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Dissemination Level		
PU	Public	x
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RE	Restricted to a group specified by the consortium (including the Commission Services)	
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Report on recommendations for foundation of measurement and statistics in Synthetic Biology

1. Introduction

Programming cellular behaviour in a predictable manner using standardized engineered parts and devices is the prime goal of current Synthetic Biology (SB) research. In order to accomplish this grand challenge it is important to create a database of standardized genetic parts (e.g. MIT registry of parts [1]). Although there are a variety of tools and techniques available for measuring and characterising engineered biological parts, there is also a need for a consensus on the role of such measurements towards part standardisation. From an engineering perspective such standardization will be legitimate if the characterization of each part in the database has been carried out under defined and repeatable measurement conditions, thus highlighting the role of analytical techniques for SB.

Microfluidics is "the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres" [2]. Microfluidic cell culture device potentially offer parallel probing of cellular behaviour with a significant reduction of resources, e.g. expensive medium and reagents, and an automated integration of all cell handling steps from culture and selection, to separation and analysis on one platform [3]. Additionally the microfluidic channels tightly regulate the transport of the fluids and soluble factors, thus enable precise spatio-temporal control over the cellular microenvironment.

2. Workshops

For the discussions on measurement units and standardisation, a sandpit workshop was held in Mallorca (20-22 October 2009)), which provided an opportunity for a high-level international discussion on promoter strength characterisation and transcription standards. To discuss the potential role of microfluidics for Synthetic Biology, UCL Biochemical Engineering hosted a mini-workshop in London (28 and 29 May 2009).

The two-day workshop in London "Microfluidics as Analytical Tool for Synthetic Biology Measurements" focussed on covering important analytical techniques relevant to promoter strength measurement and quantification, and included expert speakers both for the analytical techniques and the corresponding microfluidic implementation. The presentations of the expert speakers were followed by group discussions (Details of the workshop programme can be found in the Appendix). The workshop consisted of three sessions: Invasive analytical

techniques, non-invasive analytical techniques, high-throughput methods. 20 participants including 6 members from the EMERGENCE project attended the workshop. This group of experts and Synthetic Biology enthusiasts covered a range of disciplines from engineering, analytical chemistry, and biology.

3. Brief Summary of the Microfluidics Workshop

The presentations and discussions focussed on the quality of measurement techniques for nucleic acids and for fluorescent proteins synthesised during the gene expression cycle. It was assumed that the main measurement units for promoter strength standardisation would either the quantification of the mRNA polymerase escape rate or the determination of protein expression / specific protein activity. For the direct quantification of mRNA, reverse transcription quantitative polymerase chain reaction (RT-qPCR) was proposed as a valuable option. Fluidigms BioMark system offers high-throughput real-time qPCR in arrays of nanolitre reactors [4]. Alternatively, it seems feasible to monitor optically the levels of fluorophores that have been attached to mRNA, which may require the tailoring of the fluorophore properties. The principle of intra-cellular protein activity determination in valved microfluidic chambers has been demonstrated [5]. Since the sensitivity of fluorescence measurement scales favourably with detection volume miniaturisation [6], this technique is attractive from a microfluidics perspective.

4. Importance of Sample Preparation

Measurement techniques such as RT-qPCR and fluorescence-activated cell sorting (FACS) are routinely used to measure and quantify protein or mRNA level in the cells. Typically, the measurement protocol involves a number of steps from cell culture to the actual biochemical analysis (Figure 1). These steps are critical to achieve a reproducible analysis of the performance of engineered cells or parts. It might therefore be necessary to standardise both the measurement protocols and the steps that precede the actual analytical technique. Laboratory cell culture techniques, for example, frequently employ shake flasks. In these batch cultures, the cells are exposed to changing growth conditions (pH, dissolved oxygen tensions, temperature, nutrient and product concentration levels, etc.) over the cultivation time leading to a variability in cellular expression [7]. In order to understand the impact of these differing growth conditions on gene expression, it is necessary to precisely control them before the analytical methods are applied. Operating in chemostat mode provides a suitable solution to this challenge, because it allows the cultivation of cells in steady-state conditions. A drawback

of the chemostat operation lies in the extensive use of nutrient media and reagents at the bench scale. Additionally, bench-scale bioreactors are more labour-intensive to set up and operate than shake flasks. These two factors limit the application of 'chemostat' bioreactors.

