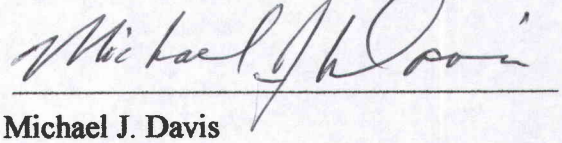
Application contains no confidential business information

Petition for Determination of Nonregulated Status for the X17-2 Line of Papaya: A *Papaya ringspot virus* – Resistant Papaya

Application contains no confidential business information

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner, which are unfavorable to the petition.



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Abbreviations and Scientific Terms

bp	base pair
CaMV 35S	35 S-cauliflower mosaic virus promoter
CP	coat protein
DNA	deoxyribonucleic acid
dsRNA	double-stranded RNA
IFAS	Institute of Food and Agricultural Sciences
Kan^r	kanamycin resistant
Kbp	kilo base pair
LB	left border in Ti plasmid
mRNA	messenger RNA
NOS -pro	nopaline synthase gene promoter
NOS -ter	nopaline synthase gene trerminator
NPTII	neomycin phosphotransferase
PRSV	*Papaya ringspot virus*
PCR	polymerase chain reaction
PTGS	post-transcriptional gene silencing
RB	right border in Ti plasmid
RNA	ribonucleic acid
T-DNA	transferred DNA
Ti plasmid	tumor-inducing plasmid
Tropical Research and Education Center	TREC
uidA	β-D-glucuronidase
University of Florida	UF

I. Rationale and Development of Papaya Line X17-2

Papaya plants have been developed at the University of Florida, Institute of Food and Agricultural Sciences, Tropical Research and Education Center, that are genetically transformed for resistance to *Papaya ringspot virus* (PRSV). PRSV is the most important papaya pathogen and a major limiting factor in commercial papaya production in the world (Conover, 1964; Gonsalves, 1998). All major production areas in the Western Hemisphere [Brazil, the Caribbean region, Mexico, and USA (Florida and Hawaii)] and Eastern Hemisphere (the Philippines, Taiwan, Thailand, and China) are affected. Once introduced to a locality, PRSV becomes established in weeds or perennial plants. PRSV has never been successfully eradicated from any region. The aphid vectors involved in dissemination of PRSV are found worldwide. Thus, even if new PRSV-free papaya trees are planted in an area where PRSV occurs, they soon become infected.

Most attempted control measures for PRSV have met with marginal success. These have included the use of insecticides against insect vectors (aphids), the removal and destruction of diseased plants, and implementation of quarantine regulations to restrict plant movement (Purcifull et al., 1984; Shukla et al., 1994). Other attempts at controlling PRSV include cross protection with a mild strain of PRSV (Yeh and Gonsalves 1994). Cross protection is very labor intensive, requiring inoculation of each plant with a mild strain of PRSV to confer marginal resistance. Plants protected from infection by severe strains, however, still exhibit mild symptoms that result in a yield reduction of 10-20% (Mau et al., 1989). Until recently, the only practical means to manage PRSV in many areas of the world has been to grow disease-tolerant varieties (Conover et al, 1986; Crane et al.1995). However, tolerance to PRSV is multigenic and difficult to breed into commercially acceptable varieties; thus, only a few tolerant varieties are available. Furthermore, the degree of tolerance in these varieties has generally proven to be inadequate. PRSV tolerance prolongs the economic life span of a crop, but the virus usually destroys the crop before it reaches its full production potential.

To overcome the problem of managing PRSV, papaya plants were genetically engineered for resistance to PRSV, and transgenic varieties are presently being grown commercially in Hawaii (Gonsalves, 1998). This technology has revitalized the Hawaiian papaya industry, which had been devastated in certain areas by PRSV (Gianessi et al., 2002; Gonsalves et al., 2004). Unfortunately, the present transgenic PRSV-resistant Hawaiian varieties cannot be grown commercially outside of Hawaii due to licensing restrictions, and even if they could be grown elsewhere, they might not be resistant to the different PRSV isolates in other geographic locations. Also, some horticultural and market characteristics of the Hawaiian varieties are not amenable for production in certain areas. For example, the varieties are intolerant to cold, which would be beneficial for production in subtropical areas, and some ethnic markets prefer larger fruit sizes than those of the Hawaiian varieties. To address this situation, we are developing papaya varieties with transgenic PRSV resistance targeted for production in the Florida, Puerto Rico, and possibly elsewhere in the Caribbean region.

Transgenic papaya expressing the CP gene of Hawaiian PRSV isolate HA 5-1 was shown to be highly resistant to PRSV isolates from Hawaii but more susceptible to a number of PRSV isolates from other geographic locations (Bau et al., 2003; Chiang et al., 2001; Gonsalves, 1998; Tennant et al., 1994; Tennant et al., 2001). In general, resistance conferred by the CP gene of a

potyvirus is RNA-mediated (Linbo and Dougherty, 1992). This resistance, which is sequence-specific and effective only when the transgene has a high homology to the CP gene of the attacking virus, is due to post-transcriptional gene silencing (PTGS) (Meins, 2000; Pang et al., 1996; Pang et al., 2000). PTGS can take place when the antisense RNA, either directly from transcription of an antisense CP transgene or indirectly from sense transgene mRNA via RNA-dependent RNA polymerases, binds to the complementary regions of viral RNA transcripts in the cytoplasm to form duplexes (Bass, 2000; Baulcombe, 1996; Dalmay et al., 2000; Sharp, 2001). These duplexes are then cleaved by dsRNA-specific nucleases into 21-23nt RNAs that interfere with transcription of homologous RNAs. Because of the homology dependence of PTGS, utilization of a PRSV CP gene of a local isolate might be a prerequisite to obtain effective PRSV resistance in transgenic papaya plants for a particular geographic region as long as genetic variation among isolates in the region is not a limiting factor. Davis and Ying (1999) examined 27 PRSV isolates from south Florida and found that the isolates were closely related based on nucleotide sequences of the CP gene and were more distantly related to 23 other isolates from throughout the world. Isolates from Puerto Rico and Mexico appeared more closely related to the Florida isolates than were isolates from more distant locations. These results indicated that transgenic PRSV-resistance based on the CP gene of a Florida isolate might be successfully used to manage PRSV in Florida and other locations in the Caribbean region.

In addition to translatable CP genes conferring resistance to potyviruses, transgene constructs with non-translatable CP genes of potyviruses have been shown to confer virus resistance in plants (Linbo and Dougherty, 1992; Smith et al., 1994; Waterhouse et al., 1998). Therefore, CP gene constructs, designed to be both translatable and non-translatable and based on the CP gene of a Florida PRSV isolate H1K, were evaluated (Davis and Ying, 2004). The CP genes in these constructs were in either the sense orientation, antisense orientation, or the sense orientation with either a frame-shift mutation or a three-in-frame stop-codon mutation. Following *Agrobacterium*-mediated transformation of somatic embryos with the different constructs, papaya plants were obtained with each construct that exhibited high levels of resistance to PRSV. Selected transgenic lines were crossed with elite papaya genotypes to initiate a breeding program for development of PRSV-resistant papaya cultivars (Davis, White, and Crane, 2003: Davis and Ying, 2004). Progeny from selected crosses are being evaluated in the field for resistance to natural infection by PRSV and agronomic performance (Table 1).

Table 1. Notifications for field evaluations of CP-mediated resistance to PRSV in papaya. All field releases were at the University of Florida, Tropical Research and Education Center, Homestead, Florida.

Notification No.	Applicant No.	Effective Date	Termination Date	Field Data Report
99-251-02n	MJD001	09/08/1999	09/01/2003	09/30/2003
03-160-02n	MJD001	06/09/2003	09/01/2004	10/27/2004
04-309-09n	MJD001	11/22/04	11/22//2005	03/01/07
06-044-01n	MJD001	3/14/06	3/14/07	N/A

The X17-2 line, containing a single insert of a CP transgene, is the subject of the present application. Although the plant of the X17-2 line was transformed with T-DNA containing the

CP gene construct with a frame-shift mutation, analyses indicate that the mutation was repaired and that the CP transgene in recent generations is both translatable and expressed. We have assigned the X17-2 line the unique identifier UFL-X17CP-6 following guidelines set forth by the Organization for Economic Co-operation and Development (http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)7). Davis and Ying (2004) have also referred to this line as FS-CP-17-2 in a report.

The X17-2 line presently consists primarily of a family of four breeding lines that are descendants of crosses between the original transgenic plant (R_0 generation) and four elite non-transgenic papaya genotypes, namely Solo Sunrise, Puerto Rico 6-65, Tainung No. 5, and Solo 40 (Davis and Ying, 2004). A process of recurrent inbreeding and selection for desired phenotypic characteristics, including resistance to PRSV, has been used to develop these advanced breeding lines and to stabilize their characteristics. They are currently in the sixth generation (R_6) of the process. In addition, some outcrosses between the advanced transgenic breeding lines and selected non-transgenic papaya genotypes have been made in an effort to produce improved breeding lines. Varieties are being developed with characteristics suitable for the green fruit and/or fresh fruit markets. Three other transgenic PRSV-resistant lines (R_6 generation) are also presently under development. One, the X26 line, was derived from a R_0 plant with an independent insertion of the same frame-shift CP transgene construct as is present in the X17-2 line, and the R_0 plant was crossed with the Experimental No. 15 non-transgenic papaya genotype. The other two lines, D6 and D95, were originally transformed with the PRSV CP gene construct with the stop-codon mutation (Davis and Ying, 2004). Three breeding lines originally crossed with the Red Lady, Solo 40 or Solo Sunrise genotypes presently make up the D6 family. The D95 line presently consists of one breeding line that was derived from a cross of the R_0 plants with the Solo Sunrise variety. Although the X26, D6, and D95 lines were originally also crossed with other elite non-transgenic genotypes, only the present breeding lines have been advanced to the present stage of development. Two-hundred fifty-two other transgenic lines (R_0 generation) were eliminated at earlier stages in the breeding program. None of the X26, D6, or D95 lines appears significantly better than similar lines in the X17-2 family. Consequently, deregulation of the X17-2 line appears to be the most rapid and economical way to produce several PRSV-resistant papaya varieties for growers in Florida and elsewhere in the Caribbean region.

In addition to this petition, we have initiated dialogs with the FDA and EPA regarding our efforts to release the X17-2 line of transgenic papaya to the public. A consultation with the FDA and EPA is currently underway.

II. The Papaya

As part of the Series On Harmonization Of Regulatory Oversight In Biotechnology No. 33 of the Organization for Economic Co-Operation and Development a consensus document on the biology of papaya is available at http://appli1.oecd.org/olis/2005doc.nsf/linkto/env-jm-mono(2005)16 .

