

COLONY ARRAYING WITH THE VIRTEK CAPS SYSTEM

version: 1.2

You will need:

Nunc OmniTray plates, with agar medium of your choice

or 384-well microtiter plates

MilliQ H₂O

70% EtOH

100% EtOH

Set-Up

Arraying Yeast The CAPS system will transfer yeast between liquid culture in microtiter plates and agar plates, or between plates of the same type. Currently, we only possess 384-pin replicators for the CAPS. Individuals using the system must initially create an agar plate of yeast arrayed in a 384-patch format using a hand-pinning tool or a 384-well microtiter plate of yeast cultures using a liquid handling instrument. In general, agar plates should be approximately 1/2 full of agar.

Machine Start-up

Compressed Air Supply The compressed air supplies the pneumatic controls of the plate gripper arm, attached to the left of the Z-axis. Turn on the house air line at the valve near the ceiling (at the back left of the machine, thin blue plastic outlet line) to bring the air pressure as high as it will go, normally around 80psi. Once the system is pressurized, the gripper arm should rise to its maximum height, away from the surface of the table. Always perform this step first to ensure that the gripper arm does not collide with anything once the motors begin moving.

Not commonly used

Vacuum Valve This is the small tan box to the left of the cabinet, which controls the vacuum used for aspirating wash solutions. It should always be switched on, as indicated by the red light on the front of the box.

Check Vacuum Traps The small Nalgene bottle on the table surface serves as the vacuum trap for aspirated liquids. First, check that the liquid level in the bottle is below the marked line and empty the trap if it is above it.

Not commonly used

Second, check the trap at the pump under the table, left-hand side. If there is a significant amount of liquid in this trap, place a small reservoir under the trap and drain it by turning the blue valve on the bottom of the trap. When the trap is empty, close the valve.

Check Humidifier Water Level At the front right of the machine, on the floor, is a plastic bucket containing the pump for the humidifier. This should be completely submerged in water (tap water is OK). Additionally, the outflow line should return to the same reservoir, to capture the water that comes out of the humidifier.

Air Filter The power switch for the air filter is found on the left side of the cabinet, high up, towards the back. The air flow into the filter is controlled by the intake port on the left side towards the back. To increase the air flow, slide the metal sheet to

expose more of the port. Air will not begin flowing until the humidifier is switched on also.

Humidifier Turn on the humidifier using the switch on the right side of the cabinet, high up, towards the back. Once the air filter and humidifier are switched on, you should be able to feel a gentle flow of air coming out of the cabinet at the gaps in the door. This does not need to be a strong flow of air, as just a little positive pressure will prevent microorganisms from entering the cabinet, but should be detectable. The humidity level in the chamber should be around 55% -- too high and the pins will stick in the head and puncture your agar, too low and the plates will dry out and your yeast will not be as happy.

Light Switch Turn on the lights in the cabinet by switching on the light switch on the front side of the cabinet, just above the doors.

Embedded Controller The motors of the robot are powered by the embedded controller, housed in a large cubic box directly underneath the table. The front of the box has a small door that flips open to reveal a round power button. Press this button to turn on the embedded controller. You should hear a beep, then a click from inside the cabinet.

Note: There is also a power switch and a circuit breaker on the back of the embedded controller -- please use the switch on the front to turn the power on and off.

Computer Boot up the computer that is under the table towards the right side.
un: Administrator
pw: yeast

Getting Started

The methods that control the CAPS are called Scripts and are composed of two parts, a *Configuration* and a set of *Tasks*.

The easiest way to begin writing a script is to modify an existing script. Since most methods involve washing the tips, a convenient method to begin to modify is a tip washing method.

In the CAT folder, there is a tip washing method called *Wash_Tips*. To access this method, select the *Shortcut to Default* folder on the desktop. Open the *CAT* folder. The *Wash_Tips* method is composed of three files, *Wash_Tips.bcr*, *Wash_Tips.sav*, and *Wash_Tips.script*. Copy all three of these files to your own folder. Now, change the name of all three files to the name of the method you want to create, e.g. *Replicate_Colonies.bcr*, *Replicate_Colonies.sav*, and *Replicate_Colonies.script*. This is the method you will modify once you launch the software.

