## How to write a PaR-PaR script

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

General guidelines for PaR-PaR scripts:

- All lines beginning with the pound character '#' are ignored by PaR-PaR. 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 PaR-PaR 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:

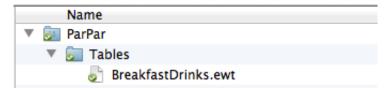
NAME BreakfastDrinks

#### Table (file)

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

TABLE BreakfastDrinks.ewt

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

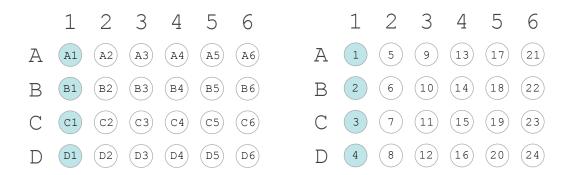
A plate and its wells are separated by a colon ':' (e.g., "PL8:A1+4, F1"). Multiple plates are separated by forward slashes '/' (e.g., "PL8:A1+4, F1/PL5:A1, C1, E1"). Plate name aliases may be used instead of the plate name (e.g. "WaterPlate:A1+4, F1" instead of "PL8:A1+4, F1").

Note: locations must not contain any space characters.

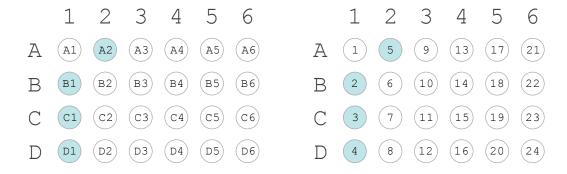


**Figure 1**. Plate well locations in letter-number notation (left), and in number-only notation (right).

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 PaR-PaR, it is possible to specify the additional (custom) methods for your robot. They are created and used on a per-experiment basis.

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

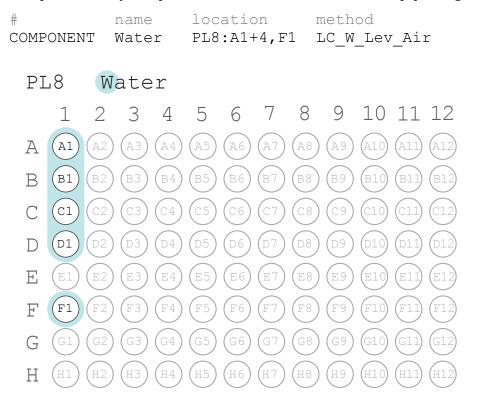


Additional methods you enter in the appeared field will be added to the current experiment. If you need one of your custom methods be the default, just click on it. Method is default when is has a color blue and a star on the right side.



## Component(s)

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



**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  $\mu$ 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  $\mu L$ )) 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: volume x repetitions 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 Bo	t 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.

#protoco.	name MyProtoc		riables Location	MyMetho	d	
# RECIPE # chai: coffee:	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
# r	ecipe:sub-reci rinks	-	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.

#use	name	value1	value2
USE	MyProtocol	PL4:A1+3	LC_W_Lev_Bot
#use	name	value1	value2
USE	MyProtocol	PL6:B3+3	DEFAULT

# Putting it all together: an example PaR-PaR script

NAME BreakfastDrinks BreakfastDrinks.ewt TABLE 11 11 11 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 chai: TeaExtract 30 Syrup 30 Water WaterVol coffee: BeanExtract 30 Milk 30 Water WaterVol lemonade: LemonJuice 15 PL7:18 45 Water WaterVol options recipe:sub-recipe location method DrinksPlate:A6+3 MIX:25x20 MAKE Drinks DEFAULT

MIX:30x10

MIX:25x20

options

options

LC W Bot Bot MIX:15x8

DEFAULT

method

DEFAULT

method

Drinks:coffee,lemonade DrinksPlate:A1+2

component destination volume (uL)

source destination

PL6:A4+10,A6 DrinkVol

volume(uL)

150

MAKE

SPREAD

Water

TRANSFER PL1:A1+3 PL6:A7+3