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(54) **Titre : PLANTE AYANT UNE INFLORESCENCE MODIFIEE**
(54) **Title: A PLANT WITH ALTERED INFLORESCENCE**

(57) **Abrégé/Abstract:**

The invention relates to genetically engineered plants with altered inflorescence. Plants such as spray carnations are transformed with a non-indigenous flavonoid 3', 5' hydroxylase (F3'5'H) and dihydroflavanol-4-reductase (DFR) in conjunction with a genetic suppressor of indigenous DFR. Preferably the substrate specificity of the indigenous DFR is different to the non-indigenous DFR in order to enhance the colour of the inflorescence.



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(54) **Title:** A PLANT WITH ALTERED INFLORESCENCE(57) **Abstract:** The invention relates to genetically engineered plants with altered inflorescence. Plants such as spray carnations are transformed with a non-indigenous flavonoid 3', 5' hydroxylase (F3'5'H) and dihydroflavanol-4-reductase (DFR) in conjunction with a genetic suppressor of indigenous DFR. Preferably the substrate specificity of the indigenous DFR is different to the non-indigenous DFR in order to enhance the colour of the inflorescence.

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A Plant with Altered Inflorescence**FILING DATA**

- 5 [0001] This application is associated with and claims priority from United States Provisional Patent Application No. 61/139,354, filed on 19 December 2008, entitled "A Plant".

FIELD

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[0002] The present invention relates generally to the field of genetic modification of plants. More particularly, the present invention is directed to genetically modified plants expressing desired color phenotypes.

15 **BACKGROUND**

[0003] Bibliographic details of the publications referred to by the author in this specification are collected at the end of the description.

- 20 [0004] Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

[0005] The flower or ornamental or horticultural plant industry strives to develop new and
25 different varieties of flowers and/or plants. An effective way to create such novel varieties is through the manipulation of flower color. Classical breeding techniques have been used with some success to produce a wide range of colors for almost all of the commercial varieties of flowers and/or plants available today. This approach has been limited, however, by the constraints of a particular species' gene pool and for this reason it is rare
30 for a single species to have the full spectrum of colored varieties. For example, the

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development of novel colored varieties of plants or plant parts such as flowers, foliage, fruits and stems would offer a significant opportunity in both the cut flower, ornamental and horticultural markets. In the flower or ornamental or horticultural plant industry, the development of novel colored varieties of carnation is of particular interest. This includes
5 not only different colored flowers but also anthers and styles.

[0006] Flower color is predominantly due to three types of pigment: flavonoids, carotenoids and betalains. Of the three, the flavonoids are the most common and contribute a range of colors from yellow to red to blue. The flavonoid molecules that make the major
10 contribution to flower color are the anthocyanins, which are glycosylated derivatives of cyanidin and its methylated derivative peonidin, delphinidin and its methylated derivatives petunidin and malvidin and pelargonidin. Anthocyanins are localized in the vacuole of the epidermal cells of petals or the vacuole of the sub epidermal cells of leaves.

15 [0007] The flavonoid pigments are secondary metabolites of the phenylpropanoid pathway. The biosynthetic pathway for the flavonoid pigments (flavonoid pathway) is well established (Holton and Cornish, *Plant Cell* 7:1071-1083, 1995; Mol *et al*, *Trends Plant Sci.* 3:212-217, 1998; Winkel-Shirley, *Plant Physiol.* 126:485-493, 2001a; and Winkel-Shirley, *Plant Physiol.* 127:1399-1404, 2001b, Tanaka and Mason, In *Plant Genetic*
20 *Engineering*, Singh and Jaiwal (eds) SciTech Publishing Llc., USA, 1:361-385, 2003, Tanaka *et al*, *Plant Cell, Tissue and Organ Culture* 80:1-24, 2005, Tanaka and Brugliera, In *Flowering and Its Manipulation, Annual Plant Reviews* Ainsworth (ed), Blackwell Publishing, UK, 20:201-239, 2006) and is shown in Figure 1. Three reactions and enzymes are involved in the conversion of phenylalanine to *p*-coumaroyl-CoA, one of the first key
25 substrates in the flavonoid pathway. The enzymes are phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate: CoA ligase (4CL). The first committed step in the pathway involves the condensation of three molecules of malonyl-CoA (provided by the action of acetyl CoA carboxylase (ACC) on acetyl CoA and CO₂) with one molecule of *p*-coumaroyl-CoA. This reaction is catalyzed by the enzyme
30 chalcone synthase (CHS). The product of this reaction, 2',4,4',6', tetrahydroxy-chalcone, is normally rapidly isomerized by the enzyme chalcone flavanone isomerase (CHI) to

produce naringenin. Naringenin is subsequently hydroxylated at the 3 position of the central ring by flavanone 3-hydroxylase (F3H) to produce dihydrokaempferol (DHK).

5 [0008] The pattern of hydroxylation of the B-ring of DHK plays a key role in determining petal color. The B-ring can be hydroxylated at either the 3', or both the 3' and 5' positions, to produce dihydroquercetin (DHQ) or dihydromyricetin (DHM), respectively. Two key enzymes involved in this part of the pathway are the flavonoid 3' hydroxylase (F3'H) and flavonoid 3', 5' hydroxylase (F3'5'H), both members of the cytochrome P450 class of enzymes.

10

[0009] F3'H is a key enzyme in the flavonoid pathway leading to the cyanidin-based pigments which, in many plant species contribute to red and pink flower color. F3'5'H leads to the production of delphinidin based anthocyanins which, in many species contribute to the purple, violet and blue flower colors.

15

[0010] Nucleotide sequences encoding F3'5'Hs have been cloned (see International Patent Application No. PCT/AU92/00334 and Holton *et al*, *Nature*, 366:276-279, 1993 and International Patent Application No. PCT/AU03/01111). These sequences were efficient in modulating 3', 5' hydroxylation of flavonoids in petunia (see International Patent Application No. PCT/AU92/00334 and Holton *et al*, 1993 *supra*), tobacco (see International Patent Application No. PCT/AU92/00334), carnations (see International Patent Application No. PCT/AU96/00296) and roses (see International Patent Application No. PCT/AU03/01111).

25

[0011] The production of the colored anthocyanins from the dihydroflavonols (DHK, DHQ, DHM), involves dihydroflavonol-4-reductase (DFR) leading to the production of the leucoanthocyanidins. The leucoanthocyanidins are subsequently converted to the anthocyanidins, pelargonidin, cyanidin and delphinidin. These flavonoid molecules are
30 unstable under normal physiological conditions and glycosylation at the 3-position, through the action of glycosyltransferases, stabilizes the anthocyanidin molecule thus

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allowing accumulation of the anthocyanins. In general, the glycosyltransferases transfer the sugar moieties from UDP sugars to the flavonoid molecules and show high specificities for the position of glycosylation and relatively low specificities for the acceptor substrates (Seitz and Hinderer, Anthocyanins. In: *Cell Culture and Somatic Cell Genetics of Plants*.

5 Constabel and Vasil (eds.), Academic Press, New York, USA, 5:49-76, 1988). Anthocyanins can occur as 3-monosides, 3-biosides and 3-triosides as well as 3, 5-diglycosides and 3,7-diglycosides associated with the sugars glucose, galactose, rhamnose, arabinose and xylose (Strack and Wray, In: *The Flavonoids - Advances in Research since 1986*. Harborne, J.B. (ed), Chapman and Hall, London, UK, 1-22, 1993).

10

[0012] Glycosyltransferases involved in the stabilization of the anthocyanidin molecule include UDP glucose: flavonoid 3-glucosyltransferase (3GT), which transfers a glucose moiety from UDP glucose to the 3-*O*-position of the anthocyanidin molecule to produce anthocyanidin 3-*O*-glucoside.

15

[0013] Many anthocyanidin glycosides exist in the form of acylated derivatives. The acyl groups that modify the anthocyanidin glycosides can be divided into two major classes based upon their structure. The aliphatic acyl groups include malonic acid or succinic acid and the aromatic class includes the hydroxy cinnamic acids such as *p*-coumaric acid, 20 caffeic acid and ferulic acid and the benzoic acids such as *p*-hydroxybenzoic acid. For example in carnation the anthocyanins exist as malylated anthocyanins (Nakayama *et al*, *Phytochemistry*, 55, 937-939, 2000; Fukui *et al*, *Phytochemistry*, 63(1):15-23, 2003).

[0014] In addition to the above modifications, pH of the vacuole or compartment where 25 pigments are localized and co-pigmentation with other flavonoids such as flavonols and flavones can affect petal color. Flavonols and flavones can also be aromatically acylated (Brouillard and Dangles, In: *The Flavonoids -Advances in Research since 1986*. Harborne, J.B. (ed), Chapman and Hall, London, UK, 1-22, 1993).

30 [0015] Carnation flowers can produce two types of anthocyanidins, depending on their genotype-pelargonidin and cyanidin. In the absence of F3'H activity, anthocyanins derived

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from pelargonidin are produced otherwise those derived from cyanidin are produced. Pelargonidin derived pigments are usually accompanied by kaempferol, a colorless flavonol. Cyanidin derived pigments are usually accompanied by both kaempferol and quercetin. Both pelargonidin and kaempferol are derived from DHK; both cyanidin and
5 quercetin are derived from DHQ (Figure 1).

[0016] The substrate specificity shown by DFR regulates the anthocyanins that a plant accumulates. Petunia and cymbidium DFRs do not reduce DHK and thus they do not accumulate pelargonidin-based pigments (Forkmann and Ruhnau, *Z Naturforsch C*. 42c,
10 1146-1148, 1987, Johnson *et al*, *Plant Journal*, 19, 81-85, 1999). Many important floricultural species including iris, delphinium, cyclamen, gentian, cymbidium, nierembergia are presumed not to accumulate pelargonidin derived pigments due to the substrate specificity of their endogenous DFRs (Tanaka and Brugliera, 2006 *supra*).

15 [0017] In carnation, the DFR enzyme is capable of metabolizing DHK to leucopelargonidin, the precursor to pelargonidin-based pigments, giving rise to apricot to brick-red colored carnations and DHQ to leucocyanidin, the precursor to cyanidin-based pigments, producing pink to red carnations. Carnation DFR is also capable of converting DHM to leucodelphinidin (Forkmann and Ruhnau, 1987 *supra*), the precursor to
20 delphinidin-based pigments. Wild-type or classically-derived carnation lines do not contain a F3'5'H enzyme and therefore do not synthesize DHM.

[0018] The petunia DFR enzyme has a different specificity to that of the carnation DFR. It is able to convert DHQ through to leucocyanidin, but it is not able to convert DHK to
25 leucopelargonidin (Forkmann and Ruhnau, 1987 *supra*). It is also known that in petunia lines containing the F3'5'H enzyme, the petunia DFR enzyme can convert the DHM produced by this enzyme to leucodelphinidin which is further modified giving rise to delphinidin-based pigments which are predominantly responsible for blue colored flowers (see Figure 1). Even though the petunia DFR is capable of converting both DHQ and
30 DHM, it is able to convert DHM far more efficiently, thus favoring the production of delphinidin (Forkmann and Ruhnau, 1987 *supra*).

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[0019] Carnations are one of the most extensively grown cut flowers in the world.

[0020] There are thousands of current and past cut-flower varieties of cultivated carnation.

5 These are divided into three general groups based on plant form, flower size and flower type. The three flower types are standards, sprays and midis. Most of the carnations sold fall into two main groups –the standards and the sprays. Standard carnations are intended for cultivation under conditions in which a single large flower is required per stem. Side shoots and buds are removed (a process called disbudding) to increase the size of the
10 terminal flower. Sprays and/or miniatures are intended for cultivation to give a large number of smaller flowers per stem. Only the central flower is removed, allowing the laterals to form a 'fan' of stems.

[0021] Spray carnation varieties are popular in the floral trade, as the multiple flower buds
15 on a single stem are well suited to various types of flower arrangements and provide bulk to bouquets used in the mass market segment of the industry.

[0022] Standard and spray cultivars dominate the carnation cut-flower industry, with approximately equal numbers sold of each type in the USA. In Japan, Spray-type varieties
20 account for 70% of carnation flowers sold by volume, whilst in Europe spray-type carnations account for approximately 50% of carnation flowers traded through out the Dutch auctions. The Dutch auction trade is a good indication of consumption across Europe.

25 [0023] Whilst standard and midi-type carnations have been successfully manipulated genetically to introduce new colors (Tanaka and Brugliera, 2006 *supra*; see also International Patent Application No. PCT/AU96/00296), this has not been applied to spray carnations. There has been absence of blue color in color-assortment in carnation, only recently filled through the introduction of genetically-modified standard-type carnation
30 varieties. However, standard-type varieties can not be used for certain purposes, such as

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bouquets and flower arrangements where a large number of smaller carnation flowers are needed, such as hand-held arrangements, and small table settings.

[0024] One particular spray carnation which is particularly commercially popular is the Cerise Westpearl line of carnations (*Dianthus caryophyllus* cv. Cerise Westpearl). The variety has excellent growing characteristics and a moderate to good resistance to fungal pathogens such as *Fusarium*. Cerise Westpearl is a sport of Westpearl. However, before the advent of the present invention, purple/blue spray carnations were not available.

[0025] White Unesco is a classically-derived carnation of the midi-type. It is white and does not normally produce anthocyanins primarily because the petals do not accumulate carnation *DFR* transcripts and so when White Unesco was transformed with *Viola F3'5'H* and a petunia *DFR* gene, over 80% of the anthocyanins produced were delphinidin based (see International Patent Application PCT/AU96/00296). Although this process has been useful in obtaining carnation lines with a purple/violet petals, it is limited to the identification of white lines that are mutant in the ability to accumulate petal carnation *DFR* mRNA or functional *DFR* enzymes in the petals but have the rest of the anthocyanin pathway intact so that the DHM produced can be converted to stable, colored anthocyanins. Of the 13 lines analyzed (see International Patent Application PCT/AU96/00296), only two were deficient in carnation *DFR* but intact in the ability to produce anthocyanins. Of the two, only one (White Unesco) resulted in the production of purple/violet petals upon the introduction of *F3'5'H* and a petunia *DFR*.

[0026] The application of a similar approach using *Viola F3'5'H* and a petunia *DFR* transformed into a colored line such as Cerise Westpearl has not yielded significant novel colored products.

[0027] There is a need, therefore, to find an alternative means of producing novel colored purple/mauve flowers using colored lines such as Cerise Westpearl.

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SUMMARY

[0028] Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to
5 imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0029] Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO). The SEQ ID NOs correspond numerically to the sequence
10 identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc.

[0030] A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

15 [0031] The present invention provides genetically modified plants exhibiting altered inflorescence. More particularly, the present invention provides genetically modified carnations and even more particularly genetically modified carnation sprays exhibiting altered inflorescence. The altered inflorescence is a color in the range of red-purple to blue such as purple and mauve to blue color in the tissue or organelles including flowers, petals,
20 anthers and styles. In one embodiment, the color is determined using the Royal Horticultural Society (RHS) color chart where colors are arranged in order of the fully saturated colors with the less saturated and less bright colors alongside. The color groups proceed through the observable spectrum and the colors referred to herein are generally in the red-purple (RHSCC 58-74), purple (RHSCC 75-79), purple-violet (RHSCC 81-82),
25 violet (RHSCC 83-88), violet-blue (89-98), blue (RHSCC 99-110) groups contained in Fan 2. Colors are selected from the range including 61A, 64A, 71A, 71C, 72A, 81A, 86A and 87A and colors in between or proximal thereto.

[0032] Hence, the present invention is directed to a genetically modified plant including its
30 progeny with purple/violet shades of color comprising a functional non-indigenous F3',5'H,

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a functional DFR in petals and genetic material which down regulates expression of a plant's indigenous *DFR* gene.

[0033] In one embodiment, the genetic material comprises sense and anti-sense nucleotide
5 sequences which correspond to the plant's indigenous *DFR* sequence (*ds plantDFR*). This induces hairpin RNAi (hpRNAi)-mediated silencing primarily *via* post-transcriptional gene silencing (PTGS). By "indigenous" is meant that an enzyme or a gene evolved in a plant, i.e. is normally resident in that plant. A "non-indigenous" enzyme or gene means that a gene or other genetic material was introduced into a plant or a parent of the plant by
10 genetic engineering or breeding practices.

[0034] In an embodiment, the plant is a carnation such as a spray carnation and the indigenous DFR is the carnation DFR. The genetic material is a chimeric construct referred to as *ds carnDFR*.

15

[0035] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous S adenosylmethionine: anthocyanin 3', 5' methyltransferase (3'5' AMT) and/or a non-indigenous flavone synthase (FNS).

20 [0036] In a further embodiment the 3'5' AMT is from *Torenia* (ThMT) and the FNS is from *Torenia* (ThFNS).

[0037] The modified plants and in particular genetically modified spray carnations comprise genetic sequences encoding at least one F3'5'H enzyme and at least one DFR
25 enzyme and express at least one *ds plantDFR* molecule. Insofar as the present invention relates to carnations, the *ds plantDFR* is *ds carnDFR* and the carnation sprays are conveniently in a Cerise Westpearl genetic background including the progenitor of Cerise Westpearl such as Westpearl. Other carnation cultivars included within the present invention are colored varieties such as Cinderella, Kortina Chanel, Vega, Artisan, Miledy,
30 Barbara, Dark Rendezvous. Other plants contemplated herein include chrysanthemums, roses, gerberas, lisianthus, tulip, lily, geranium, petunia, iris, *Torenia*, *Begonia*, *Cyclamen*,

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Nierembergia, *Catharanthus*, *Pelargonium*, orchid, grape, apple, *Euphorbia*, *Fuchsia* and other ornamental or horticultural plants.

[0038] One aspect of the present invention is directed to a genetically modified plant
5 exhibiting altered inflorescence in selected tissue, the plant comprising expressed genetic material encoding at least one F3'5'H enzyme and at least one DFR enzyme and expressing genetic material which down regulates a *DFR* gene. More particularly, the present invention provides a genetically modified plant exhibiting altered inflorescence, the plant or its progeny comprising expressed genetic material encoding at least one non-indigenous
10 F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing genetic material which down regulates expression of the plant's indigenous *DFR* gene. In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT. In a particular embodiment, the genetic material which down regulates the indigenous *DFR* gene comprises sense and anti-sense nucleotide sequence
15 corresponding to the indigenous *DFR* gene or its mRNA ("*ds plantDFR*"). The term "altered inflorescence" in this context means compared to the inflorescence of a plant (e.g. parent plant or plant of the same species) prior to genetic manipulation. The term "encoding" includes the expression of the genetic material to produce functional F3'5'H and DFR enzymes.

20

[0039] A "*ds plantDFR* molecule" is genetic material comprising both sense and anti-sense fragments of a plant is indigenous *DFR* genomic or cDNA sequence or corresponding mRNA. The *ds plantDFR* is expressed to induce hpRNAi-mediated gene silencing of an indigenous *DFR* gene. In a particular embodiment, the plant is carnation and the *ds*
25 *plantDFR* molecule is *ds carnDFR*.

