nCounter®
Prep Station

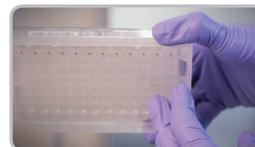## Setting Up an nCounter Hybridization Reaction

1   **Warm sealed cartridge to room temperature <u>before</u> opening.**
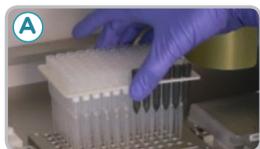
2   **Centrifuge reagent plates.**
- 2000 x g for 2 minutes.
- Check for dark pellets in row H.

3   **Configure deck.**

(A)   **Cautiously load tips** onto the deck being careful not to spill the tips.

(B)   Place reagent plates on deck — **remove lids!**

(C)   Place empty strip tubes into heater slots.

(D)   Insert tip sheaths.

(E)   Open cartridge, place on deck, and **carefully** insert electrodes by closing lid.

4   **Add sample to reaction.**
- After completion of **Steps 1–3**, remove samples from the thermocycler.
- Spin down for 1–2 seconds in a picofuge.
- Insert into holder.
- Carefully remove caps to prevent cross-contamination and close heater lid.

5   **For best results, initiate protocol immediately.**

6   **At the conclusion of the protocol, remove the cartridge and seal wells with adhesive tape to prevent evaporation.**

7   **Transfer cartridge to the Digital Analyzer or store at 4°C for up to 7 days before data collection.**

For more comprehensive information, sign in to our customer resources site (**www.nanostring.com/sign/**) and go to **Support > Customer Resources*** to view the manuals and other technical product literature. For technical support, please e-mail **support@nanostring.com** or in the U.S./Canada, call **1 888 358 6266**.

LBL-C0106-04

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Quick Start Guide

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Prep Station Routine Maintenance

● **O-Ring lubrication.**
Every two weeks, an on-screen warning icon will appear reminding you to lubricate the o-rings. Using a minimum amount of grease on gloved fingers, distribute on o-rings, and wipe away excess with a clean glove. Once the lubricate o-ring protocol has been run, the reminder will disappear.

● **Clean the deck.**
Use water and lint-free wipes to remove dust and reagent residue. Cleaners can damage the deck.

Troubleshooting Tips

● **Inactivity for more than 3 weeks**
If the Prep Station hasn't been used for more than three weeks NanoString recommends that users set up a dry run (GEN1) or calibration run (GEN2) before processing samples.

● **Temperature Sensor Error**
Shutdown the system using the touchscreen and re-start using the switch on the back of the instrument. If the error persists, contact NanoString support.

● **Tip-eject Error**
Excess o-ring grease may be the cause. Replace the tips with a new set and restart the run. If the error persists, contact NanoString support.

● **Electrode Error**
If an electrode error is encountered, follow the error correction steps on the touchscreen. Do not abort the run at this step or you will need to insert a new cartridge when re-starting the run.
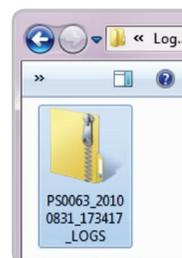
● **Aligning Bent Electrodes**
If the electrodes are not in alignment, use the touchscreen and follow the workflow under **maintenance > hardware > align** electrodes. Use tweezers or forceps to bend the electrode back into position.

● **Download Log Files**
When reporting errors to NanoString, use the touchscreen and follow the workflow under **maintenance > troubleshoot > download logs**. Use the USB drive to collect data for the last few runs in a zipped folder and e-mail to support@nanostring.com.

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Prep Station
Maintenance & Tips

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Digital Analyzer

Setting Up a Digital Analyzer Run

① Upload Reporter Library File (RLF).

Prior to using a CodeSet for the first time, it is necessary to upload its customized RLF file from the USB drive provided. The RLF file will remain on the Digital Analyzer for use in subsequent runs.

② Upload or create a CDF file.

A Cartridge Definition File, or CDF, defines sample specific data to associate with the data output and defines the parameters for the imaging instrument to use during image collection & processing. You can create a new CDF file on a PC or directly on your nCounter Digital Analyzer using the touchscreen. We recommend users create CDFs on their PCs if detailed information for each sample is required. In this case, your memory stick can be used to transfer the CDF to the imaging instrument.

②ª Apply imaging oil to sample cartridge (GEN1 nCounter System only).

- Insert the cartridge into the oiler and press the button to apply oil.
- Confirm that oil droplets were added across the entire surface of the cartridge.
- If cartridge was stored at 4°C, allow the cartridge to reach room temperature and clean surface with provided wipe before applying oil.

③ Insert cartridge into Digital Analyzer.

Press down on cartridge to ensure that it is flush with the feet of the cartridge carrier.

④ Initiate data collection.

Press "start counting" on the touch screen and follow the instructions.

⑤ Download your data.

After a run completes, a USB drive can be used to transfer data. If the Digital Analyzer is networked, it can be configured to automatically e-mail these result RCC files. If you cannot locate an RCC file after run completion, please check again later. In some cases of very high reporter counts, it may take up to several hours for the image processing to complete.

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Digital Analyzer Routine Maintenance

Clean the stage.

Use the wipes provided by NanoString to regularly clean the stage of dust and oil residue. If excess oil spills on the stage, clean immediately.

Maintain the objective.

Wipe the objective with a wipe provided by NanoString and apply a single fresh droplet of oil. Wipe in only one direction. This step should be done weekly if the system is in heavy use, or before resuming use if the system has been idle for some time.

Troubleshooting Tips

Overnight Scan Errors

Power and temperature fluctuations may cause failures. Use the maintenance menu to shut down the system and restart using the power switch on the back of the instrument. Attempt to restart data collection. If the error persists, contact NanoString support.

Repeated Re-oil and Re-scan Messages

Repeated error messages of this type may indicate a system failure. Contact NanoString support.

Download Log Files

When reporting errors to NanoString, use the touchscreen and follow the workflow under **maintenance > troubleshoot > download logs**. Use the USB drive to collect data for the last few runs in a zipped folder and e-mail to support@nanostring.com.

Digital Analyzer File Type Glossary

Reporter Library File (RLF) – This file is used during image processing to associate target identifiers with specific fluorescent barcodes.

Cartridge Definition File (CDF) – This file is created by the user to identify samples and to define all of the imaging parameters for a cartridge.

Reporter Code Count (RCC) file – This is the output of data collection and contains the counts for each target molecule.

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