A. Papaya as a Crop

The papaya is a small, tree-like herbaceous plant that is widely grown in the lowland tropics where it is frequently grown in gardens and dooryards in addition to commercial production. The fruits are most often consumed as a fresh dessert fruit, and it provides a good source of vitamins A and C (de Arriola et al., 1980). The fruits are sometimes processed into a beverage. Fruits are also eaten cooked or as raw vegetables. In southeast Asia, green fruits are often grated to produce a salad. Latex from various plant parts yields the proteolytic enzyme, papain, which is used as a meat tenderizer. The plant growth habit is perennial, but the juvenile period is short. The first fruits mature 9-12 months after plantings and then plants continue to bear fruit more or less continuously. After three to four years, the yield declines and the trees become too tall for efficient harvesting. In 2005, 6.75 million metric tons of papaya fruit were produced worldwide (FAOSTAT, 2006). Commercial papaya production in the United States occurs primarily in Hawaii, and secondarily in Puerto Rico and southern Florida.

B. Taxonomy of Papaya

The Caricaceae is a family of tropical dicot species. Until recently, the Caricaceae was thought to comprise 31 species in three genera (namely *Carica*, *Jacaratia* and *Jarilla*) from tropical America and one genus, *Cylicomorpha*, from equatorial Africa (Nakasone and Paull, 1998). However, Badillo (2000) proposed that some species formerly assigned to *Carica* were more appropriately classified in the genus *Vasconcella. A*ccordingly, the family's classification has been revised to comprise *Cylicomorpha* (2 species) and five South and Central American genera [*Carica* (1 species), *Jacaratia* (7 species), *Jarilla* (3 species), *Horovitzia* (1 species) and *Vasconcella* (21 species)(Badillo, 1971)], with *Carica papaya* the only species within the genus *Carica* (Badillo, 2000). Phylogenetic studies based on molecular techniques suggest that *Carica papaya* diverged early in the evolution of the species within Caricaceae and evolved in isolation probably in Central America (Aradhya et al., 1999; Kim et al., 2002).

C. Reproduction and Pollination of Papaya

Papaya is a polygamous species, having a mating system that is either dioecious (staminate and pistillate plants) or gynodioecious (hermaphrodite and pistillate plants). Commercially, gynodioecious lines are often preferred because of their potential for inbreeding and consequent uniformity. Hermaphrodites can be self-pollinated to homozygosity, except for sex characters, yielding gynodioecious lines that segregate in a ratio of two hermaphrodites to one female. Sex inheritance is controlled by a single locus with multiple alleles (Hofmeyer, 1967; Horovitz and Jimenez, 1967; Storey, 1976). Staminate plants (M_m/m) and hermaphrodites (Mh/m) are heterozygous, while pistillate plants (m/m) are homozygous for the recessive allele. The homozygous condition involving either dominant allele is lethal. A low out-crossing rate occurs in hermaphrodites since they tend to be andromonoecious, producing copious pollen from staminate flowers during most of the year and self-pollinate before petals open. Pistillate plants never produce anthers and are consequently obligate out-crossers. Each pollination produces hundreds of seeds, which are easily recovered from ripe fruits. In nature, pollination occurs through insects and wind.

D. Biology of the *Papaya ringspot virus*

PRSV (Murphy et al., 1995) is a member of the potyvirus group, which consists of about 160 members. The potyvirus group is the largest and most economically important group of plant viruses (Matthews, 1991). PRSV is an approved species in the genus *Potyvirus*; family *Potyviridae*. Jensen (1949a) first reported PRSV in *Carica papaya*. Synonyms for PRSV include papaya distortion mosaic virus, papaya leaf distortion virus, papaw distortion ringspot virus, papaw mosaic virus, and watermelon mosaic virus type 1 (Purcifull et al., 1986). PRSV has been divided into two types: PRSV-P type isolates that infect papaya and PRSV- W type isolates that infect watermelon (PRSV-W isolates were previously classed as watermelon mosaic virus type 1). PRSV-P infections of papaya are a major limiting factor in papaya production in Florida and many other regions where papayas are grown. PRSV-P infects papaya and cucurbits, but watermelon mosaic virus 1 (PRSV-W) infects cucurbits, but not papaya. Analysis of the coat protein genes of isolates from Australia, Thailand and the U.S.A. indicates that, in each country, PRSV-P isolates have evolved independently from local PRSV-W isolates (Bateson *et al.*, 1994). The exact nature of the difference between PRSV-P and PRSV-W isolates, other than host range, is unknown. The H1K isolate of PRSV-P from which the CP gene was obtained for construction of the CP transgene in the X17-2 line was originally obtained from a papaya plant growing near Homestead, Florida (Davis and Ying, 1999).

Viruses of the potyvirus group are flexuous rods comprised of a positive-sense RNA genome surrounded by a protein coat (capsid). The protein coat is comprised of repeating subunits of the virus coat protein (CP). The RNA genome is translated in the infected host (plant) cell to yield a polyprotein that is subsequently cleaved into at least a dozen proteins, one of which is the viral CP. The functions of some of the proteins have not been identified completely. Some of the identified functions include coat protein, nuclear inclusion body, cylindrical inclusion body, helper component involved in insect transmission, helicase, several proteases, replicase, genome-linked protein, and ATPase. During the infection process, the virus uses the host cell to make copies of the viral genome as well as the viral proteins. Then the viral RNA and CP subunits self-assemble into complete virus particles (virions) which may spread within the plant or to other plants by insect or mechanical transmission. Replication does not depend on a helper virus.

Potyviruses are easily transmitted mechanically to a relatively narrow range of hosts. The great majority are transmitted by many aphid species in a nonpersistent manner. PRSV was first reported transmitted by aphids by Jensen (1949b).

III. Description of Transformation System

The vector (pBI121fs; Figure 1) used to transfer the genes to papaya plants was constructed from the binary vector pBI121 (Clontech, San Francisco, CA), a derivative of pBIN19 (Bevan 1984), by replacing the GUS gene with a construct of the CP gene of PRSV. The complete sequences of pBIN19 (Genbank accession number U09365; Frisch et al., 1995) and pBI121 (Genbank AF485783; Chen et al., 2003) are published. The transformation elements in the vectors were derived from the plasmid pTiT37 from *Agrobacterium tumefaciens* (Zambryski et al. 1980). All tumorigenic DNA was removed in development of the plasmid vectors, and

therefore recipient plants do not develop crown gall disease. Ying et al. (1999) described the method for Agrobacterium-mediated transformation and regeneration of papaya plants from somatic embryos that was used to develop the X17-2 line. T-DNA , which included the CP gene of PRSV isolate H1K modified by incorporation of thymidine to create a frame-shift mutation and the *npt*II gene, was transferred to the genome of individual papaya somatic embryos and plants regenerated from the transformed embryos as described by Davis and Ying (2004).

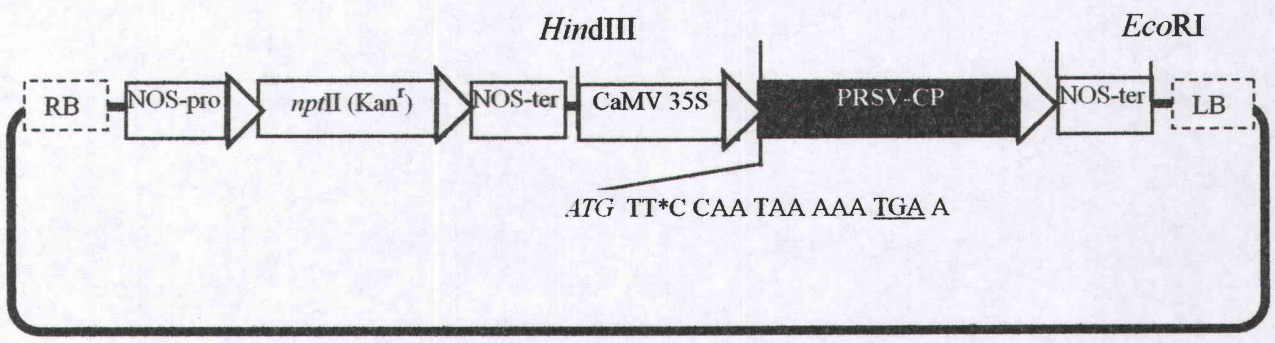


Figure 1. pBI121fs: Schematic illustration of the plasmid vector that was transferred into *Agrobacterium tumefaciens* by electroporation. Various components of the T-DNA of pBI121fs are described in Table 2. Map reflects the gene order but not the sizes. The T-DNA between the left (LB) and right (RB) borders has restriction sites for *Hin*dIII and *Eco*RI. Insertion of thymidine, as indicated by an asterisk, resulted in creation of the underlined stop codon downstream. The region outside the RB and LB is the plasmid vector backbone DNA that was not inserted into the plant DNA following transformation.

IV. Donor Genes and Regulatory Sequences

A. PRSV Coat Protien

The CP gene of PRSV isolate H1K was cloned and sequenced (Davis and Ying 2004). The sequence is deposited in Genbank as accession number AF196839. The primers used in transgene construction were: F01 (5'-AG**AACTAGT**CCCGGGTGGTCAGTCCCTT*ATG*TTC CAAAAATGAAGCTGTGGATG-3') and R01 (5'-TACAGATCTACCC TCACTATAAAATA GAAGC-3'). A BglII site in R01 is underlined, and SpeI site in F01 is in bold type, and the first initiation codon ATG is in italic type. The cloned CP gene was used as a template to amplify the modified CP by PCR. Based on reports by Cheng et al (1996, 2000) the uidA leader was more effective for transformation than a homologous virus leader and enhanced the expression of the PRSV CP. Therefore, the *uid*A leader with an initiation codon (5'-CTAGTCCCGGGTGGTCAGTCCC TT*ATG*-3') was placed at the 5' end of the PRSV CP construct by inclusion of the sequence in the F01 primer. PCR was performed and the fragment was confirmed by sequencing. The binary vector pBI121 was double-digested with *Sma*I and *Sac*I, blunted with T4 DNA polymerase and then religated with T4 DNA ligase to produce

pBI121zy. Linearized pBI121zy was produced by digestion with *Xba*I and *Bam*HI. The PRSV-CP fragment was separated by agarose gel electrophoresis, isolated from the gel, double-digested with *Bgl*II and *Spe*I, and then cloned into the linearized pBI121zy to produce pBI121fs (Figure 1) (Davis and Ying, 2004). The CP of PRSV is naturally encoded as part of a polyprotein , and the CP coding region lacks a 5'-untranslated region and a translation initation codon. The construct developed for transformation of papaya provides these sequences. Transcription of the modified coat protein gene in transformed plants is directed by the 35S promoter of CaMV and terminated by the NOS terminator sequence of *A. tumefaciens* (Table 2).

B. Neomycin Phosphotransferase Gene

The *npt*II gene from the transposon Tn5 of the bacterium *Escherichia coli* (Berg et al., 1975) was introduced into papaya as a selectable marker. The gene codes for the enzyme neomycin phosphotransferase that confers resistance to the common aminoglycoside antibiotic kanamycin. The *npt*II gene is under control of the NOS promoter and NOS terminator of *A. tumefaciens* (Table 2).

