AMYRIS

Reasonably Random Synthetic Biology at Amyris

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Overview

- Amyris is an integrated renewable products company producing advanced renewable fuels and chemicals
- Founded in 2003 on principle of social responsibility: use our know-how to address biggest health and environmental challenges
- Public company (IPO September 2010) with R&D, Manufacturing and Distribution facilities in the Emeryville, CA, Campinas, Brazil & Chicago, IL

Amyris' fouding product: Artemsinin

Artemisinin is 95% effective against malaria

The Challenge: Supplying Artemisinin Anti-Malarials

Malaria causes:
1 to 3 million deaths per year

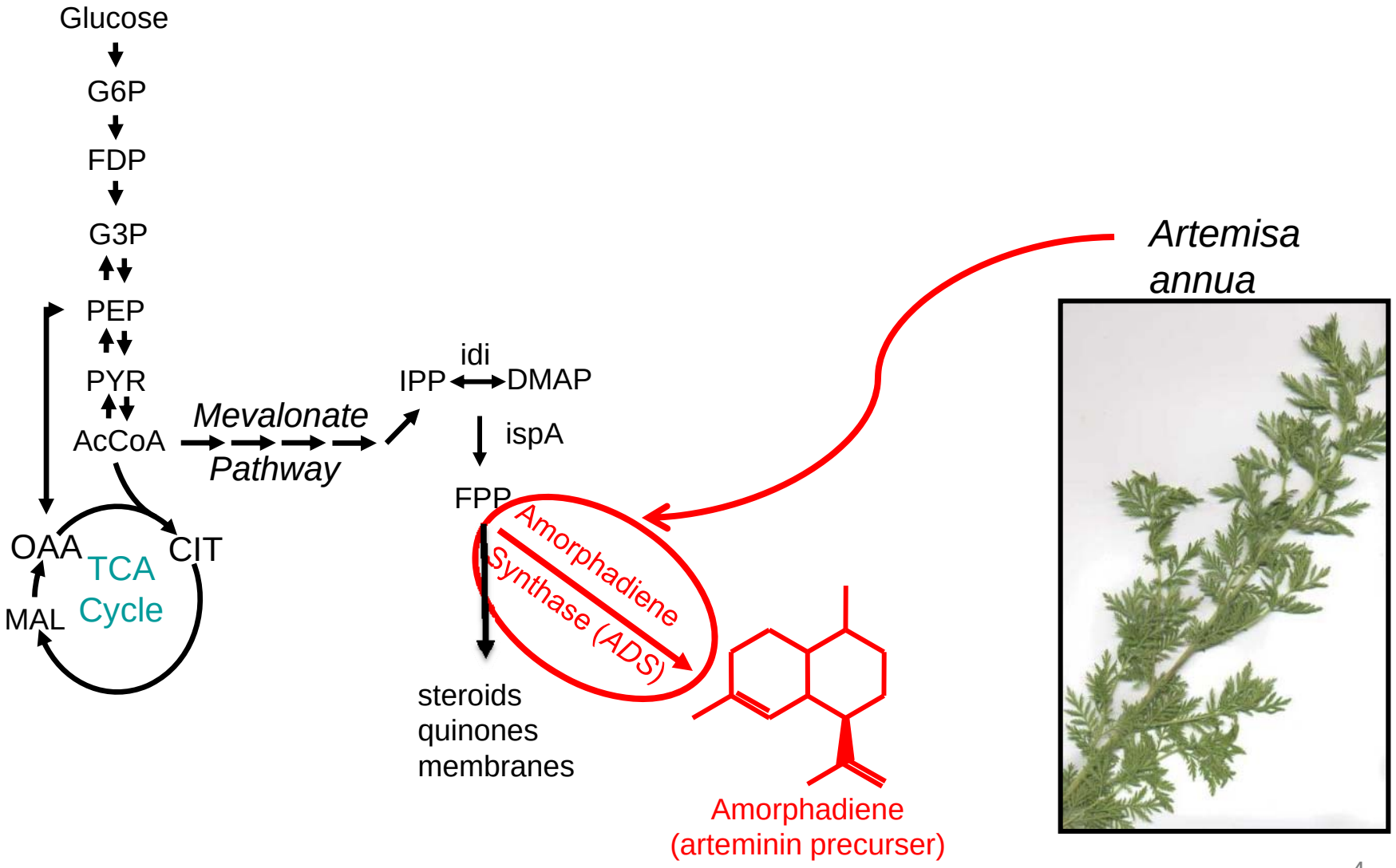
Treating malaria would require:
300 to 500 million treatments per year

Artemisinin treatments needed:
225 to 400 tons of artemisinin per year

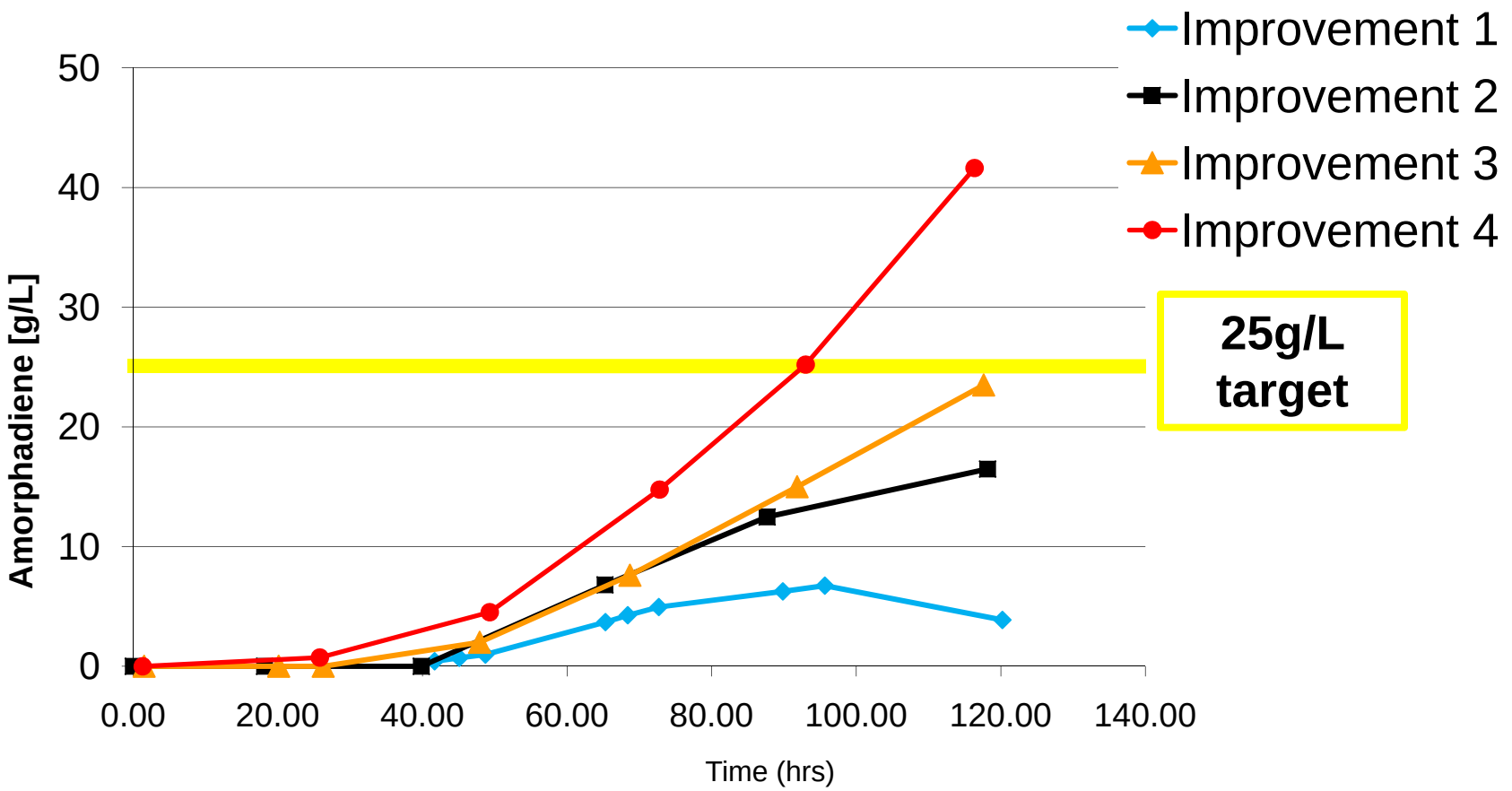
This would require:
6,000,000 tons of plant material

Total Chemical Synthesis too expensive

Non-profit effort to manufacture Artemsinin



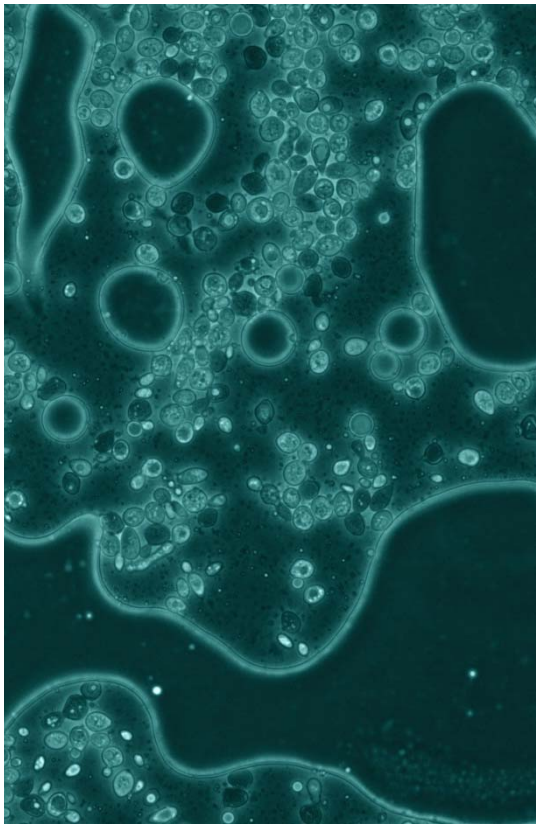
Strain performance targets reached



- Sanofi-aventis now ramping production, formulation and product stability testing
- Aim for world-wide distribution in 2012.

From drugs to fuels

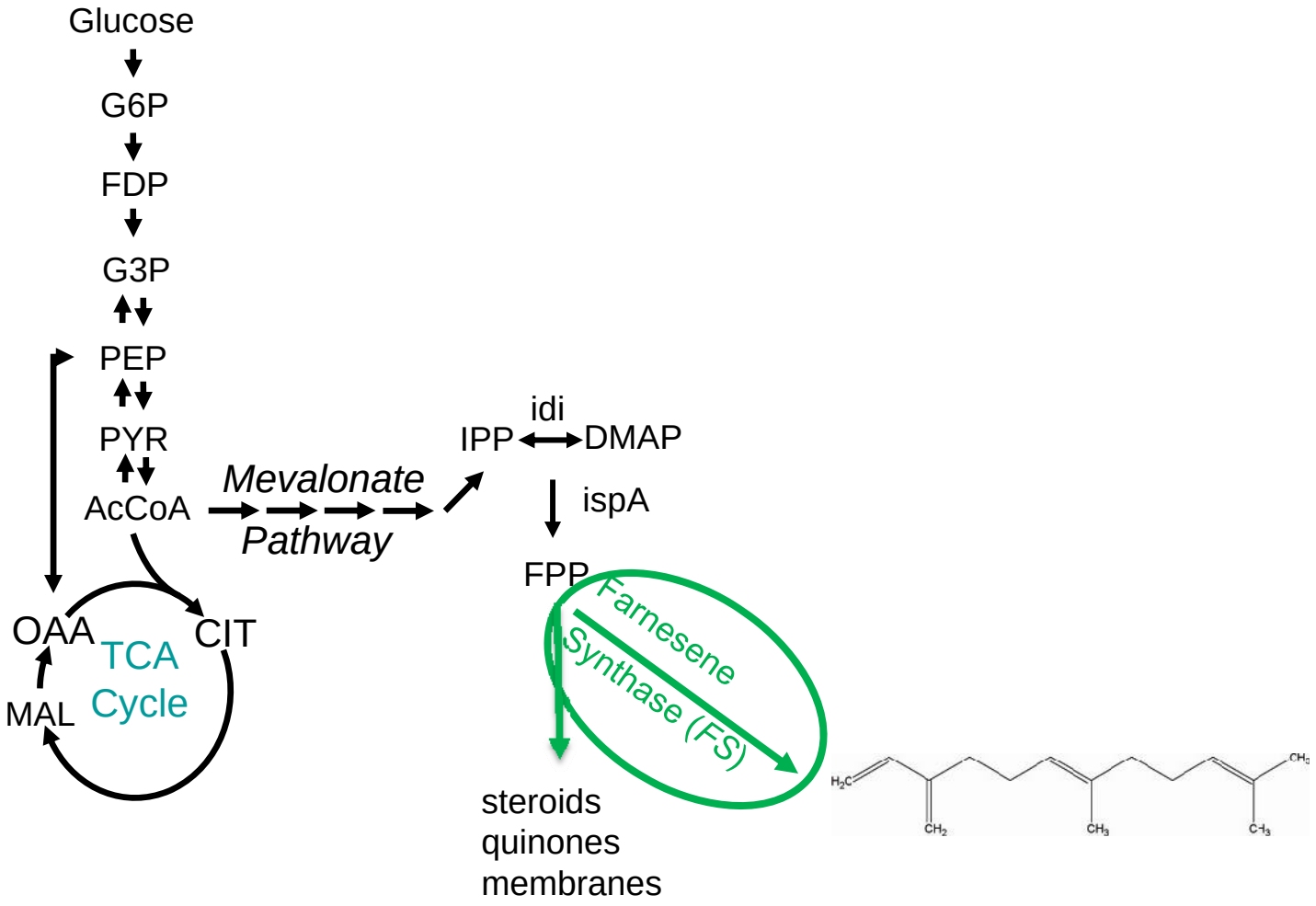
Isoprenoid technology platform capable of making more than 50,000 molecules



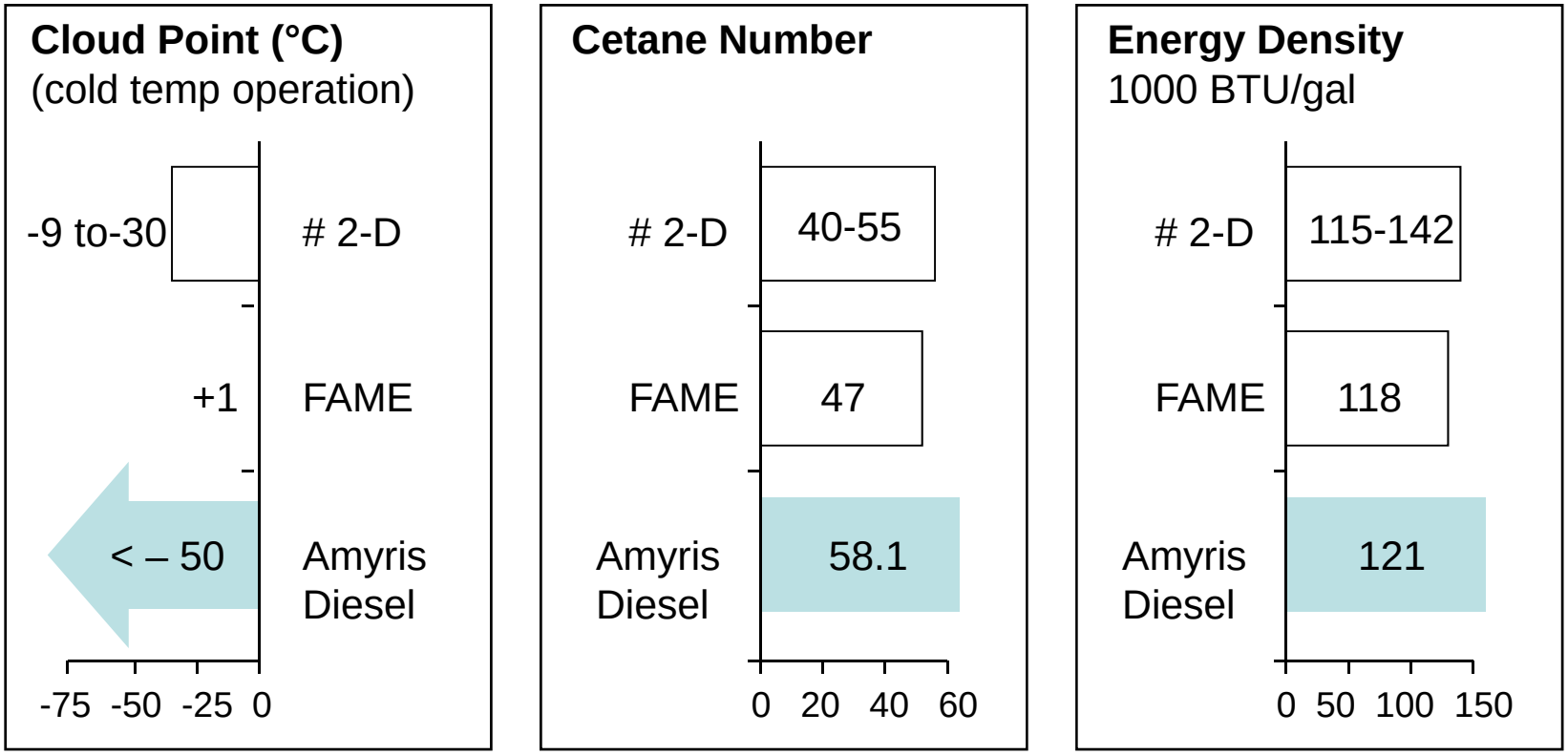
Phase-contrast micrograph of Amyris engineered microbes producing precursor to Amyris Renewable Diesel

- Hydrocarbons, not alcohols or esters
- Can be used in existing engines with no performance trade-offs
- Superior environmental profile
  - substantially lower greenhouse gas emissions than petroleum
  - No sulfur
  - Lower particulates and NOx
- Can be delivered using existing distribution infrastructure

Diesel production



Amyris Renewable Diesel: a better fuel



Additional benefits of Amyris renewable diesel compared to #2-Diesel

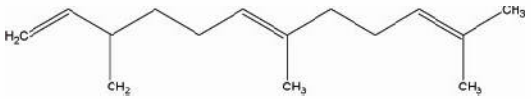
- **90%+ lower greenhouse gas emissions**
- **No sulfur & produces lower NOx and particulate emissions**
- **Registered with the EPA for 20% blends**

Note: Amyris diesel will be used in blends with conventional fuels; values shown for Amyris diesel is for our biomass derived blending component; SME = Soy Methyl Esters

>$1 Trillion dollar market accessible

fermentation

Farnesene



biology

chemistry

By combining biology and chemistry, Biofene becomes a building block of renewable products for a diverse set of applications

- Renewable diesel
  - "plug-in" fuels
  - meets or exceeds stds
  - substantially lower emissions

