DNA Extraction of Bone Samples		
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****NOTE:** This protocol allows for both nDNA QC'd reagents and Mito QC'd reagents. Any questions regarding reagent use can be directed to either the nuclear or mitochondrial DNA technical leaders.

1 Extraction Sample Set-up

1.1 Set up work area; obtain samples, reagents (0.5M EDTA and 20mg/mL Pro K and obtain two empty 50 mL conical tubes for the extraction negatives and label one as Extraction Negative 1 and the other as Extraction Negative 2.

1.2 Scan each sample into your custody.

1.3 Bone Incubation WITNESS:

1.3.1 Have a witness verify the LIMS sample label of the Extraction Negatives and samples (50 mL conical tubes).

1.4 Add 9mL 0.5M EDTA and 200 µL ProK to each tube.

1.5 Parafilm all samples and vortex thoroughly.

1.6 Place samples in shaker and incubate at 56°C at a speed of 124 RPM overnight. Fill out the Bone Incubation Run By review task.

1.7 Shaker should default at these settings.

1.7.1 To program the shaker use the "Select" button to highlight the fields on the right of the control panel. Once field is highlighted the up and down arrows can be used to set field to the appropriate number. Once samples are in the shaker, close the cover and select the "Start" button. Samples should begin shaking at set RPM's. Before opening the cover to remove samples, press the "Stop" button and allow samples to come to a stop. If shaker starts to beep after opening or closing cover hit the "Select" button once. (This beep is signaling that temperature has dropped from the setting that was selected.)

2 Clean-up

2.1 Remove tubes from shaker and set temperature to 60°C, speed at 124 RPM.

2.2 Add 1.0mL of 1.0M KOH to each tube. Dispose of all KOH tips in the amber hazardous waste bottle labeled "potassium hydroxide".

***NOTE: Eye protection must be worn when handling 1.0M KOH. Avoid contact of reagent with metal part of pipette when aliquoting from reagent container.**

2.3 Vortex thoroughly and place on shaker once it has reached 60°C for 5min.

2.4 Vortex all samples and place in large centrifuge at 2500 RPM for 3-5min.

2.5 Label 10K Amicon tubes (tops and sides) the same way the extraction sample set is labeled.

2.6 Bone Clean-up WITNESS: Have a witness verify two sets of tubes:

2.6.1 Original incubation tube – LIMS sample label

2.6.2 Amicons - side tube label (Case # Item # -)

2.7 Fill out the Bone Cleanup By review task.

2.8 Transfer the supernatant portion of the samples to Amicons. Throw away incubation tubes in the hazardous waste trash.

2.9 Spin Amicons in large centrifuge at 4000-4500 RPM for an initial 45-60min. The Eppendorf centrifuge will only reach 4000 RPM.

2.10 Continue spinning until samples are at or below the 500µL mark on the Amicon tube.

2.11 Once under 500µL, open the cap of the Amicon tube, pull out the filter portion and drain out the liquid in the bottom of the Amicon into a sink with running water.

2.12 Replace the filter in the tube. Add 5mL sterile or UltraPure water to each Amicon.

2.13 Spin again at 4000-4500 RPM for 10-15 until sample is at or below the 500µL mark on the Amicon tube.

2.14 Repeat steps 10-12 one more time for a total of 2 sterile or UltraPure water washes.

2.15 Label stratalinked 2 mL screw cap tubes with LIMS sample labels.

2.16 Tube Setup WITNESS: Have a witness verify two sets of tubes:

2.16.1 Amicons – side tube label (Case # Item # -)

2.16.2 2 mL screw cap tube – LIMS sample label

2.17 Using a 200µL pipette and sterile or UltraPure water, bring the volume of the sample in the Amicon tube up to 500µL.

2.18 Using the pipette tip, move it across the bottom of the Amicon filter to re-suspend sample with sterile or UltraPure water. Tilt the Amicon so sample collects to one side and draw up the sample, placing it into the labeled 2 mL screw cap tube. Throw away Amicon tubes when finished in the biohazard trash.

2.19 **NOTE: Samples should be processed on the EZ1 within 48 hours of extraction clean-up. If EZ1 processing cannot be done immediately after extraction, keep samples in a freezer until procedure can be performed.

3 DNA Purification of Bone Samples with EZ1 Large Volume Protocol

3.1 Place a bottle of MTL buffer in the incubator to warm it to approximately 65°C for 10 minutes.

3.2 Incubate Extraction negatives and all samples at 56°C for approximately 10 minutes. Record the thermomixer temperature and the instrument in the 'Additional Information' section. Add the instrument to the 'Instrument' box at the top of the worksheet.

3.3 During incubation:

3.3.1 Prepare Samples tubes: obtain QIAGEN 1.5 mL screw cap elution tubes and label them with tube top label and output labels.

3.3.2 Load the EZ1 instrument with the appropriate reagent strips and tips.

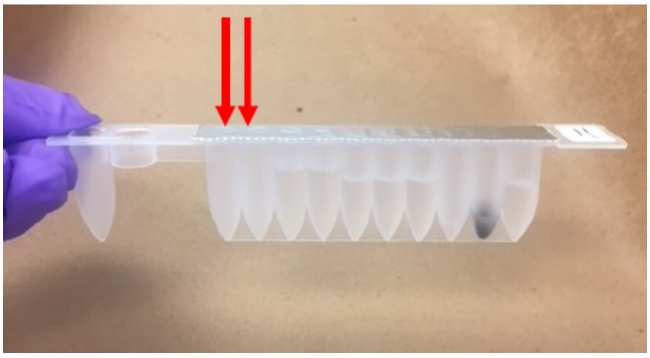
3.3.2.1 Add the EZ1 instrument to the Instrument box at the top of the worksheet. Fill out the EZ1 Setup Response in the Extraction Run By review.

