SESSION 17: RENEWABLE FUELS, CHEMICALS, AND BIO-BASED PRODUCTS IV: COMMERCIALIZATION AND ECONOMICS

SBFC Symposium on Biotechnology for Fuels and Chemicals

Joel Cherry - Amyris synthetic biology: purposeful, predictable, profitable

AMYRIS

Joel Cherry leads Amyris research and development, including various ongoing grants and collaborations. Last year, Amyris was awarded a Presidential Green Chemistry award for work on farnesane, the Amyris diesel and jet fuel. Prior to joining Amyris in 2008, Dr. Cherry was Senior Director of bioenergy biotechnology at Novozymes. During his tenure at Novozymes, he was principal investigator of the BioEnergy Project, an effort funded by the U.S. Department of Energy to reduce the cost of enzymes used in converting biomass to sugar. This work was awarded an R&D 100 Award, Scientific American Top 50 Award, and a Frost and Sullivan Emerging Technology Award. Dr. Cherry is an inventor on more than 25 issued patents, and author of three book chapters and more than 30 scientific publications. He received his B.A. in chemistry from Carleton College, and his Ph.D. in biochemistry from the University of New Hampshire focusing on transcriptional control in yeast.

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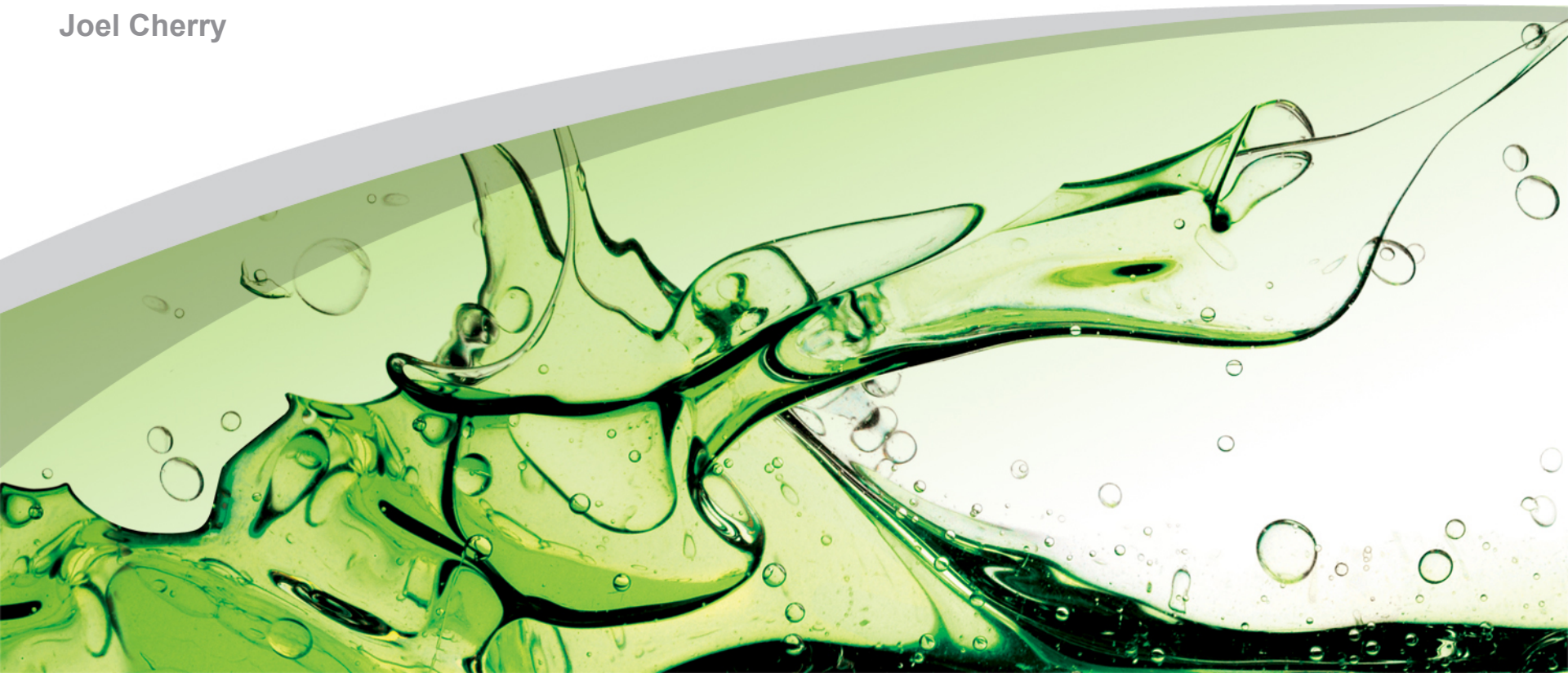
Amyris Synthetic Biology: purposeful, predictable, profitable

37th Symposium on Biotechnology for Fuels and Chemicals
San Diego, CA 30 April 2015

Joel Cherry



Cautionary Note re: Forward Looking Statements

This presentation and oral statements accompanying this presentation contain forward-looking statements, and any statements other than statements of historical facts could be deemed to be forward-looking statements. These forward-looking statements include, among other things, statements regarding numbers of malaria treatments to be manufactured in 2015, sizes of markets that may be addressed by current and potential products of Amyris, and development and introduction of potential new Amyris products, that involve risks and uncertainties. These statements and other forward-looking statements that may be provided in the presentation and/or oral statements accompanying it are based on management's estimates and current expectations and actual results and future events may differ materially due to changes in Amyris' business and various risks and uncertainties, including those associated with any delays or failures in development, production and commercialization of products, liquidity and ability to fund capital expenditures, Amyris' reliance on third parties to achieve its goals, and other risks detailed in the "Risk Factors" section of Amyris' annual report on Form 10-K filed with the SEC on March 31, 2015. Amyris disclaims any obligation to update information contained in these forward-looking statements whether as a result of new information, future events, or otherwise.

Amyris Overview: Purposeful

- ✓ Mission: Apply inspired science to deliver sustainable solutions for a growing world.
- ✓ Proven technology, multiple molecules

2014 PRESIDENTIAL GREEN CHEMISTRY AWARD WINNER

KEY COMPANY HIGHLIGHTS

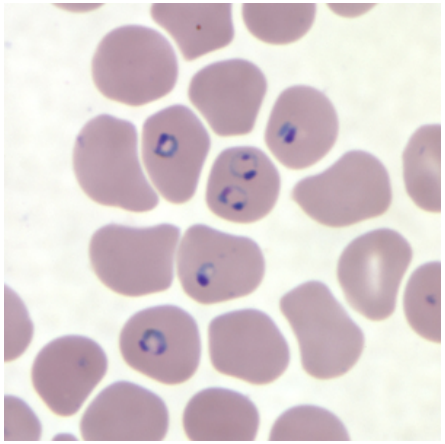
- Founded in 2003 by post-doctoral fellows from the University of California, Berkeley.
- Headquartered in the San Francisco Bay Area and with operations in Brazil
- 404 full-time employees (>25% of US employees are PhDs)
- 317 issued patents and 325 pending applications

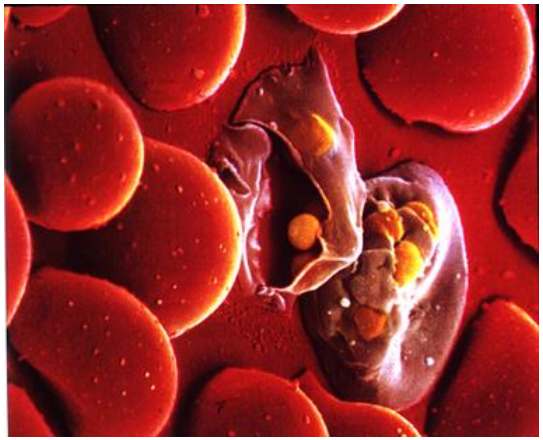
LEADING INVESTORS

TOTAL · TEMASEK HOLDINGS · KPCB · Fidelity INVESTMENTS · BIOLDING investment · TPG BIOTECH · khosla ventures · naxos · AMYRIS

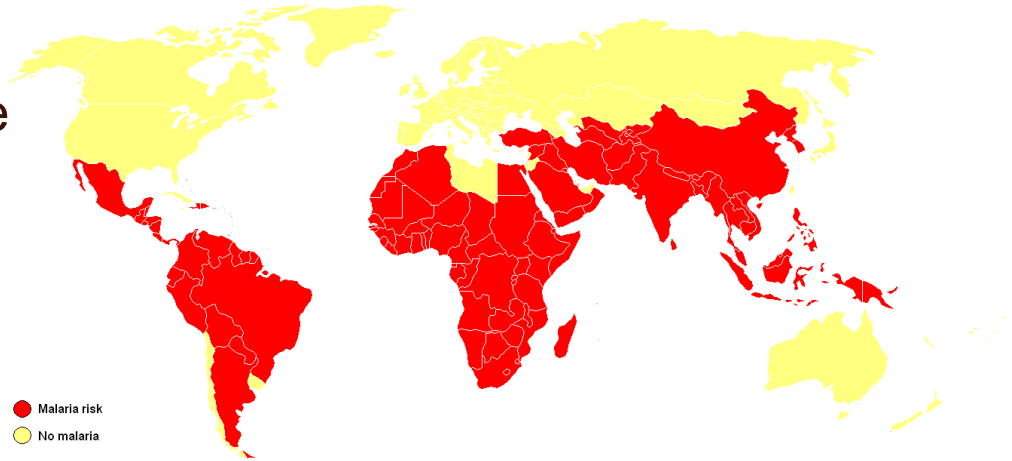
Malaria: a curable but devastating disease

