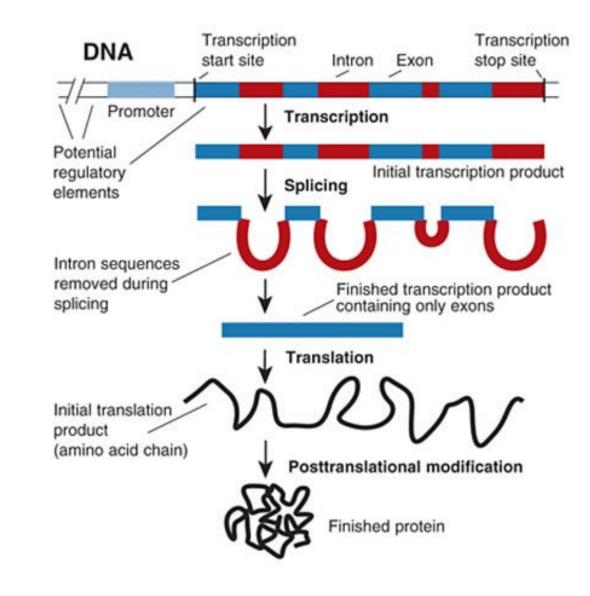
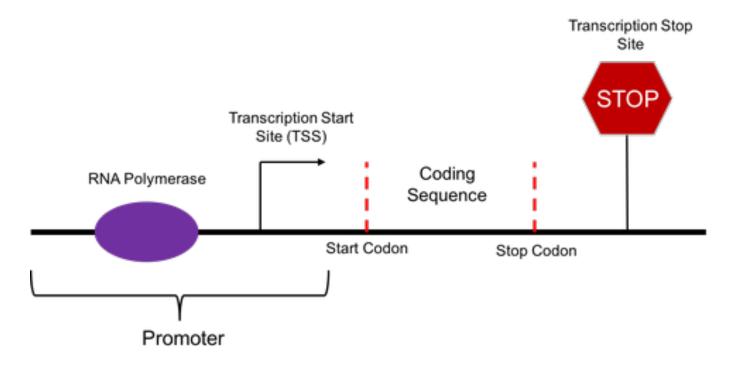
# Promoters-Cis Elements

Assoc. Prof. Dr. Ilker BUYUK





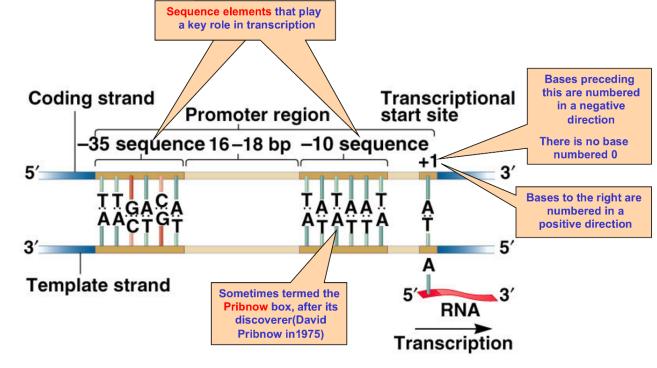
### PROMOTER

- A promoter is the main regulatory portion of a gene.
- The simplest analogy is that a promoter is a "switch" that turns a gene "on" or "off." It is the portion of the gene where cellular machinery binds before transcribing the DNA blueprint into a useful RNA.

### PROMOTER

 In genetics, Promoters are DNA sequences located in the 5' region adjacent to the transcriptional start site. RNA polymerase and accessory proteins (transcription factors) bind to the promoter to initiate production of an mRNA transcript. Interactions of proteins at the promoter regulate gene activity by activating or repressing transcription

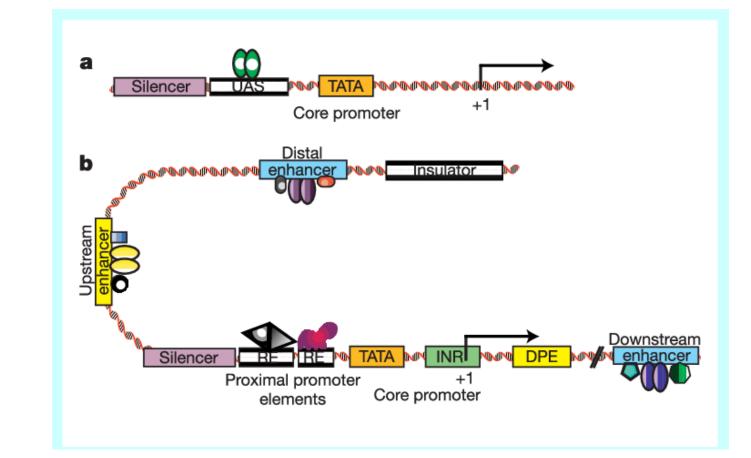
### •The bases in a promoter sequence are numbered in relation to the transcription start site.