[0040] In a particular embodiment, the plant is a spray carnation.

[0041] Accordingly, another aspect of the present invention is directed to a spray carnation
30 plant exhibiting altered inflorescence in selected tissue, the spray carnation comprising expressed genetic material encoding at least one non-indigenous F3'5'H enzyme and at

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least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

5 [0042] Yet another, aspect of the present invention is directed to a genetically modified Cerise Westpearl spray carnation plant or sport thereof exhibiting tissues of a purple to blue color, the carnation comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

10

[0043] Another aspect of the present invention is directed to a genetically modified chrysanthemum plant exhibiting tissues of a purple to blue color, the chrysanthemum comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds*
15 *chrysDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

[0044] Still another aspect of the present invention is directed to a genetically modified rose plant exhibiting tissues of a purple to blue color, the rose comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one
20 non-indigenous DFR enzyme and at least one *ds roseDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

[0045] Even yet another aspect of the present invention is directed to a genetically modified gerbera plant exhibiting tissues of a purple to blue color, the gerbera comprising
25 expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and at least one *ds gerbDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

[0046] Yet another aspect of the present invention is directed to a genetically modified
30 ornamental or horticultural plant exhibiting tissues of a purple to blue color, the ornamental or horticultural plant comprising expressed genetic sequences encoding at least

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one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least one *ds plantDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

5 [0047] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or a non-indigenous ThFNS. Reference to "purple to blue" includes mauve.

[0048] In a particular embodiment, the present invention provides a genetically modified
10 spray carnation identified herein as Cerise Westpearl (CW)/pCGP3366 and its progeny and sports. In another embodiment, the present invention provides a genetically modified spray carnation identified herein as Cerise Westpearl (CW)/pCGP3601 and its progeny and sports. In yet another embodiment, the present invention provides a genetically modified spray carnation identified herein as Cerise Westpearl (CW)/pCGP3605 and its progeny and
15 sports. Still in another embodiment, the present invention provides a genetically modified spray carnation identified herein as Cerise Westpearl (CW)/pCGP3616 and its progeny and sports. Even in yet another embodiment, the present invention provides a genetically modified spray carnation identified herein as Cerise Westpearl (CW)/pCGP3607 and its progeny and sports.

20

[0049] Progeny, reproductive material, cut flowers, tissue culturable cells and regenerable cells from the genetically plants also form part of the present invention.

[0050] The present invention further provides for the use of genetic sequences encoding at
25 least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and genetic material which down regulates a plant's indigenous *DFR* gene in the manufacture of a carnation or sports thereof exhibiting altered inflorescence including tissue having a purple to violet to blue color.

30 [0051] More particularly, the present invention is directed to the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR

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enzyme and incorporation of at least one *ds carnDFR* molecule in the manufacture of a genetically modified plant such as a spray carnation including a Cerise Westpearl carnation or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

5

[0052] The F3'5'H enzymes may be from any source. Nucleotide sequences encoding F3'5'H enzymes from *Viola* sp are particularly useful (see Table 1). Similarly, the nucleotide sequence encoding the DFR enzyme may come from any species such as but not limited to *Petunia* sp (e.g. see Table 1), iris, cyclamen, delphinium, gentian, 10 *Cymbidium*, *nierembergia*. The sense and anti-sense fragments forming the hairpin loop of the *ds carnDFR* comes from carnation. The intron in the *ds carnDFR* comes from *petunia DFR-A* intron 1 (Beld *et al*, *Plant Mol. Biol.* 13:491-502, 1989), however, any intron that is able to be processed in carnation can be used. In another embodiment no intron is used.

15 [0053] Suitable nucleotide sequences for *F3'5'H* from *Viola* sp., a *DFR* from *Petunia* sp and a *DFR* from *Dianthus* sp are set forth in Table 1.

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TABLE 1
Summary of sequence identifiers

SEQ ID NO:	NAME	SPECIES	TYPE OF SEQ	DESCRIPTION
1	<i>BPF3'5'H#40.nt</i>	<i>Viola</i> sp	nucleotide	<i>F3'5'H</i> cDNA
2	<i>BPF3'5'H#40.aa</i>	<i>Viola</i> sp	amino acid	deduced <i>F3'5'H</i> amino acid sequence
3	<i>Pet gen DFR.nt</i>	<i>Petunia</i> sp	nucleotide	<i>DFR</i> genomic clone
4	<i>Pet gen DFR.aa</i>	<i>Petunia</i> sp	amino acid	deduced <i>DFR</i> amino acid sequence
5	<i>DFRint35S F</i>		nucleotide	primer
6	<i>DFRint35S R</i>		nucleotide	primer
7	<i>ds carnDFR F</i>		nucleotide	primer
8	<i>ds carnDFR R</i>		nucleotide	primer
9	<i>Carn DFR.nt</i>	<i>Dianthus caryophyllus</i>	nucleotide	<i>DFR</i> cDNA
10	<i>Carn DFR.aa</i>	<i>Dianthus caryophyllus</i>	amino acid	deduced <i>DFR</i> amino acid sequence
11	<i>ThMT.nt</i>	<i>Torenia</i> sp.	nucleotide	<i>3'5' AMT</i> cDNA
12	<i>ThMT.aa</i>	<i>Torenia</i> sp.	amino acid	deduced <i>3'5' AMT</i> amino acid sequence
13	<i>ThFNS.nt</i>	<i>Torenia</i> sp.	nucleotide	<i>FNS</i> cDNA
14	<i>ThFNS.aa</i>	<i>Torenia</i> sp.	amino acid	deduced <i>FNS</i> amino acid sequence
15	<i>carnANS 5'</i>	<i>Dianthus caryophyllus</i>	nucleotide	<i>Carnation ANS promoter fragment</i>
16	<i>carnANS 3'</i>	<i>Dianthus caryophyllus</i>	nucleotide	<i>Carnation ANS terminator fragment</i>
17	<i>RoseCHS 5'</i>	<i>Rosa hybrida</i>	nucleotide	<i>Rose CHS promoter fragment</i>

5 [0054] BP, black pansy; nt, nucleotide; aa, amino acid; pet, petunia; carn, carnation; ThMT, S-adenosylmethionine: anthocyanin 3',5' methyltransferase from torenia; ANS, anthocyanin synthase; CHS, chalcone synthase; 3'5' AMT, S-adenosylmethionine:

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anthocyanin 3',5' methyltransferase; FNS, flavone synthase; ThFNS, flavone synthase from torenia.

[0054A] The present invention as claimed relates to:

- a cell of a genetically modified carnation or its progeny exhibiting altered
 5 inflorescence, wherein said cell is in a Cerise Westpearl background, and wherein said cell comprises at least one expressible genetic material which expresses a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions; a
 10 non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high stringency conditions; sense and anti-sense nucleotide sequences (*ds carnDFR*) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide
 15 sequence shown in SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR gene; and either a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11 or a
 20 nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:11 under high stringency conditions; or flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:13 under high stringency conditions; wherein the high stringency conditions
 25 are defined by washing in 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C; and

- a method for producing a carnation exhibiting altered inflorescence, said method comprising the steps of: introducing into regenerable cells of a carnation plant at least one expressible genetic material, wherein said carnation cells are in a Cerise Westpearl

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background, and wherein said genetic material expresses a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions; a non-indigenous

5 dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high stringency conditions; sense and anti-sense nucleotide sequences (*ds carnDFR*) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9 or a

10 nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR gene; and either a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11 or a nucleotide sequence capable of hybridizing to the

15 complementary form of the nucleotide sequence shown in SEQ ID NO:11 under high stringency conditions; or flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:13 under high stringency conditions; wherein the high stringency conditions are defined by washing in 0.2 to

20 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C, and regenerating a carnation plant therefrom.

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BRIEF DESCRIPTION OF THE FIGURES

[0055] **Figure 1** is a schematic representation of the biosynthesis pathway for the flavonoid pigments showing production of the anthocyanidin 3-glucosides that occur in most plants that produce anthocyanins. Enzymes involved in the pathway have been indicated as follows: PAL = Phenylalanine ammonia-lyase; C4H = Cinnamate 4-hydroxylase; 4CL = 4-coumarate:CoA ligase; CHS = Chalcone synthase; CHI = Chalcone flavanone isomerase; F3H = Flavanone 3-hydroxylase; DFR = Dihydroflavonol-4-reductase; ANS = Anthocyanidin synthase, 3GT = UDP-glucose: flavonoid 3-O-glucosyltransferase; Other abbreviations include: DHK = dihydrokaempferol, DHQ = dihydroquercetin, DHM = dihydromyricetin.

[0056] **Figure 2** is a diagrammatic representation of the binary plasmid pCGP3360. chimeric. The construction of pCGP3360 is described in Example 1. Selected restriction endonuclease sites are marked. Abbreviations include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from *A. tumefaciens* Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. Refer to Table 2 for a description of gene elements.

[0057] **Figure 3** is a diagrammatic representation of the binary plasmid pCGP3366. chimeric. The construction of pCGP3366 is described in Example 1. Selected restriction endonuclease sites are marked. Abbreviations include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from *A. tumefaciens* Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. In this figure "*ds carnDFR*" = the *CaMV* 35S: *ds carnDFR*: 35S 3' expression cassette. Refer to Table 2 for a description of gene elements.

[0058] **Figure 4** is a diagrammatic representation of the binary plasmid pCGP3601. chimeric. The construction of pCGP3601 is described in Example 1. Abbreviations include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from

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A. tumefaciens Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. In this figure "*ds carnDFR*" = the *CaMV 35S: ds carnDFR: 35S 3'* expression cassette. Refer to Table 2 for a description of gene elements.

5 [0059] **Figure 5** is a diagrammatic representation of the binary plasmid pCGP3605. chimeric. The construction of pCGP3605 is described in Example 1. Abbreviations include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from *A. tumefaciens* Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. In this figure "*ds carnDFR*" = the *CaMV 35S: ds carnDFR: 35S 3'* expression cassette and
10 "*ThMT*" = *CaMV 35S: ThMT: 35S 3'* expression cassette. Refer to Table 2 for a description of gene elements.

[0060] **Figure 6** is a diagrammatic representation of the binary plasmid pCGP3616. chimeric. The construction of pCGP3616 is described in Example 1. Abbreviations
15 include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from *A. tumefaciens* Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. In this figure "*ds carnDFR*" = the *CaMV 35S: ds carnDFR: 35S 3'* expression cassette. Refer to Table 2 for a description of gene elements.

20 [0061] **Figure 7** is a diagrammatic representation of the binary plasmid pCGP3607. chimeric. The construction of pCGP3607 is described in Example 1. Abbreviations include LB = Left Border from *A. tumefaciens* Ti plasmid, RB = Right border region from *A. tumefaciens* Ti plasmid, TetR = antibiotic, tetracycline resistance gene complex. In this figure "*ds carnDFR*" = the *CaMV 35S: ds carnDFR: 35S 3'* expression cassette and
25 "*ThFNS*" = *e35S 5': ThFNS: petD8 3'* expression cassette. Refer to Table 2 for a description of gene elements.

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DETAILED DESCRIPTION

5 [0062] As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a plant" includes a single plant, as well as two or more plants; reference to "an anther" includes a single anther as well as two or more anthers; reference to "the invention" includes a single aspect or multiple aspects of an invention; and so on.

10 [0063] The present invention contemplates genetically modified plants such as carnation plants and in particular spray carnations exhibiting altered inflorescence. The altered inflorescence may be in any tissue or organelle including flowers, petals, anthers and styles. Particular inflorescence contemplated herein includes a color in the range of red-
15 purple to blue color such as a purple to blue color including mauve. The color determination is conveniently measured against the Royal Horticultural Society (RHS) color chart (RHSCC) and includes colors 77A, 77B, N80B, 81A, 81B, 82A, 82B, 88D and colors in between or proximal to either end of the above range. The term "inflorescence" is not to be narrowly construed and relates to any colored cells, tissues organelles or parts thereof, as well as flowers and petals.

20

[0064] Hence, one aspect of the present invention is directed to a genetically modified plant exhibiting altered inflorescence in selected tissue, the plant comprising expressed genetic material encoding at least one F3'5'H enzyme and at least one DFR enzyme and expressing genetic material which down regulates a plant's indigenous *DFR* gene. The
25 "plant" includes a parent plant and its progeny which carry on the genetic modification. In particular, the present invention provides a genetically modified plant exhibiting altered inflorescence, the plant or its progeny comprising expressed genetic material encoding at least one non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme and at least one non-indigenous dihydroflavonol 4-reductase (DFR) enzyme and expressing genetic material
30 which down regulates expression of the plant's indigenous *DFR* gene.

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[0065] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous S-adenosylmethionine: anthocyanin 3', 5' methyltransferase (ThMT) and/or a flavone synthase (ThFNS). The genetic material which down regulates the plant's indigenous DFR gene comprises, in one embodiment, sense and anti-sense nucleotide sequences corresponding to the plant's indigenous *DFR* gene or mRNA (*ds plantDFR*).

[0066] The *ds plantDFR* molecule is a chimeric construct of sense and anti-sense genetic material from the DFR genomic DNA or cDNA corresponding to the indigenous *DFR* gene or its mRNA in the host plant. The "indigenous" DFR is the DFR normally resident in the host plant prior to genetic manipulation. A non-indigenous enzyme or gene includes a gene or other genetic material which has been introduced into a plant or a parent of the plant by genetic engineering or plant breeding practices.

15

[0067] The *ds plantDFR* molecule when expressed down-regulates *via* PTGS the *DFR* gene in the host plant. The *ds plantDFR* molecule may be from carnation (*ds carnDFR*), chrysanthemum (*ds chrysDFR*), rose (*ds roseDFR*), gerbera (*ds gerbDFR*), dianthus (*ds dianDFR*), petunia (*ds petDFR*) or from an ornamental or horticultural plant (*ds plantDFR*). Other *ds plantDFR*'s may come from lisianthus, tulip, lily, geranium, petunia, iris, *Torenia*, *Begonia*, *Cyclamen*, *Nierembergia*, *Catharanthus*, *Pelargonium*, orchid, grape, apple, *Euphorbia* or *Fuchsia*.

[0068] In a particular embodiment, the plant is a carnation. Accordingly, another aspect of the present invention is directed to a spray carnation exhibiting altered inflorescence in selected tissue, the spray carnation comprising expressed genetic material encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing of at least one *ds carnDFR* molecule. The *ds carnDFR*, when expressed, down regulates expression of the plant's indigenous *DFR* gene.

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[0069] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS.

[0070] Hence, a further aspect of the present invention is directed to a spray carnation
5 exhibiting altered inflorescence in selected tissue, the spray carnation comprising expressed genetic material encoding at least one non-indigenous F3'5'H enzyme, at least one non-indigenous DFR enzyme and at least one non-indigenous ThMT and/or ThFNS and expressing of at least one *ds carnDFR* molecule.

10 [0071] Whilst the present invention encompasses any spray carnation, a carnation of the Cerise Westpearl line is particularly useful including sports thereof. Useful sports of Cerise Westpearl include Westpearl.

[0072] Accordingly, another aspect of the present invention is directed to a genetically
15 modified Cerise Westpearl spray carnation plant line or sports thereof exhibiting tissues of a purple to blue color, the carnation comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

20

[0073] More particularly, the present invention provides a genetically modified Cerise Westpearl plant (CW)/pCGP3366 (also referred to as CW/3366 or Cerise Westpearl/3366) line exhibiting altered inflorescence, the line comprising an expressed genetic sequence encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR
25 enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

[0074] Even more particularly, the present invention provides a genetically modified Cerise Westpearl plant (CW)/pCGP3601 (also referred to as CW/3601 or Cerise
30 Westpearl/3601) line exhibiting altered inflorescence, the line comprising an expressed genetic sequence encoding at least one non-indigenous F3'5'H enzyme and at least one

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non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

5 [0075] Still more particularly, the present invention provides a genetically modified Cerise Westpearl plant (CW)/pCGP3605 (also referred to as CW/3605 or Cerise Westpearl/3605) line exhibiting altered inflorescence, the line comprising an expressed genetic sequence encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

10

[0076] Even still more particularly, the present invention provides a genetically modified Cerise Westpearl plant (CW)/pCGP3616 (also referred to as CW/3616 or Cerise Westpearl/3616) line exhibiting altered inflorescence, the line comprising an expressed genetic sequence encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

15

[0077] Yet more particularly, the present invention provides a genetically modified Cerise Westpearl plant (CW)/pCGP3607 (also referred to as CW/3607 or Cerise Westpearl/3607) line exhibiting altered inflorescence, the line comprising an expressed genetic sequence encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

20

25 [0078] In each of the above-mentioned aspects, the plant and its progeny may further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS.

[0079] Examples of Cerise Westpearl transgenic lines include #25958 (FLORIGENE Moonberry (Trade mark)) and line #25947 (FLORIGENE Moonpearl (Trade mark)).

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[0080] Additional genetically modified carnations contemplated herein include the spray carnations Westpearl, Kortina Chanel, Vega, Barbara and Artisan and the standard carnations Cinderella, Dark Rendezvous, Miledy.

5 [0081] Other genetically modified plants contemplated herein include chrysanthemums, roses, gerberas, lisianthus, tulip, lily, geranium, petunia, iris, *Torenia*, *Begonia*, *Cyclamen*, *Nierembergia*, *Catharanthus*, *Pelargonium*, orchid, grape, apple, *Euphorbia* or *Fuchsia* and other ornamental or horticultural plants.

10 [0082] Another aspect of the present invention is directed to a genetically modified chrysanthemum plant exhibiting tissues of a purple to blue color, the chrysanthemum comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds chrysDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

15

[0083] Still another aspect of the present invention is directed to a genetically modified rose plant exhibiting tissues of a purple to blue color, the rose comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and expressing at least one *ds roseDFR* molecule which
20 down regulates expression of the plant's indigenous *DFR* gene.

[0084] Yet another aspect of the present invention is directed to a genetically modified gerbera plant exhibiting tissues of a purple to blue color, the gerbera comprising expressed genetic sequences encoding at least one F3'5'H enzyme and at least one DFR enzyme and
25 expressing at least one *ds gerbDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

[0085] Yet another aspect of the present invention is directed to a genetically modified ornamental or horticultural plant exhibiting tissues of a purple to blue color, the
30 ornamental or horticultural plant comprising expressed genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one DFR enzyme and expressing at least

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one *ds plantDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene.

5 [0086] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS. The term "purple to blue color" includes mauve.

10 [0087] The *ds plantDFR*, *ds chrysDFR*, *ds roseDFR*, *ds gerbDFR*, *ds petDFR* and *ds dianDFR* comprise sense and anti-sense genomic or cDNA fragments of the gene encoding the host plant's DFR. Expression of this molecule results in down-regulation of the indigenous *DFR* gene in the host plant. Similar comments apply in relation to *ds plantDFR*'s from other host plants.

15 [0088] The genetic sequence may be a single construct carrying the nucleotide sequences encoding the F3'5'H enzymes and the DFR enzyme or multiple genetic constructs may be employed. In addition, the genetic sequences may be integrated into the genome of a plant cell or it may be maintained as an extra-chromosomal artificial chromosome. Still furthermore, the generation of a spray carnation expressing at least one F3'5'H enzyme and at least one DFR enzyme and expressing at least one *ds carnDFR* molecule may be
20 generated by recombinant means alone or by a combination of conventional breeding and recombinant DNA manipulation. The genetic sequences are "expressed" in the sense of being operably linked to a promoter and other regulatory sequences resulting in transcription and translation to produce F3'5'H and DFR enzymes.

25 [0089] Hence, another aspect of the present invention contemplates a method for producing a genetically modified plant such as a spray carnation exhibiting altered inflorescence, the method comprising introducing into regenerable cells of a plant such as a spray carnation plant expressible genetic material encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least
30 one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene and regenerating a plant therefrom or obtaining progeny from the regenerated plant.

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[0090] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS.

5 [0091] Similar methodologies are contemplated herein from chrysanthemums, rose, gerbera and ornamental plants.

[0092] The plant may then undergo various generations of growth or cultivation. Hence, reference to a genetically modified spray carnation includes progeny thereof and sister
10 lines thereof as well as sports thereof.

[0093] Another aspect of the present invention provides a method for producing a genetically modified plant such as a spray carnation line exhibiting altered inflorescence, the method comprising selecting a plant such as a spray carnation comprising expressible
15 genetic material encoding at least one non-indigenous F3'5'H enzyme and at least one DFR enzyme and incorporation of at least one *ds carnDFR* molecule which down regulates expression of the plant's indigenous *DFR* gene and crossing this plant with another plant such as a spray carnation comprising genetic material encoding the other of at least one F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least
20 one *ds carnDFR* molecule and then selecting F1 or subsequent generation plants which express the genetic material.

[0094] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS.

25

[0095] Nucleotide sequences encoding non-indigenous F3'5'H and DFR enzymes relative to a host plant may be from any source including *Viola* sp, *Petunia* sp, *Salvia* sp, *Lisianthus* sp, *Gentiana* sp, *Sollya* sp, *Clitoria* sp, *Kennedia* sp, *Campanula* sp, *Lavandula* sp, *Verbena* sp, *Torenia* sp, *Delphinium* sp, *Solanum* sp, *Cineraria* sp, *Vitis* sp, *Babiana*
30 *stricta*, *Pinus* sp, *Picea* sp, *Larix* sp, *Phaseolus* sp, *Vaccinium* sp, *Cyclamen* sp, *Iris* sp, *Pelargonium* sp, *Liparieae*, *Geranium* sp, *Pisum* sp, *Lathyrus* sp, *Catharanthus* sp, *Malvia*

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sp, *Mucuna* sp, *Vicia* sp, *Saintpaulia* sp, *Lagerstroemia* sp, *Tibouchina* sp, *Plumbago* sp, *Hypocalyptus* sp, *Rhododendron* sp, *Linum* sp, *Macroptilium* sp, *Hibiscus* sp, *Hydrangea* sp, *Cymbidium* sp, *Millettia* sp, *Hedysarum* sp, *Lespedeza* sp, *Asparagus* sp, *Antigonon* sp, *Pisum* sp, *Freesia* sp, *Brunella* sp or *Clarkia* sp, etc. For example, in one embodiment, the
5 F3'5'H enzyme comes from *Viola* sp.

[0096] The DFR may come again from the same or different plant species. For example in one embodiment the DFR enzyme comes from petunia. In another embodiment the DFR comes from iris.

10

[0097] The sense and anti-sense fragments forming the hairpin loop of the *ds carnDFR* comes from carnation (EMBL accession number Z67983, GenBank accession number gi: 1067126) or the functional equivalent from chrysanthemum, rose, gerbera or ornamental plant. Since the aim of the *ds carnDFR* is to down regulate the indigenous carnation *DFR* gene via RNAi mediated silencing various fragments of the endogenous carnation *DFR*
15 sequence may be used (see International Patent Application No. PCT/IB99/00606, Wesley *et al*, *Plant J.*, 27, 581-590, 2001, Ossowski *et al*, *Plant J.*, 53, 674-690, 2008). For example, in one embodiment a 300 bp fragment is used in a sense and anti-sense direction. The intron in the *ds carnDFR* comes from petunia *DFR-A* intron 1 (Beld *et al*, *Plant Mol.*
20 *Biol.* 13:491-502, 1989), however, any intron that is able to be processed in carnation can be used. In another embodiment, no intron is used. Again, the same comments apply for *ds plantDFR* molecules generically.

[0098] The present invention provides for the use of genetic sequences encoding at least
25 one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of genetic material which down regulates a plant's indigenous *DFR* gene in the manufacture of a carnation or sports thereof exhibiting altered inflorescence including tissue having a purple to violet to blue color.

30 [0099] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and

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incorporation of at least one *ds carnDFR* molecule in the manufacture of a spray carnation plant such as a Cerise Westpearl carnation or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

5 [0100] In another embodiment, the present invention contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least one *ds DFR* (directed at silencing of the indigenous *DFR* gene) molecule in the manufacture of a genetically modified plant selected from a rose, chrysanthemum, gerbera, tulip, lily, orchid, lisianthus, begonia,
10 torenia, geranium, petunia, nierembergia, pelargonium, iris, impatiens, cyclamen grape, apple, *Euphorbia* or *Fuchsia* or other ornamental or horticultural thereof exhibiting altered inflorescence including tissue having a purple to blue color.

[0101] In an embodiment, the plant and its progeny, further comprise genetic material
15 encoding a non-indigenous ThMT and/or ThFNS. Plant cells may require to be transformed with two or more genetic constructs each carrying one or more of the various genes. The range "purple to blue color" includes mauve.

[0102] Cut flowers, tissue culturable cells, regenerable cells, parts of plants, seeds,
20 reproductive material (including pollen) are all encompassed by the present invention.

[0103] As indicated above, nucleotide sequences encoding F3'5'H and DFR enzymes may all come from the same species of plant or from two or more different species. F3'5'H nucleotide sequence from *Viola* sp and a DFR from a *Petunia* sp and carnation are
25 particularly useful in the practice of the present invention. The nucleotide sequences encoding the F3'5'H enzymes and the DFR enzymes and the respective amino acid sequences are defined in Table 1.

[0104] Nucleic acid molecules encoding F3'5'Hs are also provided in International Patent
30 Application No. PCT/AU92/00334 and Holton *et al*, 1993 *supra*. These sequences have been used to modulate 3',5' hydroxylation of flavonoids in petunia (see International Patent

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Application No. PCT/AU92/00334 and Holton *et al*, 1993 *supra*), tobacco (see International Patent Application No. PCT/AU92/00334) and carnations (see International Patent Application No. PCT/AU96/00296). Nucleotide sequences of F3'5'H from other species such as *Viola*, *Salvia* and *Sollya* have been cloned (see International Patent Application No. PCT/AU03/01111). Any of these sequences may be used in combination with a promoter and/or terminator. The present invention particularly contemplates F3'5'H encoded by SEQ ID NO:1 and a DFR encoded by SEQ ID NO:3 and a carnation DFR (Z67983, gi: 1067126) (SEQ ID NO:9) or a nucleotide sequence capable of hybridizing to any of SEQ ID NOs:1 or 3 or 9 or a complementary form thereof under low or high stringency conditions or which has at least about 70% identity to SEQ ID NO:1 or 3 or 9 after optimal alignment.

[0105] For the purposes of determining the level of stringency to define nucleic acid molecules capable of hybridizing to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or their complementary forms, low stringency includes and encompasses from at least about 0% to at least about 15% v/v formamide and from at least about 1M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace the inclusion of formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, *J. Mol. Biol.* 5:109, 1962). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey, *Eur. J. Biochem.* 46:83, 1974). Formamide is optional in these hybridization conditions. Particular levels of washing stringency include as follows: low stringency is 6

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x SSC buffer, 1.0% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 1.0% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C.

5 [0106] Reference to at least 70% identity includes 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and 100% identity. The comparison may also be made at the level of similarity of amino acid sequences of SEQ ID NO:s:2, 4 or 10. Hence, nucleic acid molecules are contemplated herein which encode an F3'5'H enzyme or DFR having at least 70% similarity to the amino
10 acid sequence set forth in SEQ ID NOs:2 or 4 10. Again, at least 70% similarity includes 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and 100% similarity or identity.

[0107] The nucleic acid molecule encoding the F3'5'H and DFR enzymes and expression
15 of the *ds carn DFR* molecule includes one or more promoters and/or terminators. In one embodiment, a promoter is selected which directs expression of a F3'5'H and/or a DFR nucleotide sequence in tissue having a higher pH.

[0108] In an embodiment, the promoter sequence is native to the host carnation plant to be
20 transformed or may be derived from an alternative source, where the region is functional in the host plant. Other sources include the *Agrobacterium* T-DNA genes, such as the promoters for the genes encoding enzymes for biosynthesis of nopaline, octapine, mannopine, or other opines; promoters from plants, such as promoters from genes encoding ubiquitin; tissue specific promoters (see, e.g, US Patent No. 5,459,252 to Conkling *et al*; WO 91/13992 to Advanced Technologies); promoters from plant viruses (including host specific viruses), or partially or wholly synthetic promoters. Numerous promoters that are potentially functional in carnation plants (see, for example, Greve, *J. Mol. Appl. Genet.* 1:499-511, 1983; Salomon *et al*, *EMBO, J.* 3:141- 146, 1984; Garfinkel *et al*, *Cell* 27:143-153, 1983; Barker *et al*, *Plant Mol. Biol.* 2:235-350, 1983); including
25 various promoters isolated from plants (such as the Ubi promoter from the maize obi-1 gene, see, e.g, US Patent No. 4,962,028) and viruses (such as the cauliflower mosaic virus
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promoter, *CaMV 35S*). In other embodiments the promoter is *AmCHS 5'*, *RoseCHS 5'*, *carnANS 5'* and/or *petDFR 5'* (from *Pet gen DFR*) with corresponding terminators *petD8 3'*, *nos 3'*, *carn ANS 3'* and *petDFR 3'* (from *Pet gen DFR*), respectively.

5 [0109] The promoter sequences may include cis-acting sequences which regulate transcription, where the regulation involves, for example, chemical or physical repression or induction (e.g, regulation based on metabolites, light, or other physicochemical factors; see, e.g, WO 93/06710 disclosing a nematode responsive promoter) or regulation based on cell differentiation (such as associated with leaves, roots, seed, or the like in plants; see,
10 e.g. US. Patent Number 5,459,252 disclosing a root-specific promoter).

[0110] Other cis-acting sequences which may be employed include transcriptional and/or translational enhancers. These enhancer regions are well known to persons skilled in the art, and can include the ATG initiation codon and adjacent sequences.

15

[0111] The nucleic acid molecule(s) encoding at least one F3'5'H enzyme and at least one DFR enzyme and incorporation of at least one *ds carnDFR* molecule, in combination with suitable promoters and/or a terminators is/are used to modulate the activity of a flavonoid molecule in a spray carnation. Reference herein to modulating the level of a delphinidin-
20 based molecule relates to an elevation or reduction in levels of up to 30% or more particularly of 30-50%, or even more particularly 50-75% or still more particularly 75% or greater above or below the normal endogenous or existing levels of activity.

[0112] The term "inflorescence" as used herein refers to the flowering part of a plant or
25 any flowering system of more than one flower which is usually separated from the vegetative parts by an extended internode, and normally comprises individual flowers, bracts and peduncles, and pedicels. As indicated above, reference to a "transgenic plant" may also be read as a "genetically modified plant" and includes a progeny or hybrid line ultimately derived from a first generation transgenic plant.

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[0113] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least one *ds carnDFR* molecule which down regulates expression of an indigenous *DFR* gene in the manufacture of a spray carnation such as a Cerise Westpearl
5 carnation or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

[0114] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and
10 incorporation of at least one *ds chrysDFR* molecule which down regulates expression of an indigenous *DFR* gene in the manufacture of a chrysanthemum plant or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

[0115] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and
15 incorporation of at least one *ds roseDFR* molecule which down regulates expression of an indigenous *DFR* gene in the manufacture of a rose plant or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

[0116] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and
20 incorporation of at least one *ds gerbDFR* molecule which down regulates expression of an indigenous *DFR* gene in the manufacture of a gerbera plant or sports thereof exhibiting altered inflorescence including tissue having a purple to blue color.

25

[0117] The present invention also contemplates the use of genetic sequences encoding at least one non-indigenous F3'5'H enzyme and at least one non-indigenous DFR enzyme and incorporation of at least one *ds plantDFR* molecule which down regulates expression of an indigenous *DFR* gene in the manufacture of a plant exhibiting altered inflorescence
30 including tissue having a purple to blue color.

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[0118] In an embodiment, the plant and its progeny, further comprise genetic material encoding a non-indigenous ThMT and/or ThFNS. The genetic material may comprise a single or multiple constructs. The "purple to blue color" includes mauve.

5 [0119] Similar use embodiments apply to other plants as listed above.

[0120] A cultivation business model is also provided, the model comprising generating a genetically modified spray carnation plant as described herein, providing platelets, seeds, regenerable cells, tissue culturable cells or other material to a grower, generating
10 commercial sale numbers of plants, and providing cut flowers to retailers or wholesalers.

[0121] The present invention is further described by the following non-limiting Examples. In these Examples, materials and methods as outlined below were employed:

15 [0122] Methods followed were as described in Sambrook *et al*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA, 1989 or Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* 3rd edition, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA, 2001 or *Plant Molecular Biology Manual* (2nd edition), Gelvin and Schilperoot (eds), Kluwer Academic Publisher,
20 The Netherlands, 1994 or *Plant Molecular Biology Labfax*, Croy (ed), Bios scientific Publishers, Oxford, UK, 1993.

[0123] The cloning vectors pBluescript and PCR script were obtained from Stratagene, USA. pCR7 2.1 was obtained from Invitrogen, USA.

25

E. coli transformation

[0124] The *Escherichia coli* strains used were:

DH5 α

30 supE44, Δ (lacZYA-ArgF)U169, (ϕ 80lacZ Δ M15), hsdR17(r_k⁻, m_k⁺),

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recA1, endA1, gyrA96, thi-1, relA1, deoR. (Hanahan, *J. Mol. Biol.* 166:557, 1983)

XL1-Blue

supE44, hsdR17(r_k⁻, m_k⁺), recA1, endA1, gyrA96, thi-1, relA1,

5 lac⁻, [F'proAB, lacI^q, lacZΔM15, Tn10(tet^R)] (Bullock *et al*, *Biotechniques* 5:376, 1987).

BL21-CodonPlus-RIL strain

ompT hsdS(Rb- mB-) *dcm*⁺ Tet^r *gal endA* Hte [*argU ileY leuW* Cam^r]

M15 *E. coli* is derived from *E. coli* K12 and has the phenotype Nal^s, Str^s, Rif^s, Thi⁻, Ara⁺,

10 Gal⁺, Mtl⁻, F⁻, RecA⁺, Uvr⁺, Lon⁺.

[0125] Transformation of the *E. coli* strains was performed according to the method of Inoue *et al*, *Gene* 96:23-28, 1990.

15 ***Agrobacterium tumefaciens* strains and transformations**

[0126] The disarmed *Agrobacterium tumefaciens* strain used was AGL0 (Lazo *et al*, *Bio/technology* 9:963-967, 1991).

[0127] Plasmid DNA was introduced into the *Agrobacterium tumefaciens* strain AGL0 by
 20 adding 5 µg of plasmid DNA to 100 µL of competent AGL0 cells prepared by inoculating
 a 50 mL LB culture (Sambrook *et al*, 1989 *supra*) and incubation for 16 hours with
 shaking at 28°C. The cells were then pelleted and resuspended in 0.5 mL of 85% (v/v) 100
 mM CaCl₂/15% (v/v) glycerol. The DNA-*Agrobacterium* mixture was frozen by
 incubation in liquid N₂ for 2 minutes and then allowed to thaw by incubation at 37°C for 5
 25 minutes. The DNA/bacterial mix was then placed on ice for a further 10 minutes. The cells
 were then mixed with 1 mL of LB (Sambrook *et al*, 1989 *supra*) media and incubated with
 shaking for 16 hours at 28°C. Cells of *A. tumefaciens* carrying the plasmid were selected
 on LB agar plates containing appropriate antibiotics such as 50 µg/mL tetracycline or 100
 µg/mL gentamycin. The confirmation of the plasmid in *A. tumefaciens* was done by

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restriction endonuclease mapping of DNA isolated from the antibiotic-resistant transformants.

DNA ligations

- 5 [0128] DNA ligations were carried out using the Amersham Ligation Kit or Promega Ligation Kit according to procedures recommended by the manufacturer.

Isolation and purification of DNA fragments

- 10 [0129] Fragments were generally isolated on a 1% (w/v) agarose gel and purified using the QIAEX II Gel Extraction kit (Qiagen) or Bresaclean Kit (Bresatec, Australia) following procedures recommended by the manufacturer.

Repair of overhanging ends after restriction endonuclease digestion

- 15 [0130] Overhanging 5' ends were repaired using DNA polymerase I Klenow fragment according to standard protocols (Sambrook *et al*, 1989 *supra*). Overhanging 3' ends were repaired using Bacteriophage T4 DNA polymerase according to standard protocols (Sambrook *et al*, 1989 *supra*).

Removal of phosphoryl groups from nucleic acids

- 20 [0131] Shrimp alkaline phosphatase (SAP) [USB] was typically used to remove phosphoryl groups from cloning vectors to prevent re-circularization according to the manufacturer's recommendations.

Polymerase Chain Reaction (PCR)

- 25 [0132] Unless otherwise specified, PCR conditions using plasmid DNA as template included using 2 ng of plasmid DNA, 100 ng of each primer, 2 µL 10 mM dNTP mix, 5 µL 10 x Taq DNA polymerase buffer, 0.5 µL Taq DNA Polymerase in a total volume of 50 µL. Cycling conditions comprised an initial denaturation step of 5 minutes at 94°C, followed by 35 cycles of 94°C for 20 sec, 50°C for 30 sec and 72°C for 1 minute with a
30 final treatment at 72°C for 10 minutes before storage at 4°C.

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[0133] PCRs were performed in a Perkin Elmer GeneAmp PCR System 9600.

³²P-Labeling of DNA Probes

5 [0134] DNA fragments (50 to 100 ng) were radioactively labeled with 50 μ Ci of [α -³²P]-dCTP using a Gigaprime kit (Geneworks). Unincorporated [α -³²P]-dCTP was removed by chromatography on Sephadex G-50 (Fine) columns or Microbiospin P-30 Tris chromatography columns (BioRad).

10 ***Plasmid Isolation***

[0135] Single colonies were analyzed for inserts by inoculating LB broth (Sambrook *et al*, 1989 *supra*) with appropriate antibiotic selection (e.g. 100 μ g/mL ampicillin or 10 to 50 μ g/mL tetracycline etc.) and incubating the liquid culture at 37°C (for *E. coli*) or 29°C (for *A. tumefaciens*) for ~16 hours with shaking. Plasmid DNA was purified using the alkali-
15 lysis procedure (Sambrook *et al*, 1989 *supra*) or using The WizardPlus SV minipreps DNA purification system (Promega) or Qiagen Plasmid Mini Kit (Qiagen). Once the presence of an insert had been determined, larger amounts of plasmid DNA were prepared from 50 mL overnight cultures using the alkali-lysis procedure (Sambrook *et al*, 1989 *supra*) or QIAfilter Plasmid Midi kit (Qiagen) and following conditions recommended by the
20 manufacturer.

DNA Sequence Analysis

[0136] DNA sequencing was performed using the PRISM (trademark) Ready Reaction Dye Primer Cycle Sequencing Kits from Applied Biosystems. The protocols supplied by
25 the manufacturer were followed. The cycle sequencing reactions were performed using a Perkin Elmer PCR machine (GeneAmp PCR System 9600). Sequencing runs were generally performed by the Australian Genome Research Facility at the University of Queensland, St Lucia, Brisbane, Australia and at The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

30

[0137] Sequences were analyzed using a MacVector (Trade mark) application (version 9.5.2 and earlier) [MacVector Inc, Cary, North Carolina, USA].

[0138] Homology searches against Genbank, SWISS-PROT and EMBL databases were performed using the FASTA and TFASTA programs (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85(8):2444-2448, 1988) or BLAST programs (Altschul *et al*, *J. Mol. Biol.* 215(3):403-410, 1990). Percentage sequence similarities were obtained using LALIGN program (Huang and Miller, *Adv. Appl. Math.* 12:373-381, 1991) or ClustalW program (Thompson *et al*, *Nucleic Acids Research* 22:4673-4680, 1994) within the MacVector (Trade mark) application (MacVector Inc, USA) using default settings.

[0139] Multiple sequence alignments were produced using ClustalW (Thompson *et al*, 1994 *supra*) using default settings.

15 Plant transformations

[0140] Plant transformations were as described in International Patent Application No. PCT/US92/02612 or International Patent Application No. PCT/AU96/00296 or Lu *et al*, *Bio/Technology* 9:864-868, 1991. Other methods may also be employed.

[0141] Cuttings of *Dianthus caryophyllus* cv. Cerise Westpearl were obtained from Propagation Australia, Queensland, Australia.

25 Transgenic Analysis

Color coding

[0142] The Royal Horticultural Society's Color Charts, Third and/or Fifth edition (London, UK), 1995 and/or 2007 were used to provide a description of observed color. They provide an alternative means by which to describe the color phenotypes observed.

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The designated numbers, however, should be taken only as a guide to the perceived colors and should not be regarded as limiting the possible colors which may be obtained.

5 [0143] Carnation petals consist of 3 zones, the claw, corona and limb (Glimn-Lacy and Kaufman, Botany Illustrated, Introduction to Plants, Major Groups, Flowering Plant Families, 2nd ed, Springer, USA, 2006). In general only the petal limb is colored with the claw being a green color and the corona a white shade (see Figure 4). Reference to carnation petal/flower/inflorescence color generally relates to the color of the carnation petal limb.

10

Chromatographic analysis

[0144] Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) analysis was performed generally as described in Brugliera *et al*, *Plant J.* 5:81-92, 1994.

15

[0145] In general TLC and HPLC analysis was performed on extracts isolated from the petal limbs.

Extraction of anthocyanidins

20 [0146] Prior to HPLC analysis, the anthocyanin and flavonol molecules present in petal limb extracts were acid hydrolyzed to remove glycosyl moieties from the anthocyanidin or flavonol core. Anthocyanidin and flavonol standards were used to help identify the compounds present in the floral extracts.

25 [0147] Petal extracts were prepared essentially as described in Fukui *et al*, 2003 *supra*. Petal were added to 6 N HCl (0.2 mL) and boiled at 100°C for 20 min. The hydrolyzed anthocyanidins were extracted with 0.2 mL of 1-pentanol. HPLC analysis of the anthocyanidins was performed using an ODS-A312 (15 cm x 6 mm, YMC Co., Ltd, Kyoto, Japan) column, a flow rate of solvent of 1 mL min⁻¹, and detection at an absorbance of
30 600–400 nm on a SPD-M20A photodiode array detector (Shimadzu Co., Ltd). The solvent system used was as follows: acetic acid: methanol : water= 15: 20:65. Under these HPLC

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conditions, the retention time and $\lambda_{\max}$ of delphinidin were 4.0 min and 534 nm, respectively, and these values were compared with those of authentic delphinidin chloride (Funakoshi Co., Ltd, Tokyo, Japan).

- 5 [0148] The anthocyanidin peaks were identified by reference to known standards, viz delphinidin, petunidin, malvidin, cyanidin and peonidin

Stages of flower development

[0149] Carnation flowers were harvested at developmental stages defined as follows:

10

- Stage 1: Closed bud, petals not visible.
Stage 2: Flower buds opening: tips of petals visible.
Stage 3: Tips of nearly all petals exposed. "Paint-brush stage".
Stage 4: Outer petals at 45° angle to stem.
15 Stage 5: Flower fully open.

[0150] For TLC or HPLC analysis, petal limbs were collected from stage 4 flowers at the stage of maximum pigment accumulation.

- 20 [0151] For Northern blot analysis, petals were collected from stage 3 flowers at the stage of maximal expression of flavonoid pathway genes.

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EXAMPLE 1***Preparation of chimeric F3'5'H gene constructs***

[0152] A summary of promoter, terminator and coding fragments used in the preparation
5 of constructs and the respective abbreviations is listed in Table 2.

TABLE 2***Abbreviations used in construct preparations***

ABBREVIATION	DESCRIPTION
<i>CaMV 35S</i>	~0.4 kb fragment containing the promoter region from the Cauliflower Mosaic Virus 35S (<i>CaMV 35S</i>) gene - (Franck <i>et al</i> , <i>Cell</i> 21:285-294, 1980, Guilley <i>et al</i> , <i>Cell</i> , 30:763-773. 1982)
<i>35S 5'</i>	promoter fragment from <i>CaMV 35S</i> gene (Franck <i>et al</i> , 1980 <i>supra</i>) with an ~60bp 5' untranslated leader sequence (CabL) from the petunia chlorophyll a/b binding protein gene (<i>Cab 22</i> gene) [Harpster <i>et al</i> , <i>MGG</i> , 212:182-190, 1988]
<i>AmCHS 5'</i>	Promoter fragment from the <i>Antirrhinum majus</i> chalcone synthase (<i>CHS</i>) gene which includes 1.2kb sequence 5' of the translation initiation site (Sommer and Saedler, <i>Mol Gen. Gent.</i> , 202:429-434, 1986)
<i>BPF3'5'H#40</i>	<i>Viola</i> (Black Pansy) <i>F3'5'H</i> cDNA clone #40 (International Patent Application No. PCT/AU03/01111 incorporated herein by reference) (SEQ ID NO: 1)
<i>35S 3'</i>	~0.2 kb terminator fragment from <i>CaMV 35S</i> gene (Franck <i>et al</i> , 1980 <i>supra</i>)
<i>Pet gen DFR</i>	~5.3kb Petunia <i>DFR-A</i> genomic clone with it's own promoter and terminator (SEQ ID NO: 3)

ABBREVIATION	DESCRIPTION
<i>petD8 3'</i>	~0.7kb terminator region from a phospholipid transfer protein gene (<i>D8</i>) of <i>Petunia hybrida</i> cv. OGB includes a 150bp untranslated region of the transcribed region of <i>PLTP</i> gene (Holton, Isolation and characterization of petal-specific genes from <i>Petunia hybrida</i> . PhD Thesis, University of Melbourne, 1992)
<i>SuRB</i>	Herbicide (Chlorsulfuron)-resistance gene (encodes Acetolactate Synthase) with its own terminator (<i>tSuRB</i>) from <i>Nicotiana tabacum</i> (Lee <i>et al</i> , <i>EMBO J.</i> 7:1241-1248, 1988)
<i>ds carnDFR</i>	"double stranded (ds) carnation <i>DFR</i> " fragment harboring a ~0.3 kb sense partial carnation <i>DFR</i> cDNA fragment : 180bp petunia <i>DFR-A</i> intron 1 fragment (Beld <i>et al</i> , 1989 <i>supra</i>): ~0.3kb anti-sense partial carnation <i>DFR</i> fragment with the aim of formation of double stranded (hairpin loop) RNA molecule to induce RNAi-mediated silencing of the endogenous carnation <i>DFR</i> . The sequence of a complete carnation <i>DFR</i> clone (Z67983, gi: 1067126) is shown in SEQ ID NO: 9.
<i>ThMT</i>	~1.0 kb cDNA clone corresponding to S-adenosylmethionine: anthocyanin 3' 5' methyltransferase from torenia (International Patent Application No. PCT/AU03/00079 incorporated herein by reference) (SEQ ID NO: 11)
<i>ThFNS</i>	1.7 kb cDNA clone corresponding to flavone synthase from torenia (Akashi <i>et al.</i> , <i>Plant Cell Physiol.</i> 40 (11): 1182-1186, 1999, International Patent Application No. PCT/JP00/00490 incorporated herein by reference) (SEQ ID NO: 13)
<i>carnANS 5'</i>	Promoter sequence of anthocyanidin synthase (<i>ANS</i>) gene from <i>Dianthus caryophyllus</i> (See International Patent Application No. PCT/GB99/02676 incorporated herein by reference) (SEQ ID NO: 15)

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ABBREVIATION	DESCRIPTION
<i>carnANS 3'</i>	Terminator sequence of anthocyanidin synthase gene (<i>ANS</i>) from <i>Dianthus caryophyllus</i> (See International Patent Application No. PCT/GB99/02676 incorporated herein by reference) (SEQ ID NO: 16)
<i>RoseCHS 5'</i>	~2.8kb fragment containing the promoter region from a <i>CHS</i> gene of <i>Rosa hybrida</i> (see International Patent Application No. PCT/AU03/01111 incorporated herein by reference) (SEQ ID NO: 17)
<i>e35S 5'</i>	~0.7 kb fragment incorporating an enhanced <i>CaMV 35S</i> promoter (Mitsunashi <i>et al. Plant Cell Physiol.</i> 37: 49-59, 1996)

- [0153] Cerise Westpearl is a cerise colored carnation (RHSCC 57D) It typically accumulates pelargonidin-based pigments (~99% of total anthocyanin content of 1.0mg/g petal fresh weight) and therefore lacks F3'H activity and so is presumed mutant in the *F3'H* gene. HPLC analysis results on 2 flowers revealed 1.08mg/g anthocyanin (99% pelargonidin), 2.9 to 4.6 mg/g flavonols and 0.3 to 0.6mg/g dihydroflavonols accumulating in the petals of Cerise Westpearl. Cerise Westpearl is a sport of the pink colored flower Westpearl.
- 10 [0154] In order to produce novel purple/blue flowers in the spray carnation background of Cerise Westpearl, two binary vector constructs were prepared utilizing the pansy *F3'5'H* cDNA clone and petunia genomic *DFR* gene with or without a *ds carnDFR* expression cassette .
- 15 [0155] Table 3 provides a summary of chimeric F3'5'H and DFR gene expression cassettes contained in binary vector constructs used in the transformation of Cerise Westpearl (see Table 2 for an explanation of abbreviations).

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TABLE 3
Summary of Chimeric Constructs

Construct	ds plantDFR	DFR	F3'5'H	Other
pCGP3360	none	Pet gen DFR	AmCHS 5': BPF3'5'H#40: petD8 3'	
pCGP3366	CaMV35S: ds carn DFR: 35S 3'	Pet gen DFR	AmCHS 5': BPF3'5'H#40: petD8 3'	
pCGP3601	CaMV35S: ds carn DFR: 35S 3'	Pet gen DFR	AmCHS 5': BPF3'5'H#40: petD8 3'	carnANS 5':ThMT: carnANS 3'
pCGP3605	CaMV35S : ds carn DFR: 35S 3'	Pet gen DFR	AmCHS5': BPF3'5'H#40: petD8 3'	CaMV 35S:ThMT:35S 3'
pCGP3616	CaMV35S: ds carn DFR: 35S 3'	Pet gen DFR	AmCHS 5': BPF3'5'H#40: petD8 3'	RoseCHS 5':ThFNS:nos 3'
pCGP3607	CaMV35S: ds carn DFR: 35S 3'	Pet gen DFR	AmCHS 5': BPF3'5'H#40: petD8 3'	e35S 5':ThFNS:petD8 3'

- 5 NB All have ALS selectable marker gene (35S 5': SuRB)
Refer to Table 2 for a description of abbreviations and genetic elements.

[0156] The constructs pCGP3601, 3605, 3607, 3616 are all based upon pCGP3366 and have an extra expression cassette that is either a floral specific or constitutive expression of anthocyanin 3'5' methyltransferase cDNA clone from torenia (targeting methylating of the delphinidin) [pCGP3601 and 3605] or floral specific or constitutive expression of a flavone synthase cDNA clone from torenia (targeting producing of the co-pigments, flavones) [pCGP3616 and 3607].

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Preparation of the constructs

The transformation vector pCGP3360 (AmCHS 5': BPF3'5'H#40: petD8 3'; Pet gen DFR; 35S 5': SuRB)

- 5 [0157] The transformation vector pCGP3360 contains the *AmCHS* 5': *BPF3'5'H#40: petD8 3'* expression cassette and the petunia genomic *DFR-A* gene along with the 35S 5': *SuRB* selectable marker gene.

10 ***Construction of the intermediate plasmid, pCGP3356 (AmCHS 5': BPF3'5'H#40: pet D8 3')***

[0158] The plasmid pCGP3356 contains a chimeric gene consisting of *AmCHS* 5': *BPF3'5'H#40: petD8 3'* in a pBluescript backbone.

- 15 [0159] A ~1.6kb fragment harboring the *BPF3'5'H#40* cDNA clone was released from the plasmid pCGP1961 (see International Patent Application No. PCT/AU03/01111) upon digestion with the restriction endonucleases *EcoRI* and *KpnI*. The overhanging ends were repaired and the fragment was purified. The plasmid pCGP725 containing *AmCHS* 5': *petHfl: petD8 3'* in pBluescript (described in International Patent Application No. PCT/AU03/01111) was digested with the restriction endonucleases *XbaI* and *BamHI* to
- 20 release the backbone vector harboring the *AmCHS* 5' and *petD8 3'* regions. The overhanging ends were repaired and the ~4.9 kb fragment was isolated, purified and ligated with the blunt ended *BPF3'5'H#40* fragment from pCGP1961 (described above). Correct insertion of the *BPF3'5'H#40* cDNA clone in a sense orientation between the *AmCHS* 5' promoter and the *pet D8 3'* terminator was established by restriction endonuclease
- 25 analysis of plasmid DNA isolated from ampicillin-resistant transformants. The resulting plasmid was designated as pCGP3356.

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Construction of the intermediate plasmid, pCGP3357 (AmCHS 5': BPF3'5'H#40: petD8 3' in pCGP1988)

[0160] The plasmid pCGP3357 contains a chimeric gene consisting of *AmCHS* 5': *BPF3'5'H#40: petD8* 3' along with the *35S* 5': *SuRB* selectable marker gene in the pCGP1988 vector (see International Patent Application No. PCT/AU03/01111).

[0161] The plasmid pCGP3356 (described above) was digested with the restriction endonuclease *Pst*I to release a 3.5 kb fragment bearing the *AmCHS* 5': *BPF3'5'H#40: petD8* 3' expression cassette. The resulting 5'-overhang was repaired using DNA Polymerase I (Klenow fragment) according to standard protocols (Sambrook *et al*, 1989 *supra*). The fragment was purified and ligated with *Sma*I ends of the plasmid pCGP1988 (see International Patent Application No. PCT/AU03/01111). Correct insertion of *AmCHS* 5': *BPF3'5'H#40: petD8* 3' gene in a tandem orientation with respect to the *35S* 5': *SuRB* selectable marker gene cassette was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3357.

Construction of the intermediate plasmid, pCGP1472 (petunia DFR-A genomic clone)

[0162] A genomic library was made from *Petunia hybrida* cv. Old Glory Blue DNA in the vector λ 2001 (Holton, 1992 *supra*). Approximately 200, 000 pfu were plated out on NZY plates, lifts were taken onto NEN filters and the filters were hybridized with 400, 000 cpm/mL of ^{32} P-labeled petunia *DFR-A* cDNA fragment (described in Brugliera *et al*, 1994, *supra*). Hybridizing clones were purified, DNA was isolated from each and mapped by restriction endonuclease digestion. A 13 kb *Sac*I fragment of one of these clones was isolated and ligated with *Sac*I ends of pBluescriptII to create the plasmid pCGP1472. Finer mapping indicated that an ~5.3 kb *Bgl*II fragment contained the entire petunia *DFR-A* gene (Beld *et al*, 1989 *supra*).

Construction of the transformation vector, pCGP3360

[0163] The 5.3kb fragment harboring the *pet gen DFR* gene was released from the plasmid pCGP1472 upon digestion with the restriction endonuclease *Bgl*III. The overhanging ends were repaired and the fragment was purified and ligated with the repaired *Asc*I ends of the plasmid pCGP3357 (described above). Correct insertion of *pet gen DFR* gene in a tandem orientation with respect to the *AmCHS* 5': *BPF3'5'H#40: petD8* 3' and *35S* 5': *SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3360 (Figure 2).

10

The transformation vector pCGP3366 (CaMV35S: ds carn DFR: 35S 3'; Pet gen DFR; AmCHS 5': BPF3'5'H#40: petD8 3': 35S 5': SuRB)

[0164] The transformation vector pCGP3366 contains the *AmCHS* 5': *BPF3* 5'H#40: *petD8* 3' expression cassette and the petunia genomic *DFR-A* (*pet gen DFR*) genes along
15 with a *CaMV35S: ds carn DFR: 35S* 3' expression cassette and the *35S* 5': *SuRB* selectable marker gene.

Construction of the intermediate plasmid pCGP3359

[0165] A fragment bearing 180 bp of the petunia *DFR-A* intron 1 was amplified by PCR using the plasmid pCGP1472 (described above) as template and the following primers:

DFRint35S F GCAT CTCGAG GGATCC TCG TGA TCC TGG TAT GTT TTG
*Xho*I *Bam*HI

(SEQ ID NO:5)

25

DFRint35S R GCAT TCTAGA AGATCT CTT CTT GTT CTC TAC AAA ATC
*Bgl*II *Bam*HI

(SEQ ID NO:6)

30 **[0166]** The forward primer (DFRint35S F) was designed to incorporate the restriction
endonuclease recognition sites *Xho*I and *Bam*HI at the 5'-end. The reverse primer

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(DFRint35S R) was designed to incorporate *Xba*I and *Bgl*II restriction endonuclease recognition sites at the 3'-end of the 180 bp product that was amplified. The resulting 180 bp PCR product was then digested with the restriction endonucleases *Xho*I and *Xba*I and ligated with *Xho*I/*Xba*I ends of the plasmid pRTppoptcAFP (a source of the *CaMV35S* promoter and terminator fragments) (Wnendt *et al.*, *Curr Genet* 25: 510-523, 1994). Correct insertion of the petunia *DFR-A* intron 1 fragment between the *CaMV35S* and 35S 3' fragments of pRTppoptcAFP was confirmed by restriction endonuclease analysis of plasmid DNA isolated from ampicillin-resistant transformants. The resulting plasmid was designated pCGP3359.

