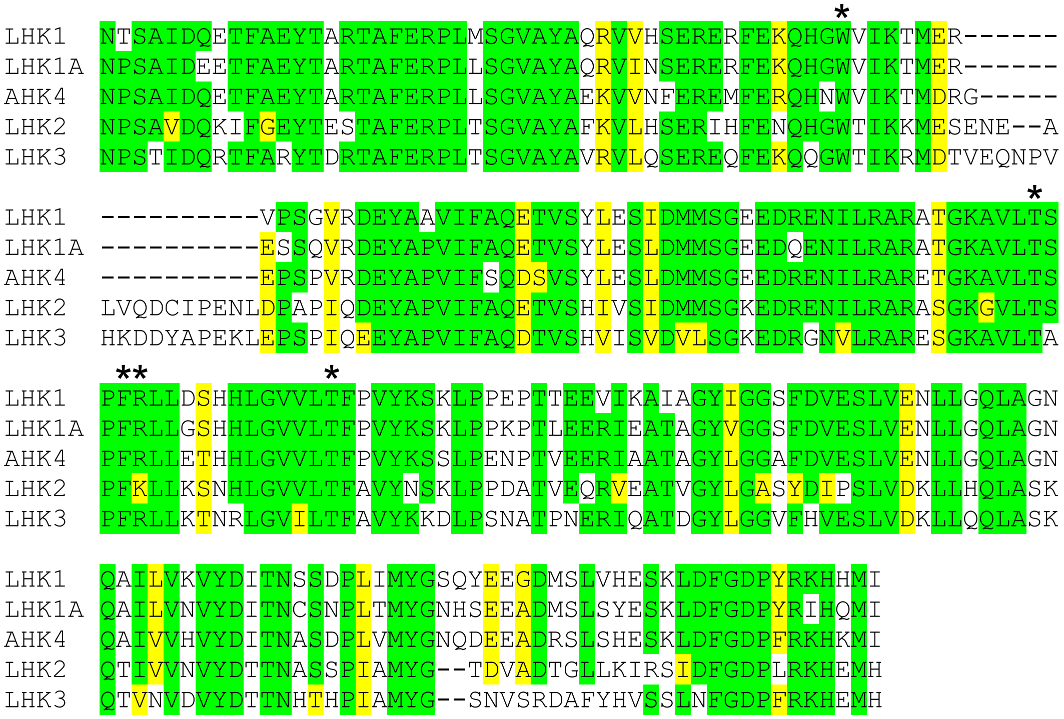

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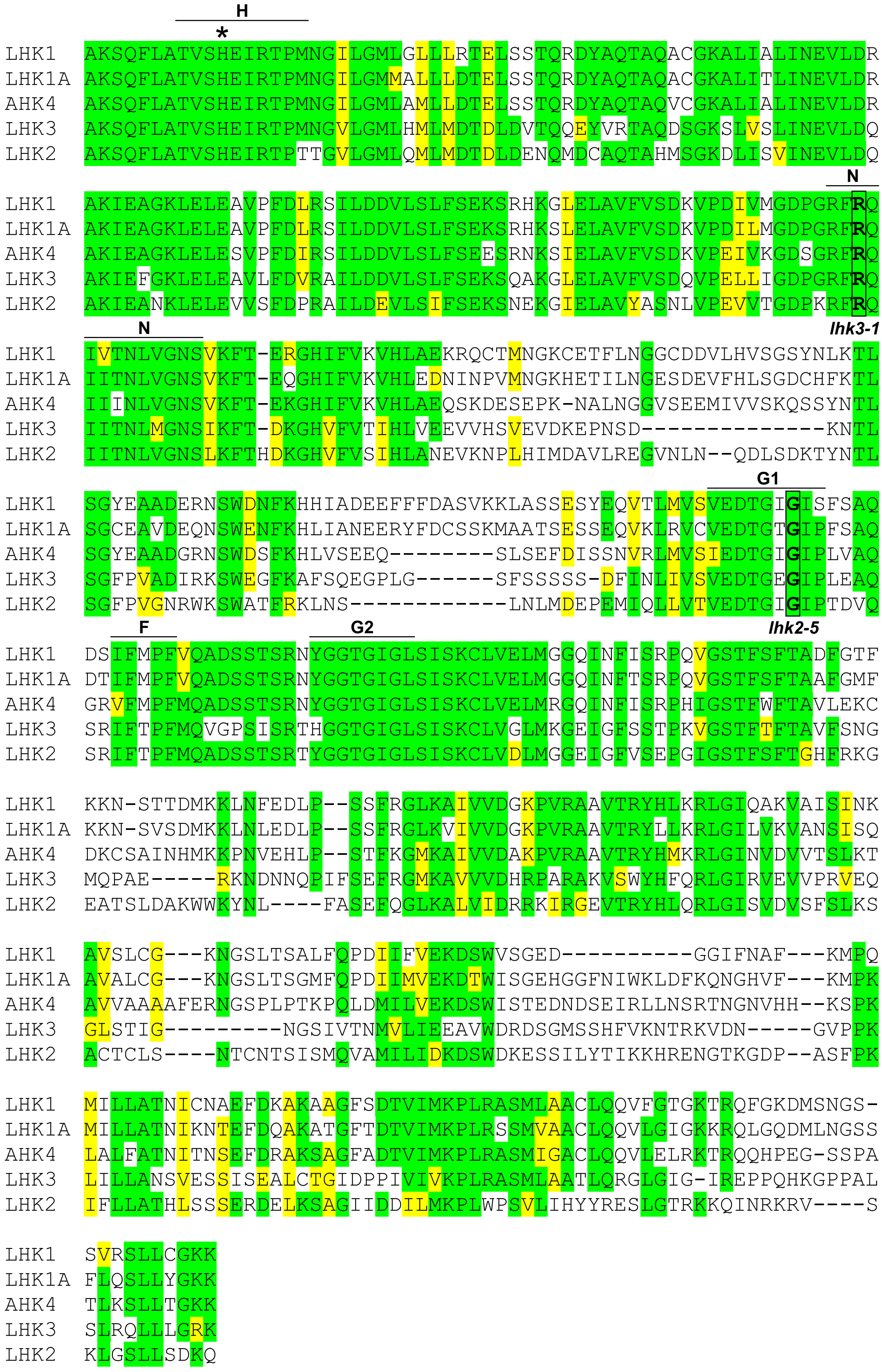
                                                    *
LHK1  NTSAIDQETFAEYTARTAFERPLMSGVAYAQRVVHSERERFEKQHGWVIKTMER------
LHK1A NPSAIDEETFAEYTARTAFERPLLSGVAYAQRVINSERERFEKQHGWVIKTMER------
AHK4  NPSAIDQETFAEYTARTAFERPLLSGVAYAEKVVNFEREMFERQHNWVIKTMDRG-----
LHK2  NPSAVDQKIFGEYTESTAFERPLTSGVAYAFKVLHSERIHFENQHGWTIKKMESENE--A
LHK3  NPSTIDQRTFARYTDRTAFERPLTSGVAYAVRVLQSEREQFEKQQGWTIKRMDTVEQNPV

                                                                 *
LHK1  -----------VPSGVRDEYAAVIFAQETVSYLESIDMMSGEEDRENILRARATGKAVLTS
LHK1A -----------ESSQVRDEYAPVIFAQETVSYLESLDMMSGEEDQENILRARATGKAVLTS
AHK4  -----------EPSPVRDEYAPVIFSQDSVSYLESLDMMSGEEDRENILRARETGKAVLTS
LHK2  LVQDCIPENLDPAPIQDEYAPVIFAQETVSHIVSIDMMSGKEDRENILRARASGKGVLTS
LHK3  HKDDYAPEKLEPSPIQEEYAPVIFAQDTVSHVISVDVLSGKEDRGNVLRARESGKAVLTA

       **           *
LHK1  PFRLLDSHHLGVVLTFPVYKSKLPPEPTTEEVIKAIAGYIGGSFDVESLVENLLGQLAGN
LHK1A PFRLLGSHHLGVVLTFPVYKSKLPPKPTLEERIEATAGYVGGSFDVESLVENLLGQLAGN
AHK4  PFRLLETHHLGVVLTFPVYKSSLPENPTVEERIAATAGYLGGAFDVESLVENLLGQLAGN
LHK2  PFKLLKSNHLGVVLTFAVYNSKLPPDATVEQRVEATVGYLGASYDIPSLVDKLLHQLASK
LHK3  PFRLLKTNRLGVILTFAVYKKDLPSNATPNERIQATDGYLGGVFHVESLVDKLLQQLASK

LHK1  QAILVKVYDITNSSDPLIMYGSQYEEGDMSLVHESKLDFGDPYRKHHMI
LHK1A QAILVNVYDITNCSNPLTMYGNHSEEADMSLSYESKLDFGDPYRIHQMI
AHK4  QAIVVHVYDITNASDPLVMYGNQDEEADRSLSHESKLDFGDPFRKHKMI
LHK2  QTIVVNVYDTTNASSPIAMYG--TDVADTGLLKIRSIDFGDPLRKHEMH
LHK3  QTVNVDVYDTTNHTHPIAMYG--SNVSRDAFYHVSSLNFGDPFRKHEMH
```

Supplemental Figure 1. **Amino-Acid Sequence Alignment of Protein Regions Containing the CHASE Domain, as Predicted for *L. japonicus* LHKs and *Arabidopsis* AHK4**. The alignment was created using CLUSTALW2 and analyzed by the BoxShade software. A threshold of ≥ 80% conservation was used. Green