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(54) **METHOD FOR PRODUCTION OF CHRYSANTHEMUM PLANT HAVING DELPHINIDIN-CONTAINING PETALS**

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(58) **Field of Classification Search**
USPC 800/282
See application file for complete search history.

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(57) **ABSTRACT**

Disclosed are: a method for producing a chrysanthemum plant having delphinidin-containing petals using a transcriptional regulatory region for a chrysanthemum-derived flavanone 3-hydroxylase (F3H) gene; and a chrysanthemum plant, a progeny or a vegetative proliferation product of the plant, or a part or a tissue of the plant, the progeny or the vegetative proliferation product, and particularly a petal or a cut flower of the plant. In the method for producing a chrysanthemum plant having delphinidin-containing petals, a flavonoid 3',5'-hydroxylase (F3'5'H) is caused to be expressed in a chrysanthemum plant using a transcriptional regulatory region for a chrysanthemum-derived flavanone 3-hydroxylase (F3H) gene.

8 Claims, 4 Drawing Sheets

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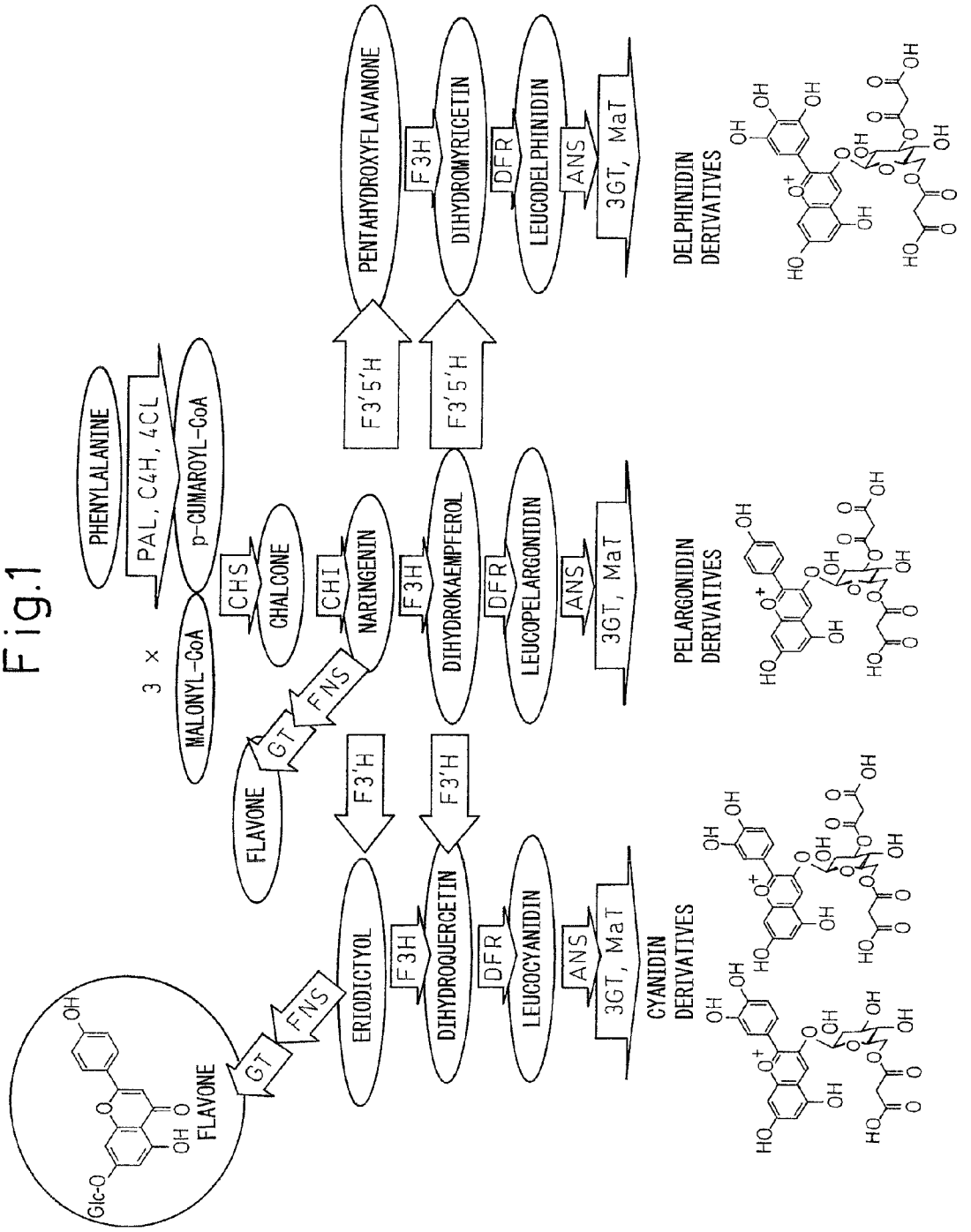
Fig.1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DIHYDROKAEMPFEROL
DFR
LEUCOPELARGONIDIN
ANS
3GT, MaT
PELARGONIDIN DERIVATIVES
F3'5'H
PENTAHYDROXYFLAVANONE
F3H
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ANS
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Fig.2

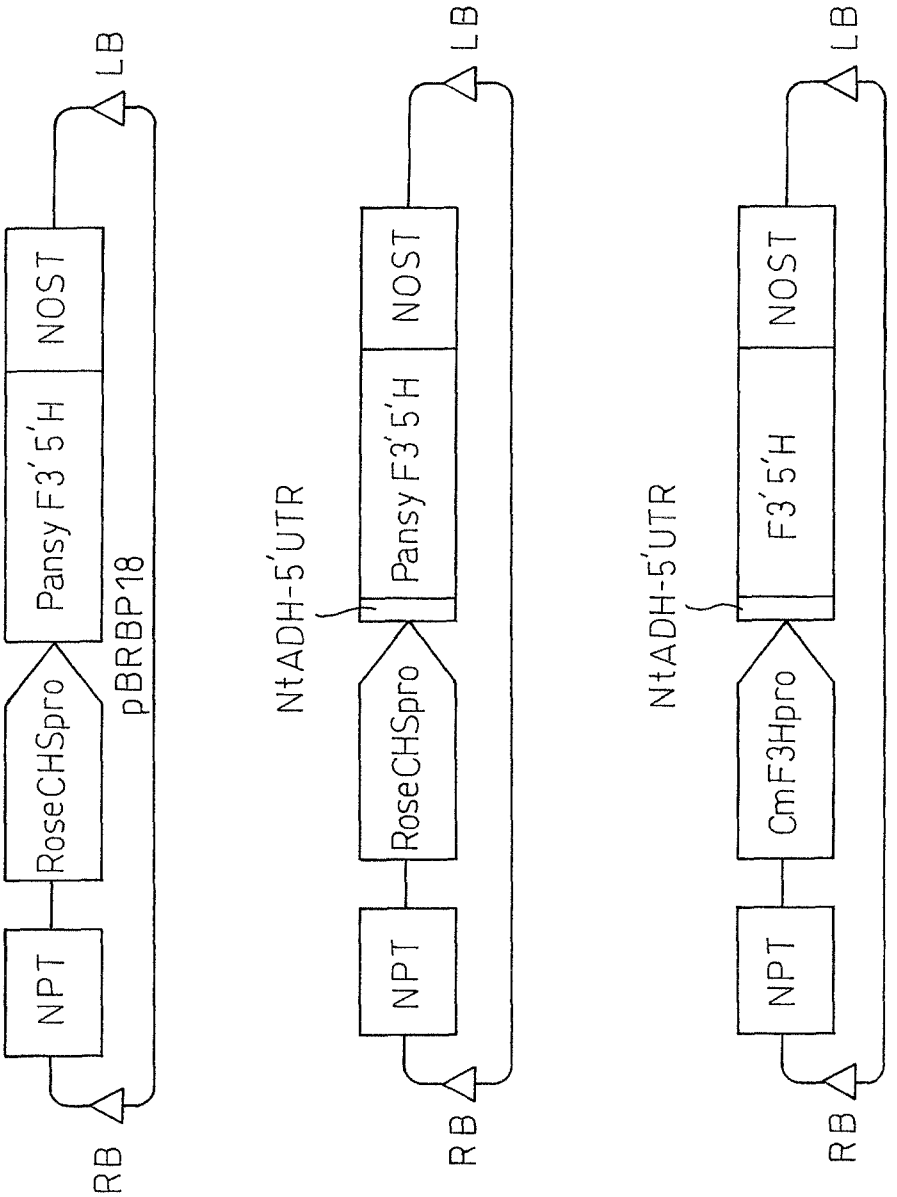
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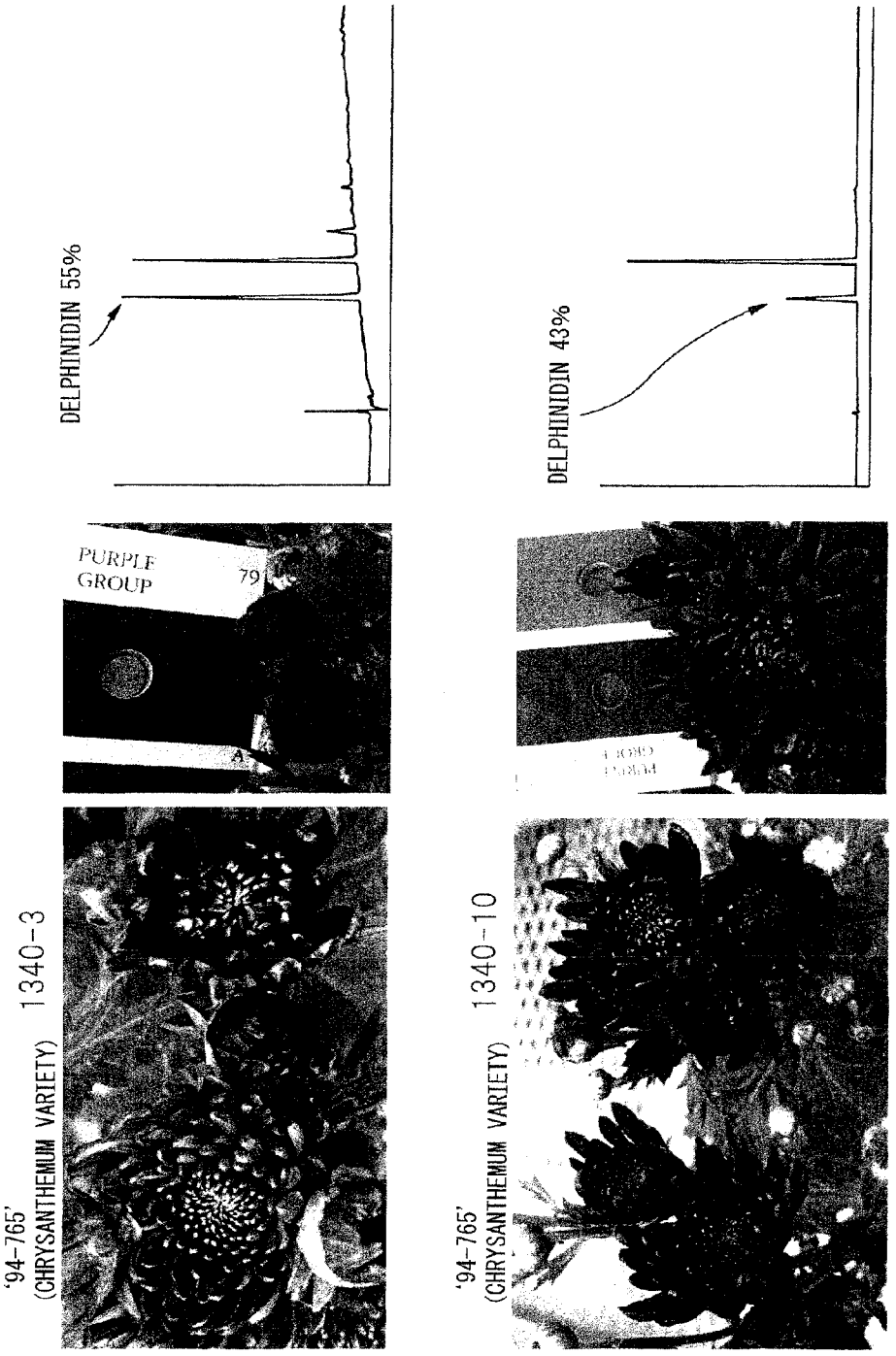
Fig.3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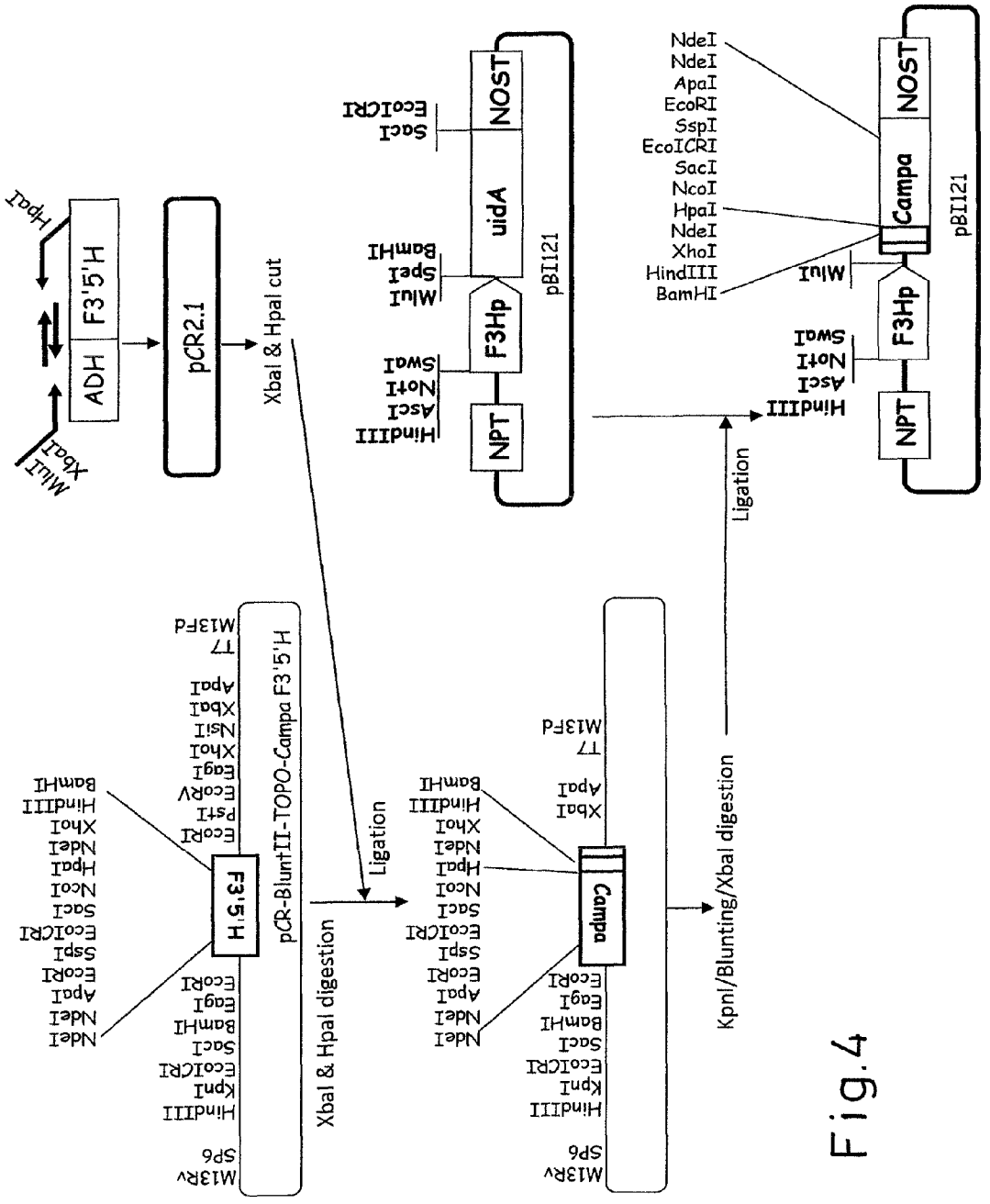
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HpaI
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XbaI
pCR2.1
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pBI121
NOST
uidA
F3Hp
NPT
SacI
EcoICRI
MluI
SpeI
BamHI
SwaI
NotI
AscI
HindIII
Ligation
Campa
NdeI
ApaI
EcoRI
SspI
EcoICRI
SacI
NcoI
HpaI
XhoI
HindIII
BamHI
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M13Fd
T7
XbaI
ApaI
NsiI
XhoI
EagI
EcoRV
PstI
EcoRI
HindIII
BamHI
NdeI
KpnI
SacI
M13Rv
SP6
XbaI & HpaI digestion
KpnI/Blunting/XbaI digestion

Fig.4

METHOD FOR PRODUCTION OF CHRYSANTHEMUM PLANT HAVING DELPHINIDIN-CONTAINING PETALS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is the National Stage of International Application No. PCT/JP2010/053904 filed Mar. 9, 2010, and claims benefit of Japanese Patent Application No. 2009-107054 filed Apr. 24, 2009, which are herein incorporated by reference in their entirety.

REFERENCE TO A SEQUENCE LISTING

A Sequence Listing containing SEQ ID NOS: 1-87 is incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to a method for producing a chrysanthemum plant containing delphinidin in the petals thereof by using the transcriptional regulatory region of chrysanthemum-derived flavanone 3-hydroxylase (F3H) gene, a nucleic acid of that regulatory region, an expression vector or expression cassette containing that nucleic acid, and a chrysanthemum plant, progeny or vegetative proliferation product thereof, or a part or tissue thereof, and particularly a petal or cut flower thereof, in which that regulatory region has been introduced.

BACKGROUND ART

The use of genetic transformation technology makes it possible to impart new traits to plants by expressing a useful gene in a target plant. A wide range of genetically modified plants produced in this manner have already been cultivated. Since regulation of gene expression is mainly controlled at the level of transcription, transcriptional regulation is the most important in terms of regulating the expression of genes. Namely, expressing a gene at a suitable time, in a suitable tissue and at a suitable strength is important for producing an industrially useful genetically modified plant. In many cases, transcription is control by a DNA sequence on the 5' untranslated region of a open reading frame. A region of DNA that determines the starting site of gene transcription and directly regulates the frequency thereof is referred to as a promoter. A promoter is located in a start codon consisting of several tens of base pairs (bp) on the 5'-untranslated region, and frequently contains a TATA box and the like. A cis element that binds various transcriptional regulatory factors is also present on the 5'-untranslated region, and the presence thereof serves to control the timing of transcription, the tissue in which transcription takes place and transcriptional strength. Transcriptional regulatory factors are classified into many families according to their amino acid sequence. For example, examples of well-known families of transcriptional regulatory factors include Myb transcriptional regulatory factor and bHLH (basic helix loop helix) regulatory factor. In actuality, the terms transcriptional regulatory factor and promoter are frequently used with the same meaning.

Anthocyanins, which compose the main components of flower color, are a member of secondary metabolites generically referred to as flavonoids. The color of anthocyanins is dependent on their color. Namely, the color becomes blue as the number of hydroxyl groups of the B ring of anthocyanidins, which is the chromophore of anthocyanins, increases. In addition, as the number of aromatic acyl groups (such as coumaroyl groups or caffeolyl groups) that modify the anthocyanin increases (namely, the wavelength of maximum absorbance shifts to a longer wavelength), the color of the anthocyanin becomes blue and the stability of the anthocyanin is known to increase (see Non-Patent Document 1).

Considerable research has been conducted on those enzymes and genes that encode those enzymes involved in the biosynthesis of anthocyanins (see, Non-Patent Document 1). For example, an enzyme gene that catalyzes a reaction by which an aromatic acyl group is transferred to anthocyanin is obtained from Japanese gentian, lavender and petunias (see Patent Document 1 and Patent Document 2). An enzyme gene involved in the synthesis of anthocyanin that accumulates in the leaves of red perilla (malonylshisonin, 3-0-(6-0-(E)-p-coumaroyl-β-D-glucopyranosyl)-5-0-(6-0-malonyl-β-D-glucopyranosyl)-cyanidin) (see Non-Patent Document 2) has previously been reported in hydroxycinnamoyl CoA: anthocyanin-3-glucoside-aromatic acyl group transferase (3AT) gene (or more simply referred to as "shiso (perilla) anthocyanin-3-acyltransferase (3AT) gene") (see Patent Document 1). Moreover, findings have also been obtained regarding the transcriptional regulation (control) of biosynthase genes of anthocyanins. Cis element sequences bound by Myb transcriptional regulatory factor and bHLH transcriptional regulatory factor are present in the transcriptional regulatory region located on the 5'-region of the start codons of these genes. Myb transcriptional regulatory factor and bHLH transcriptional regulatory factor are known to control synthesis of anthocyanins in petunias, corn and perilla (see Non-Patent Document 1).

Promoters (also referred to as transcriptional regulatory regions) responsible for gene transcription in plants consist of so-called constitutive promoters, which function in any tissue and at any time such as in the developmental stage, organ/tissue-specific promoters, which only function in specific organs and tissues, and time-specific promoters, which only express at a specific time of the developmental stage. Constitutive promoters are frequently used as promoters for expressing useful genes in genetically modified plants. Typical examples of constitutive promoters include cauliflower mosaic virus 35S promoter (also abbreviated as CaMV35S promoter) and promoters construction on the basis thereof (see Non-Patent Document 3), and Mac1 promoter (see Non-Patent Document 4). In plants, however, many genes are only expressed in specific tissues or organs or are expressed time-specifically. This suggests that tissue/organ-specific or time-specific expression of genes is necessary for plants. There are examples of genetic recombination of plants that utilize such tissue/organ-specific or time-specific transcriptional regulatory regions. For example, there are examples of protein being accumulated in seeds by using a seed-specific transcriptional regulatory region.

However, although plants produce flowers of various colors, there are few species capable of producing flowers of all colors due to genetic restrictions on that species. For example, there are no varieties of rose or carnation in nature that are capable of producing blue or purple flowers. This is because roses and carnations lack the flavonoid 3',5'-hydroxylase gene required to synthesize the anthocyanidin, delphinidin, which is synthesized by many species that produce blue and purple flowers. By transformation with the flavonoid 3',5'-hydroxylase gene of petunia or pansy, for example, which are specifies capable of producing blue and purple flowers, into these species, these species can be made to produce blue flowers. In the case of carnations, the transcriptional regulatory region of chalcone synthase gene derived from common snapdragon or

petunia is used to transcribe flavonid 3',5'-hydroxylase gene derived from common snapdragon or petunia. Examples of plasmids containing the transcriptional regulatory region of chalcone synthase gene derived from common snapdragon or petunia include plasmids pCGP485 and pCGP653 described in Patent Document 3, and examples of plasmids containing a constitutive transcriptional regulatory region include plasmid PCGP628 (containing a Mac1 promoter) and plasmid pSPB130 (containing a CaMV35S promoter to which is added E12 enhancer) described in Patent Document 4.

However, it is difficult to predict how strongly such promoters function in recombinant plants to be able to bring about a target phenotype. In addition, since repeatedly using the same promoter to express a plurality of foreign genes may cause gene silencing, it is thought that this should be avoided (see Non-Patent Document 5).

Thus, although several promoters have been used to change flower color, a useful promoter corresponding to the host plant and the objective is needed in order to further change to a different flower color.

In particular, chrysanthemum plants (also simply referred to as chrysanthemums) account for about 30% of all wholesale flower sales throughout Japan (Summary of 2007 Flowering Plant Wholesale Market Survey Results, Ministry of Agriculture, Forestry and Fisheries), making these plants an important product when compared with roses accounting for roughly 9% and carnations accounting for roughly 7%. Although chrysanthemums come in flower colors including white, yellow, orange, red, pink and purplish red, there are no existing varieties or closely related wild varieties that produce bluish flowers such as those having a purple or blue color.

Thus, one objective of the selective breeding of bluish flowers is to stimulate new demand. Chrysanthemum flower color is expressed due to a combination of anthocyanins and carotenoids. Anthocyanins are able to express various colors due to differences in the structure of the anthocyanidin serving as the basic backbone, and differences in modification by sugars and organic acids. However, there are known to be two types of anthocyanins that govern chrysanthemum flower color in which cyanidin at position 3 is modified by glucose and malonic acid (cyanidin 3-0-(6"-0-monomalonyl-β-glucopyranoside and 3-0-(3",6"-0-dimalonyl-β-glucopyranoside) (see Non-Patent Document 6). In addition, these structures are comparatively simple (see FIG. **1**). This causes the range of flower color attributable to anthocyanins in chrysanthemums to be extremely narrow. However, although the expression of bluish color is primarily the result of anthocyanins, since there is no gene that encodes the key enzyme of flavonoid 3',5'-hydroxylase (F3'5'H) in chrysanthemums, delphinidin-based anthocyanin, which produces blue color, is not biosynthesized in chrysanthemums (see FIG. **1**). Therefore, the development of a technology has been sought for controlling the expression of chrysanthemum anthocyanins using genetic engineering techniques in order to be able to produce a chrysanthemum that produces bluish flowers by modifying anthocyanin-based pigment that accumulates in chrysanthemum petals.

