

Isolation and characterisation of GtMYBP3 and GtMYBP4, orthologues of R2R3-MYB transcription factors that regulate early flavonoid biosynthesis, in gentian flowers

Takashi Nakatsuka, Misa Saito, Eri Yamada, Kohei Fujita, Yuko Kakizaki, Masahiro Nishihara

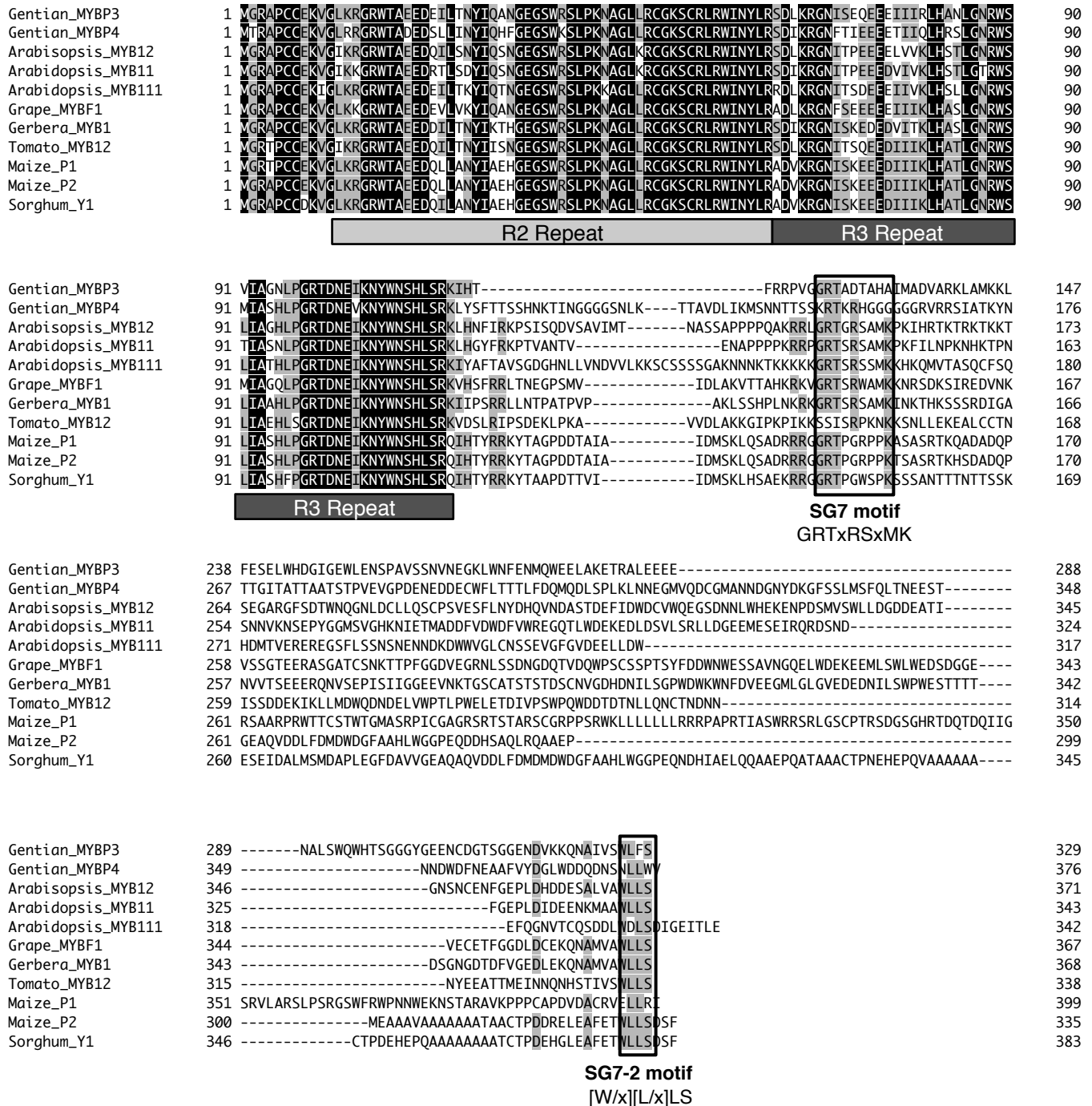


Fig. S1. Alignment of P1-orthologue proteins in higher plants.

