

## SUPPLEMENTAL MATERIALS

**Figure S1. Multiple sequence alignment of PMT.** Amino acid sequences of the PMT from *A. thaliana* (AtPMT1, Q9FR44.1; AtPMT2, Q944H0.2; AtPMT3, Q9C6B9.2), *S. oleracea* (SoPMT, AAF61950.1), *T. aestivum* (TaPMT, AAL40895.1), *C. elegans* (CePMT1, AAA81102.1; CePMT2, AAB04824.1), *H. contortus* (HcPMT1, CDJ81011.1; HcPMT2, CDJ96940.1), and *P. falciparum* (PfPMT, AAR08195.1), was performed using MultAlign. Residues in orange highlight invariant and highly conserved amino acids with variations shown in white. Residues in the MT1 and MT2 SAM/SAH binding sites are colored blue and purple, respectively. Residues in the MT1 and MT2 phosphobase binding sites are colored green and red, respectively. The box corresponds to residues of the linker helix.

AtPMT1 .....MAASYEEERDIQKNWIEHSADLTVEAMMLDSRASDLKKEERPEVLSLPPYEGKSVLELGAGIGRFT  
AtPMT2 .....MATPYKEERDIQKSYWMEHSSDLTVEAMMLDSKASDLKKEERPEVLSLPPYEGKSVLELGAGIGRFT  
AtPMT3 .....MASYGEEREIQKNWYKHSVGLSVEAMMLDSKASDLKKEERPE LAFLPPIEGTIVLE GAGIGRFT  
SoPMT .....MAASAMGVLQEREVFKYWIEHSVDLTVEAMMLDSQASDLKKEERPEVLSLPPYEGKSVLELGAGIGRFT  
TaPMT .....MDTTITVVENVFGVEVRKVKQSWZHEHSMDLTVEAMMLDSRAKDLKKEERPEVLSLPPYEGKSVLELGAGIGRFT  
CePMT1 MCLPSPPPPYPPRHSVSGVNMSTDDQSSVEDQTVAMVNVRRANFKS WDKYSKDKPDT SMML HSAE L SS RADILASLPLLNHNKDVVDIGAGIGRFT  
HcPMT1 .....MTAEVRRDSFKT WDKYSKDKPDT SMML QTAQDL AS RADILSSLPHLNHNKDVVDIGAGIGRFT  
CePMT2 .....MSSLS PRQSILY V  
HcPMT2 .....MPA ERQLIECL  
PfPMT .....

AtPMT1 GELAQKAGELIALDFIDNVIKKNESINGH.YKNVKFMCADVTSPLDKITDGSIDLIFSNNWLLMYLSDKE.VELLAERMVGV KVGGYIFFRESCHFQSGD  
AtPMT2 GELAQKAGEVIALDFIESAIQKNESVNGH.YKNVKFMCADVTSPLDKIKDGSIDLIFSNNWLLMYLSDKE.VELMAERMIGW KPGGYIFFRESCHFQSGD  
AtPMT3 TELAQKAGQVIAVDFIESVIKKNENINGH.YKNVKFLCADVTSFN NFPNESMDLIFSNNWLLMYLSDQE.VEDLAKKMLQW KVGGYIFFRESCHFQSGD  
SoPMT GELAEKASQVIALDFIESVIKKNESINGH.YKNVKFMCADVTSPLSNISPNVD IFSNNWLLMYLSDDEE.VERLVERMLKWLKPGGYIFFRESCHFQSGD  
TaPMT GELAEKAGHVIALDFI SVIKKNEEINGDIYKNITFCADVTSPELKIENSDVI VFSNNWLLMYLNDEE.VELIGRIVKWLKPGGYIFFRESCHFQSGD  
CePMT1 TVLAETARVW STDFI SFIKKN ERNAHLGNINIQVGD V...GLKMSNSVDL F NWL MYLSDEETVE.FIFNCMRWL SHGIVHLRESCE S G  
HcPMT1 TVLAETARVW STDFIESFIEKN ERNAHMGNISYQIGD V...HLQMDKESVDL F NWL MYLSDREV E.FLLNAMRWL ADGYIHLRESCE S G  
CePMT2 NK T.EGRSVSNVQVSPCQKQGYVTAFTPLTSNVQVHTSLEQLSTIRNADVLIFNNALSQI T T ADL TDFLNKNAATAIGGTVIRED.LKDCSD  
HcPMT2 HH I.KGAEPQQVGI CPQDDQRKALTEQFGSKTAT.SFCKEVDSLKNLSNLDALIVN ALDEE NDSEK D KFITAAALRSI TDGVLILR D.LSKVK  
PfPMT .....

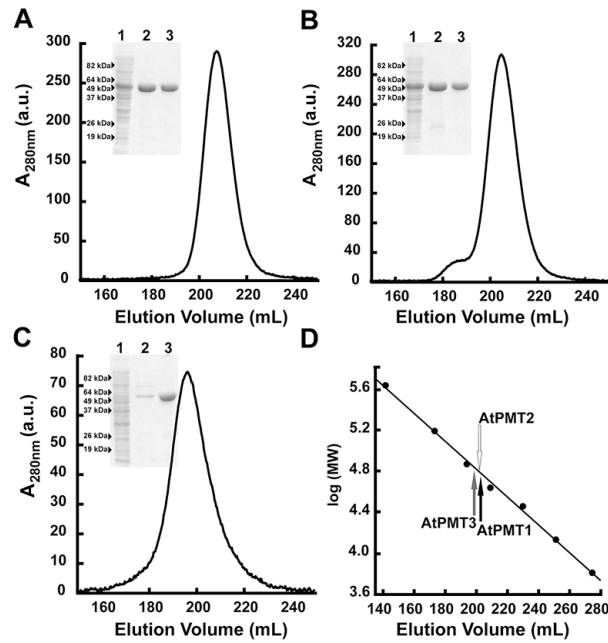
AtPMT1 SKRK.....SNPTHYREPRFYSKVFQECQTRDAAGNSFELS IGCKCIGAYVKNKKNQNICW WQKVSSE.NDRG....FORFLDNVQYKSSGILR  
AtPMT2 SKRK.....SNPTHYREPRFYKVFQECQTRDASGNSFELS VGCKCIGAYVKNKKNQNICW WQKVSVE.NDKD....FORFLDNVQYKSSGILR  
AtPMT3 NKRK.....YNPTHYREPKFYTK FKECHMNDEGNS ELSLVSKCIGAYVKNKKNQNICW WQKVSSE.NDRG....FORFLDNVQYKSSGILR  
SoPMT HRRK.....SNPTHYREPRFYTK FKECHMQDSDGNS ELSLIGCKCIGAYVKNKKNQNICSWLWQKVDSE.DDRG....FORFLDSOYKFN ILR  
TaPMT SKRK.....YNPTHYREPRFYTKVFKCHSVDQEGNSFELSLVTSKICIGAYVKNKKNQNICW WQKVKCT.EDKG....FORFLDNVQYKSTGILR  
CePMT1 RS AKSMHDTANANPTHYRFSSLYIN LRAIRYRDVD KL RFN QWSC SVPTY KRNNWROVHWAELKVPADGAKTSAELVELIKNT QNEQEAW  
HcPMT1 RL TATMHSAVDANPTHYRFSSLYIK LRAIRYRSDGKM KFD QWSC SVPTY RRCNNWROVHWAELKVPADGAKTSAELVELIKNT QNEQEAW  
CePMT2 K QV.....ARLTDY.....FDVFRRTDSDGNNTGLDLYTVDQ EHSNYVEQNFLD FIF FRKKVFA.PTDATITFRDFLDKTOY TNGTIDA  
HcPMT2 MK M.....FDVFRLE GNGN.VGFFRYAVNE GLDSVYVHQNWLF IWTLIKKPPF.KDINGVVSFRDFLDRTOY TNGTIDIFA  
PfPMT .....MTLJENLNSDKTFL NNQV TDEG KV

