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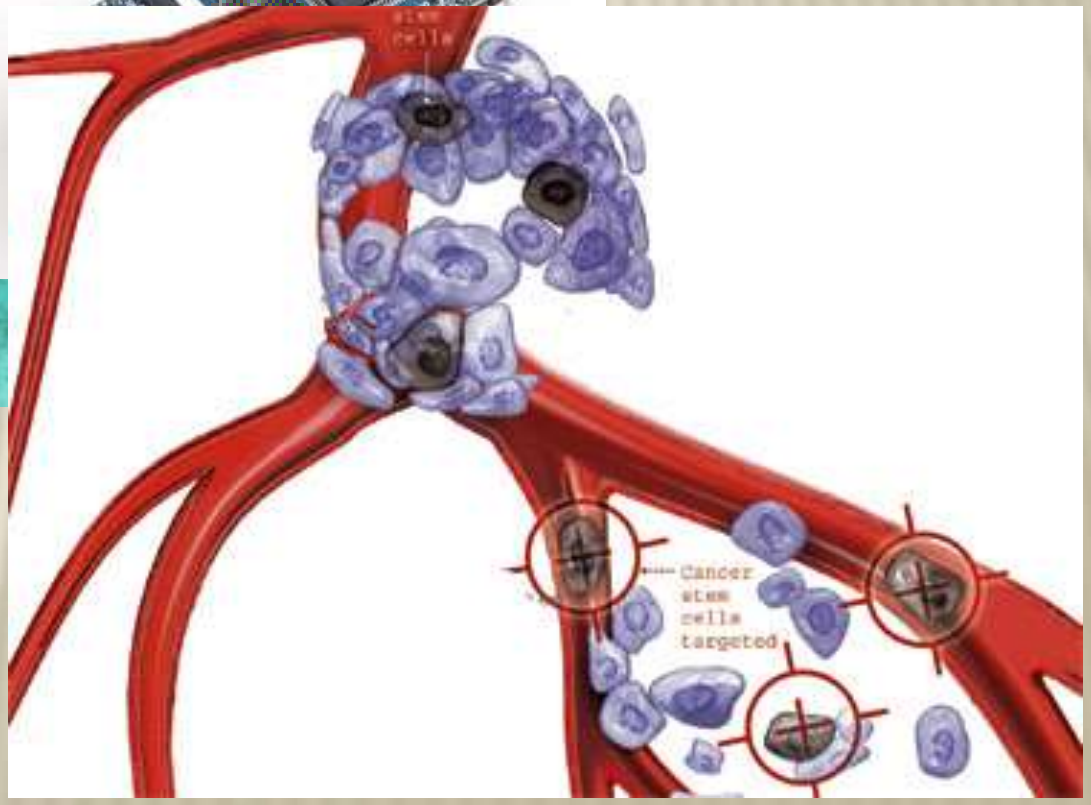
Mentored by Mark Gomelsky and Min-Hyung Ryu
Funded by Wyoming NSF EPSCoR

SYNTHETIC OPTOGENETIC SYSTEM TO STUDY BACTERIAL PATHOLOGY

SIGNIFICANCE

Treating disease non-invasively



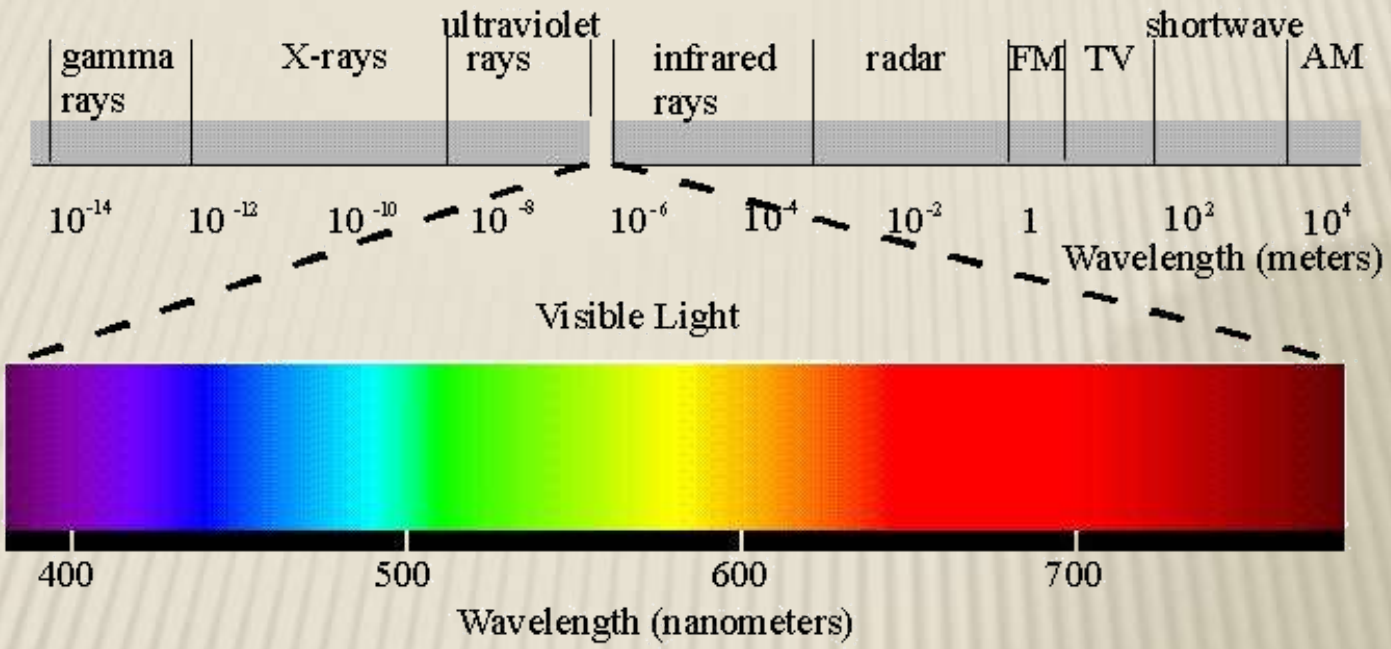
ADVANTAGES OF LIGHT

Light offers unprecedented advantages over chemicals (drugs) for regulating biological processes in vivo.

light *versus* **drugs**

- Few, if any side effects
- Spatial precision (single cells)
- Temporal control
- Reversibility

WHY NIR LIGHT?



Near Infrared Light (NIR)

- Deepest Tissue Penetration
- Harmless

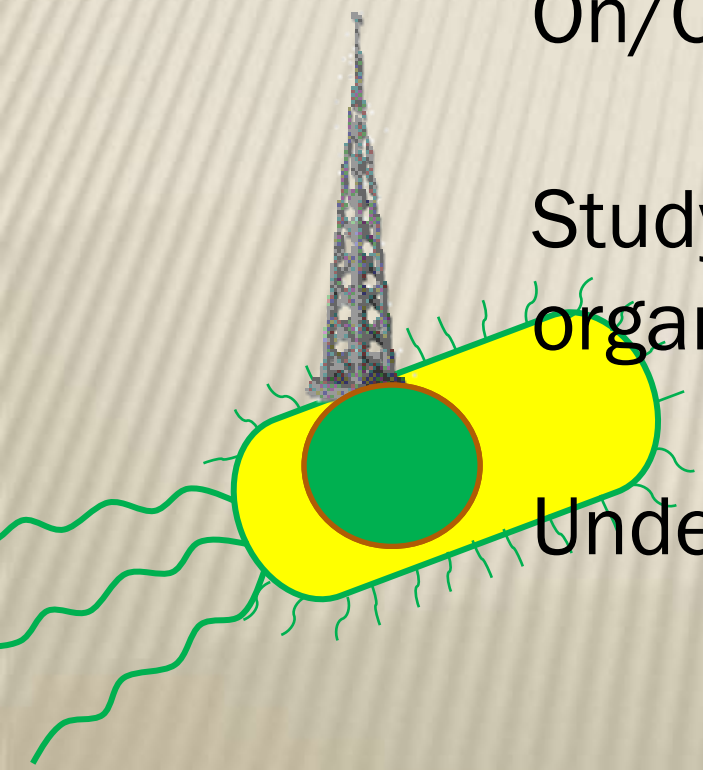
REMOTE SYSTEM TO CONTROL GENE EXPRESSION

Precise spatial and temporal control

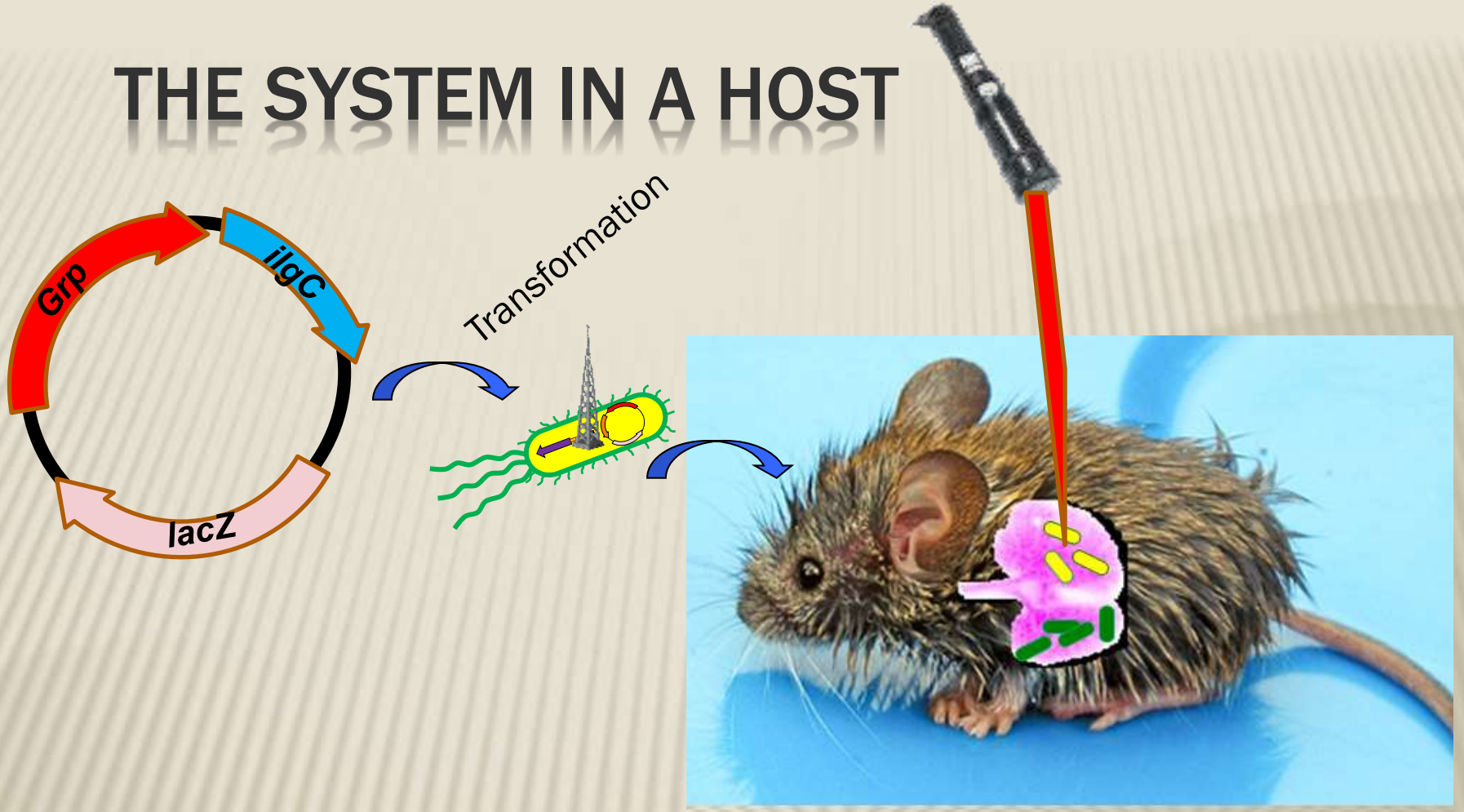
On/Off switch for gene expression

Study disease in model host organisms

Undetected by the pathogen

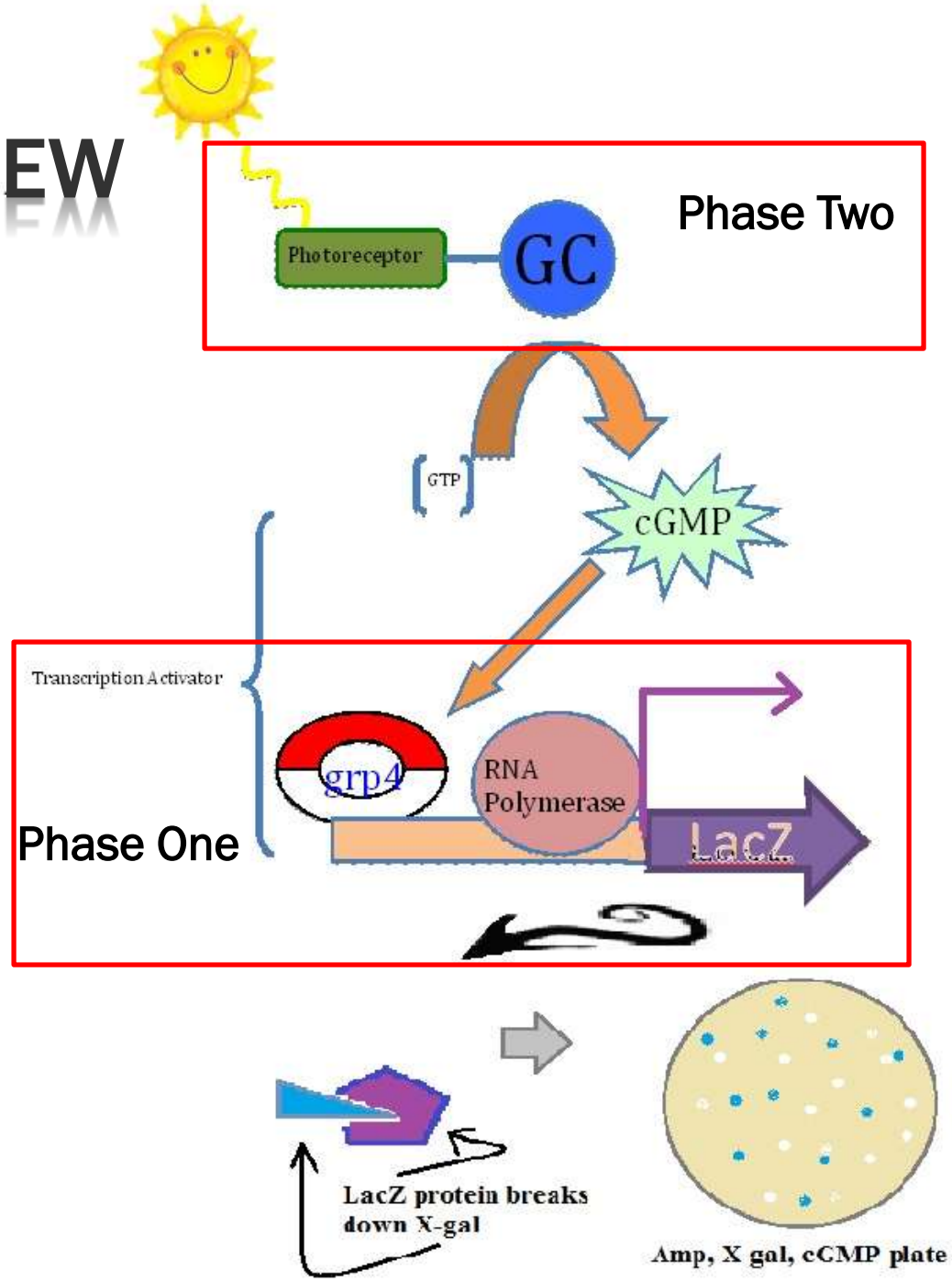


THE SYSTEM IN A HOST

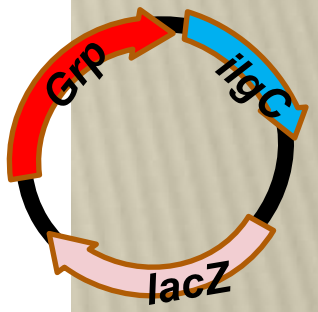


Precision photocontrol of gene expression in bacterial pathogens *in vivo*

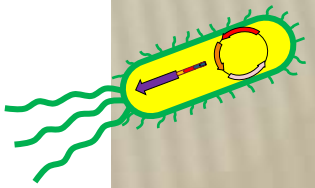
OVERVIEW



- Fusion and Mutagenic PCR on gene
- Digestion and Ligate into Plasmid



- Transfrom into E.coli

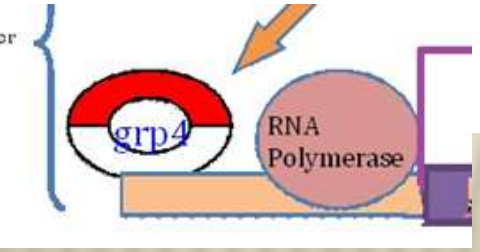


PHASE ONE: MAKING TRANSCRIPTION ACTIVATOR SENSING CGMP

cAMP binding domain native to E. coli

cGMP=
bones

ling domain
. centenum



Accomplished with Fusion PCR
Insert into plasmid by ligation
Transform into E. coli

MAKING GRP TRANSCRIPTION ACTIVATOR

grp1

Original from R. centenum

-No RNA polymerase = no transcription
-No DNA binding

Using Fusion PCR

grp2

-Binds DNA
-Binds cGMP

-No RNA polymerase = no transcription

Random Mutagenesis PCR to fix grp2

grp3.2

grp3.3

grp3.4

grp3.1

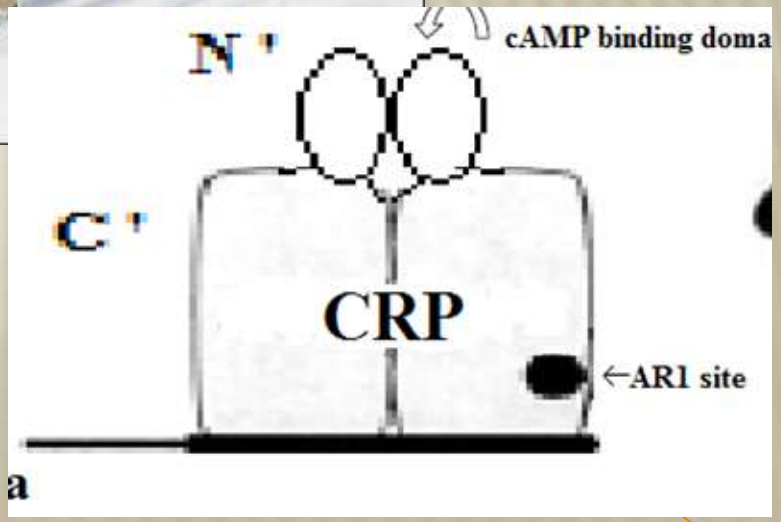
Grp3.5

AR1 site was missing

AR1 site recruits RNA polymerase for transcription

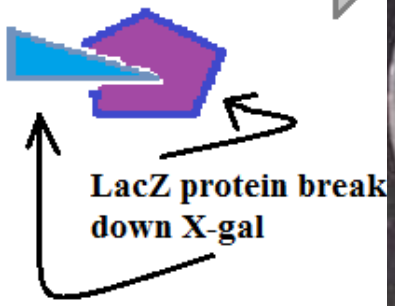
grp4

Fusion PCR to add AR1 site



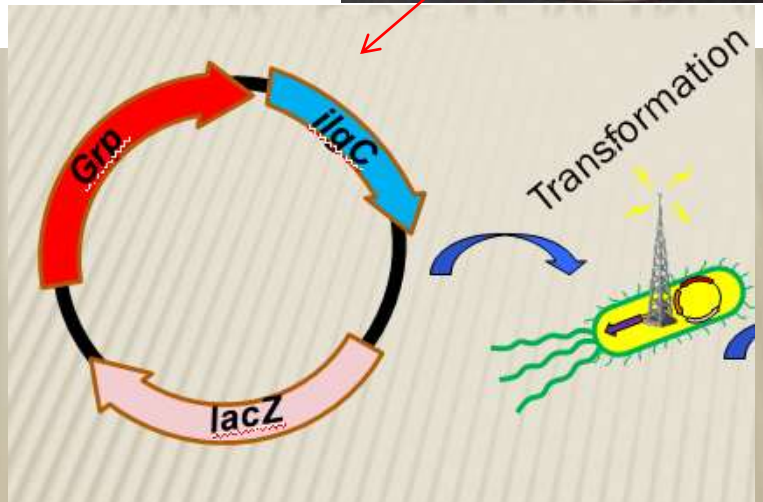
RESULTS

Binds cGMP
Binds DNA
Gene expression

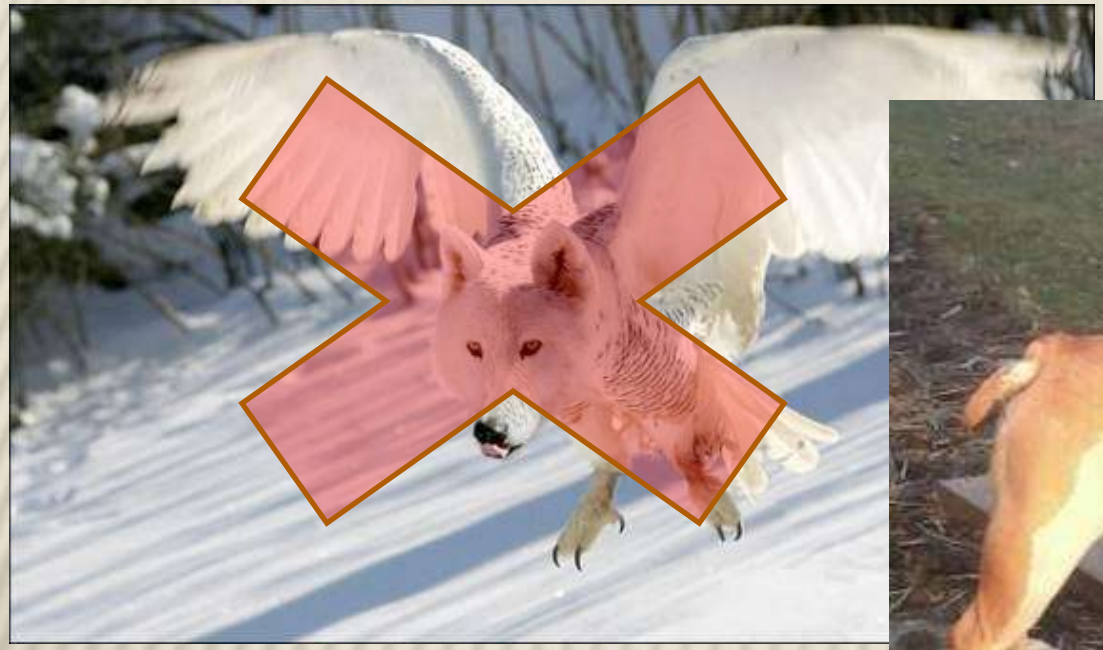


Isolate blue plasmid

re-transform to double check



NO BLUE COLONIES = NO GENE EXPRESSION



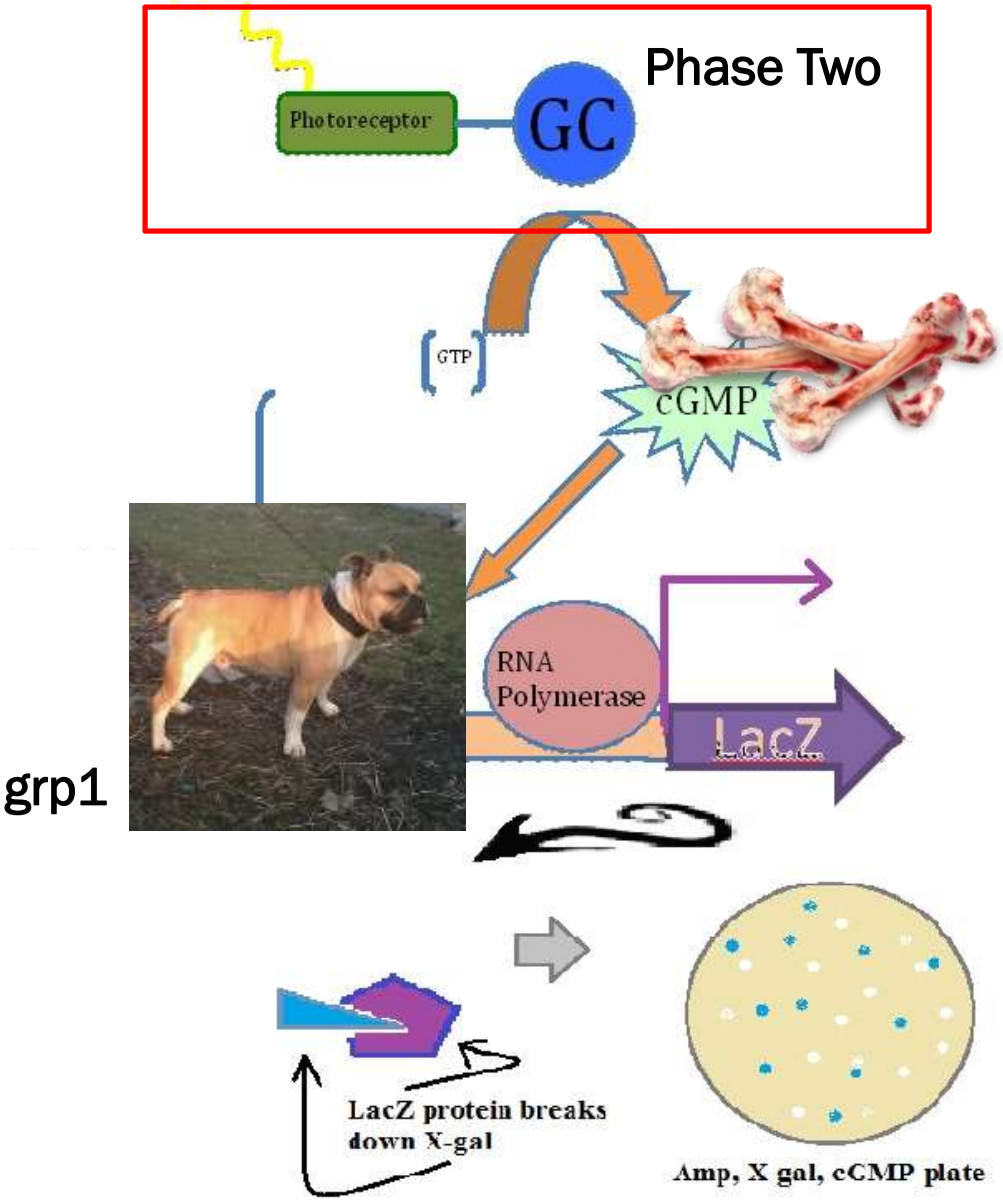
Increase cGMP production

grp1

Use R. centenum transcription activator!

FUTURE WORK NEAR IR LIGHT ACTIVATED CYCLASE

Make a strong NIR Photoactivated Guanylyl Cyclase



THANK YOU!

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Mark Gomelsky

Min-Hyung Ryu

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