

Product Information Sheet

Plant Expression Vectors (pCambia series)

Version 1.01

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A series of expression vectors for
cloning and expression of genes into plants.

Product Numbers.: M1579, M1581-84, M1586-99, M1700-10

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Important Information:

MSDS Sheets and product safety information are available by request from Marker Gene Technologies, Inc. and by accessing our web site at www.markergene.com.

Shipping and Storage

The plant expression vectors are shipped at room temperature. Store all samples of the vector at –20°C or below, once resuspended. Products are guaranteed for six months from date of shipment when stored properly.

Contents

Item	Amount
Plant Expression Vector pCambia Series, Lyophilized in TE buffer, pH 8.0	20µg

Quality Control

The plant expression vectors have been qualified by restriction endonuclease digestion and some are further qualified by transformation using an appropriate *Agrobacterium* strain in culture into *Arabidopsis thaliana* plant species and verified for activity.

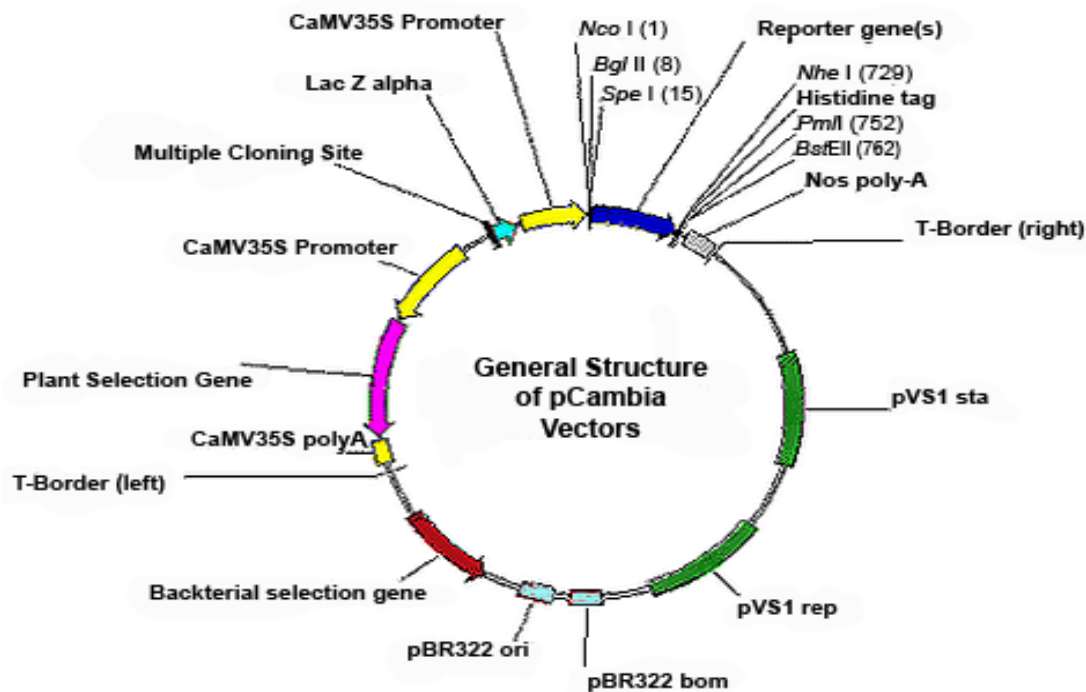
Methods

Overview

Description

The pCambia vector backbone is derived from the pPZP vectors (constructed by Hajdukiewicz, Svab & Maliga, see References). pCambia vectors offer:

- High copy number in *E.coli* for high DNA yields
- pVS1 replicon for high stability in *Agrobacterium*
- Small size, 7-12kb depending on which plasmid
- Restriction sites designed for modular plasmid modifications and small but adequate poly-linkers for introducing your DNA of interest
- Bacterial selection with chloramphenicol or kanamycin
- Plant selection with hygromycin B or kanamycin
- Simple means to construct translational fusions to *gusA* reporter genes.



Nomenclature of pCAMBIA vectors:

The four digit numbering system works as follows:

First digit - indicates plant selection: 0 for absence; 1 for hygromycin resistance; 2 for kanamycin;

Second digit - indicates bacterial selection: 1 for spectinomycin/streptomycin resistance; 2 for chloramphenicol; 3 for kanamycin;

Third digit - indicates polylinker used: 0 for pUC18 polylinker; 8 for pUC8 polylinker; 9 for pUC9 polylinker.

Fourth digit - indicates reporter gene(s) present: 0 for no reporter gene; 1 for *E.coli gusA*; 2 for *mgfp5*; 3 for *gusA:mgfp5* fusion; 4 for *mgfp5:gusA* fusion; 5 for *Staphylococcus* sp. *gusA* (GUSPlus).

Fifth digit - notes some other special feature. So far this has been used only with: pCAMBIA1305.1 and plasmids derived from it, where the .1 denotes the absence of a signal peptide from the GUSPlus™ protein; and pCAMBIA1305.2 where the .2 denotes the presence of the GRP signal peptide for *in planta* secretion of the GUSPlus™ protein.

Lagging letter - X indicates that the reporter gene lacks its own start codon and the vector is for creating fusions to the reporter; Z indicates presence of a functional *lacZa* for blue-white screening; a/b/c indicates the reading frame for fusions with the Fuse and Use vectors.

A few points about the pCAMBIA cloning strategy of exogenous genes into pCAMBIA vectors:

The pUC18 polylinker was used in some vectors, but pUC8 and pUC9 polylinkers were also used to simplify the choice of cloning enzyme. With the means of PCR, it is no longer necessary to have a large number of cloning sites. The smaller polylinkers also eliminate potential conflicts from sites such as *SphI* (which has an ATG) or *XbaI* (which has a TAG). This makes other sites in the vector more useful (such as the *SphI* site outside the right T-DNA Border, or the *SacII* site outside the left T-DNA Border).

Plant selection genes in the pCAMBIA vectors are driven by a double-enhancer version of the CaMV35S promoter and terminated by the CaMV35S polyA signal. **NOTE** that this 35S promoter can have an enhancer effect on the expression of other genes in the same cassette, so gene expression results using pCAMBIA derivatives in which portions of this promoter are still present should be interpreted with caution.

Reporter genes feature a hexa-Histidine tag at the C-terminus to enable simple purification on immobilised metal affinity chromatography resins. The sequence for this tag occurs between the first *NheI* site (there is a second *NheI* site in the pVS1-rep that we didn't eliminate) and the unique *PmlI* site. Genes of interest may be inserted in place of the reporter gene. Insertion without a stop codon and in frame at the (first) *NheI* site will append a hexa-Histidine tag to your protein of interest. Insertion without a stop codon and in frame at the *PmlI* site will append a stop codon. Insertion at the *BstEII* site will add neither a tag nor a stop codon (so you may want to ensure that a sequence inserted here contains a stop codon).

