

a

Forrest MRALAAQFSNYLCRRKVGVNLRNRFSSYNSKDELTIIEEEAERKVGWLLKTIFFVTAGVA
Essex MRALAAQFSNYLCRRKVGVNLRNRFSSYNSKDELTIIEEEAERKVGWLLKTIFFVTAGVA

Forrest GYHFFPYMGENLMQQSVSLLRVKDPLFKRMGASRLARFAVDDERRKKIVEMGGAQELLNM
Essex GYHFFPYMGENLMQQSVSLLRVKDPLFKRMGASRLARFAVDDERRKKIVEMGGAQELLNM

Forrest LSTAKDDRTRKEALHALDALSQSDEALASLHHAGAISVIRSAPNSLEDAEVEGFKLSLMK
Essex LSTAKDDRTRKEALHALDALSQSDEALASLHHAGAISVIRSAPNSLEDAEVEGFKLSLMK

Forrest RFQDLRYDVPS
Essex RFQDLRYDVPS

b

Forrest MSPAAGVSVPLLGD SKGTPPPASVPGAVFNVATSIVGAGIMSIPAIMKVLGVVPAFAMIL
Essex MSPAAGVSVPLLGD SKGTPPPASVPGAVFNVATSIVGAGIMSIPAIMKVLGVVPAFAMIL

Forrest VVAVLAELSVDFLMRFTHSGETTTTYAGVMREAFGSGGALAAQVCVIITNVGGLILYLI I I
Essex VVAVLAELSVDFLMRFTHSGETTTTYAGVMREAFGSGGALAAQVCVIITNVGGLILYLI I I

Forrest GDVLSGKQNGGEVHLGILQQWFGIHWNSREFALLFTLVFVMLPLVLYKRVESLKYSSAV
Essex GDVLSGKQNGGEVHLGILQQWFGIHWNSREFALLFTLVFVMLPLVLYKRVESLKYSSAV

Forrest STLLAVAFVIGICCGLAITLVQGKTQTPRFLPRLDYQTSFFDLFTAVPVVVTAFTHFHN
Essex STLLAVAFVIGICCGLAITLVQGKTQTPRFLPRLDYQTSFFDLFTAVPVVVTAFTHFHN

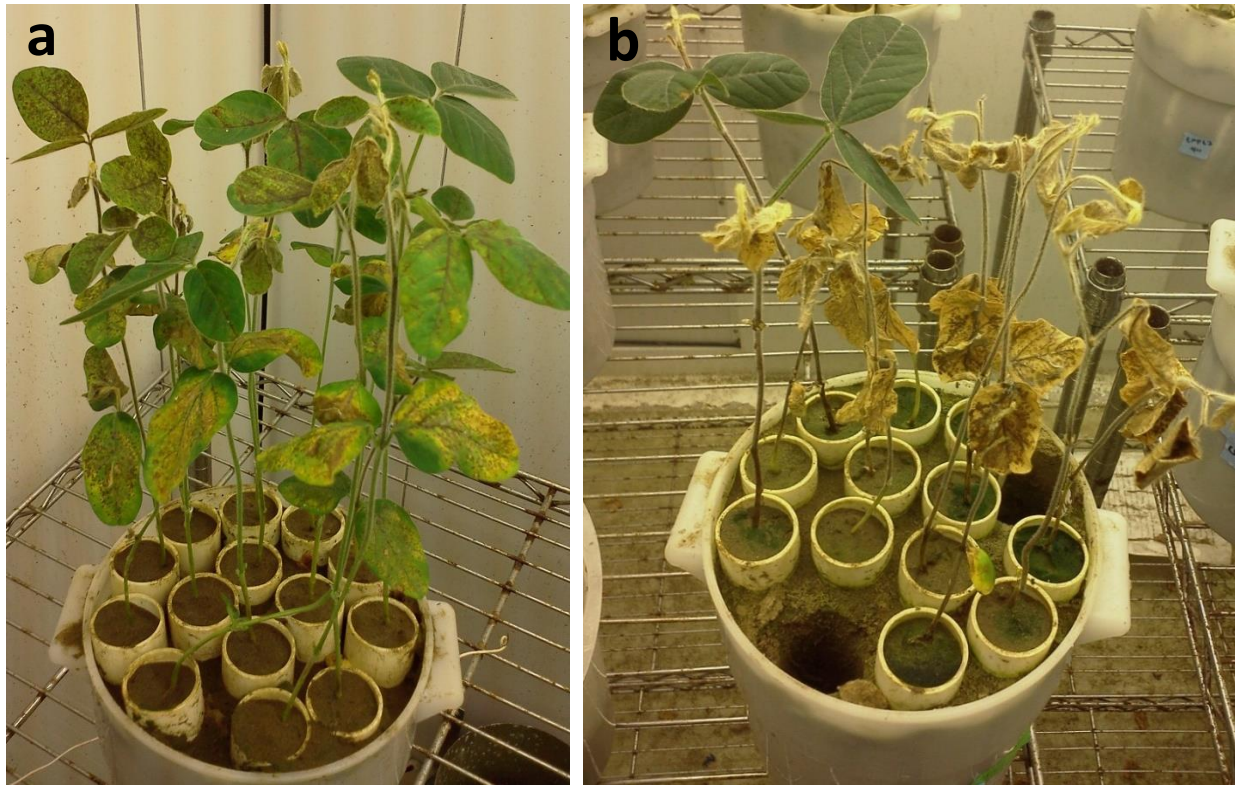
Forrest HPIGFELAKASQMTTAVRLALLLCAVIYLAIGLFGYMLFGDSTQSDILINFDQNAGSAVG
Essex HPIGFELAKASQMTTAVRLALLLCAVIYLAIGLFGYMLFGDSTQSDILINFDQNAGSAVG

