Cell-Free Expression Handbook

June 2019

myTXTL® Sigma 70 Master Mix Kit

For *in vitro* gene expression from circular templates

myTXTL® Linear DNA Expression Kit

For *in vitro* gene expression from linear and circular templates

myTXTL® T7 Expression Kit

For *in vitro* gene expression using a T7 promoter system

Please cite our products in your publication as myTXTL Sigma 70 Master Mix Kit, myTXTL Linear DNA Expression Kit, and myTXTL T7 Expression Kit from Arbor Biosciences, respectively.

All technical literature is also available at www.arborbiosci.com. Visit our website to verify that you are using the most current version of this Handbook.

CONTENTS

KIT CONTENTS

All components of the myTXTL catalog kits listed below are also available in customized size and packaging formats. Please contact us at info@arborbiosci.com for further information.

myTXTL Sigma 70 Master Mix Kit	**24 Rxn**	**96 Rxn**
Catalog No.	507024	507096
Sigma 70 Master Mix	3 x 75 µL	12 x 75 µL
P70a(2)-deGFP Positive Control Plasmid (20 nM)	1 x 35 µL	2 x 35 µL

myTXTL Linear DNA Expression Kit	**24 Rxn**	**96 Rxn**
Catalog No.	508024	508096
LS70 Master Mix	3 x 75 µL	12 x 75 µL
P70a-deGFP Linear Positive Control Fragment (80 nM)	1 x 15 µL	2 x 15 µL

myTXTL T7 Expression Kit	**24 Rxn**	**96 Rxn**
Catalog No.	505024	505096
LS70 Master Mix	3 x 75 µL	12 x 75 µL
P70a-T7rnap HP (2.4 nM)	1x 15 µL	1x 50 µL
T7p14-deGFP HP (24 nM)	1x 25 µL	1x 25 µL

Please email us at techsupport@arborbiosci.com to inquire about plasmid sequences.

SHIPPING, STORAGE AND STABILITY

myTXTL kits are shipped on dry ice. Upon receipt, myTXTL kits − and in particular myTXTL Master Mixes − must be immediately stored at –80 °C. Since the positive control DNA templates supplied with the kits are suspended in nuclease-free water, we recommend a storage temperature of at least –20 °C. For convenience, myTXTL Master Mix and positive control template can be stored together at –80 °C. Once thawed to set up a TXTL reaction, myTXTL Master Mixes should be kept on ice and used within 4 hours after thawing. Up to five thaw-and-freeze cycles are possible without loss of protein production efficiency. When stored and handled under these conditions, myTXTL kits are stable for 12 months upon arrival.

INTENDED USE

The myTXTL T7 Expression Kit, Sigma 70 Master Mix Kit, myTXTL Linear DNA Expression Kit are intended for research use only. These products are not intended for diagnosis, prevention or treatment of a disease.

SAFETY INFORMATION

When working with chemicals and reagents, always wear suitable protective laboratory equipment such as a lab coat, disposable gloves and safety glasses. For more information, please consult the appropriate safety data sheets (SDSs), which can be obtained by emailing techsupport@arborbiosci.com

QUALITY CONTROL

In accordance with Arbor Biosciences' high standards, each lot of myTXTL T7 Expression Kit, Sigma 70 Master Mix Kit, myTXTL Linear DNA Expression Kit is tested against predetermined specifications to ensure consistent product quality. Individual Certificate of Analysis (CofA) documents are available upon request by emailing techsupport@arborbiosci.com.

INTRODUCTION

Cell-free gene expression platforms allow rapid and inexpensive recombinant protein production providing a high degree of versatility and flexibility due to their open-reaction condition. This permits increased control over efficient substrate handling, and conveniently promotes precise real-time reaction monitoring and direct process optimization, all while by-passing time-consuming procedures such as bacterial transformation, clone selection and cell lysis. Their extremely user-friendly and safe handling makes them similarly well-suited for cutting-edge research in academia and biotech industry as well as student education.

myTXTL Technology Overview

myTXTL is based on a prokaryotic *in vitro* transcription (TX)-translation (TL) platform developed by Prof. Vincent Noireaux at the University of Minnesota (USA) and has been demonstrated to be valuable for various applications in protein engineering and synthetic biology (Garamella et al., 2016; www.arborbiosci.com/resources/publications/). It entirely relies on the endogenous TXTL machinery of *E. coli* employing the core RNA polymerase and *E. coli* transcription factor sigma 70 (σ^{70}) (**Figure 1**). Therefore, any endogenous *E. coli* promoter – either constitutive or inducible – is suitable for *in vitro* gene expression in myTXTL.

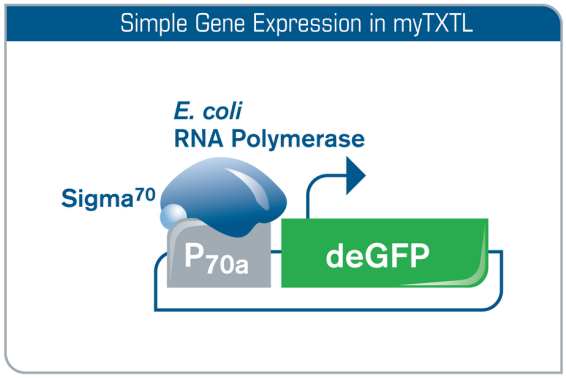

Figure 1. Gene expression in myTXTL driven by its core RNA polymerase and transcription factor sigma 70. The reporter gene depicted is an engineered version of the enhanced green fluorescent protein eGFP.

Additionally, gene expression using the popular strong T7 promoter/operator system can be enabled by a two-plasmid system (**Figure 2**): First, T7 RNA polymerase is expressed from a plasmid carrying a σ^{70} specific promoter. This allows T7 transcription of the target gene encoded on a separate plasmid.

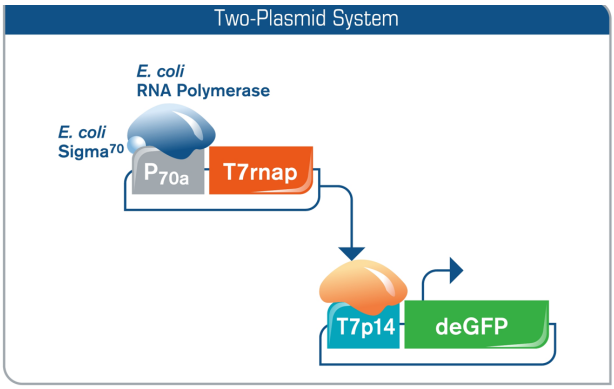

Figure 2. Co-expression of T7 RNA polymerase from a helper plasmid allows the expression of genes down-stream of a T7 promoter /operator system (T7p14-deGFP).

myTXTL Kits include an all-in-one, ready-to-use Master Mix containing *E. coli* cell extract, amino acids, and energy buffer as well as positive control template. In a typical myTXTL workflow (**Figure 3**), *in vitro* protein production starts almost instantly after mixing the DNA template and the myTXTL Master Mix in a single reaction tube. The open-reaction environment allows easy manipulation of the system and straight-forward downstream processing of the recombinant target protein. These can include either an immediate use in an activity assay or protein purification e.g. via affinity chromatography, making myTXTL ideal for high-throughput projects.

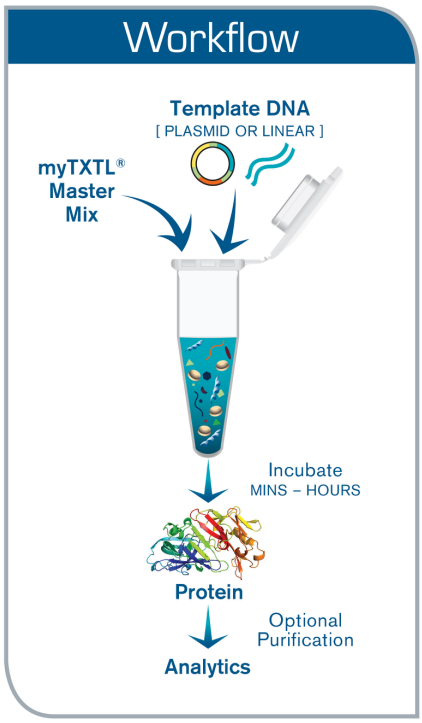

Figure 3. myTXTL workflow. *Combine either circular or linear template DNA and the ready-to-use myTXTL Master Mix to initiate in vitro gene expression.*

The **myTXTL Sigma 70 Master Mix Kit** is the perfect choice for high-yield production of soluble and membrane proteins, rapid prototyping and execution of complex multi-gene networks using plasmid DNA and RNA, the construction of synthetic minimal cells and to study cellular biology**. Our Toolbox 2.0 Plasmid Collection** offers a wide selection of promoters with different strength, various open

reading frames and options to modulate substrate turnover (complete list is available at arborbiosci.com and at the end of this handbook).

The **myTXTL Linear DNA Expression Kit** is recommended for applications that require compatibility with linear and plasmid DNA templates. It contains an engineered myTXTL Master Mix, which is based on the well-established Sigma 70 Master Mix. This makes the kit ideal for high-throughput screening of large DNA/protein libraries and allows fast iteration of design-build-test cycles with genetic parts and gene circuits. Additionally, this kit is suitable for the generation of bacteriophages.

The **myTXTL T7 Expression Kit** includes all materials required to study gene expression under a T7 promoter system and is recommended for linear and plasmid template DNA formats as it contains the engineered LS70 Master Mix of the myTXTL Linear DNA Expression Kit.

Considerations for Design and Preparation of Template Material

Design

The strength of lambda phage promoter Pr present in the P70a(2)-deGFP positive control plasmid is considered to be very similar to a standard T7 promoter, and therefore a valuable option for high-yield protein production using the endogenous *E. coli* TXTL machinery. For convenience, P70a(2)-deGFP was designed for easy sub-cloning of a target gene by using standard cloning techniques. The *deGFP* gene located between Pr and a T500 terminator is flanked by a *Nde*I and *Xho*I restriction site, respectively and can be replaced in-frame with any target gene sequence following an easy cut-and-paste procedure. The complete plasmid sequence of P70a(2)-deGFP as well as all of other plasmids is available upon request. Please refer to Technical Note TN001 Rev. 1806 “Preparation of chemo-competent KL740 cells for amplification of P_{70a} vectors” for important information regarding the handling of P70a plasmid vectors.

The optimum final concentration of the provided P70a(2)-deGFP control plasmid (3.202 kbp) in a myTXTL reaction is 5 nM (60 fmol). In general, optimum template concentration may vary depending on multiple factors such as the intended application, the strength of the promoter and RBS as well as the characteristics of the target protein. Therefore, the investigation of a concentration range of input template material in the beginning of a cell-free expression project is highly recommended. A typical concentration optimum falls within 1 nM and 20 nM final template concentration (10 -250 fmol; or 25 - 500 ng).

The absence of cell membranes in cell-free expression technology allows straightforward sample analysis and processing. For example, a target protein is directly amenable for SDS-PAGE without precipitating with TCA, ethanol or acetone and can be subjected to protein purification via affinity chromatography immediately after the incubation is completed. Additionally, an affinity tag is beneficial for detecting and quantifying the target protein in Western Blot and ELISA. To investigate

nucleic acids present or generated during the cell-free expression, standard extraction DNA and RNA protocols can be applied.

Preparation

A basic prerequisite for generating reproducible and reliable data using cell-free expression technology is the quality and purity of the input template material. It should be free of nucleases (DNases, RNases) and inhibitors of the TXTL machinery (EDTA, ethidium bromide, SDS, Cl^- ions).

Circular (plasmid) DNA Templates

For preparation of plasmid DNA, a 2-step procedure consisting of a standard, commercially available plasmid preparation kit followed by a standard PCR purification kit is recommended. The final elution should be performed in nuclease-free water to avoid interference of additional salts and ions with the well-balanced composition of the myTXTL Master Mixes. The positive control plasmid P70a(2)-deGFP provided with the kit could serve here as reference material to validate any plasmid purification procedure established at your facility.

Linear DNA Templates

Linear DNA template material is typically generated by techniques such as Golden Gate Assembly, Gibson Assembly and Assembly Polymerase Chain Reaction (PCR), and then amplified using oligonucleotides hybridizing to the 5'- and 3'-end, respectively of the desired gene construct and a high-fidelity polymerase. Prior to setting up a myTXTL reaction, the PCR product should be subjected to a PCR purification kit procedure with a final elution in nuclease-free water. Thereby, the final concentration of the template material in the myTXTL reaction can be properly determined and adjusted ensuring maximum protein yield. However, the myTXTL system also tolerates unpurified linear DNA templates directly from PCR reactions for *in vitro* gene expression, which allows even faster sample processing in (high-throughout) screening applications.

EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working in a laboratory, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs) available from the product supplier.

For *In Vitro* Gene Expression

- Nuclease-free, barrier tips and pipets capable of pipetting 0 – 100 µL
- Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
- Nuclease-free, molecular biology-grade water
- Incubator, thermo block or water bath
- Table-top microcentrifuge

- Vortex mixer

For Quantification of myTXTL Positive Control Reaction

- Fluorescence plate reader (e.g. Tecan Genios)
- Black, optical-bottom 384-well plate (e.g. Nunc)
- Phosphate-buffered saline (1x PBS)
- Recombinant eGFP (Cell Biolab, # STA-201)

For propagation of P70a plasmids and its derivatives

- *E. coli* strain KL740 cI857+ (*E. coli* Genetic Stock Center, #14222)
- Reagents, materials and instruments mentioned in Technical Note TN001 Rev. 1806 "Preparation of chemo-competent KL740 cells for amplification of P_{70a} vectors"

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PROTOCOL

General notes

- A typical myTXTL reaction has a final volume of 12 µl. Please consult with techsupport@arborbiosci.com, if changes to the final volume are desired.
- Each tube of myTXTL Master Mix contains 75 µL and is enough to prepare eight 12 µL myTXTL reactions at once.
- Avoid any condensation on the lid of the reaction vessel during incubation of the myTXTL reaction. Any changes in the reaction volume can have a considerable effect on kit performance.
- myTXTL reactions are highly sensitive to varying DNA concentration. Make sure to transfer the entire volume of DNA template to the myTXTL Master Mix.
- As the purity-grade of the template material is very crucial to generate reproducible *in vitro* expression data, we highly recommend validating the established in-house template purification procedure by using any of the myTXTL positive control or HP-grade plasmids as reference.
- To determine the background protein level, prepare a myTXTL reaction without any template DNA (use equivalent volume of nuclease-free water instead).

Protocol: myTXTL Sigma 70 Master Mix Kit

The myTXTL Sigma 70 Master Mix Kit is recommended for applications requiring cell-free gene expression from circular (plasmid) DNA templates.

Procedure

The following steps describe setting up myTXTL reactions with the positive control plasmid P70a(2)-deGFP that is supplied with the myTXTL Sigma 70 Master Mix Kit. Please refer to section ***Options for customization of a myTXTL reaction*** *below for additional information on how to optimize in vitro gene expression for your individual experiment.*

1. **Preheat incubator (or thermo block or water bath) to 29 °C.**
2. **<u>Completely</u> thaw the myTXTL Sigma 70 Master Mix and the positive control plasmid P70a(2)-deGFP on ice. Keep reagents on ice till use.**

   Note: To minimize freezing and thawing cycles, only thaw the number of reagent tubes required to set up the desired number of myTXTL reactions.
3. **Directly before use, vortex the myTXTL Sigma 70 Master Mix for 2-3 seconds and briefly spin down.** If any precipitate is visible hereafter, gently resuspend master mix solution about 10 times to ensure homogeneity. Avoid formation of bubbles and foam.
4. **Setting up a myTXTL reaction.** The recommended total volume of a myTXTL reaction is 12 µL.

   A) Set up a single myTXTL positive control reaction.
   On ice, combine components in the order depicted in Table 1 (column 1) in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Make sure to accurately transfer the entire volume of the positive control plasmid into the Sigma 70 Master Mix by resuspending it 3-4 times. Avoid formation of bubbles. The final concentration of the positive control plasmid is 5 nM.

   B) Set up multiple myTXTL positive control reactions at once (replicates).
   On ice, combine components in the order depicted in Table 1 (column 2) in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Simply scale up from volumes needed for a single myTXTL reaction and add at least 4 % of the total volume to account for pipetting errors, e.g. for eight myTXTL reactions (8x 12 = 96 µL) prepare a total volume of 100 µL. Please also read the notes in 4A).

   C) Set up a single myTXTL negative control reaction.

On ice, combine components in the order depicted in Table 1 (column 3) in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

Table 1. Pipetting scheme for setting up myTXTL positive control reactions.

Components	Single myTXTL Positive Control Reaction	Multiple (e.g. Eight) myTXTL Positive Control Reaction	Single myTXTL Negative Control Reaction
Sigma 70 Master Mix	9 µL	75 µL	9 µL
P70a(2)-deGFP Pos. Ctr. Plasmid (20 nM)	3 µL (final: 5 nM)	25 µL (final: 5 nM)	–
Nuclease-free water	–	–	3 µL
Total	12 µL	100 µL	12 µL

5. **Gently vortex the reaction mixture for 2-3 seconds and briefly centrifuge the assembled myTXTL reaction to collect the entire volume at the bottom of the tube**. The reaction should not contain any bubbles. Place back on ice.

   In the case of myTXTL reaction replicates: After centrifugation, gently resuspend the mixture 10 times, then split replicate master mix into 12 µL per reaction vessel. Add a final centrifugation step to collect the reaction on the bottom of the vessel.

6. **Incubate the myTXTL reaction(s) for up to 16 h at 29 °C.**

7. **Stop the myTXTL reaction by placing the tube(s) on ice**.

8. **Evaluate *in vitro* expression of the gene of interest.** The success of the positive control reaction can be evaluated qualitatively (visually) or quantitatively. Detailed instructions can be found in appendix section **A1.**

Options for customization of a myTXTL reaction

Based on the procedure describe above, each individual *in vitro* gene expression project might require optimization of a subset of parameters. Depending on the intended application and the characteristics of the protein of interest, parameters worth considering are:

Parameter	Options for Optimization
Incubation temperature	25 °C to 37 °C (with lower temperatures typically being advantageous).
Incubation time	5 minutes to 16 hours (or more); *In vitro* TXTL will start within the first 2-4 minutes of incubation (temperature-dependent).

Dimension of the incubation vessel	For high yield production, choose a large vessel. For example, incubate a 12 µL reaction in a 1.5 mL or 2 mL Eppendorf tube. For high-throughput applications, choose a multi-well plate and control evaporation and oxygen transfer during incubation, for example by using a multi-well plate lid and/or sealing foil.
Concentration of template material	1 – 20 nM final concentration in myTXTL reaction for initial evaluation. Fill up to the recommended final myTXTL reaction volume of 12 µL with nuclease-free water.
Supplementing additives	Addition of co-factors, chaperones, salts etc. required by the protein of interest or during an output reaction.
Switching template format: from circular to a linear fragment	In that case, supplementing the myTXTL Sigma Master Mix with purified GamS protein (Arbor Biosciences, cat # 501024 or 501096) acting as a nuclease inhibitor, will greatly enhance the yield of the protein of interest.
Promoter/RBS strength	Endogenous *E. coli* (Sigma 70-specific or others) as well as exogeneous (T7) promoters with different strength are available from Arbor Biosciences or other resources such as iGEM registry, Addgene etc.

Please also refer to our publication webpage at **https://arborbiosci.com/resources/publications/**, which is continuously updated with the newest developments in cell-free synthetic biology and biotechnology using myTXTL products. Additionally, a growing number of application and technical notes for myTXTL Cell-Free Expression products are available at **www.arborbiosci.com**.

Protocol: myTXTL Linear DNA Expression Kit

The myTXTL Linear DNA Expression Kit is recommended for applications requiring cell-free gene expression from either linear or circular (plasmid) DNA templates.

Procedure

The following steps describe setting up myTXTL reactions with the linear positive control fragment P70a-deGFP that is supplied with the myTXTL Linear DNA Expression Kit. Please refer to section ***Options for customization of a myTXTL reaction*** *below for additional information on how to optimize in vitro gene expression for your individual experiment.*

1. **Preheat incubator (or thermo block or water bath) to 29 °C.**
2. **<u>Completely</u> thaw the myTXTL LS70 Master Mix and the linear positive control fragment P70a-deGFP on ice. Keep reagents on ice till use.**

   Note: To minimize freezing and thawing cycles, only thaw the number of reagent tubes required to set up the desired number of myTXTL reactions.
3. **Directly before use, vortex the myTXTL LS70 Master Mix for 2-3 seconds and briefly spin down.** If any precipitate is visible hereafter, gently resuspend master mix solution about 10 times to ensure homogeneity. Avoid formation of bubbles and foam.
4. **Setting up a myTXTL reaction.** The recommended total volume of a myTXTL reaction is 12 µL.

   A) Set up a single myTXTL positive control reaction.

   On ice, combine components in the order depicted in Table 2 in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Make sure to accurately transfer the entire volume of the linear positive control fragment into the LS70 Master Mix by resuspending it 3-4 times. Avoid formation of bubbles. The final concentration of the linear positive control fragment is 20 nM.

   B) Set up multiple myTXTL positive control reactions at once (replicates).

   On ice, combine components in the order depicted in Table 2 in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Simply scale up from volumes needed for a single myTXTL reaction and add at least 4 % of the total volume to account for pipetting errors, e.g. for eight myTXTL reactions (8x 12 = 96 µL) prepare a total volume of 100 µL. Please also read the notes in 4A).

   C) Set up a single myTXTL negative control reaction.

On ice, combine components in the order depicted in Table 2 (column 3) in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

Table 2. Pipetting scheme for setting up myTXTL positive control reactions.

Components	Single myTXTL Positive Control Reaction	Multiple (e.g. Eight) myTXTL Positive Control Reaction	Single myTXTL Negative Control Reaction
LS70 Master Mix	9 µL	75 µL	9 µL
Linear P70a-deGFP Pos. Ctr. Frag. (80 nM)	3 µL (final: 20 nM)	25 µL (final: 20 nM)	–
Nuclease-free water	–	–	3 µL
Total	12 µL	100 µL	12 µL

5. **Gently vortex the reaction mixture for 2-3 seconds and briefly centrifuge the assembled myTXTL reaction to collect the entire volume at the bottom of the tube**. The reaction should not contain any bubbles. Place back on ice.

   In the case of myTXTL reaction replicates: After centrifugation, gently resuspend the mixture 10 times, then split replicate master mix into 12 µL per reaction vessel. Add a final centrifugation step to collect the reaction on the bottom of the vessel.

6. **Incubate the myTXTL reaction(s) for up to 16 h at 29 °C.**

7. **Stop the myTXTL reaction by placing the tube(s) on ice**.

8. **Evaluate *in vitro* expression of the gene of interest.** The success of the positive control reaction can be evaluated qualitatively (visually) or quantitatively. Detailed instructions can be found in appendix section **A1.**

Options for customization of a myTXTL reaction

Based on the procedure describe above, each individual *in vitro* gene expression project might require optimization of a subset of parameters. Depending on the intended application and the characteristics of the protein of interest, parameters worth considering are:

Parameter	Options for Optimization
Incubation temperature	25 °C to 37 °C (with lower temperatures typically being advantageous).
Incubation time	5 minutes to 16 hours (or more); *In vitro* TXTL will start within the first 2-4 minutes of incubation (temperature-dependent).

Dimension of the incubation vessel	For high yield production, choose a large vessel. For example, incubate a 12 µL reaction in a 1.5 mL or 2 mL Eppendorf tube. For high-throughput applications, choose a multi-well plate and control evaporation and oxygen transfer during incubation, for example by using a multi-well plate lid and/or sealing foil.
Concentration of template material	1 – 20 nM final concentration in myTXTL reaction for initial evaluation. Fill up to the recommended final myTXTL reaction volume of 12 µL with nuclease-free water.
Supplementing additives	Addition of co-factors, chaperones, salts etc. required by the protein of interest or during an output reaction.
Template format	The myTXTL Linear DNA Expression Kit contains the LS70 Master Mix, which is suitable for linear and circular DNA templates.
Template design	It is recommended to append a 250 bp long, non-coding flanking region upstream of the promoter and a 100 bp long, non-coding flanking region downstream of the terminator in case of linear DNA templates.
Promoter/RBS strength	Endogenous *E. coli* (Sigma 70-specific or others) as well as exogeneous (T7) promoters with different strength are available from Arbor Biosciences or other resources such as iGEM registry, Addgene etc.

Please also refer to our publication webpage at **https://arborbiosci.com/resources/publications/**, which is continuously updated with the newest developments in cell-free synthetic biology and biotechnology using myTXTL products. Additionally, a growing number of application and technical notes for myTXTL Cell-Free Expression products are available at **www.arborbiosci.com**.

Protocol: myTXTL T7 Expression Kit

The myTXTL T7 Expression Kit is recommended for applications requiring cell-free gene expression from either linear or circular (plasmid) DNA template and provides all components necessary for T7 promoter driven gene expression.

Procedure

The following steps describe setting up myTXTL reactions, in which gene expression is driven by a T7 promoter system using the positive control plasmid T7p14-deGFP and P70a-T7rnap as part of the myTXTL T7 Expression Kit. Please refer to section

Options for customization of a myTXTL reaction *below for additional information on how to optimize in vitro gene expression for your individual experiment.*

1. **Preheat incubator (or thermo block or water bath) to 29 °C.**
2. **<u>Completely</u> thaw the myTXTL LS70 Master Mix, the helper plasmid P70a-T7rnap and the positive control plasmid T7p14-deGFP on ice. Keep reagents on ice till use.**

   Note: To minimize freezing and thawing cycles, only thaw the number of reagent tubes required to set up the desired number of myTXTL reactions.
3. **Directly before use, vortex the myTXTL LS70 Master Mix for 2-3 seconds and briefly spin down.** If any precipitate is visible hereafter, gently resuspend master mix solution about 10 times to ensure homogeneity. Avoid formation of bubbles and foam.
4. **Setting up a myTXTL reaction.** The recommended total volume of a myTXTL reaction is 12 µL.

   A) Set up a single myTXTL positive control reaction.
   On ice, combine components in the order depicted in Table 3 in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Make sure to accurately transfer the entire volume of plasmid template material into the LS70 Master Mix by resuspending it 3-4 times. Avoid formation of bubbles.

   B) Set up multiple myTXTL positive control reactions at once (replicates).
   On ice, combine components in the order depicted in Table 3 in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

   Note: Simply scale up from volumes needed for a single myTXTL reaction and add at least 4 % of the total volume to account for pipetting errors, e.g. for eight myTXTL reactions (8x 12 = 96 µL) prepare a total volume of 100 µL. Please also read the notes in 4A).