Available Vectors

Product Number	Product Name
M1579	GusPlus™ No Plant Selection/Streptomycin Resistant Plant Expression Vector (pCambia0105.1R)
M1581	GusPlus™ No Plant Selection/Kanamycin Resistant Plant Expression Vector (pCambia0305.1)
M1582	Secreted GusPlus™ No Plant Selection/Kanamycin Resistant Plant Expression Vector (pCambia0305.2)
M1583	No Reporter, No Plant Selection/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia0380)
M1584	No Reporter, No Plant Selection/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia0390)
M1586	GusPlus™/lacZ Hygromycin B/Streptomycin Resistant Plant Expression Vector (pCambia1105.1)
M1587	GusPlus™ Hygromycin B/Streptomycin Resistant Plant Expression Vector (pCambia1105.1R)
M1588	No Reporter Gene Hygromycin B/Chloramphenicol Resistant Plant Expression Vector (pCambia1200)
M1589	<i>gusA</i> Hygromycin B/Chloramphenicol Resistant Plant Expression Vector (pCambia1201)
M1590	<i>gusA/lacZ</i> Hygromycin B/Chloramphenicol Resistant Plant Expression Vector (pCambia1281Z)
M1591	No Reporter Gene Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1300)
M1592	<i>gusA</i> Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1301)
M1593	<i>mgfp5</i> Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1302)
M1594	<i>mgfp5:gusA</i> Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1303)
M1595	<i>gusA:mgfp5</i> Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1304)
M1596	GusPlus™ Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1305.1)
M1597	Secretory GusPlus™ Hygromycin B/Kanamycin Resistant Plant Expression Vector (pCambia1305.2)
M1598	No Reporter Hygromycin B/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia1380)
M1599	Fusible <i>gusA</i> (ORF a) Hygromycin B/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia1381Xa)
M1700	Fusible <i>gusA</i> (ORF b) Hygromycin B/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia1381Xb)
M1701	Fusible <i>gusA</i> (ORF c) Hygromycin B/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia1381Xc)
M1702	<i>gusA/lacZ</i> Hygromycin B/Kanamycin Resistant pUC8 Plant Expression Vector (pCambia1381Z)
M1703	No Reporter Gene Hygromycin B/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia1390)
M1704	<i>gusA</i> Hygromycin B/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia1391)
M1705	Fusible <i>gusA</i> (ORF a) Hygromycin B/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia1391Xa)
M1706	Fusible <i>gusA</i> (ORF c) Hygromycin B/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia1391Xc)
M1707	<i>gusA/lacZ</i> Hygromycin B/Kanamycin Resistant pUC9 Plant Expression Vector (pCambia1391Z)
M1708	No Reporter Gene Kanamycin/Chloramphenicol Resistant Plant Expression Vector (pCambia2200)
M1709	No Reporter Gene Kanamycin/Kanamycin Resistant Plant Expression Vector (pCambia2300)
M1710	<i>gusA</i> Kanamycin/Kanamycin Resistant Plant Expression Vector (pCambia2301)

Using Plant Expression Vectors

Propagating pCambia Series Expression Vectors

If you wish to propagate and maintain the vector, we recommend using *Agrobacterium tumefaciens* or *E. coli* for transformation.

Points to consider before recombining:

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation. If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone should not contain a stop codon. The gene should also be designed to be in frame with the C-terminal epitope tag after recombination. If you do NOT wish to include the V5 epitope and 6xHis tag, please be sure that your gene contains a stop codon in the entry clone.

Concentration of Antibiotics for selection.

In bacterial system:

pCAMBIAx1xx vectors – spectinomycin/streptomycin resistance (*aadA*/aminoglycoside-3'-adenylyltransferase) in bacteria: use 50µg/mL in *E.coli* & *A.tumefaciens*

pCAMBIAx2xx vectors – chloramphenicol resistance (*cat* – chloramphenicol acetyltransferase from *E.coli* Tn-cam204) in bacteria: use 10µg/mL in *E.coli* & *A.tumefaciens*.

pCAMBIAx3xx vectors – kanamycin resistance (*aphIII/nptIII/3'5'*-aminoglycoside phosphotransferase typeIII from *Enterococcus faecalis* pJH1) in bacteria: use 50µg/mL in *E.coli* & *A.tumefaciens*.

In plant system:

pCAMBIA series	1xxx	2xxx
Resistance Gene	<i>hph</i> (From a <i>Klebsiella</i> species, via <i>E.coli</i>)	<i>nptII/aph(3')II</i> from Tn5
Transformed plant selection antibiotic	Hygromycin B	Kanamycin
Concentration in <i>Arabidopsis</i>	50µg/mL in germination medium	20-50µg/mL
Concentration in Rice	50µg/mL in 2N6 then in RGH6	Not tested
Concentration in Tobacco	50µg/mL in RMOP then in MST	Not tested

Transfection

Introduction: This section provides general information for transfecting your expression clone into the plant of choice. We recommend that you include a positive control vector and a mock transfection (negative control) in your experiments to evaluate your results.

Methods of Transformation and Transfection:

Preparation of competent cells, transformation and recovery and floral dip with *Agrobacterium tumefaciens* protocols can be found in Weigel and Glazebrook's "Arabidopsis: A Laboratory Manual" (2002).

