Got Milk?

Team Ubermensch
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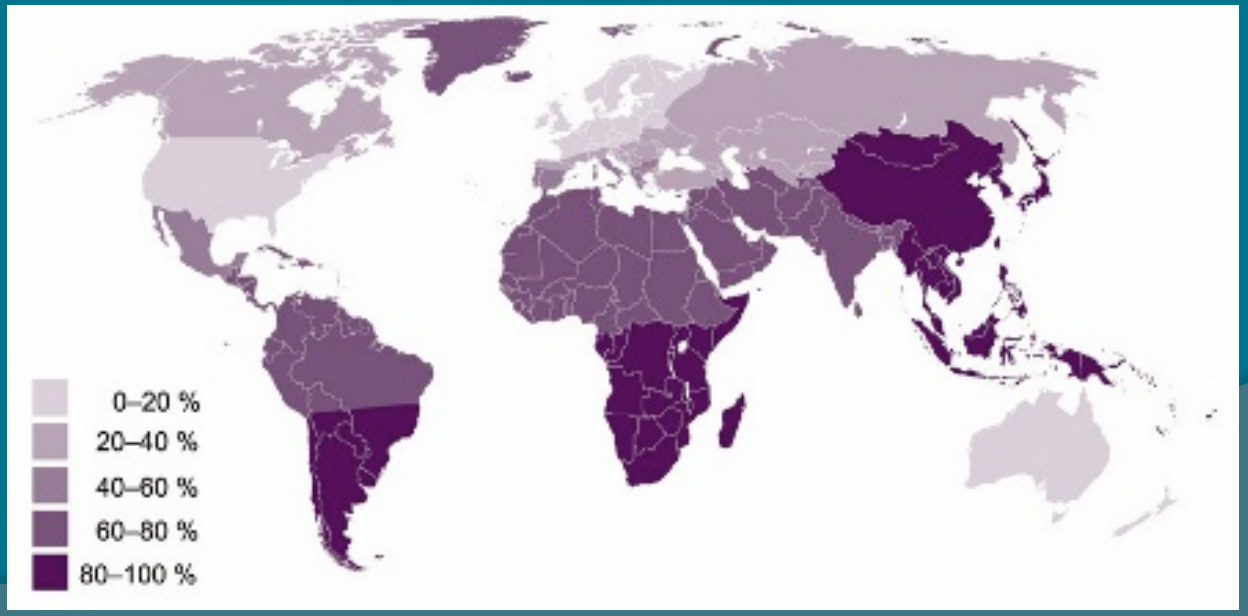
Mentors: Derek Ju and Alie Doolittle

Lactose Intolerance

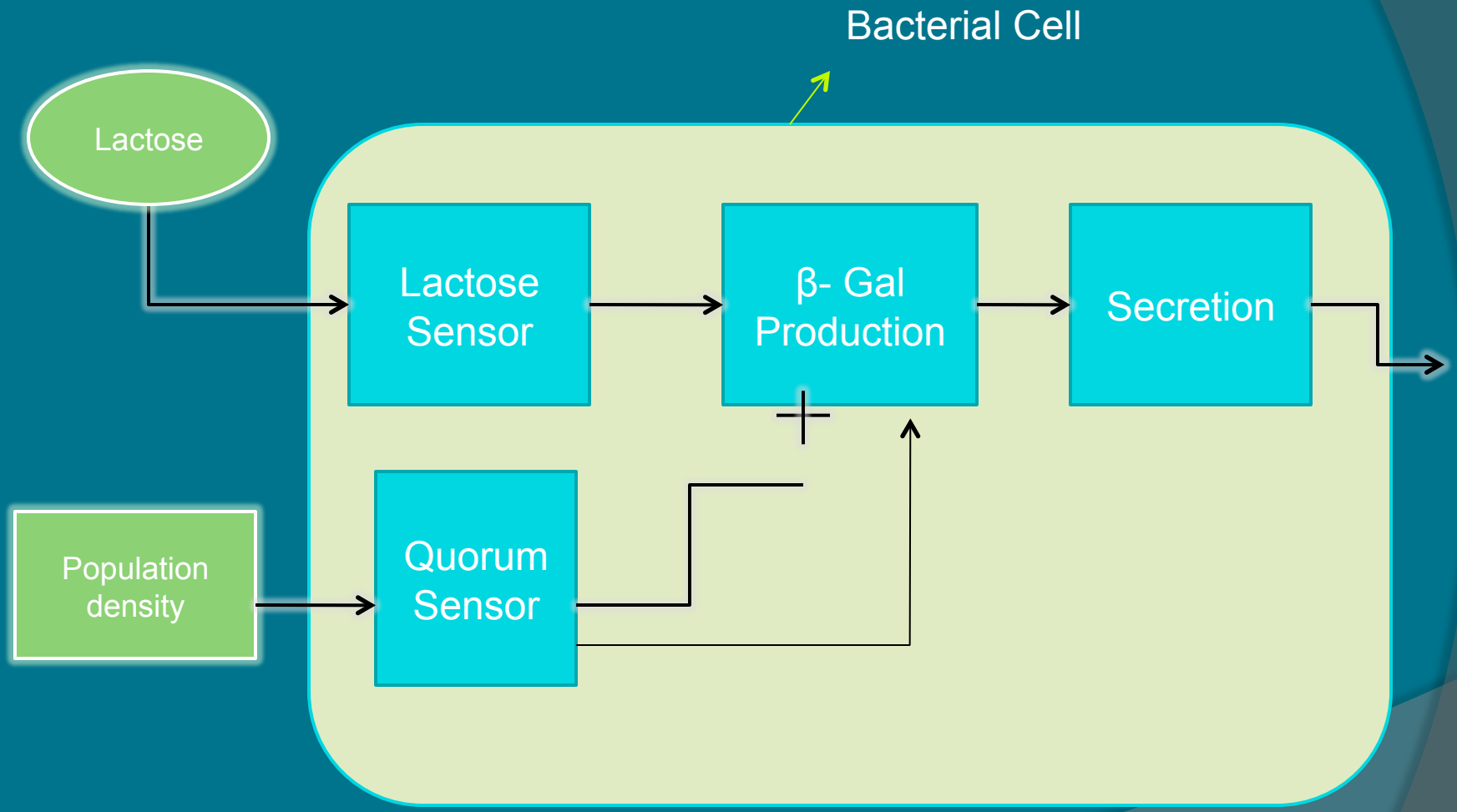
- Lack the enzyme lactase due to environmental or genetic factors
- Affects 70-75% of the worldwide population
- Varies with ethnicity



Overall System

- Bacterial production of β-Galactosidase when lactose is present in human digestive system
- Maintenance of bacterial population in the GI tract at functional numbers

