

## **Supplementary Information**

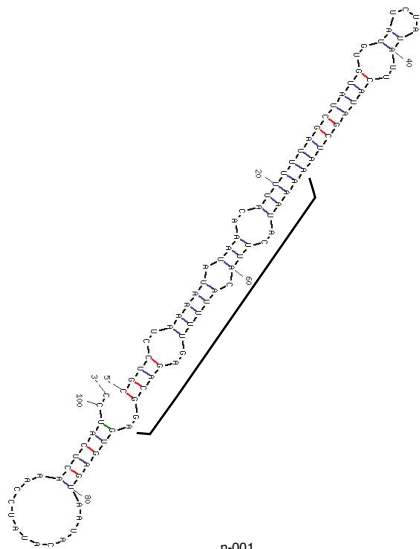
### **Identification of novel miRNAs from drought tolerant rice variety Nagina 22**

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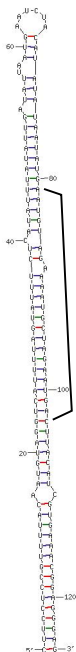
<sup>1</sup>Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, New Delhi – 110021, India

<sup>2</sup> National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi – 110067, India

\*Corresponding author



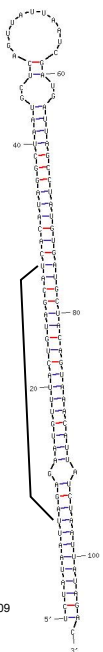
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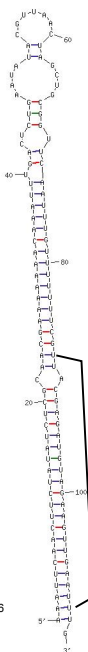
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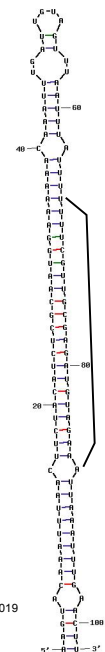
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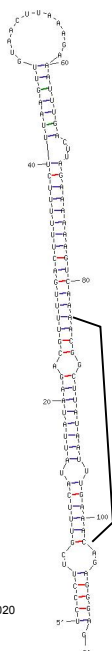
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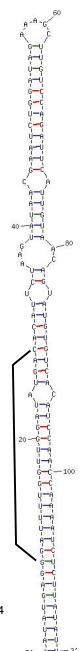
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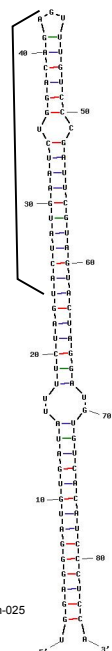
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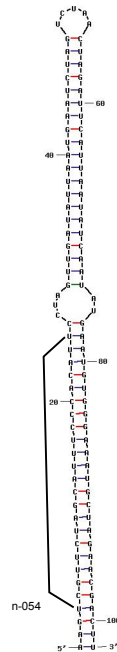
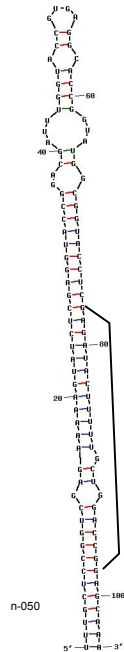
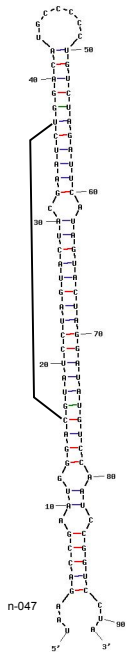
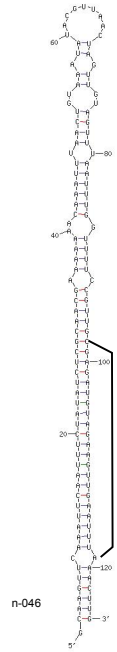
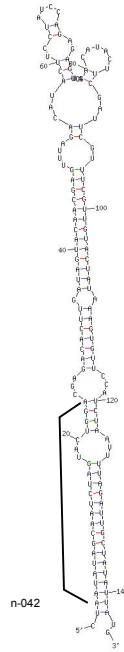
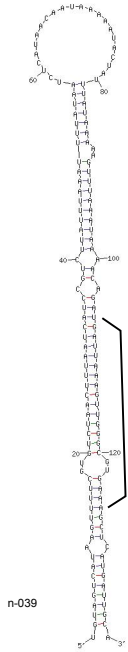
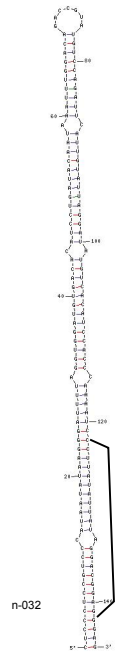
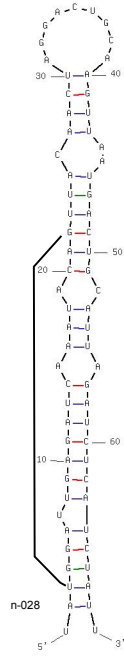
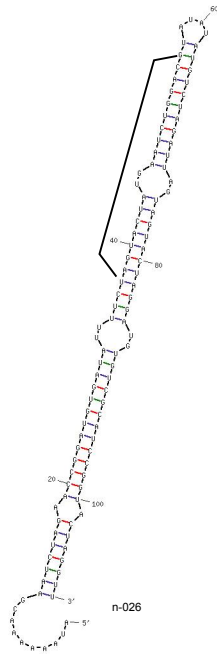
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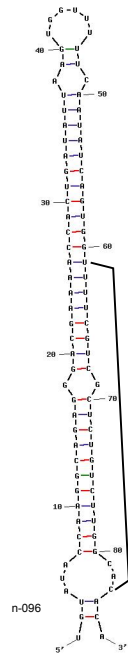
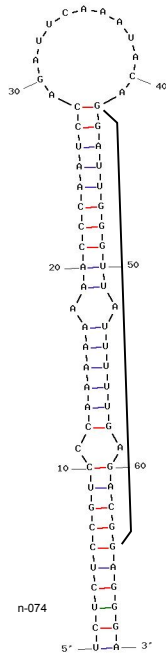
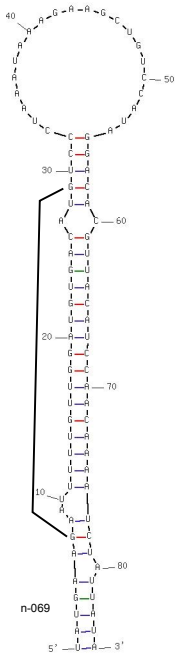
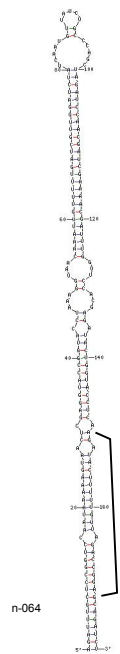
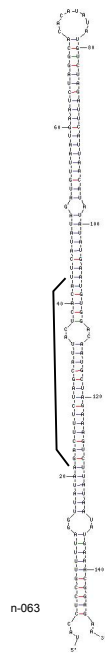
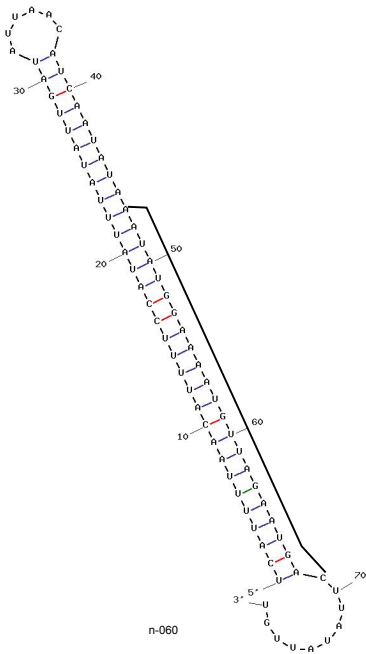
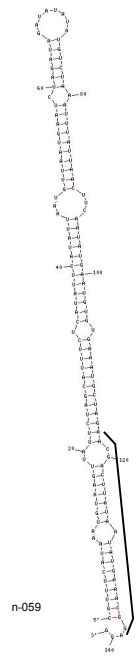
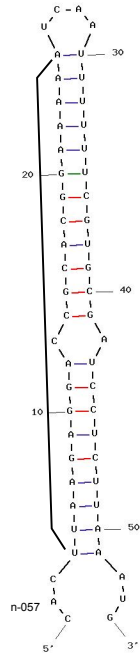
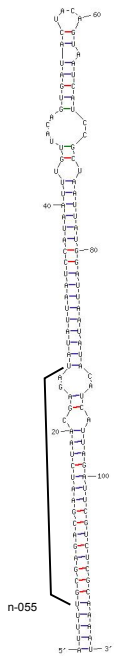


