Spatial patterning and hardwired memory: engineering biomolecules as cellular input / output devices

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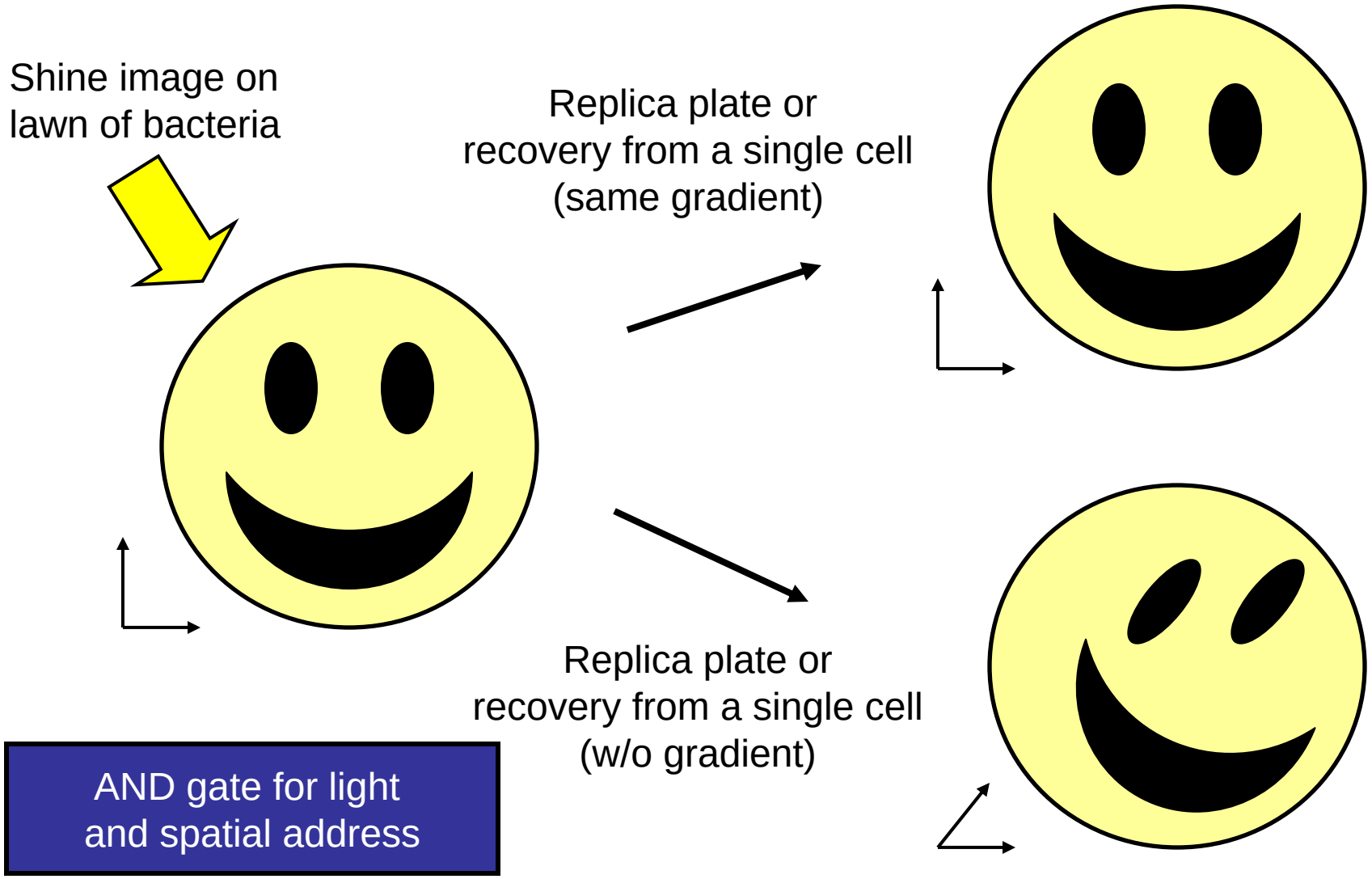
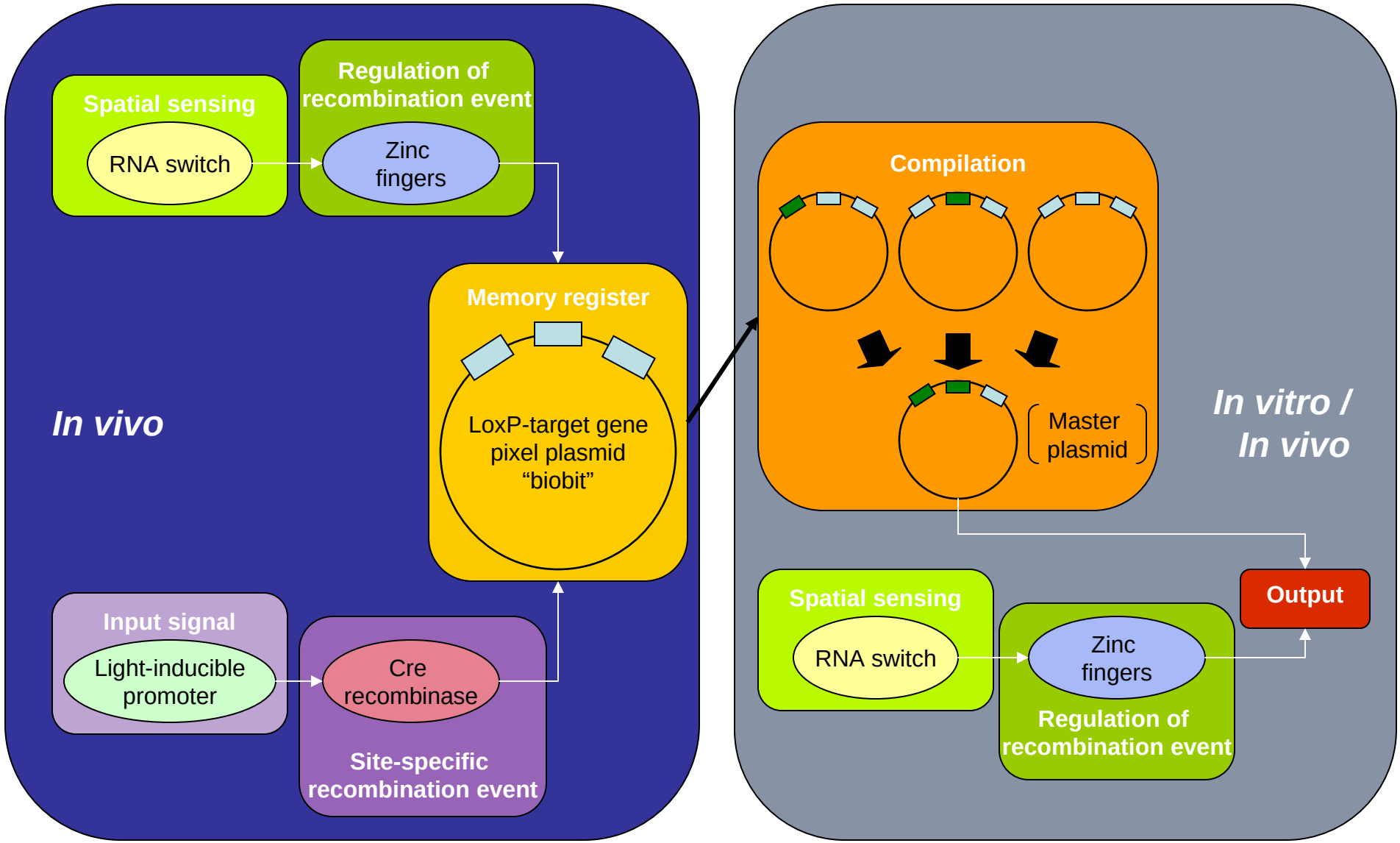
Project goal: biological memory and spatial patterning systems

- Memory and spatial patterning are key cellular behaviors
- Design-oriented approach to construct and characterize a synthetic biological circuit that will integrate spatial addressing with signal input
- *Selective recombination* is needed for multi-dimensional hardwired memory in cells
- *New molecules* that can function as programmable input / output devices are needed for advanced cellular engineering applications

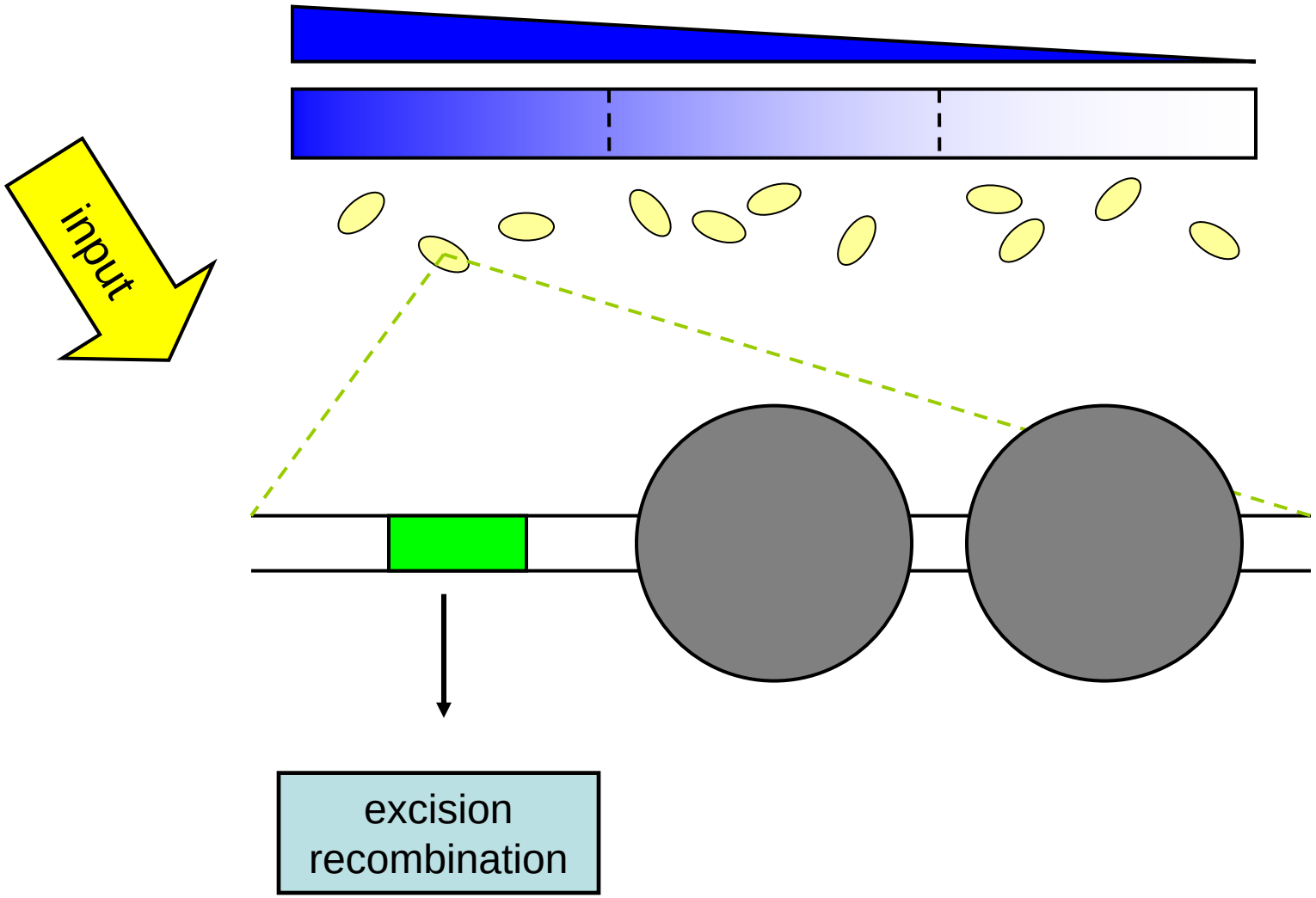
Project goal: biological memory and spatial patterning systems



