
Supplementary information

**R-loops at microRNA encoding loci
promote co-transcriptional processing of
pri-miRNAs in plants**

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Supplemental Data

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Supplemental Tables

Table S1. List of primers used in this study.

Gene (AGI)	Sequences (5' - 3')	Assay
pri-miR165a	CCATCATCACCATTACCAACC	qPCR
	CCTCAACTGAAATAGCTTAACCC	qPCR
	GTTGTCTGGATCGAGGATATTATAG	qPCR
	GTCCGAGGATACTCTCTATGATC	qPCR
	ACATGTTATTGCCTCTGATCACC	qPCR
	GCAAGAAAGATTCAAAGTCATCAC	qPCR - 5'RACE
pri-miR166b	GGATCTGTTGGGGGACGAAC	qPCR
	CCTCAAAAGAAAAATCCCTC	qPCR
	GGCTCGAGGACTCTTATTC	qPCR
	CCGACGACACTAAAACCC	qPCR
	CAATTATCACTCCCTCACAATCC	qPCR
	CACATGGATTCATAGATAGAAACC	qPCR
pri-miR168a	CATATCATAAACCTCATTTCCCA	qPCR
	CGAGCCCGATGGTGAGACTC	qPCR
	GGAACCAATTCGGCTGACAC	qPCR
	GGGATCCAATCCCTGCTCAC	qPCR
	TCCTCGAGGTGTAAAAAACTCG	qPCR
	GAGAAGCGTAGAAATCTTCCAG	qPCR
pri-miR159a	TGGTAGAGCTCCTTAAAGTT	qPCR
	TAAAGCTCCTGAGATATGCA	qPCR
	GGTCTTTACAGTTTGCTTATG	qPCR
	TAAAGCTCCTGAGATATGCA	qPCR
	CGACTCTCTATCTATCATTTCTTCC	qPCR
	GAAAGTTTTGATGAGAACGTGG	qPCR
pri-miR156a	GTGAGCACGCAAGAGAAGCAAG	qPCR
	AAGAACTGACAGAAGAGAGTGAG	qPCR

	GAGAACGAAGACAGGCCAAAG	qPCR
	AAGAACTGACAGAAGAGAGTGAG	qPCR
	ATCTTGTAGATCTCTGAAGTTGG	5'RACE
pri-miR408	GACAGGGAACAAGCAGAGCATGG	qPCR
	GAGACAAAACAGAGTCGTTTAATG	qPCR
	GAACTAACTCAAAGGAACTGGC	qPCR
	GAGACAAAACAGAGTCGTTTAATG	qPCR
	CTCTCTCATTACCGCTTTGTCTTC	qPCR
	CATGCTCTGCTTGTTCCCTGT	qPCR
pri-miR402	GGTTCATCGGAAGGAGTTAGC	qPCR
	GCAACTCAAACCTTATCTACCACG	qPCR
	GGTTCATCGGAAGGAGTTAGC	qPCR
	GAATCTGCTGTTGGAATTGAGGC	qPCR
	CATGAAAATATGGGTAAACAAACAAAGG	qPCR
pri-miR160b	CTCTGGTTCATGTTTTCCCC	qPCR
	GAACCCTTAAATATGATTGGTGG	qPCR
	CTCTGGTTCATGTTTTCCCC	qPCR
	TGCTTGACTACTCTGTACG	qPCR
pri-miR166a	TAACAAGGGTTCATTCACTGG	5'RACE
	CTGGCTCGCTCTATTCATGTTGG	qPCR
	GACGCTAAAACCCTAATCAAATC	qPCR
	GGGACGAACATAGAAAGAGAGAG	qPCR
	GCCCCTTTTTCTTTTCAGTCG	qPCR
pri-miR164b	CAGACAAATCATACCCCCAAGG	qPCR
	CACACCTTCATCATTCTCTCCG	qPCR
	CCACAAATGCGTGTATATATGC	qPCR
	CTAACTCATCCATATCATCACACTC	qPCR
MIR319b	TCTAGCACGCACAGAGAGG	qPCR
	CCATGAGTGGACCGAAGAAAGC	qPCR
MIR171a	TTTCCTTTGATATCCGCACTTTAAG	qPCR
	GATATTGGCGCGGCTCAATC	qPCR

