The first step in the biosynthesis of cocaine in *Erythroxylum coca*: the characterization of arginine and ornithine decarboxylases

Docimo Teresa[1], Reichelt Michael[1], Schneider Bernd[2], Marco Kai[3], Kunert Grit[1], Gershenzon Jonathan[1] and D'Auria John C.[1]

Max Planck Institute for Chemical Ecology

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena

[2]NMR Research Group, Max Planck Institute for Chemical Ecology, Jena

[3]Mass Spectrometry Research Group, Max Planck Institute for Chemical Ecology, Jena

Beutenberg Campus, Hans-Knöll-Straße 8 D-07745 Jena, Germany

Corresponding Author

D'Auria John C

Department of Biochemistry

Max Planck Institute for Chemical Ecology

D-07745 Jena, Germany

Supplementary Material

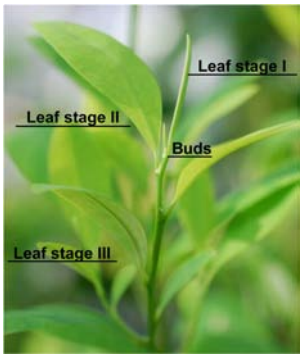

Fig 1 Developmental stages of *E .coca* leaves investigated from youngest to oldest: buds, rolled leaves (Leaf stage I), , expanding leaves (Leaf stage II) , and mature leaves (Leaf stage III).