Female Anopheles Mosquito


Plasmodium falciparum


Infected Red Blood Cells

- Affects 109 Countries Worldwide
- 216 Million cases in 2010
- ~665,000 deaths in 2010
- 1 child dies every minute of Malaria in Africa



Source: 2010 WHO malaria fact sheet

Treating Malaria : Lowering cost and stabilizing supply of ACTs

Treating malaria would require:
189 to 327 million ACT treatments per year

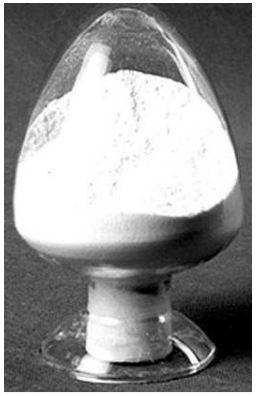
Artemisinin needed:
150 to 200 tons of artemisinin per year

- 2-3X increase in production
- Decrease/stabilization in price
- Elimination of stock-outs

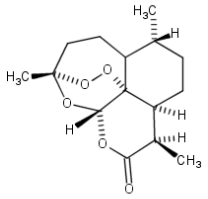
The Artemisinin Project:
Replacing inconsistent plant sources with reliable yeast production

A. annua cultivation
(10-12 month growing cycle)

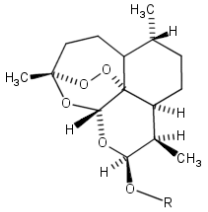
Artemisinin extraction



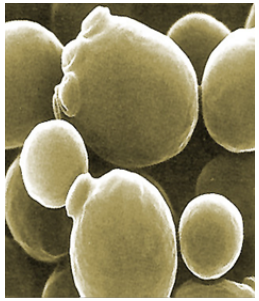
Artemisinin and Several of its Semisynthetic Derivatives



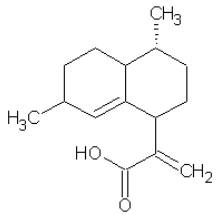
Artemisinin



Derivative	R
Artemether	methyl
Arteether	ethyl
Artesunate	-O-CO-CH$_2$CH$_2$-COO$^-$
Artelinic Acid	-O-CH$_2$-(p-phenyl)-COOH



Fermentation production
(2 week cycle)



Artemisinic acid

Artemisinic Acid

4-Step Chemical Synthesis Steps

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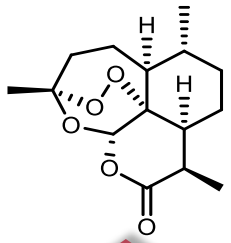
A Synthetic Biology Success Story Circa 2009:
Artemisinic acid from *S. cerevisiae* at >25g/L

Artemisinin!



Sugar

S. cerevisiae

IPP (C_5)

GPP (C_{10})

FPP (C_{15})

Acetyl-CoA

Isoprenoid Pathway

ERG9

Ergosterol

Amorphadiene

ADS

5 *A. annua* genes

4-Step Chemistry

Artemisinic Acid

22 genetic modifications led to titers of > 25 g/L artemisinic acid

Westfall *et al.*, PNAS. 2012 Jan 17

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Artemisinin in production

Sanofi is manufacturing with Amyris's yeast strain:

- **2014: >120 million treatments produced**
- **IMPACT: > 100,000 lives saved**

Nature. 2013 Apr 25;496(7446):528-32. doi: 10.1038/nature12051. Epub 2013 Apr 10.

High-level semi-synthetic production of the potent antimalarial artemisinin.

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Sanofi launches malaria drug production

17 April 2013 Mark Peplow

Like 84 Tweet 47 +1 1

Erratic supplies of a critical chemical have long denied millions of people in the developing world the malaria therapies that could save their lives. Now an effort to create a more reliable source is finally bearing fruit.

The malaria parasite carried by mosquitoes kills millions every year

On 11 April, the Paris-based pharmaceutical company Sanofi **officially launched a new production facility in Garessio, Italy**, to make **artemisinin** – the precursor to artemisinin-based combination therapies (ACTs), the most effective drugs against the deadliest malaria parasite.

Until now, the only source of artemisinin has been the sweet wormwood plant mostly grown in China and Vietnam. But variable harvests and a production cycle lasting at least 14 months have created a volatile market where prices and availability fluctuate wildly. 'Not everyone could get the drugs they needed,' says **Ponni Subbiah**, a drug-development programme leader at PATH, a global health organisation based in the US.

Sanofi expects to make about 35 tonnes of artemisinin this year, and 50–60 tonnes next year, which will meet about one-third of the global need. It will initially cost $350-400 (£230 –260) per kilogram, roughly the same as the botanical source. Pending final approval from the World Health Organization, drugs made this way should be on pharmacy shelves around the end of this year, Subbiah reckons. 'Having a more stable supply means that more people will get the drugs,' she says.

Science NOW — UP TO THE MINUTE NEWS FROM SCIENCE

Malaria Drugmakers See the Light

by Kai Kupferschmidt on 18 January 2012, 1:37 PM | 0 Comments

Email Print | +1 4 More PREVIOUS ARTICLE NEXT ARTICLE

Artemisinin, a crucial drug in the global fight against malaria, could soon become cheaper and easier to make, thanks to researchers who have found a better way to synthesize the compound. "The impact of this is hard to overestimate," says Jack Newman, an industrial chemist at Amyris Biotechnologies in Emeryville, California, who was not involved in the work. According to the World Health Organization (WHO) 655,000 people died of malaria in 2010, "and while there is a cure," Newman says, "the supply chain to make artemisinin has been a huge problem."

ENLARGE IMAGE

Bottled nature. Production of the antimalarial drug artemisinin (*inset*) still involves planting sweet wormwood and extracting the compound.

Credit: Jorge Ferreira

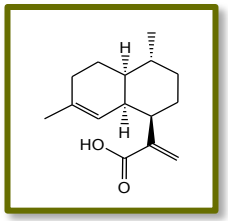
Artemisinin is naturally produced by a plant called sweet wormwood (*Artemisia annua*), which has been used for centuries in traditional Chinese medicine. In 1972, pharmaceutical scientist Tu Youyou, as part of a project for the Chinese government, identified the active compound, a discovery for which she was honored with the Lasker-DeBakey Clinical Medical Research Award last year. Since 2001, WHO has recommended that so-called artemisinin-based combination therapies (ACTs)—in which artemisinin is combined with another drug—replace older, ineffective drugs worldwide. These combinations have become a cornerstone of malaria control and are believed to have saved many lives.

The same isoprenoid platform can make 1000's of products

There are >30,000 different isoprenoids in nature

Sugar Syrup

Artemisinic Acid



Anti-Malarial Drug

p450

CPR

ADS

YEAST CELL

Mevalonate Pathway

Diesel, Jet & Chemical Precursor

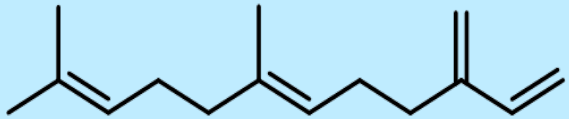
Farnesene Synthase

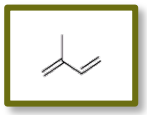
GPPS

IS

C10 Synthase

β-Farnesene ("fene")





Isoprene

Fragrance Precursors

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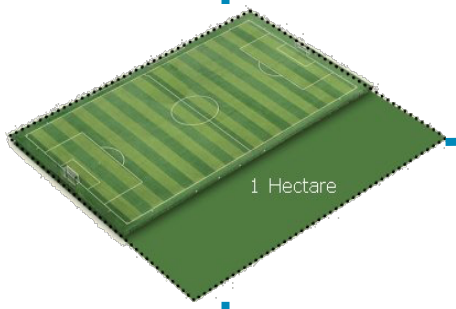
Efficient Use of Available Land

Comparison to other renewable alternatives in Brazil



Soybean Biodiesel

468 Liters/year

900 <->1000 km

Ethanol w/ Ignition improver

7.430 Liters/year

10.120 Liters/year

5.900 <-> 7.800 km

8.000 <-> 10.600km

Amyris Diesel

4.180 liters/year

5.684 Liters/year

7600<->9.500 km

10.300<-> 12.900km

With cellulosic technology

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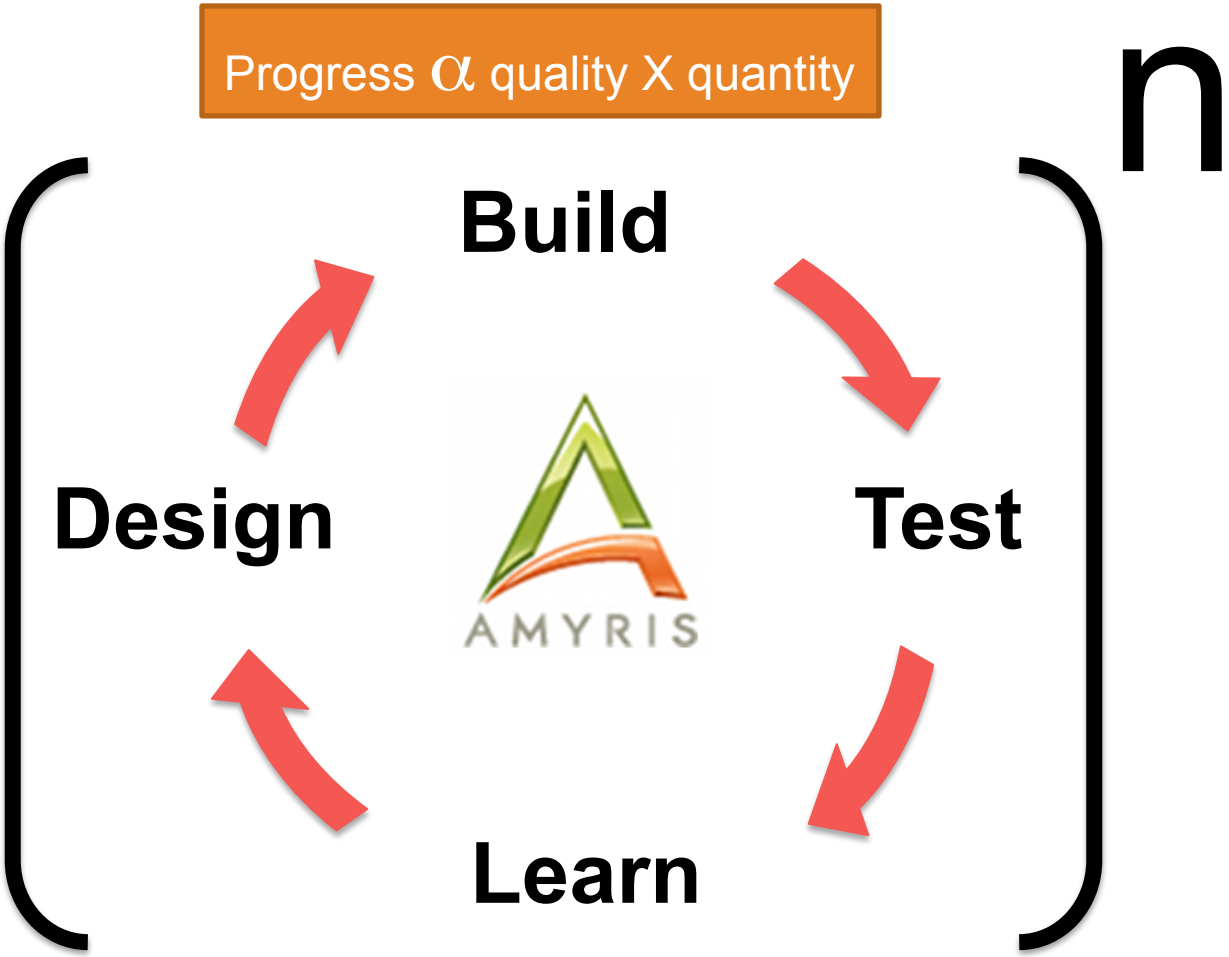
Synthetic biology can address the primary challenge:
We need to improve the engineering cycle to learn how to make better designs



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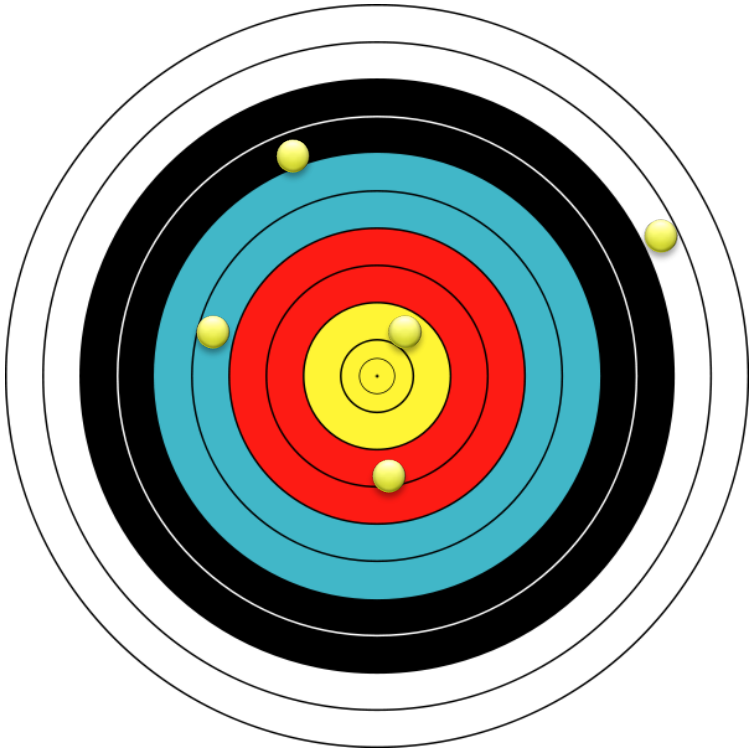
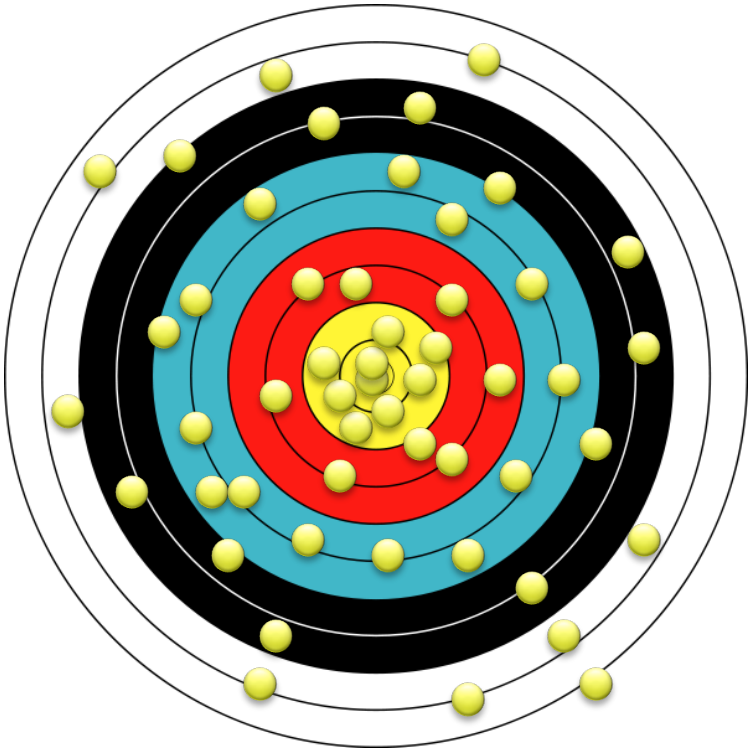
Predictable

We needed a platform technology to increase quantity *and* quality to speed development

Progress $\propto$ Number of attempts X Quality of attempts



Traditional Approaches



Amyris' Approach

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We needed a platform technology to increase quantity *and* quality to speed development

