

# **Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations**

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## **Supporting Material**

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## Assembling the MADS-box sequence dataset

To extract the MIKC type MADS-box sequence dataset from the public databases, we have used the following approach: In a first step, one representative protein sequence of each known MADS-box subfamily (according to recently published phylogenetic analyses, see for example reference 1) was chosen. The full-length protein sequence of each selected subfamily member was used to search the public databases for homologous sequences, using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>). The protein sequence dataset was built using the blastp option to search the available protein databases, while the nucleotide dataset was assembled using tblastn to search the available nucleotide databases. To ensure that even distantly related MADS-box genes would be retrieved in the homology searches, the standard settings of some search parameters of the BLAST program were changed. The expectancy value was raised to 100, and the number of homologous sequences to be displayed was increased to 500. Homologies shown in the output BLAST page were inspected visually, and MADS-box sequences were retrieved by using the sequence retrieval option and saved in batches as text files in Genbank format. These text files were subsequently imported in the GCG program using the FromGenbank function (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisc.). Because of the low stringency settings of the homology searches with the different subfamily representatives, a large proportion of the collected sequences was identified multiple times; such duplicates were removed automatically during import in GCG using the ‘remove duplicates’ option.

To yield a more comprehensive overview of the taxonomic distribution within subfamilies, the sequence identifiers were renamed manually as follows: they all start with a two- or three-letter code indicating the species name (see Species Table below for abbreviations), followed by the gene name as found in the database and terminating with the database Accession Number (according to either DNA, EST, Protein or Patent databases). Full-length sequences were aligned using the PILEUP function, followed by a manual alignment of the C-terminal regions using the Seqlab Editor of the GCG software package. To illustrate conservation of the C-terminal conserved motifs, we have displayed the alignments of the C-terminal motifs per subfamily. C-terminal motifs of sequences of which the sequence name terminates with ‘COR’ (corrected) have been identified in other reading frames than published in the database and/or beyond the proposed stopcodon. An asterisk behind a peptide motif indicates a stopcodon; motifs not terminating with an asterisk are followed by a variable number of less-conserved residues (not shown).

