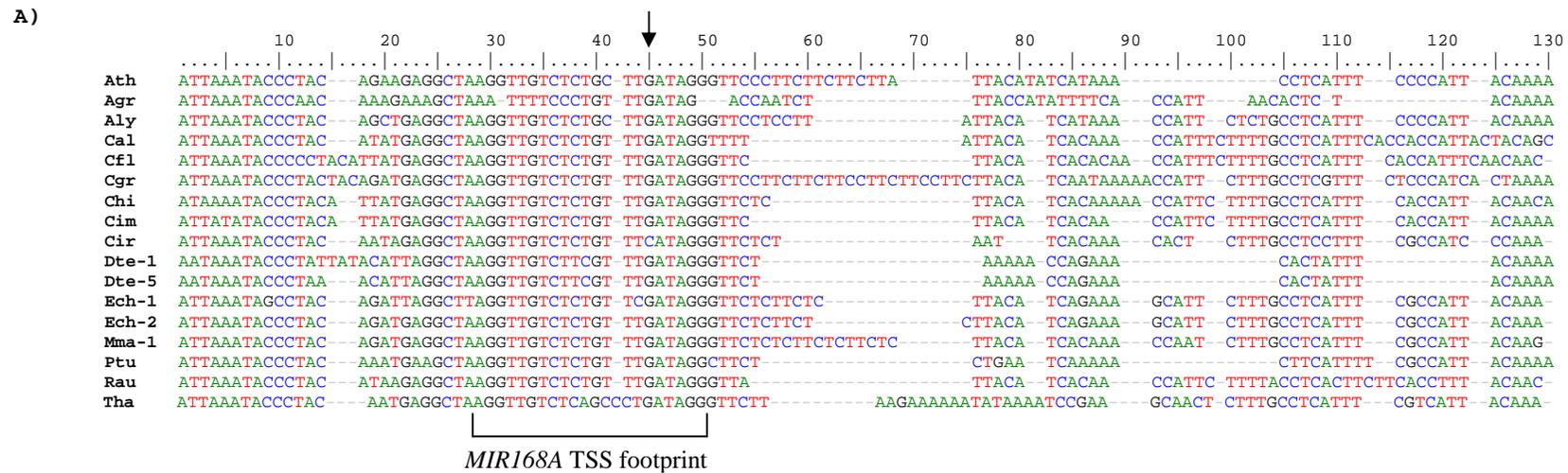


MICROSYNTENIC PHYLOGENETIC FOOTPRINTING OF *MIR168* LOCI IN BRASSICACEAE

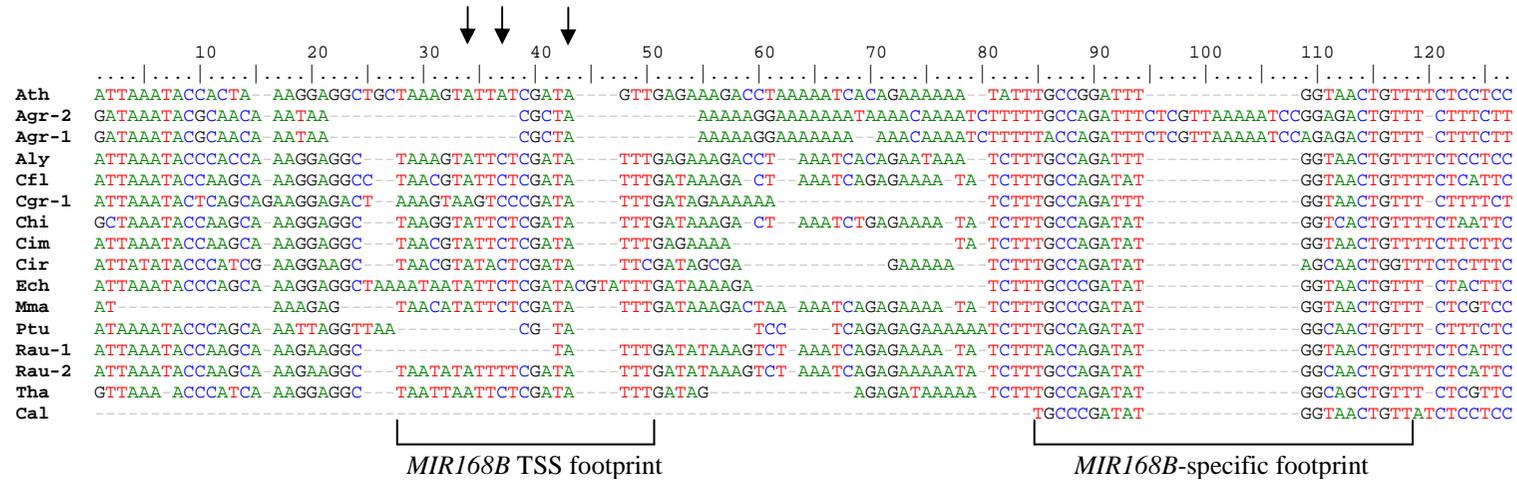
Silvia Gazzani, Mingai Li, Silvia Maistri, Eliana Scarponi, Michele Graziola, Enrico Barbaro, Jörg Wunder, Antonella Furini, Heinz Saedler and Claudio Varotto

