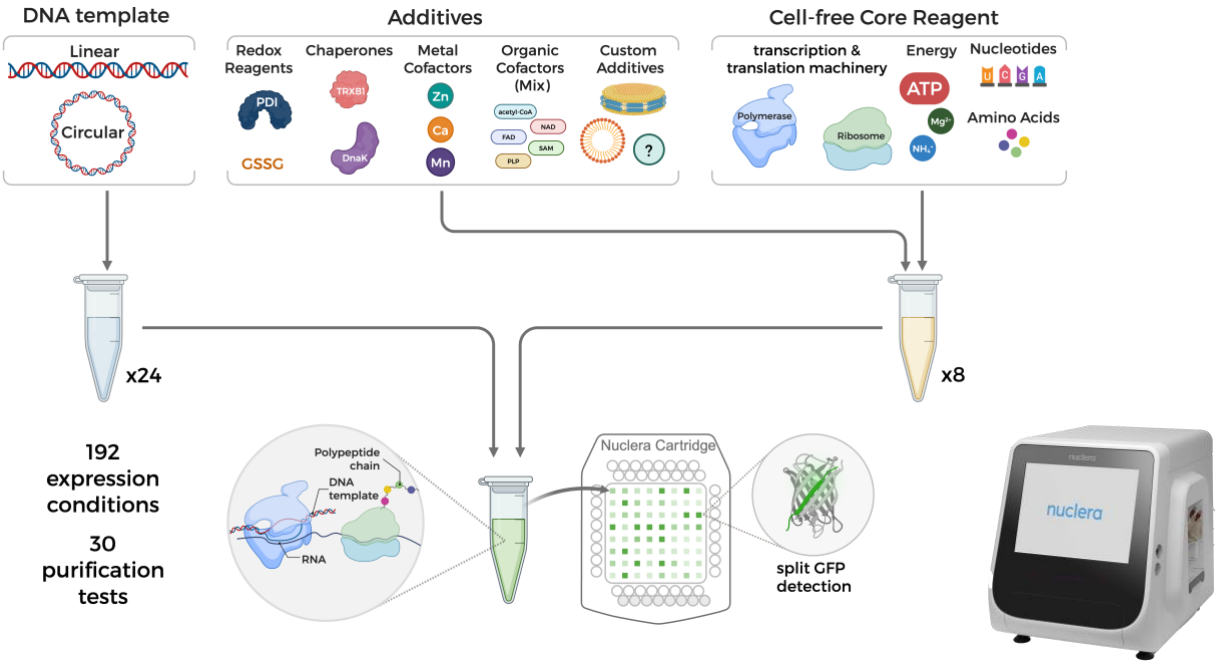
Nuclera eProtein Discovery: CMI Getting Started Guide to Cell-Free Protein Synthesis

Getting Started: Gene Design | Sample Prep: Gene Assembly | Data Collection: Screen | Data Analysis | Scale-Up | Appendix: A-Tags, B-Additives

Introduction

The eProtein Discovery system from Nuclera is a platform for Cell-Free Protein Synthesis (CFPS), also known as in vitro transcription/translation, that enables rapid protein prototyping. The system uses a digital microfluidic chip and custom reagents to screen protein expression and solubility of up to 192 conditions. Experiments begin by designing, ordering, and assembling Nuclera-compatible DNA templates (eGene Construct), using gene blocks and Nuclera megaprimers. Each eGene construct incorporates a split-Green Fluorescent Protein (GFP) peptide (GFP11) for detecting expression level, a Strep tag II for purification, and optional solubility tags. The eProtein Discovery system dispenses user-generated DNA templates, cell-free core reagents with optimization additives for gene expression onto the digital microfluidic cartridge for transcription and translation, then adds a split-GFP detection reagent. Two workflows are supported: Standard and Membrane Protein. With the Standard Workflow, 24 constructs in 8 cell-free expression conditions (192 conditions) are screened and 30 are selected for purification testing. With the Membrane Protein Workflow, 11 constructs in 8 cell-free conditions are screened for expression and purification. Nuclera scale-up reagents allow expression and purification in the µg-mg/mL range, that are reproducibly predicted from the expression and purification screen yield estimates. Alternatively, selected constructs may be adapted for cellular expression.



