Molecular Characterization of Pear 1-Aminocyclopropane-1-Carboxylate Synthase Gene Preferentially Expressed in Leaves

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Abstract

1-Aminocyclopropane-1-carboxylate (ACC) synthase catalyzes the conversion of S-adenosyl-L-methionine to ACC in the ethylene biosynthetic pathway. Lots of important ACC synthase genes have been isolated and characterized from the plant kingdom. In this study, a cDNA clone encoding putative ACC synthase was isolated from a cDNA library produced using mRNA from pear (*Pyrus pyrifolia*). The cDNA clone, designated *PpACS1* (GenBank accession No. JQ284383), comprised an open reading frame of 1, 341 bp encoding a protein of 446 amino acids that shares high similarity with the known plant ACSs. Using PCR amplification techniques, a genomic clone corresponding to *PpACS1* was isolated and shown to contain two introns with typical GT/AG boundaries defining the splice junctions. The *PpACS1* gene product shared 97% identity with an ACC synthase cluster of the plant ACS superfamily tree. RT-PCR analysis indicated that the *PpACS1* gene was preferentially expressed in pear leaves. The transcript of *PpACS1* gene was accumulated at relatively high levels in anthers. The expression signal was detected in shoot at relatively low levels, but none signal was detected in developing fruit of pear. These results suggested that the *PpACS1* may participate in the regulation of ethylene production in pear leaves and anthers.

Keywords: Pear (Pyrus pyrifolia), Ethylene, 1-Aminocyclopropane-1-carboxylate (ACC) synthase, Gene expression

1. Introduction

Ethylene is a plant hormone regulating a wide range of physiological processes such as germination, growth, development, and senescence (Klee and Tieman, 2002). As a senescing hormone, it promotes leaf-yellowing, flower and leaf abscission, climacteric fruit ripening (Yang and Hoffman, 1984). The production of ethylene in higher plants is from S-adenosyl-L-methionine (SAM) via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). The conversion of SAM to ACC is catalysed by ACC synthase, and the subsequent oxidation of ACC to ethylene is catalysed by ACC oxidase (Yang and Hoffman, 1984; Ververidis and John, 1991).

ACC synthase (ACS), the rate-limiting enzyme of the ethylene biosynthesis pathway, is encoded by a multigene family. For example, there are eight *ACS* genes in tomato, six in mung bean, five in potato, five from rice, and ten from *Arabidopsis* (Wang *et al.*, 2005). Lots of important ACC synthase genes have been isolated and characterized from the plant kingdom. For examples, *ACS* genes were identified in pear (*Pyrus communis*) (EI-Sharkawy *et al.*, 2004), Japanese pear (*Pyrus pyrifolia*) (Itai *et al.*, 1999), apple (Rosenfield *et al.*, 1996; Wang *et al.*, 2009), peach (Mathooko *et al.*, 2001; Tatsuki *et al.*, 2006), pineapple (Cazzonelli *et al.*, 1998; Botella *et al.*, 2000; Trusov *et al.*, 2006), plum (EI-Sharkawy *et al.*, 2008), cucumber (Mathooko *et al.*, 1999), watermelon (Salman-Minkov *et al.*, 2008), *Arabidopsis* (Rodrigues-Pousada *et al.*, 1993; Mayer *et al.*, 1999), tomato (Nakatsuka *et al.*, 1997), etc.

In watermelon, both *CitACS1* and *CitACS3* are expressed in floral tissue. *CitACS1* is also expressed in vegetative tissue and it may be involved in cell growth processes. The *CitACS3* gene is expressed in open flowers and in young staminate floral buds (male or hermaphrodite), but not in female flowers. *CitACS3* is also up-regulated by ACC, and is likely to be involved in ethylene-regulated anther development. The expression of *CitACS2* was not detected in vegetative or reproductive organs (Salman-Minkov *et al.*, 2008). Silencing of *ACACS2* causes delayed flowering in pineapple (Trusov *et al.*, 2006). The expression of *AtACS1* is under developmental control both in shoot and root. High expression was observed in young tissues and was switched off in mature tissues (Rodrigues-Pousada *et al.*, 1993). However, little is known so far about the expression of pear *ACS* genes during early development. Here, isolation and characterization of one pear *ACS* gene and its expression pattern during early and fruit development are reported.

2. Materials and Methods

2.1 Collection of Plant Materials

Pear (*Pyrus pyrifolia* Nakai cv.Whangkeumbae) fruit was harvested at 120, 150, and 180 day-old after full bloom from a local orchard. The other tissues (such as shoots, leaves, petals, and anthers) were derived from the same pear trees from the local orchard. These samples were frozen immediately in liquid nitrogen, and then stored at -80° C for RNA isolation.

2.2 Construction of Pear cDNA Library and Isolation of PpACS1 cDNA

Total RNA was extracted from pear tissues including shoots, leaves, petals, anthers, and fruit. Poly(A)⁺ mRNA was prepared from a pool of total RNA by using an mRNA purification kit (Qiagen). Complementary DNA was synthesized and cloned into the EcoR I–Xho I sites of the ZAP express vector and packaged using a ZAPcDNA Gigapack Gold III cloning kit (Stratagene) according to the manufacturer's instruction.

More than 3,000 cDNA clones were randomly selected from the pear cDNA library for sequencing. One PpACS clone with complete sequences was identified. The corresponding PpACS gene was amplified from the genomic DNA of pear by PCR, using Pfu DNA polymerase and gene-specific primers that were designed according to the sequence of PpACS cDNA. In total, one PpACS gene was obtained.

2.3 RNA Isolation and RT-PCR Analysis

Total RNA was isolated from shoots, leaves, petals, anthers, and developing fruit (120, 150, and 180 day-old after full bloom) of pear by the method described previously (Shi *et al.*, 2009). A 2–4 g aliquot of each pear tissue was randomly collected from 3–10 plants for RNA isolation. The concentration and purity of total RNA were identified by NanoDrop spectrophotometry and agarose gel electrophoresis. RNA samples were stored at – 80° C until use.

Expression profiling of the *PpACS1* gene in different pear tissues (such as shoots, leaves, petals, and anthers) and during different stages of fruit development (including 120, 150, and 180 day-old after full bloom fruit) was carried out by semi-quantitative RT-PCR. A pear polyubiquitin gene (*PpUBI*, GenBank accession no. AF195224) was used as a standard pear in the RT-PCRs. A two-step RT-PCR procedure was performed in the experiments using a previously described method (Shi *et al.*, 2009). First, 2 μ g of purified total RNA using Qiagen RNeasy Mini kit was reversely transcribed into cDNAs by M-MLV reverse transcriptase according to the manufacturer's instructions. The cDNAs were used as templates for RT-PCR reactions using gene-specific primers (*PpACS1* P1: 5'- CAAAGATCTTGGCCTTCCAGGCTT-3'; P2: 5'- CAGAAAGAGTAAATTGGGTTTTGTCC-3').

2.4 DNA Sequencing and Protein Analysis

The open reading frame (ORF) of *PpACS1* gene and its deduced protein sequence were analyzed using DNASTAR software (DNAStar Co, Madison, WI, USA). The conserved domain was determined by NCBI Conserved Domain Search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignment of PpACS1 with other

plant ACSs were performed using ClustalW (http: //www.ebi.ac.uk/clustalw/) and protein motif analysis using motif scan (http: //myhits.isb-sib.ch/cgi-bin/motif_scan). N-glycosylation of the putative pear ACS protein was investigated using NETNGLYC (http: //www.cbs. dtu.dk/services/NetNGlyc/). The evolutionary relationships of the ACS proteins were determined by MEGA3.1 software, based on minimum evolution from 1000 bootstrap replicates.

