

APPENDIX I: RNA CONCENTRATIONS

The concentrations of the isolated total RNA samples according to the plant sample numbers used in the laboratory are given in table I.B. An overview of the names (numbers) of the different samples is given in table I.A.

Table I.A: **The sample numbers used in the laboratory.** The table gives an overview of the sample names (numbers) used to distinguish the different plants (genotypes and treatments) in the RNA isolations, concentration measurements, cDNA synthesis and RT-PCR expression studies.

| Pop/genotype | Experiment 1 (F7) | | Experiment 2 (F10) | | |
|-----------------------|------------------------|----------|--------------------------|--------------------|----------|
| | Non-vern 14.January | 12w vern | Non-vern 16.-18.March | Non-vern 29.May | 12w vern |
| Pop 1 (VR min) | | | | | |
| 3 | 140 | 61 | 183 | 256 | 228 |
| 4 | 141 | 62 | 184 | 257 | 245 |
| 8 | 145 | 66 | 185 | 258 | 230 |
| 12 | 148 | 70 | 187 | 260 | 232 |
| 14 | 150 | 72 | 188 | 261 | 233 |
| Pop 2 (VR max) | | | | | |
| 5 | 162 | 79 | 194 | 262 | 239 |
| 6 | 163 | 80 | 195 | 263 | 240 |
| 8 | 165 | 82 | 197 | 264 | 242 |
| 11 | 168 | 85 | 199 | 266 | 229 |
| 18 | 174 | 91 | 202 | 268 | 250 |
| Grandparents | | | | | |
| BF14/16 | 182 | 137 | 224 | 270 | 254 |
| HF2/7 | 179 | Deceased | 225 | 273 | 255 |

Table I.B: **RNA concentrations.** The table shows the RNA concentrations of the different samples according to their number, given in table I.A.

| Sample | Conc. µg/µl | Sample | Conc. µg/µl | Sample | Conc. µg/µl | Sample | Conc. µg/µl | Sample | Conc. µg/µl |
|------------|----------------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|
| 140 | 2,225 | 61 | 8,62 | 183 | 3,66 | 256 | 3,645 | 228 | 6,0475 |
| 141 | 4,51 | 62 | 7,7 | 184 | 6,38 | 257 | 4,845 | 245 | 2,965 |
| 145 | 6,525 | 66 | 4,095 | 185 | 3,945 | 258 | 3,49 | 230 | 10,84 |
| 148 | 4,545 | 70 | 3,665 | 187 | 6,255 | 260 | 3,165 | 232 | 2,465 |
| 150 | 3,41 | 72 | 8,215 | 188 | 3,61 | 261 | 1,57 | 233 | 3,655 |
| | | | | | | | | | |
| 162 | 3,62 | 79 | 8,87 | 194 | 6,7025 | 262 | 2,58 | 239 | 4,355 |
| 163 | 4,405 | 80 | 7,58 | 195 | 6,64 | 263 | 6,48 | 240 | 9 |
| 165 | 3,705 | 82 | 7,59 | 197 | 7,075 | 264 | 2,23 | 242 | 3,32 |
| 168 | 4,095 | 85 | 8,955 | 199 | 5,15 | 266 | 5,6 | 229 | 5,14 |
| 174 | 4,26 | 91 | 5,04 | 202 | 3,83 | 268 | 2,905 | 250 | 6,535 |
| | | | | | | | | | |
| 182 | 4,34 | 137 | 8,67 | 224 | 6,055 | 270 | 1,685 | 254 | 7,915 |
| 179 | 1,805 | | | 225 | 7,685 | 273 | 4,87 | 255 | 0,37 |

APPENDIX II: AGAROSE GEL PHOTOGRAPHS

In this appendix some of the photographs of the agarose gels not shown in the text, but thought to be of some interest are shown.

