



Flo400 :

Users guide



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Foreword:

This high throughput lab automaton is made for ***in situ* hybridization, immunohistochemistry and fluorescent *in situ* hybridization** onto wholemount samples (400µm < X < 3cm) or Slide samples. You can also use this automation for clearing samples.

This lab automaton allows reagent cycling. It loops RNA and antibodies probes to reduce background noise and probe consumption.

This automaton is universal on all reagents, however we advise to use RNaseZap or similar for washing the device and stay in RNA free conditions.

Lexicon

Flowcell: Flow cells are the quartz tubes in which samples are contained during the experiment, there are 4 of them with the Flo 400 and they are called F1, F2, F3, and F4 in the software.

Tankers: Tankers are the different containers of the solutions used during protocols. There are 10 of them into the automaton (labeled from 1 to 10 and can contain different volumes (50, 500 or 2000 mL). Tankers 1 and 2 can be recycled: solutions are following a cycling current from tanker to flowcell to tanker to flowcell etc...

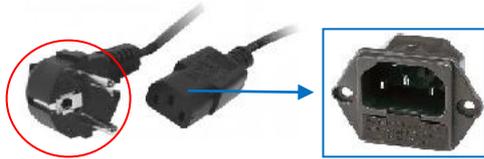
Probes: Probes defining the containers of RNA or Antibodies probes. There are 4 of them, and each one is specific to a Flowcell (P1 for F1, P2 for F2 etc...) As Tanker 1 and 2 they can be recycled.

Reagents: Reagents define the Solution used for the protocol.

Waste: It's the recipient for all chemical waste used in the experiment, It has a total volume of 2000 mL

1. Start-up:

Connect the automaton to outlet with the power cord; the end in the red circle has to be connected to outlet and the other end has to be connected to the UPS outlet at the back of the automaton.



Once the automaton connected, start the robot via the power button O/I accessible over the connection at the back of the automaton.

2. Wifi connection:

The connection to the software via your laptop is a wifi connection. To connect you, you need to search the different wifi networks with your laptop and connect you to the Flo-XXX network. You can so connect to the automaton with the wifi key.

3. Login :

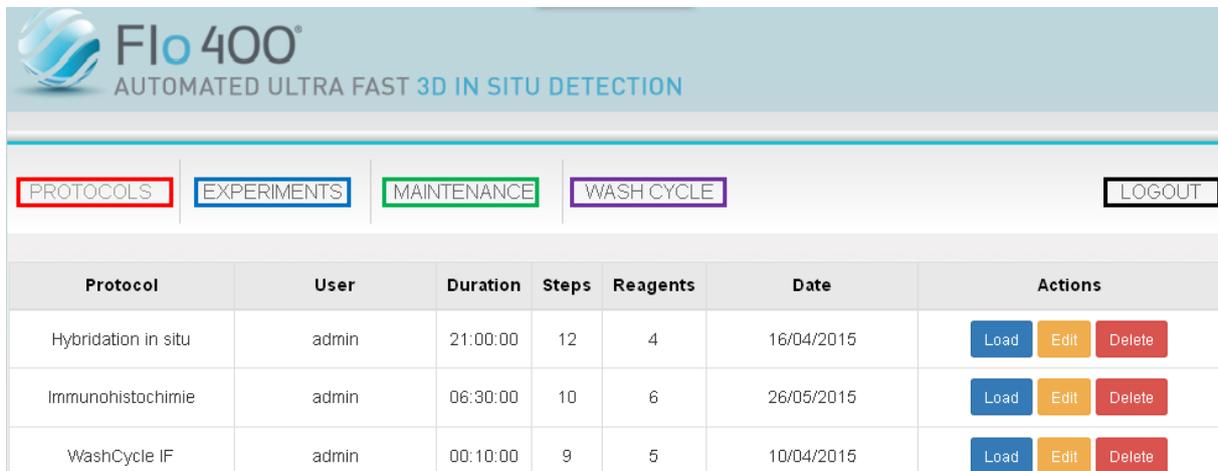
Before use, you need to be connected to the automaton's wifi and going to the URL of the website. The website is optimized for 3 different web browsers: Google Chrome  , Internet Explorer  and Mozilla Firefox .

To access the software and save data, log in via your login and password.



Welcome into Flo400's programming software.

A message "successfully logged in" appears. You are then redirected to the database of experiments.



