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DNA Extraction (solid tissues)

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We use this protocol and it's working

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Abstract

How to complete DNA extraction from solid tissues (fresh, stored in ethanol, or frozen).

Guidelines

Prepare tissues during this prep with the scissors/forceps in the drawer below the main lab bench. Be sure to use a clean petri dish and cleaned scissors/forceps for **each sample**. *Be very careful not to cross-contaminate!*

Materials

This protocol requires the Zymo Research Miniprep Extraction Kit (big yellow and green box).

Before start

Use 75% ethanol and 10% bleach to clean the lab bench, tube racks, pipettes, centrifuges (mini and large), and vortex before taking out any reagents. Turn on the hot plate (low setting; 55°C). If you're returning after sample incubation is done, place the **DNA Elution Buffer** on the hot plate and set it to the high setting (70°C). The bottle heats up faster if it is turned sideways.

Scale Protocol

1 Optionally, follow along with this supplementary tutorial video as you perform the protocol:

https://www.youtube.com/embed/nGkkiR-IlAc

Double check that you have appropriately scaled this protocol for the number of samples you need to prep (use scale option after clicking "run").

Note

*IF OPENING A NEW KIT (or prepping a new Proteinase K): To the powdered **Proteinase K**, add **1,040uL of Proteinase K Storage Buffer**. Mix (vortex) very well until clear. Store in frost-free freezer when not in use.*

Digestion

2 Create a master-mix for this step if you're running more than a few samples. To create the **Extraction Master Mix**, combine 95 µL Water + 95 µL Solid Tissue Buffer (blue) + 10 µL Proteinase K (volumes are scaled; amount for single sample would be 95 uL Water, 95 uL Solid Tissue Buffer, 10 uL Proteinase K). You may need multiple microcentrifuge tubes (or a 15-mL tube from the cabinet containing the plastic Petri dishes) to make your mix.

3 For each sample that you are extracting, label a 1.6-mL microcentrifuge tube with the corresponding lab or BIC code and aliquot **200 uL** of **Extraction Master Mix**. Using the dissection tools (scissors, forceps, and scalpel if necessary), plastic Petri dishes filled with 95% ethanol, a beaker filled with 95% ethanol to clean tools in between samples, and the glass alcohol lamp (filled with 100% ethanol) to sterilize tools in between samples, isolate a tissue subsample (< 25 mg, ethanol-stored or frozen tissue) and add it to the corresponding sample's microcentrifuge tube containing **200 uL** of **Extraction Master Mix**.

Note

Make sure to briefly dry the tissue subsample of any residual ethanol (which may interfere with downstream applications like PCR) by placing it on a kimwipe prior to inputting it into the corresponding microcentrifuge tube containing **200 uL** of **Extraction Master Mix**.

Mix thoroughy (vortex) **10-15 seconds**. Check that there is not tissue stuck to the top or sides of the tube (spin down briefly if necessary, using the mini centrifuge).

4 Incubate at 55 °C for **1-3 hrs** (can also incubate longer or overnight if needed, but beware of DNA degradation). Optional timers available here: 01:00:00 02:00:00 03:00:00

Spin-Wash-Elute

5 After removing samples from the hot plate, change the temperature setting to high (65-70°C) and keep the **DNA Elution Buffer** on the hotplate until it is required in step 10.

Mix (vortex) samples and spin down briefly if needed to remove liquid from the cap/sides of the tube. Add **400 uL Genomic Binding Buffer** to each tube. Mix thoroughly (vortex) **10-15 seconds**.
Centrifuge 12000 x g, 00:01:00 . Meanwhile, prepare a Collection Tube and Zymo-spin IIC-XLR Column for each sample (be sure to label the lid of the Column!).

6 Transfer only the aqueous **supernatant** from each tube (no insoluble debris!) from each sample's microcentrifuge tube to the appropriate labeled Column. Centrifuge 12000 x g, 00:01:00 . Discard the Collection Tube (solid waste) and the flow through (liquid waste) and place the Columns into new Collection Tubes.

7 Add **400 uL DNA Pre-Wash Buffer** to each Column (new Collection Tube). Centrifuge 12000 x g, 00:01:00 . Empty the Collection Tube into the liquid waste (don't discard the Collection Tube this time).

8 Add **700 uL Wash Buffer** to each Column. Centrifuge 12000 x g, 00:01:00 . Empty the Collection Tube into the liquid waste container (don't discard Collection Tube).

9 Add **200 uL Wash Buffer** to each Column. Centrifuge 12000 x g, 00:01:00 . Discard the Collection Tube (solid waste) and the flow through (liquid waste) and place the Columns into new, labeled microcentrifuge tubes (caps will be open in order to accommodate the Columns).

10 Add **≥ 50 uL Elution Buffer** (the buffer should be at 65-70ºC by now). Standard protocol is to use **75 uL of Elution Buffer** for a single elution. Incubate for **5 minutes** (optional timer here 00:05:00) at room temperature.

11 Centrifuge 14850 x g, 00:01:00 to elute the DNA. The eluted DNA can be used immediately for molecular-based applications or stored at < 20ºC for future use.

Note

To increase DNA yield, you may complete two elutions/incubations by repeating steps 10 and 11 instead of just one. You can also try increasing the incubation time to up to 10 minutes. Return the elution buffer to the hot plate between elution steps.

12 ***Clean the centrifuge after completing steps 1-11!!!*** Please refer to the video tutorial if you need a refresher on how to do this.