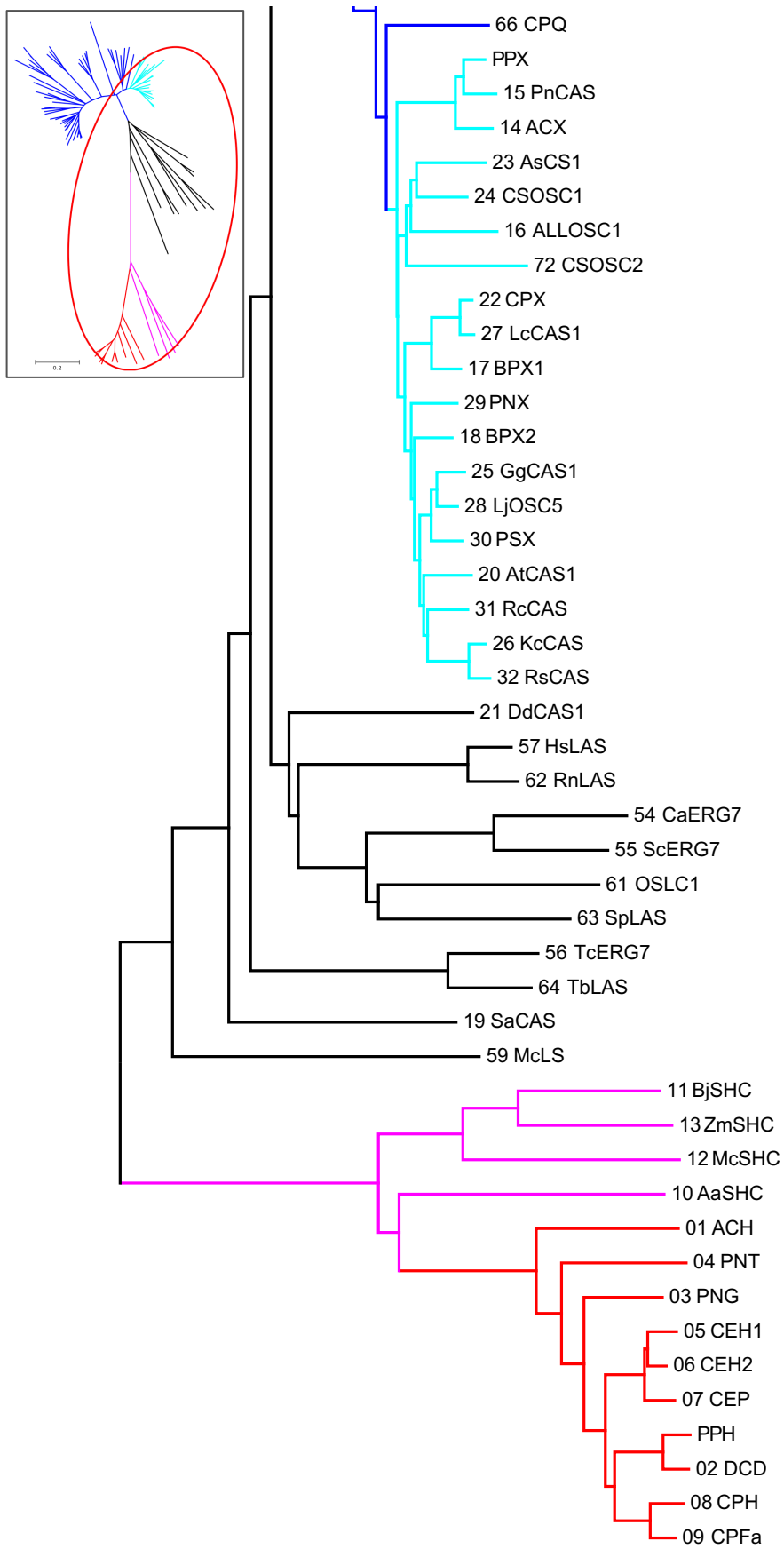


Supplementary Figure 1. Phylogenetic tree of triterpene synthases. Inlet tree shows Figure 1A. Red circle indicates expanded region.



