Synthetic Circuits.

The Basics

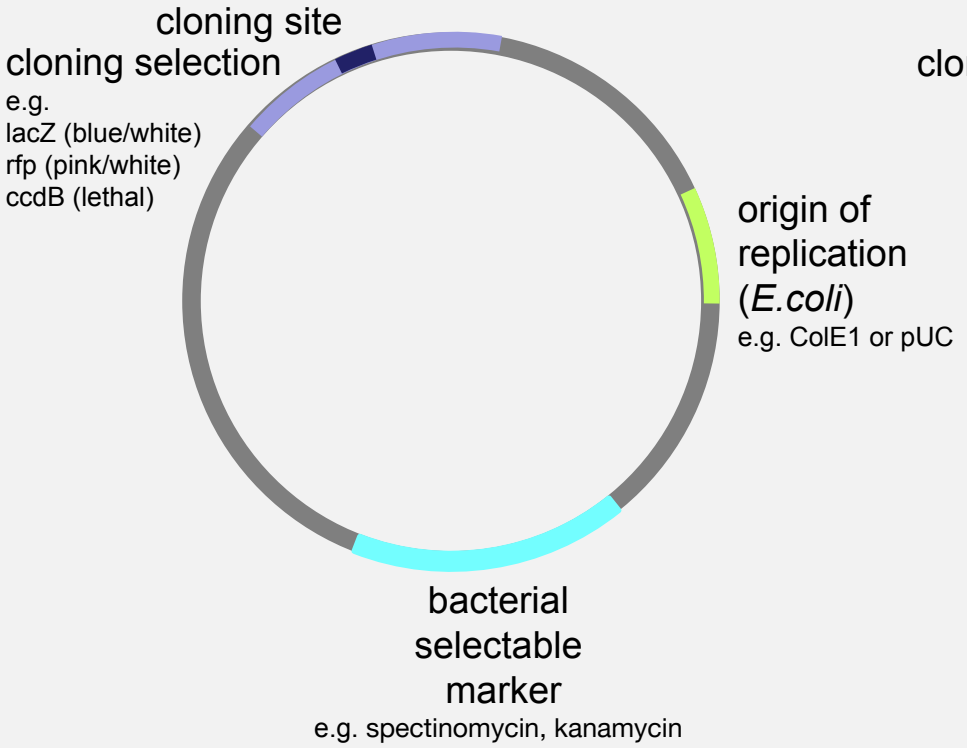
Nicola J Patron | Plant Sciences, University of Cambridge
http:// patronlab.org

