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Report on methods of monitoring Y operation biochemistry

Abstract

The use of automated robotic platforms is the state of the art solution for executing DNA editing tasks with high-throughput. Despite the obvious advantages robotics offer in terms of throughput, scalability, documentation and programmability it often suffers from critical malfunctions and inaccuracies. The success of the CADMAD vision will be determined, to a large extent, by its most vulnerable component. In order to verify that malfunctions and inaccuracies of the robotic system is not a sensitive and vulnerable component of our system we have to integrate extensive quality control measures into our production flow. The following deliverable specifies the R&S for the methods that will be used in order to monitor and control the accuracy of robotic liquid handling.

Keywords[7]:

Automation, quality control, liquid handling,

Introduction

Liquid handling robots are the technology of choice for up scaling wet lab biology. We at the CADMAD consortium are developing both software and biochemistry adapted and aimed at integration with a highly automated environment. As a result we rely heavily on the correct operation of lab robotics for proper execution of our developed methods.

In our experience lab automation robots are very high maintenance and frequently require tuning, calibration and periodical treatments. After approximately 7 years of extensively employing state of the art lab robots for various applications we are now confident that dedicated procedures for testing, tuning and calibrating of automation equipment must be developed in order to keep the production pipeline that depends on them running without detrimental error to the production process. The subject of this deliverable is to clearly define the requirements and specifications for the dedicated actions that will be taken in order to mitigate the risks associated with automation errors.

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

1. Implementation

The implementation of this deliverable is in the form of R&S for detailed robotic scripts that will be used in order to monitor the operation of our automated robotic system.

These scripts will run the robot in a dedicated mode which will sample the wide range of robotic operations, mainly various types of liquid handling, which we use in DNA editing. These include aspiration and dispensing of various types of liquids with varying properties, from various types of carriers, using different tip types, plate positions, various speeds, heights etc.

2. Results

1) Performing dedicated Y operations in real time in parallel to the construction and editing of a new DNA library. This will serve as a positive control of the entire basic construction step. This control Y operation will be a well-studied Y operation which we performed in the past and verified that works properly.
2) Developing dedicated robotic scripts for testing each and every one of the many scripts that we employ during automated DNA editing. These include:
    1) Scripts for testing the liquid handling in enzymatic reactions such as PCR, Lambda exonuclease and elongation.
    2) Scripts for testing automated DNA purification
    3) Scripts for testing automated cloning

We will define the allowed margin of error allowed for each of the processes and develop methods to monitor cases in which processing by the robot exceeds this margin.

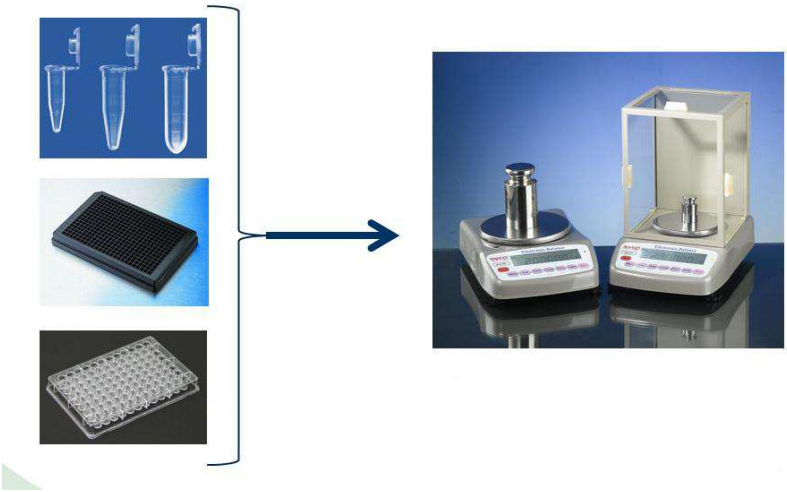
For example, we will use fluorescent materials in order to monitor the fidelity of liquid handling of the robot. This will be done by creating scripts that simulate DNA editing tasks but using a fluorescent molecule and water. The results of these scripts will then be taken to a dedicated plate reader which monitors the amount of fluorescence in each well, compares it to the expected value and computes whether the errors are within the allowed limits. Similar methods may also be developed using analytical scales which weigh the water pipetted instead of the radiation emitted for the pipetted material.

