

DNA Extraction Protocol – Gentra kit

Cell Lysis

1. Put 300 µl of Enzymatic Lysis Buffer into 1.5 ml pestle tube.
2. Place insect tissue in tube after dissection.
3. Crush this mixture with pestle.
4. Incubate at 37° C for 30 minutes.
5. Note: get 80° C incubation ready
6. Centrifuge at 14,000 rpm for 5 minutes and remove the supernatant and put in another 1.5mL tube.
7. Add 300µl of Cell Lysis Solution, pipet up and down (lyse cells).
8. Incubate at 80° C for 5 minutes (for complete cell lysis).
9. Add 5 µl of Proteinase K.
10. Vortex and incubate for 30 minutes at 55° C.

RNase Treatment

1. Add 1 µl of 100 mg/ml RNase A, mix by inverting 25 times.
2. Incubate at 37°C for 30 minutes.

Note: Make 1.5 ml tube that has 300 µl isopropyl alcohol for each extraction during incubation.

Protein Precipitation

1. Cool sample to room temp in ice.
2. Add 100 µl of protein precipitation solution.
3. Vortex 20 seconds, ice 5 minutes, centrifuge at 4°C 5 minutes (14,000 rpm), ice 5 minutes. After the centrifuge step handle tubes carefully. You DO NOT want to disturb the pellet.
4. Transfer the supernatant to a new tube with a pipet. (Keep spinning and removing to avoid floating proteins)
5. Spin this again at 14,000 rpm.

DNA Precipitation and Elution

1. Pipette the supernatant into the 1.5 ml tube containing 300µl isopropyl.
2. Invert tubes to mix. Place in freezer for at least 15 minutes. Overnight is best.
3. Centrifuge at 4°C, 5 minutes (14,000 rpm).
4. Discard supernatant.
5. Add 400 µl of 70% ethanol. Invert tubes gently to mix.
6. Let sit on ice 5 minutes. Centrifuge 5 minutes (14,000 rpm).
7. Discard supernatant. Tap tube on a clean Kim-wipe to remove remaining liquid.
8. Invert tube on a clean paper towel for one hour to dry. Pellet may not be visible at end of procedure.
9. Add 50 µl of low TE.
10. Flick tube and let sit ~1 hour at room temperature.

Storage

Short-term = 4°C

Long-term = -20°C or -80°C

Materials required

Gentra puregene tissue kit
Low TE
Isopropyl alcohol, 100% (isopropynol)
Ethyl alcohol, 70% (ethanol)

10, 20, 200 and 1000 μ L pipette tips
1.5 ml microcentrifuge tubes
1.5 ml pestle tubes
mini pestles

Equipment required

10 μ L micropipette
20 μ L micropipette
200 μ L micropipette
benchtop centrifuge capable of 20,000 x g