Viral Suppression of Host Defenses

by

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ABSTRACT

Upon detection of a pathogen, plants initiate specific signaling events designed to prevent host colonization and pathogen proliferation. Appearance of the hypersensitive response (HR), a type of programmed cell death signifies activation of active defenses in response to a one-to-one recognition of host, Resistance or R gene, and pathogen, avirulence or avr gene, encoded products. Turnip crinkle virus (TCV), however, has been shown to suppress the ability of Col-0 Arabidopsis thaliana plants to produce the HR in response to an avirulence factor. The extent of suppression was quantified by measuring cellular electrolyte leakage resulting from programmed cell death. Interestingly, cellular ion leakage levels were significantly lower in TCV-infected plants when challenged with bacteria expressing either of two bacterial effectors avrRpt2 or avrRpm1, suggesting that TCV can suppress the HR to a range of HR-inducing avirulence factors. In order to determine the viral component(s) responsible for mediating this suppression, each of the five TCV open reading frames (ORFs) was tested using an Agrobacterium tumefaciensmediated transient expression assay in Nicotiana benthamiana. Though sequencing of the five TCV clones revealed mutations in the p28, p88, and p9 clones, Agro infiltration of an HR-inducing system in conjunction with individual TCV ORFs, or combinations of, was used to gather data to determine the role each may possess in the suppression phenotype. Full-length TCV was also expressed in the presence of AvrPto/Pto to establish suppression phenotype in Nicotiana. To assay for suppression of cell death in a heterologous system, both the mutant and wild-type clones were also tested in yeast for cell-death suppression induced by hydrogen peroxide exposure.

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1 INTRODUCTION

1.1 Plant-Pathogen Interactions

Understanding the molecular basis of plant-pathogen interactions and how plants can be made more resistant to infectious and deleterious pathogens can be derived from an understanding of the plant's inherent mode of disease resistance. Plants possess numerous mechanisms to cope with the harmful effects introduced by various biotic stressors. Upon pathogenic challenge, plants may typically invoke combinations of broad and specific defense responses to confine the pathogen to the entry site. These events may include increased levels of reactive oxygen species, upregulation of genes encoding cell-wall strengthening agents and anti-microbial phytoalexins, increased levels of benzoic and salicylic acid, and the mobilization of pathogenesis-related (PR) genes (Nimchuk et al., 2003). However, initiation of specific active defense responses requires molecular recognition between a host-encoded resistance gene (R gene) and a cognate pathogen-derived avirulence factor (avr gene) in a gene-for-gene specific manner (Flor, 1971). In the absence of either gene product, the plant becomes susceptible to the pathogen and its subsequent infestation. Avirulence genes may also serve as virulence factors in the absence of a cognate R gene. Examples of this duality include avrRpt2 from Pseudomonas syringae pv. tomato, the causal agent of bacterial speck disease, which functions as an avirulence factor in resistant lines of Arabidopsis but as a virulence factor in susceptible lines (Chen et al., 2000). It is generally believed that pathogens first developed these genes to enhance their virulence on susceptible hosts, while plants

simultaneously evolved corresponding R genes as specific receptors to recognize and eliminate them (Lim and Kunkel, 2004).

To understand the molecular basis of plant-pathogen interactions, we use the plant model *Arabidopsis thaliana*, Figure 1. While *A. thaliana* is not in itself a crop plant, it is a member of the *Brassicaceae* family and is related to agriculturally significant plants such as broccoli, cabbage, and radish. *A. thaliana* provides an ideal model for research in the area of molecular genetics due to its short six-week life cycle along with the added benefit of a fully-sequenced genome. Other advantages of using *A. thaliana* include copious seed generation, making it a valuable tool in transgenic studies, and its relatively small size. With 26,000 genes on five chromosomes characterized to date, the system provides an excellent genomic resource for studying plant biology.



Figure 1: Model Plant: Wild-Type Arabidopsis Thaliana - Ecotype Columbia-0 (Col-0)

1.2 Molecular Recognition of Pathogens

To date, many cognate plant-pathogen interactions have been identified and characterized. Pto, one of the first *R*-gene encoded proteins to be characterized, recognizes the *Pseudomonas syringae* avrPto and avrPtoB proteins to initiate resistance responses in tomato (Scofield *et al.*, 1996; Martin *et al.*, 1993). The Col-0 disease

resistance locus *RPS2* mediates specific detection of the corresponding avirulence gene *avrRPT2* (Kunkel *et al.*, 1993). Likewise, the *Cf-9* gene from tomato confers resistance to strains of the leaf mold fungus *Cladosporium fulvum* expressing the cognate *Avr9* gene (Jones *et al.*, 1994). The R-protein Rpm1 recognizes two *Pst* pv. *tomato* derived effectors AvrRpm1 and AvrB (Grant *et al.*, 1995). Based on their sequences, *R* genes characterized to date fall into five main categories as shown in Table (Martin et al., 2003).

R genes falling into the classes I-III lack transmembrane segments and are thought to exert their activities from within the cytoplasmic space where they interact with avirulence factors employing the TTSS machinery for secretion into the cell. Bacterial effector proteins are typically secreted and injected through the plasma membrane into the host cell employing the Type III Secretion System (TTSS) (Hueck, 1998; Mudgett and Staskawicz, 1999). The TTSS machinery is encoded by the *hrp* (hypersensitive response and pathogenicity) locus and is required in many *R-avr* mediated interactions (Alfano and Collmer, 1996).

Class	Description	Examples
Ι	Serine/threonine kinase catalytic activity; N-terminal myristylation motif	Pto from tomato is the only member of this class
II	Contain LRR, NBS, N-terminal leucine zipper regions, and CC domains	RPM1, RPS2, Pi-ta
III	Contain LRR, NBS, N-terminal leucine zipper regions, and TIR domains homologous to animal resistance genes	TMV N gene, RPS4
IV	Extracellular LRR region; small cytoplasmic extensions	All <i>Cf</i> genes from the tomato fungus <i>Cladosporium fulvum</i>
V	Extracellular LRR region with a cytoplasmic region possessing serine/threonine kinase activity	Xa21 from Rice is the only member of this class

 Table I: Classification of R genes involved in plant disease resistance (taken from Martin et al., 2003)

Approximately 150 *R* genes in the A. thaliana genome have been identified. The majority of *R* genes characterized encode products belonging to the Class II family of proteins. The specificity of R protein function is largely thought to lie within the LRR regions, the most variable of the domains in these proteins. The NBS (nucleotide binding site) and LRR (Leucine Rich Repeat) share homology with nucleotide binding sequences and leucine-rich repeat motifs essential for protein-protein interactions, respectively while the CC (coiled-coil domain) consists of a repeated heptad sequence with interspersed hydrophobic residues (Dangl and Jones, 2001; Martin et al., 2003).

Two popular mechanistic models to describe the interaction between plants and pathogens include the receptor-elicitor theory and the guard theory. The elicitor-receptor theory places the pathogen-secreted effector in the elicitor role, while the R gene product acts as its receptor as shown in Figure 2.



Figure 2: The Receptor-Elicitor Theory

To date however, only two direct R-avr interactions have been demonstrated. The Cterminal LRR region of the Pi- ta protein from rice specifically interacts with the rice blast fungus Magnaporthe grisea secreted AvrPita in yeast-two hybrid assays and invitro binding assays. Moreover, loss of physical interaction in AvrPita mutants segregated with loss of *Pi-ta* mediated resistance in resistant rice lines (Jia et al., 2000). Likewise, the Pto protein in tomato both recognizes and physically interacts with avrPto and avrPtoB as determined using the yeast-two hybrid screening method (Kim et al., 2002). The Pto-avrPto interaction is one the best characterized pathways in plant resistance. Pto possesses serine/threonine kinase activity to initiate immunity upon direct interaction with both avirulence factors and in fact is sufficient for triggering immunity in the absence of its cognate avirulence factor when overexpressed (Tang et al., 1999). Pto autophosphorylation is required for Pto and avrPto interactions. Moreover, mutations abolishing Pto kinase activity showed an inability to form an HR (Tang et al., 1999). However, direct physical interaction between Pto and its cognate avirulence factors is not sufficient to mediate immunity. Pto-mediated resistance requires the Prf gene and other associated factors for full immunity (Salmeron et al., 1996). Though physical interaction between the Pto and avrPto comprises a key signaling event in the host immune response, it is not in itself sufficient to mediate the entire process. To date, other factors have been identified as downstream phosphorylation targets by Pto including the protein kinase Pti1 and the Pti4, Pti5, and Pti6 family of transcription factors that play a role in the induction of pathogenesis-related genes (Zhou *et al.*, 1995).

Though the receptor-elicitor theory describes mechanisms for two well-characterized systems, there exists far more compelling evidence for indirect communication between R and avr proteins in the triggering of defense activation networks. Many R gene products have been postulated to function via the "guard" theory whereby modification of a different host factor by a foreign elicitor triggers active resistance as shown in Figure 3. The R-protein serves as a surveillance mechanism to "guard" or monitor the status of the cellular targets. Once modification of the guarded protein occurs, the R protein initiates various defense responses to isolate and eliminate the pathogen.

Recent data demonstrated that the presence of avrRpt2 caused elimination of a resistancerelated protein RIN4 even in the absence of RPS2 and this ability segregated with activation of the RPS2 pathway (Mackey *et al.*, 2003, Axtell and Staskawicz, 2003). Rin4 acts as the "guarded" component serving as a negative regulator for *RPS2*-mediated defense as shown in Figure 3. In 2003, Axtell and Staskawicz demonstrated that wildtype Col-0 plants infiltrated with bacteria expressing mutant avrRpt2 alleles were unable to induce any elimination of RIN4 and that knock-down mutants of *rin4* resulted in constitutive induction of active defense pathways in the absence of an avirulence factor. Interestingly, an unrelated avr, avrRpm1 also directly interacts with and modifies Rin4. AvrRpm1 causes hyperphosphorylation of Rin4. Further, Rin4 is required for Rpm1 mediated resistance (Mackey *et al.*, 2002). Thus it appears that RIN4 may be a convergence point in the signal transduction of activated defense initiated by both avrRpm1 and avrRpt2. This would explain why transgenic Col-0 plants expressing avrRpt2 were unable to elicit the RPM1 mediated immune response when challenged with avrRpm1 (Chen et al., 2000; Ritter and Dangl, 1996).



Figure 3: The Guard Theory

In further support of the guard theory, one set of researchers concluded, after extensive searching, that there was no evidence at all for any interactions between the Cf-9 protein and its cognate avirulence factor avr9 in elicitation of resistance, suggesting the existence of a factor that mediates interactions with both (Luderer et al., 2001).

1.3 Defense Responses and Pathogen Counter-defenses

Evidence for pathogen-mediated suppression of host cell death has been demonstrated in both plant and animal systems. The *Pseudomonas* type III effector AvrPtoB was shown to inhibit host-induced cell death in the presence of the HR-inducing disease resistance gene pairs Pto/AvrPto and Cf-9/Avr9 (Abramovitch *et al.*, 2003). Interestingly, AvrPtoB was also able to suppress PCD in yeast undergoing death-inducing treatment.

The *Psg* pv. *Phaseolociola (Pph)* effector virPphA was able to suppress typical rapid HR induction, which ultimately lead to colonization of soybean by the bacteria (Jackson et al., 1999). The AvrPphF factor appeared to block HR induced by chromosomal avr effectors in *Pph* (Tsiamis et al, 2000). In mammalian cell lines, the TTSS effector YopJ from Yersinia pestis, the causal agent for bubonic plague disrupted phosphorylationbased activation of host mitogen-activated protein kinase kinases (MAPKKs) leading to suppression of host immunity (Orth et al., 1999). A number of YopJ-like proteins in other species of Yersinia as well as other plant and bacterial systems display similar interference with host defense signaling pathways, modulating the immune response and eventually contributing to virulence (refer to Orth et al., 1999). In 2002, Hay and Kannourakis compiled a comprehensive review citing evidence for viral-mediated suppression of host-initiated cell death allowing for longer viral replication cycles and increased virulence. According to the review, a number of viruses opt to disturb apoptotic-related processes by variously interfering with cellular TNF and Fas signaling, caspases involved in initiation cell death, the IFN pathway and Bcl-2-related proteins, and cell cycle and oxidative stress regulation. Likewise, expression of the baculovirus anti-apoptotic p35 gene in tomato inhibits host induced programmed cell death by acting as a strong caspase inhibitor and confers disease resistance to a broad spectrum of otherwise nectrotrophic pathogens (Lincoln *et al.*, 2002; Zhou *et al.*, 1998). Interestingly, there are no published examples of plant viruses suppressing PCD.

Establishing the mechanisms of inhibition of programmed cell death in plant systems could lead to greater understanding of the same processes in animal viruses employing similar tactics. Similarities between host initiation of programmed cell death in plant and animal systems have been identified. In 2003, Liang et al. demonstrated shared elements in the regulation and execution of ceramide-mediated PCD in plants and animals. The formation of reactive oxygen species such as superoxides and hydrogen peroxide was shared by both plant and animal systems undergoing host-induced cell death (Jabs, T., 1999). c-DNA obtained from A. thaliana was able to functionally complement a dad1 (defender against apoptotic death 1) knock-out mutant cell line of hamster tsBN7 cells in preventing apoptosis, providing one of the earliest experimental justifications for functional apoptotic-related homologues across plant and vertebrate systems (Gallois et al., 1997). A more recent example was shown by Li and Dickman (2004) whereby transgenic tobacco plants expressing the human anti-apoptotic factor bcl-2 survived lethal treatments of severe heat and cold shock and chemical stress whereas wild-type tobacco plants did not.

1.4 Turnip crinkle virus as a model

Turnip crinkle virus, a member of the *Carmovirus* genus, is a small icosahedral, positivestrand RNA virus with five ORFs (Carrington et al., 1989). The genome, as shown in Figure 4, encodes two replicase proteins, p28 and its readthrough product p88 required for in-planta replication, two intercellular movement proteins p8 and p9, and one coat protein p38 required for encapsidation of the infectious virion (Hacker *et al.*, 1992).



Figure 4: TCV Genome

Upon TCV inoculation, the TCV-susceptible line Col-0 becomes systemically infected and develops the characteristic disease symptoms of vein striping, asymmetrical mid-vein formation, leaf chlorosis and discoloration as shown in Figure 5.



Figure 5: Leaves from TCV-inoculated Col-0 plants at 10 dpi. Uninoculated, systemically infected leaf (a); inoculated leaves (b).

Viral nucleic acids are transported through intercellular conduits called plasmodesmata that are formed during incomplete cytoplasmic dissociation when plant cells undergo cytokinesis (McLean et al., 1997). Though plasmodesmata are typically used for intercellular communication and for the transport of biomolecules, viruses commonly use them for cell-to-cell movement while employing the phloem for long distance movement (Carrington et al., 1996; Samuel G., 1934). Viral translocation typically originates from older, fully expanded "source" leaves to younger, developing "sink" leaves (Leisner *et al.*, 1993)

A common feature displayed by viruses undergoing a double-stranded RNA intermediate is their ability to induce host-mediated RNA silencing as shown in Figure 6. This phenomenon has been observed and documented for a broad range of eukaryotic systems. Upon cytoplasmic replication of the virus, host detection of long double-stranded RNA triggers a pathway that results in degradation of these molecules by ribonucleases into small 21-25 nt RNAs termed small interfering RNAs or siRNAs. These shorter oligonucleotides associate with what is known as the RNA Induced Silencing Complex, or RISC, to silence complementary strand mRNA transcribed from the viral genome in a sequence specific manner. Interestingly enough, many of these same viruses that induce RNA silencing also have mechanisms to silence them. The coat protein of TCV has been shown to possess suppression of RNA silencing capabilities (Qu *et al.*, 2003).



Figure 6: Simplified schematic of RNA silencing

In *Drosophila*, the B2 protein from flock house virus is a potent inducer of hostinduced gene silencing while simultaneously suppressing it (Li *et al.*, 2002). The potyvirus helper component proteinase HC-Pro is a strong suppressor of RNA silencing and prevents accumulation of the invading nucleic acids (Anandalakshmi et al., 1998; Vance and Vaucheret, 2001). *Potato virus* X also displays suppression of RNA silencing capabilities but to a much lesser extent than HC-Pro as the viral p25 protein was shown only to suppress the mobile silencing signals (Voinnet et al., 2000).

1.5 TCV suppresses the HR

Previous work (Hammond 2001) suggested that TCV was capable of reducing the host plant's innate ability to induce the HR not only to itself but also to unrelated pathogens. Infiltrating with bacteria expressing *avrRpt2* in systemically TCV-infected Col-0 plants produced a milder tissue collapse suggesting a reduced resistance response. Likewise, TCV-resistant Di-17 plants displayed attenuated HR on symptomatic leaves after TCV challenge in plants previously inoculated with TCV. This suppression was seen though both TCV RNA and the viral coat protein were detected in the suppressed tissue. As TCV coat protein serves as the avirulence determinant conferring resistance in Di-17 plants (Zhao et al., 2000), this suggested that the HR response was suppressed in these tissues. The same uninoculated symptomatic leaves also showed high levels of PR-1 induction. It was therefore concluded that the presence of TCV in systemically infected tissues correlated with suppression of Cell death in plants, we sought to further extend previous findings that HR-lesions were visibly diminished in systemic TCV Col-0 plants.

1.6 Proposed Work

In this thesis, we show quantitative evidence that the presence of TCV interferes with the induction of the HR in the presence of the avirulent factor avrRpt2. By measuring ion leakage from leaves challenged with bacteria expressing the avirulence factors in the absence and presence of TCV, we were able to quantify the extent of cell death at equivalent time points. Moreover, we were able to extend this observation to a completely different avirulence factor, avrRpm1. avrRpm1 possesses faster kinetics of PCD induction allowing detection of peak ion fluxes much sooner than leaves infiltrated with avrRpt2.

In order to determine which viral component mediates this observed suppression, we isolated and cloned each of the five individual TCV open reading frames as well as the TCV genome into the *Agrobacterium tumefaciens* compatible pBTEX vector and transfected each construct into the Agro strain GV2260. Each of the TCV ORFs was cloned into the pBTex expression vector for use in the transient assay. Sequencing of the five TCV clones revealed mutations in the p28, p88, and p9 clones. The p28MUT contained a single amino acid change from tyrosine to cysteine due to an $A \rightarrow G$ mutation at position 59 in the sequence. The p88MUT sequence contained over five mutations while the p9MUT had two mutations resulting in significantly different residues in the amino acid sequence. As sequencing data was reviewed after performing transient assays with each construct, all experiments performed with both mutant and wild-type clones, are included. As future cloning efforts to reconstruct p28, p88 and p9 could result in evidence for suppression, the data obtained from experiments with the

mutant proteins could be valuable in determining structure-function requirements. Both the p38 and p8 clones had full consensus with the TCV coding sequence.

Each of the mutant and wild-type genes were expressed in *Nicotiana benthamiana* - a plant commonly used for transient transgene expression – in the presence of the PCDinducing combination of avrPto and Pto. Evidence and extent of suppression was determined by scoring leaves based on the percent of infiltrated area showing necrosis.

To test for activity in a heterologous system, both the mutant and wild-type TCV ORFs were cloned into the yeast compatible vector p423 to test for anti-apoptotic activity in yeast treated with H₂O₂. Yeast strain INVSc1 was transformed with each of the TCV constructs and exposed to hydrogen peroxide treatment to induce oxidative stress. Yeast survival was determined by survival of colony forming units (cfus) upon plating.

2 MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

Arabidopsis thaliana Col-0 plants were grown in Pro Mix BX soil (Premiere Horticulture Inc., Red Hill, PA) in Percival Scientific AR-60L growth chambers with a photoperiod of 16 hours and a dark period of 8 hours. Growth chamber temperature was set to 23.0°C and 21.0°C during light and dark periods, respectively. Col-0 seeds were planted in flats and subsequently covered with plastic wrap to maintain high humidity conditions through germination and before the appearance of four true leaves. Plants were watered as needed by immersing the flats in 1-L of tap water. After the first two weeks of growth, water was supplemented with 0.35g/L Miracle-Gro®. *Nicotiana benthamiana* were grown in similar soil, humidity and light/dark conditions but at a constant temperature of 28°C. Upon germination in high humidity conditions, seedlings were transplanted to individual pots. Auxiliary meristem sections were removed from developing plants to encourage enhanced growth of leaf tissue area. Plants were watered as needed and supplemented with 0.35g/L Miracle-Gro® once every two weeks after the initial four weeks of growth.

2.2 Polymerase Chain Reaction (PCR)

PCR was performed to isolate each of the five TCV open reading frames (ORFs) using the pT1D1 Δ L (Heaton *et al.*, 1989; Akgoz *et al.*, 2001) clone as template.

Table II lists the primer pairs used to amplify each of the individual ORFs. PCR reaction conditions were 1X RedTaq Polymerase Buffer (Sigma-Aldrich, St. Louis MO), 1 μ M each forward and reverse primer, 2.5 mM dNTPs, 1 unit RedTaq Polymerase (Sigma-

Aldrich, St. Louis MO), and 10 – 50 ng template DNA. All reactions were carried out in

50 µL volumes. Amplification was performed using the following thermocycler (Perkin

Elmer Cetus Gene Amp PCR System 9600) conditions:

- 1. 95°C for 5 minutes (Initial Denaturation)
- 2. 95°C for 2 minutes (Denaturation)
- 3. 68°C for 1 minutes (Annealing)
- 4. 72°C for 1 minutes (Elongation)
- 5. Repeat Steps 2 5 for 35 cycles
- 6. 72°C for 10 minutes (Final Elongation)
- 7. 4°C Hold

Table II: Primers for the Amplification of Individual TCV Open Reading Frames

Target	Forward Primer	Reverse Primer
p28MUT	5'-ATGCCTCTTCTACACACACTCAAC-3'	5'-CTAGCGGACAAAAGAGATCGC-3'
p88MUT	5'-ATGCCTCTTCTACACACACTCAAC-3'	5'-TTAGAGAGTTGTAGGGAATTCG-3'
p8	5'-ATGGATCCTGAACGAATTCCC-3'	5'-GCACTAGTTTTCCAGTCTAATG-3'
p9MUT	5'-ATGAAGGTTCTGCTAGTCACGG-3'	5'-GCACTAGTTTTCCAGTCTAATG-3'
p38	5'-ATGGAAAATGATCCTAGAGTCCGG-3'	5'-GACCAGCCCTTCTTCTG-3'

As full-length p88 encodes both p28 and p88, the p88 Δ STOP was created to produce only p88. The megaprimer method was used to change the 814G \rightarrow C for removal of the p28 leaky stop codon and to incorporate a silent mutation at 832G \rightarrow C by introducing a Sac I restriction site to test for incorporation of the mutation. The following mutagenic pair of primers was used: 5'-GTCCGCTACGGGTGCTTGCGGGAGCTCGTCGGGAGGGAGACTC-3' and 5'-CCCGACGAGCTCCCGCAAGCACCCGTAGCGGACAAAAGAGATCG-3.

Two rounds of PCR were performed – one using the p88 forward primer and the reverse mutagenic primer and the other using the p88 reverse primer and the forward mutagenic primer – to generate the "megaprimers". Thermocycler conditions were as previously

mentioned. One final round of PCR was used to create the mutant using the following reaction conditions: 1X RedTaq Polymerase Buffer (Sigma-Aldrich, St. Louis MO), 1 μ M p88 forward primer, 1 μ M p88 reverse primer, 2.5 mM dNTPs, 1 unit RedTaq Polymerase (Sigma-Aldrich, St. Louis MO), 25 ng PCR product 1, and 25 ng PCR product 2. All reactions were carried out in a 50 uL volume. Thermocycler conditions were as listed before.

2.3 Cloning

All PCR products were cloned into the pCR[®] 2.1 cloning vector (Invitrogen, Carlsbad CA) and screened for desired directionality using the universal SP6 promoter forward primer and the reverse primer of the ORF or through restriction digestion analysis when appropriate. For cloning the ORFs into the agro-compatible binary vector pBTEX, the pCR[®] 2.1 construct was digested with *Xba*I and *Kpn*I, to excise the desired fragment and ligated into compatible sites between the cauliflower mosaic virus (CaMV) 35S promoter and a downstream NOS terminator sequence. For cloning into the yeast-compatible vector p423, the pCR[®] 2.1 construct was digested with *SpeI* and *XhoI*, to excise the desired fragment and ligated into compatible sites downstream of the GAL1 promoter. The yeast vector contains a histidine synthesis and ampicillin resistance marker for selection in yeast and E. *coli*, respectively. The resultant recombinant plasmids were propagated in *E. coli* Top 10 cells and isolated using standard plasmid isolation procedures.

Each construct was sequenced by Macrogen, Inc. (Korea). Only two clones, p38 and p8, were verified as accurate. Results of each sequencing reaction are listed in Appendix B.

2.4 Preparation of Chemically Competent E. Coli

Fresh DH5 α cells were plated from glycerol stocks onto LB plates (see Table) containing no antibiotics. Cells were grown overnight in 37°C. A single colony was used to inoculate 25 ml of LB liquid media (Table) without antibiotics. Cells were grown overnight in a shaker at 37°C at 200 RPM. The next day, the culture was used to inoculate a fresh 500 ml volume of LB without antibiotics in a 1 L flask. Cells were agitated at 37°C until the A₆₀₀ was about 0.6 (after approximately 2 hours). The cells were then centrifuged at 4,000 RPM at 4°C for five minutes. The pellet was resuspended in 200 ml of ice-cold 50 mM CaCl₂ and placed on ice for 10 minutes followed by centrifugation at 4,000 RPM for five minutes at 4°C. The pellet was resuspended in 40 ml ice-cold 20% glycerol-50 mM CaCl₂. Aliquots of 1ml each were distributed to sterile microcentrifuge tubes and flash frozen in liquid nitrogen. All cells were stored in -80°C.

2.5 Transformation of E. Coli – Heat Shock

Stocks of chemically competent cells were taken from -80°C and thawed on ice. DNA was added to a 100 ul aliquot of competent cells, gently mixed with the end of a pipette tip, and kept on ice for 30 minutes. The cells were then placed in a 37°C water bath for 3 minutes for transformation. Immediately thereafter, 1 ml of LB (see Table) was added to the cells. After incubation at 37°C on a rolling drum for one hour, the cells were then spun down and plated on media containing the appropriate antibiotic for selection of transformants.

2.6 Transformation of E. Coli – Electroporation

Stocks of previously made electro competent cells were taken from -80° C and thawed on ice. Approximately 100 ng of DNA in water was added to a 20-ul aliquot of competent cells and gently mixed with the end of a pipette tip. The mixture was then pipetted into a sterile electroporation cuvette and placed into the chilled electroporation chamber. The Cell-Porator® (Life Technologies, Carlsbad CA) was set to the following conditions: voltage booster = 4 k Ω , capacitance = 330 μ F, and DC volts = low. A pulse of 2 kV was delivered to the sample. The sample was left in the cuvette for ten minutes and then transferred to 1 ml of LB media (see Table). The sample was placed in a rolling drum at 37°C for one hour. The cells were then spun down and plated on media containing the appropriate antibiotic for selection of transformants.

2.7 DNA Mini Preparation

A single colony was used to inoculate a 5-ml volume of LB media (see Table) containing the appropriate antibiotic in a loosely capped 15-ml tube. The tube was placed in a rolling drum in a 37°C incubator for 16-18 hours. Approximately 1.5 ml of the culture was placed in a microcentrifuge tube and centrifuged at 14,000 RPM in a benchtop centrifuge for 30 seconds. The supernatant was removed leaving the bacterial pellet as dry as possible. The pellet was resuspended in 100 ul of ice-cold GTE solution (50mM Glucose, 25 mM Tris-HCl - pH 8.0 and 10 mM EDTA - pH 8.0) by vigorous vortexing. To lyse the cells, 200 ul of a freshly prepared solution of 0.2 M NaOH and 1% SDS was added to the tube and the contents subsequently mixed by inverting the tube rapidly several times without any vortexing. To the lysed cells, 150 ul of ice-cold 5M

KoOAC (5 M KOAC and 3 M Glacial Acetic Acid) was added and gently vortexed in an inverted position for approximately 10 seconds. The tube was placed on ice for 5 minutes to facilitate precipitation of the detergents. After centrifugation for 5 minutes, the resulting supernatant was transferred to a new tube. Two volumes of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the supernatant and vortexed and then centrifuged for two minutes. The supernatant was transferred to a new tube without disturbing the organic layer. Double-stranded plasmid DNA was precipitated by adding two volumes of ice-cold ethanol. The mixture was then vortexed and placed in -20°C for a minimum of one hour, after which the samples were centrifuged for 30 minutes. The supernatant was removed and the pellet resuspended in 30 ul of ultradistilled sterile water. A 1-ul aliquot of RNAse enzyme was added to the nucleic acid solution and incubated for 37°C for 30 minutes to degrade RNA in the sample. For simple DNA preparations purposes, agarose gel electrophoresis was performed to verify plasmid integrity and yield. For post-ligation screening of colonies, samples were analyzed using restriction digestion analysis using 2.5 ul of DNA in a 15 ul total reaction volume.

