

Supplemental material for

Rapid detection of DNA and RNA shrimp viruses using CRISPR-based diagnostics

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Supplemental Protocol. One-pot SHERLOCK protocol for WSSV and TSV detection

Materials

- Nuclease free water
- 10X Isothermal Amplification Buffer (New England BioLabs, Cat# B0537S)
- 100 mM MgSO₄ (New England BioLabs, Cat# B1003S, supplied with M0538L)
- 10 mM dNTPs (New England BioLabs, Cat# N0447L)
- Bst 2.0 WarmStart DNA Polymerase (New England BioLabs, Cat# M0538L)
- WarmStart RTx Reverse Transcriptase (New England BioLabs, Cat# M0380L)
- 500 mM Taurine (Millipore Sigma, Cat# 86329)
- 2 M Glycine (ThermoFisher, Cat# BP381-500)
- *Aap* Cas12b enzyme (synthesized by GenScript)
- 5'FAM/TTTTT/3'IBFQ reporter (100 μM, Integrated DNA Technologies)
- SYTO-82 Fluorescent Stain (ThermoFisher, Cat# S11363)
- Sample DNA or RNA
- 10 μM Guide RNA
 - Guide RNA can be prepared ahead of time and stored at -80°C before use.
 - Prepare guide RNA as follows:
 1. Amplify guide RNA template using the following oligos and conditions:
 - *Aap Cas12b sgRNA scaffold gBlock* 5'-
TGTA AACGACGGCCAGTcatataTAATACGACTCACTATAG
GGGTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATG
GCCACTTCCAGGTGGCAAAGCCCGTTGAGCTTCTCAA
TCTGAGAAGTGGCAC GTCATAGCTGTTTCCTG-3'
 - Cas12b_sgRNA_fwd primer 5'-
CATATATAATACGACTCACTATAGGGGTCTAGAGG-3'
 - Cas12b_sgRNA_rev primer(s): Replace x's with 20 base target region 5'-
xxxxxxxxxxxxxxxxxxxxGTGCCACTTCTCAGATTTGAGAAGC
TC-3'
 - 1X HF Buffer (New England BioLabs), 200 μM dNTPs, 0.5 μM forward primer, 0.5 μM reverse primer, 0.01 ng gBlock DNA, and 1 unit Phusion DNA polymerase (New England BioLabs) in 50 μL.
 - Run reaction on a thermocycler (Applied Biosystems) with the following parameters: 98°C for 2 minutes, 30 cycles of 98°C for 10 seconds, 58°C for 30 seconds, and 72°C for 15 seconds, 72°C for 5 minutes, and a hold at 10°C.
 2. Gel purify PCR products on a 2% agarose 1x TAE gel and using the QIAquick Gel Extraction kit (Qiagen) following manufacturer's instructions.
 3. Transcribe guide RNA using the Ampliscribe T7 flash transcription kit (Lucigen)
 - In a 40 μL reaction, combine 50 ng of gel purified PCR product, 9 mM of each ribonucleotide, 9 mM of DTT, 1 μL of RiboGuard

RNase inhibitor, 4 μL of T7 RNA polymerase, and 1X transcription reaction buffer.

- Run reactions at 42°C for 4 hours
- Incubate with 2 μL of DNase I (Lucigen) at 37°C for 15 minutes
- Purify using the RNA Clean and Concentrate Kit (Zymo Research) following manufacturer's instructions
- Store purified guide RNA at -80°C.

- 10X LAMP Primer Mix

- 10X Primer mix can be prepared ahead of time and stored at -20°C before use.
- Prepare a 10x primer mix of the LAMP primers as follows:

10X Primer Mix			
LAMP Primer (100 μM)	10x concentration (μM)	Reaction Concentration (μM)	Volume (μL)
F3	2	0.2	2
B3	2	0.2	2
BIP	16	1.6	16
FIP	16	1.6	16
LF*	4	0.4	4
LB*	4	0.4	4
ddH ₂ O			56
Total			100

* If not included in LAMP primer set, replace with water

Procedure

1. Prepare sample DNA or RNA

- For synthetic target, dilutions are made as follows:
 - For TSV, combine RNA stock containing 10¹⁰ copies TSV target, nuclease free water, and specific pathogen free shrimp genomic RNA (5 ng per 20 μL one-pot reaction)
 - For WSSV, combine DNA stock containing 10⁹ copies WSSV target, nuclease free water, and specific pathogen free shrimp genomic DNA (20 ng per 20 μL one-pot reaction)
- For genomic DNA or RNA extracted from shrimp, dilutions are made as follows:

Template dilution component	WSSV	TSV
Sample DNA/RNA	0.25 ng/ μL (1ng)	2 ng/ μL (10 ng)
Pathogen free shrimp nucleic acid (DNA for WSSV, RNA for TSV)	5 ng/ μL (20 ng)	1 ng/ μL (5 ng)
Nuclease free water	variable	variable
Total volume/one-pot reaction	4 μL	5 μL

2. Prepare the master mix by combining the reagents in the following order:

One-Pot SHERLOCK Reaction component	Initial concentration	Final concentration	WSSV reaction volume (μL)	TSV reaction volume (μL)
water			3.44	1.46
Isothermal Amp Buffer	10x	1x	2	2
MgSO ₄	100 mM	8 mM	1.6	1.6
glycine	2 M	200 mM	2	
taurine	500 mM	50 mM		2
Cas12b	8.73 μ M	200 nM (150 nM for TSV)	0.458	0.344
sgRNA	10 μ M	200 nM (600 nM for TSV)	0.4	1.2
dNTPs	10 mM	1.4 mM	2.8	2.8
<i>incubate at room temperature for 15 minutes to facilitate formation of CRISPR-Cas complex</i>				
WarmStart RTx Reverse Transcriptase	15000 units/mL	150 units/mL		0.2
WarmStart Bst 2.0	8000 units/mL	320 units/mL	0.8	0.8
Primer mix	10x	1x	2	2
SYTO-82 LAMP dye	100 μ M	0.5 μ M (1 μ M for TSV)	0.1	0.2
5'FAM/TTTTT/3'IBFQ reporter	100 μ M	2 μ M	0.4	0.4
Sample DNA/RNA			4	5
Total			20	20