SEPALATA3	TTCCTTGAAGCAGATCAGAGC	qPCR
	TTAGGGTTCAGCTGGAGTGG	qPCR
U6	CGGGGACATCCGATAAAATTGG	qPCR
	CGATTTGTGCGTGTCA TCCTTG	qPCR
pri-miR163 Intron	GTTAATGTTAGTAGTTAAAAAGGATTAGTG	Probe
pri-miR163 Exon	TACGTTATCTCTTTTCATCAATTAAACC	Probe
pri-miR163 Exon/Exon	CAAAAAATTTCCGTTATCTCTTTT	Probe
pri-miR163 Loop	GGA ACTCCAGCACTTTAGTATCATC	Probe
miR163*	GAAGAGGTTGGA ACTCGATTT	Probe
miR163	ATCGAAGTTCCAAGTCCTCTTCAA	Probe
pri-miR156a Intron	GCTAATCTCACTTA ACCACGCG	Probe
pri-miR156a Exon	AGAGAGATTGAGACATAGAGAACG	Probe
pri-miR156a Exon/Exon	ACCCCTTACCTTAATATGG	Probe
pri-miR156a Loop	TGAGCACGCAAGAGAAGCAAGT	Probe
miR156a*	TGACAGAAGAGAGTGAGCA	Probe
miR156a	GTGCTCACTCTCTTCTGTCA	Probe
pri-miR156a Intron sense	GCTAATCTCACTTA ACCACGCG	Probe
pri-miR414 Exon	GTACACGTAAATCCATATGTATGTGTATGT	Probe
pri-miR393a	CCCGGTGAAGTATCCATGATAG	Probe
pri-miR156a INTRON_1	CAAGCCAGAGTTTAGATCGT	Stellaris probes
pri-miR156a INTRON_2	CATGCAAGTAGATGGCACAA	
pri-miR156a INTRON_3	TAAAGGCTAAAGGTCTCCTC	
pri-miR156a INTRON_4	TTTTTCGATACTACCCATCT	
pri-miR156a INTRON_5	ACCACGCGTTAACTAATAGA	
pri-miR156a INTRON_6	GGTTACATTGCTAATCTCAC	
pri-miR156a INTRON_7	AGGACCAGCCTATTAACAAC	
pri-miR156a INTRON_8	CTCGAGAGAGACAGAGTGGA	
pri-miR156a INTRON_9	CCTTAACATCTTATTCACGC	
pri-miR156a INTRON_10	AGCAACGACTCATTCTTATA	
pri-miR156a INTRON_11	GATGTACGAGCAAACCATTT	
pri-miR156a INTRON_12	AAGCTAATCATGCACCTCAT	
pri-miR156a INTRON_13	TCTCGCAATATTCATGTACA	

pri-miR156a INTRON_14	CCAGGGAACATGTTCTCAAG	
pri-miR156a INTRON_15	GTGTGTTTGAGAGATGCAGT	
pri-miR156a INTRON_16	CATACATTATTGGCTGCTGT	
pri-miR156a INTRON_17	CTTGTGCTACCTATTCAACA	
pri-miR156a INTRON_18	GTGGGAAGACATGACACATO	
pri-miR156a INTRON_19	TATTTTGTGCGACAAACACCA	
pri-miR156a INTRON_20	GACTCACTTACCCAAGTTAA	
pri-miR156a INTRON_21	TCATCGTGATTTTTAGTCCA	
pri-miR156a INTRON_22	GATACACATATTTGACCACA	
pri-miR156a INTRON_23	CCAAATTTCCCAATCTCTTC	
pri-miR156a INTRON_24	GTTCACCAATATTCCATGTC	
pri-miR156a INTRON_25	GCTGGAACCAACAACCTCTGA	
pri-miR156a INTRON_26	TGTGTTTGATTTTGCGTTTT	
pri-miR156a INTRON_27	ACTCTCATTTGAACATTCTT	
pri-miR156a INTRON_28	GCGTTTAGAATTGCATTTC	
pri-miR156a INTRON_29	TCTTCGATCAGAGITTGTCG	
pri-miR156a INTRON_30	TCCTACATATATCTCCGTG	
pri-miR156a INTRON_31	ACGCATTTACGAACTGTGTA	
pri-miR156a INTRON_32	GGTCTGAAAGATCCATGTGA	
pri-miR156a INTRON_33	TCTTTGCAAAGGACGACTGT	
pri-miR156a INTRON_34	CTGTAGCTTCTCTTTTTTT	
pri-miR156a INTRON_35	GTCTACTTCTTTTTTTTTTC	
pri-miR156a INTRON_36	ACCGTGAAGAATCCTACACA	
pri-miR156a INTRON_37	GTTCTTTGTAAGAAAGGGGA	
pri-miR156a INTRON_38	ATGAGTGATCTTGCAAGGAA	
pri-miR156a INTRON_39	TGCTGAAGTATGGATCTTCA	
pri-miR156a INTRON_40	GTTTGAATGGAGCTTAGACA	
pri-miR156a INTRON_41	ACCTTTGCTATTTGATGCAC	
pri-miR156a INTRON_42	GTACAAGCACGATTCATATT	
pri-miR156a INTRON_43	ATATACATGAGTGTGTGTGT	