2.5 Homology Modeling

The 3D models of the whole and domains of the deduced PpACS1 protein have been built using the 3D structure 1IAX chain 'A' as template (Lambert *et al.*, 2002) and SWISS-MODEL Workspace. Figures were generated using rw32b2a (http://www.37c.com.cn/topic/004/netguide/zip/RasMol26.zip).

3. Results

3.1 Isolation and Characterization of PpACS1 cDNA

To isolate the genes that might be involved in the regulation of ethylene production, 3, 000 cDNA clones from the pear (*Pyrus pyrifolia*) cDNA library were randomly sequenced. One *ACS* cDNA clone containing full length *ACS* cDNA sequence was identified from these clones, designated as *PpACS1* (GenBank accession no: JQ284383), encoding polypeptides consisting of 446 amino acids (50.00 kD, pI 5.55). The putative PpACS1 protein include 44 strongly basic(+) amino acids (K,R), 56 strongly acidic(-) amino acids (D,E), 147 hydrophobic amino acids (A,I,L,F,W,V), and 127 polar amino acids (N,C,Q,S,T,Y). Sequence alignment revealed that all of the predicted proteins encoded by these genes contain highly conserved Aminotransferase class I and II superfamily domain (Fig.1).

3.2 Structural Analysis of the PpACS1 Protein

Analysis of protein structure revealed that the predicted PpACS1 polypeptide contain the typical Aminotransferase class I and II domain and other conserved motifs (i.e. Aminotransferases class-I pyridoxal-phosphate attachment site, N-glycosylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site). As shown in Fig.1, the typical Aminotransferase class I and II domains (V_{51} -I₄₃₅) and Aminotransferases class-I pyridoxal-phosphate attachment sites (S₂₈₀-G₂₉₃) are highly conserved among the plant ACSs. Interestingly, three substitutions at amino acid level occurred in PpACS1 compared with other pear ACS proteins (Fig.1). At position 74, the polar Ser was substituted by a polar Cys. At position 430, the hydrophobic Ile was substituted by a hydrophobic Val. The strongly basic (+) Lys at residues 338 was substituted by a polar Asn, suggesting that PpACS1 may have a different structure and function compared with other pear ACS variants. Through PpACS1 protein, there are only one N-glycosylation site (PGSs; N in NxS/T motifs, positions 422) and cAMP- and cGMP-dependent protein kinase phosphorylation site ($R_{307}RMS_{310}$). Sequence comparison revealed that PpACS1 shares the highest similarity (97% identity) with PcACS2b (El-Sharkawy et al., 2004) and pPPACS2 (Itai et al., 1999). It shares relatively high homology (96% identity) with MdACS3a-1 and MdACS3a-2, but relatively low homology (83-95% identities) with pPPACS1 (Itai et al., 1999) and other known plant ACSs (Fig.1). This conserved structure may be related to the maintenance of ACS functions for plant development.

To understand the protein structure in detail, we predicted the 3D structure of PpACS1 from ESyPred3D (Lambert *et al.*, 2002). 3D homology model of PpACS1 has been built by using the 3D structure 1IAX chain 'A' as a template. We also used it to build the 3D structure of Aminotransferase class I and II domain. The whole modeling (Fig.2A/B) is restricted to residues 1-446 of PpACS1, and shares 54.0% identity with the template sequence (using the ALIGN program). 3D model of Aminotransferase class I and II domain (V₅₁-I₄₃₅) (Fig.2C/D) has been built using the 3D structure 1IAX chain 'A' as the template. It shares 50.9% identity with the template sequence.

3.3 Phylogenetic Relationship of PpACS Protein

To investigate the evolutionary relationships of the pear ACS proteins with apple and *Arabidopsis* ACS proteins, all of the known pear ACSs, apple ACSs, and *Arabidopsis* ACSs were selected from GenBank for phylogenetic analysis. The ACS proteins can be divided into two groups. As shown in Fig.3, these proteins obviously split into two subgroups. PpACS1, PcACS2b (El-Sharkawy *et al.*, 2004), PcACS2a (El-Sharkawy *et al.*, 2004), pPPACS2 (Itai *et al.*, 1999), MdACS3a-2, MdACS3a-1, MdACS3c (Wang *et al.*, 2009), PcACS3a, MdACS3b (Wang *et al.*, 2009) and AtACS7 (Mayer *et al.*, 1999) form one subgroup, while PbACS1B, pPPACS1 (Itai *et al.*, 1999) and PcACS1b (El-Sharkawy *et al.*, 2004) together with the *Arabidopsis* ACS8 are located in the second branch of the tree. These results suggest that the divergence could have occurred before the differentiation of the plants. PpACS1 is located on the first subgroup of the tree, which displays that PpACS1 has the closest

evolutional relationship with PcACS2b (El-Sharkawy *et al.*, 2004), and relatively close relationship with PcACS2a (El-Sharkawy *et al.*, 2004). Whereas the four ACSs, including AtACS8 (Mayer *et al.*, 1999), PbACS1B, pPPACS1 (Itai *et al.*, 1999) and PcACS1b (El-Sharkawy *et al.*, 2004), occupy another branch of the tree, which suggests that PpACS1 might diverge earlier from these ACSs during evolution.

3.4 Isolation and Characterization of PpACS1 Gene

One *PpACS* gene was amplified from the pear (*P. pyrifolia*) genome by PCR, using gene-specific primers. Sequence comparison between the cDNA and its corresponding gene revealed that *PpACS1* gene contains two introns splitting their ORF into three exons. The first intron is located within codons encoding value, and the second intron is inserted within codons encoding phenylalanine in the gene (Fig.4).

3.5 PpACS1 Gene is Preferentially Expressed in Leaves

To analyse the expression patterns of the isolated *PpACS1* gene, mRNA levels of the gene in pear tissues were quantified by RT-PCR using gene-specific primers. As shown in Fig.5, *PpACS1* showed leaf-preferential expression pattern. *PpACS1* was preferentially expressed in leaves, and moderate expression was found in anthers, but relatively weak signals were detected in shoots of pear. Little expression signal was detected in petals and developing fruit in pear.

4. Discussion

Pear and apple *ACS* genes are highly homologous throughout the protein coding regions but do show a degree of sequence divergence within the 3' untranslated regions. The data presented in Fig.1 revealed that pear ACS proteins contain the conserved structure, Aminotransferase class I and II domain and Aminotransferases class-I pyridoxal-phosphate attachment site, like all the other ACS proteins. The structure of the Plant 1-aminocyclopropane-1-carboxylate (ACC) synthase genes has been studied. For example, the pear ACC synthase gene has three exons and two introns, with all of the boundaries between these introns and exons sharing a consensus dinucleotide sequence of GT-AG.

Previous studies revealed that ACS genes display their tissue-specific or preferential expression in plants. PnACS from *Pharbitis nil* was expressed in cotyledons, petioles, hypocotyls, root and shoot apexes both in light- and dark-grown seedlings. The highest expression level of PnACS was found in the roots. The expression of PnACS is up-regulated in all the organs examined under IAA applied to the cotyledons of *P. nil* seedlings. In this case, the most IAA-responsive were the hypocotyls (Frankowski *et al.*, 2009). Likewise, our results also revealed that PpACS1 gene was predominantly expressed in leaves, moderate expression was found in anthers and shoots, but little signals were detected in developing fruit (Fig.5), suggesting that it may be involved in early growth and development of pear.

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PeACS2b MAID LEQUEQPERJEXKLASDTIGEDSPYFAGKKYDENPYHESSNPSCYLQUELAENQ 60 MAACS3a-2 MAID LEQUEQPSPGLSKI AVSDTHGEDSPYFAGKKYDENPYHESSNPSCYLQUELAENQ 60 MAACS3a-2 MAID LEQUEQPSPGLSKI AVSDTHGEDSPYFAGKKAYDENPYHESSNPSCYLQUELAENQ 60 PPACS1 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 pPACS2 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 PCACS2b VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-1 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 VSFDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 SPDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 SPDLLEHLEENSEASTROSKGSKOVSGFRENALFQDYHGLLSFRKAMASPHEQIRGGR 120 MAACS3a-2 AKPDPARVYLTAGATANELLTFI LAPGDALLVPTPYYFGFDRDLRWRTGVNIPHCE 180 PCACS2b AAFDPARVYLTAGATANELLTFI LAPGDALLVPTPYYFGFDRDLRWRTGVNIPHCE 180 MAACS3a-2 AKPDPARVYLTAGATANELLTFI LAPGDALLVPTPYYFGFDRDLRWRTGVNIPHCE 180 MAACS3a-2 AKPDPARVYLTAGATANELLTFI LAPGDALLVPTPYYFGFDRDLRWRTGVNIPHCE 180 MAACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTPNSNPLGATIQRTVLEEILDFVTQNNH 240 MAACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQNNH 240 MAACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVEEILDFVTQNNH 240 MAACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVEEXXXXXXXXXXXXXXXXXXXXXX	PcACS3a	MAIDIEQQQQPSPGLSKIALSDTHGEDSPYFAGWKAYDENPYHESSNPSGVIQMGLAENQ	60
MARCS3a-1 MAD IE OF COPPER LSK I AV SDTIEGEDSPYFAG/KAYDENPYHESSNPSGVTQWCLAENG 60 MACS3a-2 MATD IE OF COPPER LSK I AV SDTIEGEDSPYFAG/KAYDENPYHESSNPSGVTQWCLAENG 60 PPACS1 VSPD LENUE ENAL ASS WOSKOSKOG SPENAL PQDYHELLS FRAMASPEQ IEGGE 120 PPACS2 VSPD LENUE ENAL ASS WOSKOSKOG SEGRENAL PQDYHELLS FRAMASPEQ IEGGE 120 PCACS3a VSPD LENUE ENAL ASS WOSKOSKOG SEGRENAL PQDYHELLS FRAMASPEQ IEGGE 120 PCACS2b VSPD LENUE ENSEAS WOSKOSKOG KOSGRENAL PQDYHELLS FRAMASPEQ IEGGE 120 PCACS2b VSPD LENUE ENSEAS WOSKOSKOG KOSGRENAL PQDYHELLS FRAMASPEQ IEGGE 120 PCACS3 AKPDPARVVLTAGATANELLTPT IADPGDALLVPTPYYFFRAMAS PEQ IEGGE 120 PpACS1 AKPDPARVVLTAGATANELLTPT IADPGDALLVPTPYYFFRAMASPEQ IEGGE 120 PCACS3a AKPDPARVVLTAGATANELLTPT IADPGDALLVPTPYYFFRONLRWRTGVNIVPIHCE 180 MACS3a-1 AKPDP RVVLTAGATANELLTPT IADPGDALLVPTPYYFFRONLRWRTGVNIVPIHCE 180 MACS3a-2 AKPDP RVVLTAGATANELLTPT IADPGDALLVPTPYYFFRONLRWRTGVNIVPIHCE 180 MACS3a-3 AKPDP RVVLTAGATANELLTPT IADPGDALLVPTPYYFFRONLRWRTGVNIVPIHCE 180 PPACS1 SSNNFQITPQALEAAYKEAEAKNIRVRGVLTTNSSNLGATIOR VLEETID/VIVINI 140 PPACS2 SSNNFQITPQALEAAYKEAEAKNIRVRGVLTTNSSNLGATIOR VLEETID/VIVINI 140 MACS3a-2 SSNNFQITPQALEAAYKEAEAKNIRVRGVLTTNSSNLGATIOR VLEETID/VIVINI 14	PcACS2b	MAIDIEQRKQPSPRLSKIAVSDTHGEDSPYFAGWKTYDENPYHESSNPSG <u>VIQMGLAENQ</u>	60
MADDIEGRQPSPGLSKLAVSDTHGEDSPYFAGWKAYDENPYHESSNPSG <u>VTAMELEENG</u> 60 PpACS1 VSFDLLENILEENSEASNWGSKGSKOVSGFRENALFQDYHGLLSFRKAMASFMEQIRGG 120 pPACS2 VSFDLLENILEENSEASNWGSKGSKOVSGFRENALFQDYHGLLSFRKAMASFMEQIRGG 120 PcACS3a VSFDLLENILEENSEASNWGSKGSKOVSGFRENALFQDYHGLLSFRKAMASFMEQIRGG 120 MACS3a-1 VSFDLLENILEENSEASNWGSKGSKOVSGFRENALFQDYHGLLSFRKAMASFMEQIRGG 120 MACS3a-2 VSFDLLENILEENSEASNWGSKGSKOVSGFRENALFQDYHGLLSFRKAMASFMEQIRGG 120 PpACS1 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 pPACS2 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 MACS3a-1 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 AKFDPARVULTAGATAANELLTF1IADPGDALLVPTYPYGFDRDLRWRTGVNIVPIHCE 180 MACS3a-1 SSNNPQITPQALEAAYKEAEANMRRVRGVIFTNESNPLGATIQRTVLEELIDPVTQKNIH 240 PACS2 SSNNPQITPQALEAAYKEAEANMRRVRGVIFTNESNPLGATIQRTVLEELIDPVTQKNIH 240 PACS3 SSNNPQITPQALEAAYKEAEANMRRVRGVIFTNESNPLGATIQRTVLEELIDPVTQKNIH 240	MdACS3a-1	MAIDIEQRQQPSPGLSKIAVSDTHGEDSPYFAGWKAYDENPYHESSNPSG <u>VIQMGLAENQ</u>	60
PpACS1 VSPDLLENHLEENSEASINGSKGSKOYSGFRENALPOPTIGLISERKAMASPHEQIRGG 120 pPACS2 VSPDLLENHLEENSEANNGSKGSKGYSGFRENALPOPTIGLISERKAMASPHEQIRGG 120 PcACS3 VSPDLLENHLEENSEANNGSKGSKGYSGFRENALPOPTIGLISERKAMASPHEQIRGG 120 MACS3a-1 VSPDLLENHLEENSEANNGSKGSKGYSGFRENALPOPTIGLISERKAMASPHEQIRGG 120 MACS3a-2 VSPDLLENHLEENSEANNGSKGSKGYSGFRENALPOPTIGLISERKAMASPHEQIRGG 120 PpACS1 AKEPPARVVLTAGATANELLTPTIADEGDALLVPTPYPGFDRDLRWRTGVNIVPIHCE 180 pPACS2 AKEPPARVVLTAGATANELLTPTIADEGDALLVPTPYPGFDRDLRWRTGVNIVPIHCE 180 PcACS3a AKEPPARVVLTAGATANELLTPTIADEGDALLVPTPYPGFDRDLRWRTGVNIVPIHCE 180 PcACS3a AKEPPARVVLTAGATANELLTPTIADEGDALLVPTPYPGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 AKEPPI RVVLTAGATANELLTPTIADEGDALLVPTPYPGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 SSNNPQTTPQALEAAYKEAEANNRVRGVLTNPSNPLGATIQRTVLEELIDPVTQNNH 240 PPACS1 SSNNPQTTPQALEAAYKEAEANNRVRGVLTNPSNPLGATIQRTVLEELIDPVTQNNH 240 PACS3a SSNNPQTTPQALEAAYKEAEANNRVRGVLTNPSNPLGATIQRTVLEELIDPVTQNNH 240 MACS3a-1 SSNNPQTTPQALEAAYKEAEANNRVRGVLTNPSNPLGATIQRTVLEELIDPVTQNNH 240 MACS3a-1 SSNNPQTTPQALEAAYKEAEANNRVRGVLTNPSNPLGATIQRTVLEELIDPVTQNNH PACS1	MdACS3a-2	MAIDIEQRQQPSPGLSKIAVSDTHGEDSPYFAGWKAYDENPYHESSNPSG <u>VIQMGLAENQ</u>	60
PpACS1 VSPDLENNLEEBGASTWCSKGSKOVSGRENALPQDYIGLLSPRKAMASPHEQIEGGE 120 pPACS2 VSPDLEKILEENSLANWCSKGSKOVSGRENALPQDYIGLLSPRKAMASPHEQIEGGE 120 PcACS3a VSPDLEKILEENSLANWCSKGSKOVSGRENALPQDYIGLLSPRKAMASPHEQIEGGE 120 MdACS3a-1 VSPDLEKILEENSLASWCSKGSKOVSGRENALPQDYIGLLSPRKAMASPHEQIEGGE 120 MdACS3a-2 VSPDLEKILEENSLASWCSKGSKOVSGRENALPQDYIGLLSPRKAMASPHEQIEGGE 120 PpACS1 AKFDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLWRTGVNIVPIHCE 180 pPACS2 AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLWRTGVNIVPIHCE 180 PcACS3a AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLWRTGVNIVPIHCE 180 PcACS3a-1 AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLWRTGVNIVPIHCE 180 MdACS3a-2 AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLWRTGVNIVPIHCE 180 MdACS3a-3 AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 AKPDPARVULTAGATAANELLTPI LADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 SSNNPQITPQALEAAYKEAEAKMWRVGVLFTNPSNPLGATIQWTVLEELDPYTQKNIH 240 PpACS1 SSNNPQITPQALEAAYKEAEAKMWRVGVLFTNPSNPLGATIQWTVLEELDPYTQKNIH 240 MdACS3a-2 SSNNPQITPQALEAAYKEAEAKMWRVGVLFTNPSNPLGATIQWTVLEELDPYTQKNIH 240 MdACS3a-2 SSNNPQITPQALEAAYKEAEAKMWRVGVLFTNPSNPLGATIQWTVLEELDPYTQKNIH 240 MdACS3a-2 SSNNPQITPQALEAAYKEAEAKMWRVGVLFTNSNPLGATIQWTVLEELDPYTQ		↓	
pPACS2 VSPDLEKHLEENSEANWGSKGSKOVSGRENALPQVHGLLSFRAMASFMEQIEGGE 120 PcACS3a VSPDLEKHLEENSEASWGSKGSKOVSGRENALPQVHGLLSFRAMASFMEQIEGGE 120 PcACS2b VSPDLEKHLEENSEASWGSKGSKOVSGRENALPQVHGLLSFRAMASFMEQIEGGE 120 MdACS3a-1 VSPDLEKHLEENSEASWGSKGSKOVSGRENALPQVHGLLSFRAMASFMEQIEGGE 120 MdACS3a-2 VSPDLEKHLEENSEASWGSKGSKOVSGRENALPQVHGLLSFRAMASFMEQIEGGE 120 PpACS1 AKEPPARVVLTAGATAANELLTF HADPGALLVPTPYPGFDRDLWRTGVNIVPIHCE 180 PcACS2a AKEPPARVVLTAGATAANELLTF HADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PcACS3a AKEPPARVVLTAGATAANELLTF HADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PcACS3a AKEPPARVVLTAGATAANELLTF HADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PcACS3a AKEPPARVVLTAGATAANELLTF HADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-1 AKEPP RVVLTAGATAANELLTF HADPGALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PdACS1 SSNNFQITPQALEAAYKEAEKNMRVRGVI FTNPSNPLGATIQRTVLEELDFVTQNHI 240 PpACS2 SSNNFQITPQALEAAYKEAEKNMRVRGVI FTNPSNPLGATIQRTVLEELDFVTQNHI 240 PcACS3a SSNNFQITPQALEAAYKEAEKNMRVRGVI TNPSNPLGATIQRTVLEELDFVTQNHI 240 PcACS3a SSNNFQITPQALEAAYKEAEKNMRVRGVI TNPSNPLGATIQRTVLEELDFVTQNHI 240 PcACS1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVNSLSKDLGLPGFRVGTVYSVD 300 pPACS2 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVNSLSKDLGLPGFRVGTVYSVD 300 <tr< td=""><td>PpACS1</td><td>VSFDLLENHLEENCEASTWGSKGSKGVSGFRENALFQDYHGLLSFRKAMASFMEQIRGGR</td><td>120</td></tr<>	PpACS1	VSFDLLENHLEENCEASTWGSKGSKGVSGFRENALFQDYHGLLSFRKAMASFMEQIRGGR	120