10

Isolation of full-length carnation DFR cDNA clone

[0167] Isolation of a partial carnation *DFR* cDNA clone has been described in International Patent Application No. PCT/AU96/00296.

15 [0168] Around 120,000 pfus of a carnation Kortina Chanel petal cDNA library (construction of which is described in International Patent Application No. PCT/AU97/000124) were screened using the ³²P-labeled fragments of an *Eco*RI/*Xho*I partial carnation *DFR* fragment (see International PCT/AU96/00296) as a probe under high stringency hybridization washing conditions. Around 20 strongly hybridizing plaques were
20 selected and further purified. Of these one (*KCDFR#17*) contained a 1.3kb insert and represented a full-length carnation *DFR* cDNA clone with 51 bp of 5' untranslated sequence. The plasmid was designated as pCGP1547.

Construction of the intermediate plasmid pCGP3363 (*CaMV35S*: sense partial carnation *DFR*: petunia *DFR* intron 1: 35S 3')

[0169] A fragment bearing ~300 bp of the carnation *DFR* cDNA clone was amplified by PCR using the plasmid pCGP1547 (described above) as template and the following primers:

30 ds carnDFR F GCAT TCTAGA CTCGAG CGA GAA TGA GAT GAT AAA ACC
*Xba*I *Xho*I

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(SEQ ID NO:7)

ds carnDFR R GCAT AGATCT GGATCC GAG ATT GTT TTC TGC TGC G*Bgl*III *Bam*HI

5 (SEQ ID NO:8)

[0170] The forward primer (ds carnDFR F) was designed to incorporate the restriction endonuclease recognition sites *Xba*I and *Xho*I at the 5'-end. The reverse primer (ds carnDFR R) was designed to incorporate *Bgl*III and *Bam*HI restriction endonuclease recognition sites at the 3'-end of the ~300 bp product that was amplified. The resulting ~300 bp PCR product was then digested with the restriction endonucleases *Xho*I and *Bam*HI and ligated with *Xho*I/*Bam*HI ends of the plasmid pCGP3359 (described above). Correct insertion of the partial carnation *DFR* fragment in a sense direction between the *CaMV*35S and petunia *DFR* intron 1 fragment of the plasmid pCGP3359 was confirmed by restriction endonuclease analysis of plasmid DNA isolated from ampicillin-resistant transformants. The resulting plasmid was designated pCGP3363.

Construction of the intermediate plasmid pCGP3364 (CaMV35S: ds Carn DFR: 35S 3')

[0171] The amplified partial carnation *DFR* fragment described above was digested with the restriction endonucleases *Bgl*III and *Xba*I and ligated with *Bgl*III/*Xba*I ends of the plasmid pCGP3363 (described above). Correct insertion of the partial carnation *DFR* fragment in an anti-sense direction between the petunia *DFR* intron 1 and 35S 3' fragments of the plasmid pCGP3363 was confirmed by restriction endonuclease analysis of plasmid DNA isolated from ampicillin-resistant transformants. The resulting plasmid was designated pCGP3364.

Construction of the transformation vector, pCGP3366

[0172] A ~1.4 kb fragment bearing the *CaMV*35S: *ds carn DFR: 35S 3'* expression cassette was released from the plasmid pCGP3364 (described above) upon digestion with the restriction endonuclease *Pst*I. The fragment was purified and ligated with the *Pst*I ends of the plasmid pCGP3360 (described above) (Figure 2). Correct insertion of *CaMV*35S: *ds*

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carn DFR; 35S 3' expression cassette in a tandem orientation with respect to the *AmCHS* 5': *BPF3'5'H#40*: *petD8* 3', *pet gen DFR* and 35S 5': *SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3366 (Figure 3).

5

[0173] The T-DNAs of the transformation vectors pCGP3360 and pCGP3366 were introduced into the spray carnation line, Cerise Westpearl *via Agrobacterium*-mediated transformation. Transgenic cells were selected based on their ability to grow and produce roots on media containing the herbicide, chlorsulfuron. Transgenic plantlets with roots
10 were removed from media and transferred to soil and grown to flowering in temperature controlled greenhouses in Bundoora, Victoria, Australia.

[0174] The color of the petal limbs of the transgenic plants were recorded by eye using RHSCC and HPLC analysis was used to determine the anthocyanidins in the hydrolyzed
15 petal limb extracts. The results are summarized in Table 4.

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TABLE 4

Results of transgenic analysis of petals from Cerise Westpearl carnations transformed with T-DNAs containing F3'5'H and DFR gene expression cassettes.

<i>transgenes</i>	pCGP	#tg	% CC	# HPLC	% del (Range)	Av del	Del mg/g FW
<i>AmCHS 5':BP F3'5'H #40:petD8 3'; Pet gen DFR</i>	3360	38	57%	13	52 to 76%	65%	0.42 to 1.98
<i>AmCHS 5':BP F3'5'H #40:petD8 3'; Pet gen DFR; CaMV 35S: ds carnDFR: 35S 3'</i>	3366	47	94%	34	51 to 93%	84%	0.28 to 2.68

5

Transgenes = chimeric *F3'5'H* and *DFR* nucleotide sequences contained on the T-DNA

pCGP = plasmid pCGP identification number of the transformation vector used in the transformation experiment (refer to Table 3 for details)

10 #tg = total number of transgenic carnation lines produced

% CC = the percentage of the total number of events produced that had a shift in petal color towards the purple range

HPLC = number of individual events of which the anthocyanidins of hydrolyzed petal limb extracts were analyzed by HPLC. Petals for analysis were selected based on a visible shift in color of the petal from pink into the purple range.

15

% del (range) = the range in % of delphinidin detected in the hydrolyzed extracts of the petals for the population of transgenic events

Av del = the average % of delphinidin detected in the hydrolyzed extracts of the petals for the population of transgenic events

20

Del mg/g FW = the range in the amount of delphinidin (in mg/g of fresh weight) detected in the hydrolyzed extracts of the petals for the population of transgenic events

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[0175] The results suggest that of the two constructs tested (pCGP3360 and pCGP3366), pCGP3366 resulted in a higher percentage of events that produced flowers with a shift in color to the purple range. Furthermore the average delphinidin detected in the hydrolyzed
5 extracts of the petals was higher in pCGP3366 events compared to pCGP3360 events. This was presumably due to the down regulation of the endogenous carnation *DFR* by the ds carnDFR cassette *via* RNAi-mediated silencing leading to decreased competition between the endogenous DFR and the introduced F3'5'H for the DHK substrate. The introduced petunia DFR (which is not able to utilise DHK) subsequently allowed conversion DHM
10 (product of F3'5'H reaction on DHK) to leucodelphinidin and activity by the endogenous anthocyanin pathway enzymes resulted in delphinidin derived pigments accumulating in the petal tissue. To identify spray carnation lines producing petals of a novel color, the colors of petal limbs were compared to mauve/purple carnation lines already in the market place. These included the midi carnation lines FLORIGENE Moonshadow (Trade mark)
15 [82A, 82B] and FLORIGENE Moondust (76A) and the standard carnation lines FLORIGENE Moonvista (Trade mark) [81A+], FLORIGENE Moonshade (Trade mark) [81A, 82A], FLORIGENE Moonlite (Trade mark) [77D/82D, 77C, N80B] and FLORIGENE Moonaqua (Trade mark) [84A/B]. Twenty two CW/3366 lines were initially selected as being novel spray carnation lines whilst only one CW/3360 line was selected as
20 being novel spray carnation line. Further trialling with respect to petal color consistency and petal number reduced the list to 11 CW/3366 lines and no CW/3360 lines as being novel spray carnation lines with potential for new product lines (Table 5).

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TABLE 5

RHS color code of the petal limb and delphinidin levels detected in selected Cerise Westpearl/3366 lines

ACCESSION NUMBER	RHSCC NUMBER	Delphinidin levels %, (mg/g FW)
25930	77A	92% (2.2 mg/g)
25931	77A+	93% (1.7 mg/g)
25932	77A+	93% (2.3mg/g)
25946	81B/82B	84% (0.3 mg/g)
25947	77D, 78D	nd
25958	81B, 82A, N80B	81% (0.5 mg/g)
25961	77B, 88D	nd
25965	82A	85% (0.7 mg/g)
25966	81B, 82A	83% (0.4 mg/g)
25973	82b	84% (0.5 mg/g)
25976	81B	84% (0.3 mg/g)
FLORIGENE Moondust	76A	100% (0.035mg/g)
FLORIGENE Moonshadow	82A, 82B	94% (0.35 mg/g)
FLORIGENE Moonshade	81A, 82A	97% (0.6 mg/g)
FLORIGENE Moonlite	77D/82D, 77C	71% (0.06 mg/g)
FLORIGENE Moonaqua	84A/B	74% (0.07 mg/g)
FLORIGENE Moonvista	81A+	98% (1.8 mg/g)

5

Accession number = unique number given to individual transgenic event

RHSCC number = The color code of the petal limbs from the flowers of transgenic carnation lines. "+" alongside an RHSCC number highlights that the color is a darker or more intense shade of the selected code

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delphinidin levels = delphinidin levels detected in hydrolyzed extracts of petal limb tissue as determined by HPLC given in percentage of total anthocyanidins and mg/g of fresh weight of petal tissue.

nd = not done

5

[0176] Further field trial assessments in Colombia revealed that lines #25958, #25947, #25973, #25965 and #25976 produced novel spray carnation flower colors with consistent and stable colors and good plant growth characteristics. Two lines (#25958 and #25947) were selected for commercialisation. Line #25958 was subsequently named FLORIGENE
10 Moonberry (Trade mark) and line #25947 was called FLORIGENE Moonpearl (Trade mark). Both are being grown in Colombia for production of cut flowers to markets around the world.

Introduction of the transformation vector pCGP3366 into other carnation varieties

15 [0177] Due to the success in obtaining high delphinidin levels in the carnation variety, Cerise Westpearl using the construct pCGP3366 (containing at least one F3'5'H enzyme and at least one DFR enzyme and incorporation of at least one *ds carnDFR* molecule) the same genes are introduced into other colored carnation cultivars such as but not limited to Cinderella, Westpearl, Vega, Artisan, Barbara, Dark Rendezvous, Miledy, Kortina Chanel.

20

[0178] The transgenic plants are assessed for flower color as described above and lines with novel flower color (as compared to controls) are selected for commercialization.

Use of the binary vector pCGP3366 as a backbone-addition of other expression cassettes.

25 [0179] In order to shift petal color further towards the blue/purple spectrum other genes that modulated anthocyanin or flavonoid composition were added to the pCGP3366 binary vector. These included genes coding for S-adenosylmethionine: anthocyanin 3'5' methyltransferase (AMT) activity to modulate the production of methylated anthocyanins such as the production of malvidin and petunidin pigments and genes coding for flavone synthase (FNS) activity to
30 modulate the production of flavones in carnation.

Addition of AMT expression cassettes to the pCGP3366 binary construct

[0180] In an attempt to produce anthocyanins based upon malvidin (the methylated form of delphinidin) 2 new transformation vectors, pCGP3601 and pCGP3605, were prepared by addition of *AMT* expression cassettes to the transformation vector, pCGP3366 (Figure 3). The
 5 *AMT* sequence from torenia (International Patent Application No. PCT/AU03/00079) was used under the control of a floral specific promoter fragment from the *ANS* gene of carnation (*carnANS* 5') and a constitutive promoter fragment from the cauliflower mosaic virus 35S gene (*CaMV35S*).

10 *The transformation vector, pCGP3601 (carnANS 5': ThMT: carnANS 3'; AmCHS 5': BPF3'5'H#40:petD8 3'; Pet gen DFR; CaMV35S 5': ds carn DFR: 35S 3'; 35S 5': SuRB)*

[0181] The binary construct pCGP3601 contains a *carnANS* 5': *ThMT*: *carnANS* 3' expression cassette in the pCGP3366 binary construct backbone (described above) (Figure 3).

15

Construction of the intermediate plasmid, pCGP3431 (carnANS 5':ThMT: carnANS 3')

[0182] A ~1.0kb fragment bearing the torenia *AMT* cDNA clone (*ThMT*) (SEQ ID NO: 11) was released from the plasmid pTMT5 (described in International Patent Application No.: PCT/JP00/00490) upon digestion with the restriction endonucleases *EcoRI* and *Asp718*. The
 20 overhanging ends were repaired and the purified fragment was ligated with *XbaI/PstI* repaired ends of the plasmid pCGP1275 (described in International Patent Application No. PCT/AU2008/001700). Correct insertion of the *ThMT* fragment in between a promoter fragment of the carnation *ANS* gene (*carnANS* 5') and a terminator fragment of the carnation *ANS* gene (*carnANS* 3') was established by restriction
 25 endonuclease analysis of plasmid DNA isolated from ampicillin resistant transformants. The resulting plasmid was designated as pCGP3431.

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Construction of the transformation vector, pCGP3601

[0183] A 4.4kb fragment harboring the *carnANS* 5': *ThMT*: *carnANS* 3' expression cassette was isolated from the plasmid pCGP3431 (described above) upon digestion with the restriction endonuclease *Cla*I. The overhanging ends were repaired and the purified fragment

5 was ligated with the *Pme*I ends of the plasmid pCGP3366 (described above) (Figure 3). Correct insertion of the *carnANS* 5':*ThMT*: *carnANS* 3' expression cassette in a tandem orientation with respect to the *AmCHS* 5': *BPF3'5'H#40*: *petD8* 3', *pet* *gen* *DFR*; *CaMV35S* 5': *ds carn DFR*: 35S 3' and 35S 5': *SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants.

10 The resulting plasmid was designated as pCGP3601 (Figure 4).

The transformation vector, pCGP3605 (CaMV35S: ds carn DFR: 35S 3'; CaMV35S: ThMT: 35S 3'; Pet gen DFR; AmCHS 5': BPF3'5'H#40:petD8 3'; 35S 5': SuRB)

[0184] The binary construct pCGP3605 contains a *CaMV35S*: *ThMT*: 35S 3' expression

15 cassette in the pCGP3366 binary construct backbone (described above) (Figure 3).

Construction of the intermediate plasmid, pCGP3097 (CaMV35S: ThMT: 35S 3')

[0185] The plasmid pTMT5 (described in International Patent Application No. PCT/JP00/00490) was firstly linearized upon digestion with the restriction endonuclease

20 *Asp*718. The overhanging ends were repaired and a ~1.0kb fragment bearing the torenia *AMT* cDNA clone (*ThMT*) (SEQ ID NO: 11) was then released from the linearized plasmid upon digestion with the restriction endonuclease *Eco*RI. The fragment was purified and ligated with *Xba*I (repaired ends) /*Eco*RI ends of the plasmid pRTppoptcAFP (a source of the *CaMV35S* promoter and terminator fragments) (Wnendt *et al.*, 1994, *supra*). Correct insertion of the

25 *ThMT* fragment in a sense orientation between the promoter and terminator fragments of the cauliflower mosaic virus 35S gene (*CaMV35S* and 35S 3' respectively) was established by restriction endonuclease analysis of plasmid DNA isolated from ampicillin resistant transformants. The resulting plasmid was designated as pCGP3097.

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Construction of the transformation vector, pCGP3605

[0186] A ~1.6kb fragment harboring the *CaMV35S: ThMT: 35S 3'* expression cassette was isolated from the plasmid pCGP3097 (described above) upon digestion with the restriction endonuclease *Pst*I. The overhanging ends were repaired and the purified fragment was ligated with the *Pme*I ends of the plasmid pCGP3366 (described above) (Figure 3). Correct insertion of the *CaMV35S: ThMT: 35S 3'* expression cassette in a tandem orientation with respect to the *AmCHS 5': BPF3'5'H#40: petD8 3', pet gen DFR; CaMV35S: ds carn DFR: 35S 3'* and *35S 5': SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3605 (Figure 5).

Addition of FNS expression cassettes to the pCGP3366 binary construct

[0187] In an attempt to produce flavones (to act as co-pigments) and high levels of delphinidin in a Cerise Westpearl background, a further 2 transformation vectors, pCGP3616 and pCGP3607 were prepared by adding *FNS* expression cassettes to the transformation vector, pCGP3366 (Figure 3). The *FNS* sequence from torenia (International Patent Application No. PCT/JP00/00490) (SEQ ID NO: 13) was used under the control of a floral specific promoter fragment from the *CHS* gene of rose (*RoseCHS 5'*) and a constitutive promoter fragment from the cauliflower mosaic virus 35S gene (*CaMV35S*).

The transformation vector, pCGP3616 (CaMV35S: ds carn DFR: 35S 3'; RoseCHS 5': ThFNS: nos 3'; Pet gen DFR; AmCHS 5': BPF3'5'H#40:petD8 3'; 35S 5': SuRB)

[0188] The binary construct pCGP3616 contains a *RoseCHS 5': ThFNS: nos 3'* expression cassette in the pCGP3366 binary construct backbone (described above) (Figure 3).

Construction of the intermediate plasmid, pCGP3123 (RoseCHS 5': ThFNS: nos 3')

[0189] A 3.2kb fragment bearing *e35S 5': ThFNS: petD8 3'* expression cassette was released from the binary vector plasmid pSFL535 (described in International Patent Application WO2008/156206) upon digestion with the restriction endonuclease *Asc*I. The fragment was purified and ligated with the *Asc*I ends of the 2.9kb plasmid pUCAP+*Asc*I (The plasmid pUCAP/*Asc*I is a pUC19 based cloning vector with extra cloning sites specifically an *Asc*I

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recognition site at either ends of the multicloning site). Correct insertion of the *e35S 5': ThFNS: petD8 3'* expression cassette in the pUC based cloning vector was established by restriction endonuclease analysis of plasmid DNA isolated from ampicillin resistant transformants. The resulting plasmid was designated as pCGP3123.

5

Construction of the intermediate plasmid, pCGP3612 (RoseCHS 5':ThFNS: nos 3')

[0190] This plasmid pCGP3123 (described above) was linearized upon digestion with the restriction endonuclease *Bam*HI. The overhanging ends were repaired and a fragment bearing the *ThFNS* cDNA clone was then released after partial digestion of the linearized plasmid with the restriction endonuclease *Xho*I. The 1.7 kb fragment was purified and ligated with *Sma*I/*Xho*I ends of the plasmid pCGP2203 (*Rose CHS 5': BPF3'5'H#18: nos 3'* in pBluescript backbone) described in International Patent Application No. PCT/AU2008/001694. Correct insertion of the *ThFNS* fragment between the *RoseCHS* promoter and *nos* terminator was established by restriction endonuclease analysis of plasmid DNA isolated from ampicillin resistant transformants. The resulting plasmid was designated pCGP3612.

