Ionic G2 Tissue to DNA Kit Protocol

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B

Table of Contents

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Revision History

REVISION	NOTES
A	Initial release.

Safety Recommendations

For all procedures in this document, the use of appropriate personal protective equipment (PPE) is strongly recommended.

- Disposable gloves should always be worn when handling samples, reagents, fluidic chips, and any other materials that may come in contact with samples.
- Gloves should be changed immediately after any contact with the sample.

Inappropriate use of an Ionic® Purification System may cause personal injury or irreparable damage to the instrument.

- Only trained personnel should operate the Ionic system following published methods.
- All operators should review and be familiar with the *Ionic Purification System User Guide* (P/N xxxxx).
- Only Bionano qualified service engineers should service the Ionic system.

Damage to the Ionic system caused by inappropriate use, neglect to perform required maintenance, or performing inappropriate maintenance may void warranty or require services not covered by standard service contract terms.

- Do not move the instrument while it is in operation.
- Do not unplug the instrument while it is in operation.
- Do not spill liquids on any area of the instrument.
- Do not use with flammable materials or in the presence of toxic fumes.
- Do not use excessive force to open or close the system cover.
- Use only with Ionic Fluidic Chips and associated kits and protocols.

Laboratory supervisors and/or facility managers must take the necessary precautions to ensure a safe workplace and appropriate training of personnel.

- All laboratory activities should be in accordance with all national, state, and local health and safety regulations.
- Follow all applicable SDS (or MSDS) recommendations for proper handling and disposal of chemicals and reagents.
- Follow all safety guidelines for use of personal protective equipment, laboratory devices, and labware established for the laboratory where the instrument is used.

Kit Contents

Table 1: Contents provided in the Ionic® Tissue to DNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
-20ºC Reagents Box	**Lysis Buffer 1**	Lysis Buffer 1	1.66 mL	6
-20ºC Reagents Box	**RNase**	RNase A Reagent	270 µL	1
-20ºC Reagents Box	**Proteinase K**	Proteinase K Reagent	540 µL	1
-20ºC Reagents Box	**Sample Buffer**	Blank Sample Buffer	1.6 mL	1
RT Reagents Box	**Lysis Buffer 2**	Lysis Buffer 2	3.3 mL	1
RT Reagents Box	**1 - Extraction Buffer**	Extraction Buffer	12 mL	1
RT Reagents Box	**2 - Anodic Buffer**	Anodic Buffer	12 mL	1
RT Reagents Box	**3 - Separation Buffer**	Separation Buffer	18 mL	1
RT Reagents Box	**4 - Neutralization Buffer**	Neutralization Buffer	12 mL	1
RT Reagents Box	**5 - Cathodic Buffer**	Cathodic Buffer	12 mL	1
Fluidic Chip Set Box	**Ionic Fluidic Chip (Tissue)**	Fluidic Chips	N/A	6

Table 2: Reagents provided by the user.

User-supplied Reagents	For Lysate Preparation	For Purification
Fresh/frozen tissue	✓	

Table 3: Equipment provided by the user.

User-supplied Equipment (or equivalent)	For Lysate Preparation	For Purification
12-column reservoir (Agilent 204365-100)		✓
P200 multichannel pipette		✓
P200 single channel pipette	✓	✓
P20 single channel pipette		✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex mixer (adjustable speed)	✓	✓

Table 4: Labware provided by the user.