$809B

- Lubricants
  - family of base oils
  - designed to be high performance

$48B

- Consumer products
  - detergents
  - Cosmetics
  - fragrances

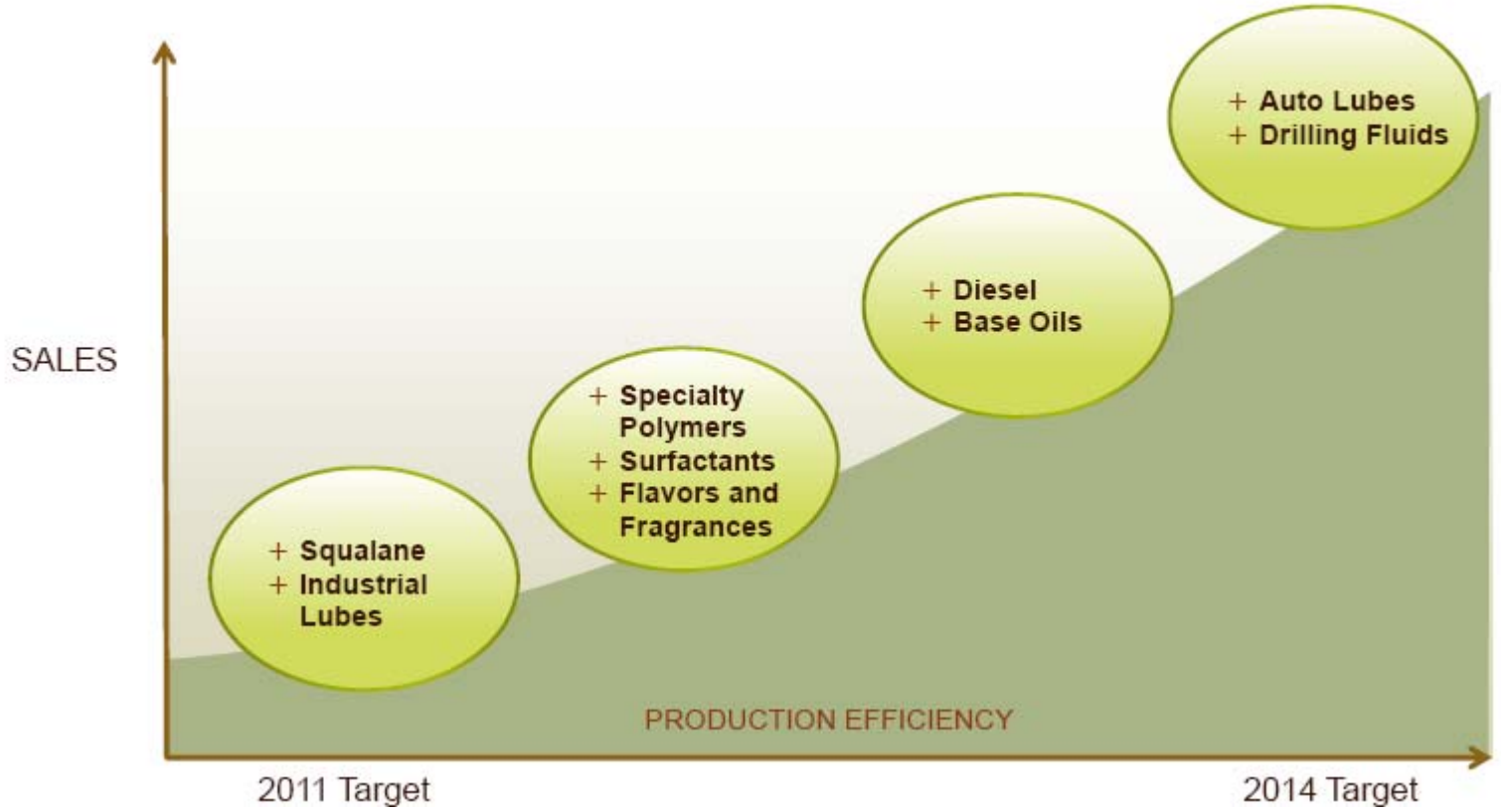
>$50B

- Polymers
  - adhesives
  - oxygen scavenger
  - toughening agent

$337B

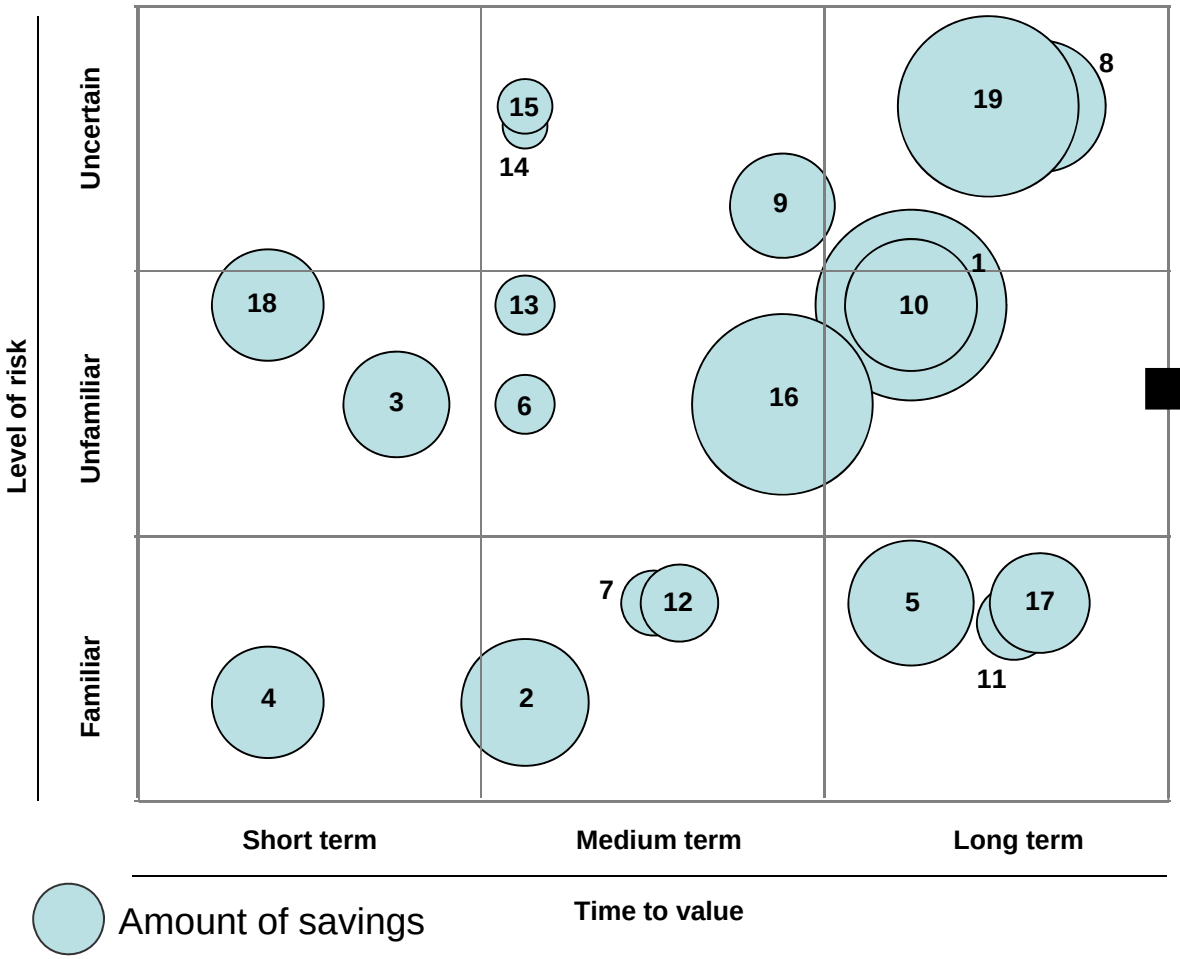
- Other applications
  - crop protection
  - many others

Lower cost of production enables access to larger markets



Low-cost production drives everything in strain R&D

Capital cost-saving opportunities



2M ton/yr plant

Engineering decisions drive strain performance criteria

Multi-parameter strain optimization problem

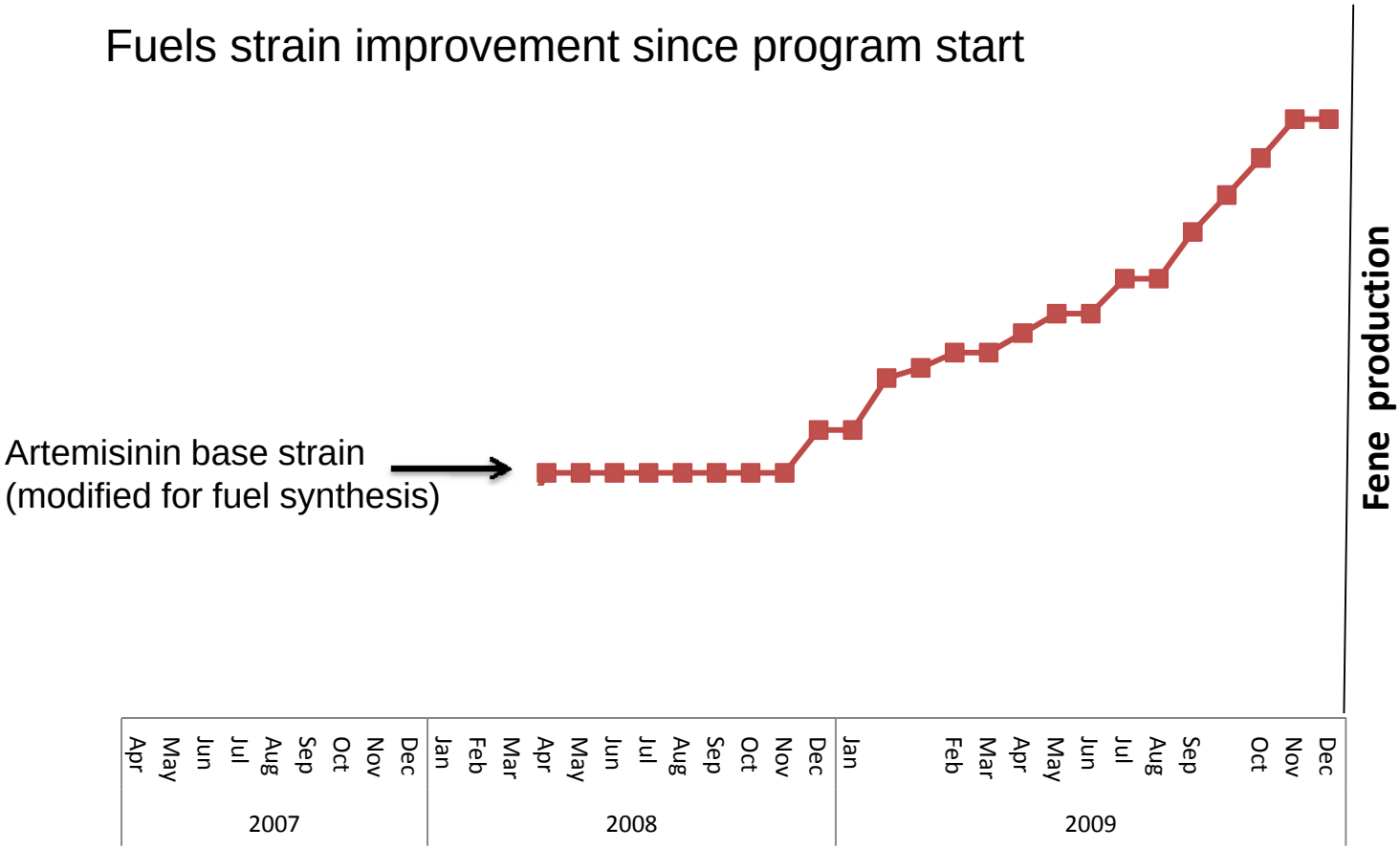
- yield
- productivity
- reduced media supplements
- temperature
- biocatalyst stability
- GMM certification

For fuel synthesis we aim to direct >90% of cell resources to the synthesis of byproducts under stringent productivity, temperature, and media conditions

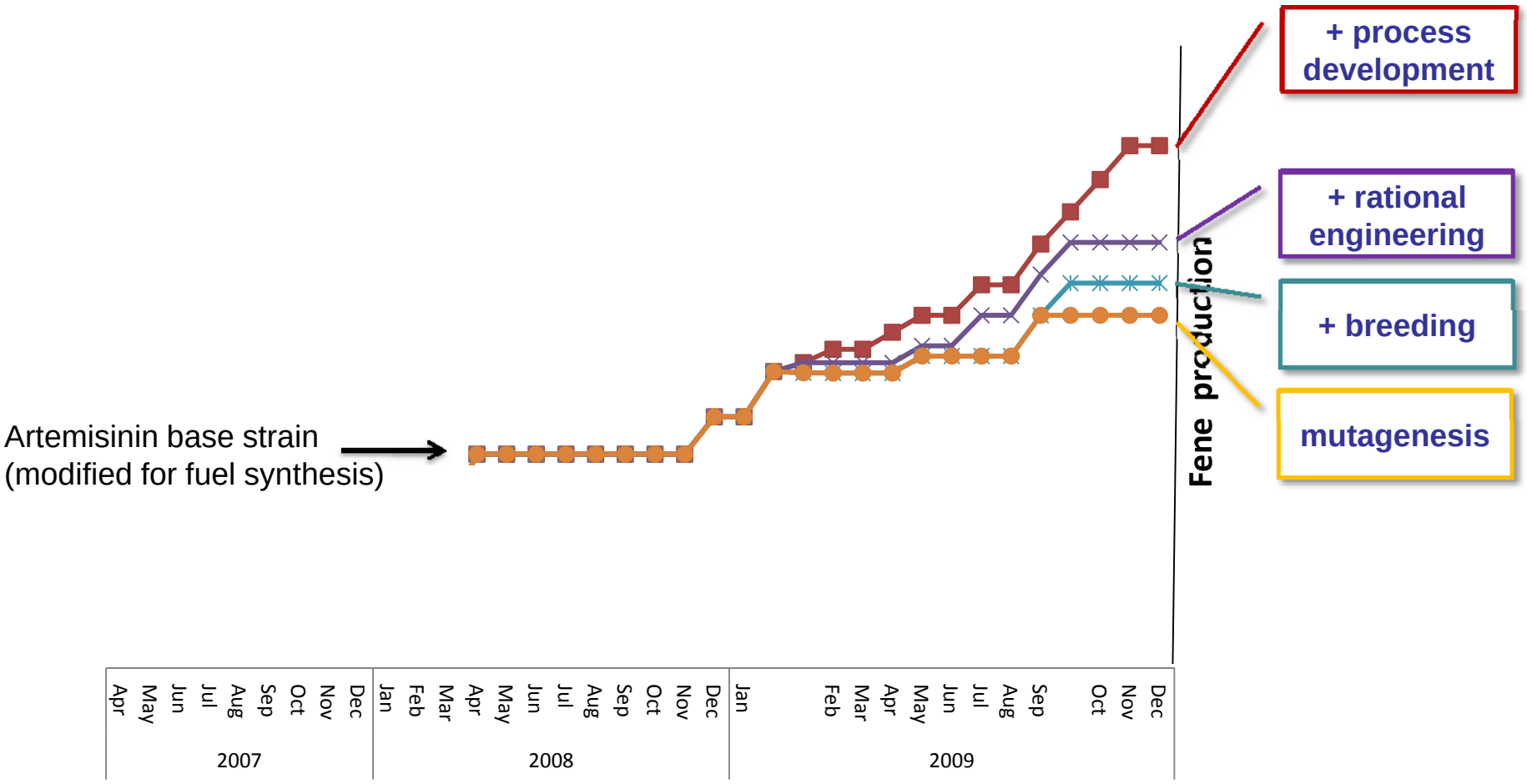
like getting a toddler to eat salad

AMYRIS

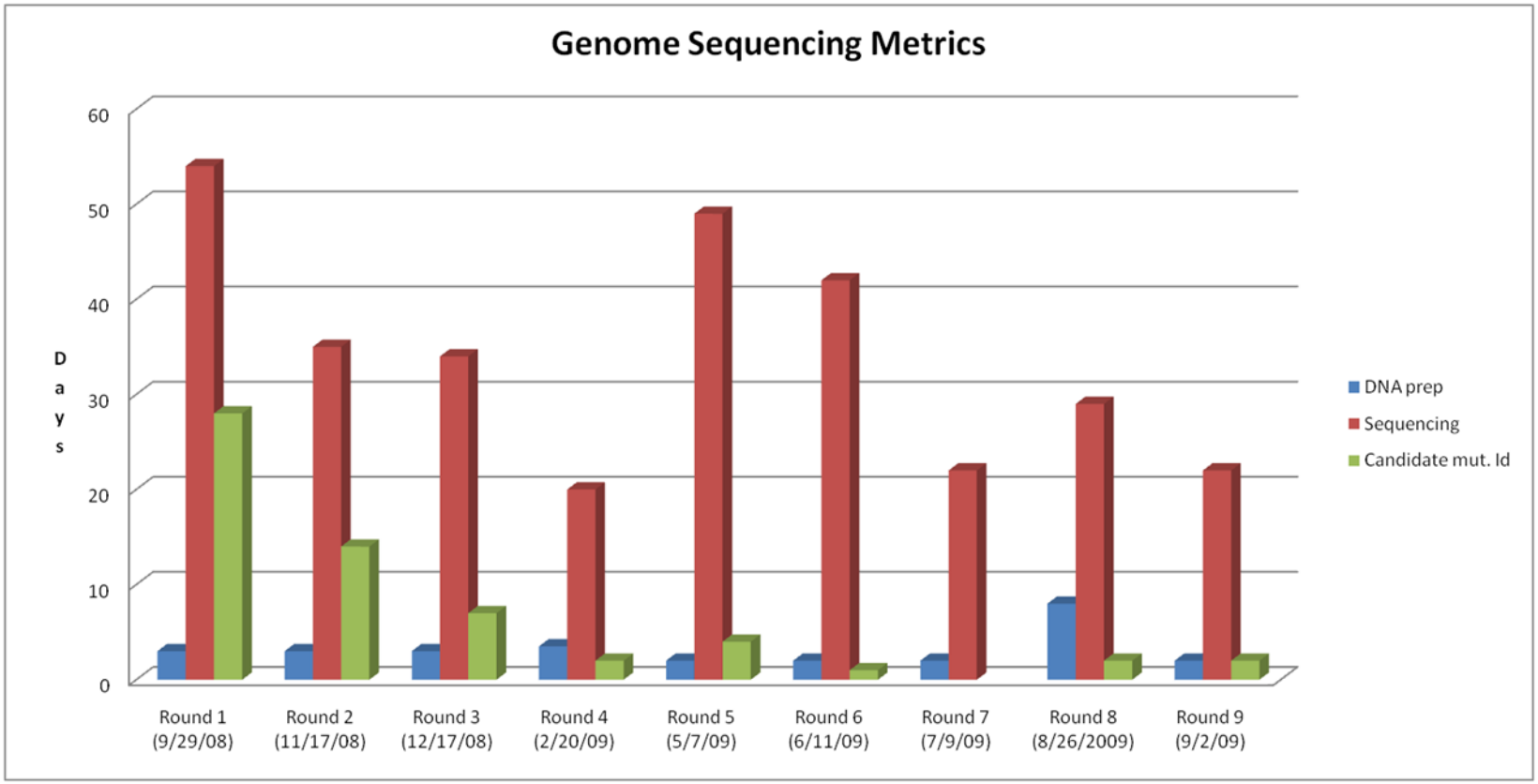
But we've made rapid progress



How we got there

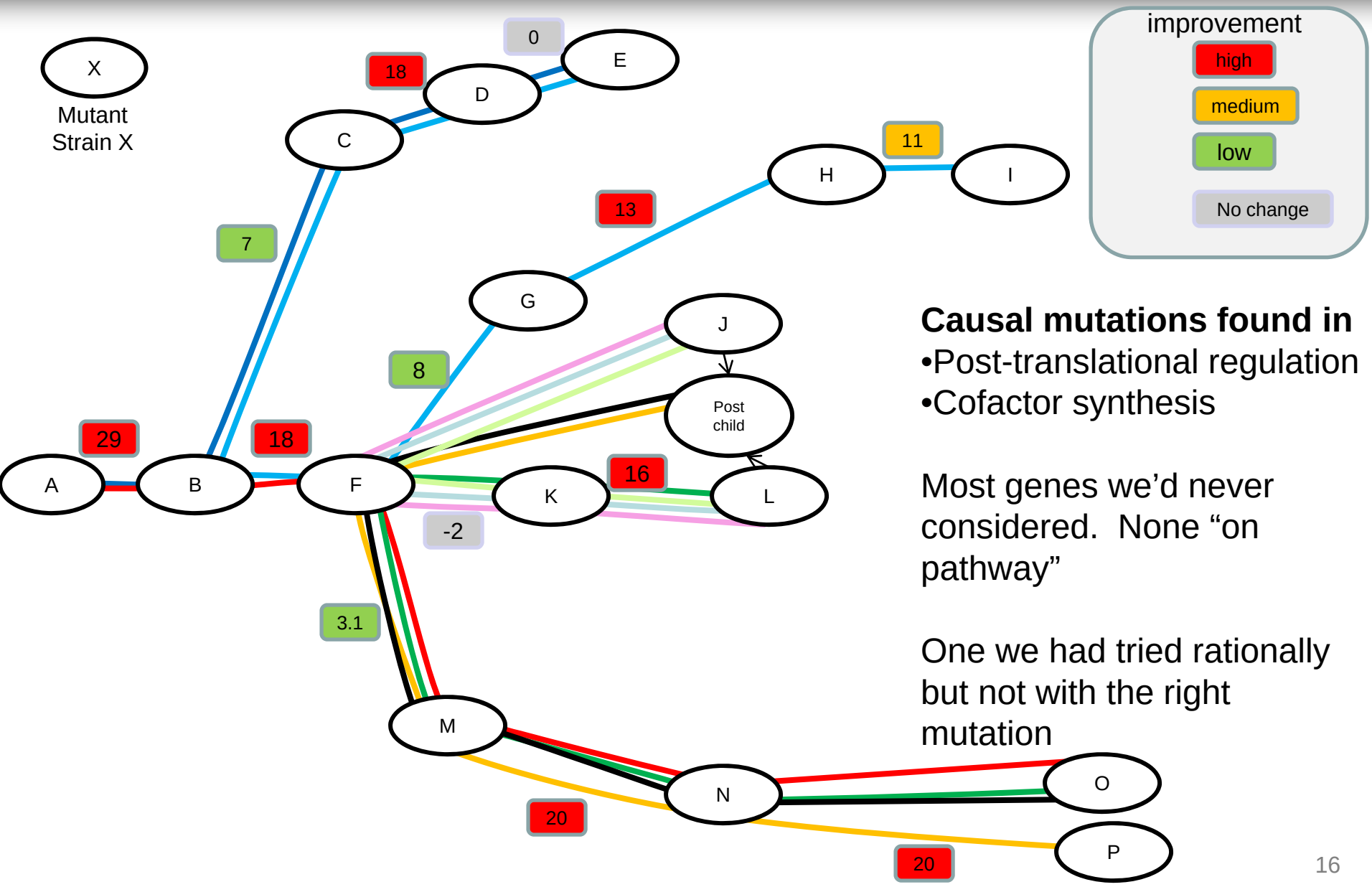


What can we learn from the mutants?



Illumina paired-end sequencing performed by Prognosys, Sequence assembly & analysis by Amyris

Mutant family tree and performance gains



Causal mutations found in

- Post-translational regulation
- Cofactor synthesis

Most genes we'd never considered. None "on pathway"

One we had tried rationally but not with the right mutation

What about rational engineering?

Synthetic Biology: the dream of plug and play biology

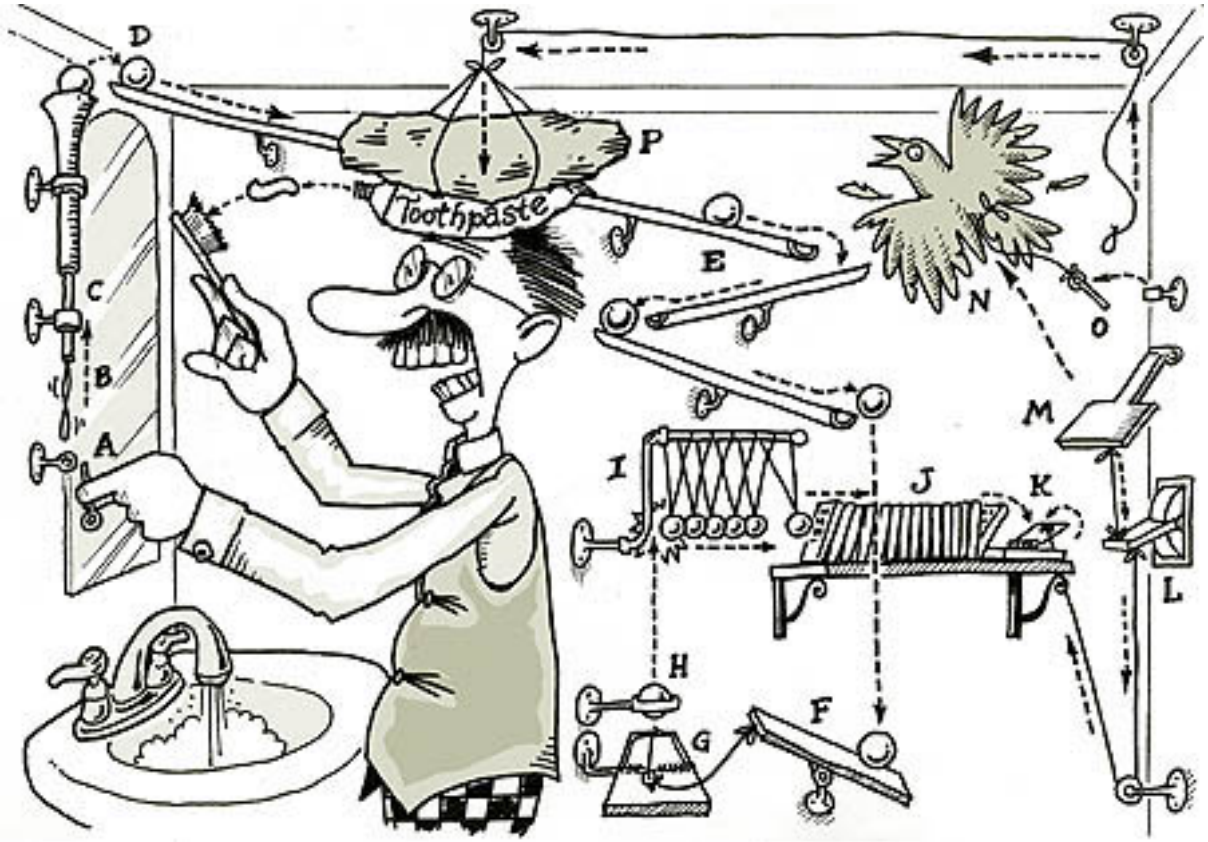
Are electronics and machines the right paradigm?