Table 2. Summary of DNA components of pBI121fs. Total size from right to left borders including border sequences is 5.3 Kb. Linker and other non-functional sequences are not included in table but account for approximately 1.9 kb between the right and left border sequences.

Genetic Element	Size (Kb)	Source	Function
RB	0.025	*Agrobacterium tumefaciens* T37 (nopaline strain)	Right border of the plasmid pTiT37 (Zambryski et al. 1980) used to initiate T-DNA transfer from *Agrobacterium tumefaciens* to the plant genome (Depicker et al., 1982; Wang et al., 1984)
NOS-pro	0.307	*Agrobacterium tumefaciens* T37	Promoter for transcription of the *npt*II gene from plasmid pTiT37 (An, 1986; Bevan et al., 1983a; Fraley et al., 1983)
*npt*II (Kan^r)	0.795	*Escherichia coli*	Neomycin Phosphotransferase type II (NPTII) enzyme (Berg et al., 1975) conferring resistance to kanamycin and thus allowing for selection of transformed plant cells (Bevan et al 1983b; Fraley et al 1983; Hoekema et al 1983; Bevan 1984)
NOS-ter	0.256	*Agrobacterium tumefaciens*	A 3' nontranslated region of the nopline synthase gene from plasmid pTiT37 involved in transcription termination and polyadenylation (Bevan, 1984)
CaMV 35S	0.835	*Cauliflower mosaic virus*	Promoter that directs transcription of the coat protein gene (Franck et al., 1983; Odell et al., 1985)

(Continued on next page)

*uid*A leader	0.024	*Escherichia coli*	Enhanced expression of PRSV CP in papaya (Cheng et al., 1996, 2000)
PRSV-CP	1.005	*Papaya ringspot virus*	Coat protein gene of PRSV H1K with frame-shift mutation (Davis and Ying, 2004)
LB	0.026	*Agrobacterium tumefaciens*	Left border sequence was derived from the Ti plasmid pTiT37 (Zambryski et al. 1980) which has been mapped in detail (Depicker et al. 1982). Its function is to delineate the DNA that is being transferred from Agrobacterium tumefaciens to the plant cells during transformation.

None of the introduced genes has any inherent plant pest characteristics or poses a risk to plant health when introduced into the modified plant.

V. Genetic Analyses and Agronomic Performance

The analysis demonstrates that the X17-2 line contains an *npt*II gene whose transcription is directed by the nopaline synthase promoter and whose termination sequences are also derived from the nopaline synthase gene, and contains the PRSV CP whose transcription is directed by the cauliflower mosaic virus 35S promoter coupled with the *uid*A leader sequence and whose termination sequences are derived from nopaline synthase. Both proteins are expressed. The T-DNA insertion sequence begins 12 and 30 nucleotide base pairs inward from the LB and RB, respectively, of pBI121fs that were originally derived from *Agrobacterium tumefaciens* T37 (Figure 1 and Table 2) and was inserted at an unidentified single location within the plant genome.

A. Insertion Analysis

Nuclear DNA of the X17-2 line was isolated from the R_0 plant using Floraclean (Q.Bio gene, Carlsbad, CA) and digested with *Eco*RI or *Hin*dIII that each cut the T-DNA once. The digested DNA was electrophoresed, transferred onto nylon membranes, and probed following the method described by Ying et al. (1996). A 555-bp fragment of the PRSV CP gene was generated by PCR using cloned PRSV H1K CP DNA as a template. The fragment was DIG-labeled (Boehringer Mannheim, Germany) and used as the hybridization probe. A single copy of the transgene construct was detected (Davis and Ying, 2004). A single copy number was also indicated in nuclear DNA from R_5 generation transgenic plants similarly digested with *Hin*dIII, blotted onto a membrane, and probed with a gel-purified, p^{32}-labelled, 1947-bp *Pst*I-fragment of pBI121 containing a portion of the *npt*II gene and the NOS terminator (appendix I). Further Southern analysis of the same DNA, either digested with *Hin*dIII or double digested with *Eco*RI and *Pst*I, and probed with the gel-purified, combined 4923-bp and 4872-bp, p^{32}-labelled, *Bgl* II and *Pst*I restriction fragments of pBI121 DNA, which contained approximately 94% the 10,432-bp vector backbone region, indicated that none of the vector backbone had been incorporated into the X17-2 and D6 lines (appendix I).

prior to the analyses. The flanking region was cloned and sequenced following a procedure modified from that of Chen et al (2003), using inverse PCR analysis (Does et al., 1991). In the procedure, total DNA from a X17-2 line plant was digested with *Taq*I, which cut the DNA on both sides of the plant DNA/T-DNA border, the resulting DNA fragment was circularized by ligation with T4 DNA ligase, and then cut with *Ssp*I to re-linearize the DNA in such a manner as to leave known vector sequences on both sides of the plant border DNA. Nested PCR, using

gtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggccacgacgggcgttcct
tgcgcagctgtgctcg*ATAGATAAAATAAATTAACCACAATATTTGATTAAAACCATGTAGAATA*
CATTTAAACACACTCACATGTAACGTGTCTTAAATCAAATTAAATGCATTGATTTGTCAGATAAA
TTAACTACATAATATTTTATCAAAAATATAAAAGATACAGATGTGGGATATAATTTTAAGCACAT
TAATATCATTCTTCATCTAGTAATACTAAAAATAAAAGGAGGTGAAAGGACCTAAAGAAAAACTC
GGATCCTTTTAACATAGAGAACAACTAGATAAAAAATTAAATTTATGAAATTAAGTGGTCAAAGG
*TTATTATTATGCAGTGCGAGATGA***ttaaactgaaggcgggaaacgacaatctg**

Figure 2. Sequence of the plant/T-DNA right border (RB) region in the X17-2 transgenic line of papaya. The RB region was amplified by PCR using forward and reverse primers sites indicated by the arrows, and the amplified DNA was then cloned in pGEM-T and sequenced. The RB region is comprised of the plant DNA (both italic and upper case letters) and the T-DNA border sequence (both bold and lower case letters). In the inverse PCR procedure used to obtain the RB region, another portion of the T-DNA (lower case letters only) was ligated to the plant DNA following restriction digestion by *Taq*I resulting in known T-DNA sequences with primer sites on both sides of the plant DNA.

primers for the known vector sequences on each side of the plant border DNA, was then used to amplify the border region DNA. The amplified border DNA region was then cloned in pGEM-T and sequenced. The original procedure of Chen et al (2003) was modified by adding the nested PCR amplification using primers that we designed: RB nested forward, 5'-GTGCCCTGAAT GAACTGC-3'; RB nested reverse, 5'- CAGATTGTCGTTTCCCGC-3'. Only one PCR product was obtained from both the primary and nested PCR reactions indicating a single insertion site of the transgenes in the plant genome (Figure 3), thus confirming the result of earlier Southern analysis.

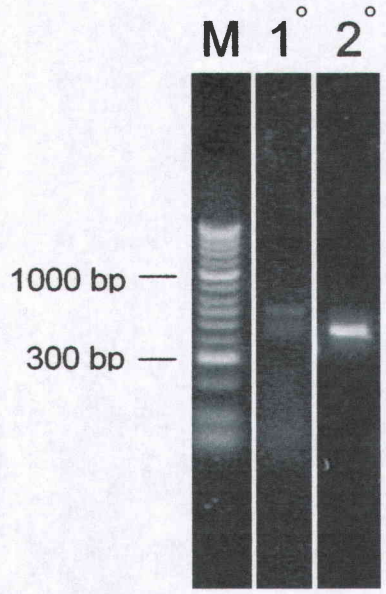
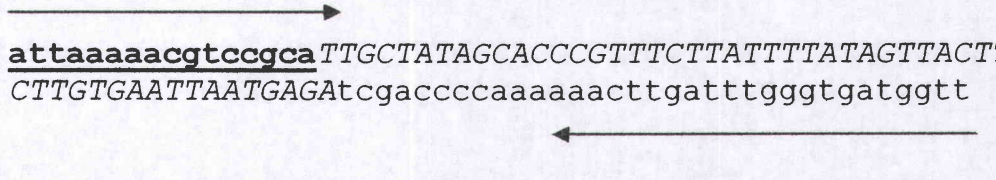


Figure 3. Results of nested PCR amplifications of the right border (RB) region of the T-DNA in the X17-2 line of transgenic papaya are shown. Single reaction products were obtained in both amplifications indicating that a single copy of the T-DNA was inserted in the plant DNA. Primary and secondary PCR reaction products were separated by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination. Molecular size markers (M) were Hyperladder II (Bioline USA, Inc.)

The left border region was also amplified, cloned, and sequenced (Figure 4).). The region was excised with *Taq*I, circularized by ligation of the ends, and linearized using the restriction enzyme *Ava*I. The primers for nested PCR amplification of the left border region analyses were the primary forward primer , 5'- AGAAAAACCACCCCAGTACA -3', primary reverse primer, 5'- TGAGTGTTGTTCCAGTTTGG -3'; nested forward primer, 5'- ATTAAAAACGTCCGCAATGT -3'; nested reverse primer, 5'- AACCATCACCCAAATCAA GT -3'. A visible amplification product was not obtained in the primary reaction, but one product was obtained in the nested PCR reaction. Again, this indicated that there is only a single insertion site of the transgenes in the genome of the X17-2 line. The border analyses, in addition to confirming the single copy number of transgene insertion, provide specific means to identify plants of the X17-2 line based on the sequences of the vector/plant DNA junctions.



attaaaaacgtccgca*TTGCTATAGCACCCGTTTCTTATTTTATAGTTACTTTTGCCTTTGT*
*CTTGTGAATTAATGAGA*tcgaccccaaaaaacttgatttgggtgatggtt

Figure 4. Sequence of the plant/T-DNA left border region in the X17-2 transgenic line of papaya. The region was amplified by PCR using forward and reverse primers sites indicated by the arrows, and the amplified DNA was then cloned in pGEM-T and sequenced. The LB region is comprised of the plant DNA (both italic and upper case letters) and the T-DNA border sequence (both bold and lower case letters). In the inverse PCR procedure used to obtain the LB region, another portion of the T-DNA (lower case letters only) was ligated to the plant DNA following restriction digestion by *Taq*I resulting in known T-DNA sequences with primer sites on both sides of the plant DNA.

A 4935 bp PCR fragment containing all but approximately 336 bp of the T-DNA insert from a R_5 generation plant of the X17-2 line was cloned and sequenced, and the expected and observed sequences were compared (Appendix II). The homology was 99.7%. Most of the deviation from expected occurred at the sites were the CP construct had been ligated into the T-DNA and appears to be of no consequence. However, the thymidine base originally incorporated into the leader sequence for the CP gene to create a frame-shift mutation (Figure 1) was missing. Consequently, the CP gene is translatable and not non-translatable as intended, and western analyses, described below and in Figure 5, indicate that the CP gene is expressed.

