



Supporting Online Material for

TOPLESS Regulates Apical Embryonic Fate in *Arabidopsis*

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Published 9 June 2006, *Science* **312**, 1520 (2006)

DOI: 10.1126/science.1123841

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Materials and methods

Plant stocks and growth conditions

The isolation of *tpl-1* was described previously in the Landsberg *erecta* (Ler) ecotype(4). Plants were grown either on soil or Murashige and Skoog salts media. For controlled temperature experiments plants were grown in a Percival growth chamber. All other plants were grown under greenhouse conditions on a 16 hour light/8 hour dark cycle.

Genetic analysis

tpl-2 through *tpl-6* alleles and *hag1-3,4* were isolated as suppressors of *tpl-1* in a high temperature suppressor screen. *tpl-1* homozygous seeds were treated with 0.2% Ethylmethane Sulphonate and sown to soil at 24°C. Upon flowering, plants were shifted to 28°C and allowed to self. 4,000 individual T2 populations were then screened for suppressors. Quadruple mutants were generated using *tpl-2* in combination with Wisconsin Knockout facility alleles of *TPR1* and *TPR4* (Wassilewskija ecotype) and a Syngenta T-DNA allele of *TPR3* (Ler ecotype). Quadruple mutants were then transformed with a *TPR2* RNAi vector containing 200 base pairs upstream of the stop codon in a modified pHANNIBAL vector(5).

Cloning and sequencing

tpl-1 was mapped to chromosome 1 using PCR based CAPS markers (4, 6). A 30 base pair deletion was found in the Ler ecotype in the last intron of *at1g15750* and used as a marker with primers TCATTAGCTCCAATCACTCATGC and GGATGTACGTTTGAATTGCTGCAC. 0/1200 chromosomes were found to be recombinant at this locus. *At1g15750* was amplified from homozygous *tpl-1* plants using

primers ATTGCAGATCTGGTGATAGTTTG and AATCAGAGAGGTAGGTGGCTCT and sequenced using internal primers. Mutations in *hag1-3* and *4* were identified by amplification of at3g54610 using primers CACCTTCACTATACTGCTTCGATGGACT and CATGTATTCCCAGTTCCAATG.

***In situ* hybridization**

TPL, *WUS*, and *HDA19* RNAs were detected with digoxigenin-labelled riboprobes using the method found at <http://www.its.caltech.edu/~plantlab/protocols/insitu.htm>. For *TPL*, 190 base pairs upstream of the start codon was used. For *HDA19*, a 490 base pair fragment starting at an internal HindIII site was used. For *WUS*, the entire coding region was used.

Microscopy

For morphological analysis, ovules were dissected and mounted in Hoyer's medium and imaged using a Leica DM5000B compound microscope. For GFP analysis, ovules were dissected into 0.5X LS media (Caisson Laboratories, Inc.; Rexburg, ID), vacuum infiltrated in 1% Fluorescent Brightener 28 (Sigma-Aldrich; St. Louis, MO), then washed 2X and mounted in 0.5X LS media. Embryos were imaged using a Leica DM IRE2 laser scanning confocal microscope. Fluorescent brightener was excited with the UV diode 405nm line, and emission was measured between at 420-470nm. GFP was excited with a 488nm argon laser line and emission was measured at 500-535nm. Images were overlaid using Leica Confocal Software (LCS) and overlapping signal is displayed as white.

