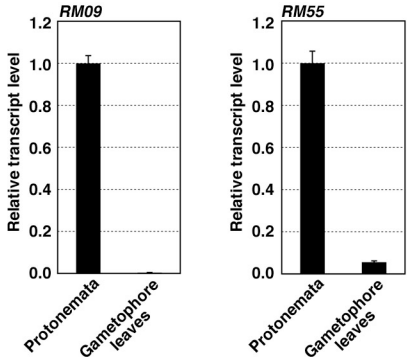
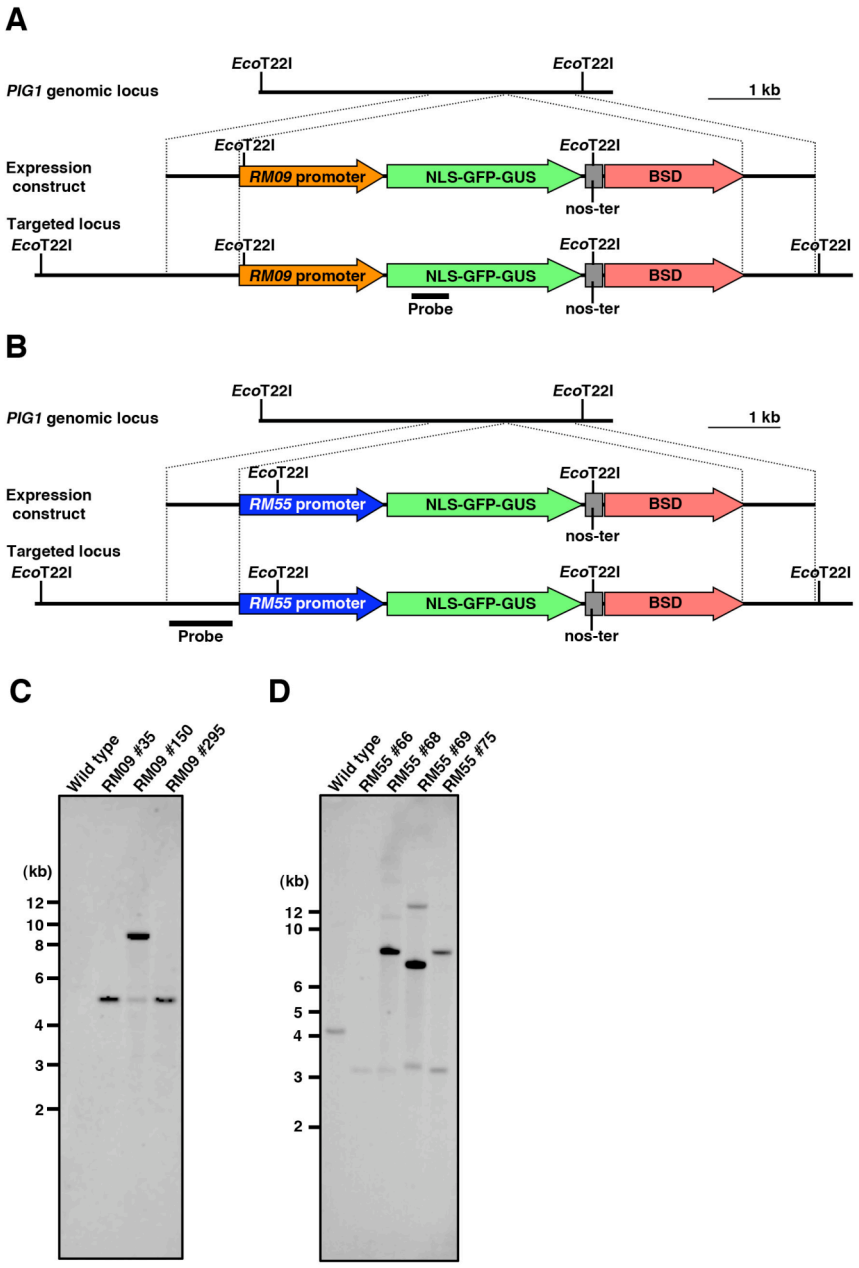


Supplemental Figure 1. Expression of Protonema-Specific Genes.

Total RNA was purified from wild-type protonemata 7 days after vegetative propagation and from the leaves of 4-week-old gametophores. *RM09* and *RM55* transcript levels were determined by qRT-PCR and normalized against α-tubulin gene (*TUA1*) transcript levels. The relative level for protonemata was taken as 1.0. Error bars indicate SE of the mean ($n = 4$).

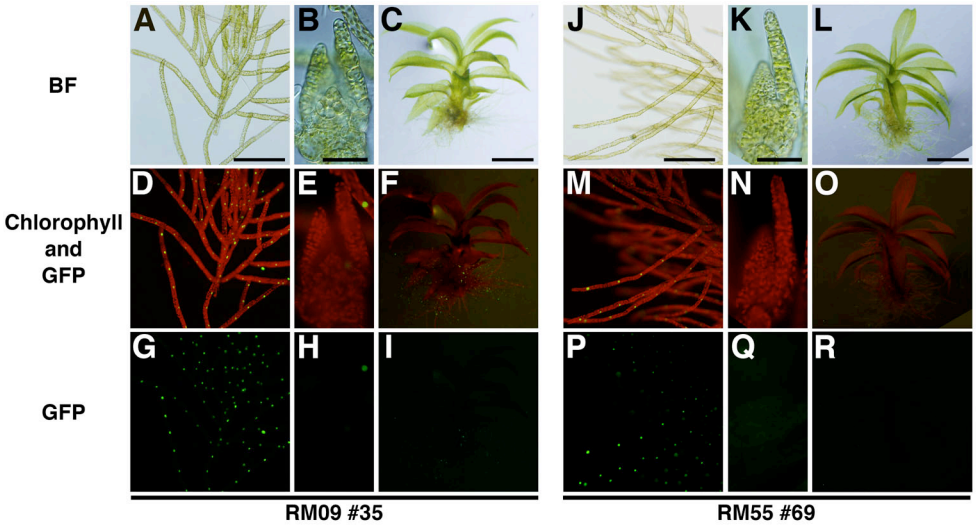
1



Supplemental Figure 2. Construction of RM09 and RM55 Lines.