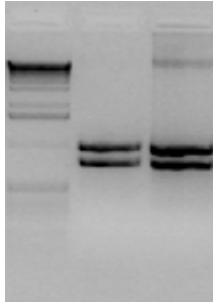


Figure II.A: ***GAPDH* amplified from genomic DNA.** The figure shows the agarose gel of the 1000bp and the 750bp *GAPDH* fragments amplified from genomic DNA from *F. pratensis* BF14/16 (lane 2) and HF2/7 (lane 3) in 40 cycles using an annealing temperature of 60°C. Lane 1 contains the 1kb DNA ladder.

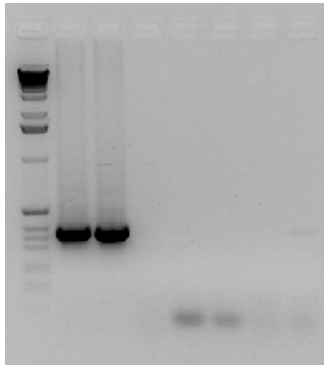


Figure II.B: **PCR products using the -RT control samples as template.** Lane 1 contains the 1kb DNA ladder. The *GAPDH* primers were used in all the PCR reactions and the templates were as follows: In lanes 2 and 3 a positive control using the cDNA bulk samples 1 and 2 respectively are shown. Lanes 5 – 8 contains the PCR product of the five -RT cDNA controls synthesised from bulks of the five batches of individual cDNA syntheses (given in table 2.1).

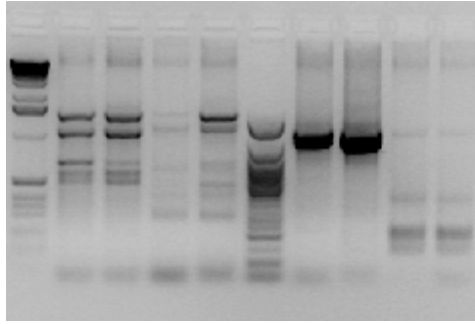


Figure II.C: Attempted amplification of *VRN1* promoter and intron1. The figure shows the agarose gel from the amplification attempts of parts of the *F. pratensis* promoter region and intron 1. Amplifications using primers for the last 1kb of the *VRN1* promoter and 55°C annealing temperature, are shown in lanes 2 – 5. The template used is BF14/16 in lanes 2 and 4, and HF2/7 in lanes 3 and 5. Lanes 2 and 3 shows the fragments amplified from LpVRN1prom_F1 and LpVRN1_344rev (shown in materials) and lanes 4 and 5 shows the fragments amplified from LpVRN1prom_F2 and FpVRN1prom_R1. The first 1kb of the *VRN1* intron 1 using 48°C annealing is attempted amplified in lanes 7 – 10. The fragments shown are amplified from BF14/16 and HF2/7 using the primers FpVRN1exon1F1/ LpVRN1exon1R1 and FpVRN1exon1F1/ LpVRN1exon1R2 (see materials) respectively. Lanes 1 and 6 show the DNA ladders, 1kb and 50bp respectively.

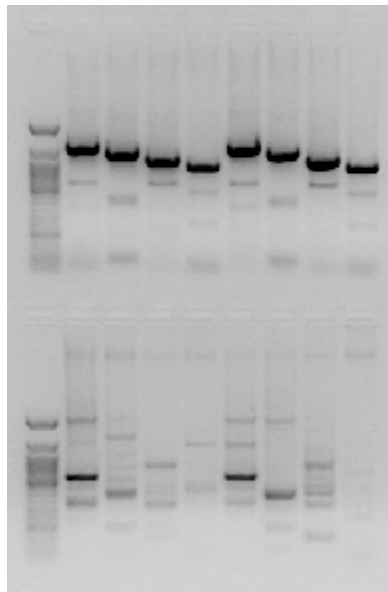


Figure II.D: FpMADS16 primer test. The figure shows amplification of *FpMADS16* using four different primer combinations consisting of two forward and two reverse primers. Amplification from F2 cDNA is shown at the top and the bottom represents the amplification attempt using P genomic DNA as template. The first lane in the top and the bottom gels shows the 50bp ladder. The next four lanes show amplification from population 1 (top) or BF14/16 (under), and the last four lanes show amplification from population 2 (top) or HF2/7 (under). For the expression study the primers amplifying the largest fragment (in lanes 2 and 6, top) were used (sequences shown in table 2.3).

