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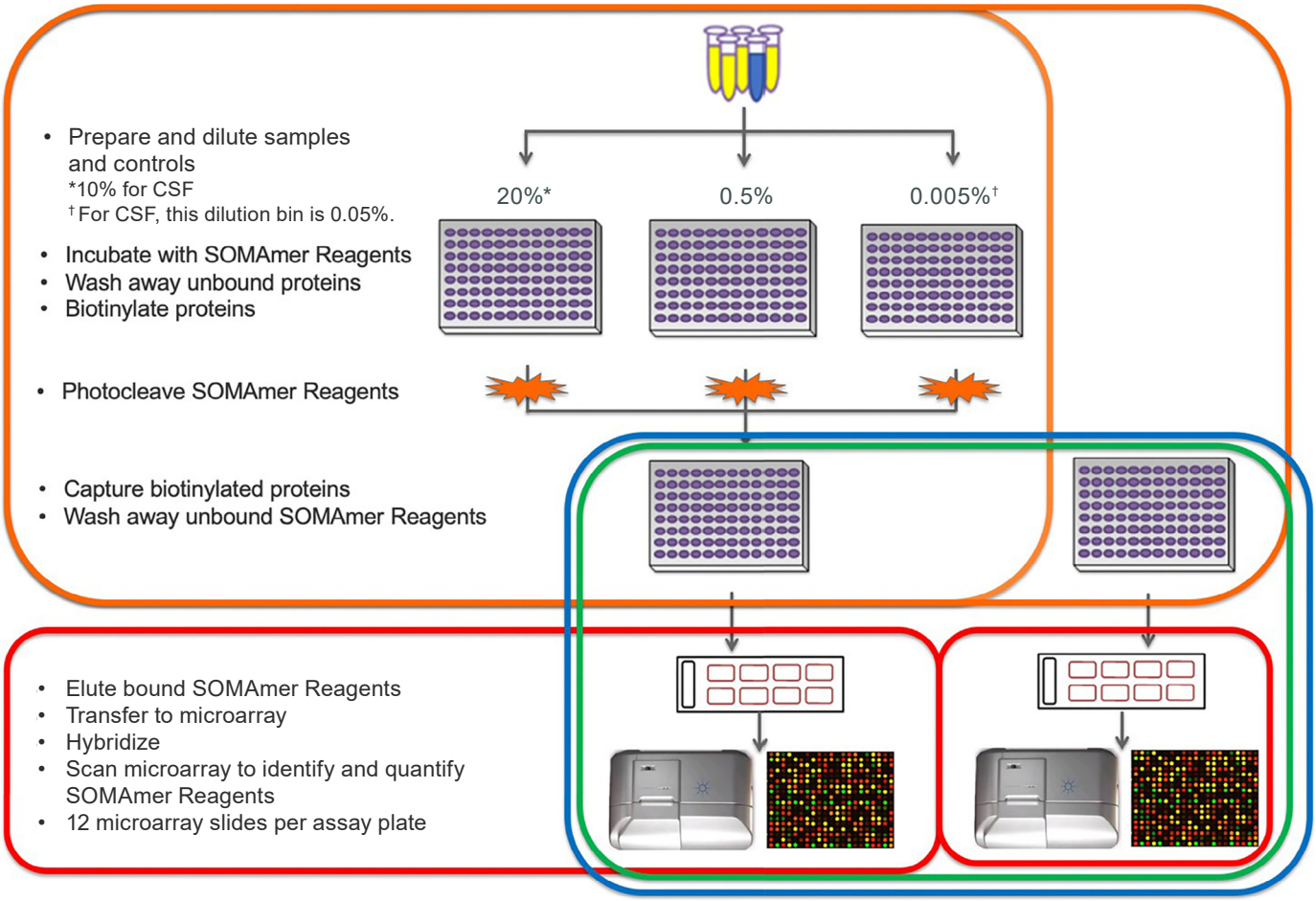
Data Standardization on the SomaScan 5K, 7K and 11K Assays

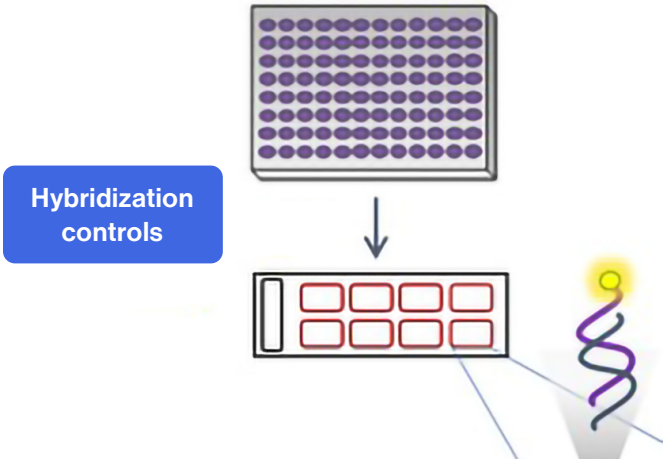
Raw SomaScan™ Assay data can include systematic biases from sources such as readout variation, pipetting error, sample heterogeneity and reagent changes. Standardization of the data helps reduce this nuisance variance. This document summarizes 5 standardization techniques employed in the SomaScan Assay for human EDTA plasma and serum (5K, 7K, 11K) and CSF (11K), plus a 6th 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 assay variance 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 Reagent 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 (for example, plasma, serum, CSF) 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 with 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 3 dilution groups.

Like the quality controls, the calibrator controls are matrix-matched samples (for example, plasma, serum, CSF) 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. This data is used in conjunction with references generated during assay qualification to calculate a scaling factor for every plate and SOMAmer Reagent.

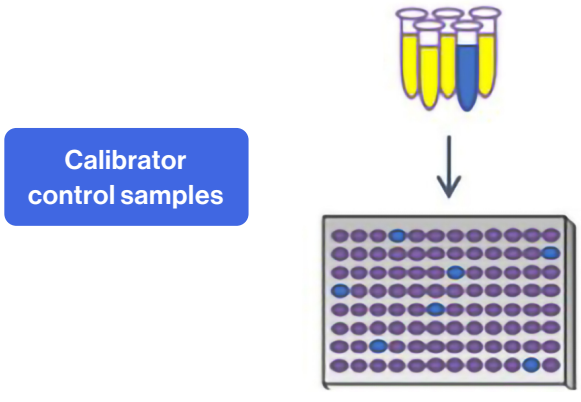


Plate scaling adjusts for total signal variation that occurs from plate to plate, typically associated with scanner intensity differences. The median value of the calibrator reference ratios is used to calculate a single scale factor for an entire plate.

Calibration reduces the variability between runs and/or entire experiments on a SOMAmer Reagent by SOMAmer Reagent basis. The ratio of the SOMAmer Reagent 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 2 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 in which all samples should have approximately the same amount of total protein.*

For samples with inherently different protein amounts (for example, heparin plasma, samples from pediatric donors, cell lysates, non-human plasma, serum, urine and CSF, and any other unique specimen type), an alternative normalization strategy called intra-study median normalization is performed. A data file that does not include the final ANML or intra-study median normalization step is also provided, which allows researchers to employ alternative normalization methods as preferred.

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