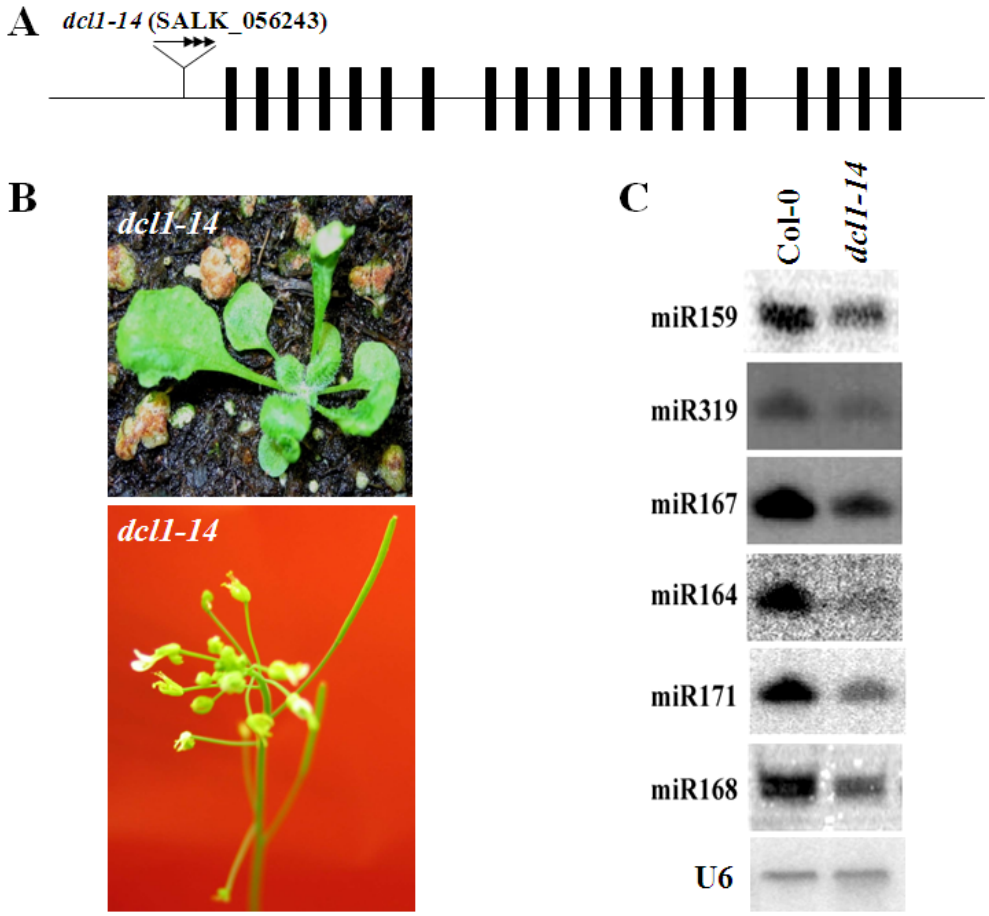
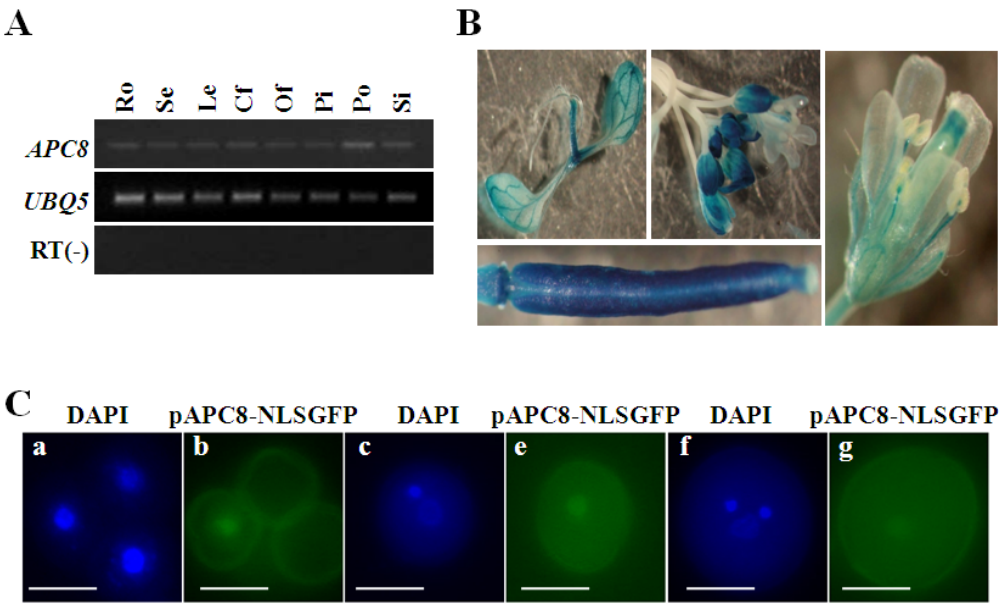



