A Genetic Circuit for Directed Evolution in vivo

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Abstract

Directed evolution is a powerful tool for answering scientific questions or constructing novel biological systems. Here we present a simple genetic circuit for in vivo directed evolution which comprises minimal elements for random mutation and artificial selection. We engineer yeast to generate the DNA mutator hAID, an essential protein in adaptive immunity, and target it specifically to a gene of interest. The target gene will be mutated and consequently evolve. By linking target gene functionality with the expression of an auxotrophic marker and a selectable chromatic marker like LacZ or GFP, positive clones can be easily isolated. This circuit may be adopted for in vivo evolution in eukaryotic systems using any genetically encoded targets. It has a variety of potential applications in academic and industrial contexts.

Mutator-AID

AID (Activation-Induced Deaminase) is crucial to antibody diversity. Briefly, AID is an enzyme that can cause deamination of an amino acid group to a carbon, resulting in a Watson-Crick mismatch. DNA repair pathways then are activated to remove the mismatch, resulting in a changed coding sequence of the hypervariable region where AID is targeted.

Target-Gal4

Structure of Gal4-DNA complex shows Pro26 in a crucial position to maintain the turning of Zinc Finger. Gal4 Pro26 is thus essentially a null-mutant. Sequence analysis on C771 region based on hAID mutagenesis hotspot suggests hAID cannot reverse the mutant at this site. Thus, all functional Gal4 derivatives must be novel gain-of-function mutants on other sites.

Advantages

We engineered a simple genetic circuit which directs in vivo gain-of-function mutagenesis on the target gene, as well as simultaneous qualitative functional assay for the target. A proof of concept experiment is performed by targeting hAID-LexA DBD fusion protein to 3'TUT of Gal4 CDS. Gain-of-function mutants were obtained from only 3 days of in vivo evolution. We conclude that in vivo directed evolution of protein is possible. Using yeast as a heterologous host, we can express, mutate, and perform functional assay on most eukaryotic proteins as well as RNA. Our yeast platform also reduced the cost of mutagenesis and assay compared to in vitro directed evolution or as well in vivo evolution like immortal B cell-based system. The assay is even simpler than yeast-two-hybrid and very robust, that it could be performed in small labs.

Perspectives

Qualitative assay such as His' phenotype could be used for initial phenotypic screen. To perform quantitative functional assay, we aim to include a "breake" component such as LacI expression under control of UAS, thus Gal4 function is inversely correlated with LexA-LexA expression. By including the "breake" component and quantitative LacZ assay, we may be able to perform progressive, asymptotic evolution of a target gene. We envision the current system could be used for de novo screening of protein interacting partners, such as small peptide ligand of transcription factor or HDAC, gain-of-function screen for antimicrobial peptide, as well as loss-of-function screen of protein-protein interaction such as searching for the interacting motif between a kinase and its substrate. Saturating mutagenesis by large scale evolution, one might be able to obtain enough information of essential structural motif for protein-protein interaction for structure-function analysis, which would be useful for drug development.

Design

The expression of human Activation-Induced Deaminase (hAID) is controlled by a lacI repressible promoter. Gal4, a yeast transcription factor, is adopted as the target to demonstrate our principle. In order to ensure specific targeting, hAID is fused with LexA DNA-binding domain, which recognizes and binds to a unique DNA sequence LexO, placed downstream of Gal4.

For yeast screening process, two different reporters are used. His3 is used to report the viability of yeast strain. Functional Gal4 is able to induce the expression of His3, which enables the yeast strain to survive on the His' medium. Another reporter gene lacZ will enable us to use blue and white screening based on X-Gal plate.

Results

Over-expression of fusion hAID with LexA DBD directs gain-of-function mutagenesis in target gene.

Left panel: After 3 days' culture in liquid His' medium, expression of hAID-LexA directs gain-of-function mutagenesis as selected on the His' plate.

Right panel: Example plate of hAID-LexA expressing strain and control strain, both were originally harboring Gal4C771T. Western blot confirmed hAID-LexA expression in the responding strain. Higher molecular weight bands are probably ubiquinated hAID-LexA.

H-2d - LexA DBD mutagenic activity on target gene depends on DNA binding and protein – DNA tertiary structure.

Left panel: A low level expression promoter pADH-LacI enables examination of H-2d-LexA activity with various linkers in between the fused domains.

A titration of linkers showed that low level expression of H-2d-LexA did not induce mutagenesis on the target Gal4 gene. Insertion of a 7aa linker GSGSHHG enhanced the mutagenesis efficiency, and another 7aa linker FGSHHG further increased it. Longer 8aa linker did not increase mutagenesis efficiency. Such results indicate that DNA-binding and protein-DNA structure configuration are essential for efficient mutagenesis.

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