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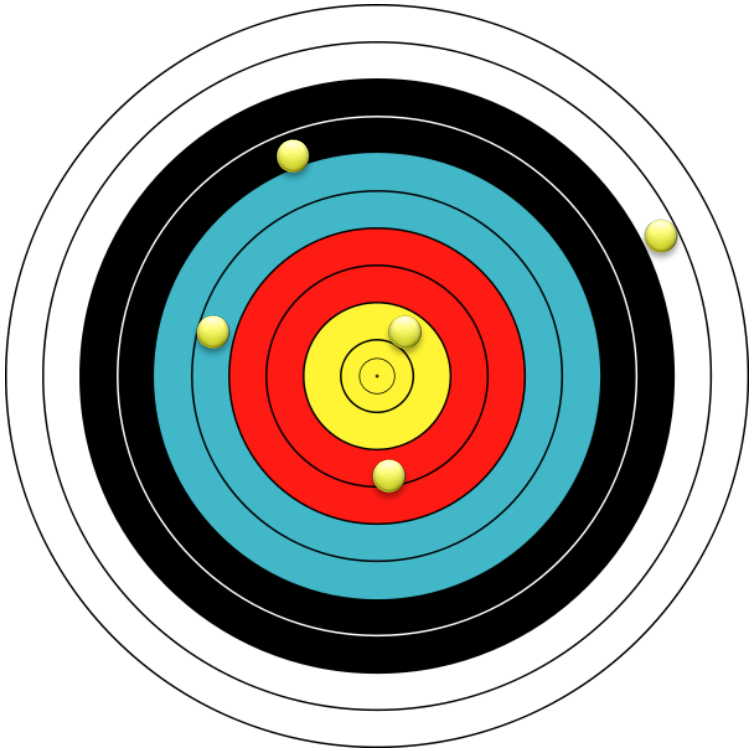
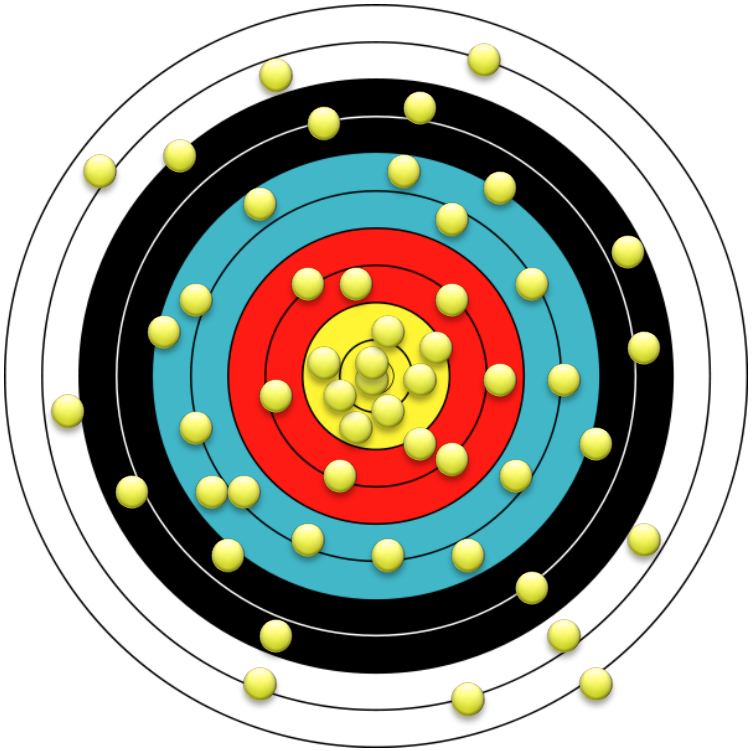
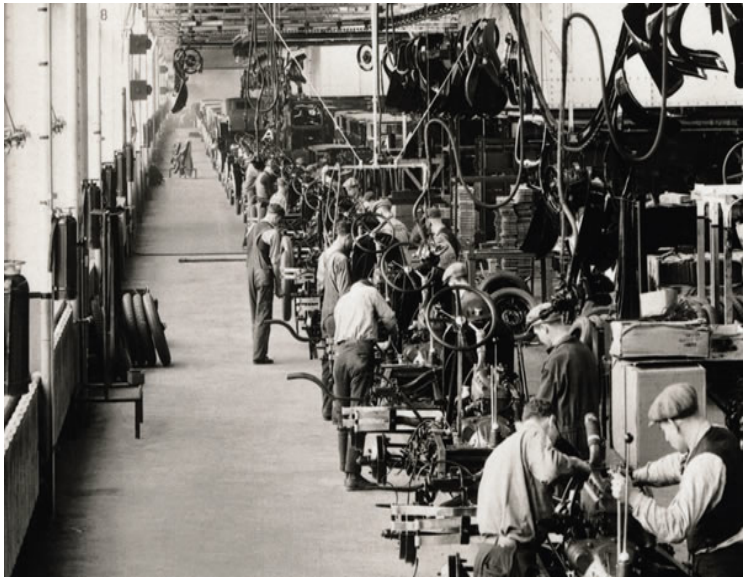


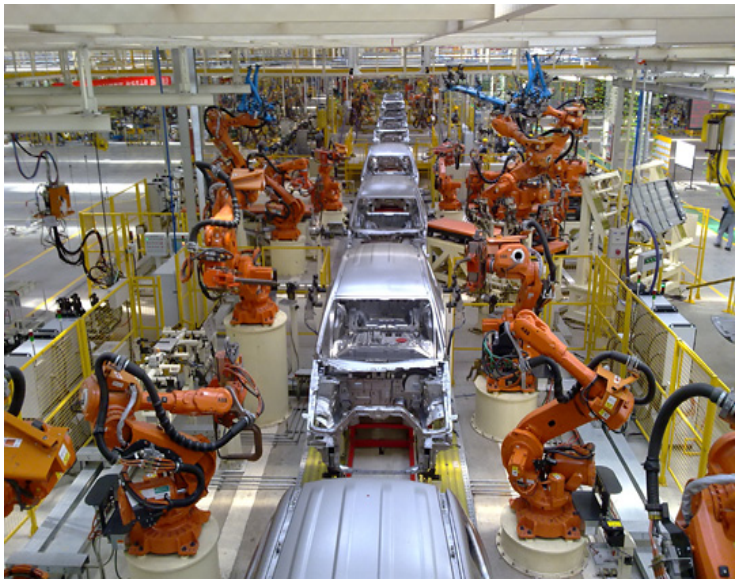
Traditional Approaches



Amyris' Approach

We learn from other industries





1920 → 2010

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Standardization is the key to efficiency

Parts Standardization

Genetic elements that can be easily interchanged using the same or similar tools

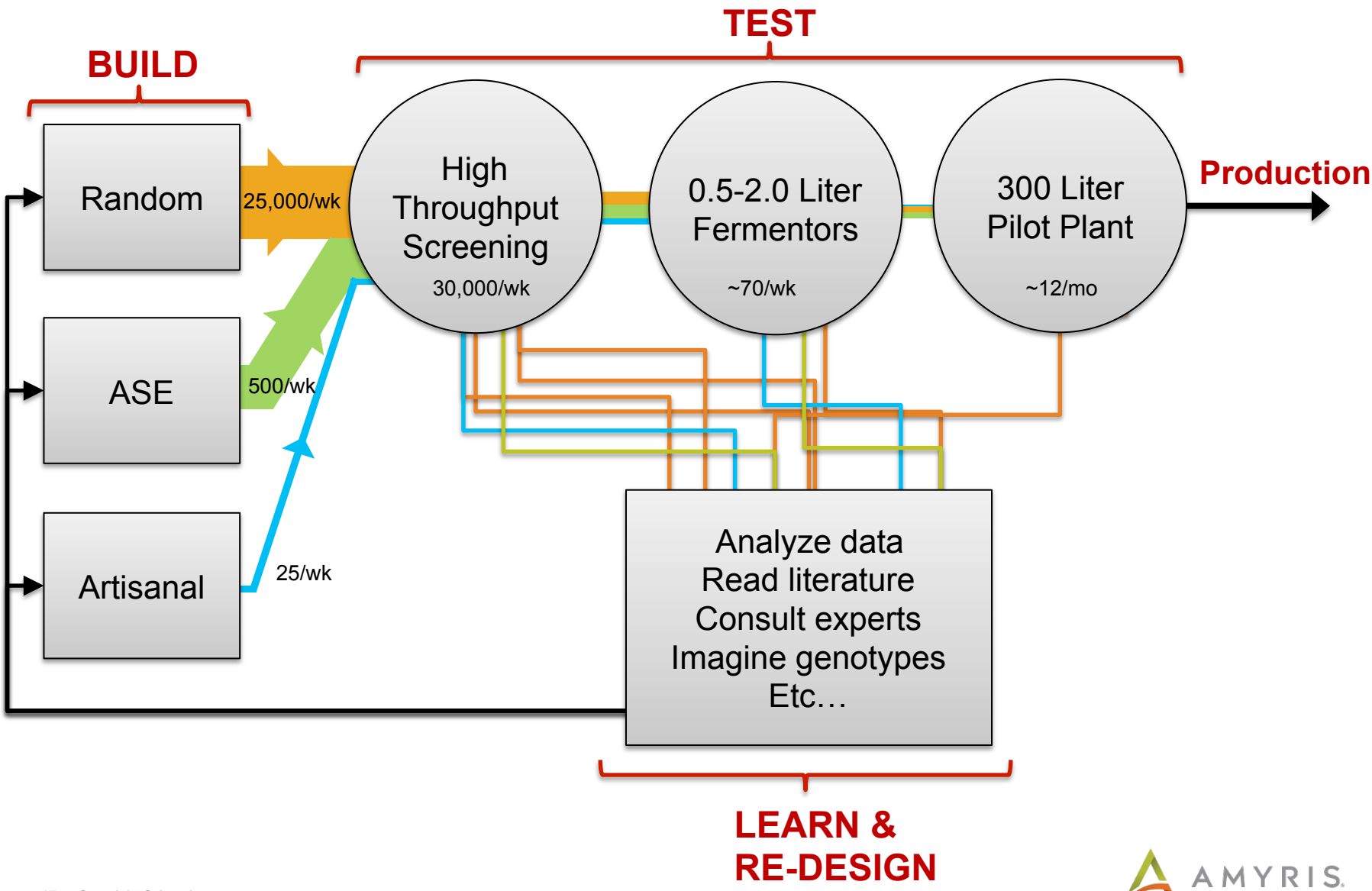
Tool Standardization

Consistent, simple and reliable enzyme and/or chemical treatments for the isolation/manipulation of genetic elements