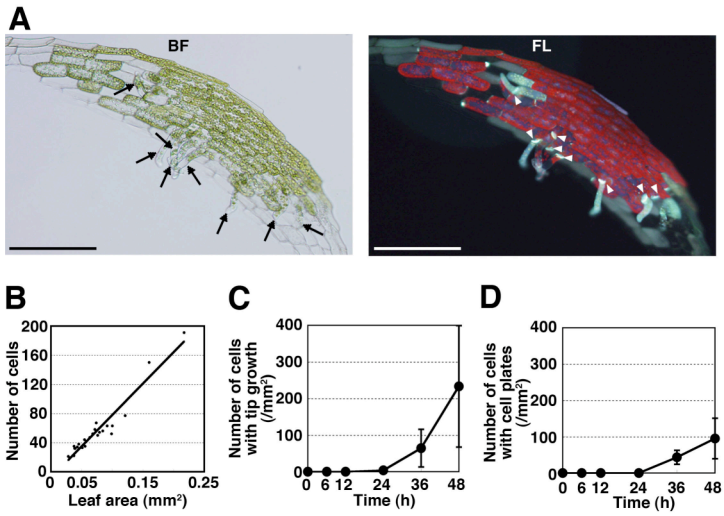
(A) and **(B)** Schematics for the insertion of the *RM09* (A) and *RM55* (B) promoter:NLS-GFP-GUS constructs into the *PIG1*-targeting locus (Okano et al., 2009). Orange and blue arrows denote putative promoters of *RM09* (XP_001784484) and *RM55* (XM_001784210), respectively. Green and red arrows, respectively, denote a fused DNA fragment of a synthetic nucleotide sequence encoding the SV40 nuclear localization signal (NLS; Kalderon et al., 1984), the *sGFP* gene (Chiu et al., 1996), and the *uidA* gene (Jefferson, 1987), and the blasticidin S deaminase expression cassette (BSD cassette).

Gray boxes denote the nopaline synthase polyadenylation signal (nos-ter; Nishiyama et al., 2001). Probes used in (C) and (D) are indicated. **(C)** and **(D)** DNA gel-blot analysis of targeted lines. Genomic DNA of wild-type, RM09 #35, #150, and #295 lines (C), and RM55 #66, #68, #69, and #75 lines (D) was digested with *Eco*T22I, blotted, and probed with the fragment indicated in (A) and (B).



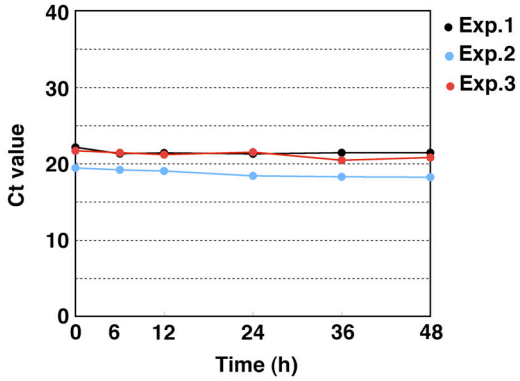
Supplemental Figure 3. Promoter Activities of the RM09 and RM55 Constructs.

Detection of the GFP signal in protonemata and gametophores of RM09 #35 **(A** to **I)** and RM55 #69 **(J** to **R)**. Bright field images (A to C, J to L) and fluorescence images of chlorophyll (red) and GFP (green) in protonemata (D and M), young gametophores with two immature leaves (E and N), and gametophores (F and O) were imaged. Note that GFP was detected in RM09 #35 and RM55 #69 in all protonemal cells but not in gametophore cells. Bars = 200 μm in (A) and (J), 100 μm in (B) and (K), 1 mm in (C) and (L).



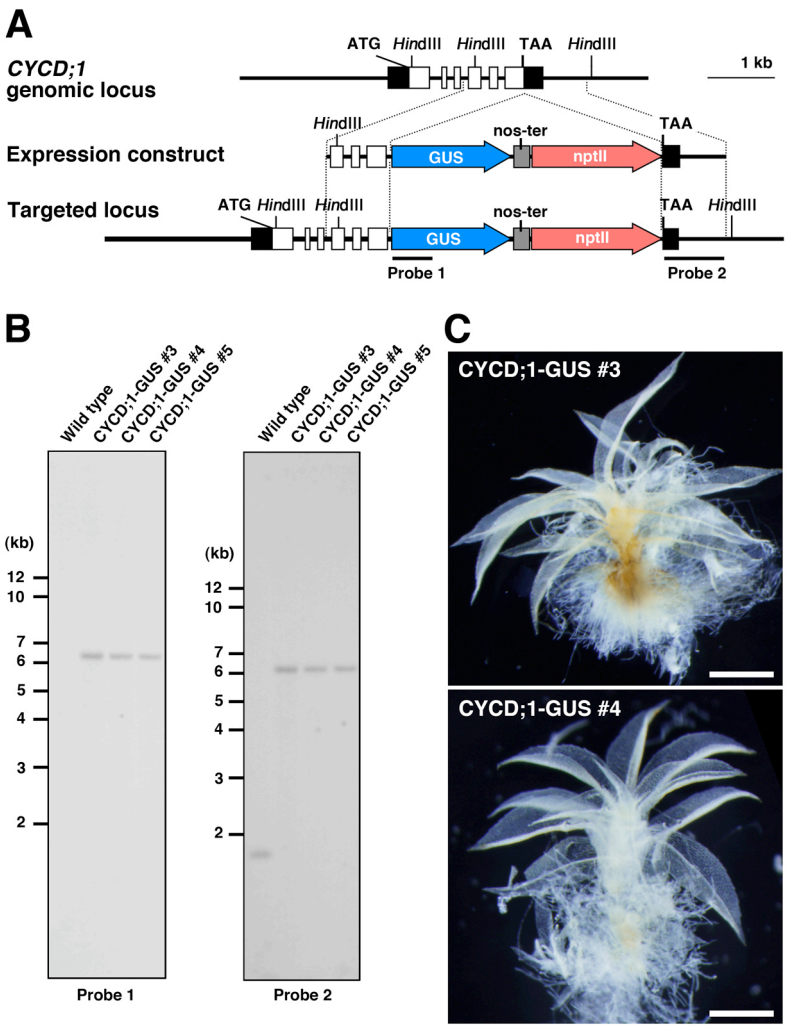
Supplemental Figure 4. Formation of Chloronema Apical Cells in a Gametophore Leaf Cut with a Homogenizer.

