Protocol 1: Alkaline Lysis

1. Material

- GTE Buffer (50 mM Glucose, 10 mM EDTA, 25 mM Tris/HCl pH8,0)
- NaOH/SDS (1% SDS, 0,2 M NaOH)
- Kac/Hac (3M KAc/2M Hac
- EtOH (70%)
- EtOH (100%)
- TE-Buffer (1x)
- ddH_2O

2. Instruments

- Haereus pico17 centrifuge (Thermo Fischer Scientific)
- Vortexer (diverse producers)

3. Experimental procedure

- Vortexing ONC (prepared the day before)
- Transferring 2 ml out of ONC in 2ml reaction tubes
- Centrifugation at 7000 rpm for 5 minutes
- Removed supernatant
- Add 2ml out of ONC and repeated centrifugation and decanting step
- Add residual volume and repeated centrifugation and decanting step
- Pellets resuspended in 90µl GTE-Buffer
- Vortexing for about 10 seconds
- Addition of 180µl NaOH/SDS
- Inverting tubes 4 times
- Addition of 135µl KAc/HAc
- Inverting 10 times
- Incubation for 5 minutes on ice
- Centrifugation at 13,300 rpm for 15 minutes
- Each supernatant transferred in one 1,5 reaction tube
- Addition of 1 ml EtOH
- Vortexing for 10 seconds
- Incubation at 13,300 rpm for 10 minutes
- Removal of ethanol
- Addition of 1ml EtOH
- Centrifugation at 13,300 rpm for 10 seconds
- Repetition of the last two steps

- Evaporation of remaining EtOH at room temperature
- Resuspending pellet in 40µl TE-Puffer