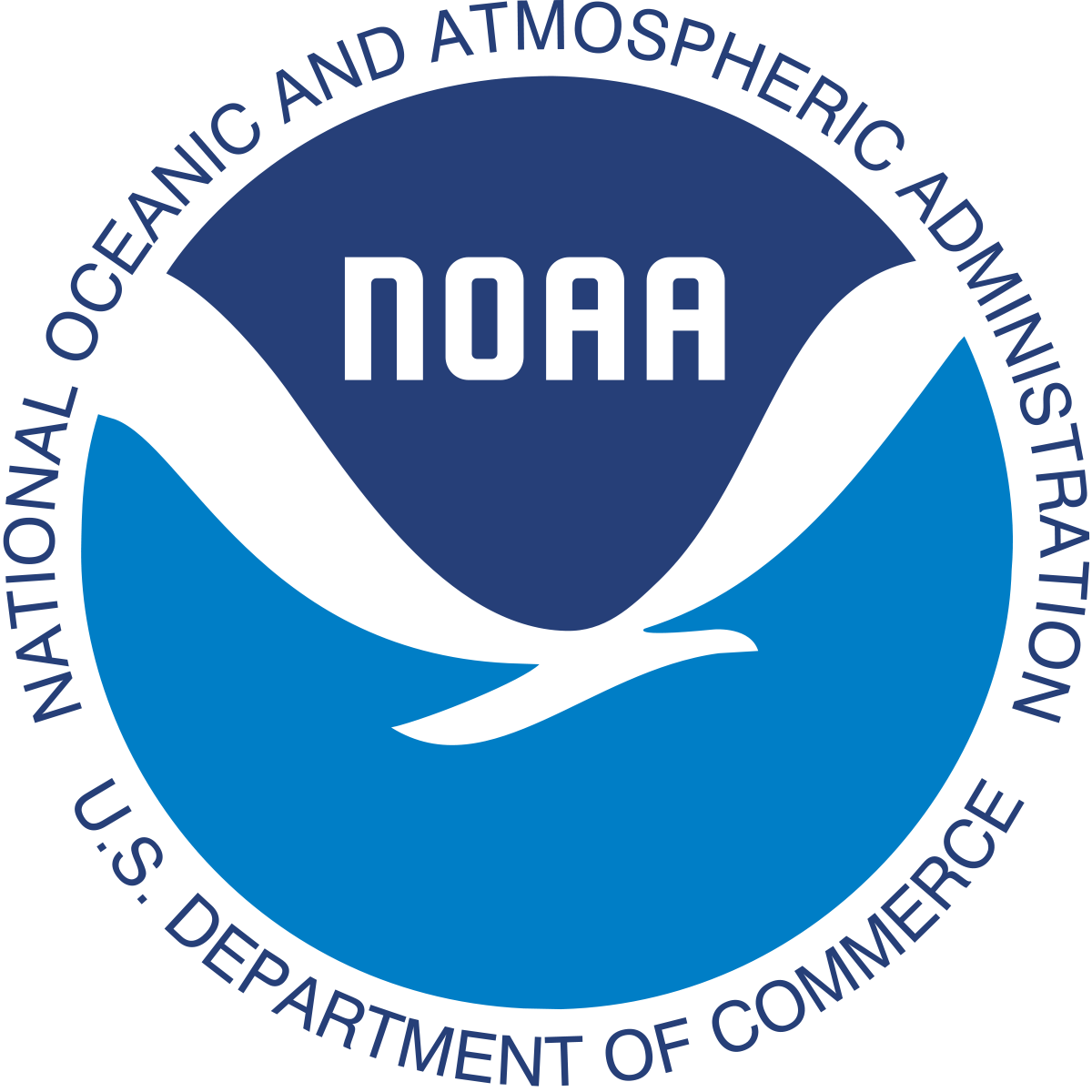
# Zymo Quick DNA Miniprep Plus Kit NOAA Protocol



## Sample Sources

### Biological Fluids

* Summarize later date

### Mammalian/Insect Cell Cultures

* Summarize later date

### Bacterial Cell Cultures

* Summarize later date

### Solid Tissues

* Summarize Later
* Overnight Proteinase K digestion @ 55oC is possible and will not affect DNA integrity
* For samples stored in DNA/RNA shield

## Pre-protocol Reagent Preparation

Add **1,040µl Proteinase K Storage Buffer** to each **Proteinase K (20mg)** tube prior to use.

