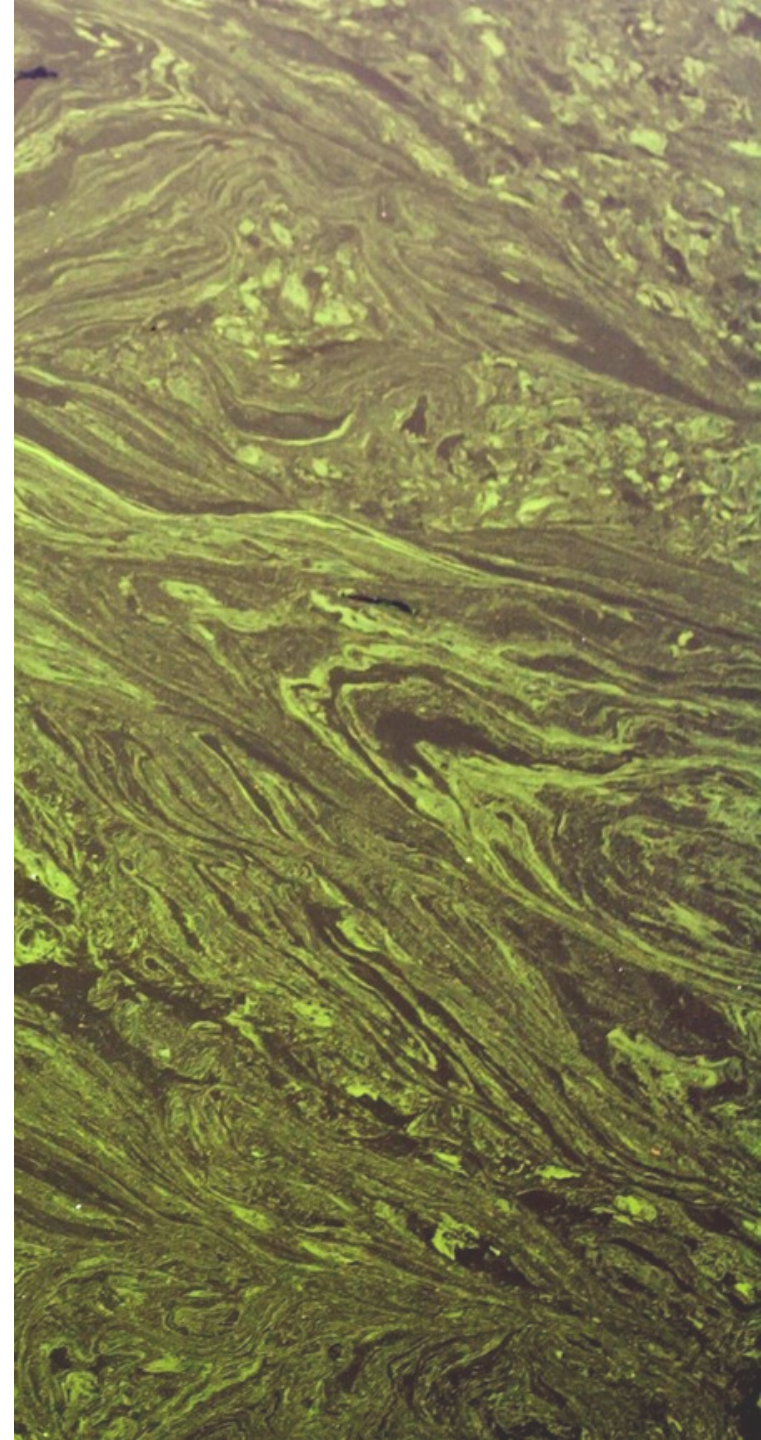


**SENSOR SYSTEM FOR DETECTING
CYANOTOXINS IN
*SACCHAROMYCES CEREVISIAE***

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ANNIKKI OLLILA AND AURA RELANDER



