

nCounter PlexSet Technology Setup Quick Reference

PlexSet technology enables multiplexed gene expression assays to be performed more efficiently and cost-effectively than ever before for projects ranging from 3 to 96 RNA or DNA targets. It enables users to run up to 96 samples in one run by multiplexing up to 8 samples per nCounter cartridge lane. It is based on molecular barcoding and digital quantification of target sequences through the use of a uniquely-designed PlexSet and the corresponding oligonucleotide probes (designed by NanoString and procured by the user) that recognize the targets of interest (Figure 1). The assay allows users to redesign and swap targets in the middle of a project, providing maximum flexibility and efficiency in their high-throughput screening.

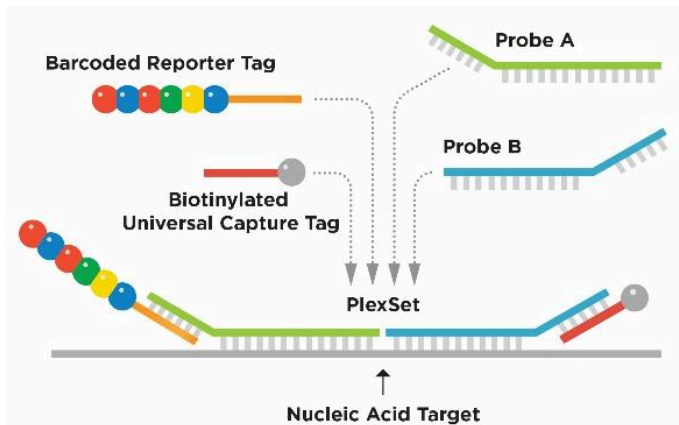


Figure 1: An existing PlexSet can be modified by reassigning Reporter Tags to new targets.

Flexible PlexSet Design

Users can modify the PlexSet assays by adding new oligonucleotide probes that enable detection of new targets. To do this, the user simply orders the new probes and re-pools them with the new configuration.

Figure 2: Example of probe swapping. Targets that have been added or replaced are indicated in blue text.

Experiment 1		Experiment 2	
Existing PlexSet		Tags Reassigned	
T001	Gene A	T001	Gene A
T002	Gene B	T002	Gene B
T003	Gene C	T003	Gene M
T004	Gene D	T004	Gene N
T005	Gene E	T005	Gene E
T006	Gene F	T006	Gene F
T007	Gene G	T007	Gene G

In consideration of the sensitive and unique nature of this design, review the **Recommended Setup for Effective Multiplexing** guidelines, below. In addition, plan to implement the two quality control measures necessary **at the onset of every project** to control for sample and system variability: **Titration** and **Calibration** (respectively). For more information on topics covered in this guide, see the *All About PlexSet Data Analysis User Manual* ([MAN-10044](#)) and the *PlexSet Reagents Manual* ([MAN-10040](#)).

Titration

Running a Titration Kit provides information on the optimal sample input amounts for the PlexSet assay. The Titration Kit contains nCounter XT TagSet reagents that correspond to the PlexSet assay (e.g. PlexSet-48 will use nCounter XT TagSet-48); this can be used to test probe attenuation strategies, if needed, but *should not* be used to combine samples or for final data analysis. Serial dilutions of control and experimental samples should be tested across lanes 1-12 of the plate at least once per study. For more information on setting up a Titration Run, see the *PlexSet Reagents Manual* ([MAN-10040](#)).

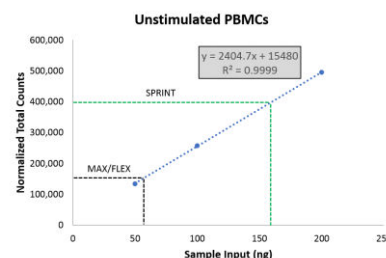


Figure 3: Example of titration kit data, and estimation of ideal sample input

Calibration

For the most accurate results with PlexSet assays, a single reference sample should be assayed across all 8 PlexSets (A through H) in a single column of your plate at least once per study and new probe set. This reference sample lane is used to calibrate potential inter-PlexSet variability. Experimental samples can be run alongside the reference samples, in other lanes of the plate and cartridge. Figure 4 shows a plate setup with the reference sample run in the first column of the first plate. Subsequent plates run with the assay need only contain experimental samples. If a new lot of PlexSet reagents is introduced to an ongoing project, a new calibration lane should be run. For more information on Calibration, see the *PlexSet Reagents Manual* ([MAN-10040](#)).

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Recommended Setup for Effective Multiplexing

You may:

- Run all 96 samples on a plate (Figure 4).
- Run less than 96 samples on a plate, with PlexSet tubes A–D (for example) on one plate (by rows) and E–H on another plate (Figure 6 and Figure 8).
- Run all 96 samples on a plate, but split the plate across multiple experiments/probe sets by columns (Figure 9).

At this time, PlexSet is NOT designed to:

- Run all 96 samples on a plate, but split the plate across multiple experiments/probe sets by rows.
- Exclude data from selected wells.

A reference sample for calibration is essential for accurate data analysis across PlexSets (Figure 4). One RLF is used for these scenarios. Reserve lane 1 of the first plate for your Calibration Sample. Subsequent PlexSet cartridges using the same lot of probe sets will not need an additional reference sample lane (Figure 4). With our current nSolver™ data analysis software, your experiments should be organized down columns (Figure 5).

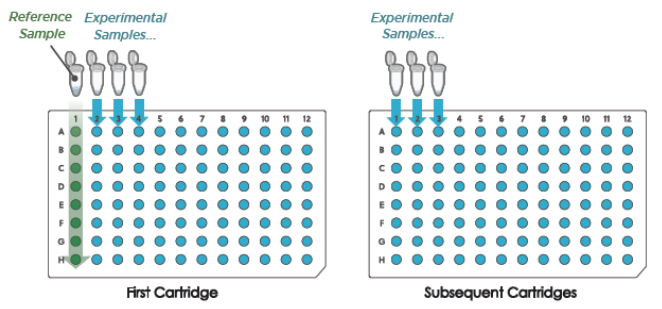


Figure 4: PlexSet layout -full plates

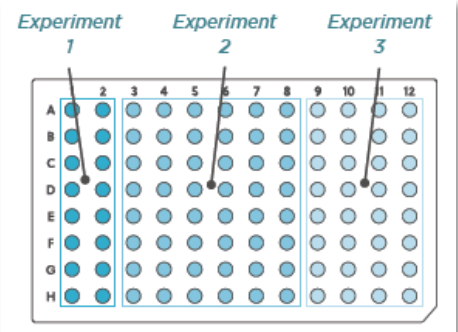


Figure 5: PlexSet samples organized in columns

Less than 96 samples can be run per cartridge; the same RLF is used for these scenarios (first and second run, Figure 6). PlexSets A–D can be run on one cartridge (first run), and PlexSets E–H can be run on another (second run). If you subsequently run a full plate with a different combination of PlexSets A through H (e.g., all PlexSets), a reference sample should be re-run across all PlexSets for calibration (third run, Figure 7).

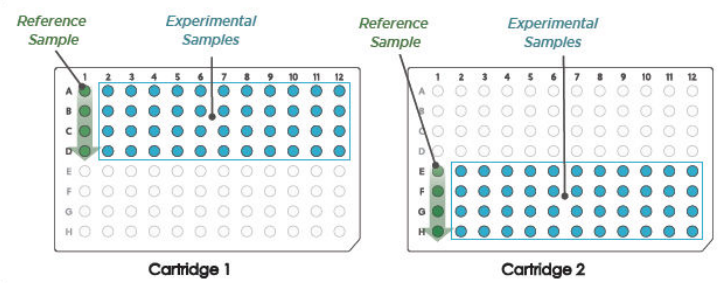


Figure 6: First and second run—partial PlexSet plates

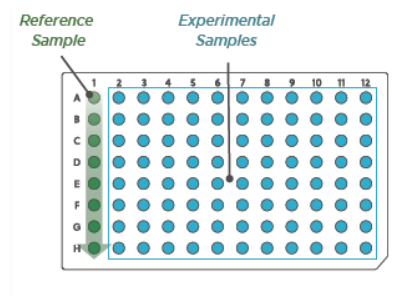


Figure 7: Third run—full PlexSet plate

PlexSet kits can be used with different probe sets, but specific configurations are required for downstream analysis. Two RLFs are used for these scenarios (one for each probe set). Running partial plates with different probe sets is possible, but the considerations above apply when running subsequent plates (Figure 8). If multiple probe sets are run on the same plate, probe sets should be organized down columns to allow downstream analysis with nSolver software (Figure 9). For more information on setting up plates in this manner, see the *PlexSet Reagents Manual* ([MAN-10040](#)).

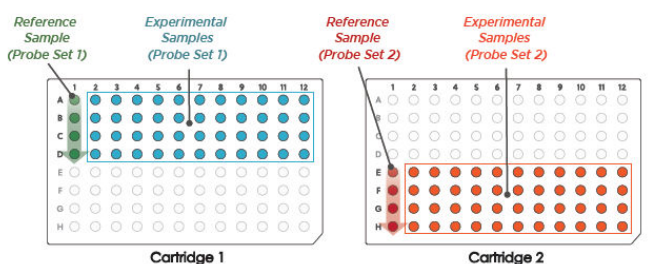


Figure 8: Partial PlexSet plates, different Probe Sets

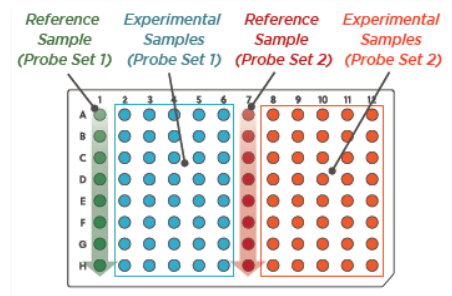


Figure 9: Different Probe Sets, organized down columns