# Chapter 9

# Genetic Transformation of Lettuce (*Lactuca sativa*) Using *Agrobacterium tumefaciens*

**Damaris Godinez-Vidal, Javier Narváez-Vásquez, and Martha L. Orozco-Cárdenas**

## Abstract

Lettuce (*Lactuca sativa* L.) plays a pivotal role in global agriculture and food supply chains as a widely cultivated commercial crop. This chapter presents a detailed protocol for transforming lettuce using *Agrobacterium tumefaciens*. The procedure includes a two-day pre-culture period of cotyledon explants, followed by a 20-min inoculation with *Agrobacterium* without explant wounding, and a two-day co-culture on the same medium. Afterward, the selection and regeneration phases under kanamycin selection led to the establishment of transgenic plants in the greenhouse. The entire process from the initial explant infection to the greenhouse cultivation of transgenic plants spans roughly 120 days, achieving an average transformation efficiency of 45%. This protocol offers a reliable method for the genetic modification of lettuce, for use in research or crop improvement.

**Key words** Lettuce, *Agrobacterium tumefaciens*, Plant transformation, Transgenesis, Kanamycin resistance gene, Neomycin phosphotransferase II (*nptII*)

## 1 Introduction

Lettuce, a crisp and refreshing leafy green, is prominent in our culinary traditions and agricultural landscapes. With its rich history dating back to ancient civilizations and widespread cultivation in modern times, lettuce is a staple vegetable cherished for its nutritional value, culinary versatility, and economic significance. From vibrant salads to flavorful wraps, lettuce adds not only color and texture but essential nutrients to our diets [1]. Rich in vitamins A, C, and K, folate, and various antioxidants, lettuce contributes to overall health and well-being. Beyond its nutritional value, lettuce plays a pivotal role in global agriculture and food supply chains. As a widely cultivated commercial crop, lettuce occupied vast acreages in fields, greenhouses, and hydroponic systems worldwide.

Claudia Stange Klein (ed.), *Agrobacterium: Methods and Protocols*, Methods in Molecular Biology, vol. 2911,
https://doi.org/10.1007/978-1-0716-4450-8_9,

While lettuce enjoys widespread consumption and cultivation, it is not immune to the challenges faced by crops. Environmental stressors, pest and disease pressures, and shifting consumer preferences underscore the importance of continual improvement in lettuce genetics [2]. Genetic transformation, a powerful tool in biotechnology, offers promising solutions to enhance lettuce varieties with traits such as disease resistance, tolerance to abiotic stresses, improved shelf life, and enhanced nutritional profiles [2–4].

Lettuce's responsiveness to tissue culture and transformation protocols, established since the late 1980s [5–8], has facilitated extensive research into transgenesis, aiming for improved crop traits and the production of biopharmaceuticals [3, 9]. The plant's short life cycle, autogamous reproduction, and sequenced genome are highlighted factors that contribute to lettuce's suitability for functional genomics studies and as a platform for molecular farming, including the production of oral vaccines and therapeutic proteins [3, 9].

This chapter outlines a detailed procedure for the transformation of lettuce using *Agrobacterium tumefaciens*, employing cotyledonary leaves as explants. The method utilizes the *Agrobacterium* strain EHA105 carrying the binary vector pART27 [10], which includes the *nptII* gene as a selective marker for plants, followed by the regeneration of genetically stable transformed plants under kanamycin selection. From the initiation of explant infection to the establishment of transgenic plants in a greenhouse setting, the protocol spans approximately 120 days (Fig. 1). We define the average transformation efficiency of our protocol as 45%, calculated by the ratio of explants that test positive for the selective marker gene via polymerase chain reaction (PCR) to the total number of infected explants.

## 2 Materials

### 2.1 Plant Material

Healthy seeds of *Lactuca sativa* (cv Conquistador) are used (*see* **Note 1**). The tissue utilized in this transformation protocol compromises cotyledons derived from lettuce seedlings [11].

### 2.2 Agrobacterium Strain and Binary Vector

This method employs electroporation-competent cells of *Agrobacterium tumefaciens* strain EHA105 (www.intactgenomics.com) and the plant transformation vector pART27 [10]. This binary vector contains the *Tn7* spectinomycin/streptomycin resistance gene as a bacterial selectable marker and the kanamycin (*nptII*) resistance gene for the selection of transformed plants.