Additional File 2

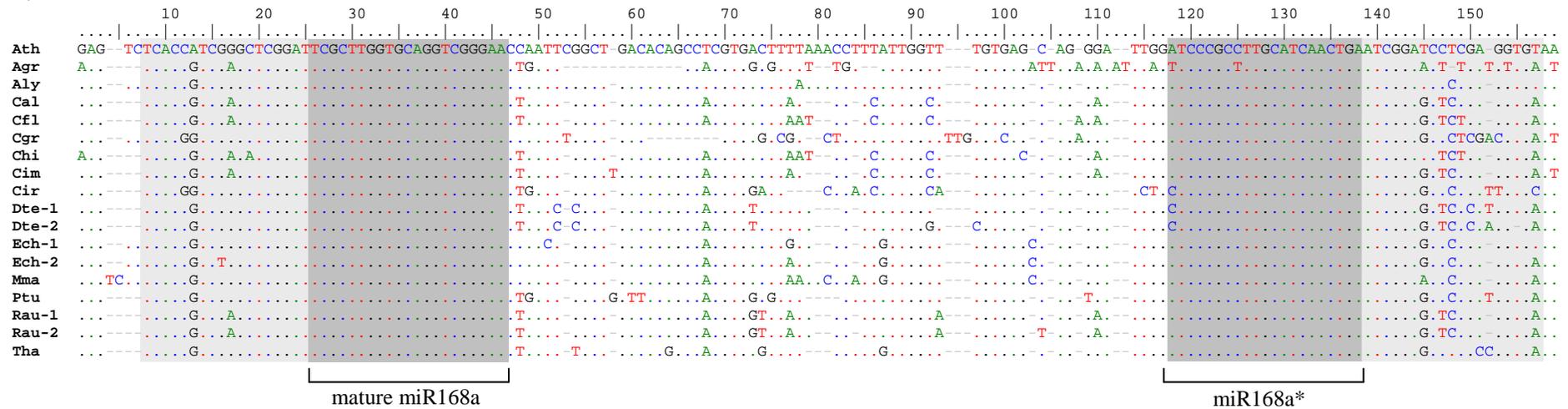
A), B) Alignment of the regions encompassing *MIR168a* and *MIR168b* TSS, respectively. Arrowheads indicate transcription start sites. C) Alignment of pre-miR168a genomic region. D) alignment of pre-miR168b genomic region. E) Alignment of the sequence stretches from miR168* to the downstream 9 bp conserved motif in *MIR168a* and *MIR168b*. F), G) Alignments of *MIR168A* and *MIR168B* genomic regions from the TSS to approximately 60 nucleotides downstream of the pre-microRNA. The alignments are reported in FASTA format and they can be visualized using programs as BioEdit Sequence Alignment Editor. In light gray are highlighted the sequences corresponding to the lower stem, while in dark grey those corresponding to the upper stem; in light blue is highlighted the 9 bp motif conserved between paralogs. For highly conserved regions dots indicate identity to the base present in the reference (first) sequence.



