Two state differentiation program: Figures booklet.

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These accompanying f gures illustrate the proposed system at several levels of abstraction. Figures 1.0, 1.1, and 2 show the system at the device level. Each box represents a device present in the engineered cells. Figures 1.0 and 1.1 do not represent the actual spacial arrangement of the devices, but rather what devices interact with each other and in what way. Arrows indicate a signal that results in activation of the device pointed to, and horizontal lines indicate an inhibitory signal. Figure 2 shows the spacial arrangement of the devices on three plasmids within an envZ- strain of E.coli.

Figures 3,4,5, and 6 represent this system at the parts level of detail, showing the three cassettes that comprise the circuit, with each part labelled with common name and biobrick registry number. Promoters are green arrows, ribosome binding sites are green triangles. Protein coding sequences are represented as blue ovals and protein products as smaller blue circles. The double terminator is shown here as a red octagon. Key protein products are indicated, as is the phosphorylation status of Cph8 and OmpR. Active transcription is indicated by an arrow over the parts being transcribed. Translational regulation is not used in this system so for simplicity in this diagram the process from gene to protein has been shortened not to include the mRNA step, proceeding from transcribed gene to protein. Figure 3 represents the system in it's quiescent state, while the cells are being grown and plated and before exposure to 660nm light. Figure 4 shows the state of the system during stimulation of undifferentiated cells with 660nm light. Figure 5 shows the state of the system during stimulation of undifferentiated cells with Xylose. Figure 6 shows the state of both cell types post differentiation.

Figure 7 is a graphical representation of the assembly steps required to create the accessory protein generator/inverter cassette.