Construction of the transformation vector, pCGP3616

[0191] A 4.9 kb fragment harboring the *RoseCHS 5': ThFNS: nos 3'* expression cassette was isolated from the plasmid pCGP3612 (described above) upon digestion with the restriction endonucleases *Bgl*II and *Not*I. The overhanging ends were repaired and the purified fragment was ligated with the *Pme*I ends of the plasmid pCGP3366 (described above) (Figure 3). Correct insertion of the *RoseCHS 5': ThFNS: nos 3'* expression cassette in a tandem orientation with respect to the *AmCHS 5': BPF3'5'H#40: petD8 3', pet gen DFR; CaMV35S: ds carn DFR: 35S 3' and 35S 5': SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3616 (Figure 6).

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The transformation vector, pCGP3607 (CaMV35S': ds carn DFR: 35S 3'; e35S 5': ThFNS: petD8 3'; Pet gen DFR; AmCHS 5': BPF3'5'H#40:petD8 3'; 35S 5': SuRB)

[0192] The binary construct pCGP3607 contains an *e35S 5': ThFNS: petD8 3'* expression cassette in the pCGP3366 binary construct backbone (described above) (Figure 3).

5

Construction of the transformation vector, pCGP3607

[0193] A 3.2kb fragment bearing *e35S 5':ThFNS: petD8 3'* expression cassette was released from the plasmid pCGP3123 (described above) upon digestion with the restriction endonuclease *AscI*. The fragment was purified and ligated with the *PmeI* ends of the plasmid pCGP3366 (described above) (Figure 3). Correct insertion of the *e35S 5': ThFNS: petD8 3'* expression cassette in a tandem orientation with respect to the *AmCHS 5': BPF3'5'H#40: petD8 3', pet gen DFR; CaMV35S: ds carn DFR: 35S 3'* and *35S 5': SuRB* genes was established by restriction endonuclease analysis of plasmid DNA isolated from tetracycline-resistant transformants. The resulting plasmid was designated as pCGP3607 (Figure 7).

15

[0194] The T-DNAs of the transformation vectors pCGP3601 (Figure 4), pCGP3605 (Figure 5), pCGP3607 (Figure 7) and pCGP3616 (Figure 6) were introduced into the spray carnation line, Cerise Westpearl via *Agrobacterium*-mediated transformation. Transgenic cells were selected based on their ability to grow and produce roots on media containing the herbicide, chlorsulfuron. Transgenic plantlets with roots were removed from media and transferred to soil and grown to flowering in temperature controlled greenhouses in Bundoora, Victoria, Australia. The results are summarized in Table 6.

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TABLE 6

A summary of the number of transgenic Cerise Westpearl that resulted in a significant shift in petal color towards the purple/violet range.

Construct	Addition to pCGP3366	#Tg	CC
pCGP3601	<i>carnANS 5': ThMT: carnANS 3'</i>	32	11
pCGP3605	<i>CaMV35S: ThMT: 35S 3'</i>	38	14
pCGP3607	<i>e35S 5': ThFNS: petD8 3'</i>	37	15
pCGP3616	<i>RoseCHS 5': ThFNS: nos 3'</i>	19	2

5

Construct = plasmid pCGP identification number of the transformation vector used in the transformation experiment

Addition to pCGP3366 = Extra expression cassette added to the pCGP3366 (Figure 3) backbone containing *AmCHS 5':BP F3'5'H #40:petD8 3'*; *Pet gen DFR; CaMV 35S: ds carnDFR: 35S 3' transgenes*

10

#Tg = total number of transgenic carnation lines produced

CC = "Color Change" -the number of events produced that had a shift in petal color towards the purple range

15 **[0195]** The transgenic plants are assessed for flower color as described above and lines with novel flower color (as compared to controls) are selected for commercialization.

[0196] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

20

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BIBLIOGRAPHY

Altschul *et al*, *J. Mol. Biol.* 215(3):403-410, 1990

Barker *et al*, *Plant Mol. Biol.* 2:235-350, 1983

Beld *et al*, *Plant Mol. Biol.* 13:491-502, 1989

Bonner and Laskey, *Eur. J. Biochem.* 46:83, 1974

Brouillard and Dangles, In: *The Flavonoids -Advances in Research since 1986*, Harborne, (ed), Chapman and Hall, London, UK, 1-22, 1993

Brugliera *et al*, *Plant J.* 5:81-92, 1994

Bullock *et al*, *Biotechniques* 5:376, 1987

Forkmann and Ruhnau, *Naturforsch C.* 42c, 1146-1148, 1987

Franck *et al*, *Cell* 21:285-294, 1980

Fukui *et al*, *Phytochemistry*.63(1), 15-23, 2003

Garfinkel *et al*, *Cell* 27:143-153, 1983

Glimn-Lacy and Kaufman, *Botany Illustrated, Introduction to Plants, Major Groups, Flowering Plant Families*, 2nd ed, Springer, USA, 2006

Greve, *J. Mol. Appl. Genet.* 1:499-511, 1983

- 59 -

Guilley *et al*, *Cell* 30:763-773, 1982

Harpster *et al*, *MGG* 212:182-190, 1988

Holton, Isolation and characterization of petal-specific genes from *Petunia hybrida*. PhD Thesis, University of Melbourne, 1992

Holton *et al*, *Nature*, 366:276-279, 1993

Holton and Cornish, *Plant Cell* 7:1071-1083, 1995

Huang and Miller, *Adv. Appl. Math.* 12:373-381, 1991

Inoue *et al*, *Gene* 96:23-28, 1990

Katsumoto *et al*, *Plant Cell Physiol.* 48(11), 1589–1600, 2007

Johnson *et al* *Plant Journal*, 19:81-85, 1999

Lazo *et al*, *Bio/technology* 9:963-967, 1991

Lee *et al*, *EMBOJ* 7:1241-1248, 1988

Lu *et al*, *Bio/Technology* 9:864-868, 1991

Marmur and Doty, *J. Mol. Biol.* 5:109, 1962

Mitsuhashi *et al*. *Plant Cell Physiol.* 37: 49-59, 1996

Mol *et al*, *Trends Plant Sci.* 3:212–217, 1998

- 60 -

Nakayama *et al*, *Phytochemistry*, 55, 937-939, 2000

Ossowski *et al*, *Plant J.*, 53, 674-690, 2008

Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85(8):2444-2448, 1988

Plant Molecular Biology Labfax, Croy (ed), Bios scientific Publishers, Oxford, UK, 1993

Plant Molecular Biology Manual (2nd edition), Gelvin and Schilperoot (eds), Kluwer Academic Publisher, The Netherlands, 1994

Salomon *et al*, *EMBO, J.* 3:141- 146, 1984

Sambrook *et al*, *Molecular Cloning:A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA, 1989

Sambrook and Russell, *Molecular Cloning:A Laboratory Manual* 3rd edition, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA, 2001

Seitz and Hinderer, Anthocyanins. In:*Cell Culture and Somatic Cell Genetics of Plants*. Constabel and Vasil (eds.), Academic Press, New York, USA, 5:49-76, 1988

Sommer and Saedler, *Mol. Gen. Genet* 202:429-434, 1986

Strack and Wray, In:*The Flavonoids - Advances in Research since 1986*. Harborne, J.B. (ed), Chapman and Hall, London, UK, 1-22, 1993

Tanaka and Mason, In *Plant Genetic Engineering*, Singh and Jaiwal (eds) SciTech Publishing Llc., USA, 1:361-385, 2003,

Tanaka *et al*, *Plant Cell, Tissue and Organ Culture* 80:1-24, 2005

Tanaka and Brugliera, *In Flowering and Its Manipulation, Annual Plant Reviews*
Ainsworth (ed), Blackwell Publishing, UK, 20:201-239, 2006)

Thompson *et al*, *Nucleic Acids Research* 22:4673-4680, 1994

Wnendt *et al.*, *Curr Genet* 25: 510-523, 1994

Wesley *et al*, *Plant J.*, 27, 581-590, 2001

Winkel-Shirley, *Plant Physiol.* 126:485-493, 2001a

Winkel-Shirley, *Plant Physiol.* 127:1399-1404, 2001b

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 27650-141 Seq 05-JUL-11 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

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61c

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Phe Ser Thr Arg Ser Ala Ala Asp Asn Gly His Asn Arg Glu Ala Ile
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Ala Ile Ser Ala Gln Asn Tyr Ala Ser Gly Lys Glu Asn Ala Pro Val
355         360         365
Ala Asn His Thr Glu Met Leu Thr Asn Val Glu Val
370         375         380

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<210> 5

<211> 37

<212> DNA

<213> artificial sequence

61f

<220>

<223> DFRint3 5S F

<400> 5

gcattctcgag ggatcctcgt gatcctggta tgttttg

37

<210> 6

<211> 37

<212> DNA

<213> artificial sequence

<220>

<223> DFRint3 5S R

<400> 6

gcattctaga agatctcttc ttgttctcta caaaatc

37

<210> 7

<211> 37

<212> DNA

<213> artificial sequence

<220>

<223> ds carnDFR F

<400> 7

gcattctaga ctgagcgag aatgagatga taaaacc

37

<210> 8

<211> 35

<212> DNA

<213> artificial sequence

<220>

<223> ds carnDFR R

<400> 8

gcatagatct ggatccgaga ttgttttctg ctgcg

35

<210> 9

<211> 1215

<212> DNA

<213> artificial sequence

<220>

<223> Carn DFR.nt - Dianthus caryophyllus

<400> 9

ctcgaatttg atttgataga cacttacaaa atacaataga tttgtaacaa aaaaagatgg

60

tttctagtac aattaacgag aactagacg gtaaacaatga cattaataag gttgggcaag

120

gcgagaccgt ctgcgtgacc ggtgcatccg gtttcattgg ttcattggctc atcatgcgac

180

61g

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tccttgagcg tggctacacc gttecgagcca cagttcgtga tcccgataac actaaaaaag 240
tgcaacattt gttggatttg ccaaagcca agactaactt gacactttgg aaagcggacc 300
tacacgaaga aggcagcttt gatgcagccg ttgatggatg caccggcgtg tttcatatcg 360
ccacacctat ggactttgag tccaaggatc ccgagaatga gatgataaaa cctacaataa 420
atggaatggt ggacatacta aagtcatgtg tgaaggccaa actaaggagg gtgggttttta 480
cgtcatctgg tggaactgtg aatgtcgaag cgactcaaaa acccgtctat gacgagactt 540
gttggagtgc tctcgacttc attcgctctg ttaagatgac tggatggatg tacttcgtgt 600
caaaaatact agcagagcaa gcagcgtgga agtacgcagc agaaaacaat ctcgaattca 660
tcagtatcat tccacccctc gttgttgggc cctttatcat gccttctatg cctcctagcc 720
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tgctcagggg agaataccct gagtacaatg tccccaccaa attcaaggac tacaaggagg 960
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ctctttccct agaacatcat ctttgtgttt tccgagtgc gcttatattt tttaaataaa 1140
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gaaaaaaaa aaaaa 1215

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<210> 10
 <211> 360
 <212> PRT
 <213> artificial sequence

<220>
 <223> Carn DFR.aa - dianthus caryophyllus

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<400> 10
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Asn Lys Val Gly Gln Gly Glu Thr Val Cys Val Thr Gly Ala Ser Gly
20          25          30
Phe Ile Gly Ser Trp Leu Ile Met Arg Leu Leu Glu Arg Gly Tyr Thr
35          40          45
Val Arg Ala Thr Val Arg Asp Pro Asp Asn Thr Lys Lys Val Gln His
50          55          60
Leu Leu Asp Leu Pro Asn Ala Lys Thr Asn Leu Thr Leu Trp Lys Ala
65          70          75          80
Asp Leu His Glu Glu Gly Ser Phe Asp Ala Ala Val Asp Gly Cys Thr
85          90          95
Gly Val Phe His Ile Ala Thr Pro Met Asp Phe Glu Ser Lys Asp Pro
100         105         110
Glu Asn Glu Met Ile Lys Pro Thr Ile Asn Gly Met Leu Asp Ile Leu
115         120         125
Lys Ser Cys Val Lys Ala Lys Leu Arg Arg Val Val Phe Thr Ser Ser
130         135         140
Gly Gly Thr Val Asn Val Glu Ala Thr Gln Lys Pro Val Tyr Asp Glu
145         150         155         160
Thr Cys Trp Ser Ala Leu Asp Phe Ile Arg Ser Val Lys Met Thr Gly
165         170         175
Trp Met Tyr Phe Val Ser Lys Ile Leu Ala Glu Gln Ala Ala Trp Lys
180         185         190
Tyr Ala Ala Glu Asn Asn Leu Glu Phe Ile Ser Ile Ile Pro Pro Leu
195         200         205

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61h

Val	Val	Gly	Pro	Phe	Ile	Met	Pro	Ser	Met	Pro	Pro	Ser	Leu	Ile	Thr
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Ala	Leu	Ser	Pro	Ile	Thr	Arg	Thr	Glu	Ser	His	Tyr	Thr	Ile	Ile	Lys
225					230					235					240
Gln	Gly	Gln	Phe	Val	His	Leu	Asp	Asp	Leu	Cys	Met	Ser	His	Ile	Phe
			245						250					255	
Leu	Tyr	Glu	Asn	Pro	Lys	Ala	Asn	Gly	Arg	Tyr	Ile	Ala	Ser	Ala	Cys
			260					265					270		
Ala	Ala	Thr	Ile	Tyr	Asp	Ile	Ala	Lys	Met	Leu	Arg	Glu	Glu	Tyr	Pro
		275					280					285			
Glu	Tyr	Asn	Val	Pro	Thr	Lys	Phe	Lys	Asp	Tyr	Lys	Glu	Asp	Met	Gly
	290					295					300				
Gln	Val	Gln	Phe	Ser	Ser	Lys	Lys	Leu	Thr	Asp	Leu	Gly	Phe	Glu	Phe
305					310					315					320
Lys	Tyr	Gly	Leu	Lys	Asp	Met	Tyr	Thr	Ala	Ala	Val	Glu	Ser	Cys	Arg
			325						330					335	
Ala	Lys	Gly	Leu	Leu	Pro	Leu	Ser	Leu	Glu	His	His	Leu	Cys	Val	Phe
			340					345					350		
Arg	Val	Thr	Leu	Ile	Phe	Phe	Lys								
		355					360								

<210> 11
 <211> 1006
 <212> DNA
 <213> artificial sequence

<220>
 <223> ThMT.nt

<400> 11
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 agacaagtgc ctatccacga gaacatccgc agctcaaaga actaaggagc gcaactgtgg 180
 acaagtatca atattggagc ttgatgaatg ttccagctga tgagggggcag ttcatttcaa 240
 tgttactgaa aattatgaac gcaaaaaaga caattgaagt tggagttttc acaggctact 300
 cactcctatc aactgctctg gctctacctg atgatggcaa aatcgttgcc attgacctg 360
 atagagaagc ttatgagact ggtttgccat ttatcaagaa agcaaactg gctcataaaa 420
 tccaatacat acaatctgat gccatgaaag tcatgaatga cctcattgct gccaagggag 480
 agaagaaga ggggagcttt gactttgggt tcgtggatgc agacaaagaa aactacataa 540
 actaccacga gaaactgttg aagctgggta aggttgagg gatcatagga tacgacaaca 600
 ctctgtggtc tggaacagtt gctgcatctg aagacgatga gaataatatg cgagactact 660
 taagagggtg cagagggcat atcctcaaac taaactcctt tctcgcaaac gatgatcgga 720
 ttgaattggc tcacctctct attggagatg gactcacctt gtgcaaactg ctcaaataat 780
 aattttcaac tttattatta ttgtttcata aaaagcattt actgctggcc tggcctggcc 840
 tgtttcagca tcttatattt ctattgttct aaatatattt gttatcttgt ttatcaactt 900
 gtctgtctta tatgttttaa agaaagatgt catgtaattg taactcgatc gggctcttgt 960
 aatattataa tgaattttat tgattttcaa aaaaaaaaaa aaaaaa 1006

<210> 12
 <211> 239
 <212> PRT
 <213> artificial sequence

61i

<220>

<223> ThMT.aa

<400> 12

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Met Lys Asp Lys Phe Tyr Gly Thr Ile Leu Gln Ser Glu Ala Leu Ala
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Lys Tyr Leu Leu Glu Thr Ser Ala Tyr Pro Arg Glu His Pro Gln Leu
      20      25      30
Lys Glu Leu Arg Ser Ala Thr Val Asp Lys Tyr Gln Tyr Trp Ser Leu
      35      40      45
Met Asn Val Pro Ala Asp Glu Gly Gln Phe Ile Ser Met Leu Leu Lys
      50      55      60
Ile Met Asn Ala Lys Lys Thr Ile Glu Val Gly Val Phe Thr Gly Tyr
65      70      75      80
Ser Leu Leu Ser Thr Ala Leu Ala Leu Pro Asp Asp Gly Lys Ile Val
      85      90      95
Ala Ile Asp Pro Asp Arg Glu Ala Tyr Glu Thr Gly Leu Pro Phe Ile
      100     105     110
Lys Lys Ala Asn Val Ala His Lys Ile Gln Tyr Ile Gln Ser Asp Ala
      115     120     125
Met Lys Val Met Asn Asp Leu Ile Ala Ala Lys Gly Glu Glu Glu Glu
      130     135     140
Gly Ser Phe Asp Phe Gly Phe Val Asp Ala Asp Lys Glu Asn Tyr Ile
145     150     155     160
Asn Tyr His Glu Lys Leu Leu Lys Leu Val Lys Val Gly Gly Ile Ile
      165     170     175
Gly Tyr Asp Asn Thr Leu Trp Ser Gly Thr Val Ala Ala Ser Glu Asp
      180     185     190
Asp Glu Asn Asn Met Arg Asp Tyr Leu Arg Gly Cys Arg Gly His Ile
      195     200     205
Leu Lys Leu Asn Ser Phe Leu Ala Asn Asp Asp Arg Ile Glu Leu Ala
      210     215     220
His Leu Ser Ile Gly Asp Gly Leu Thr Leu Cys Lys Arg Leu Lys
225     230     235

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<210> 13

<211> 1745

<212> DNA

<213> artificial sequence

<220>

<223> ThFNS.nt

<400> 13

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ccaccttctc cctcctcttc cgccgaaggg gaccaccgtc tccgcccggg cctctctccc      180
tacccataat tggccacctc cacctcctcg gcccaagact ccaccacacg ttccatgaat      240
tctcactcaa atacggccca ttgatccagc tcaagctcgg ctcgatcccg tgcgtcgtgg      300
cctcgacgcc cgagctcgcg agagagtttc ttaagacgaa cgagctcgcg ttctcctctc      360
gcaagcactc tacggccata gacatcgtea cctacgactc gtcctttgct ttctctccgt      420
acggacccta ctggaagtac atcaagaaac tgtgtacctc cgagctgctc ggagcgagga      480
acctcggaca ctttcagccc attaggaatc tcgagggtcag gtcctttctg cagcttctga      540
tgcacaagag ctttaagggc gagagtgtga atgtgacaga cgagctggtg aggctgacga      600
gcaatgtgat atcccacatg atgctgagca taaggtgctc ggaagatgaa ggcgatgctg      660

