How to write a PR-PR script

PR-PR is a high-level language that enables Biologists to more quickly design experiments that utilize liquid-handling robotics.

General guidelines for PR-PR scripts:

- All lines beginning with the pound character '#' are ignored by PR-PR. These lines can contain comments that help explain/document what is being performed in the script.
- Lines may be either tab- or space-separated. For this reason, the separated elements within a line (such as a plate name alias) should not themselves contain tab or space characters (*i.e.*, use "DrinksPlate" rather than "Drinks Plate").
- Variable definitions (*e.g.*, plate name aliases) must precede their use in the script.

A PR-PR configuration file consists of:

- 1. A link to robotic table file. In most instances, table files are created using software distributed with the robot. There may also be a set of ready-made table files to choose from.
- 2. The script itself, consisting of two logical sections: definitions and actions.

Definitions Section

(Experiment) name

The name of the experiment may optionally be specified:

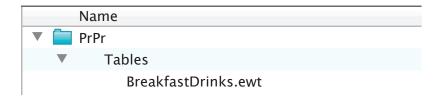
NAME BreakfastDrinks

Table (file)

The name of the robotic table file to use must be specified.

TABLE BreakfastDrinks.ewt

For the stand-alone version of PR-PR, it is necessary to include the (.ewt) table file in the "Tables" folder inside the PR-PR folder:





It is <u>very important</u> to verify that the correct table file is specified for the experiment.

Documentation Section

A documentation section, enclosed by a pair of tripled-quotation mark characters ("""), may be included in the script. This section is operationally ignored by PR-PR (much like lines beginning with the pound character '#'), but can help explain/document what is being performed in the script.

Here is an example documentation section:

```
"""
Recipe for breakfast drinks.
"""
```

Plate (name aliases)

Aliases may be specified for the plate names (that are themselves specified in the robotic table file):

```
# alias name
PLATE DrinksPlate PL4
```



Note: plate name aliases must not contain any space characters.

Locations (sources and destinations)

Locations specify plates and wells. Wells are specified as follows:

- 1. For a single well: by letter-number notation (e.g., "A1") or by number-only notation (e.g., "1").
- 2. For multiple consecutive wells: the first well, then the character '+', and then the total number of wells. For example, "A1+4" indicates the four consecutive wells starting from well A1 (*i.e.*, wells A1, B1, C1 and D1).
- 3. For multiple non-consecutive wells: the wells separated by commas ', ' (e.g., "A1, C1, E1, G1").
- 4. Consecutive and non-consecutive wells may be interspersed (e.g., "A1+4, F1").

A plate and its wells are separated by a colon ':' (e.g., "PL8:A1+4, F1"). Multiple plates are separated by forward slashes '/' (e.g., "PL8:A1+4, F1/PL5:A1, C1, E1"). Plate name aliases may be used instead of the plate name (e.g. "WaterPlate:A1+4, F1" instead of "PL8:A1+4, F1").



Note: locations must not contain any space characters.

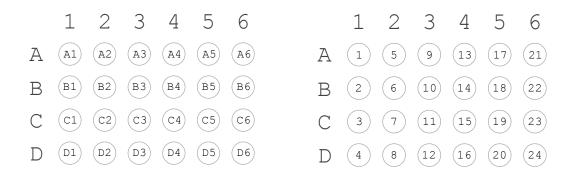


Figure 1. Plate well locations in letter-number notation (left), and in number-only notation (right).

Wells are ordered sequentially first from top to bottom, and then left to right. 'A1+4' (or '1+4'), indicates wells 'A1, B1, C1, D1' (or '1, 2, 3, 4').

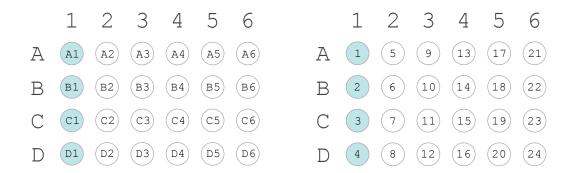


Figure 2. Plate well locations indicated by 'A1+4' (left)or '1+4' (right).

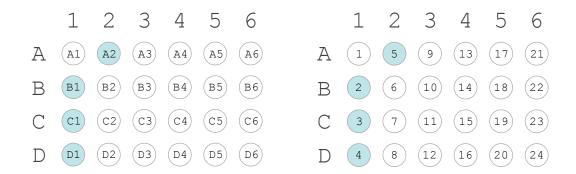


Figure 3. Plate well locations indicated by 'B1+4' (letter-number notation, left) or '2+4' (number-only notation, right). Note that for this particular plate geometry (4 rows by 6 columns), well location A2 (or 5) follows well location D1 (or 4).

