Monterey Bay Aquarium Research Institute

ESP MicroFluidics Block (MFB)

User Manual

v. 1.0.81

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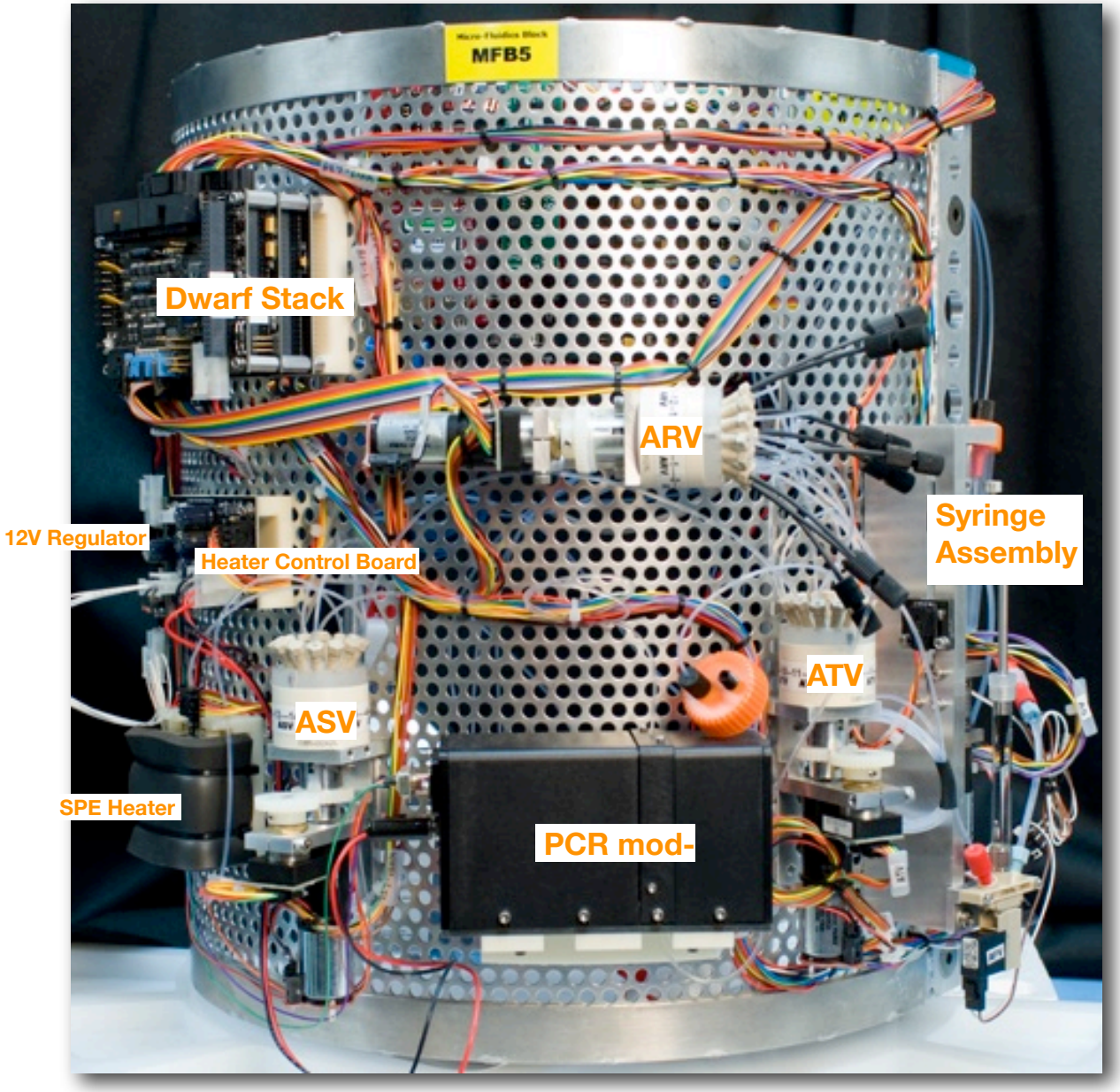
June 2, 2010

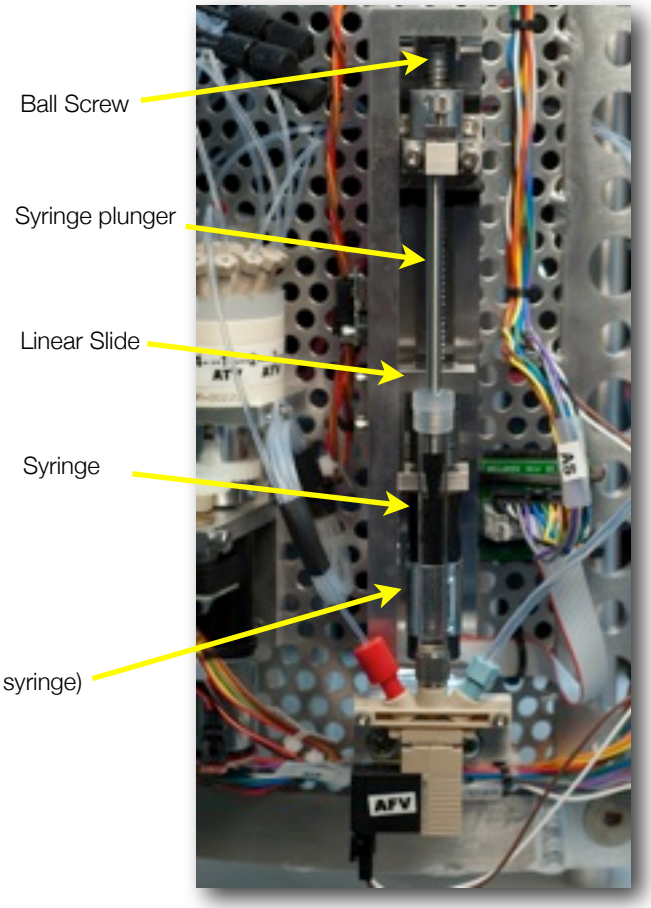
Parts Identification

Front Grill



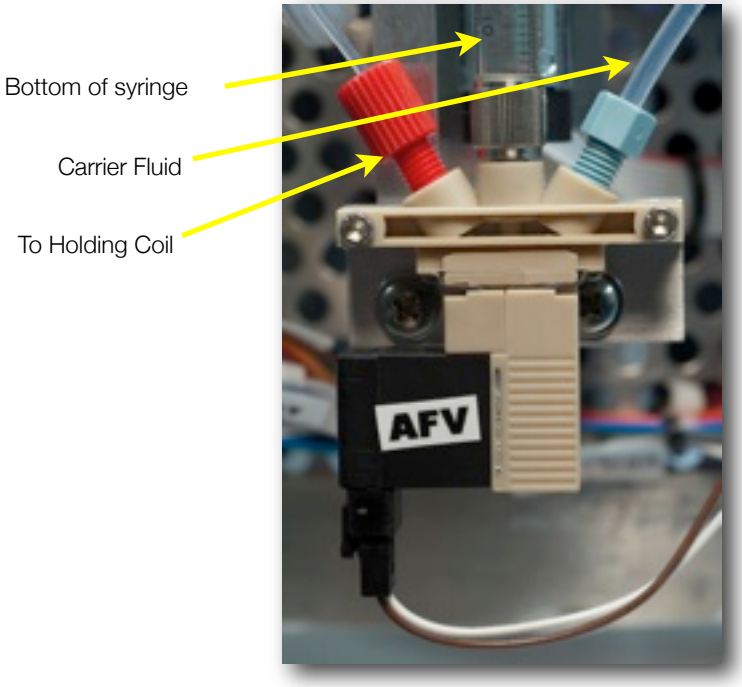
Analytical Syringe (AS)

The syringe assembly consists of a ballscrew, linear slide, coupler, syringe, motor, and home flag sensor. The syringe is a Zero Dead Volume 1.0 ml syringe from Klohn (PN 29557). The motor is a Maxon (PN 348495) 12V motor with a 512 count/rev encoder.



