

Supplementary Materials

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Title
The Importance of Conserved Serine for C-terminally Encoded Peptides Function Exertion in Apple

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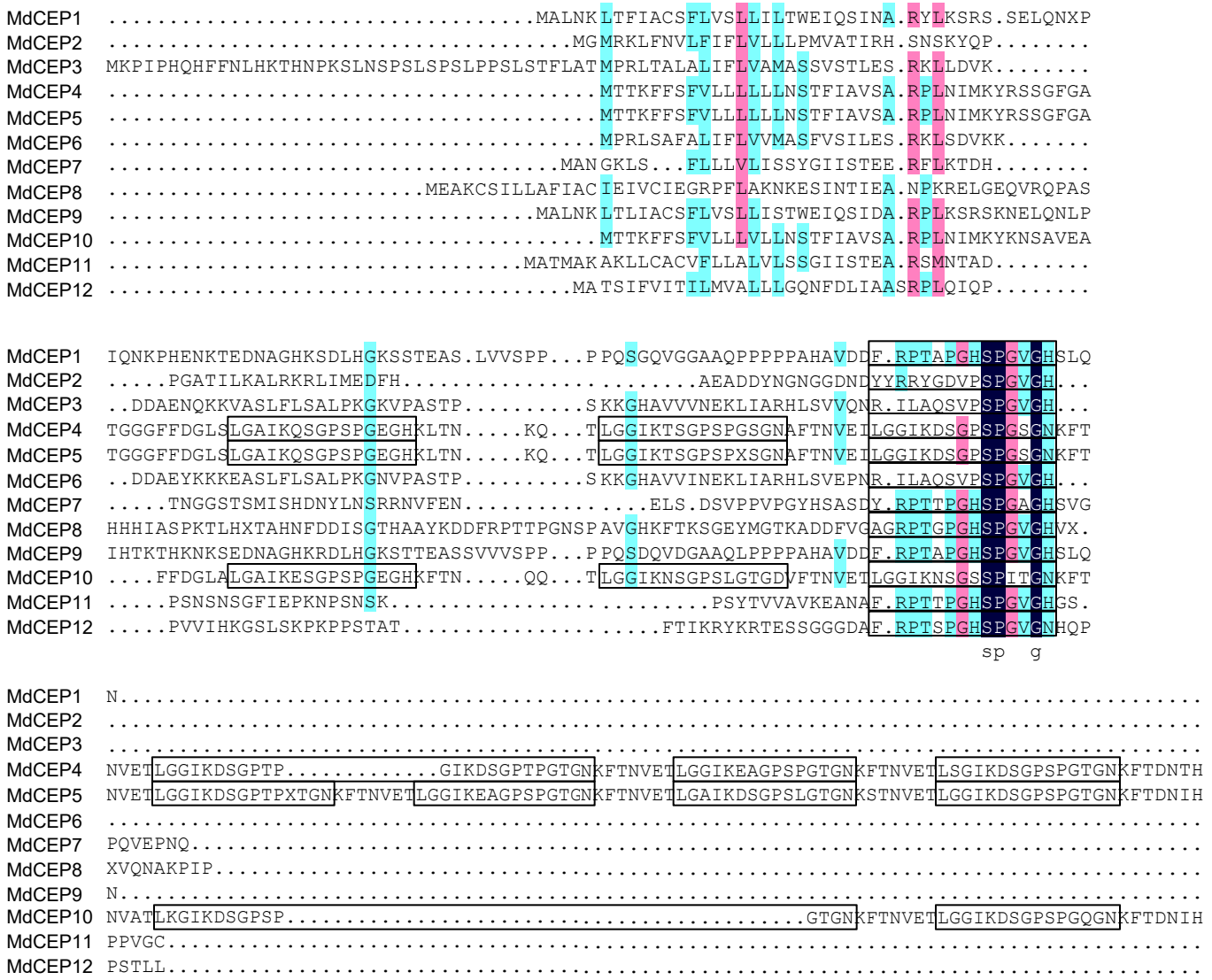
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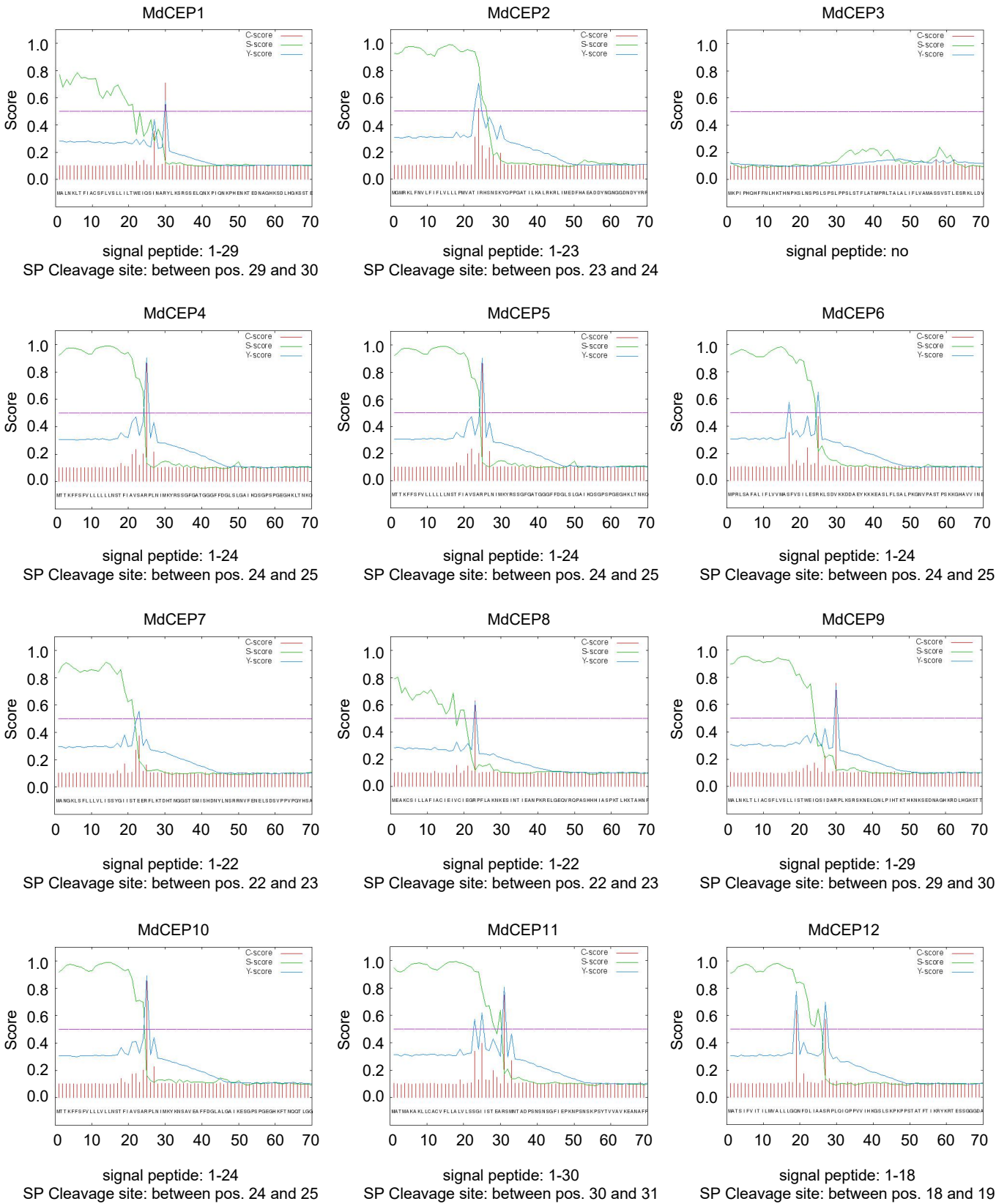
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Supplementary Figure S1



