Miniaturization and Rapid Processing of TXTL Reactions Using Acoustic Liquid Handling

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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® (**FIGURE 1**), an *E. coli*-based cell-free gene expression system entirely relying on the bacterial endogenous TXTL machinery, is a versatile and all-in-one solution for many applications, such as the screening of complex protein libraries and development of multi-stage gene circuits. For both examples, high sample throughput at an affordable cost is desirable.

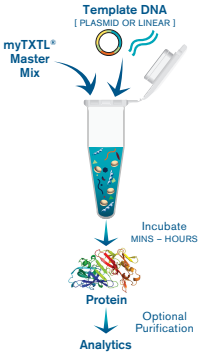


FIGURE 1: Typical workflow of *in vitro* protein production using myTXTL®.

FIGURE 2: Labcyte Echo 525 Liquid Handler

The Labcyte® Echo® 525 Liquid Handler (**FIGURE 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 (**FIGURE 3**). This simplifies experimental setup and enables a maximum degree of flexibility for study design.

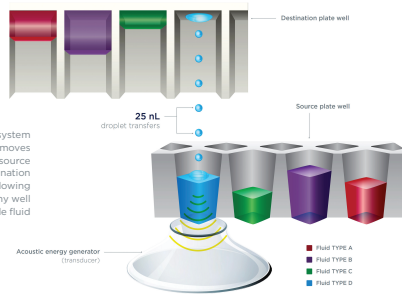


FIGURE 3: The Echo system transducer rapidly moves between wells on the source plate while the destination plate also moves, allowing rapid transfer from any well to any well for multiple fluid types.

Combining these two sophisticated platforms provides a comprehensive solution for fully-automated high-throughput *in vitro* protein production: from template preparation to product analysis. In this study, we demonstrate the ability to process Arbor Biosciences' myTXTL system in reduced volumes and to accelerate DNA input arraying using the Echo Liquid Handler. This will lower overall cost by reducing assay time and reagent consumption, while allowing for increased experimental complexity by reducing human introduced error.

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. We used the 25 nL granularity of the Echo 525 Liquid Handler to decrease the overall reaction volume by three-fold while maintaining experimental precision and reproducibility. We pursued cell-free expression of a fluorescent model protein (deGFP) at this reduced volume using two different expression systems. The initial system was a sigma factor 70 (σ70) controlled plasmid (P70a-deGFP) that utilizes the endogenous *E. coli* RNA polymerase (**FIGURE 4**). *E. coli* σ70 is present within the myTXTL Master Mix allowing the deGFP to be directly produced.

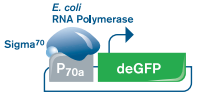


FIGURE 4: *In vitro* protein production from plasmid P70a-deGFP in myTXTL using *E. coli* σ70 transcription factor to control deGFP expression.

Alternatively, gene expression in myTXTL was optimized for the powerful T7 promoter system (**FIGURE 5**). Therefore, RNA polymerase derived from bacteriophage T7 needs to be provided to the myTXTL reaction as it is not part of the endogenous *E. coli* machinery. Either addition of T7 RNA polymerase protein or co-expression from a plasmid vector demands fine tuning of reagent concentrations for maximum protein yield. Here, the Echo 525 system with its 25 nL granularity facilitated an utmost degree of flexibility, accuracy, and experimental resolution.

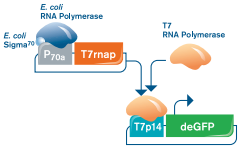


FIGURE 5: In order to express genes downstream of the T7 promoter/operator system (T7p14-deGFP), myTXTL reactions were supplemented with T7 RNA polymerase: either by using a helper plasmid encoding T7 RNA polymerase downstream of a σ70-specific promoter (P70a-T7rnap), or by addition of T7 RNA polymerase protein.

Results

ASSAY 1: *E. coli* σ70 controlled deGFP

After generating a linear standard curve at 4 µL using purified recombinant deGFP (not shown) for later protein quantification, the expression of σ70 controlled deGFP (P70a-deGFP; **FIGURE 4**) in a 4 µL total volume was investigated. The plasmid P70a-deGFP was transferred from the Echo source plate into a 384-well TXTL assay plate with a final plasmid concentration ranging from 6.25 to 2000 pM. The resulting plot demonstrated a linear response starting at 500 pM DNA. (**FIGURE 6**)

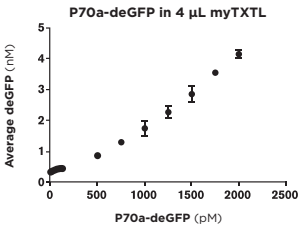


FIGURE 6: Titration of P70a-deGFP plasmid into 4 µL total reaction volume of myTXTL. Readings were taken on a BMG Labtech PHERAstar® FS and compared to a deGFP standard curve for quantitation. Each point was done in quadruplicate and the curve had an average percent CV of 7.60%.