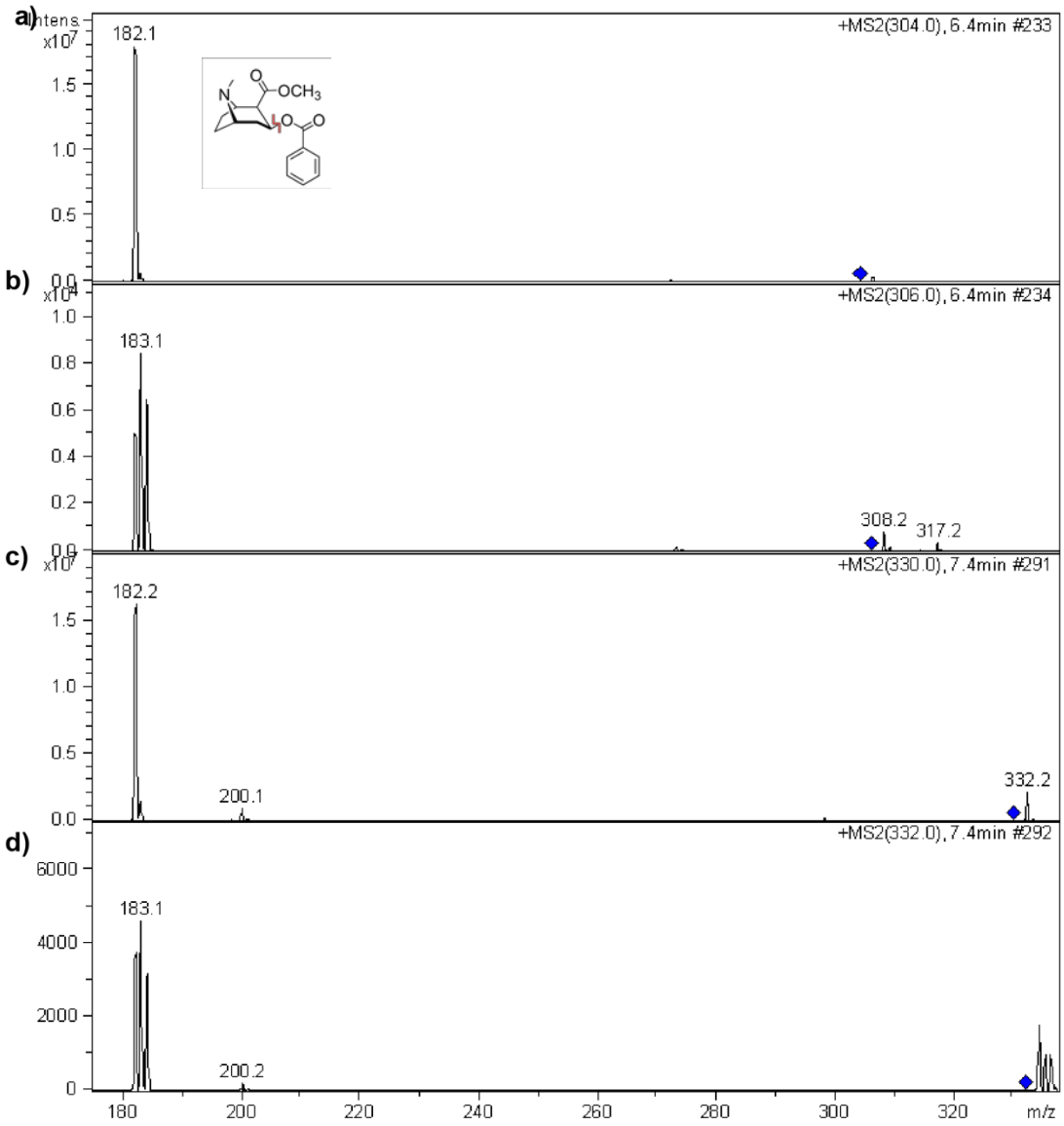

Fig2.LC-MS ion trap chromatogram comparison of methylecgonine derivatives in samples of stem cuttings treated with [U-^{13}C]glucose solution or with unlabeled glucose. a-b) MS^2 of cocaine shows ^{13}C enrichment of the methylecgonidine fragment (*m/z* 182) in a sample from unlabeled cuttings **a)** compared to one from ^{13}C-labeled cuttings **b)**. c-d) MS^2 of cinnamoyl cocaine shows ^{13}C enrichment of the methylecgonidine fragment in a sample from unlabeled cuttings **c)**compared to one from ^{13}C-labeled cuttings. **d)**

