Transformation protocol for potato

Solanum tuberosum var. Désireé

Preparing the plants

1. You need about 25 plants **3-4 weeks old** growth in MS-Medium with 2% sucrose, without antibiotics:
   5 plants/jar

Preparing the *Agrobacterium* suspension

2. On **Saturday** streak out the recombinant *Agrobacterium* on plates with YEB-media (with Amp/Rif/Kan) (in case they are not available); incubate them for two days at 28°C.
3. On **Monday**, one single colony of the *Agrobacterium* is transferred to 5 ml YEB-media (with antibiotics). Shake overnight at 28°C (the culture should be logarithmical)

Infection and co-cultivation of plant leaves with Agrobacterium

4. On **Tuesday** centrifuge bacteria culture (6500 rpm 3 min) and resuspend the pellet in 30 ml YEB-Medium without antibiotics (with $MgSO_4$ for aggressive *Agrobacterium* strains)
5. Put 10 ml of **MS-Medium with 2% Sucrose (liquid)** into each **8 petri dishes** (9 ∅)
6. Cut about **100 leaves** per construct (5 jars with 5 plants in each jar): remove the base of the leaves and additionally, cut through the middle vein 1-2 tiny cuts on lower surface
7. Add the leaves to the media, the upper surface of the leaves should have contact with the medium: about 13 leaves/Petri.
8. The leaves are sensitive to any kind of injury, burned or too harsh handled tissue will die. Therefore, never squeeze the tissue and let the forceps, after sterilisation, cool down to RT. Try to use also sharp razor blades. Use green and healthy leaves from the top of plant.
9. Give **50 µl of *Agrobacterium*** suspension into each petri dish and shake lightly for 3-5 min. on a shaker
10. Incubate them **2-3 days in the dark at RT**

Selection of transformed tissue

11. On **Friday** transfer leaves to **Callus Induction Medium (CIM)**, again leaf-upper-surface down, in contact with the medium.
12. **After 1 week** transfer the leaves to **Selection Medium (SM).**
13. Leaves contaminated with fungi must be discarded; the adjacent uninfected leaves from those plates can be transferred to selection medium containing fungicide (e.g. amphotericin) and sealed with Parafilm. They might survive. At the beginning, leaves with strong *Agrobacterium* infection should be discarded or washed in 10 mM $MgSO_4$ containing *Cefotaxime* and incubated separately on plates.
14. When callus is already formed, it is worthwhile to cut off the healthy tissue and transfer it to separate plates
15. The leaves must be transferred **to fresh medium every 10 days**.

Regeneration of transgenic plants:

16. After a couple of weeks, the first shoots are forming. It is not advisable to let them touch the top of the petri dishes or later the jars. Thus, they must be transferred to 0.25 litre jars (later to 0.5 litre).
17. After approx. 2 months the shoots are cut and transferred to **rooting medium (RM)** (max. 5 plants / 0.5 litre jar). The callus should be kept, because more shoots will form by time.
18. Shoot cuttings from the same callus (e.g. callus number 12) get the same number 12.1, 12.2, 12.3; it is advisable to number the callus from which these shoots were cut off as well in case more shoots are formed.

19. **Media for growth of *Agrobacterium***

YEB-Medium:

5 g/l Beef-Extract
1 g/l Yeast-Extract
5 g/l Peptone
5 g/l Sucrose
0.49 g/l $MgSO_4 \bullet 7H_2O$
For plates add 15 g/l Bacto Agar Difco
Autoclave. Add antibiotics to 60°C warm medium, stir well and pour immediately in plates.
If the plates contain antibiotics, they should not be kept longer than a month.

- Antibiotics: 100 mg **amp**icillin/l (Stock 100mg/ml ddH_2O) filter sterile
- 100 mg **rif**amycin/l (Stock 50mg/ml DMSO)
- 25 mg **Kan**amycin/l (Stock 50mg/ml ddH_2O) filtre sterile

20. **Media for plants**

Liquid MS- Medium

Dissolve in 900 ml ddH_2O:
4.31 g/l MS-salt (Duchefa)
20 g/l sucrose
5 ml/l Vitamin mix
Adjust pH to 5.7-5.8, about 8-10 droplets of a 1M KOH stock
Fill up to 1 l
Autoclave

Callus induction medium (CIM) MG-Medium, 5 mg/l NAA, 0.1 mg/l BAP,
250 mg/l Ticarcillin disodium/potassium-clavulanate (Duchefa),
(50 mg/l Kanamycin, 1 mg/l Hygromycin or 2 mg/l PPT)

