

USER GUIDE

Mondrian™ SP+ Cartridge

FOR USE WITH PART NOS. 8100, 8133, 8134, 8151, AND 8152

Developed and Manufactured for NuGEN by Advanced Liquid Logic

Patents, Licensing and Trademarks

©2013 NuGEN Technologies, Inc. All rights reserved. The Encore®, Ovation® and Applause® families of products and methods of their use are covered by several issued U.S. and International patents and pending applications (www.nugeninc.com). NuGEN, Ovation, SPIA, Ribo-SPIA, Applause, Encore, Prelude, Mondrian and Imagine More From Less are trademarks or registered trademarks of NuGEN Technologies, Inc. Other marks appearing in these materials are marks of their respective owners.

The Mondrian™ SP+ is covered by certain patents and trade secrets of Advanced Liquid Logic, Inc. licensed to NuGEN, Inc. Sale of the Mondrian SP+ Cartridge to NuGEN, Inc. customers comes with a limited research use only sublicense to use the Mondrian SP+ Cartridge for preparing a nucleic acid sample for analysis on a separate system by sequencing, microarray or real-time PCR. The sublicense specifically excludes the right to use the Mondrian SP+ Cartridge for biothreat detection, forensic testing, production animal health testing, and food safety testing, except for genomics research in these fields, and for human and veterinary diagnostics.

The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to modify, reverse engineer, resell, repackage or further sublicense) under these patent applications and any patents issuing from these patent applications to use this product and methods, accompanying this user guide, for research and development purposes solely in accordance with the intended use described and the written instructions provided in this user guide. No license to make or sell products by use of this product is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include or carry any right or license to use, develop or otherwise exploit this product commercially and no rights are conveyed to the buyer to use the product or components of the product for purposes including commercial services or clinical diagnostics.

Warranty

NuGEN warrants that this product meets the performance standards described in the Company's product and technical literature for a period of six months from the date of purchase, provided that the product is handled and stored according to published instructions, and that the product is not altered or misused. If the product fails to meet these performance standards, NuGEN will replace the product free of charge or issue a credit for the purchase price.

NuGEN's liability under this warranty shall not exceed the purchase price of the product. NuGEN shall assume no liability for direct, indirect, consequential or incidental damages arising from the use, results of use or inability to use its products. NuGEN reserves the right to change, alter or modify any product to enhance its performance and design.

NuGEN's products are developed, designed and sold FOR RESEARCH USE ONLY. This product is not to be used for diagnostic or therapeutic purposes, nor is it to be administered to humans or animals. Except as expressly set forth herein, no right to modify, reverse engineer, distribute, offer to sell or sell NuGEN's product is conveyed or implied by buyer's purchase of this NuGEN product. The buyer agrees to use NuGEN products accompanying the product insert in accordance with the intended use and the written instructions provided.

Table of Contents

Contents

I. Introduction	1
A. Background.....	1
B. Construction.....	1
C. Cartridges	2
II. Kit Components	3
III. Operation	4
A. Powering up the Mondrian SP+ Workstation.....	4
B. Filling the Cartridge	4
C. Cartridge Insertion	8
D. Cartridge Quality Control Check	11
E. Loading Samples and Reagents.....	14
F. Collection of Finished Product.....	15
IV. Technical Support	16
V. Appendix	17
A. Cleaning the Workstation Contact Pins	17
B. FAQs	17

I. Introduction

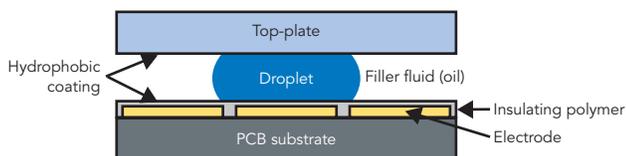
A. Background

The Mondrian™ SP+ Cartridge is used with the Mondrian SP+ System and offers a convenient 8-sample batch size, simple reagent loading and easy sample recovery. Reagents are contained in discrete droplets encased within filler fluid, isolating the reactions from the lab environment and carryover contamination.

B. Construction

When prepared for use, cartridges consist of an oil layer (Filler Fluid) sandwiched between a PCB substrate and a clear top plate. The PCB substrate is patterned with insulated electrodes. By changing the relative voltages of the patterned electrodes, aqueous droplets are manipulated to perform complex assays. Droplets are dispensed from loading ports, transported to various locations on the cartridge, mixed, incubated and collected using only software control. Figure 1 shows a cutaway side view schematic of a cartridge.

