BOIL and FREEZE DNA extraction protocol

1. Centrifuge the liquid culture, discard the supernatant
2. Froze the sample for 2 hours at -80°

3. Add 100 μl of Buffer A and place the sample in a boiling water bath for 45 segs.

4. Add 30 μl of lysozyme mix gentle by inversion. Incubate for 30 min at 37℃.

5. Add 10 μl 20% SDS and 60 μl proteinase K . Mix be gentle inversion. Incubate for 30 min. at 50 ℃.

6. Add 100 μl 20% SDS (approx 5% conc.) and 250 μl phenol:$CHCl_3$:IAA (24:24:1) (245 μl phenol and 255 μl SEVAGE [$CHCl_3$:IAA])

7. Spin at 12,000 g at 4 ℃., pipette supernatant into a fresh 1.5 ml tube.

8. **IN HOOD FROM HERE:** Extract samples with 1 volume of phenol mix, spin at full speed for 2 min at 4℃. Remove aqueous layer and add 1 vol of $CHCl_3$:IAA and mix (the DNA is with the aqueous layer. Spin at max speed for 2 min at 4℃. Remove the aqueous layer.

9. Precipitate nucleic acids by adding 24 μl of 3M sodium acetate and 1000 μl EtOH (search).

10. Centrifuge at max speed for 5 min. discard supernatant, Rinse pellet with 500 μl of 70% ethanol. Air dry on kimwipe resuspend pellet in 15 μl o elution Buffer of TE buffer.