somalogic

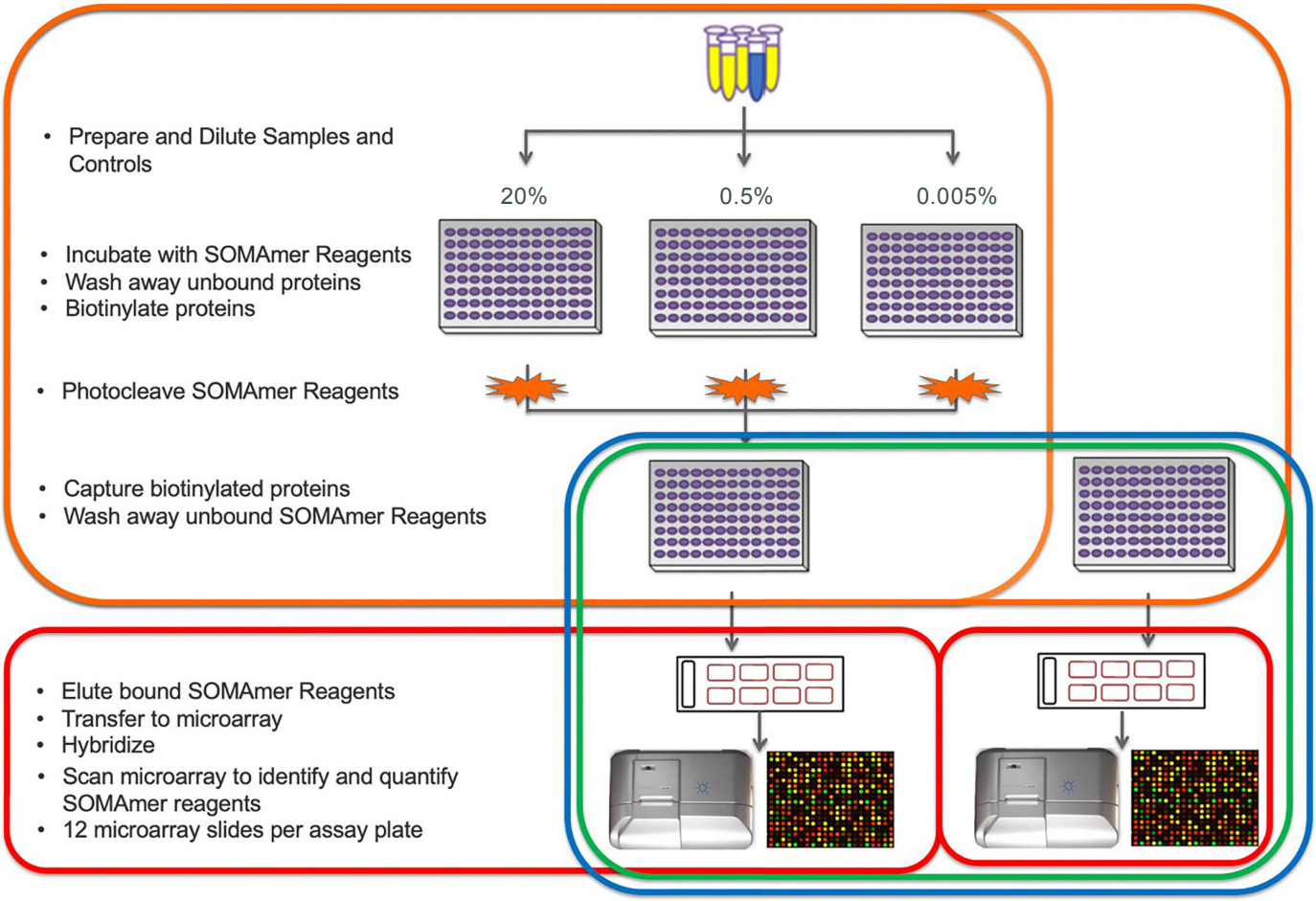
SomaScan®
v4.0, v4.1, and v5.0
Data Standardization

Raw SomaScan Assay data may contain systematic biases from many sources, such as variation introduced by the readout, pipetting errors, inherent sample variation, or consumable reagent changes. Standardization is an important tool for removing nuisance variance.

