Exonuclease III-Based High Throughput Construction of DNA Templates for In Vitro Expression

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Abstract
High throughput gene expression analysis in generating wide scale analysis of protein-protein interaction (PPI) functional assays mapping, and protein array research. Methods were selected for generating DNA templates by using Exo-LMPCR. To test Exo-LMPCR, a panel of mammalian ORFS from Escherichia coli and human were used, preparation of expression ready DNA templates (ERTs) has been a major bottleneck in the high throughput construction of DNA templates. The first lane 1 DNA products are shown in A and B. C.

Introduction
Standard methods of generating proteins for functional studies involve traditional cloning and expression in bacteria as well as yeast or viral systems. In vitro translation protocols offer the advantage of generating large quantities of homogenous protein preparations, but the efficiency of obtaining functional protein complexes remains an ongoing challenge. In vitro transcription/translation is a powerful tool to study protein expression and function, but the presence of a transcription/translation system that will accept unlimited numbers of sequences is required to generate proteins to test in the cell, the absence of efficient cloning and isolation methods for proteins, and the limitations of traditional systems for generating a combinatorial array of proteins. In this system is an additional advantage that proving protein function studies.

For ERTs to be used in in vitro transcription/translation systems, the quality of the generated transcription/translation products (DOTs) is critical. High throughput 96-well plate technology was integrated with a 96-well plate based affinity capture and detection system to quantitate and confirm the expression of the protein in vitro. Over 95% of the ORFs assessed in the first round of assays were above the detection limit and the results in vitro analysis thus far has shown 100% accuracy in the adaptor/gene junctions. Exo-LMPCR eliminates concern of internal restriction sites that are problematic in other adaptor methods.

Results
Overview of Exo-LMPCR

Figure 1. Schematic diagram of in vitro transcription/translation products of the 93 Mammalian (A) and 33 Humanized (B) PPIs. The first lane 1 DNA products are shown in A and B. C. Detection of intracellular and extracellular proteins using monoclonal Anti-c-Myc-AP in ELISA format.

High Throughput Modeled Assembly of 93 Mammalian ORFs

Figure 2. Assembly of full-length and full-length with C-terminal tagged templates (A) and functional assay of the full-length protein expression and translation in a high throughput system with detection of N-c-Myc-I (B).

Table 1: High-throughput assembly of mammalian ORFs: High-throughput assembly assay as shown in the N-c-Myc-I ELISA assay. The second lane 2 DNA products are shown in A and B. C. Exo-LMPCR eliminates concern of internal restriction sites that are problematic in other adaptor methods.

Conclusions
- Exo-LMPCR provides a unique system whereby to directly insert multiple ORFs into adapter cells containing all elements required for in vitro transcrip-
  tion/translation. Exo-LMPCR eliminates concern of internal restriction sites that are problematic in other adaptor methods.
- Exo-LMPCR offers flexibility and allows for an array of target protein libraries to be generated.
- Use of this system allows researchers to go from cDNA to expressed protein in 7 days.
- The method offers an alternative and cost-effective means to generate protein libraries at a speed unachievable by existing technologies.

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References
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