Mutation Rate as a Selection Mechanism in Continuous Evolution of Bacteriophage

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Abstract

Engineering novel proteins via continuous evolution of bacteriophage currently requires the host bacteria to be transformed with two additional functions: A mutagenesis vector to provide an elevated rate of viral mutation, and a selection mechanism to increase the production of infectious progeny [1]. I propose using a selection mechanism which simply modulates the rate of mutation by producing a mutagenesis suppressor from the desired activity of the evolving protein. Improvements in the desired activity will result in a higher percentage of faithful copies of the parent genome in the succeeding generation. A baseline mutation rate will ensure that our mechanism to lower the mutation rate will not result in stagnation, but even a small percentage of true copies of the

As the evolving protein approaches a high level of desired activity, the proportionally lowered mutation rate will ensure that more viral progeny contain exact copies of the parent genotype.

produce more exact copies of the approach a baseline rate which will allow for

There is perhaps a precedence in nature for an evolutionary selection mechanism that works soley by allowing an individual to have more clonesexact copy progeny would which are exact copies

Introduction

M-Selection

1 Introduction

Continuous evolution of bacteriophage has become a potent protein engineering tool. For example, PACE - Phage Assisted Continuous Evolution described by Esvelt,Carlson and Lui.

The continuous evolution process produces a viral genome containing a gene which has undergone many generations of mutation and selective pressure for a particular property. The generality of this approach to protein engineering is only limited by our ability to create a selection mechanism for the desired property and insert an expressable initial form of the gene into the virus.

Specifically, the PACE system requires:

- A modified viral genome missing a gene required for infectious progeny.
- A modified host genome/plasmid to induce an elevated mutation rate.

- A modified host genome/plasmid to provide a selection mechanism to provide the missing gene product in proportion to the desired activity of the evolving gene.

Effectively, the number of infectious progeny is proportional to the activity of the selection mechanism. In principle, if the current mutation of the gene produces a product with none of the desired activity, none of the infectivity protein will be produced by the host, and there will be no infectous progeny. This process involves a continuous flow of host cells through a reaction vessel and so any genotype producing less than two infectious virions per host will be washed out.

M-Selection is proposed to the selection mechanism by combining it with the mechanism for elevated mutation.

The benefits of M-Selection may include:

The virus is not crippled, but only has an additional gene or domain, similar to phage display.

The nominal (high) mutation rate can be chosen to guarantee that there will be a minimum number of non-mutant copies produced for every infection. This percentage of clones will then increase as the mutation rate is reduced.

For example, if the selection mechanism stimulated by the desired activity will consist of a repressor for the production of mutagenic polymerase II-. Positive selection is the result of reduced mutation, resulting in more clones of the selected virus relative to its mutants.

As far as we know, modulation of the mutation rate within a particular host has not been exploited in this way. This form of albeit un-natural selection is referred to as M-selection, whereby the selection mechanism consists of reducing the rate of mutation acting on a particular instance of the viral genome precisely because it is having some desired behavior.

Figure 1 shows how the combined mutagenic and selection plasmid takes the place of the AP and MP in PACE.

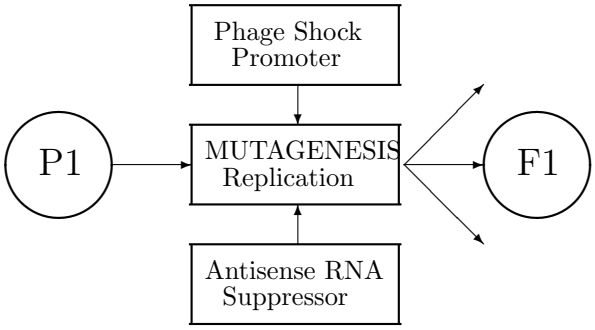

Fig 1. M-selection Plasmid

1.1 Difficulties of working with crippled Virus genome

$$\mathrm{P_Y} = \underbrace{H(Y_n) - H(Y_n|\mathbf{V}_n^Y)}_{S_Y} + \underbrace{H(Y_n|\mathbf{V}_n^Y) - H(Y_n|\mathbf{V}_n^{X,Y})}_{T_{X\to Y}}, \quad (1)$$

Multiplicity of Infection

$$\mathrm{P}\,(x) = \frac{e^{-\lambda}\lambda^{x}}{x!} \tag{2}$$

Materials and Methods

Components of Statistical Analysis

Percentage of activated genes due to Phage Shock promoter. Number of polII- (mutagenic polymerase) transcripts * error rate due to mutagenic polymerase.

Error rate of normal polII Error rate of polII-

Number of phage particles produced np(0-6 min) = 0 (six minutes before any phage appear), then production between 75-200 per hour.

Initial single-strand (virion) DNA production occurs in non-mutagenic environment, then mutants appear along with polII- production, until suppression of polII- occurs due to binding. Binding ? anti-sense RNA transcription ? polII- repression ? reduction in mutations.

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Results

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Discussion

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Conclusion

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Supporting Information

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