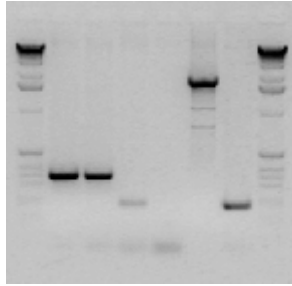


Figure II.E: **Amplification attempts of parts of *FpMADS16* including introns 2 and 3 using genomic DNA as templates.** The figure shows the results of the amplification of parts of *FpMADS16* intended to amplify intron 2 (lanes 4 and 5), intron 3 (lanes 6 and 7) and both intron 2 and 3 (lanes 2 and 3) in BF14/16 and HF2/7 respectively. In lanes 1 and 8 the 1kb DNA ladder is shown.

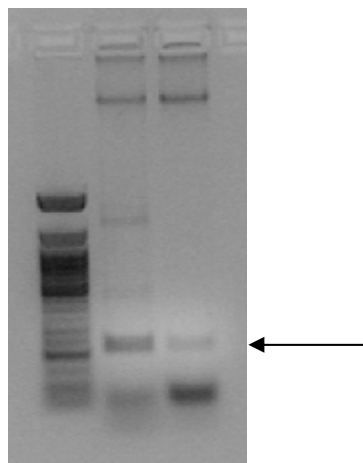


Figure II.F: **CONSTANS amplified from genomic DNA.** The figure shows the amplification of *CONSTANS* from *F. pratensis* genomic DNA of the parents BF14/16 (lane 2) and HF2/7 (lane 3) using the primers and PCR program used by Andersen et al. (2006) shown in table 2.3. The arrow identifies the *FpCO* fragments and the 50bp DNA ladder is given in lane 1.

APPENDIX III: SEQUENCES

The sequences of the genes sequenced in this experiment are shown in this appendix. If the primers (or parts of them) are included in the obtained sequences, they are underlined. The sequence lengths are given, but without both primers included, the exact length cannot be decided. Introns present are marked grey.

>*GAPDH*.genomicDNA

CAGGACTGGAGAGGTGGAAGGGCTGCTAGCTTCAACATCATTCCCAGCAGCACT
GGAGCTGCCAAGGTTAGTATAATTCAGCAGCACCGAAGTGTGTACAGTGTAATA
TGGCATCTGTGTCTATCTGACAGAATTGTATGCTTATGCATTGTGGTTCTCACTGA
TGTGTTATCCTTTATATTGATCTTGTTCTGACTTGTTAATCTTTCAACAGGCTGTTG
GCAAGGTGCTCCCAGTCCTTAACGGAAAGTTGACAGGAATGGCCTCCCGTGTCCC
AACTGTTGACGTTTCTGTTGTTGATCTGACCGTTAGACTTGAGAAGGCTGCCACCT
ATGACCAGATCAAGGCTGCGATCAAGTAAGTAATGCTACCATATAGGTGGTTTGT
ATATGGTTCTGTATATTGTTGCAGGCTTTAGTGTGTTTGCATAACATAGATGGTTT
GCTTTGGATGATTACTGATCTGCAACATCCTTTCAGGGAGGAGTCTGAGGGTAAG
CTCAAGGGCATTTTGGGTTACGTCGATGAGGACCTTGTTTCCACCGACTTCCAGG
GTGACAGCAGGTATTTGTCGTTGCTGCTTTCACTGTGCAACATAATGATTATCTGA
ATCATCTTAGTTAGCACATAACAGACCTGCTTACAAGGCATCTGAATTCACCATTA
ATGTTCTCCACTTTACCAATCACAGGTCCAGCATCTTCGATGCCAAGGCTGGGATT
GCTCTGAACGACAACCTTCNTCAAGCTTGTGTCCTGGTACGACAACGAGTGAA
-773bp

