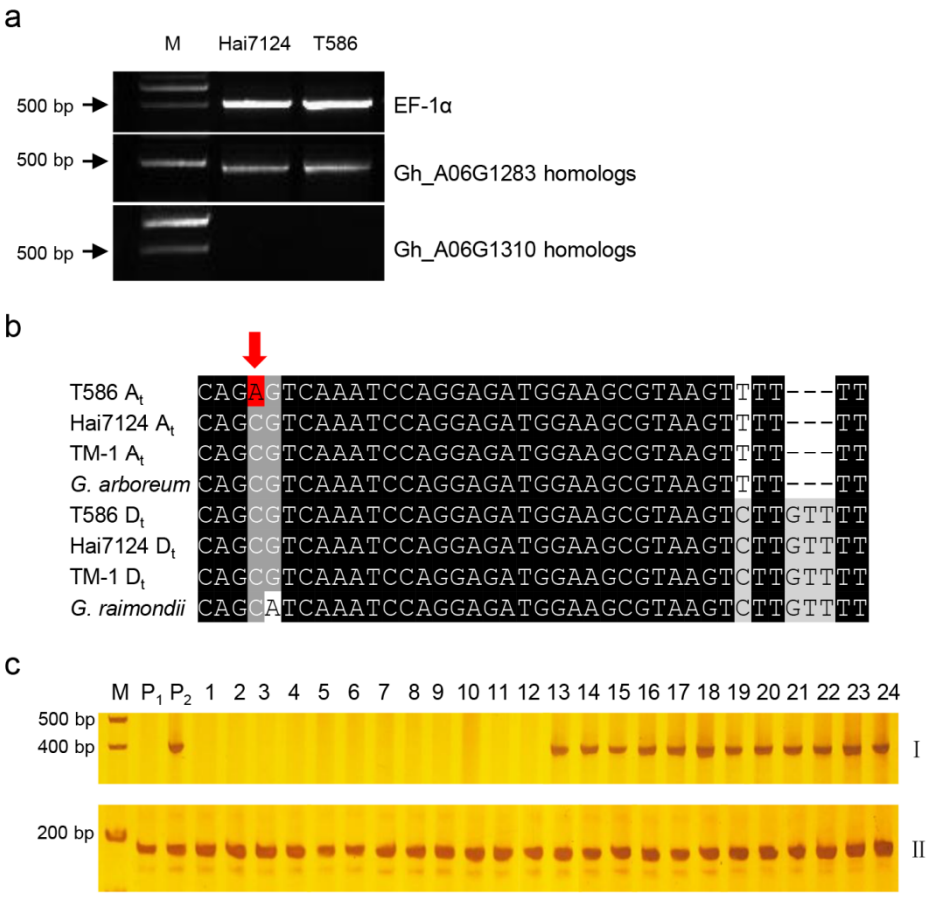
Appendix A Special primers used in this paper

Primer description	Gene name/ID	Primer ID	Primer sequences (5'-3')
SSR/SNP markers		NAU5433 F	CTTAGGATGGCCGAATAAAA
		NAU5433 R	CAAGTGCTCCACCACAAAT
		NAU5434 F	AAAAGAACTTACGGCACAGG
		NAU5434 R	AAATCACTGGCACTGGAATC
		NAU5373 F	AAGCTCCGAATGTTCAGAAT
		NAU5373 R	GAAGGGGAGGAATCGTTAAT
		S1874 F	CCCATCGGAGGGGCAAAATA
		S1874 R	AATGTAGCACTATTTTATTGGTGTCT
		S1922 F	CCTTCGGTCACCACTATCGG
		S1922 R	ACGGAAGCATGAAGAATAAAGCA
		NAU676 F	GAGTTTATTGAGAAAGGCTTTCC
		NAU676 R	CTCAAAGTGGCTGTGCTTTG
		NAU1277 F	CCCCACCAACACTACTTGCT
		NAU1277 R	GCAAAAGGCTGAGAAGGTCA
		NAU115 F	TCAACTCATACCAAATCAATTCC
		NAU115 R	CCCTGTTTTGTTCAATGGGT
Specific primer of single nucleotide polymorphism (SNP) for *HD-1* in A subgenome		S1610F	AAATGAAGCGTTATCATCGTCATACCGAGA
		S1610R	AGGAAAGCTTACTACATAGACAGAAAAGCTTAATCA
Gene special primers for RT-PCR	*Gh_A06G1310+Gh_D06G1638*	S4309F	GATGCCACCATAAGGACGAT
		S4309R	ATTGTGGCTGCCGATAGATG
	Gh_A06G1283+Gh_D06G1607	S4312F	ATCGTCATACCCAGCGTCAA
		S4312R	GGTGAGGCAAAGAAGATAAAGG
Endogenous control for RT-PCR	*EF-1α*		AGACCACCAAGTACTACTGCAC
			CCACCAATCTTGTACACATCC
Gene special primers for qRT-PCR	*HD-1 (Gh_A06G1283+ Gh_D06G1607)*	S3900F	TCTCCTTACCGTTGCTTTCCA
		S3900R	AGCATTATTGCACATTACGG
	GL1	S3944F	GCAAGTCAAAGTGTGTCATG
		S3944R	CTGGTCATTGATTCTTGAGTAG
	GL3	S3902F	GTCCAATATTAGGCTGTGAA
		S3902R	TCCACCATTTATCCCTTCAA
	TTG1	S3903F	CAATCGAAAATCCGCTTCAT
		S3903R	ACAGGTTCAATGGAAGATTC
	TRY	S3904F	GGATCATGAGACATGGAGAA

		S3904R	ACAGCATACTACAGAAAAGGC
	CPC	S3946F	GTCTCGTGTTGTTTATGATC
		S3946R	CAGGGAAGATACTTTTAATCG
	Gh_A10G0143/Gh_D10G0146	S4412F	CACTCTTGAAAGGATCAGAA
		S4412R	GAACCAAACTAATTCTCCTTG
Endogenous control for qRT-PCR	*Tubulin 1*		CCGTAAACCTTATTCCATTCCC
			AAGACCGCTGATGCTGTGAG
Gene special primers for the gDNA amplification	*GhHD-1*	S548F	AAACCACCCTAAAGCCTCC
		S548R	AGCCCTTGAAACAATACCG
		S549F	GGACCTACAGAAGCGGATAA
		S549R	GCTGATGTTGTTGGGAGTG
Gene special primers for the virus induced gene silencing assay	*GhHD-1*	S958F	GCTCTAGAAACCCTTTTCCCCCTTTATT
		S958R	CGGGATCCTTGGTCAGGATCTTGATCAT