As was previously described, although chrysanthemums are the most important flowering plant in Japan, since they are hexaploidal resulting in high ploidy and have a large genome size, in addition to having low transformation efficiency, since they may also cause silencing (deactivation) of transgenes, it is not easy to obtain genetically modified chrysanthemums capable of stable transgene expression. In chrysanthemums transformed with β-glucuronidase (GUS) gene coupled to CaMV35S promoter, the activity of the GUS gene is roughly one-tenth that of tobacco transformed with the same gene, and that activity has been reported to decrease in nearly all individuals after 12 months have elapsed following transformation (see Non-Patent Document 7). Although a promoter of a chlorophyll a/b-bound protein that favorably functions in chrysanthemums has been reported to have been obtained in order to stably express an exogenous gene in chrysanthemums, this promoter is not suitable for expressing genes in flower petals in which there is little chlorophyll present (see Non-Patent Document 8). In addition, when GUS gene coupled to tobacco elongation factor 1 (EF1α) promoter is transformed into chrysanthemums, GUS gene has been reported to be expressed in leaves and petals even after the passage of 20 months or more (see Non-Patent Document 9). Moreover, there are also examples of flower life being prolonged by expressing a mutant ethylene receptor gene in chrysanthemums (see Non-Patent Document 10), flower form being changed by suppressing expression of chrysanthemum AGAMOUS gene (see Non-Patent Document 11), and expression of exogenous genes being increased in chrysanthemums (see Non-Patent Document 12) by using a translation enhancer of tobacco alcohol dehydrogenase (see Patent Document 7).

On the other hand, although there have been examples of successful alteration of chrysanthemum flower color by genetic recombination, including a report of having changed pink flowers to white flowers by suppressing the chalcone synthase (CHS) gene by co-suppression (see Non-Patent Document 13), and a report of having changed white flowers to yellow flowers by suppressing carotenoid cleavage dioxygenase (CCD4a) by RNAi (see Non-Patent Document 14), all of these methods involve alteration of flower color by suppressing expression of endogenous genes, and there have been no successful examples of altering flower color by over-expression of exogenous genes as well as no examples of having realized a change in anthocyanin structure or an accompanying change in flower color.

Although attempts to alter flower color by over-expression of an exogenous gene have been reported that involve transformation with a gene encoding F3'5'H, which is an enzyme required for synthesis of delphinidin (see Patent Document 5 and Non-Patent Document 15), the delphinidin produced due to the action of the transfected F3'5'H gene accumulates in ray petals, and there are no reports of the production of bluish chrysanthemums. In chrysanthemums, even if F3'5'H is expressed with CaMV35S promoter, production of delphinidin is not observed (see Non-Patent Document 15). In addition, expression of a gene expressed with CaMV35S promoter is unsuitable for stable expression, and for example, ends up dissipating accompanying growth of the chrysanthemum transformant (see Non-Patent Document 7). Potato Lhca3.St.1 promoter (see Non-Patent Document 16), chrysanthemum UEP1 promoter (see Non-Patent Document 17) and tobacco EF1α promoter (see Patent Document 6 and Non-Patent Document 9), for example, have been developed for use as promoters enabling efficient and stable expression of exogenous genes in the ray petals of chrysanthemums. However, there have been no reports describing alteration of chrysanthemum flower color by over-expression of an exogenous gene using these promoters. On the basis of the above, in order to produce chrysanthemums in which flower color has been altered by genetic recombination, it is necessary to establish a technology for controlling the expression of flavonoid biosynthesis genes, including the development of a promoter suitable for chrysanthemums.

Although gene expression is mainly controlled by transcriptional regulatory regions, sequences are also known that improve translation of mRNA. For example, the omega

sequence derived from tobacco mosaic virus is known to increase the translation efficiency of heterologous genes coupled to the omega sequence both in vitro and in vivo (see Non-Patent Document 18). In addition, a sequence (ADH200) present in the 5'-untranslated region of tobacco alcohol dehydrogenase (NtADH5'UTR) is known to contribute to improved stability of the expression of heterologous genes (see Patent Document 7). In addition, in the case of coupling a 94 bp translation enhancer (ADHNF, see Patent Document 8) present downstream from this sequence to the 3'-side of CaMV35S promoter and further transformation with an expression cassette coupled with GUS gene, this sequence has been reported to contribute to increased translation efficiency in chrysanthemums (see Non-Patent Document 12). However, there are no examples of this sequence being used to change flower color by altering the structure and composition of flavonoids. Since it is necessary to express a heterologous gene in epidermal cells in which flavonoids and anthocyanins primarily accumulate in order to alter flower color, it is difficult to infer from conventional results whether or not NtADH5'UTR (ADH200 or translation enhancer ADHNF) is effective for altering flower color.

PRIOR ART DOCUMENTS

Patent Documents

Patent Document 1: WO 96/25500
Patent Document 2: WO 01/72984
Patent Document 3: WO 94/28140
Patent Document 4: WO 05/17147
Patent Document 5: U.S. Pat. No. 5,948,955
Patent Document 6: Japanese Unexamined Patent Publication No. 2004-65096
Patent Document 7: U.S. Pat. No. 6,573,429
Patent Document 8: Japanese Unexamined Patent Publication No. 2003-79372

Non-Patent Documents

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Non-Patent Document 2: Agricultural and Biological Chemistry, 53, 797-800, 1989
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Non-Patent Document 7: Plant Biotechnology, 17, 241-245, 2000
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Non-Patent Document 13: Bio/Technology, 12, 268, 1994
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Non-Patent Document 18: Nucleic Acids Research, 15, 3257-3273, 1987

SUMMARY OF THE INVENTION

Problems to be Solved by the Invention

An object to be solved by the present invention is to provide a method for producing a chrysanthemum plant containing delphinidin in the petals thereof by using the transcriptional regulatory region of chrysanthemum-derived flavanone 3-hydroxylase (F3H) gene, and a chrysanthemum plant, progeny or vegetative proliferation product thereof, or a part or tissue thereof, and particularly a petal or cut flower thereof, transformed with that regulatory region.

Means for Solving the Problems

As a result of conducting extensive studies to solve the aforementioned problems, the inventors of the present invention found that when flavonoid 3',5'-hydroxylase (F3'5'H) gene is expressed in chrysanthemum using a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) derived from chrysanthemum, a large amount of delphinidin accumulates in the petals thereof, flower color changes, and flower color changes further due to an even larger accumulation of delphinidin as a result of adding a translational enhancer derived from tobacco alcohol dehydrogenase gene, and confirmed the usefulness thereof through experimentation, thereby leading to completion of the present invention.

Namely, the present invention is as described below.

[1] A method for producing a chrysanthemum plant containing delphinidin in the petals thereof comprising the step of expressing flavonoid 3',5'-hydroxylase (F3'5'H) in a chrysanthemum plant using as a transcriptional regulatory region a nucleic acid selected from the group consisting of:

(1) a nucleic acid containing the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87;

(2) a nucleic acid able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum, and containing a nucleotide sequence in which the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87 has been modified by addition, deletion and/or substitution of one or several nucleotides;

(3) a nucleic acid able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum, and able to hybridize under highly stringent conditions with a nucleic acid composed of a nucleotide sequence complementary to the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87; and,

(4) a nucleic acid able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum, and having sequence identity of at least 90% with the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87.

[2] The method described in [1] above, wherein the flavonoid 3',5'-hydroxylase (F3'5'H) is derived from bellflower (campanula), cineraria, verbena and pansy #40.

[3] The method described in [1] or [2] above, wherein a translational enhancer derived from tobacco alcohol dehydrogenase gene is further used in addition to the transcriptional regulatory region.

[4] The method described in any of [1] to [3] above, wherein an expression vector or expression cassette is used in which the translational enhancer is coupled directly to a start codon of the F3'5'H gene.

[5] The method described in any of [1] to [4] above, wherein the content of delphinidin in the petals is 25% by weight or more of the total weight of anthocyanidins.

[6] A chrysanthemum plant, progeny thereof, or vegetative proliferation product, part or tissue thereof, containing the nucleic acid described in [1] above or produced according to the method described in any of [1] to [5] above.

[7] The chrysanthemum plant, progeny thereof, or vegetative proliferation product, part of tissue thereof, described in [6] above, which is a cut flower.

[8] A cut flower processed product using the cut flower described in [7] above.

Effects of the Invention

According to the present invention, it was determined that when flavonoid 3',5'-hydroxylase (F3'5'H) gene is expressed in chrysanthemum using the transcriptional regulatory region of flavanone 3-hydroxylase (F3H) derived from chrysanthemum, more delphinidin accumulates in the flower petals than in the case of using another promoter, and when the flower color becomes bluer, an even larger amount of delphinidin accumulates as a result of adding a translational enhancer derived from tobacco alcohol dehydrogenase gene, thereby causing the flower color to become even bluer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic diagram of the flavonoid biosynthesis pathway in transformed chrysanthemum transformed with F3'5'H gene.

FIG. **2** is a schematic diagram of a binary vector for introducing F3'5'H gene.

FIG. **3** indicates the flower color and ratio of delphinidin content in transformed individuals transformed with chrysanthemum F3Hpro::ADHNF-bellflower F3'5'H::NOSter.

FIG. **4** indicates the construction process of pBI121 chrysanthemum F3Hpro1k::ADHNF-bellflower F3'5'H::NOSter.

EMBODIMENTS OF THE INVENTION

The present invention relates to a method for producing a chrysanthemum plant containing delphinidin in the petals thereof, comprising transforming chrysanthemum with a vector containing a gene cassette that causes expression of flavonoid 3',5'-dehydroxylase (F3'5'H) by the 5'-region of a gene that encodes chrysanthemum flavanone 3-hydroxylase (F3H) (also referred to as "CmF3Hpro" or "chrysF3H5'"). The gene cassette preferably contains a translational enhancer derived from tobacco alcohol dehydrogenase gene (see bottom of FIG. **2**). The delphinidin content in the flower petals is preferably 25% by weight or more of the total weight of anthocyanidins, and the color of the flower petals is altered towards blue. The present invention also relates to a chrysanthemum plant, progeny thereof, or vegetative proliferation product, part or tissue thereof, produced according to that method or containing CmF3Hpro. The part or tissue is preferably a flower petal or cut flower.

In the present description, an "expression cassette" refers to a DNA fragment in which a promoter and a terminator are coupled to arbitrary nucleic acids.

According to the present invention, since F3'5'H gene is expressed in ray petals of chrysanthemum, and that enzyme protein is synthesized and functions, a chrysanthemum having a bluish flower color can be produced by allowing delphinidin-based anthocyanin to be synthesized and accumulate. Although accumulation of delphinidin (max. 5.4%) was confirmed in the case of using RoseCHSpro (rose chalcone synthase (CHS) gene promoter), R. rugosa DFRpro (Rugosa rose dihydroflavonol-4-reductase (DFR) gene promoter), R. rugosa F3Hpro (R. rugosa flavanone 3-hydroxylase (F3H)) or Viola F3'5'H#40pro (pansy F3'5'H gene promoter) for the promoter contained in the gene cassette used to express F3'5'H (see Table 1), this did not lead to flower color becoming bluish. Therefore, as a result of repeatedly conducting expression experiments on F3'5'H using various types of promoters in order to discover an effective promoter for enhancing accumulation of delphinidin in chrysanthemum flower petals and making flower color bluish, CmF3Hpro was determined to be an effective promoter. The use of CmF3Hpro made it possible to improve accumulation of delphinidin in comparison with the case of using other promoters (see Table 1, mean: 31.4%, max.: 80.5%), and led to the attaining of bluish flower color (see FIG. **3**, RHS color chart 79A, 77A, 72A and 72B). In addition, within the F3'5'H gene expressed by CmF3Hpro, F3'5'H derived from bellflower (delphinidin accumulation rate: max. 81%), cineraria (delphinidin accumulation rate: max. 36%), verbena and pansy (delphinidin accumulation rate: max. 27% to 28%) were found to have the ability to change chrysanthemum flower color to purple. Moreover, transformation with a gene cassette directly coupled with tobacco ADH translational enhancer (see Patent Document 8) was successful in altering flower color by enabling anthocyanin having delphinidin for the basic backbone thereof to be efficiently accumulated in ray petals of chrysanthemum (see Table 1, FIG. **3**). Furthermore, direct coupling refers to coupling without containing a surplus nucleic acid sequence between one polynucleotide and another polynucleotide.

An example of a transcriptional regulatory region according to the present invention is a nucleic acid composed of a nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87. However, a promoter composed of a base sequence in which several (1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) nucleotides has been added, deleted and/or substituted in a nucleic acid composed of a nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87 is also thought to maintain activity similar to that of the original promoter. Thus, the transcriptional regulatory region according to the present invention can also be a nucleic acid composed of a nucleotide sequence in which one or several nucleotides have been added, deleted and/or substituted in the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87 provided the nucleic acid is able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum.

The transcriptional regulatory region according to the present invention can also be a nucleic acid able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum and able to hybridize under highly stringent conditions with the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87, or a nucleic acid able to function as a transcriptional regulatory region of flavanone 3-hydroxylase (F3H) gene derived from chrysanthemum and has sequence identity of at least 90% with the nucleotide sequence indicated in SEQ ID NO. 34 or SEQ ID NO. 87.

Examples of these nucleic acids include nucleic acids composed of nucleotide sequences having sequence identity with the nucleotide sequence indicated in SEQ ID NO. 34 of preferably about 70% or more, more preferably about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98%, and most preferably about 99%.

Here, stringent conditions refer to hybridization conditions easily determined by a person with ordinary skill in the art that determined empirically typically dependent on probe length, washing temperature and salt concentration. In general, the temperature for suitable annealing becomes higher the longer the probe, and the temperature becomes lower the shorter the probe. Hybridization is generally dependent on the ability of denatured DNA to anneal in the case a complementary strand is present in an environment at a temperature close to or below the melting temperature thereof. More specifically, an example of lowly stringent conditions consists of washing and so forth in 0.1% SDS solution at 5×SSC under temperature conditions of 37° C. to 42° C. in the filter washing stage following hybridization. In addition, an example of highly stringent conditions consists of washing and so forth in 0.1% SDS at 0.1×SSC and 65° C. in the washing stage. The use of more highly stringent conditions makes it possible to obtain polynucleotides having higher homology or identity.

In the present invention, the flavonoid 3',5'-hydroxylase (F3'5'H) gene is preferably derived from bellflower (campanula), cineraria, verbena or pansy #40. In the present invention, a translation enhancer derived from tobacco alcohol dehydrogenase is preferably further used in addition to the transcriptional regulatory region. In addition, the translation enhancer is preferably directly coupled to a start codon of the F3'5'H gene in a gene cassette of an expression vector.

In the method of the present invention, the delphinidin content in the flower petals is preferably 25% by weight or more of the total weight of anthocyanidins.

The present invention is a chrysanthemum plant, progeny thereof, or vegetative proliferation product, part or tissue thereof, produced according to the method of the present invention or transformed with the aforementioned nucleic acid, and is preferably a flower petal or cut flower.

The present invention also relates to a processed product that uses the aforementioned cut flower (cut flower processed product). Here, a cut flower processed product includes, but is not limited to, a pressed flower, preserved flower, dry flower or resin-sealed product obtained by using the cut flower.

EXAMPLES

The following provides a detailed explanation of the present invention through examples thereof.

Molecular biological techniques were carried out in accordance with Molecular Cloning (Sambrook and Russell, 2001) unless specifically indicated otherwise.

The following Reference Examples 1 to 9 are examples of using a promoter other than the 5'-region of a gene encoding flavanone 3-hydroxylase (F3H) of chrysanthemum (CmF3Hpro), while on the other hand, Examples 1 to 10 are examples relating to the 5'-region of a gene encoding flavanone 3-hydroxylase (F3H) of chrysanthemum (CmF3Hpro).

Reference Example 1

Expression of F3'5'H Gene by Tobacco EF1α Promoter

pBIEF1α described in Patent Document 6 was digested with restrictases HindIII and BamHI to obtain a roughly 1.2 kb DNA fragment containing a promoter sequence of tobacco EF1α. This DNA fragment was inserted into the 5'-side of iris DFR cDNA of pSPB909 described in Patent Document 4 to obtain a plasmid pSLF339. A plasmid pSLF340 was similarly constructed in which petunia DFR cDNA (described in International Publication WO 96/36716) was inserted instead of iris DFR cDNA.

A plasmid obtained by inserting a BP40 fragment of pansy F3'5'H gene, excised by partial digestion with BamHI and XhoI from pCGP1961 described in Patent Document 4, into BamHI and SaII sites of pSPB176 (described in Plant Science, 163, 253-263, 2002) was designated pSPB575. The promoter portion of this plasmid was replaced with the promoter of the aforementioned tobacco EF1α using HindIII and BamHI to obtain pSLF338. A fragment containing iris DFR cDNA was inserted into pSLF339 digested with AscI at this AscI site. The resulting plasmid was designated pSLF346. This plasmid pSLF346 is designed to express pansy F3'5'H and iris DFR genes in plants under the control of the promoter of tobacco EF1α.

Plasmid pLHF8 containing lavender F3'5'H cDNA is described in International Publication WO 04/20637. Plasmid pSPB2772 was obtained by coupling this plasmid to the DNA fragment having the higher molecular weight among a DNA fragment obtained by digesting this plasmid with BamHI and XhoI and a DNA fragment of pSPB176 obtained by digesting with BamHI and SaII. In this plasmid, lavender-derived F3'5'H cDNA is coupled to CaMV35S promoter to which has been added E12 enhancer. This promoter portion was replaced with the aforementioned promoter of tobacco EF1α using HindIII and BamHI to obtain plasmid pSPB2778. A fragment containing petunia DFR cDNA within pSFL340 digested with AscI was inserted into this AscI site. The resulting plasmid was designated pSPB2780. This plasmid pSPB2780 is designed so as to express lavender F3'5'H and petunia DFR genes in plants under the control of tobacco EF1α promoter.

Plasmid pSPB2777 was obtained by replacing the promoter portion of plasmid pSPB748 described in Plant Biotechnol., 23, 5-11 (2006) (in which butterfly pea-derived F3'5'H cDNA is coupled to CaMV35S promoter to which has been added E12 enhancer) with the aforementioned promoter of tobacco EF1α using HindIII and BamHI. A fragment of pSLF340 digested with AscI containing petunia DFR cDNA was inserted into this AscI site. The resulting plasmid was designated pSPB2779. This plasmid pSPB2779 is designed to express butterfly pea F3'5'H and petunia DFR genes in plants under the control of the promoter of tobacco EF1α.

Each of the aforementioned plasmids pSFL346, pSPB2780 and pSPB2779 were transformed into *Agrobacterium* and then transfected into chrysanthemum variety 94-765 using this transformed *Agrobacterium*. Although anthocyanidins in flower petals of the transformed chrysanthemum were analyzed, delphinidin was not detected.

Reference Example 2

Chrysanthemum Transfected with Cineraria F3'5'H Gene Promoter

RNA was extracted based on an established method from the petals of a bud of blue Cineraria Senetti (Suntory Flowers Ltd.). A cDNA library was produced using the ZAP-cDNA® Library Construction Kit (Stratagene Corp., Catalog No. 200450) in accordance with the method recommended by the manufacturer using poly-A+RNA prepared from this RNA. This cDNA library was then screened using butterfly pea F3'5'H cDNA (Clitoria ternatea, see Plant Biotechnology, 23, 5-11 (2006)) labeled with the DIG System (Roche Applied Science) according to the method recommended by the manufacturer. Forty eight phages indicating signal were iso-

lated. Plasmids were obtained from these phages by in vivo excision according to the method recommended by the manufacturer (Stratagene).

The nucleotide sequences of the cDNA portions contained in these plasmids were determined, a Blast search was made of DNA databases, numerous genes were obtained that demonstrated homology with cytochrome P450, and these genes were able to be classified into 8 types. Among these, the entire nucleotide sequence of Ci5a18 (SEQ ID NO. 77), which was presumed to be classified as CYP75B, was determined. A pBluescript SKII-plasmid containing this sequence was designated pSPB2774.

Chromosomal DNA was extracted from a leaf of the same Cineraria, and a chromosome library was produced using the λBlueSTAR™ Xho I Half-Site Arms Kit (Novagen, on the Internet at merckbiosciences.com/product/69242). The resulting 200,000 plaques were screened using a Ci5a18 cDNA fragment labeled with DIG. This cDNA fragment was amplified using Ci5a18 as template and using primers Ci5a18F1 (SEQ ID NO. 81: 5'-CATCTGTTTTCTGCCAAAGC-3') and Ci5a18R1 (SEQ ID NO. 82: 5'-GGATTAGGAAACGACCAGG-3'). Four plaques were ultimately obtained from the resulting 17 plaques, and these were converted to plasmids by in vivo excision. When their DNA nucleotide sequences were determined, they were found to contain the same sequences. Among these, a clone designated gCi01-pBluestar was used in subsequent experiments. The cloned nucleotide sequence of gCi01-pBluestar is shown in SEQ ID NO. 79. This sequence was expected to contain a 5'-untranslated containing a sequence having promoter activity of cineraria F3'5'H, a translated region, and a 3'-untranslated region.

A roughly 5.7 kb DNA fragment excised from gCi01-pBluestar with PvuI and EcoRV (SEQ ID NO. 80) was blunted using a DNA blunting kit (Takara). This DNA fragment was then cloned into the SmaI site of pBinPLUS and designated pSPB3130. This binary vector had an nptII gene able to be used to screen the T-DNA region with kanamycin.

pSPB3130 was transformed into chrysanthemum variety 94-765 using an *Agrobacterium* method. Although anthocyanidins in the petals of the transformed chrysanthemum were analyzed, delphinidin was not detected and flower color did not change.

Reference Example 3

Production of Delphinidin Using Rose Chalcone Synthase Gene Promoter

A binary vector was constructed in which pansy-derived F3'5'H BP#18 gene was coupled to a rose-derived chalcone synthase promoter described in PCT International Patent Publication No. PCT/AU03/01111, and this binary vector was designated pBRBP18. The gene contained in this binary vector was transformed into chrysanthemum variety 94-765 as described in Reference Examples 1 and 2. When anthocyanidins in the flower petals of the transformed chrysanthemum were analyzed, although a maximum of 5.4% of delphinidin was detected with respect to all anthocyanidins, there was no change in flower color observed.

In addition, pSPB3325 (rose CHSpro::pansy #18+rose CHSp:: chrysanthemum F3'H IR) described in the ninth row from the top in Table 1 is an example of the production of delphinidin using rose chalcone synthase gene promoter, and delphinidin production in this example reached a maximum of 3.6%.

Reference Example 4

Production of Delphinidin Using Pansy F3'5'H Gene Promoter

(1) Cloning of Perilla Anthocyanin 3-Acyl Transferase Chromosome Gene

There are known to be red varieties of perilla in which anthocyanins accumulate in the leaves and green varieties in which they do not. Chromosomal DNA from the leaves of the former was prepared using a reported method (Plant Mol. Biol., December 1997, 35(6), 915-927). This chromosomal DNA was partially decomposed with Sau3AI (Toyobo), and a fraction containing a 10 kb to 15 kb DNA fragment was recovered using a sucrose density gradient method. This fragment was then inserted into the BamHI site of EMBL3 (Promega), a type of lambda phage vector, using a known method to prepare a genomic DNA library. The resulting library was screened using pSAT208 (see Plant Cell Physiol., April 2000, 41(4), 495-502), which is cDNA of anthocyanin 3-acyl transferase derived from perilla, as a probe. Screening of the library was in accordance with a previously reported method (Plant Cell Physiol., July 1996, 37(5), 711-716). Plaques that hybridized with the probe were blunted and cultured, and DNA was prepared from the resulting phage.

(2) Determination of Nucleotide Sequence of Perilla Anthocyanin 3-Acyl Transferase Chromosome Gene

10 μg of the DNA obtained above were digested with XbaI and isolated with 0.7% agarose gel followed by blotting onto Hybond-N (Amersham). When this film was hybridized in the same manner as previously described, a roughly 6.8 kb DNA fragment was found to hybridize with the probe. After digesting 20 μg of the same DNA with XbaI and isolating with 0.7% agarose gel, a roughly 6.8 kb DNA fragment was purified using a GeneClean Kit and coupled with pBluescript SKII-digested with XbaI. The resulting plasmid was designated pSPB513. The DNA sequence derived from perilla contained in this plasmid was determined by primer walking. The nucleotide sequence thereof is shown in SEQ ID NO. 4. This sequence contains a region that demonstrates high homology with anthocyanin 3-acyltransferase cDNA in the form of pSAT208, the amino acid sequence (SEQ ID NO. 6) of protein encoded by this region was observed to demonstrate substitution of 19 amino acid residues and deletion of 2 amino acid residues in comparison with the amino acid sequence encoded by pSAT208, and there were no introns observed. In addition, the sequence of the region demonstrating high homology with pSAT208 contained a 3438 bp sequence upstream from ATG that was thought to be the start codon, and a 2052 bp sequence downstream from TAA that was thought to be the stop codon thereof. A different open reading frame (ORF, SEQ ID NO. 5), which was not anthocyanin 3-acyltransferase, was present in the aforementioned 3438 bp sequence. The following experiment was conducted to amplify the transcriptional regulatory region of shiso (perilla) anthocyanin 3-acyl transferase gene, excluding this portion.

(3) Amplification of Transcriptional Regulatory Region of Shiso Anthocyanin 3-Acyltransferase Gene

PCR (25 cycles of a reaction consisting of holding for 1 minute at 95° C., 1 minute at 52° C., 2 minutes at 72° C. and

1 minute at 95° C.) was carried out using 1 ng of pSPB513 as template and two types of primers (5'-AAGCTTAACTATTATGATCCCACAGAG-3' (SEQ ID NO. 7, underline indicates HindIII recognition sequence) and 5'-GGATCCGGCGGTGTTGAACGTAGC-3' (SEQ ID NO. 8, underline indicates BamHI recognition sequence)). The amplified roughly 1.1 kb DNA fragment was digested with HindIII and BamHI.

