



**Figure S2.** Generation of constructs to express amiRNAs from authentic *OsMIR390* precursors.

(a) Design of the two overlapping oligonucleotides required for amiRNA cloning into *OsMIR390*-based vectors. Sequences covered by the forward and reverse oligonucleotides are represented with solid and dotted lines, respectively. Nucleotides of *OsMIR390* precursor, amiRNA guide strand, and amiRNA\* strand are in grey, blue, and green respectively. Other *OsMIR390* nucleotides that may be modified for preserving authentic *OsMIR390* precursor secondary structure are in red. Rules for assigning identity to positions 1 and 9 of amiRNA\* are indicated.

**Figure S2 (cont.)** (b) Diagram of the steps for amiRNA cloning in *OsMIR390* precursors. The amiRNA insert obtained after annealing the two overlapping oligonucleotides has 5'CTTG and 5'CATG overhangs and is directly inserted in a directional manner into an *OsMIR390-B/c* vector previously linearized with *BsaI*. Nucleotides of the *BsaI* sites and those arbitrarily chosen and used as spacers between the *BsaI* recognition sites and the *OsMIR390* sequence are in purple and light brown, respectively. Other details are as described in A. C, flow chart of the steps from amiRNA construct generation to plant transformation.