The conventional numbering system of promoters

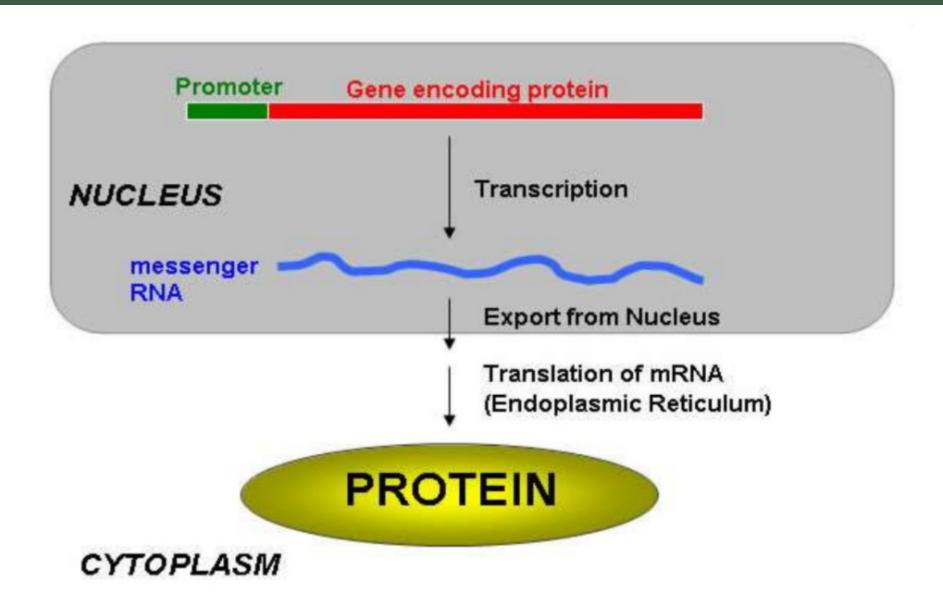
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- There is virtually an endless number of promoters, potentially as many as there are genes.
- Promoters, like genes, are made up of A's, G's, C's and T's all lined up in a certain order. Promoters can be about 100–1000 base pairs long.
- The promoter region can be short or quite long; the longer the promoter is, the more available space for proteins to bind.



## Role in transcription

 The whole process of transcription starts when the RNA polymerase binds to the promoter. RNA Polymerase has 4 subunits i-e alpha, beta, beta' and sigma. Only the Sigma Factor is required for RNA polymerase to bind to the promoter. As the RNA polymerase binds to the promoter, DNA duplex becomes unwind, base pairs are broken down and transcription bubble is appeared. This is followed by elongation phase where the RNA grows and protrudes from the bubble.



# TYPES OF **PROMOTERS USED FOR** GENE EXPRESSION

#### 1) Constitutive promoters:

- Induce the expression of the downstream located coding region in all tissues irrespective of environmental or developmental factors.
- Example: Plant pathogen promoters CaMV 35S promoter.

#### 2) Tissue Spesific Promoters:

- Operate in particular tissues and at certain developmental stages.
- Maybe induced by endogenous and exogenous factors.
- **Examples:** Tomato pz7 and pz10 gene promoters ( for ovary gene expression)

# TYPES OF PROMOTERS USED FOR GENE EXPRESSION

#### 3) Inducible promoters:

- Within this group, there are promoters modulated by presence or absence of biotic or abiotic factors such as light, oxygen levels, heat, cold and wounding. Inducible promoters are grouped as:
- a ) Chemically regulated promoters
- b) Physically regulated promoters

#### 4) Synthetic promoters :

- Promoters made by bringing together the primary elements of a promoter region from diverse origins.
- Example:
- Maize ubiquitin 1 gene (Ubi 1) core promoter
- Cytomegalovirus (CMV) promoter
- CAG promoter

### **PROMOTER REGION:**

- There are three main portions that make up a promoter: core promoter, proximal promoter, and distal promoter.
- CORE PROMOTER REGION:
- The core promoter region is located most proximally and contains the RNA polymerase binding site, TATA box, and transcription start site (TSS). RNA polymerase will bind to this core promoter region stably and transcription of the template strand will initiate.

