

SUPPLEMENTAL MATERIAL (Pierre *et al.*)

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Supp Fig. 1. T-DNA insertion in line *nmt1-1* causes *BAR:AtNMT1* transcriptional fusions and At *NMT1* overexpression.

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SUPPLEMENTAL METHODS

I- Transgene constructs cloning strategies

II- Shuffled Sc *NMT* sequence

III- List of oligonucleotide primers used in this study

a- Oligonucleotides used for cloning

b- Oligonucleotides used for RT- or real time PCR

c- Oligonucleotides used for AKIN β :GFP fusions

SupFig. 1: T-DNA insertion in line *nmt1-1* causes *BAR:AtNMT1* transcriptional fusions and *At NMT1* overexpression

Panel A: RT-PCR analysis of NMT1 transcript levels in the WT (N/N), *nmt1-1* (n/n) and NMT1/*nmt1-1* heterozygous (N/n) backgrounds. The details of the quantitative radioactive analysis have been described elsewhere (Ross et al., 2005). A value of 1 was assigned to *At NMT1* expression in the WT.

Panel B: RNA blots were analyzed as previously described (Giglione et al., 2000). We obtained mRNA from plants with a homozygous WT (N/N) or a heterozygous *NMT1/nmt1-1* background (N/n). The labeled DNA probes used corresponded to the full-length *NMT1* gene, *BAR* and the left border. The transcripts corresponding to *BAR* (0.5 kb), *NMT1* (1.6 kb) and the newly formed *BAR:AtNMT1* fusion mRNA (2.9 kb) were detected. The mRNA size ladder marker is shown on the left.

Panel C: Map of the intergenic region and the two mRNAs produced from the 35S promoter driving the *BAR* gene in the T-DNA.

References:

Giglione, C., Serero, A., Pierre, M., Boisson, B., and Meinel, T. (2000). Identification of eukaryotic peptide deformylases reveals universality of N-terminal protein processing mechanisms. *EMBO J.* **19**, 5916-5929.

Ross, S., Giglione, C., Pierre, M., Espagne, C., and Meinel, T. (2005). Functional and developmental impact of cytosolic protein N-terminal methionine excision in *Arabidopsis*. *Plant Physiol.* **137**, 623-637.

A

N/N N/n n/n Control
(no mRNA)



Signal intensity	1	8	16	0
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B**DNA probe**

NMT1

BAR

LB

N/N

N/n

N/N

N/n

N/N

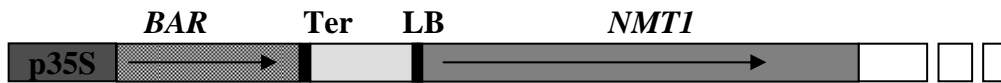
N/n

NMT1:BAR →*NMT1* →*BAR* →

← 2.7 kb

← 1.5 kb

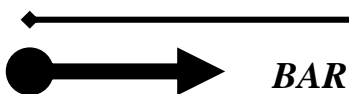
← 0.6 kb

C*NMT1::T-DNA* insertion in line *nmt1-1*

Stop codons

↑
ATG

(14)●

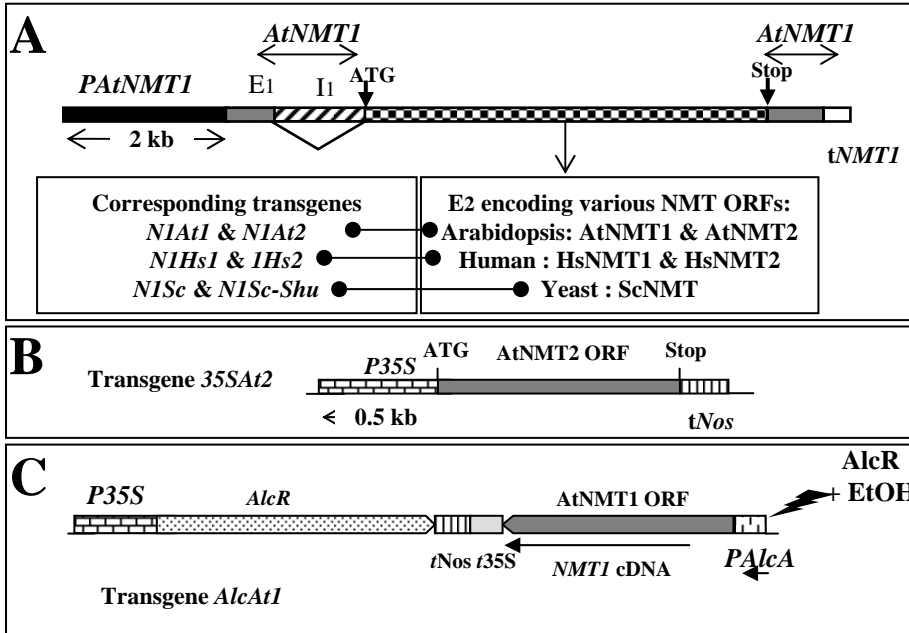
mRNAs
expressed*NMT1:BAR***SupFig.1**

SupFig. 2: Transgenes used in this study

Panel A: Map of the five types of transgene used to induce the expression of NMTs in various backgrounds. The full-length ORF of each orthologous NMT was expressed under control of the *At NMT1* promoter. Each construct contains (i) the 5' untranslated exon (E1), (ii) the first intron (I1) and (iii) the final 3' untranslated region of *At NMT1*.

Panel B: Map of a supplementary transgene used to induce NMT2 overproduction in various backgrounds. The full-length ORF of *At NMT2* was expressed under the control of the 35S promoter (*P35S*).

Panel C: Schematic diagram of the inducible NMT1 transgenic construct introduced into the *nmt1-1* line to yield the F11 line. The complete ORF of *At NMT1* was placed under the control of the *alcA* promoter (*PAlcA*). The 35S terminator (*t35S*) was used. Transcription factor *AlcR* is expressed under the control of the 35S promoter and the *Nos* terminator (*tNos*). In this construct, *At NMT1* expression is induced in the presence of EtOH vapor or watering.



SupFig.2

SupFig. 3: Sensitization of *Arabidopsis* seedlings to EtOH vapor

The glass cylinder was left open at the top to allow the air to circulate. EtOH was placed in the 1.5 ml Eppendorf tube for the times indicated in **Fig. 5** or throughout the entire life of the plant.

Glass cylinders **Opening to ambient air**



Eppendorff tubes filled with EtOH

SupFig.3

SupFig. 4: The phylogenetic tree of NMT sequences suggests that there are three main NMT families

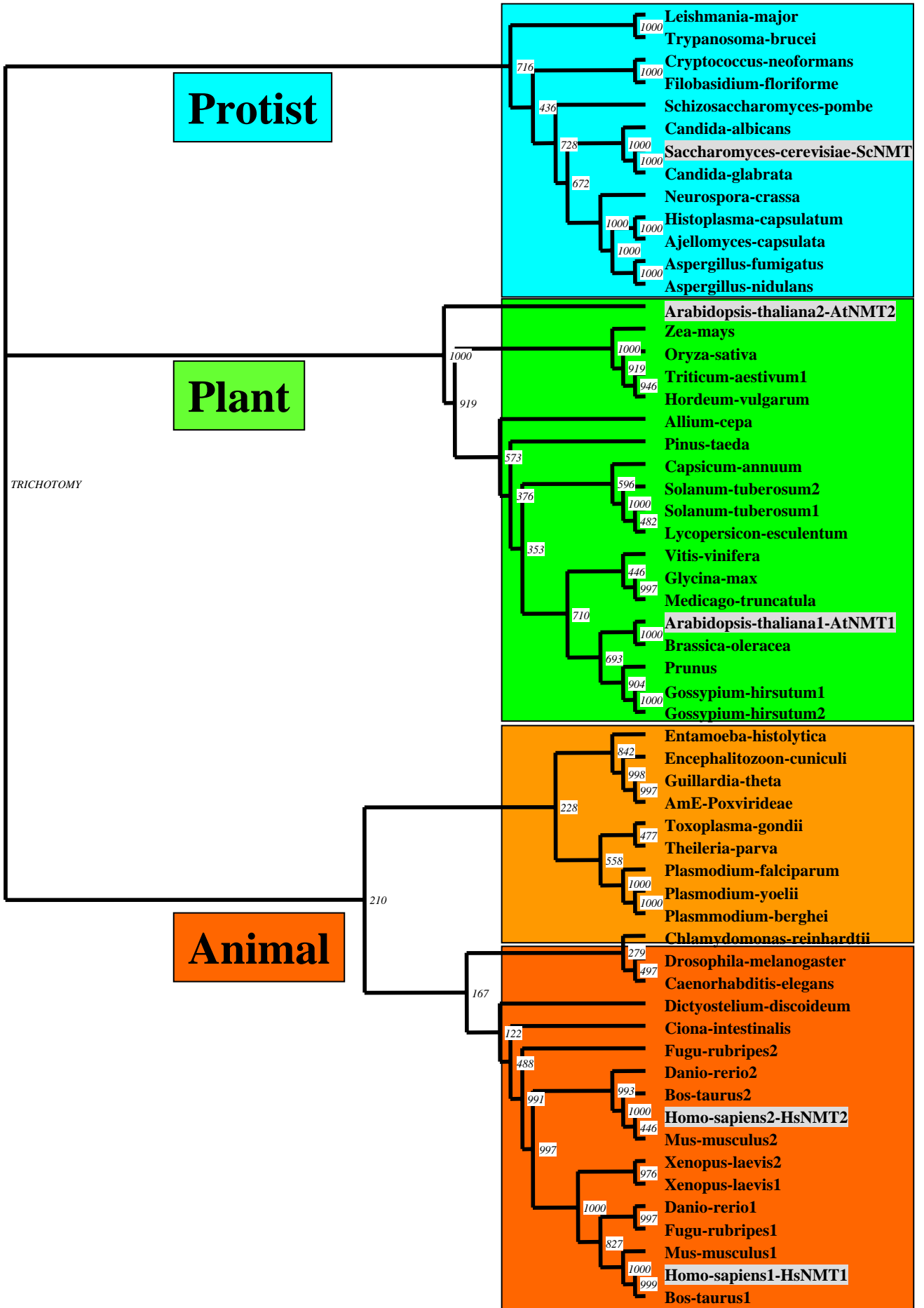
58 NMT sequences were selected as representative of sequence diversity. The sequences were extracted from completely sequenced genomes or from genomes for which sequencing is almost complete. These sequences were aligned with Clustal X (Jeanmougin et al., 1998) as shown below, manually adjusted to optimize alignment and the bootstrap tree constructed with PHYLIP. The random number generator seed was 111 and the number of bootstrap trials was 1000. The rooted phylogenetic tree was constructed with N-J Tree (Jeanmougin et al., 1998) and drawn with TreeView1.65 (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>; see Page, 1996). Internal values labeled on each node record the stability of the branch over the 1000 bootstrap replicates. The three main NMT types and classes are clustered and shown in color.