(A) Bright-field (BF) and fluorescent (FL) images of a cut gametophore leaf. After cutting in the homogenizer, leaves were removed from stems, incubated for 48 h, and then stained with aniline blue to detect callose, which is present in newly synthesized cell plates. Arrows indicate chloronema apical cells formed from leaf cells and arrowheads indicate cell plates. Some chloronema cells in the right image are out of focus on the left. Bars = 200 μm. **(B)** Correlation between the number of leaf cells and leaf area for cut leaves. The line shows a linear regression fitted to the data, having the following slope and standard error: $7.38 \pm 1.16 \times 10^2$ cells mm^{-2}. Leaf size was measured using ImageJ 1.36b (http://rsb.info.nih.gov/ij/). **(C)** and **(D)** The spatial frequency of cells with apical growth (C) and callose-positive cell plates (D) as a function of time. By 48 h, roughly 30% of gametophore cells were growing apically and 15% of cells had cell plates. Data represent counts over at least 1 mm^2 of leaf area. Error bars indicate SD for 50 replicate cut leaves.



Supplemental Figure 5. Constitutive Expression of the Alpha Tubulin Gene *TUA1* during the Reprogramming Process.

The *TUA1* transcript was detected using a critical threshold method (Livak and Schmittgen, 2001). Time indicates hours following cutting. Symbols plot the Ct value from three independent experiments. SE of the mean (n = 3) is less than 0.5 for all data.



Supplemental Figure 6. Construction of the CYCD;1-GUS Lines and Expression of the CYCD;1-GUS Protein.

(A) A schematic of the *CYCD;1* locus and its targeting for insertion. White boxes represent the *CYCD;1*-coding exons. Black boxes indicate the 5'- and 3'-untranslated exons. Blue and red arrows denote the *uidA* gene (GUS; Jefferson, 1987) and the neomycin phosphotransferase II expression cassette (nptII; Nishiyama et al., 2000), respectively. Gray boxes denote the terminator of the nopaline synthase gene (nos-ter; Nishiyama et al., 2000). Probes used in (B) are indicated. **(B)** DNA gel-blot analysis of targeted lines. Genomic DNA of the wild type and CYCD;1-GUS #3, #4, and #5 lines were digested with *Hin*dIII. **(C)** Protonemata and gametophores of CYCD;1-GUS #3 and #4 lines stained to show GUS activity. No GUS activity was detectable. Bars = 500 µm.

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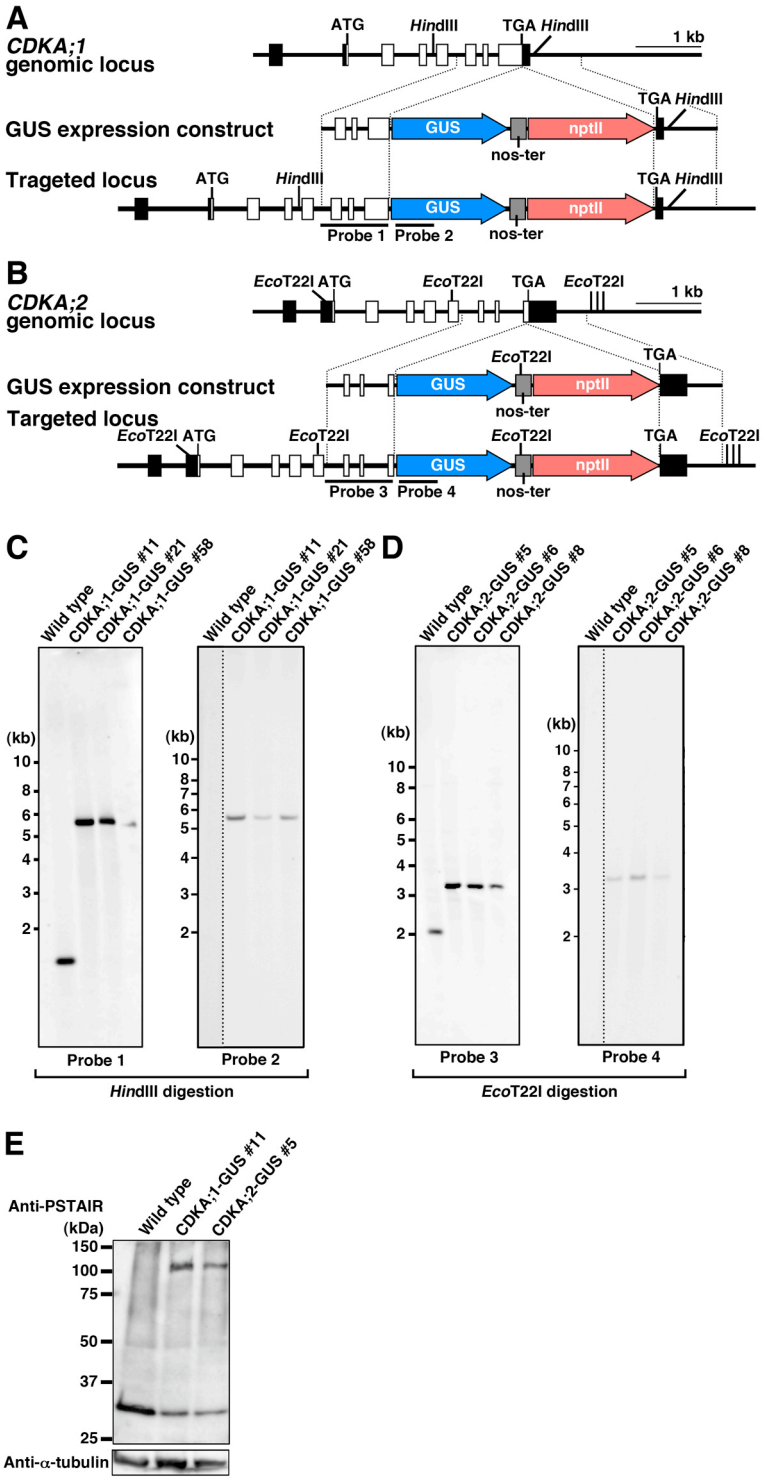
A
CDKA;1
genomic locus
ATG
HindIII
TGA HindIII
1 kb
GUS expression construct
GUS
nptII
nos-ter
Trageted locus
Probe 1
Probe 2
B
CDKA;2
genomic locus
EcoT22I ATG
EcoT22I
TGA
Targeted locus
Probe 3
Probe 4
C
Wild type
CDKA;1-GUS #11
CDKA;1-GUS #21
CDKA;1-GUS #58
(kb)
Probe 1
Probe 2
HindIII digestion
D
CDKA;2-GUS #5
CDKA;2-GUS #6
CDKA;2-GUS #8
Probe 3
Probe 4
EcoT22I digestion
E
Anti-PSTAIR
(kDa)
150
100
75
50
37
25
Anti-α-tubulin