User-supplied Labware (or equivalent)	For Lysate Preparation	For Purification
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	ü	ü
Optional: DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	ü	
Optional: DNA LoBind Plate, 96-well (Eppendorf 951032000)		ü

Protocol at a Glance

Lysate Preparation

1. Thaw Lysis Buffer 1 on ThermoMixer at 56°C for 10 minutes at 1400 rpm.
2. Add 90 µL of Proteinase K to a Lysis Buffer 1 tube to make Lysis Mix 1.
3. Collect specimen at bottom of tube by spinning at full speed for 1 minute.
4. Add 195 µL of Lysis Mix 1 to each sample tube.
5. Incubate samples on ThermoMixer:
   - 56°C for 5 hours at 1400 rpm
6. Centrifuge lysate tubes at full speed for 5 minutes.
7. Add 540 µL of Lysis Buffer 2 and 45 µL of RNase into a new tube to make Lysis Mix 2.
8. Add 65 µL of Lysis Mix 2 to eight new tubes.
9. Collect 170 µL lysis reaction from incubated lysate tubes and transfer to tube containing Lysis Mix 2.
10. Incubate at 37°C for 10 minutes.

Purification

1. Prepare Purification Buffer Reservoir.
2. Place Ionic Tissue Chip onto instrument.
3. Load Purification Buffers and prime Ionic Tissue Chip.
4. Add 200 µL of each lysate to the chip.
5. Start purification run (~70 minutes).
6. Collect ~50 µL of each extract.

Sample Requirements

This protocol is intended for the extraction and purification of DNA from up to eight fresh/frozen tissue samples in parallel and is optimized to extract the maximum yield from a minimal amount of tissue. Samples may be minced, pulverized, or OCT-embedded.

NOTE: Samples preserved with RNA stabilizers are **NOT** supported.

Bionano recommends an input of 5 mg for most downstream applications. For samples containing small amounts of DNA, up to 10 mg may be used, though care must be taken not to overload the chip.

NOTE: Overloading sample can lead to lower yields (see Figure 1). A small pilot study can be helpful in defining optimal input conditions.

Some types of tissue samples present challenges to extraction and purification that require optimization beyond the standard protocol. Contact Support@bionano.com for assistance in this case and for advice on processing samples that do not meet the requirements above.

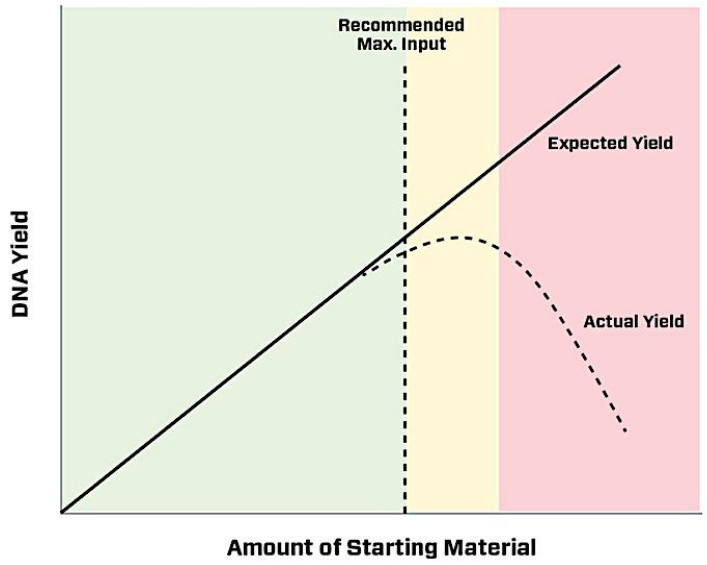

Figure 1. Impact of overloading

Lysate Preparation – Single Tube Format

Preparation before lysis protocol

Lysis Buffer 1

1. Completely thaw Lysis Buffer 1 by incubating on the ThermoMixer at 56°C for 10 minutes at 1400 rpm.

   NOTE: Lysis Buffer 1 must be at room temperature before vortexing and preparing Lysis Mix 1.

2. Add 90 µL of Proteinase K to the tube containing Lysis Buffer 1 to make Lysis Mix 1.
3. Vortex Lysis Mix 1 for 5 seconds and spin down for 10 seconds.

   NOTE: All centrifuge steps should occur at room temperature.

Samples

1. Transfer up to 10 mg of OCT-embedded, minced, or pulverized fresh-frozen tissue to a 1.5 mL or 2.0 mL nuclease-free microtube.

   NOTE: For OCT-embedded samples, OCT removal is advised, but not required.