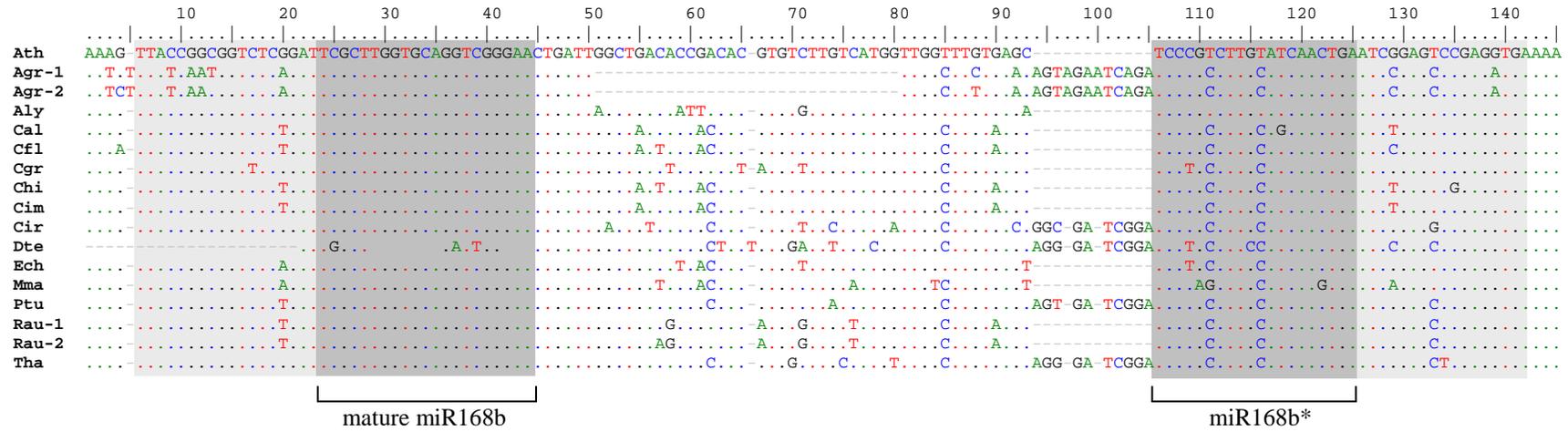
B)



