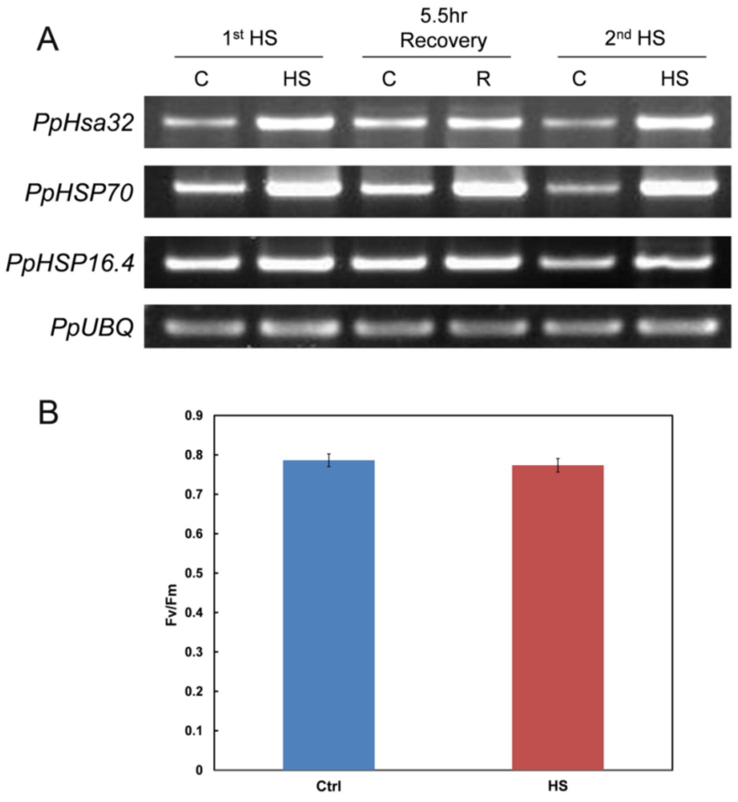
SUPPLEMENTARY FIGURES AND TABLES

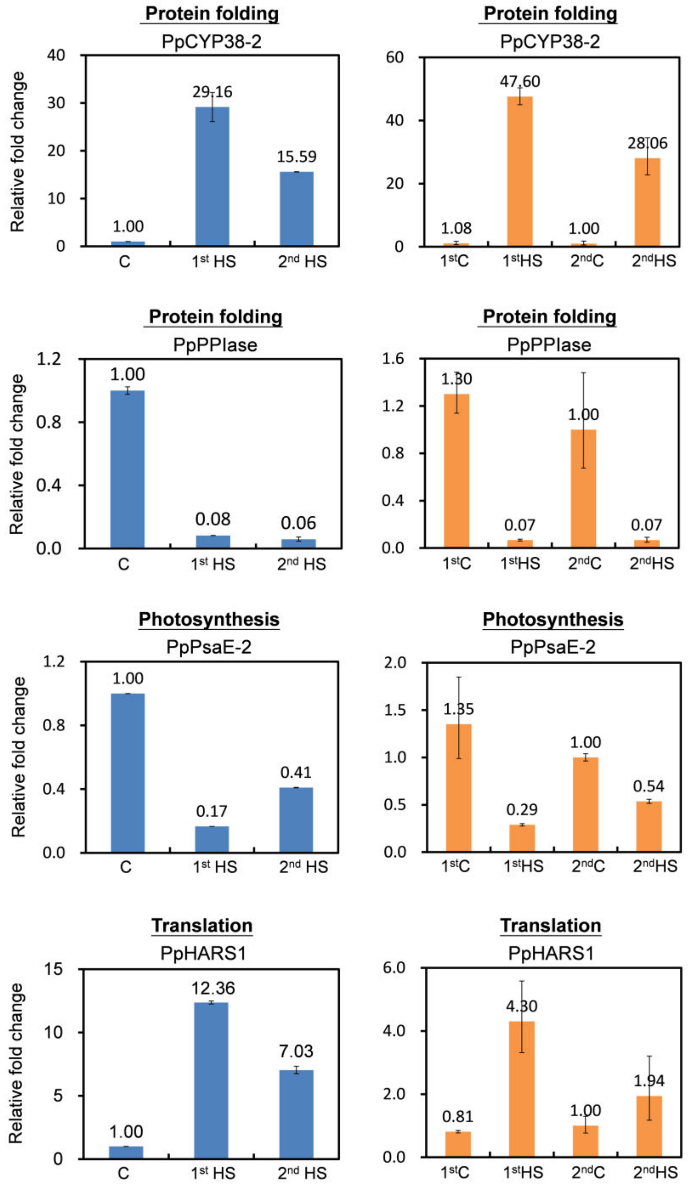
Genome-wide analysis of heat-sensitive alternative splicing in *Physcomitrella patens*

Chiung-Yun Chang, Wen-Dar Lin and Shih-Long Tu

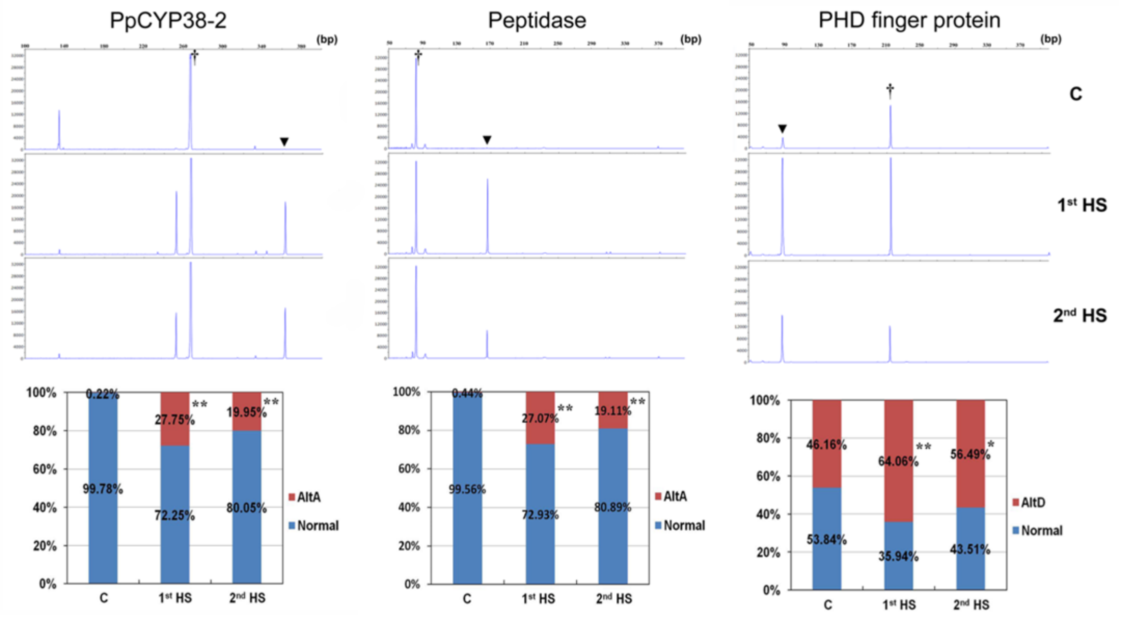


Supplemental Figure S1. Confirmation of heat shock (HS) response in *Physcomitrella*. *(A)* Expression of HS marker genes. Pooled RNA from control (C), 1st HS, 5.5 h recovery, and 2nd HS samples were used for RT-PCR reaction. Primer sets specific for *PpHsa32, PpHSP70, PpHSP16.4,* and *PpUBQ* transcripts were used. *PpUBQ* was used as a control. C, control; HS, HS-treated sample; R, 5.5 h recovered sample. *(B)* Photosynthetic efficiency of HS-treated samples. Chlorophyll *a* fluorescence of protonema was measured immediately after 2nd HS treatment. Protonema grown under normal temperature was used as the control (Ctrl).

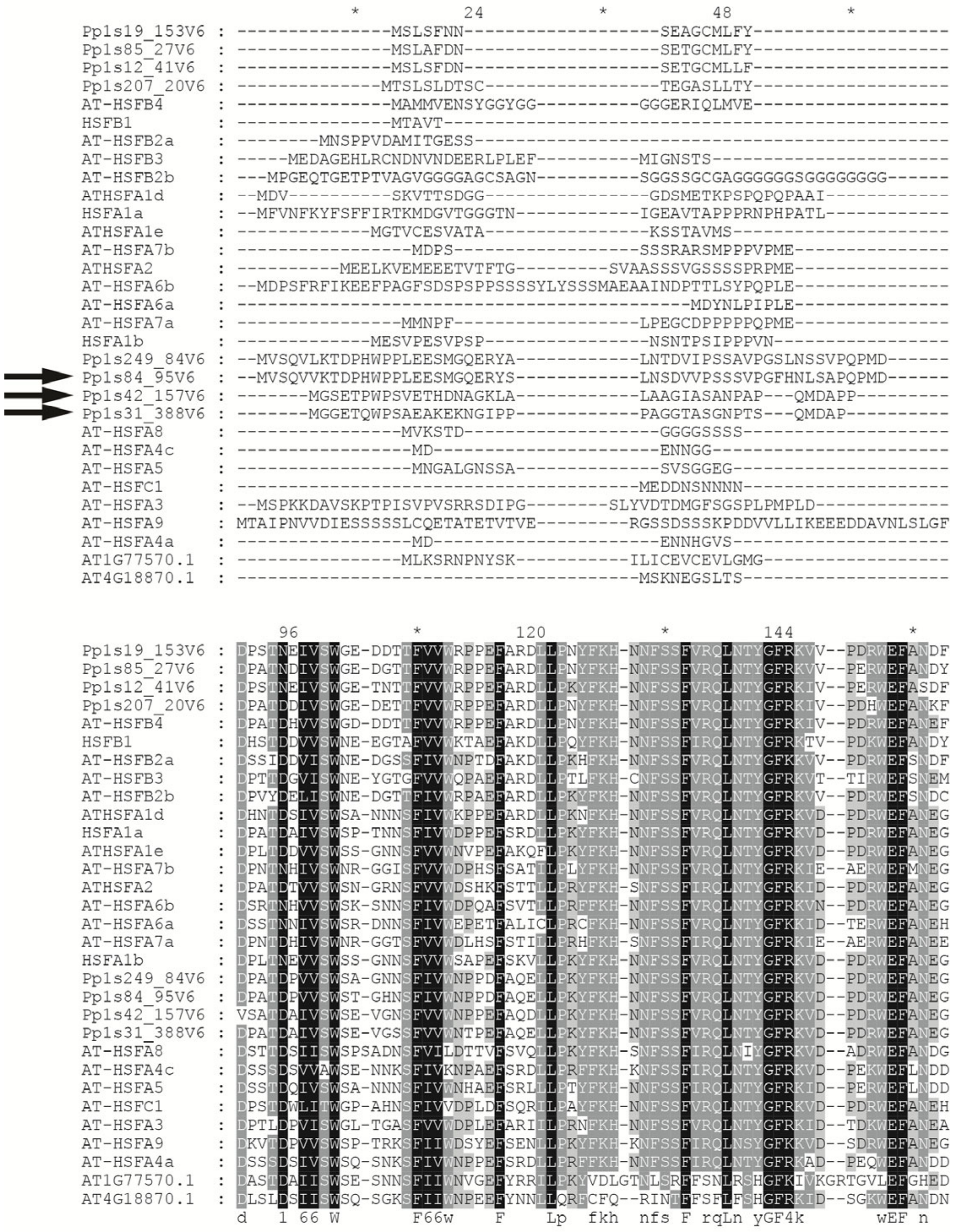
Supplemental Figure S2. Comparison of genes differentially expressed and alternatively spliced in response to 1st HS and 2nd HS. Venn diagrams show the comparison of differential expressed genes (DEGs) and heat-sensitive IR (HS-IR), AltDA (HS-AltDA) and ES (HS-ES) genes identified in 1st HS and 2nd HS samples. Numbers of DEGs and heat-sensitive AS genes are listed under each set. Numbers of specific and overlapped genes in each diagram are indicated.



Supplemental Figure S3. Validation of HS-IR events. qRT-PCR data from pooled RNA (also in the manuscript) for 4 genes were shown in the left panels (blue bars), where "C" indicates the untreated control of 2nd HS (2nd C in the right panels). qRT-PCR data from unpooled RNAs were shown in the right panels (orange bars). *PpACT2* was first used as an internal control for normalizing each qPCR reaction. Level of the IR isoforms from three biological replicates were further normalized for overall expression level of each gene and then compared with data from the control of 2nd HS (2nd C) to generate the relative IR level. Corresponding gene products of selected IR events and representative processes are shown above each graph.



Supplemental Figure S4. Validation of heat-regulated AltD and AltA sites. High-resolution RT-PCR was used for determining the expression level of selected AltD and AltA isoforms. Pooled RNA from control (C), 1st HS and 2nd HS samples were used in triplicate for RT-PCR. Primer sets designed for amplifying the AltD and AltA regions were used. DNA fragments were separated on an ABI3730 DNA Analyzer with 3 technical repeats. Integrated peak areas of RT-PCR products identified with expected sizes were used as the relative expression level. Percentage of each AS isoform was calculated by dividing by the sum of all transcripts. Significance: ** P< 0.01; * 0.01>P<0.05. †, PCR products from constitutive spliced isoforms; ▼, PCR products from alternatively spliced isoforms. Gene IDs and annotations are in Supplemental Dataset S9.



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                          *          24           *          48           *
Pp1s19_153V6 : ---------------MSLSFNN-------------------SEAGCMLFY-------------------
Pp1s85_27V6  : ---------------MSLAFDN-------------------SETGCMLFY-------------------
Pp1s12_41V6  : ---------------MSLSFDN-------------------SETGCMLLF-------------------
Pp1s207_20V6 : --------------MTSLSLDTSC-----------------TEGASLLTY-------------------
AT-HSFB4     : ---------------MAMMVENSYGGYGG----------GGGERIQLMVE-------------------
HSFB1        : ---------------MTAVT-------------------------------------------------
AT-HSFB2a    : --------MNSPPVDAMITGESS----------------------------------------------
AT-HSFB3     : -----MEDAGEHLRCNDNVNDEERLPLEF----------MIGNSTS-----------------------
AT-HSFB2b    : ---MPGEQTGETPTVAGVGGGGAGCSAGN----------SGGSSGCGAGGGGGGSGGGGGGGG------
ATHSFA1d     : --MDV----------SKVTTSDGG----------------GDSMETKPSPQPQPAAI------------
HSFA1a       : --MFVNFKYFSFFIRTKMDGVTGGGTN------------IGEAVTAPPPRNPHPATL------------
ATHSFA1e     : -------------MGTVCESVATA----------------KSSTAVMS---------------------
AT-HSFA7b    : -----------------MDPS------------------SSSRARSMPPPVPME---------------
ATHSFA2      : ----------MEELKVEMEEETVTFTG---------SVAASSSVGSSSSPRPME---------------
AT-HSFA6b    : --MDPSFRFIKEEFPAGFSDSPSPPSSSSYLYSSSMAEAAINDPTTLSYPQPLE---------------
AT-HSFA6a    : --------------------------------------------MDYNLPIPLE---------------
AT-HSFA7a    : ----------------MMNPF------------------LPEGCDPPPPPQPME---------------
HSFA1b       : -------------MESVPESVPSP----------------NSNTPSIPPPVN-----------------
Pp1s249_84V6 : --MVSQVLKTDPHWPPLEESMGQERYA------------LNTDVIPSSAVPGSLNSSVPQPMD------
Pp1s84_95V6  : --MVSQVVKTDPHWPPLEESMGQERYS------------LNSDVVPSSSVPGFHNLSAPQPMD------
Pp1s42_157V6 : -------MGSETPWPSVETHDNAGKLA------------LAAGIASANPAP---QMDAPP---------
Pp1s31_388V6 : -------MGGETQWPSAEAKEKNGIPP------------PAGGTASGNPTS---QMDAP----------
AT-HSFA8     : ----------------MVKSTD-------------------GGGGSSSS--------------------
AT-HSFA4c    : -----------------MD----------------------ENNGG-----------------------
AT-HSFA5     : -----------------MNGALGNSSA--------------SVSGGEG---------------------
AT-HSFC1     : ---------------------------------------MEDDNSNNNN--------------------
AT-HSFA3     : --MSPKKDAVSKPTPISVPVSRRSDIPG--------SLYVDTDMGFSGSPLPMPLD-------------
AT-HSFA9     : MTAIPNVVDIESSSSSLCQETATETVTVE---------RGSSDSSSKPDDVVLLIKEEEDDAVNLSLGF
AT-HSFA4a    : -----------------MD----------------------ENNHGVS---------------------
AT1G77570.1  : ----------------MLKSRNPNYSK-----------ILICEVCEVLGMG------------------
AT4G18870.1  : ---------------------------------------MSKNEGSLTS--------------------