Now, launch the colony arrayer software (VersArray colony picker & arrayer 2.1) from the shortcut in the center of the Desktop. Select **default** and click **OK**.

Running the Software

Console You can launch the *Console* by clicking its button on the toolbar: it looks like a small computer with a big "C" on its screen. This is the window you use to run scripts on the instrument and to interact with the command line. The window should open automatically when the program is launched.

Homing It is necessary to home the motors before any other commands are issued to ensure that the motion does not take the machine beyond its limits. Click the *Homing* button in the top center of the Console window. You will only be able to home the system once each time it is turned on. Once it is finished, *Homing Done!* will display in the results portion of the console window.

Joystick Controls The most simple way to move the motors around, either to get the head away from the working surface or to recover from a bug, is to use the Joystick window. Launch this window with the button on the toolbar that looks like a plane with an arrow rising vertically from it. Clicking a button once will move the selected axis a specified distance -- clicking and holding a button will cause the selected axis to jog at a defined speed. Three levels of distance/speed are available. Be careful not to move the motors beyond their physical limits. Also be certain that the replicator head is clear of obstructions on the deck when executing an X-Y move.

Control Panel Just as the Joystick window can be used to control the motor axes, the control panel can be used to control all the non-motor axes. Launch this window using the button with the two button icons on it. The gripper arm can be opened and closed, and the vacuum line can be activated. *Not commonly used: Press C5 empty to turn on the vacuum line for this system.*

Modifying the Configuration File

Again, methods are composed of two parts, a *Configuration* and a set of *Tasks*. You are now going to modify the *Configuration* portion of the method you renamed above.

Configuration This tells the CAPS what is where on the deck of the instrument. Open the configuration file associated with the script you are modifying by selecting *Define Configuration* from the *Define Run* tab. Select *Open an old file*, and press *OK*. Find the file you renamed and open the *.script* file. Select *Open*. A warning will appear -- This file contains defined tasks, do you want to overwrite? -- Select *Yes*.

The window will display a view of the deck, including the Stacker, the Cleaning Positions, and the Platforms. To change what is in each section, you must first click on it -- the bottom half of the window will change to reflect what labware, rinse basins, etc., can be placed in those positions.

Stacker

We do not have the stacker option installed on our CAPS.

Cleaning Positions

There are five wash positions on the left side of the deck. Two are specially modified spots on the deck and three are generic. The special ones are a sonicator and heated fan for drying -- their positions on the deck cannot be modified.

The other wash positions simply consist of basins that need to be manually filled and emptied. A basin of this type corresponds to a "Wash" designation in the pulldown menu. There are 3 wash positions designated in the Wash_Tips method.

Platforms

Platforms hold the labware in specific locations so that the head can be used accurately. They are the platters that the plates rest on. Several kinds of platforms are available -- we only have Type 1 platforms, which have space for 10 plates. These two platforms should be flush against one another and pushed completely to the left of the deck.

Plates

The CAPS can handle rectangular Nunc OmniTrays and other standard microtiter plates. To add plates to the platform, you will need to first select the radio button for *Platform/Stacker* under *Section* to get access to the plates. Selecting a type of plate using the radio buttons at the bottom middle of the screen will show you what plate options exist. For example, clicking the *Gel Plate* radio button will place the standard formats for agar plates into the pulldown menu. Selecting the *Plate* radio button will place the standard microtiter plate formats in the pulldown menu. Once you have selected the labware you want, click on a platform location to place the labware where you want it on the platform.