Forrest SLLNSLVRVSYALHIMLVFPLLNFSLRTNIDEVLFPPKPLATDNKRFMILTLLVLLVFSY
Essex SLLNSLVRVSYALHIMLVFPLLNFSLRTNIDEVLFPPKPLATDNKRFMILTLLVLLVFSY

Forrest LAAIAIPDIWYFFQFLGSSAVCLAFIFPGSIVLRDVKGISTRDKIIALIMIILAVVTS
Essex LAAIAIPDIWYFFQFLGSSAVCLAFIFPGSIVLRDVKGISTRDKIIALIMIILAVVTS

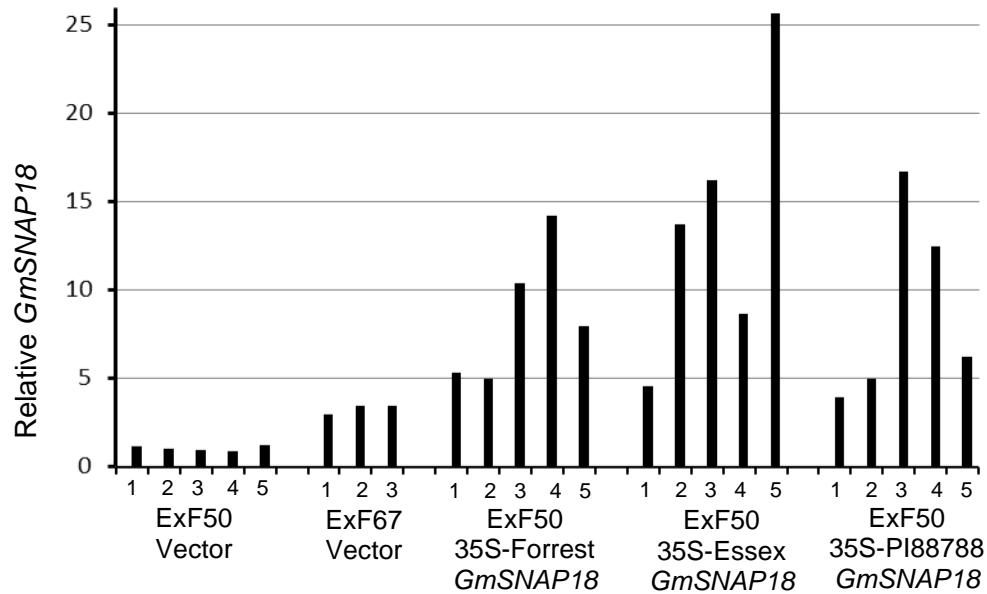
Forrest VLAISTNIYNAFSSKS
Essex VLAISTNIYNAFSSKS

Supplementary Figure 1. The predicted protein sequences of Glyma18g02570 and Glyma18g02580 in Forrest and Essex. (a) Alignment of the predicted protein sequences of Glyma18g02570 in Forrest and Essex; (b) Alignment of the predicted protein sequences of Glyma18g02580 in Forrest and Essex.