## Species Table

Species	code	Species	code
<i>Agapanthus praecox</i>	Ap	<i>Lolium temulentum</i>	Lte
<i>Akebia quinata</i>	Aq	<i>Lycopersicon esculentum</i>	Le
<i>Anemone nemorosa</i>	An	<i>Lycopodium annotinum</i>	La
<i>Antirrhinum majus</i>	Am	<i>Magnolia praecocissima</i>	Mp
<i>Aquilegia alpina</i>	Aa	<i>Malus domestica</i>	Md
<i>Aquilegia caerulea</i>	Ac	<i>Medicago sativa</i>	Ms
<i>Arabidopsis lyrata</i>	Al	<i>Michelia figo</i>	Mf
<i>Arabidopsis thaliana</i>	At	<i>Momordica charantia</i>	Mc
<i>Aranda deborah</i>	Ad	<i>Nicotinia sylvestris</i>	Ns
<i>Asarum europaeum</i>	Ae	<i>Nicotinia tabacum</i>	Nt
<i>Berberis gilgiana</i>	Bg	<i>Oncidium cv.</i>	Oc
<i>Betula pendula</i>	Bp	<i>Orchis italica</i>	Oi
<i>Brassica napus</i>	Bn	<i>Oryza sativa</i>	Os
<i>Brassica oleracea</i>	Bo	<i>Pachysandra terminalis</i>	Pt
<i>Brassica oleracea var botrytis</i>	Bob	<i>Panax ginseng</i>	Pg
<i>Brassica rapa</i>	Br	<i>Papaver californicum</i>	Pc
<i>Calycanthus floridus</i>	Cf	<i>Papaver nudicaule</i>	Pn
<i>Canavalia lineata</i>	Cl	<i>Paulownia kawakamii</i>	Pk
<i>Capsicum annuum</i>	Ca	<i>Peperomia hirta</i>	Phir
<i>Ceratopteris richardii</i>	Cr	<i>Petunia hybrida</i>	Ph
<i>Chloranthus spicatus</i>	Cs	<i>Petunia inflata</i>	Pi
<i>Chrysanthemum x morifolium</i>	Cm	<i>Petunia integrifolia</i>	Pin
<i>Cimicifuga racemosa</i>	Cra	<i>Phalaenopsis equestris</i>	Pe
<i>Clematis chiisanensis</i>	Cc	<i>Physcomitrella patens</i>	Pp
<i>Clematis integrifolia</i>	Ci	<i>Picea abies</i>	Pa
<i>Corylus avellana</i>	Cav	<i>Picea marinea</i>	Pm
<i>Cryptomeria japonica</i>	Cj	<i>Pimpinella brachycarpa</i>	Pb
<i>Cucumis sativa</i>	Cus	<i>Pinus radiata</i>	Pr
<i>Cycas edentata</i>	Ce	<i>Pinus resinosa</i>	Pres
<i>Daucus carota</i>	Dc	<i>Piper magnificum</i>	Pmag
<i>Delphinium ajacis</i>	Da	<i>Pisum sativum</i>	Ps
<i>Dendrobium grex</i>	Dg	<i>Platanus occidentalis</i>	Po
<i>Dicentra eximia</i>	De	<i>Poa annua</i>	Pan
<i>Elaeis guineensis</i>	Eg	<i>Populus balsamifera</i>	Pb
<i>Eucalyptus globulus</i>	Egl	<i>Populus tomentosa</i>	Pto
<i>Eucalyptus grandis</i>	Eug	<i>Populus tremuloides</i>	Pt
<i>Fragaria x ananassa</i>	Fa	<i>Ranunculus bulbosus</i>	Rb
<i>Gerbera hybrida</i>	Gh	<i>Ranunculus ficaria</i>	Rf
<i>Glycine max</i>	Gm	<i>Rosa rugosa</i>	Rr
<i>Gnetum gnemon</i>	Gg	<i>Rosa x hybrida</i>	Rh
<i>Gnetum parvifolium</i>	Gp	<i>Rumex acetosa</i>	Ra
<i>Gossypium hirsutum</i>	Ghi	<i>Sagittaria montevidensis</i>	Sm
<i>Helianthus annuus</i>	Ha	<i>Sanguinaria canadensis</i>	Sc
<i>Helleborus orientalis</i>	Hor	<i>Saururus chinensis</i>	Sch
<i>Hemerocallis hybrid</i>	Hh	<i>Silene latifolia</i>	Sl
<i>Hieracium piloselloides</i>	Hp	<i>Sinapis alba</i>	Sa
<i>Hordeum vulgare</i>	Hv	<i>Solanum tuberosum</i>	St
<i>Hyacinthus orientalis</i>	Ho	<i>Sorghum bicolor</i>	Sb
<i>Hydrangea macrophylla</i>	Hm	<i>Syringa vulgaris</i>	Sv
<i>Ipomoea batatas</i>	Ib	<i>Tacca chantieri</i>	Tc
<i>Ipomoea nil</i>	In	<i>Thalictrum thalictroides</i>	Tt
<i>Juglans regia</i>	Jr	<i>Trautvetteria carolinensis</i>	Tca
<i>Lilium longiflorum</i>	Ll	<i>Triticum aestivum</i>	Ta
<i>Lilium regale</i>	Lr	<i>Trollius laxus</i>	Tl
<i>Liquidambar styraciflua</i>	Ls	<i>Vitis vinifera</i>	Vv
<i>Liriodendron tulipifera</i>	Lt	<i>Zea mays</i>	Zm
<i>Lolium perenne</i>	Lp		