8

Supplemental Figure 7. Construction of the CDKA;1-GUS and CDKA;2-GUS Lines.

(A) and **(B)** Schematics of targeting for the *CDKA;1* and *CDKA;2* loci. White boxes represent the *CDKA;1*- and *CDKA;2*-coding exons. Black boxes indicate the 5'- and 3'-untranslated exons. Blue and red arrows denote the *uidA* gene (GUS; Jefferson, 1987) and the neomycin phosphotransferase II expression cassette (nptII; Nishiyama et al., 2000), respectively. Gray boxes denote the terminator of the nopaline synthase gene (nos-ter; Nishiyama et al., 2000). Probes used in (C) and (D) are indicated. **(C)** and **(D)** DNA gel-blot analysis of targeted lines. (C) Genomic DNA of the wild type and CDKA;1-GUS #11, #21, and #58 lines digested with *Hin*dIII. (D) Genomic DNA of the wild type and CDKA;2-GUS #5, #6, and #8 lines digested with *Eco*T22I. **(E)** Accumulation of CDKA;1-GUS and CDKA;2-GUS fusion proteins in gametophores. Total protein extracted from gametophores of the wild type, CDKA;1-GUS #11 line, and CDKA;2-GUS #5 line was analyzed with the anti-PSTAIR antibody. Anti-α-tubulin antibody was used as a loading control. Bands with an approximate size of 110 kDa were detected in both the CDKA;1-GUS #11 and CDKA;2-GUS #5 lines but not in the wild type.

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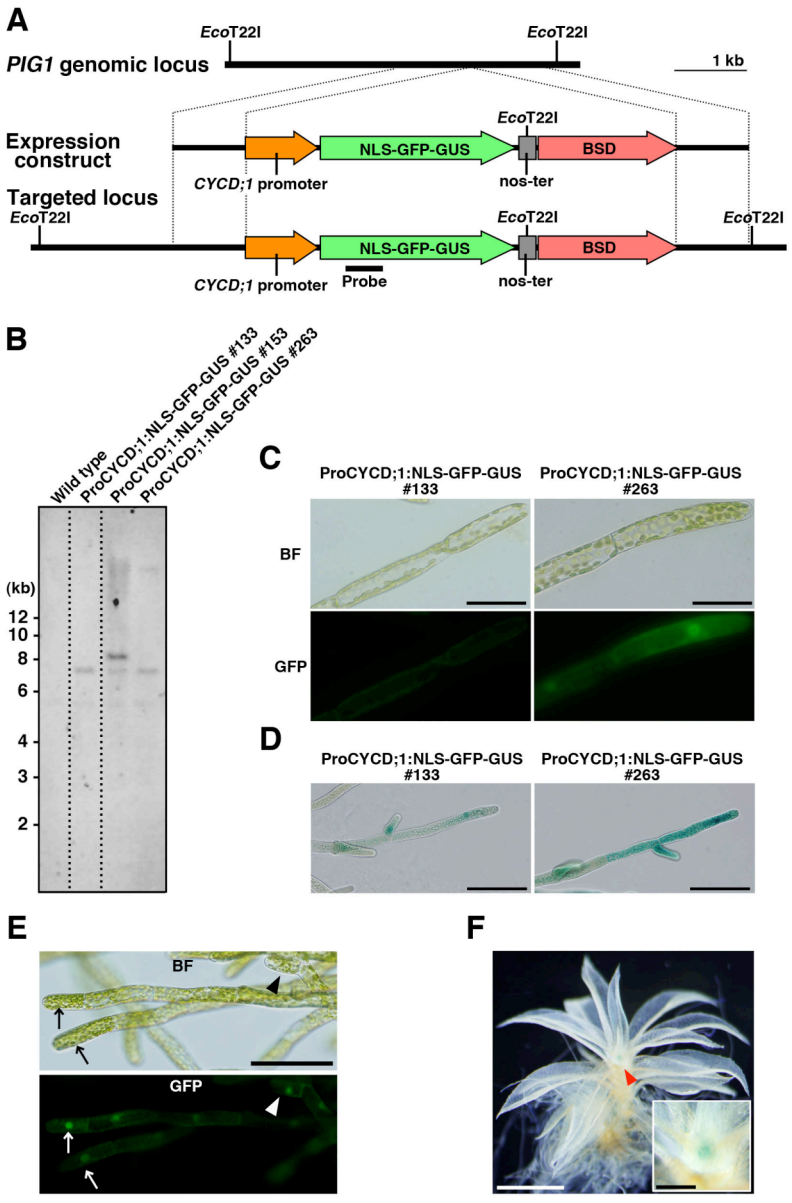
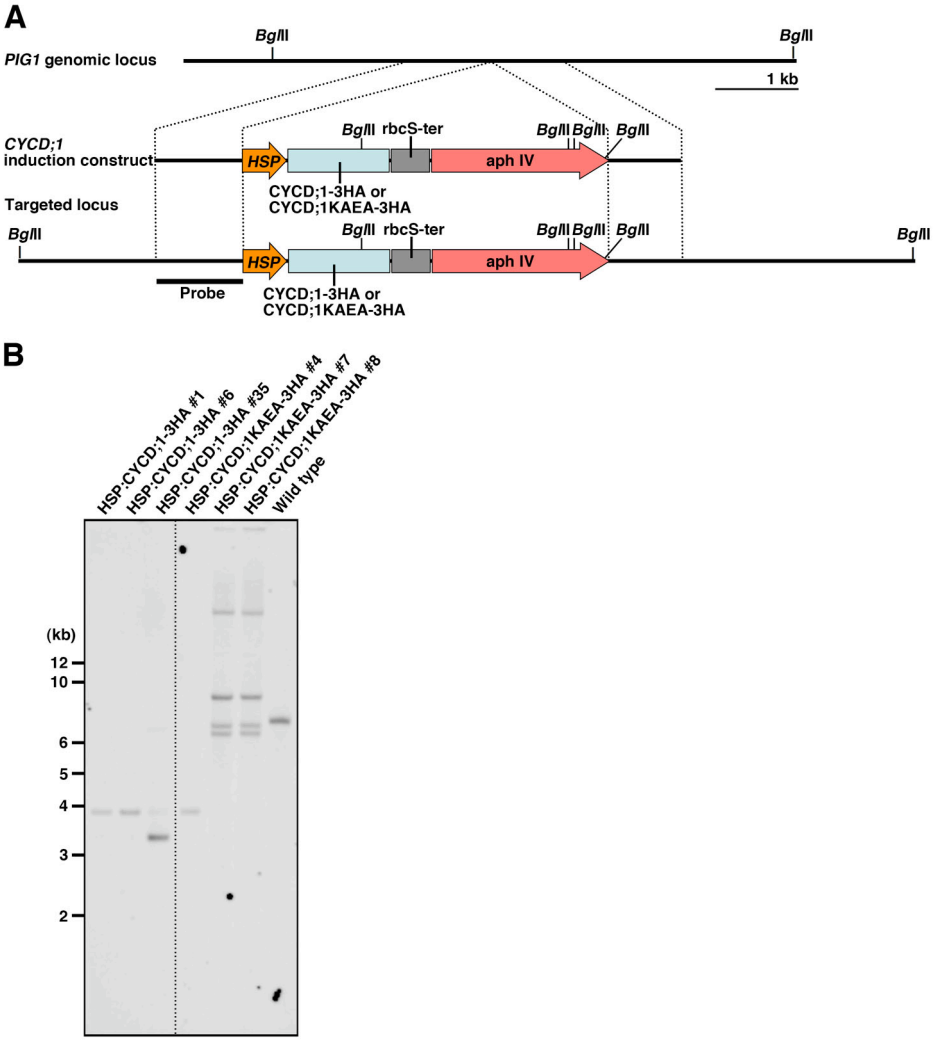


