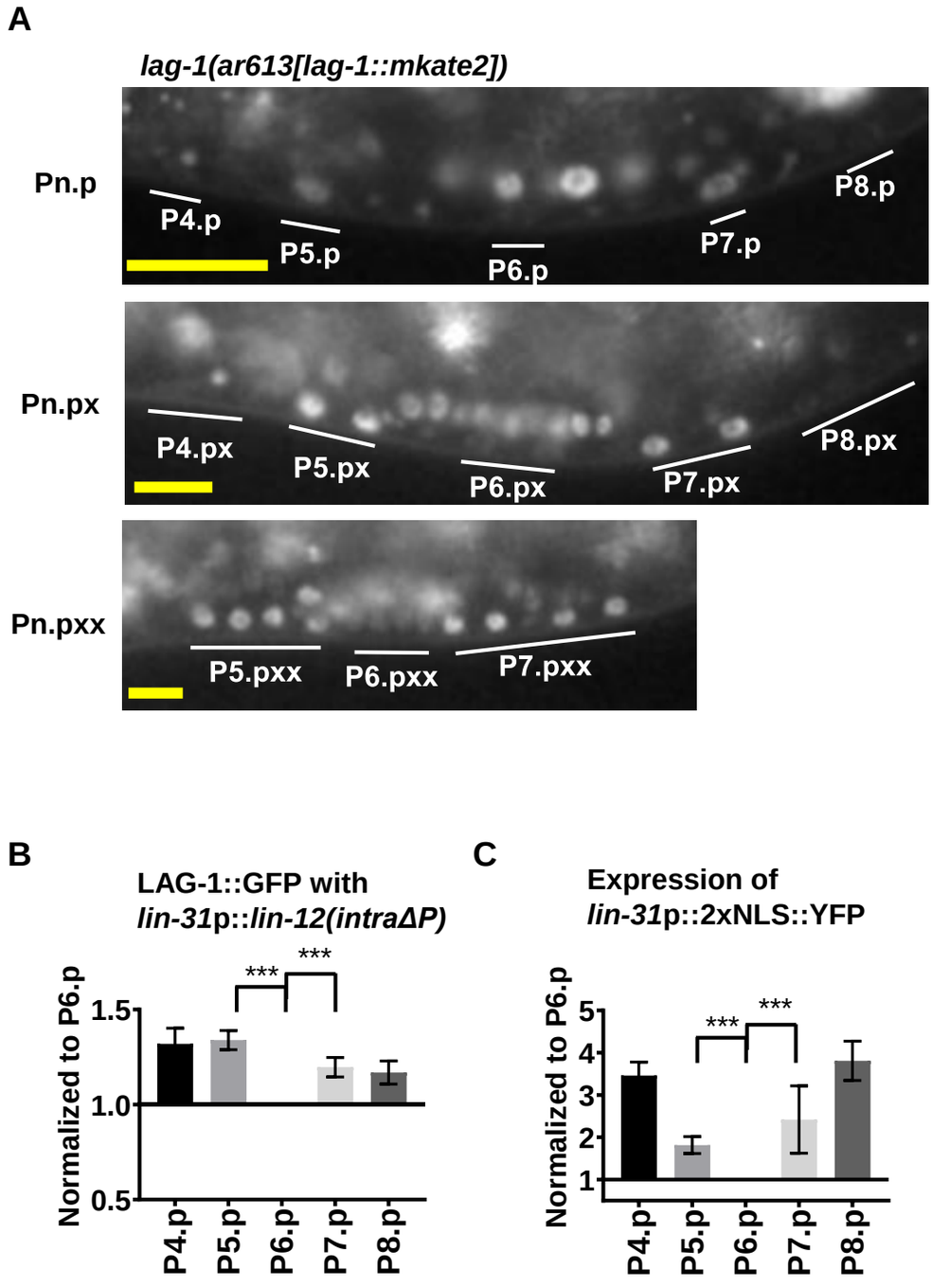

Figure S1. *lag-1(ar613[lag-1::mKate2])* expression, and additional data relevant to Figure 1.

(A) Representative image of *lag-1(ar613[lag-1::mKate2])* from L3 Pn.p stage to L3 Pn.pxx stage. VPCs are underlined and labeled. Scale bar (yellow) = 10 μm.