PeACS3a VSPDLEKHLEENSEASINGSKGKKOLGGREINALPOPTIGLLSTRAMANFHEQIERGE 120 PeACS2b VSPDLENILEENSEASINGSKGKOLGVSGFREINALPOPTIGLLSFRAMANFHEQIERGE 120 MAACS3a-1 VSPDLEKHLEENSEASINGSKGKOVSGFREINALPOPTIGLLSFRAMANFHEQIERGE 120 MAACS3a-2 VSPDLEKHLEENSEASINGSKGKOVSGFREINALPOPTIGLLSFRAMANFHEQIERGE 120 MAACS3a-2 VSPDLEKHLEENSEASINGSKGKOVSGFREINALPOPTIGLSFRAMANFHEQIERGE 120 PPACS1 AKPDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PeACS2b AKPDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PeACS3a AKPDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MAACS3a-1 AKPDPIRVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MAACS3a-2 AKPDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MACS3a-2 SINNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PPACS2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PPACS3 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PeACS2b SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MAACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEELDPVTQKNIH 240 MAACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEELDPVTQKNIH 240 MAACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEELDPVTQKNIH 240 MAACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEELDPVTQKNIH 240 MAACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEELDPVTQKNIH 240 MAACS3a-2 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS2 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS2 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS2 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS3 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS3 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSSND 300 PPACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTINFRERLRRNYDMIVEGLKSGT 360 PPACS2 LVSDEIYSGAFSSSEFISVAEILEDRQYKDAERVHITVSLSKDLGLPGFRVGTVYSND 300 PPACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTINFRERLRRNYDMIVEGLKSGT 360 PPACS2 KVV	pPPACS2	VSFDLLEKHLEENSEASNWGSKGSKGVSGFRENALFQDYHGLLSFRKAMASFMEQIRGGR	120
PeACS2b VSPDLLENLEENSEAST GSKGSKOVSGFRENALFQDYHGLLSFRKAMASPMEQIRGGR 120 MdACS3a-1 VSPDLLEKHLEENSEAST GSKGSKOVSGFRENALFQDYHGLLSFRKAMASPMEQIRGGR 120 PpACS1 AKEDPARVULTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PPACS2 AKEDPARVULTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PeACS3a AKEDP RVVLTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PeACS1 AKEDP RVVLTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 AKEDP RVVLTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 AKEDP RVVLTAGATAANELLTFI IADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PACS1 SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PACS3a-1 SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 VSDETYSGAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PACS1 LVSDETYSGAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND	PcACS3a	VSFDLLEKHLEENSEASNWGSKGSKGASGFRENALFQDYHGLLSYKKAMANFMEQIRGGR	120
MdACS3a-1 VSPDLLEKHLEENSEASNYGSKG KOVSGRERNALPOVHGLLSFRKAMANPMEQIRGGR 120 MdACS3a-2 VSPDLLEKHLEENSEASNYGSKG KGVSGRERNALPOVHGLLSFRKAMANPMEQIRGGR 120 PpACS1 AKFDPARVULTAGATAANELLTFI JAPGDALLVPTPYVPGEPDRLRWRTGVNIVPIHCE 180 PPACS2 AKFDP RVVLTAGATANELLTFI JAPGDALLVPTPYVPGEPDRLRWRTGVNIVPIHCE 180 PcACS3a AKFDP RVVLTAGATANELLTFI JAPGDALLVPTPYVPGEPDRLRWRTGVNIVPIHCE 180 PcACS3a AKFDP RVVLTAGATANELLTFI JAPGDALLVPTPYVPGEPDRLRWRTGVNIVPIHCE 180 MdACS3a-1 AKFDP RVVLTAGATANELLTFI JAPGDALLVPTPYVPGEPDRLRWRTGVNIVPIHCE 180 MdACS3a-2 AKFDP RVVLTAGATANELLTFI JAPGDALLVPTYVPGEPDRLRWRTGVNIVPIHCE 180 PpACS3 SSNNFQITPQALEAAYKEAEAKNRWRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3a LVSDE	PcACS2b	VSFDLLENHLEENSEASTWGSKGSKGVSGFRENALFQDYHGLLSFRKAMASFMEQIRGGR	120
MdaCS3a-2 VSFDLLEKHLEENSEASNWGSKGSKGVSGPRENALFQDYHGLLSFRKAMANFMEQTRGGR 120 PpACS1 AKFDPARVULTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 pPACS2 AKFDPARVULTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 PcACS3a AKFDP RVVLTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-1 AKFDP RVVLTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-2 AKFDP RVVLTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-1 AKFDP RVVLTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-2 AKFDP RVVLTAGATAANELLTF LIADPGDALLVPTPYPGFDRDLRWRTGVNI VPIHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNRVRGVL TYPSNPLGATIQRTVLEEILDFVTGKNIH 240 PcACS2 SSNNFQITPQALEAAYKEAEAKNRVRGVL TYPSNPLGATIQRTVLEEILDFVTGKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNRVRGVL TYPSNPLGATIQRTVLEEILDFVTGKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNRWRGVL TYPSNPLGATIQRTVLEEILDFVTGKNIH 240 PpACS1 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS2 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 V	MdACS3a-1	VSFDLLEKHLEENSEASNWGSKGTKGVSGFRENALFQDYHGLLSFRKAMANFMEQIRGGR	120
PpACS1 AKEPPARVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 pPPACS2 AKEPPARVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 PcACS3a AKEPP RVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MACS3a-1 AKEPP RVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MACS3a-2 AKEPP RVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MACS3a-2 AKEPP RVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MACS3a-2 AKEPP RVVLTAGATAANELLTF I IADFGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNRVRCVL FINPSNPLGATIQRTVLEEILDFVTQKNIH 240 pPPACS2 SSNNFQITPQALEAAYKEAEAKNRVRCVL FINPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNRVRCVL FINPSNPLGATIQRTVLEEILDFVTQKNIH 240 MACS3a-2 SSNNFQITPQALEAAYKEAEAKNRVRCVL FINPSNPLGATIQRTVLEEILDFVTQKNIH 240 MACS3a-2 SSNNFQITPQALEAAYKEAEAKNRVRCVL FINPSNPLGATIQRTVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVSYND 300 PcACS3a LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVSYND 300 P	MdACS3a-2	VSFDLLEKHLEENSEASNWGSKGSKGVSGFRENALFQDYHGLLSFRKAMANFMEQIRGGR	120
PpACS1 AKPDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGPDRDLRWRTGVNIVPIHCE 180 pPACS2 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGPDRDLRWRTGVNIVPIHCE 180 PcACS3 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGPDRDLRWRTGVNIVPIHCE 180 MdACS3a-1 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGPDRDLRWRTGVNIVPIHCE 180 MdACS3a-1 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGPDRDLRWRTGVNIVPIHCE 180 MdACS3a-1 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 AKFDPARVULTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PpACS2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PPACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS3 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 L			
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PeACS3a AKPDPARVVLTAGATAADELLTFI LADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PeACS2b AKFDPARVVLTAGATAANELLTFI LADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-1 AKFDPARVVLTAGATAANELLTFI LADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 MdACS3a-2 AKFDP RVVLTAGATAANELLTFI LADFGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 pPACS1 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 PeACS2 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 MdACS3a-1 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 MdACS3a-1 LVSDETYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD	pPPACS2	AKFDPARVVLTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE	180
PeACS2b AKFDP ARVULTAGATAANELLTFTI ADPGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-1 AKFDP RVVLTAGATAANELLTFTI ADPGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-2 AKFDP RVVLTAGATAANELLTFTI ADPGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 MdACS3a-1 AKFDP RVVLTAGATAANELLTFTI ADPGDALLVPTPYYPGFDRDLRWRTGVNI VPIHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEE ILDFVTQKNIH 240 PcACS2a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEE ILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEE ILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEE ILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEE ILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS2a LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTV	PcACS3a	AKFDPVRVVLTAGATAADELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE	180
