In vitro selection from designed protein scaffold library with Ribosome Display on PURE system

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Abstract

Ribosome Display (RD) on the Protein synthesis Using Recombinant Elements (PURE) system (PURE RD) was reported as a powerful method for in vitro selection of specific binders of proteins. In addition to RNAi-based RNA interference, other protein-protein interactions can be targeted through the RD method. We herein applied the PURE RD system to screen specific binders of Erk2, a cytoplasmic-membrane binding protein. Erk2 is involved in a variety of intracellular signaling pathways. In this study, we aimed to develop a new method for screening specific binders by using a PURE-based RD system. The PURE RD system has high potential compared with the conventional cell extract-based RD system. Our current research on the PURE system indicates that PURE RD has high potential to be a new standard method to screen the specific binders from the peptide and protein library such as scaffold library.

The comparison of the amount of contaminants in New and Old PURE system

The Comparison of activity of protein-synthesis between the New and Old PURE system

Model experiment of PURE RD selection with New and Old PURE system

Constitutional nuclear localization of endogenous Erk2 by the Erk2 binder containing NLS

Summary

1. We developed a new designed protein scaffold library based on the RNF8 FHA domain, and a number of Erk2 binders were selected from this library using PURE-based RD. Using this methodology, we selected binders that bound to endogenous target proteins in a strategic way by addition of NLS or NES (nuclear exporting signal) to RNF8 scaffold.

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