

# SOUTHERN

## DNA extraction

- freeze and homogenize 50 mg – 1g of plant material (mortar). Transfer ground plant material into 2 ml tube (plant material should fill tube slightly over 0.5 ml marking)
- add 1ml extraktion buffer, mix (do not vortex! dissolve material by “shaking in circles”, inverting....) inkubate 10 min at 65 °C.
- add 300 µl 5M potassiumacetate (**no** pH adjustment)
- inkubate 10 min on ice
- spin 10 min at 15000 rpm, 4°C
- transfer supernatant to new tube, add 800 µl phenol/chloroform/isoamylalcohol, mix gently by inverting tube 2-3x, incubate 1 h on shaker (gently) - (min. 15 min!!)
- spin 5 min at 12000 rpm
- **aequous phase** in new tube, add 800 µl isopropanol, mix gently by inverting tube 2-3x, inkubate 10 min at RT
- spin 10 min bei 15000 rpm
- wash pellet with 1 ml ETOH 70%
- spin 10 min bei 15000 rpm
- remove ETOH, dry pellet
- resuspend pellet in 16-20 µl sterile dH2O

## Restriction (adjust protocol if necessary)

- 15 µl DNA
- 2 µl Puffer (10 x)
- 1µl Enzym
- 1 µl RNase

37 °C o.N.

## Gelelektrophoresis

- Restriktionsansatz komplett auftragen
- 0.8 - 1%iges TAE Gel, ü.N. bei 30 V
- Gel Foto mit Linieal!

## Extraction buffer

| final conc. |                 | stock conc. | 100ml buffer vol. | 500 ml buffer vol. |
|-------------|-----------------|-------------|-------------------|--------------------|
| 100 mM      | Tris-HCl pH 8.0 | 1 M         | 10 ml             | 50 ml              |
| 50 mM       | EDTA            | 0.5 M       | 10 ml             | 50 ml              |
| 500 mM      | NaCl            | 5 M         | 10 ml             | 50 ml              |
| 1.5 %       | SDS             | 10 %        | 15 ml             | 75 ml              |
|             | H2O             |             | 55 ml             | 275 ml             |

## SOUTHERN Blot

- Place gel in tray and add 200-300 ml denaturing buffer, gently shake 45 min at room temp.
- wash gel 2x with dH<sub>2</sub>O
- add 200 – 300 ml neutralizing buffer, gently shake 45 min at room temp.
- wash gel 2x with dH<sub>2</sub>O
- build blot (use Nylon Membrane positively charged (Roche # 11417240001: € 415.15)
- transfer with 20 x SSC over night
- briefly wash membrane in 3x SSC, dry on filter paper
- UV-Crosslink
- prehybridize membrane: ~ 50 ml at 42°C min. 2-4h or over night
- label probe (Amersham Megaprime™ DNA Labeling System (GE Healthcare,#RPN1604: €447) and add to prehyb “setup”, incubate over night at 42°C
- wash membrane: 3x at 65 °C with 3xSSC + 0.1% SDS
- measure radioactivity (– when needed: add another wash step)
- place membrane between foil and place in phospho imager box + screen
- phosphoimager quantification of signals after 4h – 3d

### **Buffer:**

#### **20 x SSC:**

3M NaCl + 0.3 M Na-citrate

#### **denaturing buffer (Southern):**

1.5 M NaCl (87.6 g/L) + 0.5 M NaOH (20.05 g/L)

#### **neutralizing buffer (Southern):**

1.5 M NaCl (87.6 g/L) + 0.5 M Tris (60.7 g/L)

#### **(pre-)hybridizing buffer:**

for 500 ml:     1g     PVP (polyvinylpyrrolidone)  
                  1g     ficoll  
                  1g     BSA  
                  240ml  buffer “N”

when completely dissolved add

240ml formamide

6 ml    herring sperm DNA (heat denature prior to adding!)

store buffer at 4°C

#### **buffer “N”:**

1.8 M NaCl

100 mM NaH<sub>2</sub>PO<sub>4</sub>

10 mM EDTA

1.2 % SDS

adjust pH to pH 7.0