For each process the allowed error range will be established experimentally.

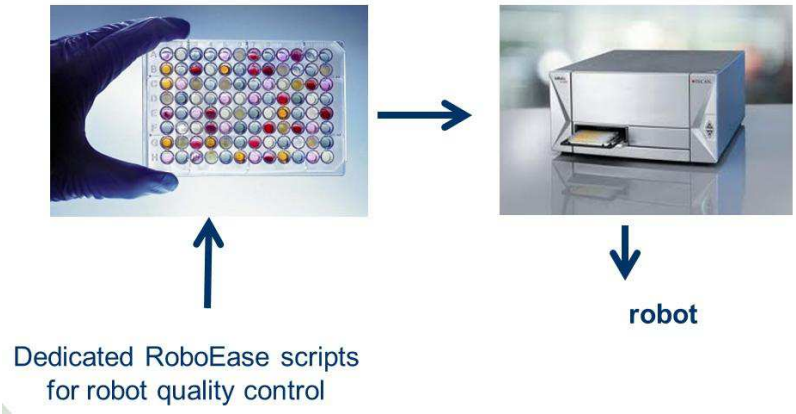
We evaluated 2 options for the monitoring of robot operations: (a) analytical scales and (b) plate reader.

We eventually chose plate reader instead of analytical scales since: (1) it offers higher throughput, automation (2) mimics the various plastic-ware used on the robot and (3) robotic plates can be used directly for plate reader measurement without .

a. – analytical scales



b. – plate reader

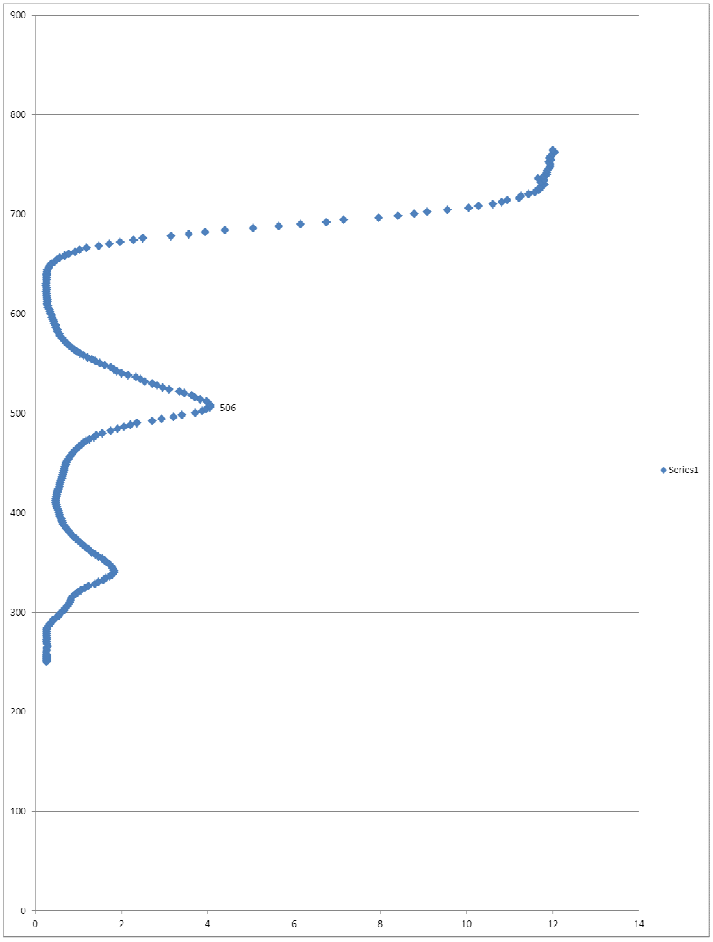


Additional information showing accomplishments made in monitoring liquid handling operations:

1) See below plate reader experiments which we used to choose an appropriate dye for monitoring liquid handling, determining the optimal wavelength for monitoring it, creating standard curves for its concentration and choosing the concentration in which its plate reader measurements is most accurate.

