



Environmental Sample Processor



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User's Manual

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Chapter 1

Introduction

The Spyglass Environmental Sample Processor—the ESP—is an electromechanical, fluidic instrument that is designed to autonomously collect water samples, concentrate suspended microorganisms and particles, apply molecular probes, and transmit data for real-time diagnostics of an aquatic environment (see Figure 1-1). The ESP also collects and preserves samples for later lab analysis. The ESP can be deployed at various depths depending on the accompanying instrument housing and infrastructure.



Figure 1-1: The Environmental Sample Processor, configured for deployment of less than 50 meters.

Currently, there are two molecular methods utilized in the ESP: sandwich hybridization and immunosorbent assays. The sandwich hybridization methodology is used to directly detect ribosomal RNA sequences diagnostic for groups of bacteria and archaea, invertebrates, and harmful algal species. An immunosorbent assay, specifically the cELISA technique, is used to detect domoic acid, an algal biotoxin.

The ESP is not limited to these methods or probes. Ongoing development is expanding the spectrum of microorganisms and the types of molecular methods used in their detection. For more information on components that extend the ESP's capabilities and probes developed since the publication of this document, please contact your Spyglass representative.

This chapter provides an introduction to how the core ESP system operates and key device components, the various types of files used and created during processing, and the protocols available at the time of this publication.

Warnings and Cautions

It is very important to follow all instructions and guidelines provided in this user's manual. Failure to do so may result in physical injury, voiding the warranty, and damage to the ESP system or surrounding equipment. For additional information on the Limited Warranty (see page i) and the Limited Warranty Statement provided with this Spyglass product. You may also request these documents from a Spyglass representative at any time.

Where appropriate, warnings and cautions have been included in this manual. The term “warning” is used to bring attention to a procedure or precaution that is important to protect the safety of the user and/or protect the ESP system from damage. The term “caution” brings attention to a procedure or precaution that should be carefully followed in order to avoid degradation in the performance of the system.

When operating the ESP or performing maintenance procedures, carefully note all warnings and cautions in this manual.

System Overview

How the ESP analyzes a sample is an orchestration of many systems and processes. This overview provides a high-level introduction to this analysis. It will also introduce various components in the ESP, which are described in “Components at a Glance” on page 4.

Protocols, Phases, and Missions

The application of a molecular methodology in the ESP is based on rigorously tested protocols. Currently, there are protocols to test for the presence of several groups of bacteria and archaea, invertebrates, and harmful algal species. Each protocol describes how the water sample is collected and filtered, what reagents and temperature are used to lyse the captured cells, and how the resulting lysate is processed and analyzed.

When the ESP performs an analysis based on one of these protocols, it is said to be *running* the protocol. Each protocol typically begins with the intake of a sample and ends with the processed results. Water sample are filtered and processed within special titanium containers called pucks. These pucks provide containment vessel for the filters and probe arrays used throughout the protocol. Several pucks are used during various stages during each protocol.

A *phase* is a series of one or more protocols. The ESP remains active for the duration of each phase. In between each phase, the ESP enters a low-power or *quiescent* state.

When the ESP operates autonomously and performs a series of phases, it is said to be running a *mission*. A mission is schedule of protocols to be performed. The mission's protocols are grouped into phases, between which the ESP enters quiescence. The mission typically ends soon after the last phase has finished.

The maximum number of phases that the ESP can run is based on the puck-carrying capacity of 132 pucks. For example, up to four (4) pucks are used in protocol for bacterial organisms (i.e., a BAC protocol). If the mission contains BAC protocols only, a total of 33 phases can be described in the mission ($132 \text{ pucks} \div 4 \text{ pucks per protocol} = 33 \text{ phases}$).

Sample Collection

Whenever a phase begins, the ESP wakes from a quiescent state. This entails powering on the necessary control electronics and moving various mechanical components to their *ready* position. If disinfectant solution was left in the intake lines by the previous phase, it must be flushed out before the first sample of the phase can begin. This is called the *initial purge*.

Once the intake is purged, *sampling* begins by loading a puck onto the collection stage. (The pucks are taken from the *Carousel*, moved to and from various locations within the ESP by the *Manipulator*.) Sampling is the process of intaking and concentrating a sample in the ESP.

There are three types of pucks that can be used for sampling: a standard volume sample puck (SVS), a high volume sample (HVS) puck, and a whole cell archive (WCR) puck. The SVS and HVS pucks are used to collect samples will be analyzed by the ESP. The WCR puck is used when collecting a sample that will be later analyzed in a laboratory. Each of these pucks contain filter media based on the target microorganism.

Note that these and other pucks used in the ESP have two openings through which fluid is allowed to pass. When a puck is in either the *Collection* or *Processing Clamp*, the clamp closes and pushes the puck down onto a *Heater Pad* to form seals around the openings. This allows fluid to pass through the puck without leakage.

With a puck secured in the Collection Clamp, the ESP measures the ambient pressure at the inlet. (The difference between the intake and the outlet pressures is monitored to determine whether the water is being drawn through the puck or if the intake has become clogged.) The *Sampling Syringe* draws in 25mL of water with each successive pull until the specified sample volume has been obtained. Particles in the water are concentrated on the filter in the puck as filtered water is expelled to the environment.

Sample Processing

Once the water has been removed, the concentrated material is ready to be processed and applied to the molecular probes.

To process a sample, a protocol specifies either a sandwich hybridization assay (SHA) or competitive enzyme-linked immunosorbent assay (cELISA). After the collection puck has been vacuumed dry, the *Collection Syringe* applies lysing reagents to the filtration residue. The puck and its contents are then heated—in place—up to 85 °C for several minutes to lyse the cells.

While the lysing is taking place, the ESP moves another puck containing a probe array into the Processing Clamp. Here, the array is prepared for lysate application. Each protocol uses an array with specific molecular probes to detect the target organisms.

After heating of the lysate is completed, the Collection Syringe draws the lysate from the collection puck. The Manipulator removes the collection puck and places it into the discard tube of the carousel. The Manipulator then retrieves a filtration puck from the Carousel and places it into the Collection Clamp. The Collection Syringe then pushes the lysate through the filtration puck while the Processing Syringe is simultaneously drawing it out. Once the lysate has passed through the filtration puck (i.e., the filtrate), the filtration puck is returned and discarded into the carousel.

The *Processing Syringe* then adds the filtrate and a series of reagents to the prepared array puck. In both SHA and cELISA methods, the addition of the final reagent results in a chemiluminescent reaction (i.e., light generating). The intensity of the reaction is proportional to the target organisms that may be present in the sample.

When the final reagent has been added, the array puck is moved to the camera-ready position. The digital CCD camera moves into position and takes one or more images. Images taken with the default resolution setting are available for data transmission. All images are stored as in Tagged Image File Format (*.tif) in the ESP system's memory and can be downloaded when the ESP is retrieved.

After the images are taken, the puck is lowered into the discard tube of the carousel.

Archival Collection

If an archive collection is part of a phase, an archival puck is loaded in the Collection Clamp and the process described in "Sample Collection" is repeated. Once the material is collected, the Collection Syringe applies a whole cell or RNA preservative to the puck, and the puck is returned to the Carousel.

Note: Based on current preservative formulations, an archive puck should be processed within 30 days of the ESP being returned to the surface and opened. Earlier processing may be necessary for deployments in higher ambient water temperatures.

Disinfection

After a phase is completed, the ESP cleans and flushes the collection and processing lines, and pumps a disinfectant into the intake line. The reagent valves as well as the collection and processing stages are also disinfected. The disinfectant remains in the intake line until the next phase begins.

Note: A small amount of disinfectant (less than 7mL) is pumped out of the ESP during the initial purge. All fluids containing chemical reagents or disinfectants generated during sample analysis are stored within the ESP until they can be properly disposed.

Components at a Glance

This section will acquaint you some of the key ESP components.

First, a few landmarks on the ESP are pointed out to provide bearings on where these components are located. Figure 1-2 shows two images of the ESP taken from opposite sides. The ESP appears as it would prior to being placed in its deployment container. The lid and the containers filled with reagents have been removed to facilitate the view into the device.

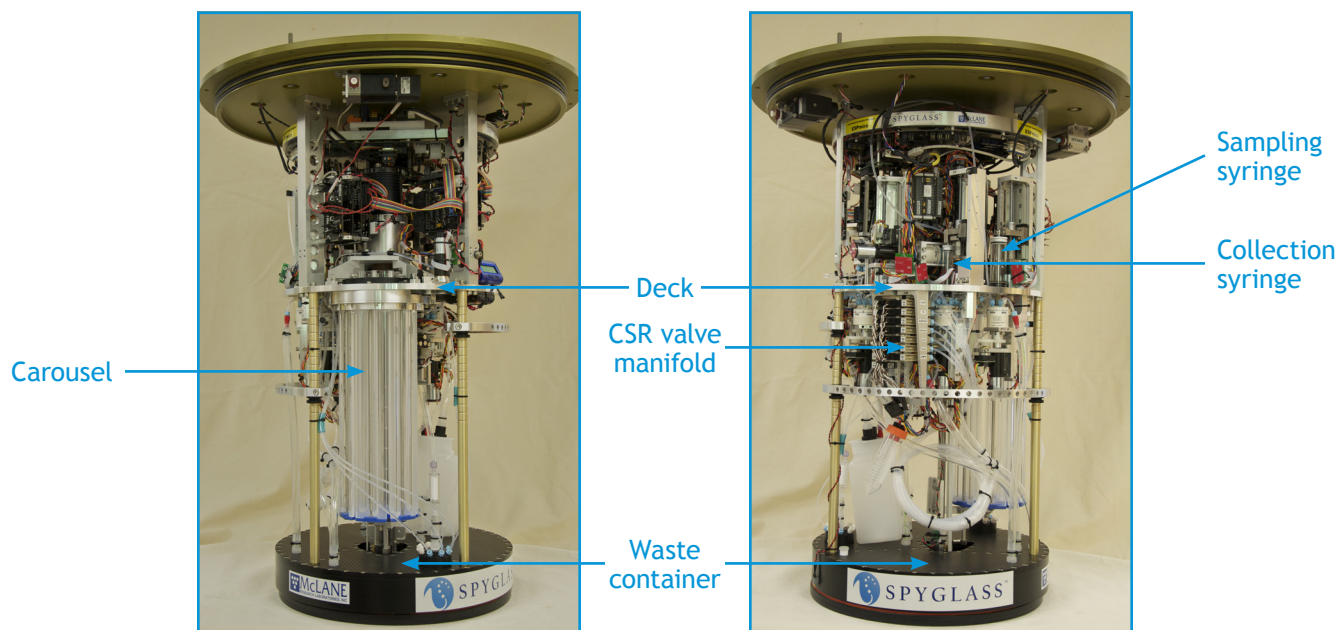


Figure 1-2: View into the ESP

Camera

The onboard CCD camera takes one or more images of each processed sample. Sensitive to low-light applications, the CCD has 1392 x 1040 pixels in a two-thirds inch format. In the ESP, the camera is set with three resolutions that can be used with a user-specified or automatic exposure. For information about image files and how to work with image settings, refer to “Image Files” on page 14 and “Image Settings” in Chapter 2.

The camera is a black cylinder, centrally located above the deck. See Figure 1-3.

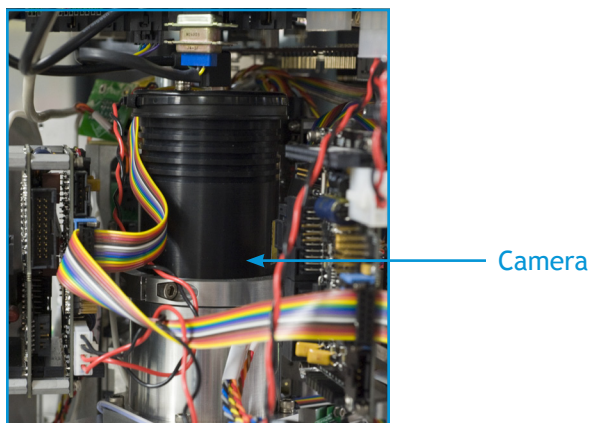


Figure 1-3: Camera

Carousel & Elevator

The Carousel is a circular arrangement of seven cylinders (or tubes) in which the pucks are held before and after processing. Each tube in the Carousel can hold 22 pucks. However, only six of these tubes contain pucks. At the beginning of a mission, it is necessary to keep one tube entirely empty. As the ESP goes through its sequence of operations and analyses, used pucks are placed into the empty tube. As that tube fills, another is emptied. The newly emptied tube becomes the current discard tube, and so on. The Carousel rotates in both directions to allow for a new puck to be retrieved or a used puck to be discarded.

A slot in each carousel tube allows the elevator to raise the stack of pucks from the bottom up. Once raised into position, the puck is retrieved from the top of the tube by the Manipulator.

The Carousel is located below the deck and is visually the largest component of the ESP. See Figure 1-4.

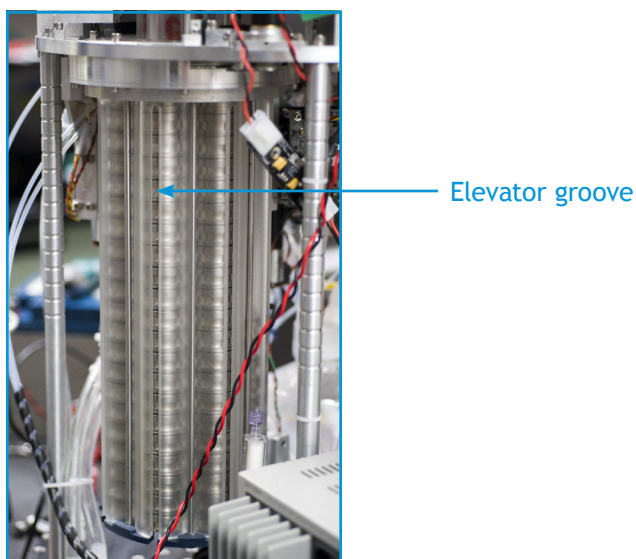


Figure 1-4: Carousel

Clamps and Heater Pads

During collection or processing, the Manipulator transfers a puck to either the Collection or Processing Clamp. The clamp lowers onto and around a puck securing on to the Heater Pad. When a puck is secured, the O-rings on the clamp and heater pad form seals with the openings in the puck through which fluid can flow leak-free. The Heater Pad also heats the puck and its contents to any temperature up to 95 °C.

A clamp and heater pad are located on either side of the flush puck garage.

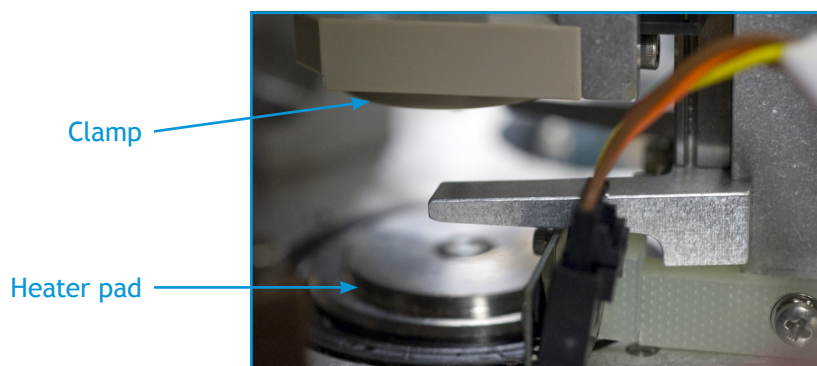


Figure 1-5: Clamp and heater pad

Collection, Processing, and Sampling Syringes

The ESP uses three syringes during the collection and processing of a sample: the Sampling Syringe, the Collection Syringe, and the Processing Syringe. Each syringe is driven by a ballscrew assembly that allows for precise positioning of the syringe plunger and, consequently, measurement of fluids drawn. See Figure 1-6.

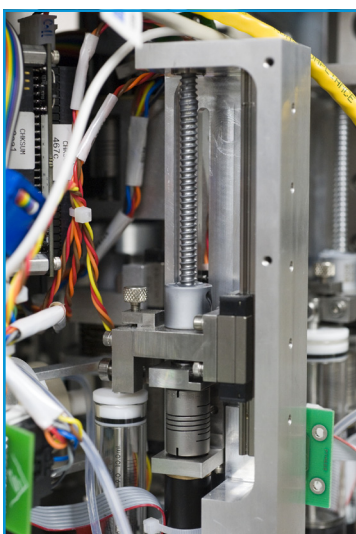


Figure 1-6: Syringe ballscrew assembly

Each syringe has a different capacity based on its function. The Sampling Syringe has a 25-mL capacity, which allows for efficient intake of water samples. After a sample is concentrated during collection and dried, its volume including any reagents added during lysing is managed by the 10-mL capacity of the Collection Syringe. The sample is further concentrated and reduced during lysate filtration and heating. This concentrate is transferred using the 2.5 mL Processing Syringe.

The syringe assemblies are located on the ESP deck. See Figure 1-7 for their orientation.

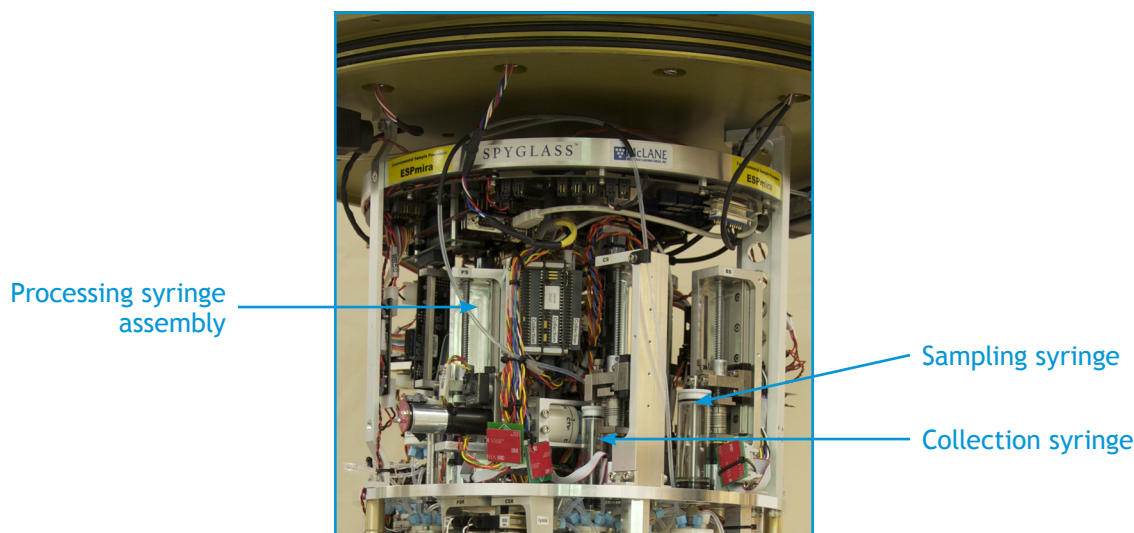


Figure 1-7: Syringe or syringe assembly locations

Communications Ports

There are four communication (comm) ports: 9-pin RS-232 connector, two USB connectors, and an Ethernet port (see Figure 1-8). The RS-232 is used for diagnostic purposes only. One USB port is connected to the low-light CCD camera. Do not insert any devices into unused connectors unless specifically instructed to do so by an authorized Spyglass technical support representative.



Figure 1-8: Communication ports

In bench-top testing, the Ethernet port is used to connect the ESP directly to a laboratory network. In deployments, the port is routed out of the pressure housing on a watertight connector to facilitate final testing. This watertight Ethernet connector is typically sealed with a dummy plug during underwater deployments.

The comm ports are located under the ESP motherboard.

Flush Puck Garage

Anytime the valves and associated lines need to be cleared of residual fluids (i.e., after a phase has completed), the ESP loads a puck made of a polyimide-based polymer onto a stage. Like its titanium counterpart, the Flush Puck has two openings through which fluid passes. Once clamped in place, the flush and clearing process takes place.

The Flush Puck is stored in a plastic housing—the *Flush Puck Garage*—located on the deck between the Collection and Processing Clamps. See Figure 1-9.

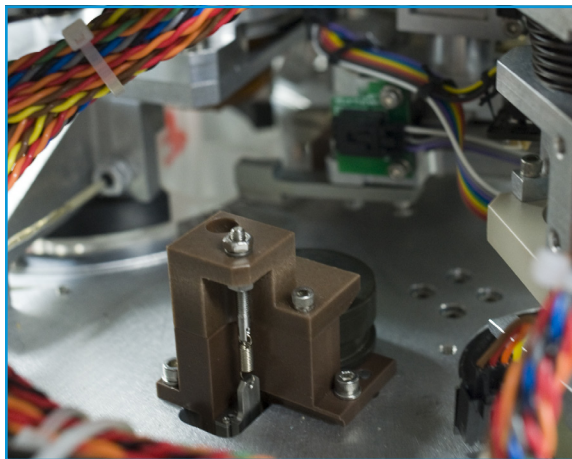


Figure 1-9: Flush puck garage

Manipulator

The Manipulator is the robotic assembly that precisely moves a puck from the carousel to a clamp and back again. Each puck has a large exterior groove in which the Manipulator grips the puck and transfers it.

The Manipulator assembly is located on the ESP deck. In Figure 1-10, the Manipulator is shown in its home position near the opening of a Carousel tube.

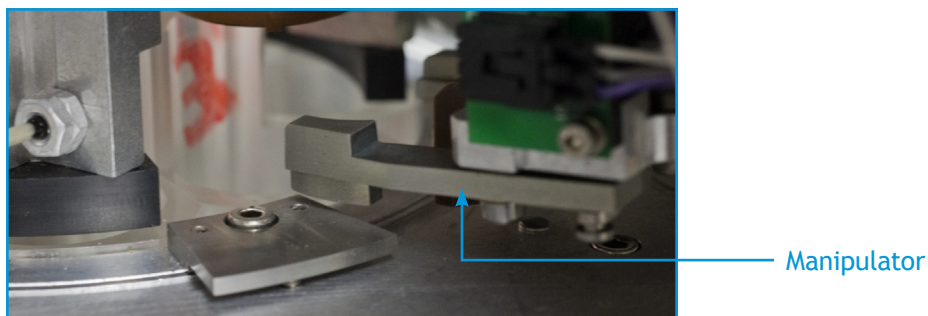


Figure 1-10: Manipulator

Pucks

The ESP uses special two-piece, titanium containers for sample collecting and processing. These reusable containers, called *pucks*, provide the solid support for filters or probe arrays. Each puck has two openings through which fluid enters and exits the pucks. While there are several types of pucks used for different purposes, all have the same external shape and size for handling in the ESP (1 3/8-inches diameter and 5/8-inch high). See Figure 1-11.



Figure 1-11: Example puck

For more information on the various type of pucks used in the ESP and how to work with them, see Chapter 3, “ESP Pucks.”

Reagents and Reagent Containers

The ESP uses a host of reagents throughout the analysis of a sample. Spyglass produces pre-packaged reagents and consumables in containers suitable for use with the ESP system (see Figure 1-12). Quality-assurance procedures including outbound quality control tests are performed on each lot of packaged reagent to ensure the end products meet rigorous quality standards.

Reagent containers are secured to the horizontal support rail, which is located to the right of the carousel below the deck.

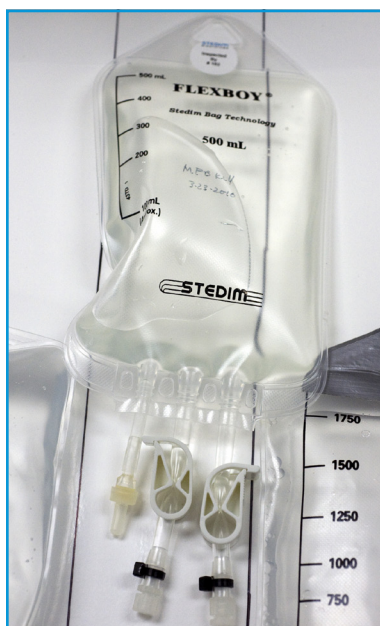


Figure 1-12: Reagent containers

Sample Intake and Exhaust Ports

Water enters and exits the ESP via ports integrated into the lid of the device (see Figure 1-13). The Sampling Syringe draws water through a collection puck, which filters out suspended particulates. Filtered water is pumped out the ESP, through its exhaust port, on each down stroke of the sampling syringe.

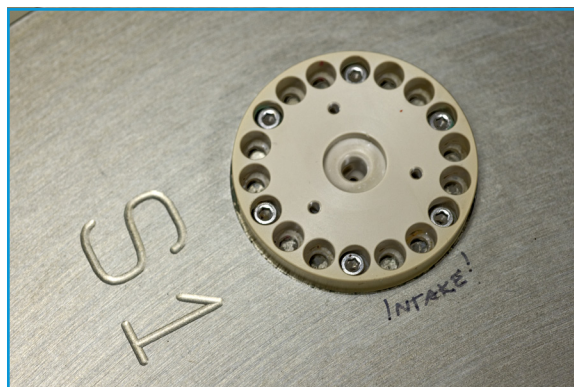


Figure 1-13: Intake port

Valves and Valve Manifold

All fluid flow through the ESP is controlled by a series of valves. The following table describes the valves used in the core ESP.

