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Supplemental Data

Noncoding Transcription by RNA Polymerase Pol IVb/Pol V Mediates Transcriptional Silencing of Overlapping and Adjacent Genes

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Supplemental Material

Chromatin Immunoprecipitation Details

Three grams of above-ground tissue of 2-week old plants was crosslinked with 0.5% formaldehyde for 10 min by vacuum infiltration, followed by addition of glycine to 80 mM. Plants were rinsed with water, frozen in liquid nitrogen, ground into powder using a mortar and pestle, suspended in 25 ml of Honda Buffer (20 mM HEPES-KOH pH 7.4, 0.44 M sucrose, 1.25% ficoll, 2.5% Dextran T40, 10 mM $MgCl_2$, 0.5% Triton X-100, 5 mM DTT, 1 mM PMSF, 1% plant protease inhibitors (Sigma)), filtered through two layers of Miracloth and centrifuged at 2000 x g for 15 min. Nuclear pellets were washed three times with 1ml of Honda buffer, resuspended in Nuclei Lysis Buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS, 1 mM PMSF, 1% Plant Protease Inhibitors) and sonicated as described (Lawrence et al. 2004). After centrifugation at 16,000 x g for 10 min., the supernatant was diluted 10-fold with 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.0, 167 mM NaCl. 25 µl of protein A agarose/salmon sperm DNA (Upstate Biologicals) and the appropriate antibody was added. Samples were then incubated overnight at 4ºC on a rotating mixer. Agarose-antibody complexes were washed five times, 5 min each, with binding/washing buffer (150 mM NaCl, 20 mM Tris-HCl pH 8.0, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1 mM PMSF) and washed twice for 5 min each with 10 mM Tris-HCl pH 8.0, 1 mM EDTA. 100µl of 10% (w/v) Chelex (Bio Rad) resin, in water, was then added to the beads and crosslinking was reversed at 99 ºC for 10 min. Samples were digested with 20 µg of proteinase K (Invitrogen) for 1h at 43 ºC followed by heat-inactivation at 95 ºC for 10 min.

RNA Immunoprecipitation Details

RNA IP was based on ChIP with the following modifications. RNase OUT RNase inhibitor (Invitrogen) was included in all buffers. IP was performed for 3h followed by four washes with Binding/Washing buffer. Immune complexes were eluted with 100 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS for 10 min at room temperature followed by a second elution at 65 ºC. Crosslinking was reversed at 65 ºC for 1h in the presence of 20 µg Proteinase K (Invitrogen). RNA was purified by extraction with acidic phenol:chloroform and ethanol precipitation.

Supplemental Table and Figures

Table S1. Oligonucleotides Used in This Study

Target	Name	Sequence (5' – 3')	Application
Actin 2 At3g18780	ACTmaiFW ACTmaiRV	TCATACTAGTCTCGAGAGATGACTCAGATCATGTTTGAG TCATTCTAGAGGCGCGCCACAATTTCCCGTTCTGCGGTAG (Herr et al, 2005)	RT-PCR
	A118 A119	GAGAGATTCAGATGCCCAGAAGTC TGGATTCCAGCAGCTTCCA	real time PCR
	A65 A66	CGAGCAGGAGATGGAAACCTCAAA AAGAATGGAACCACCGATCCAGACA	Chop-PCR
AtSN1	A122 A123	CCAGAAATTCATCTTCTTTGGAAAAG GCCCAGTGGTAAATCTCTCAGATAGA	real time PCR
AtSN1 (A)	ATS15 AtSN1-F4	ACCAACGTGCTGTTGGCCCAGTGGTAAATC AAAATAAGTGGTGGTTGTACAAGC (Herr et al, 2005)	RT-PCR Chop-PCR
AtSN1 (B)	A205 A206	TGAGAGATTTACCACTGGGCCAACA TGAGGAGCTCAACACATAAATGGCAATA	RT-PCR
AtSN1 (C)	A207 A208	CCTTTCCAAGACACCATCTCAACAAC TCCTCAACAAAAATAATTCCGAACGAC	RT-PCR
IGN5 (A)	A28 A29	TCCCGAGAAGAGTAGAACAAATGCTAAAA CTGAGGTATTCCATAGCCCCTGATCC	RT-PCR Chop-PCR
IGN5 (B)	A293 A294	CGCAGCGGAATTGACATCCTATC TCGGAAAGAGACTCTCCGCTAGAAA	RT-PCR
IGN5	A193 A194	AAGCCCAAACCATACACTAATAATCTAAT CCGAATAACAGCAAGTCCTTTTAATA	real time PCR
IGN5 bottom strand	A69 A70	TCATGCGGCCCAATAACCAACAAAAC TGAAGAAAGCCCAAACCATACACT	5' RACE
IGN5 top strand	A60 A67	TGTTGGTTATTGGGCCGCATGATACA AGCATTTGTTCTACTCTTCTCGGGAACT	5' RACE
IGN6	A30 A31	GGGACATCTATTGGGTTTAGGCTGGATG TTTGTAATTCTCAGTTCGGGTATCTGCTTG	RT-PCR Chop-PCR
	A162 A163	TTTCGCCGTCACTAACATGTAATG GAAGTAGCTTTTTCGGTCCAGTTC	real time PCR
IGN6 top strand	A62 A71	TCGGTTGCTATGTTTGCGGATCATGC CCAGCCTAAACCCAATAGATGTCC	5' RACE
IGN7	A44 A45	CATCCACAACTTCTATTGCTTTGTTTTACC TTTTCCTTTGAGTTGGTCATTGTTGTTT	RT-PCR
IGN10	A50 A51	TCTAACGCTTTGGTTGTGTATAGTGTGC ACCGGTATCTTAGTTCCTCCCACGTGTC	RT-PCR
IGN15	A110 A111	CCATAGCATAGAAACTTGGCGATATATGAA CGGAAAAGGTAAGGTGGTTGGAAAA	RT-PCR
IGN17	A114 A115	AACCCTAGCCTTTCATTAAAACCCTCTC CATAGATAGGAAACTCAATCTCTTCGCATTT	RT-PCR
solo LTR A	A221 A222	ATCAATTATTATGTCATGTTAAAACCGATTG TGTTTCGAGTTTTATTCTCTCTAGTCTTCATT	RT-PCR
solo LTR B	A217 A218	CATATAACCGAAGCCGAAGGATGTGAAA CAGAAACCTAAGGAACCATTACACGCTAAACC	RT-PCR
solo LTR C	A211 A212	ATAAAACTCGAAACAAGAGTTTTCTTATTGCTTTC TAATGGTATTATTTTGATCAGTGTTATAAACCGGA	Chop-PCR
solo LTR	A142 A143	GGATAGAGATGAATGATGGATAATGACA TTATTTTGATCAGTGTTATAAACCGGATA	real time PCR