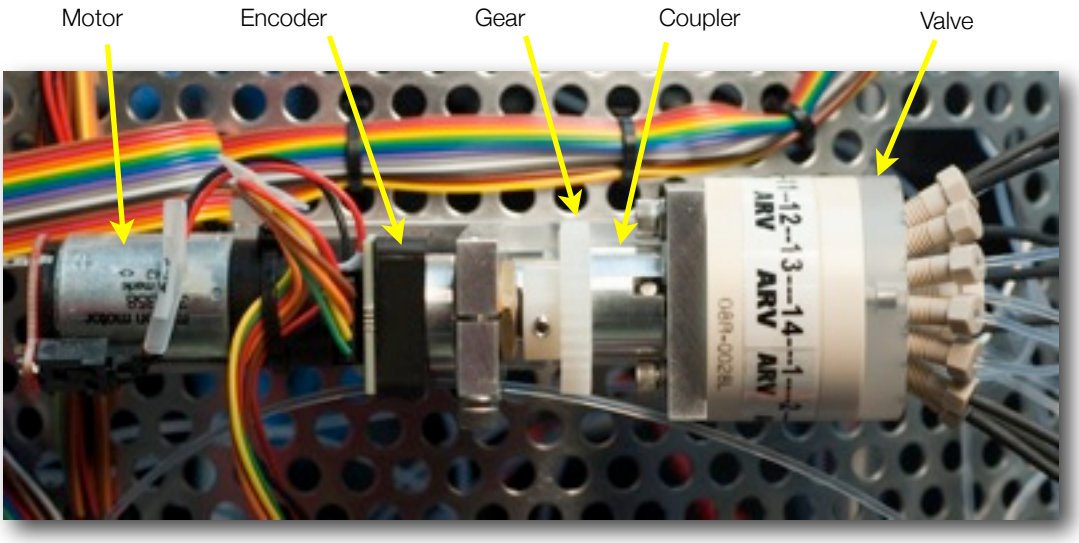
Analytical Fluidics Valve (AFV)

This is a 3-way flipper solenoid valve and manifold located at the bottom of the syringe. One side connects to the carrier fluid, the other to the holding coil, with the syringe being common between them.



Analytical Reagent Valve (ARV)

All rotary valves (either 14-port or 6-port) have the same construction; a motor connects to a geared coupler that connects to the valve. Off the geared coupler is another gear that drives the encoder. These parts are labeled for the ARV below. The ARV supplies the ATV and is mainly involved in supplying the various reagents (primers and master-mixes for PCR). Note heat-shrink tubing covering reagent tubes to protect light sensitive reagents.



Analytical Top Valve (ATV)

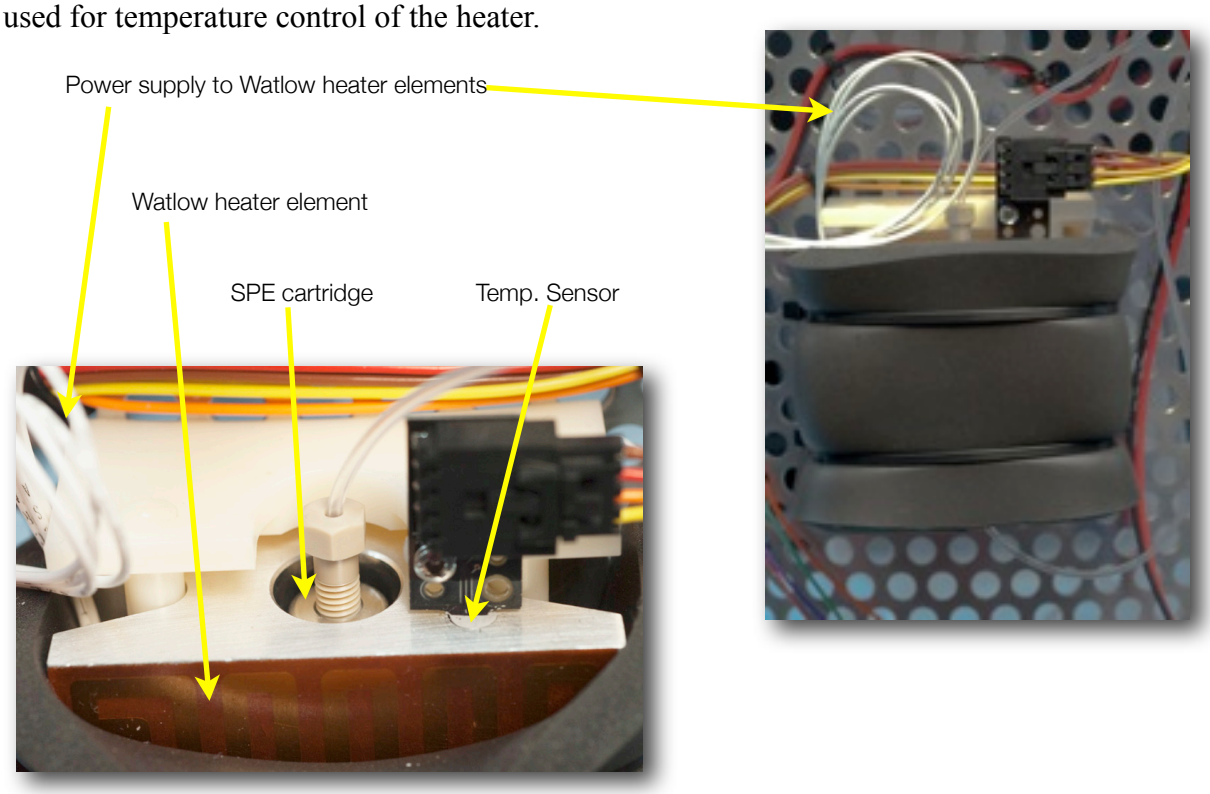
This 14 port stream-select valve is center supplied by the holding coil and operates as the central valve allowing access to the core ESP, reagents on the ARV, and modules through the ASV. The assembly of the ATV is the same as the ARV.

Analytical Sample Valve (ASV)

This 14 port stream-select valve has a special stator that accesses the mixing coil and delivers reagents to the SPE heater and Analytical Module. When performing PCR, it also serves to isolate the PCR reaction in the module during thermocycling and ensures amplified products are moved away from the central ATV valve. See the Fluidics diagram for stator configuration.

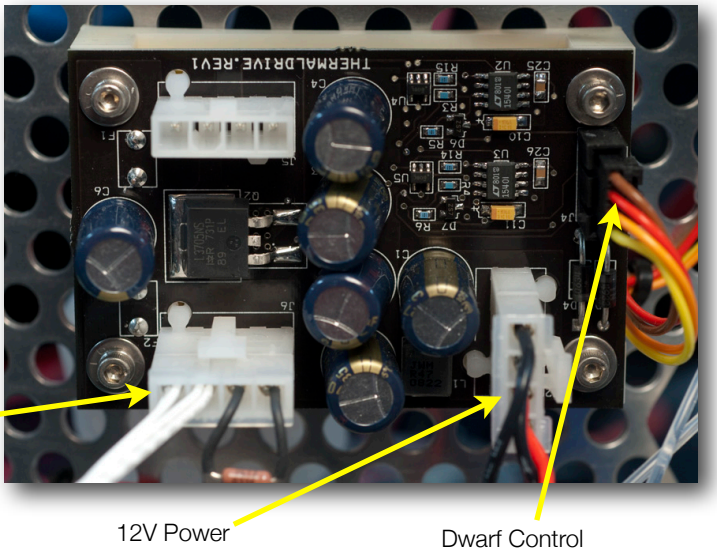
Solid-Phase Extraction (SPE)

The solid phase extraction heater consists of an insulated aluminum block with two thin-film Watlow heater elements affixed to either side. The center of the block has a column holder that contains replaceable extraction cartridges. Fluid connections to the ASV are made at the top and bottom of the column. There is also a temperature sensor potted into the block that is used for temperature control of the heater.