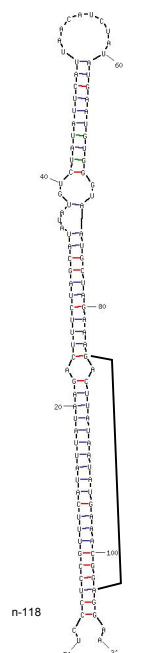
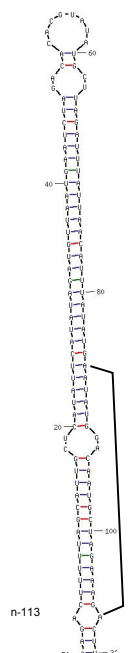
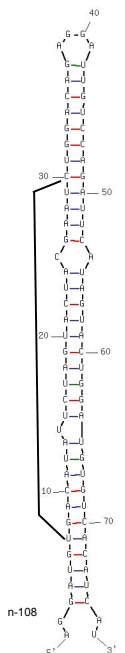
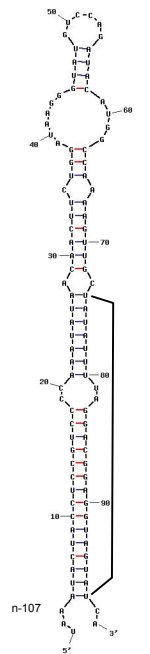
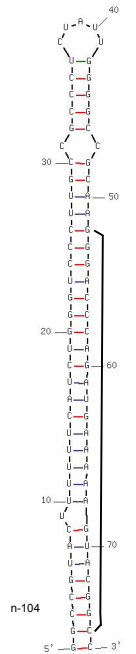
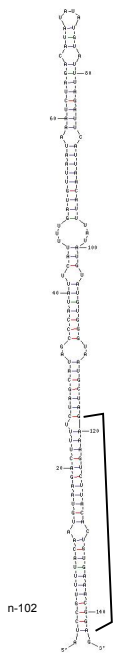
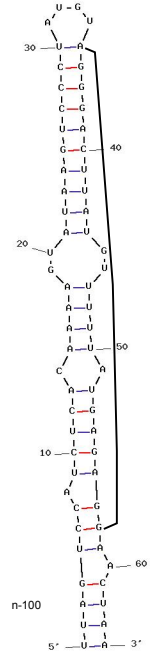
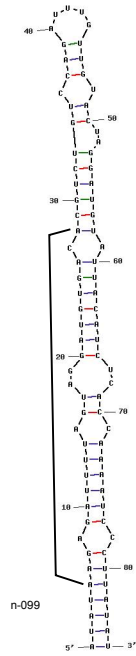
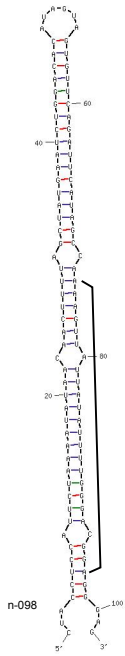
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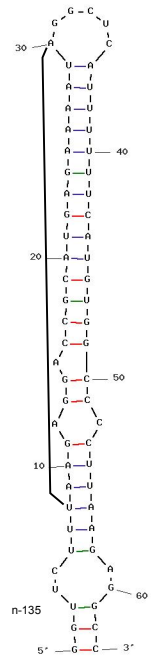
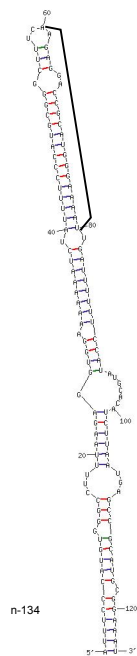
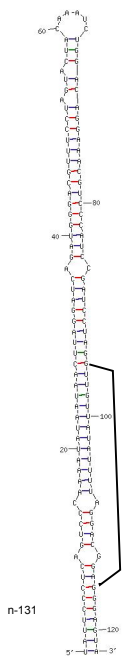
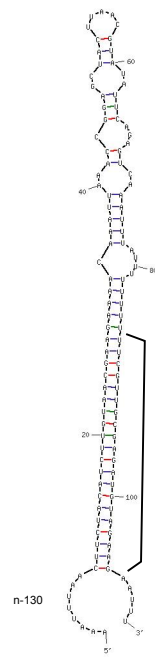
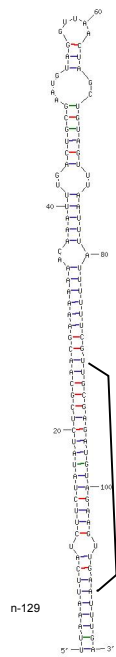
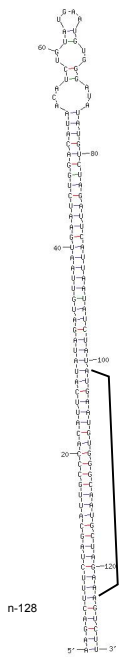
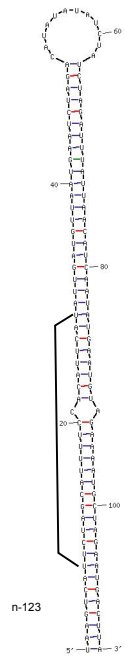
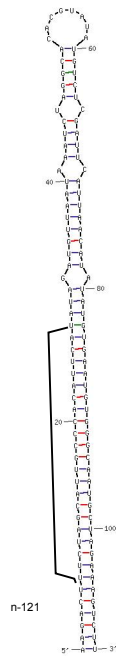
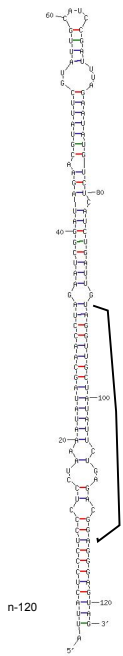


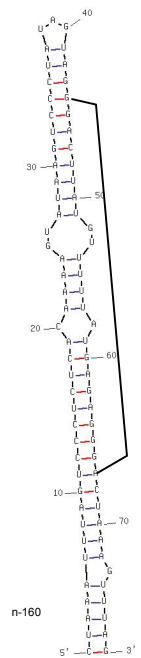
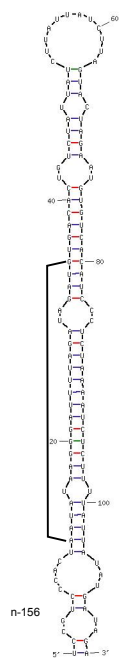
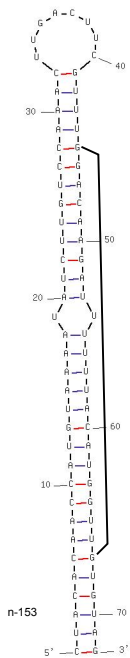
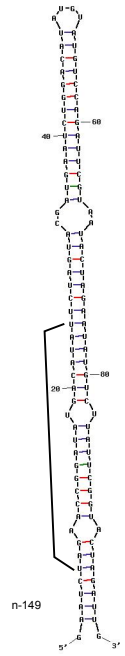
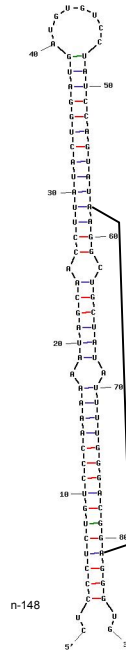
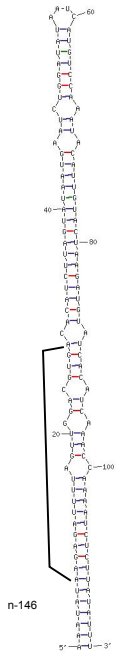
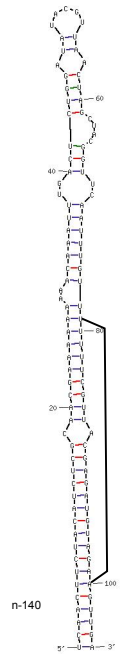
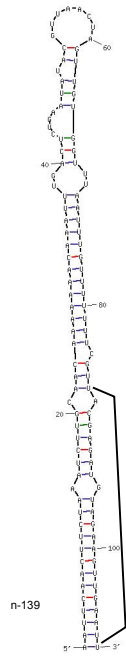
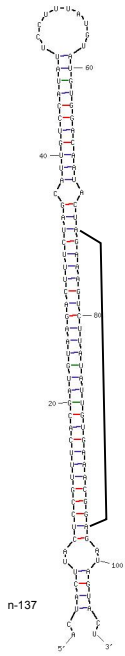
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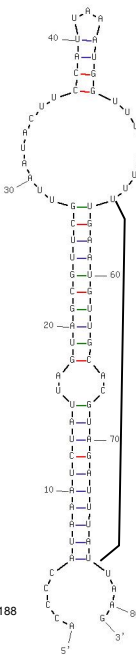
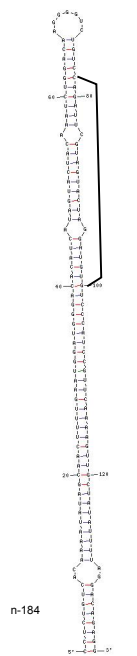
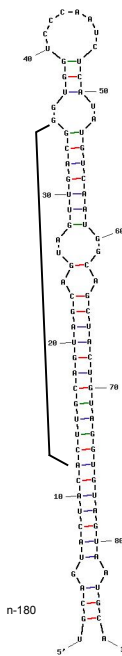
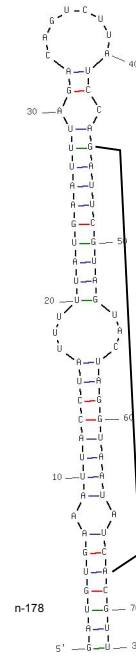
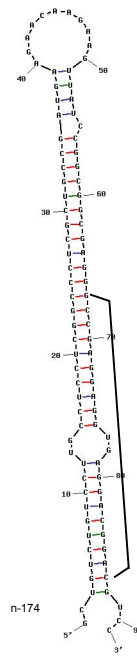
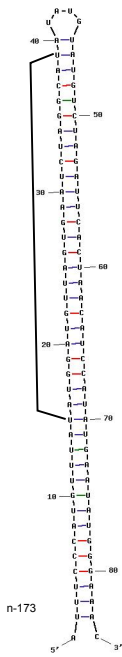
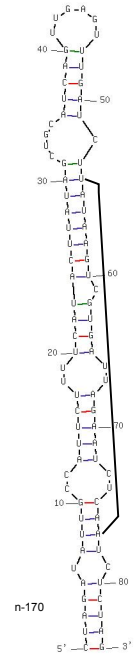
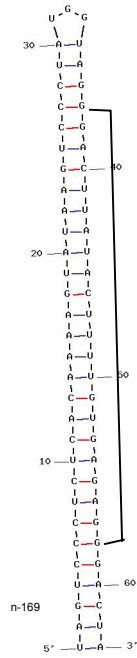
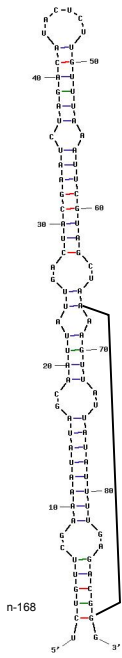




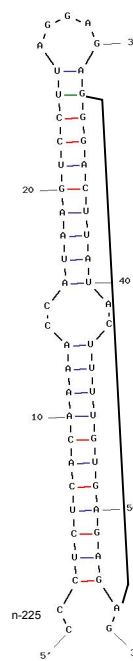
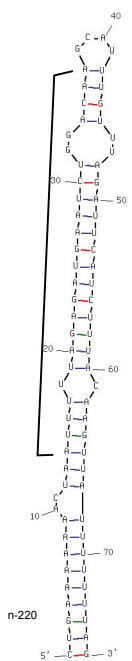
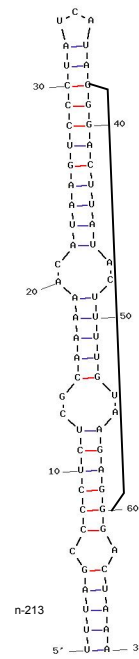
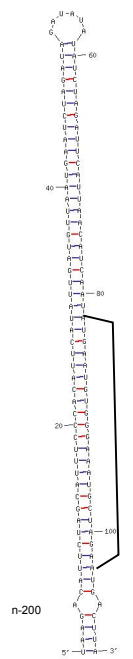
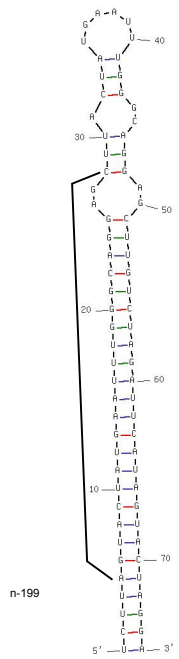
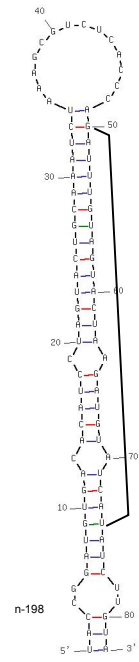
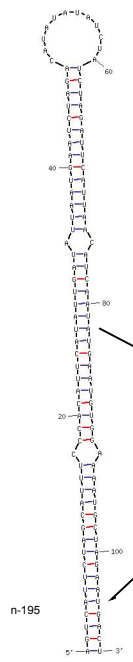
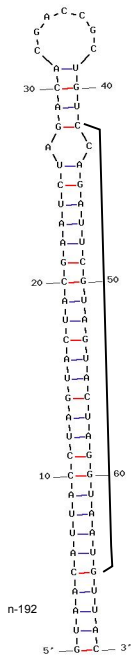




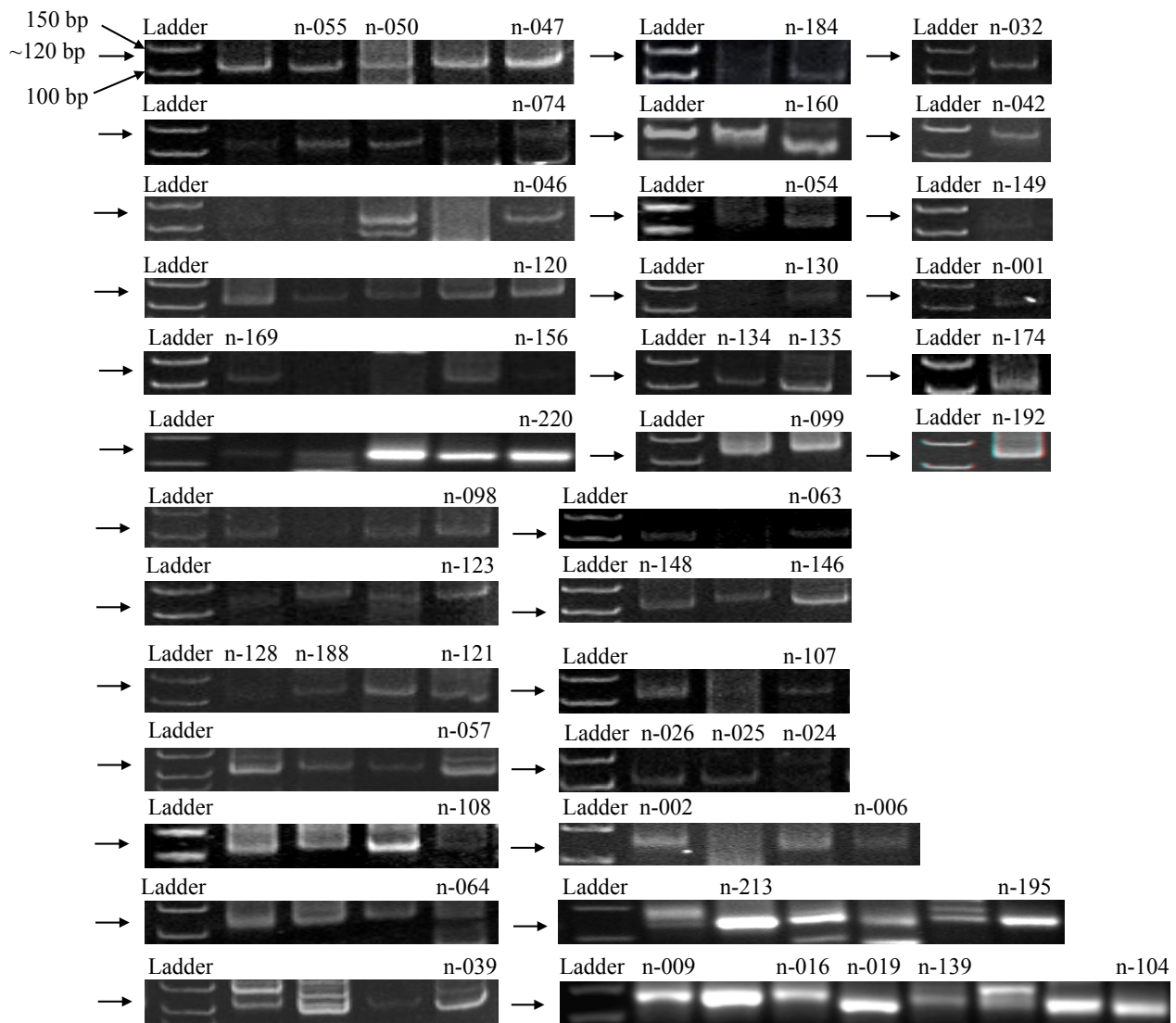




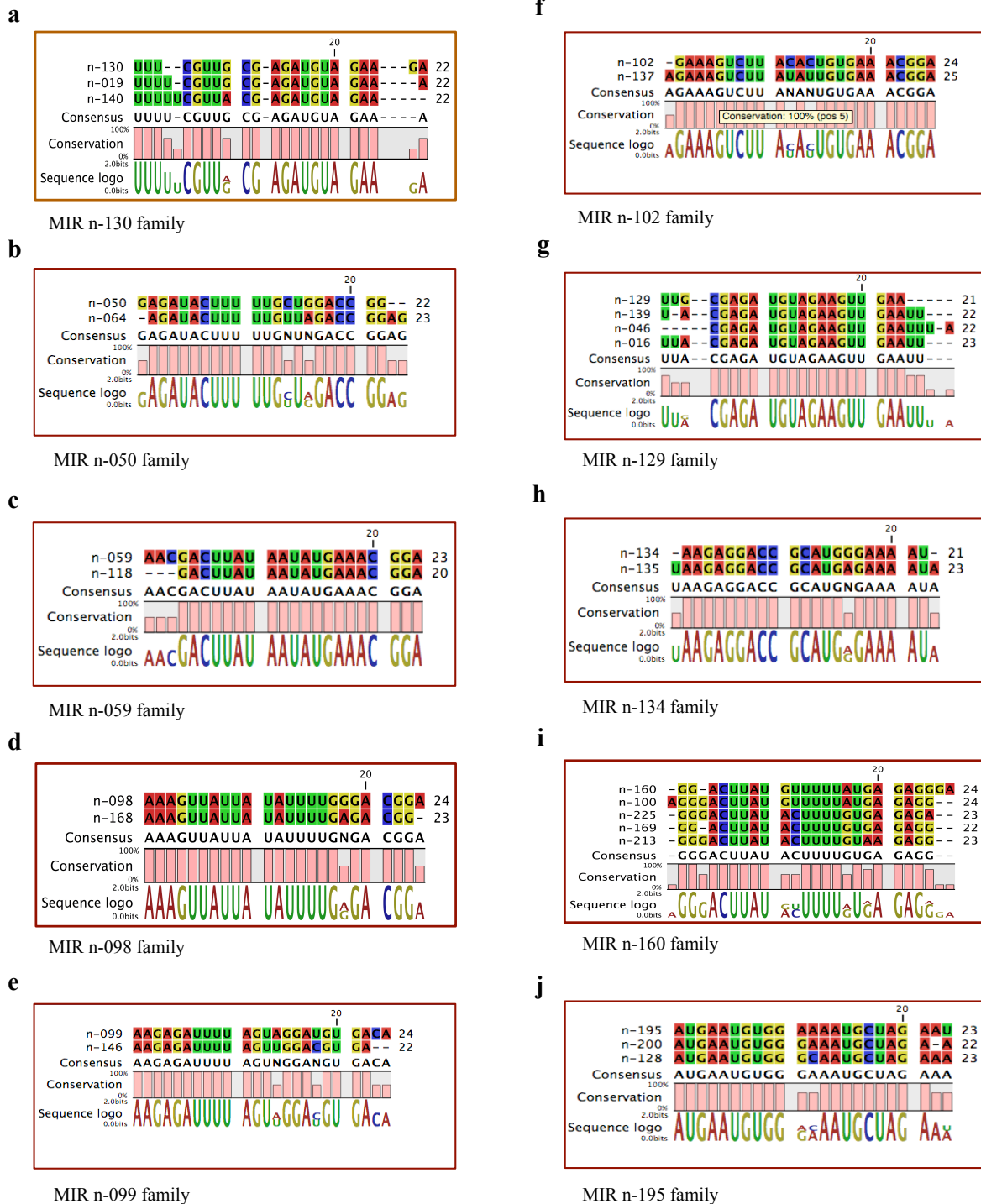




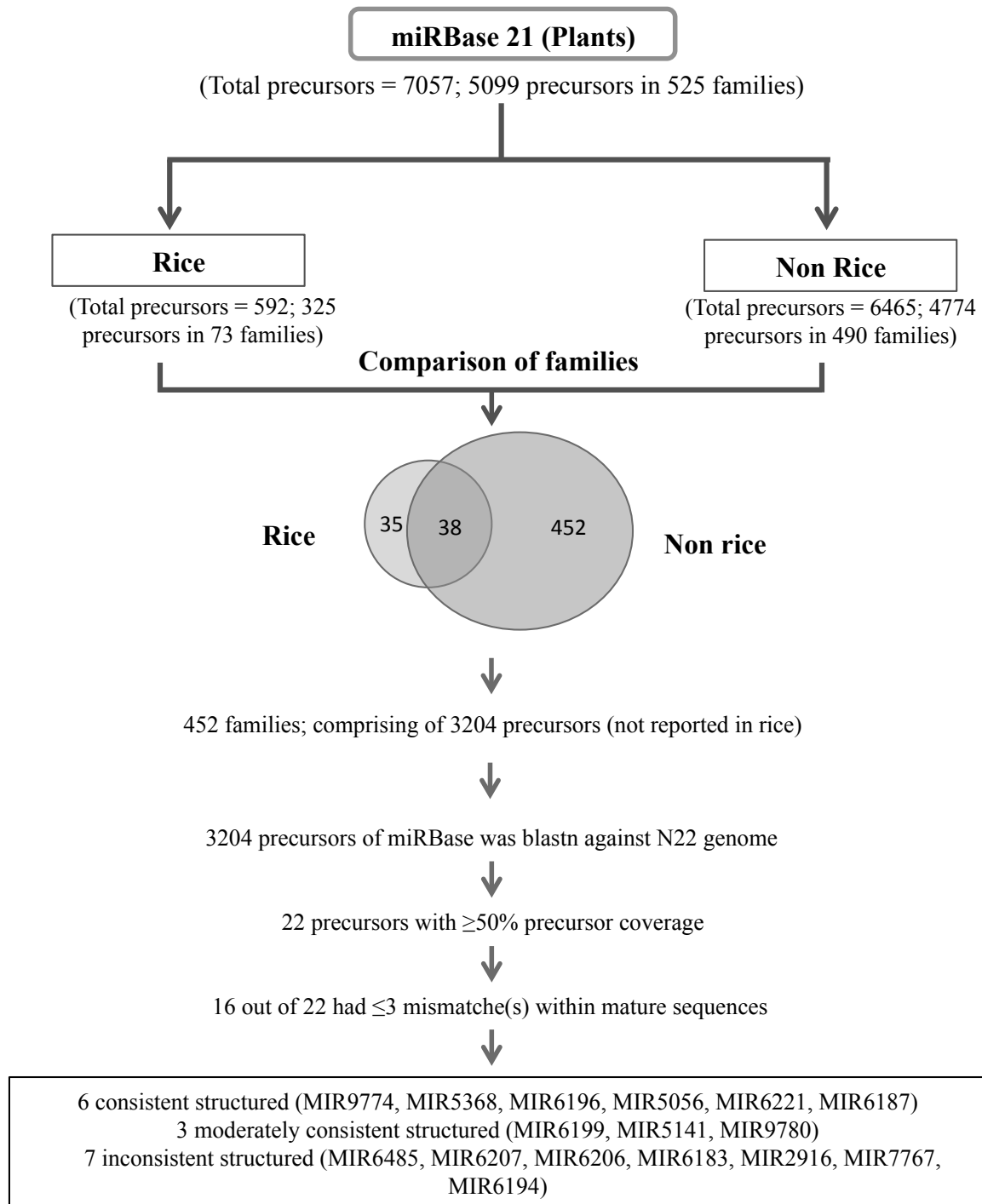
**Fig. S1.** Secondary hairpin structures of 71 novel miRNAs.



**Fig. S2 . Detection of novel miRNA candidates.** Gel profile of 47 novel miRNAs (products of qRT-PCR loaded on 6% PAGE gel). Amplified product includes ~20 bp of miRNA + 100 bp of adapter and the arrow indicates the size of ~120 bp.



**Fig. S3. Multiple sequence alignment among family members of novel miRNAs.** 27 miRNAs are distributed among 10 families based on mature sequence conservation. Alignments for MIR families (a) n-130 (b) n-050 (c) n-059 (d) n-098 (e) n-099 (f) n-102 (g) n-129 (h) n-134 (i) n-160 (j) n-195 are shown.



**Fig. S4. Flowchart for identification of putative orthologous MIR genes in N22.** Sixteen putative orthologous MIR genes are identified in N22 comprising of 6 consistent and 10 inconsistent structures.



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osa-MIR6183      GAGAAAUUUAUUGAACGAGUUGGCUGCAAGUUCGGAAGUUAUGACAAUGGCAUCAGCUCG
** *****
** *****

UUCGUCAUUGCUGUA
UUUGUCAUUGCUGUA
** *****

peu-MIR2916      UGGGGACUCGAAGACGAUCAUAUACCAUCCUAGUCUCAACCAUAAACGAUGCCGACCAGG
osa-MIR2916      UGGGGGCUCCAAGACGAUCAGAUACCGUCCUAGUCUCAACCAUAAACGAUGCCGACCAGG
**** *****

GAUUGGCGGAUGUUGCUUCUAGGAGUCCGCCA
GAUCGCGGAUGUUGCUUAUAGGACUCGCGC
** *****

rgl-MIR5141      UUGAGUAAUGCCUGAGUCGCAGCAUCAAGGUCUGAGCCAAAUUGAGCAAAGGCGGCCACU
osa-MIR5141      UUGAGUAAUGCCUGAGUCGCAGCAUCAAGGUCUGACCCAAAUUGAGCGAAGGCGGCCACU
*****

UCGCGAUAAUUGGCCAAUCCAGUUUUGAACUACCGCAUACUUGUUUCAUAGCUUUCAC
UCGCGAUAAUUGGCCAAUCCAGUUUUGAACUACCGCAGACUUGUUUCAUAGCUUUCAC
*****

UAGCGGCAGACCCGACGCGACUGACAGAUAAAGCCGACGUUA
UAGCGGCAGACCCGACGCGACUGACGGAUAAAGCCAACGUUA
*****

bdi-MIR7767      UUGAUGGCUGCCCAAGCUGAGAGCUCUCCACGACAGCUUGGACAGGAGGCCACCUGCU
osa-MIR7767      UUGAUAAACUGCCCAAGCUGAGGGCUCUCCACAACAGCUUGGACACGAGGCCACCAGU
**** *****

UGGAAGAGCUCGGAUUGAGAGGAGCAAGCAGCUUGAAGGUGGAGGACC
UGAAAGAGCUCUCCCUUGUAGCAGCAAGCUGCUUGAAGUCGGUGAAGGACC
** *****

hvu-MIR6194      CGCCACAUUUGGGUUUCAGAGAUC-----AACG-CCCAUAUAUGCCGCAUGUCAGCCUC
osa-MIR6194      -----AUUGAUGAUUCAU-GAUCUGCUUCAUUGCAGCAUAUAUGCCACAGUCAGCCUC
** * **** * * * ***** ** *****

CUGUAUGGGGAUCUGACAGACGAGGAGAAGGAAG
UUAUUGGAGACCUGACAGAUAGGAGAAGGAG
* ***** *

sbi-MIR6221      ACAGCUCACCAGCCAUCCUUAUGCGUGGUGCUCUGACUUCUGGCCCCUGCUAGCCCC
osa-MIR6221      ACAGCUCGGCAGCACAGCUUUGUACAUUGUGCUCUGACUUCUGGCCCCUGCUAGCCCC
*****