5. Microfluidic bioreactors

Microfluidic bioreactors (or microbioreactors) could overcome these challenges. The dramatic reduction in reactor volume facilitates long-term continuous culture experiments, and the potential for automation and parallelisation from microfluidics technology enables to perform a large number of experiments with little user/operator interaction. Microfluidic and mini bioreactors have been demonstrated for microbial fermentation and for mammalian cell culture. These include microbioreactors for parallelised fermentations [8], for long-term monitoring of bacterial populations in pseudo-continuously operated parallelised nanolitre reactors [9], for long-term single-cell dynamical measurements over a large population [10], for transient exposure of microbial cells to an exogenous signal in parallelised nanolitre reactors [11], and well-mixed micro-chemostats [12]. As can be seen from these examples, microfluidic bioreactors can provide a way forward for detailed analysis of cellular behaviour in small populations, and can even be employed to maintain small cell populations in controlled conditions for longer periods of time. It seems thus feasible that parallelised micro-chemostats could improve sample preparation, and thus aid in standardising the analytical protocols. Furthermore, the steps that follow cell culture and lead to either non-destructive or destructive analysis can also be performed on-chip, and a number of microfluidic devices have been realised and reviewed [13, 14, 15].

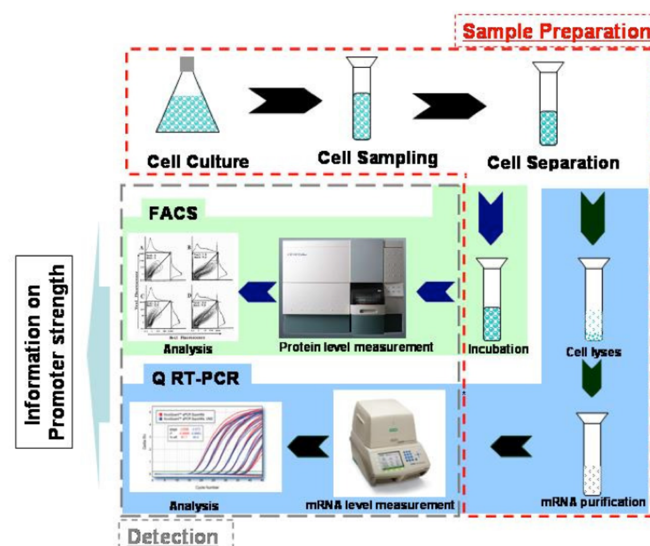

Figure 1: From cell culture to biochemical analysis

References

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Appendix

Two day Workshop:

“Microfluidics as Analytical Tool For Synthetic Biology Measurements”

28th and 29th May 2009

Venue:

Malet Place Eng. 1.20
University College London, United Kingdom

Host:

Department of Biochemical Engineering,
University College London,
Torrington Place, London, WC1E 7JE
United Kingdom

PROGRAMME

DAY I: Thursday 28th May 2009

12.30 Registration

13.00 Lunch & Networking

13.30 Welcome Note / Opportunities in Microfluidics
Dr. Nicolas Szita (University College London, UK)

13.45 EMERGENCE
Prof. Sven Panke (ETH Zurich, Switzerland)

14.00 ***Session I:***
Invasive analytical techniques for Synthetic Biology measurement and quantification
Session Chair: Prof. Sven Panke

Talk 1
Prof. Stephen A Bustin (Queen Mary, University of London, UK)
"Basic concepts of the real-time reverse transcription PCR (RT-qPCR)"

Talk 2
Dr. Philip Day (University of Manchester, UK)
"Microfluidics for quantitative cellular measurements"

15.00 **Coffee Break**

15.30 ***Session II:***
Non-Invasive analytical techniques for Synthetic Biology measurement and quantification
Session Chair: Dr. Nicolas Szita

Talk 1
Dr. Esteban Martinez (Centro Nacional de Biotecnología, Spain)
"Promoter strength measurements: tools and limitations"

Talk 2
Dr. Joshua Edel (Imperial College London, UK)
"***Molecular Isolation on the Nanoscale"***

16.30 ***Group discussion***
Session Chair: Prof. Sven Panke and Dr. Nicolas Szita
Challenges and opportunities for systematic and repeatable analytical measurements in Synthetic Biology

17.30 ***End of Day I Talk***
Dr. Nicolas Szita

DAY II: 29th May 2009

09.30 **Coffee**

09.45 **Summary of DAY I Discussion Points**

10.00 **Microfluidics for High Throughput Analytics in Synthetic Biology**
Session Chair: Dr. Frank Baganz

Talk 1
Dr. Martin Held (ETH Zurich, Switzerland)
"3 D suspension arrays for really-really fast biocatalyst screenings"

Talk 2
Prof. Wilhelm Huck (University of Cambridge, UK)
"Microdroplets in Microfluidics: a platform for single cell studies"

11.00 **Coffee Break**

11.30 **Talk 3**
Session Chair: Amol Jadhav

Dr. Sam Birtwell (University of Southampton, UK)
"High-throughput multiplexed suspension assays using encoded microparticles"

Talk 4
Dr. Nicolas Szita (University College London, UK)
"Microbioreactors for parallelised fermentation and cell analysis"

12.30 *Group Discussion*
Session Chair: Dr. Nicolas Szita, Amol Jadhav
Opportunities for High throughput microfluidics in Synthetic Biology research

13.00 *Networking & Lunch* [UCL Refectory]

14.00 *End of Workshop*