**Supplemental Figure 1.** Isolation and Characterization of *dcl1-14*.

**(A)** *DCL1* gene structure and the T-DNA insertion site of *dcl1-14*.

**(B)** 4-week old *dcl1-14* (top) and 8-week old *dcl1-14* (bottom) showed abnormal leaf development and reproductive development, respectively.

**(C)** miRNA levels in Col-0 and *dcl1-14*. Total RNAs were extracted from inflorescences. U6 was the loading control.

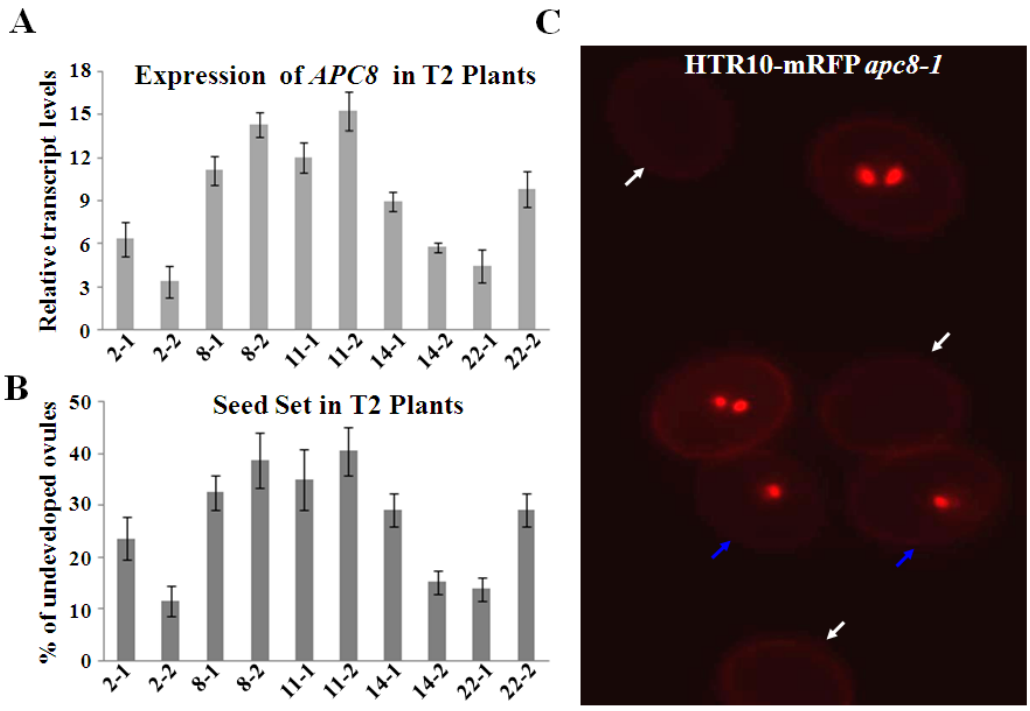



**Supplemental Figure 2.** Expression Analysis of *APC8*.

**(A)** Expression of *APC8* was determined by RT-PCR. Ro, roots; Se, seedlings; Le, leaves; Cf, closed flower buds; Of, open flowers; Pi, unpollinated pistils; Po, mature pollen; Si, siliques. The RT (−) control PCR was performed with *UBQ5* primers.

**(B)** Promoter reporter analysis (*pAPC8-GUS)* in different tissues.

**(C)** Promoter reporter analysis (*pAPC8-NLSGFP*) during male gametophyte development. Microspores or pollen at different developmental stages from at least 10 *qrt1-2* plants harboring the *pAPC8::NLS3X-GFP* construct were analyzed. **(a)**, **(c)**, **(e)** DAPI staining of unicellular microspores **(a)** bicellular pollen **(c)** and tricellular pollen **(e)**. **(b)**, **(d)**, **(f)** *pAPC8-NLSGFP* signals of unicellular microspores **(b)** bicellular pollen **(d)** and tricellular pollen **(f)**. Scale bar, 10 μm.

