Influence of 5′ UTR and N-terminal ORF Region on Protein Synthesis Efficiency in PURE*frex*®

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Abstract

PURE*frex*® is a cell-free protein synthesis system based on the PURE system, which is reconstituted only from factors involved in protein synthesis in *E. coli*. Due to the improvement of the reaction composition, the synthesis efficiency has increased up to 1 mg/mL, but it varies significantly depending on the target protein. In this presentation, we will report our findings on the influence of the 5' UTR and N-terminal region of ORF on the protein synthesis efficiency.

(1) 5' UTR

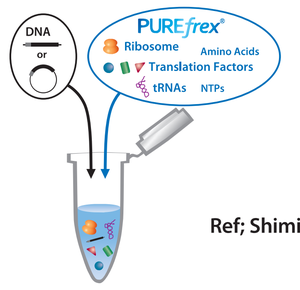
To confirm the necessity of each region in the 5' UTR derived from T7 phage currently in use, we compared the fluorescence of sfGFP synthesized from its template DNA with different 5' UTR. As a result, when either the AT-rich region or the Shine-Dalgarno (SD) sequence was completely removed, the fluorescence decreased to less than 10%. This result indicates that not only the SD sequence but also the AT-rich region in the 5' UTR is important for the efficient translation in PURE*frex*.

(2) N-terminal region of ORF

It was reported that the N-terminal region of ORF also influences the protein synthesis efficiency. First, for ten amino acids immediately below the start codon, the synthesis efficiency was higher when AT-rich codons were used than when codons commonly used in *E. coli* were used. For some proteins, changing a few codons in the N-terminal region increased synthesis efficiency more than 10-fold. Second, amino acids in the N-terminal region also affected the synthesis efficiency, especially at low temperatures. For example, replacing glycine at position 4 of sfGFP with tyrosine increased synthesis efficiency 8-fold at 23°C. These results indicate that small differences in the N-terminal sequence can cause large fluctuations in the synthesis efficiency.

1. PURE*frex*®; based on the PURE system technology

PURE*frex*® is based on the PURE system.
The PURE system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration.

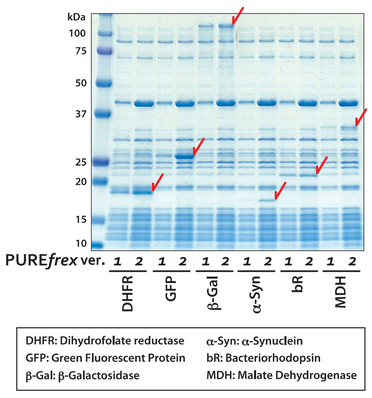


Advantage

Low level of contamination
Easy adjustment of the reagent composition
PCR product usable as a temlplate DNA

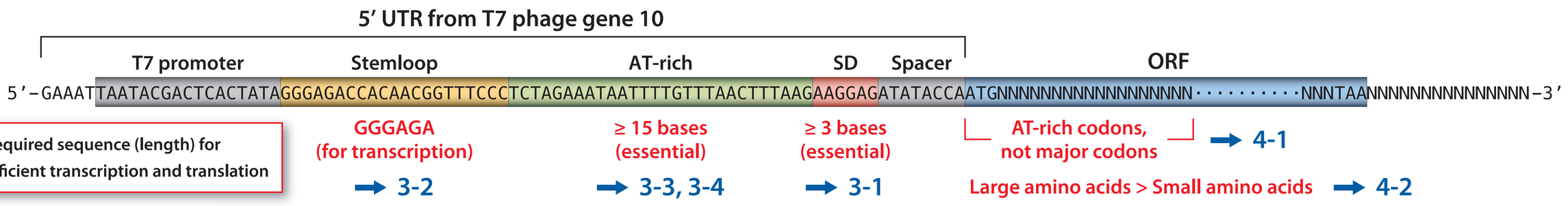
Ref; Shimizu Y. *et al.* (2001) *Nat. Biotechnol.*, vol. 19, p. 751.