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Thr	Thr	Phe	Leu	Leu	Leu	Leu	Arg	Arg	Arg	Gly	Pro	Pro	Ser	Pro	Pro
			20					25					30		
Gly	Pro	Leu	Ser	Leu	Pro	Ile	Ile	Gly	His	Leu	His	Leu	Leu	Gly	Pro
		35					40					45			
Arg	Leu	His	His	Thr	Phe	His	Glu	Phe	Ser	Leu	Lys	Tyr	Gly	Pro	Leu
	50					55					60				
Ile	Gln	Leu	Lys	Leu	Gly	Ser	Ile	Pro	Cys	Val	Val	Ala	Ser	Thr	Pro
65					70					75					80
Glu	Leu	Ala	Arg	Glu	Phe	Leu	Lys	Thr	Asn	Glu	Leu	Ala	Phe	Ser	Ser
				85					90					95	
Arg	Lys	His	Ser	Thr	Ala	Ile	Asp	Ile	Val	Thr	Tyr	Asp	Ser	Ser	Phe
			100					105					110		
Ala	Phe	Ser	Pro	Tyr	Gly	Pro	Tyr	Trp	Lys	Tyr	Ile	Lys	Lys	Leu	Cys
		115					120					125			
Thr	Tyr	Glu	Leu	Leu	Gly	Ala	Arg	Asn	Leu	Gly	His	Phe	Gln	Pro	Ile
	130					135					140				
Arg	Asn	Leu	Glu	Val	Arg	Ser	Phe	Leu	Gln	Leu	Leu	Met	His	Lys	Ser
145					150					155					160
Phe	Lys	Gly	Glu	Ser	Val	Asn	Val	Thr	Asp	Glu	Leu	Val	Arg	Leu	Thr
				165					170					175	
Ser	Asn	Val	Ile	Ser	His	Met	Met	Leu	Ser	Ile	Arg	Cys	Ser	Glu	Asp
		180						185					190		
Glu	Gly	Asp	Ala	Glu	Ala	Ala	Arg	Thr	Val	Ile	Arg	Glu	Val	Thr	Gln
		195					200					205			

61k

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Ile Phe Gly Glu Phe Asp Val Thr Asp Ile Ile Trp Phe Cys Lys Lys
 210                               215                220
Phe Asp Leu Gln Gly Ile Lys Lys Arg Ser Glu Asp Ile Gln Arg Arg
225                               230                235                240
Tyr Asp Ala Leu Leu Glu Lys Ile Ile Ser Asp Arg Glu Arg Ser Arg
                               245                250                255
Arg Gln Asn Arg Asp Lys His Gly Gly Gly Asn Asn Glu Glu Ala Lys
                               260                265                270
Asp Phe Leu Asp Met Leu Leu Asp Val Met Glu Ser Gly Asp Thr Glu
                               275                280                285
Val Lys Phe Thr Arg Glu His Leu Lys Ala Leu Ile Leu Asp Phe Phe
                               290                295                300
Thr Ala Gly Thr Asp Thr Thr Ala Ile Ala Thr Glu Trp Ala Ile Ala
305                               310                315                320
Glu Leu Ile Asn Asn Pro Asn Val Leu Lys Lys Ala Gln Glu Glu Ile
                               325                330                335
Ser Arg Ile Ile Gly Thr Lys Arg Ile Val Gln Glu Ser Asp Ala Pro
                               340                345                350
Asp Leu Pro Tyr Leu Gln Ala Ile Ile Lys Glu Thr Phe Arg Leu His
                               355                360                365
Pro Pro Ile Pro Met Leu Ser Arg Lys Ser Thr Ser Asp Cys Thr Val
                               370                375                380
Asn Gly Tyr Lys Ile Gln Ala Lys Ser Leu Leu Phe Val Asn Ile Trp
385                               390                395                400
Ser Ile Gly Arg Asn Pro Asn Tyr Trp Glu Ser Pro Met Glu Phe Arg
                               405                410                415
Pro Glu Arg Phe Leu Glu Lys Gly Arg Glu Ser Ile Asp Val Lys Gly
                               420                425                430
Gln His Phe Glu Leu Leu Pro Phe Gly Thr Gly Arg Arg Gly Cys Pro
                               435                440                445
Gly Met Leu Leu Ala Ile Gln Glu Val Val Ser Ile Ile Gly Thr Met
                               450                455                460
Val Gln Cys Phe Asp Trp Lys Leu Ala Asp Gly Ser Gly Asn Asn Val
465                               470                475                480
Asp Met Thr Glu Arg Ser Gly Leu Thr Ala Pro Arg Ala Phe Asp Leu
                               485                490                495
Val Cys Arg Leu Tyr Pro Arg Val Asp Pro Ala Thr Ile Ser Gly
                               500                505                510

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<210> 15
 <211> 2544
 <212> DNA
 <213> artificial sequence

<220>
 <223> carnANS 5'

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 tgcatttgta ctagtccgat aataattagt atcgttttca tactgatact agatcggagg 360
 tcaccatacc cgtgaagatt tttctgtgag aggaaaagaa cccaaggacg aggttcaaat 420

ctacacatgg	aaagacgcca	cgttcgtga	gttaactgac	cttgtatgtc	gccatcgctt	480
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ttggtttgag	aaagataaca	gctttacgta	tttttgtaga	tgggtgaaac	cttttcaaatt	780
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atgcgactaa	caatagtctc	cactgatcat	attctactct	tctacgttcg	atactgactg	900
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gttcttaaaa	aggtacgatc	caagtgaagc	atgttagata	tgacactttc	ttctagggac	1260
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 <212> DNA
 <213> artificial sequence

<220>
 <223> carnANS 3'

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cgttatacgg	agtactgttt	agaagacgat	atgtaataaa	tgaaatcgta	gttgtctata	180
gcttcatgat	tatcgttaca	ctattattct	tatacttcat	ctttatttta	gttaatctta	240
tcaaataactt	cgtatattgg	aaaatttcaa	aaagttactt	aaaaaataat	aaaatacacc	300
gaatttcaga	aaactcagga	atattcaggc	gtaataacat	ccgttaatac	cgaaaatatt	360
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aatctttggt	cgtaacgaat	tgcgtcctaa	atcatcataa	taatacaaat	aaaaaaatgt	480
cattttcatt	aaatttcttc	tcttctgtaa	tcatagaatt	ttattccaac	ttcatcatct	540

61m

aacttcaggt	acattttctcc	ttctctaact	tattatcatt	acttttttatt	gtttttaaatt	600
atatttttta	tgtctattaa	acgattttaa	atgatcacag	tttactctgt	acttgtcgtg	660
aataatgtgc	tgtatgcata	caacagtctt	attgggtttac	gtgaactgtt	agctgtgggt	720
gcatacaatt	tagattgaac	cgacagatat	cgcctttact	gtcggaggga	gctgttacag	780
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<210> 17
 <211> 2934
 <212> DNA
 <213> artificial sequence

<220>
 <223> RoseCHS 5'

<400> 17

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agaattctga	caaagatgtt	ttgaagaata	tccttgaatt	ctcacgtgct	tcttctgtgg	540
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CLAIMS:

1. A cell of a genetically modified carnation or its progeny exhibiting altered inflorescence, wherein said cell is in a Cerise Westpearl background, and wherein said cell comprises at least one expressible genetic material which expresses

5 a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions;

10 a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high stringency conditions;

15 sense and anti-sense nucleotide sequences (*ds carnDFR*) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR gene; and either

20 a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:11 under high stringency conditions; or

25 flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:13 under high stringency conditions;

wherein the high stringency conditions are defined by washing in 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C.

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2. A cell of a genetically modified carnation or its progeny exhibiting altered inflorescence, wherein said cell is in a Cerise Westpearl background, and wherein said cell comprises at least one expressible genetic material which expresses

5 a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions;

10 a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high stringency conditions;

15 sense and anti-sense nucleotide sequences (*ds carnDFR*) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR gene;

wherein the high stringency conditions are defined by washing in 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C; and either

20 a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11; or

flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13.

3. A cell of a genetically modified carnation or its progeny exhibiting altered
25 inflorescence, wherein said cell is in a Cerise Westpearl background, and wherein said cell comprises at least one expressible genetic material which expresses

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a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1;

a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3;

5 sense and anti-sense nucleotide sequences (*ds carn*DFR) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9, for down regulating expression of the carnation's indigenous DFR gene; and either

a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase
10 (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11; or

flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13.

4. A method for producing a carnation exhibiting altered inflorescence, said method comprising the steps of:

15 introducing into regenerable cells of a carnation plant at least one expressible genetic material, wherein said carnation cells are in a Cerise Westpearl background, and wherein said genetic material expresses

a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of
20 hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions;

a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high
25 stringency conditions;

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sense and anti-sense nucleotide sequences (*ds carn*DFR) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR gene; and either

a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:11 under high stringency conditions; or

flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:13 under high stringency conditions;

wherein the high stringency conditions are defined by washing in 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C, and

regenerating a carnation plant therefrom.

5. A method for producing a carnation exhibiting altered inflorescence, said method comprising the steps of:

introducing into regenerable cells of a carnation plant at least one expressible genetic material, wherein said carnation cells are in a Cerise Westpearl background, and wherein said genetic material expresses

a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence capable of hybridizing to a complementary form of the nucleotide sequence shown in SEQ ID NO:1 under high stringency conditions;

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a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:3 under high stringency conditions;

5 sense and anti-sense nucleotide sequences (*ds carn*DFR) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to the complementary form of the nucleotide sequence shown in SEQ ID NO:9 under high stringency conditions, for down regulating expression of the carnation's indigenous DFR
10 gene;

wherein the high stringency conditions are defined by washing in 0.2 to 2 x SSC buffer, 0.1%-1.0% w/v SDS at a temperature of at least 65°C; and either

a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11; or

15 flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13, and

regenerating a carnation plant therefrom.

6. A method for producing a carnation exhibiting altered inflorescence, said method comprising the steps of:

20 introducing into regenerable cells of a carnation plant at least one expressible genetic material, wherein said carnation cells are in a Cerise Westpearl background, and wherein said genetic material expresses

a non-indigenous flavonoid 3',5' hydroxylase (F3'5'H) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:1;

25 a non-indigenous dihydroflavonol 4-reductase (DFR) enzyme encoded by the nucleotide sequence shown in SEQ ID NO:3;

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sense and anti-sense nucleotide sequences (*ds carnDFR*) corresponding to the carnation's DFR gene and comprising a fragment or fragments of the nucleotide sequence shown in SEQ ID NO:9; and either

5 a non-indigenous S-adenosylmethionine: anthocyanin 3'5' methyltransferase (ThMT) encoded by the nucleotide sequence shown in SEQ ID NO:11; or

flavone synthase (ThFNS) encoded by the nucleotide sequence shown in SEQ ID NO:13, and

regenerating a carnation plant therefrom.

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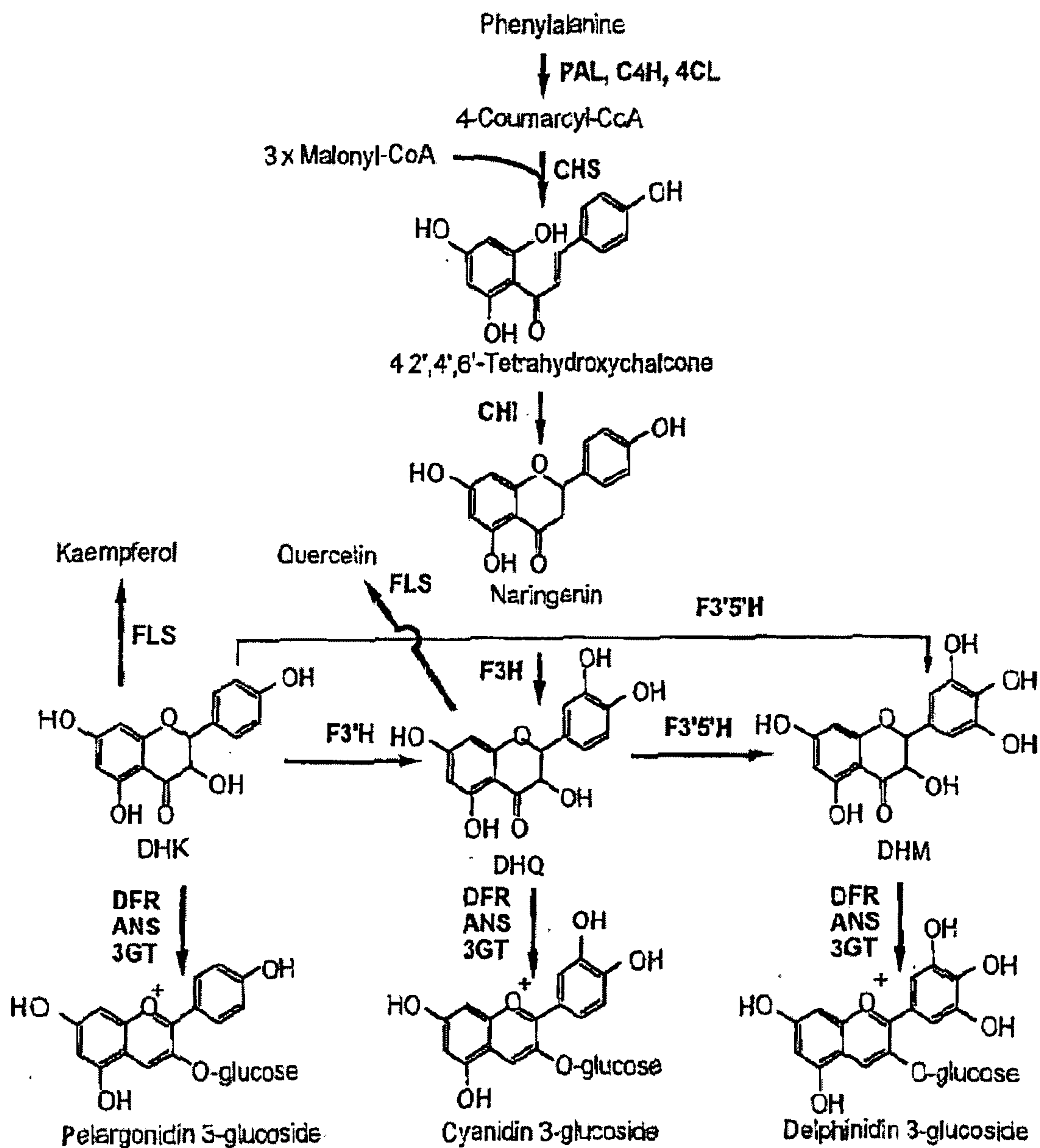


Figure 1

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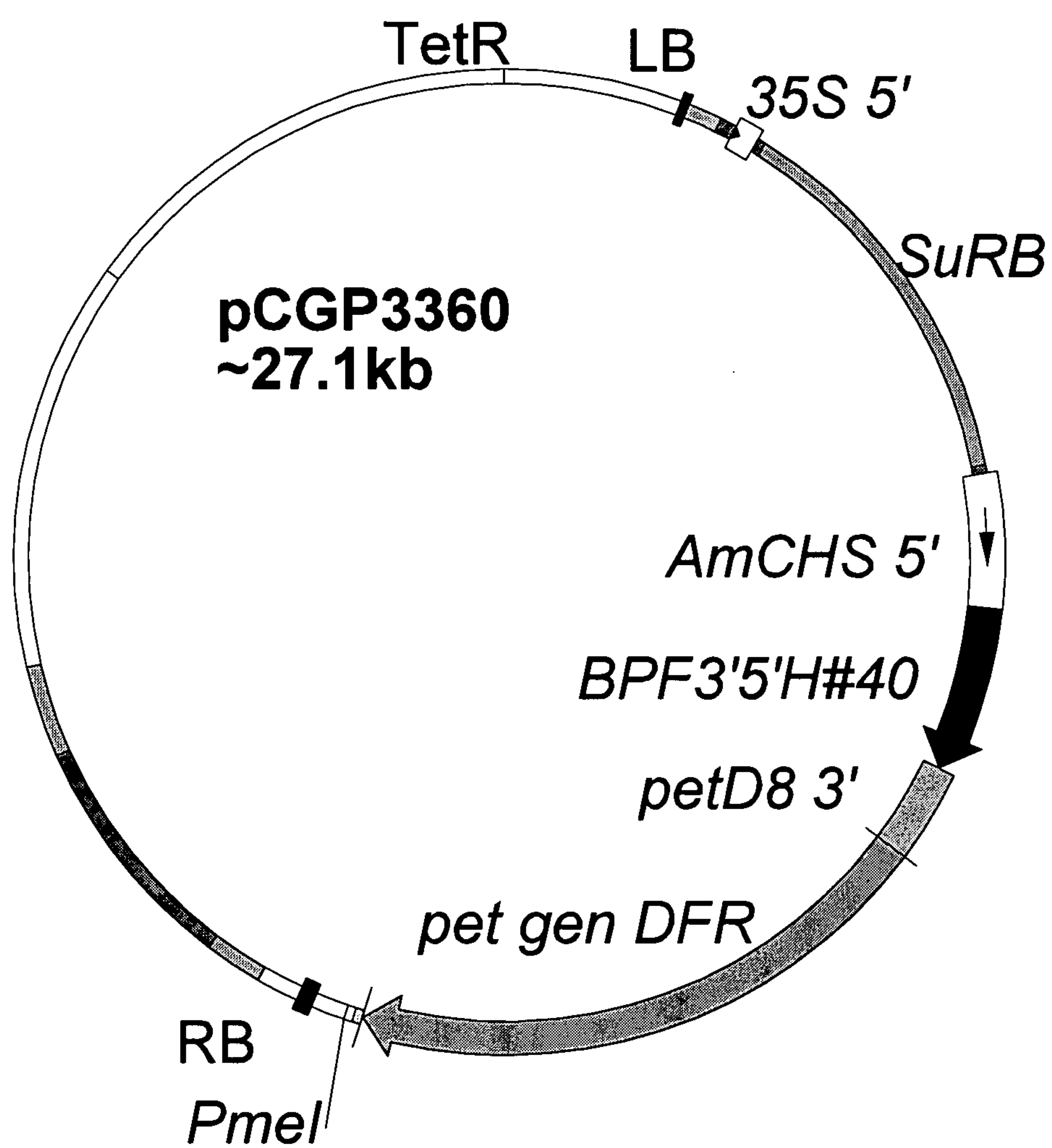