C) Set up a single myTXTL negative control reaction.
On ice, combine components in the order depicted in Table 3 (column 3) in a nuclease-free reaction vessel (e.g. 2 mL Eppendorf tube).

Table 3. Pipetting scheme for setting up myTXTL positive control reactions using a T7 promoter system.

Components	Single myTXTL Positive Control Reaction	Multiple (e.g. Eight) myTXTL Positive Control Reaction	Single myTXTL Negative Control Reaction
Sigma 70 Master Mix	9 µL	75 µL	9 µL
T7p14-deGFP HP (24 nM)	2.5 µL (final: 5 nM)	20.8 µL (final: 5 nM)	–
P70a-T7rnap HP (2.4 nM)	0.5 µL (final: 0.1 nM)	4.2 µL (final: 0.1 nM)	0.5 µL (final: 0.1 nM)
Nuclease-free water	–	–	2.5 µL
Total	12 µL	100 µL	12 µL

5. **Gently vortex the reaction mixture for 2-3 seconds and briefly centrifuge the assembled myTXTL reaction to collect the entire volume at the bottom of the tube**. The reaction should not contain any bubbles. Place back on ice.

   In the case of myTXTL reaction replicates: After centrifugation, gently resuspend the mixture 10 times, then split replicate master mix into 12 µL per reaction vessel. Add a final centrifugation step to collect the reaction on the bottom of the vessel.

6. **Incubate the myTXTL positive control reaction(s) for up to 16 h at 29 °C.**

7. **Stop the myTXTL positive control reaction by placing the tube(s) on ice**.

8. **Evaluate *in vitro* expression of the gene of interest.** The success of the positive control reaction can be evaluated qualitatively (visually) or quantitatively. Detailed instructions can be found in appendix section **A1.**

Options for customization of a myTXTL reaction

Based on the procedure describe above, each individual *in vitro* gene expression project might require optimization of a subset of parameters. Depending on the intended application and the characteristics of the protein of interest, parameters worth considering are:

Parameter	Options for Optimization
Incubation temperature	25 °C to 37 °C (with lower temperatures typically being advantageous).
Incubation time	5 minutes to 16 hours (or more); *In vitro* TXTL will start within the first 2-4 minutes of incubation (temperature-dependent).
Dimension of the incubation vessel	For high yield production, choose a large vessel. For example, incubate a 12 µL reaction in a 1.5 mL or 2 mL Eppendorf tube. For high-throughput applications, choose a multi-well plate and control evaporation and oxygen transfer during incubation, for example by using a multi-well plate lid and/or sealing foil.
Concentration of template material	1 – 20 nM final concentration of the T7 promoter plasmid carrying the gene of interest for initial evaluation. Increasing the concentration of the helper plasmid P70a-T7rnap does typically not increase yield of the protein of interest. Fill up to the recommended final myTXTL reaction volume of 12 µL with nuclease-free water.
Supplementing additives	Addition of co-factors, chaperones, salts etc. required by the protein of interest or during an output reaction.
Template format	The myTXTL T7 Expression Kit contains the LS70 Master Mix, which is suitable for linear and circular DNA templates.
Promoter/RBS strength	Promoters and RBS with different strength are available from Arbor Biosciences or other resources such as iGEM registry, Addgene etc.

Please also refer to our publication webpage at **https://arborbiosci.com/resources/publications/**, which is updated with the newest developments in cell-free synthetic biology and biotechnology using

myTXTL products. Additionally, a growing number of application and technical notes for myTXTL Cell-Free Expression products are available at **www.arborbiosci.com**.

APPENDIX

A1. Fluorescence-based Analysis of Protein Synthesis in myTXTL

For convenience, myTXTL Sigma 70 Master Mix Kits, myTXTL Linear Expression Kits and myTXTL T7 Expression Kits contain positive control template material encoding an engineered version of the enhanced green fluorescent protein (deGFP) for an easy and direct visualization of protein synthesis (Figure 4).

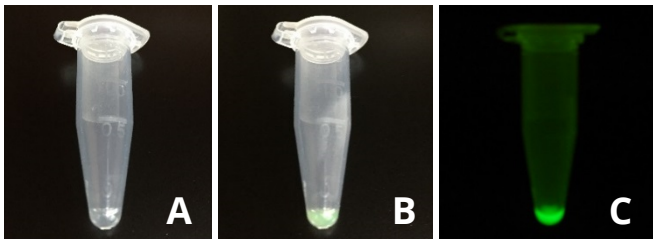

Figure 4. myTXTL reaction containing a positive control template before (A) and after (B) incubation at 29 °C. (C) deGFP fluorescence in the myTXTL reaction under UV light.

To evaluate the protein production efficiency of the myTXTL Master Mix in your lab, you can choose between two methods. After carrying out a single myTXTL positive control reaction, either perform a **(A)** qualitative (visual) read-out of your final deGFP concentration or **(B)** a quantitative analysis of your sample.

For both procedures, the myTXTL control reaction is centrifuged at > 16,300 g (or full speed) for 3 min at RT directly before sample analysis.

(A) Qualitative (visual) Analysis

Compare the intensity of (green) color in your myTXTL control reaction to the following standard eGFP color strip (Figure 5) to assess deGFP produced in your tube.

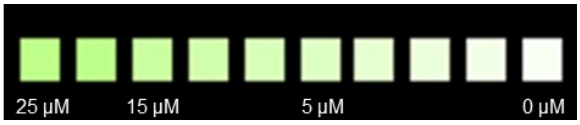

Figure 5. Color strip for the qualitative analysis of deGFP production.

(B) Quantitative Analysis

1. Prepare an eGFP standard curve (0-5 µM)

- Thaw and keep the recombinant eGFP standard (Cell Biolabs, # STA-201) on ice. Determine the molar concentration of your protein solution.
- Prepare an eGFP stock solution of 5 µM in PBS (V = 70 µL) in a 1.5 mL reaction tube.

Example: If your eGFP standard has a concentration of 30 µM, transfer 11.7 µL of the 30 µM eGFP protein solution to 58.3 µL PBS, mix thoroughly and collect mixture on the tube bottom by a short centrifugation step.

- Prepare a 2-fold dilution series of eGFP in the concentration range of 0-5 µM in 1.5 mL reaction tubes (5, 2.5, 1.25, 0.63 and 0.31 µM).

  Example: To prepare a 2.5 µM eGFP solution, transfer 35 µL of the 5 µM eGFP solution to 35 µL PBS and mix thoroughly. Then take 35 µL of the 2.5 µM eGFP solution to prepare the next dilution step. Proceed to 0.31 µM eGFP (five dilution steps).
- For each dilution, transfer 10 µL/well in triplicate into a black, optical-bottom 384-well plate. Also include a Blank measurement in triplicate using PBS only.