References:

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., and Gibson, T.J. (1998).

Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* **23**, 403-405.

Page, R.D.M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **12**, 357-358.



Plasmodium-yoelii -----
Plasmodium-berghei -----
Plasmodium-falciparum -----
Xenopus-laevis2 -----
Xenopus-laevis1 -MADQSETAAG-----DRLEEDENGHGHCSDCENEKHSNNR---GFG
Danio-erio1 -MADENETAP-----LPEKEEVEEEDHGHCSCNCEYEEHSSD---GEK
Fugu-rubripes1 -----
Homo-sapiens1-HsNMT1 -MADESETAVKPPAPPLPQMMEGNGNGHEHCSDCENEEDNSYNRGGLSPA
Bos-taurus1 -MGDESETAVKPPAPPLPQMMEGNGNGHEHCSDCENEEDNSYNRGGLSPA
Mus-musculus1 -MADESETAVKLPAPSLPLMMEGNGNGHEHCSDCENEEDNSHNRSGLSA
Homo-sapiens2-HsNMT2 -MAEDSESAAS-----QQSLELDDQDTCGIDGDNEEETEHAAG--SPG
Mus-musculus2 -MAEDSESAAS-----QQSLELDDQDTCGIDGDNEEETEHAAG--SPG
Bos-taurus2 -MAEDSESAAS-----QQSLELDDQDTCGIDGDNEEETEHAAG--SPG
Danio-erio2 -MAEDSESAAS-----QQSLELDDQDTCGIDGDNEEENEHMOC--SPG
Fugu-rubripes2 -----
Ciona-intestinalis --MEESNG-----IEEVVEVVVRVGAKEAQEKTEES-
Drosophila-melanogaster -MPNENAEDLS-----GQELKQKAKEVADASEAMLEKVVAG-----
Dictyostelium-discoideum -----
Saccharomyces-cerevisiae-ScNMT -MSEE-----DK-----
Candida-glabrata -MSE-----
Candida-albicans -MSGD-----NTGNKS-----
Histoplasma-capsulatum -MSEQEGNQSE-----HQSEHVGESEGLPNETPTTSQSTNA
Ajellomyces-capsulata -MSEQEGNQSE-----HQSEHVGESEGLPNETPTTSQSTNA
Aspergillus-fumigatus -MSD-----SK--DRKGKAP-----
Aspergillus-nidulans -MSD-----SK--DSKGKAP-----
Neurospora-crassa -----
Schizosaccharomyces-pombe -MDNE-----NNKNTK-----
Cryptococcus-neoformans -MDSS-----DNK-----
Filobasidium-floriforme -MDSS-----DNK-----
Solanum-tuberosum1 -MADD-----SKAAENH-----
Solanum-tuberosum2 -MADN-----SKSTENH-----
Lycopersicon-esculentum --ADD-----SKAAENH-----
Arabidopsis-thaliana1-AtNMT1 --ADN-----NSPPGSV-----
Brassica-oleracea -MGDE-----NSLAGSL-----
Glycina-max -MVDS-----NPSSGSP-----
Medicago-truncatula -MVDS-----NPSSGSP-----
Vitis-vinifera -----
Gossypium-hirsutum1 -MTDG-----NPPPGSP-----
Gossypium-hirsutum2 -MTDG-----NPPPGSP-----
Prunus -----
Triticum-aestivum1 -MAAP-----NNDPAA-----
Hordeum-vulgarum --APN-----NNDAAAS-----
Oryza-sativa MAAPN-----NNDAAAG-----
Zea-mays MAAPN-----SNDAAAS-----
Allium-cepa --MDL-----NPTSSTS-----
Arabidopsis-thaliana2-AtNMT2 --SDP-----KLKPVED-----
Capsicum-annuum -----
Pinus-taeda -----
Chlamydomonas-reinhardtii -----
Leishmania-major -----
Trypanosoma-brucei -----
Entamoeba-histolytica -----
Caenorhabditis-elegans -MSHG-----HSHDGAPCG-----
Toxoplasma-gondii -----
Theileria-parva -MSEIPHNT-----SLNHDKDHISSSKAREPEED-----
Encephalitozoon-cuniculi -----
Guillardia-theta -----
AmE-Poxviridae -----

Plasmodium-yoelii -----
Plasmodium-berghei -----
Plasmodium-falciparum -----
Xenopus-laevis2 -----
Xenopus-laevis1 DESGGKKKKKQKRKKE---KSGGDALEAAEAP-----
Danio-erio1 GDTGAKKKKKKQKKN---KDSGAKDAAQDPL-AK-----
Fugu-rubripes1 -----
Homo-sapiens1-HsNMT1 NDTGAKKKKKKQK---KEKGETSDSAQDQPVK-----
Bos-taurus1 NDTGAKKKKKKQK---KEKGETSDSAQDQPVK-----
Mus-musculus1 NDTGAKKKKKKQK---KEKGSMESTQDQPVK-----
Homo-sapiens2-HsNMT2 GYLGAKKKKKKQKRKKEKPNSSGGTKSDSASDSQEIKIQQPS
Mus-musculus2 GDLGAKKKKKKQKRKKEKPNSSGGTKSDSASDSQEIKIQQSSKHNAIWQI
Bos-taurus2 GDLGAKKKKKKQKRKKEKPNSSGGTKSDSASDSQEIKIQQPS
Danio-erio2 GDLGAKKKKKKQKRKKEKPNSSGGTKSDSASDSQEIK-----
Fugu-rubripes2 -----
Ciona-intestinalis -----ENKMKKKKKK-----TKEVSPEEK-D-----

Drosophila-melanogaster --LNIQDTASTNAAG---NEDAEQPDGAKN-----
Dictyostelium-discoideum -----
Saccharomyces-cerevisiae-ScNMT -----A-----
Candida-glabrata -----
Candida-albicans -----NSAP--S-----
Histoplasma-capsulatum STGTAGKGEKKSSDGPAAANPATKLTSPMAESLLELNPALR-----
Ajellomyces-capsulata STGTAGKGEKKSSDGPAAANPATKLTSPMAESLLELNPALR-----
Aspergillus-fumigatus -----EGQSSEKKGAVN---ITPQMAESLLELNPALR-----
Aspergillus-nidulans -----QPNDAEQTPGGK---LTPQAAEALLENPPLK-----
Neurospora-crassa -----
Schizosaccharomyces-pombe -----NSQDSS-----
Cryptococcus-neoformans -----AATD-----
Filobasidium-floriforme -----AATD-----
Solanum-tuberosum1 -----NLTSDSN-----
Solanum-tuberosum2 -----NQSSDDN-----
Lycopersicon-esculentum -----NLTSDSN-----
Arabidopsis-thaliana1-AtNMT1 -----EQKADQI-----
Brassica-oleracea -----EEKADQV-----
Glycina-max -----EETQPNP-----
Medicago-truncatula -----EETQPNP-----
Vitis-vinifera -----
Gossypium-hirsutum1 -----KENPDPN-----
Gossypium-hirsutum2 -----KENPDPN-----
Prunus -----
Triticum-aestivum1 -----ASASAST-----
Hordeum-vulgarum -----ASASAST-----
Oryza-sativa -----ASASATT-----
Zea-mays -----ASGTGPA-----
Allium-cepa -----QSESE-----
Arabidopsis-thaliana2-AtNMT2 -----ALVTYAK-----
Capsicum-annuum -----
Pinus-taeda -----
Chlamydomonas-reinhardtii -----AQAKD-----
Leishmania-major -----
Trypanosoma-brucei -----
Entamoeba-histolytica -----
Caenorhabditis-elegans -----GHHGDDGAGGSRPS-----
Toxoplasma-gondii -----
Theileria-parva -----LPEKLTNLKLSVNTN-----
Encephalitozoon-cuniculi -----
Guillardia-theta -----
AmE-Poxviridae -----