< Example of protein synthesis >



DHFR: Dihydrofolate reductase
GFP: Green Fluorescent Protein
β-Gal: β-Galactosidase
α-Syn: α-Synuclein
bR: Bacteriorhodopsin
MDH: Malate Dehydrogenase

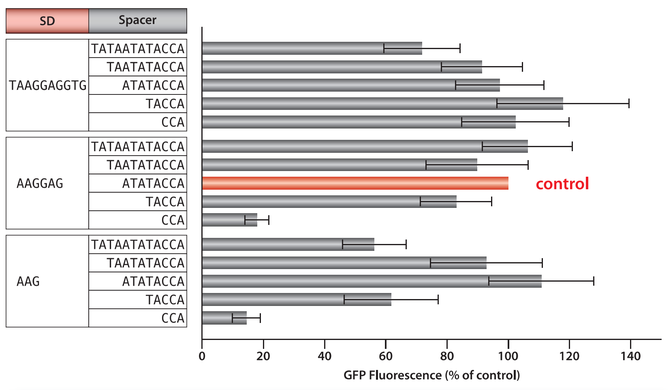
2. Construct of template DNA for PURE*frex*



3. Effect of 5′ UTR on the synthesis efficiency

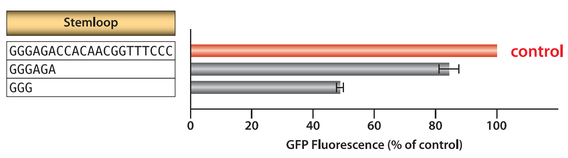
PURE*frex*®*2.1* (+GSH)
↓ + sfGFP DNA with indicated 5′ UTR
↓ incubation at 37°C for 4 h
↓ measurement of fluorescence of sfGFP

3-1. SD sequence and Spacer



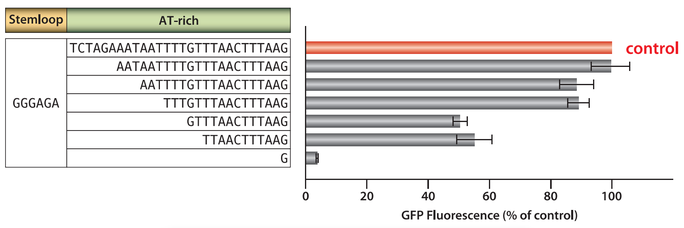
- AAG (3 bases) was enoygh for the efficient translation.
- The balance between the length of SD and spacer was important.

3-2. Stemloop



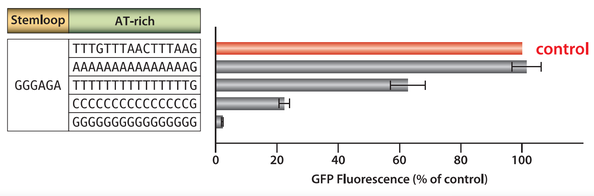
- GGGAGA, which is required for efficient transcription by T7 RNA polymerase, was sufficient.

3-3. AT-rich (Length)



- 15 bases and more was sufficient.
- AT-rich region was essential.

3-4. AT-rich (Sequence)



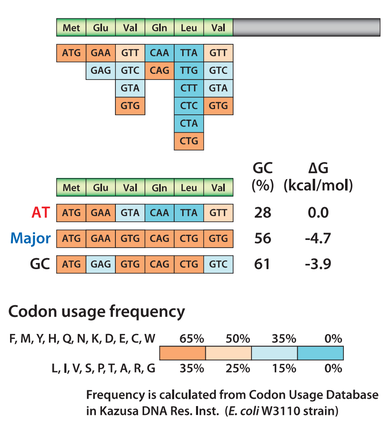
- A15 was as effective as the control, but T15 was less effective.
- In the case of C15 and G15, synthesis efficiency decreased significantly.

4. Effect of N-terminal sequence on the synthesis efficiency

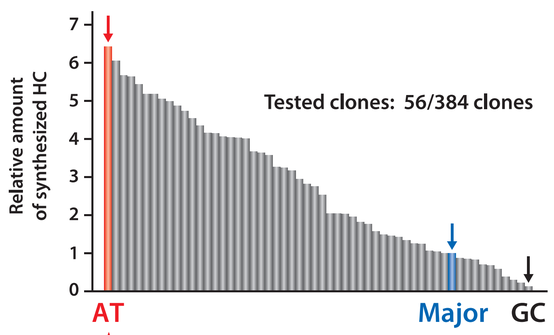
4-1. N-terminal codons

PURE*frex*®*2.1* (+GSH)
↓ + Trastuzumab HC (VH + CH1) DNA
↓ incubation at 37°C for 4 h
↓ SDS-PAGE and quantification

< N-terminal codon of Trastuzumab HC >



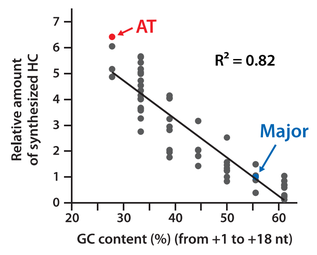
< The amount of product synthesized from the template DNA with different N-terminal codon >



