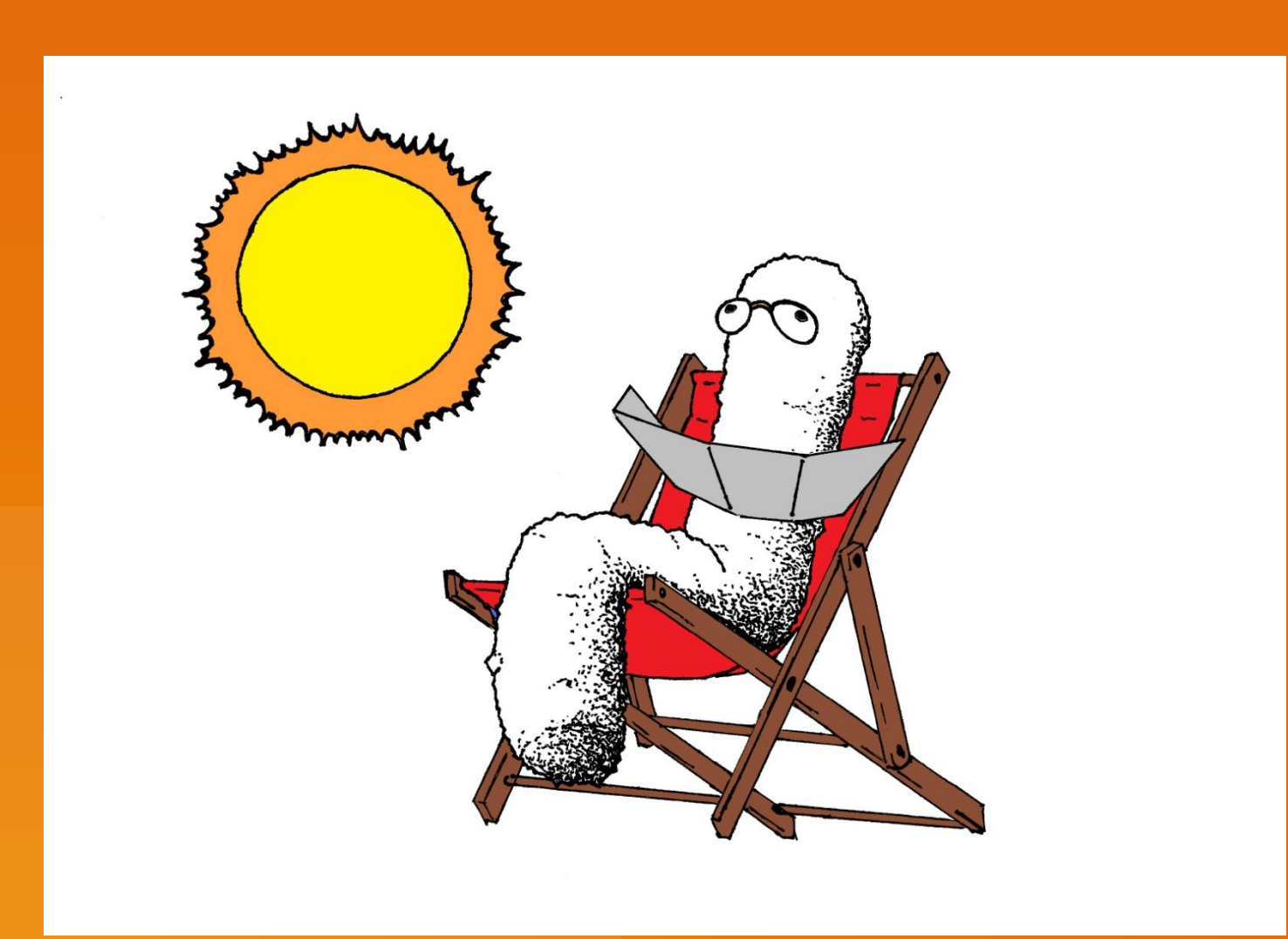
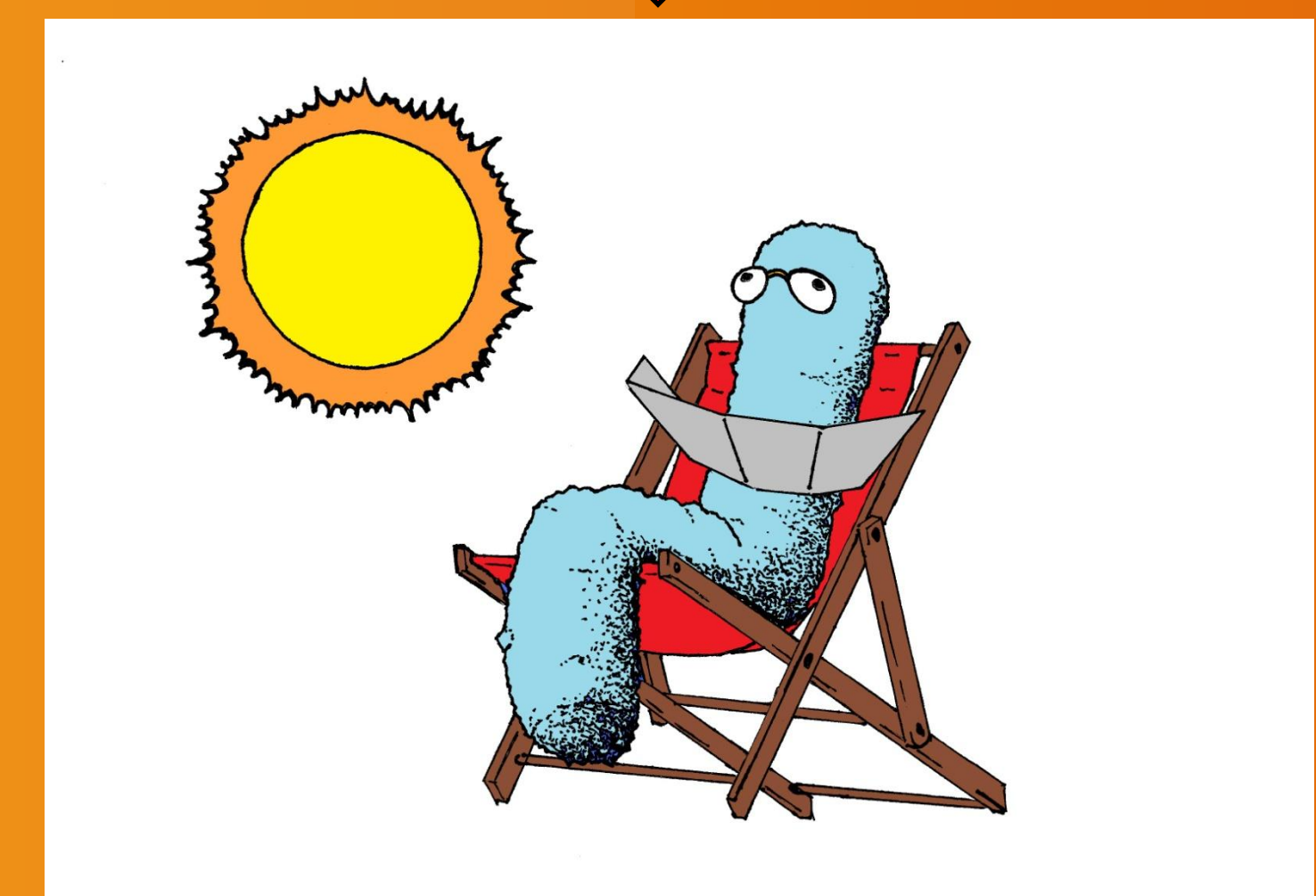
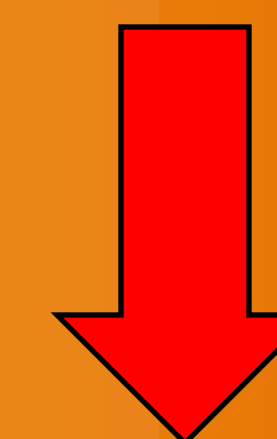


