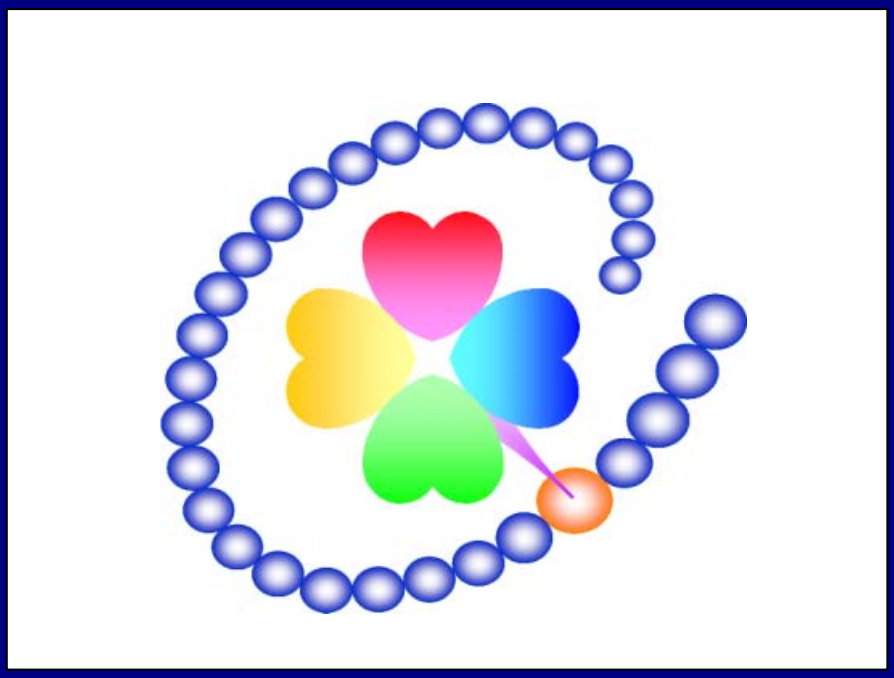


# Clover*Direct*™

tRNA Reagents for Site-Directed Protein Functionalization

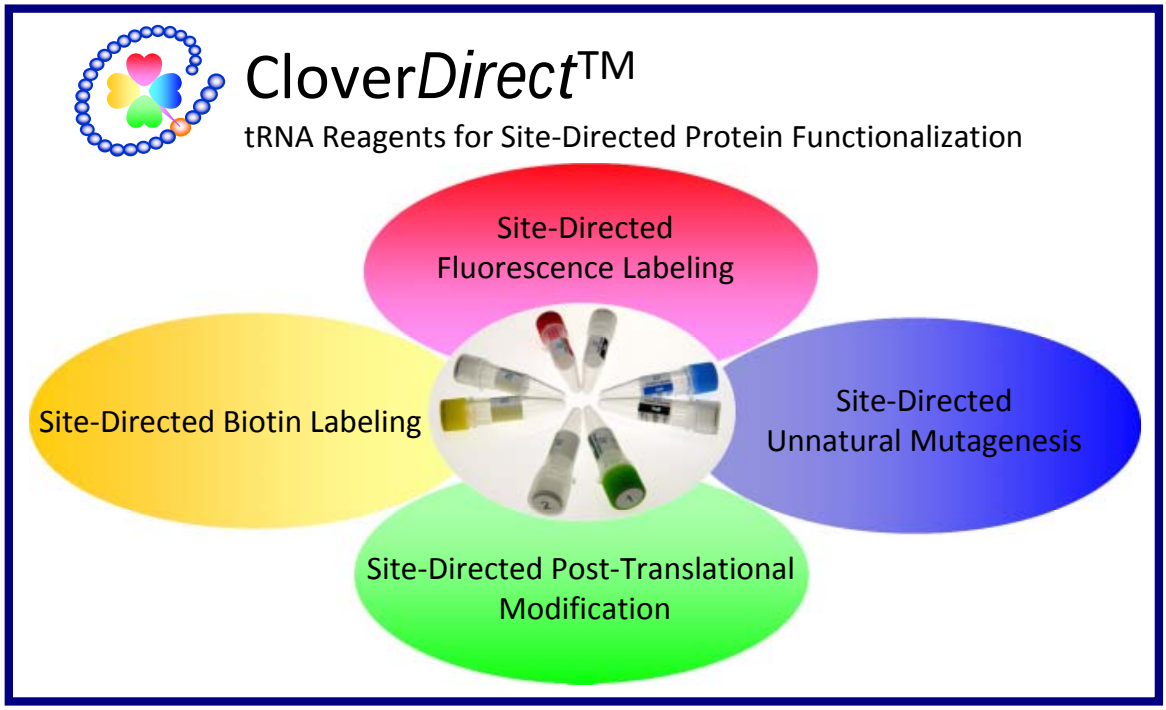
ProteinExpress

**Catalogue 2009/09**

# Contents

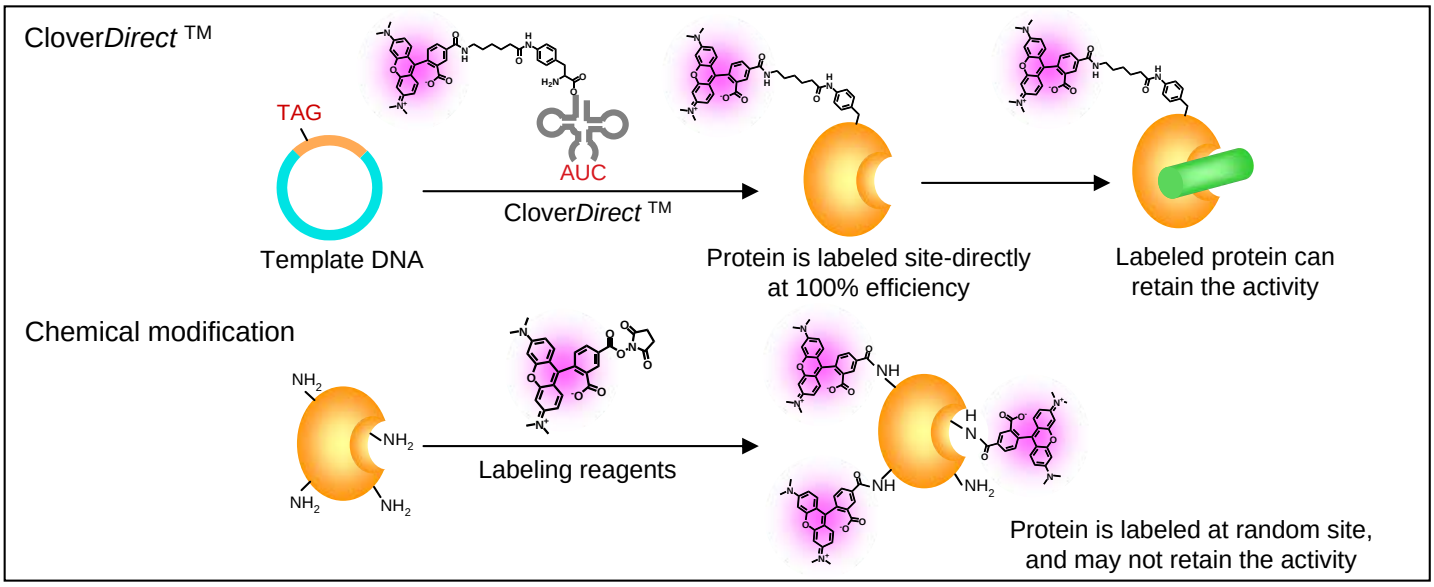
## [About Clover*Direct* ™]