MddCS3a-1 AKFDP RVUTAGATAANELLTFTIADECDALLVPTPYYPGFDRDLRWRTGVNIVPTHCE 180 MddCS3a-2 AKFDP RVUTAGATAANELLTFTIADECDALLVPTPYYPGFDRDLRWRTGVNIVPTHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 pPACS2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 pPACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 PcACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 PpACS1 KVVTTA <i>RRWS</i> SFTLISSTQHLLASMLSDKEFTGNYIKTNRERLRRYDMIVEGLKKSGI 360 PcACS2 KVVTTA <i>RRWS</i> SFTLISSTQHLLASMLSDKEFTGNYIKTNRERLRRYDMIVEGLKKSGI	PcACS2b	AKFDPARVVLTAGATAANELLTFVIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE	180
MdaCS3a-2 AKFDPTRVVLTAGATAANELLTF1IADPCDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE 180 PpACS1 SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 pPACS2 SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRVLEEILDFVTQKNIH 240 PcaCS3a SSNNFQITPQALEAAYKEAEAKNRVRGVLFTNPSNPLGATIQRVLEEILDFVTQKNIH 240 MdaCS3a-1 SSNNFQITPQALEAAYKEAEAKNRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 MdaCS3a-2 SSNNFQITPQALEAAYKEAEAKNRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 pPACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS3a LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PPACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PPACS2 KVVTTA <i>RRMSS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI <	MdACS3a-1	AKFDPVRVVLTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE	180
PpACS1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 pPACS2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 PcACS3a SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 PcACS2b SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 pPACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PPACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PcACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-1 KVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI <	MdACS3a-2	AKFDPVRVVLTAGATAANELLTFIIADPGDALLVPTPYYPGFDRDLRWRTGVNIVPIHCE	180
PpACS1 SSNNPQTPQALEAAYKEAEAKNIKVKVULTINYSNPLGATIQRIVLEEILDFVIQNNH 240 pPACS2 SSNNPQTPQALEAAYKEAEAKNIKVKGVLTINYSNPLGATIQRIVLEEILDFVIQNNH 240 PcACS3a SSNNPQTPQALEAAYKEAEAKNIKVKGVLTINYSNPLGATIQRIVLEEILDFVIQNNH 240 PcACS2b SSNNPQTPQALEAAYKEAEAKNIKVKGVLTINYSNPLGATIQRIVLEEILDFVIQNNH 240 MdACS3a-1 SSNNPQTPQALEAAYKEAEAKNIKVKGVLTINPSNPLGATIQRIVLEEILDFVTQKNH 240 MdACS3a-2 SSNNPQTPQALEAAYKEAEAKNIKVKGVLTINPSNPLGATIQRIVLEEILDFVTQKNH 240 PpACS1 LVSDETYSGSAFSSEFTSVAETLEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 pPACS2 LVSDETYSGSAFSSEFTSVAETLEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS3a LVSDETYSGSAFSSEFTSVAETLEBRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS2 LVSDETYSGSAFSSEFTSVAETLEBRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDETYSGSAFSSEFTSVAETLEBRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDETYSGSAFSSEFTSVAETLEBRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGT 360 PPACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGT 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGT 360	D 4001		0.40
PPPACS2 SSNNPQTPQALEAAYKEAEAKNMKVKVULTNYSNPLGATIQRAVLEEILDFVIQKNIH 240 PcACS3a SSNNPQTPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PcACS2b SSNNPQTPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNPQTPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNPQTPQALEAAYKEAEAKNMRVRGVLTNPSNPLGATIQRTVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 pPACS2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 PcACS3a LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 MdACS3a-1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 MdACS3a-2 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 MdACS3a-2 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVD 300 PPACS1 KVVTTA <i>RRMSS</i> FTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PPACS2 KVVTTA <i>RRMSS</i> FTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PCACS3a KVVTTA <i>RRMSS</i> FTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PCACS4 KVVTTA <i>RRMSS</i> FTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 3	PPACSI	SSNNFQ11PQALEAAYKEAEAKNMKVKGVLFINPSNPLGA11QK1VLEE1LDFV1QKN1H	240
PeaCs3a SSNNFQITPQALEAAYKEAEAAWKWGVLFINPSNPLGATIQRTVLEEILDFVTQKNIH 240 PeaCs2b SSNNFQITPQALEAAYKEAEAKNMRVRGVLFINPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-1 SSNNFQITPQALEAAYKEAEAKNMRVRGVLFINPSNPLGATIQRTVLEEILDFVTQKNIH 240 MdACS3a-2 SSNNFQITPQALEAAYKEAEAKNMRVRGVLFINPSNPLGATIQRTVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 pPACS2 LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 PcACS3a LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 PcACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 PpACS1 KVVTTA <i>ARMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>ARMSS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>ARMSS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>ARMSS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI	pPPACS2	SSNNFQ11PQALEAAYKEAEAKNMRVRGVL	240
PeaCs2b SSNNFq1TPQALEAAYKEAEAAKNMRVRGVLTNPSNPLGAT1QRTVLEE1LDFVTQKNIH 240 MdACS3a-1 SSNNFq1TPQALEAAYKEAEAKNMRVRGVLTNPSNPLGAT1QRAVLEE1LDFVTQKNIH 240 MdACS3a-2 SSNNFq1TPQALEAAYKEAEAKNMRVRGVLTNPSNPLGAT1QRAVLEE1LDFVTQKNIH 240 PpACS1 LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 pPACS2 LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 PcACS3a LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 PcACS2b LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-1 LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-2 LVSDE1YSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSND 300 PpACS1 KVVTTA <i>ARMS</i> SFTL1SSQTQHLLASMLSDKEFTGNYINTRERLRRRYDMIVEGLKKSG1 360 PcACS2b KVVTTA <i>ARMS</i> SFTL1SSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSG1 360 PcACS2b KVVTTA <i>ARMS</i> SFTL1SSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSG1 360 PcACS2b KVVTTA <i>ARMS</i> SFTL1SSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSG1 360 MdACS3a-1 KVVTTA <i>ARMS</i> SFTL1SSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSG1 360 PpACS1 ECLKGNAGLFCWMNLSPFLDEPTREELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF	PcACS3a	SSNNFQ1TPQALEAAYREAEA <mark>R</mark> NMRVRGVLFTNPSNPLGAT1QRTVLEE1LDFVTQKN1H	240
MdACS3a-1 SSNNFQTTPQALEAAYKEAEAKNMKVRGVLITNPSNPLGATIQRAVLEETLDFVTQKNIH 240 MdACS3a-2 SSNNFQTTPQALEAAYKEAEAKNMKVRGVLITNPSNPLGATIQRAVLEETLDFVTQKNIH 240 PpACS1 LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 pPACS2 LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 pcACS3a LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 pcACS2b LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-1 LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 MdACS3a-2 LVSDETYSGSAFSSSEFTSVAETLEDRQYKDAERVHTVYSLSKDLGLPGFRVGTVYSND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYT TRRERLRRRYDMIVEGLKKSGT 360 PcACS2 KVYTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTKTRRERLRRRYDMIVEGLKKSGT 360 PcACS3a KVYTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTKTRRERLRRRYDMIVEGLKKSGT 360 MdACS3a-1 KVYTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTKTRRERLRRRYDMIVEGLKKSGT 360 MdACS3a-2 KVYTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTKTRRERLRRRYDMIVEGLKKSGT 360 MdACS3a-1 KVYTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYTKTRRERLRRRYDMIVEGLKKSGT 360 MdACS3a-2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN	PcACS2b	SSNNFQ1TPQALEAAYKEAEAKNMRVRGVLFTNPSNPLGAT1QRTVLEE1LDFVTQKN1H	240
MdACS3a-2 SSNNPQITPQALEAAYKEAEAKNMRVRGVLITINPSNPLGATIQRAVLEEILDFVTQKNIH 240 PpACS1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 pcACS3a LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS3a LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PcACS2b LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSVND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PpACS1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PPACS2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPG	MdACS3a-1	SSNNFQITPQALEAAYKEAEAKNMRVRGVLITNPSNPLGATIQRAVLEEILDFVTQKNIH	240
PpACS1LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300pPACS2LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300PcACS3aLVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300PcACS2bLVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300MdACS3a-1LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300MdACS3a-2LVSDEIYSGSAFSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRvGTVYSYND300PpACS1KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PPACS2KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PcACS3aKVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PcACS3a-1KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360MdACS3a-2KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360MdACS3a-2KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PpACS2ANMSEQTLGIALTRIHNFMEKRERAC446PcACS1ANMSEQTLGIALTRIHNF	MdACS3a-2	SSNNFQITPQALEAAYKEAEAKNMRVRGVLITNPSNPLGATIQRAVLEEILDFVTQKNIH	240
PpACS1 PyBACS2 LVSDE1YSGSAFSSSEF1SVAE1LeDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSEND 300 PcACS3a LVSDEYSGSAFSSSEF1SVAE1LeDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSEND 300 PcACS3a LVSDEYSGSAFSSSEF1SVAE1LeDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 MdACS3a-1 LVSDEIYSGSAFSSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 MdACS3a-2 LVSDEIYSGSAFSSEF1SVAE1LEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PPACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 PpACS1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PcACS2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEV	PpACS1		300
PPrACS2 Evsbeitsssafsssefisvaellebreventivglexkbdglpdfrvdfvyglysdd 300 PcACS3a LVSDEWSGSAFSSSEFISVAellebreventivglexkbdglpdfrvdfvyglysdd 300 PcACS2b LVSDEIYSGSAFSSSEFISVAellebreventivglexkbdglpdfrvdfvyglysdd 300 MdACS3a-1 LVSDEIYSGSAFSSEFISVAellebreventivglexkbdglpdfrvdfvyglysdd 300 MdACS3a-2 LVSDEIYSGSAFSSEFISVAellebreventivglexkbdglpdfrvdfvyglysdd 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 pPACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 PcACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 PpACS1 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PcACS2 ECLKGNAGLFCWNNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PcACS3a ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 MdACS3a-1 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEV			200
PcACS3a LVSDENTSGSAFSSSEFISVAEIDEDRQYKDAERVHIVYSLSKDLGLPGFRVG[VYSIND 300 PcACS2b LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVG[VYSIND 300 MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVG[VYSIND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVG[VYSIND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS2a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS2b KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI 360 PpACS1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PPACS2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PcACS3a ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 MdACS3a-1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCS	pPPACS2		300
PcACS2bLVSDE1YSGSAFSSSEF1SVAEILEMRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND300MdACS3a-1LVSDE1YSGSAFSSSEF1SVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND300MdACS3a-2LVSDE1YSGSAFSSSEF1SVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND300PpACS1KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360pPACS2KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PcACS3aKVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PcACS2bKVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360MdACS3a-1KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360MdACS3a-2KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI360PpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PpACS1AMMSEQTLGIALTRIHNFMEKRERAC 446446PPACS2AMMSEQTLGIALTRIHNFMEKRERAC 446446PcACS3aAMMSEQTLGIALTRIHNFMEKRERAC 446446MdACS3a-1AMMSEQTLGIALTRIHNFMEKRERAC 446446MdACS3a-1AMMSEQTLGIALTRIHNFMEKRERAC 446446MdACS3a-2AMMSEQTLGIALTRIHNFMEKRERAC 446446MdACS3a-2AMMSEQTLGIALTRIHNFMEKRERAC 446446<	PcACS3a	LVSDE <u>V</u> YSGSAFSSSEF1SVAE1 <u>N</u> EDRQYKDAERVH1VYSLSKDLGLPGFRVGTVYSYND	300
MdACS3a-1 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 MdACS3a-2 LVSDEIYSGSAFSSSEFISVAEILEDRQYKDAERVHIVYSLSKDLGLPGFRVGTVYSYND 300 PpACS1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 pPACS2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS3a KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 PcACS2b KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYINTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-1 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRYDMIVEGLKKSGI 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRYDMIVEGLKKSGI 360 PpACS1 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PpACS2 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PcACS3a ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 MdACS3a-1 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 MdACS3a-2 ECLKGNAGLFCWNNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCH	PcACS2b	LVSDE1YSGSAFSSSEF1SVAE1LE <mark>N</mark> RQYKDAERVH1VYSLSKDLGLPGFRVGTVYSYND	300
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PeACS3aKVVTTA <i>RRMSSFTLISSQTQHLLASMLSDKEFTENYIKTNRERLRRKYDMTVEGLKKSGT</i> 360PcACS2bKVVTTA <i>RRMSSFTLISSQTQHLLASMLSDKEFTENYIKTNRERLRRRYDMTVGGLKKSGT</i> 360MdACS3a-1KVVTTA <i>RRMSSFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRRRYDMTVGGLKKSGT</i> 360MdACS3a-2KVVTTA <i>RRMSSFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRTRYDMTVGGLKKSGT</i> 360PpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420pPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PpACS1ANMSEQTLGVALTRIHNFMEKRERAC446PPACS2ANMSEQTLGIALTRIHNFMEKRERAC446PcACS3aANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446Figure 1. Sequence alignment among the pear and apple ACS proteinsFigure 1. <td>pPPACS2</td> <td>KVVITA<i>RRMS</i>SFTLISSETQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI</td> <td>360</td>	pPPACS2	KVVITA <i>RRMS</i> SFTLISSETQHLLASMLSDKEFTGNYIKTNRERLRRRYDMIVEGLKKSGI	360
PcACS2bKVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTENY1KTNRERLRRRYDMIDEGLKRSGT360MdACS3a-1KVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDM1VQGLKKSGT360MdACS3a-2KVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDM1EGLKKSGTPpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420pPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420PcACS2bECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420MdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420MdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF420PpACS1ANMSEQTLGIALTRIHNFMEKRERAC446pPACS2ANMSEQTLGIALTRIHNFMEKRERAC446PcACS3aANMSEQTLGIALTRIHNFMEKRERAC446PcACS2bANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIHNFMEKRERAC446Figure 1. Sequence alignment among the pear and apple ACS proteinsFigure 1.	PcACS3a	KVVITA <i>RRMS</i> SFTLISSQTQHLLASMLSDKEFTENYIKTNRERLRRRYDMIVEGLKKSGI	360