AtPMT1 YERVFQGG VSTGGLETTKEFVEKM NLKPGQ..KVLVGGCGIGGGDFYMAEKFDVHVVGIDLSVNMISFALERAIGL CS.VEF...EVADCTTK.HY  
AtPMT2 YERVFQGGVSTGGFETTKFVAKM.DLKPGQ..KVLVGGCGIGGGDFYMAENFDVHVVGIDLSVNMISFALERAIGLCS.VEF...EVADCTTK.TY  
AtPMT3 YERVFQGG VSTGGLETTKEFVDM.DLKPGQ..KVLVGGCGIGGGDFYMAENFDVHVVGIDLSVNMISFALE AIGLCS.VEF...EVADCTTK.EY  
SoPMT YERVFQGGVSTGGLETTKEFVSKL.DLKPGQ..KVLVGGCGIGGGDFYMAEN DVEVVGIDLSINMISFALERSIGLCA.VEF...EVADCTTK.DY  
TaPMT YERVFQGGVSTGGFETTKFVVKL.DLK PGQ..KVLVGGCGIGGGDFYMAET DVHVVGIDLSINM SFA ERAIGR CS.VEF...EVADCTTK.EY  
CePMT1 DA LDDEKVVWTDKVF SALTS..LPSNSTFFLYTPRTVSPYCHINAHTLAETFNANVWNTTEI IPEYRTSLTKSNLKDQVRVFGWNS TDSVTYQQ  
HcPMT1 DE LDNEKYSWTDKIF NAIDDEVVKNSTAVVFTPRQRSPLHVNSHLLAEKFTCNVWVNETKEYLYRTSLTKANNQDQVRVFGWNS SSSIDIWNO  
CePMT2 YEW FGVN SPGGYDENLKI KRFGD KPGQ..TMLD G GIGGGARQVA EFGVHVHGDLSNMM AIALERLHEEKDRSVK...S TD LVY.Q  
HcPMT2 YEW FGNV SPGGWQNLAI KRFG K PGQ..RMLD G GIGGGARQAA EFGLVHG DLSTNM AVALERVHKEKDRV...AV CD CEY.E  
PfPMT YEF FGENY SSGGLEATKKI SDIELNENS...KVLV D G GGGCMYINEK GAHTHGIDI SNTV MANERVSG...NNK IF...E AND LTK.E