The neutral chassis hypothesis

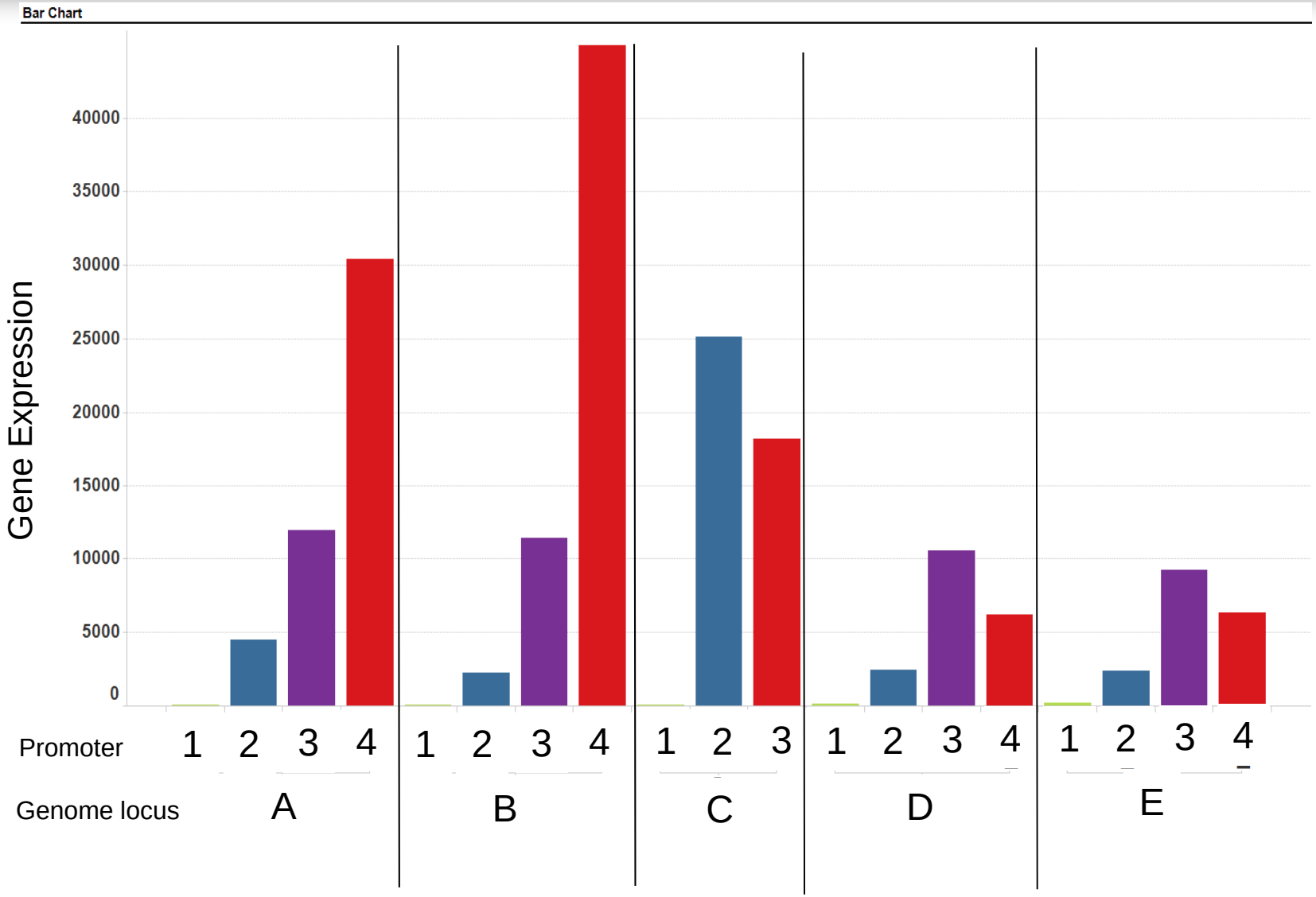
Add a little synthetic biology

Biology is designed by natural selection

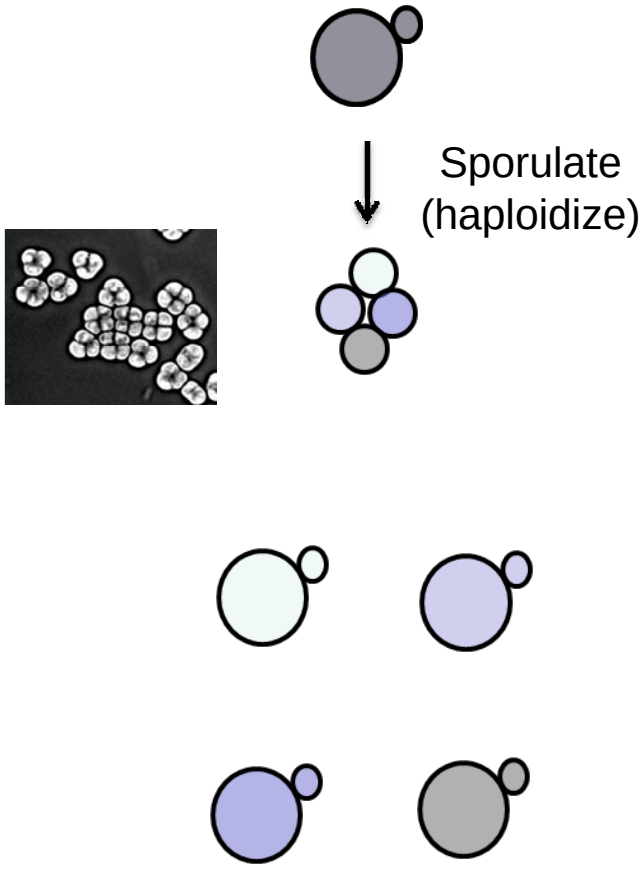
Too many parts kinda complexity



Promoter strength varies depending on its insertion site

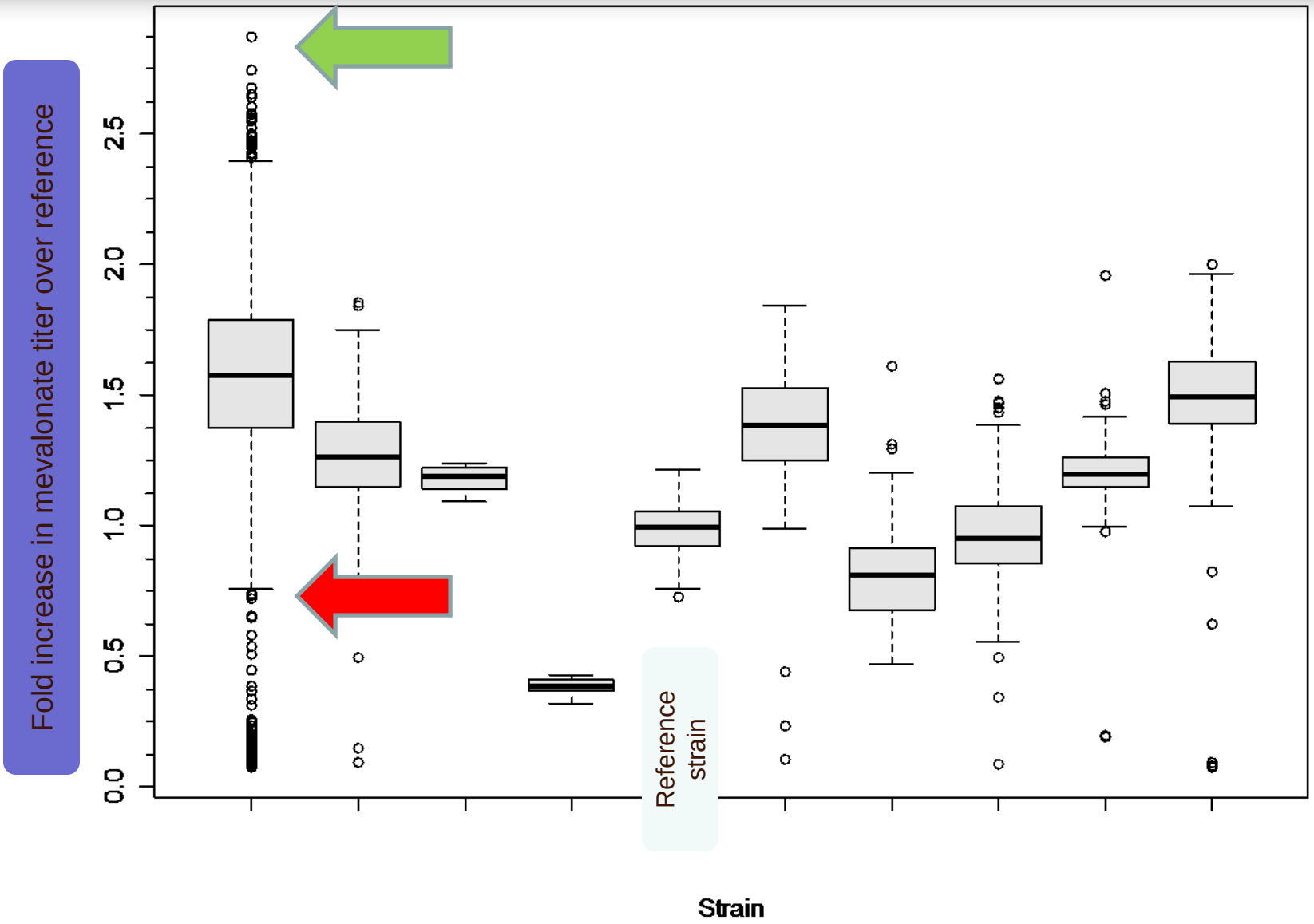


How much does diversity influence pathway production?

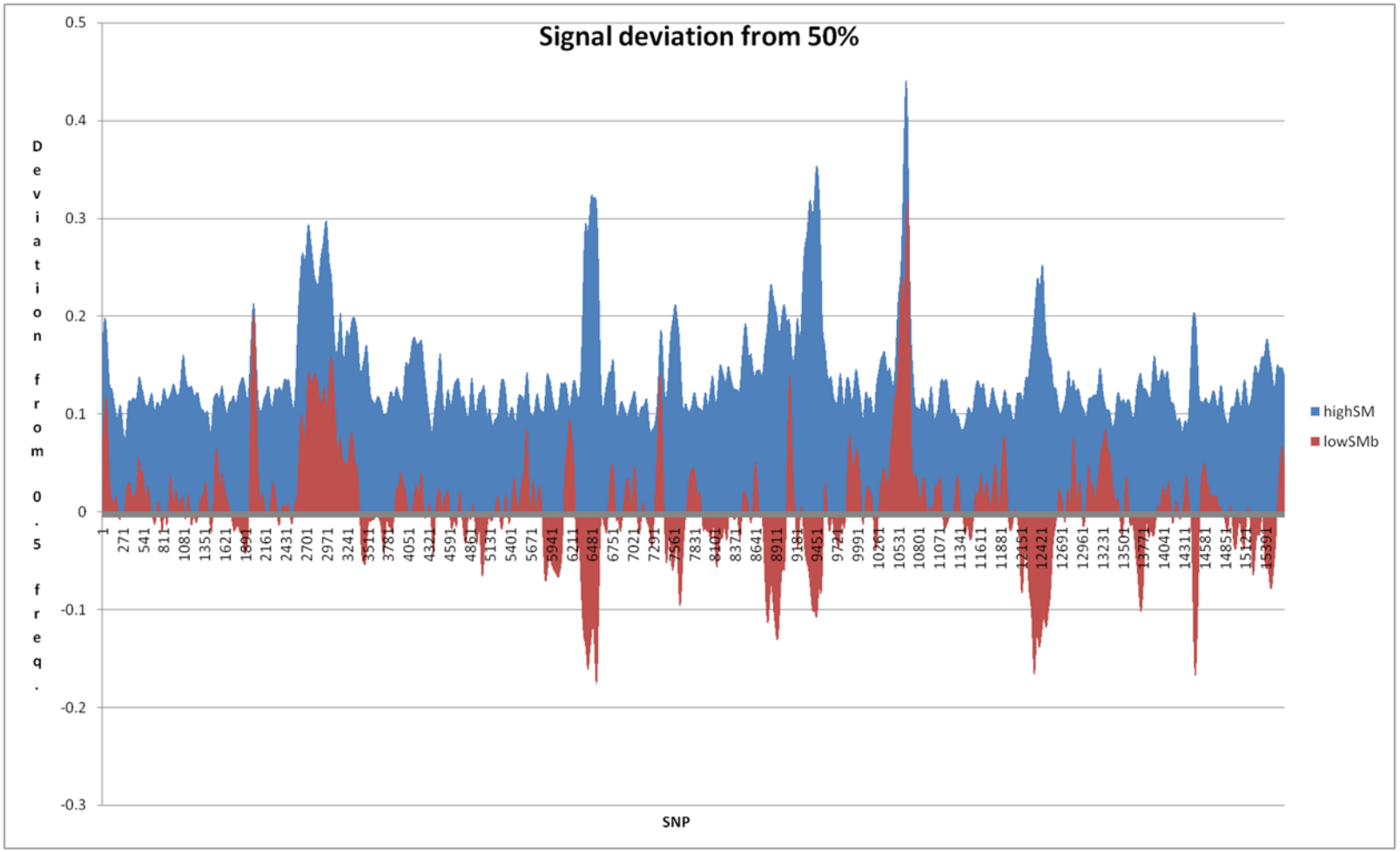


Farnesene Pathway

Impact of diversity on Mevalonate production



Frequencies for top and bottom Mevalonate pools



A practical approach

Pathway PoC → Pathway Optimization

1 *Rational*

Stoichiometry & mRNA expression

Routine

We always start here

2 *Semi-rational Random*

Enzyme kinetics

Doable but hard

We have targeted activities here when bottlenecks become clear

3 *Random*

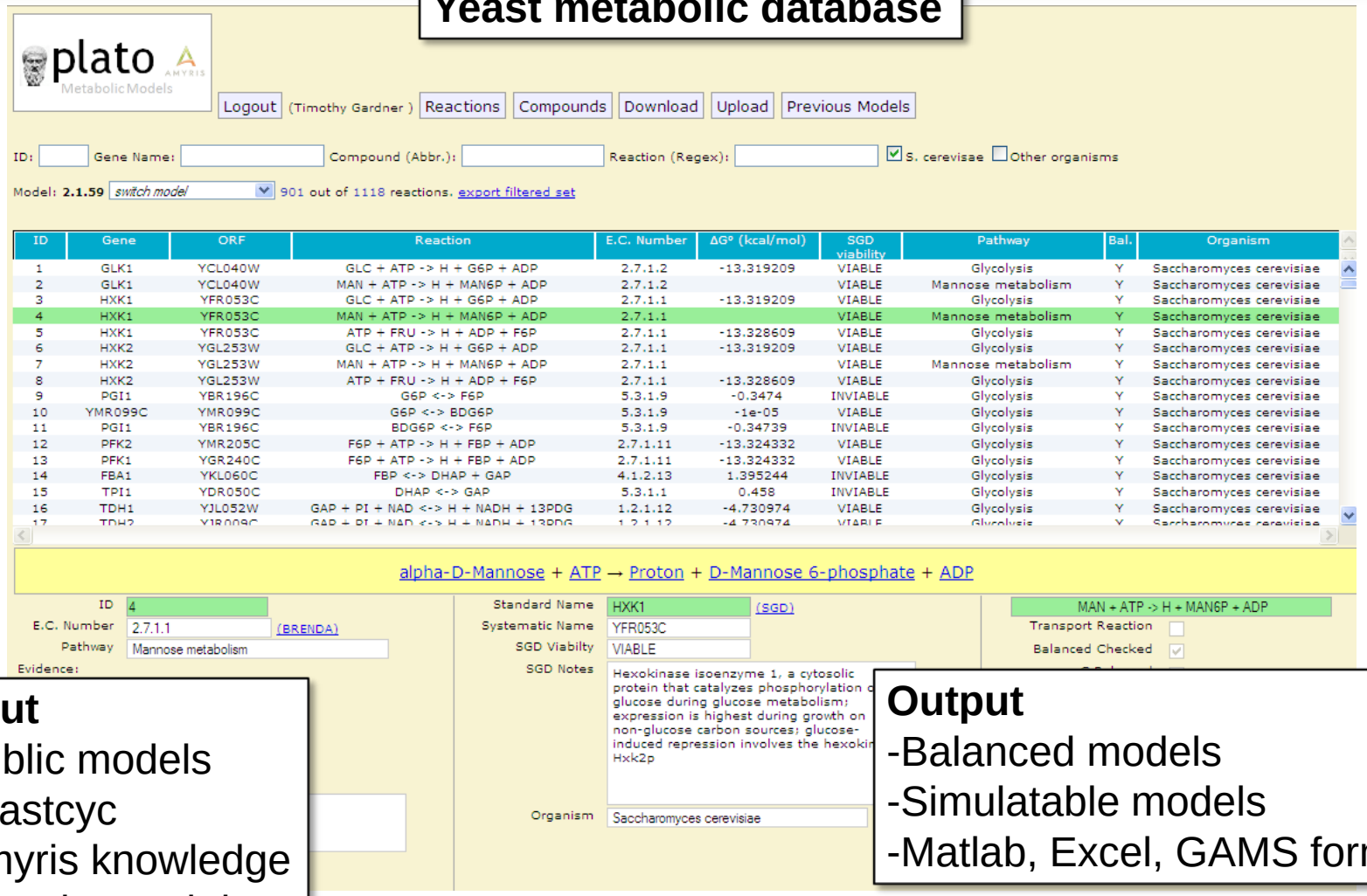
Context-effects & post-transcriptional regulation

Shooting in the dark

This is where most of the strain improvement "action" is.

1. Rational: Modeling / Isotopomers

Yeast metabolic database



ID	Gene	ORF	Reaction	E.C. Number	ΔG° (kcal/mol)	SGD viability	Pathway	Bal.	Organism
1	GLK1	YCL040W	GLC + ATP -> H + G6P + ADP	2.7.1.2	-13.319209	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
2	GLK1	YCL040W	MAN + ATP -> H + MAN6P + ADP	2.7.1.2		VIABLE	Mannose metabolism	Y	Saccharomyces cerevisiae
3	HXK1	YFR053C	GLC + ATP -> H + G6P + ADP	2.7.1.1	-13.319209	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
4	HXK1	YFR053C	MAN + ATP -> H + MAN6P + ADP	2.7.1.1		VIABLE	Mannose metabolism	Y	Saccharomyces cerevisiae
5	HXK1	YFR053C	ATP + FRU -> H + ADP + F6P	2.7.1.1	-13.328609	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
6	HXK2	YGL253W	GLC + ATP -> H + G6P + ADP	2.7.1.1	-13.319209	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
7	HXK2	YGL253W	MAN + ATP -> H + MAN6P + ADP	2.7.1.1		VIABLE	Mannose metabolism	Y	Saccharomyces cerevisiae
8	HXK2	YGL253W	ATP + FRU -> H + ADP + F6P	2.7.1.1	-13.328609	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
9	PGI1	YBR196C	G6P <-> F6P	5.3.1.9	-0.3474	INVIABLE	Glycolysis	Y	Saccharomyces cerevisiae
10	YMR099C	YMR099C	G6P <-> BDG6P	5.3.1.9	-1e-05	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
11	PGI1	YBR196C	BDG6P <-> F6P	5.3.1.9	-0.34739	INVIABLE	Glycolysis	Y	Saccharomyces cerevisiae
12	PFK2	YMR205C	F6P + ATP -> H + FBP + ADP	2.7.1.11	-13.324332	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
13	PFK1	YGR240C	F6P + ATP -> H + FBP + ADP	2.7.1.11	-13.324332	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae
14	FBA1	YKL060C	FBP <-> DHAP + GAP	4.1.2.13	1.395244	INVIABLE	Glycolysis	Y	Saccharomyces cerevisiae
15	TPI1	YDR050C	DHAP <-> GAP	5.3.1.1	0.458	INVIABLE	Glycolysis	Y	Saccharomyces cerevisiae
16	TDH1	YJL052W	GAP + PI + NAD <-> H + NADH + 13PDG	1.2.1.12	-4.730974	VIABLE	Glycolysis	Y	Saccharomyces cerevisiae

Input
-Public models
-Yeastcyc
-Amyris knowledge
-Experimental data

Output
-Balanced models
-Simulatable models
-Matlab, Excel, GAMS format

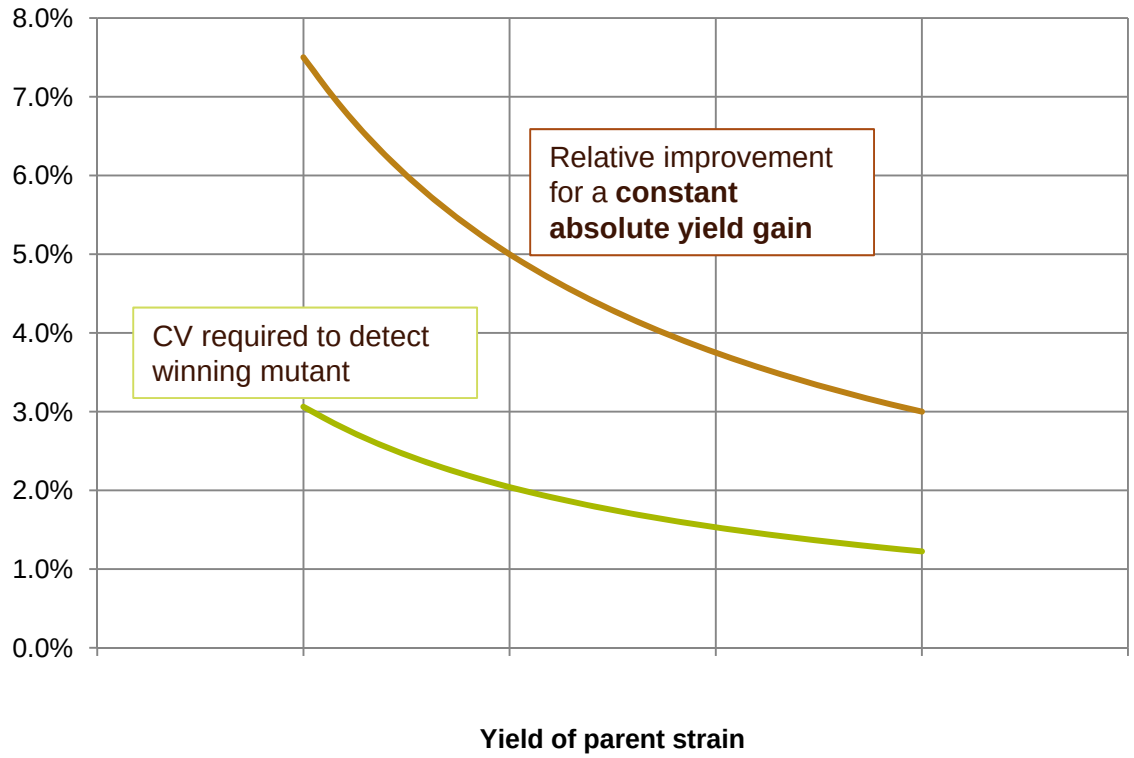
2. & 3. Industrialize strain improvement

Capacity:
Screen >70,000 strains / week
Test >40 2L fermentations / week

Continuous process improvement is critical

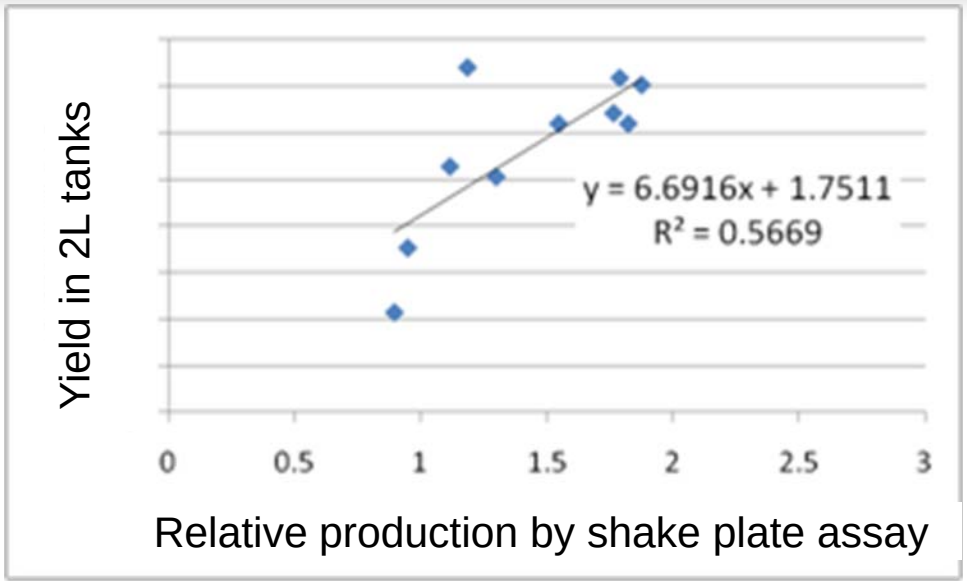
Assuming constant absolute yield gain per improved mutant strain.