Supplementary references

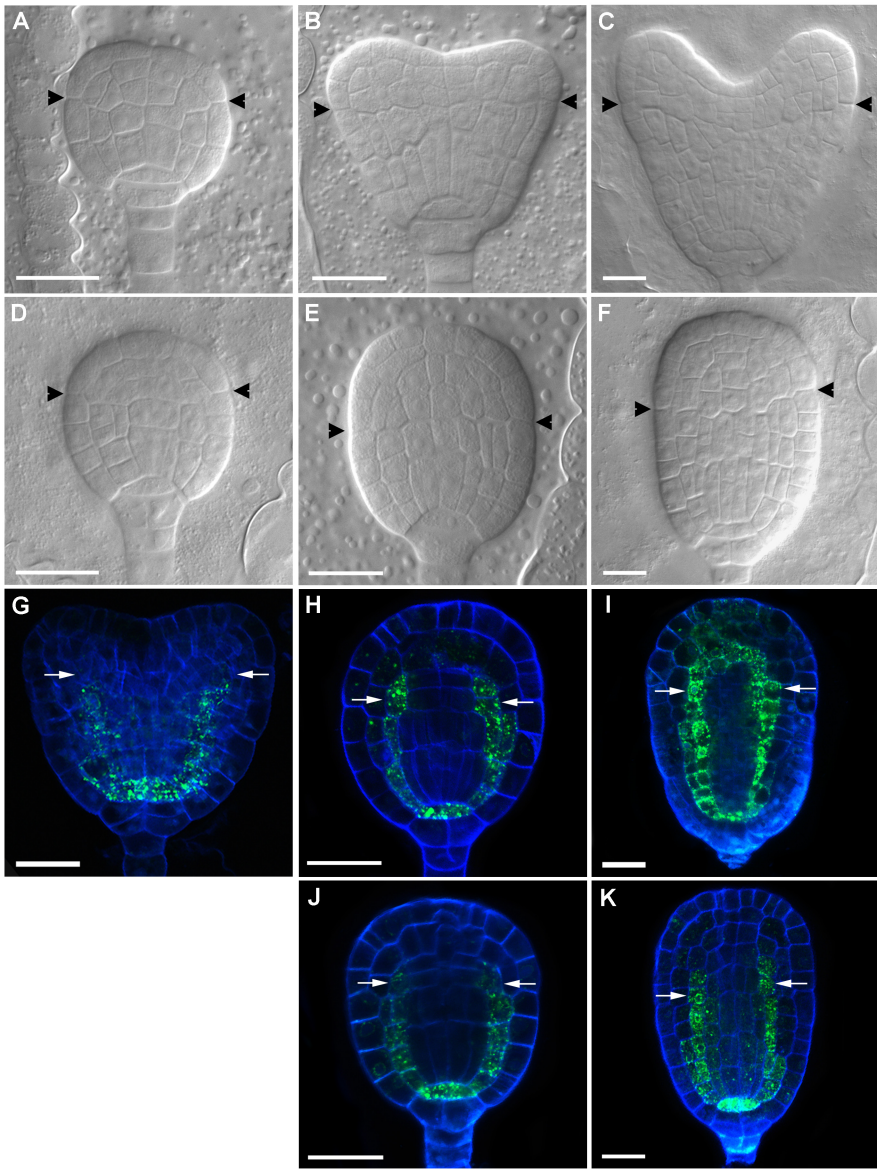
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Figure S1. Expression of a reporter for the basal marker gene *SCARECROW* (*SCR*) is misexpressed in apical tissues in *tpl-1* embryos grown at both restrictive and permissive temperatures, visualized by *pSCR::ERGFP*. There is a clearly defined morphological boundary identified by a continuous line of cell walls that delineates apical versus basal lineages in a wild type (wt) embryo, designated the O' line. Cells above the O' line will only contribute to the apical structures of the cotyledon and shoot meristem, while cells below the O' line will only contribute to the basal structures of the hypocotyl and root(1). Cleared wt (**A** to **C**) and *tpl-1* embryos (**D** to **F**) grown at the permissive temperature (21°C) illustrate that the O' line can be identified throughout early embryo development. Black arrowheads point to the epidermal cell walls that define the O' line. *SCR* is expressed in the ground tissue of the root and hypocotyl in early embryos and excluded from tissues above the O' line that contribute to apical structures(2, 3). *pSCR::ERGFP* expression in wt at the heart stage (**G**). *tpl-1* embryos grown at the restrictive temperature (29°C) show expansion of *pSCR::ERGFP* expression into apical structures by the transition stage (**H**). During the heart stage *pSCR::ERGFP* forms a pattern similar to that in the root pole (**I**), the same stage at which *tpl-1* has been shown to misexpress root cap markers(4). Transition stage *tpl-1* embryos grown at the permissive temperature (21°C) show misexpression of *pSCR::ERGFP* (**J**) similar to those grown at the restrictive temperature. Later in the heart stage, *pSCR::ERGFP* continues to misexpress in cells that would contribute to apical structures in the wild type (**K**), however; it does not form a pattern that resembles the root pole. This is consistent with our observation that these embryos will develop into monocot or tube phenotype seedlings. White arrows point to

