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# ab275761 pCambia0380 Plant Expression Vector

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Expression vector for cloning and expression of genes into plants.

This product is for research use only and is not intended for diagnostic use.

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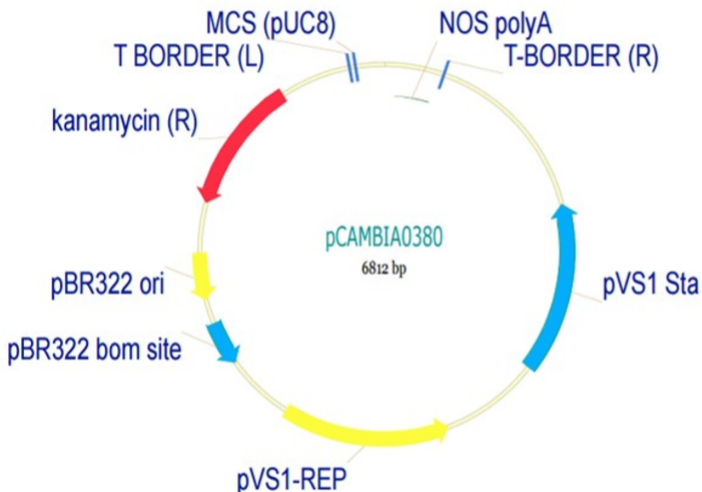
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# 1. Overview

The pCambia vector backbone is derived from the pPZP vectors. The pCambia0380 vector offers:

- High copy number in E.coli for high DNA yields
- pVS1 replicon for high stability in Agrobacterium
- Small size
- Restriction sites designed for modular plasmid modifications and small but adequate poly-linkers for introducing your DNA of interest
- Bacterial selection with kanamycin

This vectors contain a range of restriction sites on either side of the pUC8 polylinker, making it suitable for advanced construction purposes with users inserting their own promoters, selection genes, reporter genes. The only functional signals between the T-DNA borders are the start and stop codons, the histidine tag, and the NOS-poly(A) signal.



## 2. Materials Supplied and Storage

Store kit immediately on receipt. Kit can be stored for 6 months from receipt, if components have not been diluted. The plant expression vectors are shipped at room temperature. Store all samples of the vector at  $-20^{\circ}\text{C}$  or below, once resuspended.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temp
pCambia0380 Plant Expression Vector, Lyophilized in TE buffer, pH 8.0	20 $\mu\text{g}$	RT/ $-20^{\circ}\text{C}$

## 3. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)

For typical data produced using the assay, please see the assay kit datasheet on our website.

## 4. 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 immobilized 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).

## 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:**

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

## 5. Transfection

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood. Please read Notes below.

If a biological agent is being tested for its effects on enzyme activity, treat cells in culture with the agent/drug prior to use of this kit.

### 5.1 Preparation of competent *Agrobacterium tumefaciens* cells:

- 5.1.1 Inoculate 250 mL of LB medium with 750  $\mu$ L *Agrobacterium tumefaciens* (for example GV3101::pMP90).
- 5.1.2 Incubate at 28°C (Dubenoff shaking water bath) with vigorous shaking for 11 hours until the OD is approximately 0.75.
- 5.1.3 Pellet the cells by centrifugation (5000 rpm)
- 5.1.4 Wash the pellet with 2 mL sterile TE.
- 5.1.5 Centrifuge at room temperature for 10 min at 5000 rpm.
- 5.1.6 Resuspend the pellet in 20 mL LB medium. Aliquot 250  $\mu$ L of this suspension into 1.5 mL microcentrifuge tubes.

### 5.2 Transformation and recovery of *Agrobacterium tumefaciens* cells:

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

### 5.3 Floral dip with *Agrobacterium tumefaciens*:

- 5.1 Prepare Infiltration Medium (250 mL) (see recipe below in Appendix)
- 5.3.2 Prepare MS Agar Gel for plant growth (see recipe below in Appendix)
- 5.3.3 Pipette 15 – 20 mL into 25 x 250 mm sterile test tubes for each plant to be grown after dip.
- 5.3.4 Grow up one colony of transformed bacteria (See step 6.1.7) in LB media containing appropriate selection antibiotics (2 mL) overnight at 28°C (Dubenoff shaking water bath) with vigorous shaking.
- 5.3.5 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.
- 5.3.6 Pellet the bacteria by centrifugation at 6000 rpm at 25°C for 10 min.
- 5.3.7 Resuspend the bacteria in Infiltration Medium (50 mL) and swirl to ensure complete mixture of the bacteria.
- 5.3.8 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.
- 5.3.9 Dip the roots of plants in the bacterial suspension for 30 seconds.
- 5.3.10 After dipping, place the plants on agar plates (MS medium) and seal the lids with parafilm. Allow to set overnight at room temperature.
- 5.3.11 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.
- 5.3.12 Grow the plants under long-day light conditions (16 hour daylight, 8 hour darkness) for several weeks until flowering and seed pod production occurs.
- 5.3.13 Remove seed pods and dry seeds.
- 5.3.14 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  $\beta$ -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.

#### **5.4 To propagate and maintain the plasmid:**

- 5.4.1 Resuspend the vector in 20  $\mu$ l sterile water to prepare a 1  $\mu$ g/ $\mu$ L stock solution and store at  $-20^{\circ}\text{C}$ . Use the stock solution to transform *Agrobacterium tumefaciens*, or equivalent vehicle.
- 5.4.2 Select transformants on LB agar plates containing appropriate selection antibiotics.
- 5.4.3 Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

## 6. 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 assayed for GUS activity using the MarkerGene™  $\beta$ -Glucuronidase (GUS) Reporter Gene Activity Detection Kit, ab253372.

**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  $\beta$ -glucuronidase.** If you use a positive control vector, you may assay for  $\beta$ -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  $\beta$ -glucuronidase expression. See Accessory Products 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.

## 7. Recipes

### 7.1 LB (Luria-Bertani) Medium and Plates Composition:

- 1.0% Tryptone
- 0.5% Yeast Extract
- 1.0% NaCl pH 7.0

- 7.1.1 For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
- 7.1.2 Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
- 7.1.3 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.

### 7.2 LB agar plates:

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

### 7.3 4X SDS-PAGE Sample Buffer:

- 7.3.1 Combine the following reagents: 0.5 M Tris-HCl, pH 6.8, 5 ml Glycerol (100%), 4 ml  $\beta$ -mercaptoethanol, 0.8 ml Bromophenol Blue, 0.04 g SDS, 0.8 g
- 7.3.2 Bring the volume to 10 ml with sterile water.
- 7.3.3 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 ·6H<sub>2</sub>O, 0.025 mg/L cupric sulfate ·5H<sub>2</sub>O, 37.26 mg/L Na<sup>2</sup>- EDTA, 16.90 mg/L manganese sulfate-H<sub>2</sub>O, 0.250 mg/L molybdic acid sodium salt, 0.83 mg/L potassium iodide, 27.80 mg/L ferrous sulfate ·7H<sub>2</sub>O, 8.60 mg/L zinc sulfate ·7H<sub>2</sub>O) (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)

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 dH<sub>2</sub>O. Store at 4°C.

### **MS Agar Plant Growth Medium:**

1. Autoclave 10.6g Murashige & Skoog (MS) Medium and 250 mL dH<sub>2</sub>O 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.

## 8. Notes



## 9. Technical Support

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