Preparation of competent *Agrobacterium tumefaciens* cells:

1. Inoculate 250 mL of LB medium with 750 uL *Agrobacterium tumefaciens* (for example GV3101::pMP90).
2. Incubate at 28°C (Dubenoff shaking water bath) with vigorous shaking for 11 hours until the OD is approximately 0.75.
3. Pellet the cells by centrifugation (5000 rpm).
4. Wash the pellet with 2 mL sterile TE.
5. Centrifuge at room temperature for 10 min at 5000 rpm.
6. Resuspend the pellet in 20 mL LB medium. Aliquot 250 uL of this suspension into 1.5 mL microcentrifuge tubes.

Transformation and recovery of *Agrobacterium tumefaciens* cells:

1. Add 20 uL of the Vector (5 ug) to the competent GV3101::pMP90 *Agrobacterium tumefaciens* sample (250 uL) in LB medium from above.
2. Incubate on ice (0°C) for 5 minutes.
3. Incubate in liquid nitrogen (-80°C) for 5 min.
4. Incubate at 37°C (water bath) for 5 min.
5. Add LB medium (1 mL) to each vial and incubate at room temperature (with rotation) for 4 hours.
6. Streak the bacteria onto LB Agar plates containing appropriate selection antibiotics and incubate at 28°C for 3 days.
7. Pick one colony and re-streak onto an LB Agar plate containing appropriate selection antibiotics and incubate at 28°C for 2 days.

Floral dip with *Agrobacterium tumefaciens*:

1. Prepare Infiltration Medium (250 mL) (see recipe below in Appendix)
2. Prepare MS Agar Gel for plant growth (see recipe below in Appendix)
3. Pipette 15 – 20 mL into 25 x 250 mm sterile test tubes for each plant to be grown after dip.
4. Grow up one colony of transformed bacteria (See step 7 from above) in LB media containing appropriate selection antibiotics (2 mL) overnight at 28°C (Dubenoff shaking water bath) with vigorous shaking.
5. Inoculate 225 mL of LB medium with 1.25 mL of preculture from step 4 above. Incubate overnight at 28°C (Dubenoff shaking water bath) with vigorous shaking.
6. Pellet the bacteria by centrifugation at 6000 rpm at 25°C for 10 min.
7. Resuspend the bacteria in Infiltration Medium (50 mL) and swirl to ensure complete mixture of the bacteria.
8. Transfer the bacterial suspension to a sterile dipping box (the lid of a pipette tip box works well). This process is best done in a laminar flow hood under sterile conditions.
9. Dip the roots of plants in the bacterial suspension for 30 seconds.
10. After dipping, place the plants on agar plates (MS medium) and seal the lids with parafilm. Allow to set overnight at room temperature.
11. After overnight storage, rinse the plant roots with sterile water and transfer the transformed plants to sterile tubes containing the MS Agar Gel for growth.
12. Grow the plants under long-day light conditions (16 hour daylight, 8 hour darkness) for several weeks until flowering and seed pod production occurs.
13. Remove seed pods and dry seeds.
14. Replant seeds on MS Agar Gel plates and collect seeds as above. Re-growth of these plants will provide stably transformed GUS plants and seeds for use.

Positive Control:

We recommend the use of a positive control vector for plant transfection and expression which may be used to optimize recombinant protein expression levels in your particular plant. A vector that allows expression of a C-terminally tagged β -glucuronidase fusion protein that may be detected by Western blot or functional assay provides the easiest way to measure protein expression levels. Consult our technical assistance for more information about C-terminal fusion protein expression systems.

To propagate and maintain the plasmid:

1. Resuspend the vector in 20 μ L sterile water to prepare a 1 μ g/ μ L stock solution and store at -20°C. Use the stock solution to transform *Agrobacterium tumefaciens*, or equivalent vehicle.
2. Select transformants on LB agar plates containing appropriate selection antibiotics.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

Expression and Analysis

Expression of your gene of interest from the expression clone can be performed in transiently transfected cells or stable cell lines.

GUS Assay using Cell Lysates

Cell lysate from above step can be assay for GUS activity using the MarkerGene™ β -Glucuronidase (GUS) Reporter Gene Activity Detection Kit, M0877. For a detailed description please visit our website: [http:// www.markergene.com/ProductDetails.php/M0877](http://www.markergene.com/ProductDetails.php/M0877).