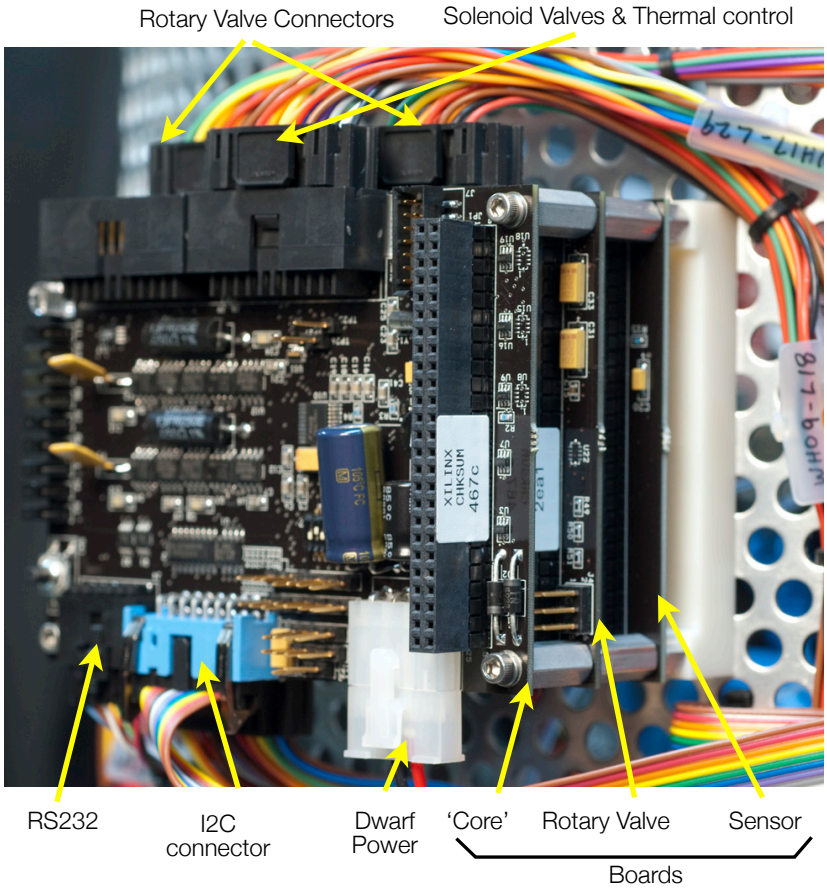
Heater control board

The Heater Control Board receives commands from the Dwarf Stack and supplies the correct amount of power to the SPE heater elements to maintain a desired temperature.



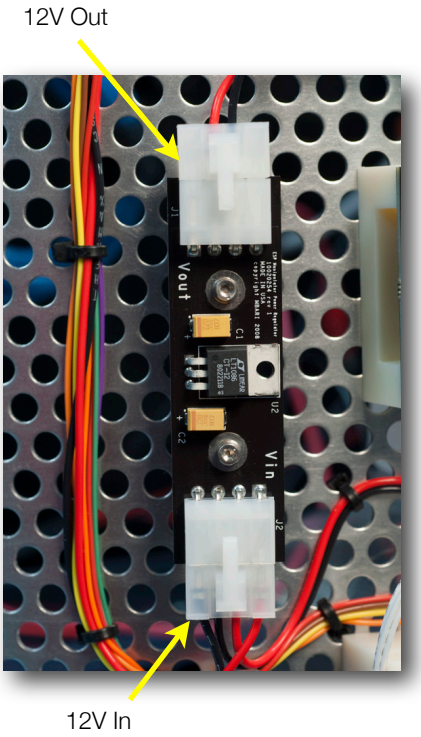
Dwarf stack

The Dwarf Stack consists of a 'Core' board, Rotary valve board and a Sensor board. The core board receives instructions from the ARM host board via the I2C network, and directly controls up to 8 solenoid valves and up to two heaters. The core board also passes RS232 from the Host to an Analytical Module. The Rotary valve board can control up to 4 DC brushed motors and their encoders. The sensor board can receive input of 1-2 temperature sensors and a pressure sensor.



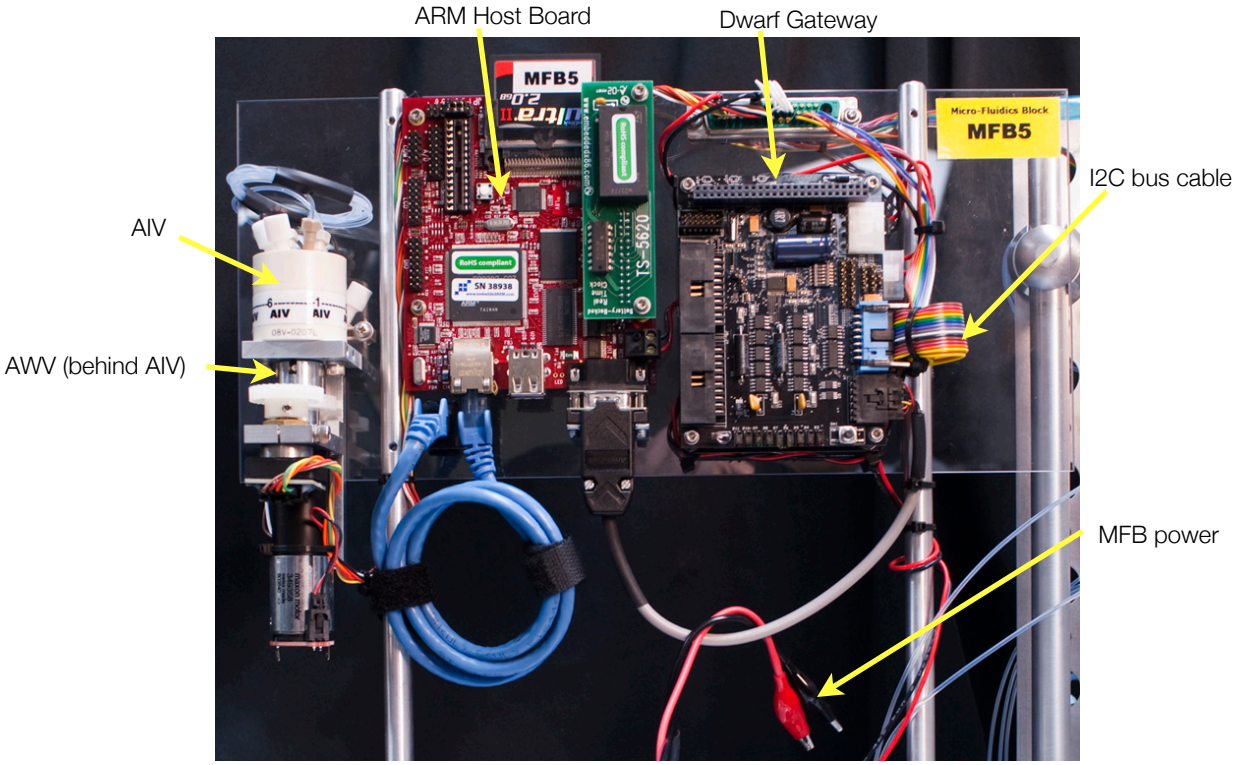
12V regulator board

The 12 Volt Regulator takes raw unregulated power in and supplies a clean 12.0 Volts out to the Dwarf stack. It also passes unregulated power to the Heater control board and to the Analytical Module.