>*GAPDH*.cDNA

CAGGACTGGAGAGGTGGAAGGGCTGCTAGCTTCAACATCATTCCCAGCAGCACT
GGAGCTGCTGTTGGCAAGGTGCTCCCAGTCCTTAACGGAAAGTTGACAGGAATGG
CCTCCGTGTCCCAACTGTTGACGTTTCTGTTGTTGATCTGACCGTTAGACTTGAG
AAGGCTGCCACCTATGACCAGATCAAGGCTGCCATCAAGGAGGAGTCTGAGGGT
AAGCTCAAGGGCATTTTGGGTTACGTCGATGAGGACCTTGTTTCCACCGACTTCC
AGGGTGACAGCAGGTCCAGCATCTTCGATGCCAAGGCTGGGATTGCTCTGAACGA
CAACTTCGTCAAGCTTGTGTCCTGGTACGACAACGAGTGAA -370bp

>*VRN1*.Intron1.consensus

AGGAAGCTCTCGAGTTCGCAACTGACTCATGGGTTAGTACCCTACATCTACATCG
CCTGCTCATTAATTTCTCTCCGATCCACCTTCGATTTCAATCCGCTCTTAGTCCGCC
GACCGCTGCTCGGACCTCCATTTCTGCGTTGTTTCGATTCATTGAGTTTGCCGCTCC
GTGTTCTTTCTGTTTCGAATTCTGCCTAGGCTGCTCGTTTCGGTTGACTAGAATC
GATGGTGGAGTAAGCTGCCCCCGCGGCTGCGATTCTGCCTTGGACCGTACGAC
GAGAGATGGGCTGCTGCGCTATTTTATGCTCCGCTATCGCCGGGGACCTTGTTTCAT

CCGCGGAAAGAAATCCTATACGGCCTGGGTCGCACCTCCCGATGGCTGGACCCAG
 TCCTACATTTGACATCTGGCAAAGCGAATCTCAAAGTAGTATGGTCAGAACTTCT
 ACCCCCGAGCCCTTCTCTGGCGGCCCGAGCCAGCCTGCTGCTCGGCGGCAGCGC
 AACTCACTGCTTTGCTGACCACCGGCGTCCAGCCACGACAAATCATCCGCTTCAT
 TTTGGGGAATTTTCCGTTCCAGACCTGCCAGCTGGCAACCAAAGTGGAAGGGGT
 GCCATAGTAAATATCTNGCTACTTTACACGCCCTTCTTTTTCTAGCTGCGGATATT
 TATTTGCGGAATTTACCTCACTCCAGTAGCAGTAGCCTGATAGTATTTGTGTACCA
 AGCTTACCCTCGTCATCTCAGTTGGCATCTTATTTGATGGCACGTCGTTGCCTGTC
 TGATCAATTACTGTGAGCTTAATTTGCTGCTTAGAACCTCATCAACTCCATTCTCT
 CAAAGCTGGCGCCCAATTTGTTGCTTCATCTCATCGGATTAGTTGTCTGGAGCTGC
 CGAAGCTCCGTAATGCATCGTTTGCCAGGAGCTGATGAAACTCGTCCGCCAGGTC
 AAATTAATTGTTTCATATGTTTTCTGCACCCCGTTCCCTTGCTATCTGCTTTCCCAG
 TTTGTAGTGAGACAGAAATTACCTACTGATTACCTGAACCTGCTACAAAATAAAT
 AGAGCTAC -1065bp