Appendix B Annotation of the candidate genes

G.hirsutum ID	*Arabidopsis* ID	*G. hirsutum* TM-1 annotation (Zhang et al. 2015)
Gh_A06G1281	AT3G20580	COBRA-like protein 10 precursor
Gh_A06G1282	AT2G07725	Ribosomal L5P family protein
Gh_A06G1283	AT4G21750	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
Gh_A06G1284	AT1G08220	
Gh_A06G1285	AT5G28237	Pyridoxal-5\'-phosphate-dependent enzyme family protein
Gh_A06G1286	AT4G04885	PCF11P-similar protein 4
Gh_A06G1287	AT1G61800	glucose-6-phosphate/phosphate translocator 2
Gh_A06G1288	AT1G61820	beta glucosidase 46
Gh_A06G1289	AT5G52580	RabGAP/TBC domain-containing protein
Gh_A06G1290	AT1G11600	cytochrome P450, family 77, subfamily B, polypeptide 1
Gh_A06G1291	AT4G04880	adenosine/AMP deaminase family protein
Gh_A06G1292	AT1G61850	phospholipases;galactolipases
Gh_A06G1293	AT4G21780	
Gh_A06G1294	AT3G20530	Protein kinase superfamily protein
Gh_A06G1295	AT4G04870	cardiolipin synthase
Gh_A06G1296	AT4G04860	DERLIN-2.2
Gh_A06G1297	AT4G21820	binding;calmodulin binding
Gh_A06G1298	AT4G04780	mediator 21
Gh_A06G1299	AT5G52890	AT hook motif-containing protein
Gh_A06G1300	AT1G11655	
Gh_A06G1301	AT1G61900	
Gh_A06G1302	AT1G62590	pentatricopeptide (PPR) repeat-containing protein
Gh_A06G1303	AT4G04640	ATPase, F1 complex, gamma subunit protein
Gh_A06G1304		
Gh_A06G1305	AT1G62050	Ankyrin repeat family protein
Gh_A06G1306	AT1G11790	arogenate dehydratase 1
Gh_A06G1307	AT1G11840	glyoxalase I homolog
Gh_A06G1308	AT4G04610	APS reductase 1
Gh_A06G1309	AT4G22010	SKU5 similar 4
Gh_A06G1310	AT2G02060	Myb_DNA-binding
Gh_A06G1311		
Gh_A06G1312	AT1G62250	
Gh_A06G1313	AT1G11860	Glycine cleavage T-protein family
Gh_A06G1314	AT4G26910	Dihydrolipoamide succinyltransferase



Appendix C Confirmation of the candidate leaf trichome gene

A, Expression detection of the candidate genes in Hai7124 and T586 by RT-PCR. The primers were gene-specific, not subgenome-specific for the candidate genes *Gh_A06G1283* homologs (*Gh_A06G1283* and *Gh_D06G1607*) and *Gh_A06G1310* homologs (*Gh_A06G1310* and *Gh_D06G1638*) in allotetraploid cotton. The expression level of *EF-1α* was used as the endogenous control (Jiang et al. 2012). B, The *Gh_A06G1283* SNP marker between T586 At and Hai7124 At (marked by an arrowhead) was developed to detect genotypes in the genetic population. C, The SNP marker distribution in the genetic population. P1, Hai7124; P2, T586. Ⅰ shows the DNA fragments of leaf trichome genes in 24 randomly selected individuals with trichomes (13-24) or without (1-12). Ⅱ shows the DNA fragments of the reference gene, *Tubulin 1* (*AF487511.1*), in the corresponding individuals.

Appendix D Sequence information of *HD-1* in different cotton materials

>*HD-1* in *G. arboretum*

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>*HD-1* in *G.raimondii*

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>*HD-1* in TM-1 A_t

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>*HD-1* in Hai7124 D_t

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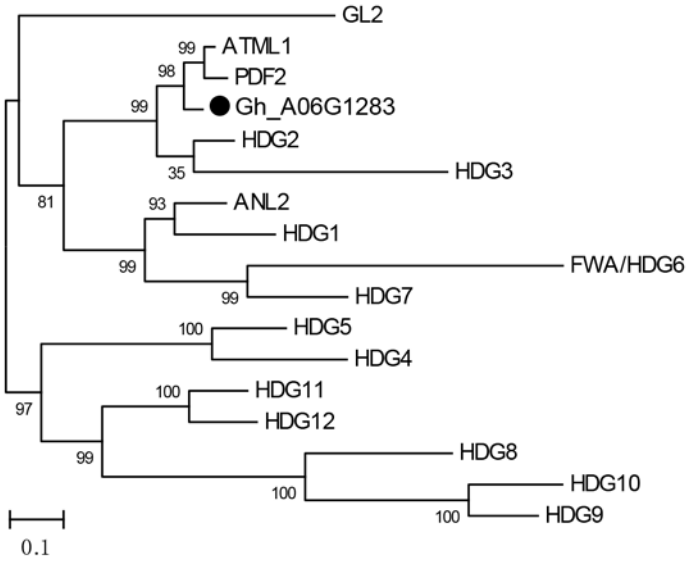
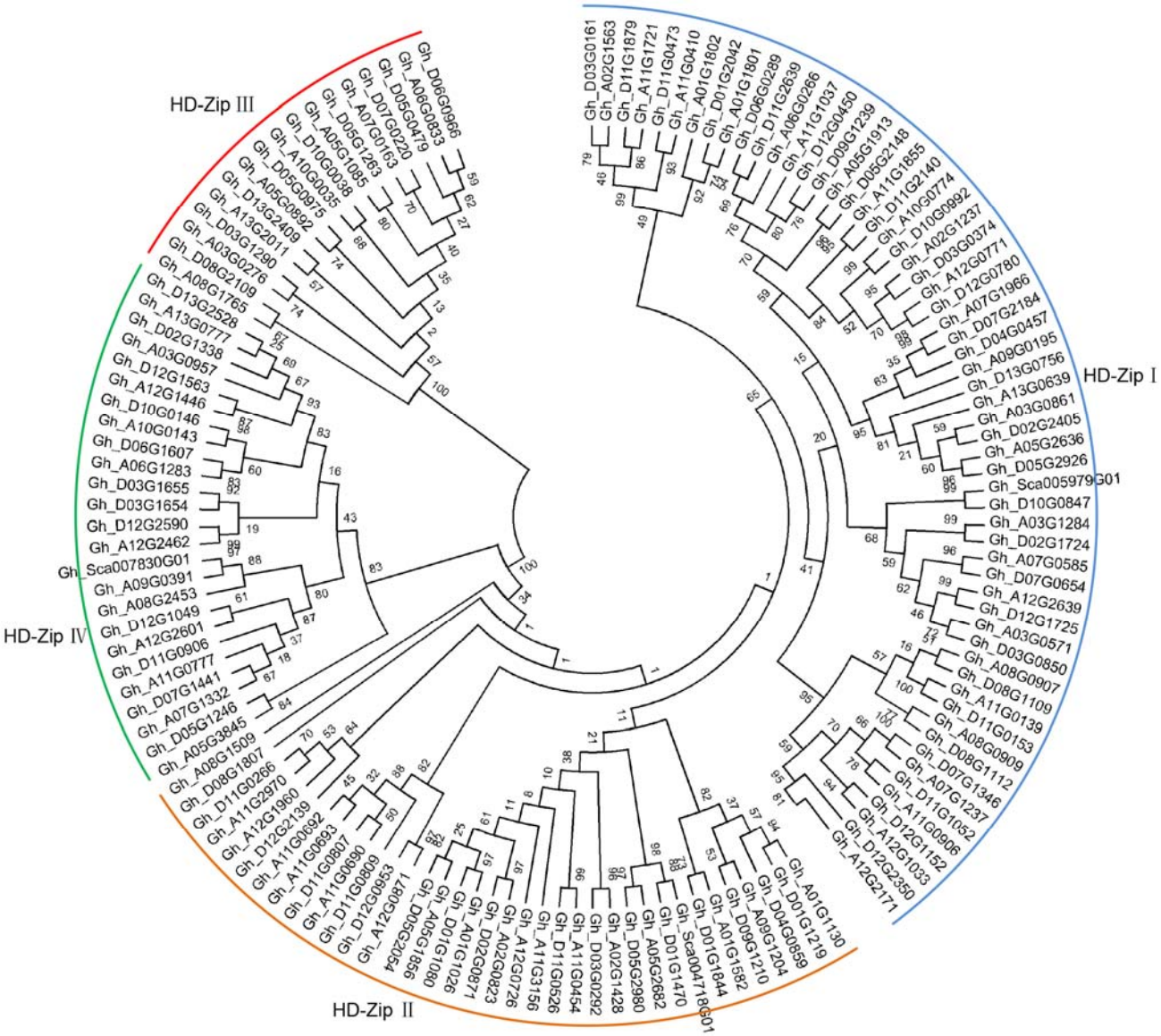


Appendix E The phylogenetic tree of the leaf trichome gene (*Gh_A06G1283*) and orthologs of HD-Zip Ⅳ genes in *Arabidopsis*

The orthologs included *GL2-Glabra2*, *ATML1-A. thaliana Meristem Layer1*, *PDF2-Protodermal Factor2*, *HDG2* to *HDG12-Homeodomain Glabrous1 to Homeodomain Glabrous12*, *ANL2-Anthocyaninless2* and *Fwa-Flowering Late* (Henriksson *et al.* 2005). The phylogenetic tree was constructed using MEGA 5.0 software (www.megasoftware.net) with the maximum likelihood method (Tamura *et al.* 2011).