Instrument Overview

The CMI has an **eProtein Discovery** Instrument from Nuclera.

Resources

See CMI Nuclera eProtein Discovery Guide to Solubility Tags, for details on Nuclera-compatible.
Additional resources are available at the CMI website, from Nuclera, and in the eProtein Discovery Software (requires login, setup during CMI training).

Applications

- Protein prototyping
- Protein expression and purification screening
- Solubility tag screening

Key Features

- Screen up to 192 conditions simultaneously
  - Standard workflow: 24 constructs in 8 cell-free conditions, with 30 purification tests
  - Membrane workflow: 11 constructs in 8 cell-free conditions, with 88 purification tests
- Reagent kits for 200 µL to 1 mL Scale-up
- Screen and Scale-up production in 48 hours

Required Supplies and Equipment

Gene Assembly – in User's Lab

- Nuclera-compatible gene fragment with 3C site (5' end) and TEV site (3' end)
  - Gene Blocks up to 3Kb can be designed using the eProtein Discovery software.
  - gBlocks from IDT can be purchased through Nuclera, for a discount.
- Nuclera eGene Prep kit (***choose one***):
  - eGene Prep Kit: Solubility Tag Screen (NC3009)
  - eGene Prep Kit: FlexiVariant Screen (NC3008)
- High fidelity PCR mastermix
  - Platinum SuperFi II PCR MasterMix, Thermo (PN 12368010) *highly recommended*
- Nuclease free water (NFW)
- PCR purification kit (column- or bead-based methods, avoid gel extraction due to yield loss)
- DNA gel electrophoresis: 1% agarose gel, DNA gel stain, loading buffer, buffer, DNA ladder
- 0.2 mL thin-walled PCR tubes or 96-well PCR plate
- *User-provided required equipment: Thermocycler, Electrophoresis apparatus and Gel doc*

eProtein Discovery Screen (Load and Run) – at CMI, eProtein Discovery Instrument

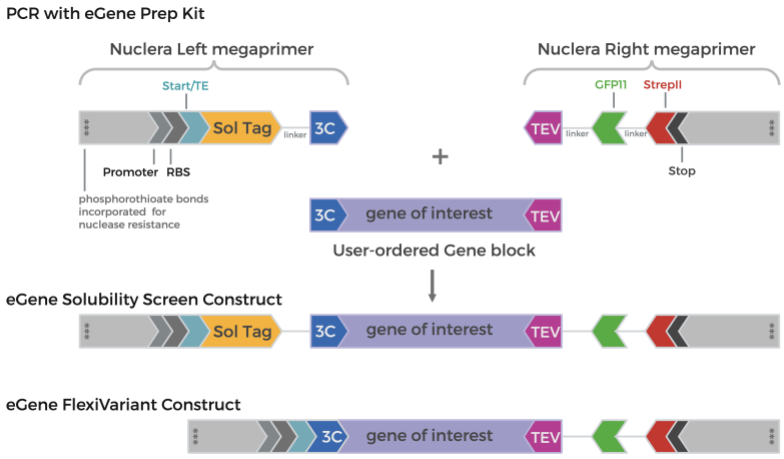
- Nuclera eProtein Discovery Cartridge with Base Fluid (NC3006)
- Nuclera Cartridge Reagent Kit (***choose one***):
  - Cartridge Reagent Kit (NC3010), Strep Beads (NC3010-2) store at 4C and other reagents at -80C
  - Cartridge Reagent Kit: Membrane Protein (NC3013)
- 96-well Transfer plate (PCR plate or V-bottom)
- *Required equipment: 30C incubator, magnetic tube rack, centrifuge (available at CMI)*

