Reproducible performance, versatility, and point-of-use optimization of NEBExpress® Cell-free *E. coli* Protein Synthesis System

B071

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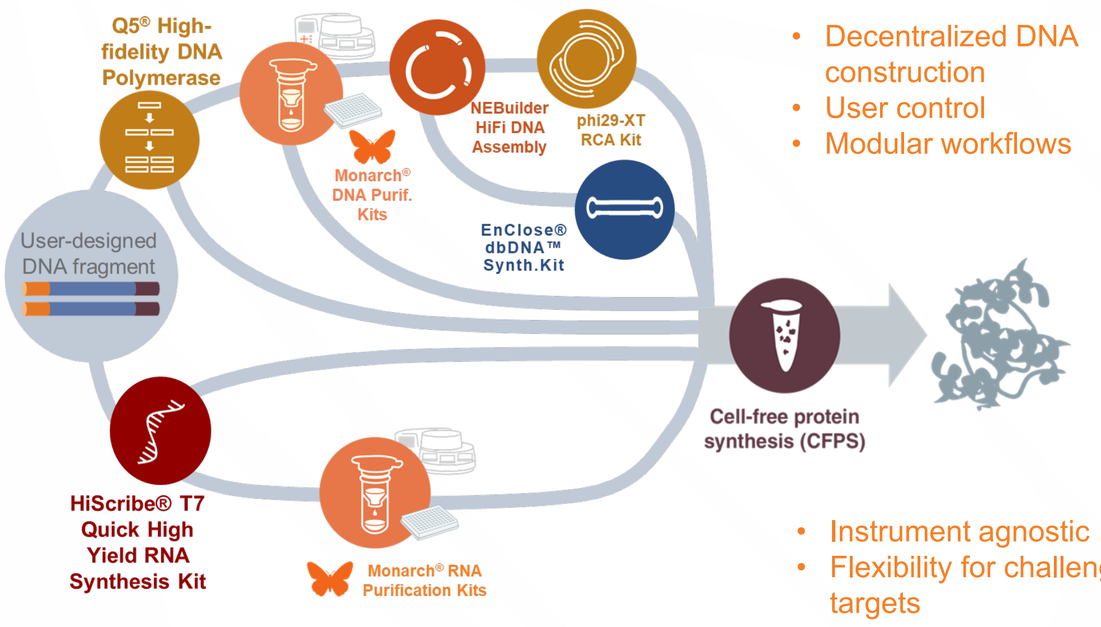
INTRODUCTION

NEBExpress® Cell-Free E. coli Protein Synthesis System can synthesize proteins of a wide range of sizes and biological origins

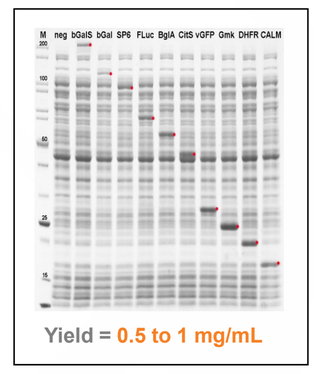
Common concerns with CFPS commercial systems:
- Do they have good productivity and consistent performance?
- Will they produce the target of choice without extensive optimization?
- Is it possible to use low amounts of linear DNA to save reagent?
- How much agitation is needed for reliable performance?
- What options exist to streamline and integrate workflows?

Project scope
- Systematic performance evaluation across targets, templates, and conditions that are amenable to HT workflows.

Our project identified:
- Practical, point of use interventions to enhance protein yield
- NEBExpress as a versatile system that maintains a consistent performance level across targets, hardware, and template input.



- Decentralized DNA construction
- User control
- Modular workflows



- Instrument agnostic
- Flexibility for challenging targets

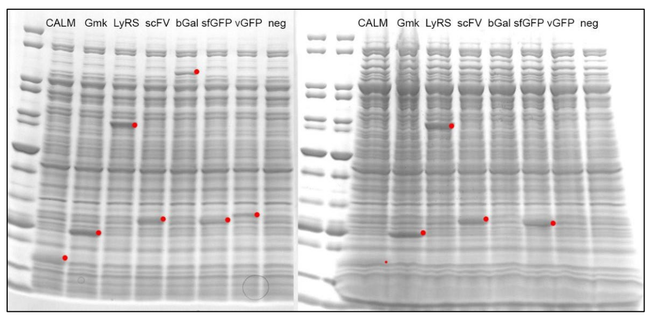
RESULTS

NEBExpress consistently supports expression of a range of fluorescent proteins (FP) and FP- enzyme fusion constructs