Wash settings

Set these by clicking on *Washing Proc* and selecting the appropriate order from the pulldown menus. The standard washing procedure is:

Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
C5	C4	Sonice	C3	Dryer	none
Wash Time: 1	Wash Time: 1	Wash Time: 8	Wash Time: 4	Wash Time: 30	Wash Time: 10
Repeat: 5	Repeat: 3	Repeat: 1	Repeat: 0	Repeat: 0	Repeat: 0
Wait Time: 0	Wait Time: 0	Wait Time: 0	Wait Time: 0	Wait Time: 0	Wait Time: 1
C3 <input type="checkbox"/> Refill <input type="checkbox"/> Refill on Repeat?	C4 <input type="checkbox"/> Refill <input type="checkbox"/> Refill on Repeat?	C5 <input type="checkbox"/> Refill <input type="checkbox"/> Refill on Repeat?			

OK Cancel

This wash protocol was optimized by Martin Jonikas and Dale Cameron (Weissman Lab). The original washing protocol was 6'. This was optimized to be a shorter washing protocol, 2.5', using YEPD and SD yeast plates with *S. cerevisiae*. This protocol should be tested and optimized as necessary to ensure efficient cleaning of the tips with other media and organisms. In

particular, at the end of every run, after the final wash, users should pin onto a sterile plate to ensure that nothing grows. This test should be done every time one uses the machine.

As noted later, the washes consist of water, 70% ethanol, and 100% ethanol. The wash solutions should be changed approximately every 10 plates, or one platter. In particular, the first wash (water) gets dirty the most quickly. The washes should be monitored and changed as they become cloudy.

Tools

Click on the tools *Configuration* button to launch the tool selection window. Most choices are not selectable, as we do not have those options installed on our instrument. The one tool that we currently have is a Replicator 384. The box beside this item should be checked.

Lids!

Do not use lids in methods. The gripper's lid removal ability is not reproducible enough to use lids in methods.

Saving

When you are finished laying out the Configuration, click the *Save As* button or simply click "OK" to save the file using the current name. You will use the Tasks interface to tell the software what to do with the described configuration.

Tasks This is a list of what the robotic arm is actually going to do with the plates you have laid out on the platform. Open the tasks file by selecting *Define Tasks* under the *Define Configuration* tab.

Load a Configuration

Select the *.script* file you just saved. Two windows will open, a configuration map (bottom) and a task editor (top). The configuration map should show the plates as you just laid them out in the configuration setup step.

Add Steps

Steps must be added to the method in order. You cannot insert or delete steps from the middle of the method list. Be careful to enter the correct settings for each step -- if you make a mistake early, you will have to go back and delete all the steps after that.

Replicate

A replicate step is used to move cells from one location to a second location. It consists of two dip steps followed by a wash step afterwards. Clicking this button changes your cursor into a black syringe-ish icon. First, click on the Configuration window on the plate to transfer from. The syringe will now turn white. Second, click on the plate to transfer to. Third, adjust the settings in the window that pops up.

Dip Level

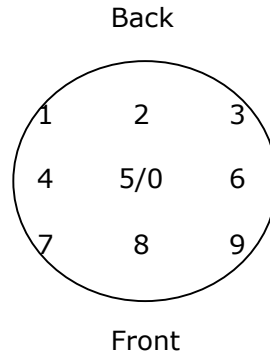
How far down the replicator will go into the plate, in mm.

Duplicate Number

How many times to touch the agar, i.e. 0 will do it just once, 1 will do it twice, and so on.

Dip Number

Where in the colony the robot will pick from. Positions 5/0 correspond to the center of the colony, 1 is the back-left, and 9 is the front-right.



Time Down/Time Up

The time the instrument spends traveling to/from the plate. Between 1500 and 3000ms is the standard time.

Dwell Time

This is the time the instrument spends in contact with the plate. 2000ms is the standard time.

Wash Step?

Check this box to perform the washing protocol that you entered into the configuration when the transfer step is finished.

When you are finished adjusting this parameters, select *OK*.

Dips

A replicate step is really just two dip steps with a wash step afterwards. If you want to do something more complicated, e.g. dip onto more than one destination plate, you can use the individual dip steps instead of a transfer.

In general with *S. cerevisiae*, one dip on a plate can be used to replicate onto three agar plates. This same plate can be dipped two more times. In general, it is probably a good idea to change the dip position (dip number) for each successive dip to insure the tips pick up cells. Thus, one *S. cerevisiae* plate can be used to replicate onto 9 agar plates total.

