Development of a 96-sample NanoString® assay, nCounter® PlexSet™ Gene Expression

#B252

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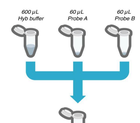
Abstract

The NanoString nCounter Analysis platform uses a novel molecular barcoding technology to measure multiplexed gene expression of up to 800 targets. The assay tabulates fluorescent barcodes to provide specific, precise digital data; it is used in a wide variety of basic research, translational medicine and *in vitro* diagnostics applications. The standard nCounter gene expression assay can be used to process 12 samples per run. We recognize an additional need for a lower gene-plex, higher sample-throughput assay which would enable researchers to quickly evaluate tens of multiplexed targets in hundreds of samples. Here we present data from a new nCounter assay, nCounter PlexSet Gene Expression, which is performed in a 96-sample format. Barcoding regents have been developed for 12-gene-plex and 24-gene-plex assays. Initial assay hybridization is performed in a 96-well plate; each column of eight assays is then combined, generating twelve sample-plexed pools. The twelve pools are processed in a single standard nCounter run on any nCounter Analysis system, and the data for the 96 individual samples are de-convoluted during data analysis. We present data showing that the sample-multiplexed nCounter PlexSet Gene Expression assay retains the specificity and precision of a standard single-sample nCounter assay, while increasing the sample throughput eight-fold. PlexSet barcoding reagents are universal and can be combined with gene-specific probe oligos to detect any target of choice. Future development includes extending the assay to 96 gene targets per sample while maintaining the throughput of 96 samples per run. The nCounter PlexSet Gene Expression assay is for research use only.

nCounter PlexSet Gene Expression Workflow

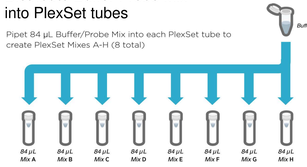
1 Create Buffer/Probe Mix

Combine 600 µL Hyb buffer with 60 µL diluted Probe A pool and 60 µL diluted probe B pool.



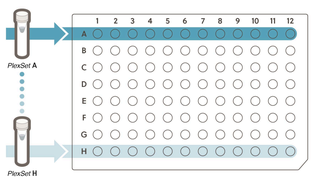
2 Distribute Buffer Probe Mix into PlexSet tubes

Pipet 84 µL Buffer/Probe Mix into each PlexSet tube to create PlexSet Mixes A-H (8 total)

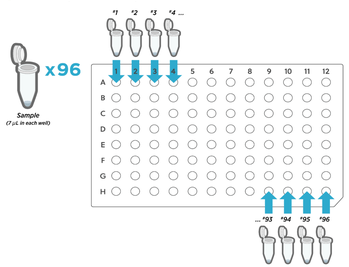


3 Distribute PlexSet master mixes into 96-well plate

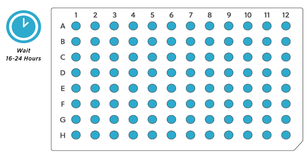
Pipet 8 µL of each PlexSet Mix into each well in that mix's row on the 96 well plate. (Mix A across row A, Mix B across row B, etc.)



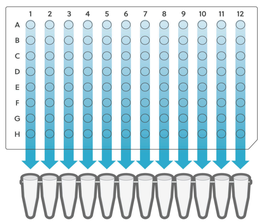
4 Add 7 µL of sample to each of the 96 wells



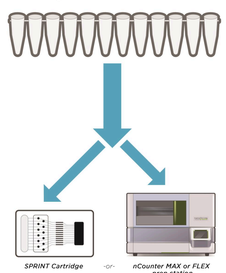
5 Hybridize overnight (16-24 hours, then 4°C hold)



6 Pool hyb reactions down columns into strip tube

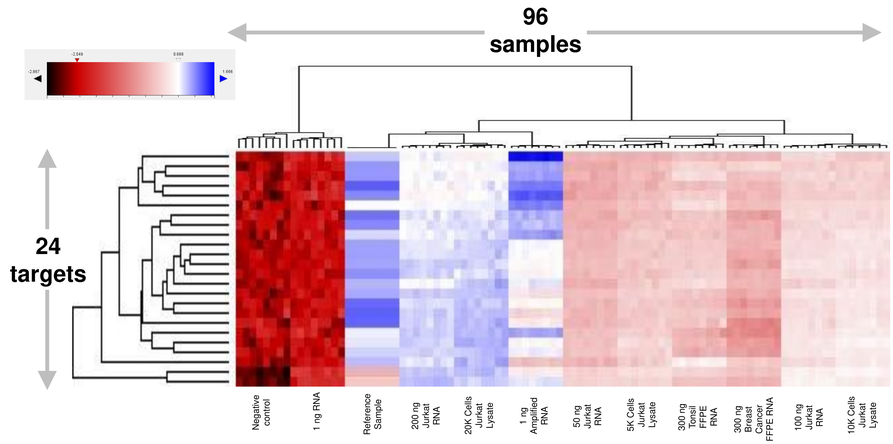


7 Process strip tube directly on either nCounter MAX or Flex Prep Station or load 35 µL of each pool into SPRINT™ cartridge



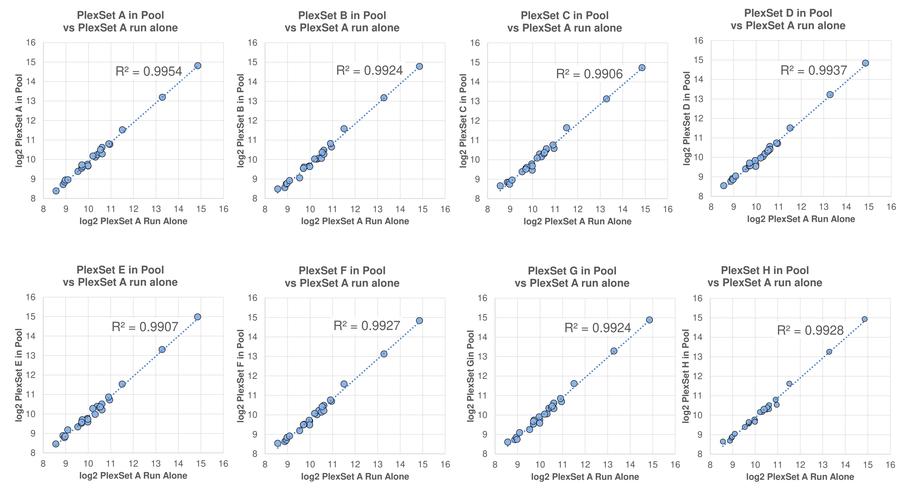
nCounter PlexSet Gene Expression has a simplified workflow. **1)** Create hybridization/probe master mix. **2)** Distribute buffer/probe master mix across eight PlexSet tubes (A through H). **3)** Distribute each PlexSet master mix across respective row. **4)** Add prepared sample to each well. **5)** Hybridize overnight. **6)** Pool hybridization down the column into a strip tube. **7)** Process strip tube on any nCounter platform.

24 targets, 96 samples: nCounter PlexSet Gene Expression assay generates 2304 data points in a single nCounter run



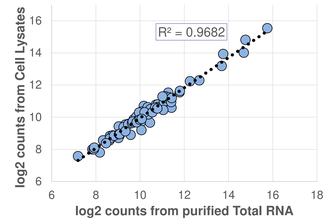
12 samples from a variety of sources (purified Jurkat Total RNA, corresponding Jurkat cell lysates, purified FFPE RNA, amplified cDNA, and a negative control) were multiplexed using the nCounter PlexSet Gene Expression assay (24 gene targets). The same samples were ran across all 8 PlexSets. Data was normalized to positive internal controls and normalized to a reference sample. Using the Euclidean distance and average linkage method an unsupervised cluster map was generated using nSolver 3.0 software. As expected, the 50 ng Jurkat RNA is clustering with 5,000 Jurkat cell lysate, 100 ng Jurkat RNA is clustering with 10,000 Jurkat cell lysate, and 200 ng Jurkat RNA is clustering with 20,000 Jurkat cell lysate.

Equivalent results are obtained when all PlexSets are used in a pool versus a single PlexSet run alone



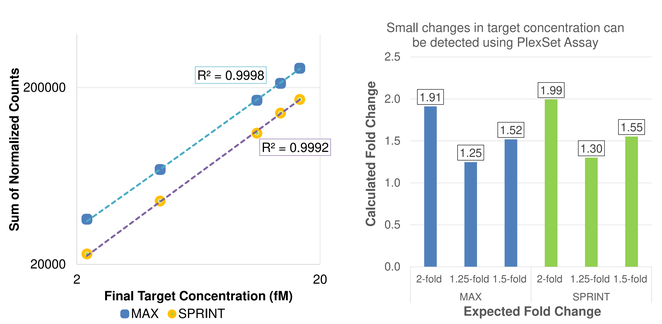
To verify that equivalent results are obtained regardless of PlexSet used or when used alone or in a pool, 24 gene targets were analyzed using lysates from 10,000 Jurkat cells. The data from PlexSet A run by itself was compared to PlexSet A through H in a multiplexing pool. Data were normalized to positive internal controls, counts $\log_2$ transformed, and the R^2 for each pairwise comparison are shown on each graph.

Equivalent results can be obtained when using purified total RNA vs whole cell lysate



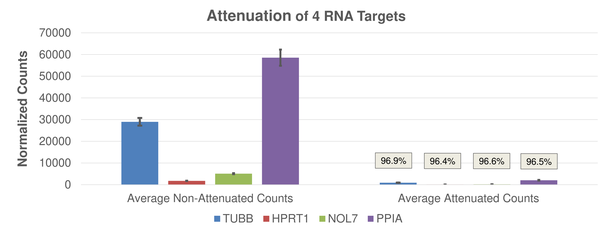
Using nCounter PlexSet Gene expression assay, purified total RNA from Jurkat cells at 50 ng, 100 ng, and 200 ng were run and compared to Jurkat whole cell lysates from 5,000, 10,000, and 20,000 cells. The R^2 = 0.9682 demonstrates that equivalent results can be obtained when using purified total RNA vs whole cell lysate.

nCounter PlexSet assay shows a strong correlation between target concentration and counts and is capable of detecting small fold-changes



Targets were titrated across all eight PlexSets at varying concentrations (2.2fM, 4.4fM, 11fM, 13.75fM, and 16.5fM) to verify sensitivity and fold-change detection of the assay on a MAX and SPRINT. Data were normalized to positive internal controls. Line graph on the left shows the strong correlation between total normalized counts and target concentration on a MAX and SPRINT with an R^2 of 0.9998 and 0.9992, respectively. Bar graph on the right shows the expected fold change (x-axis) and calculated fold change (values above each bar) of a 2-fold titration (2.2fM to 4.4fM), 1.25 fold titration (11fM to 16.5fM), and 1.5 fold titration (13.75fM to 16.5fM) on MAX and SPRINT. Difference in expected and calculated fold change is <5%.

nCounter PlexSet accommodates highly abundant targets



Attenuation enables the measurement of highly abundant targets without assay saturation. 200 ng of Jurkat RNA was run with and without 4 attenuation probes across all 8 PlexSets. Concentration of attenuation probes were designed to achieve >95% signal attenuation. Error bars are the standard deviation of counts for the same target across 8 PlexSets. Number of unattenuated counts for each target can be back-calculated during data analysis.

Future Development

- nCounter PlexSet Gene Expression with 48 and 96 gene plex

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