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² i.e. name of the person(s) responsible for the preparation of the document

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⁴ The Technical Annex of the project provides a list of deliverables to be submitted, with the following classification level:

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Abstract

In this Deliverable we have deal with developing a new high level software interface for creating and running DNA production projects on the robot.

This deliverable is divided into 2 parts:

1.Gibson Automation.

This part is about **high-level scripts for DNA synthesis tasks.**

2.Robot tuning.

This part is about **Robot configuration capabilities.**

High level command for Gibson Automation:

As the CADMAD biochemistry evolves, we face the need to serve more and more biochemical processes. For example, in the past, our robot automation software module, written in matlab, was only designed to do Y operation projects. Nowadays we are increasingly using the Gibson operation.

So we are faced with the need to redesign our automation system to support this kind of biochemical operation.

We developed a solution for supporting the Gibson operation with full automation by expanding our Matlab code that supports the traditional Y operation. To do this we used high level scripts in which Matlab writes high level functions (such as “Do Gibson assembly”) which then translate into many Roboease (CADMAD’s language for commanding the DNA editor liquid handling robot) command lines, which then translate to Tecan’s robot most basic machine language (Evoware).

Robot configuration tuning:

Our newly developed Roboease scripts for DNA editing can only be as good as the robotic liquid handling hardware that executes them. As a result, we are faced with a big challenge of tuning our robots configuration to accurately and robustly support these new high level scripts.

This effort has built upon our QC systems results which largely directed our efforts for tuning the robots configuration software.

In this deliverable we will discuss in detail our efforts in tuning the robot configurations.

Keywords⁷:

Matlab, automation, evolab, roboease, configurations

Introduction

a. Aim / Objectives:

⁷ Keywords that would serve as search label for information retrieval

The main aim of this deliverable is to enable the automation system to support new biochemical processes by optimizing its configurations and creating high level scripting for these tasks.

b. State of the Art

Not relevant

c. Innovation

This is the first time we know of that robot configuration tuning was done according to dedicated experimental QC results.

2. Implementation:

Gibson Automation : In order to keep things as simple as possible, we have described the Gibson as a variant of the traditional Y operation. By doing this we were able to use parts of the Y operation automation and re-create from scratch only the differences between them.

We had to deal solve two problems:

- A. To adapt the automation system to a new scenario in which two independent DNA editing biochemistries may be used and creating the option to tell the automation to run back and forth from using the Gibson or the Y operation upon the operators request.
- B. Design a new Roboease procedure for running the Gibson reaction.

Once we overcome these problems we focused on connecting the new Django high-level Python Web framework module to this mechanism. All these tasks have been achieved.

Robot tuning: after we have finished writing the Gibson, we had to test and tune the robot.

This phase divides into a few steps:

1. Running a script with water and colour for validation.
2. Test the results with our QC system.
3. Tune the robot configurations accordingly.

As 1 and 2 are discussed in 4.5, we will focus in 3.

The robot tuning has many different aspects. In liquid handling, the 2 major aspects are liquid classes and Labware configuration. The liquid class configuration defines the way the robot is going to handle the liquid in an operation related to a specific the liquid class. Labware defines the manner plastic-ware is set up and used on the robot.

Liquid classes:

The chief liquid class parameters are:

- Aspiration speed.
- Dispense speed.
- Delay time.
- Height of liquid aspiration.
- Height of liquid dispense.
- Leading air gap.
- Trailing air gap.
- Liquid detection.
- Calibration factor.
- Calibration offset.

Tuning a liquid class is a task of iterative optimization based on experience and trial and error. For each operation the tuning is a little bit different, but there are a few principles we have learned during our work, here are a few examples:

- Dispensing liquid into liquid is more accurate the dispensing liquid from the air.
- When dispensing liquid into liquid, it is better to make the dispense speed slow: 10-30 ul/sec.
- When dispensing liquid into liquid, it is important to exit the liquid slowly so no droplets are left on the pipetor.
- When dispensing liquid from the air, the dispense speed should increase to 100-120 ul/sec.

Labware configuration:

The labware configuration defines the dimensions of plates and wells.

It has to be tuned perfectly, otherwise the robot will not know from where to aspirate and dispense the liquid.