2. Dilute the myTXTL control reaction

- Prepare a 10-fold dilution of the centrifuged myTXTL control reaction in PBS. In a 1.5 mL reaction tube, add 4 µL myTXTL control reaction to 36 µL PBS, mix thoroughly and collect mixture on the tube bottom by a short centrifugation step.
- Transfer 10 µL/well of this diluted sample in triplicate to the same 384-well plate as the eGFP standard dilution series (see above).

3. Perform fluorescence measurement using a plate reader

- Before the fluorescence measurement, carefully tap or briefly spin down the 384-well plate to remove any air bubbles and to equally distribute each sample in the well.
- Fluorescence reader setting: Choose an excitation and emission wavelength appropriate for eGFP measurement (e.g. excitation: 488 nm, emission: 535 nm).

4. Calculate the deGFP concentration using a calibration curve (linear regression)

- Subtract the fluorescence values of the Blank (PBS only) from that of each standard protein and myTXTL control reaction.
- Plot the Blank subtracted fluorescence values of the eGFP standard (Y-axis) against their respective protein concentration (X-axis) and fit the curve to the linear regression formula ($y = m*x$) to determine the deGFP concentration in the myTXTL control reaction (Figure 6).

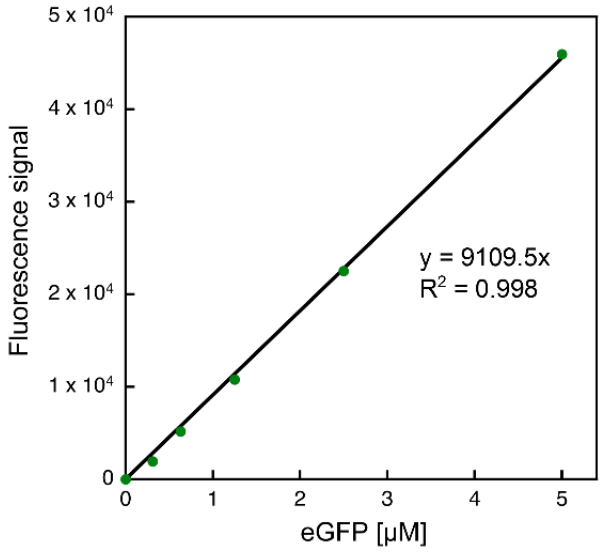

Figure 6. eGFP standard curve to evaluate protein production efficiency in myTXTL.

A2. Analysis of Protein Synthesis in myTXTL Using SDS-PAGE

In case of the target protein lacking any fluorescent properties, an SDS-PAGE analysis of myTXTL reactions is an alternative for a visual evaluation of its *in vitro* production. The 15 % SDS-PAGE gel in Figure 7 demonstrates the kinetic analysis of protein production in myTXTL over an incubation period of 16 h. Typically, only a small volume (1 – 3 µL) of the myTXTL reaction is loaded onto the SDS-PAGE gel. As myTXTL Master Mixes are derived from a *E. coli* whole cell extract, the lanes show also bacterial host cell proteins. In case the protein of interest has a molecular weight similar to a host cell protein or its *in vitro* production is less optimized, a polypeptide tag such as a His6-tag, Strep-tag® or FLAG-tag added to the N- or C-terminus of the target protein would allow protein detection via a Western Blot and/or isolation of the target protein using suitable affinity purification resins.

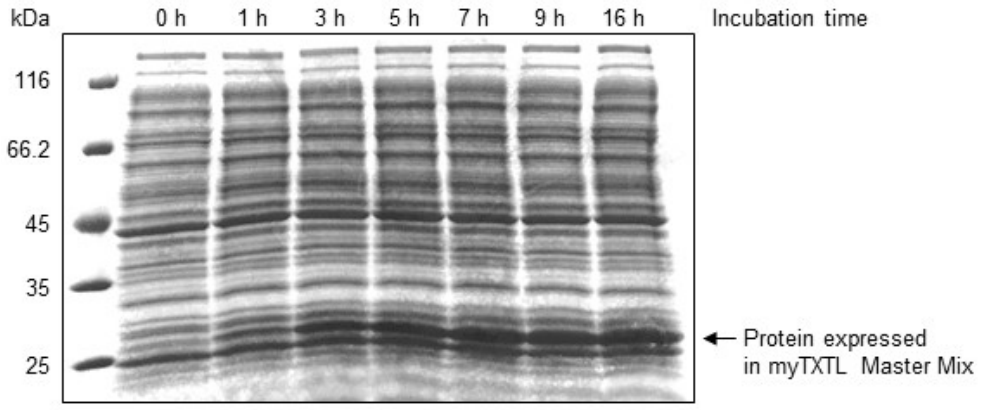

Figure 7. Kinetic analysis of deGFP expression in myTXTL Master Mix using SDS-PAGE. The gel was stained with a standard Coomassie® procedure.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may arise from using myTXTL kits. For more information, please also refer to the collection of Frequently Asked Questions (FAQ) at **arborbiosci.com/resources/faqs/**. Alternatively, Arbor Biosciences' Technical Support team is always happy to answer any questions you may have about any information or protocols in this handbook or sample and assay technologies. Contact us by phone at **+1 (734) 998-0751** or email at **techsupport@arborbiosci.com**.

Low protein yield	
a) DNA template quality insufficient	Follow recommended procedure for template preparation; use nuclease-free, molecular biology-grade water; use internal kit control template to establish in-house purification protocol
b) Template concentration not optimal	Test a template concentration within a certain range; start with 1 – 20 nM and expand from there
c) Poor promoter choice	Typically, strong promoter/RBS combination will result in high protein yield. However, if the polypeptide chain is folding slowly, a strong promoter will result in a higher degree of aggregated protein. Try out promoters of different strength.
d) Suboptimal incubation vessel	To achieve maximum yield in myTXTL, use large reactions vessel. For example, use a 1.5 or 2 mL reaction tube even to incubate a 12 µL myTXTL reaction.
e) Improper template design	If working with a N- or C-terminal peptide tag, it may be affecting the RNA structure and lowering translation levels. Try moving the fusion tag to the other terminus. If not working with a tag, try adding a tag as it can potentially increase protein solubility.
Inconsistent protein yield/results	
a) DNA template quality insufficient	Follow recommended procedure for template preparation; use nuclease-free, molecular biology-grade water; use internal kit control template to establish in-house purification protocol
b) Inaccurate product handling	Especially the myTXTL Master Mixes are highly sensitive to storage temperature. –80 °C is required for storage for more than a month. For short-term storage (< 1 month), myTXTL kits can be stored at -20 °C.