Scale-up (optional) – in User's lab or at CMI

- Scale-up kit (NC3011), Strep Beads (NC3011-1), store at 4C and other reagents at -80C
- Scale-up additives (NC3005), store at -80C
- 5 nM DNA construct generated using the Nuclera eGene prep kit
- *Required equipment: 29C incubator, magnetic tube rack, tube rotating platform, centrifuge (available at CMI)*

Getting Started: Gene Design

The first step in a Nuclera eProtein Discovery system Cell-Free Protein Synthesis (CFPS) experiment is to design a compatible gene. User-designed gene inserts must include a 5' 3C site and 3' TEV site for assembly with the Nuclera CFPS primers. Nuclera left and right megaprimers (provided in eGene Prep Kits) are required for gene assembly. The left megaprimers will incorporate a translation enhancer, and optional solubility tag, and uses the 3C site as priming site at the at the N-terminus of the gene insert. The right megaprimer uses the TEV site to prime and incorporates a split GFP peptide (GFP11) for detection and a strep tag II for purification, at the C-terminus. Two eGene Prep Kits are available from Nuclera: Solubility Tag Screen (NC3009), and FlexiVariant Screen (NC3008). Each contains sufficient primers to screen 24 constructs. The Flexivariant Screen contains a single left and right primer for 24 user-provided inserts. The Solubility Tag Screen contains eight left primers and one right primer, to screen 3 genes with all 8 solubility tags, 4 genes with 6 solubility tags, or 6 genes with 4 solubility tags. See Appendix A for Protein Sequences.



Design Tips

- Remove initial methionine and stop codons from the sequence(s).
- Avoid internal 3C and TEV sites.
- Codon-optimize for E.coli.
- For genes longer than 3Kb use "confidential sequence" feature to enter bp and protein mass.

Gene Design

1. Login to the eProtein Discovery Cloud using your email username and password.
2. Navigate to your project and go to the Proteins tab.
3. Design Gene(s) of Interest (GOI).
    - eProtein Discovery software can be used to design gene blocks up to 3Kb.
    - For longer genes, use "Confidential Sequence" feature.

    a. Click +New Protein to enter one protein or +Import Proteins to enter multiple (FASTA format).
    b. Enter protein name, label, sequence.
    c. Check compatibility.
        i. Protein will be saved as a draft allowing you to review later.
        ii. Examine the results for DNA synthesis and Protein expression compatibilities.
    d. Click ✓Finalize Protein to save and continue.
        i. Examine Possible Constructs and Structure Prediction.

4. DNA sequences with eProtein adaptors for constructs assembly (gBlock) will be created and are ready for download to order via IDT or other companies.
5. Navigate to the Experiments tab and click Design New Experiment.
6. Enter name, description (optional), and select the corresponding Experiment Workflow Version.
7. Under Design tab, follow the guided instructions provided by the software for:
   a. Selecting protein constructs. (See Appendix A for Nuclera solubility tag list and sequences).
   b. Selecting cell-free blend additives. (See Appendix B for Nuclera additive list.)
   c. Assigning protein constructs and additives to the cartridge.
   d. Purification preferences.
8. Navigate to the Progress tab and download Transfer Plate Guide. Print this document and bring it with you the day of your experiment because it is needed for plate loading.

Sample Preparation: Gene Assembly – in User's Lab

Experimental Design Tips

- Check DNA quality by gel electrophoresis (make sure you see a **single PCR product** band and that the positive and negative controls look as expected).
- Purified PCR products **must be eluted using eGene prep kit Elution Buffer** since it contains a surfactant that is essential for downstream experimental setup.
- When measuring DNA concentration using a Nanodrop:
  - blank with Elution Buffer (surfactant in the buffer causes high background absorbance).
  - measure at multiple dilutions for better accuracy.