shading indicates identical residues, whereas yellow indicates conservative substitutions. Asterisks denote conserved amino-acid positions corresponding to the Arabidopsis AHK4 receptor residues W244, T301, F304, R305 and T3017, all shown to be important for cytokinin binding (Heyl et al., 2007).



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LHK1   AKSQFLATVSHEIRTPMNGILGMLGLLLRTELSSTQRDYAQTAQACGKALIALINEVLDR
LHK1A  AKSQFLATVSHEIRTPMNGILGMMALLLDTELSSTQRDYAQTAQACGKALITLINEVLDR
AHK4   AKSQFLATVSHEIRTPMNGILGMLAMLLDTELSSTQRDYAQTAQVCGKALIALINEVLDR
LHK3   AKSQFLATVSHEIRTPMNGVLGMLHMLMDTDLDVTQQEYVRTAQDSGKSLVSLINEVLDQ
LHK2   AKSQFLATVSHEIRTPTTGVLGMLQMLMDTDLDENQMDCAQTAHMSGKDLISVINEVLDQ

LHK1   AKIEAGKLELEAVPFDLRSILDDVLSLFSEKSRHKGLELAVFVSDKVPDIVMGDPGRFRQ
LHK1A  AKIEAGKLELEAVPFDLRSILDDVLSLFSEKSRHKSLELAVFVSDKVPDILMGDPGRFRQ
AHK4   AKIEAGKLELESVPFDIRSILDDVLSLFSEESRNKSIELAVFVSDKVPEIVKGDSGRFRQ
LHK3   AKIEFGKLELEAVLFDVRAILDDVLSLFSEKSQAKGLELAVFVSDQVPELLIGDPGRFRQ
LHK2   AKIEANKLELEVVSFDPRAILDEVLSIFSEKSNEKGIELAVYASNLVPEVVTGDPKRFRQ

LHK1   IVTNLVGNSVKFT-ERGHIFVKVHLAEKRQCTMNGKCETFLNGGCDDVLHVSGSYNLKTL
LHK1A  IITNLVGNSVKFT-EQGHIFVKVHLEDNINPVMNGKHETILNGESDEVFHLSGDCHFKTL
AHK4   IIINLVGNSVKFT-EKGHIFVKVHLAEQSKDESEPK-NALNGGVSEEMIVVSKQSSYNTL
LHK3   IITNLMGNSIKFT-DKGHVFVTIHLVEEVVHSVEVDKEPNSD--------------KNTL
LHK2   IITNLVGNSLKFTHDKGHVFVSIHLANEVKNPLHIMDAVLREGVNLN--QDLSDKTYNTL

LHK1   SGYEAADERNSWDNFKHHIADEEFFFDASVKKLASSESYEQVTLMVSVEDTGIGISFSAQ
LHK1A  SGCEAVDEQNSWENFKHLIANEERYFDCSSKMAATSESSEQVKLRVCVEDTGTGIPFSAQ
AHK4   SGYEAADGRNSWDSFKHLVSEEQ--------SLSEFDISSNVRLMVSIEDTGIGIPLVAQ
LHK3   SGFPVADIRKSWEGFKAFSQEGPLG------SFSSSSS-DFINLIVSVEDTGEGIPLEAQ
LHK2   SGFPVGNRWKSWATFRKLNS------------LNLMDEPEMIQLLVTVEDTGIGIPTDVQ

LHK1   DSIFMPFVQADSSTSRNYGGTGIGLSISKCLVELMGGQINFISRPQVGSTFSFTADFGTF
LHK1A  DTIFMPFVQADSSTSRNYGGTGIGLSISKCLVELMGGQINFTSRPQVGSTFSFTAAFGMF
AHK4   GRVFMPFMQADSSTSRNYGGTGIGLSISKCLVELMRGQINFISRPHIGSTFWFTAVLEKC
LHK3   SRIFTPFMQVGPSISRTHGGTGIGLSISKCLVGLMKGEIGFSSTPKVGSTFTFTAVFSNG
LHK2   SRIFTPFMQADSSTSRTYGGTGIGLSISKCLVDLMGGEIGFVSEPGIGSTFSFTGHFRKG

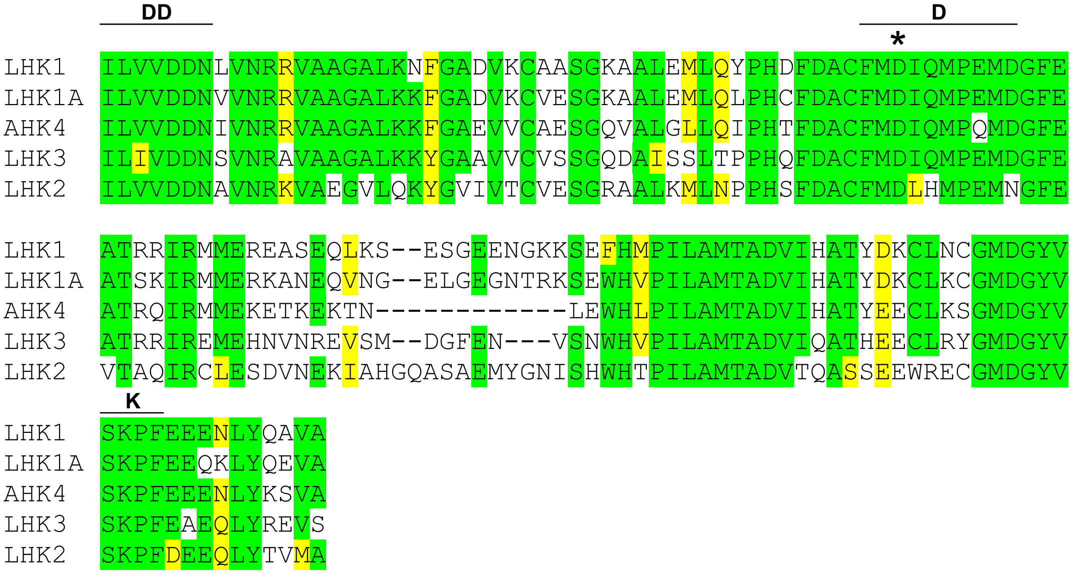
LHK1   KKN-STTDMKKLNFEDLP--SSFRGLKAIVVDGKPVRAAVTRYHLKRLGIQAKVAISINK
LHK1A  KKN-SVSDMKKLNLEDLP--SSFRGLKVIVVDGKPVRAAVTRYLLKRLGILVKVANSISQ
AHK4   DKCSAINHMKKPNVEHLP--STFKGMKAIVVDAKPVRAAVTRYHMKRLGINVDVVTSLKT
LHK3   MQPAE-----RKNDNNQPIFSEFRGMKAVVVDHRPARAKVSWYHFQRLGIRVEVVPRVEQ
LHK2   EATSLDAKWWKYNL----FASEFQGLKALVIDRRKIRGEVTRYHLQRLGISVDVSFSLKS

LHK1   AVSLCG---KNGSLTSALFQPDIIFVEKDSWVSGED----------GGIFNAF---KMPQ
LHK1A  AVALCG---KNGSLTSGMFQPDIIMVEKDTWISGEHGGFNIWKLDFKQNGHVF---KMPK
AHK4   AVVAAAAFERNGSPLPTKPQLDMILVEKDSWISTEDNDSEIRLLNSRTNGNVHH--KSPK
LHK3   GLSTIG---------NGSIVTNMVLIEEAVWDRDSGMSSHFVKNTRKVDN-----GVPPK
LHK2   ACTCLS----NTCNTSISMQVAMILIDKDSWDKESSILYTIKKHRENGTKGDP--ASFPK

LHK1   MILLATNICNAEFDKAKAAGFSDTVIMKPLRASMLAACLQQVFGTGKTRQFGKDMSNGS-
LHK1A  MILLATNIKNTEFDQAKATGFTDTVIMKPLRSSMVAACLQQVLGIGKKRQLGQDMLNGSS
AHK4   LALFATNITNSEFDRAKSAGFADTVIMKPLRASMIGACLQQVLELRKTRQQHPEG-SSPA
LHK3   LILLANSVESSISEALCTGIDPPIVIVKPLRASMLAATLQRGLGIG-IREPPQHKGPPAL
LHK2   IFLLATHLSSSERDELKSAGIIDDILMKPLWPSVLIHYYRESLGTRKKQINRKRV----S

LHK1   SVRSLLCGKK
LHK1A  FLQSLLYGKK
AHK4   TLKSLLTGKK
LHK3   SLRQLLLGRK
LHK2   KLGSLLSDKQ
```

Supplemental Figure 2. Amino-Acid Sequence Alignment of the Protein Kinase Domains, as Predicted for *L. japonicus* LHK Proteins and Compared to *Arabidopsis* AHK4. The conserved H, N, G1, F, and G2 box motifs are indicated. An asterisk denotes the presence of a conserved histidine (H) residue required for the phosphorelay. Boxed and highlighted R and G residues correspond to positions of *lhk3-1* and *lhk2-5* mutations, respectively. The alignment was created as described in the legend for the supplementary Figure 1.