	Cell-free reactions are sensitive to the input template amount/concentration and require precise and accurate pipetting.
No protein production observed	
a) Incorrect construct design	Check your template configuration (promoter sequence, ATG initiation, in frame tags, stop codon etc.)
b) myTXTL Master Mix has become inactivated	If the positive control template provided with the kit doesn't give clear visual green fluorescent protein output, the myTXTL Master Mix is very likely to have lost activity. Check the storage conditions and expiration date. Use care when freezing and thawing. Up to five freeze-and-thaw cycles are acceptable.
c) Protein doesn't fold properly/is biological inactive	Investigate if protein requires formation of disulfide bonds, co-factors or other additives. Folding helper and/or additional reagents might need to be added in addition to your DNA template. Lowering the incubation temperature to < 30 °C increases the probability of correct folding. For membrane proteins, detergents or other membrane forming additives such as Nanodiscs are required to be added to the myTXTL Master Mix.
d) Inaccurate product handling	Especially the myTXTL Master Mixes are highly sensitive to storage temperature. –80 °C is required for storage for more than a month. For short-term storage (< 1 month), myTXTL kits can be stored at -20 °C.

REFERENCES

Garamella, J., Marshall, R., Rustad, M., and Noireaux, V. (2016). The All E. coli TX-TL Toolbox 2.0: A Platform for Cell-Free Synthetic Biology. ACS Synth. Biol. *5*, 344–355.

ORDERING INFORMATION

Product	Contents	Cat #
myTXTL T7 Expression Kit - 24 Rxn	225 µL LS70 Master Mix, pTXTL-P70a-T7rnap HP 24 Rxn, pTXTL-T7p14-deGFP HP 10 Rxn	505024
myTXTL T7 Expression Kit - 96 Rxn	900 µL LS70 Master Mix, pTXTL-P70a-T7rnap HP 96 Rxn, pTXTL-T7p14-deGFP HP 10 Rxn	505096
myTXTL Sigma 70 Master Mix Kit - 24 Rxn	225 µL Sigma 70 Master Mix, P70a(2)-deGFP Positive Control Plasmid 10 Rxn	507024
myTXTL Sigma 70 Master Mix Kit - 96 Rxn	900 µL Sigma 70 Master Mix, P70a(2)-deGFP Positive Control Plasmid 20 Rxn	507096
myTXTL Sigma 70 Master Mix (bulk)	Custom volume of Sigma 70 Master Mix	507099
myTXTL Linear DNA Expression Kit - 24 Rxn	225 µL LS70 Master Mix, Linear Positive Control Fragment P70a-deGFP 10 Rxn	508024
myTXTL Linear DNA Expression Kit - 96 Rxn	900 µL Sigma 70 Master Mix, Linear Positive Control Fragment P70a-deGFP 20 Rxn	508096
myTXTL LS70 Master Mix (bulk)	Custom volume of LS70 Master Mix	508099
Related Products		
myTXTL Master Mix Supplements		
GamS Purified Nuclease Inhibitor Protein 24 Rxn	25 µL GamS protein (150 µM)	501024
GamS Purified Nuclease Inhibitor Protein 96 Rxn	80 µL GamS protein (150 µM)	501096
High-Purity (HP), Ready-to-Use Plasmids*		
pTXTL-P70a(2)-deGFP (Pos. Ctrl. Plasmid) 10 Rxns	35 µL plasmid solution (20 nM)	502138
pTXTL-P70a-deGFP HP 10 Rxns	35 µL plasmid solution (20 nM)	502117
pTXTL-P70a-T7rnap HP 24 Rxns	15 µL plasmid solution (2.4 nM).	502134
pTXTL-P70a-T7rnap HP 96 Rxns	50 µL plasmid solution (2.4 nM).	502135
pTXTL-T7p14-deGFP HP 10 Rxns	25 µL plasmid solution (24 nM)	502136

Toolbox Plasmids*		
Sigma Factor 19 specific promoters		
pTXTL-P19a-deGFP	1 µg plasmid, dry	502001
pTXTL-P19a-deCFP	1 µg plasmid, dry	502002
pTXTL-P19a-deYFP	1 µg plasmid, dry	502003
pTXTL-P19a-ntrC	1 µg plasmid, dry	502004
pTXTL-P19a-S24	1 µg plasmid, dry	502005
pTXTL-P19a-ompA-S24	1 µg plasmid, dry	502006
pTXTL-P19a-S28	1 µg plasmid, dry	502007
pTXTL-P19a-S28-ssrA	1 µg plasmid, dry	502008
pTXTL-P19a-S38	1 µg plasmid, dry	502009
Sigma Factor 24 specific promoters		
pTXTL-P24a-deGFP	1 µg plasmid, dry	502010
pTXTL-P24a-S28	1 µg plasmid, dry	502011
pTXTL-P24a-ompA-S38	1 µg plasmid, dry	502012
pTXTL-P24a-S28-ssrA	1 µg plasmid, dry	502013
pTXTL-P24a-S38	1 µg plasmid, dry	502014
Sigma Factor 28 specific promoters		
pTXTL-P28a-aH	1 µg plasmid, dry	502015
pTXTL-P28a-cI-ssrA	1 µg plasmid, dry	502017
pTXTL-P28a-aH-eGFP	1 µg plasmid, dry	502018
pTXTL-P28a-deGFP	1 µg plasmid, dry	502019
pTXTL-P28a-deGFP-ssrA	1 µg plasmid, dry	502020
pTXTL-P28a-deYFP	1 µg plasmid, dry	502021
pTXTL-P28a-flgM	1 µg plasmid, dry	502022
pTXTL-P28a-flgM-ssrA	1 µg plasmid, dry	502023
pTXTL-P28a-mApple	1 µg plasmid, dry	502025
pTXTL-P28a-mazE-ssrA	1 µg plasmid, dry	502027
pTXTL-P28a-mreB	1 µg plasmid, dry	502028
pTXTL-P28a-mreC	1 µg plasmid, dry	502029
pTXTL-P28a-ntrC	1 µg plasmid, dry	502030
pTXTL-P28a-S19	1 µg plasmid, dry	502031
pTXTL-P28a-S19-ssA	1 µg plasmid, dry	502032
pTXTL-P28a-S24-ssrA	1 µg plasmid, dry	502033
pTXTL-P28a-ompA-S24-ssrA	1 µg plasmid, dry	502034
pTXTL-P28a-S38	1 µg plasmid, dry	502035
pTXTL-P28a-S54	1 µg plasmid, dry	502036
pTXTL-P28a-T7rnap	1 µg plasmid, dry	502118
pTXTL-P28a-tetR	1 µg plasmid, dry	502037