Supplemental Figure 8. Construction and *CYCD;1* Promoter Activity of the ProCYCD;1:NLS-GFP-GUS Lines.

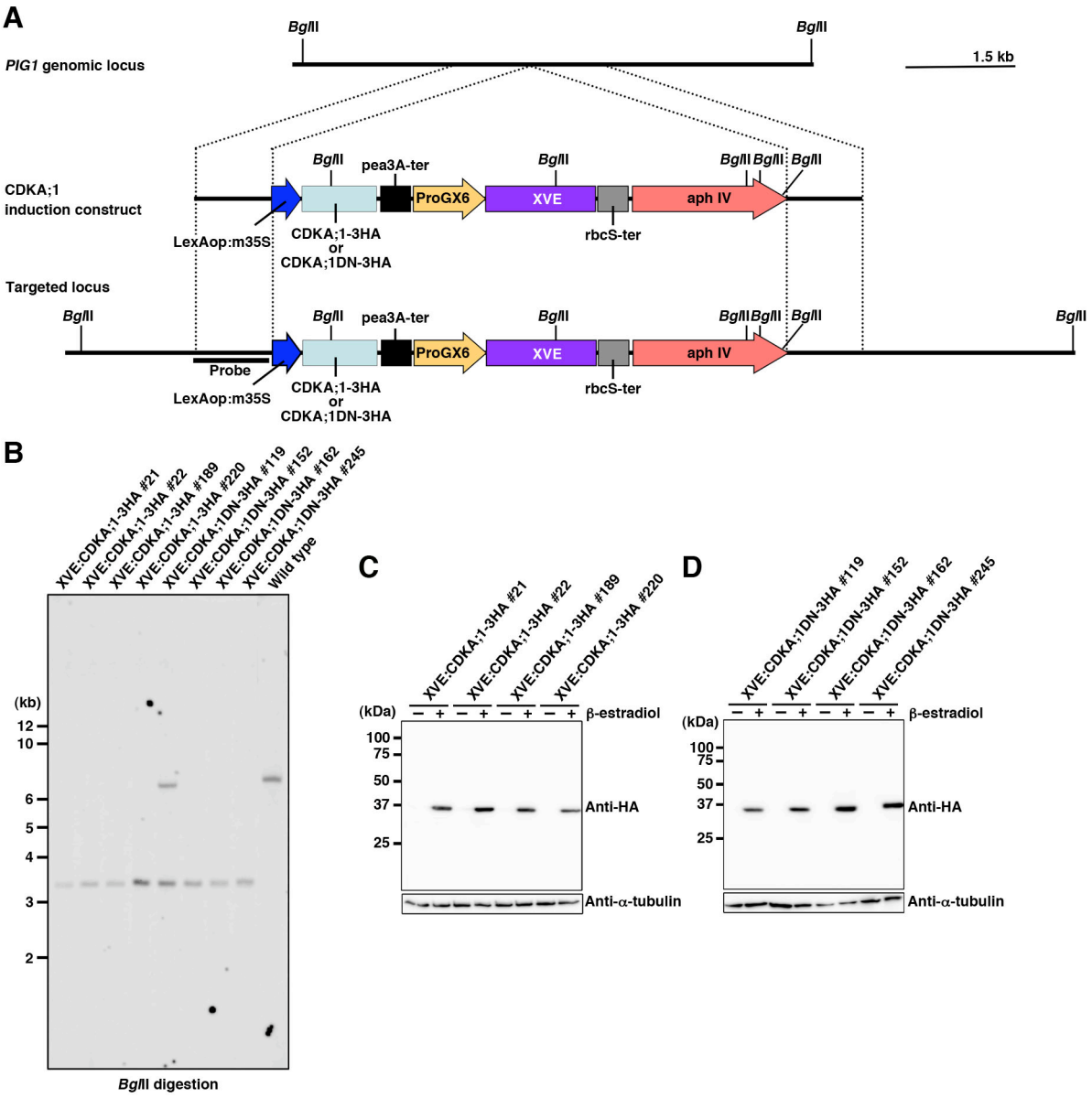
(A) A schematic for the insertion of a ProCYCD;1:NLS-GFP-GUS construct into the *PIG1*-targeting locus (Okano et al., 2009). Orange, green, and red arrows denote the *CYCD;1* promoter; the fused DNA fragment of a synthetic nucleotide sequence encoding the SV40 nuclear localization signal (NLS; Kalderon et al., 1984), the *sGFP* gene (Chiu et al., 1996), and the *uidA* gene (GUS; Jefferson, 1987); and the blasticidin S deaminase (Tamura et al., 1995) expression cassette (BSD; p35S-loxP-BSD [accession: AB537973]), respectively. Gray boxes denote the terminator of the nopaline synthase gene (nos-ter; Nishiyama et al., 2000). A probe used in (B) is indicated. **(B)** DNA