Plasmodium-yoelii -----MDGDNQKEVSRDIYQII-----
Plasmodium-berghei -----QKEISRDIYQII-----
Plasmodium-falciparum -----MNDKDFVGRDLYQLI-----
Xenopus-laevis2 -----
Xenopus-laevis1 ANTLPAERIQEIQKAIELFSVQG-GPA-----
Danio-erio1 VNSLPADKLQEIQKAIELFSVQG-GPA-----
Fugu-rubripes1 -----
Homo-sapiens1-HsNMT1 -----MNSLPAERIQEIQKAIELFSVQG-GPA-----
Bos-taurus1 -----MNSLPAERIQEIQKAIELFSVQG-GPA-----
Mus-musculus1 -----MTSLPAERIQEIQKAIELFSVQG-GPA-----
Homo-sapiens2-HsNMT2 -----KNPSVPMQKLQDIQRAMELLSACQ-GPA-----
Mus-musculus2 SAGAAMGGDTMEGEWIDL RMYHKNPTIP IQKLQDIQRAMELLSACQ-GPA-----
Bos-taurus2 -----KNSTIPVQKLQDIQRAMELLSACQ-GPA-----
Danio-erio2 -----NPAIPMQLQDIQRAMELLSTCQ-GPA-----
Fugu-rubripes2 -----EKKQEQIQRALHLFSLQG-GLP-----
Ciona-intestinalis -----SFMKVVQSALAAFEHET-S-----
Drosophila-melanogaster -----EASVSANASKQALLQAVS-DAM-----
Dictyostelium-discoideum -----MGLVELFKAS-----
Saccharomyces-cerevisiae-ScNMT -----KLENLLKLLQLN--NDD-----
Candida-glabrata -----KKIEELFEASEHE--QWG-----
Candida-albicans -----KIEELKLLAMG-----
Histoplasma-capsulatum -----SELAGMKEKATEALRQMNISD--LLT-----
Ajellomyces-capsulata -----SELAGMKEKATEALRQMNISD--LLT-----
Aspergillus-fumigatus -----NETAGMKDKKAAEAMRKMNIAB--LLT-----
Aspergillus-nidulans -----NELGGLDKDKALEALRKMNIAB--LLT-----
Neurospora-crassa -----MEAFKLLIQD--IIT-----
Schizosaccharomyces-pombe -----FSE-----GGIRELLDRLALR--SLI-----
Cryptococcus-neoformans -----EIRRALKAADLMK--ILD-----
Filobasidium-floriforme -----EIRRALKAADLMK--ILD-----
Solanum-tuberosum1 -----LSSE--SGNEVSID--SLT-----
Solanum-tuberosum2 -----LAPE--NGNEVAID--SLA-----
Lycopersicon-esculentum -----LSSE--SGNEVSID--SLA-----