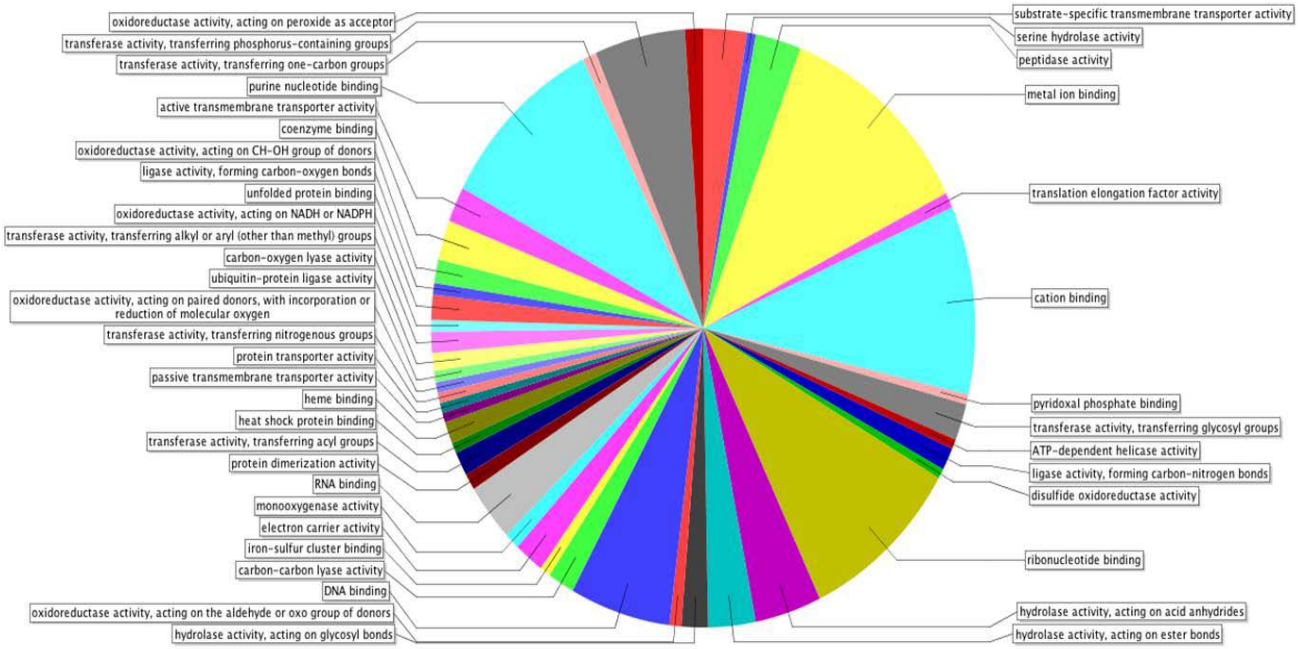

Fig3 Gene Ontology annotations for *E. coca* EST collection. Shown are percentage representations of the 3 top hierarchical annotations: molecular function, cellular component, and biological processes.

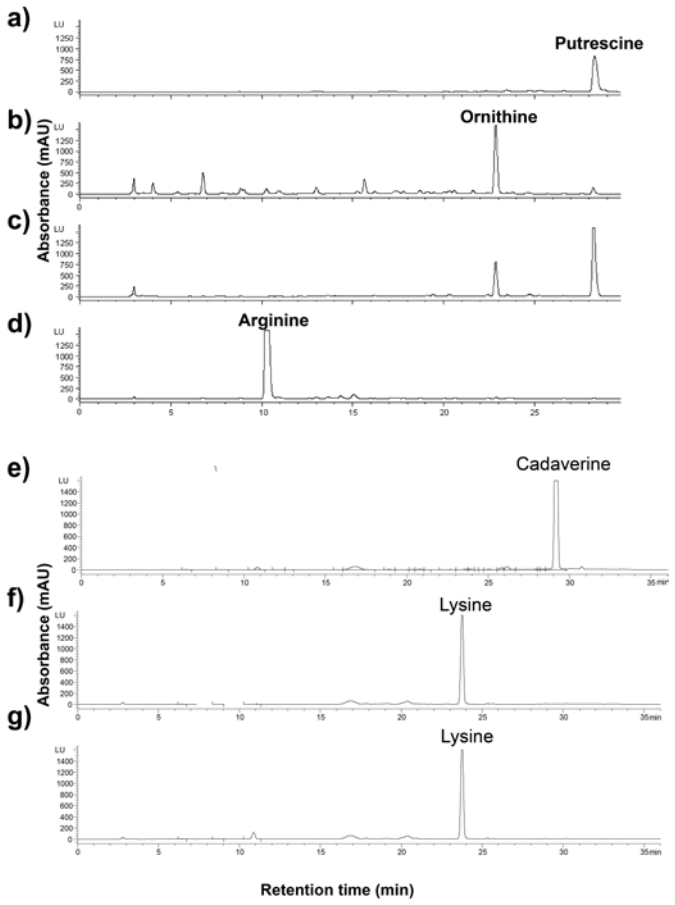

Fig 4 Demonstration of ornithine decarboxylase activity for *E. coca* ODC in *in vitro* assay.
Depicted are HPLC chromatograms with fluorescence detection of OPA derivatives for a) putrescine standard, b) extract of *E. coli* transformed with pET32 empty vector assayed with ornithine as substrate, c) His-Tag purified pET32::EcODC expressed in *E. coli* assayed with ornithine, d). His-Tag purified pET32::EcODC expressed in *E. coli* assayed with arginine e) Standard of cadaverine, product of lysine decarboxylation f) lysine Standard g) His-Tag purified *E. coca* ODC tested with lysine showing lack of activity. Retention times are; lysine, 23.7 min;,and cadaverine, 29.3 min.

