Efficient CRISPR screening with the NEW NanoString PlexSet Technology

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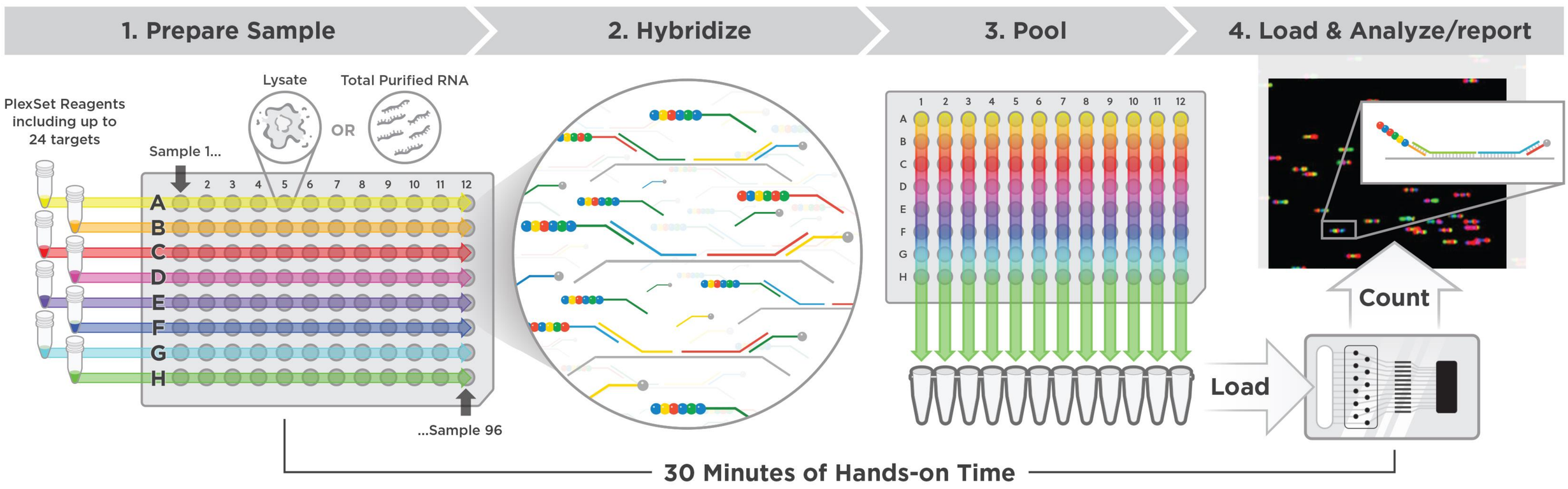


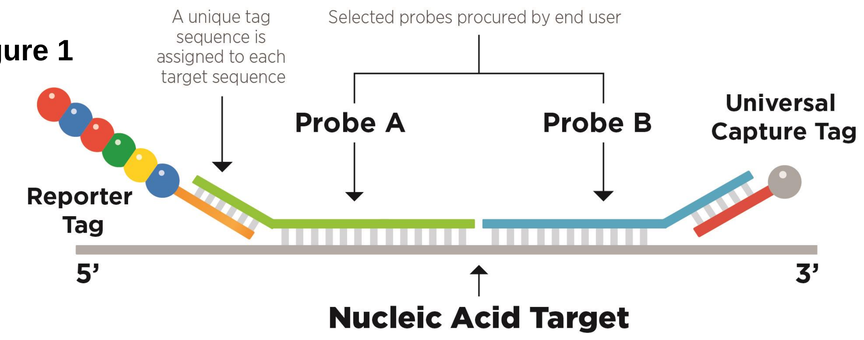
Abstract

We have developed an efficient method to screen for successful introduction of GFP fusions or other expression tags and for continued use of the multiplexed assay for high-throughput CRISPR perturbation of Myc regulatory pathways. NanoString PlexSet reagents were used for **CRISPR screening and validation directly from CRISPR gene-edited cell lysates**, or from purified total RNA. NanoString PlexSet reagents enable researchers to simultaneously confirm CRISPR hit validations and perform phenotypic functional testing for **up to 24 custom gene targets including endogenous mRNAs, synthetic constructs, fusions, splice variants and junctions for 96 samples in one run**. Like most NanoString assays, the direct digital quantification (no enzymes or amplification), flexible probe customization, technical assay controls, high sensitivity, and, cost-effective easy workflow and data analysis make this an ideal option for combined CRISPR editing screens and multiplexed gene expression assays.

Introduction

A new High-Throughput NanoString nCounter PlexSet assay is now available. PlexSet enables direct digital detection of up to 24 custom targets in 96 samples per run. Probes selected by the researcher are made with target specific sequences and tag specific sequences at 5' and 3' tailing ends (Figure 1). The selected probe sets (Probes A and B) are mixed and diluted into pools, then combined with one of the 8 unique barcoded PlexSets. These probe and PlexSet pools combined and hybridized overnight with their nucleic acid targets, with a distinct PlexSet in each row of a 96 well plate. Each of the 12 columns from the 96-well plate is then pooled for processing on the NanoString nCounter Prep Station. The Prep Station purifies the target/probe complexes and deposits them in a cartridge for data collection. Data Collection is carried out in the NanoString nCounter Digital Analyzer. Images are processed and an algorithm tabulates digital counts for each barcode class.

Figure 1



Essential Information needed	qRT-PCR	PlexSet
Sample	RNA or DNA	RNA, DNA, Cell Lysates, serum, and body fluids
Reverse Transcription	Reagents used for RNA to convert to cDNA	Not Applicable(N/A)
Nucleic Acid Assessment	Purity/Yield /RIN (RNA)/Electrophoresis	Purity/Yield /RIN (RNA)/Electrophoresis
Technical Replicates	Yes	No
Target Information	Sequence and location needed	Sequence and location needed
Splice Variant information	Needed	Needed
Multiplexing	2-5 targets	3 - 24 Targets
Primers	Sequence and Manufacture information	Sequence and Manufacture information
qPCR Protocol	Master Mix reagents and cycling conditions	N/A No Amplification preformed
Post Reaction processing	N/A no post processing preformed	Samples cleaned to remove unhybridized material
Data Acquisition	Samples quantified based on Fluorescence Intensity	Samples counted based on Barcode reads

Table 1: Comparison of assay information for both qRT-PCR and PlexSet

NanoString PlexSet Data from CRISPR studies of cMYC Regulators

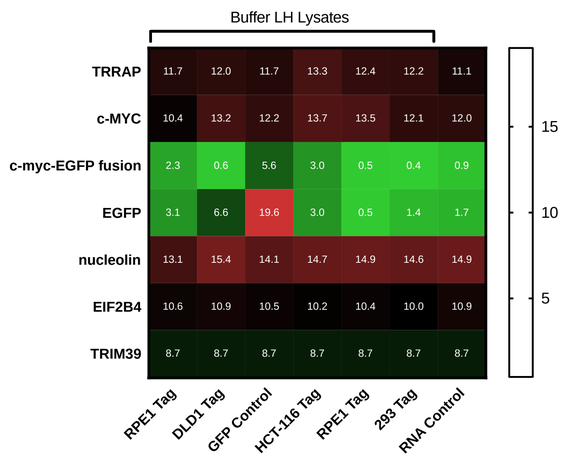
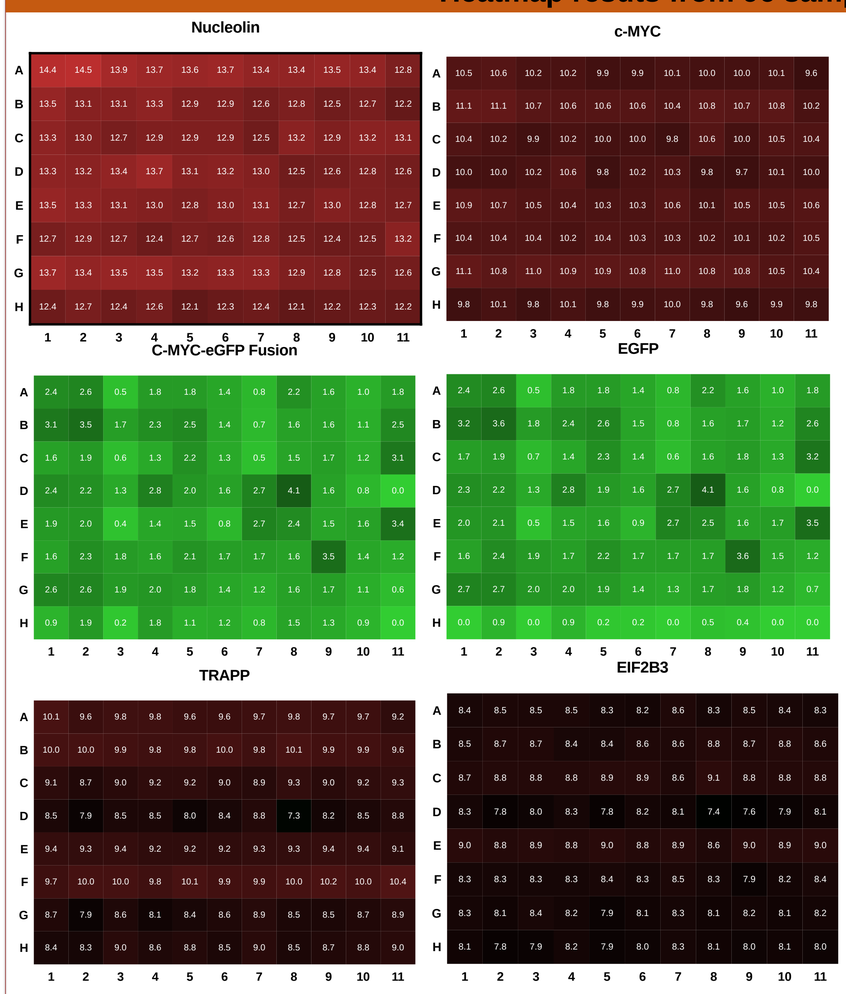


Figure 2. The resulting heat map from a titration assay using cell lysates and total RNA samples. This PlexSet assay was designed to quantify the number of CRISPR modified c-MYC-EGFP fusion cells present. Titration assays are used to establish the optimal number of cells to use for the assay.

Heatmap resuts from 96 samples



- Experiment hypothesis expected expression of precursor Nucleolin and c-MYC to be positive
- Negative screening results for C-MYC-eGFP Fusion edits
- The negative results were confirmed by qPCR and flow
- Screening results were confirmed between titration and 96 sample runs
- Both cell lysates and purified RNA gave same results

Discussion

Like most NanoString assays PlexSet is a hybridization assay that does not rely on Reverse Transcriptase (RT) or amplification for detection. The positive and negative controls are already incorporated into all PlexSet assays and the reference genes are selected by the researcher as with standard qRT-PCR experiments. The only optimization requirement is establishing sample input concentrations. This is accomplished using one tag set and the probe A and B pools in an assay.

Results From this PlexSet Assay for CRISPR edits showed

- The current tag method had negative screening
- NanoString results were accurate
- New tag methodology needed to be developed for EGFP and MYC
- Same results from purified RNA and cell lysates were demonstrated
- Experimental time utilizing PlexSet was reduced from several weeks to 1 week