the first cell above the O' line, that does not express SCR in the wild type. Cell walls were counterstained with Fluorescent Brightener 28. Scale bars: 20 μ m.

Fig. S2. A clustalW alignment of TPL and TOPLESS-RELATED (TPR) proteins. TPL shows extensive similarity to 4 other proteins in *Arabidopsis*. TPR1=At1g80490, TPR2=At3g16830, TPR3=At5g27030, TPR4=At3g15880. Identical residues are shaded black, similar residues are shaded grey, black line marks the LisH domain, dotted line marks the CTLH domain, grey lines mark the WD40 repeats and the asterisk marks the position of the asparagine to histidine mutation found in *tpl-1*.



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TPL-At1g15750 1 MSSLSRELVLILQFLDEEKFKETVHKLEQESGFFFNMKYFEDEVHNGNW
TPR1-At1g80490 1 MSSLSRELVLILQFLDEEKFKETVHKLEQESGFFFNMKYFEDEVHNGNW
TPR2-At3g16830 1 MSSLSRELVLILQFLDEEKFKESVHKLEQESGFFFNKYFEKALAGEW
TPR3-At5g27030 1 MSSLSRELVLILQFLDEEKFKESVHRLEKESGFFFNKYFDEKVLAGEW
TPR4-At3g15880 1 MSSLSRELVLILQFLDEEKFKD TVHRLEKESGFFFNMYFEDSVTAGEW

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TPL-At1g15750 51 DEVEKYLSGFTKVDDNRYSMKIFFEIRKQKYLEALDKHDRPKAVDILVKD
TPR1-At1g80490 51 DEVEKYLSGFTKVDDNRYSMKIFFEIRKQKYLEALDRHDRPKAVDILVKD
TPR2-At3g16830 51 DEVEKYLSGFTKVDDNRYSMKIFFEIRKQKYLEALDRNDRAKAVEILAKD
TPR3-At5g27030 51 DDVE TYLSGFTKVDDNRYSMKIFFEIRKQKYLEALDRQEKAKAVEILVQD
TPR4-At3g15880 51 DDVEKYLSGFTKVDDNRYSMKIFFEIRKQKYLEALDKDHAKAVDILVKE

TPL-At1g15750 101 LKVFSTFNEELFKEITQLLTLENFRENEQLSKYGDTKSARA IMLVELKKL
TPR1-At1g80490 101 LKVFSTFNEELFKEITQLLTLENFRENEQLSKYGDTKSARA IMLVELKKL
TPR2-At3g16830 101 LKVFATFNEELYKEITQLLTLENFRENEQLSKYGDTKSARS IMYTELKKL
TPR3-At5g27030 101 LRVFSTFNEELYKEITQLLTLENFRENEQLSKYGDTKTARGIMLGELKKL
TPR4-At3g15880 101 LKVFSTFNEELFKEITMLLTLENFRENEQLSKYGDTKSARGIMLGELKKL

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TPL-At1g15750 151 IEANPLFRDKLQFP TLRN SRLRTLINQSLNWQHQLCKNPRPNPDIKTLFV
TPR1-At1g80490 151 IEANPLFRDKLQFP TLRN SRLRTLINQSLNWQHQLCKNPRPNPDIKTLFV
TPR2-At3g16830 151 IEANPLFREKLAFPSFKASRLRTLINQSLNWQHQLCKNPRPNPDIKTLFL
TPR3-At5g27030 151 IEANPLFRDKLMFP TLRSSRLRTLINQSLNWQHQLCKNPRPNPDIKTLFT
TPR4-At3g15880 151 IEANPLFRDKLQFP SLKN SRLRTLINQSLNWQHQLCKNPRPNPDIKTLFV

TPL-At1g15750 201 DHSCGPPNGARAPSPVNNPLLGGIPKACGFPPLGAHGP-----FQPTAS
TPR1-At1g80490 201 DHSCRLPNDA RAPSPVNNPLLGSIPKACGFPPLGAHGP-----FQPTPS
TPR2-At3g16830 201 DHSCSPSNGARALT PVNLPVA-AVARPSNFVPLGVHGG-----PFQSNPA
TPR3-At5g27030 201 DHTCTLPNGPLAPS AVNQPV TLTTPAAYPSLGP HVVRNLDVPFP GP
TPR4-At3g15880 201 DHTCGHPNGAHTPSPTTNHLMGSVPKVGFPPLGAHGP-----FQPTPA

TPL-At1g15750 245 PV--PTPLAGWMSSPSS-VPHPAVSAGAIALG GP-SIPAALK--HPRTPP
TPR1-At1g80490 245 PV--PTPLAGWMSSPSS-VPHPAVSGGPIALGAP-SIQAALK--HPRTPP
TPR2-At3g16830 245 PAPNANALAGWMANPNPSSSVPSGVVAASPFPMQPSQVNELK--HPRAPPS
TPR3-At5g27030 250 AA-NAGALASWMAAASGASAVQA AVVTPALMPQPQNQMSILK--RPRTPP
TPR4-At3g15880 245 PL--T TSLAGWMPNPS--VQHPTVSAGPIGLGAPNSAVSMLKRERPRSP

TPL-At1g15750 289 TNA-SLDYPSADSEHVS KRTRPMGISDEVN-LGVNMLPMSFSGQAHGHSP
TPR1-At1g80490 289 SNS-AVDYPSGDS DHVSKRTRPMGISDEVN-LGVNMLPMTFPGQAHGHNQ
TPR2-At3g16830 293 NSLGLMDYQSADHEQLMKRLRSAQTSNEVT-----YP---AHS-H
TPR3-At5g27030 297 ATPGIVDYQNPDHE-LMKRLRPAPSVEEVT-----YP---APRQQ
TPR4-At3g15880 291 TNSLSMDYQTADSESVLKRPRPFGISDGVNNLPVNVLPVTYPGQSHAH--

TPL-At1g15750 337 AFKAPDDLPKTVARTLSQGSSPMSMDFHPIKQTLLLVGTNVGDIGLWEVG
TPR1-At1g80490 337 TFKAPDDLPKTVARTLSQGSSPMSMDFHPIKQTLLLVGTNVGDIGLWEVG
TPR2-At3g16830 329 PPASLDDLPRNVVSTIRQGSVVISMDFHPSHHTLLAVGCSSGEVTLWEVG
TPR3-At5g27030 333 APWSLEDLPTKAALALHQGSTVTSMEFYPMQNTLLLVGSATGEITLWELA
TPR4-At3g15880 339 ATYSTDDLPKNVSRILSQGSAIKSMDFHPVQQTMLLVGTNLGDIAIWEVG

