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(57) Abstract

The present invention provides methods for preparing nucleic acids encoding polyphenol oxidase (PPO), fragments and derivatives tereof. The present invention also provides nucleic acids encoding banana, tobacco or pineapple PPO, or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof. Vectors including such nucleic acids, methods of using such nucleic acids and transgenic plants are also provided.

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POLYPHENOL OXIDASE GENES FROM BANANA, TOBACCO & PINEAPPLE

The present invention relates to the isolation of genes encoding polyphenol oxidase (PPO) from plants.

Browning of plant tissues often occurs following injury or damage and this generally results in spoilage of fruit and vegetables. Undesirable browning also occurs during processing of plant materials to produce food or other products. Steps are taken during transport, storage, and processing to prevent these browning reactions. Often this involves the use of chemicals such as sulphur dioxide but the use of these substances is likely to be restricted in the future due to concerns about their safety and consumer acceptance. For example, the US Food and Drug Administration banned the use of sulphite for most fresh fruit and vegetables in 1986. The production of fruit and vegetable varieties with an inherently low susceptibility to brown would remove the need for these chemical treatments.

It will be understood that browning in plants is predominantly catalysed by the enzyme PPO. PPO is localised in the plastids of plant cells whereas the phenolic substrates of the enzyme are stored in the plant cell vacuole. This compartmentation prevents the browning reaction from occurring unless the plant cells are damaged and the enzyme and its substrates are mixed.

The prior art includes International Application PCT/AU92/00356 to the present applicant which describes the cloning of PPO genes from grapevine, broad bean leaf, apple fruit and potato tuber. This application recognises that PPO levels in plants may be manipulated by increasing or decreasing expression of PPO gene. The application also identifies two conserved copper binding sites in PPO genes, designated CuA and CuB. However, the method described in PCT/AU92/00356 which was used to clone the PPO genes from apple and potato involved the use of an oligo dT reverse primer for polymerase chain reaction (PCR). Whilst the method is acceptable, in some tissues, it does not give rise to a strong band of the predicted size or else it gives rise to many additional products making it difficult to resolve the PPO fragment.

Accordingly, it is an object of the present invention to overcome or at least alleviate one or more of the difficulties related to the prior art.

In a first aspect of the present invention there is provided a method for preparing nucleic acid encoding PPO, fragments and derivatives thereof, which method includes

providing

a source of a polypeptide having PPO activity,

a first primer having a sequence corresponding to a first conserved region of PPO, and

a second primer having a sequence corresponding to a second conserved region of PPO orientation;

isolating RNA from the source of polypeptide having PPO activity;

treating the RNA to construct copy DNA (cDNA) therefrom; and

amplifying the cDNA so formed using the first and second primers.

Applicant has found that the method of the present invention, which involves the use of a second primer based on PPO, means that there is less likelihood that other (non-PPO) genes are amplified. Furthermore, the method of the present invention dramatically increases the amount of genuine product formed in most cases. Moreover, the added specificity provided by the second PPO-based primer makes it possible to clone PPO more readily from certain plants in which it was difficult to obtain a clone using one primer and oligo-dT.

In a preferred aspect of the present invention there is provided a method for preparing nucleic acid encoding banana, tobacco or pineapple PPO, fragments and derivatives thereof, which method includes

providing

a source of a polypeptide having banana, tobacco or pineapple PPO activity,

a first primer having a sequence corresponding to a first conserved region of banana, tobacco or pineapple PPO, and

a second primer having a sequence corresponding to a second conserved region of banana, tobacco or pineapple PPO;

isolating RNA from the source of polypeptide having banana, tobacco or pineapple PPO activity;

treating the RNA to construct copy DNA (cDNA) therefrom; and

amplifying the cDNA so formed using the first and second primers.

The terms "nucleic acid encoding banana/tobacco/pineapple PPO" and "banana/tobacco/pineapple PPO gene" as used herein should be understood to refer to a banana, tobacco and/or pineapple PPO gene or a sequence substantially homologous therewith. For example, these terms include sequences which differ from the specific sequences given in the Examples hereto but which, because of the degeneracy of the genetic code, encode the same protein. Applicants have found that there are families of PPO genes in most plants. Thus, there are likely to be other PPO genes in banana, tobacco and/or pineapple, in addition to those which have been isolated. These could be cloned using the methods of the present invention. Thus, the terms "nucleic acid encoding banana/tobacco/pineapple PPO" and " banana/tobacco/pineapple PPO gene" should be understood to include banana, tobacco and/or pineapple PPO genes other than those specific genes that have been isolated. The terms may also include presequences such as chloroplast transit sequence as well as sequences encoding mature PPO protein.

The term "derivative" as used herein includes nucleic acids that have been chemically or otherwise modified, for example mutated, or labelled, or nucleic acids incorporating a catalytic cleavage site.

The term "fragment" includes functionally active fragments of a PPO gene which are capable of altering expression of the PPO genes. Examples of alteration of the gene may include up-regulation or down-regulation of the gene, coding of the gene, transcription of the gene, binding of the gene or stability of the gene sequence.

The source of polypeptide having PPO activity is preferably a source of polypeptide having banana or tobacco or pineapple PPO activity. The source of polypeptide having banana PPO activity may be banana peel, preferably young banana peel. More preferably the peel of young banana fruit. The source of polypeptide having tobacco PPO activity may be tobacco leaves, preferably young tobacco leaves. The source of polypeptide having pineapple PPO activity may be pineapple fruit, preferably the flesh of the pineapple fruit, more preferably the flesh of pineapple fruit exhibiting blackheart disorder.

The RNA may be isolated by any suitable method including extraction for example with a detergent such as CTAB, use of an oligo-dT spun column as described in PCT/AU92/00356 and PCT/AU96/00310 the entire disclosure of each application which is incorporated herein by reference, or use of a commercially available kit such as the PolyATtract 1000 system from Promega Corporation.

The step of treating the RNA to construct cDNA according to this aspect of the present invention may include

treating the RNA with reverse transcriptase and an adapter primer to form cDNA.

The adapter primer may be an oligonucleotide adapter primer including the following sequence or part thereof:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'

The step of treating the RNA to construct cDNA according to this aspect of the present invention may include

treating the RNA with reverse transcriptase and a reverse primer to form cDNA.

The adapter primer may be replaced with a reverse primer having a sequence corresponding to a conserved region of PPO genes including the following sequence or part thereof:

REV2 :5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'

The first primer has a sequence corresponding to a first conserved region of PPO. Preferably the first primer has a sequence corresponding to at least a portion of or in close proximity to a first copper binding site of PPO. The second primer has a sequence corresponding to a second conserved region of PPO. Preferably the second primer has a sequence corresponding to at least a portion of or in close proximity to a second copper binding site of PPO. More preferably the first primer has a sequence corresponding to at least a portion of or in close proximity to one of the CuA or CuB binding sites of PPO, and the second primer has a sequence corresponding to at least a portion of or in close proximity to the other of the CuA or CuB binding sites of PPO.

The first and second primers may be degenerate. The first primer may

include one of the following sequences or part thereof:

GEN8 : 5'-GCGAATTCGATCCIACITT[TC]GC[GT]TTICC-3'.

GEN9 : 5'GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3'.

GEN10: 5'GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3'.

The second primer may include the following sequences or part thereof

REV1 : 5'-GCCTGCAGCCACATIC[TG][AG]TCIAC[AG]TT-3'.

REV2 : 5'GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'.

The cDNA may be amplified using the polymerase chain reaction (PCR).

Those skilled in the art will appreciate that if the Cu binding sites are internal, the nucleic acid isolated will be a fragment of the PPO gene lacking 3' and 5' termini. However, it is possible to determine the complete nucleic acid sequence of the PPO gene and to prepare or isolate nucleic acid encoding such PPO or antisense to such PPO.

Accordingly, in a further aspect of the present invention there is provided a method for preparing nucleic acid encoding the 3' end of PPO, which method includes

providing

a source of polypeptide having PPO activity

a primer in sense orientation; and

an adapter primer;

isolating RNA from the source of polypeptide having PPO activity;

treating the RNA to construct cDNA therefrom; and

amplifying the cDNA so formed using the primers.

In a further aspect of the present invention there is provided a method for preparing nucleic acid encoding the 5' end of PPO, which method includes

providing

a source of polypeptide having PPO activity,

an anchor,

primers in antisense orientation; and

an anchor primer;

isolating RNA from the source of polypeptide having PPO activity;

treating the RNA to construct cDNA therefrom;

attaching the anchor to the 5' end of the cDNA so formed; and

amplifying the cDNA using the primers.

The source of polypeptide having PPO activity is preferably a source of polypeptide having banana or tobacco or pineapple PPO activity. The source of polypeptide having banana PPO activity may be banana peel, preferably young banana peel. More preferably the peel of young banana fruit. The source of polypeptide having tobacco PPO activity may be tobacco leaves, preferably young tobacco leaves. The source of polypeptide having pineapple PPO activity may be pineapple fruit, preferably the flesh of the pineapple fruit, most preferably the flesh of pineapple fruit exhibiting blackheart disorder.

The RNA may be isolated by any suitable method including extraction for example with a detergent such as CTAB, use of an oligo-dT spun column as described in PCT/AU92/00356 or PCT/AU96/00310 the entire disclosure of each patent application which is incorporated herein by reference, or use of a commercially available kit such as the PolyATtract 1000 system from Promega Corporation.

The step of treating the RNA to construct cDNA according to this aspect of the present invention may include

treating the RNA with reverse transcriptase and an adapter primer to form cDNA.

The adapter primer may be an oligonucleotide adapter primer including one of the following sequences or part thereof:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'

The adapter primer may be replaced with a reverse primer having a sequence corresponding to a conserved region of PPO genes including the following sequence or part thereof:

REV2 :5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'

The primer in sense orientation may be a banana, tobacco or pineapple PPO specific primer.

The adapter primer may include the following sequence or part thereof:

5'-GACTCGAGTCGACATCGA-3'.

The primers in antisense orientation may be banana, tobacco or pineapple

PPO specific primers.

The anchor may be of any suitable type. The anchor may be attached by ligation for example using T4 RNA ligase. The anchor primer should be capable of hybridizing with the anchor.

The cDNA may be amplified using PCR.

Those skilled in the art will appreciate that using the methods of the present invention it is possible to determine the complete nucleic acid sequence of the PPO gene of interest and to prepare or isolate nucleic acid encoding such PPO or antisense to such PPO.

In a further aspect of the present invention, there is provided a nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives thereof. Preferably the nucleic acid has the sequence shown in Fig. 1, 2, 3 or 4, fragments and derivatives thereof, and substantially homologous sequences.

In a further aspect of the present invention, there is provided a nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof. Preferably the nucleic acid has the sequence shown in Fig. 5, 6 or 7 fragments and derivatives thereof, and substantially homologous sequences.

In a further aspect of the present invention, there is provided a nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof. Preferably the nucleic acid has the sequence shown in Fig. 8, 9 or 10 fragments and derivatives thereof, and substantially homologous sequences.

The nucleic acid may be prepared by a method as hereinbefore described.

The nucleic acid may be modified, for example by inclusion of a catalytic cleavage site.

In a further aspect of the present invention there is provided a method for preparing a recombinant vector including a nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives thereof, which method includes

providing

nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives thereof; and

a vector; and

reacting the nucleic acid and the vector to deploy the nucleic acid within the vector.

In a further aspect of the present invention there is provided a method for preparing a recombinant vector including a nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof, which method includes

providing

nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof; and

a vector; and

reacting the nucleic acid and the vector to deploy the nucleic acid within the vector.

In a further aspect of the present invention there is provided a method for preparing a recombinant vector including a nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof, which method includes

providing

nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof; and

a vector; and

reacting the nucleic acid and the vector to deploy the nucleic acid within the vector.

The nucleic acid may be prepared by a method as hereinbefore described.

The nucleic acid may be modified, for example by inclusion of a catalytic cleavage site.

The vector may be a plasmid expression vector. For example Bluescript SK^+ has been found to be suitable. Alternatively, the vector may be a binary vector. The recombinant vector may contain a promoter, preferably a constitutive promoter upstream of the nucleic acid.

The cloning step may take any suitable form. A preferred form may include

fractionating the cDNA, for example on a column or a gel;

isolating a fragment of the expected size, for example from the column or gel; and

ligating said fragment into a suitable restriction enzyme site of the vector, for example the EcoRV site of a Bluescript SK^+ vector.

In order to test the clones so formed, a suitable microorganism may be transformed with the vector, the microorganism cultured and the polypeptide encoded therein expressed. The microorganism may be a strain of Escherichia coli, for example E.coli DH5 has been found to be suitable. Alternatively, appropriate vectors may be used to transform plants.

In a further aspect of the present invention there is provided a recombinant vector including a nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives thereof, which vector is capable of being replicated, transcribed and translated in a unicellular organism or alternatively in a plant.

In a further aspect of the present invention there is provided a recombinant vector including a nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof, which vector is capable of being replicated, transcribed and translated in a unicellular organism or alternatively in a plant.

In a further aspect of the present invention there is provided a recombinant vector including a nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof, which vector is capable of being replicated, transcribed and translated in a unicellular organism or alternatively in a plant.

The nucleic acid may be prepared by a method as hereinbefore described.

The nucleic acid may be modified, for example by inclusion of a catalytic cleavage site.

The vector may be a plasmid expression vector. For example Bluescript SK^+ has been found to be suitable. Alternatively, the vector may be a binary vector. The recombinant vector may contain a promoter, preferably a constitutive promoter upstream of the nucleic acid encoding banana, tobacco or pineapple

PPO or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof.

