

Genome reorganization of the *GmSHMT* gene family in soybean showed a lack of functional redundancy in resistance to soybean cyst nematode

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GmSHMT02m
GmSHMT04n
GmSHMT05c
GmSHMT06n
GmSHMT08c
GmSHMT08n
GmSHMT08m
GmSHMT09m
GmSHMT12n
GmSHMT13ch
GmSHMT14n
GmSHMT15ch
GmSHMT18m

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10 20 30 40 50 60
MVDHDLQQETQKDSYISTLCSLLNHYL PFFHSPVLSVSLFPSSRQNAHFPI SHFPFLVQP
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GmSHMT02m
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GmSHMT05c
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GmSHMT09m
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GmSHMT18m

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.....
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SHPQSN LSLGFE SSHASP...PRRSDPPV PLQLMEPQTENGNLDVESDDDDKEVEEFR
.....MAMAMA LRRLES.....SIDKPLRP
.....MAMAMA LGRLES.....EFNKPLRP
SHPQSN LSLGFE SSHASP...PRRSDPPV PLQLMEPQTENGNLDVESDDDED-KEVEEFR
.....MQACTTME SLQQQP...IWTKGLNFPKGYGSNNGFL.....PQ
.....MAMALA LRRLN.....KPFPS
.....MAMAMA LRRLES.....SIDKPLRP

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GmSHMT05c
GmSHMT06n
GmSHMT08c
GmSHMT08n
GmSHMT08m
GmSHMT09m
GmSHMT12n
GmSHMT13ch
GmSHMT14n
GmSHMT15ch
GmSHMT18m

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.....SATSIYHRMES.....ELSAQEKDKSRA...DWIKQLNDPLETIDPEIADIE
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.....MDQRVK R KIEGHQQQF-OFPE SKRVA VDSVVG.....WVWG PLGLG VADPEIFDI MQ
.....SLLAFRSBLTLIHSEFP...P-FMDPVSV.....WVWG PLGLG VADPEI HDLIE
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.....LFNAGSEL YKSSLP...DEAVYDKERP GV...TWP KQLNAPLEVV DPEIADIE
.....LFNAGSEL YKSSLP...AEAA YONEK...SCDTE LNA PLLEVV DPEIADIE
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VRFONI KPSKASHVEASLV TGKPSV PFSVPEIIG...DGSSFLDYGLSEADPDVRAID
.....NATSIY-RQES...ELSAHEKHKSR A...DWIKQLNDP LEAIDPEIADIE
.....LFNAGSEL YKSSLP...DEAVYDKERP GV...TWP KQLNASLEVV DPEIADIE

