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[3] Short name of partner(s) responsible for the deliverable

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

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 tunning.

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 tunning:

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 tunning 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 tunning the robots configuration software.

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

Keywords[7]**:**

Matlab, automation, evolab, roboease, configurations

Introduction

a. Aim / Objectives:

[7] Keywords that would serve as search label for information retrieval

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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 tunning: 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 tunning 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.

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Tunning a liquid class is a task of iterative optimization based on experience and trial and error. For each operation the tunning 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:

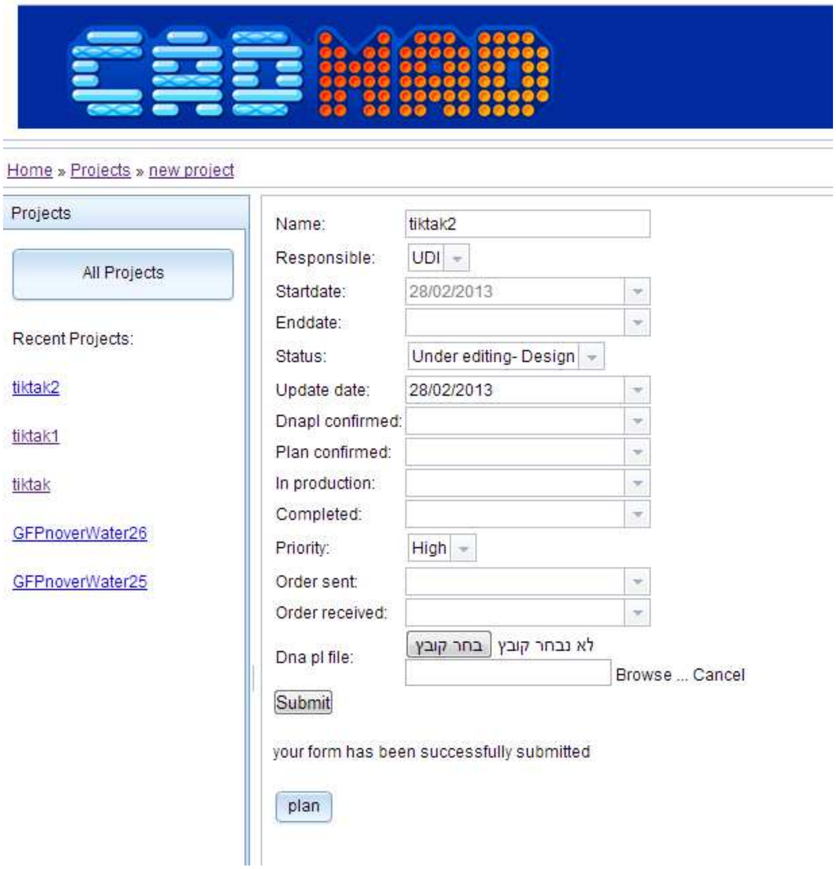
The labware configuration defines the dimensions of plates and wells.
It has to be tuned perfectly, otherwise the robot will not know from where to aspirate and dispense the liquid.

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

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:**



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Projects

Recent Projects:

- tiktak2
- tiktak1
- tiktak
- GFPnoverWater26
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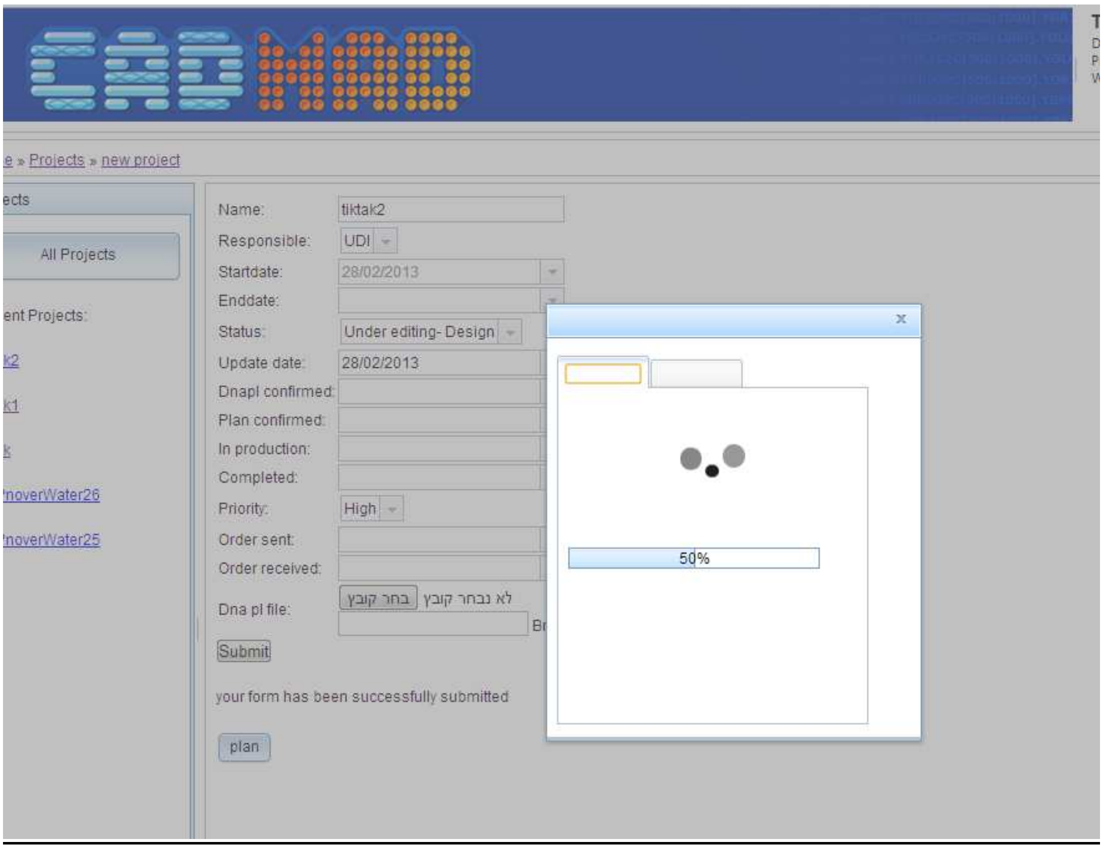
Name:	tiktak2
Responsible:	UDI
Startdate:	28/02/2013
Enddate:	
Status:	Under editing- Design
Update date:	28/02/2013
Dnapl confirmed:	
Plan confirmed:	
In production:	
Completed:	
Priority:	High
Order sent:	
Order received:	
Dna pl file:	בחר קובץ לא נבחר קובץ Browse ... Cancel

your form has been successfully submitted

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.

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



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All Projects

ent Projects:

k2

k1

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noverWater26

noverWater25

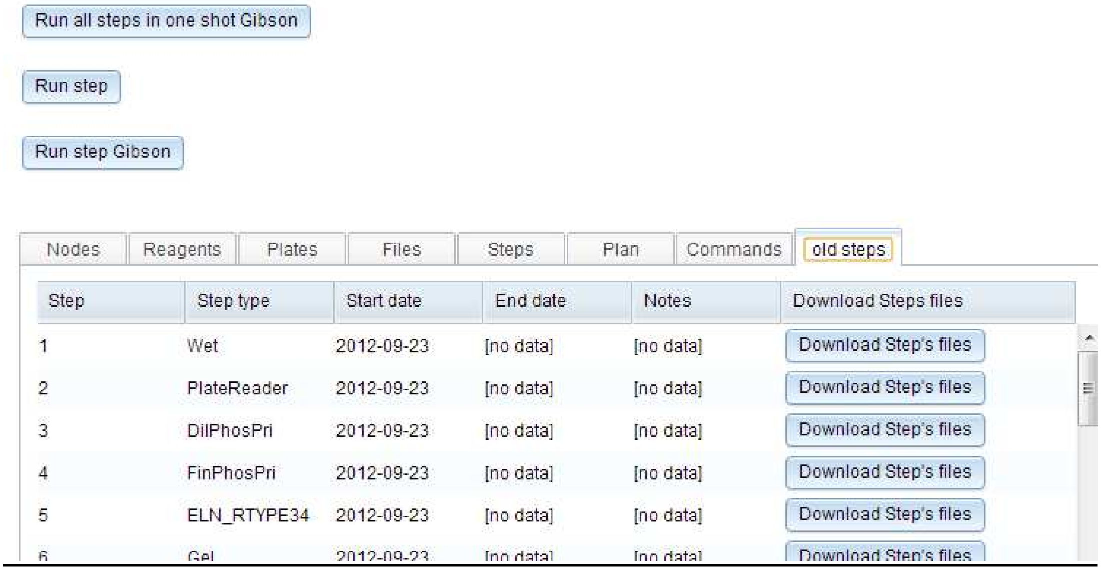
Name:	tiktak2
Responsible:	UDI
Startdate:	28/02/2013
Enddate:	
Status:	Under editing- Design
Update date:	28/02/2013
Dnapl confirmed:	
Plan confirmed:	
In production:	
Completed:	
Priority:	High
Order sent:	
Order received:	
Dna pl file:	בחר קובץ לא נבחר קובץ

Submit

your form has been successfully submitted

plan

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 wether he wants to execute the Gibson or Y operation and the Django module Instructs the Matlab module accordingly.



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

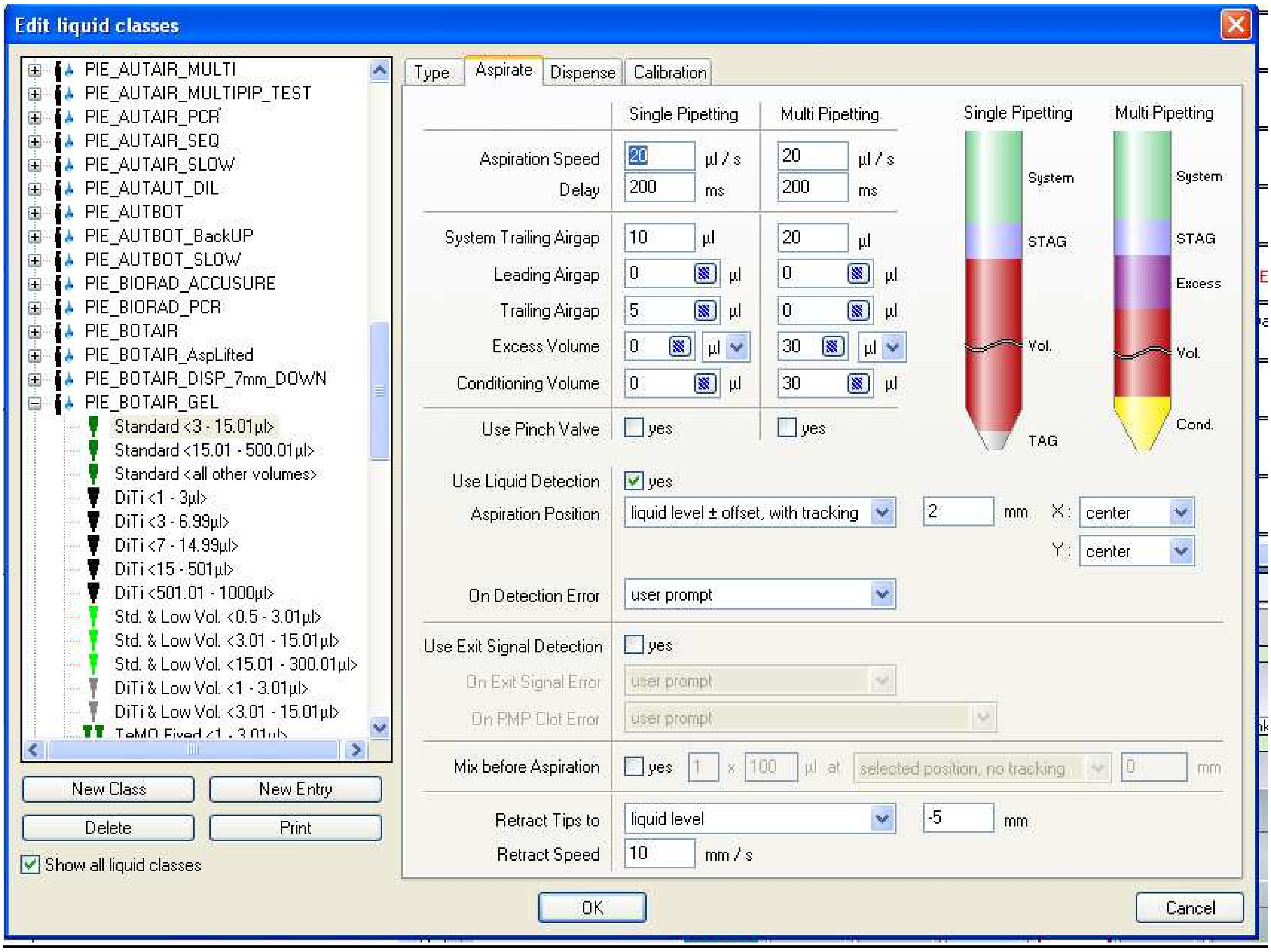
Caption: The screenshot above shows a project after all of its script steps has been created.