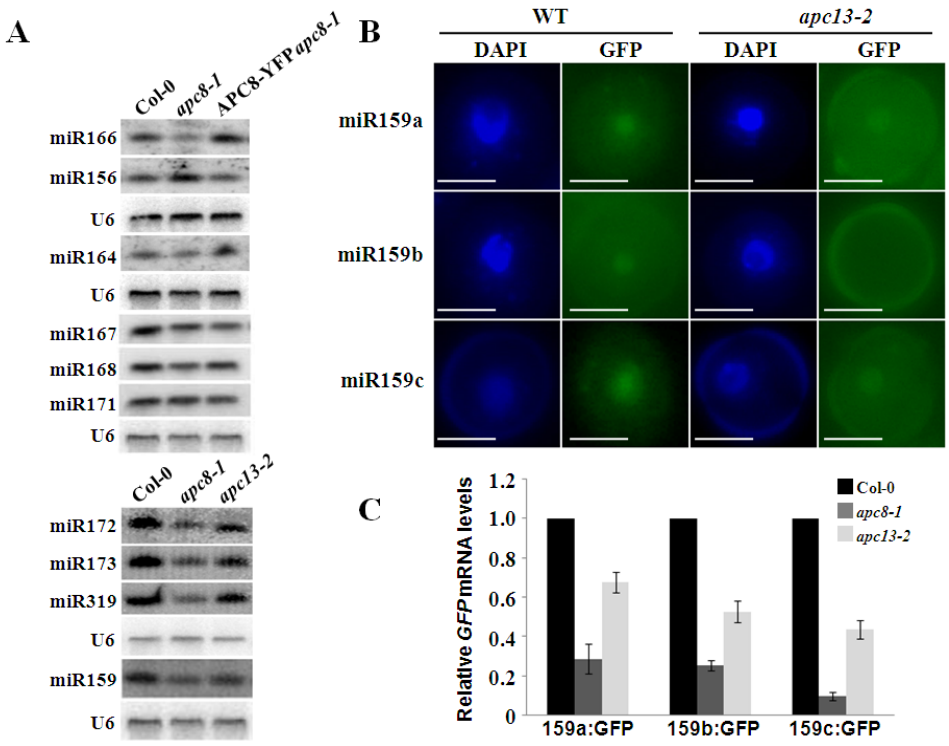



**Supplemental Figure 3.** Overexpression of APC8 in Male Germline Disrupts Seed Set Development and HTR10 Expression in *apc8*- Mature Pollen.

**(A)** Total RNA was extracted from 1-d-old open flowers of T2 progenies from five independent T1 lines (No. 2, 8, 11, 14, and 22) that ectopically expressed *APC8* driven by the HTR10 promoter, and was then used for qPCR analysis. The expression level of *APC8* in wild type was set to 1. Standard deviations were plotted from three replicates. The expression level was normalized to that of *UBQ5*.

**(B)** Seed set analysis of the plants shown in A. Standard deviations were calculated from seed counts of 10 siliques for each plant.

**(C)** Homozygous *pHTR10:HTR10-mRFP* transgenic plants were crossed into *apc8-1*, then F3 homozygotes of *pHTR10:HTR10-mRFP* in *apc8-1* were analyzed. No expression (white arrows) or single sperm-specific expression (blue arrows) was detected in *apc8-1*.

