**Zymo DNA MiniPrep Plus Extraction Protocol**

Written by B. Young

Updated: 5.30.2023 M. Studivan

All centrifugation steps are performed at **room temperature** and **15,000 x *g*** unless specified.

Prior to first use, add 1040 µl Proteinase K Storage Buffer to each Proteinase K (20 mg) tube. The final concentration of Proteinase K is ~20 mg/ml. Store at -20ºC after mixing.

Optional: Thaw all sample tubes ahead of extracts; bead-beat for 20-30 min on the vortexer or 2 min on the FastPrep at 6 m/sec. Homogenized samples can be stored at -20 ºC until extraction.

1. Once thawed, centrifuge for 1 min, then transfer 300 µL of homogenate to 2.0 mL tube.
2. Add 150 µl Solid Tissue Buffer (blue) and 10 µl Proteinase K.
3. Vortex for 10-15 sec and incubate at 55 ºC for 1 – 3 hr or until tissue solubilizes.
4. Centrifuge lysate for 1 min, then transfer 350 µL supernatant to 1.5 mL tube.
5. Add 1 volume Genomic Binding Buffer (350 µL) to the sample and vortex 10-15 sec.
6. Transfer to a Zymo-Spin IIC-XLR Column in a collection tube and centrifuge for 1 min. Transfer spin column to a **new** **collection tube**.
7. Add 400 µl DNA Pre-Washto the column and invert twice. Centrifuge for 1 min, pour out the flow-through, and dab the collection tube on a KimWipe.
8. Add 700 µL g-DNA Wash Buffer to the column and invert **only the column** twice. Centrifuge for 1 min, pour out the flow-through, and dab the collection tube on a KimWipe.
9. Add 200 µL g-DNA Wash Buffer to the filter directly (**do not invert**) and centrifuge for 1 min. Transfer spin column to a new 1.5 mL tube.
10. To elute DNA, add 50 µL of nuclease-free water heated to 60 ºC directly to the column, incubate for 5 min, and centrifuge at 19,000 x *g* for 1 min.

**MiniPrep Plus Tube Prep:**

2.0 mL tube for homogenate

1.5 mL tube for lysate

Zymo-Spin IIC-XLR Column w/ collection tube

New collection tube

1.5 mL catch tube

**Zymo OneStep PCR Inhibitor Removal Protocol**

Written by M. Studivan

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1. Add 600 µL of HRC Prep Solution to Zymo-Spin III-HRC Filter in a collection tube, centrifuge at 8,000 x *g* for 3 min, discard collection tube, and place filter in a new catch tube.
2. Transfer 50 µL of eluted DNA/RNA into prepared Zymo-Spin III-HRC Filter and centrifuge at 16,000 x *g* for 3 min.

**HRC Tube Prep:**

Zymo-Spin III-HRC Filter w/ collection tube

1.5 mL catch tube

**Zymo DNA Clean & Concentrator-5 Purification Protocol**

Written by B. Young

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All centrifugation steps are performed at **room temperature** and **13,500 x *g*** unless specified.

Prior to first use, add ethanol to buffer concentrates per instructions on bottles.

1. Add 2 volumes (100 µL) of DNA Binding Buffer to each sample and mix by vortexing.
2. Transfer to Zymo-Spin Columns and centrifuge for 30 sec. Pour out the flow-through and dab the collection tube on a KimWipe.
3. Add 200 µL of DNA Wash Buffer to the column and centrifuge for 30 sec. Pour out the flow-through, dab the collection tube on a KimWipe, and **repeat this step.** Transfer spin column to a new 1.5 mL tube.
4. Add 25 µL of nuclease-free water heated to 60 ºC directly to the filter and incubate for 1 min. Centrifuge at 14,000 x *g* for 1 min.
5. Nanodrop eluted DNA, blanking using the same DNase/RNase-Free Water used for elution.
6. Store at -20 ºC.

**DNA Clean & Concentrator-5 Tube Prep:**

Zymo Spin Column w/ collection tube

1.5 mL catch tube