

## MICROSYNTENIC PHYLOGENETIC FOOTPRINTING OF *MIR168* LOCI IN BRASSICACEAE

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### Additional File 3

A) Primers used to isolate the *MIR168a* and *MIR168b* intergenic regions from Brassicaceae species. “Upstream” and “downstream” refer to transcription direction.

Gene	Primer	Description	Sequence 5' to 3'
<i>MIR168a/b</i>	K313	from mature miR168 towards upstream sequences	AATTAGTTCCCGACCTGCACCAAGC
	K353	from mature miR168 towards downstream sequences	GCTTGGTGCAGGTCGGGA ACTAATT
<i>MIR168a</i>	K315	from upstream gene towards mature miR	CCAAAGACCAACATCACAGTTCCTA
	K355	from downstream gene towards miR	TTGGTTCAGGTTCTCAAGCTATGG
	MaLoopStar_Cons1	from miR168a* towards upstream sequences	CGATTCAGTTGATGGAAGGCGGGA
	g014	from miR168a upstream IR <sup>(a)</sup> towards mature miR	GTACAGATCACGCTCACATGG
<i>MIR168b</i>	K320	from upstream gene towards mature miR	GCTCTCTTCACAGTGGTTGGTATCTC
	5453-6s	from upstream gene towards mature miR	CGAGTCTTTAACACCGCTGGCTC
	K359	miR168b from downstream gene towards miR	CACTTCTCCTTCTTCACATATCGTCA
	MbLoopStar_Cons1	from miR168b* towards upstream sequences	CGATTCAGTTGATACAAGGCGGGA

<sup>(a)</sup> IR= intergenic region

B) Primers used for *MIR168a* and *MIR168b* expression studies.

<b>Gene</b>	<b>Primer</b>	<b>Description</b>	<b>Sequence 5' to 3'</b>
<i>MIR168a</i>	Mac-P1_For	Forward primer for <i>pMIR168a::GFP-GUS</i> construct	CACCATGCTTGTGATGGTTCGATTC
	Mac-P1_Rev	Reverse primer for <i>pMIR168a::GFP-GUS</i> construct	AGCCCGATGGTGAGACTCTACTA
<i>MIR168b</i>	Mbc-P1_For	Forward primer for <i>pMIR168b::GFP-GUS</i> construct	CACCTCTCTTCACAGTGGTTGGTATCTC
	Mbc-P1_Rev	Reverse primer for <i>pMIR168b::GFP-GUS</i> construct	GGTACCGAGACCGCCGGTAACTTTAACAATC
	Mb_RACE_1Rev	<i>MIR168b</i> RACE primer	CGAGACCGCAGGTAACCTTTAAC
	Mb_RACE_2Rev	<i>MIR168b</i> RACE nested primer	CTTTAACAATCATCGCATCTCTC

C) Primers used for phylogenetic reconstruction.

<b>Gene</b>	<b>Primer</b>	<b>Description</b>	<b>Sequence 5' to 3'</b>
<i>ITS</i>	ITS-5_For	Forward primer for <i>ITS</i>	GGAAGTAAAAGTCGTAACAAGG
	ITS-2_Rev	Reverse primer for <i>ITS</i>	GATATGCTTAAACTCAGCGGG
<i>EIF3E</i>	EIF3E_For	Forward primer for <i>EIF3E</i>	TTTGAATGTGGCAACTAYTCTRGTGCTGC
	EIF3E_Rev	Reverse primer for <i>EIF3E</i>	ACCTCTTCACACTCYTTCATCTT