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GmSHMT04n
GmSHMT05c
GmSHMT06n
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GmSHMT08n
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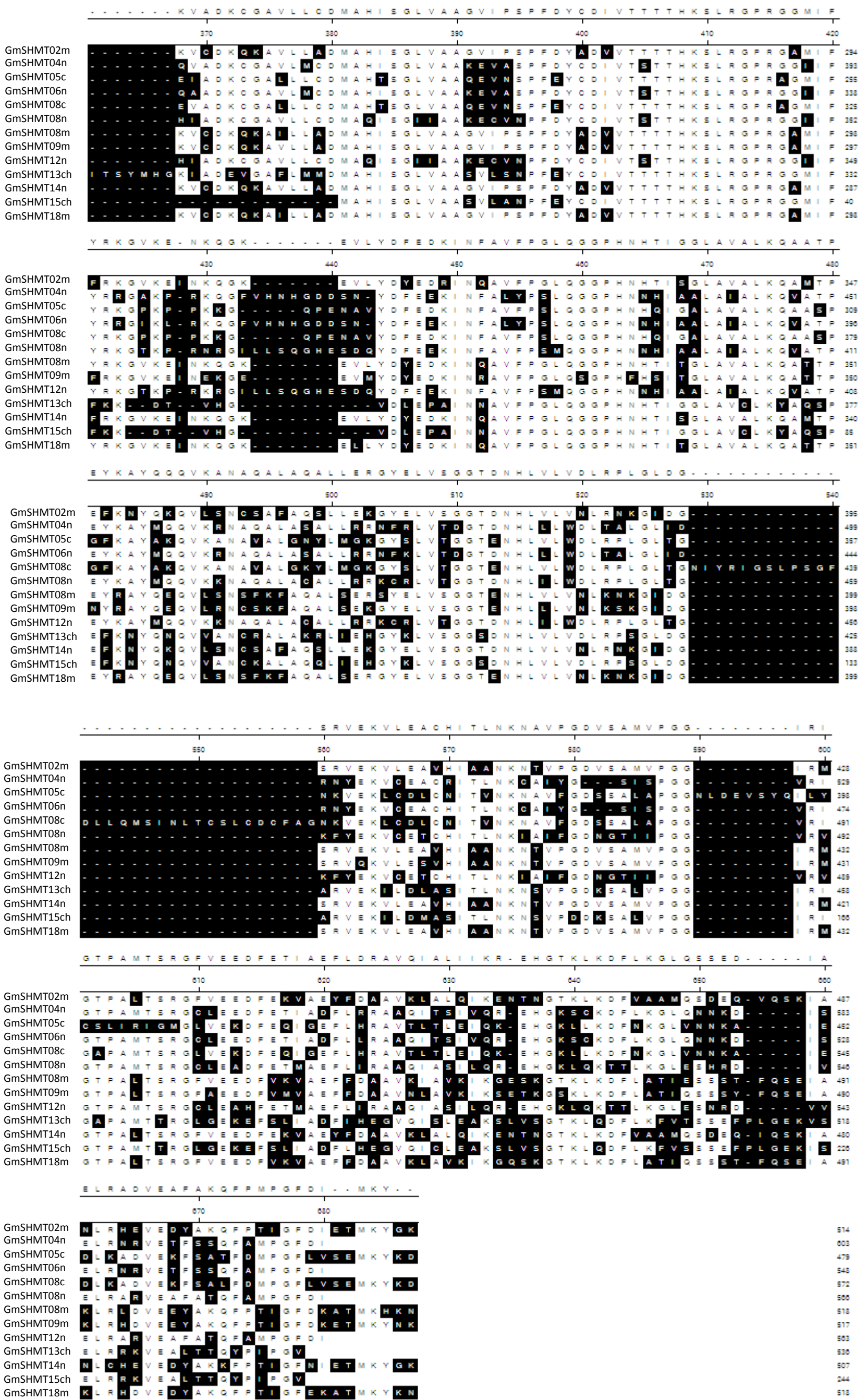
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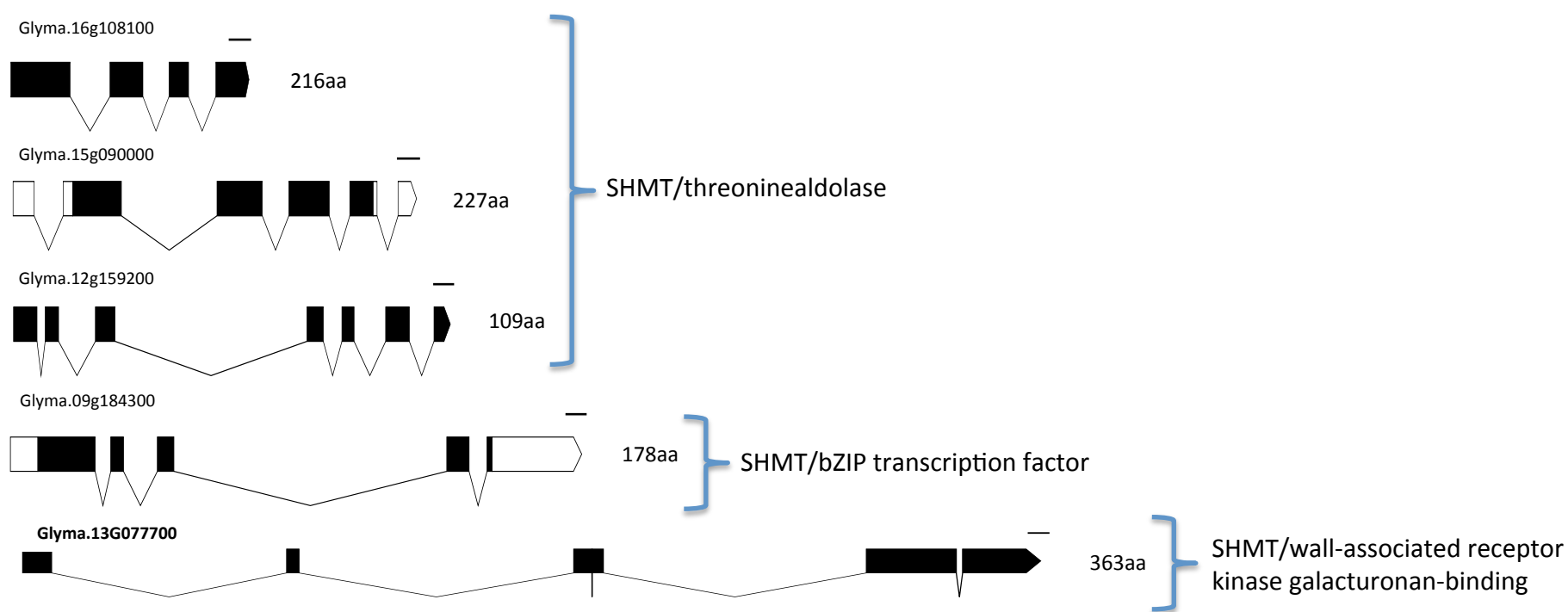
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GmSHMT04n
GmSHMT05c
GmSHMT06n
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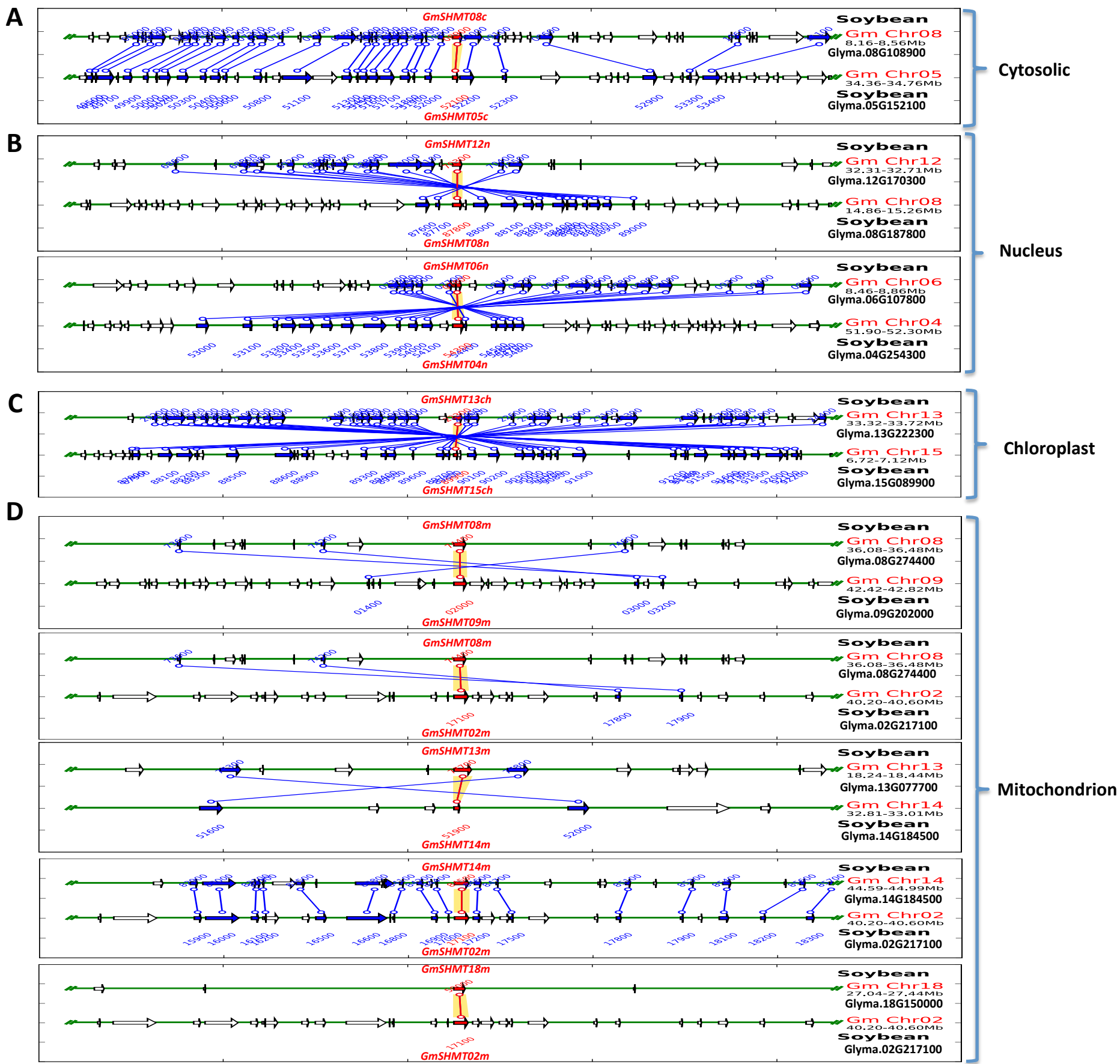
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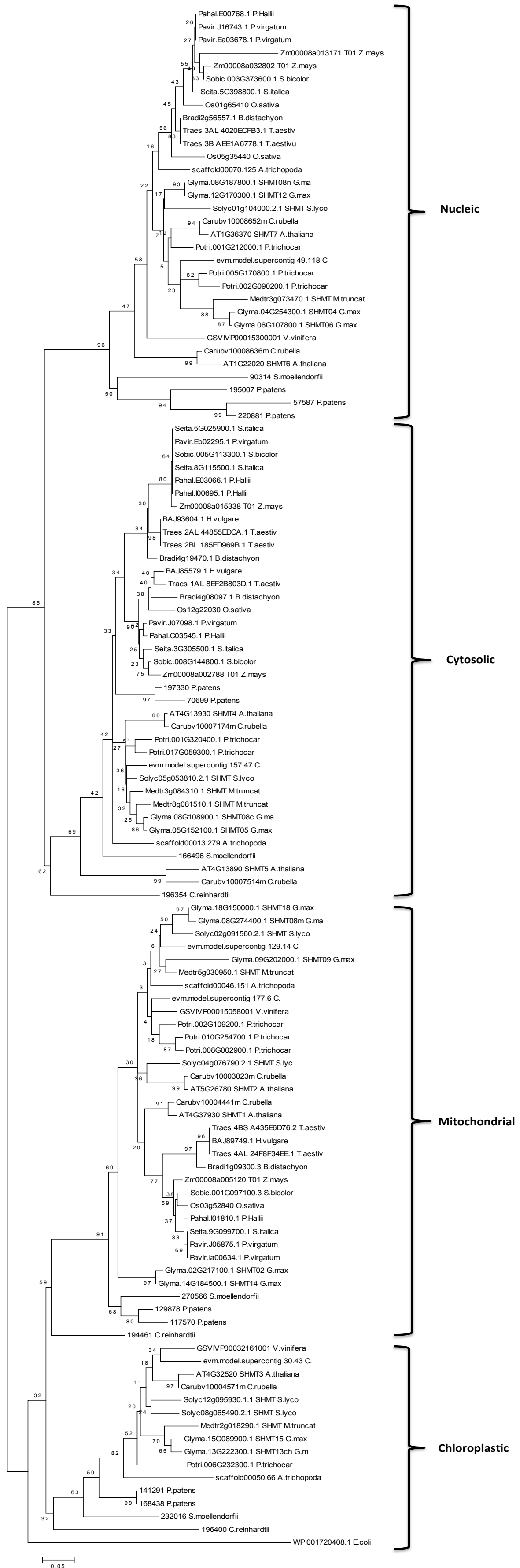
Supplementary Figure S1. Comparative analysis of GmSHMT proteins in soybean. Amino acid alignment for the predicted GmSHMT protein members in soybean. *In silico* analysis was performed using the MegAlign (DNASTAR Lasergene 8) software package and the Clustal W algorithm. All parameter values correspond to default definitions.



Supplementary Figure S2. Bifunctional GmSHMT genomic DNA structure in soybean. Black boxes represent exons, lines represent introns. White boxes represent 5' UTR and 3' UTR untranslated regions. Five GmSHMTs with multiple-bifunctional activities including a SHMT/Threoninealdolase, SHMT/Receptor Kinase, SHMT/transcription factor activity all encoding short proteins. It is presumed that they have diverged in their structure and function. Graphs were made using the Exon-Intron Graphic Maker Version 4, <http://wormweb.org/exonintron>. Scale represent 100 bp.

