Cell line screening studies require highly efficient protocols for studying many samples in parallel. We have developed a low-and-go protocol for digital gene expression profiling of 96 samples by 96 genes in parallel.

This protocol is based on nCounter PlexSet Reagents which enable multiplexing of samples and gene targets. The NanoString nCounter Analysis platform uses a novel molecular barcoding technology to measure multiplexed gene expression. The assay counts fluorescent barcodes hybridized to targets to provide precise digital data. This platform is used in a wide variety of research applications. The standard nCounter gene expression assay may be used to process 10 samples per run. We recognize an additional need for a higher sample-throughput assay which would enable researchers to quickly evaluate up to 96 high-throughput assays with the PlexSet lyse-n-go protocol. It does not require RNA purification or cDNA conversion steps, thus saving time and resources for the researcher. The data demonstrates that up to 96 high-throughput assays can be generated from crude cell lysates in an efficient, high-throughput protocol and that the cell lysate data correlates well with purified total RNA.

Analysis of IO Lymphocyte Activity Panel Across Various Primary Cells

- 7.5-25K cells from lysis protocol added to 96-pixel hybridization. 
- 50-250K total cells plated for assay, only a fraction of the lysate was used for the hybridization.
- Hierarchical clustering analysis across cell type shown.

Volcano plots for two different pairs of cells shows expected differential expression profiles.

Conclusions

- The PlexSet lyse-n-go protocol is simple and fast. It does not require RNA purification or cDNA conversion steps, thus saving time and resources.
- The data demonstrates that up to 96 high-quality, digital data points for gene expression in each of 96 samples can be generated from crude cell lysates in an efficient, high-throughput protocol.
- Cell lysate data correlates well with purified total RNA.