Methods

Methods specify the type (or class) of a liquid, as well as the pipetting method for transferring the liquid from one location to another.

The method should be one of the following:

1. LC_W_Bot_Bot	Water, aspirated from the bottom of the well, dispensed to the bottom.
2. LC_W_Bot_Lev	Water, aspirated from the bottom, dispensed to the liquid level.
3. LC_W_Bot_Air	Water, aspirated from the bottom, dispensed in air above the liquid.
4. LC_W_Lev_Bot	Water, aspirated from the liquid meniscus level, dispensed to the bottom.
5. LC_W_Lev_Lev	Water, aspirated from the liquid level, dispensed to the liquid level.
6. LC_W_Lev_Air	Water, aspirated from the liquid level, dispensed in air above the liquid.
7. DEFAULT	LC W Bot Bot; see immediately below.

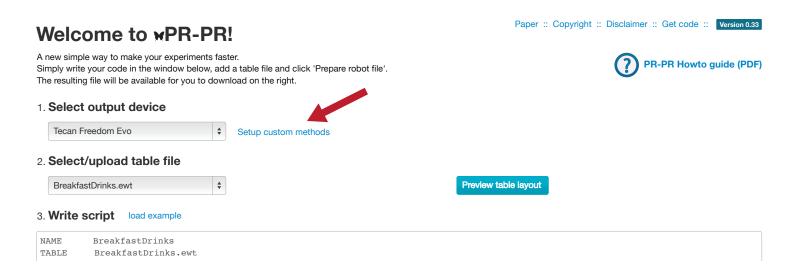
Outside of COMPONENT definition statements (see immediately below), DEFAULT refers to the method specified for the component in its definition statement. If there is no method pre-specified for the component, the method defaults to LC $\,\,$ W $\,$ Bot $\,$ Bot.

Custom methods

In the web version of PR-PR, it is possible to specify the additional (custom) methods to use. They are created and used on a per-experiment basis.

Please note, that PR-PR <u>will not</u> create additional Liquid Classes in your robot settings; you should do it yourself or ask a specialist. This feature is used to tell PR-PR which liquid classes you have (if their names are different from the default ones), so it could validate against them and correct errors in the user script.

To specify additional methods, click on the 'Setup custom methods' link on the PR-PR webpage.



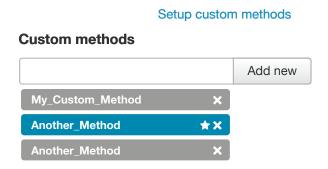
Any methods you enter in the appeared field will be added to the current experiment along with the ones existing by default. Just type in the method name and click 'Add new'.

Setup custom methods

Custom methods My_New_Method Add new My_Custom_Method X Another_Method X

The new method will be added to the list. On the screenshot below you can see the list of custom methods that have been created for this experiment.

To make one of the custom methods default (the one that will be used for components where no method is defined), click on it once. The star icon and blue color will indicate that the method is set to be default.



To delete a method, click on the cross icon on the right side of the method name.



Component(s)

Components may be specified with names, locations, and pipetting methods:

location name method COMPONENT PL8:A1+4,F1 Water LC W Lev Air PL8 Water 8 9 11 12 3 5 7 1 4 6 10 A7 A1 A4 A В7 В1 В4 В6 В8 В C C5 C1 C4 D1 \mathbb{D} E5 E7 E4 \mathbf{E} F F4 G4 G Н7 Η

Figure 4. Component location PL8: A1+4, F1 on the sample plate (8 rows by 12 columns).



Note: component names must not contain any space characters.

Volume (aliases)

Aliases may be specified for volumes (in μ L):

alias volume(uL)
VOLUME DrinkVol 50
VOLUME WaterVol 25



Note: volume aliases must not contain any space characters.

Recipe(s)

Recipes may be defined to alias specific mixtures of components, such as a PCR master mix.

The definition of a recipe begins with a RECIPE line that specifies the name of the recipe as a whole. The lines that immediately follow specify the names and contents of the sub-recipe(s) associated with the recipe (one sub-recipe per line). In the example below, the recipe Drinks has three associated sub-recipes: chai, coffee and lemonade. Each sub-recipe name is followed by a colon ':', which in turn is followed by the contents (specified in pairs of component name or locations, and volume (in µL)) of the sub-recipe. Plate name aliases, components, and volume aliases (after they have been defined) may be used when defining a recipe.

#	name					
RECIPE	Drinks					
#	component1	volume1	component2	volume2	component3	volume3
chai:	TeaExtract	30	Syrup	30	Water	WaterVol
coffee:	BeanExtract	30	Milk	30	Water	WaterVol
lemonade:	LemonJuice	15	PL7:18	45	Water	WaterVol

Actions Section

Options

Options specify what should happen after liquid has been dispensed into a well.