Figure 1. Cartridge cutaway side view.



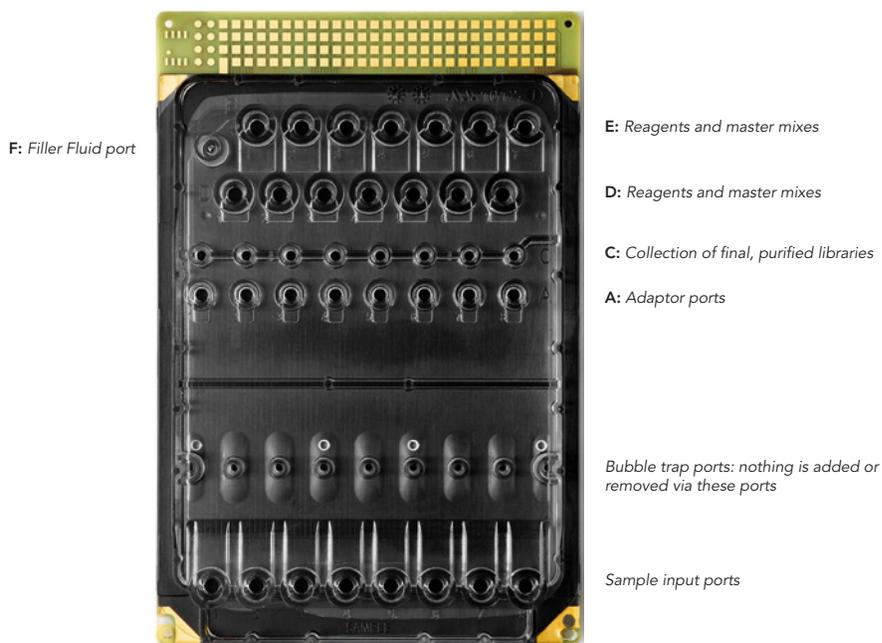
I. Introduction

C. Cartridges

Cartridges are unpacked, filled with Filler Fluid, reagents and samples, and then inserted into the deck of the workstation, where a run is initiated through the touch screen interface. Cartridges are one-time-use only. Cartridge re-use is not supported, even if a run is canceled prior to completion.

To ensure proper protocol performance, the cartridge loading procedure must be strictly followed. Consult this Mondrian SP+ Cartridge user guide and the appropriate NuGEN SP+ Library Systems user guide for the proper cartridge loading and setup procedure to achieve optimum results.

Figure 2. Top view of a Mondrian SP+ Cartridge. Sample ports along the bottom edge are labeled S1–8. Other reagent port rows are labeled A–E. Note that there is no row B. There are eight ports in rows A and C, and seven in rows D and E. The port labeled “F” is sized for a standard Luer-lock connector, through which the Filler Fluid is added to the cartridge. The cartridge electrodes are visible as rows of small gold squares at the top of the cartridge.



II. Kit Components

The Mondrian SP+ Cartridge is shipped as part of a NuGEN SP+ Library System, such as the Ovation® SP+ Ultralow Library Systems (Part Nos. 8133 and 8134) or the Encore® SP+ Complete Library Systems (Part Nos. 8151 and 8152). These kits contain all of the necessary reagents for complex sample preparation protocols on the Mondrian SP+ Workstation. Each kit also contains protocol-specific cartridge loading guides, which can be overlaid on the cartridge to help direct the addition of reagents and samples.

III. Operation

A. Powering up the Mondrian SP+ Workstation

1. Locate the instrument ON/OFF switch at the back of the instrument and turn ON.
2. Press the 'On' button (pictured below) on the front of the instrument to power up the workstation.



B. Filling the Cartridge

SP+ Cartridges must be filled with the provided Filler Fluid prior to loading with reagents. This process can be carried out on the bench top or the cartridge can be inserted into the Mondrian SP+ Workstation prior to adding filler fluid, reagents and samples.

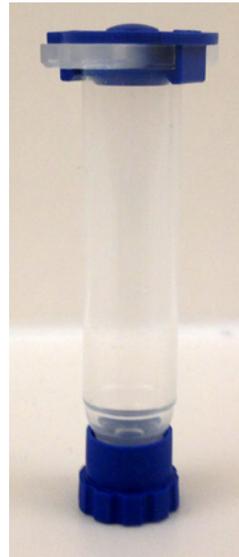
Important: Take care when removing the cartridge from the packaging. Handle the cartridge by the sides and the non-electrode end. Do not handle the cartridge by the electrodes.