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

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:



Edit liquid classes

- PIE_AUTAIR_MULTI
- PIE_AUTAIR_MULTIPIP_TEST
- PIE_AUTAIR_PCR
- PIE_AUTAIR_SEQ
- PIE_AUTAIR_SLOW
- PIE_AUTAUT_DIL
- PIE_AUTBOT
- PIE_AUTBOT_BackUP
- PIE_AUTBOT_SLOW
- PIE_BIORAD_ACCUSURE
- PIE_BIORAD_PCR
- PIE_BOTAIR
- PIE_BOTAIR_AspLifted
- PIE_BOTAIR_DISP_7mm_DOWN
- PIE_BOTAIR_GEL
  - Standard <3 - 15.01µl>
  - Standard <15.01 - 500.01µl>
  - Standard <all other volumes>
  - DiTi <1 - 3µl>
  - DiTi <3 - 6.99µl>
  - DiTi <7 - 14.99µl>
  - DiTi <15 - 501µl>
  - DiTi <501.01 - 1000µl>
  - Std. & Low Vol. <0.5 - 3.01µl>
  - Std. & Low Vol. <3.01 - 15.01µl>
  - Std. & Low Vol. <15.01 - 300.01µl>
  - DiTi & Low Vol. <1 - 3.01µl>
  - DiTi & Low Vol. <3.01 - 15.01µl>
  - TeMO Fixed <1 - 3.01µl>

☑ Show all liquid classes

Aspirate

	Single Pipetting		Multi Pipetting	
Aspiration Speed	20	µl / s	20	µl / s
Delay	200	ms	200	ms
System Trailing Airgap	10	µl	20	µl
Leading Airgap	0	µl	0	µl
Trailing Airgap	5	µl	0	µl
Excess Volume	0	µl	30	µl
Conditioning Volume	0	µl	30	µl
Use Pinch Valve	□ yes		□ yes	

Use Liquid Detection ☑ yes

Aspiration Position: liquid level ± offset, with tracking; 2 mm; X: center; Y: center

