Protocol for *P. trichocarpa* transformation

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***Agrobacterium* strain:**

Agrobacterium tumefaciens C58 w/ pBI121 construct

Plant material:

5-6 month old *Populus trichocarpa*, 150-200 cm in height, stem segments (~25cm) between 5-9 internodes (depend on trees) from greenhouse

Transformation Protocol:

1. Streak *Agrobacterium* single colony on a LB plate containing appropriate antibiotics. Incubate at 28°C for 2 days.

2. Pick up one isolated *Agrobacterium* colony to inoculate 3mL of LB medium with the appropriate antibiotics and grow for 20 hours in a shaker at 28°C, 200rpm.

3. *Agrobacterium* cells were grown for 20 hours in LB medium supplemented with the appropriate antibiotics on a shaker at 28 °C and 200 rpm. (Acetosyringone was added to LB medium)

4. Razor blade soaked in 70% ethanol for ~30 min.

5. Pick up trees 5-10 internodes in greenhouse and wash plant materials collected in 10 % Clorox solution for 20 minutes and at least three times rinse in sterile dd water.
   Notes: Keep in mind, pick up internodes varies depending on trees condition. After rinse immediately follow next step.

6. Stem segments (4 mm in length) were excised using razor blade and inoculated by swirling in an *Agrobacterium* culture for five minutes.

7. The inoculated explants were co-cultivated on CIM1 at 25 °C in darkness for two days.

8. Explants were washed four times in sterile deionized water, blotted dry on a sterile filter paper, and cultured in the dark on CIM2 at 25 °C. First, explants were subcultured twice every five days. Then, they were subcultured every 14 days until 45-60 days of co-cultivation.
   Notes: White calli should appear about 1 month in this stage, if not, prolong the time in the dark.

Agrobacterium control is crucial in this stage. Check your plate regularly. If infested, cleanup the infested explants, and transfer those good ones to a new plate.

9. Explants with putative transgenic callus were transferred in light at 25 °C onto SIM1 and subcultured every 14 days until 42 days.

10. Then explants with putative transgenic callus were inoculated on SIM3 for 1 ~ 4 months in light at 25 °C, subcultured every 20 days.

11. Putative transgenic shoots (1 cm in length) were cut off and inoculated on RM at 25 °C in low light. Whole plantlets with good root system were gained after approximately 1-1.5 months.

12. When rooted shoot reaches Magenta box top,you might confirmation by PCR and propagate copies.Later,you can move to green house with control wide type.

Agrobacterium medium:

LB, add kanamycin 50mg/L, genmycin 50mg/L, AS 20um

Culture Medium

CIM1

Basal medium: 1X MS salts and 1X vitamins
Kinetin: 0.5 mg/L
2,4-D: 0.05 mg/L
Inositol: 100mg/L
Sucrose: 30g/L
Adjust pH to 5.7 by 1N KOH, add
Agar: 6.5 g/L
Autoclaved, then add
Coconut water: 10 %

CIM2

Basal medium: 1X MS salts and 1X vitamins
Kinetin: 0.5 mg/L
2,4-D: 0.05 mg/L
Inositol: 100mg/L
Sucrose: 30g/L
Adjust pH to 5.7 by 1N KOH, add
Agar: 6.5 g/L
Autoclaved, then add filter sterilized
Kanamycin 50 mg/L
Cefotaxime 500 mg/L

SIM1

Basal medium: 1/2X MS salts and 1X vitamins

Inositol: 100mg/L
Sucrose: 30g/L
Adjust pH to 5.7 by 1N KOH, add
Agar: 6.5 g/L
Autoclaved, then add filter sterilized
Thidiazuron: 0.132 mg/L
Kanamycin 100 mg/L
Cefotaxime 500 mg/L

SIM3

Basal medium: 1X WPM
Inositol: 100mg/L
Sucrose: 30g/L
Adjust pH to 5.7 by 1N KOH, add
Agar: 6.5 g/L
Autoclaved, then add filter sterilized
Zeatin: 2.0 mg/L
Kanamycin 50 mg/L
Cefotaxime 500 mg/L

RM

Basal medium: 1 X WPM
Inositol: 100mg/L
Sucrose: 20g/L
Ca-gluconate: 650mg/L
MES: 500mg/L
Adjust pH to 5.75 by 1N KOH, add either
Agar: 6.5g/L or phytogel: 3g/L
Autoclaved