B. Mendelian Inheritance

R_0 generation of the X17-2 line was a female and crossed with five non-transgenic hermaphroditic papaya genotypes to produce the R_1 generation. Until homozygousity of the transgen was reached in a breeding line, seedlings of the R_1 and each subsequent generation were sprayed with kanamycin sulfate (2mg/ml) to detect the presence of the *npt*II gene as indicated by failure to develop bleached leaves (Davis and Ying, 2004). The segregation of the transgenes in the X17-2 line as indicated by kanamycin resistance in seedlings of the R_1 and R_3 generations was as expected for a single insertion site of the transgenes (Table 3). In the R_2 generation, although significantly different from the expected frequency of 75%, the observed frequency of 79.8% was still close. In each of the subsequent generations of the X17-2 line, selected inbred hermaphrodite plants have been perpetuated by bagging flowers to promote self-pollination and prevent cross-pollination. In the R_4 generation of the X17-2 line, 15 of 20 progenies from the R_3 selections are homozygous for the transgenes.

Table 3. Goodness of fit for phenotypic expression of resistance to kanamycin as expected for Mendelian segregation of transgenes in progeny of heterozygous parents of the X17-2 line.

Generation	No. seedlings	No. Kan^r seedlings	Expected Ratio	Chi-square value	*P*
R_1	1258	630 (50.1%)	1:1	0.0032	0.9550
R_2	911	727 (79.8%)	3:1	11.2056	0.0008
R_3	335	261 (77.9%)	3:1	1.5134	0.2186

C. Expression of Insert Genes

Resistance to kanamycin indicated expression of the *npt*II gene in the X17-2 line. The PRSV CP is also expressed but at low levels compared to those in papaya infected with PRSV. ELISA using polyclonal anti-PRSV serum indicated a possible low concentration of the PRSV CP in uninfected X17 and D6 breeding lines (Table 4). Similar results were obtained using another polyclonal PRSV antiserum (WMV-1 antiserum) obtained from Dan Purciful at the University of Florida (data not shown). Also, the 35 KDa PRSV CP was detected by Western-blot analyses using the same antisera (Figure 5). Cheng and Yeh (2000) used the same Western-blot procedure earlier to detect expression of the CP gene of PRSV with the *uid*A gene translation leader in transgenic tobacco plants with similar results. The 35 KDa size of the expressed PRSV CP in the X17 and other transgenic lines was the same as predicted from the nucleotide sequence of PRSV

RNA, although the natural CP is one of at least seven proteins that are cleaved post-translationally from the expressed polyprotein of PRSV in infected plants (Yeh et al., 1992). The CP constructs in the X17-2, D6, and D95 lines were designed to be non-translatable due to insertion of stop- codon or frame-shift mutations at the 5'-end of the CP gene, but the Western-blot results indicate that the mutations were read through or repaired resulting in expression of the CP gene.

Table 4. ELISA for PRSV coat protein in papaya leaves from transgenic and non-transgenic lines[a]. The Hawaiian 'Rainbow' contains a positive sense, translatable PRSV coat protein gene. The X17-2 and D6 lines contain the PRSV coat protein gene (H1K Florida strain). The negative control plants were non-transgenic segregants among the X17-2/Solo Sunrise progenies. The positive control plants were 'Red Lady' papaya infected with PRSV H1K. Only the positive control was inoculated with PRSV and had symptoms of the disease.

Line	No. Plants	Mean O. D.[b]	Standard Deviation
Rainbow	5	0.049	0.107
X17-2 (Solo Sunrise progenies)	5	0.222	0.125
X17-2 (Tainung No. 5 progenies)	5	0.158	0.070
X17-2 (Puerto Rico 6-65 progenies)	5	0.181	0.037
D6 (Solo Sunrise progenies)	5	0.188	0.056
Positive Control	2	2.547	0.018
Negative Control	2	0.094	0.019

[a] Newly expanded leaves were sampled, and tissue was homogenized in 0.05 M carbonate-bicarbonate buffer, pH 9.6 (1:10 wt/vol). Three, 100-µl aliquots were assayed per sample. Double antibody sandwich (DAS) ELISA was conducted using alkaline phosphatase-conjugated second antibody and p-nitrophenyl phosphate as substrate.
[b]Optical densities (O.D.) of reactions were determined at 405 nm.

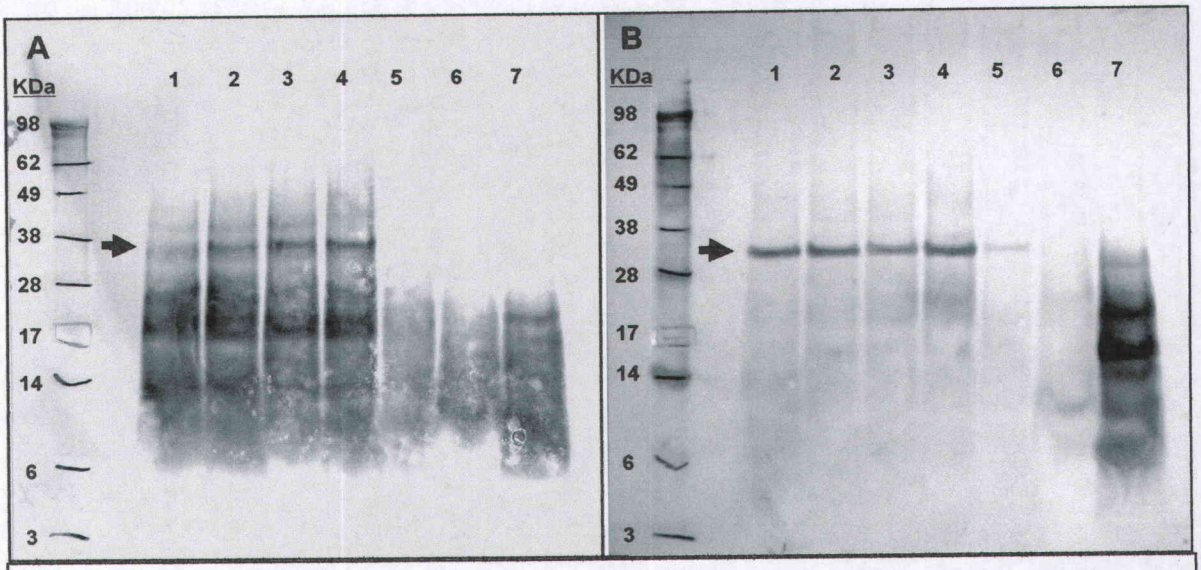


Figure 5A and 5B. Western Blots of total papaya leaf protein from the X17-2, D6, and D95 transgenic lines, and from non-transgenic Solo Sunrise papaya that was healthy or infected with PRSV H1K. Samples containing 80 µg of protein were separated by SDS-PAGE on a precast 12% NuPAGE gel with MES running buffer and transferred to PDVF membranes. In Figure 5A, the membrane was probed with anti-PRSV serum (Agdia, Inc.), and in Figure 5B, the membrane was probed with another antiserum for PRSV (D. Purciful, University of Florida). The samples were (1) X17-2 line with Solo Sunrise progenitor, (2) X17-2 line with Tainung No. 5 progenitor, (3) D6 line with Solo Sunrise progenitor, (4) D6 line with Solo 40 progenitor, (5) D95 line with Solo Sunrise progenitor, (6) Solo Sunrise, (7) Solo Sunrise infected with PRSV. The arrow in both figures indicates the location of the 35 KDa PRSV CP that was found only in transgenic lines.

D. Disease and Pest Resistance Characteristics

The X17-2 line of transgenic papaya has been field tested for seven years at the Tropical Research and Education Center, UF/IFAS, near Homestead, Florida. Field plots were observed almost daily and maintained using standard cultural practices including application of insecticides and fungicides. Common diseases and pests (Pernezny and Litz, 1993; Mossler and Nesheim, 2002) observed included powdery mildew (*Oidium caricae*), corynespora leaf spot (*Corynespra cassicola*), anthracnose (*Colletotrichum gloeosporoides*), papaya fruit fly (*Toxotrypana curvicauda*), and two-spotted mite (*Tetranychus urticae*). Transgenic plants within the field plots were compared to each other and to non-transgenic control plants. Plants with PRSV were discarded immediately upon detection to prevent further spread. No changes in susceptibility to common diseases and pests, except for PRSV, were observed when compared to non-transgenic papaya plants in the same fields.

The X17-2 line of transgenic papaya is highly resistant to natural infection by PRSV in the field (Table 5). The original transformed X17-2 plant (R_0) was crossed with five papaya genotypes, and the progenies of this or progenies of subsequent selections thereof were planted in the field and observed for the development of PRSV for approximately one year each. The incidence of PRSV infections were highest in the first generation. X17-2 plants developing PRSV were subsequently eliminated from the variety development program, and the PRSV

incidence was very low in the subsequent generations. The incidence of PRSV in plants not protected by the CP transgene was considerably higher than in the X17-2 plants in each generation. For comparison with the R_1 generation of the X17-2 line, 30 non-protected plants, consisting of five plants each of 'Red Lady', 'Puerto Rico 6-65', 'Puerto Rico 6-65 Dwarf', 'Cariflora', and 'Maradol', were planted at the same location, and 96.7% of these, all but one plant of 'Cariflora', became infected with PRSV. For the R_2 generation, 120 'Red Lady' plants were planted immediately adjacent to the planting containing the X17-2 plants, and 45% of the 'Red Lady' plants became infected with PRSV. For the R_3 generation, the 324 non-protected plants comprised of 23 named varieties or advanced breeding lines and some additional accessions were included, and 100% of these plants developed PRSV (Davis et al, 2004). The disease pressure on the R_3 generation was especially great considering the large number of infected non-transgenic plants present, and yet no incidence of PRSV was observed in all X17-2 lineages, except for four of 76 plants of the X17-2 x E15 lineage, which was subsequently eliminated from the variety development program. The R_4 and R_5 generations were severely affected by several hurricanes and many damaged plants were removed before they might have become infected by PRSV. Nevertheless, PRSV occurred in the non-protected plants but not those protected by the X17-2 transgene.

Table 5. Incidence of natural infections by PRSV in field plots of three generations of the X17-2 line of transgenic papaya containing the coat protein gene of PRSV strain H1K made non-translatable with a frame-shift mutation.