The screenshot shows the Flo400 software interface. At the top, there is a header with the Flo400 logo and the text "AUTOMATED ULTRA FAST 3D IN SITU DETECTION". Below the header is a navigation menu with four tabs: "PROTOCOLS" (highlighted in red), "EXPERIMENTS" (highlighted in blue), "MAINTENANCE" (highlighted in green), and "WASH CYCLE" (highlighted in purple). To the right of the tabs is a "LOGOUT" button. Below the navigation menu is a table with the following columns: Protocol, User, Duration, Steps, Reagents, Date, and Actions. The table contains three rows of data:

Protocol	User	Duration	Steps	Reagents	Date	Actions
Hybridation in situ	admin	21:00:00	12	4	16/04/2015	Load Edit Delete
Immunohistochimie	admin	06:30:00	10	6	26/05/2015	Load Edit Delete
WashCycle IF	admin	00:10:00	9	5	10/04/2015	Load Edit Delete

At the top of the page, you can access to the software menu including different tabs:

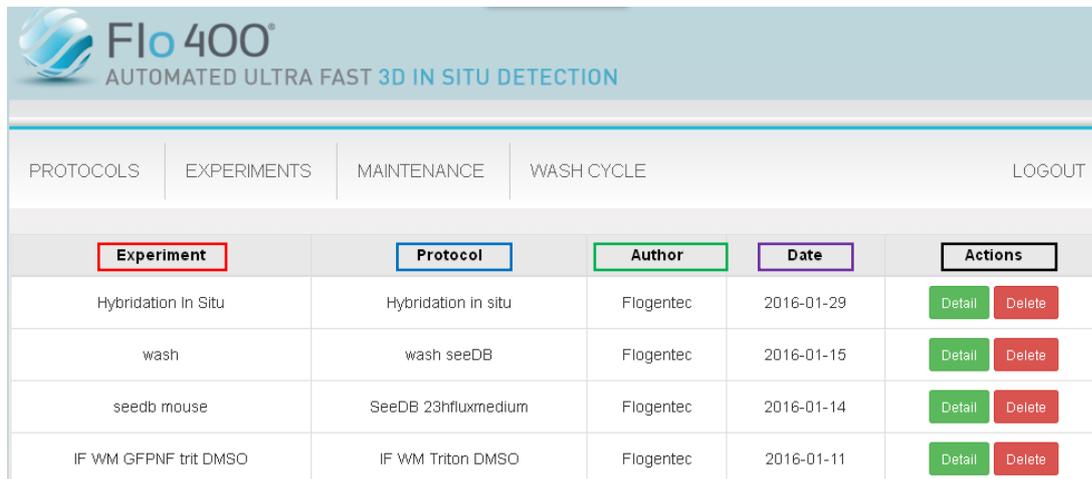
- **Protocols** (access to the database of protocols cf p)
- **Experiments** (access to the database of experiments cf p)
- **Maintenance** (access to the maintenance system of the automaton)
- **Wash Cycle** (access to the automaton washing protocol)
- **Log out** (to quit the software)

4. Software navigation:

1. Run an experiment:

i. Experiment Database:

The table below lists the different experiment saved in the automaton. Each new experiment runs on the automaton will be add to this table.



The screenshot shows the Flo 400 software interface. At the top, there is a header with the Flo 400 logo and the text "AUTOMATED ULTRA FAST 3D IN SITU DETECTION". Below the header, there is a navigation bar with tabs for "PROTOCOLS", "EXPERIMENTS", "MAINTENANCE", "WASH CYCLE", and "LOGOUT". The "EXPERIMENTS" tab is selected. Below the navigation bar, there is a table with the following columns: "Experiment", "Protocol", "Author", "Date", and "Actions". The table contains four rows of experiment data.

Experiment	Protocol	Author	Date	Actions
Hybridation In Situ	Hybridation in situ	Flogentec	2016-01-29	Detail Delete
wash	wash seeDB	Flogentec	2016-01-15	Detail Delete
seedb mouse	SeeDB 23hfluxmedium	Flogentec	2016-01-14	Detail Delete
IF WM GFPNF trit DMSO	IF WM Triton DMSO	Flogentec	2016-01-11	Detail Delete

The column **Experiment** gives you the name of the experiment.

The column **Protocol** gives you the name of the protocol used during this experiment.