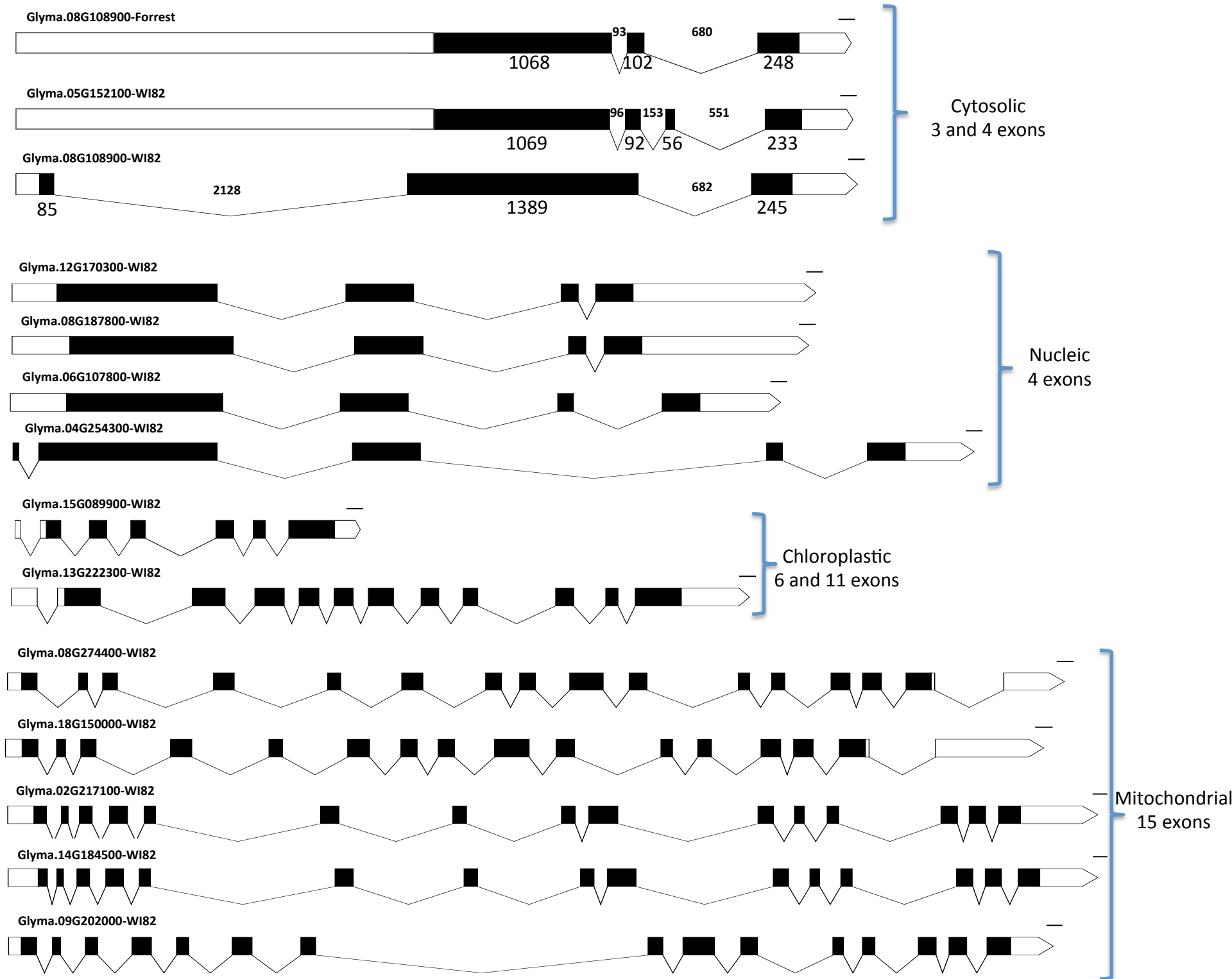


Supplementary Figure S3. Schematic representation of *GmSHMT* containing duplicated segments identified in the soybean genome. Soybean *SHMT* intragenome syntenic relationships were calculated using the Plant Genome Duplication Database. (A) Cytosolic *GmSHMTs* between chr08/chr05 belongs to a very large recent duplicated segment containing 551 additional conserved duplicated genes or anchors. (B) Nucleic *GmSHMTs* between chr12/chr08 and chr06/chr04 belong to another recent inverted duplicated segment containing 14 and 711 duplicated genes or anchors, respectively. (C) Chloroplastic *GmSHMTs* between chr13 and chr15 belongs to a fourth recent inverted duplicated segment containing 391 duplicated genes or anchors. (D) Mitochondrial *GmSHMTs* between chr08/chr09, chr08/chr02, and chr18/chr02 belongs to old duplicated segments containing 24, 10, and 7 duplicated genes or anchors. Graphs represent +/- 500 kb duplicated region centred in the *GmSHMT* genes.

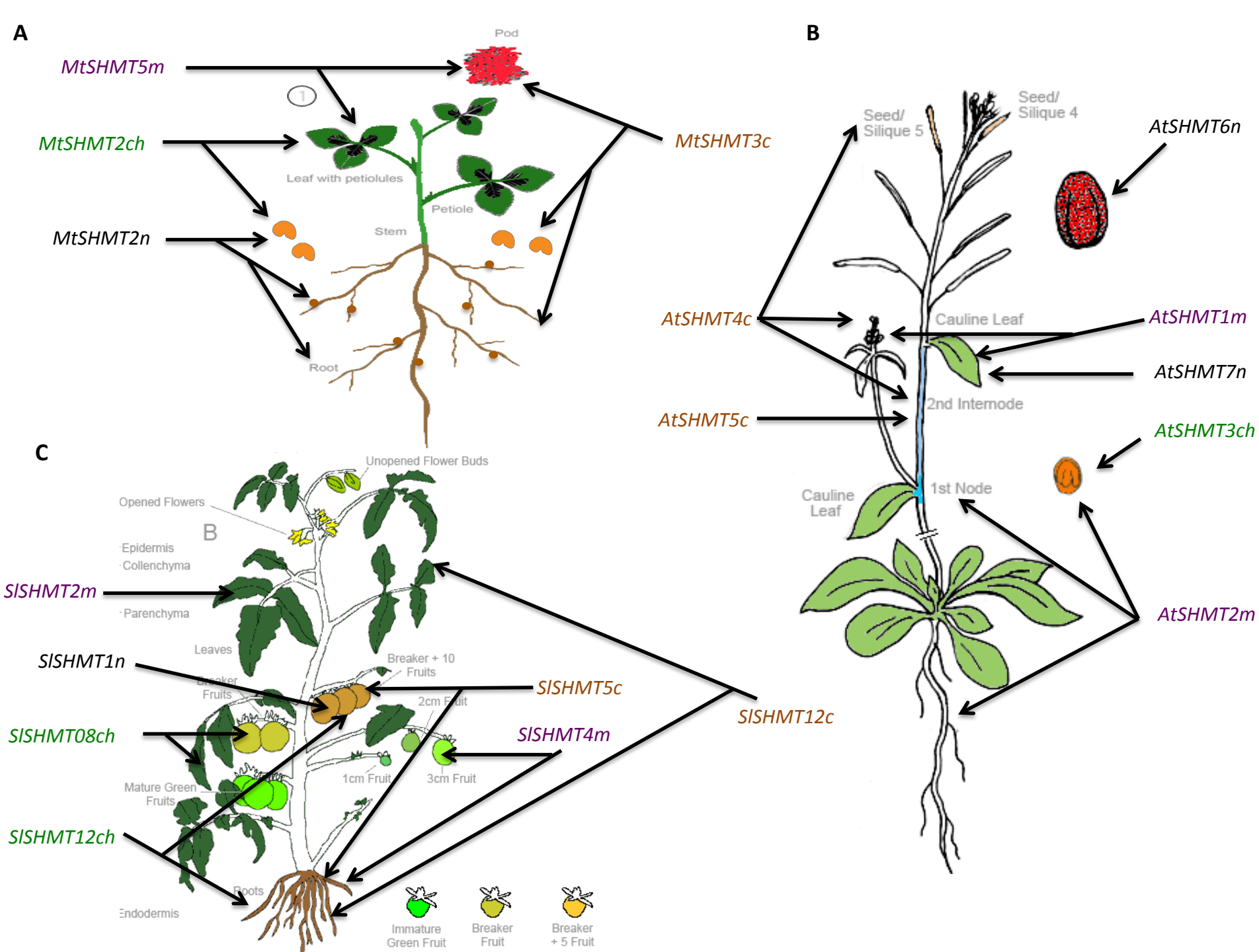


0.05

Supplementary Figure S4. Phylogenetic tree of SHMT from 22 plants species showing absence of chloroplastic-targeted SHMTs in monocots. All SHMT proteins identified in five model plants; *C. reinhardtii* (algae), *P. patens* (moss), *S. moellendorffii* (lycophyte), *O. sativa* (monocot), and *A. thaliana* (eudicot), in addition to *G. max* (soybean), in addition to other monocots and eudicots cytosolic, nucleic, chloroplastic, and mitochondrial-targeted SHMTs were included in the analysis. Extensive searches employing a considerable number of monocots including the plant model *O. sativa*, in addition to other monocots like *Z. mays*, *S. bicolor*, *S. italica*, *H. vulgare*, *P. hallii*, *P. virgatum*, and *T. aestivum* failed to identify any GmSHMT member within the chloroplastic clade. In addition, a remarkable decrease of chloroplastic-targeted GmSHMTs from all eudicots analysed was observed.