(B) LAG-1::GFP expression in the presence of *arTi102[lin-31*p::*lin-12(intraΔP)]* (data from Fig. 1D), here normalized to P6.p. Although expression is significantly higher in P5.p and P7.p compared to P6.p, this result is difficult to interpret because of patterned variation in expression that is evident upon quantification of a *lin-31*p transgene in (C). ***p<0.01

(C) Quantification of expression from *arTi88[lin-31*p::2xNLS::YFP*]* during the L3 stage shows patterned variation in expression. Expression is significantly higher in P5.p and P7.p compared to P6.p, suggesting that *lin-31*p itself is affected by spatial patterning signals. ***p<0.01

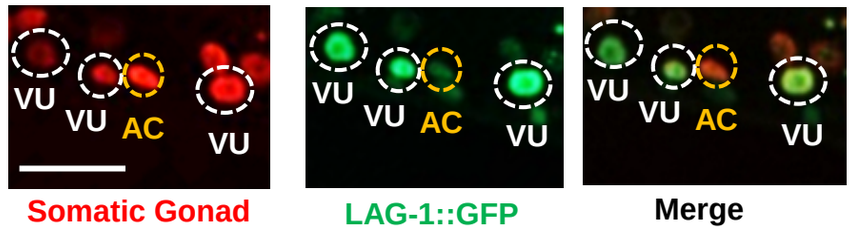

Figure S2. LAG-1::GFP in the somatic gonad
Orthogonal projection of the somatic gonad, in which the AC and VUS have already been specified. (Left) Somatic gonad is marked by *arTi112*[*ckb-3*p::mCherry::H2B]. (Middle) LAG-1::GFP is preferentially expressed in specified VUs. (Right) Merge. Scale bar = 10 µm.