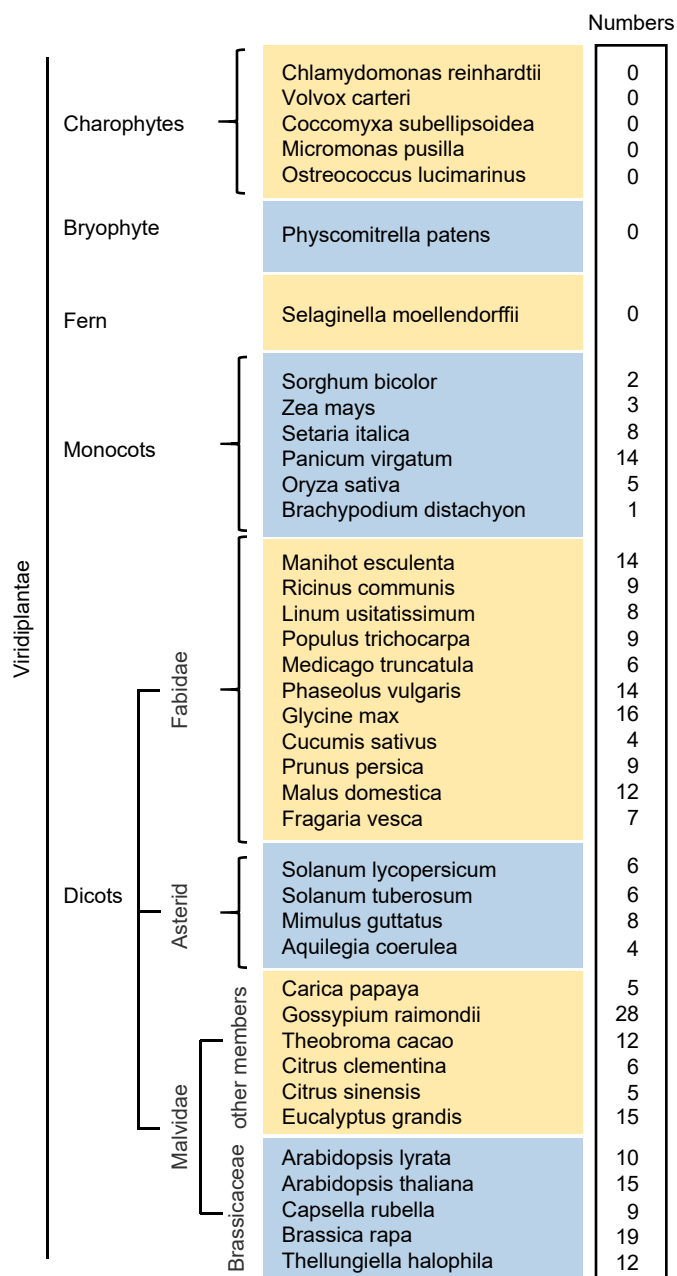
Supplementary Figure S1. Multiple sequence alignment of MdCEPs for apple. Overall conserved amino acids are in black. CEP domains are marked in open squares.

Supplementary Figure S2



Supplementary Figure S2. Signal peptide cleavage sites of MdCEPs predicated via SignalP 4.1 software. The potential cleavage sites of various MdCEPs are shown in the bottom.

Supplementary Figure S3

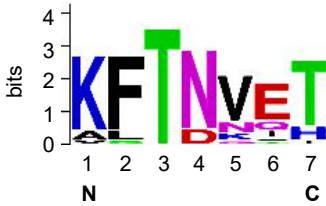
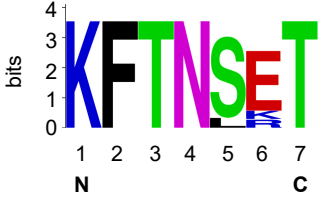
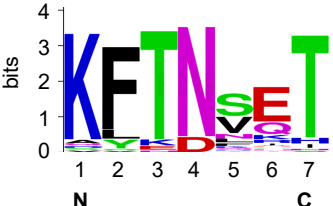


Supplementary Figure S3. The number of CEP genes in 39 plant species. Phylogenetic tree indicating the presence or absence of CEP family members in the indicated species.

Supplementary Figure S4

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Supplementary Figure S5

Species	Linker between CEP domains		WebLoGo	Sequence
<i>Malus domestica</i>	>KLTNKQT	>KFTNVET		KFTNVET
	>AFTNVET	>KFTNVET		
	>KFTNVET	>KSTNVET		
	>KFTNVET	>KFTDNIH		
	>KFTNVET	>KFTNQQT		
	>KFTDNTH	>VFTNVET		
	>KLTNKQT	>KFTNVAT		
	>AFTNVEI	>KFTNVET		
<i>Eucalyptus grandis</i>	>KFTNSET	>KFTNSKT		KFTNSET
	>KFTNSET	>KFTNSET		
	>KFTNSET	>KFTNSET		
	>KFTNSKT	>KFTNSET		
	>KFTNSET	>KFTNSET		
	>KFTNSET	>KFTNLET		
	>KFTNSET	>KFTNSRT		
	>KFTNSET	>KFTNSRT		
<i>Fragaria vesca</i>	>KFTDQRT		The number of sequences is too small to establish the logo	KFTNV_T
	>KVTNVET			
	>SFTNVDT			
<i>Solanum tuberosum</i>	>KFTNSQT		The number of sequences is too small to establish the logo	KFTNSQT
	>KFTNSQT			
	>KFTNSQI			
<i>Ricinus communis</i>	>KYKNLQT		The number of sequences is too small to establish the logo	KYKNLQT
<i>Citrus sinensis</i>	>KYNDRNT		The number of sequences is too small to establish the logo	K__D__T
<i>Citrus clementina</i>	>KYENFKT		The number of sequences is too small to establish the logo	KYENFKT
<i>Solanum lycopersicum</i>	>KFTNNQT		The number of sequences is too small to establish the logo	KFTNNQT
<i>Mimulus guttatus</i>	>KFTNVET		The number of sequences is too small to establish the logo	K_T__T
<i>Populus trichocarpa</i>	>KYKNFQS		The number of sequences is too small to establish the logo	KY_NFQ_
	>KYENFQT			
<i>Glycine max</i>	>KFTNSET		The number of sequences is too small to establish the logo	KFTNSET
All of above plants	All of above sequences			KFTNSET