Gene Assembly

1. GOI Template DNA (gBlock).
   a. Resuspend the lyophilized DNA fragment to 10 ng/µL with TE buffer or nuclease-free water.
   b. Convert DNA concentration to molarity:

$$MW_{GOI}\left(\frac{g}{mol}\right) = length\,(bp) \times 617.96\,9\left(\frac{g/mol}{bp}\right) + 36.04\left(\frac{g}{mol}\right)$$

$$[GOI]\,(nM) = [GOI]\left(\frac{ng}{ml}\right) \times 10^6/MW_{GOI}\left(\frac{g}{mol}\right)$$

   c. Prepare fresh 10 µL of the GOI template normalized to 2 nM in nuclease-free water.
2. Control Template DNA.
   - Add 1 µL of 10x Control Template to 9 µL of nuclease-free water.
3. Prepare PCR reaction mixes, always include:
   a. GOI Template DNAs with Nuclera megaprimer mix.
   b. Negative control (water).
   c. Control Template DNA.
4. PCR Assembly.
   a. Calculate elongation time for templates ~ 30s/kb.
5. Evaluate amplified DNA by agarose gel electrophoresis.
   a. Prepare 1% (w/v) agarose gel with DNA gel stain in 1x TAE or 1x TBE buffer.
   b. Prepare 12 µL samples (2 µL 6x gel loading dye + 8 µL water + 2 µL eGene DNA construct).
   c. Load 10 µL of GOI PCR products and control on the gel along with an appropriate ladder.

PCR Reaction Mix

Reagent	vol (µL)
Water (nuclease-free)	19
2x PCR mastermix	30
GOI at 2 nM, or Control	1
Nuclera megaprimer mix	10
Total volume (max)	**60**

Thermocycler parameters

PCR step	Temp [C]	Time	Cycles
Pre-incubation	98	30s	1
Denaturation	98	10s	
Annealing	60	20s	27
Elongation	72*	30s/kb	
Final elongation	72*	2min	1

Solubility Tag Screen	
Primer mix	**Length added (bp)**
P17-Strep	1,134
CUSF-Strep	1,302
FH8-Strep	1,239
TRX-Strep	1,359
ZZ-Strep	1,383
SUMO-Strep	1,341
SNUT-Strep	1,476
-Strep only*	990

   d. Check DNA quality by gel electrophoresis.
      i. make sure you see a **single PCR product** band and that the positive and negative controls look as expected.
      ii. Save image of PCR gel to share with CMI staff.
6. PCR purification.
   a. Clean PCR reactions using a DNA purification method of your choice (avoid gel extraction as it can result in significant yield loss).
   *b. **Important:** Samples **MUST** be eluted using the supplied eGene Elution Buffer (50 µL) for compatibility with the eProtein Discovery cartridge.*
7. DNA quantification.
   a. Measure the DNA concentration in ng/µL.
      - **Blanks MUST be made with eGene Elution Buffer**, provided in kit.
   b. Determine the total length of the eGene:

$$eGene\ Length\ (bp) = GOI\ (bp) + Primer\ Mix\ (bp)$$

   c. Calculate the molar conc. (nM) of eGene constructs.
   d. *Note: If the [DNA] < 5 nM, refer to the reamplification protocol in the troubleshooting section of the eGene Prep User Guide.*
8. eGene constructs normalization to 5nM, using 60 µL eGene Elution Buffer:

$$V_{eGene}\ (ul) = [eGene]_{target} \times V_{buffer}(ul)/([eGene]_{init} - [eGene]_{target})$$
$$V_{eGene}\ (ul) = 5\ nM \times 60\ ul/([eGene]_{init} - 5\ nM)$$

9. Final gel QC, run 2 µL of samples on 1% agarose gel post PCR clean-up
10. Purified and normalized eGene constructs can be stored at -20C for short term (days-weeks) or -80C for long term (months).