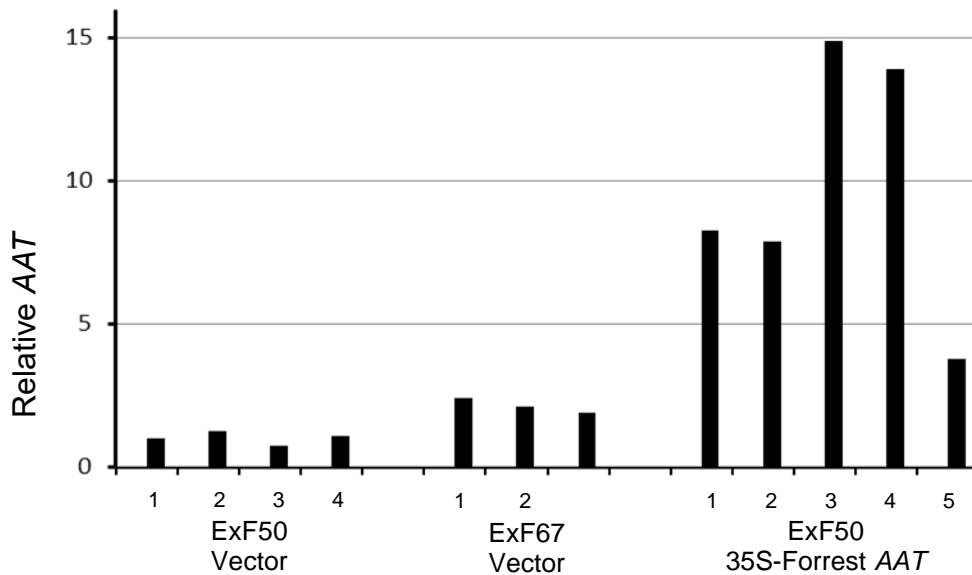


Supplementary Figure 2. Plant growth of RIL ExF67 (*GmSNAP18*⁺/*GmSHMT08*⁺) inoculated with BPMV-Forrest *GmSNAP18*. (a) 16 days post VIGS infection. (b) 30 days post VIGS infection.

