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:

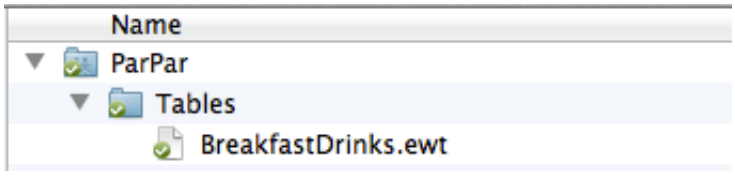
```
NAME      BreakfastDrinks
```

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.

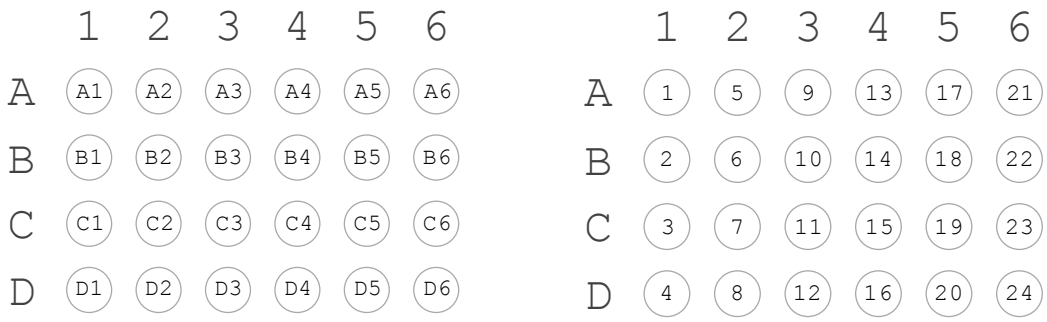


Figure 1. Plate well locations in letter-number notation (left), and in number-only notation (right).

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`').

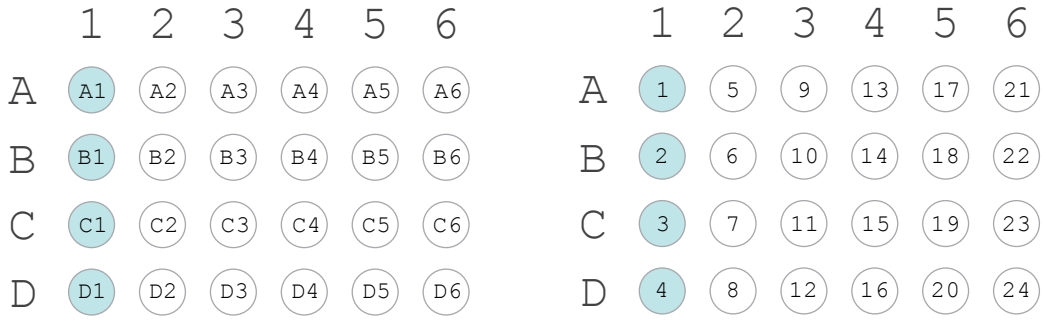


Figure 2. Plate well locations indicated by '`A1+4`' (left)or '`1+4`' (right).

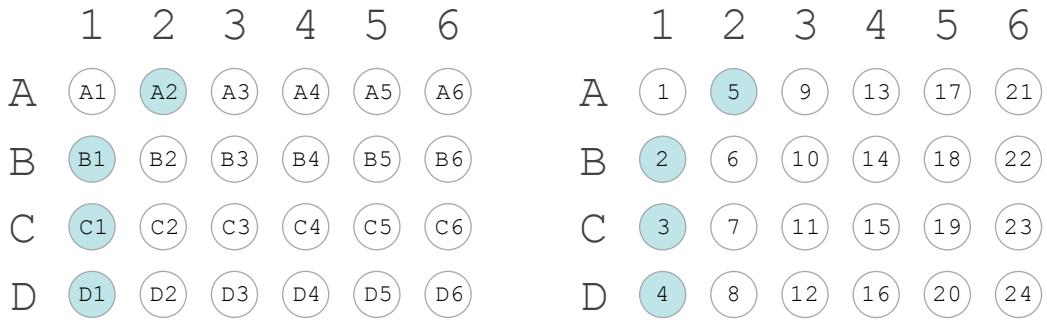


Figure 3. Plate well locations indicated by '`B1+4`' (letter-number notation, left) or '`2+4`' (number-only notation, right). Note that for this particular plate geometry (4 rows by 6 columns), well location A2 (or 5) follows well location D1 (or 4).

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_Lev_Bot` Water, aspirated from the liquid meniscus level, dispensed to the bottom.
3. `LC_W_Lev_Lev` Water, aspirated from the liquid level, dispensed to the liquid level.
4. `LC_W_Lev_Air` Water, aspirated from the liquid level, dispensed in air above the liquid.
5. `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`.

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
```

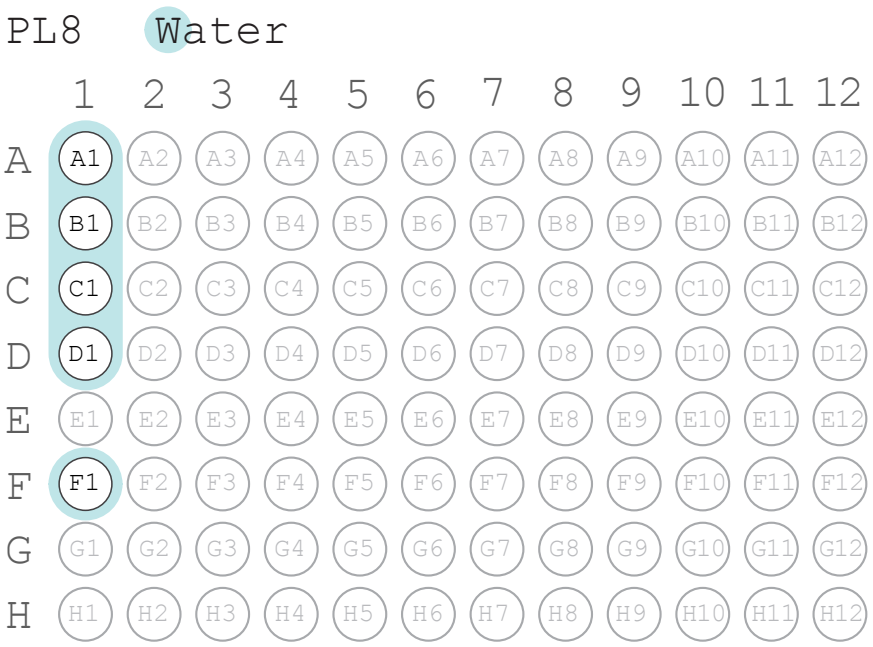


Figure 4. Component location `PL8:A1+4,F1` on the sample plate (8 rows by 12 columns).

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
```

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

#      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
```