Engineering a Real-Time Living Biosensor: DNA Damage Caused by Ultra-Violet Irradiation

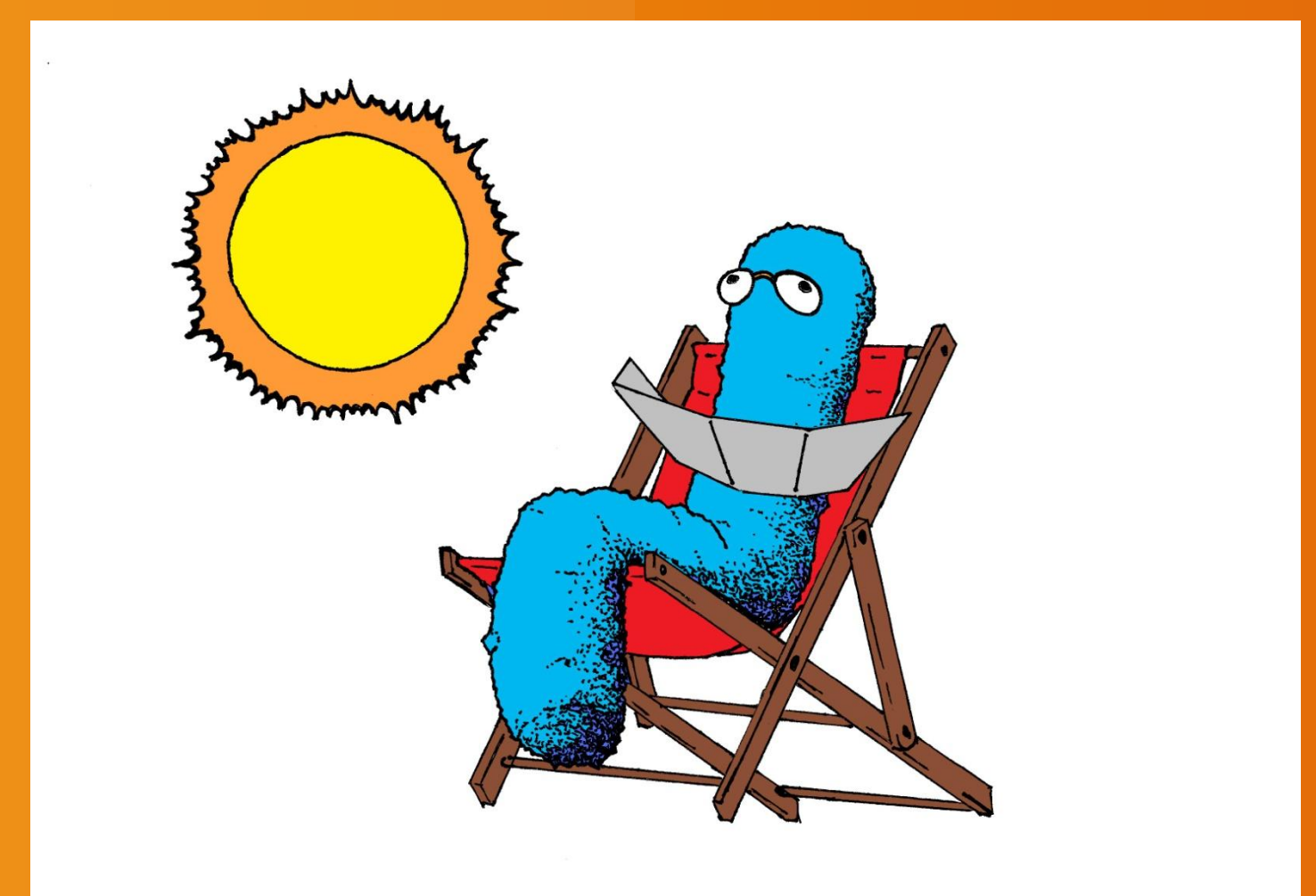
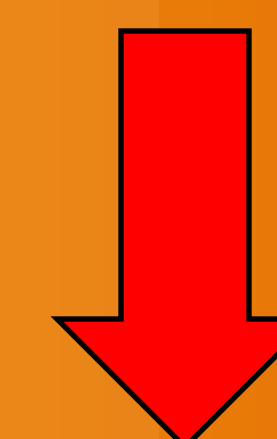
Craig Barcus¹ Jessamine Osborne² Erin Rosswurm¹ Janie Stine¹ Advisors: Dr. Jenna Rickus^{1,3,4} Dr. Kari Clase^{4,5} David Jaroch³
1: Agricultural and Biological Engineering, 2: Biological Sciences, 3: Weldon School of Biomedical Engineering, 4: Bindley Bioscience Center, 5: Industrial Technology
Purdue University, West Lafayette, IN