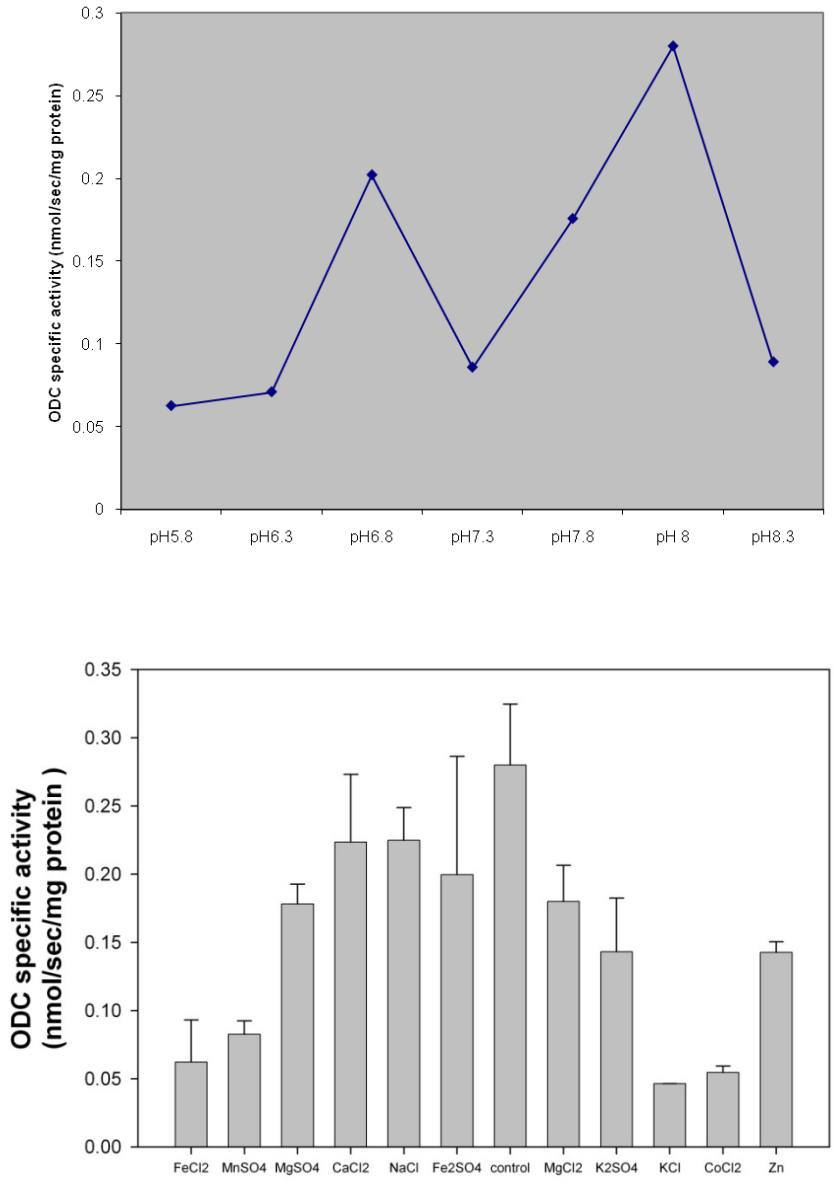

Fig5 *E. Coca* ODC activity measured at varying pH (above) and with varying monovalent and divalent ions in the assay buffer at a final concentration of 3mM(below)

