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:

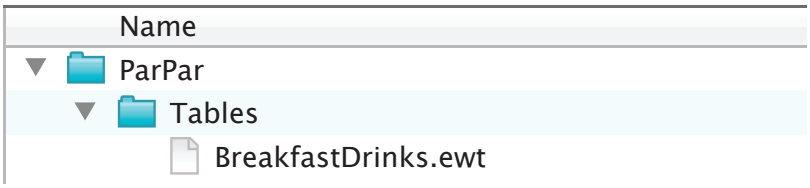
```
NAME      BreakfastDrinks
```

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 very important 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.

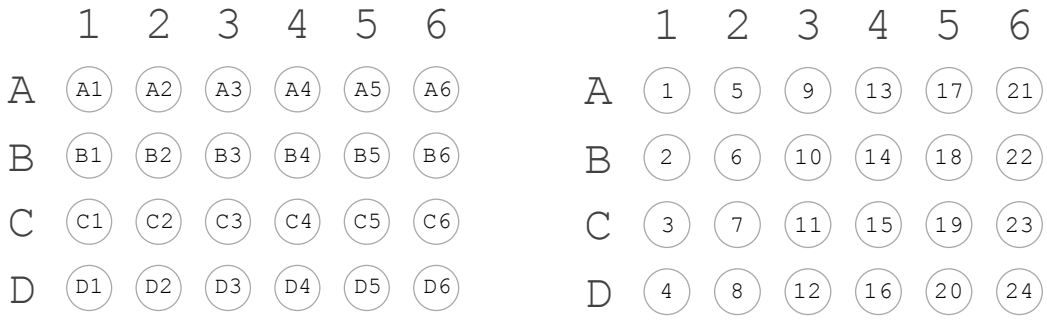


Figure 1. Plate well locations in letter-number notation (left), and in number-only notation (right).

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`').

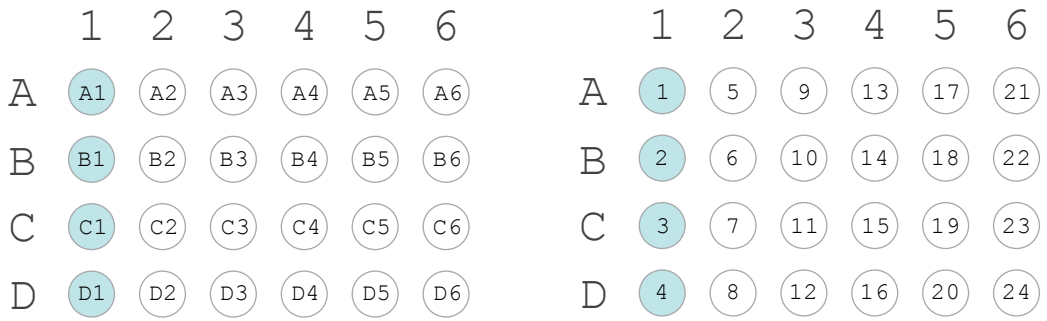


Figure 2. Plate well locations indicated by '`A1+4`' (left)or '`1+4`' (right).

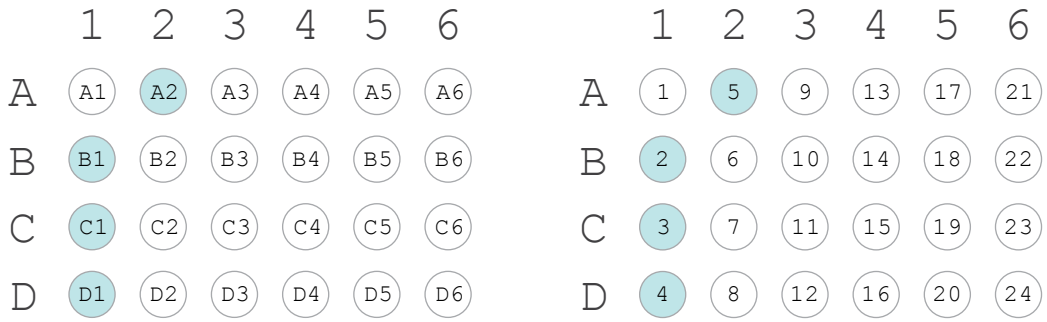


Figure 3. Plate well locations indicated by '`B1+4`' (letter-number notation, left) or '`2+4`' (number-only notation, right). Note that for this particular plate geometry (4 rows by 6 columns), well location A2 (or 5) follows well location D1 (or 4).

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.

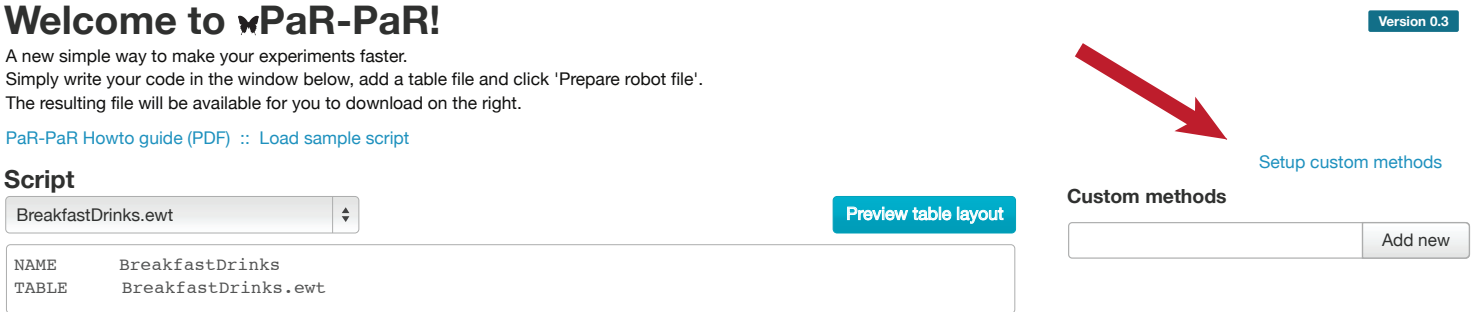
Outside of `COMPONENT` definition statements (see immediately below), `DEFAULT` refers to the method specified for the component in its definition statement. If there is no method pre-specified for the component, the method defaults to `LC_W_Bot_Bot`.

Custom methods

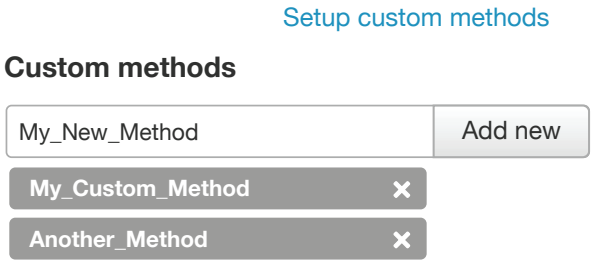
In the web version of PaR-PaR, it is possible to specify the additional (custom) methods to use. They are created and used on a per-experiment basis.

Please note, that PaR-PaR **will not** create additional Liquid Classes in your robot settings; you should do it yourself or ask a specialist. This feature is used to tell PaR-PaR 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.

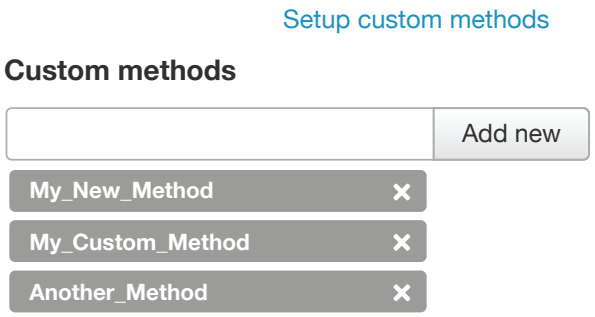
To specify additional methods, click on the 'Setup custom methods' link on the PaR-PaR webpage.



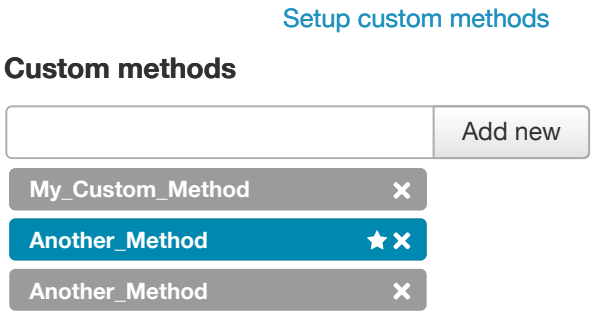
Any methods you enter in the appeared field will be added to the current experiment along with the ones existing by default. Just type in the method name and click ‘Add new’.