Clover*Direct* ™ tRNA Reagents for Site-Directed Protein Functionalization allow the incorporation of unnatural amino acids at defined positions of proteins using *in vitro* translation. Unnatural amino acids containing fluorescent groups, biotin, PEG, photo-crosslink, etc are available. Proteins with unnatural amino acids will be obtained within a few hours just by adding Clover*Direct* ™ reagents and DNA template having an amber stop codon (UAG) or a four-base codon (CGGG) to an *in vitro* translation system. Clover*Direct* ™ covers the following four applications. In addition, we provide custom services for the expression of proteins with unnatural amino acids (see page 11 for details).




### Site-Directed Fluorescence Labeling

it is not easy to incorporate fluorescent groups into proteins in a site-direct and quantitative fashion by chemical modification. Clover*Direct* ™ tRNA Reagents for Site-Directed Fluorescence Labeling allow the incorporation of fluorescent unnatural amino acids into proteins in a site-direct and quantitative fashion. Various fluorescent dyes are available including those for 488 nm, 543 nm and 633 nm excitation.



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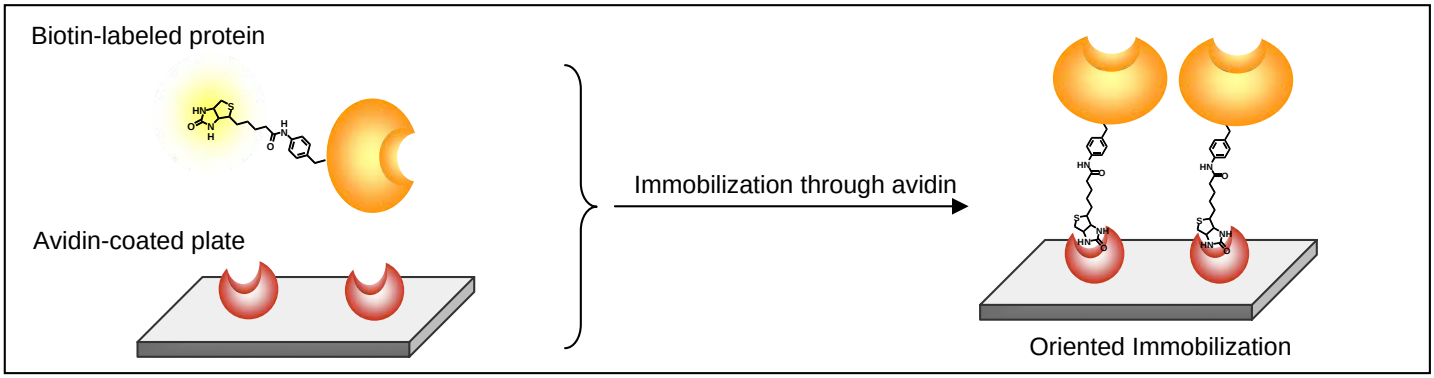
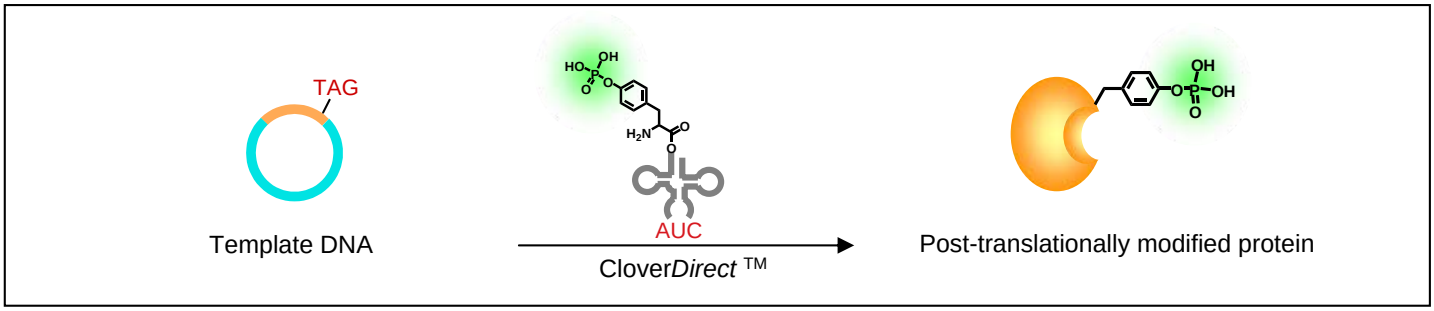
## Site-Directed Biotin Labeling

Clover*Direct* ™ tRNA Reagents for Site-Directed Biotin Labeling allow the incorporation of biotinylated unnatural amino acids into proteins in a site-direct and quantitative fashion. Labeling proteins are available for the oriented immobilization onto avidin-coated plates and beads. The biotinylated amino acids have one or two aminohexyl liners between amino acid and biotin.



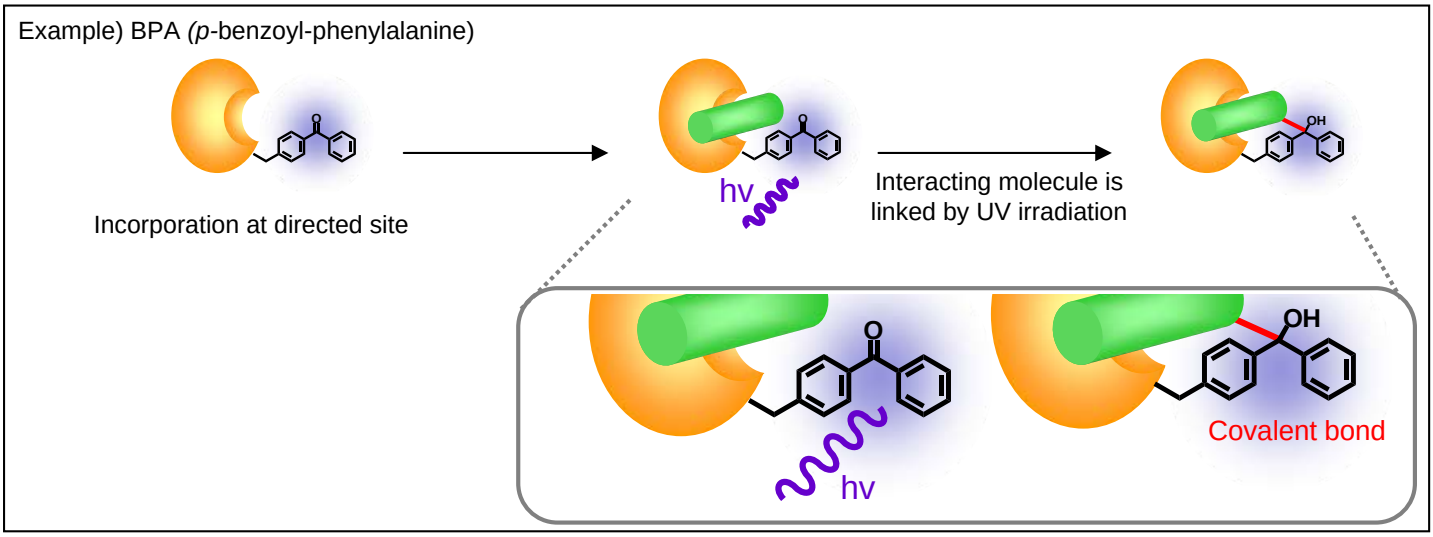

## Site-Directed Post-Translational Modification

It is not easy to prepare post-translationally modified proteins (phosphorylation, methylation, etc.). Clover*Direct* ™ tRNA Reagents for Site-Directed Post-Translational Modification allow the incorporation of modified amino acids into proteins to obtain post-translationally modified proteins in a site-direct and quantitative fashion.




## Site-Directed Unnatural Mutagenesis

By incorporation of unnatural amino acids containing functional groups, novel functional proteins can be designed and synthesized. Clover*Direct* ™ tRNA Reagents for Site-Directed Unnatural Mutagenesis allow the incorporation of unnatural amino acids with PEG, photo-crosslinking, photo-isomerizable groups, etc.

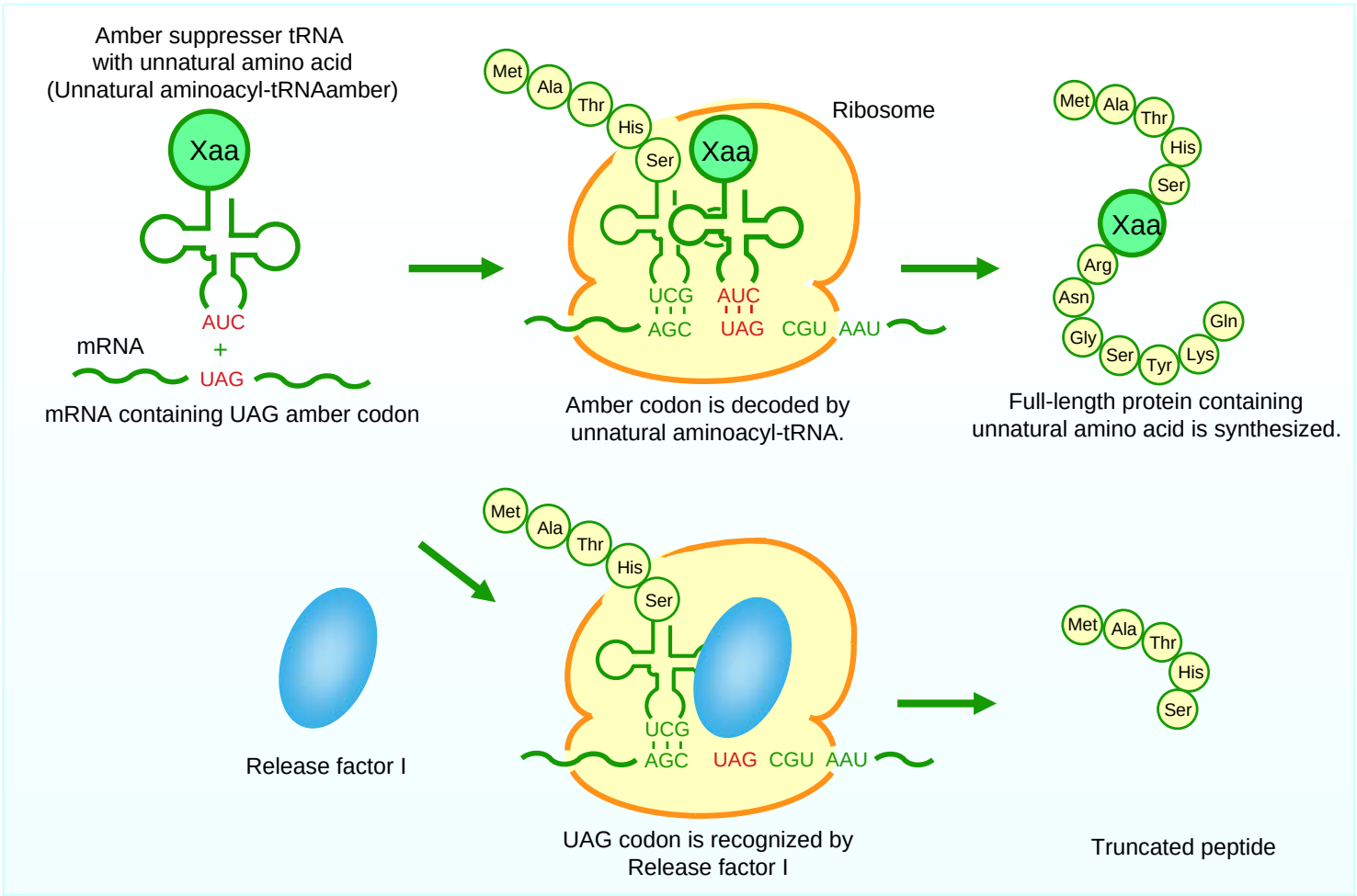


## [Principle of incorporation of unnatural amino acids]

Incorporation position of unnatural amino acids is defined by a UAG amber codon or CGGG four-base codon.
An unnatural aminoacyl-tRNA recognizes the UAG amber codon or the CGGG codon during translation. Consequently, the unnatural amino acid is incorporated at the directed site of the protein. By using two tRNAs for amber and four-base codons, dual-labeled proteins can be obtained which are available for fluorescence resonance energy transfer (FRET).

### [UAG amber codon]

If the UAG codon is recognized by the amber suppressor tRNA, full-length protein containing the unnatural amino acid is successfully synthesized. On the contrary, if the UAG codon is recognized by release factor 1 (RF1) which is one of the termination factors, the protein synthesis is terminated. Therefore, the translation product obtained as a full-length protein contains the unnatural amino acid at 100% efficiency.



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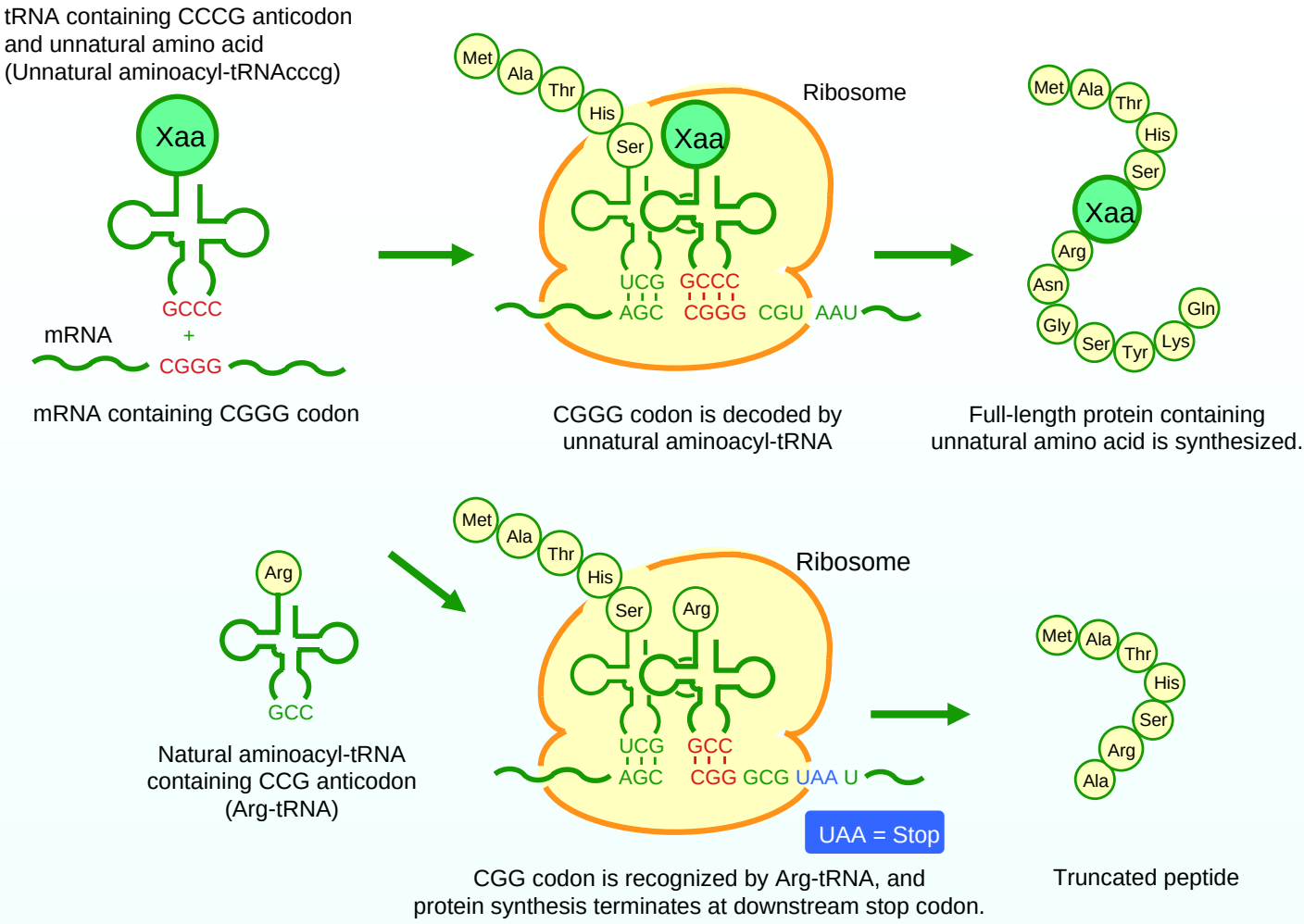
## [CGGG four-base codon]

If the CGGG codon is recognized by the four-base anticodon tRNA, full-length protein containing the unnatural amino acid is successfully synthesized. On the contrary, if the CGG is recognized as a triplet codon by Arg-tRNA, the reading frame shifts to +1 frame and a downstream stop codon terminates the protein synthesis. Therefore, the translation product obtained as a full-length protein contains the unnatural amino acid at 100% efficiency.




Incorporation at N-terminal regions (within 20 amino acid residues from the N-terminus) in response to CGGG codon sometimes results in the production of full-length proteins without unnatural amino acids, possibly because of spontaneous +1 frameshifting. In such case, ProteinExpress recommend the use of ProX™ tag, which is original peptide tag developed for the CGGG codon-mediated incorporation of unnatural amino acids.

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5'- | AUG | UCU | AAA | CAA | AUC | GAA | GUA | AAC | CGGG | UCU | AAU | GAG | -3' |
| | Met | Ser | Lys | Gln | Ile | Glu | Val | Asn | Xaa | Ser | Asn | Glu | |

Sequence of ProX™ tag

## [Product Contents]

| | |
|---|---|
| · Unnatural aminoacyl-tRNA (See note1) | X 1 |
| · tRNA dissolving buffer | X 1 |

Note 1 : One tube contains unnatural aminoacyl-tRNA sufficient for 300 μL of *in vitro* translation reaction. Once thawed, unnatural aminoacyl-tRNA can be stored at -70 °C for 2 months.

## [Equipment and reagents to be supplied by others]

Protein Expression

· Cell-free translation system(*E.coli* )
(*e.g.* RTS100 *E.coli* HY kit; Roche Applied Science, #3186156)

· Expression gene containing UAG or CGGG codon (plasmid DNA, linear DNA, or messenger RNA).
(Suitable expression vector should be used for the cell-tree translation system.)

Purification, buffer exchange, and concentration

· Affinity column and buffer for purification
(*e.g.* His SpinTrap™ kit; GE Healthcare Science , #28-9321-71)