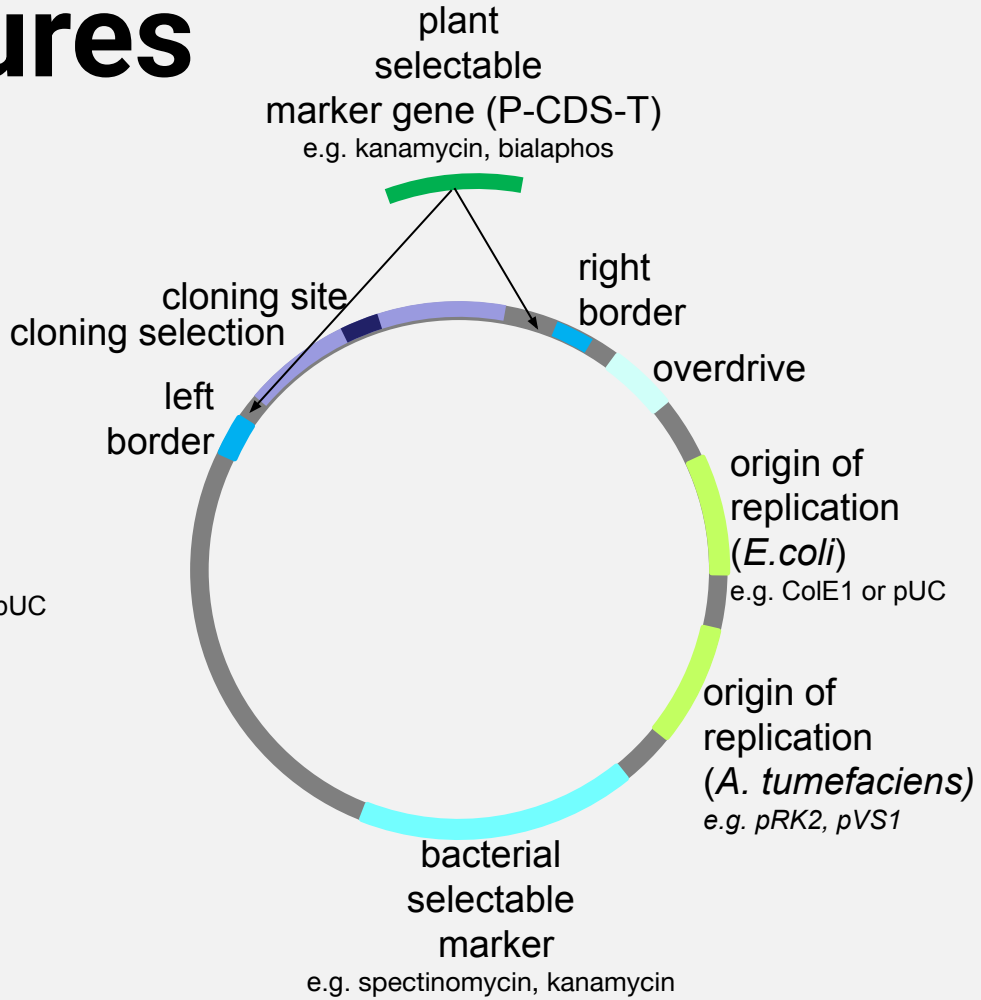
Learning Goals

To understand:

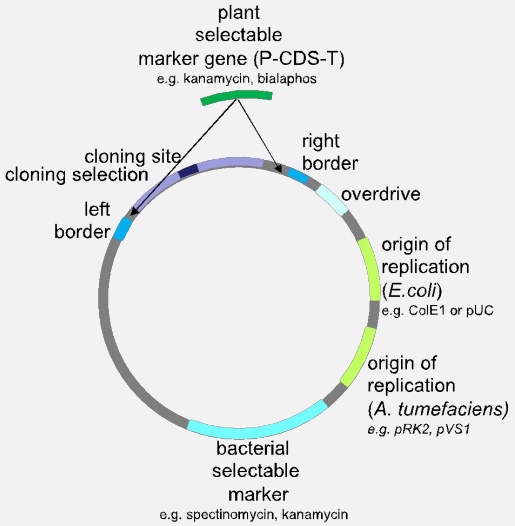
- Which sections of constructs require design input
- Plasmid backbones and how to select them
- Overexpression constructs
- Transcriptional control and how it can be manipulated
- Using plant promoters for tissue-specific expression
- Synthetic transcription factors and promoters