# *SQUAMOSA/API* SUBFAMILY

## Paleo*API* Lineage

os-mads28-osa011675  
 os-rap1b-ab041020  
 os-fdrmads6-af139664  
 os-mads14-af058697  
 zm-m15-aj430632  
 zm-m4-aj430641  
 1te-mads1-af035378  
 1p-mads1-ay198326  
 hv-mads5-aj249144  
 ta-tamads11-ab007504  
 zm-mads3-af112150  
 zm-zap1-146400  
 os-Osmads15-af058698  
 1te-mads2-af035379  
 1p-mads2-ay198327  
 hv-mads8-aj249146  
 sb-sbmads2-u32110-COR  
 dg-domads2-af198175  
 bp-mads4-x99654  
 am-defh28-ay040247  
 ph-fbp29-af335245  
 st-potm1-1-u23757  
 sc-scm1-af002666  
 1e-tdr4-aam33098  
 in-pnsah1-ab013105  
 In-PnSAH2-AB013106  
 nt-nap1-1-af009126  
 ns-nsmads1-af068725  
 nt-mads11-af385746  
 ph-fbp26-af176783  
 Ca-MADS6-AF130118  
 ph-pfg-af176782  
 bp-mads5-x99655  
 md-mads2-u78948  
 s1-s1m5-x80492  
 mp-mpmads15-q948u1  
 sa-samadsb-u25695  
 bob-fulb-aj505842  
 bob-fuld-aj505844  
 bob-fulc-aj505843  
 at-ful-u33473  
 bob-fula-aj505841

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 MPPWMLRHVNE\*~  
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## Eu*API* Lineage

at-ap1-z16421  
 pt-ap1-af034093  
 bo-boi1ap1-u67451  
 bob-ap1c-aj505846  
 bo-boi2ap1-u67452  
 bob-ap1a-aj505845  
 bo-ap1-z37968  
 sa-madsc-2-af109403  
 sa-ap1-x81480  
 bo-boical-u67454  
 bo-bocal-136926  
 bob-bobcal-136927  
 brp-aj251300  
 at-cal-136925  
 ha-ham75-af462152  
 cm-cdm111-ay173054  
 ha-ham92-ay173071  
 nt-nap1-2-af009127  
 nt-squa15-u63162  
 ns-mads2-af068726  
 ntmads5-af068724  
 1e-mads-mc-af448521  
 bp-mads3-x99653  
 ps-peam4-aj291298  
 md-mads5-aj000759  
 pt-ap1-af034094  
 am-squamosa-x63701  
 dc-mads1-aj271147

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 LYNNMKHL\*  
 SCHLGCFAT\*  
 TCHLGCF...\*  
 ECHLGCFAA\*  
 SCHLGCFGT\*  
 SCHLGCFAA\*  
 PCNLRCFA.\*

## ***TM3* SUBFAMILY**

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 pr-prmads6-u90347  
 pr-prmads8-aac27353  
 pr-prmads4-u90345  
 pr-prmads7-ab80810  
 pr-prmads9-u90344  
 pr-prmads5-u90346  
 gg-ggm1-aj132207  
 at-at5g51860  
 at-at5g51870  
 os-baa81886  
 zm-mads1-af112148  
 zm-ay104805  
 os-fdrmads8-aad38369  
 eg-opmads1-af207699  
 at-at4g22950  
 at-agl14-at4g11880  
 eg1-etc1-aad16052  
 at-agl20-at2g45660  
 sa-madsa-t10422  
 ph-fbp21-af335239  
 nt-mads1-s46526  
 ph-fbp20-af335238  
 ph-fbp28-af335244  
 pb-mads1-aac33475  
 cm-cdm36-ay173065  
 at-at5g62165-ay096509  
 ph-fbp22-af335240  
 mp-mpmads9-ab050651

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 EVETQLVIRPP  
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 DVETQLNIGPP  
 DVETDLFIGFLP  
 DVETELFIGLP  
 DVETELYIGLP  
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 DVETDLYIGLP  
 DVETELYIGWP  
 DVETGLFIGPP  
 EVVTDLFIGPP  
 DVETELFIGPP  
 DVETQLFIGLP  
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 DVETELFIGLP  
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 DVETELFIGLP

## ***STMADS11* SUBFAMILY**

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 ph-fbp25-af335243  
 hv-mads1-cab97349  
 hv-mads1-2-cab97350  
 os-cac29335  
 zm-m20-a430634  
 os-baa81880  
 zm-m26-a430693  
 zm-m19-a430633  
 zm-m21-a430635  
 bob-svpa-cad48304  
 at-svp  
 ib-aak27150  
 le-jointless-q9fuy6  
 pk-aaf22455  
 mp-mpmads1-ab050643  
 ph-fbp13-af335237  
 st-mads16-t06995  
 ib-aak27151  
 at-agl24-af005158  
 cl-af144623  
 gg-ggm12-AJ132218