Supplementary Figure 1. Continued.

| | | |
|-------|-----|--|
| PPH | 1 | M-LPYNQDF----YNEDEAIKDGHSKGAGNVTPPTLEEAIKSSQDFLLSQQYPEGYWWA |
| ACH | 1 | M-LPYNQDHH---FG-----KVAENATMPPTLDEAIERSQDFLLSLQYPEGYWWA |
| CEH1 | 1 | MALPYNQDLQNNIFAGDEAA-DDCKLGAADSRPIPSVDEAIKRTQNFLLGQOFPEGYWVG |
| CEH2 | 1 | MALPYNQDLQNNIFAGDEAA-DDCKLGAATDSRPVPSVDEAIKRTQNFLLGQOFPEGYWVG |
| AaSHC | 1 | MA-----EQLVEAPAYARTLDRAVEYLLSCQKDEGYWVG |
| | | |
| PPH | 56 | ELEANATITSEHTVILYKILGIEDECPMDKMEKYLRRMQCSHGGWELFYGDGGALSVTIES |
| ACH | 47 | ELEANVTLTAQTIMLYKILGIDHKYPIHKMKTYILRTQRAHGGWEIFYGDGGCLSTTICGA |
| CEH1 | 60 | EFESNVTIVATHTVLLYKILGIEEKYPMHKFERYLRRMQCSHGGWELSYGDGGYLSATIEBA |
| CEH2 | 60 | EFEGNVTIVATHTVLLYKILGIEEKYPMHKFERYLRRKQCSHGGWEISYGDGGYLSATIEBA |
| AaSHC | 35 | PLLSNVTMEAEFYVLLCHILDRVDRDRMEKIRRYLLHEQREDGTWALYPGGPPDLDTTIEBA |
| | | |
| PPH | 116 | YIALRLLNVPRTPDALKTALKFIVDKGGVTKSRMFTKICLALLGCFDWR SIPSLPPWVML |
| ACH | 107 | YMALRILGVKTPDVLQKALKLIHSGGGVTKSRMFTKICLALLGCYDWKIPSLPPWLVL |
| CEH1 | 120 | YIALRLLNVPRSDPALTKALQVILDGGGVTKARIFTKLYLALLGCFDWTGLPSLPPWVIL |
| CEH2 | 120 | YIALRLLNVPRSDPALTKALQVILDGGGVTKARIFTKLYLALLGCFDWTGLPSLPPWVIL |
| AaSHC | 95 | YVALKYIGMSRDEEPMQKALRFIQSQGGIESSRVFTRMWLALVGEYPWEKVPMPPEIMF |
| | | |
| PPH | 176 | LPSWFLSSIYETACWARGCVVPLIVVFDKKPVFKVSPEVSFDELYAEGREHACKTLPF-C |
| ACH | 167 | LPSWFPFSLYDTASWVRGCVVPLTIIFDKKPVYKLNPLLCLDELYSEGK GKARVHLSFIP |
| CEH1 | 180 | FPAWFLSSIYEINCPTRGCVVPLTVVLDKKPVFKVSPEVSFDELYVEGRAHACKALPF-S |
| CEH2 | 180 | FPAWFLSSIYETACWARASVVPLTVVLDKKPVFKVSPEVSFDELYTEGRGHACKALPF-S |
| AaSHC | 155 | LGKRMLNIEFGSWARATVVALSIVMSRQPVFPLPERARVPELY-ETDVPPRRRGAKGG |
| | | |
| PPH | 235 | GHLTSDFFIAVDHVFKMMERLGVVPPFRQWGI REAEKWLLERQE-DTGDYSGAYPPMFYSV |
| ACH | 227 | GDWTSNFFVGLDHVFKYENLGVVPPFRQWGIKEAERWTLE RHE-DSGDFHGIYPPMFYSI |
| CEH1 | 239 | GDWTSKLFITVDRILKMMERFGVVPPFRQRSFREAEKWLLERLEPESGQFVGAYAPMFYSV |
| CEH2 | 239 | GDWTSKLFIMVDRILKMMDRFGVVPPFRQRCFREAEKWLLERLEPESGELGVVYAPMFYSV |
| AaSHC | 214 | GGW---IFDALDRALHGYQKLSVHPFRRAEIRALDWLLER-QAGDGSWGGIOPPFYAL |
| | | |
| PPH | 294 | VCMKTLGYQVTDPPVQRALLAFKNFSIERADECSV-QSTLSPVWDTALVVRSLVESGLPP |
| ACH | 286 | VSYSLGYEITDPVVHRALES MRGFTVEREDECVV-QSCISPMWDTAFVIRSLAESGLQP |
| CEH1 | 299 | VCMKILGYEVTDPVVQRALLGFKLFTIETADECWV-QATISPVWDTAFIVRALVESGIPP |
| CEH2 | 299 | VCMKTLGYEVTDPVVQRALLGFKLFTIETADECWV-QASISPVWDTAFIVRALVESGIPP |
| AaSHC | 270 | IALKILD-MTQHPAFIKGWEGLLEYGVVELDYGGW MFQASISPVWDTGLAVLALRAAGLPA |
| | | |
| PPH | 353 | DHPALQKAGEWLIQKQITKHGDWSFKNPSG-VAGGWAFQFFNRWYPDLDDSAVVVMALDC |
| ACH | 345 | DHPALQKAGEWLLQKQATQHGNWFYKRTGR-AGGWAFQFFNRWYPDVDDSAAVSMALNA |
| CEH1 | 358 | DHPALQKAGQWLLRKQILKHGDWTFKAGSGHLAGGWAFQFYNGWYPDIDDSAAVVMALDC |
| CEH2 | 358 | DHPALQKAGQWLLQKQILKHGDWTFKAGSRHLAGGWAFQFYNRWYPDADDSAAVVMALDC |
| AaSHC | 329 | DHDRLVKAGEWLLDRQITVPGDWAVKRPNLK-PGGFAFQFDNVYYPDVDDTAVVVWALNT |

