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SomaScan™ Assay v4.1 Signaling in Non-Human Plasma

This technical note describes a series of studies to demonstrate signaling of mouse plasma and other non-human species in the SomaScan Assay.

Introduction

Animal studies are an integral tool in understanding human biology and disease. Mice are a particularly useful model organism due to their genetic, physiological, and pathophysiological similarities to humans and the ability to manipulate biologically. Mouse models are regularly used in biomedical research and serve as a precursor to clinical drug development where availability and testing on human subjects would otherwise not be possible.

The SomaScan® Assay is the only proteomic technology capable of making over 11,000 total proteomic measurements, including 10,000 unique human proteins in a single sample. Numerous published studies have demonstrated the utility of this assay in mice and other non-human samples for biomarker discovery of different diseases, understanding mechanisms of drug treatment and toxicity, and more. The results of these SomaScan studies are further supported by other experimental approaches such as RNA expression analysis and ELISA.[1-7]

SOMAmer® Reagents used in the SomaScan Assay are generated by SELEX (Systematic Evolution of Ligands by EXponential enrichment) and bind specifically to a three-dimensional epitope on their human protein target. Binding to a non-human ortholog is dependent upon the conservation of the amino acid sequence and the overall three-dimensional structure of the epitope to which the aptamer binds. Depending on the extent of the differences between the human and non-human proteins, the SOMAmer Reagent may bind with the same affinity or with reduced (or no) affinity for the non-human protein. This was demonstrated in a small internal study where the likelihood of a SOMAmer Reagent binding to its rodent homolog generally correlated with higher protein sequence similarity to the human target. Consequently, protein binding across the menu of SOMAmer Reagents will vary, at least in part, in proportion to the evolutionary distance between the two species.

SomaLogic has conducted a series of studies on the SomaScan 7K Assay to identify SOMAmer Reagents that signal in mouse plasma, rat plasma, dog plasma, and hamster plasma samples. Signaling is based on two metrics, dilution linearity and F-statistic, which are further described below for mouse, however the same concepts apply for the other tested species. This information can be used to guide researchers in understanding certain characteristics and expectations of a target signal in mouse plasma samples in the SomaScan 7K Assay. Customers interested in harnessing more content can also utilize the SomaScan 11K Assay for most non-human plasma and serum such as mouse, dog, non-human primate, cat and pig. The SomaScan 7K and 11K platforms are highly concordant within human EDTA-plasma and serum. Considering the average mouse protein amino acid sequence similarity to humans is about 90%, one can extrapolate that the proteomic coverage for mouse plasma and serum scales with the increased content of the SomaScan 11K Platform.

Protein Signaling in Mouse Plasma

Dilution groups, diluents, and control samples used in the SomaScan Assay have been optimized for human EDTA-plasma and human serum. To determine a suitable dilution and assess the dilution linearity for mouse EDTA-plasma samples, a SomaScan Assay v4.1 study (7K) was done by running a 16-point, two-fold titration series (starting at 40% dilution) of a pooled set of 12 individual mice (6 male, 6 female) from 3 different strains (BALB/c, C57BL/6 and CD-1). For the pooled EDTA-plasma mouse samples, the result was sufficiently consistent with human plasma to use the same dilution assignments: 0.005%, 0.5%, and 20%. As for the dilution linearity, analytes exhibiting a minimum of 3 consecutive dilutions that titrated linearly (within 25% of the nominal value determined at the highest dilution where linearity began) were deemed to be in the linear dilution range.

To estimate protein target signal from mouse EDTA plasma in the SomaScan Assay, over 100 individual mice (equal male and female numbers) from 4 different strains (BALB/c, C57BL/6 and CD-1, Swiss Webster) were assayed. In addition, the individual samples were pooled together and assayed over 100 times as technical replicates to estimate assay variability and calculate the F-statistic.

The population F-statistic is the ratio of variance for an individual protein measurement across the 100 mouse samples divided by the variance of the protein measurement across the pooled technical replicates. F-statistics greater than 2.41 critical value (with multiple corrections and 95% confidence) indicate that signals measured across various individuals exceeded the assay variability. In other words, a dynamic biological measurement was made rather than technical noise. Eighty-three percent, or 6,055 SOMAmer Reagents in the SomaScan Assay v4.1 content exhibited an F-statistic greater than the critical value.

Animal	High	Medium	Low
Mouse	2653	3726	910

TABLE 1 Number of SOMAmer Reagents in the SomaScan Assay v4.1 menu in high, medium, and low qualitative bins

Analytes were binned into three qualitative groups (Table 1): those in the linear dilution range with F-statistics greater than the critical value (high), those either in the linear dilution range or with F-statistics greater than the critical value (medium), and those not in the linear range and with F-statistics less than the critical value (low). Refer to Table 2 for information from similar rat, dog and hamster plasma experiments.

