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Supplemental Material to:

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Optimizing stem-loop qPCR assays through multiplexed cDNA synthesis of U6 and miRNAs

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A

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CLUSTAL 2.1 multiple sequence alignment


Oryza_sativa_cv_japonica      GTCTCTTCGGAGACATCCGATAAAATTGGAACGATACAGAGAAGATTAGC 50
Zea_mays                      GTCTCTTCGGAGACATCCGATAAAATTGGAACGATACAGAGAAGATTAGC 50
Triticum_aestivum             GCCCCTTCGGGGACATCCGATAAAATTGGAACGATACAGAGAAGATTAGC 50
Broad_bean                    ----CTTCGGGGACATCCGATAAAATTGGAACGACACAGAGAAGATTAGC 46
Medicago_truncatula           GTCCCTTTGGGGACATCTGATAAAATTGGAACGATACAGAGAAGATTAGC 50
Arabidopsis_thaliana          GTCCCTTCGGGGACATCTGATAAAATTGGAACGATACAGAGAAGATTAGC 50
                                  *** ** ****** ********************** **********


Oryza_sativa_cv_japonica      ATGGCCCCTGCGCAAGGATGACACGCACAAATCGAGAAATGGTCCAAATT 100
Zea_mays                      ATGGCCCCTGCGCAAGGATGACACGCACAAATCGAGAAATGGTCCAAATT 100
Triticum_aestivum             ATGGCCCCTGCGCAAGGATGACACGCACAAATCGAGAAATGGTCCAAATT 100
Broad_bean                    ATGGCCCCTGCGCAAGGATGACACGCACAAATCGAGAAATGGTCCAAATT 96
Medicago_truncatula           ATGGCCCCTGCGCAAGGATGACACGCACAAATCGAGAAATGGTCCAATTT 100
Arabidopsis_thaliana          ATGGCCCCTGCGCAAGGATGACACGCATAAATCGAGAAATGGTCCAAATT 100
                              ************************** ****************** **


Oryza_sativa_cv_japonica      TTT-------- 103
Zea_mays                      TTTTT------ 105
Triticum_aestivum             TTTTTGAGATT 111
Broad_bean                    TT--------- 98
Medicago_truncatula           TTT-------- 103
Arabidopsis_thaliana          TTT-------- 103
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B

Primer Sequences:

U6 cDNA synthesis primer:	5'-GTGCAGGGTCCGAGGT**TTTGGACCATTTCTCGAT-3'**
U6 qPCR forward primer:	5'-**GGAACGATACAGAGAAGATTAGCA**-3'
Universal qPCR reverse primer:	5'-GTGCAGGGTCCGAGGT-3'

Figure S1. U6 primer design. **A.** Sequence alignment of U6 sequences from multiple plant species showing a conserved region from which qPCR primers were designed. U6 sequences from multiple plant species were obtained from non-coding RNA database (http://www.ncrna.org/frnadb/), NCBI and ncRNAdb. Sequences used to design "Forward" and "Reverse" primers are underlined. **B.** Novel U6 primer sequences designed in this study for multiplexed cDNA synthesis and qPCR. The universal qPCR primer is from Varkonyi-Gasic et al (2007).