HELP

How to use BasyLiCA 1.0

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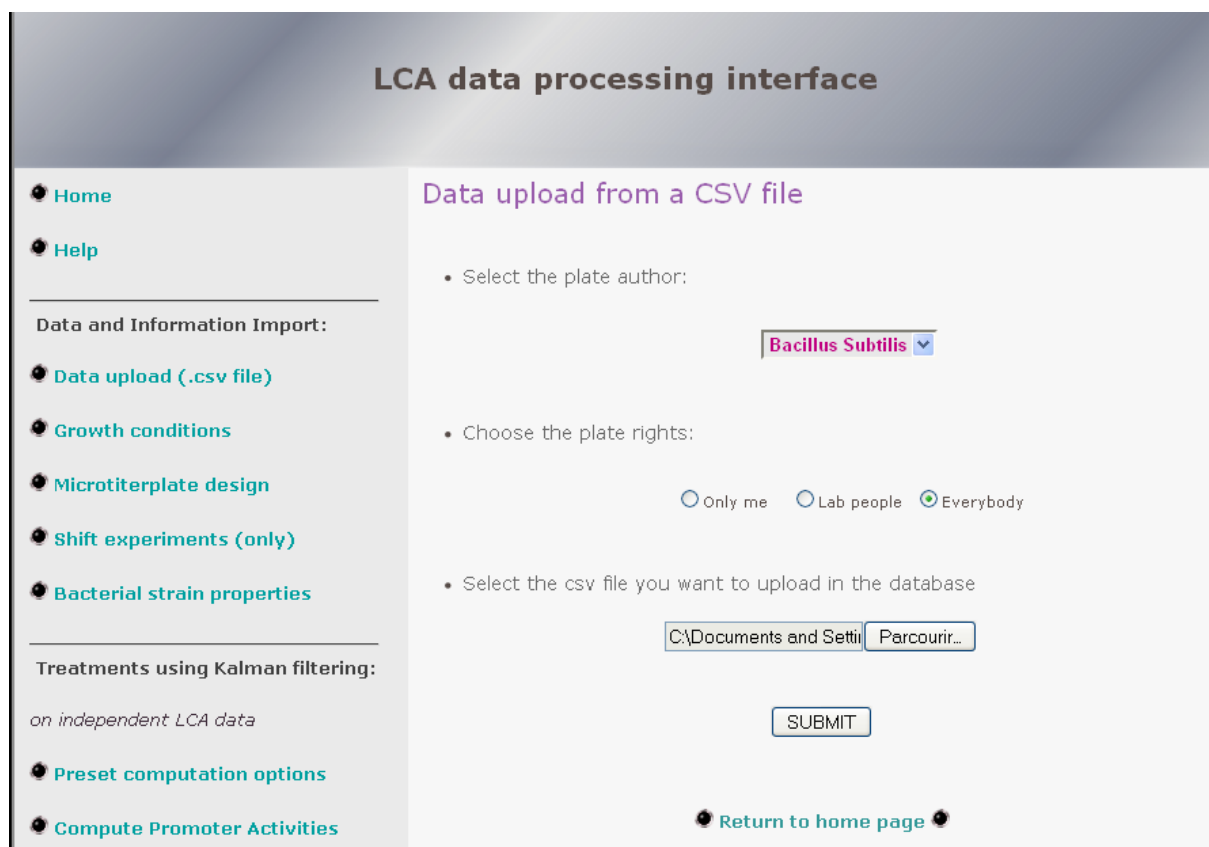# Table of figures

1. Data and information import

This section details the data and information upload procedures in BasyLiCA. Note that the user must import the data and related information into BasyLiCA in the following order:

a. **Data upload (.csv file):** upload LCA data from a CSV file built as the given .csv template file.
b. **Growth conditions:** set information related to growth conditions, media…*etc*.
c. **Microtiterplate design:** set information related to the experimental design of 96-well microtiter plate.
d. **Shift experiments (only):** set information related to an injection of a secondary compound if relevant or directly go to the next step.
e. **Bacterial strain properties:** set information related to the strain characteristics

a. Data upload (.csv file)