3.3.2.2 Remove both the tube rack and cartridge rack from the EZ1.

3.3.2.3 Obtain reagent cartridges and scan the lot number in LIMS.

****NOTE: More than one reagent lot may be used if there are not enough individual cartridges available for your full batch. Please indicate in the Extraction Run By review task comments box the additional lot number and for which samples it was used.**

3.3.2.4 Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottoms of their wells. Check that the magnetic particles are re-suspended. Also, visually inspect each reagent well. All wells except the last two must contain liquid.



3.3.2.5 Slide the EZ1 reagent cartridges into the cartridge rack, one cartridge for each sample being run. The cartridge label side should be at the blunt end of the rack, closest to the tube rack when loaded into the instrument. Place rack in EZ1.

Reagent cartridge rack with cartridges loaded

3.3.2.6 Assemble EZ1 tips and tip holders. Place them in row 2 of the EZ1 tube rack.

3.4 Once the incubation is completed, briefly centrifuge the sample tubes to remove condensation from the caps.

3.5 Remove the warmed MTL from the incubator and add 400µL of warmed MTL buffer and 1µL carrier RNA to each 2 mL sample tube, pipette mixing as needed, to ensure mixing of the MTL with the lysate.

3.6 To avoid possible precipitation, proceed directly with EZ1 DNA extraction protocol while the sample lysates are still warm.

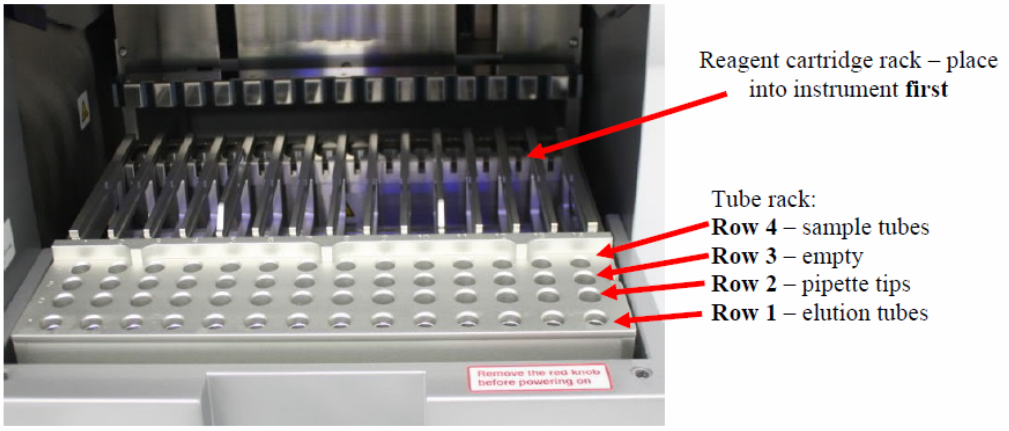
3.7 EZ1 Instrument Setup WITNESS: Have a witness verify the sample tubes, elution tubes, and loading of the Extraction negative and samples on to the EZ1.

3.7.1 Remove and discard the cap and load the sample tubes into row 4 of the EZ1 tube rack, reading the LIMS label.

3.7.2 Next, load the labeled elution tubes to row 1 of the EZ1 tube rack, reading the LIMS label and removing the screw cap as you load each sample.

3.7.3 Place tube rack into the EZ1. Make sure that the tube rack is loaded onto the instrument after the cartridge rack.

3.7.4 Witness should verify that all samples, reagents, and racks are loaded appropriately on the instrument.



EZ1 Worktable

3.8 Run the EZ1 protocol for purification of the samples:

3.8.1 If needed, press “ESC” to get to the main menu.

3.8.1.1 From the main menu press “Start” to begin a run.

3.8.1.2 When asked if you would like to create a run report, press “ESC” to select no.

3.8.1.3 Press “3” to select the large volume protocol.

3.8.1.4 Press “2” to select elution in TE.

3.8.1.5 Press “1” to select the 40µl elution volume.

3.8.1.6 The screen will display instructions to ensure the EZ1 has been loaded properly.

3.8.1.7 After checking each step, press "ENT" until the final step, and then press "Start."

3.8.1.8 The protocol run time is ~18 minutes.

3.9 After the protocol is completed, press "ENT" to continue.

3.10 Open the instrument door and remove the tube rack. Remove the 1.5 mL elution tubes, capping each sample with the labeled tube top. Discard all the used cartridges, lysate tubes, tip-holders and tips.

3.11 Fill out the Pass/Fail Response dropdown and complete the Extracted Run By review task.

3.12 Create the QUANTTRIO submission for the samples.

3.13 Verify the samples listed on the Trio Submission Worksheet click [Save] and close the Submission worksheet.

3.14 Store the extracts at 4°C and record transfer in LIMS

4 Clean the EZ1 Instrument

4.1 DO NOT USE BLEACH ON THE INSTRUMENT, ONLY 70% ETOH WITH LINT FREE WIPES. NEVER SPRAY ETHANOL DIRECTLY ON THE INSTURMENT.

4.2 Remove reagent cartridges and any waste, discard appropriately. Wipe down the inside of the instrument using a lint free wipe and 70% EtOH. Close the EZS1 door.

4.3 Follow the prompts on the screen to start a UV run, setting the time to 20 minutes.

****NOTE:** The UV lamps need a minimum switch-on time of 20 minutes. Do not interrupt a UV light cycle before 20 minutes have passed since it will reduce the lamp's lifetime. Do not touch UV lamp with your fingers. Call QA when a UV lamp needs to be replaced.