3. Run on real-time PCR thermocycler at 62°C for WSSV or 60°C for TSV for 1 hour taking a reading every 2 minutes.

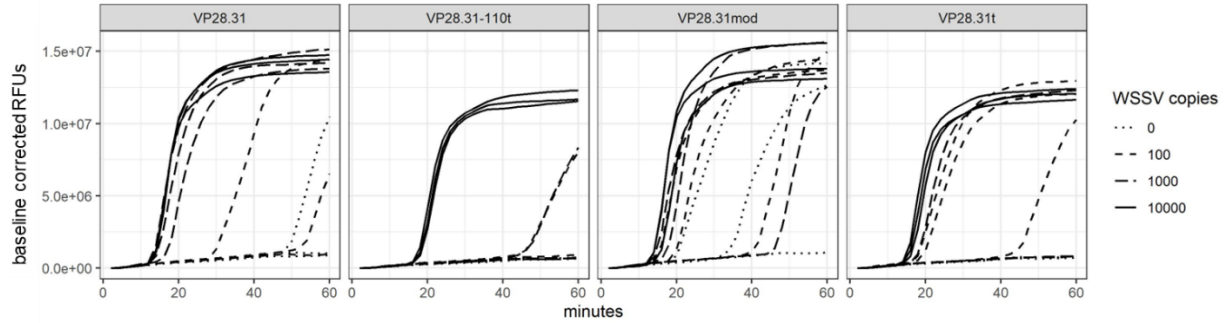


Figure S1. Amplification plots of triplicate LAMP reactions showing the effects of different modifications (indicated by plot titles and shown in **Table S1**) to the VP28.31 primer set for WSSV.

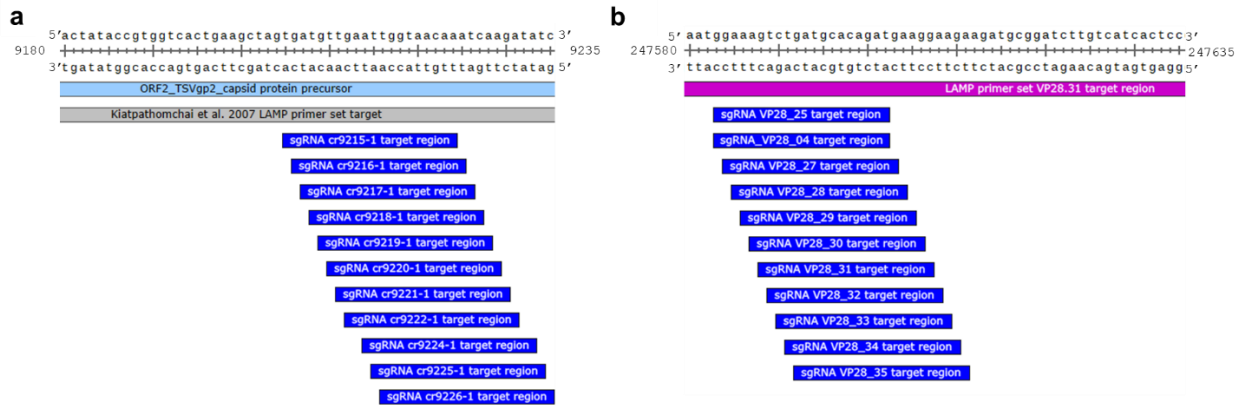


Figure S2. Diagrams of annotated consensus genome segments showing target regions of guide RNAs (dark blue) screened for **(a)** TSV or **(b)** WSSV.

