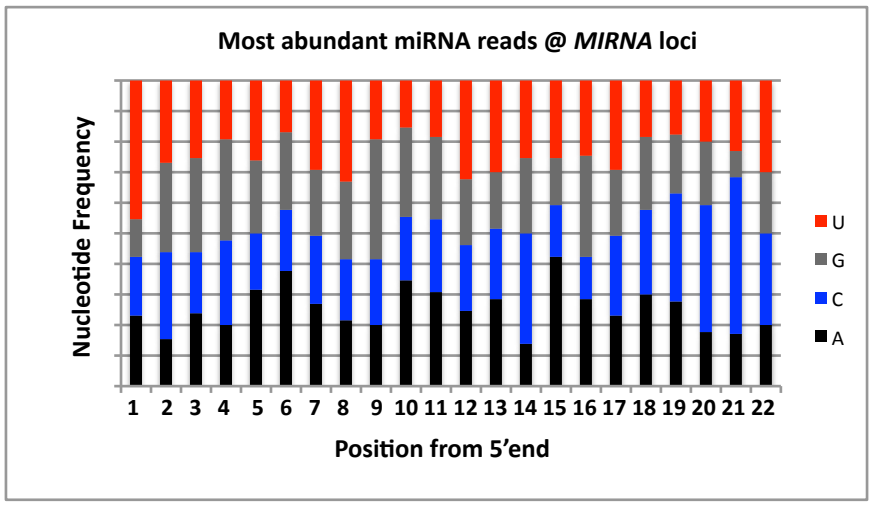
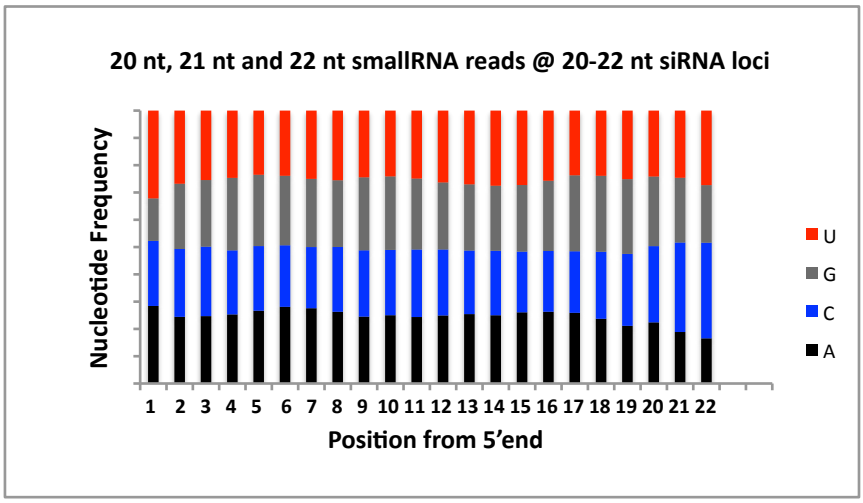
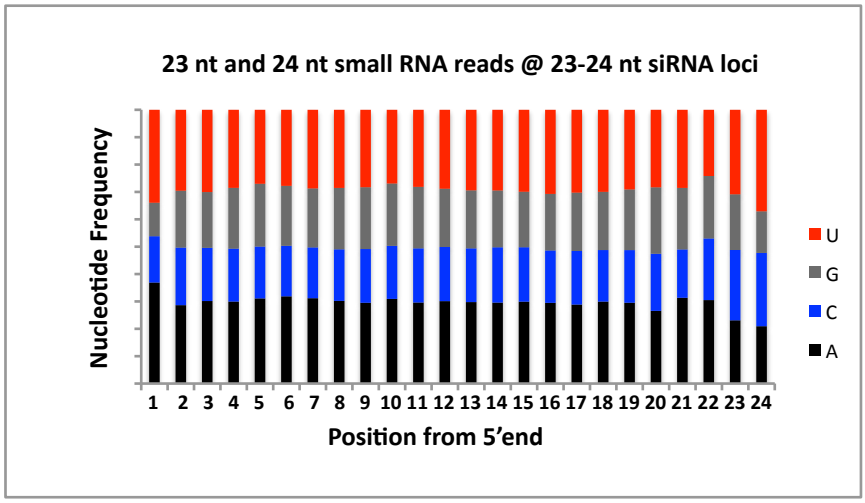
A



