**Microarray Analysis of Glycan Microarrays**

We have many years of experience analyzing defined, as well as shotgun glycan microarrays. Glycans microarrays are produced either by outside sources or in our core EGMIC. EGMIC staff can carry out assays using your glycan binding proteins (GBP) or organism(s) and assist in data analysis and interpretation.

**Technical Notes**

*Sample concentration:* The amounts of protein needed to bind to an array will depend on the affinity of protein-glycan interactions. Cholera Toxin, for example, binds strongly with extremely high signals on the microarrays at protein concentrations in the ng/ml range, while purified anti-glycan antibodies required 1 to 50 µg/ml. We find that if proteins at 200 µg/ml do not bind, they will not bind at higher concentration. Cell culture supernatant is assayed as neat, human plasma or serum is normally assayed at 1:50 dilution.

*Sample volume:* For most glycan array analyses we need minimum 70 µL of to apply on the slide after sample is diluted in binding buffer. Some arrays need 200 µL depending on the slide printing layout.

*Buffer:* Commonly used buffer to dilute your sample

1. TSM buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM CaCl2, 2 mM MgCl2, pH 7.4) with 0.05% Tween20 and 1% BSA
2. PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4 , 1.8 mM KH2PO4 , pH 7.2) with 0.05% Tween20 and 1% BSA

*Detection*: Our glycan microarray needs a detection system based on fluorescence.

* For sample labeled with fluorescent dye, no additional detection antibody is needed (one step of incubation).
* For expressed protein with a tag, we will use fluorescent labeled antibodies to the tag for detection (two steps of incubation).
* If you have antibody to your protein that you use for other assay platform (such as western blot, ELISA or flow cytometry), please send your antibody along with the sample. We will use a fluorescently labeled secondary antibody for detection (three steps of incubation).
* We have a collection of commonly used fluorescent labeled antibodies.
* Slides are scanned in an InnoScan 1100AL scanner with data processed using Mapix 8.2.5 software (Innopsys, Chicago, IL).

*Results deliverable:* The output of sample glycan binding profile is a list of glycan structures on the array and the corresponding average Relative Fluorescence of 4-6 replicate spots with a STDEV or %CV for each.  These data are in Excel spreadsheets with a histogram.

**General Protocol**

Below is a protocol to assay biotinylated lectins on a microarray slide followed by detection with fluorescent labeled Streptavidin. Lectins are commonly used for glycan array quality control because each lectin has defined specificity to terminal oligosaccharides on glycans: ConA binds mannose (Man), AAL binds fucose (Fuc), RCAI binds galactose (Gal) and SNA binds sialic acid (Neu5Aca6)

1. Prepare a cocktail of four biotinylated lectin types in TSM binding buffer (TSM buffer with 1%BSA and 0.05% Tween 20): 0.5 µg/ml biotin-ConA, 0.5 µg/ml biotin-AAL, 1 µg/ml biotin-RCAI, 1µg/ml biotin SNA.
2. Warm up a slide at room temperature (RT) for 20 min.
3. Rehydrate the slide with TSM buffer plus 0.05% Tween 20 for 5 min.
4. Apply 70 µl of diluted biotinylated lectins cocktail on slide, cover with a cover slip and incubate the slide in a dark humidified chamber for 1 hour at RT.
5. Wash the slide four times with 100 ml TSM buffer with 0.05% Tween 20, then four times with 100 ml TSM buffer.
6. A second step incubation is performed using 2 µg/ml Alexa-Flour 488 Streptavidin in TSM binding Buffer. 70 µl of 2ug/ml Alexa488 -SA is incubated on the array with a cover slip for 1 hour at RT in a dark humidified chamber. Cyanine 5 Streptavidin (1 µg/ml) could replace Alexa-Flour 488 Streptavidin.
7. Wash the slide four times with 100 ml TSM buffer with 0.05% Tween 20, then four times with 100 ml TSM buffer, then four times with 100 ml Milli-Q water.
8. Insert the slide into a clean 50 ml conical tube and centrifuge at 1000 rpm for 3 min. Or centrifuge the slide with a slide centrifuge for 20 seconds.
9. Scan the slide with InnoScan AL1100 scanner using 488 nm laser with power setting at high and gain setting at 80.
10. Process the image TIF file using Mapix 8.2.5 software (Innopsys, Chicago, IL) and fluorescent signals are quantified.
11. Data analysis is done with an Excel macro or R program.