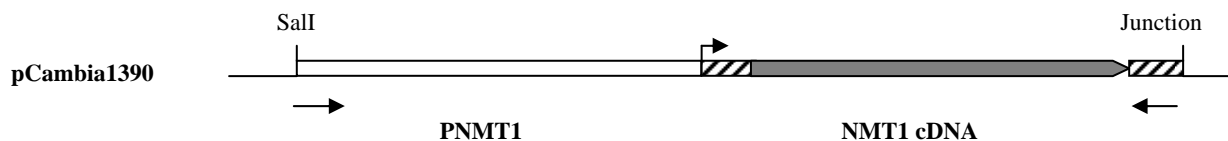
Arabidopsis-thaliana1-AtNMT1	-----VEANPLVKDDTSLE--TIV	Chlamydomonas-reinhardtii	-----RYAFWETQPVVAQFTEGSEPV--SRDGPIDKPKT
Brassica-oleracea	-----AEATPLVGDASLE--TIV	Leishmania-major	-----AAHAFWSTQPVVPT--EDTEKIVFAGPMDEPKT
Glycine-max	-----PDGNAPVSDLALE--NLA	Trypanosoma-brucei	---DKA-F-----TEHQFWSQTPVRQPGADKVGFI--MES--S
Medicago-truncatula	-----PDGNAPAESDLALD--NLA	Entamoeba-histolytica	-----
Vitis-vinifera	-----	Caenorhabditis-elegans	-----
Gossypium-hirsutum1	-----PEANPFSNDDSSLE--SIV	Toxoplasma-gondii	-----
Gossypium-hirsutum2	-----PEANPFSNDDSSLE--SIV	Theileria-parva	-----SYIPEHKFWDTQVLTKLTDVNSN--EG-GIDPNED
Prunus	-----	Encephalitozoon-cuniculi	---MG-----KIKHKFWSTQPVDRNGEAMP-----S---
Triticum-aestivum1	-----SDAALAAPEDTSIE--ALA	Guillardia-theta	-----
Hordeum-vulgarum	-----SDGAPAAPEDTSIE--ALA	AmE-Poxviridae	-----MSYWINKSICKLNYSIDN--IINTIEP---
Oryza-sativa	-----SEP--APEDTSIE--ALA		
Zea-mays	-----GE-----EDISIE--ALA		
Allium-cepa	-----SID--ALA		
Arabidopsis-thaliana2-AtNMT2	-----SSQIQLAKDDTSGG--TIV	Plasmodium-yoelii	VENVRKDEYKL-PEGYVWYVCDVNDENRKE--VYNLLTDNYVE-DDDNI
Capsicum-annuum	-----	Plasmodium-berghei	VENVRK--YKL-PEGYAWYVCDVNDENRKE--VYNLLTDNYVE-DDDNI
Pinus-taeda	-----MGPSK--VLA	Plasmodium-falciparum	VEDVRKDEYKL-PSGYAWCVCDITKENRSD--IYNLLTDNYVE-DDDNI
Chlamydomonas-reinhardtii	-----	Xenopus-laevis2	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Leishmania-major	-----MS	Xenopus-laevis1	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Trypanosoma-brucei	-----MT	Danio-erio1	---DNIREEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Entamoeba-histolytica	-----	Fugu-rubripes1	---DSIREEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Caenorhabditis-elegans	-----VNDVQALVDQLRLAGVDVS	Homo-sapiens1-HsNMT1	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Toxoplasma-gondii	-----MRVS----	Bos-taurus1	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Theileria-parva	-----SDPTPNSTTSSSIRARIF	Mus-musculus1	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Encephalitozoon-cuniculi	-----	Homo-sapiens2-HsNMT2	---DNVRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
Guillardia-theta	-----	Mus-musculus2	---DNIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
AmE-Poxviridae	-----	Bos-taurus2	---ENVRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
		Danio-erio2	---ENIRQEPYSL--PQGFMDTLDLSNAEVLKE--LYTLLNENYVE-DDDNI
		Fugu-rubripes2	---GSVRTDPYSL--PEGFRMDNLELSSQTVLRE--LCTLLSENYLE-EDDNT
		Ciona-intestinalis	---DNVREEPYSL--PPDFEWDTVNLENTKELEE--VYKLLNENYVE-DDDNI
		Drosophila-melanogaster	ISEIRALPYTL-PGGFKVTLTDLNANDLKE--LYTLLNENYVE-DDDNI
		Dictyostelium-discoideum	LDDVRKDPPLT-PPAFEWITDLCNPKPEELKE--IYLLTYHNYVE-DDDNI
		Saccharomyces-cerevisiae-ScNMT	PEDISDKPLPL-LSSFEMCSDIVDNKQLED--VYLLNENYVE-DRDAG
		Candida-glabrata	PADIPDEPLPL-LPDFEWCAIDVDEKQLED--VYLLNENYVE-DRDAS
		Candida-albicans	PEDVPNDPLPL-ISDFEWSTLDIDNQLQDE--LYKLLYDNYVE-DIDAT
		Histoplasma-capsulatum	LQVRSREPIPL-VDFGEWVTLTLDIDDEADVKE--FYELLANHYVE-DGSGM
		Ajellomyces-capsulata	LQVRSREPIPL-VDFGEWVTLTLDIDDEADVKE--FYELLANHYVE-DGSGM
		Aspergillus-fumigatus	PEKVSKEPDAL-LEGFEWATLDTNETELQE--LWDLTYHYVE-DDNAM
		Aspergillus-nidulans	PEKVSKEPDAL-LEGFEWATLDTNETELQE--LWDLTYHYVE-DDNAM
		Neurospora-crassa	VEDIPDEPIPLNLAFRFRVMTDLTDEKMQE--VEKLLYGHFVE-DDNAM
		Schizosaccharomyces-pombe	INQVPREPYRL-LKEFEWATIDVTNDNELSE--VHELLTENYVE-DATAM
		Cryptococcus-neoformans	PADVQKQEPYSL-PAGFEWSTIDINDEEQSKE--VYVLLCENYVE-DDDAM
		Filobasidium-floriforme	PADVQKQEPYSL-PAGFEWSTIDINDEEQSKE--VYVLLCENYVE-DDDAM
		Solanum-tuberosum1	LTEVKQEPYSL-PSPYEWTTCDDLSEDMCSE--VYLLTNNYVE-DDENM
		Solanum-tuberosum2	LSEVKQEPYSL-PSQYEWTTCCDMSEEMCNE--VYVLLTNNYVE-DDENM
		Lycopersicon-esculentum	LTEVKQEPYSL-PSPYEWTTCDDLSEDMCNE--VYVLLTNNYVE-DDENM
		Arabidopsis-thaliana1-AtNMT1	LSEVKQEPYSL-PSVYEWTTCCDMSEDMCSE--VYVLLKNNYVE-DDENM
		Brassica-oleracea	LSEVKQEPYSL-PAAYEWTTCCMKSDDYVCE--VYVLLKNNYVE-DDENM
		Glycine-max	LSEVKQEPYSL-PDHYEWVTCIDINSEETCDE--VYVLLAHNYVE-DDENM
		Medicago-truncatula	LSEVKQEPYSL-PNLYEWVTCIDINSEETCDE--IYVLLAHNYVE-DDENM
		Vitis-vinifera	-----
		Gossypium-hirsutum1	LSEVKQEPYSL-PSPYEWTTCDDMSEETCNE--VYVLLKNNYVE-DDENM
		Gossypium-hirsutum2	-----
		Prunus	-----CTE--VYVLLKNNYVE-DDENM
		Triticum-aestivum1	LSEVRADPYPL-PAAFEWFTCDLDD-----LYALLAHNYVE-DDENM
		Hordeum-vulgarum	LSEVRADPYPL-PAAFEWFTCDLDD-----LYALLAHNYVE-DDENM
		Oryza-sativa	LSEVRADPYPL-PAAFEWFTCDLDDDDALLTD--LYALLAHNYVE-DDENM
		Zea-mays	LSEVRAEPYPL-PAAFEWLTCDDLDDALLAD--LYSLLAHNYVE-DDENM
		Allium-cepa	LSEVKADPYNL-PLAYEWTTCDMLDDTTCLLE--VYVLLTNNYVE-DDDNI
		Arabidopsis-thaliana2-AtNMT2	VSEVKQEPYSL-LGQFEWTTCDMLSEDMCCLLE--MYNFKLEN--S-DDDQDQ
		Capsicum-annuum	-----
		Pinus-taeda	-----
		Chlamydomonas-reinhardtii	LSEVRQEPYSL-PSHYEWTCIDINKDETSIE--IYNLLTNN-----
		Leishmania-major	VQDVRQEPYSL-P--FEWCVDLTDVAHEVQVYELLSNNYVE-DDDAM
		Trypanosoma-brucei	VADIPDEPIPI-ASTFEWTTPNMEAADIIHA--IYELLRNHYVE-DDDNI
		Entamoeba-histolytica	LDAVPAEPYSL-PSTFEWSPDVANPEDLRG--VHELLRNHYVE-DSESM
		Caenorhabditis-elegans	-----EYCIWNLDNEKEMEE--VYILLKENVYKEDDDAT
		Toxoplasma-gondii	LDKVRAEPYSL-PAGFRNSVNDLSDEEQLNE--LYNLLTNNYVE-DDDSM
		Theileria-parva	---FXNEPYKL-PDGFVWCEDVDRPEELKE--VYDLSQHYVE-DDDNL
		Encephalitozoon-cuniculi	VSRVKKNP IPL-PNGFEWISLDINDEEDRQ--VYKLLSENHYVE-DGDAL
		Guillardia-theta	KHTISIEQPKL-PDGFREFDLGCVBELAN-----FLEKNYVE-DIYSG
		AmE-Poxviridae	---MKSNIKISTKIFPPELEIFCHKIKERYPIHN--IQKFLNQNYIE-DIISS
			-----HKPYNN--NYCKDNFNKVLDDENLIDLYSYLYKNY--N-----
Plasmodium-yoelii	KNARDKIK-----IDYKFWYTPQVPKINEEFSES--INEPFIADNK	Plasmodium-berghei	KNARDKIK-----IDYKFWYTPQVPKINEEFSES--INEPFIADNK
Plasmodium-falciparum	RNAKDKIK-----IDYKFWYTPQVPKINEEFSES--VNEPFIADNK	Plasmodium-falciparum	RNAKDKIK-----IDYKFWYTPQVPKINEEFSES--VNEPFIADNK
Xenopus-laevis2	-----R-----KYQFWDTPQVPKINEEITC---HGP IEPDK	Xenopus-laevis1	-----R-----KYQFWDTPQVPKINEEITC---HGP IEPDK
Xenopus-laevis1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK	Danio-erio1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK
Fugu-rubripes1	---MEEATRR-----SYQFWDTPQVPKINEEITC---HGP IEPDK	Fugu-rubripes1	---MEEATRR-----SYQFWDTPQVPKINEEITC---HGP IEPDK
Homo-sapiens1-HsNMT1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK	Homo-sapiens1-HsNMT1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK
Bos-taurus1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK	Bos-taurus1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK
Mus-musculus1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK	Mus-musculus1	KTMEEASKR-----SYQFWDTPQVPKINEEITC---HGP IEPDK
Homo-sapiens2-HsNMT2	RNIDEAAKH-----RYQFWDTPQVPKINEEITC---HGAIEPDK	Homo-sapiens2-HsNMT2	RNIDEAAKH-----RYQFWDTPQVPKINEEITC---HGAIEPDK
Mus-musculus2	RNIDEATKR-----RYQFWDTPQVPKINEEITC---HGAIEPDK	Mus-musculus2	RNIDEATKR-----RYQFWDTPQVPKINEEITC---HGAIEPDK
Bos-taurus2	RNIDEAAKH-----RYQFWDTPQVPKINEEITC---HGAIEADK	Bos-taurus2	RNIDEAAKH-----RYQFWDTPQVPKINEEITC---HGAIEADK
Danio-erio2	KNIDEATKH-----KYQFWDTPQVPKINEEITC---HGP IEPDK	Danio-erio2	KNIDEATKH-----KYQFWDTPQVPKINEEITC---HGP IEPDK
Fugu-rubripes2	RSLQAARNH-----KYHFWETQVVP--NDGVTM--HGP IIEADK	Fugu-rubripes2	RSLQAARNH-----KYHFWETQVVP--NDGVTM--HGP IIEADK
Ciona-intestinalis	---SLKEPASK-----PWKFWDTQVPKINEEITC---VGVIEADK	Ciona-intestinalis	---SLKEPASK-----PWKFWDTQVPKINEEITC---VGVIEADK
Drosophila-melanogaster	ASTROMAK-----KFAFWSTQPVTKLDEQVTT--NECIEPNKE	Drosophila-melanogaster	ASTROMAK-----KFAFWSTQPVTKLDEQVTT--NECIEPNKE
Dictyostelium-discoideum	---SLASKKPK-----GHEFWDTPQVPKINEEITC---SGPIENKT	Dictyostelium-discoideum	---SLASKKPK-----GHEFWDTPQVPKINEEITC---SGPIENKT
Saccharomyces-cerevisiae-ScNMT	TSKFTQEQK-----KAMKDKHKFWRTQPVKDFDEKVV--EEGIDKPKT	Saccharomyces-cerevisiae-ScNMT	TSKFTQEQK-----KAMKDKHKFWRTQPVKDFDEKVV--EEGIDKPKT
Candida-glabrata	HAKLTANQR-----KEMKYEKFWKTPVTKFDEEVK--EEGPIHEEKT	Candida-glabrata	HAKLTANQR-----KEMKYEKFWKTPVTKFDEEVK--EEGPIHEEKT
Candida-albicans	---QELSPAQQ-----KEMKDKHKFWRTQPVVSLSETVT--EEGIDKPKT	Candida-albicans	---QELSPAQQ-----KEMKDKHKFWRTQPVVSLSETVT--EEGIDKPKT
Histoplasma-capsulatum	GLSVNPKNQ-----KDMASFKFWQTPVIRFDDRESE--PDGPIKIVE	Histoplasma-capsulatum	GLSVNPKNQ-----KDMASFKFWQTPVIRFDDRESE--PDGPIKIVE
Ajellomyces-capsulata	GLSVNPKNQ-----KDMASFKFWQTPVIRFDDRESE--PDGPIKIVE	Ajellomyces-capsulata	GLSVNPKNQ-----KDMASFKFWQTPVIRFDDRESE--PDGPIKIVE
Aspergillus-fumigatus	GLSVSGKNQ-----KDMASFKFWQTPVIRFDETST-D--TGGPIKIID	Aspergillus-fumigatus	GLSVSGKNQ-----KDMASFKFWQTPVIRFDETST-D--TGGPIKIID
Aspergillus-nidulans	GLSLTGKNK-----KDMAAFKFWQTPVIRFDEAASNA--AGGPKIMID	Aspergillus-nidulans	GLSLTGKNK-----KDMAAFKFWQTPVIRFDEAASNA--AGGPKIMID
Neurospora-crassa	GLASSGKNR-----KDMASYKFWATQVPVQFDEKPAIF--EEGPLKIQK	Neurospora-crassa	GLASSGKNR-----KDMASYKFWATQVPVQFDEKPAIF--EEGPLKIQK
Schizosaccharomyces-pombe	EKEEAAAPP-----KTYEDFKFWKTPQVPKDFDECT--QEGPIDPNTD	Schizosaccharomyces-pombe	EKEEAAAPP-----KTYEDFKFWKTPQVPKDFDECT--QEGPIDPNTD
Cryptococcus-neoformans	GKMALGNKSGT-----KNLGEHKFWKTPVQITGSGASAPMEEGPIDDPKT	Cryptococcus-neoformans	GKMALGNKSGT-----KNLGEHKFWKTPVQITGSGASAPMEEGPIDDPKT
Filobasidium-floriforme	GKMALGNKSGT-----KNLGEHKFWKTPVQITGSGASAPMEEGPIDDPKT	Filobasidium-floriforme	GKMALGNKSGT-----KNLGEHKFWKTPVQITGSGASAPMEEGPIDDPKT
Solanum-tuberosum1	RKLQESLSL-----PKRHKFWETQPVGQFKDLGDAS--LPEGPIEPPT	Solanum-tuberosum1	RKLQESLSL-----PKRHKFWETQPVGQFKDLGDAS--LPEGPIEPPT
Solanum-tuberosum2	RKQKESLPL-----AKKHKFWETQPVGQFKDLGDSS--LPEGPIEPPT	Solanum-tuberosum2	RKQKESLPL-----AKKHKFWETQPVGQFKDLGDSS--LPEGPIEPPT
Lycopersicon-esculentum	RKVQESLSL-----SKRHKFWETQPVGQFKDLGDSS--LPEGPIEPPT	Lycopersicon-esculentum	RKVQESLSL-----SKRHKFWETQPVGQFKDLGDSS--LPEGPIEPPT
Arabidopsis-thaliana1-AtNMT1	RRFQDSMSE-----AKTHKFWETQPVGQFKDIDGTS--LPEGPIEPATP	Arabidopsis-thaliana1-AtNMT1	RRFQDSMSE-----AKTHKFWETQPVGQFKDIDGTS--LPEGPIEPATP
Brassica-oleracea	RRFQDSMSV-----EKTHKFWETQPVGQFKDIDGTS--LPEGPIEAAPT	Brassica-oleracea	RRFQDSMSV-----EKTHKFWETQPVGQFKDIDGTS--LPEGPIEAAPT
Glycine-max	QKVQESLSL-----BKRHKFWETQPVGQFKDIDGSS--LPEGPIELPIP	Glycine-max	QKVQESLSL-----BKRHKFWETQPVGQFKDIDGSS--LPEGPIELPIP
Medicago-truncatula	QKVQESLSL-----DQRHKFWETQPVGQFKDIGNPT--LPEGPIEPPTP	Medicago-truncatula	QKVQESLSL-----DQRHKFWETQPVGQFKDIGNPT--LPEGPIEPPTP
Vitis-vinifera	-----	Vitis-vinifera	-----
Gossypium-hirsutum1	RRFQDSMSS-----VKRHKFWETQPVGQFKDVGDT--LPGSIELPTP	Gossypium-hirsutum1	RRFQDSMSS-----VKRHKFWETQPVGQFKDVGDT--LPGSIELPTP
Gossypium-hirsutum2	RRFQDSMSS-----VKRHKFWETQPVGQFKDVGDT--LPGSIELPTP	Gossypium-hirsutum2	RRFQDSMSS-----VKRHKFWETQPVGQFKDVGDT--LPGSIELPTP
Prunus	-----	Prunus	-----
Triticum-aestivum1	RRVQEHMTLANNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP	Triticum-aestivum1	RRVQEHMTLANNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP
Hordeum-vulgarum	RRVQEHMTLASNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP	Hordeum-vulgarum	RRVQEHMTLASNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP
Oryza-sativa	RRVQEHMTLASNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP	Oryza-sativa	RRVQEHMTLASNP--TARRHKFWETQPVGQFKDVGADV--LPDGAIEPPSP
Zea-mays	RRVQEHMTLSSNP--AARRHKFWETQPVGQFKDVGADV--LPDGAIEPPPT	Zea-mays	RRVQEHMTLSSNP--AARRHKFWETQPVGQFKDVGADV--LPDGAIEPPPT
Allium-cepa	QKIQETLAVSSSQIQPVQHRFWETQPVQFKDLHNP--LPEGPIEPPTP	Allium-cepa	QKIQETLAVSSSQIQPVQHRFWETQPVQFKDLHNP--LPEGPIEPPTP
Arabidopsis-thaliana2-AtNMT2	GRLLCQCY--KTHKFWETQPVQFKDIDGTS--LPEGPIEPATL	Arabidopsis-thaliana2-AtNMT2	GRLLCQCY--KTHKFWETQPVQFKDIDGTS--LPEGPIEPATL
Capsicum-annuum	-----	Capsicum-annuum	-----
Pinus-taeda	GRHRMLLSLG-----KHKFWETQPGQFKDVGQNH--LPEGPIEDPTP	Pinus-taeda	GRHRMLLSLG-----KHKFWETQPGQFKDVGQNH--LPEGPIEDPTP