Schematic of a biological memory system



A three pixel system: integrating light and spatial information



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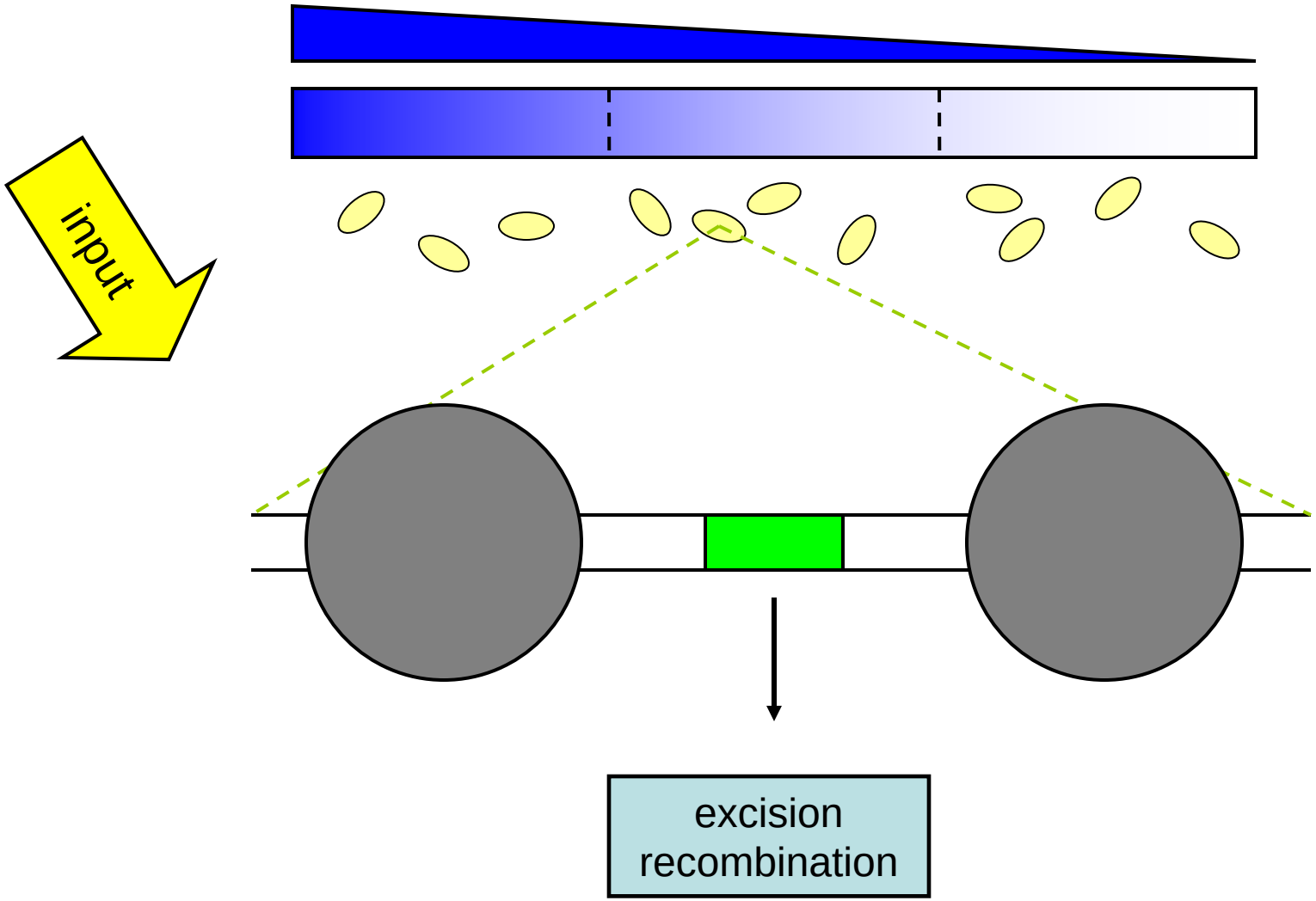
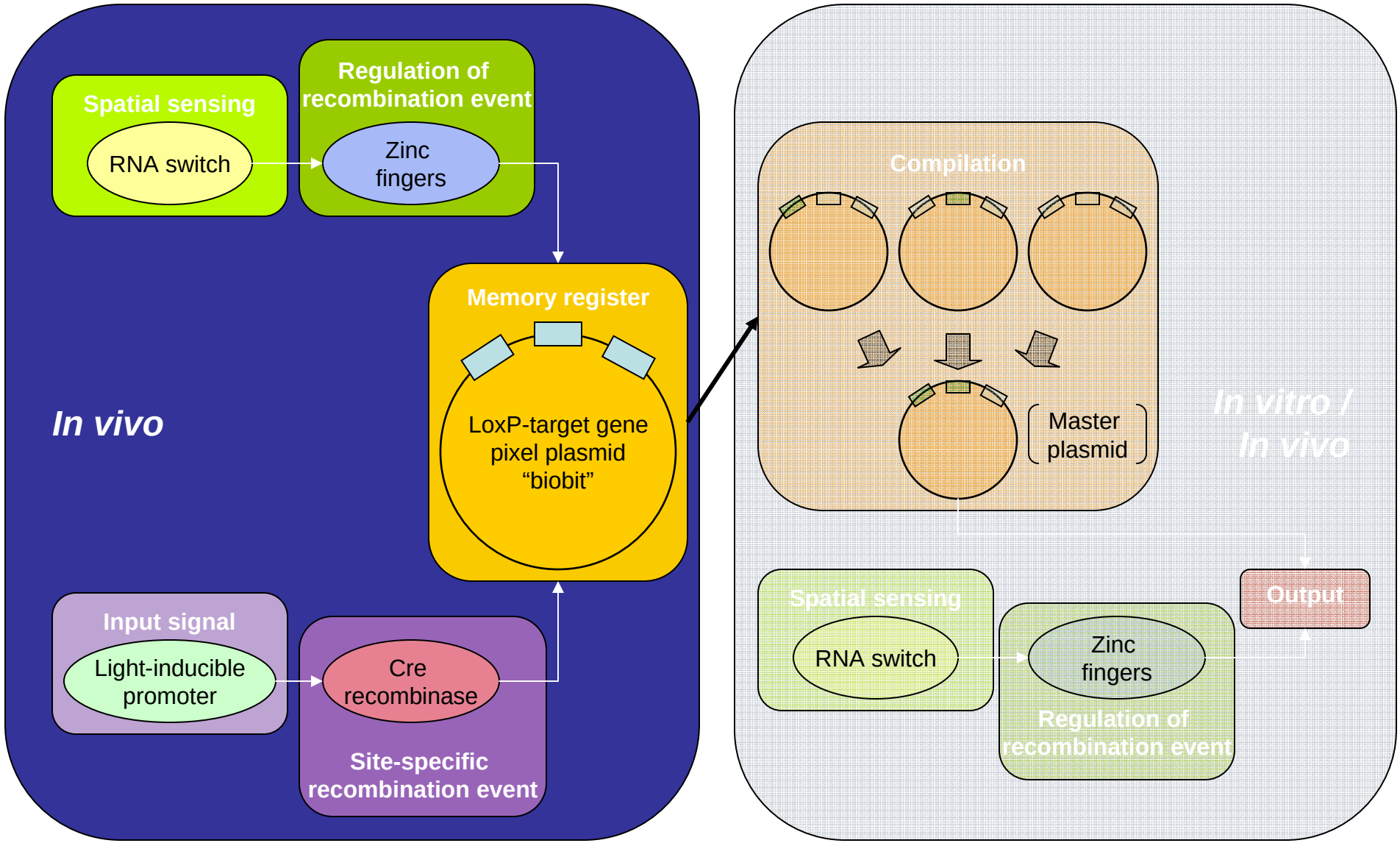
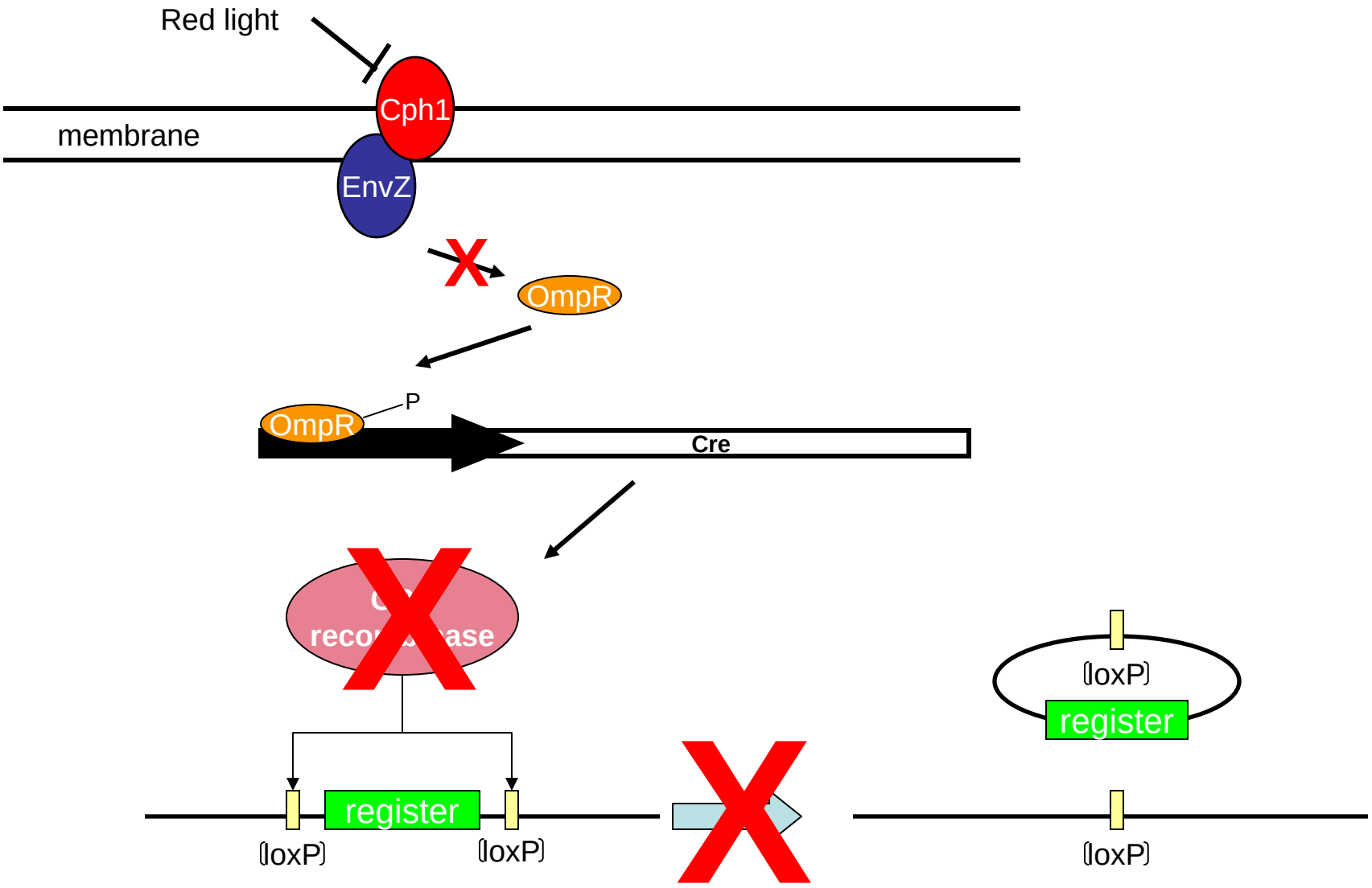


Light memory system



Light regulatable site-specific recombination

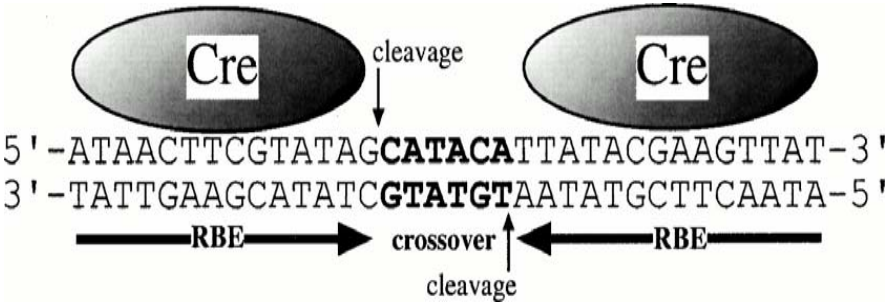


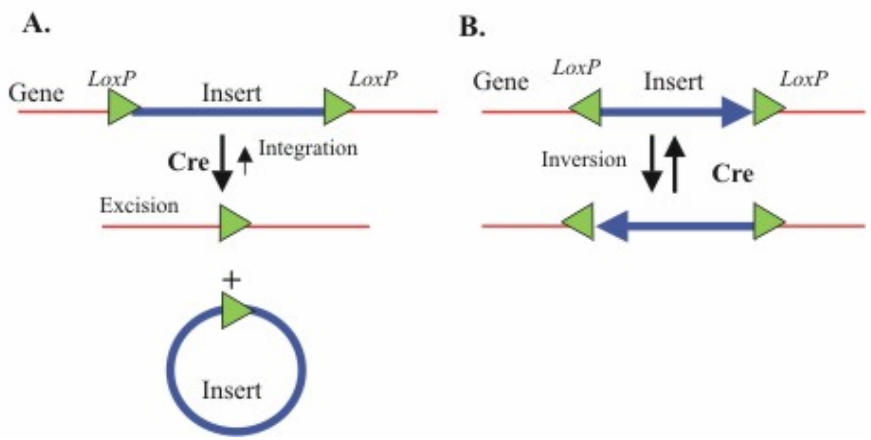
A Levskava, AA Chevalier, JJ Tabor, ZB Simpson, LA Lavery, M Levy, EA Davidson, A Scouras, AD Ellington, EM Marcotte, CA Voigt. 2005. Synthetic biology: engineering *Escherichia coli* to see light. *Nature*, 438: 441-2.

Site-specific recombination system: Cre/LoxP recombination

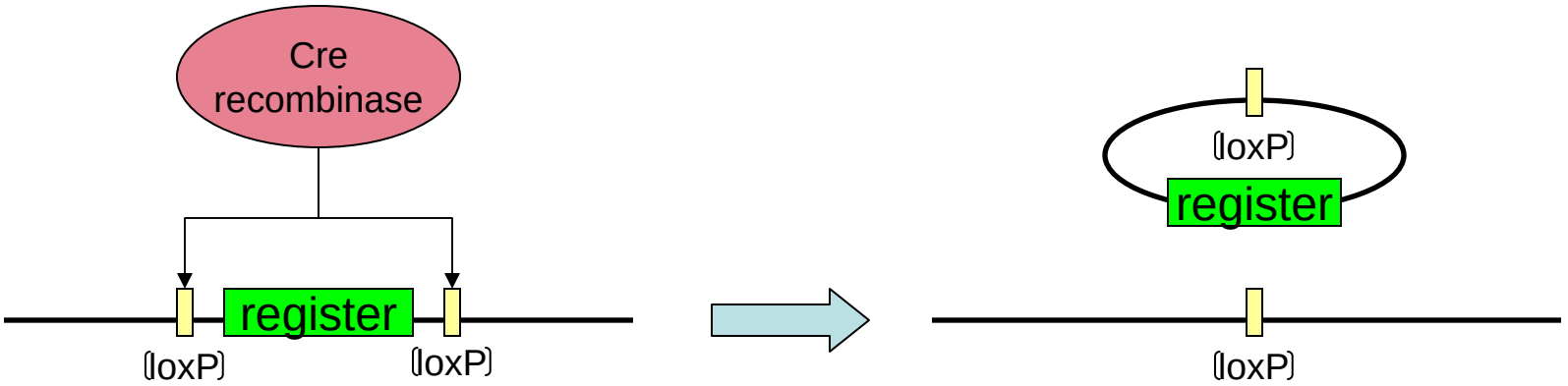
- Cre recombinase promotes recombination at 34-bp loxP sites, composed of two 13-bp recombinase binding elements (RBEs) arranged as inverted repeats flanking a 8-bp asymmetric crossover region which defines the directionality of the site

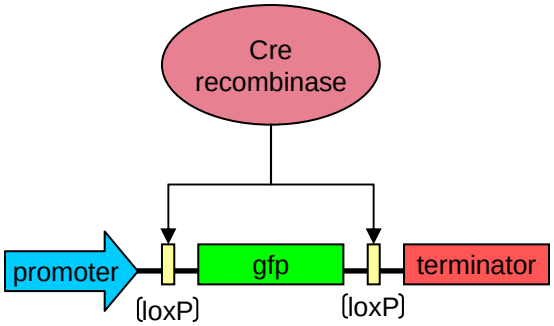
- Direct tandem repeats of loxP result in excision (A), while inverted repeats cause inversion (B)

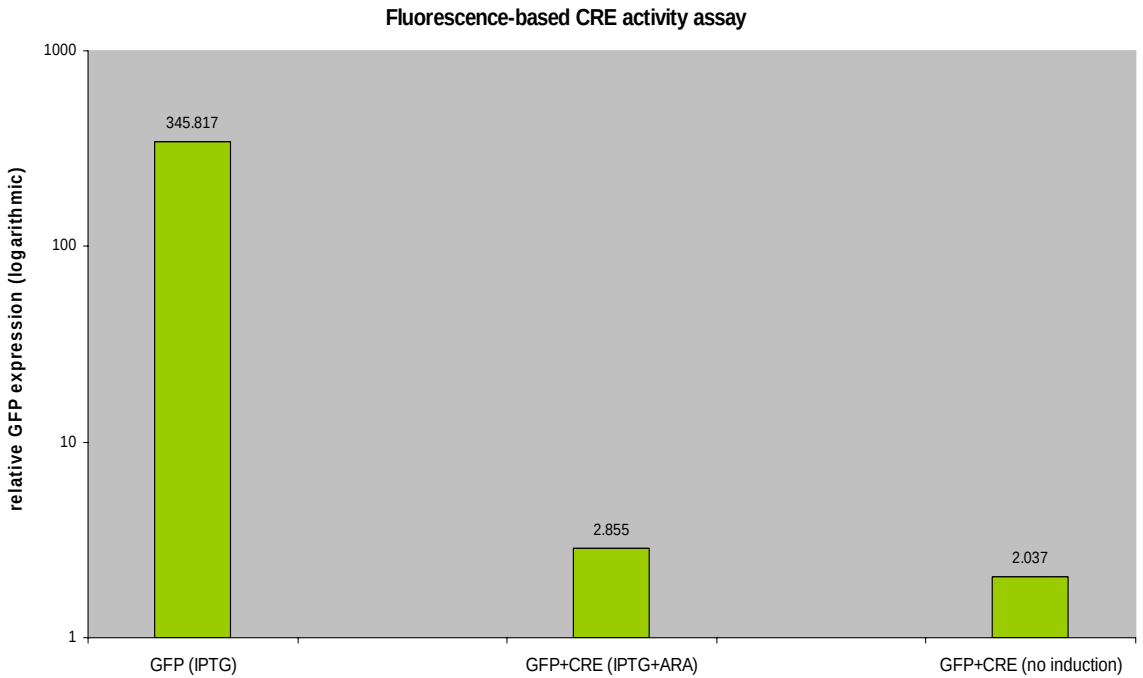




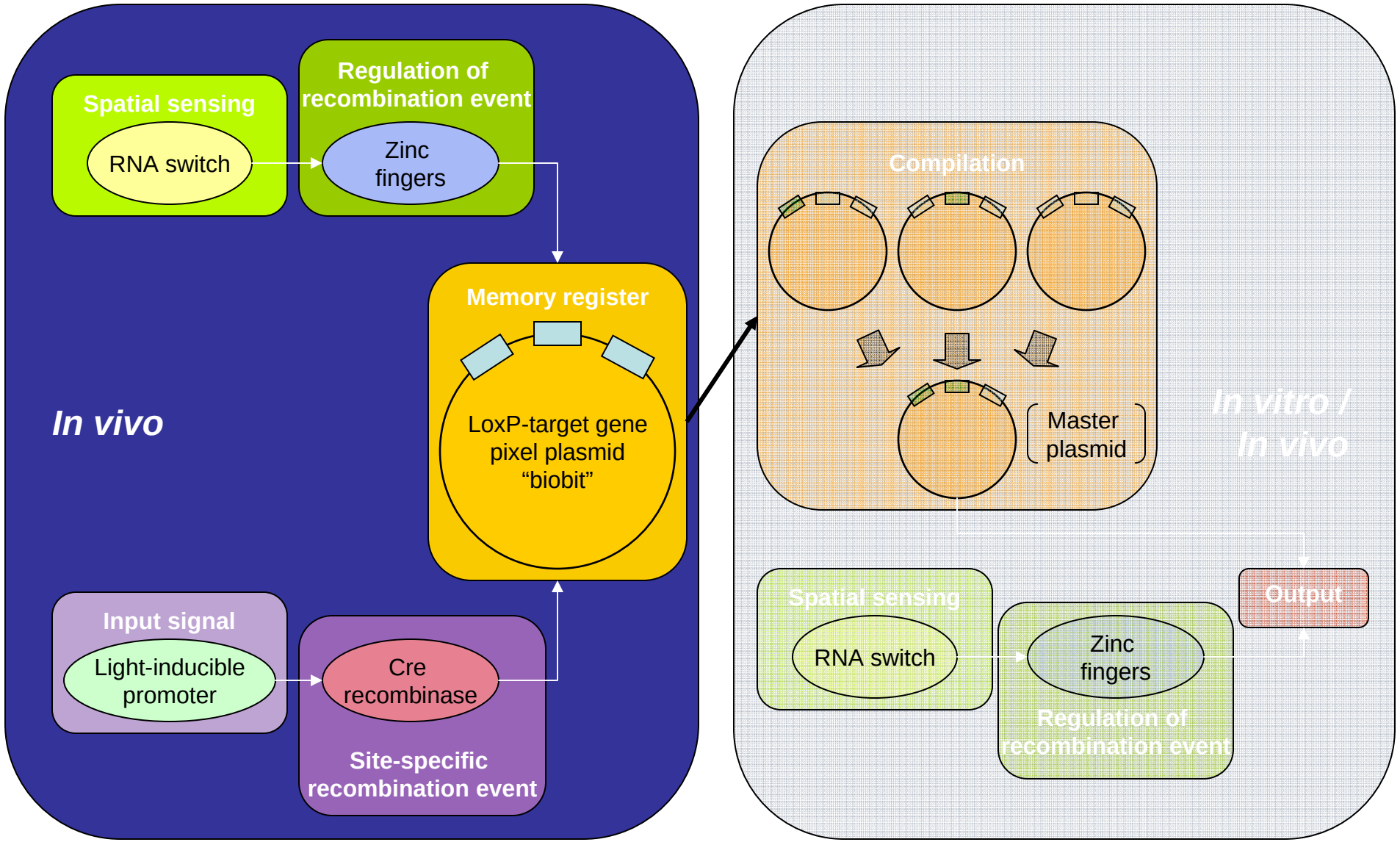
A single memory register



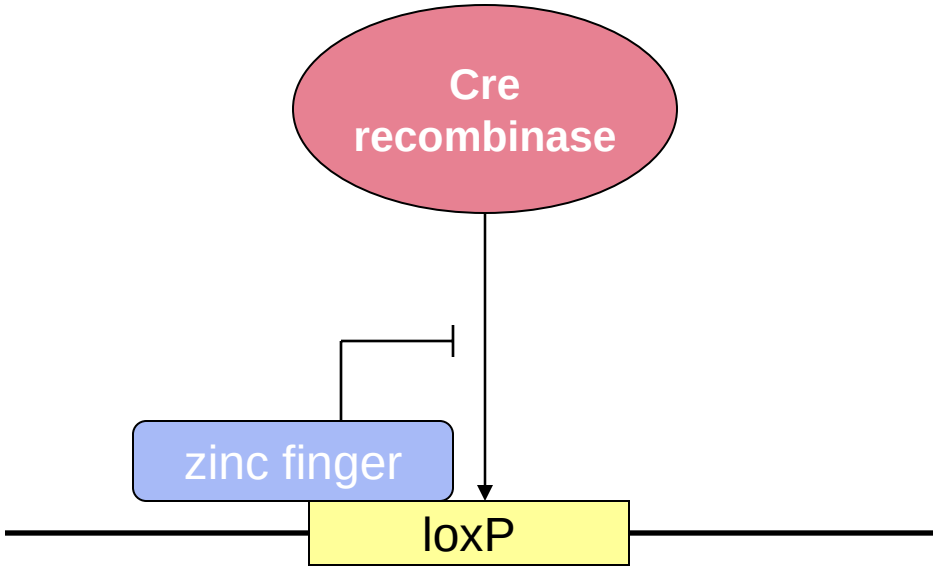




Regulatable hardwired memory

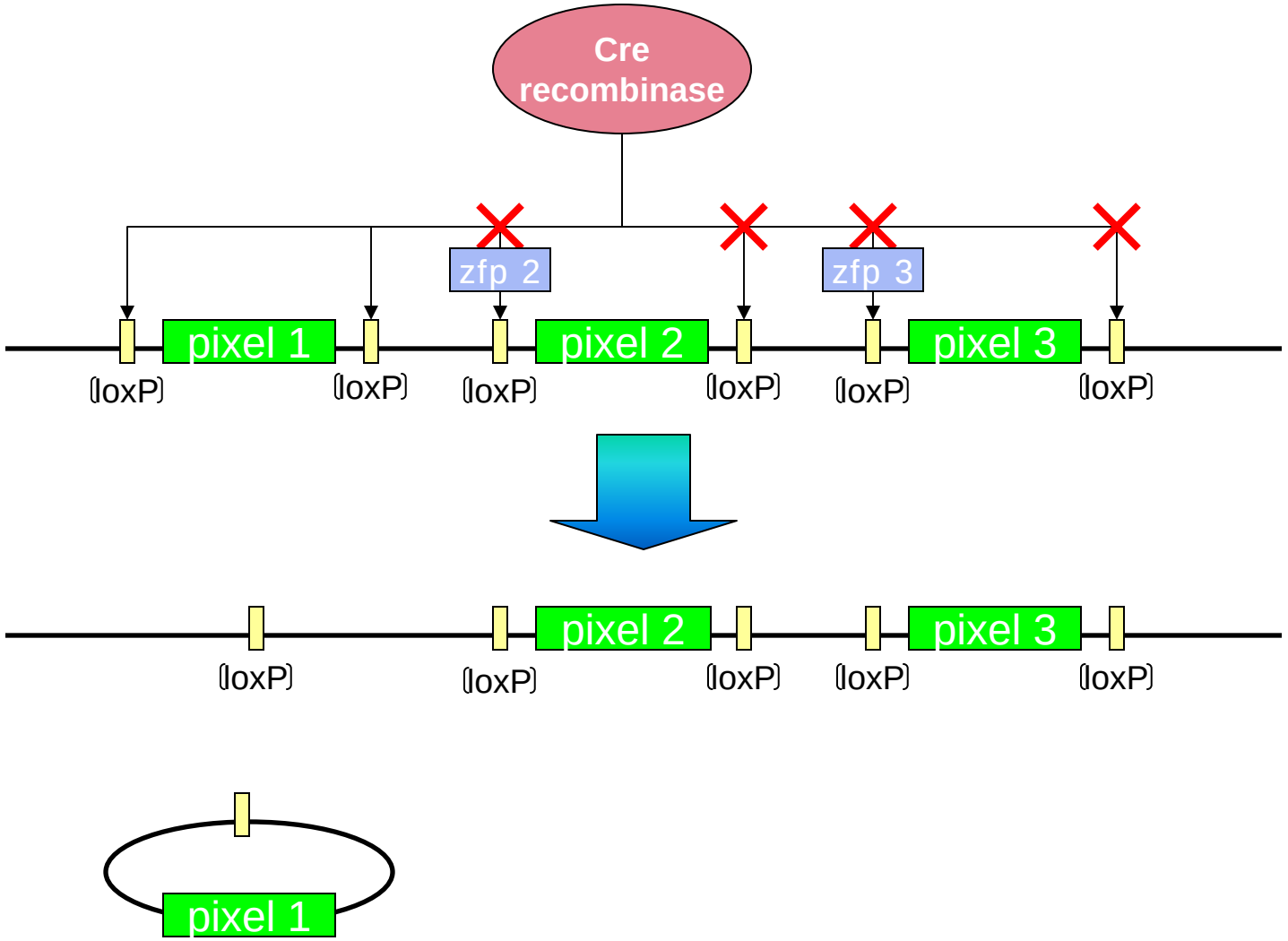


Regulation of recombination events: zinc fingers

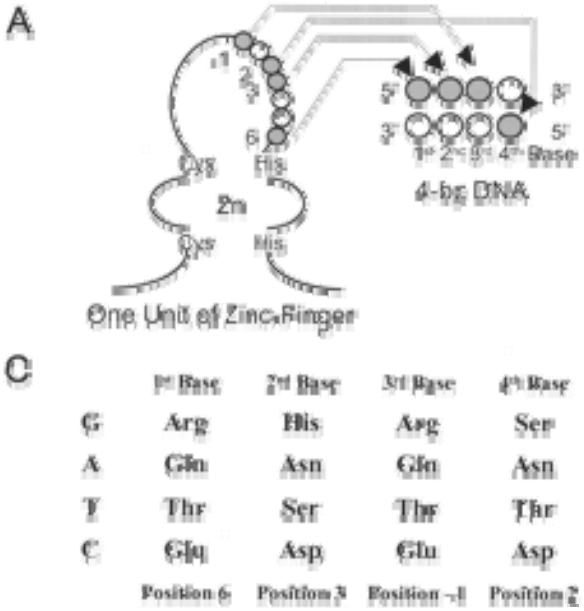


- Exhibit modularity in structure and function
- Commonly arranged as covalent tandem repeats, enabling recognition of extended asymmetrical sequences
- Each zinc finger is designed to target a specific register
- Accomplished by specific design of the DNA-binding motif to recognize a unique sequence near the LoxP site
- Overhang of the zinc finger will inhibit Cre activity on the adjacent memory register

Orthogonal zinc finger / loxP site pairs for multi-pixel systems