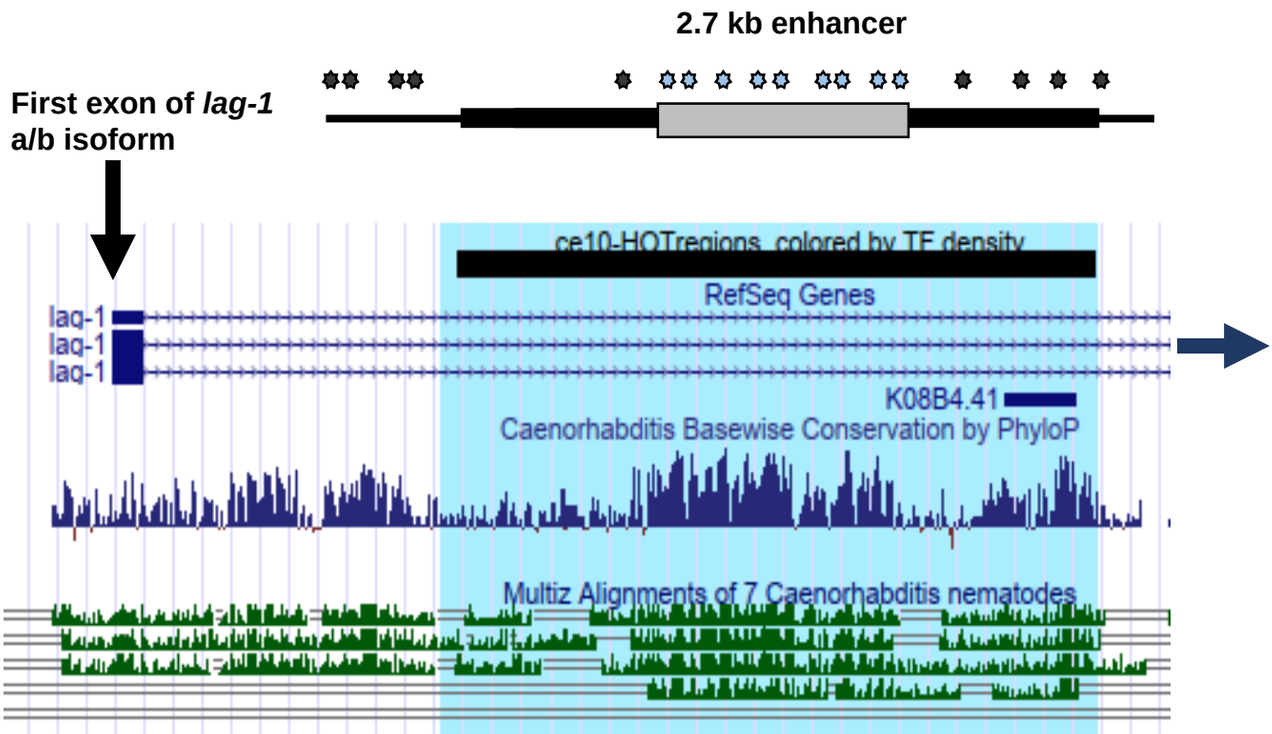

Figure S3. UCSC genome browser view of *lag-1* region used to generate the 2.7 kb enhancer. HOT region track (in black) is provided by Wreczycka, et al. (2019). Conservation tracks are shown in green.

Figure S4

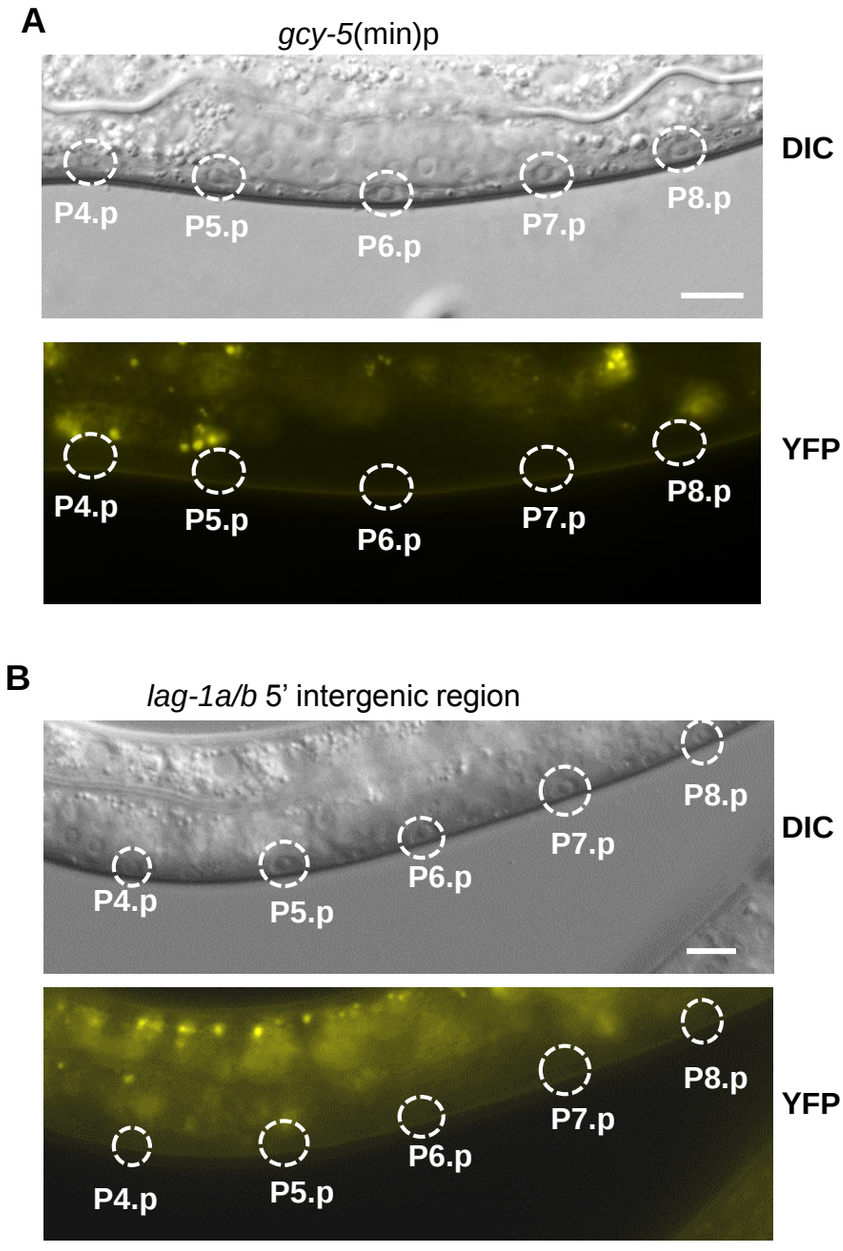

Figure S4. The *gcy-5* minimal promoter and the *lag-1* a/b 5' intergenic region do not result in detectable YFP expression in VPCs.