Each option should be one of the following:

1. MIX: volumexrepetitions Aspirate/dispense volume µL repetitions times (e.g. MIX: 25x20)



Note: options must not contain any space characters.

Make (a recipe)

The MAKE action prepares a defined recipe, or sub-recipe(s), at the specified location(s).

Sub-recipes are prepared in separate locations, and thus a location must be specified for each sub-recipe. For consecutive well locations, the sub-recipes are prepared sequentially into consecutive wells.

#	recipe:sub-recipe	location	method	options
MAKE	Drinks	DrinksPlate:A6+3	DEFAULT	MIX:25x20
MAKE	Drinks:coffee,lemonade	DrinksPlate:A1+2	DEFAULT	MIX:30x10



Note: recipe: sub-recipe(s) must not contain any space characters.

Spread (a component)

The SPREAD action distributes a single defined component (or the same liquid present in one or more source locations) to one or more destinations.

#	component	destination	volume(uL)	method	options
SPREAD	Water	PL6:A4+10,A6	DrinkVol	DEFAULT	MIX:25x20
SPREAD	PL4:A1+4	PL6:A4+10,A6	DrinkVol	DEFAULT	MIX:25x20

Transfer (liquids)

The TRANSFER action distributes liquids one-to-one from source to destination locations. A destination location must be specified for each source location.

#	source	destination	volume(uL)	method	options
TRANSFER	PL1:A1+3	PL6:A7+3	150	LC W Bot Bot	MIX:15x8

Protocols

Protocols allow reusing of both definitions and actions, when a lot of similar slightly different actions are needed. As recipes, they are first defined and then called. Protocol is located between the commands PROTOCOL and ENDPROTOCOL. You can use as many definitions and actions as you like.

#protocol PROTOCOL	name MyProtoc		riables Location	MyMethod	d	
<pre># RECIPE # chai: coffee: lemonade:</pre>	name Drinks component1 TeaExtract BeanExtract LemonJuice	volume1 30 30 15	component2 Syrup Milk PL7:18	volume2 30 30 45	component3 Water Water Water	volume3 WaterVol WaterVol WaterVol
	cipe:sub-reci inks OL	pe	location MyLocation		method MyMethod	options MIX:25x20

Protocol is then called by a command USE. The amount of values provided with the command should be the same as the amount of variables in the protocol definition.

#use protocol_name values...
USE MyProtocol PL4:A1+3 LC W Lev Bot

Variables and their values are matched one-by-one, left-to-right:

#protocol	name	variable1	variable2	
PROTOCOL	MyProtocol	MyLocation	MyMethod	
#use	name	value1	value2	
USE	MyProtocol	PL4:A1+3	LC W Lev Bot	

One protocol can be used more than once with different variable values.

value1 value2 #use name PL4:A1+3 MyProtocol LC W Lev Bot USE #use name value1 value2 MyProtocol PL6:B3+3 USE DEFAULT

Putting it all together: an example PR-PR script

#use

USE

name

MyProtocol

NAME BreakfastDrinks BreakfastDrinks.ewt TABLE ** ** ** Recipe for breakfast drinks. alias name PLATE DrinksPlate PL4 name location method COMPONENT Water PL8:A1+4,F1 LC W Lev Air LC W Lev Bot COMPONENT TeaExtract PL7:17 COMPONENT Syrup LC W Lev Bot PL7:18 COMPONENT Milk PL7:19 LC W Lev Bot COMPONENT BeanExtract PL7:20 LC W Lev Bot COMPONENT LemonJuice PL7:21 LC W Lev Bot alias volume (uL) VOLUME DrinkVol 50 WaterVol 25 VOLUME name RECIPE Drinks component1 volume1 component2 volume2 component3 volume3 Syrup chai: TeaExtract 30 30 Water WaterVol coffee: BeanExtract 30 Milk 30 Water WaterVol PL7:18 lemonade: LemonJuice 15 45 Water WaterVol variable1 variable2 variable3 #protocol name MyLocation PROTOCOL MyProtocol MyComponent MyMethod #protocol details MyComponent MyLocation 40 MyMethod SPREAD ENDPROTOCOL recipe:sub-recipe location options method MIX:25x20 MAKE Drinks DrinksPlate:A6+3 DEFAULT Drinks:coffee,lemonade DrinksPlate:A1+2 MIX:30x10 MAKE DEFAULT component destination volume(uL) method options PL6:A4+10,A6 DrinkVol MIX:25x20 SPREAD Water DEFAULT source destination volume(uL) method options TRANSFER PL1:A1+3 PL6:A7+3 150 LC W Bot Bot MIX:15x8

value1

Milk

value2

PL4:A1+3

value3

LC W Lev Bot