Arabidopsis-thalianal-AtNMT1	-----TSFQLQMNDAALIVSKQ-----	-----KGFDFVFNALDVMHN	Chlamydomonas-reinhardtii	-----LKFGMGDGO-LHYLYLNWRLGGG-HSLQPGD-----	VGL
Brassica-oleracea	-----TTFSSLMNDALIVAKQ-----	-----KGFDFVFNALDVMHN	Leishmania-major	RSFVBEQLKFGPGDGH-LRYFYFNWYAP----	KLKPSQ-----VAL
Glycina-max	-----TPLLQMLMNDVLIIVAKQ-----	-----KDFDFVFNALDVMHN	Trypanosoma-brucei	GTYLKELKFGSPGDGN-LYYFYFNWYSP----	SIPANE-----VGL
Medicago-truncatula	-----TPLLQMLMNDALIVAKQ-----	-----KDYDFVFNALDVMQN	Entamoeba-histolytica	QQYLADLLFVPGDGY-LKYLYLNWACP----	KVEPNK-----LAI
Vitis-vinifera	-----TPLLQMLMNDALIVAKQ-----	-----KDYDFVFNALDVMQN	Caenorhabditis-elegans	EKIFSDLKFGKGDGN-LQYYLYNWKCA----	DMKPSQ-----IGL
Gossypium-hirsutum1	-----TPLLQMLMNDALIVAKQ-----	-----KEFDVFNALDVMHN	Toxoplasma-gondii	KSFVEDLKFGIGDGF-LRYLYNWRCP----	EVGFFC-----LYI
Gossypium-hirsutum2	-----TPLLQMLMNDALIVAKQ-----	-----KDFDFVFNALDVMHN	Theileria-parva	SLVFKDLKFGMGDGH-LHYMFNRYVP----	DLKPSD-----VGI
Prunus	-----TPLLQMLMNDALIVAKT-----	-----EDFDFVFNALDVMQN	Encephalitozoon-cuniculi	SSFLARLGFVCGSGE-IRYLYNWKSE----	EIPRDK-----VFF
Triticum-aestivum1	-----TPLLQMLMNDALIVAKQ-----	-----KNFDFVFNALDVMEN	Guillardia-theta	KILRKIFNFKKSFGK-IRFCSHNLTIK----	TSKNR-----VIGL
Hordeum-vulgarum	-----TPLLQMLMNDALIVAKQ-----	-----KNYDFVFNALDVMEN	AmE-Poxviridae	-----K-----LISYIYNYNFN-----	NLSES-----I
Oryza-sativa	-----TPLLQMLMNDALIVAKQ-----	-----KNYDFVFNALDVMEN			
Zea-mays	-----TPLLQMLMNDALIVAKR-----	-----NNYDFVFNALDVMEN			
Allium-cepa	-----TPLLQMLMNDALIVAK-----				
Arabidopsis-thaliana2-AtNMT2	-----TSLSQLVNDALIVSKQ-----	-----KGFDFVYASDVMQN	Plasmodium-yoelii		VLL---
Capsicum-annuum	-----TSLSQLVNDALIVSKQ-----	-----FNALDVMHN	Plasmodium-berghei		VLL---
Pinus-taeda	-----TPLLQMLMNDVLIIVAKK-----	-----KDYDFVFNALDIMQN	Plasmodium-falciparum		VLL---
Chlamydomonas-reinhardtii	-----TPLLQMLVNDAMAVAAA-----	-----RGY-----D-----	Xenopus-laevis2		VL---
Leishmania-major	-----IPLHQLILDLLIVAH-----	-----RGFDVCNMVEILDN	Xenopus-laevis1		VLQ---
Trypanosoma-brucei	-----VSIITQLVNDLLIIVKL-----	-----NGFDVCNVVDIYDN	Danio-erio1		VLQ---
Entamoeba-histolytica	-----VDFKQFPKDIILICAVQ-----	-----NHCDVFNCLNISEN	Fugu-rubripes1		VLQ---
Caenorhabditis-elegans	-----VTPKQLINDSLILANR-----	-----EKFDVFNALDLMHN	Homo-sapiens1-HsNMT1		VLQ---
Toxoplasma-gondii	-----VPLKQLIEDALCLAKO-----	-----LDFDFVFNALDVMEN	Bos-taurus1		VLQ---
Theileria-parva	-----MSFKSLMEHAIYFYSKS-----	-----QGYDVYNALDLMEN	Mus-musculus1		VLQ---
Encephalitozoon-cuniculi	-----VAEMVGLDMHFS-QV-----	-----EGCDVFNCLDMMEN	Homo-sapiens2-HsNMT2		VLQ---
Guillardia-theta	-----FQLKKNFBEAKCFPKL-----	-----LKIDIFFILHGKTN	Mus-musculus2		VLQ---
AmE-Poxviridae	-----IHKNYIPNNTIYEN-----	-----YKCLKTIKLS-	Bos-taurus2		VLQ---
			Danio-erio2		LLK---
			Fugu-rubripes2		YLF CAD
			Ciona-intestinalis		VL----
			Drosophila-melanogaster		ILM---
			Dictyostelium-discoidium		VLL---
			Saccharomyces-cerevisiae-ScNMT		VML---
			Candida-glabrata		VML---
			Candida-albicans		VLL---
			Histoplasma-capsulatum		ILV---
			Ajellomyces-capsulata		ILV---
			Aspergillus-fumigatus		VML---
			Aspergillus-nidulans		VML---
			Neurospora-crassa		VMV---
			Schizosaccharomyces-pombe		VMI---
			Cryptococcus-neoformans		VML---
			Filobasidium-floriforme		VML---
			Solanum-tuberosum1		VLL---
			Solanum-tuberosum2		VLL---
			Lycopersicon-esculentum		VLL---
			Arabidopsis-thalianal-AtNMT1		VLL---
			Brassica-oleracea		VLL---
			Glycina-max		VLL---
			Medicago-truncatula		VLL---
			Vitis-vinifera		VLL---
			Gossypium-hirsutum1		ELV---
			Gossypium-hirsutum2		VLL---
			Prunus		-----
			Triticum-aestivum1		VLL---
			Hordeum-vulgarum		VLL---
			Oryza-sativa		VLL---
			Zea-mays		VLL---
			Allium-cepa		-----
			Arabidopsis-thaliana2-AtNMT2		IL---
			Capsicum-annuum		VLL---
			Pinus-taeda		VLL---
			Chlamydomonas-reinhardtii		VLM---
			Leishmania-major		VML---
			Trypanosoma-brucei		VMV---
			Entamoeba-histolytica		IL----
			Caenorhabditis-elegans		VLQ---
			Toxoplasma-gondii		SI----
			Theileria-parva		VLL---
			Encephalitozoon-cuniculi		VLP---
			Guillardia-theta		MIF---
			AmE-Poxviridae		YLF---
Plasmodium-yoelii	KSVFADLKFGEGDGT-LKYLYNWKCA---	SFDTSM-----VGI			
Plasmodium-berghei	KSVFADLKFGEGDGT-LKYLYNWKCA---	SFDTSM-----VGI			
Plasmodium-falciparum	YSVFQDLKFGEGDGS-LKYLYNWKCA---	SCHPSK-----IGI			
Xenopus-laevis2	KTFVEEGKVGIGDEN-LQYYLYNWKCP---	SMGSEK-----VGL			
Xenopus-laevis1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Danio-erio1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGSEK-----VGL			
Fugu-rubripes1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGADK-----VGL			
Homo-sapiens1-HsNMT1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Bos-taurus1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Mus-musculus1	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Homo-sapiens2-HsNMT2	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Mus-musculus2	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Bos-taurus2	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Danio-erio2	KTFLEKLFKFGIGDGN-LQYYLYNWKCP---	SMGAEK-----VGL			
Fugu-rubripes2	KAFLEPLKFRMGDVH-MQYYLYNWKCP---	DITSNK-----VGF			
Ciona-intestinalis	SSFLKQLKFNVDVDS-LHYLYNWKCP---	KISPSE-----VTL			
Drosophila-melanogaster	HVFLKDLKFGMGDGN-LHYLYNWKCP---	DIPSEK-----VGL			
Dictyostelium-discoidium	KKYFAPLKFAGDGN-LQYYLYNWKCP---	SMQPEE-----IAL			
Saccharomyces-cerevisiae-ScNMT	STFFKDLKFAVGDDG-LQYYLYNYSPT---	TKKSSE-----IGL			
Candida-glabrata	TLFLDLDLKFPGDGF-LNFYLPNRYRKPITGGI	LNPDNSNDIKRRSN-VGV			
Candida-albicans	TLFLEDLKFPGDGF-LNFYLPNRYRFPITGGI	EDQHPDTEHRSN-VGV			
Histoplasma-capsulatum	TYFLEKLFKFGDGF-LNYLYNRYRTPMDGGI	DKKTKEVVEDQTSIGV			
Ajellomyces-capsulata	PLFLEQLKFGAGDGO-LHYLYNRYRTAPIAGGV	NKDLNPKDERKRGV-VGV			
Aspergillus-fumigatus	PLFLEQLKFGAGDGO-LHYLYNRYRTAPIAGGV	NKDLNPKDERKRGV-VGV			
Aspergillus-nidulans	PLFLEQLKFGAGDGO-LHFYLYNRYRTAPIAGGV	NKDLNPKDERKRMGG-VGI			
Neurospora-crassa	ALFLEKQKFGPGDGO-LHYLYNRYRKPITGGI	QHSSTAKQGSK----			
Schizosaccharomyces-pombe	NMFLQEQKFGPGDGO-LNYLYNWNCAPIDGGQ	HSTAKQGSK----			
Cryptococcus-neoformans	NMFLQEQKFGPGDGO-LNYLYNWNCAPIDGGQ	HSTAKQGSK----			
Filobasidium-floriforme	ETFLKELKFGPGDGO-LHYLYNRYRIR---	HVLRPSE-----LGL			
Solanum-tuberosum1	ETFLKELKFGPGDGO-LHYLYNRYRIR---	HVLRPSE-----LGL			
Solanum-tuberosum2	ETFLKELKFGPGDGO-LHYLYNRYRIR---	HVLRPSE-----LGL			
Lycopersicon-esculentum	DSFLKELKFGPGDGO-LHYLYNRYRIR---	SALKPSE-----LGL			
Arabidopsis-thalianal-AtNMT1	ESFLKELKFGPGDGO-LHYLYNRYRIR---	SALKPSE-----LGL			
Brassica-oleracea	ESFLKELKFGPGDGO-LHYLYNRYRIR---	SALKPSE-----LGL			
Glycina-max	ESFLKELKFGPGDGO-LHYLYNRYRIR---	SALKPSE-----LGL			
Medicago-truncatula	ESFLKELKFGPGDGO-LHYLYNRYRIR---	HALKPSG-----LGL			
Vitis-vinifera	ESFLKELKFGPGDGO-LHYLYNRYRIR---	NGLKPSG-----LGL			
Gossypium-hirsutum1	DAFVKELKFGPGDGS-LNYDLDNRYRIR---	NLKPSE-----LGL			
Gossypium-hirsutum2	ESFLKELKFGPGDGO-PHYLYNRYRIR---	NALRPSE-----LGL			
Prunus	ESFL-----				
Triticum-aestivum1	EGFLKELKFGPGDGO-LHYLYNRYRIR---	NGIKPSE-----LGL			
Hordeum-vulgarum	EGFLKELKFGPGDGO-LHYLYNRYRIR---	NGIKPSE-----LGL			
Oryza-sativa	ESFLKELKFGPGDGO-LHYLYNRYRIR---	NGIKPSE-----LGL			
Zea-mays	ESFLKELKFGPGDGO-LHYLYNRYRIR---	NGIKPSE-----LGL			
Allium-cepa	-----				
Arabidopsis-thaliana2-AtNMT2	ESFLKELRFYPLCRQ-SHYLYNRYRIR---	NALKPSE-----LGL			
Capsicum-annuum	DSFLKELKFGPGDGO-LHYLYNRYRIR---	HVLRPSE-----LGL			
Pinus-taeda	ESFLKDLKFGPGDGO-LHYLYNRYRIR---	DALKPSQ-----LGL			

