Protocol 3: Mixi/Midi - Preparation

1. Material

- 50mM Tris + 10mM EDTA
- 0.2M NaOH + 1% SDS
- 3M potassium acetate
- phenol:chloroform:isoamyl alcohol 25:24:1
- isopropanol
- 80% ethanol
- ddH_2O

2. Instruments

- centrifuge
- vortex

3. Experimental procedure

- centrifugation of the overnight culture (10', 4500rpm = roughly 3700g)
- discard supernatant
- resuspend pellet in 200µl 50mM Tris + 10mM EDTA, transfer both suspensions into two 2ml tubes
- add 400µl 0.2M NaOH + 1% SDS, invert 5-10 times
- add 300µl 3M potassium acetate, invert 5-10 times
- add 600µl of the lower phase of phenol:chloroform:isoamyl alcohol 25:24:1, vortex thoroughly
- centrifugation (10', 13000rpm),
- transfer upper phase into two new 1.5ml tubes
- add 0.5ml isopropanol
- incubation (5-10', RT), centrifugation, (15', 13000rpm)
- discard supernatant, add 0.5ml 80% ethanol, centrifugation (10', 13000rpm)
- discard as much supernatant as possible
- incubate at 37°C with open lid for 20'
- resuspend in 50µl ddH_2O
- incubate at 37°C (10', 550rpm)
- store at -20°C