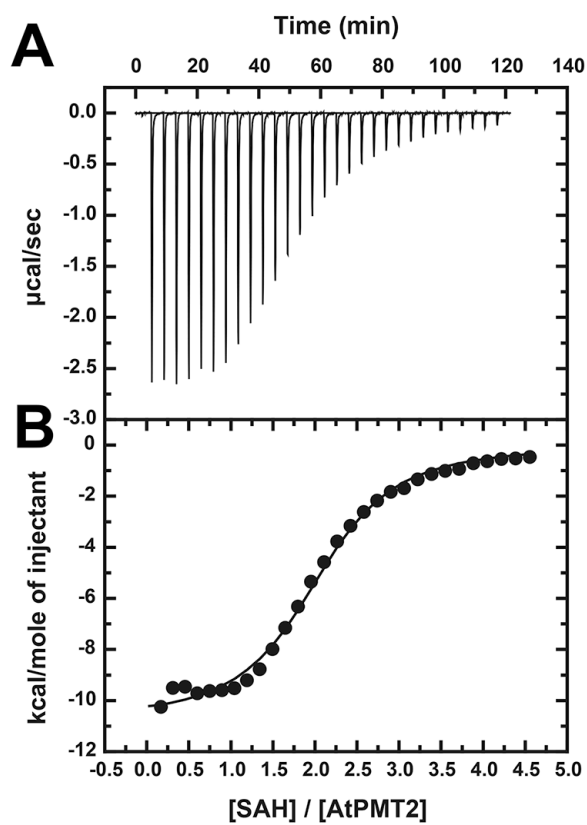
AtPMT1 PDNSFDVYSRDITLHI..QDKPALFRFP KWLKPGGKVLISDYCRSPKTP.SAEFSEYIKQRGYDLHDVQAYGQMLKDAGFTDVAEDRTDQFMQVLKR  
AtPMT2 PDNSFDVYSRDITLHI..QDKPALFRFP KWLKPGGKVLITDYCRSAETP.SPEFAEYIKQRGYDLHDVQAYGQMLKDAGFDDVAEDRTDQFVQLRR  
AtPMT3 PDN FVYSRDITLHI..QDKPALFRFP KWLKPGGKVLISDYCRSPKTP.SPFAFYIK RGYDLHDVQAYGQMLKDAGFE VIAEDRTDQFMQVLKR  
SoPMT P NSFDVYSRDITLHI..QDKPALFRFP KWLKPGGKVLISDYCRSAGTP.SAEFAAYIKQRGYDLHDVQAYGQMLKDAGFV VIAE RDTQFIQVLQK  
TaPMT A N FVYSRDITLHI..QDKPALFRFP KWLKPGGKVLISDYCRSPGTP.SEEFAAYIKQRGYDLHDVQAYGQMLKDAGFVHVAEDRTDQFLRVLER  
CePMT1 KDA FV VAT F STV..DDET..IRQLPNVMSI G K T I L E..PVDEVN.EAEMKQRIQELGYTLKS TDVTDQCIEAQ E YFK HEQLRDEKVRK  
HcPMT1 RDASFDCIVAT L ATC..DDES..IKSIASIKPEKVVILE..PVSEVD.ETSVRQR TTCG KNIT VDVTEQESLNAE.TSFIK HNL..DVE SGC  
CePMT2 EDNSFDYVSRDCIQHI..PDTEKLF SRIYKALKPGGKVLITMVGKGYGQ.SDKFKY AQRAYFLKN KEIADIANTGFV VQTE MTPRFKE LLE  
HcPMT2 E NSFDYVSRDCIQHI..KDTDKLFSRIY ALKPGGKVLITMVGVGHGTL.SESFKY SQROYLKN EQIEEIAKKTGFIDIEVE MTPRFKE LLE  
PfPMT P NNFDLIYSRDAILHI SLE KNLKLFQCKWKLKPG LITDTCATEKNWDEFKEY QORRYTLITVEEYADILTACNFK VRSKDL DY NQLEEV

AtPMT1 ELDR EKEKEEFISDFSKEDYDDIVGGWKSILERCASDEQKWGLFIANKN...  
AtPMT2 ELEK EKEKEEFISDFSEEDY DIVGGWSAKLER TASGEQKWGLFIADKK...  
AtPMT3 ELDA EKEKEEFISDFSKEDYDI GGWKSILLRSSSGEQKWGLFIANKN...  
SoPMT ELDALEQEK DFIDDFSEEDY DIVDGVKAKLVRTTEGEQKWGLFIANKN...  
TaPMT ELGE EK KEAF ADF QEDYDDIVNGWSAKLKRSSAGEQKWGLFIATK...  
CePMT1 NVLLELTH...  
HcPMT1 NYLL KASL...  
CePMT2 ERGHLEQ EAEF SKF QREKRS SGWTDKLGIEKD NWNFFLAQKFPFK  
HcPMT2 ERER EQ KETP AKFSQAYDG VSGWKSILQYIADD NWNFFAAYKPK...  
PfPMT EHKYLHE KEF KLFSEK IS DDGWSRK KDSKRMRQWGYFKATKN...

**Figure S2. Protein expression and purification of AtPMT.** Size-exclusion chromatograms are shown for AtPMT1 (**A**), AtPMT2 (**B**), and AtPMT3 (**C**). The inset in each panel shows SDS-PAGE analysis of protein purification for each protein. Samples were stained for total protein using Coomassie Blue. Triangles correspond to the indicated molecular mass markers. In each inset, the lanes are as follows: lane 1, 12.5  $\mu\text{g}$  of sonicate; lane 2, 5  $\mu\text{g}$  of affinity-purified AtPMT; lane 3, 5  $\mu\text{g}$  of size-exclusion purified AtPMT. (**D**) Molecular weight determination of AtPMT. The molecular weight calibration curve of the size-exclusion column is shown. The following standards were used: ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (27 kDa), ribonuclease A (13.7 kDa), and aprotinin (6.5 kDa). An arrow indicates the elution volume for each AtPMT.