gel-blot analysis of targeted lines. Genomic DNA of wild-type and ProCYCD;1:NLS-GFP-GUS #133, #153, and #263 lines digested with *Eco*T22I. A fragment corresponding to *sGFP* was used as a probe for DNA gel-blot analysis. Multiple copies of DNA fragments were inserted in the ProCYCD;1:NLS-GFP-GUS #153 and #263 lines. **(C)** Bright-field (BF) and fluorescence (GFP) images of chloronema apical cells of the ProCYCD;1-NLS-GFP-GUS #133 and #263 lines. **(D)** GUS activity in the chloronemata of the ProCYCD;1-NLS-GFP-GUS #133 and #263 lines. **(E)** Bright-field (top) and GFP fluorescence (bottom) images of chloronemata of the ProCYCD;1:NLS-GFP-GUS #263 line. Chloronema apical cells (arrows) and a side branch initial cell (arrowhead) are indicated. **(F)** GUS activity of the same line. A gametophore apex (red arrow) is indicated and also shown at higher magnification in the inset). Bars = 50 μm in (C), 100 μm in (D) and (E), 1 mm in (F), and 200 μm in the inset.



Supplemental Figure 9. Construction of the HSP:CYCD;1-3HA and HSP:CYCD;1KAEA-3HA Lines.

(A) A schematic for the insertion of HSP:CYCD;1-3HA or HSP:CYCD;1KAEA-3HA constructs into the *PIG1*-targeting locus (Okano et al., 2009). Orange and red arrows indicate the soybean heat-shock Gmhsp17.3B promoter (*HSP*; Saidi et al., 2005) and the aminoglycoside phosphotransferase IV expression cassette (aph IV; Hiwatashi et al., 2008), respectively. Light blue and gray boxes indicate the DNA fragment encoding CYCD;1-3HA or CYCD;1KAEA-3HA and the rbcS terminator (rbcS-ter), respectively. A probe used in (B) is indicated. **(B)** DNA gel-blot analysis of targeted lines. Genomic DNA of wild-type, HSP:CYCD;1-3HA #1, #6, and #35 lines and HSP:CYCD;1KAEA-3HA #4, #7, and #8 lines digested with *Bgl*II.



Supplemental Figure 10. Construction of the XVE:CDKA;1-3HA and XVE:CDKA;1DN-3HA Lines.

(A) A schematic for insertion of the XVE:CDKA;1-3HA or XVE:CDKA;1DN-3HA constructs into the *PIG1* targeting locus (Okano et al., 2009). Blue, orange, and red arrows denote a fused DNA fragment of the LexA operator and minimal *35S* promoter (LexAop:m35S; Zuo et al., 2000), the GX6 promoter (ProGX6; see Methods), and the aminoglycoside phosphotransferase IV expression cassette (aph IV; Hiwatashi et al., 2008), respectively. Light blue, black, purple, and gray boxes designate a DNA fragment encoding CDKA;1-3HA or CDKA;1DN-3HA, the pea rbcS3A terminator (pea3A-ter),

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a DNA fragment encoding a XVE fusion protein derived from pER8 (Zuo et al., 2000), and the rbcS terminator (rbcS-ter), respectively. A probe used in (B) is indicated. **(B)** DNA gel-blot analysis of targeted lines. Genomic DNA of the wild-type, XVE:CDKA;1-3HA #21, #22, #189, and #220 lines, and XVE:CDKA;1DN-3HA #119, #152, #162, and #245 lines digested with *Bgl*II. **(C)** and **(D)** Expression of CDKA;1-3HA and CDKA;1DN-3HA proteins in response to 1 μM β-estradiol. Total proteins from gametophores of the XVE:CDKA;1-3HA #21, #22, #189, and #220 lines and the XVE:CDKA;1DN-3HA #119, #152, #162, and #245 lines incubated with or without 1 μM β-estradiol for 24 h were analyzed with an anti-HA antibody. Anti-α-tubulin antibody was used as a loading control.

