Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	031
Title	Cell-Free Expression Screening Through Overlap Extension PCR
Research Unit	Cell-Free Protein Product
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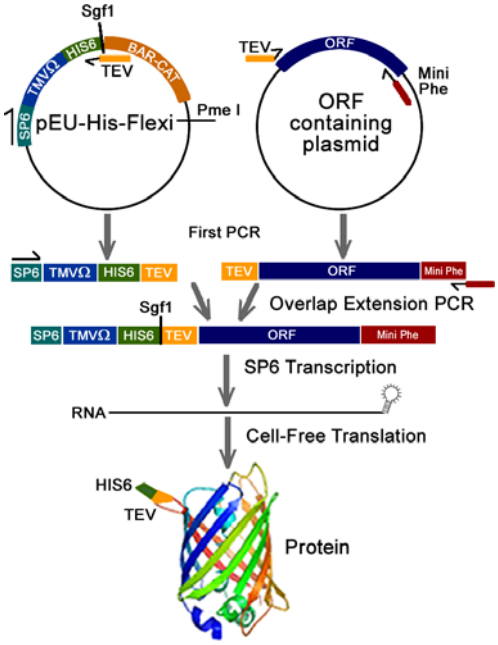

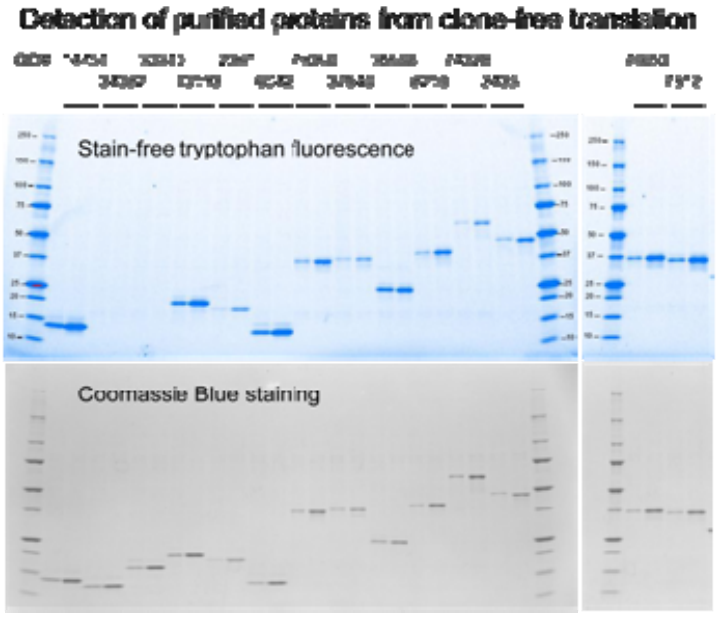

Template DNAs in transcription reaction were at 25 ng/ µL for PCR DNA or 200 ng/µl for plasmid DNA. Bi-layer 25 µL reaction/125 µL feeding buffer with WEPRO2240H. IMAC-purified samples were electrophoresed using 4-20% stain-free gels. The images by stain-free imager (upper) and CBB-stained gel (lower) are shown. Left lane: Overlap extension PCR with 3'-mini Phe construct. Right lane: Control, plasmid with ORF cloned in pEU-His-Flexi.

Summary

We have made significant progress toward implementing a clone-free translation protocol for screening new protein constructs. The protocol relies on overlap extension PCR to fuse two PCR products together. One of these products contains the SP6 promoter, the TMV omega translational enhancer, and the His6 tag from our pEU-His-Flexivector. The other PCR product contains the target ORF with the 3' extension mini-Phe. The mini-Phe forms a stem-loop structure in the RNA, which we found increases protein expression. The over-lapping sequence in these two PCR products is the TEV protease cleavage site. The resulting transcribed overlap extension PCR product achieves protein translation at ~20-30% of the level obtained from genes first cloned into the highly optimized pEU plasmid. Results obtained from study of proteins from the CESG control workgroup are shown above. This method will give a dramatic decrease in the time and expense required to test new gene constructs for the behavior of the translated membrane protein. This is because intermediate ligation, transformation, cell growth, and plasmid isolation steps will no longer be needed to perform screening experiments.

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