Deliverable D4.1

Funding Scheme: THEME [ICT-2007.8.0] [FET Open]

Paving the Way for Future Emerging DNA-based Technologies: Computer-Aided Design and Manufacturing of DNA libraries

Grant Agreement number: 265505

Project acronym: CADMAD

Deliverable number: D4.1

Deliverable name: Protocols & RoboEase Scripts for exemplary Application of DNA

Contractual Date[1] of Delivery to the CEC: M36
Actual Date of Delivery to the CEC:
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Work Package: WP4
Security[4]: Pub
Nature[5]: R
Version[6]: 0.0
Total number of pages:6

1 As specified in Annex I

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

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

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Abstract

One of the challenges of being able to rationally design complex multi-membered DNA-libraries is to evaluate which element of the library is leading to a specific desired phenotype. In order to provide the required throughput in experimentation, robotic platforms are used that allow automating the different experimental protocol. However, the programming of such robots is often laborious and system specific, which is why RoboEase was developed, a high-level language that allows facilitated programming of automation platforms even across different automation platform systems. In this deliverable, we successfully demonstrate the application of RoboEase to a typical automated analysis of the functionality conveyed by a DNA-library, specifically the multiplexed read-out of a combinatorial operon library.

Keywords[7]:

RoboEase, DNA-library analysis, laboratory automation.

Introduction

We aimed to implement a complex protocol using RoboEase for quantifying protein activity. This should demonstrate that RoboEase can be applied to real-world tasks that are relevant to DNA research.

1. Implementation

The protocol we implemented is described as follows.

1) Pre-culture

- 200 µl LB/kan50 in 96 well plate inoculation (by hand picking) with single colony
- Mix media with 100 µl pipetting (3 times up & down)
- Close plate with plastic lid
- Grow pre-culture in Tecan plate reader M200 (12 h, 37°C, 220 rpm)
- Growth curve data point every 15 min

2) Main culture

- Mix pre culture with 150 µl pipette (4 times up & down)
- Add 190 µl LB/kan50 + 10 µl pre culture in 96 well plate well
- Tip decontamination with NaClO
- Culture in Tecan plate reader M200 (2 h, 37°C, 220 rpm)
- Growth curve data point every 15 min
- Add 10 µl IPTG via air dispense (1mM stock solution, End [0.05mM])
- Incubation in Tecan plate reader M200 (4 h, 37°C, 220 rpm)
- Growth curve data point every 15 min

3) Cell lysis

- Add 20 µl Lysis buffer via air dispense (10 mg/ml Lysozyme, 20mM Tris buffer, pH8)
- Incubation on shaker (RT, 10 min, 220 rpm)
- Mix cell lysate with 150 µl pipetting (8 times up & down)
- Tip decontamination with NaClO
- Store lysed cells at -20°C (optionally)

4) Dilution

- Present 144 µl reaction buffer in well (20mM Tris buffer, pH8 or 20mM PO42- buffer, pH6.2)
- Mix lysed cells (CFX) by 150 µl pipetting steps (4 times up & down)
- Add 16 µl CFX to 144 µl reaction buffer
- Mix diluted CFX by 120 µl pipetting steps (4 times up & down)
- Additional dilution steps (repeat two steps before until required end dilution see table)