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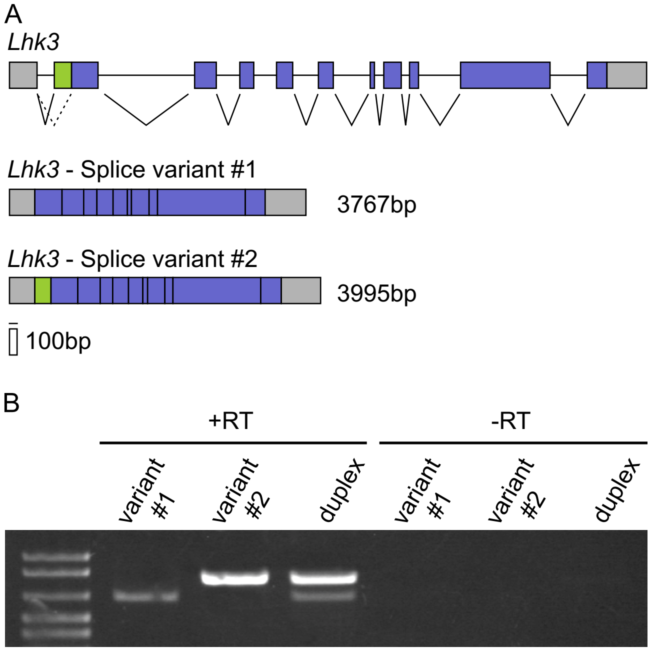
LHK1   ILVVDDNLVNRRVAAGALKNFGADVKCAASGKAALEMLQYPHDFDACFMDIQMPEMDGFE
LHK1A  ILVVDDNVVNRRVAAGALKKFGADVKCVESGKAALEMLQLPHCFDACFMDIQMPEMDGFE
AHK4   ILVVDDNIVNRRVAAGALKKFGAEVVCAESGQVALGLLQIPHTFDACFMDIQMPQMDGFE
LHK3   ILIVDDNSVNRAVAAGALKKYGAAVVCVSSGQDAISSLTPPHQFDACFMDIQMPEMDGFE
LHK2   ILVVDDNAVNRKVAEGVLQKYGVIVTCVESGRAALKMLNPPHSFDACFMDLHMPEMNGFE

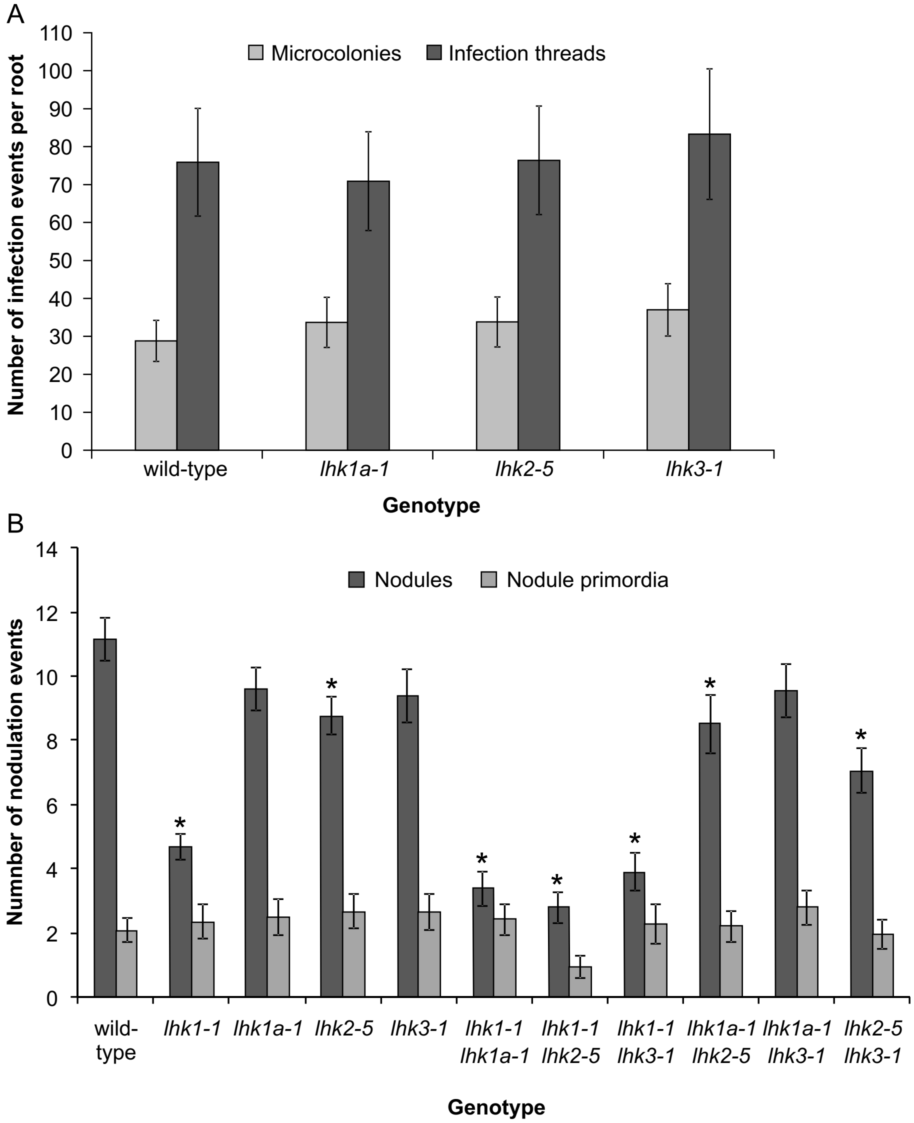
LHK1   ATRRIRMMEREASEQLKS--ESGEENGKKSEFHMPILAMTADVIHATYDKCLNCGMDGYV
LHK1A  ATSKIRMMERKANEQVNG--ELGEGNTRKSEWHVPILAMTADVIHATYDKCLKCGMDGYV
AHK4   ATRQIRMMEKETKEKTN------------LEWHLPILAMTADVIHATYEECLKSGMDGYV
LHK3   ATRRIREMEHNVNREVSM--DGFEN---VSNWHVPILAMTADVIQATHEECLRYGMDGYV
LHK2   VTAQIRCLESDVNEKIAHGQASAEMYGNISHWHTPILAMTADVTQASSEEWRECGMDGYV

LHK1   SKPFEEENLYQAVA
LHK1A  SKPFEEQKLYQEVA
AHK4   SKPFEEENLYKSVA
LHK3   SKPFEAEQLYREVS