	No. PRSV/no. plants for progeny of the given male parent:							Non-protected plants	
Generation	PR 6-65	E15	Solo 40	Solo Sunrise	Tainung No. 5	Grand total	% Infected	No. PRSV /no. control plants	% Infected
R_1	6/40	4/16	5/32	15/49	5/41	35/178	19.7	29/30	96.7
R_2	0/90	0/90	0/60	0/89	0/30	0/390	0	54/120	45.0
R_3	0/178	4/76	0/30	0/201	0/51	4/536	0.7	324/324	100
R_4	0/230	-	0/30	0/210	0/90	0/560	0	13/195	6.7
R_5	0/180	-	0/30	0/180	0/60	0/450	0	27/150	18.0

Non-transgenic papaya and transgenic papaya (R_3 generation) were mechanically inoculated with three isolates (H1A, H1C, and H1K) of PRSV, and the severity of PRSV symptoms was compared (Davis et al., 2004). The PRSV isolates were selected to represent the genetic diversity of the virus in Florida (Davis and Ying, 1999). All three PRSV isolates were originally obtained from naturally infected papaya in grower's fields in south Florida, and the sequences of their coat protein genes had been compared with those of other isolate from south Florida and other geographic locations throughout the world. The resistance of the transgenic lines to PRSV strains from locations outside Florida was not examined due to concerns regarding their possible escape in Florida. Inoculations with all three PRSV isolates from Florida clearly demonstrated that transgenic plants containing a CP transgene derived from the H1K isolate were not only resistant to the homologous PRSV isolate but also resistant to the

H1K isolate were not only resistant to the homologous PRSV isolate but also resistant to the other two PRSV isolates. All transgenic and non-transgenic plants inoculated with PRSV became infected. After inoculation, the non-transgenic varieties developed symptoms faster and to a consistently greater extent. Among the non-transgenic varieties, the 'Red Lady' variety was more tolerant to PRSV than the 'Solo Sunrise' variety, as expected based on known susceptibilities to PRSV of the varieties and confirmed by results of field experiments (Davis et al., 2004). The difference in disease severity in the 'Red Lady' and 'Solo Sunrise' varieties was much less than that between the non-transgenic varieties and PRSV-resistant transgenic lines. Both of the non-transgenic varieties eventually developed severe disease; whereas, symptoms in the PRSV-resistant lines usually never surpassed being mild. Plants of both the D6 line and X17-2 line were tested and were equally resistant to PRSV. A transgenic line with the *npt*II gene but not the CP gene reacted very similarly to the non-transgenic varieties, strongly indicating that the presence of the CP gene in the transgenic plants was largely responsible for the observed limitation to disease progress.

E. Phenotypic Characteristics

The aim of the breeding program has been to develop new papaya varieties that are not only resistant to PRSV but also well adapted for commercial production in south Florida. Each breeding line within the X17-2 transgene line has its own characteristics, because the original pollinations of the R_0 plants were outcrosses and no efforts were made to select exactly for the phenotypes of the original progenitors. Inbreeding and selection for desirable phenotypic traits has resulted in increased uniformity among the progeny of each subsequent generation within the inbred lines. Except for the resistance to PRSV, all phenotypic characteristics of the X17-2 breeding lines are generally within those observed for non-transgenic papaya varieties. The ubiquitous presence of PRSV in non-transgenic papaya, but not the transgenic PRSV-resistant transgenic papaya, in our field plots does not permit a fair comparison of most phenotypic traits between the two groups. Some information, such as nutritional characteristics of ripe fruit (Table 6), can be obtained elsewhere for comparison. However, such comparisons are limited because parameters affecting phenotypic characteristics, such as growing location, environment, fertilization, and the protocols used to measure and record these characteristic are not always the same. Nevertheless, some general observations comparing the X17-2 line of transgenic plants to their non-transgenic counterparts can be made:

a. Growth habit and ability to over-winter: Leaf, stem, and fruit morphologies of X17-2 plants are normal throughout the year. In contrast, the leaf, stem, and fruit morphology of nearby (within the same field)infected non-transgenic papaya shows typical PRSV symptoms (i.e., leaf distortion and mottling, stem streaking, and ringspots on the fruit) to greater or lesser degrees throughout the year. X17-2 plants continuously grow throughout the year; more vigorously when ambient temperatures are above 65°F and more slowly during cool weather (i.e., temperatures 50-65°F). In contrast, nearby PRSV-infected non-transgenic papaya does not grow as vigorously during warm periods and completely stops growing during cool periods. Once warm temperatures commence in the spring, X17-2 papayas resume vigorous growth whereas PRSV infected non-transgenic papaya only slowly resume growth; not growing vigorously until high temperatures in mid-summer.
b. Life-span and vegetative vigor: Papaya plants may live up to 20 years however, typically, viral infection, other diseases, or natural disasters reduce their life-span to 4 to 5 years; commercial plants are replaced after 2-3 years. The fifth generation of the X17-2

papayas has been grown for over two years with some losses due to 4 hurricanes in 2005. Those X17-2 plants surviving the hurricanes' wind damage went on to recover leaf canopy, grow, and fruit normally during 2006 and 2007. In contrast, the surviving non-transgenic papayas in nearby commercial fields showed severe PRSV symptoms and were either abandoned or replaced.

c. Number of days to onset of flowering and fruit maturity: The days from planting to first flowering and the rate of fruit development is influenced by inherent genes, environmental conditions (mostly temperature), and cultural practices. During the summer, we have not noted any difference in the days to flowering or rate of fruit maturity of the X17-2 papayas and PRSV-tolerant papaya cultivars (e.g., 'Red Lady'). During the spring, fall, and winter fruit development does appear to be slower for non-transgenic plants compared to the X17-2 papayas most likely due to the effect of the PRSV on leaf area and health.
d. Seed parameters and survival of seedlings: No differences among X17-2 line papayas and non-transgenic papayas in seed production, dormancy, percent germination, seedling survival, or seedling emergence were observed.
e. Plant survival: Papaya plant survival is greatly influenced by inherent genetic susceptibility to PRSV and thus cultivars greatly differ in their survivability. Previous comparisons by the authors have clearly demonstrated that the X17-2 papayas are resistant to PRSV whereas most of the 'Solo' types succumb to the virus; some cultivars such as 'Red Lady' show tolerance to PRSV and therefore survive but do not thrive (Davis et al., 2003; Davis et al., 2004).
f. Pollination, pollinators, self-compatibility, and seed dispersal: After X17-2 line seedling field establishment and once plants begin to flower (usually 4-5 months post planting) female plants are generally eliminated from the field since only hermaphrodites have the ability to self-pollinate (thus reducing the need for male plants) and possess the desired fruit characteristics (i.e., pear to elongated-oval shape). The fruit of hermaphroditic plants are typically derived from self-pollination because the anthers begin to dehisce and the stigmatic surfaces of the pistil are viable prior to opening of flower petals. This along with the fact that under commercial conditions a monoculture of one cultivar is grown, the potential for out-crossing with different cultivars and/or feral plants is minimal. The X17-2 line papayas are self-compatible as are all hermaphroditic papaya plants. We have observed no changes in flower morphology or potential pollinator species in our field plots. We have observed no differences in the fertility of the X17-2 line papayas compared to other commercial papaya cultivars. Papaya seeds do not shatter even if the fruit is damaged.
g. Stress adaptations: We have observed, but not been able to formally document, that hurricane wind tolerance of some breeding lines within the X17-2 transgene family appeared to be superior to non-transgenic cultivars. This was observed after the hurricane season of 2005 in which these X17-2 line papayas seemed to survive intact and withstood resetting to a vertical position post-storm better than non-transgenic papaya cultivars.

F. Cultivation of the Transgenic Plants

The target region for cultivation of the X17-2 line papayas includes south Florida, the Caribbean Region, and perhaps parts of Mexico and Central America. Possibly, the X17-2 line

papayas would not be successful outside these areas due to a lack of resistance to the PRSV strains outside these regions. We do not foresee any change in the specific ecological requirements of the farm sites for cultivation because of the basic requirements for all papaya production of warm temperatures, good quality and adequate water, well-drained soil, and best management practices. We therefore do not see any expansion of papaya production to new areas for the transgenic papaya.

As a component of U.S. fruit consumption, papaya is very low; per capita papaya consumption in the U.S. is 0.78 lb per person per year [Karst, T., 2002. The Packer (website: http://www.brookstropicals.com/pages/media8.html). Accessed 01-05-2007]. This is much lower than the 45 lbs per capita consumption of apples in the U.S. (Perez et al., 2001).

The current recommendation for commercial papaya production throughout the world is to establish new plantings away from abandoned or terminal papaya plantings that are infected with PRSV. This is a common practice because it reduces the reservoir of PRSV inoculum in the area and the potential for infection of the newly planted papaya with PRSV. Other typical cultivation practices, such as land clearing and leveling, establishment of drainage contours and/or ditches, irrigation equipment (e.g., driplines), bedding or mounding, use of natural or plastic mulch, soil fumigation, and incorporation of pre-plant fertilizer applications based on site-specific soil requirements, will not differ for X17-2 line papaya plantings from practices for non-transgenic papaya plantings. Similarly, we do not foresee any changes in the integrated pest management practices (for insects and diseases) or herbicide control practices for the X17-2 line papayas compared to non-transgenic papaya cultivars. Perhaps the only change that may occur with the use of the X17-2 line is a need for continuous harvesting of a higher amount of fruit since resistance to PRSV will favor continuous fruit production and development.

VI. Environmental consequences of Introduction of the X17-2 Line

A. PRSV

Management of PRSV in papaya by cultivation of the X17-2 line in commercial or dooryard plantings should have a positive economic impact on agriculture and for consumers without any significant deleterious effects on the environment. Extensive deployment of a single resistance gene, whether introduced into papaya by conventional breeding methods or by transformation, might select for PRSV populations that can overcome the resistance; however, the mechanism by which this might occur is not clear and the probability of it occurring is low. If necessary, the PRSV resistance in the X17-2 transgenic line might be augmented by crossing with tolerant non-transgenic papaya genotypes or with our D6 or D95 transgenic lines to produce F_1 hybrids.

The X17-2 line produces a PRSV coat protein; however, the effect of this, if any, is difficult to evaluate. The coat protein was detected by western-blot analysis, but not by ELISA, indicating that its concentration is low. In contrast, the PRSV coat protein can readily be detected by either method in infected, non-transgenic papaya plants. Since infected, non-transgenic papayas are widespread and no obvious effect of the naturally occurring PRSV coat protein on the environment has been observed, there appears to be no reason to expect that the presence of the transgenic coat protein will behave differently.

Table 6. Nutritional composition of ripe fruit pulp of the X17-2 line compared with published values for transgenic and non-transgenic papaya fruits.