MdACS3a-1 KVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDM1V0GLKRSGT 360 MdACS3a-2 KVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDM1 EGLKKSG1 360 PpACS1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 pPACS2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PcACS3a ECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PcACS2b ECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 MdACS3a-1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 MdACS3a-2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS3a-2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS2 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS2 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGVANLSPFLDEPTRECELTLWDSMLHEVKLN1SPGSSCHCSEPGWFRVCF	PcACS2b	KVVIIA <i>RRMS</i> SFILISSQIQHLLASMLSDKEFIENYIKINRERLRRRYDMI <mark>L</mark> EGLKKSGI	360
MdACS3a=2KVVTTA <i>RAMSS</i> FTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDMIEGLKRSGT360PpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420pPACS2ECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS2bECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a=1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a=2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PpACS1ANMSEQTLGJALTRIHNFMEKRERAC446PPACS2ANMSEQTLGJALTRIHNFMEKRERAC446PcACS3aANMSEQTLGJALTRIHNFMEKRERAC446MdACS3a=1ANMSEQTLGJALTRIHNFMEKRERAC446MdACS3a=2ANMSEQTLGJALTRIHNFMEKRERAC446MdACS3a=2ANMSEQTLGJALTRIHNFMEKRERAC446Figure 1. Sequence alignment among the pear and apple ACS proteins560	MdACS3a-1	KVVITARRMSSFTLISSQTQHLLASMLSDKEFTGNYIKTNRERLRIRYDMIV	360
PpACS1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420pPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PcACS3aECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420MdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF420PpACS1ANMSEQTLGIALTRIHNFMEKRERAC446PPACS2ANMSEQTLGIALTRIHNFMEKRERAC446PcACS3aANMSEQTLGIALTRINFMEKRERAC446PcACS2bANMSEQTLGIALTRINFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRIHNFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-1ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRINFMEKRERAC446MdACS3a-2ANMSEQTLGIALTRIANFMEKRERAC446Figure 1. Sequence alignment among the pear and apple ACS proteins5000000000000000000000000000000000000	MdACS3a-2	KVVTTA <i>RRMS</i> SFTL1SSQTQHLLASMLSDKEFTGNY1KTNRERLRTRYDM1 <mark>1</mark> EGLKKSG1	360
PPACS1ECEROMOGI COMMENSITE DELITINE CELTENDOMENE VIENTI OF CONSTRUCT ON TWO FpPPACS2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCFpCACS3aECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCFpCACS2bECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCFMdACS3a-1ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCFMdACS3a-2ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCFPpACS1ANMSEQTLGIALTRIHNFMEKRERACPPACS2ANMSEQTLGIALTRIHNFMEKRERACPCACS3aANMSEQTLGIALTRINFMEKRERACPcACS2bANMSEQTLGIALTRINFMEKRERACPcACS2bANMSEQTLGIALTRINFMEKRERACPcACS3a-1ANMSEQTLGIALTRIHNFMEKRERACPcACS2bANMSEQTLGIALTRIHNFMEKRERACPcACS2bANMSEQTLGIALTRIHNFMEKRERACPcACS2bANMSEQTLGIALTRIHNFMEKRERACPcACS3a-1ANMSEQTLGIALTRIHNFMEKRERACPracksa-1ANMSEQTLGIALTRIHNFMEKRERACPracksa-2ANMSEQTLGIALTRIHNFMEKRERACProdecom446PcACS3a-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2ANMSEQTLGIALTRIHNFMEKRERACPcacksa-2	PnACS1	FCI KCNAGI FCWMNI SPFI DEPTRECEI TI WDSMI HEVKI NI SPGSSCHCSEPGWERVCE	420
Princip Precktondel connector filler fil	nPPACS2	ECI KCNACI ECWANI SPEI DEPTRECELTE WDSMI HEVKI NTSPCSSCHCSEPCWERVCE	120
PcACS3a <u>ECLKGNAGLFCWMNLSPFLDEPTRESELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF</u> 420 MdACS3a-1 <u>ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF</u> 420 MdACS3a-2 <u>ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF</u> 420 PpACS1 <u>ANMSEQTLGWALTRIHNFMEKRERAC</u> 446 pPACS2 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 PcACS3a <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 PcACS2b <u>ANMSEQTLGIALTRINFMEKRERAC</u> 446 MdACS3a-1 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 MdACS3a-1 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 MdACS3a-2 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 MdACS3a-1 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 MdACS3a-2 <u>ANMSEQTLGIALTRIHNFMEKRERAC</u> 446 Figure 1. Sequence alignment among the pear and apple ACS proteins Figure 1.	PcACS3a	ECERCIVACE FORMALIST FEDER TRECELTER DISMETEVRENTIST OF SOURCE OWN RVCF	120
Pickerszb International constraints MdACS3a-1 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 MdACS3a-2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNISPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGUALTRIHNFMEKRERAC 446 pPPACS2 ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS3a ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS2b ANMSEQTLGIALTRINFMEKRERAC 446 MdACS3a-1 ANMSEQTLGIALTRINFMEKRERAC 446 MdACS3a-2 ANMSEQTLGIALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins Figure 1	PcACS2b	ECERCIVACE FORMALIST FEDER TRESELTE WDSMELIEVRENTIST GSSCHOSED OWI RVCF	420
MdAC33a-1 LCLKGAAGLPCWARLSTPLDET TRECELTLWDSMLHEVKLNTSTOSSCHCSEPGWFRVCF 420 MdACS3a-2 ECLKGNAGLFCWMNLSPFLDEPTRECELTLWDSMLHEVKLNTSPGSSCHCSEPGWFRVCF 420 PpACS1 ANMSEQTLGUALTRIHNFMEKRERAC 446 pPPACS2 ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS3a ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS2b ANMSEQTLGIALTRIKNFMEKRERAC 446 MdACS3a-1 ANMSEQTLGIALTRIKNFMEKRERAC 446 MdACS3a-2 ANMSEQTLGIALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins Figure 1	MdACS2n=1	ECTRONACT ECMANT CDEL DEDTDECELTEMDSWITTEART NTCOCCECCACAEDOREDACE	420
MUACS3a=2 ECERGNAGEPERMINESPEDEPTRECEPTINECELTEWDSMEINEVKENTSPGSSCRCSEPGWPRVCP 420 PpACS1 ANMSEQTLGUALTRIHNFMEKRERAC 446 pPPACS2 ANMSEQTLGUALTRIHNFMEKRERAC 446 PcACS3a ANMSEQTLGUALTRIHNFMEKRERAC 446 PcACS2b ANMSEQTLGUALTRIHNFMEKRERAC 446 MdACS3a=1 ANMSEQTLGUALTRIHNFMEKRERAC 446 MdACS3a=2 ANMSEQTLGUALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins Figure 1.	MdACS2a 2	ECTRONACT ECMANT CDET DEDADECET AT MD2WI TEAKI NT2DC22CTIC2EDCMEDACE	420
PpACS1ANMSEQTLGUALTRIHNFMEKRERAC446pPACS2ANMSEQTLGIALTRIHNFMEKRERAC446PcACS3aANMSEQTLGIALTRIHNFMEKRERAC446PcACS2bANMSEQTLGIALTRIK446MdACS3a-1ANMSEQTLGIALTRIK446MdACS3a-2ANMSEQTLGIALTRI446Figure 1. Sequence alignment among the pear and apple ACS proteins	MUAUSSa-2	COLVOINAGELOMMINTOLEEDELIKEOEFIEMDOMFHEAVENIOLOOPOELOMEKAOF	420
pPPACS2 ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS3a ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS2b ANMSEQTLGIALTRIKNFMEKRERAC 446 MdACS3a-1 ANMSEQTLGIALTRIHNFMEKRERAC 446 MdACS3a-2 ANMSEQTLGIALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins 1	PpACS1	A NMSE QTLG <mark>V</mark> ALTRIHNFMEKRERAC 446	
PcACS3a ANMSEQTLGIALTRIHNFMEKRERAC 446 PcACS2b ANMSEQTLGIALTRIKNFMEKRERAC 446 MdACS3a-1 ANMSEQTLGIALTRIHNFMEKRERAC 446 MdACS3a-2 ANMSEQTLGIALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins	pPPACS2	ANMSEQTLGIALTRIHNFMEKRERAC 446	
PcACS2b ANMSEQTLGIALTRIK MdACS3a-1 ANMSEQTLGIALTRIK MdACS3a-2 ANMSEQTLGIALTRIK Figure 1. Sequence alignment among the pear and apple ACS proteins	- PcACS3a	ANMSEQTLGIALTRIHNFMEKRERAC 446	
MdACS3a-1 ANMSEQTLGIALTRIHNFMEKRERAC 446 MdACS3a-2 ANMSEQTLGIALTRIHNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins	PcACS2b	ANMSEQTLGIALTRIKNFMEKRERAC 446	
MdACS3a-2 <u>ANMSEQTLGIALTRI</u> HNFMEKRERAC 446 Figure 1. Sequence alignment among the pear and apple ACS proteins	MdACS3a-1	ANMSEQTLGIALTRIHNFMEKRERAC 446	
Figure 1. Sequence alignment among the pear and apple ACS proteins	MdACS3a-2	ANMSEQTLGIALTRIHNFMEKRERAC 446	
	Figure	1. Sequence alignment among the pear and apple ACS proteins	