Supplementary Figure 2. Amino acid sequence alignment of PPH and hydroxyhopane and hopene synthases from ferns and a bacterium. Conserved residues are highlighted in pale blue. Active site residues responsible for the final deprotonation reaction are highlighted in red. ACH, hydroxyhopane synthase from *Adiantum capillus-veneris*; CEH1 and CEH2, hopene synthases from *Colysis elliptica*; AaSHC, hopene synthase from *Alicyclobacillus acidocaldarius*.

PPH 412 LKLPNEDVKNGAITRCLRWISSMQARGGGWAAYDKDSHQHWINSTPFSDIKAMLDPSTAD
 ACH 404 IKLQDDDVKKGAIKRCAEWISVMQCKDGGWAAYDCDNDREWLNCTPFGDIKAMIDPNTVD
 CEH1 418 IKLPDEDVKNGAIARCLKWLSIMQSRSGGWAAFDKNAEQHWIDSTPFSDIKAMLDSSSSD
 CEH2 418 IKLPDEDVKNGAMARCLKWLSIQSRSGGWASFDKNADQHWIDSIPFSDIKMLDSSTSD
 AaSHC 388 LRLPDERRRRDAMTKGFRWIVGMQSSNGGWGAYDVDNTSDLPNHIPECFGEVTDPPSED

PPH 472 VTARVLEMVGRLLKQ----GTSFDEAN-----VLPPESIARGLTYLRREQENEGCWFGRW
 ACH 464 VTARVLEMVGRVK-----EAGDASAILPPRAIARGLAYLRREQETEGCWYGRW
 CEH1 478 ISARVLEMVGRRLRLDLSAAGTPPARQRSALVALMPPEAIARGLAYLRRDQEEEGCWGWRW
 CEH2 478 VSARVLEMVGRLLKLDLSGAGTPLAKQORGALVALMPPEAIARGLAYLRREQEEEGCWGWRW
 AaSHC 448 VTAHVLECFGSF-----GYDDAW-----KVIRRAVEYLRREQKPDGSWFGRW