5) Hydrolysis reaction in 384 well plates and 80 µL total volume

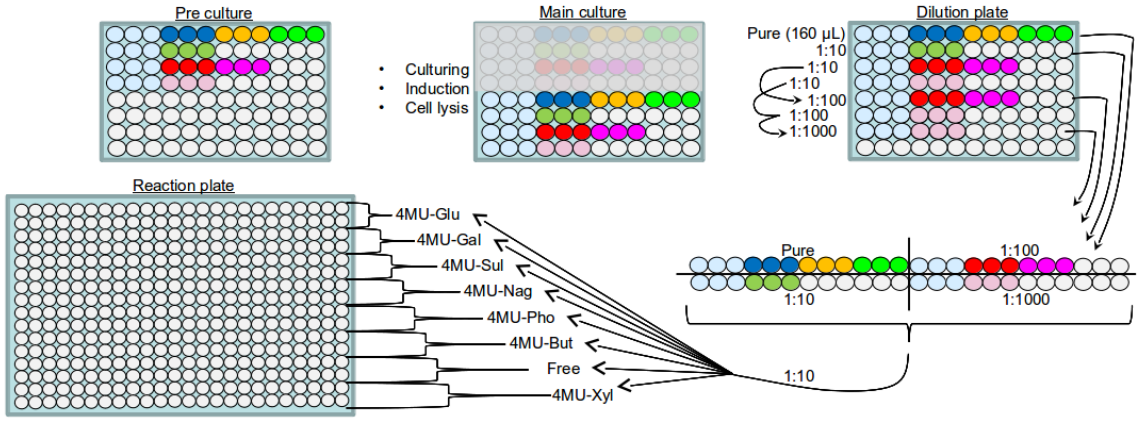
- Perform 3 replicates for each reaction
- Present 41.6 µl reaction buffer in well (20mM Tris buffer, pH8 or 20mM PO42- buffer, pH6.2)
- Add 8 µl diluted CFX
- Add 30.4 µl of corresponding 4-MU substrate (final conc., see table)
- Detection of fluorescence in Tecan plate reader M200 (λEx 365nm, λEm 445nm)

In our tests we used the following reagents.

Substrate	Stock [mM]	End [µM]	Reaction buffer
4-MU β-D-galactopyranoside (4MU-Gal)	100	50	20 mM Tris, pH 8
4-MU β-D-glucuronide (4MU-Glu)	50	100	20 mM Tris, pH 8
4-MU phosphate (4MU-PhoA)	50	50	20 mM Tris, pH 8
4-MU butyrate (4MU-But)	50	150	20 mM Tris, pH 8
4-MU N-acetyl-β-D-glucosaminide (4MU-Nag)	50	150	20 mM Tris, pH 8
4-MU α-D-xylopyranoside (4MU-Xyl)	100	150	10 mM phosphate, pH 6.2
4-MU sulfate (4MU-Sul)	100	50	20 mM Tris, pH 8

The plate arrangement is illustrated in the following figure.

	1	2	3	4	5	6	7	8	9	10	11	12
A	pET30	pET30	pET30	pET30 nagZ	pET30 nagZ	pET30 nagZ	pET30 yicI	pET30 yicI	pET30 yicI	pET30 ast	pET30 ast	pET30 ast
B	pET30	pET30	pET30	pET30 phoA	pET30 phoA	pET30 phoA						
C	pET30	pET30	pET30	pET30 gusA	pET30 gusA	pET30 gusA	pET30 aes	pET30 aes	pET30 aes			
D	pET30	pET30	pET30	pET30 lacZ	pET30 lacZ	pET30 lacZ						
E												
F												
G												
H												