   NOTE: This product is not compatible with tissue specimens that have been stored or treated with high ionic strength RNA stabilizer reagents such as RNAlater.

2. Gather tissue specimen at the bottom of the tube by centrifuging for one minute at full speed (20,000 x g, 14,000 RPM).

ThermoMixer

1. Place an appropriate heating block on the ThermoMixer to accommodate sample microtubes. Use of the ThermoMixer with its lid is recommended.
2. Create an incubation program for the Lysis Procedure with the following parameters:
   - Incubate at 56°C for 5 hours at 1400 rpm.

Preparation of Lysates

1. Add 195 µL of Lysis Mix 1 to the tissue specimen.
2. Vortex for 5 seconds and centrifuge for 10 seconds.
3. Place sample tubes into ThermoMixer and start the Lysis Procedure program created earlier.

   NOTE: Align the tube hinge to face the outside of the rotor. The pellet will form on the hinged side of the tube.

4. At the end of the program, remove the lysates from the ThermoMixer and spin them at full speed (20,000 x g, 14,000 rpm) for 5 minutes to pellet debris.
5. Set the ThermoMixer to 37°C, 0 rpm, hold.
6. Label one new 1.5 mL or 2 mL microcentrifuge per lysis reaction.
7. During the spin, add 540 µL of Lysis Buffer 2 and 45 µL RNase to a new tube labeled Lysis Mix 2.
8. Vortex for 5 seconds and briefly centrifuge.
9. Add 65 µL of Lysis Mix 2 to eight labeled tubes (one tube per original sample).

10. After the spin, aliquot 170 µL of the lysis reaction into the tube containing Lysis Mix 2 while avoiding the pelleted debris.

    NOTE: The pelleted debris may not be visible. Avoid the pellet whether visible or not, by carefully pipetting from the opposite side of the tube from the pellet.

    If samples are from a fatty tissue such as breast, also avoid the top layer of fat when transferring the lysate.

11. Vortex for 5 seconds and spin down for 10 seconds.
12. Place sample tubes into ThermoMixer set at 37°C and incubate for 10 minutes without shaking.
13. Proceed with Purification or store samples at -20°C for up to 7 days.

    NOTE: For frozen samples, thaw to room temperature, then vortex and spin briefly before proceeding with the protocol.

Purification

Prepare Buffer Reservoir

1. Label a 12-channel reservoir as shown in **Figure 2** skipping every other column to prevent purification buffer cross-contamination.

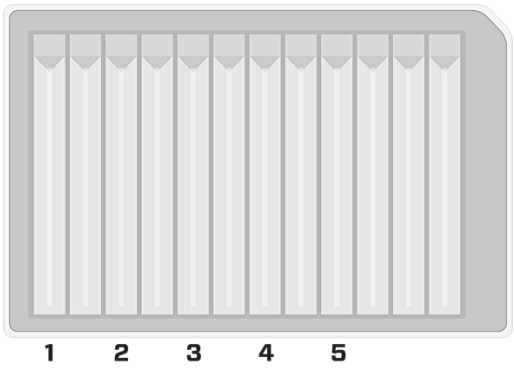

Figure 2. Reservoir column labels

2. Add purification buffers to each column according to **Table 5**.

Table 5. Reservoir buffers and volumes

Reservoir Column	Buffer from Kit	Volume
1	1 - Extraction Buffer	2.0 mL
2	2 - Anodic Buffer	2.0 mL
3	3 - Separation Buffer	3.0 mL
4	4 - Neutralization Buffer	2.0 mL
5	5 - Cathodic Buffer	2.0 mL

Set up Ionic Purification Run

1. From the Ionic Purification System start screen, press **Start** then select a User profile for the run.
2. Press the **G2 Tissue to DNA** button. The instrument cover will open.
3. When prompted, remove an Ionic Tissue chip from its packaging, handling only by the side skirting, and place on the instrument stage with the barcode on the right as shown in **Figure 3**. Gently apply pressure to all four corners of the fluidic chip simultaneously to confirm that the chip is fully seated on the stage. Press the **Arrow** on the right side of the screen to continue.