The plasmid pSPB567 described in Patent Document 4 (in which pansy-derived flavonoid 3',5'-hydroxylase gene is coupled to the 3'-side of cauliflower mosaic 35S promoter to which has been added E12 enhancer, and in which a nopaline synthase terminator is further coupled to the 3'-side thereof) was digested with PacI, and a DNA fragment containing pansy-derived flavonoid 3',5'-hydroxylase gene was cloned into the Pad site of pBin+. A plasmid in which the cauliflower mosaic 35S promoter to which E12 enhancer was added is present close to the AscI site of pBin+ in the resulting plasmid was designated pSPB575. This plasmid was then digested with HindIII and BamHI, and a DNA fragment obtained by digesting a roughly 1.1 kb DNA fragment containing the transcriptional regulatory region of perilla anthocyanin 3-acyltransferase with HindIII and BamHI was inserted therein. The resulting plasmid was designated pSFL205.

Plasmid pSFL205 was digested with HindIII and SacI, and a roughly 100 bp DNA fragment was recovered. This DNA fragment, a roughly 4 kb DNA fragment obtained by digesting pSPB513 with SacI and XbaI, and a plasmid pBin+(see Transgenic Research, 4, 288-290, 1995) digested with HindIII and XbaI were coupled to obtain plasmid pSPB3311. This plasmid pSPB3311 is a binary vector that contains the nucleotide sequence indicated in SEQ ID NO. 2, and contains the transcriptional regulatory region of perilla anthocyanin 3-acyltransferase gene and an untranslated region of the 3'-side thereof.

(4) Construction of pSPB3323

The transcriptional regulatory region of pansy flavonoid 3',5'-hydroxylase gene BP#40 (see WO 04/020637) was amplified as described below using the Takara LA PCR™ In Vitro Cloning Kit.

Chromosomal DNA was prepared from a pansy leaf using the DNA Easy Plant Kit (Qiagen). 3 μg of the chromosomal DNA were digested with restriction enzyme HindIII. The digested DNA was coupled with HindIII terminal DNA (included in Takara LA PCR™ In Vitro Cloning Kit) by reacting for 40 minutes at 16° C. using Ligation High (Takara). After diluting 4 μl of the reaction mixture with 10 μl of water and denaturing the coupled DNA by treating for 10 minutes at 94° C., the reaction mixture was cooled in ice. 5 pmol of primer C1 (5'-GTACATATTGTCGTTAGAACGCG-TAATACGACTCA-3', SEQ ID NO. 9, included in the kit as a partial sequence of HindIII cassette sequence) and 5 pmol of primer BP40-i5 (5'-AGGTGCATGATCGGACCATACTTC-3', SEQ ID NO. 10, equivalent to complementary strand of translated region of BP#40) were then added followed by repeating 30 cycles of a reaction in 25 μl of the reaction mixture consisting of 20 seconds at 98° C. and 15 minutes at 68° C. in accordance with the kit protocol. The reaction mixture was then diluted 10-fold with water. After reacting for 5 minutes at 98° C. in 25 of a reaction mixture containing 5 pmol of primer C2 (5'-CGTTAGAACGCGTAATAC-GACTCACTATAGGGAGA-3', SEQ ID NO. 11, included in kit as partial sequence of HindIII cassette sequence) and 5 pmol of primer BP40-i7 (5'-GACCATACTTCTTAGC-GAGTTTGGC-3', SEQ ID NO. 12) using 0.5 μl of this dilution as template, 30 cycles of a reaction were repeated consisting of reacting for 20 seconds at 98° C. and 15 minutes at 68° C.

The resulting DNA fragment was ligated into plasmid pCR2.1 (Invitrogen). When the nucleotide sequence of the resulting DNA was determined, the sequence was observed to have locations that did not coincide with the cDNA nucleotide sequence of BP#40. This is thought to be due to the occurrence of an error during PCR. The following procedure was carried out for the purpose of amplifying an error-free sequence.

In order to amplify a roughly 2 kb 5'-untranslated region and a 200 bp translated region of BP#40, PCR was carried out in 25 μl of a reaction mixture using 200 ng of pansy genomic DNA as template and using 50 pmol of primer BP40-i7 (SEQ ID NO. 12) and 50 pmol of primer BP40 pro-F (5'-ACT-CAAACAAGCATCTCGCCATAGG-3', SEQ ID NO. 3, sequence in 5'-untranslated region of BP#40 gene). After treating for 5 minutes at 98° C., a reaction consisting of 20 seconds at 98° C. and 15 minutes at 68° C. was repeated for 30 cycles. The amplified DNA fragment was inserted into pCR2.1. This DNA fragment contained a roughly 2.1 kbp 5'-untranslated region and a 200 bp translated region. This plasmid was designated pSFL614. The nucleotide sequence of plasmid pSFL614 is shown in SEQ ID NO. 14.

The roughly 2.1 bp 5'-untranslated region (BP40pro, SEQ ID NO. 15) contained in pSFL614 was used to transcribe BP#40 gene. At this time, the BamHI site was changed to NheI. After using 1 ng of pSFL614 as template, adding 50 pmol of primer BP40pro-HindIII-F (5'-AAG CTT GTG ATC GAC ATC TCT CTC C-3', SEQ ID NO. 16), 50 pmol of primer BP40pro-NehI-R (5'-CGA GGC TAG CTA AAC ACT TAT-3', SEQ ID NO. 17), and holding for 5 minutes at 98° C. in 25 μl of the reaction mixture, a reaction consisting of 20 seconds at 98° C. and 15 minutes at 68° C. was repeated for 25 cycles. The amplified DNA fragment was cloned into pCR2.1. This sequence was determined to be free of errors attributable to PCR by confirming the nucleotide sequence thereof. This plasmid was then digested with HindIII and NheI to obtain a 470 bp DNA fragment. This DNA fragment was designated fragment A.

After using 1 ng of pSLF614 as template, adding 50 pmol of primer BP40pro-NehI-F (5'-TTT AGC TAG CCT CGA AGT TG-3', SEQ ID NO. 18) and 50 pmol of primer BP40pro-BamHI-R (5'-GGA TCC CTA TGT TGA GAA AAA GGG ACT-3', SEQ ID NO. 19) and Ex-Taq DNA polymerase, and holding for 5 minutes at 98° C. in 25 μl of the reaction mixture, a reaction consisting of 20 seconds at 98° C. and 15 minutes at 68° C. was repeated for 25 cycles. The amplified DNA fragment was cloned into pCR2.1. This sequence was determined to be free of errors attributable to PCR by confirming the nucleotide sequence thereof. This plasmid was then digested with HindIII and NheI to obtain a 630 bp DNA fragment. This DNA fragment was designated fragment B.

The larger fragment of DNA fragments formed by digesting plasmid pSPB567 described in Patent Document 4 with HindIII and NheI was recovered, and coupled with the aforementioned fragment A and fragment B to obtain pSFL620.

After digesting pSFL620 with PacI, a roughly 3.2 kb DNA fragment was recovered. This DNA fragment was inserted into the Pad site of pBin+. The resulting plasmid was designated pSBP3317. A fragment obtained by digesting the aforementioned pSPB3311 with AscI and XbaI was cloned into the AscI and XbaI sites of pSBP3317, and the resulting plasmid was designated pSPB3323.

(5) Expression of Perilla Anthocyanin 3-Acyl Transferase Genomic Gene and Pansy F3'5'H Gene in Chrysanthemum

The pSPB3323 prepared in (4) above was introduced into *Agrobacterium* and chrysanthemum variety 94-765 (Seikoen, not sold) was transformed according to a known method using this *Agrobacterium*. Six transformed strains were acquired.

Anthocyanidins extracted according to the method described below were analyzed. Ray petals were frozen and then crushed followed by extracting 50 mg to 100 mg of the crushed petal with 500 μL of 1% hydrogen chloride-methanol, adding 500 μL of 4 N hydrochloric acid (HCl) to this extract and mixing, and hydrolyzing for 1 hour at 100° C. After cooling the solution following hydrolysis, 1 ml of 0.05 M trifluoroacetic acid (TFA) was added and mixed therein. Next, this solution was added to Sep-Pak C18 (Millipore) to adsorb the hydrolysis product. The Sep-Pak C18 was preliminarily washed with 80% acetonitrile (MeCN) and equilibrated with 0.05 M TFA. After washing the hydrolysis product adsorbed to the Sep-Pak C18 with 0.05 M TFA, the hydrolysis product was further washed with 20% MeCN and 0.05 M TFA followed by eluting the hydrolysis product with 80% MeCN and 0.05 M TFA to obtain an analysis sample.

The analysis sample was analyzed under the following conditions using high-performance liquid chromatography. An Inertsil ODS-2 column (particle diameter: 5 μm, 4.6×250 mm, GL Sciences) was used for the column, the flow rate was 0.8 ml/min, the mobile phase contained 1.5% phosphoric acid, and isocratic elution was carried out for 20 minutes using a linear concentration gradient from 5% acetic acid and 6.25% acetonitrile to 20% acetic acid and 25% acetonitrile, followed by eluting for 5 minutes with 25% acetonitrile containing 1.5% phosphoric acid and 20% acetic acid. Detection was carried out using the Agilent 1100 Series Diode Array Detector (GL Sciences) over a wavelength region of 250 nm to 600 nm, and the abundance ratios of each of the anthocyanidins was determined according to the area of optical absorbance at 530 nm.

As a result of analysis, delphinidin was detected at ratios of 0.9%, 0.8%, 1.4% and 0.6% of the total amount of anthocyanidins in transformants consisting of analyzed strains 1300-3, 1300-4, 1300-5 and 1300-6, respectively. Although this suggests that BP#40 transcriptional regulatory region of pansy governs transcription of BP#40, this did not lead to a change in flower color.

Reference Example 5

Production of Delphinidin in Chrysanthemum Using Rugosa Rose DFR Promoter

A Rugosa rose Genomic DNA library was prepared in the manner described below using the λBlueSTAR™ Xho I Half-Site Arms Kit (Novagen, on the Internet at merckbiosciences.com/product/69242). Chromosomal DNA was prepared from a young leaf of Rugosa rose using Nucleon Phytopure™ (Tepnel Life Sciences). Roughly 100 μg of chromosomal DNA was digested with restriction enzyme Sau3AI.

This DNA fragment was then partially filled in with DNA polymerase I Klenow fragment (Toyobo) in the presence of dGTP and dATP, and fractionated by sucrose density gradient centrifugation. DNA of about 13 kb was recovered and concentrated by ethanol precipitation. Roughly 180 ng of DNA were ligated for 15 hours at 4° C. with 1 μL of the λBlueSTAR™ Xho I Half-Site Arms Kit, followed by carrying out in vitro packaging to obtain a genomic library.

This library was screened using cultivated rose DFR cDNA (Plant and Cell Physiology, 36, 1023-1031, 1995) to obtain plaque indicating a signal. Plasmid pSFK710 was obtained by in vivo excision from this plaque using the method recommended by the manufacturer (Novagen). This plasmid contained a DNA sequence that closely coincided with the aforementioned cultivated rose DFR cDNA.

By carrying out PCR so as to obtain a 5'-untranslated region of a DFR translated sequence from this plasmid and facilitate coupling with heterologous genes, one of the EcoRI recognition sequences was mutated to an NheI recognition sequence followed by the addition of HindIII and BamHI recognition sequences. First, PCR was carried out in 50 μL of the reaction mixture using pSLF710 as template, using 25 pmol each of primers DFRproHindIIIF (5'-TAATAAGCTTACAGTGTAATTATC-3', SEQ ID NO. 20) and DFRproNheIR (5'-TTATGCTAGCGTGTCAAGACCAC-3', SEQ ID NO. 21), and using enzyme ExTaq DNA Polymerase (Toyobo). The PCR reaction conditions consisted of reacting for 5 minutes at 94° C. followed by repeating 30 cycles of a reaction of which one cycle consists of reacting for 30 seconds at 94° C., 30 seconds at 50° C. and 30 seconds at 72° C., and finally holding for 7 minutes at 72° C. As a result, a roughly 350 bp DNA fragment A was obtained. Similarly, a PCR reaction was carried out in 50 μL of the reaction mixture using pSFL710 as template, using 25 pmol each of primers DFRproNheIF (5'-ACACGCTAGCATAAGTCTGTTG-3', SEQ ID NO. 22) and DFRproBamHI-R (5'-GCTTGGGGATCCATCTTAGG-3', SEQ ID NO. 23), and using enzyme ExTaq DNA Polymerase (Toyobo). The PCR reaction conditions consisted of reacting for 5 minutes at 94° C. followed by repeating 30 cycles of a reaction of which one cycle consists of reacting for 30 seconds at 94° C., 30 seconds at 50° C. and 30 seconds at 72° C., and finally holding for 7 minutes at 72° C. As a result, a 600 bp DNA fragment B was obtained.

The pSPB567 described in Patent Document 4 (plasmid pUC containing CaMV35S promoter to which has been added E12 enhancer, pansy F3'5'HBP#40 and nopaline synthase terminator) was digested with BamHI and then partially digested with HindIII to couple fragment A with a fragment digested with HindIII and NheI and couple fragment B with a fragment digested with NheI and BamHI and obtain plasmid pSLF721 (containing an expression cassette of R. rugosa DFR 5':BPF3'5'H#40:nos3'. An expression cassette obtained by digesting this plasmid with Pad was introduced into the Pad site of pBinPLUS to obtain pSLF724. This plasmid was then transfected into *Agrobacterium tumefaciens* strain EHA105.

A recombinant chrysanthemum was obtained from variety 94-765 using this transformed *Agrobacterium*. The resulting strain produced delphinidin in the flower petals thereof at about 0.6% of the total amount of anthocyanidins.

In addition, other reference examples using Rugosa rose DFR promoter are shown in the second row from the top (pSPB3316 (Rugosa rose DFRpro:pansy #40+rose ANSpro:torenia 5GT, non-delphinidin-producing strain) and in the fifth row from the top (Rugosa rose DFRpro:pansy #40+Japanese gentian 3'GTpro::torenia MT, maximum delphinidin production level: 0.9%) of Table 1. Neither of these reference examples resulted in a change in flower color.

Reference Example 6

Production of Delphinidin in Chrysanthemum Using Rugosa Rose F3H Promoter

The Rugosa rose genomic DNA library produced in Reference Example 5 was screened with torenia flavanone 3-hy-

droxylase (F3H) cDNA (NCBI No. AB211958) to obtain plaques indicating signals. One of these plaques was converted to a plasmid in the same manner as Reference Example 5. This was then digested with restriction enzyme SpeI to recover a 2.6 kb DNA fragment, and plasmid pSPB804 was obtained by sub-cloning this DNA fragment to the SpeI site of pBluescript SKII-(Stratagene). This plasmid had a nucleotide sequence that demonstrates homology with F3H.

In order to amplify the 5'-untranslated region of F3H, PCR was carried out in 50 μL of a reaction mixture by using 1 ng of pSPB804 as template, using primer RrF3H-F (5'-AAGCTTCTAGTTAGACAAAAAGCTA-3', SEQ ID NO. 24) and primer RrF3H (5'-GGATCCTCTCTTGATATTTCCGTTC-3', SEQ ID NO. 25), and using Ex-Taq DNA Polymerase (Toyobo). PCR reaction conditions consisted of reacting for 5 minutes at 94° C., repeating 30 cycles of reaction of which one cycle consisted of 30 seconds at 94° C., 30 seconds at 50° C. and 30 seconds at 72° C., and finally holding for 7 minutes at 72° C. The resulting DNA fragment was inserted into pCR-TOPO (Invitrogen) to obtain plasmid pSPB811. A roughly 2.1 kb F3H 5'-untranslated region was able to be recovered from this plasmid using HindIII and BamHI. Plasmid pSFL814 (containing R. rugosa F3H 5':BFP3'5'#40:nos 3') was obtained by substituting the promoter portion of pSPB567 with the roughly 1.2 kb 5'-untranslated region of F3H using HindIII and BamHI as described in Reference Example 5. This plasmid was intoduced into *Agrobacterium tumefaciens* strain EHA105.

Although three strains of recombinant chrysanthemum were obtained from variety 94-765 using this transformed *Agrobacterium*, there were no strains in which production of delphinidin was observed in the flower petals (see Table 1).

Reference Example 7

Production of pBINPLUS Rugosa Rose F3Hpro:ADHNF-Pansy-F3'5'H#40::NOSter

A DNA fragment amplified by PCR using pSLF814 (Reference Example 6) as template and using ADH-BP40-Fd (5'-CAAGAAAAATAAATGGCAATTCTAGTCACCGAC-3', SEQ ID NO. 26) and NcoI-BP40-Rv (5'-CTCGAGCGTACGTGAGCATC-3', SEQ ID NO. 27) as primers, and a DNA fragment amplified by PCR using pB1221 ADH-221 as template and using BamHI-ADH-Fd (5'-CGCGGATCCGTCTATTTAACTCAGTATTC-3', SEQ ID NO. 28) and BP40-ADH-Rv (5'-TAGAATTGCCATTTATTTTTCTTGATTTCCTTCAC-3', SEQ ID NO. 29) as primers were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of pansy F3'5'H#40 was obtained by PCR using this mixture of DNA fragments as template and using BamHI-ADH-Fd (5'-CGCGGATCCGTCTATTTAACTCAGTATTC-3', SEQ ID NO. 30) and NcoI-BP40-Rv (5'-CTCGAGCGTACGTGAGCATC-3', SEQ ID NO. 31) as primers.

After TA-cloning this DNA fragment to pCR2.1, a roughly 600 bp DNA fragment obtained by digesting with BamHI and NcoI and a binary vector fragment obtained by digesting pSFL814 with BamHI and NcoI were ligated to obtain pBinPLUS Rugosa rose F3Hpro:ADHNF-pansy-F3'5'H#40::Noster. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

There were no individuals in which delphinidin was detected among four strains of transformants derived from chrysanthemum variety 94-765 obtained by using this transformed *Agrobacterium* (see Table 1).

Reference Example 8

Production of pBIN19 Rose CHSpro:ADH-pansy-F3'5'H#18::NOSter

A DNA fragment amplified by PCR using pB1221 ADH221 as template and using ADH KpnI Forward (5'-CGGTACCGTCTATTTAACTCAGTATTC-3', SEQ ID NO. 32) and GUS19R (5'-TTTCTACAGGACGTAACATAAGGGA-3', SEQ ID NO. 33) as primers was digested with KpnI and SmaI to obtain a roughly 110 bp tobacco ADH-5'UTR DNA fragment. This DNA fragment was ligated with a binary vector DNA fragment obtained by digesting pBRBP18 (having an expression cassette of rose CHSpro::pansy-F3'5'H#18::NOSter inserted into pBIN19) with KpnI and SmaI to obtain pBIN19 rose CHSpro::ADH-pansy-F3'5'H#18:NOSter. In this plasmid, a 38 bp spacer is present between tobacco ADH-5'UTR and pansy F3'5'H#18. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

30 strains of recombinant chrysanthemum derived from chrysanthemum variety 94-765 were obtained using this transformed *Agrobacterium*. Delphinidin was detected in the petals of five of these strains and delphinidin content reached 1.9%. However, there were no changes in flower color observed.

Reference Example 9

Production of pBI121-rose CHSpro::ADHNF-pansy-F3'5'H#40::NOSter

A DNA fragment obtained by PCR using pBRBP18 (Reference Example 3) as template, using HAPS-RhCHSpro3k-Fd (5'-CCAAGCTTGGCGCGCCTTAATTAAATTTAAATCAGCAAGAGTTGAAGAAATAG-3', SEQ ID NO. 85) and NS-RhCHSpro3k-Rv (5'-AAAGCTAGCACTAGTCATCTCGGAGAAGGGTCG-3', SEQ ID NO. 86) as primers, and using Pyrobest Polymerase (Takara), and a binary vector fragment obtained by digesting with HindIII and NheI and digesting pBI121 ADHNF with HindIII and XbaI were ligated, and the resulting binary vector was designated pBI121-RhCHSp-GUS-NOSt.

An ADHNF-pansy-F3'5'H#40 DNA fragment obtained by digesting the pCR-ADHBP40-SpeSac obtained in Example 10 with SpeI and EcoICRI was ligated to a binary vector fragment obtained by digesting pBI121-RhCHSp-GUS-NOSt with SpeI and EcoICRI to obtain pBI121-rose CHSpro::ADHNF-pansy-F3'5'H#40:: NOSter, which was used to transform *Agrobacterium tumefaciens* strain EHA105.

Although 19 strains of recombinant chrysanthemum derived from chrysanthemum variety 94-765 were obtained using this transformed *Agrobacterium*, there were no individuals in which delphinidin was detected.

Example 1

Cloning of the Promoter Region of Chrysanthemum Flavanone 3-Hydroxylase Gene

The cloned promoter region of the chrysanthemum flavanone 3-hydroxylase gene, F3Hpro1K, has the nucleic acid sequence depicted in SEQ ID NO: 34. A promoter region

having a different length was amplified in the manner described below. This portion of the chrysanthemum flavanone 3-hydroxylase gene, F3Hpro500, has the nucleic acid sequence depicted in SEQ ID NO: 87.

A DNA fragment amplified by PCR using pBluescript SK-gF3H9 as template and using HANS-F3Hpro-500Fd (5'-CCAAGCTTGGCGCGCCGCGGCCGCATTTAAAT TACTGTTCGAACCTACAAAGG-3', SEQ ID NO. 83, underline indicates sequence that anneals with DNA containing F3H promoter region) and MX-F3Hpro-Rv (5'-TTTCTAGAACGCGTTTTTTTATTTTTTCTTCACACACTTG-3', SEQ ID NO. 84, underline indicates sequence that anneals with DNA containing F3H promoter region) as primers was cloned into pCR2.1 to obtain pCR HANS-CmF3Hpro500-X. In addition, a binary vector fragment obtained by digesting pBI121 ADHNF with HindIII and XbaI and a roughly 500 bp chrysanthemum F3H promoter DNA fragment obtained by digesting pCR HANS-CmF3Hpro500-X with HindIII and XbaI were ligated to obtain pBI121 HANS-CmF3Hp500-X.

Example 2

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Bellflower F3'5'H::NOSter

Two types of primers consisting of CamF1 (5'-GTGAAGCCACCATGTCTATAG-3', SEQ ID NO. 49) and CamR1 (5'-GCATTTGCCTAGACAGTGTAAG-3', SEQ ID NO. 50) were synthesized based on the translated sequence of F3'5'H cDNA (Accession No. D14590) of bellflower (Campanula medium) registered in the GenBank DNA database. RNA was extracted from the flower petals of commercially available bellflower buds using the RNeasy Mini Plant Kit (Qiagen), and 1st strand DNA was synthesized using an RT-PCR kit. PCR was carried out using primers by using this 1st strand DNA as template. The resulting DNA fragment was cloned into pCR-TOPO II. The nucleotide sequence of the resulting clone #4 (designated as pSPB2561) was determined to be SEQ ID NO. 51.

A vector obtained by coupling tobacco ADH-5'UTR 94 bp and F3'5'H gene was constructed in the manner described below (FIG. **4**). Furthermore, the same procedure was also carried out in the subsequently described examples.

Two types of DNA fragments consisting of a DNA fragment amplified by PCR using pSPB2561 as template and using ADH-Campa-Fd (5'-CAAGAAAAATAAATGTCTATAGACATAACCATTC-3', SEQ ID NO. 53) and HpaI-Campa-Rv (5'-GTTAACATCTCTGGCACCACC-3', SEQ ID NO. 54) as primers and a DNA fragment amplified by PCR using pBI1121 ADH-221 as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and Campa-ADH-Rv (5'-GTCTATAGACATTTATTTTTCTTGATTTCCTTCAC-3', SEQ ID NO. 55) as primers, were synthesized, and a DNA fragment in which tobacco ADH-5'UTR 94 bp is directly coupled to the start codon of bellflower F3'5'H was obtained by PCR using these two types of DNA fragments as templates and using XbaI-ADH-Fd (SEQ ID NO. 42) and HpaI-Campa-Rv (5'-GTTAAC ATCTCTGGCACCACC-3', SEQ ID NO. 56) as primers. This DNA fragment was then TA-cloned into pCR2.1 followed by digesting with XbaI and HpaI, and the resulting roughly 650 bp fragment was ligated with a vector fragment obtained by digesting pSPB2561 with XbaI and HpaI to obtain pCR ADHNF-Campanula F3'5'H.

Next, pCR ADHNF-Campanula F3'5'H was digested with KpnI followed by blunting with Blunting High (Toyobo) and digesting with XbaI, and the resulting roughly 1.7 kb DNA fragment was ligated with a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hp1k-S with SpeI and EcoICRI to obtain pBI121 chrysanthemum F3Hpro1k::ADHNF-bellflower F3'5'H::NOSter. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

48 recombinant chrysanthemum strains of chrysanthemum variety 94-765 were obtained by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of 30 of these strains, and the delphinidin content reached 80.5%.

pSPB3738 was constructed from pBI121 chrysanthemum F3Hpro1k::ADHNF-bellflower F3'5'H::NOSter. This plasmid was transfected into *Agrobacterium tumefaciens* strain AGL0, and this was then used to transform the chrysanthemum variety Sei Taitan (Seikoen). Among the resulting 26 strains of recombinant chrysanthemums, a change in flower color was observed in 6 strains, and delphinidin was able to be detected by thin layer chromatography.

Example 3

Production of pIG121-Hm-chrysanthemum F3Hpro1k::ADHNF-Lisianthus F3'5'H::NOSter

Eustoma F3'5'H gene (EgF3'5'H, GenBank AB078957) cloned into pBluescript SK- was digested with XhoI followed by blunting with Blunting High (Toyobo), and the roughly 1.9 kb EgF3'5'H DNA fragment obtained by further digesting with XbaI was ligated to a pIG121-Hm binary vector obtained by digesting with XbaI and EcoICRI to obtain pIG121-Hm 35S::EgF3'5'H.

