10.1071/FP17212_AC

Supplementary Material: *Functional Plant Biology*, 2018, 45(5), 542–552.

Supplementary Material

Structural and functional characterisation of two novel durum wheat annexin genes in response to abiotic stress

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(a)

CELLO RESULTS

SeqID: Td_mahmoudi_Ann6

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Mitochondrial	0.666
N-peptide Comp.	Extracellular	0.371
Partitioned seq. Comp.	Mitochondrial	0.708
Physico-chemical Comp.	Mitochondrial	0.345
Neighboring seq. Comp.	Nuclear	0.584

CELLO Prediction:

	Mitochondrial	2.290 *
	Nuclear	1.347
	Extracellular	0.556
	Cytoplasmic	0.390
	PlasmaMembrane	0.132
	Chloroplast	0.077
	Peroxisomal	0.076
	ER	0.053
	Golgi	0.027
	Lysosomal	0.023
	Vacuole	0.015
	Cytoskeletal	0.013

(b)

SeqID: Td_mahmoudi_Ann12

Analysis Report:

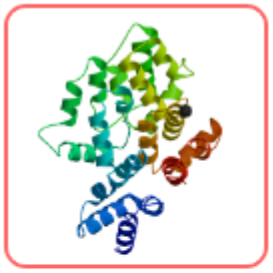
SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Cytoplasmic	0.413
N-peptide Comp.	Cytoplasmic	0.366
Partitioned seq. Comp.	Cytoplasmic	0.629
Physico-chemical Comp.	Cytoplasmic	0.508
Neighboring seq. Comp.	Cytoplasmic	0.471

CELLO Prediction:

	Cytoplasmic	2.387 *
	Mitochondrial	1.006
	Nuclear	0.783
	Extracellular	0.294
	Peroxisomal	0.194
	Chloroplast	0.151
	ER	0.063
	Golgi	0.048
	PlasmaMembrane	0.033
	Cytoskeletal	0.019
	Vacuole	0.015
	Lysosomal	0.007

Fig. S1

(a)



Model TdAnn6

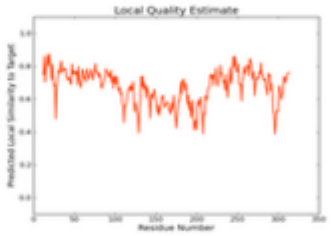
Oligo-State	Ligands
MONOMER	1 x CA

GMQE	QMEAN
0.63	-4.48

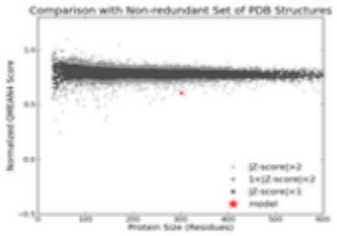
Global Quality

QMEAN	-4.48
Cβ	-2.43
All Atom	-2.01
Solvation	-0.28
Torsion	-3.97

Local Quality



Comparison



Template	Seq Identity	Coverage	Description
1mcx.1.A	33.00%		ANNEXIN I

Model-Template Alignment

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Model TdAnn6 MASLSVPPVLT-SPRHDAIALHRAFKGFGCDSTTVINILAHRDSAQRGLIMQEYRAIYHQDLYHRLSTEL  69
1mcx.1.A     ----AVSPYPTFNPSSDVEALHKAITVKGVDEATIIEILTKRTNAQRQQIKAAYLQEKGKPLDEALKKAL  100

Model TdAnn6 SGNHKDAKLLWVLEITQHNAIMLNQAHNH---DSIDLREPPPRPFSKKPSKKHTIVLYLLPRFPLCLGHD  136
1mcx.1.A     TGHLEEVALALIKTPAQFDADELRAAMKGLGTDEDTLNEILASRTNREIREINRVYKEELKRD---LAKD  167

Model TdAnn6 IRTQLRRDHQKLLL-LRTWRR------HDTEPDSSTLTYTEPAHRHGGDLLPDAVAAAIMKQVPRSERST  199
1mcx.1.A     ITSDTSGDYQKALLSLAKGDRSEDLAINDDLADTDARALYEAGERRKGTDLNVFITILTTRSYPHLRR--  235

Model TdAnn6 WLEQDVTERTYGDH-LEKGVKSETSGTFELGLLTILRCAESPARYFAKALHKAMKGLGTSDTTLIRVVVT  268
1mcx.1.A     -VFQKYSK--YSKHDMNKVLDLELKGDIENCLTVVVKCATSKPMFFAEKLHQAMKGIGTRHKTLIRIMVS  302