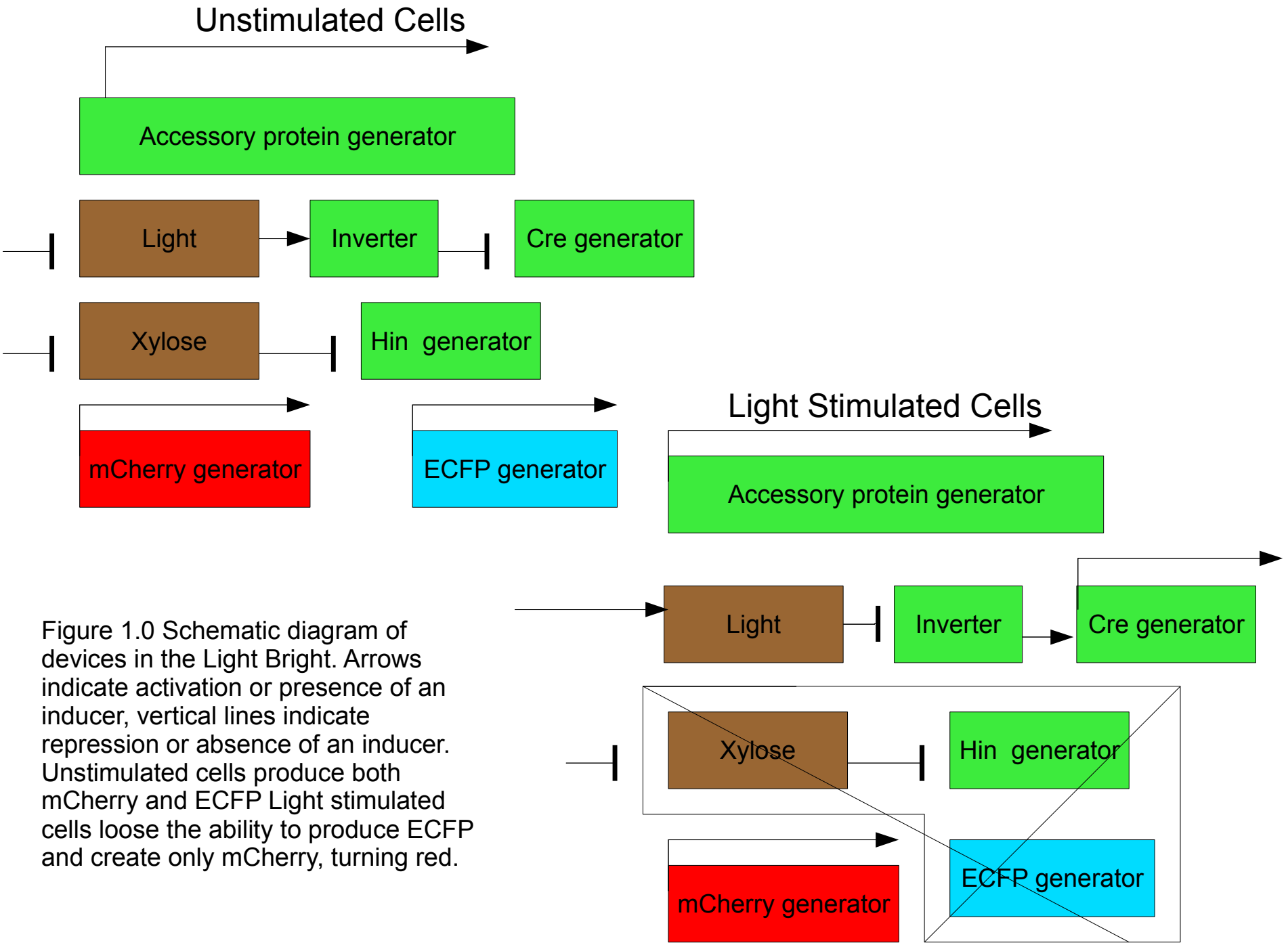
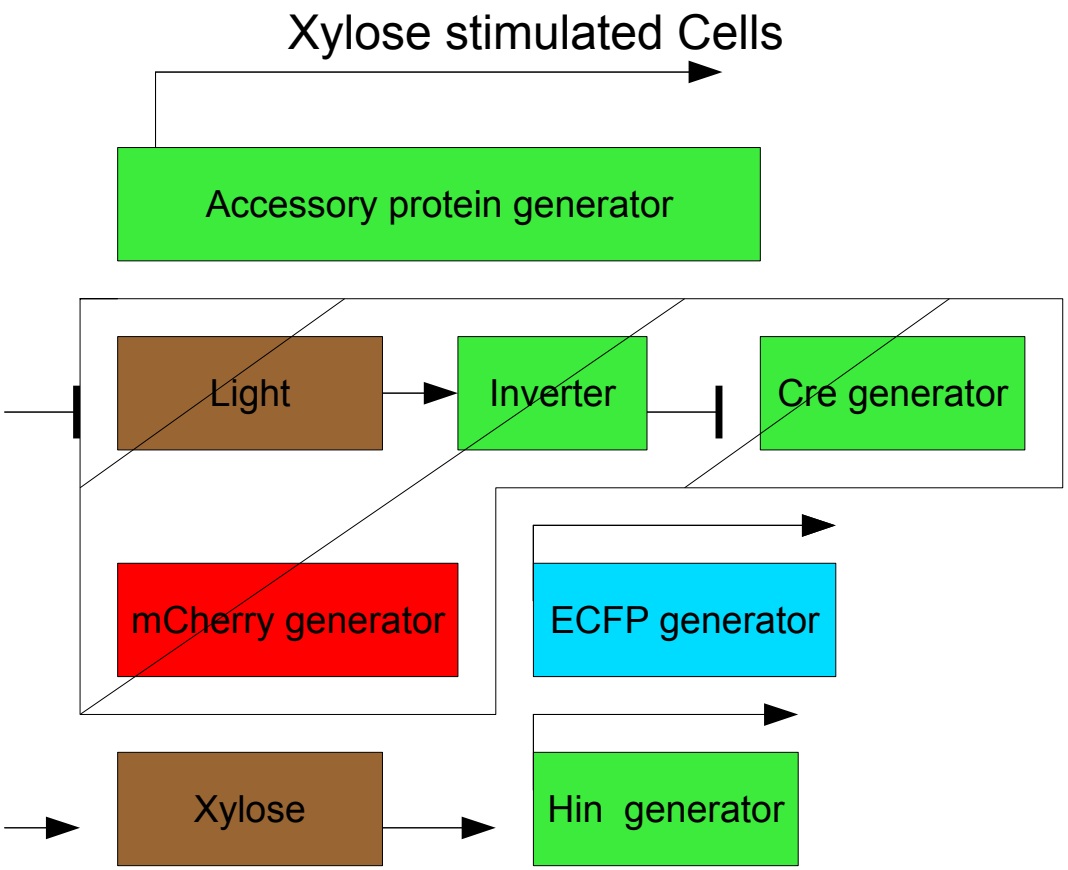
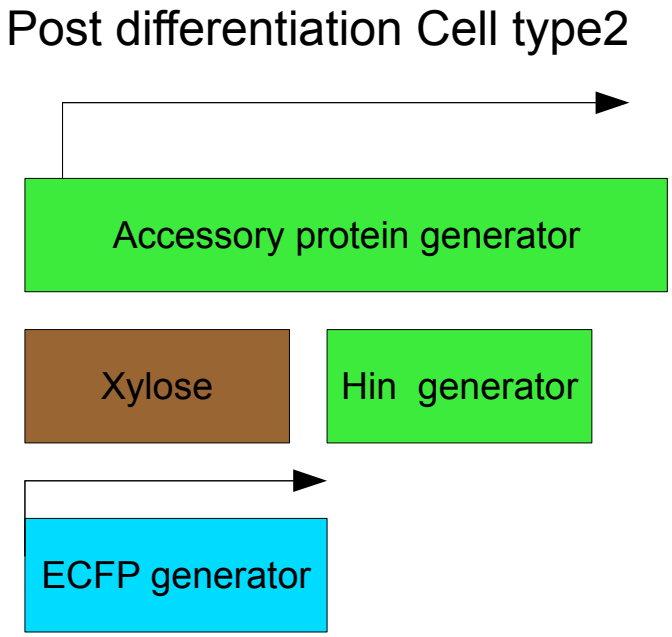