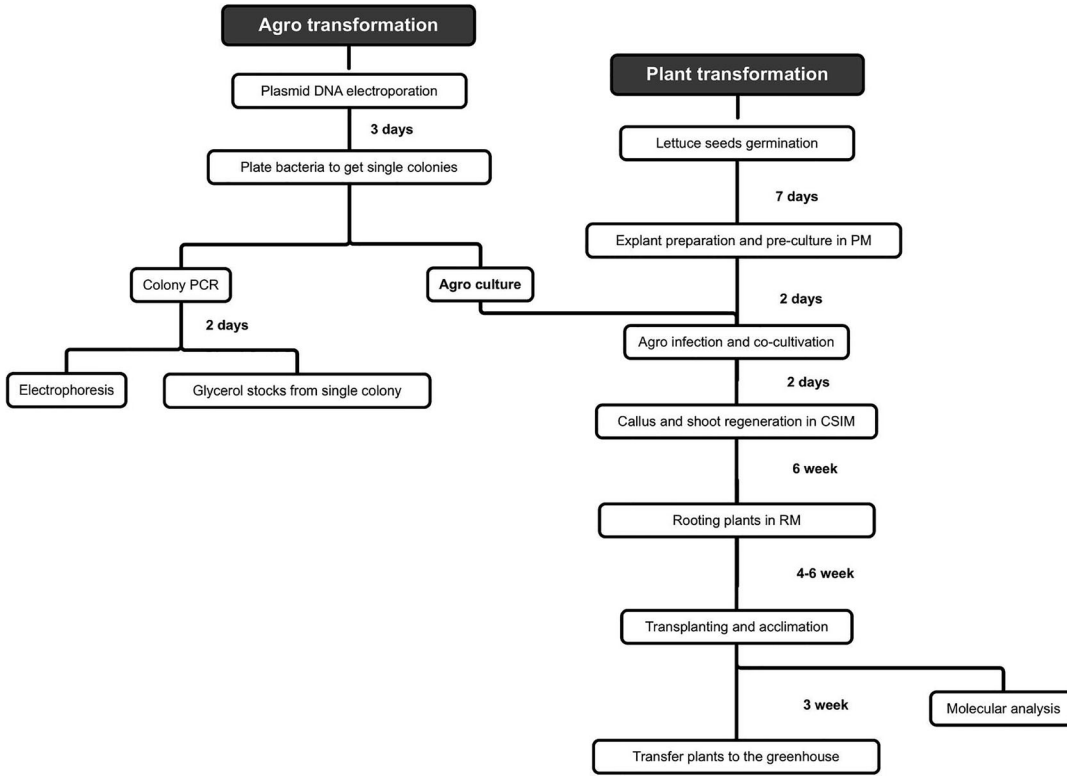


**Fig. 1** Schematic representation of *Agrobacterium*-mediated transformation of lettuce

### 2.3 Stock Solutions

1. 50 mM acetosyringone (3′,5′-dimethoxy-4′-hydroxyacetophenone): Dissolve 490.5 mg in 50 mL of dimethyl sulfoxide (DMSO).
2. Murashige and Skoog's (MS) vitamin [12] stock (1000x): Dissolve 200 mg glycine, 1 g myo-inositol, 50 mg nicotinic acid, 50 mg pyridoxine-HCl, and 100 mg thiamine-HCl in 100 mL of $ddH_2O$.
3. 100 mg/mL antibiotic stock solutions: Dissolve 1 g of each antibiotic (e.g., spectinomycin, kanamycin, cefotaxime, and carbenicillin) in 10 mL of $ddH_2O$. For 15 mg/mL rifampicin stock solution,dissolve 150 mg of rifampicin in 10 mL of $ddH_2O$. Filter sterilize antibiotic stock solutions using a 0.22 μM filter, and store them in 1 mL aliquots at −20 °C.
4. Cetyltrimethylammonium bromide (CTAB) buffer for DNA extraction: 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 3% CTAB, and 0.2% β-mercaptoethanol.

### 2.4 Other Reagents and Supplies

1. Seed sterilization solution: 10% commercial bleach (Clorox: active ingredient 7.5% sodium hypochlorite), plus two drops of Tween 20 per 50 mL of $ddH_2O$.