### **PROMOTER REGION:**

#### • PROXIMAL PROMOTER REGION:

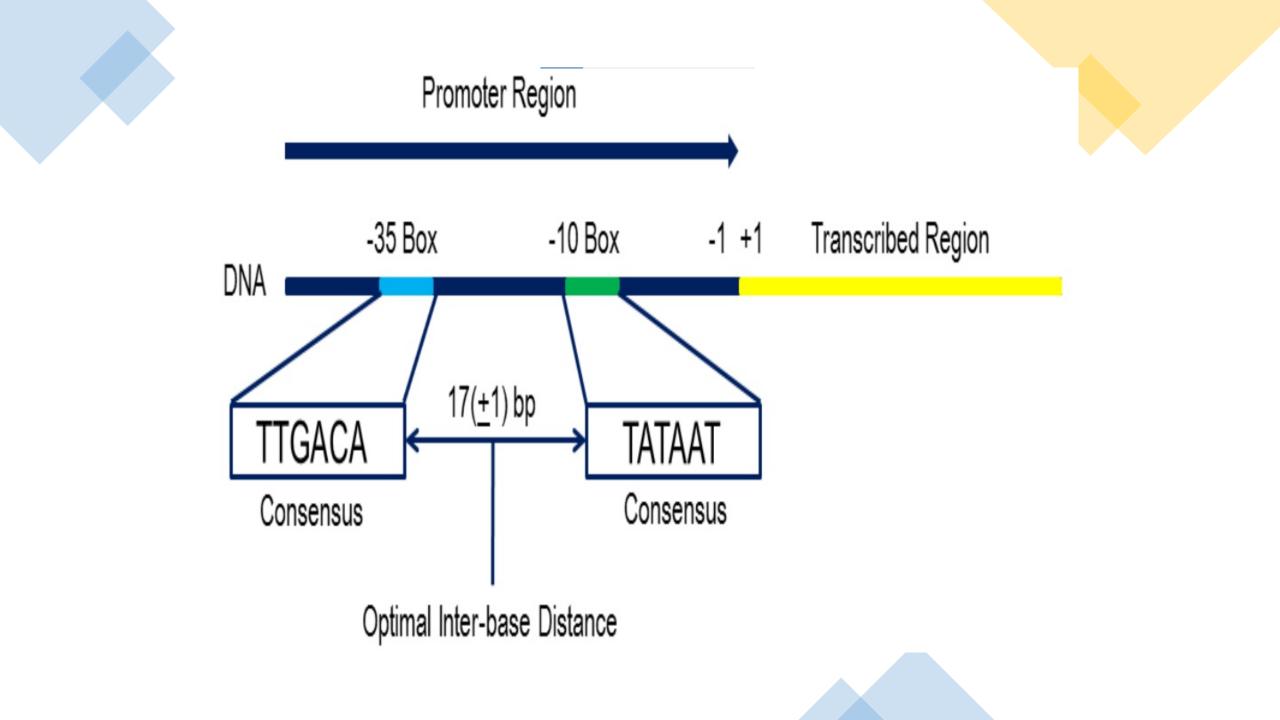
• Proximal promoter are upstream from the core promoter which contain many primary regulatory elements. The proximal promoter is found approximately 250 base pairs upstream from the TSS and it is the site where general transcription factors bind.