ACCAACACUAAGCGGGGCCAGAUUCAGAAAGCAUUGUGCAUAUAUCUGGCCUCUAGCU
ACGGAAAGUAAGCGGGGCCACAUUCAGAAAGCAUUGUGCAUAUAUCUGGCCUCUAGUU
** * * ***** ***** ***** ***** *****

GU
GU
**

tae-MIR9780      CGCCGUGGUGCCGAGGAUGCAAGGCGUGGGGAGCUGGCCAAGGCGUGCACCACCAUCA
osa-MIR9780      CGACGUGGUGCCGAGGAUGAAGACGGUGGCGGAGCUGGUCAAGGCGUGCGCCACCAUCA
** *****

UCUGGAUCGGGUCGGCGCUGCACGCGCGG
UCUGGAUCGGGUCGGCGCUGCACGCGCGC
*****

hvu-MIR6187      ACGUCGGCAUGAACAGGUUCGGCGACCUCACCCAGCGAGGUCGUCGCGCAGUUCACA
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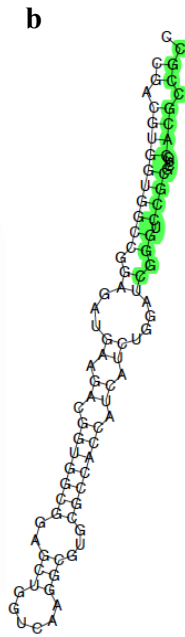
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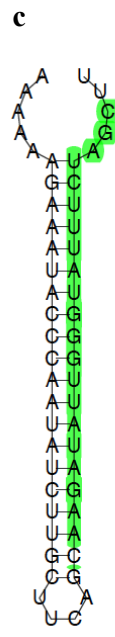
**Fig. S5. Pairwise alignment of precursor sequences of miRBase MIR genes and orthologous MIR genes from N22.** Mature sequences are highlighted in yellow and green color in the precursor sequences of parent and N22, respectively. ‘\*’ denotes the conserved nucleotides while mismatched nucleotides are indicated by space, ‘-’ denotes gaps.



