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Abstract

In this deliverable we deal with developing methods for testing the robot capabilities and developing computational tools for analysing testing results.

Purpose:

The purpose of this deliverable is to show how our work on developing experimental and computational tools for monitoring the liquid handling robots operations improves robot function in terms of accuracy and reproducibility.

Backround:

Gradually, an increasing number of actions are required from the robot. As a result, it is increasingly becoming difficult to monitor and tune the robot with existing technology: standard weight-based pipetting tests by Tecan.

What we did:

We have developed web experimental tools and an application on google app engine that tests the pipetting actions of the robot (See http://robotqcapp.appspot.com/).
In parallel, we have studied robot configuration tunning from Tecan experts and used this knowledge to reconfigure the robot according to our QC results. As a result, we developed a powerful tool to tune and maintain the robot.
In addition, we have developed a system that documents scripts that run on the robot in order to monitor and graphically report errors that arise during construction.

Keywords[7]:

Django, google app engine, google charts, robotics, web

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

Introduction

a. **Aim / Objectives:**
Develop effective QC methodologies for liquid handling robots.

b. **State of the Art :**
Standard Weight based monitoring of robot pipetting reactions (offered by Tecan).

c. **Innovation:**
Establishing a general purpose method for monitoring the accuracy of liquid handling robots.

2. Implementation:

We mapped and isolated all the pipetting actions required in order to perform our protocols. We defined 20 different pipetting operations we needed to test (See table below). We wrote a special script that tests each of these operations using a controlled dye absorbance method. The accuracy of these 20 liquid handling operations was then monitored using a desktop plate reader.

The QC system works as follows:
The robot runs a test script designed to test a single operation used in DNA editing.
For example, the script below** was designed to test the robot pipetting 44ul with a very specific features' for example: a certain type of liquid (liquid class PIE_AUTBOT), Tip type: 200ul, tip mode: keep tip, destination plastic: 96 Well PCR Plate". All the parameters mentioned above affect the pipetting operation and require testing.
After the robot finishes, we transfer the destination plate into a plate reader plate and read the absorbance with the plate reader. In addition, compare all our results to accurate manual measurement of the same operation.
Once the plate reader finishes reading and produces an Excel file of the results they are uploaded to our dedicated QC application in:
http://robotqcapp.appspot.com/ and we add a new experiment with an html form.

	done	volume	tiptype	tipmode	src plastic	dst plastic
1		150	200	keeptip	one position trough	deep well
2	V	4	200	default	deep well	plate reader plate
3		25	200	keeptip	T10 -eppendorf	Deep well
4		8	200	default	deep well	96 Well PCR Plate(tetrad)
5		16	50	default	96 Well PCR Plate(tetrad)	96 Well PCR Plate- Roche / BIORAD
6	V	4	200	MULTIPIP	T10 -eppendorf	96 Well PCR Plate(tetrad) ,96 Well PCR Plate- Roche / BIORAD
7		10.5	200	MULTIPIP	T10 -eppendorf	96 Well PCR Plate- Roche / BIORAD

8	V	8	50	default	96 Well DeepWell square	96 Well PCR Plate-Roche / BIORAD
9		16	50	default	deep well	96 Well PCR Plate-Roche / BIORAD
10	V	8	200	default	96 Well PCR Plate(tetrad)	96 Well DeepWell square
11		35	200	default	96 Well PCR Plate-Roche / BIORAD	96 Well PCR Plate(tetrad)
12		20	200	default	96 Well PCR Plate-Roche / BIORAD	96 Well PCR Plate-Roche / BIORAD
13		5	50	keeptip	T10 -eppendorf	96 Well PCR Plate(tetrad)
14	V	5	20	default	96 Well PCR Plate-Roche	96 Well PCR Plate(tetrad)
15	V	6	200	MULTIPIP	T10 -eppendorf	96 Well PCR Plate(tetrad)
16		24	200	default	96 Well PCR Plate(tetrad)	96 Well PCR Plate(tetrad)
17		36	50	default	96 Well PCR Plate-Roche / BIORAD	96 Well PCR Plate(tetrad)
18	V	5	20	default	T10 -eppendorf	96 Well PCR Plate(tetrad)
19	V	5	20	default	96 Well PCR Plate-Tetrad	96 Well PCR Plate(tetrad)
20		5	20	default	96 Well PCR Plate-Biorad	96 Well PCR Plate(tetrad)