The microorganism may be a strain of Escherichia coli, for example E.coli DH5 has been found to be suitable.

In a further aspect of the present invention there is provided a method of decreasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding banana PPO, a modified nucleic acid encoding banana PPO, or a nucleic acid antisense to banana PPO, fragments and derivatives thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

In a further aspect of the present invention there is provided a method of decreasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding tobacco PPO, a modified nucleic acid encoding tobacco PPO, or a nucleic acid antisense to tobacco PPO, fragments and derivatives thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

In a further aspect of the present invention there is provided a method of decreasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding pineapple PPO, a modified nucleic acid encoding pineapple PPO, or a nucleic acid antisense to pineapple PPO, fragments and derivatives thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

PPO activity may be decreased by the use of sense constructs

(cosuppression). Alternatively the nucleic acid may include a sequence encoding antisense mRNA to banana or tobacco or pineapple PPO or a functionally active fragment thereof. Alternatively the nucleic acid may encode banana or tobacco or pineapple PPO or a functionally active fragment thereof and incorporate a catalytic cleavage site (ribozyme). The nucleic acid may be included in a recombinant vector as hereinbefore described. In a preferred aspect, the nucleic acid may be included in a binary vector. In a further preferred aspect, the introduction of a binary vector into the plant may be by infection of the plant with an Agrobacterium containing the binary vector or by bombardment with nucleic acid coated microprojectiles. Methods for transforming banana, tobacco or pineapple with Agrobacterium are known to those skilled in the art and are described in, for example, May et al., Bio/technology (1995) 13:486-492; Michelmore et al., Plant Cell Reports (1987) 6:439-442, and Curtis et al., Journal of Experimental Botany (1994) 45:1141-1149, the entire disclosures of which one incorporated herein by reference. Methods for transforming banana, tobacco or pineapple by bombardment with DNA coated microprojectiles are known to those skilled in the art and are described in, for example, Sagi et al., Bio/technology (1995) 13:481-485, the entire disclosure of which is incorporated herein by reference.

In a further aspect of the present invention there is provided a method of increasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding banana PPO or a fragment thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

In a further aspect of the present invention there is provided a method of increasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding tobacco PPO or a fragment thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a

transgenic plant.

In a further aspect of the present invention there is provided a method of increasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding pineapple PPO or a fragment thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

The nucleic acid may be included in a recombinant vector as hereinbefore described. In a preferred aspect, the nucleic acid may be included in a binary vector. In a further preferred aspect, the introduction of the binary vector into the plant may be by infection of the plant with an Agrobacterium containing the binary vector or by bombardment with nucleic acid coated microprojectiles.

The plant may be of any suitable type. However the method is particularly applicable to banana, tobacco or pineapple.

In a further aspect of the present invention there is provided a transgenic plant, which plant contains nucleic acid capable of modifying expression of the normal banana PPO gene.

The plant may be of any suitable type. Preferably, the plant is banana.

In a further aspect of the present invention there is provided a transgenic plant, which plant contains nucleic acid capable of modifying expression of the normal tobacco PPO gene.

The plant may be of any suitable type. Preferably, the plant is tobacco.

In a further aspect of the present invention there is provided a transgenic plant, which plant contains nucleic acid capable of modifying expression of the normal pineapple PPO gene.

The plant may be of any suitable type. Preferably, the plant is pineapple.

The nucleic acid may be as hereinbefore described.

In a still further aspect of the present invention there is provided a plant vaccine including nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives thereof.

In a still further aspect of the present invention there is provided a plant

vaccine including nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof.

In a still further aspect of the present invention there is provided a plant vaccine including nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof.

The present invention will now be more fully described with reference to the accompanying Examples. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

IN THE FIGURES :

FIGURE 1:The BPPO2 cDNA nucleotide sequence and derived protein sequence encoding part of a banana PPO protein.

FIGURE 2:The BPPO8 cDNA nucleotide sequence and derived protein sequence encoding part of a banana PPO protein.

FIGURE 3:The BANPPO34 cDNA nucleotide sequence and derived protein sequence encoding part of a banana PPO protein.

FIGURE 4:The BANPPO35 cDNA nucleotide sequence and derived protein sequence encoding part of a banana PPO protein.

FIGURE 5:The TOBPPO6 cDNA nucleotide sequence and derived protein sequence encoding part of a tobacco PPO protein.

FIGURE 6:The TOBPPO25 cDNA nucleotide sequence and derived protein sequence encoding part of a tobacco PPO protein.

FIGURE 7:The TOBPPO26 cDNA nucleotide sequence and derived protein sequence encoding part of a tobacco PPO protein.

FIGURE 8:The PINPPO20 cDNA nucleotide sequence and derived protein sequence encoding part of a pineapple PPO protein.

FIGURE 9:The PINPPO2 cDNA nucleotide sequence and derived protein sequence encoding part of a pineapple PPO protein.

FIGURE 10:The PINPPOFL cDNA nucleotide sequence and derived protein sequence encoding a pineapple PPO protein.

EXAMPLE 1: Cloning Banana Peel PPO genes

Total RNA was isolated from the peel of young banana fruit. Fruit tissue (3g) was frozen and ground to a fine powder in liquid nitrogen with a coffee grinder then added to 20 ml of extraction buffer (2% hexadecyltrimethylammonium bromide (CTAB), 2% polyvinyl pyrolidone, 100 mM Tris-HCl, pH 8.0, 25 mM EDTA, 2 M NaCl, 0.05% spermidine, 2% β-mercaptoethanol) at 65°C. The extract was mixed with 20 ml of chloroform / IAA then centrifuged for 20 minutes at 5,000 RPM and the aqueous phase was re-extracted with chloroform / IAA. The aqueous phase was filtered through Miracloth and 0.25 volumes of 10 M LiCl were added then the sample was incubated overnight at 4°C before centrifuging for 20 minutes at 8,000 RPM. The supernatant was removed and the pellet was resuspended in 0.5 ml of 1 M NaCl, 0.5% SDS, 10 mM Tris, pH 8.0, 1 mM EDTA. The RNA was extracted once with an equal volume of chloroform / IAA and 2 volumes of ethanol was added. After incubation for 40 mins at -70°C the solution was centrifuged for 15 minutes at 10,000 RPM . The supernatant was removed and the pellet was rinsed with 80% ethanol, drained, and dried. The pellet was resuspended in 50 μl of sterile water.

First strand cDNA was synthesised from 10 μg total RNA with reverse transcriptase as described in Ref 2, utilising an oligo-dT primer adapter (Ref 1) :

B26 : (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3')

Oligonucleotide primers were designed based on known plant PPO DNA sequences. Comparison of a number of PPO sequences from a range of different plants allowed identification of the conserved regions of the gene, which are mostly in or near the regions which encode the two copper binding sites, CuA and CuB (2). Forward primers designed around the CuA site (GEN8, GEN9 and GEN 10) and reverse primers designed around the CuB site (REV1 and REV2) were synthesised :

GEN8 : (5'-GCGAATTCGATCCIACITT[TC]GC[GT]TTICC-3')

GEN9 : (5'-GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3')

GEN10 : (5'-GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3')

REV1 : (5'-GCCTGCAGCCACATIC[TG][AG]TCIAC[AG]TT-3')

REV2 : (5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3')

The first strand reaction was amplified by the polymerase chain reaction (PCR) essentially according to the method of Frohman (1) using GEN and REV primers, each at a final concentration of 1 μM (2). Amplification involved an initial program of 2 cycles of denaturation at 94° C for 1 min, annealing at 37° C for 2 min, a slow ramp to 72° C over 2 min and elongation at 72° C for 3 min, followed by 33 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min. A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen).

The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size were selected and their DNA sequence was determined by automated sequencing. Two putative banana PPO clones

(BPPO2 and BPPO8) were identified based on their homology to known plant PPO genes.

The 3'-end of BPPO2 was cloned using a primer designed to the sequence of BPPO2 :

BAN8F : (5'-GTTGCTCTTCTTAGGCTCGGCTTAC-3')

at a final concentration of 1 μM and a B25 adaptor primer:

B25 : (5'-GACTCGAGTCGACATCGA-3')

at a final concentration of 0.1 μM (ref 1). Amplification involved 35 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min. A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen).

The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size (1150 bp) were selected and their DNA sequence was determined by automated sequencing. Two putative banana PPO clones (BANPPO34 and BANPPO35) were identified based on their homology to known plant PPO genes. The sequence of BANPPO34 was identical to that of BPPO2.

EXAMPLE 2: Cloning Tobacco Leaf PPO genes

Total RNA was isolated from young leaves (1-3 cm long) of glasshouse grown plants. Approximately 2 g of frozen leaf material was ground to a fine powder in liquid nitrogen then extracted in 15 ml of extraction buffer (50 mM Tris-HCl, pH 9.0, 150 mM LiCl, 5 mM EDTA, 5% SDS and 0.6% β-mercaptoethanol) by shaking vigorously in a 50 ml screw cap tube for 1-2 minutes. Approximately 15 ml of phenol / chloroform / IAA (25:24:1) was added and the homogenate was

mixed then centrifuged for 15 minutes at 5,000 RPM, 4°C. The upper aqueous phase was removed and re-extracted twice with phenol / chloroform / IAA and then once with chloroform / IAA and then centrifuged for 10 minutes at 5,000 RPM, 4°C. The supernatant was removed, LiCl was added to a final concentration of 2 M and the mixture was incubated overnight at 4°C. After centrifuging for 10 minutes at 8,000 RPM, 4°C the supernatant was removed and the pellet was resuspended in 6 ml of 0.4 M LiCl then 2 ml of 8M LiCl was added and the mixture was incubated overnight at 4°C. The mixture was centrifuged for 10 minutes at 8,000 RPM, 4°C, the supernatant was removed and the pellet was resuspended in 0.5 ml of sterile water and centrifuged briefly to remove any insoluble material.

mRNA was isolated from the total RNA using a PolyATtract kit (Promega). First strand cDNA was synthesised from 10 μg total RNA or 2 μg mRNA with reverse transcriptase as described in Ref 2, utilising an oligo-dT primer adapter (Ref 1) :

B26 : (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3')

The first strand reaction was amplified by the polymerase chain reaction (PCR) essentially according to the method of Frohman (1) using GEN and REV primers described in Example 1, each at a final concentration of 1 μM (2). Amplification involved an initial program of 2 cycles of denaturation at 94° C for 1 min, annealing at 37° C for 2 min, a slow ramp to 72° C over 2 min and elongation at 72° C for 3 min, followed by 28 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min. A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen).

The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size were selected and their DNA sequence was

determined by automated sequencing. Three putative tobacco PPO clones (TOBPPO6, TOBPPO25 and TOBPPO26) were identified based on their homology to known plant PPO genes.

EXAMPLE 3: Cloning Pineapple PPO genes

Mature pineapple fruit were treated to induce blackheart disorder by holding the fruit for 17 days at 12°C then for 4 days at 25°C. Flesh showing blackheart symptoms was dissected from the fruit, frozen in liquid nitrogen and ground to a fine powder in a pre-cooled coffee grinder. To isolate total RNA 10 g of the powder was ground in a mortar and pestle then extracted with 30 ml of homogenisation buffer (100mM Tris-HCl, pH9.0, 200mM NaCl, 15 mM EDTA, 0.5% sarkosyl and 1% β-mercaptoethanol), 30 ml of phenol and 6 ml of chloroform / IAA. The mixture was stirred in a beaker, 2.1 ml of 3M NaAc (pH 5.2) was added and the mixture was kept on ice for 15 minutes then centrifuged for 15 minutes at 8,000 RPM, 4°C. The upper aqueous phase was removed and an equal volume of isopropanol was added. The mixture was incubated for 30 minutes at -70°C then centrifuged for 20 minutes at 8,000 RPM, 4°C in Corex tubes. The supernatant was removed and the pellet was rinsed with 70% ethanol and centrifuged for 5 minutes at 8,000 RPM, 4°C. The ethanol was removed and the pellet was air dried then resuspended in 0.75 ml sterile water and centrifuged to remove any insoluble material. LiCl was added to a final concentration of 3 M and the mixture was incubated overnight at -20°C then centrifuged for 30 minutes at 15,000 RPM, 4°C. The pellet was rinsed with 70% ethanol, centrifuged briefly, drained and air dried. The pellet was resuspended in 75 µl sterile water and centrifuged to remove any insoluble material.

Oligonucleotide primers were designed based on known plant PPO DNA sequences. Comparison of a number of PPO sequences from a range of different plants allowed identification of the conserved regions of the gene, which are mostly in or near the regions which encode the two copper binding sites, CuA and CuB. Forward primers designed around the CuA site (GEN8, GEN9 and

GEN 10) and reverse primers designed around the CuB site (REV1 and REV2) were synthesised :

GEN8 : (5'-GCGAATTCGATCCIACITT[TC]GC[GT]TTICC-3')

GEN9 : (5'-GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3')

GEN10 : (5'-GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3')

REV1 : (5'-GCCTGCAGCCACATIC[TG][AG]TCIAC[AG]TT-3')

REV2 : (5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3')

First strand cDNA was synthesised from 10 μg total RNA with reverse transcriptase as described in Ref 2, utilising the REV2 primer :

REV2 : (5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3')

The first strand reaction was amplified by the polymerase chain reaction (PCR) essentially according to the method of Frohman (1) using the GEN and REV primers described in Example 1, each at a final concentration of 1 μM (2). Amplification involved an initial program of 2 cycles of denaturation at 94° C for 1 min, annealing at 37° C for 2 min, a slow ramp to 72° C over 2 min and elongation at 72° C for 3 min, followed by 33 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min.

