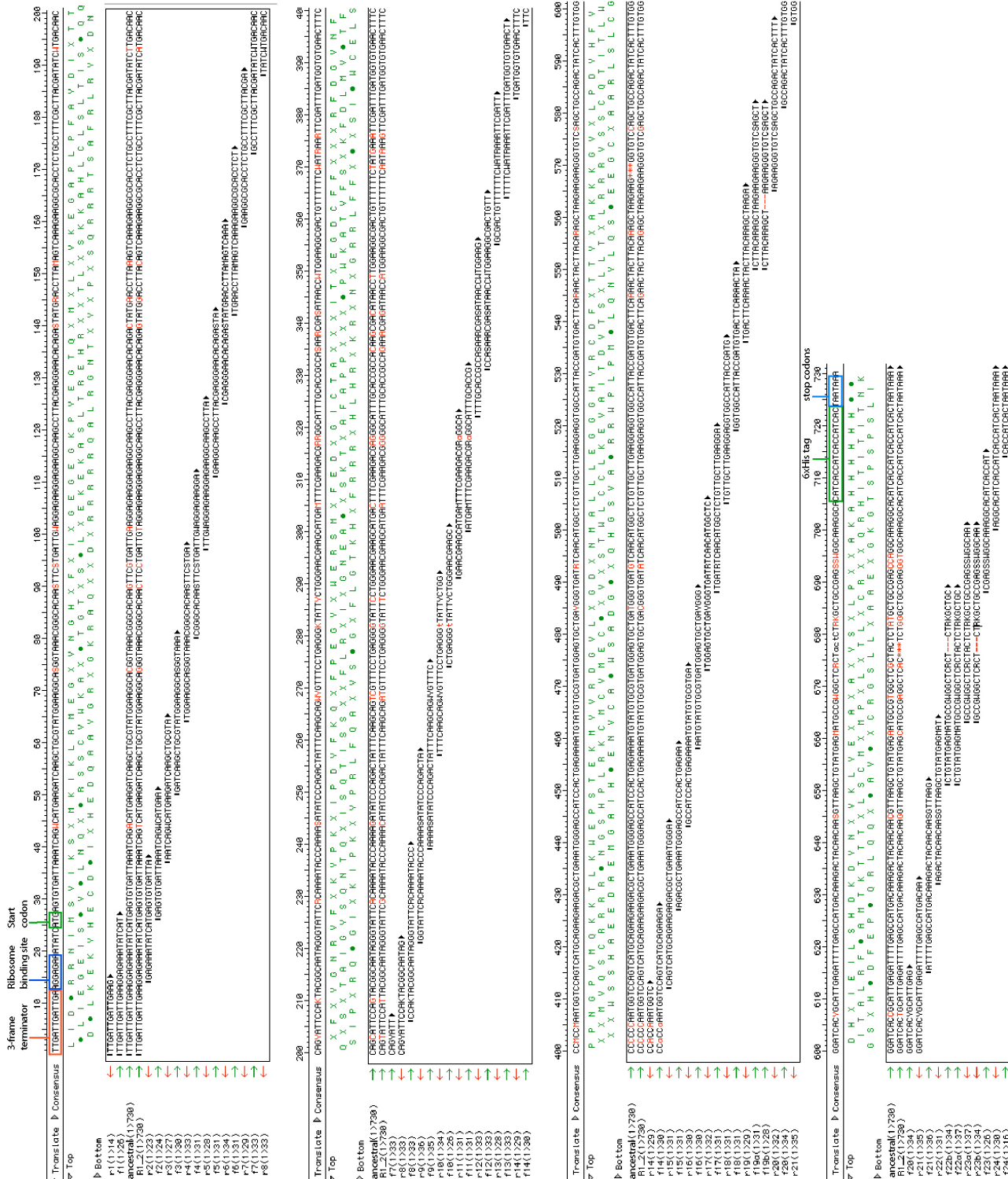
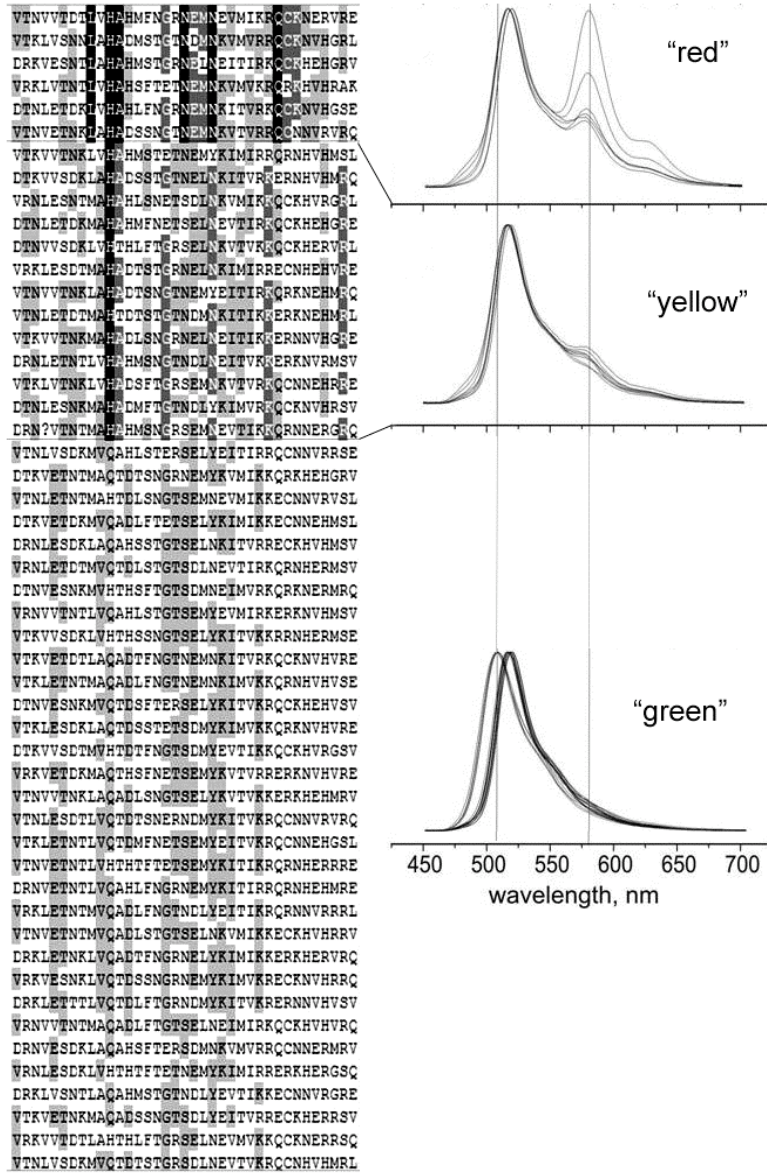


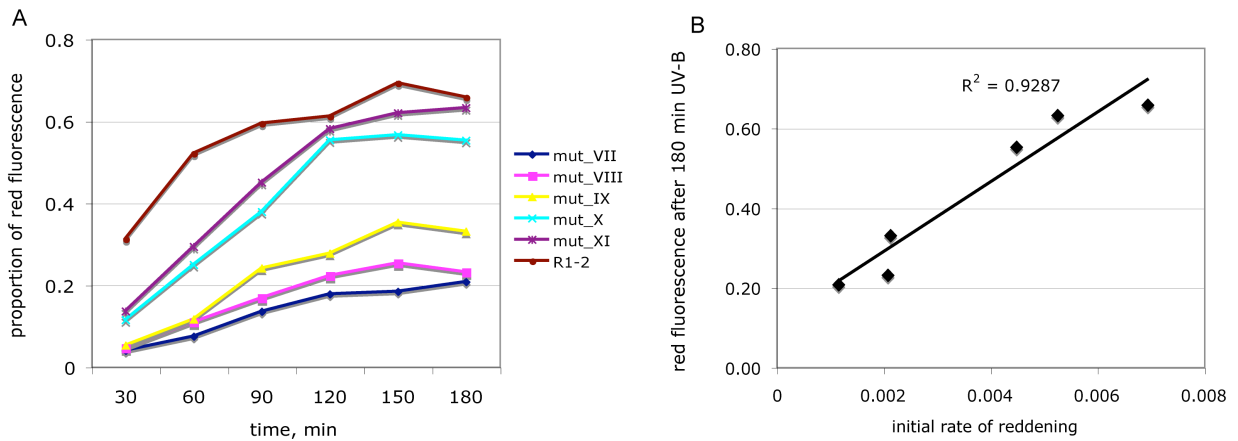
**Field and Matz 2009: Evolution of red fluorescence in GFP-like proteins from Faviina corals**  
 required a dozen mutations. **Supplementary Info**



**Figure S1.** Strategy for constructing a bacterial expression library of evolutionary intermediates. The figure shows the alignment of the ancestral protein with the extant R1-2, their consensus with translation, all the oligonucleotides used for degenerate gene synthesis, and the regulatory elements that were introduced to facilitate bacterial expression.



**Figure S2.** States of variable sites and fluorescence spectra of the subset of clones obtained from the original combinatorial library and used for association analysis. All spectra were recorded after 60 min exposure of expressing bacterial colonies to UV-B light. In the association analysis, the frequencies of site states in pooled “red” and “yellow” groups were compared to the “green” group (see Table 1). 19 “yellow-red” clones and 32 “green” clones are shown. In the complete association study, frequencies of site states in 28 “yellow-red” clones were compared to 67 “green” clones.



**Figure S3:** Maturation of the mutants from Fig. 2 A-B upon exposure to low intensity UV-B (“blacklight”). A: increase of proportion of red fluorescence in the total fluorescence spectrum of expressing bacterial colonies upon UV-B exposure. Horizontal axis: exposure time in minutes; vertical axis: proportion of red fluorescence, determined via multiple regression analysis. B: correlation between the slope of initial reddening (over the first 60 minutes, see panel A) and the maximal red fluorescence achieved over 180 min.