Nanostring SOP

Ordering the codeset:

- Identify the RefSeq IDs of the genes of interest from Illumina genome
- In case the RefSeq ID is not available for a particular probe, the corresponding RNA sequence in the Illumina genome should be identified
- Currently up to 550 transcripts can be quantitated using one codeset

Quantitation of RNA:

- It is important to have as little variation in input RNA as possible.
- RNA should be quantitated using nanodrop.
- Other sensitive techniques like Picogreen may also be used.

Nanostring setup and Run:

Preparation of samples: tRNA or cell lysates

For tRNA samples:

1. Aliquot 100ng of tRNA in 5ul volume.

Or

For cell lysate:

(2500-10,000 cells)/ul of RLT buffer, so approx.50K-100K cells in 5ul of RLT buffer may work (assuming 50K is equivalent to100ng of RNA, but again it depends on the cell type)

2. Prepare master mix of Reporter codeset (130ul) and Hybridization buffer(130ul) for 12 rxns (10% dead volume)
3. Add 20ul of mastermix to 5ul of sample, then add 5ul of Capture probe, mix, incubate at 65C for min of 15hrs and max. of 30hrs. (the lid should be set at 75C)
4. Do not store at 4C after hybridization, but immediately proceed to processing sample in nCounter Prep station.

Processing/ Running of samples in nCounter prep station: (all reagents, tips, tubes provided by vendor)

1. Take out Reagent plates and sample cartridge ½ hr before starting run, to warm to RT.
2. Spin the reagent plates at 670xg for 2min.
3. Then follow instructions on Prep station monitor for setting up all the reagents, tips, tubes etc. and then start run, takes approx. 2hrs for run to complete for 12 rxns.

4. After run take out cartridge, seal with sealing tape, either store at 4C or can go directly to reading the cartridge by putting in Digital Analyzer.

5. Before putting in cartridge for analyzing in the Digital analyzer, need to load RLF library that is created for your customized gene list.

6. Then create CDF file with your sample information on desktop and load that into the digital analyzer too.

7. When ready to load cartridge, apply optical oil to the cartridge and then load into the analyzer.

8. Select the RLF and CDF file for your samples and then start Run. Depending on the FOV (fields of view) selected takes between 2- 8 hrs for 1 cartridge.

9. When run is done, download the RCC file created for your samples and proceed to analysis.

A- Normalization Count

1- SUM all 12 (raw data) Positives and 8 (raw data) Negatives of each sample
2- Average SUM of all samples
3- Normalize factor: Average SUM divides SUM of each sample
4- Normalize Count: Data count of each gene divides to normalize factor across all samples.

B- Background Subtract (True Count Normalization):

1- Average all Normalize Count of Negatives of each sample
2- AVERAGE of all average for all genes across all samples
3- True Count Normalization: Subtract the Normalize Count of each negative to AVERAGE of all genes

C- Cut off 10

1- Average all the True Counts
2- Filter by cutting off 10 (>10)

Normalization by end user:

1. Normalize to housekeeping genes (per-chip).
    a. 20 HK genes are included in CBC codeset
    b. 3 of them could be excluded for low signals (RPS24, GNAS, RPS19) (needs to be confirmed by other experiments too)
    c. DDX5 may be excluded as well (not correlated with microarray data)
    d. Expression values are scaled to the median of selected housekeeping genes for each sample
2. Normalize to healthy controls
3. These steps can be easily done in Excel. If you prefer, you may also create a genome (or technology in GX10, there is a bug in GX10, and it is not working properly) and perform normalization in GeneSpring.

Data transformation:

1. Log base 2 transformation should be applied to the data in order to be consistence with microarray analysis

Analysis:

1. Heat map of all genes
2. Module map is not possible since not all the genes are included in the codeset