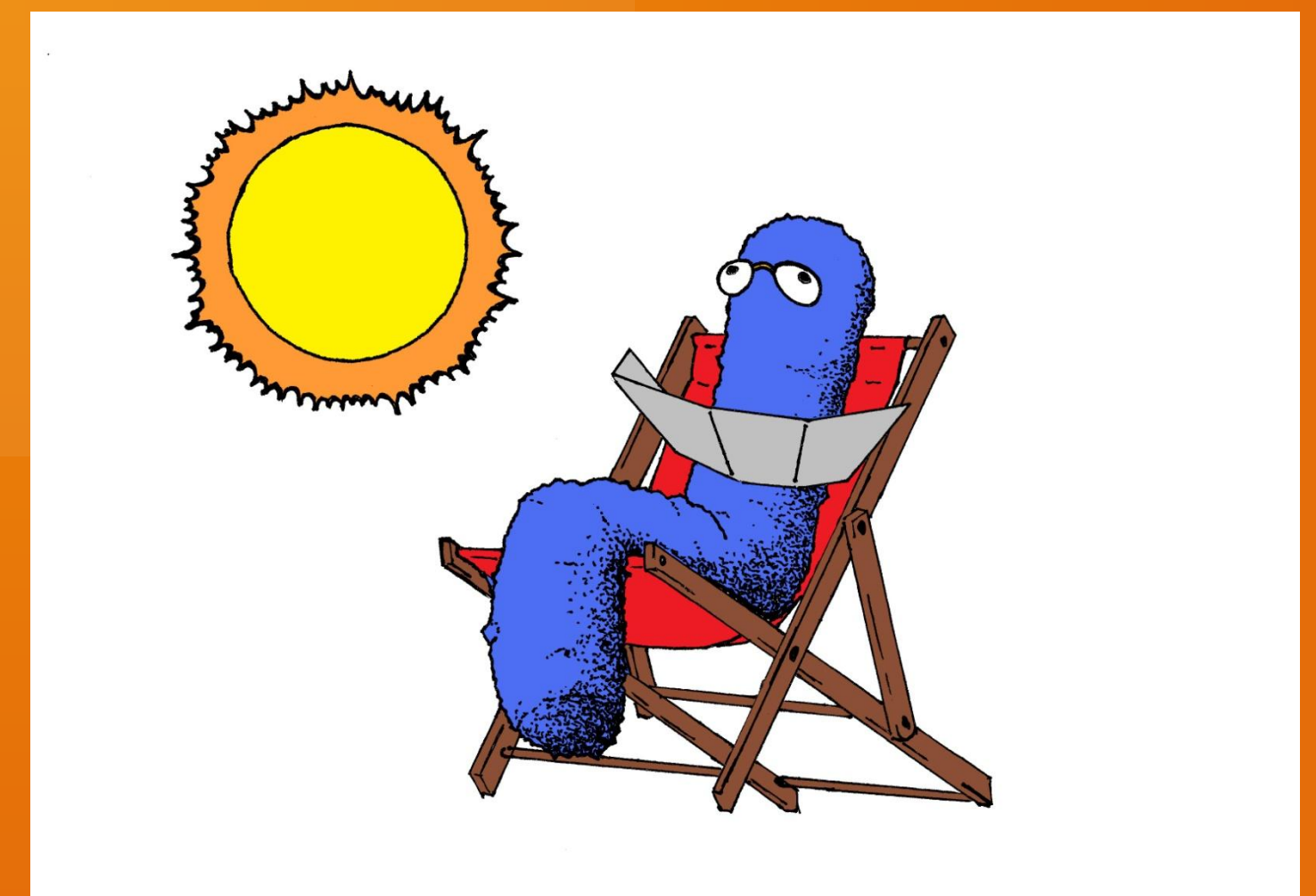
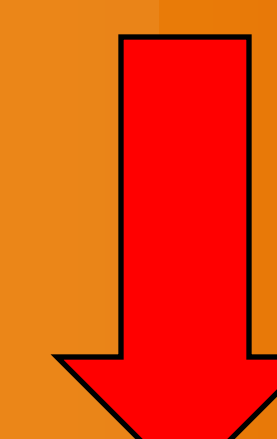
UV Exposure → DNA damage



SOS Induction → LacZ Transcribed



LacZ Reporting → X-gal Cleavage



Motivation

- 1 million Americans are diagnosed with skin cancer every year.¹
- Current products measure UV radiation level, *not* DNA damage.
- Our Goal: create a real-time sensor of DNA damage

Approach

- "If-Then" Construct: Promoter + reporter
 - Promoter: *recA* of SOS system, part J22106 (activated for extreme DNA damage)
 - Reporter: *lacZ*, part I732017 (blue/white screening on X-gal)
- If DNA is damaged extensively, *then* transcription of β-gal
- Essentially a reporter-gene assay