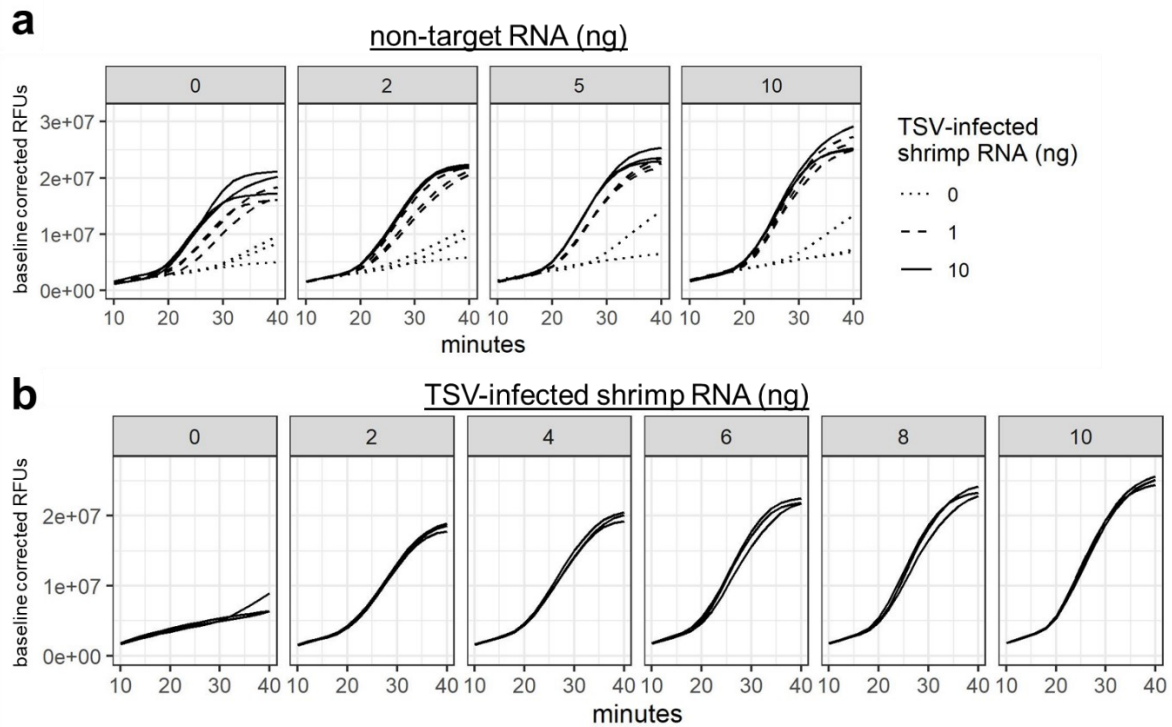
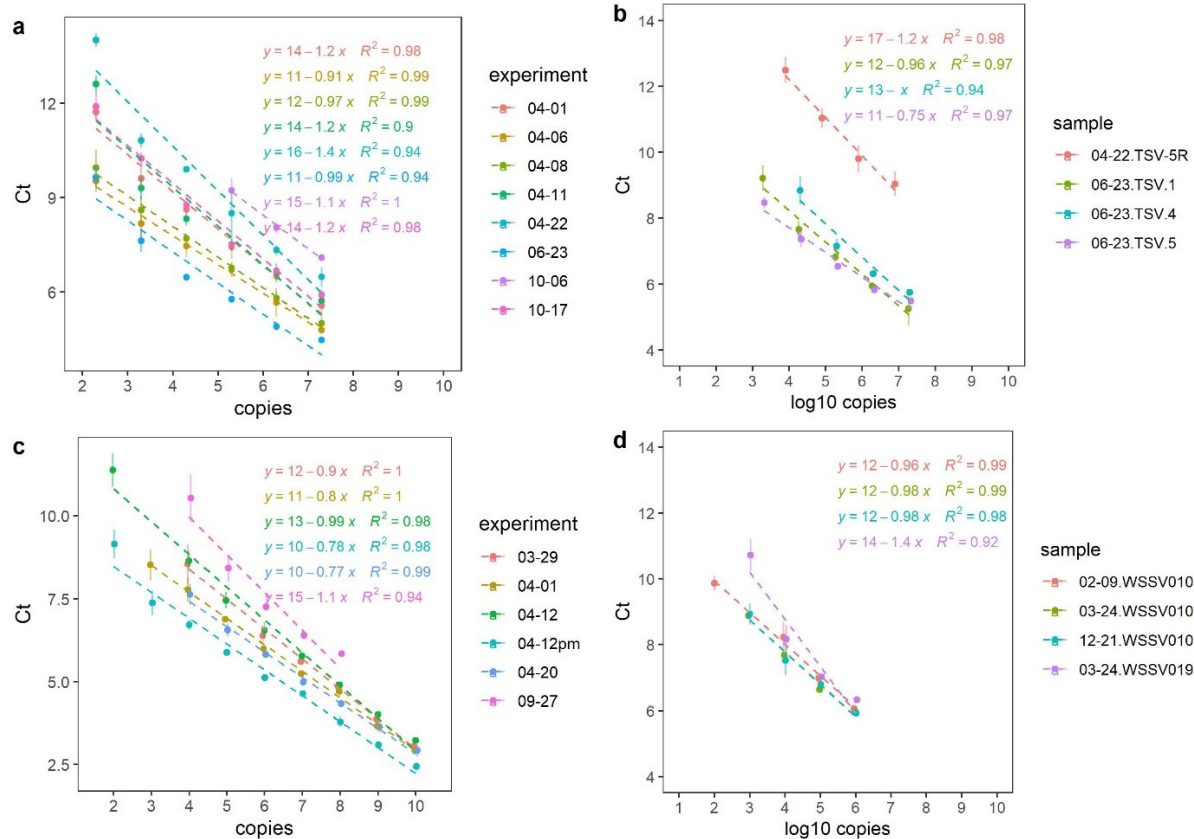


Figure S3. Optimizing amount of non-target and target RNA for the TSV SHERLOCK assay. **(a)** Cas cleavage activity plots of TSV SHERLOCK reactions containing varying amounts (ng; nanograms) of non-target RNA and varying amounts of target RNA (TSV-infected shrimp genomic RNA). **(b)** Cas cleavage activity plots of TSV SHERLOCK reactions containing varying amounts (ng; nanograms) of TSV-infected shrimp genomic RNA (gRNA).



FigureS4. Comparison of synthetic and genomic target dilution series. **(a)** TSV SHERLOCK standard curves generated with synthetic target for eight different experiments. **(b)** TSV SHERLOCK curves generated with a 10-fold dilution series of genomic target isolated from samples with high viral load. **(c)** WSSV SHERLOCK standard curves generated with synthetic target for six different experiments. **(d)** WSSV SHERLOCK curves generated with a 10-fold dilution series of genomic target isolated from samples with high viral load. Linear equations are shown for each regression model (dashed line) with color corresponding to experiment number.