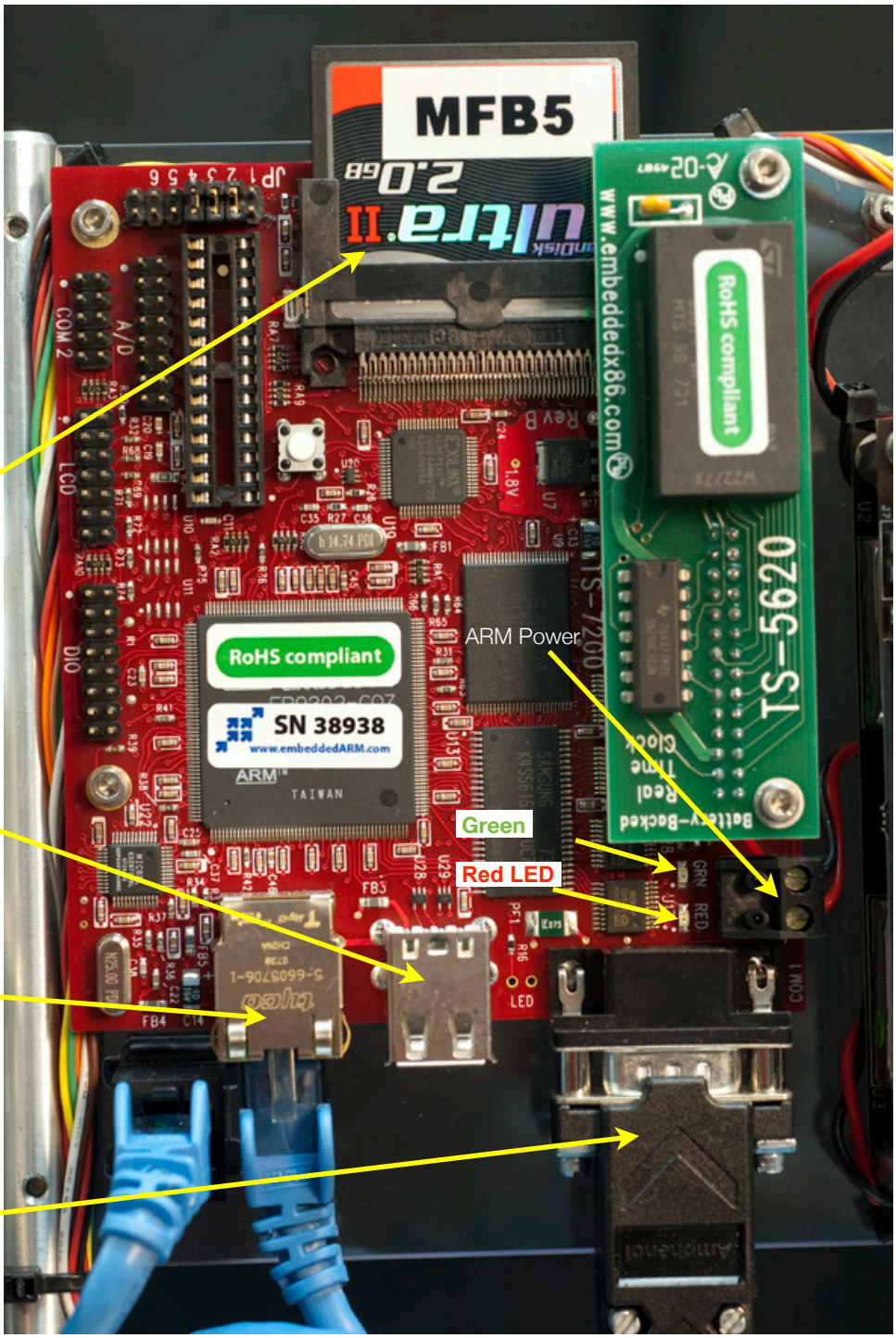
Back Burner

For the MFB to function as a stand-alone device (not connected to an ESP), several boards and valves are included and mounted behind the grill on an acrylic sheet. This is also where power is supplied to the unit (10-16VDC).



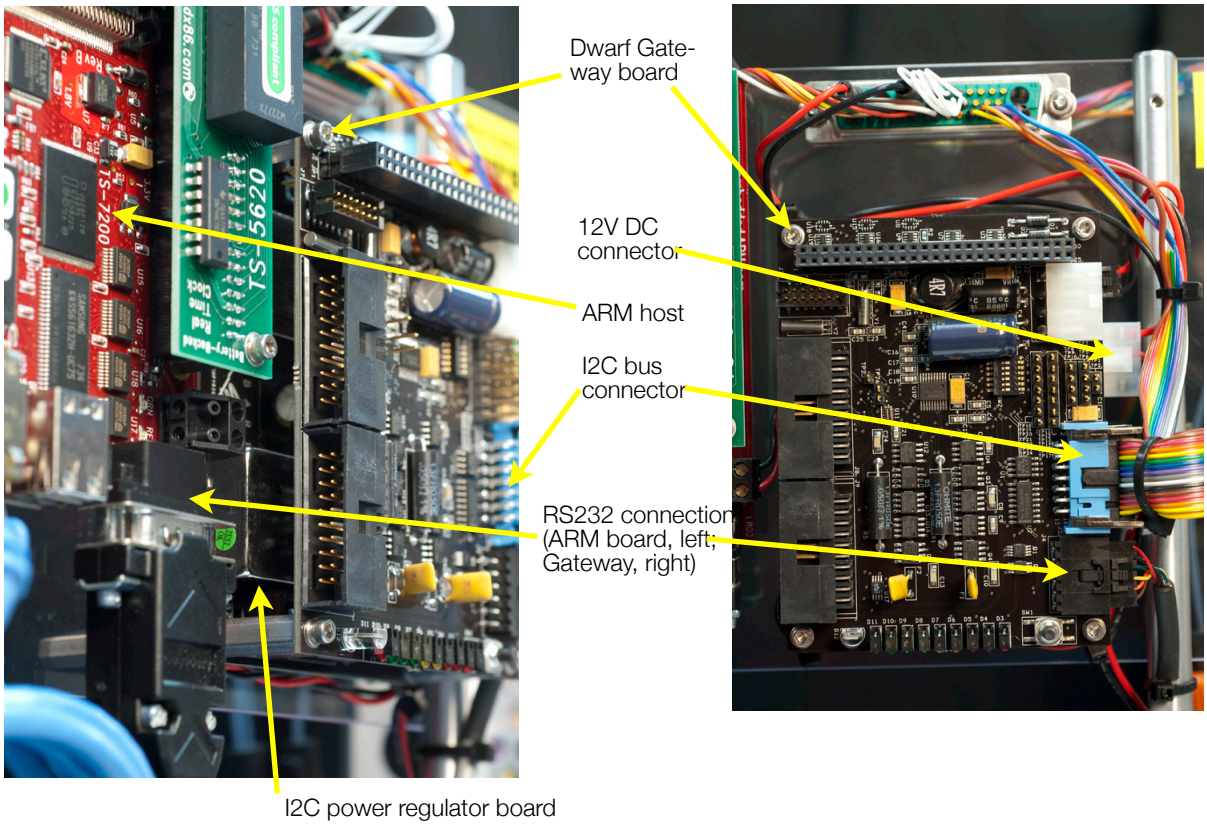
ARM Host board

The ARM host board is the main 'brain' of the MFB. It communicates via ethernet or a serial port. The operating system, Linux, lives here, and there is an on-board 2GB Compact Flash for data storage. The board also supports two USB 1.1 peripheries. 5 V DC power is supplied via the I2C Power Regulator board (located behind the Dwarf Gateway).

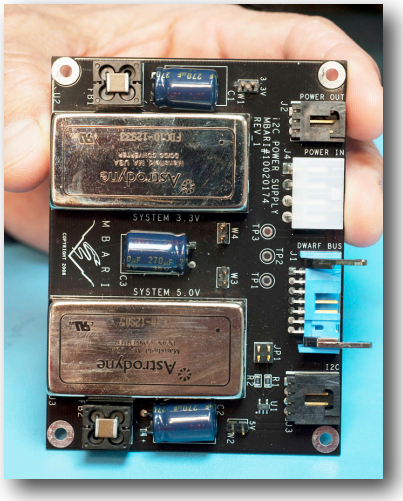


Dwarf Gateway

The gateway is a 'Core' board that has been programmed for use as a gateway to the Dwarf stack. It is connected to the Host board via a RS232 cable, and to the Dwarf stack via the I2C bus.

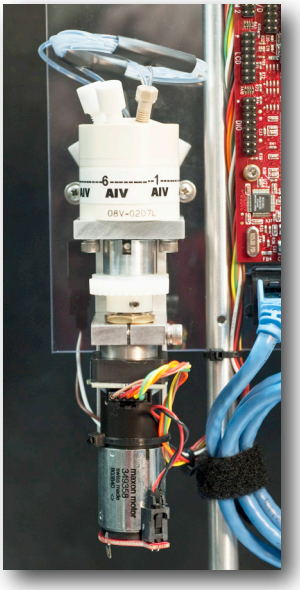


I2C power regulator board



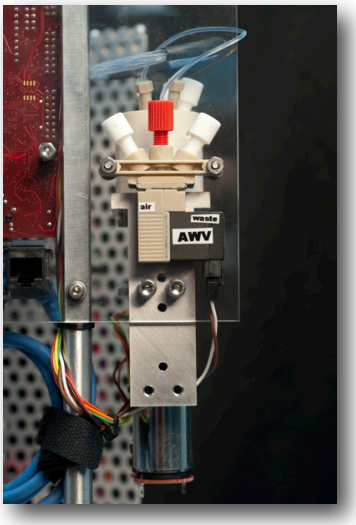
The I2C power regulator board receives 12-16 VDC power from a battery and conditions it for 3.3V and 5V system power needed by the Host board, gateway and the Dwarf stack. It supplies that power via a 14pin IDC connector that is the I2C bus cable. This board is located behind the Dwarf Gateway board.

Analytical Injection Valve (AIV)



This 6 port rotary valve is actually a 2-position valve. Position 1 allows manual filling of the lysate coil with sample. Position 2 passes the sample to the MFB.

