第 21 回 日本蛋白質科学会年会（2021 年 6 月 16 日 -18 日@オンライン）

Reconstituted cell-free protein synthesis kit
PURE*frex*®

ポスター発表　1P-80

大腸菌由来再構成型無細胞タンパク質合成系（PURE*frex*®）を用いた bicistronic な鋳型 DNA からのタンパク質合成

Protein synthesis from the bicistronic template DNA using the *E. coli*-based reconstituted cell-free protein synthesis system (PURE*frex*®)

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PURE*frex*® は、大腸菌でタンパク質合成に関与する因子だけから再構成した無細胞タンパク質合成系であり、反応液の改良により、最大で 1 mg/mL の合成産物を得ることが可能になっている。

我々は、反応液の改良だけでなく、鋳型 DNA の配列や構造が合成に与える影響についても検討している。例えば、開始コドン直後のコドンは使用頻度が高いコドンではなく、AT 含量が高いコドンを使用した場合に合成量が高くなること、5'UTR ではリボソーム結合配列だけでなく AT-rich 領域も重要であり、AT-rich 領域を除去すると合成量が 1/10 程度に減少することなどを報告している。

本発表では、上記の結果を踏まえ、2 つの遺伝子が 1 本の鋳型 DNA 上に存在する bicistronic な鋳型 DNA からの合成結果について報告する。蛍光タンパク質 mCherry の ORF の下流に、AT-rich 領域、リボソーム結合配列（SD 配列）、及び GFP の ORF を繋いだ鋳型 DNA を作製し、各合成産物の蛍光量を測定した。その結果、別々の鋳型 DNA から合成した場合と比べ、mCherry の蛍光量が増加し、GFP の蛍光量が低下した。また、GFP の 5'UTR のリボソーム結合配列を除去した DNA からは GFP はほとんど合成されなかったが、AT-rich 領域を除去しても合成量はほとんど変わらなかった。この結果から、bicistronic な鋳型 DNA の下流側の ORF の 5'UTR にはリボソーム結合配列だけが必要であることが明らかになった。

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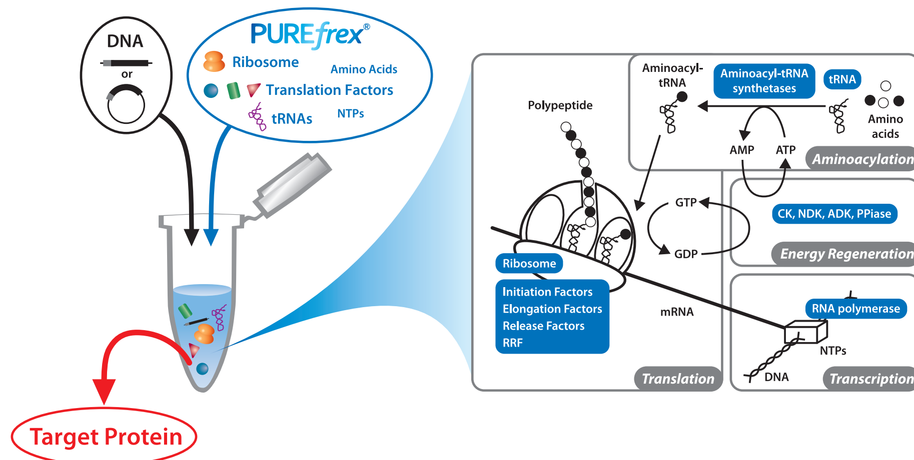
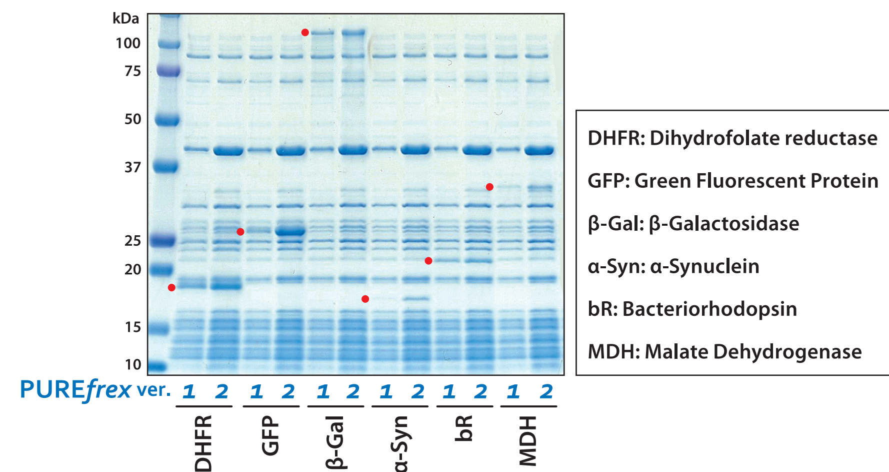
PURE*frex*® is based on the PURE system technology.