Next, two types of DNA fragments consisting of a DNA fragment amplified by PCR using pBluescript SK-EgF3'5'H as template and using ADH-EgF3'5'H-Fd (5'-CAAGAAAAATAAAT GGCTGTTGGAAATGGCGTT-3', SEQ ID NO. 40) and HpaI-EgF3'5'H-Rv (5'-GTTAACGCTGAGCCTAGTGCC-3', SEQ ID NO. 41) as primers, and a DNA fragment amplified by PCR using pBI221 ADH-221 (Satoh, J. et al. (2004), J. Biosci. Bioengineer) as template and using XbaI-ADH-Fd (5'-ACGCGTTCTAGAGTCTATTTAACTCAGTATTC-3', SEQ ID NO. 42) and EgF3'5'H-ADH-Rv (5'-TCCAACAGCCATTTATTTTTTCTTGATTTCCTTCAC-3', SEQ ID NO. 43) as primers, were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp (Satoh, J. et al. (2004), J. Biosci. Bioengineer) was directly coupled to the start codon of EgF3'5'H was obtained by PCR using the mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and HpaI-EgF3'5'H-Rv (5'-GTTAACGCTGAGCCTAGTGCC-3', SEQ ID NO. 44) as primers. After cloning this DNA fragment into pCR2.1, a roughly 1.3 kb DNA fragment obtained by digesting with XbaI and HpaI and a binary vector fragment obtained by digesting pIG121-Hm 35S::EgF3'5'H with XbaI and HpaI were ligated to obtain pIG121-Hm 35S::ADHNF-EgF3'5'H. A roughly 1.2 kb EgF3'5'H DNA fragment obtained by digesting this pIG121-Hm 35S::EgF3'5'H with HindIII and XbaI, a roughly 15 kb binary vector DNA fragment, and a DNA fragment obtained by further digesting pCR HANS-CmF3Hp1k-MNS with HindIII and SpeI were ligated to obtain PIG121-Hm chrysanthemum F3Hpro1k::ADHNF-lisianthus F3'5'H::NOSter. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

Five recombinant chrysanthemum strains derived from chrysanthemum variety 94-765 by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of one of these strains, and the delphinidin content was 4.4%.

Example 4

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Lobelia F3'5'H::NOSter

F3'5'H gene derived from the flower petals of lobelia cloned into pBluescript SK- (LeF3'5'H1, GenBank ABS221077 and LeF3'5'H4, GenBank AB221078) was digested with KpnI followed by blunting with Blunting High (Toyobo), and a roughly 1.9 kb EgF3'5'H DNA fragment obtained by further digesting with XbaI was ligated to a pIG121-Hm binary vector fragment obtained by digesting XbaI and EcoICRI to obtain pIG121-Hm 35S::LeF3'S'H1 and pIG121-Hm 35S::LeF3'5'H4.

Next, two types of DNA fragments consisting of a DNA fragment amplified by PCR using pBluescript SK-LeF3'5'H1 or pBluescript SK-LeF3'5'H4 as template and using ADH-LeF3'5'H-Fd (5'-CAAGAAAATAAATGGACGCGACAWACATTGC-3', SEQ ID NO. 45) and HpaI-LeF3'5'H-Rv (5'-GTTAACATCTCGGGCAGCACC-3', SEQ ID NO. 46) as primers, and a DNA fragment amplified by PCR using pBI121 ADH-221 as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and LeF3'5'H-ADH-Rv (5'-TGTCGCGTCCATTTATTTTTCTTGATTTCCTTCAC-3', SEQ ID NO. 47) as primers, were mixed, and DNA fragments in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of LeF3'5'H1 or LeF3'5'H4 were respectively obtained by using this mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and HpaI-LeF3'5'H-Rv (5'-GTTAACATCTCGGGCAGCACC-3', SEQ ID NO. 48) as primers.

After respectively TA-cloning these DNA fragments into pCR2.1, a DNA fragment obtained by digesting with XbaI and HpaI and a binary vector fragment obtained by digesting pIG121-Hm 35S::LeF3'5'H1 or pIG12'-Hm 35S::LeF3'5'H4 with XbaI and HpaI were respectively ligated to obtain pIG121-Hm 35S:: ADHNF-LeF3'5'H1 and pIG121-Hm 35S::ADHNF-LeF3'5'H4. A roughly 2.6 kb ADHNF-LeF3'5'H1::NOSter DNA fragment obtained by digesting these binary vectors with XbaI and EcoRV was ligated with a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hp1k-S with SpeI and EcoICRI to obtain pBI121 chrysanthemum F3Hpro1kpro::ADHNF-loberia F3'5'H1::NOSter and pBI121 chrysanthemum F3Hpro1kpro::ADHNF-eustoma F3'5'H4::NOSter.

Although 12 strains of recombinant chrysanthemum derived from chrysanthemum variety 94-765 were obtained by using *Agrobacterium* transformed with pBI121 chrysanthemum F3Hpro1kpro::ADHNF-loberia F3'5'H1::NOSter, there were no individuals obtained that contained delphinidin. In addition, although 34 strains of recombinant chrysanthemum derived from chrysanthemum variety 94-765 were obtained by using *Agrobacterium* transformed with pBI121 chrysanthemum F3Hpro1 kpro::ADHNF-loberia F3'5'H4::NOSter, there were also no individuals obtained that contained delphinidin.

Example 5

Production of pBINPLUS Chrysanthemum F3Hpro1k::ADHNF-Pansy-F3'5'H#40::NOSter

pBinPLUS chrysanthemum F3Hpro1k::ADHNF-pansy F3'5'H#40:: NOSter was obtained by ligating a roughly 1.1 kb chrysanthemum F3H promoter DNA fragment obtained by digesting pCR HANS-CmF3Hp1k-BclI with AscI and BclI, and a binary vector fragment obtained by digesting pBinPLUS Rugosa rose F3Hpro:: ADHNF-pansy F3'5'H#40:: NOSter with AscI and BamHI. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

6 recombinant chrysanthemum strains derived from chrysanthemum variety 94-675 were obtained by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of 4 of these strains, and the delphinidin content reached 26.8%.

Example 6

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Cineraria F3'5'H:NOSter and Transformation into Chrysanthemum

Two types of DNA fragments consisting of a DNA fragment amplified by PCR using the cineraria F3'S'H (pSPB2774) obtained in Reference Example 2 as template and using ADH-ScF3'5'H-Fd (5'-CAAGAAAAATAAATGAGCATTCTAACCCTAATC-3', SEQ ID NO. 57) and NdeI-ScF3'5'H-Rv (5'-CATATGTTTAGCTCCAGAATTTGG-3', SEQ ID NO. 58) as primers, and a DNA fragment amplified by PCR using pBI121 ADH-221 as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and ScF3'5'H-ADH-Rv (5'-TAGAATGCTCATTTATTTTTCTTGATTTCCTTCAC-3', SEQ ID NO. 59) as primers, were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of cineraria F3'S'H was obtained by PCR using this mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and NdeI-ScF3'5'H-Rv (5'-CATATGTTTAGCTCCAGAATTTGG-3', SEQ ID NO. 60) as primers. After TA-cloning this DNA fragment into pCR2.1, a DNA fragment obtained by digesting with XbaI and NdeI and a vector fragment obtained by digesting pSPB2774 with XbaI and NdeI were ligated to obtain pBluescript Sk^- ADHNF-cineraria F3'5'H.

Next, a roughly 1.7 kb DNA fragment obtained by digesting pBluescript Sk^- ADHNF-cineraria F3'5'H with XbaI and XhoI and a vector fragment obtained by digesting pCR2.1 with XbaI and XhoI were ligated to obtain pCR2.1 ADHNF-cineraria F3'5'H. pBI121 chrysanthemum F3Hpro1k:: ADHNF-cineraria F3'5'H:: NOSter was then obtained by ligating a DNA fragment obtained by digesting this pCR2.1 ADHNF-cineraria F3'5'H with XbaI and EcoRV with a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hp1k-S with SpeI and EcoICRI. This plasmid was introduced into *Agrobacterium* tumefaciens strain EHA105.

50 recombinant strains derived from Chrysanthemum variety 94-765 were obtained by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of 37 of these strains, and the delphinidin content reached 36.2%.

Example 7

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Japanese gentian F3'5'H::NOSter

Two types of DNA fragments consisting of a DNA fragment amplified by PCR using Japanese gentian F3'5'H cloned into pBluescript SK- (plasmid pG48 described in WO 2004/020637) as template and using ADH-Gentian-Fd (5'-CAAGAAAAATAAATGTCACCCATTTACACCACCC-3', SEQ ID NO. 61) and SalI-Gentian F3'5'H-Rv (5'-GTCGACGCTATTGCTAAGCC-3', SEQ ID NO. 62) as primers, and a DNA fragment amplified by PCR using pBI121 ADH-221 as

template and using XbaI-ADH-Fd (SEQ ID NO. 42) and Gentian-ADH-Rv (5'-AATGGGTGACATTTATTTTTCT-TGATTTCCTTCAC-3', SEQ ID NO. 63) as primers, were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of Japanese gentian F3'5'H was obtained by using this mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and SalI-Gentian F3'5'H-Rv (5'-GTCGACGCTAT-TGCTAAGCC-3', SEQ ID NO. 64) as primers. After TA-cloning this DNA fragment into pCR2.1, a roughly 400 bp DNA fragment obtained by digesting with XbaI and SalI and a vector fragment obtained by digesting pG48 with XbaI and SalI were ligated to obtain pBluescript SK-ADHNF-Japanese gentian F3'5'H.

Next, a roughly 1.8 kb DNA fragment obtained by digesting pBluescript SK-ADHNF-Japanese gentian F3'S'H with XbaI and XhoI and a vector fragment obtained by digesting pCR2.1 with XbaI and XhoI were ligated to obtain pCR2.1 ADHNF-Japanese gentian F3'5'H. pBI112 chrysanthemum F3Hpro1k::ADHNF Japanese gentian F3'S'H::NOSter was obtained by ligating a DNA fragment obtained by digesting this pCR2.1 ADHNF-Japanese gentian F3'S'H with XbaI and EcoRV and a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hp1k-S with SpeI and EcoICRI. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

Although 21 recombinant chrysanthemum strains derived from Chrysanthemum variety 94-765 were obtained by using this transformed *Agrobacterium*, there were no individuals obtained that contained delphinidin.

Example 8

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Verbena F3'5'H::NOSter

Two types of DNA fragments consisting of a DNA fragment amplified by PCR using verbena F3'5'H cloned into pBluescript SK- (pHVF7, Plant Biotechnology, 23, 5-11, 2006, DNA database accession no. ABA234898) as template and using ADH-Verbena-Fd (5'-CAAGAAAAATAAAT-GACGTTTTCAGAGCTTATAAAC-3', SEQ ID NO. 65) and NcoI-Verbena F3'5'H-Rv (5'-CCATGGAGTAAATCAG-CATCTC-3', SEQ ID NO. 66) as primers, and a DNA fragment amplified by PCR using pBI121 ADH-221 as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and Verbena ADH-Rv (5'-TGAAAACGTCATTTATTTTTCT-TGATTTCCTTCAC-3', SEQ ID NO. 67) as primers, were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of verbena F3'S'H was obtained by PCR using the mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and NcoI-Verbena F3'5'H-Rv (5'-CCATGGAGTAAAT-CAGCATCTC-3', SEQ ID NO. 68) as primers. After TA-cloning this DNA fragment into pCR2.1, pBluescript SK-ADHNF-verbena F3'S'H was obtained by ligating a roughly 700 b DNA fragment obtained by digesting with XbaI and NcoI and a vector fragment obtained by digesting pHVF7 with XbaI and NcoI.

Next, a 1.8 kb DNA fragment obtained by digesting pBluescript SK-ADHNF-verbena F3'5'H with XbaI and XhoI and a vector fragment obtained by digesting pCR2.1 with XbaI and XhoI were ligated to obtain pCR2.1 ADHNF-verbena F3'5'H. pBI121 chrysanthemum F3Hpro1k::ADHNF-verbena F3'5'H::NOSter was then obtained by ligating a DNA fragment obtained by digesting this pCR2.1 ADHNF-verbena F3'S'H with XbaI and EcoRV and a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hk1k-S with SpeI and EcoICRI. This plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105.

17 recombinant chrysanthemum strains derived from chrysanthemum variety 94-765 were obtained by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of 11 of these strains, and the maximum delphinidin content was 28.4%.

Example 9

Production of pBI121 Chrysanthemum F3Hpro1k::ADHNF-Blue Snapdragon F3'5'H::NOSter

A cDNA library was produced using mRNA obtained from the bud of a type of snapdragon (*Antirrhinum kelloggii*, blue snapdragon) using the Uni-ZAP XR Vector Kit (Stratagene) in accordance with the method recommended by the manufacturer. This library was screened according to the method described in Reference Example 2 to obtain two types of plasmids pSPB3145 and pSPB3146 respectively containing F3'5'H cDNA #1 (SEQ ID NO. 69) and F3'5'H cDNA #12 (SEQ ID NO. 71).

Two types of DNA fragments consisting of a DNA fragment amplified by PCR using pSPB3145 or pSPB3146 as template and using ADH-AkF3'5'H-Fd (5'-CAA-GAAAAATAAATGCAGATAATAATTCCGGTCC-3', SEQ ID NO. 73) and NsiI-AkF3'5'H-Rv (5'-ATGCATGTC-CTCTAACATGTATC-3', SEQ ID NO. 74) as primers, and a DNA fragment amplified by PCR using pBI121 ADH-221 as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and AkF3'5'H-ADH-Rv (5'-TATTATCTGCATTTATTTTTCT-TGATTTCCTTCAC-3', SEQ ID NO. 75) as primers, were mixed, and a DNA fragment in which tobacco ADH-5'UTR 94 bp was directly coupled to the start codon of blue snapdragon (Ak)F3'5'H #1 or #12 was respectively obtained by PCR using the mixture of DNA fragments as template and using XbaI-ADH-Fd (SEQ ID NO. 42) and NsiI-AkF3'5'H-Rv (5'-ATGCATGTCCTCTAACATGTATC-3', SEQ ID NO. 76) as primers. After TA-cloning this DNA fragment to pCR2.1, pBluescript SK-ADHNF-AkF3'5'H #1 and #12 were obtained by respectively ligating a roughly 700 b DNA fragment obtained by digesting with XbaI and NsiI and a vector fragment obtained by digesting pSPB3145 (pBluescript SK-AkF3'S'H #1) and pSBP3146 (pBluescript SK-AkF3'S'H #12) with XbaI and NsiI.

Next, roughly 700 b DNA fragments obtained by digesting pBluescript SK-ADHNF-AkF3'S'H #1 and #12 with XbaI and XhoI were ligated with a vector fragment obtained by digesting pCR2.1 with XbaI and XhoI to obtain pCR2.1 ADHNF-AkF3'5'H #1 and #12. pBI121 chrysanthemum F3Hpro1k::ADHNF-AkF3'5'H#1::NOSter and pBI121 chrysanthemum F3Hpro1k::ADHNF-AkF3'5'H#12::NOSter were obtained by respectively ligating DNA fragments obtained by digesting these pCR2.1 ADHNF-AkF3'5'H #1 and #12 with XbaI and EcoRV with a binary vector fragment obtained by digesting pBI121 HANS-CmF3Hp1k-S with SpeI and EcoICRI. These plasmids were transfected into *Agrobacterium tumefaciens* strain EHA105.

1 strain of recombinant chrysanthemum derived from chrysanthemum variety 94-765 was obtained by using this transformed *Agrobacterium*. Delphinidin was detected in the flower petals of this strain, and the delphinidin content reached 2.9%.

Example 10

Production of pBI121 Chrysanthemum F3Hpro500::ADHNF-Cineraria F3'5'H::NOSter

A binary vector DNA fragment obtained by digesting the pBI121 HANS-CmF3Hp500-X obtained in Example 1 with XbaI and EcoICRI and a DNA fragment of ADHNF-cineraria F3'5'H obtained by digesting the pCR2.1 ADHNF-cineraria F3'5'H obtained in Example 6 were ligated to obtain pBI121-chrysanthemum F3Hpro500::ADHNF-cineraria F3'5'H::NOSter, which was then introduced into *Agrobacterium tumefaciens* strain EHA105.

Seven stains of recombinant chrysanthemum derived from chrysanthemum variety Taihei were obtained by using this transformed *Agrobacterium*. Delphinidin was detected in 5 of those strains, and delphinidin content reached 25.5%.

INDUSTRIAL APPLICABILITY

According to the present invention, chrysanthemum flower color can be changed to blue by using the transcriptional regulatory region of chrysanthemum-derived flavanone 3-hydroxylase (F3H), expressing flavonoid 3'5'-hydroxylase (F3'5'H) in chrysanthemum, and allowing a large amount of delphinidin to accumulate in the flower petals. Although chrysanthemums come in flower colors including white, yellow, orange, red, pink and purplish red, since there are no existing varieties or closely related wild varieties that produce bluish flowers such as those having a purple or blue color, blue chrysanthemums produced according to the method of the present invention will lead to stimulation of new demand.

TABLE 1

Accumulation of Delphinidin in *Chrysanthemum* Transformants Introduced with Various F3'5'H Genes

Gene Cassette 1 F3'5'H				Gene Cassette 2			No. of transformants	No. of individuals analyzed for aglycones	No. of individuals containing delphinidin	Delphinidin Content** Mean (%)	Delphinidin Content** Maximum (%)	Example No.
Promoter	ADH enhancer*	F3'5'H gene origin	Terminator	Promoter	Gene	Terminator						
Rugosa rose DFR	None	Pansy #40	NOS				4	2	1	0.3	0.6	Ref. Ex. 5
Rugosa rose DFR	None	Pansy #40	NOS	Rose ANS	*Torenia* 5GT	MAS	2	1	0	0.0	0.0	Ref. Ex. 5
Rugosa rose F3H	None	Pansy #40	NOS				3	3	0	0.0	0.0	Ref. Ex. 6
Rugosa rose F3H	94 bp, direct coupled	Pansy #40	NOS				4	2	0	0.0	0.0	Ref. Ex. 7
Rugosa rose DFR	None	Pansy #40	NOS	Gentian 3'GT	*Torenia* MT	MOS	5	4	4	0.7	0.9	Ref. Ex. 5
Gerbera CHS	None	Pansy #18	NOS				2	1	0	0.0	0.0	
Pansy #40	None	Pansy #40	NOS	*Perilla* 3AT	*Perilla* 3AT	*Perilla* 3AT	6	6	4	0.6	1.4	Ref. Ex. 4
Rose CHS	None	Pansy #18	NOS				11	10	5	1.3	5.4	Ref. Ex. 3
Rose CHS	None	Pansy #18	NOS	Rose CHS	*Chrysanthemum* F3'H IR	NOS	11	11	2	0.4	3.6	Ref. Ex. 3
Rose CHS	94 bp, with spacer	Pansy #18	NOS				30	29	5	0.2	1.9	Ref. Ex. 8
Rose CHS	94 bp, direct coupled	Pansy #40	NOS				19	19	0	0.0	0.0	Ref. Ex. 9
CaMV35S	74 bp, with spacer	Pansy #40	NOS				8	5	2	0.2	0.7	
CaMV35S	74 bp, with spacer	Bellflower	NOS				11	9	9	1.5	6.9	
Chrysanthemum F3H1k	94 bp, direct coupled	Gentian	NOS				21	19	0	0.0	0.0	Ex. 7
Chrysanthemum F3H1k	94 bp, direct coupled	*Lobelia* #1	NOS				12	11	0	0.0	0.0	Ex. 4
Chrysanthemum F3H1k	94 bp, direct coupled	*Lobelia* #4	NOS				34	20	0	0.0	0.0	Ex. 4
Chrysanthemum F3H1k	94 bp, direct coupled	Blue snap-dragon	NOS				1	1	1	2.9	2.9	Ex. 9
Chrysanthemum F3H1k	94 bp, direct coupled	*Eustoma*	NOS				5	5	1	0.9	4.4	Ex. 3
Chrysanthemum F3H500	94 bp, direct coupled	*Cineraria*	NOS				7	7	5	11.9	25.5	Ex. 10
Chrysanthemum F3H1k	94 bp, direct coupled	Pansy #40	NOS				6	5	4	14.9	26.8	Ex. 5
Chrysanthemum F3H1k	94 bp, direct coupled	*Verbena*	NOS				17	12	11	8.9	28.4	Ex. 8
Chrysanthemum F3H1k	94 bp, direct coupled	*Cineraria*	NOS				50	47	37	7.5	36.2	Ex. 6
Chrysanthemum F3H1k	94 bp, direct coupled	Bellflower	NOS				48	39	30	31.4	80.5	Ex. 2

*Length of 5'UTR of tobacco ADH gene and manner of coupling to start codon of F3'5'H gene

**Ratio of delphinidin to total anthocyanidins during hydrolysis of anthocyanin accumulated in ray petals (wt %) The number of transformants for which the delphinidin content was 0 was included when determining mean values.

SEQUENCE LISTING

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aaattcggca ctaactaaat atataattta cgttttatgc actcgtaatt taaaatttca 240

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agggagtata aagttggagg ttgtggatgt ggaggagaaa gaaattaata ttttatttaa 840

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aaatctgccc agcaaaataa aataataagt aaataaataa actaaatatg tattgaatgc 960

gacatctagc aatatagcca catatatagt gcagtagcac gcagcgctcg ttactcgtca 1020

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Cys Asn Leu Ile Tyr Pro Ser Ser Pro Glu Lys Met Pro Glu Phe Arg
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65                  70                  75                  80

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            260                 265                 270

Ala Tyr Met Trp Ala Gly Val Thr Lys Ser Leu Thr Ala Asp Glu Asp
        275                 280                 285

His Asp Asp Gly Asp Ala Phe Phe Leu Ile Pro Val Asp Leu Arg Pro
    290                 295                 300

Arg Leu Asp Pro Pro Val Pro Glu Asn Tyr Phe Gly Asn Cys Leu Ser
305                 310                 315                 320

Tyr Ala Leu Pro Arg Met Arg Arg Arg Glu Leu Val Gly Glu Lys Gly
                325                 330                 335

Val Phe Leu Ala Ala Glu Ala Ile Ala Ala Glu Ile Lys Lys Arg Ile
            340                 345                 350

Asn Asp Lys Arg Ile Leu Glu Thr Val Glu Lys Trp Ser Leu Glu Ile
        355                 360                 365

Arg Glu Ala Leu Gln Lys Ser Tyr Phe Ser Val Ala Gly Ser Ser Lys
    370                 375                 380

Leu Asp Leu Tyr Gly Ala Asp Phe Gly Trp Gly Lys Ala Arg Lys Gln
385                 390                 395                 400

Glu Ile Leu Ser Ile Asp Gly Glu Lys Tyr Ala Met Thr Leu Cys Lys
                405                 410                 415

Ala Arg Asp Phe Glu Gly Gly Leu Glu Val Cys Leu Ser Leu Pro Lys
            420                 425                 430

Asp Lys Met Asp Ala Phe Ala Ala Tyr Phe Ser Ala Gly Ile Asn Gly
        435                 440                 445