Table 1-1: Valves and Valve manifolds

Valve	Description
CBV	Collection Bottom Valve. Along with the CTV, this rotary valve controls fluid flow through the collection stage. The CBV is connected to the SSV, CSV, PSV, and waste containers. The CBV is located below the deck, under the collection stage.
CSR	Collection Series Reagents. This is valve manifold for reagents and other fluids used during the lysing or archiving of a sample. These fluids are accessible to the collection syringe (CS). The CSR is located below the deck. See Figure 1.
CSV	Collection Syringe Valve. This rotary valve controls fluid flow to the collection syringe (CS). The CSV connects the collection syringe to the CSR, CTV, flush reagent, waste containers, and clean air supplies. The CSV is located below the deck, under the collection syringe assembly.
CTV	Collection Top Valve. Along with the CBV, this rotary valve controls fluid flow through the collection stage. The CTV connects to the CSV and the intake port, and is located above the collection stage clamp.
PBV	Process Bottom Valve. Along with the PTV, this rotary valve controls fluid flow through the processing stage. The PBV connects to the PSV, air supply, and waste containers. It located below the deck, under the processing stage.
PSR	Processing Series Reagents. This is a valve manifold for reagents and other fluids used during the processing of a sample. The fluids are accessible to the Processing Syringe (PS). The PSR is located below the deck.
PRV	Precious Reagent Valve. This is valve manifold for reagents and other fluids used during the processing of a sample. These fluids are accessible to the processing syringe (PS). The PRV is located below the deck.
PSV	Processing Syringe Valve. This rotary valve controls fluid flow to the processing syringe (PS). The PSV connects the processing syringe to the CBV, PSR, PBV, PTV, a mixing loop, waste containers, and air supply.
PTV	Process Top Valve. Along with the PBV, this rotary valve controls fluid flow through the processing stage. The PTV connects to the PSV, PRV, and a mixing loop. It is located above the processing syringe assembly.
SSV	Sampling Syringe Valve. This rotary valve controls fluid flow to the sampling syringe. The SSV connects the sampling syringe to the exhaust port and the CBV.

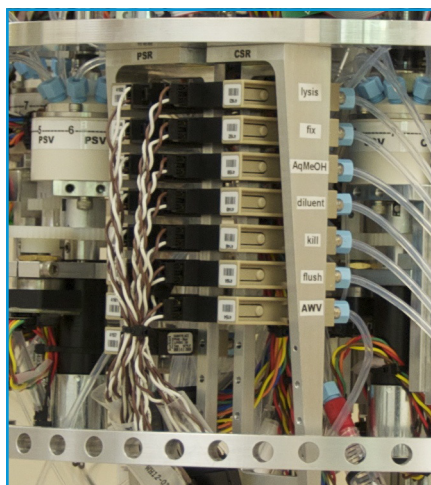


Figure 1-14: CSR manifold

For a layout of the valves, reagents and fluids, and the fluidic workflow, see Appendix C, “Fluidic Worksheet.”

Waste Containers

Environmental stewardship is the foundation of the development of the ESP. Therefore, all fluids containing chemical reagents or disinfectants generated during sample analysis are stored in the ESP until they can be properly disposed. The ESP has two waste containers built into its base with a 4.2-liter capacity (see Figure 1-2).

Files and File Formats

There are several types of files you will encounter while working with the ESP: application files, user-defined files, and several data files.

Script Files

The application used to operate the ESP is created using Ruby, an object-oriented programming language. The ESP application (`esp2`) is installed on the host computer as well as uploaded onto the ESP system memory. All files used in the ESP have the extension `*.rb` and are located in the `esp2` directory.

The ESP requires two user-defined files: the mission file and the `phasecfg.rb` file. These files are used to describe the mission and its phases. Both files are written in Ruby. For information on how to create and edit these files, see Chapter 2, “Missions & Phases.”

For more information on Ruby, see the official website: <http://www.ruby-lang.org>.

Status Files

The ESP records progress and status messages. These messages are stored in a binary log file, typically called `real.log`. When running the ESP interactively, these same progress and status messages are displayed in text form. However, when the ESP is running an autonomous mission, no host computer is available, so text status and progress messages are instead written to a file, typically called `real.out`.

The log file contains all of the messages generated while the ESP is running. Every executed movement of a component and any exception (i.e., error) are recorded to the log file. This binary file is accessible using the `dumlog` command. The `dumload` command converts the binary file into a readable text file; however, this is only recommended when troubleshooting is necessary.

The status file (*.out) provides a text record of ESP commands utilized, error messages, and other progress comments. The status file is named after the mode in which the ESP is operating. For example, deployed missions generally operate in the real mode; therefore, its status file is called `real.out`. Status files most easily accessed via the ESP's ftp server.

Note: When the ESP is running interactively during bench testing, status messages appear in the client window, but are only written to the system log file.

For information about status files and modes of operation, see Chapter 4, “Set Up & Bench-Top Operation.”

Image Files

After a probe array has been processed, one or more images are taken to capture the chemiluminescent results. These images are stored in Tagged Image File Format (*.tif).

Image files are stored in locations based on the resolution setting used. There are four resolution settings: `hires`, `midres`, `lores`, and `default`. Images taken with `hires`, `midres`, and `lores` are stored in a directory of the same name. For example, a `hires` image is stored in the `/hires` subdirectory of the esp users's FTP site (e.g., `/var/log/esp`). Images taken with the `default` setting, which has the equivalent resolution as the `midres` setting, are stored in the esp user's top FTP directory. These default images are available for data transmission during a deployed mission. Images taken with the other settings can be uploaded once the ESP has been retrieved.

Each image file is stored with a unique filename that is generated by the ESP. The image filename is written in the following format. Note that the arrows <> and curly brackets { } are not part of the filename, but are used to facilitate this explanation:

```
<protocol><date><hour>h{<volume>ml}{<exposureTime>s}{#n}.tif
```

Where:

protocol	One of the current ESP protocols (bac, da, hab, larv, or kar).
date	Date the image was taken. Represented by <code>yyymmdd</code> , where <code>yy</code> are the two digits of the year, <code>mmm</code> are three letters for the month, and <code>dd</code> are the two digits for the day of the month.
hour	The hour (h) in which the image was taken. Represented in 24-hour time. Note that any fraction of the hour is still represented by the hour alone.
volume	Actual volume sampled in milliliters (mL). This may be less than the maximum volume specified if a filter clogged.

- exposureTime* Duration of the image exposure in seconds (s). If the default exposure time is used in the phase, text for the exposure time is omitted from the filename. For automatic exposures, the actual exposure time used appears in the ESP system log and is encoded in the TIFF file's "image description" tag. It is not part of the filename since it is determined as the file is being created.
- #n* In the rare instance where an image file with the same name already exists, an integer, beginning with 2, is appended to the filename.

Here are examples of image filenames:

Filename	Explanation
da10mar2223h1000ml.tif	Automatic exposure image for a domoic acid protocol (da) taken on March 22, 2010 (10mar22) at 11 pm (23h) having sampled 1000 milliliters (1000ml).
hab10mar0518h500ml40s.tif	Image for a harmful algal bloom protocol (hab) taken on March 5, 2010 (10mar25) at 6 pm (18h). The sample volume was 500 milliliters (500ml) and the image exposure time was 40 seconds (40s).
larv10mar2206h750ml#2.tif	Image for a larval protocol (LARV) taken on March 22, 2010 (10mar22) at 6 am (06h). The sample volume was 750 milliliters (750ml). This is the second image (#2) with the same filename.

For more information on image files, see Chapter 2, "Image Settings."

ESP Protocols

At the time this document was published, the following are the current protocols developed for use with the ESP. The following table also lists the probes associated with each protocol. For more details regarding probes in development, contact your Spyglass representative.

Table 1-2: Currently available probes, by protocol

Protocol	Description
bac	Uses Sandwich Hybridization Assay (SHA) methodology and probes to detect for the presence of several bacterial clades. Probes include: - ARCTIC96BD-19 - EB750-1B7 - KTCC1119 - Marine Alpha Proteobacteria - Marine Cyanobacteria - Marine Group 1 Crenarchaea - Marine Group 2 Euryarchaea - OM60 - SAR11 - SAR86 clade III - SAR86 clades I-II
da	Uses competitive Enzyme-Linked Immunoabsorbent Assay (cELISA) methodology and antibodies to detect for the presence of domoic acid, which is associated with the genus <i>Pseudo-nitzschia</i>.
hab	Uses SHA and probes to detect for the presence of several clades associated with harmful algal blooms. Probes include: <i>Alexandrium catenella</i> <i>A. tamarense</i> <i>Heterosigma akashiwo</i> <i>Pseudo-nitzschia australis</i> <i>P. multiseriata</i> / <i>Pseudodelicatissima</i> <i>P. pungens</i>
kar	Uses SHA and probes to detect for the presence of several species of the genus <i>Karenia</i>, another harmful algae. Probes include: <i>Karenia brevis</i> <i>K. mikimoto</i> <i>K. papilionacea</i> <i>K. selliformis</i>
larv	Uses SHA and probes to detect for the presence of larva of several different marine clades. Probes include: - Clade Cirripedia, general - Clade Calanoida, subset exclusive of Acartia, Arctodiaptomus, Eudiaptomus, Tortanus and other orders in the Neocopepoda. - Clade Calanoida, subset exclusive of Acartia, Arctodiaptomus, Candacia, Eudiaptomus, Pseudocalanus, Skistodiaptomus, Tortanus and other orders in the Neocopepoda. - Genus <i>Calypptogena</i>, general - Clade Brachyura, general - Clade Crustacea, general - Clade Eukaryota, general <i>Carcinus maenas</i>, specific - Clade Siboglinidae, general - Genus <i>Mytilus</i>, general - Genus <i>Osedax</i>, specific - Clade Polychaeta, general - Clade Podoplea (all 5 orders), exclusive of Gymnoplea (contains Calanoida only). - Clade Provannidae, general

Chapter 2

Missions & Phases

As described in Chapter 1, “System Overview,” operation of the ESP begins with a mission. There are two files that you will need to create and/or edit in order to run a mission: a mission file (*.rb) and the `phasecfg.rb` file. The mission file contains information about what phases are performed during the mission and when. The `phasecfg.rb` file contains defaults for the phase such as sample volumes and settings for archives, images, and email.

Both files are located in the `mission` subdirectory of the ESP application directory (`esp2`). Both files are maintained using a text editor and Ruby, an object-oriented programming language. For more information on Ruby, see the official website: <http://www.ruby-lang.org>.

! Caution:

Use a text editor that produces UNIX-compatible ASCII text files. Do not use a word-processing application as the resulting text file may contain extraneous characters that can produce errors.

This chapter describes a mission file, the `phasecfg.rb` file, and the syntax used in both. It also describes the following:

- Creating a mission file
- Editing a `phasecfg.rb` file
- Checking a mission or `phasecfg.rb` file for syntax errors
- Changing the mission start time
- Changing the sample volume
- Changing the archive settings
- Changing the image settings
- Changing the email settings

Note: The terms “deployed” and “deployment” are used to describe a mission that is running when the ESP system is set up and operating in an aquatic environment. Under laboratory testing conditions, “benched” or “bench top” are used.

The Mission File

A mission file (*.rb) outlines the phases that the ESP will perform, and date and time each phase will begin. A mission file is required every time the ESP is deployed and when the ESP is allowed to run independently during benched testing. The filename for a mission should be unique. For example, a mission file for a deployment scheduled for June 1st, 2010 might be named 10june01.rb.

Note: The mission files are usually saved to the ESP's `mission` subdirectory.

The following text is an example of a mission file.

```
# The ESP Mission File for June 2010 Deployment
# Created by Johan Whale, johan.whale@spyglassbio.com
# First phase begins 08:00:00 PDT 6/01/2010
# Mission ends 08:00:00 PDT 6/30/2010

$bac[:wcrTime]='30:00' # overrides default fix time for BAC

mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do

  at "23:00:00 6/01/10" do # BAC 1
    initialPurge; bac [1000,75]
  end

  # other phases omitted for brevity

  at "10:30:00 6/28/10" do # LARV 5
    initialPurge; larv
  end

end # end of mission description
```

Minimally, a mission file contains the phases to be run, when each phase starts, and when the mission will terminate. A mission file can optionally contain settings that override the default values for one or more phases. (These default values are specified in the `phasecfg.rb` file explained in page 25.)

The following is the basic syntax for a mission file:

```
mission :startTube=>n, :until=>"end_time" do
    at "start_time" do
        phase
    end
end
```

Where:

- n* is the carousel tube from which the ESP begins retrieving pucks, i.e., the start tube. Note that the initial discard tube is *n*-1. Therefore, the start tube cannot be set to 1. For deployed missions, set *n*=2 as the start tube. For bench testing, *n* can be 2 – 7 as long as tube *n*-1 has enough space to accept pucks processed from tube *n*.
- end_time* is the time and date the mission will terminate. The :until symbol and *end_time* variable can be omitted for bench testing only.
- start_time* is the time and date a phase will begin.
- phase* is the description of the phase (i.e, the block of code). See “The Phase Description” on page 22.

The example mission file in also contains text—or *comments*—which has the sole purpose of enhancing the ability to understand the contents of the file. The first four lines in the example are comments. Comments are optional in code; however, they are key to organizing a mission. The text for a comment is preceded by a hash “#” mark. A comment can be located on a line by itself or it can be the last entry on a line of code. An example of a comment appearing in a line of code is:

```
at "23:00:00 6/01/10" do # BAC 1
    initialPurge; bac [1000,75]
end
```

The text, # BAC 1, that appears in the first line is a comment that indicates the phase description is for the first BAC phase in the mission. (Note that a BAC phase uses the protocol to analysis a sample for the presence of certain types of bacteria.)

The last line of code in a mission file is the `end` delimiter.

The mission Method

A mission is initiated with a method called `mission` and the symbols, `startTube` and `until`. (Methods and symbols are objects in Ruby.) Along with `mission`, `startTube` tells the ESP to orient the carousel such that the tube (specified with the `tube_#` variable) is the one from which the ESP begins taking pucks. The value entered for `tube_#` corresponds to a tube in the ESP carousel. While the start tube may vary between bench-top testing and deployments; in both cases, the start tube can never be tube 1.

! Caution:

Note that the initial discard tube is `n-1`, where `n` is number of the start tube. Therefore, the start tube cannot be set to 1.

For a deployment, `startTube` is set to 2. In bench testing, any other tube in the carousel (2 -7) can be the start tube as long as the tube (n-1) that precedes the start tube (n) has space to accommodate all of the pucks in the start tube. For example, if the benched ESP begins taking pucks from tube 4, then tube 3 must be empty or have enough space for all of the pucks that are in tube 4.

The `until` symbol and the `end_time` variable are used to specify the time and date when a mission will end. In Figure 1, the mission will end at 11:30 pm, Pacific Daylight Time, on June 30st, 2010 ('23:30:00 PDT 6/30/2010').

The end time must take into account the total time necessary to run all of the phases in the mission. With the exception of a phase that uses HAB with DA protocol (`habda`), most phases will take approximately 4 hours to run. A HABDA phase requires approximately 6 hours.

Note: The actual time that an individual phase takes to complete is dependent on the protocols used, and how long it takes to collect and filter the specified sample volume. The time needed to collect and filter a sample increase with increases in sample volume and/or increases in the amount of suspended particulates.

When deployed, the ESP operates on battery power. Therefore, it is necessary to also plan to end a mission within the battery life, which is up to 45 days.

Time & Date Format

Time and dates specified in the ESP files observe the rules used with the Ruby date class. However, it is recommend that you use the formats described here for time and date variables in the mission file.

To specify the time and date, use one of the following formats:

- `hh/mm/ss month/day/year`
- `hh/mm/ss xxx day – month – year`
- `hh/mm/ss xxx monthName day, year`

Where:

- hh/mm/ss* is the time using a 12- or 24-hour format, where *hh* is the hour, *mm* are the minutes, and *ss* are the seconds. Minutes and seconds are optional. For 12-format, AM or PM can be appended and AM is assumed if none specified. The 3-letter time zone can also be used: GMT, UMT, or the local time zone for the ESP host.
- month* is the month numeric (1-12), the English month name (January – December), or the 3-letter month abbreviation (Jan – Dec).
- day* is the numeric day of the month (1-31).
- year* is the 2- or 4-digit year.
- monthName* is the English month name or month abbreviation (see above).

Note: When entering time-date values in a mission file, the value must be enclosed in double quote marks (" ").

For example, to end a mission on midnight, June 30, 2010, you can use:

- 12 AM 06/30/10
- 24:00:00 30 - jun - 10
- 12:00 AM PDT June 30, 2010

Note: The punctuation in the first two examples above is required. However, the comma in the third example is optional.

You can also append any of the formats to the *dayofWeek*, which is the English day name (Monday – Sunday) or 3-letter day name abbreviation (Mon – Sun). For example:

Wed 11PM 6/30/10

When you specify dates, make sure to avoid errors associated with over specifying. For example, Sat 8:00 July 25, 2010 will produce an error since July 25th is actually a Sunday.

The time-date information can also contain the time zone: GMT (or UTC) or the local time zone for the ESP host. The following example is for 11:30 pm Pacific Daylight Time, June 30th, 2010:

23:30 PDT 6/30/2010

Note: The time zone needs to only be specified once in the mission file, usually in the first phase description. If a time zone is specified, it must be the same as the time zone for the ESP host. That is, do not specify EST unless the host's local time is Eastern Standard Time.

The Phase Description

A phase description is the code that is used to run a single phase. Each phase in a mission is described using the syntax:

```
at "start_time" do
    phase
end
```

Where:

start_time is the time and date that the phase will begin (see “Time & Date Format” on page 20).

phase is Ruby code that describes the phase. This code can contain one or more protocols as well as the options to override the default values associated with those protocols.

In the phase description below, beginning at 11:30 am on June 8th (6/08), the ESP will purge its internal system (`initialPurge`). The phase will use the `hab` protocol with default values for sample and archive volumes. The `end` delimiter completes the description (i.e., the code) for the phase.

```
at "11:30:00 6/08" do
    initialPurge; hab
end
```

When the ESP begins, each phase is specified in *start_time*. The start time must take into account the completion time of all preceding phases. Each phase can have a specific start time or, once the time and date for the first phase in the mission is defined, you can specify the start time for subsequent phases to begin at some amount of time after the preceding phase has completed. In the following example, the second BAC phase starts 8 hours after the first BAC phase is completed.

```
at "23:00:00 6/01/10" do
    initialPurge; bac [1100,500] # BAC 1
end
at "+8:00" do
    initialPurge; bac # BAC 2
end
```

Note: The start time for the first phase in the mission file is overridden by the mission start time. For more information about the mission start, how it affects the initial phase start time, and how to change its setting, see “Mission Start Time Setting” on page 30.

The following is a typical format for the code in *phase*. Note that a single phase description will not contain everything shown here. A semicolon must be used to separate the different parts of code.

```
initialPurge; protocol [sampVol, wcrVol]; settings;{noKill}
```

Where:

<i>initialPurge</i>	is code that commands the ESP to purge its intake system of any fluids. Required in deployed missions.
<i>protocol</i>	is the protocol the phase uses. Protocols initially installed with the ESP include <code>bac</code> , <code>da</code> , <code>hab</code> , <code>kar</code> , and <code>larv</code> . For details, see “ESP Protocols” on page 15.
<i>sampVol</i>	is the maximum volume of water (in milliliters) that the ESP collects for analysis. <i>sampVol</i> is a number greater than 10. If not specified, the default volume in the <code>phasecfg.rb</code> file is used.
<i>wcrVol</i>	is the maximum volume of water that the ESP collects for archiving. <i>wcrVol</i> is a number greater than 10. If not specified, the default volume in the <code>phasecfg.rb</code> file is used. If set to <code>nil</code> , an archive sample will not be collected.
<i>settings</i>	is optional code for the phase such as the archive, image, and email settings. If not specified, the defaults specified in the <code>phasecfg.rb</code> file are used.
<i>{noKill}</i>	is the code that commands the ESP to <u>not</u> disinfect the intake system. Generally used in bench testing only

In a deployed mission, `initialPurge` is required to purge the intake line of disinfectant that has been pushed through during the clean-up stage of a previously run phase. In the phase description, `initialPurge` appears before *protocol*.

In a benched mission, `initialPurge` may be omitted. If disinfecting the intake line is not necessary, such is the case when you are running a mission from fixed sample source, omit `initialPurge`. If `initialPurge` is omitted, the phase description must include `noKill`.

! Caution:

At the end of every mission, the intake line should be disinfected. Therefore, any subsequently run mission must purge the intake. Specify `initialPurge` in the first phase of any mission.

The *protocol* variable is the protocol the phase uses (i.e., `bac`, `da`, `hab`, `hadda`, `kar`, or `larv`) when executed. In the example above, the first phase has the values, 1100 and 500 for the sample volume (*sampVol*) and the archive volume (*wcrVol*), respectively. No such numbers appear in the second phase indicating that the default values will be used. (The default values for phases are set in “Sample Volumes Settings” on page 31)

The *settings* variable in the phase description can be archive, image, and/or emailing settings. Procedures on how to change these settings are described from page 31 to page 42.

In a bench mission, it may not be necessary to disinfect between phases. By default, the ESP disinfects the intake line after every phase. If `noKill` appears in the phase description, the ESP will not push disinfectant into the intake line at the end of the phase.

! Caution:

The intake line should be disinfected at the end of every mission, and between phases using different types of protocols (i.e., bac, hab, etc.).

The following is an example of a benched mission in which `initialPurge` is omitted from all but the first phase. The last phase does not specify `noKill`; therefore, the ESP will disinfect the intake line at the end of the mission.

```
at "10:00 6/13" do
    initialPurge; hab; {noKill} # HAB 1
end
at "+1:00" do
    hab; {noKill} # HAB 2
end
...
at "+1:00" do
    hab # final HAB
end
```

Creating a Mission File

Note: If your files are not automatically saved in a configuration management or version control system, make a back-up copy of the file before editing.

To create a mission file:

1. Open an existing mission file or open a new file in a text editor.
2. Enter the mission method and the values for `:startTube` and `:until`.
See “The mission Method” on page 20 for file details.
3. Enter a phase description for each phase to run in the mission. The order in which the phase will run is the same order in which it appears in the mission file.

See “The Phase Description” on page 22 for details. To change other mission settings, see:

- “Mission Start Time Setting” on page 30
- “Sample Volumes Settings” on page 31
- “Archive Settings” on page 32

- “Image Settings” on page 39
 - “Email Settings” on page 42
4. Enter comments throughout the file to indicate the start of the mission, to uniquely identify each phase, and to indicate any settings that differ from the defaults.

Note: Any text that appears in a line after an unquoted hash mark, #, is considered a comment in Ruby. A comment can be located on a line by itself or it can be the last entry on a line of code.

5. Save the file with the extension, .rb.

It is recommended that the file name be the date that the mission will run using the following format:

yymmdd.rb

For example, a mission that will run on June 1st, 2010 would have the following filename:

10june01.rb

After you've created the mission file, check the mission using the ESP `quick` mode (see “Checking for Syntax and Scheduling Errors” on page 46). This mode simulates the mission and can check for syntax and scheduling errors.

The phasecfg.rb File

There are two locations where options for phases are specified: the mission file or the `phasecfg.rb` file. Settings in the `phasecfg.rb` file are the defaults for all of the phases in a mission. The defaults can be overridden in the mission file for one or more phases.

Note: This file must be saved as `phasecfg.rb` and must appear in the ESP's `/mission` subdirectory.

Note: The `phasecfg.rb` file also contains settings for options and devices. Do not edit or remove these settings.

Default values for the following are set in the `phasecfg.rb` file:

- Mission start time
- Sample volume
- Archive volume, fixative (preserving reagent), number of fixative applications, and fix time
- Number of images taken, and the resolution and the exposure time for each image
- Various email settings

Initial Default Settings

The following table lists the default settings specified for each protocol as specified in the `phasecfg.rb` file initially installed with the ESP.

Table 2-1: Initial Default Archive, Image, and Email Settings by Protocol					
	BAC	DA	HAB	KAR	LARV
Sample Volume	All protocols set to use 1000mL sample volume.				
Archive Volume	1000mL	nil	50mL	50mL	1000mL
Archive Fixative	All protocols set to use a RNA stabilizing solution packaged by Spyglass.				
Archive Fixing Time	40 minutes	60 minutes	60 minutes	60 minutes	40 minutes
Archive Fix Application	2 times	3 times	3 times	3 times	2 times
Images	Three images: hires @ 160s, default @ 40s, midres @ auto.	Three images: default @ auto, midres @ 15s, hires @ 70s.	Same as BAC	Five images default @ 5s, 10s, and 40s; hires @ 160s; midres @ auto.	Same as BAC
Email	Settings that appear in the <code>phasecfg.rb</code> file initially installed with the ESP system may need to be edited. Placeholders, such <code>spyglassbio.com</code> for the domain name, are entered for file completeness.				

Default Mission Start Time

The default mission start time is specified in the `phasecfg.rb` file using the following syntax:

```
$startTime = "time"
```

Where *time* is the amount of time from when the mission is initiated to when the ESP begins the first phase. If a mission start time is specified, the start time specified for the first phase will be ignored. If time is set to `nil`, the ESP will start the first phase at the time specified in the mission file.

For deployed missions, it is recommended that `startTime` remains set to two minutes. If `startTime` is set to `nil` and the deployed mission is initiated anytime after the first phase's start time, a scheduling error is generated and the mission will not proceed. If `startTime` is set to `nil` and the mission initiates earlier than the first phase's start time, proper operation of the ESP cannot be verified until the scheduled time.

To change the mission start time, see "Mission Start Time Setting" on page 30.

Default Sample Volumes

The default sample volume is specified in the `phasecfg.rb` file apart from the other default settings using the following syntax:

```
$protocolVol = volume
```

Where:

protocol is the protocol to which the setting applies (i.e., bac, da, hab, larv, or kar).

volume is the sample volume, in milliliters (mL).

For example, the default sample volumes in the `phasecfg.rb` file initially installed with the ESP are as follows:

```
$bacVol = $daVol = $habVol = $karVol = $larvVol = 1000
$mfbbVol = 1000
$puflmVol = 2000
```

The default sample volume can be specified for an individual protocol (ex. `$mfbbVol = 1000`) or a group of protocols separated by an equal (=) sign. (Note that `$mfbbVol` and `$puflmVol` specify the default sample volume for optional ESP components. For more information about these components, contact your Spyglass representative.)

To change the sample volume, “Sample Volumes Settings” on page 31.