Sequences of pear and apple ACSs were aligned. Amino acid substitutions are highlighted in black and in grey. Arrows indicate the positions of the two introns in pear *PpACS1* genes. Aminotransferase class I and II domains are underlined. Aminotransferases class-I pyridoxal-phosphate attachment sites are boxed. N-glycosylation site is bold; cAMP- and cGMP-dependent protein kinase phosphorylation site is italic. The accession numbers of pear and apple ACS proteins in GenBank are: PpACS1 (JQ284383), pPPACS2 (BAA76388), PcACS3a (AAR12136), PcACS2b (AAR38503), MdACS3a-1 (AEP82200), and MdACS3a-2 (AEP82201). Pp: *Pyrus pyrifolia*; Pc: *Pyrus communis*; Md: *Malus domestica*



Figure 2. Three dimensional structure of the predicted PpACS1 protein from side views

(A, B) Illustration of the full PpACS1 models from cartoon and ball and stick views. (C, D) Aminotransferase class I and II domain models from cartoon and ball and stick views.



Figure 3. Phylogenetic relationship of pear ACS proteins to apple and Arabidopsis ACS proteins

The minimum evolution tree was constructed in MEGA3.1 from 1000 bootstrap replicates. The accession numbers of pear, apple and *Arabidopsis* ACS proteins in GenBank are: PpACS1 (*Pyrus pyrifolia*, JQ284383), PcACS2b (*Pyrus communis*, AAR38503), PcACS2a (*Pyrus communis*, AAL66201), pPPACS2 (*Pyrus pyrifolia*, BAA76388), MdACS3a-2 (*Malus x domestica*, AEP82201), MdACS3a-1 (*Malus x domestica*, AEP82200), MdACS3c (*Malus x domestica*, BAE94692), PcACS3a (*Pyrus communis*, AAR12136), MdACS3b (*Malus x domestica*, BAE94691), AtACS7 (*Arabidopsis thaliana*, NP_194350), AtACS8 (*Arabidopsis thaliana*, NP_195491), PbACS1B (*Pyrus x bretschneideri*, BAF44094), pPPACS1 (*Pyrus pyrifolia*, BAA76389) and PcACS1b (*Pyrus communis*, AAR38501). Pp: *Pyrus pyrifolia*; Pb: *Pyrus x bretschneideri*; Pc: *Pyrus communis*; S1: Solanum lycopersicum



Figure 4. The structure of *PpACS1* gene

Exons are denoted by black boxes. Introns, 5'-flanking regions, and 3'-UTRs are denoted by lines. The length of the intron in base pairs is indicated. The position of substitution is denoted by a diagonal line.



Figure 5. RT-PCR analysis of expression of *PpACS1* gene in pear tissues *PpUBI* was amplified as a control. 120d, 150d, and 180d refer to 120, 150, and 180 day-old after full bloom fruit.