#### • DISTAL PROMOTER REGION:

• The final portion of the promoter region is called the distal promoter which is anything further upstream from the gene. The distal promoter also contains transcription factor binding sites, but mostly contains regulatory element

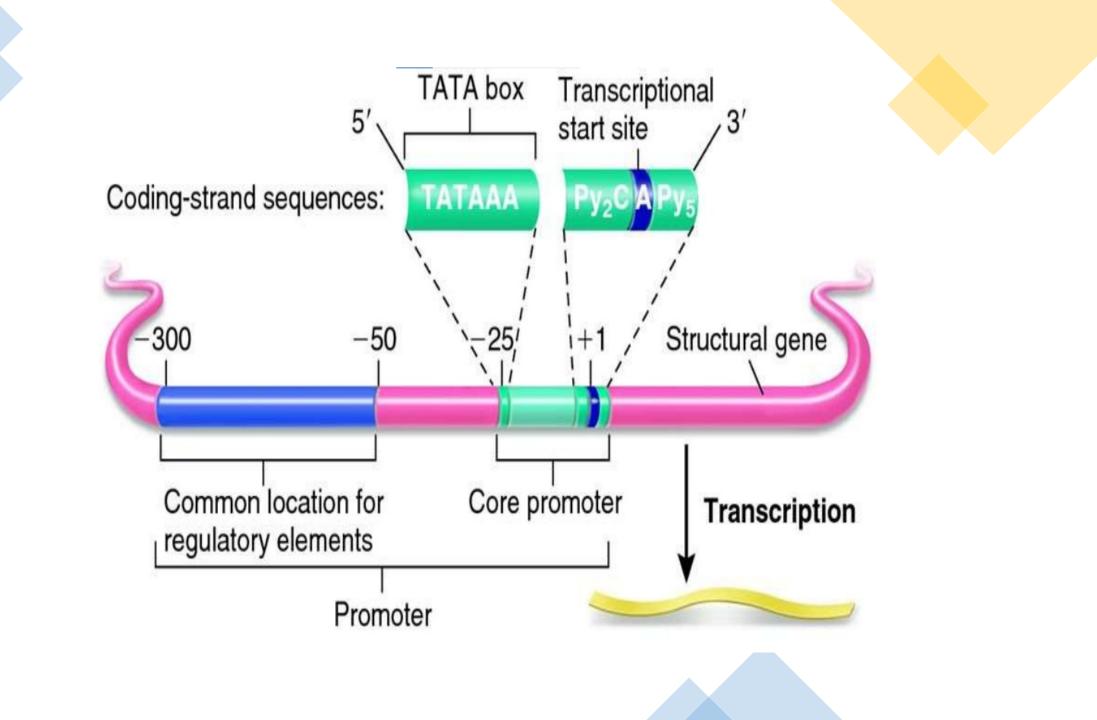
### **PROKARYOTIC PROMOTERS**

- Promoters in prokaryotic organisms are two short DNA sequences located at the -10 (10bp 5' or upstream) and -35 positions from the transcription start site (TSS).
- Their equivalent to the eukaryotic TATA box, the Pribnow box (TATAAT) is located at the -10 position and is essential for transcription initiation.
- The -35 position, simply titled the -35 element, typically consists of the sequence TTGACA and this element controls the rate of transcription.
- Prokaryotic cells contain sigma factors which assist the RNA polymerase in binding to the promoter region. Each sigma factor recognizes different core promoter sequences.



## **EUKARYOTIC PROMOTERS**

- Eukaryotic Promoters are much more complex and diverse than prokaryotic promoters. Eukaryotic promoters span a wide range of DNA sequence. There are two parts :
- The core promoter or the basal promoter
- Upstream promoter element
- Core promoter is constituted by TATA box and transcriptional start site. Initiation complex binds to the core promoter upstream elements are responsible of the regulation of transcription.



Conserved eukaryotic promoter elements	Consensus sequence
CAAT box	GGCCAATCT
TATA box	TATAA
GCbox	GGGCGG
CAP site	TAC

### **BIDIRECTIONAL PROMOTERS**

- Pairs of genes control by same promoter but located on opposite strand and opposite direction. Their TSS are separated by less than 1,000 bp.
- In general, they are rich in CpG content.
- Function of genes represented in bidirectional class are often: DNA repair genes, chaperone protein, and mitochondrial genes.
- Genes control by bidirectional promoters are often co- express, but a minority of bidirectional genes have a mutual exclusive expression.
- No correlation between length of promoter and degree of expression

### **BIDIRECTIONAL PROMOTERS**

- Bi-directional promoters are a common feature of mammalian genomes About 11% of human genes are bi-directionally paired.
- Certain sequence characteristics have been observed in bidirectional promoters:
- 1: A lack of TATA boxes
- 2:An abundance of CpG islands
- 3:Symmetry around the midpoint of dominant Cs and As on one side and Gs and Ts on the other.