A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen). The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size were selected and their DNA sequence was determined by automated sequencing. A putative pineapple PPO clone (PINPPO20) was identified based on its homology to known plant PPO genes.

First strand cDNA was also synthesised from 10 μg total RNA with reverse transcriptase as described in Dry, I.B. and Robinson, S. P (1994), utilising an oligo-dT primer adapter (Ref 1) :

B26 : (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3')

This first strand reaction was amplified by the polymerase chain reaction (PCR) essentially according to the method of Frohman, M.A. (1990) using GEN9 and GEN10 primers :

GEN9 : (5'-GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3')

GEN10 : (5'-GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3')

at a final concentration of 1 μM and a B25 adaptor primer :

B25 : (5'-GACTCGAGTCGACATCGA-3')

at a final concentration of 0.1 μM (Frohman, M.A. (1990); Dry, I.B. and Robinson, S.P. (1994)) Amplification involved a program of 33 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min.

A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen).

The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size were selected and their DNA sequence was determined by automated sequencing. Two putative pineapple PPO clones (PINPPO1 and PINPPO2) were identified based on their homology to known plant PPO genes. The sequence of PINPPO1 was found to be nearly identical to that of PINPPO20.

The 5'-end of PINPPO1 was obtained using a 5'-RACE system for rapid amplification of cDNA ends, Version 2.0, from GIBCO-BRL, according to the

manufacturer's instructions. Specific oligonucleotide primers based on the sequences of PINPPO1 and PINPPO2 were used :

PINE 1 : 5'-ATATCACCTGTCGGTACATGACGGC-3'

PINE2 : 5'-GTGCCATTGTAGTCGAGGTCAATCA-3'

A number of clones were sequenced and one, 5PINA, was found to be nearly identical to PINPPO1 in the overlapping regions.

A full-length pineapple cDNA clone was isolated using a primer designed to the 5'-end sequence of 5PINA :

5PIN1 : (5'-CCAGTGCCTGGTTTAGGTGTATTCAC-3')

and used with the B25 adaptor primer as described above to amplify cDNA prepared from blackheart-induced pineapple fruit RNA. Amplification involved a program of 33 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and elongation at 72° C for 3 min.

A sample of the amplified DNA was run on an agarose gel and stained with ethidium bromide to determine the size of the PCR products. The remainder was run on a low melting point agarose gel and the bands of interest were excised. DNA was purified from the agarose with a QIAquick PCR Purification kit (Qiagen).

The purified DNA was cloned into Eco RV-cut Bluescript SK^+ vector (Stratagene) which had been T-tailed with Taq Polymerase and the ligated DNA was introduced into E. coli DH5α by electroporation. Recombinant clones which had an insert of the predicted size (2.2kbp) were selected and their DNA sequence was determined by automated sequencing. A pineapple PPO clone (PINPPOFL) was identified based on its homology to the PINPPO20, PINPPO1 and 5PINA clones. The sequence of PINPPOFL was found to be nearly identical to that of PINPPO20, PINPPO1 and 5PINA in the overlapping regions.

REFERENCES

1. Frohman, MA (1990) in "PCR Protocols : A Guide to Methods and Applications" (MA Innis, DH Gelfrand, JJ Sninsky and TJ White, eds) Academic Press, New York pp 28-38.

2. Dry, IB and Robinson, SP (1994) "Molecular cloning and charaterisation of grape berry polyphenol oxidase". Plant Molecular Biology 26 : 495-502.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS:

1. A method for preparing a nucleic acid encoding banana, tobacco or pineapple PPO, fragments and derivatives thereof, which method includes

providing

a source of a polypeptide having banana, tobacco or pineapple PPO activity,

a first primer having a sequence corresponding to a first conserved region of banana, tobacco or pineapple PPO; and

a second primer having a sequence corresponding to a second conserved region of banana, tobacco or pineapple PPO; and

isolating RNA from the source of polypeptide having banana, tobacco or pineapple PPO activity;

treating the RNA to construct copy DNA (cDNA) therefrom; and

amplifying the cDNA so formed using the first and second primers.

2. A method according to claim 1 wherein the source of polypeptide is selected from the group including banana peel; tobacco leaves and pineapple fruit for banana, tobacco and pineapple PPO respectively.

3. A method according to claim 1 or 2 wherein the first primer has a sequence corresponding to at least a portion of or in close proximity to a first copper binding site of a banana, tobacco or pineapple PPO.

4. A method according to any one of claims 1 to 3 wherein the second primer has a sequence corresponding to at least a portion of or in close proximity to a second copper binding site of PPO.

5. A method according to claim 3 or 4 wherein the copper binding site is one of CuA or CuB binding sites of banana, tobacco or pineapple PPO.

6. A method according to any one of claims 1 to 5 wherein the first primer is selected from any one of the following sequences or part thereof:

GEN8 : 5'-GCGAATTCGATCCIACITT[TC]GC[GT]TTICC-3'.

GEN9 : 5'GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3'.

GEN10: 5'GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3'.

7. A method according to any one of claims 1 to 6 wherein the second primer is selected from one of the following sequences or part thereof:

REV1 : 5'-GCCTGCAGCCACATIC[TG][AG]TCIAC[AG]TT-3'.

REV2 : 5'GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'.

8. A method according to any one of claims 1 to 7 wherein the treating of RNA to construct cDNA includes treating the RNA with reverse transcriptase and an adapter primer to form cDNA or treating the RNA with reverse transcriptase and a reverse primer to form cDNA.

9. A method according to claim 8 wherein the adaptor primer is an oligonucleotide adapter primer including the following sequence or part thereof:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'

5'-GACTCGAGTCGACATCGA-3'.

10. A method according to claim 9 wherein the adaptor primer is replaced with a reverse primer having a sequence corresponding to a conserved region of PPO genes including the following sequence or part thereof:

REV2 :5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'

11. A method for preparing nucleic acid encoding the 3' end of PPO, which method includes

providing

a source of polypeptide having banana, tobacco or pineapple PPO activity;

a primer in sense or antisense orientation; and

an adapter primer;

isolating RNA from the source of polypeptide having PPO activity;

treating the RNA to construct cDNA therefrom; and

amplifying the cDNA so formed using the primers.

12. A method for preparing nucleic acid encoding the 5' end of PPO, which method includes

providing

a source of polypeptide having banana, tobacco or pineapple PPO activity,

an anchor,

primers in antisense orientation; and

an anchor primer;

isolating RNA from the source of polypeptide having PPO activity;

treating the RNA to construct cDNA therefrom;

attaching the anchor to the 5' end of the cDNA so formed; and

amplifying the cDNA using the primers.

13. A method according to claim 11 or 12 wherein the source of polypeptide is selected from the group including banana peel, tobacco leaves and pineapple fruit for banana, tobacco or pineapple PPO respectively.

14. A method according to any one of claims 11 to 13 wherein the step of treating RNA to construct cDNA includes an oligonucleotide adapter primer including one of the following sequences or part thereof:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'

5'-GACTCGAGTCGACATCGA-3'.

15. A method according to claim 14 wherein the adaptor primer is replaced with a reverse primer having a sequence corresponding to a conserved region of PPO genes including the following sequence or part thereof:

REV2 :5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3'

16. A nucleic acid encoding banana PPO or antisense to banana PPO, fragments and derivatives having the sequence shown in Fig. 1, 2, 3 or 4, fragments and derivatives thereof, and substantially homologous sequences.

17. A nucleic acid encoding tobacco PPO or antisense to tobacco PPO, fragments and derivatives thereof having the sequence shown in Fig. 5, 6 or 7, fragments and derivatives thereof, and substantially homologous sequences.

18. A nucleic acid encoding pineapple PPO or antisense to pineapple PPO, fragments and derivatives thereof having the sequence shown in Fig. 8, 9 or 10, fragments and derivatives thereof, and substantially homologous sequences.

19. A nucleic acid according to any one of claims 16, 17 or 18 including a catalytic cleavage site.

20. A method for preparing a recombinant vector including a nucleic acid encoding banana, tobacco or pineapple PPO or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof, which method includes

providing

nucleic acid encoding banana, tobacco or pineapple PPO or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof; and

a vector; and

reacting the nucleic acid and the vector to deploy the nucleic acid within the vector.

21. A method according to claim 20 wherein the nucleic acid is according to any one of claims 16, 17 or 18.

22. A method according to claim 20 or 21 wherein the vector is a plasmid expression vector selected from Bluescript SK^+ or a binary vector.

23. A recombinant vector including a nucleic acid encoding banana PPO or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof, which vector is capable of being replicated, transcribed and translated in a unicellular organism or in a plant.

24. A recombinant vector prepared by the method according to any one of claims 20 to 22.

25. A method of increasing the level of banana, tobacco or pineapple PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding banana, tobacco or pineapple PPO or a fragment thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

26. A method according to claim 25 wherein the nucleic acid is according to any one of claims 16, 17 or 18.

27. A method of decreasing the level of PPO activity in a plant tissue, which method includes

providing

a nucleic acid encoding banana, tobacco or pineapple PPO, a modified nucleic acid encoding banana, tobacco or pineapple PPO, or a nucleic acid antisense to banana, tobacco or pineapple PPO, fragments and derivatives

thereof; and

a plant sample; and

introducing said nucleic acid into said plant sample to produce a transgenic plant.

28. A method according to claim 27 wherein the nucleic acid is according to any one of claims 16, 17 or 18.

29. A method according to claim 26 or 27 wherein the PPO activity is decreased by using sense constructs (cosuppression) or by including a sequence encoding antisense or RNA to banana, tobacco or pineapple PPO or a functionally active fragment thereof.

30. A method according to any one of claims 27 to 29 wherein said nucleic acid is introduced into said plant sample via an *Agrobacterium* or by bombardment with a nucleic acid coated microprojectile.

31. A transgenic plant, which plant contains a nucleic acid capable of modifying expression of the normal banana, tobacco or pineapple PPO gene.

32. A transgenic plant according to claim 30 including a nucleic acid according to any one of claims 16, 17 or 18.

33. A plant vaccine including a nucleic acid encoding banana, tobacco or pineapple PPO or antisense to banana, tobacco or pineapple PPO, fragments and derivatives thereof.

34. A plant vaccine according to claim 33 including a nucleic acid according to any one of claims 16, 17 or 18.

FIGURE 1 : Sequence of BPPO2

```
                CACTGTGCGTATTGTGATGGCGCCTACGACCAGATCGGCTTCCCCAACCTCGAGCTCCAA
          1 ---------+---------+---------+---------+---------+---------+ 60
                GTGACACGCATAACACTACCGCGGATGCTGGTCTAGCCGAAGGGGTTGGAGCTCGAGGTT
    a             H   C   A   Y   C   D   G   A   Y   D   Q   I   G   F   P   N   L   E   L   Q   -

                GTCCACAACTCCTGGCTCTTCTTCCCTTGGCACCGCTTCTACCTCTACTTCCACGAGAGG
         61 ---------+---------+---------+---------+---------+---------+ 120
                CAGGTGTTGAGGACCGAGAAGAAGGGAACCGTGGCGAAGATGGAGATGAAGGTGCTCTCC
    a             V   H   N   S   W   L   F   F   P   W   H   R   F   Y   L   Y   F   H   E   R   -

                ATCCTCGGAAAGCTCATAGGCGACGACACTTTCGCCCTCCCTTTCTGGAACTGGGACGCG
        121 ---------+---------+---------+---------+---------+---------+ 180
                TAGGAGCCTTTCGAGTATCCGCTGCTGTGAAAGCGGGAGGGAAAGACCTTGACCCTGCGC
    a             I   L   G   K   L   I   G   D   D   T   F   A   L   P   F   W   N   W   D   A   -

                CCCGGCGGCATGAAGCTGCCGTCGATCTACGCCGACCCTTCGTCCTCGCTCTATGACAAG
        181 ---------+---------+---------+---------+---------+---------+ 240
                GGGCCGCCGTACTTCGACGGCAGCTAGATGCGGCTGGGAAGCAGGAGCGAGATACTGTTC
    a             P   G   G   M   K   L   P   S   I   Y   A   D   P   S   S   S   L   Y   D   K   -

                TTTCGCGACGCCAAGCACCAGCCGCCAGTCCTCGTCGACCTCGACTACAACGGAACCGAC
        241 ---------+---------+---------+---------+---------+---------+ 300
                AAAGCGCTGCGGTTCGTGGTCGGCGGTCAGGAGCAGCTGGAGCTGATGTTGCCTTGGCTG
    a             F   R   D   A   K   H   Q   P   P   V   L   V   D   L   D   Y   N   G   T   D   -

                CCTAGTTTCACCGACGCAGAGCAGATCGATCAGAACCTCAAGATCATGTACCGGCAGGTG
        301 ---------+---------+---------+---------+---------+---------+ 360
                GGATCAAAGTGGCTGCGTCTCGTCTAGCTAGTCTTGGAGTTCTAGTACATGGCCGTCCAC
    a             P   S   F   T   D   A   E   Q   I   D   Q   N   L   K   I   M   Y   R   Q   V   -

                ATCTCCAACGGCAAGACGCCGTTGCTCTTCTTAGGCTCGGCTTACCGTGCCGGCGACAAC
        361 ---------+---------+---------+---------+---------+---------+ 420
                TAGAGGTTGCCGTTCTGCGGCAACGAGAAGAATCCGAGCCGAATGGCACGGCCGCTGTTG
    a             I   S   N   G   K   T   P   L   L   F   L   G   S   A   Y   R   A   G   D   N   -

                CCAAACCCCGGCGCGGGCTCGCTCGAGAACATACCACACGGCCCCGTCCACGGGTGGACT
        421 ---------+---------+---------+---------+---------+---------+ 480
                GGTTTGGGGCCGCGCCCGAGCGAGCTCTTGTATGGTGTGCCGGGGCAGGTGCCCACCTGA
    a             P   N   P   G   A   G   S   L   E   N   I   P   H   G   P   V   H   G   W   T   -

                GGCGACAGAAGCCAACCCAATCTCGAGGACATGGGCAACTTCTACTCCGCGGGGCGCGAC
        481 ---------+---------+---------+---------+---------+---------+ 540
                CCGCTGTCTTCGGTTGGGTTAGAGCTCCTGTACCCGTTGAAGATGAGGCGCCCCGCGCTG
    a             G   D   R   S   Q   P   N   L   E   D   M   G   N   F   Y   S   A   G   R   D   -

                CCTATCTTCTTCGCCCACCATTCAAATGTCGATCGCATGTGG
        541 ---------+---------+---------+---------+-- 582
                GGATAGAAGAAGCGGGTGGTAAGTTTACAGCTAGCGTACACC
    a             P   I   F   F   A   H   H   S   N   V   D   R   M   W   -
```