                   96           *         120           *         144           *
Pp1s19_153V6 : DPSTNEIVSWGE-DDTTFVVWRPPEFARDLLPNYFKH-NNFSSFVRQLNTYGFRKVV--PDRWEFANDF
Pp1s85_27V6  : DPATNDIVSWGE-DGTTFVVWRPPEFARDLLPNYFKH-NNFSSFVRQLNTYGFRKVV--PERWEFANDY
Pp1s12_41V6  : DPSTNEIVSWGE-TNTTFVVWRPPEFARDLLPKYFKH-NNFSSFVRQLNTYGFRKIV--PERWEFASDF
Pp1s207_20V6 : DPATDDIVSWGE-DETTFVVWRPPEFARDLLPNYFKH-NNFSSFVRQLNTYGFRKIV--PDHWEFANKF
AT-HSFB4     : DPATDHVVSWGD-DDTTFVVWRPPEFARDLLPNYFKH-NNFSSFVRQLNTYGFRKIV--PDRWEFANEF
HSFB1        : DHSTDDVVSWNE-EGTAFVVWKTAEFAKDLLPQYFKH-NNFSSFIRQLNTYGFRKTV--PDKWEFANDY
AT-HSFB2a    : DSSIDDVISWNE-DGSSFIVWNPTDFAKDLLPKHFKH-NNFSSFVRQLNTYGFKKVV--PDRWEFSNDF
AT-HSFB3     : DPTTDGVISWNE-YGTGFVVWQPAEFARDLLPTLFKH-CNFSSFVRQLNTYGFRKVT--TIRWEFSNEM
AT-HSFB2b    : DPVYDELISWNE-DGTTFIVWRPAEFARDLLPKYFKH-NNFSSFVRQLNTYGFRKVV--PDRWEFSNDC
ATHSFA1d     : DHNTDSIVSWSA-NNNSFIVWKPPEFARDLLPKNFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
HSFA1a       : DPATDAIVSWSP-TNNSFIVWDPPEFSRDLLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
ATHSFA1e     : DPLTDDVVSWSS-GNNSFVVWNVPEFAKQFLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
AT-HSFA7b    : DPNTNHIVSWNR-GGISFVVWDPHSFSATILPLYFKH-NNFSSFVRQLNTYGFRKIE--AERWEFMNEG
ATHSFA2      : DPATDTVVSWSN-GRNSFVVWDSHKFSTTLLPRYFKH-SNFSSFIRQLNTYGFRKID--PDRWEFANEG
AT-HSFA6b    : DSRTNHVVSWSK-SNNSFIVWDPQAFSVTLLPRFFKH-NNFSSFVRQLNTYGFRKVN--PDRWEFANEG
AT-HSFA6a    : DSSTNNIVSWSR-DNNSFIVWEPETFALICLPRCFKH-NNFSSFVRQLNTYGFKKID--TERWEFANEH
AT-HSFA7a    : DPNTDHIVSWNR-GGTSFVVWDLHSFSTILLPRHFKH-SNFSSFIRQLNTYGFRKIE--AERWEFANEE
HSFA1b       : DPLTNEVVSWSS-GNNSFVVWSAPEFSKVLLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
Pp1s249_84V6 : DPATDPVVSWSA-GNNSFIVWNPPDFAQELLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
Pp1s84_95V6  : DPATDPVVSWST-GHNSFIVWNPPDFAQELLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
Pp1s42_157V6 : VSATDAIVSWSE-VGNSFVVWNPPEFAQDLLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
Pp1s31_388V6 : DPATDAIVSWSE-VGSSFVVWNTPEFAQELLPKYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEG
AT-HSFA8     : DSTTDSIISWSPSADNSFVILDTTVFSVQLLPKYFKH-SNFSSFIRQLNIYGFRKVD--ADRWEFANDG
AT-HSFA4c    : DSSSDSVVAWSE-NNKSFIVKNPAEFSRDLLPRFFKH-KNFSSFIRQLNTYGFRKVD--PEKWEFLNDD
AT-HSFA5     : DSSTDQIVSWSA-NNNSFIVWNHAEFSRLLLPTYFKH-NNFSSFIRQLNTYGFRKID--PERWEFLNDD
AT-HSFC1     : DPSTDWLITWGP-AHNSFIVVDPLDFSQRILPAYFKH-NNFSSFVRQLNTYGFRKVD--PDRWEFANEH
AT-HSFA3     : DPTLDPVISWGL-TGASFVVWDPLEFARIILPRNFKH-NNFSSFVRQLNTYGFRKID--TDKWEFANEA
AT-HSFA9     : DKVTDPVVSWSP-TRKSFIIWDSYEFSENLLPKYFKH-KNFSSFIRQLNSYGFKKVD--SDRWEFANEG
AT-HSFA4a    : DSSSDSIVSWSQ-SNKSFIVWNPPEFSRDLLPRFFKH-NNFSSFIRQLNTYGFRKAD--PEQWEFANDD
AT1G77570.1  : DASTDAIISWSE-SNNSFIIWNVGEFYRRILPKYVDLGTNLSRFFSNLRSHGFKIVKGRTGVLEFGHED
AT4G18870.1  : DLSLDSIISWSQ-SGKSFIIWNPEEFYNNLLQRFCFQ--RINTFFSFLFSHGFRKID--SGKWEFANDN
               d   1 66 W       F66w    F    Lp  fkh  nfs F rqLn yGF4k       wEF n
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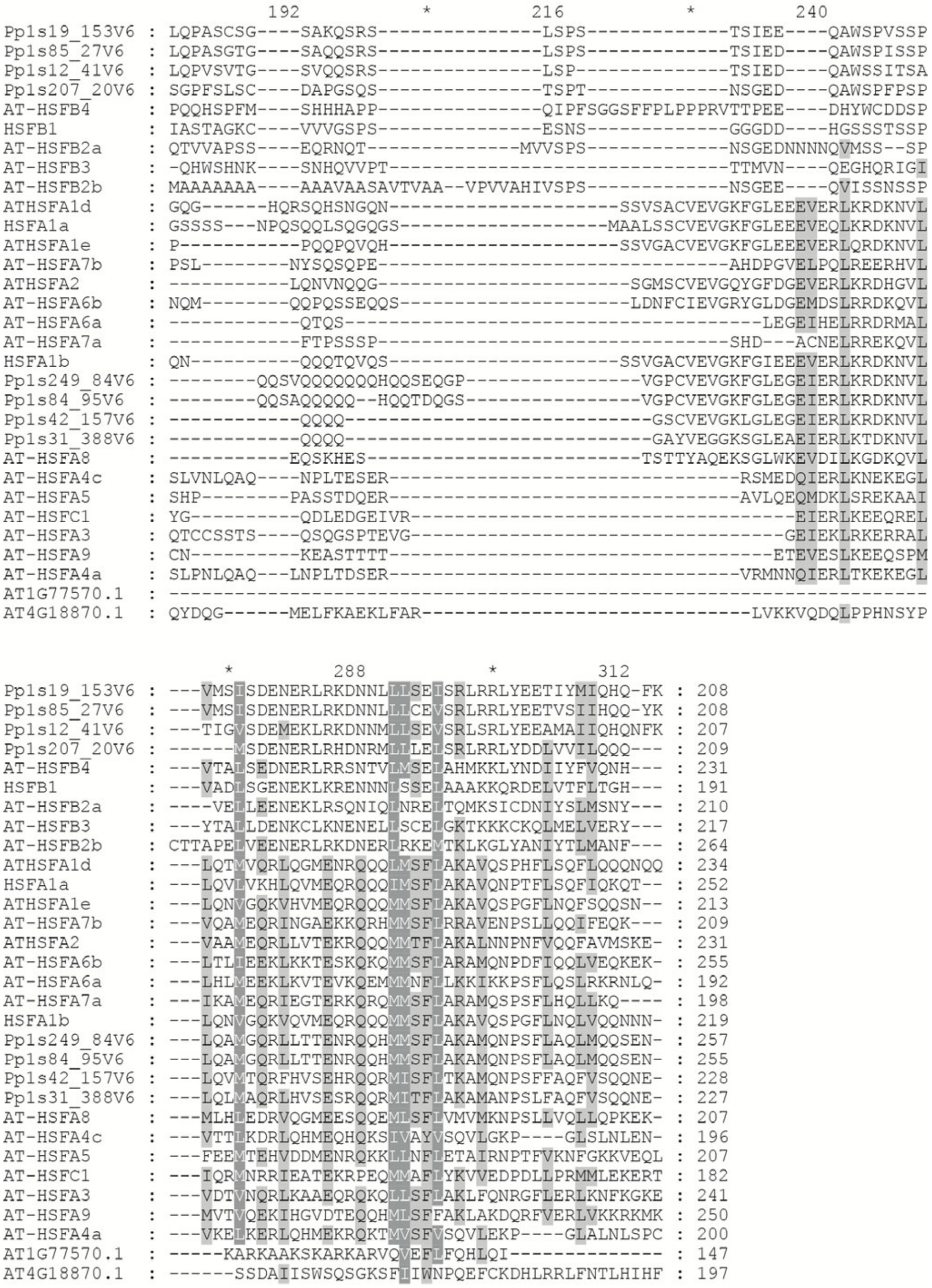

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Pp1s85_27V6  : LQPASGTG----SAQQSRS---------------LSPS-------------TSIED----QAWSPISSP
Pp1s12_41V6  : LQPVSVTG----SVQQSRS---------------LSP--------------TSIED----QAWSSITSA
Pp1s207_20V6 : SGPFSLSC----DAPGSQS---------------TSPT-------------NSGED----QAWSPFPSP
AT-HSFB4     : PQQHSPFM----SHHHAPP---------------QIPFSGGSFFPLPPPRVTTPEE----DHYWCDDSP
HSFB1        : IASTAGKC----VVVGSPS---------------ESNS-------------GGGDD----HGSSSTSSP
AT-HSFB2a    : QTVVAPSS----EQRNQT--------------MVVSPS-------------NSGEDNNNNQVMSS--SP
AT-HSFB3     : -QHWSHNK----SNHQVVPT-------------------------------TTMVN----QEGHQRIGI
AT-HSFB2b    : MAAAAAAA----AAAVAASAVTVAA--VPVVAHIVSPS-------------NSGEE----QVISSNSSP
ATHSFA1d     : GQG------HQRSQHSNGQN---------------------SSVSACVEVGKFGLEEEVERLKRDKNVL
HSFA1a       : GSSSS---NPQSQQLSQGQGS-------------------MAALSSCVEVGKFGLEEEVEQLKRDKNVL
ATHSFA1e     : P-----------PQQPQVQH---------------------SSVGACVEVGKFGLEEEVERLQRDKNVL
AT-HSFA7b    : PSL--------NYSQSQPE--------------------------------AHDPGVELPQLREERHVL
ATHSFA2      : -----------LQNVNQQG-----------------------SGMSCVEVGQYGFDGEVERLKRDHGVL
AT-HSFA6b    : NQM--------QQPQSSEQQS---------------------LDNFCIEVGRYGLDGEMDSLRRDKQVL
AT-HSFA6a    : ------------QTQS--------------------------------------LEGEIHELRRDRMAL
AT-HSFA7a    : ------------FTPSSSP--------------------------------SHD---ACNELRREKQVL
HSFA1b       : QN----------QQQTQVQS---------------------SSVGACVEVGKFGIEEEVERLKRDKNVL
Pp1s249_84V6 : --------QQSVQQQQQQHQQSEQGP-----------------VGPCVEVGKFGLEGEIERLKRDKNVL
Pp1s84_95V6  : --------QQSAQQQQQ--HQQTDQGS----------------VGPCVEVGKFGLEGEIERLKRDKNVL
Pp1s42_157V6 : ------------QQQQ----------------------------GSCVEVGKLGLEGEIERLKRDKNVL
Pp1s31_388V6 : ------------QQQQ----------------------------GAYVEGGKSGLEAEIERLKTDKNVL
AT-HSFA8     : -----------EQSKHES-------------------------TSTTYAQEKSGLWKEVDILKGDKQVL
AT-HSFA4c    : SLVNLQAQ----NPLTESER--------------------------------RSMEDQIERLKNEKEGL
AT-HSFA5     : SHP--------PASSTDQER--------------------------------AVLQEQMDKLSREKAAI
AT-HSFC1     : YG----------QDLEDGEIVR-----------------------------------EIERLKEEQREL
AT-HSFA3     : QTCCSSTS----QSQGSPTEVG----------------------------------GEIEKLRKERRAL
AT-HSFA9     : CN----------KEASTTTT-----------------------------------ETEVESLKEEQSPM
AT-HSFA4a    : SLPNLQAQ---LNPLTDSER--------------------------------VRMNNQIERLTKEKEGL
AT1G77570.1  : ---------------------------------------------------------------------
AT4G18870.1  : QYDQG------MELFKAEKLFAR------------------------------LVKKVQDQLPPHNSYP
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                    *         288           *         312
Pp1s19_153V6 : ---VMSISDENERLRKDNNLLLSEISRLRRLYEETIYMIQHQ-FK : 208
Pp1s85_27V6  : ---VMSISDENERLRKDNNLLLCEVSRLRRLYEETVSIIHQQ-YK : 208
Pp1s12_41V6  : ---TIGVSDEMEKLRKDNNMLLSEVSRLSRLYEEAMAIIQHQNFK : 207
Pp1s207_20V6 : ------MSDENERLRHDNRMLLLELSRLRRLYDDLVVILQQQ--- : 209
AT-HSFB4     : ---VTALSEDNERLRRSNTVLMSELAHMKKLYNDIIYFVQNH--- : 231
HSFB1        : ---VADLSGENEKLKRENNNLSSELAAAKKQRDELVTFLTGH--- : 191
AT-HSFB2a    : ----VELLEENEKLRSQNIQLNRELTQMKSICDNIYSLMSNY--- : 210
AT-HSFB3     : ---YTALLDENKCLKNENELLSCELGKTKKKCKQLMELVERY--- : 217
AT-HSFB2b    : CTTAPELVEENERLRKDNERLRKEMTKLKGLYANIYTLMANF--- : 264
ATHSFA1d     : ---LQTMVQRLQGMENRQQQLMSFLAKAVQSPHFLSQFLQQQNQQ : 234
HSFA1a       : ---LQVLVKHLQVMEQRQQQIMSFLAKAVQNPTFLSQFIQKQT-- : 252
ATHSFA1e     : ---LQNVGQKVHVMEQRQQQMMSFLAKAVQSPGFLNQFSQQSN-- : 213
AT-HSFA7b    : ---VQAMEQRINGAEKKQRHMMSFLRRAVENPSLLQQIFEQK--- : 209
ATHSFA2      : ---VAAMEQRLLVTEKRQQQMMTFLAKALNNPNFVQQFAVMSKE- : 231
AT-HSFA6b    : ---LTLIEEKLKKTESKQKQMMSFLARAMQNPDFIQQLVEQKEK- : 255
AT-HSFA6a    : ---LHLMEEKLKVTEVKQEMMMNFLLKKIKKPSFLQSLRKRNLQ- : 192
AT-HSFA7a    : ---IKAMEQRIEGTERKQRQMMSFLARAMQSPSFLHQLLKQ---- : 198
HSFA1b       : ---LQNVGQKVQVMEQRQQQMMSFLAKAVQSPGFLNQLVQQNNN- : 219
Pp1s249_84V6 : ---LQAMGQRLLTTENRQQHMMSFLAKAMQNPSFLAQLMQQSEN- : 257
Pp1s84_95V6  : ---LQAMGQRLLTTENRQQHMMSFLAKAMQNPSFLAQLMQQSEN- : 255
Pp1s42_157V6 : ---LQVMTQRFHVSEHRQQRMISFLTKAMQNPSFFAQFVSQQNE- : 228
Pp1s31_388V6 : ---LQLMAQRLHVSESRQQRMITFLAKAMANPSLFAQFVSQQNE- : 227
AT-HSFA8     : ---MLHLEDRVQGMEESQQEMLSFLVMVMKNPSLLVQLLQPKEK- : 207
AT-HSFA4c    : ---VTTLKDRLQHMEQHQKSIVAYVSQVLGKP----GLSLNLEN- : 196
AT-HSFA5     : ---FEEMTEHVDDMENRQKKLLNFLETAIRNPTFVKNFGKKVEQL : 207
AT-HSFC1     : ---IQRMNRRIEATEKRPEQMMAFLYKVVEDPDLLPRMMLEKERT : 182
AT-HSFA3     : ---VDTVNQRLKAAEQRQKQLLSFLAKLFQNRGFLERLKNFKGKE : 241
AT-HSFA9     : ---MVTVQEKIHGVDTEQQHMLSFFAKLAKDQRFVERLVKKRKMK : 250
AT-HSFA4a    : ---VKELKERLQHMEKRQKTMVSFVSQVLEKP----GLALNLSPC : 200
AT1G77570.1  : -----KARKAAKSKARKARVQVEFLFQHLQI-------------- : 147
AT4G18870.1  : ------SSDAIISWSQSGKSFIIWNPQEFCKDHLRRLFNTLHIHF : 197
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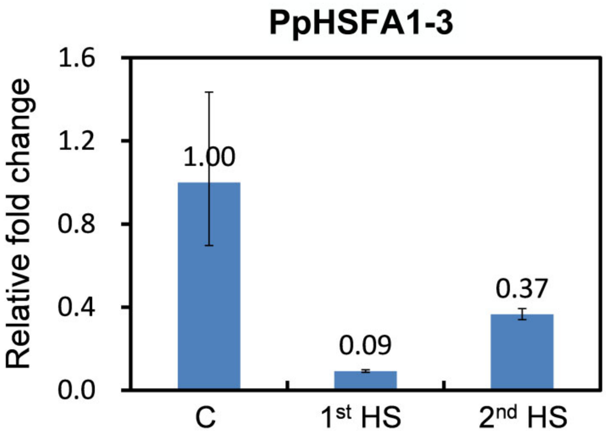
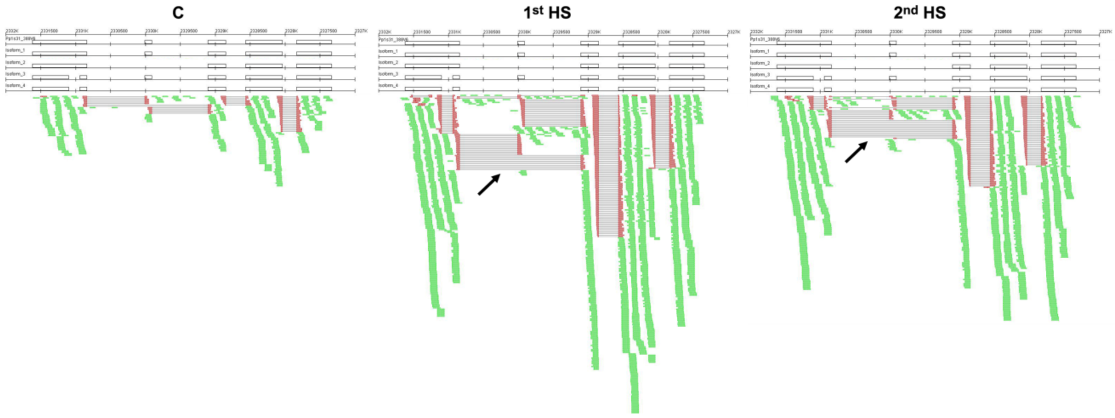
Supplemental Figure S5. Multiple sequence alignment of *Arabidopsis* and *Physcomitrella* heat shock transcription factors (HSFs). DBD and HR-A/B domains of HSF proteins derived from *Arabidopsis thaliana* (AT) and *Physcomitrella patens* (Pp) were used. The genes annotated as *PpHSFA1-1* (Pp1s31_388V6), *PpHSFA1-2* (Pp1s42_157V6) and *PpHSFA1-3* (Pp1s84_95V6) in this study is indicated by arrows.



Supplemental Figure S6. Validation of heat-regulated IR on *PpHSFA1-3* transcripts. Pooled RNA from control (C), 1st HS and 2nd HS samples was analyzed in triplicate for quantitative RT-PCR (qRT-PCR). Primer sets designed for *PpHSFA1-3* IR isoforms, total transcripts of the corresponding gene, and the *PpACT2* were used. *PpACT2* was first used as an internal control for normalizing each qPCR reaction. Level of the IR isoform were further normalized for overall expression level and then compared with data from the HS control to generate the relative IR level. Annotations and gene IDs are in Supplemental Dataset S9.



Supplemental Figure S7. Mapping results for *PpHSFA1-1* from control (C), 1st HS and 2nd HS samples. Arrows indicate reads supporting exon 3 (E3) skipping.

Supplemental Table S1. Mapping statistics of RNA-sequencing

	C	**1st HS**	**2nd HS**	**Total**
Sequence reads	32,458,315	42,737,188	42,727,285	117,922,788
Mapped reads	22,455,889	23,692,513	21,633,044	67,781,446
Mapping percentage (%)	69.18	55.44	50.63	57.48
Intragenic reads	20,568,144	21,327,252	19,778,224	61,673,620
Exonic reads	15,606,490	16,654,203	15,144,324	47,405,017
Intronic reads	288,738	214,236	209,328	712,302
Splice junction reads	4,127,768	3,972,194	3,949,534	12,049,496
Exon-intron bridge reads	545,148	486,619	475,038	1,506,805
Intergenic reads	1,887,745	2,365,261	1,854,820	6,107,826

Supplemental Table S2. Splicing junctions (SJs) and alternative splicing events identified from BLAT and TopHat mapping tools.

	BLAT				TopHat			
	All	**C**	**1st HS**	**2nd HS**	**All**	**C**	**1st HS**	**2nd HS**
SJs	124,713	89,866	87,339	81,730	110,438	83,346	85,354	81,750
IR	37,559	24,711	16,941	17,645	41,007	23,786	17,857	19,265
AltDA	31,953	10,590	13,451	11,276	20,051	7,547	11,483	9,927
ES	1,347	671	730	707	1,414	704	864	857

SJs, Splicing junctions. IR, intron retention. AltD/A, alternative donor/acceptor site. ES, exon skipping.

Supplemental Table S3. Premature termination codon (PTC) prediction of IR events

	HS-IR events
IR events	1279
IR transcripts	2020
IR transcripts with retained intron in protein coding region	1556
PTC in the retained intron	1529
PTC in the downstream region	18
IR transcripts without PTC	9
Percentage of IR transcripts with PTC	98.26%

Supplemental Table S4. Primers used in this study

Gene product	Gene ID	Primer name	Sequence (5' to 3')
RT-PCR: HS marker genes			
PpHsa32	Pp1s251_75V6	Hsa32-F	CCATGGAGGAAAAGCTTATTC
		Hsa32-R	TCACGCAAAGTGGATGCTTCG
sHSP16.4	Pp1s27_332V6	sHsp-F	GCTTCTCGCGATCTTATTCCGCAG
		sHsp-R	CCAGTTGTACCTTCACGTCGGCTG
PpHSP70	Pp1s115_168V6	Hsp70-2-F	CCAAGCTGTAGTGAACCCTG
		Hsp70-2-R	CCTCCGCAGTCGCTGTAATA
PpUBQ	Pp1s46_189V6	PpUBQ-RT-F	CAGCGTCTGATTTTCGCTGG
		PpUBQ-RT-R	CGTCCACTATCAGTACGAAC
qRT-PCR: Intron retention			
KH domain protein	Pp1s87_162V6	87_162 Int2--qF	CCATGTCGTGAGAAAGTGCATT
		87_162 Int2--qR	ACCACCGTGCTGTTGGTTGTAG
		87_162 con--qF	ATCATCAACCTCAACATGGTGG
		87_162 con--qR	CTCCTTTTCCAATAACAAGCCC
PpPTB2	Pp1s326_1V6	Q_02-11_Pp1s326_1V6 (F)	TGACCTGAAGCGTTAGCAACCT
		Q_02-11_Pp1s326_1V6 (R)	CAAAGCCAAAGGCAGAAAACAC
		QC_02-11_Pp1s326_1V6 (F)	ATATTTGCTGCCTGACCACGTT
		QC_02-11_Pp1s326_1V6 (R)	ATGGAGCCACCGGAAGATATG
PpSRP34A	Pp1s28_193V6	28_193Int1-qF	CATGCCCGGTTAGATTTGG
		28_193Int1-qR	AAAGACGTAGGAGCAGTTCGG
		28_193V6-con-qF	TACTCGAAGCCGTTCACGTAGC
		28_193V6-con-qR	AGATCGGTTACGCGGAGACTTG
PpS-ACP-DES	Pp1s354_37V6	354_37 Int3--qF	TTCCAATTTCTTGCAGGTCG
		354_37 Int3--qR	TCTTGCCGTCGACAAATCC
		354_37 con--qF	GGCCGCTTGGAAGTAGTTATG
		354_37 con--qR	TGTGTCTCCGTTGGTCGTTTA
PpRPL37aB	Pp1s76_74V6	Pp1s76_74V6-q2F	GGCAGGGTGTTGTATGTGTTG
		Pp1s76_74V6-q2R	CAATTCCGGCTTTCTTCGTG
		Pp1s76_74V6-qCF	TCACTATGACTAAGCGCACGAA
		Pp1s76_74V6-qCR	CCTCAAACTGGCACCATAACG

