Miniaturization and Optimization of a Two-Plasmid Expression Pathway in myTXTL® Cell-Free Protein Expression Kit (Arbor Biosciences) Using Labcyte Echo® Acoustic Technology

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INTRODUCTION

In vitro transcription (TX)-translation (TL) is a rapidly developing technology with vast potential for synthetic biology and bio-engineering. myTXTL® (**FIG. 1**), an *E. coli* based cell-free protein expression system entirely relying on the endogenous TXTL machinery, is a versatile and all-in-one solution for many applications, such as the development of multi-stage gene circuits, and screening of complex protein libraries. For both examples, high sample throughput at an affordable cost is desirable.

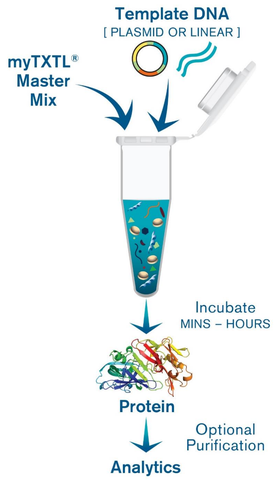

FIG 1. myTXTL® workflow.

FIG 2. Labcyte Echo® 525.

The Labcyte Echo® 525 Liquid Handler (**FIG. 2**) allows rapid, accurate and contact-free transfer of volumes at a nanoliter scale with acoustic sound. Its integrated Dynamic Fluid Analysis technology enables the Echo® system to easily adapt to various types of fluid without the need for calibration, which makes it ideal for handling multi-component solutions (**FIG 3**). This simplifies experimental setup and enables a maximum degree of flexibility for study design.

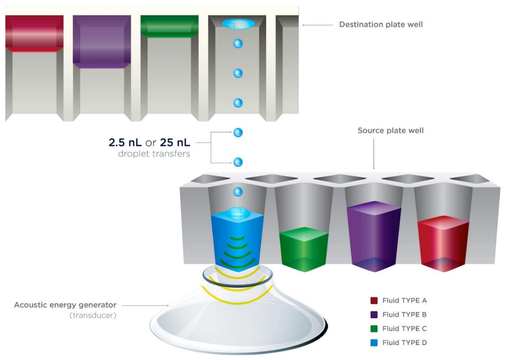

FIG 3. Acoustic droplet ejection (ADE) technology of the Echo® Liquid Handler.

Combining these two sophisticated platforms provides a comprehensive solution for fully-automated high-throughput *in vitro* protein expression: from template preparation to product analysis. In this study, we demonstrate the ability to process Arbor Biosciences' myTXTL® system in very low volumes and to accelerate DNA titration and dispensing using the Labcyte Echo® Liquid Handler. This method will lower overall cost by reducing assay time and reagent consumption, while increasing experimental complexity and minimizing the risk of contamination and human errors.

EXPERIMENTAL DESIGN

A typical myTXTL® reaction has a total volume of 12 µL and consists of a ready-to-use myTXTL® Master Mix and a nucleotide template. Here, we pursued cell-free expression of a model protein (eGFP mutant) under transcriptional control of the T7 system. This requires the presence of T7 RNA polymerase, which was supplied to the myTXTL® reaction encoded on a plasmid (**FIG 4**).

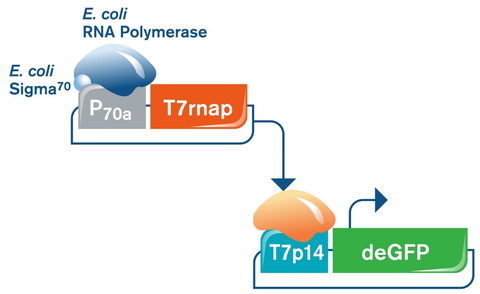

FIG 4. *In vitro* protein production in myTXTL® using the T7 expression system.

RESULTS

Assay 1. Miniaturization of myTXTL® reaction

In the course of generating a standard curve for quantification of deGFP produced *in vitro*, the Echo® 525 Liquid Handler was able to accurately dispense a deGFP standard at different concentrations for volumes between 1 µL and 12 µL (**FIG 5**).

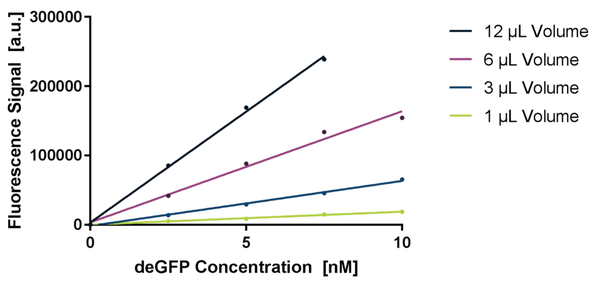

FIG 5. deGFP standard curve set up by acoustic transfer. deGFP fluorescence in the 384-well plate was acquired in a PHERAstar® reader at 485 nm (ex) and 520 nm (em).

Assay 2. Optimization of a two-plasmid myTXTL® reaction

Using acoustic liquid transfer, reagents were arrayed into 384-well plates to allow multiplexing of the dual-plasmid circuit (**TABLE 1**). Then, plates were incubated at 29 °C using a Labcyte MicroClime® lid to prevent evaporation, and fluorescence signal of deGFP produced in myTXTL® was continuously recorded over 16 hours.

TABLE 1. Experimental scheme for evaluating a two-plasmid myTXTL® reaction using Echo® 525 Liquid Handler for reagent transfer.

384-well plate matrix for multivariance DOE		P70a-T7rnap [pmol]											
		100	75	50	37.5	25	10	7.5	5	2.5	1.25	0.62	GFP STD Curve
		1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24
T7p14-deGFP [nM]	6.75	A+B											
	5.63	C+D											
	4.5	E+F											
	3.38	G+H											
	2.25	I+J											
	1.13	K+L											
	0.56	M+N											
	0.28	O+P											

Reagent	Acoustic Transfer Range
T7p14-deGFP	25-600 nL
P70a-T7rnap	25-400 nL
deGFP Standard	50-1000 nL
myTXTL® Master Mix	3000 nL
diH_2O	0-950 nL

Maximum production of deGFP was observed in the presence of 4.5 nM T7p14-deGFP and 37.5 pmol P70a-T7rnap (**FIG 6**). At higher concentration of either plasmid, total deGFP yield was reduced. Alterations in the concentration of P70a-T7rnap were most pronounced over the time course of the experiment (**FIG 7**).

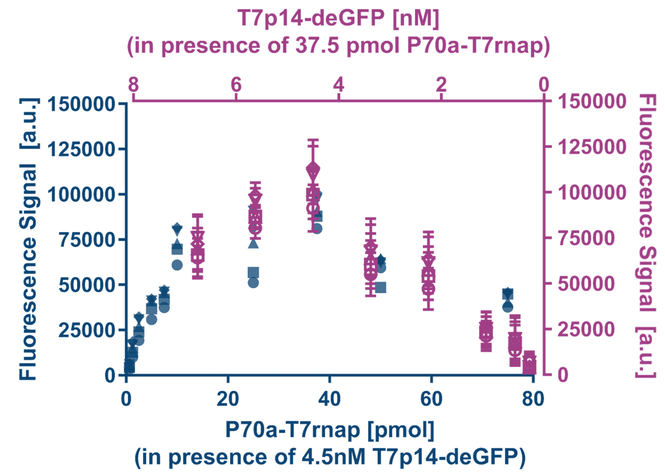

FIG 6. deGFP expression in myTXTL® as a function of plasmid concentration. Either T7p14-deGFP (blue) or P70a-T7rnap (purple) were kept constant, while the concentration of the corresponding plasmid was titrated over several magnitudes. Data recorded after 4 h (circle), 4.5 h (square), 5 h (triangle), 5.5 h (diamond) and 6 h (inverted triangle) is plotted.

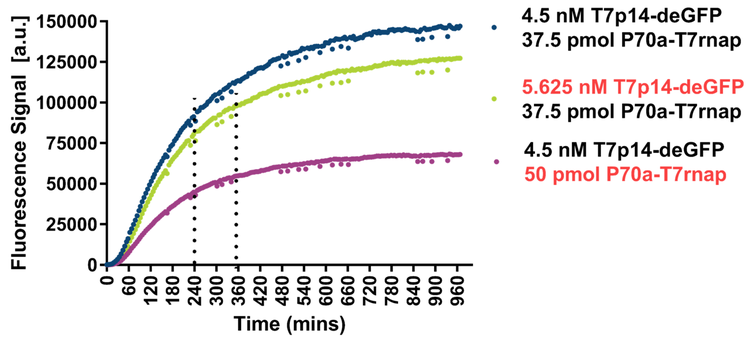

FIG 7. Kinetic of deGFP production in myTXTL® under three different reaction conditions of the dual-plasmid system. The 4-6 h window is marked with dotted lines. Fluorescence signal was acquired every 5 min.

DISCUSSION

Arbor Biosciences' myTXTL® Master Mix combined with the Labcyte Echo® gives the ability to obtain fluorescent protein readout from a multivariable DNA input within a matter of hours. Rapid arraying, in combination with the reduced DNA and reagent demand, allows for a large increase in the number of possible experimental conditions as seen in Table 1. More work with acoustically arrayed myTXTL® will be performed to further highlight the flexibility of both systems in building a biological circuit.

HIGHLIGHTS

- **Echo® 525 Liquid Handler reproducibly and reliably transfers myTXTL® Master Mix.**
- **Miniaturization reduces overall assay costs by a factor of three due to lowered reagent consumption.**
- **The speed of arraying allows for time-dependent reads of multiple plasmid inputs in a myTXTL® reaction.**