Reactions incubated on 96 well plates without shaking, compatible with basic laboratory instrumentation

Endpoint fluorescent **signal from vGFP, sfGFP, stGFP, mScarlett, Juniper, Charteuse** *(mChart)*, **Guanylate kinase-Charteuse** *(Gmk-Chart)*, **Bacillus α-amylase-Charteuse** *(AMY-Chart)*. ***G1*** *and* ***G2*** *correspond competitor products, 30C, 6h, 125 ng plasmid DNA, sealed PCR 96-well plates (w/o shaking).*

NEBExpress can synthesize all tested targets from purified linear DNA



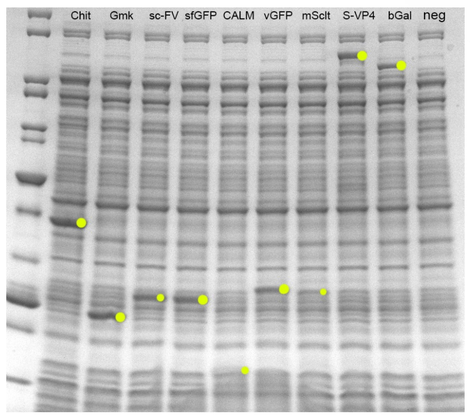
CFPS Conditions: 25 µL reaction, 30C, 3h incubation, 125 ng purified linear DNA, sealed PCR 96-well plates (w/o shaking).

SDS-PAGE from 2uL of CFPS reaction.
- FL targets: **vGFP, sfGFP**
- Eukaryotic enzyme: **Calmodulin** (*CALM*)
- Enzymes: **Lysyl tRNA synthetase** (*LysRS*), **Guanylate Kinase** (*GmK*)
- Antibody Fragment: ***svFV***.

- **NEB**
- 7/7 expressed
- All bands strong yields

- **System G1**
- 5/7 targets expressed
- Some bands are low yield

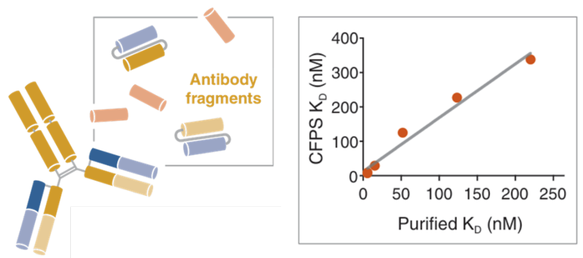
NEBExpress can synthesize viral fusion proteins, active enzymes, antibody fragments, eukaryotic proteins, chaperones, etc.



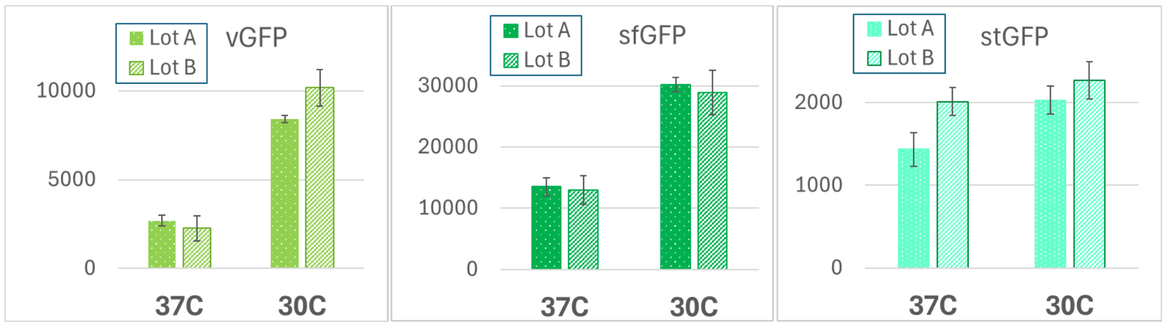
- **NEBExpress: 9 of 9 targets** (2 low yield)
- **Competitor G1:** 7 of 9 targets (5 low yield)
- **Competitor G2:** 6 of 9 targets (2 low yield)

CFPS Conditions: 25 µL reaction, 30C, 3h incubation, 125 ng plasmid, sealed PCR 96-well plates (w/o shaking).

SDS-PAGE from 2uL of CFPS reaction.
- FL targets: ***vGFP, sfGFP, mScarlett***
- Eukaryotic: **Chitinase from *P. falciparum*** (*Chit*), **Calmodulin** (CALM)
- Enzymes: **Guanylate Kinase** (*GmK*), **βGalactosidase** (*bGal*)
- Viral proteins: **VP4 capping enzyme** (fusion protein) (*S-VP4*)
- Antibody Fragment: ***scFV*** :Kd was determined directly (using crude reaction) and demonstrates high correlation to purified fragments.