The position of the R2R3-DNA binding domain is indicated below the alignment. The conserved SG7-1 and SG7-2 domains are highlighted by black boxes.

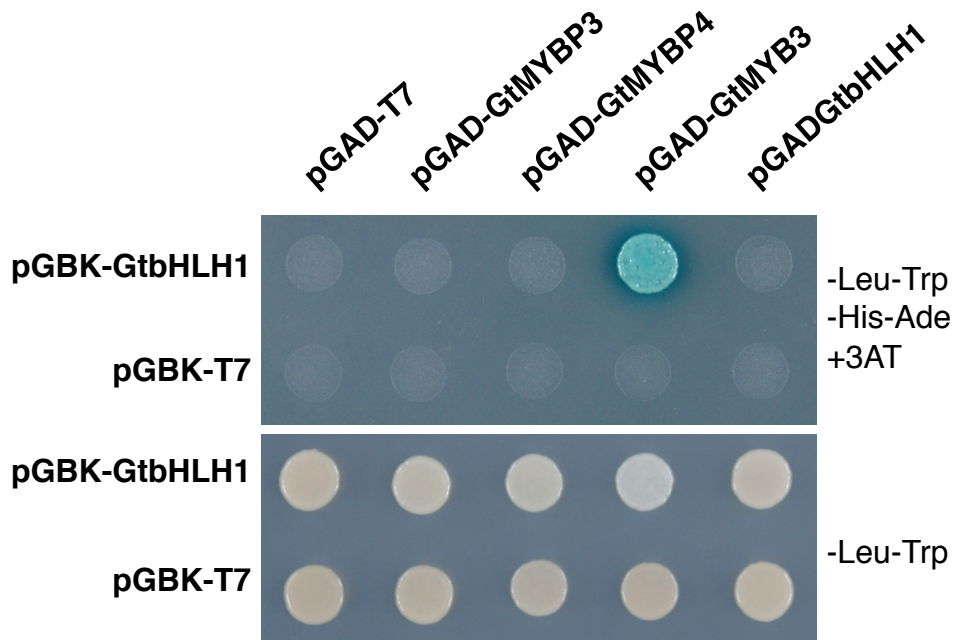


Fig. S2. Yeast two-hybrid analysis to examine the protein-protein interaction between GtMYBs and GtbHLH1.

The GtbHLH1 protein was fused to the GAL4 binding domain (BD) and assayed for its ability to bind the GtMYBs and GtbHLH1 fused to the GAL4 activation domain (AD). The transformed yeasts were grown on quadruple dropout medium (without leucine, tryptophan, histidine and adenine, upper) supplemented with 15 mM 3AT and on double dropout medium (without leucine and tryptophan, lower) at 30°C for 3 days. Protein-protein interactions are shown by yeast growth on quadruple dropout medium.