#### • Sub-genomic promoters

• A sub-genomic promoter is a promoter added to a virus for a specific heterologous gene, resulting in the formation of mRNA for that gene alone.

#### • Detection of promoters

• A wide variety of algorithms have been developed to facilitate detection of promoters in genomic sequence, and promoter prediction is a common element of many gene prediction methods. A promoter region is located before the -35 and -10 Consensus sequences. The closer the promoter region is to the consensus sequences the more often transcription of that gene will take place.

### **COMMONLY USED PROMOTERS**

- The most commonly used promoters are:
- Lac (Lactose)
- Trp, (Tryptophan)
- Tac, (invitro combo of Lac and Trp)
- T 7 System
- All of these bind to the sigma-70 factor for transcription.

### Why the interest in promoters?

- The interest in promoters stems from the myriad opportunities for controlling gene expression.
- The study and understanding of the function of their multiple components and the factors associated with their performance have opened up the possibility of modulation of the expression of genes in homologous organisms as well as in heterologous organisms, where foreign promoters together with genes of interest are inserted.
- Promoters are regarded as **molecular biological tools crucial** for the regulation of the expression of genes of interest.
- As such, they have a huge influence in follow-on research and development in biotechnology.

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• The National Center for Biotechnology Information provides access to genomic information in order to advance science. The genome database currently comprises of 16,326 species and can be sorted by kingdom, group and subgroup.

	Phaseolus vulgaris	Physical position on <i>P.</i> <i>vulgaris</i> genome			Protein 1		Molecular weight	Instability	Aliphatic		Stable or	NCBI Accession	
ID	Genomic Database Identifier	Chr.	Start position (bp)	End Position (bp)	length (aa)	pI	weight (Da)	index	index	GRAVY	unstable	NCBI Accession No.	
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Pvul-BLEC-1	Phvul.001G239200.1.p	1	49.244.673	49.246.082	345	5.28	37510.23	28.22	89.77	-0.126	stable	XP_007163496.1	

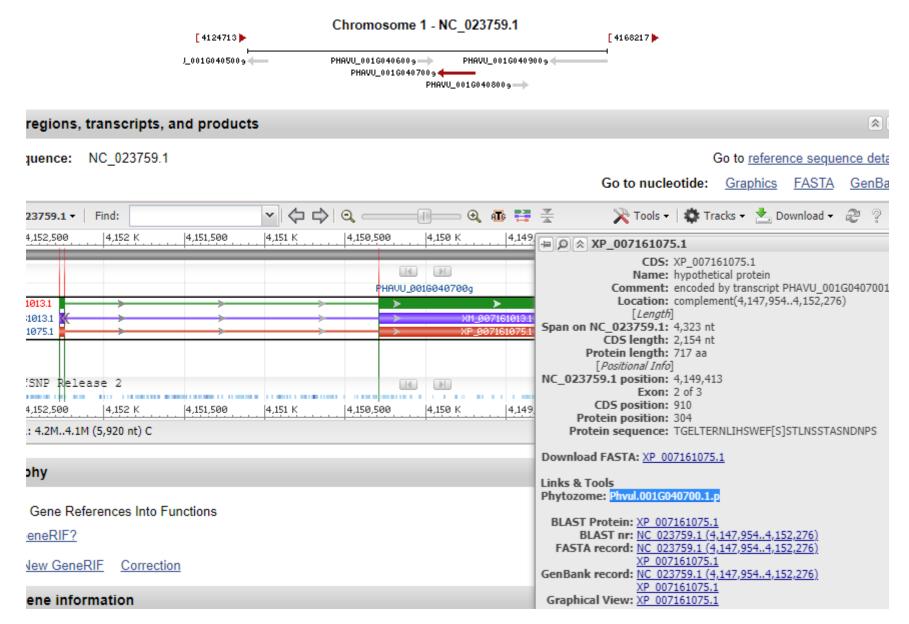
#### Phaseolus vulgaris cultivar G19833 chromosome 1, whole genome shotgun sequence

NCBI Reference Sequence: NC\_023759.1

GenBank FASTA

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#### Chromosome: 1; NC\_023759.1 (4147723..4152276, complement)