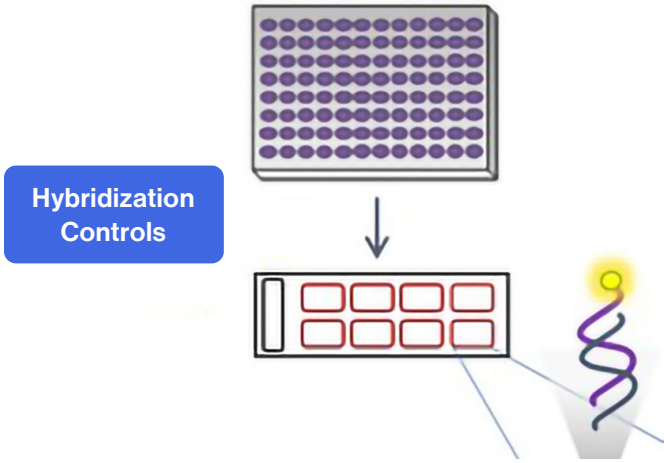
This document briefly explains five techniques used to standardize SomaScan v4.0, v4.1, and v5.0 Assay results and a sixth quality control step.

A schematic of the SomaScan Assay is illustrated below with colored boxes indicating the source of variation eliminated by each data standardization technique:

1. **Hybridization normalization** controls for variability in the readout of individual microarrays using 12 control SOMAmer® reagents added to the eluate just prior to readout.
2. **Intraplate median signal normalization** controls for total signal differences caused by assayvariance between individual Calibrator Controls within an assay run.
3. **Plate scaling** accounts for plate-by-plate total signal variation typically associated with scanner intensity differences.
4. **Calibration** removes the variation between assay runs within and across experiments on a SOMAmer-by-SOMAmer reagent basis.
5. **Adaptive normalization** to a reference controls for total signal differences between individual samples and is an optional step that is dependent on the study design and expectations.
6. **Quality control check** uses replicate Quality Controls (QC) that are pooled matrix-matched samples (e.g., plasma, serum) run in the SomaScan Assay alongside clinical samples to quantify the quality of each assay run by determining the accuracy of the median replicate signal for each SOMAmer reagent compared to the reference. QC check is performed after steps 1-5 above have been applied.



Hybridization normalization mitigates bias caused by differential readout conditions (eluate transfer, hybridization, wash, scan) in individual microarrays. Control SOMAmer reagents are not exposed to protein but added during the transfer of SOMAmer reagents to the Agilent slide. The control signals are then used to calculate a single scaling factor that is applied to all spots in the microarray.



Intraplate median signal normalization was developed to account for differences in total signal between replicate control samples. It uses measurements from Calibrator Controls to calculate a sample-based scaling factor for each of the three dilution groups.

Like the Quality Controls, the Calibrator Controls are matrix-matched samples (e.g., plasma, serum) that are run in replicate in the SomaScan Assay alongside clinical samples. Plate scaling and calibration adjustments require data from invariant controls that are run in replicate in all assays. These data are used in conjunction with references generated during assay qualification to calculate a scaling factor for every plate and SOMAmer reagent.

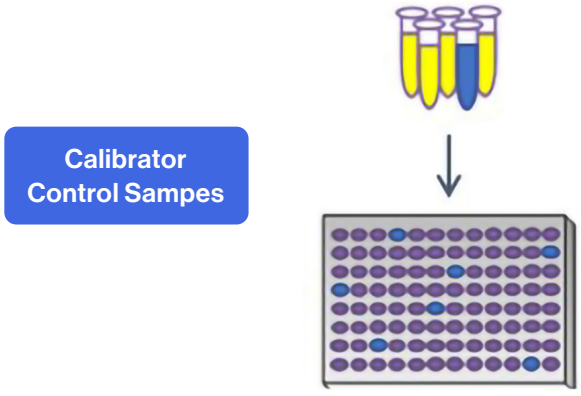


Plate scaling adjusts for total signal variation that occurs from plate to plate, typically associated with scanner intensity differences. The median value of the calibrator reference ratios is used to calculate a single scale factor for an entire plate.

Calibration reduces the variability between runs and/ or entire experiments on a SOMAmer-by-SOMAmer reagent basis. The ratio of the SOMAmer-specific reference value to the median value of the Calibrator Controls is the calibration scale factor for the SOMAmer reagent across the run.

Adaptive normalization to a reference, Adaptive Normalization by Maximum Likelihood (ANML), adjusts for inter-sample technical and biological variability in total signal within and between runs. A scale factor for each set of SOMAmer reagents within a dilution bin is computed using only sample values within two population standard deviations of the normal reference; the process is iterated to convergence and maximizes the probability that a sample's RFU measurements come from the reference distribution. The scale factor for each dilution bin is then applied to their respective SOMAmer reagents. This step is applied to QC and individual samples.

Note: *Normalization to a reference standardizes the overall signal from every sample and is appropriate for experiments where all samples should have approximately the same amount of total protein.*

For samples with inherently different protein amounts (e.g., Duchenne Muscular Dystrophy or leukemic blood),median signal normalization to a reference will mute variations in protein levels that may be of interest. For further details, please refer to the SomaLogic Technical Note SL00000063.

SL00000442 Rev5: 2023-12

SomaScan v4.0_v4.1_and v5.0 Data Standardization