Supplemental methods

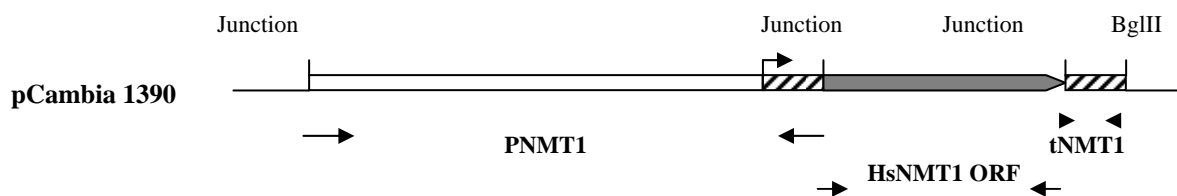
I- Transgene constructs cloning strategies

N1At1 (PNMT1:AtNMT1)



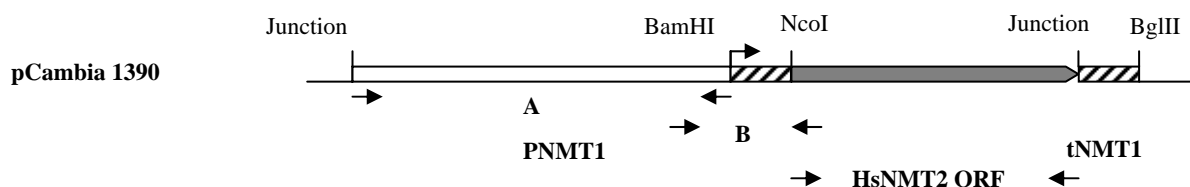
The *NMT1* gene (2090 bp for promoter and 1995 bp for cDNA) was amplified from genomic DNA with N5F9 and N5R4 primers and digested with SalI. The fragment was inserted into the binary vector pCambia1390 between the SalI and SmaI restriction sites.

N1Hs1(PNMT:HsNMT1)



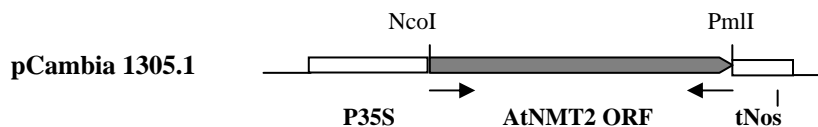
- The 3' end of *NMT1* gene was amplified from genomic DNA with N5F20 and N5R16 (BglII site) primers and cloned into SmaI and BglII sites of the binary vector pCambia1390.
- The promoter and the 5' non coding region of *AtNMT1* (2550bp) were amplified with PN1Xho (XhoI site) and N5R22 (StuI site) primers, digested by XhoI and cloned into SalI/EcoRV digested pBluescript II KS+ vector (Stratagene).
- *N1Hs* ORF was amplified from a cDNA clone with N1HF2 (DraI site) and N1HR2 (XmaI site) primers, digested and cloned into StuI / XmaI sites of the previous construct.
- The resulting construct was cloned after XhoI/SmaI digestion into SalI/SmaI sites of the binary vector containing the 3' non coding region of *AtNMT1*.