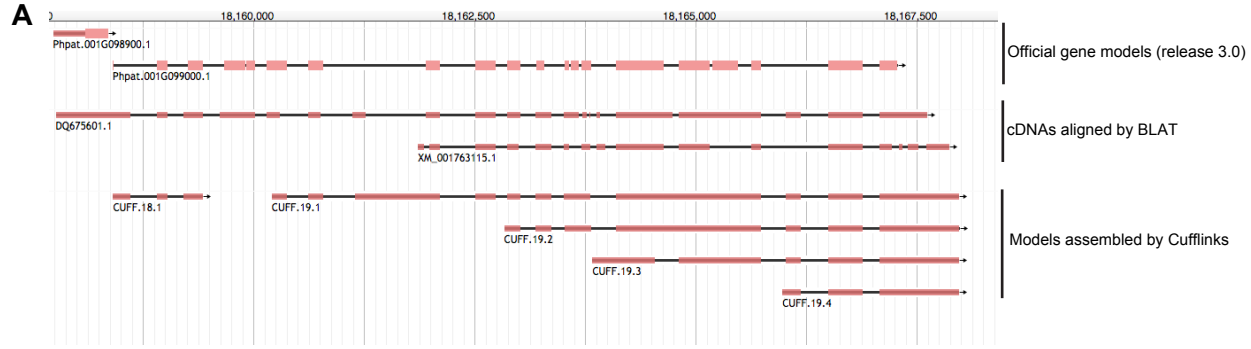
B

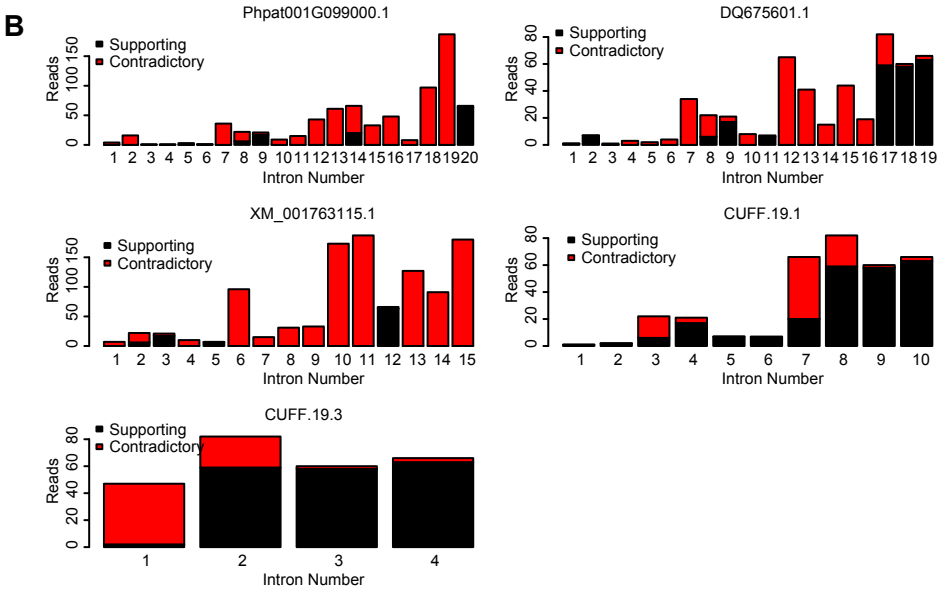


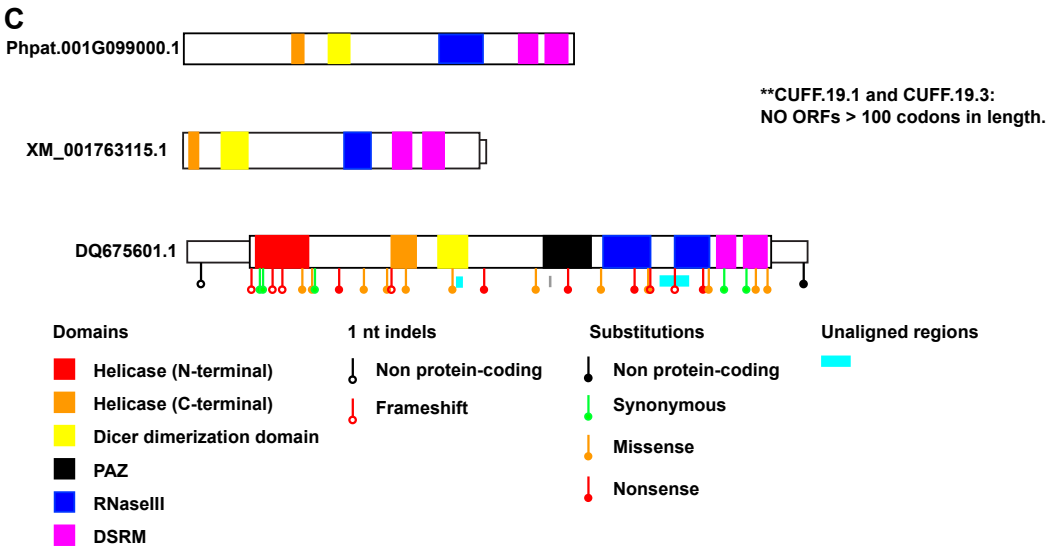
C



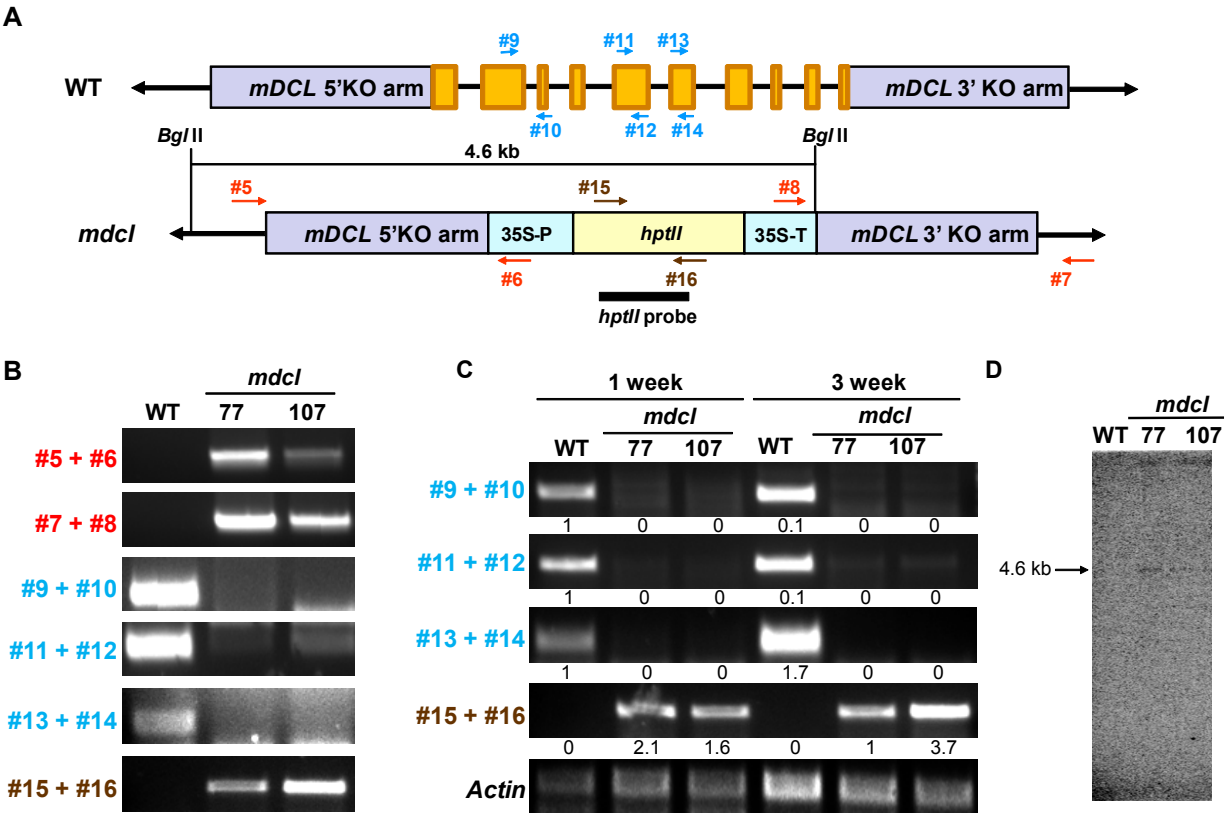
Supplemental Figure 1. Nucleotide frequency of *Physcomitrella patens* small RNAs for each position. Only distinct small RNA sequences were considered in this analysis.
Frequency of each nucleotide for each position in **(A)** most abundant miRNA reads at *MIRNA* loci **(B)** small RNA reads mapped to 20-22 nt siRNA loci and **(C)** 23-24 nt siRNA loci.