Default Archive, Image, and Email Settings

The default settings for archive samples, images, and email are specified in the `phasecfg.rb` file using the following syntax:

```
$protocol = {
  :assay=>:assay,
  :wcrVol=>volume,
  :wcrTime=>'duration',
  :wcrFixes=>[:fixative]*number,
  :image=>[image1, ... imageN],
  :email=>:HABfans.denotes(news_type + ['address1', ... 'addressN'])
}
```


Where:

<i>protocol</i>	is the protocol for which the settings apply (bac, da, hab, larv, or kar)
<i>assay</i>	is the analytical procedure used in the protocol (bac, da, hab, larv, or kar).
<i>volume</i>	is the volume, in milliliters (mL), used for archive collection. If set to nil, an archive sample <i>will not</i> be collected.
<i>duration</i>	is the total time that the ESP fixes the archive sample.
<i>fixative</i>	is the fixative (i.e., preserving reagent) used to fix the archive sample. In standard ESP configurations, this is a RNA stabilizing solution packaged by Spyglass (rnal). To change this setting, contact Spyglass technical support.
<i>number</i>	is the number of times the fixative is applied to the archive sample. If <i>number</i> is greater than 1, the duration of each application is <i>duration/number</i> . For example, if the total time is 40 minutes and the fixing reagent is applied twice, the duration of each application is 20 minutes.
<i>image1...N</i>	is the information for each image taken. This information can contains resolution (hires, midres, lores, default) and or exposure time (in seconds).
<i>news_type</i>	is the type of progress information emailed to recipients. See “Email Settings” on page 42.
<i>address1...</i>	is the email address for additional recipients. See “Email Settings” on page 42.

For example, the default settings for the HAB protocol might be as follows:

```
$hab = { :assay=>:hab,
          :wcrVol=>50,
          :wcrTime=>'40:00',
          :wcrFixes=>[:rnal]*2,
          :image=>[:hires,160, :default,40, :midres,:auto],
          :email=>:LARVfans.denotes(GoodNews+[ ])
        }
```

In the example above, HAB protocol uses the HAB assay (:assay=>:hab). The default volume used to collect archive samples is 50 milliliters (:wcrVol=>50) and the archive sample will be fixed twice using the fixative solution (:wcrFixes=>[:rnal]*2) over a period of 40 minutes (:wcrTime=> '40:00'). Three images are taken using three different resolutions and exposure times (:image=>[:hires,160, :default,40, :midres,:auto]), and email is sent to the wardens and Henry Limpet (:email=>:LARVfans.denotes(GoodNews+[henry.limpet@spyglassbio.com])).

As with the default sample volume, if more than one protocol uses the same settings, you can use the following syntax to abbreviate the code:

```
$protocol2 = $protocol1.dup.update :diff_setting
```

Where:

protocoll is the phase with the defaults specified.

protocol2 is the phase that uses the settings specified for *protocoll*.

diff_setting is one or more defaults that differ from those for *protocoll*.

For example, in the following, the KAR protocol uses many of the same defaults used in HAB (as seen on page 7). The exceptions are the assay, which is `kar`, and there is no additional email addressees.

```
$kar = $hab.dup.update :assay=>:kar,
  :email=>:KARfans.denotes(GoodNews+[ ])
```

Editing the phasecfg File

Edit the `phasecfg` file when you want to change the default settings for the phases in any mission. Otherwise, edit the mission file or individual phase descriptions instead. You can also create a special protocol in a mission file that can be used by phases in that mission. See “Adding a Protocol Description to a Mission File” on page 45.

Note: If your files are not automatically saved in a configuration management or version control system, make a back-up copy of the file before editing.

To edit the phasecfg file:

1. Open the existing `phasecfg.rb` file in a text editor.
2. Enter the changes to the default settings.

The default settings are used in a phase when no other settings appear in the phase description. See “The `phasecfg.rb` File” for details. How to change a default setting is described in the following sections:

- “Mission Start Time Setting” on page 30
- “Sample Volumes Settings” on page 31
- “Archive Settings” on page 32
- “Image Settings” on page 39
- “Email Settings” on page 42

3. Enter comments to indicate the changes you are introducing to the file.

Note: Any text that appears in a line after an unquoted hash mark, `#`, is considered a comment in Ruby. A comment can be located on a line by itself or it can be the last entry on a line of code.

5. Save the changes. The file must always be saved with the filename and the extension, `phasecfg.rb`.

After you’ve edited the file, check the mission using the ESP `quick` mode (see “Checking for Syntax and Scheduling Errors” on page 46). This mode simulates the mission and can check for syntax and scheduling errors.

Mission Start Time Setting

There are two ways to change the mission start time. You change the default setting in the `phasecfg.rb` file. In a mission file, you can specify a new start time for that mission only.

Note: If you want to delay the start of a mission until the time specified for the first phase, it is recommended that you specify a mission start time in the current mission file (see page 30).

To change the default mission start time

1. Open the `phasecfg.rb` file in a text editor.

The following is default start time in the `phasecfg.rb` file initially installed with the ESP software:

```
$startTime = "+0:02" #2 minute initial delay overrides specified start time
```

2. Using the following syntax, enter a new start time:

```
$startTime = "time"
```

Where *time* is the delay between when the mission is initiated and when the ESP begins the first phase. If a mission start time is specified, the start time specified for the first phase will be ignored.

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the start time for the current mission

1. Open the mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to enter the new start time for the mission:

```
$startTime = "time"
```

Where *time* is the delay between when the mission is initiated and when the ESP begins the first phase. If a mission start time is specified, the start time specified for the first phase will be ignored. If time is set to `nil`, the ESP will start the first phase at the time specified in the mission file

For example, the following mission will start at the time specified for the first phase (23:00:00 6/01/10).

```
$startTime = 'nil'

mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do

  at "23:00:00 6/01/10" do # BAC 1

    initialPurge; bac [1000,75]
```

3. Save the file.
4. Check the mission file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Sample Volumes Settings

There are several ways to change the volume used for sample collection. You can change the default setting in the `phasecfg.rb` file. In a mission file, you can also change the volume for every phase based on a protocol or you can change it for one single phase.

Note: The default sample volume is used when a sample volume has been specified in a mission file. A setting in the description for a phase takes precedence over any setting in the mission file or in the `phasecfg.rb` file.

To change the default sample volume

1. Open the `phasecfg.rb` file in a text editor.
2. Using the following syntax, enter a new volume—in milliliters (mL)—for the protocol:

`$protocolVol = volume`

The default sample volume can be specified for one protocol or a group of protocols separated by an equal (=) sign.

In the following example, all of the protocols use the same default sample volume of 1000 mL.

```
#default sample volumes for various phases
$bacVol = $daVol = $habVol = $karVol = $larvVol = 1000
```

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the sample volume for phases based on the same protocol

1. Open the mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to enter the sample volume used for every phase based on that protocol:

`$protocolVol = volume`

For example, all HAB phases in the following mission will have a sample volume to 3000 mL.

```
$habVol = 3000
```

```
mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do
```

All phases in this mission will use this setting unless a phase description specifies a different setting.

3. Save the file.
4. Check the mission file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the sample volume for a single phase

1. Open a mission file in a text editor.
2. In the phase description, the sample volume to use is entered immediately following the *protocol* reference. Enter a number greater than 10 for the volume in milliliters (mL).

```
protocol [sampVol, wcrVol];
```

For example, the following LARV phase will use 1100 mL as its sample volume and 500 mL for its archive volume.

```
at "23:00:00 6/03/10" do
```

```
  initialPurge; larv [1100, 500]
```

```
end
```

If you are planning to use the default archive volume setting, you do not have to specify a value for *wcrVol*. For example:

```
larv 1100
```

Note: To create a negative control during bench testing, the sample volume can be set to `nil`.

3. Save the file.
4. Check the mission file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Archive Settings

You can change the volume used to collect an archive sample, how many times the fixative is applied to the archive, and how long the archive is fixed.

Note: You can also specify that an archive sample is not collected. For more information, see “To specify that an archive sample is not collected” on page 35.

Changing the Archive Volume

There are several ways to change the volume used for archive collection. You can change the default setting in the `phasecfg.rb` file. In a mission file, you can also change the volume for every phase based on a protocol or you can change it for one single phase.

Note: The default setting is used when no archive volume has been specified in a mission file. A setting in the phase description takes precedence over any setting in the mission file or in the `phasecfg.rb` file.

! Caution:

Do not set the archive volume to zero (0). Although the ESP does not collect an archive sample, a WCR puck is still required for the phase. To command the ESP to not collect an archive, set `wcrVol` or *wcrVol* to `nil`. See “To specify that an archive sample is not collected” on page 35.

To change the default archive volume

1. Open the `phasecfg.rb` file in a text editor.

In the files initially installed with the ESP, the default settings are located under the following comment:

```
# default settings for archive, image, and email
```

2. Use the following syntax to enter a new volume—in milliliters (mL)—for the protocol.

```
:wcrVol=>volume,
```

Unless otherwise specified in the mission file, a phase based on this protocol will use this setting.

The following shows the default set to 1200 mL for phases using the HAB protocol.

```
$hab= {
  :assay=>:hab,
  :wcrVol=>1200,
  :wcrTime=>'40:00',
  :wcrFixes=>[:rnl]*2,
  :image=>[: hires,160, :default,40, :midres,:auto],
  :email=>:HABfans.denotes(GoodNews + [ ])
}
```

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the archive volume for phases based on the same protocol

1. Open a mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to enter the sample volume used for the phases based on that protocol.

```
$protocol[:wcrVol]=volume
```

In the following example, the archive volume for HAB phases is set to 3000 mL.

```
$hab[:wcrVol]=3000 # override default HAB archive volume;

mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do
```

All phases in this mission will use this setting unless a phase description specifies a different setting.

3. Save the file and check for syntax and scheduling errors.
- See “Checking for Syntax and Scheduling Errors” on page 46.

To change the archive volume for a single phase

1. Open a mission file in a text editor.
2. In the phase description, the archive volume (as well as the sample volume) to use is entered immediately following the *protocol* reference. Enter a number greater than 10 for the volume in milliliters (mL).

```
protocol [sampVol, wcrVol]
```

Note: If you specify an archive volume in the phase description, you must also include the sample volume even if you are using the default sample volume setting.

In the following example, this particular LARV phase uses 1100 mL for the sample volume and 500 mL for the archive volume.

```
at "23:00:00 6/03/10" do

  initialPurge; larv [1100, 500]

end
```

3. Save the file and check for syntax and scheduling errors.
- See “Checking for Syntax and Scheduling Errors” on page 46.

To specify that an archive sample is not collected

The BAC, HAB, KAR, and LARV protocols originally installed in the ESP software have `wcrVol` set to collect an archive sample (that is, a volume is specified). In the DA protocol, `wcrVol` set to `nil`, which specifies that an archive will not be collected during a phase using DA.

! Caution:

Do not set the archive volume to zero (0). Although the ESP does not collect an archive sample, a WCR puck is still required for the phase. To command the ESP to not collect an archive, set `wcrVol` or *wcrVol* to `nil`.

- To change the default settings, open the `phasecfg.rb` file in a text editor. In the default archive volume setting for a protocol, enter the following:

```
:wcrVol=>nil
```

- To change the settings for all phases using the same protocol, open the mission file in a text editor. Before the line of code that initiates a mission, enter the following:

```
$protocol[:wcrVol]=nil
```

- To change the setting for one phase, open the mission file in a text editor. In the phase description, enter `nil` for the *wcrVol* setting. Note that a value for *sampVol* must also be specified.

```
protocol[sampVol, nil]
```

Changing the Archive Fixing Time

There are several ways to change the amount of time a fixative is applied to an archive sample. You can change the default setting in the `phasecfg.rb` file. In a mission file, you can also change the setting for every phase based on a protocol or you can change them for one single phase.

Note: A default archive setting is used when no archive setting has been specified in a mission file. A setting in the description for a phase takes precedence over any setting in the mission file or in the `phasecfg.rb` file.

To change a default archive fixing time

- Open the `phasecfg.rb` file in a text editor.

In the files initially installed with the ESP, the default settings are located under the following comment:

```
# default settings for archive, image, and email
```

- Using the following syntax, enter the total amount of time (*hh:mm:ss*) that the fixative is applied to the archive. Unless otherwise specified, a phase based on this protocol will use this time when processing an archive sample.

```
:wcrTime=>'duration',
```

In the following example, the default fix time has been set to 40 minutes.

```
$hab= {
    :assay=>:hab,
    :wcrVol=>1200,
    :wcrTime=>'40:00',
    :wcrFixes=>[:rnal]*2,
    :image=>[:hires,160, :default,40, :midres,:auto],
    :email=>:HABfans.denotes(GoodNews + [ ])
}
```

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the archive fix time for phases based on the same protocol

1. Open a mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to change the fix time used for phases based on that protocol:

```
$protocol[:wcrTime]='duration'
```

In the following example, the fix time for HAB phases is set to 1 hour and 10 minutes.

```
$hab[:wcrTime]='1:10:00' # override default HAB archive fix time;
mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do
```

All phases in this mission will use this setting unless a phase description specifies a different setting.

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the archive fix time for a single phase

1. Open a mission file in a text editor.
2. After the protocol reference in the phase description, enter the new archive fix time (`wcrTime`) using the following syntax:

```
$protocol.dup.update :wcrTime=>'duration'
```

Note: `$protocol.dup.update` copies the default settings for the protocol (specified in the `phasecfg.rb` file) and then merges them with any new setting (ex. `wcrTime`) that follows. If `dup` is missing from the code, the default setting will be “updated” for all subsequent phases with the same protocol.

For example, the fix time for this particular LARV phase is 1 hour and 10 minutes.

```
at "23:00:00 6/03/10" do
    initialPurge; larv [1100, 500]; $larv.dup.update :wcrTime=>'1:10:00'
end
```

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Changing the Archive Fix Application

There are several ways to change how many times a fixative is applied to an archive sample. You can change the default setting in the `phasecfg.rb` file. In a mission file, you can also change the setting for every phase based on a protocol or you can change them for one single phase.

Note: A default setting is used when no archive setting has been specified in a mission file. A setting in the description for a phase takes precedence over any other setting in the mission file or in the `phasecfg.rb` file.

To change a default archive fix application

1. Open the `phasecfg.rb` file in a text editor.

In the files initially installed with the ESP, the default settings are located under the following comment:

```
# default settings for archive, image, and email
```

2. Using the following syntax, enter the number of many times the fixative is applied to the archive. Unless otherwise specified, a phase based on this protocol will use this time when processing an archive sample.

```
:wcrFixes=>[:rnal]*number
```

In the following example, the fixative, `rnal`, is applied twice (2):

```
$hab= {
    :assay=>:hab,
    :wcrVol=>1200,
    :wcrTime=>'40:00',
    :wcrFixes=>[:rnal]*2,
```

```

:image=>[:hires,160, :default,40, :midres,:auto],
:email=>:HABfans.denotes(GoodNews + [ ])
}

```

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the archive fix application for phases based on the same protocol

1. Open a mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to change the fix application used for phases based on that protocol:

```
$protocol[:wcrFixes=>[:rnal]*number
```

In the following example, the fix time for HAB phases is set to 1 hour and 10 minutes.

```

$hab[:wcrFixes=>[:rnal]*2 # override default HAB fix application;

mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do

```

All phases in this mission will use this setting unless a phase description specifies a different setting.

3. Save the file.
4. Check the mission file for syntax errors.

To change the archive fix application for a single phase

1. Open a mission file in a text editor.
2. After the protocol reference in the phase description, enter the number of times the fixative is applied using the following syntax:

```
$protocol.dup.update :wcrFixes=>[:rnal]*number
```

Note: `$protocol.dup.update` copies the default settings for the protocol (specified in the `phasecfg.rb` file) and then adds the new setting (ex. `wcrFixes`). If `dup` is missing from the code, the default setting will be “updated” for all subsequent phases with the same protocol.

In the following example, `rnal` is applied twice (2) during this particular LARV phase.

```

at "23:00:00 6/03/10" do
  initialPurge; larv [1100, 500]; $larv.dup.update :wcrFixes=>[:rnal]*2
end

```

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Image Settings

You can specify the number of images taken, the resolution and exposure of each image, and the sequence in which the images are taken. The syntax used for the image settings allows you to specify all of these settings in the same line of code.

The following is the syntax used for the image settings:

```
:image=>[image1, ... , imageN],
```

The symbol, `:image`, is followed by a hash (i.e., list) of values. These values can be the resolution (`: hires`, `: midres`, `: lores`, or `: default`) or the exposure time (specified in seconds or `: auto`). If a value in the hash is a resolution, the ESP expects the next value to be the exposure time. For example,

```
:image=>[: hires,160, : default,40, : midres,: auto],
```

In the example above, three images are specified. The ESP camera takes the first image using high resolution with an exposure time of 160 seconds (`: hires,160`), and the second image with the default resolution with a 40-second exposure (`: default,40`.)

If a number appears in the hash and it is not preceded by a resolution, the ESP interprets this as the exposure time for another image based on the last resolution specified.

Note: If a resolution is not initially specified in the hash, the ESP camera uses the default resolution. Once a resolution is specified, to use the default setting again, `: default` must be specified in the hash.

For example,

```
:image=>[10, 20 : hires, 120, : default, : auto]
```

In this example, the ESP camera takes two images taken use the default resolution, one with a 10-second exposure and the second with a 20-second exposure. The third image is taken using high resolution and a 120-second exposure. The last image is taken using the default resolution using the automatic exposure time.

All images are saved in the Tagged Image File Format (*.tif) and stored in ESP memory. Refer to “Image Files” on page 14 for information about image filenames and directories.

Image Resolution

Table 2-2 lists the available resolutions for the ESP camera.

Table 2-2: Available Image Resolution		
Setting	Image Format (Pixels)	Directory
hires	1392 x 1040	/hires
midres	696 x 520	/midres
lores	348 x 260	/lores
default	696 x 520 (same as midres)	the top level directory

Image Exposure Time

The exposure time is specified in seconds or using `auto` for automatic exposure. The automatic exposure calculates exposure time such that the brightest spot in the image will be near the maximum intensity the image can capture without saturating any part of the image.

Changing Image Settings

There are several ways to change the image settings. You can change the default setting in the `phasecfg.rb` file. In a mission file, you can also change the settings for every phase based on a protocol or you can change them for one single phase.

Note: The default image settings are used when no other setting has been specified in a mission file. A setting in the description for a phase takes precedence over any other setting in the mission file or in the `phasecfg.rb` file.

To change the default image settings

1. Open the `phasecfg.rb` file in a text editor.

In the files initially installed with the ESP, the default settings are located under the following comment:

```
# default settings for archive, image, and email
```

2. Using the following syntax, enter the images to be taken for the protocol. Unless otherwise specified, each phase based on this protocol will use the following syntax:

```
:image=>[image1, ... , imageN],
```

In the following example, the image settings specifies three images using three different resolutions.

```
$hab= {
```

```

:assay=>:hab,
:wcrVol=>1200,
:wcrTime=>'40:00',
:wcrFixes=>[:rnal]*2,
:image=>[:hires,160, :default,40, :midres,:auto],
:email=>:HABfans.denotes(GoodNews + [ ])
}

```

3. Save the file.
4. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the image settings for all phases based on the same protocol

1. Open the mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to change the image settings for phases based on that protocol:

```
$protocol[:image=>[image1, ... , imageN]
```

In the following example, the image settings specifies three images using three different resolutions.

```

$hab[:image]=>[:hires,160, :default,40, :midres,:auto];

mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do

```

For this example, all HAB phases in this mission will use this setting unless a phase description specifies a different setting.

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the image settings for a single phase

1. Open the mission file in a text editor.
2. After the protocol reference in the phase description, enter the image settings using the following syntax:

```
$protocol.dup.update :image=>[image1, ... , imageN]
```

Note: `$protocol.dup.update` copies the default settings for the protocol (specified in the `phasecfg.rb` file) and then adds the new setting (ex. `image`). If `dup` is missing from the code, the default setting will be “updated” for all subsequent phases with the same protocol.

In the following example, three images using three different resolutions will be taken for this particular LARV phase.

```
at "23:00:00 6/03/10" do
    initialPurge; larv [1100, 500]; $larv.dup.update :image=>[: hires,160, :de-
        fault,40, :midres,:auto]
end
```

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Email Settings

You can specify how status emails are sent, to whom the messages are sent, and the text that appears in the message. These settings are specified in the `phasecfg.rb` file.

The `phasecfg.rb` initially installed with the ESP application has the following setting that specify emails will be sent through an email server. The ESP must be connected to the server via Ethernet cable. Do not change this setting unless the ESP is being deployed.

```
Email.server = 'mail'
```

The status of a phase is automatically sent to the wardens of the ESP. The wardens are email addresses specified in the `phasecfg.rb` file. Wardens and the type of messages they receive are specified using the following code:

```
Email.domain = 'domain_name'
```

and

```
wardens = [recipient1', ... , 'recipientN']

:BadNews.denotes mail.new wardens, :Subject=>'bad_news'

:GoodNews.denotes mail.new wardens.dup, :Subject=>'good_news'
```

Where:

- domain_name* is the domain of the email addressees. For example, spyglassbio.com.
- recipient1...N* is a warden of the ESP. A warden will receive all of the status messages for each phase in the mission. Enter the email name for each warden. This name is appended with the domain specified with the *domain_name* setting for the complete email address.
- bad_news* is the text that appears in the subject line of the email message. Enter text that indicates there is a problem with the phase and it was not completed.
- good_news* is the text that appears in the subject line of the email message. Enter text that indicates the phase has completed successfully.

The wardens can only be changed in the `phasecfg.rb` file; however, additional addresses may be added in the mission file. In the default settings for a given protocol, you can specify who else will receive status messages using the following syntax:

```
:email=>:protocolfans.denotes(GoodNews+['address1', ... , 'addressN'])
```

Where:

- protocol* is the protocol for which the specified recipients will receive messages (i.e., BAC, DA, HAB, LARV, or KAR). The protocol must be entered with all capital letters. When a phase based on this protocol is run, a status message will be sent.
- address1...N* is the complete email address for an email recipient. If no addresses are specified, only the wardens will be sent the message.

Note: Many of the email settings that appear in the `phasecfg.rb` file initially installed with the ESP system may need to be edited. Placeholders, such as `spyglassbio.com`, are entered for file completeness. Edit these settings accordingly.

To change the ESP wardens

1. Open the `phasecfg.rb` file in a text editor.
2. To change the email domain, use the following syntax:

```
Email.domain = 'domain_name'
```

For example:

```
Email.domain = 'spyglassbio.com'
```

3. To change the email recipient name, use the following syntax. Enter a name for each additional recipient of the status message. The name is appended with the domain name specified above.

```
wardens = ['recipient1', ... , 'recipientN']
```

For example:

```
wardens = ['richard.moby', 'johan.whale', 'santiago.marlin']
```

The complete email address will be `richard.moby@spyglassbio.com`, etc.

4. Save the file.
5. Check the mission using the new `phasecfg.rb` file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To change the text for a status message

1. Open the phasecfg.rb file in a text editor.
2. Enter the text for the status message using the following syntax:

```
:BadNews.denotes mail.new wardens, :Subject=>'bad_news'
```

or

```
:GoodNews.denotes mail.new wardens.dup, :Subject=>'good_news'
```

This text appears in the subject line of the email.

For example:

```
:BadNews.denotes mail.new wardens, :Subject=>'Problem with phase'
```

3. Save the file.
4. Check the mission using the new phasecfg.rb file for syntax errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To add an email recipient for all phases based on the same protocol

1. Open the mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax to change the email settings for phases based on that protocol:

```
$protocol[:email]=>:protocolfans.denotes(GoodNews+['address1', ... , 'addressN'])
```

In the following example, the image settings specifies three images using three different resolutions:

```
$hab[:email]=>BACfans.denotes GoodNews+[henry.limpet@spyglassbio.com];
```

```
mission :startTube=>2, :until=>"23:30 PDT 6/30/2010" do
```

For this example, an email will be sent to Henry Limpet for every HAB phases that completes successfully in this mission (unless otherwise specified in a phase description).

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

To add an email recipient for a single phase

1. Open the mission file in a text editor.
2. After the protocol reference in the phase description, enter the additional email addresses using the following syntax:

```
$protocol.dup.update :email=>:protocolfans.denotes(GoodNews+['address1', ... , 'addressN'])
```

Note: `$protocol.dup.update` copies the default settings for the protocol (specified in the `phasecfg.rb` file) and then adds the new setting (ex. `email`). If `dup` is missing from the code, the default setting will be “updated” for all subsequent phases with the same protocol.

For example:

```
at "09:00:00 07/02/2010" do

  initialPurge; bac; $bac.dup.update :email=>BACfans.denotes
  (GoodNews+[henry.limpet@spyglassbio.com];

end
```

3. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Adding a Protocol Description to a Mission File

If you are changing a number of default settings for a phase that will be used many times in the mission, you can add a protocol description to the mission file and then refer to it in the phase description.

1. Open the mission file in a text editor.
2. Before the line of code that initiates a mission, use the following syntax:

```
$myprotocol = {

  :assay=>:assay,

  :wcrVol=>volume,

  :wcrTime=>'duration',

  :wcrFixes=>[:rnal]*number,

  :image=>[image1, ... imageN],

  :email=>:HABfans.denotes(news_type + ['address1', ... 'addressN'])

}
```

Where:

myprotocol is the name for the modified protocol. For example, `specialkar`, which might represent a protocol where the archive settings are different than the defaults.

See Default Archive, Image, and Email Settings” for information on other settings.

For example, the KAR protocol initially installed in the ESP software is described in the `phasecfg.rb` file as follows:

```
$karVol = 1000
```

and

```
$kar = $hab.dup.update :assay=>:kar,
  :image=>[5, 10, 40, :hires,160, :midres, :auto],
  :email=>:KARfans.denotes(GoodNews+[ ])
```

If you want to run several phases based on the KAR protocol always with the same changes, you might enter the following into the mission file:

```
$specialkar = {
  :assay=>:kar,
  :wcrVol=>1100,
  :wcrTime=>'40:00',
  :wcrFixes=>[:rnal]*2,
  :image=>[:hires,160, :midres,60],
  :email=>:KARfans.denotes(GoodNews+['ligeia.siren@spyglassbio.com'])
}
```

3. In the phase description, refer to the new protocol.

For example:

```
at "06:00:00 07/04/2010" do
  initialPurge; specialkar 1100
end
```

Note that the sample volume was set in the phase description.

4. Save the file and check for syntax and scheduling errors.

See “Checking for Syntax and Scheduling Errors” on page 46.

Checking for Syntax and Scheduling Errors

The ESP has several modes of operation. One mode, `quick`, allows you to check the mission and `phasecfg.rb` files for syntax and scheduling errors.

To check the mission and/or phasecfg file:

1. On the ESP host machine, make sure the files are located in the `mission` subdirectory of the ESP application directory (`eps2`). (The host machine is the computer set up to communicate with the ESP system.)
2. Start the ESP program.

See “Starting the ESP Application” on page 78.

3. At the ESP prompt, type the following and press ENTER:

```
ESP=quick esp mission_name.rb
```

Where *mission_name* is the filename of the mission script.

4. If there are any syntax errors, edit the mission and/or `phasecfg.rb` files accordingly, and then repeat step 3.
5. If there are no syntax errors, check the log file to confirm that the mission is running the desired protocols on schedule with the expected options.

See “Viewing the Progress Messages” on page 87.

6. Upload the mission file and/or `phasecfg.rb` file to the ESP.

See “Uploading Files to the ESP” on page 79.



Chapter 3

ESP Pucks

The ESP uses special two-piece, titanium containers for sample collecting and processing. These reusable containers, called *pucks*, provide the solid support for filters or probe arrays. While there are several types of pucks, all have the same external shape and size for handling in the ESP.

This chapter provides the following information:

- What types of pucks are used in the ESP
- What pucks are required for a given phase
- How to open a puck
- How to clean the pucks and the internal components
- How to assemble a puck for a given phase
- How to create a carousel loading map
- How to load pucks into the ESP carousel



WARNING:

Performing these procedures described in this chapter may involve contact with harsh or hazardous chemicals. Observe the necessary precautions and wear the appropriate protective clothing, gloves, and eye protection at all times.

! Caution:

The procedures described in this chapter call for specific products have been tested with the ESP. The use of a product other than the one specified may affect the quality in performance and/or data. See Appendix B, “Supplies,” for details.

Puck Types

The ESP uses different types of pucks during different stages of sample collection and processing. While the overall exterior dimensions are the same between all of the pucks, the internal configuration and contents vary depending on the puck’s functionality.

A puck is comprised of two pieces: a puck top and a puck bottom. These pieces are snapped together and held in place by an O-ring. A second O-ring is used to seal the puck. There are openings in the top and bottom pieces through which fluid enters and exits the puck. The size of the opening in the puck top varies between the puck types; the openings in the puck bottoms are the same size.

Figure 3-1 shows the variety of pucks, each open to display its interior configuration. The top row in the figure shows the bottom pieces position above its corresponding top piece. An assembled puck is shown on its side above the ruler.

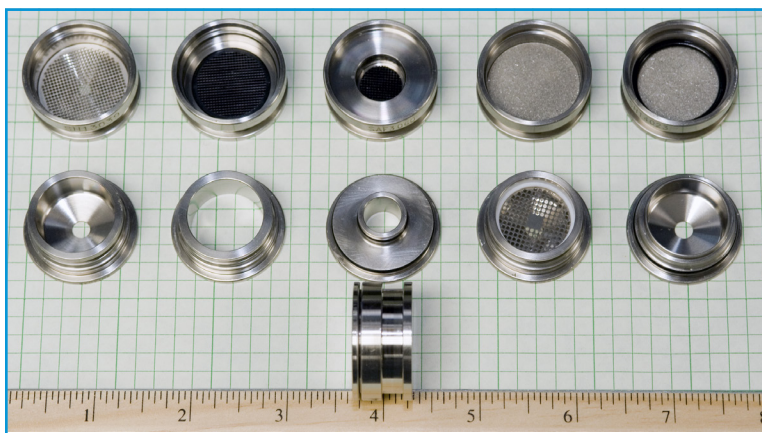


Figure 3-1: Interior view of ESP pucks

The outside edge of the top and bottom of each puck is engraved with a series of letters and digits. The first three letters represent the puck type and the remaining characters provided a unique ID number. See “Identifying Pucks” on page 53.

The following are the available ESP pucks that are described in the following sections.

- Standard-volume sample
- High-volume sample
- Whole cell archive
- Large array format
- Small array format

Standard Volume Sample Puck

A standard-volume sample puck (SVS) is used during sample collection and during lysate filtration. See Figure 3-2. For either collection or filtration, the SVS puck is assembled using an SHT top and an SH1 bottom. Note that the three-letter designation identifies the puck type and is engraved on an outside edge of each top and bottom. See “Identifying Pucks” on page 53 for more information.

For a standard-volume sample collection, the puck is loaded with a membrane filter through which 25 ml to 2 liters of water are passed. (For a sample volume greater than 2 liters, the HVS puck is used.) The filter porosity (0.22 μm to 5 μm) is determined by the protocol used in the phase. The majority of phases use this puck for sampling.

When used in lysate filtration, the SVS puck contains a 0.22 μm membrane filter through which the lysate is drawn. This configuration is used during lysate filtration for all current protocols.

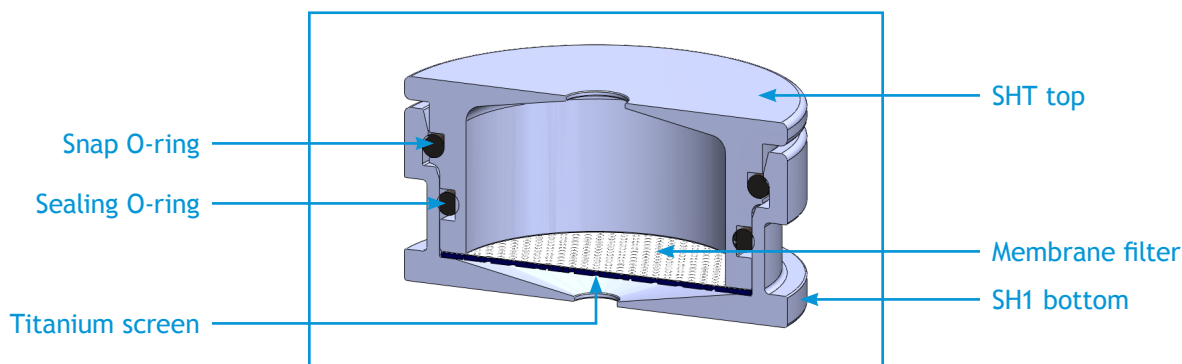


Figure 3-2: Standard volume sample puck, SVS, cross section

High-Volume Sample Puck

A high-volume sample puck (HVS) is used during sample collection when the environmental occurrence of a target microorganism is low, requiring a larger volume of water to be collected (one to 10 liters). A HVS puck is assembled using a HV2 top and an SH1 bottom, and is loaded with a titanium filtration frit (see Figure 3-3). Note that the three-letter designation identifies the puck type and is engraved on an outside edge of each top and bottom. See “Identifying Pucks” on page 53 for more information.

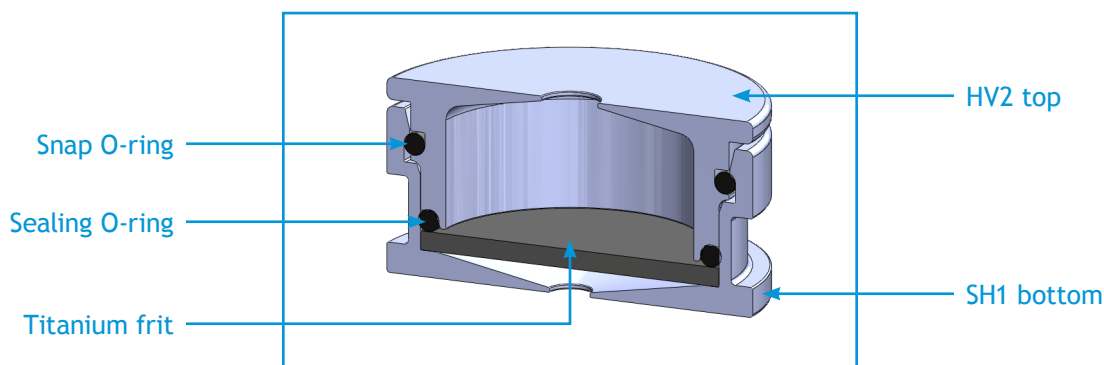


Figure 3-3: High volume sample puck, HVS, cross section

Whole Cell Archival Puck

The whole cell archival puck (WCR) is used for archive sample collection. A WCR puck is assembled using a WCR top and WCR bottom. Note that the three-letter designation identifies the puck type and is engraved on an outside edge of each top and bottom. See “Identifying Pucks” on page 53 for more information.

A WCR puck contains one or more membrane filters supported by a titanium frit through which 25 ml to 2 liters of water are passed (see Figure 3-4). The porosity of the membrane filters are based on the protocol used in the phase. This puck is used only during the archival collection process in which whole cells are collected and preserved for future laboratory testing. A preservative is added during the archival process.

Note: Based on current preservative formulations, an archive puck should be processed within 30 days of the ESP being returned to the surface and opened. Earlier processing may be necessary for deployments in higher ambient water temperatures.

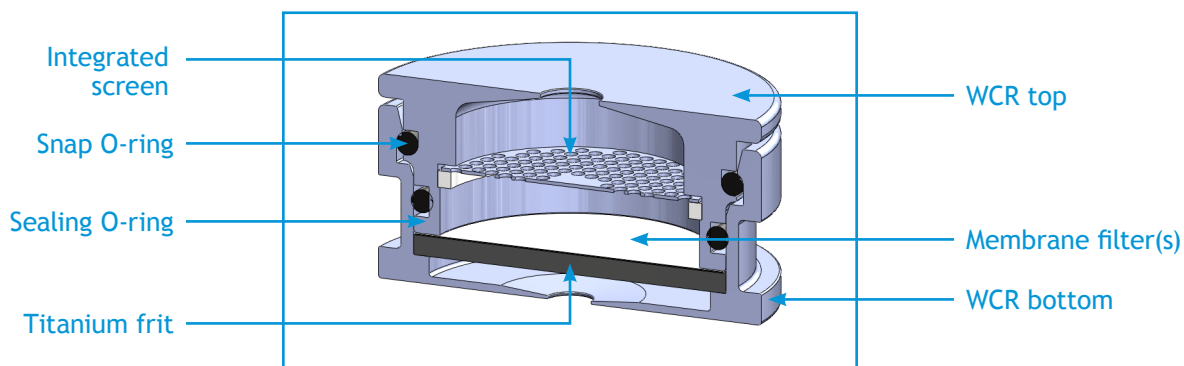


Figure 3-4: Whole cell archive puck, WCR, cross section

Large array format

A large array format puck (LAF) is used during sample processing. A LAF puck is assembled from an LAF top and an LAF bottom. Note that the three-letter designation identifies the puck type and is engraved on an outside edge of each top and bottom. See “Identifying Pucks” on page 53 for more information.

A LAF puck contains a probe array on a nitrocellulose membrane supported by a polysulfone disc. Pre-configured arrays have been designed for several algal, bacterial, and larval organisms. See Figure 3-5.

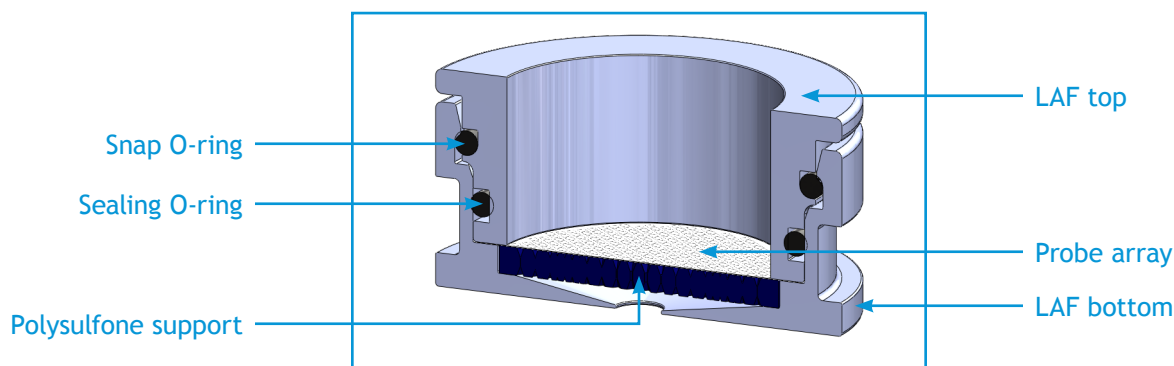


Figure 3-5: Large array format puck, cross section

Small array format

A small array format puck (SAF) is used during the sample processing. A SAF puck is assembled from an SAF top and an SAF bottom. Note that the three-letter designation identifies the puck type and is engraved on an outside edge of each top and bottom. See “Identifying Pucks” on page 53 for more information.

A SAF puck contains an probe array on a nitrocellulose membrane supported by a polysulfone disc. See Figure 3-6. Currently, small format arrays have only been designed for phases running the domoic acid (da) protocol.

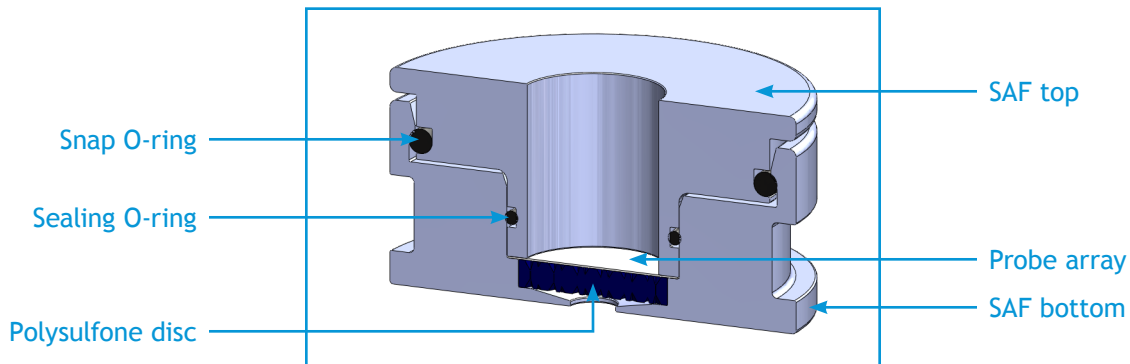


Figure 3-6: Small array format puck, cross section

Identifying Pucks

Each puck has a serial number that indicates the type of puck it is, the date it was manufactured, and provides a 4-digit identifier. See Figure 3-7 below. The format for the serial number is:

TTT MY-SSSS

Where:

- TTT* is the three-letter puck type: HV2, LAF, SAF, SH1, SHT, or WCR. Note that HV2 and SHT appear on tops only, and SH1 appear on bottoms only.
- MY* is the month (M) and year (YY) in which the puck was manufactured. The month is represented by a number, 1 – 9 for January to September, or the letters, A, B, or C for October, November, and December, respectively. The year, YY, is represented by two numbers for the year.
- SSSS* is a four-digit serial number for the puck (0000 – 9999).

The following are examples of serial numbers:

SHT 410-1234 is a serial number for an SHT puck top that was manufactured in April, 2010 (410).

SH1 410-4321 is a serial number for a SH1 bottom that was manufactured in April, 2010.

WCR A10-1008 is a serial number for a WCR puck top or bottom that was manufactured in October, 2010 (A10).

Note that the pucks may also have an additional three-letter designation, such as MRL, which is a manufacturing origination code.



Figure 3-7: Engraved serial number

Pucks Required in a Phase

Each phase requires a certain number of pucks. The following table outline which pucks are required and which pucks are optional for a given phase.

Table 3-1: Pucks used in a phase		
Phase/Protocol	Puck Type	Comment
BAC DA HAB KAR LARV	SVS or HVS for sample collection	Required.
	LAF or SAF with appropriate array for sample processing	Required. Use LAF for BAC, HAB, KAR, and LARV phases. Use SAF for DA.
	SVS for lysate filtration	Required
	WCR for whole cell archive collection	By default, a WCR is collected for the BAC, HAB, KAR, and LARV phases, and not for the DA phases. To change the settings, see Appendix 2, "Archive Settings."
HABDA	SVS or HVS for HAB collection	Required.
	LAF with HAB array	
	SVS for lysate filtration	
	WCR for HAB whole cell archive collection	By default, a WCR is collected for the HAB portion of the phase only. To change these settings, see Appendix 2, "Archive Settings."
	SVS or HVS for DA collection	Required.
	SAF with DA array	
	SVS for lysate filtration	
Short sh2 protocol	LAF or SAF with appropriate array	Required.

Opening a Puck

Pucks must be opened prior to cleaning and assembling.

Supplies

You will need the following supplies to open a puck:

- ESP Puck Separator
- Gloves, disposable, powder-free
- Protective clothing and eyewear

**WARNING:**

Do not use any other metal implement other than the separator. Metal-to-metal contact may damage the puck rendering it useless. Using a damaged puck in the ESP may produce erroneous results or result in damage to the ESP system.

To open a puck:

1. Place the ESP Puck Separator on a level surface, orienting it so you are able to place one hand on the lever and your other hand over the separator jaws.

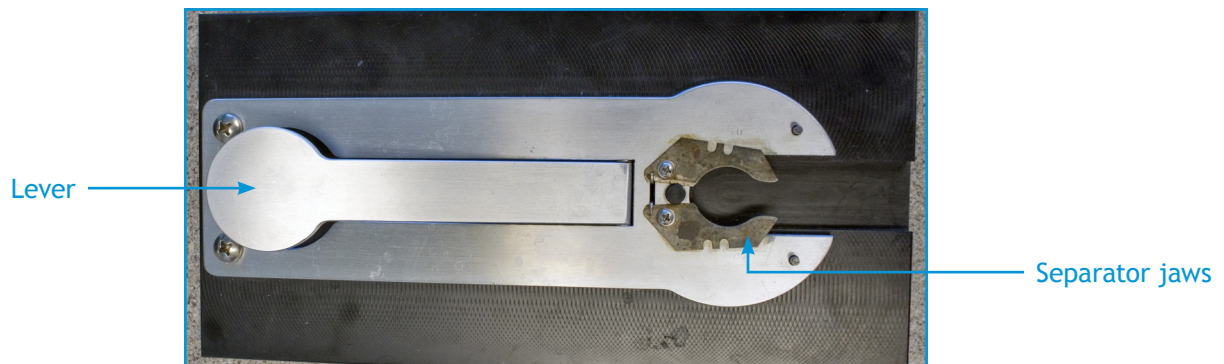


Figure 3-8: ESP Puck Separator

2. With the top of the puck facing up, place the puck in the slot and slide the puck into the jaws of the separator.
3. Place one hand over the puck to keep the puck top from popping off.
4. Place your other hand on the device lever. With a steady motion, push down on the lever. See Figure 3-9.

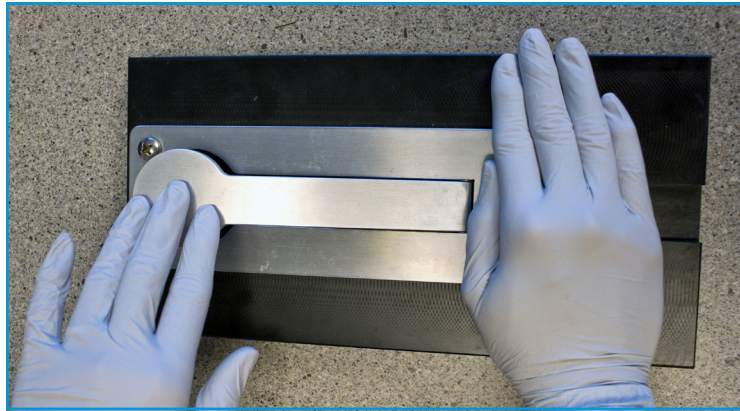


Figure 3-9: Hand position while opening puck

5. If the puck does not open on the first attempt, slide the puck out of the jaws and rotate it 90 degrees. Replace the puck into the jaws. Repeat steps 3 and 4 until the puck pops.
6. Dispose of any fluid in the puck into a hazardous waste container.
7. Remove and discard the following items. Figure 3-10 shows how to press and squeeze the O-ring up along the groove in order to remove it.
 - Sealing and snap O-rings
 - Membrane filters
 - Probe arrays



Figure 3-10: Grasping O-ring

8. Remove and place the following components in separate plastic containers for cleaning:
 - Puck tops and bottoms
 - Titanium frits
 - Polysulfone discs
 - Titanium screens

Once the pucks have been opened and their contents removed, they are ready to be cleaned.

Cleaning Pucks and Puck Components

The process of cleaning and loading the pucks is a multi-day process. For instance, in the case of cleaning titanium frits, the frits must be soaked overnight prior to completing the cleaning process. Read through this entire section and plan accordingly.

Supplies

You will need the following supplies to clean the pucks and their reusable components. For items marked with an asterisk (*), refer to Appendix B, “Supplies,” for the required or recommended manufacturer and part number.

- cSt 1000 silicone lubricant*
- Desiccant packages (for storing LAF and SAF pucks only)
- Ethanol, 200 proof (absolute), for molecular biology
 - 70% solution made with high-quality RO water
- Gloves, disposable, powder-free
- Hydrochloric acid
 - 10% solution made with high-quality RO water
- Lint-free cloth *
- Plastic bags, quart-sized resealable
- Plastic containers that fit inside the sonicator to be used for cleaning
- Protective eyewear and clothing
- Ultrasonic cleaner or sonicator
- Ultrasonic cleaning solution*
- Water
 - High-quality RO (standard RO passed through an ultra filter)
 - Tap

Cleaning Pucks

Before cleaning, pucks must be opened and emptied of all contents.

To clean pucks

1. If necessary, open the pucks.

See “Opening a Puck” on page 55.

2. Examine the inside and outside of the top and bottom halves of the puck. If the puck appears damaged, do not use the puck. Return the puck to Spyglass for recycling.



WARNING:

Do not use pucks that have deep scratches (those that can be felt when a fingernail drawn over it), dings, and dents. Using a damaged puck in the ESP may produce erroneous results or result in damage to the ESP system.

3. Using tap water, carefully rinse the pucks.
 4. Place pucks in a plastic container arranging them so they are in a single layer and do not touch one another.
- Figure 3-11



Figure 3-11: Pucks arranged for cleaning

5. Place the container in the sonicator and fill the container with the diluted cleaning solution. Fill to ¼" above the top of the pucks.

Note: Use a solution of one part Cole-Parmer Micro-90 cleaning solution to six parts tap water.

6. Turn on the sonicator and run at full power for 15 minutes.

Note: Do not use heat during sonication.

7. Remove the container from the sonicator and drain.
8. Repeat steps 5 to 7, substituting tap water for the diluted cleaning solution.
9. Using RO water, carefully rinse the pucks.
10. Repeat steps 5 to 7, substituting RO water for tap water.

 **WARNING:** Do not sonicate using ethanol. To do so may result in a combustion hazard. Consult the sonicators user manual for safe use of the sonicator.

11. In another clean plastic container, place and arrange the pucks as in step 4. Using 70% ethanol solution, fill the container to ¼" above the top of the pucks. Allow the pucks to soak for 20 minutes.

Note: The 70% ethanol solution should be made with high-quality RO water. Run RO through an ultra filter.

12. Drain and rinse the pucks with high-quality RO water.
13. Place the pucks on a lint-free cloth and allow to dry completely. Alternatively, place in a 50 °C oven until dry.

Cleaning Titanium Frits

The titanium frits used for filtration and for support can be reused. However, they must be cleaned prior to reuse.

Note: Cleaning the titanium frits require a minimum 8-hour pre-soak before sonication.

To clean the titanium frits

1. Examine each titanium frit and make sure it is free of all nicks, scratches, and dents. If the frit is damaged in any way, return it to Spyglass for recycling.
2. Place in an appropriate container and fill with 10% HCL solution to to ¼" above the top of the frits.
3. Allow to soak for at least 8 hours.
4. Continue by following steps 3 through 13 in "Cleaning Pucks" on page 58.

Cleaning Polysulfone Supports & Titanium Screens

The polysulfone supports can be reused multiple times; however, they must be cleaned prior to reuse.

To clean the supports

1. Examine each support:
 - For a polysulfone support, make sure it is free of all nicks, scratches, and dents. If the support is marred in any way, return it to Spyglass for recycling.
 - For the titanium screens, hold the screen eyelevel and make sure it has not been distorted. If the screen is marred, bent, or distorted in any way, return it to Spyglass for recycling.
2. Continue by following steps 3 through 13 in “Cleaning Pucks” on page 58.

Assembling the Pucks

When assembling pucks, observe standard aseptic techniques to avoid contamination.

Supplies

You will need the following supplies to assemble the pucks. For items marked with an asterisk (*), refer to Appendix B, “Supplies,” for the required or recommended manufacturer and part number.

- Canned air duster, petroleum-distillates free
- cSt 1000 silicone lubricant*
- Desiccant packages (for LAF and SAF puck storage)
- ESP probe arrays*
- ESP pucks*
- Gloves, disposable, powder free
- Membrane filters*
- Plastic storage containers with secure-fitting lids (such as Ziplock®)
- Polysulfone supports*
- Sealing O-rings*
- Snap O-rings*
- Titanium frits (for HVS and/or WCR pucks)*
- Titanium support screens*
- Vise, non-marring

Keeping an Assembly Log

The serial number engraved on the ESP pucks does not contain information regarding the contents of the puck. Therefore, it is essential to keep a record of what each prepared puck contains. To do so, use a lab notebook to keep a record of the date the puck was prepared, its serial number, the type of phase in which it will be used, and any changes to its contents. For example:

<i>Date</i>	<i>Puck ID</i>	<i>Contents</i>
June 10, 2010	SH/T 410 3456	hab SVS 5 μ m
"	SH/T 410 2389	hab SVS 5 μ m
June 10, 2010	SH/T 410 1903	hab SVS 0.22 μ m
June 11, 2010	SH/T 410 1204	lyfi SVS 0.22 μ m

In the example above, the content description, *hab SVS 5 μ m*, means that the standard volume sample (SVS) puck is prepared for use in a HAB phase, and it contains a 5- μ m filter. Note the content description, *lyfi*, indicates the puck is prepared for use in lysate filtration.