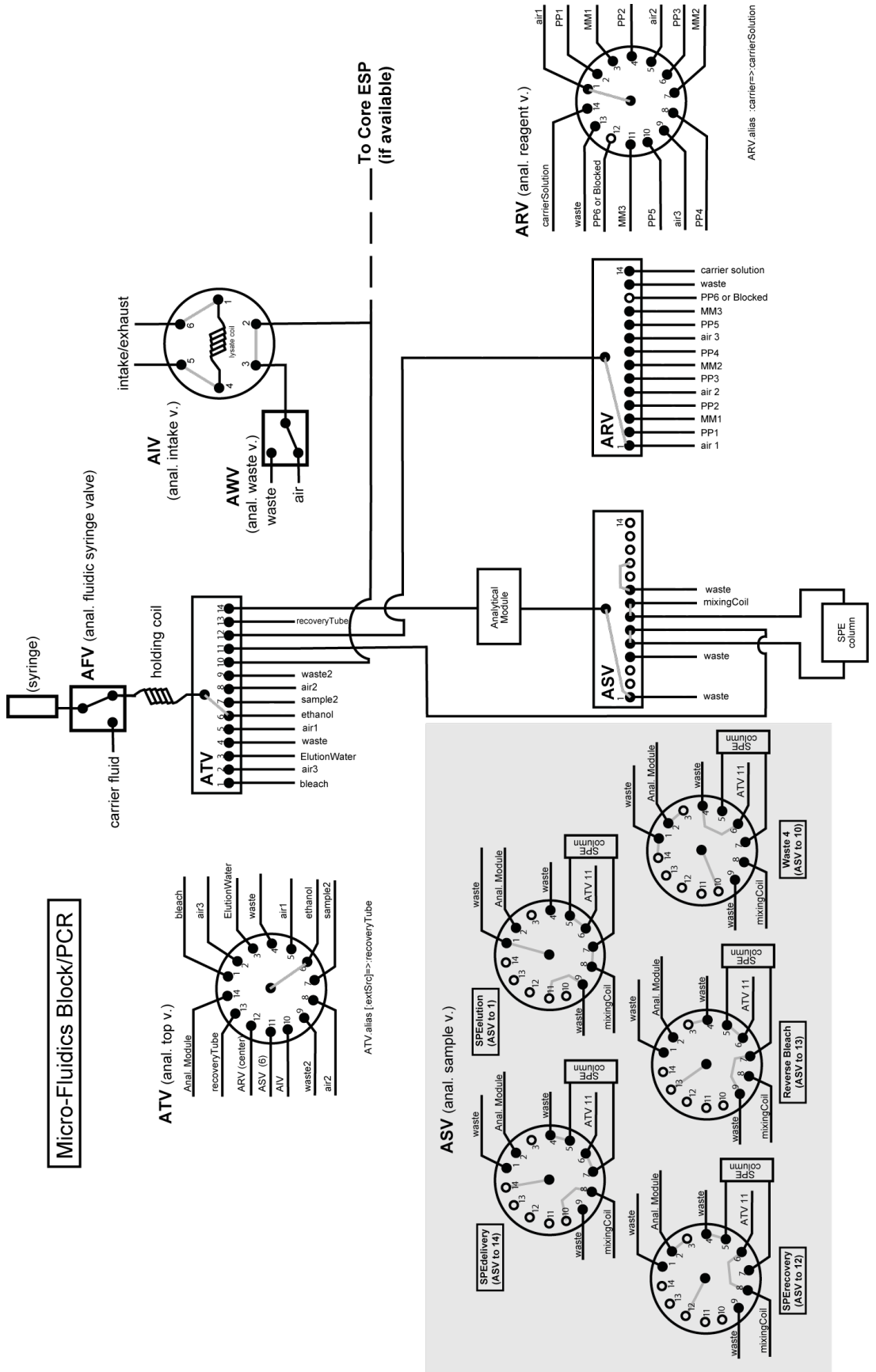
Analytical Waste Valve (AWV)



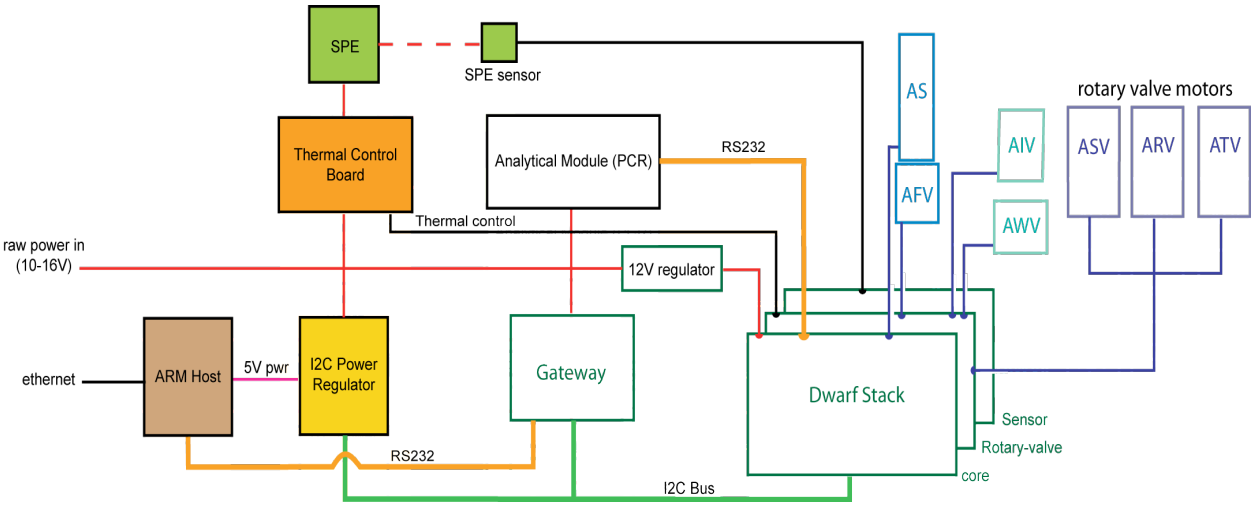
Located behind the AIV, this 3 way flipper solenoid valve and manifold provides access to i.) clean air, which ensures an uncontaminated sample is delivered to the MFB, or ii.) waste, in order to decontaminate the injection loop in preparation for the next sample.

Fluidics Pathway

The schematic on the next page shows valve and syringe arrangement. All valves have a 'center-to-single-port' stator except the ASV and AIV. Reagents on the ARV represent primer/probe (PP) and master mix (MM) arrangements determined by the user.


Micro-Fluidics Block/PCR
(syringe)
AFV (anal. fluidic syringe valve)
carrier fluid
holding coil
intake/exhaust
AIV
(anal. intake v.)
lysate coil
AWV
(anal. waste v.)
waste
air
To Core ESP
(if available)
ATV
recoveryTube
waste2
air2
sample2
ethanol
air1
waste
ElutionWater
air3
bleach
ARV
carrier solution
waste
PP6 or Blocked
MM3
PP5
air 3
PP4
MM2
PP3
air 2
PP2
MM1
PP1
air 1
ASV
waste
mixingCoil
waste
waste
Analytical Module
SPE column
ARV (anal. reagent v.)
carrierSolution
waste
PP6 or Blocked
MM3
PP5
air3
PP4
air1
PP1
MM1
PP2
air2
PP3
MM2
ARV.alias :carrier=>:carrierSolution
ATV (anal. top v.)
Anal. Module
recoveryTube
ARV (center)
ASV (6)
AIV
waste2
air2
bleach
air3
ElutionWater
waste
air1
ethanol
sample2
ATV.alias [:extSrc]=>:recoveryTube
ASV (anal. sample v.)
SPEdelivery (ASV to 14)
SPEelution (ASV to 1)
Reverse Bleach (ASV to 13)
SPErecovery (ASV to 12)
Waste 4 (ASV to 10)
Anal. Module
waste
mixingCoil
ATV 11
SPE column

Electronics Layout



Power Requirements

Battery power supply

Powersonic PS-12120

A PowerSonic PS-12120 F2 12 Volt, 12 Amp.Hr. battery is used to provide un-interrupted power to the instrument.

Battery charger

Xenotronix HPX-30

A Xenotronix HPX-30 Sealed lead acid battery Charger is used to keep the battery at full capacity. It outputs 12 Volts, and 2 Amps.

Communications

Establishing communications

Start-up

The host computer is normally plugged into a wired Ethernet network via the RJ-45 jack on ARM processor on the back-burner lucite panel. It should integrate itself automatically into any network that supports both DHCP and DDNS.

If your network supports DHCP, once you have unpacked and visually inspected the MFB for damage, you should plug it into your network via a Cat 5 Ethernet cable before applying power to the MFB. When power is applied, a pair of tiny red and green light emitting diodes on the component side of the host computer board will blink briefly. One of the lights should remain solidly green if the board is working properly. If the lights continue to flash in a rapid, alternating pattern, the computer is failing to start. In this case, remove power and try again. If the problem persists, call MBARI.