N1Hs2 (PNMT1:HsNMT2)



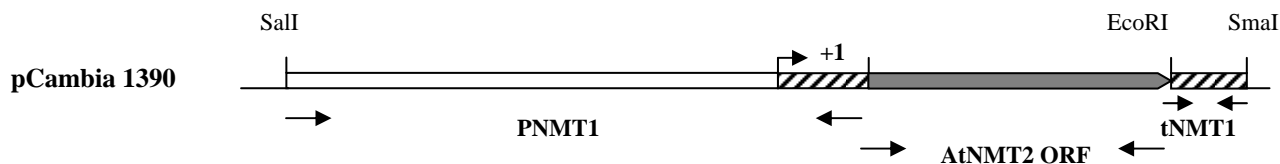
- *N2Hs* ORF was amplified from a cDNA clone with NHF2 (NcoI site) and NHR1 (EcoRI site) primers. The resulted fragment was cloned after EcoRI digestion into EcoRI / SmaI sites of pBluescript II KS+.
- The 5' *NMT1* non coding region (B) was amplified from genomic DNA with N5F14 (SacI site) and N5R15 (NcoI site) primers and cloned after digestion into NcoI / SacI sites of the previous vector containing the *N2Hs* ORF.
- The DNA fragment *N2Hs* ORF/ 5' end of *NMT1* was excised from pSK+ by EcoRI / BamHI digestions and inserted after filling in EcoRI site, into the modified binary vector pCambia 1390 containing the 3' non coding region of *AtNMT1* (N1Hs1 cloning).
- The *NMT1* promoter region (2080 bp) was amplified with PN1Xho (XhoI site) and N5R8 primers and cloned after XhoI/BamHI digestion into SalI/BamHI sites of the previous binary vector.

35SAt2 (P35S:NMT2)



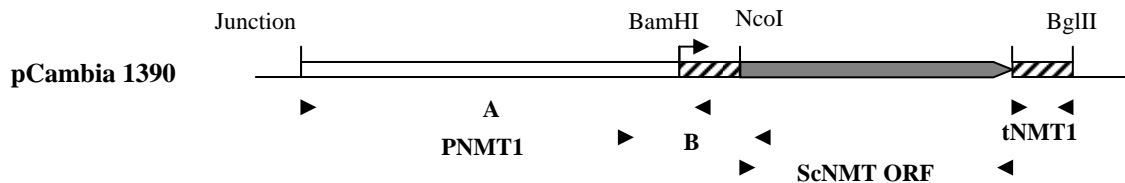
- The *NMT2* ORF was amplified from a cDNA clone with N2-Nco and N2R-Pml primers and digested with NcoI and PmlI. The resulting fragment was inserted into the binary vector pCambia 1305.1 after removal of the *Gus* gene by the same digestions.

N1At2 (PNMT1:AtNMT2)



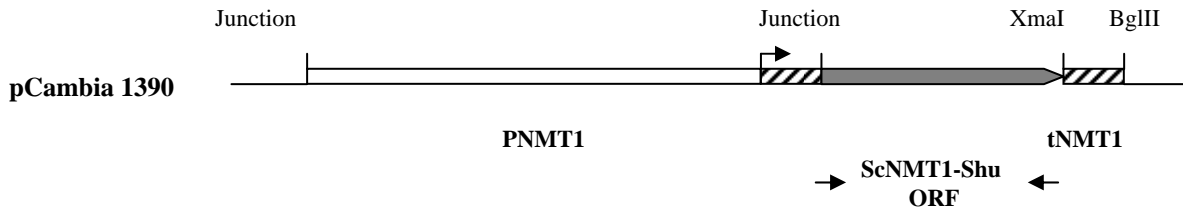
- The 3' end of *NMT1* gene was amplified from genomic DNA with N1-Eco and N1R-Xma primers; the resulting fragment was digested by EcoRI and cloned into EcoRI and SmaI sites of the binary vector pCambia 1390.
- The *NMT2* ORF was amplified from a cDNA clone with N2+1 and N2R-Eco primers and digested by EcoRI. The promoter and the 5' non-coding region of *NMT1* were amplified from genomic DNA using PN1-Xho and PN1R primers and digested by XhoI.
- The two fragments were ligated together first and the resulted construct was subcloned into pBluescript II KS+ (XhoI/EcoRI). The construct was finally cloned in the SalI/EcoRI sites of the previously modified binary vector.

N1Sc (PNMT1:ScNMT)



- The 3' end of *NMT1* gene was amplified from genomic DNA with N5F20 and N5R16 (BglIII site) primers and cloned into SmaI and BglIII sites of the binary vector pCambia 1390.
- Fragment A from *NMT1* promoter was amplified with PN1Xho (XhoI site) and N5R8 primers and cloned after XhoI/BamHI digestion into SalI / BamHI sites of the previous cloning vector.
- N1Sc coding region was amplified from a pQE60 expression vector (Boisson & Meinel 2003) derived from pBB331 with N1ScF1 (NcoI site) and N1ScR1 (SnaBI site) primers. The amplification product was inserted into a previous pBluescript II KS+ construct containing the 5' *NMT1* non coding region (B) and the ORF of *N2HS*. The *N2HS* gene was removed by NcoI/EcoRV digestion and replaced by *N1Sc*.
- The resulting pSK construct was digested by BamHI and SnaBI and cloned into BamHI/Sma sites of the pCambia construct containing the promoter and the 3' end of *NMT1*.

N1Sc-Shu (*PNMT1*:*ScNMT-Shu*)



- N1Sc-Shu (see sequence in &II) was amplified from the pBluescript provided by US-Biological with N1ScF21 (DraI site) and N1ScR1 (XmaI site) primers and digested. The resulted fragment was inserted into a previous pBluescript II KS+ construct containing the N1Hs1. The *HsNMT1* ORF was removed by StuI / XmaI digestion and replaced by *N1Sc-Shu* ORF.
- The resulting construct was cloned after XhoI/SmaI digestion into Sall/SmaI sites of the modified binary vector pCambia 1390 containing the 3' non coding region of *AtNMT1* (N1Hs1 cloning).

II- Shuffled Sc *NMT* sequence (*ScNMT-Shu*)

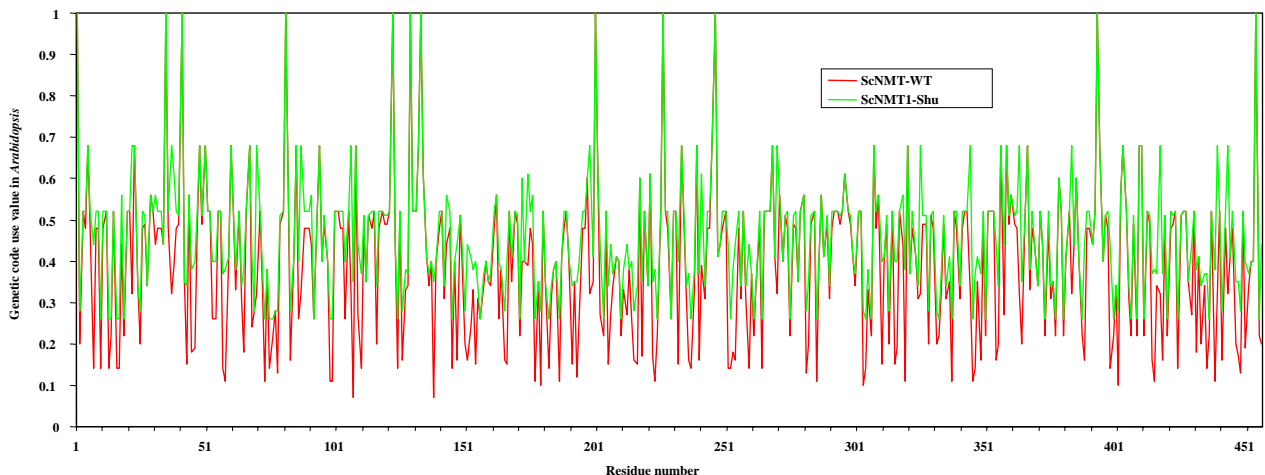
a- Shuffled reverse translation of *ScNMT* ORF (456aa) to a 1365 base sequence of most likely codons obtained with *Arabidopsis thaliana* genetic code

