

Quick Start Guide

nCounter[®] Pro System

The nCounter Pro Analysis System rapidly immobilizes and counts samples which have hybridized to nCounter barcodes. This document provides a concise, picture-guided work flow for processing samples and scanning cartridges through an nCounter Pro system after overnight hybridization. Additional information may be required and can be found by consulting the nCounter Pro Analysis System User Manual, or by contacting support@nanosttring.com.

1 Prepare Consumables on the Prep Station

1. Remove one blank sample cartridge (figure A) from -20°C storage. Allow it to warm to room temperature (**~20 minutes**), then remove it from the foil package.
2. Remove two prep plates (figure B) from 4°C storage. Centrifuge at **2000g** for **2 minutes**, allow the plates to warm to room temperature (**~20 minutes**), and confirm the presence of magnetic beads in only the last row of the plate (row H).



figure A



figure B

2 Load Consumables on the Prep Station

1. Place the prep plates on the deck (figure C) with green labels forward, discarding the lids.
2. Load the tips (figure D). The metal rack can be removed from the prep station for easier loading.
3. Place the tip sheaths in the slots located in front of the tip rack (figure E).
4. Remove the foil from the cartridge.

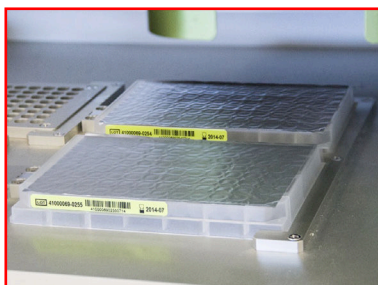


figure C

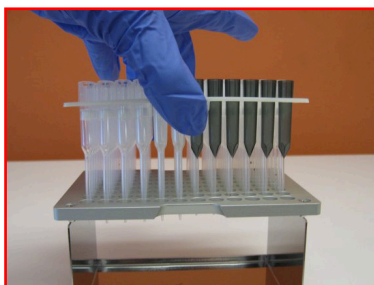


figure D

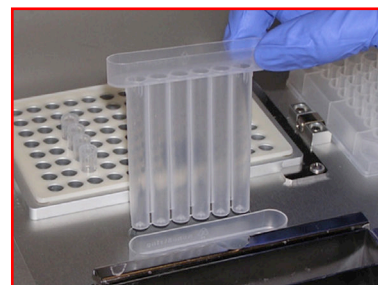


figure E

3

Load Consumables on the Prep Station (Continued)

- Place the cartridge under the electrode fixture (figure F). The notch in the cartridge should be in the back left and the barcode sticker should be on the right (figure G). A properly aligned cartridge will be tilted with the right side higher than the left; the right side should be able to bounce up and down with light pressure from your index finger.
- Lower the electrode fixture to insert the electrodes into the cartridge (figure G).
- Place empty NanoString-provided strip tubes into the heater block (figure H).
- Uncap the sample strip tubes and place them into the metal tube holder, aligning the notches of the strip tubes with the metal pegs (figure H).
- Close the heater lid (figure H).
- Click **Start Processing** to initiate the run.

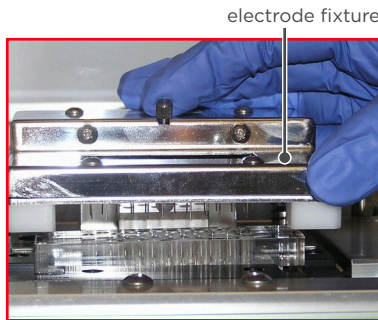


figure F

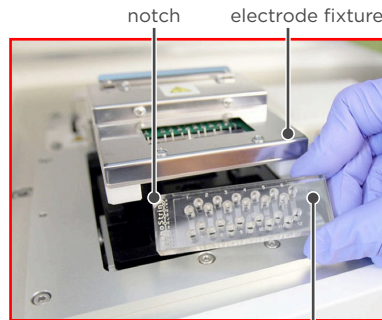


figure G

barcode sticker

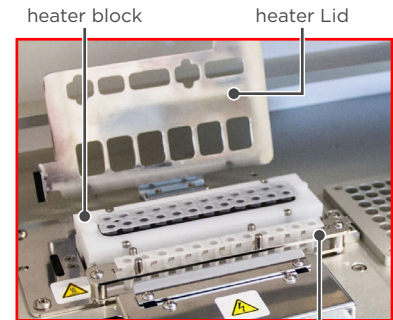


figure H

metal tube holder

4

Validate Deck Layout on the Prep Station

The Prep station will automatically check to confirm that consumables are placed correctly on the deck. Wait approximately **10 minutes** and ensure that the 'System Processing' message appears, indicating that validation is complete and the run is starting. If an error occurs, an error message will appear, providing a toll-free support number to call with questions.