The column **Author** gives you the name of the author of the experiment.

The column **Date** indicates when the experiment was run.

The column Action allows you to:

- Access to the selected experiment (**Detail** button)
- Delete an experiment (**Delete** button)

To run an experiment saved in the automaton, clic on the detail button next to the desired experiment.

ii. Detail of an experiment:

Up of the page, the software's breadcrumb (steps processing) allows you to access to the different steps of experiment setting:

- Configuration cf p [10](#)
- Protocol cf p [11](#)
- Experiment (activated tab)

EXPERIMENT		REAGENTS		
Protocol	Disco 2h10	Reagent	Tank	Volume (mL)
Experiment	Flogentec	THF50%	T3	24
Author	Flogentec	THF80%	T4	24
Date	29/01/2016	THF 100%	T1	50
Total duration	2:15:08	THF 100%	T2	50
		DCM	P1	20

[Save](#) [Report](#) [History](#)

The **Experiment** bloc recaps all the data about the experiment:

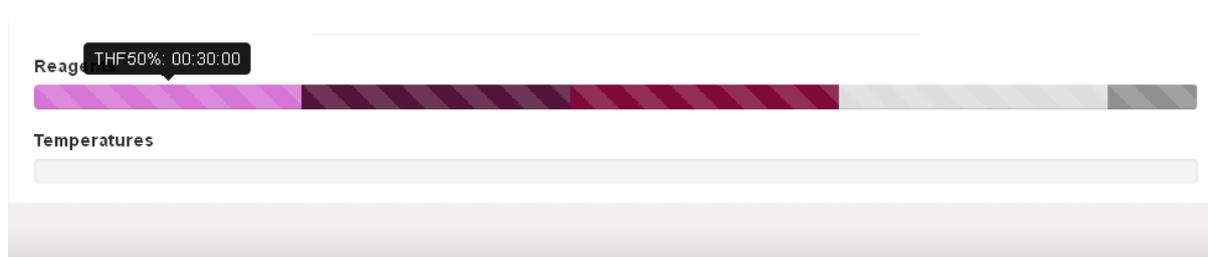
- The associated protocol (Protocol)
- The name of the experiment (Experiment)
- The author of the experiment (Author)
- The date (Date)
- The total duration of the experiment (Total Duration)

You can change this information to start a new experiment.

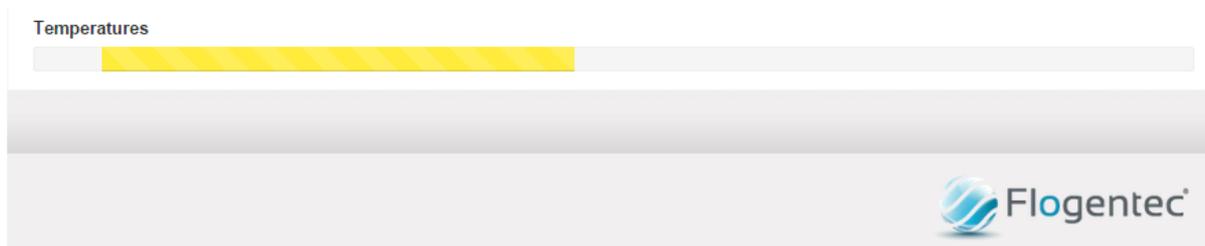
The **Reagent** Bloc recaps all solutions needed for this experiment:

- Column reagent gives the name of the solution
- Column Tank is the number of the tank containing the solution
- Column Volume gives the needed volume (mL) for running this experiment.
- The 3 button below are here to:
 - Save new parameters of the experiment (Save button)
 - Access to the report of the experiment (Report cf p 13)
 - Access to the history of experiments (history)

The colorful diagram below recaps experiment's steps. Each color corresponds to a reagent. By clicking on color segment, you can access to the name of the reagent and the duration of the step.



The diagram below indicates Temperature uses during the experiment. By clicking on the color part, you can access to the set-up temperature of the protocol and the set-up temperature of each flowcell (first number corresponding to Flowcell 1, the second one to flowcell 2 etc...).



iii. Run an experiment:

If you are agree with all these parameters, you can load the protocol by clicking on the button **load protocol**.