   NOTE: Remove the Ionic Tissue chip from its packaging and place chip directly on the instrument stage to minimize accumulation of static electricity. Avoid contact with the top and bottom surfaces of the fluidics chip.

Figure 3. Proper handling and placement orientation of an Ionic Tissue chip.

4. Enter Run Name, Chip ID, and Reagent Lot and press the **Arrow** on the right side of the screen to continue.

   NOTES:

   Chip ID is located on the label on the top surface of the Ionic Tissue chip. Include both the P/N and L/N for the chip:

   - Reagent Lot number is located on the G2 Tissue to DNA Kit room temperature box label.
   - Barcodes can be scanned into the software using a handheld barcode reader connected to the instrument USB port.

5. Using a P200 multichannel pipette and the proper pipetting technique shown in **Figure 4**, add the appropriate volume of each purification buffer as shown on the screen (also see **Figure 5**), working left to right (1 to 5), to the fluidic chip**.**

   NOTE: Do not remove the plastic film from the sample wells at this point as the fluidic chip will not prime correctly without these wells covered

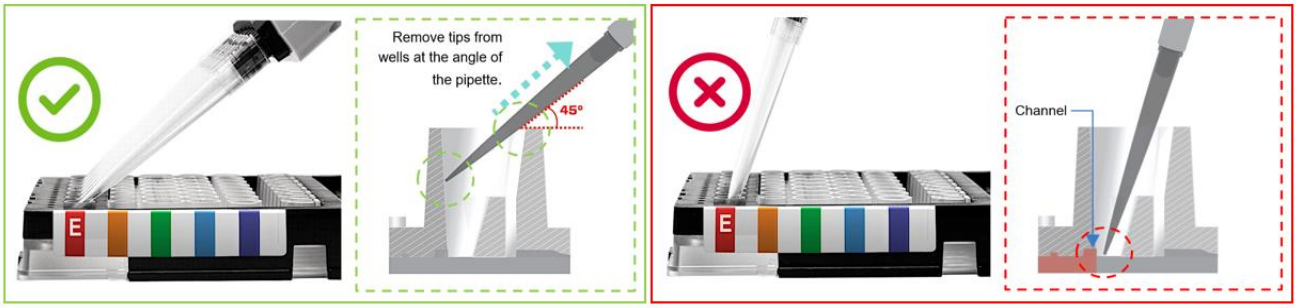

Figure 4. Proper (left) and improper (right) pipetting techniques for fluidic chip buffer wells.

NOTE: Correct technique is important to ensure the buffers prime correctly into the fluidic chip.

- Visually inspect tips to ensure each contains the same volume prior to adding to the fluidic chip.
- Rest pipette tips on the top right of the wells and dispense against the left wall no more than halfway into the well with the pipette at an angle of ~45° relative to the chip surface.
- Smoothly dispense at a steady speed to the first stop only then remove tips from the well by dragging the tips against the top right wall of the wells.
- Always avoid contact with the bottom of the wells when dispensing.
- Change tips after each dispense.

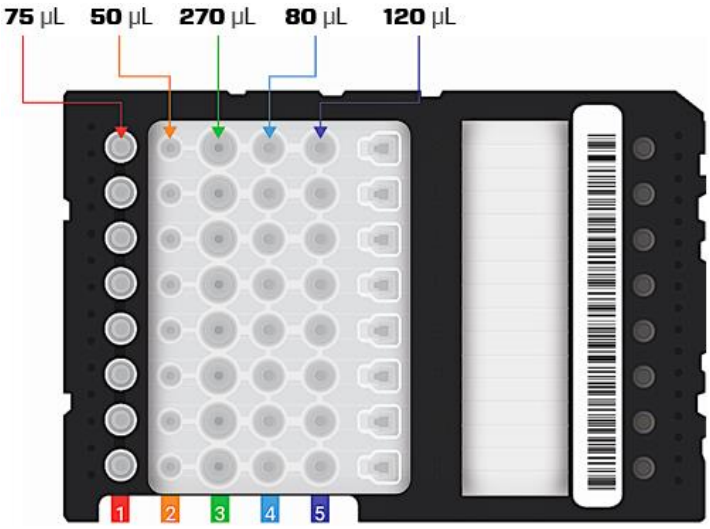

Figure 5. G2 Tissue to DNA purification buffer dispense volumes.

