Cre Stoplight with Living Colors® is a faster, brighter reporter for Cre recombinase.

Drago A Guggiana-Nilo¹, Anne Marie Quinn²§, Thomas E. Hughes ¹

¹Department of Cell Biology and Neuroscience, Montana State University, Bozeman MT 59715
²Montana Molecular, LLC, Bozeman MT 59715
§Corresponding author

Email address:
Anne Marie Quinn: amq@montanamolecular.com
Introduction

Cre recombinase is a powerful and widely used tool for manipulating the genome. The enzyme targets areas of the chromosome that are flanked by specific loxP recognition sites and can either delete genes, or release new gene expression in a transgenic animal. Cre can introduce mutations that can be specifically activated (the inducible knockout), or targeted to specific cells. Many new genetic strains of mice have become available that either express Cre recombinase in particular cells and tissues, or that harbor the loxP sites that Cre recognizes (1).

The Cre enzyme is a 38kD recombinase that recognizes a 34 bp sequence, the loxP site, in the target DNA. By a series of staggered cuts, recombination occurs between the two loxP sites and the enzyme releases the DNA. When the loxP sites are positioned in opposite orientations, the intervening DNA is inverted, and when the sites are oriented head to tail, the DNA in between is excised. Cre is powerful because the reaction it catalyzes is robust, it does not require additional proteins, it can manipulate large regions of the genome, and it can be targeted to specific cell types (2). The ability to detect exactly which cells have been manipulated by Cre, in any experimental setting, is essential to verifying and interpreting Cre-mediated events.

Using color as a reporter

The Cre Stoplight plasmid switches color to report Cre activity by conditionally expressing two different fluorescent proteins (3). The original reporter uses the red fluorescent protein DsRed (4) followed by a transcription terminator. These two elements are flanked by loxP and loxH sites, which are followed by
eGFP (enhanced Green Fluorescent Protein). Cells transiently transfected with only the original Cre Stoplight produce red fluorescence. However, if Cre is also expressed, DsRed is excised, along with the transcription terminator, and eGFP is expressed. The loxP and variant loxH sites make this reaction irreversible (5). The original Cre Stoplight has been widely used since it was published (6-8) and we recently reengineered the plasmid to use Living Colors® fluorescent proteins.

**Cre Stoplight with Living Colors® is brighter, faster and more useful.**

We improved the original Cre Stoplight by 1) using optimized fluorescent proteins that mature quickly and produce strong fluorescence, 2) swapping the arrangement of the fluorescent proteins, 3) removing superfluous elements of the plasmid. The original Cre Stoplight expresses a tetrameric DsRed (9) in the absence of Cre. The arrangement of red as the default and green as the Cre indicator limits the utility of the original reporter in the context of cell lines and animal strains that already contain a green fluorescent protein (10). To address this, we inverted the order of the fluorescent proteins such that green fluorescence would be expressed in cells without Cre, and red would indicate functional Cre. After testing a series of fluorescent proteins, we identified Zsgreen1 (4) and Mcherry (11) to be the brightest combinations in this expression context. The original Cre Stoplight also contained a variety of arbitrary tags, cloning sites, and vector sequence that had accumulated during the history of the plasmid. We removed all of these extra elements to create a more compact and efficient reporter system.
Conclusions

A new and improved version of the Cre Stoplight reporter is now available that is brighter, faster and more compatible with current techniques. Cre Stoplight with Living Colors® employs ZsGreen1 and mCherry to yield bright fluorescence 24 hours post transfection. This new version of the original reporter detects Cre activity both qualitatively and quantitatively in living cells when co-expressed with the Cre enzyme.

References


---

**Figure 1. Cre Stoplight 2.4 with Living Colors® is a brighter option to the original Cre Stoplight.**

Above: DNA map of the improved Cre Stoplight.

Below: testing Cre Stoplight 2.4. 500 ng of the Cre Stoplight 2.4 were co-transfected into HEK 293 cells with varying amounts of the Cre expression vector, and imaged at different times after transfection. A: Control experiment, no Cre expression vector added, 24 hours after transfection; B: 100 pg of Cre
expression vector added, 24 hours after transfection; C: 1 μg of Cre expression vector added, 24 hours after transfection; D: 1 μg of Cre expression vector added, 48 hours after transfection.