(A) Representative image of a transgene inserted in the LG I containing only [*gcy-5*(min)p::2xNLS::YFP]. No expression was observed in the L3 VPCs.

(B) Representative image of the intergenic region 5' to the *lag-1* ATG (of isoforms a/b) fused to *2xnls-yfp*. No expression was observed in the L3 VPCs.

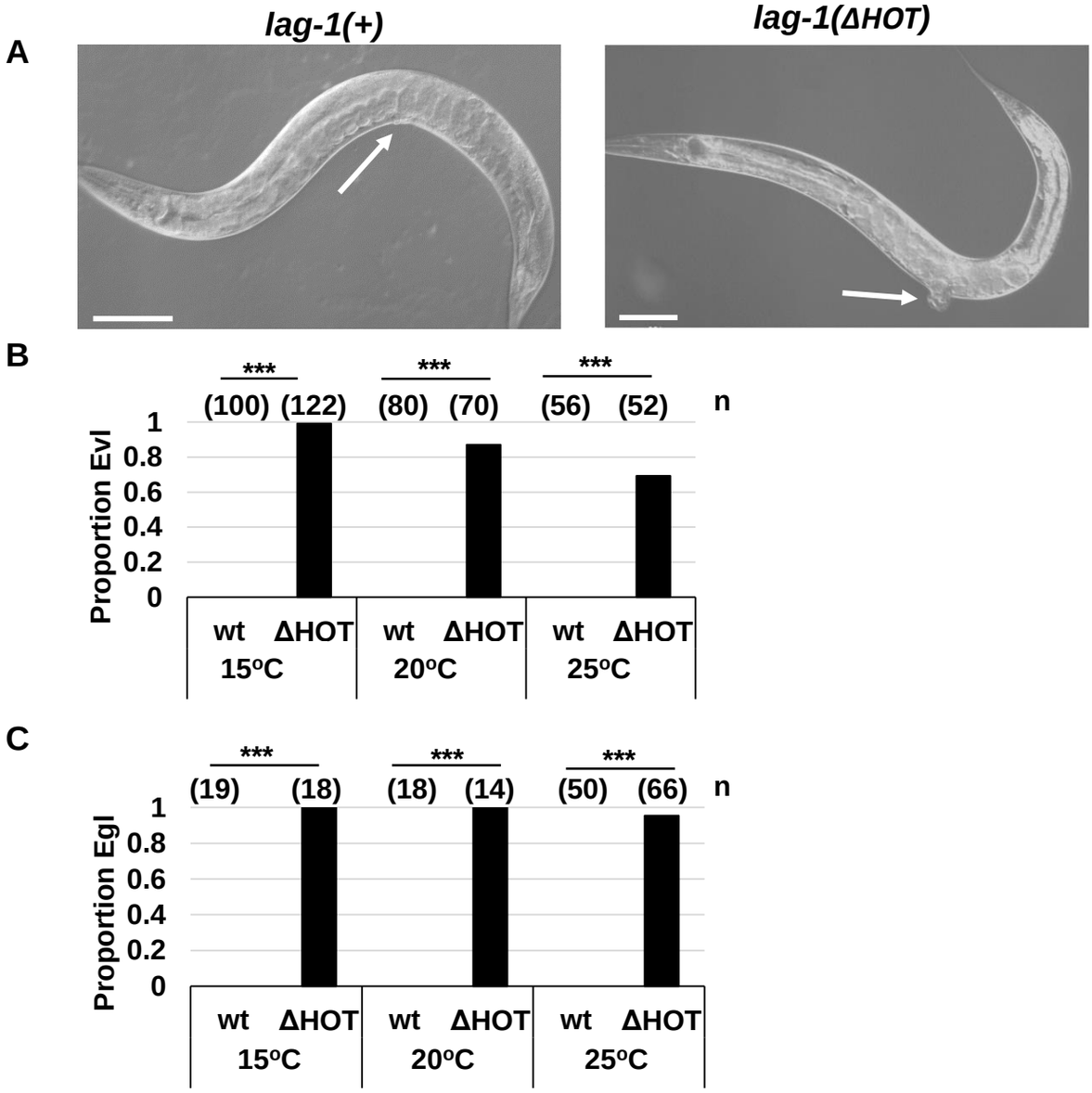

Figure S5. Deletion of the HOT region results in an egg-laying defective (Egl) phenotype and a cold-sensitive abnormal vulval eversion (Evl) phenotype. In addition, no difference in AC marker expression was observed between *lag-1::gfp* and *lag-1(ΔHOT)::gfp*.

(A) Representative images of adult hermaphrodite of *lag-1::gfp* compared to *lag-1(ΔHOT)::gfp* . Scale bar denotes 100 µm.

(B) Temperature dependence of the Evl phenotype. All comparisons between *lag-1::gfp* and *lag-1(ΔHOT)::gfp* were significant ($p<0.001$, Fisher's Exact Test).

(C) Temperature dependence of the Egl phenotype. All comparisons between *lag-1::gfp* and *lag-1(ΔHOT)::gfp* were significant ($p<0.001$, Fisher's Exact Test).

Table S1

Strain List	
Strain	**Genotype**
GS8732	*lag-1(ar611[lag-1::gfp]); jccTi1*[*lin-31*p::mCherry::H2B]
GS8762	*arTi102*[*lin-31*p::*lin-12(intraΔP)*] *lag-1(ar611[lag-1::gfp]); jccTi1*[*lin-31*p::mCherry::H2B]
