Agrobacterium transformation

- Preculture
- 24h culture in 5 ml LB agro medium
- Centrifugation (5 min)
- pellet + resuspension medium:
  - 10 mM MES/KOH pH5.6
  - 10 mM MgCl2
  - 150 µM acetosyringone
- DO600 = 0.2-0.3

Day 1: Agroinfiltration

Day 3 to day 5:
Cell wall digestion (2-6 hours, 30°C) in 1X enzyme solution

Observation

Nicotiana benthamiana

Enzyme solution (for 100ml of 5X stock in proto medium)
- 0.2g Macerozyme R-10
- 0.5g Driselase
- 1.0g Cellulase “Onuzuka”

Centrifugation for 10 minutes at 4000 rpm
Froze at -20°C

Protoplast medium (for 1 litre)
- 4.3g/l Murashige and Skoog Plant Salt Mix (ICN Biomedicale)
- 0.5g/l MES KOH to 5.6
- 20g sucrose
- 80g mannitol