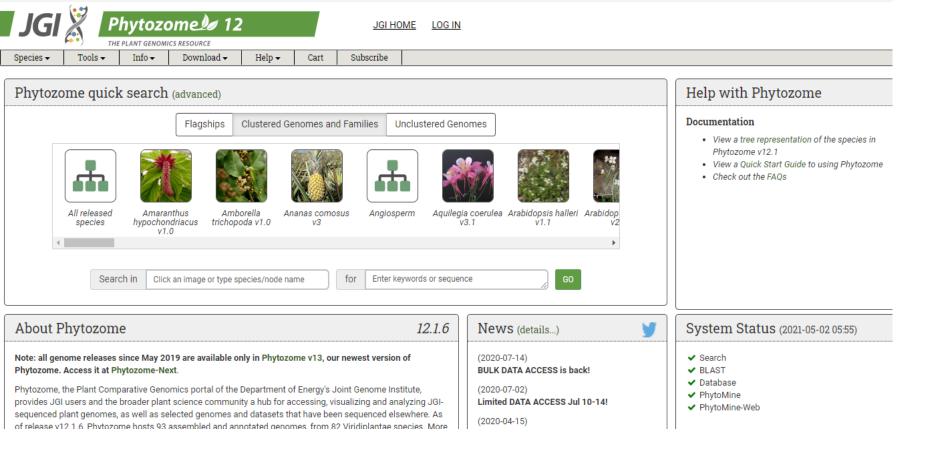
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Dosya Düzen Biçim Görünüm Yardım

>ref|NC\_023759.1|:c4150299-4149498 Phaseolus vulgaris cultivar G19833 chromo
shotgun sequence

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 Phytozome is a plant comparative genomics portal wherein families of related genes representing the modern descendants of ancestral genes are constructed at key phylogenetic nodes. Currently Phytozome provides access to 58 sequenced and annotated green plant genomes.

1. Select a Target	1 species selected 🗙	2. Build your query GO
Target set:         Phytozome 12.1         Pre-release spe           Target type:         Ancestor nodes         Species	cies	Search type: Keyword BLAST
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<u>https://phytozome.jgi.</u> <u>doe.gov/pz/portal.html</u> <u>#!search?show=BLAST</u>	AAAACTCCACCTTTAAAA	g sequence: upstream: downstream: 0 Submit Chr01:43361064338606 forward CCAATAAGAAAAAAGAAAAAAGTAGAGGAGATCAAAGACCAAACCTCCAATTGAGTCAAAGAAAAGATCTTAAATTAATCC ATAATCTTATTCCTATGACTCCATATCTCACTCACTAGTCATCGCAATCAAAATAAAAGCAGCAGAAAACACTTCTAATCGC ATTACTCGTAGTGACAATCTTATTTTCCAACATCCTCCAAGCTGTAACCTAAGCTGAAGGCAAAACCTTAATCCTCCAAAAC

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CDS sequence Peptide sequence

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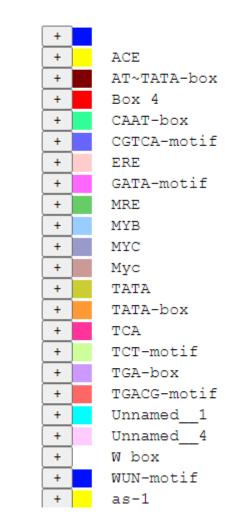
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Motifs Found

+	ААААААТ <mark>САА</mark>	TAAAAGGATA	TCCACATAAC	ATACTCTTAC	<mark>GTCА</mark> ТААТАТ	TTTTTTT <mark>TATA</mark>	ATATGTGA <mark>GA</mark>
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-	ATCCTCTATC	TGTTCATACA	GTTTGTTTAG	AATGGCACCT	TAGGTTAATT	GTTAGATTCT	CTTTAATAG
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-	ATAGTATACA	CAGTAAACCT	CCTACTATCT	CTGGTATTTT	GTTAAATAAA	TTATTAAAAA	AAGGTTTAG
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+ AATATAAGGA TATACTAATA TTGCTTATCA TACCATATTA TTTTAAAATT TTTGTTTTCA GAATTTTAAA - TTATATTCCT ATATGATTAT AACGAATAGT ATGGTATAAT AAAATTTTAA AAACAAAAGT CTTAAAATAT