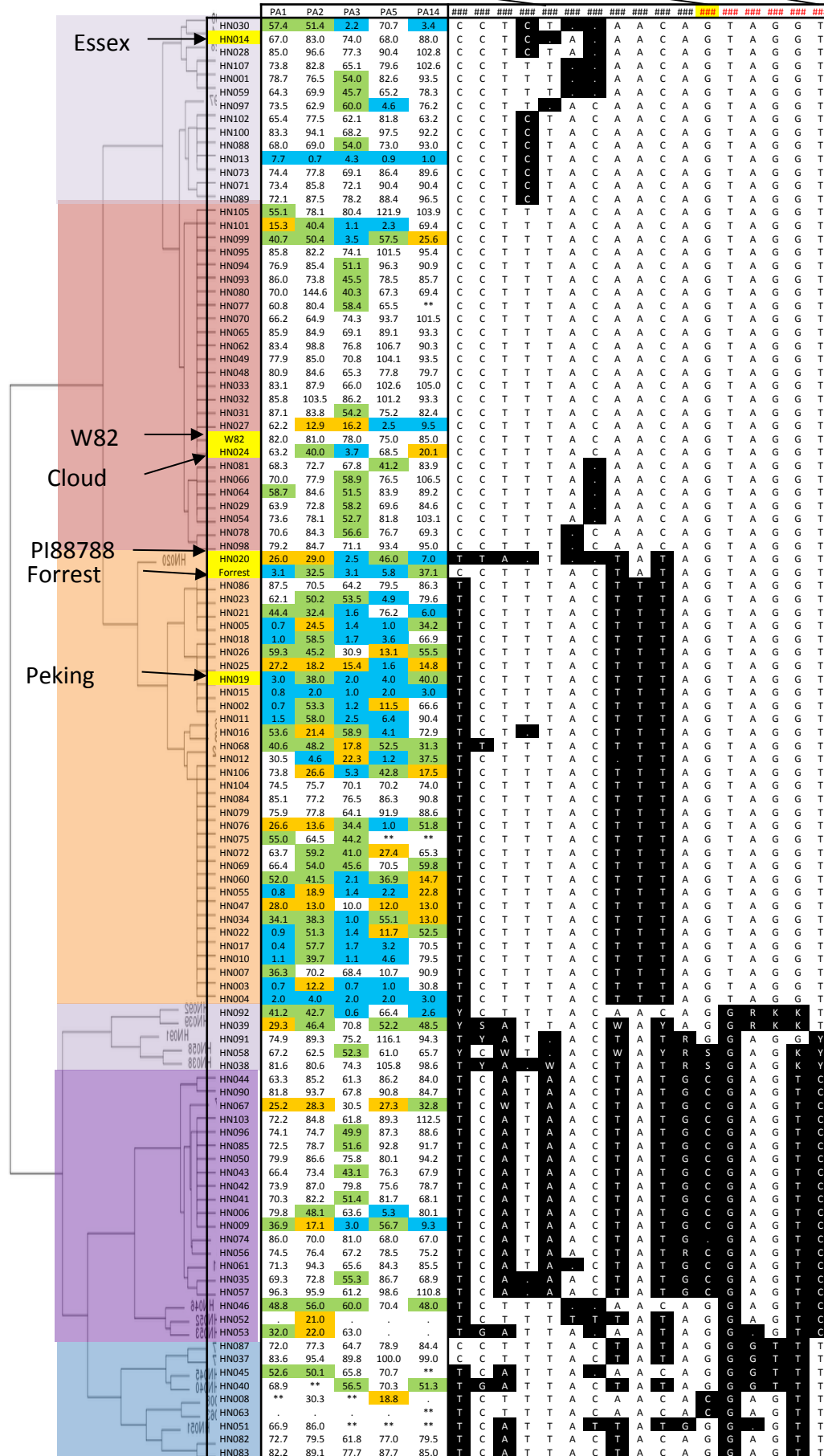
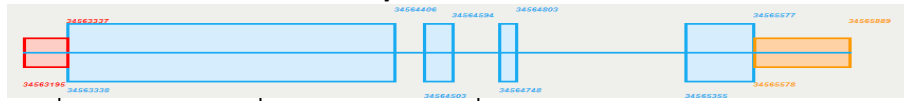


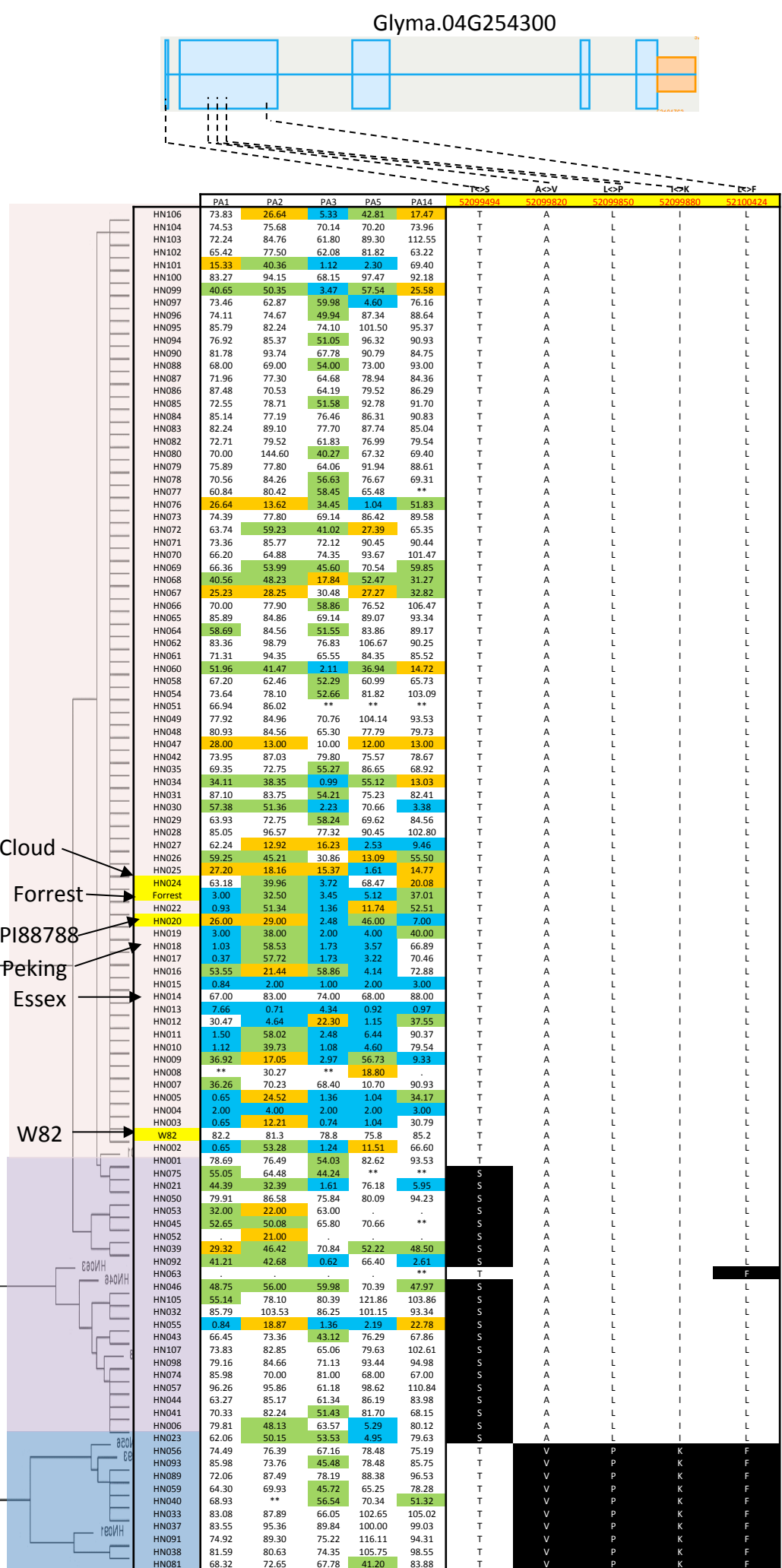
Supplementary Figure S5. *GmSHMT* genomic DNA structure in soybean. Black boxes represent exons, lines represent introns. White boxes represent 5' UTR and 3' UTR untranslated regions. Nucleic *GmSHMT*s has 4 Exons, mitochondrial *GmSHMT*s has 15 exons. Chloroplasmic *GmSHMT13* has 11 exons, however the truncated *GmSHMT15* contains 6 exons only. Cytosolic *GmSHMT*s present different numbers of exons, whereas the *GmSHMT05* represent 4 exons, the *GmSHMT08* contained 3 exons only. Graphs were made using the Exon-Intron Graphic Maker Version 4, <http://wormweb.org/exonintron>. Scale represent 100 bp.



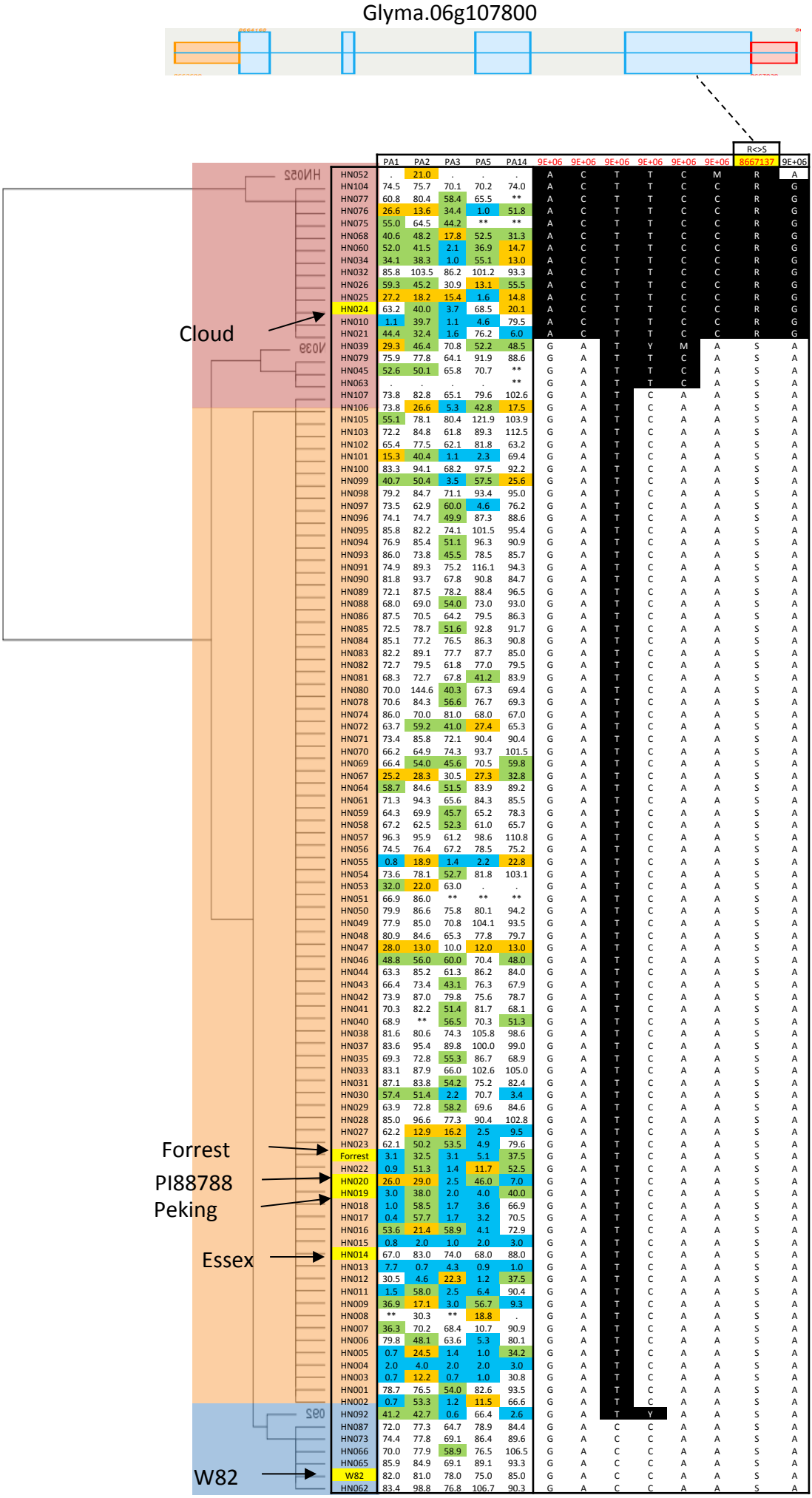
Supplementary Figure S6. Expression pattern of *Medicago truncatula* (A), *Arabidopsis thaliana* (B), and *Solanum lycopersicum* (C) SHMT gene members *in planta*, compiled from the public bio-analytic resource for plant biology database (<http://bar.utoronto.ca>) (Winter et al. 2007).