High Level System Overview

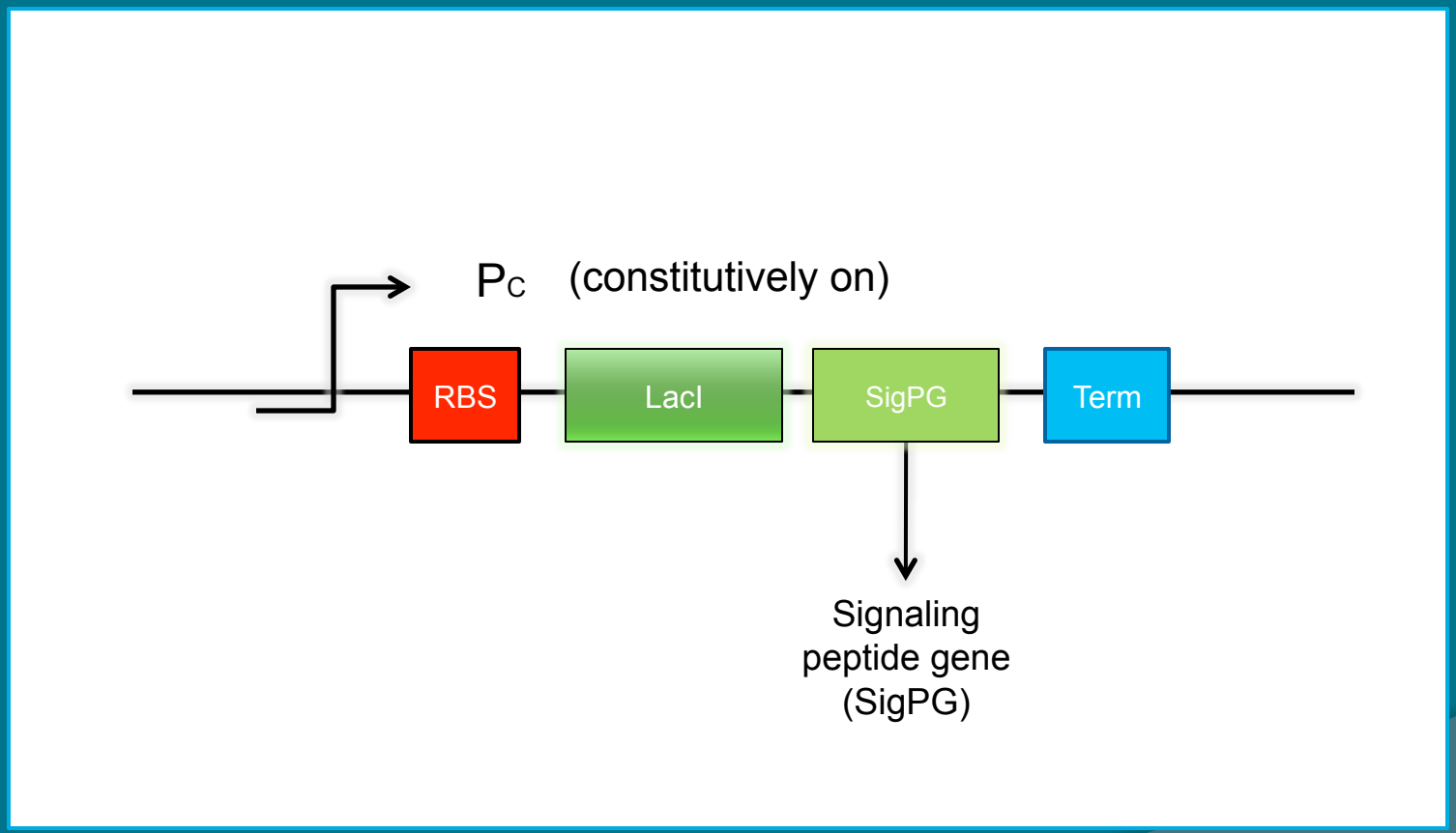


Inputs and Outputs

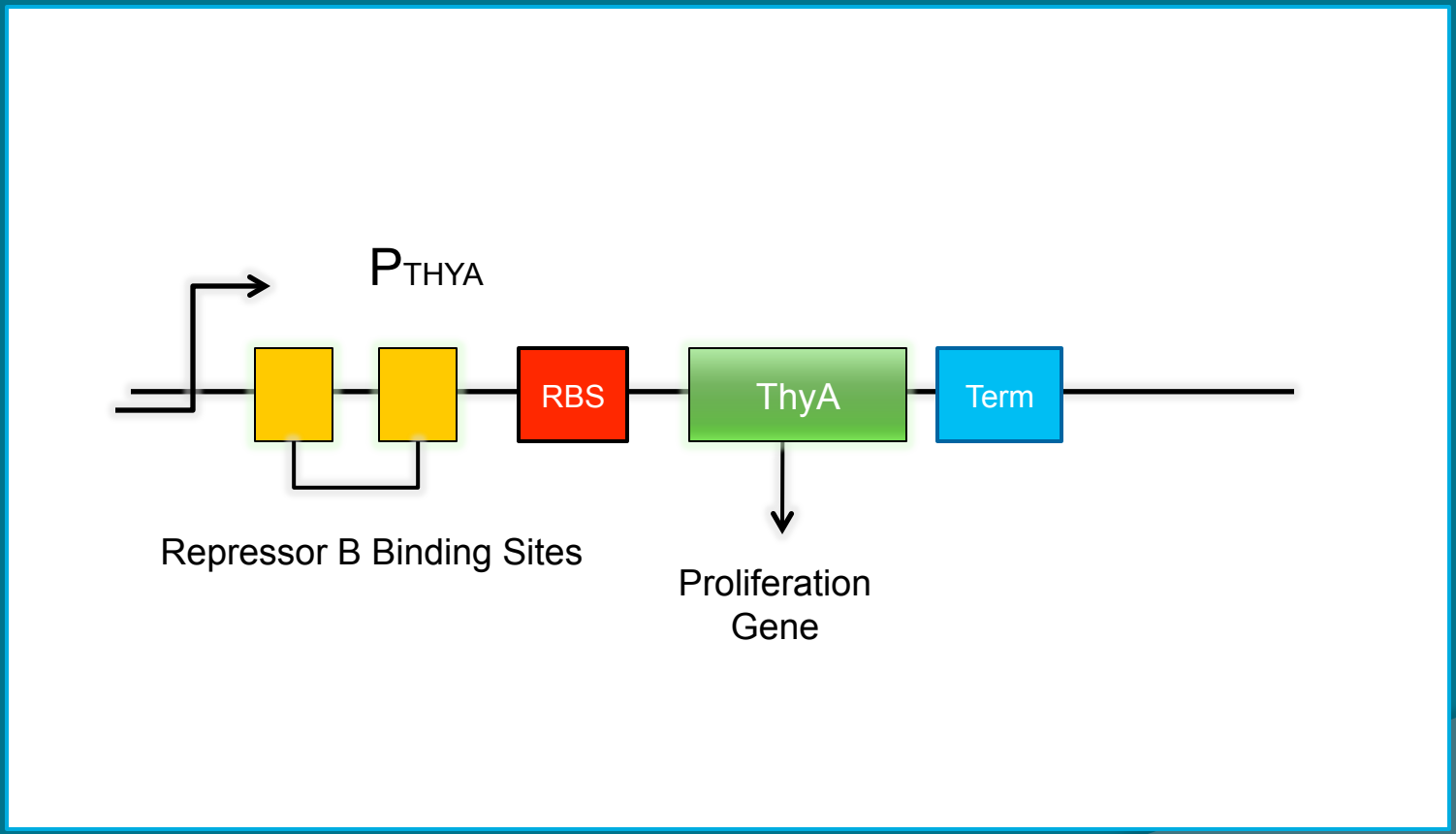
	Lactose On	Lactose Off
High Population	Produce β-Gal	Normal Replication
Low Population	Stop producing β-Gal, Normal Replication	Normal Replication