LHK2   SKPFDEEQLYTVMA
```

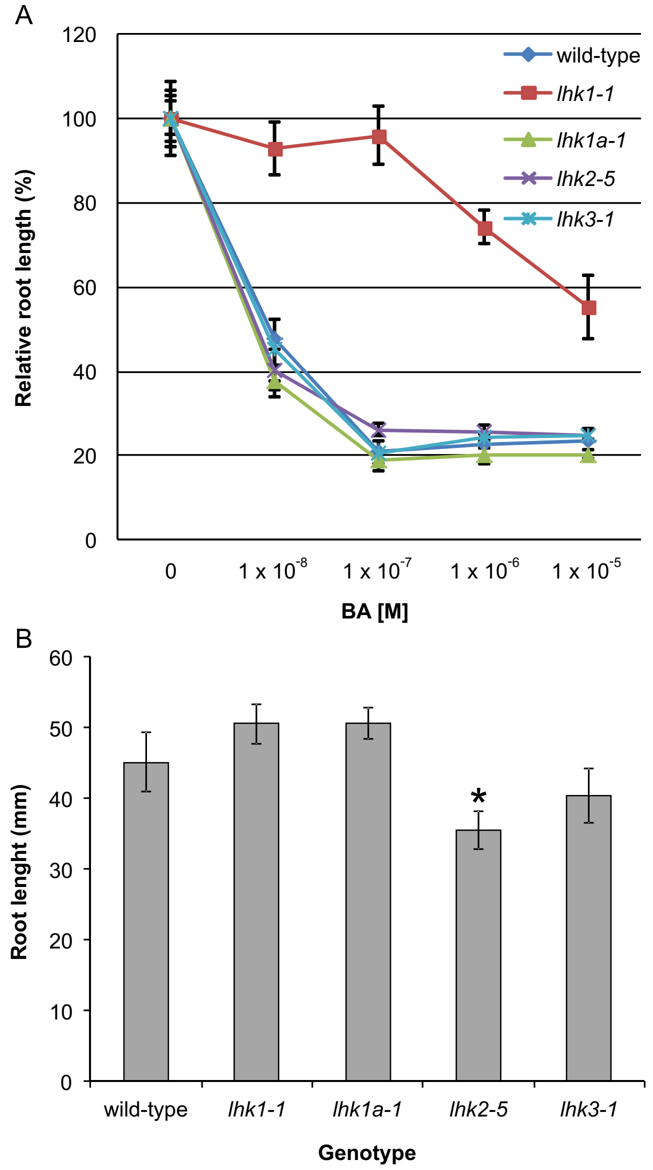
Supplemental Figure 3. Amino-Acid Sequence Alignment of the Receiver Domains, as Predicted for *L. japonicus* LHK Proteins and *Arabidopsis* AHK4. The conserved DD, D, and K motifs are indicated. An asterisk denotes the presence of a conserved aspartic acid (D) residue required for the phosphorelay. The alignment was created as described in the legend for the supplemental Figure 1.



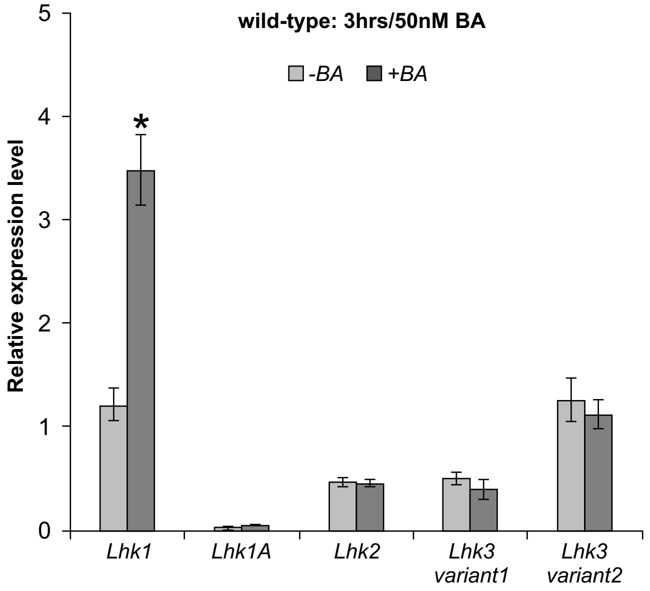
Supplemental Figure 4. Alternative Splicing of *Lhk3*. (**A**) Alternative splicing at the intron 1/exon 2 junction of the *Lhk3* locus results in the production of two *Lhk3* splice variants (named *Lhk3* - splice variant #1 (dashed line) and #2, differing by 228bp (green box). (**B**) RT-PCR and transcript-specific primers were used to detect and sequence these two splice variants from the *L. japonicus* nodule RNA.