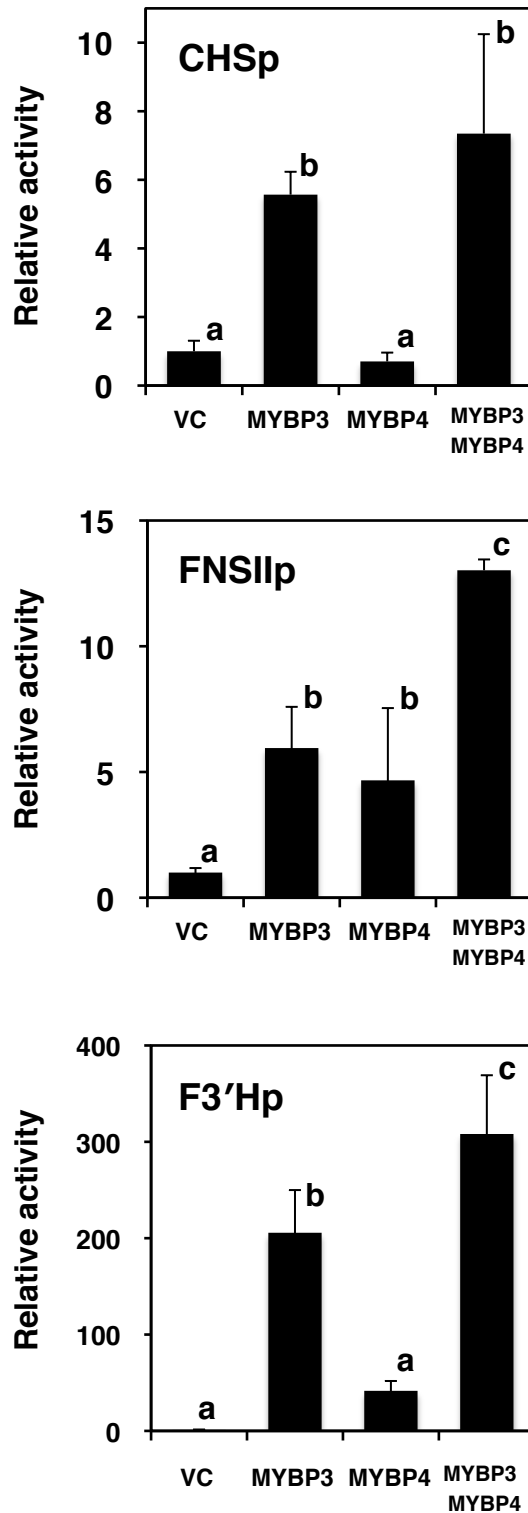


Fig. S3. Effect of co-transfection of GtMYBP3 and GtMYBP4 on promoter activities of *GtCHS*, *GtFNSII*, *GtF3'H*.

Transient expression assays were performed using protoplasts from Arabidopsis T87 cells, as described in Fig. 4B. Letters are the results from Tukey's multiple comparisons test where different letters represent a significant difference at $P < 0.05$.