On Detection Error: user prompt

Use Exit Signal Detection □ yes

On Exit Signal Error: user prompt

On PMP Clot Error: user prompt

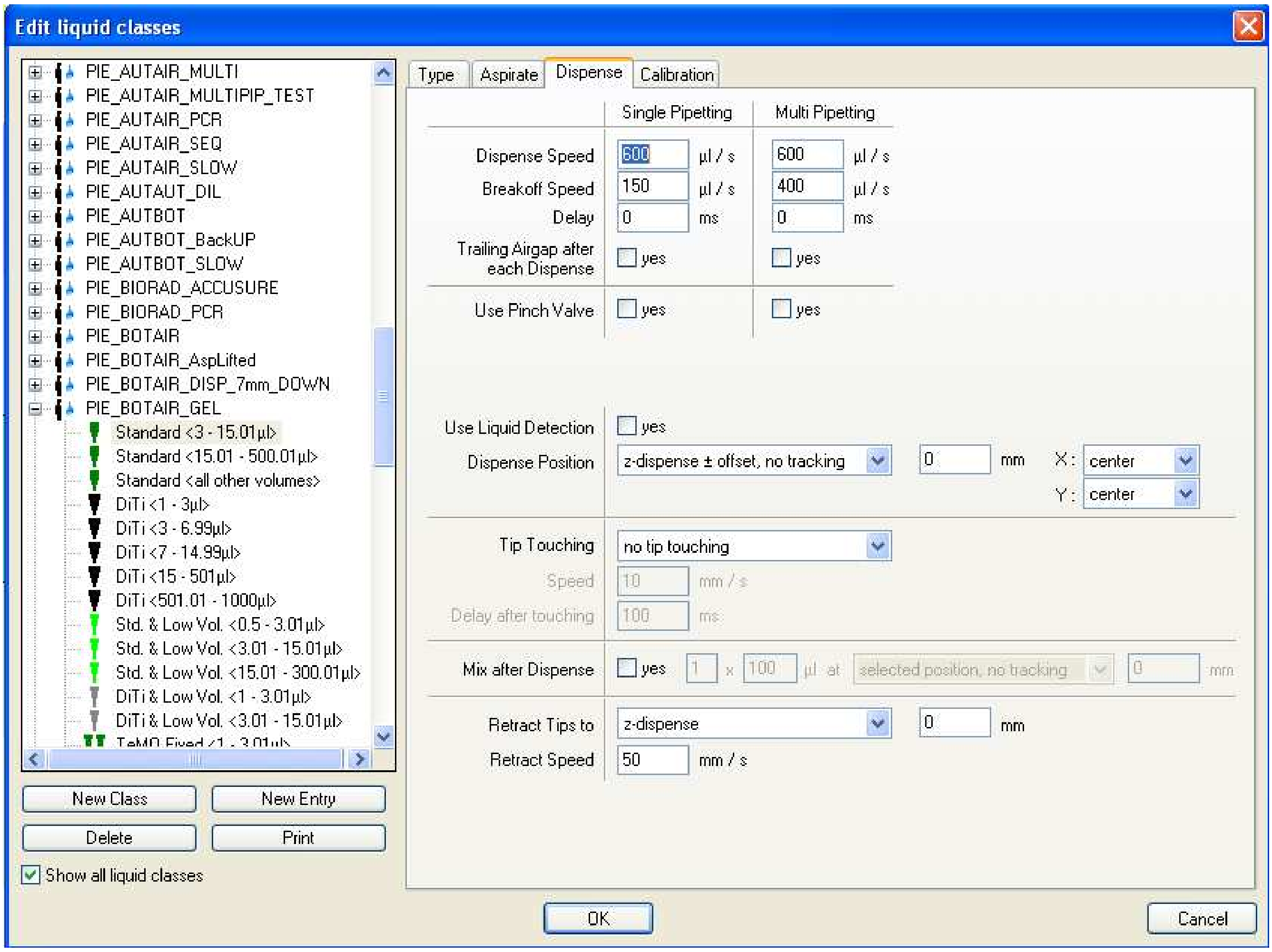
Mix before Aspiration □ yes 1 x 100 µl at selected position, no tracking 0 mm

Retract Tips to: liquid level; -5 mm

Retract Speed: 10 mm / s

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 configures for each and every script we run.