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 SDTSLKLGLH~  
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 SDTSLKLGLP~  
 SDTFLKLGLP~  
 SITSLKLGLP~  
 SDTSLKLGLP~  
 SDTSLKLGLP~  
 SDTSLKLGLP~  
 SDTSLHLGLP~

## AGAMOUS SUBFAMILY

rr-baa90744	DQISLQLV*	Nt-AG-T03592	DQPSLQLV*
rr-baa90745	DQISLQLV*	Bn-AG-A43484	DQTALQLV*
md-mads-cac80858	DQISLQLV*	At-AG-P17839	DQTALQLV*
Cav-mads1-aad03486	DQMALQLV*	pres-aad01266	EQTTLQLG*
jr-cac38764	DQMALQLV*	pa-dal2-t14847	EQTTLQLG*
rh-aad00025	DQISLQLV*	pe-mads1-af234617	QQTALQLG*
fa-stag1-af168468	DQVSLQLV*	pm-sag1a-aac97157	EQTTLQLG*
cus-cum1-aac08528	DNMALQLV*	ce-cyag-af492455	DQAALQLG*
pb-ptag1-aac06237	DQMALQLV*	Gg-GGM3-AJ132209	EQTALHLG~
Ra-s57586	NQTPLQLV*	mp-mpmads11-bab70746	DQTALHLG~
gh-gaga1-caa08800	DQTPLQLV*	mp-mpmads2-bab70737	EQTALQLG*
gh-gaga2-caa08801	DQTPLQLV*	ho-hag1-aad19360	QQTALQLG*
Ha-HAM45-aa018228	DQTPLQLV*	Os-bab32985	QPTTLQLG~
Ha-HAM59-aa018229	DQTPLQLV*	Os-MADS3-s59480	QPTTLQLG~
Cm-aa022984	DQTPLQLV*	zm-ucsd78a-aab81103	QPTTLQLG~
s1-s1m1-caa56655	DQTTLQLN*	hv-hvag1-af486648	QPTALQLG~
pin-pag11-aaa68001	DQTALQLV*	Le-TAGL11-aam33102	DHKR~~~~~*
dc-mads4-cac81071	QHVPLQLV*	Ph-fbp7-caa57311	DKKSLDLE*
Le-TAGL1-AY098735	DQTPLQLV*	Ph-Fbp11-caa57445	DKKSLQLE*
Bn-SHP1-aa00646	DQPPQLQLV*	Mc-aa020104	DKKMLHLG*
At-agl1-SHP1-P29381	DQPPQLQLV*	cus-cum10-aac08529	DKKMLHLG*
At-agl5-SHP2-P29385	DQPPQLQLV*	Ghi-GHMADS2-aan15183	DKKILHLG*
ph-pmads3-q40885	DQPPQLQLV*	Vv-MADS5-af373604	DKKVLHLG*
le-tag1-aaa34197	DQPPIQQLV*	Md-MADS10-caa04324	DKKNLHLG*
ls-aad38119	DQTPLQLV*	AT-AGL11-Q38836	DKKILHLG*
Am-FAR-cab42988	DQPLQLQLV*	zm-zag1-jq2289	DRKDFNDQ~
vv-MADS1-af265562	DQTALQLV*	hv-hvag2-af486649	DRKTLNSV~
Am-plena-A44343	DQTALQLV*	zm-zag2-caa56504	ATELNLY~
Ph-fbp6-X68675	DQTALQLV*	OsMADS13-AF151693	PTELNLGY~
pg-gag2-caa86585	DQTALQLV*	os-agamous-bab90168	QTALHLGY~
pb-ptag2-aac06238	DQFLS~~~*	Ho-MADS1-aaf08830	QTALHLGY~