DEVICES

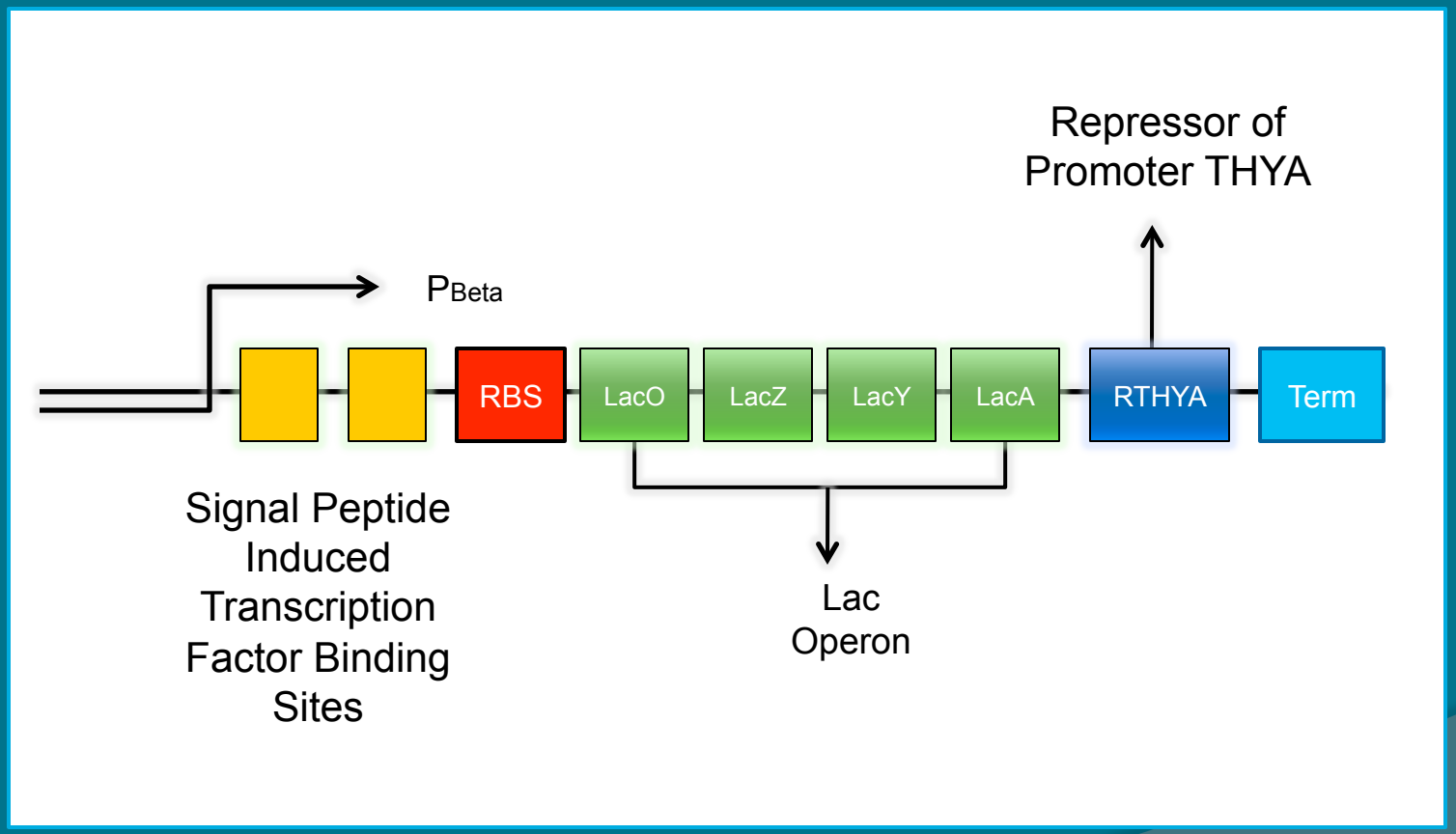
Lactose Sensor



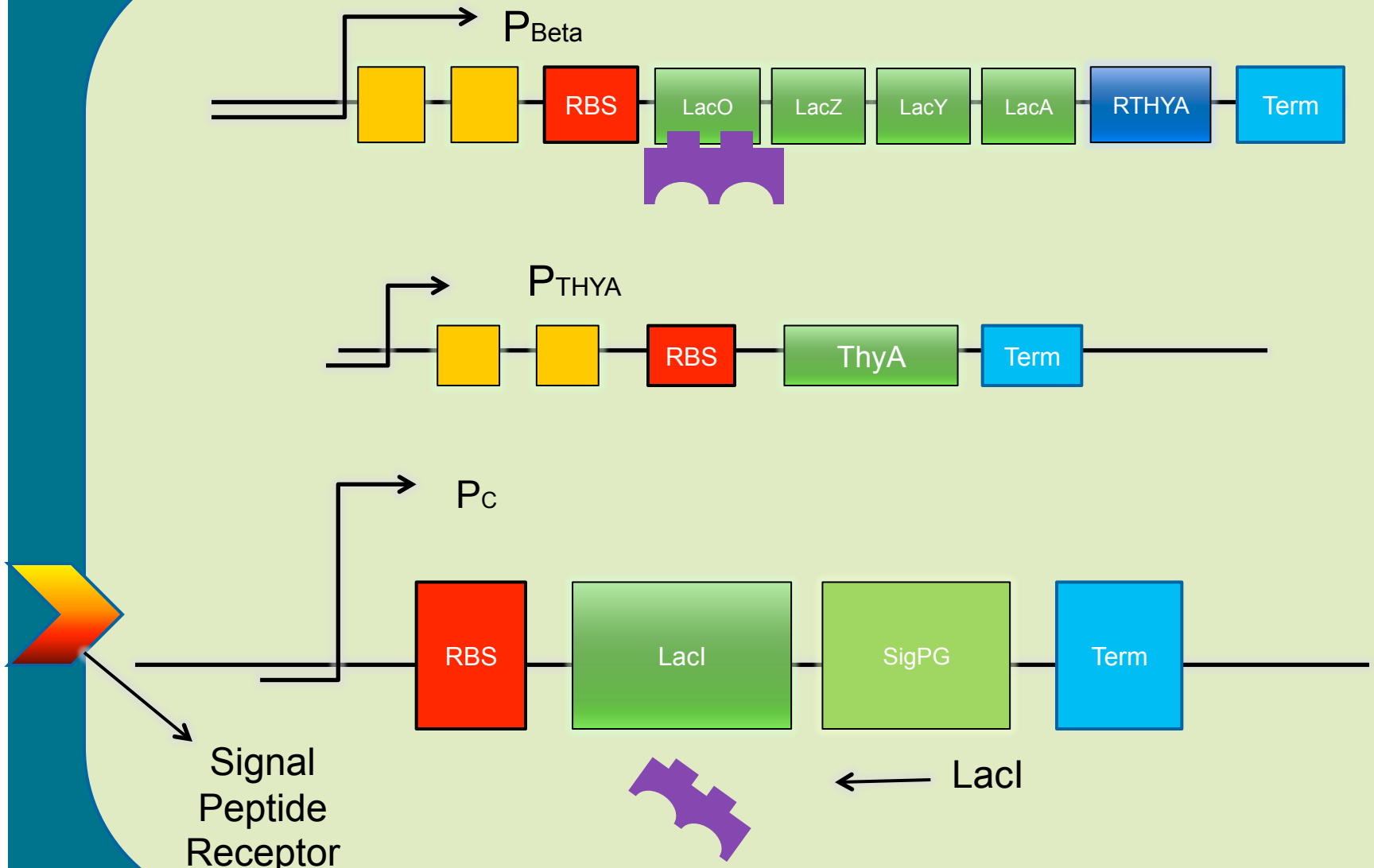
Quorum Sensor



β-Galactosidase Production



System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RBS
LacO
LacZ
LacY
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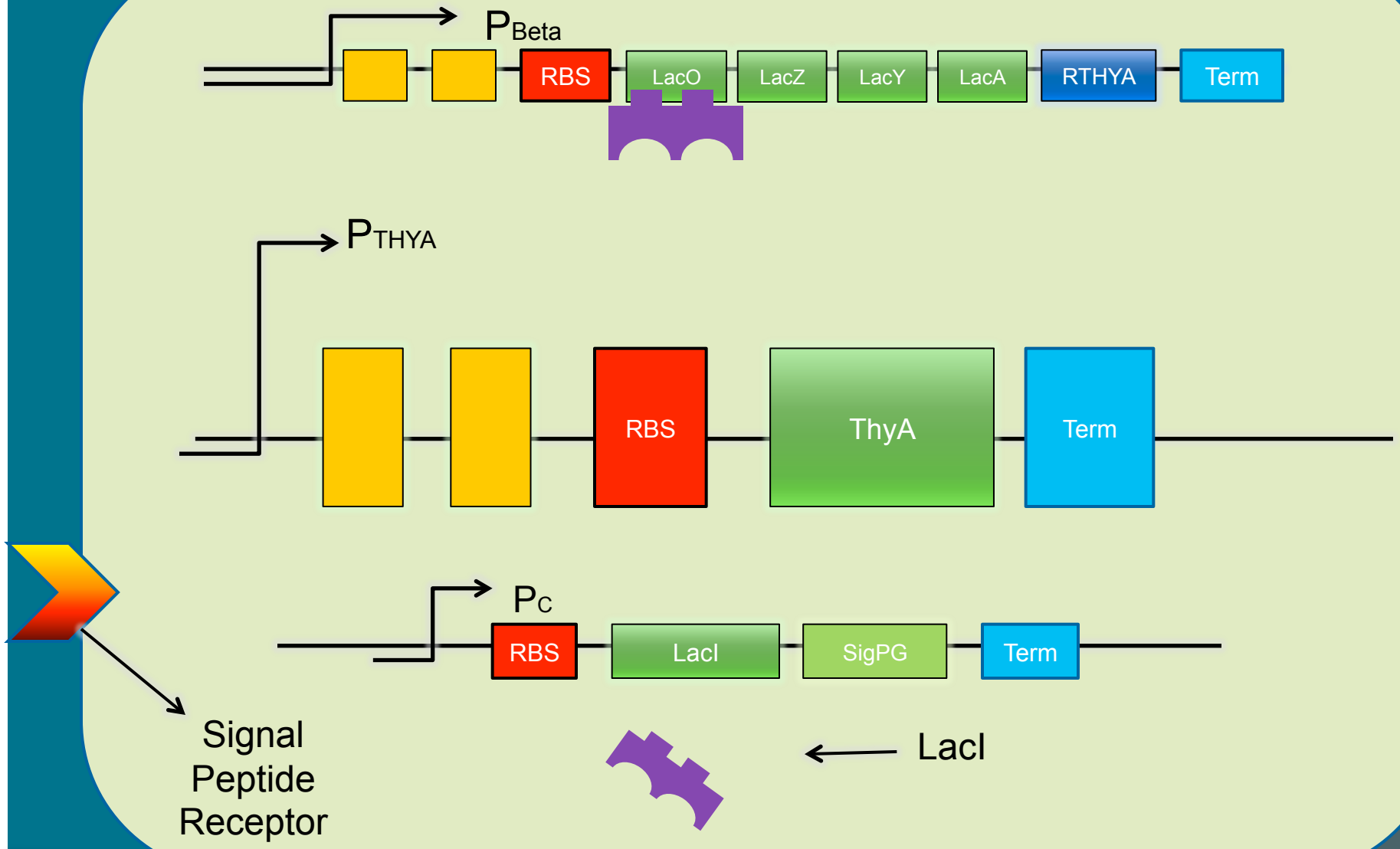
SigPG
Term
Signal
Peptide
Receptor
LacI

System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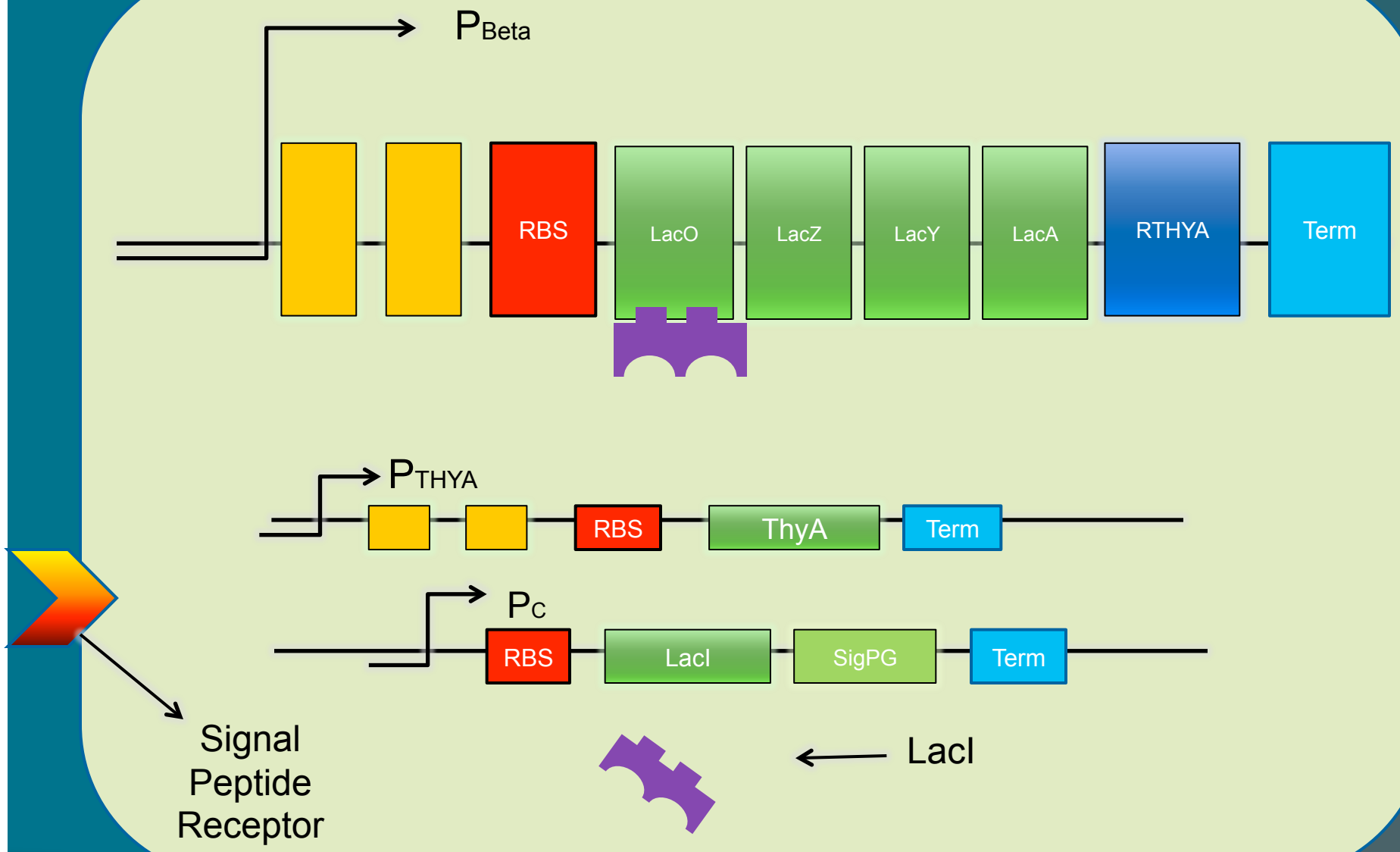
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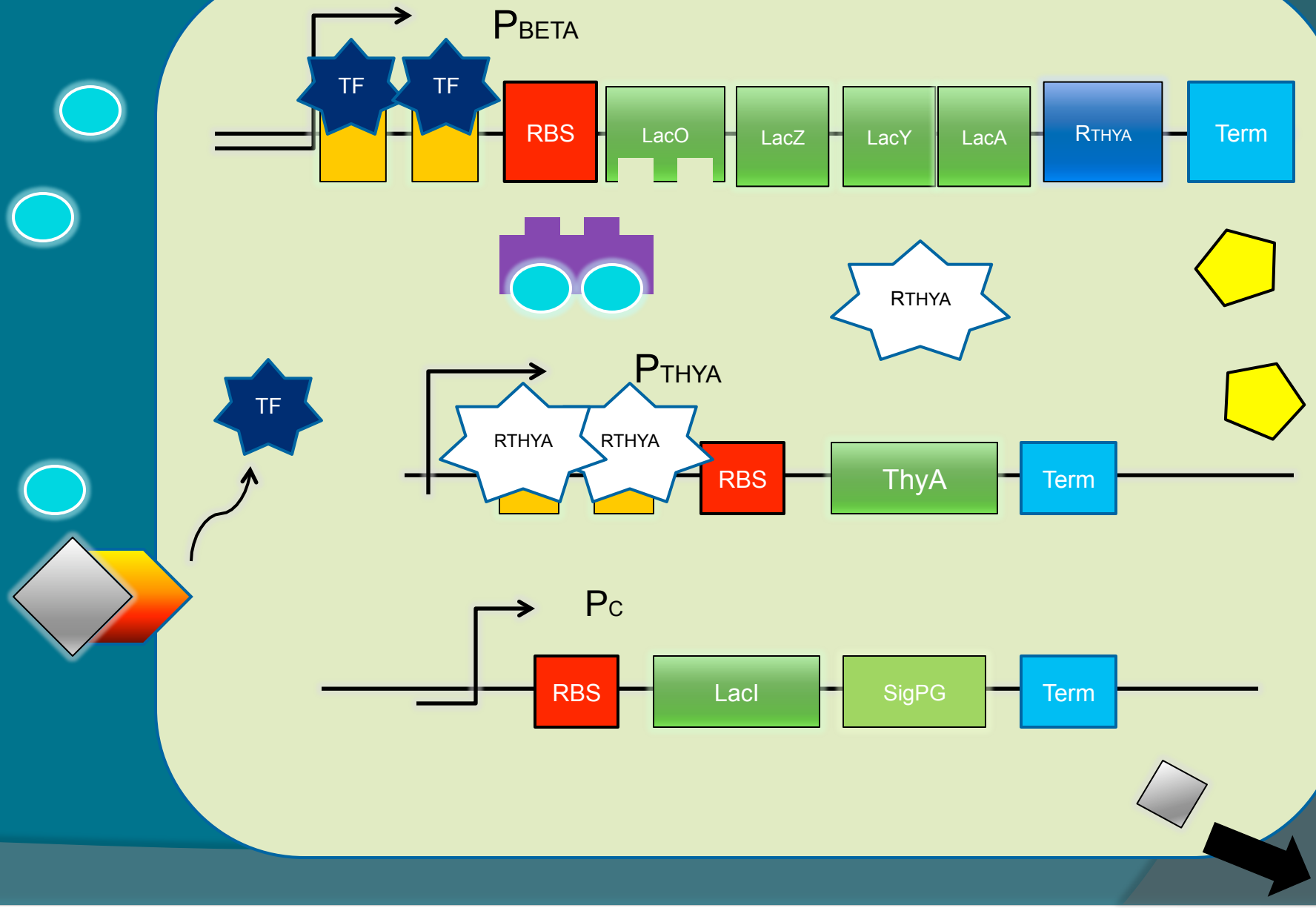
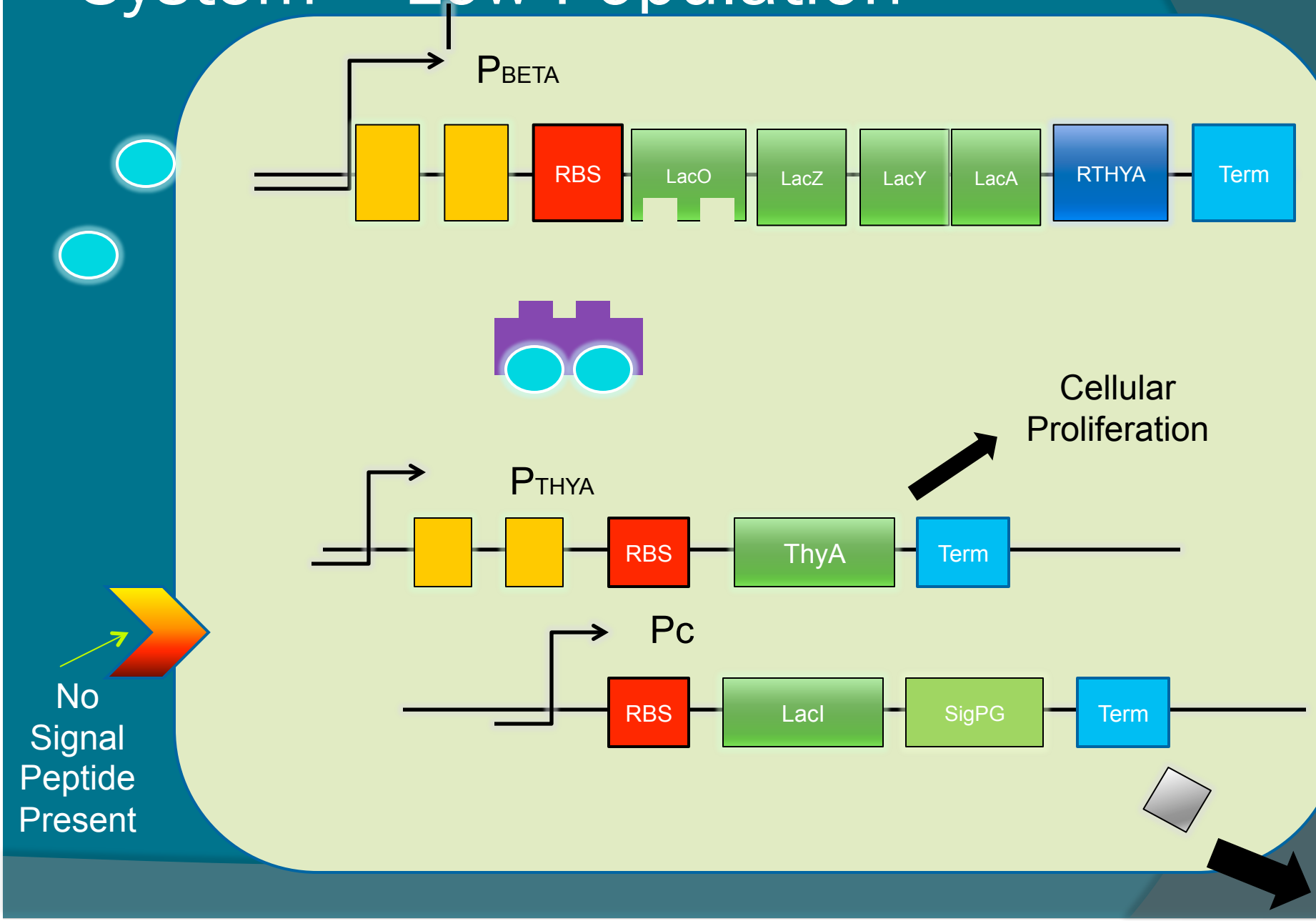
System - High Population

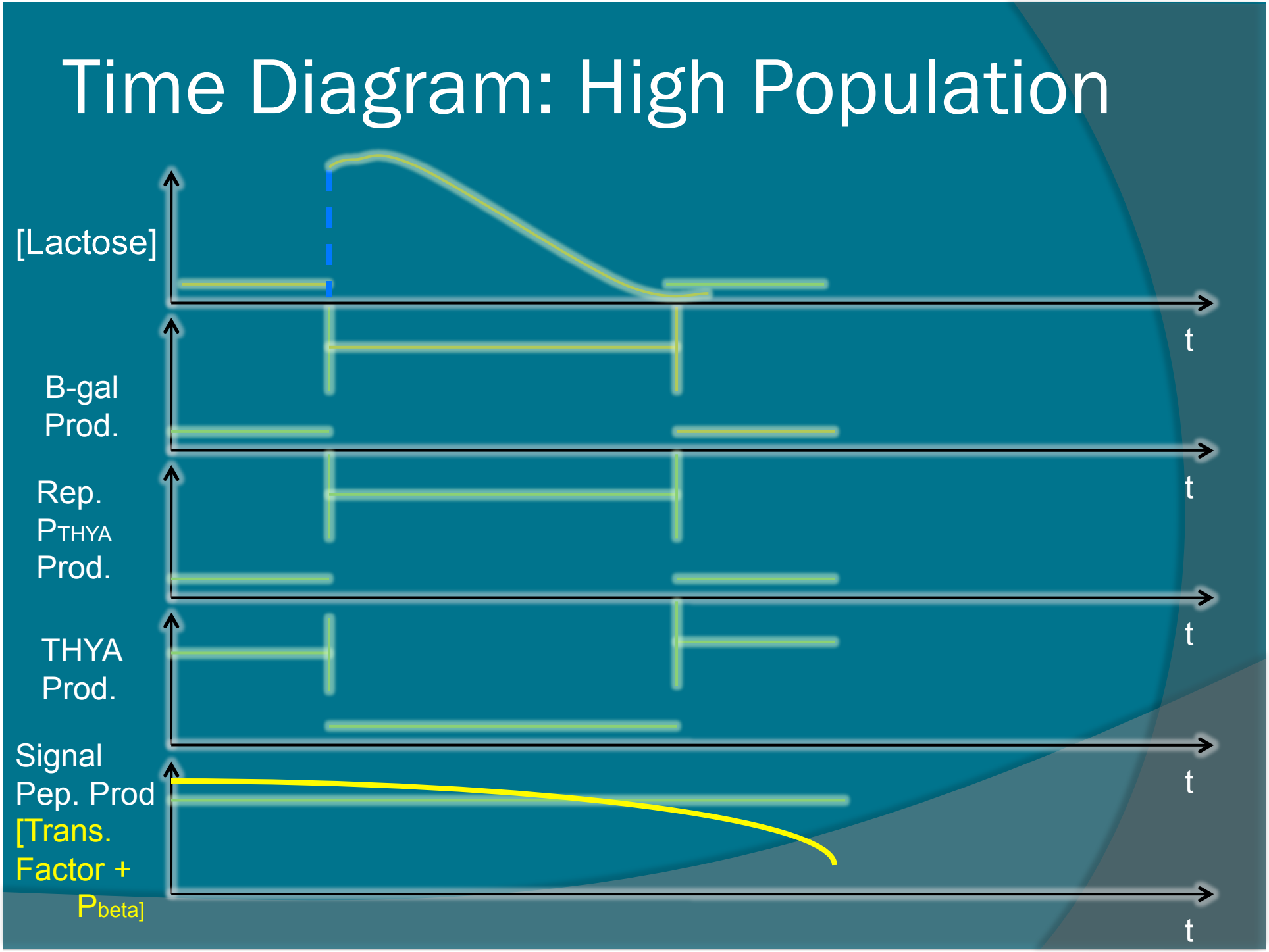


System – Low Population

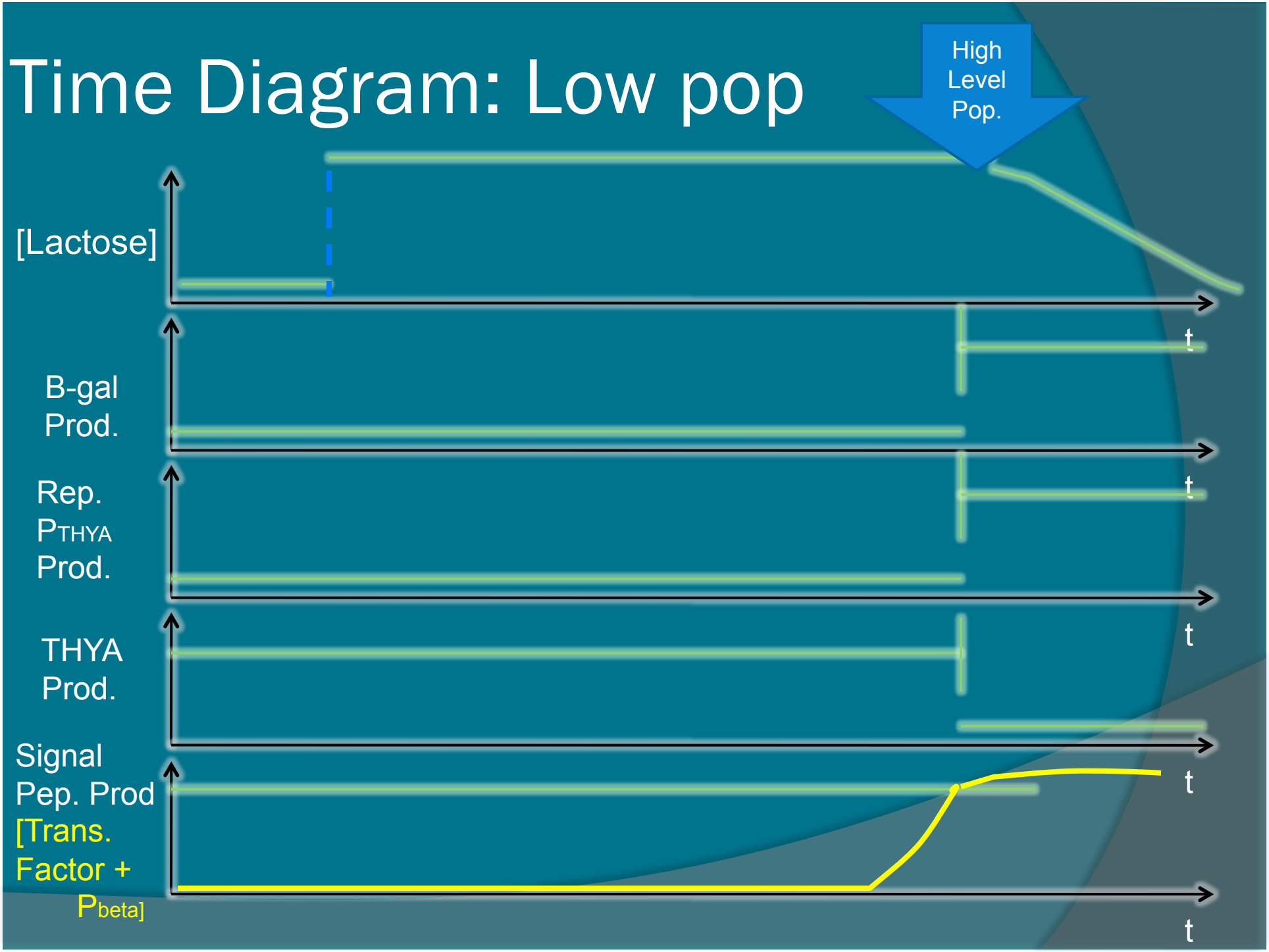


TIME DIAGRAM

Time Diagram: High Population



Time Diagram: Low pop



TESTING/DEBUGGING

Strategy for Testing: general cloning

- Restriction Enzyme Digest and Gel Electrophoresis

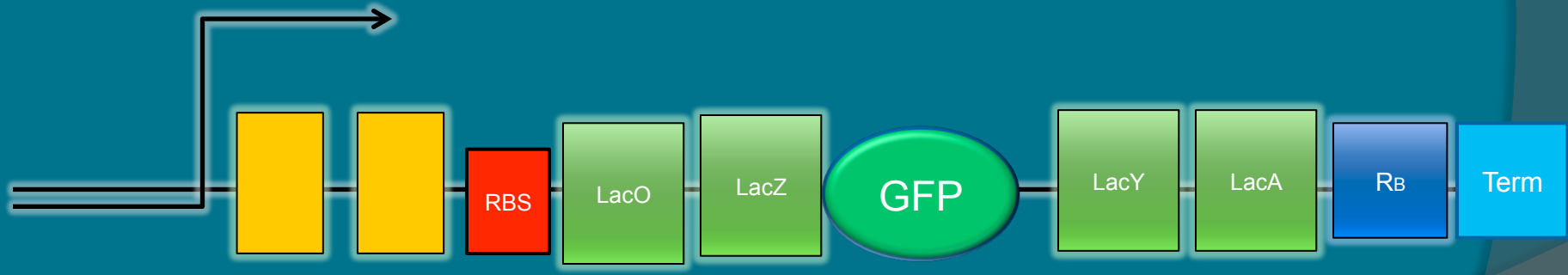
- Sequence plasmid after each insertion to check transformation of DNA
  - Check for mutations