Content in the high category consists of 2,653 SOMAmer Reagents whose signals were shown to titrate linearly for at least 3 consecutive dilutions and had distinguishable signals between the group of individual mice that were tested. Content in the medium category consists of 3,726 analytes whose signals were shown to titrate linearly for at least 3 consecutive dilutions but did not show significantly greater signal variance in the group of individual mice compared to the variability of the analyte in the assay itself. Also in this medium category are analytes whose signals were discriminated across the group of individual mice from the assay variability but were not in the linear dilution range. Content in the low category (910 SOMAmer Reagents) did not signal in the linear range pooled sample, nor was it possible to measure changes that were greater than the assay variability across individual subjects.

It is possible that proteins not meeting the F-statistic critical value are biologically stable across individual mice, but true biological changes (that are greater than the assay variance) may be measurable in various disease states or treatments. Conversely, the pooled sample which is used to determine whether an analyte is in the linear range may not be indicative of a population, and so those analytes that were not found to be in the linear dilution range but still had measurable signal changes across a large set of individual mice (a significant F-statistic) can be just as valuable demonstrations of signaling.

Protein Sequence Identity Between Mice and Human

While the F-statistic and linearity do not directly address SOMAmer Reagent specificity to the target, the ability to detect robust differences across a set of samples (per the F-stat) skews toward proteins with higher sequence similarity, agreeing with the concept of epitope conservation across species and that the reagent is binding to a protein (Figure 1).

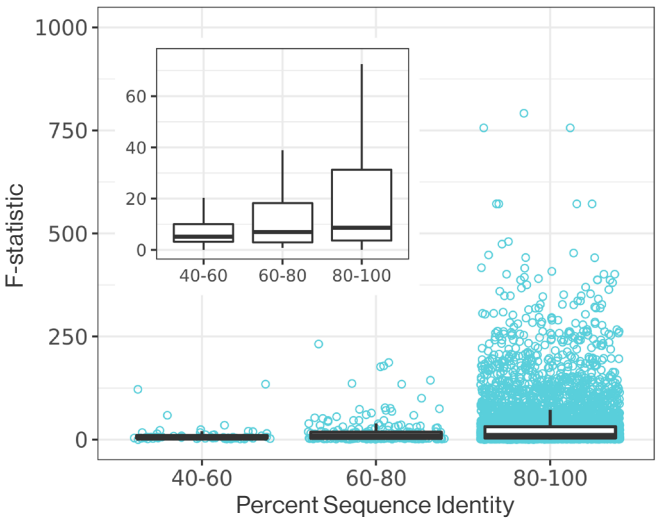

FIGURE 1 Sequence identity between mice and humans vs. F-statistic.

Most proteins have a high sequence identity between mice and humans (90% average). One can see that higher sequence identity (>80%) tended to be associated with higher F-statistics and a larger range of F-statistical values. Generally, those proteins with low sequence identity (<60%) had a lower F-statistical value, suggesting the lack of a robust SOMAmer Reagent measurement across individuals may be due to a lack of availability of its conformational epitope in the mouse ortholog. Since a SOMAmer Reagent is selected against human proteins, it makes sense that when the three-dimensional epitope is conserved in a mouse protein, there is greater likelihood it can bind to the mouse ortholog.

Conclusion

SomaLogic has demonstrated that a large portion of the SomaScan Assay v4.1 content (88% in the high- and medium-qualitative bins) was shown to signal in mouse plasma samples based on the metrics described above, which agrees with the percent sequence identity assessment performed in Figure 1. This information offers a deeper level of assessment to investigators who are interested in harnessing multiplexed proteomics in mouse samples for biomarker discovery, understanding molecular mechanisms of disease, assessing drug candidates, and more.

It is important to note that the target signal may vary depending on mouse strains, populations, or experimental conditions and disease state used in other studies. The qualitative bins are based on thresholds established for the dilution linearity and F-statistic, however content in each bin can fluctuate and special consideration of the unique characteristics of each mouse study should be taken into account. It is at the user's discretion to decide the prioritization of signaling metrics based on their specific needs. The F-statistic and quality category metrics for each SOMAmer Reagent in mouse plasma can be found in the Menu Query Tool (accessible at https://menu.somalogic.com/). Similar information for the other species listed in Table 2 can be provided upon request by contacting techsupport@somalogic.com.

Animal Model	Percent of Reagents in Each Qualitative Signaling Bin			Percent of Signaling Content	Average Protein Sequence Similarity to Human
	High	Medium	Low	High + Medium	
Mouse	36%	51%	12%	88%	90%
Rat	18%	53%	28%	72%	86%
Dog	29%	57%	14%	86%	88%
Hamster	36%	52%	11%	89%	87%

TABLE 2 Summary of SomaScan 7K Assay content in high, medium, and low qualitative bins for multiple species

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