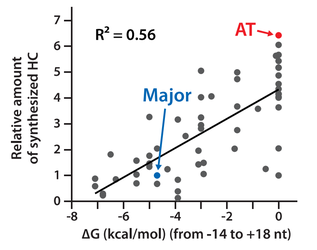
Yield: 0.6 mg/mL

AT-rich codons > Major codons @ 5′-terminus of ORF

< Relationship between protein synthesis and GC content >



< Relationship between protein synthesis and secondary structure of mRNA >



4-2. N-terminal amino acid sequence

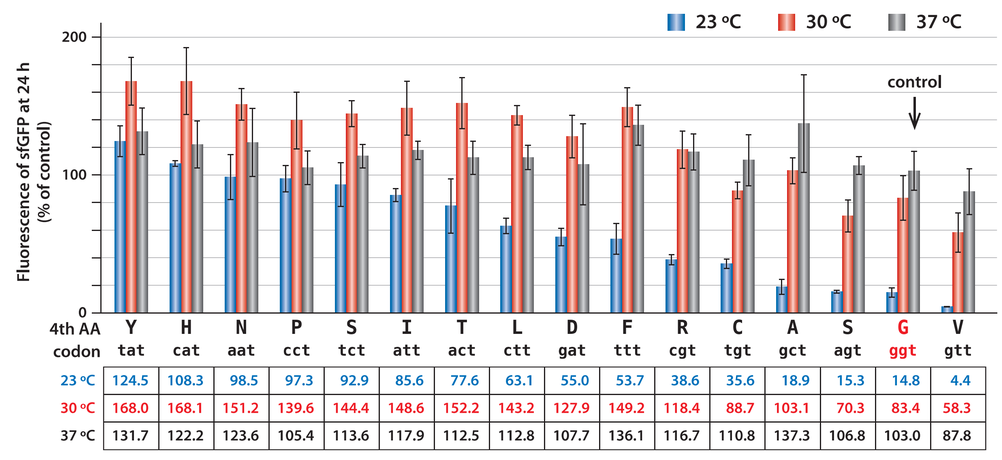
PURE*frex*®*2.1* (+ GSH)
↓ + sfGFP DNA with indicated 4th amino acid
↓ incubation at 23, 30 or 37 °C for 24 h
↓ Measurement of fluorescence of sfGFP

< N-terminal sequence of sfGFP >

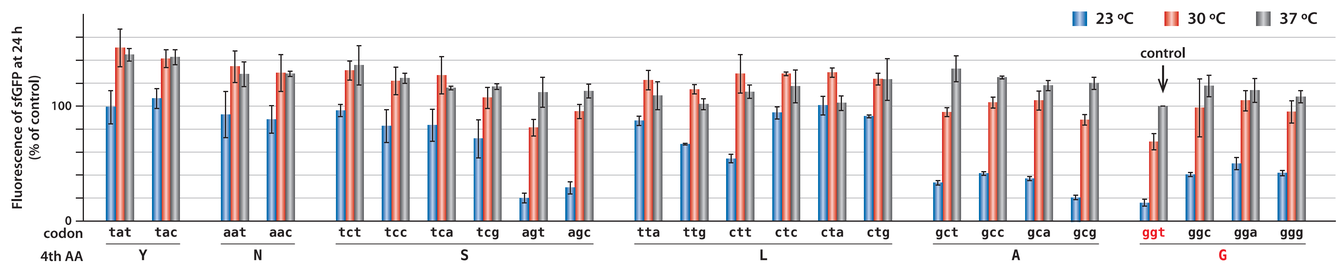
M S K G E E
atgtctaaaggtgaagaa

other amino acids
(NNT or synonymus codon)

< Effects of 4th amino acid >



< Effects of synonymus codon >



The amount of synthesized sfGFP, especially at 23 °C, differed depending on the 4th amino acid.
When the 4th amino acid was a large amino acid such as Tyr or His, the amount of sfGFP was increased compared to Gly.
The influence of amino acids was greater than that of synonymous codons.

Conclusion

5′ UTR

- Not only SD sequence but also AT-rich region in 5′ UTR is very important for efficient translation reaction.

N-terminal sequence

- For the N-terminal codon, AT-rich codons increase the synthesis efficiency more than commonly used codons.
- Large amino acids in the N-terminal region increase the protein synthesis efficiency, especially at low temperatures.