2. 50-mL conical centrifuge tubes.
3. Eppendorf 1.5-mL tubes.
4. 17-mm x 100-mm polypropylene tubes.
5. Genesys 30 spectrophotometer.
6. Bio-Rad (Micropulser) electroporator.
7. NanoDrop spectrophotometer.
8. Molecular Imager Gel Doc XR+ Imaging System.
9. Greenhouse standard open flat with drainage hole: McConkey, Cat # EJPFONH.
10. Clear Humi-dome 7″ (plastic, transparent): McConkey, Cat # HYFCKDOME-50.
11. Jiffy peat pellets 42 mm: McConkey, Cat # JPA703.

### 2.5 Culture Media

1. SOC media: 5 g/L yeast extract, 20 g/L tryptone, 0.6 g sodium chloride (NaCl), 0.19 g potassium chloride (KCL), and 2.4 g magnesium sulfate (MgSO4). Adjust pH to 7.5 with 1 M sodium hydroxide (NaOH). Prepare aliquots of 1 mL, and store them at −20 °C until use.
2. YEP media: 10 g/L yeast extract, 10 g/L peptone, and 5 g NaCl. Adjust pH to 7.0 with 1 M NaOH. For YEP semi-solid plates, add bacto agar 15 g/L before sterilization. After the autoclave cycle, wait until YEP media cools to less than 55 °C before adding the antibiotics rifampicin (12.5 mg/L) and spectinomycin (100 mg/L) for bacterial selection.
3. Germination media: 4.3 g/L Murashige and Skoog's (MS) inorganic salts [12], 30 g/L sucrose, 100 mg/L myo-inositol, and 1 mL/L MS vitamin stock (1000x), adjust pH to 5.7 with 1 M KOH, and add 8 g/L agar before autoclaving.
4. Pre-culture media (PM): 4.3 g/L MS inorganic salts, 30 g/L sucrose, 100 mg/L myo-inositol, 1 mL/L MS vitamin stock (1000x), 0.1 mg/L indole-3-acetic acid (IAA), and 1 mg/L N-6(2-isopentenyl)-adenine (2ip). Adjust pH to 5.8 with 1 M KOH, and add agar 8 g/L before sterilization.
5. *Agrobacterium* dilution media (ADM): 4.3 g/L MS inorganic salts, 30 g/L sucrose, 1 mL/L MS vitamin stock solution (1000x), and pH 5.8.
6. Callus and shoot induction selection media (CSIM): 4.3 g/L MS inorganic salts, 30 g/L sucrose, 100 mg/L myo-inositol, 1 mL MS vitamins (1000x), 0.1 mg/L indole-3-acetic acid (IAA), and 0.5 mg/L kinetin. Adjust pH to 5.8 with 1 M KOH, and add agar 8 g/L before sterilization. After autoclaving, shake and let the medium cool down to <55 °C, and then, add the antibiotics cefotaxime (250 mg/L) and carbenicillin

(500 mg/L) for the control of *Agrobacterium* and kanamycin (100 mg/L) for the selection of transformed plant cells. Pour medium into 100 × 15-mm Petri dishes.

7. Rooting media (RM): Schenk and Hildebrandt (SH) inorganic salts [13], 30 g/L sucrose, 100 mg/L myo-inositol, and 1 mL/L MS vitamins (1000x). Adjust pH to 5.8 with 1 M KOH, and add agar 8 g/L before sterilization. After autoclaving, shake and let the medium cool down to <55 °C, and then, add 125 mg/L cefotaxime, 250 mg/L carbenicillin, and 50 mg/L kanamycin.

