iGEM 2007

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Diauxie Elimination:
Increasing bioenergy efficiency

Bio-Dosimeter : Bacterial
Radiation Sensing

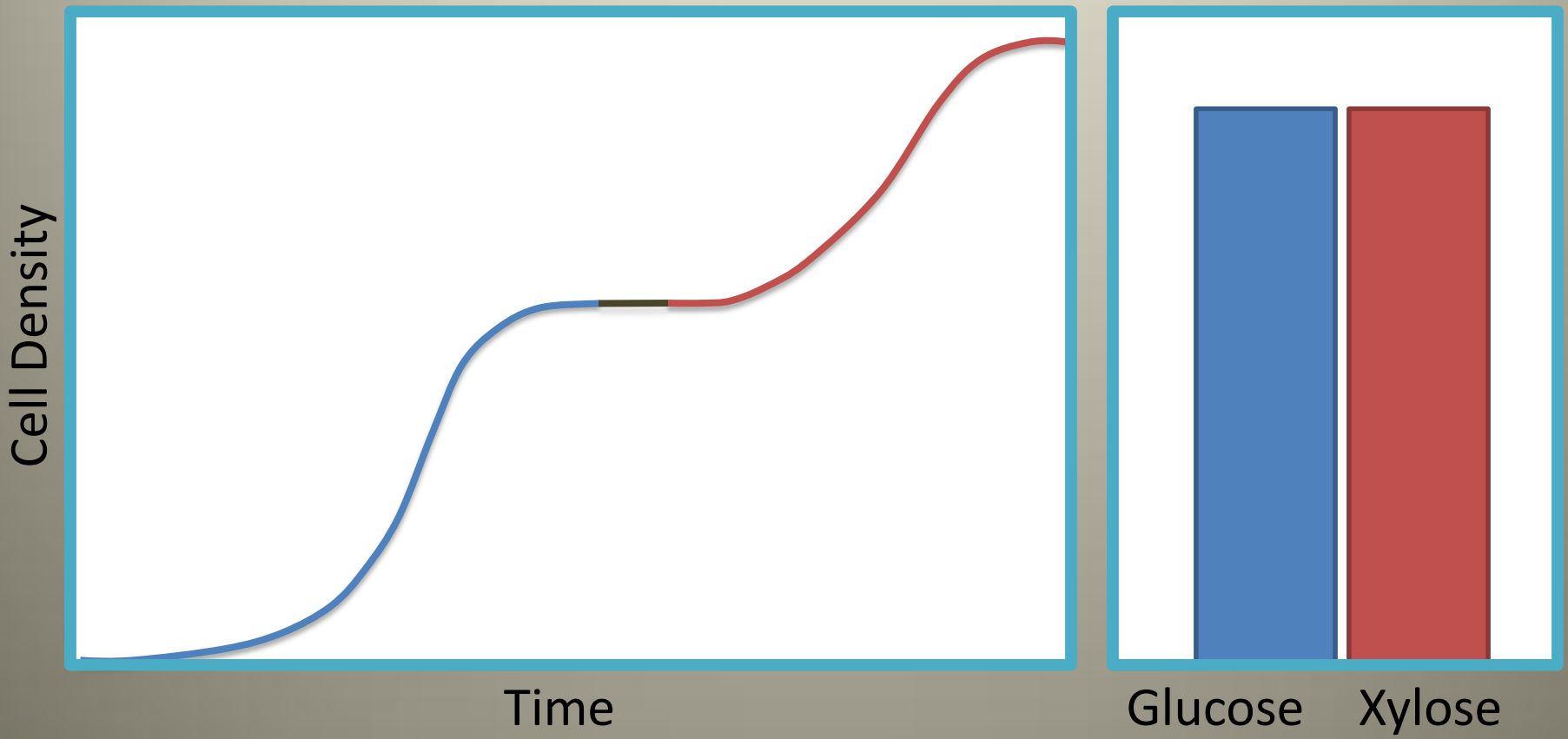
Diauxie Background

- Traditional energy sources are not sustainable
- Biomass is a renewable resource
- Process still not efficient enough to be widely used

bioreactor

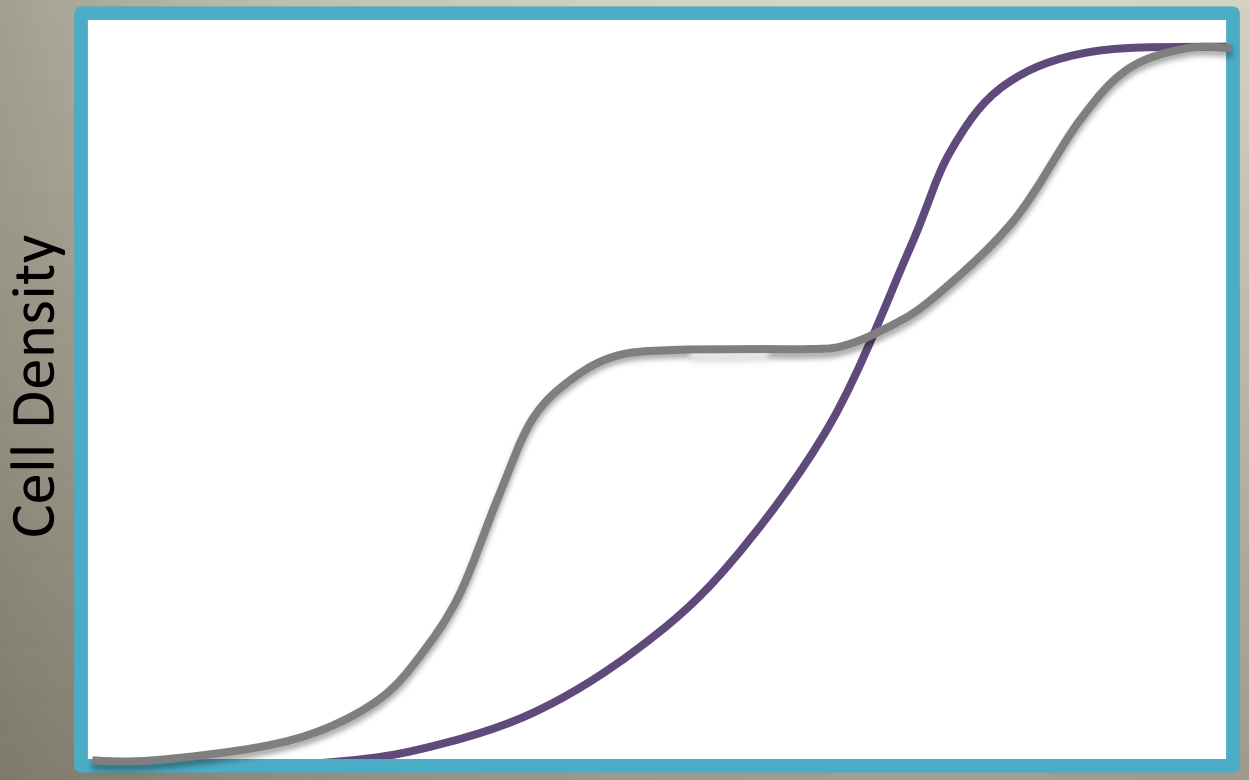
Diauxie Background

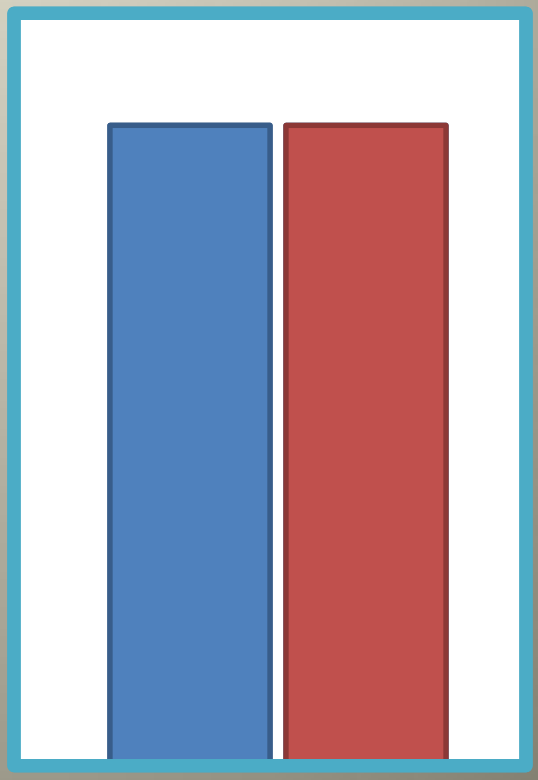
- Diauxie – cells growing in sugar mixtures will metabolize them one at a time.



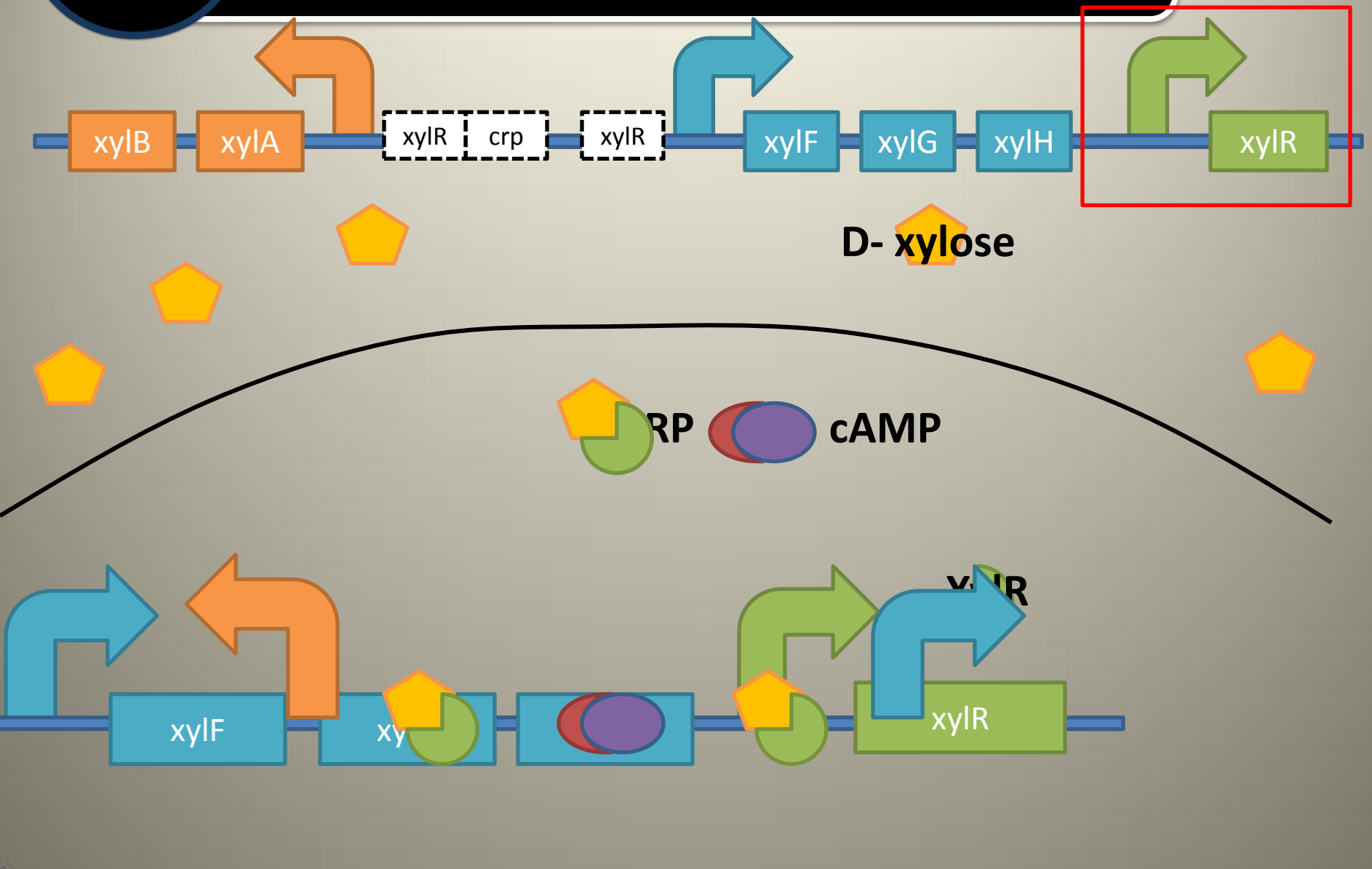
Eliminating Diauxie

- Eliminating Diauxie could increase the efficiency of bioreactors..

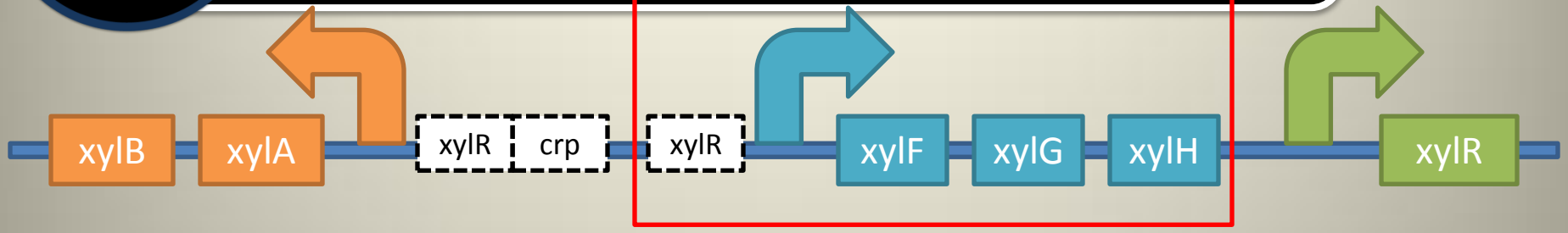


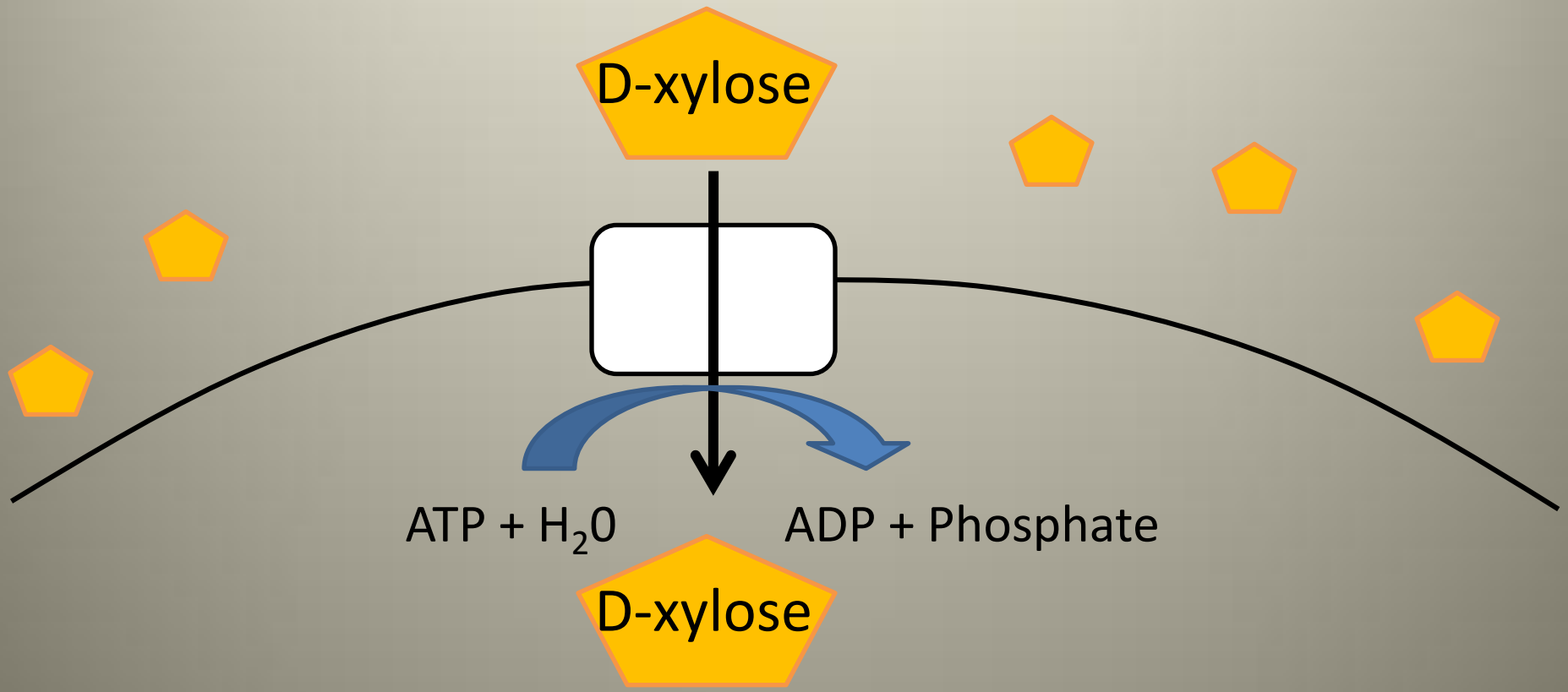


Xylose Metabolism



Xylose Metabolism





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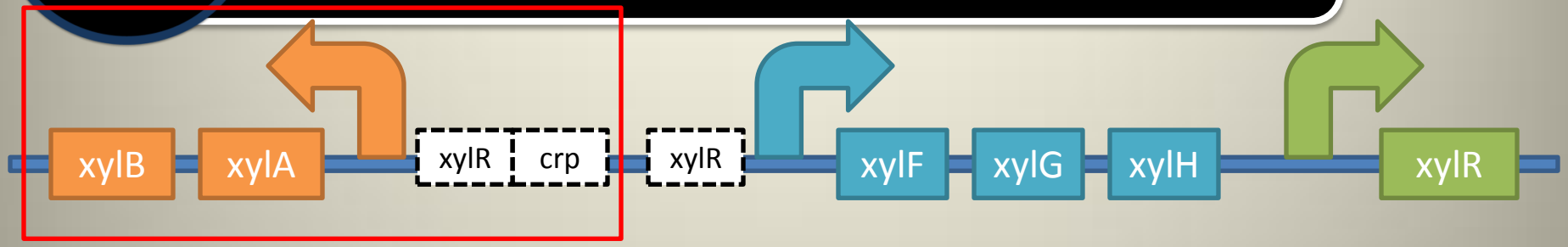
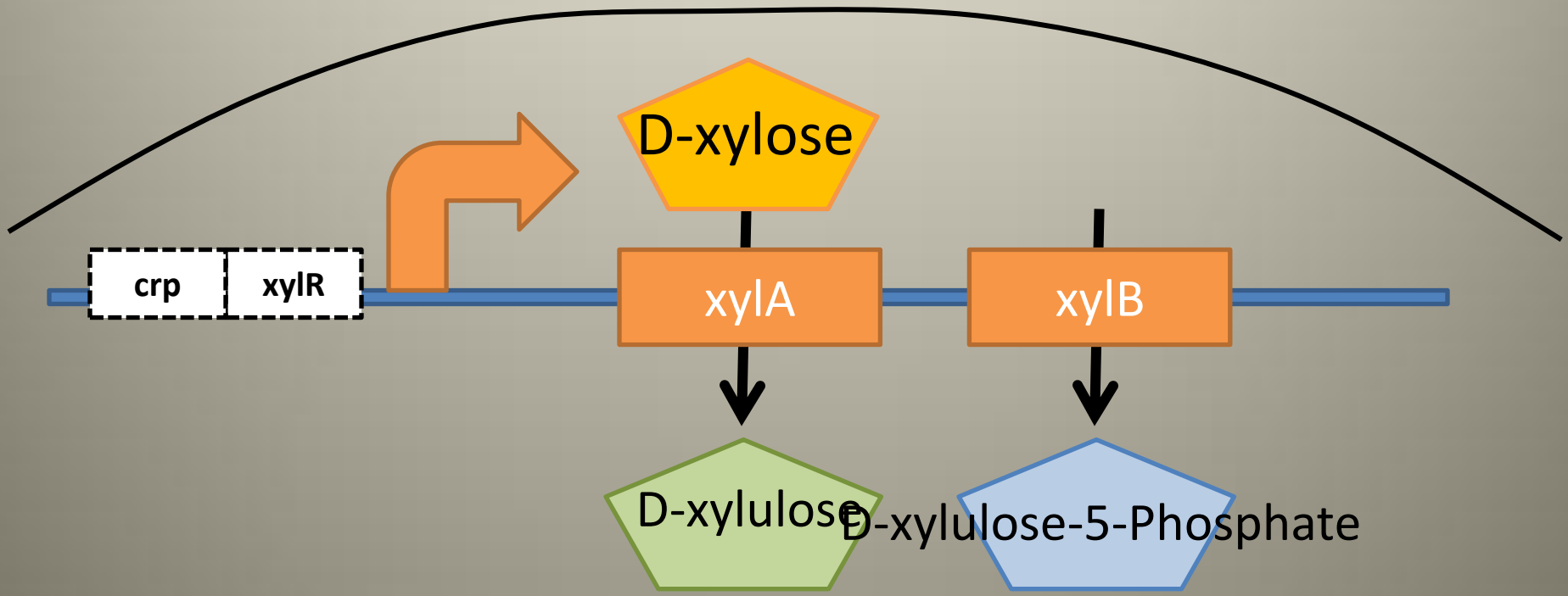
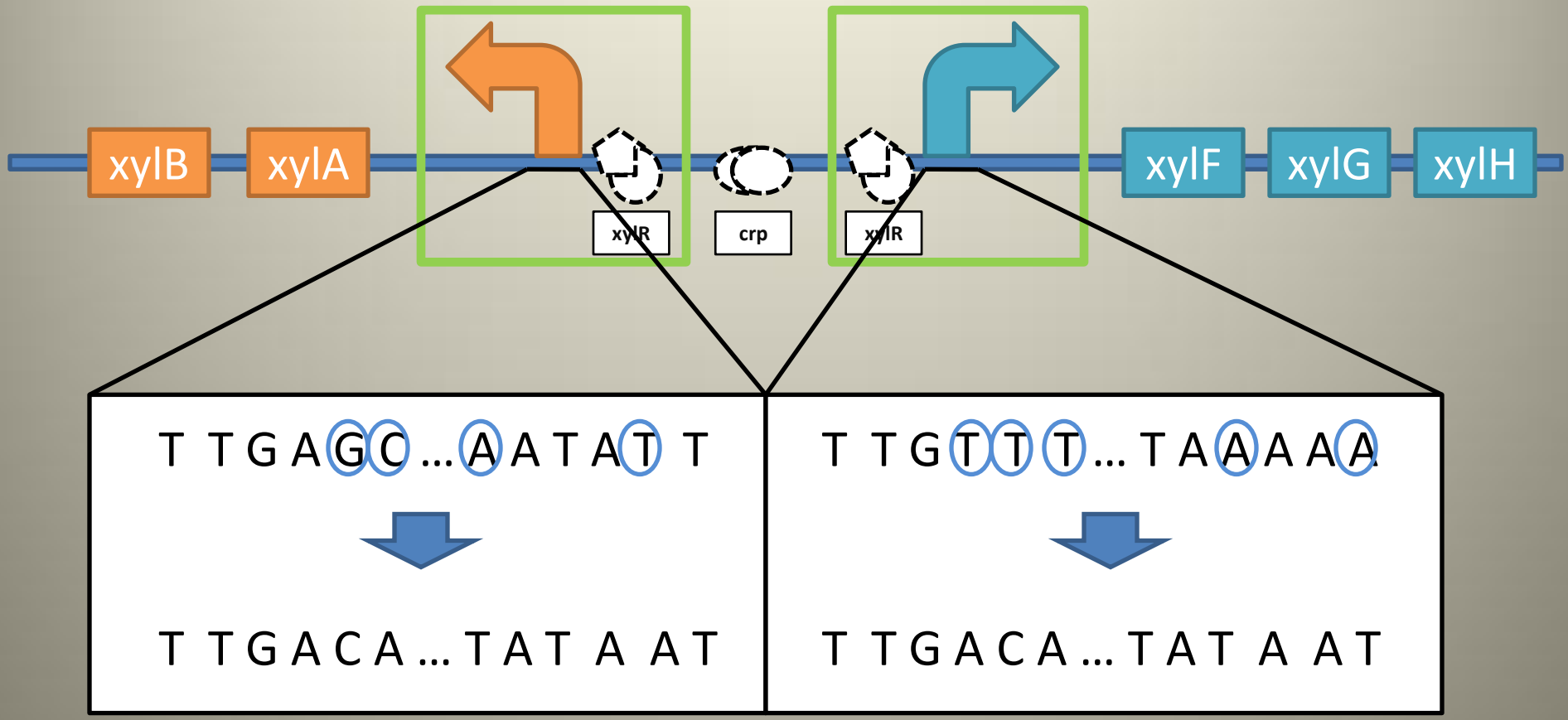
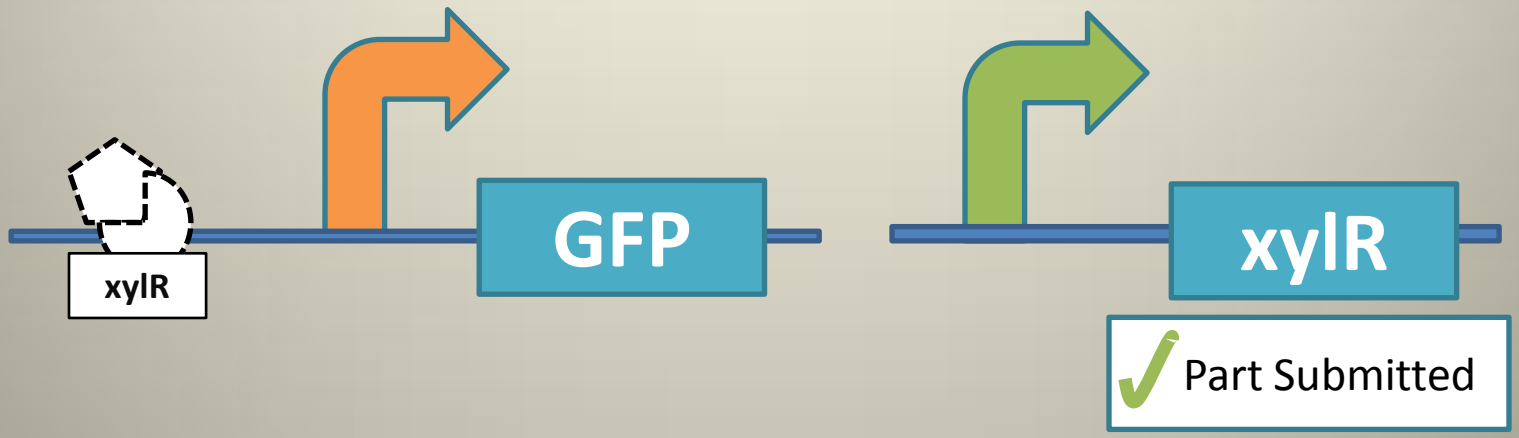


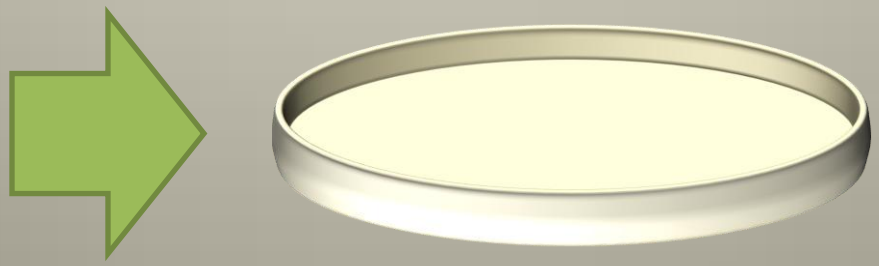


Strategy 1 – Regulatory Region



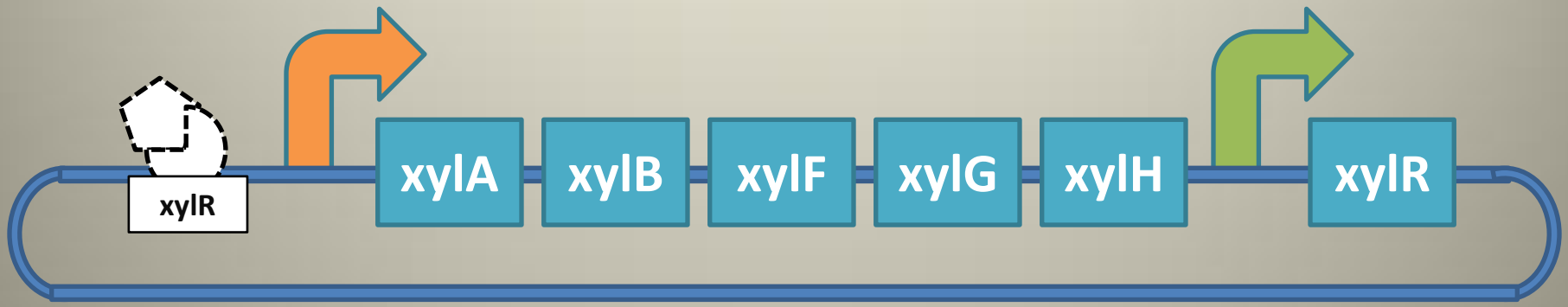
Strategy 1 – Testing



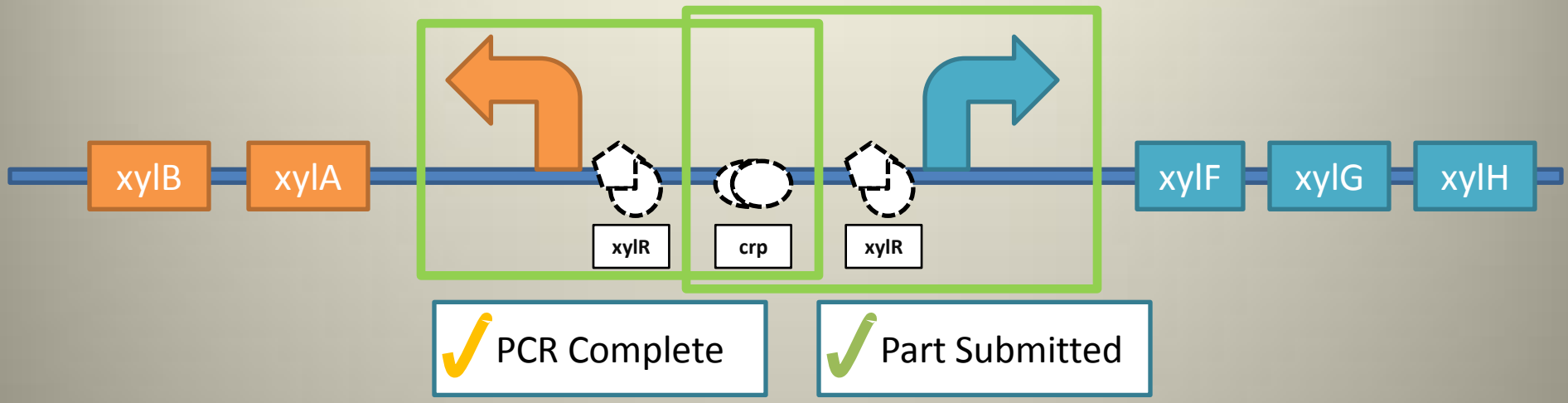


M9 minimal media w/ Xylose & Glucose

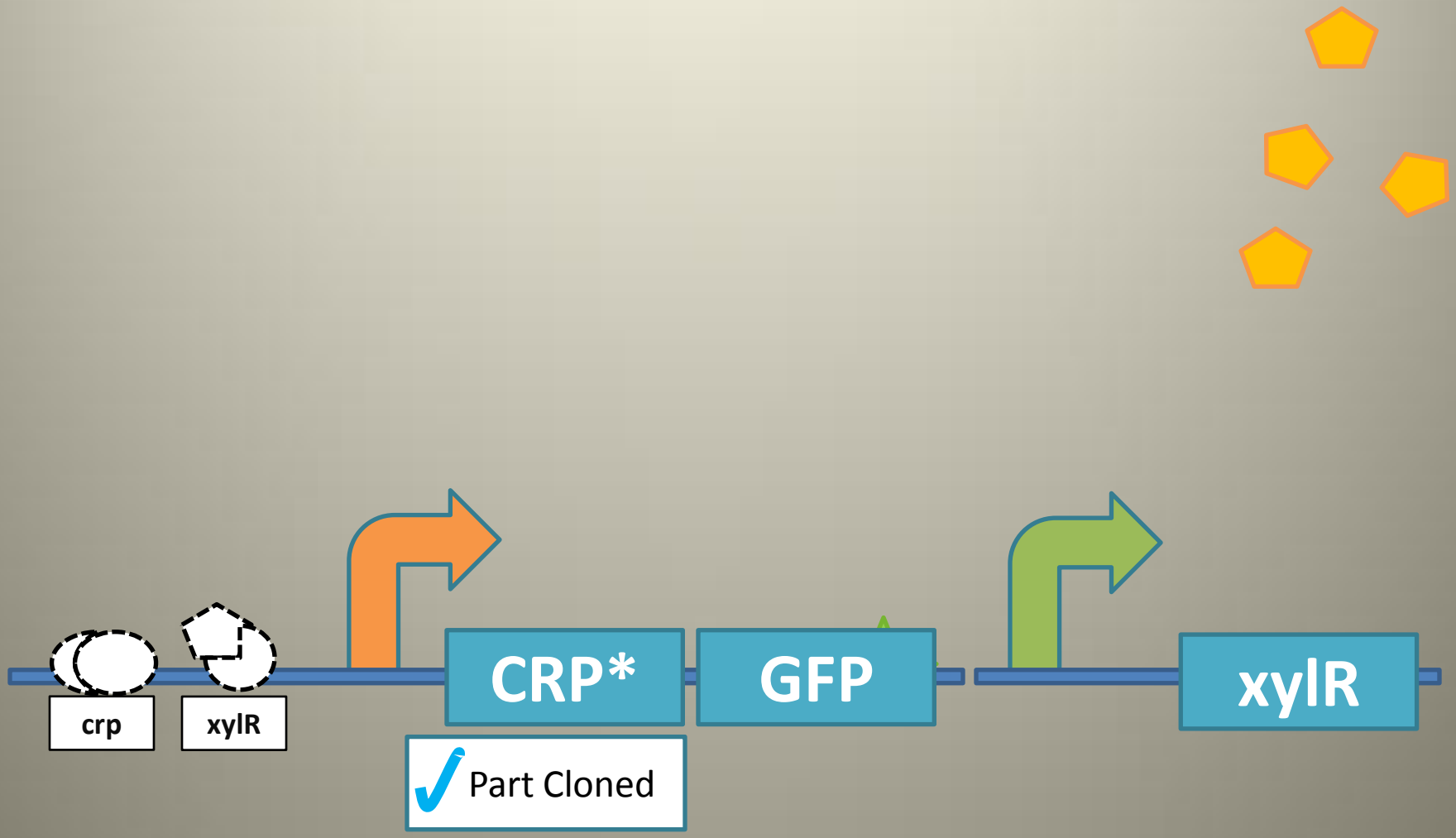
Strategy 1 – Final Construct



Strategy 2- CRP*



Strategy 2 - Testing



Strategy 2- Final Construct

Progress

Part Name	Bio-Brick Number	Status
XylR	BBa_I741005	Submitted
F Promoter w/ CRP site	BBa_I741018	Submitted
A Promoter w/ CRP site	BBa_I741019	PCR Complete
F Promoter	BBa_I741020	PCR Complete
A Promoter	BBa_I741021	PCR Complete
CRP*	BBa_I741023	Cloned

Constructs	Bio-Brick Number	Status
HSL Detector	BBa_I741001	Available + Working
IPTG Detector	BBa_I741002	Available + Working
HSL Producer	BBa_I741003	Available + Working
XylF Promoter + Reporter	BBa_I741102	Ligated

Bio-Dosimeter :
Biological Radiation Sensing

Introduction: Dosimetry

- Exposure to radiation or radioactive material must be quantified to avoid harmful/lethal doses of radiation
- For individuals with high exposure, electrometer dosimeter pen is an expensive and accurate way to measure dose
- Relatively Inexpensive photographic or thermoluminescent dosimeter badges also exist

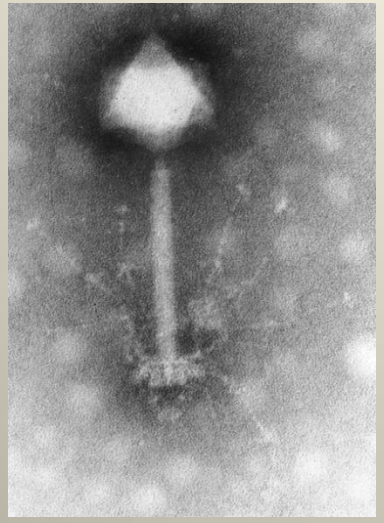
Expanding Need for Dosimeters

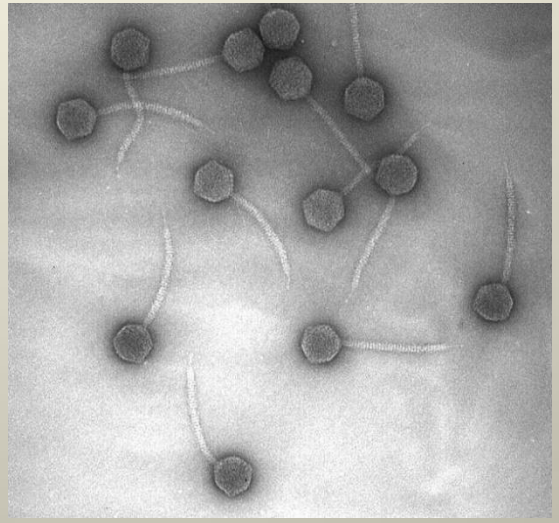
- Expanding need for radiation detection
  - Homeland security
- Possible exposure to radioactive material
  - Yucca Mountain
- Organisms could be used as a first warning dosimeter
- A biological dosimeter would be cheap, easy to read, easy to maintain, but probably inaccurate

Theory of a Bio-Dosimeter

- Radiation's harmful effects stem from the genetic damage caused by ionizing radiation
- Biological organisms already have very advanced genetic repair mechanisms
- Most direct implementation of a biosensor would monitor the genetic damage accumulated by a bacterial cell and emit a signal after a critical threshold

λ Phage Lysogeny



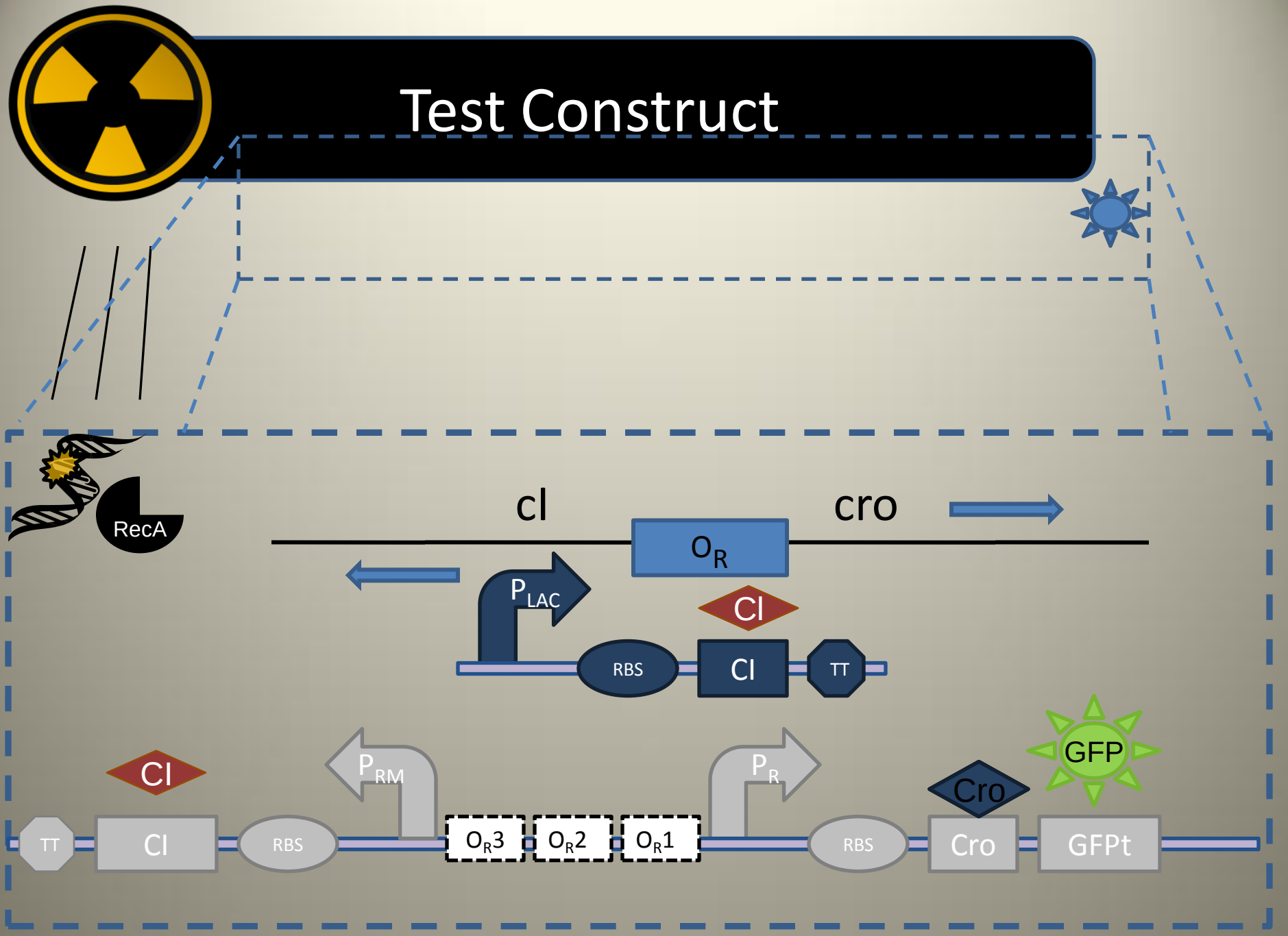


- Genetic Damage to bacterial host triggers λ lysogen's entrance into lytic phase
  - Bacterial RecA recognizes mutations, cleaves λ repressor
- By utilizing key regions of the λ genome, we assemble a dosimeter 'switch' that would throw after a certain dosage of radiation

The λ Control Region

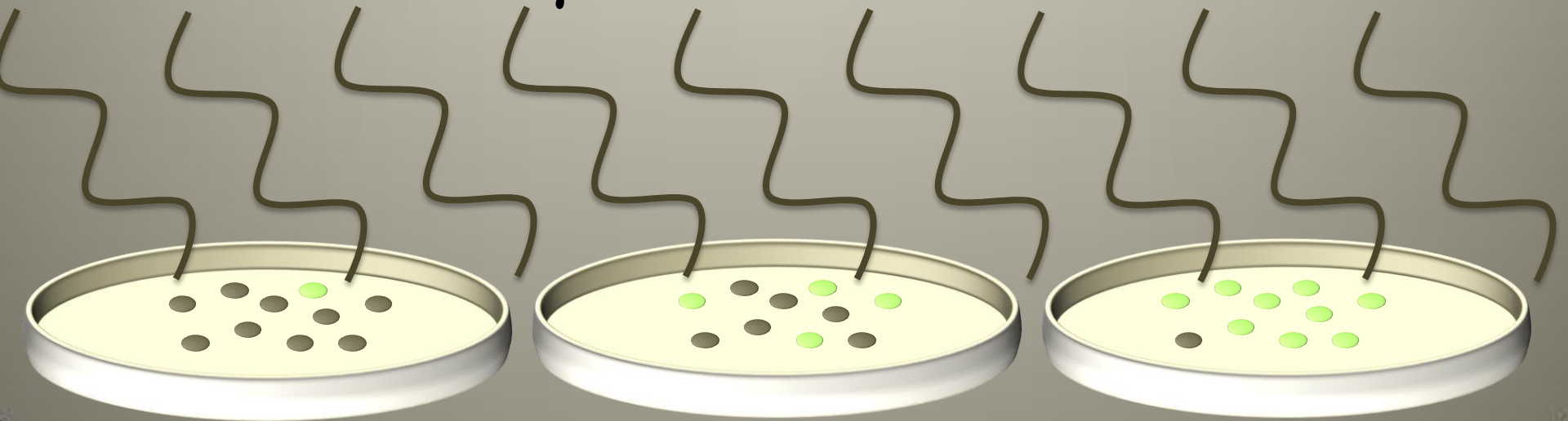
- The lambda phage maintains lysogeny through a single bidirectional operator (O_R)
  - Operator is controlled by two proteins that bind to three sites (O_R1, O_R2, and O_R3)
- O_R contains two promoters P_{RM} and P_R that transcribe a repressor (CI) and anti-repressor (Cro), respectively

Test Construct



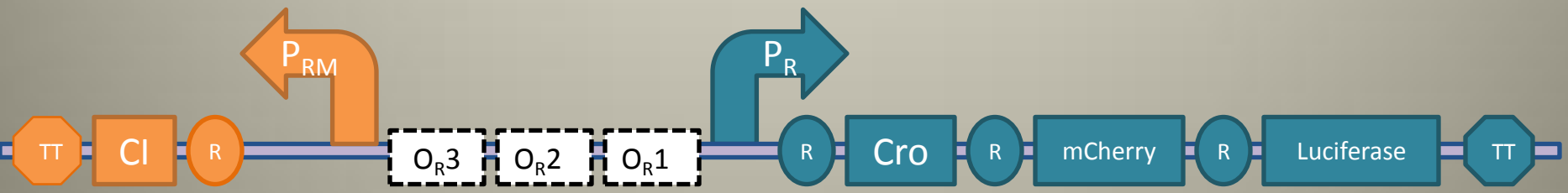
Threshold adjustment with RBS Strength

- Trigger on cellular level is all or nothing
- Test different Cro RBS strengths to set the threshold dose
  - Maximum repressor/antirepressor concentration will not change, only replacement rate



Final Dosimeter Construct

- Readable in most lighting conditions
- Output could be changed for different applications



Progress

Part Name	Bio-Brick Number	Status
Repressor CI (no LVA)	BBa_I741110	Ligated ✓
Antirepresor Cro	BBa_I741111	Ligated ✓
O_R	BBa_I741109	Ligated ✓

Acknowledgements

Project ad

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