A
18,160,000
18,162,500
18,165,000
18,167,500
Phpat.001G098900.1
Phpat.001G099000.1
DQ675601.1
XM_001763115.1
CUFF.18.1
CUFF.19.1
CUFF.19.2
CUFF.19.3
CUFF.19.4
Official gene models (release 3.0)
cDNAs aligned by BLAT
Models assembled by Cufflinks

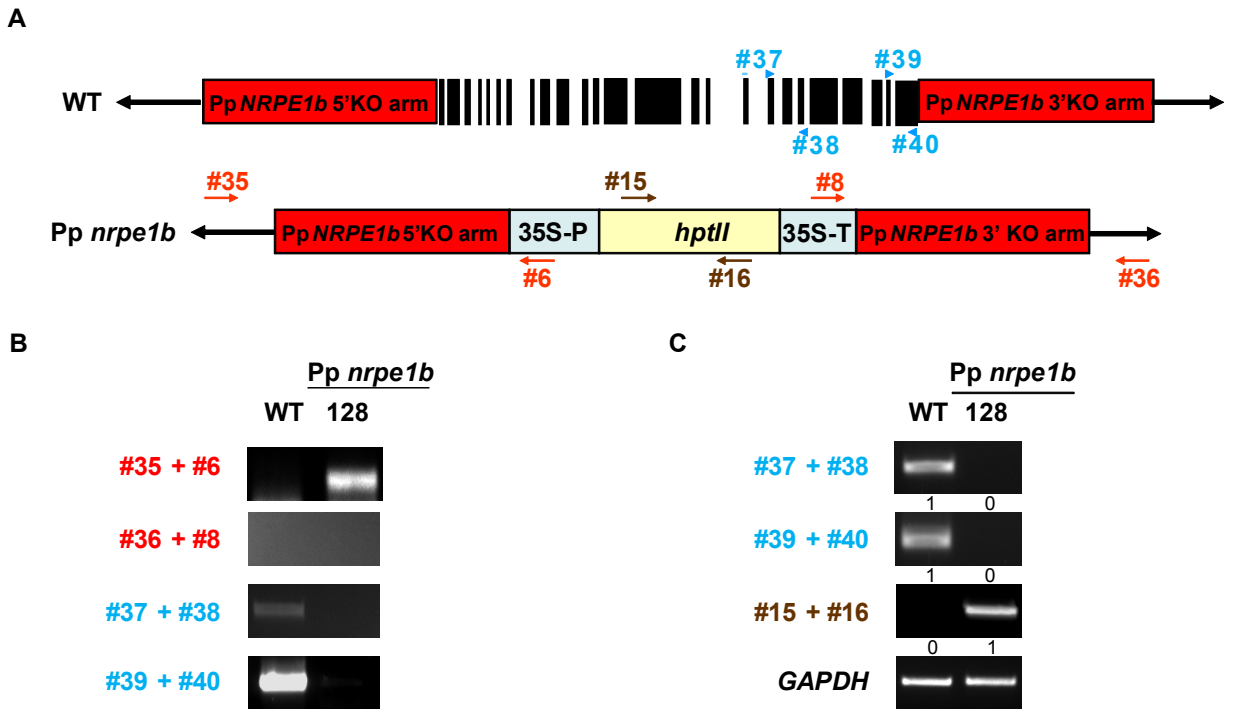

B
Phpat001G099000.1
DQ675601.1
XM_001763115.1
CUFF.19.1
CUFF.19.3
Supporting
Contradictory
Reads
Intron Number


C
Phpat.001G099000.1
XM_001763115.1
DQ675601.1
**CUFF.19.1 and CUFF.19.3:
NO ORFs > 100 codons in length.
Domains
Helicase (N-terminal)
Helicase (C-terminal)
Dicer dimerization domain
PAZ
RNaseIII
DSRM
1 nt indels
Non protein-coding
Frameshift
Substitutions
Non protein-coding
Synonymous
Missense
Nonsense
Unaligned regions