Table S2. IDs of the datasets used in this study.

Study	Ref.	Series Accession	Samples analyzed	Description

Transient genome-wide adaptations of nascent RNAPII transcription triggered by low temperature	{Kindgren, 2020 #56}	GSE131733	GSM3814845 GSM3814846 GSM3814849 GSM3814850	Plant native elongating transcripts sequencing (plaNET-Seq) was used to determine the genomic positions of transcriptionally engaged RNAPII in Col-0 seedlings which were exposed to cold (4 °C) for 0h, 3h or 12h.
Organismal Benefits of Transcription Speed Control at Gene Boundaries	{Leng, 2020 #57}	GSE133143	GSM3900879 GSM3900880 GSM3900881 GSM3900882	Plant native elongating transcripts sequencing (plaNET-Seq) was used to determine the genomic positions of transcriptionally engaged RNAPII in wild type and nrpb2-Y732F seedlings.
R-loop landscapes in Arabidopsis	{Xu, 2020 #71}	GSE116232	GSM3214368 GSM3214369 GSM3214344 GSM3214345 GSM3214346 GSM3214347 GSM3214348 GSM3214349 GSM3214382 GSM3214383 GSM3214328 GSM3214329	ssDRIP-seq in Arabidopsis to profile genome-wide R-loop levels providing a first-hand R-loop atlas during Arabidopsis development and in response to various environmental factors.
Genome-wide maps of R-loops in Arabidopsis	{Xu, 2017 #42}	GSE95765	GSM2525600	ssDRIP-seq in 12-day-old Arabidopsis seedlings for genome-wide identification of R-loops.

Table S3. Deeptools parameters used to scale different regions for metagen analyses using ComputeMatrix and plotProfile tools.

Mode	Genome regions	Score files (.bw) from:	--regionBodyLength	--upstream	--downstream	--binSize	Figure
scale-regions	pri-miRNAs (previously scaled and sorted by processing type)	PlaNET-seq (5' nt)	1000 bp	500 bp	500 bp	1	2B
scale-regions	pri-miRNAs (previously scaled and sorted by processing type)	PlaNET-seq (3' nt)	1000 bp	500 bp	500 bp	1	2D, S2B-C, 3A-D, S3A-D, S3F-H, S3J, 5C
scale-regions	Mature miRNAs (miRNA-3p or miRNA-5p)	plaNET-seq (5' nt)	20 bp	20 bp	20 bp	1	2C
scale-regions	Mature miRNAs (miRNA-3p or miRNA-5p)	plaNET-seq (3' nt)	20 bp	20 bp	20 bp	1	2G, S2D
scale-regions	Mature miRNA-3p	plaNET-seq (3' nt and whole_read)	40 bp	20 bp	20 bp	1	3E
scale-regions	pri-miRNAs (previously scaled)	PlaNET-seq (3' nt)	200 bp	100 bp	100 bp	1	3G, S3E
scale-regions	pri-miRNAs	PlaNET-seq (3' nt)	1000 bp	0 bp	0 bp	1	6G
scale-regions	pri-miRNAs	PlaNET-seq (3' nt)	1000 bp	500 bp	500 bp	1	6H
scale-regions	Two windows: 1) TSS to the nucleotide before miRNA-5p 2) pri-miRNA (previously scaled)	ssDRIP-seq	1) 1000 bp 2) 1000 bp	1) 300 bp 2) 0 bp	1) 0 bp 2) 300 bp	Default: 10	6B, 6D, S6

scale- regions	TSS to the nucleotide before miRNA-5p	ssDRIP-seq	1000 bp	300 bp	0 bp	Default: 10	6l
scale- regions	Coordinates regions including polycistronic miRNA duets or miRNAs encoded within protein coding genes	ssDRIP-seq	1000	300 bp	300 bp	Default: 10	6G-H