**Extraction and purification protocol from serpentine (low-biomass) rocks**

**Brazelton Lab, October 2016**

**Recipes**

**Low Biomass Rock DNA Extraction Buffer (different from the one uses for Sterivex)**

for 45 mL:

0.03M Tris-HCl (pH 8) 1.35 mL of 1.0 M

0.01M EDTA 0.135 g

0.02M EGTA 0.342 g

0.1M KH2PO4 (pH 8) 0.54 g

0.8M guanidine HCl 3.44 g

0.5% Triton-X 100 0.225 mL (225 μL) of 100%

Add Ultrapure water to ~40 mL

Add NaOH to pH 10

Add Ultrapure water to 45 mL

Use 0.2 μm filter and a syringe to remove possible spores

Boil the buffer in microwave for 2.5 minutes with Med-Hi power in a 1000ml glass bottle, tight the lid in order to not losing any liquid during evaporation.

Aliquot into sterile microfuge tubes.

**20% Sodium pyrophosphate solution:** 2 g sodium pyrophosphate decahydrate, 10 mL Ultrapure water

For 10 mL:

Add the provided amount of sodium pyrophosphate decahydrate and water into the 15 mL falcon tube and vortex it pretty well and place it in the 70°C incubator for ∼10 mins. Vortex it occasionally during this time. After it dissolve completely, filter-sterilize and boil it along with the DEB buffer.

**Extraction Protocol**

**Hammering, grinding, preparing samples**

1. Remove sample from freezer. Use rock hammer to crush a small sized chunk of rock into chunks as small as possible under the hood. Use whirlpak bags to avoid contamination. Crush 15-20 grams of sample.
2. Use the flame sterile mortar and pestle to crush the sample into a fine powder. Do this for all 15-20 grams.
3. Pour crushed sample into 50 mL falcon tube, mix well and store at -80 C. This is your working sample.
4. When ready, measure out 0.5 g of sample into 2 mL sterile tubes.
5. Add 1000 µL DEB, 150 µL 20% sodium pyrophosphate solution, and 150 µL dATP to each tube.
6. Shaking them and vortexing them couple of times before put them in the fridge.
7. Store at 4ºC few hours-overnight to allow sample to become immersed in the buffer. Occasional vortexing is recommended during this period. (You can go directly to freeze/thaw if you are in a hurry.)

***Possible Stopping Point. Store at -20ºC after the initial overnight period at 4ºC***

**Lysis**

8. Remove tubes from fridge, vortex them and invert them couple of times.

9. Put them in -80ºC freezer for 30 minutes.

10. Remove the tubes from the freezer and place them in Thermo Mixer at 54º C with 600 rpm speed for 45’-1 h.

11. Increase the temperature to 75º C and the speed to the maximum (1500 rpm) and let them stay in the Thermo Mixer for another 30 minutes.

\*\*\*Beating the tubes (40”) without beads\*\*\*

12. Centrifuge them with MiniSpin for 3 mins at 6,100 g/rcf.

Transfer the supernatant (fluid) to a new sterile 2 mL tubes. (do this really carefully and make sure that your tip doesn’t touch the muddy precipitate)

***Possible Stopping Point. Store at -20ºC***

**Washing sample with VivaSpin column (100,000 MW)**

13. Before starting: ﬁlter sterilize TE with 0.2 μm syringe ﬁlter and boil it.

14. Keep the TE and ultra-pure water in incubator at 65ºC.

15. Load the samples into the columns.

16. If the volume of your samples would be less than 2 mL, add 65ºC TE to bring total volume up to 2 mL.

17. Assemble the columns as it is shown in the manual.

18. Spin for 35 minutes at 2,500 g (**not rpm**).

19. Empty filtrate container and refill the columns with 2 mL 65ºC TE.

20. Spin again for 35 minutes at 2,500 g.

21. Empty the container again and refill the columns with 2 mL 65ºC ultra-pure water.

22. Spin for 30 mins at 2,500 g.

23. Remove filtrate tube, invert the concentrator body and insert the recovery cap (the smaller one) into the tube.

24. Spin for 2,500 g for 5 mins.

25. The recovery caps can be sealed for the storage or you can transfer your clean sample into a new sterile microfuge tube.

***Tip:***

\*\*\*The column can be pre-rinsed with ultra-pure water before loading your sample.\*\*\*

**Measuring conductivity:**

26. Dilute the sample with ultra-pure water. The total amount of sample should be up to 5 mL for each Aurora run . So, you can add 4 mL or more of water to the sample. (usually after the washing step you will get something ~200 µL)

27. Open the protection lid of the conductivity meter. Pour some drops of the diluted sample (~100 µL) on the sensor.

28. Press the MEAS button to enter the measurement mode.

29. When 😊 lights up, the measurement is completed.

30. To lock the measured value, press the MEAS button again.

31. The sample conductivity needs to be ⪯100 µS/cm for DNA clean-up run with Aurora.

32. After measurement, clean the sensor with DI water and dry it by tapping it gently on some kimwipes.

33. Turn off the power and close the protection cover before storage.

\*\*\***Make sure to store the sensor without any moisture\*\*\***

***Tips:***

1. If you are going to use the conductivity meter after a long storage, you need to put some drops of the moistening solution. Let it sit for 10 mins then wash the sensor with DI water. After this treatment you can turn the meter on.
2. If you want to recalibrate the meter, you should pour the 1.41 mS/cm standard solution. Press the CAL button over 2 seconds. When the CAL and 😊 sign light up, the calibration is completed. Clean the sensor with DI water before loading your sample.

**Measuring DNA concentration:**

34. For preparing the Qubit Working Solution, the Qubit reagent need to be diluted 1:200 in Qubit buffer. 200 µL of Working Solution is required for each sample.