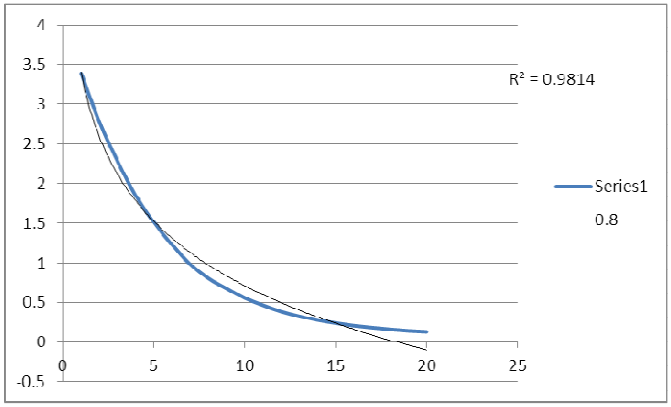
Determining an optimal wavelength measurement for a green dye using an absorbance scan in a plate reader

used for monitoring the operation of the robots liquid handling:

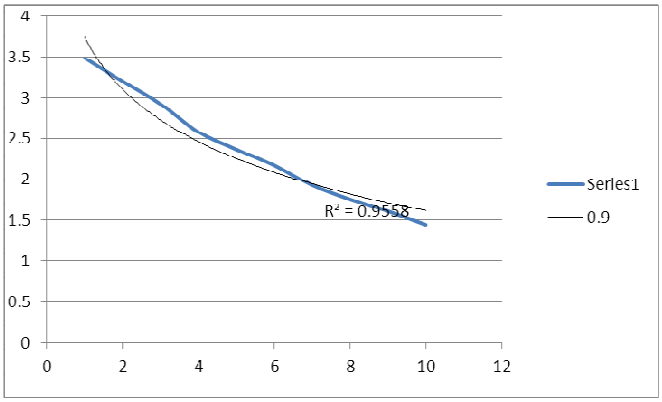


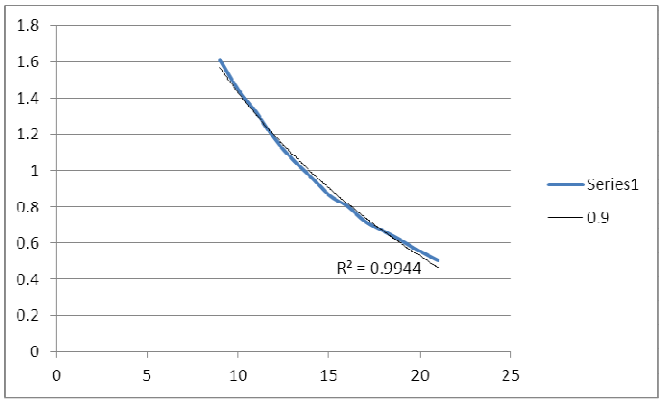
We chose 506 nm as the wavelength in which to perform liquid handling monitoring since it exhibited the highest signal to noise ratio, as evident from the graph.

Comparison of a 0.8X dilution series over 20 dilutions manually in order to create a standard curve of the green dye. The results (R^2= 0.98) show good adherence to the corresponding mathematical function.



We then continued to to make an additional 0.9X dilution series over 20 dilutions and analyzed different concentrations to determine which is most accurately read in the plate reader. Below is this standard curve analyzed seperately for dilutions 1-10 and dilutions 10-20, with the 10-20 dilutions showing a higher R^2 value (0.9558 compared to 0.9944).





Our results show that measurements are most accurate between dilutions 10-20 and we are designing our scripts so that liquid handling will be monitored using these concentrations.

2) See below an exemplary dedicated script for monitoring a wide range of liquid handling operations routinely used in the Y operation. The scripts below is divided (in yellow)

into the different parts that test distinct operations on the robot. For example, the first part (Wetting reagents in yellow) tests the accuracy of the automated wetting of primers that are shipped to us from a commercial provider freeze dried. Each such subscript generates at least 48 repetitions of the same operation so that we have sufficient statistics on the accuracy of the operation. The results will be read in a plate reader and automated computational tools (to be developed) will be used in order to analyze the results.

SCRIPT

PROMPT NEED TO BE ON THE TABLE: P4- DEEP WELL FOR REAGENTS, P7 - ROCHE PLATE, P8- BIORAD, TP1 - TETRAD PLATE.
PROMPT NEED 10 PLATES OF PLATE READER - 9 POSITION: P3,P5,P6,P11,P13,P14,P15,TP2,S1,S2.(there will be one change of plate)

###################### Wetting reagents ############################
THIS SCRIPT CHECKS THE WETTING OF PRIMERS. NEED TO BE THERE ALREADY COLOR.

DIST_REAGENT2 DDW P4:A1+48 DDW_LIST DEFAULT
TIPTYPE:1000,TIPMODE:KEEPTIP
MIX_WELLS P4 A1+48 WET_MIX_TIMES WET_MIX_VOL PIE_MIX_AUT

###################### Preparing reagents for plate reader (196 + 4 instead Of 55 ul+ 4 from the original script ##################################
196 UL OF DDW AND 4 UL OF COLOR DILUTION (700 UL DDW + X UL OF COLOR)
DIST_REAGENT2 DDW P15:A1+48 196 PIE_TROUGH_AUTAIR
TIPTYPE:1000,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS P4:A1+48 P15:A1+48 4 PIE_AUTAIR_LowVol
MIX:PIE_MIX:10x45
######################Dilution of Primer +Phosphorylation ##########################

THIS SCRIPT CHECKS THE PHOSPHO MIX (156 UL OF DDW AND 44 UL OF COLOR)
DIST_REAGENT2 DDW P5:A1+48 156 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
DIST_REAGENT2 PHOS_MIX P5:A1+48 44 PIE_AUTBOT TIPTYPE:200,TIPMODE:KEEPTIP

##Elongation ####################

FIRST WE CHECK THE TEMPLATE FROM BOTH SOURCE PLATE ONE DEEP WELL AND ONE TETRAD PLATE.
THERE ARE TWO TYPE OF SCRIPT FOR ELONGATION- AUT_ELN_OLIGO AND AUT_ELN

##CHECKING TEMPLATE NUMBER 1 - 192 UL(WATER) + 8 TEMPLATE - COLOR DILUTION (700 UL DDW + X UL OF COLOR) TP2: A1+48
DIST_REAGENT2 DDW TP2:A1+48 192 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
PREPARE_LIST ELN_LIST1 TP2 A1+48 PIE_AUTAIR_DISP_7mm_DOWN TIPTYPE:200,MIX:5x10

##CHECKING TEMPLATE NUMBER 2 - 184 UL(WATER) + 16(TEMPLATE - COLOR) TP2: A7+48
DIST_REAGENT2 COLOR TP1:A1+48 100 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
DIST_REAGENT2 DDW TP2:A7+48 184 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS TP1:A1+48 TP2:A7+48 16 PIE_BOTBOT_SLOW TIPTYPE:50

SECOND WE CHECK THE MIX OF ELONGATION - NEED TO CHECK IF THE LIQUID CLASS PIE_BIORAD_ACCUSURE EFFECT THE ACTION EVEN TOUGH IT IS A TETRAD PLATE.
196 DDW + MIX - 4 UL OF COLOR (S1:A1+48)

DIST_REAGENT2 DDW S1:A1+48 196 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP

#DIST_REAGENT2 DDW TP1:A7+48 100 DEFAULT
TIPTYPE:1000,TIPMODE:KEEPTIP
DIST_REAGENT2 ELN_Mix_X5 S1:A1+48 4 PIE_BIORAD_ACCUSURE
TIPTYPE:AUTO,TIPMODE:MULTIPIP,MIX:PIE_MIX:5x7

Dilution of elongation

##192+8
CHECK DILUTION OF ELONGATION 192 UL OF WATER AND 8 UL (COLOR) OF ELONGTION PRODUCT.
DIST_REAGENT2 DDW P6:A1+48 192 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS TP1:A1+48 P6:A1+48 8 PIE_BOTBOT_SLOW
TIPTYPE:200,MIX:PIE_MIX_AUT:6x200