		Information Source[a]									
		Univ. of Florida	Univ. of Florida	Univ. of Florida	Univ.of Florida	Univ. of Hawaii	Univ. of Hawaii	USDA	NZICFR**	NZICFR**	Morton
		X17-2[b] (16B8)	X17-2[b] (16B9)	X17-2[b] (16B14)	X17-2[b] (16D5)	55-1[b]	63-1[b]	Orange-fleshed papaya	Australian origin	Papua NG origin	Malaysian origin
Nutrient	Unit	Value per 100 g ripe raw papaya pulp									
Moisture	g	86	86	86	89	87	88	89	89	87	86 to 93
Ash	g	0.49	0.51	0.43	0.50	ND	ND	0.61	ND	ND	0.31 to 0.66
Total dietary fiber	g	1.3	1.8	1.4	2.4	ND	ND	1.8	2.3	0.8	0.5 to 1.3
Calories	kcal	54	55	54	44	ND	ND	39	30	50	23 to 26
Calories from fat	kcal	1	0	0	3.6	ND	ND	ND	ND	ND	ND
Total fat	g	0.07	0.04	0.04	0.4	ND	ND	0.14	0.1	0.1	.05 to .96
Saturated fatty acids	g	0.04	0.02	0.02	0.16	ND	ND	0.043	ND	ND	ND
Monounsaturated fatty acids	g	0.01	0.01	0.01	ND	ND	ND	0.038	ND	ND	ND
Polyunsaturated fatty acids	g	0.02	0.01	0.01	ND	ND	ND	0.031	ND	ND	ND
Carbohydrate	g	12.6	12.8	13.4	9.7	ND	ND	9.81	7	12	6.2 to 6.8
Total sugars	g	11.0	10.7	10.4	7.0	11.1 to 14.2**	11.0 to 12.5**	5.9	ND	ND	ND
Protein	g	0.657	0.780	BDL	0.5	ND	ND	0.61	0.4	0.5	0.08 to 0.34
Beta Carotene *	mcg	882	954	816	584	326	242	276	910	710	4.5 to 676
Vitamin A ***	IU	1470	1600	1370	974	ND	ND	1094	250	197	ND
Vitamin C	mg	68.9	72.8	71.0	41.3	41.3 to 53.6	44.7 to 63.1	61.8	60.0	73.0	35.5 to 71.3
Sodium	mg	52.9	9.5	9.5	7.16	2	2	3	7	6	ND
Calcium	mg	11.4	12.1	10.8	13.5	16	17	24	28	24	12.9 to 40.8
Iron	mg	0.65	0.75	0.37	0.14	0.21	0.28	0.10	0.5	0.7	0.25 to 0.78

[a]USDA National Nutrient Database for Standard Reference: http:// http://www.ars.usda.gov/ba/bhnrc/ndl
New Zealand Institute for Crop and Food Research
Morton" is Julia Morton. The values are from her book Fruits of Warm Climates
[b]Transgenic line: 16B8, 16B9, 16B14 & 16D5 are breeding selections of the X17-2 transgenic line; 55-1 & 63-1 are 'Sunset'-derived transgenic lines
"ND" = No Data; BDL=Below Detectable Limits
* Where Beta Carotene values are given in IU, this calculation was used: IU x 0.6 = micrograms
** Values are "Total Soluble Solids" - refractometer method
*** Morton's Vitamin A values are listed only as "Carotene".

B. Appearance of PRSV-Resistant Weeds

There is no evidence for interspecific hybridization of cultivated papaya with wild relatives in nature. However, some wild relatives have traits of interest, such as resistance to PRSV and papaya bunchy top disease, cold tolerance, and monoecious habit that are attractive in terms of papaya improvement. However, early attempts to manually intercross papaya with wild relatives yielded only nonviable seeds, illustrating the reproductive isolation of *Carica papaya* from other members of the Caricaceae (Mekako and Nakasone, 1975; Sawant, 1958). Advanced techniques, such as embryo rescue (Litz and Conover, 1983; Magdalita et al., 1997; Manshardt and Wenslaff, 1989) and protoplast fusion (Litz and Conover, 1979; Litz and Conover, 1980; Jordan et al., 1986) have been attempted to obtain interspecific hybrids. Some hybrids have been obtained by embryo rescue, but not protoplast fusion. Although hybrids have generally had low

vigor and viability and/or were infertile, some have been produced that exhibit resistance to PRSV and other diseases (Drew et al., 1998). Nevertheless, *Carica papaya* is generally considered incompatible with its wild relatives. Furthermore, the wild relatives are not common in Florida or the Caribbean regions.

C. Weediness of the X17-2 Papaya Line

Feral papaya plants are occasionally found growing in proximity to commercial or dooryard crops. Papaya is not considered a weed. There is no evidence indicating that PRSV is a major limiting factor in the development of either natural, or escaped populations of papaya. Therefore, there is no reason to suspect that introduction of the X17-2 line would result in papaya becoming a weed or becoming more competitive in natural areas and upsetting the ecological balance.

D. Vertical and Horizontal Transfer of the Transgenes

Outcrossing could occur from the transformed line to other domestic papayas following transfer of pollen by either wind or insects. This kind of gene transfer happens in nature constantly. Seed producers, therefore, would have to segregate production of transgenic and non-transgenic seed or take other measures, such as covering flowers to prevent outcrossing, to prevent cross-pollination.

Horizontal gene transfer is difficult to predict much less evaluate with respect to environmental consequences. Considerable research on the *npt*II gene has shown that it is safe for both the environment and the consumer (Fuchs, 1993). The issue of recombination of all or a portion of the CP gene with that of another virus is discussed below.

E. Issues Related to Potential Effects of CP Gene-Mediated Virus Resistance on Virus Infections

Three distinct interactions, transcapsidation, synergy, and recombination, have been observed to occur when two plant viruses simultaneously infect a cell, and their importance concerning the biosafety of crop plants made virus resistant through coat protein gene-mediated protection has been discussed in a consensus document by the Organization for Economic Co-operation and Development [OCDE/GD(96)162; http://www.olis.oecd.org/olis/1996doc.nsf/LinkTo/ocde-gd(96)162].

Transcapsidation occurs when two different strains (or two viruses) simultaneously infect a single plant cell and the genome of one virus becomes encapsidated by the coat protein of the second virus. The possibility of this happening with the transgenic PRSV CP is purely speculation, since there is no evidence that this has occurred in plants naturally infected by PRSV much less those containing the CP transgene. Synergy occurs when two viruses simultaneously naturally infect a plant and symptoms are more severe than when either of the viruses infects the plant singly. Since the potyvirus CP genes are not involved in synergism, it is, therefore, unlikely that infection of a plant of the X17-2 line with any other virus would result in a synergistic interaction.

Recombination occurs when there is an exchange of nucleotide sequences between two nucleic acid molecules. The persistence of a recombined viral genome will depend upon its fitness to replicate within the host cell, to replicate in the presence of parental viruses, to spread systemically within the host, or its successful transmission to new host plants. Analyses of the genomes of viruses indicates that such recombination events have occurred and is probably an important mechanism for virus change over evolutionary time frames. The stability of many

viruses during recent times indicates that the rate of stable recombinations in nature is probably low. We know of no reason why the presence of the X17-2 CP transgene would increase the opportunity for recombination. The CP gene in the X17-2 line was obtained from a virus that regularly infects papaya. The engineered CP sequence, if available at all for recombination, is unlikely to pose the potential for generating novel recombinants in comparison with natural mixed infections in the recipient plant.

VII. Adverse Consequences of Introduction

We are not aware of any potential adverse consequences of the introduction that pose a threat to humans or the environment. By controlling PRSV, diseases due to other viruses that infect papaya might become more evident because they are not "masked" by PRSV symptoms. Also, other diseases might become more prevalent due to the increased longevity of papaya plantings without PRSV, but the economic benefit of controlling PRSV should be well worth these risks. We cannot foresee any possible adverse effects on other non-target organisms, such as insects, pollinators, etc., because the transgenes are not known to have any toxic properties.

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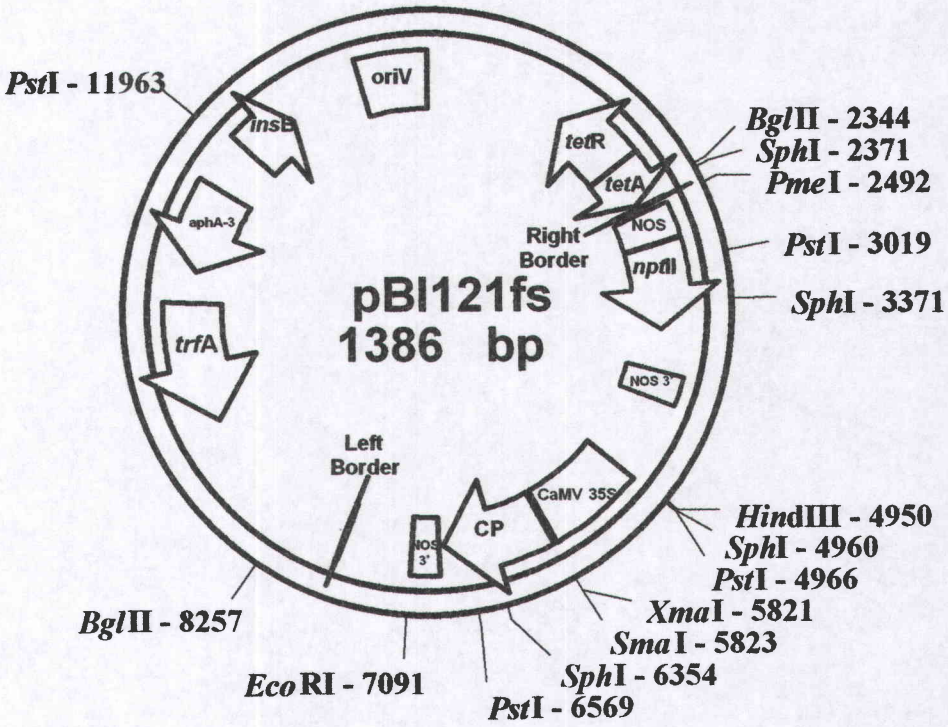
Application contains no confidential business information

Appendix I

Map of pBI121fs and Southern Blots

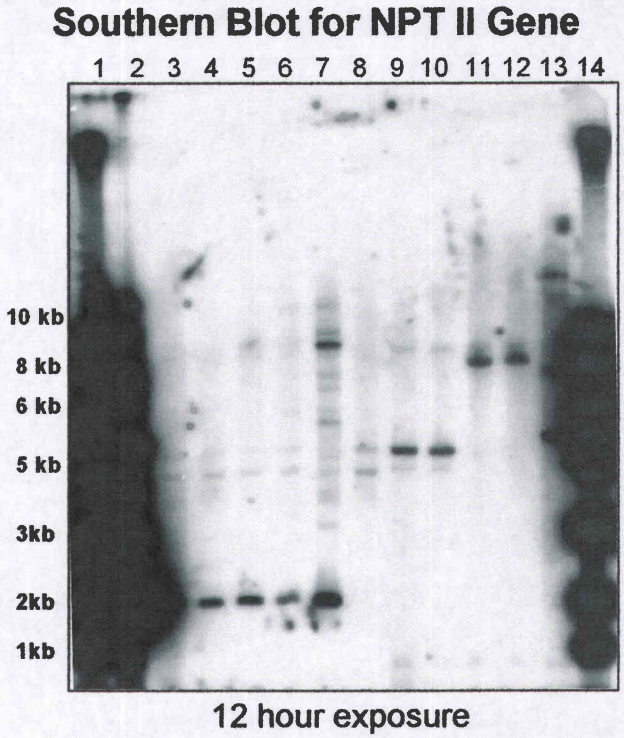


NOS	nopaline synthase promoter
*npt*II	neomycin phosphotransferase resistance gene
NOS 3'	nopaline synthase terminator
CaMV 35S	cauliflower mosaic virus promoter
CP	PRSV coat protein gene
*tet*A	tetracycline efflux protein gene
*tet*R	tetracycline repressor protein gene
oriV	origin of replication
*ins*B	IS1 insertion sequence transposase gene
alpha-3	*aph(3')-III* (synonym:*nptIII*), aminogycoside antibiotic resistance gene
*trf*A	gene encoding a trans-acting product essential for vegetative plasmid replication
borders	delimit T-DNA and vector backbone DNA