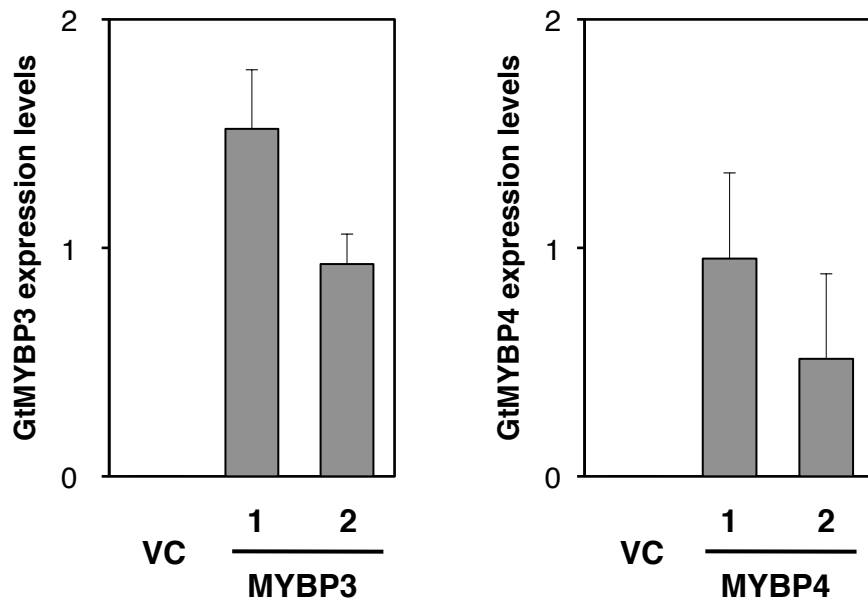
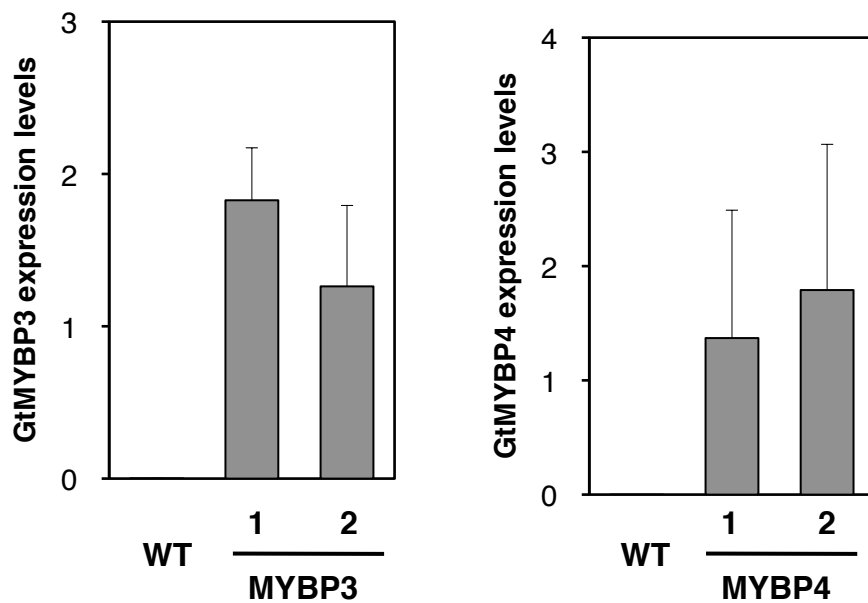
A**B**

Fig. S4. Confirmation of the expression of transgenes in transgenic Arabidopsis and tobacco.

The expressions of *GtMYBP3* and *GtMYBP4* were investigated in transgenic 5-day-old Arabidopsis seedlings (A) and in transgenic tobacco petals (B). VC indicates vector control.