This part is devoted to the insertion of the CSV file. The user must define (Figure 1):

- The plate authorship: The authors displayed in the list corresponds to the first name and the last name of the users in the database (see Figure 19)
- The plate rights (Only me/Lab people/Everybody)
- The location of the CSV file on the present computer

Figure 1: Data upload from a csv file

File format:

To be correctly imported and stored in BasyLiCA, the filename must be formatted as displayed on the Figure 2:

1 letter: "**P**" for plate

2 character combinations of double-digit: "**04-07-11**" for the experiment day in American format

1 letter: "**U**" for user

1 digit: "**1**" corresponding to how many times the identical microtiter plate is being assayed in the same conditions

P04-07-11U1FGPrt1.csv

Comma-Separated Variables file extension

1 digit: "**1**" stands for the part number being loaded

3 letters: "**Prt**" stands for part (of importance when your files are split into two (or more) parts due to the experimental design)

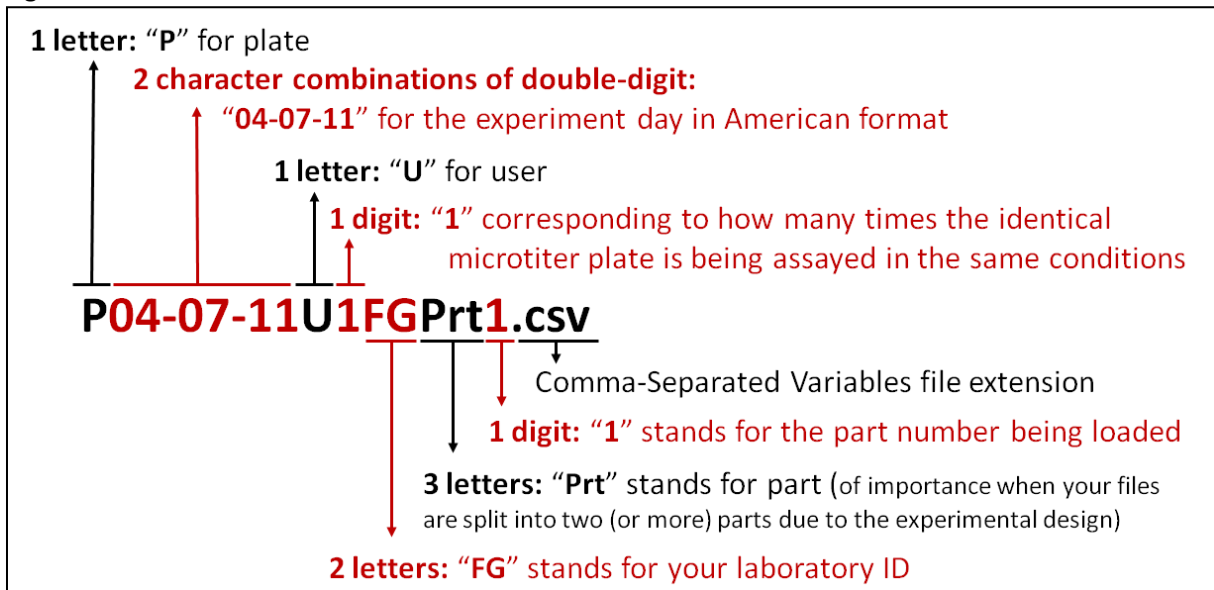**2 letters:** "**FG**" stands for your laboratory ID

Figure 2 : The file name is a concatenation of several pieces of information. In black are the characters (letters or digits) that cannot be changed; in red the variables that the user must set. Please note that if for an experiment you generated 2 files chronologically following each other, BasyLiCA may load them separately and then automatically assembles the related datasets. You simply have to first import the "Prt1" file followed by the "Prt2" file.