Results:

Below we present several screenshots of the system we have developed which demonstrate how the new Gibson Automation runs:

a. Project planning by the Django module:

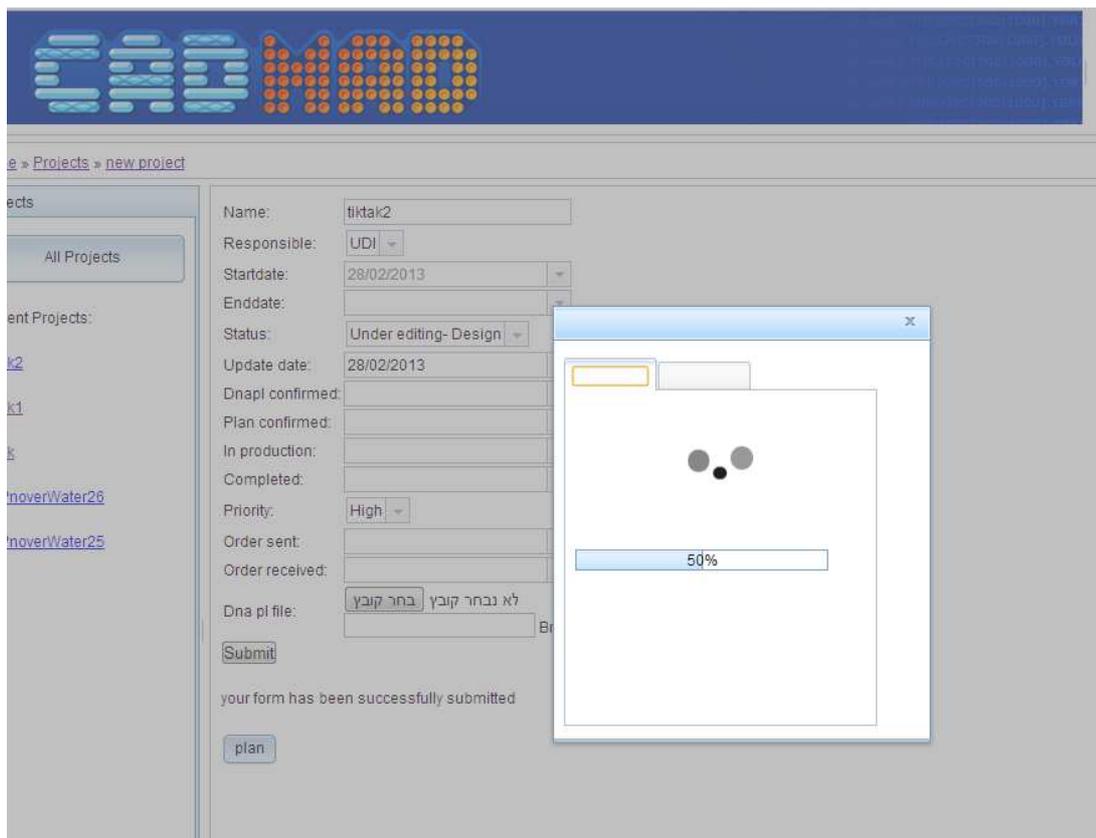
The screenshot shows a web interface for creating a new DNA manufacturing project. At the top, there is a blue header with the 'Gibson' logo. Below the header, there is a navigation breadcrumb: Home » Projects » new project. The main content area is divided into two sections. On the left, there is a sidebar with a 'Projects' section containing an 'All Projects' button and a 'Recent Projects' list with links to 'tiktak2', 'tiktak1', 'tiktak', 'GFPnoverWater26', and 'GFPnoverWater25'. The main form area contains the following fields:

- Name:
- Responsible:
- Startdate:
- Enddate:
- Status:
- Update date:
- Dnapi confirmed:
- Plan confirmed:
- In production:
- Completed:
- Priority:
- Order sent:
- Order received:
- Dna pl file: לא נבחר קובץ

Below the form is a button. A message below the button reads: "your form has been successfully submitted". At the bottom of the form area is a button.

Caption:

The above screen shot is an HTML form we developed for creating a new DNA manufacturing project. It is written in python – a Django framework.



Caption:

The screenshot above shows a progress bar of the planning stage in our system. It shows the system in the step following the planning phase and prior to the automation phase. The user chooses whether he wants to execute the Gibson or Y operation and the Django module instructs the Matlab module accordingly.