>ScNMT/Res-At

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ATGTCTGAAGAAGATAAGGCTAAGAAGCTTGAAAATCTTCTTAAGCTTCTTCAACTAATAATGATGATACTTCTAAGTTTACTCA
AGAACAAAAGAAGGCTATGAAGGATCATAAGTTTTGGAGAAGCTCAACCTGTTAAGGATTTTGATGAAAAGGTTGTTGAAGAAGG
ACCTATTGATAAGCCTAAGACTCCTGAAGATATTTCTGATAAGCCTCTTCTCTTCTTTTGAATGGTGTCTATTGATGTT
GATAATAAGAAGCAACTGAAGATGTTTTTGTCTTCTTAATGAAAATTATGTTGAAGATAGAGATGCTGGATTAGATTTAATTA
TACTAAGGAATTTTTAATTGGGCTCTTAAGTCTCCTGGATGGAAGAAGGATTGGCATATTGGAGTTAGAGTTAAGGAACTCAA
AAGCTTGTGCTTTTATTCTGCTATTCCTGTTACTCTTGAGGATTAGAGGAAAGCAAGTTCCTTCTGTTGAAAATTAATTTCTTTG
TTCATAAGCAACTTAGATCTAAGAGACTTACTCCTGTTCTTATTAAGGAAATTAAGTAGAAGGTTAATAAGTGTGATATTTGGCAT
GCTCTTTATACTGCTGGAATTGTTCTTCTGCTCTGTTTCTACTTGTAGATATACTCATAGACCTCTTAATTGGAAGAAGCTTTAT
GAAGTTGATTTTACTGGACTTCTGATGGACATACTGAAGAAGATATGATTGCTGAAAATGCTCTTCTGCTAAGACTAAGACTGC
TGGACTTAGAAAAGCTTAAGAAGGAAGATATTGATCAAGTTTTTGAACTTTTTAAGAGATATCAATCTAGATTTGAACCTTATC
TTTTACTAAGGAAGAATTTGAACATAATTTATTGGAGAAGAATCTCTTCTCTTGATAAGCAAGTTATTTTTCTTATGTGTTG
ACAACCTGATGGAAGATTACTGATTTTTTTCTTTTTATTCTTCTTTTACTATTCTTAATAATACTAAGTATAAGGATCTTGG
AATTGGATATCTTTATTATGCTACTGATGCTGATTTTCAATTTAAGGATAGATTTGATCCTAAGGCTACTAAGGCTCTTAAGAC
TAGACTTTTGAACTTATTTATGATGCTTGTATTCTTGCTAAGAATGCTAATATGGATGTTTTTAATGCTCTTACTTCTCAAGATA
TACTCTTTTTCTTGATGATCTTAAGTTTGGACCTGGAGATGGATTTCTTAATTTTTATCTTTTTAATTATAGAGCTAAGCCTATT
GGAGACTTAATCTGATAATCTAATGATATTAAGAGAAGATCTAATGTTGGAGTTGTTATGCTTTGA
  
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B- Comparison of *A. thaliana* codon use between normal (WT) and shuffled (Shu) ScNMT DNA sequences



III- List of oligonucleotide primers used in this study

a- Oligonucleotides used for cloning

Oligonucleotide name	Sequence	Feature
NHF2	GCCGCCATGGCGGAGGACAGCGAGTCTGCG	NcoI site
NHF4	AGGCTGGCTCCTGCAGTGGCACTGTG	
NHR1	TGTCAGAATTCTAGAAATAAAATCCATCTA	EcoRI site
NHR2	CTTTGGAATGGCAGTTCAGAAATCATTAA	
NHR3	TACCCGTTTCGATATCAACTTCTTATGA	
N1-Eco	TTATAGAATTCACAACCTGATTTGATGGTATC	EcoRI site
N1HF2	TCAGCCTGTTTAAATGAACTCTTTGCCAGCAGAGAGGA	DraI site
N1HR2	CGCACTGGTCCC GGTTATTGTAGCACCAGTCCA	XmaI site
N1ScF1	CGCACCGATAACAGATCCTTGAATAGAGA	NcoI site
N1ScF2	GCCTTTATCTCAGCCATACCGTAACA	
N1ScF21	GGAATCTTTTTAAATGTCTGAAGAAGATAA	DraI site
N1ScR1	CTATATACGTATCGTCTTTTCAGCCACTACA	SnaBI site
N1ScR21	GAGTCCC GGGTCAAAGCATAACA ACTCCAA	XmaI site
N1ScR2	TCGTCTCGTAATTTCTTTAATTAGAACAGGT	
N1R-Xma	CGACAACCCGGGAAACGTAGCGACCTAGTTCG	SmaI site
N2+1	TGTCAGATCCAAA ACTGAAGCCAGTGGAGG	
N2HR1	TGTCAGAATTCTAGAAATAAAATCCATCTA	
N2-NcoI	TTGACGATCAACATGGCAGATCCAAA ACT	NcoI site
N2R-Eco	GGTTTTAAGAATTCTAAAGAATAAGCCCAAGT	EcoRI site
N2R-Pml	GAGGAATCACGTGCTACACATTGGTTTAATG	PmlI site
N5F3	TTCGTTCACTGCATAGTGATGGAATGAAA	
N5F5	CTGGAACCCTAAATCTCGAATCTGGTAA	
N5F9	GAAGGGTCGACCTAGAATCTCTTGGTTACATT	Sal I site
N5F14	TCAGAAGAGCTCACTTGTTCTGGAACCCTA	SacI site
N5F20	GCGGAACCCGGGCTTGTCTCTTATAA	
N5R4	GTAGCGACCTAGTTCGGCTATAACAGGC	
N5R8	CGCACCGATAACAGATCCTTGAATAGAGA	
N5R10	GAACTATGGTTTCCAGCGAAGTATCATCCT	
N5R13	GGCCGTAGCTACTTCGTGACCTAAAAATGCA	
N5R15	TCTGCCATGGCCTACAAA ACTTAT	NcoI site
N5R16	GCGTGTAAGATCTGGATTGGTGCGTGCGAT	BglII site
N5R21	GATACCATCAGATCTAGTTGTTGAGCTTATAA	Bgl II site
N5R22	GTTATCTGCCAAGGCCTACAATTACAAA ACTTATA	StuI site
PN1R	TTTCTACAATTACAAA ACTTATAGAGCTAAG	
PN1-Xho	GGGATGACCTCGAGTCTCTTGGTTACATTCTC	XhoI site

b- Oligonucleotides used for RT- or real-time PCR

Gene to be amplified	Oligonucleotide name	Sequence
AlcA-AtNMT1	AlcA2	AGAGGACGACCTGCAGGTCGACGGA
	N5R8	CGCACCGATAACAGATCCTTGAATAGAGA
Actin	ActF	GGTAACATTGTGCTCAGTGGTGG
	ActR2	TGCCACGACCTTAATCTTCATGCTGCT
AtNMT1	N5F2	TCCTTCTGTTTACGAGTGGACGACATGT
	NR6	CTCCAATATGCCAGCTCTGGTAATAACC
	N5F5	CTGGAACCCTAAATCTCGAATCTGGTAA
	N5R10	GAACTATGGTTTCCAGCGAAGTATCATCCT
AtNMT2	N2F	GCACTTCAACCCGAAGCTTTTGACGA
	N2R9	TTCCAACCTATGGTTCCACCTGAAGTATCAT
AtMAP1A	487	CTAGAACACCTAAGCTTCCACAC
	MAT52	ATTCGGTTCAACTTTGGGAGTCCCATC
AtMAP2A	MAT69	CGCTGAAAAATCAGTTCCTAATGTTAG
	MAT55	CCTTGGTAACCTCAAAGGTACATGACCCA
AtMAP2B	MAT54	ATTCCCTACAGGATGCTCTTTGAATTGG
	MAT68	GTTGAAGGCAACTGTAAATGCACAG
EF	EF4	GATGATCTGCTGTTGTAACAAGATGGA
	EF7	ACCCTCAAATCCAGAGATGGGCACAAAT
N1At2 & 35S NMT2	N2F1	CCCAATTGAGCCCGCAACTTTGGTG
	NR6	CTCCAATATGCCAGCTCTGGTAATAACC
HsNMT1	N1HF3	GACATTCCAGTAGTGCACCAGCTCCTCA
	N1HR3	GGAAATCTGTCACCTCTCCGTTTGC GTTCT
HsNMT2	NHF4	AGGCTGGCTCCTGCAGTGGCACTGTG
	NHR3	TACCCGTTTCGATATCAACTTCTTATGA
PR1	F	GGAGCTACGCAGAACA ACTAAGA
	R	CCCACGAGGATCATAGTTGCAACTGA
ScNMT1	N1ScF2	GCCTTTATCTCAGCCATACCGTAACA
	N1ScR2	TCGTCTCGTAATTTCTTTAATTAGAACAGGT

b- Oligonucleotides used for RT- or real-time PCR (following)

Gene to be amplified	Oligonucleotide name	Sequence
AKIN β 1	1K1F4	ACTGGCGGTGCCACCTCAGCTTCAT
	K1R6	TACTATGGATTGAGGAGTCCATCCTTGCTCT
AKIN β 2	K2F1	TAACCATGTGCTCCTCCTGAGCTTATGGGT
	K2R1	GAAGGATTAGGGATATGAATTCATCAGGTCTTTGAAGT
AKIN β 3	K3F2	CTATATCGAGAACCGAGACGCCCAAGA
	K3R2	GCTCCCTCTTCTTTGGACAGGCTT

c- Oligonucleotides used for AKIN β 1: and AKIN β 2:GFP fusions

Oligonucleotide name	Sequence	Feature
AK1F	TGCATCTAGATGGGAAATGCGAACGGCA	XbaI site
AK1FG ₂ A	TGCATCTAGATGGCAAATGCGAACGGCA	XbaI site, G ₂ A
AK1RM	ACGTGGATCCGTTTCTGCAGCTTCTT	BamHI site
AK2F	TGCATCTAGATGGGTAACGTGAACGCGA	XbaI site
AK2FG ₂ A	TGCATCTAGATGGCTAACGTGAACGCGA	XbaI site, G ₂ A
AK2RM	ACGTAGATCTGCAGCCGACTTCTTGT	BglII site