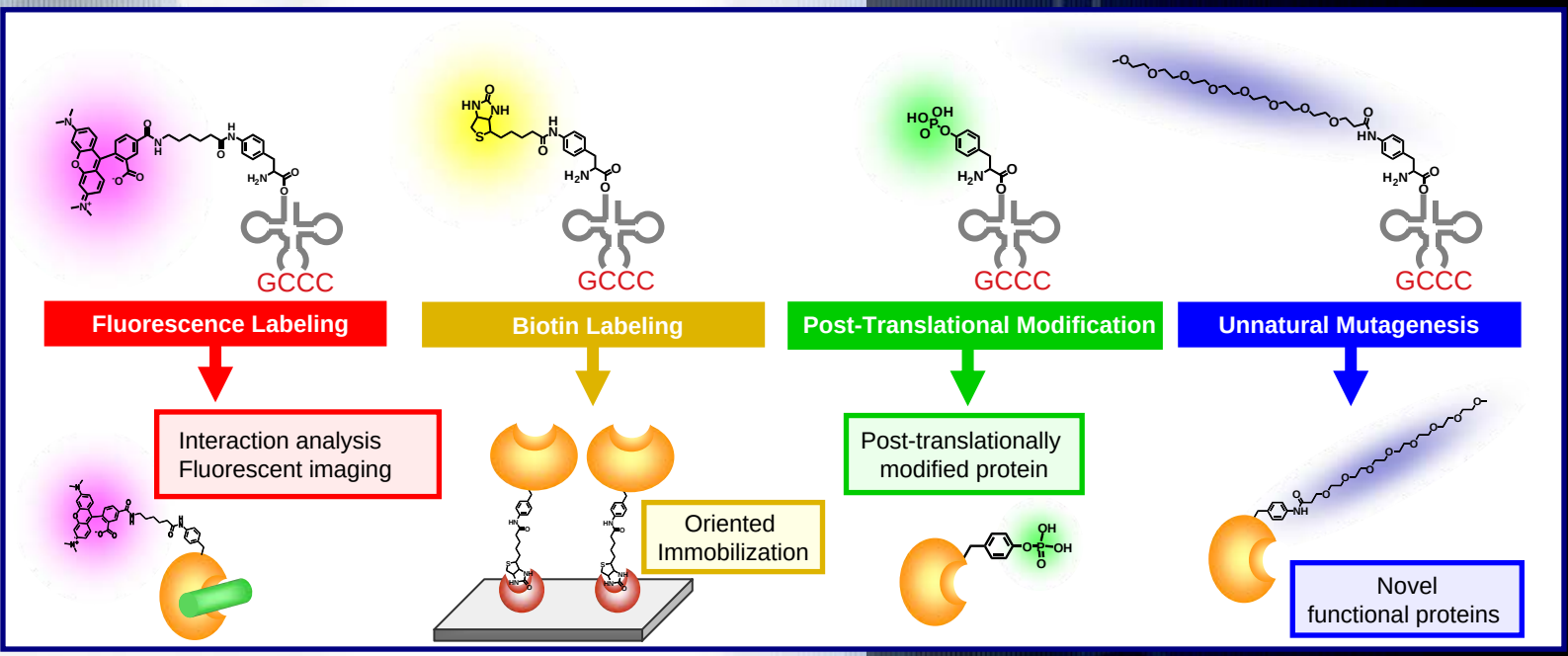
CloverDirect™

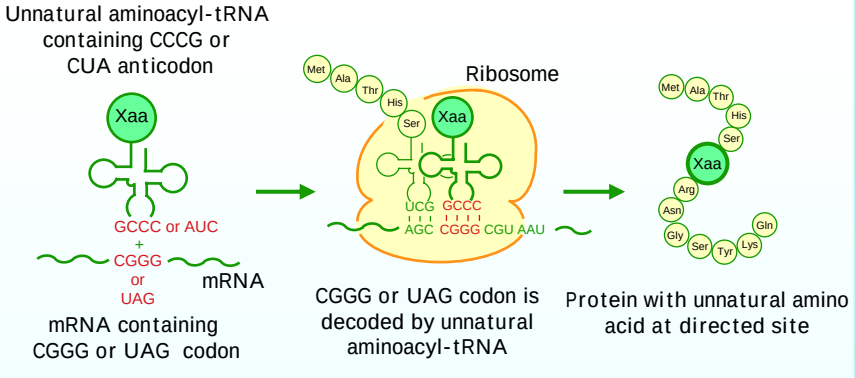
tRNA Reagents for Site-Directed Protein Functionalization

Expression of proteins with unnatural amino acids using four-base codon (CGGG) or amber stop codon (UAG)

New Tool For Protein Research

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 system. Various unnatural amino acids are available for fluorescence labeling, biotin labeling, post-translational modification, and unnatural mutagenesis of proteins.





[Principle]

If CGGG or UAG codon is recognized by unnatural aminoacyl-tRNA, full-length protein with unnatural amino acid is successfully synthesized. On the contrary, if CGGG or UAG codon is recognized by natural translation factor (arginyl-$tRNA_{CCG}$ or release factor 1), the protein synthesis is terminated. Therefore, the translation product obtained as a full-length protein contains the unnatural amino acid at 100% efficiency.

[Features]

Accurate and Quantitative : Incorporation position of unnatural amino acids is defined by CGGG four-base codon or UAG amber codon. An unnatural aminoacyl-tRNA recognizes the CGGG or UAG codon during translation to introduce unnatural amino acids into proteins in a site-directed and quantitative fashion.

Fast and Easy: Proteins with unnatural amino acids can be obtained within a few hours just by adding Clover*Direct* ™ reagents and DNA or mRNA having CGGG or UAG codon to an *in vitro* translation system.

Flexible: Various unnatural amino acids containing fluorescent groups, biotin, PEG, photo-cross-linker, *etc.* are available.

[Product Contents]

· Unnatural aminoacyl-tRNA X 1
· tRNA dissolving buffer X 1

One tube contains unnatural aminoacyl-tRNA sufficient for 300 µL of *in vitro* translation reaction.

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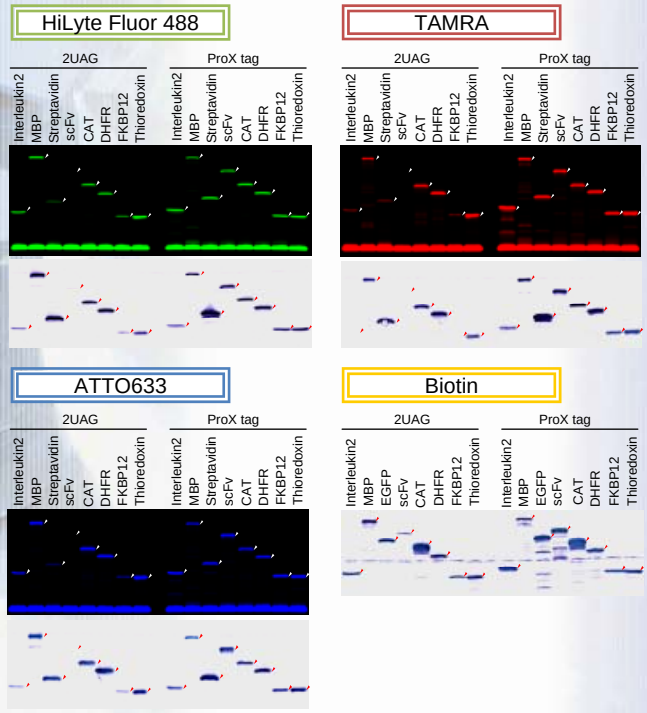
[Expression of site-directly labeled proteins]

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.



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 images (Top) are visualized with Ex and Em wavelengths listed below:

HiLyte Fluor488	Ex : 488nm / Em : 520 nm
TAMRA	Ex : 532nm / Em : 580 nm
ATTO633	Ex : 635nm / Em : 670 nm

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

[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).

[Applications of site-directly labeled proteins]

Site-directly fluorescent-labeled proteins are available to the following analyses.

- Protein interaction analysis using Single Molecule fluorescence detection system (MF20 / FluoroPoint-Light; OLYMPUS).
- Conformation analysis of protein by inter- or intra-molecular fluorescence resonance energy transfer (FRET).
- Functional analysis by cell imaging.
- Interaction analysis by protein array.
- Expression analysis by fluorescent detection of SDS-PAGE.

[Product List]

Site-Directed Fluorescence Labeling

- CloverDirect ™ CR110-X-AF-tRNA
  [5-CR110-X : Abs/Em = 498/521nm]
- CloverDirect ™ HiLyte Fluor™ 488-AF-tRNA
  [HiLyte FluorTM 488 : Abs/Em = 497/525nm]
- CloverDirect ™ TAMRA-X-AF-tRNA
  [5(6)-TAMRA-X : Abs/Em = 546/575nm]
- CloverDirect ™ ATTO 633-AF-tRNA
  [ATTO633 : Abs/Em = 629/657nm]
- CloverDirect ™ ATTO 655-X-AF-tRNA
  [ATTO655-X : Abs/Em = 633/684nm]

Site-Directed Biotin Labeling

- CloverDirect ™ Biotin-AF-tRNA
  [Biotin]
- CloverDirect ™ Biotin-X-AF-tRNA
  [Biotin-X]
- CloverDirect ™ Biotin-XX-AF-tRNA
  [Biotin-XX]

Sire-Directed Post-Translational Modification

- CloverDirect ™ Tyr(PO_3H_2)-tRNA
  [*O*-phospho-Tyr]
- CloverDirect ™ Lys(Me)-tRNA
  [ε-methyl-Lys]
- CloverDirect ™ Lys(Me_2)-tRNA
  [ε-dimethyl-Lys]
- CloverDirect ™ Lys(Ac)-tRNA
  [ε-acetyl-Lys]

Site-Directed Unnatural Mutagenesis

PEGylated amino acids
- CloverDirect ™ PEG4-AF-tRNA
  [Methyl-PEG4]
- CloverDirect ™ PEG8-AF-tRNA
  [Methyl-PEG8]
- CloverDirect ™ PEG12-AF-tRNA
  [Methyl-PEG12]

Cross-linking amino acids
- CloverDirect ™ BPA-tRNA
  [*p*-benzoyl-phenylalanine]
- CloverDirect ™ AcPhe-tRNA
  [*p*-acetyl-phenylalanine]

Photo-isomerizable amino acid
- CloverDirect ™ azoAla-tRNA
  [*p*-phenylazophenyl-alanine]

We provide custom services for the synthesis of unnatural aminoacyl-tRNAs and the expression of proteins with unnatural amino acids.

ProteinExpress

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