production role	liquid class	liquid type	frequency
wetting of primers	PIE_AUTAIR_LowVol	ddw	rare
prepering reagents for platereader	PIE_AUTAIR_LowVol	ddw	rare
dil of primeres after phosphorilation	PIE_AUTBOT	ddw	rare
template elongation	PIE_AUTAIR_DISP_7mm_DOWN	ddw	popular
template elongation for PCR	PIE_BOTBOT_SLOW	ddw	
CHECK THE MIX OF ELONGATION	PIE_LMB	Enzyme	
CHECK THE MIX OF PCR	PIE_LMB	enzyme	
CHECKING THE template	PIE_AUTAIR_PCR	ddw	
CHECKING THE PRIMER.	PIE_AUTAIR_PCR	ddw	

checking the dilution of Elongation	PIE_BOTBOT_SLOW	ddw	
checking the unite of single strand	PIE_BOTAIR	ddw	
checking unit finales	PIE_BOTBOT	ddw	
checking the LB syber	PIE_BOTAIR_GEL	LB_syber	popular
checking the template of the gel	PIE_BOTAIR_GEL_FOR_SRC_PLATE	ddw	popular
checking the mix of Lambda	PIE_LMB	enzyme	
checking the template of Lambda	PIE_BOTBOT_SLOW	ddw	
checks the unification of lambda template before zymo	PIE_BOTBOT_SLOW	ddw	
checking the template of the gel	PIE_BOTAIR_GEL	loading buffer	popular
checking the template of the gel	PIE_BOTAIR_GEL_FOR_SRC_PLATE	ddw	popular
checking the template of the gel	PIE_BOTAIR_GEL	ddw	popular

Caption:

The above tables show all the robot operations that resulted from our mapping of unique DNA editing operations that require monitoring. Each row represents a unique robot operation performed during DNA editing.

We had to test each operation several times against our QC system in order to tune it.

Below is an exemplary script:

```
**

###########  DOCS  ###############################
DOC
DNE Script
Project Name: OctTask
Created on 24-Jan-2010 13:39:35
ENDDOC

###########  TABLE  ###############################
TABLE TABLE_DNE

###########  HEADER  ###############################
OPTION UPDATE_VOL_DB
OPTION VERIFY_TABLE

#GLOBAL REAGENTS

REAGENT COLOR T10 1 PIE_AUTBOT 4#src
REAGENT DDW          BUF12 1 PIE_TROUGH_AUTAIR 8

LABWARE PCR P11 "96 Well PCR Plate"#dest
LABWARE trough BUF12 "1 pos trough"

############ END OF DEFAULT HEADER ########################


##########################################################################
SCRIPT


############################################################################
#########


DIST_REAGENT2 DDW P11:A1+96 106 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP#dispensing water to dst
DIST_REAGENT2 COLOR P11:A1+96 44 PIE_AUTBOT
TIPTYPE:200,TIPMODE:KEEPTIP#dispensing color to
```

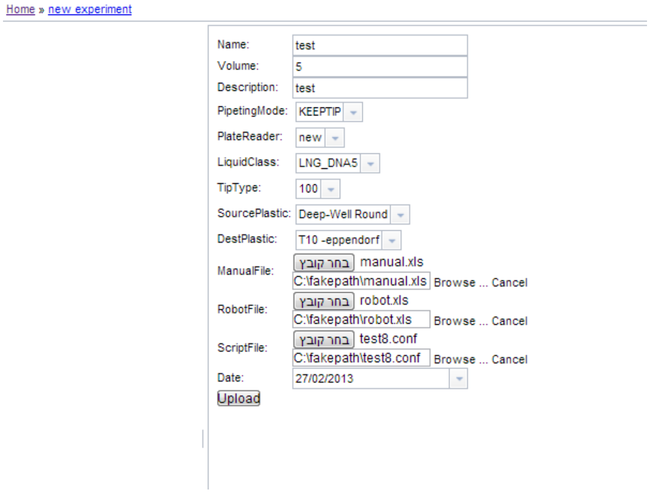
ENDSCRIPT

Explanation:
The script above is a sample script for robot testing.
In this script we dispense 106 ul of water from trough to PCR plate and 44ul from Eppendorf tube into PCR plate. Then we pass the liquid from the PCR plate into plate reader plate and measure its absorbance.

The output of these scripts are many 96 well plates with predefined amounts of dye in each well, corresponding to the amount of dye the robot should have pippeted into those wells in each of the script. The plates are then measures in a UV absorbance plate reader and the absorbance of the dye in each well is used in order to compute the volume that the robot has pippeted in each well, using standard curves that we have created:

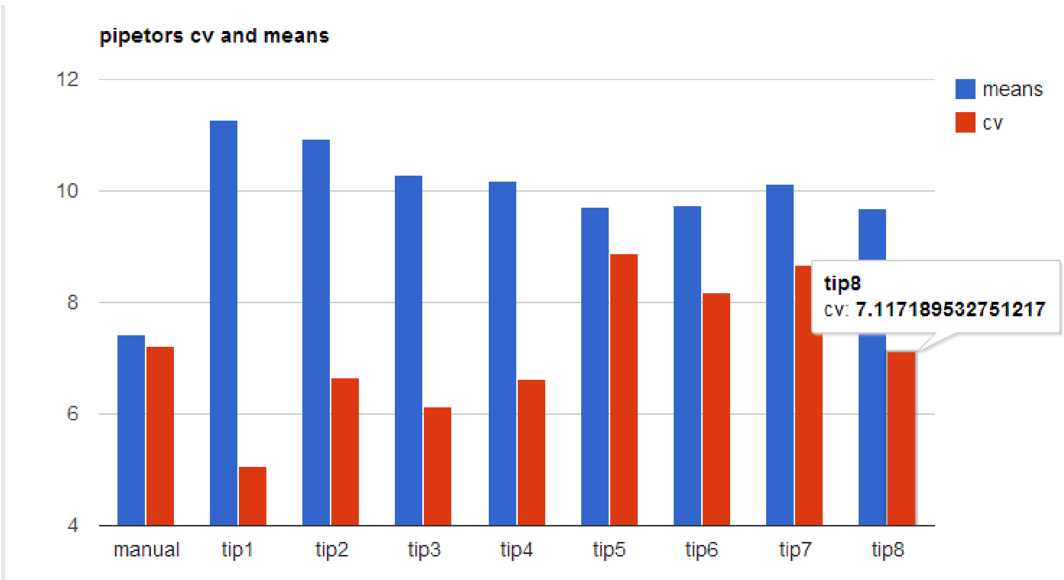
Caption:
The Tecan pate reader and 96 well plates we use to experimentally determine the robot;s pippeting accuracy and reporoducibility.

Below is a new experiment definition page in the online application we developed for monitoring the robot using dedicated scripts:


Home » new experiment

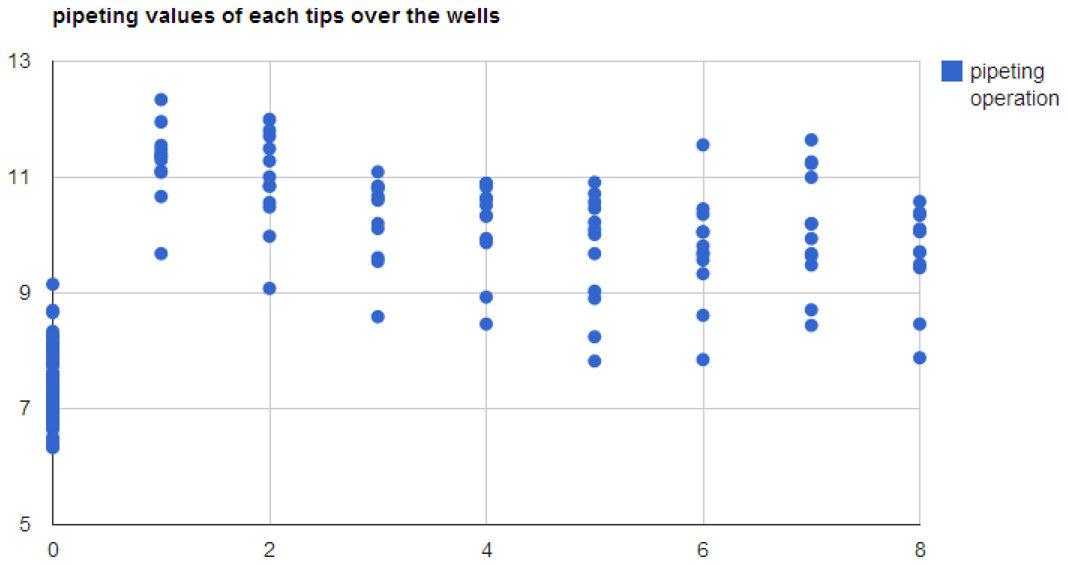
Name: test
Volume: 5
Description: test
PipetingMode: KEEPTIP
PlateReader: new
LiquidClass: LNG_DNA5
TipType: 100
SourcePlastic: Deep-Well Round
DestPlastic: T10 -eppendorf
ManualFile: בחר קובץ manual.xls C:\fakepath\manual.xls Browse ... Cancel
RobotFile: בחר קובץ robot.xls C:\fakepath\robot.xls Browse ... Cancel
ScriptFile: בחר קובץ test8.conf C:\fakepath\test8.conf Browse ... Cancel
Date: 27/02/2013
Upload

Caption:
The screenshot above is of a HTML form from the QC website for a new experiment. Here the user defines parameters for a new experiment:
Name, volume , description, liquid class, pipetting mode, liquid class , source plastic, dest plastic, manual plate reader report, robot plate reader report, script file.



Caption:
The report above is reproduced in google charts new client side technology.
X axis is tips. Y axis is volumes and CVs.
Its purpose is to show for each tip its average volume of pipetting and the CV average value of it. CV means: Coefficient of variation. This is the same standard Tecan is using to define accuracy in their tests.
The most right column stands for a manual operation being done in parallel

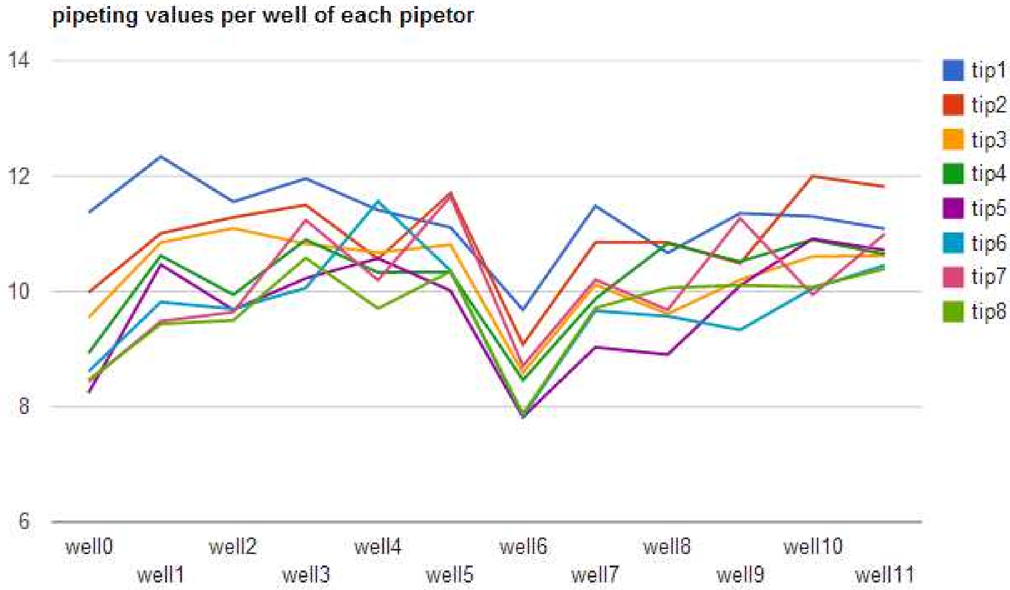
To the robot operations, in order to have a better perspective and Calibration.



Caption:

The above chart represents isolated pippeting volumes of each tip.

X axis is tips. Y axis is volumes. The most right column stands for the manual operation. This gives us a good view on the accuracy of each tip.



Caption:

X axis stands for wells. Y axis stands for volumes. Each line stands for tip
So, for each tip one can see the volumes in each well.
This information is important because sometimes a specific well has problems.
For example, you can see that for all tips, well 6 had a low volume. This information could not be achieved without this kind of chart.

After a few tests are made on the same operation, we can see a history of these tests because all the data is saved on google data store and represented on a searchable table:

Home » experiments

Name	Description	Pipeting Mode	Liquid Class	Tip Type	Source Plastic	Dest Plastic	plate reader	date	Edit Experiment details
test	test	KEEPTIP	PIE_BOTBOT_MAYA	50	Plate-Reader	T10 - eppendorf	old	2013-02-26	Edit Experiment Details
test	test	KEEPTIP	LNG_CE	200	Deep-Well Round	T10 - eppendorf	old	2013-02-26	Edit Experiment Details
test	test	KEEPTIP	LNG_DDW	100	T10 - eppendorf	Deep-Well Round	old	2013-02-26	Edit Experiment Details
test21	test21	KEEPTIP	PIE_BOTAIR_GEL	200	T10 - eppendorf	Bio-Rad	old	2013-02-26	Edit Experiment Details
test	test	KEEPTIP	LNG_DNA5	100	Deep-Well Round	T10 - eppendorf	new	2013-02-27	Edit Experiment Details

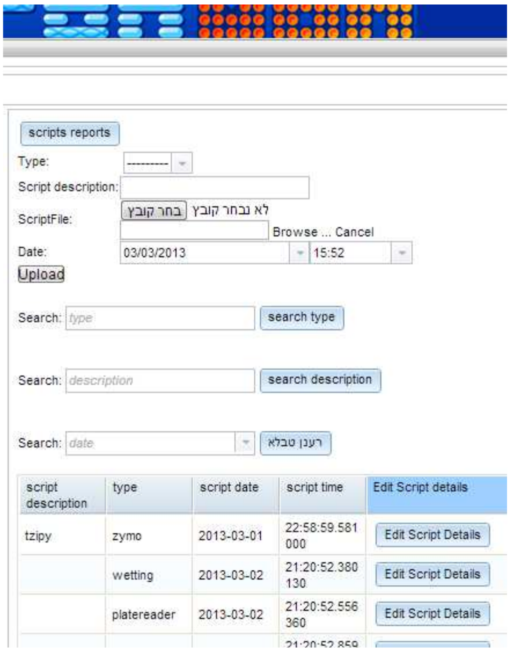
Caption:

The screenshot above shows a dynamic, searchable html table from our web site at
http://robotqcapp.appspot.com/

Each row stands for an experiment. If you click the right most column, you will be able to see the details and reports of the experiment you clicked on.

