Supplemental Table 1. Oligonucleotide primers used in this study.

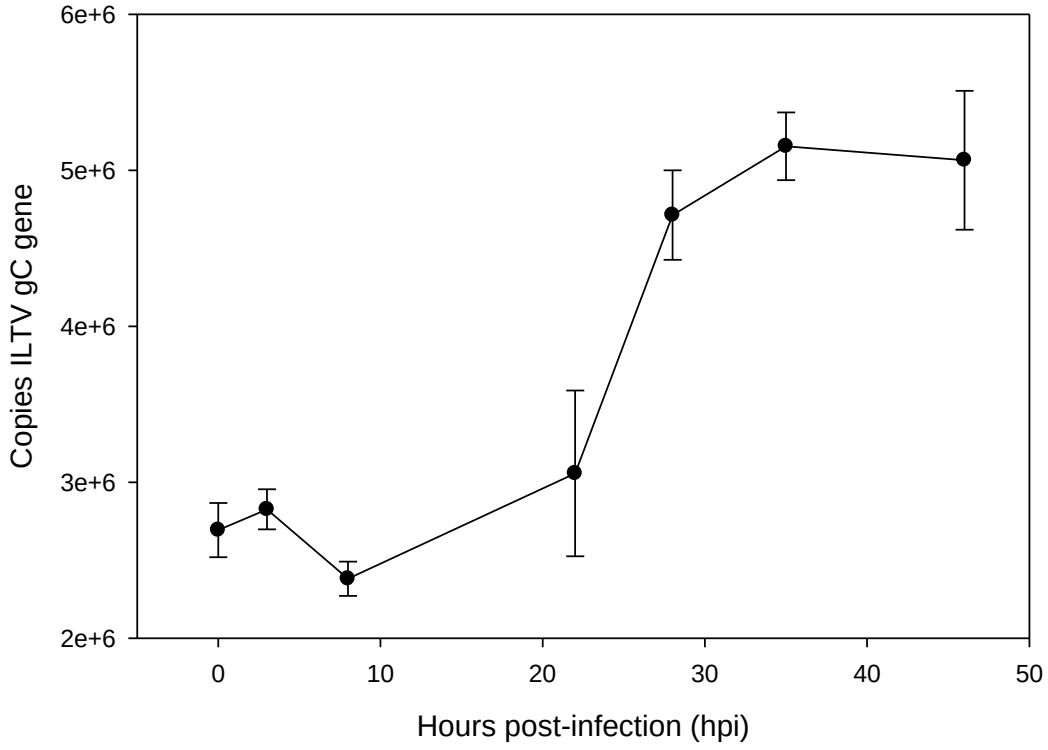
Primer name	Purpose	Genome positions (TR_S)	Sequence	Amplicon size (bp)	Annealing temp. (^oC)
5T-R	qRT-PCR iltv-miR-I5 region	146,476 - 146,495	CACTCCGAAATCGGAAAAGC	242	55
5T-F		146,689 - 146,706	GGCGCGCTCACGATCTTC		
Protect-F	qRT-PCR "protected" area	146,743 – 146,761	ATTCGGGACCGCGTGTACC	191	55
Protect-R	4079F – 3907R	146,913 – 146,933	GAGATGATGCGGAGAACTTCC		
GAPDH-5' Exon 7	qRT-PCR endogenous control	Not applicable	GGAAAGTCATCCCTGAGCTG	81	55
GAPDH-3' Exon 8		Not applicable	GGTCAACAACAGAGACATTGG		55
4079R1	Modified RACE, cDNA synthesis	146,913 - 146,933	GAGATGATGCGGAGAACTTCC	301 [a]	55
53pGSP1	Modified RACE, 1^oPCR Reverse	146,884 - 146,899	TGGGATCGGACCAGGA	267 [a]	55
53pGSP2	Modified RACE, 2^oPCR Reverse	146,826 - 146,844	CCACGCAGCGACGGCCCTA	212 [a]	55
RNA adapter	Modified RACE, ligation	Not applicable	GUUCAGAGUUCUACAGUCCGAC	n/a	n/a
DNASh-Adapt	Modified RACE, 1^oPCR Forward	Not applicable	GTTCAGAGTTCTACAGTCCGAC	212 [a]	55
DNASh-mod2	Modified RACE, 2^oPCR Forward	Not applicable[b]	GTTCAGAGTTCTACAGTCCGAC**CG**	212 [a]	55
DNASh-mod7	Modified RACE, 2^oPCR Forward	Not applicable[b]	GTTCAGAGTTCTACAGTCCGAC**CGTCTTCT**	212 [a]	55
63ptarget-F-SpeI	Cloning pMiR-Report-I5-I6-target	146,290 - 146,316	GTAGGTCACTAGTAATGGACAGGCGA	409	60
53ptarget-R-SacI	(WT and mutated derivative)	146,685 - 146,708	CCGGCGAGCTCACGATCTTCTTTC		60
53ptarget-R-SacI	Cloning pmiR-Report-I5-target	146,685 - 146,708	CCGGCGAGCTCACGATCTTCTTTC	331	60
53ptarget-F-SpeI	(WT and mutated derivative)	146,377 – 146,402	TTAACGGGAGGACACTTCTCTCTGTC		

[a] Predicted amplicon size if RACE amplification results from adapter ligated to nt#10 in iltv-miR-I5-3p binding site.