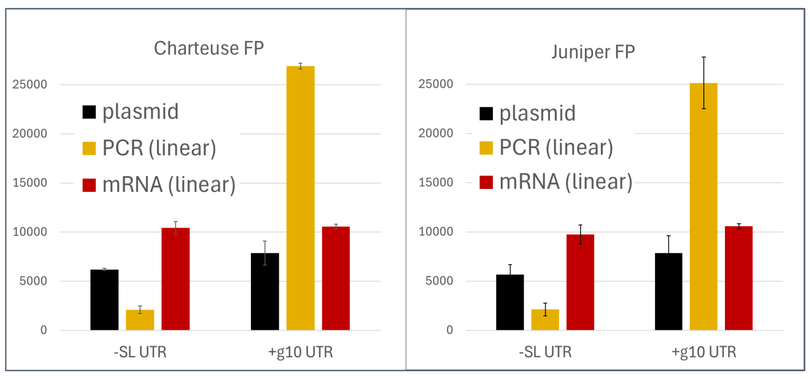


NEBExpress is designed for high reproducibility: similar productivity profiles shown through testing independent lots



Endpoint fluorescent signal from vGFP , sfGFP, and stGFP.
CFPS conditions: 25 µL reaction, 30C, 3h, 125 ng plasmid DNA, sealed PCR 96-well plates (w/o shaking).

NEBExpress CFPS solves template amplification challenges with multiple input template options



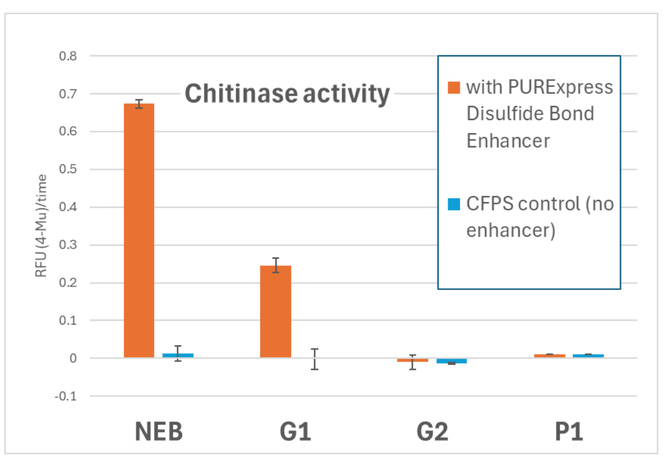
NEBExpress tolerates crude, unpurified, inputs such as PCR or mRNA synthesis reactions.

Using linear DNA, the effect of flanking regions (-SL or g10 5'UTR) is evident.

In contrast, mRNA input gives similar yields, regardless of genetic context

CFPS Conditions: 25uL reaction, 30C, 3h, 125 ng plasmid DNA, linear DNA, or in sealed PCR 96-well plates (w/o shaking) Linear DNA: 2 µL of a PCR reaction were used as template (amplification from 1ng of DNA performed under standard conditions with Q5® High-Fidelity 2X Master Mix). mRNA: 1 µL of a transcription reaction was used as template (mRNA synthesis from 20ng of DNA under standard conditions with HiScribe® T7 Quick High Yield RNA Synthesis Kit)

Correctly fold target proteins with multiple disulfide bonds with the addition of Disulfide Bond Enhancer



CFPS Conditions: 25 µL reaction, 30C, 6h, 125 ng plasmid DNA, sealed PCR 96-well plates (w/o shaking). All reactions were supplemented with PURExpress Disulfide Bond Enhancer (unless a folding supplements was provided in kit)

Chitinase activity: 1 µL of CFPS reaction was mixed with 200 µL of 40 µM 4-MU-chitotrioside in 200 mM NaCl, NaPO4 pH 6.0. Reactions were incubated at 37C for 30min. Free 4-Methylumbelliferone was measured at em 513 - ex 532.

P1, G1 and G2 correspond to competitor products.

SUMMARY

Our project identified:
Generalizable performance benchmarks:
- Synthesis of 18 different proteins under variable conditions.
- Practical, point of use interventions to enhance protein yield
  - Different flanking regions
  - Linear v Plasmid DNA v mRNA
  - Temperature/time

- NEBExpress® supports consistent protein synthesis across diverse targets, templates, and reaction formats.
- This flexibility allows users to implement simple, point-of-use optimizations to improve outcomes for specific applications.
- Standardized workflows enable rapid iteration without specialized equipment.