Run all steps in one shot Gibson

Run step

Run step Gibson

Nodes	Reagents	Plates	Files	Steps	Plan	Commands	old steps
Step	Step type	Start date	End date	Notes	Download Steps files		
1	Wet	2012-09-23	[no data]	[no data]	Download Step's files		
2	PlateReader	2012-09-23	[no data]	[no data]	Download Step's files		
3	DilPhosPri	2012-09-23	[no data]	[no data]	Download Step's files		
4	FinPhosPri	2012-09-23	[no data]	[no data]	Download Step's files		
5	ELN_RTYPE34	2012-09-23	[no data]	[no data]	Download Step's files		
6	Gel	2012-09-23	[no data]	[no data]	Download Step's files		

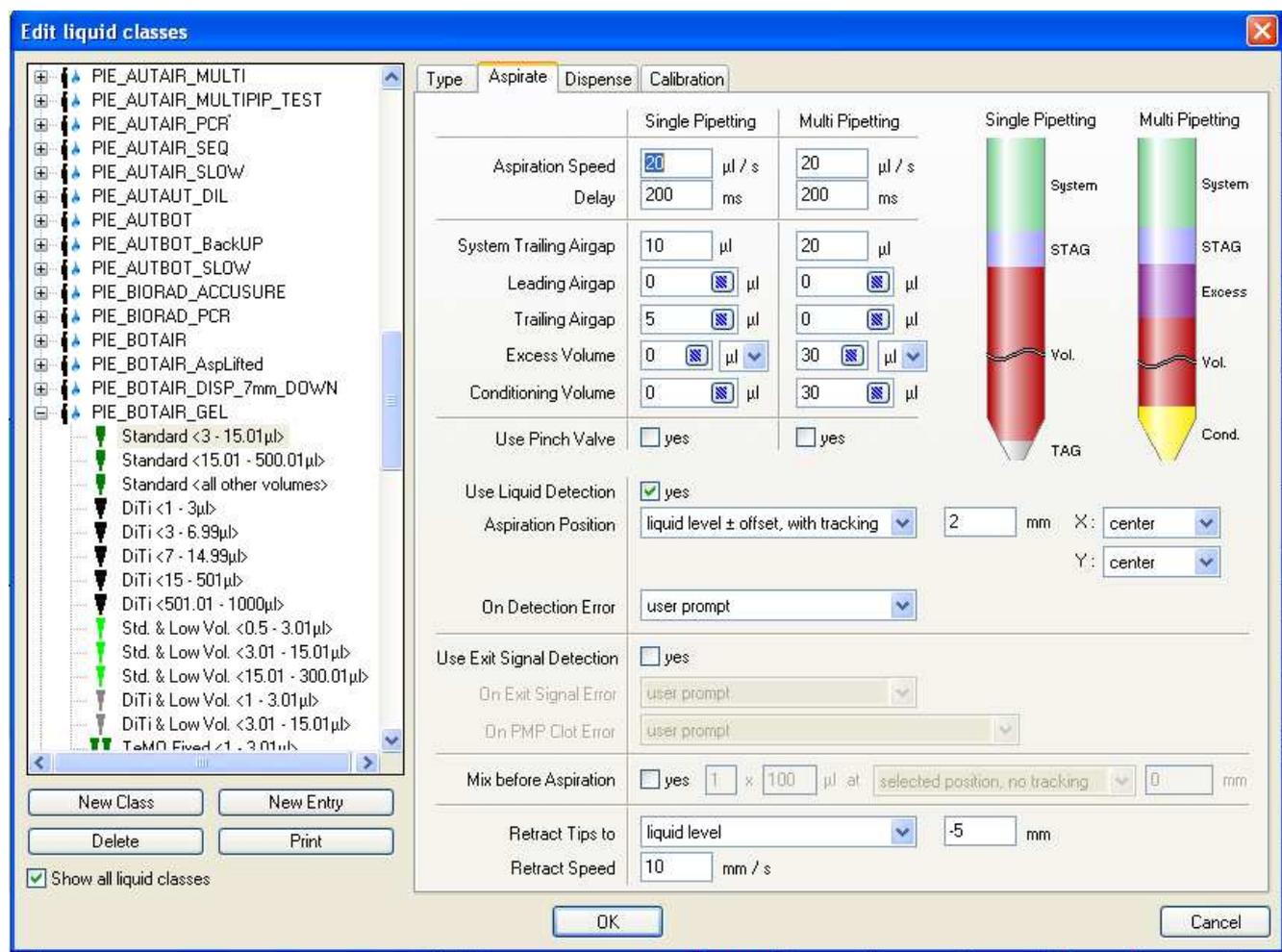
Caption: The screenshot above shows a project after all of its script steps has been created.

