iGEM Paris Bettencourt Collaboration (MINI-INTERLAB)

Title
Analysing the variability of cell-free expression systems with an interlab experiment
Team
Paris_Bettencourt 2018
Team location
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Overview
E coli based *in vitro* transcription-translation (TXTL) expression platforms allow rapid and inexpensive protein synthesis with the highest versatility and flexibility due to their open-reaction character. These cell-free expression systems allow high-yield production of soluble and membrane proteins, rapid prototyping of gene networks using plasmid DNA and RNA, including the construction of synthetic minimal cells. The possibility of automated miniaturization makes it ideal system for screening of libraries and allows fast iteration of design-build-test cycles of genetic parts and gene circuits. However, it is tedious and time-consuming, with huge batch to batch variations of the resulting product.

Following into the footsteps of the iGEM interlab study to minimize measurement variations in synthetic biology, we propose a mini-interlab collaboration to measure the amount of GFP produced via cell-free expression. We selected a GFP expressing plasmid (**BBa_I20270**) from the iGEM 2018 distribution kit. Following a standardized protocol, we plan to express it in our homemade and/or Arbor_biosci myTXTL expression system. With a plate reader we will be able to measure fluorescence and note/compare results of various iGEM teams that participate in this collaboration.

This allows us to do an inter-lab experiment that tests the efficiency of various cell free expression systems and the technical variability when executed via various teams with good reproducibility. It's a short experiment that takes 2 days upon following the standardized protocol. There is no exchange of materials involved, all the teams that use cell-free expression systems are invited to join this mini-interlab experiment.

Requirements
Either of the following cell-free expression systems
- Arbor bioscience (myTXTL sigma70)
- Homemade cell free system (PMID: 24084388, Noireaux et. al)

Negative control plasmid **BBa_R0040** 2018 Kit Plate 6 or 7
Positive control plasmid **BBa_I20270** 2018 Kit Plate 6 or 7
Nuclease free water
Fluorescence standard curve - Fluorescein Protocol (Calibration 3 protocol from Interlab)
Fluorescence Plate reader, 384 or 96 well plates

Method
Prepare plasmids at 2 concentrations (5 nM and 2.5 nM)
Prepare cell free extracts (equal concentrations and equal volumes)

	PLATE DESIGN	A	B	C	D	E	F	G	H
1	BBa_I20270	5nM		5nM		5nM		BLANK	
2	BBa_I20270		2.5 nM		2.5 nM		2.5 nM		BLANK
3	BBa_R0040	5nM		5nM		5nM		BLANK	
4	BBa_R0040		2nM		2nM		2.5 nM		BLANK

Record results with plate reader at settings calibrated during the interlab calibration.
Analyze the GFP expression with reference to the Fluorescein standard curve obtained from the interlab experiment.

(For a detailed protocol and related queries, do not hesitate to contact our team)

REFERENCES

- myTXTL reference:

Table 1. Pipetting scheme for myTXTL® reactions using plasmid DNA as template.

	Single myTXTL® rxn	Single myTXTL® eGFP control rxn	e.g. Eight myTXTL® rxns
Sigma 70 Master Mix	9 µL	9 µL	75 µL
Template DNA	X µL (final: 5 nM)	–	X µL (final: 5 nM)
P70a-deGFP ctr. plasmid (20 nM)	–	3 µL (final: 5 nM)	–
Nuclease-free water	X µL	–	X µL
Total	12 µL	12 µL	100 µL

- iGEM INTERLAB: http://2018.igem.org/Measurement/InterLab