G2 Ionic FFPE Complete Protocol

NOTE: Use one Ionic® FFPE to Pure RNA Kit and one Ionic® FFPE to Pure DNA Kit

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C

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bionano

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Revision History

REVISION	NOTES
A	Initial release
B	Changed amount of Proteinase K added during the Preparation of Lysates section to 135 ul.
C	Complete using G2 FFPE to DNA Kit and G2 FFPE to RNA Kit

NOTE: This protocol now requires the use of two kits:
One Ionic® FFPE to Pure RNA Kit
One Ionic® FFPE to Pure DNA Kit

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 encounter 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.
- 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 in 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 one Ionic® FFPE to Pure RNA Kit and one Ionic® FFPE to Pure DNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
-20ºC Reagents Box	Lysis Buffer 1	Lysis Buffer 1	1.35 mL	12
-20ºC Reagents Box	Proteinase K	Proteinase K Reagent	810 µL	2
-20ºC Reagents Box	DNase I	DNase I Reagent	120 µL	1
-20ºC Reagents Box	RNase	RNase A Reagent	120 µL	1
-20ºC Reagents Box	Sample Buffer	Blank Sample Buffer	1.6 mL	2
RT Reagents Box	Lysis Buffer 2	Lysis Buffer 2	3.8 mL	2
RT Reagents Box	1 - Extraction Buffer	Extraction Buffer	12 mL	2
RT Reagents Box	2 - Anodic Buffer	Anodic Buffer	12 mL	2
RT Reagents Box	3 - Separation Buffer	Separation Buffer	18 mL	2
RT Reagents Box	4 - Neutralization Buffer	Neutralization Buffer	12 mL	2
RT Reagents Box	5 - Cathodic Buffer	Cathodic Buffer	12 mL	2
RT Reagents Box	Mineral Oil	Mineral Oil	15 mL	2
Fluidic Chip Set Box	Ionic Fluidic Chip	Fluidic Chips	N/A	12

Table 2: Reagents provided by the user.

User-supplied Reagents	For Lysate Preparation	For Purification
FFPE sections or scrolls	✓	

Table 3: Equipment provided by the user.

User-supplied Equipment (or equivalent)	For Lysate Preparation	For Purification
Razors/scalpels (if using slides)	✓	
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)	✓	✓
Optional: DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	✓	
Optional: DNA LoBind Plate, 96-well (Eppendorf 951032000)		✓

Protocol at a Glance

Lysate Preparation

	1. **Centrifuge FFPE tissue sample tubes for 2 minutes**
	2. Add 300 µL of mineral oil
	3. Prepare Lysis Mix 1 and add 325 µL to each sample
	4. Incubate samples on ThermoMixer. • 65°C for 5 mins at 1000 rpm • 60°C for 1 hour at 500 rpm • 70°C for 1 hour at 0 rpm
	5. Transfer 155 µL of lysate for RNA processing into a separate tube then proceed with RNA purification
	6. Incubate remaining lysate for DNA processing containing mineral oil on Thermomixer then proceed to DNA purification. • 70°C for 7 hours at 1000 rpm • Hold at 8°C

RNA Purification

	1. Prepare RNA Lysis Mix 2 and add 65 µL to each tube for RNA purification.
	2. Incubate on ThermoMixer • 20°C for 10 mins at 300 rpm
	3. Prepare Purification Buffer Reservoir
	3. Place Ionic Fluidic Chip onto instrument
	4. Load Purification Buffers and prime Ionic Fluidic Chip
	6. Add 200 µL of each lysate to the chip
	7. Start purification run (~70 minutes)
	8. Collect ~50 µL of each extract

DNA Purification

	1. Centrifuge tubes for DNA processing for 5 minutes and transfer lysates to new tubes
	2. Prepare DNA Lysis Mix 2 and add 65 µL to each tube
	3. Incubate on ThermoMixer • 20°C for 10 mins at 300 rpm
	4. Prepare Purification Buffer Reservoir
	5. Place Ionic Fluidic Chip onto instrument
	6. Load Purification Buffers and prime Ionic Fluidic Chip
	7. Add 200 µL of each lysate to the chip
	8. Start purification run (~70 minutes)
	9. Collect ~50 µL of each extract

Sample Requirements

This protocol is intended for the independent extraction and purification of RNA and DNA from up to eight Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples in parallel and is optimized to recover the maximum amount of RNA and DNA from a minimal amount of tissue. Bionano recommends a single 10 µm section of FFPE tissue (scroll or slide-mounted) with an area of 50–300 mm^2 for most downstream applications. For this protocol, a 10 µm section of FFPE tissue with dimensions of 1 inch x 1 inch (25.4 mm x 25.4 mm) and a mass of < 12 mg is typical.