This record is useful as a cross reference when you are completing a carousel loading map and begin loading the pucks into the carousel.

Preparing the O-rings

Each puck assembly requires two O-rings: one sealing O-ring and one snap O-ring. Refer to the following table for the size of the O-ring required for use in a given puck type (Figure 3-12).

Table 3-2: O-ring specifications		
Puck Type	Sealing O-ring	Snap O-ring
HVS	2-020, 50 duro Buna-N	2-021, 90 duro Buna-N
LAF		2-021, 70 duro Buna-N
SAF	0453, 70 duro Buna-N	
SVS	2-020, 50 duro Buna-N	
WCR		

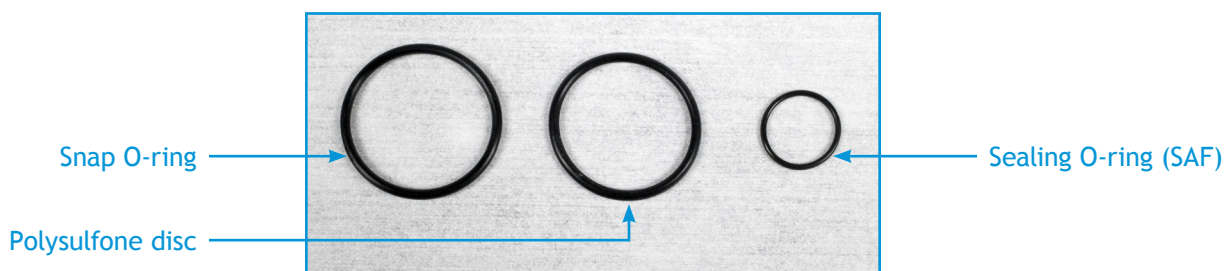


Figure 3-12: Puck O-rings

To prepare the O-rings

1. Working in a clean, lint-free environment, label a clean, resealable plastic bag with the size of O-ring to prepare.
2. Place the O-rings into the bag.
3. Squeeze a small amount of the silicone lubricant into the bag.
4. Seal the bag, removing excess air. Rub the bag between your fingers to distribute the lubricant and coat the O-rings completely.

Keep the O-rings in the bag until ready for use. At ambient temperatures, the prepared O-rings can be stored in this manner indefinitely.

Assembling a LAF, SAF, SVS, or WCR Puck

The assembly for the pucks is very similar from type to type. Before assembly, make sure the puck top and bottom pieces are clean and thoroughly dried.

To assemble a LAF, SAF, SVS, or WCR puck

1. On a clean, lint-free cloth, place the top and bottom sections of a puck. Engraved on the outside edge of each puck top and bottom is a series of letters and numbers. The first three letters represent the puck type. For a given puck type, the 3-letter puck ID on the top and bottom must match as follows:

Table 3-3: Top and bottom code by puck type		
Puck Type	Top	Bottom
LAF	LAF	LAF
SAF	SAF	SAF
SVS	SHT	SH1
WCR	WCR	WCR

! Caution:

Dust is detrimental to sample processing, especially in the LAF and SAF pucks. To remove any dust particles, use a recommended canned air duster. Lightly spray air over the puck surfaces.

2. Into the puck bottom, place a support. Refer to the following table for the type of support required for a given puck type (Figure 3-13).

Table 3-4: Support by puck type	
Puck Type	Support
LAF	Large polysulfone disc
SAF	Small polysulfone disc
SVS	Titanium screen
WCR	10 μ m titanium frit

Note: Make sure the support is clean and free of damage. Screens must be flat and show no signs of warping. Polysulfone discs must be free of dents and deep scratches.

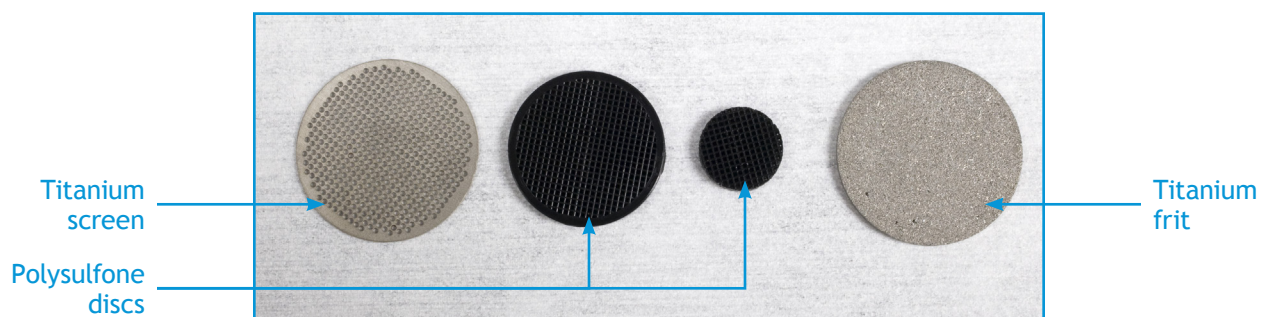


Figure 3-13: Types of puck support

3. For a LAF puck, place a large array with the marked side face up on top of the support. Then go to step 8.
4. For a SAF puck, place a small array with the marked side face up on top of the support. Then go to step 8.
5. For a SVS puck used in sample collection, place the appropriate membrane on top of the support screen. Then go to step 8.

The following are suggested porosities for a given protocol. Use the porosity appropriate for the organism for which you are testing.

Table 3-5: Durapore membrane porosity by protocol	
Protocol	Filter Porosity
BAC	0.22 µm
DA	0.65 µm
HAB	0.65 µm
KAR	5.0 µm
LARV	0.65 µm

For example, if you are assembling a SVS for a HAB phase and using the recommend filter, place a 0.65 µm membrane on top of the support screen.

- For a SVS puck used in lysate filtration, place a 0.22 µm Durapore membrane on top of the support screen, then go to step 8.
- For a WCR puck, place the appropriate membrane(s) on top of the support screen. Then go to step 8.

The following are suggested combinations of filters to be used for a given protocol. Use the porosity appropriate for the organism for which you are testing.

Note: The membranes are listed the order in which they are placed into the puck. The Metrical membrane also helps to retain moisture in the WCR. The 5-µm Durapore and the 1.2-µm Isopore membranes are used a pre-filters.

Table 3-6: WCR membranes and porosity by protocol	
Protocol	Filter Type & Porosity
BAC	0.45 µm Metrical, 0.22 µm Durapore, 5 µm Durapore
HAB*	0.45 µm Metrical, 1.2 µm Isopore
LARV	
KAR	

- Remove one prepared snap O-ring and one sealing O-ring from the resealable bags. Inspect the O rings to make sure they are defect free. Place the O-rings on another clean, lint-free cloth and lightly blot to remove excess lubricant.

Refer to the following table for the appropriate sizes.

Table 3-7: O-ring specifications for LAF, SAF, SVS and WCR pucks		
Puck Type	Sealing O-ring	Snap O-ring
LAF	2-020, 50 duro Buna-N	2-021, 70 duro Buna-N
SAF	0453, 70 duro Buna-N	
SVS	2-020, 50 duro Buna-N	
WCR		

**WARNING:**

The snap and sealing O-rings appear similar in size. Do not mix up the sizes. Using the incorrect O-ring will result in improper assembly. Using an improperly assembled puck may result in fluid leaking into and damaging the ESP system.

- Place the snap O-ring in the topmost groove in the puck top (see Figure 3-14). Note that the snap O-ring is the larger diameter of the two O-rings used.



Figure 3-14: Close up of O-ring grooves in puck top

- Place the sealing O-ring in the groove below the snap O-ring.
- Using a clean, lint-free cloth, remove any excess lubricant from the surfaces below the sealing O ring of the puck top.
- Place puck top into the puck bottom. Make sure the top and bottom are aligned level. Place two fingers on the puck top. With evenly distributed pressure, push down on the top until you feel the top snap into place.
- Make sure the closure formed between the top and bottom is uniform all the way around the puck (Figure 3-15). If the groove is not uniform, open the puck, replace the sealing O-ring, and close it again.



Figure 3-15: Uniform closure in properly assembled puck

14. Using a clean, lint-free cloth, wipe the outside of the puck to remove any excess lubricant and store the puck in a clean plastic container fitted with a lid. Label the container with the puck type and phase.

Note: For prepared LAF and SAF pucks, add a desiccant packet to the storage container.

Recommendation: Store the prepared pucks separately by type and by contents. For example, use one container for SVS pucks assembled for BAC collection and another for SVS pucks assembled for lysate filtration (abbreviated as *lyfil*).

Assembling a HVS Puck

The procedure to assemble a high-volume sample puck varies from the other pucks. How the O-rings are positioned and the puck contents are different.

To assemble a HVS puck

1. On a clean, lint-free cloth, place the top and bottom sections of a puck. Engraved on the outside edge of each puck top and bottom is a series of letters and numbers. The first three letters represent the puck type. For a given puck type, the 3-letter puck ID on the top and bottom must match as follows:

Puck Type	Top 3-letter code	Bottom 3-letter code
HVS	HV2	SH1

! Caution:

Dust is detrimental to sample processing, especially in the LAF and SAF pucks. To remove any dust particles, use a recommended canned air duster. Lightly spray air over the puck surfaces.

2. Into the puck bottom, place a 5- μ m titanium frit.

3. Remove one prepared snap O-ring and one sealing O-ring from the resealable bags. (Refer to the following table for the appropriate sizes.) Inspect the O-rings to make sure they are defect free. Place the O-rings on another clean, lint-free cloth and lightly blot to remove excess lubricant.

Table 3-8: O-ring specifications for HVS pucks		
Puck Type	Sealing O-ring	Snap O-ring
HVS	2-020, 50 duro Buna-N	2-021, 90 duro Buna-N

**WARNING:**

The snap and sealing O-rings appear similar in size. Do not mix up the sizes. Using the incorrect O-ring will result in improper assembly. Using an improperly assembled puck may result in fluid leaking into and damaging the ESP system.

6. Place the snap O-ring in the groove in the puck top. Figure 3-16.

Unlike the other puck tops that have two grooves for the O-rings, the top of an HV2 puck has only one groove. The sealing O-ring sits inside the puck bottom.



Figure 3-16: Single groove on HVS top

7. Place the sealing O-ring in the puck bottom on top of the frit.
8. Using a clean, lint-free cloth, remove any excess lubricant from the edge of the puck top that is inserted into the puck bottom.
9. Place puck top into the puck bottom. Make sure the top and bottom are aligned level. Then place the puck into the vise. Turn the vise handle until the top snaps into place.
10. Make sure the closure formed between the top and bottom is uniform all the way around the puck (Figure 3-17). If the groove is not uniform, open the puck, replace the sealing O-ring, and close it again.



Figure 3-17: Uniform closure in properly assembled puck

11. Using a clean, lint-free cloth, wipe the outside of the puck to remove any excess lubricant and store the puck in a clean plastic container fitted with a lid. Label the container with the puck type and phase.

Carousel Loading Map

As described in Chapter 1, the ESP carousel contains seven tubes, six of which hold the pucks used in a mission. Each tube has the capacity to hold 22 pucks. The order in which pucks are loaded into the carousel is critical for the safe operation of the ESP. If the sequences of pucks retrieved for a phase does not match the order of operation defined by a protocol, the phase will not proceed correctly. In the worst case, the incorrect order of the pucks can lead to the ESP flooding internally and being irreparably damaged.



WARNING:

The order in which the pucks are loaded into the Carousel is crucial to the safe operation of the ESP system. If the type of puck retrieved is not the type called for in the active protocol, fluid may leak into the ESP damaging the system.

To assist in the process of loading the ESP correctly, it is recommended that a document be created that provides a reference to the mission, its phases, and the pucks used. This document is referred to as the *carousel loading map*. This map should be used to provided the following information:

- Each phase in the mission
- The protocol for the phase
- List of pucks required for the phase
- The order in which the pucks are retrieved
- The identification numbers for the assembled pucks

Once the map is completed, you will have a visual representation of the mission to be run. You will also see how many pucks of a given type you will need for the mission. Most importantly, you will have a reference for the order in which the pucks are load into the ESP.

Creating a Carousel Loading Map

You can create the map using a word processor or a spreadsheet program. The following is an example of a spreadsheet used to create a carousel loading map. This example shows the information for tubes 2 and 3.

phase	tube 2	puck #	puck ID	phase	tube 3	puck #	puck ID
HAB 1	collection SVS 5 µm	1		Cont.	lyfil SVS 0.22 µm	1	
	array LAF hab	2			archive WCR 0.22µm	2	
	lyfil SVS 0.22 µm	3		BAC #	collection SVS 0.22 µm	3	
	archive WCR 0.22 µm	4			array LAF bac	4	
HAB 2	collection SVS 5 µm	5			lyfil SVS 0.22 µm	5	
	array LAF hab	6			archive WCR 0.22µm	6	
	lyfil SVS 0.22 µm	7		HAB 5	collection SVS 5 µm	7	
	archive WCR 0.22 µm	8			array LAF hab	8	
HAB 3	collection SVS 0.22 µm	9			lyfil SVS 0.22 µm	9	
	array LAF hab	10			archive WCR 0.22 µm	10	
	lyfil SVS 0.22 µm	11		LARV 1	collection HVS 5 µm	11	
	archive WCR 0.22 µm	12			array LAF larv	12	
HAB 4	collection SVS 5 µm	13			lyfil SVS 0.22 µm	13	
	array LAF hab	14			archive WCR 1.2µm	14	
	lyfil SVS 0.22 µm	15		BAC 5	collection SVS 0.22 µm	15	
	archive WCR 0.22 µm	16			array LAF bac	16	
BAC 1	collection SVS 0.22 µm	17			lyfil SVS 0.22 µm	17	
	array LAF bac	18			archive WCR 0.22µm	18	
	lyfil SVS 0.22 µm	19		LARV 2	collection HVS 5 µm	19	
	archive WCR 0.22µm	20			array LAF larv	20	
BAC 2	collection SVS 0.22 µm	21			lyfil SVS 0.22 µm	21	
	array LAF bac	22			archive WCR 1.2µm	22	

Tubes 2 through 7 in the ESP carousel are each represented by a column in the spreadsheet (i.e., tube 2). Each puck in the tube is listed in descending order from 1 to 22. The ESP starts retrieving pucks beginning with the first puck in the start tube. In this example, this is puck #1 in tube 2. Through the various operations in a mission, the ESP continues retrieving pucks from 1 to 22. When the tube is empty, pucks from the next tube are retrieved (i.e., tube 3) and so on.

In the example map, each phase is described using four columns in the spreadsheet: phase, tube number, puck number, and puck ID. The “phase” column provides a description of the phase in the order it will occur in a mission. The “tube number” column lists the type of pucks required for the phase. The “puck #” column lists the position that the puck will occupy in the carousel tube; puck #1 being the topmost position in the tube. The last column, the “puck ID” column will contain the unique ID number for each assembled puck loaded into the carousel. This will be entered by hand.

The first phase in the example map is called “HAB #1.” This phase uses the protocol for harmful algal bloom (HAB). This HAB 1 corresponds to HAB 1 in the mission file.

phase	tube 2	puck #	puck ID
HAB 1	collection SVS 5 µm	1	
	array LAF hab	2	
	lyfil SVS 0.22 µm	3	
	archive WCR 0.22 µm	4	

HAB 1 requires four pucks, which are listed by puck type and in the order in which the ESP retrieves the pucks from the tube. The details on the pucks required for a phase are discussed earlier in this chapter.

! Caution:

The pucks are listed for a phase in the order in which the ESP retrieves them from the carousel and not in the order in which they are used during sample processing. An array puck must be prepared prior to the lysate being added into it. This process, called *blocking*, takes place while the ESP is collecting and lysing the sample. Once the array is blocked, it is ready for the filtered lysate to be added to it.

Carousel Loading Map Conventions

When creating a carousel loading map, observe the following guidelines:

- Each phase in the mission file is identified in the map.
- The order in which a phase appears in the map is the same order it appears in the mission script (*.rb)
- The order of the pucks is the order in which the ESP retrieves the pucks.
- The pucks for a phase can span two tubes. In the example, BAC #2 begins in tube 2 with puck 21 and continues into tube 3, puck 1 and 2.
- Each puck used in the mission is identified with its unique puck ID number.

Loading Pucks into the Carousel

Note: It is highly recommend that another person verify the carousel loading map and the work performed for this procedure.

To load pucks into the carousel

1. Ready the ESP.

See “Readying the ESP” on page 79.

2. If necessary, remove the carousel access gate. Unthread the two thumb screws located at the ends of the gate and remove gate. See Figure 3-18.

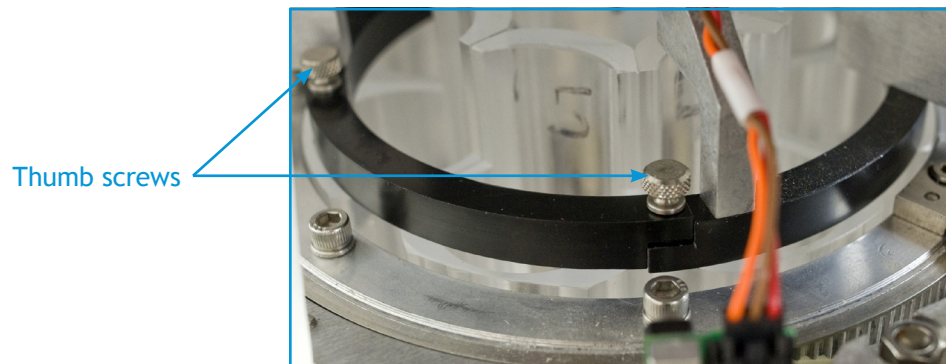


Figure 3-18: Carousel access gate

3. At the ESP prompt, type `access` and the number of the tube to load. For example, to load tube 2, type the following and press ENTER:

```
access 2
```

! Caution: Do not load pucks into tube 1. This is a discard tube.

4. Using your carousel loading map, organize the prepared pucks in the order that they will be loaded into the tube. For each puck, write its ID numbers in the carousel loading map.

For example, the first puck to be loaded into tube 2 is a LAF with a HAB array. The ID number for the LAF puck assembled for a HAB phase is LAF 410-1257. This number is verified in the log or record for the puck assembly and then written into the carousel loading map.

phase	tube 2	puck #	puck ID
HAB 1	collection SVS 5 μm	1	
	array LAF hab	2	
	lyfil SVS 0.22 μm	3	
	archive WCR 0.22 μm	4	
<i>rows omitted from example</i>			
BAC 2	collection SVS 0.22 μm	21	
	array LAF bac	22	LAF 410-1257

**WARNING:**

Dropping the pucks into the tube may damage the pucks, dislodge their contents, and/or damage the carousel. This support must be able to bare the weight of an entire stack of pucks.

- Place a wooden or plastic support into the elevator groove and all the way into the tube. Move the stick to the top of the tube. See Figure 3-19.

Support inserted
into elevator groove



Figure 3-19: Support for loading pucks

- Loading the tube from the bottom up, take a prepared puck, verify it against the carousel loading map, and place the puck in the tube on top of the stick (Figure 3-20). Lower the support just enough to allow next puck

to be placed in the tube. Place the next puck on top of the stack. Repeat this process until the tube is full (i.e., 22 pucks have been loaded). Gently remove the support from the bottom of the stack.

Note: The stick must be able to bear the weight of all of the pucks are they are loaded into the tube.

This method of loading the pucks minimizes the chance of particles being shed into the pucks. If pucks are lowered into the tube one at a time, each time the support is drawn out from under a puck, particles may be shed from the support or the puck's underside into the puck below. Since the stack is being supported from the very bottom, when the stick is removed, any material shed falls to the bottom of the carousel.

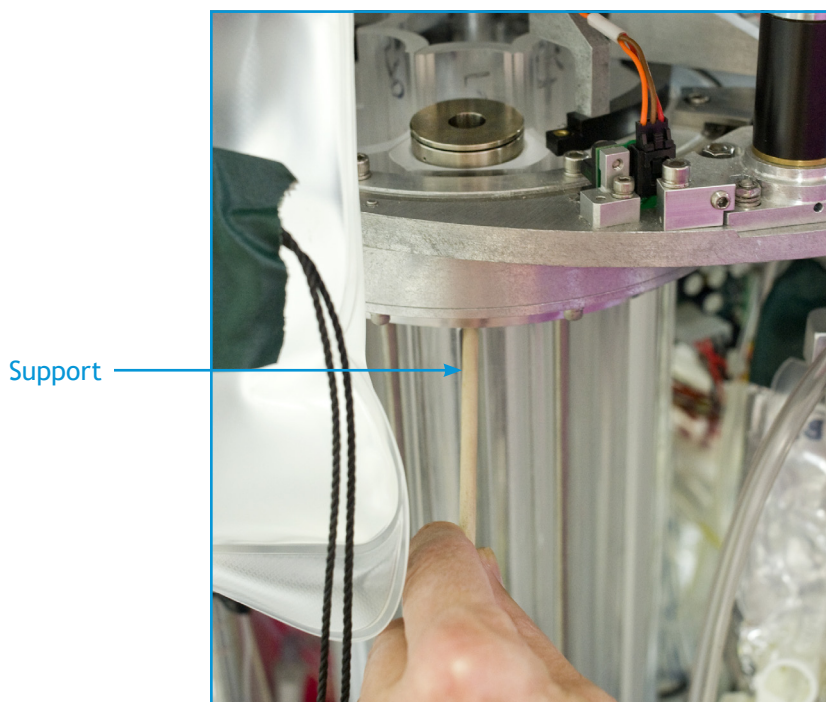


Figure 3-20: Raising stack to load a puck

7. Repeat steps 5 through 7 until all of the necessary pucks have been loaded. ##steps
8. Reinstall the access gate.

Puck Assembly Quick Reference

Note: Do not use this reference until you are familiar with the procedures in “Assembling the Pucks” on page 60. The components are listed from top to bottom.

Puck Type	Process Used In	Phases/Protocols	Assembly Components
SVS	Standard-volume sample collection	All phases	SHT puck top 2-021, 70 duro snap O-ring 2-020, 50 duro sealing O-ring 0.22 µm Durapore membrane titanium screen SH1 puck bottom
HVS	High-volume sample collection	All phases	HV2 puck top 2-021, 90 duro snap O-ring 2-020, 50 duro sealing O-ring 5 µm titanium frit SH1 puck bottom
LAF	Array processing	BAC HAB LARV KAR	LAF puck top 2-021, 70 duro snap O-ring 2-020, 50 duro sealing O-ring large format array large polysulfone disc LAF puck bottom
SAF	Array processing	DA	SAF puck top 2-021, 70 duro snap O-ring 0.453, 70 duro sealing O-ring Small format array small polysulfone disc SAF puck bottom
WCR	Archive collection	BAC	WCR puck top 2-021, 70 duro snap O-ring 2-020, 50 duro sealing O-ring 5 µm Durapore membrane 0.22 µm Durapore membrane 0.45 µm Metrical membrane 10 µm titanium frit WCR puck bottom
		HAB LARV KAR	WCR puck top 2-021, 70 duro snap O-ring 2-020, 50 duro sealing O-ring 1.2 µm Isopore membrane 0.45 µm Metrical membrane 10 µm titanium frit WCR puck bottom

Chapter 4

Set Up & Bench-Top Operation

This chapter describes how to set up and operate the ESP in a laboratory setting.



WARNING:

Performing these procedures may involve contact with harsh or hazardous chemicals. Wear the appropriate protective clothing, gloves, and eye protection at all times. Use disposable, powder-free gloves.

The following procedures are described in this chapter:

- Setting up the ESP
- Starting the ESP Application
- Uploading Files to the ESP
- Readyng the ESP
- Running a Short sh2 Protocol
- Setting Up Pressurized Sampling
- Running a Benched Mission
- Running a Benched Mission
- Viewing the Progress Messages
- Shutting Down the ESP

Setting up the ESP

To set up the ESP for operation you must connect the ESP to a power supply and establish communications.

To power up the ESP

! Caution: Connect the Ethernet cable before connecting the power supply.

1. Plug the Cat 5 Ethernet cable from the network to the ESP. See Figure 4-1.

If necessary, disconnect the cable from the external connection to the ESP lid.



Figure 4-1: Ethernet connector

! Caution: When power is connected to the ESP, the unused plug is energized. To avoid a shorting the battery, insert a dummy plug into the unused plug.

2. Connect the ESP to its power supply.

The power supply is commonly a 12-volt 40.0 AH, rechargeable battery with a transformer-type battery charger. See Figure 4-2.



Figure 4-2: Power Supply

The power supply can be connect to the ESP using one of two plugs. See Figure 4-3.

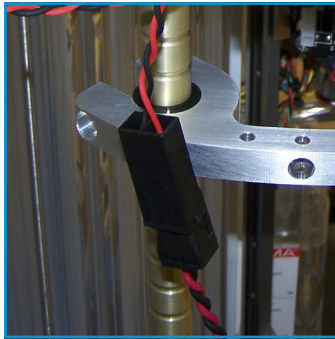


Figure 4-3: Power connector

Now, you can establish communication with the ESP.

To establish communication with the ESP

1. From the computer connected to the same network as ESP, open a connection to the ESP.
 - If you are using a Linux-based machine, open a terminal window.
 - If you are using a Mac-OS X-based machine, go to Applications > Utilities > Terminal.
2. At the terminal prompt, type the following and press ENTER:

```
ssh user@device_name
```

Where:

user is user name.

device_name is the name of the ESP.

For example, if the user name is `esp` and the device is `spyglass`, you would enter:

```
ssh esp@espspyglass
```

Next, you are prompted for a ESP password.

3. Enter your password and press ENTER.

A prompt is returned and verifies communication with the ESP. This is the Linux operating system's "esp" user prompt. The text for the prompt is the text entered after the `ssh` command followed by `:~$`. For example:

```
esp@espspyglass:~$
```

Note: The Linux esp user prompt is referred to in this section as the \$ prompt.

The following ESP-related commands are available at the \$ prompt. You can use these commands start a mission, a client session, or access files on the ESP.