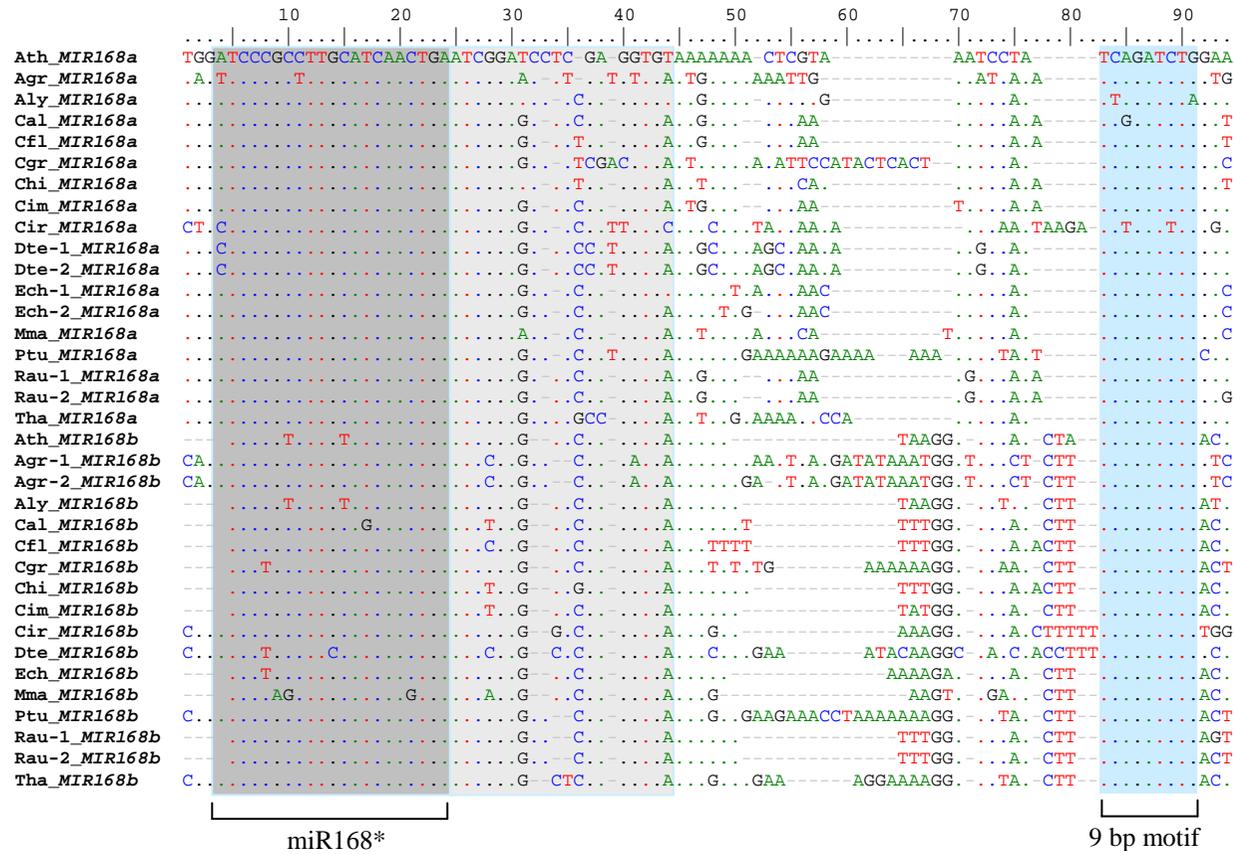
C)



D)



E)



F)

>Ath_MIR168a

GATAGGGTTCCT-----TCTTCTTCTTATTA-CAT-----ATC--ATAAACCTCATT--CCCCATTAC-AAAACCTAGA-----ACTCCTTCTCTTCTTCTTTCTTCATA-----TCCCTG-----TCT-----
-----AAAGGGATT-----ATT-ATGATAGT--AGAG--TCTCACCATCGGGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCTCGTGACTTTTAAACCTTTATTGGTT--TGTGAGC--
AG-GGA--TTGGATCCCGCCTTGCATCAACTGAATCGGATCCTCGA-GGTGTA--AAAA-----ACTCGTAAATCCTA-TCAGATCTG----GAAGATT-----TCT--ACGC--T-----T-CTCC-TTCTTT-
ATAT

>Agr-1_MIR168a

GATAGACCAATCT-----TTACCATATTTT-CACCATT----AACACTC-T-----AC-AAAACCTAAA---ACTCTTC-TCTCTCTCCTC-TTTTTTCACTTTCTCTC-----TACA-----
TAGAGAGA-----TTGAT----AAAG---TCACCGTCGAGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCTGATT-----CCACGTGGCGTTTT--TGTATTGGTT--
TGTGAGATTAGAGAAATTTAGTTCCCGCTTTCATCAACTGAATCGGAAC-TTGTGTTGAATGAAAAA-----TTGAAATCAAA-TCAGATCTGGT--GGAGATA-----TCT-----
TACTC-TCTCTTT-CTTT

>Aly_MIR168a

GATAGGGTTCCTC-----CTTATTACATC---ATAAACCTATT--CTC-TGCCTCATT--CCCCATTAC-AAAACCTAAA-----TCTCCTTCTCTTCTCTCTT-----CT-----TCT-----
AAAGAGAGAGAGAGAGAG-----ATT-ATGATAT--AAGAG--TCTCACCCTCGGGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCTCGTGACTTTAAACCTTTATTGGTT--
TGTGAGC--AG-GGA--TTGGATCCCGCCTTGCATCAACTGAATCGGATCCCCGA-GGTGTA--GAAA-----ACTCGTGAATCCAA-TTAGATCTA----GAAGAAA-----TCT--ACTC--T-----T-
CTCC-TTCTTT-ATCT

>Cal_MIR168a

GATAGG-----TTTTATTA-CATC---ACAAACCATTCTTTTGCCTCATTTCACCACCATTAC-TACAGCTTCA-----TCTCTTATCTTTCTTCTCACAAGCCTTCG-----
TTTCAGTTTCTTGAGAGCGGAGGAGAGATTACG--ATAGATGAT----AGAG---TCACCGTCGAGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCACGTGACTTAT---
CCTCATTTGGCT--TGTGAGC--AG-GAA--TTGGATCCCGCCTTGCATCAACTGAATCGGAGCTCCGA-GGTGAA----AGAA-----ACTCAAAATCCAAA-TCGGATCTG-----GATGATT-----
TAGAAGAAATCT--TCTC--T-----T-TTCT-TTCTTT-TTA

>Cfl_MIR168a

GATAGGGTTCCT-----A-CATC--ACACAACCATTCTTTTGCCTCATT--CACCATTTC-AACAACCTCT-----CTCTTCTCTTATCTTTTCTTCTCACAAGTCTTCG-----
TTTCAGTTTCTTGAGAGCA-AGAAAGA-TTACG---TTAGATGAT----AGAG---TCACCGTCGAGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCACGTGACTTAAT--
CCTCATTTGGCT--TGTGAGC--A-AGAA--TTGGATCCCGCCTTGCATCAACTGAATCGGAGCTCTGA-GGTGAA----AGAA-----ACTCAAAATCCAAA-TCAGATCTG-----GATGATT-----TTT-----

>Cgr_MIR168a

GATAGGGTTCCTTCTTCTTCTTCTTCTTCTTCA-CATCAATAAAAAACCATT--CTTTGCCTCGTT--CTCCCATCTAAAAACCTAAA----GGGAGACTCCTTCTCCTCCTTATCTCTC-----TCTCTG-----
-----TCT-----AGAGAGA-TTCTT--ATATATAAACAGCGAGAG--TCTCACGGTCGGGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTTGGC-TGA-----CGTGAGTCGTCT---
CTTTATTGGTTTTGTGTCAGC--AGAGGA--TTGGATCCCGCCTTGCATCAACTGAATCGGAGCCCTCGACGTGAAT---AAAAAACATTCCATACTCACTAATCCAA-TCAGATCTG----GACC-----
TAT--ACTCGTT-----T-CTCC-TTCTTT-TTA-

>Chi_MIR168a

GATAGGGTTCCT-----TA-CATCA-CAAAAACCATT-CTTTGCCTCATT--CACCATTAC-AACACCT-----TCCTTCTCTTATCTT-----TCTTCG-----
TTTCAGTTTCTTGAGAGCAATAAAAAGATTTAC--GATAGATGAT----AAAG---TCACCGTCGAGATCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCACGTGACTTAAT--
CCTCATTTGGCT--TGTGACC--AG-GAA--TTGGATCCCGCCTTGCATCAACTGAATCGGATCTCTGA-GGTGAAA----TAAA-----CTCAAAATCCAAA-TCAGATCTG-----GATGGTT-----
TTTGAAGAAATCTGAAATC--T-----T-CTCC-TTCTTT-TTAT

>Cim_MIR168a

GATAGGGTTCCT-----A-CATCA---CAACCATT-CTTTTGCCTCATT--CACCATTAC-AAAACCT-----TCTCTTCTTATCTCTTCTTCTC-ACAAGTCTTCG-----
TTTCAGTTTCTTGAGAGCA-AGAAAGA-TTACC---ATAGATGAT----AGAG---TCACCGTCGAGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCAATTCGGC-TGA-CACAGCCACGTGACTTAT---
CCTCATTTGGCT--TGTGAGC--AG-GAA--TTGGATCCCGCCTTGCATCAACTGAATCGGAGCTCCGA-GGTGAAA----TGAA-----ACTCAATATCCAAA-TCAGATCTG----GAAGATT-----
TAGAAGAAATCT--ACTC--T-----T-CTCC-TTCTTT-CTAT