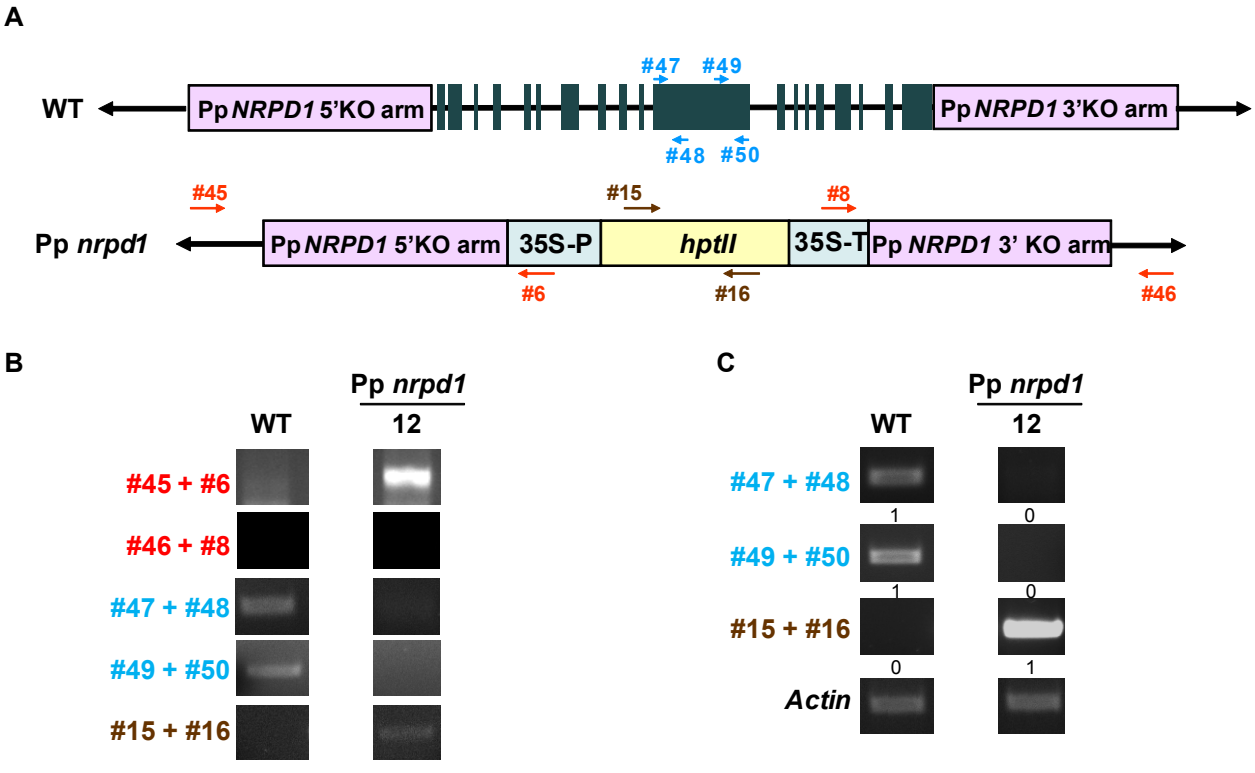
Supplemental Figure 2. Discrepancies in Pp *DCL1b* annotations.
(A) Genome-browser snapshot of the *DCL1b* locus, showing various mRNA models. The region is roughly Chr01:18157500..18167930 **(B)** Barplots showing tallies of supporting and contradictory RNA-seq reads for each intron of the indicated gene models. RNA-seq data were from a merger of SRR435295 and SRR435296 (Chen et al., 2012), aligned to the reference genome using tophat (version 2.0.9) with non-default setting -I 5000 (using bowtie2 version 2.1.0 as the underlying alignment engine). Contradictory reads were those which had at least one base aligned to the intron, while confirmatory reads were those which spanned the annotated splice sites exactly. The RNA-seq alignments are downloadable from http://plantsmallrnagenes.psu.edu/Physcomitrella_patens/data/RNA-seq/Chen_et_al_WT.bam Note that the majority of introns for the first three models are unsupported by the empirical data. **(C)** Schematics, to scale, of inferred protein sequences from the indicated mRNA models. Protein domains were based on searches of the PFAM database (version 27; http://pfam.xfam.org/) under default parameters. mRNA model DQ675601.1 had multiple mismatches to the reference genome. The consequences of those edits are shown. Note that none of the gene models can make a full-length functional DCL protein. Phpat001G09900.1 and XM_001763115.1 lack the N-terminal Helicase domain, the PAZ domain, and one of the RNase III domains, while DQ675601.1 contains multiple frameshifts and premature stop codons.None of the the Cufflinks-assembled mRNAs have any ORFs longer than 100 codons.



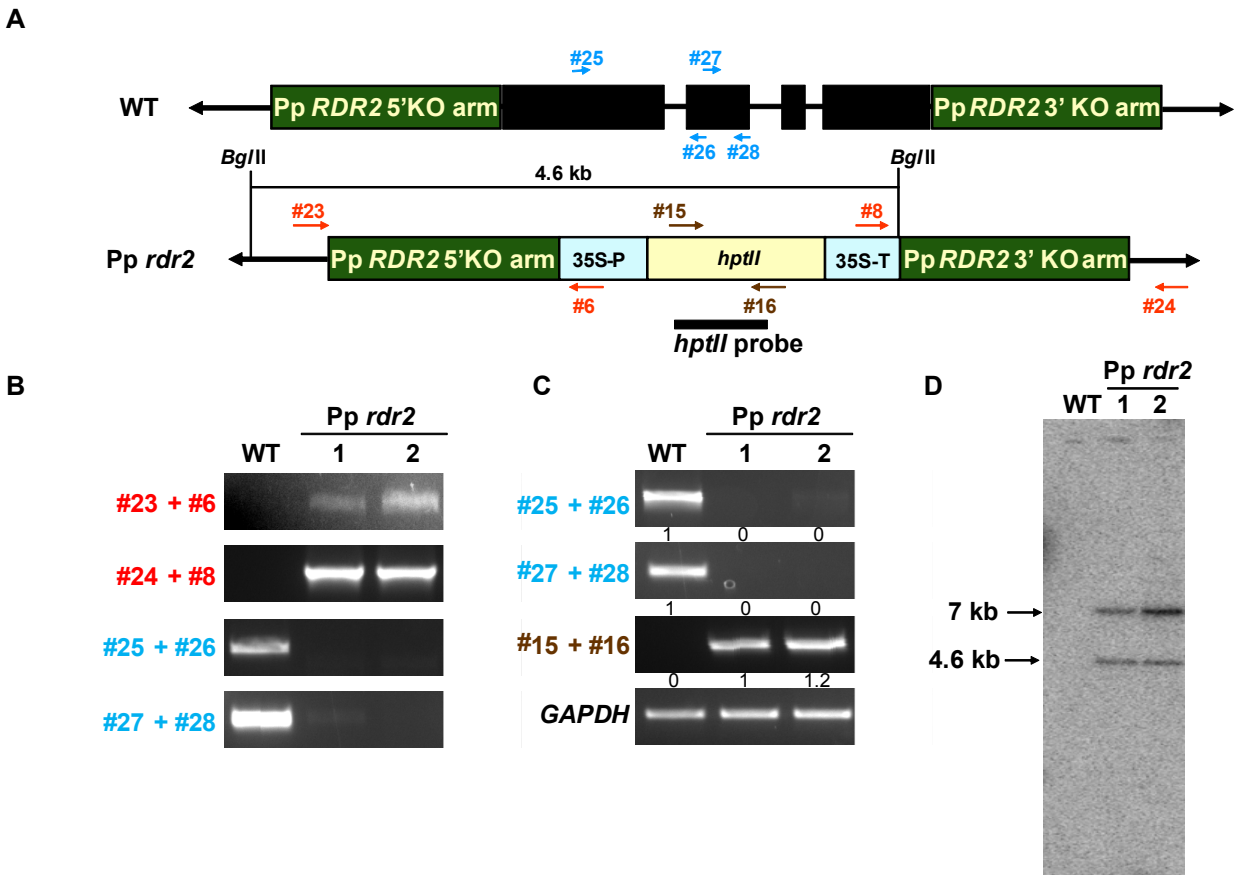
Supplemental Figure 3. Targeted knock-out of *mDCL*.
(A) Schematic of *mDCL* knock-out by homologous recombination. 77 and 107 correspond to different alleles of the *mdcl* mutant. The numbered arrows indicate approximate locations of primers (Supplemental Table 3). 35S-P, CaMV 35S promoter; 35S-T, CaMV 35S terminator. **(B)** Genotyping of transformed plants by genomic DNA PCRs using the indicated primer sets. **(C)** Transcript analysis by RT-PCR using indicated primer sets. *Actin* served as a control. Numbers below PCR fragments indicate relative (to *Actin*) intensities of bands compared to 1-week old WT, except for primer set 15-16, which was normalized to the value of 3-week *mdcl* line 77. **(D)** DNA blot analysis of *mdcl* mutant plants. BglII digested genomic DNA was blotted and hybridized with an *hptII* probe.