Glyma.05G152100.1

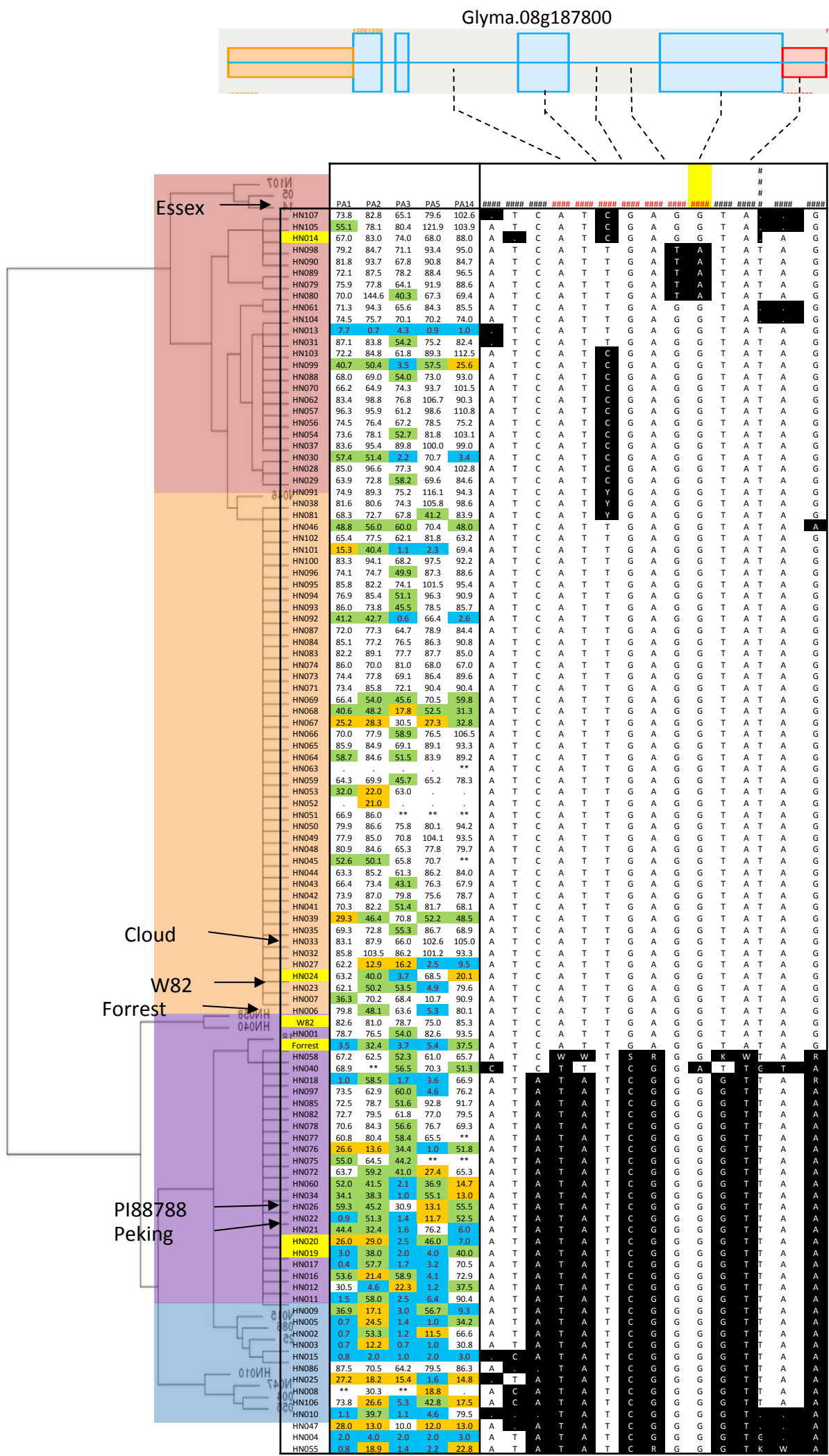


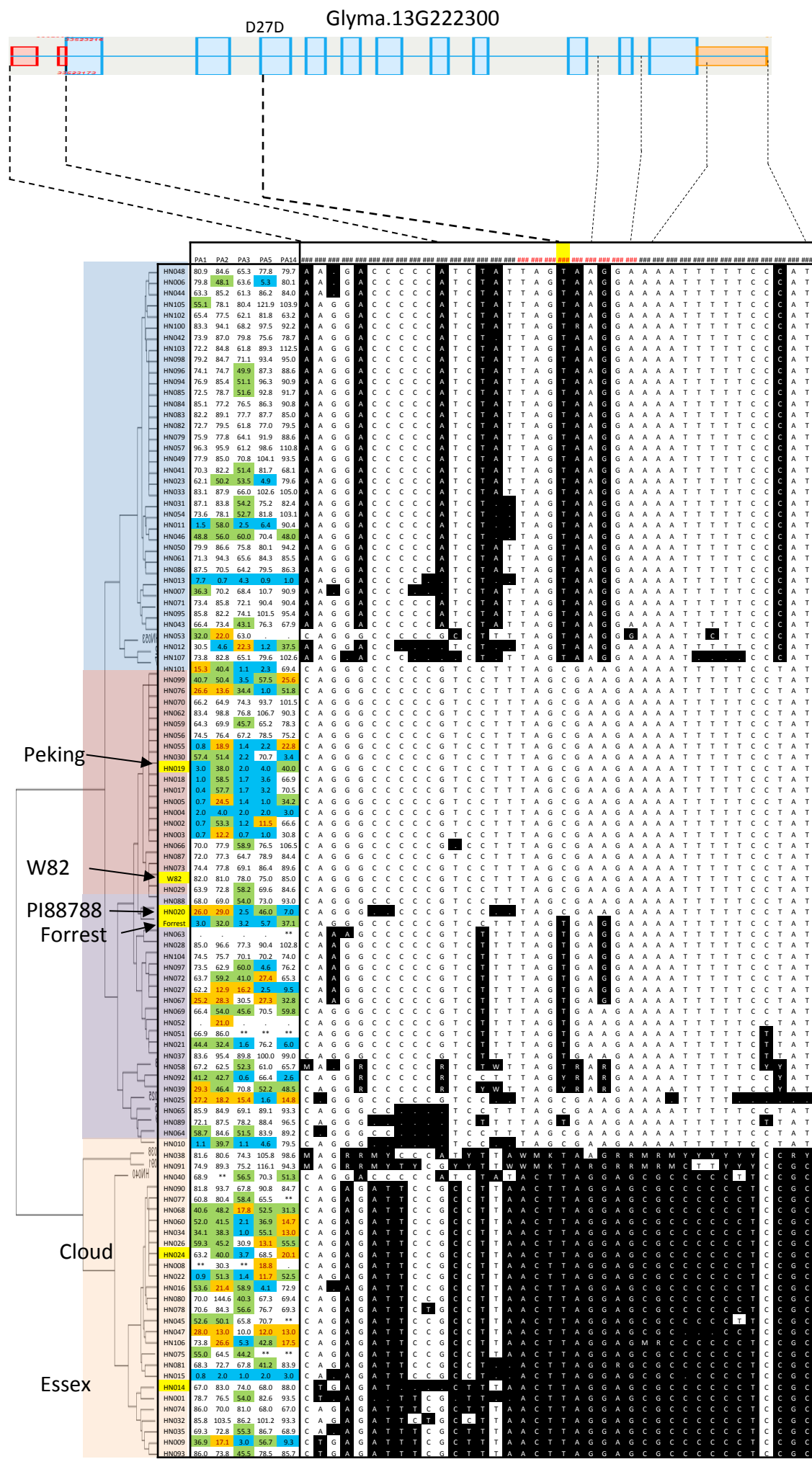


Supplementary Figure S8. Haplotype clustering and correlation with soybean cyst nematode female index of the nucleic-localized *GmSHMT04n* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.04g254300* (*GmSHMT04n*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).