Figure 1.0 Schematic diagram of devices in the Light Bright. Arrows indicate activation or presence of an inducer, vertical lines indicate repression or absence of an inducer. Unstimulated cells produce both mCherry and ECFP Light stimulated cells loose the ability to produce ECFP and create only mCherry, turning red.





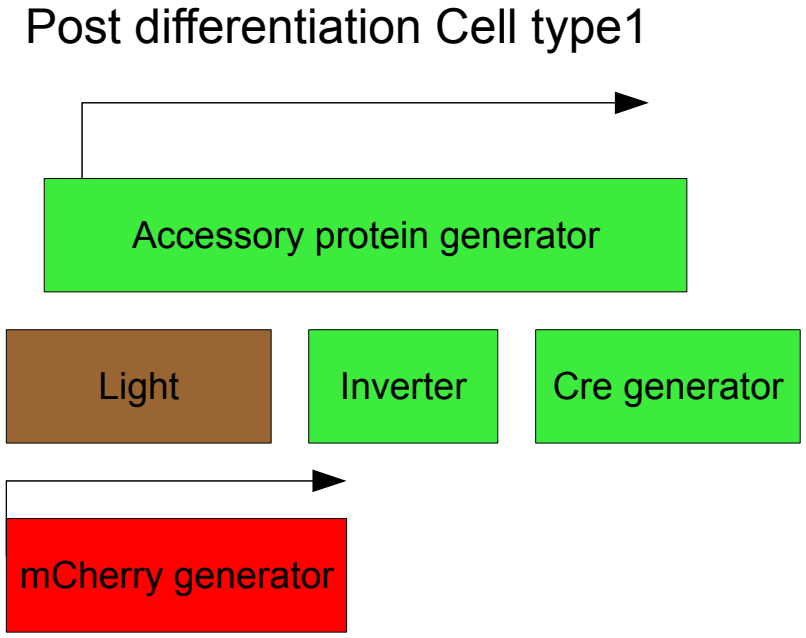


Figure 1.1. Schematic diagram of devices in the Light Bright continued. Cells not previously stimulated by light are sensitive to Xylose. Adding Xylose causes these cells to loose the ability to produce mCherry and produce only ECFP, turning blue.
Post differentiation, every cell on the plate is either blue or red, and cannot change its colour.

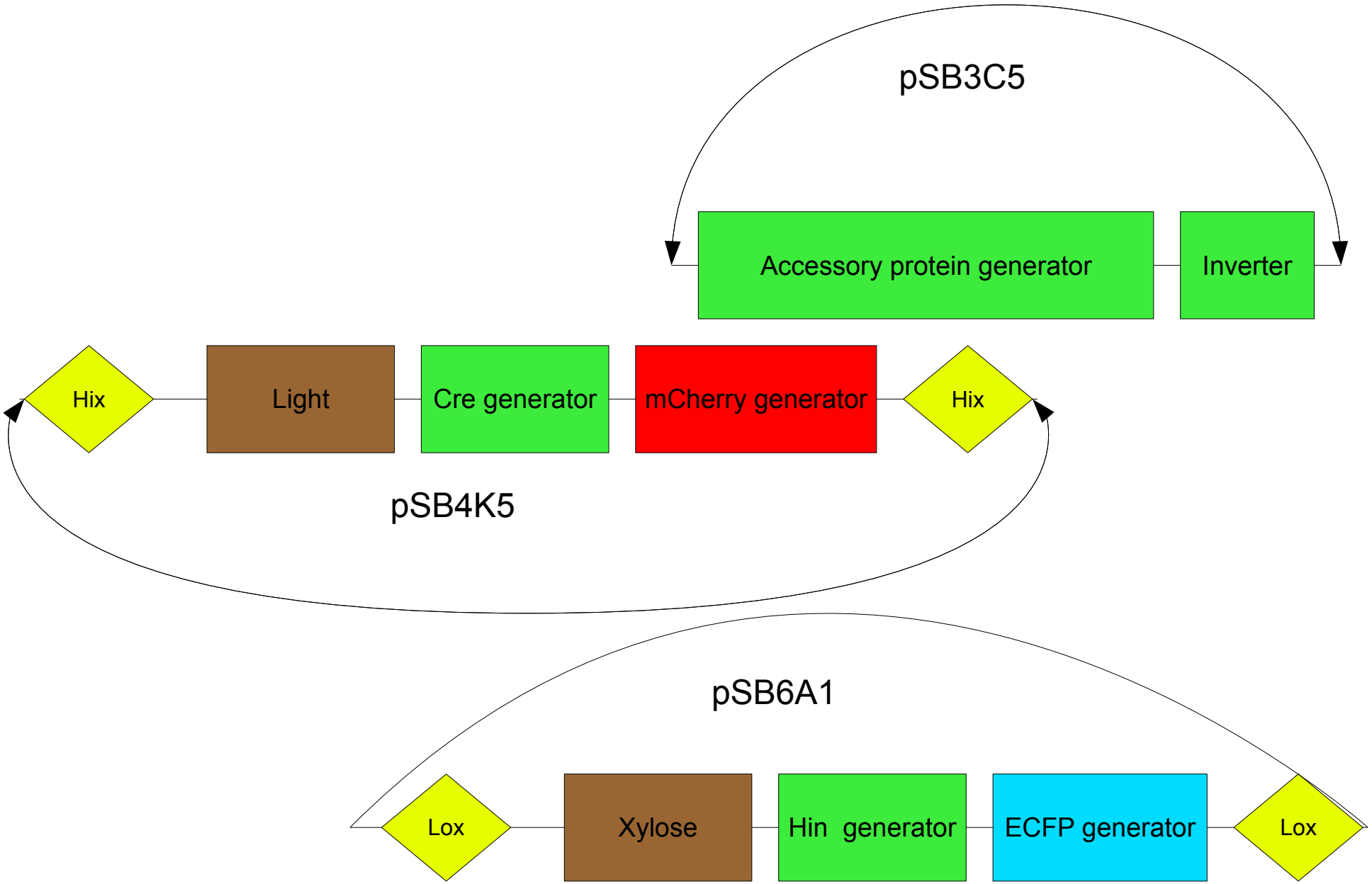


Figure 2. Diagram depicting the physical order of devices on the three plasmids in the final construct *E.coli.*

