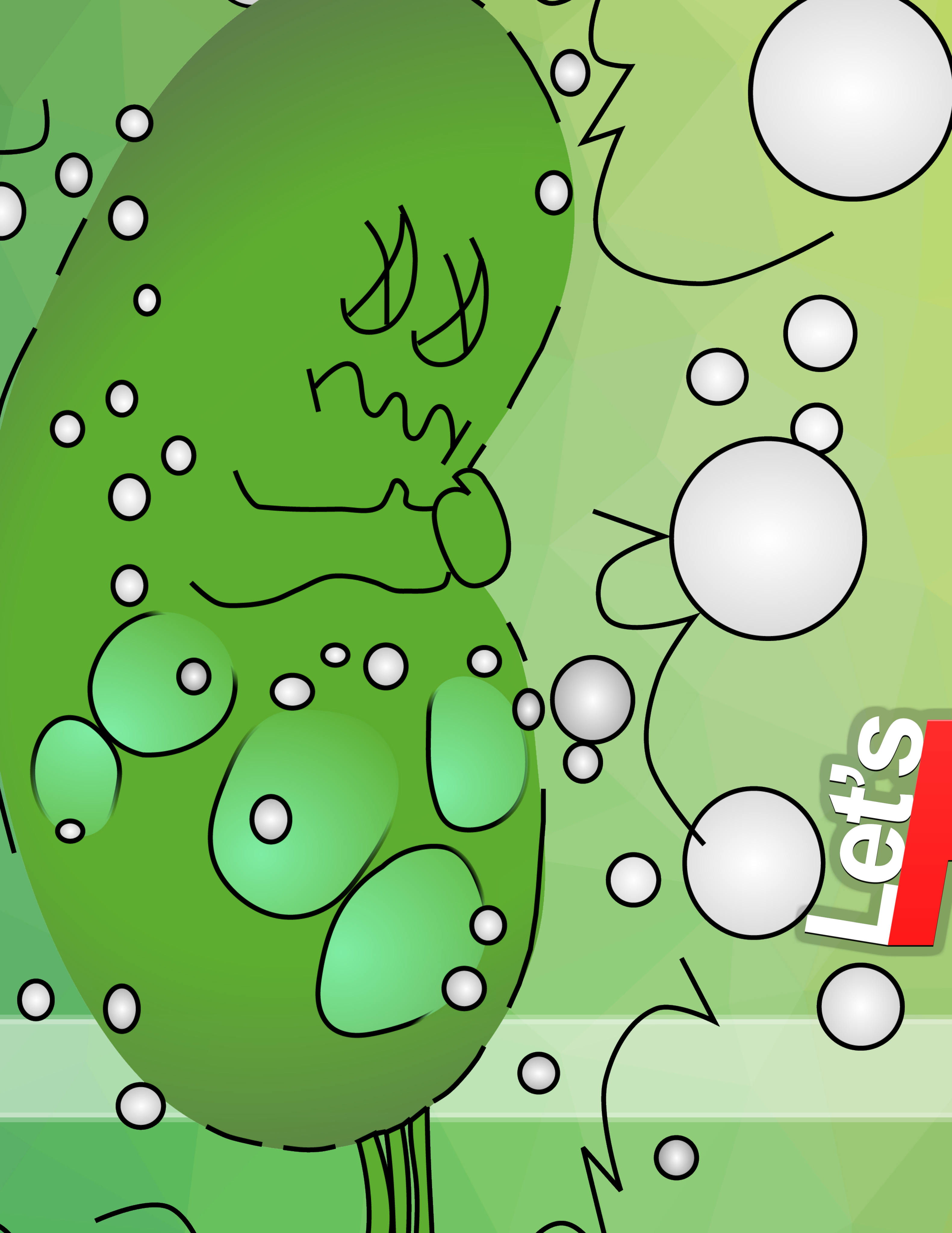

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Let's PLAy Project

Bioproduction of Poly-Lactic acid

Plastic waste – a worldwide problem

280 million tons

- **1000 years** for a plastic bottle
- **up to 450 years** for a plastic bag

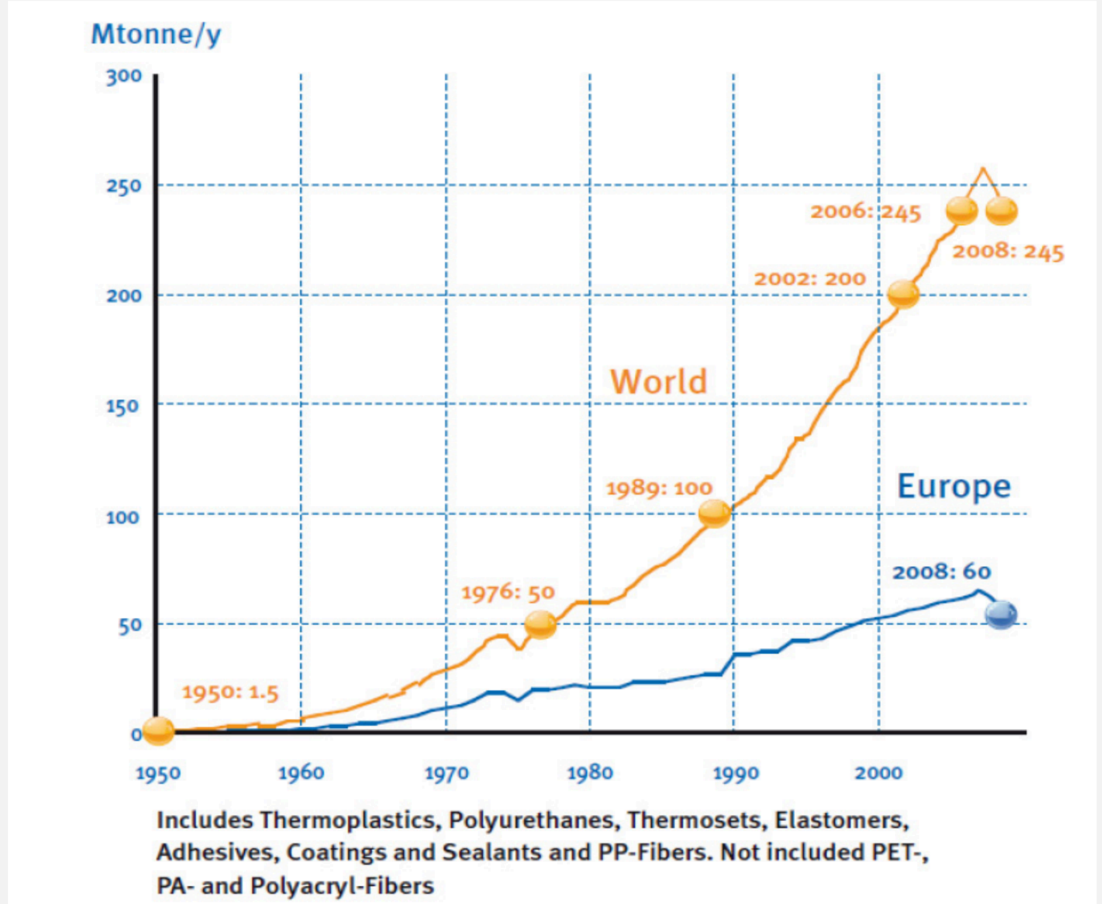


Figure 1. World Plastics Production 1950-2008. From *The Compelling Facts about Plastic*, PlasticsEurope (2009), p33.

Our team

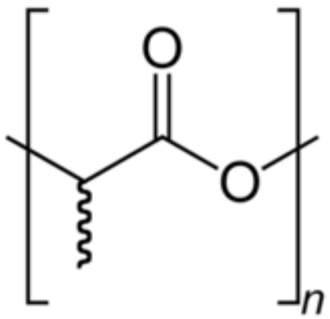
GENOPOLE
VIVRE L'INNOVATION

iSSB

université evry val-d'essonne

iGEM EVRY 2016

SynBio for PLA Bioproduction



Poly-lactic acid

- Biodegradable polymer
- Thermoplastic

- Biosynthesis less expensive than chemical synthesis
- Conducted by metabolic engineering in *E. coli* by

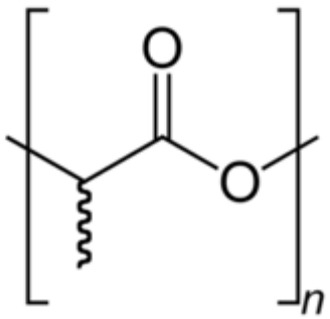
Jung *et al.*, 2010

Characteristic / Strain	*E. Coli*
Genetic manipulation	
Safety	
Lactate production	
In vivo polymerization	

High	Medium	Low

Reference: Jung, Y.K., Kim, T.Y., Park S.J. & Lee S.Y. Metabolic Engineering of *Escherichia coli* for the Production of PLA and Copolymers. *Biotech and Bioeng* 105:1, 161-171 (2010)

SynBio for PLA Bioproduction



Poly-lactic acid

- Biodegradable polymer
- Thermoplastic

Our chassis: ***Pseudomonas putida KT2440***

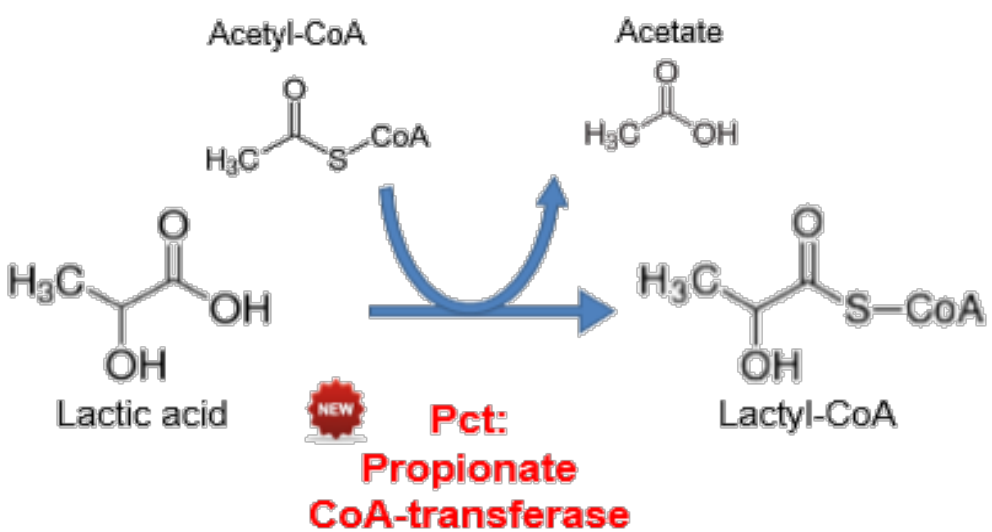
Table 1. Common chassis strains and their characteristics

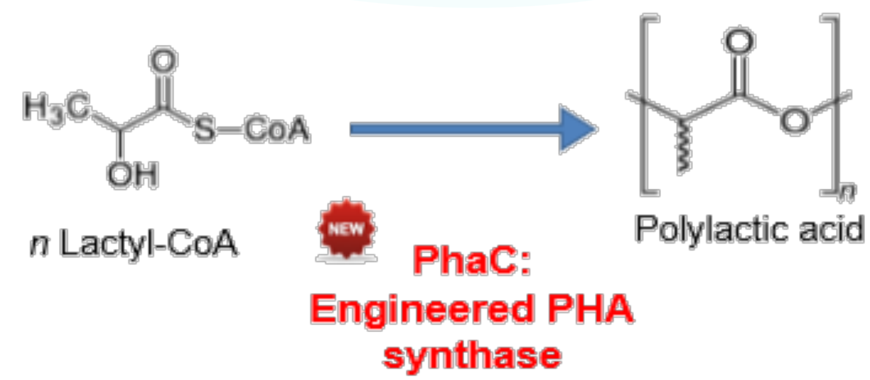
Characteristic / Strain	*E. Coli*	*B. Subtilis*	*P. putida KT2440*
Genetic manipulation			
Safety			
Lactate production			
In vivo polymerization			

High	Medium	Low

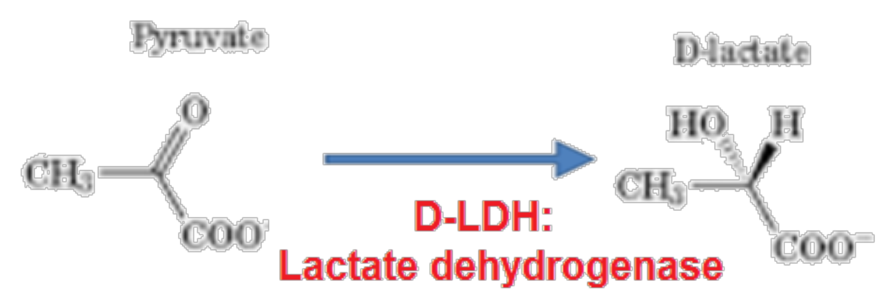
Implementation of pathway

- Enabling bioproduction of PLA in *P.putida*:
  - **Pct**
  - **PhaC**



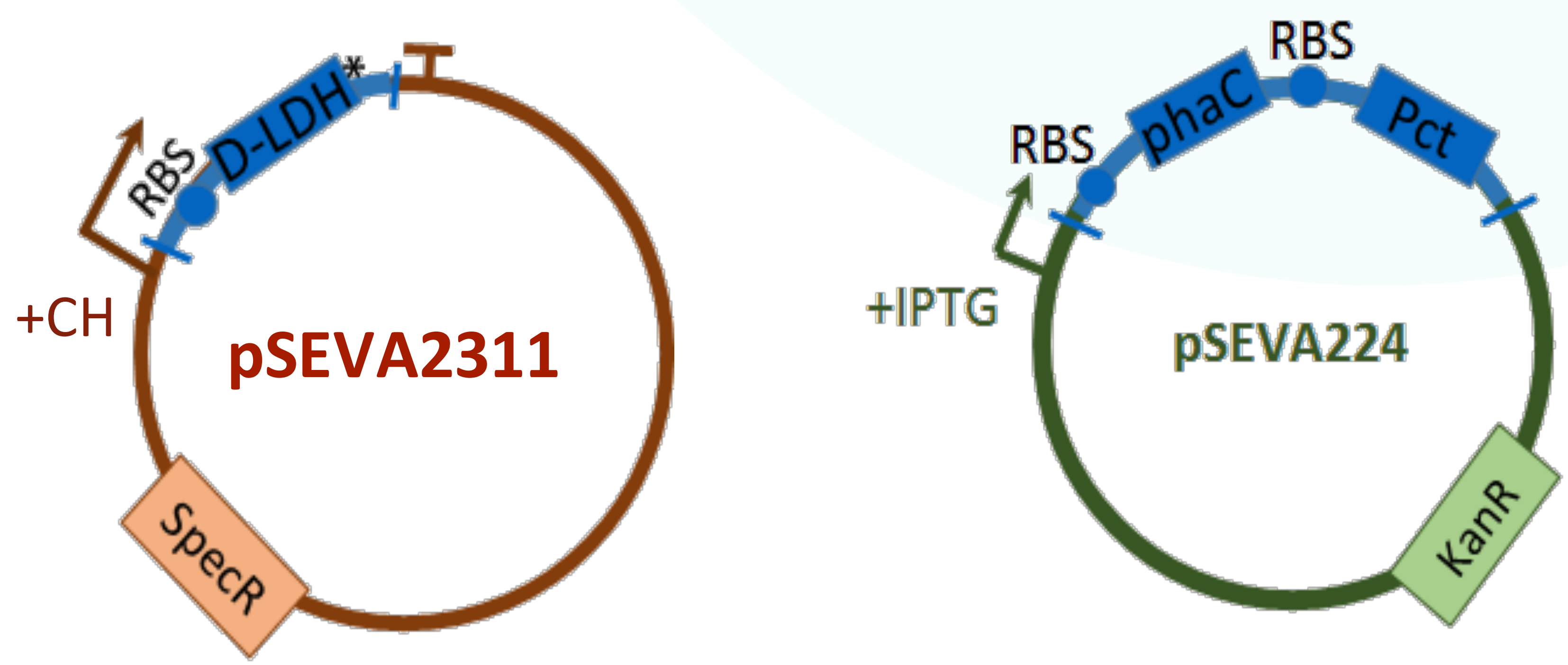


- Boosting the metabolic flux by increasing the quantity of precursor (an **evolved lactate dehydrogenase** (D-LDH*))
  - **D-LDH**



Implementation of pathway

Situation of genes in plasmids pSEVA



- **Inducible devices** - Cyclohexanone and IPTG
- **Designed for *Pseudomonas putida*** - De Lorenzo's lab (Spain)
- **Step-wised optimization for metabolic engineering applications**
    - Fermentation and Bio-polymerization

Results

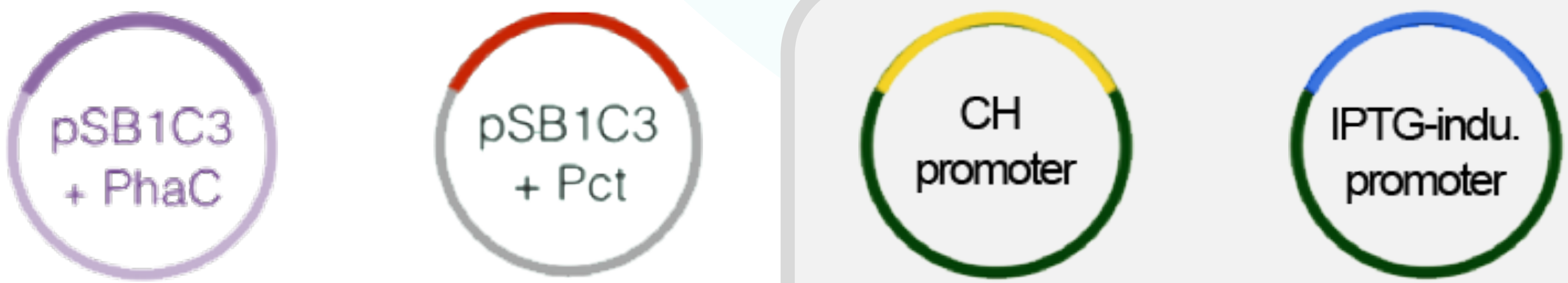
- Different cloning strategies used (restriction enzymes, Gibson assembly)

Improvements!

New additions!

Results

- Different cloning strategies used (restriction enzymes, Gibson assembly)



Improvements!

New additions!

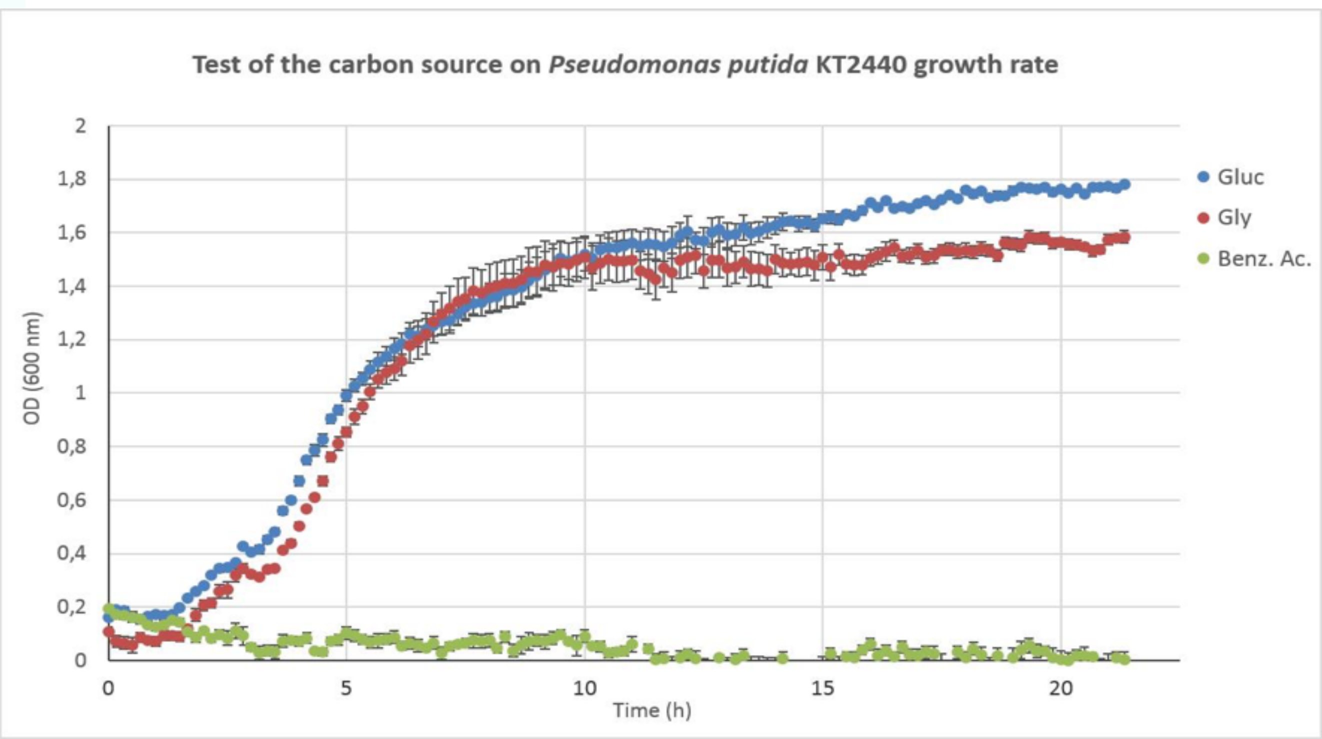
- Biobricks ✔
- Expression in *Pseudomonas putida* ✗

Results

- Different cloning strategies used (restriction enzymes, Gibson assembly)

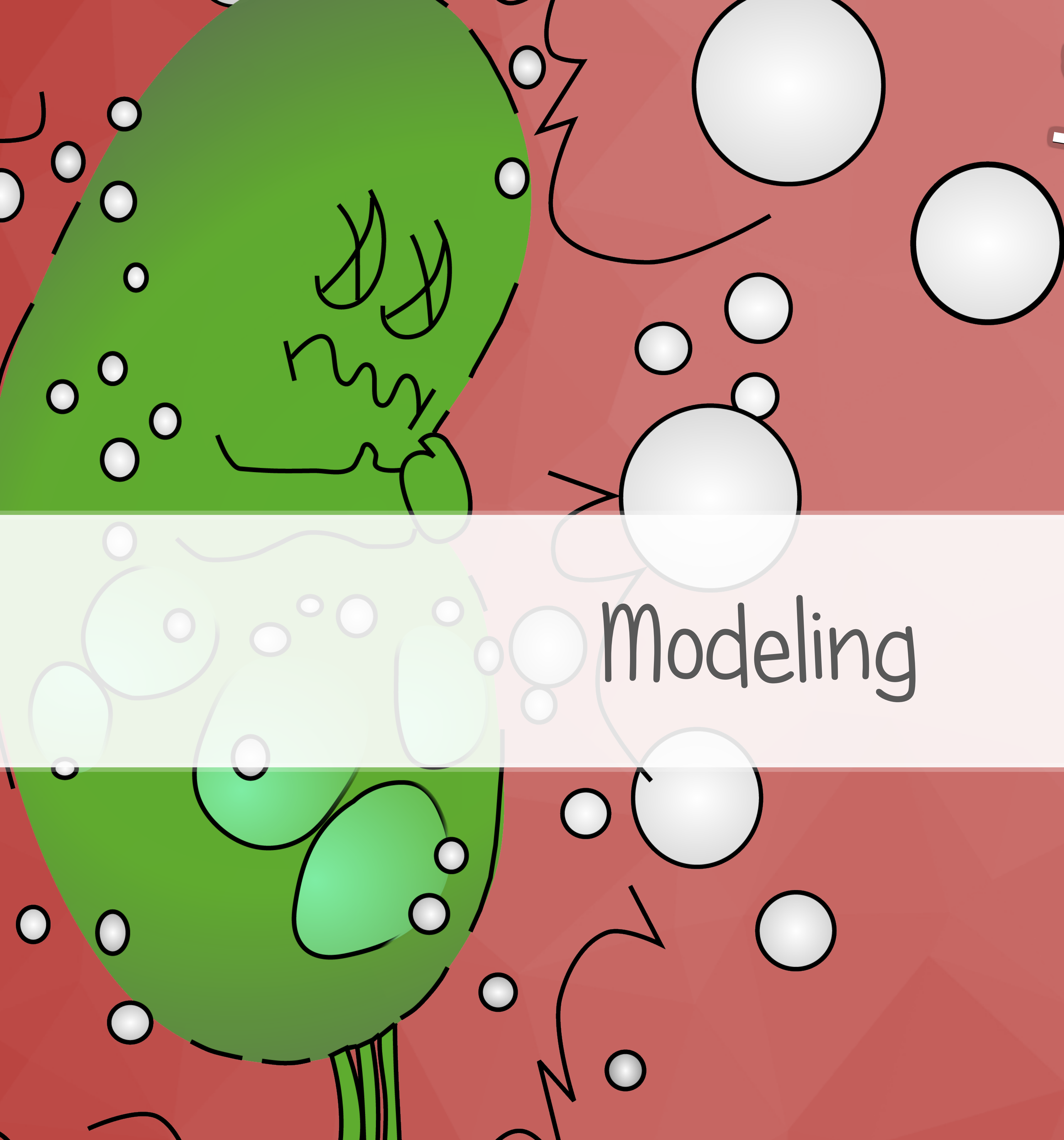
Improvements!

New additions!



Pseudomonas putida in Glycerol	0,206 h^{-1} = 0,0034 min^{-1}
Pseudomonas putida in Glucose	0,212 h^{-1} = 0,0035 min^{-1}

Let's PLAy Project



Modeling

In-silico improvements

Flux Balance Analysis (FBA)

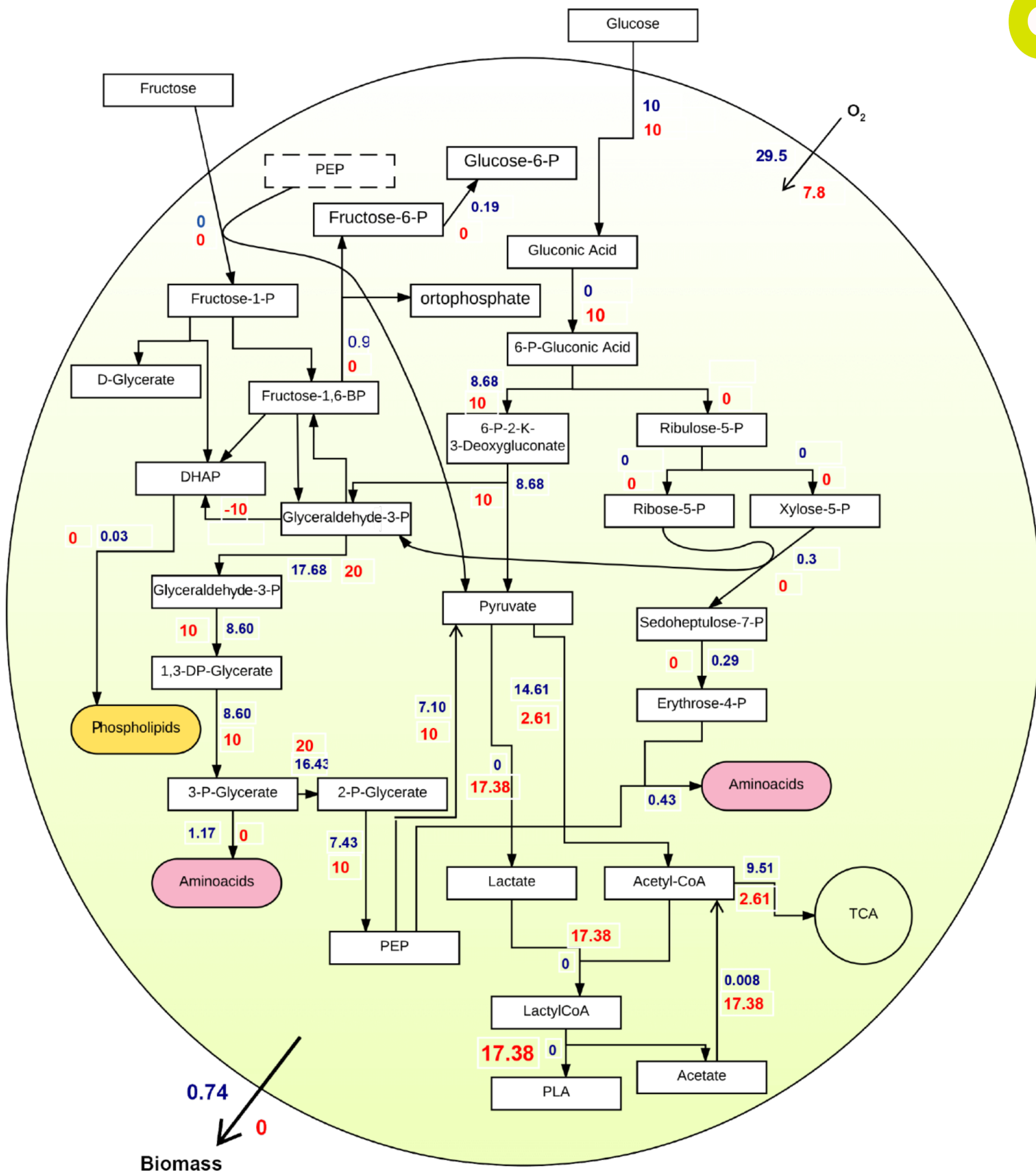
In-silico improvements

<u>Flux Balance Analysis (FBA)</u>

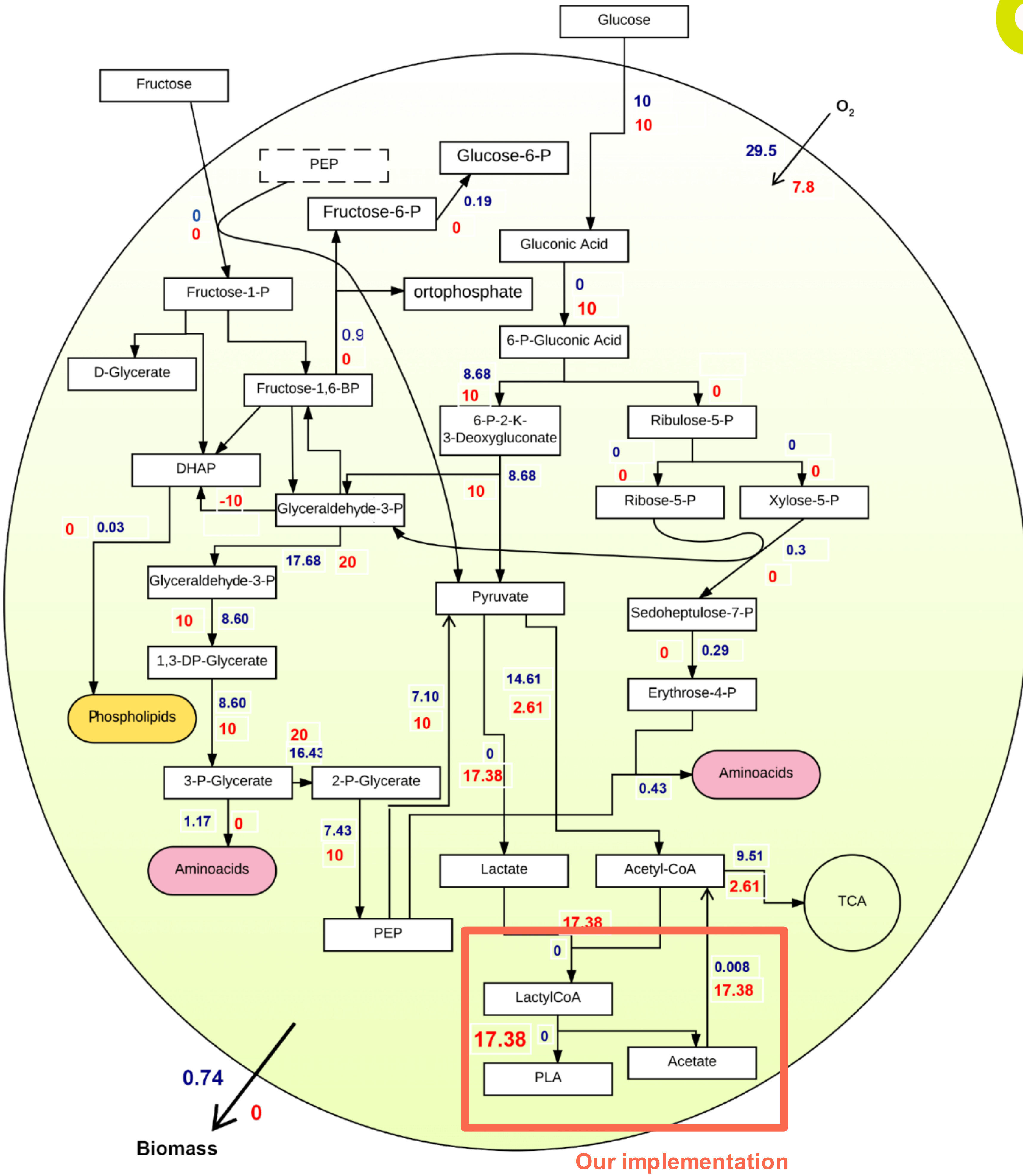
<u>Dynamic regulation system</u>

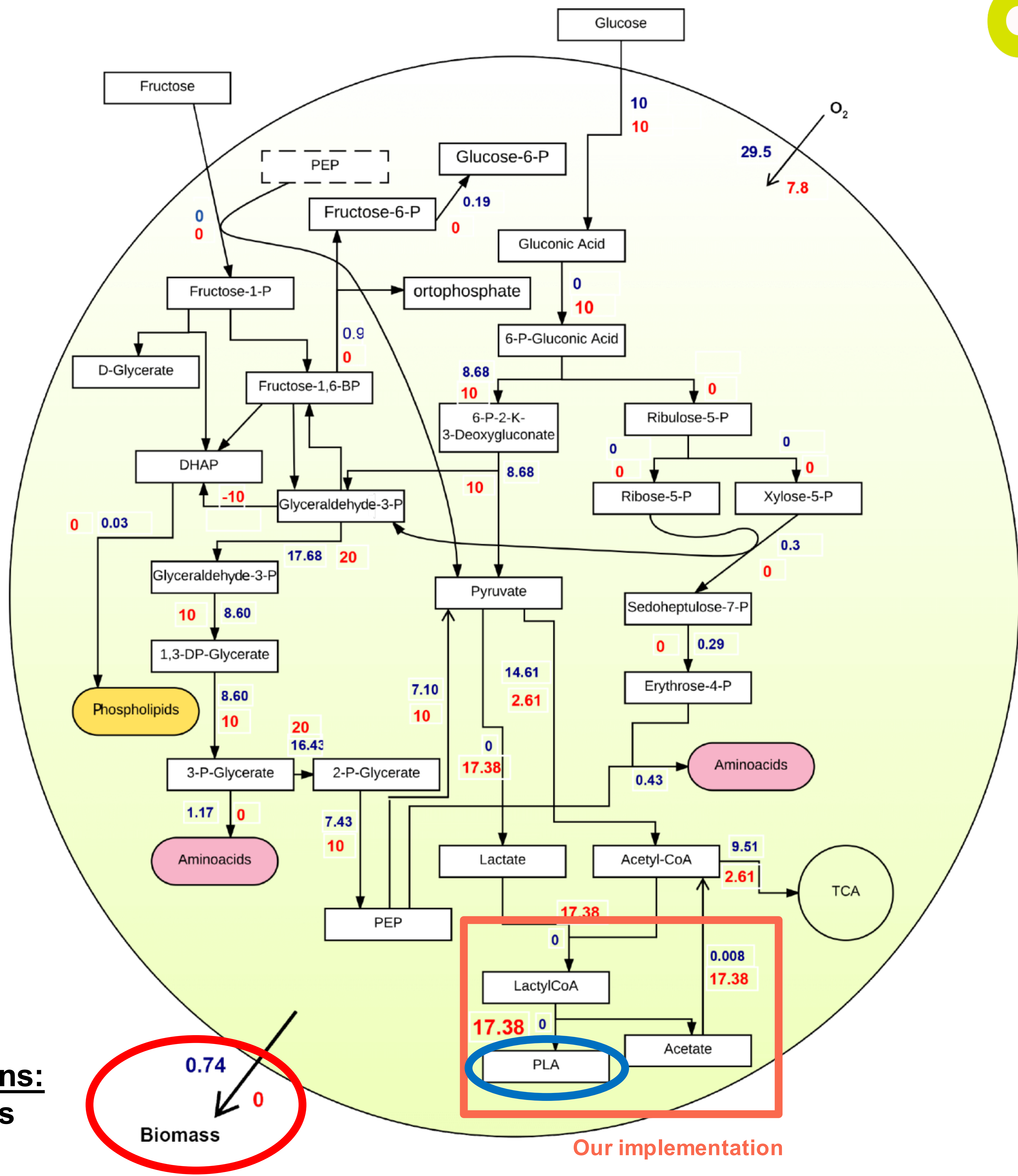
→ Kappa-based modeling

→ Electronic circuit



Glucose
Fructose
PEP
Glucose-6-P
Fructose-6-P
Gluconic Acid
ortophosphate
Fructose-1-P
6-P-Gluconic Acid
D-Glycerate
Fructose-1,6-BP
6-P-2-K-3-Deoxygluconate
Ribulose-5-P
DHAP
Glyceraldehyde-3-P
Ribose-5-P
Xylose-5-P
Glyceraldehyde-3-P
Pyruvate
Sedoheptulose-7-P
1,3-DP-Glycerate
Erythrose-4-P
Phospholipids
Aminoacids
3-P-Glycerate
2-P-Glycerate
Aminoacids
Lactate
Acetyl-CoA
TCA
PEP
LactylCoA
PLA
Acetate
O_2
Biomass
10
10
29.5
7.8
0.19
0
0
0
0
10
0.9
0
8.68
10
0
-10
8.68
10
0
0
0
0
0
0.03
17.68
20
0.3
0
10
8.60
0
0.29
14.61
2.61
7.10
10
8.60
10
20
16.43
0
17.38
0.43
1.17
0
7.43
10
9.51
2.61
17.38
0
0.008
17.38
17.38
0
0.74
0


Glucose
Fructose
PEP
Glucose-6-P
Fructose-6-P
Gluconic Acid
O$_2$
29.5
7.8
0.19
0
10
10
Fructose-1-P
ortophosphate
6-P-Gluconic Acid
D-Glycerate
Fructose-1,6-BP
0.9
8.68
10
6-P-2-K-3-Deoxygluconate
Ribulose-5-P
Ribose-5-P
Xylose-5-P
DHAP
-10
Glyceraldehyde-3-P
0.03
17.68
20
Pyruvate
0.3
Sedoheptulose-7-P
0.29
Erythrose-4-P
1,3-DP-Glycerate
8.60
Phospholipids
7.10
14.61
2.61
16.43
17.38
3-P-Glycerate
2-P-Glycerate
Aminoacids
0.43
1.17
7.43
Lactate
Acetyl-CoA
9.51
TCA
PEP
LactylCoA
0.008
Acetate
PLA
0.74
Biomass
Our implementation

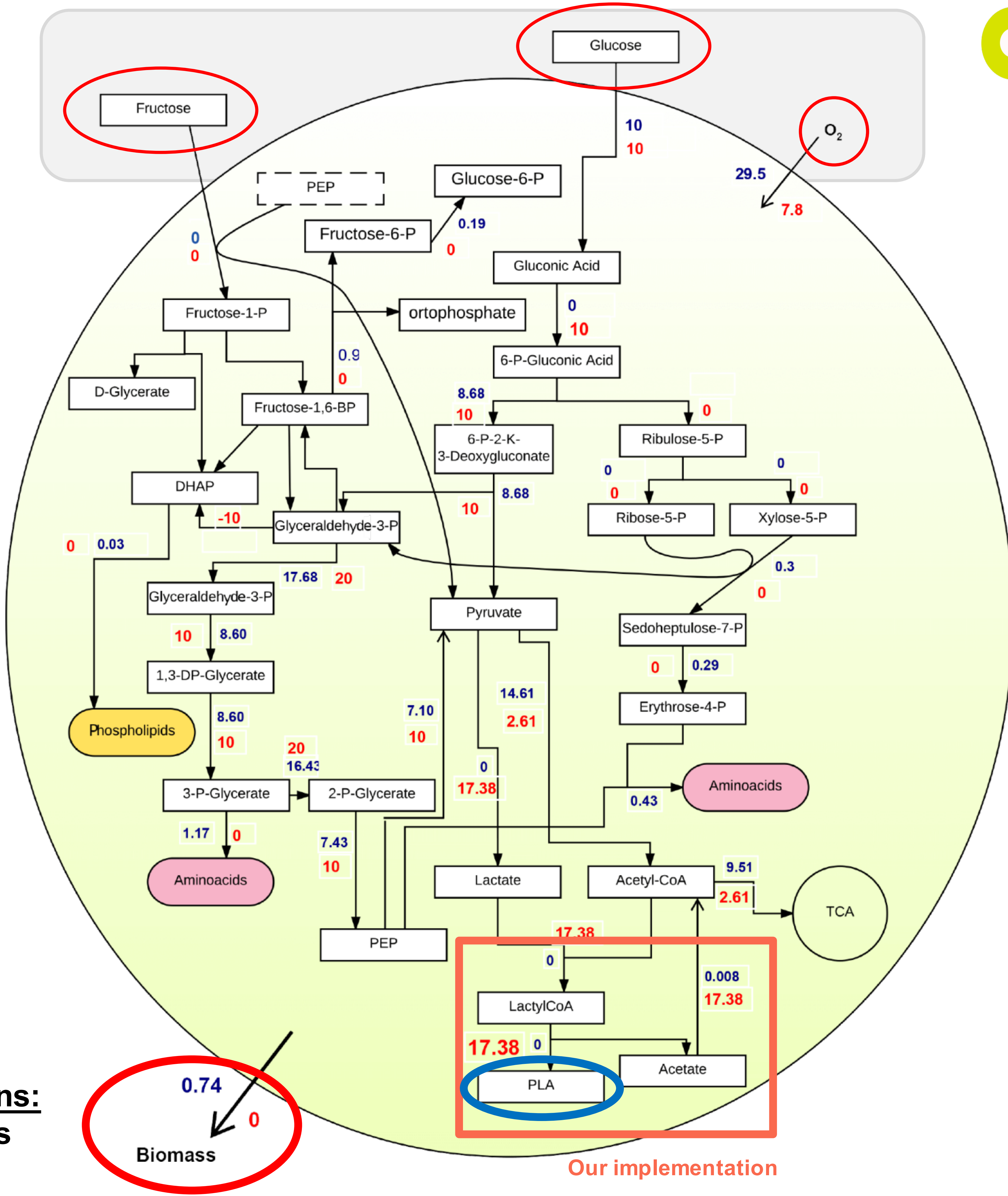


Objective functions:

In blue: biomass

In red: PLA

Growth conditions



Objective functions:

In blue: biomass

In red: PLA

Playing with FBA

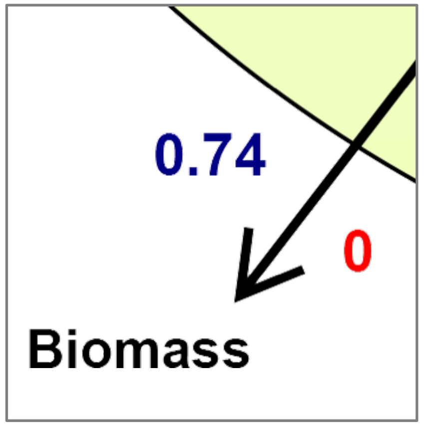
Glucose

Fructose

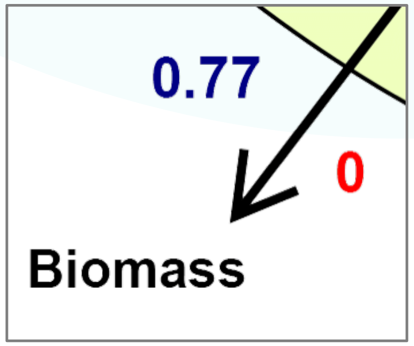
Playing with FBA

Objective functions:
In blue: biomass
In red: PLA

Glucose

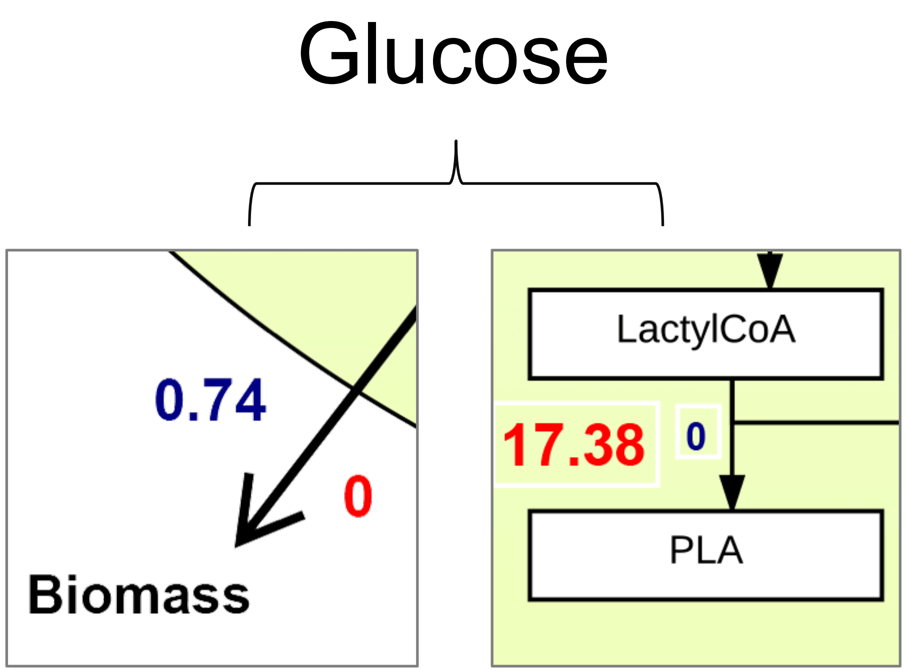


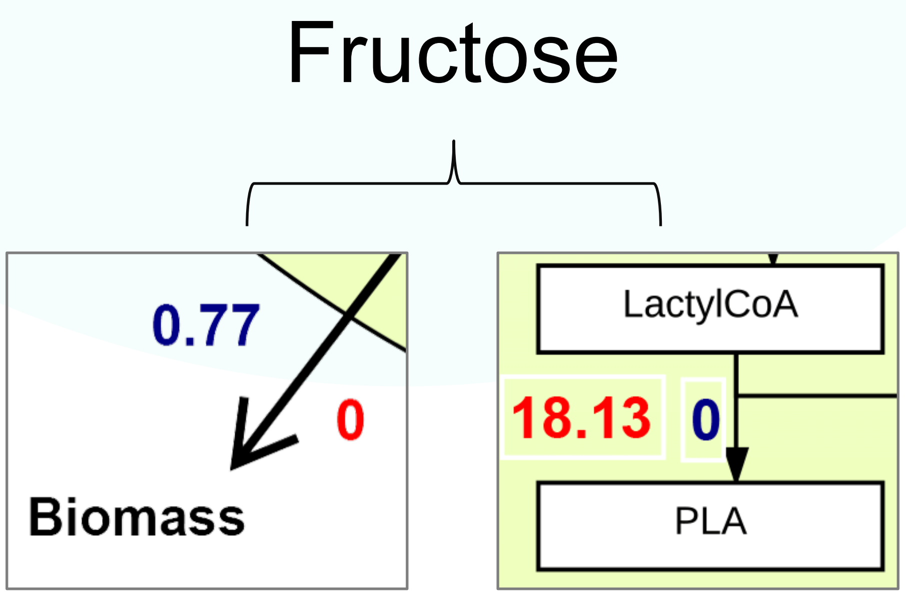
Fructose



Playing with FBA

Objective functions:
In blue: biomass
In red: PLA

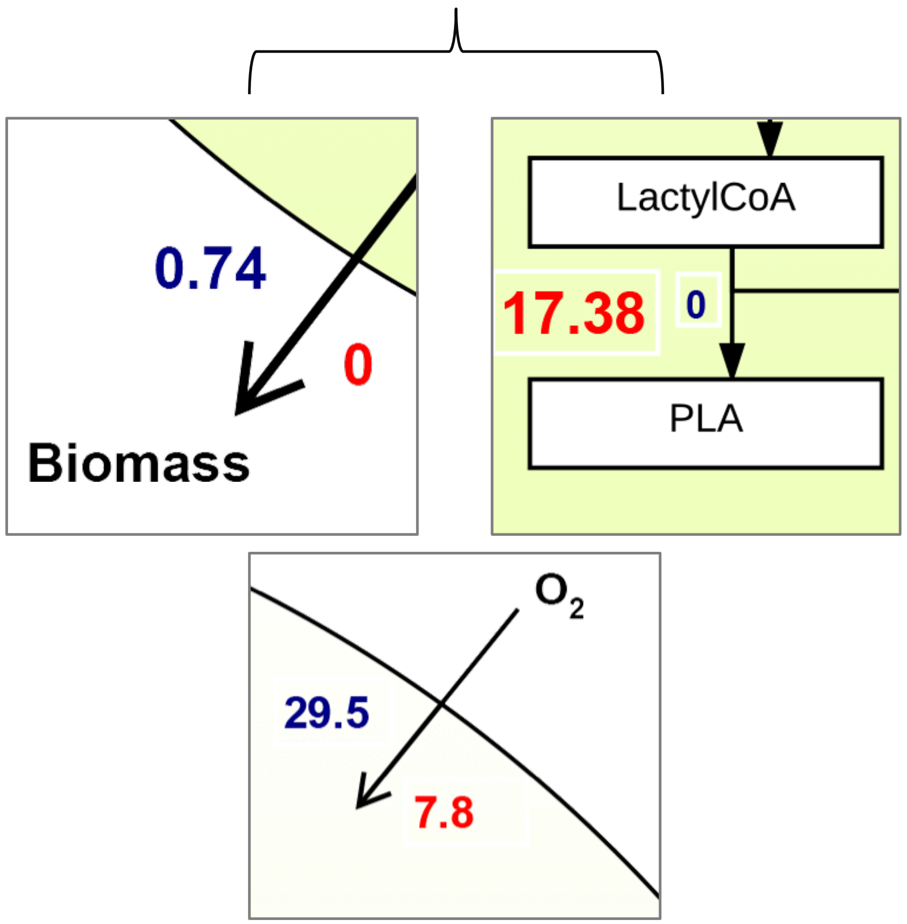




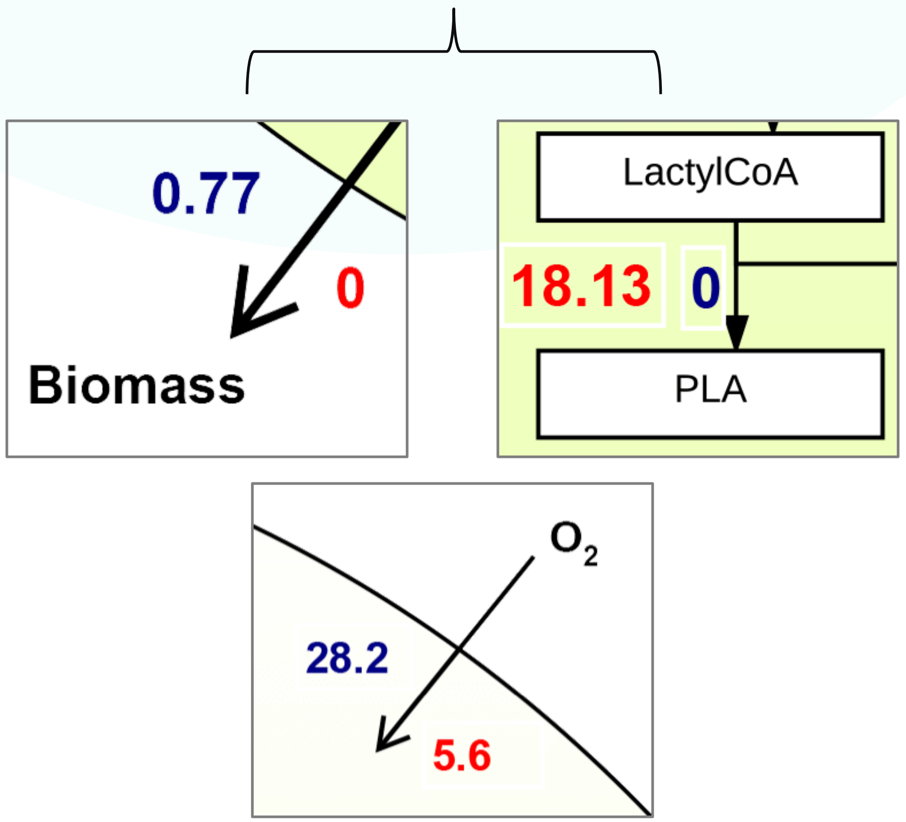
Playing with FBA

Objective functions:
In blue: biomass
In red: PLA

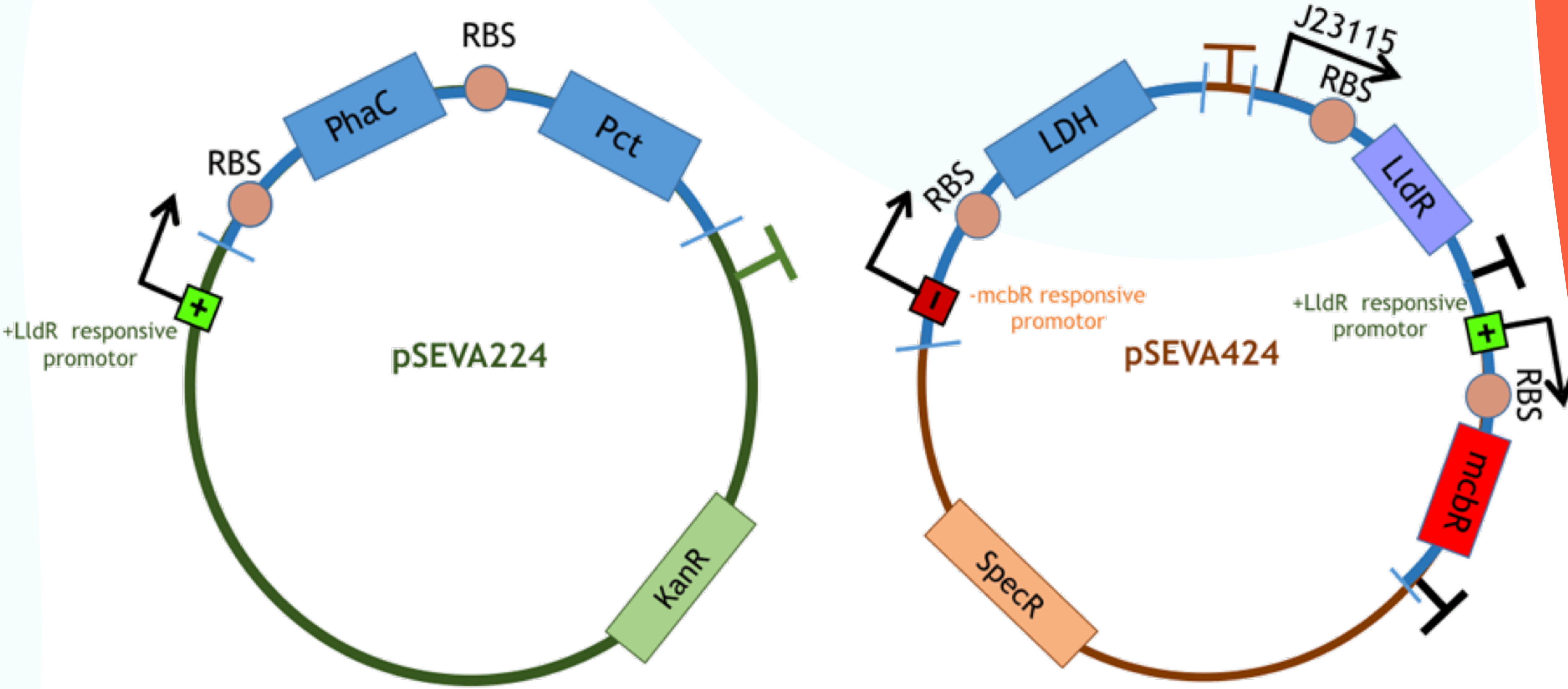
Glucose



Fructose

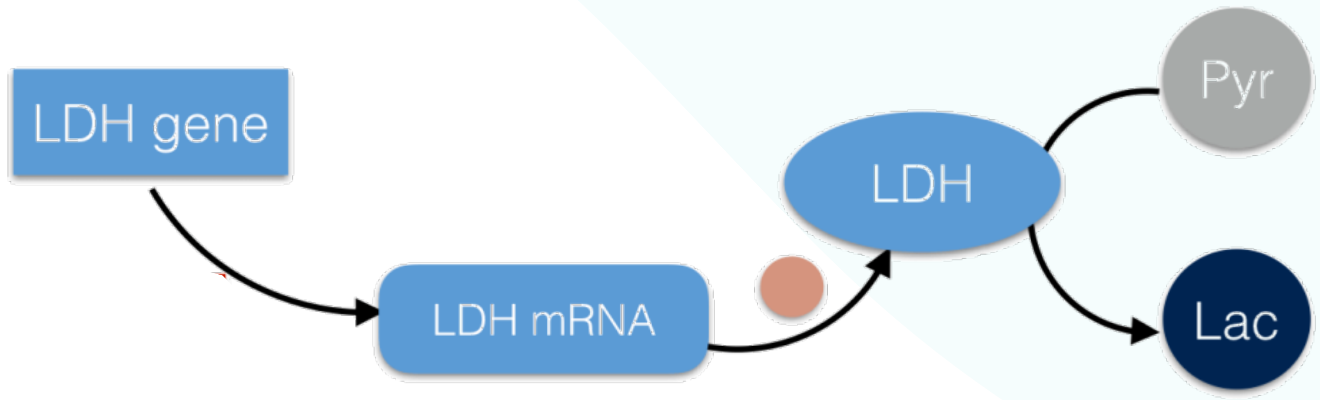


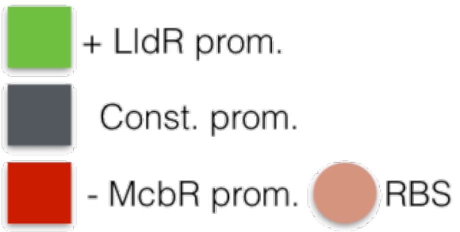
Dynamic regulation – general design



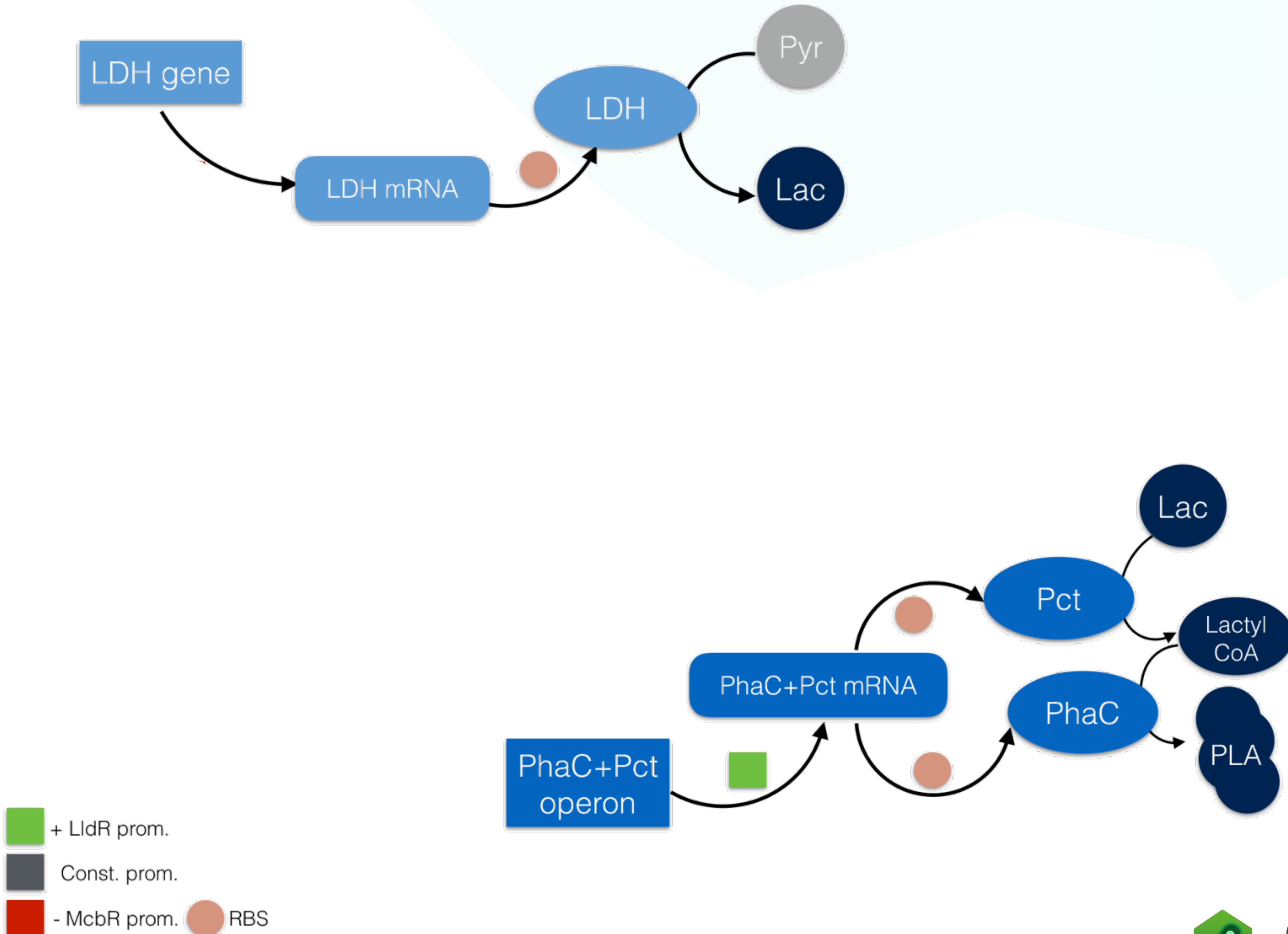
Goal: optimization of cell metabolism

Kappa-based modeling

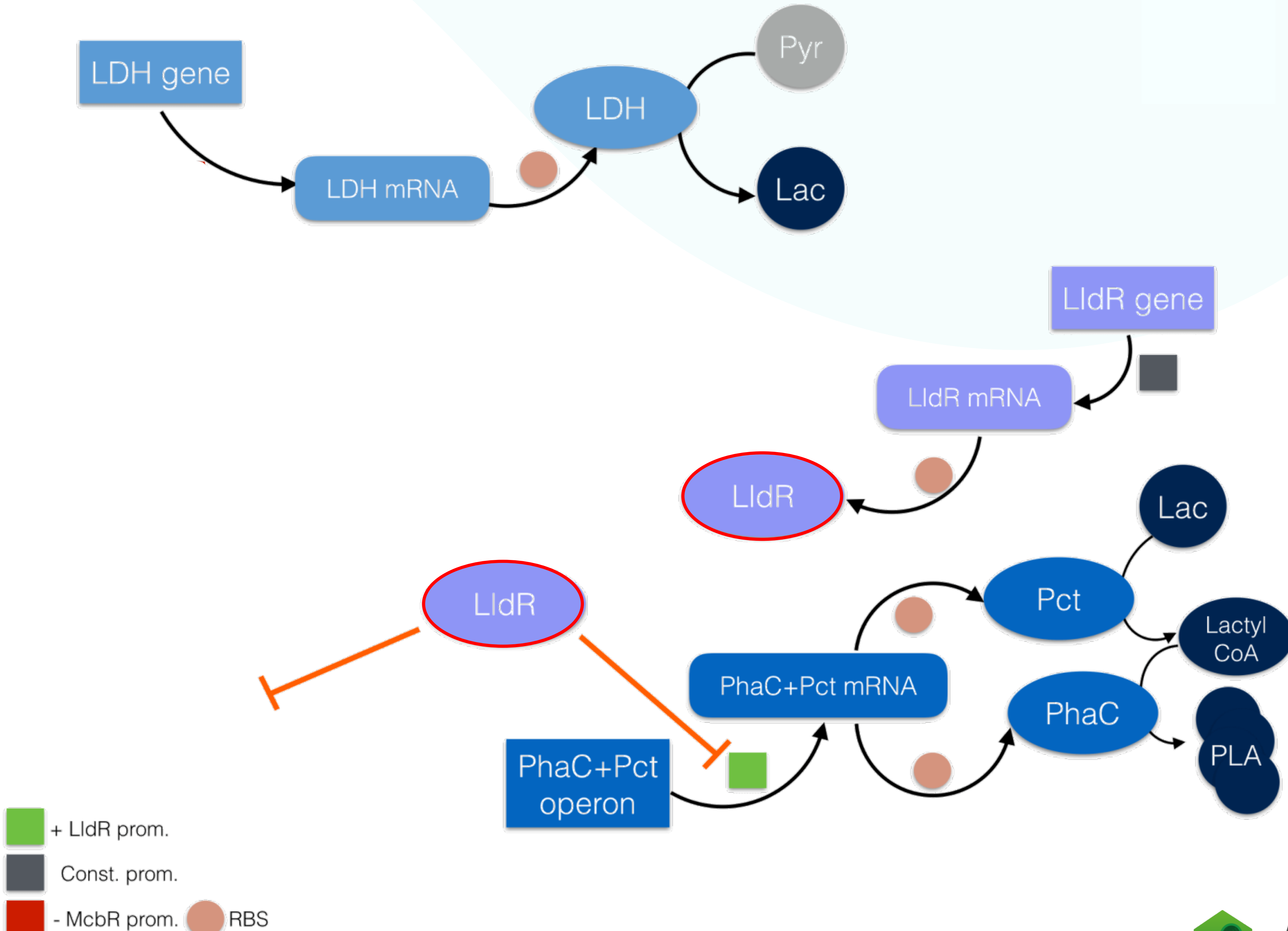




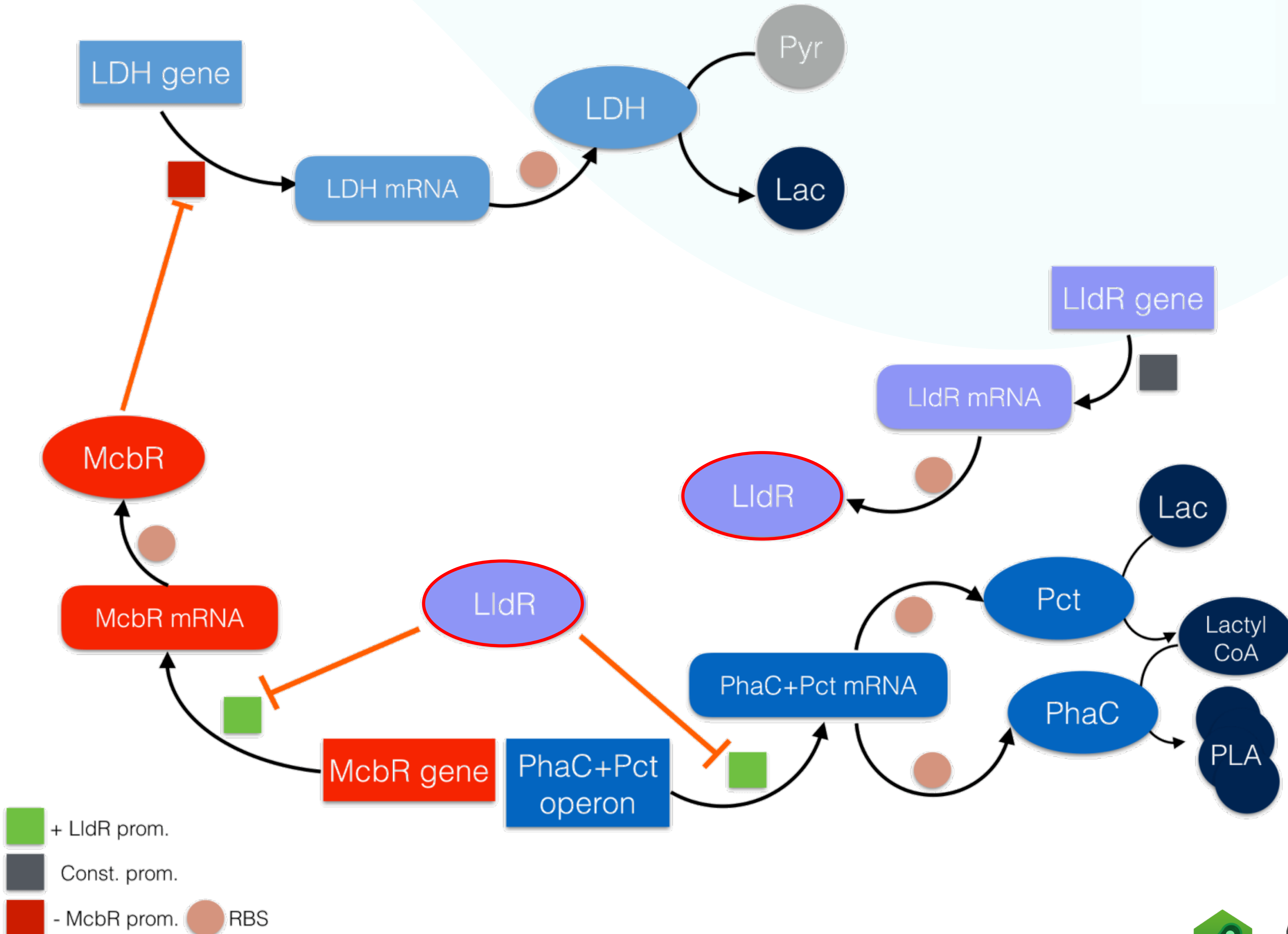
Kappa-based modeling



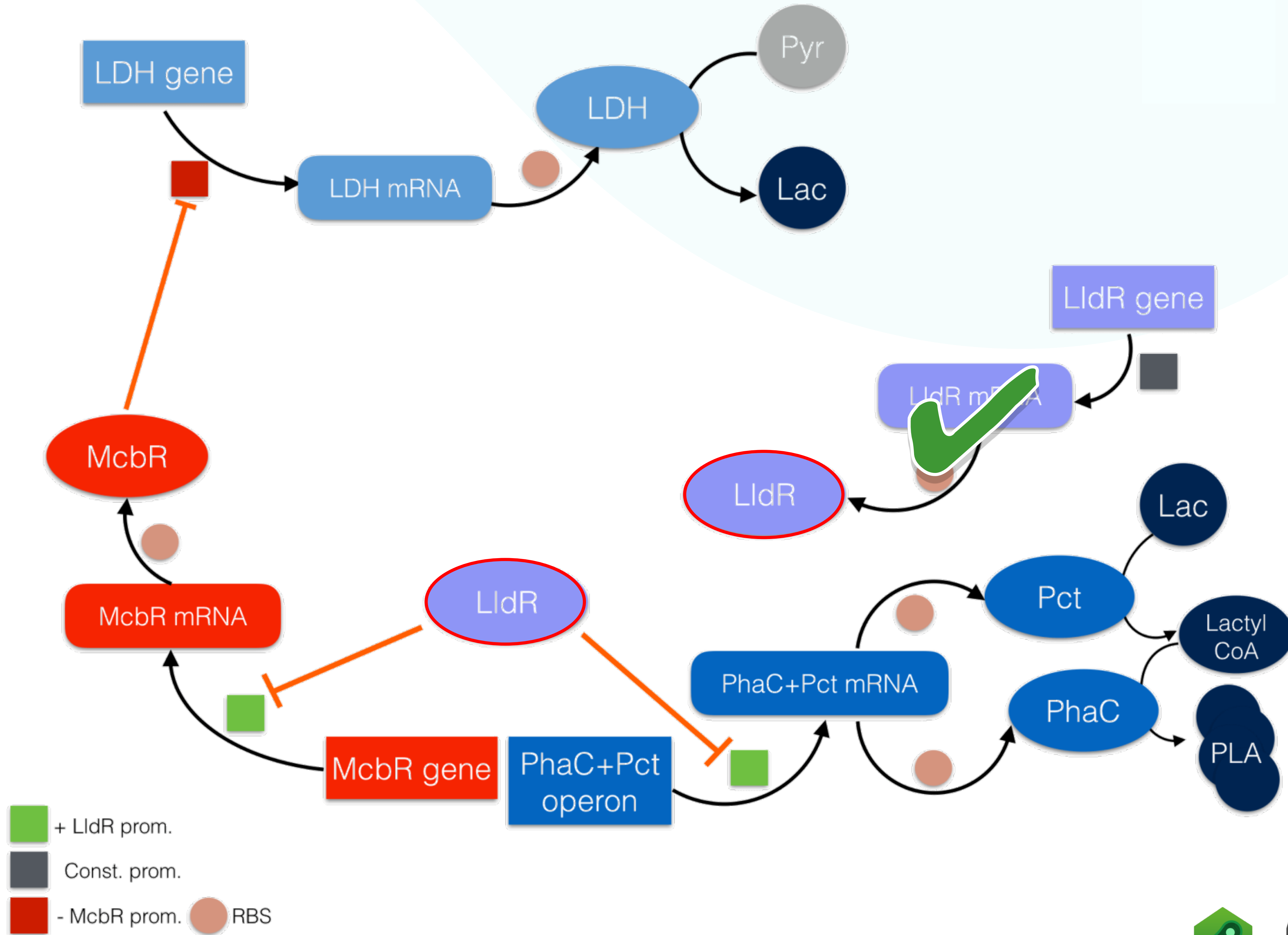
Kappa-based modeling



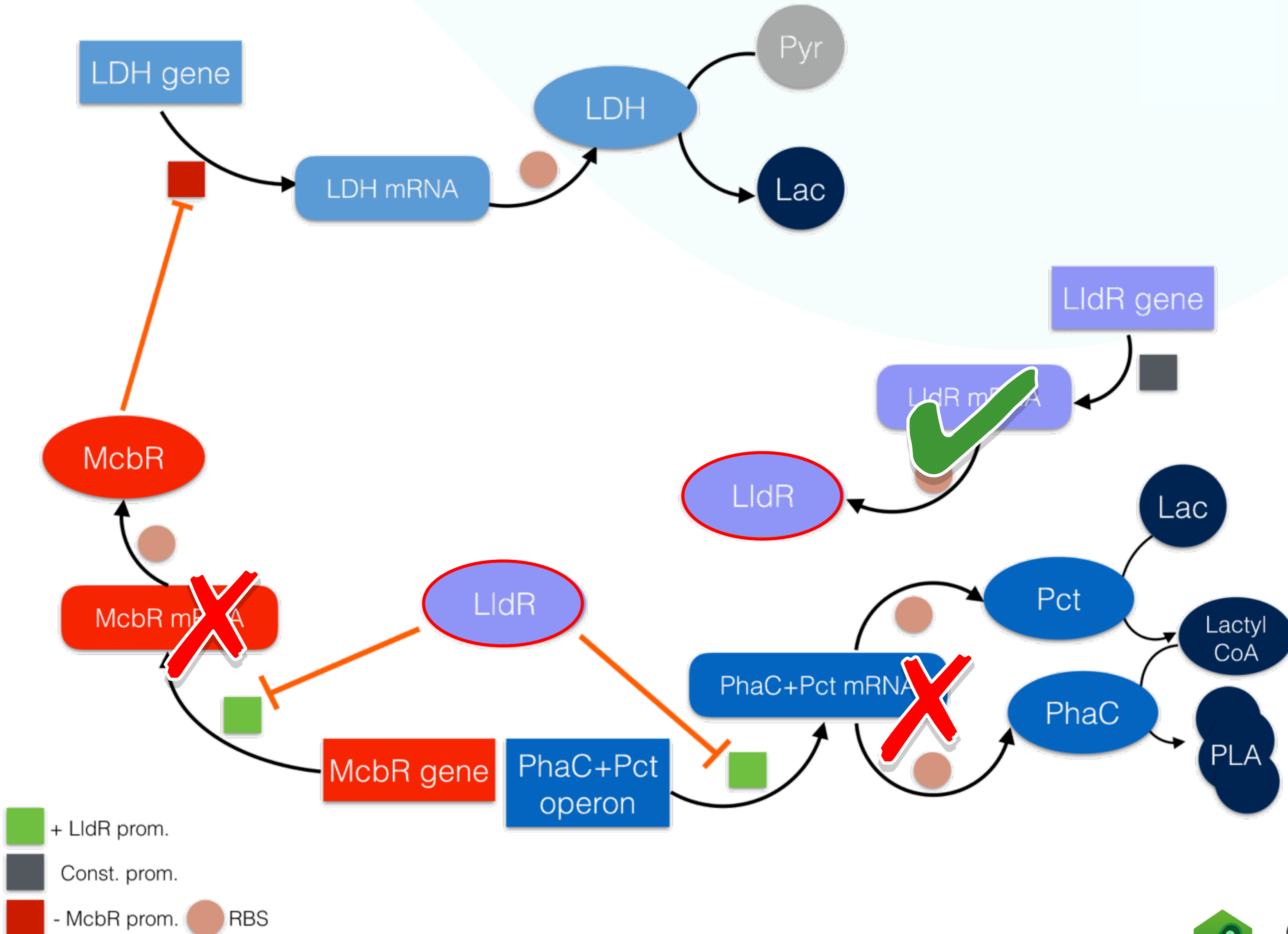
Kappa-based modeling



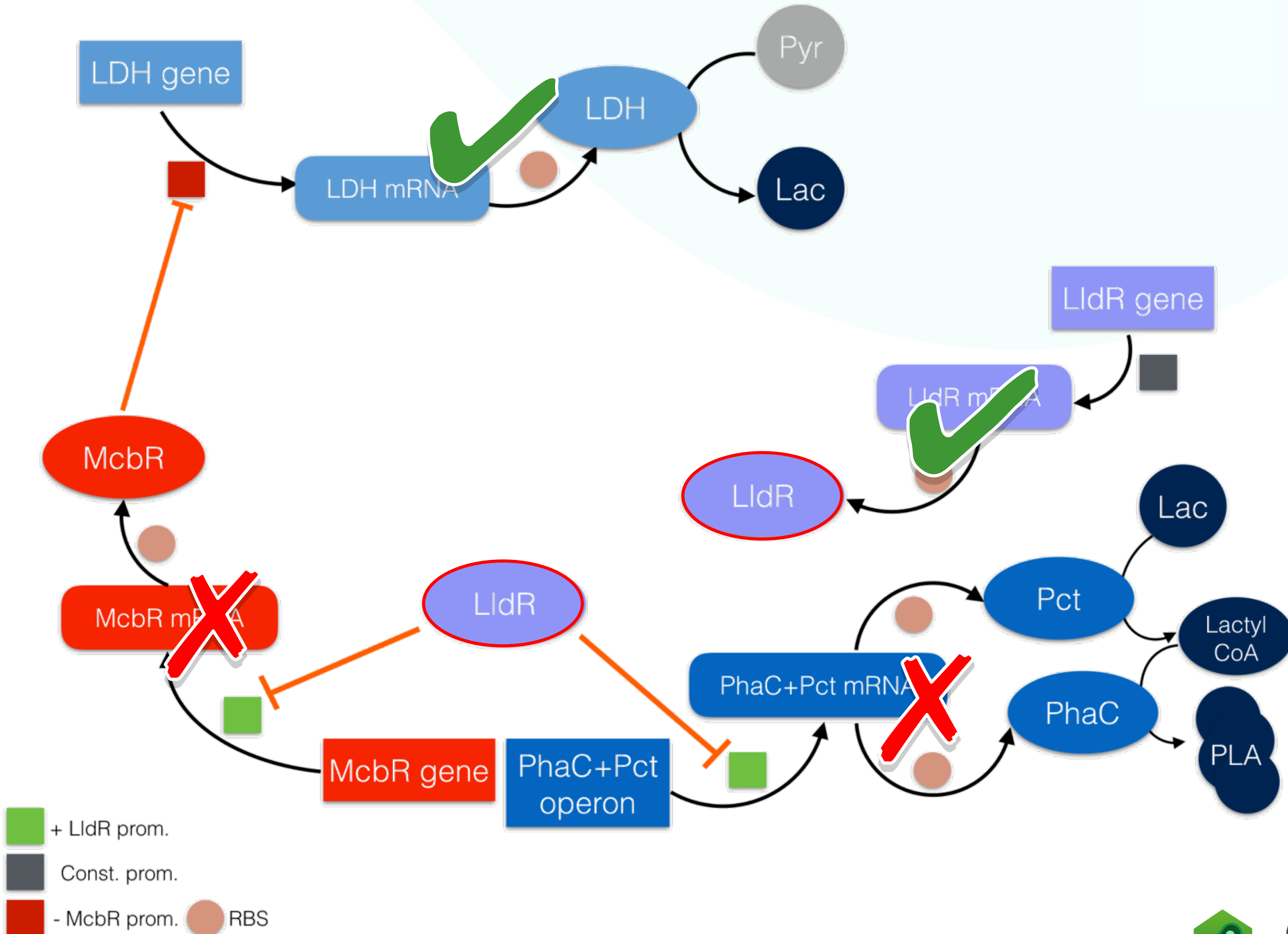
Kappa-based modeling



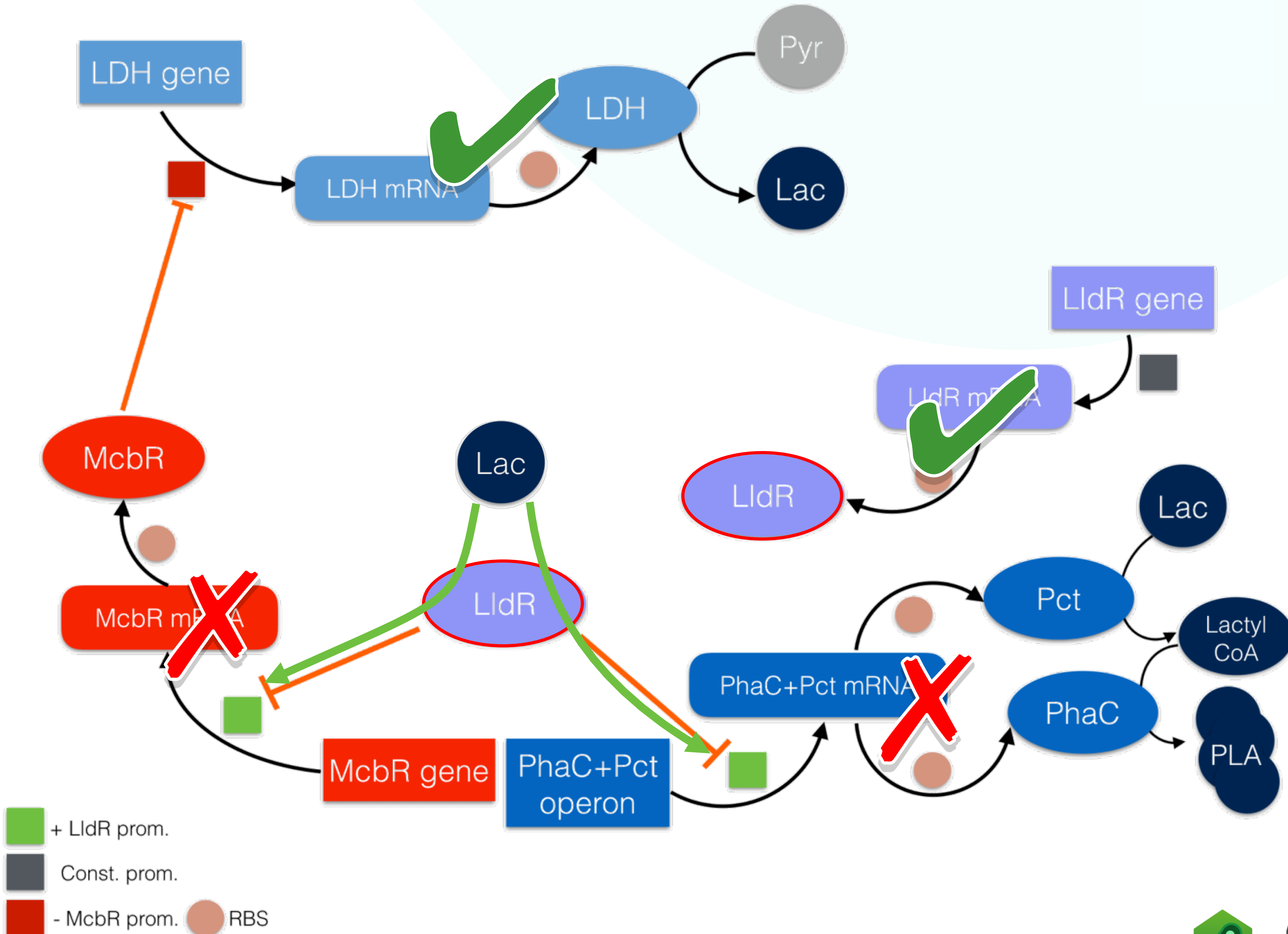
Kappa-based modeling



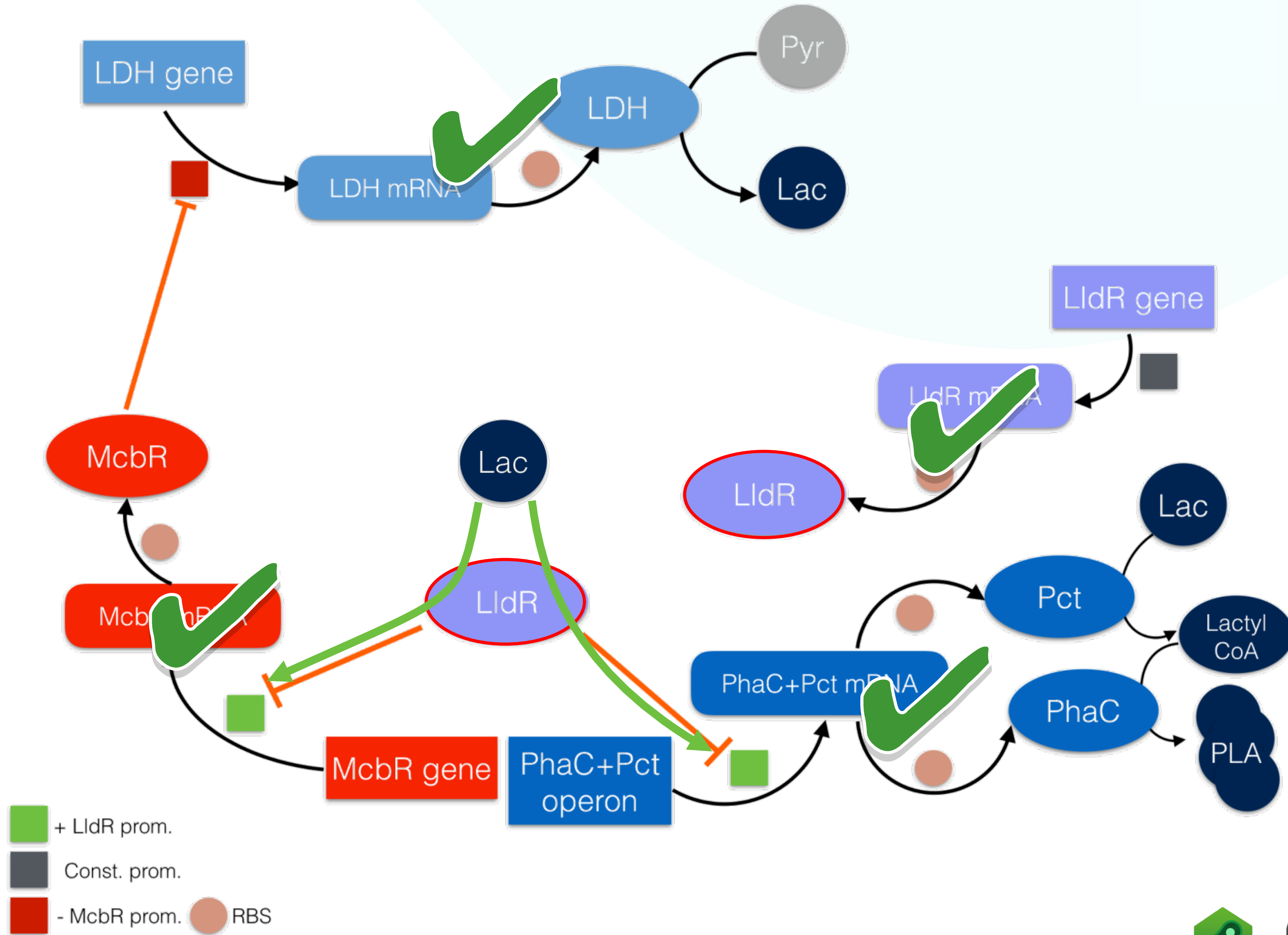
Kappa-based modeling



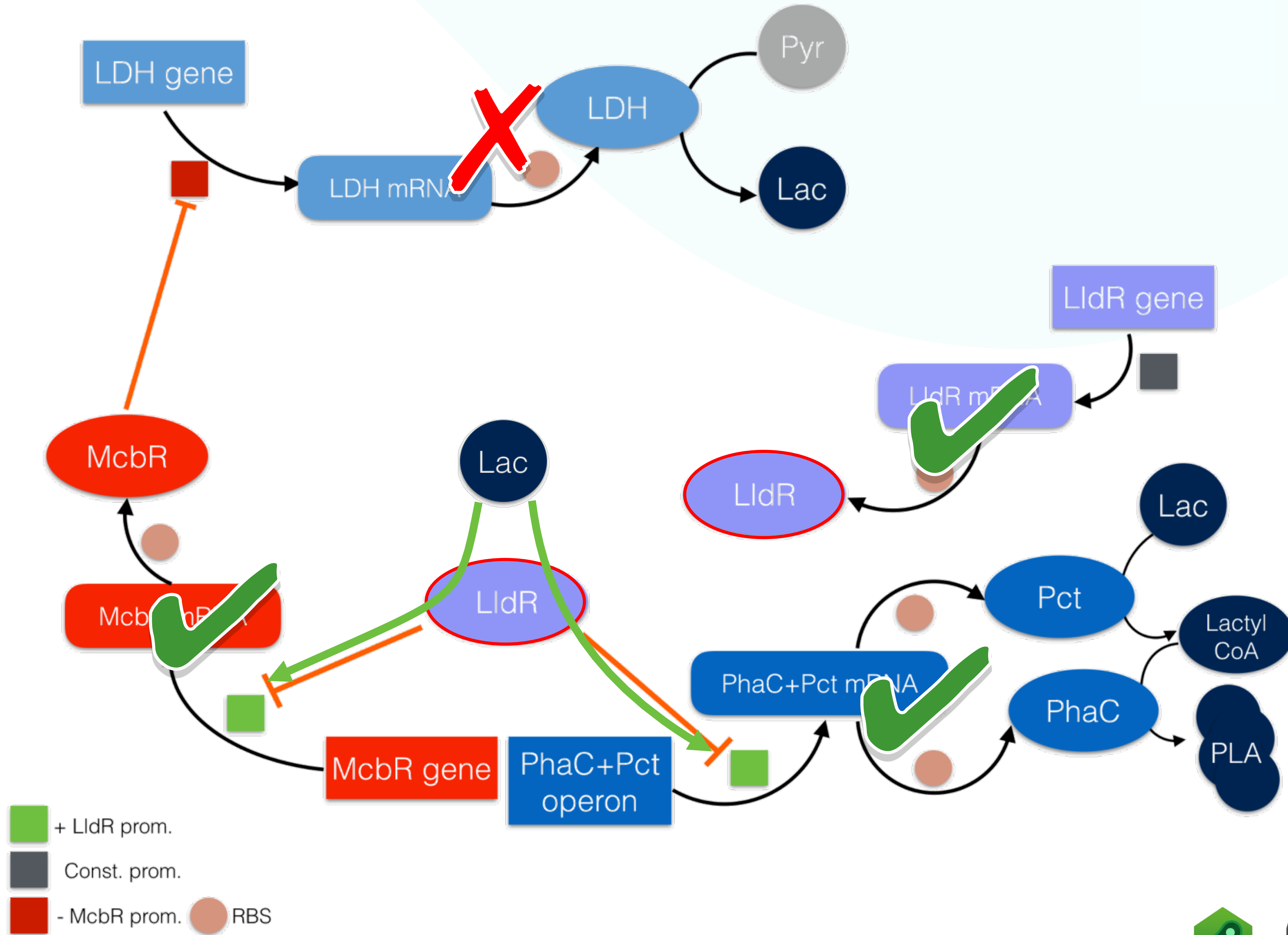
Kappa-based modeling



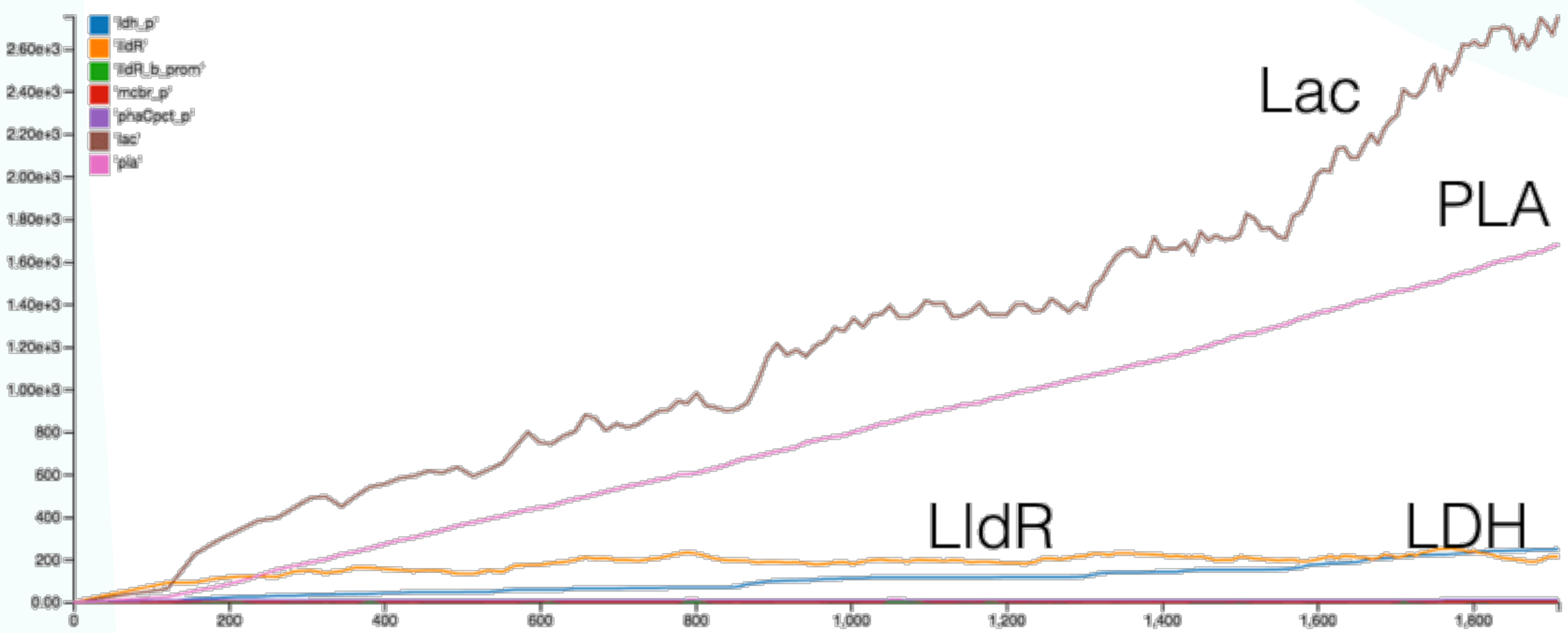
Kappa-based modeling



Kappa-based modeling



Kappa-based modeling

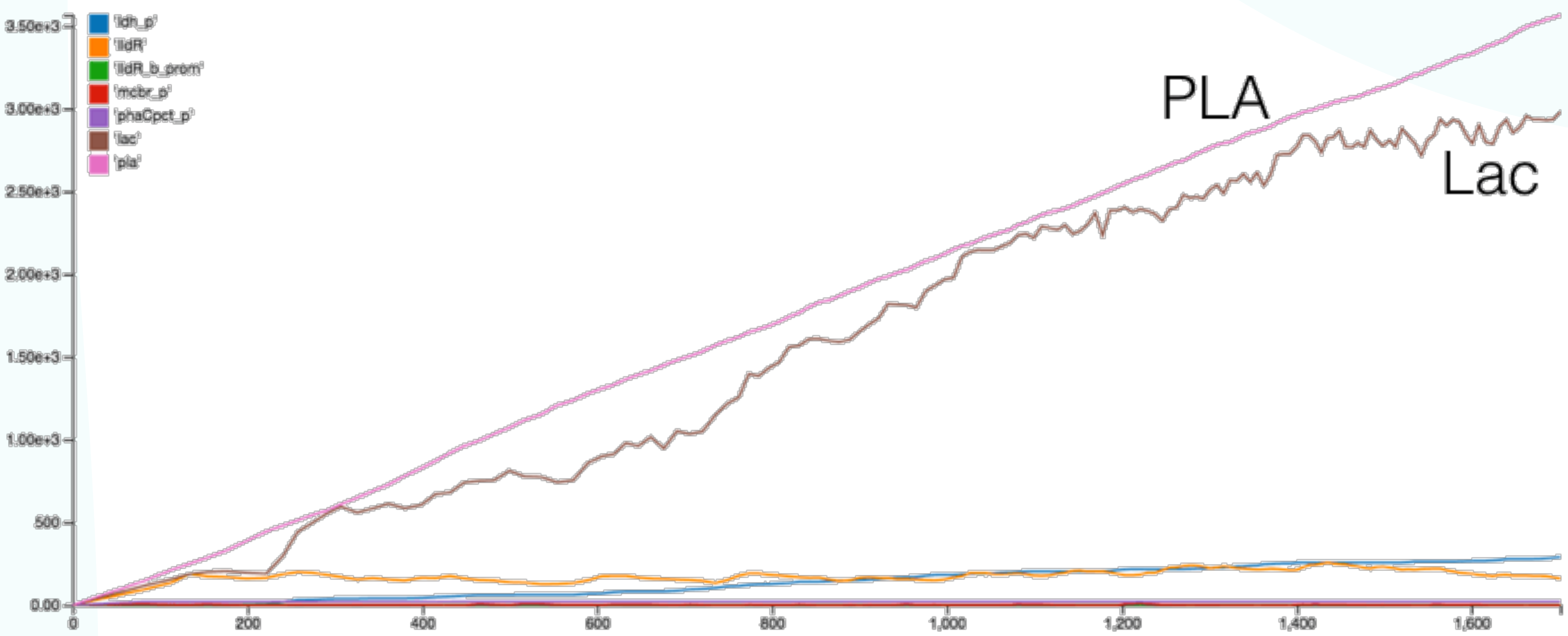


Species	Synthesis rate
lldR mRNA	0,5
lldR protein	0,5

Strong promoter and RBS

Lactate and PLA are being produced, but not all the lactate is directed towards PLA

Kappa-based modeling

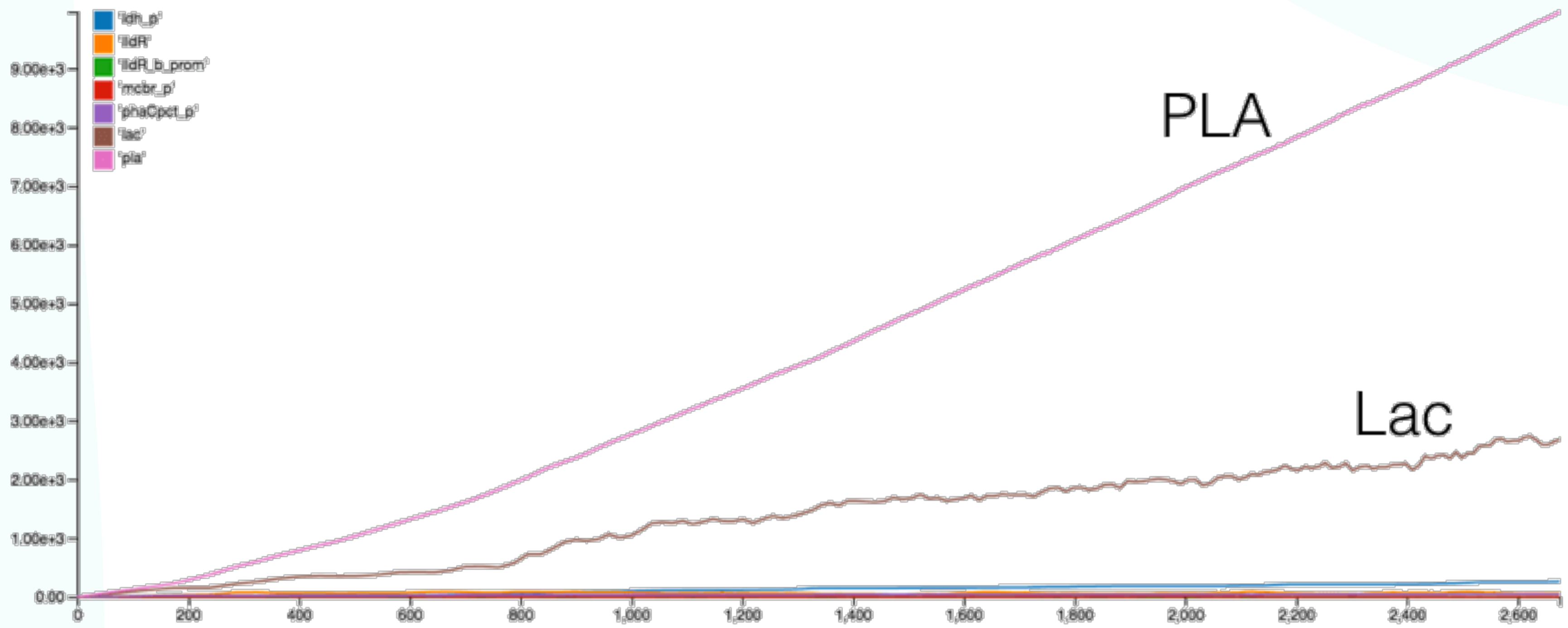


Species	Synthesis rate
lldR mRNA	**0,15**
lldR protein	0,5

Weak promoter and strong RBS

The PLA production increased, but still a lot of lactate is being lost

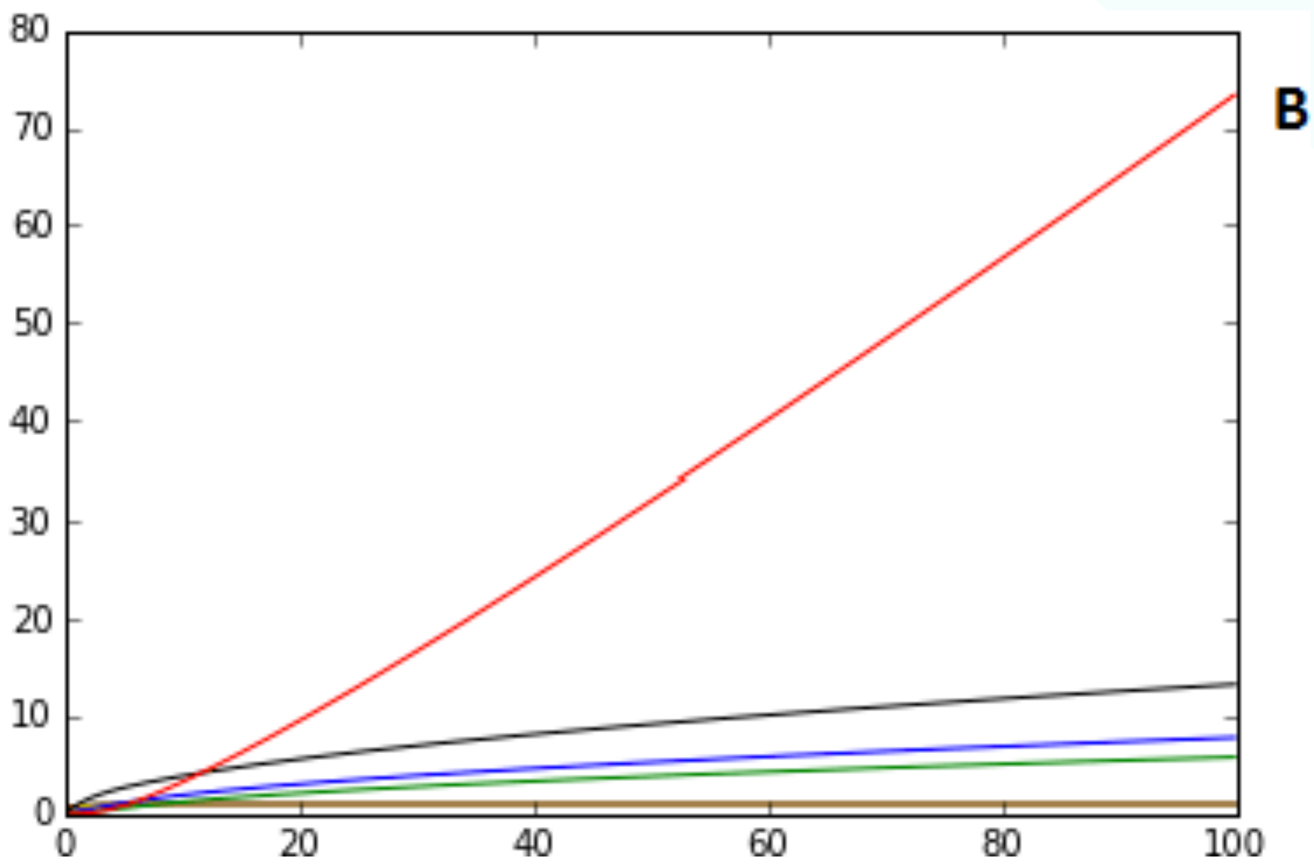
Kappa-based modeling



Species	Synthesis rate
lldR mRNA	0,15
lldR protein	0,15

Weak promoter and weak RBS

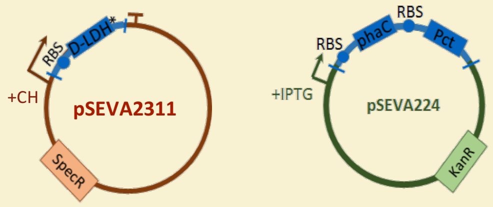
Dynamic circuit – general design



All the elements are stable over time, except PLA which is continuously increasing.

Modeling

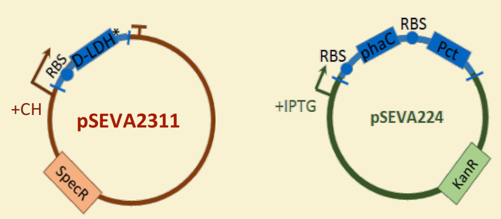
Basic design



Modeling

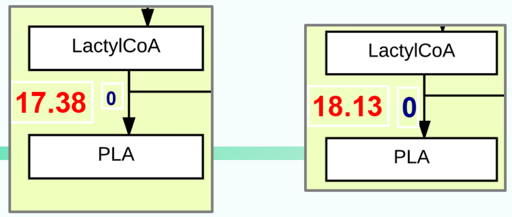
FBA

Basic design



Dynamical modeling

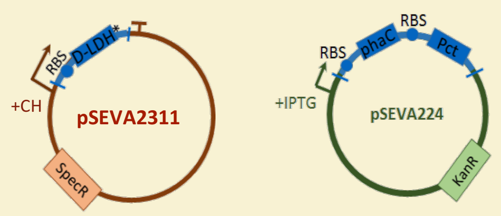
Modeling



FBA

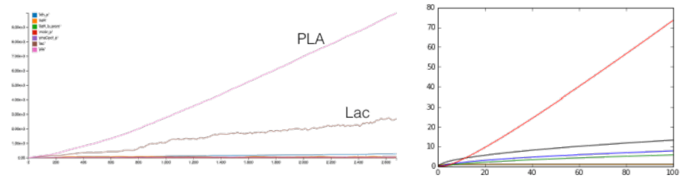
Improvement of growth conditions

Basic design

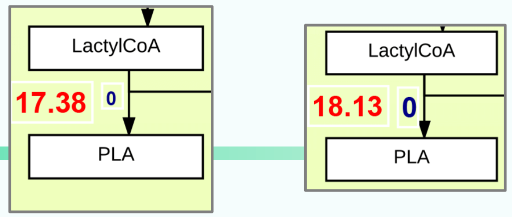


Dynamical modeling

Optimization by genetic elements of control



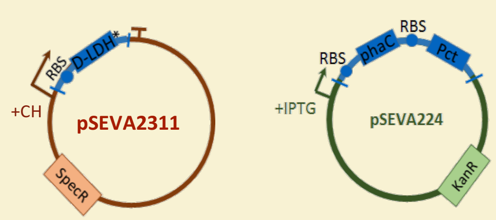
Modeling



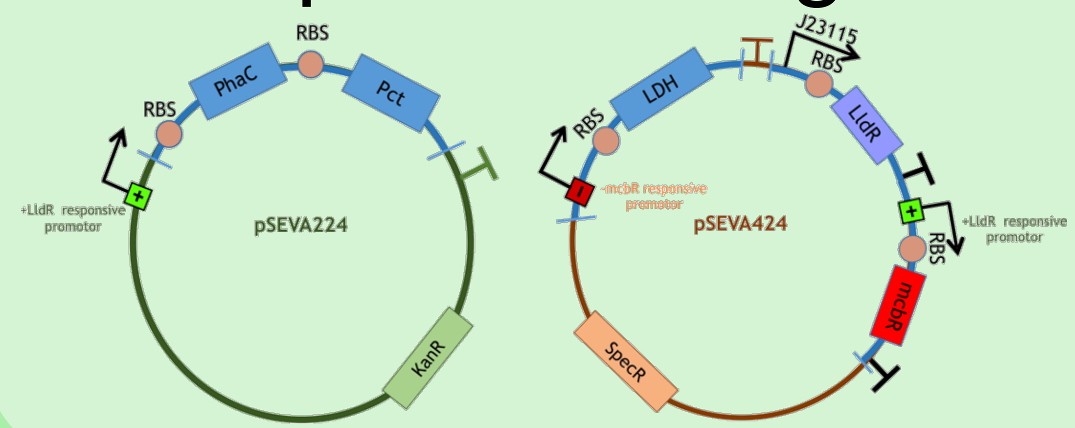
FBA

Improvement of growth conditions

Basic design

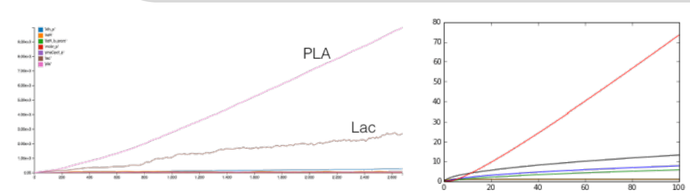


Improved design



Dynamical modeling

Optimization by genetic elements of control

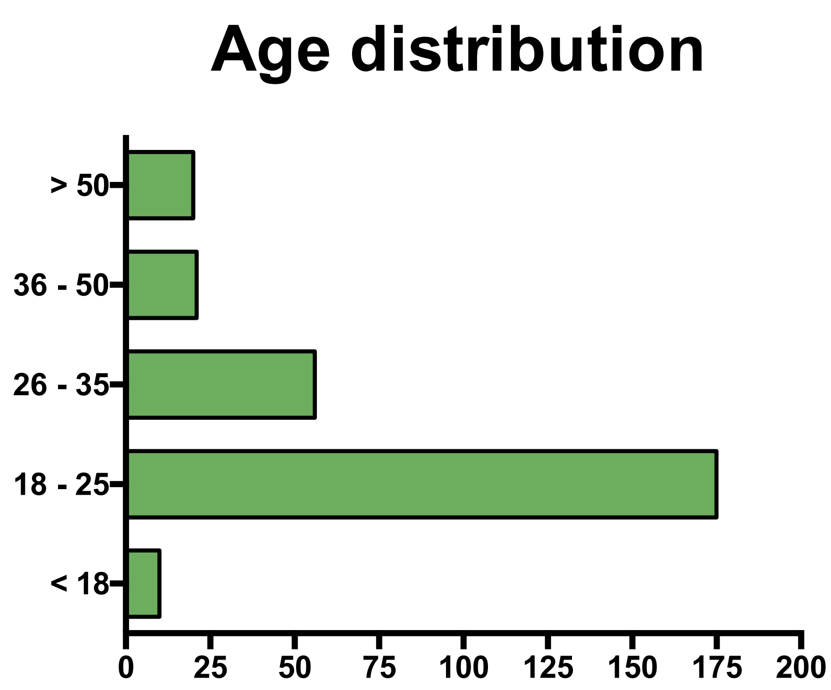


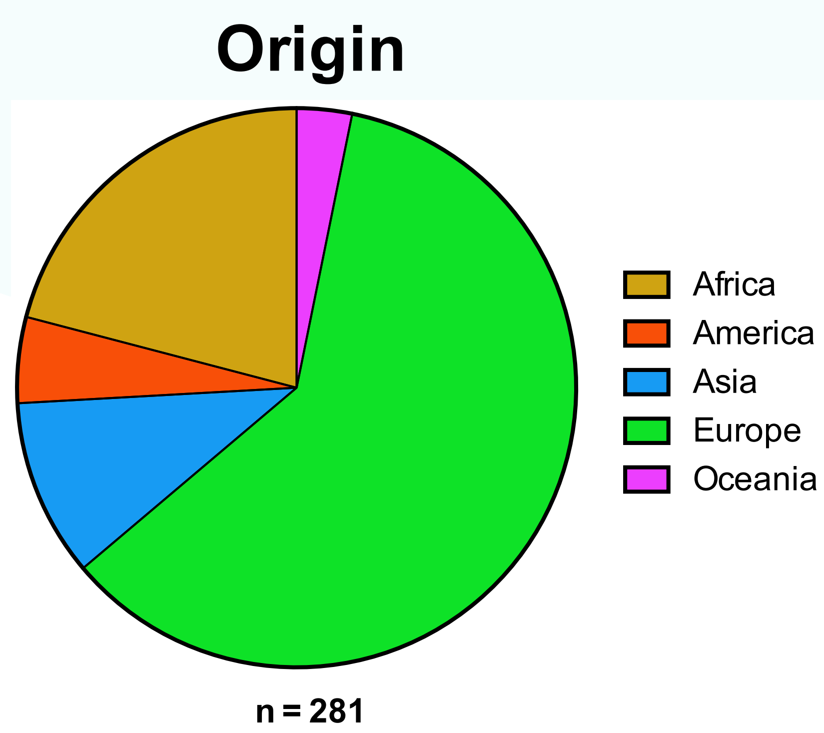
Human practices

Are people interested in PLA?

Survey responses

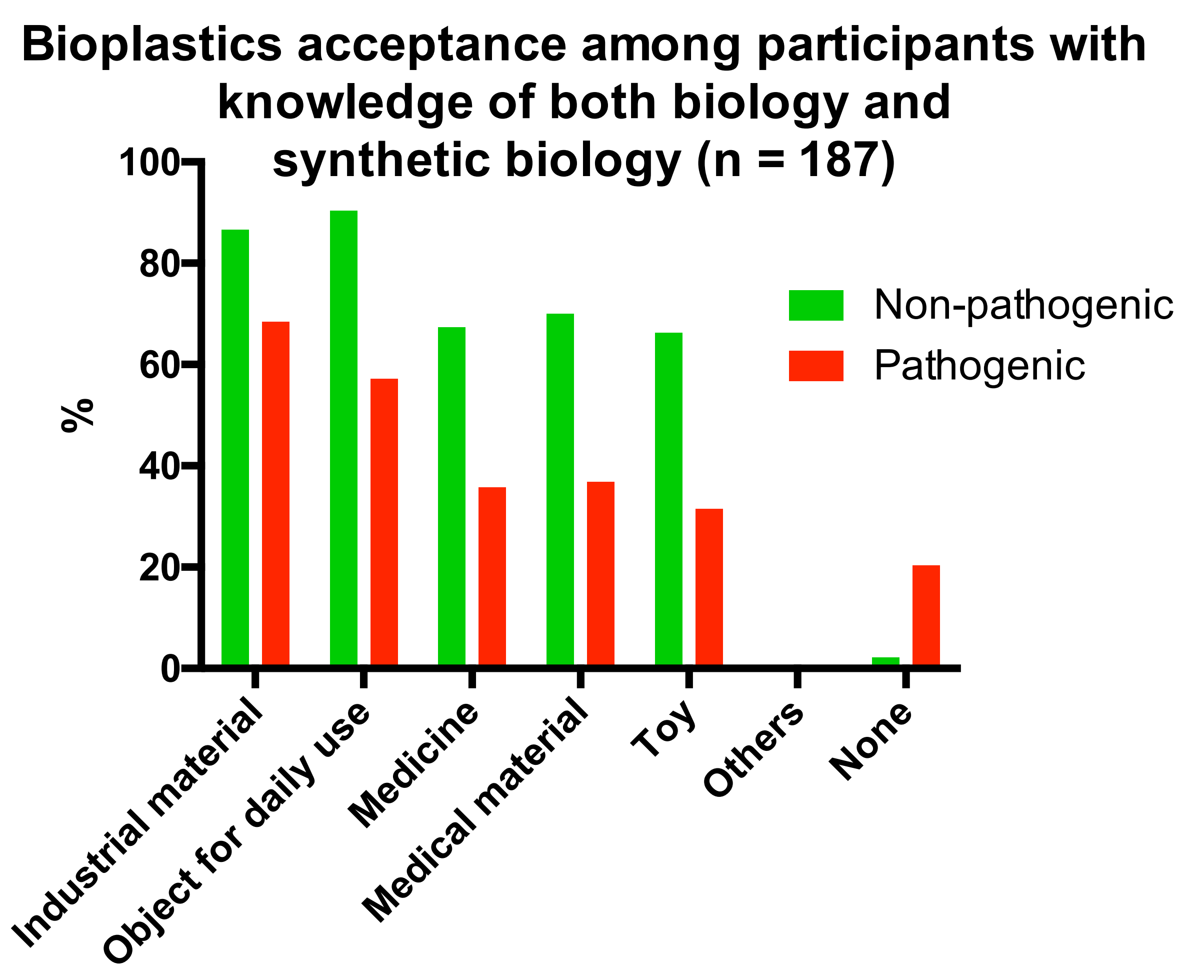
281 responses

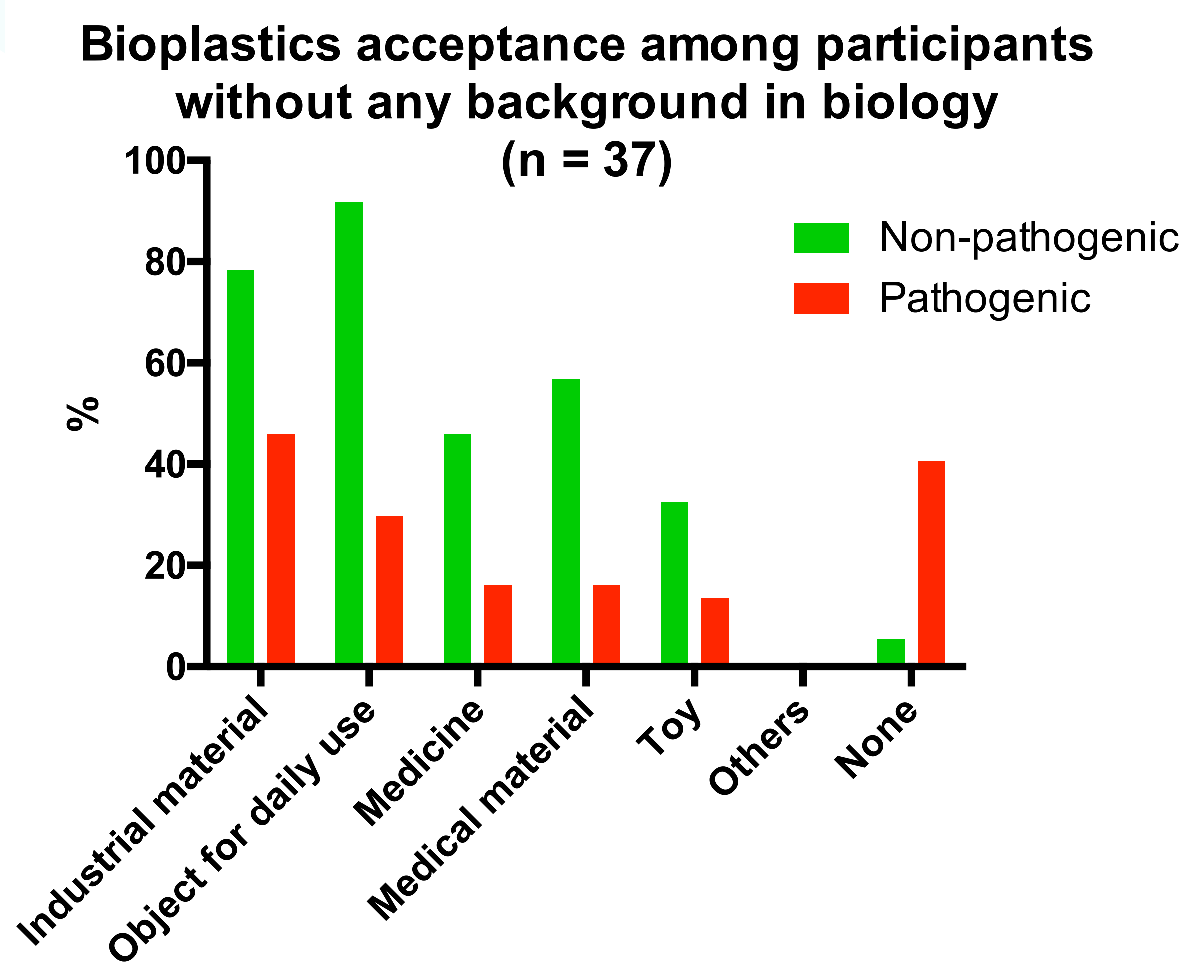




88.3% of respondents would choose bioplastics over petroleum-based equivalents

Bioplastics acceptance





Bioplastics acceptance

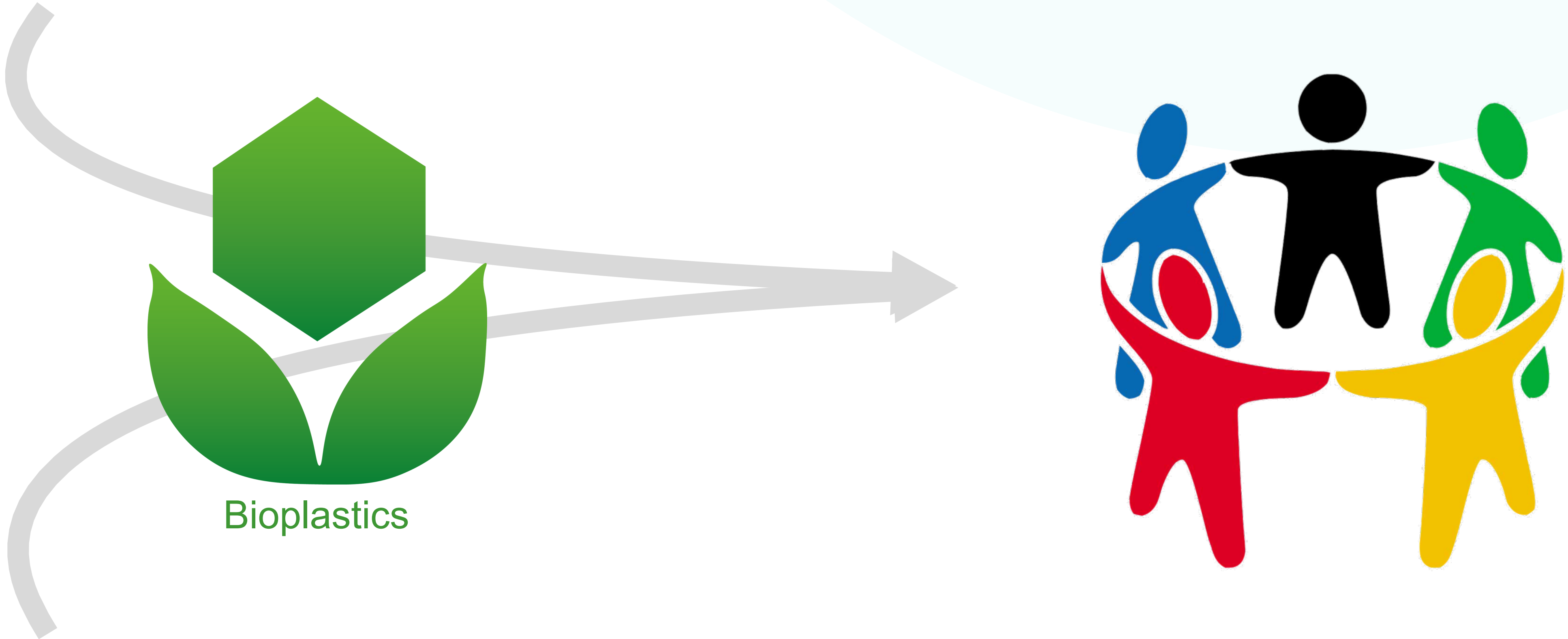
« Bacteria », « Pathogenic » = blurred knowledge

But

People are **interested in bioplastics**

Our Human Practices Objectives

1. Communicate efficiently on SynBio



2. Act towards more bioplastic consumption

Communicating efficiently on bioplastics

Physical meetings

Meeting people with different backgrounds

Physical meetings

Meeting people with different backgrounds

Physical meetings

Meeting people with different backgrounds

université
PARIS-SACLAY

Physical meetings

Meeting people with different backgrounds

université PARIS-SACLAY

iGEM EVRY 2016

Physical meetings

Meeting people with different backgrounds

université
PARIS-SACLAY

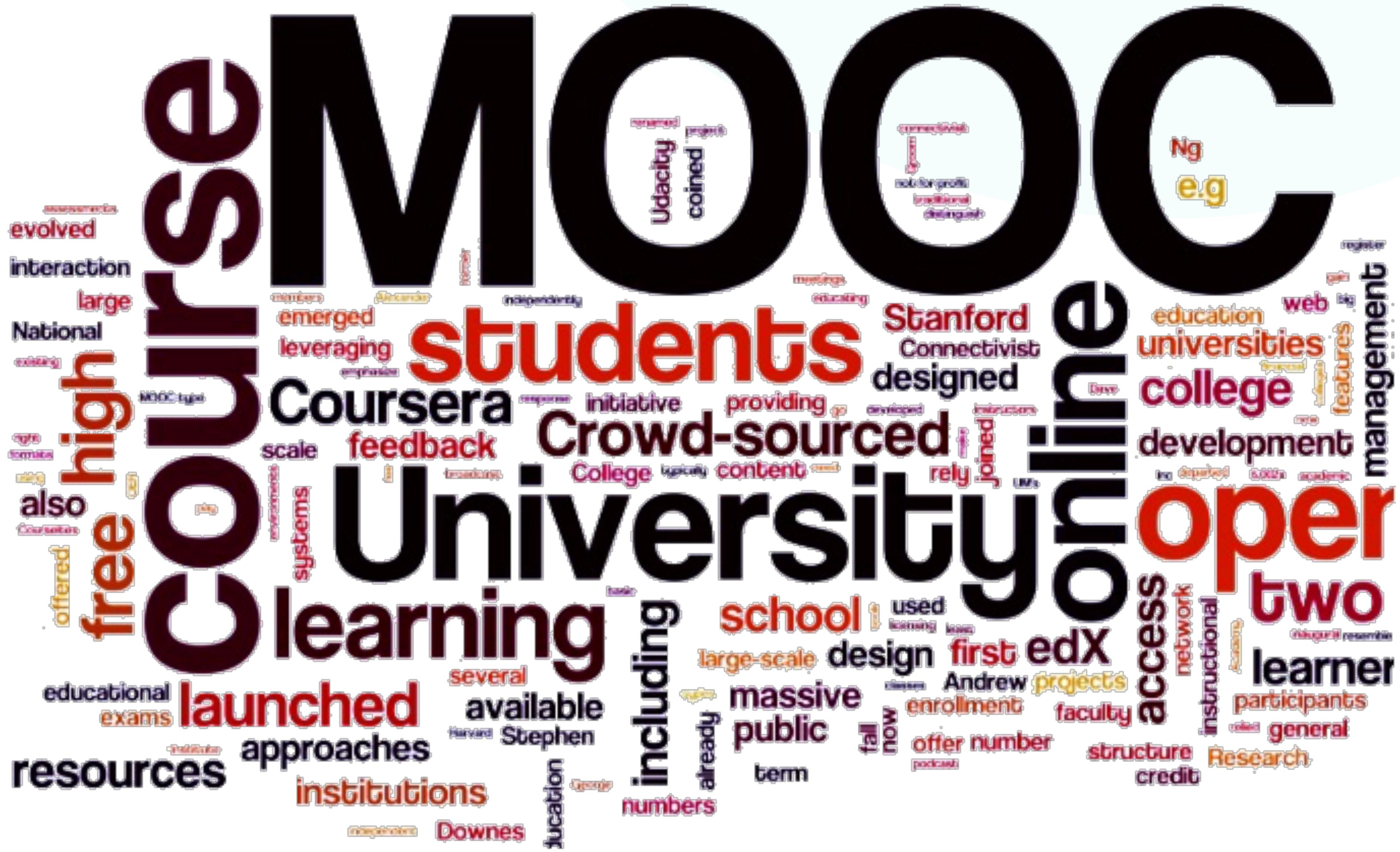
fête de
la Science

Uneven SynBio practice

http://2016.igem.org/Community/Map

How can we communicate on a wider scale ?

Massive Online Open Course



How can we communicate on a wider scale?

MOOC Experiments

High Schools
Not an easy public

University students
More reachable

Discussing on MOOCs

Souleymane Bachir Diagne

French and Philosophy Professor @ Columbia University

Former Professor at Cheikh-Anta-Diop University (Senegal)

Digital divide: gaps in access to the Internet

Discussing on MOOCs

Digital divide: gaps in access to the Internet

JERRY
DO IT TOGETHER

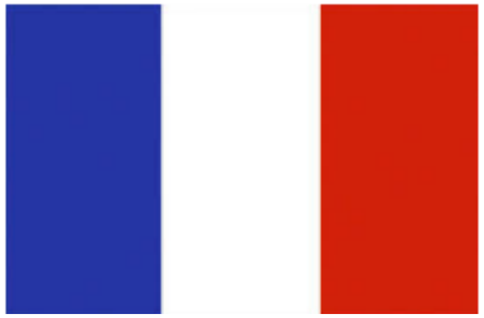
http://www.youandjerrycan.org/

Acting for more bioplastic consumption

Plastic bags – French and European legislations

European Directive: reducing plastic bag consumption

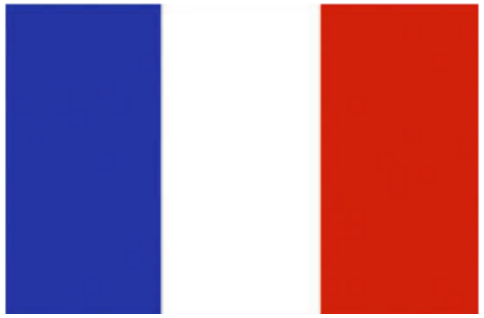
French legislation: a progressive ban on petroleum-based plastic bags



Plastic bags – French and European legislations

European Directive: reducing plastic bag consumption

French legislation: a progressive ban on petroleum-based plastic bags



Could banning petroleum-based plastic affect bioplastic prices?

Stakeholders point of view

Paris City Council
Cabinet of deputy Mayor for sustainable development

Artaxerkes
A bioplastic factory Liaison operating in Africa and Europe

Biofutura
Importer, wholesaler and online shop of sustainable products.

Reducing prices

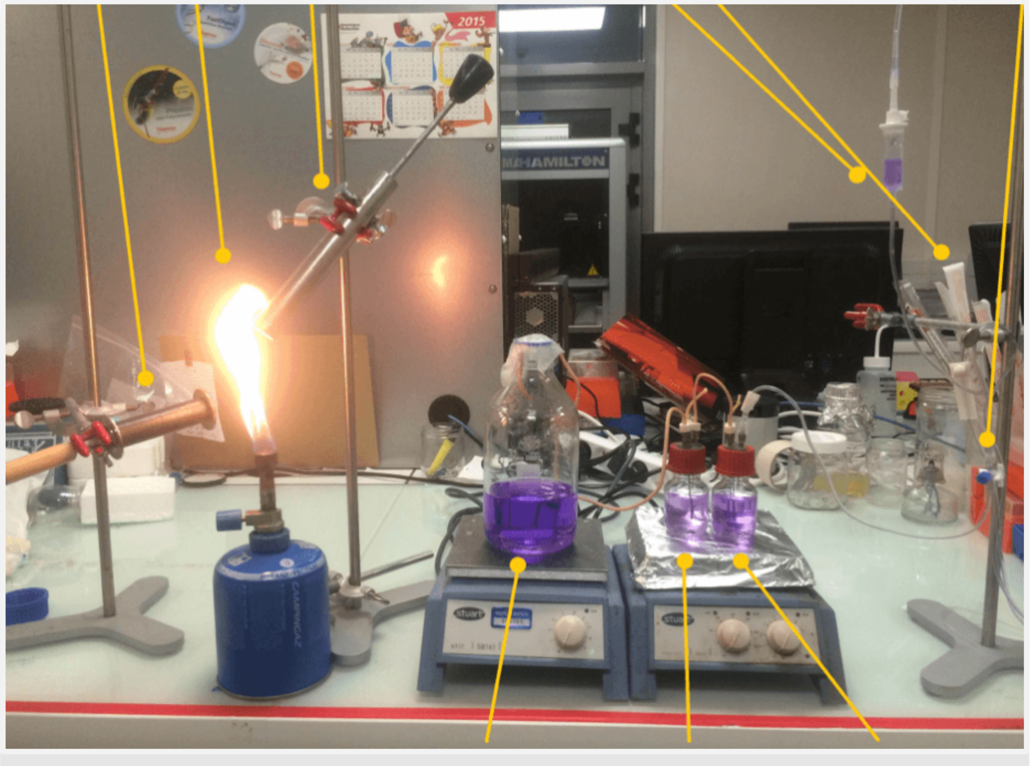
Increase of demand + increase of competition

Economies of scales - Bioprocess

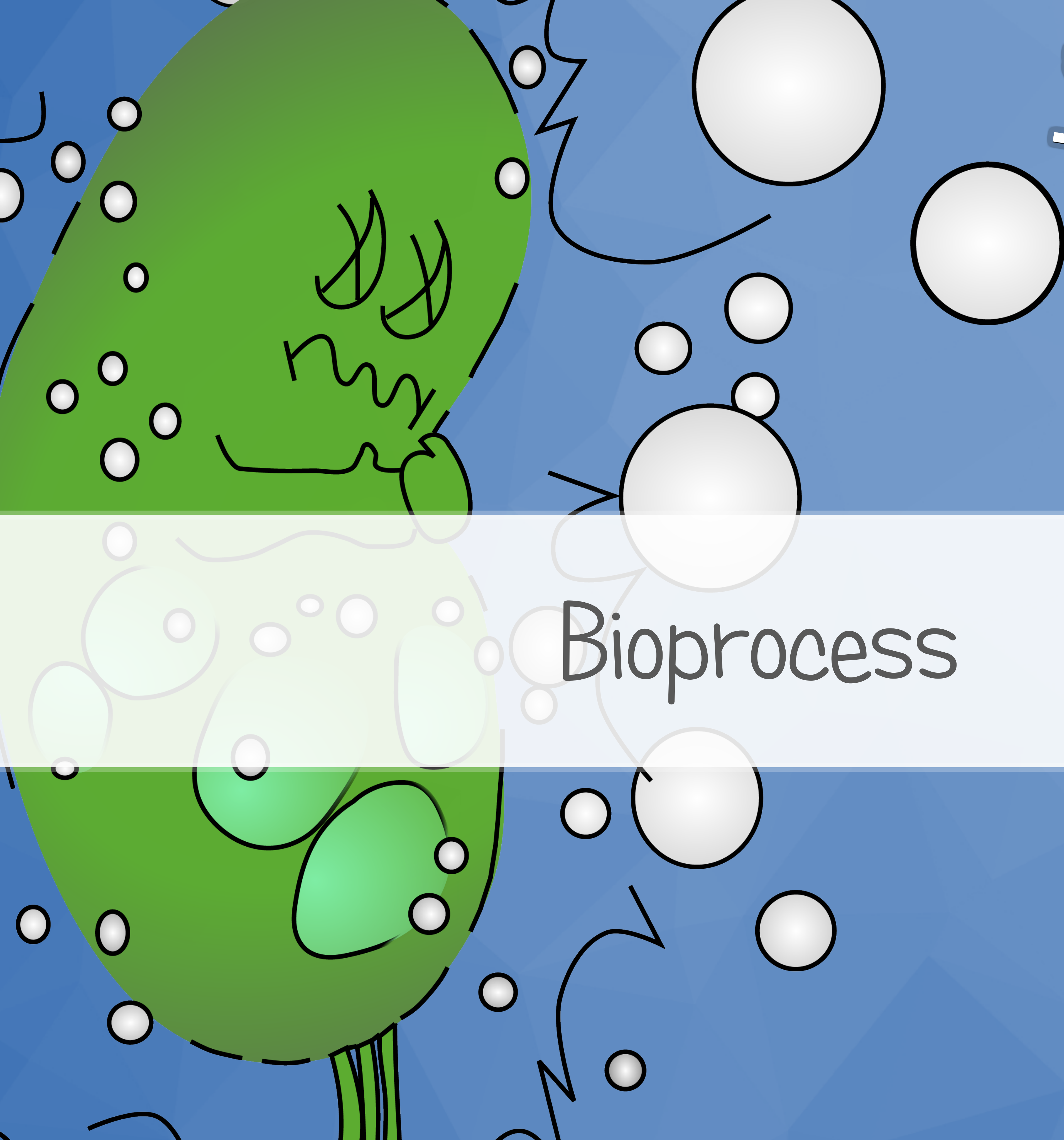
Reducing prices

Increase of demand + increase of competition

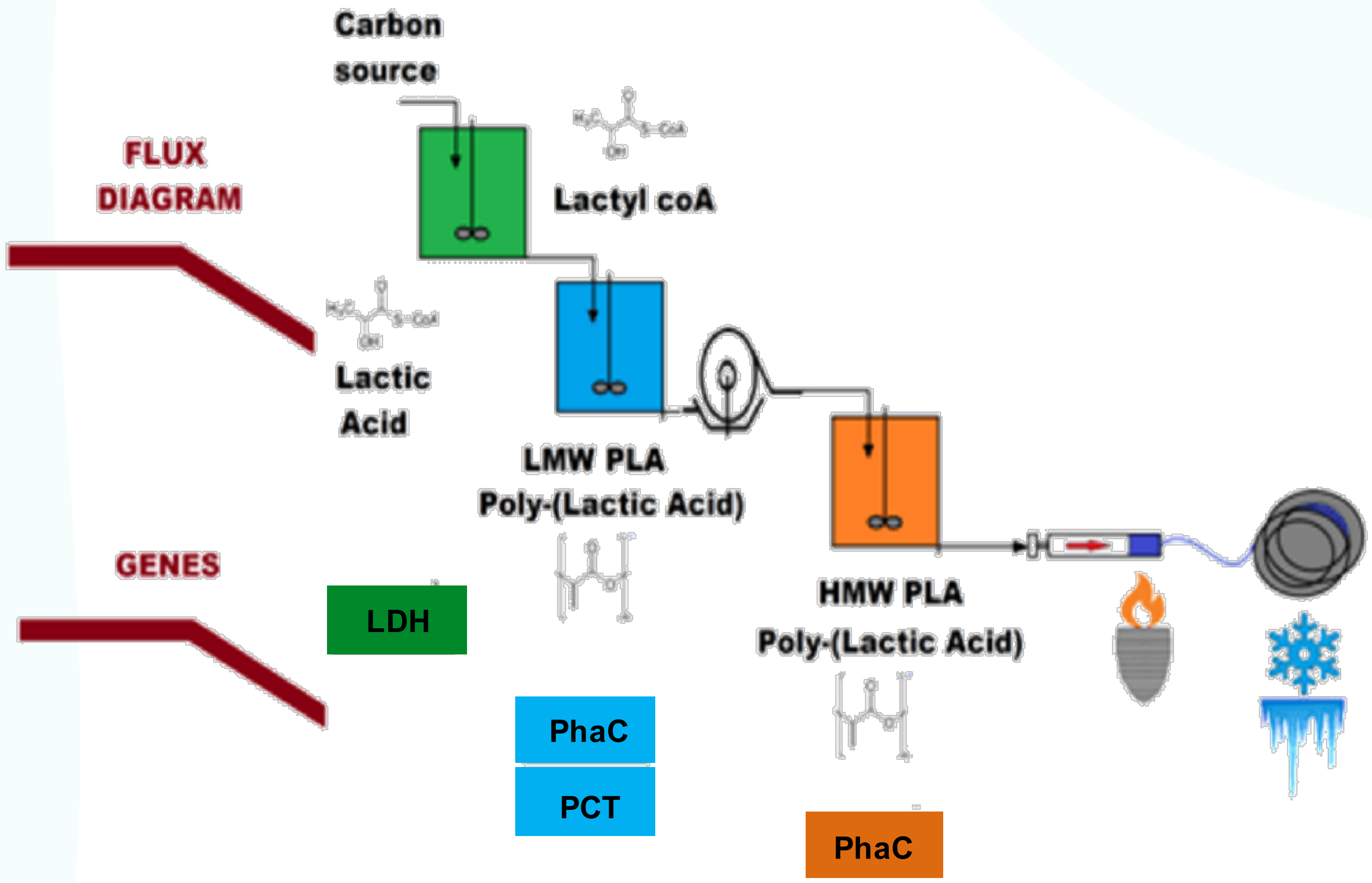
Economies of scales - Bioprocess



Bioprocess



Bioprocess diagram



FLOW ON

BIOMASS & PRECURSOR

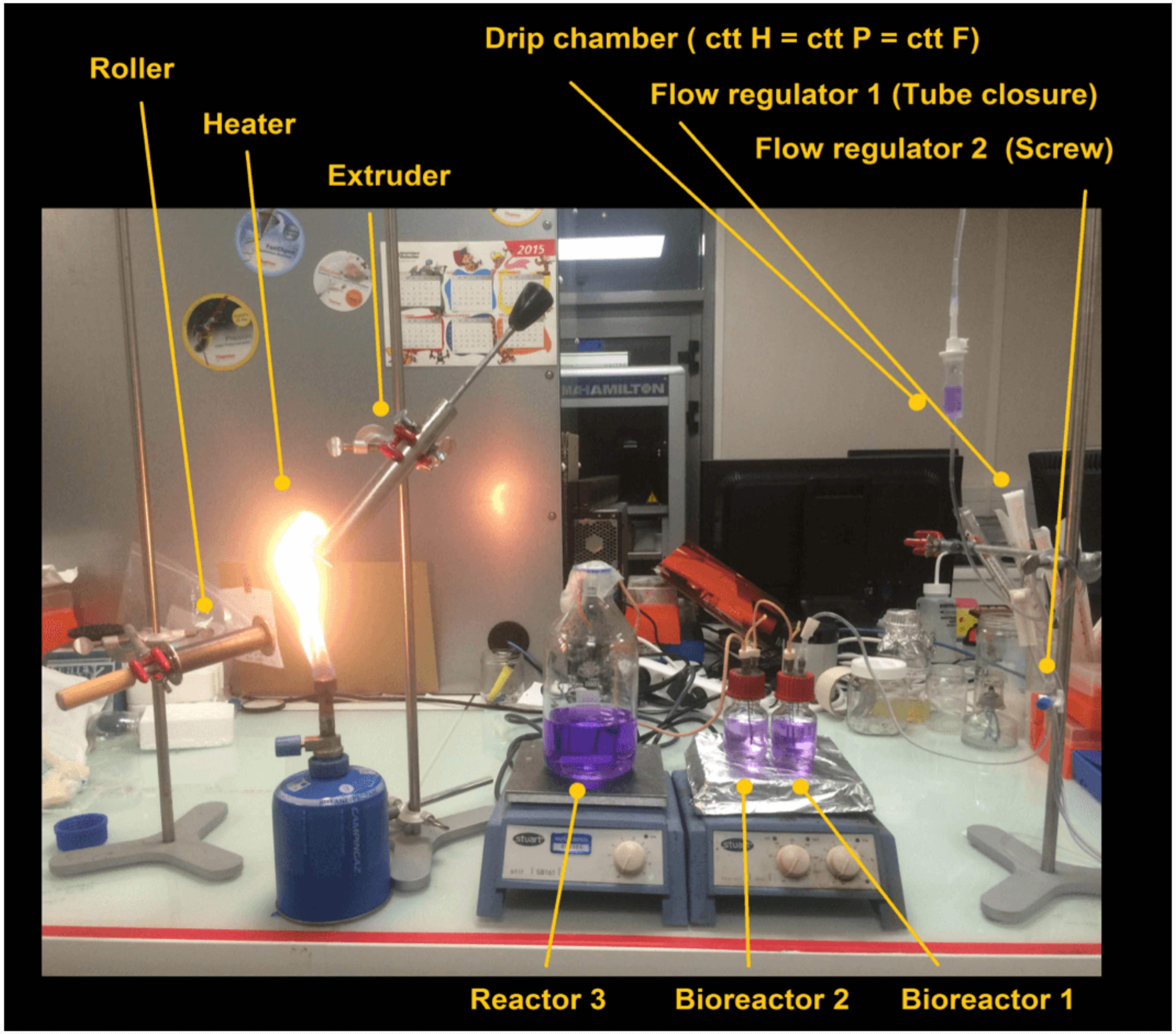
FLOW ON

BIO POLYMERIZATION

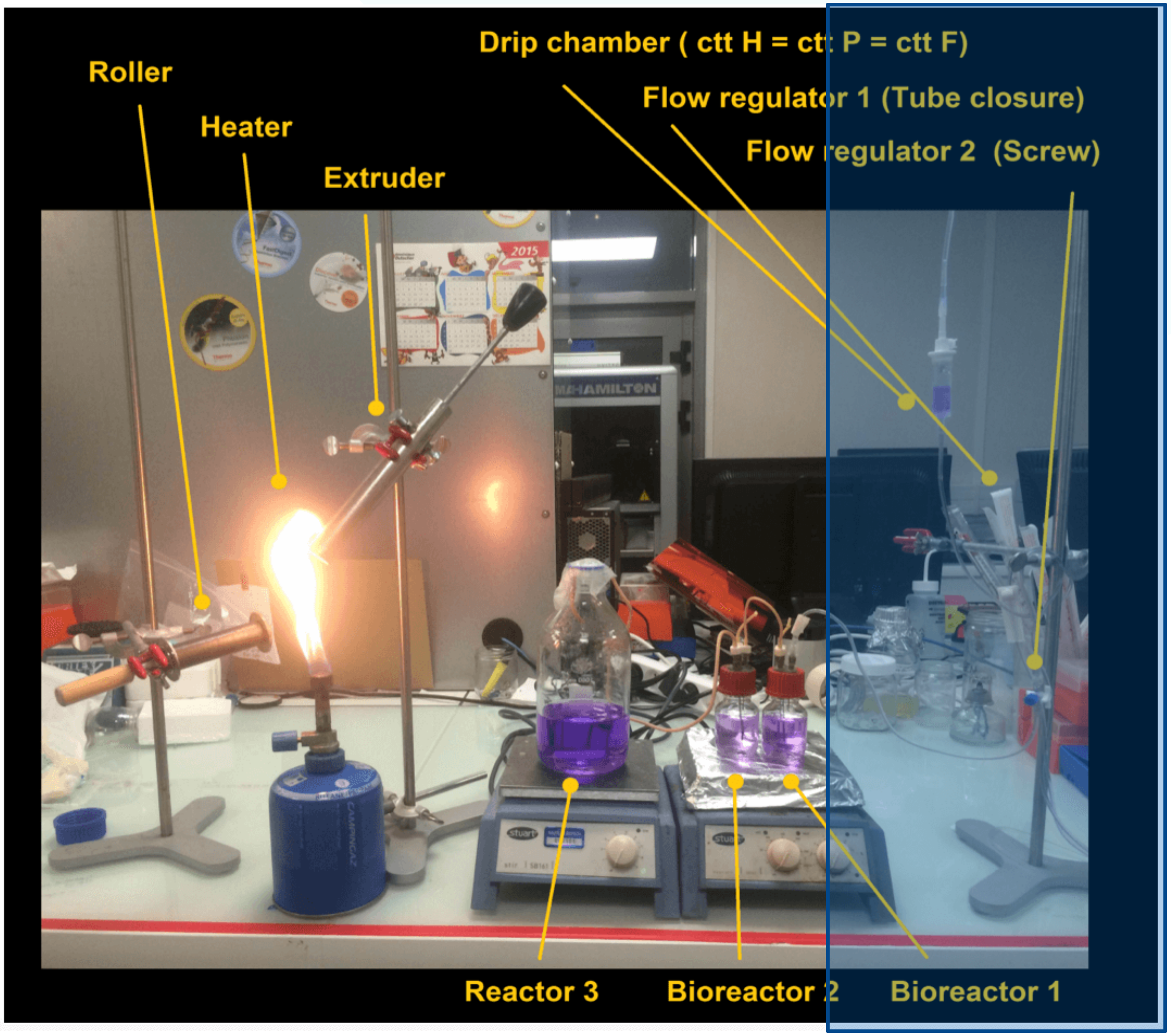
CELL DISRUPTION

CELL-FREE POLYMERIZATION | **CHEMICAL POLYMERIZATION**

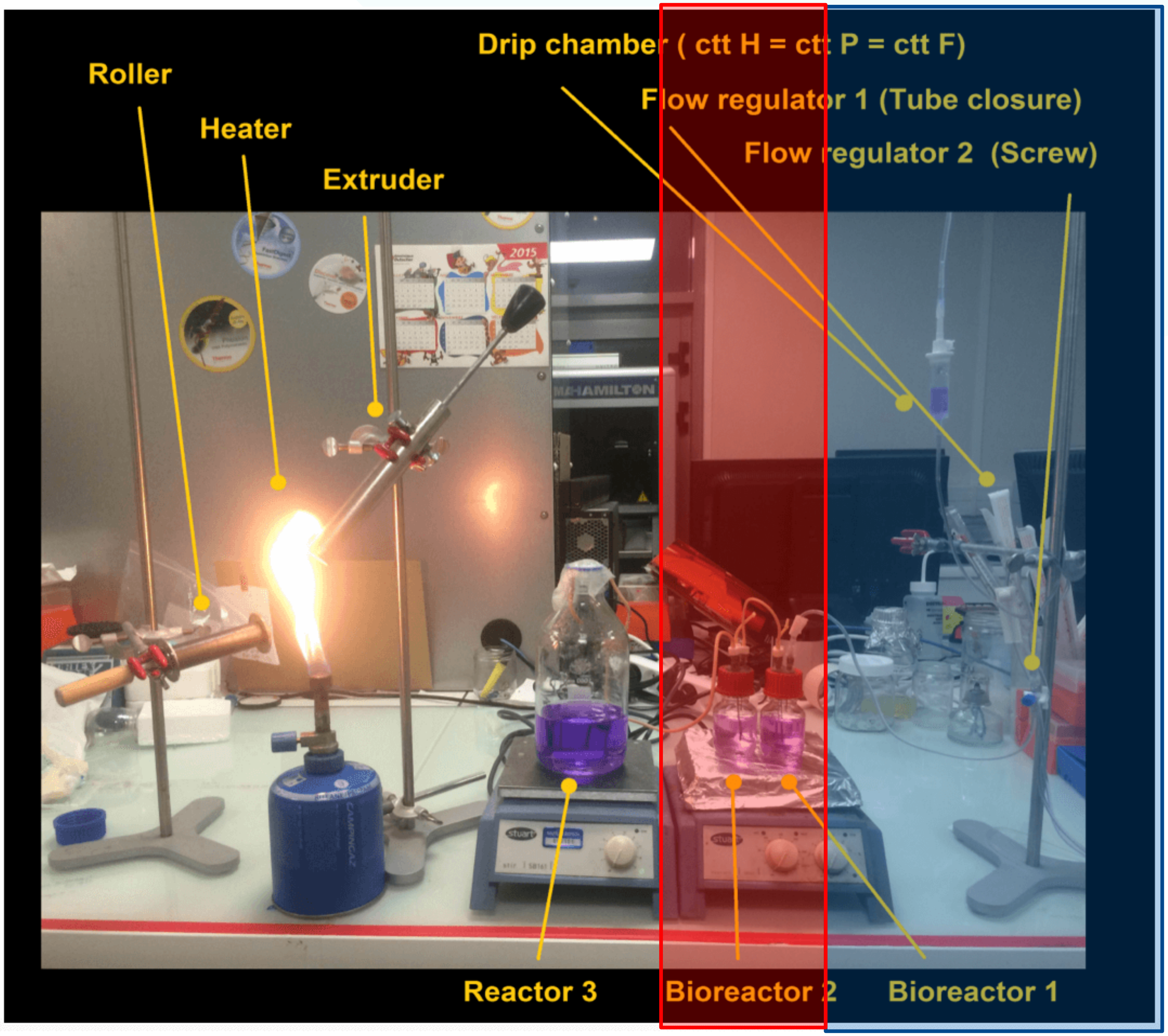
Bioprocess – the whole picture



Bioprocess – the whole picture



Bioprocess – the whole picture



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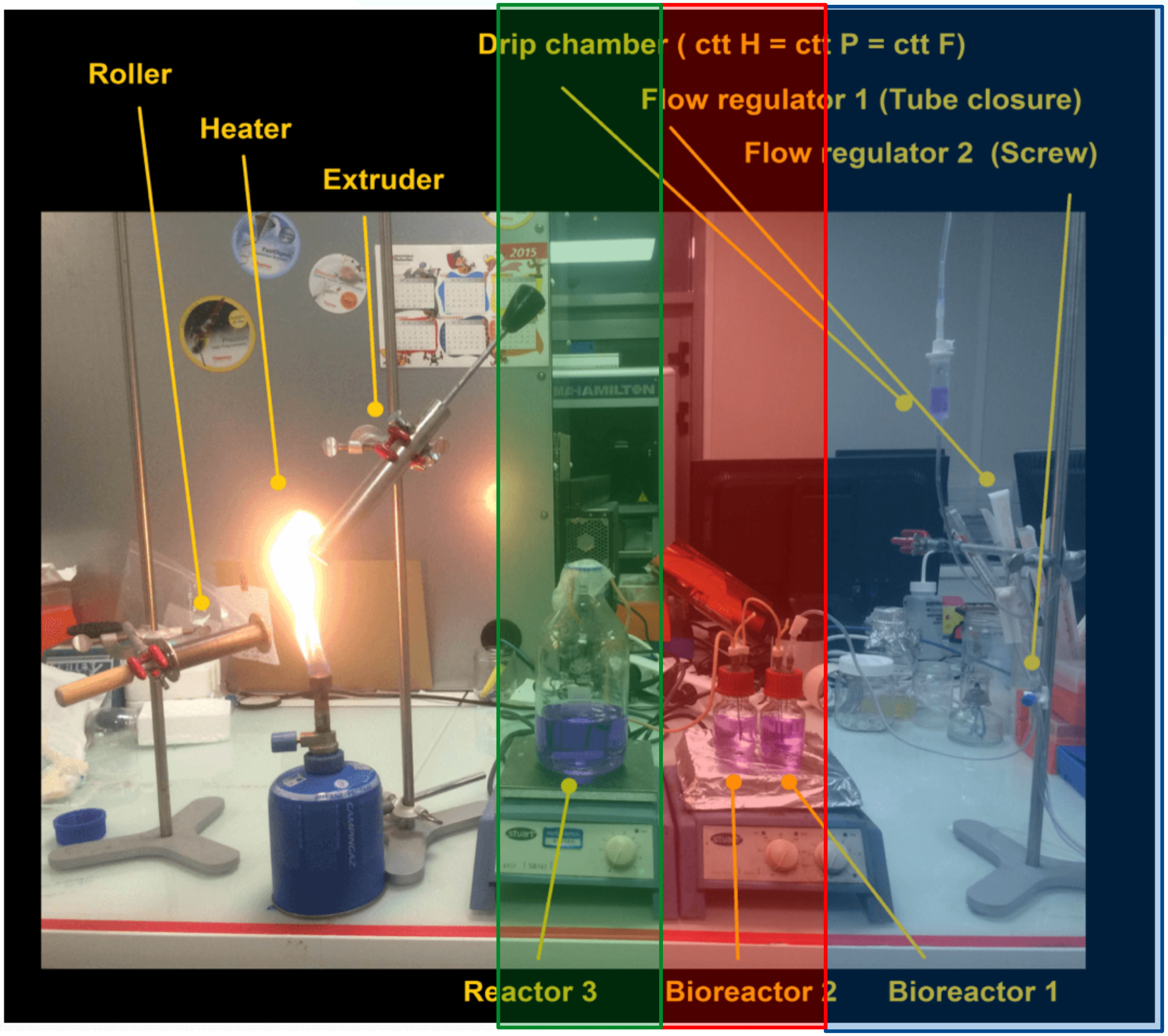

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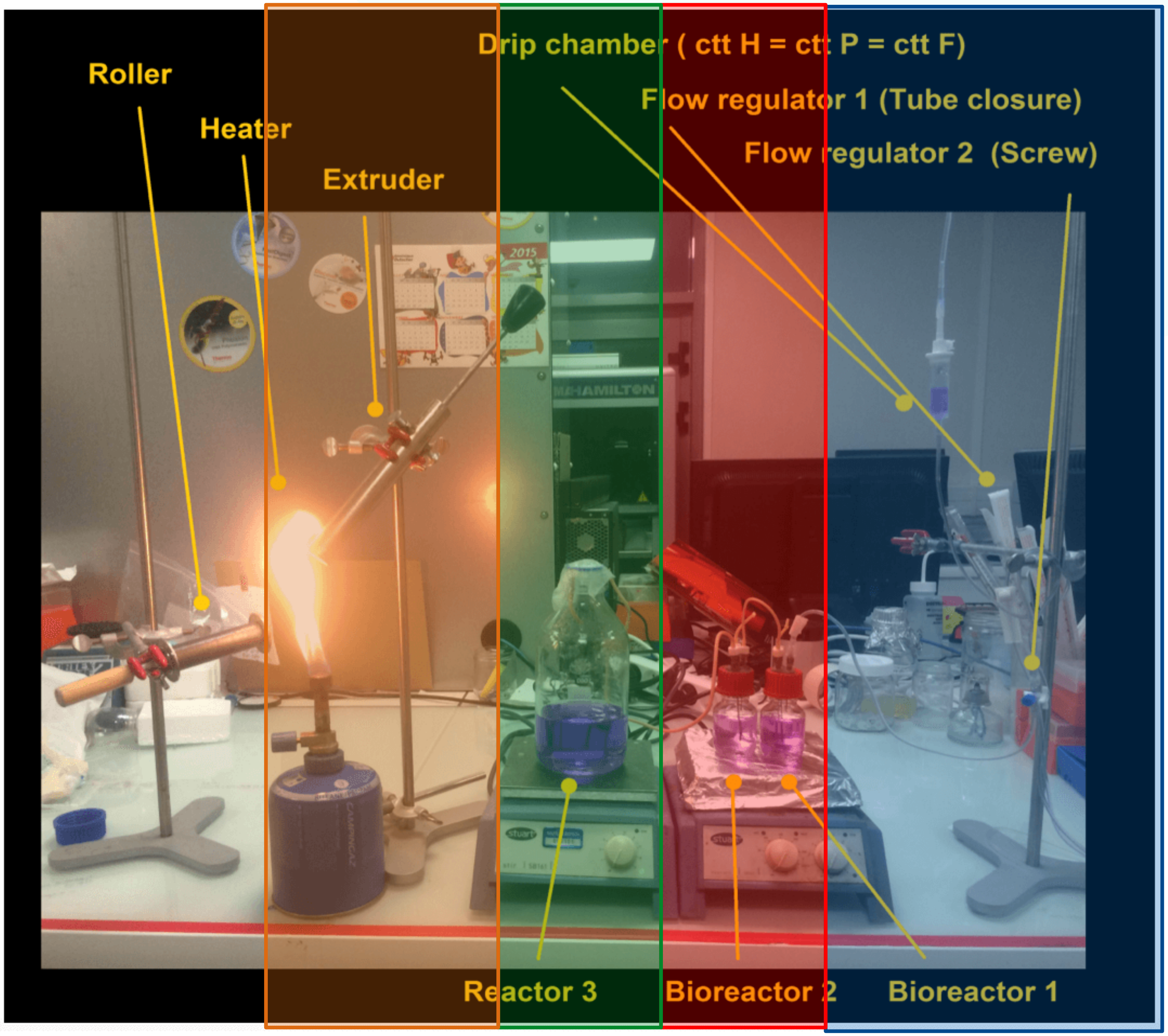
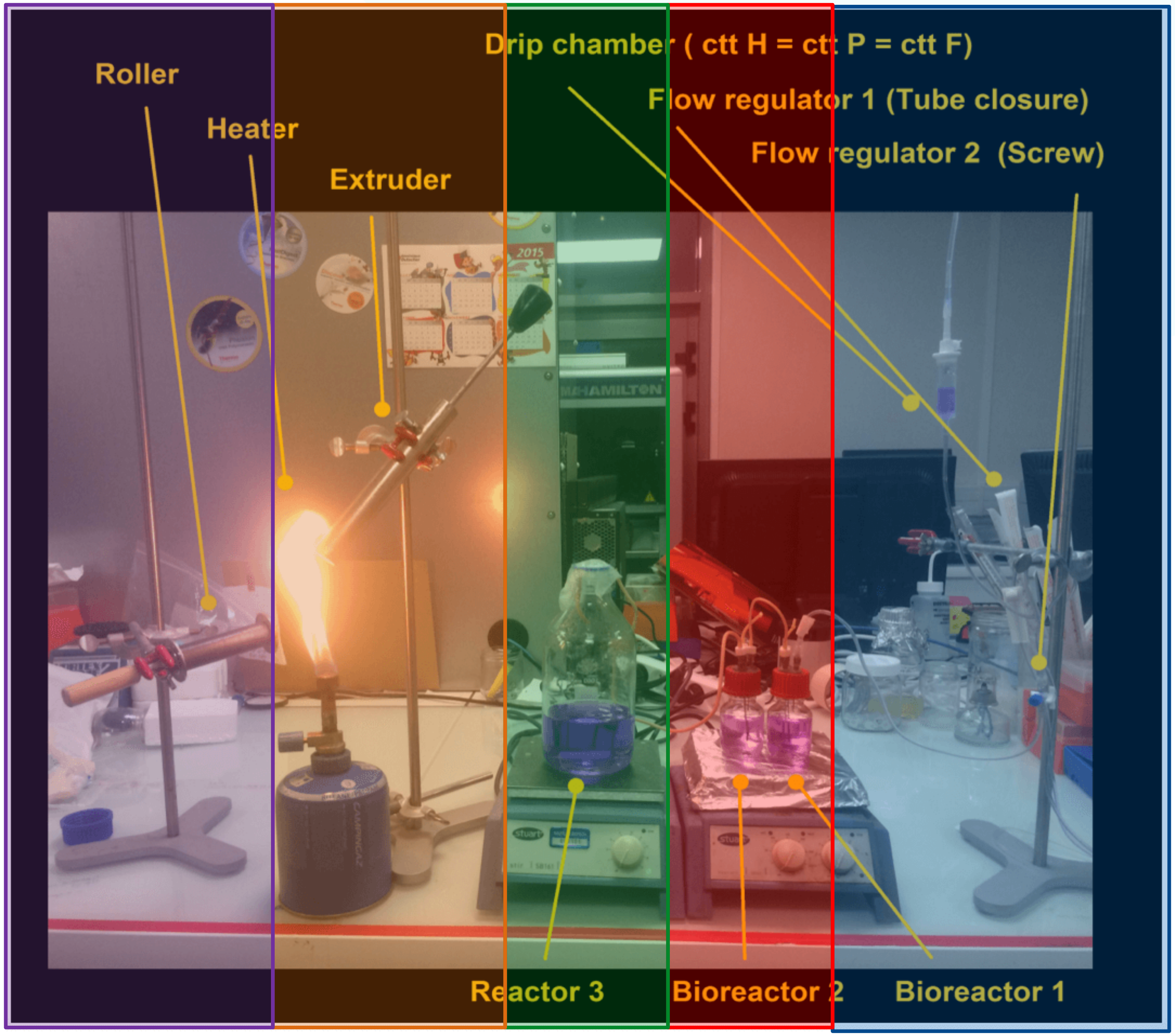
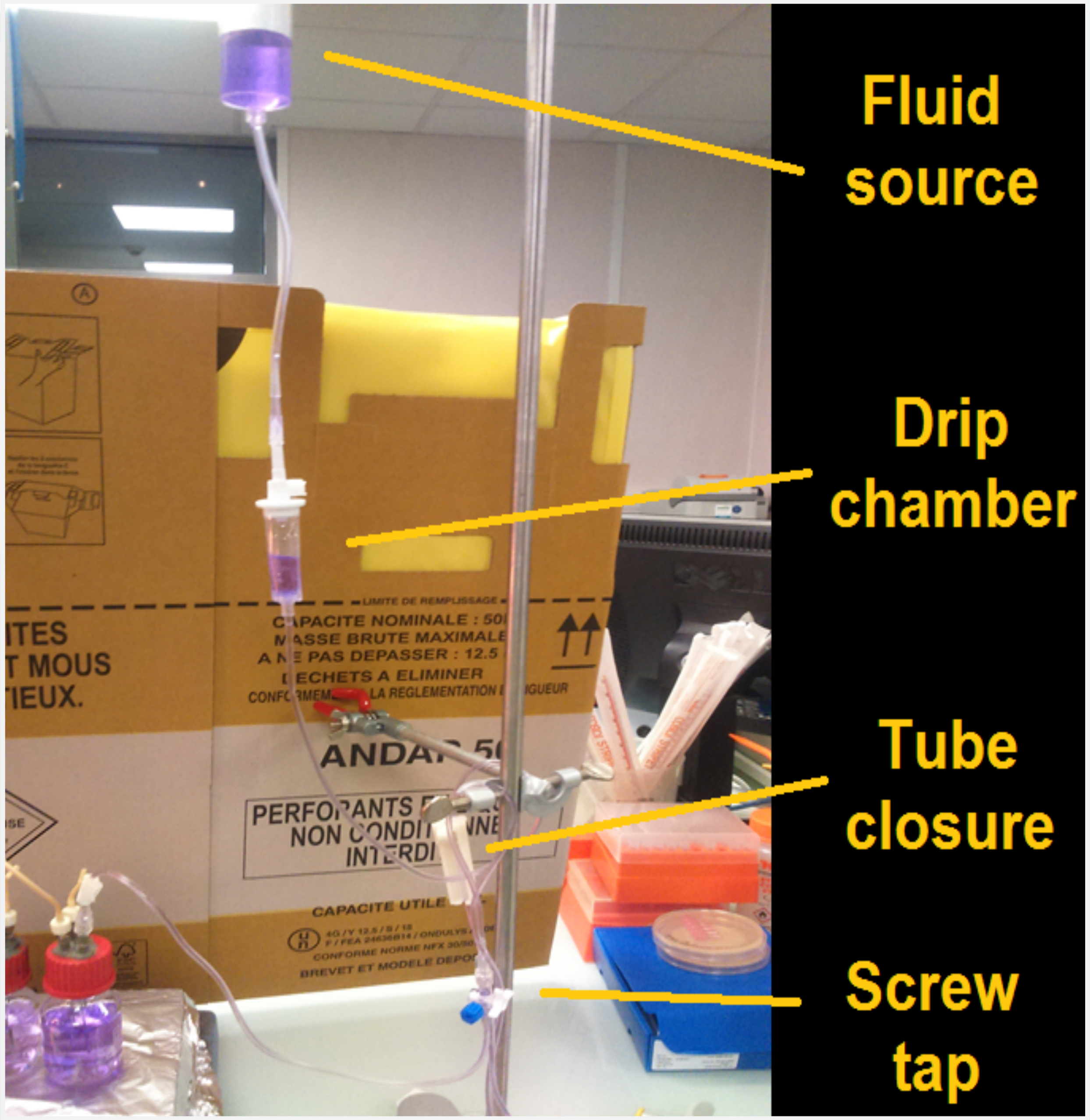


Bioprocess – the whole picture



Bioprocess – DIY Continuous Pump



Incompressible flow equation

$$\frac{P}{\rho g} + H + \frac{V^2}{2g} = K$$

Fluid velocity in the drip chamber

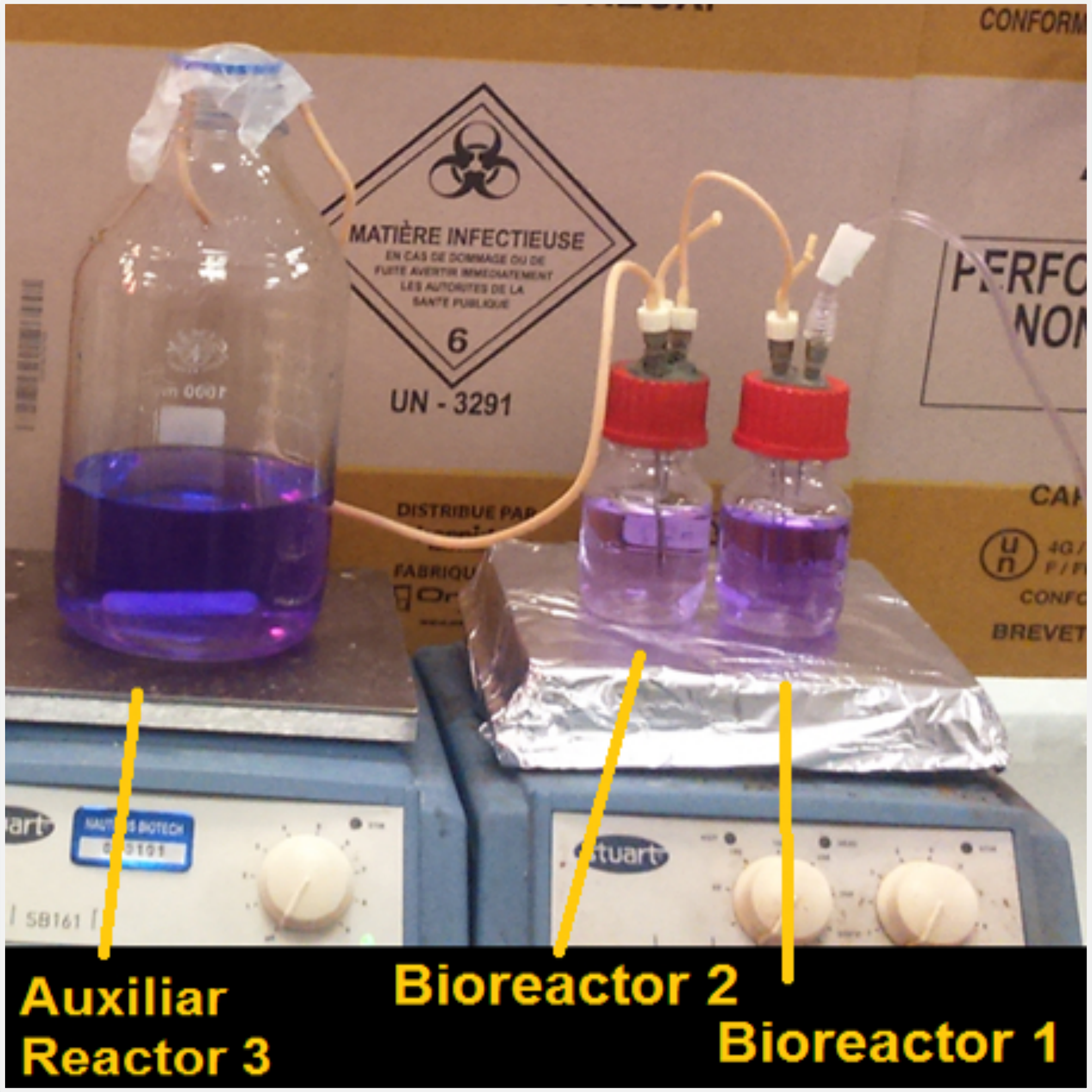
$$V = (2gH)^{\frac{1}{2}}$$

Steady & Continuous flow rate:

- Fluid velocity
- Area of the tube

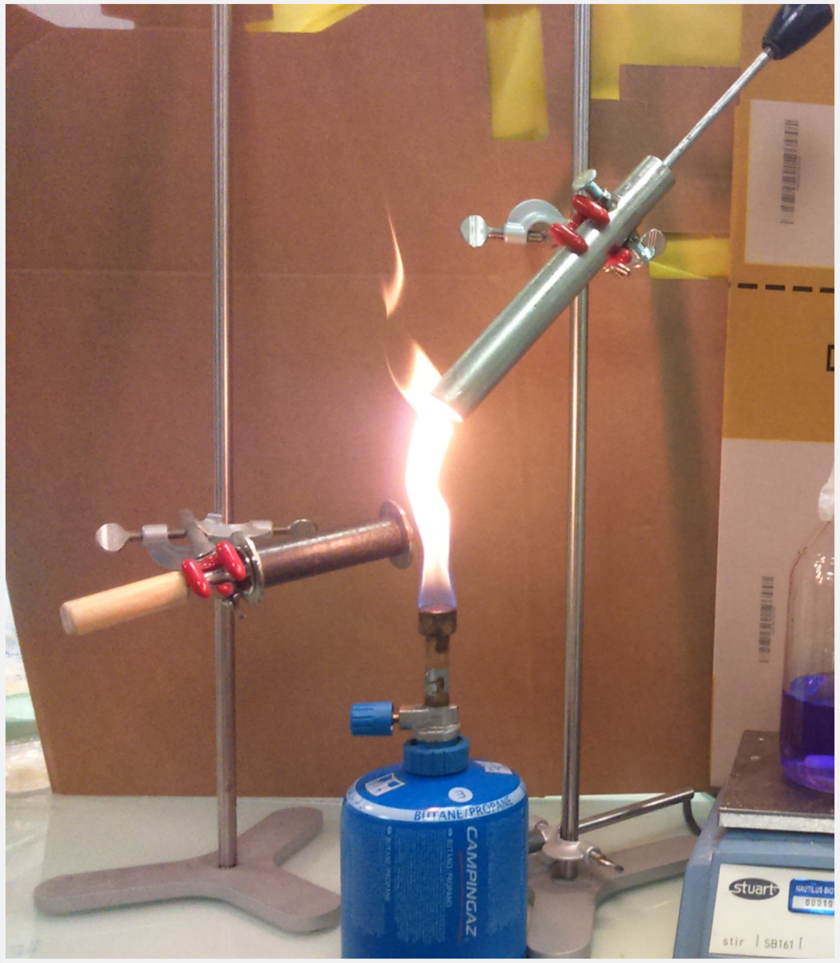
$$F = V \cdot A$$

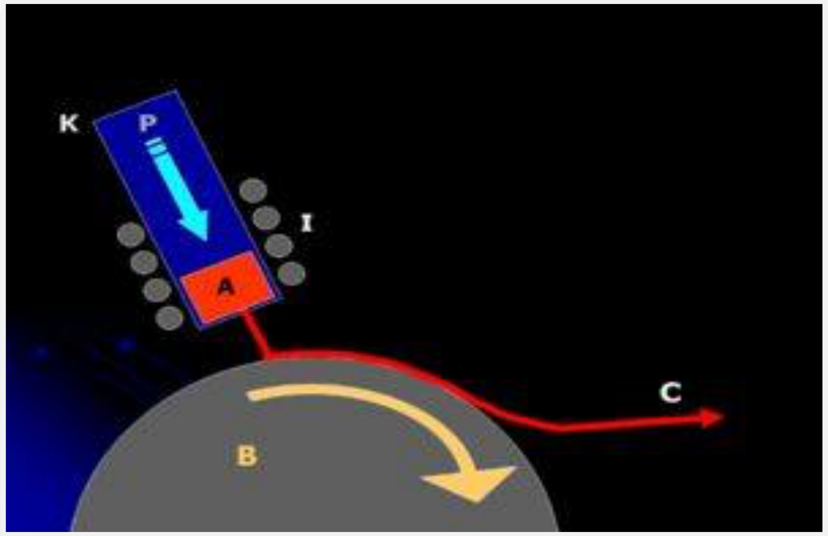
Bioprocess – DIY Continuous Bioreactor(s)



PARAMETERS FOR THE BIOREACTOR		
Parameter	Max growth rate* (μ_{max}) (min^{-1})	Max flow (F) (mL/min)
Bioreactor 1 (1 - LDH)	**0.0035**	**< 0.17**
Bioreactor 2 (PCT & PhaC)	**0.0035**	**< 0.17**
Auxiliar Reactor 3 (Extension)	**-**	**-**

Bioprocess – DIY PLA Extruder & Roller





DIY-PLA-Extruder

- Piston
- Cylinder
- 1.5 mm hole
- Heater

Made out of INOX material

- Heat-resistant
- Durable
- Standard
- Easy to use

Collaborations

Frank & Stain

ecolibrium

- HPLC and MS (collab. with Bettencourt's team)

- Plotting growth rates – iGEM Imperial College London

The European Experience

IGEM IONIS

THE EUROPEAN EXPERIENCE 2016

iGEM EVRY 2016

Quick summary – take home message

Designed a bioproduct using variety of SynBio tools

BioBricks:
2 improved coding seq. (Pct, PhaC)
2 new inducible promoters

The plastic problem

Investigated about plastic beyond the lab and provided tools for raising awareness

Improved and integrated by developing a fully DIY bioprocess

Our sponsors

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Acknowledgements

Students:

Advisors:

and to all other previous members and PIs!

Acknowledgements

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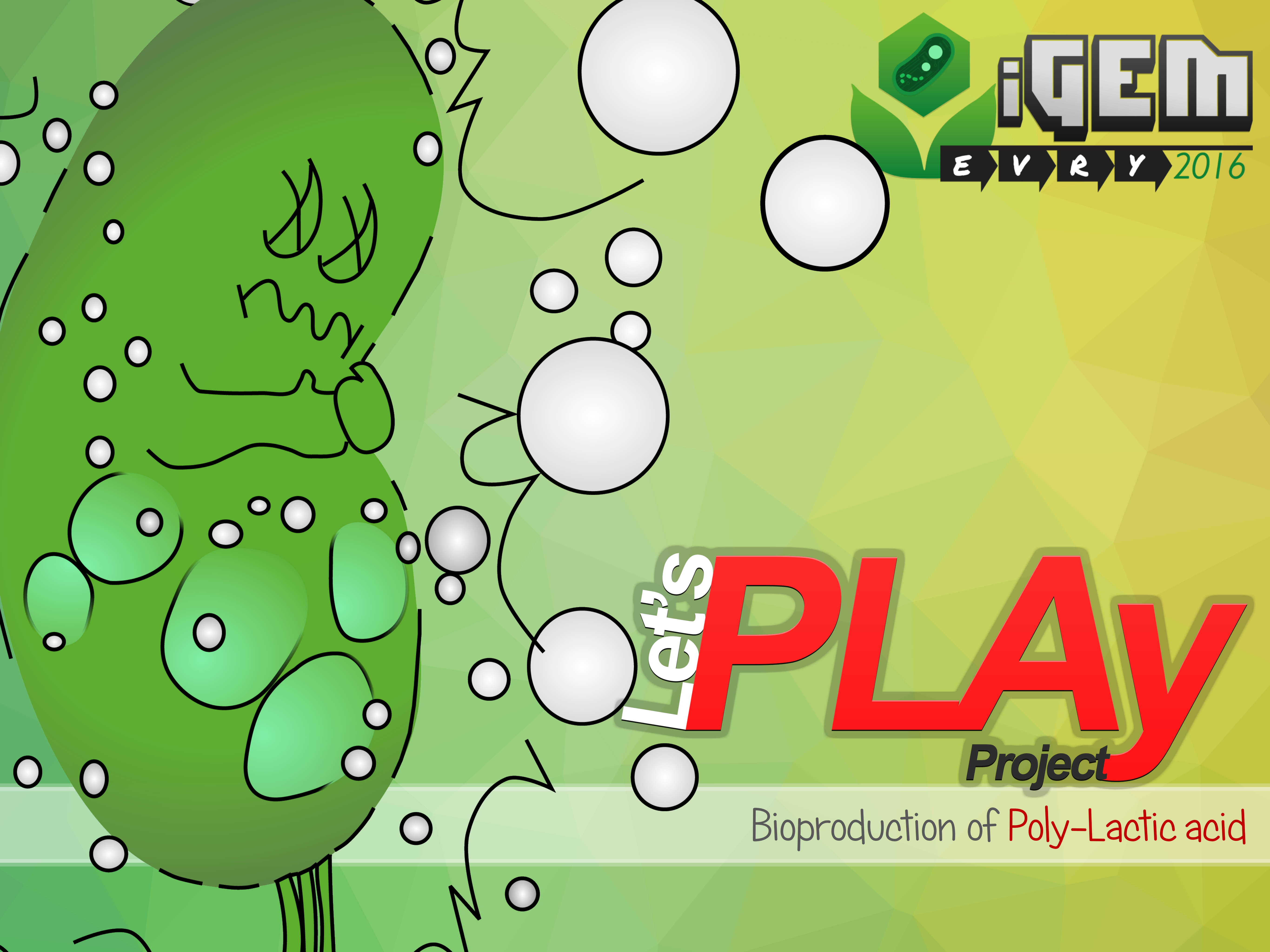
To all researchers and members from host institutions that helped

To all external people or associations that contributed to the project development, human practices, and the supporters of our crowdfunding campaign

*find them in **Attributions** in our wiki!*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