Command	Description
dumplog	Creates an ASCII text file from the binary log file that is generated when the ESP is running a process.
esp	Starts the ESP application, which allows you to operate the ESP interactively.
espclient	Opens a client window, which allows you to interactively execute ESP commands during an active ESP process.
showlog	Allows you to view the log file for the currently active ESP process.
start	Allows you to run a mission in the background, diverting messages that would normally appear in the terminal window.

To display available options for these commands, type the following and press ENTER:

```
command --help
```

Where *command* is the name of the command. For example, `dumplog --help` will return the options and examples for the `dumplog` command.

Starting the ESP Application

In order to run a short process, a single protocol, or directly manipulate a component in the ESP, you must start the ESP software application to run the ESP interactively.

To start the ESP application

1. Set up the ESP.

See “Setting up the ESP” on page 76

2. At the \$ prompt, type the following and press ENTER:

```
esp
```

Allow 30 seconds for the ESP application to start and to return the ESP application prompt. A series of messages precede the prompt. These are status messages issued as various components of the ESP are being initialized. The ESP application prompt in the following format is returned:

```
ESPdevice_name:001:0>
```

Where *device_name* is the name of the computer on which the ESP software has been installed. For example:
ESPspyglass:001:0>

Note: The ESP application prompt is referred to in this section as the ESP prompt.

You can now operate the ESP interactively.

Uploading Files to the ESP

In order for the ESP to run a mission, the mission file and the `phasecfg.rb` file must be uploaded to the ESP's flash memory. You can use the secure file protocol in Linux or an FTP program to upload the files.

The following procedure uses a FTP program.

To upload a mission:

1. Set up the ESP.
See “Setting up the ESP” on page 76
2. Start the FTP program.
3. Where prompted for the server, enter the device name for the ESP.
4. Where prompted for the user and password, enter the ESP user name and password.
5. Once connected, select and upload the mission and/or `phasecfg.rb` file.

You can now run the mission.

Readying the ESP

Before you run a short sh2 process or protocol, or perform certain maintenance procedures, you must ready the ESP. This ensures that various components are in their ready or home state.

To ready the ESP

1. Set up the ESP and start the ESP application.
See “Setting up the ESP” on page 76 and “Starting the ESP Application” on page 78.
2. At the ESP prompt, type the following and press ENTER:

```
ESP.ready!
```

This commands various ESP components to return to their starting position or home state. Text similar to the following will appear in the terminal window as the ESP readies its components:

```
ESPspyglass:001:0> ESP.ready!  
  
=> CSV = Collection Syringe Valve at waste2  
  
CTV = Collection Top Valve at spare  
  
CBV = Collection Bottom Valve at CSV
```

```

PSV = Processing Syringe Valve at PSR
PBV = Processing Bottom Valve at waste2
... lines omitted
CH = Collection Puck Heater at 18.0C
PH = Processing Puck Heater at 18.0C
Intake = Outer Intake Valve closed
Exhaust = Outer Exhaust Valve closed
ESPspyglass:002:0> PSV.PSR
PSV.dial :PSR
=> Processing Syringe Valve at PSR ESPspyglass:003:0>

```

If there are no errors returned, the ESP is ready for operation.

To verify the ESP is ready

- If you want to verify whether the ESP is in a ready state, type the following and press ENTER:

```
ESP.ready?
```

If the ESP is ready, the following is returned:

```
=> true
```

Running a Short sh2 Protocol

All currently available protocols have an abbreviated version used in bench testing only. This protocol is referred to as *short sh2*, and only processes and applies a prepared (i.e., lysed) sample to an array. The collecting, filtering, and lysing processes are not performed during a short sh2. Since these processes do not take place in a short sh2, only one puck is required: a large format array puck (LAF) or small format array puck (SAF).

A short sh2 runs interactively. The ESP loads an array puck from the carousel and then blocks the array (i.e., application of the blocking agent and heating the puck). While the array is blocked, you are prompted to load the prepared sample. After a short delay, the sample is drawn through the disconnected Air Clean line by the Processing Syringe. The Processing Syringe adds the sample and then the processing reagents to the puck. When processing is complete, the puck is moved to the camera-ready position, the images are taken, and the puck is lowered into the discard tube.

Unless otherwise specified, each short sh2 will run using the default image settings for the protocol on which it is based. In addition to the image settings, you can specify different times for the sampling delay and blocking time.

Short sh2 Syntax

A short sh2 is initiated using the following syntax:

```
protocol :delay=>sec, :blockDelay='min', :image=>[image1, ... imageN]
```

Where:

- protocol* is the short sh2 protocol to run. See Table 4-1 below.
- sec* is the time from when you press Enter to acknowledge the “load sample” message to when the Processing Syringe begins to pull the sample. If `:delay` is not specified, the default setting of 10 seconds is used.
- min* is the blocking time. If `:blockDelay` is not specified, the default setting of 8 minutes is used.
- image1...N* is the information for each image taken. This information can contains resolution (`hires`, `midres`, `lores`, `default`) and or exposure time (in seconds). If images are not specified, the default settings are used. See “Initial Default Settings” on page 26 for details. For information on how to specify images, see “Image Settings” on page 39.

The following example may be used to initiate a short sh2 to process a BAC array with a 15-second sampling delay and a 15-minute blocking time. Also specified are two high-resolution images with exposure times of 120 and 150 seconds.

```
shortBACsh2 :delay=>15, :blockDelay=>'15:00', :images=>[ :hires, 120, 150]
```

The following are the currently available short protocols:

Table 4-1: Currently available short sh2 protocols	
Protocol	Type of Array Processed
shortBACsh2	BAC
shortDASH2	DA
shortHABsh2	HAB
shortLARVsh2	LARV
shortKARsh2	KAR

Supplies

You will need the following items to run a short process:

- an ESP access tube
- LAF pucks prepared with appropriate arrays
- 1.1 to 1.2 mL of prepared sample for each short sh2

To run a short sh2 process

1. Set up the ESP and start the ESP application.

See “Setting up the ESP” on page 76 and “Starting the ESP Application” on page 78.

2. Load the prepared LAF pucks into the carousel.

See “Assembling the Pucks” on page 60 and “Loading Pucks into the Carousel” on page 71.

3. Locate the line to the “Air Clean” port on the Processing Syringe Valve (PSV). This line connects the PSV to the Clean Air container.

To find port locations, refer to the Appendix C, “Fluidic Worksheet.”

4. Follow the line from the port to the in-line connector (see Figure 4-4). The connector is located between the PSV and the Clean Air container.

For example, if “Air Clean” is located on port 5, trace the line from the valve port to the connectors located in-line.

5. Unscrew the connector to disconnect the line.

! Caution:

In the course of running several short sh2 protocols, residue from the sample lysate may begin to accumulate in and around the connectors. This residue may contain harsh or hazardous chemical. Wear protective eyewear and gloves, and use caution when removing the residue.

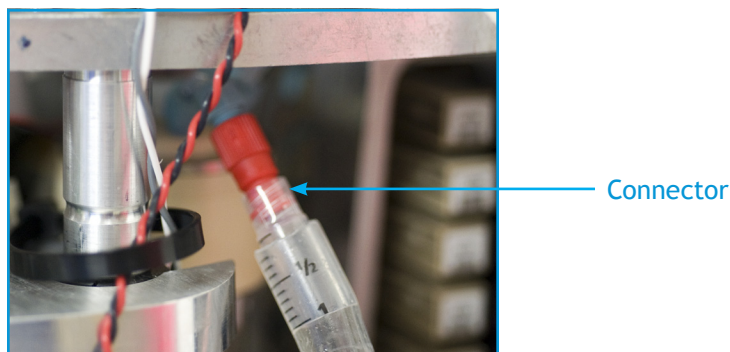


Figure 4-4: Connection in Air Clean line

6. Using a sterile swab dampened with RO water, clean any residue that may have formed around the interior of connector.
7. Connect an ESP access tube to the portion of the Air Clean line leading to the PSV. See Figure 4-5.

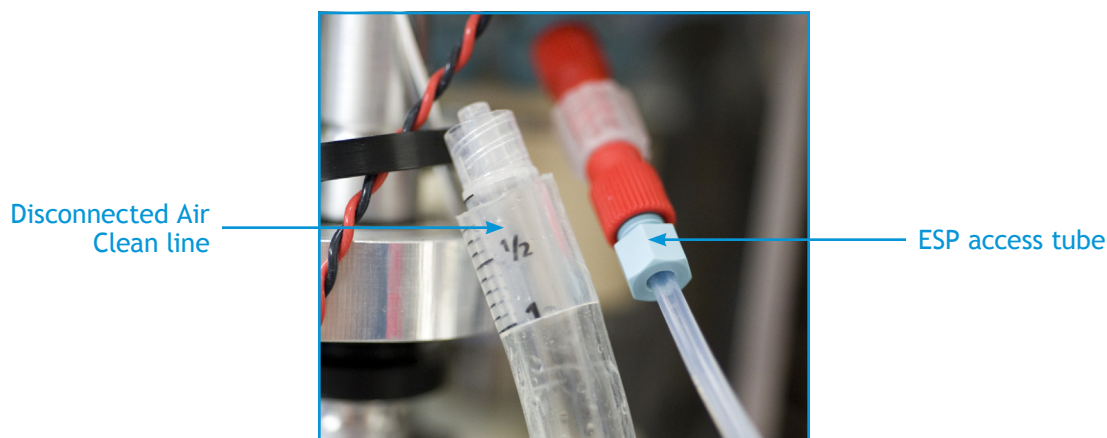


Figure 4-5: ESP access tube connected to Air Clean line

! Caution: The sample tube must not contain more than 1.2 mL of fluid.

8. Have 1.1 to 1.2 mL of the prepared sample readily at hand.
9. Specify the tube from which the ESP will begin taking the array pucks (i.e., the start tube). At the ESP prompt, type the following and press ENTER:

```
startTube n
```

Where *n* is the number of the start tube. The ESP will return the tube number as confirmation.

Note: For the start tube *n*, tube *n*-1 must be empty or have enough space for all of the pucks that are in tube *n*.

For example, if the start tube is 4, type `startTube 4`. When you press ENTER, the ESP returns:

```
=> 4
```

10. Specify the type of short process to run. At the ESP prompt, type the command and any desired settings for the short sh2 to run and press ENTER.

See “Short sh2 Syntax” on page 80 for more information.

Once the short sh2 is initiated, the carousel moves the start tube. The ESP loads an array puck into the Processing Clamp and begins the blocking process. The terminal window displays several messages indicating the activity of several components such as valve and Manipulator movements. Additionally, processing status messages such as the following are displayed:

...

@14:54:17.44 Loading sh2 puck from tube 4 into Processing Clamp

...

@14:54:43.15 Heating sh2 puck from tube 4 in Processing Clamp to 28.0C for 2:00:00

...

These messages indicate that an array puck has been taken from the specified tube, placed into the Processing Clamp, and is being heated.

Approximately 10 minutes from when you initiated the short process, a message appears prompting you to load the sample. For example:

Load sample by 15:11:20.43

Press enter, then quickly connect sample tube to PSV.air->

The time noted in the “Load sample by” message is when the blocking process is completed and the array is ready for the application of the sample.

11. Press ENTER and quickly insert the access tube into the sample tube.

From the time you press ENTER, you have 10 seconds before the processing syringe begins to draw. (The 10 second delay is the default setting.) The syringe pulls 1 mL of sample into the line. After a delay of 5 seconds, the sample in the line and remaining portion in the tube are pulled into the syringe.

After the sample is drawn, one of the following messages is returned:

Waiting for blocking to complete.

or

You are late! Blocking ran long. Continuing...

The first message occurs if you have completed step 11 within the blocking time. If you exceeded the time, the second message appears.

Note: Exceeding the blocking time does not stop the process; however, the results may not be accurate. It is recommended that you allow the short process to complete rather than stopping it. Commanding a process to stop, stops all of the activity in the ESP immediately. As a result, various components may have not returned to a ready state. Before starting another process, you will need to recover the system. See Appendix A, “Troubleshooting.”

12. After the sample is drawn, disconnect the access tube and reconnect the clean air line.

The ESP adds the sample and then the processing reagents to the puck.

13. Cover the ESP with a static-free camera shroud or turn off the ambient lighting so the room is dark.

Note: The camera is designed to operate optimally in lighting conditions experienced when the ESP is placed in its container and deployed. Ambient lighting in the lab environment may interfere with the image exposure.

When processing is complete, the puck is moved to the camera-ready position and the images are taken.

After the images are taken and the puck is lowered into the discard tube, the following message is returned to indicate that the short process has completed:

```
Finished short protocol assay
```

14. Rinse the access tube with RO water and set aside.

The short sh2 is now complete.

Setting Up Pressurized Sampling

You can operate the ESP with pressure kettles to simulate the depth at which the ESP will be deployed.

Supplies

- 3 ESP sample tubes: one for intake purge, two for pressure kettles
- Water sample
- Pressure kettles, one each for the intake and exhaust ports
- Stir bar and magnetic stirrer
- Container to collect purged disinfectant
- Paper towels

To set up the ESP for pressurized sampling

1. Set up the ESP.

See “Setting up the ESP” on page 76.

2. Upload the mission file to the ESP.

See “Uploading Files to the ESP” on page 79

3. Attach a sampling tube to the Intake Port. Place the free end of the tube into a catch container.



Figure 4-6: Sampling tube connected to Intake Port

4. Clear the intake line of any disinfectant. To do so, type the following at the ESP prompt and press ENTER:

```
purgeIntake
```

5. After the intake has been purged, disconnect the sampling tube.
6. Set up the pressure kettles according to the manufacturer's instructions. If desired, place a stir bar in the kettle with the sample and place the kettle on a magnetic stirring unit.
7. Make sure the valves from both pressure kettles are closed.
8. Attach a sampling tube from the pressure kettle containing the sample to the Intake Sample Port.
9. Attach a sampling tube from the Exhaust Port to the exhaust pressure kettle.

! Caution: The pressure applied to the system should not exceed 60 psi.

10. Based on the anticipated depth at which the ESP deployment, apply the appropriate pressure—not to exceed 60 psi—to the system.
11. Open the valve from the pressure kettle to the intake port.
12. Check for water leaks in and around the pressure kettles, and in the ESP. If any leaks present, immediately turn off pressure and check connections. Wipe up any water that may have leaked onto or into the ESP.
13. Bleed the intake line of all visible air.

Note: Have paper towels on hand to immediately absorb any water that leaks out during this step.

- a. Slightly loosen the connection between the sampling tube and intake port until air and sample water begins to leak out.
 - b. Allow air and water to continue to leak out until all visible air in the line has been removed.
 - c. Tighten the connection between the sampling tube and the intake port.
 - d. Wipe up any water present.
5. Open the valve from the pressure kettle to the exhaust port.

You can now start a mission.

Running a Benched Mission

The ESP runs a mission autonomously. That is, once initiated, the mission will run all of the phases without prompting.

To run a mission

1. Set up the ESP.
See “Setting up the ESP” on page 76.
2. If desired, set up the ESP for pressurized sampling.
See “Setting Up Pressurized Sampling” on page 85.
3. At the \$ prompt, type the following and press ENTER:

```
start esp mission
```

Where

mission is the filename for the mission to run.

A “Good News” message is returned indicating that the mission will begin. Then the ESP enters a quiescent state before starting the first phase. The duration of this state is specified with the mission start time (“Mission Start Time Setting” on page 30). After powering on the necessary control electronics and moving various mechanical components to their ready position, the ESP starts the first phase of the mission.

Progress messages for missions initiated with the `start` command do not appear in the terminal window. A mission started in this manner runs in background to prevent stoppages due to a communication interruption. These message can be viewed in another terminal window. See “Viewing the Progress Messages” on page 87.

Viewing the Progress Messages

When a short sh2 is running, the progress messages are displayed in the terminal window from which the protocol was initiated. For a mission, the progress messages can be displayed in another terminal window.

For both a short sh2 or a mission, progress messages are also written to a log file.

Viewing Messages for An Active Mission

When a mission is initiated with the `start` command, the process runs in background to prevent the mission from stopping due to a communication interruption. To view the messages, you must first open another terminal window and log onto the ESP running the mission.

To view the progress messages for an active mission

1. Open another terminal window and establish communications with the ESP.

See “To establish communication with the ESP” on page 77.

2. The `$` prompt, type the following and press ENTER:

```
showlog
```

The messages in the log file for the mission are displayed in the terminal window. The log file may be quite large, so use options for the `showlog` command to limit the number of lines displayed. For example, to display 50 lines of the log, enter the following:

```
showlog -n50
```

More information on options for `showlog`, type `showlog --help`.

Outputting the Log File

Creating a text file from the log allows you use a familiar text editor to review and search the messages. Reviewing the log file created when you check a mission for syntax and scheduling errors allows you to verify the mission is running the desired protocols with the expected options. If there is a stoppage in a process, the output file will allow you determine the state of various components and recover the system accordingly.

To output the log file

1. If running a mission, open another terminal window and establish communications with the ESP.

See “To establish communication with the ESP” on page 77.

2. Change the directory to the location of the log file.

- At the `$` prompt, type the following and press ENTER:

```
cd /var/log/user
```

Where *user* is the name used to establish communications with the ESP and initiate the mission.

3. Create a text file from the log messages.

- At the `$` prompt, type the following and press ENTER:

```
dumplog || name.txt
```

Where *name* is the text used to identify the log file output, i.e., the name of the mission file. For more information on options for `dumplog`, type `dumplog --help`.

4. Open the text file in the text editor and review.

Stopping an Active Process

The instances in which you will stop an active process include, but are not limited to, when an incorrect puck being loaded or when fluid leakage occurs. Stopping an active process will result in various components having not returned to a ready state. Before starting another process, you will need to recover the system. See Appendix A, "Troubleshooting."

! Caution:

Perform this procedure only when necessary. After a stoppage, you must recover the system before starting another process or mission.

To stop a mission

1. Establish communication with the ESP. Since the ESP is running, you must connect as an esp client. At the \$ prompt, type the following and press ENTER:

```
espClient "name"
```

Where:

name is the text to identify who is stopping the mission.

2. At the prompt, type the following and press ENTER:

```
abort! "reason"
```

Where:

reason is the text that will appear in terminal output and log files. The text must be enclosed within double quote marks (" ")

All activity in the ESP will stop immediately. In order to run another mission, you must first recover the system (see Appendix A).

To stop a short sh2 protocol

- In the terminal window in which the short sh2 was initiated, do one of the following:

Press CTRL + C

or

Type `stop` and press ENTER.

All activity in the ESP will stop immediately. In order to run another short sh2, you must first recover the system. See Appendix A, "Troubleshooting."

Shutting Down the ESP

To shut down the ESP, the ESP must be in a safe state. That is, all processes have completed.

1. Make sure the ESP is not running a mission or protocol.
 - a. If necessary, open another terminal window and establish communications with the ESP.

See “To establish communication with the ESP” on page 77.

- b. At the prompt, type `ESP.ready?` and press ENTER.
3. At the ESP prompt, type `exit` and press ENTER.

The ESP application is now closed.

4. At the \$ prompt, type `exit` and press ENTER.

Communication to the ESP is now ended.

5. If desired, turn off the power supply.

Chapter 5

Maintaining & Restocking the ESP

To ensure that the ESP system continues to operate smoothly, routine maintenance must be performed. For more information on a particular ESP component, see Chapter 1, “Components at a Glance.”

 **WARNING:** Electrostatic discharge (ESD) may damage the ESP circuitry. Ensure that ESD protection, such as an antistatic or ESD wrist strap, is being used when performing these procedures.

 **WARNING:** Performing these procedures may involve contact with harsh or hazardous chemicals. Wear the appropriate protective clothing, gloves, and eye protection at all times. Also use disposable, powder-free gloves.

! Caution: The following procedures call for specific products that have been tested with the ESP. The use of a product other than the one specified may affect the quality in performance and/or data. See Appendix B, “Supplies,” for details.

This chapter provides instructions on how to perform the following maintenance procedures:

- Cleaning the Clamps and Heater Pads
- Emptying the Clean-air Containers
- Lubricating Ballscrews for the Clamp and Syringes
- Lubricating Syringe Plungers
- Maintaining the Flush Puck Garage
- Replacing Air Filters
- Replacing the O-rings on the Clamps and Heater Pads
- Restocking Reagents

In addition to the routine maintenance described in this chapter, the thermal units and pressure systems should be calibrated annually. Notification will be sent to schedule periodic software updates. Contact your Spyglass representative for details.

Cleaning the Clamps and Heater Pads

Residue from reagents used in the ESP can accumulate on the clamps as well as the heater pads. To maintain proper function of the ESP, this residue must be removed regularly.

Supplies

You will need the following additional supplies:

- Foam-tipped swabs
- Inspector mirror
- RO water

Maintenance

Schedule: Clean the clamps and heater pads before every deployment. For bench-top usage, inspect the surfaces and pads every 10 phases, and clean as needed.

To clear the clamps and heater pads:

1. Using a inspection mirror, check the underside of the clamps located above the processing and collection stage. See Figure 5-1.

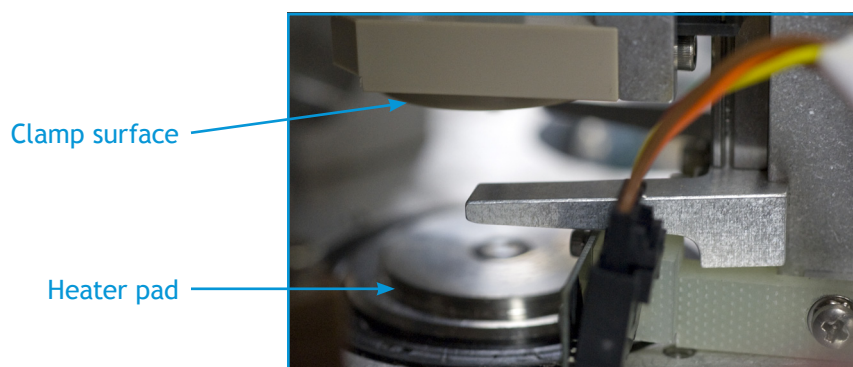


Figure 5-1: Clamp surface and heater pad

2. With distilled water, lightly dampen the foam-tipped swab. Wipe any residue from the clamp surfaces and heater pads. Discard the swab when it becomes soiled.
3. Repeat step 2 until the surfaces are free of residue.

The maintenance for the clamps and heater pads is now completed.

Emptying the Clean-air Containers

The clean-air containers may collect small amounts of reagent fluid used during sample processing. Remove any accumulated fluid to avoid backflow into the air line. A small clean-air container is suspended below the Collection Series Reagents valve manifold (CSR) and the Processing Series Reagents valve manifold (PSR). Each one of these containers has a volume of 50mL. A large container sits on top of ESP base and has a volume of 1000mL.

Schedule: Empty the clean-air container before every deployment. For bench-top usage, inspect the container after every 10 phases, and empty as needed.

Supplies

You will need the following additional supplies:

- Hazardous waste container

To empty a clean-air container:

1. Unscrew a container from its cap. See Figure 5-2.

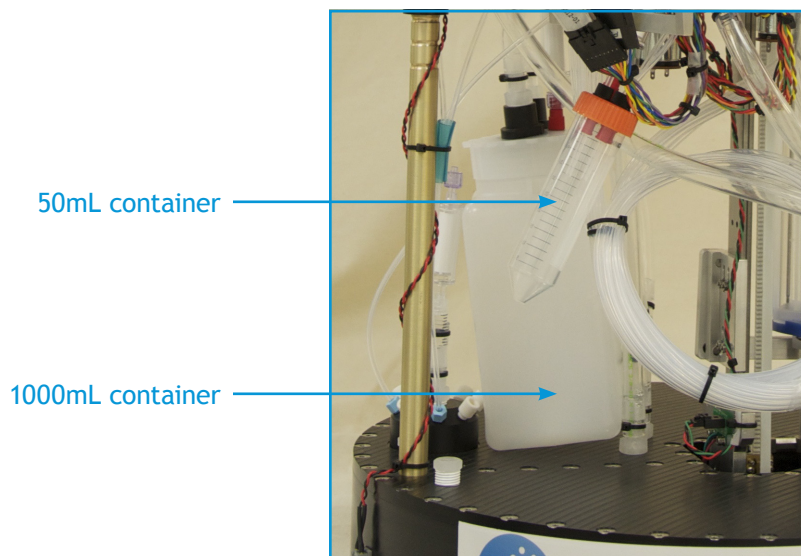


Figure 5-2: Clean air containers

2. Pour fluid into the appropriate waste container.
3. Screw the container back into the cap. Check to make sure it is properly threaded and tighten.
4. Repeat steps 1 through 3 for the two remaining clean-air containers.

The maintenance for the clean-air containers is now completed.

Emptying the Waste Containers

The reagents used in sample processing are disposed of into two waste containers located in the base of the ESP. The carrying capacity for the `waste1` and `waste2` containers is 3.2 L and 0.98 L, respectively.

If the waste capacity of any of the containers is exceeded, the waste fluid may back up into the waste tubes and vent filters.

Supplies

You will need the following additional supplies:

- 9/16-inch box wrench
- Hazardous waste container
- Teflon thread sealing tape, 1/2-inch wide
- Vacuum flask set up:
 - Büchner or vacuum Erlenmeyer flask, 5-liter capacity
 - Flex tubing, sizes to connect vacuum flask to vacuum pump
 - One-hole stopper
 - 10-mL pipette tube
 - Vacuum pump

Maintenance

A vacuum flask set up is required to extract waste from the ESP waste containers. Waste fluids are hazardous and require proper disposal.

Schedule: Empty the waste containers before every deployment. For bench-top usage, inspect the containers after every 10 phases, and empty as needed.

To empty the waste containers:

1. Disconnect the ESP from its power supply.
2. Securely place the single-hole stopper into the mouth of the vacuum flask. Connect the flex tube to the stopper and the plastic pipette. Connect the hose barb to the vacuum source. See Figure 5-3.

