# Genomic extraction E. coli

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## Needed:

- o/n culture
- TE Buffer (10T/1E)
- lysozyme [50 mg/ml]
- SDS 10
- 5M NaCl
- 100
- Chloroform:Phenol
- isopropanol
- 70% EtOH
- MilliQ water
- Some serious protective gear!

## Step-by-step

1. Pellet the o/n culture.
2. Wash the pellet with TE buffer twice to remove media.
3. Resuspend in TE buffer and ad 2 µl lysosyme. Mix thoroughly.
4. Incubate for 60 min at 37 °C.
5. After incubation ad SDS so final conc. is 1%. Mix thoroughly and let tube sit for a few mins.
6. OPTIONAL: One can ad 6 µl [10 mg/ml] Proteinase K when adding the SDS. If Proteinase is added incubate the tube in 37 degrees until liquid is clear.
7. Ad 100 µl 5M NaCl and mix until mixture becomes foggy.
8. Incubate mixture at 65 degrees C for 3 min.
9. Ad equal volume of 100% chloroform and mix thouroughly.
10. Centrifuge at 10 000 x g for 5 min. And transfrer supernatant to new tube.
11. Ad equal volume chloroform:phenol to the supernatant and mix until emulsion occurs.
12. Centrifuge at 14000 x g for 5 min and transfer supernatant to a new tube.
13. Ad equal volume of chloroform to the supernatant and centrifuge at 10 000 x g for 5 min.
14. Transfer the supernatant to a new tube and ad 0.7 volume of isopropanol. Mix gently until percipitate is seen. Let stand at room temp. for a few mins.
15. Centrifuge at 13 000 x g for 30 min. Discard the isopropanol.
16. Wash the pellet with 70% EtOH and centrifuge at 10 000 x g fro 10 min. Discard the EtOH and dry the pellet.
17. Resuspend the pellet in 50 µl MilliQ water.