Soon after the boot LEDs finish blinking, you should be able to observe two other blinking LEDs on the RJ-45 jack attached to the host computer board. Note that there are no LEDs on the MFB's Lucite panel. Follow the patch cable from that jack to the host board to see the network status LEDs. At least one of these should be illuminated and you should see irregular blinking corresponding to network activity. If these LEDs are still dark after 20 seconds, there is most likely a problem with the network connection. Check cables and routers. Try plugging a laptop or other computer into the same cable you intended to use for the MFB. If the other computer works, but the MFB shows no network lights, try another cable and/or try plugging directly into the RJ-45 connector on the MFB host computer, bypassing the patch cable to the Lucite panel.

If the network is plugged in when the MFB host is first powered on or reset, it should be ready to contact 30 seconds after you first observe network activity on its LEDs. However, if the network is plugged in after the host computer as started booting, the network integration process may take as long as five minutes. During this process it queries your network router for a unique IPv4 address and requests that it be associated with the unique name assigned to the MFB at the time of its manufacture. This "hostname" will be displayed on a prominent label affixed to the MFB's Lucite panel.

You can test whether the MFB is accessible a number of ways. In all the examples below the MFB's hostname is to be substituted for 'hostname' in the text:

- Point a web browser on your network at the URL: FTP://hostname
- Telnet to the host with the command: telnet hostname
- Ping the hostname with the command: ping hostname
- SSH into the host with the command: ssh mfb@hostname

Note that commands are to be entered on the command line of a computer on the same LAN. SSH is available under Mac OSx, Linux and Unix, but may not available under Microsoft Windows unless it had been installed as a 3rd party add on. The ping command on Linux and Unix will continue testing until aborted (typically with 'Control-C'). If either telnet and/or ssh prompt for login or password you are ready to begin using the instrument.

The MFB host computer supports two networking protocols to log in to it and issue Linux commands. The telnet protocol is not secure, but supported on just about every networked computer. The ssh protocol, or Secure SHell, is supported by all Linux, Unix and Mac OSx computers. Use ssh if your computer supports it.

Telnet always prompts for both a username and a password. SSH infers the user name from parameters input on the command line and prompts only for the password. Here's a typical command dialog for Telnet starting a telnet session: (typed text is in ***bold italics)***

> > ***telnet MFB3***
> Trying 134.89.10.238...
> Connected to mfb3.shore.mbari.org.
> Escape character is '^]'.
>
> MBARI ESP Embedded Linux 1.7 -- 12/2/08 brent@mbari.org
> MFB3 login: ***mfb***
> Password: ***mclane***
> Using esp2local, ESPhome=/home/mfb/esp2
> mfb@MFB3:~$

Here's a typical command dialog for starting an ssh session:

> > ***ssh mfb@MFB3***
> The authenticity of host 'mfb3 (134.89.10.238)' can't be established.
> RSA key fingerprint is ae:05:da:5f:4a:05:a1:7b:b8:be:2b:64:4a:00:a4:06.
> Are you sure you want to continue connecting (yes/no)? ***yes***
> Warning: Permanently added 'mfb3,134.89.10.238' (RSA) to the list of known hosts.
> mfb@mfb3's password: ***mclane***
> Using esp2local, ESPhome=/home/mfb/esp2
> mfb@MFB3:~$

Both the Telnet and SSH sessions function identically once started. One may have any reasonable number of either session type opened simultaneously.

If the above commands indicate that the MFB host computer is not reachable, it may be that your network supports DHCP but does not support DDNS. This means that the MFB can only be identified by its numeric IP address of the form 11.22.33.44 This numeric address is typically assigned by your network router box. Search your router's configuration web pages for information relating to DHCP assignment on your local network. You may find an entry for the MFB host name listed there. If you do, try substituting the IP address your router assigned in place of hostname in the above commands. If that works, you can identify the

MFB host by this number. However, beware that it may change whenever the MFB is removed from the network or powered off.

Accessing MFB

To start the esp application that controls the MFB from the host's Linux command prompt:

esp@*hostname*:~$***esp***

Allow 30 seconds for the esp application to start and issue the esp command prompt:

```
@20:48:47.63 <MAIN> Copyright 2008 MBARI
/home/mfb/esp2/mfb/MFB3/configure.rb
$Revision: 1.1 $by $Author: brent $on $Date: 2008/12/06 05:47:00 $
@21:04:10.64 SPE.stop
@21:04:10.70 SPE.configure SPEconfig
@21:04:10.83 AS.coast
@21:04:10.87 AS.configure ASconfig
@21:04:11.05 PCR.pidtemp= 50,0.1,0,300,25,25
@21:04:11.15 PCR.caltemp= 4.803,0.0371,0
@21:04:11.19 All dwarves are running firmware version 2.59
ESPMFB3:001:0>
```

You should see messages indicating that various mechanical components of the MFB are being configured and initialized. If you see messages indicating that parts are "not responding" or "MISSING", execute the shutdown procedure described below and check cabling, etc., then power up, log in and try starting the esp application again.

The esp application is written in the programming language Ruby and its command prompt is a variant of Interactive Ruby or irb. It supports command auto-completion with the <TAB> key and command history recall using up and down arrow keys.

Ready the instrument - When the MFB is switched on, one must calibrate its Analytical Syringe and other components before using it. The command to do that is:

>***MFB.ready!*** (MFB.ready! will home the syringe and flush 1 ml of carrier fluid through the syringe and out the ATV waste port. The MFB is left with the syringe at empty, ATV at waste, ARV between carrier and air1, and ASV at waste1.)

>***MFB.ready?*** This returns true if all axis are homed, false if not.

To quit the ESP application and return to the Linux command prompt:

ESPMFB3:003:0> ***quit***
mfb@MFB3$

--or--

ESPMFB3:003:0>***exit***

Running the Instrument

LeTter-Case Matters!!

Individual commands

Syringe Movements

Before moving the syringe, always check the position of the valves!

- To initialize the home sensor
  >***AS.home.to :home***

- Configuration names for syringe speeds:
  We sample at 64 tics/sec, thus 1 tic = 0.015625 sec

  ASslow0: maxSpeed = 200 counts/tic = 10 ul/s

  ASslow1: maxSpeed = 100 counts/tic = 5 ul/s
  --ASslow1 is used majority of time to get & deliver reagents

  ASslow2: maxSpeed = 20 counts/tic = 1ul/s
  --ASslow2 is used to maintain the integrity of reactions separated from the carrier fluid by air bubbles or AS moves <10µl. If ASslow2 is used the syringe must be above the 0 or empty position. The stiction of the syringe at empty (or 0) does not allow metering of accurate volumes.

  ASconfig: maxSpeed = 350 counts/tick = 17.5 ul/s
  ASnorm: maxSpeed = 400 counts/tic = 20 ul/s
  ASfast: maxSpeed = 750 counts/tic = 37.5 ul/s

- To fill syringe (default speed ASnorm)
  >AS.fill

- To empty syringe (default speed ASnorm)
  >***AS.empty***

- To move at a 'custom' speed (in ul/s)
  >***AS.config.maxSpeed=17***
  >***AS.configure***

- To change syringe speed on the fly
  >***AS.to position, configurationname*** (e.g., ***AS.to .5, ASfast***)
  >AS.pull volume, configurationname (e.g., ***AS.pull 0.3, ASslow1***)

  For AS.fill and AS.empty, no comma is needed
  >***AS.fill ASfast***

- To move to a absolute position on the syringe (e.g., 0.6ml)
  >***AS.to volume*** (e.g., **AS.to 0.6**)

- To push a relative volume of fluid
  >***AS.push volume*** (in mL) (e.g., **AS.push 0.01**)

- To pull a relative volume of fluid
  >***AS.pull volume*** (in mL) (e.g., **AS.pull 0.01**)

- To stop the syringe
  Either >***control-C*** (if the last command was a syringe move)
  or >***AS.stop*** (if it is doing something else also)

- To release the syringe motor from holding a position
  >***AS.coast***

- To query the raw encoder counts for calibration purposes
  >***AS.rawPosition***

- To change syringe speed (use configuration names above)
  > ***AS.reconfigure configurationname*** (e.g., **AS.reconfigure ASslow1**)

- To fill the syringe full of carrier solution (1 ml)
  >***fillSyringeFull***

- To fill the syringe half full of carrier solution (0.5ml)
  >***fillSyringeHalf***

- To pull a volume a bleach from the ATV into the syringe.
  >***bleach(volume)***
  (Note: this protocol pushes 15 ul of air back into the valve port)

- To safely pull a volume of ethanol
  >***EtOHwash(volume)***
  (Note: this protocol pushes 15 ul of air back into the valve port)

Removing Air Bubble from Syringe

Take a 60cc syringe with a short line of hose (end fitting should screw into AFV valve) and fill with 25ml of water and evacuate all air. Remove the holding coil from the AFV valve. Hook up a short line to the 60cc syringe filled with water.