FIGURE 2 : Sequence of BPPO8

```
                TTGCCGTTTTGGAATTGGGACGCGCCCGGCGGCATGAAGCTGCCGTCGATCTACGCCGAC
              1 ---------+---------+---------+---------+---------+---------+ 60
                AACGGCAAAACCTTAACCCTGCGCGGGCCGCCGTACTTCGACGGCAGCTAGATGCGGCTG
a                L  P  F  W  N  W  D  A  P  G  G  M  K  L  P  S  I  Y  A  D  -

                CCTTCGTCCTCGCTCTATGACAAGTTTCGCGACGCCAAGCACCAGCCGCCGGTCCTCGTC
             61 ---------+---------+---------+---------+---------+---------+ 120
                GGAAGCAGGAGCGAGATACTGTTCAAAGCGCTGCGGTTCGTGGTCGGCGGCCAGGAGCAG
a                P  S  S  S  L  Y  D  K  F  R  D  A  K  H  Q  P  P  V  L  V  -

                GACCTCGACTACAACGGAACCGACCCTAGTTTCACCGACGCAGAGCAGATCGATCAGAAC
            121 ---------+---------+---------+---------+---------+---------+ 180
                CTGGAGCTGATGTTGCCTTGGCTGGGATCAAAGTGGCTGCGTCTCGTCTAGCTAGTCTTG
a                D  L  D  Y  N  G  T  D  P  S  F  T  D  A  E  Q  I  D  Q  N  -

                CTCAAGATCATGTACCGGCAGGTGATCTCCAACGGCAAGACGCCGTTGCTCTTCTTAGGC
            181 ---------+---------+---------+---------+---------+---------+ 240
                GAGTTCTAGTACATGGCCGTCCACTAGAGGTTGCCGTTCTGCGGCAACGAGAAGAATCCG
a                L  K  I  M  Y  R  Q  V  I  S  N  G  K  T  P  L  L  F  L  G  -

                TCGGCTTACCGTGCCGGCGACAACCCAAACCCCGGCGCGGGCTCGCTCGAGAACATACCA
            241 ---------+---------+---------+---------+---------+---------+ 300
                AGCCGAATGGCACGGCCGCTGTTGGGTTTGGGGCCGCGCCCGAGCGAGCTCTTGTATGGT
a                S  A  Y  R  A  G  D  N  P  N  P  G  A  G  S  L  E  N  I  P  -

                CACGGCCCCGTCCACGGGTGGACTGGCGACAGAAGCCAACCCAATCTCGAGGACATGGGC
            301 ---------+---------+---------+---------+---------+---------+ 360
                GTGCCGGGGCAGGTGCCCACCTGACCGCTGTCTTCGGTTGGGTTAGAGCTCCTGTACCCG
a                H  G  P  V  H  G  W  T  G  D  R  S  Q  P  N  L  E  D  M  G  -

                AACTTCTACTCCGCGGGGCGCGACCCTATCTTCTTCGCCCACCATTCAAATGTCGATAGC
            361 ---------+---------+---------+---------+---------+---------+ 420
                TTGAAGATGAGGCGCCCCGCGCTGGGATAGAAGAAGCGGGTGGTAAGTTTACAGCTATCG
a                N  F  Y  S  A  G  R  D  P  I  F  F  A  H  H  S  N  V  D  S  -

                ATGTGG
            421 ------ 426
                TACACC
a                M  W  -
```

FIGURE 3 : Sequence of BANPPO34

```
          GTTGCTCTTCTTAGGCTCGGCTTACCGTGCCGGCGACAACCCAAACCCCGGCGCGGGCTC
        1 ---------+---------+---------+---------+---------+---------+ 60
          CAACGAGAAGAATCCGAGCCGAATGGCACGGCCGCTGTTGGGTTTGGGGCCGCGCCCGAG
  b         L  L  F  L  G  S  A  Y  R  A  G  D  N  P  N  P  G  A  G  S  -

          GCTCGAGAACATACCACACGGCCCCGTCCACGGGTGGACTGGCGACAGAAACCAACCCAA
       61 ---------+---------+---------+---------+---------+---------+ 120
          CGAGCTCTTGTATGGTGTGCCGGGGCAGGTGCCCACCTGACCGCTGTCTTTGGTTGGGTT
  b         L  E  N  I  P  H  G  P  V  H  G  W  T  G  D  R  N  Q  P  N  -

          TCTCGAGGACATGGGCAACTTCTACTCCGCGGGGCGCGACCCTATCTTCTTCGCCCACCA
      121 ---------+---------+---------+---------+---------+---------+ 180
          AGAGCTCCTGTACCCGTTGAAGATGAGGCGCCCCGCGCTGGGATAGAAGAAGCGGGTGGT
  b         L  E  D  M  G  N  F  Y  S  A  G  R  D  P  I  F  F  A  H  H  -

          TTCAAACGTCGACCGCATGTGGTACTTGTGGAAGAAGCTCGGCGGGAAGCATCAGGACTT
      181 ---------+---------+---------+---------+---------+---------+ 240
          AAGTTTGCAGCTGGCGTACACCATGAACACCTTCTTCGAGCCGCCCTTCGTAGTCCTGAA
  b         S  N  V  D  R  M  W  Y  L  W  K  K  L  G  G  K  H  Q  D  F  -

          TAACGATAAGGACTGGCTCAACACCACCTTCCTCTTCTACGACGAGAATGCTGACTTAGT
      241 ---------+---------+---------+---------+---------+---------+ 300
          ATTGCTATTCCTGACCGAGTTGTGGTGGAAGGAGAAGATGCTGCTCTTACGACTGAATCA
  b         N  D  K  D  W  L  N  T  T  F  L  F  Y  D  E  N  A  D  L  V  -

          TCGAGTCACCCTCAAGGACTGCTTGCAGCCGGAGTGGCTTCGTTACGATTACCAAGACGT
      301 ---------+---------+---------+---------+---------+---------+ 360
          AGCTCAGTGGGAGTTCCTGACGAACGTCGGCCTCACCGAAGCAATGCTAATGGTTCTGCA
  b         R  V  T  L  K  D  C  L  Q  P  E  W  L  R  Y  D  Y  Q  D  V  -

          CGAGATCCCGTGGCTGAAGACCCGGCCGACTCCCAAAGCCTTGAAGGCGCAGAAAACCGC
      361 ---------+---------+---------+---------+---------+---------+ 420
          GCTCTAGGGCACCGACTTCTGGGCCGGCTGAGGGTTTCGGAACTTCCGCGTCTTTTGGCG
  b         E  I  P  W  L  K  T  R  P  T  P  K  A  L  K  A  Q  K  T  A  -

          AGCGAAAACACTGAAAGCTACAGCAGAGACGCCGTTCCCGGTGACGCTGCAATCCGCGGT
      421 ---------+---------+---------+---------+---------+---------+ 480
          TCGCTTTTGTGACTTTCGATGTCGTCTCTGCGGCAAGGGCCACTGCGACGTTAGGCGCCA
  b         A  K  T  L  K  A  T  A  E  T  P  F  P  V  T  L  Q  S  A  V  -

          GAGCACGACGGTGAGGAGGCCCAAGGTATCGAGGAGCGGCAAGGAGAAGGAAGAGGAAGA
      481 ---------+---------+---------+---------+---------+---------+ 540
          CTCGTGCTGCCACTCCTCCGGGTTCCATAGCTCCTCGCCGTTCCTCTTCCTTCTCCTTCT
  b         S  T  T  V  R  R  P  K  V  S  R  S  G  K  E  K  E  E  E  E  -

          GGAGGTCCTCATCGTGGAGGGGATCGAGTTCGACCGCGACTACTTCATAAAGTTCGACGT
      541 ---------+---------+---------+---------+---------+---------+ 600
          CCTCCAGGAGTAGCACCTCCCCTAGCTCAAGCTGGCGCTGATGAAGTATTTCAAGCTGCA
  b         E  V  L  I  V  E  G  I  E  F  D  R  D  Y  F  I  K  F  D  V  -

          CTTCGTGAACGCCACCGAGGGTGAGGGCATCACGCCGGGCGCCAGCGAGTTCGCGGGCAG
      601 ---------+---------+---------+---------+---------+---------+ 660
          GAAGCACTTGCGGTGGCTCCCACTCCCGTAGTGCGGCCCGCGGTCGCTCAAGCGCCCGTC
  b         F  V  N  A  T  E  G  E  G  I  T  P  G  A  S  E  F  A  G  S  -

          CTTCGTCAACGTCCCGCACAAGCACAAGCACAGCAAGAAGGAGAAGAAGCTGAAGACGAG
      661 ---------+---------+---------+---------+---------+---------+ 720
          GAAGCAGTTGCAGGGCGTGTTCGTGTTCGTGTCGTTCTTCCTCTTCTTCGACTTCTGCTC
  b         F  V  N  V  P  H  K  H  K  H  S  K  K  E  K  K  L  K  T  R  -
```

```
          GCTCTGCCTGGGGATCACTGACCTGCTCGAGGACATCGGGGCGGAGGACGACGACAGCGT
      721 ---------+---------+---------+---------+---------+---------+ 780
          CGAGACGGACCCCTAGTGACTGGACGAGCTCCTGTAGCCCCGCCTCCTGCTGCTGTCGCA
  b         L  C  L  G  I  T  D  L  L  E  D  I  G  A  E  D  D  D  S  V  -

          GCTCGTCACCATCGTCCCGAAAGCCGGAAAGGGCAAGGTGTCGGTCGCCGGCCTCCGCAT
      781 ---------+---------+---------+---------+---------+---------+ 840
          CGAGCAGTGGTAGCAGGGCTTTCGGCCTTTCCCGTTCCACAGCCAGCGGCCGGAGGCGTA
  b         L  V  T  I  V  P  K  A  G  K  G  K  V  S  V  A  G  L  R  I  -

          CGATTTCCCAAATTGAAGTAATACTATATATTTCTACTACCTATCAAGGAAAATAAAAGC
      841 ---------+---------+---------+---------+---------+---------+ 900
          GCTAAAGGGTTTAACTTCATTATGATATATAAAGATGATGGATAGTTCCTTTTATTTTCG
  b         D  F  P  N  *  S  N  T  I  Y  F  Y  Y  L  S  R  K  I  K  A  -

          CGCACCATCGTAACAAAAAAAAAAA
      901 ---------+---------+----- 925
          GCGTGGTAGCATTGTTTTTTTTTTT
  b         A  P  S  *  Q  K  K  K   -
```

MAP of: bpo34 check: 1607 from: 1 to: 925 August 22, 1997

FIGURE 4 : Sequence of BANPPO35

```
          GTTGCTCTTCTTAGGCTCGGCTTACCGTGCCGGTGACCAGCCTAACCCCGGCGCGGGATC
        1 ---------+---------+---------+---------+---------+---------+ 60
          CAACGAGAAGAATCCGAGCCGAATGGCACGGCCACTGGTCGGATTGGGGCCGCGCCCTAG
  b         L  L  F  L  G  S  A  Y  R  A  G  D  Q  P  N  P  G  A  G  S  -

          CATCGAGAACATGCCGCACAACAACGTGCACTTGTGGACCGGCGACCGCACCCAGCCCAA
       61 ---------+---------+---------+---------+---------+---------+ 120
          GTAGCTCTTGTACGGCGTGTTGTTGCACGTGAACACCTGGCCGCTGGCGTGGGTCGGGTT
  b         I  E  N  M  P  H  N  N  V  H  L  W  T  G  D  R  T  Q  P  N  -

          CTTCGAGAACATGGGCACCTTCTACGCGGCGGCGCGCGACCCCATCTTCTTCGCCCACCA
      121 ---------+---------+---------+---------+---------+---------+ 180
          GAAGCTCTTGTACCCGTGGAAGATGCGCCGCCGCGCGCTGGGGTAGAAGAAGCGGGTGGT
  b         F  E  N  M  G  T  F  Y  A  A  A  R  D  P  I  F  F  A  H  H  -

          CGCCAACATCGACCGAATGTGGTACCTGTGGAAGAAGCTCAGCAGGAAGCACCAGGACTT
      181 ---------+---------+---------+---------+---------+---------+ 240
          GCGGTTGTAGCTGGCTTACACCATGGACACCTTCTTCGAGTCGTCCTTCGTGGTCCTGAA
  b         A  N  I  D  R  M  W  Y  L  W  K  K  L  S  R  K  H  Q  D  F  -

          CAATGACTCGGACTGGCTCAAAGCTTCCTTCCTCTTCTACGACGAGAACGCCGACTTAGT
      241 ---------+---------+---------+---------+---------+---------+ 300
          GTTACTGAGCCTGACCGAGTTTCGAAGGAAGGAGAAGATGCTGCTCTTGCGGCTGAATCA
  b         N  D  S  D  W  L  K  A  S  F  L  F  Y  D  E  N  A  D  L  V  -

          TCGGGTCACGGTCAAGGACTGCTTGGAGACCGAGTGGCTGCGCTACACGTACCAAGACGT
      301 ---------+---------+---------+---------+---------+---------+ 360
          AGCCCAGTGCCAGTTCCTGACGAACCTCTGGCTCACCGACGCGATGTGCATGGTTCTGCA
  b         R  V  T  V  K  D  C  L  E  T  E  W  L  R  Y  T  Y  Q  D  V  -

          GAAGATCCCATGGGCGAACACCCGACCGACTCCCAAGCTCGCCAAGGCGAGGAAAGCCGG
      361 ---------+---------+---------+---------+---------+---------+ 420
          CTTCTAGGGTACCCGCTTGTGGGCTGGCTGAGGGTTCGAGCGGTTCCGCTCCTTTCGGCC
  b         K  I  P  W  A  N  T  R  P  T  P  K  L  A  K  A  R  K  A  G  -

          CAGCAGATCGCTGAAAGCCACCGCGGAGGTGCAGTTCCCTGTGACGCTGGAATCCCCGGT
      421 ---------+---------+---------+---------+---------+---------+ 480
          GTCGTCTAGCGACTTTCGGTGGCGCCTCCACGTCAAGGGACACTGCGACCTTAGGGGCCA
  b         S  R  S  L  K  A  T  A  E  V  Q  F  P  V  T  L  E  S  P  V  -

          CAAAGTGACGGTGAAGAGGCCCAAGGTGGGGAGGAGCGGCAAGGAGAAGGAAGATGAGGA
      481 ---------+---------+---------+---------+---------+---------+ 540
          GTTTCACTGCCACTTCTCCGGGTTCCACCCCTCCTCGCCGTTCCTCTTCCTTCTACTCCT
  b         K  V  T  V  K  R  P  K  V  G  R  S  G  K  E  K  E  D  E  E  -

          GGAGATACTCATAGTGGAGGGGATCGAGTTCGACCGCGACTACTTCATCAAGTTCGACGT
      541 ---------+---------+---------+---------+---------+---------+ 600
          CCTCTATGAGTATCACCTCCCCTAGCTCAAGCTGGCGCTGATGAAGTAGTTCAAGCTGCA
  b         E  I  L  I  V  E  G  I  E  F  D  R  D  Y  F  I  K  F  D  V  -

          CTTCGTGAACGCGACGGAGGGCGACGGCATCACGGCCGGGGCCAGTGAGTTCGCCGGCAG
      601 ---------+---------+---------+---------+---------+---------+ 660
          GAAGCACTTGCGCTGCCTCCCGCTGCCGTAGTGCCGGCCCCGGTCACTCAAGCGGCCGTC
  b         F  V  N  A  T  E  G  D  G  I  T  A  G  A  S  E  F  A  G  S  -

          CTTCGTGAACGTCCCGCACAAGCACAAGCACCGCAAGGATGAGAATAAGCTGAAGACGAG
      661 ---------+---------+---------+---------+---------+---------+ 720
          GAAGCACTTGCAGGGCGTGTTCGTGTTCGTGGCGTTCCTACTCTTATTCGACTTCTGCTC
  b         F  V  N  V  P  H  K  H  K  H  R  K  D  E  N  K  L  K  T  R  -
```

```
          GCTGTGTCTGGGAATCACCGACCTGCTCGAGGACATCGGCGCGGAGGACGACGACAGCGT
     721  ---------+---------+---------+---------+---------+---------+  780
          CGACACAGACCCTTAGTGGCTGGACGAGCTCCTGTAGCCGCGCCTCCTGCTGCTGTCGCA
b           L  C  L  G  I  T  D  L  L  E  D  I  G  A  E  D  D  D  S  V  -

          GCTCGTCACCATCGTGCCGAAGGCAGGCAAAGGAAAGGTGTCCGTCGGCGGTCTTCGGAT
     781  ---------+---------+---------+---------+---------+---------+  840
          CGAGCAGTGGTAGCACGGCTTCCGTCCGTTTCCTTTCCACAGGCAGCCGCCAGAAGCCTA
b           L  V  T  I  V  P  K  A  G  K  G  K  V  S  V  G  G  L  R  I  -

          TGACTTTTCCAAGTGAGGAAATAAAAGAATTCACGTGCCGTGCCTGCTTTCAATGTACGA
     841  ---------+---------+---------+---------+---------+---------+  900
          ACTGAAAAGGTTCACTCCTTTATTTTCTTAAGTGCACGGCACGGACGAAAGTTACATGCT
b           D  F  S  K  *  G  N  K  R  I  H  V  P  C  L  L  S  M  Y  E  -

          ATAAAATAAGAGTGCATCATCACCGACCATGGTTCTACTTTAAAAAAAAAAAAAAAAAAA
     901  ---------+---------+---------+---------+---------+---------+  960
          TATTTTATTCTCACGTAGTAGTGGCTGGTACCAAGATGAAATTTTTTTTTTTTTTTTTTT
b           *  N  K  S  A  S  S  P  T  M  V  L  L  *  K  K  K  K  K     -

MAP of: bpo35  check: 1637  from: 1  to: 960 August 25, 1997
```

FIGURE 5 : Sequence of TOBPPO6

```
          GATCCGACGTTTGCGTTGCCATATTGGAACTGGGATCATCCAAAGGGCATGCGTTTGCCA
        1 ---------+---------+---------+---------+---------+---------+ 60
          CTAGGCTGCAAACGCAACGGTATAACCTTGACCCTAGTAGGTTTCCCGTACGCAAACGGT
    a      D  P  T  F  A  L  P  Y  W  N  W  D  H  P  K  G  M  R  L  P   -

          CACATGTTTGATCAACCAAACGTGTACCCTGATCTTTACGATCCAAGACGTAACCAAGAA
       61 ---------+---------+---------+---------+---------+---------+ 120
          GTGTACAAACTAGTTGGTTTGCACATGGGACTAGAAATGCTAGGTTCTGCATTGGTTCTT
    a      H  M  F  D  Q  P  N  V  Y  P  D  L  Y  D  P  R  R  N  Q  E   -

          CACCGCGGTTCTGTAATCATGGACCTTGGTCATTTTGGTCAAGACGTGAAAGGAACTGAC
      121 ---------+---------+---------+---------+---------+---------+ 180
          GTGGCGCCAAGACATTAGTACCTGGAACCAGTAAAACCAGTTCTGCACTTTCCTTGACTG
    a      H  R  G  S  V  I  M  D  L  G  H  F  G  Q  D  V  K  G  T  D   -

          TTGCAAATGATGAGCAATAACCTTACTCTAATGTATCGTCAAATGATTACCAATTCACCA
      181 ---------+---------+---------+---------+---------+---------+ 240
          AACGTTTACTACTCGTTATTGGAATGAGATTACATAGCAGTTTACTAATGGTTAAGTGGT
    a      L  Q  M  M  S  N  N  L  T  L  M  Y  R  Q  M  I  T  N  S  P   -

          TGTCCACAACTCTTTTTCGGTAAGCCATATTGTACGGAAGTTGGACCCAAACCAGGGCAG
      241 ---------+---------+---------+---------+---------+---------+ 300
          ACAGGTGTTGAGAAAAAGCCATTCGGTATAACATGCCTTCAACCTGGGTTTGGTCCCGTC
    a      C  P  Q  L  F  F  G  K  P  Y  C  T  E  V  G  P  K  P  G  Q   -

          GGAGCTATTGAAAACATCCCTCATACTCCTGTCCACATTTGGGTTGGTAGTAAGCCTAAT
      301 ---------+---------+---------+---------+---------+---------+ 360
          CCTCGATAACTTTTGTAGGGAGTATGAGGACAGGTGTAAACCCAACCATCATTCGGATTA
    a      G  A  I  E  N  I  P  H  T  P  V  H  I  W  V  G  S  K  P  N   -

          GAGAATAACTGTAAAAACGGTGAAGATATGGGAAATTTCTATTCAGCTGGTAAGGATCCT
      361 ---------+---------+---------+---------+---------+---------+ 420
          CTCTTATTGACATTTTTGCCACTTCTATACCCTTTAAAGATAAGTCGACCATTCCTAGGA
    a      E  N  N  C  K  N  G  E  D  M  G  N  F  Y  S  A  G  K  D  P   -

          GCTTTCTATAGTCACCATGCAAATGTAGATCGCATGTGGACAATATGGAAAACATTAGGA
      421 ---------+---------+---------+---------+---------+---------+ 480
          CGAAAGATATCAGTGGTACGTTTACATCTAGCGTACACCTGTTATACCTTTTGTAATCCT
    a      A  F  Y  S  H  H  A  N  V  D  R  M  W  T  I  W  K  T  L  G   -

          GGAAAACGCAAGGACATCAACAAGCCAGATTATTTGAACACTGAGTTCTTTTTCTACGAC
      481 ---------+---------+---------+---------+---------+---------+ 540
          CCTTTTGCGTTCCTGTAGTTGTTCGGTCTAATAAACTTGTGACTCAAGAAAAAGATGCTG
    a      G  K  R  K  D  I  N  K  P  D  Y  L  N  T  E  F  F  F  Y  D   -

          GAAAA
      541 ----- 545
          CTTTT
    a      E     -
```

FIGURE 6 : Sequence of TOBPPO25

```
                TGCACTGTGCGTATTGCAACGGTGCTTACAAAATTGGTGGCAAAGAGTTACAAGTCCATT
          1 ---------+---------+---------+---------+---------+---------+ 60
                ACGTGACACGCATAACGTTGCCACGAATGTTTTAACCACCGTTTCTCAATGTTCAGGTAA
    c             H  C  A  Y  C  N  G  A  Y  K  I  G  G  K  E  L  Q  V  H  F -

                TCTCGTGGCTTTTTTTCCCTTTTCATAGATGGTACTTGTACTTCTATGAAAGAATCTTGG
         61 ---------+---------+---------+---------+---------+---------+ 120
                AGAGCACCGAAAAAAAGGGAAAAGTATCTACCATGAACATGAAGATACTTTCTTAGAACC
    c             S  W  L  F  F  P  F  H  R  W  Y  L  Y  F  Y  E  R  I  L  G -

                GCTCTTTAATTAATGATCCTACTTTTGGTTTGCCATATTGGAACTGGGACCATCCAAAGG
        121 ---------+---------+---------+---------+---------+---------+ 180
                CGAGAAATTAATTACTAGGATGAAAACCAAACGGTATAACCTTGACCCTGGTAGGTTTCC
    c             S  L  I  N  D  P  T  F  G  L  P  Y  W  N  W  D  H  P  K  G -

                GCATGCGTATACCTCCCATGTTCGATCGTGAAGGGTCTTCCCTTTACGACGAAAAACGTA
        181 ---------+---------+---------+---------+---------+---------+ 240
                CGTACGCATATGGAGGGTACAAGCTAGCACTTCCCAGAAGGGAAATGCTGCTTTTTGCAT
    c             M  R  I  P  P  M  F  D  R  E  G  S  S  L  Y  D  E  K  R  N -

                ACCAAAGTCACCGTAATGGAACCATAATTGATCTTGGTCATTTCGGTCAAGAAGTCCAAA
        241 ---------+---------+---------+---------+---------+---------+ 300
                TGGTTTCAGTGGCATTACCTTGGTATTAACTAGAACCAGTAAAGCCAGTTCTTCAGGTTT
    c             Q  S  H  R  N  G  T  I  I  D  L  G  H  F  G  Q  E  V  Q  T -

                CAACTCAACTGCAGCAGATGACTAATAACTTAACTATAATGTATCGTCAAATGATAACTA
        301 ---------+---------+---------+---------+---------+---------+ 360
                GTTGAGTTGACGTCGTCTACTGATTATTGAATTGATATTACATAGCAGTTTACTATTGAT
    c             T  Q  L  Q  Q  M  T  N  N  L  T  I  M  Y  R  Q  M  I  T  N -

                ATGCTCCTTGCCCCTTGCTCTTCTTTGGTCAGCCTTACCCTCTAGGAACTGATCCCAGTC
        361 ---------+---------+---------+---------+---------+---------+ 420
                TACGAGGAACGGGGAACGAGAAGAAACCAGTCGGAATGGGAGATCCTTGACTAGGGTCAG
    c             A  P  C  P  L  L  F  F  G  Q  P  Y  P  L  G  T  D  P  S  P -

                CAGGGATGGGCACTATTGAAAACATCCCTCATACTCCTGTCCACATTTGGGTTGGTAGTA
        421 ---------+---------+---------+---------+---------+---------+ 480
                GTCCCTACCCGTGATAACTTTTGTAGGGAGTATGAGGACAGGTGTAAACCCAACCATCAT
    c             G  M  G  T  I  E  N  I  P  H  T  P  V  H  I  W  V  G  S  R -

                GGCTTGATGAGAATAATACGAAACACGGTGAGGATATGGGTAATTTTTACTCGGCCGGTT
        481 ---------+---------+---------+---------+---------+---------+ 540
                CCGAACTACTCTTATTATGCTTTGTGCCACTCCTATACCCATTAAAAATGAGCCGGCCAA
    c             L  D  E  N  N  T  K  H  G  E  D  M  G  N  F  Y  S  A  G  L -

                TAGACCCGCTTTTCTATTCCCATCACGCCAATGTGGACCGGATGTGGTCCGAGTGGAAAG
        541 ---------+---------+---------+---------+---------+---------+ 600
                ATCTGGGCGAAAAGATAAGGGTAGTGCGGTTACACCTGGCCTACACCAGGCTCACCTTTC
    c             D  P  L  F  Y  S  H  H  A  N  V  D  R  M  W  S  E  W  K  A -

                CCTTAGGAGGGAAAAGAAGGGATCTCACGCACAAAGATTGGTTGAACTCCGAGTTCTTTT
        601 ---------+---------+---------+---------+---------+---------+ 660
                GGAATCCTCCCTTTTCTTCCCTAGAGTGCGTGTTTCTAACCAACTTGAGGCTCAAGAAAA
    c             L  G  G  K  R  R  D  L  T  H  K  D  W  L  N  S  E  F  F  F -

                TCTACGATGAAAA
        661 ---------+--- 673
                AGATGCTACTTTT
    c             Y  D  E     -
```

FIGURE 7 : Sequence of TOBPPO26

```
                TGCATTGTGCGTATTGCAACGATGCTTACACAATGGGTGACCAAAAGTTACAAGTTCACC
          1     ---------+---------+---------+---------+---------+---------+ 60
                ACGTAACACGCATAACGTTGCTACGAATGTGTTACCCACTGGTTTTCAATGTTCAAGTGG
    c             H  C  A  Y  C  N  D  A  Y  T  M  G  D  Q  K  L  Q  V  H  Q -

                AATCGTGGCTTTTCTTCCCGTTTCATAGATGGTACTTGTACTTCTACGAGAGAATCTTGG
         61     ---------+---------+---------+---------+---------+---------+ 120
                TTAGCACCGAAAAGAAGGGCAAAGTATCTACCATGAACATGAAGATGCTCTCTTAGAACC
    c             S  W  L  F  F  P  F  H  R  W  Y  L  Y  F  Y  E  R  I  L  G -

                GCTCCCTCATCGATGATCCAACTTTTGCTCTGCCATATTGGAACTGGGACCATCCAAGCG
        121     ---------+---------+---------+---------+---------+---------+ 180
                CGAGGGAGTAGCTACTAGGTTGAAAACGAGACGGTATAACCTTGACCCTGGTAGGTTCGC
    c             S  L  I  D  D  P  T  F  A  L  P  Y  W  N  W  D  H  P  S  G -

                GCATGCGTTTGCCTGCTATGTTCGATGTCGAAGGTTCTTCCCTCTACGATGCAAGACGTA
        181     ---------+---------+---------+---------+---------+---------+ 240
                CGTACGCAAACGGACGATACAAGCTACAGCTTCCAAGAAGGGAGATGCTACGTTCTGCAT
    c             M  R  L  P  A  M  F  D  V  E  G  S  S  L  Y  D  A  R  R  N -

                ATCCACATGTCCGTAATGGAACCATAATCGATCTTGGTTTTTTCGGTGATGAAGTCAAAA
        241     ---------+---------+---------+---------+---------+---------+ 300
                TAGGTGTACAGGCATTACCTTGGTATTAGCTAGAACCAAAAAAGCCACTACTTCAGTTTT
    c             P  H  V  R  N  G  T  I  I  D  L  G  F  F  G  D  E  V  K  T -

                CTAATGAAATACAGATGATAACTAACAACTTAATTCTAATGTATCGTCAAATGATAACTA
        301     ---------+---------+---------+---------+---------+---------+ 360
                GATTACTTTATGTCTACTATTGATTGTTGAATTAAGATTACATAGCAGTTTACTATTGAT
    c             N  E  I  Q  M  I  T  N  N  L  I  L  M  Y  R  Q  M  I  T  N -

                ATGCTCCATGCCCGCTGTTGTTCTTCGGAGAGCCTTACAGATTCGGATCTAAACCCAATC
        361     ---------+---------+---------+---------+---------+---------+ 420
                TACGAGGTACGGGCGACAACAAGAAGCCTCTCGGAATGTCTAAGCCTAGATTTGGGTTAG
    c             A  P  C  P  L  L  F  F  G  E  P  Y  R  F  G  S  K  P  N  P -

                CGGGGCAGGGAACCATTGAAAACATTCCTCATACTCCGGTTCACATTTGGACTGGTACTG
        421     ---------+---------+---------+---------+---------+---------+ 480
                GCCCCGTCCCTTGGTAACTTTTGTAAGGAGTATGAGGCCAAGTGTAAACCTGACCATGAC
    c             G  Q  G  T  I  E  N  I  P  H  T  P  V  H  I  W  T  G  T  V -

                TGCGGTGTACGGATTTGGGTAATTGTGTGCCATCATACGGTGAGGATATGGGTAATTTCT
        481     ---------+---------+---------+---------+---------+---------+ 540
                ACGCCACATGCCTAAACCCATTAACACACGGTAGTATGCCACTCCTATACCCATTAAAGA
    c             R  C  T  D  L  G  N  C  V  P  S  Y  G  E  D  M  G  N  F  Y -

                ACTCAGCTGGTTTAGACCCAGTTTTTTACAGCCACCACGCCAATGTGGACCGCATGTGGA
        541     ---------+---------+---------+---------+---------+---------+ 600
                TGAGTCGACCAAATCTGGGTCAAAAAATGTCGGTGGTGCGGTTACACCTGGCGTACACCT
    c             S  A  G  L  D  P  V  F  Y  S  H  H  A  N  V  D  R  M  W  N -

                ATGAATGGAAAGCACTAGGAGGGAAAAGAAGGGATCTCACAGACAATGATTGGTTAAACT
        601     ---------+---------+---------+---------+---------+---------+ 660
                TACTTACCTTTCGTGATCCTCCCTTTTCTTCCCTAGAGTGTCTGTTACTAACCAATTTGA
    c             E  W  K  A  L  G  G  K  R  R  D  L  T  D  N  D  W  L  N  S -

                CGGAGTTCTTTTTCTACGACGAAAA
        661     ---------+---------+----- 685
                GCCTCAAGAAAAAGATGCTGCTTTT
    c             E  F  F  F  Y  D  E     -
```

FIGURE 8 : Sequence of PINPPO20

```
                TGCATTGTGCGTACTGCGACGGCGCGTATGACCAAATCGGCTTCCCCGATCTCGAGATCC
          1 ---------+---------+---------+---------+---------+---------+ 60
                ACGTAACACGCATGACGCTGCCGCGCATACTGGTTTAGCCGAAGGGGCTAGAGCTCTAGG
    c             H  C  A  Y  C  D  G  A  Y  D  Q  I  G  F  P  D  L  E  I  Q -

                AGATCCACAACTCGTGGCTCTTCTTTCCTTGGCACCGGTTCTACCTCTACTTCAACGAGC
         61 ---------+---------+---------+---------+---------+---------+ 120
                TCTAGGTGTTGAGCACCGAGAAGAAAGGAACCGTGGCCAAGATGGAGATGAAGTTGCTCG
    c             I  H  N  S  W  L  F  F  P  W  H  R  F  Y  L  Y  F  N  E  R -

                GCATACTCGGGAAACTTATCGGCGACGACACGTTCGCGCTGCCTTTCTGGAACTGGGACG
        121 ---------+---------+---------+---------+---------+---------+ 180
                CGTATGAGCCCTTTGAATAGCCGCTGCTGTGCAAGCGCGACGGAAAGACCTTGACCCTGC
    c             I  L  G  K  L  I  G  D  D  T  F  A  L  P  F  W  N  W  D  A -

                CGCCGGGGGGCATGCAGTTCCCGTCTATCTACACGGACCCTTCATCCTCGCTATATGACA
        181 ---------+---------+---------+---------+---------+---------+ 240
                GCGGCCCCCCGTACGTCAAGGGCAGATAGATGTGCCTGGGAAGTAGGAGCGATATACTGT
    c             P  G  G  M  Q  F  P  S  I  Y  T  D  P  S  S  S  L  Y  D  K -

                AGCTGCGTGATGCGAAGCACCAGCCGCCGACTTTGATTGACCTCGACTACAATGGCACCG
        241 ---------+---------+---------+---------+---------+---------+ 300
                TCGACGCACTACGCTTCGTGGTCGGCGGCTGAAACTAACTGGAGCTGATGTTACCGTGGC
    c             L  R  D  A  K  H  Q  P  P  T  L  I  D  L  D  Y  N  G  T  D -

                ATCCTACCTTCTCCCCTGAAGAACAGATTAACCACAACCTCGCCGTCATGTACCGACAGG
        301 ---------+---------+---------+---------+---------+---------+ 360
                TAGGATGGAAGAGGGGACTTCTTGTCTAATTGGTGTTGGAGCGGCAGTACATGGCTGTCC
    c             P  T  F  S  P  E  E  Q  I  N  H  N  L  A  V  M  Y  R  Q  V -

                TGATATCCAGTGGAAAGACACCAGAGCTGTTTATGGGCTCAGCGTACCGCGCCGGTGACC
        361 ---------+---------+---------+---------+---------+---------+ 420
                ACTATAGGTCACCTTTCTGTGGTCTCGACAAATACCCGAGTCGCATGGCGCGGCCACTGG
    c             I  S  S  G  K  T  P  E  L  F  M  G  S  A  Y  R  A  G  D  Q -

                AGCCTGACCCCGGCGCAGGTTCTGTAGAGCAGAAGCCGCACGGCCCGGTGCATGTGTGGA
        421 ---------+---------+---------+---------+---------+---------+ 480
                TCGGACTGGGGCCGCGTCCAAGACATCTCGTCTTCGGCGTGCCGGGCCACGTACACACCT
    c             P  D  P  G  A  G  S  V  E  Q  K  P  H  G  P  V  H  V  W  T -

                CAGGTGATCGCAACCAGCCCAATCGCGAAGACATGGGCACGCTCTACTCGGCGGCGTGGG
        481 ---------+---------+---------+---------+---------+---------+ 540
                GTCCACTAGCGTTGGTCGGGTTAGCGCTTCTGTACCCGTGCGAGATGAGCCGCCGCACCC
    c             G  D  R  N  Q  P  N  R  E  D  M  G  T  L  Y  S  A  A  W  D -

                ACCCCGTTTTTTTCGCACACCACGGCAACATCGACCGCATGTGGTACGTGTGGAGGAACC
        541 ---------+---------+---------+---------+---------+---------+ 600
                TGGGGCAAAAAAAGCGTGTGGTGCCGTTGTAGCTGGCGTACACCATGCACACCTCCTTGG
    c             P  V  F  F  A  H  H  G  N  I  D  R  M  W  Y  V  W  R  N  L -

                TTGGCGGCAAGCACCGCAACTTCACCGACCCCGACTGGCTCAACGCGTCCTTCCTGTTCT
        601 ---------+---------+---------+---------+---------+---------+ 660
                AACCGCCGTTCGTGGCGTTGAAGTGGCTGGGGCTGACCGAGTTGCGCAGGAAGGACAAGA
    c             G  G  K  H  R  N  F  T  D  P  D  W  L  N  A  S  F  L  F  Y -

                 ACGACGAAAA
        661 ----------+ 670
                TGCTGCTTTT
    c             D  E     -
```