PCR

FIRST WE CHECK THE MIX. MIX- 10.5 UL (COLOR) + TEMPLATE 8 UL (WATER) + PRIMER
DIST_REAGENT2 DDW P3:A1+48 189.5 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
DIST_REAGENT PCR_Mix_X5 P3 A1+48 10.5 PIE_AUTAIR_PCR
TIPTYPE:200,TIPMODE:MULTIPIP

CHEACKING THE TEMPLATE.
192 UL OF WATER + 8 UL OF TEMPLATE (COLOR - DIL 192 DDW + 8 COLOR)
DIST_REAGENT2 DDW P3:A7+48 192 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
PREPARE_LISTPCR1 P3 A7+48 PIE_AUTAIR_PCR
TIPTYPE:50,MIX:LCWMX:3xMIX_VOLUME

CHECKING THE PRIMER. CHECKING IT FROM PLATE OF DW (P4) OF WETTING REAGENTS.

184 UL OF WATER + 16 UL PRIMER (COLOR) .
DIST_REAGENT2 DDW P5:A7+48 184 DEFAULT TIPTYPE:1000,TIPMODE:KEEPTIP
PREPARE_LIST PCR1 P5 A7+48 PIE_AUTAIR_PCR
TIPTYPE:50,MIX:LCWMX:3xMIX_VOLUME

################################## unite SINGLE STRAND ####################
PROMPT REFILL THE COLOR TUBES IN T10 22+3
DIST_REAGENT2 DDW P7:A1+48;P8:A1+48 100 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP
#THIS LINE IS FOR NEXT SCRIPT -GEL
#THIS SCRIPT CHECKS THE UNIT FINALES 0F 35 UL COLOR AND 165 UL DDW.
#IT CHECKS THIS SCRIPT FOR THE ROCHE(P7) & BIORAD (P8) PLATE
DIST_REAGENT2 COLOR P7:A7+48;P8:A7+48 85 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP
DIST_REAGENT2 DDW P6:A7+48;P13:A1+48 165 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS P7:A7+48 P6:A7+48 35 PIE_BOTAIR TIPTYPE:200
TRANSFER_LOCATIONS P8:A7+48 P13:A1+48 35 PIE_BOTAIR TIPTYPE:200

#################### unite finales - DIFFERENT LIQUID CLASS ################
PROMPT FILL IN THE COLOR TUBES T10 22*3 AGAIN
DIST_REAGENT2 COLOR P7:A7+48;P8:A7+48 85 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP

DIST_REAGENT2 DDW S1:A7+48;P11:A1+48 180 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS P7:A7+48 S1:A7+48 20 PIE_BOTBOT
TRANSFER_LOCATIONS P8:A7+48 P11:A1+48 20 PIE_BOTBOT

################################## GEL ################################
#PROC Source_Loc Target_Loc

#CHECK THE LB SYBER - 195 UL DDW + 5 UL LB SYBER (COLOR)
DIST_REAGENT2 DDW P14:A1+96 195 DEFAULT TIPTYPE:200,TIPMODE:KEEPTIP
DIST_REAGENT2 LB_SYBR P14:A1+48 5 PIE_AUTAIR_GEL
    TIPTYPE:50,TIPMODE:KEEPTIP

#CHECK THE TEMPLATE - 195 UL DDW + 5 UL TEMPLATE (COLOR)
IT CHECKS THE BIORAD PLATE AND THE ROCHE PLATE.
TRANSFER_LOCATIONS P7:A7+48 P14:A7+48 5 PIE_BOTAIR_GEL TIPTYPE:20
TRANSFER_LOCATIONS P8:A7+48 P13:A7+48 5 PIE_BOTAIR_GEL TIPTYPE:20

LAMBDA

#CHEACKING THE LAMBDA MIX - 6 UL.
DDW 194 UL + MIX (COLOR) 6 UL
DIST_REAGENT2 DDW S2:A1+48 194 DEFAULT TIPTYPE:200,TIPMODE:KEEPTIP
DIST_REAGENT2 Lambda_Mix_X5 S2:A1+48 6 PIE_LMB
TIPTYPE:AUTO,TIPMODE:MULTIPIP