<210> SEQ ID NO 4
<211> LENGTH: 6835
<212> TYPE: DNA
<213> ORGANISM: Perilla frutescens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1608)..(2330)
<223> OTHER INFORMATION: Other ORF
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (3439)..(4785)
<223> OTHER INFORMATION: SAT208 ORF
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6815)..(6815)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 4

ccccaaaaac cttgattagg gtgatggttc acgtagtggg ccatcgccct gatagacggt     60

ttttcgccct ttgacgttgg agtccacgtt ctttaatagt ggactcttgt tccaaactgg    120

aacaacactc aaccctatct cggtctattc ttttgattta taagggattt tgccgatttc    180

ggcctattgg ttaaaaaaat gagctgattt aacaaaaatt taacgcgaat tttaacaaaa    240

tattaacgct tacaatttcc attcgccatt caggctgcgc aactgttggg aagggcgatc    300

ggtgcgggcc tcttcgctat tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt    360
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aagttgggta acgccagggt tttcccagtc acgacgttgt aaaacgacgg ccagtgagcg    420

cgcgtaatac gactcactat agggcgaatt gggtaccggg ccccccctcg aggtcgacgg    480

tatcgataag cttgatatcg aattcctgca gcccgggggа tccactagtt ctagaagatg    540

aagagacaaa acatcgacta cttgcccttg tgtttgggca aaattaaatt aatgtaattg    600

taattgtgag atgtgtgtta gtaattatgc tatgtgtgtg ttagtaatta tgagatgtgt    660

gtgtttgtaa ttttgagatg tcttttcctc actttataaa taattaatgt attttatgca    720

tatctatttc tcttattctt ttcatacaaa cctgcatgca taagtctcaa tcatgcattg    780

gattctttat gccttgtcaa tttctttttg tacaaacctc atgcatctca atcatgcatt    840

ggattcttat actctcattt caatttatat gcaagagtaa agctaagtat atcacatgca    900

ttggattcca ctttatatca aattgatttc ttgataaatc acatgctttt gtcagccatc    960

acatgcattg gattccactt tatatcaaat taatttcttg ataaatcaca tacttttgtc   1020

ggccatttca tgcattggat tccactttat atcaaattga ttttttgata aatcacatgc   1080

ttttgtcggc tagcccatgc tttgtctata catatctcag aaaatgcaca tcaaaagaaa   1140

ctcaaacaaa atcctcaata ccttaccaca tctttcaact tcactttaga aaaatgtctg   1200

cacatgaaaa ttctgatgtt gaatcaaact caagttctaa ttattctgat tctaacgaac   1260

ttgatgaatg gctagagcga ggttatgaaa aatatcgtga agttgatagt ataatccaga   1320

atgtgctcat aaataatccc aatctggttg taggagctca aacttctaca gtcagaagaa   1380

ggtattgtga tagggaacgt gagaatggtg aagagcgttt gatgaaagac tattttgtct   1440

ctaatccaac gtattctcca gagctcttcc gacgatgatt tcacatgcag aaatcacttt   1500

ttcttcgtat agtggaggcc gttactacca atgatgacta ttttcaacag aggccaaatt   1560

gcacgggtag aaaaggtctt tcaccattgt aaaaatgtac aggagct atg agg gta     1616
                                                  Met Arg Val
                                                  1

ttg gct tat ggg gca tca gcc gat gtc gtt gat gaa tac tta cga atg     1664
Leu Ala Tyr Gly Ala Ser Ala Asp Val Val Asp Glu Tyr Leu Arg Met
    5                  10                  15

agt gca acg gta aca aga gat gct gtc atc cat ttc gta gaa ggt gtc     1712
Ser Ala Thr Val Thr Arg Asp Ala Val Ile His Phe Val Glu Gly Val
20                  25                  30                  35

att tca tgc ttc agt gac aca tat ctt agg aag cct aat caa caa gat     1760
Ile Ser Cys Phe Ser Asp Thr Tyr Leu Arg Lys Pro Asn Gln Gln Asp
                40                  45                  50

ttg gca aga cta ctc tat gtt gga gag caa cgt ggt ttt cct ggc atg     1808
Leu Ala Arg Leu Leu Tyr Val Gly Glu Gln Arg Gly Phe Pro Gly Met
            55                  60                  65

att ggt agt att gat tgc atg cac tgg gaa tgg aca aat tgt cct aat     1856
Ile Gly Ser Ile Asp Cys Met His Trp Glu Trp Thr Asn Cys Pro Asn
        70                  75                  80

gcc tgg gca ggg caa ttt aca ggg aga agt gga aag tca aca atc att     1904
Ala Trp Ala Gly Gln Phe Thr Gly Arg Ser Gly Lys Ser Thr Ile Ile
    85                  90                  95

ttg gaa gct gtt gca tca tat gat tta tgg ata tgg cat gcg ttt ttt     1952
Leu Glu Ala Val Ala Ser Tyr Asp Leu Trp Ile Trp His Ala Phe Phe
100                 105                 110                 115

gga aca tca ggt gcg tgc aat gat att aat gtt ctc cac ggt tct cca     2000
Gly Thr Ser Gly Ala Cys Asn Asp Ile Asn Val Leu His Gly Ser Pro
                120                 125                 130

att ttt agt gat gtt tta gaa ggt cga gca cca cat gtt agt tac atc     2048
Ile Phe Ser Asp Val Leu Glu Gly Arg Ala Pro His Val Ser Tyr Ile
            135                 140                 145
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gtc aat ggt cgc caa aat gat aga gca tat tat ctc acc gat ggc ata     2096
Val Asn Gly Arg Gln Asn Asp Arg Ala Tyr Tyr Leu Thr Asp Gly Ile
        150                 155                 160

tat cct tca tgg gct gca ttt gta aag tca atc aca tct cct atg act     2144
Tyr Pro Ser Trp Ala Ala Phe Val Lys Ser Ile Thr Ser Pro Met Thr
    165                 170                 175

cga aag tat aag ttg ttt gtt caa cac caa gaa gct gct aga aaa gat     2192
Arg Lys Tyr Lys Leu Phe Val Gln His Gln Glu Ala Ala Arg Lys Asp
180                 185                 190                 195

gta gaa cgg gcc ttt gga gtt cta caa gct cgt ttt gca ttt att cga     2240
Val Glu Arg Ala Phe Gly Val Leu Gln Ala Arg Phe Ala Phe Ile Arg
                200                 205                 210

cgt cca tgt ctt gtt tgg gac aag gtt ttg atg gga aaa att atg atg     2288
Arg Pro Cys Leu Val Trp Asp Lys Val Leu Met Gly Lys Ile Met Met
            215                 220                 225

gct tgt atc atc ata cac aat atg att gtg gag gat gaa tga             2330
Ala Cys Ile Ile Ile His Asn Met Ile Val Glu Asp Glu
        230                 235                 240

gacacatacc taaactatta tgatcccaca gagtttttga cagatgagtc ttcaggagga   2390

gatgctgaac cttttcacta ctctactgaa cgcatcacaa gtttatcggc ttatatgact   2450

aatagggatc aacttcacaa cagagaggct catagagctc ttaaagagga tttgatcgag   2510

cacatatgga aaaaattcgg cactaactaa atatataatt tacgttttat gcactcgtaa   2570

tttaaaattt catgtgtctc attgtagttt atttaattat gttttcactc ttgtaatttt   2630

tattttgttg tgaagtaaat tatgaattta taattatatg ggtaattttt tgataattat   2690

gcaattaaaa ataattaata ttttttaaat gcaagagaaa aatgttattt taataacatg   2750

ttcttattaa aaaataaaat gataaatatt ttatgtaggt tgggagaaaa tgaaaaaata   2810

atattttatt tgaaggttgg gttggatgag gtcactgatg ggagtataaa taatactccc   2870

tccgtcccat aattattgtc cattattcct ttttgggatg tcccaaaatt atagtcctat   2930

tctaaattgg gattgtattt aaatattctt ttacaaatat aaccctattt gatatagtat   2990

gaatgcaatt aatatagtaa aaaaataagg gcaatatagg ataattattg taaattgtat   3050

atttccaata catattaaat gtgatttctt aatctgtgtg aaaataggaa gtggactata   3110

attatgggac ggagggagta taaagttgga ggttgtggat gtggaggaga aagaaattaa   3170

tattttattt aaagattgga ttaaaggagg tcactgatgt gggtagtctt agaggaaatg   3230

tagtcttaga ggaaatctgc ccagcaaaat aaaataataa gtaaataaat aaactaaata   3290

tgtattgaat gcgacatcta gcaatatagc cacatatata gtgcagtagc acgcagcgct   3350

cgttactcgt cagtcgtcaa agaatggtaa gtatagaaaa gcatctttaa ataacacacc   3410

aaaaaccaca gctacgttca acaccgcc atg acc acc acc gtg atc gaa acg      3462
                              Met Thr Thr Thr Val Ile Glu Thr
                                              245

tgt aga gtt ggg cca ccg ccg gac tcg gtg gcg gag caa tcg ttg ccg     3510
Cys Arg Val Gly Pro Pro Pro Asp Ser Val Ala Glu Gln Ser Leu Pro
    250                 255                 260

ctc aca ttc ttc gac atg acg tgg ctg cat ttt cat ccc atg ctt cag     3558
Leu Thr Phe Phe Asp Met Thr Trp Leu His Phe His Pro Met Leu Gln
265                 270                 275                 280

ctc ctc ttc tac gaa ttc cct tgt tcc aag caa cat ttc tca gaa tcc     3606
Leu Leu Phe Tyr Glu Phe Pro Cys Ser Lys Gln His Phe Ser Glu Ser
                285                 290                 295

atc att cca aaa ctc aaa caa tct ctc tct aaa act ctc ata cac ttc     3654
Ile Ile Pro Lys Leu Lys Gln Ser Leu Ser Lys Thr Leu Ile His Phe
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           300                 305                 310

ttc cct ctc tca tgc aat tta atc tac cct tca tct ccg gag aaa atg     3702
Phe Pro Leu Ser Cys Asn Leu Ile Tyr Pro Ser Ser Pro Glu Lys Met
        315                 320                 325

ccc gag ttt cgg tat cta tcg ggg gac tcg gtt tct ttc act atc gca     3750
Pro Glu Phe Arg Tyr Leu Ser Gly Asp Ser Val Ser Phe Thr Ile Ala
    330                 335                 340

gaa tct agc gac gac ttc gat gat ctc gtc gga aat cgc gca gaa tct     3798
Glu Ser Ser Asp Asp Phe Asp Asp Leu Val Gly Asn Arg Ala Glu Ser
345                 350                 355                 360

ccc gtt agg ctc tac aac ttc gtc cct aaa ttg ccg cag att gtc gaa     3846
Pro Val Arg Leu Tyr Asn Phe Val Pro Lys Leu Pro Gln Ile Val Glu
                365                 370                 375

gaa tct gat aga aaa ctc ttc caa gtt ttc gcc gtg cag gtg act ctt     3894
Glu Ser Asp Arg Lys Leu Phe Gln Val Phe Ala Val Gln Val Thr Leu
            380                 385                 390

ttc cca ggt cga ggc gtc ggt att gga ata gca acg cat cac acc gtt     3942
Phe Pro Gly Arg Gly Val Gly Ile Gly Ile Ala Thr His His Thr Val
        395                 400                 405

agc gat gcc ccg tcg ttt ctc gcc ttt ata acg gct tgg gct tgg atg     3990
Ser Asp Ala Pro Ser Phe Leu Ala Phe Ile Thr Ala Trp Ala Trp Met
    410                 415                 420

agc aaa cac att gaa gat gaa gat gaa gag ttt aaa tct ttg cca gtt     4038
Ser Lys His Ile Glu Asp Glu Asp Glu Glu Phe Lys Ser Leu Pro Val
425                 430                 435                 440

ttc gat aga tcc gtc ata aaa tat ccg acg aaa ttt gac tcg att tat     4086
Phe Asp Arg Ser Val Ile Lys Tyr Pro Thr Lys Phe Asp Ser Ile Tyr
                445                 450                 455

tgg aaa aag gcg cta aaa ttt cct ttg caa tct cgt cat ccc tca tta     4134
Trp Lys Lys Ala Leu Lys Phe Pro Leu Gln Ser Arg His Pro Ser Leu
            460                 465                 470

ccg acg gac cgc att cga acc acg ttc gtt ttc acc caa tcc gaa att     4182
Pro Thr Asp Arg Ile Arg Thr Thr Phe Val Phe Thr Gln Ser Glu Ile
        475                 480                 485

aag aaa ttg aag ggt tcg att cag tcc aga gtt cca agt tta gtc cat     4230
Lys Lys Leu Lys Gly Ser Ile Gln Ser Arg Val Pro Ser Leu Val His
    490                 495                 500

ctc tca tct ttt gta gcg att gca gct tat atg tgg gct ggc gta acg     4278
Leu Ser Ser Phe Val Ala Ile Ala Ala Tyr Met Trp Ala Gly Val Thr
505                 510                 515                 520

aaa tca ctc aca gca gat gaa gac cac gac gac ggg gat gca ttt ttc     4326
Lys Ser Leu Thr Ala Asp Glu Asp His Asp Asp Gly Asp Ala Phe Phe
                525                 530                 535

ttg att ccg gtc gat cta agg cca cga tta gat ccg cca gtt ccc gaa     4374
Leu Ile Pro Val Asp Leu Arg Pro Arg Leu Asp Pro Pro Val Pro Glu
            540                 545                 550

aat tac ttc ggg aac tgc tta tcg tac gcg ctg ccg aga atg cgg cgg     4422
Asn Tyr Phe Gly Asn Cys Leu Ser Tyr Ala Leu Pro Arg Met Arg Arg
        555                 560                 565

cga gag ctg gtg gga gag aaa ggg gtg ttt ctg gcg gct gag gca atc     4470
Arg Glu Leu Val Gly Glu Lys Gly Val Phe Leu Ala Ala Glu Ala Ile
    570                 575                 580

gcg gcg gag atc aaa aaa agg atc aac gac aag aga ata tta gaa acg     4518
Ala Ala Glu Ile Lys Lys Arg Ile Asn Asp Lys Arg Ile Leu Glu Thr
585                 590                 595                 600

gtg gag aaa tgg tcg ctg gag att cgt gaa gcg ttg cag aaa tca tat     4566
Val Glu Lys Trp Ser Leu Glu Ile Arg Glu Ala Leu Gln Lys Ser Tyr
                605                 610                 615

ttt tcg gtg gca gga tcg agc aag cta gat ctt tac ggt gca gat ttt     4614
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Phe Ser Val Ala Gly Ser Ser Lys Leu Asp Leu Tyr Gly Ala Asp Phe
            620                 625                 630

gga tgg ggg aag gcg aga aag caa gaa ata ttg tcg att gat ggg gag     4662
Gly Trp Gly Lys Ala Arg Lys Gln Glu Ile Leu Ser Ile Asp Gly Glu
        635                 640                 645

aaa tat gca atg acg ctt tgt aaa gcc agg gat ttc gaa gga gga ttg     4710
Lys Tyr Ala Met Thr Leu Cys Lys Ala Arg Asp Phe Glu Gly Gly Leu
    650                 655                 660

gag gtt tgc ttg tct ttg cct aag gac aaa atg gat gct ttt gct gct     4758
Glu Val Cys Leu Ser Leu Pro Lys Asp Lys Met Asp Ala Phe Ala Ala
665                 670                 675                 680

tat ttt tca gcg gga att aat ggt taa taaatgtatg taattaaact           4805
Tyr Phe Ser Ala Gly Ile Asn Gly
                685

aatattatta tgtaacaatt aattaagtgt tgagtaacgt gaagaataat atcttttacc   4865

tattatatat ttatgagttg gttcaaataa aatcacttca tttattgtat taaccgttta   4925

gtgttcttct caccatattt tggtgctatt ttttaaaaaa tgtttttttt attgtatttt   4985

agtattaatt gttttaccac taaaattaca gtaaaatgca agatagttta atttttacat   5045

ttacatatga aacacattct ctttataacc aacctctcta tatatataat atgtgtgtat   5105

gtatgtatac acatgtatga atactagaaa tatatcttaa accatccatc cttcaaaaat   5165

ttcggggcca tattgcatgg tgacattata atatttgata atttcttcga acacgttatt   5225

aattcaattt aataattcta ataaaaagac gctcagacaa tatatgtaga taggatcggc   5285

ccaaaggggt gtctgggtgg gctgtcgccc atgggccccg aaatcttagg ggcaaaaaaa   5345

aaaaaattca ttatacctag ggcaaaaaaa ttaccgctct tcacttctct gcctctctcc   5405

ctcatccctc gttcctcctc tctcttccct atgtacgcct ctttcactcc ctccccctct   5465

ctcagttctc tatcacttgt attttgtatt gaaaacttgt tgaaaactaa accaaaaata   5525

gaaaaaggta tagaaaattt gaaaacaaag gttgtttttt tgtgttgctg cagttcccaa   5585

acttgccgag ttgccgactt gccgtgttga attgttatat atgttaaaag cctaaaatat   5645

atcctttcag aattgagatg gattgttgta actatcaggt ttttttttatt gagaatttta   5705

gatcaattag ttatcttgta attttttatt ctttttaata caatactccc tccatcccaa   5765

tagcaaggtc cccttgctat tgggcacggg tattaaggag gaggattatt ataatgaaaa   5825

ttaatataaa gtaagtggat tccactttat taaggaatat tataatcaaa agtaatataa   5885

agtaagtgga ttccacttta attaggacac taattatttt ctttttggt atgagacttt   5945

gctattggga catcccaaaa aggcaaaaga gaccttgcta ttaggacggt ggacgtgctg   6005

ccgaggcacg caaattaatt tacctttcct cttctatact aactcgtagt agcggcgagt   6065

aaaggtcgaa ccctcaagga gcaattgaac tagatgtgct attagaaata aaataaacac   6125

aagtgagagg ggagtttttg gtttcaattt aactaaaact aattatgaaa atgaaaaaac   6185

aaatataaaa cataaacagg tagacgaaat atgataaaga tagaattcta gttctcggtt   6245

cagttatcac ctttctccaa gtatttcatg aataatgcaa cgcctctttt catacaactt   6305

agaatcgatg tccaaaggtt aatatcaagc tttatttacc taattgtctc gtacgattag   6365

ttaactaaaa caagctcttt aattaactct actcaattag ataacctaga ataagctctc   6425

tagagcggcc gccaccgcgg tggagctcca gcttttgttc cctttagtga gggttaattg   6485

cgcgcttggc gtaatcatgg tcatagctgt ttcctgtgtg aaattgttat ccgctcacaa   6545

ttccacacaa catacgagcc ggaagcataa agtgtaaagc ctggggtgcc taatgagtga   6605
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gctaactcac attaattgcg ttgcgctcac tgcccgcttt ccagtcggga aacctgtcgt 6665

gccagctgca ttaatgaatc ggccaacgcg cggggagagg cgggttgcgt attgggccgc 6725

tcttccgctt ccttggttac ttgactcgct gcgctcggcc gtcggctgcg gcgagcggta 6785

tcaagctcac tcaaaggcgg taataccggn tatccacaga atcaggggat 6835

<210> SEQ ID NO 5
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Perilla frutescens

<400> SEQUENCE: 5

Met Arg Val Leu Ala Tyr Gly Ala Ser Ala Asp Val Val Asp Glu Tyr
1 5 10 15

Leu Arg Met Ser Ala Thr Val Thr Arg Asp Ala Val Ile His Phe Val
20 25 30

Glu Gly Val Ile Ser Cys Phe Ser Asp Thr Tyr Leu Arg Lys Pro Asn
35 40 45

Gln Gln Asp Leu Ala Arg Leu Leu Tyr Val Gly Glu Gln Arg Gly Phe
50 55 60

Pro Gly Met Ile Gly Ser Ile Asp Cys Met His Trp Glu Trp Thr Asn
65 70 75 80

Cys Pro Asn Ala Trp Ala Gly Gln Phe Thr Gly Arg Ser Gly Lys Ser
85 90 95

Thr Ile Ile Leu Glu Ala Val Ala Ser Tyr Asp Leu Trp Ile Trp His
100 105 110

Ala Phe Phe Gly Thr Ser Gly Ala Cys Asn Asp Ile Asn Val Leu His
115 120 125

Gly Ser Pro Ile Phe Ser Asp Val Leu Glu Gly Arg Ala Pro His Val
130 135 140

Ser Tyr Ile Val Asn Gly Arg Gln Asn Asp Arg Ala Tyr Tyr Leu Thr
145 150 155 160

Asp Gly Ile Tyr Pro Ser Trp Ala Ala Phe Val Lys Ser Ile Thr Ser
165 170 175

Pro Met Thr Arg Lys Tyr Lys Leu Phe Val Gln His Gln Glu Ala Ala
180 185 190

Arg Lys Asp Val Glu Arg Ala Phe Gly Val Leu Gln Ala Arg Phe Ala
195 200 205

Phe Ile Arg Arg Pro Cys Leu Val Trp Asp Lys Val Leu Met Gly Lys
210 215 220

Ile Met Met Ala Cys Ile Ile Ile His Asn Met Ile Val Glu Asp Glu
225 230 235 240

<210> SEQ ID NO 6
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: Perilla frutescens

<400> SEQUENCE: 6

Met Thr Thr Thr Val Ile Glu Thr Cys Arg Val Gly Pro Pro Pro Asp
1 5 10 15

Ser Val Ala Glu Gln Ser Leu Pro Leu Thr Phe Phe Asp Met Thr Trp
20 25 30

Leu His Phe His Pro Met Leu Gln Leu Leu Phe Tyr Glu Phe Pro Cys
35 40 45

Ser Lys Gln His Phe Ser Glu Ser Ile Ile Pro Lys Leu Lys Gln Ser

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    50                  55                  60

Leu Ser Lys Thr Leu Ile His Phe Phe Pro Leu Ser Cys Asn Leu Ile
65                  70                  75                  80

Tyr Pro Ser Ser Pro Glu Lys Met Pro Glu Phe Arg Tyr Leu Ser Gly
                85                  90                  95

Asp Ser Val Ser Phe Thr Ile Ala Glu Ser Ser Asp Asp Phe Asp Asp
            100                 105                 110

Leu Val Gly Asn Arg Ala Glu Ser Pro Val Arg Leu Tyr Asn Phe Val
        115                 120                 125

Pro Lys Leu Pro Gln Ile Val Glu Glu Ser Asp Arg Lys Leu Phe Gln
    130                 135                 140

Val Phe Ala Val Gln Val Thr Leu Phe Pro Gly Arg Gly Val Gly Ile
145                 150                 155                 160

Gly Ile Ala Thr His His Thr Val Ser Asp Ala Pro Ser Phe Leu Ala
                165                 170                 175

Phe Ile Thr Ala Trp Ala Trp Met Ser Lys His Ile Glu Asp Glu Asp
            180                 185                 190

Glu Glu Phe Lys Ser Leu Pro Val Phe Asp Arg Ser Val Ile Lys Tyr
        195                 200                 205

Pro Thr Lys Phe Asp Ser Ile Tyr Trp Lys Lys Ala Leu Lys Phe Pro
    210                 215                 220

Leu Gln Ser Arg His Pro Ser Leu Pro Thr Asp Arg Ile Arg Thr Thr
225                 230                 235                 240

Phe Val Phe Thr Gln Ser Glu Ile Lys Lys Leu Lys Gly Ser Ile Gln
                245                 250                 255

Ser Arg Val Pro Ser Leu Val His Leu Ser Ser Phe Val Ala Ile Ala
            260                 265                 270

Ala Tyr Met Trp Ala Gly Val Thr Lys Ser Leu Thr Ala Asp Glu Asp
        275                 280                 285

His Asp Asp Gly Asp Ala Phe Phe Leu Ile Pro Val Asp Leu Arg Pro
    290                 295                 300

Arg Leu Asp Pro Pro Val Pro Glu Asn Tyr Phe Gly Asn Cys Leu Ser
305                 310                 315                 320

Tyr Ala Leu Pro Arg Met Arg Arg Arg Glu Leu Val Gly Glu Lys Gly
                325                 330                 335

Val Phe Leu Ala Ala Glu Ala Ile Ala Ala Glu Ile Lys Lys Arg Ile
            340                 345                 350

Asn Asp Lys Arg Ile Leu Glu Thr Val Glu Lys Trp Ser Leu Glu Ile
        355                 360                 365

Arg Glu Ala Leu Gln Lys Ser Tyr Phe Ser Val Ala Gly Ser Ser Lys
    370                 375                 380

Leu Asp Leu Tyr Gly Ala Asp Phe Gly Trp Gly Lys Ala Arg Lys Gln
385                 390                 395                 400

Glu Ile Leu Ser Ile Asp Gly Glu Lys Tyr Ala Met Thr Leu Cys Lys
                405                 410                 415

Ala Arg Asp Phe Glu Gly Gly Leu Glu Val Cys Leu Ser Leu Pro Lys
            420                 425                 430

Asp Lys Met Asp Ala Phe Ala Ala Tyr Phe Ser Ala Gly Ile Asn Gly
        435                 440                 445
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<210> SEQ ID NO 7
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial

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<220> FEATURE:
<223> OTHER INFORMATION: HindIII containing primer

<400> SEQUENCE: 7

aagcttaact attatgatcc cacagag 27

<210> SEQ ID NO 8
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BamHI containing primer

<400> SEQUENCE: 8

ggatccggcg gtgttgaacg tagc 24

<210> SEQ ID NO 9
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer C1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: primer C1

<400> SEQUENCE: 9

gtacatattg tcgttagaac gcgtaatacg actca 35

<210> SEQ ID NO 10
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40-i5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40-i5

<400> SEQUENCE: 10

aggtgcatga tcggaccata cttc 24

<210> SEQ ID NO 11
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer C2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer C2

<400> SEQUENCE: 11

cgttagaacg cgtaatacga ctcactatag ggaga 35

<210> SEQ ID NO 12
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40-i7
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40-i7

<400> SEQUENCE: 12

gaccatactt cttagcgagt ttggc 25

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<210> SEQ ID NO 13
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40pro-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40pro-F

<400> SEQUENCE: 13

actcaaacaa gcatctcgcc atagg 25

<210> SEQ ID NO 14
<211> LENGTH: 2369
<212> TYPE: DNA
<213> ORGANISM: Viola x wittrockiana
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Plasmid pSFL614