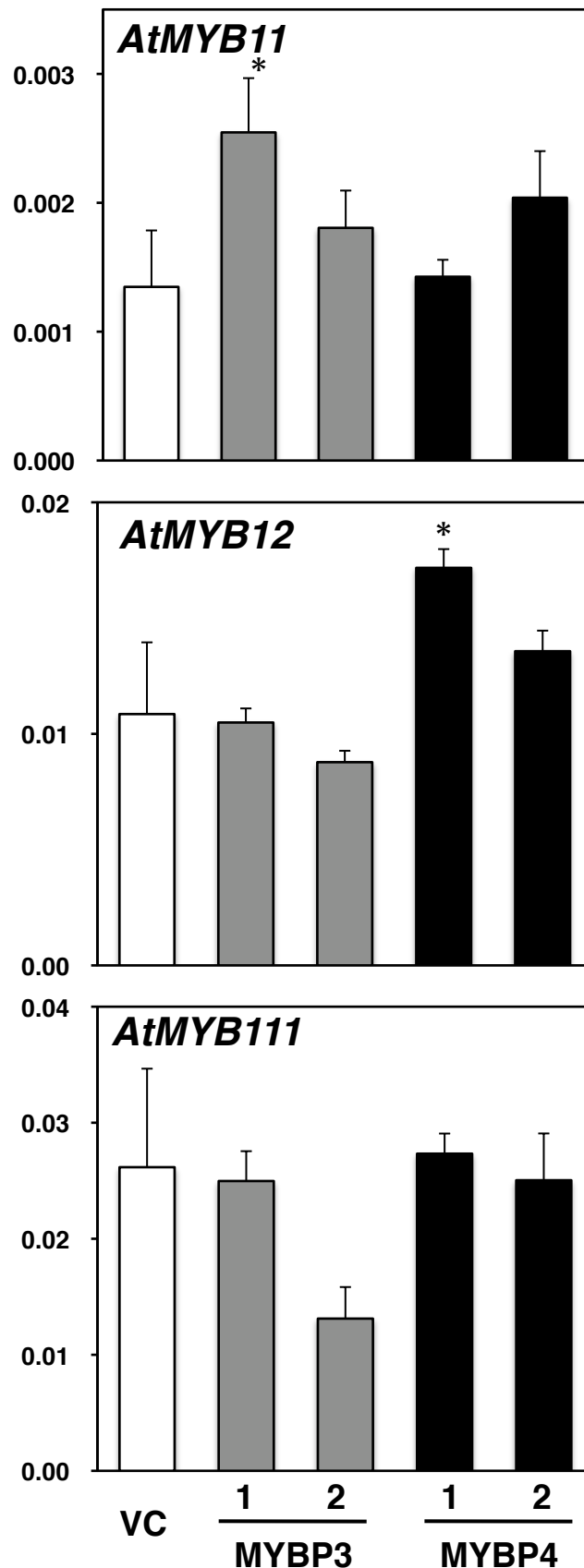


Fig. S5. Expression analyses of endogenous flavonol-specific transcription factor genes in transgenic Arabidopsis.

The effects of *GtMYBP3* and *GtMYBP4* overexpression on endogenous flavonol-specific R2R3MYB genes were investigated using qRT-PCR analyses in vector control and 5-day-old transgenic seedlings. The two independent transgenic lines shown in Fig. 6 were analysed. Asterisks (*, $P < 0.05$) represent statistically significant differences between the means for vector control and transgenic lines, as judged by Student's *t*-test.

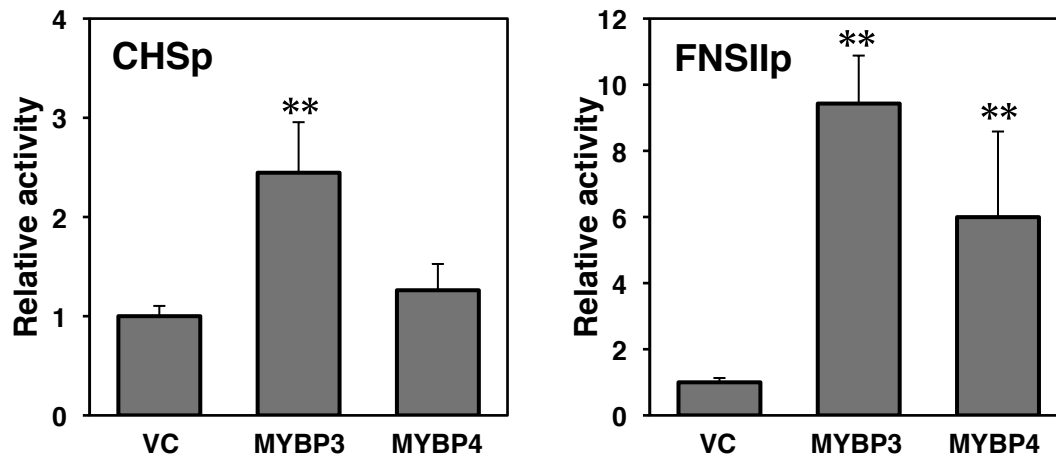


Fig. S6. Transient expression assay in gentian mesophyll protoplast.

The effect of GtMYBP3 and GtMYBP4 on promoter activities of two early flavonoid biosynthetic genes, *GtCHS* and *GtFNSII*, were investigated in mesophyll protoplasts of gentian. Transient expression assays were performed by the dual-Glo luciferase assay system, as described in Fig. 4B. Asterisks (**, $P < 0.01$) represent statistically significant differences between the means for negative control (pBI221) and tested genes, as judged by Student's *t*-test.

Table S1. Primers used in this study.

		Forward	Reverse
Degenerate PCR	Set 1	GRB TDM GRA ARG GTK CWT GGA	GCW ATH ARD GAC CAY CTR TT
	Set 2	AAR WSI TGY MGI YTI MGI TGG AYN AAY TA	CCA RTA RTT YTT IAM YTC RTT RTC
Probe	MYBP3	ATG CAA ATC TGG GTA ACA GGT GGT	CGT CTC TTT CGC TAA TTC TTC CCA
	MYBP4	TTC ATC CAA GCG GAC CAA GC	CCA TCC CCT CAT TAT TTA GC
Inverse PCR	FNSII pro	CGT TGT ATT GTA GTC TCC ACC CCT GAA CTA GC	GGA GGA TTG AGA GGA CGA AAA TGG AAG CAG AG
	F3'H pro	TCC TTA TAC GAG CAA TAG CGA GTG GCG GTC AT	TGA GGA AGA TGA AGA TGA AGA CGA CGA AAA GG

Table S2. Primers used for quantitative RT-PCR analyses

	Primer sequence (5' - 3')	
	Forward	Reverse
Arabidopsis		
<i>AtCHS</i>	CTAAGGATCTCGCCGAGAACA	CGGCTGTGATCTCAGAGCAG
<i>AtCHI</i>	GTCACCGGCCTCCTCCA	TGGATATCAAGGCCTCGGAC
<i>AtF3H</i>	TGAAGGAGCGTTTGTCGTCA	TGAACCTCCCATGCTCAAAA
<i>AtF3'H</i>	CCTCCACCTCCGACTAGGGT	TGCTCGGCCACGGATTA
<i>AtFLS</i>	CAACATTCCGAGGTCCAACG	TCTTCGTCTGGGATCGCTTAG
<i>AtDFR</i>	CGAGATGACGGCAGCTTTG	AGCGGCGACATGGAAGAC
<i>AtMYB11</i>	TCGCCAATAACCGTCGAGAAT	CGGATCTGCTGGTTCTTCCA
<i>AtMYB12</i>	CGTAAAACGAAGAAAACGTCTGC	GCTTCTTTATCAGCCCCAGCT
<i>AtMYB111</i>	CAATGTTTCTCACAACCTAAGGAGC	CCAAAGACTCTCCTTCAAAAATTACCA
<i>AtACT2</i>	ACCCGATGGGCAAGTCATC	CGAGGGCTGGAACAAGACTTC
Tobacco		
<i>NtPAL</i>	TGCTTAACCACAATGTCACTCCA	CGAGATCACCAGAGGCGGT
<i>NtCHS</i>	GCCGGTGGCACGGTACT	ACTCGAGCGCCCTTGTTGT
<i>NtCHI</i>	TGAAGCAGTGCTGGAATCCA	TTTCGGCGATACTACACTTTGC
<i>NtF3H</i>	TTTTTACCCAAAGTGTCACAGC	GGGTGATGGTTCCTGGATCA
<i>NtF3'H</i>	TGGATTAACCCATTCATTTGGAT	TTCCAAAAGGCTCAACACTTCTC
<i>NtDFR</i>	TGAGTTTAAAGGCATCGATAAGGA	GAATTGAAACCCCATATCCGTC
<i>NtANS</i>	TCCTCCACAATATGGTGCCTG	GGGTGTCCCAATATGCATG
<i>NtFLS</i>	GGCCTAAAAATCCTCCCTCCT	TTCTCCACAACCTTCTCGCAGC
<i>NtUBQ</i>	AAGATTCAGGACAAGGAAGGCA	AGCTGCTTACCTGCGAAAATCA