Materials List for:

Protocols for Implementing an *Escherichia coli* Based TX-TL Cell-Free Expression System for Synthetic Biology

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Materials

Name	Company	Catalog Number	Comments
2xYT	MP biomedicals	3012-032	
3-PGA	Sigma-Aldrich	P8877	
ATP	Sigma-Aldrich	A8937	
Bacto-agar	BD Diagnostics	214010	
Bead-beating tubes (polypropylene microvials)	BioSpec	522S	
Beads, 0.1mm dia.	BioSpec	11079101	
BL21 Rosetta 2 *E. coli* strain	Novagen	71402	
Bradford BSA Protein Assay Kit	Bio-rad	500-0201	
cAMP	Sigma-Aldrich	A9501	
Chloramphenicol	Sigma-Aldrich	C1919	
CoA	Sigma-Aldrich	C4282	
CTP	USB	14121	
Cuvettes, 1.5ml	Fisher	14-955-127	
DTT	Sigma-Aldrich	D0632	
Folinic acid	Sigma-Aldrich	F7878	
GTP	USB	16800	
HEPES	Sigma-Aldrich	H6147	
K-glutamate	Sigma-Aldrich	G1149	
Mg-glutamate	Sigma-Aldrich	49605	
Micro Bio-Spin Chromatography Columns	Bio-Rad	732-6204	
NAD	Sigma-Aldrich	N6522	
Nunc 384-well optical bottom plates	Thermo-Scientific	142761	
Nunc sealing tape	Thermo-Scientific	232701	
PEG-8000	Promega	V3011	
Potassium phosphate dibasic solution	Sigma-Aldrich	P8584	
Potassium phosphate monobasic solution	Sigma-Aldrich	P8709	
RTS Amino Acid Sampler	5 Prime	2401530	
Slide-A-Lyzer Dialysis Cassettes, 10k MWCO (Kit)	Thermo-Scientific	66382	

Spermidine	Sigma-Aldrich	85558	
Tris base	Fischer	BP1521	
tRNA (from *E. coli*)	Roche Applied Science	MRE600	
UTP	USB	23160	
1L Centrifuge Bottle	Beckman-Coulter	A98813	This is specific for Avanti J-series; obtain equivalent size for centrifuge in use.
4L Erlenmeyer Flask	Kimble Chase	26500-4000	
Avanti J-26XP Centrifuge	Beckman-Coulter	393127	Or 1L-capable centrifuge equivalent.
Forma 480 Orbital Shaker	Thermo Scientific	480	Or chest-size 6x4L shaker equivalent.
JLA-8.1000 Rotor	Beckman-Coulter	363688	Or 1L-capable, 5000 x g rotor equivalent for centrifuge.
Mini-Beadbeater-1	BioSpec	3110BX	

Supplemental Material 1. Recipes for Items.

Chloramphenicol, 34 mg/ml: Prepare 0.51 g chloramphenicol and add ethanol to 15 ml. Filter sterilize (0.22 µM), aliquot to 1 ml tubes, store at -20 °C for later use.

2xYT+P+Cm agar plate: Prepare 1.24 g 2xYT, 1.6 ml potassium phosphate dibasic solution @ 1 M, 0.88 ml potassium phosphate monobasic solution @ 1 M, 0.6 g agar, and water to 40 ml. Autoclave. Let cool to 50 °C and add 40 µl Cm. Aliquot 25 ml into a 100x15 mm petri dish, and let cool for an hour.

2xYT+P media: Prepare 124 g 2xYT, 160 ml potassium phosphate dibasic solution @1 M, 88 ml potassium phosphate monobasic solution @ 1 M, and water to 4 L. Aliquot out into 2x1.88 L and 0.24 L. Autoclave.

Tris base, 2 M: Prepare 60.57 g Tris base and water to 250 ml. Sterilize, store at RT for later use.

DTT, 1 M: Prepare 2.31 g DTT and water to 15 ml. Filter sterilize (0.22 µM), aliquot to 1 ml tubes, store at -20 °C for later use.

S30A buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, 50 ml Tris at 2M, acetic acid (to pH 7.7), and water to 2 L. Autoclave, store at 4 °C, add 4 ml 1 M DTT before use.

S30B buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, Tris at 2 M (to pH 8.2), and water to 2 L. Autoclave, store at 4 °C, add 2 ml 1 M DTT before use.

HEPES: Prepare 1.91 g HEPES (MW 238.21), KOH (to pH 8), and water to 4 ml.

tRNA: Prepare 30 mg of tRNA and water to 600 µl.

CoA: Prepare 30 mg of CoA (MW 767.53) and water to 600 µl.

NAD: Add 34.83 mg of NAD (MW 663.43), Tris at 2 M (to pH 7.5-8), and water to 300 µl. (Add 27 µl of Tris at 2 M to bring the solution to pH 7.5-8).

cAMP: Add 42.80 mg of cAMP (MW 329.22), Tris at 2 M (to pH 8), and water to 200 µl. (Add 73 µl of Tris at 2 M to bring the solution to pH 8).

Folinic Acid (33.9 mM): To 20 mg of solid folinic acid calcium salt (MW 511.5), add 1.15 ml water.

Spermidine: Prepare 23.55 µl of spermidine (MW 145.25) and water to 150 µl. Prepare at room temperature after melting briefly at 37 °C.

3-PGA: Add 1.03 g of 3-PGA (MW 230.02), Tris at 2 M (to pH 7.5), and water to 3.2 ml. (Add 1.73 ml of Tris at 2 M to bring the solution to pH 7.5).

Nucleotide Mix: Add 145 mg of ATP dipotassium salt dihydrate (MW 619.4), 133 mg of GTP disodium salt (MW 567.14), 79.4 mg of CTP disodium salt dihydrate (MW 563.16), 82.6 mg of UTP trisodium salt dihydrate (MW 586.12), KOH at 15% dilution (to pH 7.5), and water to 1.5 ml. (Add 353 µl of KOH at 15% dilution to bring the solution to pH 7.5).

Supplemental Material 2. Bradford Assay.

1. Remove Bradford agent from 4 °C and set at room temperature.
2. Prepare 50 µl BSA Standard at 1 mg/ml and at 0.1 mg/ml.
3. Prepare 40 µl 20x dilution of extract from step 1.47.
4. Add 800 µl water to 7 cuvettes.
5. Prepare standard cuvettes for 0 mg/ml, 1 mg/ml (10 µl 0.1 mg/ml BSA), 2 mg/ml (20 µl 0.1 mg/ml BSA), 4 mg/ml (4 µl 1 mg/ml BSA), 6 mg/ml (6 µl 1 mg/ml BSA).

6. Prepare experimental cuvettes for 2 µl of sample and 4 µl of sample.
7. Add 200 µl of Bradford agent to each cuvette and mix well by pipetting. Incubate at room temperature for at least 10 min.
8. Produce standard curve at OD 595nm using cuvettes from step 6.5. Reject standard curve if $r^2 < 0.95$.
9. Determine extract concentration at OD 595nm using cuvettes froms tep 6.6.

Supplemental Material 3. Buffer calibration spreadsheet.

See TXTL_e(template)_calibration_JoVE.xlsx.

Supplemental Material 4. Cell-free expression run spreadsheet.

See TXTL _JoVE.xlsx.