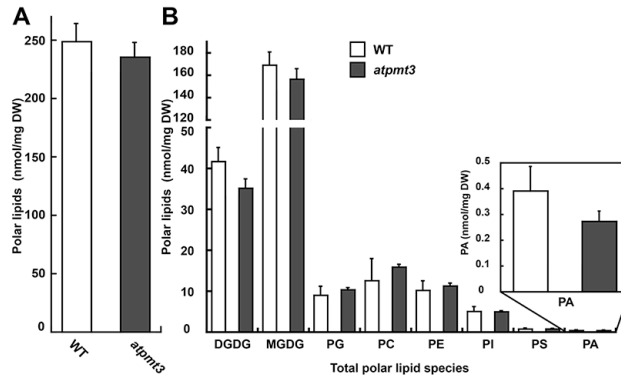


**Figure S3. Isothermal titration calorimetry of SAH binding to AtPMT2. (A)** Titration of AtPMT2 with SAH. ITC data are plotted as heat signal ( $\mu\text{cal sec}^{-1}$ ) versus time (min). The experiment consisted of 30 injections of SAH into a solution containing AtPMT2 at 20 °C. **(B)** Integrated heat response per injection from (A) is plotted as normalized heat per mol of injectant. The solid line represents the fit to the data.



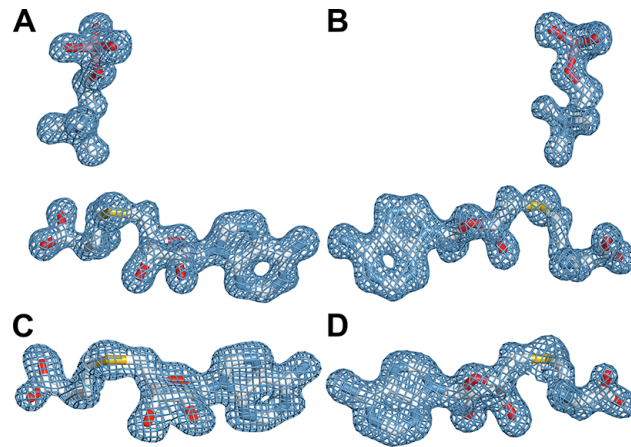
**Figure S4. Polar lipid profiling of wild-type and *atpmt3* T-DNA knockout line.**

**(A)** Total polar lipid levels for wild-type (white) and *atpmt3* (grey) plants are shown as nmol per mg dry weight (DW). Analysis of polar lipid content was performed as described in the experimental methods. **(B)** Comparison of total polar lipid digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) species in wild-type (white) and *atpmt3* knockout (grey) plants. The inset is a close-up of the changes in phosphatidic acid (PA). All values in panels A and B are shown as mean  $\pm$  standard deviation ( $n = 6-8$ ).



**Figure S5. Electron density for active site ligands in AtPMT1 and AtPMT2.**

**(A)** Electron density for pCho (top) and SAH (bottom) in the AtPMT1 MT1 domain active site is shown as a  $2F_o - F_c$  omit map ( $1.5 \sigma$ ). **(B)** Electron density for pCho (top) and SAH (bottom) in the AtPMT1 MT2 domain active site is shown as a  $2F_o - F_c$  omit map ( $1.5 \sigma$ ). **(C)** Electron density for SAH in the AtPMT2 MT1 domain active site is shown as a  $2F_o - F_c$  omit map ( $1.25 \sigma$ ). **(D)** Electron density for SAH in the AtPMT2 MT2 domain active site is shown as a  $2F_o - F_c$  omit map ( $1.25 \sigma$ ).



**Table S1. Summary of sequences used to generate phylogenetic tree.** The AtPMT1 sequence was used as a BLAST query to identify 153 sequences encoding putative PMT (see Fig. 8).

|        |                               |                |           |               |                                      |                               |                 |
|--------|-------------------------------|----------------|-----------|---------------|--------------------------------------|-------------------------------|-----------------|
| Type I | <i>Gossypium arboreum</i>     | KHG16117.1     | [eudicot] | Type I        | <i>Xenopus laevis</i>                | NP_001087172.1                | [frogs & toads] |
| Type I | <i>Gossypium hirsutum</i>     | XP_016666825.1 | [eudicot] | Type I        | <i>Strongylocentrotus purpuratus</i> | XP_794381.2                   | [sea urchins]   |
| Type I | <i>Gossypium raimondii</i>    | KJB30122.1     | [eudicot] | Type I        | <i>Branchiostoma floridae</i>        | XP_002594089.1                | [lancelets]     |
| Type I | <i>Halostachys caspica</i>    | AHX22003.1     | [eudicot] | Type II       | <i>Eimeria acervulina</i>            | CDI77742.1                    | [apicomplexans] |
| Type I | <i>Ipomoea nil</i>            | XP_019169742.1 | [eudicot] | Type II       | <i>Eimeria maxima</i>                | CDJ58552.1                    | [apicomplexans] |
| Type I | <i>Jatropha curcas</i>        | KDP23922.1     | [eudicot] | Type II       | <i>Eimeria mitis</i>                 | CDJ34460.1                    | [apicomplexans] |
| Type I | <i>Juglans regia</i>          | XP_018857980.1 | [eudicot] | Type II       | <i>Eimeria necatrix</i>              | CDJ67597.1                    | [apicomplexans] |
| Type I | <i>Lupinus angustifolius</i>  | OIV94185.1     | [eudicot] | Type II       | <i>Eimeria tenella</i>               | CDJ43178.1                    | [apicomplexans] |
| Type I | <i>Lycium barbarum</i>        | AGI56231.1     | [eudicot] | Type II       | <i>Plasmodium cynomolgi</i>          | PCYB_122070                   | [apicomplexans] |
| Type I | <i>Malus domestica</i>        | XP_008360927.1 | [eudicot] | Type II       | <i>Plasmodium falciparum</i>         | PF3D7_1343000                 | [apicomplexans] |
| Type I | <i>Manihot esculenta</i>      | OAY46532.1     | [eudicot] | Type II       | <i>Plasmodium knowlesi</i>           | PKH_121150                    | [apicomplexans] |
| Type I | <i>Medicago truncatula</i>    | AES76054.1     | [eudicot] | Type II       | <i>Plasmodium vivax</i>              | PVX_083045                    | [apicomplexans] |
| Type I | <i>Morus notabilis</i>        | EXB69095.1     | [eudicot] | Type II (MT1) | <i>Ascaris suum</i>                  | ERG79882.1                    | [nematodes]     |
| Type I | <i>Musa acuminata</i>         | XP_009385384.1 | [eudicot] | Type II (MT1) | <i>Bursepelencbus xylophilus</i>     | BUX.s01513.225                | [nematodes]     |
| Type I | <i>Nelumbo nucifera</i>       | XP_010274723.1 | [eudicot] | Type II (MT1) | <i>Caenorhabditis brenneri</i>       | CBN10892                      | [nematodes]     |
| Type I | <i>Nicotiana attenuata</i>    | OIT04993.1     | [eudicot] | Type II (MT1) | <i>Caenorhabditis briggsae</i>       | CBG02363                      | [nematodes]     |
| Type I | <i>Nicotiana sylvestris</i>   | XP_009782224.1 | [eudicot] | Type II (MT1) | <i>Caenorhabditis elegans</i>        | NP_494990.2                   | [nematodes]     |
| Type I | <i>Nicotiana tabacum</i>      | XP_016437407.1 | [eudicot] | Type II (MT1) | <i>Caenorhabditis japonica</i>       | CJA07932                      | [nematodes]     |
| Type I | <i>Noccaea caerulea</i>       | JAU57851.1     | [eudicot] | Type II (MT1) | <i>Caenorhabditis remanei</i>        | CRE11930                      | [nematodes]     |
| Type I | <i>Phaseolus vulgaris</i>     | ESW24678.1     | [eudicot] | Type II (MT1) | <i>Caenorhabditis sp.11</i>          | Csp11.Scaffold630.g21423.t1   | [nematodes]     |
| Type I | <i>Phoenix dactylifera</i>    | XP_008799221.1 | [eudicot] | Type II (MT1) | <i>Caenorhabditis sp.5</i>           | Csp5_scaffold_01891.g23737.t1 | [nematodes]     |
| Type I | <i>Populus euphratica</i>     | XP_011039130.1 | [eudicot] | Type II (MT1) | <i>Haemonchus contortus</i>          | CDJ81011.1                    | [nematodes]     |
| Type I | <i>Populus tomentosa</i>      | APR64075.1     | [eudicot] | Type II (MT1) | <i>Necator americanus</i>            | ETN83758.1                    | [nematodes]     |
| Type I | <i>Populus trichocarpa</i>    | EEF06229.2     | [eudicot] | Type II (MT1) | <i>Pristionchus pacificus</i>        | PPA22786                      | [nematodes]     |
| Type I | <i>Prunus mume</i>            | XP_008240152.1 | [eudicot] | Type II (MT1) | <i>Strongyloides stercoralis</i>     | US 2007/0009981 A1 ID no: 10  | [nematodes]     |
| Type I | <i>Prunus persica</i>         | XP_007209941.1 | [eudicot] | Type II (MT2) | <i>Ascaris suum</i>                  | GS_23928                      | [nematodes]     |
| Type I | <i>Pyrus betulifolia</i>      | AER10513.1     | [eudicot] | Type II (MT2) | <i>Bursepelencbus xylophilus</i>     | BUX.s01143.358                | [nematodes]     |
| Type I | <i>Pyrus x bretschneideri</i> | XP_009360079.1 | [eudicot] | Type II (MT2) | <i>Caenorhabditis brenneri</i>       | CBN22056                      | [nematodes]     |
| Type I | <i>Raphanus sativus</i>       | XP_018432929.1 | [eudicot] | Type II (MT2) | <i>Caenorhabditis briggsae</i>       | CBG08775                      | [nematodes]     |
| Type I | <i>Ricinus communis</i>       | XP_002532097.1 | [eudicot] | Type II (MT2) | <i>Caenorhabditis elegans</i>        | NP_504248.1                   | [nematodes]     |