EXPERIMENT		REAGENTS		
Protocol	Disco 2h10	Reagent	Tank	Volume (mL)
Experiment	Flogentec	THF50%	T3	24
Author	Flogentec	THF80%	T4	24
Date	29/01/2016	THF100%	T1	50
Total duration	2:15:08	THF100%	T2	50
		DCM	P1	20

Save Report History

Reagents

Temperatures

Load Protocol

The downloading bar appears.

The screenshot shows the Flogentec interface with a 'Loading protocol ...' dialog box. The dialog box contains a progress bar at 10%, 'Commands : 2/20', and 'Errors : 0'. In the background, there is a table with the following data:

EXPERIMENT		REAGENTS		
Protocol	Disco 2h10	Reagent	Tank	Volume (mL)
Experiment	Flogentec	THF50%	T3	24
Author	Flogentec	THF80%	T4	24
Date		THF100%	T1	50
Total duration				50
				20

Below the table, there is a 'Reagents' section with a progress bar showing approximately 30% completion.

Please wait during the download. Once the protocol is 100% loads, click on close button to close the download window and then click on the execute button.

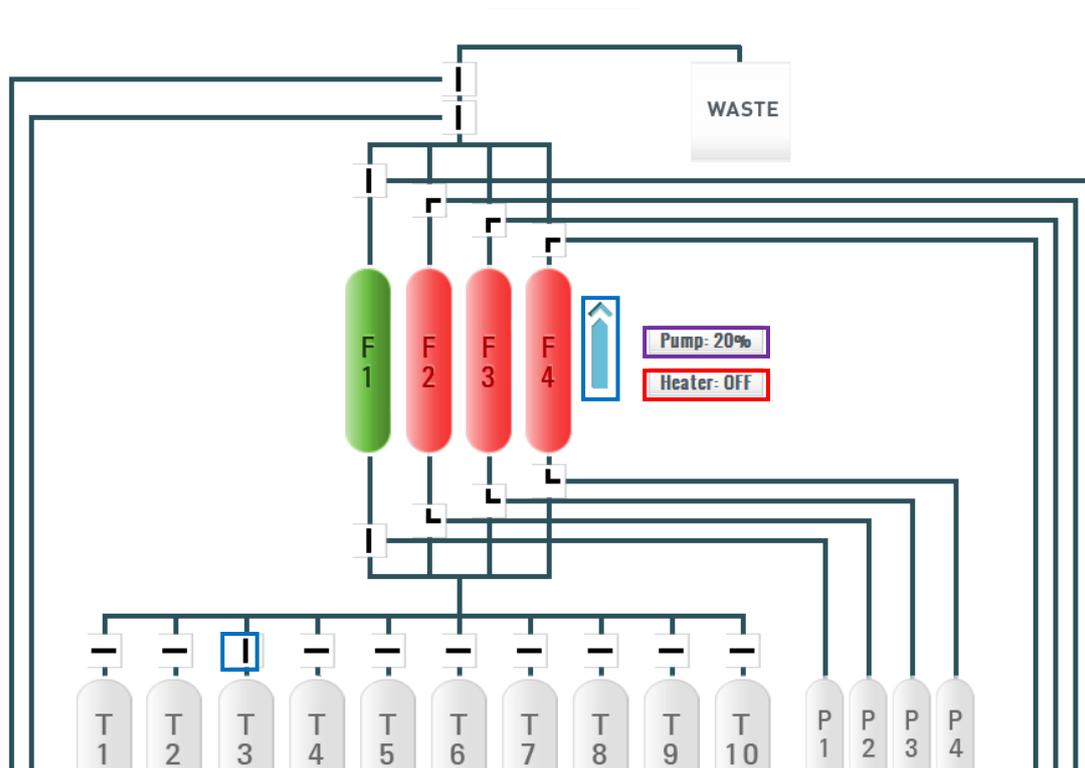
Protocol loaded

The screenshot shows the 'Protocol loaded' dialog box. It features a blue progress bar at 100%, 'Commands : 20/20', and 'Errors : 0'. A 'Close' button is highlighted with a red box.

A progression bar appears showing you the evolution of the experiment.

The screenshot shows the Flogentec interface with a progression bar. The 'Reagents' section has a progress bar with a red and white striped pattern. The 'Temperatures' section has a grey progress bar. The 'Progress' section has a blue progress bar at 0%. Below the bars are 'State' and 'Stop' buttons.

You can access at any time to the system state to check temperatures and reagents distribution by clicking onto the green button.