Restriction enzymes: *Bgl*II, *Eco*RI, *Pst*I, *Sph*I, *Sma*I, *Xma*I, *Hin*dIII, *Pme*I

Backbone probe: *Bgl*II / *Pst*I fragments from 0-2344 bp to 8257-13867 bp
*npt*II probe: *Pst*I fragment from 3019 bp to 4966 bp

Application contains no confidential business information



Probe: Gel purified and p^{32}-labelled 1947-bp DNA restriction fragment of pBI121 digested with *Eco*RI and *Pst*I containing a portion of the *npt*II gene and the NOS terminator

Lane:

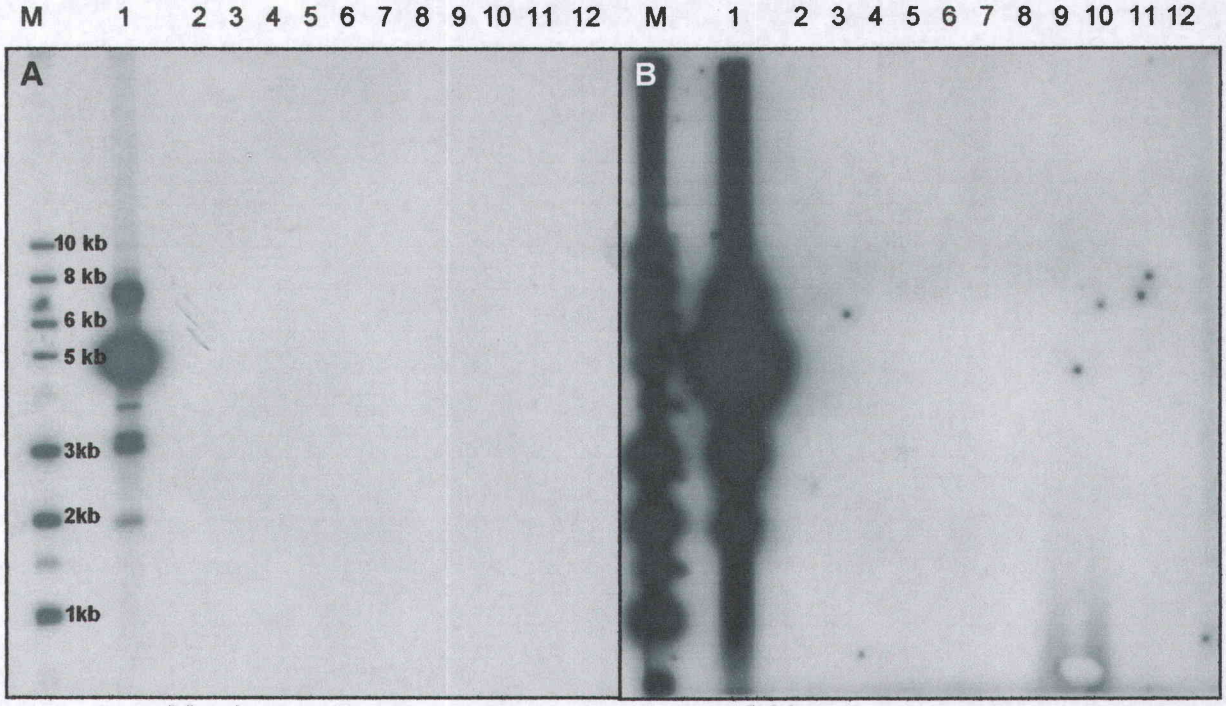
1 molecular size markers (sizes indicated at left margin)
2 pBI121 digested with *Eco*RI and *Pst*I showing 1947 bp DNA fragment
3 X17-2 transgenic line (Solo Sunrise male progenitor) DNA digested with *Eco*RI and *Pst*I
4 X17-2 transgenic line (Tainung No. 5 male progenitor) DNA digested with *Eco*RI and *Pst*I
5 D6 transgenic line (Solo Sunrise male progenitor) DNA digested with *Eco*RI and *Pst*I
6 D6 transgenic line (Solo 40 male progenitor) DNA digested with *Eco*RI and *Pst*I
7 D95 transgenic line (Solo Sunrise male progenitor) DNA digested with *Eco*RI and *Pst*I
8 Non-transgenic Solo Sunrise DNA digested with *Eco*RI and *Pst*I
9 X17-2 transgenic line (Solo Sunrise male progenitor) DNA digested with *Hin*dIII
10 X17-2 transgenic line (Tainung No. 5 male progenitor) DNA digested with *Hin*dIII
11 D6 transgenic line (Solo Sunrise male progenitor) DNA digested with *Hin*dIII
12 D6 transgenic line (Solo 40 male progenitor) DNA digested with *Hin*dIII
13 D95 transgenic line (Solo Sunrise male progenitor) DNA digested with *Hin*dIII
14 molecular size markers (sizes indicated at left margin)

Results:

The X17-2 and D6 transgenic lines have a single copy of the *npt*II gene, and the D95 transgenic line has three copies as indicated with the *Hin*dIII digests of total DNAs. A 1947-bp DNA restriction fragment containing a portion of the *npt*II gene and the NOS terminator was indicated in DNA from transgenic lines digested with *Eco*RI and *Pst*I . Faint bands are most likely the result of non-specific absorption of the probe.

...

Southern Blot for Vector Backbone



Probe: Gel purified and p^{32}-labelled 4923-bp and 4872-bp DNA restriction fragments of pBI121 digested with *Bgl* II and *Pst*I containing approximately 94% the 10,432-bp vector backbone region

Lane:

M Molecular size markers (sizes indicated in panel A)
1 pBI121 digested with *Eco*RI and *Pst*I showing 1947 bp DNA fragment
3 D6 transgenic line (Solo Sunrise male progenitor) DNA digested with *Eco*RI and *Pst*I
4 D6 transgenic line (Solo Sunrise male progenitor) DNA digested with *Hin*dIII
5 D6 transgenic line (Solo 40 male progenitor) DNA digested with *Eco*RI and *Pst*I
6 D6 transgenic line (Solo 40 male progenitor) DNA digested with *Hin*dIII
7 Non-transgenic Solo Sunrise DNA digested with *Eco*RI and *Pst*I
8 Non-transgenic Solo Sunrise DNA digested with *Hin*dIII
9 X17-2 transgenic line (Solo Sunrise male progenitor) DNA digested with *Eco*RI and *Pst*I
10 X17-2 transgenic line (Solo Sunrise male progenitor) DNA digested with *Hin*dIII
11 X17-2 transgenic line (Tainung No. 5 male progenitor) DNA digested with *Eco*RI and *Pst*I
12 X17-2 transgenic line (Tainung No. 5 male progenitor) DNA digested with *Hin*dIII

Results: The X17-2 and D6 transgenic lines do not contain detectable vector backbone DNA. Gel-purification of the probe DNA was not complete leaving some other vector DNA at lower concentrations; however, none of these DNA hybridized with DNA from the plants.

Appendix II

Comparison Between Expected Sequence And Observed Sequence Of X17-2 T-DNA

A 4935 bp PCR fragment containing all but approximately 336 bp of the T-DNA incert from a R_5 generation plant of the X17-2 line was cloned and sequenced, and the expected and observed sequences were compared. The homology was 99.7%.

miscel.	1..26
	/note ="plant DNA border region"
promoter	55..309
	/note="NOS"
gene	374..1168
	/gene="*npt*II"
	/product="neomycin phosphotransferase II"
terminator	155..1810
	/note="NOS"
promoter	2510..3344
	/note="CaMV 35S"
gene	3352..4360
	/gene="PRSV X17-2 coat protien construct"
terminator	4378..4630
	/note="NOS"

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X17-2 expected  GGTTATTATTATGCAGTGCGAGATGATTAAACTGAAGGCGGGAAACGACAATCTGATCAT 60
X17-2 observed  GGTTATTATTATGCAGTGCGAGATGATTAAACTGAAGGCGGGAAACGACAATCTGATCAT
                ************************************************************

X17-2 expected  GAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTT 120
X17-2 observed  GAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTT
                ************************************************************

X17-2 expected  TACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGA 180
X17-2 observed  TACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGA
                ************************************************************

X17-2 expected  GTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAG 240
X17-2 observed  GTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAG
                ************************************************************

X17-2 expected  GTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTC 300
X17-2 observed  GTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTC
                ************************************************************

X17-2 expected  CCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATC 360
X17-2 observed  CCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATC
                ************************************************************

X17-2 expected  TGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGT 420
X17-2 observed  TGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGT
                ************************************************************


X17-2 expected  GGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT 480
```

```
X17-2 observed   GGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT
                 ************************************************************

X17-2 expected   GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGC 540
X17-2 observed   GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGC
                 ************************************************************

X17-2 expected   CCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCC 600
X17-2 observed   CCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCC
                 ************************************************************

X17-2 expected   TTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGA 660
X17-2 observed   TTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGA
                 ************************************************************

X17-2 expected   AGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCAT 720
X17-2 observed   AGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCAT
                 ************************************************************

X17-2 expected   GGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCA 780
X17-2 observed   GGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCA
                 ************************************************************

X17-2 expected   AGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGA 840
X17-2 observed   AGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGA
                 ************************************************************

X17-2 expected   TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGC 900
X17-2 observed   TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGC
                 ************************************************************

X17-2 expected   GCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATAT 960
X17-2 observed   GCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATAT
                 ************************************************************

X17-2 expected   CATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGA 1020
X17-2 observed   CATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGA
                 ************************************************************

X17-2 expected   CCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG 1080
X17-2 observed   CCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
                 ************************************************************

X17-2 expected   GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTT 1140
X17-2 observed   GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTT
                 ************************************************************

X17-2 expected   CTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAA 1200
X17-2 observed   CTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAA
                 ************************************************************

X17-2 expected   GCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTG 1260
X17-2 observed   GCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTG
                 ************************************************************

X17-2 expected   GGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATG 1320
X17-2 observed   GGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATG
                 ************************************************************

X17-2 expected   CTGGAGTTCTTCGCCCACGGGATCTCTGCGGAACAGGCGGTCGAAGGTGCCGATATCATT 1380
X17-2 observed   CTGGAGTTCTTCGCCCACGGGATCTCTGCGGAACAGGCGGTCGAAGGTGCCGATATCATT
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X17-2 expected  ACGACAGCAACGGCCGACAAGCACAACGCCACGATCCTGAGCGACAATATGATCGGGCCC 1440
X17-2 observed  ACGACAGCAACGGCCGACAAGCACAACGCCACGATCCTGAGCGACAATATGATCGGGCCC
                ************************************************************