#CHEACKING THE TEMPLATE OF LAMBDA - 24 UL.
#IT CHECKS THE SCRIPT FOR BIORAD(P8) & ROCHE(P7) PLATE
DIST_REAGENT2 DDW S2:A7+48;P15:A7+48 176 DEFAULT
TIPTYPE:200,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS P7:A7+48 S2:A7+48 24 PIE_BOTBOT_SLOW
TIPTYPE:200,MIX:PIE_MIX_SLOW:4x25
TRANSFER_LOCATIONS P8:A7+48 P15:A7+48 24 PIE_BOTBOT_SLOW
TIPTYPE:200,MIX:PIE_MIX_SLOW:4x25

################################## ZYMO reaction cleanup ######################

```
# FEW LINES IN THE SCRIPT THAT DOESNT INVOLVE COLONGH
#164 UL DDW (INSTED OF 100 UL IN THE ORIGINAL SCRIPT) + 36 UL TEMPLATE.
PROMPT REPLACE PLATE ON P15 TO A NEW PLATE.

#IT CHECKS THE SCRIPT FOR BIORAD(P8) & ROCHE(P7) PLATE
DIST_REAGENT2 DDW P11:A7+48;P15:A1+48 164 PIE_PB
TIPTYPE:1000,TIPMODE:KEEPTIP
TRANSFER_LOCATIONS P7:A7+48 P11:A7+48 36 PIE_BOTBOT_SLOW
TIPTYPE:50,MIX:PIE_MIX_AUT:7x45
TRANSFER_LOCATIONS P8:A7+48 P15:A1+48 36 PIE_BOTBOT_SLOW
TIPTYPE:50,MIX:PIE_MIX_AUT:7x45

##########################################################################
#LABWARE TEST_WetReagent P4 "96 Well DeepWell square"
#LABWARE TEST_phos_mixPCR P5 "96 Well PlateReader"
#LABWARE TEST_RDR1 P15 "96 Well PlateReader"
#LABWARE TEST_DilELN_UnitSS P6 "96 Well PlateReader"
#LABWARE TEST_PCR P3 "96 Well PlateReader"
#LABWARE TEST_PCRROCHE1 P7 "96 Well PCR Plate- Roche"
#LABWARE TEST_BIORAD1 P8 "96 Well PCR Plate -Biorad"
#LABWARE TEST_GEL P14 "96 Well PlateReader"
#LABWARE TEST_MixELN_UnitFINALES S1 "96 Well PlateReader"
#LABWARE TEST_LMB S2 "96 Well PlateReader"
#LABWARE TEST_ZYMO P11 "96 Well PlateReader"
#LABWARE TEST_TETRAD TP1 "96 Well PCR Plate"
#LABWARE TEST_ELN_2TEMP TP2 "96 Well PlateReader"

ENDSCRIPT
```

3. Conclusions

The R&S listed specify the set of actions that will be taken throughout the CADMAD project in order to ensure that our basic operational hardware, namely the automated robotic systems, will remain in top form and will not hinder our efforts to complete the construction and editing

of complex DNA libraries. We are confident that taking extreme measures to ensure the reliability of the automated hardware is the only path forward in a system in which multiple factors may impede the execution of a task.

4. References

1) Linshiz G., Ben Yehezkel T, Kaplan S., Gronau I., Ravid S., Adar R., Shapiro E. , Recursive construction of perfect DNA molecules and libraries from imperfect oligonucleotides Mol Syst Biol. 2008;4:191.
2) T. Ben Yehezkel, Linshiz G., Buaron H, Kaplan S., Shabi U., Shapiro E., De novo DNA synthesis using single molecule PCR E Nucleic Acids Res. 2008 Oct;36(17) 2 Uri Shabi , et al., & Ehud Shapiro Processing DNA Molecules as Text Systems and synthetic biology, 2010.
3) Ben Yehezkel T, Nagar S, Mackrants D, Marx Z, Linshiz G, Shabi U, Shapiro E Computer-aided high-throughput cloning of bacteria in liquid medium Biotechniques. 2011 Feb;50(2):124-7