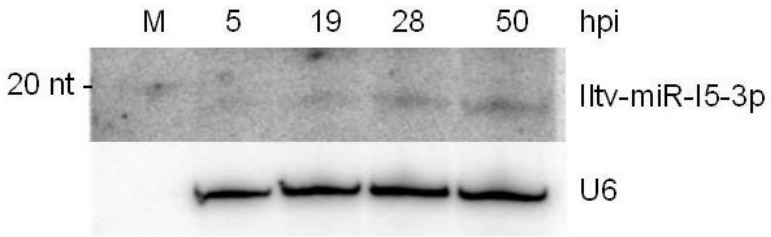
[b] Bold type base pairs "cg" in DNASh-mod2 and "cgtcttct" in DNASh-mod7 are predicted to bind to nt#1-9 and 8-9, respectively, of complement to iltv-miR-I5-3p, at genome positions 146633-146640, in the TR_S.

Supplemental Figure 1. ILTV growth and microRNA production in LMH cells. (A) ILTV growth curve. For each time point, three wells of ILTV-infected LMH cells were harvested, and total DNA was isolated from the combined cell culture medium and cell monolayer. Each well was subjected to three qPCR reactions for the ILTV gC gene as described previously (Callison et al., 2007). Data are the averages of gC gene copies (+/- SE) per standard volume for three biological replicates. (B) Northern blot analysis of RNA from ILTV-infected LMH cells. Cells were plated in 6-well plates and infected at an MOI of 0.01. RNA was extracted at 5, 19, 28, and 50 hours post-transfection (hpi). The RNA was probed with ^{32}P-labelled oligonucleotides antisense to iltv-miR-I5-3p. The cellular small RNA U6 was probed on the same blot as a loading control. Approximate nucleotide size as indicated by the 10-bp DNA Step Ladder (Promega).

(A)



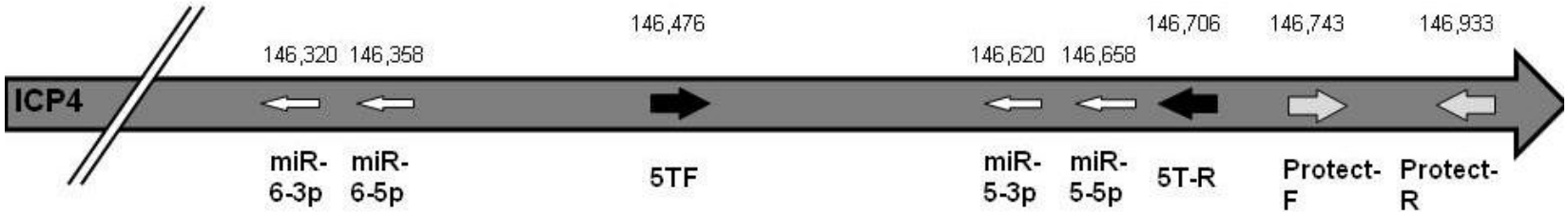
(B)



Callison, S. A., Riblet, S. M., Oldoni, I., Sun, S., Zavala, G., Williams, S., Resurreccion, R. S., Spackman, E., and Garcia, M. (2007). Development and validation of a real-time Taqman PCR assay for the detection and quantitation of infectious laryngotracheitis virus in poultry. *J. Virol. Methods* **139**(1)**,** 31-38.

Supplemental Figure 2. Quantitative RT-PCR of three regions within the coding region of ICP4. LMH cells were co-transfected with a plasmid expressing ICP4 (pRc-ICP4) and 300 nM microRNA mimics (iltv-miR-I5 or a negative control mimic) or 600 nM mimic pool (iltv-miR-I5 plus its antagomiR or 600 nM negative control). Panel A: Total RNA was subjected to qRT-PCR for the ICP4 gene, using two different pairs of primers. The primer pair flanking the iltv-miR-I5 target region is designated 5T. Primers flanking the area outside of microRNA target sites are designated "Protect F-R." Panel B: qRT-PCR values are normalized to cellular GAPDH, and the values are expressed as average relative quantities (RQ, 2^{-ddCt}) +/- standard deviation (SD) of three PCR replicates. The p-values result from Student's t-test comparison of microRNA mimic treatments to 300 or 600 nM negative control treatments, with significant difference ($p<0.05$) from the negative control indicated with grey highlighting.