Appendix F Phylogenetic relationships of HD-Zip genes in tetraploid cotton TM-1

The gene IDs and amino acid sequences of HD-Zip members were downloaded from http://mascotton.njau.edu.cn/ (Zhang *et al.* 2015). The phylogenetic tree was constructed using MEGA 5.0 software (www.megasoftware.net) with the maximum likelihood method (Tamura *et al.* 2011). Different colors represented the four HD-Zip subgroups.

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                          200                                                          *
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Gh_D12G2590     : ----------------------------------------------------------------------------------------------
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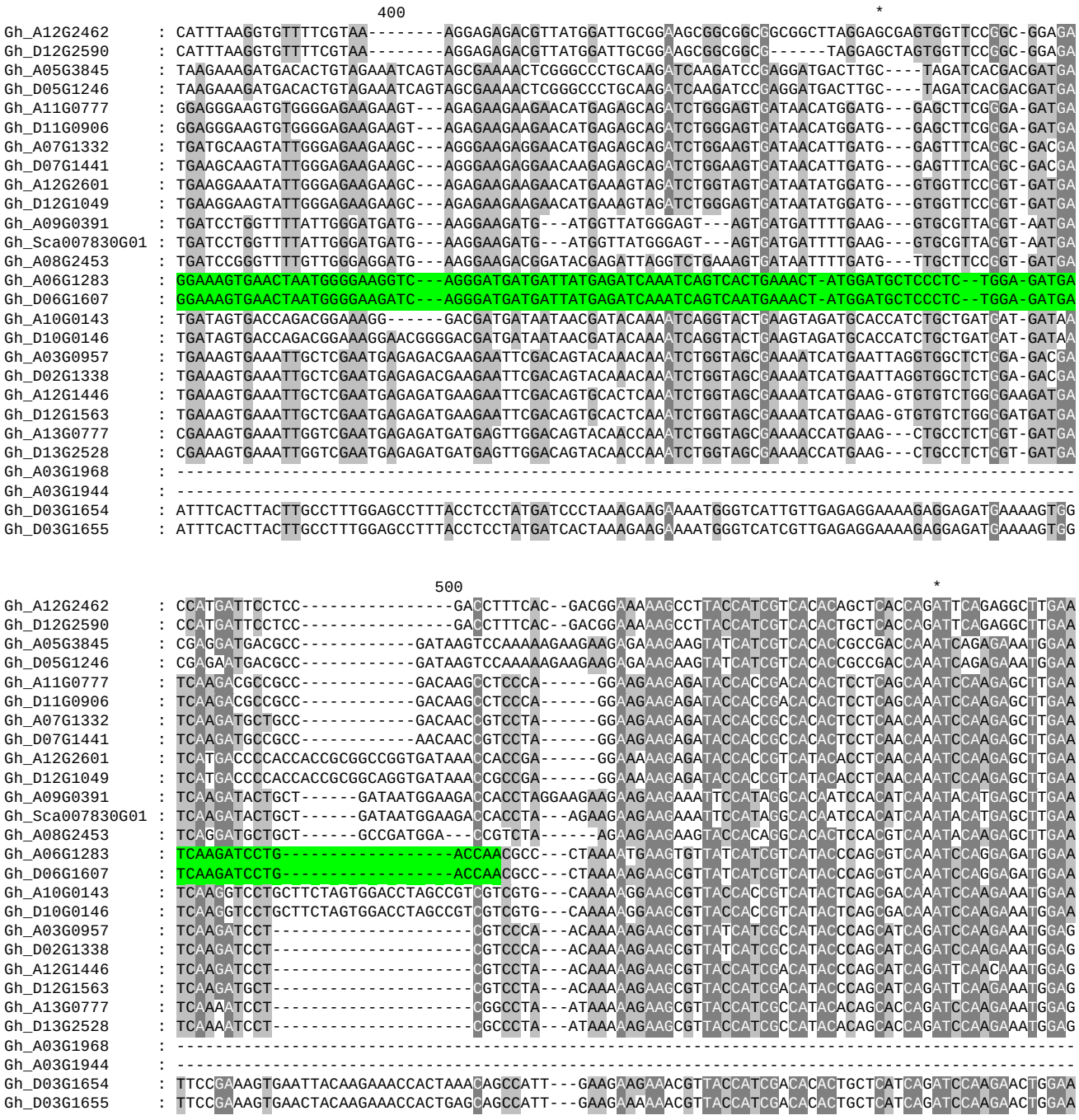
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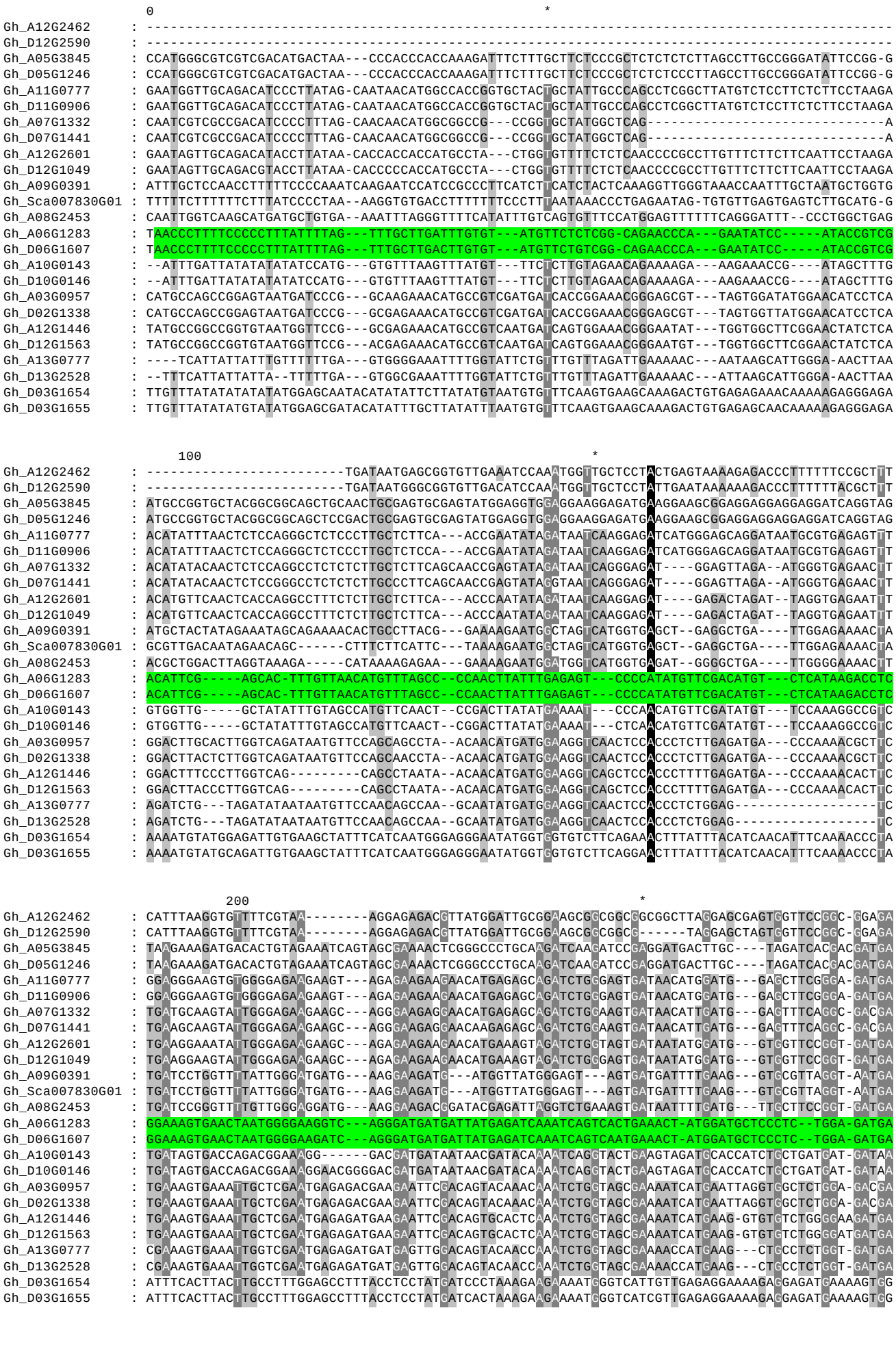