# AGL2 SUBFAMILY PART I

sa-samadsd-Y08626	Y	M	L	G	W	L	P	~	~
at-agl9-SEP3-at1g24260	Y	M	L	G	W	L	P	~	~
ph-fbp2-m91666	Y	M	A	G	W	L	P	*~	
ns-mads3-af068722	Y	M	A	G	W	L	P	*~	
le-tdr5-x60480-COR	Y	M	A	G	W	L	P	*~	
vv-mads4-af373603	Y	M	P	G	W	L	P	*~	
am-defh200-s71757	Y	I	S	G	W	L	P	*~	
bp-mads1-cab95648	Y	M	S	G	W	M	P	*~	
eug-egm1-af029975-COR	F	M	P	G	W	F	P	*~	
cm-cdm44-ay173057	Y	M	P	G	W	Y	Q	*~	
ps-t06543	Y	M	G	G	W	L	P	*~	
am-defh72-s71756	Y	N	M	T	G	W	L	P	*
ad-x69107	Y	M	P	P	G	W	L	G	~
at-agl3-aa136250	F	F	P	G	W	M	V	*~	
bob-agl3a-cad48302	F	F	P	G	W	M	V	*~	
AT-agl2-SEP1-m55552	Y	I	P	G	W	M	L	*~	
bob-sep1a-cad48303	Y	I	P	D	W	M	L	*~	
at-agl4-SEP2-at3g02310	Y	I	P	G	W	M	L	*~	
cus-cagl2-af135962	F	I	P	G	W	M	L	*~	
vv-mads2-af373601	F	I	P	G	W	M	L	*~	
pt-magl2-af185574	F	I	P	G	W	M	L	*~	
pt-magl4-af185574	F	I	P	G	W	M	L	*~	
pt-af034095	F	I	P	G	W	M	L	*~	
md-mads1-t17023	F	I	P	G	W	M	L	*~	
md-mdmads8-caa04919	F	I	P	G	W	M	L	*~	
md-mdmads9-caa04920	F	I	P	G	W	M	L	*~	
nt-mads4-af068723	F	I	P	G	W	M	L	*~	
ph-fbp9-af335236	F	I	P	G	W	M	L	*~	
ca-mads2-af129875	F	I	H	G	W	M	L	*~	
ph-fbp23-af335241	F	I	H	G	W	M	L	*~	
eug-egm3-aac78284	F	I	P	G	W	M	L	*~	
md-mads7-aj00076	Y	I	P	G	W	M	L	*~	
md-mads3-u78949	Y	I	P	G	W	M	L	*~	
md-mads6-aj000760	Y	I	P	G	W	M	L	*~	
dc-cmb1-q39685	F	A	Q	G	W	M	L	*~	
md-mads4-u78950	F	F	P	G	W	M	L	*~	
mp-mpmads13-bab70747	Y	M	P	G	W	L	V	*~	
fa-RIN-af484683	F	I	P	G	W	M	L	*~	
Le-Lemadsrin-AF448522	V	V	P	G	W	M	L	*~	
ph-fbp4-af335234	V	L	P	G	W	M	L	*~	
dc-mads5-cac81072	V	I	P	G	W	M	L	*~	
le-tagl2-aam33104	M	I	P	G	W	M	L	*~	
le-tm29-cac83066	M	I	P	G	W	M	L	*~	
ph-pmads12	M	V	P	G	W	M	L	*~	
ph-fbp5-af335235	I	I	P	G	W	M	L	*~	
am-defh49-s78015	L	V	P	G	W	M	L	*~	
ha-ham137-ay173072	Q	M	Q	G	W	P	A	*~	
gh-grcd1-aj400623	Q	M	Q	G	W	P	A	*~	
cm-cdm77-ay173058	Q	M	Q	G	W	P	A	*~	

## *AGL2* SUBFAMILY PART II (monocotyledons)

Zm-m24-AJ430638  
 Zm-m31-AJ43060  
 os-baa81882  
 ta-bj219318  
 ta-bj276769  
 hv-hvmads7-aj249145  
 ta-bj218990  
 zm-zmm8-y09303  
 zm-zmm14-cab85962  
 os-osmads1-1hs-s53306  
 ta-bj313906  
 ta-bj265532  
 ta-bj211160  
 zm-zmm3-y09301  
 os-osmads5-u78890  
 zm-bg837363  
 zm-zmm7-caa70485  
 os-osmads8-u78892  
 sb-sbmads1-u49734-cor  
 ta-bq902720  
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 ta-bj275311  
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 os-fdrmads1-AF141966