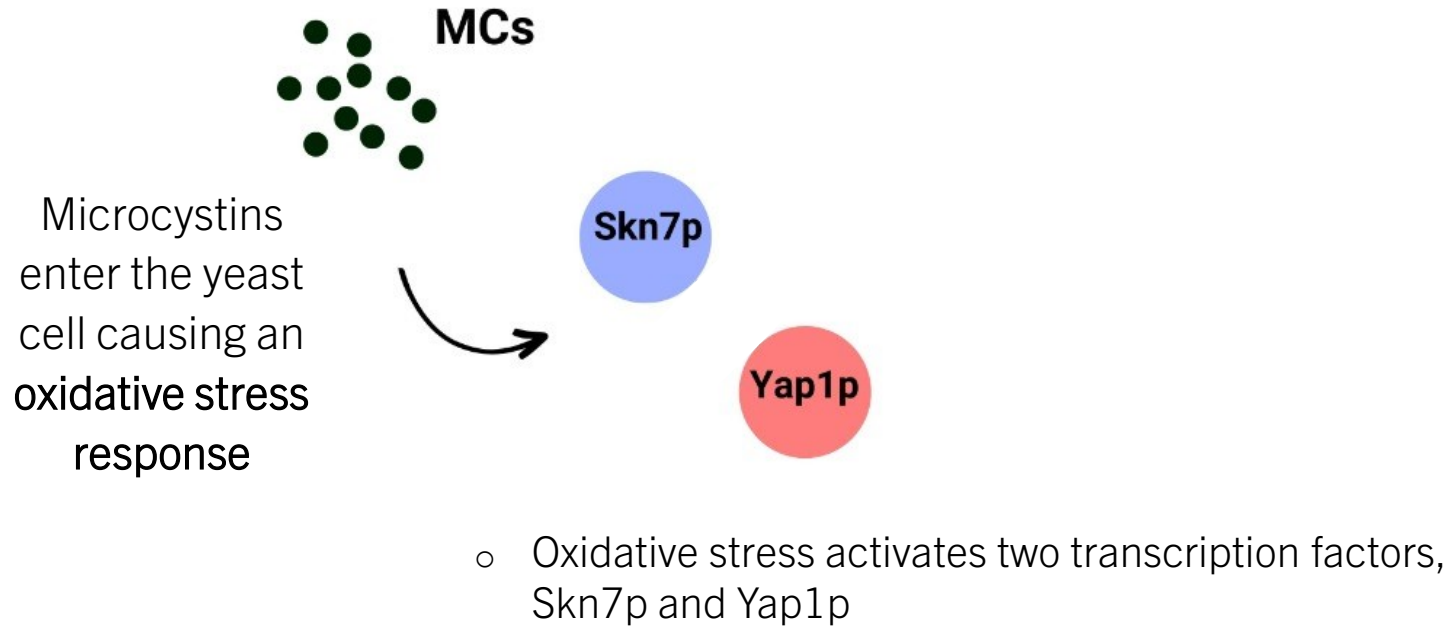
WHAT AND WHY?

- Cyanobacteria (blue-green algae) are a problem in water systems
- Cyanobacteria produce hepatotoxins called microcystins by blooming during warm weather
- Microcystins can cause health risks such as headache and diarrhea to humans and animals
- With this sensor, the microcystins can be detected