The robot erros system:

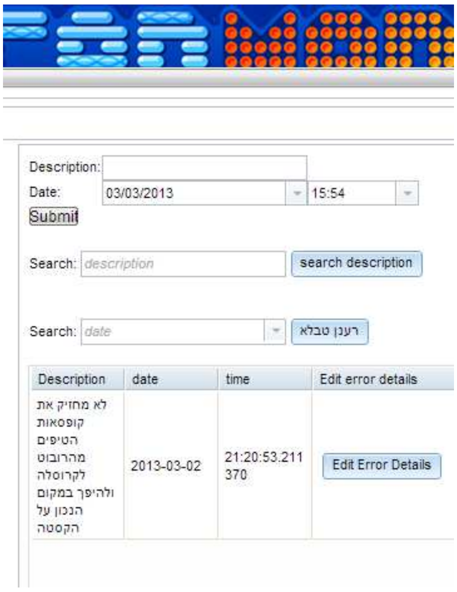
We recognized the need to document the history of scripts that run on the robot as a tool for production monitoring and debugging. As a result, we developed a feature on our web application where robot runs are documented, as well as their errors (if they occur) so we monitor and fix them:



Caption:

In the screenshot above we can see from top to bottom:

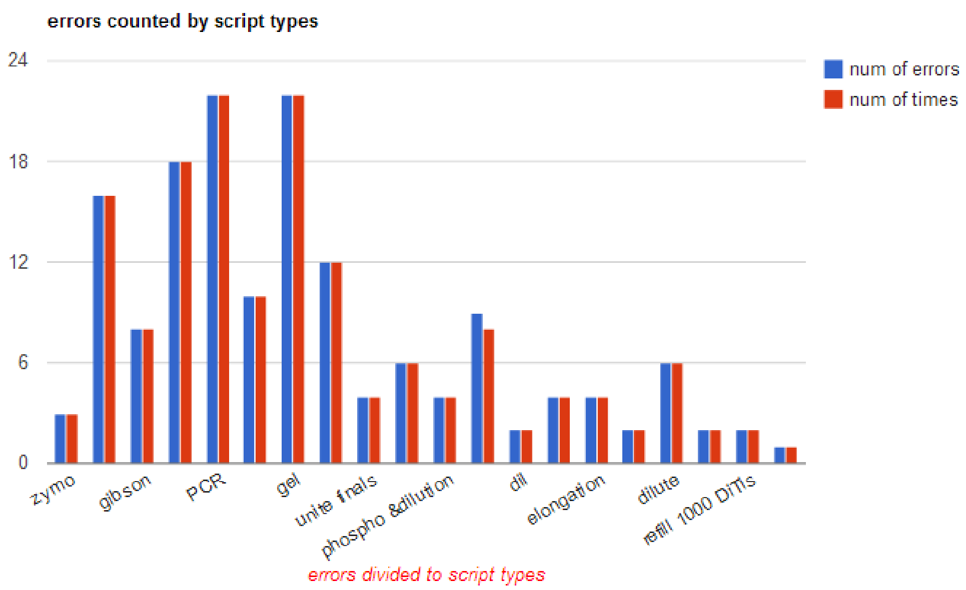
1. A button to view graphic scripts reports.
2. Below we can see a form to create a new script and upload a file.
3. Below there are 3 search criteria:
    1. Type of script ran on the robot
    2. Script description.
    3. Scripts run date.



Caption:
In the screenshot above we can see a web page of a script. This page contains from top to bottom:

1. A new error form
2. Two search criteria.
3. Table of errors connected to the script.

In this way we can view all script's errors.



Caption:

Finally, in the screenshot above we can view a chart written in google charts technology displaying basic statistics about the running if various acripts on the robot. X axis is for script type. Blue is for number of errors in the script type. Red is for number of times this type of script has run. Y axis is for number of errors. Using this tool we can see which script is more error prone and monitor is performance over time.

3. Results

The major result of the work described above is that is that we are able to monitor and tune every pipetting machine much better than we could before and, as a result, our robot is more tuned and production ready.

4. Conclusions

Much is achieved and much more to achieve.

Our plans are

- Continue developing the QC system so we can monitor all robot operations and write them to our database.
- Make a mathematical standard for quality of experiment and draw a chart of progress over time of this experiment.
- Publish our application so more labs could enjoy it.

5. Abbreviations

List all abbreviations used in the document arranged alphabetically.

QC	Quality control
Tecan	A liquid handling robot