+ A<mark>AACCTAA</mark>TA CACTTTTATA ATTTTTCATT TATACTTA<mark>AT ATAA</mark>ATTTTA CAAAGTGAAA AGTAATTTTT - TTTGGATTAT GTGAAAATAT TAAAAAGTAA ATATGAATTA TATTTAAAAT GTTTCACTTT <mark>TCATTAAA</mark>AA

+ TATTCTTTGA ATTCAAAACT TTGCATGATG GA<mark>ATATAA</mark>TA TTAAAAAGAA <mark>AAATTACTA</mark>A AGA<mark>CATTTG</mark>T - ATAAGAAACT TAAGTTTTGA AACGTACTAC CTTATATTAT AATTTTTCTT TTTAATGATT TCTGTAAACA

+ TTAAATTGTT GAGTTAAACT T<mark>TATTTAAA</mark>C AGCAACCTGC GTGAA<mark>CAAAT</mark> TCAGAACAAA CTTATTCATT - AATTTAACAA CTCAATTTGA AATAAATTTG TCGTTGGACG CACTTGTTTA AGTCTTGTTT GAATAAGTAA

#### TATA-box

Site Name	Organism	Position	Strand M	Matrix score.	sequence
TATA-box	Brassica napus	12	+	6	ATTATA
TATA-box	Arabidopsis thaliana	61	+	8	TAAAGATT
TATA-box	Brassica juncea	40	-	7	TATAAAT
TATA-box	Helianthus annuus	265	-	6	TATAAA
TATA-box	Arabidopsis thaliana	19	-	5	TATAA
TATA-box	Arabidopsis thaliana	185	+	4	TATA
TATA-box	Arabidopsis thaliana	42	-	5	TATAA
TATA-box	Arabidopsis thaliana	266	-	5	TATAA
TATA-box	Arabidopsis thaliana	14	+	4	TATA
TATA-box	Arabidopsis thaliana	119	+	4	TATA
TATA-box	Helianthus annuus	41	-	6	TATAAA
TATA-box	Pisum sativum	264	-	7	TATAAAA
TATA-box	Arabidopsis thaliana	20	+	4	TATA
TATA-box	Brassica oleracea	184	+	6	ATATAA
TATA-box	Arabidopsis thaliana	43	+	4	TATA
TATA-box	Arabidopsis thaliana	267	+	4	TATA
TATA-box	Arabidopsis thaliana	13	-	5	TATAA
TATA-box	Brassica napus	18	+	6	ATTATA
TATA-box	Brassica napus	117	+	6	ATTATA
TATA-box	Brassica napus	539	+	6	ATATAT
TATA-box	Arabidopsis thaliana	118	-	5	TATAA
TATA-box	Arabidopsis thaliana	540	+	6	TATATA
TATA-box	Brassica napus	541	+	6	ATATAT
TATA-box	Arabidopsis thaliana	542	+	4	TATA
TATA-box	Brassica oleracea	640	+	6	ATATAA
TATA-box	Arabidopsis thaliana	641	-	4	TATA
TATA-box	Brassica napus	699	+	6	ATTATA
TATA-box	Arabidopsis thaliana	700	-	5	TATAA
TATA-box	Arabidopsis thaliana	701	-	4	TATA
TATA-box	Arabidopsis thaliana	764	-	8	TAAAGATT
TATA-box	Brassica oleracea	772	+	6	ATATAA
TATA-box	Arabidopsis thaliana	773	-	4	TATA
TATA-box	Arabidopsis thaliana	781	-	4	TATA
TATA-box	Pisum sativum	834	-	7	TATAAAA
TATA-box	Helianthus annuus	835	-	6	TATAAA
TATA-box	Arabidoneie thaliana	836	-	5	татаа

core promoter element around -30 of transcription start core promoter element around -30 of transcription start