Plasmid Features





Selecting plasmids

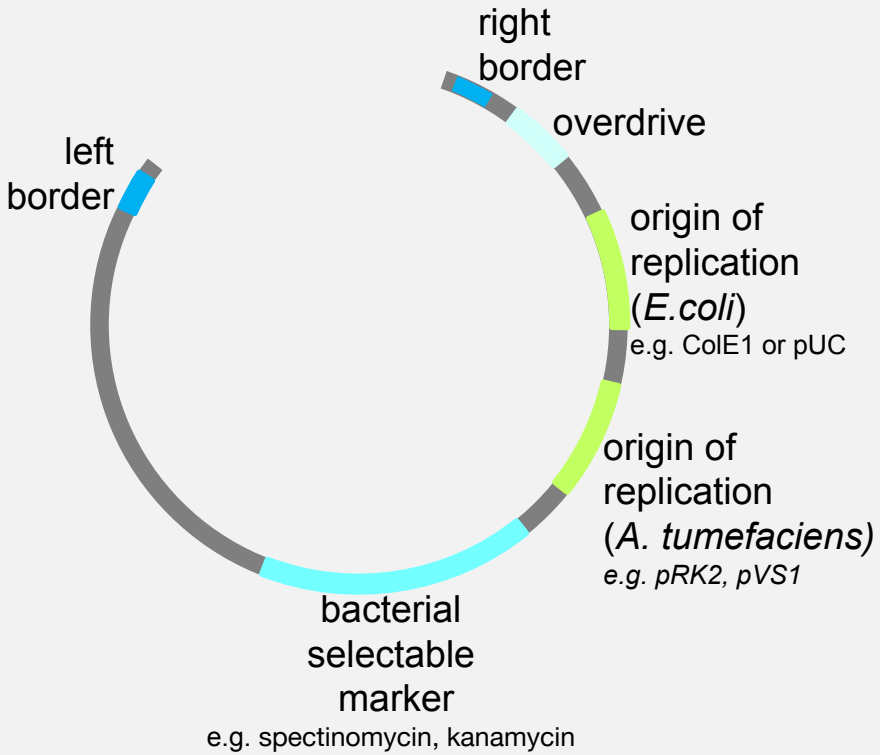


- Delivery method (protoplasts, *Agrobacterium*)
- Cloning site / method – Gateway, Golden Gate etc.
- Other features

Overexpression Constructs

e.g.
*CaMV53S$_P$_**G.O.I.**_35s$_T$*

(plant selectable marker)
(e.g. *NOS$_P$_NPTII_OCS$_T$)*



Where?

When?

how often?

Time-limited?

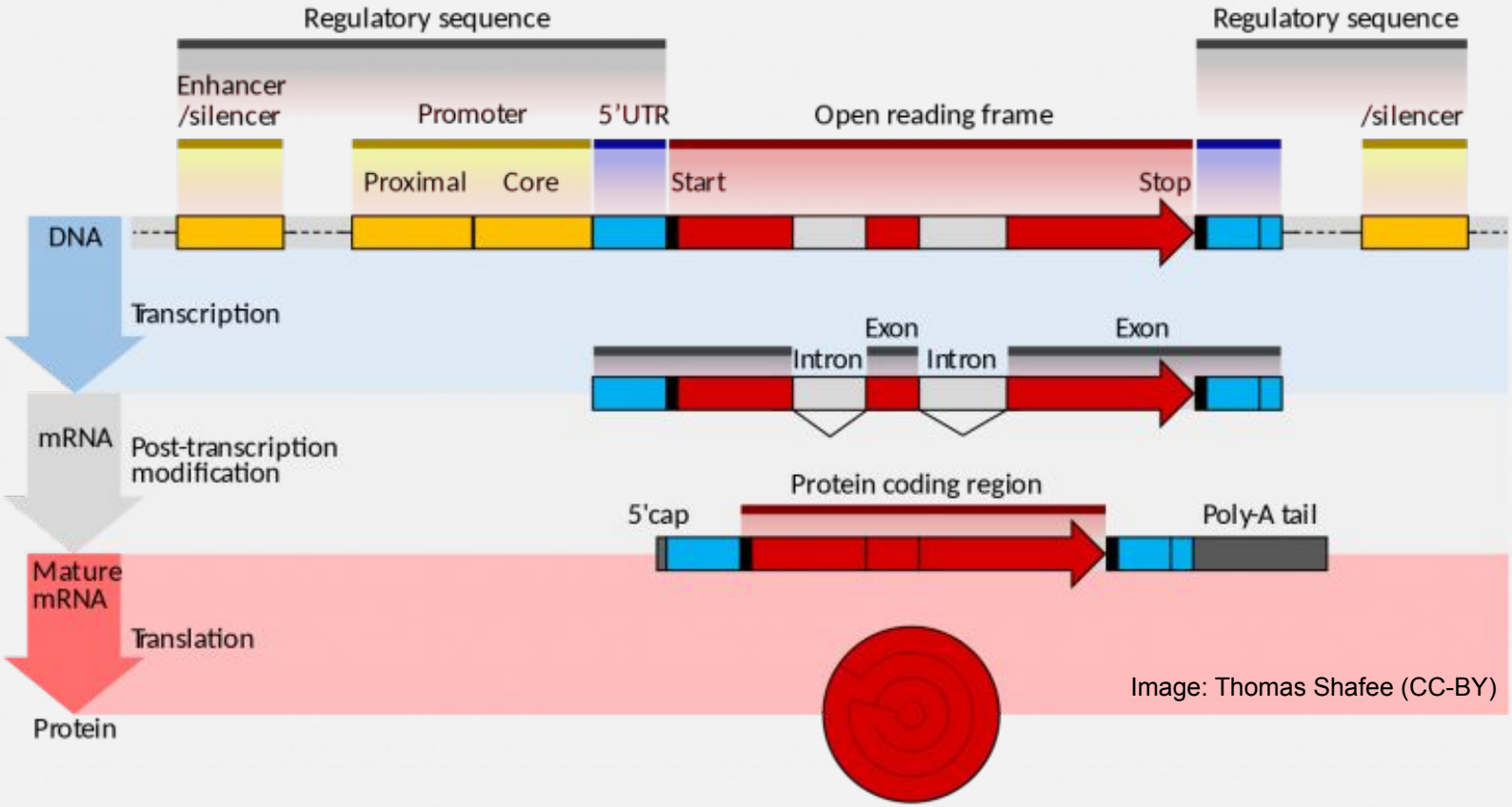
How much?

Periodical/cyclic?

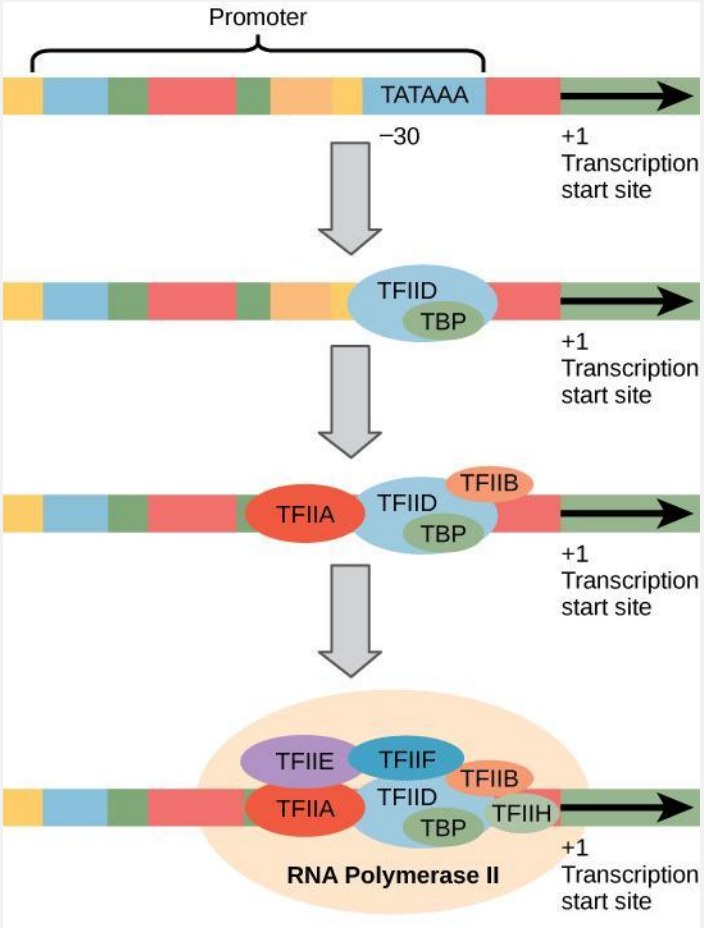
In response to?

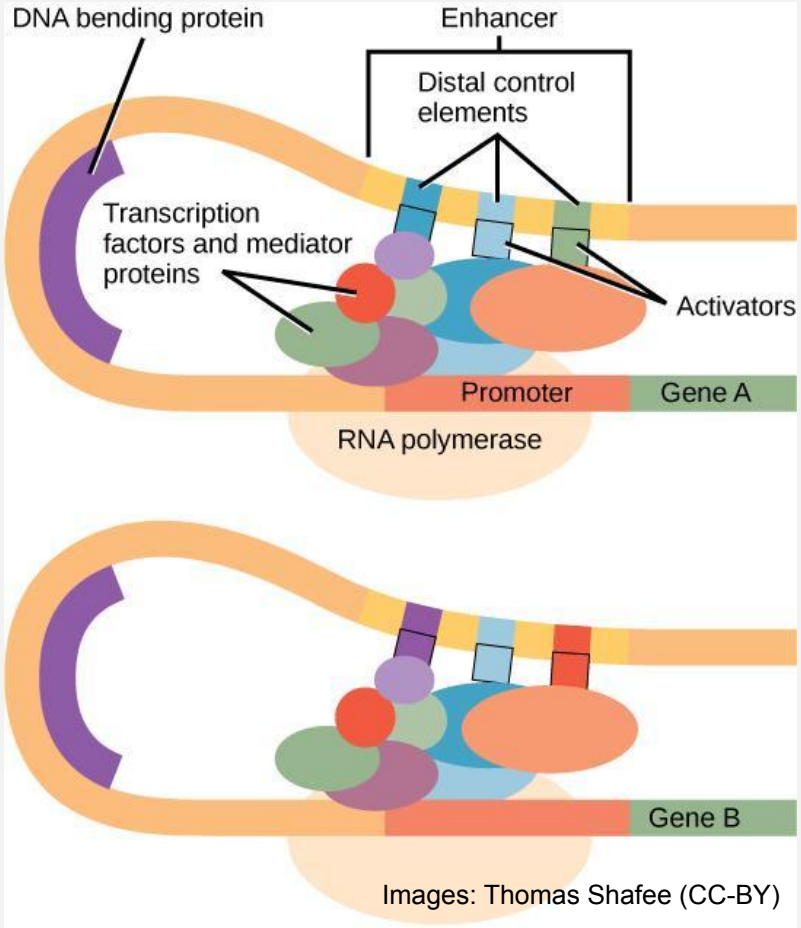
How quickly?