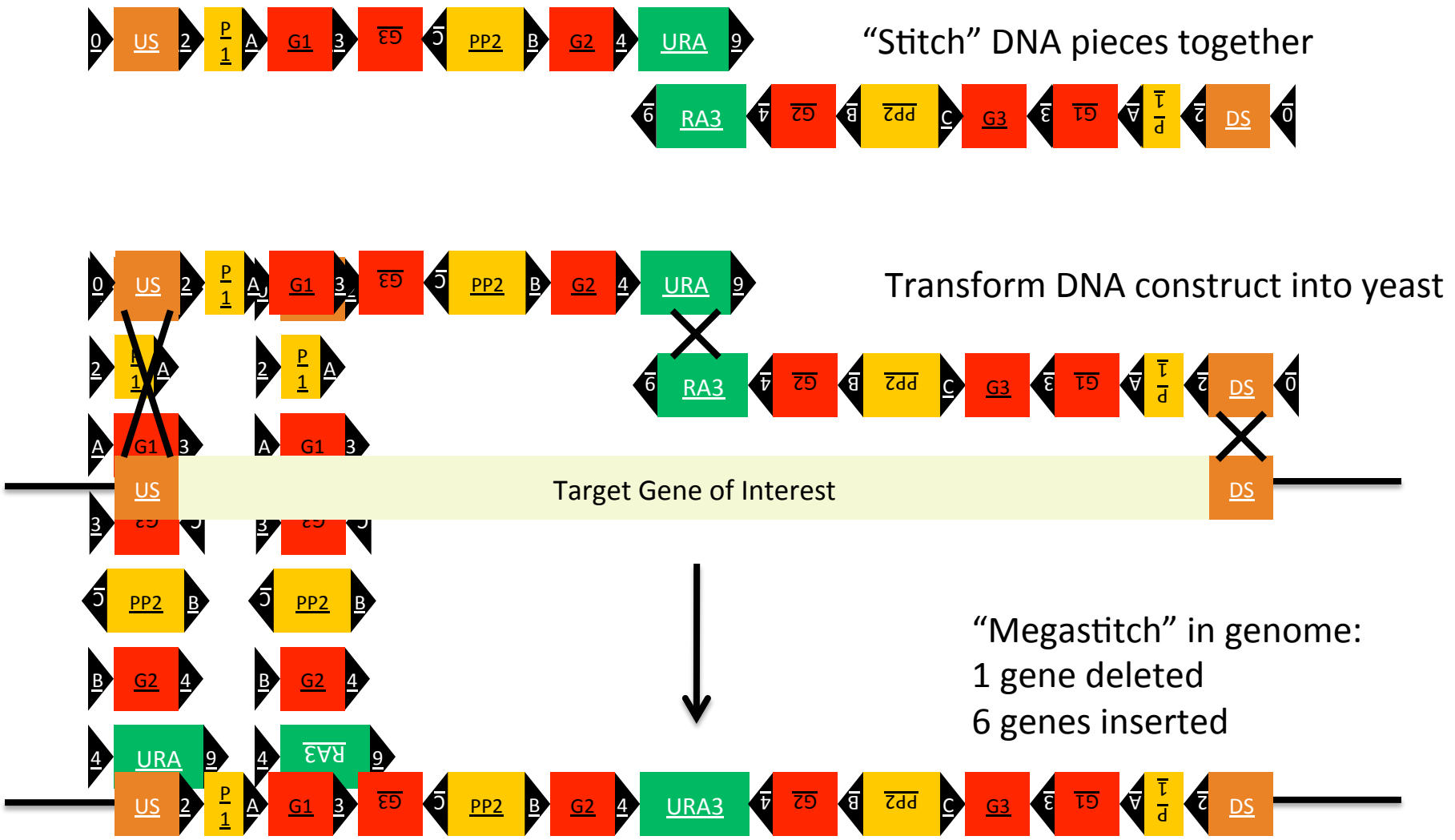
Process Standardization

Consistent, simple and reliable methods for the insertion and deletion of genetic elements

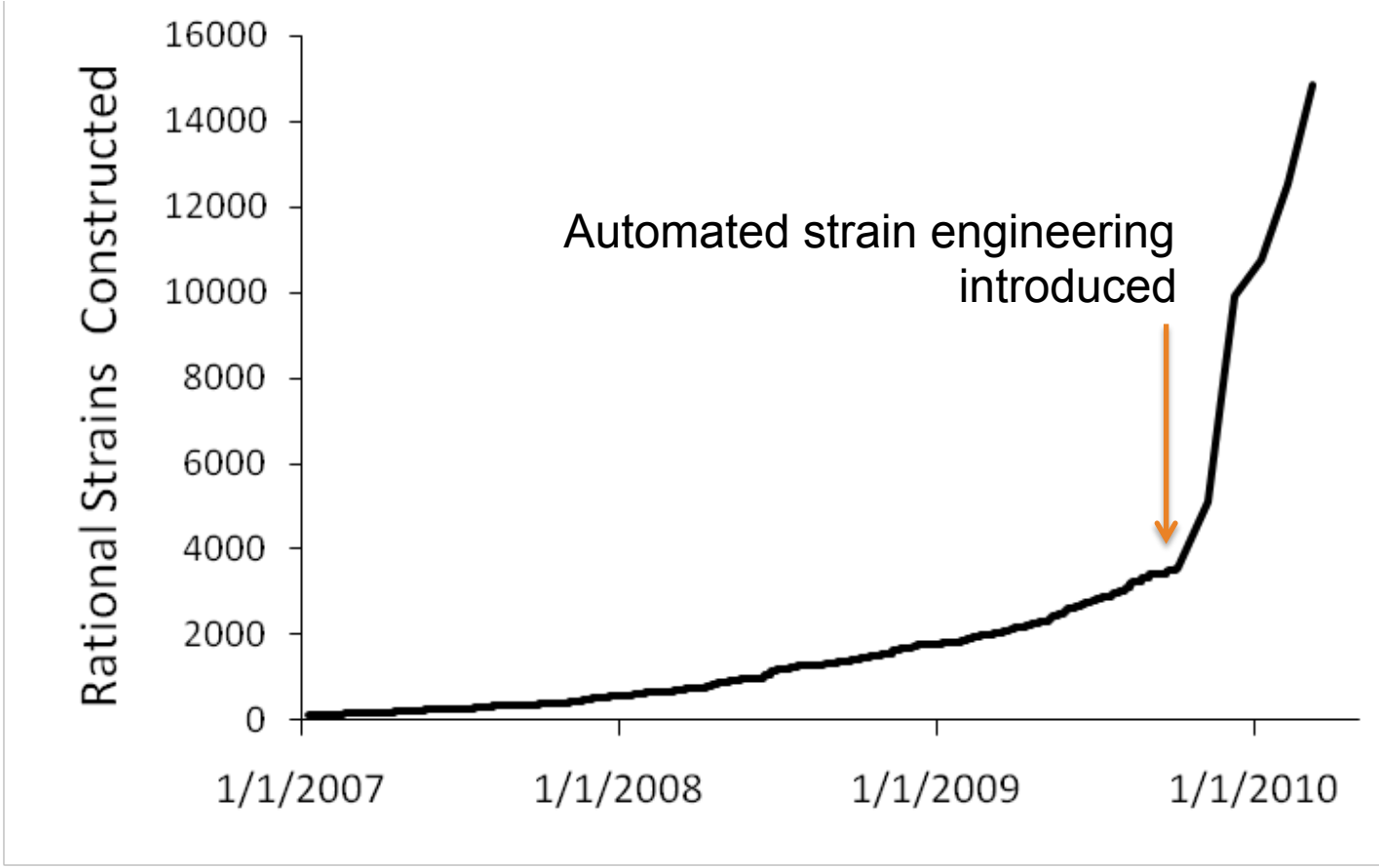
Design-Build-Test-Learn applied to biology



