PRODUCT DATA SHEET
nCounter® Gene Expression and CNV: Panel-Plus and CodeSet-Plus

nCounter®
Panel-Plus and CodeSet-Plus

Product Highlights

• 30 unique Reporter Probes are universally compatible with all custom CodeSets and Panels

• Compatible with all Gene Expression and CNV products, including Single Cell applications

Applications

• Customize a Panel
  Add up to 30 of your favorite genes or a collection of specific controls

• Update a large CodeSet
  Add new genes as you go to keep your CodeSet updated

• Create specific control sets
  Create multiple Plus control sets for use with different sample or cell types

• Finalize your gene list more quickly
  Add more genes later

nCounter® Panel-Plus and CodeSet-Plus Product Overview

The nCounter® Panel-Plus and CodeSet-Plus products from NanoString Technologies add a new level of flexibility to nCounter Gene Expression and CNV experiments. Researchers can now customize any NanoString off-the-shelf panel kit or add genes to any custom CodeSet by utilizing up to 30 unique Reporter Probes exclusively formulated for use in Panel-Plus and CodeSet-Plus products.

Panel-Plus enables any off-the-shelf panel kit to be customized with up to 30 additional genes of interest. Customizing a panel kit with a Panel-Plus product allows researchers to leverage the bioinformatics knowledge incorporated into every nCounter panel kit and provides unprecedented flexibility to tailor a comprehensive list of genes to your exact needs. In addition, combining a panel kit combined with Panel-Plus will allow you to survey a comprehensive collection of genes for a lower cost per sample when compared to the same gene set being ordered as a custom CodeSet.

CodeSet-Plus provides researchers the ability to update custom CodeSets that may be used to analyze large sample sets over many months. Genes of interest that become known during ongoing research or from a new publication can now easily be added to a custom CodeSet. This flexibility means that a custom CodeSet sufficient to analyze all the samples in a large study can be purchased with confidence. Purchasing a larger custom CodeSets provides a more cost effective solution versus purchasing multiple smaller custom CodeSets.
nCounter® Panel-Plus and CodeSet-Plus Product Overview

Plus CodeSets contain Reporter (Reporter-Plus) and Capture (Capture-Plus) Probes for up to 30 targets and can be combined with any custom CodeSet or pre-designed Panel prior to overnight hybridization. Using a Plus product with either a CodeSet or Panel is simple and requires few additional steps as outlined in the diagram below.

NOTE: After hybridization, all subsequent steps are performed as described in the nCounter Prep Station and Digital Analyzer Manuals. For specific recommendations on sample input see the nCounter RNA or DNA User Manual. For a detailed protocol see the nCounter Panel-Plus and CodeSet-Plus Protocol.

1. Create Reporter and Plus Reagent Master Mix
   - Add 26 μL to Reporter CodeSet
   - Thaw 12 reactions on ice. Invert to mix and spin down.
   - Add 130 μL to Reporter CodeSet
   - Invert to mix and spin down.

2. Set Up Hybridization Reaction
   - To each tube, add:
     - 22 μL Master Mix
     - Sample
     - RNase-free Water (if needed)
     - Minimum 27 μL total volume per tube
   - Complete Hybridization Reactions
     - 1. Cap tube, mix by inverting strip
     - 2. Briefly spin down
     - 3. Incubate at 65°C

3. Create Capture ProbeSet and Plus Reagent Mix
   - Add 14 μL to Capture ProbeSet
   - Thaw 12 reactions on ice.
   - Invert to mix and spin down.

4. Complete Hybridization Reactions
   - To each tube, add:
     - 6 μL Capture ProbeSet and Plus Reagent Mix
   - * 27 μL is the standard Gene Expression and CNV hybridization set-up volume. Up to 37 μL can be used for Single Cell assays that include MTE volumes up to 15 μL per sample.
Performance of Panel-Plus Gene Sets with Pre-designed Panels

Standard Gene Expression and Single Cell Analysis

Two experiments were performed by adding Panel-Plus genes to the Human Cancer Reference Kit. Data in each figure below demonstrate that addition of CodeSet-Plus probes does not impact performance of original nCounter panel probes or single cell panel probes.

Gene Expression

\[ R^2 = 0.99953 \]

Single Cell Gene Expression

\[ R^2 = 0.99641 \]

Human reference total RNA (100 ng) was hybridized with either the nCounter Cancer Reference Kit alone (Original CodeSet) or with the nCounter Cancer Reference Kit and a 30-gene Panel-Plus (Original CodeSet with Plus). Assays were performed in triplicate for each condition tested and normalized to internal positive controls. Average count values for each triplicate measurement were determined and plotted for all nCounter Cancer Reference Kit probes in the Original CodeSet alone (y-axis) and Original CodeSet with Plus (x-axis).

Design and Ordering Information

When a custom nCounter Panel-Plus or CodeSet-Plus is ordered, probes are designed for every gene sequence in the request. Two separate vials for custom Plus genes are shipped along with a unique Add-in Library File (ALF) file that includes the additional genes. The Plus products will not include controls but are tested prior to shipment with the nCounter standard CodeSet controls included in all custom CodeSets and panels. Plus products are always used with either a panel or a custom CodeSet and do not require the purchase of additional Master Kit reagents.

Plus products can be ordered from three separate gene categories and are offered with purchases of Panels or Custom CodeSets for 48 reactions or more.

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<thead>
<tr>
<th>Description</th>
<th>Number of Genes</th>
<th>Number of Reactions</th>
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<tr>
<td>Panel-Plus</td>
<td>6 - 11 genes</td>
<td>48 minimum</td>
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<tr>
<td></td>
<td>11 - 20 genes</td>
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<td></td>
<td>21 - 30 genes</td>
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<tr>
<td>CodeSet-Plus</td>
<td>6 - 10 genes</td>
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Frequently Asked Questions

1. Can I use multiple Plus products with a Panel or Custom CodeSet?

No. Multiple Plus products cannot be mixed or combined in a reaction. Only one Plus product may be used in conjunction with a set of specific reactions. It is possible to use different Plus products on separate occasions with different Panels or CodeSets.

2. Can I use the Plus product as I would a custom CodeSet?

No. Plus products are not the same as custom CodeSets. Plus products have been formulated to be mixed with Panel or Custom CodeSet Reporter and Capture Probe Mixes. Plus products also do not include the necessary controls for proper normalization.

3. How do I include the new genes in my data analysis?

To include the new genes, please contact NanoString Bioinformatics at bioinformatics@nanostring.com. Include in your message the name of the Plus product and original CodeSet that will be combined. A new Reporter Library File (RLF) containing both will be sent within 24 hours.

4. How do I analyze the combined data?

After scanning on the Digital Analyzer, data may be imported into nSolver™ Analysis Software for downstream analysis. Please see the nSolver Analysis Software Manual for additional information on the nSolver application.

5. Can I purchase a Panel-Plus for my miRNA, miRGE™, or Plex™ experiment?

Not at this time. Plus products are specially formulated to be universally compatible with all CNV and Gene Expression CodeSets.