Generalised Gene Structure



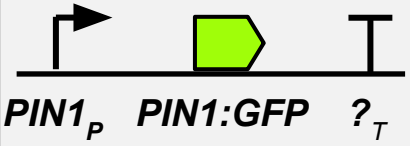
Recruitment of regulatory proteins





Images: Thomas Shafee (CC-BY)

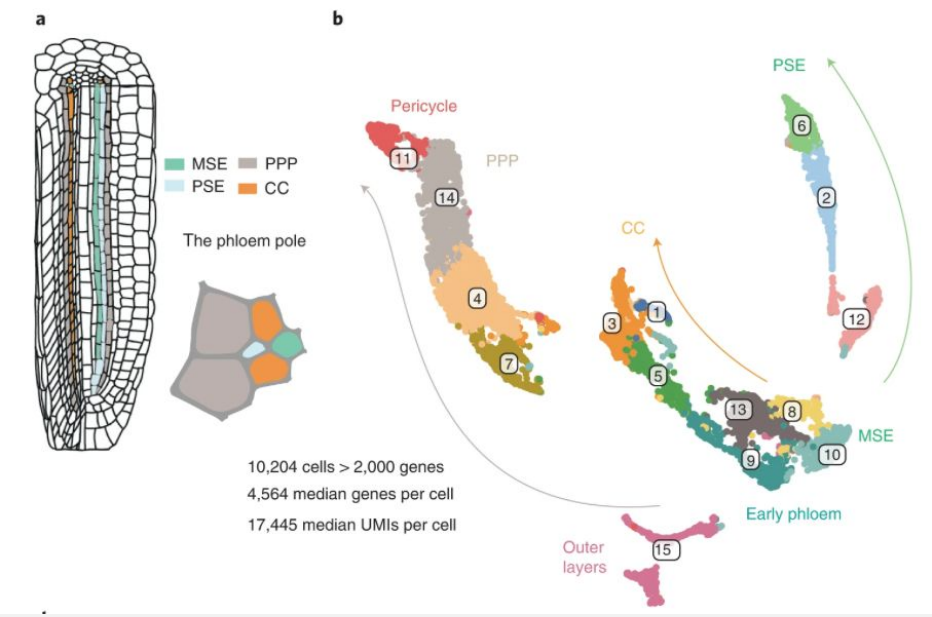
Using Plant Promoters - spatial control

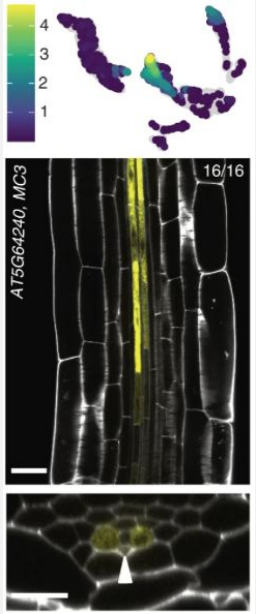


Red = Propidium iodide
Green = GFP

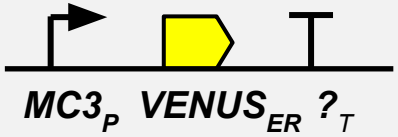
Raya-González et al 2014 PMID: 24784134

Using Plant Promoters - spatial control

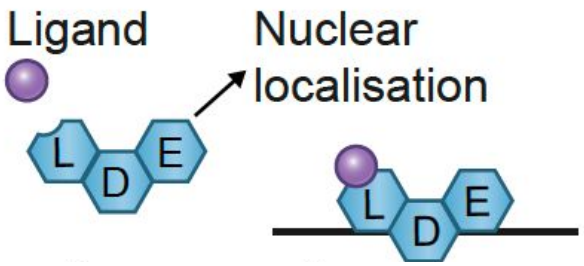




METACASPASE 3 (MC3, At5g64240)
Identification of novel companion-cell genes from single-cell transcriptomics, validated in reporter lines.
Otero et al 2022. PMID: 35927456



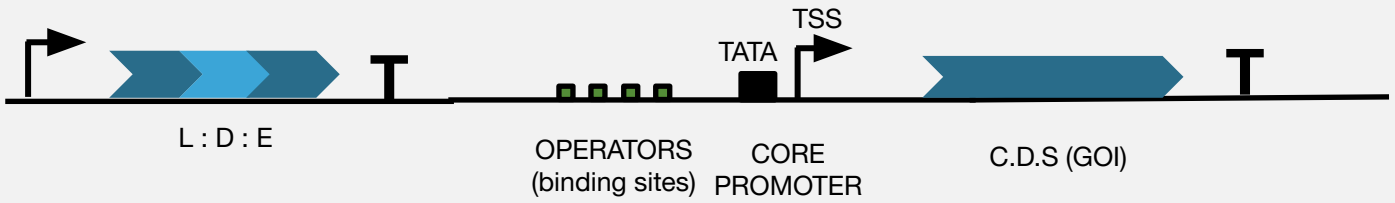
Inducible control – external, chemical



L = ligand binding domain (e.g., estradiol)
D = DNA-binding domain (e.g., LexA)
E = effector domain (e.g., VP16)

Tune amplitude by modulating:

- # binding sites in Psyn
- DNA-binding affinity
- type/identity effector domains



e.g. Zuo et al 2000. PMID: 11069700.

Inducible control - optogenetics

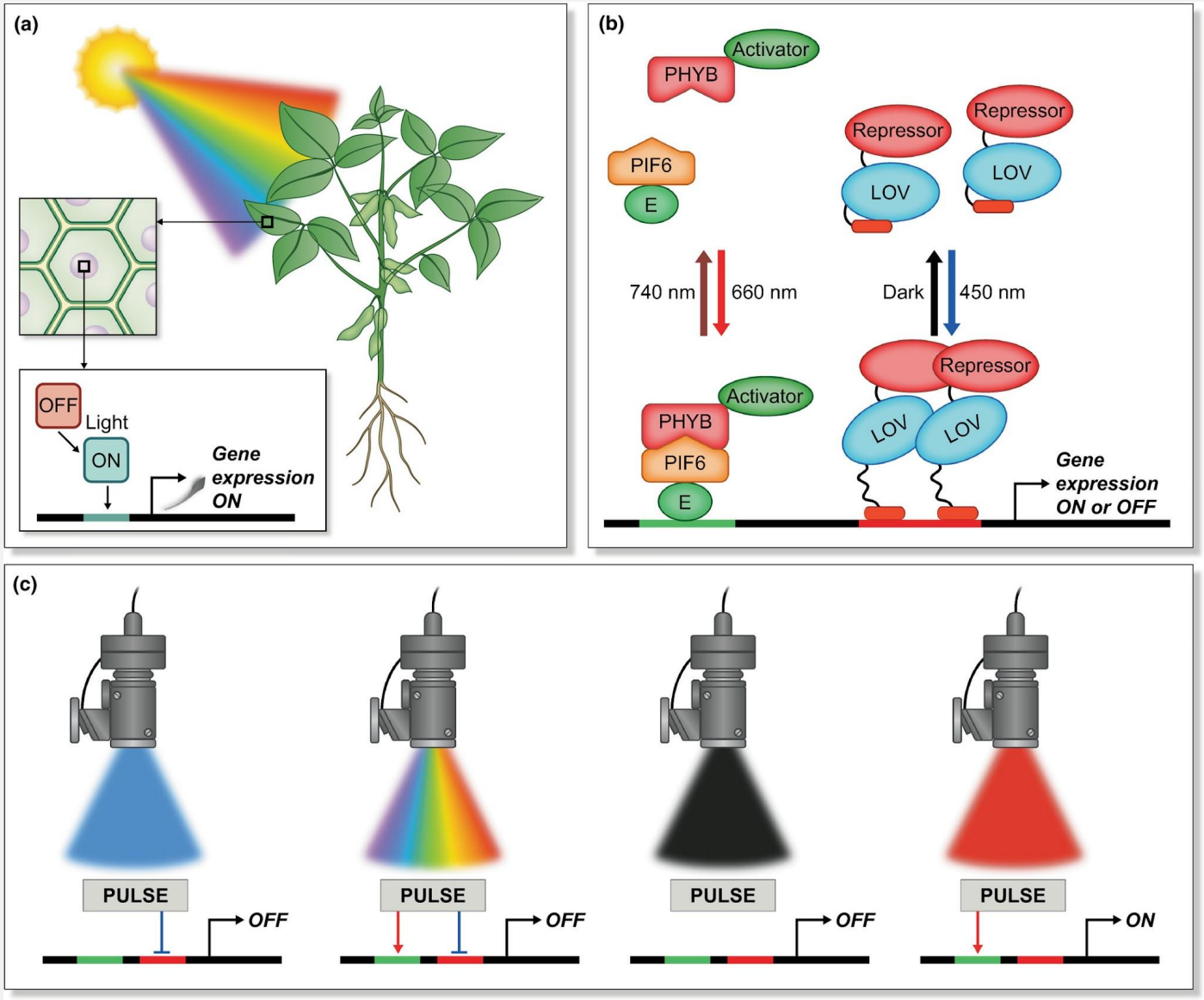
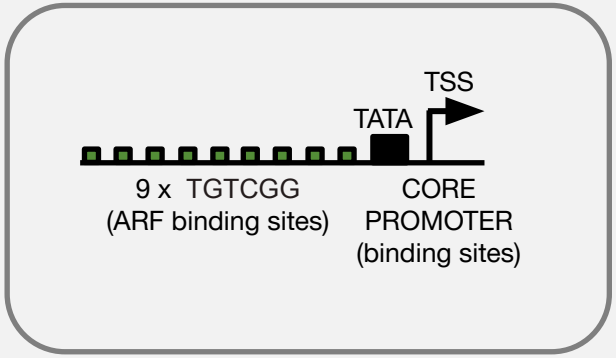
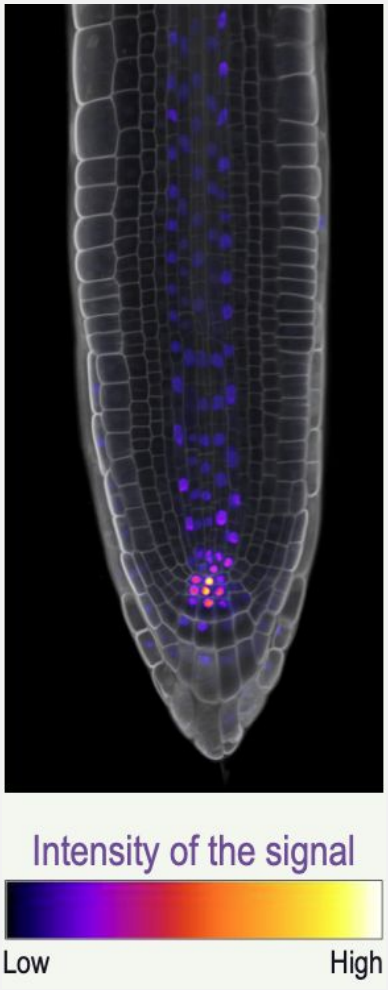


Figure: Christie and Zurbriggen (2021) New Phytol.

Inducible - endogenous



DR5v2 (Liao et al, 2015. PMID: 25643149)
Auxin-inducible expression / Auxin biosensor



END OF PART.

Synthetic Circuits. The Basics

- Which parts of constructs require design input
- Plasmid backbones and how to select them
- Overexpression constructs
- Transcriptional control and how it can be manipulated
- Using plant promoters for tissue-specific expression
- Synthetic transcription factors and promoters

Manipulating Endogenous Expression