pTXTL-P28a-tetR-ssrA	1 µg plasmid, dry	502131
pTXTL-P28a-tetO1-deGFP-ssrA	1 µg plasmid, dry	502132
pTXTL-P28a-venus-mreB	1 µg plasmid, dry	502038
pTXTL-P28a-venus-mreB-18L	1 µg plasmid, dry	502039
Sigma Factor 32 specific promoters		
pTXTL-P32a-deGFP	1 µg plasmid, dry	502040
Sigma Factor 38 specific promoters		
pTXTL-P38a-deGFP	1 µg plasmid, dry	502041
pTXTL-P38a-ntrC	1 µg plasmid, dry	502042
pTXTL-P38a-S19	1 µg plasmid, dry	502043
pTXTL-P38a-S19-ssrA	1 µg plasmid, dry	502044
pTXTL-P38a-S28	1 µg plasmid, dry	502045
pTXTL-P38a-S28-ssrA	1 µg plasmid, dry	502046
pTXTL-P38a-S54	1 µg plasmid, dry	502047
Sigma Factor 54 specific promoters		
pTXTL-P54a-deGFP	1 µg plasmid, dry	502048
pTXTL-P54a-S19	1 µg plasmid, dry	502049
pTXTL-P54a-S24	1 µg plasmid, dry	502050
pTXTL-P54a-S28	1 µg plasmid, dry	502051
pTXTL-P54a-S38	1 µg plasmid, dry	502052
Sigma Factor 70 specific promoters		
pTXTL-P70a-broccoli	1 µg plasmid, dry	502119
pTXTL-P70a-clpXP	1 µg plasmid, dry	502053
pTXTL-P70a-cI	1 µg plasmid, dry	502120
pTXTL-P70a-cI-ssrA	1 µg plasmid, dry	502054
pTXTL-P70a-deCFP	1 µg plasmid, dry	502055
pTXTL-P70a-deGFP	1 µg plasmid, dry	502056
pTXTL-P70a-deGFP-ssrA	1 µg plasmid, dry	502057
pTXTL-P70a-deYFP	1 µg plasmid, dry	502058
pTXTL-P70a-dmVenus	1 µg plasmid, dry	502059
pTXTL-P70a-dTomato	1 µg plasmid, dry	502060
pTXTL-P70a-luc	1 µg plasmid, dry	502121
pTXTL-P70a-mApple	1 µg plasmid, dry	502061
pTXTL-P70a-mmCherry	1 µg plasmid, dry	502063
pTXTL-P70a-mGapt1	1 µg plasmid, dry	502064
pTXTL-P70a-mGapt-deGFP	1 µg plasmid, dry	502065
pTXTL-P70a-mRuby	1 µg plasmid, dry	502066
pTXTL-P70a-S19	1 µg plasmid, dry	502067
pTXTL-P70a-S19-ssrA	1 µg plasmid, dry	502068

pTXTL-P70a-S24	1 µg plasmid, dry	502069
pTXTL-P70a-ompA-S24	1 µg plasmid, dry	502070
pTXTL-P70a-ntrC	1 µg plasmid, dry	502071
pTXTL-P70a-S28	1 µg plasmid, dry	502072
pTXTL-P70a-S28-ssrA	1 µg plasmid, dry	502073
pTXTL-P70a-S28-ybaQ	1 µg plasmid, dry	502074
pTXTL-P70a-S32	1 µg plasmid, dry	502075
pTXTL-P70a-S32-ssrA	1 µg plasmid, dry	502076
pTXTL-P70a-S38	1 µg plasmid, dry	502077
pTXTL-P70a-ompA-S38	1 µg plasmid, dry	502078
pTXTL-P70a-S54	1 µg plasmid, dry	502079
pTXTL-P70a-tagRFPT1	1 µg plasmid, dry	502080
pTXTL-P70a-T3rnap	1 µg plasmid, dry	502081
pTXTL-P70a-T7rnap	1 µg plasmid, dry	502082
pTXTL-P70a-U3-deGFP	1 µg plasmid, dry	502083
pTXTL-P70a-U4-deGFP	1 µg plasmid, dry	502084
pTXTL-P70a-U5-deGFP	1 µg plasmid, dry	502085
pTXTL-P70a-venus	1 µg plasmid, dry	502086
pTXTL-P70b-broccoli	1 µg plasmid, dry	502122
pTXTL-P70b-deGFP	1 µg plasmid, dry	502087
pTXTL-P70b-mGapt1	1 µg plasmid, dry	502088
pTXTL-P70b-U3-deGFP	1 µg plasmid, dry	502090
pTXTL-P70b-U4-deGFP	1 µg plasmid, dry	502091
pTXTL-P70c-deGFP	1 µg plasmid, dry	502092
pTXTL-P70c-mGapt1	1 µg plasmid, dry	502093
pTXTL-P70c-U3-deGFP	1 µg plasmid, dry	502095
pTXTL-P70c-U4-deGFP	1 µg plasmid, dry	502096
pTXTL-P70d-deGFP	1 µg plasmid, dry	502097
pTXTL-P70e-broccoli	1 µg plasmid, dry	502123
pTXTL-P70e-deGFP	1 µg plasmid, dry	502124
pTXTL-PLlacO1-deGFP	1 µg plasmid, dry	502125
pTXTL-PLlacO1-deCFP	1 µg plasmid, dry	502126
pTXTL-PLlacO1-tetR	1 µg plasmid, dry	502127
pTXTL-PLtetO1-deGFP	1 µg plasmid, dry	502098
pTXTL-PLtetO1-deGFP-ssrA	1 µg plasmid, dry	502099
pTXTL-PLtetO1-deGFP-ybaQ	1 µg plasmid, dry	502100
pTXTL-PLtetO1-lacO1	1 µg plasmid, dry	502128
pTXTL-PLtetO1-tetR	1 µg plasmid, dry	502129
pTXTL-PLtetO1-mmCherry-ssrA	1 µg plasmid, dry	502133

pTXTL-pC-mGapt	1 µg plasmid, dry	502102
pTXTL-pCa-deGFP	1 µg plasmid, dry	502103
pTXTL-T3p-deGFP	1 µg plasmid, dry	502104
T7 promoters		
pTXTL-T7p7-deGFP	1 µg plasmid, dry	502105
pTXTL-T7p7-mGapt	1 µg plasmid, dry	502106
pTXTL-T7p8-deGFP	1 µg plasmid, dry	502107
pTXTL-T7p8-mGapt	1 µg plasmid, dry	502108
pTXTL-T7p11-deGFP	1 µg plasmid, dry	502109
pTXTL-T7p11-mGapt	1 µg plasmid, dry	502110
pTXTL-T7p14-deGFP	1 µg plasmid, dry	502111
pTXTL-T7p14-mGapt	1 µg plasmid, dry	502112
pTXTL-T7p14-aH	1 µg plasmid, dry	502113
pTXTL-T7p14-aH-eGFP	1 µg plasmid, dry	502114
pTXTL-T7p14-luc	1 µg plasmid, dry	502130
pTXTL-T7p16-deGFP	1 µg plasmid, dry	502115
pTXTL-T7p16-mGapt	1 µg plasmid, dry	502116

* Plasmid sequences available upon request at techsupport@arborbiosci.com.

HANDBOOK REVISION HISTORY

Document	Date	Changes
myTXTL-Handbook 06/2019	June 2019	Created document

myTXTL-Handbook-v01