>fillSyringeHalf; ATV.to :waste; AS.empty; fillSyringeHalf
(Commands separated by semicolons allow you to string along commands)

As the syringe is emptying, pull on the 60cc syringe to evacuate all contents of the AS syringe (create a vacuum in the barrel of the syringe) and hold it until the AS has emptied and switched back to the carrier fluid on the AFV valve. When the valve flips to the carrier it should fill with water and then fill to half. If the air bubble has not disappeared, repeat the above line. Once the bubble has disappeared, reattach the holding coil to the ATV and

>3.times {fillSyringeFull; ATV.to :waste; AS.empty ASfast}

Valve Movements

There are six valves: ATV, ASV, ARV, AIV, AFV, and AWV

- To find the current position of any valve
  >valvename (e.g., **ATV**)

- To find out the names of the valve ports
  >***valvename.legend*** (the result is a list of encoderValues mapped to port numbers and names)

- To move to a port on any valve
  >***valvename.to portnumber*** (e.g., **ATV.to 4**) or
  >***valvename.to :portname*** (e.g., **ATV.to :waste**)
  >valvename.portname (e.g., **ATV.waste)**

- To block off flow by moving valve exactly between ports x and y:
  >valvename.dialBetween portnumber, nextportnumber (e.g., **ARV.dialBetween 1,2** or **ARV.dialBetween :carrier, :air1**)

- To move the valve in a specific direction:

>***valvename.to portnumber,:direction*** (e.g., **ATV.to 8,:up**) (e.g., **ATV.to 8,:down**)

- Another way to move valves, crossing or avoiding certain ports:
  >valvename.to portnumber, :via => :portname
  :avoiding => portname

- To get the encoder position of the Valve
  >***valvename.rawPosition*** (e.g., **ATV.rawPosition**)

- To rotate the valve by x
  >***valvename.advance x***
  >***valvename.retard x***

(Note: x may be signed and/or fractional, thus >**valvename.retard -1.5** is equivalent to >**vavlvename.advance 1.5**)

SPE Commands

Commands for working with the Solid-Phase Extraction (SPE) component of the MFB:

- To determine the temperature on the SPE heater
  >SPE

- To access the raw data of the SPE heater
  >SPE.status

- Set the SPE heater for a specified time to a temperature
  >***SPE.hold "hour:min:sec", temp***

- Turn off the SPE heater
  >***SPE.stop***

- To prepare a new SPE column for extraction
  >***columnBurnIn***

- To run solid phase extraction from the AIV:
  >runSPE (this defaults to the injection loop on the AIV)

At the end of this protocol, the extracted nucleic acid will be primed to the ATV.11, bleach will remain parked in the SPE extraction column, and ATV-AIV-lysate coil will contain (from the ATV valve), air, water, air, bleach.

- To run solid phase extraction from sample line on the ATV.

You must first prime the lysate to the ATV valve. Prior to priming, dip the end of the tube in 1 ml of 20% (v/v) Chlorox bleach (unscented), followed by a dip in two tubes of 1 ml clean water. Place the tube in the modified lysate.

>ATV.to :valveposition
>AS.pull 0.15, ASslow0
>ATV.to :waste
>AS.empty
>runSPE :valveposition

At the end of the protocol, the extracted nucleic acid will be primed to the ATV, bleach will remain parked in the SPE extraction column, and the line that the lysate was primed to will contain (from the valve), air, water, air, bleach.

- To recover the lysate from ATV.13 (:extSrc) after the extraction
  >deconEluateRecoveryLine :extSrc
  >recover (volume)
  Either the whole extract can be recovered or multiple aliquots of the sample. The extract is recovered through ATV.13 (:extSrc) into 1.5 ml epi tubes.

- To decontaminate the SPE module and ready it for the next sample
  >deconSPE
  Note: deconSPE defaults to decontaminating the AIV line.

- If lysate was loaded via an external port on the ATV.
  >deconSPE :portname

At the end of the decontamination, the following have been aired out: ATV-ASV line, column, mixing coil, and lysate delivery line.

PCR Commands

- To turn the PCR fan on/off
  >***PCR.fan = :on*** >***PCR.fan = :off***

- To query the PCR.state
  >***PCR.status***

- To set PCR to a temperature
  >***PCR.temp = tempdesired***

- Returns the current PCR program
  >PCR.program

- Loads a new PCR program

>PCR.program :programname
Programs are found/created in the MFB/MFB#/configure.rb file

- To run the PCR program
  >PCR.run

- To visualize PCR data
  >PCR.sample

Once a new run is initialized the data from the previous run is lost.

- Stopping PCR
  >PCR.abort (stops any PCR run in progress)
  >PCR.off (stops any PCR run in progress and turns off PCR heating element)

Cleaning Commands

- To decon the AIV Lysate line (done automatically in deconSPE or fullDecon)
  >***deconLysateSA*** (if MFB is in Stand-Alone mode)
  >deconLysateINT (if MFB is mated to the core ESP [i.e., INTegrated])

- To decon after Solid Phase Extraction
  >***deconSPE*** (defaults to cleaning AIV injection loop)
  >deconSPE :lysateSource (deconSPE includes the decontamination of the lysate delivery line)

- To decontaminate between PCR runs
  >pcrDecon

- To decontaminate after running solid phase extraction and PCR
  >***fullDecon*** (defaults to AIV injection loop)
  >fullDecon :portName

- To clean the Eluate recovery line or lysate line on ATV
  >***deconEluateRecoveryLine :portName***

- To clean the PCR line when the tubing has been replaced or contaminated (This is a more thorough cleaning that concentrated on the PCR line)
  >***cleanPCRline*** (rinses with water followed by pcrDecon)
  >waterClean (pcrDecon followed by water washes running ;bleachProfile)

Configuring MFB

Configuration of the MFB is handled in 3 separate files:

preconfig.rb
postconfig.rb
configure.rb.

The pre- and post- configuration files are found in esp2/mfb. Configure.rb can be found in esp2/mfb/<hostname>

The preconfig.rb and postconfig.rb are conditions that apply to all MFBs.
The configure.rb contains specific configuration information for each individual instrument.

---

preconfig.rb contains configuration information for the type of valves on the MFB.

postconfig.rb identifies each valve name and type and provides the default configuration names for the valve ports on the MFB.

configure.rb is located in esp2/mfb/MFB#. Machine specific configurations are located here. These include parameters for solid phase extraction, building and positioning the pcr reaction, and decontamination found in protocol scripts. It also contains calibration information for the SPE heater, analytical syringe, PCR module. The PCR reaction types and their thermocycle conditions are also found here. Within this file you can reconfigure the valve names on the ports.

Below is the current set-up for running PCR (default found in postconfig.rb)

```
unless ARV.positions > 0
 ARV.with AxisMap.new(Valve::FourteenPosition)
 ARV.rename :air1=>1, :PP1=>2, :MM1=>3, :PP2=>4, :air2=>5, :PP3=>6, :MM2=>7,
:PP4=>8, :air3=>9, :PP5=>10, :MM3=>11, :waste=>13, :carrierSolution=>14
 ARV.alias  :carrier=>:carrierSolution
End
```

Copy and move to configure.rb for appropriate MFB, then update the port names. Note that ARV.2 has two names, Reagent1 and HybSol. Multiple names for a single port can also be made using aliases (see ARV.alias :carrier=>:carrierSolution).

```
unless ARV.positions > 0
```

```
 ARV.with AxisMap.new(Valve::FourteenPosition)
 ARV.rename :air1=>1, [:Reagent1, :hybSol=>2], :Reagent2=>3, :Reagent3=>4, :Rea-
gent4=>5, :Reagent6=>6, :Reagent7=>7, Reagent=>8, :Reagent9=>9, :Reagent10=>10,
:Reagent11=>11, :waste=>13, :carrierSolution=>14
 ARV.alias  :carrier=>:carrierSolution
End
```

ARV Protocols

Cleaning pigtails for PCR reagents

To bleach and rinse the lines which connect to reagent coils on the ARV:
You will need two Stedim bags: a 50ml bag filled with 20% bleach and a 150ml Stedim bag with clean water, a 0.2 um Sterevix filter and a short piece of tubing to attach the bag to the ARV port.

To clean the ARV port:

>***cleanPigTail (port)***

e.g., cleanPigTail (:PP1)

The protocol will prompt the user to attach reagents (20% bleach or water) or air filter to the pigtail via the short piece of tubing. Once each step is completed, the user presses enter/return to continue with the script. Upon completion a carrot is returned (>), the pigtail is clean, aired out and ready to receive the reagent. The user can add a filter (GV 0.2um 13mm filter) or MicroClave connector (ICU Medical Inc, B3300) to the end of the pigtail so it remains clean until the reagent is attached.