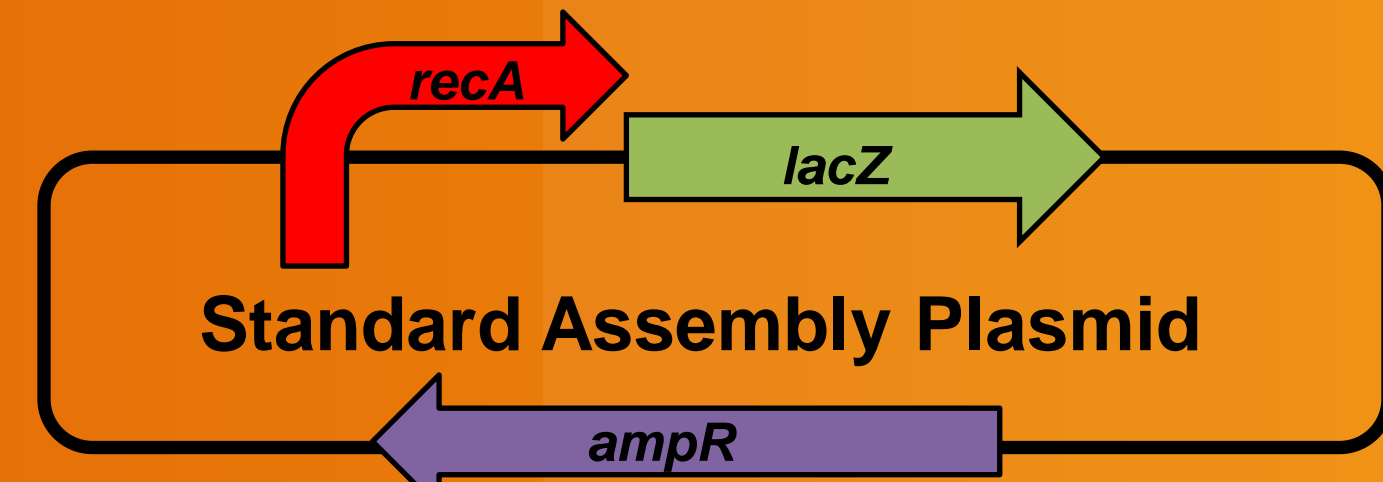
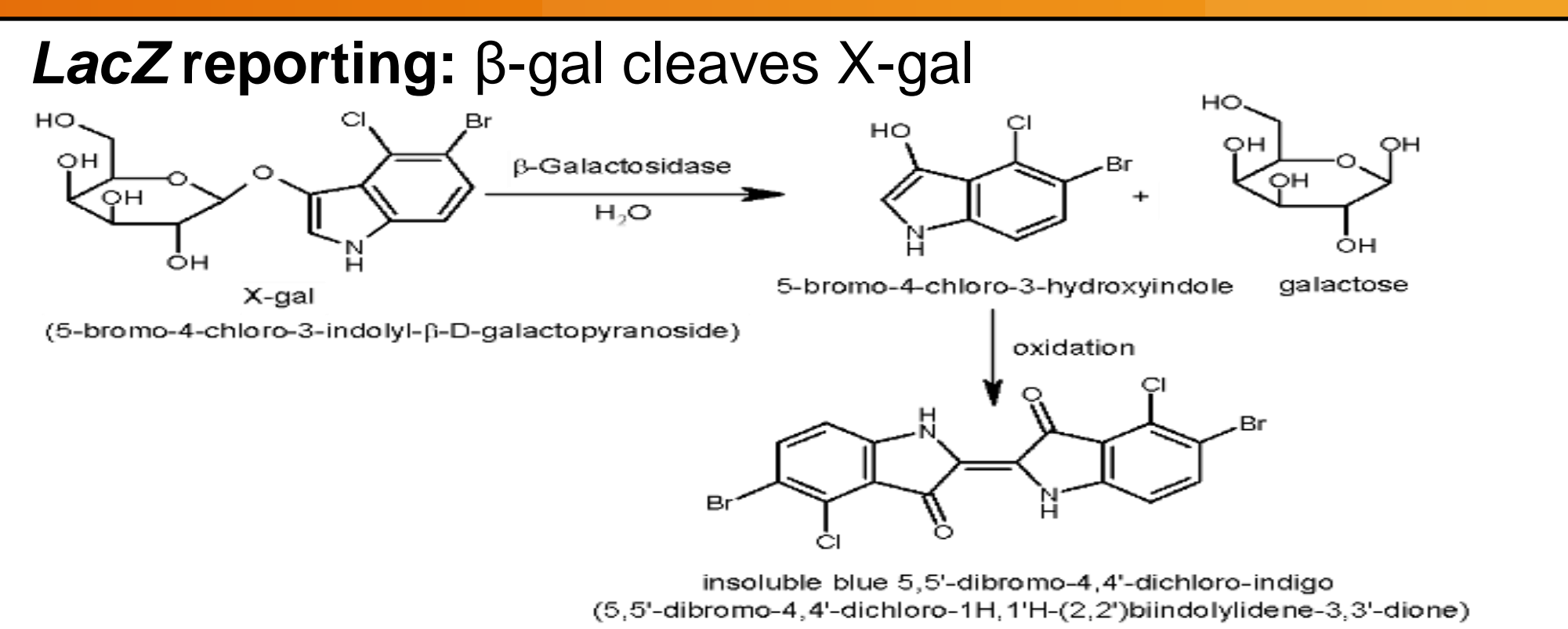
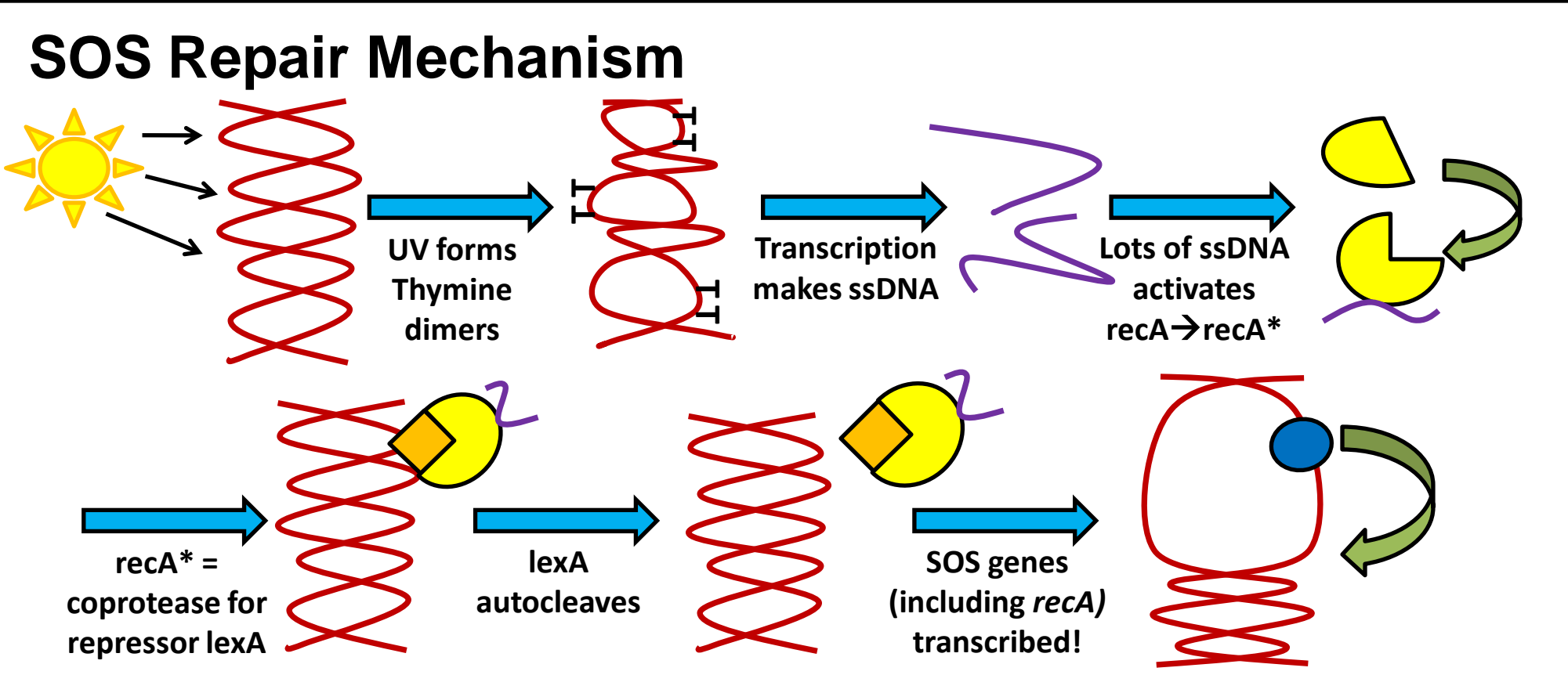