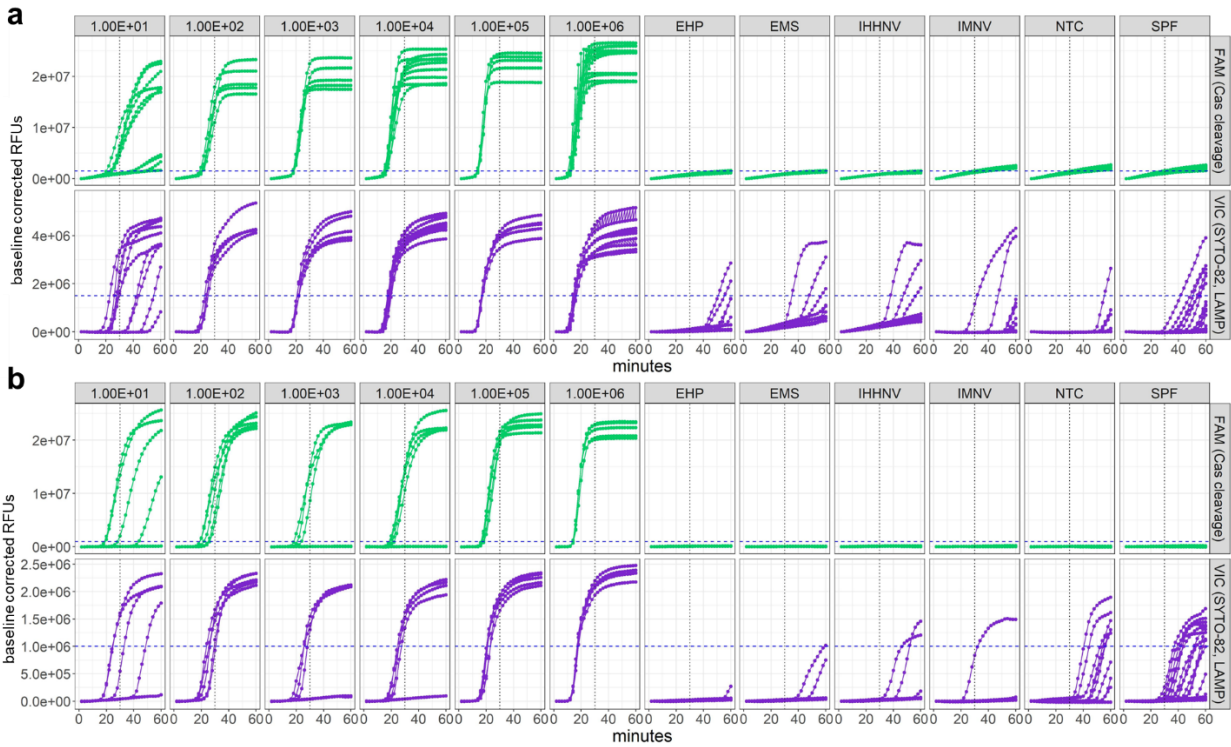


Figure S5. Non-specific LAMP signal does not give rise to Cas cleavage signal. (a) TSV and (b) WSSV SHERLOCKv2 assay fluorescence shows only samples containing target sequence produce a Cas cleavage signal (green) above the CT (blue dashed line), and non-specific LAMP signal (purple) produced in samples without target does not lead to Cas cleavage. Plots titled 1e+01 through 1e+06 indicate viral copies present in each reaction; ‘NTC’ signifies no template control; SPF signifies specific-pathogen free shrimp samples; EHP, EMS, IHNV, and IMNV signify sample infected with nontarget viruses.

Table S1. Consensus genome, gBlock oligos, primers, sgRNA, and reporter oligo sequences used for developing TSV and WSSV assays. Bold indicates oligos used in final versions of assays.

Virus	Oligo type	Name	LAMP Primer Mix Name	Notes	Sequence (5'-3')
TSV	LAMP Primer	TSV_F3	TSV		CAATTGAAATTCTGAGATTAGATC
TSV	LAMP Primer	TSV_FIP	TSV		CTAGCTTCAGTGACCACGGTATAGTTTTATTTTGTAGTCCAAAGCTCCA
TSV	LAMP Primer	TSV_LF	TSV		TACCTGGAGTAAATTTAC
TSV	LAMP Primer	TSV_B3	TSV		GGTACATATCGAGCCACTC
TSV	LAMP Primer	TSV_BIP	TSV		GCGAACCCATGCGGGTATAGTTTTCAATGCGACCAATGACTG
TSV	LAMP Primer	TSV_LB	TSV		ATGAAGAACCGCCAGTTAAGCAA
TSV	LAMP Primer	TSV_P123L7_F3	TSV_P123L7		GTCCAGCTTTCACTTTTGG
TSV	LAMP Primer	TSV_P123L7_FIP	TSV_P123L7		ACAAATTCATGGCACCTCAACCCCTGCCGAATTGGCTTAA
TSV	LAMP Primer	TSV_P123L7_LF	TSV_P123L7		TGACGGGTCTACACCTGAAGG
TSV	LAMP Primer	TSV_P123L7_BIP	TSV_P123L7		TGGTGCATTTATCTCAACAGTTTTCTCTCAGAGTAAAAACCTG
TSV	LAMP Primer	TSV_P123L7_B3	TSV_P123L7		CTCTAATAGACCTTAGTGAGCA
TSV	LAMP Primer	TSV_P73L39_F3	TSV_P73L39		TTCCAAGTCTTCCCTCAA
TSV	LAMP Primer	TSV_P73L39_FIP	TSV_P73L39		ATCACCAGCACTTGAAGCAATCAATTAGTAATTTGGAAAGGACG
TSV	LAMP Primer	TSV_P73L39_LB	TSV_P73L39		TCACTTTTGGACCTGCCGA
TSV	LAMP Primer	TSV_P73L39_BIP	TSV_P73L39		GACCACACTTTCTCTCAATTGGCCTGAAGGGTGTTAAATTAA GCC
TSV	LAMP Primer	TSV_P73L39_B3	TSV_P73L39		ACAATGACGGGTCTACAC
TSV	LAMP Primer	TSV_P215L25_F3	TSV_P215L25		GTTGAACAACAGTGATCCAT
TSV	LAMP Primer	TSV_P215L25_FIP	TSV_P215L25		TTGAGGTTGCAAAAACAAAAGATGGGTACATATGTCCAGTGT CAGG
TSV	LAMP Primer	TSV_P215L25_LB	TSV_P215L25		GATGCATTGACGCGGCTTTAG
TSV	LAMP Primer	TSV_P215L25_BIP	TSV_P215L25		GCACGTATGTCCCCAAGTCTACTTTAACATCTACATAAACGC ATAG
TSV	LAMP Primer	TSV_P215L25_B3	TSV_P215L25		CGATGCGTGCAAACCTCAT
WSSV	LAMP Primer	VP28_F3	VP28	initial design	CTCCGATTCTGTGACTG
WSSV	LAMP Primer	VP28_B3	VP28	initial design	CCACACCTTGAATGTTCCCT
WSSV	LAMP Primer	VP28_FIP	VP28	initial design	GCCCAAGGTGTCGCTGTCAAACCTGAGGTTGGATCAGGCTAC
WSSV	LAMP Primer	VP28_BIP	VP28	initial design	CTTGTCATCACTCCCGTGGAGGAAGGTGAGATTCTGCCCA
WSSV	LAMP Primer	VP28_LF	VP28	initial design	GGACACATCAGTCATCTTG
WSSV	LAMP Primer	VP28_LB	VP28	initial design	GCCGAGCACTCGAAGTG
WSSV	LAMP Primer	VP28.1_F3	VP28.1	initial design	GGTTGGATCAGGCTACT