Build: An example of a common DNA design

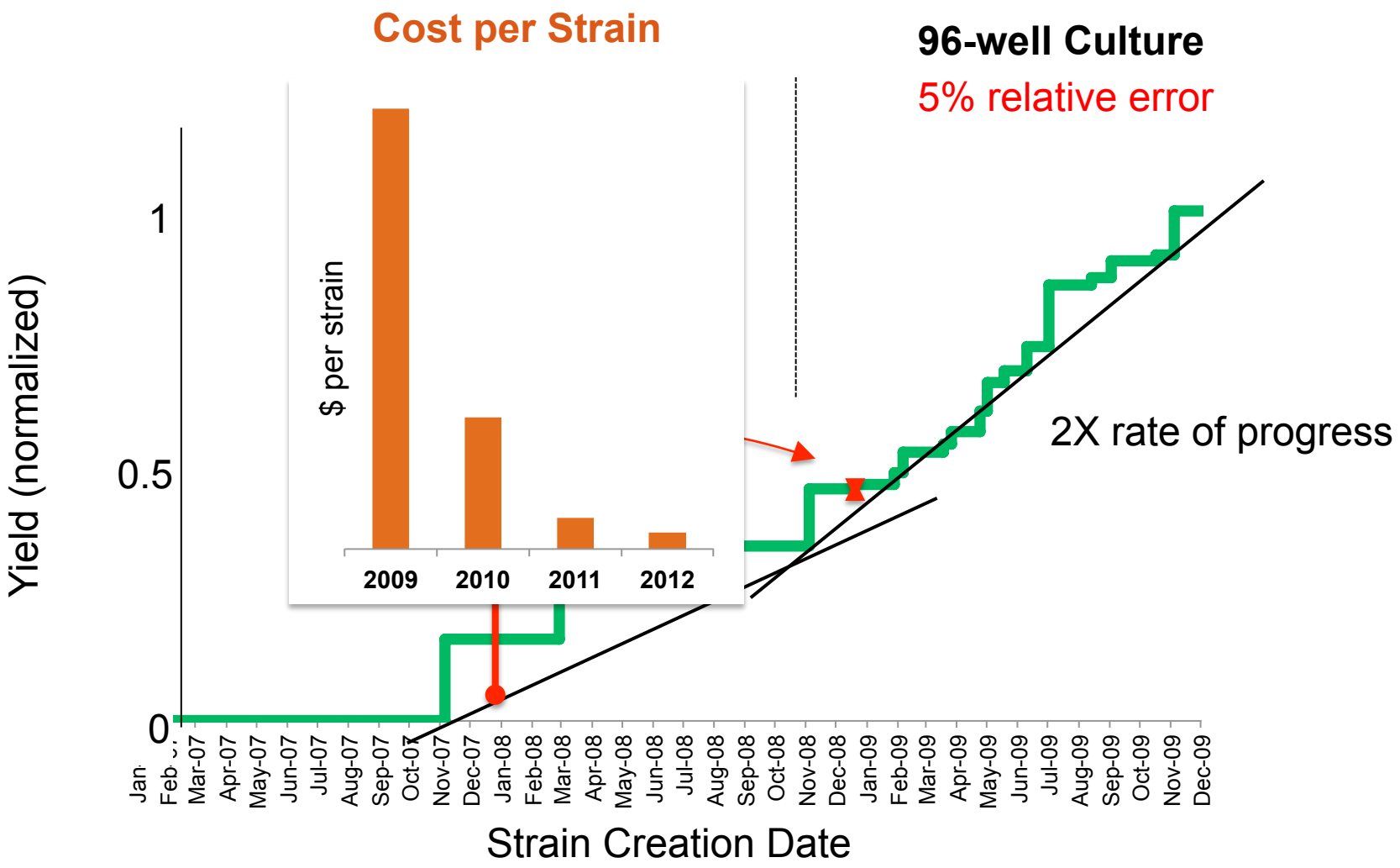


One measure of ASE's contribution

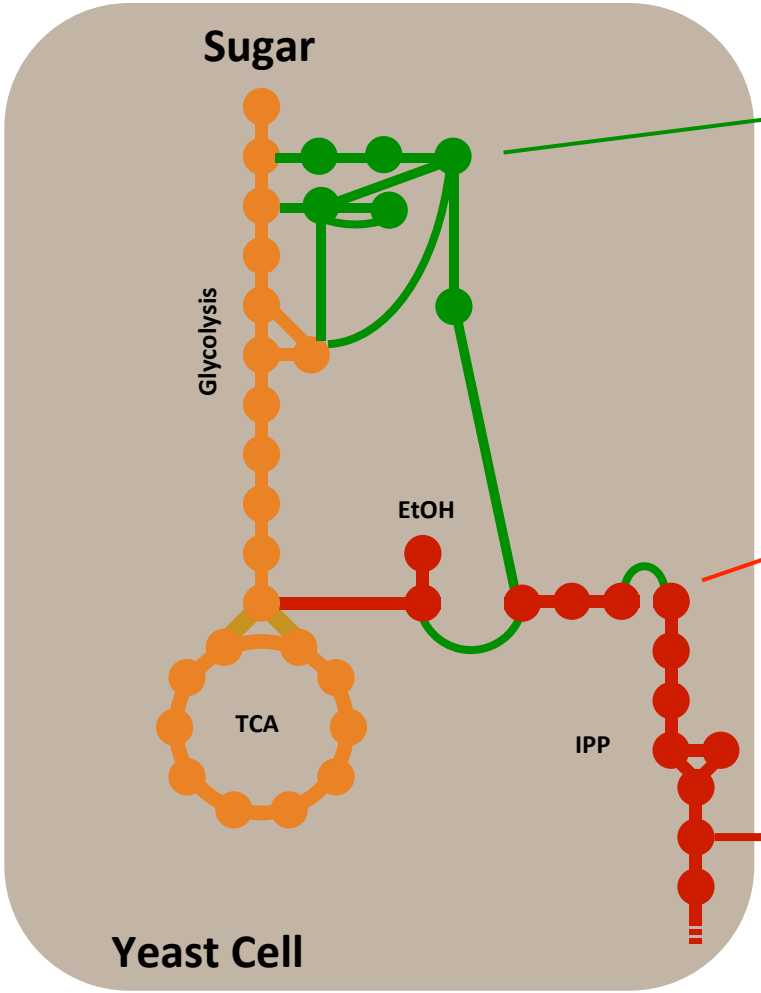


Currently running 81st cycle of ASE!

Predictable, part 1:
Impact of 100 strains/wk to 30,000 strains/wk



Design-Build-Test-Learn:
2nd generation pathway enables high yields in isoprenoid production



Amyris Gen 2 Pathway
29.8% max yield potential

Four novel enzymes required for Gen 2

- PK
- PTA
- ADA
- HMGR

Amyris Gen 1 Pathway
23.4% max yield potential

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Models show higher theoretical maximums

GLUCOSE

Central metabolism	Maximum theoretical yield from glucose g/g	Maximum theoretical yield from O_2 mol/mol	Maximum theoretical productivity g/L/hr
Native	23.8%	13.2%	2.7
Synthetic	29.8%	59.0%	10.8

25% increase
chemical limit is
32.5% mass yield

4x increase!

Acetyl-phos

PK

PTA

AcO

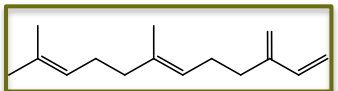
ADA

Acetyl-CoA

NADH-HMGR

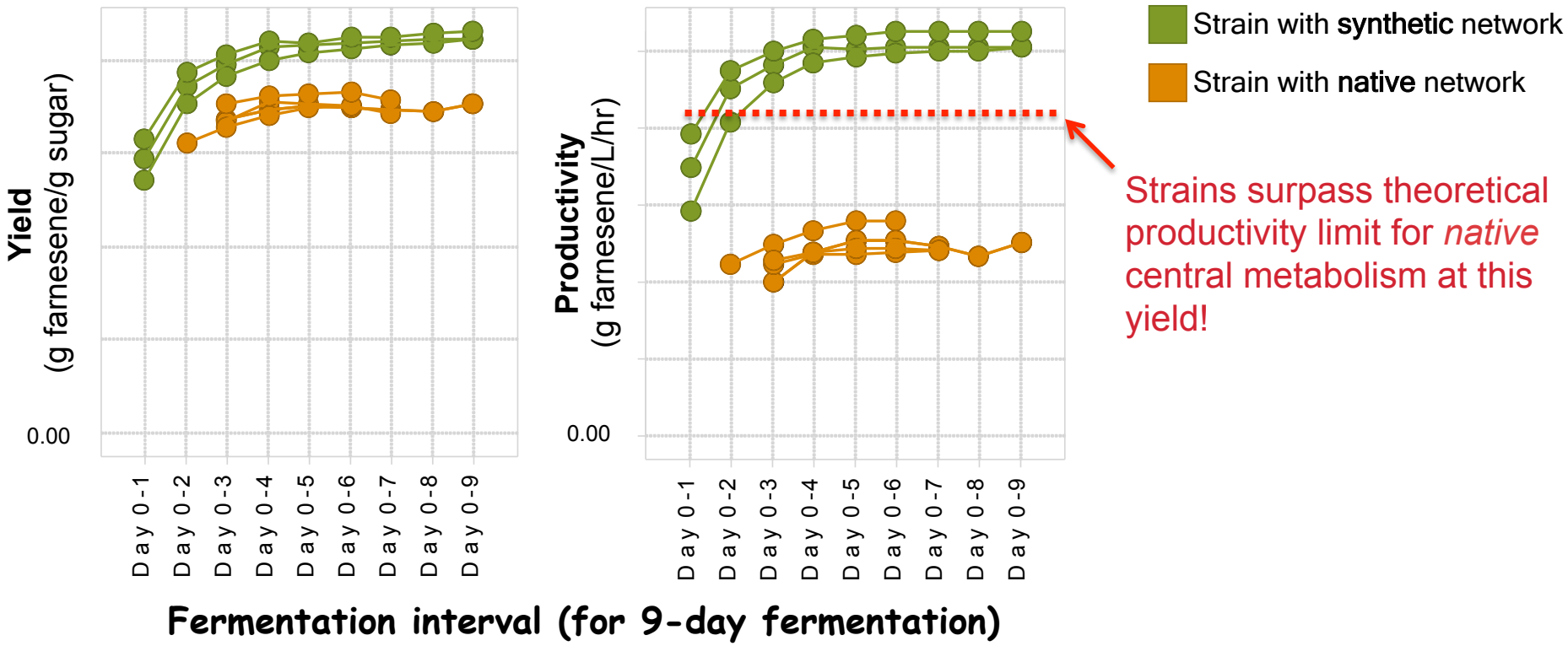
FPP

FS



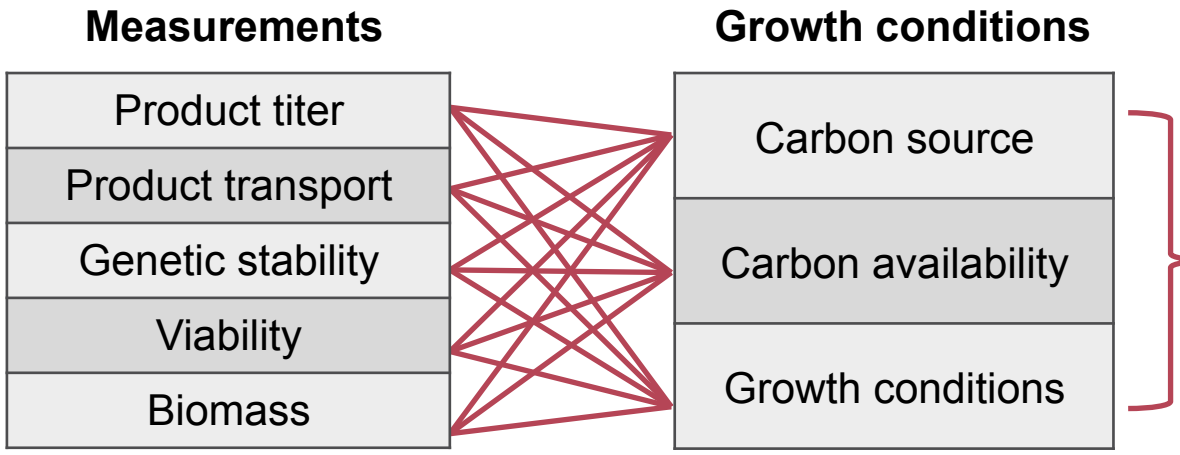
1 FARNESENE

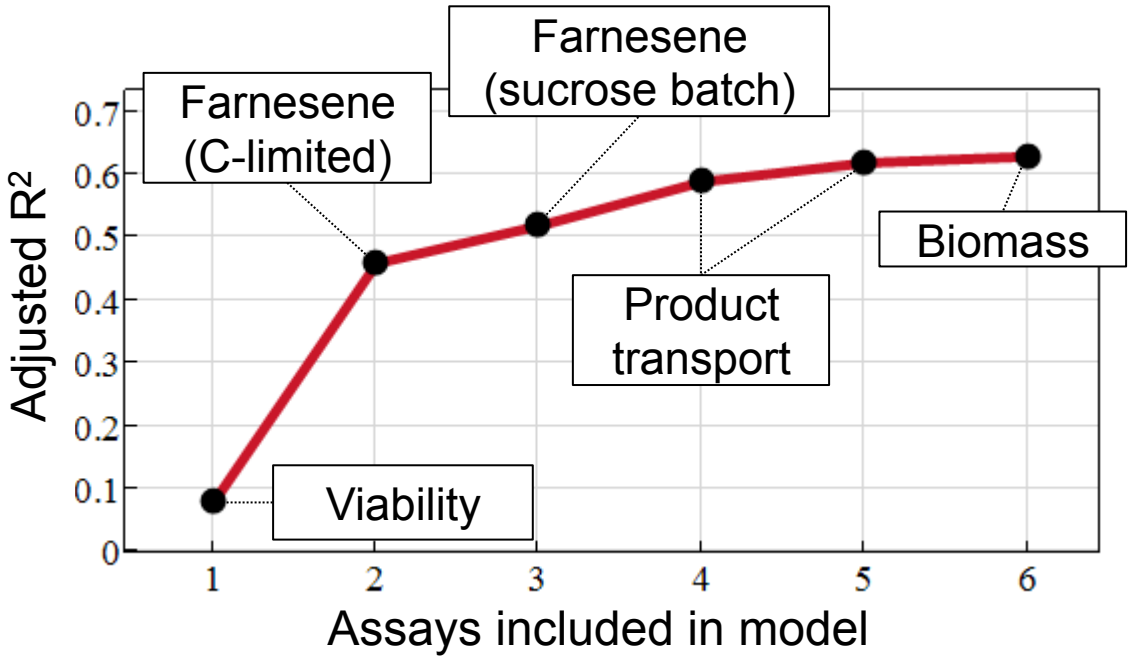
2nd Generation Farnesene strains in action



In addition to many clever and dedicated people, Automated Strain Engineering was critical to achieving this quickly.

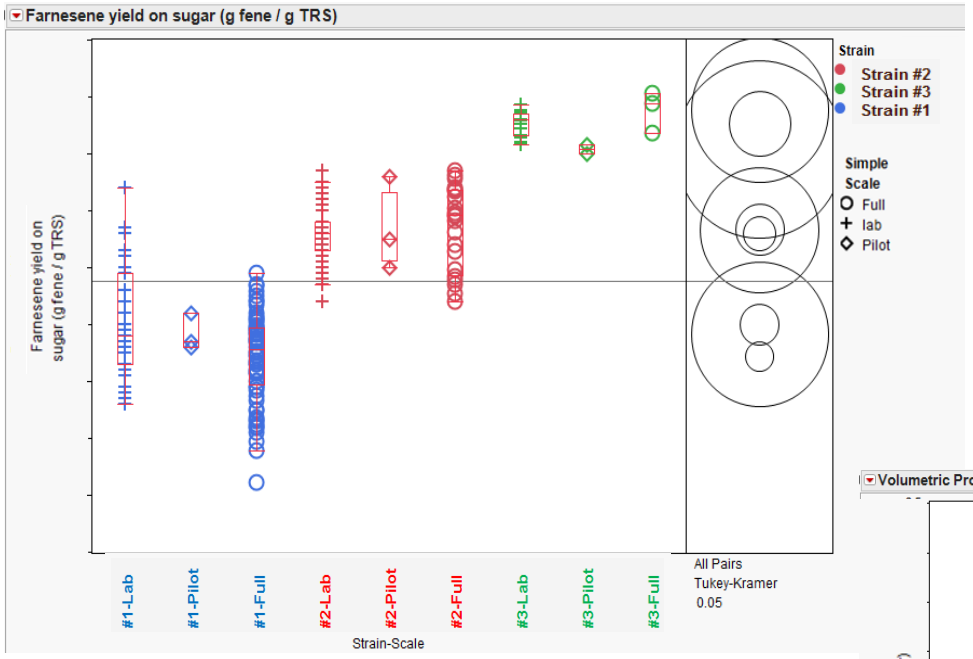
Predictable, part 2:
Chasing the correlation between small scale testing and full-scale production





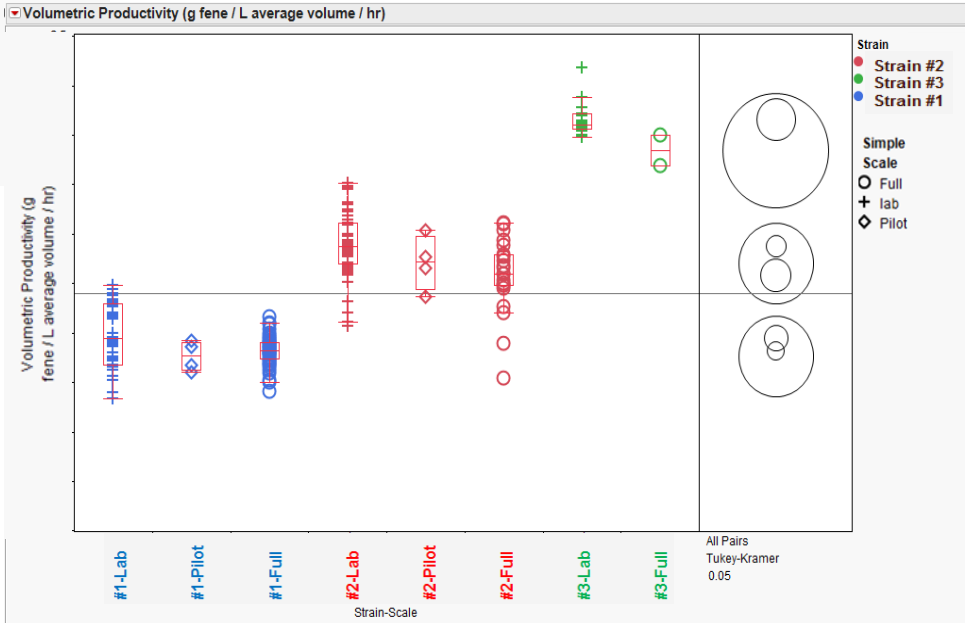
Combining models using multivariate methods identified sets of assays able to predict fermentor yield, productivity, etc.

Similar results upon scale-up to the 100-200m^3 fermentors



YIELD

PRODUCTIVITY



2L results remain predictive of 200 m^3 performance

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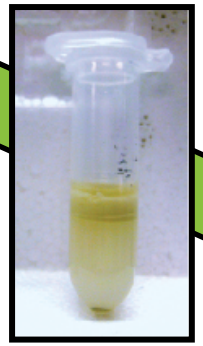
Farnesene: What did it take to get to manufacturing?

Spring 2007



Drop of farnesene collected from a 2L fermentor

~2010

Liters of fene

2011-12

Millions of liters of fene made at CMOs

January 2013
Amyris plant in Brazil
6 x 200,000L fermentors

Genotype of a recent fene-producing strain:

- **Removed 27,679 bps of DNA**
- **Added 81,994 bps of DNA**
- **Mutated ~400 genes (in random mutagenesis): 17 mutant genes are known to be beneficial**

Amyris Cane Diesel in Brasil
>2 million liters of product, >17 million miles

Sao Paulo Buses with Cane Diesel blend

Rio Volksbus running 100% Cane Diesel

Commercial Flights with Partner Airlines
GOL, Lufthansa, Air France and KLM

Commercial Pan America flight by GOL Linhas Aéreas Inteligentes on July 31, 2014

Boeing 737-800 powered by CFM engines

Route: Orlando, USA to São Paulo, Brazil

SPONSORS: AMYRIS. TOTAL BOEING BID Morgan Stanley

Commercial flight in Europe by Lufthansa on September 15, 2014

A320 powered by CFM engines

Route: Frankfurt to Berlin

Weekly Air France flights service since September 17, 2014

A321 powered by CFM engines

Route: Toulouse to Paris

Commercial trans-continental select flight service by KLM first on December 23, 2014