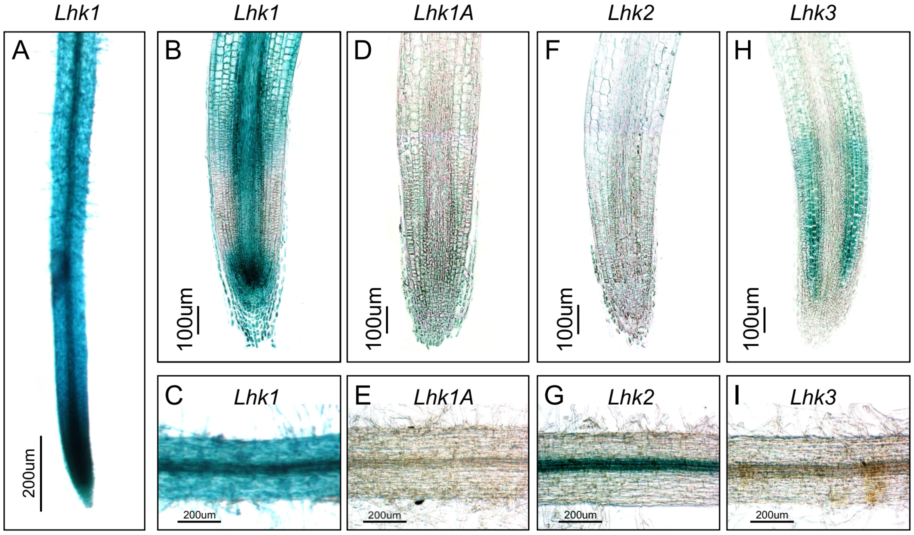
Supplemental Figure 5. Bacterial Infection and Nodule Formation are Mostly Unaffected by *lhk1a-1*, *lhk2-5* and *lhk3-1* Mutations. (**A**) The number of microcolonies and infection threads was scored in *lhk* single mutants at 7 dai with the *M. loti* strain tagged with the *hemA*::*LacZ* reporter and this was compared to the *L. japonicus* Gifu wild-type control. In all cases, values reported are the mean ± 95% CI (n = 10). (**B**) Nodule and nodule primordia formation in *lhk1-1*, *lhk1a-1*, *lhk2-5*, and *lhk3-1* single and the corresponding double mutants were scored 21 dai. In all cases, values reported represent the mean ± 95% CI (n = 29-50). An asterisk denotes significant differences between the mutant and wild-type control (Student's t-Test, P<0.05).



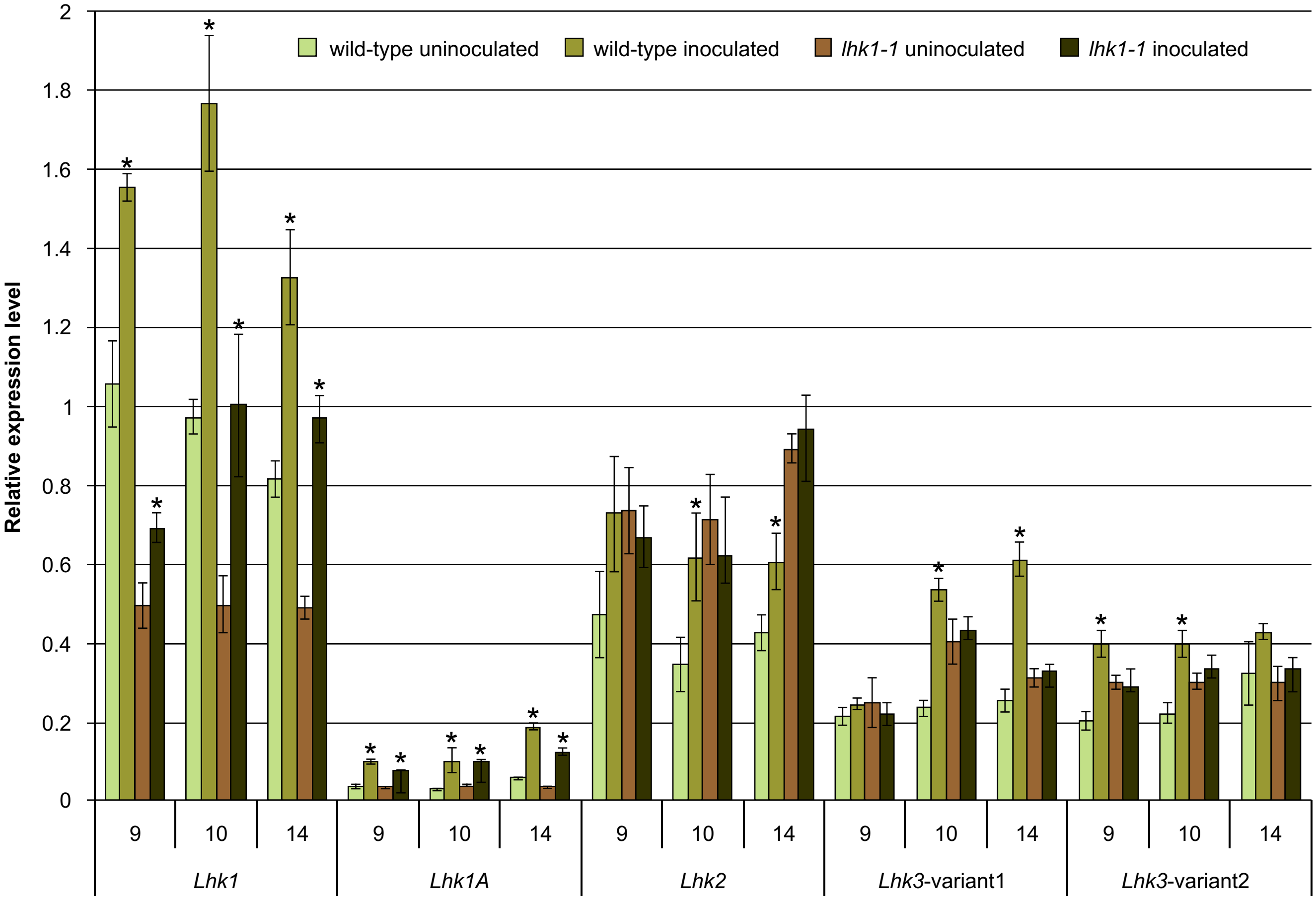
Supplemental Figure 6. Responses of the *L. japonicus* Wild-Type and *lhk* Mutant Roots to Exogenous Cytokinin and Root Growth. (**A**) Plants were grown on the surface of agar plates in the dark, in the presence of increasing concentrations of cytokinin (BA). The root length was measured 7 days after sowing. The relative root length is given, where the length of control roots that were grown in the absence of BA was set as 100%. BA: 6-benzylaminopurine. (**B**) The average length of control, untreated roots for given genotypes are shown. Values in panels (**A**) and (**B**) represent the mean ± 95% CI ($n \geq 10$). An asterisk denotes significant difference with the wild-type value (Student's t-Test, $P<0.05$).



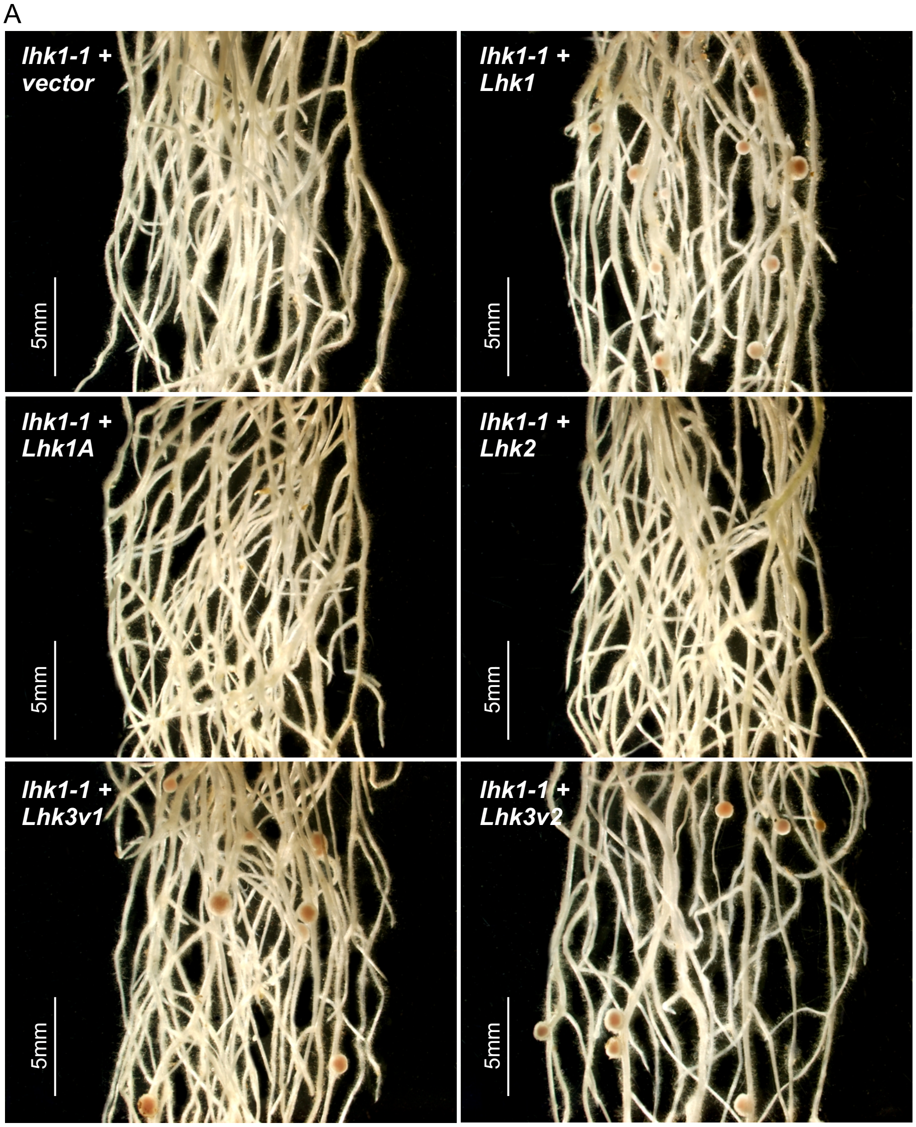
Supplemental Figure 7. Ectopic cytokinin Increases the Steady-State Level of the *Lhk1* mRNA. The relative steady-state levels of *Lhk1, 1A, 2 and 3* transcripts in un-treated (-BA) and cytokinin treated (+BA) *L. japonicus* roots are given. Values (mean ± SE of three biological replicates) for *L. japonicus* wild-type roots that were incubated in H_2O or H_2O supplemented with 50nM BA for 3 hours are shown. An asterisk denotes significant difference (Student's t-Test, $P<0.05$).



Supplemental Figure 8. Activities of *Lhk1, 1A, 2* and *3* Promoters in Uninoculated *L. japonicus* Roots. Whole mounts (**A, C, E, G** and **I**) and 35 µm thick longitudinal sections (**B, D, F** and **H**) are shown. *Lhk1*$_{pro}$*:GUS* activity in *L. japonicus* root (**A**), root apical region (**B**) and a root segment within a zone susceptible to *M. loti* infection approx. 2-5 mm above the root tip (**C**). Activities of *Lhk1A*$_{pro}$*:GUS* (**D** and **E**)**,** *Lhk2*$_{pro}$*::GUS* (**F** and **G**) and *Lhk3*$_{pro}$*:GUS* (**H** and **I**) reporter constructs in the corresponding apical and root susceptible zones. All images represent specimens collected at 7 or 14 days after sowing.