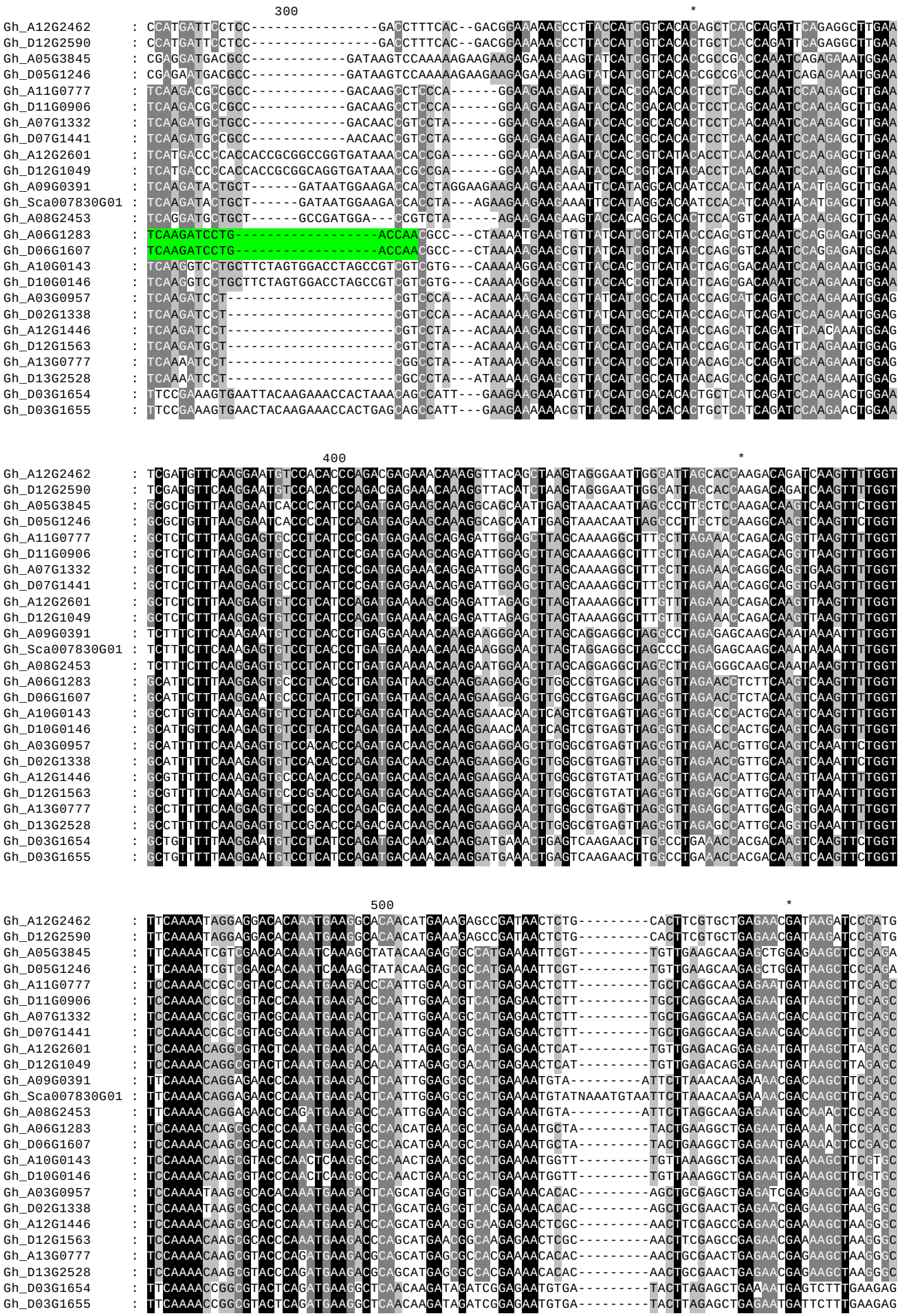

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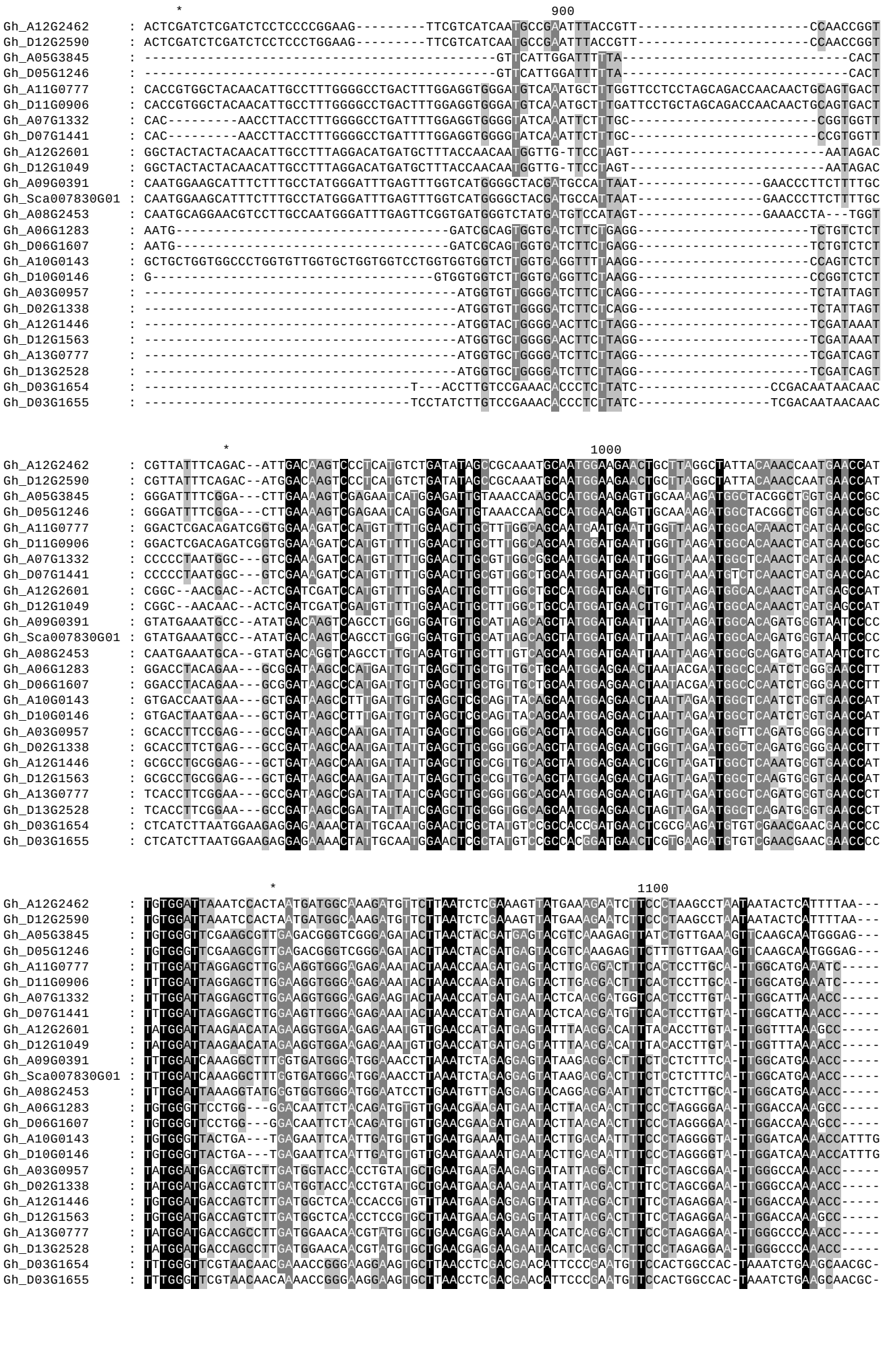