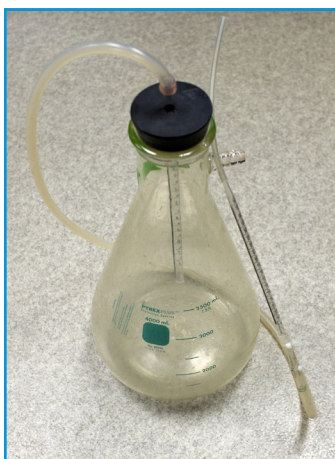


Figure 5-3: Example vacuum flask set up

3. Using a 9/16-inch box wrench, loosen the nut for the air-filter assembly from the manifold to the first waste container to be emptied. See Figure 5-4.

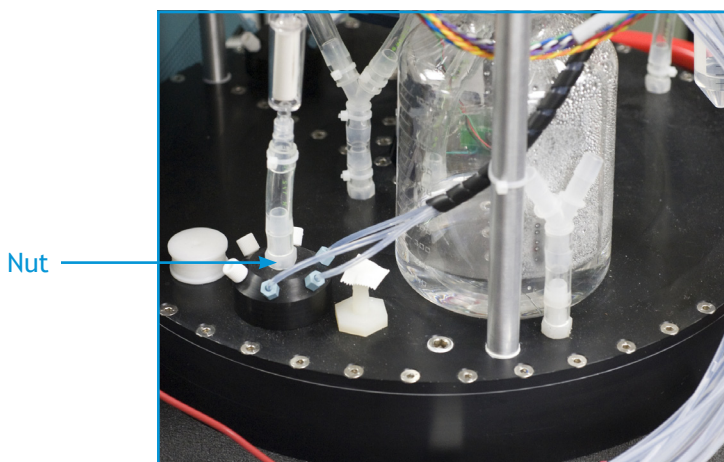


Figure 5-4: Opening to waste container

4. Start the vacuum pump and insert the flex tube into the container to empty it. Check the flask periodically to make sure it does not overfill. Empty the flask as needed.



WARNING:

Do not attempt the next step alone. Tilt the ESP from the lid. Do not grab another location of the ESP to tilt it.

5. When the waste container is nearly empty, securely grab the lid of the ESP and tilt the entire instrument slightly to allow the remaining waste to be collected. Suction the remaining waste into the flask.
6. If necessary, change the air filter. See “Replacing Air Filters” on page 105.
7. Using the Teflon thread sealing tape, wrap the threads of the exhaust port three (3) times. Reattach the air-filter assembly to the manifold. Check to make sure it is properly threaded and tightened.

8. Repeat steps 3 through 7 for the second waste container.
9. Turn off the vacuum, remove the stopper, and dispose of waste fluid in the appropriate hazardous waste container.

The maintenance for the waste containers is now completed.

Lubricating Ballscrews for the Clamp and Syringes

Ballscrews are used to move the Collection and Processing clamps, as well as the plungers in the Sampling, Collection, and Processing Syringes. Lubricant must be applied regularly to ensure the mechanisms operate smoothly.

Supplies

You will need the following additional supplies:

- Haynes® LubriFilm heavy-duty sanitary lubricant

Maintenance

Lubricant is applied to the shaft of the ballscrew and the related mechanism is commanded to travel up and down the shaft. Movement ensures the lubricant enters the nut and coats the bearings.

Schedule: Lubricate the ballscrews before every deployment. For bench-top usage, lubricate the ballscrews every 30 days.

To lubricate a ballscrew:

1. Apply a small amount of lubricant to the shaft of the ballscrew, just above the nut. See Figure 5-5.

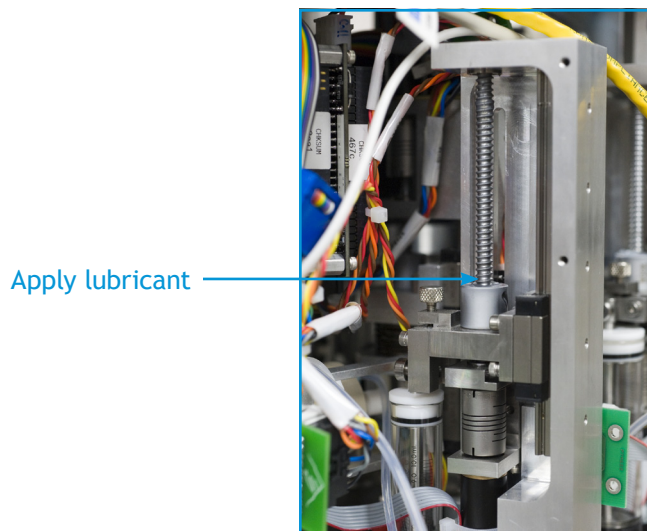


Figure 5-5: Ballscrew

2. For syringe ballscrews, apply a small amount of lubricant to the underside of the nut.

3. Repeat steps 1 and 2 for each ballscrew mechanism.

Now, command the mechanism to move, which spreads the lubricant through the ballscrew assembly.

To move the clamp

1. Ready the ESP.

See “Readying the ESP” on page 79.

When the ESP is ready, the clamps are in the open position.

2. To close a clamp without having a puck in place, enter one of the following and press ENTER:

- CC.to :closed
- PC.to :closed

Where:

CC is the collection clamp

PC is the processing clamp

The clamp will close.

3. Now, open the clamp. Enter one of the following and press ENTER:

- CC.open
- PC.open

4. Wipe away any excess lubricant that may have pooled on top of the nut.

5. Repeat steps 2 through 4 for the remaining clamp.

To move the plunger in a syringe

1. Ready the ESP.

See “Readying the ESP” on page 79.

When the ESP is ready, the plunger is located at the bottom of the syringe cylinder.

2. Enter the following sequence of commands to move the plunger through the syringe chamber.

Step	At ESP prompt, enter:	Result
a. Set the syringe valve to <code>air</code> .	<code>SVAL.air</code>	Air is used to avoid using fluid unnecessarily.
b. Command the syringe to fill.	<code>SYR.fill</code>	The plunger moves up, filling the syringe with air.
c. Send the air in the syringe back to the air container.	<code>SYR.empty</code>	The plunger moves down, pushing the air out.

Where:

`SVAL` is a syringe valve. Must be entered with all capital letters: `CSV` for Collection Syringe Valve, `PSV` for Processing Syringe Valve, `SSV` for Sampling Syringe Valve.

`SYR` is the syringe to move. Must be entered with all capital letters: `CS` for Collection Syringe, `PS` for Processing Syringe, `SS` for Sampling Syringe.

3. Wipe away any excess lubricant that may have pooled on top of the nut.
4. Repeat steps 2 through 3 for the remaining syringes.

The maintenance for the ballscrews is now completed.

Lubricating Syringe Plungers

The plungers in the Collection Syringe (CS), the Processing Syringe (PS), and Sampling Syringe (SS) need to be lubricated to ensure proper function of the syringes.

Supplies

You will need the following additional supplies:

- Disposable, sterile transfer pipette
- Dow-Corning® cSt 1000 silicone lubricant

Maintenance

Lubricant is placed regularly in the syringe chamber above the top of the plunger. After lubricant is in the syringe chamber, command the syringe to move to coat the plunger with lubricant.

Schedule: Lubricate the syringe plungers before every deployment. For bench-top usage, inspect the containers after every 10 phases, and lubricate as needed.

To lubricate a syringe plunger:

1. If no vent hole is present in the top of the white Teflon syringe stopper, pull out the stopper. See Figure 5-6.



Figure 5-6: Syringe

! Caution: Do not overfill the chamber with lubricant.

2. Fill a pipette with the silicone lubricant. Insert the pipette into the syringe chamber through vent hole or the opened top. Slowly drop the lubricant onto the plunger top. Allow the lubricant to settle between drops. Continue until there is approximately 1/16 inch of lubricant above the top of the plunger. Measure from the top of the plunger to the top of the meniscus formed by the lubricant.
3. If removed in step 1, replace the stopper and wipe away any excess lubricant from the outside surface.
4. Command the plunger to move. See the steps in “To move the plunger in a syringe” on page 97.
5. Wipe away any excess lubricant that may have leaked through the top of the plunger.

The maintenance for the syringe plungers is now completed.

Maintaining the Flush Puck Garage

The Flush Puck is used each time the ESP disinfects and flushes its internal plumbing. This puck is stored in a plastic housing (i.e., the *Flush Puck Garage*) located between the camera and the processing stage. The Flush Puck is held in the garage by the tension of a ball-detent assembly. If the tension is too light, the puck may fall out place. If the tension is too high, the robotic arm will not be able to remove the puck for use.

The residue from reagents used in the ESP will accumulate on Flush Puck and various components in the Flush Puck Garage. If the residue is not removed routinely, the Flush Puck will not function properly.

In addition to cleaning various components, you will also need to lubricate all moving parts.

Supplies

You will need the following additional supplies:

- Ball-end Allen wrenches, straight handle, in the following sizes:
 - 1.5 mm
 - 3/32 inch
- Dow Corning cSt 1000 silicone lubricant

Maintenance

In order to maintain the Flush Puck Garage, you will disconnect it from the ESP, disassemble it, clean several of the components, reassemble and test it.

Schedule: Perform maintenance on the Flush Puck Garage before every deployment. For bench-top usage, inspect the ball detent every 10 phases and clean as needed. Depress the ball with your finger. If the ball does not readily pop up or appears to stick, then the assembly requires cleaning.

To disconnect the Flush Puck Garage:

1. Using a 3/32" ball-end Allen wrench, unthread the three screws that secure the base of the Flush Puck Garage to the ESP. See Figure 5-7.

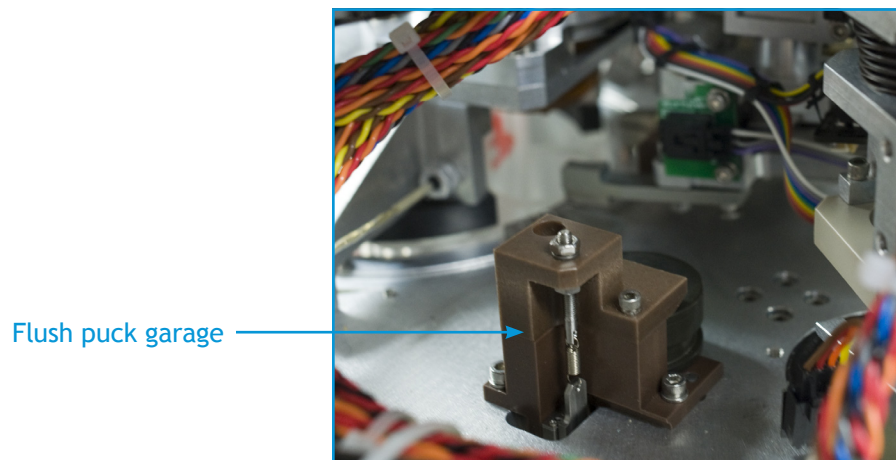


Figure 5-7: Flush puck garage

2. Lift and remove the garage assembly from the ESP. Remove the Flush Puck from the garage.

See Figure 5-8. Note that the ball detent assembly is seen on the left side. It is comprised of the tensioning screw with nuts and lock washer, the tension spring, lifter arm, and the slide. The assembly also contains the ball and ball spacer, which are located inside the garage body.

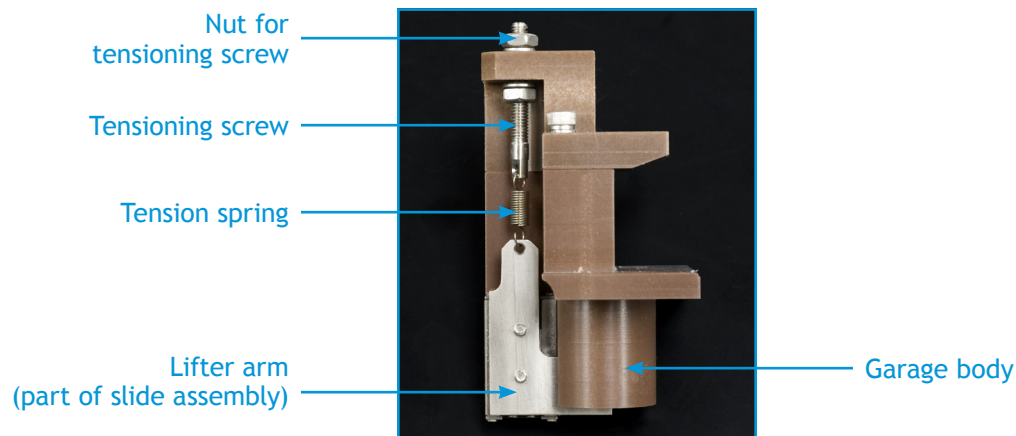


Figure 5-8: Flush puck garage removed from ESP

To disassemble the Flush Puck Garage:

1. Unthread the nut and remove the lock washer at the top of the tensioning screw.
2. Using a 1.5-mm ball-end Allen wrench, unthread the two screws and lock washers to free the slide assembly. Remove the slide assembly from the plastic garage housing.

! Caution:

Handle the tension spring carefully to avoid distorting the spring and damaging it.
Do not disassemble the slide.

3. Unhook the tension spring from the lifter arm (part of the slide assembly). The spring can remain attached to the tensioning screw.
4. Remove the ball and ball spacer from inside of the slot in the garage body.

Figure 5-9 shows the completely disassembled Flush Puck Garage.

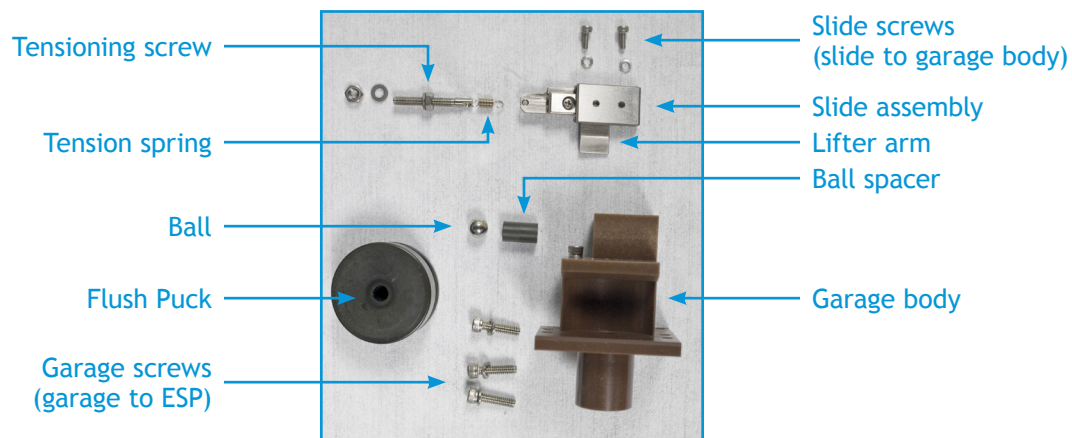


Figure 5-9: Disassembled Flush Puck Garage

To clean the Flush Puck Garage components

! Caution: Do not use any type of abrasive to clean the components.

1. Using soap and tap water, clean the garage body, the puck, the ball, the ball spacer, and the lifter arm.

! Caution: Do not clean the slide assembly.

2. After cleaning and rinsing the components, rinse thoroughly with RO water.
3. Allow components to air dry or place in a 50 °C oven until dry.

Once the components are clean and dry, you can lubricate the necessary parts.

To lubricate the components

1. Using a small amount of silicone lubricant, apply a very thin coat on the ball and ball spacer.
2. Also apply a small amount of the lubricant to the rails of the slide assembly and the slot in the garage body in which the rail moves.
3. Wipe off excess lubricant from the garage body.

Now, reassemble the Flush Puck Garage.

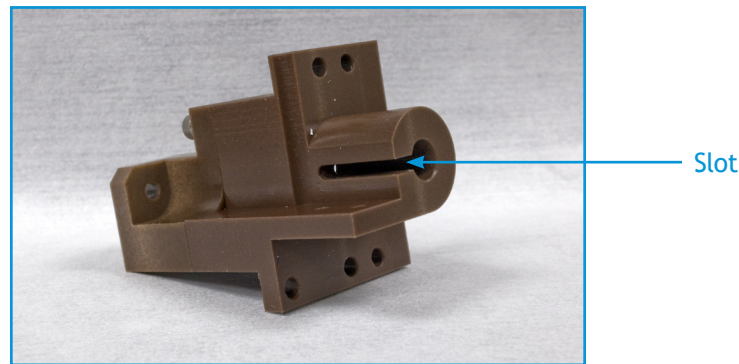


Figure 5-10: Slot in garage body

To reassemble the Flush Puck Garage

1. Place the lubricated ball into the slot of the garage body. Next, place the ball spacer into the slot making sure the end with the conical depression is toward the ball.
2. Reattach the spring to the lifter arm. If necessary, reattach the spring to the tensioning screw.
3. Reattach the slide assembly to the garage body:
 - a. Position the slide assembly by placing the lifter arm into the slot of the garage body. Align the screw holes of the slide to those in the garage body.
 - b. Using the 1.5-mm, ball-end Allen wrench, thread the screw with a lock washer into one of the holes. Thread until the slide is just attached to the garage body. Do not tighten it down. Repeat with the remaining screw and lock washer.
 - c. Check the alignment of the slide. Adjust as needed to ensure the lifter arm moves smoothly along the groove of the garage body. Tighten the screws until the two ends of each lock washer are aligned. Do not over-tighten; snug is tight enough.
4. Put the washer on the tensioning screw. Thread the nut just until the assembly is secure. Tighten slightly.
5. Adjust the tension to make sure the Flush Puck is secure in the garage.
 - a. Place the Flush Puck into the garage. Invert the garage so that the edge with the puck is facing down.
If the tension is appropriate, the puck will remain in place. If the puck falls out, increase the tension slightly and test again.
 - b. If necessary, repeat the previous step just until the Flush Puck remains in the garage.
3. Using the 3/32-inch, ball-end Allen wrench, reattach the garage to the ESP.
4. When placing the Flush Puck into the garage, make sure it is oriented correctly. The groove in the puck must be nearest to the stage. See Figure 5-11.

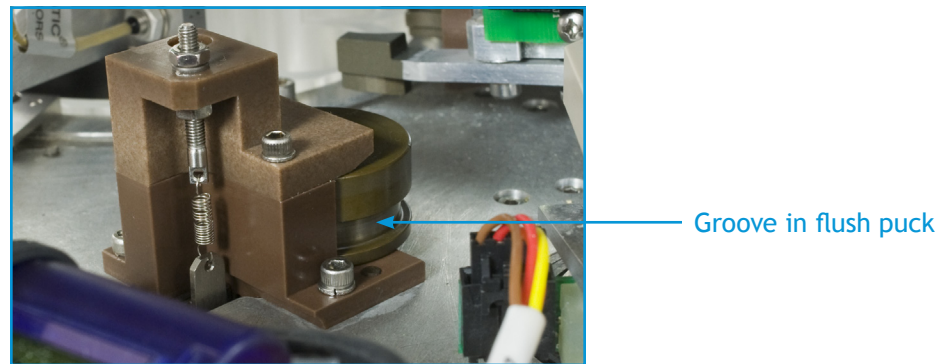


Figure 5-11: Flush Puck properly oriented

To ensure the tension is not too high, command the ESP to retrieve the Flush Puck.

To test the Flush Puck Garage

1. Ready the ESP.

See “Readying the ESP” on page 79.

2. At the ESP prompt, type the following and press ENTER:

```
CC.loadFlushPuck
```

The Manipulator will retrieve the Flush Puck from the garage and move it to the collection stage. If the tension is too high and the clamp will have difficulty removing the Flush Puck from the garage, decrease the tension.

3. To return the Flush Puck to the garage, type the following and press ENTER:

```
FlushPuck.garage
```

The Flush Puck is returned to the garage.

4. If necessary, decrease the tension slightly and repeat steps 2 and 3.

The maintenance for the Flush Puck Garage is now completed.

Note that `FlushPuck` can be abbreviated to `Flush`. Therefore, the commands above can be entered as follows:

- `CC.loadFlush`
- `Flush.garage`

Replacing Air Filters

Air filters are connected to each waste container manifold, the waste bottle, and the clean air containers. As fluids enter these containers, air is displaced and passed through these filters.

Supplies

You will need the following additional supplies:

- Air filters, Millipore™ Sterivex™ GV 0.22 µm Filter Unit, part no. SVGVL10RC
- Cable ties

Maintenance

Moisture and chemicals can build up in an air filter. Each air filter should be examined regularly and replaced when moisture is visible inside the filter's plastic cylinder. When a filter is replaced, it must be secured in place using a cable tie.

Schedule: Replace the air filters before every deployment. For bench-top usage, inspect the air filter every 10 phases. If the air filter appears damp, replace it.

To replace an air filter

1. Carefully cut the cable tie to free the air filter from the exhaust tube. See Figure 11.

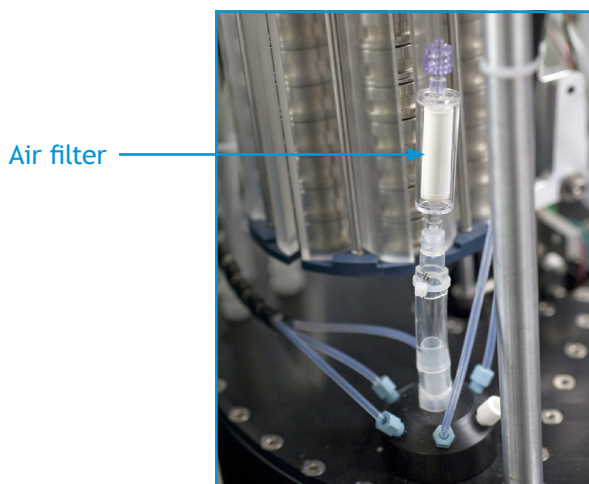


Figure 5-12: Air filter attached to exhaust tube

2. Remove and dispose of the air filter in the appropriate hazardous waste container.
3. Insert a new filter into the exhaust tube and secure with a cable tie.

The maintenance for the air filters is now completed.

Replacing the O-rings on the Clamps and Heater Pads

O-rings are located on the Collecting and Processing clamps, and the heater pads on the collecting and processing stages. They provided a seal between the valve and puck openings, and prevent fluids from leaking out of the pucks.

Supplies

You will need the following additional supplies:

- Inspection mirror, angled
- O-rings – Marco Rubber & Plastic Products, Inc., Buna-N (Nitrile), standard AS 568
 - Clamp, inner – 2-010, 70 durometer
 - Clamp, outer – 2-020, 70 durometer
 - Heater pad – 2-008, 70 durometer
- Foam-tipped swabs

Maintenance

O-rings are subjected to chemicals and heat, which cause them to wear and harden. If they are not routinely replaced, leakage may occur, which may damage the ESP instrumentation.

Schedule: Replace the O-rings on the clamps and heater pads every deployment. For bench-top usage, inspect the O-rings every 10 phases. If there is any indication of wear or hardening, replace the O-ring.

To replace an O-ring

! Caution: Do not use any type of metal instrument to remove or place the O-ring. Metal-to-metal contact may damage the ESP.

1. Using an inspection mirror, check O-rings located on the underside of the clamps above the processing and collection stage. Check the O-rings on the heater pads. (See Figure 5-13.) Touch them and compare them to a new O-ring. If any O-rings appear worn or hardened, replace them.

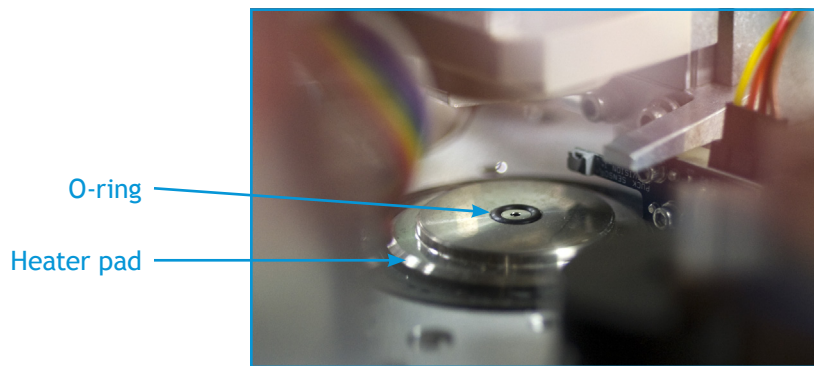


Figure 5-13: O-ring in heater pad

2. Remove the O-ring from the clamp and from the heater pad, and discard. You can use the slant edge of an orange stick or similar implement.
3. Clean the grooves in the clamps and heater pads of any debris. Dry the grooves with a clean foam-tipped swab.
4. Place a new O-ring into the groove. See the following table for appropriate sizes. Run your finger around the groove to push the O-ring into place. Make sure that the O-ring is seated evenly in the groove.

Location	O-ring Specification
Processing clamp, outer	2-020, 70 durometer
Processing clamp, inner	2-010, 70 durometer
Collection clamp	2-010, 70 durometer
Heater pads	2-008, 70 durometer

5. To ensure the fit of the O-ring, command the ESP to load the Flush Puck.
 - a. If necessary, ready the ESP.
See “Readying the ESP” on page 79.
 - b. At the ESP prompt, type one of the following commands and press ENTER:

Command	Description
<code>CC.loadFlushPuck</code>	Use when replacing the O-rings for the collection stage (CC).
<code>PC.loadFlushPuck</code>	Use when replacing the O-rings for the processing stage (PC).

The clamp will retrieve the Flush Puck from the garage and move it to the specified stage.

- c. Allow the Flush Puck to remain in place for one (1) minute.
- d. To return the Flush Puck to the garage, type the following and press ENTER:

```
FlushPuck.garage
```

The Flush Puck is returned to the garage.

6. Repeat steps 1 through 5 to replace the O-rings for the remaining clamp and heater pad.

The maintenance for the clamps and heater pads is now completed.

Note that `FlushPuck` can be abbreviated to `Flush`. Therefore, the commands above can be entered as follows:

- `CC.loadFlush` or `PC.loadFlush`
- `Flush.garage`

Restocking Reagents

In order to change a reagent bag, it is necessary to access the line between a syringe and the reagent bag. Access is controlled by valves between a syringe and the reagents. For a standard ESP configuration, there are valves for the collection and processing syringes and three manifolds. Manipulating the valves is accomplished by sending commands interactively from the ESP host to the ESP instrument.

