of 395 genes were thought to relate to reactions required for cell proliferation such as DNA metabolism, transcriptional regulation, nuclear or chromatin structures, RNA processing, protein metabolism, signal transduction, cell cycle regulation and germ line formation (Table 1). Table 2 contains the detailed information about genes carrying DRE and/or DRE-1M sequences.

Further analysis showed that 5'-aATCGATA-3' and 5'-TATCGATt-3' were highly scored (57 and 58, respectively out of 536 copies), while 5'-gATCGATA-3' and 5'-TATCGATc-3' were in lower score (11 and 9, respectively).

## Number of DRE and DRE-1M sequences in core promoter regions

In core promoter regions (from -60 to +40 with respect to transcription initiation sites (+1)), we found 97 of DREs and 124 of DRE-1Ms. Further analysis showed that 5'-aATCGATA-3' and 5'-TATCGATt-3' were highly scored (18 and 19 respectively out of 124 copies). Contrary to this, 5'-gATCGATA-3' and 5'-TATCGATc-3' were in lower score (2 and 3 respectively).

## DISCUSSION

Involvement of the DRE/DREF system in the regulation of a considerable variety of genes has been suggested by the results of the present study, even if some of scored genes may not be eventually regulated by DREF. The result of immunological staining experiment using anti-DREF antibody strongly suggests most of the genes are under the regulation of DREF.

The statistical probability of the appearance of any specific 8-bp sequence is one in about 65.5 kb (=4<sup>8</sup>). The frequency of the 8-bp DRE sequence within 1 kb upstream regions from transcription start sites is 200 copies in 159 genes, which shows one DRE per every 0.8 kb depending on the assumption that DREs locate evenly through one kb regions. Thus, the occurrence of DREs in the scored genes is higher than 80-times of the accidental frequency. Such concentration of DRE sequence has not been found in the databases of other organisms including vertebrates. Thus, most of these DREs are thought to function in transcriptional activation. Our analysis showed that more than 83% (167/200) of the DRE sequences were located within 0.3 kb from transcription start sites, and about 50% (97/200)



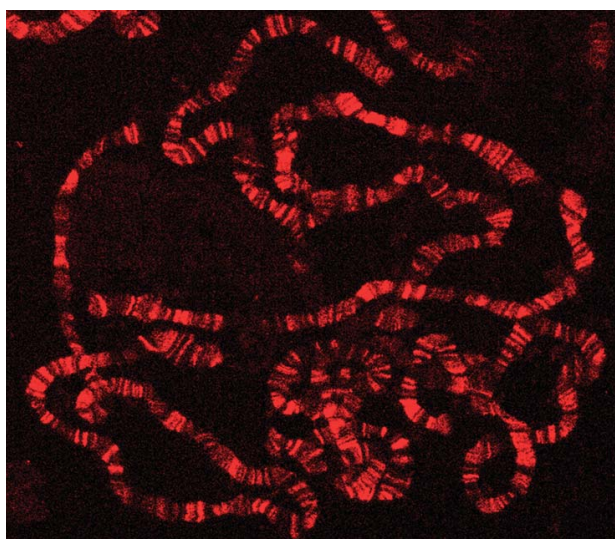


Fig. 1. Immunological detection of DREF in polytene chromosomes. The chromosome from the salivary glands of the third-instar larva was stained using the specific monoclonal antibody. Red bands indicate localizations of DREF.

of those were in the core promoter region. Including DRE-1Ms, total 221 sequences were also found in core promoter region. Ohler *et al.*<sup>37)</sup> performed a computational analysis of core promoters in the *Drosophila* genome and found 277 DRE's in core promoter region, and reported [WATCGATW] as a consensus sequence. This is the second highest among various regulator elements.

It is important to see that the genes found in the genome screening are eventually targets of DREF. For supporting evidence, immunostaining of salivary gland polytene chromosome with the anti-DREF monoclonal antibody was performed to find out several hundreds of antibody-stained bands through the chromosomes (Fig. 1). These results suggest that many genes are under the regulation of DREF in the salivary gland. We compared the anti-DREF binding sites and the cytogenetic maps of the genes found in the genome screening. For examples, the locus for the *skpA* gene (X;1B14), the SNF1A gene (X;2A1), the CG15636 gene (2L; 25A1) and the Rfc40 gene (3L; 64A7-8) were matched with the sites where staining by anti-DREF antibody was detected. The *skpA* gene carries two DREs, and is reported that encodes a component of the SCF complex which functions in combination with the ubiquitin-conjugating enzyme UbcD1 involved in cell cycle regulation<sup>18, 38)</sup>. Knock down of DREF expression using the RNA interference method resulted

in the down regulation of *skpA*.

It is also interesting whether other organisms carry the regulatory system like the DRE/DREF. Recently, a human homologue (hDREF/KIAA0785) of *Drosophila* DREF was identified by BLAST search<sup>39)</sup>. Amino acid sequences corresponding to three regions highly conserved between two *Drosophila* species also proved to be very similar in the hDREF/KIAA0785 polypeptide. A consensus binding sequence (5'-TGTCG(C/T)GA(C/T)A-3') for hDREF/KIAA0785 partially overlapped with that for the *Drosophila* DRE (5'-TATCGATA-3'). RNAi experiments targeting hDREF/ KIAA0785 resulted in inhibition of S phase entry and reduction of mRNA for histone H1, whose gene contains a consensus sequence for hDREF/KIAA0785. Twenty-two out of 79 human ribosomal protein (RP) genes contain sequences similar to the hDREF-binding sequence (hDRE) within 200-bp regions upstream of their transcriptional start sites. Electrophoretic mobility shift assays and chromatin immunoprecipitation analysis indicated that hDREF binds to hDRE-like sequences in the RP genes both *in vitro* and *in vivo*<sup>40)</sup>. These data suggest that hDREF is also an important transcription factor for cell proliferation.

In conclusion, most of DRE and/or DRE-1M containing genes identified in this study might be regulated by the DRE/DREF system, and therefore DREF is possibly thought to be one of master key transcriptional regulators required for cell proliferation. Large amounts of experiments are necessary to confirm how many of the scored genes are truly regulated by the DRE/DREF system.

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