>Cir_MIR168a

CATAGGGTTCCT-----A-ATTC---ACAAA-CACT--CTTTGCCTCCTTT--CGCCATCCC-AAA-CCTAAA-----
CTTCTCTCTCTCTTCTTCTTCTCTCTGTTCTGTCTTCTGTTCTTATTTTGTTCCTCAGATTTCT-----AGAGAGAGA-TTG-----TGTGAT----AGAG----
TCACGGTTCGGCTCGGATTCGCTTGGTGCAGGTTCGGGAACCTGATT-GGC-TGA-CACAGCCACGTGGATT---CCCATATTGGCA---TGTGAGC--AG-GGA--
TCTGCTCCCGCCTTGCATCAACTGAATCGGAGCCCGATTGTGCAAA---CAAA-----TATCAAAAATCAAA-TAAGATCTGATTTGGGAGATA-----TAT--ACTC--T-----CCT-CTCC-TATTTC-ATTT

>Dte-2_MIR168a

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-----AGAGAC-----TATGGT-----AGAG---TCACCGTCGGGCTCGGATTCGCTTGGTGCAGGTTCGGGA-CTAATC-CGC-TGA-CACAGCCACGTGCTTTT---ACTTATTGGTT---GTGAGC--AG-GGA--
TTGGCTCCCGCCTTGCATCAACTGAATCGGAGCTCCCATGGTGAAGC--AAAA-----GCCAAAAAGCCAA-TCAGATCTG----GAAAAATATATCTTTGTGTGTGTCT--ATGT--TGATGCATAT-CTTC-
TTCTGT-ATAT

TTACCGGCGGTCTCTGATTCGCTTGGTGCAGGTCGGGAACTGATTGGCTAATACCACCAC-GTGTCTTGTCATGGTTGGCTTGTAAGC-----
TCCCGCCTGCATCAACTGAATCCGAGTCCGAGGTGAAAATTTTTTT-----GGATCCAAAC---TTTCAGATC--TGACACTTCTTCGTT-ACTCTT-----AAGGTAAATTTTGAT-----
ATTTTGTTT---AT
>Cgr-1_MIR168b
ATTAAATACTCAGCAGAAGGAGACT--AAAGTAAGTCCCGATA---TTTGATAGAAAAA-----TCTTTGCCAGATT-----GGTAACTGTTTCTTTCTCTCTTTTCATCTTTTTTTTTTC-
TTTCTAATGAGAAAGAGAGAAAGAGAGA-----TACGATGAA--GGTTACAAAAAAAAG-TTACCGGCGGTTTCGGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTGACTCCGACAT-
ATGTTTTGTCATGGTTGGCTTGTGAGC-----TCCTGCCTGCATCAACTGAATCGGAGTCCGAGGTGAAAAATATGAAAAAA-----GGATCA-AAC---TTTCAGATC--TGACTCGATCTT----CATCTT--
-----ACGGTAGAATCTTTAT-----ATTTTTTGTGAAA
>Chi_MIR168b
GCTAAATACCAAGCA-AAGGAGGC--TAAGGTATTCTCGATA---TTTGATAAAGA-CT--AAATCTGAGAAAA-TA-TCCTTGCCAGATAT-----GGTCACTGTTTTCTAATTCTTCT-----
CCTCTTTCATATT-TTCCATTGAGAGAGATAAGAGCAAAAAG-----TACGATGAT--GATA-----AAG-
TTACCGGCGGTCTCTGATTCGCTTGGTGCAGGTCGGGAACTGATTGGCTAATACCACCAC-GTGTCTTGTCATGGTTGGCTTGTAAGC-----
TCCCGCCTGCATCAACTGAATCTGAGTCGGAGGTGAAAAAAATTT-----GGATCCAAAC---TTTCAGATC--TGACACGTTCTTCGTTCACTCTT-----AAGGTAAATATTGAT-----
ATTTGGTTT---AT
>Cim_MIR168b
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TTCCATTGAGAGAGATAGAAAAAAG-----TACGATGAT--GATA-----AAG-TTACCGGCGGTCTCTGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTAACACCACCAC-
GTGTCTTGTCATGGTTGGCTTGTGAGC-----TCCCGCCTGCATCAACTGAATCTGAGTCCGAGGTGAAAAAATAT-----GGATCC-AAC---TTTCAGATC--TGACACATTCTTCGTC-ACTCTT----
-----AAGGTAAATCTTGAT-----ATTTTGTTT---AT
>Cir_MIR168b
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TTACCGGCGGTCTCGGATTCGCTTGGTGCAGGTCGGGAACTGATTGACTGTCACCGCCAC-GTGTTTTCTCATGATTGGCTTGTGACCGGCGATCGGA--
TCCCGCCTGCATCAACTGAATCGGAGGCCGAGGTGAAAAGAAAA-----GGATCC-AACTTTTTTCAGATCTGTGGCTCAATCTTCTTCTGACTCTT-----AAGGTAGAAGCTTGAA-----
ATCTTGTTT--AAC
>Dte_MIR168b