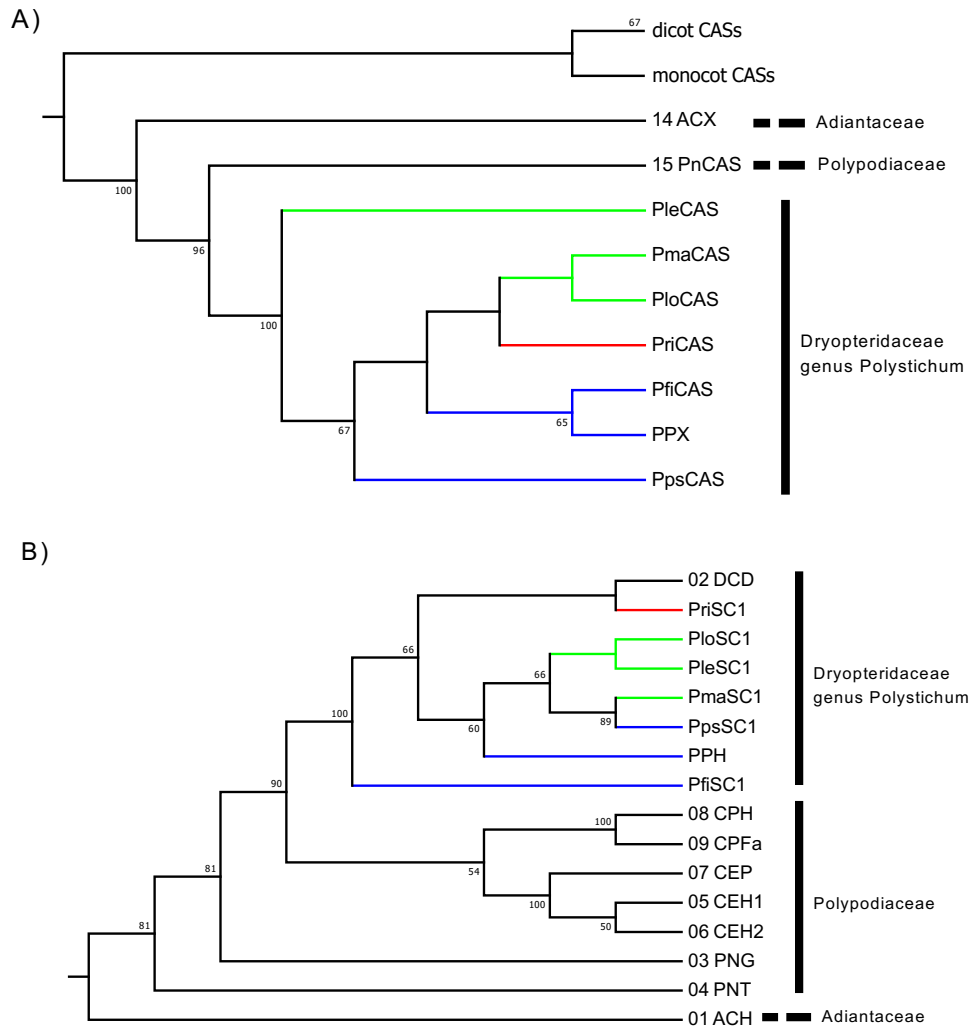
PPH 523 GVNYIYGTCGDLVALSLVAPMAHAEEIARGARWL VQVQNMHG-----
 ACH 512 GVNYIYGTS GALMALALVAPSTHKEEIERGARWLVEVQNKRGTKGANGYSH TNGAREGGV
 CEH1 538 GVNYIYGTCGALIALSLVAPTTHREEE IARGAKWL VQVQNR LGNN-----
 CEH2 538 GVNYIYGTCGALMALSLVAPTTHREEE IARGAKWL VQVQNR LGNN-----
 AaSHC 490 GVNYLYGTGAVVSALKA V GIDTREP YIQKALDWVEQH QNP-----

PPH 565 -----K KINGPQDGGWGETCF SYNDPALRGQGDVSTASQTAWALQGLLAAGDALGKYE V
 ACH 572 AMNGNCKNMGAPEDGGWGETCF SYNDITLKRNEVSTVSQTAWALQGLLAAGDALGKYE V
 CEH1 582 -----KVLHGPQDGGWGETCLS YNDPALKAQSDASTASQTAWALQGLLAAGDALGKYE V
 CEH2 582 -----KVLHGPQDGGWGETCLS YNDPALKEQSDASTASQTAWALQGLLAAGDALGKYE V
 AaSHC 530 -----DGGWGEDCRSYEDPAYAGKG-ASTPSQTAWALMALIAGGRA-----ES

PPH 619 ESIEQGVQYLLPTQRKDGSWHEPHFTGGGFPIHFYLRHYHYPQHFTLGFLARYRTRMEAS
 ACH 632 ESIEHGVQYLLSTQRKDGSWCEKHFTGGGFPRFFYIRYHLYAGHFPLSALARYRDRVRAG
 CEH1 636 EAMEQGVQYLLATQRKDGSWHESQFTGCGSPIHFYFRYHYA QHFPLSALARYRTRILQV
 CEH2 636 EAMEQGVQYLLATQRKDGSWHESQFTACGFPMHFYLYKYHYA QHFPLSALARYRTRILQA
 AaSHC 572 EAARRGVQYL VETQRPDGGWDEPY YTGTFPGDFYLYGTYMYRHVFPTLALGRYKQAIERR

PPH 679 KIKPPIP
 ACH 692 KMAK
 CEH1 696 SHTN
 CEH2 696 SHTN

Supplementary Figure 2. Continued.



Supplementary Figure 3. Phylogenetic tree of CAS clade (A) and fern SC clade (B).