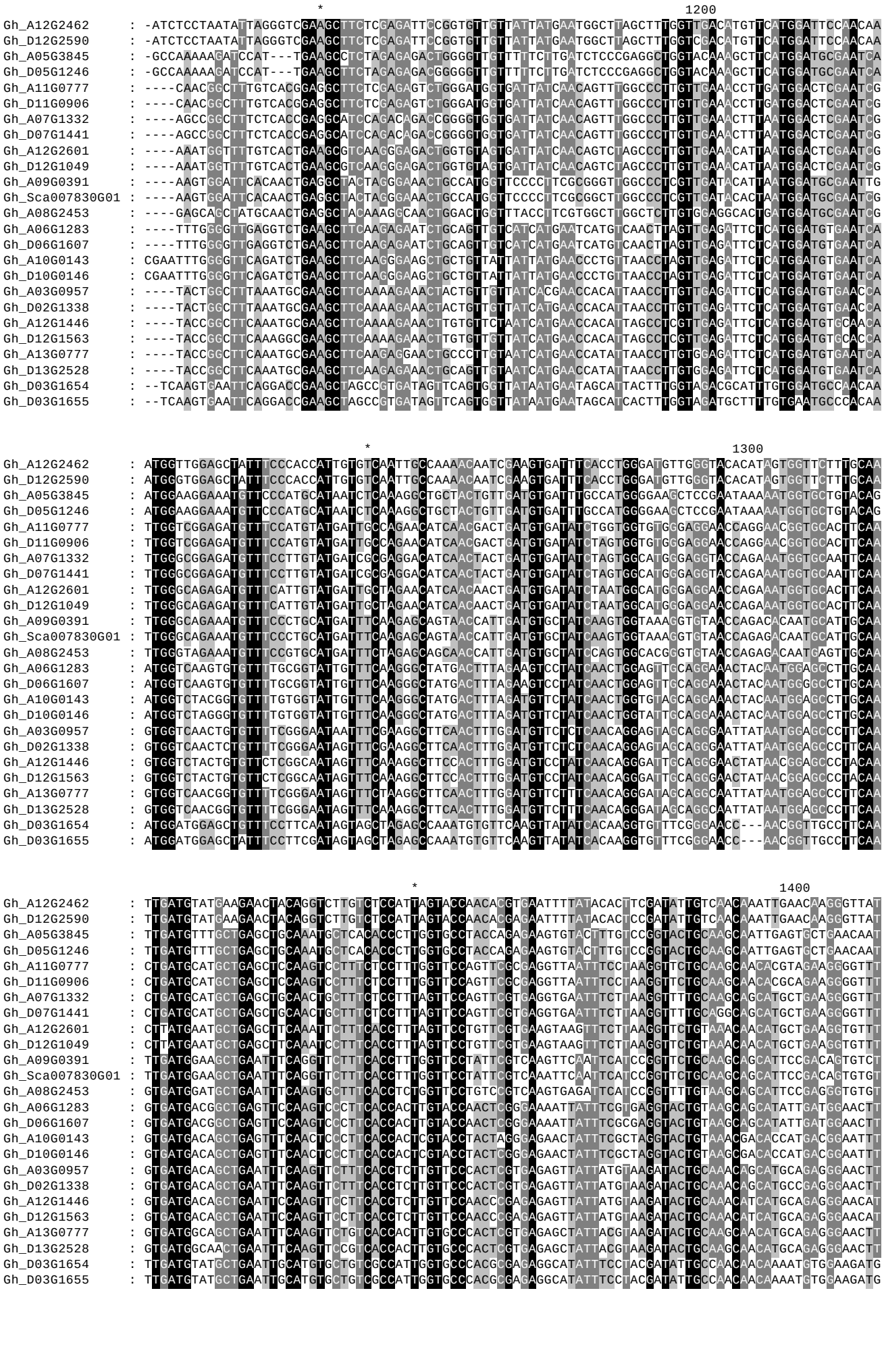
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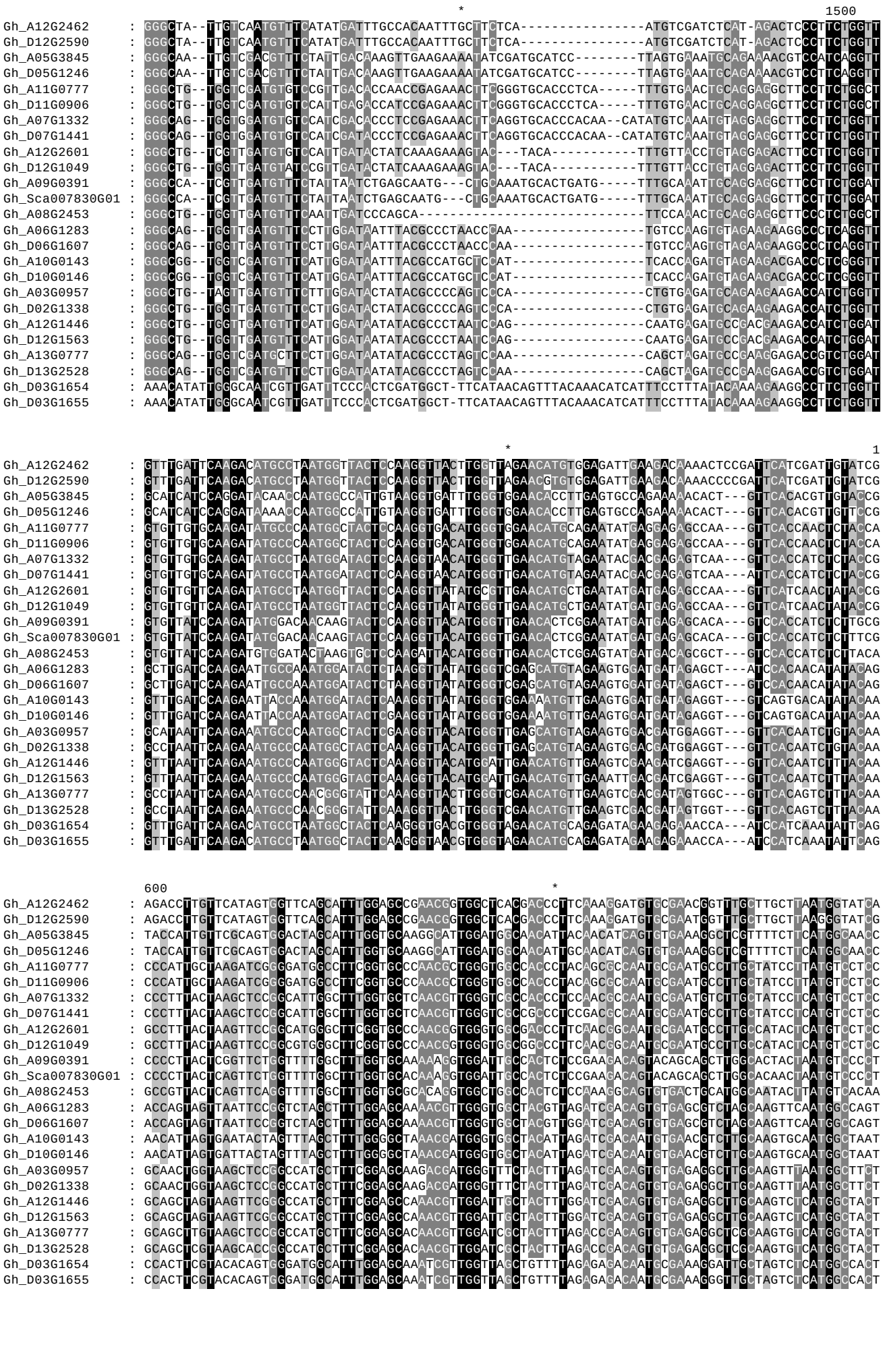
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