<400> SEQUENCE: 14

actcaaacaa gcatctcgcc aatggttctc taaattttct tctactctca tctcacgtgg 60

tttccgccaa tctgtctctg attacagcct tttcacatat gtcaaaggtt cagttagtgt 120

ttttgtcctt gtttatgtcg acgatataat cgttactggc aacaatctag atgccatttc 180

tgagactaaa caattcctcg caaattcatt ctctattaaa gatctcggca ctcttcgata 240

ttttcttgga atcgaagtat ctcgttctac gaaaggtatt ttcttatgtc aacgaaaata 300

cactctcgat attctctcag attctggtca ccttggatgt cgaccttctc catttcccat 360

ggagcaacat cttcatctac ttcctgatga tggtacacca ctacccgacc catccattta 420

tcgacgtctg gttggtcgac tactttactt gactgtcact cgtcctgata ttcaatatgc 480

agtgaatact cttagtcaat tcatgcaact tcctcgttcg acccatctcg atgcggcaaa 540

tcgagttctc cgatatctca aaggatcagt tggtaaagga atcctccttt cggccactag 600

tcctctttca cttgttggtt ttgctgattc tgactgggct ggttgtccaa ctactcgtcg 660

ttcaactact ggctacatta ccatgcttgg ttcaagtcct atctcttgga aaactaaaaa 720

gcaacccact gtctctcgat cttctgccga agccgaatat cgatcactcg ctgctctcac 780

ttcagagata cagtggcttc attatctact ctcggatctc ggttttcccc ctcaacaacc 840

gattaccgtt cattgtgaca accaagctgc tatacacatc gctaataatc cggttttcca 900

tgaacgaaca aagcacattg agctcgattg tcactttgtt cgtgaaaaaa ttatttctgg 960

tctcgtctcc accagttatt tgcgttcctc agatcaactt gctgatattt tcacaaaacc 1020

acttggtgca gatgcattta atcaccttat ttccaagttg ggcgtgatcg acatctctct 1080

cccggctcca acttgacggg gggtgttaaa cgtatacaag attttctaat cttgtatatt 1140

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tccttagtat caggaaagtt agttgtagat attattttat atttcaaatc tgtatctaat 1260

acttgcctat ataaaggcca actaatcaat gaaatgaaca catcaatttt ctcaatttct 1320

cattctctgt tttcatatct attctctatt ttcacatttt ctgaaaagaa agatgcttga 1380

catgatcaga gacagttctt tcttcttcat actttcgtac taaacttctc ctggtccgca 1440

actaatcttc catcattttc ttgtgatctt cacttgagga tagtctctag aaaacggcac 1500

ggtcacgctg gataagtgtt taggatccct cgaagttgag ttgcatgaat tttgcgggta 1560

cgcaagtgac ttgactctta tcttggacgt cttatatgct cgaccaaatg ttggccaagt 1620

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cgggatgctc gggttaagcc tctcttaggt caagtttatg agcgaacccc tttctttgag 1680

ggctctttat ttgccaactc gtctgccatt aaagttctat tagagctcta atgctgtgta 1740

tgtggctacc gatcaccttc attctcagag gaatcctctt ttcgaatttc tggtactttg 1800

aaactagctg cttcaatttc agccactcga attaaacact aaaacagaac attgagagga 1860

acgggccctc ttccaaatat agaaagaaac agataatgtc aaaagacaca tcaactaggt 1920

cgagatacct gctcacatgc atcacatcta accaactcga gtcggacgag aaatgagttc 1980

gtaactcgat gataataagg caaaggtcta aaaccacatt cggttggtgg ttgtgttcat 2040

ggaccgatca cgtgccctaa cctaaccccc gcatccatcc accaacagct agtcctcgcc 2100

gagtccccca aagttcctat ttatatcact aaagtccctt tttctcaaca tagacatgca 2160

aacacgagac aacatggcaa ttctagtcac cgacttcgtt gtcgcggcta taattttctt 2220

gatcactcgg ttcttagttc gttctctttt caagaaacca acccgaccgc tccccccggg 2280

tcctctcggt tggcccttgg tgggcgccct ccctctccta ggcgccatgc ctcacgtcgc 2340

actagccaaa ctcgctaaga agtatggtc 2369

<210> SEQ ID NO 15
<211> LENGTH: 1102
<212> TYPE: DNA
<213> ORGANISM: Viola x wittrockiana
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: BP40pro
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(6)
<223> OTHER INFORMATION: HindIII
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1097)..(1102)
<223> OTHER INFORMATION: BamHI

<400> SEQUENCE: 15

aagcttgtga tcgacatctc tctcccggct ccaacttgac ggggggtgtt aaacgtatac 60

aagattttct aatcttgtat atttgatttt ctaatatctt gtatatttga ttttctatta 120

tcttgtattt gaacttttgt atttccttag tatcaggaaa gttagttgta gatattattt 180

tatatttcaa atctgtatct aatacttgcc tatataaagg ccaactaatc aatgaaatga 240

acacatcaat tttctcaatt tctcattctc tgttttcata tctattctct attttcacat 300

tttctgaaaa gaaagatgct tgacatgatc agagacagtt ctttcttctt catactttcg 360

tactaaactt ctcctggtcc gcaactaatc ttccatcatt ttcttgtgat cttcacttga 420

ggatagtctc tagaaaacgg cacggtcacg ctggataagt gtttagctag cctcgaagtt 480

gagttgcatg aattttgcgg gtacgcaagt gacttgactc ttatcttgga cgtcttatat 540

gctcgaccaa atgttggcca agtcgggatg ctcgggttaa gcctctctta ggtcaagttt 600

atgagcgaac ccctttcttt gagggctctt tatttgccaa ctcgtctgcc attaaagttc 660

tattagagct ctaatgctgt gtatgtggct accgatcacc ttcattctca gaggaatcct 720

cttttcgaat ttctggtact ttgaaactag ctgcttcaat ttcagccact cgaattaaac 780

actaaaacag aacattgaga ggaacgggcc ctcttccaaa tatagaaaga aacagataat 840

gtcaaaagac acatcaacta ggtcgagata cctgctcaca tgcatcacat ctaaccaact 900

cgagtcggac gagaaatgag ttcgtaactc gatgataata aggcaaaggt ctaaaaccac 960

attcggttgg tggttgtgtt catggaccga tcacgtgccc taacctaacc cccgcatcca 1020

tccaccaaca gctagtcctc gccgagtccc ccaaagttcc tatttatatc actaaagtcc 1080

ctttttctca acatagggat cc 1102

<210> SEQ ID NO 16
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40pro-HindIII-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40pro-HindIII-F

<400> SEQUENCE: 16

aagcttgtga tcgacatctc tctcc 25

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40pro-NheI-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40pro-NheI-R

<400> SEQUENCE: 17

cgaggctagc taaacactta t 21

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40pro-NheI-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: BPpro-NheI-F

<400> SEQUENCE: 18

tttagctagc ctcgaagttg 20

<210> SEQ ID NO 19
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP40pro-BamHI-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Primer BP40pro-BamHI-R

<400> SEQUENCE: 19

ggatccctat gttgagaaaa agggact 27

<210> SEQ ID NO 20
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: DFRproHindIIIF

<400> SEQUENCE: 20

taataagctt acagtgtaat tatc 24

<210> SEQ ID NO 21

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: DFRproNheIR

<400> SEQUENCE: 21

ttatgctagc gtgtcaagac cac 23

<210> SEQ ID NO 22
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: DFRproNheIF

<400> SEQUENCE: 22

acacgctagc ataagtctgt tg 22

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: DFRproBamHI-R

<400> SEQUENCE: 23

gcttggggat ccatcttagg 20

<210> SEQ ID NO 24
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer RrF3H-F

<400> SEQUENCE: 24

aagcttctag ttagacaaaa agcta 25

<210> SEQ ID NO 25
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer RrF3H-R

<400> SEQUENCE: 25

ggatcctctc ttgatatttc cgttc 25

<210> SEQ ID NO 26
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-BP40Fd

<400> SEQUENCE: 26

caagaaaaat aaatggcaat tctagtcacc gac 33

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NcoI-BP40-Rv

<400> SEQUENCE: 27

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ctcgagcgta cgtgagcatc 20

<210> SEQ ID NO 28
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BamHI-ADH-Fd

<400> SEQUENCE: 28

cgcggatccg tctatttaac tcagtattc 29

<210> SEQ ID NO 29
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BP40-ADH-Rv

<400> SEQUENCE: 29

tagaattgcc atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 30
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BamHI-ADH-Fd

<400> SEQUENCE: 30

cgcggatccg tctatttaac tcagtattc 29

<210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NcoI-BP40Rv

<400> SEQUENCE: 31

ctcgagcgta cgtgagcatc 20

<210> SEQ ID NO 32
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH KpnI Forward

<400> SEQUENCE: 32

cggtaccgtc tatttaactc agtattc 27

<210> SEQ ID NO 33
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: GUS19R

<400> SEQUENCE: 33

tttctacagg acgtaacata aggga 25

<210> SEQ ID NO 34
<211> LENGTH: 1047
<212> TYPE: DNA

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<213> ORGANISM: Chrysanthemum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Flavanone 3-hydroxylase (F3H) promoter
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (1)..(23)
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (1023)..(1047)

<400> SEQUENCE: 34

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taaagagggt aggcgtctag ggtttcggta ttcctttcca ttatcctttt cattcatcct 180

ttcatttcat agtattcatc tctaatgaga gtctagacac acgatcatag cgtgtgtata 240

atagttgtag tagtttttt gttttaatta ataaagaaaa ccttattatt agtgatgttg 300

attgtgtttt taatcattcc gctgttttca atcaattgat atcactcata ccctagttga 360

gtcccgatct tgttttcaac aattggtttc agagcctcgt ggctctcgat ctagggttta 420

taagattttc atgtaattag ggtttatact ctaattcatc tattgcagca gatttgaaaa 480

gaaaagaggc agcagatggg gaattgatca catggctact gttcgaacct acaaaggaat 540

atcaatacga gggctcaatt attgtctcgg attcaatgaa ttcacaaggt aaataaacgc 600

ggtactcttt tcattggtcc ttcgttttat ttgtttgaca attaattggg atggctggcg 660

tgtataattc tcaatacatg tctgatttaa tatgtgattg gttgacattc atgtgaaatt 720

aatatactca ttttatgatt acaaagaccc acgatgtata attaattcca atcttgtgga 780

atgggatcca ttgtgaaccg gtgcatgatt gttacggtgg ggattacttt tgattggttc 840

agcattatca tataaccccc gttcaacgga tgcatgctac attggtacgt atacatatac 900

gattcacgtg tggtagttga taactagcgc gatacgcccc caccccatat ttcttcaatt 960

ttctctacaa atacccatgc caaccttacg aaacactcat tcccctctac tcatagacgc 1020

accaagtgtg tgaagaaaaa ataaaaa 1047

<210> SEQ ID NO 35
<211> LENGTH: 2346
<212> TYPE: DNA
<213> ORGANISM: Chrysanthemum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: pBluescript SK-F3H9 which contains Flavanone
3-hydroxylase (F3H) promoter
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (1092)..(1114)
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (2114)..(2138)

<400> SEQUENCE: 35

aagctttcgg gggatgaatt cttcgctacc acgtaattcc ccatggcttt gagtctaact 60

taaagactcg gtaataaaga aaagtgctct ttcgggagac aacccgaatt atccttttta 120

tttcttttat ttcacttttt gtttggtttt gtgtttttaa cattttgcag gaattggaga 180

agaattcaca agtgactaaa acggggacct gttctgtcca acagttacgg cgtaactcat 240

cctcatggtt acgccgtaac atatcctcag tagcgattct ctccaataca taaaccatta 300

cggcgtaatc ccattctatg gttacgccgt agctattctc cagtagcaaa ttgccagttt 360

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atttttccta tattaacaca ataaccttgt attagttttt aaatgaactt tgcgggtatg 480

ttccatgtaa gccctcatga gactacactc gtccacttgg gacaccaagt ggtttaaaat 540

gcttgttgca tatgctaaat gcaaccgtga ttcctacgaa agtgagttag atttcttttt 600

gtttttgttt ttatttttct ttttagaatt atgcttgttg gttagtgtga tatcagggaa 660

tgaagtttgc tcgtggatgc ttaagcaaag gcacgattct cttcgtaggc cttctttctt 720

tttaagagca aatttcaggg aagttctcgc tctaattcta ctttctcttc acctttattt 780

aacgtttagt acaaaaggga ctttgtacat cttaagtggg ggggacggga gtagaattat 840

tacttgaact taattgccct cgtttttcta gtttattttg aaaaattatg ccatttttaa 900

aattttggca tgtttttctt aagctaacta gattagacct tagccgagca ctttataacc 960

cttgatattt tatggtgaga ttagctttat ccgtttctaa ttatttaccc aaatccacta 1020

aattattaga gtgtcggtag cttgtaaact ttagaacttg gtctttgtgt tgggaattgt 1080

cgagttgaag attacaaaac catgtgcaag aatgaagaaa gaagaaacaa tgagggtcta 1140

atatgtaata gttagcttag cttttctagt aagctaaatt tagggttttt atgtaacctc 1200

cctctcttat ataaagaggg taggcgtcta gggtttcggt attcctttcc attatccttt 1260

tcattcatcc tttcatttca tagtattcat ctctaatgag agtctagaca cacgatcata 1320

gcgtgtgtat aatagttgta gtagtttttt tgttttaatt aataaagaaa accttattat 1380

tagtgatgtt gattgtgttt ttaatcattc cgctgttttc aatcaattga tatcactcat 1440

accctagttg agtcccgatc ttgttttcaa caattggttt cagagcctcg tggctctcga 1500

tctagggttt ataagatttt catgtaatta gggtttatac tctaattcat ctattgcagc 1560

agatttgaaa agaaaagagg cagcagatgg ggaattgatc acatggctac tgttcgaacc 1620

tacaaaggaa tatcaatacg agggctcaat tattgtctcg gattcaatga attcacaagg 1680

taaataaacg cggtactctt ttcattggtc cttcgtttta tttgtttgac aattaattgg 1740

gatggctggc gtgtataatt ctcaatacat gtctgattta atatgtgatt ggttgacatt 1800

catgtgaaat taatatactc attttatgat tacaaagacc cacgatgtat aattaattcc 1860

aatcttgtgg aatgggatcc attgtgaacc ggtgcatgat tgttacggtg gggattactt 1920

ttgattggtt cagcattatc atataacccc cgttcaacgg atgcatgcta cattggtacg 1980

tatacatata cgattcacgt gtggtagttg ataactagcg cgatacgccc ccaccccata 2040

tttcttcaat tttctctaca aatacccatg ccaaccttac gaaacactca ttcccctcta 2100

ctcatagacg caccaagtgt gtgaagaaaa aataaaaaat ggcacctata tccttgaaat 2160

gggacgataa ttcgctgcat gaaaaccggt tcgtccgtga tgaggacgag cggcctaagg 2220

tgccatacaa caagtttacc aacgagattc ccgttatctc acttaaggga attgacgatg 2280

tggaagagag tagcggtggt atcaaatcac gtagggccga gatttgtgag aagataataa 2340

aagctt 2346

<210> SEQ ID NO 36
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HANS-F3Hpro1k-Fd

<400> SEQUENCE: 36

ccaagcttgg cgcgccgcgg ccgcatttaa atttacaaaa ccatgtgcaa gaatg 55

<210> SEQ ID NO 37
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: SNM-F3Hpro-Rv

<400> SEQUENCE: 37

actagtgcta gcacgcgttt tttatttttt cttcacacac ttg 43

<210> SEQ ID NO 38
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NSM-F3Hpro-Rv

<400> SEQUENCE: 38

gctagcacta gtacgcgttt tttatttttt cttcacacac ttg 43

<210> SEQ ID NO 39
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BclI-CmF3Hp-Rv

<400> SEQUENCE: 39

ttttgatcat tttttatttt ttcttcacac agtg 34

<210> SEQ ID NO 40
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-EgF3'5'H-Fd

<400> SEQUENCE: 40

caagaaaaat aaatggctgt tggaaatggc gtt 33

<210> SEQ ID NO 41
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-EgF3'5'H-Rv

<400> SEQUENCE: 41

gttaacgctg agcctagtgc c 21

<210> SEQ ID NO 42
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: XbaI-ADH-Fd

<400> SEQUENCE: 42

acgcgttcta gagtctattt aactcagtat tc 32

<210> SEQ ID NO 43
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:

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<223> OTHER INFORMATION: EgF3'5'H-ADH-Rv

<400> SEQUENCE: 43

tccaacagcc atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-EgF3'5'H-Rv

<400> SEQUENCE: 44

gttaacgctg agcctagtgc c 21

<210> SEQ ID NO 45
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-LeF3'5'H-Fd

<400> SEQUENCE: 45

caagaaaaat aaatggacgc gacawacatt gc 32

<210> SEQ ID NO 46
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-LeF3'5'H-Rv

<400> SEQUENCE: 46

gttaacatct cgggcagcac c 21

<210> SEQ ID NO 47
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: LeF3'5'H-ADH-Rv

<400> SEQUENCE: 47

tgtcgcgtcc atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-LeF3'5'H-Rv

<400> SEQUENCE: 48

gttaacatct cgggcagcac c 21

<210> SEQ ID NO 49
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: CamF1

<400> SEQUENCE: 49

gtgaagccac catgtctata g 21

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<210> SEQ ID NO 50
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: CamR1

<400> SEQUENCE: 50

gcatttgcct agacagtgta ag 22

<210> SEQ ID NO 51
<211> LENGTH: 1585
<212> TYPE: DNA
<213> ORGANISM: Campanula medium
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Cline #4 pSPB2561
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(1577)