FIGURE 9 : Sequence of PINPPO2

```
                TTGCCGTTTTGGAATTGGGACGCGCCGGGGGGCATGCAGATCCCGGCCATCTACGCCGAC
          1 ---------+---------+---------+---------+---------+---------+ 60
                AACGGCAAAACCTTAACCCTGCGCGGCCCCCCGTACGTCTAGGGCCGGTAGATGCGGCTG
a                L  P  F  W  N  W  D  A  P  G  G  M  Q  I  P  A  I  Y  A  D  -

                GCTTCGTCCCCGCTCTACGACAAGCTGCGCAATGCGAAGCACCAGCCGCCGACTTTGGTC
         61 ---------+---------+---------+---------+---------+---------+ 120
                CGAAGCAGGGGCGAGATGCTGTTCGACGCGTTACGCTTCGTGGTCGGCGGCTGAAACCAG
a                A  S  S  P  L  Y  D  K  L  R  N  A  K  H  Q  P  P  T  L  V  -

                GACCTCGACTACAACGGCACCGACCCGACCTTCACCCCTGAGCAGCAGATCGCCCACAAC
        121 ---------+---------+---------+---------+---------+---------+ 180
                CTGGAGCTGATGTTGCCGTGGCTGGGCTGGAAGTGGGGACTCGTCGTCTAGCGGGTGTTG
a                D  L  D  Y  N  G  T  D  P  T  F  T  P  E  Q  Q  I  A  H  N  -

                CTCACCATCATGTACCGACAGGTGATATCCGGCGGGAAGACGCCGGAGTTGTTTATGGGC
        181 ---------+---------+---------+---------+---------+---------+ 240
                GAGTGGTAGTACATGGCTGTCCACTATAGGCCGCCCTTCTGCGGCCTCAACAAATACCCG
a                L  T  I  M  Y  R  Q  V  I  S  G  G  K  T  P  E  L  F  M  G  -

                GCGGCGTACCGCGCGGGCGACGCGCCAGACCCGGGCGCAGGCACTCTAGAGCTCGTGCCG
        241 ---------+---------+---------+---------+---------+---------+ 300
                CGCCGCATGGCGCGCCCGCTGCGCGGTCTGGGCCCGCGTCCGTGAGATCTCGAGCACGGC
a                A  A  Y  R  A  G  D  A  P  D  P  G  A  G  T  L  E  L  V  P  -

                CACAACACGATGCATTTGTGGACCGGCGACCCCAACCAACCCAACGACGAAGACATGGGC
        301 ---------+---------+---------+---------+---------+---------+ 360
                GTGTTGTGCTACGTAAACACCTGGCCGCTGGGGTTGGTTGGGTTGCTGCTTCTGTACCCG
a                H  N  T  M  H  L  W  T  G  D  P  N  Q  P  N  D  E  D  M  G  -

                ACGTTCTACGCGGCGGCGCGGGACCCCATCTTCTTCGCCCACCACGGCAACGTCGACCGC
        361 ---------+---------+---------+---------+---------+---------+ 420
                TGCAAGATGCGCCGCCGCGCCCTGGGGTAGAAGAAGCGGGTGGTGCCGTTGCAGCTGGCG
a                T  F  Y  A  A  A  R  D  P  I  F  F  A  H  H  G  N  V  D  R  -

                ATGTGGTACGTGTGGCGGAAACTCGGGGGCACGCACCGCGATTTCACCGACCCCGACTGG
        421 ---------+---------+---------+---------+---------+---------+ 480
                TACACCATGCACACCGCCTTTGAGCCCCCGTGCGTGGCGCTAAAGTGGCTGGGGCTGACC
a                M  W  Y  V  W  R  K  L  G  G  T  H  R  D  F  T  D  P  D  W  -

                CTCAACGCGTCCTTCCTCTTCTACGACGAGAACGCGCAGCTCGTCCGCGTCAAAGTAAAG
        481 ---------+---------+---------+---------+---------+---------+ 540
                GAGTTGCGCAGGAAGGAGAAGATGCTGCTCTTGCGCGTCGAGCAGGCGCAGTTTCATTTC
a                L  N  A  S  F  L  F  Y  D  E  N  A  Q  L  V  R  V  K  V  K  -

                GACTGCTTGAGCGCCGACGCGCTGCGGTACACGTACCAGGACGTCGACATCCCGTGGATC
        541 ---------+---------+---------+---------+---------+---------+ 600
                CTGACGAACTCGCGGCTGCGCGACGCCATGTGCATGGTCCTGCAGCTGTAGGGCACCTAG
a                D  C  L  S  A  D  A  L  R  Y  T  Y  Q  D  V  D  I  P  W  I  -

                AGTGCGAAGCCGACGCCGAAGAAAACACCGGGGGGCGCTGCGCCTTCCACGACAGAGGCT
        601 ---------+---------+---------+---------+---------+---------+ 660
                TCACGCTTCGGCTGCGGCTTCTTTTGTGGCCCCCCGCGACGCGGAAGGTGCTGTCTCCGA
a                S  A  K  P  T  P  K  K  T  P  G  G  A  A  P  S  T  T  E  A  -

                ATATTTCCGGTGGTGCTGGATAAGCCGGTGAGCTCTACGGTGGCGAGGCCGAAGACGGGG
        661 ---------+---------+---------+---------+---------+---------+ 720
                TATAAAGGCCACCACGACCTATTCGGCCACTCGAGATGCCACCGCTCCGGCTTCTGCCCC
a                I  F  P  V  V  L  D  K  P  V  S  S  T  V  A  R  P  K  T  G  -
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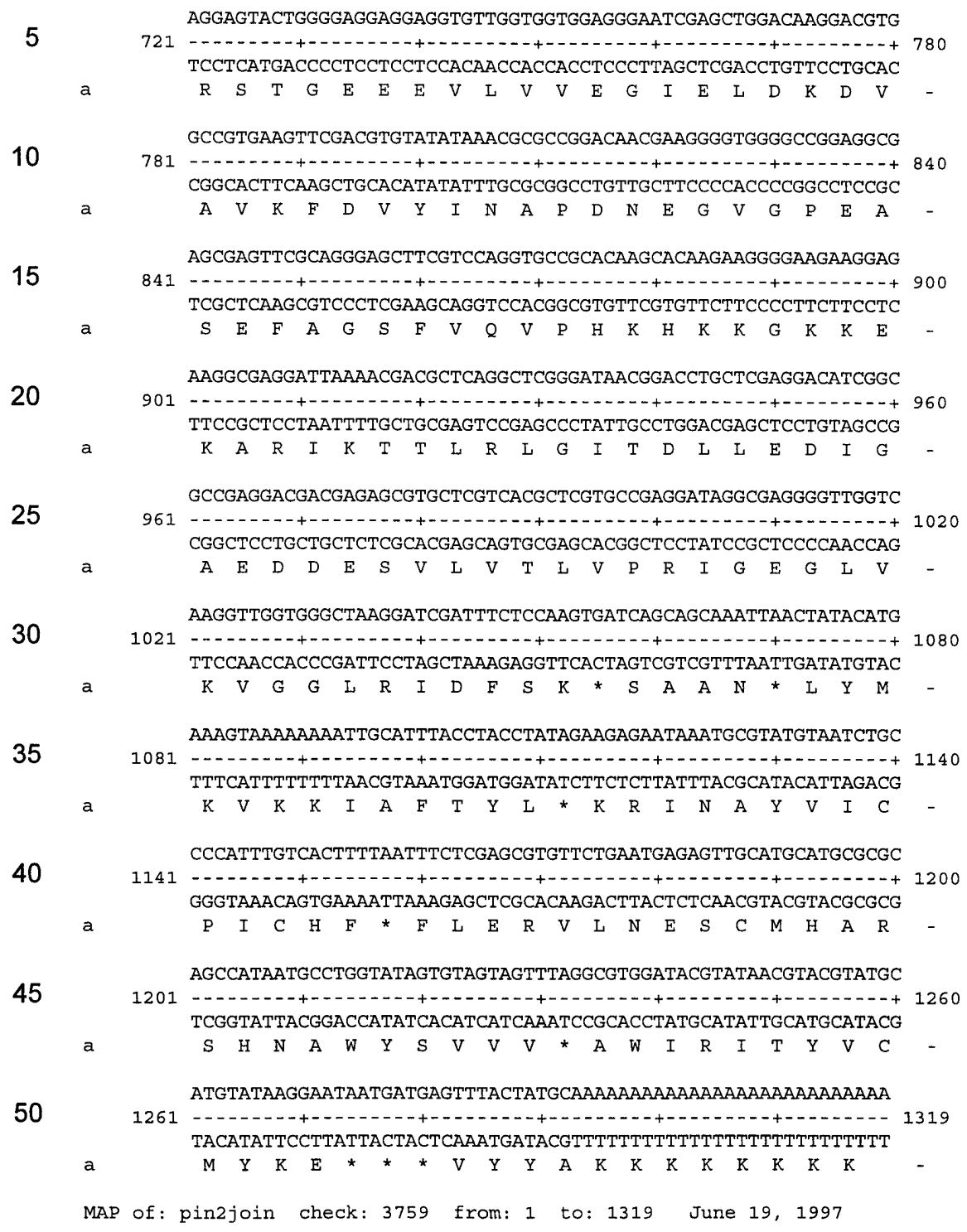

MAP of: pin2join check: 3759 from: 1 to: 1319 June 19, 1997

FIGURE 10 : Sequence of PINPPOFL

```
          CGGTATCGATAAGCTTGATCCAGTGCCTGGTTTAGGTGTATTCACTATGGCCACCCTCTC
        1 ---------+---------+---------+---------+---------+---------+ 60
          GCCATAGCTATTCGAACTAGGTCACGGACCAAATCCACATAAGTGATACCGGTGGGAGAG
  b         G  I  D  K  L  D  P  V  P  G  L  G  V  F  T  M  A  T  L  S  -

          TAAACTAGCTTCCCAACCAATAACACCTCCACTCTCCCCGCTCCCTCCTTTGCATGCTCC
       61 ---------+---------+---------+---------+---------+---------+ 120
          ATTTGATCGAAGGGTTGGTTATTGTGGAGGTGAGAGGGGCGAGGGAGGAAACGTACGAGG
  b         K  L  A  S  Q  P  I  T  P  P  L  S  P  L  P  P  L  H  A  P  -

          TTCTCTCACCAAAAGCTTCACCACCACCTTCCTCTCCCCTGTAGGGGTCCCAAACCACCC
      121 ---------+---------+---------+---------+---------+---------+ 180
          AAGAGAGTGGTTTTCGAAGTGGTGGTGGAAGGAGAGGGGACATCCCCAGGGTTTGGTGGG
  b         S  L  T  K  S  F  T  T  T  F  L  S  P  V  G  V  P  N  H  P  -

          CGTCATAAGATCTCATGCAAATCTAAGGAGCAACAAGAGAATGCCGACAAGCCTGCGGGC
      181 ---------+---------+---------+---------+---------+---------+ 240
          GCAGTATTCTAGAGTACGTTTAGATTCCTCGTTGTTCTCTTACGGCTGTTCGGACGCCCG
  b         V  I  R  S  H  A  N  L  R  S  N  K  R  M  P  T  S  L  R  A  -

          CGCATCGCCCGCCGCGACCTACTCCTGGGCCCTCGGCGGGCTTTACGGTGCCACCACTGG
      241 ---------+---------+---------+---------+---------+---------+ 300
          GCGTAGCGGGCGGCGCTGGATGAGGACCCGGGAGCCGCCCGAAATGCCACGGTGGTGACC
  b         A  S  P  A  A  T  Y  S  W  A  L  G  G  L  Y  G  A  T  T  G  -

          GCTCGGCCTCAACCGTCGAGCGGCCGCCGCCCCTATCCTGGCTCCCGACCTCTCAACTTG
      301 ---------+---------+---------+---------+---------+---------+ 360
          CGAGCCGGAGTTGGCAGCTCGCCGGCGGCGGGGATAGGACCGAGGGCTGGAGAGTTGAAC
  b         L  G  L  N  R  R  A  A  A  A  P  I  L  A  P  D  L  S  T  C  -

          TGGGCCGCCTGCCGACCTCCCTGCCTCCGCCCGACCGACAGTTTGCTGCCCGCCATACCA
      361 ---------+---------+---------+---------+---------+---------+ 420
          ACCCGGCGGACGGCTGGAGGGACGGAGGCGGGCTGGCTGTCAAACGACGGGCGGTATGGT
  b         G  P  P  A  D  L  P  A  S  A  R  P  T  V  C  C  P  P  Y  Q  -

          ATCCACCATCATCGACTTCAAGCTCCCCCCGCGATCTGCTCCGCTTCGCGTCCGGCCTGC
      421 ---------+---------+---------+---------+---------+---------+ 480
          TAGGTGGTAGTAGCTGAAGTTCGAGGGGGGCGCTAGACGAGGCGAAGCGCAGGCCGGACG
  b         S  T  I  I  D  F  K  L  P  P  R  S  A  P  L  R  V  R  P  A  -

          GGCCCACTTGGTTGACGCCGACTACCTGGCCAAGTATAAGAAGGCGGTCGAGCTCATGAG
      481 ---------+---------+---------+---------+---------+---------+ 540
          CCGGGTGAACCAACTGCGGCTGATGGACCGGTTCATATTCTTCCGCCAGCTCGAGTACTC
  b         A  H  L  V  D  A  D  Y  L  A  K  Y  K  K  A  V  E  L  M  R  -

          GGCCCTGCCGGCCGACGACCCGCGCAACTTCGTACAGCAAGCGAAAGTGCACTGTGCGTA
      541 ---------+---------+---------+---------+---------+---------+ 600
          CCGGGACGGCCGGCTGCTGGGCGCGTTGAAGCATGTCGTTCGCTTTCACGTGACACGCAT
  b         A  L  P  A  D  D  P  R  N  F  V  Q  Q  A  K  V  H  C  A  Y  -

          TTGCGACGGCGCGTATGACCAAATCGGCTTCCCCGATCTCGAGATCCAGATCCACAACTC
      601 ---------+---------+---------+---------+---------+---------+ 660
          AACGCTGCCGCGCATACTGGTTTAGCCGAAGGGGCTAGAGCTCTAGGTCTAGGTGTTGAG
  b         C  D  G  A  Y  D  Q  I  G  F  P  D  L  E  I  Q  I  H  N  S  -

