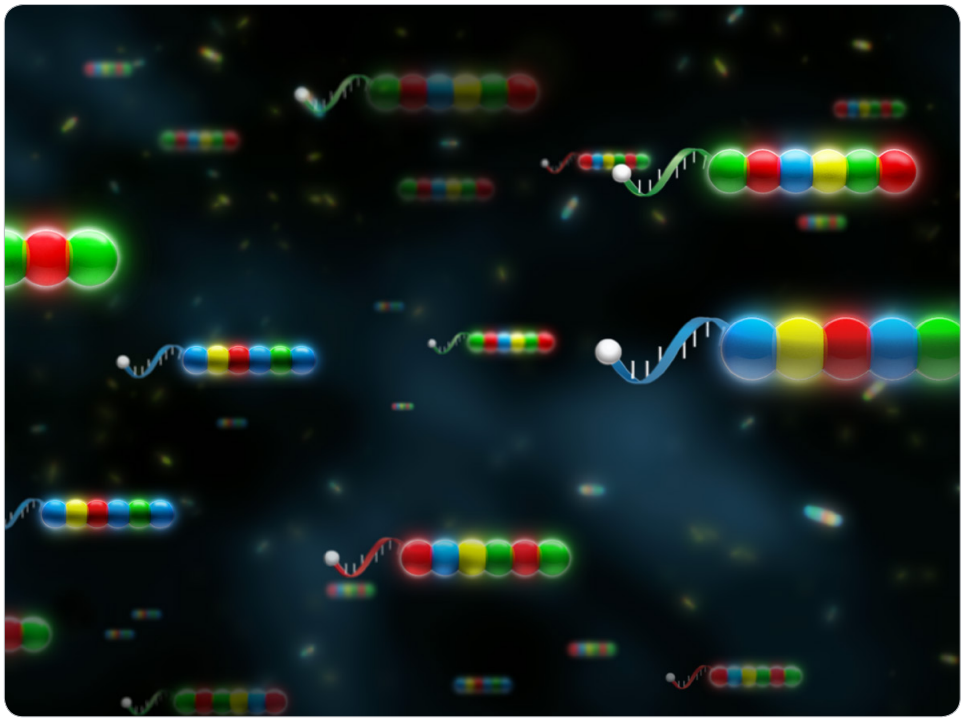
nCounter®
Panel-Plus and CodeSet-Plus



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:** Utilize results from your experiments and 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® Gene Expression Panel-Plus and CodeSet-Plus Product Overview

The NanoString nCounter® Panel-Plus and CodeSet-Plus products add a new level of flexibility to nCounter 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.

The Panel-Plus product 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 using 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.

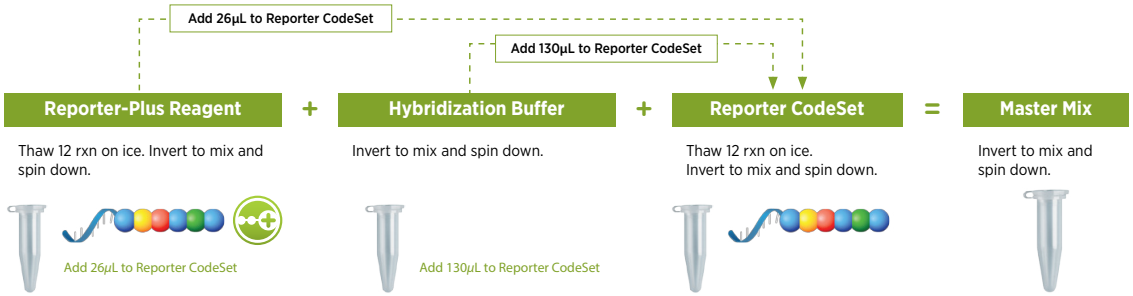
The CodeSet-Plus product provides researchers the ability to update custom Codesets that may be used to analyze large sample sets over many months. Genes of interest that become known from ongoing research, or from a new publication, can now easily be added to a custom CodeSet. This flexibility means that a custom CodsSet 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.

Setting up nCounter Panel-Plus or CodeSet-Plus Reactions

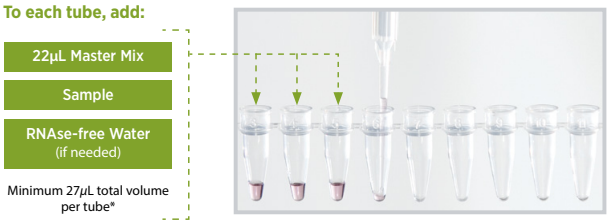
Plus CodeSets contain Reporter (Reporter-Plus) and Capture (Capture-Plus) Probes for up to 30 targets and can be combined with any Custom CodeSet or Panel prior to overnight hybridization. Using a Plus product with either a Panel or Custom CodeSet is simple and requires minimal additional steps as outlined 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.

