Hybridization

1. Prepare the 10X aCGH blocking agent by adding 1350 ul of nuclease free water to the lypholized product in the tube provided.
2. Leave the blocking agent at room temperature for 1 hour. Vortex and spin the sample. You can aliquot and store at -20 degrees Celsius.
3. Thaw your labeled samples on ice in the dark if stored at -20.
4. Prepare a hybridization master mix by mixing 5.5 ul of 10X aCGH blocking agent and 27.5 ul of 2X Hi-RPM hybridization buffer per sample in a separate tube.
5. Measure the volume of the reference sample and divide that by the number of test samples +1. This volume will be the volume of reference that you will mix to each test sample. For eg., If after purification you have 25ul of labelled reference for 8 reactions (Remember 1 column was used to purify 8 labelled references), the to each test sample you will add 25/9 ul of reference sample. (The volume is divided by 9 instead of 8 in order to consider pipetting errors).
6. Add the volume calculated from step 5 + aCGH blocking agent per sample + 2X Hi-RPM hybridization buffer per sample. Subtract this from a total volume of 55 ul and that is the volume of the test sample that will be in each hybridization reaction.
7. If the test samples contain more volume than what is calculated in step 6, vacuum centrifuge the samples, (refer to Vacuum procedures), for 15 minutes.
8. Measure the volumes of each of the test samples again using a pipette and bring up each of the test samples to the desired volume calculated in step 6 with nuclease free water.
9. In an 8 strip PCR tubes, add the following: 33 ul of the hybridization master mix, the appropriate amount of reference sample calculated in step 5, and all of the test sample in one tube. Pipette to mix carefully. Place a fresh cap, spin them down and place in the thermocycler in the AGLB program to incubate at 95 degrees Celsius for 3 minutes and at 37 degrees Celsius for 30 minutes.

Note: For the next steps, be sure your lab bench is clean and executed in the dark! You may want to practice on an old slide first to get used to it.

10. Clean a hybridization chamber with water and dry it. Disassemble the apparatus. Place a new gasket slide (with 8 gaskets) on the base of the hybridization chamber apparatus, so that the Agilent label is on top and left side.
11. Take out the PathoChip array slide from the dessicator, and mark the slide number on the label outside the slide box for the slide you are taking out for use.
12. Quickly, remove the samples from thermocycler, spin the samples down and remove the cap. Carefully pipette up 45 ul of the sample and place the tip in the very center of the gasket chamber. Very steadily and slowly, pipette out all 45 ul, slowly dragging it. Do not let the liquid touch the gasket. Try to dispense the liquid to the centre of each gasket. (I have observed if the liquid touches the gasket, there is a chance of leak during hybridization).
13. Put the samples 1 to 4 in the order from left to right in the upper row of the gasket slide, and samples 5-8 from left to right in the lower row of the gasket slide.

Agilent	1	2	3	4
	5	6	7	8

14. Cover the gasket slide containing the hybridization mix with a microarray slide making sure that the Agilent label on the array slide faces the Agilent label on the gasket slide. Use the tips of your fingers to carefully maneuver it down. Do not touch the surface of the microarray slide or gasket slide. Do this carefully by making sure that both ends of the microarray slide are placed on top of

the gasket slide at the same time instead of one end first than the other. This is to ensure that the sample do not leak outside the gasket chamber.

15. Place the hybridization top on the base containing the gasket and array slide and screw it with a clamp by twisting it tightly. Once tightened sufficiently, vertically rotate the chamber to observe the mobility of the hybridization mix., i.e to see if the hybridization mix covers the entire array when the hybridization chamber rotates and also to look for any leakages.

Get another hybridization chamber for balance and place in the incubation oven at 65 degrees Celsius for 40 hours rotating at 20 rpm.