博士論文（要約）

Ribosome display microarray for high-throughput quantitative screening of whole protein library

タンパク質ライブラリーの網羅的かつ定量的スクリーニングのためのリボソームディスプレイマイクロアレイ

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June 2016

Abstract

Proteins are the main biomolecular catalysts, structural elements and signaling molecules of biological cells and tissues. Function of gene can be known by analysing these biological proteins. Protein microarray technology is one of the method used to study proteins in a high-throughput mode. However, protein microarray technology has some difficulties such as the instability of immobilized protein molecules and unavoidable modification of template DNA. In contrast, the ribosome display method widely used to engineer proteins makes stable and functional protein for selection but the proteins are not spatially addressable limiting the application of this method to selection of protein based on qualitative analysis. To eliminate the above mentioned limitations, this study reports the development of the ribosome display microarray technology which integrates the conventional ribosome display technology with the microarray technology. By integrating these technologies, the disadvantages present when each of these technologies is applied individually will be amended by the intrinsic properties of the other method.

Several techniques were developed and tested in this study in order to successfully fabricate ribosome display microarray. Firstly, a new ribosomal stalling method that was suitable for stalling ternary complexes formed on solid surfaces was introduced. This new technique of stalling ribosomes using a covalent bonding generated by a photo-crosslinker was successfully demonstrated by fabricating a high density ribosome display microarray. The use of the new technique to stall ternary complexes has an added advantage over conventional ribosome display method where the native sequence of the starting DNA template does not have to be modified. The microarray was fabricated using the microintaglio printing method which produces a much higher density microarray when compared to other conventional protein microarray methods.

One of the requirements for a high-performance and quantifiable ribosome display microarray is a stable immobilization chemistry and a suitable solid surface for making the microarray. For this purpose, a comparative study of biomolecule immobilization chemistry on two types of solid surface, gold-coated substrate and APTES-functionalized glass substrate, was conducted to check suitability for on-chip protein screening. The stability of probe DNA molecules on gold-coated substrate and APTES-functionalized glass surface was tested against a range of temperature and pH, and it was observed DNA molecules immobilized on APTES-functionalized substrate were more stable than those immobilized on gold-coated substrate. Uniformity of DNA molecules immobilized on the substrate was also checked as this is an

important factor in determining accurate quantification of protein activity.

One of the advantage of generating the ternary complexes in a microarray format is it enables users to screen, analyze and select desired biomolecules on the same platform. Two methods was tested to recover genotype material from the microarray. The first method involves direct recovery of mRNA from the mRNA microarray surface. In the second method, DNA-bound magnetic beads that were generated using emulsion PCR and were used as a template to generate the mRNA array, was recovered instead. Both these methods were compared in terms of efficiency of recovery (signal-to-noise ratio), and the recovery of DNA-bound magnetic beads had no noise. Finally, the newly proposed ribosome microarray method displaying functional GFPuv4 which displays its native fluorescence upon synthesis was demonstrated.

Finally a comprehensive discussion on the merits and demerits of the proposed method as well as an idea for future work was presented. The merits are: (1) the use of DNA/RNA with native sequence and without any modification, (2) the applications of ribosome display method can be extended beyond the conventional screening of binding proteins, to include enzymatic assay, (3) enables direct functional quantification and comparison between different mutant proteins without any downstream processing such as sequencing, and (4) enables immediate collection the genotype material encoding the protein of interest. The demerits are: (1) the size of DNA library that can be screened in a single experiment is much larger in the conventional method (10^7 versus 10^{15}), (2) the proposed method requires the use of cell-free system without release factors when using DNA with stop codon, and (3) the cell-free system used in this study may not be able to synthesize difficult proteins and in some cases, are not able to carry out certain post-translation modification to proteins.