Supplementary Figure S5. Multiple sequence alignments of adjoining sequence between CEP domains in group 2.
The adjoining sequences (KFTNVET) between CEP domains in group 2 showed high amino acid similarity (KFTNVET), indicating that the proteolytic processing might be identical in some higher land plants.

Supplementary Table S1. Genome-wide analysis of MdCEPs in apple.

Gene id	protein length	pI	Mw	CDS length	exon no.	Genomic location
MDP0000143462	191	9.52	19277.57	576	2	MDC035751.16:9289..9911
MDP0000143463	204	9.49	20643.54	615	1	MDC035751.16:12043..12657
MDP0000146517	96	5.87	10384.43	291	1	MDC001634.432:141..431
MDP0000187706	81	9.1	9183.6	246	1	MDC010707.221:2870..3115
MDP0000213875	119	7.01	12673.43	360	1	MDC017493.407:10804..11163
MDP0000254055	96	9.92	10378.21	291	1	MDC029559.30:7325..7615
MDP0000306151	156	9.44	16109.15	471	1	MDC018097.432:5007..5477
MDP0000375783	89	8.99	9088.43	270	1	MDC018728.84:5624..5893
MDP0000770985	92	10.62	9746.36	279	1	MDC005909.470:7191..7469
MDP0000804928	135	10.46	14510.03	408	1	MDC015524.135:32478..32885
MDP0000886456	141	7.82	15122.71	426	1	MDC022708.143:7353..7778
MDP0000886459	121	7.98	12978.62	366	1	MDC022708.143:15790..16155

Supplementary Table S1. The detailed information including protein length, molecular weight, isoelectric point (pI), CDS length, number of exons and genomic location are shown in the table.

Supplementary Table S2. References of bioinformatics software, website and databases.

Bioinformatic software and databases	Websites	References
Phytozome	http://www.phytozome.net/	Goodstein et al. 2012
ExPASy Proteomics Server	http://expasy.org/	Gasteiger et al. 2003
Plant-CARE Server	http://bioinformatics.psb.ugent.be/webtools/plantcare/html/	Jia et al. 2015
MUSCLE program	http://www.drive5.com/muscle/	Edgar 2004
MEGA7	http://www.megasoftware.net/	Tamura. et al. 2011
Gene Structure Display Server	http://gsds.cbi.pku.edu.cn/	Hu. et al. 2015
Treeview	http://gijgo.com/tree/	Zhang. et al. 2013
I-TASSER	http://zhanglab.ccmb.med.umich.edu/I-TASSER/	Yang. et al. 2015
BAR	http://bar.utoronto.ca/	Kiana Toufighi. et al. 2005
DNAMan	https://www.lynnon.com/pc/framepc.html	Jyrki Kaukonen. et al. 2000
TAIR10	https://www.arabidopsis.org/	Tanya Z. et al. 2015
Genevestigator	https://genevestigator.com/gv/	Hruz T. et al. 2008
TMHMM Server v. 2.0	http://www.cbs.dtu.dk/services/TMHMM/	S. Moller. et al. 2001
SUBA4	http://suba.live/	Hooper CM. et al. 2017
SignalP4.1	http://www.cbs.dtu.dk/services/SignalP/	Nielsen H. 2017
PLAZA comparative genomics tool	http://bioinformatics.psb.ugent.be/plaza/	Van Bel. et al. 2017
Uniprot databases	http://uniprot.org/blast/	Wu J. et al. 2014
Camelina sativa database	http://www.camelinadb.ca/	Kagale, S. et al. 2014
Brassica Database	http://brassicadb.org/brad/index.php/	Cheng F. et al. 2011
GDDH13 Version 1.1	https://iris.angers.inra.fr/gddh13/the-apple-genome-downloads.html	Nicolas Daccord. et al. 2017

Supplementary Table S3. Primers used in this study.