Detecting Recombinant Fusion Proteins:

To detect expression of your recombinant fusion protein by Western blot analysis, you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies available from Invitrogen or Amersham Biosciences or an antibody to your protein of interest.

Assay for β -glucuronidase:

If you use a positive control vector, you may assay for β -glucuronidase expression by Western blot analysis or activity assay using cell-free lysates (Miller, 1972). X-GlcU staining, or fluorescence detection are common methods of analysis. Marker Gene offers several reagents and kits for fast and easy detection of β -glucuronidase expression. See Accessory Products (page 3) for more information about these products.

Purification of Recombinant Fusion Proteins:

The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows for purification using a metal-chelating resin (available from Invitrogen). Note: Other purification methods may also be suitable including affinity chromatography.

Accessory Products

Additional products that may be used with the Plant Expression Vectors are now available from Marker Gene.

Ordering information is provided below.

Product	Unit Size	Catalog no.
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Expression of your recombinant fusion protein can be detected using:

MarkerGene™ β -Glucuronidase (GUS) Reporter Gene Activity Detection Kit	1 kit	M0877
4-Methylumbelliferyl β -D-Glucuronide (MUGlcU)	25 mg	M0240
Carboxyumbelliferyl β -D-Glucuronide (CUGlcU)	10 mg	M0256
Fluorescein di- β -D-Glucuronide, di-methyl ester	5 mg	M0969

Inhibition of cloned GUS activity can be afforded using:

Phenethyl-1-thio- β -D-Glucopyranosiduronic Acid	10 mg	M0261
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Appendix: Recipes

LB (Luria-Bertani) Medium and Plates Composition:

1.0% Tryptone

0.5% Yeast Extract

1.0% NaCl

pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic if needed. Store at room temperature or at +4°C.

LB agar plates:

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic if needed, and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C.

4X SDS-PAGE Sample Buffer:

1. Combine the following reagents:

0.5 M Tris-HCl, pH 6.8, 5 ml

Glycerol (100%), 4 ml

β-mercaptoethanol, 0.8 ml

Bromophenol Blue, 0.04 g

SDS, 0.8 g

2. Bring the volume to 10 ml with sterile water.

3. Aliquot and freeze at -20°C until needed.

Infiltration Medium:

½ MS salts (MS salts: 1650.00 mg/L ammonium nitrate, 332.02 mg/L calcium chloride anhydrous, 180.70 mg/L magnesium sulfate anhydrous, 1900.00 mg/L potassium nitrate, 170.00 mg/L potassium phosphate monobasic, 6.20 mg/L boric acid, 0.025 mg/L cobalt chloride·6H₂O, 0.025 mg/L cupric sulfate·5H₂O, 37.26 mg/L Na²⁺ EDTA, 16.90 mg/L manganese sulfate·H₂O, 0.250 mg/L molybdic acid sodium salt, 0.83 mg/L potassium iodide, 27.80 mg/L ferrous sulfate·7H₂O, 8.60 mg/L zinc sulfate·7H₂O) (Sigma, St. Louis, M0654)

1X Gamborg's B5 Vitamin (Sigma, St. Louis, G1019)

5% Sucrose (w/v)

0.044 μM 6-benzylaminopurine (BAP) (stock solution 1mg/mL DMSO)

0.05% Silwet L77 (Lehle Seeds)

1. For 250mL Infiltration media, mixed 12.5mL 10X MS salts, 0.25mL Gamborg's B5 Vitamin 1000X, 12.5mg Sucrose, 2.5μL 1mg/mL stock solution of Benzylaminopurine (BAP), 12.5μL Silwet L77, 224.73mL dH₂O. Store at +4°C.

MS Agar Plant Growth Medium:

1. Autoclave 10.6g Murashige & Skoog (MS) Medium and 250 mL dH₂O on the liquid cycle for 20 minutes at 15 psi.
2. After autoclaving, cool to ~55°C, adjust to pH 5.7 with 1N NaOH.
3. Autoclave solution, again, on the liquid cycle for 20 minutes at 15 psi.
4. After autoclaving, cool to ~55°C, and pour into 10 cm plates.
5. Let harden, store at 25°C.

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