**Supplementary to**

**Expression of mouse small interfering RNAs in lettuce using artificial microRNA technology**

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**Supplementary Figure S1**



**Supplementary Figure S1**. PCR scheme to produce artificial microRNA constructs. (A) The original rice micro RNA 528 sequences (green) were replaced by C3 and CF7 artificial microRNA sequences (red) during the first PCR using three pairs of primers. Primer sequences are shown in Supplementary Table S1. Yellow sequences are complementary to primers and multiple cloning sites are shown in blue. (B) Three DNA fragments were amplified during PCRs and were combined by fusion PCR using G-4368 and G-4369 primers to obtain one DNA sequence. (C) Final DNA sequence ready for subsequent cloning.

**Supplementary Figurer** **S2**



**Supplementary Figure S2.** Absolute quantification of transgene copy number in*C3siRNA-* and *CF7siRNA*-expressing lettuces using quantitative real-time PCR. (A) Standard curve showing Ct values plotted against a series of 5-fold DNA dilutions. (B) Transgene copy number results using standard curve for primary C3 and CF-7 artificial microRNA backbones. Data were analyzed using Ct(samples)=slope(standard.curve)×log(copy.number)+intercept(standard.curve) formula.

**Supplementary Figure S3**



**Supplementary Figure S3.** Quantitative real-time PCR analysis of mature C3 and CF7 artificial microRNAs (amiRNAs) in*C3siRNA-* and *CF7siRNA*-expressing lettuces using stem-loop primer. (A) Fold change expression of mature C3 amiRNA in different transgenic lines compared to line with the lowest expression level of C3 amiRNA. (B) Fold change expression of mature C7 amiRNA in different transgenic lines compared to line with the lowest expression level of C7 amiRNA. *TIP-41* (Tonoplastic intrinsic protein 41) was used as internal control and data were analyzed by Livak method. Primer sequences are shown in Table 1.

**Supplementary Table S1**

**Supplementary Table S1.** Primer sequences for siRNA cloning.

