5MEMORANDUM FOR MicroLab, Berry Center 320/115

SUBJECT: Standard Operating Procedure for Assist Plus PowerWater Kits

REFERENCES:

1. Quick-Start Protocol: DNeasy PowerWater Kit
2. SOP for Assist Plus and ViaFlo Electronic Pipette

1. Purpose: To provide a standard for personnel practices within the Ecology and Biodiversity Laboratory Department. Updates to SOP will be provided as needed by authorized laboratory personnel, as instructed by supervisor (see signature block for supervisor).

2. Scope: This SOP of the Ecology and Biodiversity Laboratory Department Supervisor and Authorized Designees details the instructions as seen in the Quick-Start Protocol of the DNeasy PowerWater Kit to the instructions as seen on the **Assist Plus and ViaFlo machines**. All personnel who are running samples with the PowerSoil Kits on the Assist Plus machine should be familiar with this SOP and all related references.

**The Bold Black Ink are the instructions to be followed when running a DNeasy Power Water (50) or (100) on the AssistPlus robot.** All other text is for reference only. It is mostly taken from the manual instructions of the kit itself.

**Note: Only Load Partial Tip Boxes on Assist Plus as FIRST Box!! It is your only chance to tell the robot that a box is not full. Empty columns must be furthest left.**

**3.** **Sample Preparation (Quick-Start Protocol Steps 1-4)**

* + **Perform all centrifugation steps at room temperature (15-25°C)**
  + Solution PW1 must be warmed at 55C for 5-10 minutes to dissolve precipitates prior to use. PW1 should be used while still warm
    - **Heat Solution PW1 for 10 minutes**
  + **Gather 48 or fewer randomized samples from each site for the study**
    - **The researcher should provide them loaded with water filters/samples**
    - **Scan barcodes into tracking document**
  + **Label 5 Collection Tubes and 1 MB Spin Column (Filter) with the same identifier as initial sample, one set for each sample.**
  + **One “kit blank” without any sample added should be run for each kit lot number.** 
    - **Record Lot Number Used**
  + **Save All Files in the proper folder found in the directory:**
    - Windows: [\\petalibrary.arcc.uwyo.edu\commons\EPSCoR\_Micro\MicroLab](file:///\\petalibrary.arcc.uwyo.edu\commons\EPSCoR_Micro\MicroLab)
    - Mac: <smb://petalibrary.arcc.uwyo.edu/commons/EPSCoR_Micro/MicroLab>
  + **Always return tubes in the same order they were removed!!**
    - **Use marks on vortex adaptors and centrifuges as position one and add clockwise.**
    - **Remove in the same order**

**4. ViaFlo Procedures and Actions(Quick-Start Protocol Steps 5-9, QSP 5-9):**

* + **Turn on Integra 1250 ul Pipette by pressing “Run”**
  + **Scroll down to “Custom Programs” and press center button**
  + **Press center button to select “Power\_Water\_A”**
  + QSP5. Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube
    - **Press “Run” to Aspirate 1 ml from PW1**
    - **Press “Run” to Dispense 1 ml PW1 to 5 ml PowerWater DNA Bead Tube**
      * + **Keep pipette tip above dispense tube, discard if any splashing or touching of tube occurs**
    - **Repeat steps a and b until all samples have PW1**
  + **QSP6. Secure tubes horizontally to a vortex adaptor**
  + **QSP7. Vortex at maximum speed for 5 minutes. Centrifuge the tubes 4000 x rcg (g) for 1 minute. Add first sample to “1” spot and follow arrow clockwise around**
  + 8. Transfer the supernatant to a clean 2ml collection tube
    - **Press backwards arrow to move back a menu on pipette**
    - **Scroll down to “Power\_Water\_B”, Press center button to select it**
    - **Press “Run” to Aspirate 650 ul of “supernatant” place tip into beads lightly**
    - **Press “Run” to Dispense 650 ul into the correctly labeled 2 ml collection tube**
  + **QSP9. Centrifuge at 13,000 g (rcf) for 1 min, Add first sample to “1” spot and follow arrow clockwise around**