## *AGL6* SUBFAMILY

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 ph-pmads4-baa94287  
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 gg-ggm9-cab44455  
 gp-gpmads3-baa85630  
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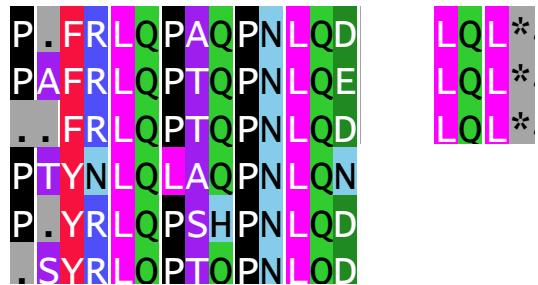


## GLO/PI SUBFAMILY

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## Bs SUBFAMILY

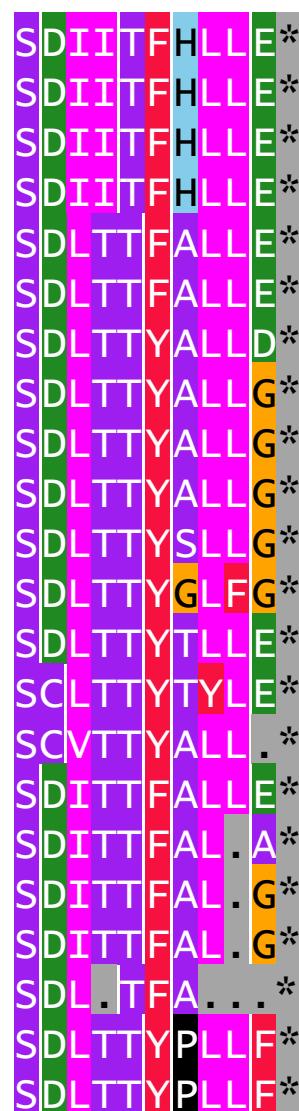
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 am-defh21-cac85225



## DEF/AP3 SUBFAMILY

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 bo-boi1ap3-u67453  
 at-ap3-ay087369  
 am-deficiens-x52023  
 Sv-svap3-af052869  
 Hm-hmap3-af230702  
 Hp-hpdef2-af180365  
 Hp-hpdef1-af180364  
 gh-gdef2-aj009725  
 cm-cdm115-ao22985  
 cm-cdm19-ay173064  
 jr-ap3-j313089  
 ra-d1-u28482  
 s1-s1m3-x80490  
 ph-gp-x69946  
 Nt-ntdef-x96428  
 1e-ap3-af052868  
 st-stdef4-x67508  
 dc-mads3-aj271149  
 ms-nmh7-141727  
 gm-ax478039



# DEF/AP3 SUBFAMILY

## PaleoAP3 Lineage

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 oc-ao45824  
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 rb-rbap3-2-aad31697  
 rf-rfap3-2-af130870  
 an-ap3-3type2-ao26491  
 an-ap3-3type2-ao26492  
 hor-ap3-3a-ao26524  
 hor-ap3-3b-ao26525  
 t1-ap3-3type1-ao26542  
 t1-ap3-3type2-ao26543  
 aa-ap3-3-ao26499  
 ac-ap3-3-ao26503  
 cra-ap3-3-ao26512  
 rf-ap3-3-ao26531  
 bg-ap3-1-ao26505  
 os-ab003323  
 Os-osmads16-af077760  
 Ta-tamads51-ab007506  
 zm-silky1-af181479  
 hh-mads1-af209729  
 11-mads1-af503913  
 Lr-1rdef-ab071378  
 tc-ap3-af230706  
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 aq-ap3-1type2-ao26488  
 po-ap3-1-ao26529  
 po-ap3-2-ao26530  
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 aq-ap3-2\_type\_1-ay162839  
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 Sc-scap3-af130868  
 pn-pnap3-2-af052874  
 cs-ap3-af230701  
 mf-mfap3-af052877

YNQHYV\*  
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 HELRLA\*  
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 YGFQLA~  
 YNLQLA\*  
 SSLQLA\*  
 YNLRLA\*  
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 YFGVMH\*  
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 NDLRLA\*  
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 cra-ap3-2-ao26511  
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 pb-ptd-aac13695  
 pto-ptap3-ao49713  
 le-tdr6-x60759  
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 hm-tm6-af230703  
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 pa-dal12-af158541

HDLRLA\*  
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