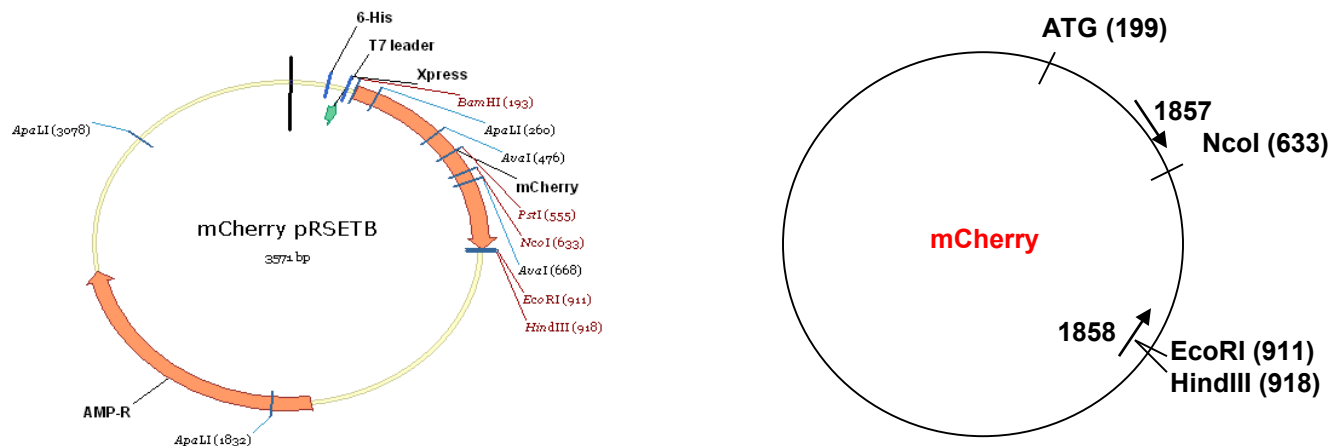


## Supplementary Figure 2.

### 1) Remove stop codon at C-terminus of mCherry sequence

Supplementary Fig. 2. Removal of C-terminal stop codon in mCherry sequence in mCherry-pRSETB vector. See Materials and Methods for explanation.



Forward primer #1857 (bp 610-632)

Reverse primer #1858 (bp 924-891) – removes stop codon (TAA → TAC) and inserts extra bp's to separate EcoRI and HindIII restriction sites

		<b>EcoRI</b>	<b>HindIII</b>	
<b>Original mCherry</b>	891	5' -GGACGAGCTGTACAAG	<b>TAA</b> GAATTTCGAAGCTTG-3'	924
<b>sequence:</b>	891	3' -CCTGCTCGACATGTTTCATTC	TTAAGCTTCGAAC-5'	924

		↓ <b>EcoRI</b>	<b>HindIII</b>	
<b>Stop codon removed</b>		5' -GGACGAGCTGTACAAG	<b>TAC</b> GAATTCTCCCTGGAAGCTTG-3'	
<b>and bp's inserted:</b>		3' -CCTGCTCGACATGTTTCATGCTTAAGAGGGACCTTCGAAC-5'	← <b>#1858</b>	

#### Instructions:

- PCR out the mCherry fragment using primers #1857 and #1858
- Gel purify the PCR fragment
- Digest both the PCR fragment and the mCherry-pRSETB with NcoI and HindIII
- Ligate the PCR fragment into the digested mCherry-pRSETB
- Sequence to determine that the stop codon has been removed