Bauer Core Standard Protocol		
Title: Cytek Aurora		
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1. Purpose

This protocol provides instructions for running samples on the Cytek Aurora

2. Materials

Filtered samples
5mL polystyrene tubes or 96/384 round bottom plate
Bleach
Contrad
Distilled Water

PREPARING THE INSTRUMENT

1. Computer should be on and logged in, if not the password is Welcome1!
2. Turn on the Instrument by pressing power button on the side.
3. Fill sheath tank with MilliQ Water and dump waste down the drain, refilling with ~500mL of Bleach
4. On the desktop find Spectroflow, open the software and login in with your account
5. Make a new dH2O tube by adding 3mL of water into polystyrene tube.
6. Once the software is connected remove the old water tube and replace with the new one
7. Open any experiment (this can be new or old) and run the water for 30-45 minutes
    a. Be mindful if running on High this may deplete the tube before time is complete
    b. This is important to warm up all the lasers correctly
8. After the water clean, stop running the sample and go to the QC tab
9. Create a new tube of QC beads if there is not one in the fridge, this is done by adding 1 or drops in 1mL of dH2O.
10. Remove the water tube from the SIP, wait for the backflush to complete before placing the QC tube on.
    a. Vortex the tube before placing on
11. Run the QC keeping an eye to make sure that the events/second are at least 150
12. Once QC is completed remove the tube and you can start running samples
13. If QC fails, follow these steps
    a. Try rerunning with new beads, making sure they are concentrated enough
    b. Run water for another 10 minutes
    c. Perform a flow cell clean with just water

RUNNING SAMPLES

1. Create a new experiment adding all the appropriate flours to the experiment
    a. It is important to be accurate with the right flours for example using AF647 vs APC
    b. If flours are not in the experiment add all the ones possible then create the experiment, then go to "Library" and add the missing color to the "Library" then you can go back to the experiment and add the missing color
2. Create reference groups based on the controls you have
    a. Make sure each reference control has a negative fraction in the tube
        i. If no negative is present add additional negatives to the list
    b. All controls should be treated the same as the samples
        i. Eg. if the samples are fixed then the controls need to be fixed
3. No voltages need to be adjusted (excluding scatter) unless the parameters are off scale
    a. Adjust the whole detector module when reducing the voltage to ensure that the spread is continuous across all channels
4. Record all the single stain controls in the Reference Group
5. Once reference group is finished Unmixing is available
6. Select the right peak for each flour, using the guide as an example
    a. If dye is not in the guide determine the brightest peak and select that channel
7. Live unmixing allows for the data already ran to be unmixed whereas unmix save and open creates a new template for new unmixed files

PLATE MODE

1. Make sure the plate loader is on
    a. If not, the power button is to the right of the machine by the lever
2. Remove the current tube on the sip and bring the lever up and towards you
3. Change the sample delivery to "Plate" under the acquisition settings tab
4. Eject the plate and then select the plate type and wells to run
5. Since this is vacuum based it doesn't run the set volume desired if it hits the criteria, for instance if 10,000 events were recorded before it hit 100uL it will not run the remaining volume
6. Note there is a large amount of dead volume, please add 75uL into your calculations
    a. If you need 100uL ran increase the well volume to 170uL
7. When finished with the plate change it back to tube mode and retract both the plate holder and mixer. The cleaning can be performed in tube mode.
8. Be sure to replace the tube of water once completed with everything.

CLEANING THE SYSTEM

1. Place the new bleach tube onto the sip and run on High for 2 minutes
2. Run dH2O on High for 2 minutes
3. Leave tube of water on the SIP and just log out of the software
4. If last user, please shut off the instrument
    a. Follow the commands in the "Fluidic Shutdown" menu under the QC tab

b. Shutdown the instrument after fluidic shutdown

BIOSAFETY

1. Closed toed shoes and clothing that goes below the knee is always required in the lab
2. Lab coat and gloves are required for BL2 samples and chemicals
3. Goggles must be worn in addition to a lab coat when pouring chemicals greater than 500mL
    a. Chemicals cannot be disposed of down the sink
4. Instrument cleanup is required after running BL2 samples which consists of 10 minutes of bleach followed by 10 minutes of water
5. Spray down the workstation with 70% ethanol when finished with the machine