Rational zinc finger design



T Sera, C Uranga. 2002. Rational design of artificial zinc finger proteins using a nondegenerate recognition code table. *Biochemistry*, 41: 7074-7081.

6-finger binding motif for LoxP site

Position: -1 2 3 6 (reverse)
Finger 1: Gln-Ser-Gln-Thr
Finger 2: Gln-Asp-Thr-Asn
Finger 3: Thr-Asp-Arg-Asn
Finger 4: Thr-Asn-Thr-Thr
Finger 5: Gln-His-Glu-Thr
Finger 6: Gln-Ser-Gln-Ser

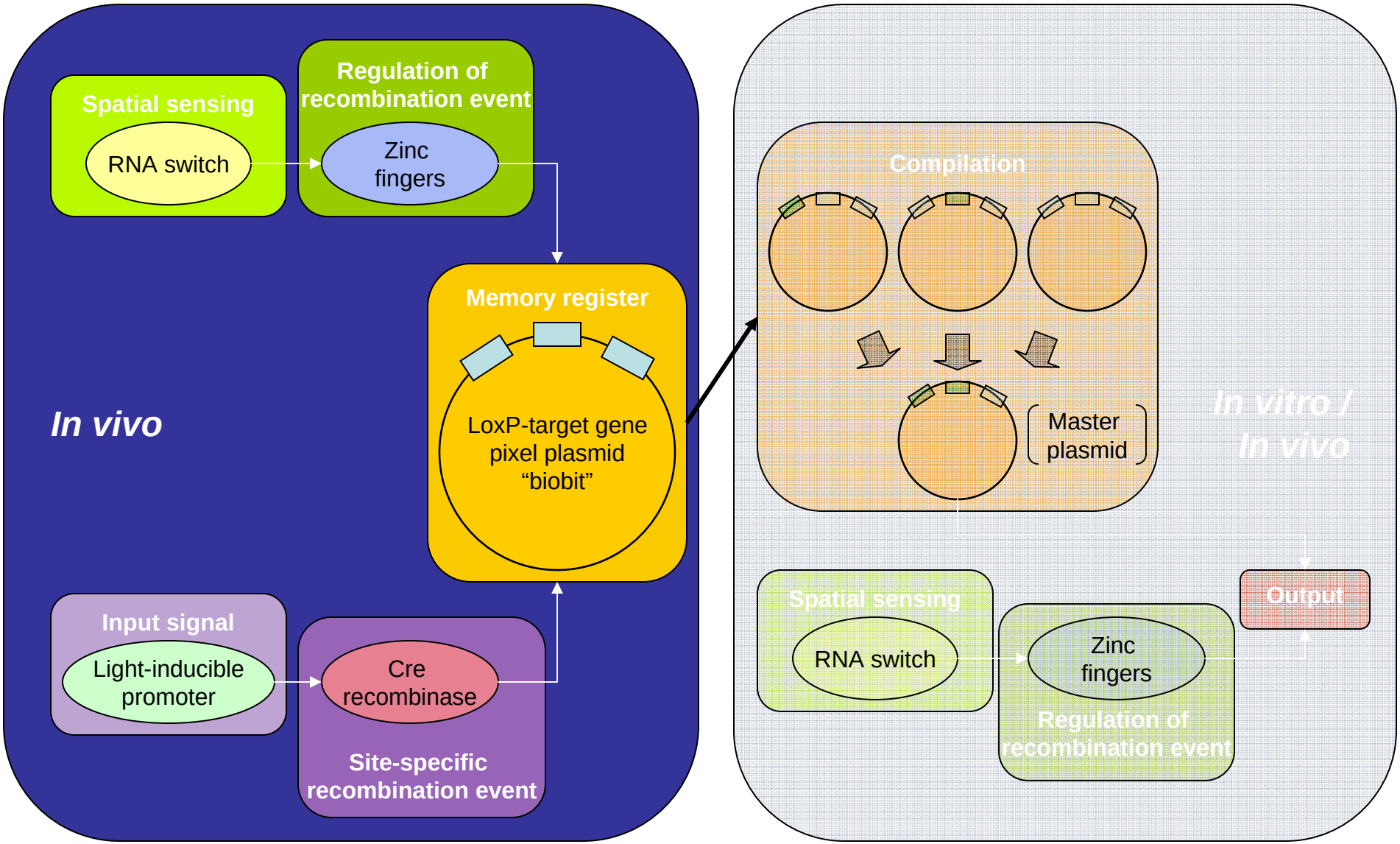
Berg's protein-based LoxP binding zinc finger:

ZFP1:

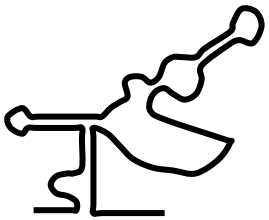
mekp ykcpecgksf sqssqlqthqrth tgekp ykcpecgksf sqsdtlqnhqrth tgekp ykcpecgksf stsdrlsnhqrth lrqkdgerp ykcpecgksf stsntlqthqrth tgekp ykcpecgksf sqshelqthqrth tgekp ykcpecgksf sqssqlsshqrthqnkk

- Rationally designed 6-finger zinc finger proteins to target specific nucleotide sequences using a nondegenerate recognition code table
- Initial zinc finger designed to bind to the entire LoxP sequence to test Cre inhibition
- Matching transfer functions of zinc fingers and Cre were determined to be critical for successful design

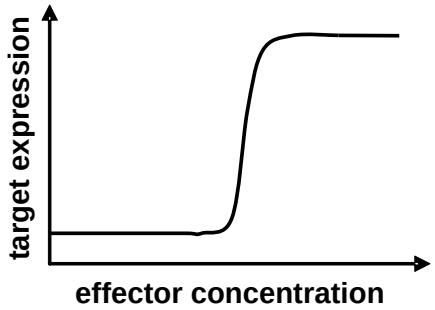
Spatial sensing module

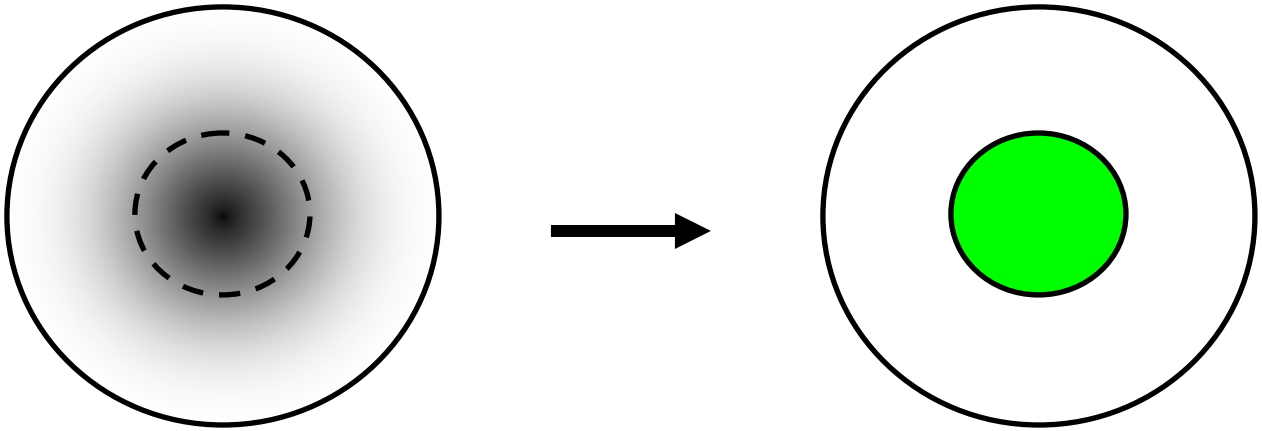


Spatial patterning of a single concentration cut-off

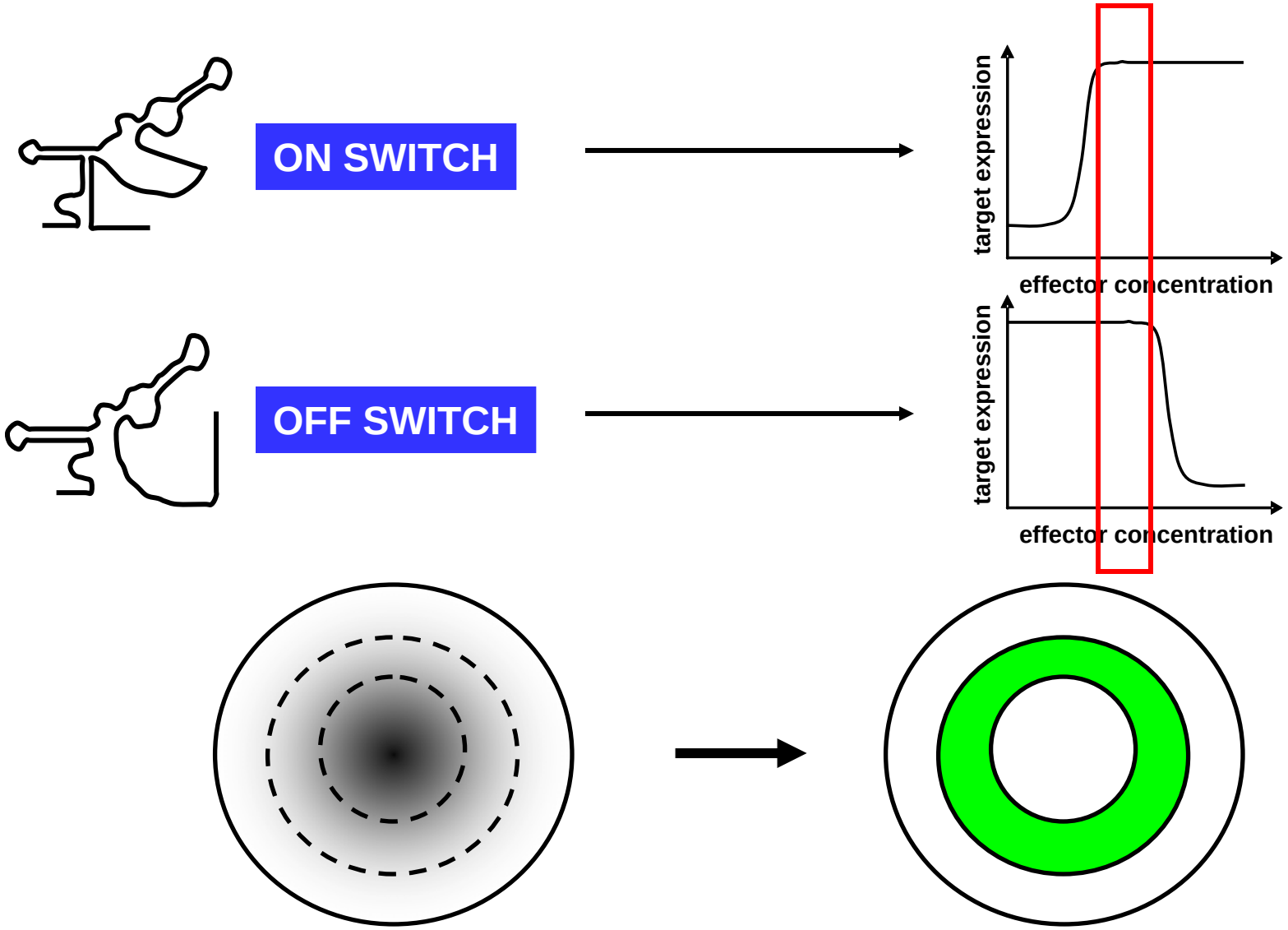


ON SWITCH





Spatial patterning of concentration ranges



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