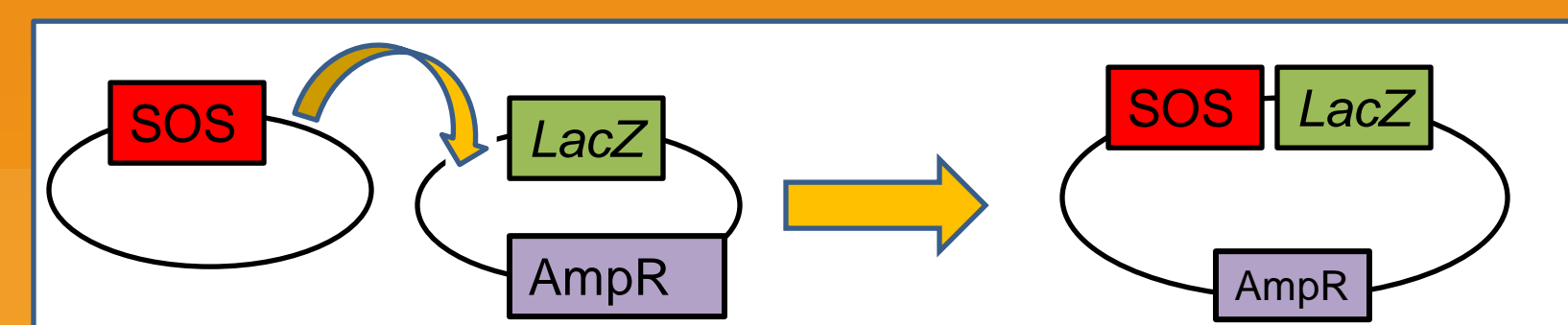
Figure 1: Basic construction of the part we wish to use.

Mechanisms

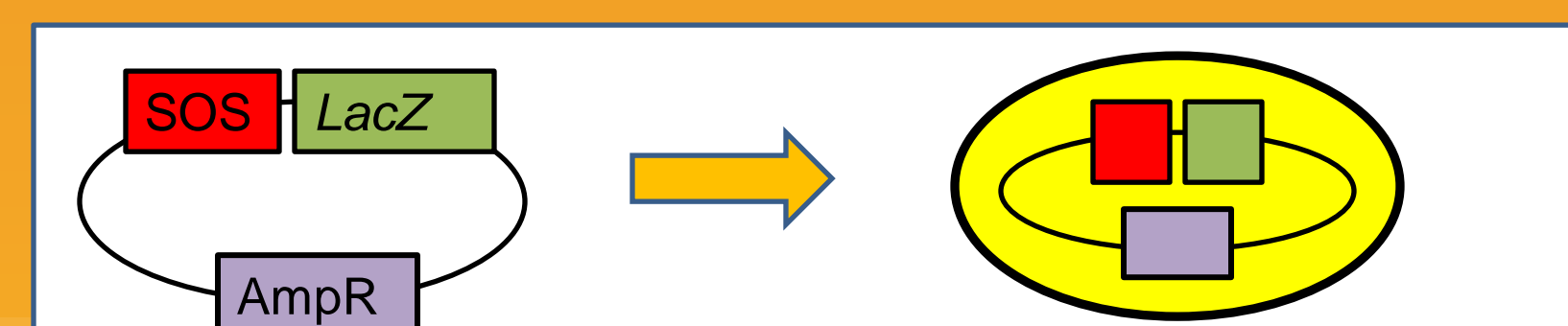


Building and Testing the System

- Ligation with T4 ligase to create engineered plasmid



- Transformation of clone into *lac*-competent cells



- Plate on Ampicillin/X-gal
- Dose with UV light to test abilities
- Miniprep/Digest to check successful assembly

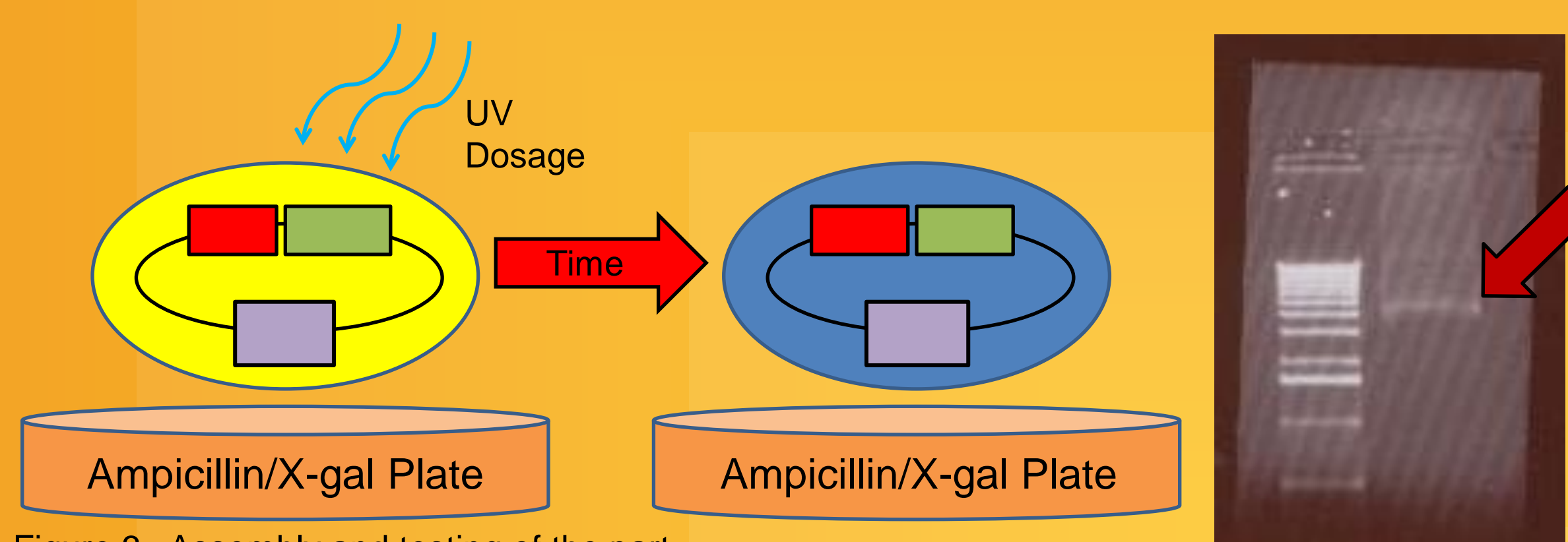


Figure 3: Assembly and testing of the part.

Modeling the SOS System

Assumptions:

- The UV irradiation is instantaneous, with a dosage of 5 J / m².
- The bacteria are not undergoing DNA repair at the time of irradiation.
- Thymine dimer formation by adjacent thymines is the major DNA damage occurring.
- The equilibrium point between RecA and RecA* is considered to be full induction of the SOS system.

$$\frac{d[LexA]}{dt} = \frac{a_L}{1 + K_L * C_L} - b_L * C_{R^*} * C_L - e_L * C_L$$

$$\frac{d[recA]}{dt} = \frac{a_R}{1 + K_R * C_L} - e_R * C_R - b_R * C_R * C_S + b_{R^*} * C_{R^*}$$

$$\frac{d[recA^*]}{dt} = b_R * C_R * C_S - b_{R^*} * C_{R^*}$$

$$[C_S] = \frac{S}{6 * 10^8}$$

- a_i is rate of production of i in absence of repression,
- b_i is the binding constant of component i to its activator,
- e_i is the inverse rate of breakdown of component i.
- L relates to LexA, R to RecA, R* to RecA* and S to ssDNA
- K_i is the binding constant of component i to the LexA gene.
- C_s is the concentration of single stranded DNA.

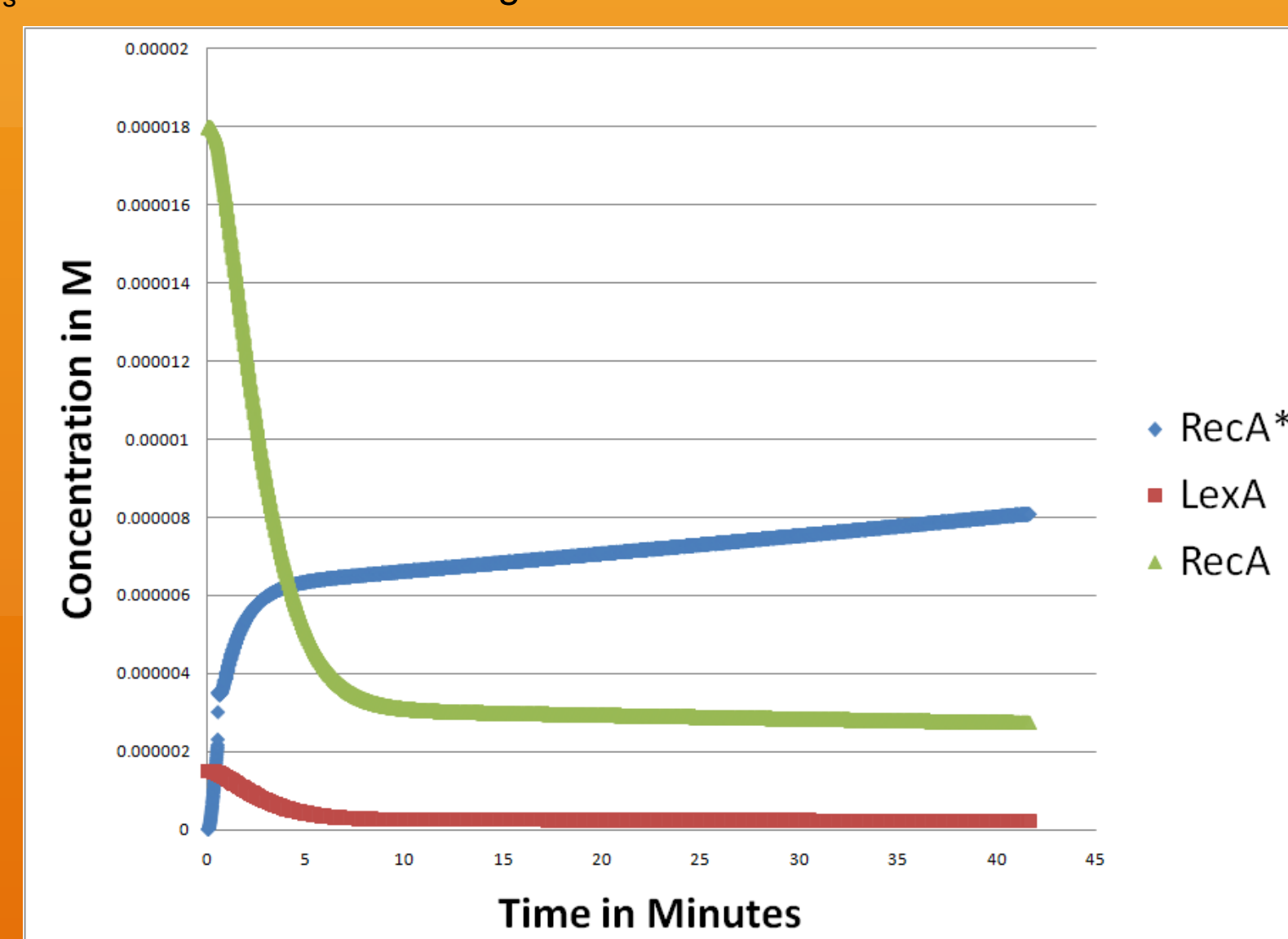


Figure 4: The numerical solution to the above differential equations. RecA and RecA* are equal at 4.2 minutes. This becomes time 0 for Xgal cleavage.

Predicted Response Time: Tens of Minutes

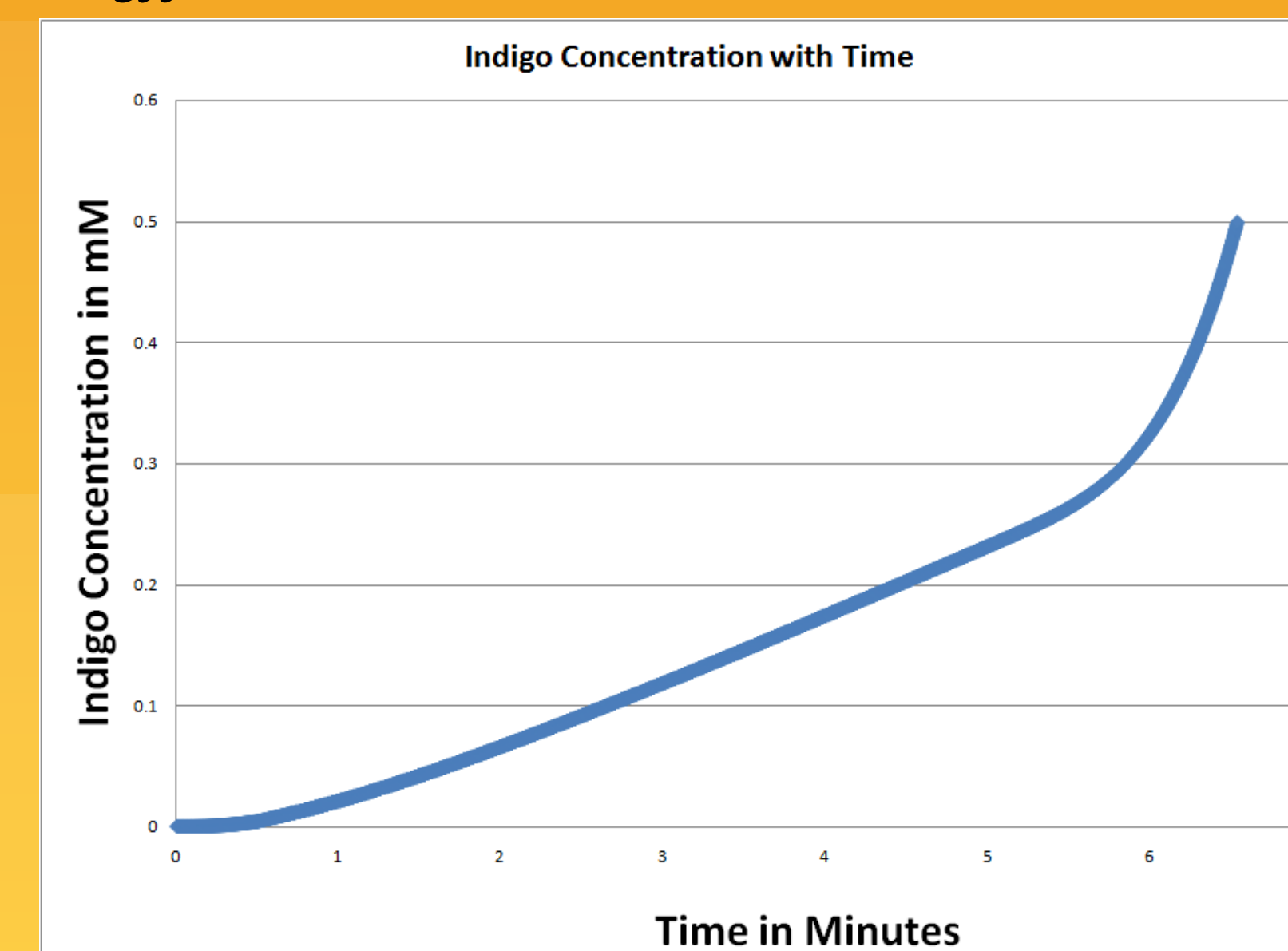
Assumptions:

- The bacteria have been on the X-gal plate sufficiently long for the X-gal to be in equilibrium with the cell.
- There is no diffusion limitation with this system.
- Half-life of beta-gal is 60 minutes. (Bachmair et al. 1986).
- The amount of X-gal within the cell exceeds K_m (500mM to 0.2 mM) so that the rate of indigo formation is V_{max}.
- V_{max} is a function of K_{cat} and the concentration of the enzyme.

$$\frac{d[indigo]}{dt} = \frac{V_m * [Xgal]}{K_m + [Xgal]} \rightarrow \frac{d[indigo]}{dt} = V_m \quad V_m = K_{cat} * [\beta gal]$$

$$\frac{d[indigo]}{dt} = K_{cat} * [\beta gal]$$

Equation solved using Runge-Kutta 4th order in Mathcad



Next Steps

- Perform additional experiments comparing UV radiation to SOS signal
- Compare and refine theoretical model
- Perform experiments with bacteria under UV with different SPF levels
- Develop a sustainable bacterial gel with a shelf life of >3 months
- Create a bio-sensor patch

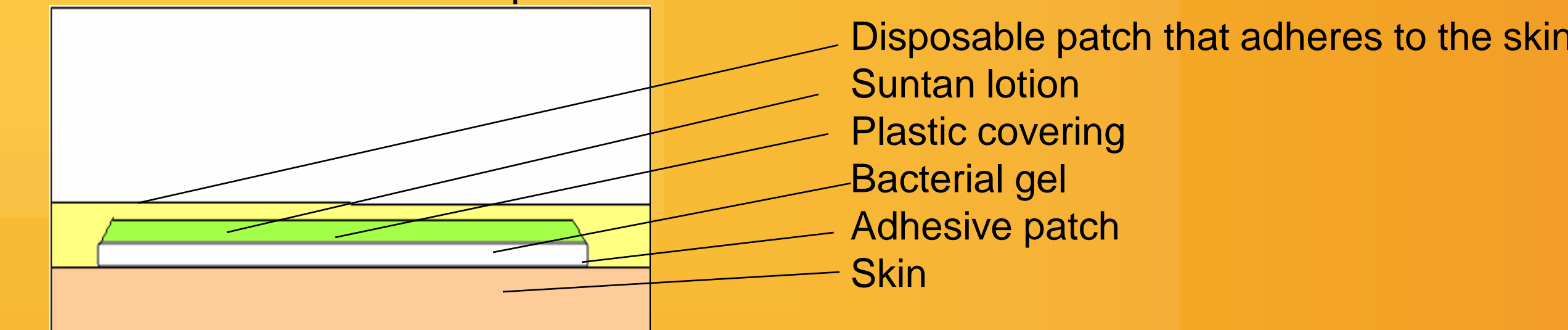


Figure 7: Theoretical utilization of this technology

Safety

- Negligible safety issues with project:
 - Pre-engineered *E. coli* cannot sustain a population outside lab environment
 - Biological Safety Level I only (low risk)
- Project reviewed and approved by the Institutional Biosafety Committee (IBC)
 - IBC oversees rDNA research at Purdue
 - No characteristics of protocol are considered hazardous