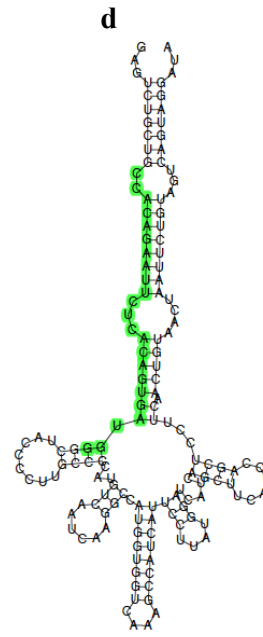
osa-MIR6187



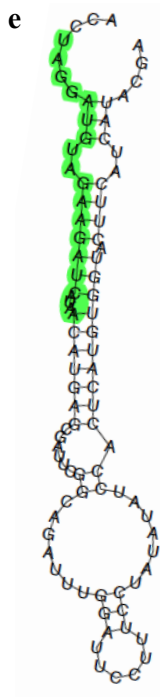
osa-MIR9780



osa-MIR9774



osa-MIR6199



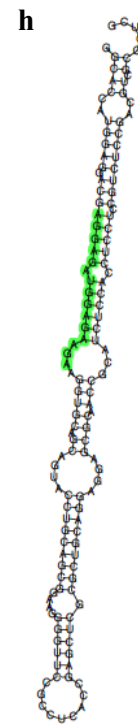
osa-MIR6485



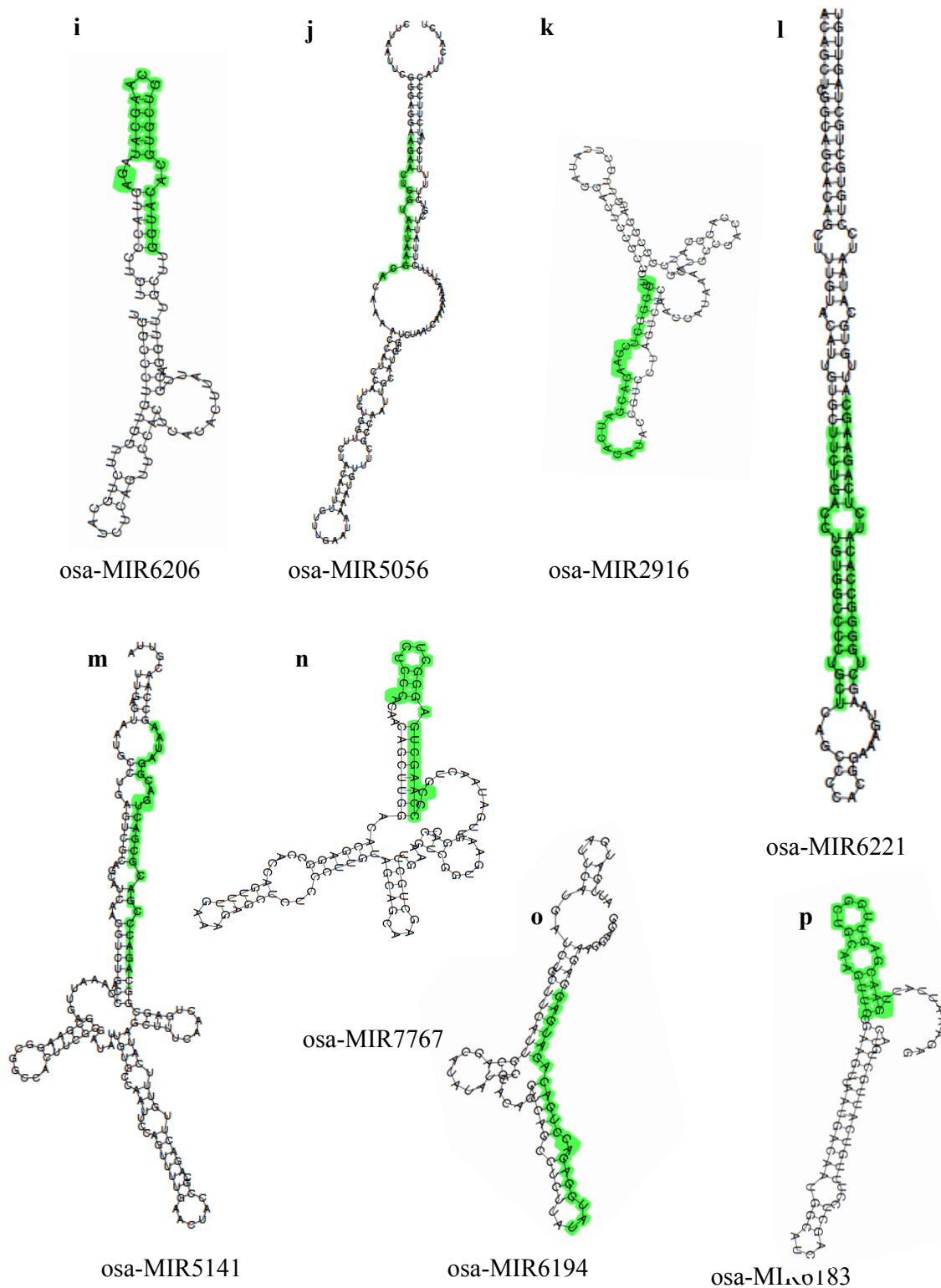
osa-MIR5368



osa-MIR6207

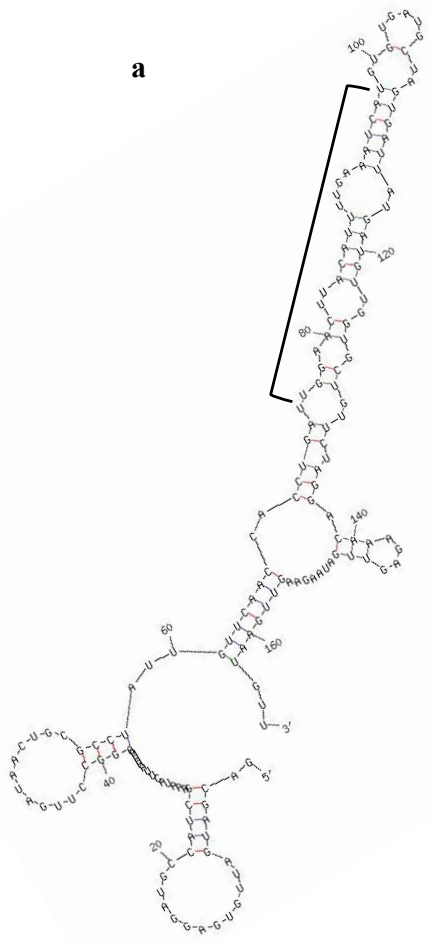


osa-MIR6196

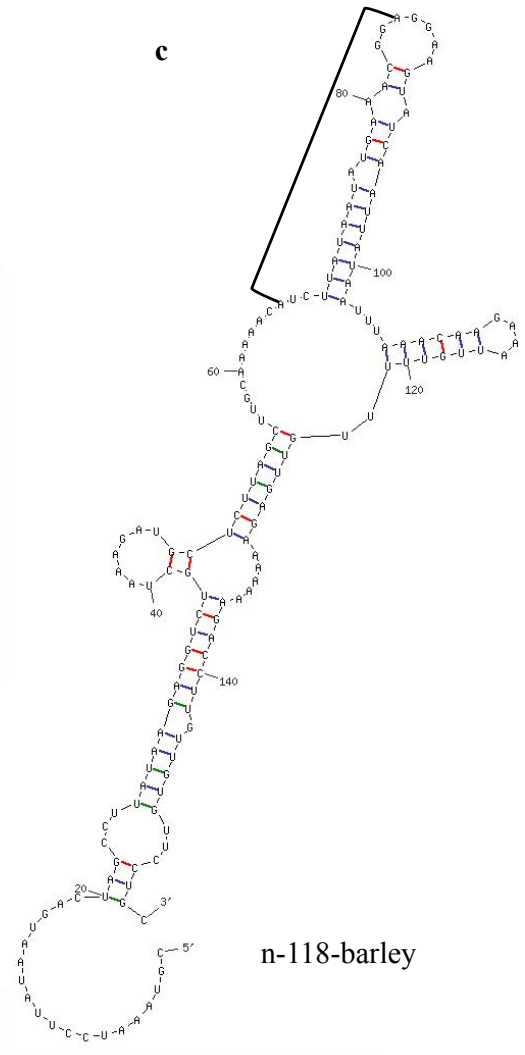


**Fig. S6. Secondary hairpin structures of 16 putative orthologous MIR genes in N22.** Secondary hairpin foldbacks of (a) MIR6187 (b) MIR9780 (c) MIR9774 (d) MIR6199 (e) MIR6485 (f) MIR5368 (g) MIR6207 (h) MIR6196 (i) MIR6206 (j) MIR5056 (k) MIR2916 (l) MIR6221 (gives rise to both -5p and -3p) (m) MIR5141 (n) MIR7767 (o) MIR6194 and (p) MIR6183.

