MAGPix Assay Notes.

Training session with Sabrina Hawthorne.

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Present

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MACHINE PREP

1. Remember, the MAGPix is a fluids based machine. It needs to be kept clean, and it needs to be calibrated and flushed regularly in order to work properly.
2. Turn it off in the back (hard power off in the back) when not in use. This will help GREATLY in instances of power outages. A power outage (with the instrument on) will cause a probe height malfunction.
3. Turn instrument on (button in front), and start-up software. There is no warm up time needed.
4. Press log-in (there is no log-in ID for software, you can leave that blank).
5. Click on the Maintenance tab.
6. Go to commands and routines.
7. Our lab tends to use the instrument 1/week or so, meaning we need a more thorough cleaning than the “daily routine”. Go to Enhanced Start-Up routine.
8. Click eject and fill up the strip well reservoirs with each of the liquids outlined on the screen, and run the cleaning. Cleaning routine takes about 5min.
9. If any issues, check the probe. Take the probe out, and flush with water to make sure fluids go through.
10. Probe can also be cleaned more thoroughly by putting in a water bath sonicator.
11. Full troubleshooting guides are included on the computer associated with the MAGPix, including tutorial videos, in case issues arrive. A technical number is also on the desktop
12. Go to Auto Maintenance
13. Go to calibration/verification
14. When you get a new lot number for the calibration kit, you will need to “import kit”.
15. Vortex each of the bead vials, and add 6 drops of each bead vial into the strip well as designated on the software. 5 drops is not OK, but 7 is.
16. Add strip well to MAGPix and run.
17. Make sure calibration has cleared.
18. Calibration should be run every week. Software will remind you!

MACHINE PREP, DAY 2

1. May need to run calibration again, especially if delta T > 5C (from last calibration to when you’re running your assay).
2. Set up protocols (method for a kit) and batches (data for each separate experiment).
3. If you haven’t run your kit before, create a new protocol. Add volume (from instrument settings in manual), 96-well plate, type of data analysis. Can do qualitative (for fold-change) or none (to get raw data in Excel file). Need standards to compare to if you do quantitative.
4. Hit next and add beads that you’re looking at in your experiment (from manual again). Change names to the actual names.
5. Hit next and set up 96 well plate. Only need to add the controls and not your samples into this. If you chose none for data analysis, do not need to add controls to wells on the program. Label as unknowns instead.
6. Go to batches, create new batch from existing protocol. Give it a name – this will be your data file. Select your protocol and click next.
7. Add samples – make them all unknowns with 1 replicate. Save it!
8. Once plate is ready (from Assay Prep, Day 2), go to Run, and run assay, with your batch and protocol selected.

SAMPLE PREP

1. Collect samples with MAGPix provided lysis buffer, plus protease inhibitors. MAGPix lysis buffer already includes Sodium OrthoVanadate.
2. Run the BCA protein assay.
3. From the BCA, obtain a protein concentration, in ug/uL for each of your samples.
4. From your concentrations, identify the least concentrated (i.e. most dilute) sample. Take this concentration and multiply it by 12.5uL (this is the maximum volume of sample you can load in the MAGPix assay). This will give you the total protein to be loaded for all your samples.
5. Ideally, you want to be able to load >=5ug of total protein for every sample. This is not always possible, but this is the goal to shoot for. If you are under this amount, make an adjustment for next time to reduce your lysis buffer volume during lysis, and therefore increase your concentration of lysate.
6. If you have a few samples that are very dilute, don’t bother running them. Identify another ‘minimum concentration’ sample, and re-do steps 4 and on.
7. For all remaining (non-min) samples, divide the total protein to be loaded, in ug, by the sample concentration, in ug/uL. This will tell you how many microliters of each of the other samples to load in each well.
8. Also, for each well, take 12.5uL – the value found in #7. This will tell you how many uL of lysis buffer you will need to queue each sample to come to a total volume of 12.5uL in each well.
9. Create a sample map for your samples. After some wells are taken by positive and negative controls, there is room for a total of 86 samples. You can either use your own technical replicates from you experiment, or run each of your lysates twice as a technical replicate.

NOTE: this was written for the 48-680MAG kit – 9-plex ERK multi-pathway signaling kit. Each kit will come with its own pamphlet, which are slightly different. The kit-accompanied pamphlet should always be followed.

ASSAY PREP, DAY 1

1. Get kit from the fridge, and allow all reagents to warm up to room temperature.
2. Thaw lysates on ice on bench top.
3. Sonicate beads for 15s, and vortex for 30s and room temperature.
4. NOTE: for pretty much any reagent, vortex reagent briefly (1-2s) immediately before use.
5. Pre-wet the assay plate with 50uL of assay buffer for ~10min. Cover plate so dust doesn’t get in wells. Make sure to save all unused assay before for subsequent steps.
6. Dilute beads to 1X by combining 150uL of beads with 2.85mL of Assay buffer 2 into the provided mixing bottle.
7. Dump the assay buffer in the sink, and knock rest of liquid out onto a kimwipe.
8. For all the wells that will have sample, add 12.5uL of Assay Buffer 2.
9. For each sample, add the needed amount of lysis buffer to queue all samples to 12.5uL. This was calculated in SAMPLE PREP step 8.
10. Now, add your lysate to each well, as calculated in SAMPLE PREP #7.
11. Seal your plate with a plate sealer (provided in kit). Cover in foil, and shake, O/N in the cold room on an orbital shaker at ~200rpm. Ramp up slowly to avoid the liquid hitting the lid.

ASSAY PREP, DAY 2

1. NOTE: all day 2 times are exact. i.e. 1 hr = 1 hr! not 1hr15min. Keep these timings closely monitored.
2. Get your plate from the cold room. Allow assay buffer to warm up to room temperature before beginning.
3. Dilute 150uL of biotinilyated antibody (labeled biotin) into 2.85mL of Assay Buffer 2 into a new mixing bottle.
4. Put your plate onto the magnet. Make sure to line up correctly. Hold plate down as you clamp the plate into place. Allow plate to sit on magnet for 1min before starting washes.
5. Decant the solution off of the plate into a waste tray (or sink).
6. GENTLY press the plate onto a kimwipe to remove excess waste.
7. Wash beads with 100uL of Assay Buffer 2. 2x.
8. Add 25uL of diluted detection antibodies to wells.
9. Seal with plate sealer and shake at room temperature at 175rpm for 1h.
10. Make streptavidin-PE before this hour is up. Vortex antibody and dilute from 25x to 1x in assay buffer 2. 2.88ml assay buffer 2 + 120ul of streptavidin-PE in a new mixing bottle.
11. Add plate to magnet, sit for 1 min, and then decant solution.
12. Add 25uL of Strep-PE solution to each well, and incubate on plate shaker for 15min at 175rpm. Add solution quickly and evenly to ensure that 15min doesn’t turn into 17min.
13. DO NOT REMOVE strep-PE solution or wash.
14. Add 25uL signal amplification buffer. Do this quickly, but cleanly, to keep times even at 15min. Again, timing is important in these steps.
15. Seal plate with plate sealer and foil, and shake at RT at 175rpm for 15min.
16. Place the plate on the magnet, sit for 1min, and then decant off the amplification buffer.
17. Take off the plate, and add 150uL of Assay Buffer 2. Volumes here don’t have to be exact, just move quickly, and make sure not to touch the sides of the wells with your pipette tip (beads are in solution).
18. Seal plate back up, cover in foil, and shake at RT at 175rpm for 5min.
19. After 5min, add plate to MAGPix and run your sample.