N.B. The plate identifier (plate ID) used by the database to store and query information is built from the file name and the plate will be referred by its ID for all further treatments. From the previous example, the corresponding plate ID becomes "**P04-07-11U1FG**".

➢ The format of the file to be uploaded must strictly correspond to the example file provided with the database.

The file must contain 5 tables:

- Optical Density (OD), named OD600. ➔ **1 table**.
- Fluorescence (GFP), named GFP. ➔ **1 table**.
- Initial OD600 and absorbance at 900, 977 nm measurements for each well, named ODi600, ODi900, ODi977, respectively. ➔ **3 tables**.
- The file content must end by the word **END**.

The names (OD 600, GFP, ODi600, ODi900, ODi977, END) are used as separators in the software. The BasyLiCA database will refuse any misspelling and is case-sensitive. Note that the values must be numbers and different from zero.

N.B. Be aware of the following points:

- The CSV format (Comma Separator Values), depends on local parameters, the column separator is by default the comma or semicolon. The semicolon must be chosen.
- In the data tables, NULL values (*i.e.* missing data in a field) are allowed. However, all filled-in fields must have the same number of kinetic data points and cannot only be partially filled in.
- The time format is in international standard notation, hh:mm:ss for hours:minutes:seconds (Be careful that if you generate your files with excel, although data can be visible as **XX**:XX:XX, they might be saved in the .csv format as **X**:XX:XX).

Plate rights:

The options: "Only me" prevents access to the given microtiterplate related data from any other users (even a super-user).

"Lab people" similarly prevents access from anyone who does not belong to the lab user (even a super-user).

"Everybody" gives access to the plate related data to everyone.

b. Growth conditions and plate characteristics

Figure 3: Plate characteristics

Figure 3 shows the fields describing the plate characteristics. Some fields are mandatory (*) while others are optional.

- Choose in the list the plate ID,
- Tick off and/or fill in the different fields with respect to their respective units.

If the field "plate opening" is set to "yes", it means that the lid of the plate has been removed (once or many more times) to allow injection of any compound. Such fields are meant for standardization purposes.

c. Microtiterplate design

In order to facilitate the storage of data related information, BasyLiCA proposes two possibilities to register information associated with each well of the microtiter plate (see Figure 4):

- by either copying the well characteristics of a plate, which has already been inserted in the database, later on called the "reference plate".
- or by defining new well characteristics from scratch.

Figure 4: Two choices to design the well characteristics

You can combine the two options by copying information associated with several wells from a reference plate and by defining others from scratch. Note that for one specific well, you cannot only copy a piece of information, so that the new well related information will become identical to that of the reference plate.

Well characteristics from a reference plate:
When choosing to copy information from a reference plate, the user only needs to select the reference plate ID, the new plate ID and the wells of the reference plate that are identical to those of the new plate by ticking them off (see Figure 5).

Figure 5: Well characteristics from a reference plate

Well characteristics from scratch:

When choosing to start from scratch, the user needs to define all the well characteristics (see Figure 6); mandatory fields are indicated by (*):

- **Plate ID**
- **Validation** of the well: this option is meant to store information related to the strain being grown in the well and the type of experiment being performed (*i.e.* "the strain in this well has been validated" means that users can genetically identify the strain, which is opposed to "the strain has not been validated" in the case for example of a collection screening after mutagenesis for which strains will be identified, sequenced or validated afterward).
- **Strain ID:** the name of the strain in the well
- **Background** well: background wells are used to correct for the natural fluorescence stemming either from the plate or the micro-organism being grown. Usually background wells are the wells in which strains without any fluorescent markers were grown.
- **Set of replicates:** if the well belongs to a set of replicates, the name of the set (*e.g.* R1) is defined. By default, the name is "UX" where X refers to the well number (*i.e.* U1 to U96 for the 96 wells of the microtiterplate).
- **Medium:** name: *e.g.* M9
- **Volume** of the well in μL
- **Product/Concentration:** the name of the major carbon source (such as glucose or a mixture of carbon source such as casein hydrolysate) added to the medium and its concentration
- A second product or carbon source of interest can be defined

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Figure 6: Well characteristics from scratch

Then, the user must select all the wells having the same characteristics, *i.e.* the biological replicates (see Figure 7). On Figure 7, the wells B9, C9, E9, F9, G9 are replicates and then automatically get the same characteristics. Note that you can only tick off wells for which cells exhibited growth and fluorescence; others were automatically discarded.

Figure 7: Well characteristics from scratch (2)

d. Shift experiments (only)

In the case of transition experiments including the addition of one (or more) compound(s) such as drugs or alternative substrates, BasyLiCA also proposes two possibilities to fill in information related to the injection (*e.g.* injection time, compounds and concentrations…, see Figure 4):

- by either copying the injection characteristics of a reference plate, which has already been inserted in the database.
- or by defining new injection characteristics from scratch.

BasyLiCA let you free to combine the two options by copying the injection characteristics of several wells from a reference plate and by completing the others from scratch. Note that if you choose to copy the injection-related information from a "reference plate", you cannot only copy a part of it. The "new plate" resulting injection will be identical to that of the reference plate.

Figure 8: Two choices to define the injection characteristics

Injection characteristics from a reference plate:
When choosing to copy information from a reference plate, the user needs to identify the:

- **Reference plate ID**
- **Injection number** of the reference plate that you want to duplicate (*e.g.* there are two injections (different added products or different times of injection) recorder for the reference and you can duplicate only the first or second injection, or both injections).
- **New plate ID**
- **Injection number** of the new plate (Identically as for the reference plate, you may have performed several injections in your newly recorded plate).
- **Wells-related checkbox table:** tick off the wells of the reference plate for which the injection characteristics are identical to those of the plate you are creating (see Figure 9).

Note that the injection characteristics will be identical for both plates. Otherwise, the user has to define the injection characteristics from scratch.

Figure 9: Injection characteristics from a reference plate

Injection characteristics from scratch:
When choosing to fill in injection-related characteristics from scratch, the user needs to specify the following information (see Figure 10, mandatory fields are indicated by *):

- **Plate ID**
- **Current injection number**
- **Injection time** in international standard notation, hh:mm:ss (for hours:minutes:seconds)
- **Injected volume** in µL
- **Injected product:** the name of the injected product
- **Product concentration** in g/L
- **Wells related checkbox table:** tick off the wells of the reference plate exhibiting identical injection properties

Figure 10: Injection characteristics from scratch

e. Bacterial strain properties

BasyLiCA may store a large amount of information related to the strains being analyzed. Therefore, we recommend users to properly fill in the following fields (mandatory fields are indicated by *) corresponding to the bacterial strain characteristics (see Figure 11 and Figure 12):

- **Strain author:** to who belongs this strains
- **Strain ID:** the identifier of the strain, which has to correspond to the strain ID in the well characteristics (field "Strain ID" on Figure 6).
- **Clone number:** (a letter) referring to the clone (in case of biological replicates, for which strains were created or obtained by two different means).
- **Gene name:** the name of the gene of interest (Figure 13A., gene of interest being either *gene A, B* or *C*)
- **Strain rights:** who can access the strain related datasets.
- **Strain status:** official/non official
- **Fusion promoter:** the name of the promoter of the reported gene (Figure 13A., the gene of interest can be *gene B*, but the fusion can be placed upstream promoter of *gene A*, so the fusion promoter name could "*gene B*").
- **Fusion type:** 5'/3' defines the location of the fusion (*i.e.* 5' Figure 13B. and C. *versus* 3', Figure 13D.)
- **Gene reporter:** the gene name of the fluorescent protein (Figure 13A., the *xfp* gene)
- **Laboratory ID:** defines the laboratory to which belongs the strain

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- **Sequencing:** if the strain has been sequenced or not
- **Ectopic locus:** name of the locus where the fusion is inserted (if it is not at the reported gene locus)
- **Recombination sequence**
- **Strain validation:** "validated" means that the strain is able to grow and to express the fluorescent protein.
- **General comments**
- **Background:** the master strain (e.g. *B. subtilis* 168, which is a widely used background for *B. subtilis*)
- **Fluorescent protein fusion reporter:** the name of the fluorescent protein if different from its corresponding gene name (*e.g.* GFP, YFP)
- **Translational fusion protein:** (for translational fusion only, Figure 13E.), the name of protein with the translational fusion
- **Fusion comments**

Figure 11: Strain characteristics

Figure 12: Strain characteristics (2)

A. RBS RBS RBS Terminator P₁ gene A gene B P₂ gene C

B. RBS RBS P₁ xfp P₁ gene A

C. RBS RBS Terminator P₂ xfp P₂ gene C

D. RBS RBS Terminator P₂ gene C xfp

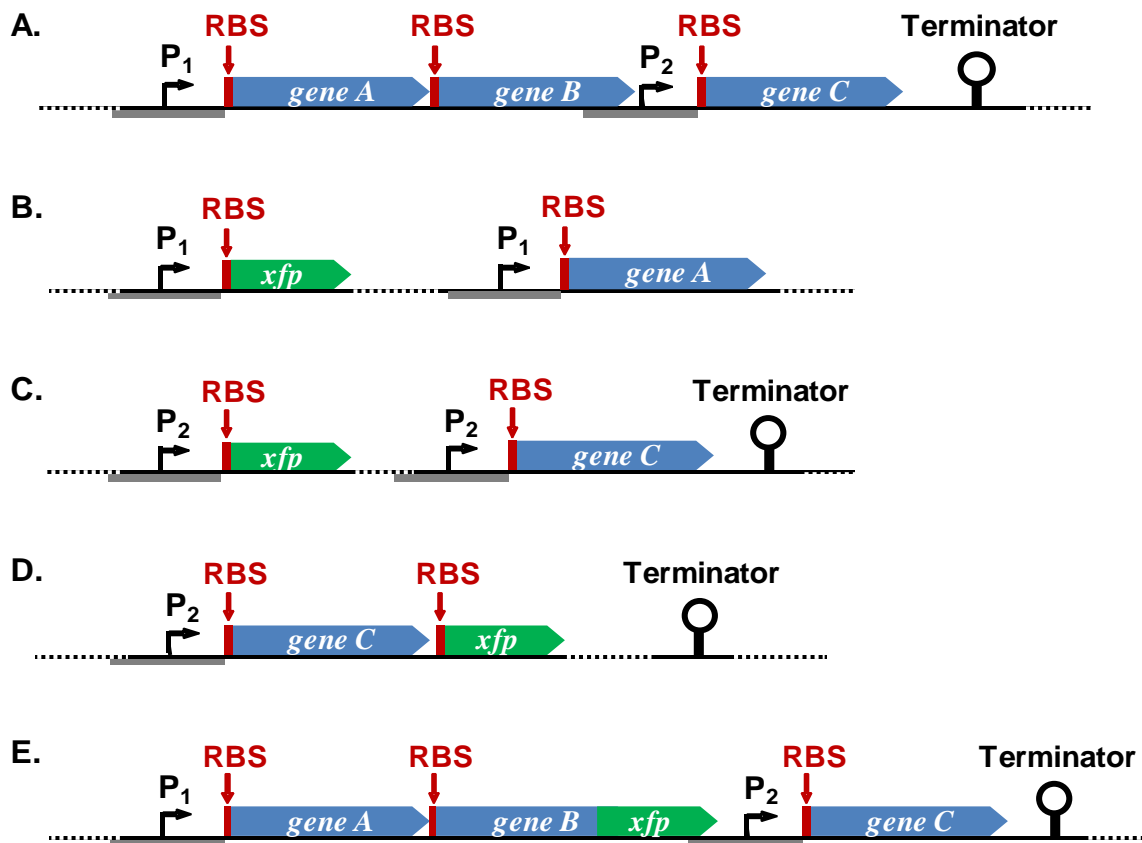**E.** RBS RBS RBS Terminator P₁ gene A gene B xfp P₂ gene C