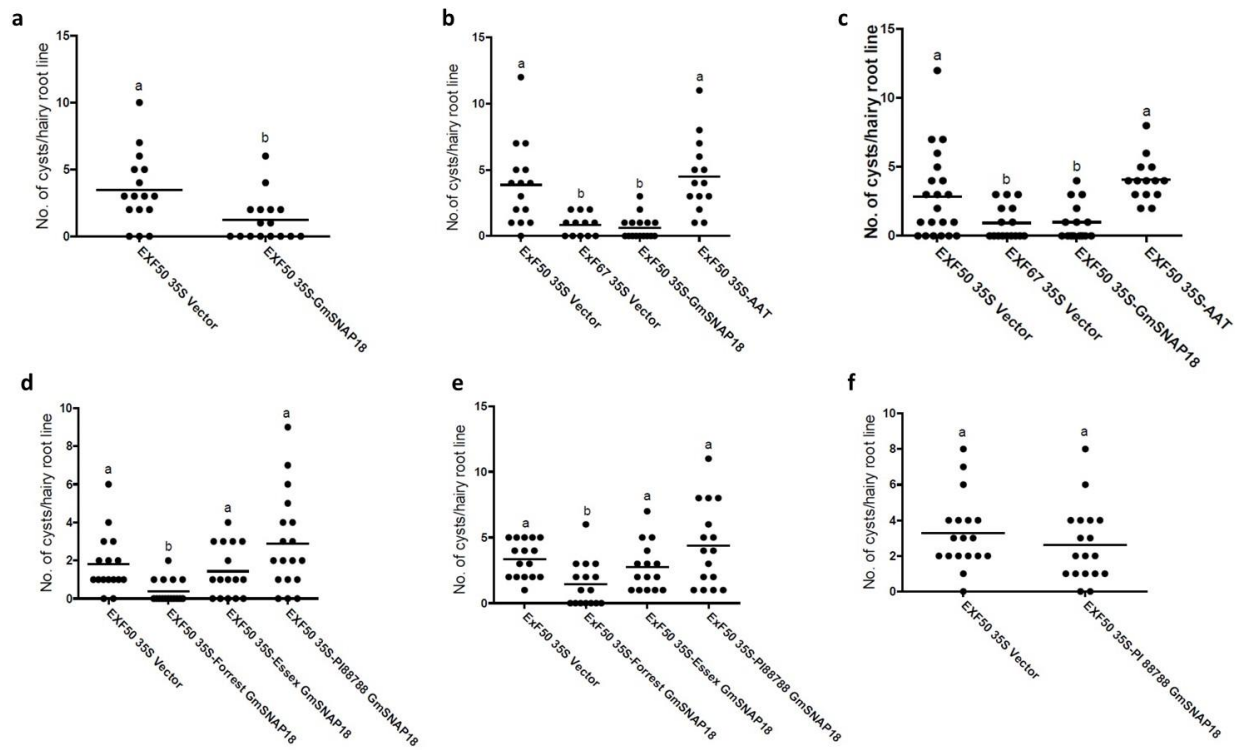
a



b



Supplementary Figure 3. Expression analysis of transgenic hairy roots. (a) Expression of Forrest *GmSNAP18* in ExF50 (*GmSNAP18*⁻/*GmSHMT08*⁺) *GmSNAP18* transgenic hairy roots. Numbers denote independent lines used for the analysis. Data is relative to the Forrest *GmSNAP18* expression in one of the ExF50 vector control roots. (b) Expression analysis of AAT in ExF50 AAT transgenic hairy roots. Numbers denote independent lines used for the analysis. Data is relative to the expression in one of the ExF50 vector control roots. ExF67 (*GmSNAP18*⁺/*GmSHMT08*⁺) was taken as the control. The qPCR primers used for the expression analysis are listed in the primer table (Supplementary Table 1).



Supplementary Figure 4. Replicate experimental data for nematode infection assays. (a) SCN infection of RIL ExF50 transgenic hairy roots expressing Forrest *GmSNAP18*. (b) and (c) SCN infection of RIL ExF50 transgenic hairy roots expressing Forrest *GmSNAP18* or AAT. (d), (e) and (f) SCN infection of RIL ExF50 transgenic hairy roots expressing *GmSNAP18* of Forrest, Essex or PI 88788. *GmSNAP18* refers to Forrest-*GmSNAP18*, if not indicated otherwise. Significantly different experimental results obtained by an unpaired *t*-test are denoted by different letters ($P < 0.05$, range from 0.04 to 0.0004). Each dot denotes cyst number from an independent hairy line ($n \geq 12$).

Supplementary Table 1 The primers used in this study except the primers employed for RSE-Seq. Some of primers referred to the Supplementary References, and the left were designed and developed in this study.

I. Primers for screening of *rhg1-a* recombinants and genotyping of *Rhg4*

rhg1

Sat_210 ⁴	Forward: GCGCCAGCAACAAAGTTCCTGACAAA Reverse: GCGCATGCAAATGAAATAATAA
Satt309 ⁴	Forward: GCGCCTTCAAATTGGCGTCTT Reverse: GCGCCTTAAATAAAACCCGAAACT
SIUC-SAT143 ¹	Forward: TGTTACTTAGTAATTATGAAG Reverse: AATAATGATTTGTTGATCGAT