Data Collection: eProtein Discovery Screen

General Care and Maintenance

- The eProtein Discovery Instrument will generally be powered on.
- Remove and dispose of used cartridge and base fluid after each run.

Experimental Design Tips

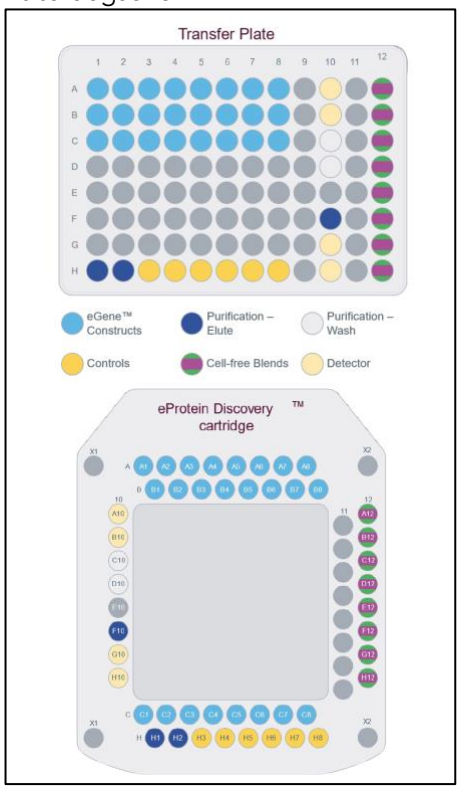
- Cartridge fluid must be degassed at 30C for at least 1 hr before run time.
- Plate setup for cartridge loading should be done right before experimental startup.
  - Do not let a filled plate sit idle for more than an hour.
- Take care to avoid air bubbles during loading of the cartridge.

Startup

1. Assemble reagents and consumables
   a. Normalized eGene DNA (as above).
   b. Nuclera eProtein Discovery Cartridge and Nuclera Cartridge Reagent Kit.
2. Book instrument in PPMS calendar.
   - Must be confirmed by CMI staff in PPMS. Share assembly gel for staff review.
3. Login to the instrument using your Nuclera PIN.
4. Empty the Waste Fluid vial located at the side of the instrument.
5. Degas Base fluid, **at least 1 hour before starting run.**
   a. Take Base Fluid vial from eProtein Discovery Cartridge box, remove seal, and loosen the rubber stopper.

b. Place the loosened vial in 30C incubator located under the instrument to degas for 1+ hr.

eProtein Discovery Screen



6. Prepare your reagents and supplies.
   a. Thaw elution buffer (blue lid), wash buffer (white lid), and strep purification beads (orange lid) at room temperature.
   b. Thaw all other reagents on ice.
      i) label tube lids to avoid accidentally mixing them up during load.
   c. Spin down all tubes before mixing and pipetting steps.
7. Prepare working stock of Strep purification beads.
   a. Gently resuspend beads and transfer 90 µL into a 1.5 mL tube.
   b. Wash beads 3 times:
      i) Place tube with Strep Beads on the magnetic rack and capture for 1 min.
      ii) Remove all supernatant and discard liquid.
      iii) Remove tube with Strep Beads from the magnetic rack.
      iv) Resuspend beads in 100 µL Wash Buffer by gentle pipetting.
      v) Repeat twice.
   c. Final resuspension of Strep beads (15 µL 30% Strep Beads working dilution):
      i) After 3rd wash, spin down tube and capture for 1 min on magnetic rack.
      ii) Remove all supernatant and discard liquid.
      iii) Spin down tube again, place it back on the magnetic rack and remove the residual buffer.
      iv) Remove tube from the magnetic rack.
      v) Gently resuspend beads in 10.5 µL Wash Buffer.
      vi) Keep the beads in the tube at room temperature. **Do not store on ice**.
8. Prepare the transfer plate for reagent loading.
   a. Attach plate label (provided in the Cartridge Reagent Kit) to a 96 plate (Transfer Plate) to mark reagent loading areas.
   b. Place plate on ice, and allow it to cool for 5 min before loading.
9. Load the transfer plate with reagents:
   a. Load 5 µL eGene constructs into rows A, B and C first, in the correct order (**must match** eProtein Discovery software experimental design).
   b. Load 10 µL Blank Buffer and Controls (located in row H).
   c. Load 10 µL Elution Buffer to wells H1 and H2.
   d. Load 16 µL Elution Buffer to well F10.
   e. Load 16 µL Detection Protein and Wash buffer (both located in column 10).
   f. Finally, add 16 µL Cell-free Core to column 12 (12A-12H) and 2 µL Additive 1 and 2 µL Additive 2, in the correct order.
10. Start Experiment on eProtein Discovery Instrument.
    a. Select your finalized experiment on the instrument.
    b. User interface will guide you through cartridge setup:
       i) Register eProtein Discovery Cartridge.
       ii) Prime Base Fluid.
       iii) Step-by-step loading of constructs and reagents into eProtein Discovery Cartridge from transfer plate.

iv) Quality checks prior to screen run.

c. **Important note:** all ports of the eProtein Discovery Cartridge (except column 11 and X-ports) must be loaded with sample or reagents.
   i) If you have fewer than 24 DNA constructs, replace unused wells with Blank Buffer.
   ii) If you have fewer than 8 Cell Free Blends, replace unused wells with Additive Buffer.

d. Monitor the progress of your run by either checking the status in the eProtein Discovery Cloud software under Experiments (or at the instrument).

Instrument Shutdown

1. Clean up around the instrument.
2. Dispose of cartridge.
   a. Remove the cartridge from the instrument.
   b. Carefully drain the excess oil by tilting the cartridge against paper towels.
   c. Discard the cartridge in the sharps container.
3. Empty the Waste Fluid vial located at the side of the instrument.
   - Waste should be collected in a 50 mL tube and discarded as biohazard waste.
4. Logoff from eProtein Discovery Instrument.
5. Keep the instrument power ON.

Data Analysis

1. Login to the eProtein Discovery Cloud and navigate to Experiments and go to the results tab.
2. Download the report. The following files should be inspected:
   a. _video.mp4: movie timeline showing droplet distribution and mixing.
   b. _report.csv: contains additional data not reported in the experiment results tab.
   c. Key_frames folder allows inspection of individual frames, for technical support.
3. Inspect your results including the Data Analytics tab to identify construct(s)/additives combinations that yield best expression and purification. Download figures from the Data Analytics tab, as they are not included in the report folder.
4. Scale-up analysis (optional).
   a. Click on the Scale-Up Data tab and chose which construct/additives combination you wish to pursue at the desired yield. Adjust volume or desired yield to see predicted results.
   b. Download the eRecipe CSV which has the formulations needed for the scale-up experiment follow-up.

Scale-up (Optional)

- Instructions below are for a 200 µL CFPS reaction. Use table to adjust for other reaction volumes.
- Maximum recommended reaction volume: 500 µL per tube.
- Wash buffer: 100 mM Tris pH 8, 150 mM NaCl.
- Elution buffer: 100 mM Tris pH 8, 150 mM NaCl, 50 mM biotin, 0.05% non-ionic detergent (12.5kDa).
  - Elution buffer contains detergent that absorbs intensely at 280 nm.
  - Consider preparing your own elution buffer without detergent to avoid interference downstream.

1. CFPS Scale-up Expression Reaction
    a. Prepare CFPS expression reaction (200 µL):
        i. 120 µL Cell Free Reagent
        ii. 15 µL Additive 1
        iii. 15 µL Additive 2
        iv. 50 µL 5 nM DNA
    b. Incubate the reaction at 29C overnight (15-18hr).
    c. Reserve 3µL CFPS reaction to run on SDS-PAGE (label: crude CFPS).