Model TdAnn6 RAEVDMQYIKAEYHKKYKRSLADAIRSIHEKSGNFRTFLLLTLVGRDR  316
1mcx.1.A     RSEIDMNDIKACYQKLYGISLCQAI--LDETKGDYEK-ILVALGGD-  346
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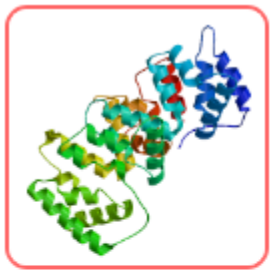
Fig. S2

(b)



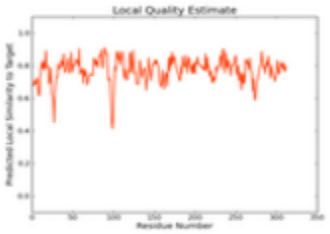
Model_TdAnn12

Oligo-State	Ligands	GMQE	QMEAN
MONOMER	None	0.83	-0.87

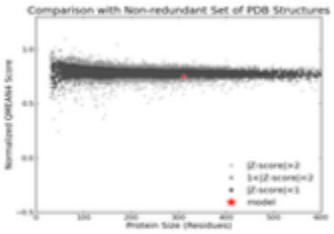
Global Quality

QMEAN	-0.87
Cβ	-0.26
All Atom	0.46
Solvation	1.55
Torsion	-1.15

Local Quality



Comparison



Template	Seq Identity	Coverage	Description
1n00.1.A	64.31%		annexin Gh1

Model-Template Alignment

```
Model_TdAnn12 MATLKVPSNVPALADDCDNLRKAFQGWGTNEALIISILGHRDAAQRRAIRKHYADTYGEELLRSITDEIS  70
1n00.1.A      -ATLTVPTTVPSVSEDCEQLRKAFSGWGTNEGLIIDILGHRNAEQRNLIRKTYAETYGEDLLKALDKELS  75

Model_TdAnn12 GDFERAVILWTLDPAERDAVLANETAKKWHPGNPVLVEIACARGSKQLFAVRQAYHDRFKRSLEEDVAAH 140
1n00.1.A      NDFERLVLLWALDPAERDALLANEATKRWTSSNQVLMEIACTRSANQLLHARQAYHARYKKSLEEDVAHH 145

Model_TdAnn12 VTGDFRKLLVPLVSSHRYEGPELNTRLAHSEAKLLHEKIEHKAYGDDEVIRILTTRSKAQLLATFNNYND 210
1n00.1.A      TTGDFHKLLLPLVSSYRYEGEEVNMTLAKTEAKLLHEKISNKAYSDDDVIRVLATRSKAQINATLNHYKN 215