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



Edit liquid classes

- PIE_AUTAIR_MULTI
- PIE_AUTAIR_MULTIPIP_TEST
- PIE_AUTAIR_PCR
- PIE_AUTAIR_SEQ
- PIE_AUTAIR_SLOW
- PIE_AUTAUT_DIL
- PIE_AUTBOT
- PIE_AUTBOT_BackUP
- PIE_AUTBOT_SLOW
- PIE_BIORAD_ACCUSURE
- PIE_BIORAD_PCR
- PIE_BOTAIR
- PIE_BOTAIR_AspLifted
- PIE_BOTAIR_DISP_7mm_DOWN
- PIE_BOTAIR_GEL
  - Standard <3 - 15.01µl>
  - Standard <15.01 - 500.01µl>
  - Standard <all other volumes>
  - DiTi <1 - 3µl>
  - DiTi <3 - 6.99µl>
  - DiTi <7 - 14.99µl>
  - DiTi <15 - 501µl>
  - DiTi <501.01 - 1000µl>
  - Std. & Low Vol. <0.5 - 3.01µl>
  - Std. & Low Vol. <3.01 - 15.01µl>
  - Std. & Low Vol. <15.01 - 300.01µl>
  - DiTi & Low Vol. <1 - 3.01µl>
  - DiTi & Low Vol. <3.01 - 15.01µl>
  - TeMO Fixed <1 - 3.01µl>

☑ Show all liquid classes

Type | Aspirate | Dispense | Calibration

	Single Pipetting		Multi Pipetting	
Dispense Speed	600	µl / s	600	µl / s
Breakoff Speed	150	µl / s	400	µl / s
Delay	0	ms	0	ms
Trailing Airgap after each Dispense	□ yes		□ yes	
Use Pinch Valve	□ yes		□ yes	