function

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ID	DEVELOPMENT																
Phvul-GASA-1	AAGAA-motif			0	02-site												
Phvul-GASA-2				(	02-site	GCN4_					LIGHT	•					
Phvul-GASA-3		HD-Zip 1					Box 4			G-box	GATA-motif						
Phvul-GASA-4		HD-Zip 3					Box 4 Box 4	AT1-m AT1-m		G-box		GA-motif	GT1-motif GT1-motif				
Phvul-GASA-5							Box 4 Box 4	AT1-m		G-box			GT1-motif			TCT-motif	
	AAGAA-motif						Box 4	AT1-m		G-box			GT1-motif			TCT-motif	
Phvul-GASA-6	AAGAA-motif		CAT-b	ox (	02-site	GCN4_	Box 4			G-Box	GATA-motif	GT1-motif		Gap-box		TCT-motif	chs-CMA1
Phvul-GASA-7	AAGAA-motif						Box 4	AT1		Chay			GT1-motif				chs-CMA1
Phvul-GASA-8	AAGAA-motif					GCN4_	Box 4 Box 4	AT1-m	otif	G-box							chs-CMA1
Phvul-GASA-9	AAGAA-motif			0	02-site		Box 4	AT1-m	otif	G-box	GATA-motif		GT1-motif			TCT-motif	
Phvul-GASA-10			CAT-b	ox (	02-site		Box 4			G-Box		GA-motif		Gap-box		TCT-motif	
Phvul-GASA-11	AAGAA-motif				02-site		Box 4	4.74	- 115	G-Box	GATA-motif	GA-motif	GT1-motif			TOT	
Phvul-GASA-12	AAGAA-motif				02-site	GCN4	Box 4 Box 4	AT1-m	otif	G-box		GA-motif	GT1-motif GT1-motif			TCT-motif	
_					oz-site		Box 4						GT1-motif			TCT-motif	chs-CMA1
Phvul-GASA-13						GCN4_	Box 4			G-box	GATA-motif	GA-motif	GT1-motif			TCT-motif	
Phvul-GASA-14	AAGAA-motif	HD-Zip 1					Box 4			G-box			GT1-motif				
Phvul-GASA-15	AAGAA-motif					GCN4_	Box 4 Box 4	AT1-m	otif	G-box			GT1-motif GT1-motif				chs-CMA1
Phvul-GASA-16	AAGAA-motif		ENVIRONMENTAL	STRESS			B07.4	Altein	oth			HORMON					CH3-CIVIA1
Phvul-GASA-17	AAGAA-motif		MYC	W box	WUN-motif			ABRE		ERE							
Phvul-GASA-18	AAGAA-motif						ich repeats	ABRE		ERE							chs-CMA1
Phvul-GASA-19	AAGAA-motif		MYC				ich repeats	4.0.05	P-box	ERE	00704	41 <b>6</b>		TOACO			
-		MDC	MYC		WUN-motif		ich repeats	ABRE ABRE	P-box P-box	ERE ERE	CGTCA-mo			TGACG-	•		
Phvul-GASA-20	a the second second second	MBS MBS	MYC MYC		WUN-motif WUN-motif	TC-N	ich repeats	ABRE	P-DOX	EKE					-		
Phvul-GASA-21	AAGAA-motif		MYC		WUN-motif				P-box	ERE		TATC-box	TCA-element				
Phvul-GASA-22	AAGAA-motif						ich repeats	ABRE		ERE	CGTCA-mo	tif		TGACG-	•		
Phvul-GASA-23	AAGAA-motif	MBS	MYC MYC	W box W box		TC-r	ich repeats	ABRE ABRE	P-box	ERE ERE	CGTCA-mo	tif TATC-box		TGACG-			
		MBS	MYC	W box	WUN-motif			ABRE	P-00X	ERE	CGTCA-mc		TCA-element	TGACG-			
		MBS	MYC			TC-r	ich repeats	ABRE		ERE	CGTCA-mo		TCA-element	TGACG-	-		
			MYC	W box		TC-r	ich repeats	ABRE		ERE	CGTCA-mo	tif	TGA-element TCA-element	TGACG-	·		
		MBS	MYC	W box	WUN-motif				P-box ERE						-		
		MBS	MYC MYC	W box		TC-r	ich repeats	TGA-box		ERE	CGTCA-mo		TCA-element	TGACG TGACG-	-		
			MYC					ABRE		ERE	Corca-mo		TCA-element	TGACG			
			MYC					ABRE		ERE			TCA-element				
		MBS	MYC		WUN-motif				P-box	ERE		TATC-box					
			MYC			TC-r	ich repeats	ABRE		ERE	CGTCA-mo	tif TATC-box	TCA-element	TGACG-	•		
		MBS	MYC	Wbox		TC-r	ich repeats	ABRE		ERE		TATC-box	TCA-element		-		
			MYC	W box			•	ABRE	P-box	ERE		-					