Dot Blot Assay

- Dot Blot Apparatus
- Forceps
- Bio Dot SF Filter Paper
- Zeta-Probe Blotting Membranes
- Container to hold blot
- MilliQ water
- Multichannel pipeter
- Trough for multichannel
- Blocking solution (PBS pH 7.4, consisting of 10% SDS and 1mM EDTA)
- PBS (10% SDS, 0.1%SDS)
- ECF reagent

1. Place two pieces of Bio Dot SF filter paper in milliQ water.
2. Take a membrane and cut the bottom right corner. This is necessary so as to know the orientation of the Blot after imaging. Place membrane in milliQ water.
3. Attach unassembled Dot Blot Apparatus to pump. Turn on pump and ensure that vacuum is directed towards Dot Blot. Under vacuum, assemble the Dot blot in the following order; gasket support plate, gasket, two pieces of Filter Paper, and membrane.
4. Screw on the sample template with attached screws in a diagonal fashion (top right, bottom left, bottom right, top left). Tighten the screws as tightly as possible. If the apparatus is not tightly sealed, the wells will leak.
5. Turn the valve towards air (away from the Dot Blot) and turn off the vacuum. Using the multichannel pipeter, pipet 180l of milliQ water into each well. Turn vacuum all the way up and turn valve to Dot Blot. Push on Dot Blot and check to see that all of volume is drained from each well. Turn valve away from Dot Blot and turn off vacuum. Some wells drain slower than others. Be cautious of the first column.
6. Once you are confident that the wells are draining properly, refill the wells with 180l water. If not doing serial dilutions for samples, place 1g of sample into a designated well.
7. Make sure to include a serial dilution of the DNA 55mer biotin oligo. The DNA oligo should be in solution at 100ng/l. In small PCR tubes, serially dilute sample in two fold dilutions from 100ng - 3.125ng (six dilutions). Place 1l in each well of serial dilution in a single row.
8. Pipet 500ng of each sample in triplicate where possible. The volume in each well should be approximately the same for wells to drain properly, so if pipetting in a large volume (a dilute sample), remove the same volume of water from the well as you will insert sample.
9. Turn on the vacuum. Turn the valve towards Dot Blot. Wait for all wells to drain. Turn valve away from Dot Blot and turn off vacuum.
10. Unscrew sample template and pull off membrane. Good suction is evident from the membrane and filter paper sticking to the sample template.
11. Place membrane on saran wrap and put into UV photocrosslinker. Apply autocrosslinking 2X.
12. Block the membrane in blocking solution for 20 min with rotation on “The Belly Dancer”, with a setting ~5-6.
   a. Did the SDS crash out of solution? Streptavidin isn’t picky. Wash it with two more batches of blocking solution (5 min), or until it stops crashing out. Then proceed with probing, even if the probing solution crashes out after about 5-10 min.
13. Probe membrane in blocking solution with Streptavidin-AP at 1:1000 dilution for 15 min.
14. Wash the membrane in PBS (0.1%SDS), two times each, for 10 minutes each time.
15. Expose the blot to ~2.9 ml of ECF reagent, but make sure entire membrane is covered. A good way to do this is to pipet over the membrane for 5-10 min exposure time to ECF. Make sure to do this to the side containing the RNA.
16. Image the membrane on the Typhoon. Place the membrane RNA side down and use the setting “BSN” at 100microns resolution and 750mV. Analyze using ImageQuant. Export values to file and save on jump drive.
17. Use ImageJ and R to analyze.

References: