<table>
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<tr>
<th><strong>Title</strong></th>
<th>Characterization of mutant red fluorescent protein (AsRed2)</th>
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<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Huang, Junjie</td>
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<td><strong>Citation</strong></td>
<td>Huang, J. (2009, March). Characterization of mutant red fluorescent protein (AsRed2). Presented at Discover URECA @ NTU poster exhibition and competition, Nanyang Technological University, Singapore.</td>
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<td><strong>Date</strong></td>
<td>2009</td>
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<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10220/8996">http://hdl.handle.net/10220/8996</a></td>
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Characterization of Mutant Red Fluorescent Protein (AsRed2)

Introduction

- **AsRed2**
  - Commericially 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
  - Ultimate objective:
    To improve the fluorescence intensity and expression reproducibility of AsRed2 to suit laboratory applications
  - Previous work:
    A library of mutant AsRed2 was generated by random mutagenic PCR.
  - Aim of this project:
    To characterize selected mutants from the library in order to develop suitable applications of the mutant AsRed2

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.

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

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Open Reading Frame</th>
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<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>
</tbody>
</table>

Next Steps

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