PURE*frex*™ Technical information

PURE system technology is a reconstituted cell-free protein synthesis system invented by Professor Takuya Ueda in the University of Tokyo (Ref.1, 2).

This system consists of factors for transcription, translation, aminoacylation, and energy regeneration. All proteinous factors and ribosome are highly purified individually and are assembled with substrates, such as amino acids, NTPs, and tRNAs in the buffer. Concrete factors are described in Fig.3. Translational factors includes followings; IF1, IF2, and IF3 as initiation factors, EF-Tu, EF-Ts, and EF-G as elongation factors, RF1, RF2, and RF3 as release factors, ribosome recycling factor (RRF), 20 kinds of aminoacyl-tRNA synthetase (ARS), methionyl-tRNA transformylase.

Described above, the PURE system is a reaction system consisting of a translation system only, not like other cell-free protein synthesis system using cell lysates. It indicates that the PURE system has no unknown protein and can be adjusted its composition as you like.

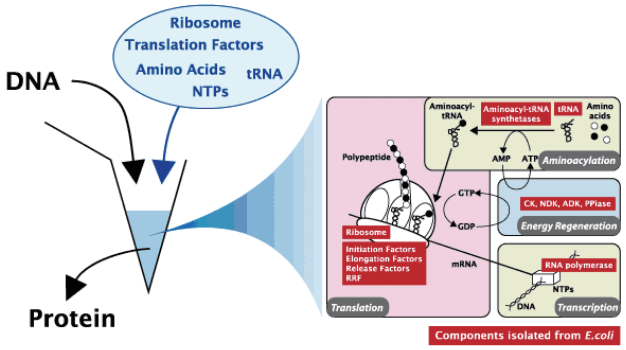

Figure 3
Outline of protein synthesis in the PURE system

Template DNA

PCR products, circular DNA, and linear DNA are available as the template DNA for PURE*frex*™. The template DNA must contain a T7 promoter and ribosome binding site (SD sequence) upstream of the gene of interest, shown in Fig. 4. The gene of interest must contain an ATG initiation codon and a stop codon.
All stop codons, amber, ocher and opal, are available.
More than 10 nucleotides are necessary following the stop codon.
To generate template DNA using circular DNA, T7 terminator is necessarily following the gene of interest.
To generate template DNA using linear DNA, including PCR product and digested circular DNA by restriction enzyme, T7 terminator is not necessary in the downstream of the stop codon.

Figure 4
Construct of the template DNA for PURE*frex*™

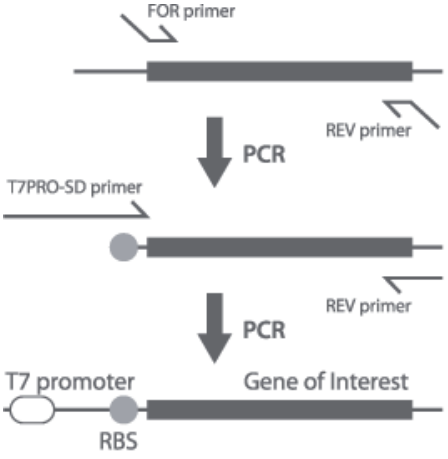

Table 2
Primers used for amplification of the template DNA

Primer	Sequence
FOR primer	5′-AAGGAGATATACCA-ATG-N(10-20)-3′ (RBS)
REV primer	5′-GGATTAGTTATTCA-TTA-N(10-20)-3′ (more than 10 any nucleotides)
T7PRO-SD primer	5′-GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC (T7 promoter) CCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCA-3′ (RBS)

Figure 5
Example of preparation of the template DNA using PCR

To generate template DNA using PCR product, overlap extension PCR can be used by steps shown in Fig.5 with primers shown in Table 2.