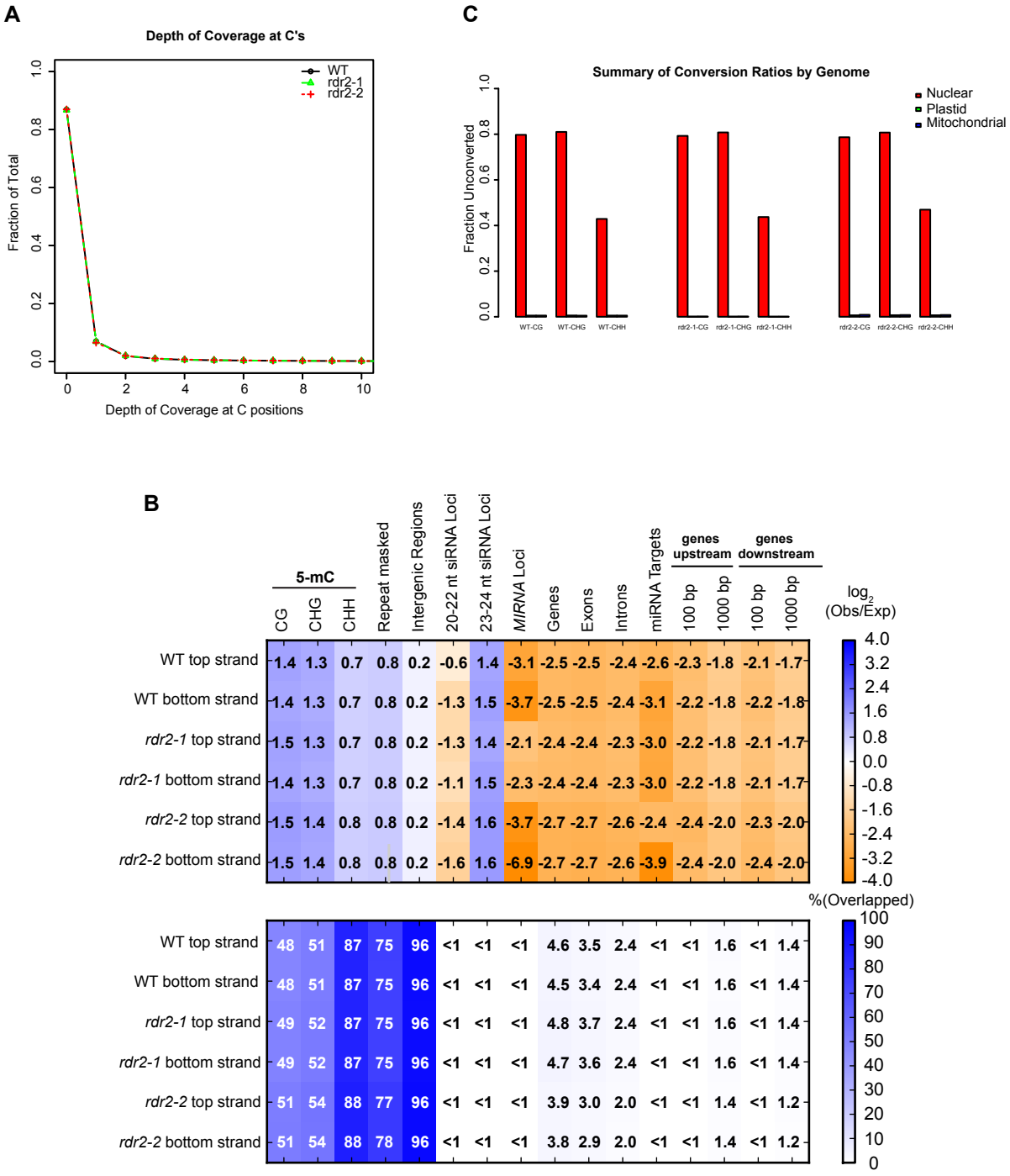
Supplemental Figure 4. Targeted knock-out of Pp *NRPE1b*
(A) Schematic of Pp *NRPE1b* knock out by homologous recombination. The numbered arrows indicate approximate locations of primers (Supplemental Table 3). 35S-P, CaMV 35S promoter; 35S-T, CaMV 35S terminator. **(B)** Genotyping of transformed plants by genomic DNA PCRs using the indicated primer sets. '128' referes to the isolated mutant line. **(C)** Transcript analysis by RT-PCR using indicated primer sets. *GAPDH* served as a control. Numbers below PCR fragments indicate relative intensity (normalized to *GAPDH*) compared to that of WT (primer sets 37-38 and 39-40) or mutant (primer set 15-16).