List of project steps created by the automation, showed on our website

In order to actually produce them we simply click the "run all steps in one shot Gibson".

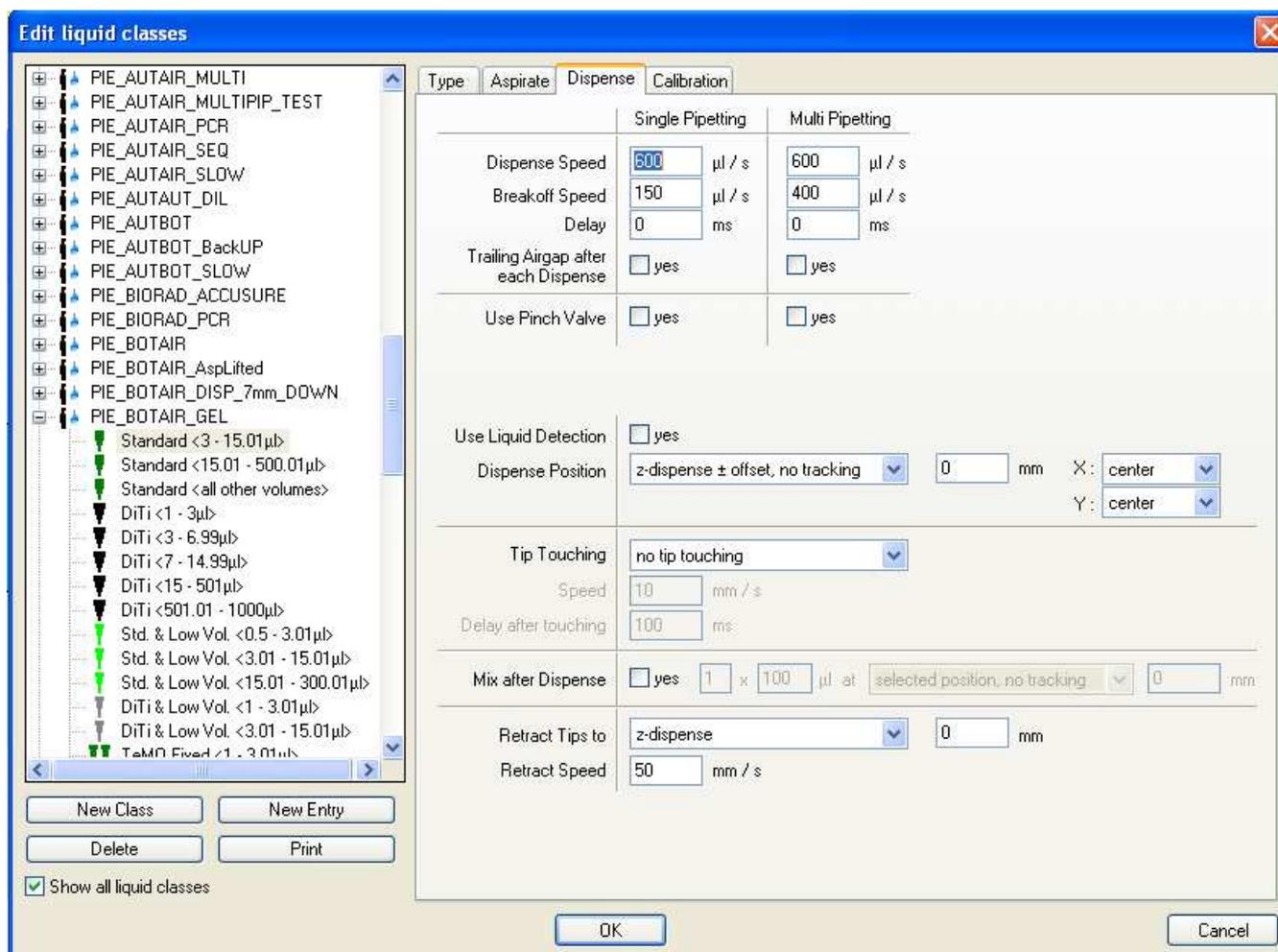
Liquid classes and lab-ware configuration:

The following are figures describe the liquid handling parameters that were optimized during robot re-configuration:



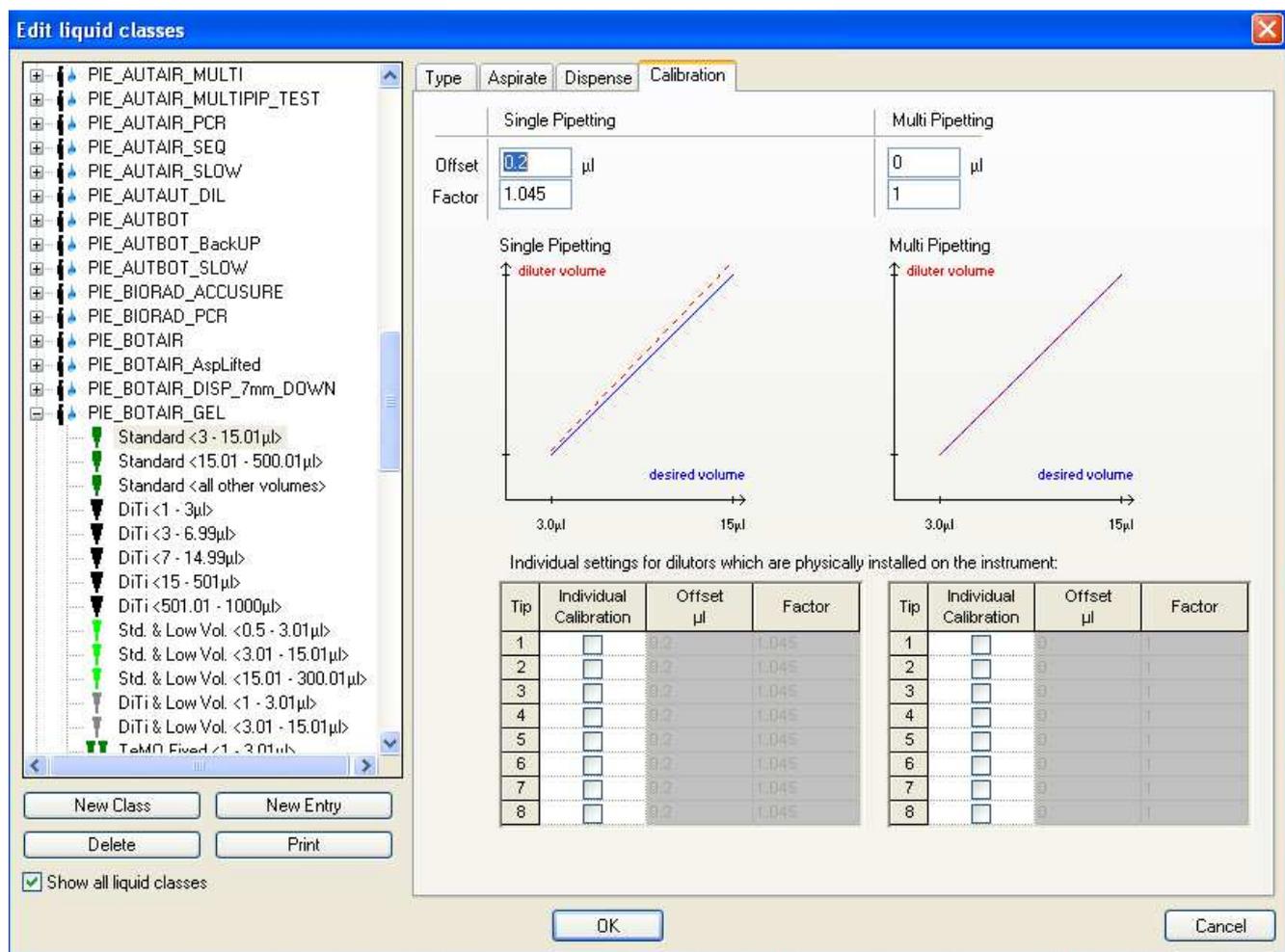
Caption:

In the screenshot above, we see the liquid class aspiration parameters. Each parameter exists twice: 1 for single pipetting and one for multi pipetting. The most important parameters are: aspiration speed, delay, aspiration position, trailing air gap. All these parameters were configured for each and every script we run.