Pp EF1B/RPS6	Pp1s359_29V6	Pp1s359_29V6-qF	TTTGTGGATGTGTAGGCCATCA
		Pp1s359_29V6-qR	CCCCTGTTGAACATCTCAACGT
		Pp1s359_29V6-qCF	ACCAAGCCAGAGAGCCAAAAG
		Pp1s359_29V6-qCR	ATCATCGTCCCGGTTCAGTCT
PpHARS1	Pp1s38_401V6	38_401 Int11--qF	TGCTTT CACAGTGTTCTTCCGT
		38_401 Int11--qR	ATCGCAAGAGATCGTCGAACAG
		38_401 con--qF	TTTATACCCGCAAAGCTGGTGA
		38_401 con--qR	TTCTCCCACCTTTGTCCTCAA
PpATG8	Pp1s209_115V6	209_115 Int4--qF	ATATGAGGCGCAGAAAACTGG
		209_115 Int4--qR	AAACCCTACAAGCGCCCAT
		209_115 con--qF	TCAAGCAAGAGCATCATCTGGA
		209_115 con--qR	TGTCGCTTTTCTCCGCCTT
PpRPS27	Pp1s63_161V6	Q_03-1_Pp1s63_161V6 (F)	GCTTATGTCCGTCTTGTGTTGC
		Q_03-1_Pp1s63_161V6 (R)	AAGCATCCTTGACACTTCACATC
		QC_03-1_Pp1s63_161V6 (F)	GAAGTGTCAAGGATGCTTCAGC
		QC_03-1_Pp1s63_161V6 (R)	AAGCACTGTGGAGCAACTTCC
PpCYP38-2	Pp1s90_50V6	90_50 Int1--qF	CGCCTCCTCGGATATGAATTC
		90_50 Int1--qR	GGTGCGCCATAGAGATTGAAGT
		90_50 con--qF	CTGGCAAAGTGCGAACTGTTC
		90_50 con--qR	TGTTGCCTTGTACTTGCCAAG
PpPPIase	Pp1s46_106V6	46_106 Int9--qF	GGAGAAGAAACAGCGCAGTGTG
		46_106 Int9--qR	GCACTCCACACTACTTCCTGCA
		46_106 con--qF	CCGTGCTTTTGGGAATGAG
		46_106 con--qR	CAAGCTCCTCTTTCTCCCAACA
PpRING/U-box	Pp1s157_62V6	62 Ubox Int1--qF	TGATGTGAAGTGTGCATTGCTG
		62 Ubox Int1--qR	GACAGAAACCGCATTCCAATTC
		62 Ubox con--qF	CAAACGCCGATTTCGAAGAG
		62 Ubox con--qR	CATACGCCTTTTGAGCAGCAT
Pp PsaE-2	Pp1s334_17V6	Pp1s334_17V6-qF	TTTAACGACACCGGCAAGGTT
		Pp1s334_17V6-qR	AACGTCGAGGAGGAGATGGAAC
		Pp1s334_17V6-qCF	TGTCAAGGAAGAGGCTAAGCCA
		Pp1s334_17V6-qCR	CCTCAGAACCTTAACGACGCTG
PpUROD	Pp1s114_123V6	Pp1s114_123V6-qF	GCTGCGATGGTTCTGTTGTACA
		Pp1s114_123V6-qR	GCCAAATCCACATTCTCAGAGC
		Pp1s114_123V6-qCF	TTGCGTGAGGAGGTCGGTAAT

		Pp1s114_123V6-qCR	TGATCCGCCCTCGACTATGTAG
PpTBP1	Pp1s246_34V6	TBP Int7--qF	CTGTGGACCTTGCCAAACATC
		TBP Int7--qR	CAAGAACCAACCCCAACTTCC
		TBP con--qF	CGCATCATTCAAAAGCTTGG
		TBP con--qR	GGAACTTCACGTCACATGATCC
PpRBCS2B	Pp1s251_44V6	Pp1s251_44V6-qF	CTCTTACTTGCCCCCATTGTCC
		Pp1s251_44V6-qR	AGAGACAGGGGACTTACCACGT
		Pp1s251_44V6-qCF	GAGTTCCACCTTGTTCTCCAGC
		Pp1s251_44V6-qCR	GAACTTCGTCATGCCAATGG
PpHSFA1-3	Pp1s84_95V6	84_95 Int2---qF	GATTACGGCAACAGCAGCAGA
		84_95 Int2---qR	GCGGAAAATTCAAGTACCTGCA
		84_95 Con---qF	AACTTTTCCAGCTTCGTTCGG
		84_95 Con---qR	CCGGATCAACCTTACGGAATC
High-resolution RT-PCR			
Peptidase	Pp1s47_94V6	47_94FAM---F	CATTTACGGCACACGCTCTCC
		47_94---R	CGCCAATACTGGTGAATCAGC
PpCYP38-2	Pp1s90_50V6	90_50---F	GACAACCCGAATGTGAAAGATG
		90_50 FAM---R	CTCAAGCGTGGCACCGTAG
PHD finger protein	Pp1s384_37V6	384_37FAM---F	ACCACTATGACGTGCAGAGC
		384_37---R	CTGTTGCATTCCAGCATCAC
PpHSFA1-1	Pp1s31_388V6	31_388E2--F	CATCGGCTGAAGCGAAAGAG
		31_388E2--FAM_R	CTCAATCTCCGCCTCAAGTC