MEMORANDUM FOR MicroLab, Berry Center 320/115

SUBJECT: Standard Operating Procedure for Assist Plus PowerPlant HTP 96 well plate kits

REFERENCES:

1. Quick-Start Protocol: DNeasy PowerPlant HTP kit (this is the sheet contained in the kit)
2. SOP for Assist Plus

1. Purpose: To provide a standard for personnel practices within the Genome Technologies Laboratory. 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 provides more details to assist with the interpretation of the **Quick-Start Protocol** and the prompts provided by the **Assist Plus machine**. All personnel who are running samples with the PowerPlant Kits on the Assist Plus machine should be familiar with this SOP and all related references.

**Note: If you want to load a tip box that is not full, then do this as the first box. Empty columns must be furthest left. If you load a partially full box later then you will get an error when the robot runs out of tips. If that happens, not a big deal, just add more tips.**

3. Procedures and Actions:

**I. Assist Plus Machine Setup:**

1. **Open box of pipette tips, set on stand below arm with lid facing out**
   * **Slot A1 of pipette tray goes in back left corner of stand below arm**
2. **Select correct size reservoir and set on the deck**
   * **Ensure reservoir is parallel to pipette arm and seated firmly**
   * **Ensure reservoir of all sizes are available (10, 25, and 100 mL). More of these should be available in the laboratory. If you start to run low on these then let your supervisor know, so they can buy more.**

II. **Sample Preparation:**

1. Notes Before Starting
   1. Perform all centrifugation steps at room temperature (15-25°C)
   2. If Solution SL has precipitated, heat at 60°C until precipitate dissolves
      * You can do this by simply setting the container in some warm water.
   3. **Make sure that samples have been randomized by treatment group, we don’t want to extract all control samples from one kit and all treatment samples from a different kit. If you aren’t sure then ask your supervisor**
      * The researcher probably will have provided you with tubes filled with weighed and ground leaves. If not, then talk to your supervisor to learn what else needs to be done before extraction can begin. You cannot extract DNA from an intact leaf.
      * Scan barcodes into tracking document (as of 11/1/19 we aren’t doing this yet)
   4. **One “kit blank” without any sample added should be run for each kit lot number.** 
      * Record Lot Number Used, or some other identifier that explains which box the blank goes to. There should be a blank for every extraction kit.
   5. **Make sure that a spreadsheet exists that has the sample identifiers with which wells and which plate those samples are assigned. This is CRITICAL. If we don’t know which sample is in which well then the extraction will not provide useful DNA and will waste samples. The spreadsheet should also have sample weights, which extraction kit was used for each sample, and the date extracted. If you are not sure about any of this, then ask your supervisor.**
2. Instructions
3. **Labelling and keeping track of which sample is in which well is more important than anything else. If in doubt about anything involving sample tracking or labelling, stop and ask your supervisor.**
   1. **Doublecheck that a spreadsheet exists linking sample to well before beginning.**
   2. Clients/researchers should have provided tubes arrayed into 96 well tube racks. These should already be ground up and weighed. Get these tubes out of freezer to thaw.
   3. Add 45 ml of PowerBead Solution to a Falcon tube and then add 300 ul of RNAse to that tube. Check with supervisor to see if the clients want to use PSS. Make another tube that has PSS (4 ml) and SL (5 ml).
   4. Wear gloves: Add 410 ul of the PowerBead/RNAse solution to each tube. Then add 90 ul of the PSS/SL solution. If PSS is not needed, then add 450 ul of the PowerBead/RNAse solution. If you have a little extra solution, just dump it into the waste container. Clean the tubes with super clean water.
   5. Make sure the tubes are sealed really well and **LABELLED** on the sides. Then put them in the tissue lyser and shake them for 8 min. It is best to check after shaking for a few seconds to make sure nothing is leaking. If there is a leak, remove that sample, put tape over the leak and shake the sample up by hand.
   6. Flip the plates over so that the samples closest to the lyser are now farthest from the lyser. Shake another 8 min.
   7. Centrifuge the tubes at 4500 rcf for 9 min. Make sure the centrifuge is on rcf, not rpm.
   8. After centrifugation, array the samples back into 96 well tube racks. Doublecheck that each sample is in the well that it is supposed to be. This is really important, because if a sample gets swapped into the wrong well then we will never know from this point forward.
   9. Label a 1ml collection plate (these plates are in the extraction kit) with which plate of samples will go into that plate. Make sure the plate is aligned properly so sample A1 goes into the correct well. Wear gloves: pipette supernatant (about 400–600ul) from each of the spun tubes into its respective well. Try to avoid getting much of the pelleted tissue. If you get a little it is ok, but try not to get much. Pro tip: be very careful pipetting. Hold open tubes away from the open wells so that if you drop a tube it won’t contaminate anything. Cover open wells of the plate with chemwipes. Try to move the tip of the pipette over the chemwipes, so that it never hovers over an open well. **ANYTHING you can do to avoid possible contamination or mix ups at this step is super important.**
   10. Now to the robot. Turn robot on, make sure tips are in place. Fill a 25 ml reservoir with about 20 ul of IR. Navigate to the program called “PowerPlantIRx2” and run it. The program will prompt you where to add the plate (left side or right). The robot will then add 175 ul to each well. Add more IR before the robot moves to the second plate (it should prompt you). **This is step 5 of the QuickStart Protocol.**
   11. Seal the collection plate with sealing tape (contained in kit). Make sure seal is really tight so that no well-to-well contamination occurs during vortexing.
   12. Vortex the plates for about 5 seconds. Use the plate adaptor on the vortexer. Don’t go crazy here or contamination can occur.
   13. Incubate the 1 ml collection plate at 4C for 10 min. Just stick the plate into the fridge and set a timer for 10 min.
   14. Centrifuge plates at 4500 rcf for 9 min. Note that the plate centrifuge defaults to rpm upon startup, so doublecheck that you are in rcf mode.
   15. Label 2ml collection plates (in kit). Follow robot prompts. The robot will move the supernatant from the 1ml plate into the 2ml plate, but first we will add some solvents to the 2ml plate.
   16. The robot will add 600 ul of PB to each well of the 2ml plate. Therefore you will need to fill a 100ml reservoir with roughly 65 ml of PB, for each plate. Dump any remaining PB into the waste.
   17. Ethanol will also get added to each well of the 2ml plate. Fill the same 100 ml reservoir that you used for step 16 with roughly 65 ml of ethanol. We are using 100% ethanol. Dump any remaining ethanol into the waste.
   18. Follow robot prompts. **Make sure that the 2ml plate is labelled and oriented properly.** The robot will now move the samples from the 1ml plate into the 2ml plate and mix by pipetting. Watch out for leaks here as this would cause cross-contamination. Pro tip: I like to cover the unused wells with a small square of a plastic bag that provides an impermeable barrier for any random drops.
   19. Label a QIAamp 96 plate and put it on top of an S block. Note that we reuse S blocks, therefore the S blocks may be opened already.
   20. Follow prompts. The robot will now move samples from the 2ml plates into the QIAamp plates. KEEP the half full 2ml plates. It is good to cover them with chemwipes.
   21. Cover the QIamp plate with airpore tape and centrifuge at 4500 rcf for 5 min.
   22. Discard the flow through into the waste (use the funnel to avoid making a mess).
   23. We now repeat the past couple steps to make sure we get all of our sample. Follow robot prompts. What will happen is that the QIAamp plates will come out of the centrifuge and be placed back on the robot with the associated 2ml plate. Then the robot will move the remaining sample from the 2ml plate to the QIAamp plate. Apply airpore tape and centrifuge. This will be done for both plates.
   24. **MAKE SURE ETHANOL HAS BEEN ADDED TO IW**. If not then add 106 ml of ethanol to the IW and label the bottle to say that ethanol has been added.
   25. Add 55 ml of IW to a clean 100 ml reservoir.
   26. Add 500 ul of IW to each well of the QIAamp plate, do this for both plates.
   27. Apply airpore tape and centrifuge at 4500 rcf for 3 min. Discard flow through
   28. Add 55 ml of ethanol to the same 100 ml reservoir.
   29. Add 500 ul of ethanol to each well of the QIAamp plate. Refill the ethanol, and do the same to the second plate.
   30. Apply airpore tape and centrifuge at 4500 rcf for 3 min. Discard flow through.
   31. Centrifug at 5900 rcf or so for 7 min. This is to try and dry the ethanol off the plates.
   32. **LABEL** some elution microtube racks (in kits) with the plate numbers. Label the lids and both sides of the racks. Carefully set the QIAamp plates on their respective racks. I try to be careful here to avoid possible cross contamination.
   33. Remove the airpore tape and let air dry for 10 min at room temperature.
   34. Add 12 ml of EB to a 10 ml reservoir (it is ok if it is full). Pipette 100 ul of EB into each well of the QIAamp plate. Do this for both plates. You will need to refill with EB before the second plate.
   35. Check and make sure the EB hasn’t stuck to the side of the well. I sometimes lightly tap the elution microtube rack/QIAamp rack lightly on the desk to get any EB that has beaded up on the sides of the wells to slide down onto the filter. Incubate for room temperature for at least 2 min.
   36. Centrifuge at 4500 rcf for 3 min. Remove and discard the Airpore sheet. The liquid in the elution microtube racks is your DNA!
   37. Seal the microtube racks with the rubber caps. Make sure everything is labeled with the client name, the date, and what the samples are (e.g. plant tissue, sediment, soil). Place the microtube racks in the -80 freezer.
   38. Clean up your workspace. Clean the counters with water or ethanol and make sure the robot is clean. Discard spent tips into the sharps container.

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**

**Most problems can be solved by pressing “Run” after trying to resolve the obstruction. For instance, some times the machine will pick up the whole tip box. You can remove the tip box and then press run and it should start where it left off.**

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 PowerPlant 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: