Modelling and synthetic biology for two step fermentation

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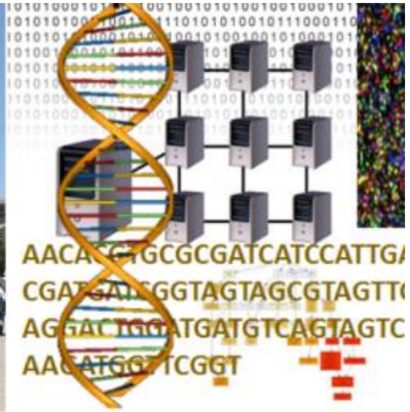
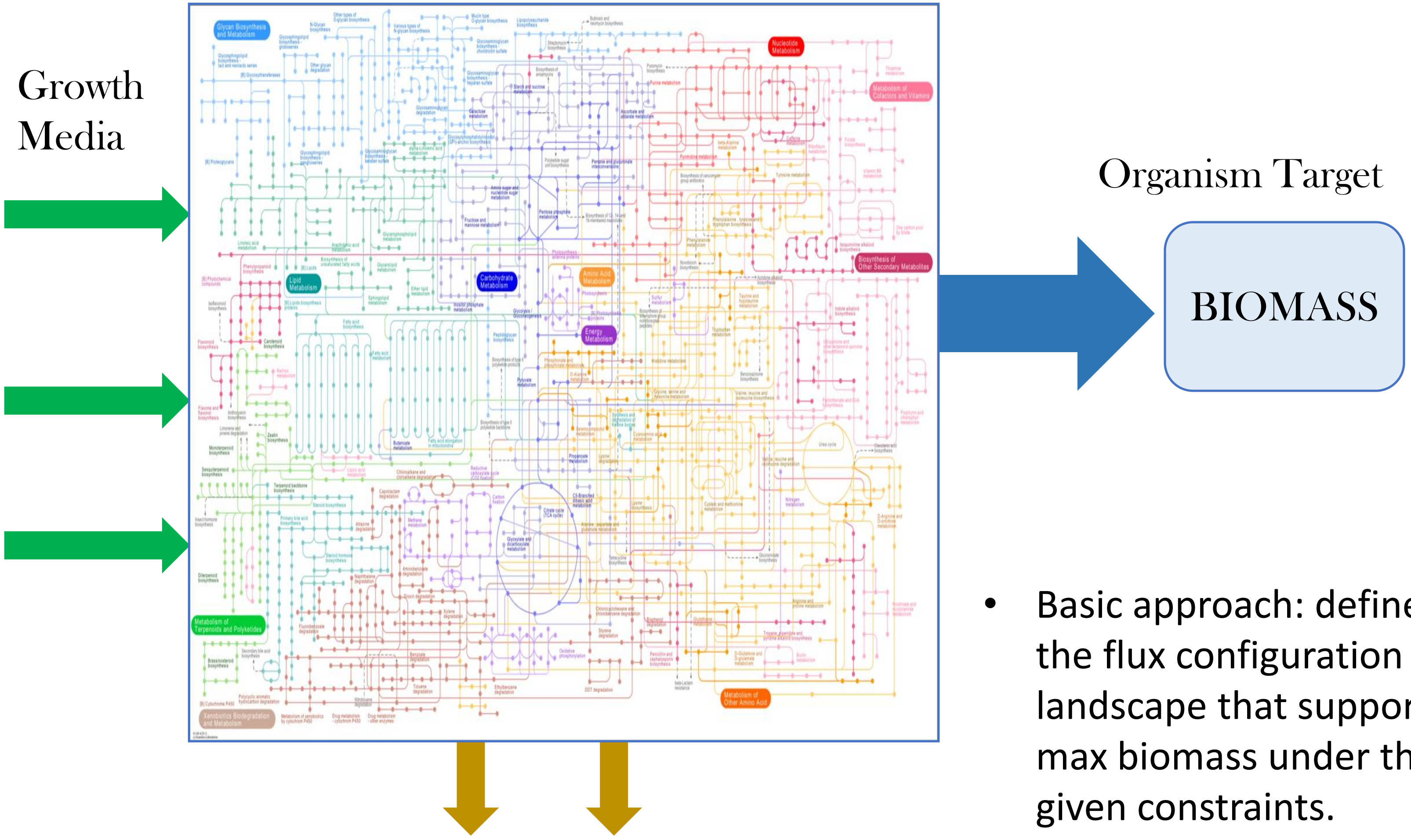
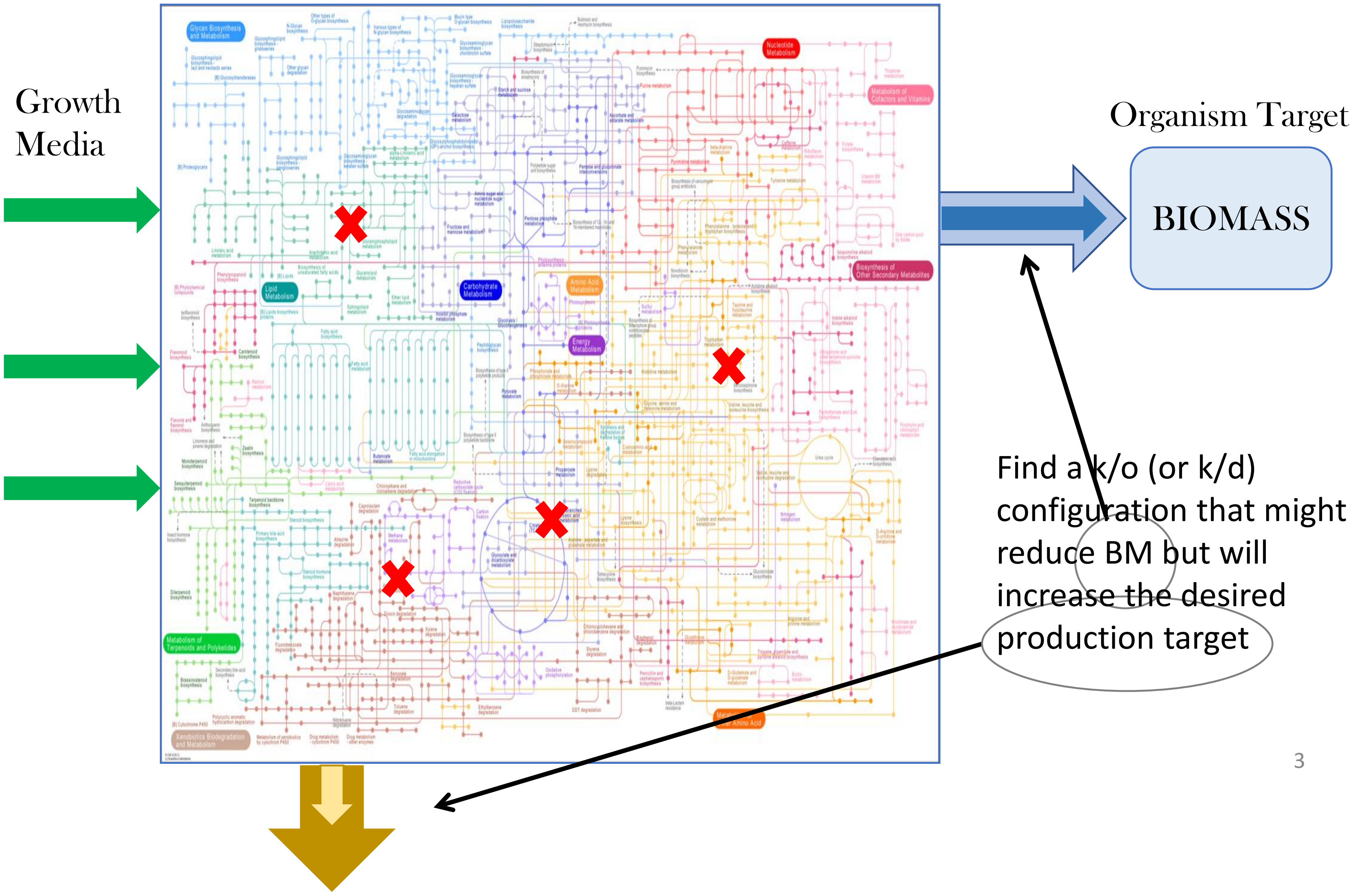


Constraints based modelling (Pallson and others)

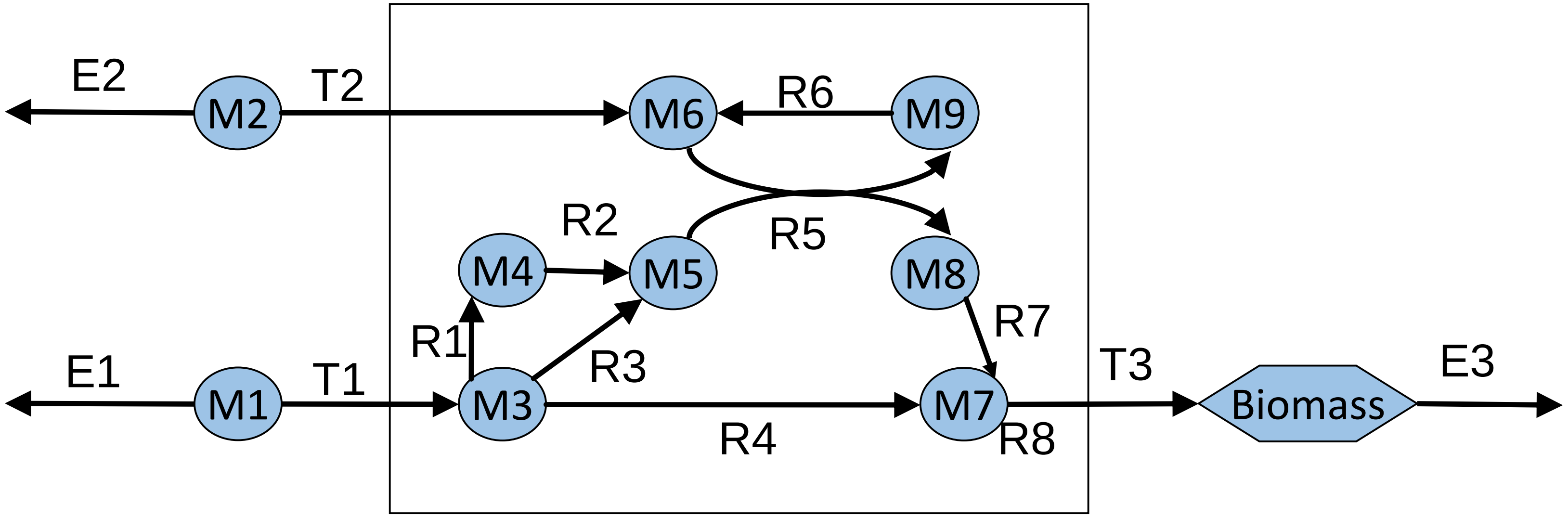


- Basic approach: define the flux configuration landscape that supports max biomass under the given constraints.
- Media composition is a constraint, for this purpose

Metabolic Engineering by Reaction KnockOuts



Toy-network example



S=

	E1	E2	E3	T1	T2	T3	R1	R2	R3	R4	R5	R6	R7	R8
M1	**-1**	0	0	**-1**	0	0	0	0	0	0	0	0	0	0
M2	0	**-1**	0	0	**-1**	0	0	0	0	0	0	0	0	0
M3	0	0	0	**1**	0	0	**-1**	0	**-1**	**-1**	0	0	0	0
M4	0	0	0	0	0	0	**2**	**-2**	0	0	0	0	0	0
M5	0	0	0	0	0	0	0	**1**	**1**	0	**-1**	0	0	0
M6	0	0	0	0	**1**	0	0	0	0	0	**-1**	**1**	0	0
M7	0	0	0	0	0	**-1**	0	0	0	**1**	0	0	**1**	**-1**
M8	0	0	0	0	0	0	0	0	0	0	**1**	0	**-1**	0
M9	0	0	0	0	0	0	0	0	0	0	**1**	**-1**	0	0
Biomass	0	0	**-1**	0	0	**1**	0	0	0	0	0	0	0	**1**

$$\max_{\vec{v}}\{v_{Biomass}\} \equiv \max_{\vec{v}}\{v_{E3}\}$$

subject to:

$$S*\vec{v}=0 \qquad (1)$$

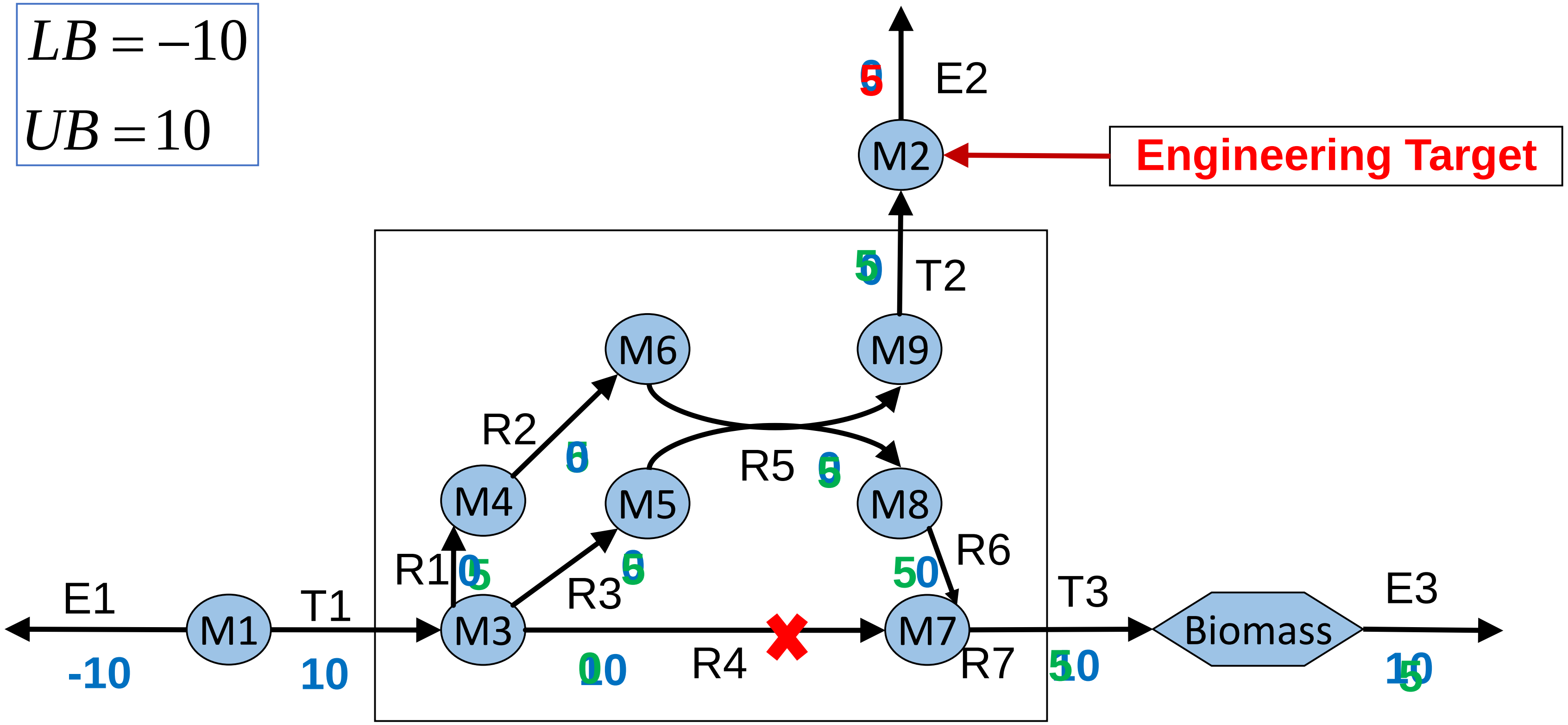
$$\vec{v}_{lb} \le \vec{v} \le \vec{v}_{ub} \qquad (2)$$

Metabolic Engineering by Reaction KnockOuts

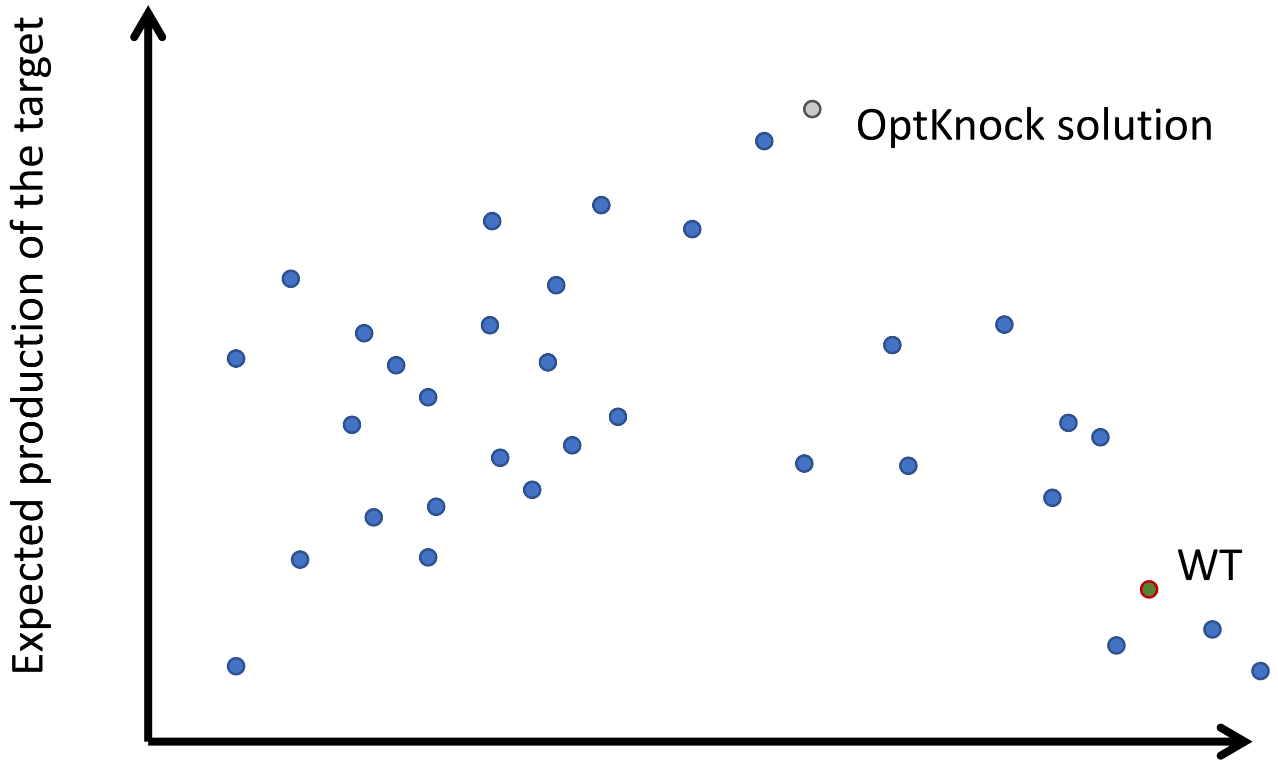
Toy-network example

$$LB = -10$$

$$UB = 10$$

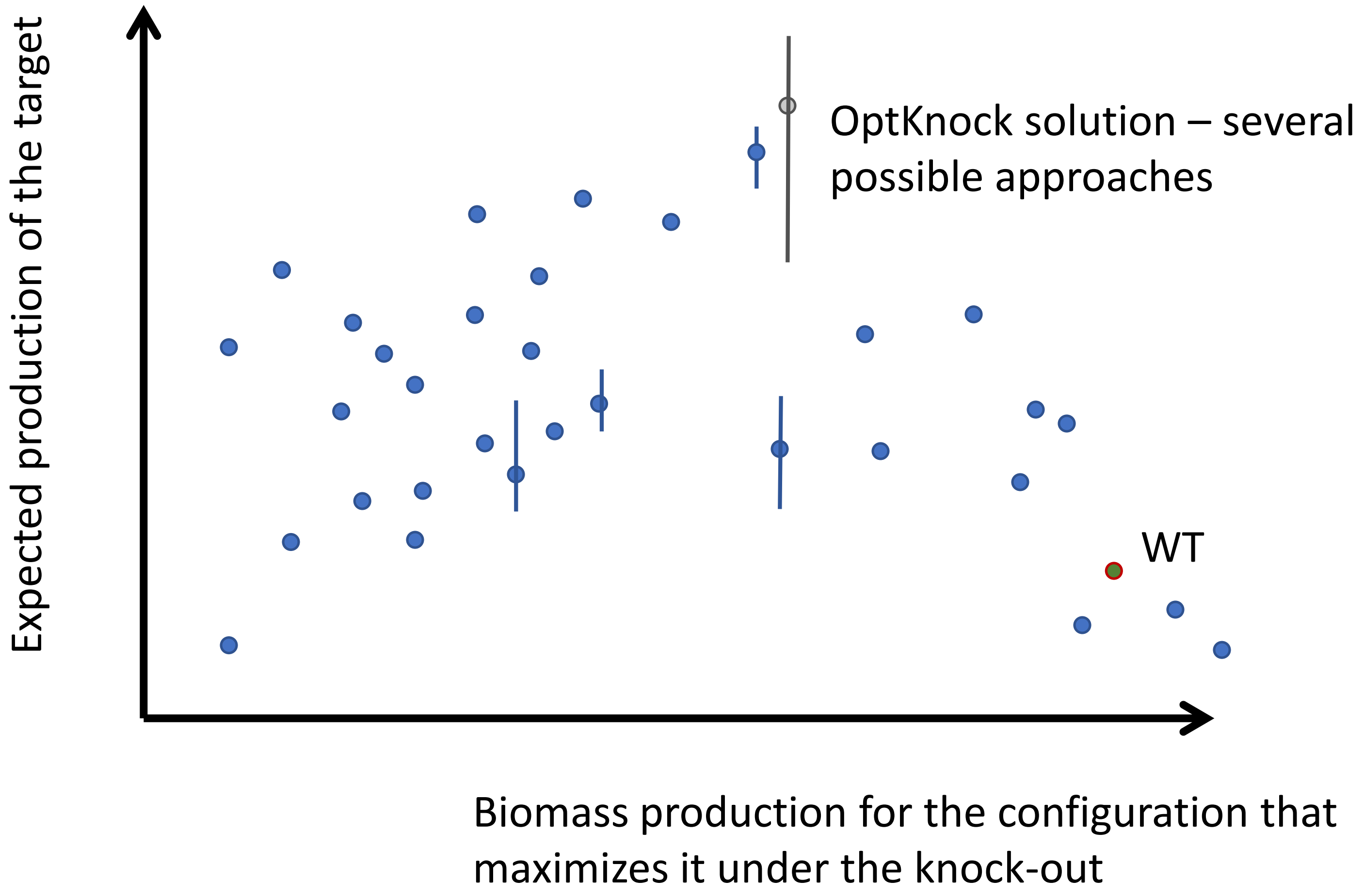


An abstract view of OptKnock…

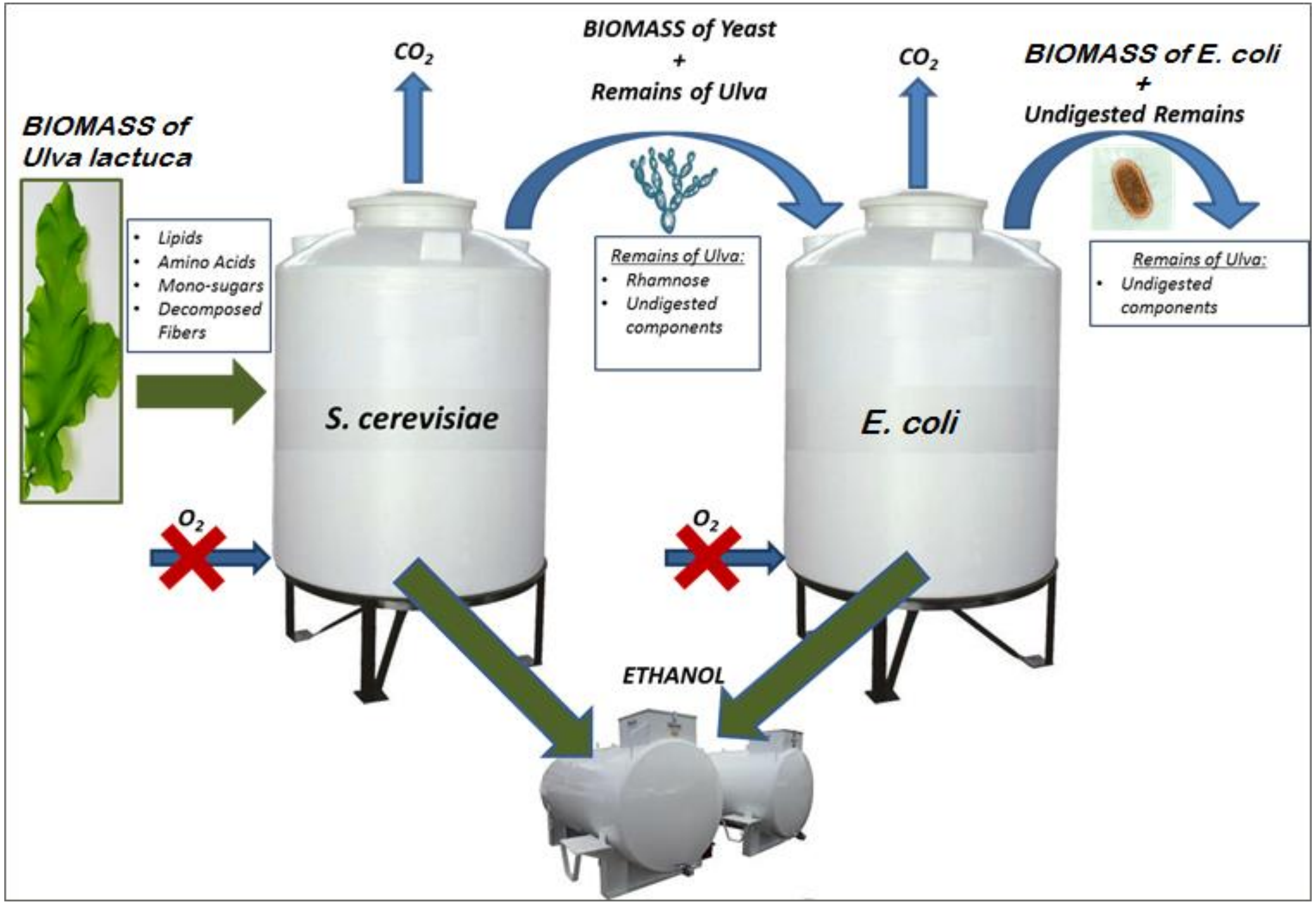


Biomass production for the configuration that maximizes it under the knock-out

An abstract view of OptKnock … cont



Multi-organisms systems



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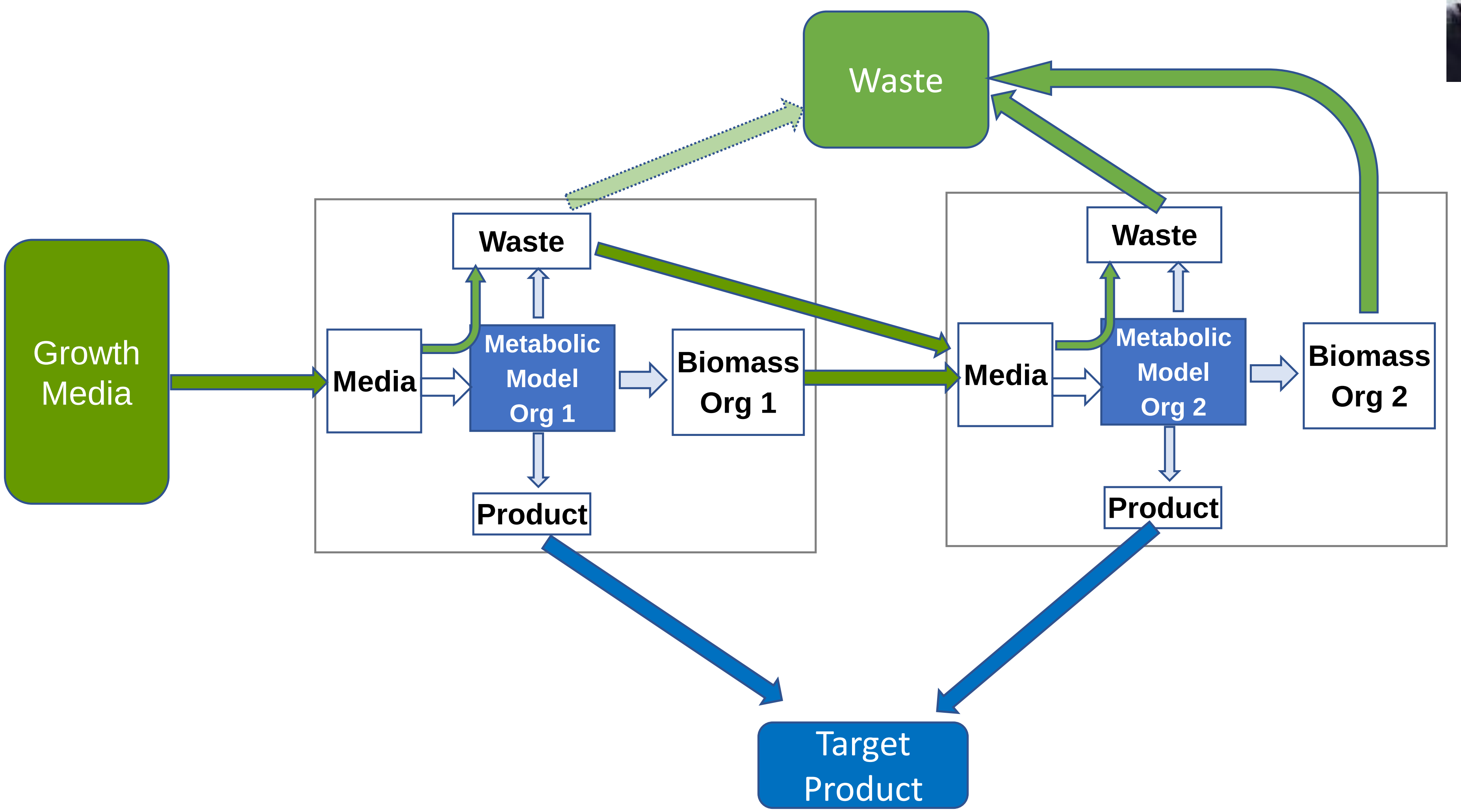
BioLego – Backend (Two-Step)

Edward Vitkin



How can this be actually done? – challenges in the BioLego approach

- Typical full-cell model is complex
  - More than 1,000 metabolites
  - More than 2,000 reactions
- Most exact models are manually reconstructed. Each organism is reconstructed by a different group. Stitching models is an engineering challenge.
- Automatically generated models don’t have sufficient quality.
- Growth media used to simulate/validate reconstruction is standard and simple (single Carbon/Nitrogen source). For example – glucose minimal media. We need to adapt and tune models for more realistic media scenarii.

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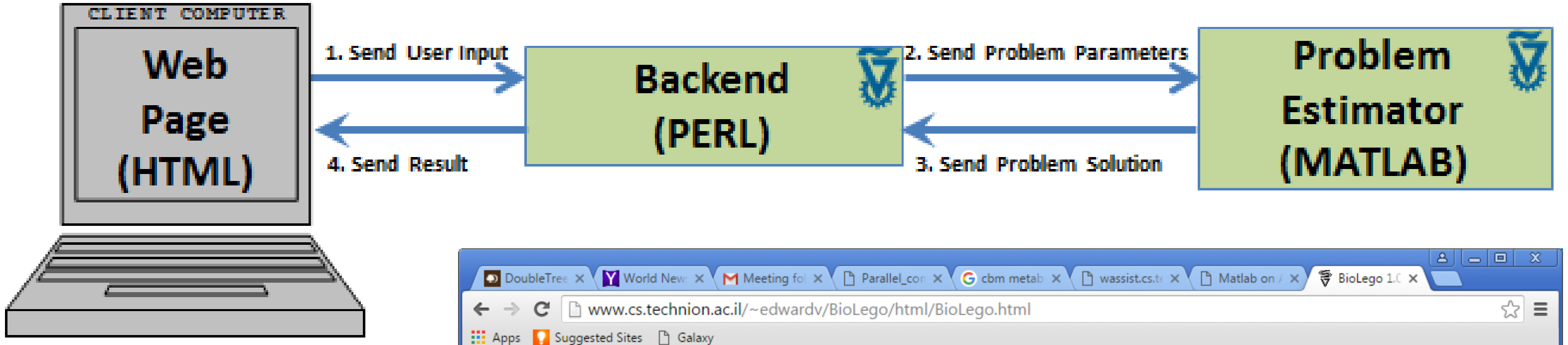
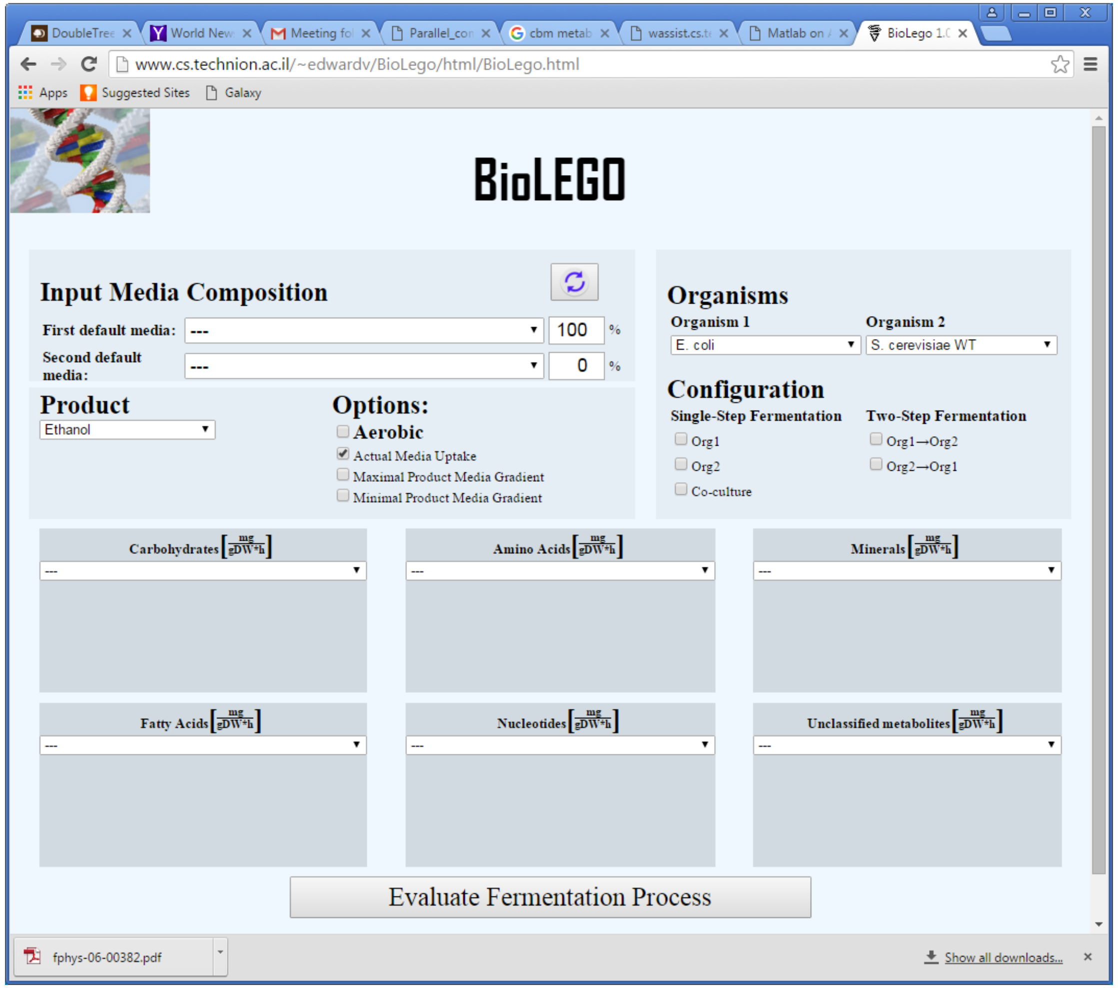
Service structure – current



Edward Vitkin

- Limitation of a single processor system: can not do (even single) k/os in both organisms in reasonable time.
- Each LP run is several seconds on a complicated model.
- Solutions:
  + More clever multiple LPs
  + Parallelization –use clusters or cloud services
- Coming release will be deployed on Microsoft Azure

Microsoft Azure



Modeling Results – Wild Type

Alex Golberg

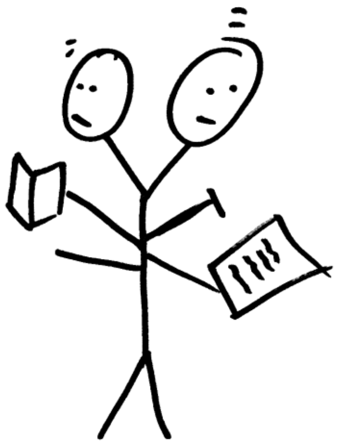
Organism	Ulvan Decomposition	Xylose Uptake	Rhamnose Uptake	Other Sugars Uptake	Min [mg/gDW] Ethanol	Max [mg/gDW] Ethanol	Min [C] Utilization	Max [C] Utilization
S. cerevisiae	+	+	-	+	250	250	30.5%	30.5%
	+	-	-	+	194	201	23.6%	24.5%
	-	+	-	+	235	235	28.6%	28.6%
	-	-	-	+	188	196	22.9%	23.8%
E. coli	+	+	+	+	126	126	15.4%	15.4%
	-	+	+	+	124	125	15.1%	15.2%

Table 2: *ONE-STEP Fermentation results*

First Organism	Second Organism	Ulvan Decomp-osition	Yeast Xylose Uptake	Yeast Rhamnose Uptake	Yeast Other Sugars Uptake	Min [mg/gDW] Ethanol	Max [mg/gDW] Ethanol	Min [C] Utiliz-ation	Max [C] Utiliz-ation
S. cerevisiae	*E. coli*	+	+	-	+	250	250	30.5%	30.5%
		+	-	-	+	220	228	26.8%	27.7%
		-	+	-	+	235	235	28.6%	28.6%
		-	-	-	+	207	217	25.3%	26.4%
E. coli	*S. cerevisiae*	+				126	130	15.4%	15.8%
		-				124	127	15.1%	15.5%

Table 3: *TWO-STEP Fermentation results*

Oligo Library Synthesis and applications



Moving from reading DNA to WRITING DNA

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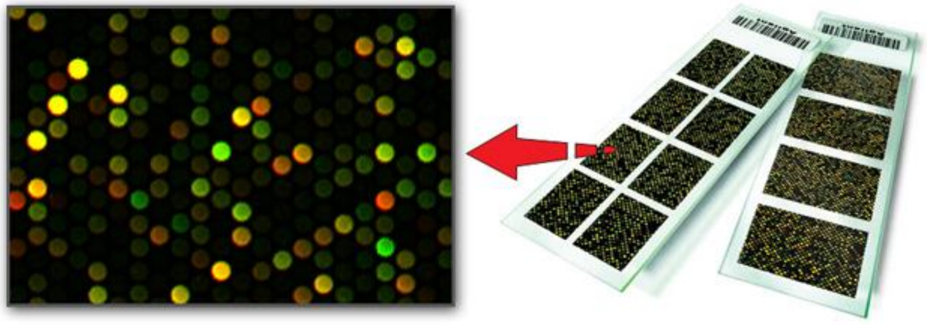
Microarrays

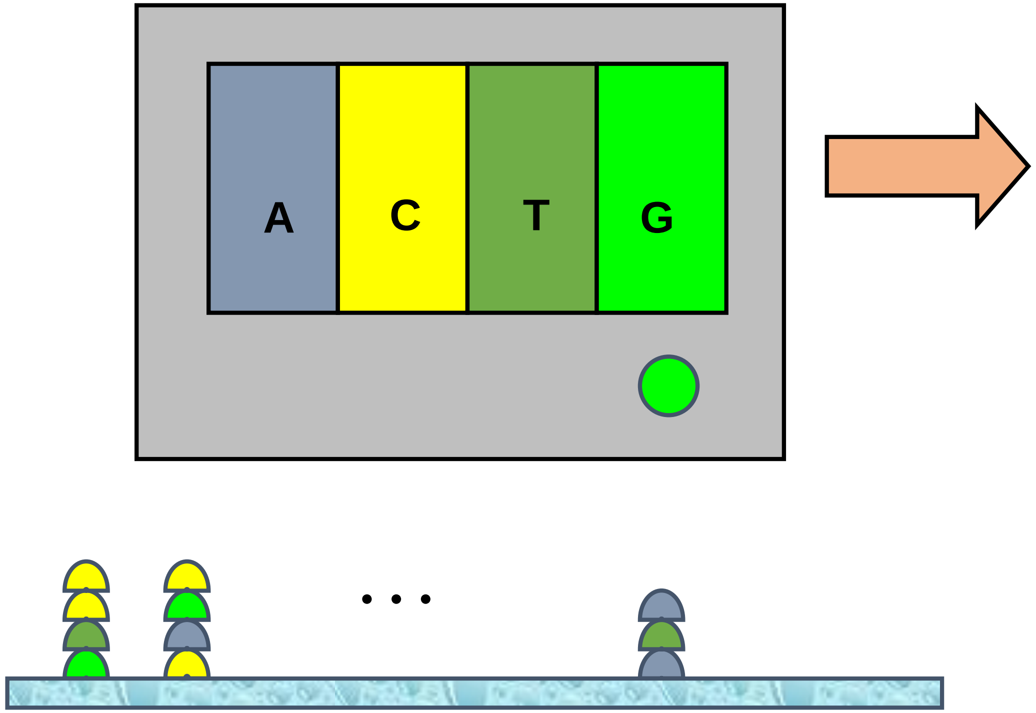
DeRisi, Iyer, Brown, Science 1997 (cDNA)
Golub et al, Science 1999 (Affymetrix)
Bittner et al, Nature 2000 (Inkjet by Agilent/HP)

- Hybridization with specific DNA probes occurs on small glass support (e.g microscope slide size)
- Hybridization is detected via fluorescence of the target sample
- Fluorescence levels at any given position is used to derive biological information/data (e.g SNP, gene expression, copy number)

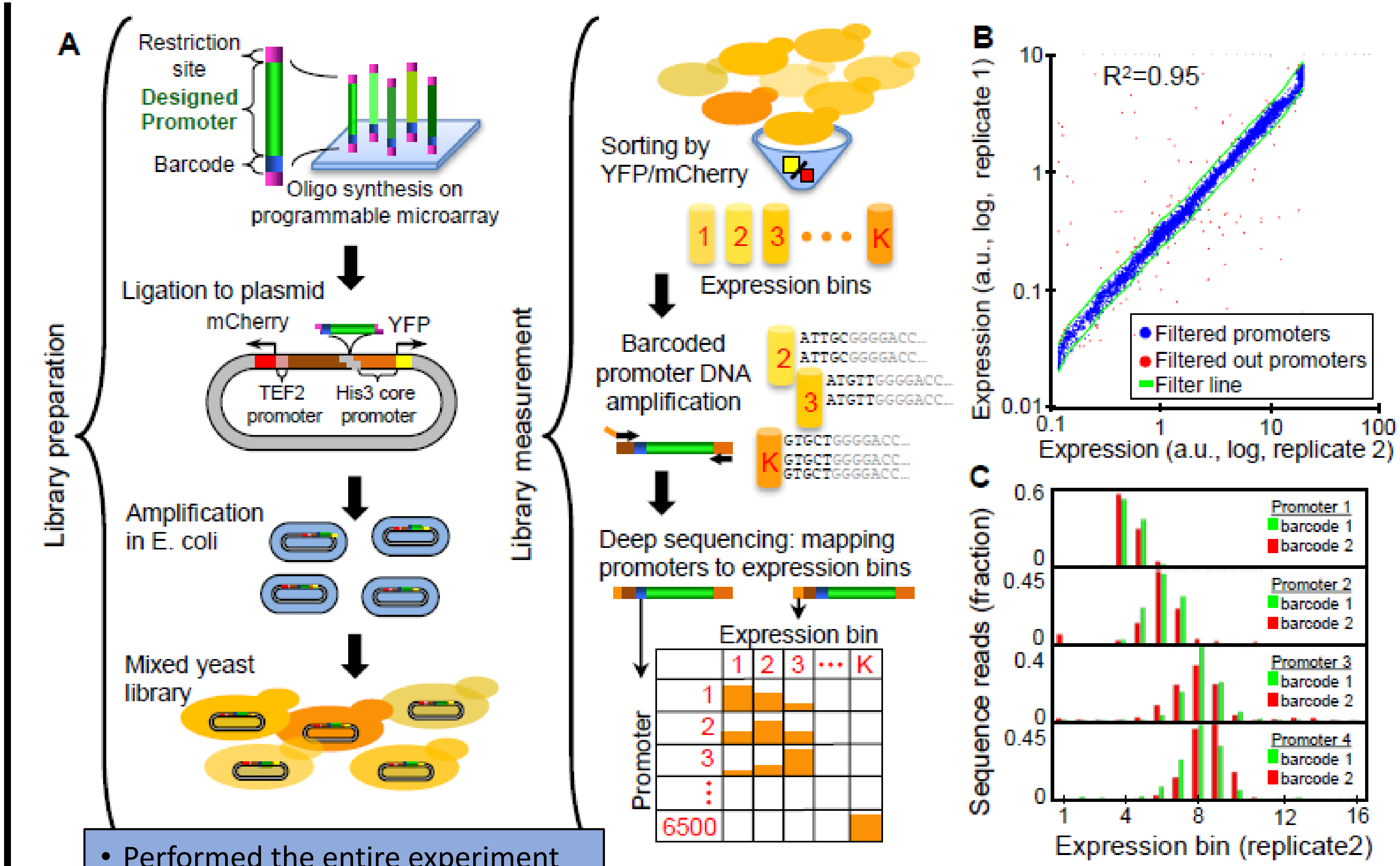


SurePrint *in-situ* Process

Agilent microarray features are DNA oligonucleotide probes synthesized in situ with ink-jet technology (Nature Biotech 2004).



Synthetic promoters: Overview of the study



- Performed the entire experiment twice (1 year, 1 month)

Sharon et al, Nat Biotech 2012

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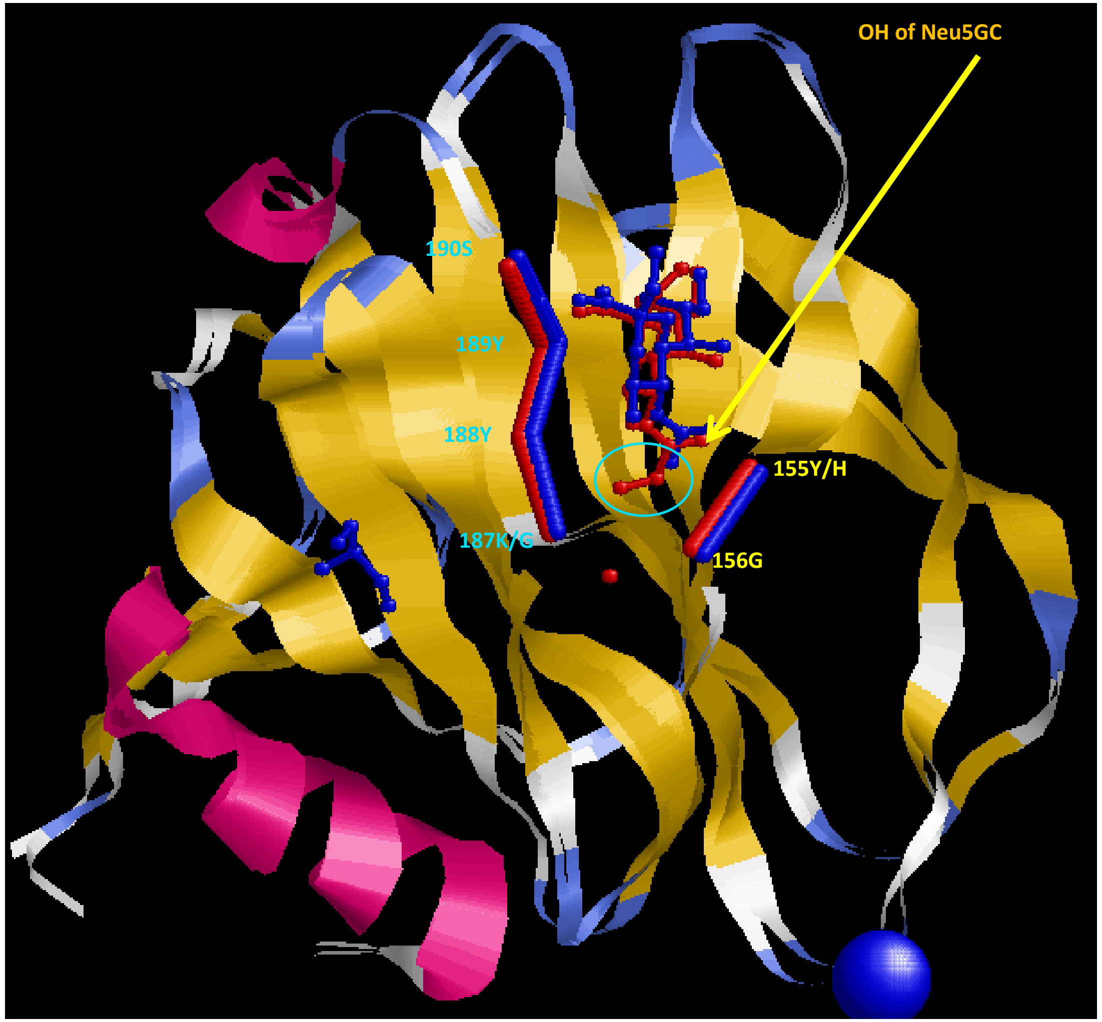
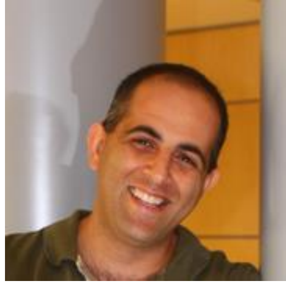
Protein design