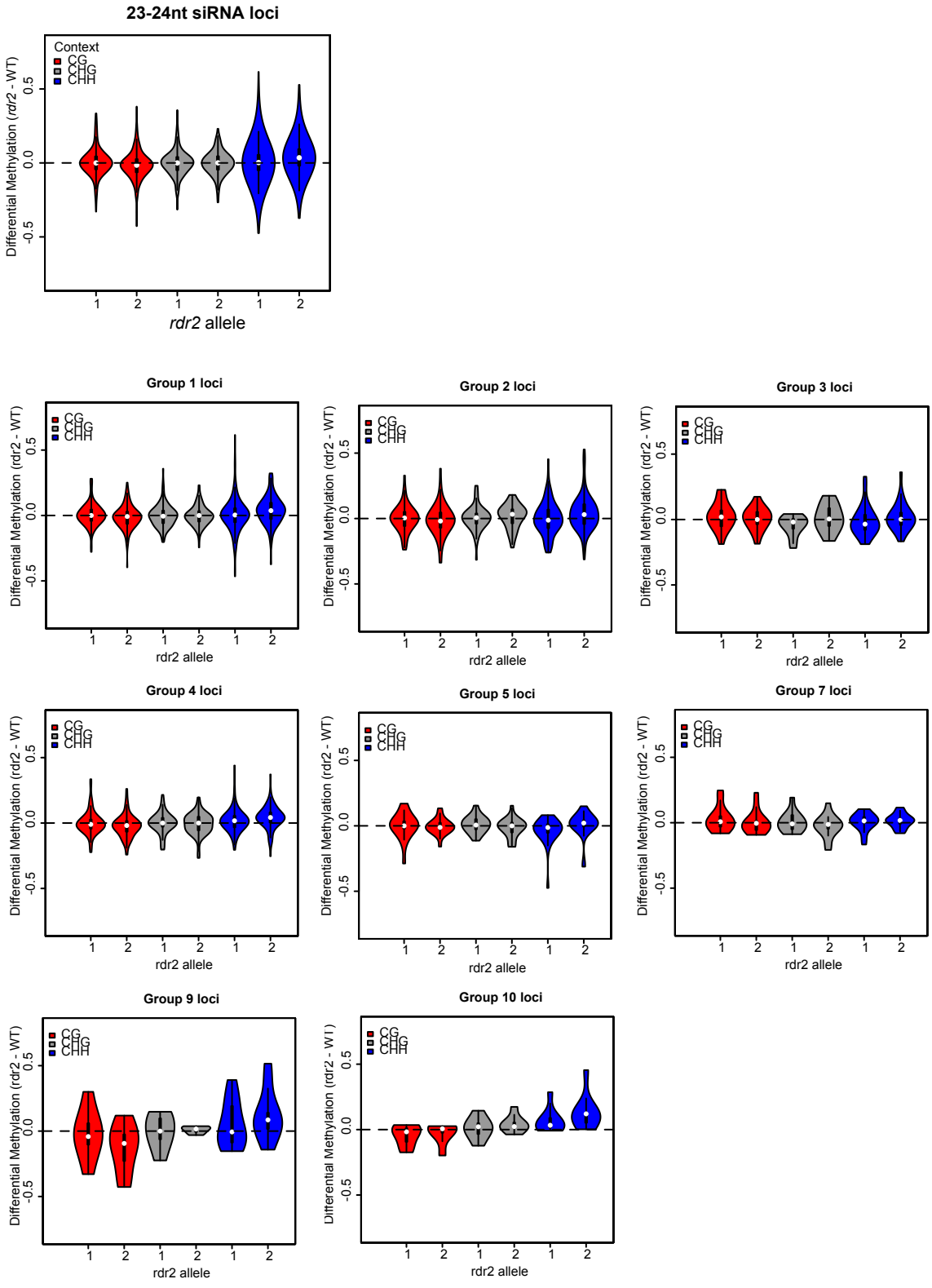
Supplemental Figure 5. Targeted knock-out of Pp *NRPD1*.
(A) Schematic of Pp *NRPD1* knock out by homologous recombination. The numbered arrows indicate approximate locations of primers (Supplemental Table 3). 35S-P, CaMV 35S promoter; 35S-T, CaMV 35S terminator. **(B)** Genotyping of transformed plants by genomic DNA PCRs using the indicated primer sets. Both 5' and 3' recombination in line 12 was confirmed. **(C)** Transcript analysis by RT-PCR using indicated primer sets. *Actin* served as a control. Numbers below PCR fragments indicate relative intensity (normalized to *Actin*) compared to that of WT (primer sets 47-48 and 49-50) or mutant (primer set 15-16).



Supplemental Figure 6. Targeted knock-out of Pp *RDR2*.
(A) Schematic of Pp *RDR2* knock out by homologous recombination. The numbered arrows indicate approximate locations of primers (Supplemental Table 3). 35S-P, CaMV 35S promoter; 35S-T, CaMV 35S terminator. **(B)** Genotyping of transformed plants by genomic DNA PCRs using the indicated primer sets. '1' and '2' refer to two independent lines **(C)** Transcript analysis by RT-PCR using indicated primer sets. *GAPDH* served as a control. Numbers below PCR fragments indicate relative intensity (compared to *GAPDH*) compared to that of WT (primer sets 25-26 and 27-28) or mutant line 1 (primer set 15-16). **(D)** DNA blot analysis. BglII digested genomic DNA was blotted and hybridized with an *hptII* probe. The 7kb band corresponds to the fragment size for the tandem repeat of the targeting cassette. This result shows the vector was inserted into a single site with a tandem repeat in the genome.



Supplemental Figure 7. Overview of bisulfite-seq libraries. **(A)** Overall depth of coverage at cytosines in the nuclear genome. **(B)** Heatmap showing log2 (observed overlapped bases / expected overlapped bases) for each of the pair-wise comparisons shown (top), and percentages of covered regions that overlap indicated regions (bottom). **(C)** Summary of conversion ratios by genome and library.



Supplemental Figure 8. Analysis of DNA methylation at 23-24 nt siRNA loci in Pp *rdr2* mutants. Differential DNA methylation between the indicated Pp *rdr2* alleles and the wild-type for the indicated loci. The 'groups' refer to groups shown in Figure 6D. Violin plots show a Tukey boxplot (line=median, box edges=1st and 3rd quartiles, whiskers=1.5 IQR) surrounded by a twinned kernel density plot to visually show the distribution of the data.

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Supplemental Table 1: *Physcomitrella patens* small RNA-seq libraries.