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

Concept of PURE*frex*



Example of protein synthesis using PURE*frex*



Advantages of PURE*frex*

- Low level of contamination
- Easy to adjust composition
- Usable of PCR product as a template DNA

▶ Synthesis efficiency depends on the target proteins.

- How does the nucleotide sequence of the template DNA affect the synthesis efficiency?
- What is the optimum nucleotide sequence for synthesizing with PURE*frex*?

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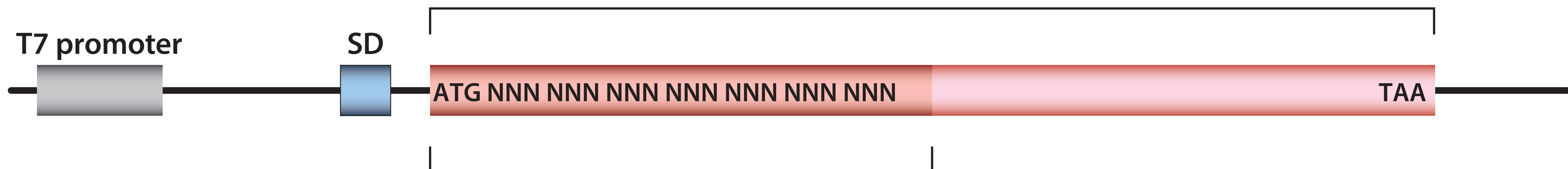
Optimum codon of the template DNA for PURE*frex*

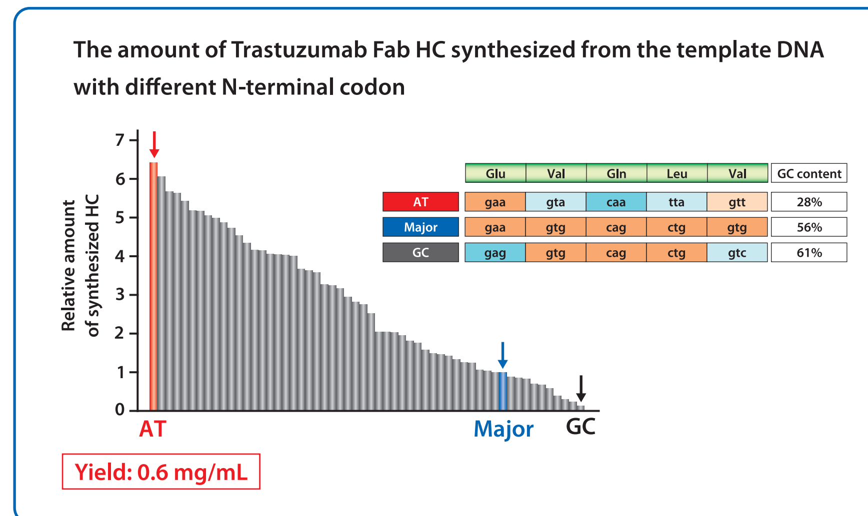
▶ **Multiple codons shoud be used for each amino acid according to their usage frequency in *E. coli*.**

Entire ORF



6 -10 aa at N-terminus

▶ **AT-rich codons shoud be used regardless of their usage frequency.**



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Effect of deletion of 5′ UTR in the template DNA for PURE*frex*

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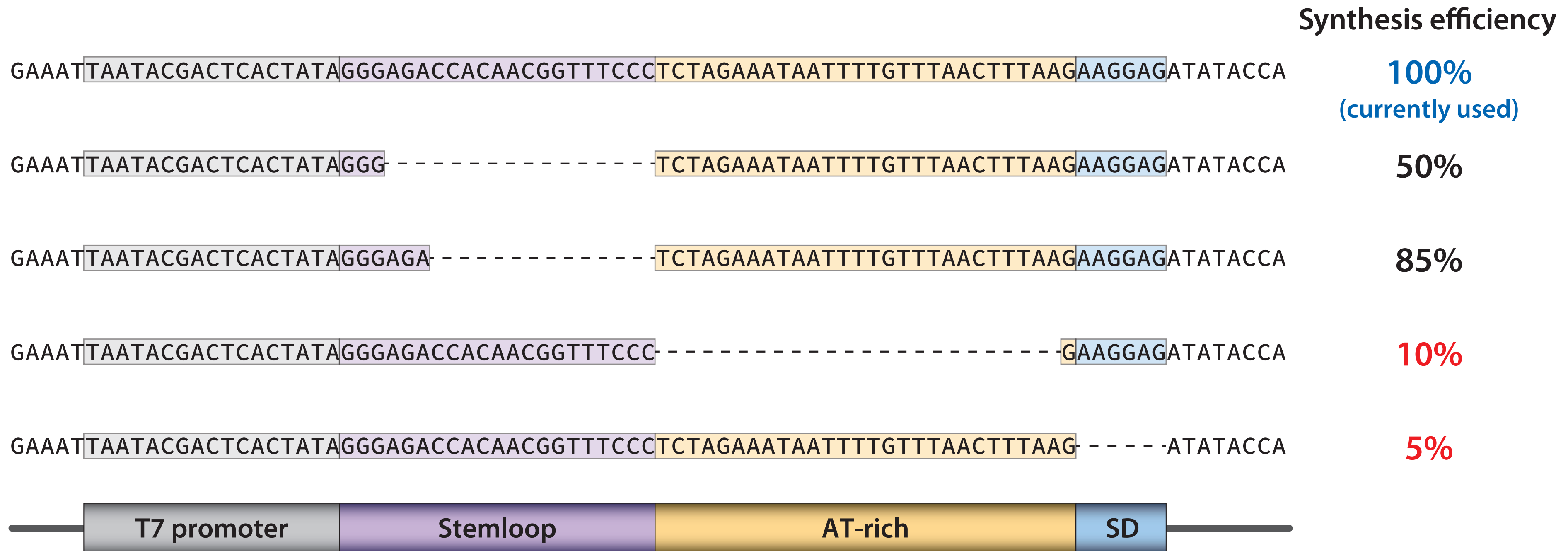

- ▶ Stemloop structure is not required. GGGAGA is required for efficient transcription by T7 RNAP.
- ▶ More than 10 nt is required for efficient translation in PURE*frex*.
- ▶ More than 3 nt is required for efficient translation in PURE*frex*.