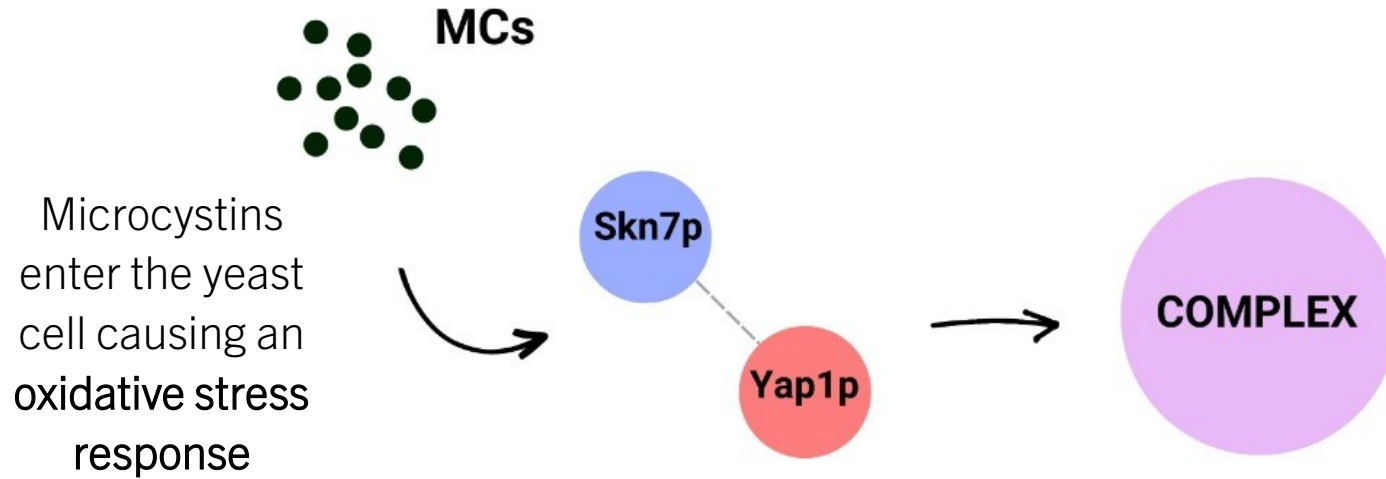
MICROCYSTIN EFFECTS ON YEAST

- Toxicity mechanism not fully known:
 - inhibition of protein phosphatases 1 & 2a and production of reactive oxygen species (ROS) involved
- Oxidative stress: increased activity of transcription factors → genes of oxidative stress response activated
- *S. cerevisiae* displays similar MC toxicity effects that occur in higher eukaryotes
- *S. cerevisiae* VL3 strain has a transporter for MC import

MICROCYSTIN SENSOR FUNCTION

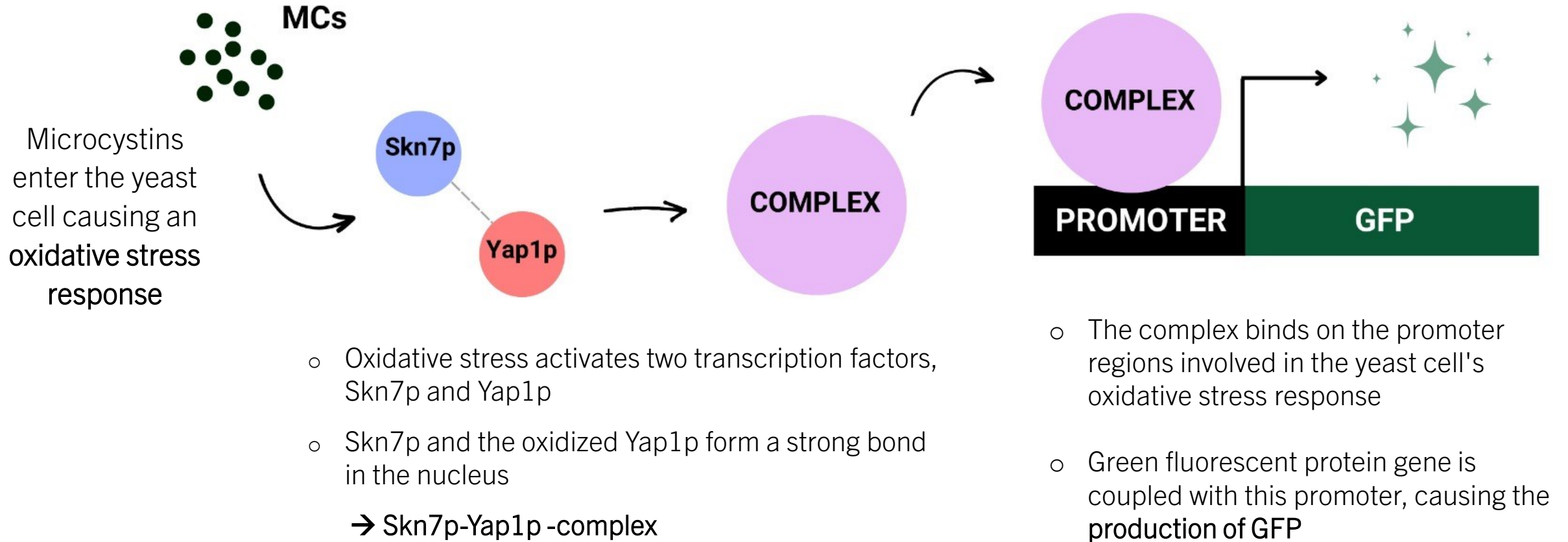


MICROCYSTIN SENSOR FUNCTION



- Oxidative stress activates two transcription factors, Skn7p and Yap1p
 - Skn7p and the oxidized Yap1p form a strong bond in the nucleus
- Skn7p-Yap1p -complex

MICROCYSTIN SENSOR FUNCTION



MICROCYSTIN SENSOR FUNCTION

- OSR transcription factors: Yap1p and Skn7p
- Yap1p and Skn7p have binding sites in the promoter areas of OSR genes
 - Skn7p binds to oxidative stress response element (**OSRE**) and Yap1p to Yap1 response element (**YRE**)
- One of these genes: thioredoxin peroxidase (**TSA1**)
 - Has one of the best characterized promoter regions that Yap1p and Skn7p bind to

TSA1
(260BP)



IDENTIFICATION: GFP

- Green fluorescent protein
- Emits green fluorescence under UV light

Detection in laboratory:

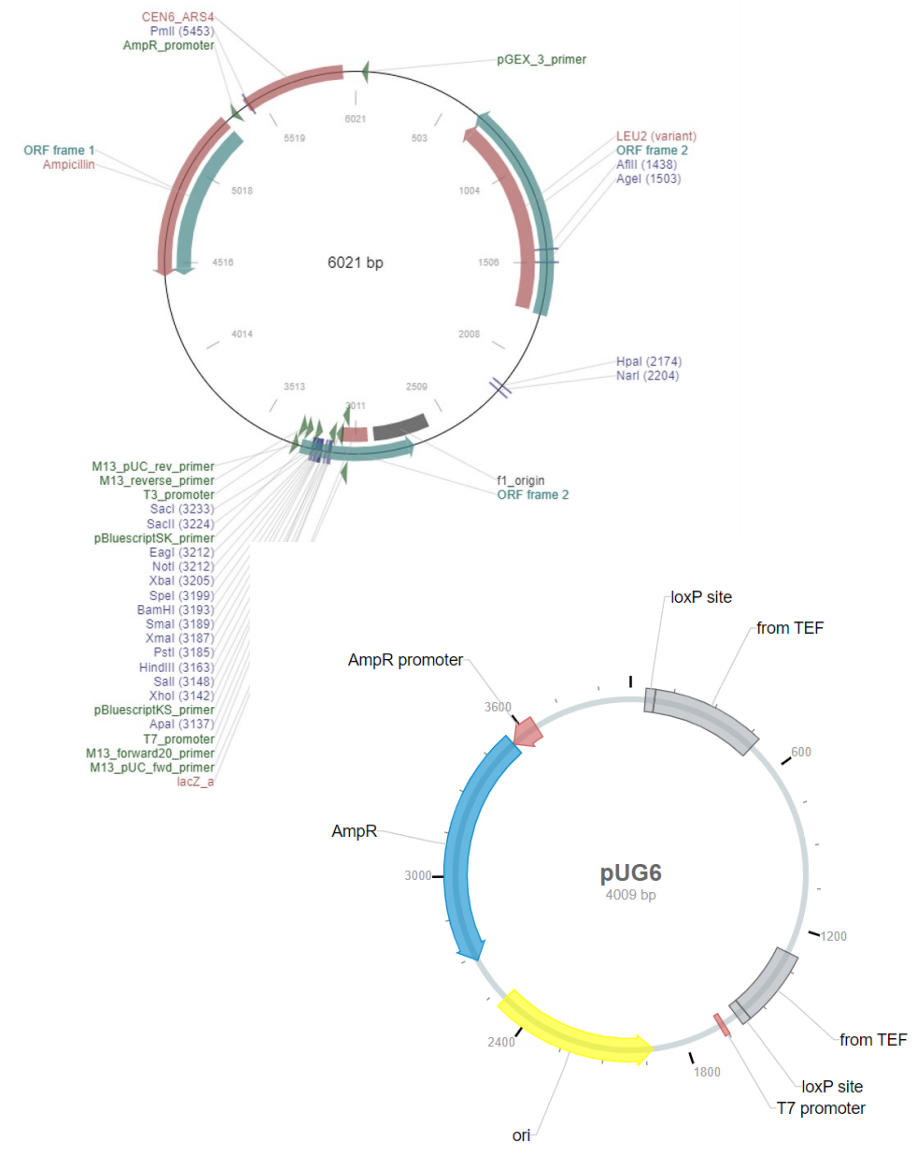
- Flow cytometry
- Fluorescent microscopy

TRUTH TABLE

Microcystine	GFP
1	1
0	0

SELECTED PARTS

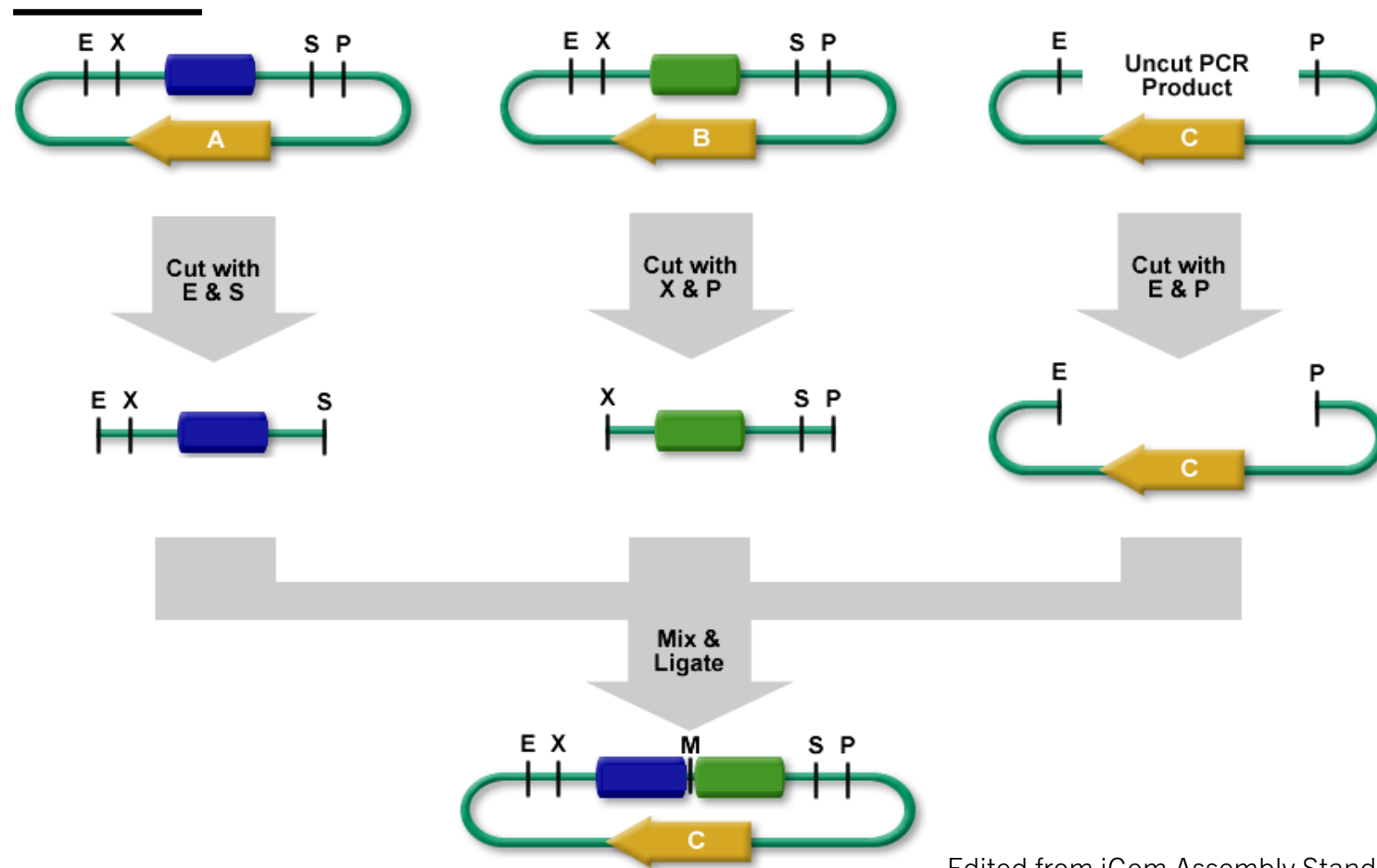
- Fluorescence expression: yeGFP (BBa_K3402000)
- Promoter: TSA1 (BBa_K1907003)
- Plasmid backbone for promoter: pRS415
- Shuttle vector: pUG6



ASSEMBLY

- Chosen BioBricks parts must belong to the same assembly standard
 - ensures compatibility and trouble-free assembly
- RFC[10] is the most commonly used assembly standard: well tested and documented, great diversity

ASSEMBLY: RFC[10]



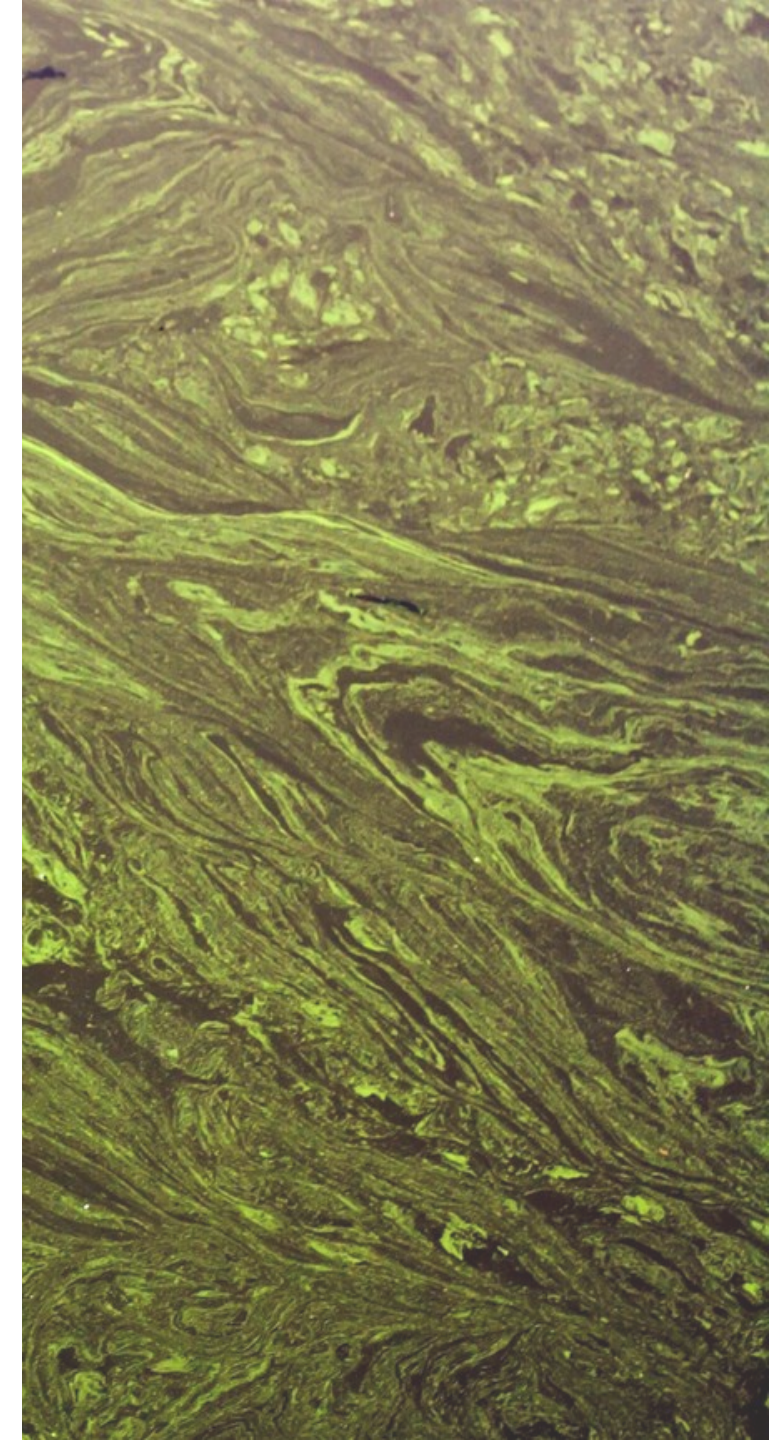
SOURCES:

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THANK YOU!
QUESTIONS?