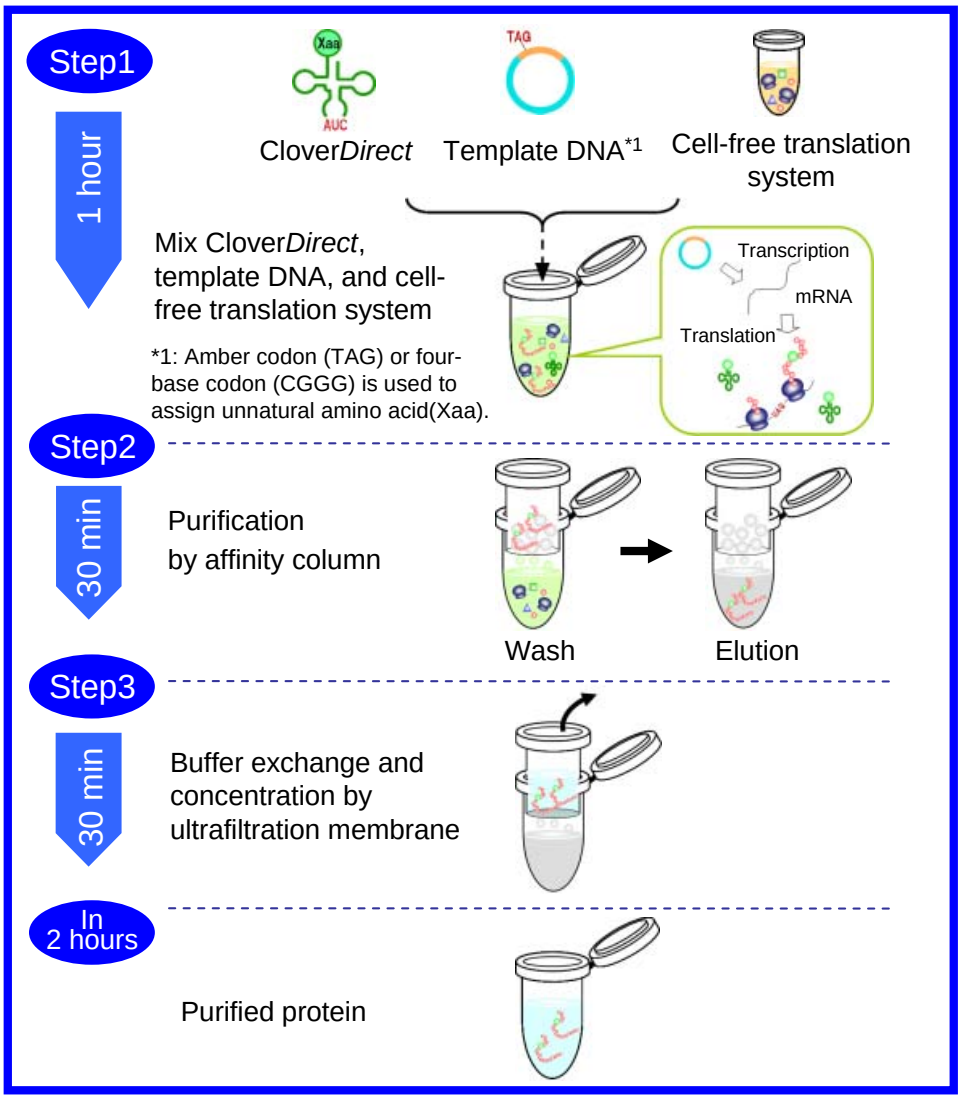
· Ultrafiltration membrane and buffer
(*e.g.* ULTRAFREE®-0.5 Centrifugal Filter Devices 10k; Millipore , UFV5BC00)

## [Brief protocol]

Step 1 Protein Expression
Dissolve unnatural aminoacyl-tRNA with tRNA buffer, and mix with template DNA and cell-free translation system. The mixture is incubated for 1 hour to synthesize protein containing unnatural amino acid.

Step 2 Purification
Reaction mixture includes several proteins derived from cell-free translation system and unnatural amino acid which is not incorporated into protein. Full-length protein containing unnatural amino acid can be isolated by purification for C-terminal tag such as His tag.

Step 3 Buffer exchange and concentration (optional)
Buffer exchange and concentration can be done by ultrafiltration membrane. Purified protein containing unnatural amino acid can be directly used for the downstream experiment.

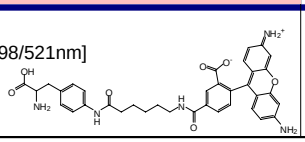



Technical Overview of Clover*Direct* ™

## [Points to note]

1. Protein expression
Confirm your protein can be expressed in *E.coli* cell-free translation system.
In case of very low expression of a wild-type gene that does not contain UAG codon or CGGG codon, optimization of nucleotide sequence (codon usage, addition of N-terminal tags, etc.) is required to improve the expression.
ProteinExpress provides custom service for protein expression using Clover*Direct* ™ including gene construction for efficient expression.

2. Incorporation site-dependency
Some unnatural amino acids are allowed to be incorporated only at the N-terminal region (within 20 amino acid residues from the N-terminus). Please check the product list for details.

| Pin-point Fluorescence Labeling | Site-dependency (*1) N-terminal | Site-dependency (*1) Internal, C-terminal | codon | Q'ty (translation) | Product No |
|---|---|---|---|---|---|
| **CR110-X-AF** [5-CR110-X : Abs/Em = 498/521nm]  | ○ | × | Amber | 300 µL | CLD1001 |
| | | | | 5 X 300 µL | CLD2001 |
| | | | CGGG | 5 X 300 µL | CLD2002 |

○ : Available
△ : Available in some sites
× : Unavailable

N-terminal region = within 20 amino acid residues from the N-terminus

Incorporation at N-terminal regions (within 20 amino acid residues from the N-terminus) in response to CGGG codon sometimes results in the production of full-length proteins without unnatural amino acids, possibly because of spontaneous +1 frameshifting. In such case, ProteinExpress recommend the use of ProX™ tag, which is original peptide tag developed for the CGGG codon-mediated incorporation of unnatural amino acids.

| 5'- | AUG | UCU | AAA | CAA | AUC | GAA | GUA | AAC | CGGG | UCU | AAU | GAG | -3' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Met | Ser | Lys | Gln | Ile | Glu | Val | Asn | Xaa | Ser | Asn | Glu | |

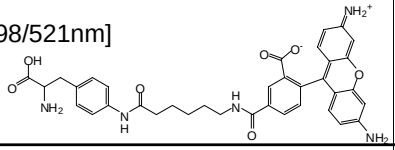
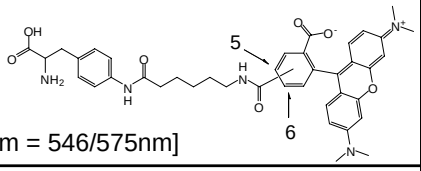
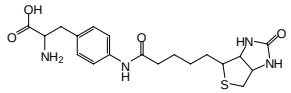
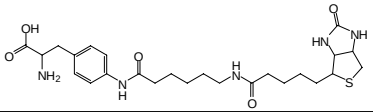
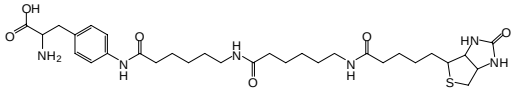
Sequence of ProX™ tag

3. Custom Services
ProteinExpress provides custom services for the synthesis of unnatural aminoacyl-tRNAs (fluorescent-labeled, functional amino acids, etc.), which allows the expression of proteins with your requested unnatural amino acids.

● Custom Services for Unnatural Aminoacyl-tRNA……p.11

ProteinExpress also provides custom services for the expression of proteins with unnatural amino acids including construction of recombinant DNA, cell-free translation, and purification of proteins.

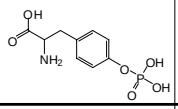
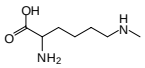
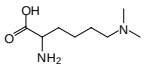
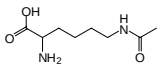
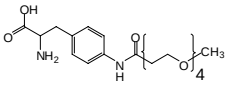
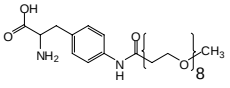
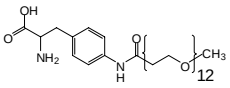
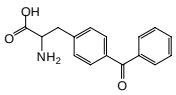
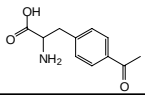
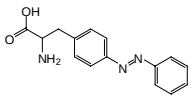
● Custom Service for Protein Expression using Clover*Direct* ™……p.11

| Site-Directed Fluorescence Labeling | Site-dependency (*1) N-terminal | Site-dependency (*1) Internal, C-terminal | codon | Q'ty (translation) | Product No |
|---|---|---|---|---|---|
| **CR110-X-AF**<br>[5-CR110-X : Abs/Em = 498/521nm]  | ○ | × | Amber | 300 µL | CLD1001 |
| | | | | 5 X 300 µL | CLD2001 |
| | | | CGGG | 5 X 300 µL | CLD2002 |
| **HiLyte Fluor™ 488-AF**<br>[HiLyte Fluor™ 488 : Abs/Em = 497/525nm]<br>Not Available | ○ | × | Amber | 300 µL | CLD01 |
| | | | | 5 X 300 µL | CLD05 |
| | | | CGGG | 5 X 300 µL | CLD2004 |
| **TAMRA-X-AF**  <br>[5(6)-TAMRA-X : Abs/Em = 546/575nm] | ○ | × | Amber | 300 µL | CLD02 |
| | | | | 5 X 300 µL | CLD06 |
| | | | CGGG | Contact to OLYMPUS | |
| **ATTO633-AF**<br>[ATTO633 : Abs/Em = 629/657nm]<br>Not Available | ○ | × | Amber | 300 µL | CLD03 |
| | | | | 5 X 300 µL | CLD07 |
| | | | CGGG | 5 X 300 µL | CLD2008 |
| **ATTO655-X-AF**<br>[ATTO655-X : Abs/Em = 633/684nm]<br>Not Available | ○ | × | Amber | 300 µL | CLD1009 |
| | | | | 5 X 300 µL | CLD2009 |
| | | | CGGG | 300 µL | CLD1010 |
| | | | | 5 X 300 µL | CLD2010 |
| **Site-Directed Biotin Labeling** | | | | | |
| **Biotin-AF**<br>[Biotin]  | ○ | ○ | Amber | 5 X 300 µL | CLD2101 |
| | | | CGGG | 5 X 300 µL | CLD2102 |
| **Biotin-X-AF**<br>[Biotin-X]  | ○ | ○ | Amber | 5 X 300 µL | CLD2103 |
| | | | CGGG | 5 X 300 µL | CLD2104 |
| **Biotin-XX-AF**<br>[Biotin-XX]  | ○ | △ | Amber | 300 µL | CLD04 |
| | | | | 5 X 300 µL | CLD08 |
| | | | CGGG | 5 X 300 µL | CLD2106 |

*1 : Incorporation site-dependency

[Please inquire about the price]

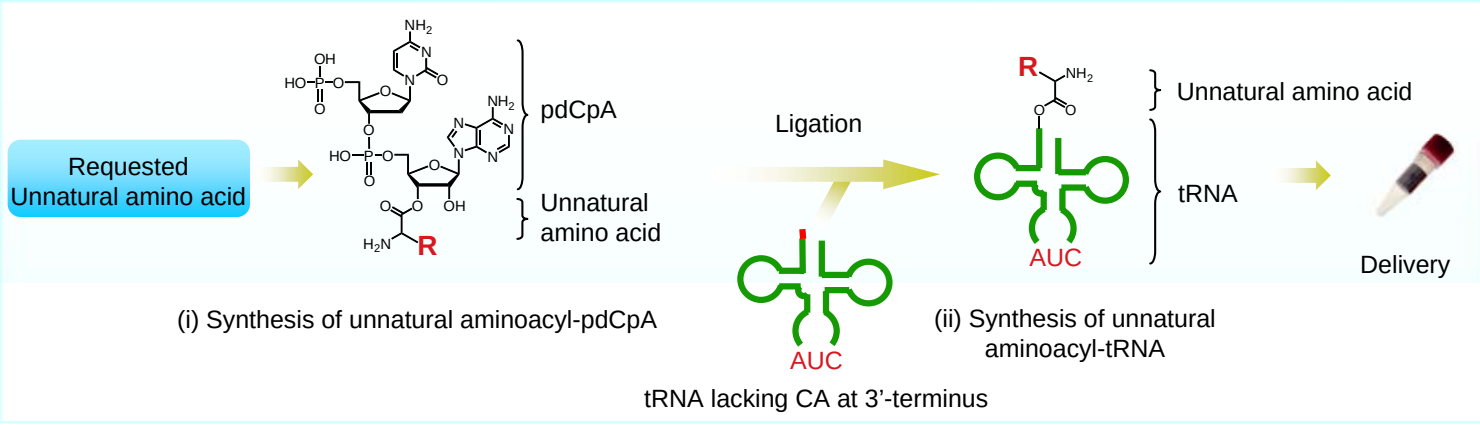
Some unnatural amino acids are incorporated in a incorporation site-dependent manner.
(N-terminal region = within 20 amino acid residues from the N-terminus.)
○ : Available
△ : Available in some sites
× : Unavailable

| Site-Directed Post-Translational Modification | | Incorporable site (*1) N-terminal | Incorporable site (*1) Internal, C-terminal | codon | Q'ty (translation) | Product No |
|---|---|---|---|---|---|---|
| **Tyr($PO_3H_2$)** [*O*-phospho-Tyr] |  | ○ | △ | Amber | 5 X 300 µL | CLD2201 |
| | | | | CGGG | 5 X 300 µL | CLD2202 |
| **Lys(Me)** [ε-methyl-Lys] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2203 |
| | | | | CGGG | 5 X 300 µL | CLD2204 |
| **Lys($Me_2$)** [ε-dimethyl-Lys] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2205 |
| | | | | CGGG | 5 X 300 µL | CLD2206 |
| **Lys(Ac)** [ε-acetyl-Lys] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2207 |
| | | | | CGGG | 5 X 300 µL | CLD2208 |
| **Site-Directed Unnatural Mutagenesis** | | | | | | |
| **PEGylated amino acids** | | | | | | |
| **PEG4-AF** [Methyl-PEG4] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2301 |
| | | | | CGGG | 5 X 300 µL | CLD2302 |
| **PEG8-AF** [Methyl-PEG8] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2303 |
| | | | | CGGG | 5 X 300 µL | CLD2304 |
| **PEG12-AF** [Methyl-PEG12] |  | ○ | △ | Amber | 5 X 300 µL | CLD2305 |
| | | | | CGGG | 5 X 300 µL | CLD2306 |
| **Cross-linking amino acids** | | | | | | |
| **BPA** [ρ-benzoyl-phenylalanine] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2321 |
| | | | | CGGG | 5 X 300 µL | CLD2322 |
| **AcPhe** [ρ-acetyl-phenylalanine] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2323 |
| | | | | CGGG | 5 X 300 µL | CLD2324 |
| **Photo-isomerizable amino acid** | | | | | | |
| **azoAla** [ρ-phenylazophenyl-alanine] |  | ○ | ○ | Amber | 5 X 300 µL | CLD2331 |
| | | | | CGGG | 5 X 300 µL | CLD2332 |

[Please inquire about the price]

## [Custom Services for Unnatural Aminoacyl-tRNA]

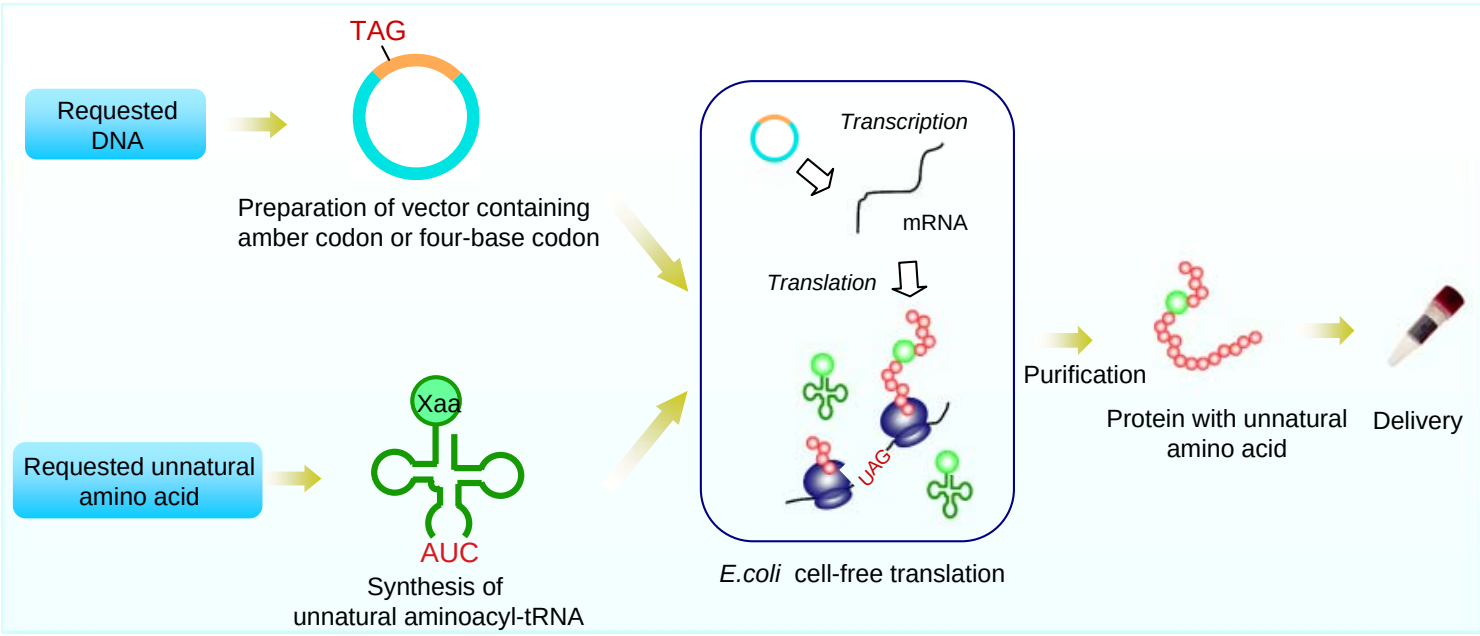
ProteinExpress provides custom services for the synthesis of unnatural aminoacyl-tRNAs (fluorescent-labeled, functional amino acids, etc.), which allows the expression of proteins with your requested unnatural amino acids.




**Custom Synthesis of Unnatural Aminoacyl-tRNA**

## [Custom Service for Proteins with Unnatural Amino Acids]

ProteinExpress provides custom services for the expression of proteins with your requested unnatural amino acids at requested positions, including artificial gene synthesis, cell-free translation, and protein purification.



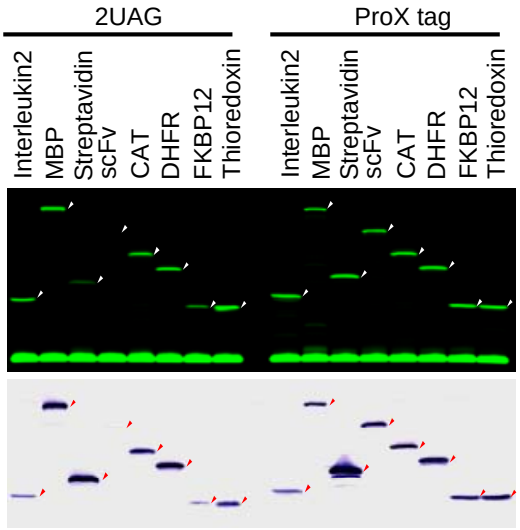

**Custom Service for Protein with Unnatural Amino Acids**

## [Expression of site-directly labeled proteins]

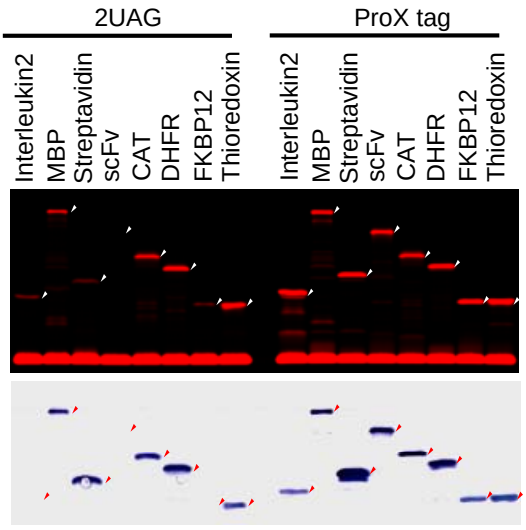
Fluorescent- and biotin-labeled unnatural amino acids are incorporated into eight prokaryote and eukaryote proteins. The site-directly fluorescent-labeled proteins can be visualized on SDS-PAGE using a laser-based fluorescence scanner. The proteins are also detectable by an antibody against tag peptide or biotin. A 0.25 ~ 1 µL of translational reaction mixture is sufficient for the detection.

· Labeled unnatural amino acids that are not incorporated into proteins are detected at the bottom of SDS-PAGE gel.

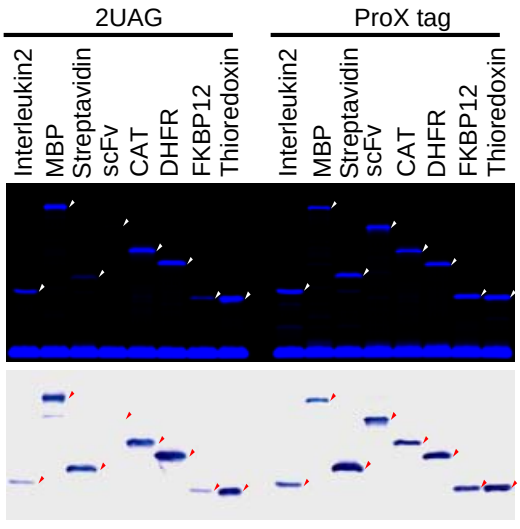
HiLyte Fluor 488



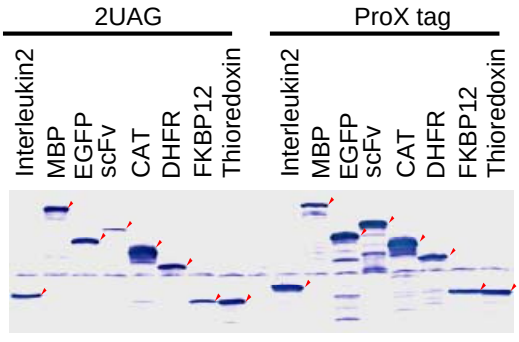

TAMRA




ATTO633




Biotin




2UAG : UAG codon is inserted after initiator AUG codon.
ProX tag : ProX tag is fused to the N-terminus.
Applied volume: 0.25 µL of translational reaction mix
Fluorescence image (Top) are visualized with Ex and Em wavelengths listed below:

| | |
|---|---|
| HiLyteFluor488 | Ex : 488nm / Em : 520 nm |
| TAMRA | Ex : 532nm / Em : 580 nm |
| ATTO633 | Ex : 635nm / Em : 670 nm |