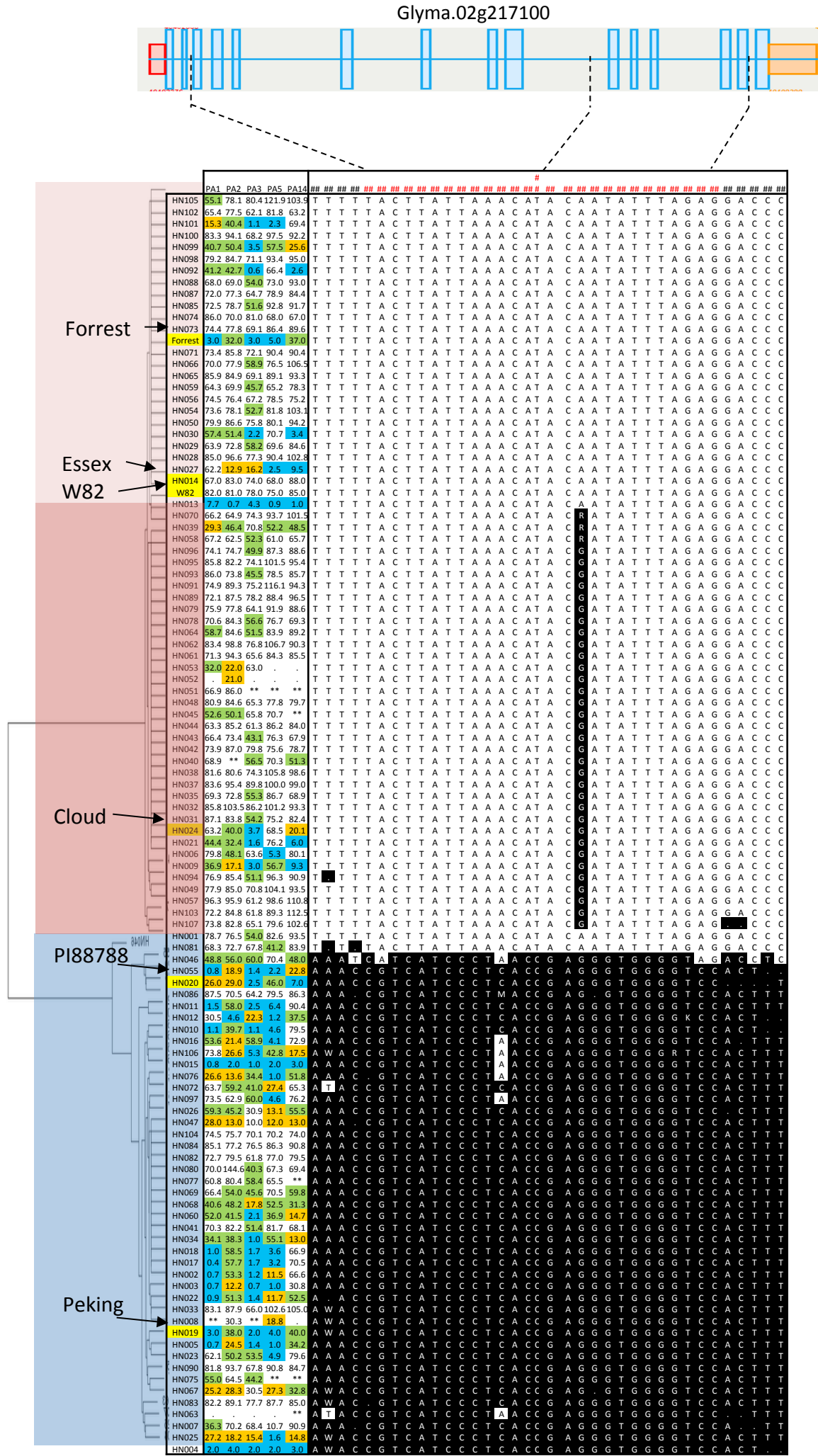


Supplementary Figure S9. Haplotype clustering and correlation with soybean cyst nematode female index of the nucleic-localized *GmSHMT06n* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.06g107800* (*GmSHMT06n*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).