5

Cover Cartridge after Run on the Prep Station

Upon completion of the run, use the clear adhesive film supplied in the Prep Pack to seal the holes on the top of the sample cartridge. Do not leave processed cartridges uncovered overnight, as evaporation of liquid in the wells results in data loss. Cartridges can be scanned immediately, or stored in the dark at **4°C** for up to **1 week**.

6

Upload RLF to the Digital Analyzer

- Retrieve the green NanoString USB (figure I). Every CodeSet from NanoString is shipped with an RLF (Reporter Library File) which can be found on the green NanoString USB inside the -80°C CodeSet box. The RLF contains barcode definitions for the group of probes which comprise the CodeSet.
- Upload the RLF. Insert the USB into the port off the right side of the Digital Analyzer touch screen, and follow the menu commands from the Main Menu: **Upload Files > upload RLF**.

NanoString recommends uploading the RLF from each new shipment of CodeSet to ensure that the most up to date version is used.



figure I

7 Initiate Scan on the Digital Analyzer

1. Click **Start Counting** on the Main Menu and select a stage position on the touch screen.
2. Insert the cartridge in the corresponding stage position while holding it lengthwise. Close the magnetic clip and push down to ensure that the cartridge is flat (figure J).
3. Under **Cartridge Definition Mode**, select **Create New**. This option is for use with generic sample names (which can then be properly annotated during data analysis). If custom sample names are to be used, refer to the Creating a CDF section of the nCounter Analysis System User Manual.
4. Enter a **Cartridge ID** (figure K). This will be automatically prepended by a date stamp.
5. Select **FOV count** for scanning (figure K). Higher FOV settings will result in longer scan times and generally generate less noisy data. For example, at High (280 FOV), the scan will typically take ~2½ hours and at Max (555 FOV), it will typically take ~5 hours.
6. Click the **Samples** button and select all sample tubes.
7. Enter a generic **Sample ID** (figure L) to be used with all samples (individual samples will be differentiated by lane numbers).
8. Click **Library RLF** (figure L) and select the Reporter Library File which corresponds with the CodeSet being run.
9. Click **Done** (figure L) to return to the Cartridge information screen, then **Done** again to return to the Select Stage Position Screen. Click **Done** a third time if no additional scans will be programmed.
10. Click **Start**.

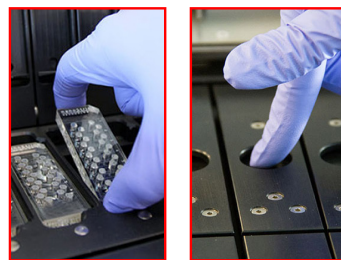


figure J

figure K

figure L

#9

8 Download Data from the Digital Analyzer

1. Insert a USB drive into the Digital Analyzer USB Port.
2. Once the scan has completed, click the **Download Data** button (figure M) from the touch screen. This button is also available on the Main Menu.
3. Select the file corresponding to the Cartridge ID, and click **Download Data** (figure N).

If data has already been downloaded or emailed from the scanner, it will be moved into the older data folder on the scanner. To access this data, select the older data check box.

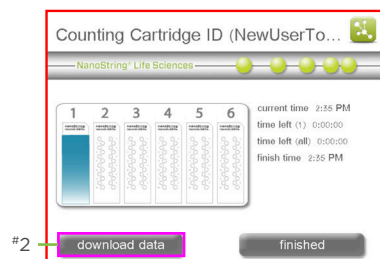


figure M

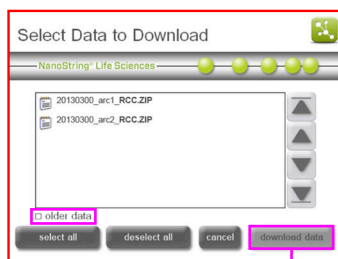


figure N

#3

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Additional Customer Resources

For more comprehensive information, visit us at nanosttring.com and go to **Support > Product Support** to view manuals and other technical product literature.

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