## 3 Methods

### 3.1 Agrobacterium Transformation

1. *A. tumefaciens* cells are transformed by electroporation with the respective binary vector.
2. Thaw the electrocompetent EHA105 *Agrobacterium* cells on ice.
3. Place a 1.5-mL microfuge tube and either a 0.1 or 0.2 cm electroporation cuvette on ice.
4. In the cold 1.5-mL microfuge tube, mix 40 μL of the EHA105 *Agrobacterium* cells with 1 to 2 μL of plasmid DNA (10 ng/μL). DNA should be in a low-ionic strength buffer such as TE. Mix well, and incubate on ice for ~1 min (*see* **Note 2**).
5. Set the BioRad micropulser to "EC1" when using 0.1-cm cuvettes.
6. Transfer the mixture of cells and DNA to the cold electroporation cuvette, and tap the suspension to the bottom. Place the cuvette in the chamber slide. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber. Pulse once.
7. Remove the cuvette from the chamber, and immediately add 1 mL of SOC medium to the cuvette. Quickly but gently resuspend the cells with a sterile Pasteur pipette (*see* **Note 3**).
8. Transfer the cell suspension to a 17-mm × 100-mm polypropylene tube, and incubate at 28 °C for 1 h, shaking at 225 rpm.
9. Place 100 μL of the transformed cells on a solid YEP medium with the respective selection antibiotics, and incubate the plate at 28 °C until single colonies are observed (*see* **Note 4**).
10. Reconfirm *Agrobacterium* transformation by colony PCR.

### 3.2 Colony PCR

1. PCR enables the efficient amplification of specific DNA fragments from a single colony of bacteria such as *E. coli* or *Agrobacterium*. Prepare regular PCR reactions (25 μL) using a set of

primers to amplify regions from the gene of interest, a selection, or a reporter gene present in the binary transformation vector.

2. After *Agrobacterium* transformation, pick up 3–5 independent colonies using the tip of a sterile toothpick or a pipette tip.
3. Immerse and shake the tip in the PCR reaction mix within the PCR tube.
4. Transfer the tip into a 17-mm x 100-mm polypropylene tube with 3 mL of YEP medium with the corresponding selection antibiotic, and incubate with shaking at 225 rpm and 28 °C overnight.
5. After the PCR amplification is completed (*see* **Note 5**), the resulting PCR products are analyzed by agarose gel electrophoresis.
6. Positive colonies with expected PCR amplicons and capable of growing in the selection YEP medium are selected and stored as glycerol stocks at −80 °C for later use in plant transformation (*see* **Note 6**).

### 3.3 Preparation of Agrobacterium Cells for Plant Transformation

1. Two days before transformation, streak *Agrobacterium* from a frozen glycerol stock on solid YEP media containing the appropriate antibiotics, and incubate the plate at 28 °C until single colonies are observed.
2. From a single colony, inoculate a PCR reaction tube previously prepared per colony, and inoculate 3 mL of liquid YEP media with the appropriate antibiotics. Incubate the 3 mL YEP culture at 28 °C, shaking at 225 rpm, and let it grow overnight.
3. Take 100 μL of this culture to inoculate 25 mL of liquid YEP media containing the appropriate antibiotics and 80 μM acetosyringone.
4. Incubate at 28 °C overnight, with shaking at 225 rpm.
5. Next day, spin down the culture at 5000 rpm (2152× *g*) for 10 min, and resuspend the pellet in 10 mL of ADM with 80 μM acetosyringone.
6. Determine the $OD_{600}$ of resuspended *Agrobacterium* cells, and adjust the $OD_{600}$ to 0.6 with ADM (*see* **Note 7**).

### 3.4 Explant Preparation for Transformation

1. Sterilize lettuce seeds with a sterilization solution for 15 min.
2. Rinse seeds three times with sterile $ddH_2O$.
3. Plant seeds on germination media, and incubate for 10–13 days at 25 °C with a 16 h light photoperiod (18 μmol $m^{-2}$ $s^{-1}$ provided by daylight fluorescent tubes).

4. After seven days, cotyledon explants from germinated seedlings are cut into squares of ~0.25 $cm^2$ and placed upside down over PM media.
5. Incubate explants for 2 days at 25 °C and a 16 h photoperiod (18 μmol $m^{-2}$ $s^{-1}$, daylight fluorescent tubes).

### 3.5 Agrobacterium Infection and Co-cultivation

1. After two days, cotyledon explants are transferred to a 50 mL conical tube with 40 mL of the diluted *Agrobacterium* cell suspension.
2. The cotyledons are incubated with the *Agrobacterium* cells for 20 min at 25 °C at 50 rpm.
3. Drain the liquid ADM medium with *Agrobacterium*, and transfer the explants over two sterile paper towels to quickly remove the excess liquid medium with *Agrobacterium* cells (*see* **Note 8**).
4. The infected cotyledons are transferred back to the PM plates with filter paper and incubated under the same conditions for two days (*see* **Note 9**).