2.8 DNA Maxi Preparation

A single colony was used to inoculate 100 ml of LB media (see Table) in a 500 ml Erlenmeyer flask. The culture was incubated in a 37°C shaker for 16-18 hours with vigorous shaking. Cells were poured into a large centrifuge tube and were spun down at 3,500 RPM for 15 minutes using the Sorvall RC-5B centrifuge (Sorvall GSA rotor). After removal of the supernatant, cells were resuspended in 6 ml of GTE buffer (50mM

Glucose, 25 mM Tris-HCl - pH 8.0 and 10 mM EDTA - pH 8.0). To lyse the cells, 12 ml of a freshly prepared solution of 0.2 M NaOH and 1% SDS was added and gently mixed. The cells were placed on ice for 5 minutes after which 8 ml of ice-cold KOAC (pH 4.8) (5 M KOAC and 3 M Glacial Acetic Acid) was added to precipitate the detergent. The cells were then iced for 20 minutes and centrifuged at 3,500 RPM for 15 minutes. The supernatant was poured through a 3-ply layer of cheesecloth into a new centrifuge tube. To precipitate DNA, 14 ml of ice-cold isopropanol was added. Immediately thereafter, the sample was centrifuged at 10,000 RPM for five minutes. After careful removal of the supernatant, the resulting pellet was dissolved in 200 ul TE buffer and transferred to a microcentrifuge tube. To the tube, 10M NH₄OAc was added to make a final concentration of 2M NH₄OAc. The sample was placed on ice for 10 minutes and then spun down at 14,000 RPM in a benchtop centrifuge for 10 minutes. The supernatant was transferred to a fresh tube. After adding two volumes of ethanol, the sample was placed in -20°C for one hour to overnight to precipitate double-stranded DNA. The solution was then spun down in a microcentrifuge for 10 minutes with resuspension of the pellet in 100 ul TE buffer. 1 ul of a 10 ug/ml solution of RNAse A was added to the DNA and incubated for 37°C for 30 minutes. After incubation, a 1/10 volume of 3M NaOAc pH 5.2 and two volumes of ice-cold ethanol were added to precipitate DNA. The solution was placed in -20°C for 30 minutes after which 42.7 ul of 5M NaCl and 37 ul of 30% polyethylene glycol (PEG)/1.5 M NaCl was added. The sample was vortexed and placed on ice for 30 minutes, and then spun down for 10 minutes. The pellet was resuspended in 100 ul of ultra-filtered water and 100 ul of 2x proteinase K (PK) buffer and placed in a 37°C water bath for 30 minutes. To complete the DNA prep, one volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the solution, vigorously vortexed and then spun down for two minutes. The aqueous phase was then transferred to a new tube to which two volumes of ice-cold ethanol were added. The solution was placed in -20° C for a minimum of one hour. The sample was spun down for five minutes and the pellet resuspended in 100 ul of ultra-pure water. DNA was quantified at A₂₆₀ using a spectrophotometer and also checked for protein contamination at A₂₈₀. Minimal protein contamination was ensured at a A₂₆₀/A₂₈₀ ratio of 1.8 – 2.0.

2.9 In-vitro transcription

TCV-B was transcribed from pT1d1Δl. 12 ug of plasmid was linearized with *XbaI*. Two volumes of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the restriction digest, vortexed and centrifuged at 14,000 RPM for two minutes. The supernatant was transferred to a new tube where 1/10 volume of 3 M NaOAC was added with vigorous vortexing, followed by two volumes of ice-cold ethanol. The DNA was placed in -20°C for one hour to precipitate DNA. The DNA was then centrifuged at 14,000 RPM for 30 minutes. The pellet was resuspended in 15 ul of DEPC-treated water. Transcription was executed using 50 units of T7 RNA Polymerase (N.E. Biolabs, Beverly MA), 100 units of RNasin Ribonuclease Inhibitor (Promega, Madison WI), and 1mM of each ribonucleotide in 100 uL of 1x transcription buffer (N.E. Biolabs, Beverly MA) at 37 C for 2.5 hours. Afterwards, 15 units of RQ1 RNAse-free DNAse (Promega, Madison WI) was added. The reaction was incubated at 37°C for 30 minutes. To retrieve the RNA, phenol/chloroform/isoamyl alcohol (25:24:1) extraction was performed followed by

ethanol precipitation. After recovery of the pellet, RNA was resuspended in 50 ul DEPCtreated water and quantified using the spectrophotometer.

2.10 Viral Inoculation of TCV

Infectious BBM-TCV, consisting of total RNA extracted form TCV-infected turnip, was resuspended in 1X inoculation buffer (0.05M glycine, 0.03 M K₂HPO₄ and 1% celite) to a final inoculum concentration of 0.2 μ g/ μ l. Inoculations were performed at 24-26 dpp on older, fully expanded leaves. A sterile glass stirring rod was briefly dipped into 1- μ l aliquots of the viral suspension and rubbed onto the adaxial side of several oldest leaves while using a stiff platform for abaxial support to provide adequate inoculum penetration. Plants were left in the growth chamber for 10 days to allow progression of TCV.

N. benthamiana plants were inoculated in a similar manner. However, instead of inoculating with BBM virus, the plant was inoculated with in-vitro transcribed RNA.

2.11 Bacterial Infiltration

Fresh plates of *Pseudomonas syringae* pv. *glycinea* were streaked from glycerol stocks. A single colony was used to inoculate a 5-ml culture of fresh NYG media (see Table III) supplemented with 50 µg/ml kanamycin and allowed to grow overnight at 28°C. A 200ul aliquot was used inoculated to a fresh sub-culture to an A600 of 0.5-0.9. Samples were centrifuged at 3,000 RPM (Sorvall GSA rotor) for 10 minutes at 4°C. The pellet was resuspended in 10 ml of 10mM MgSO4 and re-centrifuged. The pellet was finally resuspended in 10 mM MgSO4 and samples were diluted to an A600 of 0.2. Bacterial samples were syringe- infiltrated into symptomatic leaves of TCV and mock-inoculated plants at 10 days post inoculation.

2.12 Ion Leakage Assays

Four-6mm diameter leaf punches were obtained from symptomatic leaves harvested 18 hours post infiltration and 5 hours post infiltration for avrRPT2 and avrRPM1, respectively. Leaf discs were subsequently floated on 2 ml ultra-pure water with abaxial sides towards the solution. Samples were incubated at room temperature for 4 hours and measurements obtained with a Cole-Parmer® 19815-00 Basic Conductivity Meter recently calibrated with Traceable One-Shot[™] Conductivity Calibration Standard (Control Company, Friendswood TX).

2.13 Preparation of Electro-competent Agrobacterium

GV2260 cells were plated and incubated at 30°C for two days. Several colonies were then used to inoculate a 5 ml volume of LB (see Table) supplemented with 100 mg/l rifampicin and 50 mg/l kanamycin. The culture was left to grow overnight on a rolling drum at 30°C. The next day, a 200-400 ul aliquot of the culture was used to inoculate 500 ml of LB supplemented with 50 mg/l rifampicin. The flask was placed in a 28-30°C shaker at approximately 400 RPM until an A₆₀₀ of about 0.5 was attained (usually 16-18 hours). To harvest the cells, the flask was chilled on ice for 30 minutes and then transferred to centrifuge bottles and centrifuged in the Sorvall RC-5B centrifuge (Sorvall GSA rotor) at 4,000 RPM for 15 minutes and 4°C. The supernatant was discarded and cells resuspended in 500 ml of 1 mM Hepes pH 7.4 and spun again at the same conditions. The cells were the resuspended in 250 ml 1mM Hepes pH 7.4 and spun again at the same conditions. The cells were once again resuspended in 10 ml of 1 mM Hepes pH 7.4 and spun down one final time at the same conditions. The cells were resuspended in 2 ml of ice-cold 10% glycerol. The competent cells were distributed into 40 ul aliquots per microcentrifuge tube, placed in liquid nitrogen, and then stored in -80°C until needed.

2.14 Agrobacterium Transformation

Cells were thawed at room temperature and then immediately placed on ice. About 25 ul of the cells was transferred to a sterile microcentrifuge tube. Approximately 80 ng of DNA was added to the cells in a volume not exceeding 2 ul. The cells and DNA were gently mixed and then pipetted into a sterile electroporation cuvette. The Cell-Porator® (Life Technologies, Carlsbad CA) was set to the following conditions: voltage booster = $4 \text{ k}\Omega$, capacitance = 330μ F, and DC volts = low. The cuvette was placed into the chilled electroporation chamber and pulsed at 1.4 kV. 1 mL of LB (Table) was immediately added to the cells. The cells were left to incubate at room temperature, with no agitation, for one hour. The cells were then plated on LB/kan/rif plates and incubated for 2-3 days in 30°C. After isolated colonies appeared, transformants were used to inoculate 5 ml cultures of LB/rif/kan. Cultures were grown overnight at 30°C to make glycerol stocks. Transformants were verified using colony screening via PCR and then grown overnight in 5 ml of LB supplemented with the appropriate antibiotic. Cultures were used to make 20% glycerol stocks to be stored in -80°C.

2.15 Agrobacterium-Mediated Transient Expression Assay

Agro strains were streaked onto LB plates (see Table) containing 50 mg/l kanamycin and 100 mg/l rifampicin and grown for two days in 30°C. Plates were kept for no more than two weeks at a time. New plates were always streaked from glycerol stocks. To start the cultures, a 5 ml volume of LB supplemented with 100 mg/l rifampicin and 50 mg/l kanamycin was inoculated with a smear of colonies and grown overnight in a rolling drum at 28-30°C. The cultures were then placed in a 15 ml Falcon tube and spun down at 4,000 RPM for five minutes in the Sorvall RC-5B centrifuge (Sorvall GSA rotor) and then resuspended in 5 mL of induction medium (400 ml water, 4.88g MES, 2.5 g glucose, and 0.12 g NaH₂PO₄ and 25 mL AB salts (20 g NH₄Cl, 6g MgSO₄-7H₂O, 3g KCl, 0.2g CaCl₂, and 0.05g FeSO₄-7H₂O); 500 ul of 200mM fresh acetosyringone in DMSO prepared JUST prior to use) and spun again. The pellet was resuspended in 5 ml of induction media. The culture was used to inoculate a 50 ml volume of induction media, 50 mg/l kanamycin (rifampicin is not required at this step), in a 250 ml flask and was then cultured overnight in the large shaker at 30°C by simply turning the shaker heater switch off. The next day, the cultures were spun down in 50 ml Falcon tubes at 4,000 RPM for five minutes and resuspended in 40 ml of 10 mM MES + 200 uM fresh acetosyringone in DMSO. The cultures were spun down again at 4,000 RPM for five minutes and then resuspended in 30 ml of 10 mM MES + 200 uM acetosyringone. To find the absorbance of the culture, a 1:10 dilution was made and used to make a final cell suspension absorbance of 0.3 using 10 mM MES + 200 uM acetosyringone as the diluting agent. For cell death suppression assays, HR-inducing agents such as the Pto and avrPto combination were mixed at a 1:1 ratio, while the candidate or known suppressor

or empty vector was mixed at a final ratio of 1:1 to the R-avr volume. Cases requiring coinfiltrations of more than one TCV ORF were mixed at equal ratios. For example, 3ml of 0.3 OD agro containing p28MUT and 3 ml of 0.3 OD agro containing p88MUT would be added to 3 ml of 0.3 OD agro containing avrPto and 3 ml of 0.3 OD agro containing Pto for a final solution volume of 12 ml and a final OD of 0.3

For the transient expression assay, middle-aged leaves about the width of a hand length were selected. Small circles about the size of a quarter were made followed by a needle poke to the center of each circle. A 1-ml syringe was used to pressure infiltrate the Agro into the hole so as to cover the tissue demarcated by the marker. Several duplicates of samples were made at varying leaf positions as leaf position does tend to affect the transient assay. Plants were moved to 24 hour light and moderate temperature conditions and monitored daily for transient expression phenotypes. For RNA extraction, leaves were harvested 36 hpi. Leaves were scored 7 dpi for final analysis of cell death suppression or progression.

2.16 RNA Isolation from Plant Tissue

Leaves were harvested from plants, weighed, and flash frozen in liquid nitrogen and placed in -80° C until preparation. Concert Plant RNA Reagent (Invitrogen) was used for RNA isolation from *Arabidopsis thaliana* and *Nicotiana benthamiana* leaves. After isolation, RNA was resuspended in DEPC-water with A₂₆₀ quantification of yield using the spectrophotometer. RNA samples were always stored at -80° C.

DEPC water was prepared by adding 200 ul of DEPC to 100 ml water. After vigorous shaking, the solution was autoclaved. All solutions used in the RNA prep were made

from autoclaved DEPC water. Note: Only autoclaved DEPC water may be used to prepare buffers to prevent ethylation of titratable groups and loss of buffer conditions.

2.17 RNA Gel Electrophoresis

RNA samples were run on a 1.2% agarose denaturing gel. To prepare the gel, agarose was first melted in distilled water. After cooling the superheated mixture, 1X gel running buffer (0.2M MOPS, 50 mM NaOAc and 1 mM EDTA) and 2.2 M 37% formaldehyde were added to the agarose solution. The gel was poured and allowed to solidify. After solidification, the gel was placed into an electrophoresis chamber containing1X gel running buffer. Approximately 10 ug of RNA was used per lane. 15 ul of denaturation buffer (1V 10X gel running buffer, 1.75V 37% formaldehyde and 5V 99% formamide) was added per 5 ul of RNA. The solution was mixed well and incubated at 55°C for 30 minutes. After denaturing the sample, 1/10V loading buffer (50% glycerol, 1mM EDTA and 0.4% bromophenol blue) was added to the solution. The samples were loaded onto the gel and run until the bromophenol blue marker migrated 90% of the full length of the gel.

To visualize RNA integrity, the gel was placed in a 0.1% Ehidium Bromide/DEPC water solution and gently agitated for 10 minutes. The gel was then placed under UV light, with minimal exposure, using the UV transilluminator to check for intact ribosomal RNA bands. The gel was destained by gentle agitation in DEPC water for 10 minutes. Occasionally the gel was saved for no more than 24 hours before blotting.

2.18 Northern Blots

After gel electrophoresis, the gel was rinsed with several washes of DEPC water. The gel was then allowed to soak in alkaline transfer buffer (0.01M NaOH and 3M NaCl) for 10 minutes. While soaking the gel, the membrane was prepared for subsequent RNA transfer. A section of positively charged nylon membrane was cut to a size approximately 1 mm larger than the gel in both dimensions. The membrane was then carefully placed onto a container of DEPC water. After the membrane was fully wetted, the membrane was pushed into the water and allowed to soak for five minutes.

After retrieving the gel from the alkaline transfer buffer, the gel was moved to a clean surface where a sharp scalpel was used to cut away unused portions of agarose and to cut one corner of the gel as a positional marker. A similar cut was made into the nylon membrane after soaking to mark orientation of the gel.

To assemble the capillary transfer system, a 3 cm stack of lab grade folded-paper towels was constructed. Sections of paper towel were cut approximately one inch greater than the gel in both dimensions. On the side, five pieces of filter paper cut to the size of the gel were set to soak in alkaline transfer buffer. To construct the second layer, two pieces of the wetted filter paper were placed on top of the first layer. The nylon membrane was then added as the third layer. The trimmed gel was then placed on top of the membrane as the fourth layer taking care to align the cut corners of the membrane and gel. Four 0.5 inch-strips of parafilm were cut and each placed underneath the four sides of the perimeter of the gel to make a seal between the gel and membrane. At this point, care was taken to ensure that no air bubbles resided between the membrane and the gel.
The top of the gel was then wetted with transfer buffer and the remaining three wetted sections of filter paper were added on top of the gel. Two long 2-in X 24-in strips of filter paper were wetted in transfer buffer and placed on top of the gel with either end of the strip immersed in transfer buffer (see Figure 7). A thin glass plate was placed on top of the strips to prevent evaporation of buffer. A 100-g weight was finally placed on top of the entire assembly. One hour was typically sufficient for transfer of RNA to the membrane. After one hour the system was dismantled and the membrane placed in 5X SSC (22g NaCl, 11g sodium citrate in 400 mL water, pH to 7.0, top to 500 ml) for five minutes with gentle agitation.



Figure 7: Capillary Transfer System

After soaking in 5X SSC, the membrane was transferred to a piece of 3 mm filter paper to dry. Upon complete drying, the membrane was placed onto a new piece of filter paper and placed into the FB-UVXL-1000 UV crosslinker (Fisher Scientific, Hampton NH), to fix the sample to the membrane, and set to "Optimal Crosslink". After irradiation, the membrane was placed into a box containing 3 ul of a 400 ug/ml ethidium bromide solution for staining. The membrane was gently agitated for five minutes and then visualized using the UV transilluminator with minimal UV exposure. After observation

of the ribosomal bands, the membrane was destained by immersion in DEPC water and gentle agitation for 20 minutes. The membrane was then allowed to dry on a piece of 3 mm filter paper and was then wrapped in plastic wrap and stored in 4°C.

2.19 Northern Hybridization

To prepare a probe for hybridization, 25 ng of purified DNA fragment was placed in a microcentrifuge tube. To the DNA, 24 ul of sterile water was added. The DNA was then randomly primed following using the Prime-it II kit (Stratagene, La Jolla CA). The reaction was incubated at 37°C for 30 minutes. After incubation, 150 ul of water was added to the reaction and the probe was ready for spin column purification. NucAway[™] Spin Columns (Ambion, Austin TX) were used to recover purified probe DNA. After spin column purification, the probe was pipetted to a fresh microcentrifuge tube. Two scintillation vials were partially filled with scintillation fluid. A 2 ul-aliquot of purified probe was added to one vial and both vials were placed into the scintillation counter for measurement of radioactivity. Typical acceptable counts for a functional probe were around 20,000 cpm/ul. Probes possessing counts less than 10,000 were typically deemed inefficient and were not used.

In preparation for hybridization, 1% BSA was freshly added to 10 ml of hybridization solution (0.5 M NaPO4, 1 mM EDTA and 7% SDS). The solution was poured into a glass hybridization tube followed by the membrane, RNA side facing into the tube, ensuring that the membrane was fully covered by solution. The tube was placed into the micro hybridization chamber and rotated for two hours at 65°C. The purified probe was kept on ice behind a polycarbonate shield in the interim.

After pre-hybridization of the membrane, the tube was opened and the solution discarded. A fresh 10 ml volume of hybridization buffer was prepared as described above and added into the tube. The probe was then boiled for 5 minutes in a water bath and then pipetted into the tube. The amount of probe to add should yield a final concentration of approximately 1 x 10^6 cpm solution. The tube was placed into the hybridization chamber and left to rotate for 18 hours at 65° C.

After hybridization, the membrane was retrieved from the tube and immersed into Wash Solution #1 (1X SSC and 0.1% SDS) for ten minutes, with gentle agitation, at room temperature. After Wash #1, the membrane was returned to the tube along with Wash Solution #2 (0.5 SSC and 0.1% SDS). The tube was placed back into the chamber and rotated at 60 C for ten minutes. The membrane was then left to dry on plastic wrap. A Geiger Counter was used after each wash step to ensure removal of unhybridized probe. The dried membrane was placed into a cassette and exposed to a panel from the BAS 1000 (Fujix, Kyoto Japan) phosphorimager for two hours. The panel was then developed in the phosphorimager for image capture and analysis.

2.20 RT-PCR

Using RNA harvested from leaves agro-infiltrated with TCV ORFs, 1 uL 50 uM oligo dT, 2 ug total RNA and 1 uL10 mM dNTPs were added to a microcentrifuge tube and brought to 13 uL with RNAse-free water or DEPC water (for samples containing the agro-infiltrated full-length TCV construct, Spe I primer was used in lieu of oligo dT). The tube was then placed in 65° C for 5 minutes and then spun down and placed on ice. To the tube, 4 uL of 5X First Stand Buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15

mM MgCl₂], 1 uL 0.1 mM DTT, 1 uL RNASEOUT[™] Ribonuclease Inhibitor (Invitrogen, Carlsbad CA) and 1 uL SuperScript[™] II Reverse Transcriptase (Invitrogen, Carlsbad CA) was added. The tube was placed in a 50° C water bath for one hour and then spun down and placed on ice. The enzyme was deactivated by placing the tube in 70° C for 15 minutes. For amplification of the resulting cDNA, standard PCR was performed using an internal primer pair and the RT reaction as template. Typically 1 uL of the RT reaction in a 50-uL total PCR reaction volume was sufficient for amplification. Analysis was performed using standard gel electrophoresis.

2.21 Yeast Transformation

Fresh INVSc1 (Invitrogen, Carlsbad CA) yeast strains were streaked onto YPD plates (see Table) from glycerol stocks and allowed to grow for 2-3 days at 30°C. A single colony was then used to inoculate 10 ml of YPD media in a 25-50 ml flask. The culture was grown overnight in a 30°C shaker at 400 RPM. The 10 ml culture was used to inoculate 25 ml of fresh YPD in a 100 ml flask. The flask was placed in a 30°C shaker for 1-2 hours until A₆₀₀ as about 1. The culture was then transferred to a 50 ml falcon and spun down at 3,000 RPM in the Sorvall RC-5B (Sorvall GSA rotor) at 4°C for 5 minutes. The cells were then resuspended in 40 ml of ice-cold sterile water and spun at 2,500 RPM at 4°C for 5 minutes. The wash was repeated with 20 ml sterile ice-cold water at 2,500 RPM for 5 minutes. The pellet was resuspended in 5 ml ice-cold 1 M glycerol. The culture was then centrifuged a final time at 2,000 RPM at 4°C for five minutes. The cells were resuspended in 150 ul of ice-cold 1 M glycerol and transferred to a sterile 1.5 ml microcentrifuge tube and kept on ice until ready for transformation. A 20 ul aliquot of

yeast cells were transferred to a sterile microcentrifuge. Approximately 5 ug of DNA in distilled water was added to the cells in a volume no more than 5 ul. The mixture was carefully pipetted into a sterile electroporation cuvette and placed into the chilled Cell-Porator® (Life Technologies, Carlsbad CA) chamber and set to the following conditions: voltage booster = 4 kO, capacitance = 330 μ F, and DC volts = low. Cells were electroporated at 1.5 kV. Immediately thereafter, 1 ml of 1M ice-cold glycerol was added and the cells left to incubate at room temperature for one hour without agitation. The cells were then plated on SD plates and incubated at 30°C.

2.22 Yeast Cell Death Assays

Wild-type INVSc1 cells were transformed with the appropriate constructs just prior to performing each experiment. Transformants were then selected and grown in 10 mL SD media, lacking histidine (see Table I), and 2% glucose and grown overnight to select for the presence of the plasmid. The cells were then centrifuged at 3,500 RPM for five minutes and resuspended in SD medium, lacking histidine, containing 2% galactose and 1% raffinose. The cells were centrifuged again at 3,500 RPM for five minutes and resuspended in 10 ml of SD/gal/raff/-his media to induce expression from the GAL1 promoter in p423.

The yeast cells were induced for six hours with agitation at 30°C. After six hours, cells were diluted to an A_{600} of 0.05 in a total volume of 10 ml of SD/gal/raff/-his and were stressed by either chemical treatment or heat shock treatment. For chemical treatment, cells were treated with 3 mM H₂O₂ final concentration in the medium and incubated at 30°C with vigorous shaking at 800 RPM for 6 hours. For heat stress, yeast cells were

incubated at 37°C for 30 minutes with vigorous shaking at 800 RPM, then transferred to a stationary water bath at 50°C for 30 minutes, and then returned to 30°C with vigorous shaking at 800 RPM for 6 hours. Following treatments, cell viability was determined by plating five serial ten-fold dilutions, using SD-/gluc media as the diluting agent, of each sample on SD/-his plates. To ensure cell viability, both wild-type and transformed cells were grown at 30°C for six hours with agitation at 400 RPM. Wild-type yeast cells were also subject to heat treatment and chemical treatment to ensure cell susceptibly to stressful conditions.

Media	Liquid	Solid
LB	10.0 g tryptone 10.0 g sodium chloride 5.0 g yeast extract	10.0 g tryptone 10.0 g sodium chloride 5.0 g yeast extract 20.0 g agar
NYG	5.0 g Bacto [™] Proteose Peptone #3 (BD, Sparks MD) 3.0 g yeast extract 80.0 mL 25% glycerol	5.0 g Bacto [™] Proteose Peptone #3 (BD, Sparks MD) 3.0 g yeast extract 80.0 mL 25% glycerol 15.0 g agar
YPD	10.0 g yeast extract 20.0 g Bacto™ Proteose Peptone #3 (BD, Sparks MD) 20.0 g dextrose	10.0 g yeast extract 20.0 g Bacto [™] Proteose Peptone #3 (BD, Sparks MD) 20.0 g dextrose 20.0 g agar
SD	26.7 g Minimal SD Base (Clontech, Palo Alto CA) 0.77 g -His DO Supplement (Clontech, Palo Alto CA)	26.7 g Minimal SD Base (Clontech, Palo Alto CA) 0.77 g -His DO Supplement (Clontech, Palo Alto CA) 20.0g agar

Table III: Media Recipes - Based on 1L total volume

3 RESULTS

3.1 TCV suppresses formation of HR to avrRpt2

Col-0 plants infiltrated with *P. syringae* strains expressing either endogenous or plasmidencoded avrRpt2 typically form visual HR lesions within 24 hours (Shapiro et al., 2001). In previous experiments, TCV-infected Col-0 plants showed a marked reduction in lesion size or severity. In order to quantify the apparent suppression of the HR, an ion leakage assay approach was used, as electrolyte leakage is a common hallmark of PCD (Rate and Greenberg, 2001).

Before commencing experiments investigating TCV-mediated suppression of avrRpt2 induced PCD, it was necessary to establish the time of peak ion flux in avrRpt2-induced PCD in the absence of TCV. Accordingly, Col-0 plants were infiltrated with *Psg* carrying the avrRpt2-expressing vector. Leaf punches were harvested at 14, 16, 18 and 20 hpi. Maximum conductivity for each of the three samples was apparent at 18 hours post infilatration, as shown in Table IV. Leaves from all subsequent ion leakage assays involving avrRpt2 mediated PCD were scored at 18 hpi consistent with the time-trace experiment detailed in Table IV.

In order to assay the effect of TCV on ion leakage, three-week old Col-0 plants were inoculated with TCV. Upon appearance of viral symptoms, the uninoculated but symptomatic leaves were challenged with one of the following: buffer, *Psg* carrying an empty vector, or *Psg* carrying a vector encoding avrRpt2. Equivalent size and age leaves were infiltrated on all mock-TCV inoculated plants. Samples representing negative controls were mock-bacterial and mock-viral inoculated with 10 mM MgSO₄ infiltration

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buffer and inoculation buffer, respectively. After bacterial infiltration, leaf punches were taken from infiltrated tissue and analyzed for conductivity.

Table IV: Time course data for determination of peak ion flux upon avrRpt2induced PCD. Three-week old Col-0 plants were either mock-infiltrated with 10 mM MgSO4, bacterially-infiltrated with Psg harboring an empty vector, or bacterially-infiltrated with Psg carrying a plasmid expressing avrRpt2. All infiltrations were done with 10^7 cfu/mL. Four 6-mm leaf punches were taken at the indicated time points and assayed for conductivity.

Time (hpi)	Mock Infiltrated		Psg (Empty)		Psg (avrRpt2)		
14	42.6	43.3	85.2	73.9	74.4	32.7	
16	49.3	46.6	32.2	38.5	89.5	108.5	
18	44.5	47.2	43	48.6	140.7	101.2	
20	43.7	33.7	28.5	32.1	156.3	30.2	

Figure 8 shows the results of three independent trials. Untreated leaves show a low level of ion leakage due to the mechanical damage to cells at the cut site. Mock bacterial infiltration with bacteria carrying an empty vector show a level of ion leakage not significantly different than the untreated leaves. This is true for both the TCV infected plants and the plants not inoculated with virus. A large increase in conductivity was seen in the leaves infiltrated with the *avr* and that were not infected with TCV. This large increase is characteristic of tissue undergoing programmed cell death. In contrast, TCV-infected leaves leaf samples challenged with *avrRpt2* showed a significant decrease in conductivity providing evidence of inhibition of cell death in leaves harboring the virus.

Averaging the data from the three independent experimental trials, the conductivity for HR suppressed samples was consistently half that of its virus free counterpart as shown

in Figure 9. In fact, after subtracting the background seen in untreated tissue, the TCVinfected tissue showed a four-fold decrease conductivity.



Figure 8: Quantification of AvrRpt2-induced Programmed Cell Death by Ion Leakage. Three week-old Col-0 plants were inoculated with 0.2 ug/uL TCV. After 10 days, plants were either mock-infiltrated or Psg infiltrated with 10mM MgSO₄ or 10^7 cfu/ml, respectively. Four 6-mm leaf discs were taken 18 hours post infiltration and analyzed for conductivity. Plot shows conductivity values obtained from three independent trials. Each trial consisted of three samples of four leaf discs each. Blue and yellow bars show ion leakage data for leaves infiltrated 10 days post-viral inoculation, while red bars shows data for leaves infiltrated 13 days post-viral inoculation.



Figure 9: Statistical Analysis of avrRpt2-induced PCD Quantification in Col-0. Bars represent the average of three independent trials consisting of nine total samples. Letters above bars represent distinct significance groups as determined by the Tukey-Kramer Method for multiple sample comparisons. Each group differs from the others at a confidence level of P = 0.05. All statistics were performed using NCSS statistical software.

3.2 TCV suppresses formation of HR to avrRpm1

In order to determine if TCV could suppress the HR to a different avr, a similar set of experiments were carried out - the only difference being the avr carried on the plasmid in these experiments was avrRpm1. To establish HR suppression, Col-0 plants were either mock inoculated or inoculated with TCV. Ten days post viral inoculation, the symptomatic leaves and equivalent sized leaves on mock-inoculated plants, were infiltrated with one of three samples: 10 mM MgSO4, *Psg* carrying an empty vector, or *Psg* carrying a vector encoding avrRpm1. Since the kinetics of HR to avrRpm1 is much

quicker than avrRpt2, conductivity and tissue collapse was monitored at 5 hpi rather than 18 hpi (Shapiro et al., 2001).

As predicted, leaf samples subjected to *P. syringae* strains expressing *avrRpm1* displayed the highest levels of conductivity as shown in Figure 10.



Figure 10: Quantification of AvrRpm1-induced Programmed Cell Death by Ion Leakage. Three week-old Col-0 plants were inoculated with 0.2 ug/uL TCV. At 10 dpi, plants were then either infiltrated with 10mM MgSO4 or infiltrated with or 10^7 cfu/ml of *Psg*. Four 6-mm leaf discs were taken 5 hours post infiltration and analyzed for conductivity. Plot shows conductivity values obtained from two independent trials. Each trial consisted of three samples of four leaf discs each (a). Pictures of *Psg* avr infiltrated leaves at 5 hpi from plants mock-inoculated (b) or virus infected (c).

In contrast, the leaves that harbored virus showed a much smaller increase in conductivity levels upon infiltration with *Psg* carrying the *avr*, and in fact, showed values similar to all the other groups. Averaging the data from the two independent experimental trials, the conductivity for HR suppressed samples was consistently 2.5 times less that of its virus free counterpart as shown in

Figure 11. After subtracting the background seen in untreated tissue, the difference is four-fold.