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- Dr. Jenna Rickus
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- Bindley Bioscience Center
- Oncological Sciences Center

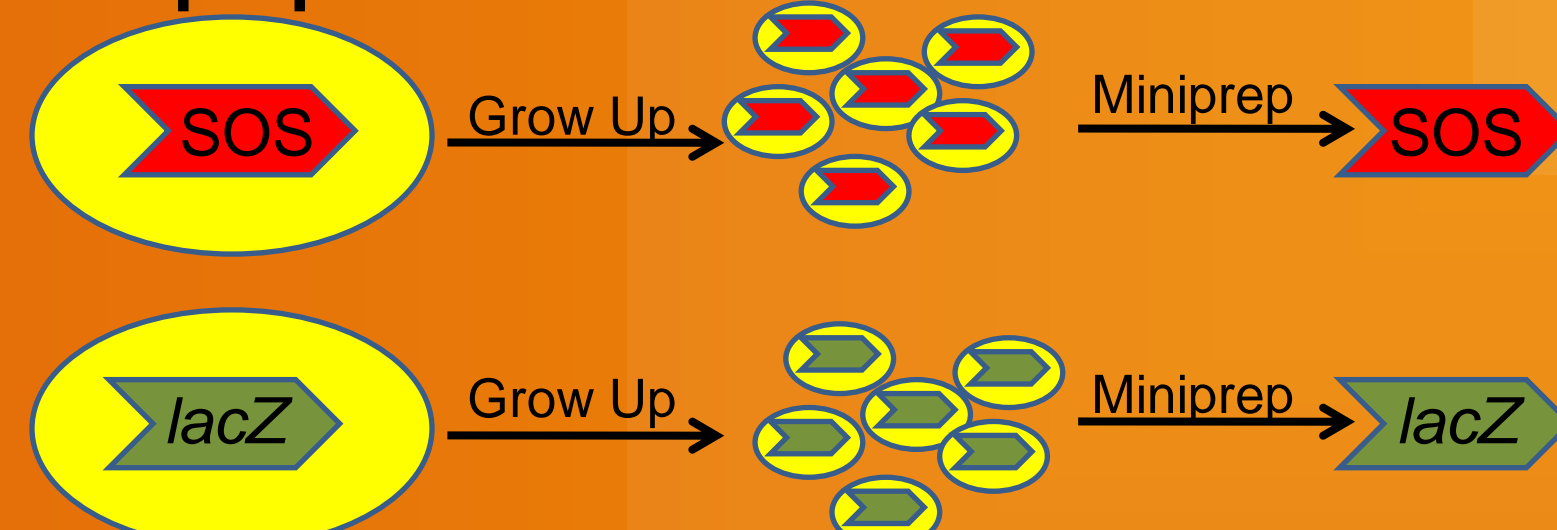
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Standard Assembly of the Part

- Received stabs of TOP10 cells from iGEM:
 - LacZ (I732017) and *recA* promoter (J22106)
 - Parts on standard pSB1A2 plasmids (AmpR)

- Grow up to make glycerol stocks
- Mini-prep to extract DNA



- Digestion:
 - SOS: EcoRI & SpeI
 - LacZ: EcoRI & XbaI
 - Specific Enzymes → SOS in front of LacZ
- Purify from 1% agarose gel

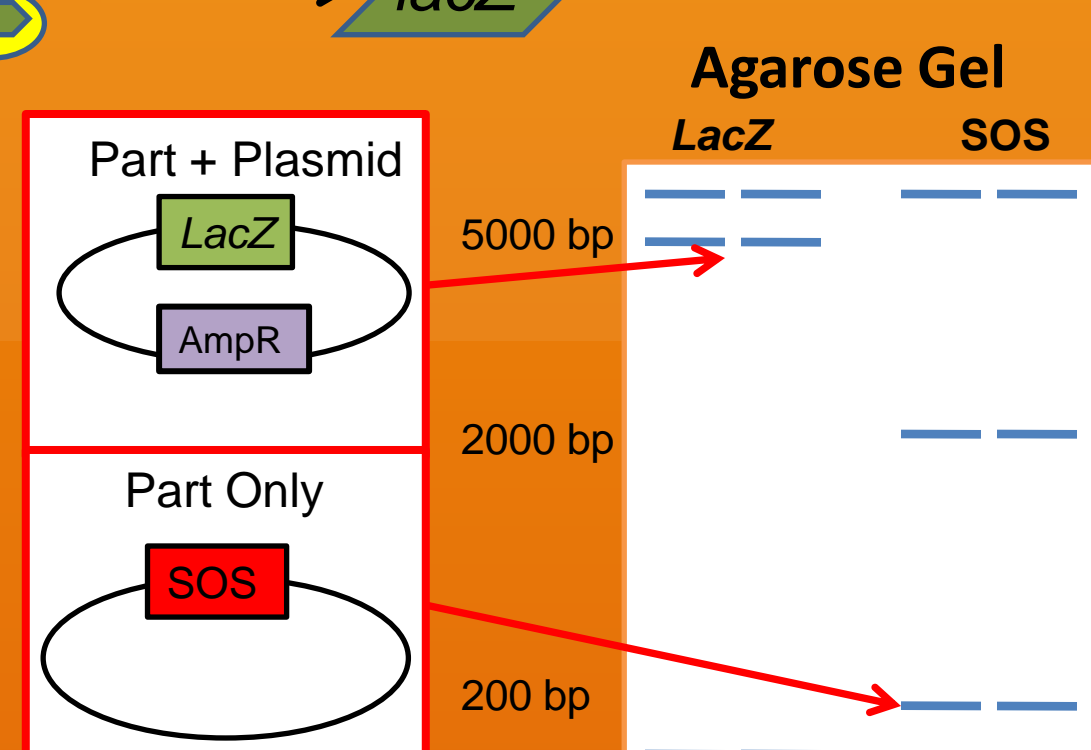


Figure 2: The construction and purification of our parts.