### 3.6 Callus and Shoot Regeneration

1. Once two days have passed, the cotyledons are transferred to the CSIM medium with selection antibiotics for callus formation and shoot induction and incubated under a 16 h photoperiod (50–70 μmol $m^{-2}$ $s^{-1}$, daylight provided by fluorescent tubes) at 25 °C (*see* **Note 10**).
2. The explants must be sub-cultured every two weeks. After six weeks, when shoots are elongated (~2 cm long), gently cut them off, remove the browned calli at the base of the shoot and any vitrified tissue (*see* **Note 11**), and transfer the shoots onto fresh rooting media (Fig. 2a–e).

### 3.7 Rooting Plants

1. The elongated shoots are cultured in rooting RM media [6] and incubated as before (*see* **step 1** of Subheading 3.6).
2. Once the shoots have grown multiple roots (~3 cm long), the plantlets can be transferred to soil for acclimation (Fig. 2f).

### 3.8 Transplanting and Acclimation

1. Gently remove well-rooted seedlings from the sterile container, and place them on a tray with $ddH_2O$.
2. Carefully remove the agar medium remaining attached to the roots by slightly shaking and pressing the agar with your fingers and using a squirt bottle with $ddH_2O$.
3. Transfer each plantlet to a Jiffy peat pellet, placed within a flat tray covered with a transparent dome to maintain the humidity during the first week (Fig. 2g). Each putative transgenic (T0) plant should be independently labeled.