<400> SEQUENCE: 51

```
gtgaagccac c atg tct ata gac ata acc att ctc tta tgt gaa ctt gtt      50
             Met Ser Ile Asp Ile Thr Ile Leu Leu Cys Glu Leu Val
             1               5                   10

gct gca att tca ctc tac tta tta acc tac tat ttc att tgt ttc ctc       98
Ala Ala Ile Ser Leu Tyr Leu Leu Thr Tyr Tyr Phe Ile Cys Phe Leu
    15                  20                  25

ttc aaa ccc tct cat cat cac cac cac ctc cct ccc ggc cca acc gga      146
Phe Lys Pro Ser His His His His His Leu Pro Pro Gly Pro Thr Gly
30                  35                  40                  45

tgg ccg atc att gga tcc ctt cct ctc tta ggc act atg cca cat gtt      194
Trp Pro Ile Ile Gly Ser Leu Pro Leu Leu Gly Thr Met Pro His Val
                50                  55                  60

tcc tta gcc gac atg gcc gta aaa tac ggg cct ata atg tac cta aaa      242
Ser Leu Ala Asp Met Ala Val Lys Tyr Gly Pro Ile Met Tyr Leu Lys
            65                  70                  75

ctt ggt tca aag ggc acc gtc gtg gcc tca aat cca aaa gcc gcc cga      290
Leu Gly Ser Lys Gly Thr Val Val Ala Ser Asn Pro Lys Ala Ala Arg
        80                  85                  90

gca ttc ttg aaa tcc cat gat gcc aat ttt tct aac cgt ccg att gat      338
Ala Phe Leu Lys Ser His Asp Ala Asn Phe Ser Asn Arg Pro Ile Asp
    95                  100                 105

ggg ggg ccc acc tac ctc gcg tat aat gca caa gac atg gtt ttt gca      386
Gly Gly Pro Thr Tyr Leu Ala Tyr Asn Ala Gln Asp Met Val Phe Ala
110                 115                 120                 125

gaa tat ggc cca aaa tgg aag ctt ttg cga aag cta tgt agc ttg cac      434
Glu Tyr Gly Pro Lys Trp Lys Leu Leu Arg Lys Leu Cys Ser Leu His
                130                 135                 140

atg tta ggc ccg aag gca ctc gag gat tgg gct cat gtc aga gtt tca      482
Met Leu Gly Pro Lys Ala Leu Glu Asp Trp Ala His Val Arg Val Ser
            145                 150                 155

gag gtc ggt cat atg ctc aaa gaa atg tac gag caa tcg agt aag tcc      530
Glu Val Gly His Met Leu Lys Glu Met Tyr Glu Gln Ser Ser Lys Ser
        160                 165                 170

gtg cca gtg gtg gtg cca gag atg tta act tat gcc atg gct aat atg      578
Val Pro Val Val Val Pro Glu Met Leu Thr Tyr Ala Met Ala Asn Met
    175                 180                 185

att gga cga atc ata ctc agt cga cgc cct ttt gtt atc acg agc aaa      626
Ile Gly Arg Ile Ile Leu Ser Arg Arg Pro Phe Val Ile Thr Ser Lys
190                 195                 200                 205

tta gac tcg tct gct tct gct gct tct gtt agt gaa ttc caa tat atg      674
Leu Asp Ser Ser Ala Ser Ala Ala Ser Val Ser Glu Phe Gln Tyr Met
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                210                 215                 220

gtt atg gag ctc atg agg atg gca ggg ttg ttc aat att ggt gat ttc      722
Val Met Glu Leu Met Arg Met Ala Gly Leu Phe Asn Ile Gly Asp Phe
            225                 230                 235

ata cca tat att gcg tgg atg gat ttg caa ggc att caa cgc gat atg      770
Ile Pro Tyr Ile Ala Trp Met Asp Leu Gln Gly Ile Gln Arg Asp Met
        240                 245                 250

aag gtt ata cag caa aag ttt gat gtc ttg ttg aac aaa atg atc aag      818
Lys Val Ile Gln Gln Lys Phe Asp Val Leu Leu Asn Lys Met Ile Lys
    255                 260                 265

gaa cat aca gaa tcc gct cat gat cgt aaa gat aat cct gat ttt ctt      866
Glu His Thr Glu Ser Ala His Asp Arg Lys Asp Asn Pro Asp Phe Leu
270                 275                 280                 285

gat att ctt atg gcg gct acc caa gaa aac acg gag gga att cag ctt      914
Asp Ile Leu Met Ala Ala Thr Gln Glu Asn Thr Glu Gly Ile Gln Leu
                290                 295                 300

aat ctc gta aat gtt aag gcg ctt ctt ttg gat tta ttc acg gcg ggc      962
Asn Leu Val Asn Val Lys Ala Leu Leu Leu Asp Leu Phe Thr Ala Gly
            305                 310                 315

acg gat aca tcg tcg agt gtg atc gaa tgg gca cta gcc gaa atg ttg     1010
Thr Asp Thr Ser Ser Ser Val Ile Glu Trp Ala Leu Ala Glu Met Leu
        320                 325                 330

aac aat cga cag atc cta aac cgg gcc cac gaa gaa atg gac caa gtc     1058
Asn Asn Arg Gln Ile Leu Asn Arg Ala His Glu Glu Met Asp Gln Val
    335                 340                 345

att ggc aga aac aga aga cta gaa caa tct gac ata cca aac ttg cca     1106
Ile Gly Arg Asn Arg Arg Leu Glu Gln Ser Asp Ile Pro Asn Leu Pro
350                 355                 360                 365

tat ttc caa gcc ata tgc aaa gaa aca ttc cga aaa cac cct tcc acg     1154
Tyr Phe Gln Ala Ile Cys Lys Glu Thr Phe Arg Lys His Pro Ser Thr
                370                 375                 380

ccc tta aac ctc cca aga atc tca aca gaa gaa tgt gaa gtc gaa gga     1202
Pro Leu Asn Leu Pro Arg Ile Ser Thr Glu Glu Cys Glu Val Glu Gly
            385                 390                 395

ttt cgc ata ccc aaa aac act aga cta ata gtg aac ata tgg gca ata     1250
Phe Arg Ile Pro Lys Asn Thr Arg Leu Ile Val Asn Ile Trp Ala Ile
        400                 405                 410

ggg aga gac cct aaa gtg tgg gaa aat cca ttg gat ttt acc ccg gaa     1298
Gly Arg Asp Pro Lys Val Trp Glu Asn Pro Leu Asp Phe Thr Pro Glu
    415                 420                 425

cga ttc ttg agt gaa aaa cac gcg aaa att gat ccg cga ggt aat cat     1346
Arg Phe Leu Ser Glu Lys His Ala Lys Ile Asp Pro Arg Gly Asn His
430                 435                 440                 445

ttt gag tta atc cca ttt ggg gcg gga cgg agg ata tgt gca ggg gct     1394
Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Ala
                450                 455                 460

aga atg gga gcg gcc tcg gtc gag tac att tta ggt aca ttg gtg cac     1442
Arg Met Gly Ala Ala Ser Val Glu Tyr Ile Leu Gly Thr Leu Val His
            465                 470                 475

tca ttt gat tgg aaa ttg cct gat gga gtt gtg gaa gtt aat atg gaa     1490
Ser Phe Asp Trp Lys Leu Pro Asp Gly Val Val Glu Val Asn Met Glu
        480                 485                 490

gag agc ttt ggg ata gca ttg cag aaa aag atg cct ctt tct gct att     1538
Glu Ser Phe Gly Ile Ala Leu Gln Lys Lys Met Pro Leu Ser Ala Ile
    495                 500                 505

gtt act cca aga ttg cct cca agt gct tac act gtc tag gcaaatgc        1585
Val Thr Pro Arg Leu Pro Pro Ser Ala Tyr Thr Val
510                 515                 520
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<210> SEQ ID NO 52
<211> LENGTH: 521
<212> TYPE: PRT
<213> ORGANISM: Campanula medium

<400> SEQUENCE: 52

Met Ser Ile Asp Ile Thr Ile Leu Leu Cys Glu Leu Val Ala Ala Ile
1 5 10 15

Ser Leu Tyr Leu Leu Thr Tyr Tyr Phe Ile Cys Phe Leu Phe Lys Pro
20 25 30

Ser His His His His His Leu Pro Pro Gly Pro Thr Gly Trp Pro Ile
35 40 45

Ile Gly Ser Leu Pro Leu Leu Gly Thr Met Pro His Val Ser Leu Ala
50 55 60

Asp Met Ala Val Lys Tyr Gly Pro Ile Met Tyr Leu Lys Leu Gly Ser
65 70 75 80

Lys Gly Thr Val Val Ala Ser Asn Pro Lys Ala Ala Arg Ala Phe Leu
85 90 95

Lys Ser His Asp Ala Asn Phe Ser Asn Arg Pro Ile Asp Gly Gly Pro
100 105 110

Thr Tyr Leu Ala Tyr Asn Ala Gln Asp Met Val Phe Ala Glu Tyr Gly
115 120 125

Pro Lys Trp Lys Leu Leu Arg Lys Leu Cys Ser Leu His Met Leu Gly
130 135 140

Pro Lys Ala Leu Glu Asp Trp Ala His Val Arg Val Ser Glu Val Gly
145 150 155 160

His Met Leu Lys Glu Met Tyr Glu Gln Ser Ser Lys Ser Val Pro Val
165 170 175

Val Val Pro Glu Met Leu Thr Tyr Ala Met Ala Asn Met Ile Gly Arg
180 185 190

Ile Ile Leu Ser Arg Arg Pro Phe Val Ile Thr Ser Lys Leu Asp Ser
195 200 205

Ser Ala Ser Ala Ala Ser Val Ser Glu Phe Gln Tyr Met Val Met Glu
210 215 220

Leu Met Arg Met Ala Gly Leu Phe Asn Ile Gly Asp Phe Ile Pro Tyr
225 230 235 240

Ile Ala Trp Met Asp Leu Gln Gly Ile Gln Arg Asp Met Lys Val Ile
245 250 255

Gln Gln Lys Phe Asp Val Leu Leu Asn Lys Met Ile Lys Glu His Thr
260 265 270

Glu Ser Ala His Asp Arg Lys Asp Asn Pro Asp Phe Leu Asp Ile Leu
275 280 285

Met Ala Ala Thr Gln Glu Asn Thr Glu Gly Ile Gln Leu Asn Leu Val
290 295 300

Asn Val Lys Ala Leu Leu Leu Asp Leu Phe Thr Ala Gly Thr Asp Thr
305 310 315 320

Ser Ser Ser Val Ile Glu Trp Ala Leu Ala Glu Met Leu Asn Asn Arg
325 330 335

Gln Ile Leu Asn Arg Ala His Glu Glu Met Asp Gln Val Ile Gly Arg
340 345 350

Asn Arg Arg Leu Glu Gln Ser Asp Ile Pro Asn Leu Pro Tyr Phe Gln
355 360 365

Ala Ile Cys Lys Glu Thr Phe Arg Lys His Pro Ser Thr Pro Leu Asn
370 375 380

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Leu Pro Arg Ile Ser Thr Glu Glu Cys Glu Val Glu Gly Phe Arg Ile
385                 390                 395                 400

Pro Lys Asn Thr Arg Leu Ile Val Asn Ile Trp Ala Ile Gly Arg Asp
                405                 410                 415

Pro Lys Val Trp Glu Asn Pro Leu Asp Phe Thr Pro Glu Arg Phe Leu
            420                 425                 430

Ser Glu Lys His Ala Lys Ile Asp Pro Arg Gly Asn His Phe Glu Leu
        435                 440                 445

Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Ala Arg Met Gly
    450                 455                 460

Ala Ala Ser Val Glu Tyr Ile Leu Gly Thr Leu Val His Ser Phe Asp
465                 470                 475                 480

Trp Lys Leu Pro Asp Gly Val Val Glu Val Asn Met Glu Glu Ser Phe
                485                 490                 495

Gly Ile Ala Leu Gln Lys Lys Met Pro Leu Ser Ala Ile Val Thr Pro
            500                 505                 510

Arg Leu Pro Pro Ser Ala Tyr Thr Val
        515                 520


<210> SEQ ID NO 53
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-Campa-Fd

<400> SEQUENCE: 53

caagaaaaat aaatgtctat agacataacc attc                                  34


<210> SEQ ID NO 54
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-Campa-Rv

<400> SEQUENCE: 54

gttaacatct ctggcaccac c                                                21


<210> SEQ ID NO 55
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Campa-ADH-Rv

<400> SEQUENCE: 55

gtctatagac atttatttt cttgatttcc ttcac                                  35


<210> SEQ ID NO 56
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: HpaI-Campa-Rv

<400> SEQUENCE: 56

gttaacatct ctggcaccac c                                                21


<210> SEQ ID NO 57
<211> LENGTH: 33
<212> TYPE: DNA
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-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-ScF3'5'H-Fd

<400> SEQUENCE: 57

caagaaaaat aaatgagcat tctaacccta atc 33

<210> SEQ ID NO 58
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NdeI-ScF3'5'H-Rv

<400> SEQUENCE: 58

catatgttta gctccagaat ttgg 24

<210> SEQ ID NO 59
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ScF3'5'H-ADH-Rv

<400> SEQUENCE: 59

tagaatgctc atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 60
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NdeI-ScF3'5'H-Rv

<400> SEQUENCE: 60

catatgttta gctccagaat ttgg 24

<210> SEQ ID NO 61
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-Gentian-Fd

<400> SEQUENCE: 61

caagaaaaat aaatgtcacc catttacacc accc 34

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: SalI-GentianF3'5'H-Rv

<400> SEQUENCE: 62

gtcgacgcta ttgctaagcc 20

<210> SEQ ID NO 63
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Gentian-ADH-Rv

<400> SEQUENCE: 63

aatgggtgac atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: SalI-GentianF3'5'H-Rv

<400> SEQUENCE: 64

gtcgacgcta ttgctaagcc 20

<210> SEQ ID NO 65
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-Verbena-Fd

<400> SEQUENCE: 65

caagaaaaat aaatgacgtt ttcagagctt ataaac 36

<210> SEQ ID NO 66
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NcoI-Verbena-F3'5'H-Rv

<400> SEQUENCE: 66

ccatggagta aatcagcatc tc 22

<210> SEQ ID NO 67
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Verbena-ADH-Rv

<400> SEQUENCE: 67

tgaaaacgtc atttattttt cttgatttcc ttcac 35

<210> SEQ ID NO 68
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NcoI-VerbenaF3'5'H-Rv

<400> SEQUENCE: 68

ccatggagta aatcagcatc tc 22

<210> SEQ ID NO 69
<211> LENGTH: 1755
<212> TYPE: DNA
<213> ORGANISM: Antirrhinum kellogii
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: F3'5'HcDNA#1 pSPB3145
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (73)..(1602)

<400> SEQUENCE: 69

ttcggcacga gggtaccttt agtatgttca atctctagtt ttttattaat cacaactcaa 60

tagataatcg tc atg cag ata ata att ccg gtc ctc ctg aag gag ctc acc 111
Met Gln Ile Ile Ile Pro Val Leu Leu Lys Glu Leu Thr

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             1               5                  10

gta gca gca tta ctc tat gtt ttc act aac att ctc atc cgc tca ctt      159
Val Ala Ala Leu Leu Tyr Val Phe Thr Asn Ile Leu Ile Arg Ser Leu
    15                  20                  25

ctc aca aga ccc tgt cac cgt ctc ccg cca ggg cca aga ggc ttt cca      207
Leu Thr Arg Pro Cys His Arg Leu Pro Pro Gly Pro Arg Gly Phe Pro
30                  35                  40                  45

gta gtc ggc gct ctt cca ctc cta ggc agc atg cca cac gtg gcg ctc      255
Val Val Gly Ala Leu Pro Leu Leu Gly Ser Met Pro His Val Ala Leu
                50                  55                  60

gcc aaa atg tcc aaa act tat ggt ccc gtc ata tac cta aaa gta ggc      303
Ala Lys Met Ser Lys Thr Tyr Gly Pro Val Ile Tyr Leu Lys Val Gly
            65                  70                  75

gca cac ggc atg gca gtg gcc tca act cct gaa tcc gcc aaa gcg ttc      351
Ala His Gly Met Ala Val Ala Ser Thr Pro Glu Ser Ala Lys Ala Phe
        80                  85                  90

ctc aaa acc cta gac acc aac ttc tcc aac cgc ccg cca aat gcc ggt      399
Leu Lys Thr Leu Asp Thr Asn Phe Ser Asn Arg Pro Pro Asn Ala Gly
    95                  100                 105

gcc act cac ctg gct tat aac tca caa gac atg gtg ttt gcc gcc tac      447
Ala Thr His Leu Ala Tyr Asn Ser Gln Asp Met Val Phe Ala Ala Tyr
110                 115                 120                 125

ggc ccg agg tgg aga ttg ctt aga aag ttg agc aat ctc cac atg ttg      495
Gly Pro Arg Trp Arg Leu Leu Arg Lys Leu Ser Asn Leu His Met Leu
                130                 135                 140

ggg act aag gct tta gac gat tgg gca aat gtt agg gtt tcg gag gtt      543
Gly Thr Lys Ala Leu Asp Asp Trp Ala Asn Val Arg Val Ser Glu Val
            145                 150                 155

gga tac atg tta gag gac atg cat ggg gca agt ggc cgc gga gag gcg      591
Gly Tyr Met Leu Glu Asp Met His Gly Ala Ser Gly Arg Gly Glu Ala
        160                 165                 170

gtg ggt gtg ccg ggg atg ttg gtg tac gca atg gct aat atg ata gga      639
Val Gly Val Pro Gly Met Leu Val Tyr Ala Met Ala Asn Met Ile Gly
    175                 180                 185

cag gtg ata ctt agt cgg cgt gtt ttc gtg acg aga gga gaa gaa ttg      687
Gln Val Ile Leu Ser Arg Arg Val Phe Val Thr Arg Gly Glu Glu Leu
190                 195                 200                 205

aac gag ttt aag gat atg gtg gtg gag ctc atg act tcg gct gga tat      735
Asn Glu Phe Lys Asp Met Val Val Glu Leu Met Thr Ser Ala Gly Tyr
                210                 215                 220

ttc aat att ggt gat ttt att ccg tct ttt gct tgg atg gat ttg caa      783
Phe Asn Ile Gly Asp Phe Ile Pro Ser Phe Ala Trp Met Asp Leu Gln
            225                 230                 235

gga ata gag aag gga atg aag ggc ttg cac aaa aag ttt gat gat ttg      831
Gly Ile Glu Lys Gly Met Lys Gly Leu His Lys Lys Phe Asp Asp Leu
        240                 245                 250

atc agt aga atg ttg gag gaa cac ctg gcg tca gct cat atc cga aag      879
Ile Ser Arg Met Leu Glu Glu His Leu Ala Ser Ala His Ile Arg Lys
    255                 260                 265

gag aaa cct gat ttt ctt gat gtc att ttg gct aat cgt gat act ttg      927
Glu Lys Pro Asp Phe Leu Asp Val Ile Leu Ala Asn Arg Asp Thr Leu
270                 275                 280                 285

gag gga gag agg ctt acc act tct aac atc aag gct ctt tta ctg aac      975
Glu Gly Glu Arg Leu Thr Thr Ser Asn Ile Lys Ala Leu Leu Leu Asn
                290                 295                 300

ttg ttc acc gcc ggt acg gat aca tct tcg agc aca ata gag tgg gcg     1023
Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Ile Glu Trp Ala
            305                 310                 315

ctg gcg gag atg ata aaa aac ccg gcg atc ctc aag aaa gca cac gat     1071
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Leu Ala Glu Met Ile Lys Asn Pro Ala Ile Leu Lys Lys Ala His Asp
        320                 325                 330

gaa atg gat caa gtc gta ggc cgg aat cga cgt tta atg gag tcg gac     1119
Glu Met Asp Gln Val Val Gly Arg Asn Arg Arg Leu Met Glu Ser Asp
    335                 340                 345

ata ccc aaa ctt cca tac cta caa gcg ata tgc aag gaa tca ttt cgt     1167
Ile Pro Lys Leu Pro Tyr Leu Gln Ala Ile Cys Lys Glu Ser Phe Arg
350                 355                 360                 365

aag cac cct tcc act cct tta aat ctg ccc cga atc tct tca caa gca     1215
Lys His Pro Ser Thr Pro Leu Asn Leu Pro Arg Ile Ser Ser Gln Ala
                370                 375                 380

tgc acg gtg aac ggt tac tac ata ccg aag aac acg agg ctc aac gtc     1263
Cys Thr Val Asn Gly Tyr Tyr Ile Pro Lys Asn Thr Arg Leu Asn Val
            385                 390                 395

aac ata tgg gcg atc gga agg gat ccc aac gtg tgg gag aat ccc ctg     1311
Asn Ile Trp Ala Ile Gly Arg Asp Pro Asn Val Trp Glu Asn Pro Leu
        400                 405                 410

gaa ttc aac ccc gac agg ttc atg tcc ggt aag aat gca aag ctc gat     1359
Glu Phe Asn Pro Asp Arg Phe Met Ser Gly Lys Asn Ala Lys Leu Asp
    415                 420                 425

ccg aga gga aat gat ttt gaa ctc att ccg ttc ggg gct ggt cga agg     1407
Pro Arg Gly Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg
430                 435                 440                 445

att tgt gcg gga gcg agg atg ggg ata gtt ctt gtg gaa tat ata ttg     1455
Ile Cys Ala Gly Ala Arg Met Gly Ile Val Leu Val Glu Tyr Ile Leu
                450                 455                 460

gga agt ttg gtg cat tct ttt gat tgg aaa ttg ccc gaa gga gtg aag     1503
Gly Ser Leu Val His Ser Phe Asp Trp Lys Leu Pro Glu Gly Val Lys
            465                 470                 475

gag atg aat ttg gat gag gct ttt ggg ctt gct ttg caa aaa gct gtt     1551
Glu Met Asn Leu Asp Glu Ala Phe Gly Leu Ala Leu Gln Lys Ala Val
        480                 485                 490

cct ctt gca gca atg gtt act ccg agg ttg cct tca aat tgt tat gct     1599
Pro Leu Ala Ala Met Val Thr Pro Arg Leu Pro Ser Asn Cys Tyr Ala
    495                 500                 505

cct taagtaatag tatttaagtg cgtcggaata tcgaagtcta tatgattttc          1652
Pro
510

ttgtgcttgt ttctatccac tatgttgtaa gaattcatct ccgatcctct ggtggtcatg   1712

gctatatatc gtaattcttt ttcgaaaaaa aaaaaaaaaa aaa                     1755


<210> SEQ ID NO 70
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Antirrhinum kellogii

<400> SEQUENCE: 70

Met Gln Ile Ile Ile Pro Val Leu Leu Lys Glu Leu Thr Val Ala Ala
1               5                   10                  15

Leu Leu Tyr Val Phe Thr Asn Ile Leu Ile Arg Ser Leu Leu Thr Arg
            20                  25                  30

Pro Cys His Arg Leu Pro Pro Gly Pro Arg Gly Phe Pro Val Val Gly
        35                  40                  45

Ala Leu Pro Leu Leu Gly Ser Met Pro His Val Ala Leu Ala Lys Met
    50                  55                  60

Ser Lys Thr Tyr Gly Pro Val Ile Tyr Leu Lys Val Gly Ala His Gly
65                  70                  75                  80

Met Ala Val Ala Ser Thr Pro Glu Ser Ala Lys Ala Phe Leu Lys Thr
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                85                  90                  95

Leu Asp Thr Asn Phe Ser Asn Arg Pro Pro Asn Ala Gly Ala Thr His
            100                 105                 110

Leu Ala Tyr Asn Ser Gln Asp Met Val Phe Ala Ala Tyr Gly Pro Arg
        115                 120                 125

Trp Arg Leu Leu Arg Lys Leu Ser Asn Leu His Met Leu Gly Thr Lys
    130                 135                 140

Ala Leu Asp Asp Trp Ala Asn Val Arg Val Ser Glu Val Gly Tyr Met
145                 150                 155                 160

Leu Glu Asp Met His Gly Ala Ser Gly Arg Gly Glu Ala Val Gly Val
                165                 170                 175

Pro Gly Met Leu Val Tyr Ala Met Ala Asn Met Ile Gly Gln Val Ile
            180                 185                 190

Leu Ser Arg Arg Val Phe Val Thr Arg Gly Glu Glu Leu Asn Glu Phe
        195                 200                 205

Lys Asp Met Val Val Glu Leu Met Thr Ser Ala Gly Tyr Phe Asn Ile
    210                 215                 220

Gly Asp Phe Ile Pro Ser Phe Ala Trp Met Asp Leu Gln Gly Ile Glu
225                 230                 235                 240

Lys Gly Met Lys Gly Leu His Lys Lys Phe Asp Asp Leu Ile Ser Arg
                245                 250                 255

Met Leu Glu Glu His Leu Ala Ser Ala His Ile Arg Lys Glu Lys Pro
            260                 265                 270

Asp Phe Leu Asp Val Ile Leu Ala Asn Arg Asp Thr Leu Glu Gly Glu
        275                 280                 285

Arg Leu Thr Thr Ser Asn Ile Lys Ala Leu Leu Leu Asn Leu Phe Thr
    290                 295                 300

Ala Gly Thr Asp Thr Ser Ser Ser Thr Ile Glu Trp Ala Leu Ala Glu
305                 310                 315                 320

Met Ile Lys Asn Pro Ala Ile Leu Lys Lys Ala His Asp Glu Met Asp
                325                 330                 335

Gln Val Val Gly Arg Asn Arg Arg Leu Met Glu Ser Asp Ile Pro Lys
            340                 345                 350

Leu Pro Tyr Leu Gln Ala Ile Cys Lys Glu Ser Phe Arg Lys His Pro
        355                 360                 365

Ser Thr Pro Leu Asn Leu Pro Arg Ile Ser Ser Gln Ala Cys Thr Val
    370                 375                 380

Asn Gly Tyr Tyr Ile Pro Lys Asn Thr Arg Leu Asn Val Asn Ile Trp
385                 390                 395                 400

Ala Ile Gly Arg Asp Pro Asn Val Trp Glu Asn Pro Leu Glu Phe Asn
                405                 410                 415

Pro Asp Arg Phe Met Ser Gly Lys Asn Ala Lys Leu Asp Pro Arg Gly
            420                 425                 430

Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala
        435                 440                 445

Gly Ala Arg Met Gly Ile Val Leu Val Glu Tyr Ile Leu Gly Ser Leu
    450                 455                 460

Val His Ser Phe Asp Trp Lys Leu Pro Glu Gly Val Lys Glu Met Asn
465                 470                 475                 480

Leu Asp Glu Ala Phe Gly Leu Ala Leu Gln Lys Ala Val Pro Leu Ala
                485                 490                 495

Ala Met Val Thr Pro Arg Leu Pro Ser Asn Cys Tyr Ala Pro
            500                 505                 510
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<210> SEQ ID NO 71
<211> LENGTH: 1811
<212> TYPE: DNA
<213> ORGANISM: Antirrhinum kellogii
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: F3'5'cDNA#12 pSPB3146
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (86)..(1615)

<400> SEQUENCE: 71

```
gatactaaaa accatccaaa ttaagtacct ttagtatgtt caatctctag ttttttttatt     60

aatcacaact caatagataa tcgtc atg cag ata ata att ccg gtc ctc ctg      112
                           Met Gln Ile Ile Ile Pro Val Leu Leu
                           1               5

aag gag ctc acc gta gca gca tta ctc tat gtt ttc act aac att ctc      160
Lys Glu Leu Thr Val Ala Ala Leu Leu Tyr Val Phe Thr Asn Ile Leu
10                  15                  20                  25

atc cgc tca ctt ctc aca aga ccc cgt cac cgt ctc ccg cca ggg cca      208
Ile Arg Ser Leu Leu Thr Arg Pro Arg His Arg Leu Pro Pro Gly Pro
                30                  35                  40

aga ggc ttt cca gta gtc ggc gct ctt cca ctc cta ggc agc atg cca      256
Arg Gly Phe Pro Val Val Gly Ala Leu Pro Leu Leu Gly Ser Met Pro
            45                  50                  55

cac gtg gcg ctc gcc aaa atg tcc aaa act tat ggt ccc gtc ata tac      304
His Val Ala Leu Ala Lys Met Ser Lys Thr Tyr Gly Pro Val Ile Tyr
        60                  65                  70

cta aaa gta ggc gca cac ggc atg gca gtg gcc tca act cct gaa tcc      352
Leu Lys Val Gly Ala His Gly Met Ala Val Ala Ser Thr Pro Glu Ser
    75                  80                  85

gcc aaa gcg ttc ctc aaa acc cta gac acc aac ttc tcc aac cgc ccg      400
Ala Lys Ala Phe Leu Lys Thr Leu Asp Thr Asn Phe Ser Asn Arg Pro
90                  95                  100                 105

cca aat gcc ggt gcc act cac ctg gct tat aac tca caa gac atg gtg      448
Pro Asn Ala Gly Ala Thr His Leu Ala Tyr Asn Ser Gln Asp Met Val
                110                 115                 120

ttt gcc gcc tac ggc ccg agg tgg aga ttg ctt aga aag ttg agc aat      496
Phe Ala Ala Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys Leu Ser Asn
            125                 130                 135

ctc cac atg ttg ggg act aag gct tta gac gat tgg gca aat gtt agg      544
Leu His Met Leu Gly Thr Lys Ala Leu Asp Asp Trp Ala Asn Val Arg
        140                 145                 150

gtt tcg gag gtt gga tac atg tta gag gac atg cat ggg gca agt ggc      592
Val Ser Glu Val Gly Tyr Met Leu Glu Asp Met His Gly Ala Ser Gly
    155                 160                 165

cgc gga aag gtg gtg ggt gtg ccg ggg atg ttg gtg tac gca atg gct      640
Arg Gly Lys Val Val Gly Val Pro Gly Met Leu Val Tyr Ala Met Ala
170                 175                 180                 185

aat atg ata gga cag gtg ata ctt agt cgg cgt gtt ttc gtg acg aga      688
Asn Met Ile Gly Gln Val Ile Leu Ser Arg Arg Val Phe Val Thr Arg
                190                 195                 200

gaa gaa gaa ttg aac gag ttt aag gat atg gtg gtg gag ctc atg act      736
Glu Glu Glu Leu Asn Glu Phe Lys Asp Met Val Val Glu Leu Met Thr
            205                 210                 215

tcg gct gga tat ttc aat att ggt gat ttt att ccg tct ttt gca tgg      784
Ser Ala Gly Tyr Phe Asn Ile Gly Asp Phe Ile Pro Ser Phe Ala Trp
        220                 225                 230

atg gat ttg caa gga ata gag aag gga atg aag ggt ttg cac aaa aag      832
Met Asp Leu Gln Gly Ile Glu Lys Gly Met Lys Gly Leu His Lys Lys
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    235                 240                 245

ttt gat gat ttg atc agt aga atg ttg aag gaa cac ctg gcg tca gct      880
Phe Asp Asp Leu Ile Ser Arg Met Leu Lys Glu His Leu Ala Ser Ala
250                 255                 260                 265

cat atc cga aag gag aaa cct gat ttt ctt gat gtc att ttg gct aat      928
His Ile Arg Lys Glu Lys Pro Asp Phe Leu Asp Val Ile Leu Ala Asn
                270                 275                 280

cgt gat act ttg gag gga gag agg ctt acc act tct aac atc aag gct      976
Arg Asp Thr Leu Glu Gly Glu Arg Leu Thr Thr Ser Asn Ile Lys Ala
            285                 290                 295

ctt tta ctg aac ttg ttc acc gcc ggt acg gat aca tct tcg agc aca     1024
Leu Leu Leu Asn Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr
        300                 305                 310

ata gag tgg gcg ctg gcg gag atg ata aaa aac ccg gcg atc ctc aag     1072
Ile Glu Trp Ala Leu Ala Glu Met Ile Lys Asn Pro Ala Ile Leu Lys
    315                 320                 325

aaa gca cat gat gaa atg gat caa gtc gta ggc tgg aat cga cgt tta     1120
Lys Ala His Asp Glu Met Asp Gln Val Val Gly Trp Asn Arg Arg Leu
330                 335                 340                 345

atg gag tcg gac ata ccc aaa ctt cca tac cta caa gcg ata tgc aag     1168
Met Glu Ser Asp Ile Pro Lys Leu Pro Tyr Leu Gln Ala Ile Cys Lys
                350                 355                 360

gaa tca ttt cgt aag cac cct tcc act cct tta aat ctg ccc cga atc     1216
Glu Ser Phe Arg Lys His Pro Ser Thr Pro Leu Asn Leu Pro Arg Ile
            365                 370                 375

tct tca caa gca tgc acg gtg aac ggt tac tac ata ccg aag aac acg     1264
Ser Ser Gln Ala Cys Thr Val Asn Gly Tyr Tyr Ile Pro Lys Asn Thr
        380                 385                 390

agg ctc aac gtc aac ata tgg gcg atc gga agg gat ccc aat gtg tgg     1312
Arg Leu Asn Val Asn Ile Trp Ala Ile Gly Arg Asp Pro Asn Val Trp
    395                 400                 405

gag aat ccc ctg gaa ttc aac ccc gac agg ttc atg tcc ggt aag aac     1360
Glu Asn Pro Leu Glu Phe Asn Pro Asp Arg Phe Met Ser Gly Lys Asn
410                 415                 420                 425

gca aag ctc gat ccg aga gga aat gat ttt gaa ctc att ccg ttc ggg     1408
Ala Lys Leu Asp Pro Arg Gly Asn Asp Phe Glu Leu Ile Pro Phe Gly
                430                 435                 440

gct ggt cga agg att tgt gcg gga gcg agg atg ggg ata gtt ctt gtg     1456
Ala Gly Arg Arg Ile Cys Ala Gly Ala Arg Met Gly Ile Val Leu Val
            445                 450                 455

gaa tat ata ttg gga agt ttg gtg cat tct ttt gat tgg aaa ttg ccc     1504
Glu Tyr Ile Leu Gly Ser Leu Val His Ser Phe Asp Trp Lys Leu Pro
        460                 465                 470

gaa gga gtg aag gag atg aat ttg gat gag gct ttt ggg ctt gct ttg     1552
Glu Gly Val Lys Glu Met Asn Leu Asp Glu Ala Phe Gly Leu Ala Leu
    475                 480                 485

caa aaa gct gtt cct ctt gca gca atg gtt act ccg agg ttg cct tca     1600
Gln Lys Ala Val Pro Leu Ala Ala Met Val Thr Pro Arg Leu Pro Ser
490                 495                 500                 505

aat tgt tat gct cct taagtaatag tatttaagtg cgtccgaata tcgaagttta     1655
Asn Cys Tyr Ala Pro
                510

tatgattttc ttgtgcttgt ttctatccac tatgttgtaa gaattcatct ccgatcctct   1715

ggtggtcatg gctatatatc gtaattcttt ttctatgtcg tactaatatc aatcaattat   1775

attttcaaac ttttttctaa aaaaaaaaaa aaaaaa                            1811


<210> SEQ ID NO 72
<211> LENGTH: 510
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-continued

<212> TYPE: PRT
<213> ORGANISM: Antirrhinum kellogii

<400> SEQUENCE: 72

Met Gln Ile Ile Ile Pro Val Leu Leu Lys Glu Leu Thr Val Ala Ala
1 5 10 15

Leu Leu Tyr Val Phe Thr Asn Ile Leu Ile Arg Ser Leu Leu Thr Arg
20 25 30

Pro Arg His Arg Leu Pro Pro Gly Pro Arg Gly Phe Pro Val Val Gly
35 40 45

Ala Leu Pro Leu Leu Gly Ser Met Pro His Val Ala Leu Ala Lys Met
50 55 60

Ser Lys Thr Tyr Gly Pro Val Ile Tyr Leu Lys Val Gly Ala His Gly
65 70 75 80

Met Ala Val Ala Ser Thr Pro Glu Ser Ala Lys Ala Phe Leu Lys Thr
85 90 95

Leu Asp Thr Asn Phe Ser Asn Arg Pro Pro Asn Ala Gly Ala Thr His
100 105 110

Leu Ala Tyr Asn Ser Gln Asp Met Val Phe Ala Ala Tyr Gly Pro Arg
115 120 125

Trp Arg Leu Leu Arg Lys Leu Ser Asn Leu His Met Leu Gly Thr Lys
130 135 140

Ala Leu Asp Asp Trp Ala Asn Val Arg Val Ser Glu Val Gly Tyr Met
145 150 155 160

Leu Glu Asp Met His Gly Ala Ser Gly Arg Gly Lys Val Val Gly Val
165 170 175

Pro Gly Met Leu Val Tyr Ala Met Ala Asn Met Ile Gly Gln Val Ile
180 185 190

Leu Ser Arg Arg Val Phe Val Thr Arg Glu Glu Glu Leu Asn Glu Phe
195 200 205

Lys Asp Met Val Val Glu Leu Met Thr Ser Ala Gly Tyr Phe Asn Ile
210 215 220

Gly Asp Phe Ile Pro Ser Phe Ala Trp Met Asp Leu Gln Gly Ile Glu
225 230 235 240

Lys Gly Met Lys Gly Leu His Lys Lys Phe Asp Asp Leu Ile Ser Arg
245 250 255

Met Leu Lys Glu His Leu Ala Ser Ala His Ile Arg Lys Glu Lys Pro
260 265 270

Asp Phe Leu Asp Val Ile Leu Ala Asn Arg Asp Thr Leu Glu Gly Glu
275 280 285

Arg Leu Thr Thr Ser Asn Ile Lys Ala Leu Leu Leu Asn Leu Phe Thr
290 295 300

Ala Gly Thr Asp Thr Ser Ser Ser Thr Ile Glu Trp Ala Leu Ala Glu
305 310 315 320

Met Ile Lys Asn Pro Ala Ile Leu Lys Lys Ala His Asp Glu Met Asp
325 330 335

Gln Val Val Gly Trp Asn Arg Arg Leu Met Glu Ser Asp Ile Pro Lys
340 345 350

Leu Pro Tyr Leu Gln Ala Ile Cys Lys Glu Ser Phe Arg Lys His Pro
355 360 365

Ser Thr Pro Leu Asn Leu Pro Arg Ile Ser Ser Gln Ala Cys Thr Val
370 375 380

Asn Gly Tyr Tyr Ile Pro Lys Asn Thr Arg Leu Asn Val Asn Ile Trp
385 390 395 400

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Ala Ile Gly Arg Asp Pro Asn Val Trp Glu Asn Pro Leu Glu Phe Asn
                405                 410                 415

Pro Asp Arg Phe Met Ser Gly Lys Asn Ala Lys Leu Asp Pro Arg Gly
            420                 425                 430

Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala
        435                 440                 445

Gly Ala Arg Met Gly Ile Val Leu Val Glu Tyr Ile Leu Gly Ser Leu
    450                 455                 460

Val His Ser Phe Asp Trp Lys Leu Pro Glu Gly Val Lys Glu Met Asn
465                 470                 475                 480

Leu Asp Glu Ala Phe Gly Leu Ala Leu Gln Lys Ala Val Pro Leu Ala
                485                 490                 495

Ala Met Val Thr Pro Arg Leu Pro Ser Asn Cys Tyr Ala Pro
            500                 505                 510


<210> SEQ ID NO 73
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: ADH-AkF3'5'H-Fd

<400> SEQUENCE: 73

caagaaaaat aaatgcagat aataattccg gtcc                                34


<210> SEQ ID NO 74
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NsiI-AkF3'5'H-Rv

<400> SEQUENCE: 74

atgcatgtcc tctaacatgt atc                                            23


<210> SEQ ID NO 75
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AkF3'5'H-ADH-Rv

<400> SEQUENCE: 75

tattatctgc atttattttt cttgatttcc ttcac                               35


<210> SEQ ID NO 76
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NsiI-AkF3'5'H-Rv

<400> SEQUENCE: 76

atgcatgtcc tctaacatgt atc                                            23


<210> SEQ ID NO 77
<211> LENGTH: 1667
<212> TYPE: DNA
<213> ORGANISM: Cineraria
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Ci5a18
<220> FEATURE:
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<221> NAME/KEY: CDS
<222> LOCATION: (39)..(1550)

<400> SEQUENCE: 77

```
gaattactaa ccaattctta cgttgtcaag taaataaa atg agc att cta acc cta     56
                                          Met Ser Ile Leu Thr Leu
                                          1               5

atc tgc acc ttc atc act ggt ttg atg ttc tat ggg ttg gtt aat ttg      104
Ile Cys Thr Phe Ile Thr Gly Leu Met Phe Tyr Gly Leu Val Asn Leu
            10                  15                  20

ctt agc cgt cgc gct agc cgt ctt cct cca ggt cca acc cca tgg cca      152
Leu Ser Arg Arg Ala Ser Arg Leu Pro Pro Gly Pro Thr Pro Trp Pro
        25                  30                  35

atc atc ggc aac cta atg cac ctt ggt aaa ctt cca cat cac tcg ctg      200
Ile Ile Gly Asn Leu Met His Leu Gly Lys Leu Pro His His Ser Leu
    40                  45                  50

gcg gac ttg gcg aaa aag tat ggt ccg ttg ata cat gtc cga cta ggg      248
Ala Asp Leu Ala Lys Lys Tyr Gly Pro Leu Ile His Val Arg Leu Gly
55                  60                  65                  70

tcc gtt gat gtt gtg gtg gcc tcg tct gcg tcc gtt gct ggg cag ttt      296
Ser Val Asp Val Val Val Ala Ser Ser Ala Ser Val Ala Gly Gln Phe
                75                  80                  85

tta aag gtg cac gat gcg aat ttt gcc aac agg cca cca aat tct gga      344
Leu Lys Val His Asp Ala Asn Phe Ala Asn Arg Pro Pro Asn Ser Gly
            90                  95                  100

gct aaa cat atg gcg tat aat tat cat gat atg gtg ttt gcg ccg tat      392
Ala Lys His Met Ala Tyr Asn Tyr His Asp Met Val Phe Ala Pro Tyr
        105                 110                 115

ggt cca agg tgg cga atg ctt cga aag atg tgc tcc atg cat ctg ttt      440
Gly Pro Arg Trp Arg Met Leu Arg Lys Met Cys Ser Met His Leu Phe
    120                 125                 130

tct gcc aaa gca ctc act gat ttt cgt caa gtt cga cag gag gag gta      488
Ser Ala Lys Ala Leu Thr Asp Phe Arg Gln Val Arg Gln Glu Glu Val
135                 140                 145                 150

atg ata ctc acg cgc gtt ttg gcc ggg act gaa caa tcg gca gtg aaa      536
Met Ile Leu Thr Arg Val Leu Ala Gly Thr Glu Gln Ser Ala Val Lys
                155                 160                 165

cta gat caa caa ctt aac gtg tgc ttc gca aac aca tta tcc cga atg      584
Leu Asp Gln Gln Leu Asn Val Cys Phe Ala Asn Thr Leu Ser Arg Met
            170                 175                 180

atg tta gac agg aga gta ttt gga gac ggt gat cca aag gcg gac gac      632
Met Leu Asp Arg Arg Val Phe Gly Asp Gly Asp Pro Lys Ala Asp Asp
        185                 190                 195

tac aag gat atg gtg gtt gag ttg atg act ttg gcc gga caa ttc aac      680
Tyr Lys Asp Met Val Val Glu Leu Met Thr Leu Ala Gly Gln Phe Asn
    200                 205                 210

atc ggt gac tac att cct tgg ctt gac ttg ctt gac cta caa ggc att      728
Ile Gly Asp Tyr Ile Pro Trp Leu Asp Leu Leu Asp Leu Gln Gly Ile
215                 220                 225                 230

gtc aaa agg atg aag aaa gtt cat tct caa ttc gat tcg ttc ctt gac      776
Val Lys Arg Met Lys Lys Val His Ser Gln Phe Asp Ser Phe Leu Asp
                235                 240                 245

acc atc att gat gaa cat act att ggc acg ggc cgt cat gtt gac atg      824
Thr Ile Ile Asp Glu His Thr Ile Gly Thr Gly Arg His Val Asp Met
            250                 255                 260

tta agc aca atg att tca ctc aaa gat aat gcc gat gga gag gga ggg      872
Leu Ser Thr Met Ile Ser Leu Lys Asp Asn Ala Asp Gly Glu Gly Gly
        265                 270                 275

aag ctt tcg ttc atc gag atc aaa gct ctt cta ctg aac tta ttc tca      920
Lys Leu Ser Phe Ile Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ser
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    280                 285                 290

gcg gga acg gac acg tca tct agt acc gtg gaa tgg gga ata gcg gaa      968
Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Gly Ile Ala Glu
295                 300                 305                 310

ctc att cgc cac cca cag cta atg aaa caa gcg caa gaa gaa atg gac     1016
Leu Ile Arg His Pro Gln Leu Met Lys Gln Ala Gln Glu Glu Met Asp
                315                 320                 325

att gta att gga aaa aac cgg ctt gta aca gaa atg gac ata agc caa     1064
Ile Val Ile Gly Lys Asn Arg Leu Val Thr Glu Met Asp Ile Ser Gln
            330                 335                 340

cta aca ttc ctc caa gcc att gtg aaa gaa acg ttt aga ctc cac ccc     1112
Leu Thr Phe Leu Gln Ala Ile Val Lys Glu Thr Phe Arg Leu His Pro
        345                 350                 355

gcg acg cca ctt tcc ctg cca agg att gca tcg gaa agc tgt gag gtc     1160
Ala Thr Pro Leu Ser Leu Pro Arg Ile Ala Ser Glu Ser Cys Glu Val
    360                 365                 370

aag ggg tat cat gtt cct aag gga tcc ata ctc ttt gtt aac gtg tgg     1208
Lys Gly Tyr His Val Pro Lys Gly Ser Ile Leu Phe Val Asn Val Trp
375                 380                 385                 390

gcc att gct cga caa tca gaa ttg tgg acc gac cca ctt gaa ttt cgg     1256
Ala Ile Ala Arg Gln Ser Glu Leu Trp Thr Asp Pro Leu Glu Phe Arg
                395                 400                 405

cct ggt cgt ttc cta atc cca gga gaa aaa cct aat gtt gaa gtg aag     1304
Pro Gly Arg Phe Leu Ile Pro Gly Glu Lys Pro Asn Val Glu Val Lys
            410                 415                 420

cca aat gat ttc gaa att gta cca ttc ggg gga gga cga agg att tgt     1352
Pro Asn Asp Phe Glu Ile Val Pro Phe Gly Gly Gly Arg Arg Ile Cys
        425                 430                 435

gca ggt atg agc ctc gga ttg aga atg gtc aat ttg ctt att gca aca     1400
Ala Gly Met Ser Leu Gly Leu Arg Met Val Asn Leu Leu Ile Ala Thr
    440                 445                 450

ttg gtt caa gcc ttt gat tgg gaa ttg gct aat ggg tta gag cca gaa     1448
Leu Val Gln Ala Phe Asp Trp Glu Leu Ala Asn Gly Leu Glu Pro Glu
455                 460                 465                 470

aag ctt aac atg gaa gaa gtg ttt ggg att agc ctt caa agg gtt caa     1496
Lys Leu Asn Met Glu Glu Val Phe Gly Ile Ser Leu Gln Arg Val Gln
                475                 480                 485

ccc ttg ttg gtg cac ccg agg cca agg tta gcc cgt cac gta tac gga     1544
Pro Leu Leu Val His Pro Arg Pro Arg Leu Ala Arg His Val Tyr Gly
            490                 495                 500

acg ggt taaggaaata aactgcctgt ttgtaagata aatctgtttg aatttatgta      1600
Thr Gly

ttaaatagtt atgctaagaa ctatttttac aaataaaagt atattggttt gaaaaaaaaa   1660

aaaaaaa                                                            1667


<210> SEQ ID NO 78
<211> LENGTH: 504
<212> TYPE: PRT
<213> ORGANISM: Cineraria

<400> SEQUENCE: 78

Met Ser Ile Leu Thr Leu Ile Cys Thr Phe Ile Thr Gly Leu Met Phe
1               5                   10                  15

Tyr Gly Leu Val Asn Leu Leu Ser Arg Arg Ala Ser Arg Leu Pro Pro
            20                  25                  30

Gly Pro Thr Pro Trp Pro Ile Ile Gly Asn Leu Met His Leu Gly Lys
        35                  40                  45

Leu Pro His His Ser Leu Ala Asp Leu Ala Lys Lys Tyr Gly Pro Leu
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    50                  55                  60

Ile His Val Arg Leu Gly Ser Val Asp Val Val Val Ala Ser Ser Ala
65                  70                  75                  80

Ser Val Ala Gly Gln Phe Leu Lys Val His Asp Ala Asn Phe Ala Asn
                85                  90                  95

Arg Pro Pro Asn Ser Gly Ala Lys His Met Ala Tyr Asn Tyr His Asp
            100                 105                 110

Met Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Met
        115                 120                 125

Cys Ser Met His Leu Phe Ser Ala Lys Ala Leu Thr Asp Phe Arg Gln
    130                 135                 140

Val Arg Gln Glu Glu Val Met Ile Leu Thr Arg Val Leu Ala Gly Thr
145                 150                 155                 160

Glu Gln Ser Ala Val Lys Leu Asp Gln Gln Leu Asn Val Cys Phe Ala
                165                 170                 175

Asn Thr Leu Ser Arg Met Met Leu Asp Arg Arg Val Phe Gly Asp Gly
            180                 185                 190

Asp Pro Lys Ala Asp Asp Tyr Lys Asp Met Val Val Glu Leu Met Thr
        195                 200                 205

Leu Ala Gly Gln Phe Asn Ile Gly Asp Tyr Ile Pro Trp Leu Asp Leu
    210                 215                 220

Leu Asp Leu Gln Gly Ile Val Lys Arg Met Lys Lys Val His Ser Gln
225                 230                 235                 240

Phe Asp Ser Phe Leu Asp Thr Ile Ile Asp Glu His Thr Ile Gly Thr
                245                 250                 255

Gly Arg His Val Asp Met Leu Ser Thr Met Ile Ser Leu Lys Asp Asn
            260                 265                 270

Ala Asp Gly Glu Gly Gly Lys Leu Ser Phe Ile Glu Ile Lys Ala Leu
        275                 280                 285

Leu Leu Asn Leu Phe Ser Ala Gly Thr Asp Thr Ser Ser Ser Thr Val
    290                 295                 300

Glu Trp Gly Ile Ala Glu Leu Ile Arg His Pro Gln Leu Met Lys Gln
305                 310                 315                 320

Ala Gln Glu Glu Met Asp Ile Val Ile Gly Lys Asn Arg Leu Val Thr
                325                 330                 335

Glu Met Asp Ile Ser Gln Leu Thr Phe Leu Gln Ala Ile Val Lys Glu
            340                 345                 350

Thr Phe Arg Leu His Pro Ala Thr Pro Leu Ser Leu Pro Arg Ile Ala
        355                 360                 365

Ser Glu Ser Cys Glu Val Lys Gly Tyr His Val Pro Lys Gly Ser Ile
    370                 375                 380

Leu Phe Val Asn Val Trp Ala Ile Ala Arg Gln Ser Glu Leu Trp Thr
385                 390                 395                 400

Asp Pro Leu Glu Phe Arg Pro Gly Arg Phe Leu Ile Pro Gly Glu Lys
                405                 410                 415

Pro Asn Val Glu Val Lys Pro Asn Asp Phe Glu Ile Val Pro Phe Gly
            420                 425                 430

Gly Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly Leu Arg Met Val
        435                 440                 445

Asn Leu Leu Ile Ala Thr Leu Val Gln Ala Phe Asp Trp Glu Leu Ala
    450                 455                 460

Asn Gly Leu Glu Pro Glu Lys Leu Asn Met Glu Glu Val Phe Gly Ile
465                 470                 475                 480
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Ser Leu Gln Arg Val Gln Pro Leu Leu Val His Pro Arg Pro Arg Leu
                485                 490                 495

Ala Arg His Val Tyr Gly Thr Gly
            500


<210> SEQ ID NO 79
<211> LENGTH: 8552
<212> TYPE: DNA
<213> ORGANISM: Cineraria
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: gCi01-pBluestar

<400> SEQUENCE: 79

gcggccgcgg atcccgggaa ttctcgatcc agccatgtgt ctagtacaac catacagacg     60

attaaaaaaa aaactttaaa ccacaaaacg ggttttgcaa acgaagaaat tgcctcaaaa    120

catttccata tggagtttag ggacagagtg cgtttgctac attaaacaac tcttttataa    180

aaaaacatag cggtacgaga atgacccact aaccgttcat gtccattggc aaaagttact    240

attgtgagtc ttgtatatac atttaaaaaa aaagaatata tcagttccat aaagggcctg    300

aaacataagt aagaatatat caatgacgtt cggttcggtt tttggtttat ataaagagaa    360

cttgatttga aaaattacga gaataacaaa tatttggggt gtcattttat aaaatatcaa    420

aattttaaaa ctattttaca aaatgttaac aagtaagttg ttttttttt ttttttcaca    480

agcagttgaa acagtttttg ttgaacgtga agttatagct ttacttgaag tttgatattt    540

tggcatcttg acactacatg tctttctagt gtgaccctta tcttacaact atcacatgtc    600

aacggttttg tctgtccttt ggatagtata cggtctttgt tttaggacgt ctcgcatgtg    660

tcctctctat ggtggtgggt tgatcgtatg aatccttagg atcgtagcca tttagaagga    720

tttccgacat ggaatatcaa tcatgtatat gtacgtttat aattctcggc gttgaaccaa    780

tgttgtgtcg aactcccgac atcgttcatt tccaaatgtg ttaaaactgt tgtaaggtgt    840

gaacaaggta taccatattt ttgccaaact tgacaactgc attttttttа tcatgttgtc    900

acacacctca tacatttatc attaactggt atgactttcc atccaaactt gacaagctct    960

taaccatttg gcgacatcta aactattcga tagtgactta attcgtaagt taatgcacaa   1020

atgtcgacaa catattccgc gagtcgcatc tggtatggaa cacaaatgga tcaagagggc   1080

taaaacccat gaggttagaa aattttactt ccaagttcaa gttcatttga tacaagcact   1140

gcaaaatcat tctgcagact aatctaaatc ttattcttcc agagatgata agttagtttg   1200

cagctcggtt tttatgtttt cttgatacgt ttatctgtag atgtgatcga aatgatagta   1260

cacgcgctta ttttttgtag tcgtatcgca tatgttagtt aaaaagtctg aaactaactt   1320

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Phe Arg Leu His Pro Ala Thr Pro Leu Ser Leu Pro Arg Ile Ala Ser
    355                 360                 365

gaa agc tgt gag gtc aag ggg tat cat gtt cct aag gga tcg ata ctc     4733
Glu Ser Cys Glu Val Lys Gly Tyr His Val Pro Lys Gly Ser Ile Leu
370                 375                 380                 385

ttt gtt aac gtg tgg gcc att gct cga caa tca gaa ttg tgg acc gac     4781
Phe Val Asn Val Trp Ala Ile Ala Arg Gln Ser Glu Leu Trp Thr Asp
                390                 395                 400

cca ctt gaa ttt cgg cct ggt cgt ttc cta atc cca gga gaa aaa cct     4829
Pro Leu Glu Phe Arg Pro Gly Arg Phe Leu Ile Pro Gly Glu Lys Pro
            405                 410                 415

aat gtt gaa gtg aag cca aat gat ttc gaa att gta cca ttc ggg gga     4877
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Asn Val Glu Val Lys Pro Asn Asp Phe Glu Ile Val Pro Phe Gly Gly
        420                 425                 430

gga cga agg att tgt gca ggt atg agc ctc gga ttg aga atg gtc aat     4925
Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly Leu Arg Met Val Asn
    435                 440                 445

ttg ctt att gca aca ttg gtt caa gcc ttt gat tgg gaa ttg gct aat     4973
Leu Leu Ile Ala Thr Leu Val Gln Ala Phe Asp Trp Glu Leu Ala Asn
450                 455                 460                 465

ggg tta gag cca gaa aag ctt aac atg gaa gaa gtg ttt ggg att agc     5021
Gly Leu Glu Pro Glu Lys Leu Asn Met Glu Glu Val Phe Gly Ile Ser
                470                 475                 480

ctt caa agg gtt caa ccc ttg ttg gtg cac ccg agg cca agg tta gcc     5069
Leu Gln Arg Val Gln Pro Leu Leu Val His Pro Arg Pro Arg Leu Ala
            485                 490                 495

cgt cac gta tac gga acg ggt taaggaaata aactgtctgt ttgtaagatg       5120
Arg His Val Tyr Gly Thr Gly
        500

aatctgtttg aatttatgta ttaaatagtt atgctaagaa ctatttttac aaataaaagt   5180

atattggttt gattgttctc gcttagcctt tgctaaatct tagatagatg agttgtataa   5240

cacatcatca ttaactcaca tcacgtggta acgatttgtt tttgagttaa aatttttaaa   5300

gaaaggaaag aaagagaaag taaatataaa aaaatttgtg ttcccgagaa gttttttacg   5360

aaggaagagg ggagaaagag agagaatttt agagaaattt tgagtatttt acaacaaaaa   5420

tcatcctctc atttttggga tgatttggag gatctttttt ctttcttttc cttcgtccac   5480

ttcacctccc tttctttcca aaaaaatctc ggaaacatag cgtaatgata aacaaaaacc   5540

aataaaaatg agcaggagca aaccctagaa ggacgaaatc ttgaaaattt attctaagat   5600

ttttaaaaaa aacttggcag ttggaaaggg cggcggat                           5638


<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: forward primer Ci5a18F1

<400> SEQUENCE: 81

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<210> SEQ ID NO 82
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer Ci5a18R1

<400> SEQUENCE: 82

ggattaggaa acgaccagg                                                19


<210> SEQ ID NO 83
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<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 83

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<400> SEQUENCE: 84

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<400> SEQUENCE: 85

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<210> SEQ ID NO 86
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer NS-RhCHSpro3k-Rv

<400> SEQUENCE: 86

aaagctagca ctagtcatct cggagaaggg tcg 33

<210> SEQ ID NO 87
<211> LENGTH: 531
<212> TYPE: DNA
<213> ORGANISM: Chrysanthemum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: CmF3Hpro500
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (1)..(21)
<220> FEATURE:
<221> NAME/KEY: primer_bind
<222> LOCATION: (507)..(531)

<400> SEQUENCE: 87

tactgttcga acctacaaag gaatatcaat acgagggctc aattattgtc tcggattcaa 60

tgaattcaca aggtaaataa acgcggtact cttttcattg gtccttcgtt ttatttgttt 120

gacaattaat tgggatggct ggcgtgtata attctcaata catgtctgat ttaatatgtg 180

attggttgac attcatgtga aattaatata ctcattttat gattacaaag acccacgatg 240

tataattaat tccaatcttg tggaatggga tccattgtga accggtgcat gattgttacg 300

gtggggatta cttttgattg gttcagcatt atcatataac ccccgttcaa cggatgcatg 360

ctacattggt acgtatacat atacgattca cgtgtggtag ttgataacta gcgcgatacg 420

cccccacccc atatttcttc aattttctct acaaatacc atgccaacct tacgaaacac 480

tcattcccct ctactcatag acgcaccaag tgtgtgaaga aaaaataaaa a 531

The invention claimed is:

1. A method for producing a chrysanthemum plant containing delphinidin in the petals thereof comprising expressing flavonoid 3',5'-hydroxylase (F3'5'H) using a transcriptional regulatory region; wherein the chrysanthemum plant is transformed with an expression vector or expression cassette comprising a gene encoding F3'5'H and the transcriptional regulatory region; wherein the F3'5'H is derived from bellflower (campanula), cineraria, verbena, or pansy; and wherein the transcriptional regulatory region is

a nucleic acid containing the nucleotide sequence indicated in SEQ ID NO: 34 or SEQ ID NO: 87.

2. The method according to claim 1, wherein a translation enhancer derived from tobacco alcohol dehydrogenase is further used in addition to the transcriptional regulatory region.

3. The method according to claim 2, wherein the translation enhancer is coupled directly to a start codon of the F3'5'H gene.

4. A chrysanthemum plant, or a progeny, a vegetative proliferation product, a part, or a tissue thereof, transformed by the method according to claim 1.

5. A chrysanthemum plant, or a progeny, a vegetative proliferation product, a part, or a tissue thereof according to claim 4, which is a cut flower.

6. A cut flower processed product made from the cut flower according to claim 5, wherein said cut flower processed product comprises a F3'5'H gene sequence from bellflower (campanula), cineraria, verbena, or pansy operably linked to a transcriptional regulatory sequence, and wherein the transcriptional regulatory region is

a nucleic acid containing the nucleotide sequence indicated in SEQ ID NO: 34 or SEQ ID NO: 87.

7. The method according to claim 1, wherein the content of delphinidin in the petals is 25% by weight or more of the total weight of anthocyanidins, and wherein a translation enhancer derived from tobacco alcohol dehydrogenase is further used in addition to the transcriptional regulatory region.

8. The method according to claim 3, wherein the content of delphinidin in the petals is 25% by weight or more of the total weight of anthocyanidins.

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