Western blotting (Bottom) are visualized by anti-His tag antibody (for fluorescent amino acids) and anti-biotin antibody (for biotin).

## [Applications for site-directly fluorescent labeled proteins]

The site-directly fluorescent-labeled proteins are available to the following measurements.

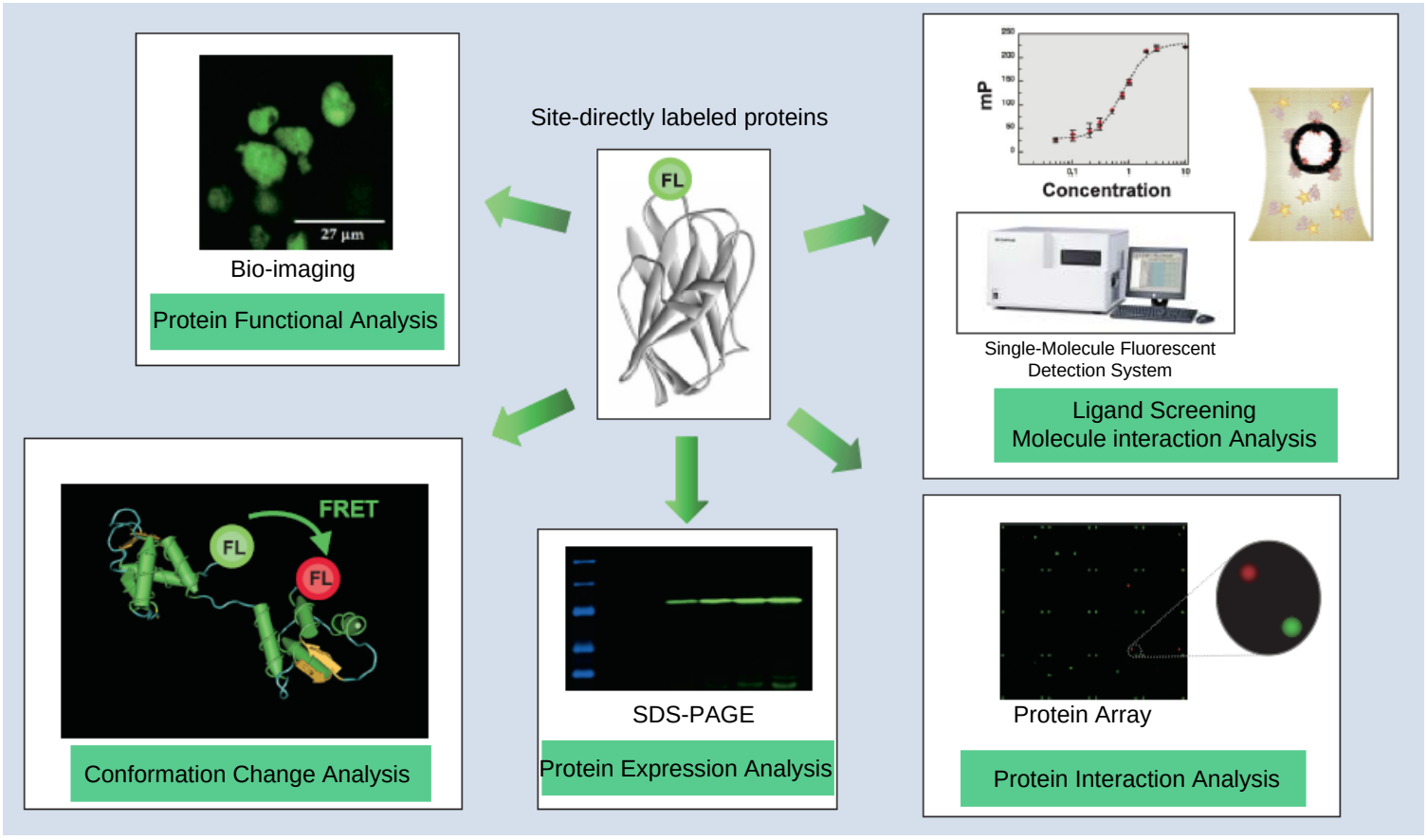
·Interaction analysis using single molecule fluorescence analysis
Single Molecule fluorescence detection system (MF20 / Fluor Point-Light MF20; OLYMPUS)

·Conformation analysis of protein by inter- or intra-molecular fluorescence resonance energy transfer (FRET)

·Functional analysis in cell imaging

·Interaction analysis in protein array

·Expression analysis by in-gel fluorescent detection of SDS-PAGE



## [References]

1) FRET analysis of protein conformational change through position-specific incorporation of fluorescent amino acids
Daisuke Kajihara, Ryoji Abe, Issei Iijima, Chie Komiyama, Masahiko Sisido, Takahiro Hohsaka
*Nature Methods.,* 3, 923-929 (2006).

2) Position-specific incorporation of biotinylated non-natural amino acids into a protein in a cell-free translation system
Takayoshi Watanabe, Norihito Muranaka, Issei Iijima, Takahiro Hohsaka
*Biochem. Biophys. Res. Commun.,* 361, 794-799 (2007)

3) Comprehensive screening of amber suppressor tRNAs suitable for incorporation of non-natural amino acids in a cell-free translation system
Hikaru Taira, Yosuke Matsushita, Kenji Kojima, Kaori Shiraga, Takahiro Hohsaka
*Biochem. Biophys. Res. Commun.*, 374, 304-308 (2008).

4) Efficient Incorporation of Nonnatural Amino Acids with Large Aromatic Groups into Streptavidin in In Vitro Protein Synthesizing Systems
Takahiro Hohsaka, Daisuke Kajihara, Yuki Ashizuka, Hiroshi Murakami, Masahiko Sisido
*J. Am. Chem. Soc.*, 121, 34-40 (1999).

## [Questions about Products]

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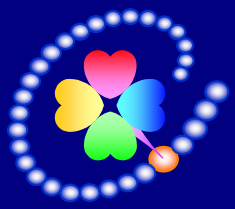

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