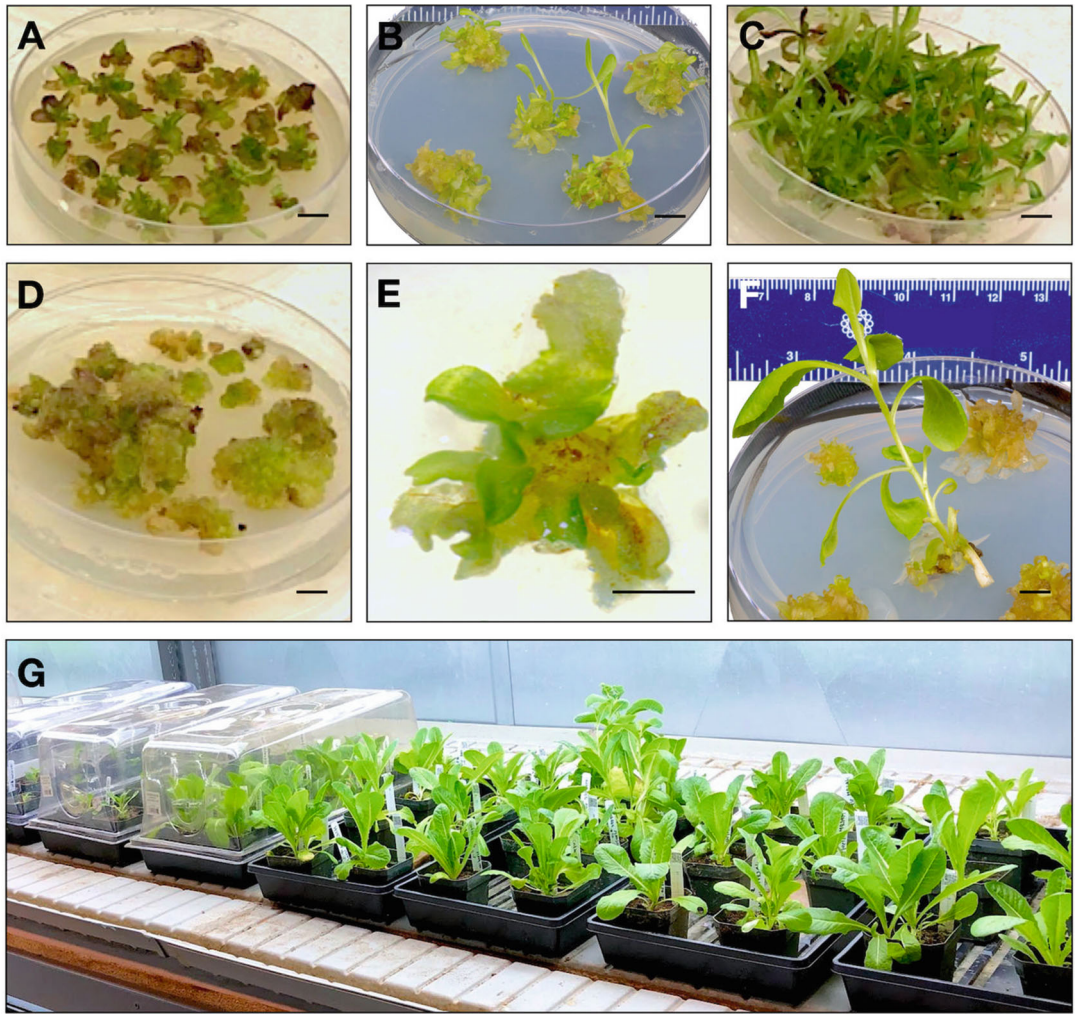


**Fig. 2** Callus formation and shoot regeneration from cotyledon explants after *Agrobacterium*-mediated transformation. (**a**) Shoot initiation after four weeks on CSIM selection medium. (**b**, **c**) Shoot elongation and proliferation after six weeks on CSIM. (**d**, **e**) Callus and shoot showing vitrified tissue (*see* **Note 11**). (**f**) Root induction in shoots 2 weeks after transfer to RM selection medium. (**g**) Acclimation and growth of transgenic lettuce plants four weeks after soil transfer. Scale bars: 1 cm

4. The plantlets are grown at 22 °C under a 16 h-light-8 h-dark photoperiod (~100 μmol $m^{-2}$ $s^{-1}$, daylight provided by fluorescent tubes).
5. Once the plants are well established (after about 3 weeks), transfer them to larger pots (e.g., 6.5″) with Sunshine Universal soil mix, and water daily with a half-strength Hoagland's solution.
6. Grow plants to maturity in a greenhouse with a temperature of ~25 °C, relative humidity of ~70%, and natural light conditions.

### 3.9 DNA Extraction for Molecular Analyses

1. For DNA extraction, collect 100 mg of leaves from every plant in duplicate, flush freeze in liquid $N_2$, and store tissue at −80 °C.
2. Grind the tissue with liquid $N_2$ in a mortar, transfer the tissue powder to a 1.5-mL microfuge tube, and add 450 μL of CTAB extraction buffer.
3. Incubate the suspension for 60 min at 65 °C.
4. Add 450 μL of chloroform-to-isoamyl alcohol (1:1) to each tube, and shake the tubes vigorously using a vortex mixer for 1 min.
5. Transfer the upper aqueous phase to a newly labeled microfuge tube for DNA precipitation.
6. Add 450 μL of cold isopropanol to each tube or a volume of the total upper aqueous phase obtained. Mix gently, inverting several times. Incubate the tubes at −20 °C for 30 min.
7. Centrifuge the tubes at 14,000 rpm for 10 min.
8. Discard the supernatant without disturbing the pellet.
9. Wash the pellet with 1 mL of cold 70% ethanol by vortexing briefly to ensure washing of the pellet, and then, centrifuge the tubes at 14,000 rpm for 5 min.
10. To resuspend the DNA pellet, add 30–50 μL of TE or nuclease-free water to each tube. The DNA has to be resuspended by pipetting up and down or by incubation at 65 °C for 10 min.
11. Measure the DNA concentration, and check quality using a NanoDrop spectrophotometer.
12. Store the DNA at −20 °C until further use.

### 3.10 Detection of Transgenes Using PCR Analysis

1. Polymerase chain reaction (PCR) is a widely used technique to reconfirm the presence of a transgene (e.g., a reporter or selective marker gene) in genomic DNA isolated from putative transgenic plants regenerated after *Agrobacterium*-mediated transformation. Prepare the PCR reaction mix by adding the following components to a PCR tube for every plant tissue sample to be screened (a master mix is normally prepared by multiplying the volume of each component by the number of samples):
   (a) 12.5 μL of Mango Mix Taq DNA polymerase mix (*see* **Note 12**).
   (b) 1.0 μL of 10 μM forward stock solution primer (*see* **Note 13**).
   (c) 1.0 μL of 10 μM reverse stock solution primer.
   (d) DNA template (10–100 ng/rx).
   (e) X μL PCR-grade water to reach the final volume of 25 μL.
   (f) Mix the contents of the tube thoroughly by pipetting up and down.