Rhg4

Satt632 ⁴	Forward: GGGCTATGAAGGGAATGGAAAGGA Reverse: CCCATATTGAAGATTTGAAGTAAT
Sat_162 ⁴	Forward: GCGTGGTTTTTCGCTGGATATA Reverse: GCGCATTCGTAACATATTTTTTAC
GMES6186 ³	Forward: AGCGGGAATTGAAGGTTTT Reverse: GGAATCTCATCTGAAATAATGGA

II. Primers for high density genetic mapping of *rhg1-a* recombinants and haplotyping of soybean lines

rhg1

Sat_210 ⁴	Forward: GCGCCAGCAACAAAGTTCCTGACAAA Reverse: GCGCATGCAAATGAAATAATAA
5601	Forward: TTA CTTTTGGTCAGCATT TGGC Reverse: TATTGTTGATATATTATATTGTCC
5701	Forward: ACCTTTTTGCAGTATTTATGC Reverse: CTAGGTA ACTCTTTTAGCCGTGA
580	Forward: GACCAAGGTGAAAAGCAAAGCA Reverse: ATAGTAGGCTGCCAGGCACCG
590	Forward: ATGGCCGATCAGTTATCGAAGGG Reverse: ACCCAGGAGAAGAGATATCAATA
600	Forward: GAAATGCATGGTTTGAAGTAAAC Reverse: GACAGAGTTAATGTCTATAGTAT
610	Forward: GTTTGGTTTGGTTACTTGAAAATC Reverse: GGCATCATCACAACCCAACT
10	Forward: CTCTAACCATTTGACTTGTTC Reverse: ATGAGTCTATGACCATACCCT
12	Forward: CAAAAGTCTGACTTAAAAGTCTC Reverse: GTCGTCTGTAGACTGATACAG
122452	Forward: CGCAAAC TTTATTGTGTTCC TAGTC

TMD1	Reverse: TGACCCACCTGATAAAATGTAGT Forward: AAAC TAGTCCATTTTGATGGA Reverse: AGCATTTGTATTCTCATCAAG
Satt309 ⁴	Forward: GCGCCTTCAAATTGGCGTCTT Reverse: GCGCCTTAAATAAAACCCGAAACT
375912	Forward: TGCTCTCAACTCATGCTCCTAC Reverse: ACTTGGTGCTTCTTGGATTGTG
122592	Forward: GCCGTCTTCTTTTTCTTTTTCACTTTCTAA Reverse: TGTTGGCTTTTCCTTTTCATTTATCT
120	Forward: TTTGGTTGATGTTGTTGCATC Reverse: GGGAAATAAGAAAAACAATGCT
SIUC-SAT143 ¹	Forward: TGTTACTTAGTAATTATGAAG Reverse: AATAATGATTTGTTGATCGAT
SIUC-SAT185	Forward: CAGAGTCAAGTTTGAAC TTCAT Reverse: AATCAAGTGATCATTG TACTG
375752	Forward: GCTGCCTCAATTCAACAATCC Reverse: TTGGTGTCATGTCTTGCTCTC

***GmSHMT08* sequencing**

SHMT ¹	Forward: ACAACACTCTCTTCTCTCGC Reverse: CAGATTATGAGTTTTGGCCTG
SHMT2 ¹	Forward: CAGGCCAAAAC TCATAATCTG Reverse: CGTTACCAATTCGCACTCCA
SHMT3 ¹	Forward: TAATTTGGTTGGAGAACAATG Reverse: CTAATCCTTGACTTCATTTTC

***GmSNAP18* sequencing**

SNAP1	Forward: CGCTTATGAATCTTCTTCTTCTTC Reverse: GCATGTAGTGGTAACAAC T GAGAC
SNAP2	Forward: GGTTTTGGAGTGGGCTGAATC Reverse: ACCCAGGAGAAGAGATATCAATA
SNAP3	Forward: GCAGGAGCAAATTATTTTGCTGTC Reverse: GAATTTGATGACACGTACAATAAATG
SNAP4	Forward: CACTGTGTAAAGTTAATTTTTTGCTTAC Reverse: CCAATTC AATTA AAACCAAAGCAGG