35. Ensure all reagents are at room temperature.

36. Add 198 µL of the Working Solution and 1 µL of your sample to a 500 µL thin-walled PCR tube.

37. Vortex the tube for 2-3 second.

38. Incubate the tube for 2 mins at room temperature.

39. Insert the tube in the Invitrogen Qubit 2.0 fluorometer, select “DNA” and “dsDNA high sensitivity” setting.

40. Select “no” when asked whether to read new standards.

41. Press “read next sample”.

42. Select “calculate stock concentration”.

43. Select “2 µL” for stock volume.

44. Do the math for final volume of your sample for the total DNA concentration.

\*\*\***Make sure to store the dye in the dark all the time\*\*\***

**Purification with Aurora instrument**

45. Soak the cartridge and dams in 10% household bleach (5% sodium hypochlorite) for thirty minutes. Do not soak any items longer than 30 minutes; this can damage the equipment.

46. Wash the cartridge and the dams with Milli-Q water thoroughly.

**\*\*\*\*Do not use ethanol to clean cartridge; it will cause the cartridge to crack\*\*\*\***

47. Place both cartridge and dams under the UV hood for 30 minutes, or until dry.

48. Insert dams into their proper locations (see “Aurora Reusable Cartridge Handling Manual”) and pour molten agarose into the designed gap between the dams as it is shown in the manual.

49. Be sure to check for, and correct, any abnormalities such as **bubbles** in the molten gel.

50. Expose the cartridge with molten agarose to UV for 15-20 minutes until agarose sets.

51. Once gel is ready, gently remove dams by slightly pulling out, and then up.

52. Check extraction well to ensure that no agarose is present, which could affect DNA yield and concentration.

53. Fill each buffer chamber with 5 ml of 0.25x TBE Buffer, except for the chamber to the left of the extraction well, which will be filled with 4 ml of buffer.

54. Place lid on cartridge.

55. Pipette 60 ul of 0.25x TBE buffer into the extraction well, and immediately seal with PCR tape, using the extraction well sealing tool to ensure a strong seal.

56. Turn on Thermocube (next to the Aurora) and press “start”.

57. The Thermocube can take a while to set. Make sure the “\*” on the cube turns into a “+” or “-” when the start button is pushed. The asterisk means the thermocube temperature control is not active.

58. Don’t forget to check the level of the water in the Thermocube’s tank. Add more water if it needs.

59. Open the cartridge drawer on the Aurora instrument.

60. Place approximately 1 ml of Milli-Q water on the cold plate of the Aurora to ensure good thermal contact between the cartridge and cold plate.

61. Place the cartridge onto the cold plate oriented so the concentration gel is on the left hand side (see “Aurora Reusable Cartridge Handling Manual”).

62. Load the sample that passed the required amount of conductivity (⪯100 µS/cm) into the sample chamber in the cartridge.

***Tip:***

**\*\*\***As an added precaution to reduce the risk of DNA contamination, cut a piece of PCR tape large enough to cover the sample chamber.

Remove the white backing from the adhesive film and carefully lay it over the sample chamber from left to right, while leaving a small air gap between the concentration gel and the sample chamber to relieve any pressure during the run. Use a scalpel blade and forceps to cut away and remove any tape that may be covering any of the electrodes.

To seal the tape, use a PCR paddle or a flat object to seal the edges, take care to avoid any wrinkles or bubbles.\*\*\*

63. Close the drawer and select the desired protocol.

64. Select the “106-0001-CA-D\_AURORA\_DNA\_CLEAN-UP\_PROTOCOL”. This protocol recovers DNA molecules 0.3-50 kb in length.

65. Create an experiment folder for the run if you want to save the logs.

66. The run will complete in 4 hours based on the selected protocol.

67. If you run the Aurora over night or the time that you won’t be around after the run to obtain your cleaned sample, you can choose the same protocol with “wait-time” option. This option allows you to determine the time that you will be available for obtaining your sample. It basically postpones the focus process (the last step that concentrates and leads the DNA into the extraction well).

68. The initial conductivity check done by the Aurora may also takes a while, in which errors can occur. It is ideal to wait around for a short amount of time to make sure the run continues properly.

***Tip:***

**\*\*\***When run is complete, extract purified DNA as soon as possible. DNA will begin to be reabsorbed by the gel if left too long.

If you’re late to extract the sample, a block of refocusing can be done to try and recover any DNA loss that may have occurred.

Normal focus block is done for about an hour and a half, but the Focus Block protocol can be adjusted to desired length. The longer the focus, the (theoretically) higher DNA yield.\*\*\*

69. When run is complete, open the drawer.

70. Examine the concentration gel and ensure that there are no large bubbles present. Presence of large bubbles indicates that the gel overheated during the run, which will adversely affect the quality of the output.

71. Using forceps to remove the PCR tape covering the extraction well.

72. Transfer concentrated DNA from this well using a micropipette.

Typically, two pipetting steps are required in order to extract most of the liquid from the extraction well. Be sure to extract any sample that is suspended on the walls of the extraction well. **The expected output volume is 50-60 µl, but may vary from 40-70 µl depending on sample and run conditions.**

73. Decant and dispose of the buffer from the cartridge. Using the cleaning brush, gently break up and remove the gel from the cartridge and rinse it with Milli-Q water.

***Tip:***

**\*\*\*\***It is very important to take care **not to scratch the bottom layer of plastic** in the cartridge. Do not use brushes or other cleaning implements with sharp edges or points.

The layer of plastic separating the gel and buffer in a cartridge from the aluminum plate on the bottom of the cartridge is very thin, and if it is scratched, it may be necessary to replace the cartridge.**\*\*\*\***

**Please see the Aurora User Manual and Aurora Reusable Cartridge Handling Manual for troubleshooting information**