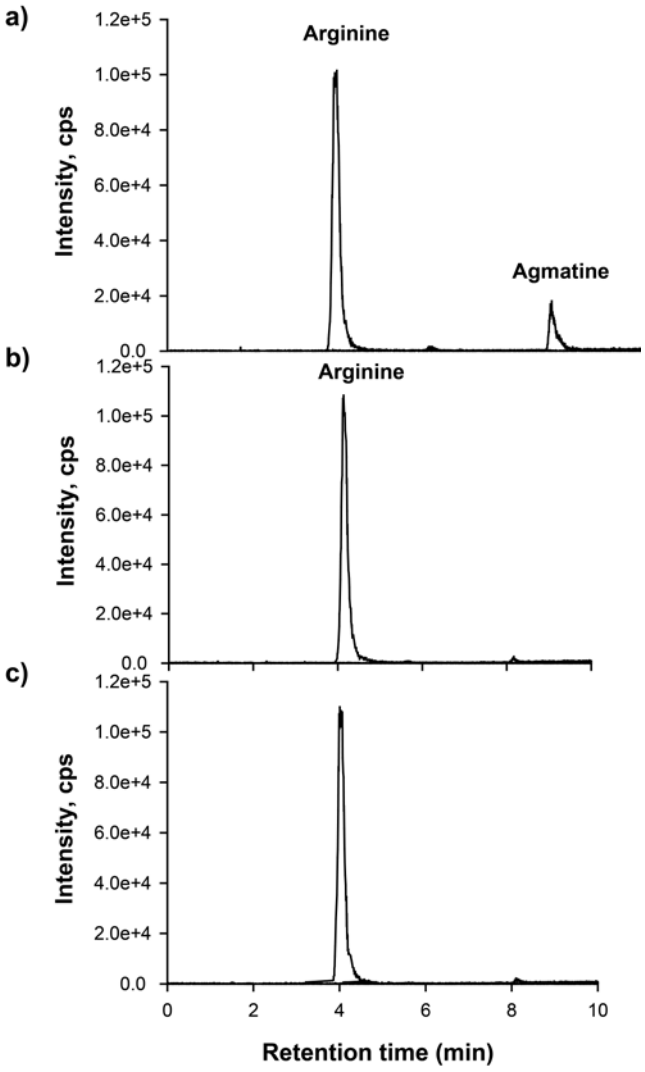

Fig6. Demonstration of arginine decarboxylase activity for *E. coca* ADC in *in vitro* assay. Depicted are LC chromatograms of the assay with MS triple quadrupole detection of **a)** purified pET32:: *EcADC* expressed in *E. coli*, **b)** extract of *E. coli* transformed with pET32 empty vector, and **c)** pET32::*EcADC* boiled enzyme.

Table 1 Relative stability ranking of internal reference genes made according to a previously described method (Vandesompele et al., 2002)

Rank	Genes	geNorm
1	PeX4	**1.447**
2	PP2AA2	**0.725**
3	EF1α	**0.822**
4	ATPTB1	**0.752**
5	APT2	**1.086**
6	Actin	**0.982**
7	11242	**0.741**
8	10131	**0.734**
9	6402	**0.722**