CLUSTAL W (1.83) multiple sequence alignment VRN1 intron 1 HF2/7 and BF14/16

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HF2/7          -----GTTTCGAGTGC GCA-CTGACTCATGGTGTAGTACCCTACATNNACATCGCCTGTC 53
BF14/16       NGNAAGTCTCGAGTTCGCAACTGACTCATGGT-TAGTACCCTACATCTACATCGCCTG-C 58
                *****  ****  *****
HF2/7          TCATTNATTNNTCTCCGATCCACCTTCGGTTTCATTCCGCTCTTANGTCCGCCGACCGAC 113
BF14/16       TCATTAATTTCTCTCCGATCCACCTTCGATTTTCATTCCGCTCTTAG-TCCGCCGACCG-C 116
                *****  ***  *****
HF2/7          TGNTCCGACCTCCATTTCTGCGTTGTTTCGATTCATTGAGTTTGCCGCTCCGTGTTCTTTTC 173
BF14/16       TGCTCCGACCTCCATTTCTGCGTTGTTTCGATTCATTGAGTTTGCCGCTCCGTGTTCTTTTC 176
                **  *****
HF2/7          CTGTTTTCGAATTTCTGCCTCGGCTGCTCGTTTTCGGTTGACTANAATCGATGGTGGAGTAAG 233
BF14/16       CTGTTTTCGAATTTCTGCCTTAGGCTGCTCGTTTTCGGTTGACTAGAATCGATGGTGGAGTAAG 236
                *****  *****
HF2/7          CTGCCCCCGCGGCTGCNATTCCTGCCTTGACCGTACGACGAGAGATGGGCTGCTGCGC 293
BF14/16       CTGCCCCCGCGGCTGCNATTCCTGCCTTGACCGTACGACGAGAGATGGGCTGCTGCGC 296
                *****  *****
HF2/7          TATTTTATGNTCCGCTATCGCCGGGGACCTTGTTCATCCGCGGAAAGAAATCCTATACGG 353
BF14/16       TATTTTATGCTCCGCTATCGCCGGGGACCTTGTTCATCCGCGGAAAGAAATCCTATACGG 356
                *****  *****
HF2/7          CCTGGGTGCGCACCTCCCGATGGCTAGGACCCAGTCCTACATTTGACATCTGGNAAAGCGA 413
BF14/16       CCTGGGTGCGCACCTCCCGATGGCT-GGACCCAGTCCTACATTTGACATCTGGCAAAGCGA 415
                *****  *****
HF2/7          ATCTCAAAGTAGTATGGTCAGAACTTCTACCCCGAGCCCTTCTCTGGNGGNCCGAGCCC 473
BF14/16       ATCTCAAAGTAGTATGGTCAGAACTTCTACCCCGAGCCCTTCTCTGGCGGCCGAGCCC 475
                *****  *****
HF2/7          AGCCTGCTGCTCGGNGGCAGCNCAACTCACTGCTTTGCTGACCACCGGCGTCCAGCCACG 533
BF14/16       AGCCTGCTGCTCGGCGGCAGCGCAACTCACTGCTTTGCTGACCACCGGCGTCCAGCCACG 535
                *****  *****
HF2/7          ACAAATCATCCGCTTCATTTTGGGGAATTTTTCGCTCCAGACCTGCCAGCTGGCAACC 593
BF14/16       ACAAATCATCCGCTTCATTTTGGGGAATTTTTCGCTCCAGACCTGCCAGCTGGCAACC 594
                *****  *****

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HF2/7 AAAGTGGAAGGGGTGCCATAGTAAATATCNGCTACTTTACACGCCCTTCTTTTCTAGC 653
 BF14/16 AAAGTGGAAGNGGGGCCATAGTNAANATCTNGNNACTTTACACGNCCNTCTTTTCTAGN 654
 ***** ** ***** ** ** * ***** ** *****

HF2/7 TCGGATATTTATTTTCGNGAATTTACCTC--ACTCCAGTAGCAGTAGCCTGATAGTATTT 711
 BF14/16 NGCGGANATNTATTTNGCGAATNTACCTCTCACTCCAGTAGCAGTAGCCTGATAGTATTT 714
 ***** ** ***** * ***** ***** *****