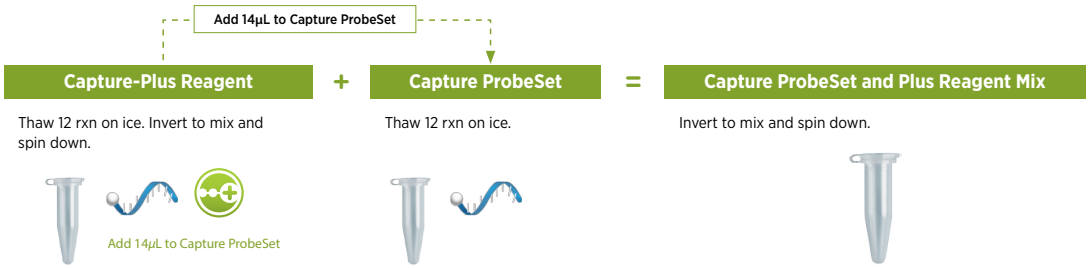
1 Create Reporter and Plus Reagent Master Mix



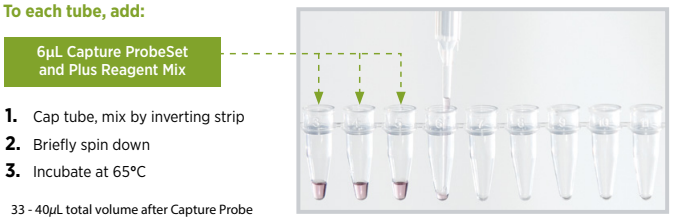
2 Set Up Hybridization Reaction



3 Create Capture ProbeSet and Plus Reagent Mix



4 Complete Hybridization Reactions



*27µL is the standard Gene Expression and CNV hybridization set-up volume.
Up to 37µL can be used for Single Cell reactions that include MTE volumes up to 15µL per sample.

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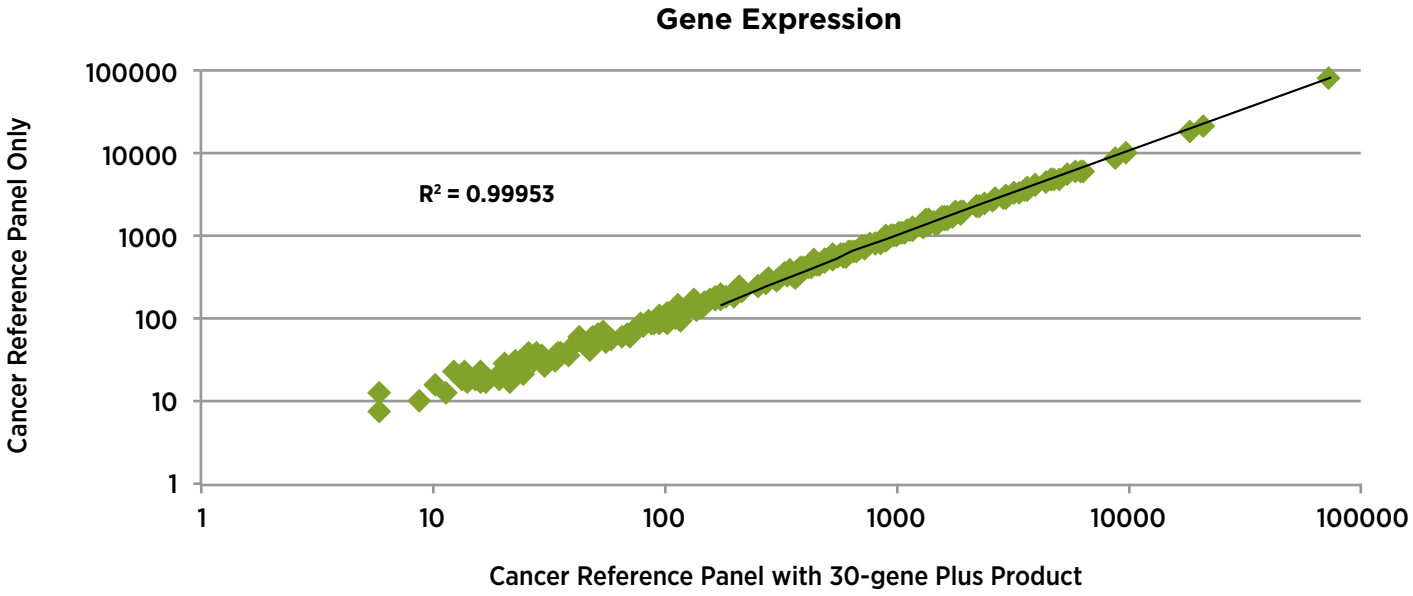
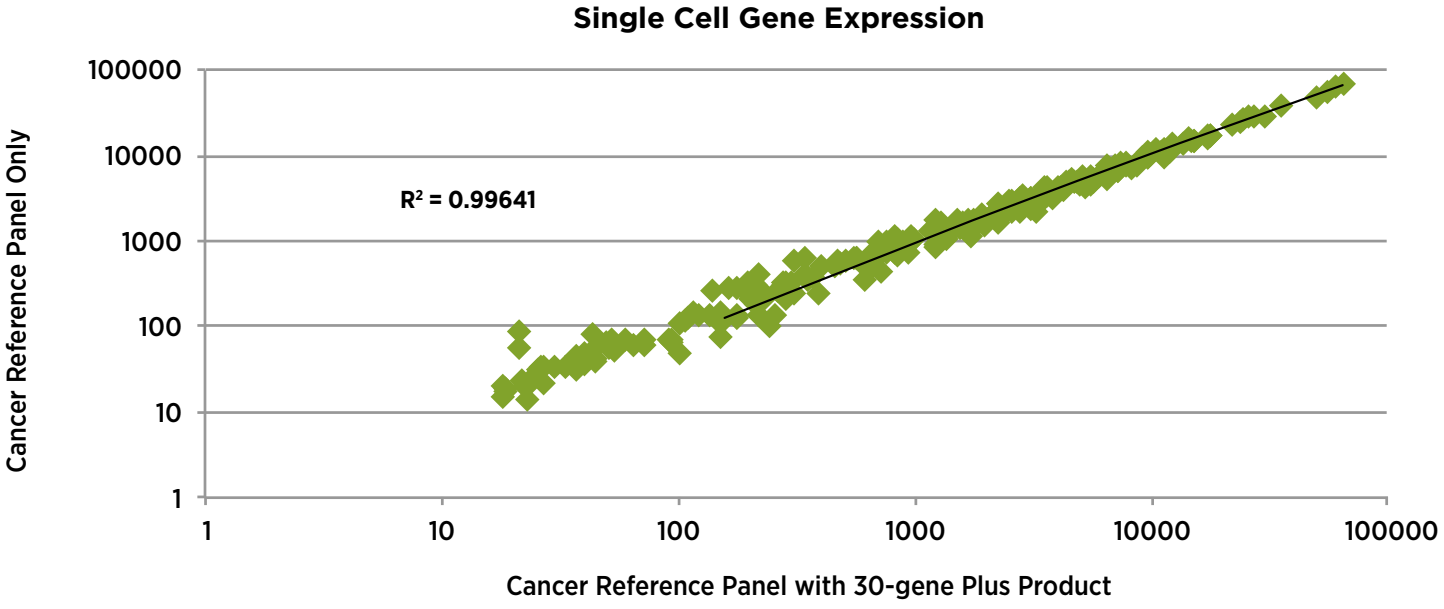
Performance of Panel-Plus Gene Sets with Pre-designed Panels
Standard Gene Expression and Single Cell Analysis

Two experiments were performed by adding Panel-Plus genes to the Cancer Reference Panel. 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.



Human Reference Total RNA (100ng) was hybridized with either nCounter Cancer Reference Panel (Original CodeSet) alone or nCounter Cancer Reference Panel with 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 Panel probes in the Original CodeSet alone (y-axis) and Original CodeSet with Plus (x-axis).



Human Reference Total RNA (100pg) was converted to cDNA and enriched via a Multiplexed Target Enrichment (MTE) with primers appropriate for downstream hybridization according to the nCounter Single Cell Expression Assay Protocol for Total RNA. The enriched cDNA was hybridized with either nCounter Cancer Reference Panel CodeSet (Original CodeSet) alone or nCounter Cancer Reference Panel with 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 Panel 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 are ordered probes are designed for every gene sequence in the request. Two separate vials for custom Plus genes are shipped along with a unique .ALF (Add-in Library File) 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 3 separate gene categories and are offered with purchases of Panels or Custom CodeSets of 48 reactions or more.

Description	Number of Genes	Number of Reactions
Panel-Plus	6 - 10 genes 11 - 20 genes 21 - 30 genes	48 minimum
CodeSet-Plus	6 - 10 genes 11 - 20 genes 21 - 30 genes	48 minimum

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 a CodeSet.

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 RLF containing both will be sent to you 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 Manual (MAN-C0019) for additional information on the nSolver application. Note: nSolver does not support cross-RLF analysis. In order to compare data generated with a CodeSet-Plus + the original CodeSet versus the original CodeSet alone, all data must be exported to a 3rd party analysis application such as Microsoft Excel.

5. **Can I purchase a Panel-Plus for my miRNA and miRGE™ or Plex²™ experiment?**
   Not at this time. Plus products are specially formulated to be universally compatible with all CNV and Gene Expression CodeSets.

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