TPL-At1g15750	387	SRERLVQKTFKVWDLSCSMPLQAALVKEPVVSVNRVIWSPDGSFLGVAY
TPR1-At1g80490	387	SRERLVQKTFKVWDLSCSMPLQAALVKEPVVSVNRVIWSPDGSFLGVAY
TPR2-At3g16830	379	SREKVVTEPFKIWNMAACSVIFQGSIVKEPSISVTRVAWSPDGNLLGVSF
TPR3-At5g27030	383	ARERLVS RPFKIWDMSNCSHQFQALIAKETPI SVTRVAWSPDGNFI GVAF
TPR4-At3g15880	389	SREKLVSR SFKVWDLATCTVNLQASLASEYTA AVNRV V WSPDGGL LGVAY

TPL-At1g15750	437	SRHIVQLYSYHGGEDMRQHLEIDAHVGGVNDISFSTPNKQLCVITCGDDK
TPR1-At1g80490	437	SRHIVQLYSYHGGEDMRQHLEIDAHVGGVNDIAFSTPNKQLCVITCGDDK
TPR2-At3g16830	429	TKHLIHVYAYQG-SDLRQHLEIDAHVGGVNDLAFAHPNKQMCVVTCGDDK
TPR3-At5g27030	433	TKHLIQLYAFSGPNDLRQHTEIDAHVGAVNDLAFANPNRQLCVITCGDDK
TPR4-At3g15880	439	SKHIVHIYSYHGGEDLRNHLEIDAHAGNVNDLAFS QPNQQLCVVTCGEDK

TPL-At1g15750	487	TIKVWDAATGVKRHTFEGHEAPVYSVCPHYKENIQFIFSTALDGKIKAWL
TPR1-At1g80490	487	TIKVWDAATGVKRYTFEGHEAPVYSICPHYKENIQFIFSTALDGKIKAWL
TPR2-At3g16830	478	LIKVDLS-GKKLFTFEGHEAPVYSICPHQKENIQFIFSTALDGKIKAWL
TPR3-At5g27030	483	LIKVDVS-GRKHFTFEGHDAPVYSICPHYKENIQFIFSTALDGKIKAWL
TPR4-At3g15880	489	TIKVDAVTGNKLHTFEGHEAPVYSVCPHQKENIQFIFSTAVDGKIKAWL

TPL-At1g15750	537	YDNMGS RVDYDAPGRWCTTMAYSADGTRLFSCGTSKDGESFIVEWNESEG
TPR1-At1g80490	537	YDNMGS RVDYFAPGRWCTTMAYSADGTRLFSCGTSKDGESFIVEWNESEG
TPR2-At3g16830	527	YDNVGS RVDYDAPGQWCTTMLYSADGSR L FSCGTSKEGDSFIVEWNESEG
TPR3-At5g27030	532	YDNLGS RVDYDAPGKWCTRMLYSADGTRLFSCGTSKDGD S FIVEWNESEG
TPR4-At3g15880	539	YDNMGS RVDYDAPGRSCTSMAYCADG-----TSKEGESFIVEWNESEG

TPL-At1g15750	587	AVKRTYQGFHKRSIGVVQFDTTKNRYLAAGDDFSIKFWDMDAVQLLTAID
TPR1-At1g80490	587	AVKRTYQGFHKRSIGVVQFDTTKNRYLAAGDDFSIKFWDMDTIQLLTAID
TPR2-At3g16830	577	AIKRTYLGFRKKSAGVVQFDTTRNRF L AVGEDNQIKFWMNDNTNLLTVVE
TPR3-At5g27030	582	SIKRTYKEFQKLAGVVQFDTSKNHFLAVGEDGQIKFWMNNINVLSTD
TPR4-At3g15880	582	AVKRTYLG LGKRSVGVVQFDTMKNKFLVAGDEFQVKFWDMDSDLLSSTA