HF2/7 GTGTACCAAGCTTACCCTCGTCATCTCAGTTGGCATCTTATTTGATGGCACGTCGTTGCC 771
 BF14/16 GTGTACCAAGCTTACCCTCGTCATCTCAGTTGGCATCTTATTTGATGGCACGTCGTTGCC 774

HF2/7 TGTCTGATCAATTACTGTGAGCTTAATTTGCTGCTTAGAACCTCATCAACTCCATTCTCT 831
 BF14/16 TGTCTGATCAATTACTGTGAGCTTAATTTGCTGCTTAGAACCTCATCAACTCCATTCTCT 834

HF2/7 CAAAGCTGGCGCCCAATTTGTGTGCTTCATCTCATCGGATTAGTTGTCTGGAGCTGCCGAA 891
 BF14/16 CAAAGCTGGCGCCCAATTTGTGTGCTTCATCTCATCGGATTAGTTGTCTGGAGCTGCCGAA 894

HF2/7 GCTCCGTAATGCATCGTTTGGCAGGAGCTGATGAAACTCGTCCGCCAGGTCAAATTAATT 951
 BF14/16 GCTCCGTAATGCATCGTTTGGCAGGAGCTGATGAAACTCGTCCGCCAGGTCAAATTAATT 954

HF2/7 GTT-CATATGTTTTCTGCACCCCGTTCCCTTGCTATACTGCTTTCCCAGTTTGTNGTG 1010
 BF14/16 GTTGCATATGTTTTCTGCACCCCGTTCCCTTGCTAT-CTGCTTTCCCAGTTTGTNGTG 1013
 *** ***** **

HF2/7 AGACAGAAATTACCTACCTGATTACCTGAACCTGCTACAAAATAAATAGAGCTAC--- 1065
 BF14/16 AGACAGAAATTACCTAC-TGATTACCTNA-CCTGCTACAAA-AAATAGAGCGACNNNC 1069
 ***** ***** * ***** ***** **

>*FpMADS16.cDNA*

GTGCGCGTTGAGGTTTCCCGGCGGGCCGGTCAAGGGCGGAGATGGCGCGGGAGAGG
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 CGGAGGCGCGGGCTCTTCAAGAAGGCCGAGGAGCTCGGCGTGCTCTGCGACGCC
 GACGTCGCGCTCGTCGTCTTCTCCTCCACCGGCAAGCTCTCCCAGTTCGCAAGCTC
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 TTTGAATGAACAACCTCGCTGAAGCAAGTCTTCACCTTAGACACATGAGAGGTGAG
 GAACTTGGGGGACTGAGTGTGGGAACTGCAGCAGATGGAAAAGGATCTTGAA
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 AGTGACCTCCAACAAAAGGGCACACAGCTGGCAGAAGAGAATATGCGCTTGAGA
 AACCAATGCCTCAGGTGCCAACGGCCGGCATGATGGCTGTCCTGAAGATGTTT
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 CGACGGTTCTGATATATCCCTGAAACTAGCGTTGCCTTGGAAGTAAGGATCATGA
 GGAGACCACCATGATGAGACGACGGAGTTGCCCTTGGTAGAGAGGATTGCTCAAT
 GCTGTGGAGACTCCAAGCTAGACTGACTGGAATGGTCCCATATTAGAAACCAGAT
 CAGTTTACCCGAATTGTCATCGTTAAGTCTGCATGCGTATGGTGCAGTTGTTACC
 TTTGCTTGGGTCTTACCCAACCTTGTAGCGTAGTTGTGTGTACGTAAGTCTTCTT
 CACCTGCGTCACGTATCATTCTGCGAAGTACGT -963bp

>*FpMADS16.F5R5.cDNA*

ATGAACAACCTCGCTGAAGCAAGTCTTCACCTTAGACACATGAGAGGTGAGGAAC
TTGGGGGACTGAGTGTTGGGGAAGTGCAGCAGATGGAAAAGGATCTTGAAACAG
GACTACAGAGTGTGCTTTGTACAAAGGACCAACAATTCATGCAACAGATCAGTGA
CC