GS9052	*lin-12(n941); lag-1(ar611[lag-1::gfp]); jccTi1*[*lin-31*p::mCherry::H2B]*; arEx1442*
GS9122	*hlh-2(ar614); lag-1(ar611[lag-1::gfp]); jccTi1*[*lin-31*p::mCherry::H2B]
GS8555	*lag-1(ar613[lag-1::mKate2])*
GS9295	*lag-1(ar611[lag-1::gfp]) jjIs3900*[*hlh-8*p::NLS::mCherry]
GS9047	*lag-1(ar611[lag-1::gfp]); arTi112*[*ckb-3*p::mCherry::H2B]
GS9233	*arSi35; jccTi1*[*lin-31*p::mCherry::H2B]
GS9236	*arSi59; jccTi1*[*lin-31*p::mCherry::H2B]
GS9252	*arSi60; jccTi1*[*lin-31*p::mCherry::H2B]
GS9235	*arSi55; jccTi1*[*lin-31*p::mCherry::H2B]
GS9179	*arSi53*
GS9181	*arSi55*
GS9264	*arSi74*
GS9296	*arSi81*
GS9354	*arSi59; arTi145*[*ckb-3*p::mCherry::H2B]
GS9355	*arSi60; arTi145*[*ckb-3*p::mCherry::H2B]
GS9293	*arSi59; jjIs3900*[*hlh-8*p::NLS::mCherry]
GS9294	*arSi60; jjIs3900*[*hlh-8*p::NLS::mCherry]
GS9397	*lag-1(ar611ar647); jccTi1*[lin-31p::mCherry::H2B]
GS9441	*lag-1(ar611ar647[lag-1(ΔHOT)::gfp]*); *arIs222*[*lag-2*p::2xNLS::tagRFP]
GS9442	lag-1(ar611*[lag-1::gfp]*); *arIs222*[*lag-2*p::2xNLS::tagRFP]
GS8741	*lag-1(ar613[lag-1::mKate2]); arTi22*[*hlh-2(prox)*p::gfp::h2b]

Table S2

Targeting sequence used for CRISPR/Cas9	
Target	**Sequence**
lag-1::gfp insertion	ATGGTGTCGTCTACTCGTC
lag-1 C-terminal insertions	CGAGAGTGGAATCTAGTAAT
lag-1 HOT deletion	TTGAGGTTCCCATGATGCTC, GTATAATCCGTTGAAGATTG