NOTE: The Separation Buffer should be added using two pipette transfers with fresh tips for each transfer.

6. After loading purification buffers press the **Arrow** on the right side of the screen to continue. The instrument cover will close, and the fluidic chip will prime.

   NOTE: Priming takes approximately 4 minutes.

   NOTE: Samples should be loaded within 10 minutes of priming completion.

7. During fluidic chip priming, vortex lysates for 5 seconds and pulse spin for 10 seconds. Hold on ice.

8. When priming is complete press the **Arrow** on the right side of the screen to continue. Press **OK** to confirm samples have been vortexed. The instrument cover will open.
9. Enter sample naming information (See Ionic User Manual for additional information on sample naming options) and press the **Arrow** on the right side of the screen to continue. Confirm run information is correct and press the **Arrow** on the right side of the screen to continue.
10. While firmly holding the chip by the side skirt, carefully remove the plastic film from the sample wells using the pull tab and press **OK** to continue (**Figure 6**).

Figure 6. Remove the plastic film from the sample wells.

11. Using a P200 pipette and keeping the tip in contact with the bottom of the tube, slowly aspirate and transfer 200 µL of lysate to each sample well.

    NOTE: Insert tip no more than halfway into the well at the position noted in **Figure 7** and smoothly dispense at a steady speed to the first stop only.

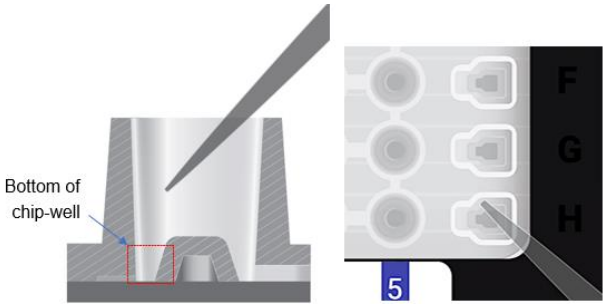

Figure 7. Proper Sample Well lysate loading positioning.

12. Press **Begin Run**. The instrument cover will close, and the purification process will begin.

    NOTE: The G2 Tissue to DNA purification run will proceed for approximately 65 minutes and the instrument will indicate when the run has completed.

    IMPORTANT: Upon completion, extracts should be collected within 1 hour.

Collect DNA Extracts

1. Once the purification run has completed, click the **Arrow** on the right side of the screen to continue. The instrument cover will open.
2. Using a P200 pipette set to 60 µL, aspirate each purified DNA extract (~50 µL) by placing the tip at the *bottom* of the left wall of each Extraction Buffer well and transfer to a LoBind microcentrifuge tube or LoBind 96-well microplate.

   NOTE: As demonstrated in **Figure 7**, the Extraction Buffer well has a small ledge. Be sure to navigate the pipette tip to the left of the small ledge to reach the well bottom.

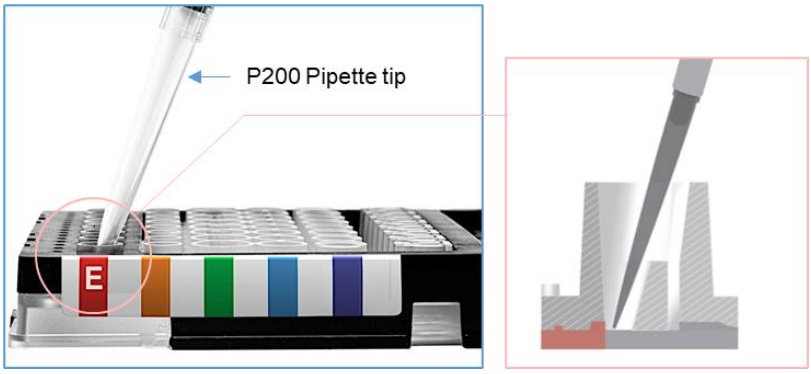

Figure 7. Aspirating the DNA extract from the Extraction Well

3. Using a P20 pipette set to 10 µL, aspirate any remaining DNA extract from the microchannel on the right side of the Extraction Buffer well and combine with the extract from Step 2. See **Figure 8**.

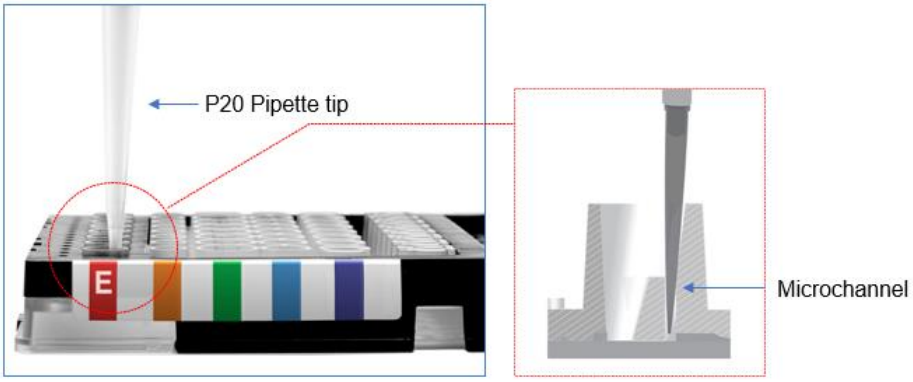

Figure 8. Recovery of any remaining DNA from the Extraction Well microchannel

4. Press the **Arrow** on the right side of the screen and confirm the extracts have been collected by clicking **OK**.
5. Remove the chip from the instrument stage and click the **Arrow** on the right side of the screen to continue. The instrument cover will close.
6. Press **Finish** to set up another purification run or return to the Home screen.
7. Vortex DNA extracts and pulse spin before using in downstream assays.

   NOTE: Store DNA extracts on ice for same-day use and -20°C for long term storage.

Troubleshooting

Ionic Purification System Feedback

The Ionic Purification System provides post-purification feedback for each sample lane as described in **Table 6**.

Table 6. Run-status icon description.

Run-status Icon	Description
	A green checkmark indicates a successful purification run for that lane. Collect samples by continuing to Step 3 below.
	A yellow warning indicates that a purification run abnormality has been detected for that lane. • Please collect the sample from the Extraction Buffer Well • If additional information is needed, please Save System Logs as described below and contact support@bionano.com
	An orange recycle icon indicates that the purification run for that lane did not initiate. • Please recover the sample from the Sample Input well as described below. • This sample is not lost and can be run on a new chip.

Recovery of Non-Initiated Lysate

If the Ionic system displays an orange recycle icon (shown above). Upon completion of a purification run, follow the steps below to recover the non-initiated sample so it can be run on a new Ionic fluidic chip.

1. Before collecting non-initiated lysate(s), collect all purified extracts as described above in the Collect DNA Extracts section.
2. Remove the Ionic fluidic chip from the instrument stage and place on a level surface. Cover columns 1–5 with a plastic adhesive film leaving the Sample Well column uncovered as shown in **Figure 9**. Ensure a tight seal for each well.

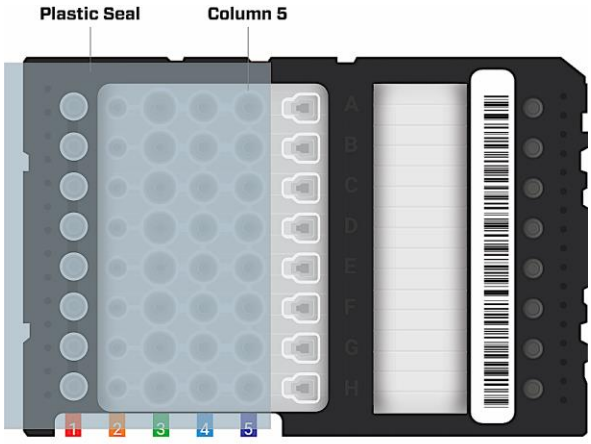

Figure 9. Proper plastic seal application for sample recovery

3. Set a 200 µL pipette to 200 µL. While using the thumb/forefinger to reinforce the seal on the well in Column 5, aspirate slowly from the bottom right side of the Sample Well of the non-initiated sample where the well and channel meet (see **Figure 10**) and transfer to a new microtube.

   NOTES: Typically, between 75–125 µL is expected to be recoverable from a non-initiated sample.

   - Aspirating from the bottom right side of the sample well allows lysate to be collected from the sample well, and potentially to the right, the sample channel.
   - Aspiration of fluid from the channel between the Cathodic Buffer (5) and the Sample Well should be avoided.
   - Ensuring a proper seal on the Cathodic Buffer (5) well will minimize the amount of buffer that is aspirated from the channel to the left of the sample well.

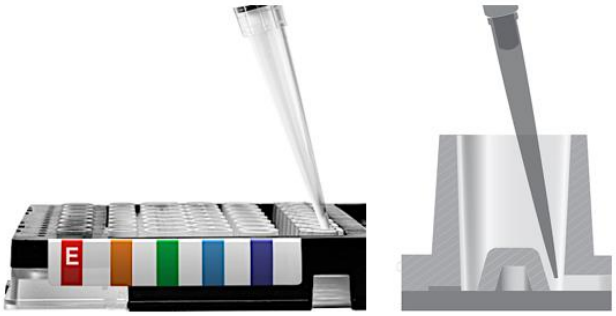

Figure 10. Sample recovery technique

4. Add a sufficient volume of Tissue Sample Buffer to the sample to bring the total volume to ~210 µL.
5. Store recovered samples at -20°C until they are ready to be re-run. Then, thaw recovered samples, vortex, spin down, place samples on ice and proceed directly to purification. When re-running the sample, always use the same protocol from the original run.

If errors are encountered while running the instrument, follow the steps in Save System Logs and then email the log file to support@bionano.com. Support will be in contact within 48 hours to follow up.

Save System Logs

The **Save System Logs** maintenance screen is used to save system log files to a USB flash drive. Bionano Support can use the system log files to diagnose problems with the instrument.

NOTE: The USB flash drive must be in a FAT32 format. It is recommended to have a minimum of 1 GB of available space on the flash drive.

Cases where this function should be used:

- If the Run results are not as expected
- If the self-test fails
- If Support personnel request a system log

1. Press the **Save System Logs** button to save system log files to a USB device, as shown in **Figure 11**.

Figure 11. Save System Logs screen - Step 1

2. Insert a USB drive into the USB slot located at the bottom-right on the front of the instrument.
3. Once a valid USB drive is detected, the next screen is displayed, shown in **Figure 12**. The definitions in **Table 7** are helpful here. Press **Write** to begin the transfer.

Figure 12. Save System Logs screen - Step 2

Table 7. Save System Logs screen.

Call Out	Screen Component	Definition
1	Write button	A button to copy the system log to the USB drive inserted in the USB slot in the font of the instrument. The system log file is copied to the root directory of the USB drive
2	Help icon	Loads the Help screen

4. A status bar is displayed on the touchscreen. The status bar may seem inactive for larger log files. Wait for the system log to be saved to the USB flash drive.
5. After the system log file is saved to the USB flash drive, press the button on the left side to return to the **Maintenance & Service** screen.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

TYPE	CONTACT
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