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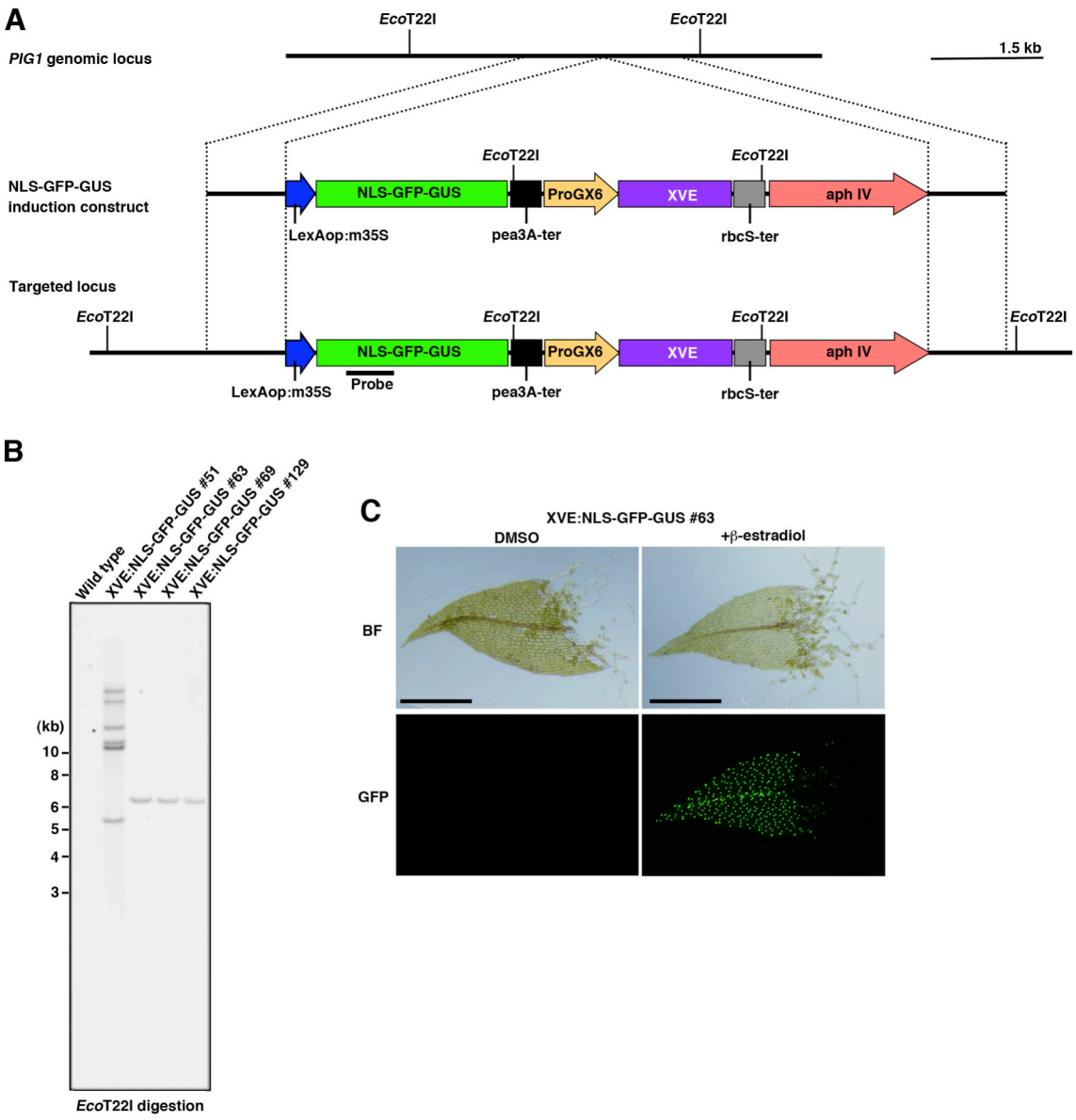

Supplemental Figure 11. Construction of the XVE:NLS-GFP-GUS Line.

(A) A schematic for insertion of the XVE:NLS-GFP-GUS construct into the *PIG1* targeting locus (Okano et al., 2009). Blue, orange, and red arrows denote a fused DNA fragment of the LexA operator and minimal *35S* promoter (LexAop:m35S; Zuo et al., 2000), the GX6 promoter (ProGX6; see Methods), and the aminoglycoside phosphotransferase IV expression cassette (aph IV; Hiwatashi et al., 2008), respectively. Black, purple, and gray boxes designate the pea rbcS3A terminator (pea3A-ter), a DNA fragment encoding a XVE fusion protein derived from pER8 (Zuo et al., 2000), and the rbcS terminator (rbcS-ter), respectively. Green boxes denote the *NLS-GFP-GUS* fusion gene

(NLS-GFP-GUS) composed of a synthetic nucleotide sequence encoding the SV40 nuclear localization signal (NLS; Kalderon et al., 1984), the *sGFP* gene (Chiu et al., 1996), and the *uidA* gene (GUS; Jefferson, 1987). A probe used in (B) is indicated. **(B)** Genomic DNA of the wild-type and XVE:NLS-GFP-GUS #51, #63, #69, and #129 lines digested with *Eco*T22I. A fragment corresponding to *sGFP* was used as a probe. **(C)** Expression of the NLS-GFP-GUS protein in excised leaves. Bright-field (BF) and fluorescence (GFP) images of excised leaves of the XVE:NLS-GFP-GUS #63 line incubated for 72 h with or without 1 μM β-estradiol. Bars = 500 μm.

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Supplemental Table 1. Primer Sequences for qRT-PCR.

Gene name		Sequence
CDKA;1	(F)	5’-TTGCCGTCCATCGTGTTTT-3’
	(R)	5’-GGAGTACCAGCTCCGCACAA-3’
CDKA;2	(F)	5’-TGATTCGGCTTTTGGAGTGTT-3’
	(R)	5’-ACGCGCGAAGCAAGGA-3’
CDKB;1	(F)	5’-AGGTGGGCACCGTGGTT-3’
	(R)	5’-GCCAGCTAACGATGTCAA-3’
CYCB;1	(F)	5’-CGTGCTGTCTCCGGTCTCTT-3’
	(R)	5’-CCTGGTAGCGAACATGGTTGT-3’
CYCB;2	(F)	5’-TCCGAGGCTCAGATCAAGGA-3’
	(R)	5’-TCACTGGCCTTGCTGTGAAG-3’
CYCD;1	(F)	5’-AGAGCGCCAGCCTATAAAGTG-3’
	(R)	5’-CAACGGAAACTGCCAGTGAA-3’
CYCD;2	(F)	5’-CCGCAGAGCCCTATTGGA-3’
	(R)	5’-CTTCAGTGGCGGAGCTCAA-3’
KRP	(F)	5’-TCCCCGCGATCTCCATTAC-3’
	(R)	5’-GTTGAAGTAGCGCACGACACTT-3’
HFO	(F)	5’-GCTCGTCGTGGTGGAGTGA-3’
	(R)	5’-CGCCTCGAGTCTCCTCGTAT-3’
E2F;1	(F)	5’-TGCCCATTAACTCCAGGACATT-3’
	(R)	5’-GAGTCGGTGCACTGCTTGTG-3’
E2F;2	(F)	5’-AGCTCTGGCCCCACCAA-3’
	(R)	5’-GCAGGCGCTGTTCTCATGAT-3’
E2F;3	(F)	5’-GCAGCATCCCAGACCTCTGA-3’
	(R)	5’-TGATCCCGCCCACAAAGT-3’
E2F;4	(F)	5’-GTTCCCATGGAGACTCTACCTGAT-3’
	(R)	5’-TGCCATCGACAGATGAAGATG-3’
DP;1	(F)	5’-AAAAGCTAAGGCGAAGCATGAGA-3’
	(R)	5’-ATAGGAAACAAGGGACCGAAAAC-3’
DP;2	(F)	5’-CCCCCTGTTCCAGGTATCCT-3’

	(R)	5’-CTGTGACCCACTATGAGACGTTCT-3’
DP;3	(F)	5’-CCCAGATGGGTTGGATCATG-3’
	(R)	5’-GGGAATCCTCTCCAAAATTGC-3’
RBR;1	(F)	5’-AAGCAGCCGCAGTGCAA-3’
	(R)	5’-CAACCGACCCGTCAAGGA-3’
RBR;2	(F)	5’-CGCGCTCTCGTAATCACAAA-3’
	(R)	5’-GCGAGTGCAGGACATTCCA-3’
RBR;3	(F)	5’-GTGGGCTGGCTTACTTGGAA-3’
	(R)	5’-GCGCAAATTCGCTGCAA-3’
TUA1	(F)	5’-CGTAGGAGGGACCAGTTTGG-3’
	(R)	5’-TGCATTCATCCCCGAGTCA-3’
RM09	(F)	5’-TGCACCTACGACGGAATACG-3’
	(R)	5’-CGATCGGAGACGAAATAGTGTTATC-3’
RM55	(F)	5’-GCAATTTGGCGACAGCTTTAG-3’
	(R)	5’-AGCCGGCACCCAAGAAG-3’

(F): Forward primer

(R): Reverse primer

Supplemental Table 2. **List of the *Physcomitrella patens* Cell Cycle Regulator and Other Genes Used in This Study.**

Gene family	Gene name	Accession number
CDKA	*CDKA;1*	AJ515321
	CDKA;2	AB547329
CDKB	*CDKB;1*	AB548666
CYCD	*CYCD;1*	AJ428953
	CYCD;2	AB547332
CYCB	*CYCB;1*	AB547330
	CYCB;2	AB547331
E2F	*E2F;1*	AB547337
	E2F;2	AB547338
	E2F;3	AB547339
	E2F;4	AJ428951
DP	*DP;1*	AB547340
	DP;2	AB547341
	DP;3	AB547342
RBR	*RBR;1*	AB547334
	RBR;2	AB547335
	RBR;3	AB547336
ICK/KRP	*KRP*	AB547333
Histone H4	*HFO*	AB547911
Alpha Tubulin	*TUA1*	AB096718

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Supplemental Table 3. Primers for Plasmid Construction.

Primers for gene targeting

Target gene		Sequence
CYCD;1	5’	(F) 5’-GGGGCGGCCGCTTCGGTCCGATCAGCGTTCACTCTGGG-3’
	5’	(R) 5’-GGGGGGATCCATAGGCTGGCGCTCTCTACGTGGAGAC-3’
	3’	(F) 5’-GGGGAATTCTAAAGTGTCCATCGAACCGAATTTTTTTC-3’
	3’	(R) 5’-GGGGTCGACAAACGCAGAGTTCACTCCACTGGGTC-3’
CDKA;1	5’	(F) 5’-GGGGCGGCCGCTTGTGAGACGAGAGAACGTGGAAG-3’
	5’	(R) 5’-GGGGGATCCAGGGTACAAGACCGATATCCTTGA-3’
	3’	(F) 5’-GGGATCGATTTCTTGTATGCAACTTGTATGATACTGT-3’
	3’	(R) 5’-GGGGGATCCAACTCGGCCTGTAGTTAGACGTA-3’
CDKA;2	5’	(F) 5’-GGGGCGGCCGCATTCTCTCCAGGCACTGC-3’
	5’	(R) 5’-GGGGGATCCACGGCACCAGACCGACATCCTTG-3’
	3’	(F) 5’-CCCCTCGAGTCTGTTCCTGCAATATACCTG-3’
	3’	(R) 5’-CCCGGTACCTGAGTAAACAGAAGCATGCATGAT-3’

(F): Forward primer

(R): Reverse primer

Primers for promoter-reporter lines

Primer name	Sequence
CYCD1proF	5’-ACCTTTGTGTGCTAAATGTATGTATTCCAAACTGG-3’
CYCD1proR	5’-GCTTGCCAAGCAATCAACACTGGGAGACAT-3’
RM09F	5’-GATTTGGAATTGGTTGATTTATGT-3’
RM09R	5’-AGTGGCCATCTTGGTCGATTGCCTGAT-3’
RM55F	5’-CAAAGGGTGAACCAATCCTATAAC-3’
RM55R	5’-TTGCGACATTTCCCTTGTTTCGTGT-3’