III. Primers for cDNA sequencing

Glyma18g02570-cDNA	Forward: ATGCGCGCCCTAGCAGCTCA Reverse: TCATGATGGCACATCATATCTGAG
Glyma18g02580-cDNA	Forward: ATGTCTCCGCGCCGGAGA Reverse: TCATGACTTGCTACTAAAAGCATTAT
Glyma18g02590-cDNA	Forward: CGCTTATGAATCTTCTTCTTCTTC Reverse: CCAATTC AATTA AAACCAAAGCAGG

IV. Primers for subcloning

GmSNAP18-F (Forrest/Essex/PI88788)	AAAAAAGCAGGCTAT ATG GCC GAT CAG TTA TCG AA
GmSNAP18-Forrest-R	AAGAAAGCTGGGTCTCA AGT AAT AAC CTC ATA CTC CTC
GmSNAP18-PI88788-R	AAGAAAGCTGGGTC TCA AGT AAT AGC CTC ATG CTG CTC
GmSNAP18-Essex-R	AAGAAAGCTGGGTCTCA AGT AAG ATC ATC CTC CTC
GmAAT-F	AAAAAAGCAGGCTTA GAAGGAGATAGAACCATGTCTCCGGCCCGGAGT
GmAAT-R	AAGAAAGCTGGGTC TTA TGA CTT GCT ACT AAA AGC ATT
attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT
attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGT

V. Primers for VIGS

GmSNAPVIGS-F	CGCGGATCCCTCGAGATCCAACATTTTCAGGAACACG
GmSNAPVIGS-R	CGCGGATCCCCAGTTCCTGATATCGTTCTAA

VI. Primers for qRT-PCR

GmSNAP18-RT-F	ACAAGGCTGGAGCGACATAC
GmSNAP18-RT-R	AGCAATGTGCAGCATCGACA
qGm18AAT-F	GGTTTGGAATTCCTACTGGTGGAA
qGm18AAT-R	CAATACCAATGGAAGCATAACAAAGA
GmUbiquitin-F	GTGTAATGTTGGATGTGTTCCC
GmUbiquitin-R	ACACAATTGAGTTCAACACAAACCG

Supplementary Table 2 Direct identification of *rhg1* candidate gene *GmSNAP18* conferring resistance to SCN by analysis of the polymorphisms possibly associated with the alleles underlying resistance to SCN. The results indicate that gene Glyma18g02590 (*GmSNAP18*) is the candidate gene most possibly associated with the resistance to SCN at *rhg1*, in which those 6 SNPs (C163208A, C163225G, G164965C, G164968T, G164968C and C164974A) and 4 insertions (A164972AGGT and A164972AGGC) are all located within the exons, leading to the amino acid (AA) changes of predicted GmSNAP18 protein.

Gene	SNPs/Insertions	Total AA changes	AA (nucleotide) changes in Forrest/Peking	AA (nucleotide) changes in PI88788
Glyma18g02353	1	1	1	-
Glyma18g02420	1	1	1	-
Glyma18g02450	3	3	3	-
Glyma18g02520	1	1	-	1
Glyma18g02590 (<i>GmSNAP18</i>)	10	9	3+2 D208E (C163225G) D286Y (G164968T) D287E and -288V (A164972AGGT) L289I (C164974A)	4+2 Q203K (C163208A) E285Q (G164965C) D286H (G164968C) D287E and -288A (A164972AGGC) L289I (C164974A)
Glyma18g02650	1	1	1	-
Glyma18g02660	1	1	1	-
Glyma18g02681	1	1	1	-
Glyma18g02690	3	3	3	-
Glyma18g02700	1	1	1	-
Glyma18g02720	2	2	-	2
Glyma18g02741	1	1	-	1

Supplementary Table 3 The primers specific for the enrichment of the 300 kb regional genomic DNA segment at *rhg1* by RSE.