Library	Genotype	Strain	Number of Mapped Reads	GEO GSE	GEO GSM	Libraries Re-sequenced
1	Wild-type	Gransden 2004	29,985,916	GSE44900	GSM1093595	-
2	Wild-type	Gransden 2004		GSE44900	GSM1194292	Re-run of Library 1
3	Wild-type	Gransden 2004	31,363,125	GSE44900	GSM1093596	-
4	Wild-type	Gransden 2004		GSE44900	GSM1194293	Re-run of Library 3
5	Wild-type	Gransden 2004	10,487,292	GSE44900	GSM1194296	-
6	Wild-type	Gransden 2004	12,655,162	GSE44900	GSM1194297	-
7	Wild-type	Gransden 2009	19,593,167	GSE44900	GSM1093597	-
8	Wild-type	Gransden 2009		GSE44900	GSM1194294	Re-run of Library 7
9	Wild-type	Gransden 2009	24,811,895	GSE44900	GSM1093598	-
10	Wild-type	Gransden 2009		GSE44900	GSM1194295	Re-run of Library 9
11	rdr2-1	Gransden 2004	22,023,647	GSE51419	GSM1245155	-
12	rdr2-1	Gransden 2004		GSE51419	GSM1245157	Re-run of Library 11
13	rdr2-1	Gransden 2004	29,305,900	GSE51419	GSM1245156	-
14	rdr2-1	Gransden 2004		GSE51419	GSM1245158	Re-run of Library 13
15	rdr2-2	Gransden 2004	16,328,990	GSE51419	GSM1245159	-
16	rdr2-2	Gransden 2004	17,532,117	GSE51419	GSM1245160	-
17	rdr6-19	Gransden 2004	12,771,158	GSE51419	GSM1245161	-
18	rdr6-19	Gransden 2004	10,799,819	GSE51419	GSM1245162	-
19	rdr6-35	Gransden 2004	12,976,974	GSE51419	GSM1245163	-
20	rdr6-35	Gransden 2004	14,889,594	GSE51419	GSM1245164	-
21	dcl3-5	Gransden 2004	11,234,135	GSE51419	GSM1245131	-
22	dcl3-5	Gransden 2004	12,369,785	GSE51419	GSM1245132	-
23	dcl3-10	Gransden 2004	22,572,662	GSE51419	GSM1245133	-
24	dcl3-10	Gransden 2004	12,409,186	GSE51419	GSM1245134	-
25	dcl4-1	Gransden 2004	8,549,049	GSE51419	GSM1245135	-
26	dcl4-1	Gransden 2004	15,871,722	GSE51419	GSM1245136	-
27	mdcl-77	Gransden 2009	25,765,988	GSE51419	GSM1245137	-
28	mdcl-77	Gransden 2009		GSE51419	GSM1245141	Re-run of Library 27
29	mdcl-77	Gransden 2009	24,688,556	GSE51419	GSM1245138	-
30	mdcl-77	Gransden 2009		GSE51419	GSM1245142	Re-run of Library 29
31	mdcl-107	Gransden 2009	39,984,388	GSE51419	GSM1245139	-
32	mdcl-107	Gransden 2009		GSE51419	GSM1245143	Re-run of Library 31
33	mdcl-107	Gransden 2009	25,387,913	GSE51419	GSM1245140	-
34	mdcl-107	Gransden 2009		GSE51419	GSM1245144	Re-run of Library 33
35	nrpe1b_128	Gransden 2004	29,803,961	GSE51419	GSM1245145	-
36	nrpe1b_128	Gransden 2004		GSE51419	GSM1245149	Re-run of Library 35
37	nrpe1b_128	Gransden 2004	26,708,434	GSE51419	GSM1245146	-
38	nrpe1b_128	Gransden 2004		GSE51419	GSM1245150	Re-run of Library 37
39	nrpd1_12	Gransden 2004	13,482,499	GSE51419	GSM1245153	-
40	nrpd1_12	Gransden 2004	15,407,223	GSE51419	GSM1245154	-

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Supplemental Table 2: Summary of whole-genome bisulfite-seq libraries

Sample	SRA Accession	Total Pairs	Pairs Passed Quality Control	Concordantly Aligned Pairs	Pairs flagged as PCR Duplicates	Accepted Pairs
Wild-type	*SRR2013850*	222,081,099	211,783,332	156,513,930	153,613,143	2,900,787
rdr2-1	*SRR2013877*	235,045,740	224,132,719	202,792,076	199,510,574	3,281,502
rdr2-2	*SRR2013879*	259,564,115	245,949,602	199,155,549	195,904,139	3,251,410

Supplemental Data. Coruh et al. Plant Cell (2015) 10.1105/tpc.15.00228

Supplemental Table 3. Oligonucleotide sequences used in this study.