Table S3

Primers for homology template		
Target	**F**	**R**
lag-1::gfp insertion 5' homology arm	TTCAGTTGACGACGAACGTGG	final codon of *lag-1* (*lag-1* Sarov fosmid was used as the template so C-terminal GFP was used for R primer)
lag-1::gfp insertion 3' homology arm	GACATGATGTATCTCGGATTTTGTGGAAC	GCTTGTTGTTCTCATCTCTGCCAC
lag-1 C-terminal insertions 5' homology arm	AGGACAACCGGCGATGTTGAG	GTAATTGGACACAATTCTGCACGGTC
lag-1 C-terminal insertions 3' homology arm	TAGATTCCACTCTCGCGGGATTACTG	GAATCGGATGCGTGGATAGTTGATAATTTATCTG
lag-1 HOT deletion ssODN repair	CGGAAGTACAGCTAAAATGTGTGAGATCTAGGTTAGTTCCCATGATGCTCTGGGCAATTTCGCACATGAC	

Table S4

Transgenes made using *ttTi4348*				
Strain	**Genotype**	***ttTi4348* Transgene**	**Plasmid**	**Figure**
GS9233	***arSi35***; *jccTi1*	lag-1enhancer (2.7kb) + gcy-5(min)p::2xnls-yfp	pKL77	4B
GS8999	***arSi35***			4E
GS9236	***arSi59***; *jccTi1*	lag-1enhancer (1.6kb) + gcy-5(min)p::2xnls-yp	pKL107	4C
GS9293	***arSi59***; *jjIs3900*			5A
GS9354	***arSi59***; *arTi145*			5B
GS9252	***arSi60***; *jccTi1*	lag-1enhancer (1.6kb ΔLBS) + gcy-5(min)p::2xnls-yfp	pKL108	4D
GS9355	***arSi60***; *arTi145*			5C
GS9294	***arSi60***; *jjIs3900*			5D
GS9264	***arSi74***	lag-1enhancer (2.7kb Δnon-conserved LBS) + gcy-5(min)p::2xnls-yfp	pKL131	4F
GS9296	***arSi81***	lag-1enhancer (2.7kb Δconserved LBS) + gcy-5(min)p::2xnls-yfp	pKL110	4G
GS8992	***arSi31***	gcy-5(min)p::2xnls-yfp	pKL78	S4A
GS9353	***arSi86***	lag-1p(gene-to-gene 1.4kb)::2xnls-yfp	pKL139	S4B
GS9235	***arSi55***; *jccTi1*	lag-1enhancer(conserved +400bp) + gcy-5(min)p::2xnls-yfp	pKL103	S4C
GS9250	***arSi70***	lag-1enhancer(1.6kb enhancer Δnon-HOT LBS) + gcy-5(min)p::2xnls-yfp	pKL122	S4D
GS9179	***arSi53***	lag-1enhancer(conserved) + gcy-5(min)p::2xnls=yfp	pKL96	S4E

Table S5

Primers used in enhancer analysis		
Transgene	**F Primer**	**R Primer**
arSi35	TTCCCATCCTAGTTTTTCCCACAC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi59	CGTCATCGTCCTCTGTCGC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi60	CGTCATCGTCCTCTGTCGC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi74	TTCCCATCCTAGTTTTTCCCACAC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi81	TTCCCATCCTAGTTTTTCCCACAC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi31	GCAGATACCAACAAGATTAAAACTTCAAAC	TTTTCATCAGAATAAGTAATTTTTCGAAAACAATAAATAG
arSi86	AACTTTATTTTTAGAAAAGCGAATTTTACCTTCA	CTGAAATTTCTGAATGTTATTTTCATCAATTATAAC
arSi55	AAAATTACATTCCGCACTGCCAG	TTAGGCTTAGTAATGTTGTTTTCTAAGCC
arSi70	CGTCATCGTCCTCTGTCGC	AAATTCAAATACTGGCATAGAATATATAACTAGTTTTC
arSi53	CGTCATCGTCCTCTGTCGC	TTTCTTAGTACTTTTCAATCTTCCCACCAG