Washington University in St. Louis
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SIMPLE, UNIVERSAL DNA MOTIFS FOR MODULATING PROTEIN PRODUCTION

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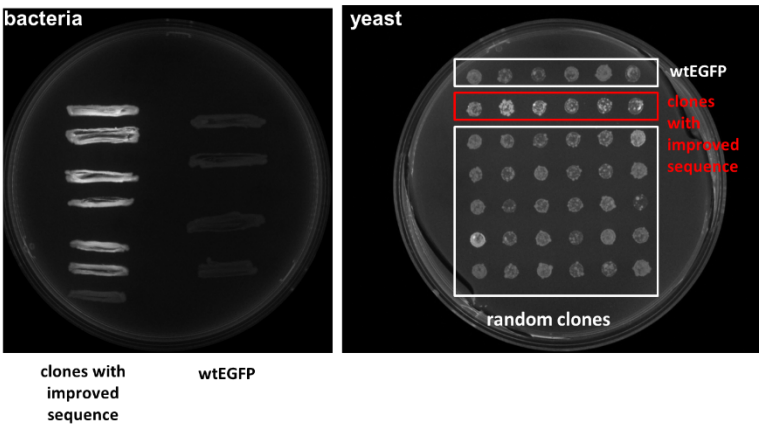
Technology Description

Researchers in Prof. Sergej Djuranovic's laboratory have discovered short DNA coding motifs that can increase or decrease expression of recombinant proteins by 3-4 orders of magnitude in a variety of organisms. Together, these universal tools can provide tightly controlled, tunable protein levels in a broad range of applications.

- Three amino acids inserted into a precise position near the N-terminus of the mRNA can increase expression beyond the effects of folding kinetics. This improves overall translational efficiency, increasing the abundance of protein. (T-016819, T-017416)
- Poly(A) tracks inserted into the coding regions can stall or terminate translation prematurely, decreasing protein expression. (T-016608)

The motifs have similar effects on expression rates for all proteins tested, regardless of the protein being expressed or the expression vector used. Furthermore, this approach is expected to be universal across most organisms and has been demonstrated in bacteria, yeast, tetrahymena and mammalian cells.



A short motif (three amino acids inserted near the N-terminal) increases expression in different genes and in different organism

Stage of Research

The inventors demonstrated the technology in a fluorescent protein test system as well as in other recombinant proteins (human G-alpha subunit, regulator of G-proteins (RGS), human growth hormone (HGH), Pfu polymerase) using in vitro and cell-based assays. They demonstrated higher fluorescence as well as folding kinetics in engineered forms of eGFP, GFP, YFP, mCherry, mCardinal, mEos2 and Dendra2. A simple change in three amino acids in these and other experiments showed:

- 100x increases of fluorescence in eGFP
- 100% increase in expression of human growth hormone

- 500% increase in expression of Pfu DNA polymerase

The inventors also have data for eGFP in HeLa lysates and continue to explore this technology in additional proteins and expression systems.

Applications

- **Synthetic biology** – controlling expression of recombinant proteins with end user applications such as:
  - industrial proteins (e.g., enzymes for chemicals or food production)
  - agriculture (e.g., protein production in transgenic plants and animals)
  - biomaterials (e.g., expressing enzymes in organisms that make bioplastics)
  - drug manufacturing (e.g., producing therapeutic proteins or antibodies)
  - bioenergy (e.g., increasing expression of enzymes that generate biofuel)
  - genome engineering
- **Research tools**:
  - manufacturing fluorescent proteins and other recombinant proteins used as research reagents
  - protein expression kits or vectors for basic research use

Key Advantages

- **Tunable expression** – different motifs can be used to either increase or decrease expression of protein of interest
- **Highly increased protein expression** – for the motif designed to increase expression:
  - demonstrated 3-4 order of magnitude increase in multiple recombinant proteins
  - improves folding of fluorescent proteins
  - could lower cost of production
- **Universal system** – can be applied to a range of proteins in a variety of organisms
  - motifs have similar effects on expression rates for all proteins tested, regardless of the protein being expressed or the expression vector used
  - demonstrated in bacteria (*E. coli*), yeast (*S. cerevisiae*), *Tetrahymena* and mammalian cells (HeLa and RRL lysates)
  - conserved ribosome structure suggests that the same mechanism will apply in other organisms
- **Simple, fast methods** – a short sequence of three amino acids is inserted into the coding sequence through standard DNA cloning techniques

Publications

- Verma M, Choi J, Cottrell KA, Lavagnino Z, Thomas EN, Pavlovic-Djuranovic S, ... & Djuranovic S. (2019). Short translational ramp determines efficiency of protein synthesis. *Nature Communications*, 10: 5774.
- Arthur LL, Pavlovic-Djuranovic S, Koutmou KS, Green R, Szczesny P, & Djuranovic S. (2015). Translational control by lysine-encoding A-rich sequences. *Science Advances*, 1(6): e1500154.
- Koutmou KS, Schuller AP, Brunelle JL, Radhakrishnan A, Djuranovic S, Green R. (2015). Ribosomes slide on lysine-encoding homopolymeric A stretches. *eLife*, 4:e05534.

Patent Applications

- US 20200048634
- US 20190390205

Related Websites: Djuranovic Profile & Lab