Figure 11: Statistical Analysis of avrRpm1-induced PCD Quantification in Col-0. Bars represent the average of two independent trials consisting of six total samples. Letters above bars represent unique significance groups as determined by the Tukey-Kramer Method for multiple sample comparisons. Each group differs from the others at a confidence level of P = 0.05. All statistics were performed using NCSS statistical software.

3.3 Pto/AvrPto Challenge of TCV infected *Nicotiana benthamiana*

With the eventual goal to determine the viral component responsible for reducing or suppressing the HR, we began developing a new system for monitoring the TCV suppression of HR. A transient assay in *Nicotiana benthamiana* was recently used to demonstrate the ability of avrPtoB to suppress the HR (Abramovitch et al, 2003). In order to utilize this system effectively, it was first necessary to determine if TCV could suppress the HR in these plants. As it had previously been shown that TCV could systemically infect *N. Benthamiana* (Lin and Heaton, 1999), four-week old plants were inoculated with in vitro transcribed TCV genomic RNA. At 22 dpi, viral symptoms were clearly visible on younger leaves. Symptoms of infections included leaf wrinkling, stunted growth, rough texture and vein coloring causing a mottled appearance.



Figure 12: TCV infection in *Nicotiana benthamiana* at 22 dpi. Four week-old plants were either mock inoculated or TCV inoculated with 0.2 ug/uL RNA. Mock-Inoculated plants (a) and (b) TCV inoculated plants (c) and (d).

Symptomatic and asymptomatic leaves of TCV-infected and control plants were then infiltrated with a mixture of *Agrobacterium tumefaciens* carrying two different plasmids. One encoded for avrPto while the other encoded for Pto. Under normal conditions, co-expression of these two proteins results in a visible HR throughout the infiltrated area (Abramovitch et al., 2003)

Three different types of leaf tissue were infiltrated – symptomatic young leaves and middle-aged leaves, and older, uninoculated leaves. Older leaves were included as a control since virus was not anticipated to be present in these tissues allowing, measurement of PCD activity in the absence of virus. Symptomatic young and middleaged leaves were infiltrated to assay for HR suppression as these leaves harbor active virus.

These experiments were evaluated two different ways – electrolyte leakage measurements taken within two days following infiltration and visual inspection of the tissue after several days. For the ion leakage test, leaf punches were taken at 30 hpi and 48 hpi. These time points were selected because older leaves were showing visible signs of tissue collapse.

Figure 13 shows conductivity readings for the two time points.



Figure 13: Quantification of AvrPto/Pto induced Programmed Cell Death in *N. benthamiana* plants. Four week-old plants were inoculated with 0.2 ug/uL in vitro transcribed TCV. Plants were then either mock-infiltrated or Agro infiltrated with 10mM MES or with bacteria at $A_{600} = 0.3$, respectively. Four 6-mm leaf discs were taken analyzed for conductivity. Plot shows conductivity values obtained at 30 hours (blue bars) and 48 hours (red bars) post infiltration.

As expected, older leaves revealed the highest conductivity levels in both TCV and control plants. In the virus free plant, the peak of conductivity is at 30 hpi and decreases considerably at 48 hpi. The TCV-infected older leaves show a different pattern with a higher level of ion leakage at 48 hpi but the difference is not so great between the time points. Interestingly, the younger and middle-aged leaves of the virus-free plant showed little ion leakage at either time point. However, by 4 dpi visual inspection showed significant tissue collapse. Isolating the time point at which peak ion flux occurs in these younger mock-inoculated tissues is crucial before extending the analyses to include TCV. Overall, the range of conductivity values in the TCV-infected plant was higher and much broader indicating that the virus may variably affect the PCD pathway as visual inspection of younger and middle-aged TCV-infected leaves showed variable levels of lesion formation, from 5 - 100%, when scored 7 dpi.

3.4 Transient Expression of TCV in *Nicotiana Benthamiana*

The entire TCV-genome was cloned into the *Agrobacterium* compatible plant expression vector pBTex for use in the transient assay system. The clone was sequenced and checked. The p28, p9, and p38 ORFs had full consensus. The p88 ORF contains a single amino acid change from glutamic acid to glutamine. Moreover, p88 and p8 will need to be resequenced as their sequence data was incomplete.

The full-length TCV clone was then tested to check for recapitulation of a viral infection. After infiltration of *N. benthamiana* plants with *Agro* carrying the vector, samples were harvested 2, 4, and 6 dpi from Agro-infiltrated leaves. To determine whether the expressed virus was spreading from infiltrated tissue to uninfiltrated tissue, sections of leaves proximal to infiltration sites were harvested at 4 and 6 dpi. RT-PCR was performed on RNA extracted from these samples to detect for presence of TCV. Figure 14 shows the results of the RT-PCR analysis.



Figure 14: RT-PCR of Total RNA extracted from tissue infiltrated with TCV carrying the full-length TCV expressing vector. 1-kB DNA Ladder (Lane 1); Infiltrated leaf 2 dpi (Lane 2); Uninfiltrated and infiltrated leaf 4 dpi (Lanes 3 and 4); Uninfiltrated and infiltrated leaf 6 dpi (Lanes 5 and 6); negative PCR control (Lane 7).

As indicated by the figure, TCV transcript was detectable at 2 dpi. Movement of TCV to uninfiltrated sections of tissue was detected at 4 and 6 dpi. Active replication of the virion in infiltrated tissue was also evident as intensity of amplified cDNA increased from 4 to 6 dpi.

3.5 Detection of Individual TCV ORFs in N. Benthamiana

Each construct was transfected into *Agrobacterium* and infiltrated into *N. benthamiana* leaves. To determine if the constructs were being properly transcribed inplanta, RNA gel blot analysis and RT-PCR were performed on total RNA extracted from infiltrated tissue. As the p28MUT:pBTEX clone was the first construct made, a gel blot analysis was used to detect p28MUT transcript. Figure 15 shows detection of p28MUT transcript in infiltrated leaves.



Figure 15: RNA gel blot detection of p28MUT transcript in total RNA harvested from N. benthamiana leaves 36 hpi. Ethidium bromide stained RNA denaturing gel showing 50 ng pT1D1 Δ L DNA (Lane 1) and 10 ug total RNA from leaves infiltrated with agro carrying pBTEX expressing p28MUT (Lane 2) (a); RNA gel blot of the same gel blotted and hybridized with a p28 probe (Lane 2) (b).

To test for the presence of p8, p9MUT, p38 and p88MUT transcript, RT-PCR was used. Total RNA was extracted from *N. benthamiana Agro*-infiltrated with each of the

TCV ORFs. The RNA was then used as template for first strand synthesis by reverse transcription. The resulting cDNA was used as template for amplification using traditional PCR and primer pairs specific for each transcript as shown in Figure 16.



Figure 16: RT-PCR detection of TCV ORF transcripts in total RNA harvested from *N. benthamiana* leaves 36 hpi. 1-kB DNA Ladder (Lane 1) and amplified cDNA from leaves infiltrated with Agro carrying pBTEX expressing p88MUT (Lane 2) (a); 1-kB DNA Ladder (Lane 1), amplification of cDNA from leaves infiltrated with Agro carrying pBTEX expressing p8 (Lane 2), p9MUT (Lane 3) and p38 (Lane 4) (b).

3.6 Transient Expression of Individual TCV ORFs in *N. benthamiana*

As previously discussed, *N. benthamiana* plants infiltrated with *Agro* expressing AvrPto and Pto display localized necrotic lesions – evidence of the HR and a functional R-avr interaction. Each of the TCV constructs was evaluated for anti-PCD activity in the presence of AvrPto/Pto-mediated PCD. Additionally, combinations of each of the constructs were also tested to see if the suppression phenotype required the presence of more than one component. For almost all of the systems tested, a variable extent of lesion formation was observed in the transient assay system, as shown in Figure 17-Figure 19.



Figure 17: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ in combination with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. Leaves were scored for suppression phenotypes 7 dpi. p28MUT(a) p88MUT(b) p8(c) p9MUT(d) p38(e) p28MUT,p88MUT(f) p28MUT,p8(g) p28MUT,p9MUT(h). Three different plants were tested. Leaves 1 and 2 were taken from one plant, while leaf 3 was taken from a second plant and leaves 4 and 5 were taken from a third plant.

The percentage of infiltrated area that resulted in tissue collapse varied from 5% to 100% in the five distinct samples expressing the same construct or combination of

constructs. The only combinations that showed consistency in observable phenotype was the p88MUT and p9MUT pair, panel (j) of Figure 18, and the p88MUT and p38 pair, panel (k) of Figure 18, which resulted in 90-100% tissue collapse in all five leaf samples. Tables B-1 through B3 in Appendix B show progression of necrosis from 4 dpi – 7 dpi.



Figure 18: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with combinations of *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. p88MUT, p8(i) p88MUT, p9MUT(j) p88MUT, p38(k) p28MUT, p38(l) p8MUT, p9MUT(m) p8, p38(n) p9MUT, p38(o). Three different plants were tested. Leaves 1 and 2 were taken from one plant,

while leaf 3 was taken from a second plant and leaves 4 and 5 were taken from a third plant.

Minimal cell death was also observed for the p28MUT, p38 and p88MUT combination, panel (d) in Figure 19. Leaves scored at 7 dpi from this combination showed no more than a 10% total collapse of infiltrated tissue across both samples. However, samples containing these three components in addition to a fourth construct, showed a more variable percentage of collapse. The p28MUT, p38, p88MUT and p8 combination, as shown in Panel (k) showed almost 75–100% lesion formation in the infiltrated sites. However, the p28MUT, p38, p88MUT and p9MUT sample, Panel (l) showed reduced cell death phenotype with only 15-30% of the infiltrated area showing lesions. Table B-4 in Appendix B shows progression of necrosis from 4 dpi – 7 dpi.



Figure 19: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with combinations of *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$.

p8,p9MUT,p38(a) p28MUT,p88MUT,p8(b) p28MUT,p88MUT,p9MUT(c) p28MUT,p88MUT,p38 p28MUT,p8,p38(e) p28MUT,p9MUT,p38(f) (d) p88MUT,p9MUT,p38MUT(h) p88MUT,p8,p38(g) p88MUT,p8,p9MUT,p38(i) p28MUT,p8,p9MUT,p38MUT p28MUT,p88MUT,p8,p38 (j) (k) p28MUT,p88MUT,p9MUT,p38 (l) and p28MUT,p88MUT,p8,p9MUT (m). All samples were scored 7 dpi. All leaves were taken from the same plant.

Controls for the transient expression assays are shown in Figure 20-Figure 22 shows the results from ten separate co-infiltrations of *Agrobacterium* carrying the vector expressing avrPto and Pto on a single leaf from the same plant containing leaves 1 and 2 in Figure 17 and Figure 18. At 7 dpi, only mild tissue collapse could be observed in each of the ten samples where full tissue collapse was expected. This is consistent with data from these same leaves infiltrated with TCV ORFs and displayed a much reduced severity of necrosis when compared with leaves from different plants. Plant to plant variation in induction of PCD-pathways may be accountable for this observation.



Figure 20.: Controls for Transient Expression Assays of TCV ORFs *N.* benthamiana. Eight-week old *N. Benthamiana* plants were infiltrated with Agrobacterium carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. The leaf was scored 7 dpi for cell death phenotypes. All samples are from the same leaf.

Figure 21 shows the results from twelve separate co-infiltrations of *Agrobacterium* carrying the vector expressing avrPto and Pto in combination with the anti-PCD effector

avrPtoB on a single leaf from the same plant containing leaves 4 and 5 from Figure 17-Figure 18. As expected, leaf tissue was completely suppressed for cell death in each of the twelve samples at 7 dpi.

Figure 22 shows each of the TCV ORF constructs along with avrPtoB, avrPto, and Pto constructs individually expressed in N. benthamiana plants. As expected, only tissue infiltrated with *Agrobacterium* carrying the vector expressing avrPto results in induction of an HR – consistent with previous findings (Abramovitch et al, 2003).



Figure 21: Controls for Transient Expression Assays of TCV ORFs *N.* benthamiana. Eight-week old *N. Benthamiana* plants were infiltrated with Agrobacterium carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$ in combination with Agrobacterium carrying the vector expressing avrPtoB at $A_{600} = 0.3$. The leaf was scored 7 dpi for cell death phenotypes. All samples are from the same leaf.



Figure 22: Controls for Transient Expression Assays of TCV ORFs N. benthamiana. Eight-week old N. Benthamiana plants were infiltrated with Agrobacterium carrying vectors expressing p8(a) p9MUT(b) p28MUT(c)

avrPtoB(d) avrPto(e) Pto(f) p38(g) and p88MUT(h) at $A_{600} = 0.3$. The leaf was scored 7 dpi for cell death phenotypes. All samples are from the same leaf.

3.7 Expression of TCV ORFs in Sacharomyces cerevisiae

To check for PCD suppression in a heterologous system, both the mutant and wild-type TCV ORFs were cloned into the yeast-compatible vector p423. The vector p423 contains a histidine selection marker along with a GAL1 galactose-inducible promoter. Prior to each yeast cell death assay, the constructs were transfected into competent INVSC1 yeast cells and selected using histidine-free media.

Transformants were cultured overnight, induced and then subjected to stress by including 3 mM H_2O_2 in their growth media after induction. After the six-hour H_2O_2 exposure, 20-uL aliquots of culture and each of 5 ten-fold serial dilutions of each of the samples were plated on His-free media to determine yeast survival. Untreated samples were also plated to compare viability with the treated cells.

Yeast transformed with p38 showed the greatest colony growth in dilution 3 of H_2O_2 treated cells when compared with the positive control avrPtoB. Likewise, yeast carrying the empty vector, along with the yeast samples carrying each of the TCV ORFs, showed a reduced extent of yeast survival for the same dilution. Untreated cells showed very similar concentrations as all constructs produced several colonies at the 5th dilution. However, the H_2O_2 -treated cells showed a wide range of survival depending upon the construct it contained. Empty vector and vector encoding p28MUT and p88MUT showed 10 or so cfu by the 3rd dilution. In contrast, avrPtoB and p38 had about ten times for cfus.



Figure 23: Survival of S. cerevisiae strain INVSC1 expressing TCV ORFs undergoing oxidative stress. Untreated samples (a) and 3 mM H₂O₂ (b). Transformants were grown overnight and induced with galactose for six hours. Cells were then diluted to $A_{600} = 0.05$ and incubated for six hours at 30°C in SD/-HIS or SD/-HIS supplemented with 3 mM H₂O₂. Samples were then plated along with five ten-fold dilutions to test for yeast survival.

4 DISCUSSION

In this thesis, we have demonstrated that *A. thaliana* plants systemically infected with *Turnip crinkle virus* are suppressed in their ability to initiate a PCD-response when challenged with the cognate avirulence factor avrRpt2. Moreover, we were able to extend these findings to an entirely different avirulence factor, avrRpm1, which displays faster kinetics in the induction of the PCD pathway. Infiltration of TCV-infected leaves with either *avr* resulted in a significant four-fold difference in conductivity levels when compared with non-TCV infected leaves undergoing equivalent treatments.

We propose that TCV may be involved in the modification of a host factor that is common between both the Rps2 and Rpm1 mediated PCD pathways as both Rps2 and Rpm1 initiate active defense in a Rin4 dependent manner. In fact, it has been shown that RPS2 knockout Col-0 plants overexpressing avrRpt2 were unable to mount an HR to avrRpm1 due most likely to avrRpt2-mediated elimination of Rin4 (Chen et al., 2000). The fact that Rps2 and Rpm1 both associate with Rin4 leads to the assertion that these two disease resistance proteins evolved out of necessity to monitor different pathogeninduced modifications of Rin4 (Mackey et al., 2003). Interaction between plant virus proteins and host factors is not uncommon as the p8 movement protein was shown to directly interact with the Arabidopsis protein Atp8 in both yeast-two hybrid and in-vitro binding assays. As Atp8 was shown to possess two putative transmembrane fragments, TCV has the capability to exploit host factors to promote its own virulence (Lin and Heaton, 2001).

We have also demonstrated that both the mutant and wild-type TCV constructs was transcribed in-planta. Moreover, full-length TCV expressed by agrobacterium was not only expressed, but was able to reproduce endogenous viral movement as it was detected in uninfiltrated tissue.

The results of the ion leakage assay in N. benthamiana plants challenged with avrPto and Pto were inconclusive. Though an increase in conductivity levels in older leaves was evident at 24 hpi with respect to untreated and mock tissue, peak ion flux for younger and middle-aged leaves was not determined. Before repeating the experiment with TCV infected plants, it is recommended that a time trace analysis be performed on healthy N. Benthamiana plants challenged with the AvrPto and Pto scheme to isolate the time point for maximum electrolyte leakage. The infiltrated leaves should be monitored for appearance of tissue collapse every twelve hours as lesions on these tissues were detectable by 4 dpi. Tracking of lesion formation using tables similar to those in Appendix B would reveal the time span in which these younger leaves invoke their PCD programs. Once that time point is established, leaves can be then be assayed for comparative assessment of ion leakage from both TCV and mock-TCV plants undergoing PCD.

The p28-WT, p88-WT, and p9-WT clones will need to be reconstructed as soon as possible. If future experiments with these clones reveal HR suppression, data from the mutant constructs could provide some insight into key residues required for function. I wish the next person that constructs these clones the best of luck in showing that one of these ORFs plays a role in HR suppression.

The ORFs from the full-length TCV were mostly fine except for a single glutamic acid to glutamine change in the p88 ORF. Moreover, p8 and p88 sequencing reactions

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were incomplete, so it is recommended that these two ORFs be resent for sequencing to ensure full consensus.

In the meantime, transient assays involving the p38 and p8 constructs will need to be repeated with more precise experimental parameters. As the plant-to-plant response in the HR severity under similar treatments was quite variable, there may be a concentration threshold for the agrobacterium to efficiently translocate T-DNA to the host genome. When testing three-way and four-way expression of TCV components, the overall concentration of the individual factors may need to be optimized to ensure sufficient delivery of each.

Incorporating one of the R-avr schemes that we used for quantification of HR suppression may offer some new revelations in the use of the transient expression system. If TCV is operating through modification of a signaling component essential to both the RPS2 and RPM1 pathways, then we may not be able to observe any visible affect on HR induction in completely different systems such as avrPto-Pto and Avr9-Cf9. This seems to be a very promising alternative as we now have solid evidence that TCV interferes with both Rps2 and Rpm1 mediated immunity.

As Abramovitch et al. (2003) were able to show anti-apoptotic activity of avrPtoB in both plants and yeast, we tried expressing each of the TCV ORFs in yeast undergoing oxidative stress. Though we were only able to try the assay once, yeast expressing p38 showed stronger survival rates with respect to avrPtoB. Performing the yeast apoptosis assay to duplicate the suppression phenotype of avrPtoB may help in optimizing the assay protocol for use with the TCV constructs. Inclusion of p8 and p9-WT will also need to be performed in future experiments.

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APPENDIX A

SEQUENCES OF TCV CLONES p28MUT Sequence

Consensus #1	ATGCCTCTTCTAC	CACACACTCAAC	CACAGCGCTC	GCAGT GGGACT	CCTAGGAGCC	AGGTGCTACC	CTGAGGTTCAA	ACCTT
	10	20	30	40	50	60	70	80
TCVbGenome.txt SP6_Forward T7Promoter Reverse	ATGCCTCTTCTAC ATGCCTCTTCTAC ATGCCTCTTCTAC	CACACACTCAAC CACACACTCAAC CACACACTCAAC	CACAGCGCTCC CACAGCGCTCC CACAGCGCTCC	GCAGT GGGACT GCAGT GGGACT GCAGT GGGACT	CCTAGGAGCC CCTAGGAGCC	AGGTACTACCO AGGTGCTACCO AGGTGCTACCO	CTGAGGTTCAA CTGAGGTTCAA GTGAGGTTCAA	ACCTT ACCTT ACCTT
Concensus #1	CTTCCCCCTCCCT				CCTCTCTTT			ACTCT
Consensus #1	90	100	110	120	130	140	150	16(
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	CTTGGGGCTGCCT CTTGGGGGCTGCCT CTTGGGGGCTGCCT	GACTACGTGGC GACTACGTGGC GACTACGTGGC	TCACATGAAG TCACATGAAG TCACATGAAG	GAATGTAGTAC GAATGTAGTAC GAATGTAGTAC	GGTCTGTTTT CGGTCTGTTTT CGGTCTGTTTT	CCAGGGATCT CCAGGGATCT CCAGGGATCT	GGGCT AGT AGT GGGCT AGT AGT GGGCT AGT AGT GGGCT AGT AGT	AGTGT AGTGT AGTGT
Consensus #1	CCTCCGACACAGT	CGGTGTCAGGC	GGACGTATA	GTAATAGAGGT	CAGATAGGTA	GTAGTCTCGG	GTGTATACTAG	CCGTT
	170	180	190	200	210	220	230	24(
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	CCTCCGACACAGT CCTCCGACACAGT CCTCCGACACAGT	CGGT GT CAGGC CGGT GT CAGGC CGGT GT CAGGC	GGGACGTATAC GGGACGTATAC GGGACGTATAC	GT AAT AGAGGT GT AAT AGAGGT GT AAT AGAGGT	°CAGATAGGTA °CAGATAGGTA °CAGATAGGTA	GTAGTCTCGG GTAGTCTCGG GTAGTCTCGG	GTGTATACTAG GTGTATACTAG GTGTATACTAG	CCGTT CCGTT CCGTT
Consensus #1	CCGGAT AGCGGGC	GCGGATATAGAA	ATAGACCTA	GATAGGTTGGT	AGGAACGGAA	GAGGAAGCCA	CATCCTGTTTG	GTGGA
	250	260	270	280	290	300	310	32(
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	CCGGATAGCGGGC CCGGATAGCGGGC CCGGATAGCGGGC	CGGATATAGAA CGGATATAGAA CGGATATAGAA	ATAGACCTAC ATAGACCTAC ATAGACCTAC	GATAGGTTGGT GATAGGTTGGT GATAGGTTGGT	`AGGAACGGAA `AGGAACGGAA `AGGAACGGAA	GAGGAAGCCA GAGGAAGCCA GAGGAAGCCA	CATCCTGTTTG CATCCTGTTTG CATCCTGTTTG	GTGGA GTGGA GTGGA
Consensus #1	GGCGGT AGGT AGT	ACCGCAGATGT	CCCCAGGAG	GAGAGTTCGTC	CAAAAGGGGGCG	GTTTGCTATG	CATGCCGTCAA	CGCAG
	330	340	350	360	370	380	390	400
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	GGCGGT AGGT AGT GGCGGT AGGT AGT GGCGGT AGGT AGT	ACCGCAGATGT ACCGCAGATGT ACCGCAGATGT	°CCCCAGGAGG °CCCCAGGAGG °CCCCAGGAGG	GAGAGTTCGTC GAGAGTTCGTC GAGAGTTCGTC	XAAAAGGGGCG XAAAAGGGGCG XAAAAGGGGCG	GTTTGCTATG GTTTGCTATG GTTTGCTATG	CATGCCGTCAA CATGCCGTCAA CATGCCGTCAA	CGCAG TGCAG CGCAG
Consensus #1	CAAAGCTGCACTT	TTGTGGCGTCC	CAAAACCCA	CTGAAGCGAAT	CGACTAGCGG	TCTCAAAATG	GCTTGTCCAAT	ACTGC
	410	420	430	440	450	460	470	480
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	CAAAGCTGCACTT CAAAGCTGCACTT CAAAGCTGCACTT	TTGTGGCGTCC TTGTGGCGTCC TTGTGGCGTCC	CAAAACCCAG CAAAACCCAG CAAAACCCAG	CTGAAGCGAAT CTGAAGCGAAT CTGAAGCGAAT	CGACTAGCGG CGACTAGCGG CGACTAGCGG	ТСТСААААТG ТСТСААААТG ТСТСААААТG	GCTTGTCCAAT GCTTGTCCAAT GCTTGTCCAAT	ACTGĊ ACTGC ACTGC
Consensus #1	AAAGAGAGACATC	TCGTAGACAG	CACATCAGA	ACGATAGTCAA	TACGGCTCTT	CCTAGAGTGT	FCACGCCTGAC	GCGGA
	490	500	510	520	530	540	550	560
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	AAAGAGAGACATC AAAGAGAGACATC AAAGAGAGACATC	TCGTAGACAGC TCGTAGACAGC TCGTAGACAGC	CACATCAGA/ CACATCAGA/ CACATCAGA/	ACGATAGTCAA ACGATAGTCAA ACGATAGTCAA	TACGGCTCTT ATACGGCTCTT ATACGGCTCTT	CCTAGAGTGT CCTAGAGTGT CCTAGAGTGT	FCACGCCTGAC FCACGCCTGAC FCACGCCTGAC	GCGGA GCGGA GCGGA
Consensus #1	AGACATTCAGGTC	GTGCTGGATTI	GCACAGTGT	AGAGCACACG	GACCACCGCAA	CGCCCTAGCC	GAAGCAGGCAA	AGTGC
	570	580	590	600	610	620	630	64(
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	AGACATTCAGGTC AGACATTCAGGTC AGACATTCAGGTC	GTGCTGGATTT GTGCTGGATTT GTGCTGGATTT	GCACAGTGT / GCACAGTGT / GCACAGTGT /	AAGAGCACACG AAGAGCACACG AAGAGCACACG	JACCACCGCAA JACCACCGCAA JACCACCGCAA	CGCCCTAGCCO CGC <mark>T</mark> CTAGCCO CGCCCTAGCCO	GAAGCAGGCAA GAAGCAGGCAA GAAGCAGGCAA	AGTGC AGTGC AGTGC
Consensus #1	GGAAGTGGTGGGT	CAATCTCGCGA	ATGCATCCCAT	GACTGGGAGG	TCGTGGTCCA	GGGCTTGGAG	GCGATTATGCC	GACTG 72(
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	GGAAGT GGT GGGT GGAAGT GGT GGGT GGAAGT GGT GGGT	CAATCTCGCGA CAATCTCGCGA CAATCTCGCGA	ATGCATCCCAT ATGCATCCCAT ATGCATCCCAT	I GACT GGGAGG I GACT GGGAGG I GACT GGGAGG	TCGTGGTCCA TCGTGCTCCA TCGTGGTCCA	GGGCTTGGAGG GGGCTTGTAGG GGGCTTGGAGG	GCGATTATGCC GCGATTATGCC GCGATTATGCC	GACTG GACTG GACTG
Consensus #1	CCTGACGACCAGC	GGATCTCTTT	GTCCGCTAG					
	730	740	750					
TCVbGenome.txt SP6_Forward T7Promoter_Reverse	CCTGACGACCAGC CCTGACGACCAGC CCTGACGACCAGC	CGATCTCTTT CGATCTCTTT CGATCTCTTTT	GTCCGCTAG GTCCGCTAG GTCCGCTAG					

p28MUT Translated

Consensus #1

TCVbGenome.txt SP6_Forward T7Promoter_Reverse

MP L L HT L NT AL AVGL	LGARCYPEV	QTFLGLPDYVO	GHMKNVVRS VI	F QGS GL VVVS S	DTVGVRGTYS	NRGQI
10	20	30	40	50	60	70
MP L L HT L NT A L A V G L	L GAR Y Y P E V	QTFLGLPDYVO	GHMKN VVRS VI	F QGS GL VVVS S	DTVGVRGTYS	NRGQI
MP L L HT L NT AL AVGL	LGARCYPEV	QTFLGLPDYVO	GHMKNVVRS VI	F QGS GL VVVS S	DT VGVRGT YS	NRGQI
MPLLHILNIALAVGL	L GDKU I KE V	QIFLGLFDIV	JHMKNVVKSVI	r QUSUL V V V S S	DIVGVKGIIS	NKGQI
GSSLGCILAVPDSGA	DIEIDLDRL	VGTEEEATSCI	VEAVGSTAD	VPRRRVRQKGR	FAMHAVNAAK	KL HF C G
80	90	100	110	120	130	140
GS S L G C I L A V P D S G A	DI EI DL DRL	VGTEEEATSCI	VEAVGSTAD	VPRRRVROKGR	FAMHAVNAAK	LHFCG
GSSLGCI LAVPDSGA	DI EI DL DRL	VGTEEEATSCI	VEAVGSTAD	VPRRRVRQKGR	FAMHAVNAAK	CLHFCG
GSSLGCILAVPDSGA	DI EI DL DRL	VGTEEEATSCI	LVEAVGS TAD	V P R R R V R Q K G R	FAMHAVNAAK	CL HF C G
VP KP TE ANRLAVS KV	VLVQYCKERH	VVDSHIRTI VI	NTALPRVFTPI	DAEDI QVVLDL	HS VRAHDHRN	JALAEA
150	1		I	r í	1	
1.10	160	170	180	190	200	210
VPKPTEANRI AVSKV			180 ITAL PRVETPI	190	200	
VP KP TE ANRLAVS KV VP KP TE ANRLAVS KV	160 VLVQYCKERH VLVQYCKERH	170 VVDSHIRTIVN VVDSHIRTIVN	180 TALPRVFTPI NTALPRVFTPI	190 J DAEDI QVVLDL DAEDI QVVLDL	200 HS VR AHDHRN HS VR AHDHRN	210 NALAEA NALAEA
VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV	160 VLVQYCKERH VLVQYCKERH VLVQYCKERH	170 VVDSHIRTIVN VVDSHIRTIVN VVDSHIRTIVN	180 NTALPRVFTP1 NTALPRVFTP1 NTALPRVFTP1	190 J DAEDI QVVLDL DAEDI QVVLDL DAEDI QVVLDL	200 HS VR AHDHRN HS VR AHDHRN HS VR AHDHRN	210 NALAEA NALAEA NALAEA
VP KP TE ANRLAVS KV VP KP TE ANRLAVS KV VP KP TE ANRLAVS KV GK VR K WWVNLAMHP N	160 ML VQYCKERH ML VQYCKERH ML VQYCKERH	170 VVDSHIRTIV VVDSHIRTIV VVDSHIRTIV RRLCRLPDDO	180 NTALPRVFTPI NTALPRVFTPI NTALPRVFTPI	190 J DAEDI QVVLDL DAEDI QVVLDL DAEDI QVVLDL	200 HS VR AHDHRN HS VR AHDHRN HS VR AHDHRN	210 JALAEA JALAEA JALAEA
VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV GK VR KWWVNL AMHP N	160 M. VQYCKERH M. VQYCKERH M. VQYCKERH MT GRS WS RAW	170 VVDSHI RTI V VVDSHI RTI V VVDSHI RTI V RRLCRLPDDQ	180 NTALPRVFTPI NTALPRVFTPI NTALPRVFTPI	190 DAEDI QVVLDL DAEDI QVVLDL DAEDI QVVLDL	200 HS VRAHDHRM HS VRAHDHRM HS VRAHDHRM	210 NALAEA NALAEA NALAEA
VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV VP KP TE ANRL AVS KV GK VR KWWVNL AMHP N 220	160 MLVQYCKERH MLVQYCKERH MLVQYCKERH MTGRSWSRAW 230	170 VVDSHIRTIVN VVDSHIRTIVN VVDSHIRTIVN RRLCRLPDDQ/ 240	180 NTALPRVFTPI NTALPRVFTPI NTALPRVFTPI AISFVR- 250	190 DAEDI QVVL DL DAEDI QVVL DL DAEDI QVVL DL	200 HS VRAHDHRN HS VRAHDHRN HS VRAHDHRN	210 NALAEA NALAEA NALAEA
ISO VP KP TE ANRLAVS KV VP KP TE ANRLAVS KV VP KP TE ANRLAVS KV GK VR KWWVNL AMHP N 220 GK VR KWWVNL AMHP N CK VR KWWNL AMHP N	160 ML VQYCKERH ML VQYCKERH ML VQYCKERH MT GRS WS RA W 230 TT GRS WS RA W	170 VVDSHIRTIVN VVDSHIRTIVN VVDSHIRTIVN RRLCRLPDDQ/ 240 RRLCRLPDDQ/ RRLCRLPDDQ/	180 NT AL P R VF T P I NT AL P R VF T P I NT AL P R VF T P I NI S F VR - 250 250 XI S F VR .	190 DAEDI QVVLDL DAEDI QVVLDL DAEDI QVVLDL	200 HS VRAHDHRN HS VRAHDHRN HS VRAHDHRN	210 NALAEA NALAEA NALAEA