2. Transfer the PCR reaction mix to a PCR tube.
3. Place the PCR tubes in a thermal cycler, and run the following PCR program:
   (a) Initial denaturation: 95 °C for 5 min.
   (b) Denaturation: 95 °C for 30 s.
   (c) Annealing: Use the appropriate annealing temperature for your primers (typically 55–65 °C), and incubate for 30 s.
   (d) Extension: 72 °C for the time determined by the expected size of the amplicon (usually 1 min per kb).
   (e) Repeat steps b–d for the desired number of cycles (typically 25–35 cycles).
   (f) Final extension: 72 °C for 5 min.
4. After the PCR amplification is complete, the resulting PCR products are analyzed using agarose gel electrophoresis and/or DNA sequencing (Fig. 3).

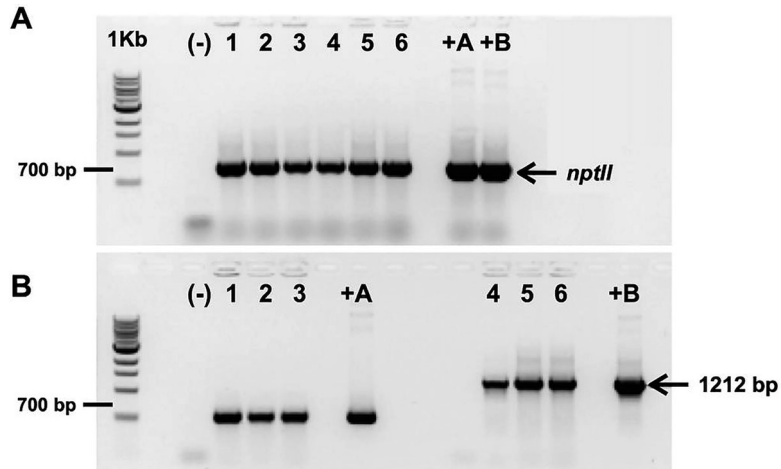


**Fig. 3** PCR analysis of bacterial colonies and putative transgenic lettuce plants. (**a**) Colony PCR for the detection of kanamycin-resistant (*nptII*) genes in *Agrobacterium* transformed by electroporation with plasmids A and B. Lanes 1–3 represent independent *Agrobacterium* colonies transformed with plasmid A, and lanes 4–6 represent colonies transformed with plasmid B. (**b**) Detection of transformed genes A and B in genomic DNA isolated from regenerated plants. Lanes 1–3 are plants transformed with a plasmid containing gene A, and lanes 4–6 correspond to plants transformed with a plasmid containing gene B. (−) non-template control; (+A) plasmid A; (+B), plasmid B

## 4 Notes

1. *Lactuca sativa* cv. Conquistador is recommended for its high efficiency of transformation. However, other lettuce cultivars can also be transformed using this method, albeit with varying efficiencies. The lettuce seeds were obtained from the Lettuce Genetic Resource Center (LGRC), University of California Davis (www.ucdavis.edu).
2. It is best to mix the plasmid and cells in a microfuge tube since the narrow gap of the cuvettes prevents uniform mixing. Be aware to always keep the cuvette in ice even once electroporation occurs. Little temperature changes can affect the transformation efficiency and the recovery of *Agrobacterium* cells.
3. The period between applying the pulse and transferring the cells to the outgrowth medium is crucial for recovering *Agrobacterium* cells. Delaying in transfer by even 1 min causes a threefold drop in transformation. This decline continues to a 20-fold drop by 10 min. Check and record the pulse parameters; the time constant should be close to 5 milliseconds, and the field strength can be calculated as actual volts (kV) cuvette gap (cm).
4. Depending on the *Agrobacterium* strain, colonies can be observed 2–3 days after transformation.
5. The PCR program should include an initial denaturing cycle at 95 for 5 min to break the bacterial cells for the release of the plasmid DNA template.
6. To establish the glycerol stock, mix an equal volume of 60% glycerol with the bacteria culture, prepare aliquots in a 1.5-mL microfuge tube, and store at −80 °C.
7. For the final dilution, use the equation, $C_1V_1 = C_2V_2$. Always use freshly prepared ADM medium to dilute *Agrobacterium*.
8. Because the cotyledons are very fragile, handle them carefully and quickly to avoid dehydration.
9. The filter paper (e.g., Whatman #1) helps control *Agrobacterium* overgrowth that may kill the tissue.
10. If *Agrobacterium* overgrowth is observed, carefully remove the explants from the plate, place them in a 50-mL conical tube, and gently rinse two to three times with sterile $ddH_2O$. Then, add 50 mL of antibiotic solution containing 250 mg/L cefotaxime and 500 mg/L carbenicillin, incubate with gentle shaking for 15 min, and rinse two to three times with sterile $ddH_2O$. Pad-dry the explants on sterile filter paper, and transfer them to fresh SHIM selection media plates.