          GTGGCTCTTCTTTCCTTGGCACCGGTTCTACCTCTACTCCAACGAGCGCATACTCGGGAA
      661 ---------+---------+---------+---------+---------+---------+ 720
          CACCGAGAAGAAAGGAACCGTGGCCAAGATGGAGATGAGGTTGCTCGCGTATGAGCCCTT
  b         W  L  F  F  P  W  H  R  F  Y  L  Y  S  N  E  R  I  L  G  K  -
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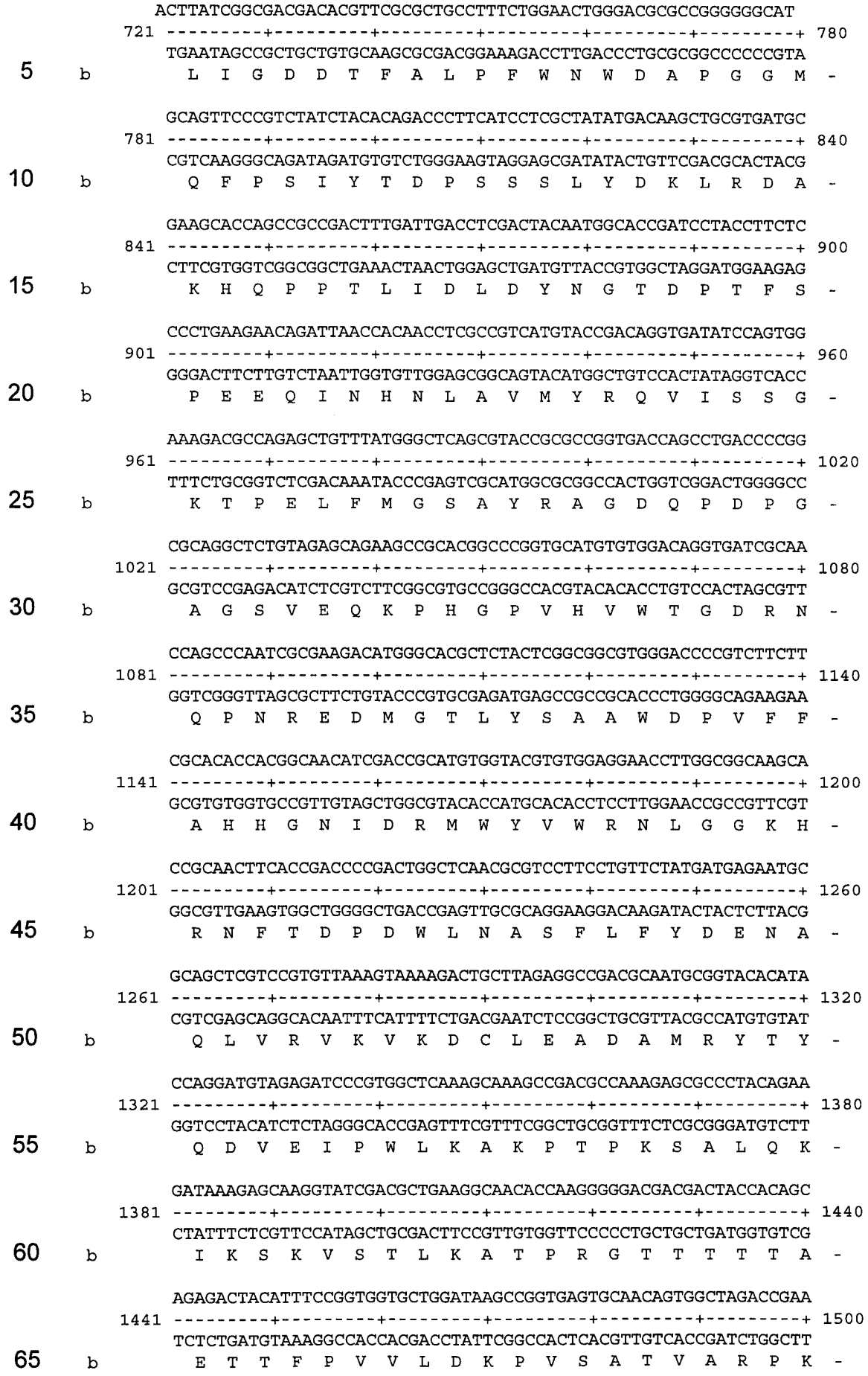
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b L I G D D T F A L P F W N W D A P G G M -
GCAGTTCCCGTCTATCTACACAGACCCTTCATCCTCGCTATATGACAAGCTGCGTGATGC
781 840
CGTCAAGGGCAGATAGATGTGTCTGGGAAGTAGGAGCGATATACTGTTCGACGCACTACG
b Q F P S I Y T D P S S S L Y D K L R D A -
GAAGCACCAGCCGCCGACTTTGATTGACCTCGACTACAATGGCACCGATCCTACCTTCTC
841 900
CTTCGTGGTCGGCGGCTGAAACTAACTGGAGCTGATGTTACCGTGGCTAGGATGGAAGAG
b K H Q P P T L I D L D Y N G T D P T F S -
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901 960
GGGACTTCTTGTCTAATTGGTGTTGGAGCGGCAGTACATGGCTGTCCACTATAGGTCACC
b P E E Q I N H N L A V M Y R Q V I S S G -
AAAGACGCCAGAGCTGTTTATGGGCTCAGCGTACCGCGCCGGTGACCAGCCTGACCCCGG
961 1020
TTTCTGCGGTCTCGACAAATACCCGAGTCGCATGGCGCGGCCACTGGTCGGACTGGGGCC
b K T P E L F M G S A Y R A G D Q P D P G -
CGCAGGCTCTGTAGAGCAGAAGCCGCACGGCCCGGTGCATGTGTGGACAGGTGATCGCAA
1021 1080
GCGTCCGAGACATCTCGTCTTCGGCGTGCCGGGCCACGTACACACCTGTCCACTAGCGTT
b A G S V E Q K P H G P V H V W T G D R N -
CCAGCCCAATCGCGAAGACATGGGCACGCTCTACTCGGCGGCGTGGGACCCCGTCTTCTT
1081 1140
GGTCGGGTTAGCGCTTCTGTACCCGTGCGAGATGAGCCGCCGCACCCTGGGGCAGAAGAA
b Q P N R E D M G T L Y S A A W D P V F F -
CGCACACCACGGCAACATCGACCGCATGTGGTACGTGTGGAGGAACCTTGGCGGCAAGCA
1141 1200
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GCAGCTCGTCCGTGTTAAAGTAAAAGACTGCTTAGAGGCCGACGCAATGCGGTACACATA
1261 1320
CGTCGAGCAGGCACAATTTCATTTTCTGACGAATCTCCGGCTGCGTTACGCCATGTGTAT
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b Q D V E I P W L K A K P T P K S A L Q K -
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b I K S K V S T L K A T P R G T T T T T A -
AGAGACTACATTTCCGGTGGTGCTGGATAAGCCGGTGAGTGCAACAGTGGCTAGACCGAA
1441 1500
TCTCTGATGTAAAGGCCACCACGACCTATTCGGCCACTCACGTTGTCACCGATCTGGCTT
b E T T F P V V L D K P V S A T V A R P K -

```
          GGCCAGGAGGAGTGGGAAGGAGAAGGAAGAAGAGGAGGAGGTGTTGGTGGTGGAGGGAAT
      1501 ---------+---------+---------+---------+---------+---------+ 1560
5         CCGGTCCTCCTCACCCTTCCTCTTCCTTCTTCTCCTCCTCCACAACCACCACCTCCCTTA
      b     A  R  R  S  G  K  E  K  E  E  E  E  E  V  L  V  V  E  G  I  -

          CGAGTTGGAGAAGGACGTGTTCGTGAAGTTTGATGTGTATATAAACTCGCCGGAGCACGA
      1561 ---------+---------+---------+---------+---------+---------+ 1620
10        GCTCAACCTCTTCCTGCACAAGCACTTCAAACTACACATATATTTGAGCGGCCTCGTGCT
      b     E  L  E  K  D  V  F  V  K  F  D  V  Y  I  N  S  P  E  H  E  -

          AGGGGTGGGGCCGGAGGCGAGTGAGTTCGCAGGGAGCTTCGTCCACGTGCCACACAAGCA
      1621 ---------+---------+---------+---------+---------+---------+ 1680
15        TCCCCACCCCGGCCTCCGCTCACTCAAGCGTCCCTCGAAGCAGGTGCACGGTGTGTTCGT
      b     G  V  G  P  E  A  S  E  F  A  G  S  F  V  H  V  P  H  K  H  -

          CAAGAAGGCGAAGAAGGGGAAGGAGATGGCCAGGATGAACACAAGGCTTAAGCTCGGGAT
      1681 ---------+---------+---------+---------+---------+---------+ 1740
20        GTTCTTCCGCTTCTTCCCCTTCCTCTACCGGTCCTACTTGTGTTCCGAATTCGAGCCCTA
      b     K  K  A  K  K  G  K  E  M  A  R  M  N  T  R  L  K  L  G  I  -

          AACGGACCTGCTCGAGGACATCGGCGCTGAGGACGACGAGAGCGTGCTCATCACGCTCGT
      1741 ---------+---------+---------+---------+---------+---------+ 1800
25        TTGCCTGGACGAGCTCCTGTAGCCGCGACTCCTGCTGCTCTCGCACGAGTAGTGCGAGCA
      b     T  D  L  L  E  D  I  G  A  E  D  D  E  S  V  L  I  T  L  V  -

          GCCCAGGAGCGGCAAGGGAATGGTGAAGGTTGGAGGGCTAAGGATTGATTTCTCCAAGTG
      1801 ---------+---------+---------+---------+---------+---------+ 1860
30        CGGGTCCTCGCCGTTCCCTTACCACTTCCAACCTCCCGATTCCTAACTAAAGAGGTTCAC
      b     P  R  S  G  K  G  M  V  K  V  G  G  L  R  I  D  F  S  K  *  -

          ATGAGCATATTGTGAAGAGAAAATTTGCATTTACCGCCCTATAGAATCGAAAAATTGCGT
      1861 ---------+---------+---------+---------+---------+---------+ 1920
35        TACTCGTATAACACTTCTCTTTTAAACGTAAATGGCGGGATATCTTAGCTTTTTAACGCA
      b     *  A  Y  C  E  E  K  I  C  I  Y  R  P  I  E  S  K  N  C  V  -

          ATATGTCCCATTATTGTTTTTTTTATTCTTCAAGCGTATTCAGAATAAGAGTTGCGTGCA
      1921 ---------+---------+---------+---------+---------+---------+ 1980
40        TATACAGGGTAATAACAAAAAAAATAAGAAGTTCGCATAAGTCTTATTCTCAACGCACGT
      b     Y  V  P  L  L  F  F  L  F  F  K  R  I  Q  N  K  S  C  V  H  -

          TGCACGCATGCAGCCATGTTGTTGTAGTCGATATGTGGGGTATGTTTGGATCAGGGATAA
      1981 ---------+---------+---------+---------+---------+---------+ 2040
45        ACGTGCGTACGTCGGTACAACAACATCAGCTATACACCCCATACAAACCTAGTCCCTATT
      b     A  R  M  Q  P  C  C  C  S  R  Y  V  G  Y  V  W  I  R  D  N  -

          TGATGTGAACTTTGAATTAATTATTACACTCTGAGAATAAATTAGAGAGTTTATTATGCA
      2041 ---------+---------+---------+---------+---------+---------+ 2100
50        ACTACACTTGAAACTTAATTAATAATGTGAGACTCTTATTTAATCTCTCAAATAATACGT
      b     D  V  N  F  E  L  I  I  T  L  *  E  *  I  R  E  F  I  M  Q  -

          AGTTGCTTGGTGTAATAGATATTCAACATTGTTTCCTATACATCTTTTTTTGGAAGAAAA
      2101 ---------+---------+---------+---------+---------+---------+ 2160
55        TCAACGAACCACATTATCTATAAGTTGTAACAAAGGATATGTAGAAAAAAACCTTCTTTT
      b     V  A  W  C  N  R  Y  S  T  L  F  P  I  H  L  F  L  E  E  K  -

          AAAAAAAAAAAAAAAATCGAT
      2161 ---------+---------+- 2181
60        TTTTTTTTTTTTTTTTAGCTA
      b     K  K  K  K  K  S     -
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MAP of: apineppofl check: 1756 from: 1 to: 2181 May 19, 1998 10:35

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00362

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/53, A01H 1/00, 5/00.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WPAT, Chemical Abstracts: Key words - see electronic darabase box below.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Medline, JAPIO, USPM, EMBL, GENEBANK, SWISS-PROT, PIR: Key words - see electronic darabase box below.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, USPM, Chemical Abstracts and Medline Key words: Polyphenol oxidase and (tobacoo or banana or pineapple);
EMBL, GENEBANK, SWISS-PROT, PIR: Sequences as defined in figures 1-10

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 97/29193 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 14 August 1997, See entire document, especially the examples.	1-16, 18-34.
X, Y	WO 96/37617 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 28 November 1996, See entire document, especially the examples and figures 1-3.	1-16, 18-34.
X	*Plant Molecular Biology* **26**: 495-502, 1994 Dry I. B. and Robinson S. P. "Molecular cloning and characterisation of grape berry polyuphenol oxidase" See espeially pages 499-500.	1-16, 18-34.
X Y	*Plant Molecular Biology* **27**: 429-43 3, 1995. Boss P. K. *et al* "An apple polyphenol oxidase CDNA is up-regulated in wounded tissues" See especially page 430 (EMBL acc no. L29450).	16, 18. 1-15, 19-34.

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search
16 July 1998

Date of mailing of the international search report
20 JUL 1998

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00362

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/02195 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 4 February 1993 See especially pages 8, 9.	1-16, 18-34.
X Y	*Plant Physiol* (1995) **109**: 525-53 1. Thygesen P. W. et al "Polyphenol oxidase in potato A multigene family that exhibits differential expression patterns" See especially page 528. (EMBL accession nos. U22921 and U22922)	17. 1-15, 19-34.
X Y	*Plant Molecular Biology* **21:** 1035-1051, 1993. Newman S. M. et al "Organisation of the toato polyphenol oxidase gene family" See especially pages 1042-1043. (EMBL accession no. Z12837)	17. 1-15, 19-34.
X Y	*Plant Molecular Biology* **21:** 59-68, 1993. Hunt M. D. et al "CDNA cloning and expression of potato polyphenol oxidase" See especially pages 62, 63. (EMBL accession no. M95196)	17. 1-15, 19-34.
P, X	EMBL accession nos D87669 and D87670 Published on 2 August 1997.	16, 18.
P, X	EMBL accession no Y12501. Published on 25 February 1998	17.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 98/00362

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	97/29193	AU	14330/97				
WO	96/37617	AU	56803/96	EP	832244		
WO	9302195	AU	23126/92	CA	2112998	EP	599868
		NZ	243594				

END OF ANNEX