1. Use scissors to cut open the Mondrian SP+ Cartridge pouch. Remove the Mondrian SP+ Cartridge.
Note: Do **not** attempt to tear open the pouch as this may damage the SP+ Cartridge.
2. Lay the cartridge on a level bench top and place the appropriate SP+ Library Systems Cartridge Loading Guide on the cartridge.
3. Remove the empty Filler Fluid tube and the SP+ Filler Fluid bottle from the NuGEN SP+ Library System kit and set both items on the bench top.

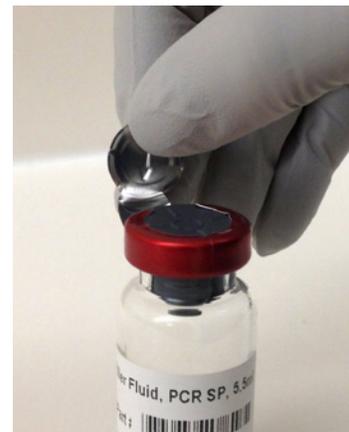
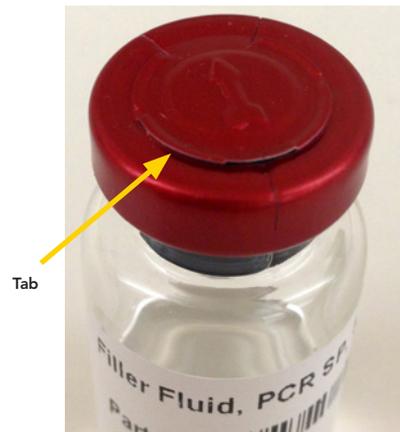


The SP+ Filler Fluid has been degassed and packaged in a sealed, single-use vial. Do not open the vial until ready for use.

III. Operation

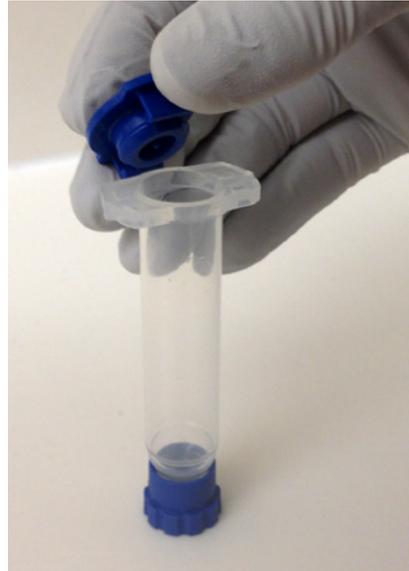


4. Carefully grasp the tab on the red aluminium seal of the SP+ Filler Fluid bottle and pull up and back to break the seal, as shown below.

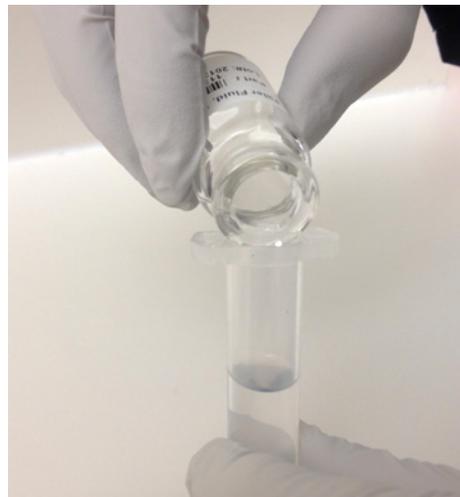


5. Pull the tab until it breaks completely and remove the aluminium seal from the bottle. Leave the grey rubber stopper in place.
6. Remove the top cover from the empty Filler Fluid tube, leaving it capped at the bottom. Set the cap aside. Important: **Do not discard the top cover.**

III. Operation



7. Remove the rubber stopper from the bottle of SP+ Filler Fluid.
8. Making sure the Filler Fluid tube is capped at the bottom, slowly pour the SP+ Filler Fluid into the empty Filler Fluid tube. Be careful not to pour too quickly as this will introduce bubbles into the fluid. Air bubbles entering the cartridge may interfere with droplet movement.



9. Once the entire aliquot of SP+ Filler Fluid is added, recap the Filler Fluid tube.
10. Invert the Filler Fluid tube and gently twist off the bottom cap of the Filler Fluid tube, exposing the Luer-lock tip, as shown below.

III. Operation

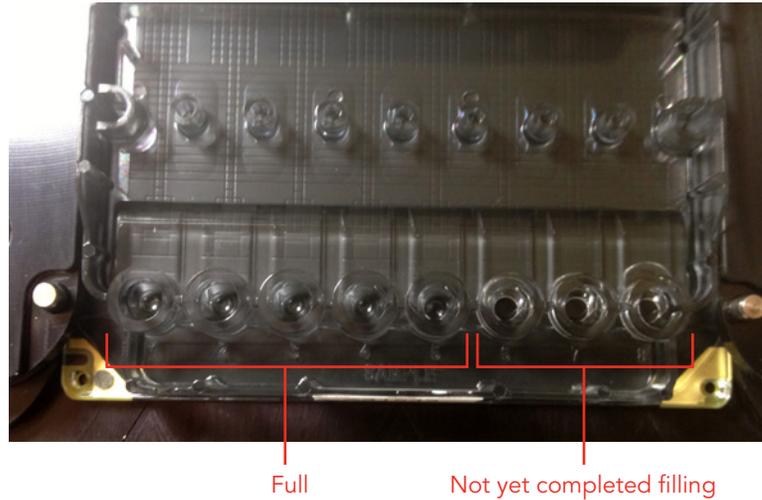


11. Insert the tip of the Filler Fluid tube into the port marked "Filler Fluid" on the SP+ Cartridge while gently twisting the tube in a clockwise direction to seat the tip of the Filler Fluid tube into the cartridge port. Remove the top cover from the Filler Fluid tube, allowing the entire volume of Filler Fluid to flow into the cartridge. This process will take approximately 3–5 minutes.



During the cartridge filling process, you will begin to see the filler fluid rising in the sample and reagent ports. You should see filler fluid in all sample and reagent ports by the end of the filling process.

III. Operation



12. Once the entire volume of Filler Fluid has been delivered, carefully remove the Filler Fluid tube from the SP+ Cartridge.
13. Inspect the cartridge to make certain that Filler Fluid has risen into each of the sample and reagent ports. Occasionally some of the ports may fail to properly fill due to surface tension. If this is the case, the cartridge may be tilted slightly to encourage the Filler Fluid to enter the ports. Alternatively, a pipet tip may be used to break the surface tension in affected ports.

C. Cartridge Insertion

1. Raise the lid of the Mondrian SP+ Workstation to reveal the cartridge deck.



III. Operation

2. With the cartridge lever in the up position, as shown above, hold the cartridge level by the plastic top cover with ports facing up and the gold colored electrodes pointing towards the workstation. Carefully insert the cartridge into the cartridge deck flush with the surface of the deck, allowing the side rails to guide it into place.

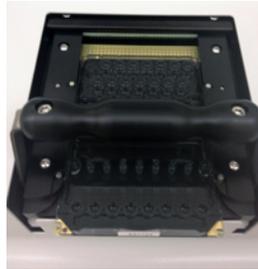


3. If you encounter any resistance to the cartridge moving into the deck, inspect for foreign objects that may be impeding the cartridge and remove them. If the cartridge appears to be catching on the edge of the heater bars or Peltier cooling plate, you may gently press down on them to allow the cartridge to move forward. The heater bars and cooling unit are designed to be slightly flexible in order to engage the cartridge. However, do not press too hard on the heater bars or Peltier unit as they may become misaligned.
4. Confirm that the cartridge is fully inserted into the deck. When the cartridge is properly inserted, the electrodes are no longer visible (Figure 3) and the three guide arrows etched into the front of the deck should be flush with the edge of the cartridge (Figure 4).

III. Operation

Figure 3. Inserting the Mondrian SP+ Cartridge. A) shows a cartridge improperly inserted into the workstation. Note the visible electrodes. B) shows a cartridge correctly inserted.

A

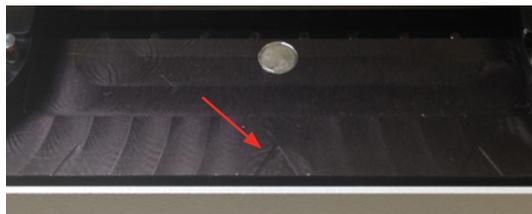


B

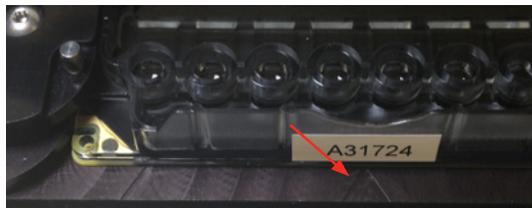


Figure 4. A close up view of the cartridge alignment marks etched into the deck of the Mondrian SP+ Workstation. A) no cartridge is inserted. The alignment marks are clearly visible. B) the cartridge is correctly inserted. The points of the guide arrows align with the edge of the cartridge.

A



B



5. Pull the locking lever forward as illustrated below to engage the control electronics and lock the cartridge into place.

III. Operation



Note: Once a run has started, do not move the locking lever as this will result in an unrecoverable interruption of the running protocol. The Mondrian SP+ Workstation will not run with the lever in the up position.

D. Cartridge Quality Control Check

The Mondrian SP+ Cartridge QC protocol allows confirmation of the basic functionality of the Mondrian SP+ Cartridge prior to use. We highly recommend running this protocol with each cartridge prior to adding samples and reagents.

The Cartridge QC loading process can be carried out on the bench top or the cartridge can be inserted into the Mondrian SP+ Workstation prior to adding filler fluid and elution buffer.

1. Place the Cartridge Loading Guide, provided with each Mondrian SP+ Cartridge, on the cartridge.

Note: It is possible to place the loading guide on the cartridge in only one orientation.

2. Remove the Elution Buffer reagent tube from the room temperature storage components of a NuGEN SP+ Library System kit.
3. Load 50 μ L of Elution Buffer into port E5 of the Mondrian SP+ Cartridge. Insert the pipette tip into the port all the way to the bottom of the cartridge. If the tip contacts the bottom, withdraw the pipette tip slightly to allow space for dispensing. Slowly depress the plunger to dispense the reagent, but do not depress the plunger completely (blow out) as this could introduce bubbles into the cartridge.

Important: Do NOT add any samples or other reagents to the cartridge at this time. Ensure that only Elution Buffer has been loaded.

III. Operation

4. If the cartridge is not already inserted into the Mondrian SP+ Workstation, carefully transport the cartridge to the Mondrian SP+ Workstation and insert the cartridge into the deck.
5. Pull the cartridge lever of the Mondrian SP+ Workstation forward to the locked position and close the lid of the workstation.
6. Select 'Run' on the touch screen menu, choose the Mondrian SP+ Cartridge QC protocol from the list of protocols and then select 'Next' to proceed to the Protocol Information screen.
7. Select 'Next' to proceed to the Run Information screen.
8. **Optional:** Enter run details in the Run Information screen. We highly recommend recording the serial number of the cartridge in the Run Information screen. The cartridge serial number can be found on the front of each cartridge for easy reference.
9. Select 'Next' and then select 'Start Run'.

The Mondrian SP+ Cartridge QC protocol will take about 15 minutes to complete. During this test, Elution Buffer droplets will be dispensed from the E5 port and transported around the cartridge prior to being returned to the port. The purpose of this test is to confirm the basic performance of the cartridge.

III. Operation

At the end of the protocol, the instrument will display the Run Complete screen and one of the following messages:

MESSAGE	MEANING	NEXT STEP
Mondrian™ SP+ Cartridge passed. Continue to intended protocol.	No errors were detected. Droplet transport was normal. Cartridge is ready to run a protocol	Press "OK" on the Run Complete screen to return to the main menu. Proceed to section D, "Loading Samples and Reagents" in this user guide or follow the instructions in the appropriate NuGEN SP+ Reagent System.
Mondrian™ SP+ Cartridge failed. Remove cartridge from instrument deck and set aside prior to contacting NuGEN Technical Support.	A problem was detected with droplet transport within the cartridge.	<p>Press 'OK' on the Run Complete screen to return to the main menu. Remove the cartridge from the deck and set it aside (do not discard). Clean the contact pins on the workstation following the protocol in Appendix C: Cleaning the Workstation Contact Pins.</p> <p>Repeat the Cartridge QC protocol from step 4 (above) one more time. Do not add any additional Filler Fluid or Elution Buffer to the cartridge.</p> <p>If the message after the second Mondrian SP+ Cartridge QC protocol is:</p> <ul style="list-style-type: none"> • 'Mondrian SP+ cartridge passed,' proceed as described for passing cartridges (above). • 'Mondrian SP+ Cartridge failed' or 'Mondrian SP+ cartridge status is undetermined,' do not use the cartridge. Contact NuGEN Technical Support.
Mondrian SP+ Cartridge status is undetermined. Please consult the Mondrian SP+ Cartridge user guide or appropriate NuGEN SP+ Library Systems user guide for further instructions.	The results are inconclusive.	<p>Press 'OK' on the Run Complete screen to return to the main menu. Remove the cartridge from the deck and set it aside (do not discard). Clean the contact pins on the workstation following the protocol in Appendix C: Cleaning the Workstation Contact Pins.</p> <p>Repeat the Cartridge QC protocol from step 4 (above) one more time. Do not add any additional Filler Fluid or Elution Buffer to the cartridge.</p> <p>If the message after the second Mondrian SP+ Cartridge QC protocol is:</p> <ul style="list-style-type: none"> • 'Mondrian SP+ cartridge passed,' proceed as described for passing cartridges (above). • 'Mondrian SP+ Cartridge failed' or 'Mondrian SP+ cartridge status is undetermined,' do not use the cartridge. Contact NuGEN Technical Support.

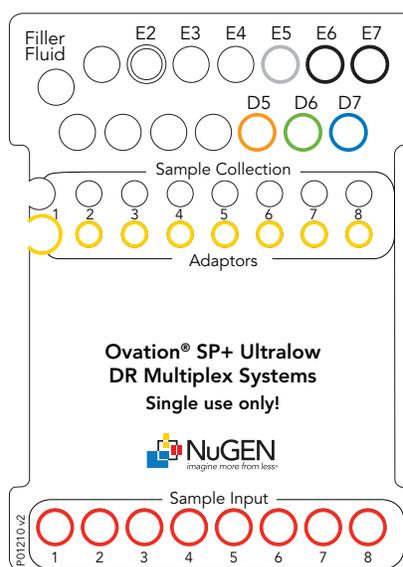
III. Operation

E. Loading Samples and Reagents

Protocols for Mondrian SP+ products can be found in the product specific user guides which can be downloaded from www.nugeninc.com/mondriansupport. Many SP+ sample preparation protocols may appear to be similar, so make certain you are working from the appropriate user guide.

Use the product-specific SP+ cartridge loading guides to aid in proper placement of reagents and samples (Figure 5).

Figure 5. An example cartridge loading guide.



Follow the recommendations below to load reagents into their appropriate cartridge ports.

- Use a 10 or 20 μL pipette to add all reagent master mixes whose volume is less than 10 μL as well as the adaptors.

Important: It is critical that a 10 μL pipette be used for adding DR Multiplex Adaptor Ligation Mixes. Do not use 2 μL pipettes to add reagents to the cartridge as they will not generate sufficient force to expel the reagents from the pipette tip into the filler fluid-containing cartridge.

- Use a 100 or 200 μL pipette for adding any reagent of a volume greater than 20 μL .

III. Operation

- Pipet samples and reagents carefully to avoid overflow of the Filler Fluid from the port.
- When adding samples and reagents, insert the pipette tip all the way to the bottom of the cartridge. If the tip contacts the bottom, withdraw the pipette tip slightly to allow space for dispensing. Slowly depress the plunger to dispense the reagent, but do not depress the plunger completely (blow out) as this could introduce bubbles into the cartridge.

Refer to the Mondrian SP+ Workstation User Manual and the appropriate NuGEN SP+ Library System user guide for guidance on protocol selection and starting the sample preparation run.

F. Collection of Finished Product

Note: A short instructional video on 'Mondrian Sample Collection' is available on the Mondrian Support page at www.nugeninc.com/mondriansupport.

1. Once the protocol run has completed, raise the lid and prepare all necessary materials for sample collection from the cartridge as directed in the appropriate NuGEN SP+ Library System user guide.
2. Use a 100 or 200 μL pipette and set it to a volume of 20 μL . Depress the plunger on the pipette and insert the tip all the way to the bottom of the sample collection port, perpendicular to the cartridge so as to make a seal between the cartridge and the pipet tip.
3. Maintaining the seal formed between the pipette tip and the bottom of the cartridge, release the plunger. Immediately lift the pipette slightly off the bottom of the cartridge to release the seal, rapidly drawing Filler Fluid and the sample droplet into the pipette tip.
4. Examine the pipette tip to ensure that the appropriately sized droplet is suspended in the Filler Fluid in the pipette tip.
5. Dispense the collected sample into the sample vessel as directed in the user guide for your particular protocol. If no sample droplet is observed, dispense the contents of the pipette tip into the sample collection vessel and repeat the sample collection procedure for the port.

Note: The droplet occasionally breaks into pieces and becomes more difficult to observe. The excess Filler Fluid collected during the procedure will not interfere with downstream molecular processes.

6. Remove the cartridge from the workstation and dispose of in accordance with local regulations for laboratory waste.

IV. Technical Support

For help with any of our products, please contact NuGEN Technical Support at 650.590.3674 (direct) or 888.654.6544, option 2 (toll-free, U.S. only). You may also send faxes to 888.296.6544 (toll-free) or email techserv@nugeninc.com.

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216 (Fax) or email europe@nugeninc.com.

In all other locations, contact your NuGEN distributor for technical support.

V. Appendix

A. Cleaning the Workstation Contact Pins

The pins on the workstation/cartridge interface may become dirty and cause performance issues. If this happens, you will need to clean the interface pins.

Materials

- Lint-free wipes such as Kimwipes or Super PolX 1200 Wipers. Do not use cotton or any material that may leave particles behind
- Isopropyl alcohol
- Canned air

Procedure

1. Turn the workstation off and unplug from the power source.
2. Soak the wipe in isopropyl alcohol.
3. Firmly rub all pins with the wipe.
4. Wait 2 minutes.
5. Blow the area dry with canned air.
6. Plug the workstation back in and turn it on.

Note: We recommend cleaning the pins once a week, after any prolonged storage of the workstation, after a Filler Fluid spill, or if the Mondrian SP+ Cartridge QC protocol returns a 'Mondrian SP+ Cartridge Failed' or a 'Mondrian SP+ Cartridge Status Undetermined' message.

B. FAQs

Q1. Can the Mondrian SP+ Cartridges be used with the Mondrian SP Workstation?

No. The Mondrian SP+ Cartridges can be run only on the Mondrian SP+ Workstation. Running an SP+ Cartridge on an SP Workstation will result in a failed experiment and loss of samples.

Q2. Can the Mondrian SP Cartridges be used with the Mondrian SP+ Workstation?

Yes. The Mondrian SP+ Workstation is fully backward-compatible and will run all NuGEN SP protocols.

Q3. Does NuGEN sell the Mondrian SP+ Cartridges as a stand-alone?

No. Mondrian SP+ Cartridges can be purchased only as part of a NuGEN SP+ Library System.

V. Appendix

Q4. I use the Library Prep Method 1 protocol on the SP Workstation and I want to upgrade to the SP+ Workstation. Can I continue to use this protocol?

Yes. The Mondrian SP+ Workstation supports the Library Prep Method 1 protocol on Mondrian SP Universal Cartridges.

Please visit www.nugeninc.com/mondriansupport for more information and to download the correct protocol file for the Mondrian SP+ Workstation.

NuGEN Technologies, Inc.

Headquarters USA

201 Industrial Road, Suite 310
San Carlos, CA 94070 USA
Toll Free Tel: 888.654.6544
Toll Free Fax: 888.296.6544
custserv@nugeninc.com
techserv@nugeninc.com

Europe

P.O. Box 109
9350 AC Leek
The Netherlands
Tel: +31-13-5780215
Fax: +31-13-5780216
europe@nugeninc.com

For our international distributors contact information, visit our website

www.nugeninc.com



©2013 NuGEN Technologies, Inc. All rights reserved. The Encore®, Ovation® and Applause® families of products and methods of their use are covered by several issued U.S. and International patents and pending applications (www.nugeninc.com). NuGEN, Ovation, SPIA, Ribo-SPIA, Applause, Encore, Prelude, Mondrian and Imagine More From Less are trademarks or registered trademarks of NuGEN Technologies, Inc. Other marks appearing in these materials are marks of their respective owners.

For research use only.

M01344 v1