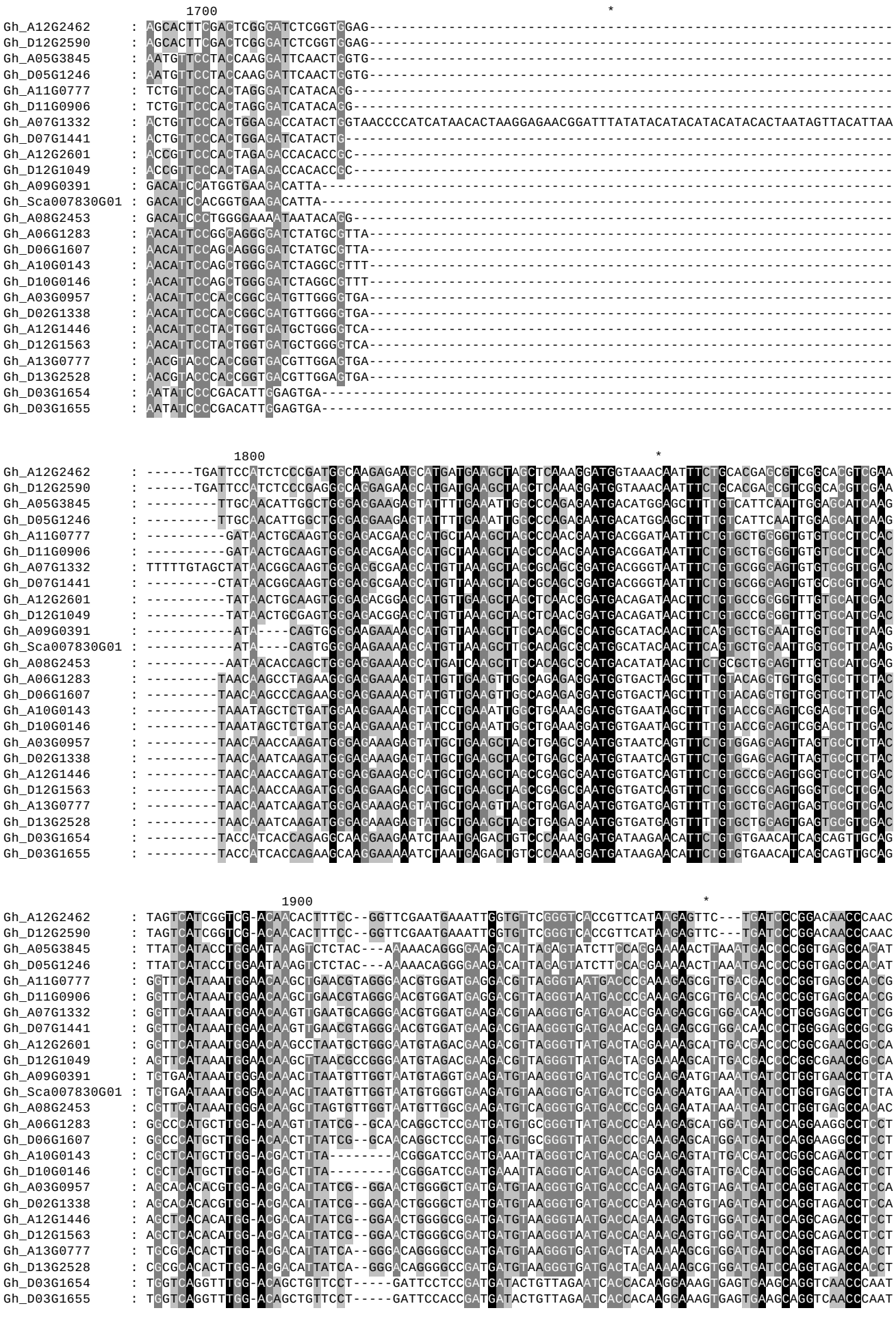
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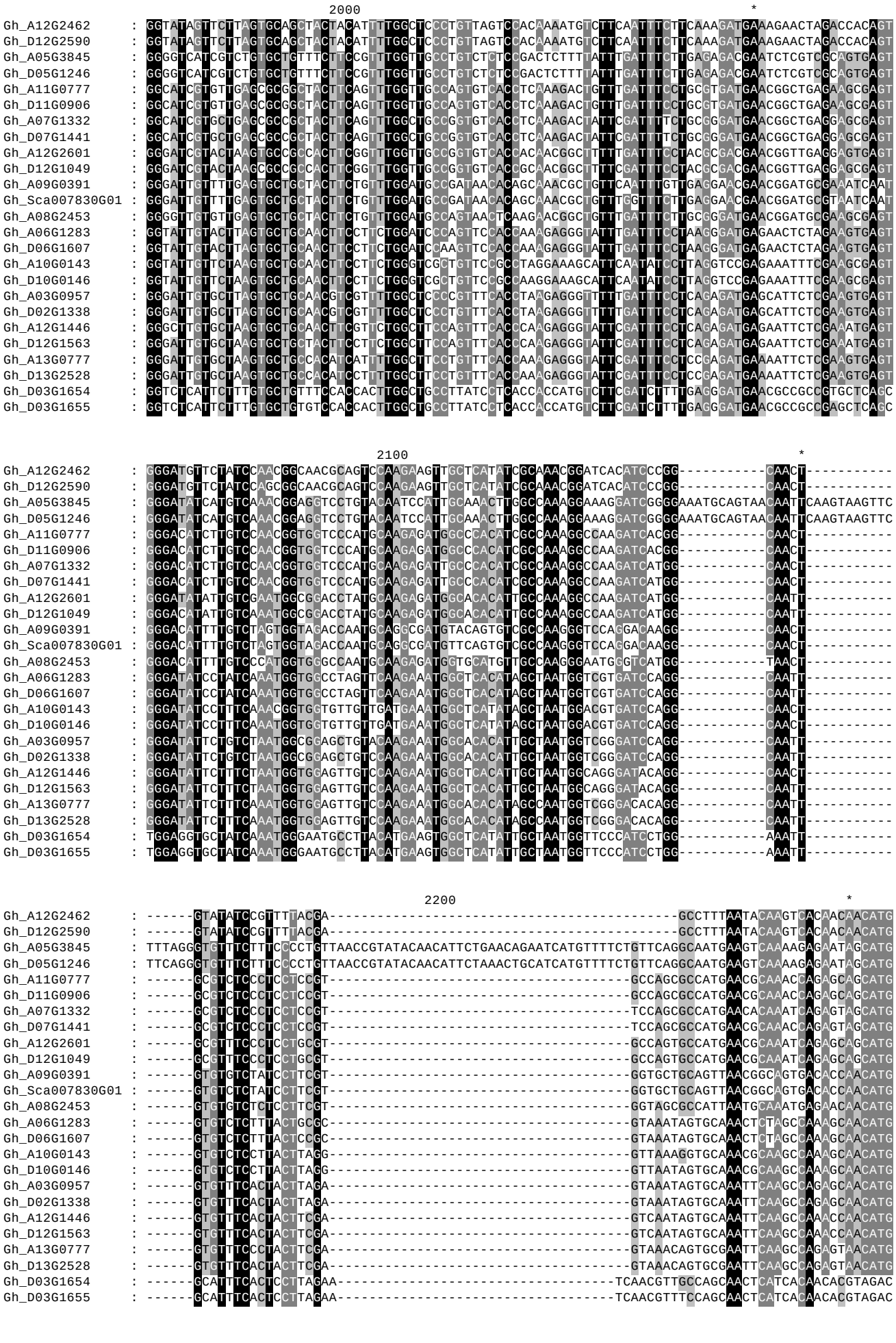