TPL-At1g15750	637	GDGGLQASPRIRFNKEGSL LAVSGNENVIKIMANS DGLRL LHTFENISSE
TPR1-At1g80490	637	ADGGLQASPRIRFNKEGSL LAVSANDNM IKVMANS DGLRL LHTVENLSSE
TPR2-At3g16830	627	AEGGLPNLPRLRFNKDGNLLAVTTADNGFKILANTDGLRTLRAFEARSFE
TPR3-At5g27030	632	AEGGLPALPHLRFNKDGNLLAVTTADNGFKILANPAGFRSLRAMETPASE
TPR4-At3g15880	632	AEGGLPSSPCLRTINKEGTL LAVSTTDNGIKILANAEGSRILHSMANRGLD

TPL-At1g15750	687	SSKPAINSIAA-----AAAAAATSAGHADRSANVVS IQGMNGDSRN
TPR1-At1g80490	687	SSKPAINSIP-----MVERPASVVSIPGMNGDSRN
TPR2-At3g16830	677	ASKASIDMKVSTS---AMASSISPAIGKIEHMDAGSPARPTPIPNGIEAM
TPR3-At5g27030	682	TMRTPVDFKAVPG---APVASVN-----CKVERGSPVRHSQMLNGVDPS
TPR4-At3g15880	682	SSRAPPGSVAKGPIVGTFGTPNSSTGMSLSMGERSGPVASVTGLNGDNRS

TPL-At1g15750	728	MVDVKPVITEESNDKSKIWKLTEVSEPSQCRSLRLPEN-LRVAKISR LIF
TPR1-At1g80490	717	MVDVKPVITEESNDKSKVWKLTEVGEPSQCRSLRLPEN-MRVTKISR LIF
TPR2-At3g16830	724	SRTMEKPRNLDSVDKSKPLELTEIVDPTQCRQVTMPDSKDSVSKVARLLY
TPR3-At5g27030	723	-----KSRI DDSTDKPKSWQLAEILDPSQCFQATLPDTAGSSTKV VQLLY
TPR4-At3g15880	732	LPDVKPRIADDAE-KSKTWKLTEISERSQLRTLRLPDT-LLPARVVKLIY

TPL-At1g15750	777	TNSGNAILALASNAIHL	LWKWQRNERNATGKATASLPPQWQPASGILMT
TPR1-At1g80490	766	TNSGNAILALASNAIHL	LWKWQRNDRNATGKATASLPPQWQPASGILMT
TPR2-At3g16830	774	TNSGVGVLAALGSNGVQRL	LWKWIRNEQNPTGKATASVTPQHWQPNISGILMA
TPR3-At5g27030	768	TNSGAGILALGSNGIQRL	LWKWVPNEQNPSGKATATVVPQHWQPNISGILLMT
TPR4-At3g15880	780	TNSGGAILALAEENAAHK	LWKWQKSERNLLGKANSNVPPQLWQPSGVLMT

TPL-At1g15750	827	NDVAETNP	EAVPCFALS	KNDSYVMSASGGKISLFNMMTFKTMATFMPPP
TPR1-At1g80490	816	NDVAETNP	EAVPCFALS	KNDSYVMSASGGKISLFNMMTFKTMATFMPPP
TPR2-At3g16830	824	NDVP-ENPEGS	VPCIALSKNDSYVMSACGGKVSLFNMMTFKVMTTTFMPPP	
TPR3-At5g27030	818	NDVSGVNL	ENAPCIALSKNDSYVMSAAGGVSLFNMMTFKVMTTTFMPPP	
TPR4-At3g15880	830	NDTREGNKE	DVPCFALS	KNDSYVMSASGGKISLFNMMTFKTMTTTFMAPP

TPL-At1g15750	877	PAATFLAFHPQDNNIIAIGMDDSTIQIYNVRVDEVKSKLKGHSKRITGLA
TPR1-At1g80490	866	PAATFLAFHPQDNNIIAIGMDDSTIQIYNVRVDEVKSKLKGHSKRITGLA
TPR2-At3g16830	873	PASTFLAFHPQDNNIIAIGMEDSSIHIYNVRVDEVKITKLKGHQKHITGLA
TPR3-At5g27030	868	PASTFLAFHPQDNNVIAIGMEDSTIHIYNVRVDEVKSKLKGHQKRITGLA
TPR4-At3g15880	880	PAATSLAFHPQDNNIIAIGMDDSSIQIYNVRVDEVKSKLKGHQKRVITGLA

