qbio
quantitative
biology

SynBio Practicals Guide Part 2

2021-2022

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Introduction

In this practice we will explore the impact of varying regulatory elements on bacterial gene expression using a range of quantitative techniques (plate reader, cytometry) and gene expression systems (living cells and cell-free).

We will perform data collection in vivo and in vitro to compare the different collection methods and data analysis using different freely available softwares and Python scripts.

Students will have to present the results of their analysis during a final oral exam in January 2022.

Schedule

We have to complete the table below properly

Date	Day		#hours	Time	Subject	Where
23 November	TUE	Day 1	4	13:00-17:00	Microbiology basics and good lab practices	CBS
24 November	WED	Day 2	4	9:00-13:00	DNA extraction and sequencing	CBS
25 November	THU	Day 3	4	13:00-17:00	Fluorescence measurement and sequencing results analysis	CBS
26 November	FRI	Day 4	4	13:00-17:00	Fluorescence measurement and sequencing results analysis	CBS
29 November	MON	Day 5	3	9:00-12:00	Cell free: Introduction and constructs amplification	CBS
29 November	MON	Day 5	3	14:00-17:00	Cell free: Introduction and constructs amplification	CBS
30 November	TUE	Day 6	4	13:00-17:00	DNA analysis and quantification	CBS
1st December	WED	Day 7	4	13:00-17:00	Running a cell free experiment	CBS
2 December	THU	Day 8	4	13:00-17:00	Data processing (python)	CBS
3 december	FRI	Day 9	4	13:00-17:00	Data processing and analysis (python)	CBS

Activities

Day 5
Cell-free : Introduction and constructs amplification

General concepts

Presentations and discussions about cell-free systems

Resources:

Videos

PCR basics:
PCR 1

Agarose gel:

Agarose gel preparation

Agarose gel loading

Reading

Cell free hints

Cell-free gene expression; Garenne, D., Haines, M.C., Romantseva, E.F. *et al. Nat Rev Methods Primers* 1, 49 (2021). https://doi.org/10.1038/s43586-021-00046-x
https://www.nature.com/articles/s43586-021-00046-x.pdf

Differentially optimized cell-free buffer enables robust expression from unprotected linear DNA in exonuclease-deficient extracts. Angelo Cardoso Batista, Antoine Levrier, Paul Soudier, et al., bioRxiv 2021.09.07.459228;
doi: https://doi.org/10.1101/2021.09.07.459228

For more informations:

For more infos on applications of cell free :

Silverman, A. D., Karim, A. S., & Jewett, M. C. (2020). Cell-free gene expression: an expanded repertoire of applications. *Nature Reviews Genetics*, *21*(3), 151-170.

For infos on the protocol used for cell-free mix preparation :

Sun, Z. Z., Hayes, C. A., Shin, J., Caschera, F., Murray, R. M., & Noireaux, V. (2013). Protocols for implementing an Escherichia coli based TX-TL cell-free expression system for synthetic biology. *Journal of visualized experiments: JoVE*, (79).

Practical part

Group 1: Morning 9h-12h
Group 2: Afternoon 14-17h

Feedback on data analysis attempts
Presentation of cell-free experimental workflow

- PCR from plasmid template (Day5)
- PCR check on an electrophoresis gel (Day6)
- DNA Concentration determination and adjustment using Qbit machine (Day6)
- Cell-Free reaction setup (Day7)

Day 5 : PCR setup for linear cell free with DNA from miniprep :

1. Identification of the best primer pair using Benchling
2. Preparation of PCR master Mix
3. Preparation of individual reactions
4. Thermocycler Set Up and start

Link to working spreadsheet for Group1

Link to working spreadsheet for Group2

Day 6
DNA quality and quantification

General concepts

Ressources

Using the Qbit:
Qbit Thermofisher

Practical part

Gel analysis of PCR :

1. Pour the Gel (30-50 ml) choosing right size and right comb based on the number of samples
2. Add immediately 4 µl of Sybr Safe Dye to the melted gel, mix it and let it solidify (30 min)
3. Prepare your DNA for the electrophoresis : mix 1 µl DNA + 4 µl water + 1 µl 6X loading dye
4. Load the gel with the chosen ladder and your samples (6µl for small wells or 12 µl for large wells)
5. Run the gel at 100 V for around 35 min
6. Image the gel and identify potential band size

DNA quantification using qBit

Adjustment of DNA concentrations.

To run Cell-Free characterization, all the constructions have to be diluted to the same molar concentration. The final concentrations generally used are usually either 100 nM or 50 nM but in the case where one of your samples is already at a concentration < 50 nM, dilute your other DNAs to the same concentration.

Use same Xls protocol
Link to working spreadsheet for Group1

Link to working spreadsheet for Group2

Day 7
Running a cell free experiment

General concepts

The cell-free characterization of our linear DNA construct will be proceeded using the following protocol :

Cell free protocol

Practical part

Link to working spreadsheet for Group1

Link to working spreadsheet for Group2

Organization of end of practical work

- Micropipettes cleaning
- Store your Micropipettes on the maximum volume
- Clean up the lab
- Get rid of useless samples, solutions
- Store anything useful

Days 8 and 9

Data analysis

General concepts

Here you will process your data using python and possibly other tools.

Practical part

1. *For plate reader and cell-free analysis-see Luca's class.*

2. *For flow-cytometry, these python packages process FCS files. Have a look and give it a try...*

FCS parser: script to convert FCS files to dataframes.

https://github.com/eyurtsev/fcsparser

Flowkit:

Python toolkit for flow cytometry analysis and visualization.

https://www.frontiersin.org/articles/10.3389/fimmu.2021.768541/full

https://github.com/whitews/FlowKit