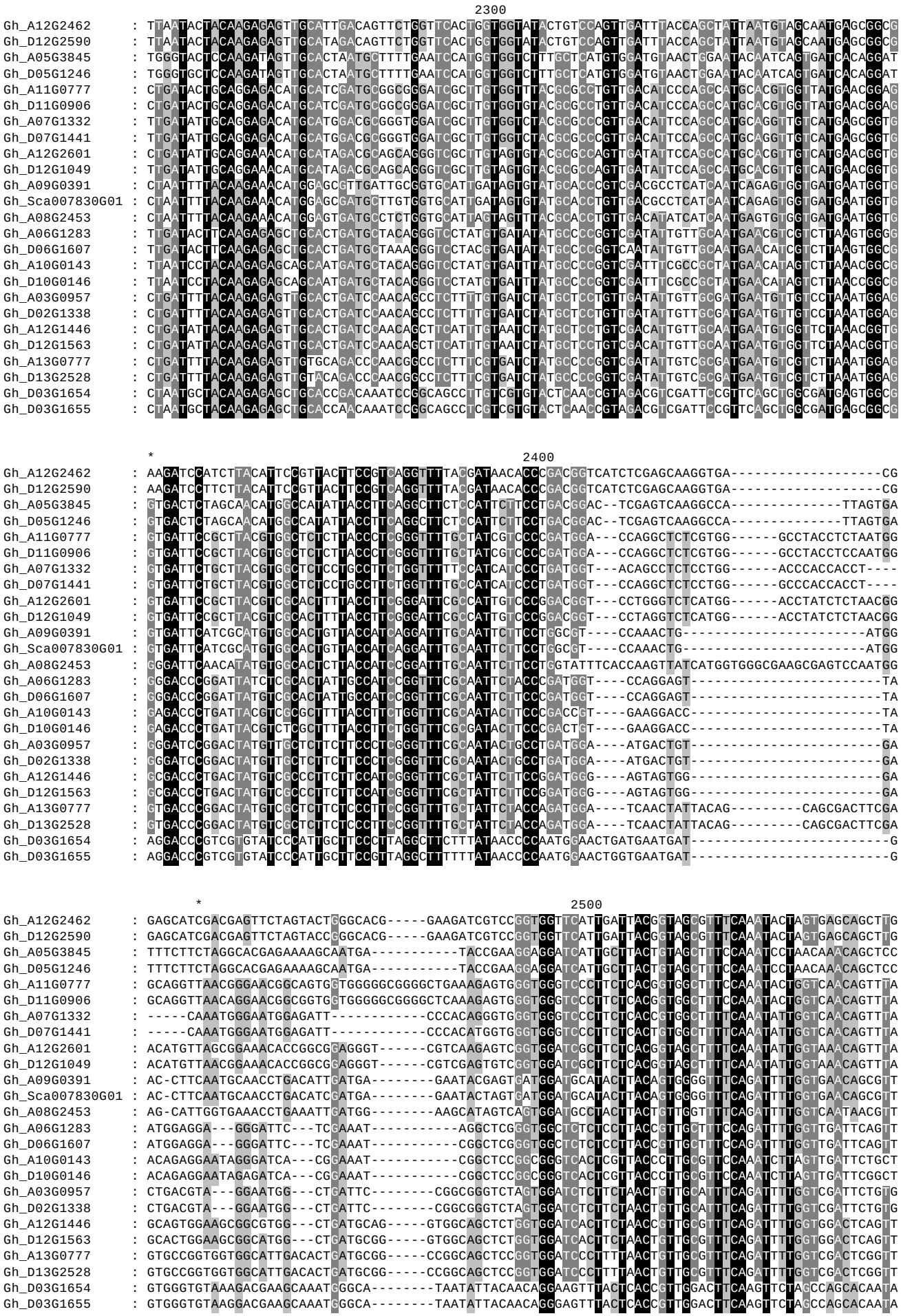
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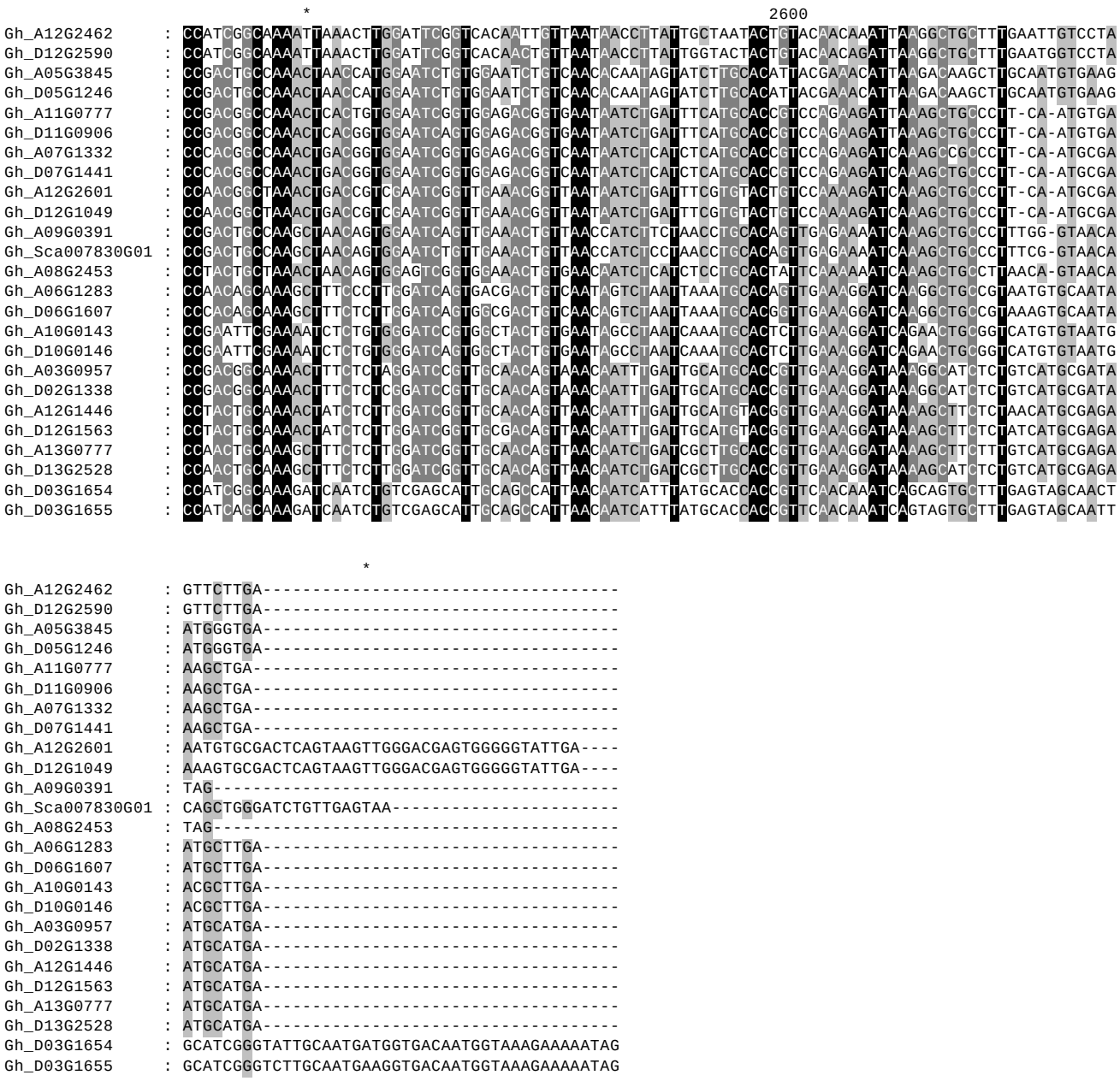
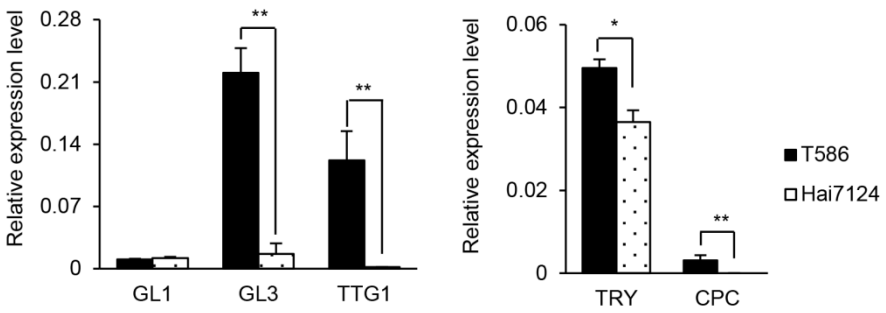