WSSV	LAMP Primer	VP28.1_B3	VP28.1	initial design	CCAGTGATGTTGATCTTTCTTGATG
WSSV	LAMP Primer	VP28.1_FIP	VP28.1	initial design	CCATTGCGGATCTTGATTTTGTCAAGATGACTGATGTGTC
WSSV	LAMP Primer	VP28.1_BIP	VP28.1	initial design	GCCGAGCACTCGAAGTCACACCTTGAATGTTCCCTCA
WSSV	LAMP Primer	VP28.1_LF	VP28.1	initial design	GGTGTGCTGTCAAAG
WSSV	LAMP Primer	VP28.1_LB	VP28.1	initial design	GTGGGGCAGAATCTCA
WSSV	LAMP Primer	VP28.31_F3	VP28.31	initial design	AGGCTACTTCAAGATGAC
WSSV	LAMP Primer	VP28.1_B3	VP28.31	initial design	CCAGTGATGTTGATCTTTCTTGATG
WSSV	LAMP Primer	VP28.31_FIP	VP28.31	initial design	CAGACTTCCATTGCGGATTGATGTGTCTTTGACAG
WSSV	LAMP Primer	VP28.1_BIP	VP28.31	initial design	GCCGAGCACTCGAAGTCACACCTTGAATGTTCCCTCA
WSSV	LAMP Primer	VP28.31_LF	VP28.31	initial design	ATTTGCCCAAGGTGTCG
WSSV	LAMP Primer	VP28.1_LB	VP28.31	initial design	GTGGGGCAGAATCTCA
WSSV	LAMP Primer	VP28.1_F3	VP28.110	initial design	GGTTGGATCAGGCTACT
WSSV	LAMP Primer	VP28.1_B3	VP28.110	initial design	CCAGTGATGTTGATCTTTCTTGATG
WSSV	LAMP Primer	VP28.110_FIP	VP28.110	initial design	GACTTCCATTGCGGATCTTGACTGATGTGTCTTTGAC
WSSV	LAMP Primer	VP28.110_BIP	VP28.110	initial design	AGGGCCGAGCACTCGAACACACCTTGAATGTTCCCTC
WSSV	LAMP Primer	VP28.110_LF	VP28.110	initial design	TGCCAAGGTGTCGCT
WSSV	LAMP Primer	VP28.110_LB	VP28.110	initial design	GTGACTGTGGGGCAGA
WSSV	LAMP Primer	VP28.31t_FIP	VP28.31	optimization	CAGACTTCCATTGCGGATTTTTGATGTGTCTTTGACAG
WSSV	LAMP Primer	VP28.1t_BIP	VP28.31	optimization	GCCGAGCACTCGAAGTTTTTACACCTTGAATGTTCCCTCA
WSSV	LAMP Primer	VP28_110_BIPt	VP28.110	optimization	AGGGCCGAGCACTCGAATTTTACACCTTGAATGTTCCCTC
WSSV	LAMP Primer	VP28_31mod_FIP	VP28.31	optimization	GCATCAGACTTCCATTGCGGGTGTCTTTGACAGCG
WSSV	LAMP Primer	VP28_F3	VP28_VP2 8.1	initial design	CTCCGATTCTGTGACTG
WSSV	LAMP Primer	VP28_FIP	VP28_VP2 8.1	initial design	GCCCAAGGTGTCGCTGTCAAAGTGGATCAGGCTAC
WSSV	LAMP Primer	VP28_LF	VP28_VP2 8.1	initial design	GGACACATCAGTCATCTTG
WSSV	LAMP Primer	VP28.1_B3	VP28_VP2 8.1	initial design	CCAGTGATGTTGATCTTTCTTGATG
WSSV	LAMP Primer	VP28.1_BIP	VP28_VP2 8.1	initial design	GCCGAGCACTCGAAGTCACACCTTGAATGTTCCCTCA
WSSV	LAMP Primer	VP28.1_LB	VP28_VP2 8.1	initial design	GTGGGGCAGAATCTCA
NA	Cas12b sgRNA scaffold gBlock	Cas12b_m13-T7-Handle	NA	Cas12b conserved handle with T7 promoter and m13 fwd (5' TGT AAA ACG ACG GCC AGT) and m13 rev (5' CAG GAA ACA GCT ATG AC)	TGTAAAACGACGGCCAGTcatataTAATACGACTCACTATAG GGGTCTAGAGGACAGAAATTTTCAACGGGTGTGCCAATGG CCACTTCCAGGTGGCAAAGCCCGTTGAGCTTCTCAAATCT GAGAAGTGGCAC GTCATAGCTGTTCTCTG

NA	gRNA template primer	Cas12b_sgRNA_fwd	NA		CATATATAATACGACTCACTATAGGGGCTAGAGG
TSV	gRNA template primer	TSV_cr9215-1	NA		ttgttaccattcaacatcaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9216-1	NA		ttgttaccattcaacatcaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9217-1	NA		attgttaccattcaacatcaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9218-1	NA		gattgttaccattcaacaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9219-1	NA		tgattgttaccattcaacGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9220-1	NA		ttgattgttaccattcaaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9221-1	NA		cttgattgttaccattcaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9222-1	NA		tcttgattgttaccattcaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9224-1	NA		tatcttgattgttaccatGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9225-1	NA		atatcttgattgttaccaaGTGCCACTTCTCAGATTGAGAAGCTC
TSV	gRNA template primer	TSV_cr9226-1	NA		gatattcttgattgttaccGTGCCACTTCTCAGATTGAGAAGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_25	NA		ATCTGTGCATCAGACTTCCGTGCCACTTCTCAGATTGAGAAGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_V P28_27	NA		CATCTGTGCATCAGACTTCCGTGCCACTTCTCAGATTGAGAAGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_28	NA		TCATCTGTGCATCAGACTTGTGCCACTTCTCAGATTGAGAAGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_29	NA		TTATCTGTGCATCAGACTTGTGCCACTTCTCAGATTGAGAAGCTC

WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_30	NA		CTTCATCTGTGCATCAGACTGTGCCACTTCTCAGATTTGAGA AGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_31	NA		CCTTCATCTGTGCATCAGACGTGCCACTTCTCAGATTTGAGA AGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_32	NA		TCCTTCATCTGTGCATCAGAGTGCCACTTCTCAGATTTGAGA AGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_33	NA		TTCTTCATCTGTGCATCAGGTGCCACTTCTCAGATTTGAGA AGCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_34	NA		CTTCCTTCATCTGTGCATCAGTGCCACTTCTCAGATTTGAGAA GCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_35	NA		TCTTCCTTCATCTGTGCATCGTGCCACTTCTCAGATTTGAGAA GCTC
WSSV	gRNA template primer	Cas12b_sgRNA_rev_VP 28_04	NA		GGAAAGTCTGATGCACAGATGTGCCACTTCTCAGATTTGAG AAGCTC
TSV	gBlock	P215L25	NA		CCTAGGTCTAGGGCGGCacaagggaccttagcatgacccttagatccagctacgatgcgtgcaaacctcttaacatcatataaacgcatagatctaaacgccgtctgaatgcatctgacactatgaatagactggggacatacgtgcctggaattacgtttgaggttgcacaaacaaagatgggtgctatgaatgtattgccttaccctgacactggacatagtacttgatggatcactgtgttcaacctaataaaattcaataactcaggCCCTATAGTGAGTCGTATTACTCTATTTTTATAGGTTAATGTCATGATGGTTTCTTAGACGTCGGAATTGCC TGT
TSV	gBlock	TSV-9098-9375	NA		CCTAGGTCTAGGGCGGCagatcgcaagaatacgtctgataagcttattgggtacatcagaccactctacggcaaatcgaccaatgactgattgcttaactggcggttctcatctatacccgcatgggttcgcggttacgctcatttactgtgatcttgattgttaccattcaacatcactagctcagtgaccacggatagttacctggagtaaatcactggagcttggactcaaaatcgtacttaattcagaaattcaattgctctccacgaattGCCCTATAGTGAGTCGTATTACTCTATTTTTATAGGTTAATGTCATGATGGTTTCTTAGACGTCGG AATTGCCTGT
TSV	gBlock	TSV-9807-10157	NA		CCTAGGTCTAGGGCGGCactaatgtcttaatagaccttagtgagcaggcatttatctctcagagtaaaaaccctgccgcgcaacgcggttagggaaaactgttgagataaatcaccatgaatgacaaattcggcactcaaccacgcgtcaaatgacgggtctacacctgaaggtgttaaatgaaccaattcggcaggtccaaaagtgaagctggacatcctgccaattgagagaaagtgtggtcatcaccagcactgcaagcacaagtaactcatttccgtcctttcaaaactaattgataaataccttaattgagggaaagactggaatcaataactaaattttctgctCCCTATAGTGAGTCGTATTACTCTATTTTTATAGGTTAATGTCATGATGTTTCTTAGACGTCGGAATTGCCTGT
WSSV	gBlock	VP28_gBlock	NA	synthetic target for WSSV VP28	CTCCGATTCTGTGACTGCTGAGGTTGGATCAGGCTACTTC AAGATGACTGATGTGTCCTTTGACAGCGACACCTTGGGCAA AATCAAGATCCGCAATGGAAAGTCTGATGCACAGATGAAGG AAGAAGATCGGATCTTGTCATCACTCCCGTGGAGGGCCGA GCACTCGAAGTACTGTGGGCAGAAATCTACCTTTGAGGG AACATTCAAGGTGTGG

WSSV	gBlock primer	VP28-1_gBlock_B3	NA	primer for amplifying WSSV VP28 synthetic target for VP28.31 primer set	CCAGTGATGTTGATCTTTCTTGATGTGTTGTTCCACACCTTGAATGTTCCC
NA	reporter	FAM_5bpDNA_Report er	NA		/56-FAM/TTTTT /3IABkFQ/
NA	reporter	FAM_FluorDNA_Reporter	NA		/56-FAM/TTTTTTTTTTTTTTTTTTTT/3IABkFQ/
NA	reporter	Fluor_DNA_Reporter	NA		/5HEX/TTTTTTTTTTTTTTTTTTTT/3IABkFQ

> TSV | consensus genome | tsv_consensus_20201004

TTTAAAAGTCGTGCGTGGCTTCACCACGCACGATCAGTACTATCAGTTAACCACTCTTGAATAT
GCTCAATGACCCTATTCAACACTGGTGTCTCTTAGTACATTATTTTAGCACTTAACGTGCATGA
GTTTTGCCCATTTCTTTCAAAAATGAGTATTCGAGGAGACGTCCCCTCCCGTCTTATTTCAA
CCGTAGACTCGACATCTATTGGTGGACATTTAATTCCAGTCGCCGTAAGTTGCTTCTGCCCCGC
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CACACTATAGGAGTTTCAGGAAATGTTGTTACTAATACAGATATTGTTTATACAGGATATGCAG
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GCACGTAATTATGTGTTGTTGTGTGAGGAAATGAGCGAGAAAATATTCCTGTAATTTTCCAGG
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AGCATCCAATATGTATACAGCTCGAGATGTAGCGTCTAAAAATTTGTTAGATCAGCTACATAAC
ACCCCAATGTGCACATGCACTCTCTGCATTCCTCCCGTATGAGAATTTCCCTTGTGAAGCTC
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GTACCCACATGTTTACTTTATCCACACTCCCCAGAGATCAGGTGAAACGCTAGAGTTGGCTAA
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ggggaattatcttcaacactattaagtttggtgcataagatagccgccctgaactacttgat
tataaattgttatatcgatgctatttcagaacaaagatctcctataaaaattgtgattagggca
gaccaacatcccgtctccagtgtaagacgatacatgaagtcctgggtccagttggcacgaaaatg
gcagccgttcgtgaaggattttctcacgtcaactcgtctcaccttactggtacttttccaaatc

gtcgtggatctacattgaaactaaaatctctttcgtggggatcacttccatccttttaagagaa
gagaggcactctgtaaacaggagaccaatctgaatagtagccttaaagctgttctatTTTTTct
tctagagtgggtaccgctctgcggtgcattcggtacacctccaaatccgTTTTcctttacttgaa
tcgattttgctccttcattttcccagagcgcacatcagaatcgtacctgTcgtcgaacgaatcttc
acctcccattttatTTTatTTTcctctatatgataaaaaccacacctaAAAtgtctaataTTTta
ctacactgaaaa

Table S2. Composition of LAMP primer evaluation reactions, Cas sgRNA evaluation reactions, one-pot SHERLOCK reactions, 10X LAMP primer mix, and sample dilutions used in one-pot SHERLOCK reactions.

a) Composition of LAMP primer evaluation reactions.

