**Figure 1** Simplified illustration of unspliced mRNA precursor with four exons (I, II, III, and IV) and three exons (shown as lines). Specific primer positions within the exon were shown as blocked boxes and their sequences were indicated with the same color.

**Figure 2** PCR amplicon product from (a) mature male flower cDNA using *EgHd3a* flanking primer (X1 and X2 at around 550 bp and X3 at 654bp) and (b) direct PCR from *E. coli* colony from cloning result, every electrophoresis hole related to one colony.

**Figure 3** PCR optimization for amplification of three mRNA specimens with X1, X2 and X3 specific primers at different annealing temperatures. (a) Amplification of X1 and X2 cloned fragment using X1 specific primer. (b) Amplification of X1 and X2 cloned fragment using X2 specific primer. (c) Amplification of X1 fragment, X2 fragment and cDNA using X3 specific primer. (d) Amplification of cDNA using X1, X2 and X3 specific fragment.

**Figure 4** Attachment location of primer (a) forward *EgHd3a*-X1 in the sequence *EgHd3a*-X2/*EgHd3a*-X3 (left) and *EgHd3a*-X1 (right), (b) forward *EgHd3a*-X2/ *EgHd3a*-X3 in the sequence *EgHd3a*-X1(left) and *EgHd3a*-X2 (right), (c) reverse *EgHd3a*-X1/*EgHd3a*-X2 in the sequence *EgHd3a*-X3 (left) and *EgHd3a*-X1/*EgHd3a*-X2 (right), and (d) reverse *EgHd3a*-X3 in the sequence *EgHd3a*-X1/*EgHd3a*-X2 (left) and *EgHd3a*-X3 (right).

**Table 1** Standard curve data for qPCR parameters (regression and PCR efficiency) and melting points of amplicon products using three primers.