2. Results

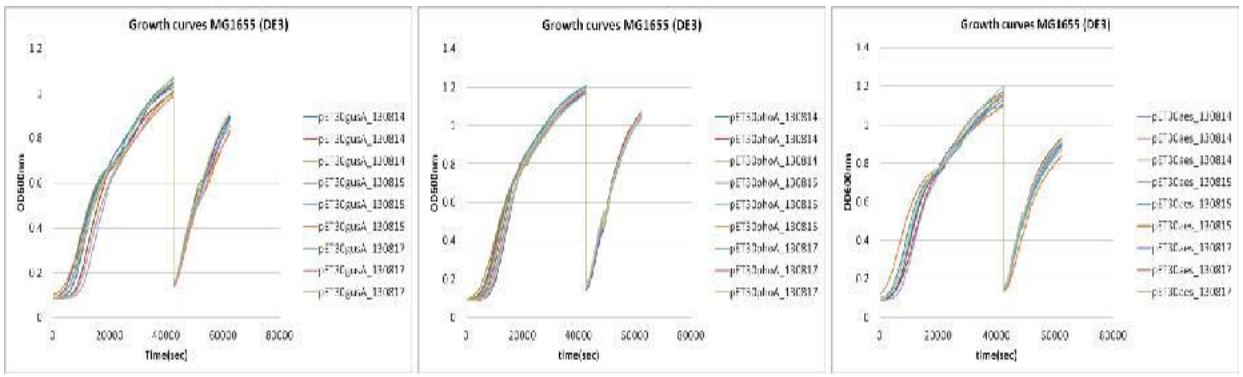
The protocol described above was translated into the following RoboEase script.

```
labware:
  sourcePlate1:
    model: "96 Deep Well Plate"
  culture:
    model: "Nunc F96 MicroWell"
  dilution:
    model: "Nunc F96 MicroWell"
  reaction:
    model: "384 Well Plate"

reagents:
  media: sourcePlate1(A01|H02)
  iptg: sourcePlate1(A03)
  lysisBuffer: sourcePlate1(A04)
  reactionBuffer: sourcePlate1(A05|H07)
  substrate4MUGlu: sourcePlate1(A12)
  substrate4MUGal: sourcePlate1(B12)
  substrate4MUSul: sourcePlate1(C12)
  substrate4MUNag: sourcePlate1(D12)
  substrate4MUPho: sourcePlate1(E12)
  substrate4MUBut: sourcePlate1(F12)
  substrateFree: sourcePlate1(G12)
  substrate4MUXyl: sourcePlate1(H12)

programs:
  readerProgram1:
    description: |
      Grow pre-culture in Tecan plate reader M200 (12 h, 37 C, 220 rpm).
      Growth curve data point every 15 minutes.
  readerProgram2:
    description: |
      Culture in Tecan plate reader M200 (2 h, 37 C, 220 rpm).
```

```
      Growth curve data point every 15 minutes.
  readerProgram3:
    description: |
      Incubation in Tecan plate reader M200 (4 h, 37 C, 220 rpm).
      Growth curve data point every 15 minutes.
  readerProgram4:
    description: |
      Detection of fluorescence in Tecan plate reader M200 (λEx 365nm, λEm
445nm).
  shakerProgram1:
    description: |
      Incubation on shaker (RT, 10 min, 220 rpm)

protocol:
- // Add media to upper rows
- distribute reagent.media labware.culture(A01xD12) 200ul
- // Inoculate by operator
- prompt "Please inculate culture plate."
- // Pre culture
- readPlate readerProgram1 labware.culture
- // Add media to lower rows
- distribute reagent.media labware.culture(E01xH12) 190ul
- // Inoculate
- transfer labware.culture(A01xD12) labware.culture(E01xH12) 10ul
mixBefore=4x150ul
- // Main culture growth
- readPlate readerProgram2 labware.culture
- // Add IPTG
- distribute reagent.iptg labware.culture(E01xH12) 10ul
- // Main culture expression
- readPlate readerProgram3 labware.culture
- // Add lysis buffer
- distribute reagent.lysisBuffer labware.culture(E01xH12) 20ul
- // Shake
- shakePlate shakerProgram1 labware.culture
- // Mix and lyse
- mixWithPipetter labware.culture(E01xH12) 4x150ul
- // Create dilutions from culture plate to dilution plate
- dilute:
    allOf:
    - source: reagent.reactionBuffer
    - oneOf:
      - source: labware.culture(E01-E12)
        destination: labware.dilution(A01-A12)
        factor: 1
      - source: labware.culture(F01-F12)
        destination: labware.dilution(B01-B12)
        factor: 10
      - source: labware.culture(G01-G12)
        destination: labware.dilution(C01-D12)
        factor: [10, 100]
      - source: labware.culture(H01-H12)
        destination: labware.dilution(E01-G12)
        factor: [10, 100, 1000]
    volume: 160ul
    mixBefore: true
- // Create titration series from dilution plate to reaction plate
- titrate:
    allOf:
    - source: reactionBuffer
```

```
      amount: 41.6ul
    - source: dilution(A01-A12+B01-B12+D01-D12+G01-G12)
      amount: 8ul
    - source: [substrate4MUGlu, substrate4MUGal, substrate4MUSul,
substrate4MUNag, substrate4MUPho, substrate4MUBut, substrateFree,
substrate4MUXyl]
      amount: 30.4ul
    replicates: 1
    destination: reaction(A01xP24)
- // Main culture growth
- readPlate readerProgram4 labware.reaction
```

Below we show several measurements from experiments run on the Tecan robot. We see the variation of replicates performed on separate days. While deviation is high, cell growth is uniform. We have determined that some of the deviation is due to problems with the robot itself, and will repeat the experiments once maintenance has been performed.



3. Conclusions

RoboEase was used to implement a complex protocol for measuring protein expression, demonstrating that it can be used for complex tasks as well as simple ones. The RoboEase script is reasonably portable because lab-specific settings are maintained in separate files, letting each lab determine, for example, how the `readPlate` command should be executed.