No.	Primers (5'>3')	No.	Primers (5'>3')
Gm18-1	ctctatcgactctcagtctc	Gm18-31	cctggaacaagccatgttc
Gm18-2	acatacaatataagcctgcgaag	Gm18-32	cgtgacacacctctgtctaa
Gm18-3	cgccttgtgcaaaagaagat	Gm18-33	cccaaactgaaagcgtgaag
Gm18-4	actaacgccacttaccacca	Gm18-34	gtgtaatgaatggacggctcc
Gm18-5	tgactacgatgtcactgac	Gm18-35	agagtattatgaacggcgtct
Gm18-6	ccaatceccacctcaggtaa	Gm18-36	gacttcgcaaatccaagacg
Gm18-7	ccagactcaagtctaagctc	Gm18-37	ggggtgtcaagacaatctag
Gm18-8	gaacgacactgatatgctgg	Gm18-38	gacacaaaaggctgtttcc
Gm18-9	ctagtgtctgcatcatcaca	Gm18-39	gcagttcttagctgttctg
Gm18-10	ccgaagttaggaaagaaggg	Gm18-40	cctgaatttctggtaaagcaag
Gm18-11	gaactcttgaatgcttctcga	Gm18-41	gcagctattccagttctgtt
Gm18-12	gctctataaatagagcattcgtagtct	Gm18-42	ggggagttaaacacctcctc
Gm18-13	gttggtccctagaatatctga	Gm18-43	ccttatcatcaaccgcaaca
Gm18-14	gcatcggaatgaacgctaac	Gm18-44	gaacagcacggctatcatca
Gm18-15	cgaggatcaggatgattttg	Gm18-45	ctcactttggtagtagctc
Gm18-16	cctgtgtagtaactagtaac	Gm18-46	gtagccatggatcgaatgaag
Gm18-17	cacgtacacccatattctga	Gm18-47	gtgtctcagaaccagttag
Gm18-18	agtaggggtacgggtatgag	Gm18-48	aacggataacaagacagaaagaa
Gm18-19	ctttcgttttcttagcacatgtctt	Gm18-49	gttacacgagtcaggaccat
Gm18-20	gcttctagaagtaaaactaacga	Gm18-50	aacaaggttgcctgcaagtc
Gm18-21	cattcaggcactactaaactgttgc	Gm18-51	ctttgcattagcgagaaaatttgg
Gm18-22	ctcaaggaggaggaaagtgat	Gm18-52	tcatcttctgatggaggct
Gm18-23	tttatcatggcctaggcctacca	Gm18-53	ccggacttaggataactaaaacaag
Gm18-24	ccatcacggcatctttaaatttacc	Gm18-54	catgggaggaatgaacgctgga
Gm18-25	ccgtatgagaccgcatatt	Gm18-55	cgtaaccttactttataccatac
Gm18-26	tgatgatttttagcggctcag	Gm18-56	gggcttagcttaggcattata
Gm18-27	tgcgttaagagatgatccag	Gm18-57	ccgaggtggactttgattctct
Gm18-28	acatcaggcaattcccgtag	Gm18-58	gtccctcaaaggcattcttt
Gm18-29	cagcattcctcatatggagtg	Gm18-59	cgagtggactaagtctctta
Gm18-30	cattaaataggtcgcctaca	Gm18-60	tccgtgaatgattgacaagg

Supplementary References

1. Liu, S., Kandath, P.K., Warren, S.D., Yechel, G., Heinz, R., Alden, J., Yang, C., Jamai, A., Ei-Mellouki, T., Juvale, P.S., Hill, J., Baum, T.J., Cianzio, S., Whitham, S.A., Korkin, D., Mitchum, M.G. & Meksem, K. A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. *Nature* **492**, 256-260 (2012).
2. Hyten, D.L., Choi, I.Y., Song, Q., Shoemaker, R.C., Nelson R.L., Costa, J.M., Specht, J.E. & Cregan, P.B. Highly variable patterns of linkage disequilibrium in multiple soybean populations. *Genetics* **175**, 1937-1944 (2007).

3. Hwang T.Y., Sayama, T., Takahashi, M., Takada, Y., Nakamoto, Y., Funatsuki, H., Hisano, H., Sasamoto, S., Sato, S., Tabata, S., Kono, I., Hoshi, M., Hanawa, M., Yano, C., Xia, Z., Harada, K., Kitamura, K. & Ishimoto, M. High-density integrated linkage map based on SSR markers in soybean. *DNA Res.* **16**, 213-225 (2009).
4. <http://soybase.org>