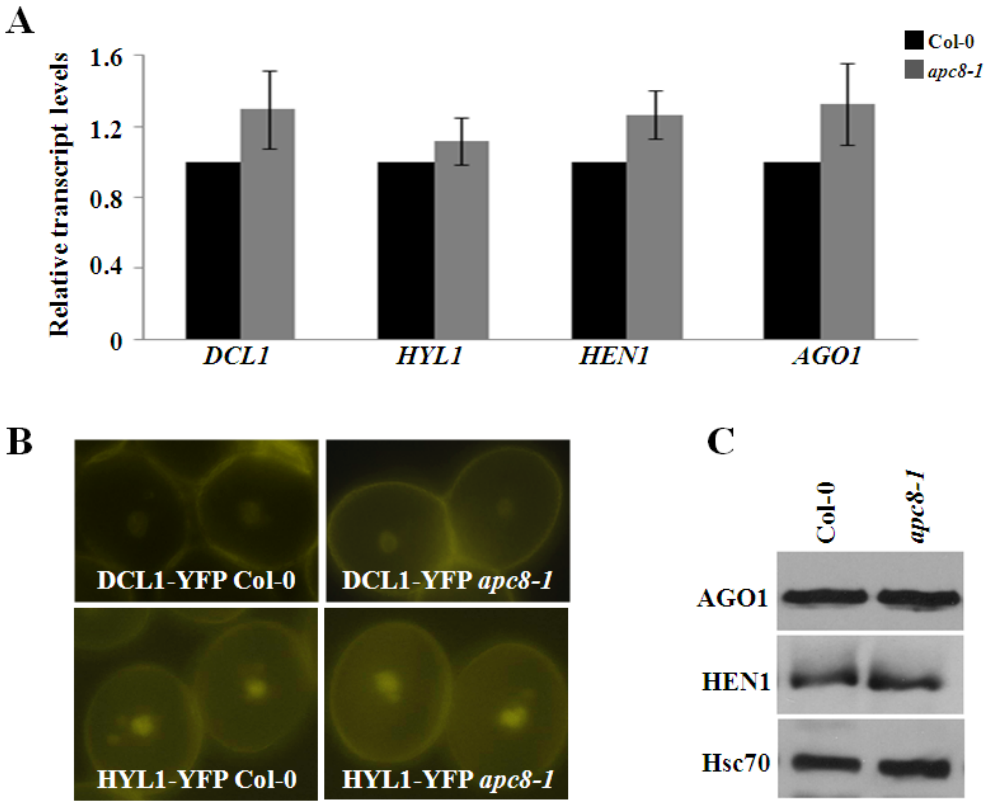



**Supplemental Figure 4.** Mature miRNA Levels and *MIR159* Transcription in *apc/c* Mutants.

**(A)** More miRNA accumulation in Col-0, *apc8-1*, *apc13-2*, and APC8 *apc8-1* (the *pAPC8:APC8-YFP* construct was introduced into *apc8-1*). Total RNAs were extracted from inflorescences. U6 was the loading control.

**(B)** *ProMIR159a, b, c-NLSGFP* reporter in uninucleate microspores from WT and *apc13-2* plants. Fifteen individual T1 plants in WT or the *apc13-2* background were examined. Scale bar, 10 μm.

**(C)** The accumulation of *GFP* mRNA from the pollen of a representative transgenic plant with single copy insertion for each genotype in Figure 5C and Supplemental Figure 4B was determined by qPCR. The *GFP* mRNA levels were normalized to those of *UBQ5* and compared to Col-0. Standard deviations were calculated from three technical replicates. 3 more independent transgenic plants with single copy insertion for each genotype yielded nearly identical results.