Protein synthesis from mono- and bicistronic template DNA

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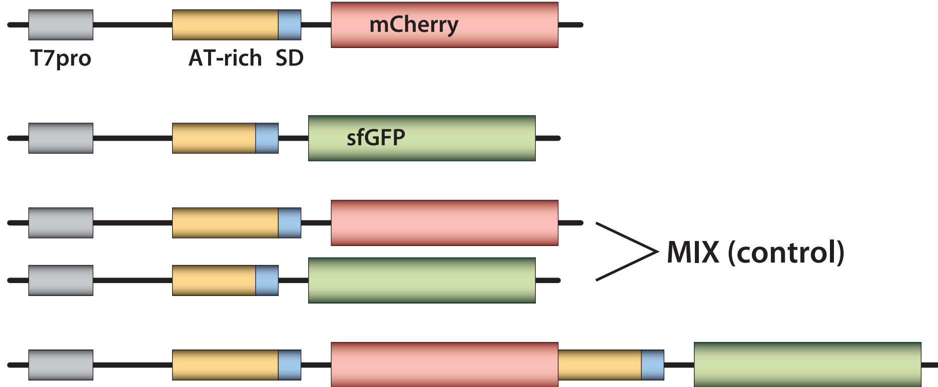
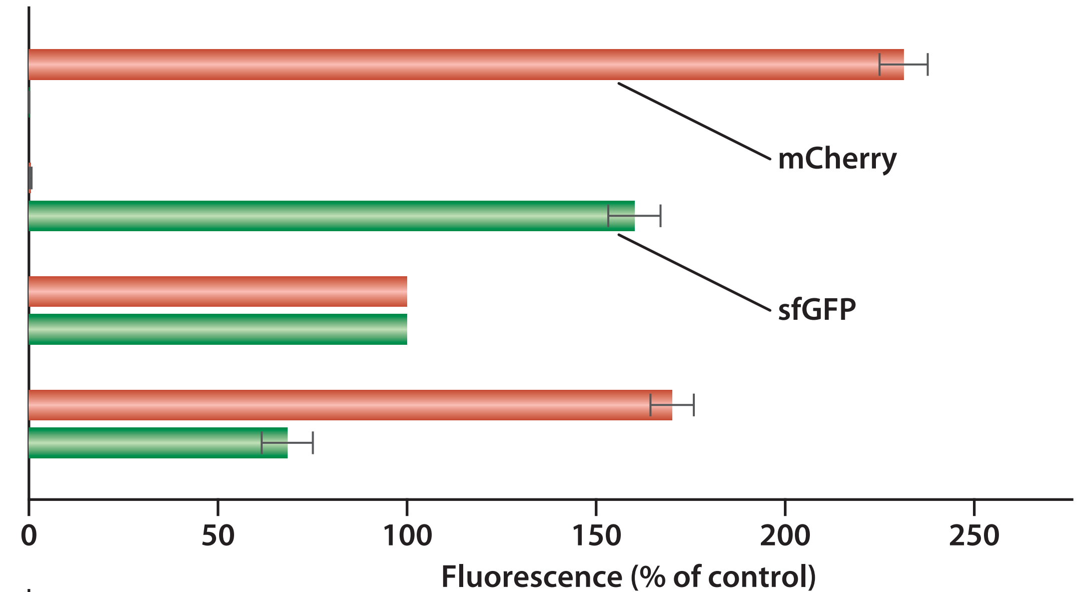
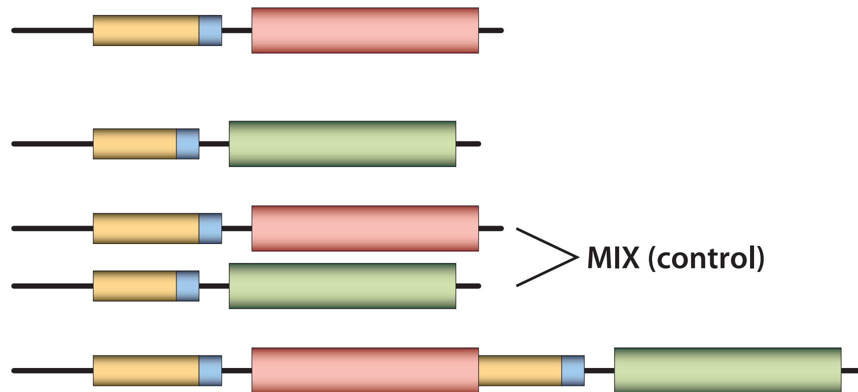
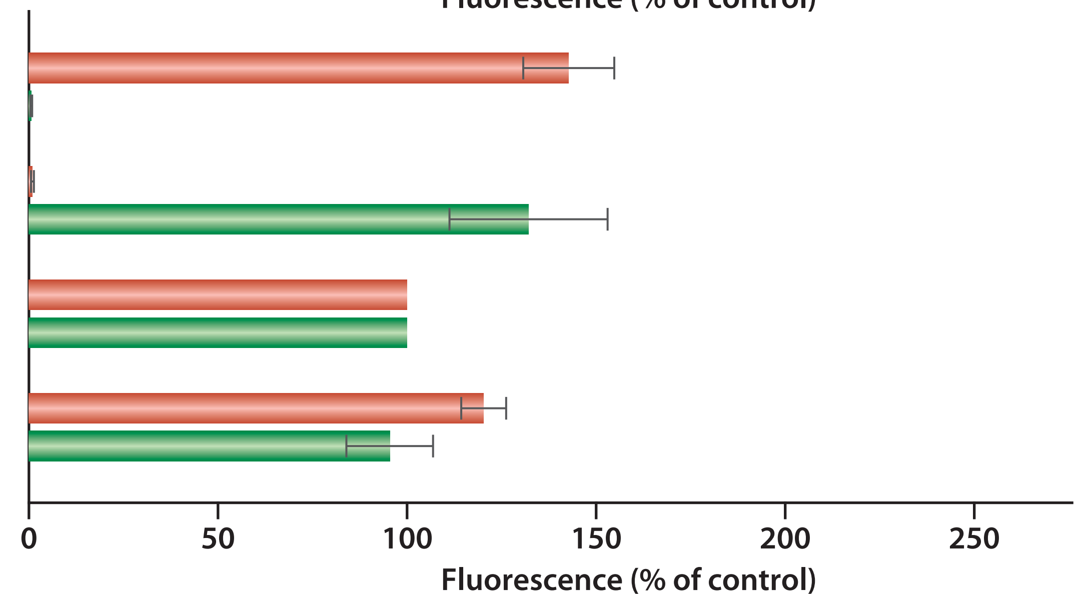
Synthesis from DNA





Synthesis from mRNA





▶ Protein synthesis from the upstream gene (mCherry) was up, while the downstream gene (sfGFP) was down when bicistronic template DNA was used.

