Version 1 Last updated 7 September 2020

pCambia Plant Expression Vectors

For cloning and expression of genes into plants

These products are for research use only and are not intended for diagnostic use.

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1. Overview

The pCambia vector backbone is derived from the pPZP vectors.

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. Reporter genes feature a hexa-Histidine tag at the C-terminus to enable simple purification on immobilized metal affinity chromatography resins.

abID	Product name	Features (Reporter, Plant /Bacterial resistance, Polylinker)
ab275550	pCambia1304 Plant Expression Vector	*mgfp5*:*gusA,* Hygromycin B /Kanamycin
ab275753	pCambia1301 Plant Expression Vector	*gusA,* Hygromycin B /Kanamycin
ab275754	pCambia1300 Plant Expression Vector	No Reporter Gene, Hygromycin B /Kanamycin
ab275755	pCambia2301 Plant Expression Vector	*gusA,* Kanamycin /Kanamycin
ab275758	pCambia2300 Plant Expression Vector	No Reporter Gene, Kanamycin /Kanamycin
ab275760	pCambia1302 Plant Expression Vector	*mgfp5,* Hygromycin B /Kanamycin
ab275761	pCambia0380 Plant Expression Vector	No Reporter Gene, No Plant Selection /Kanamycin, pUC8
ab275762	pCambia1305.1 Plant Expression Vector	GusPlus™, Hygromycin B /Kanamycin
ab275764	pCambia1303 Plant Expression Vector	*gusA*:*mgfp5,* Hygromycin B /Kanamycin
ab275765	pCambia1105.1 Plant Expression Vector	GusPlus™/*lacZ*, Hygromycin B /Streptomycin
ab275766	pCambia1305.2 Plant Expression Vector	Secretory GusPlus™, Hygromycin B /Kanamycin
ab275767	pCambia1390 Plant Expression Vector	No Reporter Gene, Hygromycin B /Kanamycin, pUC9
ab275769	pCambia1380 Plant Expression Vector	No Reporter, Hygromycin B /Kanamycin, pUC8

abID	Product name	Features (Reporter, Plant /Bacterial resistance, Polylinker)
ab275770	pCambia1381Z Plant Expression Vector	*gusA/lacZ,* Hygromycin B /Kanamycin, pUC8
ab275771	pCambia0390 Plant Expression Vector	No Reporter Gene, No Plant Selection /Kanamycin, pUC9
ab275772	pCambia 1105.1R Plant Expression Vector	GusPlus™, Hygromycin B / Streptomycin
ab275773	pCambia1201 Plant Expression Vector	*gusA*, Hygromycin B / Chloramphenicol
ab275774	pCambia1391 Plant Expression Vector	*gusA*, Hygromycin B /Kanamycin, pUC9
ab275775	pCambia0305.2 Plant Expression Vector	Secreted GusPlus™, No Plant Selection /Kanamycin
ab275776	pCambia1391Z Plant Expression Vector	*gusA/lacZ*, Hygromycin B /Kanamycin, pUC9
ab275777	pCambia0105.1R Plant Expression Vector	GusPlus™, No Plant Selection /Streptomycin
ab275778	pCambia1200 Plant Expression Vector	No Reporter Gene, Hygromycin B /Chloramphenicol
ab275779	pCambia1281Z Plant Expression Vector	*gusA/lacZ*, Hygromycin B /Chloramphenicol
ab275780	pCambia2200 Plant Expression Vector	No Reporter Gene, Kanamycin / Chloramphenicol

Δ Note: For China and Japan see links in Appendix section 9.2.

2. Storage and Stability

Store all samples of the vector at –20°C or below, once resuspended.

3. 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.

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 - Z indicates presence of a functional *lacZa* for blue-white screening.

5. Cloning 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 $CaMV_{35}S$ promoter and terminated by the CaMV35S polyA signal.

Δ 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).

6. Using Plant Expression Vectors

6.1 Propagating pCambia Series Expression Vectors:

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

6.2 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.

7. Transfection

7.1 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.

7.2 Preparation of competent *Agrobacterium tumefaciens* cells:

7.2.1 Inoculate 250 mL of LB medium with 750 µL *Agrobacterium tumefaciens* (for example GV3101::pMP90).

7.2.2 Incubate at 28°C (Dubenoff shaking water bath) with vigorous shaking for 11 hours until the OD is approximately 0.75.

7.2.3 Pellet the cells by centrifugation (5000 rpm).

7.2.4 Wash the pellet with 2 mL sterile TE.

7.2.5 Centrifuge at room temperature for 10 minutes at 5000 rpm.

7.2.6 Resuspend the pellet in 20 mL LB medium. Aliquot 250 µL of this suspension into 1.5 mL microcentrifuge tubes.

7.3 Transformation and recovery of *Agrobacterium tumefaciens* cells:

7.3.1 Add 20 µL of the Vector (5 µg) to the competent GV3101::pMP90 *Agrobacterium tumefaciens* sample (250 µL) iin LB medium from above.

