

5' _____ 3' microRNA

1. Poly (A) Tailing of microRNA



5' _____ AAAAAAAAAAAAAAAAAAAAAA 3'

Poly (A) tailed microRNA

2. Hybridization of Reverse Transcription Oligo dT primer



5' _____ AAAAAAAAAAAAAAAAAAAAAA 3'
TTTTTTTTTTTTTTTTTTTTTT 5'

3. Reverse Transcription of Tailed microRNA



5' _____ AAAAAAAAAAAAAAAAAAAAAA 3'
←----- TTTTTTTTTTTTTTTTTTTTTT 5'

4. Degradation of RNA and purification of cDNA copy of miRNA



3' _____ TTTTTTTTTTTTTTTTTTTTTT 5'

5. Poly (dT) Tailing of cDNA copy of the microRNA



3' TTTTTTTTTTTTTTTTTTTTTT _____ TTTTTTTTTTTTTTTTTTTTTT 5'

6. Addition of T7 Promoter synthesis reagents



Blocked Extension
3' ←----- TTTTTTTTTTTTTTTTTTTTTT _____ TTTTTTTTTTTTTTTTTTTTTT 5'
AAAAAAAAAAAAAX----->

7. T7 Promoter Synthesis via template directed extension of the 3' end of the cDNA copy of the miRNA



5' ----->
3' T7 Promoter AAAAAAAAAAAAAA TTTTTTTTTTTTTTTTTTTTTT _____ TTTTTTTTTTTTTTTTTTTTTT 5'

8. Purification of amplified 5'/3' poly dA miRNA



5' = AAAAAAAAAAAAAAAAAAAAAA _____ AAAAAAAAAAAAAAAAAAAAAA 3'
microRNA