The optimal amount of starting material will be informed by the FFPE tissue thickness, cross-sectional area, and cellularity. If a 10 µm section yields <1 µg of RNA or DNA with traditional kits (bead- or column-based), increasing the input FFPE tissue amount for RNA purification on the Ionic Purification System is acceptable. In such cases, this protocol can accommodate FFPE tissue sections totaling 20 µm in thickness (e.g., 2 x 10 µm or 4 x 5 µm). Care must be taken to avoid overloading since adding too much input FFPE tissue can lead to lower than expected RNA or DNA recovery (see **Figure 1**). A small pilot study is helpful in defining optimal input conditions as some FFPE tissue samples present challenges to extraction and purification requiring optimization beyond the standard protocol. Contact support@bionano.com for guidance on processing samples that do not meet the above requirements.

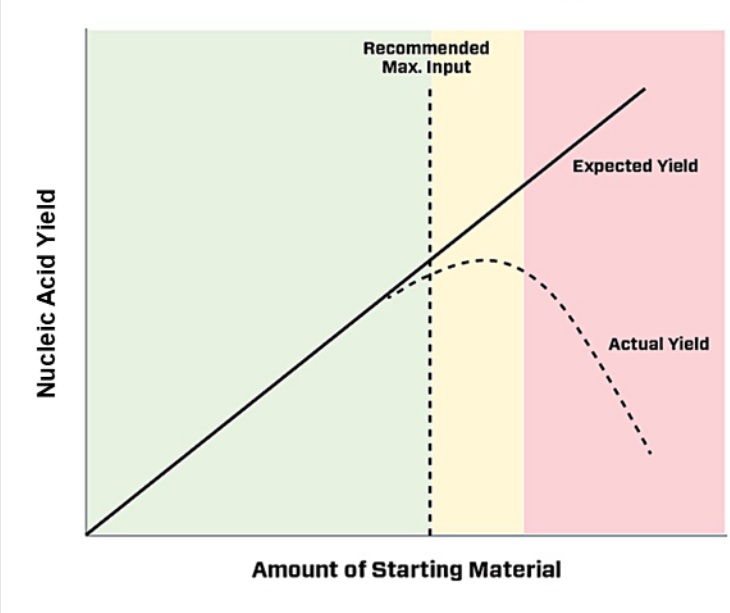

Figure 1. Impact of overloading

Lysis Procedure

Overview of the Approach

The Ionic FFPE Complete Kit provides DNA and RNA from the same starting FFPE sample by splitting the sample after lysis into two tubes, digesting the non-target nucleic acid, and then purifying the lysates on the Ionic Purification System. A summary of this procedure is shown in **Figure 2**.

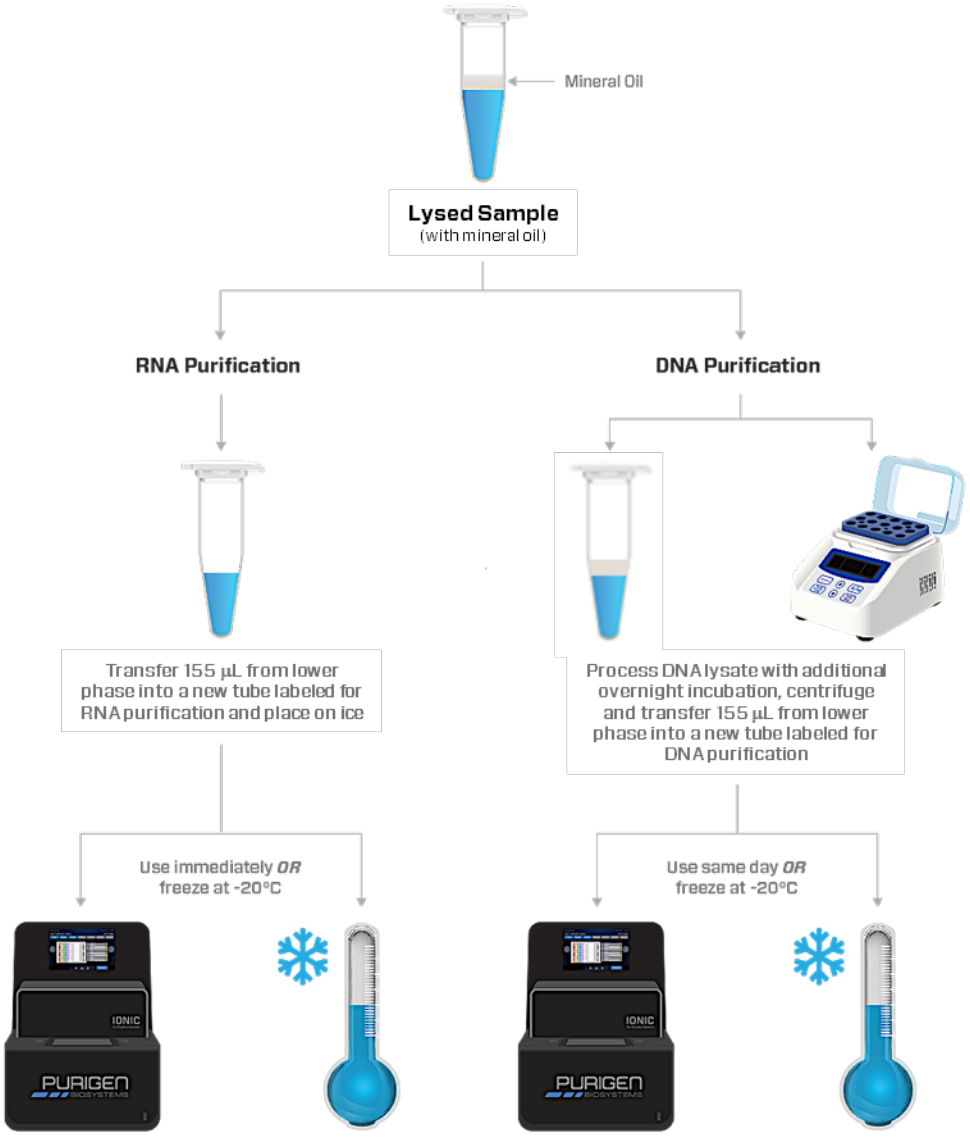

Figure 2. Overview of purification approach and pause points for the FFPE Complete Kit

Preparation of Lysates

1. Transfer each FFPE tissue section into a 1.5 mL LoBind Eppendorf tube.
2. Centrifuge tubes containing FFPE tissue sections at maximum speed (>10,000x g) for 2 minutes to move tissue to the bottom of the tube. Hold at room temperature.
3. Place two tubes of Lysis Buffer 1 on the ThermoMixer and incubate at 56°C for 10 minutes at 1000 rpm. If a precipitate is present after incubation, place tube back on ThermoMixer until buffer is clear.
4. Vortex the Lysis Buffer 1 tubes for 3 seconds, pulse spin and immediately proceed to the next step. Hold at room temperature.
5. Remove Proteinase K from the freezer, flick three times and pulse spin. Hold on ice.
6. To prepare Lysis Mix 1, add 135 µL of Proteinase K directly to each Lysis Buffer 1 tube. Vortex for 3 seconds and pulse spin. Hold at room temperature.
7. Add 300 µL of mineral oil to each sample tube from Step 2.
8. Using a P200 pipette, add 325 µL of prepared Lysis Mix 1 to each sample tube with two 162.5 µL transfers. Change tips between each dispense.
   NOTE: If a precipitate is present in Lysis Mix 1, heat at 56°C until the solution is clear before adding to tubes. Do not vortex sample tube containing mineral oil and Lysis Mix 1.
9. Place sample tubes into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 65°C for 5 minutes at 1000 rpm.
   - Incubate at 60°C for 1 hour at 500 rpm.
   - Incubate at 70°C for 1 hour at 0 rpm.
10. After the program is completed, remove tubes from the ThermoMixer, pulse spin, and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the DNase treatment below.

    NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase.
11. With a P200 pipette set to 155 µL, gently pipette mix the lower nucleic acid-containing lysate 10 times.
12. Aspirate 155 µL of lysate and transfer to a new tube for RNA processing. Hold these RNA lysates on ice.
    NOTE: To preserve RNA integrity, proceed immediately to DNase treatment. Alternatively, store lysate tubes at -20°C for up to 7 days. Frozen lysates must be thawed on ice prior to proceeding to DNase treatment.
13. Hold tubes containing mineral oil and the remaining lysate from Step 11 at room temperature for DNA processing. Proceed with the Processing DNA Lysates section below immediately after the RNA lysates have completed the DNase treatment and the ThermoMixer is available.

DNase Treatment of RNA Lysates

1. Remove DNase I from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare ‘RNA Lysis Mix 2’, combine 630 µL of Lysis Buffer 2 and 20 µL of DNase I in a 1.5 mL tube. Invert ‘Lysis Mix 2’ ten times to mix and pulse spin. Hold on ice.
3. Add 65 µL of ‘RNA Lysis Mix 2’ to each lysate tube for RNA processing from Step 12 of the Preparation of Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

Processing DNA Lysates

1. Place lysates for DNA processing from Step 13 of the Preparation of Lysates section above into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 70°C for 7 hours at 1000 rpm.
   - Hold at 8°C.
2. After the program is completed, remove tubes from ThermoMixer and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the RNase treatment below.

   NOTE: If mineral oil layer is solidified, place at 20°C or higher until mineral oil is no longer solid.
3. Centrifuge tubes at maximum speed (>10,000x g) for 5 minutes.

   NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase. If mineral oil layer is solidified, place at 20°C or higher until mineral oil is no longer solid.
4. Using a P200 pipette with the tip touching the bottom of the tube, slowly aspirate and transfer 155 µL of lysate from the lower phase of each tube into new microtubes avoiding any pelleted material that may be present. Hold lysate tubes on ice.

   NOTE: A minimal amount (~5-10 µL) of mineral oil may be aspirated during transfer of the lysate and does not impact the purification process.

   To preserve DNA integrity, proceed immediately to the next step. Alternatively, store lysate tubes at -20°C for up to 7 days. Frozen lysates should be thawed on ice prior to proceeding to RNase treatment.

RNase Treatment of DNA Lysates

1. Remove RNase from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare DNA Lysis Mix 2, combine 630 µL of Lysis Buffer 2 and 20 µL of RNase in a 1.5 mL tube. Invert Lysis Mix 2 ten times to mix and pulse spin. Hold on ice.
3. Add 65 µL of DNA Lysis Mix 2 to each lysate tube for DNA processing from Step 4 of the Processing DNA Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

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.

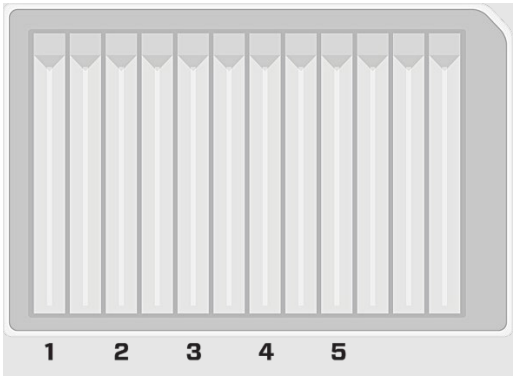

Figure 2. Reservoir column labels

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 FFPE Complete** button. The instrument cover will open.
3. When prompted, remove a fluidic chip from its packaging, handling only by the side skirting, and place it 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 fluidic 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 fluidic chip.

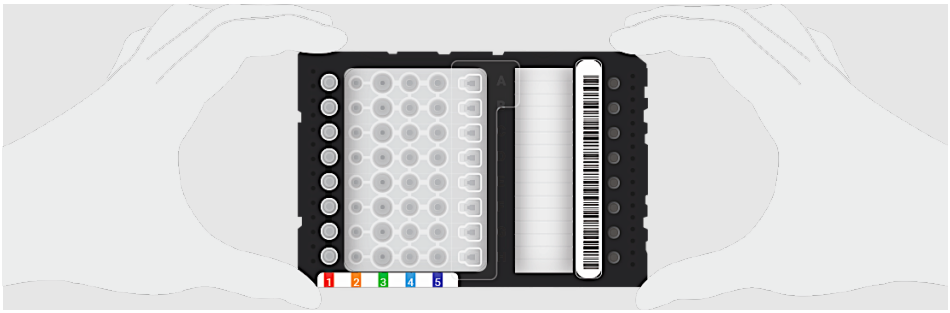

Figure 3. Proper handling and placement orientation of an Ionic fluidic chip.

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 fluidic chip: Include both the P/N and L/N for the chip:

     P/N: S0223 Ionic® Fluidic Chip
     L/N: P12345560001

   - Reagent Lot number is located on the G2 FFPE to RNA 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

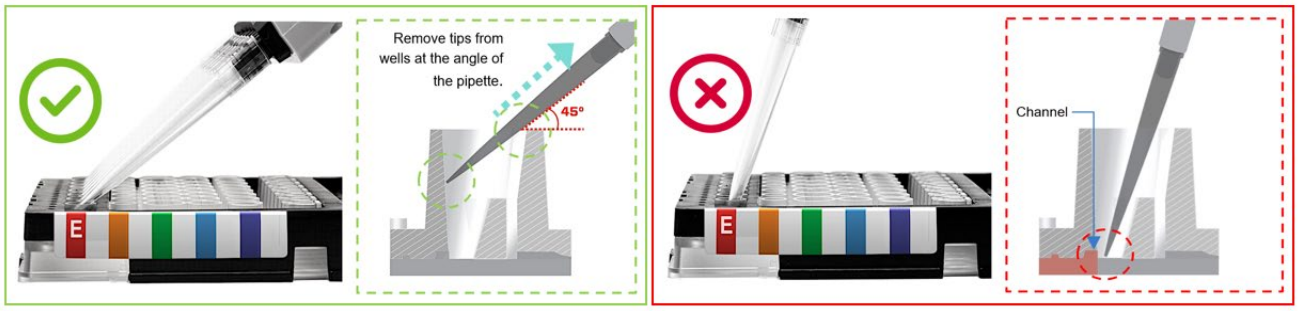

Figure 4. Proper (left) and improper (right) pipetting techniques for fluidic chip buffer wells.

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.

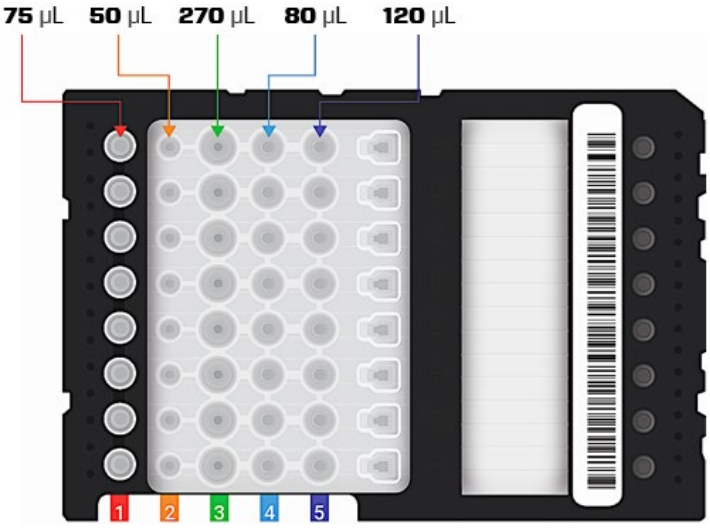

Figure 5. G2 FFPE To RNA purification buffer dispense volumes.

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.

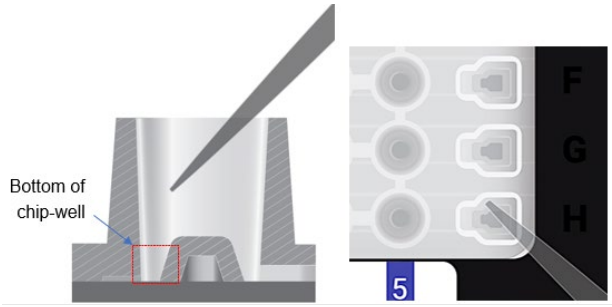

Figure 7. Proper Sample Well lysate loading positioning.

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

    NOTE: The G2 FFPE Complete purification run will proceed for approximately 70 minutes and the instrument will indicate when the run has completed.

    NOTE: Upon completion, extracts should be collected within one hour.

Collect RNA or 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 RNA 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.

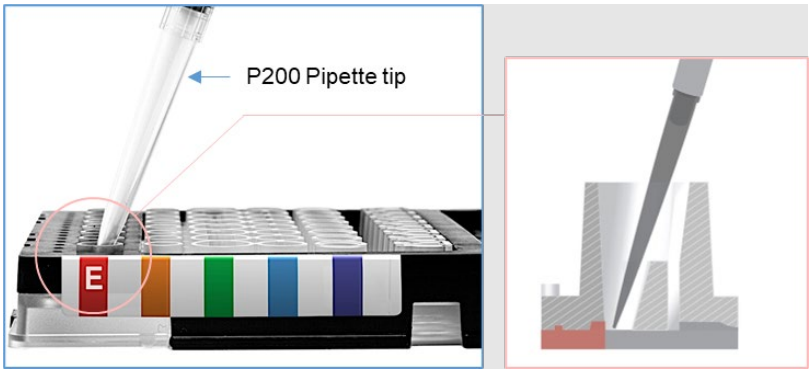

Figure 7. Aspirating the RNA extract from the Extraction Well

3. Using a P20 pipette set to 10 µL, aspirate any remaining RNA or 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**.

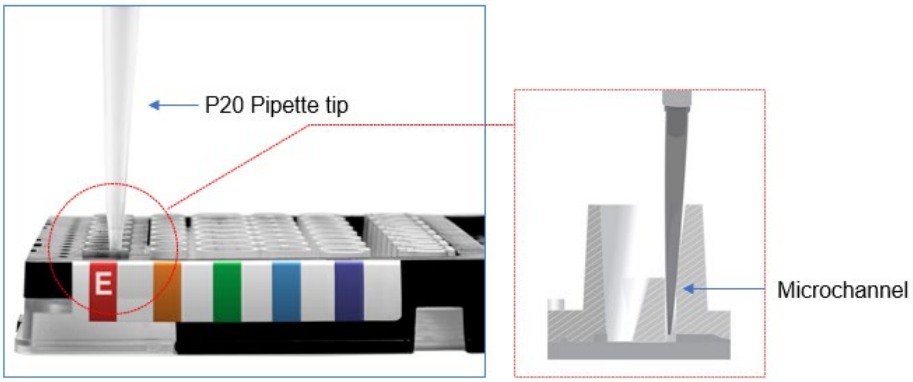

Figure 8. Recovery of any remaining RNA from the Extraction Well microchannel

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 RNA or DNA extracts and pulse spin before using in downstream assays.

   NOTES: Store RNA extracts on ice for same-day use and -80°C for long term storage.

   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 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 RNA or 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**.
3. Ensure a tight seal for each well.

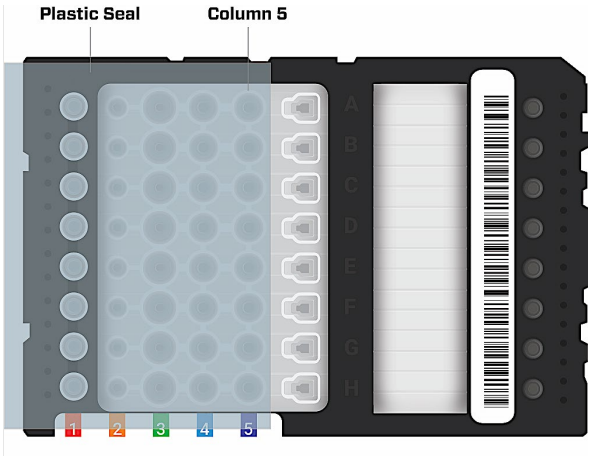

Figure 9. Proper plastic seal application for sample recovery

4. 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.
   - Avoid Aspiration of fluid from the channel between the Cathodic Buffer (5) and the Sample Well.
   - 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.

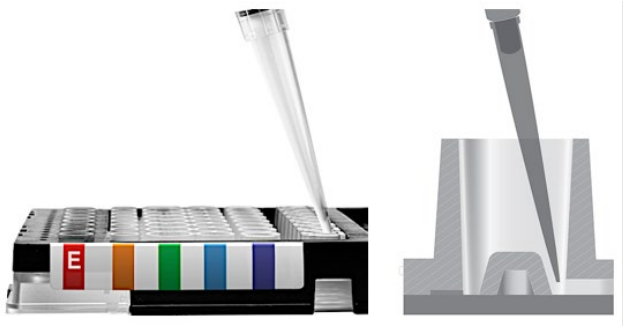

Figure 10. Sample recovery technique

5. Add a sufficient volume of FFPE Sample Buffer to the sample to bring the total volume to ~210 µL.
6. 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
Email	support@bionano.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663
Website	www.bionano.com/support
Address	Bionano Genomics, Inc. 9540 Towne Centre Drive, Suite 100 San Diego, CA 92121