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NanoString nCounter Gene Expression CodeSet RNA Hybridization Protocol V.1

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Mackenzie Bolen[1], Malu G. Tansey[1]

[1]University of Florida

Mackenzie Bolen
University of Florida

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We use this protocol and it's working

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Abstract

NanoString’s patented molecular barcodes provide a true digital detection technology capable of highly multiplexed analysis. CodeSet chemistry uses a Reporter CodeSet and a Capture ProbeSet to bind to the target. Excess probes are removed, and hybridized probes bind to the cartridge. The target-probe complexes are immobilized and aligned on the cartridge, and the barcodes are counted on nCounter® systems.

Attachments

PDF

MAN-10056-05-Gene-Ex...

1.2MB

Guidelines

This manual includes instructions for setting up hybridization reactions for gene expression assays that utilize NanoString’s standard CodeSet chemistry for off-the-shelf XT panels as well as custom-designed XT assays.

Materials

Items provided by NanoString:
item, Description, Storage
1. Reporter CodeSet, Barcoded Reporter Probes, At or below -80°C
2. Capture ProbeSet, Capture Probes, At or below -80°C
3. Reporter Plus CodeSet (optional), Barcoded Reporter Plus Probes, At or below -80°C
4. Capture Plus ProbeSet (optional), Capture Plus Probes, At or below -80°C
5. Hybridization Buffer, Supplied with nCounter Master Kits and SPRINT Reagent Packs, RT (15–25°C)
6. Panel Standard (Optional), Optional sample used for Calibration, At or below -20°C

Additional materials required but not provided by NanoString:
Item, Manufacturer, Part number
1. Thermal Cycler, Various, Various
2. Microfuge or picofuge, Various, Various
3. NanoDrop or QuBit*, ThermoFisher, Various
4. Bioanalyzer 2100*, Agilent, G2939BA
5. 12-tube PCR hybridization strip, Various, Various
6. Multi-channel pipettor, Various, Various
7. Pipettes for 0.5–10, 2–20, 20–200 µL*, Rainin, Various
8. 96-well clear polystyrene round-bottom plates*, Corning, 351177
9. Disposable gloves, Various, Various
10. RNeasy® Mini Kit* (optional), QIAGEN 74104, 74106
11. Proteinase K Solution (20 mg/mL)**, ThermoFisher, AM2546

nCounter Hybridization

1 Pre-heat the thermal cycler to 65°C with a heated lid at 70°C.

1.1 NOTE: If you are using cell lysates, see MAN-10051, Preparing RNA and Lysates from Fresh
Frozen Samples.
NOTE: If you are not using Panel Plus or CodeSet Plus, use the CodeSet Hybridization Setup.

2 Remove Reporter CodeSet, Capture ProbeSet, Reporter Plus and Capture Plus tubes from the freezer
and thaw at room temperature. Invert or flick the tube several times to mix well and briefly spin down
reagents.

2.1 IMPORTANT: After it has thawed, inspect the tube of Reporter CodeSet to make sure no colored precipitate is present. If you see a colored precipitate, heat the entire tube to 75°C for 10 minutes and cool at room temperature before using.

3 Create a hybridization master mix by adding the following reagents to the tube containing the
Reporter CodeSet (Table 5). Do not remove the Reporter CodeSet from the tube, add components
directly into the CodeSet tube. Do not add the Capture ProbeSet or Capture Plus to the master mix.

3.1 IMPORTANT: If using crude whole cell lysates as sample input, add Proteinase K to the CodeSet Master Mix at a final concentration of 200 µg/mL (for 20 mg/ml Proteinase K solution in the final hybridizatiation volume of 18 µl, add 2.5 µL into the 14-reaction hybridization Master Mix).

4 Flick or invert the tube repeatedly to mix then briefly spin down the Master Mix.

5 Label a 12-tube PCR hybridization strip. If necessary, ensure the strip will fit in a microfuge or picofuge
by cutting both the strip tube and its lid in half prior to setting up the reactions, taking care not to
crack the tubes.

6 Prepare hybridization reactions using a fresh tip for pipetting into each well:

6.1 Add 10 µL of Master Mix to each well of a strip tube.

6.2 Add 5 µL of sample (or diluted Panel Standard) to each tube containing Master Mix. If you are using a Panel Standard, see Panel Standard Usage for details.

6.3 Mix the Capture ProbeSet and Capture Plus tubes by inverting or flicking, and briefly spin down the contents.

6.4 Create a Master Capture by adding 14 µL of the Capture Plus directly into the Core Capture ProbeSet tube containing 28 µL. Mix by inverting or flicking, and briefly spin down the contents.

6.5 Add 3 µL of Master Capture to each tube. NOTE: Final hyb volume for Core CodeSet + Panel/CodeSet Plus will be 18 µL.

6.6 Cap the strip tubes tightly and mix by inverting the tubes several times and flicking to ensure complete mixing.

6.7 Spin briefly and immediately place the tubes in a pre-heated 65°C thermal cycler.

7 Incubate hybridization reactions for at least 16 hours. Maximum hybridization time should not exceed 48 hours.

8 Incubate at 4°C once desired hyb time is reached and process the following day. Do not leave the reactions at 4°C for more than 24 hours or increased background may result.

8.1 NOTE: Counts continue to accumulate with time at 65°C, with total counts typically increasing 5% per hour between 16 and 24 hours. Although a 16-hour incubation is adequate for most purposes, a longer incubation increases sensitivity by increasing counts without significantly increasing background.

9 Once the hybridization reactions have been removed from the thermal cycler, proceed immediately to an nCounter Prep Station or SPRINT as described in the nCounter Analysis System User Manual (MAN-C0035) or nCounter SPRINT User Manual (MAN-10017).

Preparing the RLF

10 All nCounter Plus reagents are accompanied by an add-in library file (ALF), which specifies the association between each Plus reagent and its target. Information from the ALF must be merged with the reporter library file (RLF) from the original CodeSet prior to scanning on the Digital Analyzer. Failure to merge an ALF with the original RLF will result in no count information being collected for targets of Plus reagents.
Counts that are not scanned are not recoverable on MAX/FLEX instruments. To obtain a merged RLF file, email NanoString at bioinformatics@nanostring.com. Include both the ALF for your Plus product and the RLF for the CodeSet that you want to spike into the Plus product. A new merged RLF will be generated and emailed to the requestor's address that contains all probe information for both the Plus product and the original CodeSet.

10.1 IMPORTANT: Failure to use the merged RLF during the subsequent scan will result in incomplete barcode scanning and the loss of data from a subset of the included probes.

Protocol references

NanoString nCounter Gene expression protocol identifier: MAN-10056-05-Gene-Expression-Hybridization-Protocol