The organization of the flow of reagents through the ESP is shown in the ESP Fluidic Worksheet. Figure 5-14 shows the portion of the worksheet containing information the Processing Syringe (PS) and processing series reagents. The valve leading to the processing syringe is called the Processing Syringe Valve (PSV), and the valve manifold for the reagents is referred to as the PSR (Processing Series Reagents).

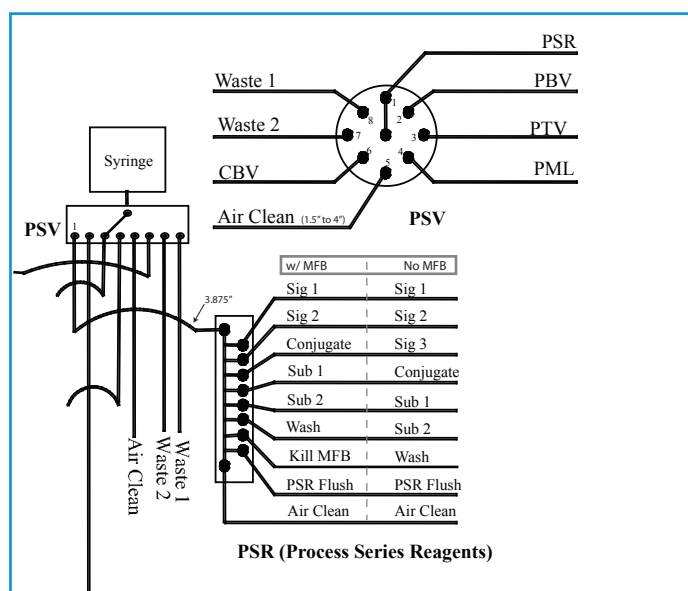


Figure 5-14: PSV and PSR, close up of ESP Fluidics Worksheet

The reagents connected to the PSR valve manifold are listed in the following table. Note these are reagents for the core ESP device (i.e., without the MFB component).

Table 5-1: Reagents and Air on Processing Series Reagent (PSR) manifold	
Reagent	Valve
Signal reagent for HAB protocol	sig1
Signal reagent for BAC protocol	sig2
Signal reagent for LARV protocol	sig3
Conjugate reagent	conjugate
Substrate reagent 1	sub1
Substrate reagent 2	sub2
Wash used in blocking arrays	wash
PSR flush used to flush the manifold	flush
Clean air	air

For the complete fluidics worksheet and the reagents used in the ESP, refer to Appendix C, “Fluidic Worksheet.”

Supplies

To restock the reagents, You will need the following additional supplies:

- Two 50-mL sterile containers with lids (such as Corning® sterile centrifuge tubes, 50 mL, part no. 430829). Fill with one of the following:
 - Ethanol, 70% solution made with molecular-grade water and passed through a 0.2 µm filter
 - Molecular-grade water
- Cable ties
- Disposable, sterile pipette
- ESP access tube
- ESP reagents
- RNase Zap® RNase decontamination solution, in spray bottle

Maintenance

In addition to accessing the line, you will need to clear the line, prime it with the new reagent, and then flush and clear the manifold.

Schedule: Restock all of the reagents for every deployment. For bench-top usage, inspect the reagent bags every 10 phases, and replace as necessary.

! Caution: To avoid cross contamination, use standard sterile techniques when handling the reagents.

To access reagent line

1. Ready the ESP.

See “Readying the ESP” on page 79.

2. Access the valve on the manifold for the desired reagent. At the ESP prompt, type the following and press ENTER:

```
MAN.select :svalve
```

Where:

MAN is the name of a valve manifold. Must be entered with all capital letters: CSR for Collection Series Reagents manifold, PSR for Processing Series Reagents manifold, and PRV for Precious Reagents Valve manifold.

svalve is the series valve on the manifold to access. Must be entered with all lowercase letters. Refer to the Fluidics Worksheet in Appendix B for details.

For example, to access the PSR valve for the HAB signal reagent (sig1), you would type the following and press ENTER:

```
PSR.select :sig1
```

Note that the command above can be abbreviated to PSR.sig1. This abbreviation is used in the remaining steps.

3. Set the valve on the syringe to access the manifold specified in step 3.

- At the ESP prompt, type the following and press ENTER:

```
SVAL.MAN
```

Where:

SVAL is a syringe valve. Must be entered with all capital letters: CSV for Collection Syringe, PSV for Processing Syringe.

MAN as described above.

For example, to establish the line between the processing syringe (PS) to the processing series reagent manifold (PSR), you would type the following and press ENTER:

```
PSV.PSR
```

Now the line from the syringe to the reagent bag is open.

To verify the correct line is open

1. Unclip the empty reagent bag and dispose of it in the appropriate waste container. Take care not to drip any reagent remaining in the bag and line.
2. Attach a clean access tube to the reagent intake line (see Figure 5-15).

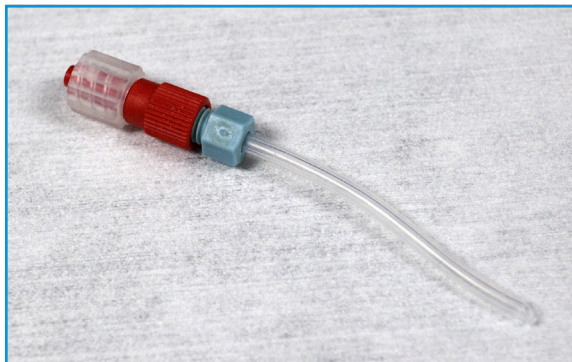


Figure 5-15: ESP access tube

3. Command the syringe to draw 0.5 mL. At the ESP prompt, type the following and press ENTER:

```
SYR.pull 0.5
```

Where:

SYR is the syringe to command. This command must be entered with all capital letters: CS for Collection Syringe, PS for Processing Syringe.

MAN as described above.

You should observe the fluid moving in the line that has been disconnected from the bag, which also introduces an air slug into the line.

For example, to command the processing syringe to draw 0.5 mL, enter the following:

```
PS.pull 0.5
```

Once you've verified the correct reagent line, you need to clean the line of the old fluid.

To clear the reagent line

Enter the sequences of commands to draw the remaining reagent through the line and then clean the line.

Step	At ESP prompt, enter:	Result
1. Place the end of the access tube into the container filled with molecular-grade water, and command the syringe to fill.	<i>SYR.fill</i>	The syringe draws any reagent remaining in the line as well as water.
2. Send the fluid in the syringe to the waste container	<i>SVAL.waste1</i>	The syringe valve moves to waste container #1.
	<i>SYR.empty</i>	The syringe pushes the fluid out into the waste container.
3. Return the syringe valve back to access the manifold.	<i>SVAL.MAN</i>	
4. Repeat steps 1 through 3, using the molecular-grade water again.		
5. Remove the access tube from the water and introduce an air slug into the line.	<i>SYR.pull 0.5</i>	The air slug provides a barrier between the water and the next solution, ethanol. This prevents the ethanol from diluting and become less effective as a cleaning agent.
6. Using the 70% ethanol solution, repeat steps 1 through 3. Then repeat step 5.		
7. Using the molecular-grade water again, repeat steps 1 through 3.		

For example, to complete steps 1 through 4 for the Processing Syringe using water, you would place the access tube into the container with molecular-grade water. Then you would enter the following sequence of commands:

```
PS.fill
```

```
PSV.waste1
```

```
PS.empty
```

```
PSV.PSR
```

```
PS.pull 0.5
```

After completing all of the steps above, connect the new reagent bag.

To connect the reagent bag

1. Disconnect the access tube.
2. Using RNAseZap, spray the connector on the reagent line.

3. Fill a disposable pipette with the molecular-grade water and rinse the connector. Flick the connector to shake off any excess water.
4. Remove the cap from the appropriate connector on the reagent bag.

The reagent bag has two ports, each with different type of connectors: a female and a male. See Figure 5-16. Check the end of the reagent line to determine which cap to remove. Match female to male, and vice versa.



Figure 5-16: Male and female connectors on reagent bag

5. Using the RNaseZap, spray the opening of the reagent bag connector.
6. Fill a disposable pipette with the molecular-grade water and rinse the connector. Flick the connector to shake off any excess water.
7. Connect the bag to the reagent line.
8. Open the flow restrictor clip on the bag.
9. Holding the bag with the ports pointing down, tap the ports of the bag to remove any trapped air bubbles and then secure the bag to the support rail with a cable tie.
10. Check the connection between the reagent line and bag to make sure it is secure and not leaking.

After the bag is connected, prime the line with the new reagent fluid. To completely prime the line, approximately 5 mL of the reagent is used.

To prime the line

Enter the sequences of commands to prime the line.

Step	At ESP prompt, enter:	Result
1. Fill the processing syringe with the new reagent.	<i>SYR.fill</i>	The syringe pulls the reagent through the line and fills its chamber.
2. Send the fluid in the syringe to the Waste 1 container.	<i>SVAL.waste1</i>	The syringe valve moves to the line for the waste container #1.
	<i>SYR.empty</i>	The syringe pushes the fluid out into the waste container.
3. Return the syringe valve back to access the manifold.	<i>SVAL.MAN</i>	The valve moves back to PSR.
4. Repeat steps 1 through 3 again.		

For example:

PS.fill

PSV.waste1

PS.empty

PSV.PSR

The line is now primed and you must clear and flush the PSR manifold. Next, the manifold must be flushed of reagents to prevent cross contamination.

To flush and clear the manifold

Enter the sequences of commands to flush and clear the manifold.

Step	At ESP prompt, enter:	Result
1. Empty the manifold of any fluid by filling it with air.	<i>MAN.air</i>	The manifold valve to Air Clean opens.
	<i>SYR.fill</i>	The syringe draws air through the manifold and the line, and then fills its chamber.
	<i>SVAL.waste1</i>	The syringe valve moves to the waste #1 container.
	<i>SYR.empty</i>	The syringe pushes the fluid out into the waster container.
2. Flush and clear the manifold.	<i>MAN.flush</i>	The manifold valve to the Flush reagent opens.
	<i>SYR.pull 1</i>	The syringe pulls one (1) mL of flush.
	<i>MAN.air</i>	The manifold valve Air Clean opens.
	<i>SYR.fill</i>	The syringe draws air, which also pulls the 1 mL of flush through the manifold and the line. The manifold and line are left empty of fluid.
	<i>SVAL.waste1</i>	The syringe valve moves to the Waste #1 container.
	<i>SYR.empty</i>	The syringe pushes the 1 mL of flush and air into the container.

For example, step 1 would be:

PSR.air

PS.fill

PSV.waste1

PS.empty

And step 2 would continue with:

```
PSR.flush
```

```
PS.pull 1
```

```
PSR.air
```

```
PS.fill
```

```
PSV.waste1
```

```
PS.empty
```

The reagent is now ready for use.

Appendix A

Troubleshooting

A stoppage in an interactive process (such as a short sh2 protocol) can occur as the result of one of the following conditions:

- A user-specified stoppage
- An interruption in the communication between the ESP and the host computer
- An interruption in power
- A mechanical failure

In any of the above instances, the interactive ESP process will stop immediately. How to recover from the stoppage depends on where in the process that the ESP stopped. Ultimately, the ESP needs to be returned to a ready state.

This appendix contains information on how to recover the ESP system after a stoppage during an interactive process. In order to accomplish this safely, first determine the cause of the stoppage.



WARNING:

Do not attempt to move any component or the puck before accessing the state of the ESP. If a stoppage occurs when a puck is clamped onto the collection or processing stage, the puck and its contents may be under pressure. Attempting to move a pressurized puck may result in hazardous materials being sprayed out of the puck.



WARNING:

Electrostatic discharge (ESD) may damage the ESP circuitry. Ensure that ESD protection, such as an antistatic or ESD wrist strap, is being used when performing these procedures.



WARNING:

Performing these procedures may involve contact with harsh or hazardous chemicals. Wear the appropriate protective clothing, gloves, and eye protection at all times. Also use disposable, powder-free gloves.

! Caution:

Do not turn off the machine unless instructed to do so by a Spyglass technical representative.

To determine the cause of the stoppage

1. If the stoppage is due to a user-directed command, go to “Recovery Flowchart” on page 118. Otherwise, proceed to step 2.

2. From the terminal window in which the process was initiated, at the ESP prompt, press ENTER.

If a new ESP prompt is returned, the stoppage may be due to a network interruption. Go to step 3.

If a new prompt is not returned, the stoppage may be due to a power failure or mechanical error. Go to step 4.

3. Check the Ethernet connection.

If the connection was interrupted, contact your network administrator to reestablish it. If the interruption is momentary, the ESP process may continue once the connection is reestablished.

If the process does not continue, go to step 4.

4. Open and scan the log file from the bottom up to see if there is a mechanical error message (“Outputting the Log File” on page 88).

The following is the format for the most common mechanical error messages:

Axis overCurrent

Axis Speed

Axis Trajectory

Axis Timeout

Where *Axis* is a component in the ESP. (In Ruby, the term *axis* refers to an object involving movement.) See “Abbreviations” on page 123 for a list of component abbreviations.

If one of these mechanical errors appears in the log file, contact Spyglass technical support for assistance.

If no such error appears in the log file, the stoppage may be due to a software error. Go to step 5.

5. Scan the log file from the bottom up to see if there is a software error message.

The following is the format for a software error:

SoftErr: *text*

Where *text* is a specific software error message.

Go to “Recovery Flowchart” on page 118 to recover the system. After recovery is complete, exit and restart the ESP application.

Recovery Flowchart

Refer to the following table for the action to take to recover a particular component. The following actions require you to set up the ESP and start the ESP application (page 76 and page 78).

! Caution:

Take precautions to avoid pushing fluids into the wrong locations. Empty and flush the processing syringe before attempting to recover any other component in the system.

ESP Component	State	Action
Processing Syringe	Plunger not in ready position	Empty and flush the Process Syringe (see page 120).
	Fluid in syringe	
	Fluid in the access tube or the line to the Processing Syringe Valve (PSV)	
Puck	In carousel tube in which it was loaded initially	No action required. Puck is unused and may be used in another short process.
	In manipulator	Fluid may be in Processing Syringe or lines. Perform the following steps in order: 1. Empty and flush Processing Syringe (see page 120). 2. Recover the Manipulator assembly (see page 122). 3. Perform a puck count (see page 121).
	Clamped into processing stage	Fluid may be in Processing Syringe and inside puck. Perform the following steps in order: 1. Empty and flush the Processing Syringe (see page 120). 2. Empty the puck (see page 120). 3. Unload the puck (see page 121). 4. Perform a puck count (see page 121).
	In the camera-ready position	Puck has been processed and image may or may not have been taken. Perform the following steps in order: 1. Empty and flush the Processing Syringe (see page 120). 2. Discard puck (see page 120). 3. Perform a puck count (see page 121).
	In carousel discard tube	No action required. The puck was discarded after the short process completed.
Manipulator	Not in ready state, but does not have puck	Fluid may be in Processing Syringe or lines. Perform the following steps in order: 1. Empty and flush the Processing Syringe (see page 120). 2. Recover the Manipulator assembly (see page 122).
	Not in ready state and is holding puck	Fluid may be in Processing Syringe or lines. Perform the following steps in order: 1. Empty and flush the Processing Syringe (see page 120). 2. Recover the Manipulator assembly (see page 122).
Carousel	In between tubes	Recover the carousel (see page 122).
	Positioned to access the start tube or discard tube, and elevator is raised	

Recovering the Processing Syringe

Anytime there is a stoppage during an interactive process, make sure to empty and flush the Processing Syringe before attempting to recover any other component in the ESP.

To empty and flush the Processing Syringe

1. Set the Processing Syringe Valve (PSV) to access a waste port. At the ESP prompt, enter the following:

```
PSV.waste1
```

2. Empty the Processing Syringe (PS). Enter the following:

```
PS.empty
```

3. Flush the Processing Syringe. Enter the following:

```
PS.flush
```

The syringe is flushed and emptied, and the plunger is returned to a ready state.

Recovering a Puck

This section describes the recovery procedures for components related to puck handling.

To discard a puck in the camera-ready position

When a puck is in the camera-ready position, the Elevator is raised in the Carousel such that any pucks in the discard tube provide a platform for the camera-ready puck.

- At the ESP prompt, type the following and press ENTER:

```
Puck.finishImage
```

To empty a clamped puck

4. Ensure the puck is empty. See page 120).

5. At the ESP prompt, enter the following:

```
PS.recoverReagent
```

Now unload the puck.

To unload a puck in the Manipulator

1. Ensure the Processing Syringe is empty. See page 120.
2. Ensure the puck is empty. See page 120).
3. Ensure the Carousel is in a ready state. See page 122.
4. Place the puck into the Collection Clamp, close the clamp, and then command the ESP to unload the puck. To do so, enter the following sequence of commands:

```
Arm.to CC
```

```
CC.close
```

```
CC.unloadPuck n, :type
```

Where:

n is the tube in which to discard the puck.

type is the type of puck being discarded (e.g., LAF, SVS, etc.). The text entered for type will appear in the log file and in the status returned in the terminal window.

To unload a puck from the Processing Clamp

1. Ensure the Processing Syringe is empty. See page 120.
2. Ensure the puck is empty. See page 120).
3. At the ESP prompt, enter the following:

```
PC.unloadPuck n, :type
```

Where:

n is the tube in which to discard the puck.

type is the type of puck being discarded. The text entered for type will appear in the log file and in the status returned in the terminal window. For example, in short processes, the type is either LAF or SAF.

4. Use a foam-tipped swab to clean any fluid that make have leaked out from the puck.

To perform a puck count

If the stoppage was due to loss of power, count the pucks in the tubes from which the ESP was retrieving and discarding pucks.

1. Ensure the ESP components are in a ready state. Refer to the “Recovery Flowchart” on page 118.
2. At the ESP prompt, enter the following:

```
Puck.count n1...n2
```

Where:

n1 is the number of the discard tube.

n2 is the number of the tube from which the ESP was retrieving new pucks.

Recovering the Manipulator Assembly

The Manipulator assembly is made up of the Forearm, Elbow, and Hand. You will need to recover each part individually.

To recover the Manipulator assembly

1. Ready the forearm. At the ESP prompt, enter the following:

```
Forearm.home.to :retract
```

2. Ready the elbow. Enter the following:

```
Elbow.home.to :clear
```

3. To recover the hand, do one of the following:

- If there is a puck in the Manipulator, see “To unload a puck in the Manipulator” on page 120.

Or

- If the hand is empty, enter the following:

```
Hand.home.to :home
```

The Manipulator is now in a ready state.

Recovering the Carousel

The Carousel rotates clockwise and counter-clockwise to move a tube into position. The Elevator raises a stack of puck to allow the Manipulator to retrieve a puck from or discard a puck to the tube. You may need to recover both components after a stoppage.

To recover the Carousel

1. If the Elevator is raised, lower it. At the ESP prompt, enter the following:

```
SE.home.to :down
```

2. Position the Carousel to access the appropriate tube. That is, if you are moving the Carousel to discard a puck, the tube should be the current discard tube. Enter the following:

```
SC.home.to n
```

Where:

n is the tube to access.

The Carousel is now in a ready state. You can retrieve or discard a puck.

Abbreviations

The following table lists the more common abbreviations that appear in the ESP messages.

Abbreviation	Description	Abbreviation	Description
ab	Antibody	PBV	Process Bottom Valve
abwash	Antibody wash solution	PC	Processing Clamp
Air	Air line from waste container	PH	Processing Heater
Air Clean	Air line from Clean Air container	PML	Processing Mixing Loop
AqMethanol	Aqueous Methanol Solution	PR	Precious Reagent
bac	Bacteria protocol	PRV	Precious Reagent Valve
CBV	Collection Bottom Valve	PS	Processing Syringe
CC	Collection Clamp	PSR	Process Series Reagents
CH	Collection Heater	PSR Flush	Flush solution specific to PSR
conj	Conjugate Solution	PSV	Process Syringe Valve
CTV	Collection Top Valve	PTV	Process Top Valve
CS	Collection Syringe	SC	Shuttle Carousel
CSR	Collection Series Reagents	SE	Shuttle Elevator
CSV	Collection Syringe Valve	sh1	Sandwich Hybridization stage 1
CSV Flush	Flush solution specific to CSV	sh2	Sandwich Hybridization stage 2
da	Domoic acid protocol	sig1, 2, 3	Signal solutions
flush	Flush solution	SS	Sample Syringe
hab	Harmful Algal Bloom protocol	SSV	Sample Syringe Valve
kill	Kill Solution	wash	Wash/buffer
larv	Larval invertebrates protocol	wastel, 2	Waste lines
lyfil	Lysate Filtration	wcr	Whole Cell Archival
Mix Loop	Line for Mixing 2+ Solutions		



Appendix B

Supplies

To service the ESP or its components, you will need the items listed in this appendix. Items listed under “ESP-Specified Products” can be ordered directly through Spyglass Biosecurity, Inc.

ESP-Specified Products

! Caution:

The following products have been tested in the procedures and protocols used with the ESP. The use of a product other than the one specified may affect the quality in performance and/or data.

- Air filters – Millipore™ Sterivex™ GV 0.22µm Filter Unit, part no. SVGVL10RC
- Cleaning solution – Cole-Parmer® Micro-90 cleaning solution, part no. EW-18100
- cSt 1000 silicone lubricant – Dow Corning®, part no. 360 Medical Fluid 1000 cst.
- ESP access tubes – Spyglass Biosecurity, Inc.
- ESP probe arrays – Spyglass Biosecurity, Inc.
 - BAC, part no. *tbd*
 - DA, part no. *tbd*
 - HAB, part no. *tbd*
 - LARV, part no. *tbd*
 - KAR, part no. *tbd*
- ESP Puck Separator – Spyglass Biosecurity, Inc., part no. *tbd*
- ESP Pucks – Spyglass Biosecurity, Inc.
 - Standard-volume sample (SVS), part no. *tbd*
 - High-volume sample (HVS), part no. *tbd*
 - Large array format (LAF), part no. *tbd*

- Small array format (SAF), part no. *tbd*
- Whole cell archive (WCR), part no. *tbd*
- ESP sample tubes – Spyglass Biosecurity, Inc., part no. *tbd*
- Lint-free cloth – ITW Texwipe® Technicloth® II, part no. TX1109
- LubriFilm heavy-duty sanitary lubricant – Haynes®, part no. M2550.0000
- Membrane filters
 - Millipore™ Durapore™, 25mm, 100 qty/pk
 - 0.22 µm, part no. GVWP 025 00
 - 0.65 µm, part no. DVPP 025 00
 - 5.0 µm, part no. SVLP 025 00
 - Millipore Isopore™, 25mm, 100 qty/pk
 - 1.2 µm, part no. RTTP 025 00
 - Pall Corp., Metrical®, 25 mm, 100 qty/pk
 - 0.45µm, part no. GN-6 63068
- O-rings – Marco Rubber & Plastic Products, Inc., Buna-N (Nitrile), standard AS 568 except *
 - Clamp, inner – 2-010, 70 durometer
 - Clamp, outer – 2-020, 70 durometer
 - Heater pad – 2-008, 70 durometer
 - Sealing for HSV, LAF, SVS, WCR pucks
 - 2-020 , 50 durometer,
 - *Sealing for SAF pucks
 - 0.453ID x .039", 70 durometer
 - Snap for LAF, SAF, SVS, WCR
 - 2-021, 70 durometer
 - Snap for HSV
 - 2-021, 90 durometer
- Polysulfone supports
 - 25mm for LAF pucks – Pall Corp., part no. 87265
 - 13mm for SAF pucks – Spyglass Biosecurity, part no. *tbd*

- Titanium frits, for support or filtration, 25mm, 10 µm – Chand Eisenmann Metallurgical, part no. 102375
- Titanium support screens, 25mm – Photofabrication Engineering, Inc., part no. 1002432-00
- RNase Zap® RNase decontamination solution – Applied Biosystems™, part no. AM9780)

Additional Supplies

In addition to the ESP-specified products, you will need to acquire the items listed in this section. Manufacturer and part number information is provided as guidance only.

- Ball-end Allen wrenches, straight handle, in the following sizes:
 - 0.05 inch
 - 3/32 inch
- Box wrench, in the following size:
 - 9/16-inch
- Cable ties
- Calipers
- Canned air duster, petroleum-distillate free (such as Fisherbrand® Super Friendly Air' it™)
- Desiccant packages (for LAF and SAF puck storage only)
- Disposable gloves, exam grade, powder-free
- Disposable, sterile transfer pipettes
- ESD (electrostatic discharge) wrist strap
- Ethanol, 200 proof (absolute), for molecular biology
- Filtration system with 2-µm porosity filter
- Hazardous waste containers
- Hydrochloric acid
- Inspection mirror, angled head, approximately 1 to 2 inches in diameter
- Plastic containers with lids, sterile, ~ 50 mL (such as Corning® sterile centrifuge tubes, part no. 430829)
- Plastic storage containers with secure-fitting lids (such as Ziplock®)
- Plastic storage bags, quart-sized resealable (such as Ziplock)
- Pipettes, sterile, disposable
- Protective eyewear and clothing
- Static-free shroud (to cover ESP during image exposure)

- Swabs, foam tipped (do not use cotton or other materials that may shed)
- Teflon thread sealing tape, 1/2-inch wide
- Ultrasonic cleaner or sonicator
- Vacuum flask set up (see “Example vacuum flask set up” on page 95 in Chapter 5)
 - 10-mL, plastic pipette
 - Büchner or vacuum Erlenmeyer flask, approximately 4000 to 5000 mL
 - Silicone flex tubing, size to connect pipette to vacuum flask to vacuum pump
 - One-hole stopper
 - Vacuum pump
- Vise, non-marring
- Water
 - Molecular-grade water (such as Sigma-Aldrich®, part no. W4502)
 - Reverse-osmosis water
 - Household quality
 - High-quality (such household-quality RO through an ultra filter)

Appendix C

Fluidic Worksheet

The fluidics worksheet is located on the last page of this document. For the most current fluidic worksheet, use the worksheet provided with the ESP system. If you wish to print out this worksheet, choose a Page Scaling option that allows you to tile and print the page across multiple pages.