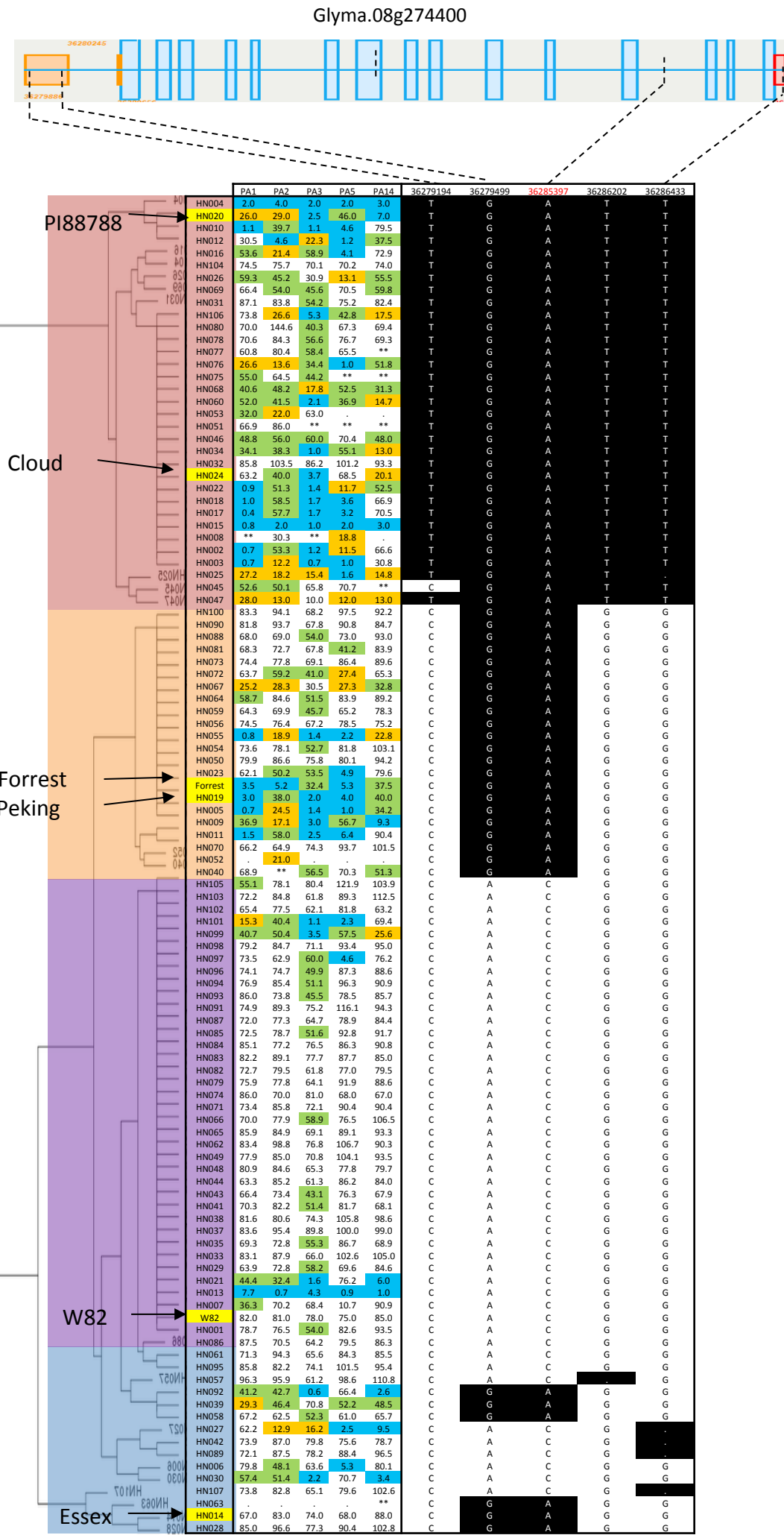




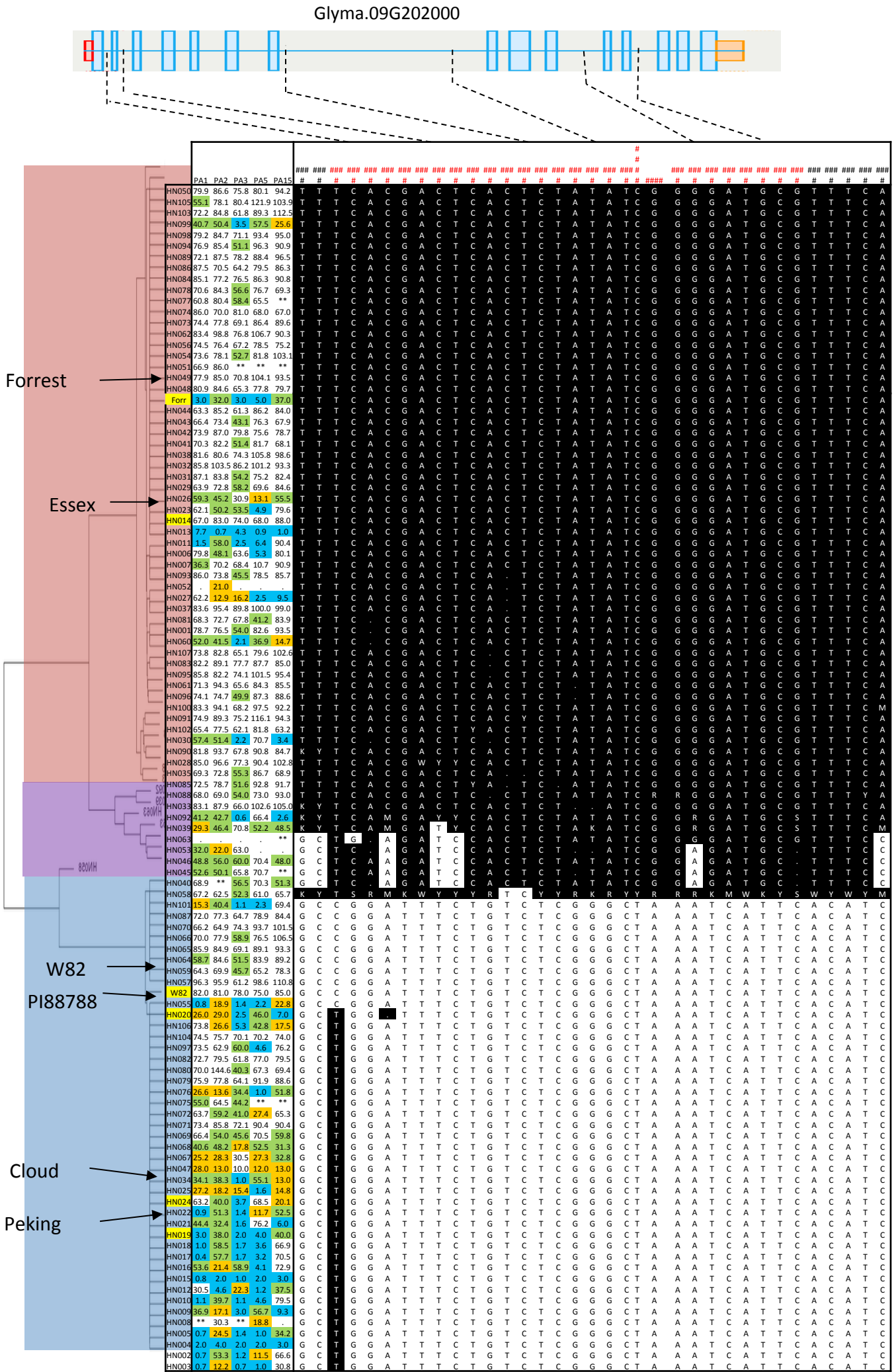
Supplementary Figure S12. Haplotype clustering and correlation with soybean cyst nematode female index of the chloroplastic-localized *GmSHMT13ch* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.13g222300* (*GmSHMT13ch*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).



Supplementary Figure S13. Haplotype clustering and correlation with soybean cyst nematode female index of the mitochondrial-localized *GmSHMT02m* in the 106 soybean lines. The 106 soybean lines included non-domesticated, semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.02g217100* (*GmSHMT02m*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).

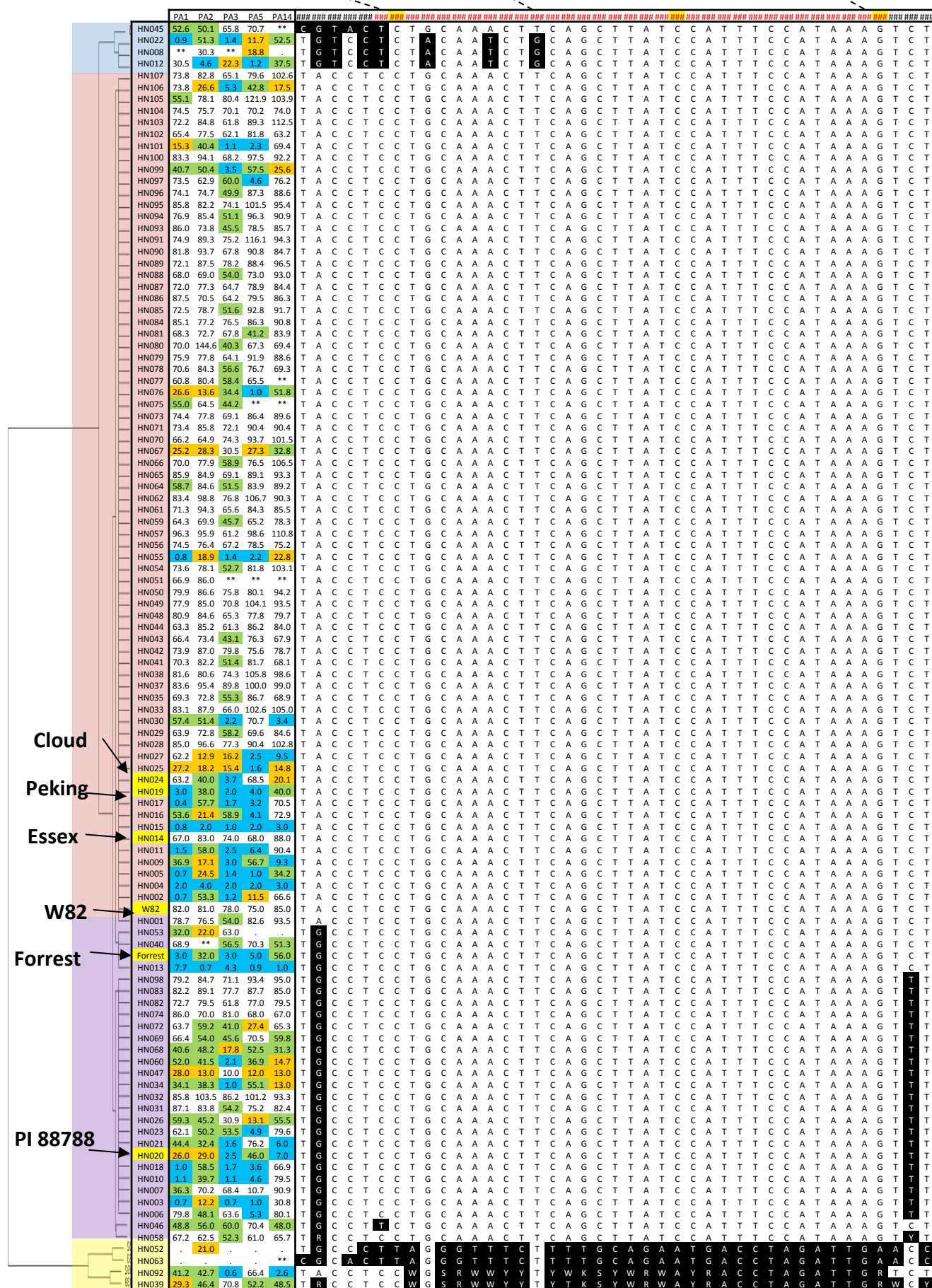
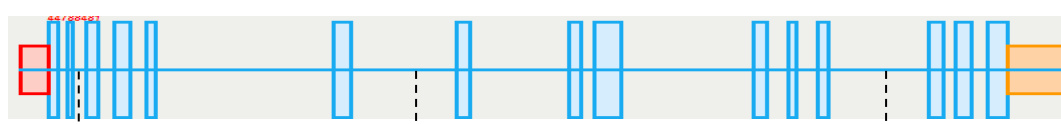


Supplementary Figure S14. Haplotype clustering and correlation with soybean cyst nematode female index of the mitochondrial-localized *GmSHMT08m* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.08g274400* (*GmSHMT08m*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).



Supplementary Figure S15. Haplotype clustering and correlation with soybean cyst nematode female index of the mitochondrial-localized *GmSHMT09m* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.09g217100* (*GmSHMT09m*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).

Glyma.14g184500



Supplementary Figure S17. Haplotype clustering and correlation with soybean cyst nematode female index of the mitochondrial-localized *GmSHMT14m* in the 106 soybean lines. The 106 soybean lines included non-domesticated; semi-domesticated, and elite domesticated introductions belonging to the USDA soybean collection. Schematic graphs shows the position of SNP/indel for *Glyma.14g184500* (*GmSHMT14m*) gene. SNP in black background were specific to Peking-type of resistance, and clustered with soybean lines carrying resistance to three SCN Hg-types; 0, 2.7, and 2.5.7. Blue box represents exon, blue bar represents intron, orange box represents promoter region, grey box represents 3' or 5' UTR. SNPs were positioned relative to the genomic position in W82. SNP position in red text showing non-synonymous SNPs leading to amino acid change. Female Index in blue (FI<10, resistant to SCN), in green (10<FI<30, Moderate resistance), in orange (30<FI<60, Moderate susceptibility), in white (60<FI, Susceptible to SCN).

Supplemental Table S1. *GmSHMTs* intragenome syntenic relationship calculations showing independent duplicate blocks containing the genomic pairs of *GmSHMT* family members with their corresponding number of conserved genes or anchors. Soybean genome duplicated chromosomal segments containing *GmSHMTs* were calculated using the Plant Genome Duplication.

Gene A	Chromosome	Length (AA)	Locus position (Mb)	Gene B	Chromosome	Length (AA)	Locus position (Mb)	Score	E-Value	Block	Ka	Ks	Ka/Ks	Conserved Anchors
GmSHMT06n	Ch06	584	8.46-8.86	GmSHMT04n	Ch04	603	51.90-52.30	28247.0	7e-172	huge	0.02	0.17	0.11	711
GmSHMT08c	Ch08	471	8.16-8.56	GmSHMT05c	Ch05	479	34.36-34.76	21948.0	2e-135	huge	0.02	0.20	0.1	551
GmSHMT13ch	Ch13	536	33.32-33.72	GmSHMT15ch	Ch15	244	6.72-7.12	15580	3e-37	large	0.02	0.12	0.16	391
GmSHMT02m	Ch02	514	40.20-40.60	GmSHMT14m	Ch14	507	44.59-44.99	9852.0	2e-158	huge	0.02	0.10	0.16	248
GmSHMT08m	Ch08	518	36.08-36.48	GmSHMT09m	Ch09	517	42.42-42.82	921.0	0.0	large	0.07	0.53	0.13	24
GmSHMT08n	Ch08	566	14.86-15.26	GmSHMT12n	Ch12	563	32.31-32.71	558.0	0.0	large	0.01	0.16	0.06	14
GmSHMT08m	Ch08	518	36.08-36.48	GmSHMT02m	Ch02	514	40.20-40.60	361.0	1e-52	Small	0.11	1.64	0.06	10
GmSHMT02m	Ch02	514	40.20-40.60	GmSHMT18m	Ch18	518	27.04-27.44	226.0	3e-137	Small	0.10	1.88	0.05	7
GmSHMT13m	Ch13	363	18.24-18.44	GmSHMT14m	Ch14	507	32.81-33.01	929.0	0.0	large	0.16	0.29	0.55	24

Supplementary Table S2. Number of *SHMT* genes identified in select algae, moss, lycophyte, basal angiosperms, monocotyledonous, and dicotyledonous species available in *Phytozome* (www.phytozome.net). Number of Exons in mitochondrial, chloroplastic, nucleic, and cytosolic targeted SHMTs from all analyzed species is shown.

Species	Number of SHMTs	Number of Exons			
		Mitochondrion	Chloroplast	Nucleic	Cytosol
<u>Green Algae</u>					
<i>Chlamydomonas reinhardtii</i>	3	16	12		8
<u>Moss</u>					
<i>Physcomitrella patens</i>	14	14	11	4	2
<u>Lycophyte</u>					
<i>Selaginella moellendorffii</i>	4	15	10	4	4
<u>Basal Angiosperm</u>					
<i>Amborella trichopoda</i>	8	15	7	4	4
<u>Monocotyledons</u>					
<i>Hordeum vulgare</i>	3	15	–	4	4
<i>Brachypodium distachyon</i>	4	15	–	4	4
<i>Sorghum bicolor</i>	4	15	–	4	4
<i>Oryza sativa</i>	5	15	–	4	4
<i>Setaria italica</i>	5	15	–	4	4
<i>Panicum hallii</i>	5	15	–	4	4
<i>Panicum virgatum</i>	8	15	–	4	4
<i>Triticum aestivum</i>	13	15	–	4	4
<i>Zea mays</i>	13	15	–	4	4
<u>Dicotyledons</u>					
<i>Carica papaya</i>	5	15	9	4	4
<i>Citrus sinensis</i>	6	15	11	4	4
<i>Cucumis sativus</i>	6	15	11	4	4
<i>Arabidopsis thaliana</i>	7	15	11	4	4 or 3
<i>Solanum lycopersicum</i>	7	15	11	4	4
<i>Capsella rubella</i>	7	15	11	4	4
<i>Fragaria vesca</i>	8	15	11	4	4
<i>Prunus persica</i>	8	15	11	4	4
<i>Populus trichocarpa</i>	9	15	11	4	4
<i>Eucalyptus grandis</i>	10	15	11	4	4
<i>Solanum tuberosum</i>	11	15	11	4	4
<i>Vitis vinifera</i>	11	15	11	4	4
<i>Medicago truncatula</i>	12	15	11	4	4
<i>Glycine max</i>	18	15	11	4	4 or 3

Supplementary Table S3. Primers used for genotyping, expression (qRT-PCR), cloning, subcellular localization, sequencing and EcoTILLING.

Gene	Gene model	Primers	Primer Sequences	Purpose
GmSHMT08c	Glyma08g11490	GmSHMT08-RT-Fw	TAACCTCGCCGTGTTCCCTT	qRT-PCR
		GmSHMT08-RT-Rv	TGTTTCGCGTAGGCCTTAAA	
		GmSHMT08-Fw	ACAACACTCTCTCTCTCGC	Genotyping
		GmSHMT08-Rv	CAGATTATGAGTTTTGGCCTG	
		GmSHMT05-RT-Fw	GTTAATAAGAACGCAGTTT	qRT-PCR
		GmSHMT05-RT-Rv	CAATTCTGATAAGAGAACAGT	
GmSHMT05c	Glyma05g28490	GmSHMT05-Fw1	GCACACATAGGCACTGGGCAC	Genotyping
		GmSHMT05-Rv1	CTTATCCGCAATTTCCCTGAAGC	
		GmSHMT05-Fw2	AAACGCTTCAGGGAAATTGCGG	Genotyping
		GmSHMT05-Rv2	GCAAGATCTGTATTCTAGCACATAAAAAG	
		GmSHMT05-Fw3	GCGAATTGGTAACCAACTTATTTC	Genotyping
		GmSHMT05-Rv3	ATAAACCCCTACCCCTCCCAGAATGG	
		GmSHMT05-SL-Fw	TATAAAGCTTTTAAAGACGCACACATAGGCAC	Subcellular Localization
		GmSHMT05-SL-Rv	TATAGTCGACTATCCTTGTACTTCAITTCAGA	
GmSHMT06n	Glyma06g11300	GmSHMT06-RT-Fw	GCAGCAGGTGAAGAGAAATGC	qRT-PCR
		GmSHMT06-RT-Rv	TGACACGCCTCACAGACCTTT	
		GmSHMT06-SL-Fw	TATAGAATTCATGGACTCCCAACCGGGTCTC	Subcellular Localization
		GmSHMT06-SL-Rv	TATAGTCGACTAATATCGAATCCTGGCATTGC	
GmSHMT04n	Glyma04g43360	GmSHMT04-RT-Fw	GGCAGCTCAGATTACCAGCAT	qRT-PCR
		GmSHMT04-RT-Rv	ATATCGAAGCCTGGCATTGC	
GmSHMT12n	Glyma12g29171	GmSHMT12-RT-Fw	GTTGTGCCCGCTTTTACAAA	qRT-PCR
		GmSHMT12-RT-Rv	ATGATGCCACGATCCAAACC	
GmSHMT08n	Glyma08g20050	GmSHMT08b-RT-Fw	TGCAGCTGTCAGGTTTTACA	qRT-PCR
		GmSHMT08b-RT-Rv	ATGCATTAGCTGGGATGGT	
		GmSHMT08b-SL-Fw	TATAGAATTCATGGACTCTCTCACCCCTCAA	Subcellular Localization
		GmSHMT08b-SL-Rv	TATAGTCGACTAATGTCAAAAACCCGGCATGGC	
GmSHMT14m	Glyma14g36280	GmSHMT14-RT-Fw	GGTTCCTGGAGGCATTCTGA	qRT-PCR
		GmSHMT14-RT-Rv	AACCTTGACAGCCGATCAAAG	
GmSHMT02m	Glyma02g38160	GmSHMT02-RT-Fw	GCAAAAAGGAGGTGCAACACTT	qRT-PCR
		GmSHMT02-RT-Rv	TGGTGCAGATGCTAGCTTTACA	
GmSHMT08m	Glyma08g37270	GmSHMT08c-RT-Fw	GGGAGAGAGCAAAGGAACAAAAG	qRT-PCR
		GmSHMT08c-RT-Rv	TTCTTGTGCTTCATGGTTGCTT	
GmSHMT13ch	Glyma13g29410	GmSHMT08c-RT-Fw	CCTAGTTCCAGGAGGCATTTCG	qRT-PCR
		GmSHMT08c-RT-Rv	CTTGGAGCTTCGTTCTGAGA	
GmSHMT18m	Glyma18g27710	GmSHMT18-RT-Fw	GGGAACCCCTGCTCTTACTTCT	qRT-PCR
		GmSHMT18-RT-Rv	TGGCGGAGCTTTGCTATCTC	
GmSHMT09m	Glyma09g33480	GmWEMBL-RT-Fw	GGATTGCTGAGGAGGATTTTG	qRT-PCR
		GmWEMBL-RT-Rv	GGCGAAGCTTTGCAATCTCT	
Gm-Ubiquitin	Glyma20g27950	GmUBI20-RT-Fw	GTGTAATGTTGGATGTGTTCCC	qRT-PCR
		GmUBI20-RT-Rv	ACACAATTGAGTTCAACACAAACCG	

Supplementary Table S4. The GenBank accessions of the serine hydroxymethyltransferase (SHMT; EC 2.1.2.1) alleles deposited at NCBI

Line	Amino acid change	GenBank accession
SHMT08_F6266	E61K	KX881924
SHMT08_F6756	M125I	KX881925
SHMT08_F1927	G132D	KX881926
SHMT08_F1817	R257Q	KX881927
SHMT08_F1336	L299F	KX881928
SHMT08_F1801	N368T	KX881929
SHMT08_F891	A302V	KX881930
SHMT08_F427	G71D	KX881931
SHMT08_F1460	G326E	KX881932
SHMT08_Forrest_WT	-	KX881923
SHMT08_Essex_WT	-	KX881933