Use Liquid Detection □ yes

Dispense Position: z-dispense ± offset, no tracking; 0 mm; X: center; Y: center

Tip Touching: no tip touching

Speed 10 mm / s

Delay after touching 100 ms

Mix after Dispense □ yes 1 x 100 µl at selected position, no tracking 0 mm

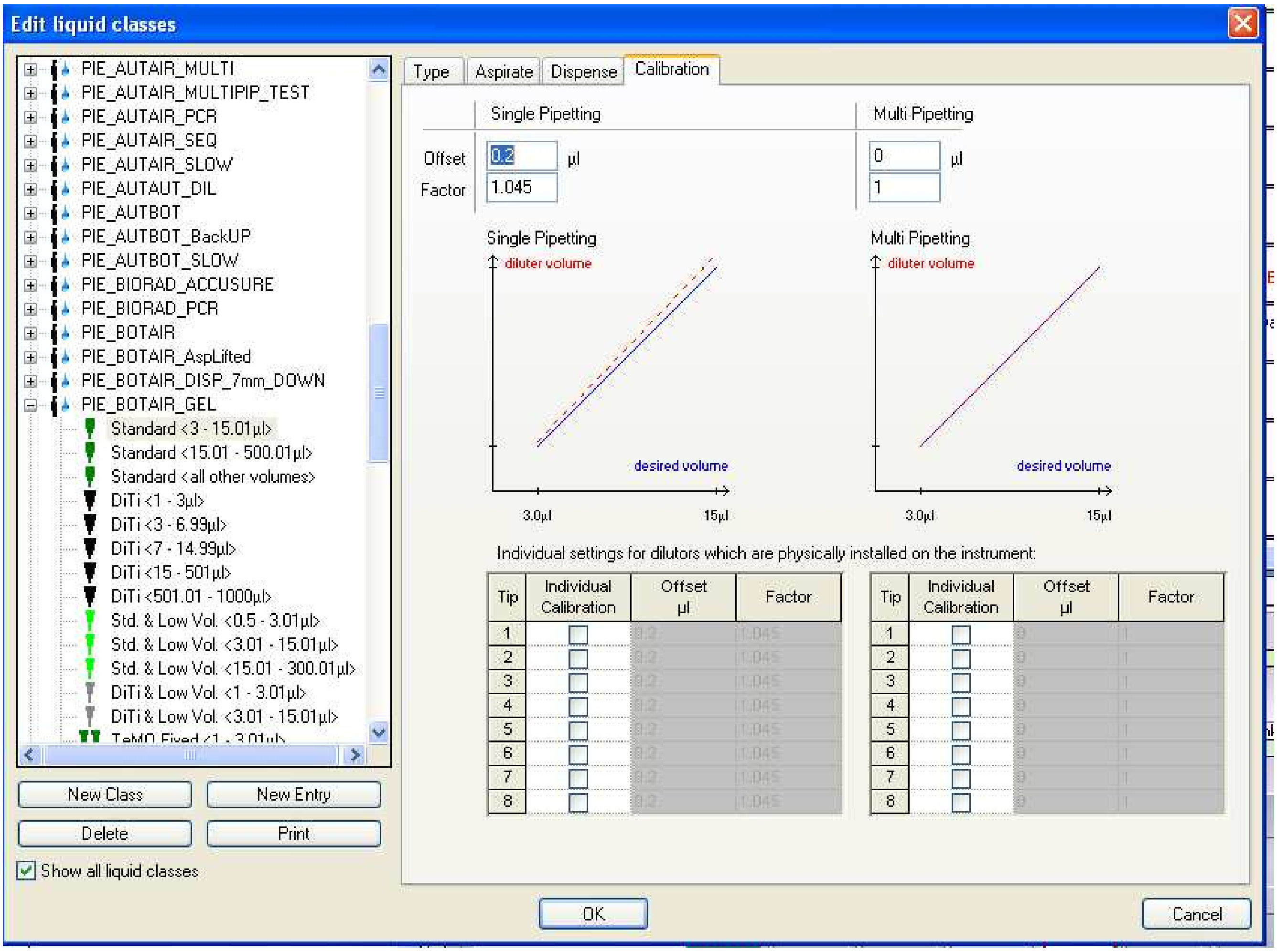
Retract Tips to: z-dispense; 0 mm

Retract Speed 50 mm / s

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.

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



Edit liquid classes

- PIE_AUTAIR_MULTI
- PIE_AUTAIR_MULTIPIP_TEST
- PIE_AUTAIR_PCR
- PIE_AUTAIR_SEQ
- PIE_AUTAIR_SLOW
- PIE_AUTAUT_DIL
- PIE_AUTBOT
- PIE_AUTBOT_BackUP
- PIE_AUTBOT_SLOW
- PIE_BIORAD_ACCUSURE
- PIE_BIORAD_PCR
- PIE_BOTAIR
- PIE_BOTAIR_AspLifted
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- PIE_BOTAIR_GEL
  - Standard <3 - 15.01µl>
  - Standard <15.01 - 500.01µl>
  - Standard <all other volumes>
  - DiTi <1 - 3µl>
  - DiTi <3 - 6.99µl>
  - DiTi <7 - 14.99µl>
  - DiTi <15 - 501µl>
  - DiTi <501.01 - 1000µl>
  - Std. & Low Vol. <0.5 - 3.01µl>
  - Std. & Low Vol. <3.01 - 15.01µl>
  - Std. & Low Vol. <15.01 - 300.01µl>
  - DiTi & Low Vol. <1 - 3.01µl>
  - DiTi & Low Vol. <3.01 - 15.01µl>
  - TeMO Fixed <1 - 3.01µl>

New Class | New Entry

Delete | Print

☑ Show all liquid classes

Type | Aspirate | Dispense | Calibration

	Single Pipetting		Multi Pipetting	
Offset	0.2	µl	0	µl
Factor	1.045		1	

Individual settings for dilutors which are physically installed on the instrument:

Tip	Individual Calibration	Offset µl	Factor
1	□	0.2	1.045
2	□	0.2	1.045
3	□	0.2	1.045
4	□	0.2	1.045
5	□	0.2	1.045
6	□	0.2	1.045
7	□	0.2	1.045
8	□	0.2	1.045

Tip	Individual Calibration	Offset µl	Factor
1	□	0	1
2	□	0	1
3	□	0	1
4	□	0	1
5	□	0	1
6	□	0	1
7	□	0	1
8	□	0	1

OK | Cancel

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.

