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Toehold testing/ PURE system protein production

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Protocol status: Working
We use this protocol and it's working

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Abstract

Our Toehold switches are tested using NEB PURExpress and OnePot PURE system (our own cell-free protein expression system, search OnePot for detailed protocols). Our goal is to define a concentration of Toehold switch and trigger DNA sequence which has a high protein (sfGFP in our case)expression but low or no leakage at same time.

Guidelines

Both OnePot and PURExpress solution are sensible to temperature and freezing & thawing effect. Keep the solution On ice while micro plate preparation will extend their working life. OnePot solution are not available online, it is produced using our own protocols, it won't be mentioned in the material list, but the usage will be discussed in the steps page.

Materials

MATERIALS

- PURExpress In Vitro Protein Synthesis Kit - 10 rxns (25 microliter) **New England Biolabs Catalog #**E6800S
- DNAse/RNAse free distilled water **Thermo Fisher Scientific Catalog #**10977023
- RNAse inhibitor Murine **NEB Catalog #**M0314S
- Trigger DNA
- Toehold DNA **IDT**
- Chill-out™ Liquid Wax **Bio-Rad Laboratories Catalog #**CHO1411

Troubleshooting

Safety warnings

Centrifuge has to be balanced, by applying the weight of another micro plate with same prototype on the other side.

Before start

Warm the micro plate reader, keep it at 37 °C . If the user is not familiar with micro plate reader, please set the plate reader protocol first before start, the procedure of setting sfGFP detection is enclosed in another protocol. Separating the droplet in the wells during preparation is recommended, therefore centrifuge is necessary in the end of pipetting, keep centrifuge at 4 °C in order to avoid undesired expression before the test.

1 Take a clean 384-well micro plate, mark the using wells on the plastic cover, when finished put the cover underneath the micro plate, the marks should be seen though the wells, so it reduces the risk of miss pipetting.

2 Make OnePot premix, an supplemental step if using OnePot system and having experiments more than 5 reactions. Energy solution has to keep separately all the time. The OnePot energy solution and OnePot premix reaction volume is referring to the NEB PURExpress solution A and B accordingly in following steps.

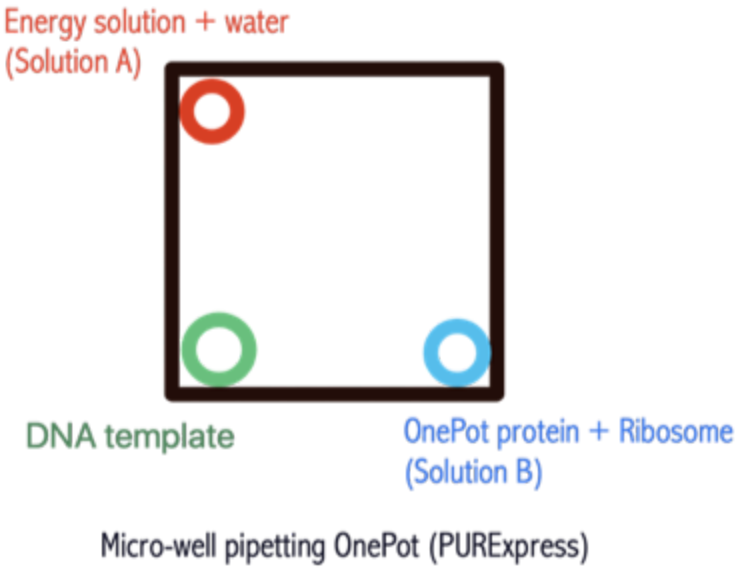
2.1 OnePot protein solution : OnePot ribosome solution = 13 : 18. (The concentration of the solution is indicated in the OnePot protocol)
For a 5 µL reaction a 1.5 µL of premix is needed.
(exp. 5 x 1.5 µL premix contains 4.64 µL of OnePot protein solution and 6.43 µL of OnePot ribosome solution)
Tips: when making the premix, always make one extra reaction volume to make sure that in the end, there won't be short of premix solution.

3 There is limited space in a 5 µL reaction, after solution A and solution B there will be only 1.5 µL space for both toehold and trigger. It's better to dilute or concentrate them to the desired concentration before adding. The following steps are referring to a [M] 5 nanomolar (nM) of toehold switch and [M] 2 micromolar (µM) of trigger dsDNA as an example.

4 Pipetting:

		Reference/ rxn concentration (5µL)	Toehold_1 (+)	Toehold_ 2 (+)	Toehold_ 3 (+)	Toehold_1 (-)	Toehold_ 2 (-)	Toehold_ 3 (-)
	H2O	fill till 5 µL	/	/	/	1	1	1
	A/Energy	2	2	2	2	2	2	2
	B/Premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	Toehold_1	5nM	0.5	/	/	0.5	/	/
	Toehold_ 2	5nM	/	0.5	/	/	0.5	/

	Toehold_3	5nM	/	/	0.5	/	/	0.5
	BN_trigger	2uM	1	1	1	/	/	/
	RNase inhibitor	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Total:	5	5	5	5	5	5	5



Micro-well pipetting OnePot (PURExpress)

Order of adding the reagents: Solution A (Energy solution) + water → Solution B (OnePot premix) → DNA template

5 Balance the centrifuge, 4000 rpm 00:01:00 .

6 Add 35 µL of BIO-RAD Chillout liquid wax to each well. Place the wells gently inside the plate reader, check the setting of the plate reader, start the measurement.

7 When the measurement is done, export the data.