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Effect of deletion of 5’ UTR of the downstream gene

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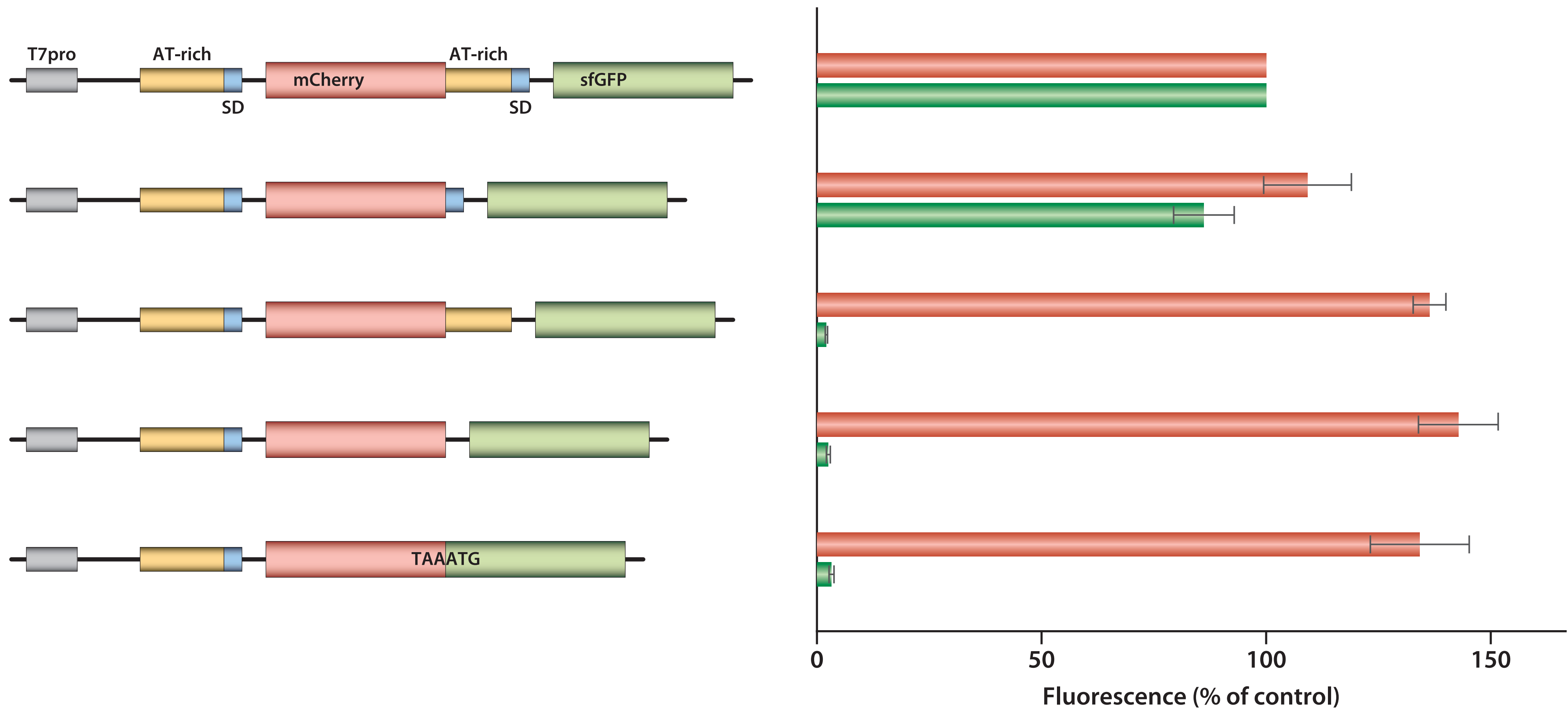

▶ SD sequence was required, but AT-rich region was not, for translation of the downstream gene.