Supplemental Figure 9. All Four *Lhks* Respond to *M. loti* Inoculation. The relative steady-state levels of all four *Lhk* transcripts in uninoculated roots and roots of the same age harvested 9, 10 and 14 days after sowing are given. Note that plants were inoculated with *M. loti* 7 days after sowing such that each of the time points (i.e. 9, 10 and 14) reflect the measurements at 2, 3 and 7 dai, respectively, for the inoculated samples. Average values for three biological replicates ± SE are given. Asterisks denote significant differences between the corresponding inoculated and un-inoculated samples (Student's t-Test, $P<0.05$).



B

	Total # of plants	# Nodulated plants	Nodules/ nodulated plant
lhk1-1 + vector	32	0	0
lhk1-1 + Lhk1	20	16	17.8 ± 2.0
lhk1-1 + Lhk1A	29	0	0
lhk1-1 + Lhk2	3	0	0
lhk1-1 + Lhk3 variant #1	30	25	15.8 ± 2.7
lhk1-1 + Lhk3 variant #2	21	10	5.8 ± 1.4

Supplemental Figure 10. *Lhk3* Functionally Replaces *Lhk1*. The five *Lhk* cDNAs (*Lhk1*, *Lhk1A*, *Lhk2*, *Lhk3v1* and *Lhk3v2*) were expressed under the control of *CaMV* 35S promoter in the *lhk1-1* mutant background via *A. rhizogenes*-mediated hairy-root transformation. (**A**) Representative images of transgenic hairy roots are shown at 21 dai with *M. loti*. (**B**) Quantitative data for plants used and nodule frequency obtained are given. For *Lhk2*, only 3 plants were scored due to an apparent suppression of hairy root formation. In all cases, values represent the mean ± 95% CI (confidence interval).

Supplemental Table 1. Amino Acid Conservation among *L. japonicus* LHK Proteins and with Their Presumed *Arabidopsis* Counterparts.

Name	Length	Name	Length	Identity (%)	Similarity (%)
LHK1	993	LHK1A	997	80	86
LHK1	993	LHK2	1218	52	67
LHK1	993	LHK3	986	49	66
LHK1	993	AHK4	1057	68	78
LHK1A	997	LHK2	1218	53	68
LHK1A	997	LHK3	986	51	68
LHK1A	997	AHK4	1057	69	79
LHK2	1218	LHK3	986	54	69
LHK2	1218	AHK2	1176	59	71
LHK3	986	AHK3	1036	68	80

Supplemental Table 2. A list of *lhk1a*, *2* and *3* Mutant Alleles as Identified by a TILLING Approach. gDNA: genomic DNA mutation for a given locus; ORF change: the resulting alteration to the open reading frame. Adenine in the predicted ATG initiation codon was set as 1 for defining the position of a given mutation in gDNA. Similarly, a predicted initiating methionine residue was set as 1 to calculate the position of any given amino-acid change.

Line number	Allele name	Mutation (gDNA)	ORF change
SL761-1	*lhk1a-1*	G_{4439} - A	W_{565} - STOP
SL1481-1	*lhk1a-2*	C_{4808} - T	L_{689} - F
SL3496-1	*lhk1a-3*	C_{4808} - T	L_{689} - F
SL4275-1	*lhk1a-4*	G_{4725} - A	G_{661} - E
SL5603-1	*lhk1a-5*	G_{4929} - A	S_{729} - N
SL4559-1	*lhk1a-6*	C_{4388} - T	H_{549} - T
SL871-1	*lhk1a-7*	G_{3934} - A	*None - Intronic*
SL1169-1	*lhk1a-8*	C_{4504} - T	*None - synonomous*
SL3064-1	*lhk1a-9*	C_{4031} - T	*None - Intronic*
SL4389-1	*lhk1a-10*	G_{4933} - A	*None - synonomous*

Line number	Allele name	Mutation (gDNA)	ORF change
SL4340-1	*lhk2-1*	G_{5499} - A	A_{517} - T
SL4236-1	*lhk2-2*	G_{5532} - A	D_{528} - N
SL404-1	*lhk2-3*	G_{5547} - A	E_{533} - K
SL577-1	*lhk2-4*	G_{5551} - A	G_{534} - D
SL965-1	*lhk2-5*	G_{5769} - A	G_{605} - R
SL1482-1	*lhk2-6*	G_{5817} - A	A_{621} - T
SL2025-1	*lhk2-7*	G_{5836} - A	R_{627} - Q
SL5335-1	*lhk2-8*	G_{5899} - A	G_{648} - E
SL80-1	*lhk2-9*	G_{5908} - A	G_{651} - E

Line number	Allele name	Mutation (gDNA)	ORF change
SL390-1	*lhk2-10*	G_{6051} - *A*	D_{699} - *N*
SL1428-1	*lhk2-11*	C_{5645} - *T*	*None - synonomous*
SL1535-1	*lhk2-12*	C_{5248} - *T*	*None - Intronic*
SL314-1	*lhk2-13*	C_{5335} - *T*	*None - synonomous*
SL6570-1	*lhk2-14*	C_{5691} - *T*	*None - synonomous*
SL5401-1	*lhk2-15*	G_{5260} - *A*	*None - Intronic*

Line number	Allele name	Mutation (gDNA)	ORF Change
SL201-1	*lhk3-1*	G_{4558} - A	R_{561} - Q
SL577-1	*lhk3-2*	G_{5219} - A	V_{596} - I
SL1972-1	*lhk3-3*	G_{6057} - A	S_{875} - N
SL780-1	*lhk3-4*	C_{4782} - T	None - Intronic
SL1767-1	*lhk3-5*	G_{4973} - A	None - Intronic