This diagram recap:

- Activated **Flowcells**
- Reagents **Flow direction**
- Pump **Outflow**
- Set **Temperature**

iv. Stop an experiment:

You can also stop the experiment by clicking on the button stop, at this point; a window appears demanding if you really want to stop the experiment. Click on **Yes** if you want to stop it, otherwise click on **No**.



If you want edit configuration of the experiment, click on the configuration tab on the breadcrumb.

2. Flo400 configuration:

The **pump** bloc permits you to set-up different speed for the pump.

The **Flowcell** bloc permits you to activate flowcells and to set-up temperatures for each:

The activated flowcells are in green and disabled in red.

For each Flowcells you can set up 4 different temperatures, Click on the scrolling tab of the selected flowcell and press on the wanted temperature.

The **Reagents** bloc is here to save and parameter the solution you will used for this experiment and to define tankers:

- Name your reagent with filling in the blank in the reagent column.
- Select the Tanker of the solution with the column Tank
- The capacity of cycling the tanker is indicated by the column can cycle.
- The column color gives you the possibility to define a color for each reagent; this color will be used for the experiment's recap diagram.
- With the column action you can:
 - Edit a saved reagent (Edit)
 - Delete a reagent (Deleted)
 - Save a new reagent (add reagent)

Once all is ready, you can save all by clicking on the button Save or go back to the parameters by clicking on the button Cancel.

The screenshot displays the configuration interface for the Flo400 system. It is divided into three main sections: Pump, Flowcells, and Reagents.

PUMP: This section features three horizontal sliders for setting pump speeds. The 'Low' slider is set to 20%, the 'Medium' slider is set to 50%, and the 'High' slider is set to 70%.

FLOWCELLS: This section contains a table for configuring four flowcells (FC1, FC2, FC3, FC4) across four tanks (T1, T2, T3, T4). Each cell in the table shows a temperature value. Below the table are 'Save' and 'Cancel' buttons.

	FC1	FC2	FC3	FC4
T1	41 °C	40 °C	0 °C	0 °C
T2	0 °C	0 °C	0 °C	0 °C
T3	0 °C	0 °C	0 °C	0 °C
T4	0 °C	0 °C	0 °C	0 °C

REAGENTS: This section displays a table of reagents with their respective tanks, cycling capabilities, and colors. Below the table are input fields for adding a new reagent.

Reagent	Tank	Can cycle	Color	Actions
PBS	T1	Yes	Green	Edit Delete
alpha-DIG	T2	Yes	Blue	Edit Delete
DMTP	T3	No	Cyan	Edit Delete

Below the table, there are input fields for 'Reagent' and 'Tank', and an 'Add reagent' button.

If all the parameters are ready, you can edit or create a protocol by clicking on the tab protocol on the breadcrumb.

REAGENTS				
Reagent	Tank	Can cycle	Color	Actions
THF50%	T3	No		Valid Cancel
THF80%	T4	No		Edit Delete
THF100%	T1	Yes		Edit Delete
THF100%	T2	Yes		Edit Delete
DCM	P1	Yes		Edit Delete
Reagent	Tank			Add reagent

To define the color of your reagent, click on the color area, and choose the desired color for your reagent thanks to the color palette.

3. Save a Protocol:

i. Database :

To access to the database of protocols click on the protocols button on the menu. You may **create**, **load**, **delete** or **edit** one. If you have created a new one, with the **Create** button, click on **Edit** to fully write the protocol. A recap of the protocol is visible with the name, the user which creates this protocol, the total duration of the protocol, the number of steps and reagents and the date.

PROTOCOLS						
PROTOCOLS	EXPERIMENTS	MAINTENANCE	WASH CYCLE	LOGOUT		
Protocol	User	Duration	Steps	Reagents	Date	Actions
Hybridation in situ	admin	21:00:00	12	4	16/04/2015	Load Edit Delete
Immunohistochimie	admin	06:30:00	10	6	26/05/2015	Load Edit Delete
WashCycle IF	admin	00:10:00	9	5	10/04/2015	Load Edit Delete
Name	admin				29/01/2016	Create

ii. Writing the protocol

If you have created a new protocol or if you have edited one, you are redirected to the following page:

FIo 400[®]
AUTOMATED ULTRA FAST 3D IN SITU DETECTION

PROTOCOLS | MAINTENANCE | BUY / NEEDS | LOGOUT

STEPS PROCESSING | CONFIGURATION > PROTOCOL > EXPERIMENT 4

1 → Name: Hybridation in situ | Steps: 13
 Date: 2015-04-16 | Reagents: 4
 Author: admin | Duration: 17:15:00

3 → Save Cancel

Order	Step	Duration	Reagent	Cycling	Actions
▼	Pump High	00:00:00			Edit Delete
▲▼	Flow	00:15:00	PBS	No	Edit Delete
▲▼	Heater T1	00:00:00			Edit Delete
▲▼	Flow	08:00:00	mRNA	Yes	Edit Delete
▲▼	Heater OFF	00:00:00			Edit Delete
▲▼	Flow	00:30:00	PBS	Yes	Edit Delete
▲▼	Pump Low	00:00:00			Edit Delete
▲▼	Flow	01:00:00	DMTP	No	Edit Delete
▲▼	Heater T2	00:00:00			Edit Delete
▲▼	Flow	07:00:00	alpha-DIG	Yes	Edit Delete
▲▼	Pump OFF	00:00:00			Edit Delete
▲▼	Flow	00:30:00	PBS	No	Edit Delete
▲	Heater OFF	00:00:00			Edit Delete

Action ▼ Hour ▼ Min ▼ Sec ▼ Reagent ▼ Cycling ▼ Add step 2

Flogentec[®]

You can name your protocol (1).

Choose actions to execute via the different tab and the Add step button (2):

Step : Select the work to do in this step (flow, pump high/medium/low, heater T1/2/3/4, heater OFF).

Duration: Set-up the duration of the step.

Reagents: Select reagent you will use during this step.

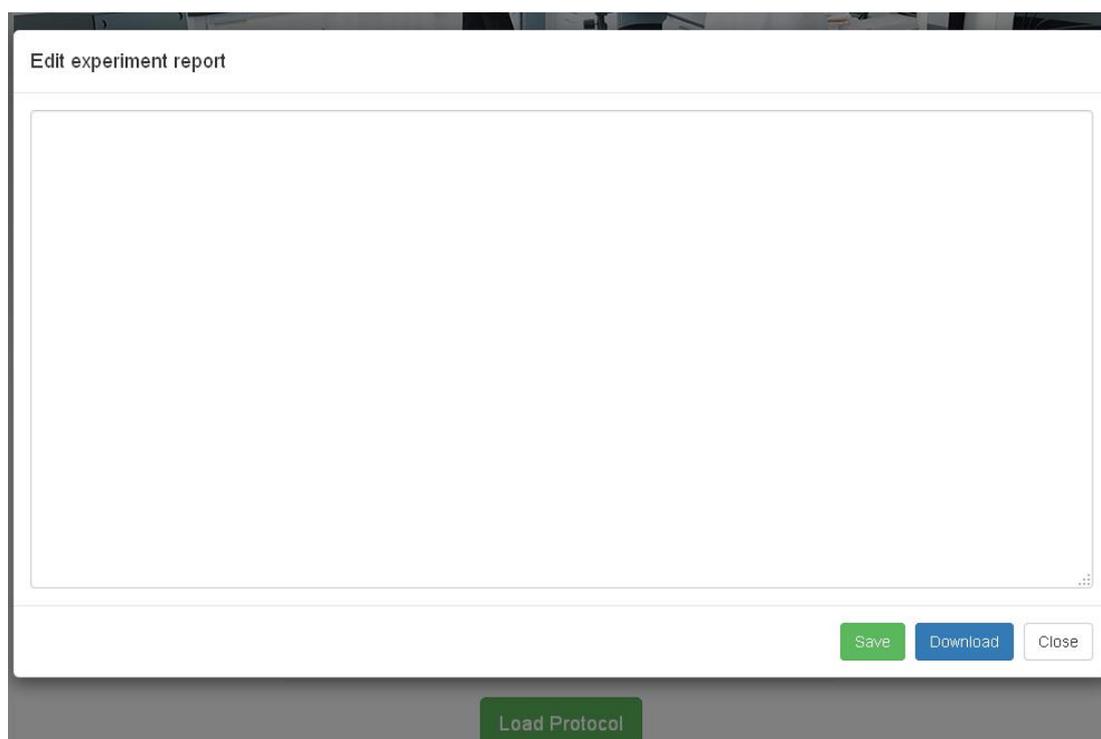
Cycling: If you need to cycle your reagents switch on Yes.

