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Correction to Light-Inducible Recombinases for Bacterial Optogenetics

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After publication, a reader alerted us to a mistake in the sequence of the Opto-Cre-Vvd construct, where we had erroneously placed a stop codon between the Vvd photodimer and the cCre sequence. Although, in principle, the design should not work with the stop codon in place, in practice the construct does function well, as described in the original manuscript. Our hypothesis is that in the original construct, even minimal stop codon read-through between Vvd and cCre produces sufficient Vvd-cCre to allow for recombination. In the original design, we had Opto-Cre-Vvd on a high copy number plasmid (ColE1 origin).

We removed the stop codon from between Vvd and cCre and transferred the system to a low copy number plasmid (SC101 origin). We denote this new design Opto-Cre-Vvd-2. We have repeated all the key results from the original manuscript with Opto-Cre-Vvd-2, including light-induction time courses for DNA excision and RFP expression at the single-cell and population levels (Figure 1). In all cases, the performance of Opto-Cre-Vvd-2 is nearly identical to the Opto-Cre-Vvd results described in the original manuscript.

We note that we also tested the construct with the stop codon between Vvd and cCre removed on a high copy number plasmid (ColE1 origin). This variant does exhibit blue light inducible recombination but has impaired growth and recombination relative to the Opto-Cre-Vvd and Opto-Cre-Vvd-2 designs (Figure 2a,b). Another variant with cCre removed entirely showed no recombination (Figure 2c).

The original Opto-Cre-Vvd system works as described in the manuscript and can continue to be used as is; however, moving forward we suggest users consider working with Opto-Cre-Vvd-2 as an alternative. Both plasmids are available on AddGene: https://www.addgene.org/Mary_Dunlop/.

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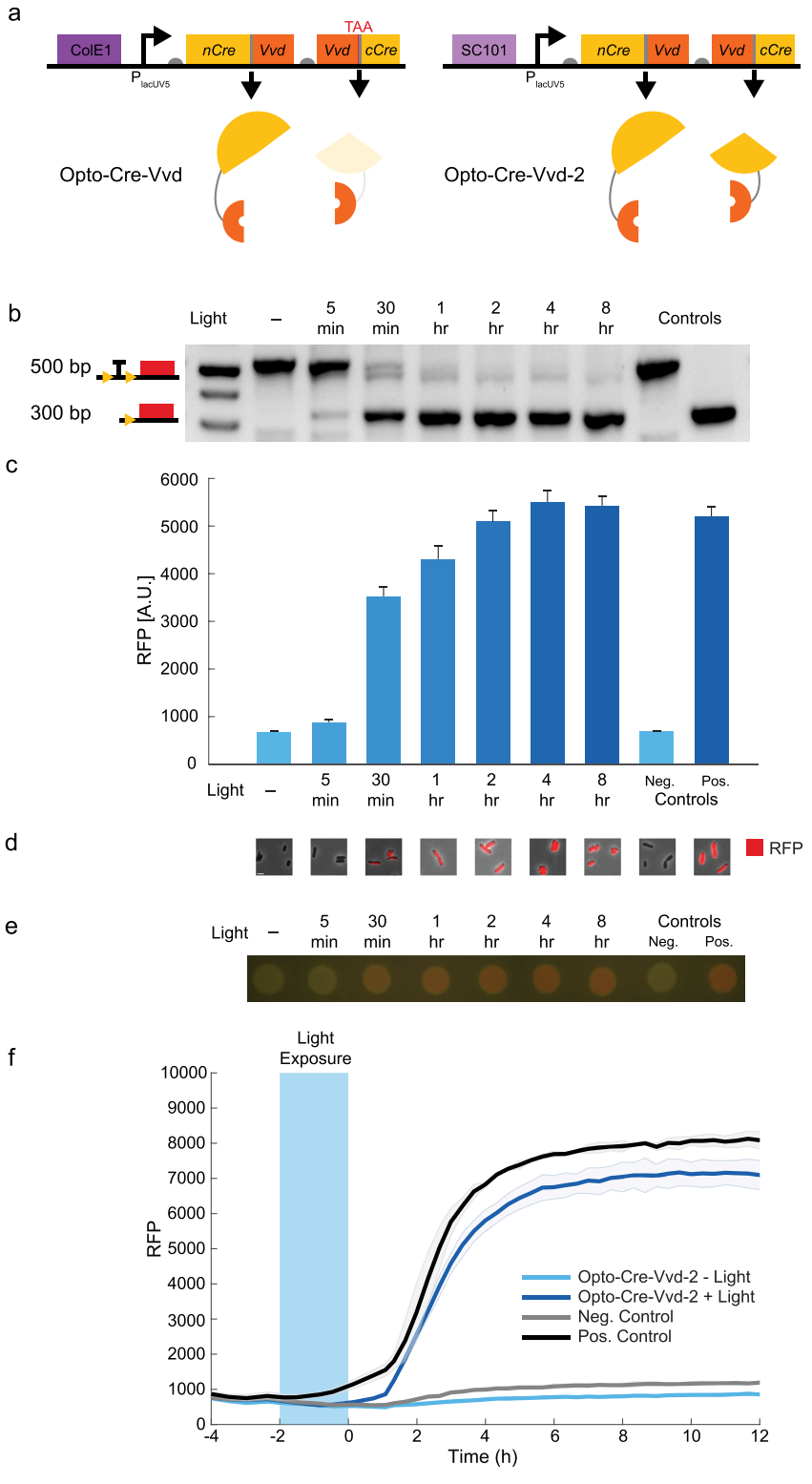

Figure 1. Opto-Cre-Vvd-2 performance with blue light induction. (a) Opto-Cre-Vvd has a stop codon between Vvd and cCre and is on a high copy number plasmid (ColE1). Opto-Cre-Vvd-2 does not have the stop codon and is on a low copy number plasmid (SC101). (b) DNA gel image showing reporter bands with and without transcription terminator excision. (c) Single-cell fluorescence microscopy averages of RFP values. Error bars show standard error around the mean ($n \approx 100$ cells per sample). (d) Representative microscopy images (scale bar = 2 μm). (e) Samples of culture spotted on agar plates of Opto-Cre-Vvd-2 exposed to different durations of blue light. (f) Opto-Cre-Vvd-2 activation in real-time, with the blue bar indicating light exposure. Shaded error bars represent standard deviation around the mean from plate reader data ($n = 3$ wells).

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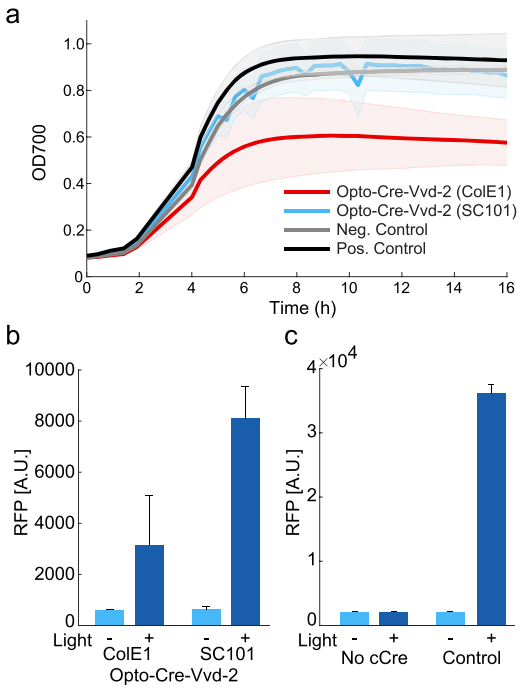

Figure 2. (a) Growth curve data for cultures with reporter and Opto-Cre-Vvd-2 for the ColE1 or SC101 origin of replication, no plasmid, or the reporter plasmid only. Shaded error bars show standard deviation around the mean from plate reader data (n = 3 wells). (b) RFP values of Opto-Cre-Vvd-2 on the ColE1 or SC101 origin plasmids grown overnight after a 2 h light exposure. (c) RFP values for the Opto-Cre-Vvd construct without cCre grown overnight after a 2 h light exposure. Error bars show standard deviation around the mean from plate reader data (n = 3 wells).

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