Model_TdAnn12 TFGHPITKDLKADPKDEFLKTLRAVIRCFTCPDRYFEKVARVAIAGNGTDENSLTRVITTRAEVDLKLIK 280
1n00.1.A      EYGNDINKDLKADPKDEFLALLRSTVKCLVYPEKYFEKVLRLAINRRGTDEGALTRVVCTRAEVDLKVIA 285

Model_TdAnn12 EAYQKRNSVPLEKAVAGDTSGDYESMLLALLGKE 314
1n00.1.A      DEYQRRNSVPLTRAIVKDTHGDYEKLLLVLAG-- 317
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Fig. S2

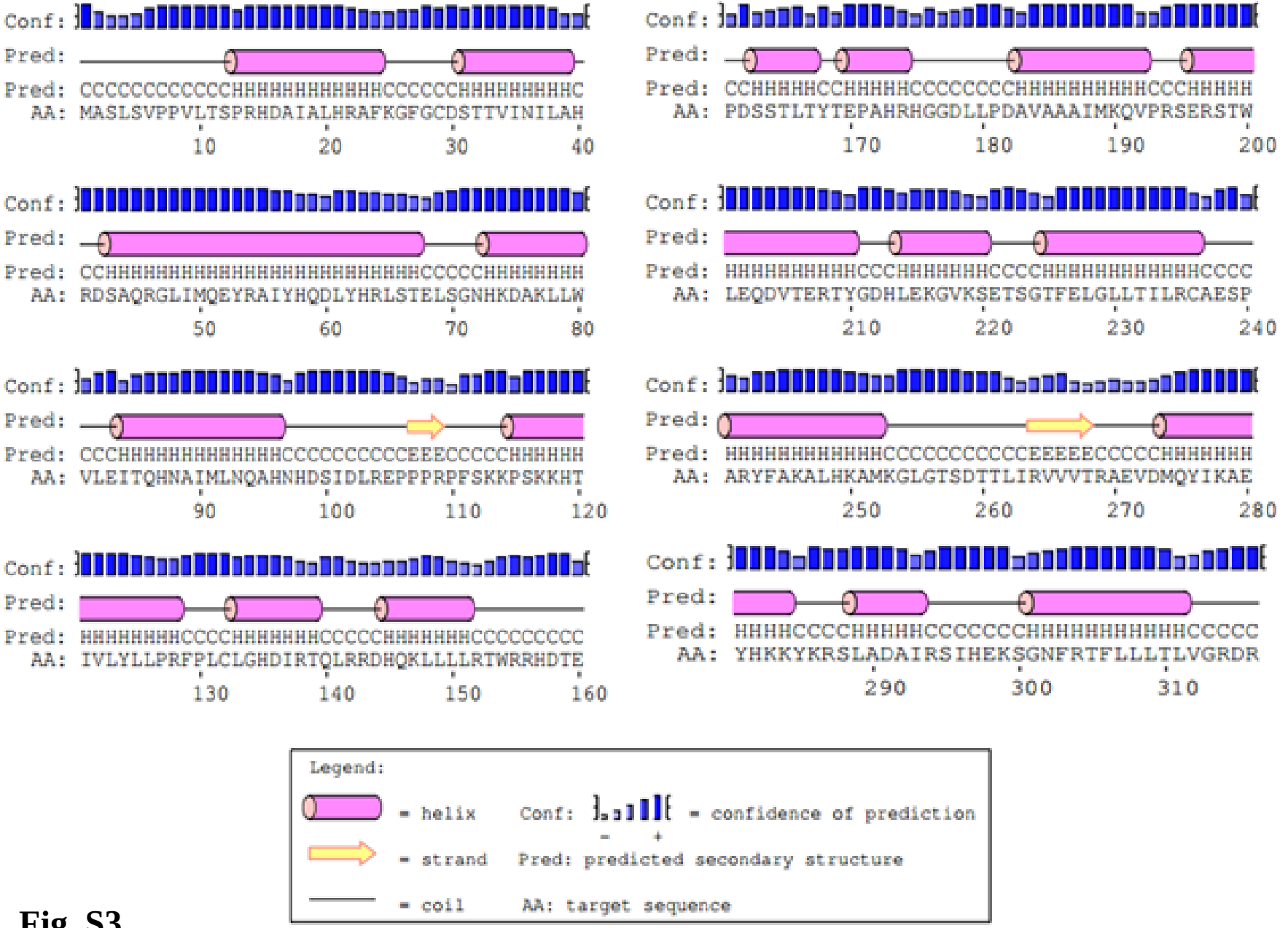
Conf:
Pred:
Pred: CCCCCCCCCCCCHHHHHHHHHHHHCCCCCCHHHHHHHHHC
AA: MASLSVPPVLTSPRHDAIALHRAFKGFGCDSTTVINILAH
10 20 30 40
Pred: CCHHHHHCCHHHHHCCCCCCCCHHHHHHHHHHCCCHHHHH
AA: PDSSTLTYTEPAHRHGGDLLPDAVAAAIMKQVPRSERSTW
170 180 190 200
Pred: CCHHHHHHHHHHHHHHHHHHHHHHHHHHCCCCCHHHHHHHH
AA: RDSAQRGLIMQEYRAIYHQDLYHRLSTELSGNHKDAKLLW
50 60 70 80
Pred: HHHHHHHHHHCCCHHHHHHHCCCCHHHHHHHHHHHHCCCC
AA: LEQDVTERTYGDHLEKGVKSETSGTFELGLLTILRCAESP
210 220 230 240
Pred: CCCHHHHHHHHHHHHHCCCCCCCCCCEEECCCCCCHHHHHH
AA: VLEITQHNAIMLNQAHNHDSIDLREPPPRPFSKKPSKKHT
90 100 110 120
Pred: HHHHHHHHHHHHCCCCCCCCCCCCEEEEECCCCCHHHHHHH
AA: ARYFAKALHKAMKGLGTSDTTLIRVVVTRAEVDMQYIKAE
250 260 270 280
Pred: HHHHHHHCCCCHHHHHHHCCCCCHHHHHHHCCCCCCCCC
AA: IVLYLLPRFPLCLGHDIRTQLRRDHQKLLLLRTWRRHDTE