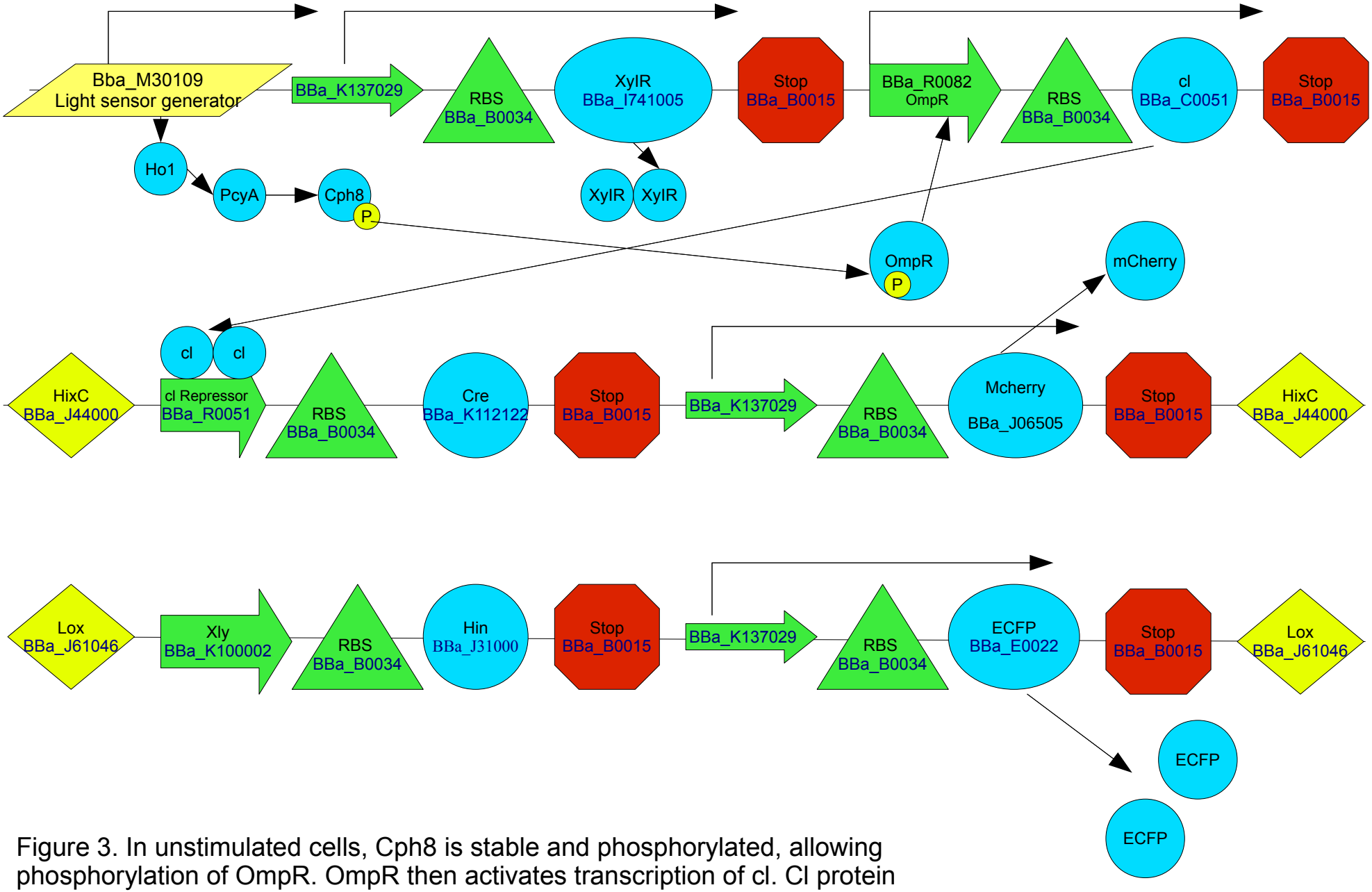


Figure 3. In unstimulated cells, Cph8 is stable and phosphorylated, allowing phosphorylation of OmpR. OmpR then activates transcription of cl. Cl protein represses Cre transcription. The Xly promoter is inactive as XylR is not bound to xylose. Both ECFP and mCherry are produced.

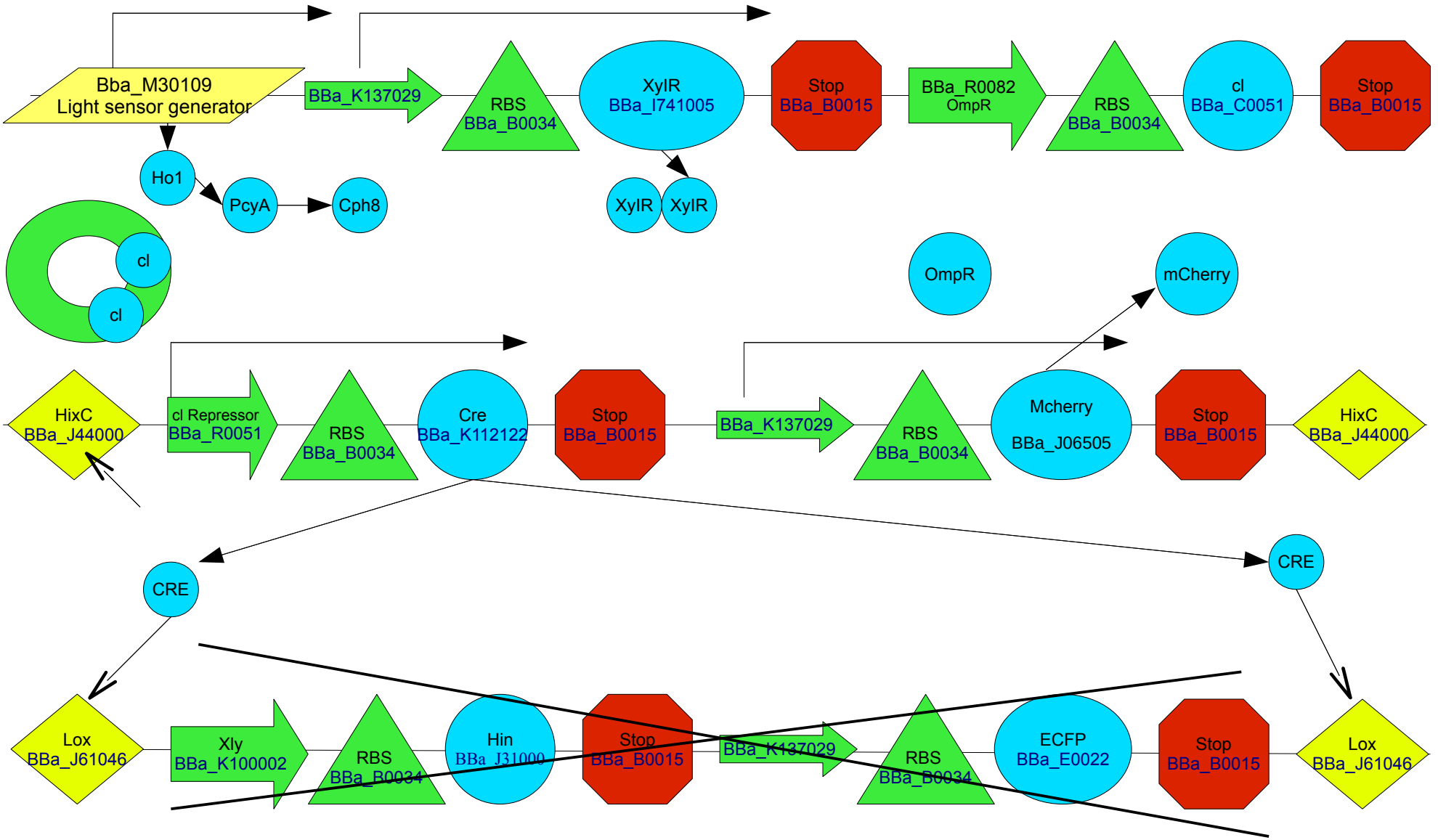
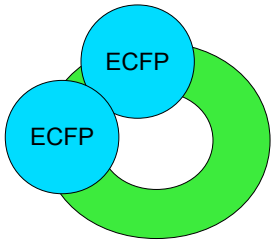


Figure 4. In cells stimulated by light at 660nm the EnvZ domain of Cph8 is unstable and cannot phosphorylate OmpR. Transcription of the cl repressor is halted, cl is degraded and the cl promoter can activate transcription of Cre. Cre protein splices out the ECFP and Hin generators. Excess ECFP protein is degraded, and mCherry production is not affected. The stimulated cells become red.



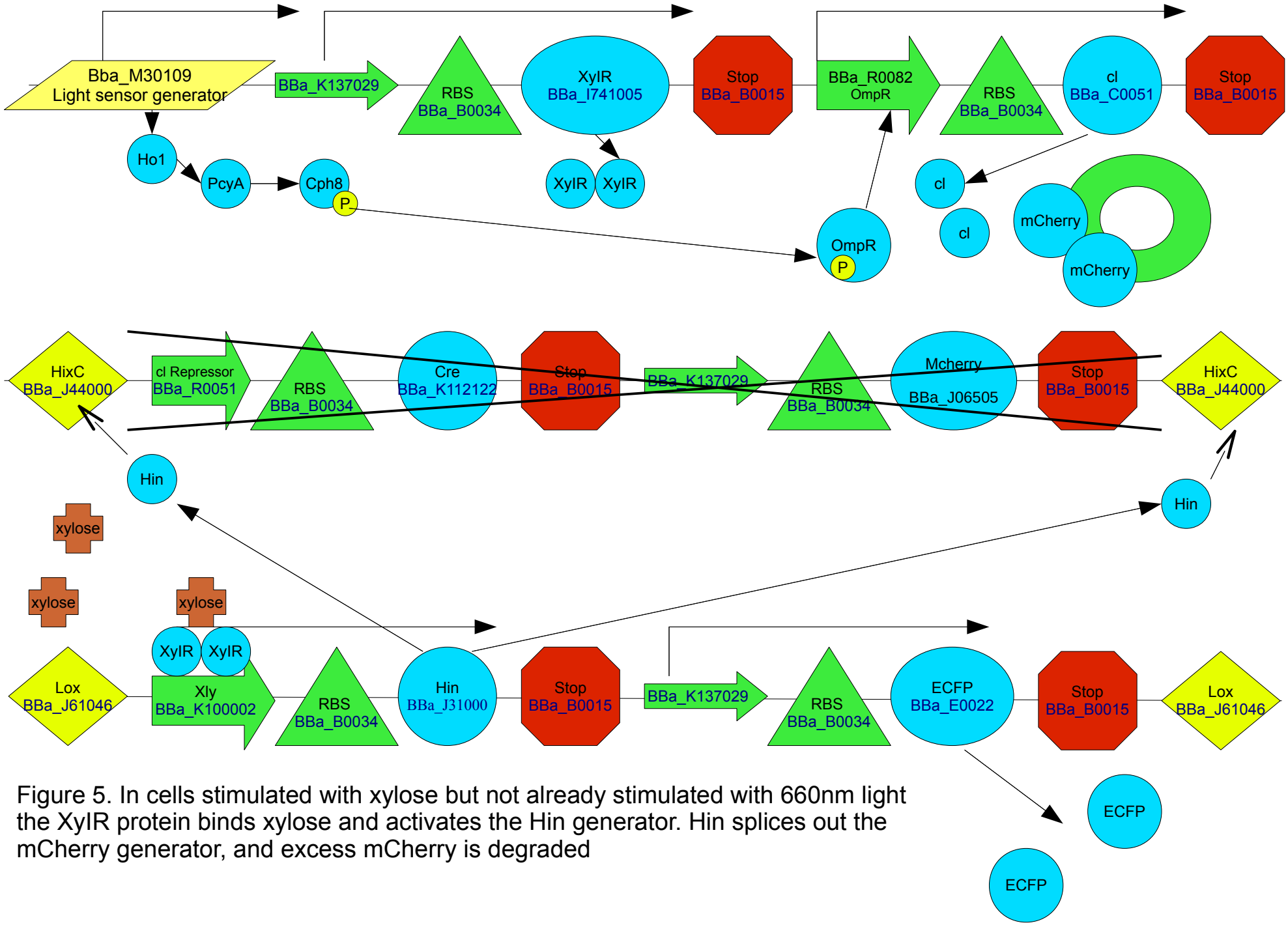


Figure 5. In cells stimulated with xylose but not already stimulated with 660nm light the XylR protein binds xylose and activates the Hin generator. Hin splices out the mCherry generator, and excess mCherry is degraded

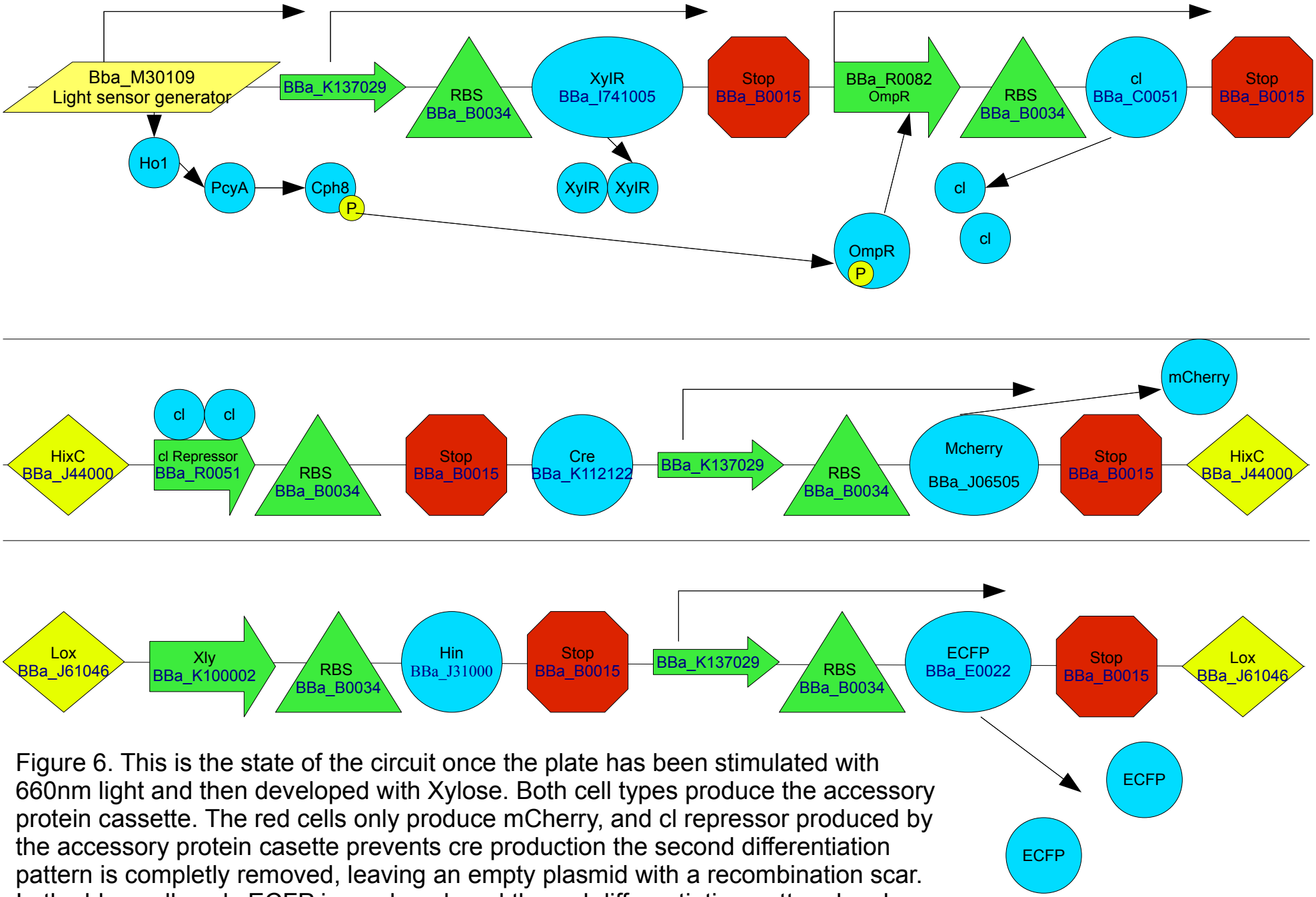


Figure 6. This is the state of the circuit once the plate has been stimulated with 660nm light and then developed with Xylose. Both cell types produce the accessory protein cassette. The red cells only produce mCherry, and cl repressor produced by the accessory protein casette prevents cre production the second differentiation pattern is completly removed, leaving an empty plasmid with a recombination scar. In the blue cells only ECFP is produced, and the red differentiation pattern has been removed. If there is still xylose in the media Hin would still be transcribed, but that shouldn't be a problem.

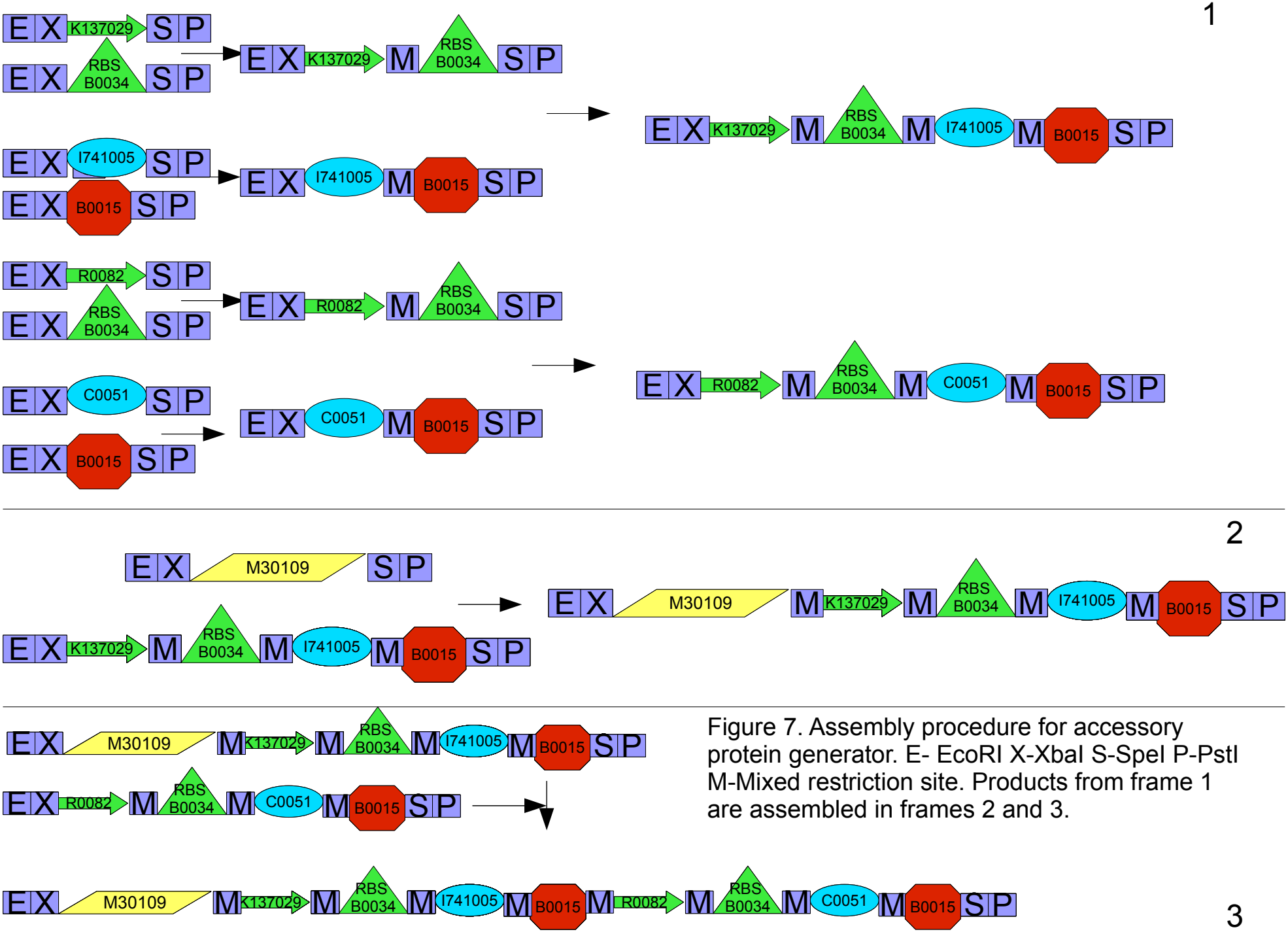


Figure 7. Assembly procedure for accessory protein generator. E- EcoRI X-XbaI S-SpeI P-PstI M-Mixed restriction site. Products from frame 1 are assembled in frames 2 and 3.