Washes

If you do use some Dip steps, don't forget to follow them with a Wash step. All washes will use the standard wash configuration that you entered in the Configuration window.

Saving Scripts The tasks are automatically saved when you click the *OK* button in the task editor window. They are saved to the same .script file that houses the configurations. Any changes you make to the configuration will obliterate your tasks.

Running a Script

After you have created a list of tasks for your platform configuration, return to the Console window to run them.

Open Tasks

Use this button to select your .script file. Once the file is open, the list of tasks should be visible in the window in the upper left-hand corner of the window.

Fill the Wash Reservoirs

Before beginning, clean all of the wash reservoirs with warm water. The sonicator cannot be removed fully; simply lift it up, pour its contents into another receptacle, and wipe it out with a damp paper towel. Then fill the reservoirs with the appropriate solutions. The typical wash protocol has the wash solutions in the following order,

BACK OF CAPS

HEATER
SONICATOR FILLED WITH 70% ETHANOL
100% ETHANOL
70% ETHANOL
WATER

Some people like to submerge a sponge in the water wash basin to help shear away cells from the pins. These sponges can be bought from VWR (catalog number 58542-246, 5-1/2" x 3-5/8" x 1-1/8"). They need to be trimmed to fit the basin: ~7.5cm x ~12.5cm x ~1.5cm. You can trim the thickness by cutting each sponge to half its thickness using a scalpel. If you do not use a sponge, some cellular debris may be deposited on the plates – although generally this debris does not grow into a colony. The sponges can be reused for several runs. The sponge should be thoroughly rinsed each time the wash solution is changed.

In general, the tips are sterilized by the ethanol washes. Be sure to wash the tips one round before beginning your protocol to insure that they have been sterilized. After the first plate, the tips are washed after each manipulation, so they will be sterile for all subsequent plates.

Place Labware on Platform

Place all of your plates on the deck in the locations given in the configuration. Be very careful to place the plates pushed into the appropriate corner -- i.e. away from the springs in the position. The springs are not strong enough to force the plate into the corner, so you need to give each a nudge. Also be careful about the orientation of the plates. Always place the plates in the same orientation, e.g. notched edges towards the front. This will insure that the alignment of the pins to the colonies will always be the same.

Command Line

There are a number of commands that are very important for the operation of the CAPS, but have no buttons to send that command to the robot. Therefore, you will need to learn these fun commands.

abort

Stop the motion dead in its tracks. It will also abandon its place in your current list of tasks. You would then need to begin the whole script over again.

ready

Returns the replicator head to the ready position. Useful for getting the gantry out of the way of the plates and the deck.

Note: Using this command can break the pins if there are any obstacles in the way. For example, if the head is in a wash basin when the ready command is executed, the head will move to the ready position using a diagonal move out of the wash basin to its final destination. The pins will bend as they come in contact with the side of the wash basin. This would be catastrophic since the pins are no longer made.

show vel

Show the current speed setting for the robot as a fraction of full speed.

vel ##

Set the current speed to the number input. This command takes a percentage as an argument, not a decimal fraction as the command above. Do not run the machine faster than 30% of maximum speed.

Run

Once everything is set up, to run your script, select *Run Tasks*. Do NOT select Run).

Shutting Down

Simply go through the process for turning on the robot in the reverse order. Also, please turn on the UV light for approximately 10-15 minutes to decontaminate the area for the next user.

Ordering Information

Hand Pinning Tool

V&P Scientific, Inc. (800) 455-0644

VP 408FH

MULTI-BLOT E-CLIP FLOATING PIN REPLICATOR - 96 PINS

\$990

Guide for Hand Pinning Tool

V&P Scientific, Inc. (800) 455-0644

VP 381

LIBRARY COPIER - REGISTERS REPLICATOR TO MICROPLATES

\$75

Nunc Omni-Tray, with lid

Nalge Nunc International

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