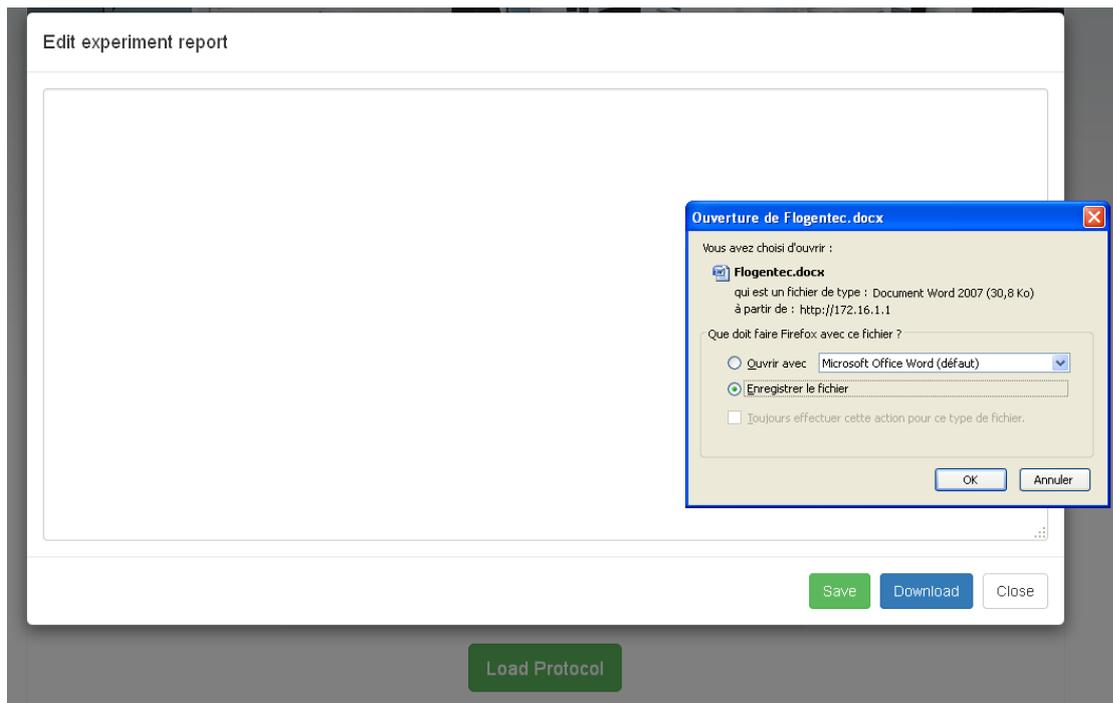
Action: Modifying or deleting the step selected.

Once protocol set-up click on SAVE (3). You can then access to the experiment page thanks to the breadcrumb and the button EXPERIMENT (4).

4. Edit a report :

At the end of the experiment, you can sum-up your results into a report where you can import your images. The protocol can then be saved or exported into a word document. Every experimental report can be load during experiment loading via the report button.





EXPERIMENT REPORT

Experiment	Protocol	Author	Date
Test	Hybridation in situ	test	11/04/2015

Order	Reagent	Duration	Temperature (°C)
0	PBS	00:15:00	
1	Probe	08:00:00	T1 : 41°C, 40°C, 0°C, 0°C
2	PBS	00:30:00	
3	DMTP	01:00:00	
4	alpha-DIG	07:00:00	T2 : 0°C, 0°C, 9°C, 0°C
5	PBS	00:30:00	T2 : 0°C, 0°C, 9°C, 0°C

Comments :
Test hybridation in situ



5. Device maintenance :

To access to the maintenance system, click on **MAINTENANCE**. You can see the different loops effectuated by reagents.



You can test the device and change connections between tankers and Flowcells thanks to square connection . It is also possible to test flows created via HIGH, MEDIUM and LOW button. You can stop the running experiment by click on the STOP button.

6. Automate washing (WASH CYCLE) :

This tab allows running a wash of the system.

The washing protocol is saved into the automaton and includes necessary volumes to a pump function at 100% value.

7. Contact :

If needs, a remote maintenance can be performed by our society. You can contact us at the following address:

www.flogentec.com

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Flogentec[®] Company remains at your disposal for any requests.