**5. Assist Plus Procedures and Actions:**

**I. Assist Plus Machine Setup and Instructions(Assist Plus Steps 1-41, QSP 10-23):**

* **Open pipette tip rack, set on stand below arm with lid facing out**
  + **Slot A1 of pipette tray goes in back left corner of stand below arm**
* **Select correct size reservoir (10 mL to start) and set on first stand**
  + **Ensure reservoir is parallel to pipette arm and seated firmly**
  + **Ensure reservoir of all sizes are available (10, 25, and 100 mL)**
* **Set sample 1.5-2 mL tube racks on middle and right stands**
* **Ensure 1250 ul Voyager Pipette is on Assist Plus**
* **Start AssistPlus Program**
  + **Press Back Arrow to get to “Main Menu”**
  + **Press center button to select “Assist Plus”**
  + **Press center button to select “VIALAB Programs”**
  + **Scroll down to “PowerWaterC”, Press Center Button to select**
  + **Press “Run” or flashing arrow button on Assist Plus base to home and proceed between steps**
* **Add spun 2 ml tubes to left rack, labeled clean tubes to right rack**
  + **AP2“Samples Left Clean Tubes Right”**
* **Add 11 ml Solution IRS to 10 ml reservoir WITH 1 INSERT** 
  + **AP3“Load 10 ml Reservoir with 10 ml IRS”**
* AP4, QSP10 Avoiding the pellet, transfer the supernatant to a clean 2 ml . . .
* AP5, AP6, QSP11 Add 200 ul of Solution IRS and **Vortex briefly. Incubate at 4C for 5 min**
* AP7, QSP12 “**Centrifuge 13000 rcf 1 minute”, Start at “1” spot and follow arrow clockwise around**
* AP8 **Add sample tubes to the right and clean tubes to the left rack**
* AP9 **Load 100 ml Reservoir with 36 ml PW3**
* AP10, QSP14 (13 and 14 are reversed deliberately!) Add 650 ul PW3 to empty tubes
* AP11, QSP13 Transfer 825 ul of supernatant to tubes with PW3, mix via pipette
* AP12 **Load Spin Columns into right rack. MATCH ORDER!!!**
* QSP15, AP13 Load 650 ul of supernatant onto a MB Spin Column. **Leave Left Tubes Alone**
* AP15 “**Centrifuge 13000 rcf 1 minute”, Start at “1” spot and follow arrow clockwise around**
* AP16 **Discard FlowThrough**
* AP17 **Load Spin Columns on Right**
* AP18 Load 650 ul of supernatant onto a MB Spin Column. **Leave Left Tubes Alone**
* AP20 “**Centrifuge 13000 rcf 1 minute”, Start at “1” spot and follow arrow clockwise around**
* AP21 **Discard FlowThrough**
* AP22 **Load Spin Columns on Right**
* AP23 Load 650 ul of supernatant onto a MB Spin Column.
* AP24 “**Centrifuge 13000 rcf 1 minute”, Start at “1” spot and follow arrow clockwise around**
* AP25 **Discard FlowThrough**
* AP26, QSP 16 **Place Filter in Clean 2ml Tubes**
* AP27 **Load 100 ml Reservoir with 36 ml PW4 (Shake before adding)**
* AP28 Add 650 ul of Solution PW4
* AP29 “**Centrifuge 13000 rcf 1 minute”, Start at “1” spot and follow arrow clockwise around**
* AP30 **Discard FlowThrough**
* AP31 **Load 100 ml Reservoir with 36 ml Ethanol**
* AP32, QSP18 Add 650 ul EtOH
* AP33 **Centrifuge 1300 rcf 1 minute, Start at “1” spot and follow arrow clockwise around**
* AP34 **Discard FlowThrough**
* AP35, QSP19 **Centrifuge 13000 rcf 2 minutes, Start at “1” spot and follow arrow clockwise around**
* AP36, QSP20 **Place Filter in Clean Tubes**
* AP37 **Load 10 ml Reservoir with 5 ml EB**
* AP38 Add 100 ul EB
* AP39 **Centrifuge 13000 rcf 1 minute, Start at “1” spot and follow arrow clockwise around**
* AP40 **Save FlowThrough. This is the DNA.**

IV. Troubleshooting, Care and Maintenance, Safety Precautions

**If Assist Plus prompts “Tips Still Detected” when tips are absent, wipe off both sensor sides with 95% EtOH**

1. Refer to SOP for Assist Plus Reservoir for all troubleshooting, care/maintenance, and safety precautions as needed.
2. Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety).
3. Technical Assistance: support.qiagen.com.
4. The DNeasy PowerSoil Kit can be stored at room temperature (15-25°C) until the expiry date provided on the box label.

4. Signature Block

The following contact is the current assigned supervisor for the Ecology and Biodiversity Laboratory Department. Any questions regarding the information contained within this SOP or any problems found with the Assist Plus machine or any associated components should be directed to the following: