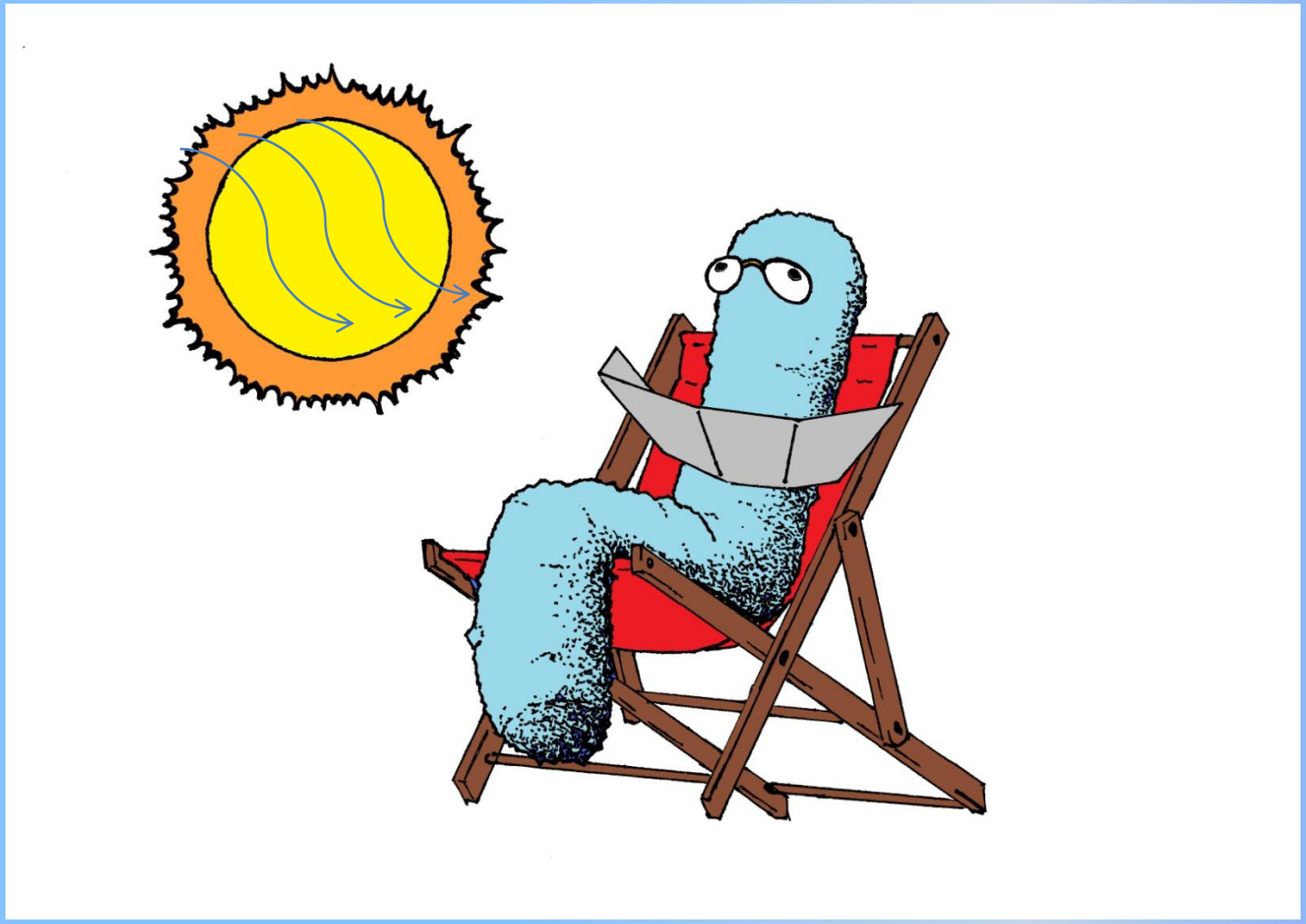
Engineering a Real-Time Living Biosensor: DNA Damage Caused by Ultra-Violet Irradiation

Craig Barcus, Jessamine Osborne,
Erin Rosswurm, Janie Stine

Purdue University, West Lafayette, IN

Concept



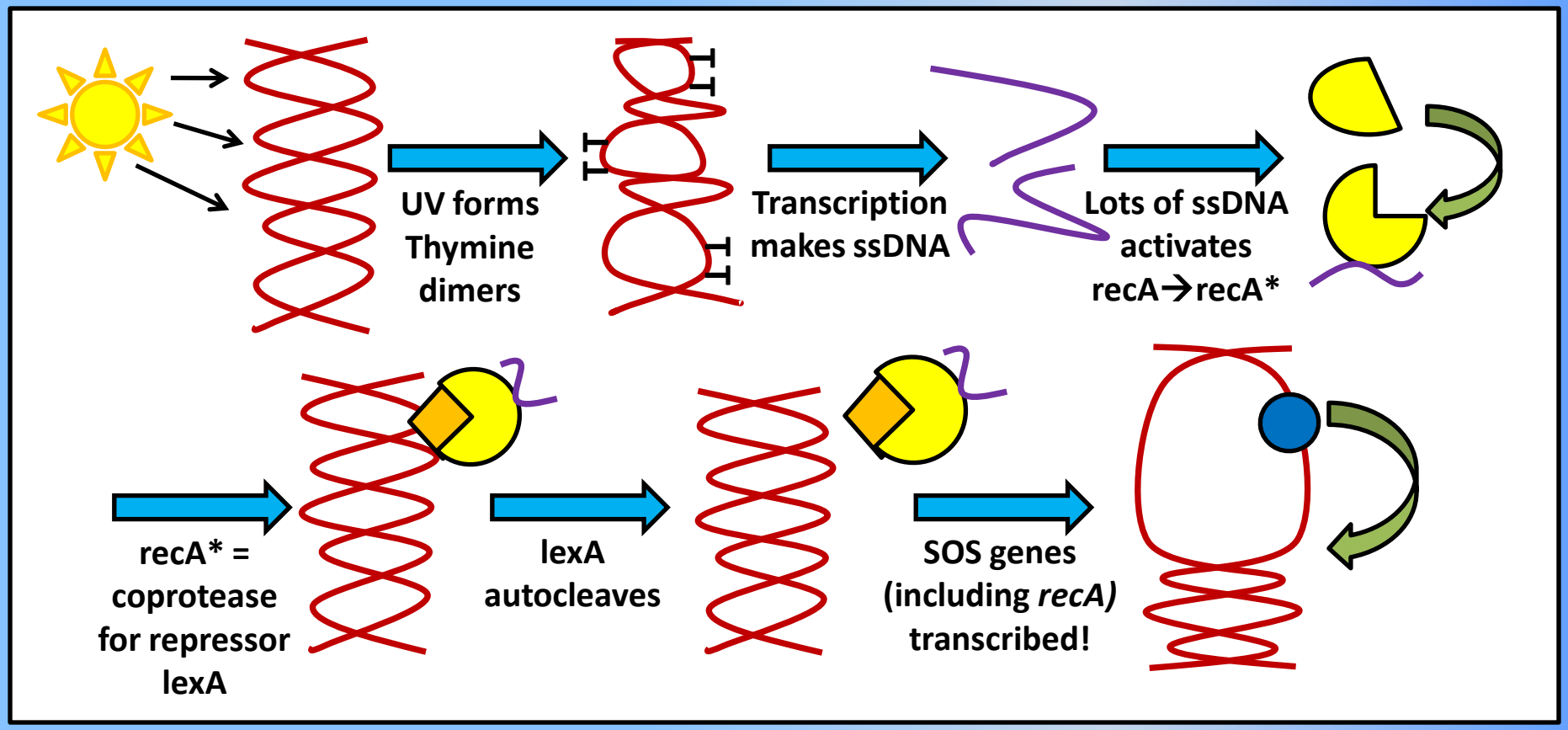
UV Irradiation from the Sun Activates *lacZ* pathway, cleaving X-gal

Why we want to create this construct

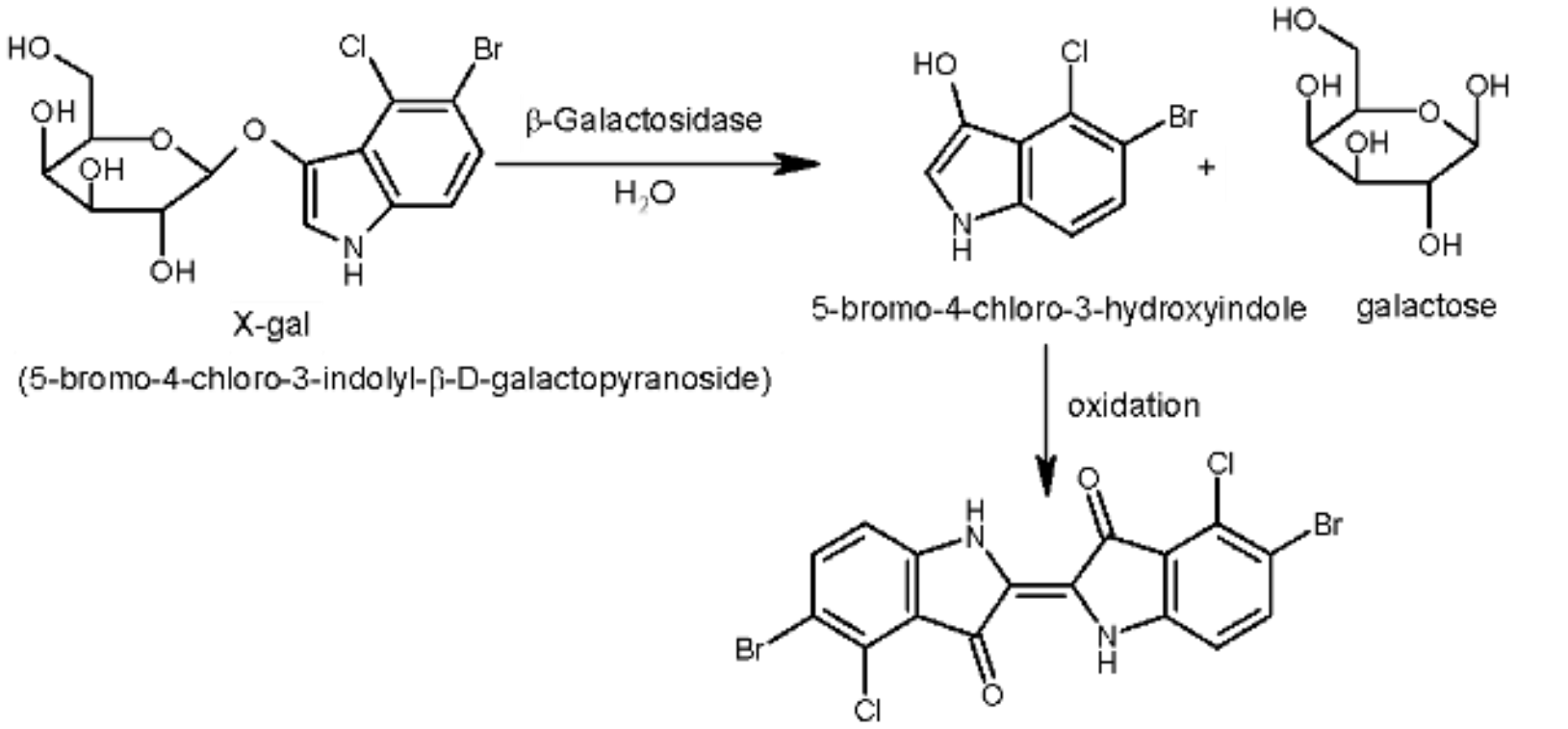
- 1 million Americans are diagnosed with skin cancer each year[1]
- Current products measure UV radiation level, *not* DNA damage
- Our Goal: create a real-time sensor of DNA damage

[1] American Academy of Dermatology, *Skin Cancer Fact Sheet*, 2008

SOS Induction and Repair Mechanism



LacZ Reporting: Cleavage of X-Gal



X-gal

(5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)

5-bromo-4-chloro-3-hydroxyindole

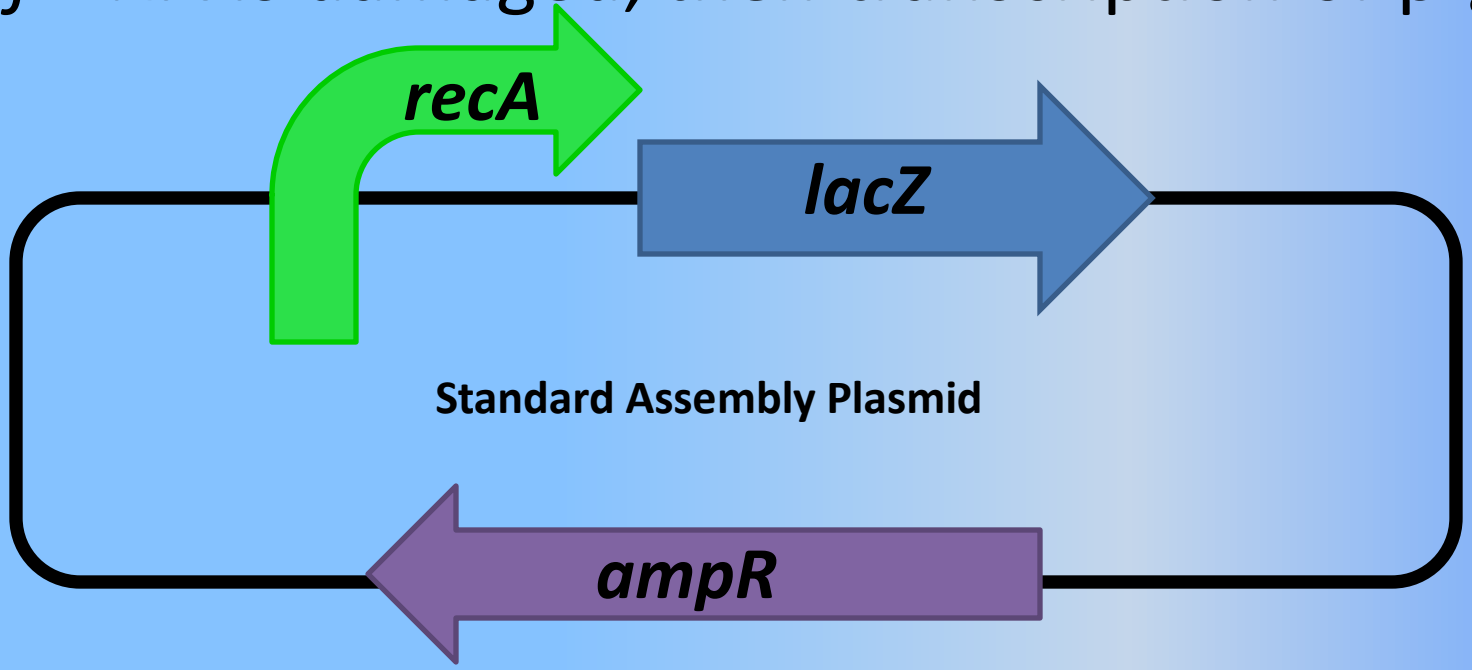
galactose

insoluble blue 5,5'-dibromo-4,4'-dichloro-indigo

(5,5'-dibromo-4,4'-dichloro-1H,1'H-(2,2')biindolylidene-3,3'-dione)

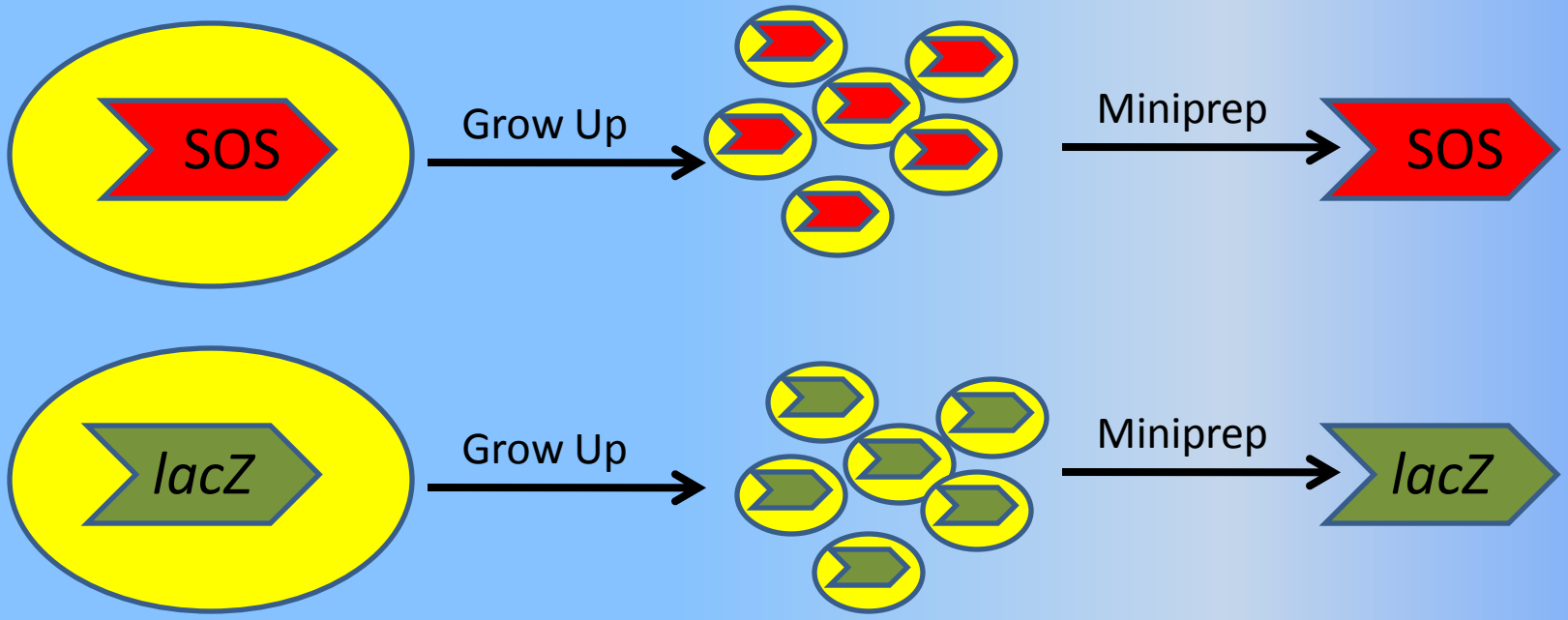
How we are going to create this construct

- "If-Then" Construct: Promoter + Reporter
  - Promoter: *recA* of SOS system (extreme DNA damage)
  - Reporter: *lacZ* + X-gal plate (blue/white screening)
- *If* DNA is damaged, *then* transcription of β-gal



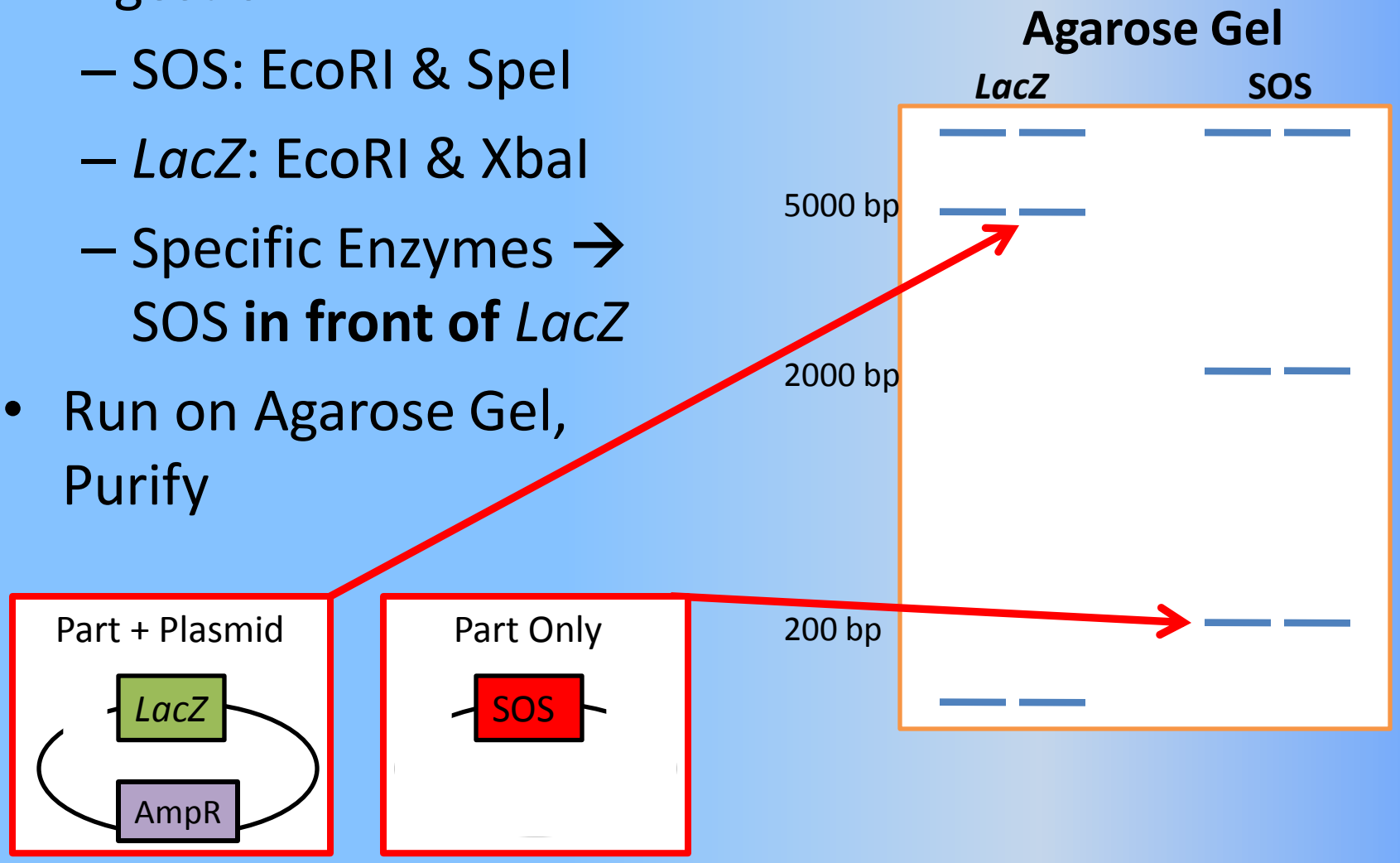
Isolating the Sub-Parts

- Stabs of TOP10 cells:
  - *LacZ* (I732017) and *recA* promoter (J22106)
  - Parts on standard pSB1A2 plasmids (AmpR)
- Grow up → glycerol stocks
- Mini-prep: extract DNA



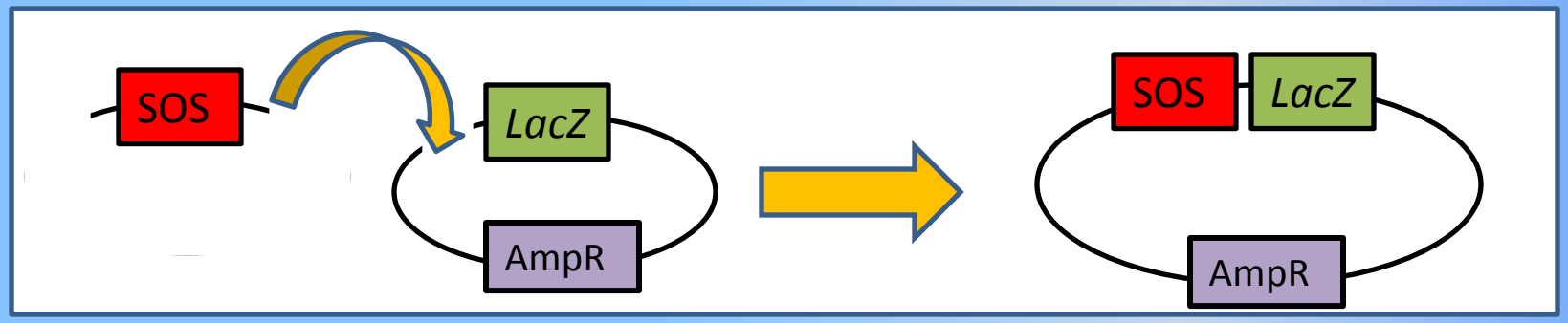
Standard Assembly of the Part

- Digestion:
  - SOS: EcoRI & SpeI
  - *LacZ*: EcoRI & XbaI
  - Specific Enzymes → SOS **in front of** *LacZ*
- Run on Agarose Gel, Purify

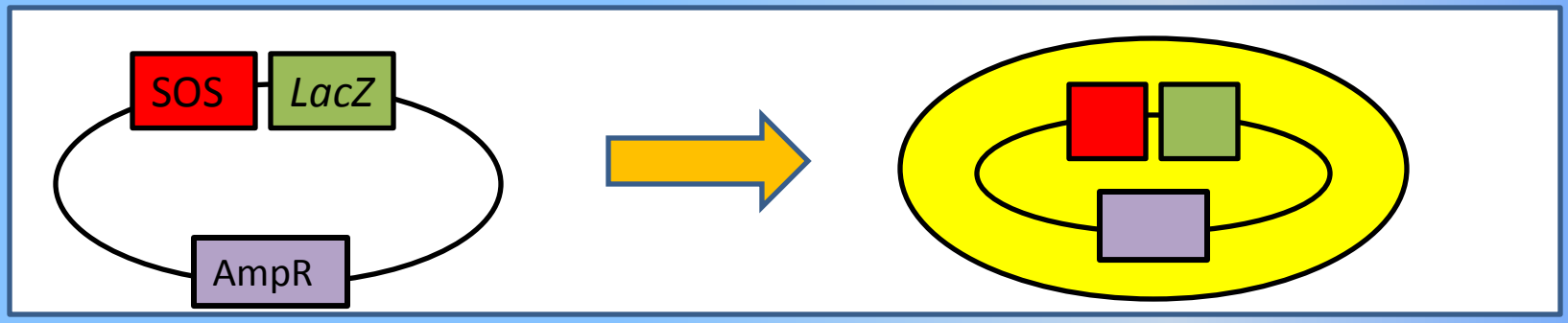


Building the System

- Ligation: Make our part

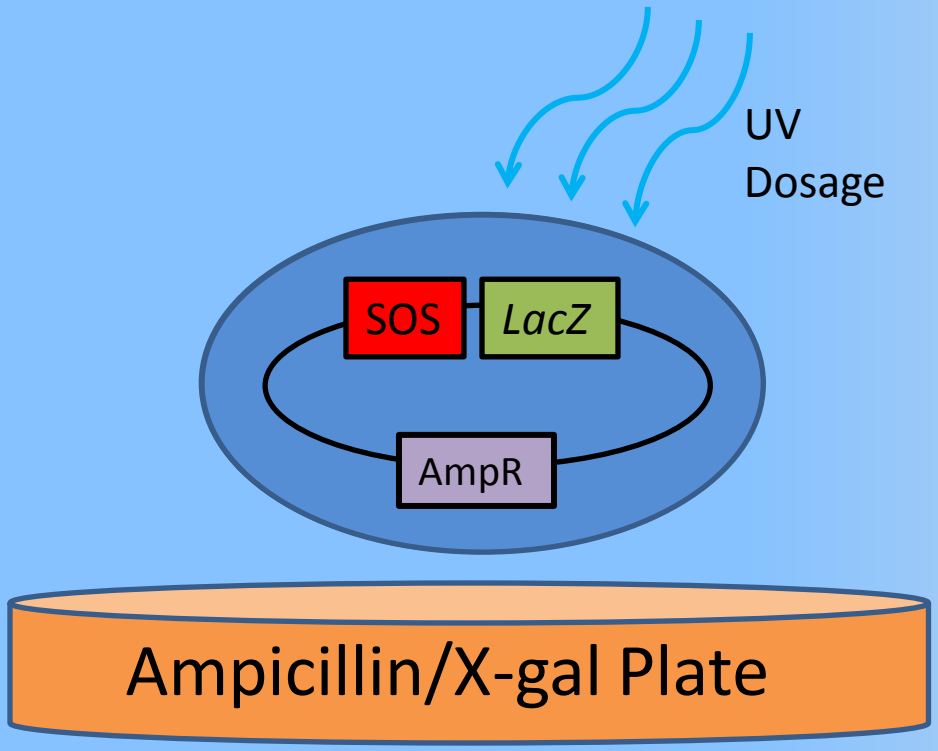


- Transformation
  - Use *lac*- chemically competent cells



Testing the System

- Plate on Ampicillin/X-gal
- Dose with UV light



- Miniprep/Digest to check assembly

Differential Equation Model of the SOS System

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

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

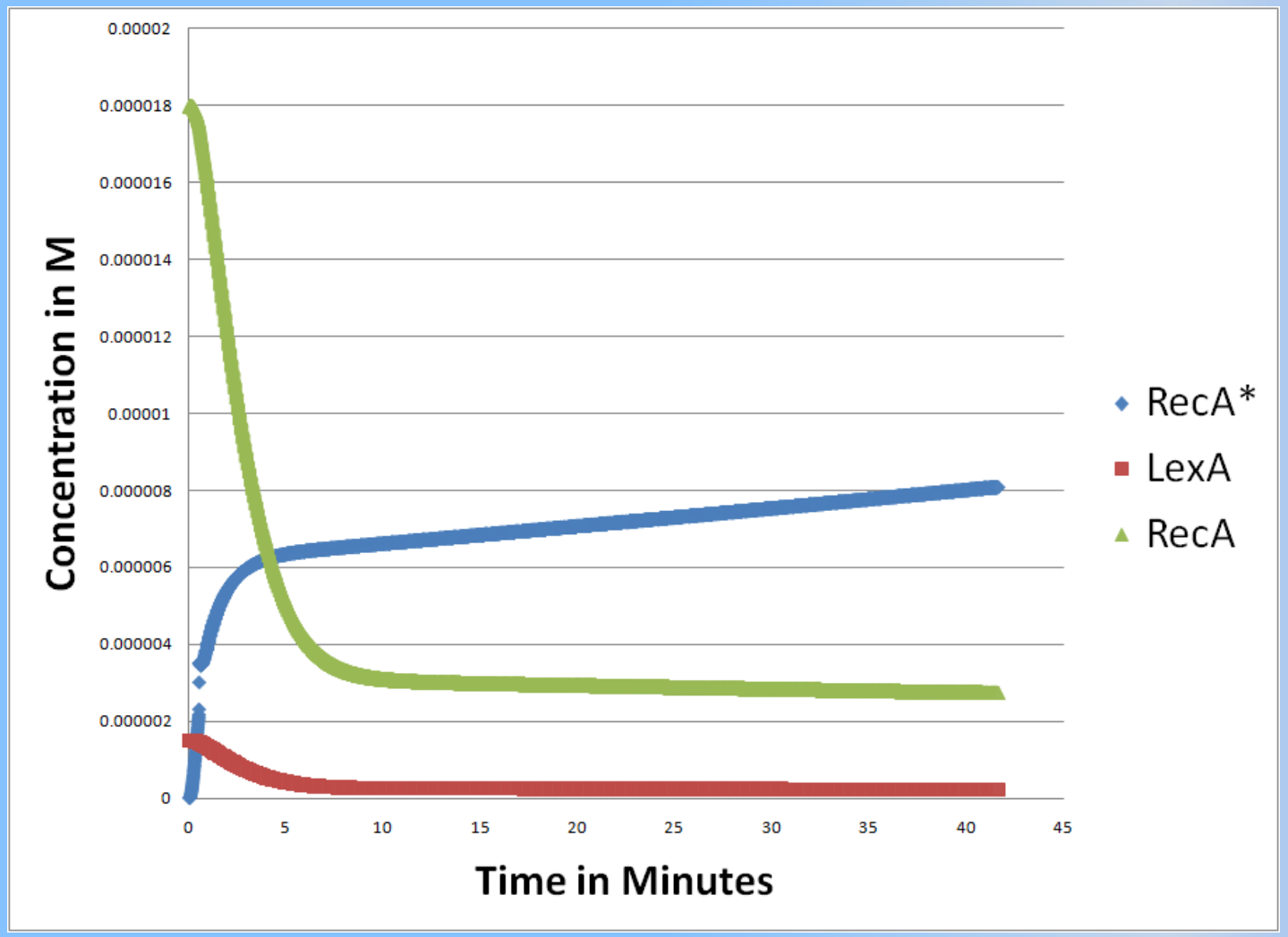
$$\frac{d[recA^*]}{dt} = b_R * C_R * C_S - b_{R^*} * C_{R^*}$$

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

- 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

Aksenov et al. 1997.

Numerically Modeling the System



- Assume that SOS repair begins when RecA concentration equals RecA*, which correlates to 4.2 minutes after UV irradiation.

- Assumed that rate of protein synthesis for both SOS and β-galactosidase is equivalent.

Modeling β-Gal cleavage of X-Gal

Assumptions

•The bacteria have been on the plate sufficiently long as to be in equilibrium with the X-gal plate (7.478*10^-16 mol per cell).

•The concentration of X-gal is constant at 0.5M within the cell at all times.

•The concentration of X-gal is sufficiently large to have the rate of change of indigo be equal to Vmax.

•Vmax is equal to Kcat multiplied by the concentration of β-galactosidase.

•The concentration of β-galactosidase is equal to that of RecA* since transcription of both genes occurs at the same time.

Kinetic parameters derived from Sharp et al. 1969. X-gal concentration from bios.niu.edu/johns/recdna/blue_white.htm. Uninduced level of β-Gal from Stryer, 2006.

Rate Equations for the Formation of the Indigo Color

$$\frac{d[indigo]}{dt} = \frac{Vm*[Xgal]}{Km+[Xgal]} \Rightarrow \frac{d[indigo]}{dt} = V_{\max}$$

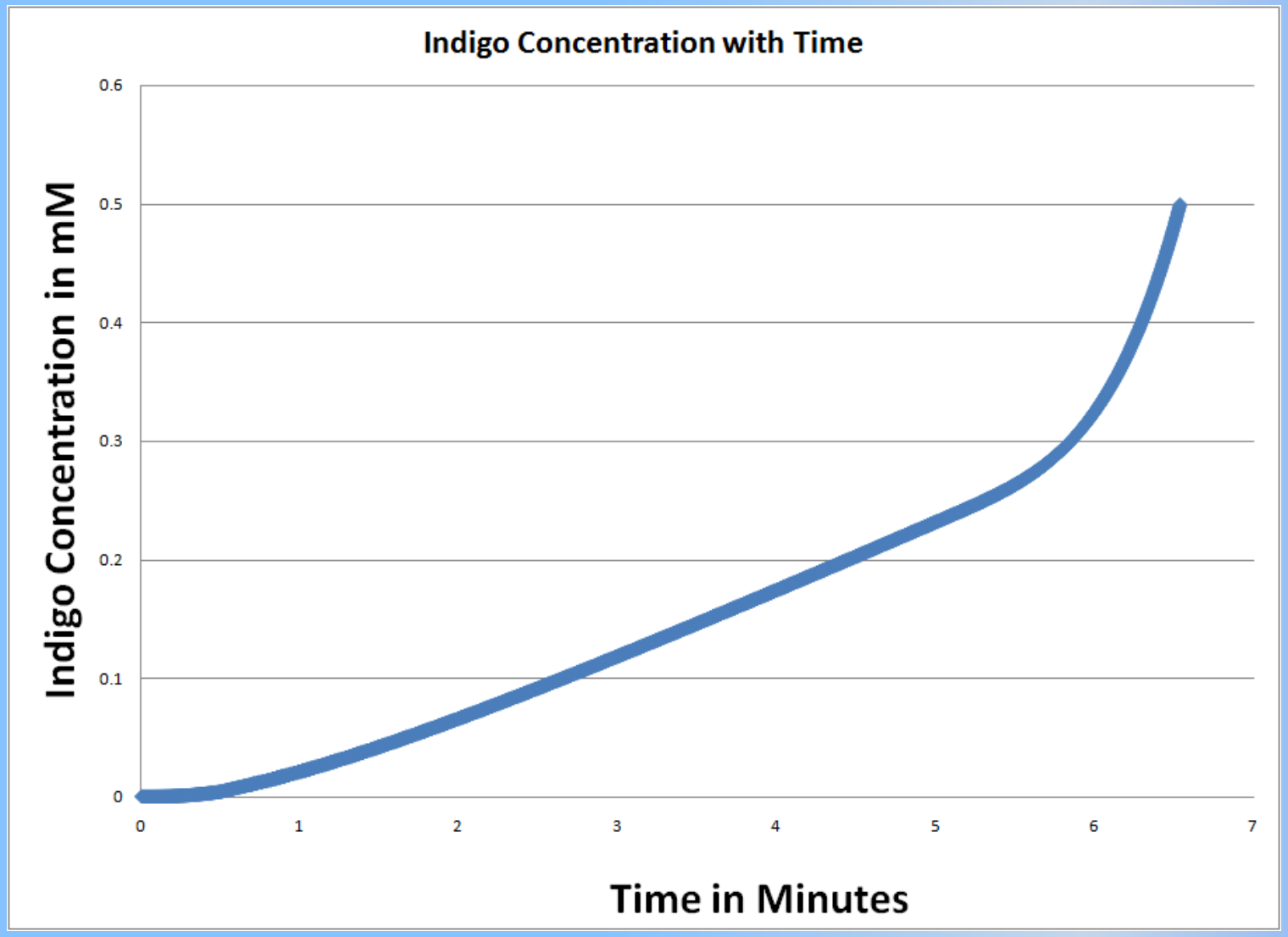
$$Vm = K_{cat} *[\beta gal]$$

$$[\beta gal] = [recA*]$$

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

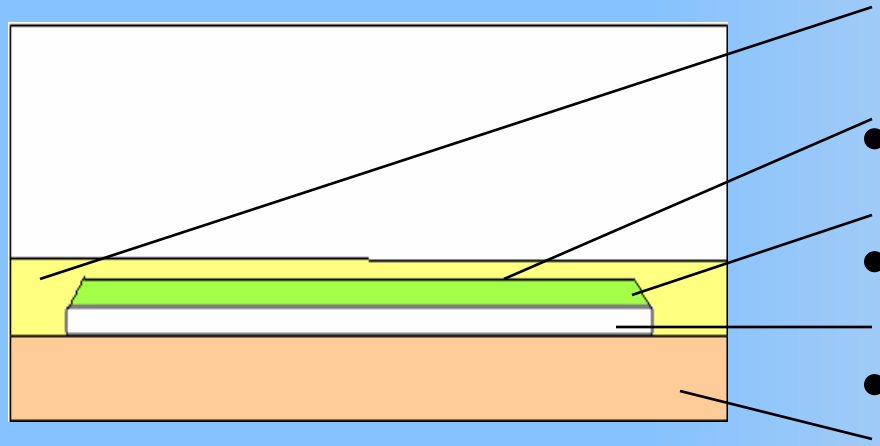
K_{cat} = 1.52*10^{-5} Molar Xgal / molecule β-gal * second
[recA*] is derived from the above model of SOS induction.

Simulating the Concentration of Indigo as a Function of Time



- We see that as the SOS system continues to be transcribed, more and more enzyme becomes produced, leading to a rapid formation of Indigo

Implementation



- Disposable patch that adheres to the skin
- Suntan lotion
- Plastic covering
- Bacterial gel
- Adhesive patch
- Skin

Next Steps

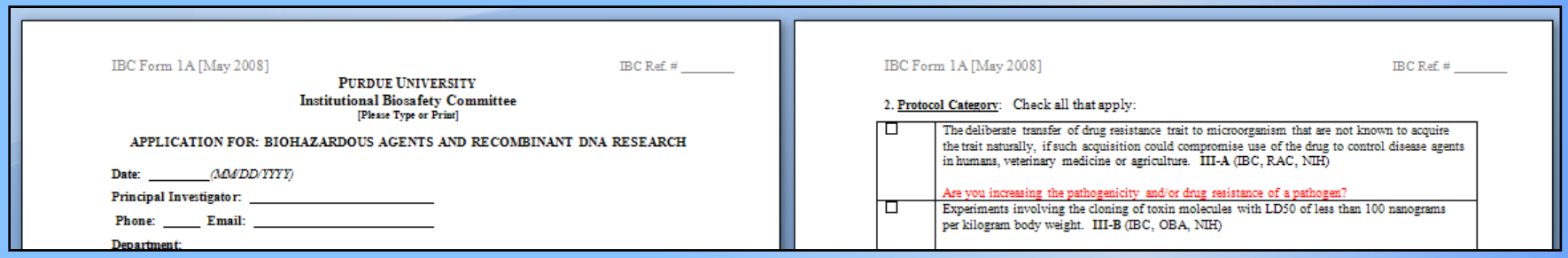
- Perform experiments comparing UV radiation to SOS signal
- Compare and refine theoretical model
- Perform experiments with bacteria under UV with different SPF numberings
- Develop a sustainable bacterial gel with a shelf life of >3 months

Biological Safety

- Negligible safety issues with project:
  - *E. coli* cannot sustain a population outside lab
  - Biological Safety Level I

Project reviewed and approved by the Institutional Biosafety Committee (IBC)

- IBC oversees rDNA research at Purdue
- Protocol not considered hazardous



IBC Form 1A [May 2008] IBC Ref. # _____

PURDUE UNIVERSITY
Institutional Biosafety Committee
[Please Type or Print]

APPLICATION FOR: BIOHAZARDOUS AGENTS AND RECOMBINANT DNA RESEARCH

Date: _____(MM/DD/YYYY)
Principal Investigator: _____
Phone: _____ Email: _____
Department:

IBC Form 1A [May 2008] IBC Ref. # _____

2. Protocol Category: Check all that apply:

☐	The deliberate transfer of drug resistance trait to microorganism that are not known to acquire the trait naturally, if such acquisition could compromise use of the drug to control disease agents in humans, veterinary medicine or agriculture. III-A (IBC, RAC, NIH) Are you increasing the pathogenicity and/or drug resistance of a pathogen?
☐	Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight. III-B (IBC, OBA, NIH)

Club Plans

- Recruit more members for IGEM
- Become more involved in IBE (Institute of Biological Engineering)
- Possibly continue to work on this project
  - Modify the bacteria to match mammalian cell damage and repair mechanisms

Materials and Expertise Obtained from the following:

- Biology Teaching Labs
- Simran Banga
- Bob Stephenson
- Dr. Kari Clase
- Dr. Luo
- Dr. Jenna Rickus
- Bindley Bioscience Center

Funding Provided By:

- College of Agriculture: Office of Academic Programs, International Programs in Agriculture, Agricultural Research Programs
- College of Engineering: Office of the Dean
- Bindley Bioscience Center
- Oncological Sciences Center at Purdue
- Department of Agricultural and Biological Engineering

References

- Aksenov, S. V., E. A. Krasavin, et al. (1997). "Mathematical model of the SOS response regulation of an excision repair deficient mutant of Escherichia coli after ultraviolet light irradiation." Journal of Theoretical Biology **186(2): 251-260.**
- Berg, J. T., JL. Stryer, L. (2006). Biochemistry. New York, NY, W.H. Freeman and Company.
- bios.niu.edu/johns/recdna/blue_white.htm.
- Sharp, A. K., G. Kay, et al. (1969). "KINETICS OF BETA-GALACTOSIDASE ATTACHED TO POROUS CELLULOSE SHEETS." Biotechnology and Bioengineering **11(3): 363-&.**
- Warner, J. B. and J. S. Lolkema (2002). "LacZ-promoter fusions: the effect of growth." Microbiology-Sgm **148: 1241-1243.**

Questions?