|        |                             |                |           |               |                                |                              |             |
|--------|-----------------------------|----------------|-----------|---------------|--------------------------------|------------------------------|-------------|
| Type I | <i>Salicornia europaea</i>  | ABG57185.1     | [eudicot] | Type II (MT2) | <i>Caenorhabditis japonica</i> | CJA04052                     | [nematodes] |
| Type I | <i>Sesamum indicum</i>      | XP_011072995.1 | [eudicot] | Type II (MT2) | <i>Caenorhabditis remanei</i>  | CRE18566                     | [nematodes] |
| Type I | <i>Solanum lycopersicum</i> | XP_004241642.1 | [eudicot] | Type II (MT2) | <i>Haemonchus contortus</i>    | CDJ96940.1                   | [nematodes] |
| Type I | <i>Solanum pennellii</i>    | XP_015080167.1 | [eudicot] | Type II (MT2) | <i>Meloidogyne hapla</i>       | MhA1_Contig1162.frz3.gene5   | [nematodes] |
| Type I | <i>Solanum tuberosum</i>    | XP_006346642.1 | [eudicot] | Type II (MT2) | <i>Meloidogyne javanica</i>    | US 2007/0009981 A1 ID no: 12 | [nematodes] |
| Type I | <i>Spinacia oleracea</i>    | Q9M571.1       | [eudicot] | Type II (MT2) | <i>Necator americanus</i>      | ETN82894.1                   | [nematodes] |
| Type I | <i>Suaeda japonica</i>      | BAC57432.1     | [eudicot] | Type II (MT2) | <i>Pristionchus pacificus</i>  | PPA16775                     | [nematodes] |
| Type I | <i>Suaeda liaotungensis</i> | ABK42071.1     | [eudicot] |               |                                |                              |             |

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