**Glycan and Custom Microarray Printing**

**General Protocol**

1. *Planning:* 
   1. Contact EGMIC to get an initial consult on your printing project.
   2. Provide us with details such as a printing sample list, type of slides, array amount, and a timeline.
2. *Source plate preparation*
   1. Samples to be printed should be free of precipitates. Pass it through a Mini-filter unit or by centrifuging at 10,000 rpm for 5 min and transfer the supernatant only to a clean tube.
   2. Load the source plate according to a source plate map. The minimum printing volume requirement is 10 µl. Seal the plate with film and centrifuge at 2500 rpm for 5 min.

* Sample is normally printed at 100 µM for glycan and 200 µg/ml for proteins. For example, 5 µl glycans at 200 µM and 5 µl 200 mM phosphate buffer (pH8.5) are loaded into a well of 384 well conical plate.
* Consider including positive and negative controls for glycan and protein.
* In addition, consider including gridding spots, choosing from Biotin-BSA, Cy5- or Alexa488- Streptavidin, Cy5- or Alexa488-Hydrazide.
* Filtered 0.5% Tween 20 is included in the source plate and used as wash detergent for pins.

1. *Slide preparation*
   1. Schott Nexterion NHS slides are stored at -20oC. Warm up slide at room temperature inside the desiccator container with vacuum applied for at least 20 min.
   2. Epoxy slides and nitrocellulose coated slides are stored at room temperature. They can be used right away.

1. *Aushon2470 Printer operation* 
   1. Refill wash tank with Milli-Q water, empty waste tank, refill the water in humidifier.
   2. Clean pins with acetone, change pin configuration, and load pin.
   3. Edit the source plate and design run configuration
   4. Remove the seal from the source plate, cover with a plastic lid, snap the plate in the plate holder and put the plate holder into the plate loading deck.
   5. Place slides on the slide loading platen and put the platen on the slide loading deck.
   6. Start the print. Print report and a gal file are created automatically after printing completion.
   7. Monitor the printing process. The printer will automatically pause if the wash tank is low or the waste tank is full. Refill wash tank with Milli-Q water and empty waste tank and resume the printing. If other error occurs, contact our staff and we will contact technical support from the vendor.
   8. When printing is completed, remove source plate and slides, close software, and turn off Printer power. Post printing treatment on the slide see section 5 below.
   9. Seal the plates and store the source plates at -20oC. When preparing to use it again, thaw the plate in room temperature, centrifuge, add water to each well to bring the final volume to 10 µl, sonicate in the water bath for 10 min, and centrifuge.
2. *Slide treatment after printing.*
   1. *Overall procedure*
      1. Place the slides in a chamber in which the slides can be placed horizontally and have a piece of paper towel of appropriate size to block between the slide and the lid. Place the chamber in a 55oC water bath to get a humidified environment for 1 hour.
      2. Dry the slides at room temperature overnight.
      3. Block slides at room temperature for 1 hour. See section 5.2 for details.
      4. Slides are ready for use or stored at -20oC for long-term storage.
   2. *Blocking Procedure*
      1. Wash slides 4 times in 100 ml of PBS buffer (pH7.5) with 0.05% tween20.
      2. Wash slides 4 times in 100 ml Milli-Q water.
      3. Block each slide in 30-50 ml blocking solution for 1 hour at room temperature.

* The blocking solution for NHS slide is 50mM ethanolamine in 50mM sodium borate, pH8.0.
* The blocking solution for Epoxy slide is 50mM ethanolamine in 20 mM Tris, pH 8.0.
* The blocking buffer for Nitrocellulose slide is 4% BSA (Bovine Serum Albumin) in PBS (pH7.4) with 0.05% Tween20.
  + 1. Wash slides 4 times in 100 ml of PBS buffer (pH7.5) with 0.05% tween20.
    2. Wash slides 4 times in 100 ml Milli-Q water.
    3. Centrifuge slides in a 50 ml conical tube at 1000 rpm for 3 minutes to dry or spin the slide in a slide centrifuge device for 20 seconds to dry.