11. Vitrification, also known as hyperhidrosis, occurs when plant tissues become excessively hydrated and take on a translucent, glass-like appearance. This condition impairs normal shoot growth and development, making the plants less viable when transferred to soil or less capable of normal physiological functions. If overgrowth of crystal-like calli or hyperhydrated leaf tissue is noted, this tissue must be removed and the calluses transferred to a fresh medium. Improving air exchange or in vitro ventilation of vessels, i.e., with the use of vented (perforated) lids, helps reduce the incidence of hyperhydricity.
12. MangoMix™ is a 2x reaction mix containing MangoTaq™ DNA Polymerase, $MgCl_2$, and ultra-pure dNTPs manufactured by Meridian Bioscience.
13. For primer design, use the region of interest in your DNA template. Using software to design the primers is essential, as it considers factors like GC content, secondary structure, and specificity. Ensure the primers have appropriate melting temperatures (Tm) and avoid self-complementarity or primer-dimer formation.

## Acknowledgements

The authors wish to acknowledge Andres Narváez and Serine Kousi for their technical assistance. This work was supported by the Plant Transformation Research Center of the University of California Riverside.

### References

1. Lal B, Bhandari S, Upadhyay A, Singh P, Singh VP (2024) Lettuce: A dive into its nutritional value and economic significance. AGBIR 40: 954–956
2. Pan W, Liu X, Li D, Zhang H (2022) Establishment of an efficient genome editing system in lettuce without sacrificing specificity. Front Plant Sci 13:930592
3. Mohebodini M, Jalali-Javaran M, Alizadeh H, Mahboudi F, Yarbakht M (2014) *Agrobacterium*-mediated transformation of lettuce (*Lactuca sativa* L.) to express IgG-binding protein a and human pro-insulin as a fusion protein. J Hortic Sci Biotechnol 89:719–725
4. Sharma S, Gautam N, Thakur AK, Srivastava DK (2023) Transgenic lettuce (*Lactuca sativa* L.) harboring chitinase gene expressed resistance against a devastating fungus, *Sclerotinia sclerotiorum*. Vegetos 36:1265–1274
5. Michelmore R, Ellen M, Seely S, Landry B (1987) Transformation of lettuce (*Lactuca sativa*) mediated by *Agrobacterium tumefaciens*. Plant Cell Rep 6:439–442
6. Teng WL, Liu YJ, Soong TS (1992) Rapid regeneration of lettuce from suspension culture. Hortic Sci 27:1030–1032
7. Curtis IS, Power JB, Blackhall NW, de Laat AMM, Davey MR (1994) Genotype-independent transformation of lettuce using *agrobacterium tumefaciens*. J Exp Bot 279: 1441–1449
8. Lim W, Park J, Park S (2011) Re-evaluation of the effects of growth regulators on callus induction and shoot regeneration in *Agrobacterium*-mediated transformation of lettuce. Acta Physiol Plant 33:1631–1637
9. Darqui FS, Radonic LM, Beracochea VC, Hopp HE, López Bilbao M (2021)

Peculiarities of the transformation of Asteraceae Family species: the cases of sunflower and lettuce. Front Plant Sci 12:767459

10. Gleave AP (1992) A versatile binary vector system with a T-DNA organizational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Mol Biol 20:1203–1207
11. Fallah-Ziarani M, Haddad R, Garoosi G, Jalali M (2013) *Agrobacterium* transformation of cotyledonary leaf of lettuce (*Lactuca sativa* L.) by the GCHI gene. IJGPB 2:47–55
12. Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant 15:473–497
13. Schek RU, Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canad J Bot 50:199–204