Bacterial insulators

A synthetic oligo library and sequencing approach reveals an insulation mechanism encoded within bacterial σ54 promoters





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- Oz Solomon

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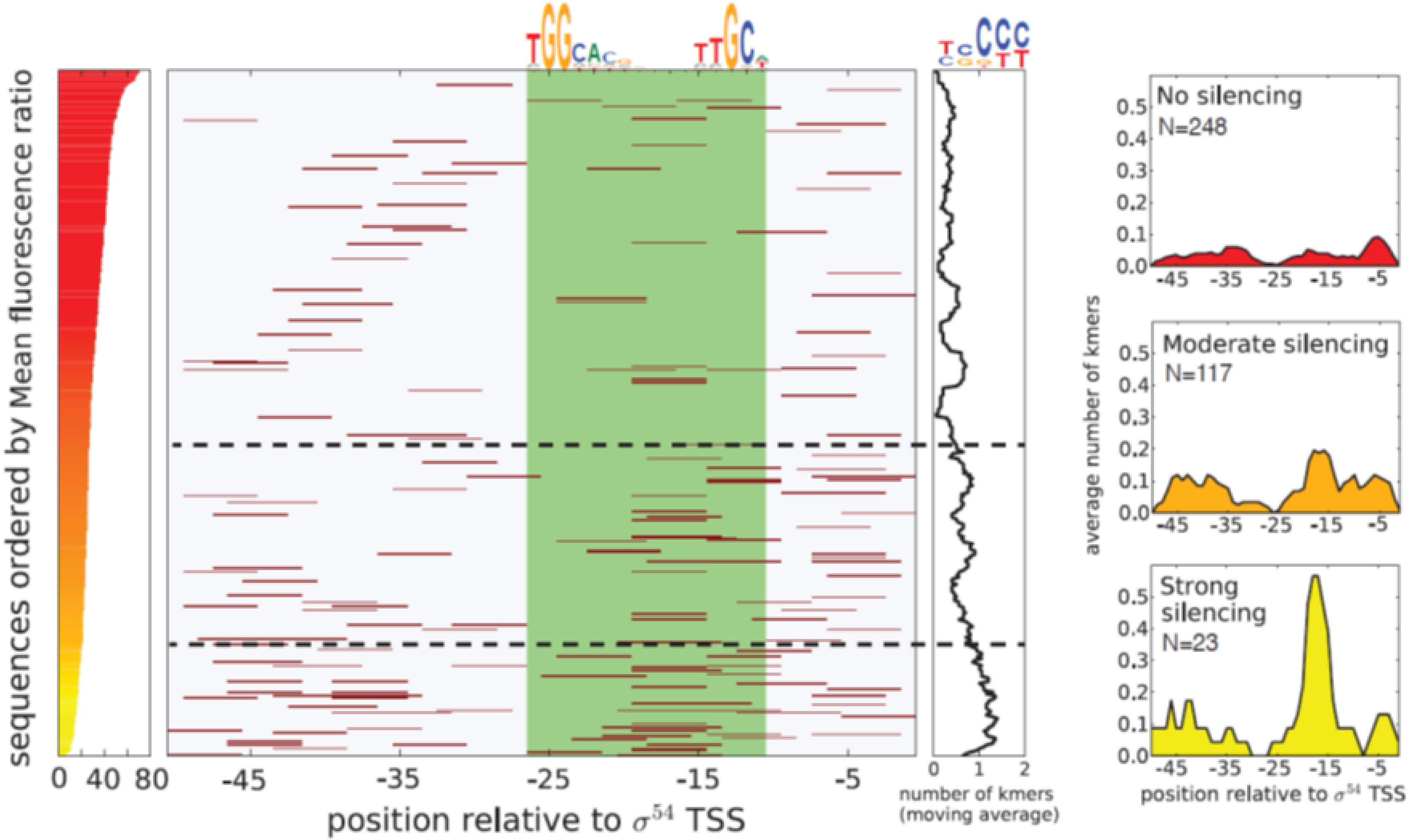

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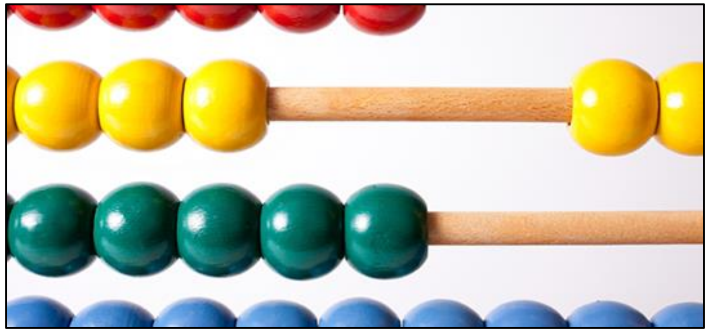
Summary



- Modelling can guide the selection of ulva species and of fermentation configurations and species for more efficient biorefineries
- Two step fermentation offers better production rates
- BioLEGO – a web based design and modelling service
- Fully designed OLs can be used to
    - Study sequence determinants of regulation and of protein function and efficiency
    - Suggest species modifications and/or guide selection in cultivation

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THANKS!

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