LAMP Reaction component	Initial Concentration	Final Concentration	WSSV reaction volume (μL)	TSV reaction volume (μL)
Isothermal Amp Buffer (NEB)	10x	1x	2	2
MgSO4 (NEB)	100 mM	8 mM	1.6	1.6
glycine	2 M	200 mM	2	
taurine	500 mM	50 mM		2
water			4.3	5.8
dNTPs (NEB)	10 mM	1.4 mM	2.8	2.8
WarmStart RTx Reverse Transcriptase (NEB)	15000 units/mL	300 units/mL		0.4
WarmStart Bst 2.0 (NEB)	8000 units/mL	320 units/mL	0.8	0.8
Primer mix	10x	1x	2	2
Template			4	2
SYTO-82 LAMP dye (Thermo)	100 μM	0.5 μM (1 μM for TSV)	0.1	0.2
5' FAM-polyT(5) reporter (IDT)	100 μM	2 μM	0.4	0.4
Total			20	20

b) Composition of Cas sgRNA evaluation reactions.

Cas/gRNA evaluation reaction component	Initial Concentration	Final Concentration	WSSV reaction volume (μL)	TSV reaction volume (μL)
NE Buffer 2.1 (NEB)	10x	1x	1	0
Isothermal Amp Buffer (NEB)	10x	1x	0	2
MgSO ₄ (NEB)	100 mM	8 mM	0	1.6
Cas12b (GenScript)	8.73 μM	200 nM (250 nM for TSV)	0.229	0.570
sgRNA	5 μM (2.5 μM for TSV)	400 nM (250 nM for TSV)	0.8	2
water			4.571	7.83
Template			3	5
5' HEX-polyT(20) reporter (IDT)	5 μM	200 nM	0.4	0
5' FAM-polyT(5) reporter (IDT)	50 μM	2.5 μM	0	1
Total			10	20

c) Composition of one-pot SHERLOCK reactions.

One-Pot SHERLOCK Reaction component	Initial Concentration	Final Concentration	WSSV reaction volume (μL)	TSV reaction volume (μL)
Isothermal Amp Buffer	10x	1x	2	2
MgSO ₄	100 mM	8 mM	1.6	1.6
glycine	2 M	200 mM	2	
taurine	500 mM	50 mM		2
Cas12b	8.73 μM	200 nM (150 nM for TSV)	0.458	0.344
sgRNA	10 μM	200 nM (600 nM for TSV)	0.4	1.2
water			3.44	2.46
dNTPs	10 mM	1.4 mM	2.8	2.8
Incubate for 15 minutes at room temperature then add...				
WarmStart RTx Reverse Transcriptase	15000 units/mL	150 units/mL		0.2
WarmStart Bst 2.0	8000 units/mL	320 units/mL	0.8	0.8
Primer mix	10x (20x for TSV)	1x	2	1

DNA/RNA			4	5
SYTO-82 LAMP dye	100 μ M	0.5 μ M (1 μ M for TSV)	0.1	0.2
5' FAM-polyT(5) reporter	100 μ M	2 μ M	0.4	0.4
Total			20	20

d) Composition of 10X primer mix.

10x Primer Mix component (100 μ M)	10x concentration	Reaction Concentration	Volume (μ L)
F3	2 μ M	0.2 μ M	2
B3	2 μ M	0.2 μ M	2
BIP	16 μ M	1.6 μ M	16
FIP	16 μ M	1.6 μ M	16
LF	4 μ M	0.4 μ M	4
LB	4 μ M	0.4 μ M	4
Water			56
Total			100

e) Composition of sample dilutions used in SHERLOCK reactions.

Sample dilution component	WSSV	TSV
Sample DNA/RNA	1ng	10 ng
Pathogen free shrimp nucleic acid (DNA for WSSV, RNA for TSV)	20 ng	5 ng
water	variable	variable
Total volume/one-pot reaction	4 μ L	5 μ L

Table S3. Viral concentrations estimated by qPCR, SHERLOCKv1, and SHERLOCKv2 assays ('NA', not detected).

Target	Sample Name	qPCR predicted copies/ μ L (Mean)	qPCR predicted copies/ μ L (Std Dev)	SHERLOCKv1 predicted copies/ μ L (mean)	SHERLOCKv1 predicted copies/ μ L (Std Dev)	SHERLOCKv2 predicted copies/ μ L (Mean)	SHERLOCKv2 predicted copies/ μ L (Std Dev)
TSV	Val.1	9.449E+06	2.946E+04	0.000E+00	0.000E+00	3.667E+06	1.241E+06
TSV	Val.2	6.475E+02	8.442E+01	0.000E+00	0.000E+00	NA	NA
TSV	Val.4	2.040E+09	4.584E+08	0.000E+00	0.000E+00	1.715E+08	5.258E+07
TSV	Val.5	9.907E+06	3.761E+05	0.000E+00	0.000E+00	1.131E+06	1.548E+05
TSV	Val.6	2.943E+04	1.010E+03	0.000E+00	0.000E+00	4.000E+03	3.287E+03
TSV	Val.7	2.340E+03	2.518E+02	0.000E+00	0.000E+00	1.150E+02	7.981E+01
TSV	Val.8	9.453E+02	1.844E+02	0.000E+00	0.000E+00	4.200E+01	3.861E+01
TSV	Val.9	9.985E+03	4.586E+02	0.000E+00	0.000E+00	1.198E+01	1.288E+01

TSV	Val.10	4.252E+03	2.525E+02	0.000E+00	0.000E+00	2.465E+02	1.626E+02
TSV	Val.12	2.001E+01	6.875E+00	0.000E+00	0.000E+00	NA	NA
TSV	Val.13	7.932E+05	2.680E+04	0.000E+00	0.000E+00	8.302E+04	8.499E+04
TSV	Val.14	4.780E+03	1.094E+03	0.000E+00	0.000E+00	8.364E-01	8.537E-01
TSV	Val.15	1.947E+04	6.245E+02	0.000E+00	0.000E+00	5.203E+02	6.394E+02
TSV	Val.16	2.503E+05	9.313E+03	0.000E+00	0.000E+00	2.252E+05	1.353E+05
TSV	Val.18	8.489E+03	1.669E+03	0.000E+00	0.000E+00	1.435E+03	8.932E+02
TSV	Val.19	1.298E+04	2.445E+02	0.000E+00	0.000E+00	1.404E+03	7.166E+02
TSV	Val.20	4.244E+03	1.343E+03	0.000E+00	0.000E+00	4.699E+02	2.488E+02
TSV	Val.21	9.617E+07	5.850E+06	0.000E+00	0.000E+00	1.667E+07	1.775E+07
TSV	Val.22	3.308E+04	1.023E+03	0.000E+00	0.000E+00	2.837E+02	3.080E+02
TSV	Val.23	5.702E+03	2.979E+02	0.000E+00	0.000E+00	5.226E+02	1.716E+02
TSV	Val.25	8.645E+02	9.886E+01	0.000E+00	0.000E+00	NA	NA
TSV	Val.26	5.215E+06	1.170E+05	0.000E+00	0.000E+00	1.168E+06	1.211E+06
TSV	Val.28	6.022E+03	1.445E+02	0.000E+00	0.000E+00	6.070E+00	4.024E+00
TSV	Val.29	8.226E+07	3.939E+06	0.000E+00	0.000E+00	8.163E+05	2.325E+05
TSV	Val.30	1.832E+04	8.273E+01	0.000E+00	0.000E+00	1.398E+02	8.417E+01
TSV	Val.31	5.549E+03	2.953E+02	0.000E+00	0.000E+00	2.279E+02	1.174E+02
TSV	Val.32	1.044E+02	5.121E+00	0.000E+00	0.000E+00	1.046E-03	9.807E-04
TSV	Val.33	5.150E+03	5.260E+02	0.000E+00	0.000E+00	NA	NA
TSV	Val.34	3.735E+03	3.124E+02	0.000E+00	0.000E+00	4.455E+02	2.415E+02
TSV	Val.35	3.080E+07	1.027E+06	0.000E+00	0.000E+00	6.240E+06	7.671E+06
WSSV	Pvan_001	1.261E+06	4.825E+04	1.777E+06	6.257E+05	5.467E+06	1.360E+06
WSSV	Pvan_002	1.718E+06	5.008E+04	1.669E+06	5.453E+05	4.610E+06	1.110E+06
WSSV	Pvan_003	1.437E+07	4.244E+06	9.002E+06	7.179E+06	9.668E+07	6.755E+06
WSSV	Pvan_004	8.136E+05	8.335E+04	3.494E+05	1.934E+05	1.781E+06	5.254E+05
WSSV	Pvan_005	1.491E+07	9.171E+05	1.180E+07	6.902E+06	7.704E+07	9.320E+06
WSSV	Pvan_006	2.455E+05	1.066E+04	3.294E+05	5.066E+04	1.162E+06	8.754E+04
WSSV	Pvan_007	1.990E+06	6.025E+04	2.411E+06	6.014E+05	8.351E+06	3.014E+06
WSSV	Pvan_008	8.500E+06	1.241E+06	8.461E+06	9.652E+06	4.689E+07	4.589E+06
WSSV	Pvan_009	2.043E+07	2.886E+06	1.453E+07	5.325E+06	5.841E+07	1.437E+07
WSSV	Pvan_010	3.483E+07	5.419E+06	3.315E+07	5.680E+07	1.292E+09	2.086E+08
WSSV	Pvan_011	7.553E+02	1.339E+02	2.282E+03	4.922E+02	1.355E+03	1.869E+03
WSSV	Pvan_012	8.253E+02	8.739E+01	1.709E+03	7.888E+02	NA	NA
WSSV	Pvan_013	9.842E+02	5.217E+02	2.258E+03	8.400E+02	7.382E+02	5.447E+02
WSSV	Pvan_014	3.520E+02	4.074E+02	5.704E+02	1.858E+02	1.695E+02	1.682E+02
WSSV	Pvan_015	2.770E+06	6.137E+05	2.963E+06	1.853E+05	5.662E+04	3.025E+04
WSSV	Pvan_016	3.145E+06	2.963E+05	3.008E+06	1.441E+06	2.550E+04	2.349E+04
WSSV	Pvan_017	7.379E+06	4.678E+05	3.945E+06	3.114E+05	3.251E+05	3.747E+05
WSSV	Pvan_018	5.524E+06	2.616E+05	1.211E+07	2.421E+07	5.117E+04	5.783E+04
WSSV	Pvan_019	5.898E+07	1.231E+07	6.127E+07	1.210E+08	3.249E+07	9.313E+06

WSSV	Pvan_020	2.851E+05	1.344E+04	4.409E+01	1.516E+01	7.085E+03	2.078E+03
WSSV	Pvan_021	2.651E+07	4.818E+05	1.278E+07	1.324E+07	6.260E+07	1.339E+07
WSSV	Pvan_022	2.378E+06	2.214E+05	1.323E+07	2.645E+07	1.401E+05	8.987E+04
WSSV	Pvan_023	5.685E+06	8.777E+05	1.094E+07	2.152E+07	8.905E+04	4.511E+04
WSSV	Pvan_024	6.622E+05	1.041E+05	6.296E+05	1.090E+06	3.659E+02	4.427E+02
WSSV	Pvan_025	7.149E+02	1.300E+02	9.502E+03	1.892E+04	5.317E+02	4.620E+02
WSSV	Pvan_026	5.440E+01	2.210E+00	3.462E+01	1.709E+00	NA	NA
WSSV	Pvan_027	4.713E+02	2.112E+01	8.036E+03	1.563E+04	4.571E+03	6.644E+03
WSSV	Pvan_028	2.509E+07	7.114E+05	4.033E+07	6.985E+07	3.000E+07	1.250E+07
WSSV	Pvan_029	3.811E+01	7.100E-01	3.440E+01	1.733E+00	NA	NA
WSSV	Pvan_030	4.017E+02	1.276E+02	7.409E+02	1.216E+03	1.842E+03	3.875E+02
WSSV	Pvan_031	5.157E+07	1.085E+07	5.494E+07	5.642E+07	6.302E+07	2.063E+07
WSSV	Pvan_032	3.280E+05	3.823E+04	1.353E+05	1.617E+05	1.325E+05	4.616E+04
WSSV	Pvan_033	4.521E+07	1.392E+07	2.271E+07	1.533E+07	1.521E+08	4.032E+07
WSSV	Pvan_034	2.561E+06	4.552E+05	2.179E+06	3.746E+06	1.760E+04	1.003E+04
WSSV	Pvan_035	6.993E+06	8.791E+05	5.778E+06	9.374E+06	1.386E+04	6.544E+03