Scale-Up calculations

CFPS Reagents (per tube)	% of total reaction
Cell Free Reagent	60%
Additive 1	7.5%
Additive 2	7.5%
5nM DNA	25%
Total volume	0.2-0.5mL
Strep Beads	**2X CFPS volume**

2. Prepare working stock of Strep purification beads:
    a. Resuspend beads by gentle pipetting and transfer 400 µL into 1.5 mL tube.
    b. Wash beads 3 times:
        i. Place the tube with Strep Beads on the magnetic rack and capture for 1 min.
        ii. Remove all the supernatant and discard the liquid.
        iii. Remove the tube with Strep Beads from the magnetic rack.
        iv. Resuspend the beads in equal volume Wash Buffer (400 µL) by gentle pipetting.
        v. Repeat 2-4 times
    c. Final resuspension of Strep beads:
        i. After 3rd wash, spin down the tube and capture for 1 min on magnetic rack.
        ii. Remove all the supernatant and discard the liquid.
        iii. Spin down the tube again, place it back on a magnetic rack and remove the residual buffer (remove as much liquid as possible without disturbing beads).
        iv. Keep the beads in the tube at room temperature. **Do not store on ice**.
3. Purification of Scale-up
    a. Transfer CFPS reaction to the tube containing beads and resuspend.
    b. Incubate for 30min at room temperature using a tube rotator.
    c. Place tube on the magnet to pellet beads
    d. Remove supernatant (unbound contaminant proteins) and reserve to run on an SDS-PAGE gel.
    e. Wash beads
        i. Remove tube from magnet and resuspend beads in 400µL wash buffer
        ii. Place on magnet, pellet beads, remove supernatant.
        iii. Repeat wash step 3 times.
    f. Resuspend beads in 125-250uL elution buffer and place tube on a rotator for 10 min.
    g. Place tube on magnet, pellet the beads, and transfer the supernatant into a new tube (label: purified protein). Reserve supernatant to run on an SDS-PAGE gel.
4. Run SDS-PAGE, including the following:
    a. BSA or another protein standard at a range of concentrations (0.125-1 mg/mL)
    b. Crude CFPS
    c. Unbound contaminant proteins
    d. Purified protein

Book instrument time and Report Problems through the PPMS system: https://ppms.us/hms-cmi.

- rates are based on ***booked time***.

Contact cmi@hms.harvard.edu with questions.

last edited: 2025-07-31
Ahmad Almawi, Kelly Arnett

Appendix A

eGene Constucts: Protein Sequences

eGene Solubility Screen Construct (translated)

MSKEKR-SolTag-GGGSEGGGSEGGGSE-LEVLFQGP-Gene-ENLYFQS-
GGGGSGGGGSGGGGS-GETIQLQEHAVAKYFTE-EAAAKEAAAKEAAAK-WSHPQFEK*

eGene Flexivariant Construct (no solubility tag)

MSKEKR-LEVLFQGP-Gene-ENLYFQS-GGGGSGGGGSGGGGS-GETIQLQEHAVAKYFTE-
EAAAKEAAAKEAAAK-WSHPQFEK*

MSKEKR	translation enhancer
LEVLFQGP	3C
ENLYFQS	TEV
GETIQLQEHAVAKYFTE	split GFP (GFP11)
WSHPQFEK	strep tag II

