Additional file 1: Figure S1

Raw Data (35nt)

Removing low quality reads
(Quality value less than 20)

Perl script

Trimming of 3’ adaptor

Perl script

Small RNAs (1-35 nt)

Small RNAs (18-25 nt)

Blast search against miRBase
19.0
miRNA criteria 1

a) Query reads exactly match the reference miRNAs
b) Reads with 1–2 nt extended or shortened at the 5′ end of the known miRNAs.
c) Reads with 1–4 nt extended or shortened at the 3′ end of the known miRNAs.

CLC genomic workbench

Yes  No

Known miRNAs  Further analysis 1
miRNA criteria 1

a) Query reads exactly match the reference miRNAs

```
5'  3'
5'  3'
```

b) Reads with 1–2 nt extended or shortened at the 5’ end of the known miRNAs.

Extended

```
5'  3'
5'  3'
```

Shortened

```
5'  3'
5'  3'
```

c) Reads with 1–4 nt extended or shortened at the 3’ end of the known miRNAs.

Extended

```
5'  3'
5'  3'
```

Shortened

```
5'  3'
5'  3'
```
miRNA criteria 2

a) Reads with first ten nucleotides identical to those of known miRNAs (including reverse complement and 5’ shift)

Yes

No

Further analysis 2

18-25 nt Unidentified small RNAs
miRNA criteria 2

a) Reads with first ten nucleotides identical to those of known miRNAs (including reverse complement and 5' shift)

5' shift

: reference known miRNA

: reverse complement - reference known miRNA

: query read
miRNA criteria 3

a) Reads with first sixteen nucleotides identical to those of known miRNAs (including reverse complement and 5’ shift)

Yes          No

Known miRNAs         Further analysis 3
miRNA criteria 3

a) Reads with first sixteen nucleotides identical to those of known miRNAs (including reverse complement and 5' shift)

5' shift

: reference known miRNA
--- : reverse complement - reference known miRNA
--- : query read
Further analysis 3

FASTA v36

miRNA criteria 4

a) Sequence with Smith-Waterman score of at least 70

(Parameters: +5/-4)

Yes No

Known miRNAs Putative miRNAs