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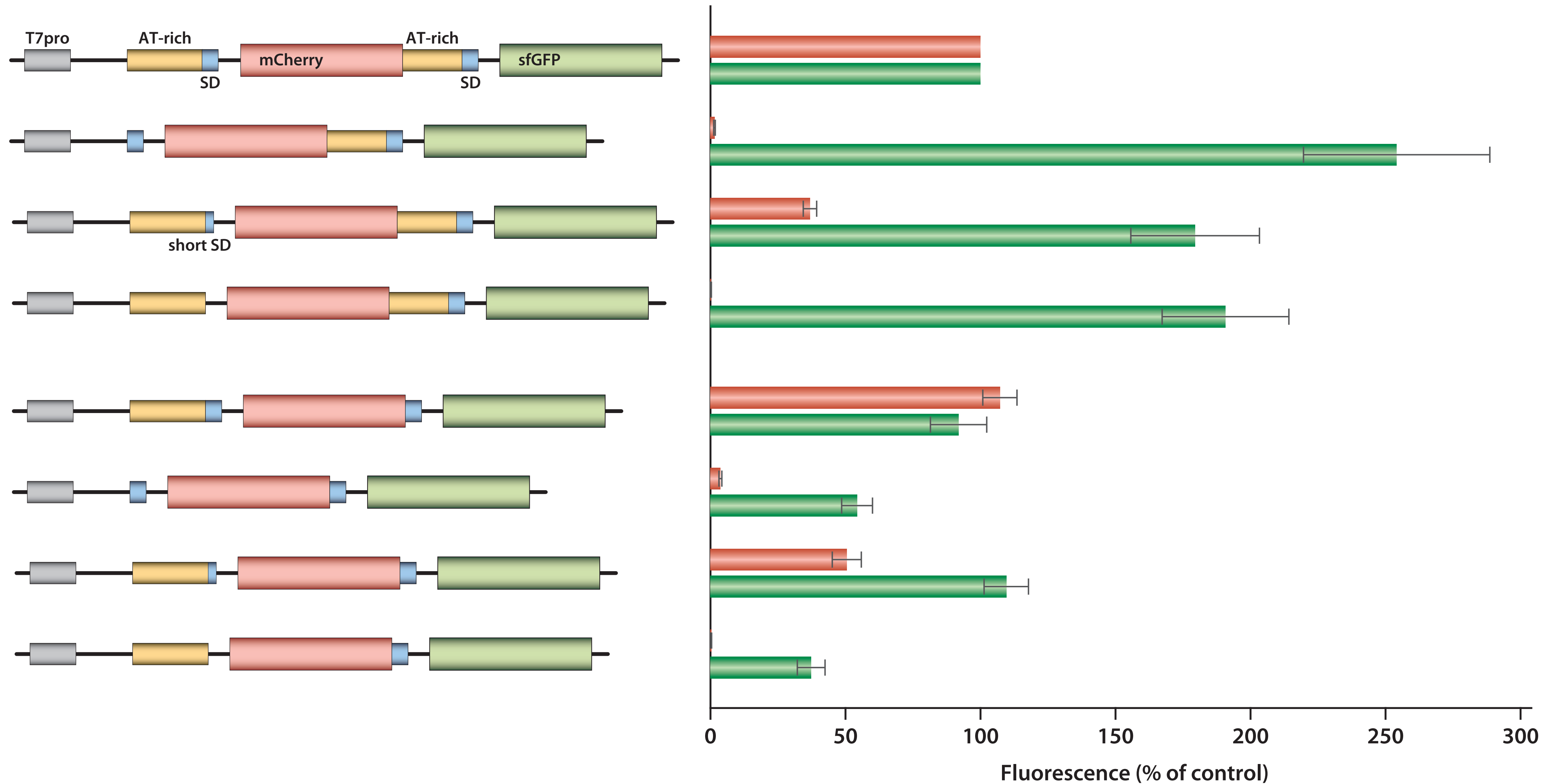
Effect of deletion of 5' UTR of the upstream gene



▶ **Deletion of AT-rich region or SD sequence in the upstream gene increased translation of the downstream gene only with AT-rich region in the 5' UTR.**

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Translational coupling in UAAUG (stop and start codon)