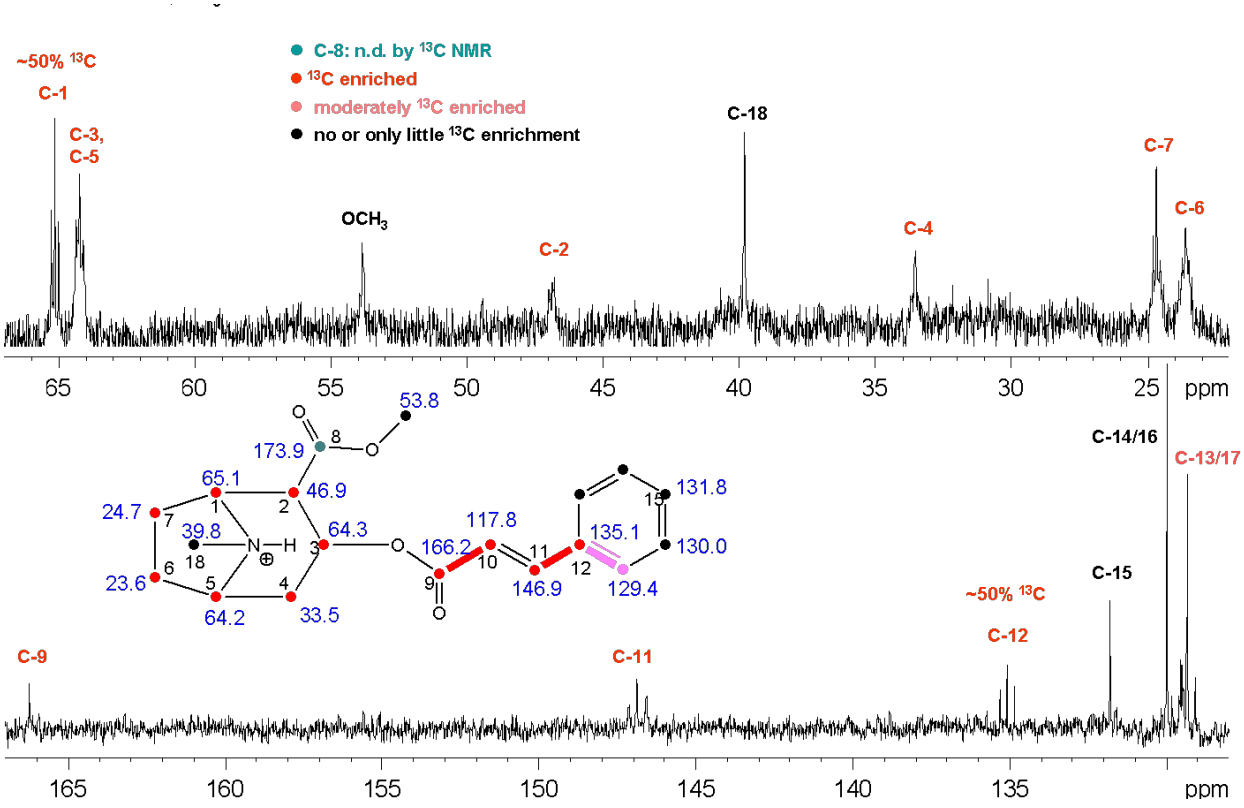

Fig7 Detection of ^{13}C incorporation in cinnamoyl moiety of cinnamoyl cocaine after [U-^{13}C]glucose feeding. Depicted is the ^{13}C-NMR spectrum with signals colored for relative degree of enrichment.