130 140 150 160
Pred: HHHHCCCCHHHHHCCCCCCCHHHHHHHHHHCCCCC
AA: YHKKYKRSLADAIRSIHEKSGNFRTFLLLTLVGRDR
290 300 310
Legend:
= helix
= strand
= coil
Conf: - + = confidence of prediction
Pred: predicted secondary structure
AA: target sequence

Fig. S3

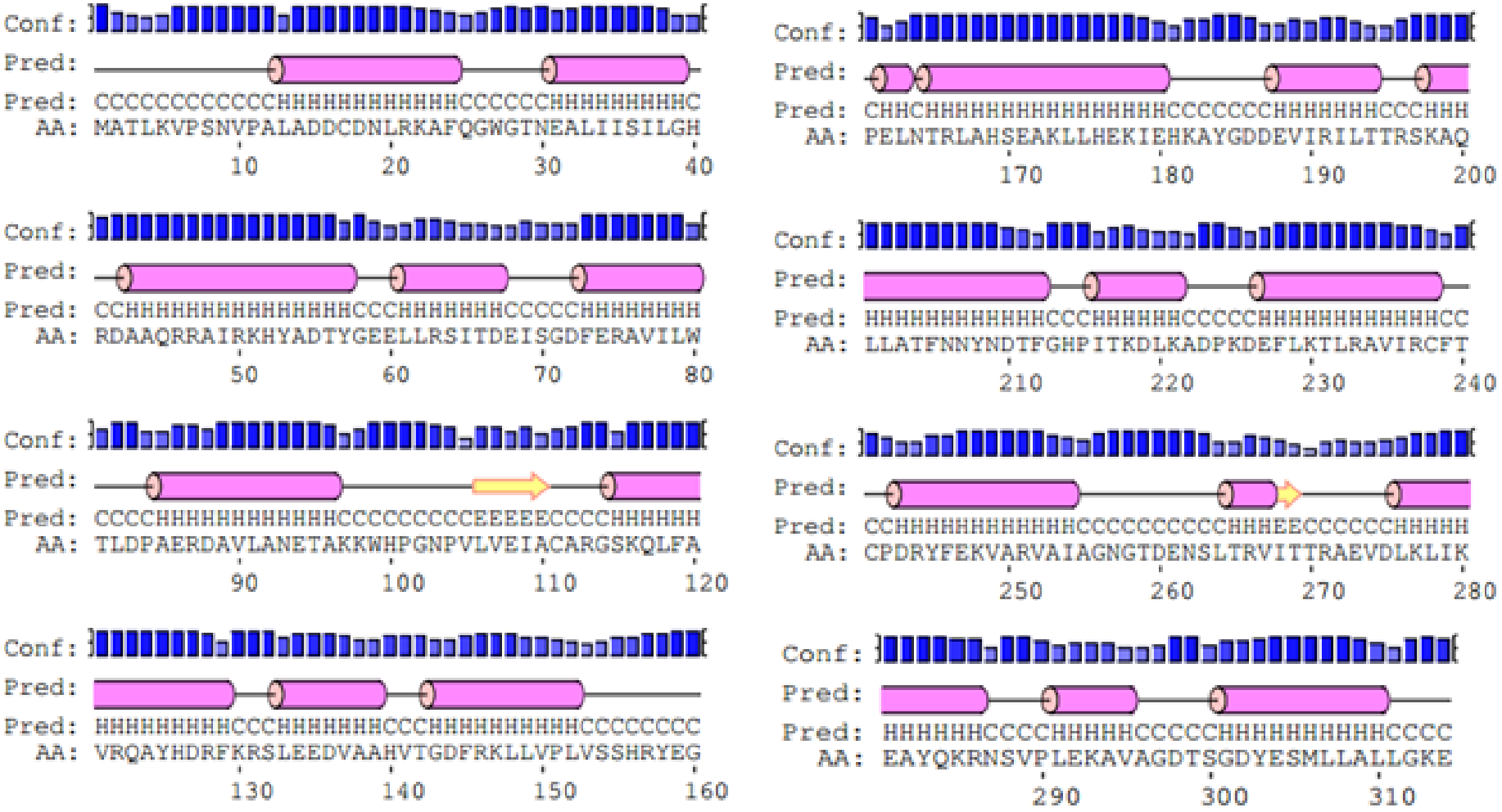
Conf:
Pred:
Pred: CCCCCCCCCCCCHHHHHHHHHHHHHCCCCCCHHHHHHHHHC
AA: MATLKVPSNVPALADDCDNLRKAFQGWGTNEALIISILGH
10 20 30 40
Pred: CCHHHHHHHHHHHHHHHCCCHHHHHHHCCCCCHHHHHHHH
AA: RDAAQRRAIRKHYADTYGEELLRSITDEISGDFERAVILW
50 60 70 80
Pred: CCCCHHHHHHHHHHHHCCCCCCCCCEEEEECCCCHHHHHH
AA: TLDPAERDAVLANETAKKWHPGNPVLVEIACARGSKQLFA
90 100 110 120
Pred: HHHHHHHHHCCCHHHHHHHCCCHHHHHHHHHHCCCCCCCC
AA: VRQAYHDRFKRSLEEDVAAHVTGDFRKLLVPLVSSHRYEG
130 140 150 160
Pred: CHHCHHHHHHHHHHHHHHHCCCCCCCHHHHHHHCCCHHH
AA: PELNTRLAHSEAKLLHEKIEHKAYGDDEVIRILTTRSKAQ
170 180 190 200
Pred: HHHHHHHHHHHHCCCHHHHHHCCCCCHHHHHHHHHHHCC
AA: LLATFNNYNDTFGHPITKDLKADPKDEFLKTLRAVIRCFT
210 220 230 240
Pred: CCHHHHHHHHHHHHCCCCCCCCCCHHHEECCCCCCHHHHH
AA: CPDRYFEKVARVAIAGNGTDENSLTRVITTRAEVDLKLIK
250 260 270 280
Pred: HHHHHHCCCCHHHHHCCCCCHHHHHHHHHHCCCC
AA: EAYQKRNSVPLEKAVAGDTSGDYESMLLALLGKE