  -Knockout- Auxotrophs

  -Antibiotic resistance

Strategies for Testing: β-Gal Production/Function

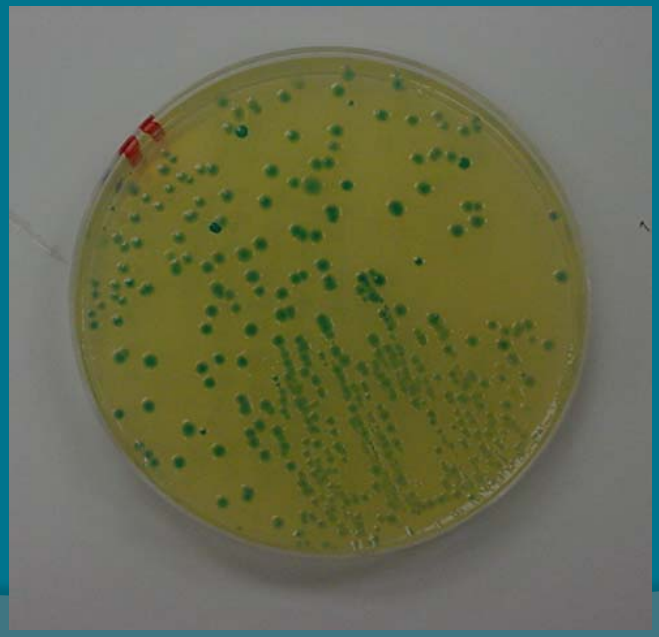
- Test for Devices
  - GFP-tag a component in each device
  - Fluorescence indicator
  - Promoter activity

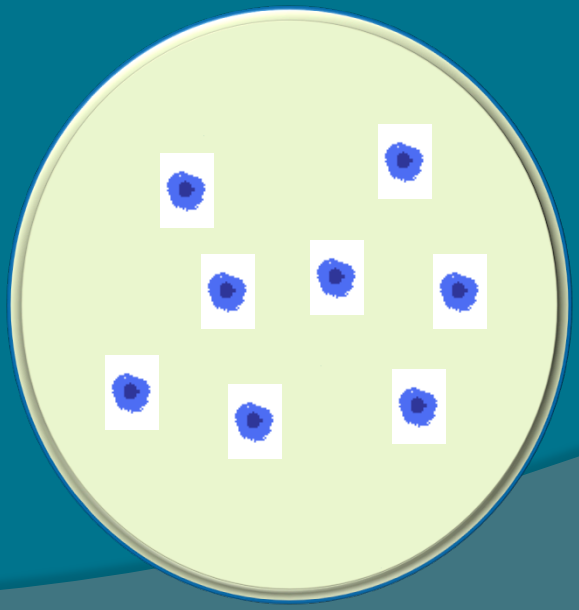


-Protein activity: isolate proteins and test activity *in vitro*

Strategies for Testing: Secretion

- Grow on X gal culture
- The agar plate around the colonies with correct gene should turn blue





http://delliss.people.cofc.edu/virtuallabbook/SpreadingPlates/SpreadingExamples.html

Strategies for Testing: Quorum Sensing

- Signal Peptide production?
  - Tag it with GFP
- Signal Peptide interacts with receptor→ transcription factor?
  - Insert Signal peptide, tag transcription factor with GFP: does it produce the transcription factor?
- Transcription factor + P_{beta}?: should produce β-Gal

Equations

- RNApol + Pc → LacI mRNA + sigPG mRNA
- LacI mRNA + ribosome → LacI
- LacI mRNA → decay
- SigPG mRNA + ribosome → SigPG
- SigPG mRNA → decay
- LacI + LacO → LacILacO
- LacILacO + Allolactose → LacIAllolactose + LacO
- SigPG + receptor → SigPGreceptor + TF
- TF + P_{beta} → TFP_{beta}
- TF + TFP_{beta} → $2TFP_{beta}$
- $2TFP_{beta}$ + RNApol → β-Gal mRNA + R_{THYA} mRNA
- β-Gal mRNA + RNApol → β-Gal

Equations

- β-Gal mRNA → decay
- R_{THYA} mRNA + ribosome→ R_{THYA}
- R_{THYA} mRNA → decay
- R_{THYA} + P_{THYA} → RP_{THYA}
- R_{THYA} + RP_{THYA} → $2RP_{THYA}$
- $2RP_{THYA}$ → RP_{THYA} + R_{THYA}
- RP_{THYA} → R_{THYA} + P_{THYA}
- P_{THYA} + RNApol → ThyA
- $2TFP_{beta}$ → TF + TFP_{beta}
- TFP_{beta} → TF + P_{beta}
- SigPGreceptor → SigPG + receptor
- TF → decay
- R_{THYA} → decay
- LacI → decay

Cost – Biological Parts

- Most needed proteins are relatively common, most of our peptide parts will be found in the registry of biological parts.
- 3 promoters, ~45 bp each, $.40/bp
- 5 pairs of primers, 20-40 bp each, $.40/bp
- DNA synthesis, $400
- $255 for a strain of Lactobacillus acidophilus

Grand Total: ~$1,000*

*Unless we learn that we need proteins that are not in the registry.

Benefits

People will be able to ingest lactose containing products without regular ingestion of lactase pills or lactose-free products!

Unanswered Questions

1) How do you get the synthetic genes into the genome?
2) How do you isolate the appropriate signal peptide, transcription factor and promoter that it would interact with?
3) Can we rely on the natural bacterial secretory pathway?
4) Can we assume that the chassis will not over-proliferate due to the natural competition in lower GI?
5) Kill switch: should we have one in order to eliminate those with too many mutations on the inserted genes?

Buildable?

- Yes!

Cost?

- Reasonable!

Safe/Secure?

- Yes! (BL1 microorganism)

Go or No Go?

GO!

References

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