p88MUT sequence Consensus #1 70 80 90 100 110 120 TCVbGenome.txt SP6 FORWARD T A C C C T G A G G T T C A A A C C T T C T T G G G G C T G C C T G A C T A C G T G G G T C A C A T G A A A A T G T A Consensus #1 130 140 150 160 170 180 TACCCT GAGGTT CANACCTT CTT GGGGCT GCCT GACT AC GT GGGT CACAT GAAGAAT GTA TACCCT GAGGTT CAAACCTT CTT GGGGCT GCCT GACTAC GT GGGT CACAT GAAGAAT GTA TCVbGenome.txt SP6 FORWARD G T A C G G T C T G T T T T C C A G G G A T C T G G G C T A G T A G T A G T G T C C T C C G A C A G T C G G T G T C Consensus #1 190 200 210 220 230 240 GT AC GGT CT GT TT T C C AGGGAT CT GGG CT AGT AGT AGT GT CT CC GAC A C AGT C GGT GT C GT AC GGT CT GT TT T C C AGGG AT CT GGG CT AGT AGT AGT AGT CT CC C G AC A C AGT C GGT GT C TCVbGenome.txt SP6_FORWARD Consensus #1 A G G G G G A C G T A T A G T A A T A G A G G T C A G A T A G G T A G T A G T C T C G G G T G T A T A C T A G C C G T T 250 260 270 280 290 300 AAT AGAGGTCAGATAGGTAGT A G GT AGCCGT AGGGGGGACGT TCVbGenome.txt AGT TCTCGGG A G G G G A C G T A T A G T A A T A G A G G T C A G A T A G G T A G T A G T C T C G G G T G T A T A C T A G C C G T T SP6_FORWARD Consensus #1 310 320 330 340 350 360 GAT TAG AG GGA AGG TCVbGenome.txt TAGC AGAA GGA C C G G A T A G C G G G G C G G A T A T A G A A T A G A C C T A G A T A G G T T G G T A G G A A C G G A A G A G SP6_FORWARD Consensus #1 G C C A C A T C C T G T T T G G T G G A G G C G G T A G G T A G T A C C G C A G A T G T C C C C A G G A G A G A G T T 370 380 . 390 400 410 420 GCCACATCC TGGTGGAGGCGGTAGGTAGTACCGCAGATGTCCCCAGGAGGAGAGT TCVbGenome.txt TGT SP6_FORWARD G C C A C A T C C T G T T T G G T G G A G G C G G T A G T A G T A C C G C A G A T G T C C C C A G G A G G A G A G T T Consensus #1 C G T C A A A A G G G G C G G T T T G C T A T G C A T G C C G T C A A C G C A G C A A G C T G C A C T T T T G T G G C 430 440 450 460 470 480 TCVbGenome txt C G T C A A A A G G G G C G G T T T G C T A T G C A T G C C G T C A A C G C A G C A A A G C T G C A C T T T C G T C A A A A G G G G C G G T T T G C T A T G C A T G C C G T C A A C G C A G C A A A G C T G C A C T T T GTGGC SP6 FORWARD TGTGGC G T C C C A A A A C C C A C T G A A G C G A A T C G A C T A G C G G T C T C A A A A T G G C T T G T C C A A T A C T G C Consensus #1 490 500 510 520 530 540 G T C C C A A A A C C C A C T G A A G C G A A T C G A C T A G C G G T C T C A A A A T G G C T T G T C C A A T A C T G C G T C C C A A A A C C C A C T G A A G C G A A T C G A C T A G C G G T C T C A A A A T G G C T T G T C C A A T A C T G C TCVbGenome txt SP6 FORWARD Consensus #1 A A A G A G A C A T G T C G T A G A C A G C C A C A T C A G A A C G A T A G T C A A T A C G G C T C T T C C T A G A 560 570 600 550 580 590 TCVbGenome.txt SP6 FORWARD Consensus #1 G T G T T C A C G C C T G A C G C G G A A G A C A T T C A G G T C G T G C T G G A T T T G C A C A G T G T A A G A G C A 620 610 630 640 650 660 G T G T T C A C G C C T G A C G C G G A A G A C A T T C A G G T C G T G C T G G A T T T G C A C A G T G T A A G A G C A G T G T T C A C G C C T G A C G C G G A A G A C A T T C A G G T C G T G C T G G A T T T G C A C A G T G T A A G A G C A TCVbGenome.txt SP6_FORWARD C A C G A C C A C C G C A A C G C C C T A G C C G A A G C A G G C A A A G T G C G G A A G T G G T G G G T C A A T C T C Consensus #1 680 670 690 700 710 720 C A C G A C C A C C C C C T A G C C G A G C A G G C A A G T G C G G A A G T G G T G G G T C A A T C T C C A C G A C C A C C G C A A C G C C C T A G C C G A A G C A G G C A A A G T G C G G A A G T G G T G G G T C A A T C T C TCVbGenome.txt SP6 FORWARD GCGATGCATCCCATGACTGG. AGGTCGTGGTCCAGGGCTTG. Consensus #1 730 740 750 760 GCGATGCATCCCATGACTGGGAGGTCGTGGTCCAGGGCTTGG GCGATGCATCCCATGACTGG-AGGTCGTGGTCCAGGGCTTGA TCVbGenome.txt

SP6_FORWARD

Consensus #1 Majority	GGGCGATTAT GGGCGATTAT	GCCGACTGCC	CTGACGACCAC CTGACGACCAC	GCGATCTCT1 GCGATCTCT1	TTGTCCGCTA TTGTCCGCTA	GGGGTGCTTG GGGGTGCTTG	GCGGGAGCTGG GCGGGAGCTGG	TCGGGAGGGA TCGGGAGGGA
	770	780	790	800	810	820	830	840
TCVbGenome.txt Nru_Forward	A <mark>GGCGATTAT</mark> G <mark>AGCGATTAT</mark>	GCCGACTGCC GCCGACTGCC	CTGACGACCAC CTGACGACCAC	GCGATCTCT1 GCGATCTCT1	TTGTCCGCTA TTGTCCGCTA	GGGGTGCTTG GGGGTGCTTG	CGGGAGCTGG CGGGAGCTGG	TCGGGAGGGA TCGGGAGGGA
Consensus #1 Majority	GACTCAAATC GACTCAAATC	TCCAGGGGTC	GAAAACCCGGC GAAAACCCGGC	CATGCGCGTC	GTTCCCGTTAG GTTCCCGTTAG	CAAATCCCCC	GAAGGTTCGA GAAGGTTCGA	CGCATCTTCC CGCATCTTCC
	850	860	870	880	890	900	910	920
TCVbGenome.txt Nru_Forward	GACTCAAATC GACTCAAATC	TCCAGGGGTC TCCAGGGGTC	GAAAACCCGGC GAAAACCCGGC	CATGCGCGTC CATGCGCGTC	GTTCCCGTTAG GTTCCCGTTAG	CAAATCCCCC CAAATCCCCC	GAAGGT T CGA GAAGGT T CGA	CGCATCTTCC CGCATCTTCC
Consensus #1 Majority	ATATCTGTGC ATATCTGTGC	AATGGGCAAT	GGTTTAGACT	TTGGAGTCCA	СААСААСТСА СААСААСТСА	CTCAACAATT	T GAGAAGAGG T GAGAAGAGG	GTTGATGGAA GTTGATGGAA
	930	940	950	960	970	980	990	1000
TCVbGenome.txt Nru_Forward	ATATCTGTGC ATATCTGTGC	AATGGGCAAT AATGGGCAAT	GGTTTAGACT GGTTTAGACT	TTGGAGTCCA TTGGAGTCCA	СААСААСТСА ССААСААСТСА	СТСААСААТТ СТСААСААТТ	T GAGAAGAGG T GAGAAGAGG	GTTGATGGAA GTTGATGGAA
Consensus #1 Majority	AGAGTCTTT AGAGTCTTTT	ACGTTGAAGA ACGTTGAAGA	ATGCGCAGAAC ATGCGCAGAAC	GCAATTGAAAC GCAATTGAAAC	CAGCCCCCCA	ACCGATCCCA	GGGATTTTCG	GGAAGTTGAG GGAAGTTGAG
	1010	1020	1030	1040	1050	1060	1070	1080
TCVbGenome.txt Nru_Forward	AGAGTCTTTT AGAGTCTTTT	ACGTTGAAGA ACGTTGAAGA	ATGCGCAGAAC ATGCGCAGAAC	GCAATTGAAAC GCAATTGAAAC	CAGCCCCCCA CAGCCCCCCA	ACCGATCCCA	GGGATTTTCG	IGGAAGTTGAG IGGAAGTTGAG
Consensus #1 Majority	TGGGATTCGC TGGGATTCGC	GAGACGATTGC GAGACGATTGC	GTCAGGTTGGC GTCAGGTTGGC	CGGAAATCAT CGGAAATCAT	ACCCCTGTGC	CTCGGGAGAA CTCGGGAGAA	ATACCCGTCG	TTCTACAAGG TTCTACAAGG
	1090	1100	1110	1120	1130	1140	1150	1160
TCVbGenome.txt Nru_Forward	TGGGATTCGC TGGGATTCGC	AGACGATTGC AGACGATTGC	GTCAGGTTGGC GTCAGGTTGGC	CGGAAATCAT CGGAAATCAT	ACCCCTGTGC ACCCCTGTGC	CTCGGGAGAA CTCGGGAGAA	ATACCCGTCG	TTCTACAAGG TTCTACAAGG
Consensus #1 Majority	GCAGGAGGGC GCAGGAGGGC	CACCATATAC	CAAAAGGCTT CAAAAGGCTT	TGGATTCTCT TGGATTCTCT	ACATGACAGC	CCGGTATCCC	GGAAGGACGC GGAAGGACGC	AGAACTCAAA AGAACTCAAA
	1170	1180	1190	1200	1210	1220	1230	1240
TCVbGenome.txt Nru_Forward	GCAGGAGGGC GCAGGAGGGC	CACCATATAC CACCATATAC	CCAAAAGGCTT CCAAAAGGCTT	TGGATTCTC1 TGGATTCTC1	ACATGACAGC ACATGACAGA	ACGGTATCCC CCGGTATCCC	GGAAGGACGC GGAAGGACGC	AGAACTCAAA AGAACTCAAA
Consensus #1 Majority	ACATTCGTGA ACATTCGTGA	AGGCAGAAAA	AGATCAATTTC AGATCAATTTC	CACGGCTAAGA	AAGACCCGGC	TCCACGGGTC	ATCCAGCCGA	GGGACCCACG
	1250	1260	1270	1280	1290	1300	1310	1320
TCVbGenome.txt Nru_Forward	ACATTCGTGA ACATTCGTGA	AGGCAGAAAA AGGCAGAAAA	AGATCAATTTC AGATCAATTTC	CACGGCTAAGA CACGGCTAAGA	AAGACCCGGC AAGACCCGGC	TCCACGGGTC TCCACGGGTC	ATCCAGCCGA ATCCAGCCGA	GGGACCCACG GGGACCCACG
Consensus #1 Majority	ATATAATATT ATATAATATT	GAGGTTGGGA GAGGTTGGGA	AATACTTGAA AATACTTGAA	ACCGTACGAC	САССАТТТАТ САССАТТТАТ	ATCGGGCAAT ATCGGGCAAT	TGACGCTATG TGACGCTATG	T GGGGT GGGC T GGGGT GGGC
	1330	1340	1350	1360	1370	1380	1390	1400
TCVbGenome.txt Nru_Forward	АТАТАА <mark>С</mark> АТТ АТАТААТАТТ	GAGGTTGGGA GAGGTTGGGA	AATACTTGAA AATACTTGAA	ACCGTACGAC ACCGTACGAC	БСАССАТТТАТ БСАССАТТТАТ	ATCGGGCAAT ATCGGGCAAT	TGACGCTATG TGACGCTATG	TGGGGTGGGC TGGGGTGGGC
Consensus #1 Majority	CCACTGTGCT CCACTGTGCT	GAAAGGATAC	CGAT GT GG GG G CGAT GT GG GG GG	GAGCTTGGAAA GAGCTTGGAAA	CATTATGAGT	AACACCTGGG AACACCTGGG	атаааттссо атаааттссо	GAAAACGTGT GAAAACGTGT
	1410	1420	1430	1440	1450	1460	1470	1480
TCVbGenome.txt Nru_Forward	CCACTGTGCT CCACTGTGCT	GAAAGGATAC GAAAGGATAC	CGAT GT GG GG C CGAT GT GG GG GC	GAGCTTGGAAA GAGCTTGGAAA	ACATTATGAGT ACATTATGAGT	AACACCTGGG AACACCTGGG	ATAAATTCCG ATAAATTCCG	GAAAACGTGT GAAAACGTGT
Consensus #1 Majority	GCGATAGGAT GCGATAGGAT	TTGACATGAA TTGACATGAA	AGAGATTCGAC AGAGATTCGAC	CAGCACGTAT	CCGTGGACGC	CCTACGATGG	GAACACAGTG GAACACAGTG	TATACAACGC
	1490	1500	1510	1520	1530	1540	1550	1560
TCVbGenome.txt Nru_Forward	GCGAT AGGAT GCGAT AGGAT	TTGACATGAA TTGACATGAA	AGAGATTCGAC AGAGATTCGAC	CAGCACGTAT CAGCACGTAT	CCGTGGACGC CCGTGGACGC	CCTACGATGG CCTACGATGG	GAACACAGTG GAACACAGTG	TATACAACGC TATACAACGC
Consensus #1 Majority	G G							
TCVbGenome.txt	G							

TCVbGenome.txt Nru_Forward
TCVbGenome.txt T7Promoter_Reverse

Consensus #1

TCVbGenome.txt T7Promoter_Reverse

Consensus #1

TCVbGenome.txt T7Promoter_Reverse

Consensus #1

TCVbGenome.txt T7Promoter_Reverse

Consensus #1

TCVbGenome.txt T7Promoter Reverse

Consensus #1

TCVbGenome.txt T7Promoter_Reverse

GGCTTTAAC	TGTCCCGAG	TTGGCACAGC	FGCTAACTTG	GCAGTTGACC	AACAAGGGGGT	TGGGAGAGCCT
1570	1580	1590	1600	1610	1620	1630
GGCTTTAAC GGCTTTAAC	TGTCCCGAG TGTCCCGAG	TTGGCACAGC TTGGCACAGC	FGCTAACTTG FGCTAACTTG	GCAGTTGACC. GCAGTTGACC	AACAAGGGGGI AACAAGGGGGI	TGGGAGAGCCT TGGGAGAGCCT
CCGATGGCT	TTATCAAAT	ACCAAGTTGA	r GGTTGTCGC	ATGTCCGGAG	ATGTTAACACA	GCCTTGGGCAA
1640	1650	1660	1670	1680	1690	1700
CCGATGGCT CCGATGGCT	TTATCAAAT. TTATCAAAT.	ACCAAGTTGA ACCAAGTTGA	TGGTTGTCGCA TGGTTGTCGCA	ATGTCCGGAG ATGTCCGGAG	AT GT T ÀACACA AT GT T AACACA	AGCCTTGGGCAA AGCCTTGGGCAA
CTGCCTACT	GGCTTGCTC	TATCACCAAG	TACTTAATGA	AGGGAATCAA	ATGCAAATTAA	TCAACAATGGA
1710	1720	1730	1740	1750	1760	1770
CTGCCTACT CTGCCTACT	GGCTTGCTC GGCTTGCTC	TATCACCAAG TATCACCAAG	ΓΑСΤΤΑΑΤΘΑ. ΓΑСΤΤΑΑΤΘΑ.	AGGGAATCAA AGGGAATCAA	ATGCAAATTA ATGCAAATTA	ATCAACAATGGA ATCAACAATGGA
GACGATTGT	GTGCTGTTC	TTCGAAGCTG	ATGAAGTCGA	CAGGGTGCGC	GAAAGGCTGCA	TCATTGGATCG
1780	1790	1800	1810	1820	1830	1840
GACGATTGT GACGATTGT	GTGCTGTTC GTGCTGTTC	TTCGAAGCTG. TTCGAAGCTG.	ATGAAGTCGA ATGAAGTCGA	CAGGGT GCGC CAGGGT GCGC	GAAAGGCTGCA GAAAGGCTGCA	ATCATTGGATCG ATCATTGGATCG
ACTTTGGGT	TTCAATGCA	TAGCGGAAGA	ACCACAATAC	GAATTGGAGA	AAGTTGAATTI	TGCCAGATGTC
1850	1860	1870	1880	1890	1900	1910
ACTTTGGGT ACTTTGGGT	TTCAATGCA TTCAATGCA	TAGCGGAAGA. TAGCGGAAGA.	ACCACAATAC ACCACAATAC	GAATTGGAGA GAATTGGAGA	AAGTTGAATT1 AAGTTGAATT1	TGCCAGATGTC TGCCAGATGTC
CCCTATTT	CGATGGTGA	AGGGTGGGTC	ATGGTCAGAA	ACCCCCGTGT	GAGCCTCTCCA	AGGACAGCTAC
1920	1930	1940	1950	1960	1970	1980
CCCTATTTT CCCTATTTT	CGATGGTGA CGATGGTGA	AGGGTGGGTC. AGGGTGGGTC.	ATGGTCAGAA. ATGGTCAGAA.	ACCCCCGTGT	GAGCCTCTCCA GAGCCTCTCCA	AGGACAGCTAC AGGACAGCTAC
. GCACCACA	CAATGGGCG	AATGAGAAAG	ATGCAGCCAG	ATGGTTGGCT	GCCATCGGAGA	AGT GT GGCT T GG
1990	2000	2010	2020	2030	2040	2050
A <mark>GCACCACA</mark> TGCACCACA	CAATGGGCG CAATGGGCG	AATGAGAAAG. AATGAGAAAG.	ATGCAGCCAG. ATGCAGCCAG.	ATGGTTGGCT ATGGTTGGCT	GCCATCGGAGA GCCATCGGAGA	AGT GT GGCTT GG AGT GT GGCTT GG
CTATTGCAG	GTGGCGTAC	CAGTGTTACA	ATCATATTAT	ГСТТБССТБА	AGAGGAATTT	GGACCCCTGGC
2060	2070	2080	2090	2100	2110	2120
CTATTGCAC CTATTGCAC	GT GGCGT AC GT GGCGT AC	CAGTGTTACA. CAGTGTTACA.	ΑΤCΑΤΑΤΤΑΤ΄ ΑΤCΑΤΑΤΤΑΤ΄	FCTTGCCTGA FCTTGCCTGA	AGAGGAATTT1 AGAGGAATTT1	GGACCCCTGGC GGACCCCTGGC
CGGGGACTA	CAAGAAGAA	GATGCAAGAT	GTTTCCTTTG	ATAGTGGATT	CTACAGGTTAT	CCAAGAACGGG
2130	2140	2150	2160	2170	2180	2190
CGGGGACTA CGGGGACTA	CAAGAAGAA CAAGAAGAA	GAT GCAAGAT GAT GCAAGAT	ЭТТТССТТТБ. ЭТТТССТТТБ.	ATAGTGGATT ATAGTGGATT	CTACAGGTTA1 CTACAGGTTA1	CCAAGAACGGG CCAAGAACGGG
ATGAGGGGC	CAGCAAAGAC	GTGTCCCAAG	ATGCTAGGTT	CAGCTTTTAC	CGGGGGGTTCGC	GCTACACTCCAG
2200	2210	2220	2230	2240	2250	2260
AT GAGGGGC AT GAGGGGC	CAGCAAAGAC CAGCAAAGAC	GTGTCCCAAG. GTGTCCCAAG.	ATGCTAGGTT ATGCTAGGTT	CAGCTTTTAC CAGCTTTTAC	CGGGGGGTTCGC CGGGGGGTTCGC	GCTACACTCCAG GCTACACTCCAG
ACGAGCAGC	GAAGCGCTTG	AGGAGTACTA	CGACAACCT.	AACTGCTCT	GTGAGTGGGAG	CCCCACGGGATA
2270	2280	2290	2300	2310	2320	2330
ACGAGCAGC ACGAGCAGC	GAAGCGCTTG GAAGCGCTTG	AGGAGTACTA AGGAGTACTA	CGACAACCTG CGACAACCT <mark>C</mark>	C <mark>AACTGCTCT</mark> GAACTGCTCT	GT GAGT GGGAC GT GAGT GGGAC	CCCCACGGGATA CCCCACGGGATA
TAAAGAAGA	ACTTAGTGA	TAGATGGATC	CTGAACGAAT	ГСССТАСААС	ТСТСТАА	
2340	2350	2360	2370	2380	2390	
TAAAGAAGA TAAAGAAGA	ACTTAGTGA	TAGATGGATC TAGATGGATC	TGAACGAAT TGAACGAAT	ICCCTACAAC ICCCTACAAC	TCTCTAA TCTCTAA	

Consensus #1		MP L L I	HT L N'	TALAV	p88 GLLG	MUT Aryy	' Tran pevqt	slated	DYVO	HMKN	VVRS	VFQG	SGLVV	VSSD	ΓVGV
				10		20		30		4	0		50		60
TCVbGenome.txt SP6_FORWARD		MP L L I MP L L I	HTLN' HTLN'	T AL AV T AL AV	GLLG/ GLLG/	ARYY ARYY	P E VQT P E VQT	F L G L P F L G L P		H MK N V H MK N V	V R S V R S	VF QG VF QG	S GL V V S GL V V	VSSD1 VSSD1	F V G V F V G V
Consensus #1		RGTY	SNRG	QIGSS	LGCII	LAVP	DS GAD	IEIDL	DRLV	GTEEH	EATS	CLVE	AVGST	ADVPI	RRV
TCVbGenome.txt SP6_FORWARD		R G T Y: R G T Y:	S NR G S NR G	70 QI GS S QI GS S	LGCI I LGCI I	80 LAVP LAVP	DS GAD DS GAD	90 I EI DL I EI DL	, DRLV , DRLV	l GTEEI GTEEI	00 E A T S E A T S	CLVE. CLVE.	110 AVGSTA AVGSTA	ADVP I Advp i	120 RRRV RRRV
Consensus #1		RQKG	RFAM	HAVNA	AKLHI	FCGV	РКРТЕ	ANRLA	VSKV	VL VQY	CKER	HVVD	SHI RTI	VNT	ALPR
TCVbGenome.txt SP6_FORWARD		RQKG RQKG	R F A M R F A M	130 HAVNA HAVNA	AKLHI AKLHI	140 FCGV FCGV	Р К Р Т Е Р К Р Т Е	150 ANRLA ANRLA	VS KV VS KV	l VL VQYO VL VQYO	60 CKER CKER	HVVD HVVD	170 S HI R T I S HI R T I	VNT A	180 ALPR ALPR
Consensus #1		VFTP	DAED		DLHSV	VRAH	DHRNA	LAEAC	GKVRK	WWVNI	AMH	P MT G	RSWSRA	4 W	
TCVbGenome.txt SP6_FORWARD		VFTP VFTP	DAE DI DAE DI	I QVVL I QVVL	DLHSV DLHSV	VRAH VRAH	DHR NA DHR NA	LAEAC LAEAC	G K V R K G K V R K	2. WWV NI WWV NI	20 A MH A MH	P MT G P MT G	230 R S WS R A R S WS R A	A W A	
Consensus #1	ERLC	R L P	DDQ.	AISF	VR-	GCL	RELV	GRET	r q i s	RGE	NPA	MRV	FPLA	NPP	KVR
TCVbGenome.txt Nru_Forward	770 R R L C E R L C	780 R L P R L P	790 D D Q D D Q	800 A I S F A I S F	810 V R . V R .	820 G C L G C L	830 RELV RELV	840 GRET GRET	850 F Q I S F Q I S	860 RGE RGE	870 N P A N P A	880 M R V M R V	890 FPLA FPLA	900 N P P N P P	910 K V R K V R
Consensus #1	RIFH	ICG	MGN	GLDF	GVH	NNS	LNNL	RRGI	MER	VFY	VED	A Q K	QLKP	APQ	PIP
TCVbGenome.txt Nru_Forward	920 R I F H R I F H	930 I C G I C G	940 MGN MGN	950 GLDF GLDF	960 G V H G V H	970 N N S N N S	980 LNNL LNNL	990 R R G I R R G I	1000 MER MER	1010 VFY VFY	1020 V E D V E D	1030 A Q K A Q K	1040 Q L K P Q L K P	1050 A P Q A P Q	1060 P I P P I P
Consensus #1	GIFG	KLS	GIR	RRLV	RLA	G N H	TPVP	REKY	YPSF	YKG	RRA	TIY	QKAL	DSL	H D S
TCVbGenome.txt Nru_Forward	G I F G G I F G	K L S K L S	GIR GIR	R R L V R R L V	R L A R L A	G N H G N H	T P V P T P V P	REKY REKY	TISO Y P S F Y P S F	Y K G Y K G	R R A R R A	TIY TIY	Q K A L Q K A L	DSL DSL	HDS HDR
Consensus #1	TVSR	K D A	ELK	TFVK	AEK	INF	TAKK	DPAR	PRVI	QPR	D P R	YNI	EVGK	YLK	PYE
TCVbGenome.txt Nru_Forward	1220 TVSR PVSR	1230 K D A K D A	1240 E L K E L K	1250 TFVK TFVK	1260 A E K A E K	1270 I N F I N F	1280 T A K K T A K K	1290 DPAF DPAF	1300 P R V I P R V I	1310 Q P R Q P R	1320 D P R D P R	1330 Y N I Y N I	1340 EVGK EVGK	1350 Y L K Y L K	1360 PYE PYE
Consensus #1	HHLY	RAI	DAM	WGGP	TVL	KGY	DVGE	LGNI	MSN	TWD	KFR	ктс	AIGF	DMK	RFD
TCVbGenome.txt Nru_Forward	1370 HHLY HHLY	1380 R A I R A I	1390 D A M D A M	1400 WGGP WGGP	1410 TVL TVL	1420 K G Y K G Y	1430 DVGE DVGE	1440 L G N I L G N I	1450 M S N M S N	1460 T W D T W D	1470 KFR KFR	1480 K T C K T C	1490 AIGF AIGF	1500 D M K D M K	1510 R F D R F D
Consensus #1	<u>Q H V S</u> 1520	<u>V D A</u> 1530	L R W	<u>E H S V</u> 1550	<u>Y N A</u> 1560										
TCVbGenome.txt Nru Forward	Q H V S Q H V S	V D A V D A	L R W L R W	E H S V E H S V	Y N A Y N A										

Consensus #1	GFNC	PELA	AQL	LTWQ	LTN	KGV	GRA	SDGF	ΙΚΥ	QVD	GCRM	SGD	VNT	ALGN	CLL
	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700	1710
TCVbGenome.txt T7Promoter_Reverse	G F N C G F N C	PELA PELA	A Q L A Q L	L T WQ L T WQ	L T N L T N	K G V K G V	GRA GRA	S D G F S D G F	I K Y I K Y	Q V D Q V D	G C R M G C R M	S G D S G D	VNT. VNT.	A L G N A L G N	C L L C L L
Consensus #1	ACSI	ткүі	LMK	<u>бікс</u>	KLI	N N G	DDC	VLFF	EAD	EVD	RVRE	RLH	HWI	DFGF	QCI
	1720	1730	1740	1750	1760	1770	1780	1790	1800	1810	1820	1830	1840	1850	1860
TCVbGenome.txt T7Promoter_Reverse	ACSI ACSI	Т К Ү I Т К Ү I	L MK L MK	GIKC GIKC	K L I K L I	N N G N N G	DDC DDC	VLFF VLFF	E A D E A D	E V D E V D	R V R E R V R E	RLH RLH	H WI I H WI I	DFGF DFGF	Q C I Q C I
Consensus #1	AEEP	QYEI	LEK	VEFC	QMS	PIF	DGE	GWVN	<u>IVRN</u>	PRV	<u>s l s k</u>	DS Y	STT	Q WA N	EKD
	1870	1880	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
TCVbGenome.txt T7Promoter_Reverse	A E E P A E E P	Q Y E I Q Y E I	L E K L E K	VEFC VEFC	Q M S Q M S	PIF PIF	D G E D G E	G W V N G W V N	IVRN IVRN	P R V P R V	S L S K S L S K	DSY DSY	STT CTT	Q WA N Q WA N	E K D E K D
Consensus #1	AARW	LAAI	GE	CGLA	IAG	GVP	VLQ	SYYS	СЬК	RNF	GPLA	G D Y	ккк	MQDV	SFD
	2020	2030	2040	2050	2060	2070	2080	2090	2100	2110	2120	2130	2140	2150	2160
TCVbGenome.txt T7Promoter_Reverse	A A R W A A R W	LAAI LAAI	G E G E	C G L A C G L A	I A G I A G	G V P G V P	V L Q V L Q	S Y Y S S Y Y S	C L K C L K	R N F R N F	G P L A G P L A	G D Y G D Y	К К К К К К	MQDV MQDV	S F D S F D
Consensus #1	SGFY	RLSI	KNG	MRGS	K D V	SQD	ARF	SFYR	GFG	YTP	DEQE	ALE	ЕҮҮ	DNLE	LLC
	2170	2180	2190	2200	2210	2220	2230	2240	2250	2260	2270	2280	2290	2300	2310
TCVbGenome.txt T7Promoter_Reverse	SGFY SGFY	RLSI RLSI	K N G K N G	MRGS MRGS	K D V K D V	SQD SQD	ARF ARF	S F Y F S F Y F	l G F G l G F G	Y T P Y T P	D E Q E D E Q E	ALE ALE	E Y Y E Y Y	DNLQ DNLE	L L C L L C
Consensus #1	EWDP	TGY	КЕЕ	LSDR	WIL	NEF	РТТ	L -							
	2320	2330	2340	2350	2360	2370	2380	2390							
TCVbGenome.txt T7Promoter_Reverse	E WD P E WD P	T G Y I T G Y I	K E E K E E	L S D R L S D R	WI L WI L	NEF NEF	PTT PTT	L. L.							

Consensus #1	АТ G G A T C C T	р8 Sequ баасбаатт	IENCE C C C T A C A A C T	СТСТААGСGА	C
	2360	2370	2380	2390	2400
TCVbGenome.txt SP6_Forward	A	G A A C G A A T T G A A C G A A T T	C C C T A C A A C T C C C T A C A A C T	C T C T A A G C G A C T C T A A G C G A	C A G C G A C G C A A C C A G C G A C G C A A C
Consensus #1	A G G A A A A C G	GAAGAAAGG	CGGAGAGAAA	A G T G C G A A G A	A G A G A T T G G T A G
TCVbGenome.txt SP6_Forward	A G G A A A A C G A G G A A A A A C G	G A A G A A A G G G A A G A A A G G G A A G A A A G G	I C G G A G A G A A A C G G A G A G A A A	I A G T G C G A A G A A G T G C G A A G A	I A G A G A T T G G T A G A G A G A T T G G T A G
Consensus #1	CTAGCCACG	CGGCTAGCT	CTGTTTTAAA	CAAGAAAAGA	AATGAAGGTTCT
TCVbGenome.txt SP6_Forward	2460 C T A G C C A C G C T A G C C A C G	C G G C T A G C T C G G C T A G C T C G G C T A G C T	2480 C T G T T T T A A A C T G T T T T A A A	2490 C A A G A A A A G A C A A G A A A A A G A	2500 A A T G A A G G T T C T A A T G A A G G T T C T
Consensus #1	<u>GCTAGTCAC</u> 2510	GGGGGGTACT 2520	T G G G T T A T T G	TTGCTGATAA 2540	AGTGGAAGTCTC 2550
TCVbGenome.txt SP6_Forward	G C T A G T C A C G C T A G T C A C	G G G G G T A C T ' G G G G G G T A C T '	T G G G T T A T T G T G G G T T A T T G	T T G C T G A T A A T T G C T G A T A A	A G T G G A A G T C T C A G T G G A A G T C T C
Consensus #1	AATCAACTT 2560	CAACTTCTA 2570	ATCAGAAATG 2580	T C A G T G C C C G 2590	A C G T C C C C G T G G 2600
TCVbGenome.txt SP6_Forward	A A T C A A C T T A A T C A A C T T	С А А С Т Т С Т А . С А А С Т Т С Т А .	A T C A G A A A T G A T C A G A A A T G	T C A G T G C C C G T C A G T G C C C G	A C G T C C C C G T G G A C G T C C C C G T G G
Consensus #1	GTAATATAT 2610	<u>GCTTTCTAC</u>	A A C T C T C T C T C T C T C T C T C T	CACTGGTCCT	CCTACTTTGTCA 2650
TCVbGenome.txt SP6_Forward	G T A A T A T A T G T A A T A T A T A T	GCTTTCTAC. GCTTTCTAC.	A A C T C T C T C T C T C T C T C T C T	C A C T G G T C C T C A C T G G T C C T	C C T A C T T T G T C A C C T A C T T T G T C A
Consensus #1	TCTGATTCC 2660	<u>T G A A A T C A A</u> 2670	ACCGATTCAC 2680	A C A T C C T A C A 2690	A C A C A C A C G A C T 2700
TCVbGenome.txt SP6_Forward	T	Т <u>G A A A T C A A</u> . Т <u>G A A A T C A A</u> .	A C C G A T T C A C . A C C G A T T C A C .	A C A T C C T A C A A C A T C C T A C A	A C A C A C A C G A C T A C A C A C A C G A C T
Consensus #1	CATCGAAGC	AGCAACACA	TAAGCATCAA	CACTGGAAAT	GGAAAATGA
	2710	2720	2730	2740	2750
TCVbGenome.txt SP6_Forward	C A T C G A A G C C A T C G A A G C	A G C A A C A C A ′ A G C A A C A C A ′	T A A G C A T C A A (T A A G C A T C A A (C A C T G G	G G A A A A T G A G G A A A A T G A

p8 Translated

Consensus #1	MDP	ERII	PYNS	LSD	SDAT	GKR	K K G	GEKS	A K K	RLV	ASH	AAS S	VLN	KKR	NEGS
	2360	2370	2380	2390	2400	2410	2420	2430	2440	2450	2460	2470	2480	2490	2500
TCVbGenome.txt	MDP	ERII	P Y N S	LSD	S D A T	GKR	KKG	GEKS	AKK	RLV	ASH	AAS S	VLN	KKR	NEGS
SP6_Forward	MDP 1	ERII	P Y N S	LSD	S D A 1	GKR	KKG	GEKS	A K K	RLV	AS H	AAS S	VLN	KKR	N E G S
Consensus #1	ASH	GGT	WVI V	A D K	VEVS	5 I N F	NF -	$S \in MS$	VPD	VPV	G N I	CFLQ	L S L	TGP	PTLS
	2510	2520	2520	25.40	25.50	2560	2570	2590	2500	2,000	2(10	2(20)	2(20	2(40	0(50
	2510	2520	2530	2540	2550	2560	2570	2580	2590	2600	2610	2620	2630	2640	2650
TCVbGenome.txt	ASH	GGT	WVI V	A D K	VEVS	SINF	NF.	SEMS	V P D	VPV	GNI	CFLQ	LSL	TGP	PTLS
SP6_Forward	ASH	G G T '	WVI V	A D K	VEV S	SINF	NF.	SEMS	V P D	VPV	GNI	CFLQ	LSL	TGP	PTLS
_															
Consensus #1	SDS-	- NQ	ГDSH	ILQ	HTRI	IEA	АТН	КНQН	WK W	ΚMΝ					
	-														
	2660	2670	2680	2690	2700	2710	2720	2730	2740	2750					
TCVbGenome.txt	S D S	NQ	T D S H	ILQ	HTRI	LIEA	ATH	KHQH	WKW	KMN					
SP6_Forward	SDS.	NQ	Г <mark>D</mark> S Н	ILQ	H T R I	LIEA	АТН	кнүн	WK W	KMN					

p9MUT Sequence

Consensus #1	ATGAAGGTT	CTGCTAGTC	ACGGGGGTACI	T T G G G T T A T T G	GTTGCTGATAAA
	2500	2510	2520	2530	2540
TCVbGenome.txt SP6_Forward	AT GAAGGT T AT GAAGGT T	CTGCTAGTCA CTGCTAGTCA	ACGGGGGGTACI ACGGGGGGTACI	F T G G G T T A T T C F T G G G T T A T T C	GTTGCTGATAAA GTTGCTGATAAA
Consensus #1	GTGGAAGTC	TCAATCAACT	TTCAACTTCT	AATCAGAAAT	GTCAGTGCCCGA
	2550	2560	2570	2580	2590
TCVbGenome.txt SP6_Forward	GT GGAAGT C GT GGAAGT C	T C A A T C A A C T T C A A T C A A C T	Г Т С А А С Т Т С Т / Г <mark>С С А А С Т Т С Т /</mark>	А А Т С А G A A A T (А А Т С А <mark>G A A A T (</mark>	GT C A GT G C C C G A GT C A GT G C C C G A
Consensus #1	CGTCCCCGT	GGGTAGTATA	ATGCTTTCTA	CAACTCTCTC	<u>CACTGGTCCTC</u>
	2600	2610	2620	2630	2640
TCVbGenome.txt SP6_Forward	CGTCCCCGT CGTCCCCGT	GGGT A <mark>A T A T A</mark> GGGT A GT A T A	ATGCTTTCTAC ATGCTTTCTAC	CAACTCTCTCT CAACTCTCTCTCT	CACTGGTCCTC CACTGGTCCTC
Consensus #1	CTACTTTGT	CATCTGATT	CCTGAAATCAA	ACCGATTCA	CACATCCTACAA
	2650	2660	2670	2680	2690
TCVbGenome.txt SP6_Forward	CTACTTTGT CTACTTTGT	CATCTGATTC CATCTGATTC	CCTGAAATCA/ CCTGAAATCA/	ACCGATTCA ACCGATTCA	CACATCCTACAA CACATCCTACAA
Consensus #1	CACACACGA	CTCATCGAA	GCAGCAACAC	ATAAGCATCAA	ACACTGGAAATG
	2700	2710	2720	2730	2740
TCVbGenome.txt SP6_Forward	CACACACGA CACACACGA	СТСАТСБАА СТСАТСБАА СТСАТСБАА	GCAGCAACAC/ GCAGCAACAC/	ATAAGCATCA ATAAGCATCA	ACACTGGAAATG ACACTGGAAATG
Consensus #1	GAAAATGA 2750				
TCVbGenome.txt SP6_Forward	GAAAAT GA GAAAAT GA				
		p9MUT Tr	anslated		

Consensus #1	MKVL	LVT	GVL	GLL	LLII	ĸwĸs	QST	STSN	Q К С	QCPT	r s p v	V V V Y	AFYN	SLSI	L V L
		-		1		1		1	1		1		l		
	2500	2510	2520	2530	2540	2550	2560	2570	2580	2590	2600	2610	2620	2630	2640
									I				I		
TCVbGenome.txt	MKVL	LVT	GVL	GLL	LLI	KWKS	QST	STSN	QKC	QCPI	FSPV	V V I Y	AFYN	ISLSI	L V L
SP6_Forward	MKVL	LVT	GVL	GLL	LLI	KWKS	QST	P T S N	ОКС	Q C P 1	r s p v	VVVY	AFYN	ISLSI	V L
Consensus #1	LLCH	ILI P	EIK	РІН	TSYN	ΝΤΗΟ	S S K	QQHI	S I N	TGNO	ЭК-				
		-				1	1	-	1						
	2650	2660	2670	2680	2690	2700	2710	2720	2730	2740	2750				
TCVbGenome.txt	LLCH	LIP	EIK	PIH	TSYN	NTHD	S S K	QQHI	SIN	TGNO	<mark>ЭК</mark> .				
SP6_Forward	LLCH	ILI P	EIK	РІН	TSYN	NTHD	S S K	QQHI	S I N	TGNO	<mark>ЭК</mark> .				

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1 TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1 TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

p38 Sequence

G G

AAGTGGCAGAAGAAGGGC

		AGTCCGG	2770	2780	TGGCGC	2790	G <mark>G G </mark> C G /		GTGGCA	2810
				NANANG	ATTTAT	ANNCA	т <mark>G G</mark> G C (GATAA	G T G G C A	GAAGAA
TGGTCAACC	CTAACCAG	CAGACAG	AACAGAG	cocco	CGCAGC	GATGG	GGATCA	AGCT	стстсс	TGTGGC
2820	2830	2840	28	50	2860		2870		2880	2
T G G T C A A C C T G G T C A A C C)	C A G A C A G A C A G A C A G A	A A C A G A (A A C A G A (CCGCCCG CCGCCCG	C G C A G C C G C A G C	G A T G G G A T G G	G G A T C / G G A T C /	A G C T (A G C T (стстсс стстсс	T G T G G C T G T G G C
	EA A A GT G A C	терестр	GIGCIC							GCCTCG
2900	2910	0	2920	2930		2940	GCGAGG	2950	CALLLA	2960
C C T G T G C A G C C T G T G C A G	GAAAGT GAC GAAAGT GAC	Т С G G C T G / Т С G G C T G /	A GT G C T C G	CGGTGGC	C C T T G C C C T T G C	CTACC	G C G A G (G C G A G (GTTTC GTTTC	С А С С С А С А С С С А	<u>сстсс</u> сстсс
								<mark>C</mark>	САСССА	GCCTCG
TCTACTGCC	AGGGACGG	CATAACCA	AGAAGCG	GTTCTGA	ACTGAT	CACAA	CCTTGA	AGAA	GAACAC	TGACAC
T C T A C T G C C	AGGGACGG	CATAACC	A G A A G C G G	GTTCTGA	ACTGAT	САСАА	CCTTG/	AGAA	GAACAC	Т G A C A C
T C T A C T G C C T C T A C T G C C)	CATAACCA CATAACCA	A G A A G C G (A G A A G C G (G Т Т С Т G А G Т Т С Т G А	A C T G A T A C T G A T	C A C A A C A C A A	C	A	G A A C A C G A A C A C	T G A C A C T G A C A C
CCTAAGTAC	ACCACAGE	төтөстт		GCGAACC	CGGAAC	ATTCA	ACCAGO	TCAT	TAAGGA	<u>GGCGGC</u>
3050	306	0 TGTGCTT	3070	3080	CGGAAC	3090	ACCAG	3100	ТААССА	3110
CCTAAGTAC	ACCACAGC	TGTGCTT	ACCCAA ACCCAA	G C G A A C C	CGGAAC	ATTCA	ACCAGO	CTCAT	T A A G G A T A A G G A	GGCGGC
TATGAAAA	TACCGATT	CACGTCA	CTCAGATI	TAGGTA	стсссс	CATGA	GCCCTI	CAAC	CACCGG	AGGCAA
3120	3130	3140	31	50	3160		3170		3180	3
Т А Т G А А А А А Т А Т G А А А А А	. Т <u>А С С G А Т Т</u> А Т А С С G А Т Т	C A C G T C	C T C A G A T I C T C A G A T I	T A G G T A T T A G G T A	стсссс стсссс	C A T G A C A T G A	G C C C T 1 G C C C T 1	CAAC CAAC	C A C G G	A G G C A A A G G C A A
ΤΑΤ GΑΑΑΑΑ	TACCGATT	CACGTCA	C T C A G A T 1	TAGGTA	стсссс	CATGA	<u> </u>	CAAC	C A C C G G	AGGCAA
GCTCTGGCA	TTCGACCG	AGATGCA	GCCAAACO	2220	CAACGA	2240	СТТССС	2250	CAACAT	AGAGGG
GCTCTGGCA	TTCGACCG	A G A T G C A G	GCCAAAC(T C C G C C	CAACGA	CCTCG	сттссс		СААСАТ	A G A G G G
G C T C T G G C A G C T C T G G C A	L T T C G A C C G A T T C G A C C G	A G A T G C A G A G A T G C A G	G C C A A A C (G C C A A A C (C C C C G C C C C C C G C C	C A A C G A C A A C G A	. C C T C G . C C T C G	С Т Т С С (С Т Т С С (СТСТА СТСТА	С А А С А Т С А А С А Т	AGAGGG AGAGGG
GTATCTAGC	сотоссто	GACAGGG	TTATT	GACCGT	CCCAAC	AGATT	стасто	GACCG	CTTTGT	G G C G G A
	3280	3290	33		3310	AGATT	3320	ACCG	3330	GGCGGA
GTATCTAGC	GTGCCCTG	GACAGGG	TTATTT	T G A C C G T	CCCAAC	AGATT	С Т А С Т (С Т А С Т (GACCG	СТТТ БТ СТТТБТ	GGCGGA
ATCAGCGAT	CCAAAGCT	TGTCGAT	TTCGGCA	А G C T C A T	CATGGC	САССТ	ACGGC	CAAGG	AGCCAA	TGATGC
3350	336	0	3370	3380		3390		3400		3410
A T C A G C G A T A T C A G C G A 1	C C A A A G C T C C A A A G C T	T G T C G A T ' T G T C G A T '	T T C G G C A A T T C G G C A A	A G C T C A T A G C T C A T	C A T G G C C A T G G C	C A C C T C A C C T	A C G G C (A C G G C (CAAGG. CAAGG.	A G C C A A A G C C A A	T G A T G C T G A T G C
ATCAGCGAT	CCAAAGCT	TGTCGAT	F T C G G C A /	АСТСАТ	CATGGC	CACCT	A C G G C (CAAGG.	AGCCAA	TGATGC
3420	3430	3440	34	50	<u>CAAGAA</u> 3460	CAGAA	3470	CAAC	3480	GCCCA
CAACTCGGT	GAAGTGCG	AGTCGAG	TACACCG	I I G C A G C T	CAAGAA	CAGAA	CTGGCT			CGCCCA
CAACTCGGT	GAAGTGCG	AGTCGAG	FACACCG	GCAGCT	CAAGAA	CAGAA	CTGGCI	ГСААС	CAGCGA	CGCCCA
GGGGACTTC	GCAGGTGT	TAAGGAC	GGACCCA	GGCTGGT	CTCATG	GTCCA	AGACC	AGGG	GACAGC	TGGGTG
	3510	0 <u> </u>	3520 G G A C C C A G	3530	TTCATG	3540	AGACC	3550	GACAGC	3560 T G G G T G
G G G G A C T T C G G G G A C T T C	GCAGGTGT GCAGGTGT	T A A G G A C O T A A G G A C O	G G A C C C A G G G A C C C A G	G G C T G G T G G C T G G T	CTCATG	GTCCA	A G A C C / A G A C C /	A G G G A G G G	G A C A G C G A C A G C	T G G G T G T G G G T G
	CATTTCT	CGGAACC	GGAAACT	тстсатт	<u>GACAT</u> T	<u>G T T C </u> T	ACGAG	AGGC	<u>G C C G G</u> T	<u>стсб</u> б
CACGATTGT		3590	36	00	3610		3620		3630	2
<u>C A C G A T T G T</u> 3570	3580			гстсбтт	GACATT	GTTCT	ACGAGA	AGGC	GCCGGT	CTCGGG
<u>C A C G A T T G 1</u> 3570 C A C G A T T G T C A C G A T T G T	3580 <u>CATTTTCT</u> CATTTTCT	C	G G A A A C T 1 N							
<u>CACGATTG1</u> 3570 CACGATTG1 CACGATTG1 CACGATTG1	3580 CATTTTCT CATTTTCT CATTTTCT	C G G A A C C C C G G A A C C C C G G A A C C C	G G A A A C T 1 N G G A A A C T 1	гстсдтт	GACATT	GTTCT	A C G A G A	AGGC	GCCGGT	CTCGGC
<u>CACGATTG1</u> 3570 <u>CACGATTG1</u> CACGATTG1 CACGATTG1 <u>CACGATTG1</u> <u>GAAAACGCA</u> 3650	3580 CATTTTCT CATTTTCT CATTTTCT AGACGCCTC 3660	C G G A A C C C C G G A A C C I C G G A A C C C T G A C T T C T	3 G A A A C T ⁷ N 3 G A A A C T ⁷ T C G G T C C ⁷ 3670	F C T C G T T F G G G A G A 3680	<mark>G A C A T T</mark> A G C C G C	GTTCT AGCAG 3690	<mark>A C G A G 7</mark> G T A G T (A G G C G G T C C A . 3700	<mark>G C C G G T</mark> A T G G G C	СТСGGC АGGAGT 3710
<u>C A C G A T T G 1</u> 3570 <u>C A C G A T T G T</u> C A C G A T T G T C A C G A T T G T G A A A A C G C A 3650 <u>G A A A A C G C A</u>	3580 CATTTCT CATTTCT CATTTCT CATTTCT GACGCCTC GACGCCTC	CGGAACC CGGAACC CGGAACC TGACTTC 0 TGACTTC	3 G A A A C T ⁷ N 3 G A A A C T ⁷ F C G G T C C ⁷ 3670 F C G G T C C ⁷	F C T C G T T F G G G A G A 3680 F G G G A G A	G A C A T T A G C C G C A G C C G C	G T T C T A G C A G 3690 A G C A G	<mark>A C G A G 7</mark> G T A G T (G T A G T (AAGGC GTCCA 3700 GTCCA	<mark>G C C G G T</mark> A T G G G C A T G G G C	C T C G G G A G G A G T 3710 A G G A G T
<u>C A C G A T T G 1</u> 3570 C A C G A T T G T C A C G A T T G T C A C G A T T G T G A A A A C G C A G A A A A C G C A G A A A A C G C A	3580 CCATTTTCT CCATTTTCT CATTTTCT CATTTTCT CATCTTCT CATCTCC CACCCCCCCC	CGGAACC CGGAACC CGGAACC TGACTTC 0 TGACTTC TGACTTC	3 G A A A C T N F C G G T C C 3670 F C G G T C C	F C T C G T T F G G G A G A 3680 F G G G A G A F G G G A G A	G A C A T T A G C C G C A G C C G C A G C C G C	G T T C T A G C A G 3690 A G C A G	A C G A G / G T A G T (G T A G T (G T A G T (A A G G C A G T C C A T 3700 G T C C A G T C C A	G C C G G T A T G G G C A T G G G C A T G G G C	CTCGGC AGGAGT 3710 AGGAGT

GTAGCAGAAAGGGGACAAGGCGNA<mark>AAA</mark>- - T<mark>GTCACA</mark>NTN<mark>GA</mark>NNCNA

TCVbGenome.txt CP_Forward CP_Reverse Consensus #1

<u>.</u>

ATTTAG

TCVbGenome.txt CP_Forward CP_Reverse

71

Consensus #1	pso i fansialeu
	2750 2760 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
TCVbGenome.tx	xtMENDPRVRKFASDGAQWAI <mark>KWQKKGWSTLTSRQKQTARAAMGIKLSPVAQPVQKV</mark>
CP_Forward	XXXXXXXXXXKRFI NMGD <mark>K WQKKGWSTLTSRQKQTARAAMGI KLSP VAQP VQKV</mark>
CP_Reverse	****
Consensus #1	T P I S A P V A I A V P E V S T A P D G I T P S G S E I I T T I K K N T D T E P K V T T A VI N P
	2910 2920 2930 2940 2950 2960 2970 2980 2990 3000 3010 3020 3030 3040 3050 3060 307
TCVbGenome.tz	AT R L S A P VAL A Y R E V S T Q P R V S T A R D G I T R S G S E L I T T L K K N T D T E P K Y T T A V L N P
CP_Forward	TRLS AP VALAYREVS TOPRVS TARDGI TRS GSELI TTLKKNTDTEPKYTTAVLNP
CP_Reverse	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
Consensus #1	<u>SEPGTFNQLIKEAAQYEKYRFTSLRFRYSPMSPSTTGGKVALAFDRDAAKPPPND</u>
	3080 3090 3100 3110 3120 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230
TCVbGenome ty	TSEPGTENOLI KEAAOYEKYRETSI. RERYSPMSPSTTGGKVAL AFDRDAAKPPPND
CP Forward	S E P G T F N Q L I KE A A O Y E K Y R F T S L R F R Y S P MS P S T T G G K V A L A F D R D A A K P P N D
CP_Reverse	S E P G T F NQLI KE A AQYE KYR F T S L R F R Y S P MS P S T T G G K V A L A F D R D A A K P P N D
Consensus #1	LASLYNIEGCVSSVPWIGFILTVPIDSTDRFVADGISDPKLVDFGKLIMATYGQG
	3240 3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360 3370 3380 3390 340
TCVbGenome.tz	TLASLYNI EGCVSSVPWTGFILTVPTDSTDRFVADGISDPKLVDFGKLIMATYGQG
CP_Forward	LASLYNIEGCVSSVPWTGFILTVPTDSTDRFVADGISDPKLVDFGKLIMATYGQG
CP_Reverse	L A S L Y NI E G C V S S V P WT G F I L T V P T D S T D R F V A D G I S D P K L V D F G K L I MAT Y G Q G
Conconque #1	AND A A OL GEVENEVT VOLKNET GETE DA OL GDE A GVEDGERL VS WS ETEGTA GWE
Collselisus #1	ANDAAQLOEVKVETTVQLKNKTOSTSDAQTODFAOVKDOFKLVSWSKTKOTAOWE
	3410 3420 3430 3440 3450 3460 3470 3480 3490 3500 3510 3520 3530 3540 3550 3560
TCVbGenome.tz	ANDAAQL GE VR VE YT VQL KNRT GS TSDAQI GDF AG VKDGP RL VS WS KT KGT AG WE
CP_Forward	ANDAAQL GE VR VE YT VQL KNR T GS T S DAQI GDF AG VKDGP R L V S WS K T K G T AG WE
CP_Reverse	ANDAAQL GE VR VE YT VQL KNR T GS T S DAQI GDF AG VKDGP R L V S WS K T K G T A G WE
Consensus #1	H D C H F L G T G N F S L T L F Y E K A P V S G L E N A D A S D F S V L G E A A A G S V Q W A G V K V A E R G
	3570 3580 3590 3600 3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720 373
TCVbGenome.tz	THDCHFLGTGNFSLTLFYEKAPVSGLENADASDFSVLGEAAAGSVQWAGVKVAERG
CP_Forward	HDCHFLGT
CP_Reverse	HDCHFLGTGNFSLTLFYEKAPVSGLENADASDFSVLGEAAAGSVQWAGVKVAERG
Consensus #1	Q G X K X V T X E X
	3740 3750 3760 3770 3780 3790
TCVbGenome.tx	K <mark>tQ G V K MV T</mark> T <mark>E</mark> E Q P K G K WQ A L R I .
CP_Forward	
CP_Reverse	QGEK XVIIET

p38 Translated

Full-length TCV p28 ORF

Consensus #1	ATGC	стсттс	TACAC	ACAC	ΓСΑΑС	CAGCO	i C T C G C	AGTGG	GACTO	CTAG	GAGCC	AGGTA	CTACCC
		70		80		90		100		110		120	
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	ATGCO ATGCO ATGCO	СТСТТС СТСТТС СТСТТС	СТ А С А С СТ А С А С СТ А С А С	ACAC ACAC ACAC	Г С А А С А Г С А А С А Г С А А С А	ACÁGCO ACAGCO ACAGCO	ICTCGC ICTCGC ICTCGC	CAGTGG CAGTGG CAGTGG	GACTO GACTO GACTO	CCTAGO CCTAGO CCTAGO	GAGCCA GAGCCA GAGCCA	A G G T A A G G T A A G G T A	CTACCC CTACCC CTACCC
Consensus #1	TGAG	GTTCAA	ACCTT	CTTG	задсто	GCCTGA	CTACO	GT GGGT	CACAT	GAAGA	ATGT	AGTAC	GGTCTG
	130		140		150		160		170		180		190
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	T G A G O T G A G O T G A G O	G Т Т С А А G Т Т С А А G Т Т С А А	ACCTT ACCTT ACCTT	C T T G (C T T G (C T T G (366CT (366CT (366CT (6 C C T G A 6 C C T G A 6 C C T G A	CTACC CTACC CTACC) T G G G T) T G G G T) T G G G T	CACAI CACAI CACAI	[G A A G	A T G T A A T G T A A T G T A	A G T A C A G T A C A G T A C	G G T C T G G G T C T G G G T C T G
Consensus #1	<u>T T T T C</u>	200	GATCTC	210	AGTAGI	220	ССТСС	230	AGTCC	<u>240</u>	CAGGG	<u>GGACG</u> 250	TATAGT
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	T T T T C T T T T C T T T T C	CCAGGO CCAGGO CCAGGO	Б А Т С Т С Б А Т С Т С Б А Т С Т С Б А Т С Т С	GGCT / GGCT / GGCT /	4 G T A G 1 4 G T A G 1 4 G T A G 1	AGTG1 AGTG1 AGTG1	CCTCC CCTCC CCTCC	CGACAC CGACAC CGACAC	AGTCC AGTCC AGTCC	GGT GT C GGT GT C GGT GT C	CAGGG CAGGG CAGGGG	G G A C G G G A C G G G A C G	T A T A G T T A T A G T T A T A G T
Consensus #1	<u>AATAO</u> 260	GAGGTC	270	GGTA	<u>3 T A G T C</u> 280	CTCGGC	<u>T G T A 1</u> 290	ACTAG	CCGT1 300	CCGGA	ATAGCO 310	GGGGC	GGATAT 320
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	AATA AATA AATA AATA	GAGGT (GAGGT (GAGGT (CAGATA CAGATA CAGATA	.GGTA .GGTA .GGTA	GTAGTO GTAGTO GTAGTO	CTCGGC CTCGGC CTCGGC	T GT A 1 T GT A 1 T GT A 1	` A C T A G ` A C T A G ` A C T A G	CCGT1 CCGT1 CCGT1	C C G G A C C G G A C C G G A	ATAGCO ATAGCO ATAGCO	6666C 6666C 6666C	GGATAT GGATAT GGATAT
Consensus #1	AGAA	ATAGAC	CTAGA	TAGG	Γ Τ G G T A	AGGAAC	GGAAG	GAGGAA 1 360	GCCAC	370	GTTT	GGTGG	AGGCGG
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	AGAA AGAA AGAA	ATAGAC ATAGAC ATAGAC	C T A G A C T A G A C T A G A	TAGG TAGG TAGG	Г Т G G T A Г Т G G T A Г Т G G T A	GGAAC GGAAC GGAAC	GGAAC GGAAC GGAAC	JAGGAA JAGGAA JAGGAA	GCCAC GCCAC GCCAC	CATCCI CATCCI CATCCI	GTTT GTTT GTTT	GGTGG GGTGG GGTGG	AGGCGG AGGCGG AGGCGG
Consensus #1	TAGG	ΓΑGΤΑC	CGCAG	AT GT (CCCCAC 410	GGAGGA	GAGT1	CGTCA	AAAGO	GGGCGC	GTTTG	CTATG	CATGCC 450
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	TAGG TAGG TAGG	Г А G T A C Г А G T A C Г А G T A C	CGCAC CGCAC CGCAC	ATGTO ATGTO ATGTO		}	GAGT1 GAGT1 GAGT1	` C G T C A ` C G T C A ` C G T C A	AAAGO AAAGO AAAGO	366C60 366C60 366C60	GTTTG GTTTG GTTTG	С Т А Т G С Т А Т G С Т А Т G	CATGCC CATGCC CATGCC
Consensus #1	GTCA	ACGCAC 460	CAAAG	470	ACTTTI	<u>GTGGC</u> 480	GTCCC	2 A A A A C 490	CCACI	GAAGC 500	CGAAT	<u>CGACT</u> 510	AGCGGT
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	G T C A A G T C A A G T C A A	ACGCAC ACGCAC ACGCAC	GCAAAG GCAAAG GCAAAG	CTGC CTGC CTGC	А С Т Т Т Т А С Т Т Т Т А С Т Т Т Т	GT GGC GT GGC GT GGC	GTCCC GTCCC GTCCC	CAAAAC CAAAAC CAAAAC	CCAC1 CCAC1 CCAC1	FGAAGO FGAAGO FGAAGO	CGAAT (CGAAT (CGAAT (CGACT CGACT CGACT	AGCGGT AGCGGT AGCGGT
Consensus #1	CTCA	AAATGO	GCTTGT	CCAA	TACTGO	CAAAGA	GAGAG	CATGTC	GTAG	ACAGCO	CACAT	CAGAA	CGATAG
	520		530										
TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse	CTCA				540		550		560		570		580
	CTCA	A A A T G C A A A T G C A A A T G C	CTTGT CTTGT CTTGT	CCAA CCAA CCAA	540 F A C T G C F A C T G C F A C T G C	CAAAGA CAAAGA CAAAGA	550 GAGAC GAGAC GAGAC	CATGTC CATGTC CATGTC	560 GTAGA GTAGA GTAGA	ACAGCO ACAGCO ACAGCO	570 CACATO CACATO CACATO	C A G A A C A G A A C A G A A	580 CGATAG CGATAG CGATAG
Consensus #1	CTCAA	A A A T G C A A A T G C A A A T G C T A C G G C 590	CTTGT CTTGT CTTGT CTTGT	CCAA CCAA CCAA CCAA CCTAG	540 FACTGO FACTGO FACTGO AGTGT	CAAAGA CAAAGA CAAAGA CCACGC 610	550 GAGAC GAGAC GAGAC	CATGTC CATGTC CATGTC CGCGGA 620	560 GTAGA GTAGA GTAGA	$\frac{ACAGCO}{ACAGCO}$	570 CACATO CACATO CACATO	CAGAA CAGAA CAGAA TGCTG 640	580 CGATAG CGATAG CGATAG GATTTG
Consensus #1 TCVbGenome.txt TCVBtex_Forward p288ev_Feverse	<u>ТСАА</u> <u>ТСАА</u> <u>ТСАА</u> <u>ТСАА</u>	A A A T GC A A A T GC A A A T GC T A C G GC 590 T A C G GC T A C G GC T A C G GC		CCAA CCAA CCAA CCAA CCAA 600 CTAG	540 FACTGC FACTGC ACTGC AGTGT1 AGTGT1	CAAAG/ CAAAG/ CAAAG/ CACGC 610 CACGC CACGC	550 GAGAC GAGAC CTGAC	CATGTC CATGTC CATGTC CGCGGA 620 CGCGGA	560 GTAGZ GTAGZ GTAGZ AGACZ	ACAGCO ACAGCO ACAGCO ATTCAC 630 ATTCAC ATTCAC	570 CACATO CACATO CACATO GGTCG GGTCG	CAGAA CAGAA CAGAA TGCTG 	580 CGATAG CGATAG CGATAG GATTTG GATTTG GATTTG GATTTG
Consensus #1 TCVbGenome.txt TCVbIex_Forward p28Rev_Reverse Consensus #1	<u>ТСАА</u> ТСАА ТСАА ТСАА САСА	A A A T G G A A A T G G A A A T G G T A C G G G T A C G G G T A C G G G G T G T A A		CCAA CCAA CCAA CCAA CCAA CCAA 600 CTAG CTAG	540 FACTGC FACTGC AGTGTT AGTGTT AGTGTT	CAAAGA CAAAGA CAAAGA CCACGC CACGC CCACGC CCACGC	550 GAGAC GAGAC CTGAC CTGAC CTGAC CTGAC	CATGTC CATGTC CATGTC CGCGGA CGCGGA CGCGGA CGCGGA	560 GTAG/ GTAG/ GTAG/ AGAC/ AGAC/ AGAC/	ACAGCO ACAGCO ACAGCO 630 ATTCAC ATTCAC ATTCAC	570 CACATO CACATO CACATO GGTCG GGTCG GGTCG	CAGAA CAGAA CAGAA TGCTG 640 TGCTG TGCTG TGCTG	580 CGATAG CGATAG CGATAG GATTTG GATTTG GATTTG GATTTG GATTTG
Consensus #1 TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse Consensus #1	<u>ТСАА</u> <u>ТСАА</u> <u>ТСАА</u> <u>ТСАА</u> <u>САСА</u> <u>650</u>	A A A T GC A A A T GC T A C G G C 590 T A C G G C T A C G G C T A C G G C G T G T A A	CTTGT CTTGT CTTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CGAGCA 660	CCAA CCAA CCAA CCAA CCAA CCAA CCAA CCA	540 FACTGC FACTGC AGTGTTT AGTGTTT AGTGTTT AGTGTTT ACCACC 670	CAAAGA CAAAGA CAAAGA CACGC CACGC CACGC CACGC CACGC	550 GAGAC GAGAC CTGAC CTGAC CTGAC CTGAC CTGAC	ATGTC ATGTC ATGTC CGCGGA 620 CGCGGA CGCGGA CGCGGA	560 GT A G / GT A G / GT A G / GT A G / A G A C / 690	ACAGC ACAGC ACAGC 630 4 TTCAC ATTCAC ATTCAC	570 570 CACATC CACATC CACATC CACATC GGTCG GGTCG GGTCG GGTCG AAGTG 700	CAGAA CAGAA CAGAA I GCTG G G G G G G G C G G C G G A A C G G A A A A	580 CGATAG CGATAG CGATAG GATTTG GATTTG GATTTG GATTTG GATTTG GATTTG GATTG 710
Consensus #1 TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse Consensus #1 TCVbGenome.txt TCVbGenome.txt TCVbGex_Forward p28Rev_Reverse	TCAA TCAA TCAA TCAA TCAA TCAA TCAA CACAA 650 CACAA CACAA CACAA	AAATGC AAATGC AAATGC 590 I ACGGC I ACGGC I ACGGC GTGTAA GTGTAA	CTTGT CTTGT CTTGT CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC	CCAA CCAA CCAA CCAA CCAA CCAG CCACG CCACG CCACG	540 FACTGO FACTGO FACTGO AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTGTT AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGTG AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC 	CAAAG CAAAG CAAAG CAAGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CACGG CAAAG	550 GAGAC GAGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CCTGAC CCCGAC GCCC1 GCCC1 GCCC1	CATGTC ATGTC CATGTC CGCGGA CCGGGA CGCGGA CGCGGA CGCGGA CGCGGA CGCGGA CGCCG AGCCG AGCCG	560 GTAG/ GTAG/ GTAG/ AGAC/ AGAC/ AGAC/ AGAC/ AGAC/ AAGC/ AAGC/ AAGC/ AAGC/	ACAGCO ACAGCO ACAGCO 630 ATTCAO ATTCAO ATTCAO AGGCAA AGGCAA	570 570 CACAT (CACAT (CACAT (CACAT (GGT CG ⁷ GGT CG ⁷ GGT CG ⁷ GGT CG ⁷ AAGT G(AAGT G(AAGT G(AAGT G	CAGAA CAGAA CAGAA CAGAA CAGAA GCTG CGGAA CGGAA CGGAA CGGAA	580 CGATAG CGATAG CGATAG GATTTG GATTTG GATTTG GATGG GTGGTG GTGGTG GTGGTG GTGGTG
Consensus #1 TCVbGenome.txt TCVbIex_Forward p28Rev_Reverse Consensus #1 TCVbGenome.txt TCVbGenome.txt TCVbGex_Forward p28Rev_Reverse Consensus #1	СТСАЛ ТСАЛ ТСАЛ ТСАЛ ТСАЛ ТСАЛ САСАЛ 650 САСАЛ САСАЛ САСАЛ САСАЛ САСАЛ САСАЛ САСАЛ САСАЛ САСАЛ	AAATGC AAATGC S90 TACGGC TACGGC TACGGC GTGTAA GTGTAA GTGTAA GTGTAA TCTC 720	CTTGT CTTGT CTCTTG CTCTTC CTCTTC CTCTTC CTCTTC CTCTTC CGAGCA GAGCA GAGCA CGCGAT	CCAA CCAA CCAA CCAA CCAA CCAA CCACG CCACG CCACG CCACG CCACG CCACG	540 FACTGG FACTGG FACTGG AGTGTT AGTGTT AGTGTT AGTGTT ACCACC 670 670 670 670 670 670 670 670 670 670	CAAAGA CAAAGA CCACGC 610 CCACGC CCACGC CCACGC CCACGC CGCAAC CGCAAC CGCAAC CGCAAC CGCAAC	550 GAGAC GAGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC CTGAC	AT GT C CAT GT C AT GT C CGC GGA 620 GC GGGA GC GGGA CGC GGA AGC C G AGC C G A	560 GT A G / GT A G / GT A G / A G A C / A G A C / A G A C / A G A C / 690 A A G C / A A G C /	ACAGC ACAGCC ACAGCC 630 ATTCAC ATTCAC ATTCAC AGGCAA AGGCAA AGGCAA AGGCAA AGGCAA AGGCAA	570 CACATC CACATC CACATC GGTCG GGTCG 700 AAGTGG AAGTGG AAGTGG	CAGAA CAGAA CAGAA FGCTG G40 FGCTG FGCTG FGCTG CGGAA CGGAA CGGAA CGGAA CGGAA T770	580 CGATAG CGATAG CGATAG GATTTG GATTTG GATTTG GTGGTG 710 GTGGTG GTGGTG GTGGTG GTGGTG GTGGTG
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Full-Length TCV Translated p28 ORF

TCVbGenome.txt
TCVBtex_Forward
p28Rev_Reverse

Consensus #1

Consensus #1

TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse

Consensus #1

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Consensus #1

TCVbGenome.txt TCVBtex_Forward p28Rev_Reverse

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NKGQ	IGSSL	GCILA	A V P D S	GADIE	IDLDK	LVGIEE	EAISC	LVEAV	GSIAD	VPKKK	VRQKC	JKFAMHA
N R G Q	I GSSL	. GCILA	A V P D S	GADI E	IDLDR	LVGTEE	EATS C	LVEAV	GSTAD	VPRRR	VRQKC	F A MHA
VNAA	KLHFC	CGVPKP	ΡΤΕΑΝ	RLAVS	K WL V Q	YCKERH	IVVDSH	IRTIV	NTALP	RVFTP	DAEDI	QVVLDL
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VNAA	KL HF C	GVPKP	TEAN	RLAVS	K WL V Q	YCKERH	IVVDS H	IRTIV	NTALP	RVFTP	DAEDI	QVVLDL
V N A A	KLHF C	GVPKP	TEAN	RLAVS	KWL VQ	YCKERH	IVVDS H	IRTIV	NTALP	RVFTP	DAEDI	QVVLDL
HSVR	AHDHR	NALAF	AGKV	R K WWV	NI. A MH	P MT G R S	WSRAW	RRLCR	LPDDO	ALSEV	R -	
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650	660 670	680	690 700	0 710	720 730	0 740	750 760	770	780 790	800	810	
HSVR	AHDHR	NALAE	AGKV	PKWWV	NI A MH	PMTGRS	WSPAU	PPICP	I P D D O	ALSEV	P	
HSVR	AHDHR	NALAL	AGKV	R K WWV	NL A MH	PMTGRS	WSRAW	PRICR		ALSEV	D.	
		INALAT	ACKV		NL A MIL		UE OCU		VTN	AT ST V	R.	
пзук	АПОНК	INAL AP	CAUKV	KKWWV	NLAMH	PMTGKS	wr QG <mark>v</mark>	VK K X U K	AIN			

Full-Length TCV p88 ORF

1	ATGCCTC	TTCTACA	CACACTO	AACACA	GCGCTC	GCAGTG	GGACT	CCTAG	GAGCC	AGGTA	CTACCC
	70)	80	90		100		110		120	
ne.txt orward	A T G C C T C A T G C C T C	T T C T A C A T T C T A C A	CACACTC CACACTC	AACACA	GCGCTC GCGCTC	GC A GT G GC A GT G	GGACT (GGACT (C C T A G C C T A G	GAGCC. GAGCC.	AGGTA AGGTA	CTACCC CTACCC
1	$\frac{TGAGGTT}{120}$	CAAACCT	TCTTGGG	GCTGCC	TGACTA	CGTGGG	TCACA	ΓGAAG	AATGT	AGTAC	GGTCTG
ne.txt	TGAGGTT		TCTTGGG	GCTGCC	TGACTAC	CGTGGG	TCACA	FGAAG	AATGT	AGTAC	GGTCTG
orward	TTTTCC	CAAACCI			TGACTAG			GAAG	AAIGI		
1	20	0	210	22()	230	CAGICO	240	CAGGG	250	IAIAGI
ne.txt orward	Т Т Т Т С С А Т Т Т Т С С А	GGGATCT GGGATCT	GGGCTAG	T AGTAG	T G T C C T (T G T C C T (CCGACA	C A G T C O C A G T C O	GGTGT GGTGT	CAGGG CAGGG	GGACG GGACG	T A T A G T T A T A G T
1	AATAGAG	GTCAGAT	AGGTAGT	AGTCTC	GGGTGT	АТАСТА	GCCGT	TCCGG	ATAGC	GGGGC	GGATAT
	260	270		280	290		300		310		320
ne.txt orward	A À T A G A G A A T A G A G	GT C A G A T GT C A G A T	AGGTAGT AGGTAGT	A G T C T C A G T C T C	GGGTGT GGGTGT	АТ АСТ А АТ АСТ А	GCCGT GCCGT	ГССGG ГССGG	ATAGC ATAGC	GGGGC GGGGC	GGATAT GGATAT
1	AGAAATA	GACCTAG	ATAGGTT	GGTAGG	AACGGA	AGAGGA	AGCCA	CATCC	Т G T T T	GGTGG	AGGCGG
ne txt		0 GACCTAG) AACGGA		AGCCA		тотт		AGGCGG
orward	AGAAATA	GACCTAG	ATAGGTT	GGTAGG	AACGGA	AGAGGA	AGCCA	CATCC	Т G T T T	GGTGG	AGGCGG
1	TAGGTAG 390	TACCGCA 400	GATGTCC	CCAGGA 410	GGAGAG 420	TCGTC	AAAAG 430	GGGCG	<u>G T T T G</u> 440	CTATG	CATGCC 450
ne.txt orward	T A G G T A G T A G G T A G	T A C C G C A T A C C G C A	GATGTCC GATGTCC	CCAGGA CCAGGA	GGAGAG GGAGAG	T T C G T C T T C G T C	AAAAG AAAAG	GGGCG GGGCG	G T T T G G T T T G	C T A T G C T A T G	C A T G C C C A T G C C
1	GTCAACG	CAGCAAA	GCTGCAC	TTTTGT	GGCGTC	CCAAAA	CCCAC	TGAAG	CGAAT	CGACT	AGCGGT
	46	0	470	480)	490		500		510	
ne.txt orward	G T C A A C G G T C A A C G	CAGCAAA CAGCAA <mark>A</mark>	GCTGCAC GCTGCAC	T T T T GT T T T T GT	GGCGTC GGCGTC	CCAAAA CCAAAA	CCCAC CCCAC	Г GAAG Г GAAG	C G A A T C G A A T	CGACT. CGACT.	AGCGGT AGCGGT
1	CTCAAAA	TGGCTTG	TCCAATA	CTGCAA	AGAGAGA	ACATGT	CGTAG	ACAGC	CACAT	CAGAA	CGATAG
ne txt	520	530	ТССААТА	540	550	ACATGT	560	ACAGC	570	CAGAA	580
orward	СТСАААА	TGGCTTG	TCCAATA	CTGCAA	AGAGAGA	ACATGT	CGTAG	ACAGC	CACAT	CAGAA	CGATAG
1	TCAATAC	GGCTCTT	CCTAGAG	TGTTCA	CGCCTG	ACGCGG	AAGACA	ATTCA	GGTCG	TGCTG	GATTTG
ne.txt orward	59 T C A A T A C T C A A T A C	0 GGCTCTT GGCTCTT	600 CCTAGAG CCTAGAG	T GTTCA T GTTCA) <mark>CGCCTG</mark> / CGCCTG/	620 ACGCGG ACGCGG	AAGACA AAGACA	630 A T T C A A T T C A	GGTCG GGTCG	640 TGCTG TGCTG	G A T T T G G A T T T G
1	CACAGTG	TAAGAGC	ACACGAC	CACCGC	AACGCC	CTAGCC	GAAGC	AGGCA	AAGTG	CGGAA	GTGGTG
	650	660		670	680		690		700		710
ne.txt orward	C À C A G T G C A C A G T G	T A A G A G C T A A G A G C	ACACGAC ACACGAC	CACCGC CACCGC	AACGCC AACGCC	CTAGCC CTAGCC	GAÁGCA GAAGCA	A G G C A A G G C A	AAGT G AAGT G	C G G A A C G G A A	GT G GT G GT G GT G
1	GGTCAAT	CTCGCGA	TGCATCC	CATGAC	TGGGAG	GTCGTG	GTCCA	GGGCT	TGGAG	GCGAT	TATGCC
	72	0	730	740)	750		760		770	
ne.txt orward	G G T C A A T G G T C A A T	CTCGCGA CTCGCGA	T G C A T C C T G C A T C C	CATGAC CATGAC	T G G G A G (T G G G A G (GT C GT G GT C GT G	GTCCA GTCCA	GGGCT GGGCT	T G G A G T G G A G	GCGAT GCGAT	T A T G C C T A T G C C
1	GACTGCC	TGACGAC	CAGGCGA	тстстт	ттөтсс	GCTAGG	GGTGC	TTGCG	GGAGC	TGGTC	GGGAGG
	•	,					•				840
ne.txt	780	790		800	810		820		830		
orward	780 <mark>G A C T G C C</mark> G A C T G C C	790 T G A C G A C T G A C G A C	C A G G C G A C A G G C G A	800 .T <u>C T C T T T</u> .T <mark>C T C T T T</mark>	810 TTGTCCC TTGTCCC	G C T A G G G C T A G G	820 GGTGCT GGTGCT	T T G C G T T G C G	830 GGAGC GGAGC	T G G T C T G G T C	GGGAGG GGGAGG

Consensus #1

TCVbGenome.txt TCVBtex_Forwar

Consensus #1

TCVbGenome.txt TCVBtex_Forward

Consensus #1

TCVbGenome.txt TCVBtex_Forward G A G G A G

TCVbGenome.txt p88rev_Reverse

Consensus #1

TCVbGenome.txt p88rev_Reverse Consensus #1

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Consensus #1

TCVbGenome.txt p88rev_Reverse

Consensus #1

TCVbGenome.txt p88rev_Reverse

Consensus #1

TCVbGenome.txt p88rev_Reverse

TGGGA	ACACAGTG	TATACAACGC	GGGCTTTAAC	TGTCCCGAG	TTGGCACA
TGGGA					
TGGGA	A C A C A G T G	TATACAACGC	G G G C T T T A A C	T G T C C C G A G	TTGGCACA
GCTGC	TAACTTGG	CAGTTGACCA	ACAAGGGGGT	TGGGAGAGC	CTCCGATG
C C T C C '					
G C T G C '	TAACTTGG	CAGTTGACCA	. A C A A G G G G G T	TGGGAGAGC	CTCCGATG
GCTTT	ATCAAATA	CCAAGTTGAT	GGTTGTCGCA	TGTCCGGAG	ATGTTAAC
GCTTT					
G С Т Т Т .	АТСАААТА	CCAAGTTGAT	GGTTGTCGCA	TGTCCGGAG	АТ G T T A A C
ACAGC	CTTGGGCA	ACTGCCTACT	GGCTTGCTCT	ATCACCAAG	TACTTAAT
ACAGC					
ACAGC	C T T G G G C A	АСТ ССТАСТ	GGCTTGCTCT	A T C A C C A A G	ГАСТТААТ
GAAGG	GAATCAAA	TGCAAATTAA	TCAACAATGG	AGACGATTG	тдтдстдт
	1750	1760	1770	1780	1790
G A A G G (G A A G G (G A A T C A A A G A A T C A A A	T G C A A A T T A A T G C A A A T T A A		A G A C G A T T G ' A G A C G A T T G '	T G T G C T G T T G T G C T G T
тсттс	GAAGCTGA	TGAAGTCGAC	AGGGTGCGCG	AAAGGCTGC	ATCATTGG
	1800	1810	1820	1830	1840
ТСТТС (ТСТТС (G A A G C T G A G A A G C T G A	Т G A A G T C G A C Т G A A G T C G A C	A G G G T G C G C G A G G G T G C G C G	A A A G G C T G C A A A A G G C T G C A	A T C A T T G G A T C A T T G G
ATCGA	стттбббт	ттсаатбсат	AGCGGAAGAA	ССАСААТАС	GAATTGGA
	1850	1860	1870	1880	1890
A T C G A (C T T T G G G T	ТТСААТ ССАТ	A G C G G A A G A A	CCACAATAC	G A A T T G G A G A A T T G G A
GAAAG	TTGAATTT	тессадатет	ССССТАТТТ	CGATGGTGA	AGGGTGGG
0.1.1.1.0	1900	1910	1920	1930	1940
GAAAG	TTGAATTT	TGCCAGATGT	CCCCTATTT	CGATGGTGA	AGGGTGGG
GAAAG		IUUUAUAIUI		CGAIGGIGA.	
					A 1 · A 1 · I · A 1 · I ·
ICAIGO	GTCAGAAA 1950	CCCCCGTGTG 1960	I A G C C T C T C C A	1980	1990
TCATGO	<u>G T C A G A A A</u> 1950 <mark>G T C A G A A A</mark>	<u>CCCCCGTGTG</u> 1960 <u>CCCCCGTGTG</u>	А <u><u><u></u></u><u></u><u></u><u></u> 1970 А <u><u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u></u></u>	1980 A G G A C A G C T A	1990 A C A G C A C C
T C A T G O T C A T G O	<u>G T C A G A A A</u> 1950 G T C A G A A A G T C A G A A A	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A	1980 A G G A C A G C T A A G G A C A G C T A	1990 A C A G C A C C A C A G C A C C
T C A T G O T C A T G O A C A C A A	<u>G T C A G A A A</u> 1950 <u>G T C A G A A A</u> G T C A G A A A A T <u>G G G C G A</u> 2000	<u>C C C C C G T G T G</u> <u>1960</u> <u>C C C C C G T G T G</u> <u>C C C C C G T G T G</u> <u>A T G A G A A A G A</u> <u>2010</u>	1970 A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A A G C C T C T C T C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C C A G C C A G C C A G C C A G C C A G C C A G C C C A G C C C A G C C C C	1980 A G G A C A G C T A A G G A C A G C T A T G G T T G G C T G 2020	$\begin{array}{c} 1990 \\ A C A G C A C C \\ A C A G C A C C \\ G C C A T C G G \\ 2040 \end{array}$
T C A T G O T C A T G O A C A C A .	<u>G T C A G A A A</u> 1950 <u>G T C A G A A A</u> <u>G T C A G A A A</u> <u>G T C A G A A A</u> <u>A T G G G C G A</u> <u>2000</u>	C C C C C G T G T G 1960 C C C C C G T G T G C C C C C G T G T G A T G A G A A A G A 2010 A T G A G A A A G A	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A T G C A G C C A G A 2020 T G C A G C C A G A	A G G A C A G C T A 1980 A G G A C A G C T A A G G A C A G C T A T G G T T G G C T G 2030 T G G T T G G C T G G C T A	1990 A C A G C A C C A C A G C A C C G C C A T C G G 2040 G C C A T C G G
Т С А Т G (Т С А Т G (А С А С А . А С А С А . А С А С А .	G T C A G A A A 1950 G T C A G A A A G T C A G A A A A T G G G C G A 2000 A T G G G C G A A T G G G C G A	C C C C C C G T G T G 1960 C C C C C C G T G T G C C C C C C G T G T G C C C C C G T G T G A T G A G A A A G A 2010 A T G A G A A A G A A T G A G A A A G A	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C A G A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A	A G G A C A G C T A 1980 A G G A C A G C T A G G A C A G C T T G G T T G G C T 2030 I G G T T G G C T G G T T G G C T	1990 A C A G C A C C A C A G C A C C G C C A T C G G 2040 G C C A T C G G G C C A T C G G
T C A T G O T C A T G O A C A C A C A A A C A C A C A A A C A C	G T C A G A A A 1950 G T C A G A A A G T C A G A A A A T G G G C G A 2000 A T G G G C G A A T G G G C G A G T G G C C T T G	C C C C C G T G T G 1960 C C C C C G T G T G C C C C C G T G T G C C C C C G T G T G 2010 A T G A G A A A G A 2010 A T G A G A A A A G A G C T A T T G C A G	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A T G C A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A G C C G C A G C C A G A G G C G G C C A G A G G G G C G T A C C	A G G A C A G C T A 1980 A G G A C A G C T A A G G A C A G C T A T G G T T G G C T A 2030 T G G T T G G C T A T G G T T G G C T A A G T T G G T T A C A A	1990 A C A G C A C C A C A G C A C C G C C A T C G G 2040 G C C A T C G G G C C A T C G G A T C A T A T T
T C A T G C T C A T G C A C A C A A A C A C A A A C A C A A C A C	G T C A G A A A 1950 G T C A G A A A G T C A G A A A 2000 A T G G G C G A A T G G G C G A A T G G G C G A G T G G C T T G 2050 G T G G C T T G	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A . T G C A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A 2020 T G C A G C C A G A G C T G C A G C C A G A G T G G C G T A C C 2070 G T G G C G T A C C 2070	1980 A G G A C A G C T A A G G A C A G C T A A G G A C A G C T A 2030 T G G T T G G C T A G G T T G G C T A A G T G T T A C A A 2080 A G T G T T A C A A	1990 A C A G C A C C A C A G C A C C G C C A T C G G 2040 G C C A T C G G G C C A T C G G G C C A T C G G A T C A T A T T 2090 A T C A T A T T
T C A T G G T C A T G G A C A C A A A C A C A A A G A G T G A G A G T G	G T C A G A A A 1950 G T C A G A A A G T C A G A A A G T C A G A A A A T G G C C G A A T G G G C G A A T G G G C G A G T G G C C T T G G T G G C C T T G G T G G C C T T G	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A G C C A G C C A G A T G C A G C C A G A C A G C C A G C C A G A T G C A G C C A G A T G C A G C C A G C C A G A T G C A G C C A G C C A G A T G C A G C C A G C C A G A C G T G G C G T A C C Q070 G T G G C G T A C C G T G G C G T A C C G T G G C G T A C C G T G G C G T A C C	A G G A C A G C A G C A G C A G C A G C A G C A G C A G C A G C C C T A G G C T A G A G C T G G T T G G T T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G G G G G G G T G G T G G G G G G G G G G G G G G	1990 A C A G C A C C A C A G C A C C A C A G C A C C G C A T C G G 2040 G C C A T C G G G C C A T C G T A T T 2090 A T C A T A T T A T C A T A T T
T C A T G G T C A T G G A C A C A . A C A C A . A C A C A . A G A G T G A G A G T G A T T C T	G T C A G A A A 1950 G T C A G A A A G T C A G A A A 2000 A T G G G C G A A T G G G C G A A T G G G C T T G 2050 G T G G C T T G G T G G C T T G G T G C C T G A A	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A G C A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A 2020 T G C A G C C A G A T G C A G C C A G A C T G C A G C C A G A C T G C A G C C A G C A G A C T G C G C G T A C C 2070 G T G G C G T A C C G T G G C G T A C C G T G G C C T G T A C C G G A C C C C T G G	A G G C A G A G A G A G A G A G A G A G A G A G A G A G A G A G A G A G A G A G A G T G G T T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G T G G G G T G G T G	$\begin{array}{c} 1990 \\ A C A G C A C C \\ A C A G C A C C \\ A C A G C A C C \\ 2040 \\ G C C A T C G G \\ 2040 \\ G C C A T C G G \\ G C C A T C G G \\ A T C A T A T T \\ 2090 \\ A T C A T A T A T T \\ A C A A G A A G \\ A A G \\ A G A A G \\ A A G \\ A G A G \\$
T C A T G G T C A T G G A C A C A A A C A C A A A G A G T G A G A G T G A G A G T G A T T C T	G T C A G A A A 1950 G T C A G A A A G T C A G A A A 2000 A T G G G C G A A T G G G C G A A T G G G C T T G 2050 G T G G C T T G G T G G C T T G G T G C C T G A A 2100 T G C C T G A A	C C C C C G T G T G 1960 C C C C C G T G T G C C C C C G T G T G 2010 A T G A G A A A G A 2010 A T G A G A A A G A A T G A G A A A G A G C T A T T G C A G 2060 G C T A T T G C A G G C T A T T G C A G G C T A T T G C A G G C T A T T T T T T 2110 G A G G A A T T T T	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A A G C C T C T C C A T G C A G C C A G A 2020 T G C A G C C A G A 2020 T G C A G C C A G A C G T G G C G T A C C 2070 G T G G C G T A C C 2070 G T G G C G T A C C 2070 G G G G C G T A C C 2070 G G G G C G T A C C 2070 G G G C G C G T A C C 2070 G G G C G C G T A C C 2020 G G G C G C G T A C C G G G C G C C C C T G G 3120 320 32120	$\begin{array}{c} 1980 \\ A \ G \ G \ A \ C \ A \ G \ C \ I \ C \ C \ C \ C \ C \ C \ C \ C$	$\begin{array}{c} 1990 \\ \hline \\ A C A G C A C C \\ \hline \\ A C A G C A C C \\ \hline \\ C A C A G C A C C \\ \hline \\ C C A C A G C A C C \\ \hline \\ C C A C A T C G G \\ \hline \\ C C A T C A T C G G \\ \hline \\ C C A T C A T A T T \\ \hline \\ \hline \\ 2090 \\ \hline \\ A T C A T A T T \\ \hline \\ A C A A G A A G \\ \hline \\ 2140 \\ \hline \\ \hline \\ A C A A G A A G \\ \hline \\ \hline \\ A C A A G A A G \\ \hline \\ \hline \\ A C A A G A A G \\ \hline \\ \hline \\ C A A G A A G \\ \hline \\$
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T C A T G G T C A T G G A C A C A A C A C A A C A C A A C A C A A C A C A A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G A G A G T G C G G G A T G C G G G A T G A C C G G G A A C C G G G A	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c} A \ G \ C \ C \ T \ C \ T \ C \ A \\ 1970 \\ A \ G \ C \ C \ T \ C \ T \ C \ C \ A \\ G \ C \ C \ T \ C \ T \ C \ C \ A \\ 2020 \\ \hline T \ G \ C \ A \ G \ C \ C \ C \ A \ G \ A \\ T \ G \ C \ A \ G \ C \ C \ C \ A \ G \ A \\ T \ G \ C \ A \ G \ C \ C \ C \ C \ A \ G \ A \\ C \ C \ C \ C \ C \ C \ C \ C \ C \ C$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} 1990 \\ \hline \\ 1990 \\ \hline \\ A C A G C A C C \\ \hline \\ G C A C A G C A C C \\ \hline \\ 2040 \\ \hline \\ G C C A T C G G \\ \hline \\ 2040 \\ \hline \\ G C C A T C G G \\ \hline \\ G C C A T C G G \\ \hline \\ G C C A T C G G \\ \hline \\ G C C A T C G G \\ \hline \\ A T C A T A T T \\ \hline \\ 2090 \\ \hline \\ A T C A A G A A G \\ \hline \\ A C A A G A A G A A G \\ \hline \\ 2140 \\ \hline \\ A C A A G A A G A A G \\ \hline \\ C A G A A G A A G \\ \hline \\ C A G C T T T T \\ \hline \\ C A G C T T T T \\ \hline \\ 2240 \\ \hline \\ C A G C T T T T \\ \hline \\ A G G C T T T T \\ \hline \\ A G G A G T A C \\ \hline \\ \end{array}$
T C A T G G T C A T G G A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A G A G T G A T T C T A A G A T G A A G A T G A A G A T G C G G G A C G G G A A C C G G G A C C G G G	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c} A \ G \ C \ C \ T \ C \ T \ C \ A \\ 1970 \\ A \ G \ C \ C \ T \ C \ T \ C \ C \ A \\ G \ C \ C \ T \ C \ T \ C \ C \ A \\ 2020 \\ \hline T \ G \ C \ A \ G \ C \ C \ C \ A \ G \ A \\ C \ C \ A \ G \ C \ C \ C \ A \ G \ A \\ C \ C \ A \ G \ C \ C \ C \ A \ G \ A \\ C \ C \ C \ C \ C \ C \ A \ G \ A \\ C \ C \ C \ C \ C \ C \ C \ C \ C \ C$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} 1990 \\ \hline 1990 \\ \hline A C A G C A C C \\ \hline 1990 \\ \hline A C A G C A C C \\ \hline C A C A G C A C C \\ \hline C A C A G C A C C \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A T C G G \\ \hline C C A A G A A G \\ \hline C A C A A G A A G \\ \hline C A A G A A G A A G \\ \hline C A A G A A G A A G \\ \hline C C A A G G A A G A A G \\ \hline C C A A G G A A G A A G \\ \hline C C A A G G A A G A A G \\ \hline C C A A G G A A G A A G \\ \hline C C A G C T T T T \\ \hline C C A G C T T T T \\ \hline C A G C C T T T T \\ \hline C A G C C T T T T \\ \hline C A G C C T T T T \\ \hline C A G C C T T T T \\ \hline C 2240 \\ \hline C A G C C T T T T \\ \hline C A G C A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline \end{array}$
I C A T G G T C A T G G A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A C A C A A G A G T G A T T C T A A G A T G A A G A T G A A G A T G C G G G A C G G G A C G G G A A C C G G A C C G G A C C G G T A C G A	$\begin{array}{c} \mathbf{G} \ \mathbf{T} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{A} \ \mathbf{A} \ \mathbf{A} \\ 1950 \\ \mathbf{G} \ \mathbf{T} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{A} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{G} \ \mathbf{T} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{A} \ \mathbf{G} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{A} \ \mathbf{A} \\ 2000 \\ \mathbf{A} \ \mathbf{T} \ \mathbf{G} \ \mathbf{G} \ \mathbf{G} \ \mathbf{C} \ \mathbf{G} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{C} \ \mathbf{G} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{G} \ \mathbf{C} \ \mathbf{G} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{A} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{C} \\ \mathbf{C} \ \mathbf{C} \ \mathbf{A} \ \mathbf{G} \ \mathbf{C} \ C$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c} A \ G \ C \ C \ T \ C \ T \ C \ A \\ 1970 \\ A \ G \ C \ T \ C \ T \ C \ C \ A \\ G \ C \ T \ C \ T \ C \ C \ A \\ 2020 \\ \hline T \ G \ C \ A \ G \ C \ C \ A \ G \ C \\ C \ A \ G \ C \ C \ A \\ G \ C \ C \ A \ G \ C \ C \ A \\ G \ A \ C \ C \ C \ C \ A \\ G \ A \ C \ C \ C \ C \ C \ A \\ G \ C \ C \ C \ C \ C \ C \ C \ C \ C \$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} 1990 \\ \hline 1990 \\ \hline 1990 \\ \hline A C A G C A C C \\ \hline G C A G C A C C \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 2040 \\ \hline G C C A T C G G \\ \hline 1 C C A T A T T \\ \hline 1 C A T A T T \\ \hline 1 C C A T A T A T T \\ \hline 1 C C A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G A A G A A G \\ \hline 2140 \\ \hline A C A A G C T T T T \\ \hline 2240 \\ \hline C A G C A G C T T T T \\ \hline 1 T \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A G G A G T A C \\ \hline 2290 \\ \hline A C A C A G C T T T T \\ \hline A C A C A C \\ \hline A C A G C A G T A C \\ \hline A C A C \\ \hline A C A G C A G T A C \\ \hline A C A C \\ \hline A C A G C A G T A C \\ \hline A C A C \\$
T C A T G G T C A T G G A C A C A A A C A C A A A C A C A A A C A C A A A C A C A C A A C A C A C A A C A C A C A A C A C A C A A G A G T G A G A G T C T A T T C T T A T T C T T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A G A T C T A A C A G A T C C G G A A C C G G G A A C C G G G A A C C G G G A T A C G A C	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A G C C T C T C C A 1970 A G C C T C T C C A A G C C T C T C C A G C C T C T C C A G C C A G C A G A 2020 T G C A G C C A G A T G C A G C C A G A T G C A G C C A G A G T G G C G T A C C 2070 G T G G C G T A C C 2070 G T G G C G T A C C G T G G C G T A C C G T G G C C C T G G 2120 G G A C C C C T G G G G A C C C C T G G 2120 G G A C C C C T G G G T G G C G T A C C 2120 G G A C C C C T G G G T G G C G T A C C 2120 G G A C C C C A A G A 2120 G G A C C C C A A G A 2120 T A G T G G A T T C 2170 T A G T C C C A A G A Y A G T C C C A A G A Y A G T C C C A A G A Y A G T C C C A A G A Y A G T C C C A A G A Y A G T C C C A A G A Y A G T C C C A A G A Y A G	$\begin{array}{c} 1980 \\ \hline A \ G \ A \ C \ A \ G \ C \ I \ A \ G \ C \ I \ A \ G \ C \ I \ A \ G \ C \ I \ A \ G \ G \ A \ C \ A \ G \ C \ I \ A \ G \ G \ A \ C \ A \ G \ C \ I \ A \ G \ G \ A \ C \ A \ G \ C \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ A \ C \ A \ G \ C \ I \ A \ G \ G \ G \ I \ I \ A \ C \ G \ G \ G \ G \ A \ C \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ G \ G \ I \ I \ A \ G \ I \ I \ A \ G \ G \ I \ I \ A \ G \ I \ I \ A \ G \ I \ A \ G \ I \ A \ G \ I \ A \ G \ I \ A \ G \ I \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ I \ A \ A \ G \ A \ A \ G \ A \ A \ G \ A \ A$	$\begin{array}{c} 1990\\ A C A G C A C C\\ 1990\\ A C A G C A C C\\ C A C A G C A C C\\ 2040\\ G C C A T C G G\\ 2040\\ G C C A T C G G\\ G C C A T C G G\\ G C C A T C G G\\ 2090\\ A T C A T A T T\\ 2090\\ A T C A T A T T T\\ 2090\\ A T C A T A T A T T\\ A C A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ A C A A G A A G A A G\\ 2140\\ C A G C A G A A G A A G\\ 2140\\ C A G C A G A A G A A G\\ 2140\\ C A G C T T T T\\ 2240\\ C A G C T T T T\\ 2240\\ C A G C T T T T\\ 2240\\ C A G C T T T T\\ 2240\\ C A G C T T T T\\ 2240\\ C A G C T T T T\\ 2290\\ A G G A G T A C\\ 2290\\ A G G A G T A C\\ 2290\\ A G G A G T A C\\ A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G G A G T A C\\ 300\\ C A G C T C T C C\\ 300\\ C A G C C C C C C C C C C C C C C C C C$

TCVbGenome.txt TCV-BTEX-mid5-5



TCV Construct Translated p88 ORF

MP L I	HTLNT	ALAVG	LLGAR	YYPEV	/QTFLG	LPDYV	G H MK N	VVRSV	FQGS	GLVVVS	SDTVG	VRGTYS
70	80	90 100	110	120 130	140	150 160	170	180 190	200	210 220	230	240 250
MP Ĺ I MP L I	L H T L N T L H T L N T	A Ĺ A V G A L A V G	L L <mark>G A R</mark> L L <mark>G A R</mark>	YYPEV YYPEV	/ Q T F L G / Q T F L G	L P D Y V L P D Y V	G H MK N G H MK N	VVRSV VVRSV	/ F Q G S / F Q G S	G L Ý V Ý S G L V V V S	S DT VG S DT VG	VRGTYS VRGTYS
NRGO	QIGSSL	GCILA	VPDSG	ADIEI	DLDRL	VGTEE	EATSC	LVEAV	GSTA	DVPRRR	VRQKG	RFAMHA
260	270 280	290	300 310	320	330 340	350	360 370	380	390 400) 410	420 430	440 45
N R G (N R G (QI GSSL QI GSSL	GCILA GCILA	VPDSC VPDSC	ADI EI ADI EI	DL DR L DL DR L	VGTEE VGTEE	E A T S C E A T S C	L VE A V L VE A V	/ G S T A] / G S T A]	D V P R R R D V P R R R	VRQKG VRQKG	R F A MH A R F A MH A
VNAA	AKLHFC	G V P K P	TEANR	LAVSE	KWL VQY	CKERH	VVDSH	IRTIV	NTAL	PRVFTP	DAEDI	QVVLDL
460	470 	480 490	500 TEANR	510 520	530	540 550	560	570 580	590	600 610	620	630 640
VNAA	AKL HF C	GVPKP	T E <mark>A N R</mark>	LAVSI	CWL VQY	CKERH	VVDS H	IRTIV	/NTAL	PRVFTP	DAEDI	QVVLDL
HSVE	RAHDHR	NALAE	AGKVR	KWWVN	NLAMHP	MT G R S	WS R A W	RRLCF	RLPDD	QAISEV	R - GCL	RELVGR
HSVI	RAHDHR	NALAE	AGKVR	K WWV1		MT G R S	WS R A W	RRLCF		QAISFV	R. GCL	RELVGR
HS VI	RAHDHR	NALAE	AGKVR	KWWVN	N L A MH P	MT G R S	WS R A W	RRLCF	RLPDD	QAISFV	R. GCL	RELVGR
E												
E												
<u>v</u>	VE HS V	YNAG	FNCP	ELAC	QLLTW	Q L T N	KGVG	RASI	OGFI	KYQVD	GCRM	IS GDVN
	1550	15601	570 1	580	15901600	1610	16201	630	1640	16501660	1670	16801690
V	VE HS V	YNAG VNAC	FNCP	ELAC	QLLTW	QLTN	KGVG	RASI	DGFI I	KYQVD	GCRM	IS GDVN
• •	VEHSV	INAG	FNCP	ELA	ZLLIW	QLIN	KUVU	KASI	JGFI	K I Q V D	GURM	IS GD V N
<u>1</u>	TALGN		CSIT	KYLN	AKGI K	CKLI	NNGD	DCVI	FFE	ADEVD	RVRE	RLHHW
=	1700	1710	720 1	730	17401750	1760	17701	780	1790	18001810	1820	18301840
ו ד	ALGN ALGN	CLLA CLLA	CSI T	KYLN KYLN	AKGI K AKGI K	CKLI	NNGD	DCVI	JFFE. JFFE.	A D E V D A D E V D	RVRE	R L H H W
I	DFGF		ЕЕРС	YELE	EKVEF	COMS	PIFD	GEGV	VV MV I	RNPRV	SLSK	DSYST
_	1850	18601	870 1	880	18901900	1910	19201	930	1940	19501960	1970	19801990
I	DFGF	QCIA	E E P Q	YELE	KVEF	C Q MS	P I F D	GE G V	VV MV I	R N P R V	SLSK	DS YS T
I	DF GF	QCI A	EEPQ	YELE	EKVEF	C Q MS	PIFD	GEGV	VV MV I	R N P R V	SLSK	DSYST
1	QWAN	EKDA	A R WL	AALO	GECGL	AIAG	GVPV	LQSY	YSCI	LKRNF	GPLA	GDYKK
_	2000	20102	020 2	2030	20402050	2060	20702	080	2090	21002110	2120	21302140
ך ד	F Q WA N F O WA N	E K D A E K D A	A R WL	AAI (GECGL GECGL	AI AG	G V P V G V P V		YSCI	L <mark>K R</mark> N F L K R N F	GPLA GPLA	GDYKK GDYKK
L	MODY	e e de	CEVD	LCVN		e k dv	C O D A	DECI	ND CI	ECVED	DEOE	ALEEV
<u>r</u>	2150	<u>3 F D 3</u> 1	170 CF 1K		1000200	2210	<u>3 QDA</u>		2240	F G Y I P	2270	
k		S F D S	GEYR		JGMRG	S K D V	S OD A	RESE		$\frac{22502260}{1}$		
k	C MQ D V	SFDS	GFYR	LSKN	G MR G	SKDV	SQDA	RFSF	YRGI	FGYTP	DEQE	ALEEY
١	/ DNL E	LLCE	WD P									
_	2300	23102	320									
N	(DNL Q	LLCE	WD P									
, V	(DNLE	LLCE	WDP									
	LSX											
	2390											
	L S N											
	Q <mark>S</mark> X											

TCVbGenome.txt TCVBtex_Forward

Consensus #1

TCVbGenome.txt TCVBtex_Forward

Consensus #1

TCVbGenome.txt TCVBtex_Forward

Consensus #1

TCVbGenome.txt TCVBtex_Forward

Consensus #1

TCVbGenome.txt TCVBtex_Forward Consensus #1

TCVbGenome.txt p88rev_Reverse

Consensus #1

TCVbGenome.txt TCV-BTEX-mid5-5

TCV Construct P8 ORF

Consensus #1 Majority

TCVbGenome.txt Mid5-Forward Consensus #1 Majority

TCVbGenome.txt

Mid5-Forward

2200	2270	2290	2200	2100
2360	2370	2380	2390	2400
ATGGATCC	TGAACGAATT	CCCTACAACI	ATCTCNGCG	ACAGCGACG
			n <mark>i ci c</mark> n <mark>oco</mark> i	
AGGAAAAC	GGAAGAAAGG	GCGGAGAGAAA	AGTGCGAAG	AAGAGATTG
AGGAAAAC	GGAAGAAAGC		I	AAGAGAIIG
2410	2420	2430	2440	2450
AGGAAAAC	GGAAGAAAGC	GCGGAGAGAAA	AGTGCGAAGA	AAGAGATTG
AGGAAAAC	GGAAGAAAGC	GCGGAGAGAAA	AGT GC GAAG2	AAGAGATTO
CTAGCCAC	GCGGCTAGCT	СТ G T T T T A A A	CAAGAAAAG	AAATGAAGG
CTAGCCAC	GCGGCTAGCT	CTGTTTTAA	CAAGAAAAG	AAATGAAGG
2460	2470	2480	2490	2500
CTAGCCAC	GCGGCTAGCT	CTGTTTTAA	CAAGAAAAGA	AAATGAAGG
CTAGCCAC	GCGGCTAGCT	СТ G T T T T A A A	ACAAGAAAAGA	AAAT GAAGO
GCTAGTCA	CGGGGGGTACT	TGGGTTATTO	GTTGCTGATA	AAGTGGAAG
GCTAGTCA	CGGGGGTACT	TGGGTTATTO	GTTGCTGATA	AAGTGGAAG
2510	2520	2530	2540	2550
GCTAGTCA	CGGGGGTACT	TGGGTTATTC	GTTGCTGATA	AAGTGGAAG
GCTAGTCA	CGGGGGGTACT	T G G G T T A T T C	GTTGCTGATA/	AAGT GGAAC
	тсласттста			
AATCAACT	TCAACTTCTA	A		
2560	2570			
	<u>2570</u>			

TCV Construct Translated p8 ORF

Consensus #1	XXXXXX	XXXXI	SDXDA	ΓGKR	KKGGEKS	AKKR	RLVASHA	ASS	VLNKKRI	NEGS
Majority	XXXXXX	XXXXI	SDXDA	ΓGKR	KKGGEKS	AKKR	R L V A S H A	ASS	VLNKKRI	NEGS
		1	1 1	1	1 1		1 1			
	23602370	2380	23902400	2410	24202430	2440	24502460	2470	24802490	2500
TCVbGenome.txt	MDPERI	P Y N S <mark>I</mark>	. S D S D A 1	GKR	K K G G E K S	AKKR	RL VAS HA	ASS	VLNKKRI	NEGS
Mid5-Forward	XXXXXX	XXXX <mark>I</mark>	SEX <mark>DA</mark>	Γ <mark>G K R</mark> I	K K G G E K S	AKKR	RLVASHA	ASS'	VLNKKRI	NEGS
Consensus #1	ASHGGT	WVI VA	ADKVEVS	SINFI	NF-					
Majority	ASHGGT	WVI VA	DKVEVS	SINF	N F					
	25102520	2530	25402550	2560	2570					
TCVbGenome.txt	ASHGGT	WVI VA	DKVEVS	SINFI	NF.					
Mid5-Forward	ASHGGT	WVI VA	ADKVEVS	SI NFI	NF					

TCV Construct

		P9 O	RF		
Consensus #1 Majority	A T G A A G G T T C A T G A A G G T T C	CTGCTAGTCA CTGCTAGTCA	ACGGGGGGTACT ACGGGGGGTACT	T G G G T T A T T G T G G G T T A T T G	GTTGCTGATAAA GTTGCTGATAAA
	2500	2510	2520	2530	2540
TCVbGenome.txt Mid5-Forward	A T G A A G G T T C A T G A A G G T T C	CT G C T A G T C A CT G C T A G T C A	ACGGGGGGTACT ACGGGGGGTACT	T G G G T T A T T (T G G G T T A T T (GTT GCT GAT AAA GTT GCT GAT AAA
Consensus #1 Majority	G T G G A A G T C T G T G G A A G T C T	CAAT CAAC CAAT CAAC	ГТСААСТТСТА ГТСААСТТСТА	ATCAGAAAT ATCAGAAAT	GTCAGTGCCCGA GTCAGTGCCCGA
	2550	2560	2570	2580	2590
TCVbGenome.txt Mid5-Forward	G T G G A A G T C T G T G G A A G T C T	СААТ СААС СААТ СААС СААТ СААС	ГТСААСТТСТА ГТСААСТТСТА	ATCAGAAAT ATCAGAAAT	GTCAGTGCCCGA GTCAGTGCCCGA
Consensus #1 Majority	CGTCCCCGTC CGTCCCCGTC	GGTAATATA GGTAATATA	ATGCTTTCTAC ATGCTTTCTAC	AACTCTCTCT	TCACTGGTCCTC TCACTGGTCCTC
	2600	2610	2620	2630	2640
TCVbGenome.txt Mid5-Forward	CGTCCCCGTC CGTCCCCGTC	GGTAATATA GGTAATATA	ATGCTTTCTAC ATGCTTTCTAC	AACTCTCTCT AACTCTCTCTC	TCACTGGTCCTC TCACTGGTCCTC
Consensus #1 Majority	С Т А С Т Т Т G Т С С Т А С Т Т Т G Т С	CAT CT GAT T C	CCT GAAAT CAA CCT GAAAT CAA	ACCGATTCA	CACATCCTACAA CACATCCTACAA
	2650	2660	2670	2680	2690
TCVbGenome.txt Mid5-Forward	С Т А С Т Т Т G Т С С Т А С Т Т Т G Т С	CATCTGATT(CATCTGATT(CCT GAAAT CAA CCT GAAAT CAA	ACCGATTCA ACCGATTCA	CACATCCTACAA CACATCCTACAA
Consensus #1 Majority	CACACACGAC CACACACGAC	CTCATCGAA CTCATCGAA	GCAGCAACACA GCAGCAACACA	TAAGCATCA TAAGCATCA	A C A C T G G A A A T G A C A C T G G A A A T G
	2700	2710	2720	2730	2740
TCVbGenome.txt Mid5-Forward	C A C A C A C G A C C A C A C A C G A C	CTCATCGAA CTCATCGAA	GCAGCAACACA GCAGCAACACA	Т А А <u>G</u> С А Т С А А Т А А <u>G</u> С А Т С А А	A C A C T G G A A A T G A C A C T G G A A A T G
Consensus #1 Majority	GAAAATGA GAAAATGA 2750				
TCVbGenome.txt Mid5-Forward	GAAAAT GA GAAAAT GA				

TCV Translated p9 ORF

Consensus #1 Majority	$\frac{MK V L}{MK V L}$	L V T L V T 2510	GVLG GVLG		LIKY LIKY 2540	WKSQS WKSQS	TSTS TSTS 0 257	NQKC NQKC	Q C P Q C P	T S P W T S P W 2600	VI YAF VI YAF 261006	YNSLS YNSLS 20 2630	L V L L V L 2640
TCVbGenome.txt Mid5-Forward	MK V L MK V L	L VT L VT	G V L G G V L G			WKSQS WKSQS	TSTS TSTS	NQKC NQKC	QCP QCP	T S P W	VI YAF VI YAF	YNSLS YNSLS	L V L L V L
Consensus #1 Majority	LLCH <u>LLCH</u> 2650	LIP LIP 2660	EI KP EI KP 26702	ІНТ <u>ІНТ</u> 2680	S Y N 1 S Y N 1 2690	Г Н D S S Г Н D S S 2700271	КQQH <u>КQQH</u> 0 272	ISIN <u>ISIN</u> 20 273	T G N <u>T G N</u> 502740	GK - <u>GK -</u> 2750			
TCVbGenome.txt Mid5-Forward	L L C H L L C H	LIP LIP	EI KP EI KP	I HT I HT	S Y N I S Y N I	THDS S THDS S	KQQH KQQH	I S I N I S I N	T G N T G N	GK. GK.			

TCV Construct

ATGGAAAATGAT	p38 ORF	' CGGAAGTTCG(CATCTGATGG	CGCCCAA
ATGGAAAATGAT	CCTAGAGTCC	GGAAGTTCGG	CATCTGATGG	CGCCCAA
2750	2760	2770	2780	2790
A	CCTAGAGICC	GGAAGTTCGC	CATCTGATGG CATCTGATGG	CGCCCAA
GGCGATAAAGTG	GCAGAAGAAG	GGGCTGGTCA	ACCCTAACCA	GCAGACA
GGCGATAAAGTG	GCAGAAGAAG	GGCTGGTCA	ACCCTAACCA	<u>GCAGACA</u>
GGCGATAAGTG	GCAGAAGAAG	2820 GGGCTGGTCAA		2840 J GCAGACA
GGCGAT <mark>AAAGTG</mark>	GCAGAAGAAC	GGCTGGTCA	ACCCTAACCA	GCAGACA
AACAGACCGCCC	GCGCAGCGAT	GGGGGATCAAG	GCTCTCTCCTC	GT GGC GC
2850	2860	2870	2880	2890
AACAGACCGCCC	GCGCAGCGAT	GGGGGATCAAG	GCTCTCTCCTC	GT GGC GC
AACAGACCOCCC	OTCLOTOCOAL	TCACTCCTC		
CCTGTGCAGAAA	GTGACTCGGC	TGAGTGCTCC	CGGTGGCCCT	I GCCTAC I GCCTAC
2900	2910	2920	2930	2940
C C T G T G C A G A A A C C T G T G C A G A A A	GTGACTCGGC GTGACTCGGC	TGAGTGCTCC TGAGTGCTCC	CGGTGGCCCT CGGTGGCCCT	Г G C C T A C Г G C C T A C
CGAGGTTTCCAC	CCAGCCTCGC	GTCTCTACT	GCCAGGGACG	GCATAAC
CGAGGTTTCCAC	CCAGCCTCGC	GTCTCTACT	GCCAGGGACG	GCATAAC
CGAGGTTTCCAC	CCAGCCTCGC	GTCTCTACT	GCCAGGGACG	GCATAAC
GAAGCGGTTCTG	AACTGATCAC	CAACCTTGAAG	GAAGAACACT	GACACTO
3000	3010	3020	3030	3040
GAAGCGGTTCTG	AACTGATCAC	CAACCTTGAAC	GAAGAACACT	GACACTG
GAAGCGGTTCTG	AACTGATCAC	CAACCTTGAAG	JAAGAACACT	GACACTG
CCTAAGTACACC CCTAAGTACACC	ACAGCTGTGC ACAGCTGTGC	CTTAACCCAAC CTTAACCCAAC	GCGAACCCGG GCGAACCCGG	4 A C A T T C 4 A C A T T C
3050	3060	3070	3080	3090
CCTAAG <mark>T</mark> ACACC CCTAAGTACACC	ACAGCTGTGC ACAGCTGTGC	ТТ ААСССАА ТТ ААСССАА (GCGAACCCGG GCGAACCCGG	4 A C A T T C 4 A C A T T C
CCAGCTCATTAA	GGAGGCGGCC			TCACGTO
CCAGCTCATTAA	GGAGGCGGCC	CAGTATGAA	AATACCGAT	TCACGTC
3100	3110	3120	3130	3140
CCAGCTCATTAA CCAGCTCATTAA	GGAGGCGGCC GGAGGCGGCC	CAGTATGAAA CAGTATGAAA	AATACCGAT AAATACCGAT	T C AC G T C T C AC G T C
TCAGATTTAGGT	ACTCCCCCAT	GAGCCCTTCA	ACCACCGGA	GGCAAGG
TCAGATTTAGGT	ACTCCCCAT	CAGCCCTTCA	ACCACCGGA	GGCAAGC
TCAGAT TTAGGT	ACTCCCCAT	GAGCCCTTCA	ACCACCGGA	GGCAAGG
T C A G A T T A G G T	ACTCCCCAT	GAGCCCTTCA	ACCACCGGA	GGCAAGG
GCTCTGGCATTC GCTCTGGCATTC	GACCGAGAT C	CAGCCAAACC	CTCCGCCCAA CTCCGCCCAA	CGACCTC
3200	3210	3220	3230	3240
GCTCTGGCATTC	GACCGAGATO		CTCCGCCCAA	CGACCTC
TTOCCTOTACA	CATACACCC	TOTOTATOTATOT	COCTOCCAN	
TTCCCTCTACAA	CATAGAGGGI CATAGAGGGI	TGTGTATCTA	AGCGTGCCCT	GACAGG
3250	3260	3270	3280	3290
TTCCCTCTACAA TTCCCTCTACAA	CATAGAGGGT CATAGAGGGT	TGTGTATCTA TGTGTATCTA	A GC GT GC CC T (A GC GT GC CC T (GGACAGO GGACAGO
TTATTTTGACCG	TCCCAACAGA	TTCTACTGA	CCGCTTT	
TTATTTGACCG	TCCCAACAGA	TTCTACTGAC	CGCTTT	

TCVbGenome.txt HindIII_Reverse

Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse

TCVbGenome.txt HindIII_Reverse Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