ASSAY 2A: Exogenous T7 RNA Polymerase controlled deGFP

As depicted in **FIGURE 5**, the plasmid T7p14-deGFP requires the activity of T7 RNA polymerase to generate a fluorescent response. To that end, purified T7 RNA polymerase was added exogenously to the 4 µL myTXTL reaction. Both, the polymerase and plasmid additions were varied over a 384-well assay plate (**FIGURE 7**). Peak production of deGFP (0.78 nM) was found to be at 1 Unit of T7 RNA polymerase and 2000 pM T7p14-deGFP.

Exogenous T7 RNA Polymerase and T7p14-deGFP in 4 µL myTXTL

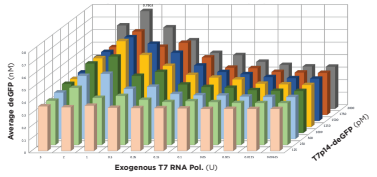


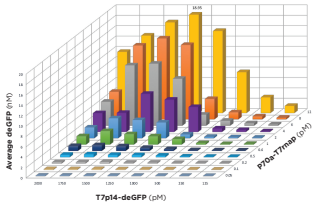
FIGURE 7: Multi-variable titration of the T7p14-deGFP plasmid and exogenous T7 RNA polymerase into 4 µL myTXTL reactions after 2.3 h of incubation. The concentration of produced deGFP calculated from the protein-specific fluorescence signal, which was acquired on a BMG Labtech PHERAstar FS (λEx = 485nm, λEm = 520 nm) are displayed. Each point was done in quadruplicate and the curve had an average percent CV of 14.8%. Addition of 1 Unit of T7 RNA polymerase led to maximum deGFP yield.

ASSAY 2B: *In vitro* T7 RNA Polymerase controlled deGFP

T7 promoter controlled deGFP (T7p14-deGFP) was also generated within the myTXTL reaction by simultaneous addition of the P70a-T7rnap helper plasmid. Peak production was found to be at 1250 pM T7p14-deGFP and 12 pM P70a-T7rnap with an average deGFP concentration of 18.95 nM (**FIGURE 8**). This corresponds to a 21.8 times higher deGFP yield than achieved through the exogenous T7 RNA polymerase addition strategy above.

Plasmid Derived T7 RNA Polymerase (P70a-T7rnap) and T7p14-deGFP in 4 µL myTXTL

FIGURE 8: Multi-variable titration of plasmids T7p14-deGFP and P70a-T7rnap into 4 µL myTXTL reactions after 7.5 h of incubation. Readings were taken on a BMG Labtech PHERAstar FS (λEx = 485 nm, λEm = 520 nm). Each point was done in quadruplicate and the plot had an average percent CV of 14.51%.



Conclusion

High-throughput liquid handling of Arbor Biosciences' myTXTL Master Mixes allows assaying hundreds to thousands of gene template constructs simultaneously and enables to investigate many experimental setups within a matter of hours. Meanwhile, efficient *in vitro* gene expression systems, like myTXTL, allow reducing total reaction volumes, and therefore reagent cost, while still generating a sufficient protein amount for downstream analysis and functional assays. The assay optimization run on the Echo 525 Liquid Handler delivered robust results at lowered reagent consumption of three-fold and saved preparation time of many days compared to a manual reaction setup.

Summary

- **The Echo 525 Liquid Handler reliably transfers the myTXTL Master Mix and plasmid inputs with the required nanoliter granularity while maintaining a high level of precision and accuracy.**
- **The ability to rapidly array in a 384-well format was crucial to providing the desired data depth for assay optimization. TXTL reactions are time sensitive and manual sample handling at such low volumes would generate far less reproducible results.**
- **The overall reaction volume was able to be reliably decreased by three-fold from the manufacturer recommended volume reducing reagent cost.**

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