- **S/N will drop as yield increases.**
- **So too must CV.**

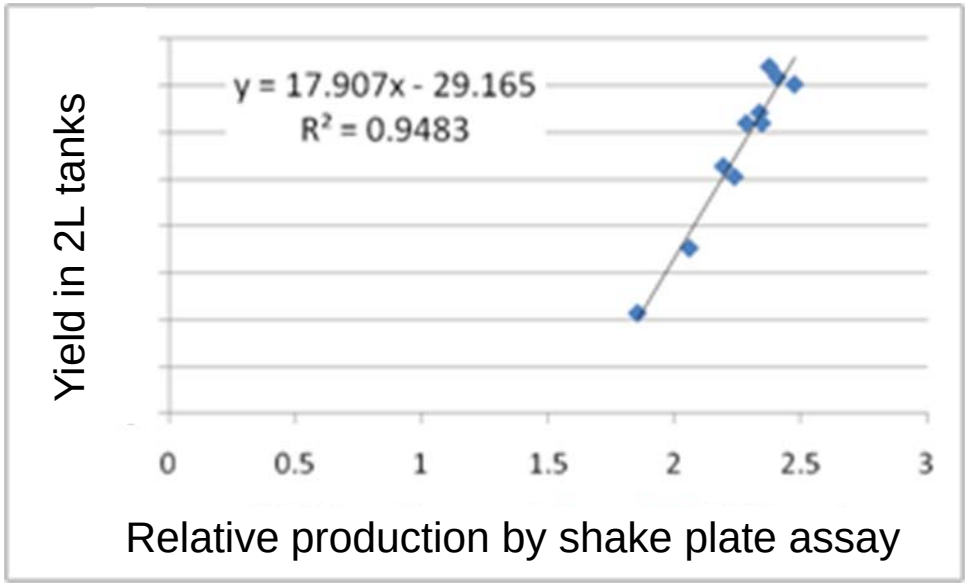


The value of process control

Original strain screening assay



New assay



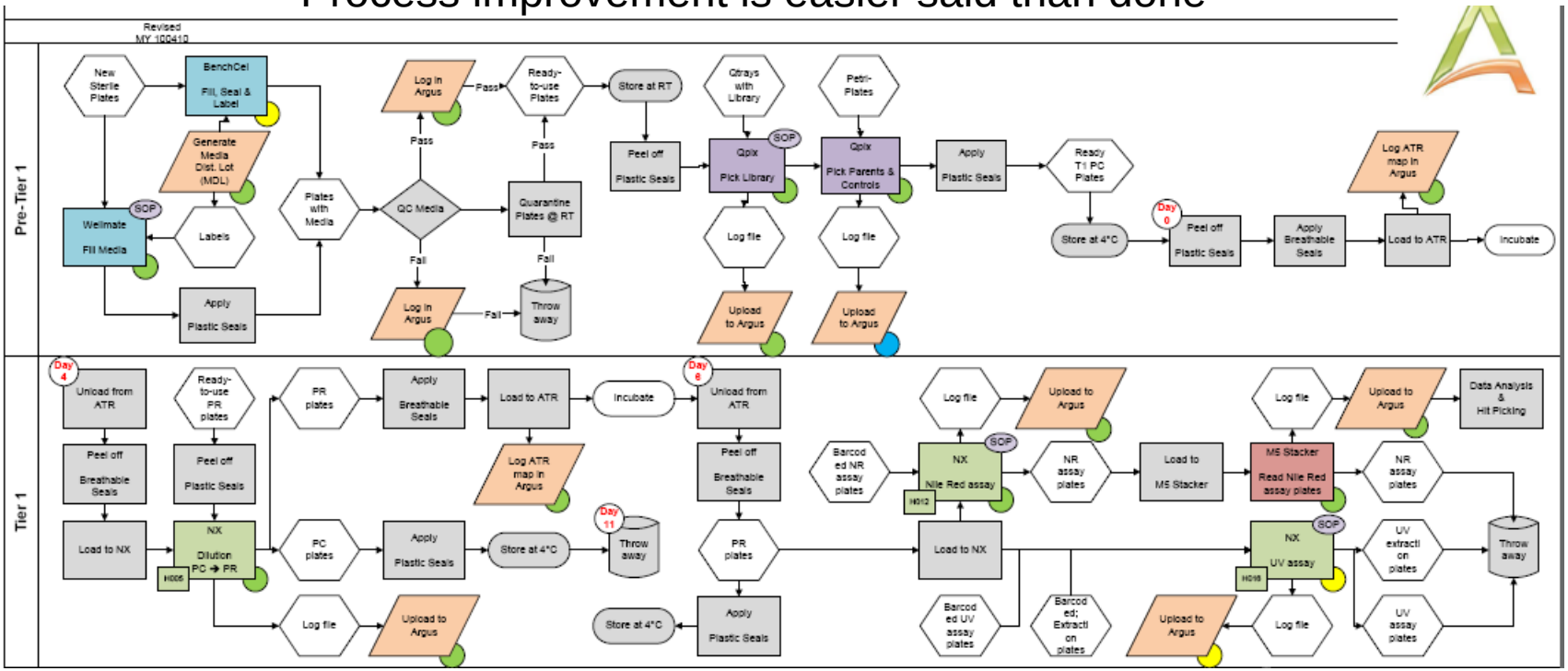
The reward:

