Title
Characterization of mutant red fluorescent protein (AsRed2)

Author(s)
Huang, Junjie

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Characterization of Mutant Red Fluorescent Protein (AsRed2)

Introduction

- Commercially available red fluorescent protein
- Employed as a transcriptional reporter in Escherichia coli in multi-species background
- Inadequacies observed when cloned in E. coli:
  1. Low fluorescence intensity;
  2. Inconsistent expression level

Methods

- Fluorescence measurement of E. coli cultures using Infinite™ M200 multi-mode monochromator-based microplate reader (Tecan).
  - Excitation/emission wavelengths: 570nm/600nm; gain setting: 95.
- Plasmid extraction from mutant AsRed2 (Miniprep) → DNA sequencing → Sequence alignment with wildtype AsRed2 and analysis

Results and Discussion

A. Discrepancy of fluorescence properties of the same mutant AsRed2 under different promoters

- Observation: The same mutant AsRed2 performs differently relative to wildtype when fused downstream of different promoters.
- Inference:
  - Change in amino acid sequence of the mutant protein is not the sole determinant of the change in fluorescence properties.
  - Compatibility between the promoter and the AsRed2 gene probably plays a role.

B. Effects of point mutations on fluorescence properties

- Observation: Substantial changes in fluorescence properties were observed even with no or only one amino acid change.

Next Steps

Fluorescence properties

- Mutations:
  - Amino acid changes
  - Codon usage changes
  - Mutations before the open reading frame (may affect mRNA stability)

Table 1. Point mutations and corresponding amino acid changes in selected mutants

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Open Reading Frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H</td>
<td>962 T to C</td>
</tr>
<tr>
<td>7</td>
<td>966 T to A</td>
</tr>
<tr>
<td>4C</td>
<td>978 T to G, 992 G to A, 1001 A to C</td>
</tr>
<tr>
<td>2D</td>
<td>962 T to G</td>
</tr>
<tr>
<td>L110hA</td>
<td>982 G to A</td>
</tr>
<tr>
<td>1048 C to A</td>
<td>Silent</td>
</tr>
<tr>
<td>992 G to A</td>
<td>Silent</td>
</tr>
<tr>
<td>1001 A to C</td>
<td>Silent</td>
</tr>
<tr>
<td>1066 C to T</td>
<td>1288 C to T</td>
</tr>
<tr>
<td>1211 Y to F</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Comparison of fluorescence intensity between wildtype and mutant AsRed2 in 24h cultures. Plasmids containing wildtype or mutant AsRed2 gene fused downstream of 4 different promoters were introduced into E. coli. The promoterless AsRed2 served as negative control. Data shown are mean ± SD of 25 replicates.

Figure 2. Comparison of Fluorescence intensities between wildtype and a series of mutant AsRed2 at different time points. Both wildtype and mutant genes are fused downstream of promoter P_{PA1/04/03}. Data shown are means of replicates.