Figure 13 : Bacterial genomic organisation (A.) illustrating the possible transcriptional (B., C., D.) and translational (E.) fluorescent (*xfp* gene for *gfp*, *yfp*…) **fusions. A.** The *ABC* operon is composed of 3 genes (*A*, *B* and *C*), each of them having their own RBS. The expression of the entire operon is controlled by a promoter, P_1, and terminated by a terminator loop. Besides, there is an internal promoter, P_2, which can also be switch on under some conditions. **B.** The transcriptional fusion is placed under the control of the P_1 promoter and integrated either at an ectopic locus or at the *gene A* locus. **C.** The transcriptional fusion is placed under the control of the P_2 promoter and integrated either at an ectopic locus or at the *gene C* locus. When inserted at the locus, the *ABC* operon is disrupted. **D.** The transcriptional fusion is placed downstream *gene C* and reflects the combined expression mediated by both P_1 and P_2 promoters. **E.** Expression of the translational fusion between *gene B* and *xfp* is under the control of the P_1 promoter.

2. LCA data treatment using Kalman filtering

LCA data are treated by discrete Kalman filter to compute the promoter activity from OD and fluorescence curves. Two versions of the discrete Kalman filter are proposed.

- The first one considers the LCA data independently from each other. The Kalman filter is applied to each well leading to the estimations of OD, fluorescence, and growth rate and promoter activity for each well.
- The second one uses the biological replicates indicated on the plate. The optical density and the growth rate are estimated for each replicate. Then a common promoter activity taking into account all replicates is estimated from the fluorescence curves and the estimated OD.

For both filters, the treatment occurs in two steps: 1- a preset computation and 2- a final computation. The preset computation must be executed before proceeding to the final computation otherwise a warning message is displayed. During the preset computation step, the wells that do not exhibit correct growth properties (later on called "bad wells" as opposed to "good wells") are identified. The final step then allows better estimation of promoter activities after removal of those bad wells. However the final computation is not required and users may directly utilize the data generated after the preset computation step.

a. Preset computation options

To proceed to the preset computation, the user must set:

- The plate to analyze in the menu from the plate ID.
- The sampling time in minutes of the filter. Here the user should choose the sampling time of the LCA data.
- The smoothing degree and the data quality with 2 sliders (Figure 14).

Figure 14: Preset computation options for discrete Kalman filter on independent LCA (this interface is identical to the one of replicated LCA data)

At the end of the preset steps, the user can download a pdf file and a CSV file containing the raw and estimations of the promoter activities, the growth rate, OD and fluorescence for every well.

b. Compute promoter activities on LCA data

In the final computation of the promoter activities, the user can discard the bad wells identified during the preset computation step by ticking them off (see Figure 15). As for the preset computation step, to proceed to the final computation, the user must set:

- The plate to analyze in the menu from the plate ID
- The sampling time of the filter
- The smoothing degree and the data quality with the 2 sliders

Figure 15: Final computation of promoter activities on replicated LCA data (this interface is identical to the one of independent LCA data)

When using replicated LCA data, a table summarizing the set of replicates of your plate is displayed (see Figure 16).

Figure 16: Set of replicates

At the end of the computation steps, the user can download a pdf file and CSV files containing the raw and estimations of the promoter activities, the growth rate, OD and fluorescence for all wells.

