

MicroBAR Operating Instructions

Rev. 2013-06-21

Making PDMS Chips

- 1) The chip mold is fabricated using standard SU-8 lithography techniques
- 2) Chips are molded from Sylgard 184 PDMS with a 10:1 curing weight ratio
- 3) After molding, remove the chips from the wafer and cut along the edges with a sharp straight razor blade. Note that initially we cut a little beyond the edge of the chip and we make the final edge cut after the "floor" of the chip is bonded.
- 4) A 4 mm hole punch is used to create the inlet wells (Harris Unicore, Ted Pella). Make sure to clean the punch with Eliminase.
- 5) The bottom of the chip is cut from Stockwell Elastomerics .010" Thick HT6240 sheets.
- 6) Both the PDMS channels and the "floor" sheet are treated with Eliminase to remove DNA, DNase, and RNase and then are rinsed with ultrapure water.
- 7) The two chip parts are bonded using oxygen plasma.
- 8) After bonding, the edges are cut to the precise dimensions

Setting up a Chip

- 1) Currently, the LAMP reaction mix is prepared off-chip under sterile conditions. Template is added last.
- 2) Positive and negative reaction mixes are split into two tubes: one for the thermocycler and one for the MicroBAR
- 3) The MicroBAR cartridge is vacuum treated for > 1 hr prior to loading.
- 4) At this point, cartridges may be sealed for future use using a commercial food sealer (although they should be vacuum treated first with a more powerful vacuum as the food sealer isn't powerful enough to enable full vacuum loading).
- 5) 20 uL of each reaction mix is loaded into each inlet immediately after it is taken out of vacuum. It takes about 30 minutes for the reaction mix to fully load into all chambers.

Operating the MicroBAR

- 1) Make sure you have a fully-charge battery or you have the instrument plugged in to wall power (note that we added a power plug in lieu of the LiPo battery so that the instrument could be powered continuously with a 5V wall-wart power supply. The power supply you use should have a rating of at least 2A.
- 2) During powerup, you should see the lights go through all 16 brightness levels, the run and bat lights flash once, and you should hear two beeps.
- 3) Plug the box into the USB port
- 4) Fire up the UI
- 5) Often, it doesn't connect the first time. Close the UI, unplug the USB and plug it back in again. That almost always fixes it.
- 6) Set your assay parameters (note that we almost always use the defaults, which are given in parentheses):
 - a. Step time (2000 ms)
The time between samples. Note that internally, the MicroBAR averages 100 samples per sample that it returns.
 - b. Stabilization steps (10)
The photodetector amplifier needs a little time to reach a steady state value, so we let the instrument run for a certain number of steps before we start recording.
 - c. Run steps (5000)
This determines the length of the assay. Run steps * step time = total assay time (although actually it's a little off due to the way it calculates things).
 - d. Temp (60 C)
This is the run temperature
 - e. LEDs (1)

Sets the light level. These are logarithmically spaced intensity values from 1-15 (0 is OFF, 1 is lowest intensity). Values above 1 tend to saturate the detectors, so we stick with 1.

f. Start Well (1)

If you only want to sample a subset of wells, you specify the start and stop here.

g. End Well (96)

Stop well to sample.

- 7) Click "Set Assay Params" and verify that the unit responds with the correct parameters (the unit's state is printed on the right side)
- 8) Set the appropriate output directory. Note that it must already exist. Don't include a filename here, that will be automatically added (.log)
- 9) Insert chip and line it up with the phototransistors. Make sure that the bottom of the chip sits flat and the PDMS "wets" the surface of the ITO/glass slide. This will ensure that it doesn't flex during heating.
- 10) Close the lid gently and make sure it completely seals (might have to wiggle it back and forth a bit)
- 11) Click Start Assay and wait
- 12) The unit will emit 3 long beeps when it's done
- 13) To view the data, load the Matlab script and change the filename to your log file. It takes a long time to parse the log files, but it saves them as a MAT file so you can do this again and it's much faster.
- 14) Specify the colors of the wells you want to plot. PLOT_COLORS tells the script what color to print (standard matlab 1-letter abbreviations). If you set a well to 0, it won't print.