Figure 2

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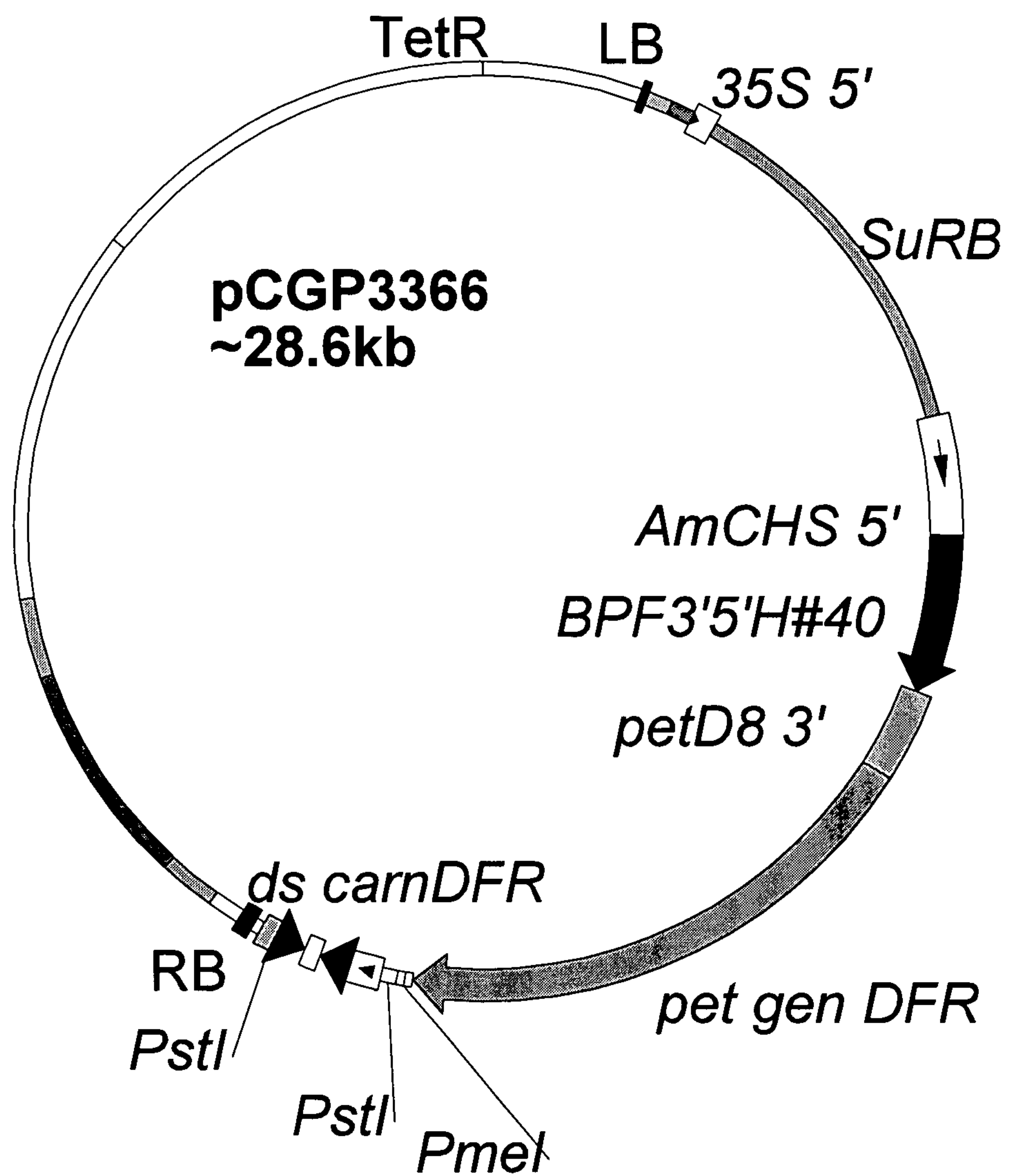


Figure 3

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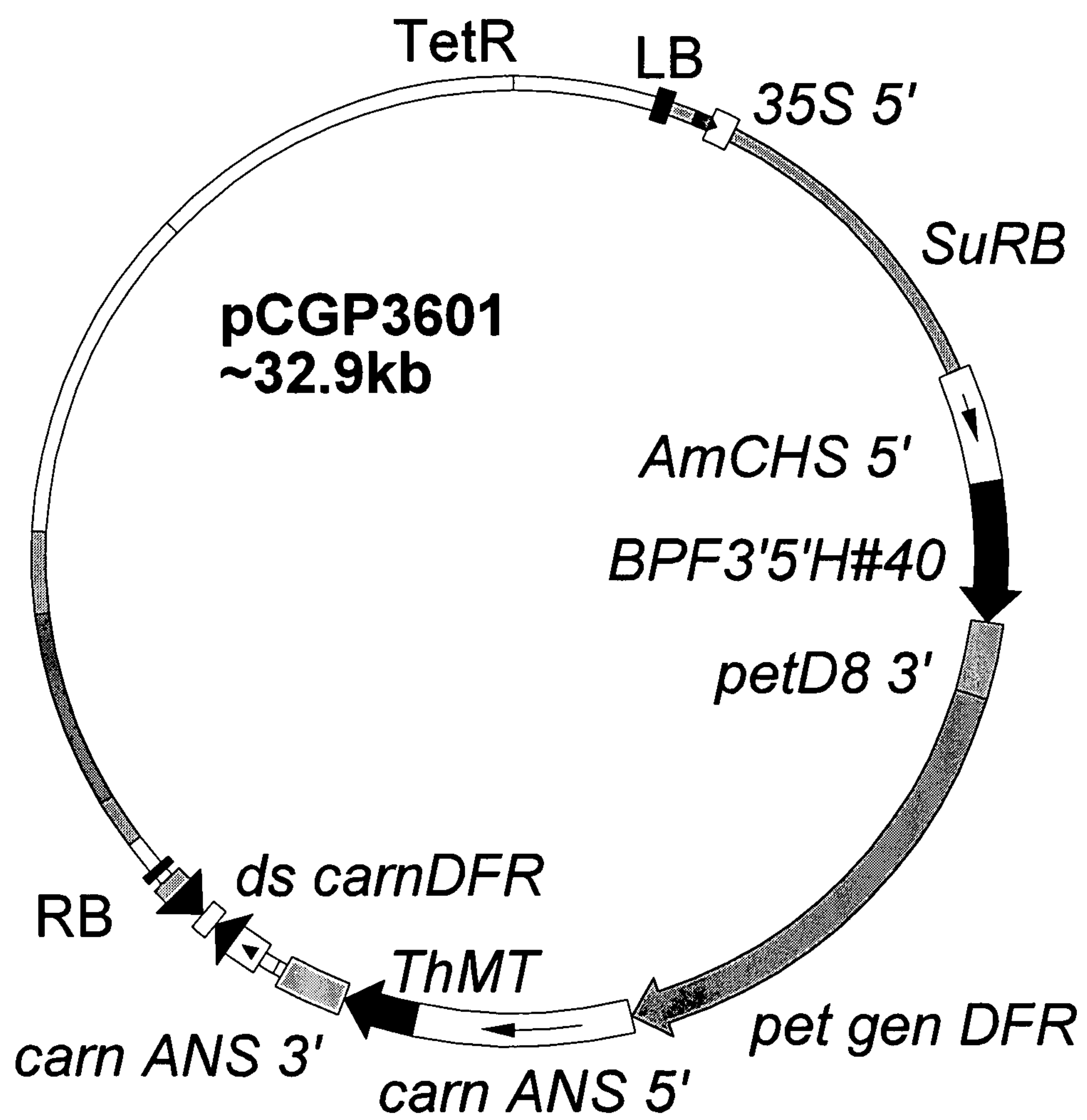


Figure 4

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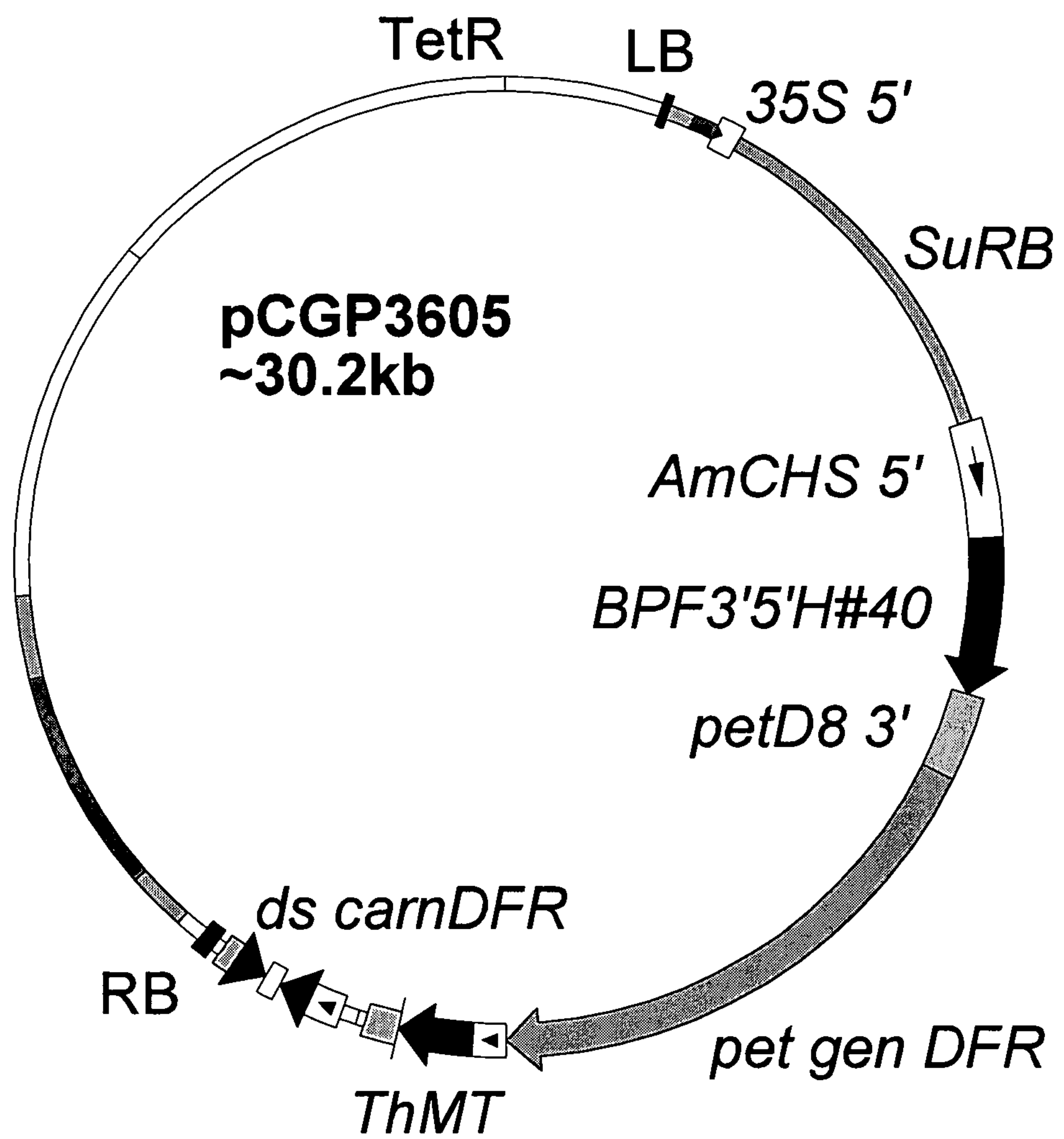


Figure 5

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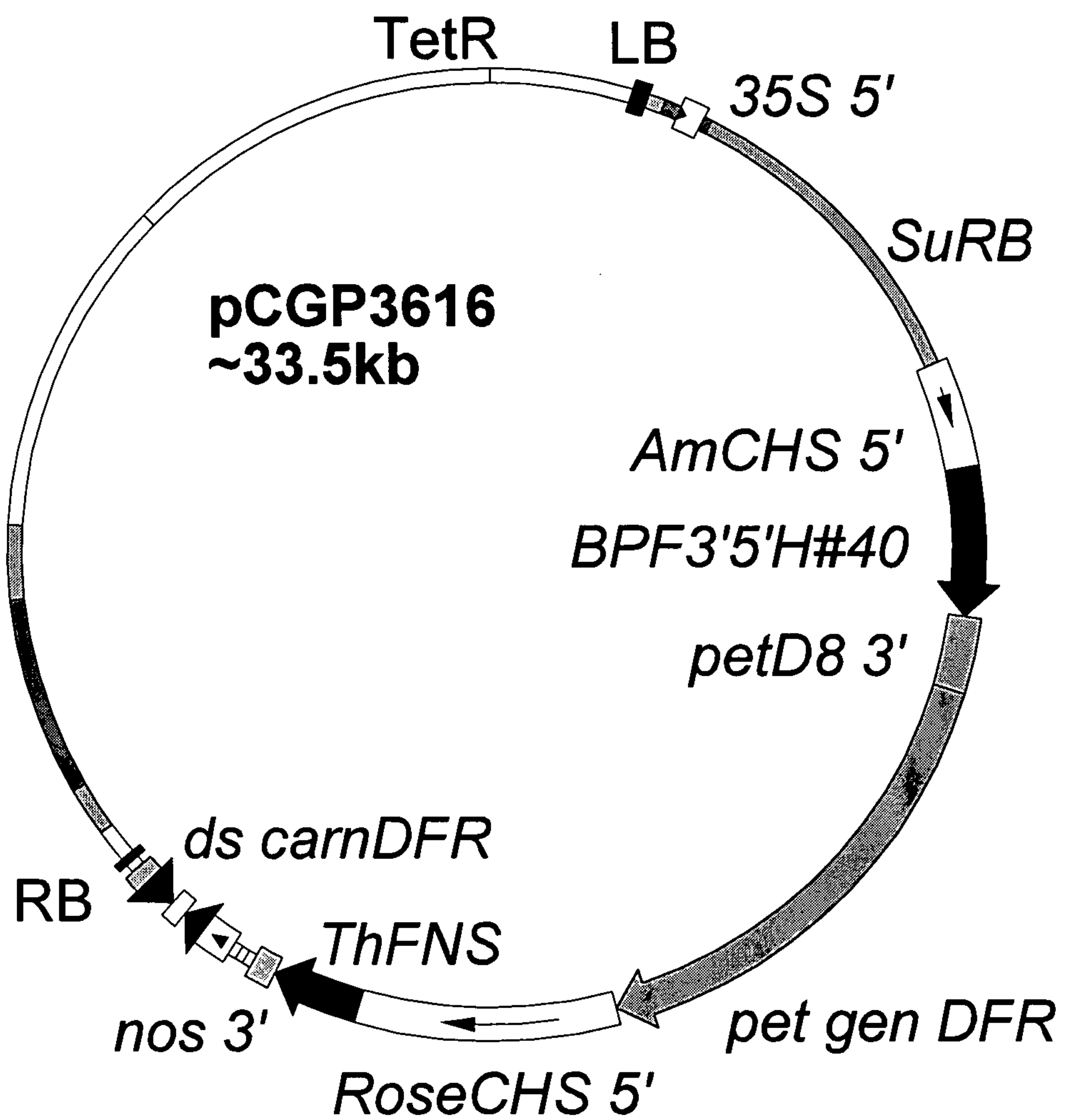


Figure 6

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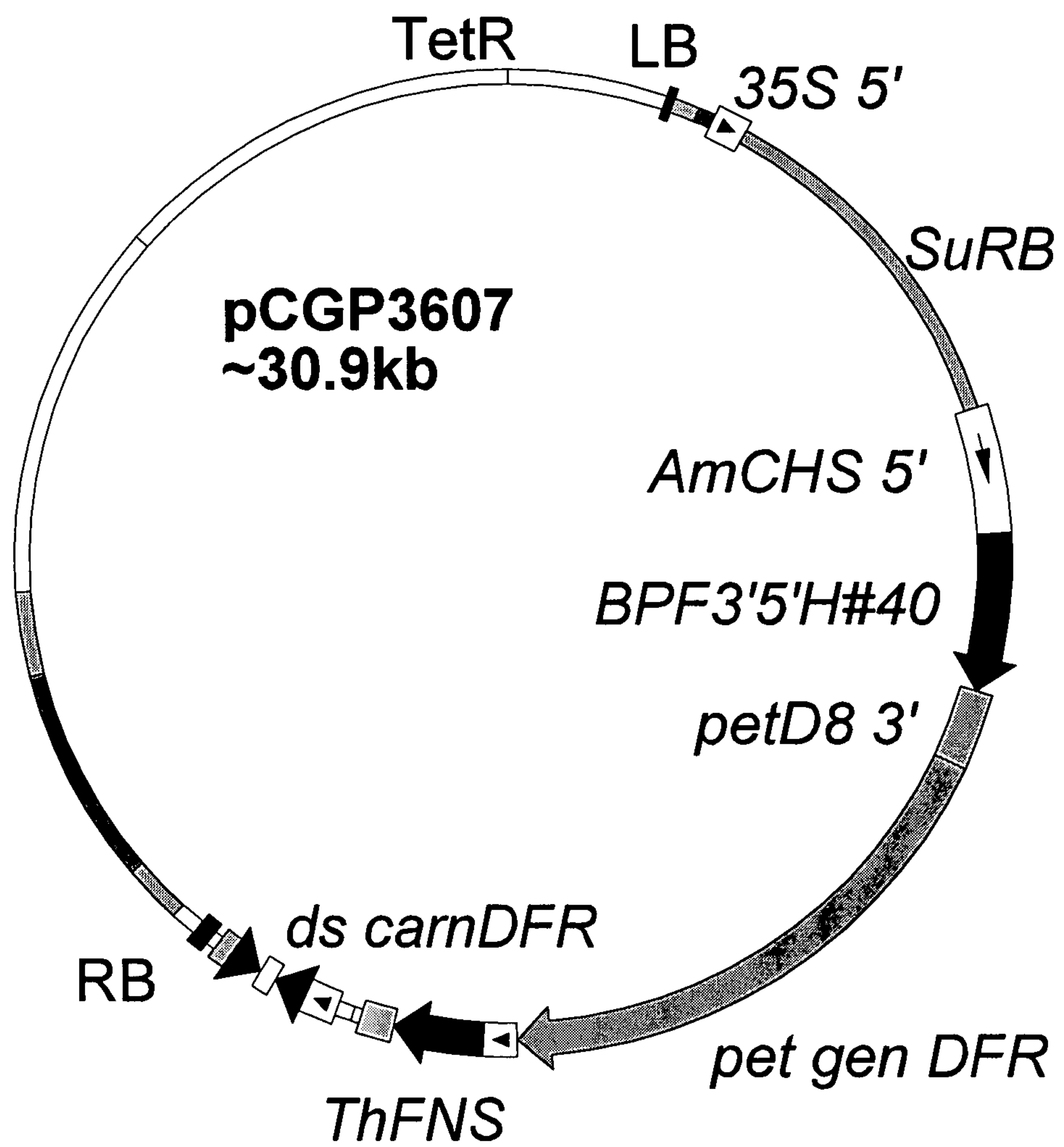


Figure 7