Consensus #1 Majority

3340 3350 3360 3370 GI GGCGGAI GGI ALICAGE GATECAAAGCI LOTE GATTI EGOCAAGCI GI GGCGGAI GGI ALICAGE GATECAAAGCI LOTE GATTI EGOCAAGCI CATGGCCACCT ACGGCCAAGGAGCCAATGAT GCCGCCCAACTCGGTC CATGGCCACCT ACGGCCAAGGAGCCAATGAT GCCGCCCAACTCGGTC CATGGCCACCT ACGGCCAAGGAGCCAATGAT GCCGCCCAACTCGGTC CATGGCACCT ACGGCCAAGGAGCCAATGAT GCCGCCCAACTCGGTC CATGGCAACTCAAGACCGT GCAGCT CAAGAACAGAACT GGCT CAACC GGCGAGT CGAGT ACACCGT GCAGCT CAAGAACAGAAC	010000	GGAIGGIA	<u> </u>	AAAGCTTGTC	GATTTCGGCAA	GCT
GT GGC GGA T GGT AT C AGC GAT C C AAAG C T TGT C GAT TT TC GGC AAGC T GT GGC GGA T GGT AT C AGC GAT C C AAAG C T TG T C GAT TT TC GGC AAGC T CAT GGC C AC CT A C GGC C AA GG AG C C AAT GAT GC C GC C		3340	3350	3360	3370	
CATGGCC ACCT ACGGCC AAGG AGCC AAT GAT GCCGCCC AACT CGGT C CATGGCC ACCT ACGGCC AAGG AGCC AAT GAT GCCGCCC AACT CGGT C 3300 3400 3410 3420 CATGGC ACT ACGCC AAGG AGC AAT GAT GCCGCC CAACT CGGT C CATGGC ACT ACGCC AAGG AGC AAT GAT GCCGCC CAACT CGGT C TGCGAGT CGAGT AC ACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACCCGT GC AGCT CAAG AACAG AACT GGCT C AACC 1GCGAGT CGAGT ACCCGT GC AGGT GT T AAGG ACGGACCCAGGCT 1GCGAGT CGAGT ACCCGT GC AGGT GT T AAGG ACGGACCCAGGCT 1GCGGAGT CGAGT ACCCGT GC AGGT GT T AAGG ACGGACCCAGGCT 1GCGGAGT CGAGT ACCCGT GGCAGCT GGGT GGG AGCACCG AGCT 1GCGGAGT CGAGT GGGGAC T CGCAGGT GGT AGGACGGACCCAGGCT 1CCATGGT CCAAGACCAAGGGGACAGCT GGGT GGG AGCACGAT TGT C 1TCATGGT CCAAGACCAAGGGGACAGCT GGGT GGGAGCACGAT TGT C 1TCTCGGAACCGGAAACT T CT CGT TGACAT T GT T CT ACGAGAAGGCC 1TCT CGGAACCGGAAACT T CT CGT TGACAT T GT T CT ACGAGAAGGCC 1TCT CGGAACCGGAAACT T CT CGT TGACAT T GT T CT ACGAGAAGGCC 1TCT CGGAACCGGAAACT T CT CGT TGACAT T GT T CT ACGAGAAGGCC 1TCT CGGGGCT AGAAAACGCAAGCAGC CT CT GACT T CT CG GT C CT GGC 1CT CGGGGCT AGAAAACGCAAGCAGC CT CT GACT T CT CG GT CCT GGC 1CT CGGGGCT AGAAAACGCAAGCAGC CT CT GACT T CT CG GT C CT GGC 1CT CGGGGCT AGAAAACGCAAGCAGC CT CT GACT T CT CG GT C CT GGC 1CT CGGGGCT AGAAAACGCAAGCAGC CT CT GACT T CT CG GT C CT GGC 1GC CGGGGCT AGAAAACGCAAGCAGC CT CT GGCAGGAAGGT AGCAAAA 1GC CAAGGCGT GAAAAT GGT C CAAT GGGCAGGCAGCCAAAGGGT AGAAA 1GC CAAGGCGT GAAAAT GGT C CAACT GGGGAGCAGCCAAAGGGT AAA 1GC CAAGCACT CA	GT GGC (GT GGC (G G A T G G T A' G G A T G G T A'	FCAGCGATCCA FCAGCGATCCA	A A G C T T G T C G A A G C T T G T C G	G A T T T C G G C A A G A T T T C G G C A A	AGCT AGCT
3390 3400 3410 3420 CAT GGCC ACCT ACGCCCAAGGAGCCAATGAT GCCGCCCAACT CGGT CAT GGCACT ACGCCCAAGGAGCCAATGAT GCCGCCCAACT CGGT CGCGAGT CGAGT ACACCGGT GCAGCT CAAGAACAGAAC	CATGG CATGG	CCACCTAC	GGCCAAGGAG GGCCAAGGAG	CCAATGATGC CCAATGATGC	CGCCCAACTCC	G G T G G G T G
CAT GGC CACC TACGGC CAAGGAGC CAAT GAT GC GC CCAAC T CGGT C CAT GGC ACC TACGGC CAAGGAGC CAAT GAT GC GC CC CAAC T CGGT GGC AGT CGAGT AC AC CGT GC AGCT CAAGAA CAGAAC T GGC T CAACC TGCGAGT CGAGT AC ACC GT GC AGCT CAAGAA CAGAAC T GGC T CAACC 3440 3450 3460 3470 TGCGAGT CGAGT AC AC CG T GC AGCT CAAGAA CAGAAC T GGC T CAACC GGC CC AGAT T GGGGAC TT CGC AGGT GT T AAGGAC GGAC		3390	3400	3410	3420	
TGCGAGTCGAGTACACCGTGCAGCTCAAGAACAGAACTGGCTCAACA 1440 3450 3460 3470 TGCGAGTCGAGTACACCGTGCAGCTCAAGAACAGAACTGGCTCAACA GACACTGGCTCAACGTGCAGCTCAAGAACAGAACTGGCTCAACA TGCGAGTCGAGTACACCGTGCAGCTCAAGAACAGAACAG	C A T G G G C A T G G G	CCACCTAC CCACCTAC	GGCCAAGGAG GGCCAAGGAG	CCAATGATGC CCAATGATGC	CGCCCAACTCC CGCCCAACTCC	G G T G G G T G
3440 3450 3460 3470 IGCGAGT CGAGT ACACCGIGCAGCT CAAGAACAGAACIGGCT CAACC IGCGAGT CGAGT ACACCGIGCAGCT CCAAGACAGAACIGGCT CAACC IGCGAGT CGAGT ACACCGIGCACCT CGCAGGT GT TAAGGACGGACCCAGGCT GACGCCCAGAT TGGGGACT TCGCAGGT GT TAAGGACGGACCCAGGCT 3490 3500 3510 3490 3500 3510 GACGCCCAGAT TGGGGACT TCGCAGGT GT TAAGGACGGACCCAGGCT GACGCCCAGAT TGGGGACAT CGCAGGT GT TAAGGACGGACCCAGGCT GACGCCCAGAT TGGGGACAC TCGCAGGT GT TAAGGACGGACCCAGGT TG TC GACGCCCAGAT TGGGGACACAAGGGGACAGCT GGGT GGG	T G C G A G T G C G A G	G T C G A G T A G T C G A G T A	CACCGTGCAG	CTCAAGAACA CTCAAGAACA	G A A C T G G C T C A G A A C T G G C T C A	ACC
TO CGAGT CGAGT ACACCG TG AGCT CAAGAACAGAAC		3440	3450	3460	3470	
GACGCCAGATTGGGGACTTCGCAGGTGTTAAGGACGGACCCAGGCT GACGCCAGATTGGGGACTTCGCAGGTGTTAAGGACGGACCCAGGCT 3490 3500 3510 3520 GACGCCCAGATTGGGGACTTCGCAGGTGTTAAGGACGGAC	T G C G A C T G C G A C	GT C G A GT A GT C G A GT A	CACCGTGCAG CACCGTGCAG	CTCAAGAACAO CTCAAGAACAO	GAACTGGCTCA GAACTGGCTCA	ACC ACC
3490 3500 3510 3520 GACGCCCAGATTGGGGACTTCGCAGGTGTTAAGGACGGAC	GACGCO	CCAGATTG	GGGACTTCGC GGGACTTCGC	A G G T G T T A A G G A G G T G T T A A G G	GACGGACCCAC	а а а а а а а а а а
GAC GC C C A GATT GG G GA C T C GC A GG T G T T A A G G A C G G A C C A G G C T G G G A G C C A G G C T G G G A G C C A G G C C A G G T G T C A A G G G G A C C A A G G G G A C A G C T G G G T G G G G G G A C C G A T T G T C T C A T G G T C C A A G A G C G G A G C T G G G T G G G G G G A G C A C G A T T G T C T C A T G G T C C A A G A G G G G A C A G C T G G G T G G G G G G A G C A C G A T T G T C T C T G G T G C A A G A C C A A G G G G A C A G C T G G G T G G G G G G A G C A C G A T T G T C T C G G T G G G T G G G G G A G C A C G A T T G T C T C G G G A G C A C G A T T G T C T C G G G A G C A C G A T T G T C T C G G G A G C A C G A G C G G A A C T T C T C T C G T T G A C A T T G T C T A C G A G A A G G G A A C T T C T C G T T G A C A T T G T C T A C G A G A A G G C C T T C T C G G A A C C G G A A A C T C T C G T T G A C A T T G T C T A C G A G A A G G C C T T C T C G G A A C C G G A A A C T C T C G T T G A C A T T G T C T A C G A G A A G G C G T C T G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G A A G C C G A A A C T C T C G T G A C A T T G T C T C C G G T C C T G G A C C T T C T C G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C T T C C G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C T T C C G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C T C T G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C T C C A T G G C A G G A G G T A G C A G A A A C G C A G A G C C C T T G A C T T C T C G G T A G A A A C G C A G A C G C C T T G A C T T C T C G G T A G A A A C G C A G A C G C C T T G A C T T C T C G G T A G A A A C G C A G A C G C T T C T C G G T A G A A A C G C A G A C G C T T C T C G G T A G A A A C G C A G A G C C T T G A C T T C T C G G T A G A A A C G C A G A G C C T T G A C T T C T C G G T A G A A A C G C A G A G C T C T G A G G T A G C A G C A G C A G A G T A G C A G C A G C A G C A G C A G C A G		3490	3500	3510	3520	
TT CAT GGT CCAAGAC CAAGGGGA CAGC T GGGT GGG	GACGC (GACGC (CCAGATTG CCAGATTG	GGGACTTCGC GGGACTTCGC	A G G T G T T A A G G A G G T G T T A A G G	GACGGACCCAC GACGGACCCAC	G G C T G G C T
11 CATGGTCCAAGACCAAGGGGACAGCTGGGTGGGAGCACGATTGTC 3540 3550 3560 3570 TTCATGGTCCAAGACCAAGGGGACAGCTGGGTGGGAGCACGATTGTC TTCATGGTCCAAGACCAAGGGGACAGCTGGGTGGGAGCACGATTGTC TTCTCGGAACCGGAAACTTCTCGTTGACATTGTTCTACGAGAAGGCC 3590 3600 3610 3620 TTCTCGGGAACCGGAAACTTCTCGTTGACATTGTTCTACGAGAAGGCC 3590 3600 3610 3620 TTCTCGGGAACCGGAAACTTCTCGTTGACATTGTTCTACGAGAAGGCC GTCTCGGGGCTAGAAACGCAGCGCCCTCTGACTTCTCGGTCCTGGC GTCTCGGGGCTAGAAAACGCAGACGCCTCTGACTTCTCGGTCCTGGC 3640 3650 3660 3670 GTCTCGGGGCTAGAAAACGCAGAGCGCCTCTGACTTCTCGGTCCTGGC GTCTCGGGGCTAGAAAACGCAGAGCGCCTCTGACTTCTCGGTCCTGGC 3640 3650 3660 3670 GTCTCGGGGCTAGAAAACGCAAGCAGCCCTCTGACTTCTCGGTCCTGGC GCCGCAGCAGGTAGTGTCCAATGGGCAGGCAGGAGTGAAGGTAGCAGAAA AGCCGCAGCAGGTAGTGTCCAATGGGCAGGCAGGCAGGAGTAGCAGAAA 3690 3700 3710 3720 AGCCGCGCAGGCAGGTAGTGTCCAATGGGCAGGCAGGCAG	TTCAT	GGTCCAAG.	ACCAAGGGGA	CAGCTGGGTG	GGAGCACGATI	GTC
TT CAT GGT CC AA GA CC AA GG GG AC AG CT GG GG GG GG AG CA CG AT TG T C TT CAT GGT CC AA GA CC T T C T C GT T GA CAT T GT T C T A C G A G A A G C C TT C T C GG AA C C GG AA A C T T C T C G T T GA C A T T G T T C T A C G A G A A G C C T C T C GG AA C C GG AA A C T T C T C G T T G A C A T T G T T C T A C G A G A A G C C 3590 3600 3610 3620 TT C T C GG AA C C GG AA A C T T C T C G T T G A C A T T G T T C T A C G A G A A G G C T C T C GG AA C C GG AA A C T T C T C G T T G A C A T T G T T C T A C G A G A A G G C T C T C GG AA C C G G AA A C T C T C G T T G A C A T T G T T C T A C G A G A A G G C GT C T C GG GG C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G GT C T C GG GG C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C GG GG C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C GG GG C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C GT C T C G G G G C T A G A A A C G C A G A C G C C T C T G A C T T C T C G G T C C T G G C A G C C G C A G C A G G T A G T G T C C A A T G G C A G G A G T A G C A G A A A A G C C G C A G C A G G T A G T G T C C A A T G G C A G G A G T A G C A G A A A A G C C G C A G C A G G T A G T T C C A A T G G C A G C A G C C A A A G G C A G C A G C A A A T G G T C A C A A C T G A G G A G C C A A A G G C A G C A A A T G G T C A C A A C T G A G G A G C A G C C A A A G G C T A A A T G G T C A C A A C T G A G G A G C C A A A G G C T A A A T G T C A C A A C T G A G G A G C A G C C A A A G G G T A A A T G T C A C A A C T G A G G A G C A G C C A A A G G G T A A A T G T C A C A A C T G A G G A G C A G C C A A A G G C T A A A T G T C A C A A	TICAT	3540	3550	3560	3570	GIC
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GACAAGGCGTGAAAATGGTCACAACTGAGGAGCAGCCAAAGGGTAAA GACAAGGCGTGAAAATGGTCACAACTGAGGAGCAGCCAAAGGGTAAA CAAGCACTCAGAATTTAG <u>CAAGCACTCAGAATTTAG</u> 3790 CAAGCACTCAGAATTTAG	TTCTCC GTCTCC GTCTCC GTCTCC GTCTCC AGCCGC AGCCGC AGCCGC GACAAC	3590 GGAACCGG GGAACCGG GGGGCTAG GGGGCTAG GGGGCTAG GGGGCTAG CAGCAGGT AGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGCAGT CAGT CAGCAGT CAGCAGT CAGCAGT CAGT CAGT CAGCAGT C	A A A C T T C T C G ' 3600 A A A C T T C T C G ' A A A C G C A G A (A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (36700 A G T G T C C A A T (3700 A G T G T C C A A T (A T G G T C C A A (TTGACATTGT 3610 TTGACATTGT TGACATTGT CGCCTCTGAC CGCCTCTGAC 3660 CGCCTCTGAC GGCAGGAGT GGCAGGAGT GGCAGGAGT GGCAGGAGT GGCAGGAGC ACTGAGGAGCA	T C T A C G A G A A A 3620 F C T A C G A G A A O F C T A C G A G A A O F C T A C G A G A A O F C T A C G A G A A O F C T A C G G T C C T T T C T C G G T C C T 3670 T T C T C G G T C C T GA A G G T A G C A C GA A G G T A G C A C 3720 J A A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C	GGC G GGC G GGC G GGC G GGC G GGG G GGG G GGG G GGG G GAAAA JAAAA
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CAAGCACTCAGAATTTAG 3790 CAAGCACTCAGAATTTAG		3590 GGAACCGG GGAACCGG GGGGCTAG GGGGCTAG GGGGCTAG GGGGCTAG CAGCAGGT AGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT GGCGTGAA 3740 GGCGTGAA	<u>A A A C T T C T C G '</u> <u>3600</u> <u>A A A C T C T C G G</u> <u>A A A C T C T C G G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A G A G</u> <u>A A A C G C A C A G</u> <u>A A A C G C C A A T G G T C C A A T G G T C A C A J</u> <u>A T G G T C C A C A J</u> <u>A T G G T C C A C A J</u> <u>A T G G T C C A C A J</u> <u>A A T G G T C A C A J</u> <u>A A T G G T C A C A J</u> <u>A A T G G T C A C A J</u> <u>A A T G G T C A C A J</u> <u>A A T G G T C A C A J</u> <u>A A T G G T C A C A J</u>	TTGACATTGT 3610 TGACATTGT TGACATTGT TGACATTGT CGCCTCTGAC CGCCTCTGAC GGCCTCTGAC GGCCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGC ACTGAGGAGC ACTGAGGAGC	F C T A C G A G A A A 3620 F C T A C G A G A A O F C T A C G A G A A O F C T A C G A G A A O F C T A C G A G A A O F T C T C G G T C C T 3670 F T C T C G G T C C T G A G G T A G C A O G A A G G T A G C A O G A A G G T A G C A O G A A G G T A G C A O G A A G G T A G C A O G A A G G T A G C A O G A G C C A A A G G G T A G C C A A A G G G T 3770 A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T	GCCC GCCC GCCC GCCC GCCC GCCC GCCC GCC
	TTCTCC TTCTCC GTCTCC GTCTCC GTCTCC AGCCGC AGCCGC AGCCGC GACAAC GACAAC CAAGC	3590 GGAACCGG GGAACCGG GGGGCTAG GGGGCTAG GGGGCTAG GGGGCTAG CAGCAGGT CAGCAGGT CAGCAGGT CAGCAGGT GGCGTGAA GGCGTGAA GGCGTGAA CAGAGA	A A A C T T C T C G ' 3600 A A A C T C T C G ' A A A C G C A G A (A A A C G C A G A (A A A C G C A G A (3650 A A A C G C A G A (3650 A A A C G C A G A (A A A C G C A G A (3650 A A A C G C A G A (A G T G T C C A A T (A G T G T C C A A T (A G T G T C C A A T (A G T G T C C A A T (A G T G T C C A A T (A T G G T C A C A, A T G G T C A C A, 3750 A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T G G T C A C A, A T T G T C A C A,	TTGACATTGT 3610 TTGACATTGT TGACATTGT TGACATTGT CGCCTCTGAC CGCCTCTGAC 3660 CGCCTCTGAC GGCCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT ACTGAGGAGC ACTGAGGAGC	T C T A C G A GA A G 3620 F C T A C G A GA A C I C T A C G A GA A C I C T A C G A GA A C I C T A C G A GA A C I T C T C G G T C C T T C T C G G T C C T I T C T C G G T C C T GA A G G T A G C A C GA A G G T A G C A C 3720 GA A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C GA A G G T A G C A C 3720 GA A G G T A G C A C GA A G G T A G C A C GC C A A A G G G T GC C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T	GGCG GGCG GGCG GGGG GGGG GGGG GGGG GGG
	TTCTCC GTCTCC GTCTCC GTCTCC AGCCGC AGCCGC AGCCGC GACAAC GACAAC GACAAC CAAGCZ	3590 GGAACCGG GGAACCGG GGGGCTAG GGGGCTAG GGGGCTAG GGGGCTAG CAGCAGGT AGCAGGT AGCAGGT GGCGTGAA GGCGTGAA GGCGTGAA GCGTGAA ACTCAGAA 2700	A A A C T T C T C G G' 3600 A A A C T T C T C G G' A A C T T C T C G G' A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A G A G A A A C G C A A A C G C A A A C G C A A A C G C A A A C G C A A A C G C A A A C G C A A A C G C A C A	ITGACATTGT 3610 ITGACATTGT ITGACATTGT ITGACATTGT ITGACATTGT CGCCTCTGAC CGCCTCTGAC GGCCTCTGAC GGCCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGT GGGCAGGAGCA ACTGAGGAGCA ACTGAGGAGCA	T C T A C G A G A A A 3620 T C T A C G A G A A A T C T A C G A G A A A T C T A C G A G A A A T C T C G G T C C T 3670 T C T C G G T C C T G A A G G T A G C A C G A A G G T A G C A C 3720 G A A G G T A G C A A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T A G C C A A A G G G T	GGCG GGCG GGCG GGGG GGGG GGGG GGGG GGG

TCVbGenome.txt RC-TCV-BTEX-Btex3

TCVbGenome.txt RC-TCV-BTEX-Btex3

Consensus #1 Majority

Consensus #1 Majority

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3 Consensus #1 Majority

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Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3

TCV Construct Translated p38 ORF

MENDPRVRKFASDGAQWAIKWQKKGWSTLTSRQKQTARAAMGIKLSPVAQ MENDPRVRKFASDGAQWAIKWQKKGWSTLTSRQKQTARAAMGIKLSPVAQ 280@810 2820 283@840 2750 2760 27702780 2790 2850 28602870 2880 2890 MENDPRVRKFASDGAQWAI KWQKKGWSTLTSRQKQTARAAMGI KLSPVAQ MENDP R V R K F A S D G A Q WA I K WQ K K G W S T L T S R Q K Q T A R A A MG I K L S P V A Q P VOK VTRLS AP VALAYREVS TOPRVSTAR DGI TRSGSELI TTLKKNTDTE P VQKVTRLS AP VALAYREVS TQP RVS TARDGI TRS GS ELI TTL KKNTDTE 2900 2910 292@930 2940 295@960 2970 298@990 3000 30108020 3030 304(P VOK VT R L S A P VAL A Y R E V S T O P R V S T A R D G I T R S G S E L I T T L K K N T D T E P VQK VT R L S A P VAL A Y R E V S T Q P R V S T A R D G I T R S G S E L I T T L K K N T D T E P K Y T T A V L N P S E P G T F N Q L I K E A A Q Y E K Y R F T S L R F R Y S P MS P S T T G G K V P K Y T A V L N P S E P G T F N Q L I KE A A Q Y E K Y R F T S L R F R Y S P M S P S T T G G K V 3120 30708080 31008110 313@140 3150 31608170 3050 3060 3090 3180 3190 KYTTAVLNP SEPGTF NOLI KEAAQYEKYRFTSLRFRYSPMSPSTTGGKV P K Y T T A V L N P S E P G T F NQLI KE A AQYE KYR F T S L R F R Y S P MS P S T T G G K V ALAF DR DAAKP P P NDL AS L YNI E GC VS S VP WT GF I L T VP T DS T DR F ALAF DR DAAKP P P NDL AS L YNI E GC VS S VP WT GF I L T VP T DS T DR F 3210 322@230 3240 325@260 3270 3280B290 3300 3200 33108320 3330 AL AF DR DA AK P P P N DL AS L Y NI E G C V S S V P W T G F I LTVP TDSTDRF A L A F D R D A A K P P P N D L A S L Y N I E G C V S S V P W T G F I L T V P T D S T D R F VADGI S DP KL V DF GKL I MAT Y G Q G A N D A A Q L G E V R V E Y T V Q L K N R T G S T S VADGI S DP KL V DF GKL I MAT Y G Q G ANDAAQL G E V R V E Y T V Q L K N R T G S T S 334@350 3360 33703380 3390 340@410 3420 34308440 3450 346@470 348 VADGI S DP KL V DF GKLI MATYGOGANDAAOL GE VR VE YT VOLKNRTGS T S VADGI S DP KL V DF GKL I MAT Y G Q G A N D A A Q L G E V R V E Y T V Q L K N R T G S T S DAQI GDF AGVKDGP RLVS WS KTKGT AG WE HDC HFLGTGNFSLTLFYEKAP DAQI GDF AGVKDGP RLVS WS KTKGT AGWE HDCHFLGTGNF SLTLFYEKAP 34906500 3510 352@530 3540 355@560 3570 35808590 3600 361@620 363 DAQI GDF AGVKDGP RL VS WS KTKGT AG WEHDCHFLGTGNF SLTLF YEKAP DAQI GDF AGVKDGP RL VS WS KTKGT AG WEHDCHFLGTGNF SLTLF YEKAP VS GL E NADAS DF S VL GE AAAGS VQ WAGVKVAE R GQ G VK MVT T E E Q P K G K W VS GLENADAS DFS VLGEAAAGS VQWAGVKVAERGQGVKMVTTEEQPKGKW 364@650 3660 367@680 3690 370@710 3720 373@740 3750 37608770 378 VS GLE NADAS DF S VLGE AAAGS VQ WAG VK VAE R GQG VK MVT T E E QP K GK W VS GLE NADAS DF S VLGE AAAGS VQ WAG VK VAE R GQG VK MVT T E E QP K GK W QALRI -QALRI -3790

Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

Consensus #1 Majority

TCVbGenome.txt HindIII_Reverse

Consensus #1 Majority

TCVbGenome.txt HindIII Reverse

Consensus #1 Majority

TCVbGenome.txt HindIII Reverse

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3

Consensus #1 Majority

TCVbGenome.txt RC-TCV-BTEX-Btex3 QALRI

OALRI

APPENDIX B

HR TRACKING TABLES

Table B-1: Transient Expression Assay in *N. benthamiana*. Eight-week *old N. Benthamiana* plants were infiltrated with *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ in combination with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. Number indicates % of infiltrated site displaying HR lesions (tissue collapse). Three different plants were tested. Leaves 1 and 2 were taken from one plant, while leaf 3 was taken from a second plant and leaves 4 and 5 were taken a third plant.

	Day 4	Day 5	Day 6	Day 7
Leaf 1				
p28MUT	5	10	12	20
p88MUT	30	75	100	100
p88MUT	5	20	45	75
p9	2	15	20	25
p38	20	50	90	90
Leaf 2				
p28MUT	10	10	10	10
p88MUT	5	10	10	10
p88MUT	5	10	10	10
p9	5	10	10	10
p38	5	10	10	10
Leaf 3				
p28MUT	15	90	100	100
p88MUT	25	65	100	100
p88MUT	100	100	100	100
p9	100	100	100	100
p38	100	100	100	100
Leaf 4				
p28MUT	10	10	25	60
p88MUT	65	100	100	100
p88MUT	100	100	100	100
p9	80	100	100	100
p38	85	90	100	100
Leaf 5				
p28MUT	100	100	100	100
p88MUT	100	100	100	100
p88MUT	100	100	100	100
p9	100	100	100	100
p38	100	100	100	100

Table B-2: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ in combination with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. Number indicates % of infiltrated site displaying HR lesions (tissue collapse). Three different plants were tested. Leaves 1 and 2 were taken from one plant, while leaf 3 was taken from a second plant and leaves 4 and 5 were taken a third plant.

	Day 4	Day 5	Day 6	Day 7
Leaf 1				
p28MUT,p88MUT	8	50	90	90
p28MUT,p8	5	12	15	15
p28MUT,p9	5	5	5	5
p88MUT,p8	5	8	10	10
p88MUT,p9	10	15	35	70
p88MUT,p38	55	80	95	100
p28MUT,p38	20	30	40	50
p8,p9	65	75	85	95
p8,p38	10	35	75	80
p9,p38	10	15	20	35
Leaf 2				
p28MUT,p88MUT	5	5	5	5
p28MUT,p8	5	10	10	10
p28MUT,p9	8	10	10	10
p88MUT,p8	10	60	95	100
p88MUT,p9	5	10	50	60
p88MUT,p38	5	10	60	95
p28MUT,p38	5	5	5	5
p8,p9	5	8	10	10
p8,p38	10	10	12	15
p9,p38	2	2	2	2
Leaf 3				
p28MUT,p88MUT	60	80	100	100
p28MUT,p8	100	100	100	100
p28MUT,p9	95	100	100	100
p88MUT,p8	100	100	100	100
p88MUT,p9	50	100	100	100
p88MUT,p38	80	90	95	100
p28MUT,p38	80	100	100	100
p8,p9	90	100	100	100
p8,p38	100	100	100	100
p9,p38	80	100	100	100

Table B-3: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ in combination with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. Number indicates % of infiltrated site displaying HR lesions (tissue collapse). Three different plants were tested. Leaves 1 and 2 were taken from one plant, while leaf 3 was taken from a second plant and leaves 4 and 5 were taken a third plant.

	Day 4	Day 5	Day 6	Day 7
Leaf 4				
p28MUT,p88MUT	100	100	100	100
p28MUT,p8	100	100	100	100
p28MUT,p9	80	100	100	100
p88MUT,p8	100	100	100	100
p88MUT,p9	100	100	100	100
p88MUT,p38	80	100	100	100
p28MUT,p38	100	100	100	100
p8,p9	95	100	100	100
p8,p38	100	100	100	100
p9,p38	75	100	100	100
Leaf 5				
p28MUT,p88MUT	100	100	100	100
p28MUT,p8	95	100	100	100
p28MUT,p9	60	80	90	100
p88MUT,p8	100	100	100	100
p88MUT,p9	95	100	100	100
p88MUT,p38	100	100	100	100
p28MUT,p38	100	100	100	100
p8,p9	100	100	100	100
p8,p38	100	100	100	100
p9,p38	100	100	100	100

Table B-4: Transient Expression Assay in *N. benthamiana*. Eight-week old *N. Benthamiana* plants were infiltrated with *Agrobacterium* carrying a vector expressing each of the TCV ORFs at an $A_{600} = 0.3$ in combination with *Agrobacterium* carrying vectors expressing AvrPto and Pto, each at an $A_{600} = 0.3$. Number indicates % of infiltrated site displaying HR lesions (tissue collapse). Three different plants were tested. Both leaves were taken from the same plant.

	Day 4	Day 5	Day 6	Day 7
Leaf 1				
P8,p9,p38	5	8	10	10
P8,p28MUT,p88MUT	5	15	30	70
P9,p28MUT,p88MUT	5	10	25	35
P28MUT,p38,p88MUT	2	2	2	2
P8,p28MUT,p38	40	60	70	90
P9,p28MUT,p38	5	10	15	50
P8,p38,p88MUT	5	10	15	15
P9,p38,p88MUT	8	15	15	80
P8,p9,p38,p88MUT	10	10	15	25
P8,p9,p28MUT,p38	2	2	5	15
P8,p28MUT,p38,p88MUT	95	100	100	100
P9,p28MUT,p38,p88MUT	10	12	15	30
P8,p9,p28MUT,p88MUT	30	65	75	85
Leaf 2				
P8,p9,p38	15	15	15	20
P8,p28MUT,p88MUT	2	2	5	5
P9,p28MUT,p88MUT	5	10	12	15
P28MUT,p38,p88MUT	5	8	8	10
P8,p28MUT,p38	5	6	10	10
P9,p28MUT,p38	8	20	25	55
P8,p38,p88MUT	15	20	20	30
P9,p38,p88MUT	20	80	95	95
P8,p9,p38,p88MUT	5	5	10	15
P8,p9,p28MUT,p38	20	35	40	45
P8,p28MUT,p38,p88MUT	15	50	65	75
P9,p28MUT,p38,p88MUT	5	10	15	15
P8,p9,p28MUT,p88MUT	15	35	40	50