Supplementary Table 1. Sequence information used in the phylogenetic analysis

| | Accession No. | Clone | Origin | Function |
|----|---------------|------------------|--|--------------------|
| 1 | AB368376 | ACH | <i>Adiantum capillus-veneris</i> | hydroxyhopane |
| 2 | AB429303 | DCD | <i>Dryopteris crassirhizoma</i> | dammaradiene |
| 3 | AB530327 | PNG | <i>Polypodiodes niponica</i> | germanicene |
| 4 | AB530326 | PNT | <i>Polypodiodes niponica</i> | tirucalladiene |
| 5 | LC074708 | CEH1 | <i>Colysis elliptica</i> | hop-22(29)-ene |
| 6 | LC074709 | CEH2 | <i>Colysis elliptica</i> | hop-22(29)-ene |
| 7 | LC074710 | CEP | <i>Colysis elliptica</i> | a-polypodatetraene |
| 8 | LC074711 | CPH | <i>Colysis pothifolia</i> | hop-22(29)-ene |
| 9 | LC074712 | CPF _a | <i>Colysis pothifolia</i> | fern-9(11)-ene |
| 10 | M73834 | AaSHC | <i>Alicyclobacillus acidocaldarius</i> | hop-22(29)-ene |
| 11 | X86552 | BjSHC | <i>Bradyrhizobium japonicum</i> | |
| 12 | Y09978 | McSHC | <i>Methylococcus capsulatus</i> | |
| 13 | X80766 | ZmSHC | <i>Zymomonas mobilis</i> | |
| 14 | AB368375 | ACX | <i>Adiantum capillus-veneris</i> | CAS |
| 15 | AB530328 | PnCAS | <i>Polypodiodes niponica</i> | |
| 16 | AB025353 | ALLOSC1 | <i>Allium macrostemon</i> | |
| 17 | AB055509 | BPX1 | <i>Betula platyphylla</i> | |
| 18 | AB055510 | BPX2 | <i>Betula platyphylla</i> | |
| 19 | AJ494839 | SaCAS | <i>Stigmatella aurantiaca</i> | |
| 20 | U02555 | AtCAS1 | <i>Arabidopsis thaliana</i> | |
| 21 | AF159949 | DdCAS1 | <i>Dictyostelium discoideum</i> | |
| 22 | AB116237 | CPX | <i>Cucurbita pepo</i> | |
| 23 | AJ311790 | AsCS1 | <i>Avena strigosa</i> | |
| 24 | AB058507 | CSOSC1 | <i>Costus speciosus</i> | |
| 25 | AB025968 | GgCAS1 | <i>Glycyrrhiza glabra</i> | |
| 26 | AB292609 | KcCAS | <i>Kandelia candel</i> | |
| 27 | AB033334 | LcCAS1 | <i>Luffa cylindrica</i> | |
| 28 | AB181246 | LjOSC5 | <i>Lotus japonicus</i> | |
| 29 | AB009029 | PNX | <i>Panax ginseng</i> | |
| 30 | D89619 | PSX | <i>Pisum sativum</i> | |
| 31 | DQ268870 | RcCAS | <i>Ricinus communis</i> | |
| 32 | AB292608 | RsCAS | <i>Rhizophora stylosa</i> | |