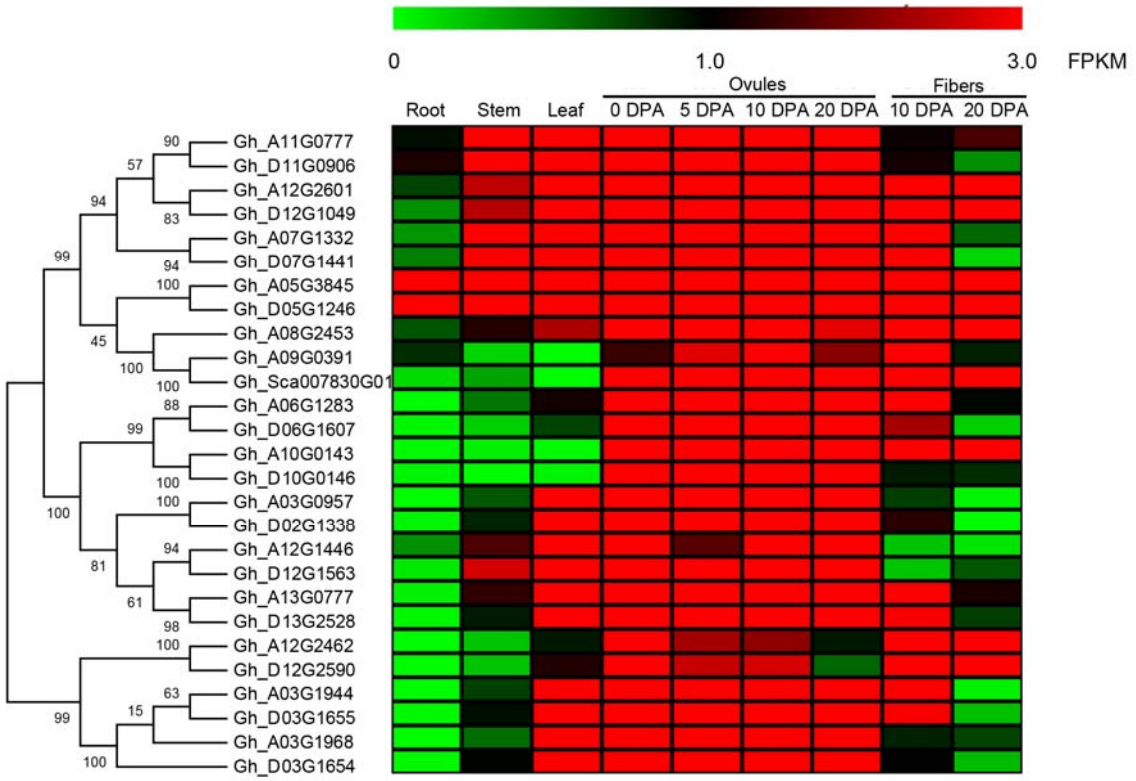
Appendix G Sequence alignments of all the HD-Zip Ⅳ genes in TM-1

A total of 261 bp (marked by green shadows) was selected for gene silencing (99 bp before the start codon “ATG” and 159 bp after the start codon “ATG”). The alignment was carried out with ClustalX 1.83 software (Higgins *et al.* 1996).



Appendix H Expression patterns of genes involved in trichome formation

The orthologous genes of Arabidopsis related to trichome formation were retrieved in cotton by the BLASTp method. The expression levels of the *Tubulin 1* gene (*AF487511.1*) were used as the endogenous control. The relative expression level was calculated using the $2^{-\triangle CT}$ method (Livak and Schmittgen. 2001) and the values±SD indicated three replicates. * and ** denoted significant differences at 5% and 1% levels, respectively.



Appendix I Expression patterns of HD-Zip Ⅳ genes in different tissues of TM-1

TM-1 RNA-Seq data were downloaded from http://mascotton.njau.edu.cn/ (Zhang *et al*. 2015). The fragments per kilobase of exon model per million mapped reads (FPKM) method with Cufflinks (http://cufflinks.cbcb.umd.edu/) was used to calculate expression levels. The tissues here included roots, stems, leaves and ovules from different days post anthesis (DPA) (0, 5, 10, and 20 DPA), and fiber tissues (10 and 20 DPA).