7.3.2 Incubate on ice (0°C) for 5 minutes.

7.3.3 Incubate in liquid nitrogen (-80°C) for 5 minutes.
7.3.4 Incubate at 37oC (water bath) for 5 minutes.
7.3.5 Add LB medium (1 mL) to each vial and incubate at room temperature (with rotation) for 4 hours.
7.3.6 Streak the bacteria onto LB Agar plates containing appropriate selection antibiotics and incubate at 28°C for 3 days.
7.3.7 Pick one colony and re-streak onto an LB Agar plate containing appropriate selection antibiotics and incubate at 28°C for 2 days.

7.4 Floral dip with *Agrobacterium tumefaciens*:

7.4.1 Prepare Infiltration Medium (250 mL) (see recipe below in Appendix).
7.4.2 Prepare MS Agar Gel for plant growth (see recipe below in Appendix).
7.4.3 Pipette 15 – 20 mL into 25 x 250 mm sterile test tubes for each plant to be grown after dip.
7.4.4 Grow up one colony of transformed bacteria (See step 5.3.7 from above) in LB media containing appropriate selection antibiotics (2 mL) overnight at 28°C (Dubenoff shaking water bath) with vigorous shaking.
7.4.5 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.
7.4.6 Pellet the bacteria by centrifugation at 6000 rpm at 25oC for 10 minutes.
7.4.7 Resuspend the bacteria in Infiltration Medium (50 mL) and swirl to ensure complete mixture of the bacteria.
7.4.8 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.
7.4.9 Dip the roots of plants in the bacterial suspension for 30 seconds.
7.4.10 After dipping, place the plants on agar plates (MS medium) and seal the lids with parafilm. Allow to set overnight at room temperature.
7.4.11 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.
7.4.12 Grow the plants under long-day light conditions (16 hour daylight, 8 hour darkness) for several weeks until flowering and seed pod production occurs.

7.4.13 Remove seed pods and dry seeds.

7.4.14 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.

7.5 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.

7.6 To propagate and maintain the plasmid:

7.6.1 Resuspend the vector in 20 µL sterile water to prepare a 1µg/µL stock solution and store at -20°C. Use the stock solution to transform *Agrobacterium tumefaciens,* or equivalent vehicle.

7.6.2 Select transformants on LB agar plates containing appropriate selection antibiotics.

7.6.3 Prepare a glycerol stock of a transformant containing plasmid for long-term storage. Pipette 15 – 20 mL into 25 x 250 mm sterile test tubes for each plant to be grown after dip.

8. Expression and Analysis

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

8.1 GUS Assay using Cell Lysates

Cell lysate from above step can be assayed for GUS activity using the Beta-Glucuronidase (GUS) Reporter Gene Activity Detection Kit, ab253372.

8.2 Detecting Recombinant Fusion Proteins:

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

8.3 GUS Assay using Cell Lysates

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. X-GlcU staining, or fluorescence detection are common methods of analysis.

8.4 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.

Δ Note: Other purification methods may also be suitable including affinity chromatography.

9. Appendix:

9.1 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·$6H_20$, 0.025 mg/L cupric sulfate·$5H_20$, 37.26 mg/L Na_2- EDTA, 16.90 mg/L manganese sulfate-H_20, 0.250 mg/L molybdic acid sodium salt, 0.83 mg/L potassium iodide, 27.80 mg/L ferrous sulfate·$7H_20$, 8.60 mg/L zinc sulfate·$7H_20$)
1X Gamborg's B5 Vitamin
5% Sucrose (w/v)
0.044 µM 6-benzylaminopurine (BAP) (stock solution 1mg/mL DMSO)
0.05% Silwet L77 (Lehle Seeds)

For 250 mL Infiltration media, mixed 12.5 mL 10X MS salts, 0.25 mL Gamborg's B5 Vitamin1000X, 12.5 mg Sucrose, 2.5 µL 1 mg/mL stock solution of Benzylaminopurine (BAP), 12.5 µL Silwet L77, 224.73 mL dH_20. Store at +4°C.

MS Agar Plant Growth Medium:

1. Autoclave 10.6 g Murashige & Skoog (MS) Medium and 250 mL dH_20 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.

9.2 Links

abID	For China	For Japan
ab275550	https://www.abcam.cn/ab275550	https://www.abcam.co.jp/ab275550
ab275753	https://www.abcam.cn/ab275753	https://www.abcam.co.jp/ab275753
ab275754	https://www.abcam.cn/ab275754	https://www.abcam.co.jp/ab275754
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ab275758	https://www.abcam.cn/ab275758	https://www.abcam.co.jp/ab275758
ab275760	https://www.abcam.cn/ab275760	https://www.abcam.co.jp/ab275760
ab275761	https://www.abcam.cn/ab275761	https://www.abcam.co.jp/ab275761
ab275762	https://www.abcam.cn/ab275762	https://www.abcam.co.jp/ab275762
ab275764	https://www.abcam.cn/ab275764	https://www.abcam.co.jp/ab275764
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ab275780	https://www.abcam.cn/ab275780	https://www.abcam.co.jp/ab275780

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)