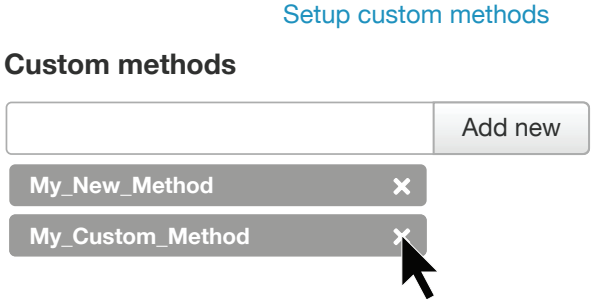
The new method will be added to the list. On the screenshot below you can see the list of custom methods that have beed created for this experiment.



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:

```
#           name     location      method
COMPONENT   Water    PL8:A1+4,F1   LC_W_Lev_Air
```

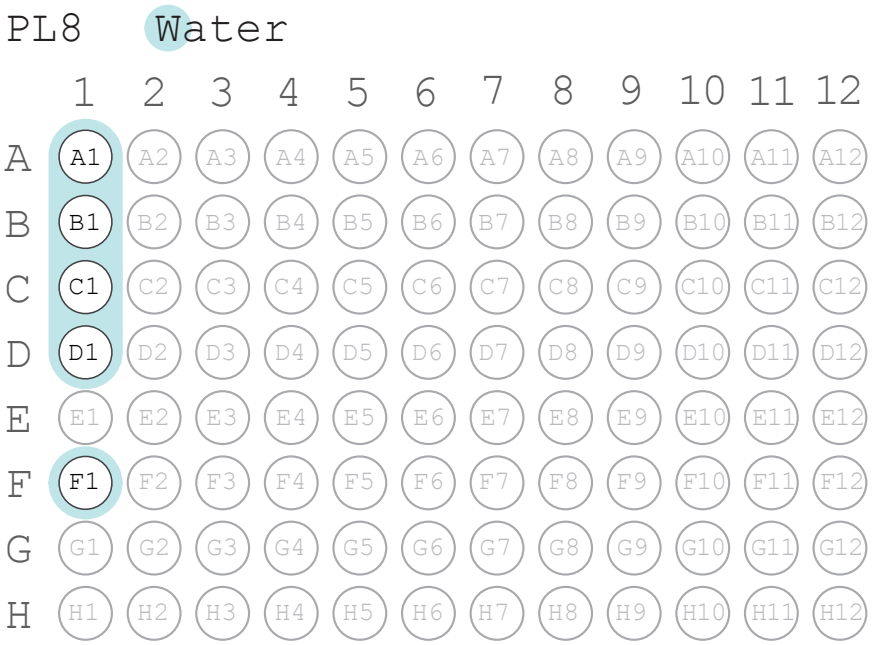


Figure 4. Component location `PL8:A1+4,F1` on the sample plate (8 rows by 12 columns).

Note: component names must not contain any space characters.

Volume (aliases)

Aliases may be specified for volumes (in μL):

```
#         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:`*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_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      name           variables...
PROTOCOL       MyProtocol     MyLocation     MyMethod

#          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

#       recipe:sub-recipe        location           method       options
MAKE    Drinks                   MyLocation         MyMethod     MIX:25x20
ENDPROTOCOL
```

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
TABLE     BreakfastDrinks.ewt

"""
Recipe for breakfast drinks.
"""

#         alias         name
PLATE     DrinksPlate   PL4

#         name          location     method
COMPONENT Water         PL8:A1+4,F1  LC_W_Lev_Air
COMPONENT TeaExtract    PL7:17       LC_W_Lev_Bot
COMPONENT Syrup         PL7:18       LC_W_Lev_Bot
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
VOLUME    WaterVol      25

#         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


#protocol     name           variable1      variable2     variable3
PROTOCOL      MyProtocol     MyComponent    MyLocation    MyMethod
#protocol_details
SPREAD    MyComponent      MyLocation      40       MyMethod
ENDPROTOCOL


#      recipe:sub-recipe       location           method       options
MAKE   Drinks                  DrinksPlate:A6+3   DEFAULT      MIX:25x20

MAKE   Drinks:coffee,lemonade  DrinksPlate:A1+2   DEFAULT      MIX:30x10

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

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

#use          name          value1     value2        value3
USE           MyProtocol    Milk       PL4:A1+3      LC_W_Lev_Bot
```