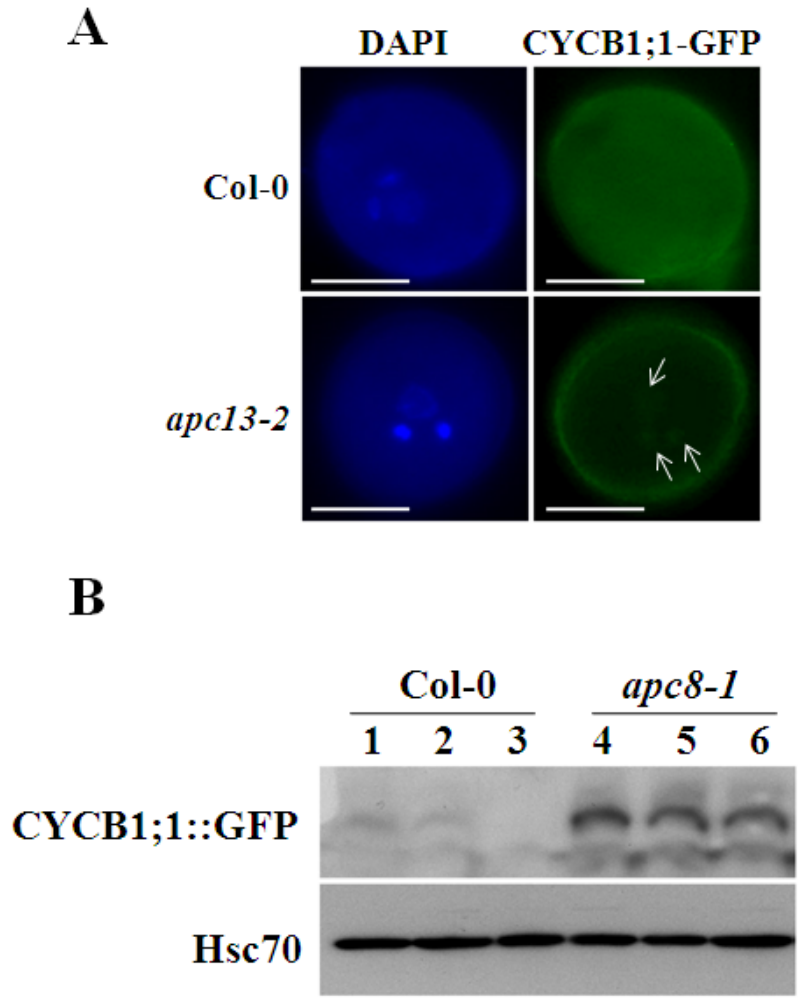



**Supplemental Figure 5.** Expression of Known miRNA Biogenesis Genes in *apc8-1*.

**(A)** Expression of known critical miRNA biogenesis genes in *apc8-1* was determined by qPCR.

**(B)** DCL1-YFP and HYL1-YFP in *apc8-1*. pDCL1:DCL1-YFP and pHYL1:HYL1-YFP transgenic plants were crossed into *apc8-1*, then F3 homozygotes harboring transgenes were analyzed. One example relative to each genotype was shown here.

**(C)** HEN1 and AGO1 accumulation in *apc8-1* by immunoblotting. Total protein was extracted from inflorescences, Hsc70 was the loading control. Two biological replicates were done for each.




**Supplemental Figure 6.** Increased *CYCB1;1* Expression in *apc8-1* and *apc13-2*.

**(A)** Accumulation of CYCB1;1-GFP in mature pollen from *apc13-2*. Arrows showed accumulated CYCB1;1-GFP in VN and SN. Scale bar, 10 μm.

**(B)** CYCB1;1-GFP levels in WT and *apc8-1* by immunoblotting. *pCYCB1;1:CYCB1;1-GFP* was introduced into WT (1, 2, and 3, Col-0) and *apc8-1* (4, 5, and 6), then total protein was extracted from inflorescences of individual T3 plants with single copy insertion. Hsc70 was the loading control. At least two biological replicates were done for each.

**Supplemental Table 1.** Primers Used in This Study.

| | | |
|---|---|---|
| APC8F2 | TTCGACCAGGTTGAAATCATG | Genotyping |
| APC8R2 | TTGTTCAGTTTCAAAGCTCTCC | Genotyping |
| APC8F1 | CACCCTGTCATACATAACCAACCAAC | Complementation |
| APC8R1 | AATAGGAAAATGCTCGAGATCC | Complementation |
| APC8R7 | AGCAGAAGCAGAAACTGAGAG | Promoter analysis |
| APC8F3 | CGCGGATCCGCGATGGTCTCTAAAGAGTGTTGCCG | Yeast complementation |
| APC8R3 | CGCGGATCCGCGCTAAATAGGAAAATGCTCGAGATCC | Yeast complementation |
| APC8F11 | CGGGTAGAAGATATGGAGCTCTATTCTAATGTTCTG | D to E |
| APC8R11 | CAGAACATTAGAATAGAGCTCCATATCTTCTACCCG | D to E |
| APC8F6 | GTTACCTTCCGTTGGCTTCTC | RT-PCR |
| APC8R6 | CTCAGGTCTGTACTTATCAG | RT-PCR |
| miR159aF1 | CACCCTCCGGAACTCTAATCGGAT | Promoter analysis |
| miR159aR1 | CTTCCATCGTCAGATCAAGATC | Promoter analysis |
| miR159bF1 | CACCCCCAGGTGGATCTTTTATATT | Promoter analysis |
| miR159bR1 | GAAGAGTGAAGCCATTAAAGG | Promoter analysis |
| miR159cF1 | CACCTAATTCCGGTTCGAGCAAATA | Promoter analysis |
| miR159cR1 | CGAAGAGAAGAGAATGATATG | Promoter analysis |
| miR159aF2 | GGCTTTTACTCTTCTTTGGATTGA | qPCR |
| miR159aR2 | CACGCTAAACATTGCTTCGGA | qPCR |
| miR159bF2 | ATGGCTTCACTCTTCTTTGGATTG | qPCR |