Purpose	Name	Accession number of genes	Sequence (5'-3')
Transgenic callus Vector, pRI 101-AN DNA	AtCEP1 F	AT1G47485	GTCGACATGGGAATGTCGAATAGGTCAGT
	AtCEP1 R	AT1G47485	GGATCCTCAATGTCGCCCCGTTAGAGT
	MdCEP1 F	MDP0000213875	GTCGACATGGCGACCATGGCCAAA
	MdCEP1 F	MDP0000213875	GGATCCTCAACATCCAACAGGAGGG
	MdCEP ^s F	MDP0000213875	GTCGACATGGCGACCATGGCCAAA
	MdCEP ^s F	MDP0000213875	GGATCCTCAACATCCAACAGGAGGGGCTTCCATGACCAACACCAG GACAGTGGCCTGGT
	MdCEP ^g F	MDP0000213875	GTCGACATGGCGACCATGGCCAAA
	MdCEP ^g F	MDP0000213875	GGATCCTCAACATCCAACAGGAGGGGCTTCCATGTGCAACACCAGG ACTGTGG
	MdCEP ^{sg} F	MDP0000213875	GTCGACATGGCGACCATGGCCAAA
	MdCEP1 ^{sg} F	MDP0000213875	GGATCCTCAACATCCAACAGGAGGGGCTTCCATGTGCAACACCAGG ACAGTGGCCTGGT
qRT-PCR in <i>Malus domestica</i>	Md CEP1 F	MDP0000213875	ACTGAAGCATCATTAGTTGTATC
	Md CEP1 R	MDP0000213875	GCCTGAAGTCATCTACGG
	Md CEP2 F	MDP0000187706	ATCAGGCACAGTAATAGCA
	Md CEP2 R	MDP0000187706	TAATAGTCGTTATCTCCACCAT
	Md CEP3 F	MDP0000804928	AAACCCACAATCCCAAATC
	Md CEP3 R	MDP0000804928	TAGAGTTGACACAGATGAGG
	Md CEP4 F	MDP0000143462	TCGGAGGGATTAAGGATTC
	Md CEP4 R	MDP0000143462	CCACCAAGAGTCTCAACA
	Md CEP5 F	MDP0000143463	AGCGATTAAGGACTCTGG
	Md CEP5 R	MDP0000143463	TTATTGATGGATGTTGTCTGTAA
	Md CEP6 F	MDP0000254055	AGGCTTCTCTGTTTCTAAGT
	Md CEP6 R	MDP0000254055	GATTTGGTTCAACGCTAAGA
	Md CEP7 F	MDP0000146517	ATCAGCCATGACAATTATCTC
	Md CEP7 R	MDP0000146517	CTACTTGAGGTCTACAGAA
	Md CEP8 F	MDP0000886456	TGGCAAAGAACAAAGAGTC
	Md CEP8 R	MDP0000886456	GACTAGCAATGTGGTGATG
	Md CEP9 F	MDP0000886459	TCATCTCAACCTGGGAAAT
	Md CEP9 R	MDP0000886459	GCATTATCCTCCGATTTGTT
	Md CEP10 F	MDP0000306151	TGGAGCCATCAAGGAATC
	Md CEP10 R	MDP0000306151	CCACCTAGAGTCTCAACATT
	Md CEP11 F	MDP0000375783	GTTCTCTCTTCTGGAATCATTT
	Md CEP11 R	MDP0000375783	CTGGGTTTGGAATTAGATGG
	Md CEP12 F	MDP0000770985	AGCGTTACAAGAGGACTG
	Md CEP12 R	MDP0000770985	GTACTIONGGTGGCTGATGATT
	18s rRNA F	DQ912698.1 (gene bank)	ACACGGGGAGGTAGTGACAA
	18s rRNA R	DQ912698.1 (gene bank)	CCTCCAATGGATCCTCGTTA
	MdNRT2.1 F	MD11G1141700 F	GTGTGCTGTACTCTTCCT
	MdNRT2.1 R	MD11G1141700 R	CACGCTCAGACCTACTATT