

Supplementary File 3. Updated criteria for plant miRNA annotation and criteria for 23-nt and 24-nt miRNAs.

Criteria for normal miRNAs (Axtell and Meyers, 2018):

1. One or more miRNA/miRNA* duplexes with two-nucleotide 3' overhangs, excluding secondary stems or large loops in the miRNA/miRNA* duplex and limiting precursor length to 300 nucleotides.
2. Confirmation of both the mature miRNA and its miRNA* only by sRNA-seq.
3. miRNA/miRNA* duplex contains ≤ 5 mismatched bases, and has at most one asymmetric bulge containing at most 3 bulged nucleotides.
4. $\geq 75\%$ of reads from exact miRNA or miRNA*, including one-nucleotide positional variants of miRNA and miRNA* when calculating precision.
5. Novel annotations should meet all criteria in at least two sRNA-seq libraries (biological replicates).
6. Homology-based annotations should be noted as provisional, pending actual fulfillment of all criteria by sRNA-seq.
7. No RNAs < 20 nucleotide or > 24 nucleotides should be annotated as miRNAs. Annotations of 23- or 24-nucleotide miRNAs require extremely strong evidence.

Criteria for 23- and 24-nt miRNAs: Beside all criteria above, the following 2 requirements added.

1. Reads corresponding to mature miRNA with RPM (reads per million) ≥ 20 .
2. miRNA/miRNA* duplex contains at least 1 mismatched bases or bulge.
3. The miRNA* must have corresponding reads.

References

Axtell, M.J. and Meyers, B.C. Revisiting Criteria for Plant MicroRNA Annotation in the Era of Big Data. *Plant Cell* 2018;30(2):272-284.