Caption:

In the screen shot above we see the liquid class dispense configurations. The most important parameters are: dispense speed, breakoff speed, delay and dispense position. All these parameters were configured for each and every script we run.



Caption:

In the above screenshot we see the calibration part of the liquid class. It is divided into 2 parts: namely Single and multi-pipetting. For each part there are 2 parameters: Offset and factor.

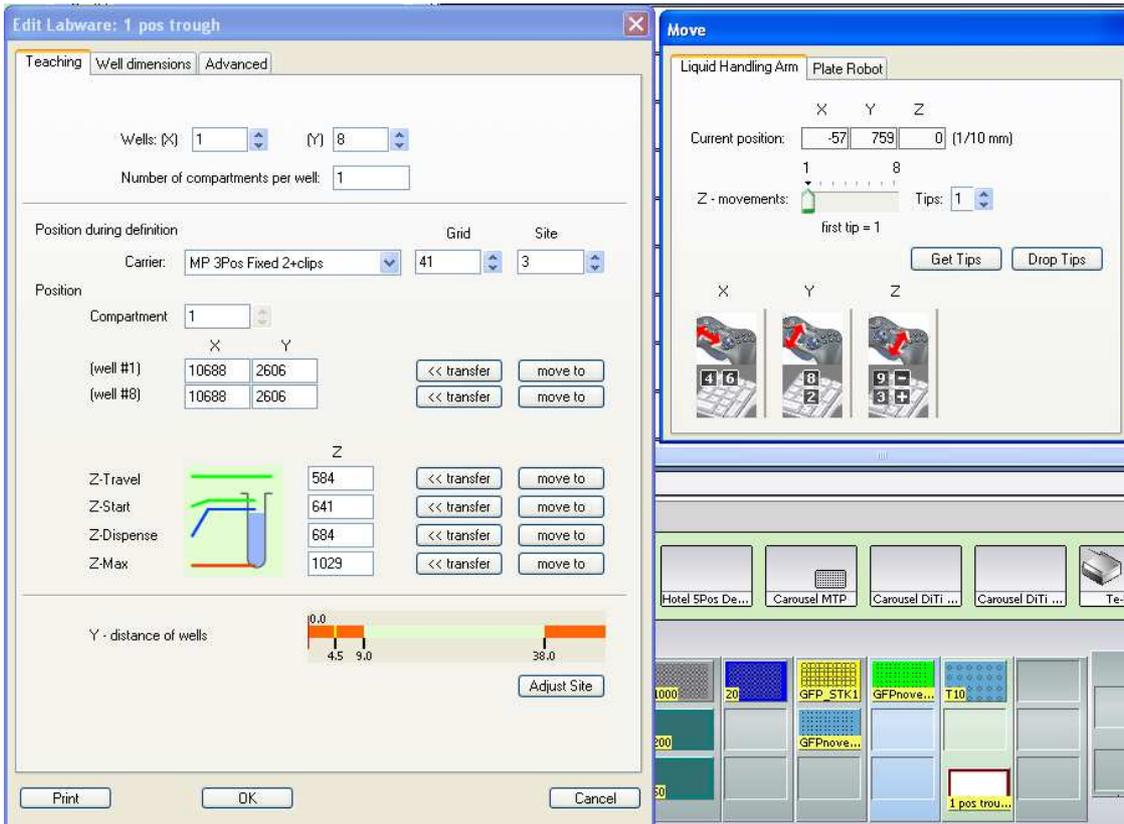
The offset is meant to add or subtract a steady amount of liquid for each pipetting operation.

For example: $7 + 0.1$ where 7 is the amount of liquid to aspirate and 0.1 is the offset.

The factor is meant to multiply the amount of liquid by factor.

For example: $7 * 1.01$ where 7 is the volume to be aspirates and 1.01 is the factor.

As stated above, all these parameters were configures for each and every script we run.



Caption:

The screenshot above shows the configuration window of the labware. The right part determines the well dimensions, grid position, allowed Carriers. The right part is for tuning the vector of the LIHA (liquid handling arm) to fit in the labware.

3. Conclusions:

The configuration of the DNA editor is now optimized for each and every script of our DNA editing system.