X17-2 expected  GGCGTCCACATCAACGGCGTCGGCGGCGACTGCCCAGGCAAGACCGAGATGCACCGCGAT 1500
X17-2 observed  GGCGTCCACATCAACGGCGTCGGCGGCGACTGCCCAGGCAAGACCGAGATGCACCGCGAT
                ************************************************************

X17-2 expected  ATCTTGCTGCGTTCGGATATTTTCGTGGAGTTCCCGCCACAGACCCGGATGATCCCCGAT 1560
X17-2 observed  ATCTTGCTGCGTTCGGATATTTTCGTGGAGTTCCCGCCACAGACCCGGATGATCCCCGAT
                ************************************************************

X17-2 expected  CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATG 1620
X17-2 observed  CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATG
                ************************************************************

X17-2 expected  ATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATG 1680
X17-2 observed  ATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATG
                ************************************************************

X17-2 expected  ACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG 1740
X17-2 observed  ACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
                ************************************************************

X17-2 expected  ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG 1800
X17-2 observed  ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG
                ************************************************************

X17-2 expected  TTACTAGATCGGGCCTCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTGGCGG 1860
X17-2 observed  TTACTAGATCGGGCCTCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTGGCGG
                ************************************************************

X17-2 expected  CTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGG 1920
X17-2 observed  CTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGG
                ************************************************************

X17-2 expected  TTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAATAA 1980
X17-2 observed  TTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAATAA
                ************************************************************

X17-2 expected  GGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACT 2040
X17-2 observed  GGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACT
                ************************************************************

X17-2 expected  TGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGG 2100
X17-2 observed  TGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGG
                ************************************************************

X17-2 expected  CCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCA 2160
X17-2 observed  CCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCA
                ************************************************************



X17-2 expected  AGTCGGTGACGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCT 2220
X17-2 observed  AGTCGGTGACGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCT
                ************************************************************

X17-2 expected  CCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCCCAATACGCAAACCGCCTCTCCC 2280
X17-2 observed  CCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCCCAATACGCAAACCGCCTCTCCC
                ************************************************************

X17-2 expected  CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG 2340
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X17-2 observed   CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCTGACTGGAAAGCGGG
                 ****************************************** **************

X17-2 expected   CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACA 2400
X17-2 observed   CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACA
                 ************************************************************

X17-2 expected   CTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG 2460
X17-2 observed   CTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGCGAGCGGATAACAATTTCACACAGG
                 ********************************** ************************

X17-2 expected   AAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCCCCAGATTAGCCTT 2520
X17-2 observed   AAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCCCCAGATTAGCCTT
                 ************************************************************

X17-2 expected   TTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTTACGCAGCAGGTCTC 2580
X17-2 observed   TTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTTACGCAGCAGGTCTC
                 ************************************************************

X17-2 expected   ATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAATACCTTCCCAAGAAGGTT 2640
X17-2 observed   ATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAATACCTTCCCAAGAAGGTT
                 ************************************************************

X17-2 expected   AAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTT 2700
X17-2 observed   AAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTT
                 ************************************************************
X17-2 expected   CTCAAGATCAGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAGG 2760
X17-2 observed   CTCAAGATCAGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAGG
                 ************************************************************

X17-2 expected   CAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAAAGGCCATGGAG 2820
X17-2 observed   CAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAAAGGCCATGGAG
                 ************************************************************

X17-2 expected   TCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAAAGACTGGCGAACAGTTCATA 2880
X17-2 observed   TCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAAAGACTGGCGAACAGTTCATA
                 ************************************************************

X17-2 expected   CAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGAC 2940
X17-2 observed   CAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGAC
                 ************************************************************

X17-2 expected   ACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAG 3000
X17-2 observed   ACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAG
                 ************************************************************

X17-2 expected   ACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT 3060
X17-2 observed   ACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT
                 ************************************************************

X17-2 expected   CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGAT 3120
X17-2 observed   CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGAT
                 ************************************************************

X17-2 expected   AAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCA 3180
X17-2 observed   AAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCA
                 ************************************************************

X17-2 expected   CCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT 3240
X17-2 observed   CCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
                 ************************************************************

X17-2 expected   TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC 3300
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X17-2 observed   TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC
                 ************************************************************

X17-2 expected   CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACGGGGGACTCTAG----- 3360
X17-2 observed   CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACGGGGGACTCTAGAGGAT
                 ************************************************************

X17-2 expected   -TCCCGGGTGGTCAGTCCCTTATGTTCCAAAAATGAAGCTGTGGATGCTGGTTTGAATGA 3420
X17-2 observed   CTCCCGGGTGGTCAGTCCCTTATGT-CCAAAAATGAAGCTGTGGATGCTGGTTTGAATGA
                  ************************ *********************************

X17-2 expected   AAAGCTCAAAGAAAAAGAAAAACAGAAAGAAAAAGAAAAAGAAAAACAAAAAGAAAAAGA 3480
X17-2 observed   AAAGCTCAAAGAAAAAGAAAAACAGAAAGAAAAAGAAAAAGAAAAACAAAAAGAAAAAGA
                 ************************************************************

X17-2 expected   CGATGCTAGTGACGGAAATGATGTGTCGACTAGCACAAAAACTGGAGAGAGAGATAGAGA 3540
X17-2 observed   CGATGCTAGTGACGGAAATGATGTGTCGACCAGCACAAAAACTGGAGAGAGAGATAGAGA
                 ****************************** ******************************

X17-2 expected   TGTCAATGTTGGGACCAGTGGAACTTTCACTGTTCCGAGAATTAAATCATTCACTGATAA 3600
X17-2 observed   TGTCAATGTTGGGACCAGTGGAACTTTCACTGTTCCGAGAATTAAATCATTCACTGATAA
                 ************************************************************

X17-2 expected   GATGATTTTACCGAGAATTAAGGGAAAGACTGTCCTTAATTTGAATCATCTTCTTCAGTA 3660
X17-2 observed   GATGATTTTACCGAGAATTAAGGGAAAGACTGTCCTTAATTTGAATCATCTTCTTCAGTA
                 ************************************************************

X17-2 expected   TAATCCGCAACAAATTGACATTTCTAACACTCGTGCCACTCAGTCACAATTTGAGAAATG 3720
X17-2 observed   TAATCCGCAACAAATTGACATTTCTAACACTCGTGCCACTCAGTCACAATTTGAGAAATG
                 ************************************************************
X17-2 expected   GTATGAGGGAGTGAGGAGTGATTATGGCCTGAATGATAATGAGATGCAAGTGACGCTAAA 3780
X17-2 observed   GTATGAGGGAGTGAGGAGTGATTATGGCCTGAATGATAATGAGATGCAAGTGACGCTAAA
                 ************************************************************

X17-2 expected   TGGCTTGATGGTTTGGTGTATCGAGAATGGTACATCTCCAGACATATCTGGTGTCTGGGT 3840
X17-2 observed   TGGCTTGATGGTTTGGTGTATCGAGAATGGTACATCTCCAGACATATCTGGTGTCTGGGT
                 ************************************************************

X17-2 expected   TATGATGGATGGGGAAACCCAAGTTGATTATCCAATCAAGCCTTTGATTGAGCATGCTAC 3900
X17-2 observed   TATGATGGATGGGGAAACCCAAGTTGATTATCCAATCAAGCCTTTGATTGAGCATGCTAC
                 ************************************************************

X17-2 expected   TCCGTCATTTAGGCAAATTATGGCTCACTTTAGTAACGCGGCAGAAGCATACATTGCGAA 3960
X17-2 observed   TACGTCATTTAGGCAAATTATGGCTCACTTTAGTAACGCGGCAGAAGCATACATTGCGAA
                 * ************************************************************

X17-2 expected   AAGAAATGCCACTGAGAGGTACATGCCGCGGTATGGAATCAAGAGGAATTTGACTGACAT 4020
X17-2 observed   GAGAAATGCCACTGAGAGGTACATGCCGCGGTATGGAATCAAGAGGAATTTGACTGACAT
                  ************************************************************

X17-2 expected   TAGCCTCGCCAGATACGCTTTCGATTTCTATGAGGTAAATTCGAAAACACCTGATAGAGC 4080
X17-2 observed   TAGCCTCGCCAGATACGCTTTCGATTTCTATGAGGTAAATTCGAAAACACCTGATAGAGC
                 ************************************************************

X17-2 expected   TCGCGAAGCTCACATGCAGATGAAAGCTGCAGCACTGCGAAATGCTAGTCGCAGAATGTT 4140
X17-2 observed   TCGCGAAGCTCACATGCAGATGAAAGCTGCAGCACTGCGAAATGCTAGTCGCAGAATGTT
                 ************************************************************

X17-2 expected   TGGTATGGACGGCAGTGTTAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAGA 4200
X17-2 observed   TGGTATGGACGGCAGTGTTAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAGA
                 ************************************************************

X17-2 expected   TGTCAATAGAGACATGCACTCTCTCCTGGGTATGCGCAACTGAATACTCGCGCTTGTGTG 4260
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X17-2 observed   TGTCAATAGAGACATGCACTCTCTCCTGGGTATGCGCAACTGAATACTCGCGCTTGTGTG
                 ************************************************************

X17-2 expected   TTTGTCGAGCCTGGCTCGACCCTGTTTCACCTTATAGTACTATATAAGTTTTAGAATACA 4320
X17-2 observed   TTTGTCGAGCCTGACTCGACCCTGTTTCACCTTATAGTACTATATAAGTATTAGAATACA
                 ************* ********************************** **********

X17-2 expected   GTGTGGCTGTGCCACCGCTTCTATTTTATAGTGAGGGTAGATCCCCCGAATTTCCCCGAT 4380
X17-2 observed   GTGTGGCTGTGCCACCGCTTCTATTTTATAGTGAGGGTAC-TAGCCCGAATTTCCCCGAT
                 ************************************  *  ****************

X17-2 expected   CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATG 4440
X17-2 observed   CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATG
                 ************************************************************

X17-2 expected   ATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATG 4500
X17-2 observed   ATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATG
                 ************************************************************

X17-2 expected   ACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG 4560
X17-2 observed   ACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
                 ************************************************************

X17-2 expected   ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG 4620
X17-2 observed   ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG
                 ************************************************************

X17-2 expected   TTACTAGATCGGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG 4680
X17-2 observed   TTACTAGATCGGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG
                 ************************************************************

X17-2 expected   CGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGA 4740
X17-2 observed   CGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGCAATAGCGA
                 ************************************************ ********

X17-2 expected   AGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGCCCGCTCCTT 4800
X17-2 observed   AGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGCCCGCTCCTT
                 ************************************************************

X17-2 expected   TCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATC 4860
X17-2 observed   TCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATC
                 ************************************************************

X17-2 expected   GGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTG 4920
X17-2 observed   GGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTG
                 ************************************************************

X17-2 expected   ATTTGGGTGATGGTT 4935
X17-2 observed   ATTTGGGTGATGGTT
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