Figure S1. The chromosomal contexts of *IGN7* and *IGN15* loci at which Pol V-dependent transcripts have been identified (Fig 1F). Shown are open reading frames (ORF), repetitive elements (TE repeats) and small RNAs from the MPSS database (sRNA). Single copy genes are marked in white, retrotransposons in grey and transposons in black. Data were obtained from http://chromatin.cshl.edu/cgi-bin/gbrowse/arabidopsis5/.

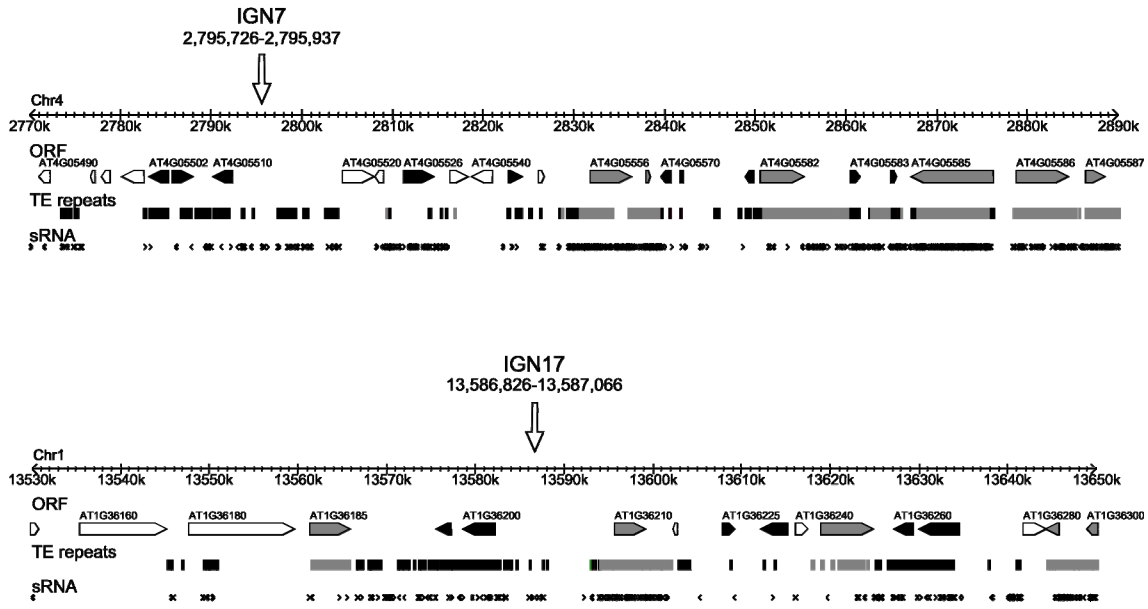

Figure S2. 5’ ends of Pol V-dependent transcripts identified by 5’RACE. The terminal nucleotides of cloned 5’ RACE products are marked with short arrows and n indicates the number of independent clones obtained for each 5’ end. 5’ RACE primers as well as nested primers used for amplification are marked with long arrows. Annotations above the DNA sequence refer to top strand-specific RACE clones and those below the DNA sequence refer to bottom strand clones.