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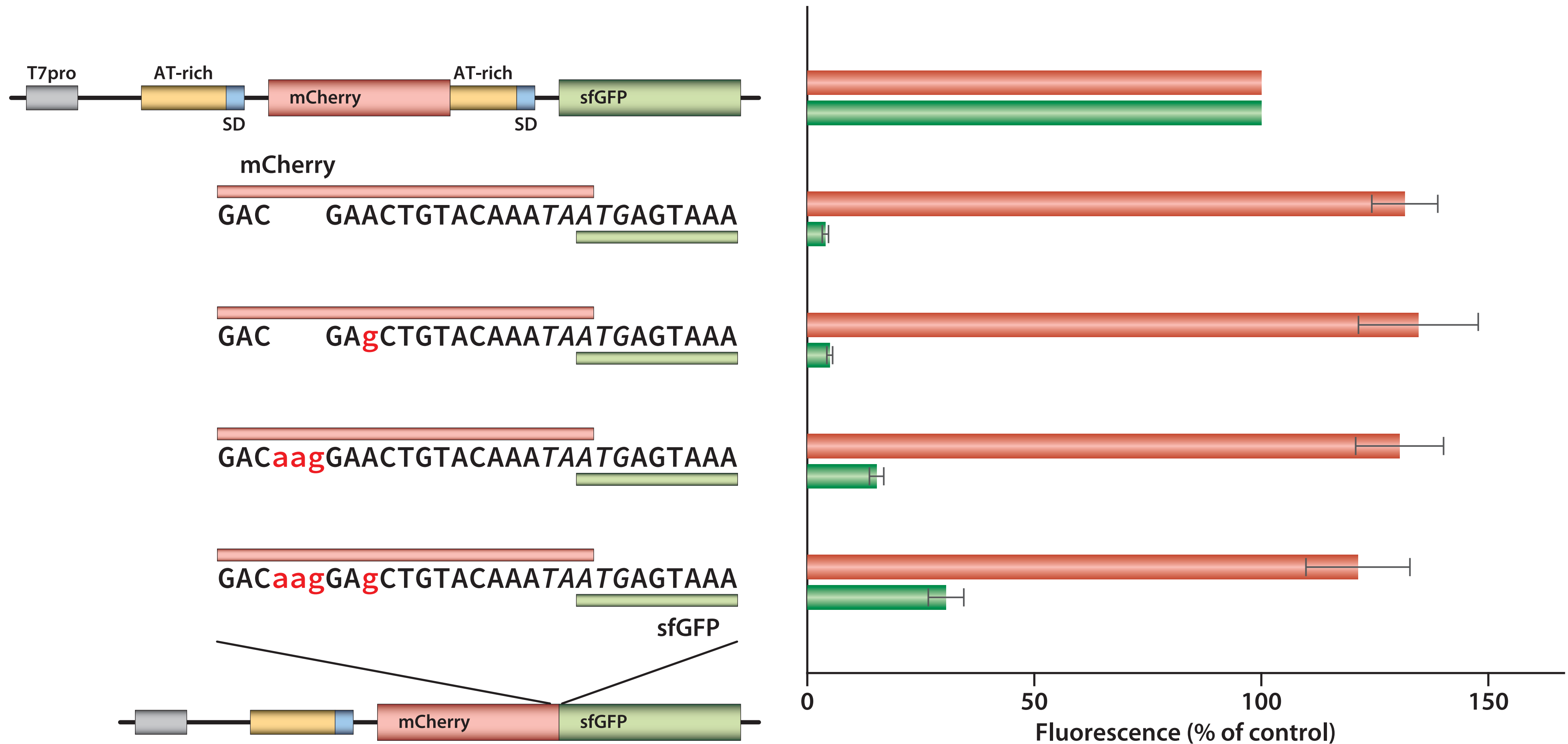

▶ Stronger SD sequence resulted in the increase of translation of the downstream gene.

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Summary

Protein synthesis from the bicistronic DNA using PURE*frex*

- Protein synthesis from the upstream gene (mCherry) was up, while the downstream gene (sfGFP) was down when bicistronic template DNA was used.
- SD sequence was required, but AT-rich region was not, for translation of the downstream gene.
- Deletion of AT-rich region or SD sequence in the upstream gene increased translation of the downstream gene only with AT-rich region in the 5' UTR.
- Stronger SD sequence resulted in the increase of translation of the downstream gene.

SD sequence, not AT-rich region, is important for efficient translation of the downstream gene.