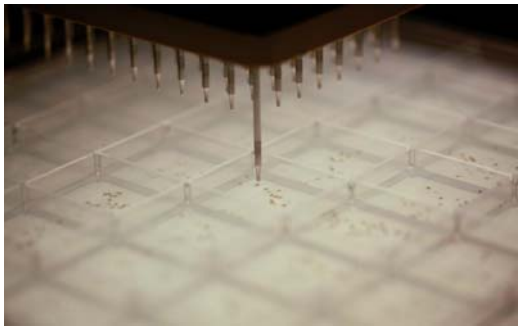
Overall screening process CV <4%

Enables detection of 4% improvements w/ 5% FN and 5% FPs through 2 tiered screen

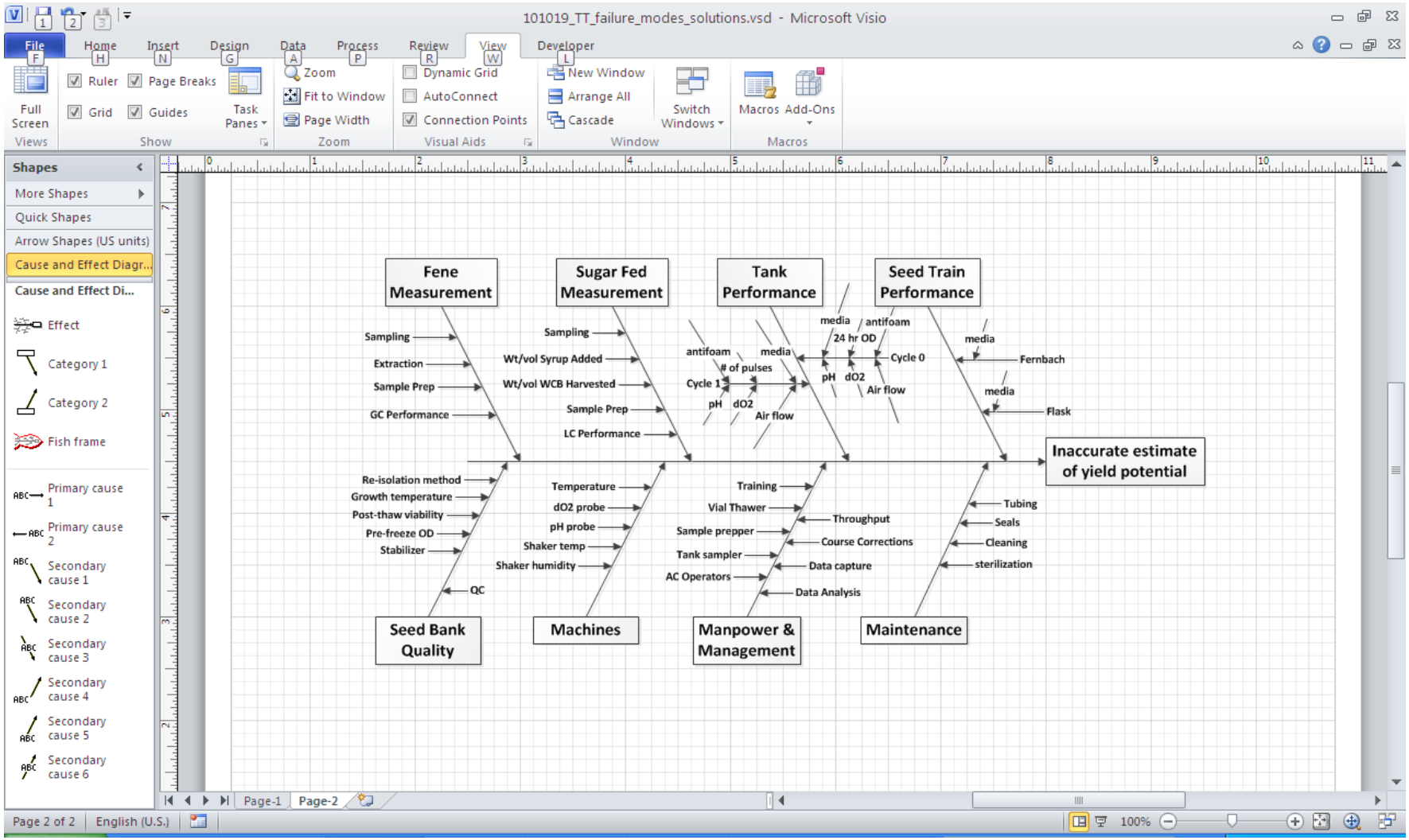
HT screening pipeline

Process improvement is easier said than done



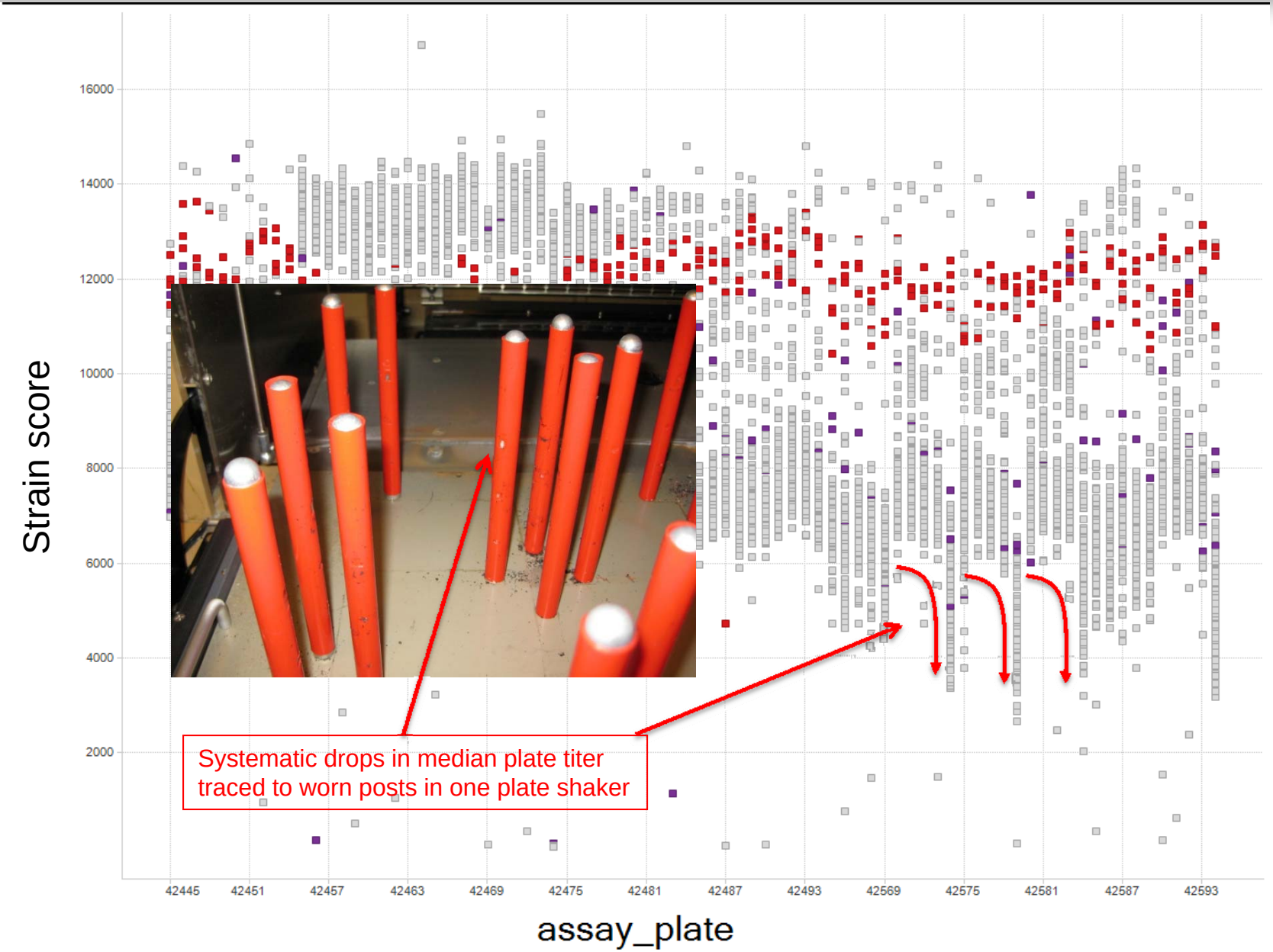


Diagnosing sources of variation



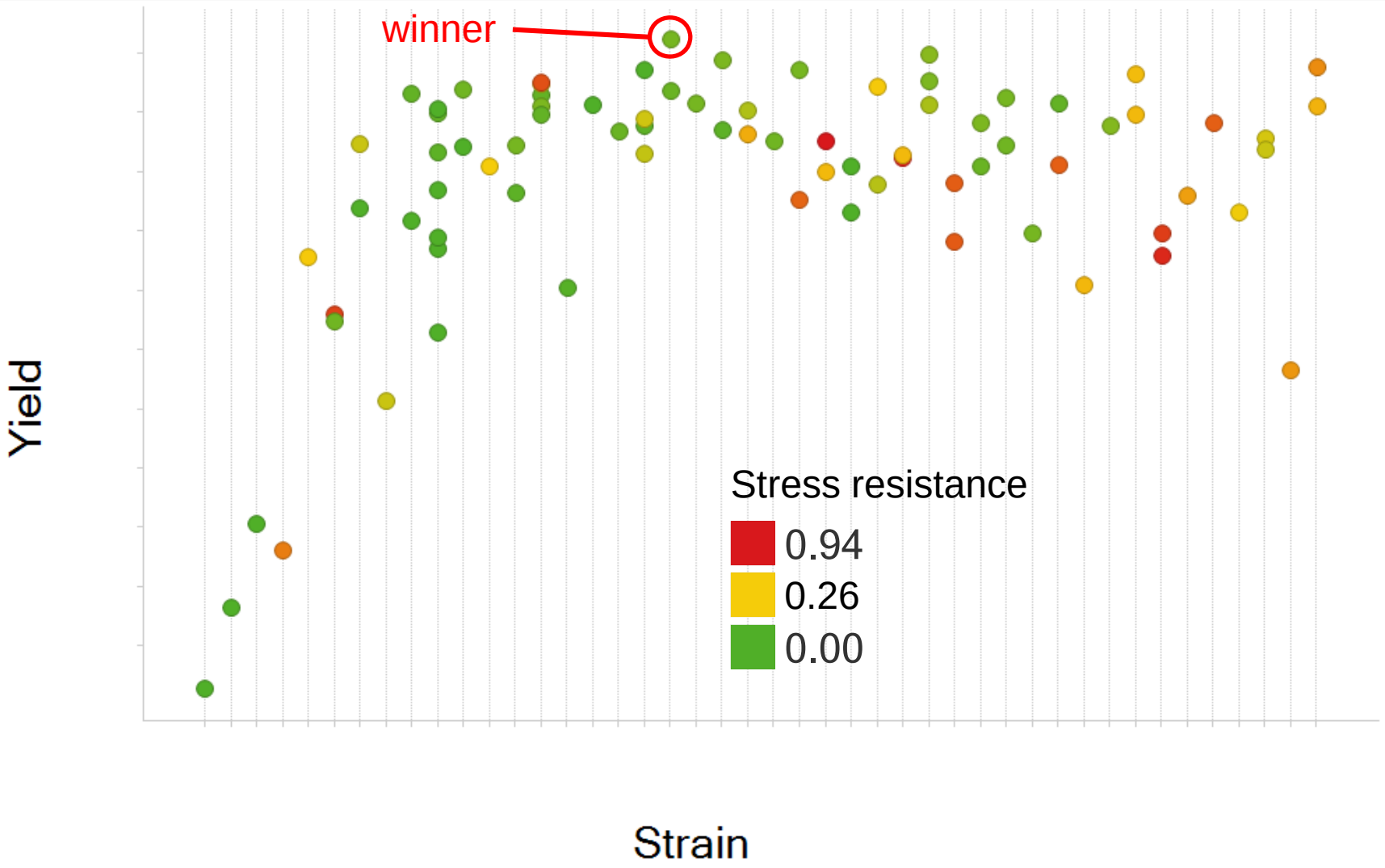
Better decisions via informatics integration

LIMS systems is identifying and eliminating sources of error

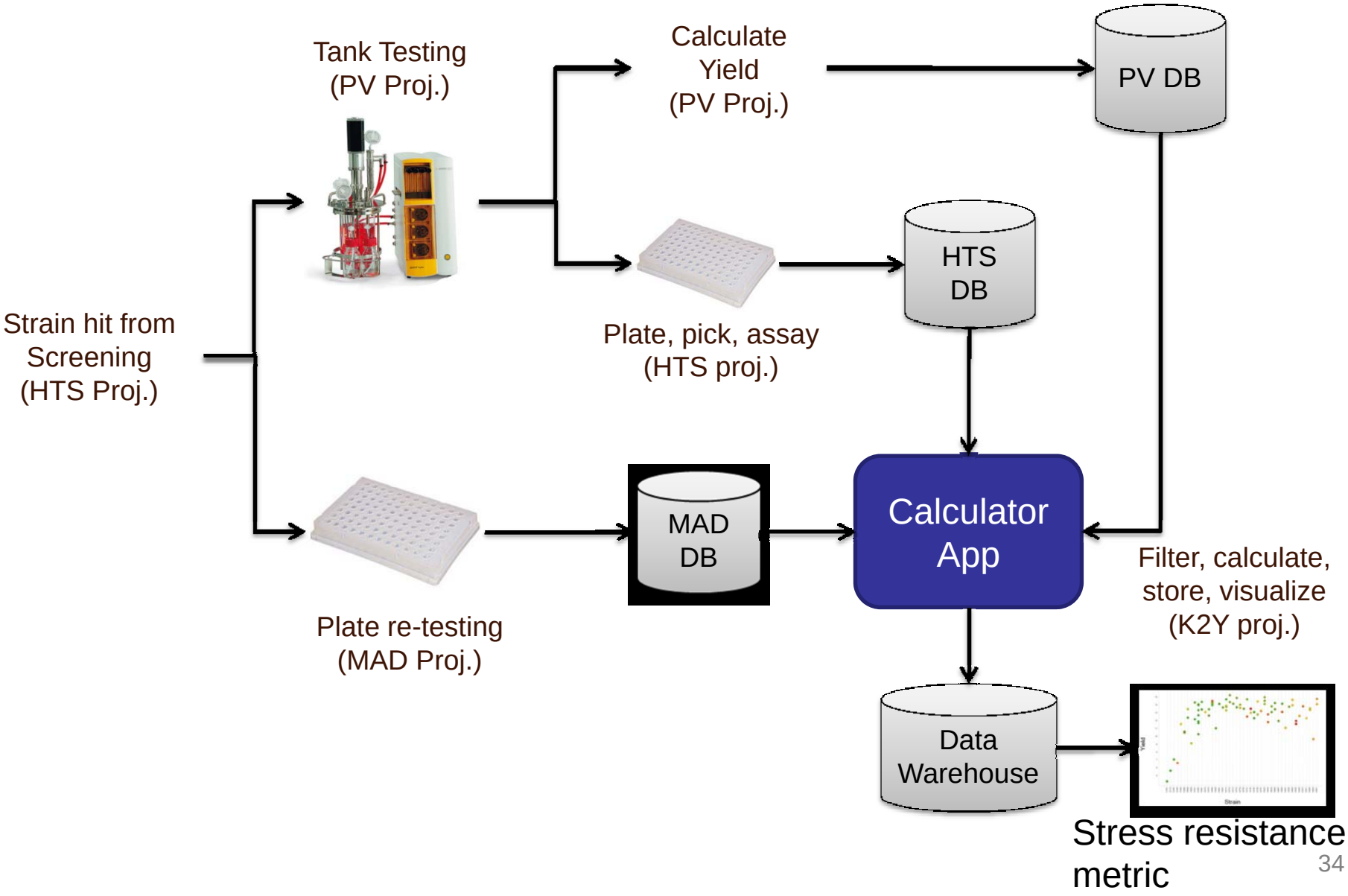


Multivariate optimization – picking winners

Informatics integration is critical to good decisions (get data out of silos)



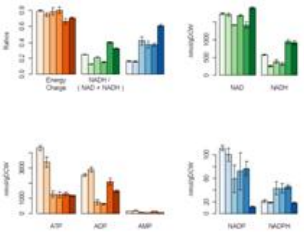
Informatics integration enables assessment of stress resistance



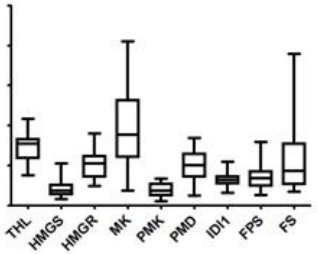
Let the data guide

Use empirical data mining to guide library construction, screening conditions, process dev.

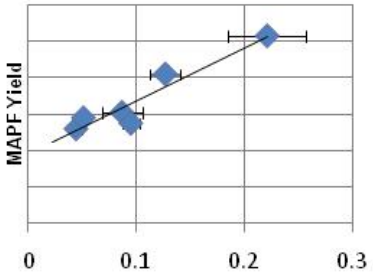
Energy Charge



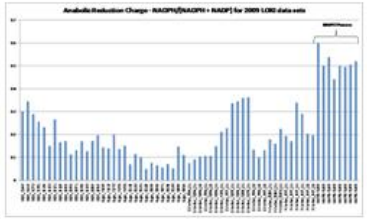
MRM Analysis



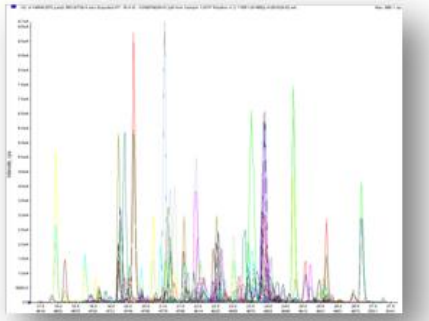
Flux Leader Board



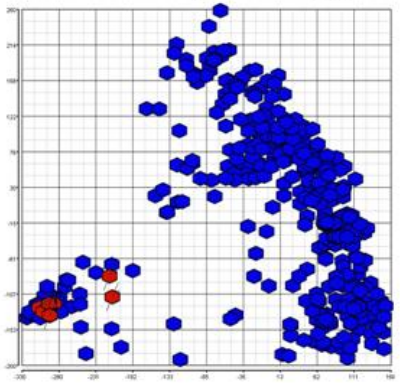
Metabolite Monitoring



Proteomic Analysis



T-Omic Clustering of Physiology



Conclusions

- **Rational engineering gets the ball rolling**
- **Industrialization enables rapid strain optimization**
  - Harnessing nature's way of "thinking": randomness and diversity
  - We are doing in 4 years what used to take 12
- **Continuous process improvement is critical to the success of an industrial platform**
  - Informatics is fundamental
  - Data mining & omics is fundamental

AMYRIS

Thanks to the >200 folks in R&D contributing to our success