Nuclera Solubility Tags

Sol Tag	Description	Added DNA [bp]	Added protein [kDa]	Tag Sequence
P17	P17 protein (3.8KDa) from tail of T7 phage; this hydrophilic sequence can increase solubility and thermostability	1134	12.85 (124aa)	KNESSTNATNTKQWRDETKGFRDEAKRFKN TAG
CUSF	Cation efflux system protein CusF (9.9KDa), periplasmic component of CusCBFA complex; forms beta-barrel structure.	1302	18.95 (180aa)	ANEHHHETMSEAQPQVISATGVVKGIDLES KKITIHHDPIAAVNWPEMTMRFTITPQTKM SEIKTGDKVAFNFVQQGNLSLLQDIKVSQ
FH8	FH8 (7.5KDa) is a highly soluble and thermal stable small antigen secreted by F. hepatica, whch can improve solubility.	1239	15.57 (159aa)	PSVQEVEKLLHVLDRNGDGKVSAEELKAFA DDSKCPLDSNKIKAFIKEHDKNKDGKLDLK ELVSILSS
TRX	Thioredoxin (11.7KDa) from E. coli can increase the solubility of heterologous proteins.	1359	20.71 (199aa)	SDKIIHLTDDSFDTDVLKADGAILVDFWAE WCGPCKMIAPILDEIADEYQGKLTVAKLNI DQNPGTAPKYGIRGIPTLLLFKNGEVAATK VGALSKGQLKEFLDANLA
ZZ	IgG-binding domain ZZ (13.2KDa) of Protein A from S. aureus.	1383	22.11 (206aa)	VDNKFNKEQQNAYYEILHLPNLNEGQRNAF IQSLKDDPSQSANLLAEAKKLNDAQAPKVD NKFNKEQQNAFYEILHLPNLNEEQRNAFIQ SLKDDPSQSANLLAEAEKLNDAQAPK
SUMO	Human Small Ubiquitin-like Modifier (11.5KDa), can solubilize otherwise insoluble proteins.	1341	20.54 (193aa)	SEEKPKEGVKTENDHINLKVAGQDGSVVQF KIKRHTPLSKLMKAYCERQGLSMRQIRFRF DGQPINETDTPAQLEMEDEDTIDVFQQQTG GVPESSLAGHSF
SNUT	Solubility enhancing Ubiquitous Tag (16.7KDa), derived from portion of trans-peptidase sortase (SrtA)1 found in S. aureus.	1476	25.79 (238aa)	KPHIDNYLHDKDKDERIEQYDKNVKEQASK DKKQQAKPQIPKDKSKVAGYIEIPDADIKE PVYPGPATPEQLNRGVSFAEENESLDDQNI SIAGHTFIDRPNYQFTNLKAAKKGSMVYFK VGNETRKYKMTSIRDVKPTDVEVLDGS
No Tag	= Flexivariant primer mix. For the creation of protein that is untagged at the N-terminus.	990	7.89 (88aa)	

Appendix B

Nuclera Additives

- included in Cartridge Reagent Kits (NC3010, NC3013), Scale-up additive Kit (NC3005)

Name	Description	Characteristics
Additive buffer	HEPES buffer pH 7.5 and surfactant	CFPS reaction buffer, dilution normalization
PDI + GSSG Mix	Protein disulfide isomerase and oxidized glutathione	Chaperone and redox modification to oxidizing environment to support disulfide bond formation
TrxB1	Thioredoxin reductase	Protects proteins from oxidative aggregation and inactivation and acts as a reductase in redox regulation
DnaK Mix	Chaperone	DnaK mix Chaperone mix to support folding and prevent aggregation
$ZnCl_2$	Zinc chloride solution	Cofactor can be required for folding, stability, or activity
$CaCl_2$	Calcium chloride solution	Cofactor can be required for compaction, folding, stabilization, or activity
$MnCl_2$	Manganese chloride solution	Cofactor for metalloenzymes for structure and activity
Cofactor Mix	NAD, acetyl CoA, FAD, SAM, & PLP	Cofactors that assist in folding, stability and activity
GSSG	Oxidized glutathione	Redox modification to oxidizing environment
3C protease	3C protease solution	Protease to cleave off the N-terminal solubility tag at the specific amino acid sequence (LEVLFQ/GP

- Users may also use custom additives of their own, such as detergent or nanodiscs for membrane protein.