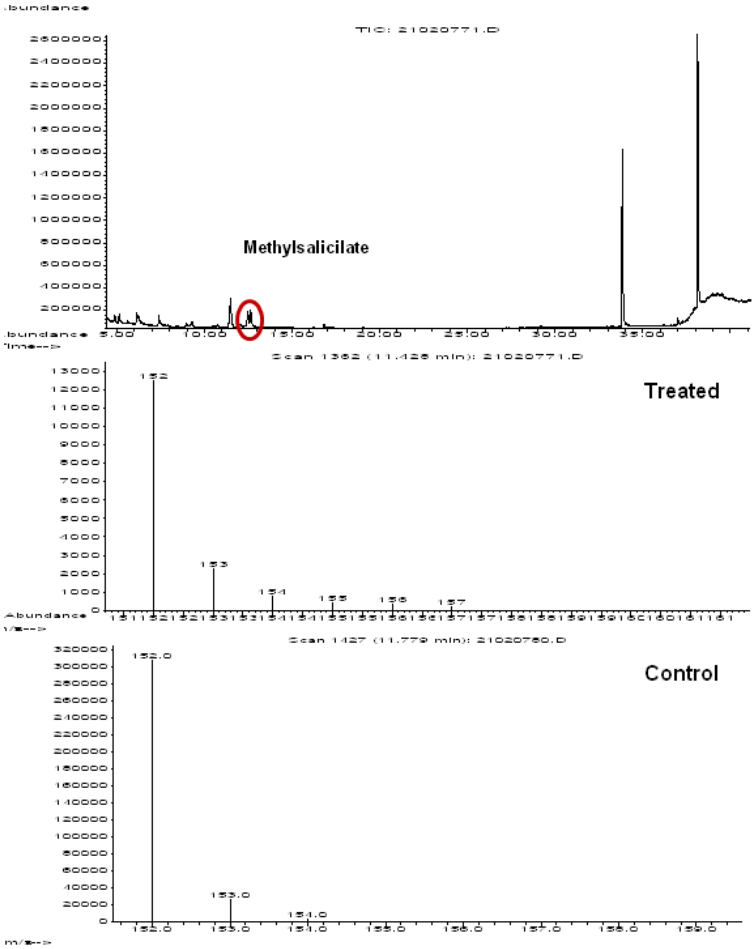

Fig8 Detection of ^{13}C-label in methyl salicylate in rolled leaves after feeding with a ^{13}C glucose solution compared with being fed with unlabeled glucose. Shown is the GC-MS total ion trace (top) and mass spectrum of methyl salicylate from [^{13}C]glucose-fed (middle) and glucose-fed (bottom) plants.

Table 2 Description of Primers used for PCR and qPCR

Gene	Accession. number	Sequence	Orientation	Purpose
NtODC	AAB65826	CCTCTCTCTTTTCTTCCTTTGTTTGG	For	Library screening
NtODC		CCATCAAAACCACGGTAGTAATTCC	Rev	
AttbODC	JF909553	CTGGTTCCGCGTGGTTCCATGCCAACTTT	For	Cloning/PCR
AttbODC		CAAGAAAGCTGGGTCCTACGGATTGGAATAGGCA	Rev	
qODC		GAAGAGGAACGAAAGCAAAGAGC	For	qPCR
qODC		CGGGAGAGACACGGAGAGAC	Rev	
pYesODC		CTGGTTCCGCGTGGTTCCAAAATGCCAACTTT	For	Cloning/PCR
PYesODC		CAAGAAAGCTGGGTCCGGATTGGAATAGGCAA	Rev	
ADC	JF909552	ATGGACGGTTGGGGTGCT	For	Sequencing
ADC		AACCGAAATGACCCGCATGT	Rev	
AttbADC		CTGGTTCCGCGTGGTTCCATGGACGGTTG	For	Cloning/PCR
AttbADC		CAAGAAAGCTGGGTCTCAAGCACAACAATAAGA	Rev	
qADC		GCAGCGGTGGAGGCAGAG	For	qPCR
qADC		CTGCTGCTGCTGCTGCTGA	Rev	
Reference genes for qPCR				
qEF1α	JN020156	TGGAGGTATTGACAAGCGTGTGATTGAGAG	For	qPCR
qEF1α		TTTGACACCAAGAGTGAAAGCAAGAAGAGC	Rev	
qACTIN	JN020155	GGATTTCCAAAGGTGAATACGATG	For	qPCR
qACTIN		TTGAACCAGCAAAGTTGAATAAGC	Rev	
q10131	JN020153	TGGAAGGGTAGTGGGGTAACAATG	For	qPCR
q10131		GAGCGTAGTCGTCAGAGAAGGC	Rev	
q6409	JN020150	GAAGAGACAAGTGGTGGGGTGAG	For	qPCR
q6409		AGAAGAGAGCAAAGAGGAAGAGTGG	Rev	
q11242	JN020151	ACATTACCAAAGCAGGCTCATACG	For	qPCR
q11242		TACATCTTCTCACCACCAACACAGC	Rev	
qAPT2	JN020149	ACTCAGAGAGCGAGAGAGGATGTTT	For	qPCR
qAPT2		TCAACTCCAGCAACCACAGAAATG	Rev	

qPEX4	JN20157	GTCGGTTCTTTAGCAAGGTCAGTG	For	qPCR
qPEX4		CGTGGTGGCGGTGGTGG	Rev	
qTIP41	JN20154	TGCTCCTGTTATGGGTCCTGAAG	For	qPCR
qTIP41		CATCTGGGTCCTCACTCAACTCC	Rev	
qPTPB	JN20152	CCGATTGAAGCCATAACAGGAGAC	For	qPCR
qPTPB		CTGGTGCTGGTCCTGTGGG	Rev	