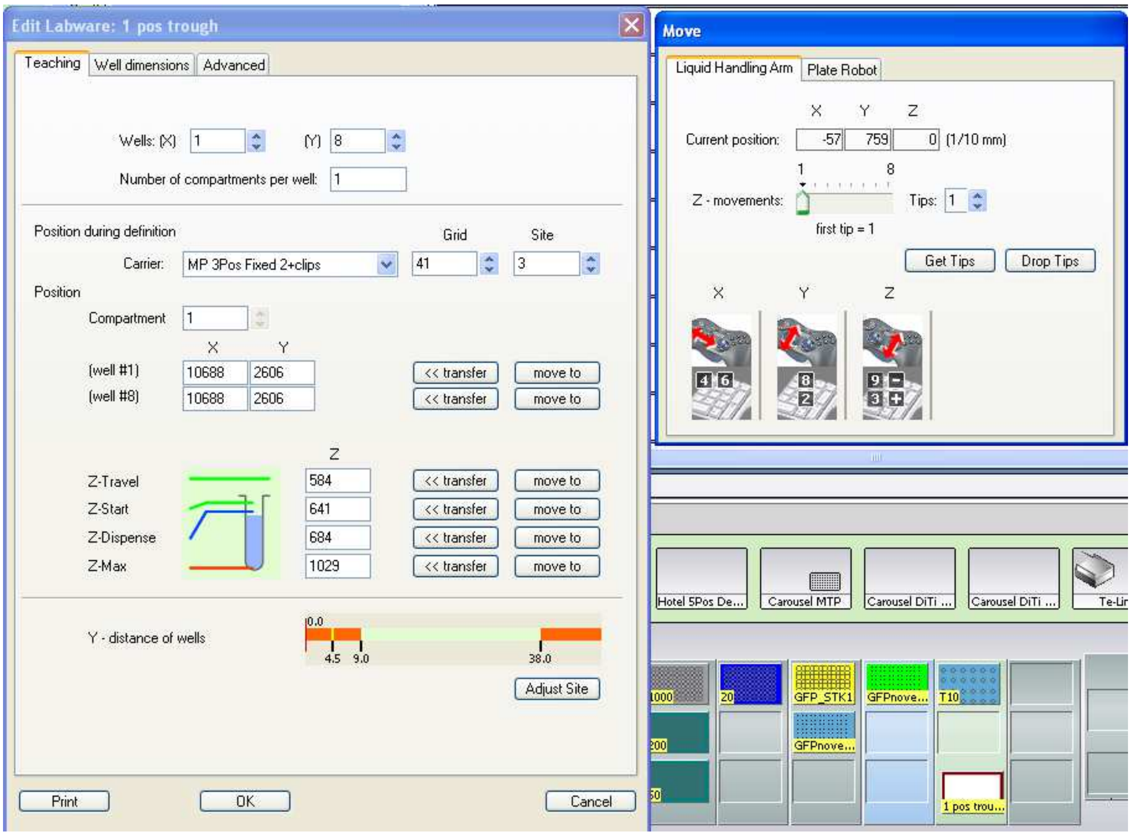
For example: 7 + 0.1 where 7 is the amount of liquid to aspirate and 0.1 is the offset.

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.

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



Edit Labware: 1 pos trough

Wells: (X) 1 (Y) 8

Number of compartments per well: 1

Position during definition

	Carrier:	Grid	Site
	MP 3Pos Fixed 2+clips	41	3

Position

Compartment 1

	X	Y
(well #1)	10688	2606
(well #8)	10688	2606

	Z
Z-Travel	584
Z-Start	641
Z-Dispense	684
Z-Max	1029

Y - distance of wells

Move

Current position: X -57 Y 759 Z 0 (1/10 mm)

Z - movements: 1 8

first tip = 1

Tips: 1

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 tunning 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.

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