

Supplemental Experimental Procedures

Rapid and scalable preparation of bacterial lysates for cell-free gene expression

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Autolysate production

2xYT medium

- 1) 1900 ml deionized water
- 2) 62 g 2xYT dry medium (EMD Millipore, #4.85008).
- 3) mix until the dry medium powder is completely dissolved
- 4) add deionized water to 2 liters
- 5) Autoclave on liquid cycle, 30 minutes

2xYTP medium

- 1) 1900 ml deionized water
- 2) 62 g 2xYT dry medium (EMD Millipore, #4.85008).
- 3) 5.99 g potassium phosphate monobasic (F.W. 136.09)
- 4) 13.93 g potassium phosphate dibasic (F.W. 174.18)
- 5) mix until all components are completely dissolved
- 6) add deionized water to 2 liters
- 7) Autoclave on liquid cycle, 30 minutes

2xYTPG medium

- 1) 400 ml 2xYTP liquid medium
- 2) 7.2g D-glucose (F.W. 180.16)
- 3) mix until glucose is dissolved
- 4) filter sterilize (0.2um filter)

S30A buffer

50 mM Tris-HCl (pH7.7), 60 mM potassium glutamate, 14 mM magnesium glutamate. Final pH was checked and adjusted to 7.7 if necessary.

GamS purification

GamS lysis buffer

50 mM Tris-HCl, pH 8.2 (from 1M stock solution, pH 8.2 at room temperature)

500 mM NaCl

5 mM imidazole (from 5M stock solution, pH 8 at room temperature)

1 mM Phenylmethylsulfonyl fluoride

0.1% (v/v) 2-mercaptoethanol

GamS wash buffer

50 mM Tris-HCl, pH 8.2 (from 1M stock solution, pH 8.2 at room temperature)

500 mM NaCl

20 mM imidazole (from 5M stock solution, pH 8 at room temperature)

0.1% (v/v) 2-mercaptoethanol

GamS elution buffer

50 mM Tris-HCl, pH 8.2 (from 1M stock solution, pH 8.2 at room temperature)

500 mM NaCl

300 mM imidazole (from 5M stock solution, pH 8 at room temperature)

0.1% (v/v) 2-mercaptoethanol

GamS dialysis buffer

50 mM Tris-HCl, pH 8.2 (from 1M stock solution, pH 8.2 at room temperature)

100 mM NaCl

1 mM dithiothreitol

2% (v/v) dimethyl sulfoxide

PCR Primers

Name	Sequence (5' to 3')
pBEST-promoter-fw	tctggcgaatcctctgacc
pBEST-promoter-rev	ggtatatctccttcttaaagttaacaaaattattgc
pBEST-terminator-fw	cacatgttctttcctgcggtatcc
pBEST-terminator-rev	accgccgccgggatctaac
pBEST-cat.fw	gcaataatTTTgtttaactttaagaaggagatataccatggagaaaaaatcactggatataccacc
pBEST-cat.rev	gtagatcccggcggcggtttacgccccgccctgcca
pBEST-Rluc.fw	gcaataatTTTgtttaactttaagaaggagatataccatgacttcgaaagttatgatccagaaca
pBEST-Rluc.rev	gtagatcccggcggcggtttattgttcattttgagaactcgctca

Cell-free expression premix

1) 14x Solution I

Total volume = **4 ml**

Concentration	units	name	MW (g/mol)	add weight (mg)
700	mM	HEPES (free acid)	238.3	667.2
21	mM	ATP (Sigma A8937)	619.39	52.0
21	mM	GTP (USB 16800)	567.14	47.6
12.6	mM	CTP (USB 14121)	563.16	28.4
12.6	mM	UTP (USB 23160)	586.12	29.5
2.8	mg/ml	tRNA (Roche MRE600, cat number 10109541001)		11.2
3.64	mM	CoA (Sigma C4282)	767.53	11.2
4.62	mM	NAD (Sigma N6522)	663.43	12.3
10.5	mM	cAMP (Sigma A9501)	329.21	13.8
0.95	mM	Folinic acid (Sigma F7878)	511.5	1.9
14	mM	Spermidine (Sigma 85558)	145.25	8.1
420	mM	3-PGA (Sigma P8877)	230.02	386.4

First resuspend HEPES in 2 ml water, adjust pH to ~8.0 w/ KOH

Add all the other components

Add water to final volume

Adjust pH to 7.6 w/ KOH

Sterile filter

Aliquot and freeze at -80C.

2) 4x amino acid solution

Prepared in deionized water from dry amino acid powder.

Concentration: leucine 20mM, all the other amino acids 24 mM.

Adjust final pH to ~6.8 w/ potassium hydroxide

Aliquot and freeze at -80C.

3) 2.5x premix

Volume (ul)	Stock concentration (x)	reagent
714.3	14	Solution I
2500.0	4	aminoacid mix
500.0	20	40% w/v PEG-8000
303.0	33	2M K-glutamate
25.0	400	1M Mg-glutamate
100.0	100	100 mM DTT

Final pH = 7.5

Aliquot and freeze at -80C.

4) Premix with extra magnesium glutamate and PEG 8000

Reagent	Volume (ul)
premix 2.5x	380
magnesium glutamate, 1M	4.75
PEG 8000, 40% (w/v)	36.1

In 20 ul reaction use: 8.9ul premix + 8ul autolysate + 3.1 ul DNA or other reagents

Note: in the above example 5 mM extra magnesium glutamate and 1.5% (w/v) extra PEG8000 are added to the base 2.5x premix. Since these concentrations are critical to TXTL reaction performance it is **very** important to optimize additional magnesium glutamate and PEG8000 concentrations for new batches of autolysate or 2.5x premix. Too low or too high concentrations of these components may result in poor TXTL reaction performance. We typically screened the following concentrations: 0-10 mM magnesium glutamate extra, 0-3% (w/v) PEG8000 extra.