After cleaning all ports, decon the ARV using

>***pcrDecon***

Reagents Coils: Assembly, Cleaning and Loading

PCR reagent coils consist of

PFA 1/32" Industrial Tubing Natural (Cole Parmer #06371-00))
Cheminert Flangeless Tube end Fittings 1/16"OD, (Vici, Valco Instruments Co. Inc CFL-1R)
¼-28 Female to Female Luer Fitting (UpChurch #P678)
Microclave Connectors (ICU Medical Inc, B3300)

Coils length determined by the volume of PCR reagent loaded + additional 50 µl. For the above tubing:

Coil Length = (Volume in ml +0.05)/(3.142*(0.0396875^2))

Final Assembly should be in this order: Microlave connector, Female to Female Luer Fitting, Cheminert Flangless Tube end Filttings, Appropriate Tubing Length, Cheminert Flangless Tube end Filtting, Female to Female Luer Fitting, Microclave connector, Air filter.

Cleaning Coils

Need:

2, 10cc Syringes
1, 60cc Syringe
20% Chlorox Bleach (Unscented)
Water (Sigma)

IF reusing a coil: remove the air filter.
IF new coil, you do not need to attach the microclave connector until after the cleaning process.

Using a 10cc syringe attached to one end and a 10cc syringe barrel in a catch container, push ~8ml of 20% bleach through the coil. Once finished, attach the 60cc syringe and flush with 60 ml of water. Air out the coil with repeated pushes of air with the 60cc syringe. Once clean, inspect the ends and add sterile microclave connectors. The coil is now ready to receive the reagent.

Loading Reagents into Coils

Need:

2, 3cc Syringes per Coil
PCR reagents

To one end of the coil attach a 3cc syringe barrel. To the other end a 3cc syringe. Pipet the volume of PCR reagent to be loaded into the upright 3cc barrel. Using the 3cc syringe attached at the other end, slowly draw the reagent into the coil being careful not to introduce bubbles into the line. The reagent should be drawn to the base of the fitting. Remove the syringes and discard. To one end, a 0.2µm Sterevix filter should be attached. Make sure to protect the reagents from light. Coil ready for loading in instrument.

Recovering Reagents from Coils

Need:

1, 3cc Syringe per Coil
Sterile Eppendorf Tube

With the air filter still attached, withdraw the reagent from the coil into the syringe. Remove syringe and empty into a sterile eppendorf tube.

Loading PCR reagents onto MFB

After cleaning pigtails and loading reagents into coils, the MFB is ready to receive the reagent coils. It's best to load in reverse order (MM3, PP5, PP4, MM2, PP3, PP2, MM1, PP1). At the end of each pigtail a ¼-28 Female to Male Luer Fitting (UpChurch #P765) should be attached to a Cheminert flangeless tube end fitting to accept the Microclave connector.

First, air out the line between the ATV and ARV.

>ATV.to :ARV
>ARV.to :air1
>AS.pull 0.2, ASslow1

Hook up the clave connector to the ARV pigtail at the appropriate port. During the syringe pull watch the front and/or back end of the reagent to make sure that you can see it move through the coil.

>ATV.to :ARV
>ARV.to :PortName
>AS.pull 0.120, ASslow1

After the pull, check to see if the reagent has moved into the ARV-ATV line. You may see part of the reagent poking into the ARV-ATV line either before or after the below air pull.

>ARV.to :air (Move to the nearest airport)
>AS.pull 0.03, ASslow1

If no reagent is visible, go back to the appropriate reagent port and pull 20µl

>ARV.to :PortName
>AS.pull 0.02, ASslow1

Then, repeat the air pull. Continue with the 20µl pulls until you see the reagent slug in the ARV-ATV line. Once primed, you can move to the next reagent. If you run out of travel on the syringe, send the ATV to waste and empty the syringe.

>ATV.to :waste
>AS.empty
>ATV.to :ARV

After all reagents have been primed, run a PCR cleanup protocol followed by a series of negative control PCR reactions using elution water. If possible watch the PCR reactions being built to check their integrity.

>ATV.to :waste
>AS.empty
>pcrDecon
>shortmfb ([ASSAYS], [:elutionWater]) {}.finish

Recovering PCR Reagents from MFB

Fill the holding coil with air from the ARV. Then push air into each PCR reagent line so that the front of the PCR reagent is observed in the coil. Then move to the next reagent on the ARV and repeat the 150 µl push. If the syringe reaches empty, you must refill with air from the ARV.

>ATV.to :ARV
>ARV.to :air1
>AS.fill ASslow1
>ARV.to :PortName
>AS.push 0.15, ASslow1

Once the reagent is in the coil you can disconnect it from the pig tail and recover the reagent from the coil (see Recovering Reagents from coils). When the reagent coils have been removed you should then clean the pig tails with 20% bleach and water (see Cleaning pigtails for PCR reagents).

PCR Reaction Building Protocols

These commands refer to building a reaction 'slug' trapped between two small air bubbles from reagents on the ARV and ATV. The slug is then moved into position for thermocycling and analysis via the PCR module. Data stored in *.pcr files is comma delineated and can be opened in Microsoft Excel. PCR profiles and locations of the primers and probes are found in mfb/MFB#/configure.rb.

To build a slug and position it in the PCR module:

>pcrSlug(template, mastermix, primerprobe, endAir)
e.g., pcrSlug (ASV, :MM1, :PP2, :air2)

To build a slug, position it, thermocycle and create a file with data

>pcrMission (template, assay)
e.g., pcrMission (:elutionWater, G1Ar)

If the slug is already positioned in the module,

>pcrMission (nil, assay)
e.g., pcrMission(nil, G1Ar)

(Runs the thermocycling protocols and save the data to a file.)

Alternatively,

>PCR.program :programname
>PCR.run
>PCR.sample

(note data displayed on screen only)

The above protocols require decontamination between reactions.

>pcrDecon

To run combinations of solid phase extraction, multiple PCR profiles and templates with the appropriate decontamination protocol between, use the following variations of the shortmfb command. The commands incorporate the pcrMission, pcrSlug, and SPE protocols. The basic structure of the command is below followed by examples of how it works. A file containing the PCR data is saved for each reaction.

>shortmfb ([profiles], [templates]) {lysatesource}.finish

e.g., ***shortmfb ([IPC, SAR11], [:elutionWater]) {}.finish***

Builds a reaction for the IPC using elution water as the template, positions it, themocycles, stores the data, runs a pcrDecon. Then repeats the above for a reaction with SAR11 primers.

e.g. ***shortmfb ([IPC, SAR11], [:ASV]) {:extSrc}.finish***

Does a solid phase extraction from lysate primed to the ATV valve at position ATV.extSrc, followed by a PCR for the IPC using the eluted DNA (primed to the ATV from the ASV), does a pcrDecon, then a PCR for SAR11 from the eluted DNA, then a fullDecon.

e.g. ***shortmfb ([IPC, SAR11], [:ASV, :elutionWater]) {:extSrc}.finish***

Does a solid phase extraction from lysate primed to the ATV valve at position ATV.extSrc, followed by a PCR for the IPC using the eluted DNA (primed to the ATV from the ASV), does a pcrDecon, then a PCR for SAR11 from the eluted DNA, then a fullDecon. Then, does an IPC PCR reaction with elution water as the template, runs a pcrDecon, then does a SAR11 PCR reaction with elution water as the template followd by a pcrDecon.

Reagents for Solid Phase Extraction

Reagents are located on the valve positions specified:

20% Clorox Bleach (ATV.1)

Clorox Brand Bleach unscented diluted in water (Sigma #W4502)
0.2µm filtered

Elution Water (ATV.3) and Carrier (ARV.14 and AFV)

Water (Sigma #W4502)

ESTE Buffer 50 ml (EtOHwash, ATV.6)

13.3 ml Water (Sigma #W4502)
0.2 ml 0.5M EDTA (Sigma #E7889)

0.5 ml 1.0M Tris-pH 7.8 (Sigma #T2569)
1.0 ml 4M NaCl (Sigma #S5150)
35ml 200 proof Ethanol

0.1µm filter into Stedim Bag.

Solid Phase Extraction Dilution Buffer (SPEdil, on core **ESP PRV)**

100µl 3M NaAcetate pH 5.2 (Sigma # S7899)
800µl 200 proof Ethanol