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>Ech_MIR168b
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TTTCCAATGAGAGACAGAGAGAGAGA-----TACGATGAT--GGTTA-----AAG-TTACCGGCGGTCTCAGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTGACATCACCAC-
GTGTTTTGTCATGGTTGGTTGTGAGT-----TCCTGCCTGCATCAACTGAATCGGAGTCCGAGGTGAAAAA-----GAATCC-AAC---TTTCAGATC--TGACAT-----CCTCTT-----
---ATGGTACAATCTTGAA-----ATTTTTTTT---AT
>Mma_MIR168b
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GAGATACGATGA-----TG-GATGAT--TGTTA-----AAG-TTACCGGCGGTCTCAGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTGATACCACCAC-
GTGTCTTGAATGGTTGTCTTGTGAGT-----TCCAGCTTGCATCAAGTGAATCAGAGTCCGAGGTGAAAAGAA-----GTATGA-TAC---TTTCAGATC--TGACAC-----AATCTT-----
-ATGGTACAATCTTGAA-----ATTTTATTT---AC
>Ptu_MIR168b
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GATGAGAGAGAAGAAAGATGAGAGA-----TACGATGAT--GATA-----AAG-TTACCGGCGGTCTCTGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTGACACCGCCAC-
GTGTCTTATCATGGTTGGCTTGTGAGCAGTGCATCGGA--TCCCGCCTGCATCAACTGAATCGGAGCCCAGGTGAAAAGAAGAAGAAACCTAAAAA--GGATCT-AAC---TTTCAGATC--
TGACTCAATCTTTGTTCTCTT-----AAGGTATAATCTTGAATCAATCTTG--TTTATGTT-----
>Rau-1_MIR168b
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TTTCAATAAGAGAGAGAAAAAAG-----TACGATGAT--GATA-----AAG-TTACCGGCGGTCTCTGATTTCGCTTGGTGCAGGTCGGGAACTGATTGGCTGACGCCGACAC-
ATGTGTTGTTATGGTTGGCTTGTGAGC-----TCCCGCCTGCATCAACTGAATCGGAGCCCAGGTGAAAAAATTT-----GGATCC-AAC---TTTCAGATC--TGAGTCACTTCTTCTT-ACGCTT----
-----AAGGTATAATCTTGAT-----ATTTTGTTT---AT
>Tsa_MIR168b
GTTAAA-ACCCATCA-AAGGAGGC--TAATTAATTCTCGATA---TTTGATAG-----AGAGATAAAAA-TCTTTGCCAGATAT-----GGCAGCTGTTT-CTCGTTCTCTG-----TTCTCTTATCTT-
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