290 300 310
Legend:
= helix
= strand
= coil
Conf: - +
= confidence of prediction
Pred: predicted secondary structure
AA: target sequence

Fig. S4

Table S1. The primers used in PCR and real time qPCR assays

Primers	Sequences
TaAnn6_F	5’-CATCATGCAGGAGTACAGG-3’
TaAnn6_R	5’-GTACTTCTTGTGGTACTCG-3’
TaAnn12_F	5’-CTACGGCGAGGAGCTGCT-3’
TaAnn12_R	5’-CTAGTGAGGGAGTTCTCAT-3’
R5Ann6	5’-CGATAGGCGATGGTAGAGGTCC-3’
R3Ann6	5’-TGGTCACCAGGGCGGAGGTGG-3’;
R5Ann12	5’-GCCCTCTCGAAGTCGCCGGA-3’
R3Ann12	5’-GCCAGGGTGGCCATTGCAGG-3’
TdAnn6A-F	5’-ATGGCGAGCCTGAGCGTGCCTC-3’
TdAn6A-R	5’-TTAGCGGTCGCGGCCGACGAGT-3’
TdAn12A-F	5’-ATGGCGACGCTCAAGGTCCCCT-3’
TdAn12A-R	5’-TCACTCCTTCCCCAGGAGGGCA-3’
qActinTd-F	5’-TACTCCCTCACAACAACCG-3’
qActinTd-R	5’-AGAACCTCCACTGAGAACAA-3’
qTdAnn6-F	5’-TGTATTTGCTCCCGAGGTTC-3’
qTdAnn6-R	5’-TGATGATCACGCCTTAGCTG-3’
qTdAnn12-F	5’-CACAAGGTTGGCTCATTCAG-3’
qTdAnn12-R	5’-GAATGTTGCAAGCAGCTGAG-3’
TdAnn6A-FN	5’-CACCATGGCGAGCC TGAGCGTGCCTC-3’
TdAnn6A-R-WS	5’-GCGGTCGCGGCCGACGAGTG-3’
TdAnn12A-FN	5’-CACCATGGCGACGCTCAAGGTCCCCT-3’
TdAnn12A-R-WS	5’-CTCCTTCCCCAGGAGGGCAAG-3’
18SF	5’-GCAAGTCTGGTGCCAGCAGCC-3’
18SR	5’-CTTCCGTCAATTCTTTTAAG-3’