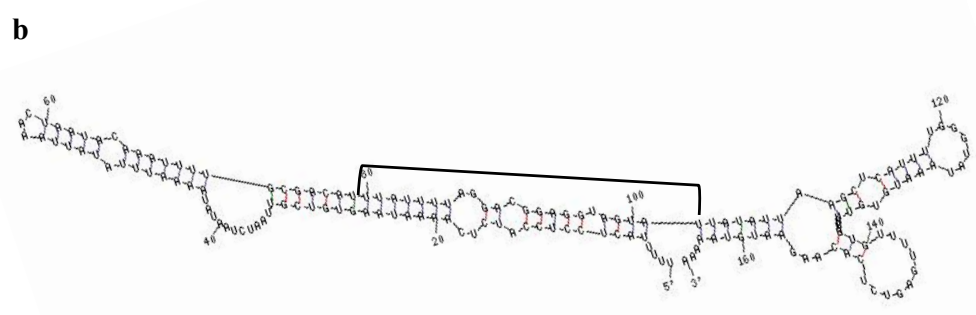




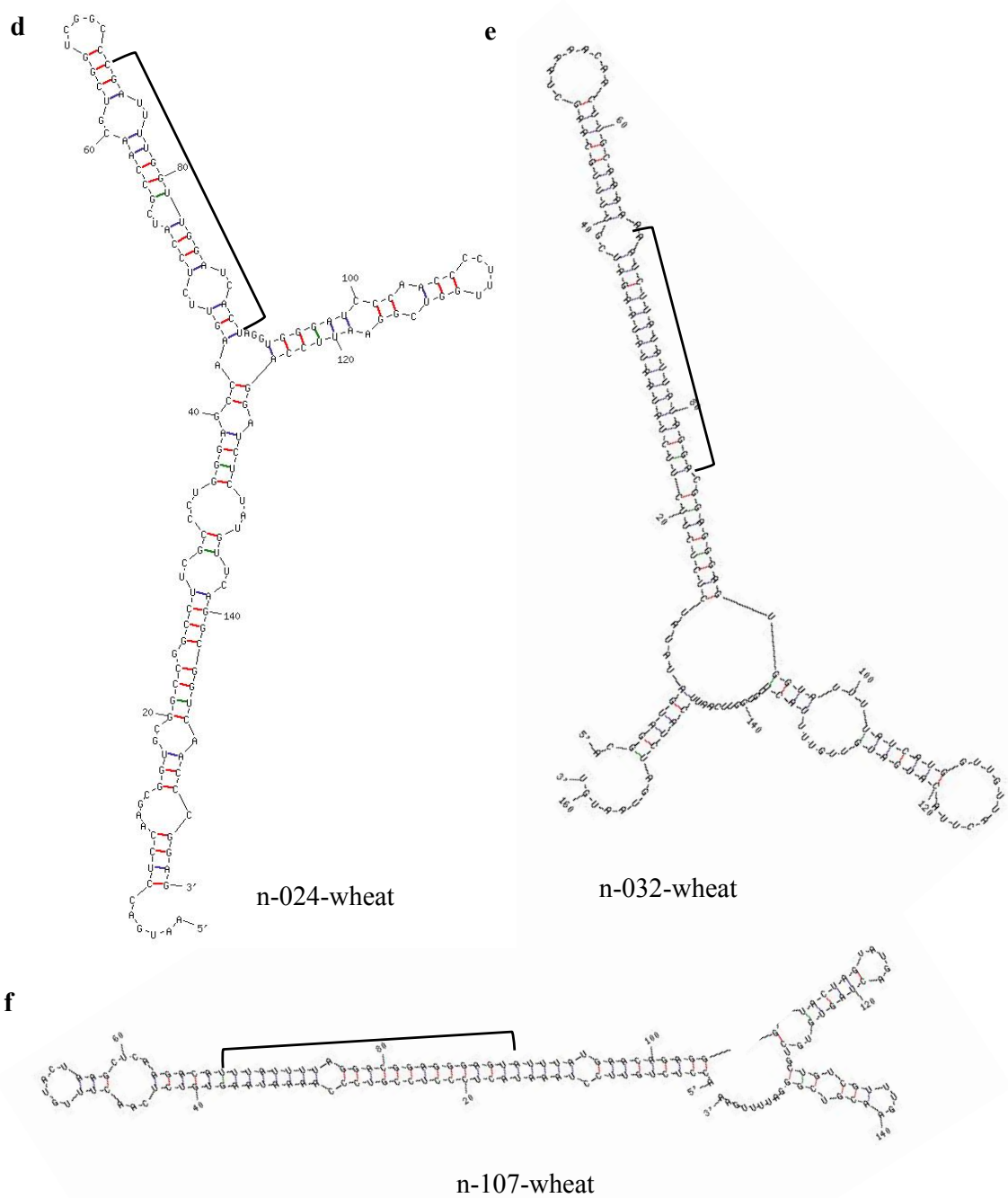
n-006-barley



n-118-barley



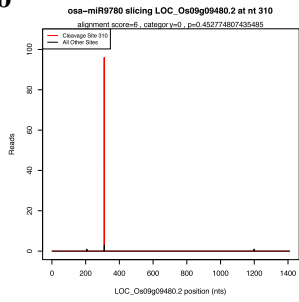
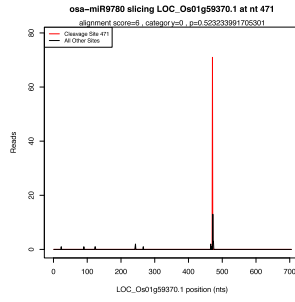
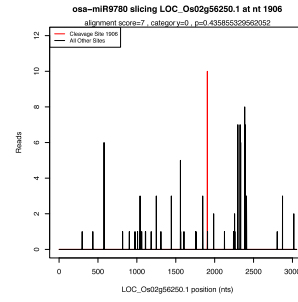
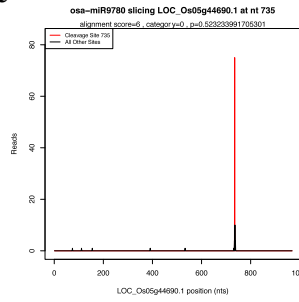
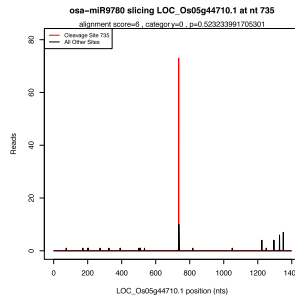
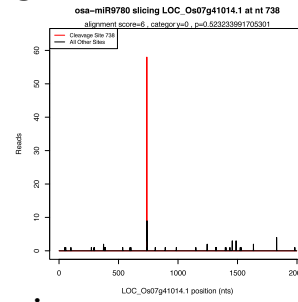
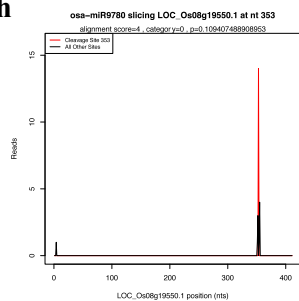
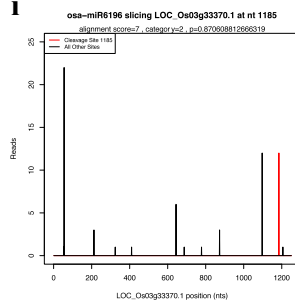
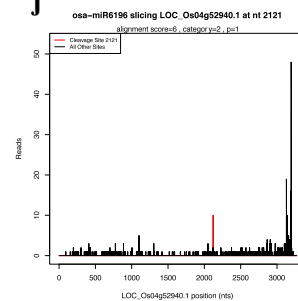
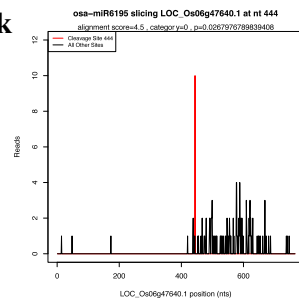
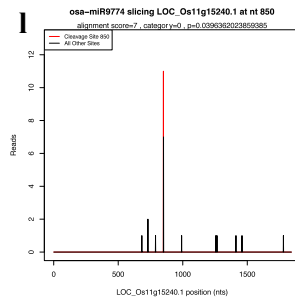
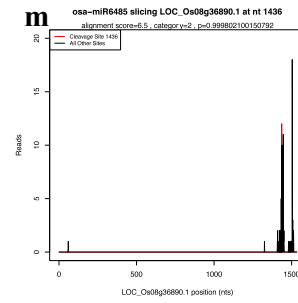
n-107-barley

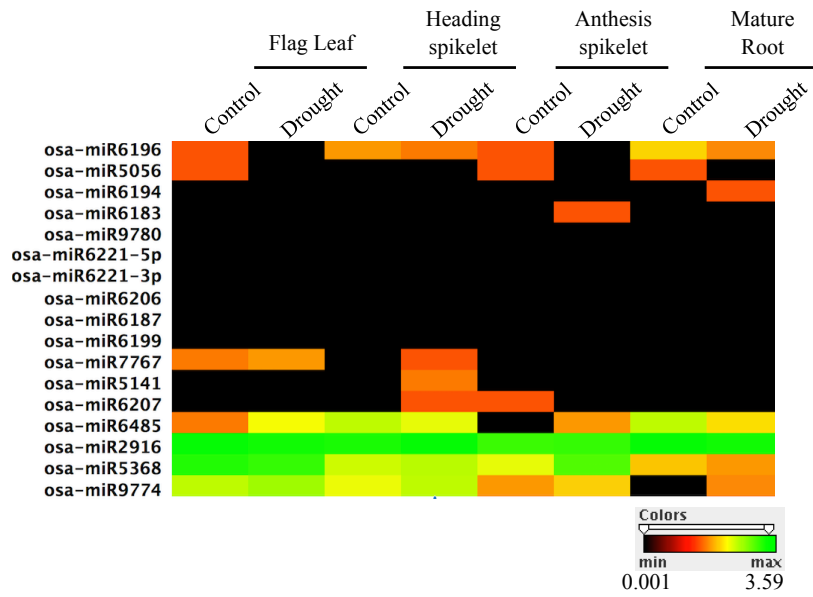


**Fig. S7. Secondary hairpin structures of the novel orthologous miRNA loci in other plants.** Secondary hairpin structures of (a) n-006, (b) n-107, (c) n-118 in barley (d) n-024, (e) n-032, (f) n-107 in wheat.

**a**

	Degradome reads $\geq 10$	Category $\leq 2$	p-value $\leq 0.05$	Degradome reads $\geq 10$ , category $\leq 2$ and p-value $\leq 0.05$
No. of orthologous miRNA:target pair	10:38	15:450	7:10	1:1

**b****c****d****e****f****g****h****i****j****k****l****m**

**n**

**Fig. S8. Characterization of orthologous miRNAs.** (a) Table showing number of miRNA:target pairs for orthologs based on different PARE criteria. T-plots of osa-miR9774 cleaving (b) LOC\_Os11g15240, (c) LOC\_Os01g59370 (d) LOC\_Os02g56250 (e) LOC\_Os05g44690 (f) LOC\_Os05g44710 (g) LOC\_Os07g41014 (h) LOC\_Os08g19550 (i) osa-miR6196 cleaving LOC\_Os03g33370 and (j) LOC\_Os04g52940 (k) osa-miR6195 cleaving LOC\_Os06g47640 (l) osa-miR9774 cleaving LOC\_Os11g15240 (m) osa-miR6485 cleaving LOC\_Os08g36890. The vertical red line indicates the site of cleavage. (n) Heat map of orthologous miRNAs in 8 N22 sRNA libraries.