Selection medium (SM) MG-Medium, 1.4 mg/l Zeatin riboside, 20 µg/l GA_3,
20 mg/l NAA, 250 mg/l Ticarcillin disodium/potassium-clavulanate (Duchefa),
(50 mg/l Kanamycin, 3 mg/l Hygromycin or 2 mg/l PPT)

Rooting medium (RM)

MG-Medium, 250 mg/l Ticarcillin disodium/potassium-clavulanate (Duchefa),
(50 mg/l Kanamycin, 3 mg/l Hygromycin or 2 mg/l PPT)

MG- Medium

Dissolve in 900 ml ddH_2O:
4.31 g/l MS-salt (Duchefa)
16 g/l glucose
5 ml/l Vitamin mix
Adjust pH to 5.7-5.8, about 8-10 droplets of a 1M KOH stock
Add agar direct into the bottle
Fill up to 1 l
Autoclave, cool to 60°C (hand warm), add hormones and/or antibiotics, stir well and pour immediately in petri dishes or jars.

In general:

- Store medium always at 4° C. Let it warm to RT before usage.
- Do not use medium, which is older than a month due to decrease in the activity of hormones and antibiotics
- If fungal infection occurs, add amphotericin (5 mg/l) to the medium

Stocks

α -Naphtalenacetic acid (NAA) (MW 186.2; # N-0640 Sigma stored at RT)
stock concentration: 1 mg/ml
add 1/10 vol. 0,1M NaOH, then 9 /10 vol. ddH_2O
filter sterile (0.2 µm)
store 1 ml aliquots at 4°C or for longer at -20°C

6-Benzylaminopurin (BAP) (MW 225,3; # B-3408 Sigma, powder, stored at RT)
stock concentration: 1 mg/ml
add 1/10 vol. 0,1M HCl or 0,1M NaOH, then 9/10 vol. sterile ddH_2O)
filter sterile (0.2 µm)
store 1 ml aliquots at 4°C or for longer at -20°C

Amphotericin B Fungicide (A-2411 Sigma; durable 3 days at 37°C, powder, stored at 4°C)
Stock concentration: 5 mg/ml
Dissolve in DMSO
Store 1ml aliquots at -20°C

Basta (PPT) Herbicide (AgrEvo, 183 g/l Glufosinate, stored at RT: poison cabinet)
stock concentration: 10 g/l
dilute in ddH_2O
filter sterilise (0.2 µm)
store in 100 ml bottle in RT in room 218

Gibberellic acid (GA_3) (MW 346,4; # G-7645 Sigma, powder, stored at RT)
stock concentration: 20 µg/ml
add ddH_2O
filter sterilise (0.2 µm)
store 1 ml aliquots at -20°C

Hygromycin B (H) (Duchefa Bioch. 2 ml solution: 502 mg/ml, H0192 - 1 g = 107 €, durable 2 years at 4°C; from Sigma the powder is stable at least 5 years if stored at 2-8°C)
stock concentration: 15 mg/ml
dilute in ddH_2O
filter sterile (0.2 µm)
store 1 ml aliquots at 4°C - freezing should be avoided
http://www.sigmaaldrich.com/catalog/product/sigma/H9773?lang=de&region=DE

Kanamycin (KAN) (K-1377 Sigma, oder Duchefa K0126, salt stored at RT)
stock concentration: 50 mg/ml
Dilute in ddH_2O (60 mg Kan-salt contains approx. 50 mg Kan !!!)
filter sterile (0.2 µm)
store 1ml aliquots at -20°C

Ticarcillin disodium/potassium-clavulanate (TiCla) Anti-bacterial (T0190 Duchefa Bioch., stored at 4°C, 10 g = € 150)
stock concentration: 250 mg/ml
add sterile ddH_2O
filter sterilise (0.2 µm) and store 1 ml aliquots at -20°C

Vitamin mix (stock stored in 25 ml aliquots at -20°C)

Nicotine acid	0.1 g/l	final conc. in 1 l medium	0.5 mg/l
Pyridoxine-HCl	0.1 g/l	final conc. in 1 l medium	0.5 mg/l
Thiamine-HCl	0.02 g/l	final conc. in 1 l medium	0.1 mg/l
Glycine	0.4 g/l	final conc. in 1 l medium	2.0 mg/l
myo-Inositol	20 g/l	final conc. in 1 l medium	100 mg/l

Zeatin riboside (ZR) (MW 351,4; # CAS 6025-53-2 Duchefa, powder, stored at 0°C)
stock concentration: 1.4 mg/ml
add 1/10 vol. 0.1M HCl, then 9/10 vol ddH_2O
filter sterilise (0.2 µm) and store 1 ml aliquots at -20°C