3. Plate visualization and rights

a. Plate layout

A picture of the plate summarizing its main characteristics can be produced (see Figure 17) and provide the following information:

- Well number
- Strain
- Gene name
- Medium
- Product/Carbon source…
- Injected product

The plate is identified by its ID, which is defined by the CSV filename the user uploaded. You can choose the background color if you want to print it.
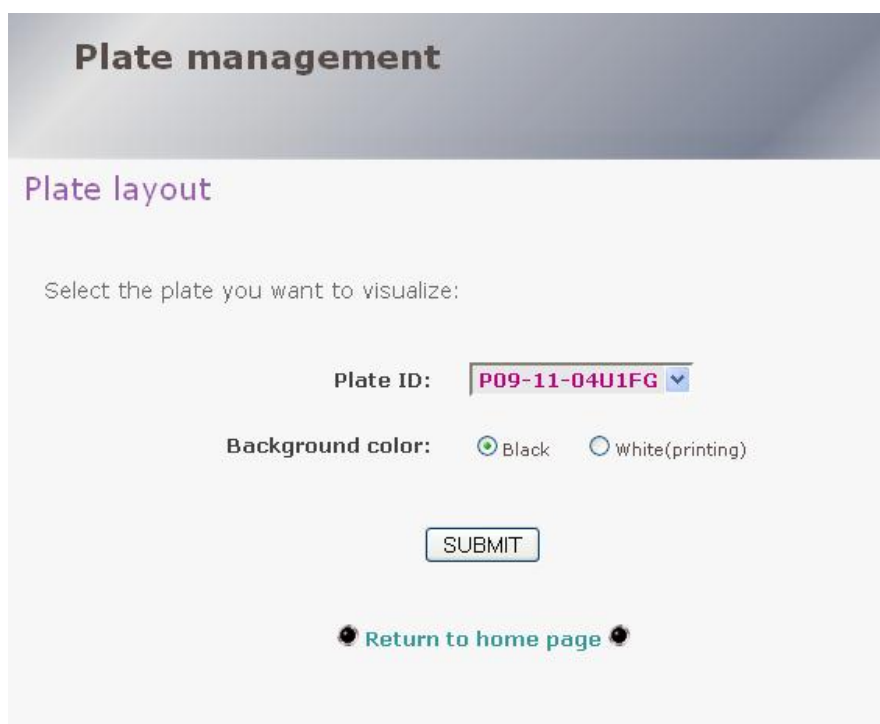

Figure 17: Plate layout

b. Change plate rights (to restrict accessibility)

This section allows you to change the rights of a plate (see Figure 18). The options:

"**Only me**" prevents access to the given microtiterplate related data from any other users (even a super-user).

"**Lab people**" similarly prevents access from anyone who does not belong to the lab user (even a super-user).

"**Everybody/Public**" gives access to the plate related data to everyone.

Figure 18: Change plate rights

4. Administrator part (for super user only)

This section is only available for users with administrative rights on BasyLiCA.

a. Add or delete a user

You can add a user (see Figure 19) by defining the new user login, password, first name, last name, email and laboratory. The administrator must define the user rights, either limited user, either super user (or administrator). All the fields are mandatory.

Pay attention to the rights of the user. An administrator (or super-user) can delete or add plates, strains or users while a simple user can only enter a plate and the associated information (well, injection, clone) and launch computations.

Figure 19: Add a user

Figure 20: Delete a user

A super-user can delete any other user (see Figure 20). **Be careful:** if a user is deleted from the database, its own plates will not be accessible anymore!

b. Change users rights

Here you can change the user rights (see Figure 21) (limited user or super user). **Be careful:** do not remove the rights to all super-users!

Figure 21: Change user rights

c. Delete a plate or a strain

You can delete a plate (see Figure 22) or a strain (see Figure 23) from the database based on their ID.

Figure 22: Delete a plate

Figure 23: Delete a strain

NB1: if a plate is deleted, all the characteristics related to the plate are also deleted (*i.e.* wells and injection characteristics).
NB2: If a strain is used in a plate, it cannot be deleted.

5. General remarks - FAQ

- **Edge well convention:** note that due to evaporation of the wells on the periphery of the plate (A1 to A12, H1 to H12 and B1, C1, D1, E1, F1, G1, B12, C12, D12, E12, F12, G12) must be empty (without a strain).
- **Output format:** All graphics generated can be downloaded in pdf format, and the corresponding datasets in CSV file. The picture of the plate can be downloaded in PNG format (click with the right button on the picture, "save as the picture" to download it)