>*FpMADS16.F5R5.genomic*

ATGAACAACCTCGCTGAAGCAAGTCTTCACCTTAGGTTATATTTTGTTCATTCTGG
CTTTGTGCGATTAAGTAAAAGTCTTACTACAAAGTGGTAATATTGTTAACCTTC
AACGTCATGTTGTACATTTATAGACACATGAGAGGTGAGGAACTTGGGGGACTGA
GTGTTGGGGAAGTGCAGCAGATGGAAAAGGATCTTGAAACAGGACTACAGAGTG
TGCTTTGTACAAAGGTAGGGTATTCTGAATTTAATTCTTCTTAACCTTGGTTTACA
TTGCATGCACAAATGTCAATTGTTTCACACGACATGTTTCTGCAGGACCAACAAT
TCATGCAACAGATCAGTGACC

>*PHYC*

GAGGCGTCCGGTTTCTGTTTATGAAGAACAAGTGC GGATGATATGNCCNTTGTG
CTGCTANNCTGTGAAGCTCATT CAGGATGACAACCTACCACAGCCTATCAGCCT
CTGTGGCTCCAGCATGAGGGCACCCATGGCTGCCATGCCAGTACATGGCCAAC
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AGGATGGAGACACCGGAAATGACCAGCAGCAGCCCAAAGGCAGGAAGCTGTGG
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ATGCTTGCGAATTCCTCTTGCAAGTATTCGCCATACAGCTCAACAAGGAGGTGGA
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CTGCGAAAGAGATCAAGTGGGGTGGAGCTAAGCATGAACCAGGTGATGCAGATG
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CAGCTAATTTTTCGTGGCTCCCTGCAAGATGAAGATGCCAACGACAACAATGTAA
GGACCATCGTTGAAGCTCCATCTGATGATATGAAGAAGATACAGGGGCTACTTGA
ACTGAGAGTTGTGACAAATGAGATGGTCCGCCTAATCGAGACAGCAACTGCTCCT
ATCTTGGCTGTTCGACATTGCTGGTAACATAAATGGATGGAATAATAAAGTCGCGG
AGATTACTGGATTACCCACCATGGAAGCCATAGGGATGGCTCTGGTAGATGTTGT
TGAGGGTGATTTTGTGGAAGTGGTTAAACAGATCTTGA ACTCAGCTACTACAAGG
TTGTCGCTATGTTCTTGAATTTCTTTTTTATAANAANCCAGATTAGTTATAACTT
GTCCATTATTTTGTACA -1171bp

>*RUBQ2*

GCTCCAACACAAAGACACTTACTGACCACCACGGAGACGGAGCACCAGGTGGAG
GGTAGACTCCTTATGGATGTTGTAGTCNNCAAGGGTGCGGNCATCCTCCAGCTGC
TTGCCAGCAAAGATGAGGCGCTGCTGGTCCGGGGGAATGCCCTCCTTGTCTGGA
TCTTAGCCTTGACGTTGTCAATGGTGT CAGAGGACTCCACCTCAAGGGTGATGGT
CTTGCCGGTCAAGGTCTTGACGAATATCTGCATACCACCACGGAGNCGGAGCACC
AGGTGGAGGGTAGACTCCTT -294bp

>*CONSTANS*

CGCGAAAATATCAGAAGCGGAAATGGAAGTGGACCAGATGTTCTCAGCTGCAGC
TCTGTCTGACAGTAGCTACAGTACTGTTCCATGGTTTTAATGAGACTCTATGAGAC
ATTACATTAGCATATATATGTACTTACCAGAACAATAAGGTCCAGTGCAAGCAGT
TTAGGTAGATCGGTGCTCTGAATAATTGTGTGGTATGCGAACCTTAATTGATAAG
GTA -223bp