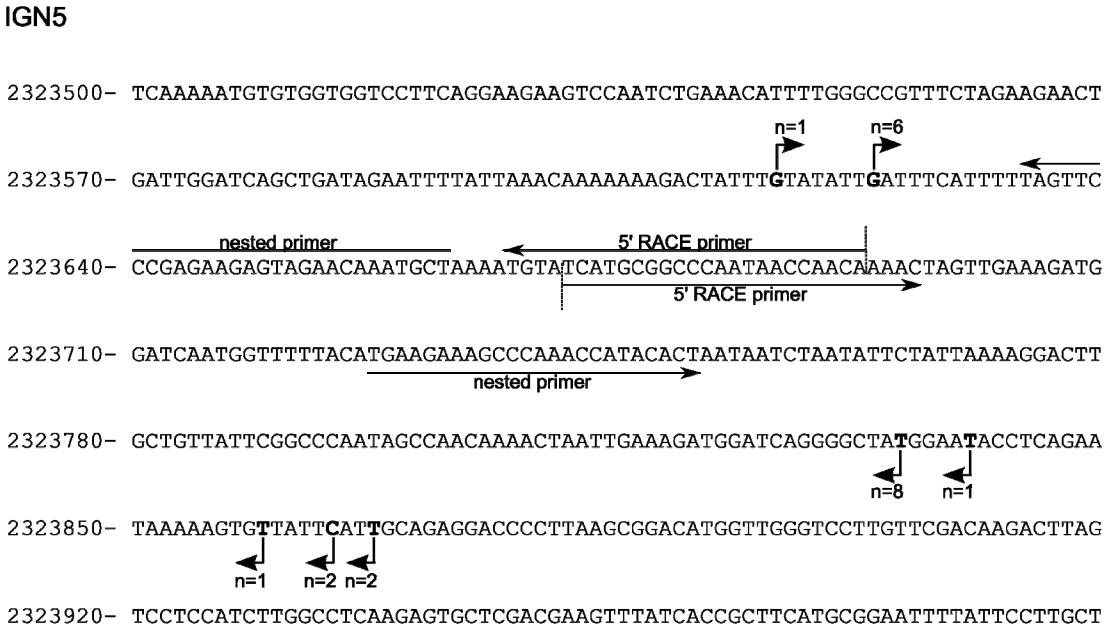

Figure S3. The chromosomal contexts of the *AtSN1* and *solo LTR* loci tested in our study. Shown are open reading frames (ORF), repetitive elements (TE repeats) and small RNAs in the MPSS database (sRNA). Single copy genes are marked in white, retrotransposons in grey and transposons in black. Data were obtained from http://chromatin.cshl.edu/cgi-bin/gbrowse/arabidopsis5/.

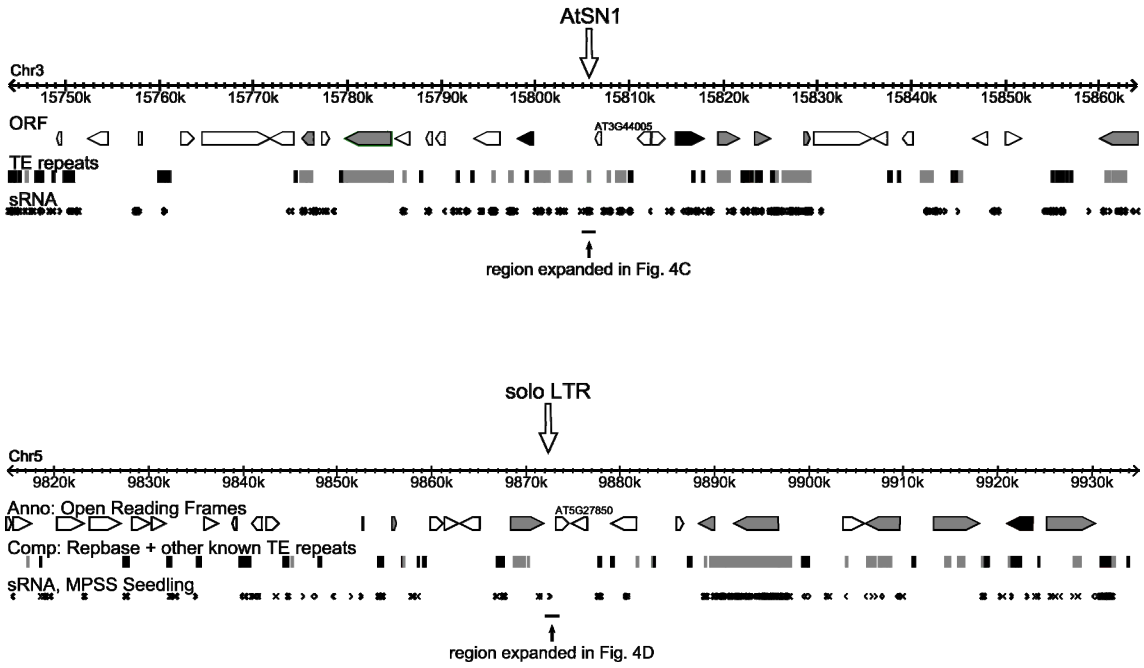

Figure S4. Quantitative PCR of control reactions in which no antibody was included in the chromatin immunoprecipitation (ChIP) experiments shown in Fig. 4F and Figs. 5A-C. Mean values for reactions performed in triplicate are essentially baseline in all cases.