Supplementary Table 1. continued

| | Accession No. | Clone | Origin | Function | |
|----|---------------|-----------|----------------------------------|-----------|-----|
| 33 | EU330197 | AaBAS | <i>Artemisia annua</i> | bAS | |
| 34 | AJ311789 | AsbAS1 | <i>Avena strigosa</i> | | |
| 35 | AJ430607 | Mtbas1 | <i>Medicago truncatula</i> | | |
| 36 | AB289585 | BgbAS | <i>Bruguiera gymnorrhiza</i> | | |
| 37 | AB055512 | BPY | <i>Betula platyphylla</i> | | |
| 38 | AY520818 | CabAS | <i>Centella asiatica</i> | | |
| 39 | AB206469 | EtAS | <i>Euphorbia tirucalli</i> | | |
| 40 | AB037203 | GgbAS1 | <i>Glycyrrhiza glabra</i> | | |
| 41 | AB181244 | LjOSC1 | <i>Lotus japonicus</i> | | |
| 42 | AY836006 | OXA1 | <i>Aster sedifolius</i> | | |
| 43 | AB009030 | PNY1 | <i>Panax ginseng</i> | | |
| 44 | AB014057 | PNY2 | <i>Panax ginseng</i> | | |
| 45 | AB034802 | PSY | <i>Pisum sativum</i> | | |
| 46 | DQ915167 | SvBS | <i>Vaccaria hispanica</i> | | |
| 47 | AB289586 | BgLUS | <i>Bruguiera gymnorrhiza</i> | | LUS |
| 48 | AB055511 | BPW | <i>Betula platyphylla</i> | | |
| 49 | AB116228 | GgLUS1 | <i>Glycyrrhiza glabra</i> | | |
| 50 | AB025343 | OEW | <i>Olea europaea</i> | | |
| 51 | AB181245 | LjOSC3 | <i>Lotus japonicus</i> | | |
| 52 | DQ268869 | RcLUS | <i>Ricinus communis</i> | | |
| 53 | AB025345 | TRW | <i>Taraxacum officinale</i> | | |
| 54 | L04305 | CaERG7 | <i>Candida albicans</i> | LAS | |
| 55 | U04841 | ScERG7 | <i>Saccharomyces cerevisiae</i> | | |
| 56 | AF285826 | TcERG7 | <i>Trypanosoma cruzi</i> | | |
| 57 | D63807 | HsLAS | <i>Homo sapiens</i> | | |
| 58 | AB247155 | LSS | <i>Arabidopsis thaliana</i> | | |
| 59 | AE017282 | McLS | <i>Methylococcus capsulatus</i> | | |
| 60 | AB244671 | LjOSC7 | <i>Lotus japonicus</i> | | |
| 61 | AF327881 | OSLC1 | <i>Cephalosporium caerulens</i> | | |
| 62 | D45252 | RnLAS | <i>Rattus norvegicus</i> | | |
| 63 | U41368 | SpLAS | <i>Schizosaccharomyces pombe</i> | | |
| 64 | AF226705 | TbLAS | <i>Trypanosoma brucei brucei</i> | | |
| 65 | AB257562 | At4g15340 | <i>Arabidopsis thaliana</i> | arabidiol | |

Supplementary Table 1. continued

| | Accession No. | Clone | Origin | Function |
|----|---------------|-------------|-----------------------------|------------------|
| 66 | AB116238 | CPQ | <i>Cucurbita pepo</i> | cucurbitadienol |
| 67 | AB058643 | LcIMS1 | <i>Luffa cylindrica</i> | isomultiflorenol |
| 68 | AB265170 | PNA | <i>Panax ginseng</i> | dammarenediol |
| 69 | AB017064 | THA1 | <i>Arabidopsis thaliana</i> | thalianol |
| 70 | AL161541 | BARS1 | <i>Arabidopsis thaliana</i> | multifunctional |
| 71 | AC002986 | CAMS1 | <i>Arabidopsis thaliana</i> | |
| 72 | AB058508 | CSOSC2 | <i>Costus speciosus</i> | |
| 73 | AF489920 | F1019.4 | <i>Arabidopsis thaliana</i> | |
| 74 | AB257507 | KcMS | <i>Kandelia candel</i> | |
| 75 | AF478454 | LjAMY1 | <i>Lotus japonicus</i> | |
| 76 | U49919 | LUP1 | <i>Arabidopsis thaliana</i> | |
| 77 | AB013391 | MRN1 | <i>Arabidopsis thaliana</i> | |
| 78 | AF478453 | MtAMY1 | <i>Medicago truncatula</i> | |
| 79 | AB291240 | OEA | <i>Olea europaea</i> | |
| 80 | AB034803 | PSM | <i>Pisum sativum</i> | |
| 81 | AB263203 | RsM1 | <i>Rhizophora stylosa</i> | |
| 82 | AB263204 | RsM2 | <i>Rhizophora stylosa</i> | |
| 83 | AC007260 | T30F21.16 | <i>Arabidopsis thaliana</i> | |
| 84 | AY091131 | YUP8H12R.43 | <i>Arabidopsis thaliana</i> | |
| 85 | AB116239 | CPR | <i>Cucurbita pepo</i> | |
| 86 | AB206470 | EtOSC | <i>Euphorbia tirucalli</i> | |
| 87 | AF478455 | LjAMY2 | <i>Lotus japonicus</i> | |
| 88 | AB244670 | LjOSC6 | <i>Lotus japonicus</i> | |
| 89 | AB009031 | PNZ | <i>Panax ginseng</i> | |
| 90 | AB025346 | TRV | <i>Taraxacum officinale</i> | |