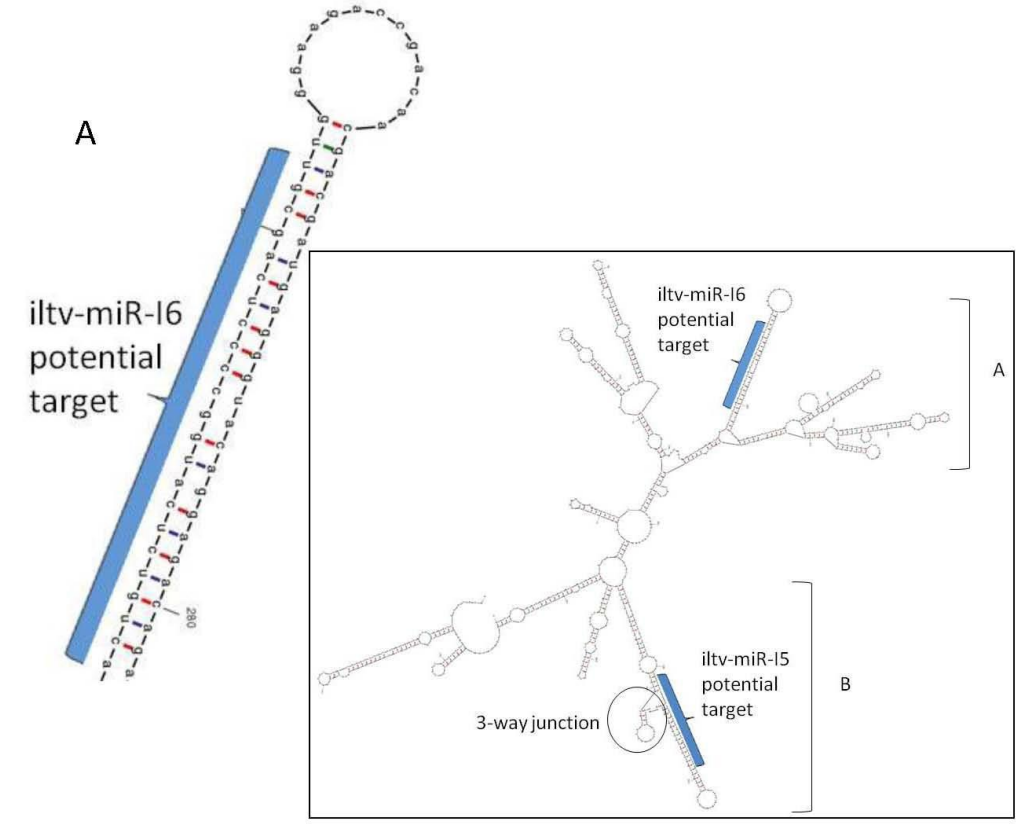
A.

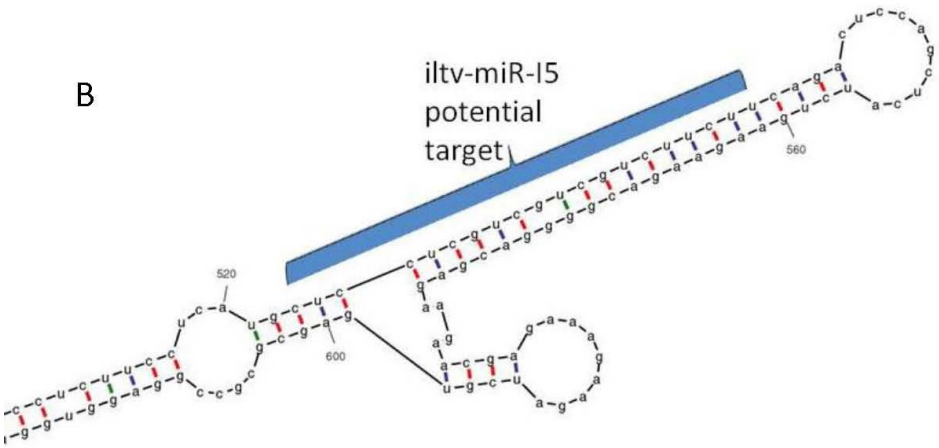


B.

MicroRNA target Region	Primers used	Treatment	RQ	SD	p value
iltv-miR-I5 target region	5T-F, 5T-R	pRcICP4+300 nM Negative miR	1.00	0.08	
		+600 nM Negative miR	1.000	0.11	
		+miR-I5	0.48	0.03	**0.01**
		+miR-I6	0.90	0.07	0.33
		+miR-I5+antimiR-I5	0.86	0.06	0.17
"protected" area, no target	Protect-F, Protect R	pRcICP4+300 nM Negative miR	1.00	0.17	
		+600 nM Negative miR	1.00	0.07	
		+miR-I5	0.68	0.11	0.18
		+miR-I6	0.81	0.16	0.41
		+miR-I5+antimiR-I5	0.84	0.13	0.16

Supplemental Figure 3. Secondary structure of putative target sites for iltv-miR-I5 and iltv-miR-I6 within the ICP4 coding region. An 800-nucleotide portion of the ICP4 coding region (genome positions 146,100 to 146,899) was subjected to *in silico* folding using mFold (Zuker, 2003), Potential target sites to which either iltv-miR-I6 or iltv-miR-I5 are 100% complementary are marked with blue brackets (inset). The putative secondary structure opposite the iltv-miR-I5 target sequence (three-way junction) is indicated with a black circle. (A) Region containing potential target site for iltv-miR-I6. (B) Region containing potential target site for iltv-miR-I5.





Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31**(13)**,** 3406-3415.