Number	Use	Sequence (5'→3')
1	mDCL KO vector construction, 5'KO arm (Forward)	CGCCTAGGATTTAAATAGATGTGTATTAATTACACCAACAC
2	mDCL KO vector construction, 5'KO arm (Reverse)	CGAAGCTTAATGATGATACAGGGGTGACAACGG
3	mDCL KO vector construction, 3'KO arm (Forward)	CGAGATCTCTTTATAGAAGGCATCTAGGAAGTC
4	mDCL KO vector construction, 3'KO arm (Reverse)	CGACGCGTATTTAAATTACAATAGATTAATTTTCATACAAA
5	mDCL KO identification of checking for 5' recombination (Forward)	ACCTCCAACGAGATGAGAACTACGC
6	KO identification checking for 5' recombination, 35S Promoter Internal (Revei	AGATAGCTGGGCAATGGAATCCGA
7	mDCL KO identification of checking for 3' recombination (Reverse)	AATATCCGCGCAGGTTAAGTTCCTAGC
8	KO identification checking for 3' recombination, 35S Terminator Internal (Forv	GGGTTTCGCTCATGTGTTGAGCAT
9	mDCL KO genotyping (Internal Forward1)	GAAGCACTCGATGGTGGTGG
10	mDCL KO genotyping (Internal Reverse1)	ACTGCAGATGTTCGCCGTACGTAG
11	mDCL KO genotyping (Internal Forward2)	GGGCAAGTCATTGGACTCAAAACC
12	mDCL KO genotyping (Internal Reverse2)	CTTCCTCTTGGTACACCGCTC
13	mDCL KO genotyping (Internal Forward3)	GCATGTGAAGGGAACCACTCATAC
14	mDCL KO genotyping (Internal Reverse3)	CGTCTTGGTATTTAGCAGTTCAGC
15	Identification of hptII gene in mutants (Forward)	TGTTTATCGGCACTTTGCATCGGC
16	Identification of hptII gene in mutants (Reverse)	AGCTGCATCATCGAAATTGCCGTC
17	Actin (Forward)	ATCTGGAATGGTCAAGGCCGGTTT
18	Actin (Reverse)	TCATCTTCTCCCTGTTCGCCTTCG
19	RDR2 KO vector construction, 5'KO arm (Forward)	CAAGCTTGGGACAAGGGAAGAGGTTCTCAAA
20	RDR2 KO vector construction, 5'KO arm (Reverse)	AACTCGAGACACCCACCACATTCCTCAGTCAT
21	RDR2 KO vector construction, 3'KO arm (Forward)	CCAGATCTACTGCTACACAGCGAGGATTTCTG
22	RDR2 KO vector construction, 3'KO arm (Reverse)	CCACGCGTTCAAGCAATGGGATAGGAGGCCAA
23	RDR2 KO identification of checking for 5' recombination (Forward)	GAGAGATGCAGTTTCGCAGCAGTA
24	RDR2 KO identification of checking for 3' recombination (Reverse)	TGGCTATATGTATGGTAATAAGGGACC
25	RDR2 KO genotyping (Internal Forward1)	ACAATGATCAGGGCATGGATGGGA
26	RDR2 KO genotyping (Internal Reverse1)	ACCCGCTGCGAGCATATCTATCAA
27	RDR2 KO genotyping (Internal Forward2)	TGATAGATATGCTCGCAGCGGGTT
28	RDR2 KO genotyping (Internal Reverse2)	AAACCAAGCAGTCAACCATGTGCC
29	GAPDH F	CCTCTTGCAAAGGTGATCAACGAC
30	GAPDH R	ACCACACGGTTGCTGTAACCCCAC
31	NRPE1a KO vector construction, 5'KO arm (Forward)	GGAAGCTTCCGGAAGAATTTGGCTAATCCGCA
32	NRPE1a KO vector construction, 5'KO arm (Reverse)	GGCTCGAGCGAGCGATAAGCATTAAAGCAACG
33	NRPE1a KO vector construction, 3'KO arm (Forward)	GGAGATCTTGCGTGAAACCTATTTGAGATGGA
34	NRPE1a KO vector construction, 3'KO arm (Reverse)	GGACGCGTGCCACAAGTCCAAGACATTAGAACT
35	NRPE1a KO identification of checking for 5' recombination (Forward)	TCTGTTGTTGCTGATGCAGGTCAG
36	NRPE1a KO identification of checking for 3' recombination (Reverse)	GTGTCTTCAAGCTAGACATATTTAGAAATGG
37	NRPE1a KO genotyping (Internal Forward1)	GGGACAAATTTTCTTTTGTGTCAGTTA
38	NRPE1a KO genotyping (Internal Reverse1)	AATACCAAACCAAGTCTCTGTGAG
39	NRPE1a KO genotyping (Internal Forward2)	AACTTGGTGGCAGGCTTTCTGACG
40	NRPE1a KO genotyping (Internal Reverse2)	TCAAGATCCTCATGATCAATAGGC
41	NRPD1 KO vector construction, 5'KO arm (Forward)	GGCCTAGGTGTCATTTAGGATAGTGCGGG
42	NRPD1 KO vector construction, 5'KO arm (Reverse)	GGCTCGAGCCTTCAAGCACAAAAACAAAG

43	NRPD1 KO vector construction, 3'KO arm (Forward)	GGAGATCTGATTGGTTACCTTCGCAATGCCAT		
44	NRPD1 KO vector construction, 3'KO arm (Reverse)	GGACGCGTGCAATTTGATGGCTCCTTGT		
45	NRPD1 KO identification of checking for 5' recombination (Forward)	TGTGAAGGCAGTTAATGGTGA		
46	NRPD1 KO identification of checking for 3' recombination (Reverse)	GGAGATGGATACTATGATTGATGG		
47	NRPD1 KO genotyping (Internal Forward1)	AGATACATGAAGGGGCATATTTTAGC		
48	NRPD1 KO genotyping (Internal Reverse1)	GTCGTTCAATATTAAAAGCCGTGAC		
49	NRPD1 KO genotyping (Internal Forward2)	TTGGATAAGGTTGCTGTCGATAGG		
50	NRPD1 KO genotyping (Internal Reverse2)	ACCATACCGTGATGATAAAGTGTG		
51	Small RNA gel blot of ppt-miR156 (probe)	GTGCTCACTCTCTTCTGTCA		
52	Small RNA gel blot U6 (probe)	TTGTGCGTGTCATCCTTGCGCA		
53	Small RNA gel blot SBP3 up target region F (probe)	GTATCCCTGCCCTTCAACTTCAGGTTGGTTTTATGTTTGTCGAAACAGCT		
54	Small RNA gel blot SBP3 up target region R (probe)	AGCTGTTTCGACAAACATAAAACCAACCTGAAGTTGAAGGGCAGGGATAC		
55	Small RNA gel blot SBP3 down target region F (probe)	TGAGTCTGTGGGGCTGAATTGTGGGCTAGCTGCGACTGGTTACGGGGCTC		
56	Small RNA gel blot SBP3 down target region R (probe)	GAGCCCCGTAACCAGTCGCAGCTAGCCCACAATTCAGCCCCACAGACTCA		
57	Small RNA gel blot HD-ZIPIII up target site F (probe)	CAACGCAAGGAAGCAACAAGGCTGGTCAGTGTTAATGCAAAGCTGACAGC		
58	Small RNA gel blot HD-ZIPIII up target site R (probe)	GCTGTCAGCTTTGCATTAACACTGACCAGCCTTGTTGCTTCCTTGCGTTG		
59	Small RNA gel blot HD-ZIPIII down target site F (probe)	GATTACTGTACTTTGAGATACACTACAATTTTGGAGGATGGAAACCTGGT		
60	Small RNA gel blot HD-ZIPIII down target site R (probe)	ACCAGGTTTCCATCCTCCAAAATTGTAGTGTATCTCAAAGTACAGTAATC		
61	Sequencing primer A*	ACACTCTTTCCCTACACGACGCTCTTCCGATCT		
62	Sequencing primer B*	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT		
63	Illumina PE PCR primer A	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT		
64	Illumina PE PCR primer B	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT		
65	RT3 Forward	AACCATGGTCTTCTRTTTCTATGGAYTTCATCA		
66	RT3 Reverse	CCAAAATCTTGATACAAATTGAGT		
67	RT6 Forward	TATGATTGCGCCATAGAMTTRSARGAAGGA		
68	RT6 Reverse	AAAATATCATCYARRTAGATGACAACAAA		
69	Pp EF1-alpha Forward	CGACGCCCCTGGACATC		
70	Pp EF1-alpha Reverse	CCTGCGAGGTTCCCGTAA		
*	C's were synthesized as 5-methyl Cytosine			