TPL-At1g15750	927	FSNVNLNVLVSSGADAQLCVWNT	DGWEKQ	RSKVLPLPQGRPNAPS	DTRVQ
TPR1-At1g80490	916	FSNVNLNVLVSSGADAQLCVWNT	DGWEKQ	KSKVLQIPQGRSTSSLS	DTRVQ
TPR2-At3g16830	923	FSTALN	ILVSSGADAQLFFWTAD	SWEKKKSSAIQLPPGK--APVGDTRVQ	
TPR3-At5g27030	918	FSTALN	ILVSSGADAQICFWSIDTWEKRKSVAIQMPAGK--AANGDTRVQ		
TPR4-At3g15880	930	FSNVNLNVLVSSGADS	QLCVWSMDGWEKQ	ASKQIQIPSGHSPNPLA	HTRVQ

TPL-At1g15750	977	FHQDQAHFLVVHETQLAIYET	TKLECMKQWAVRESL-APITHATFSCDSQ
TPR1-At1g80490	966	FHQDQVHFLVVHETQLAIYET	TKLECMKQWPVRESA-APITHATFSCDSQ
TPR2-At3g16830	971	FHNDQIQQLLVSHETQLAIYDASKMECIHKWVPQEALS	SPITSASYSCNSQ
TPR3-At5g27030	966	FHVDQLRILVVHETQLAVFDASKMECIQWIPQDLS	APISSAVYACNSQ
TPR4-At3g15880	980	FHQDQIHVLVHASQLAIYEAPKLENMKQWIPKES	-GSVTDVAVYSCDSQ

TPL-At1g15750	1026	LVYASFMDATVCVFSSANLRLRCRVNPSAYLPA-SLSNSNVHPLVIAAHP	
TPR1-At1g80490	1015	LIYTSFMDATICVFSSANLRLRCRVNPSAYLPA-SLSNSNVHPLVIAAHP	
TPR2-At3g16830	1021	LVYASFADGNIAVFDAESLRLRCRIAPSAYMPQPTPNSAPIFPQVITAHP	
TPR3-At5g27030	1016	LIYTIFRDGNIGVEDADSLRLRCRISPSAYLPQ---GNOGLSPLVVAHP	
TPR4-At3g15880	1029	SIYAAFDGGSVSI	LTATTLQLKCRIGPNSYLPNPS-SRVYPATVAAHP

TPL-At1g15750	1075	QEPNMF	AVGLSDGGVHIFE	PLESE	GKMGVAPPAENG	SAS----	GAPTAPS
TPR1-At1g80490	1064	QESNM	FAVGLSDGGVHIFE	PLESE	GKMGVAPPPENG	SAS----	AVTATPS
TPR2-At3g16830	1071	QEPNQ	LAVGLSDG	SVKVI	EPSELSRRWGV	GVAAGSDK	AGTENGRPSSSA
TPR3-At5g27030	1063	QDPNQ	FAVGLNDG	SVKMM	EPT	EGEGKWM	IIPPSE-----AINSPST
TPR4-At3g15880	1077	SEP	NQFAVGLT	DGGVHV	IEPPG	PEGKMG	ISAPPENGAGP----SVSSAP-

TPL-At1g15750	1121	VGASASDQ	PQR
TPR1-At1g80490	1110	VGASASDQ	PQR
TPR2-At3g16830	1121	ANNSSSDQ	IQR
TPR3-At5g27030	1104	TSNQ	TPEQLQR
TPR4-At3g15880	1122	----	GSDQQPR