| | | |
|---|---|---|
| miR159bR2 | CCTACTCAAGATCCATCATCCAT | qPCR |
| miR159cF2 | AGCTCCTTTTCTTCTTCTCTTAAT | qPCR |
| miR159cR2 | CGTCTTCTCGTAAATAAACAACATT | qPCR |
| miR159aF3 | AGCTCTACTTCCATCGTCAGAT | ChIP-PCR |
| miR159aR3 | CTCTCTATCTATCATTTCTTCC | ChIP-PCR |
| miR159bF3 | GACTCCCTCTTTCTTTCTCATT | ChIP-PCR |
| miR159bR3 | CTTATATATGGTTCGAAACAGG | ChIP-PCR |
| miR159bF4 | GGTTCTTTCTGAGTCAAATTGT | ChIP-PCR |
| miR159bR4 | GAGCTCTTCCTCTTCTTCTTCC | ChIP-PCR |
| miR159cF3 | GACAATAAGATTTACTGCCAAA | ChIP-PCR |
| miR159cR3 | GTTACACTTGAAGAACGAATGT | ChIP-PCR |
| miR166aF1 | TGGCTCTCTCCACTACTCAA | ChIP-PCR |
| miR166aR1 | GACAACAGTCCCCTCAAAA | ChIP-PCR |
| miR167aF1 | CGACCCTTAAACTCTCCATAA | ChIP-PCR |
| miR167aR1 | ACTTCACCGTAGCAGATCAA | ChIP-PCR |
| miR171aF1 | TGCTTTGGTAGTAGATGAGGTT | ChIP-PCR |
| miR171aR1 | CGTGTGTGGTCAGGTAAGAT | ChIP-PCR |
| Actin2F1 | GAGAGATTCAGATGCCCAGAAGTC | ChIP-PCR |
| Actin2R1 | TGGATTCCAGCAGCTTCCA | ChIP-PCR |
| CYCB1;1F1 | CACCGGAAGCTTCATCTTATTCTTTTACC | Protein fusion |
| CYCB1;1R1 | AGCAGATTCAGTTCCGGTCAAC | Protein fusion |
| CYCB1;1F2 | CTTGACAGTTCCGACTCATT | qPCR |
| CYCB1;1R2 | AATAGCCAGTGTGATGCTTG | qPCR |
| miR156 | GTGCTCACTCTCTTCTGTCA | Probe |
| miR159 | TAGAGCTCCCTTCAATCCAAA | Probe |
| miR164 | TGCACGTGCCCTGCTTCTCCA | Probe |
| miR166 | GGGGAATGAAGCCTGGTCCGT | Probe |
| miR167 | TAGGTCATGTTGGCAGTTTCA | Probe |
| miR168 | ATTCGGTTGGTGCAGGGCGGG | Probe |
| miR171 | GATATTGGCGCGGCTCAATCA | Probe |

| | | |
|---|---|---|
| miR172 | CTCCGTAGTAGTTCTAAGTGT | Probe |
| miR173 | GTGATTTCTCTCTCGAAGCGAA | Probe |
| miR319 | GGGAGCCTCCCTTCAGTCCAA | Probe |
| U6 | AGGGGCCCATGCTAATCCTTCCTC | Probe |
| DUO1F | CTGACGAAGAGAGGACTGTG | RT-PCR, qPCR |
| DUO1R | AGATTTGGGATTGAAACTCG | RT-PCR, qPCR |
| DUO3F | CTCTGACGATGAGATGATGGA | RT-PCR, qPCR |
| DUO3R | CCAAACTGTGAGATGGTCGTT | RT-PCR, qPCR |
| UBQ5F | GGTGCTAAGAAGAGGAAGAAT | RT-PCR, qPCR |
| UBQ5R | CTCCTTCTTTCGGTAAACGT | RT-PCR, qPCR |
| GFP_F | GCAGGAGAGAACCATCTTC | qPCR |
| GFP_R | CGTTGTGGGAGTTGTAGTTGT | qPCR |
| DCL1F | ATTGGGAAAATAGTGACAGC | qPCR |
| DCL1R | TCAAAGGTTGTTGCTTACAG | qPCR |
| HYL1F | CAGTAAAGAGGAGGAAGTCG | qPCR |
| HYL1R | ACACCAGAGGAAACATCAGT | qPCR |
| HEN1F | GTTCCATCCTTGAGTTTGAC | qPCR |
| HEN1R | GTTTTCTTCTTGGGTTTCTG | qPCR |
| AGO1F | TGGACCACCGCAGAGACAAT | qPCR |
| AGO1R | CATCATACGCTGGAAGACGACT | qPCR |