* + Final concentration of Proteinase K is ~20mg/ml
  + Store at -20oC after mixing
  + Can aliquot out, each reaction uses 10µl Proteinase K

## For Samples Stored in DNA/RNA Shield

1. For each 300µl sample prepared in DNA/RNA Shield, add **150µl Solid Tissue Buffer** (Blue) and **10µl Proteinase K**
2. Vortex 15 seconds and incubate @ 55oC for 1-3 hours.
   * N.B., overnight is possible and will increase effectiveness of digestion and DNA recovery.
3. Centrifuge at >12,000g for 1 minute
4. Transfer aqueous supernatant to clean microcentrifuge tube
5. **Add 1 volume Genomic Binding Buffer** to the sample
   * If 300µl supernatant, add 300µl Genomic Binding Buffer
6. Vortex 15 seconds
7. Transfer mixture to **Zymo-spin IIC-XLR column** in a collection tube.
8. Centrifuge at >12,000g for 1 minute.
   * If lysate still visible on matrix, centrifuge for another minute OR until completely cleared.
9. Discard collection tube with the flow through.
10. Add **400µl DNA-Pre-Wash Buffer** to the spin column in a new collection tube
11. Centrifuge at >12,000g for 1 minute
12. Empty collection tube
13. Add **700µl g-DNA Wash Buffer** and centrifuge at >12,000g for 1 minute
14. Empty collection tube of flow through
15. Add **200µl g-DNA Wash Buffer** and centrifuge at >12,000g for 1 minute
16. Discard collection tube with flow through
17. Transfer Spin column to a clean microcentrifuge tube
18. Add **>50µl DNA Elution Buffer or water** directly to the matrix.
    * Can elute in as little as 35µl for highly concentrated DNA, see Figure on last page for more information on working out elution volume.
    * IF using **water**, make sure pH > 6.0
    * **DNA Elution Buffer** = 10mM Tris-HCL, pH 8.5, 0.1mM EDTA
19. Incubate at room temperature for 5 minutes
20. Centrifuge at top speed for 1 minute to elute DNA
    * Total yield can be improved by eluting in 60-70oC DNA Elution Buffer
    * Total yield can be improved by reloading the first run elution to the spin column, incubating for 3 minutes at room temperature, and centrifuging at max speed for 1 minute.
21. Store eluted DNA @ -20/-80oC

## Solid Tissue Sample Processing

1. Add coral sample to microcentrifuge tube
2. Add **95µl water**, **95µl** **Solid Tissue Buffer** (Blue), and **10µl Proteinase K**
3. Vortex 15 seconds
4. Incubate tube **@ 55oC for 1-3 hours**
   * N.B., overnight is possible and will increase effectiveness of digestion and DNA recovery.
5. After incubation, mix thoroughly
6. Centrifuge at >12,000g for 1 minute and transfer aqueous supernatant to a clean microcentrifuge tube
   * Avoid transferring lipid layer and pelleted cellular debris.
7. Add **2 volumes Genomic Binding Buffer** to the supernatant.
   * If 200µl supernatant, add 400µl Genomic Binding Buffer
8. Vortex for 15 seconds
9. Transfer mixture to **Zymo-spin IIC-XLR column** in a collection tube.
10. Centrifuge at >12,000g for 1 minute.
    * If lysate still visible on matrix, centrifuge for another minute OR until completely cleared.
11. Discard collection tube with the flow through.
12. Add **400µl DNA-Pre-Wash Buffer** to the spin column in a new collection tube
13. Centrifuge at >12,000g for 1 minute
14. Empty collection tube
15. Add **700µl g-DNA Wash Buffer** and centrifuge at >12,000g for 1 minute
16. Empty collection tube of flow through
17. Add **200µl g-DNA Wash Buffer** and centrifuge at >12,000g for 1 minute
18. Discard collection tube with flow through
19. Transfer Spin column to a clean microcentrifuge tube
20. Add **>50µl DNA Elution Buffer or water** directly to the matrix.
    * Can elute in as little as 35µl for highly concentrated DNA, see Figure on last page for more information on working out elution volume.
    * IF using **water**, make sure pH > 6.0
    * **DNA Elution Buffer** = 10mM Tris-HCL, pH 8.5, 0.1mM EDTA
21. Incubate at room temperature for 5 minutes
22. Centrifuge at top speed for 1 minute to elute DNA
    * Total yield can be improved by eluting in 60-70oC DNA Elution Buffer
    * Total yield can be improved by reloading the first run elution to the spin column, incubating for 3 minutes at room temperature, and centrifuging at max speed for 1 minute.
23. Store eluted DNA @ -20/-80oC