A330 powered by CFM engines

Route: Amsterdam, NL to San Francisco, USA

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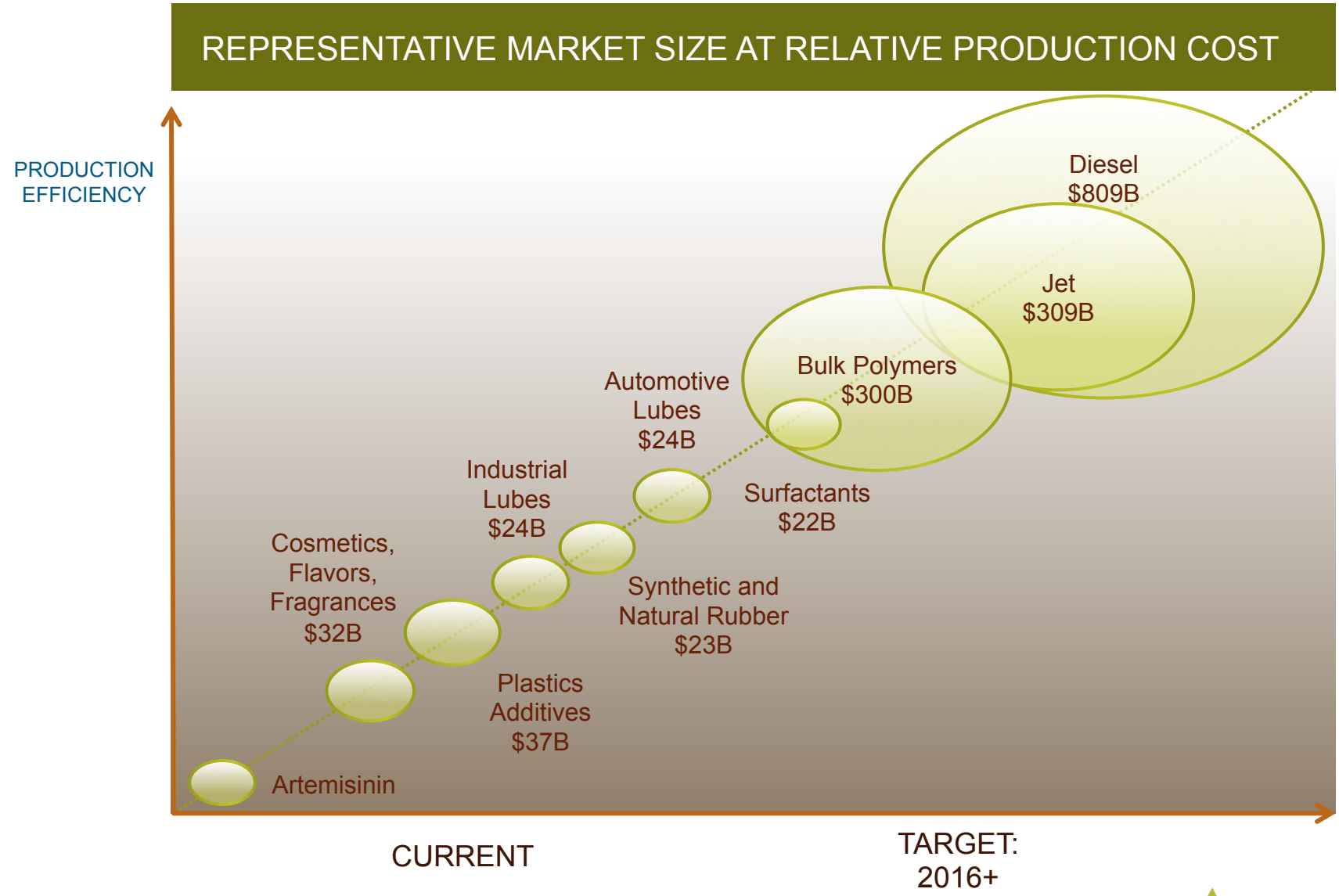
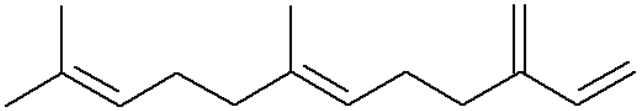
Technology Improvement Expands Addressable Markets



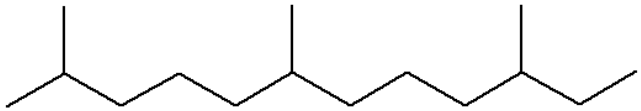
Farnesene: base molecule for many products

Farnesene (Fene)



+ 4 H_2 —catalyst→

Farnesane (Fane)



Diesel Fuel
Jet Fuel

Fermentation Product Made by Amyris-Engineered Yeast

Various chemical reactions

Emollients

Lubricants

Polymers

Fragrances

AMYRIS

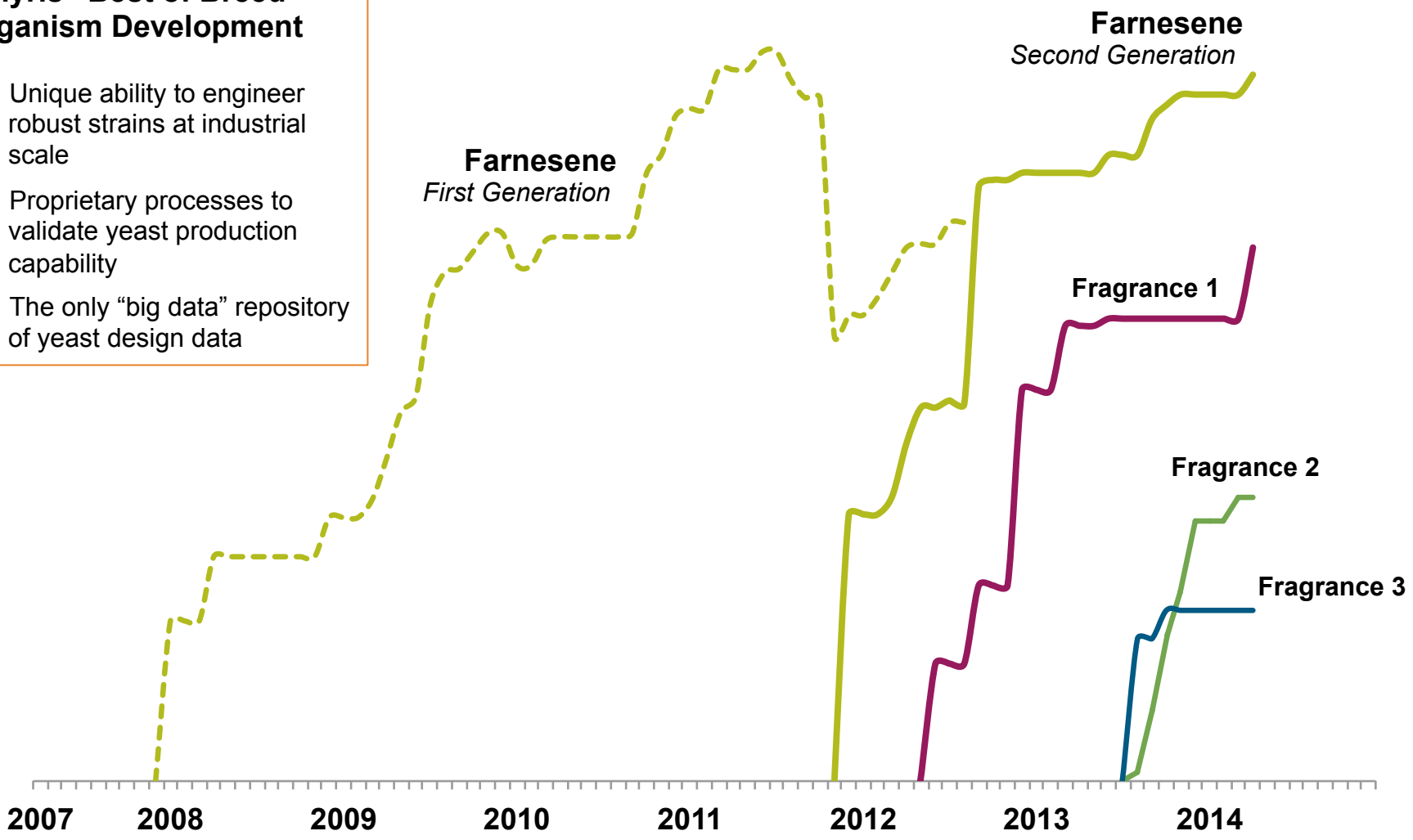
Direct to consumer…today!

First Consumer Product Launch

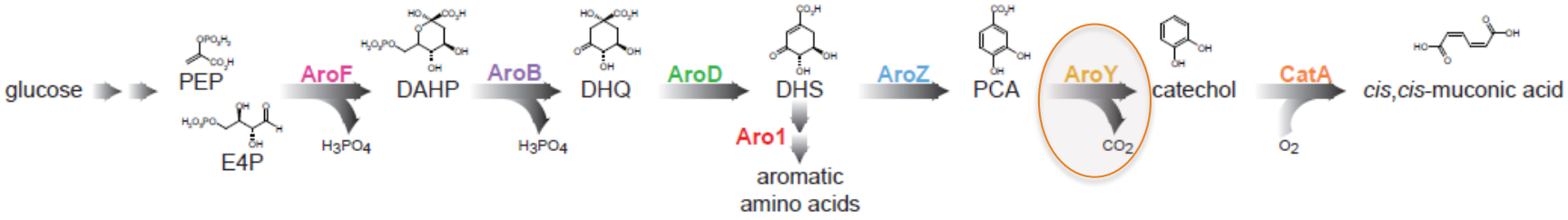
Historical Strain Development Performance

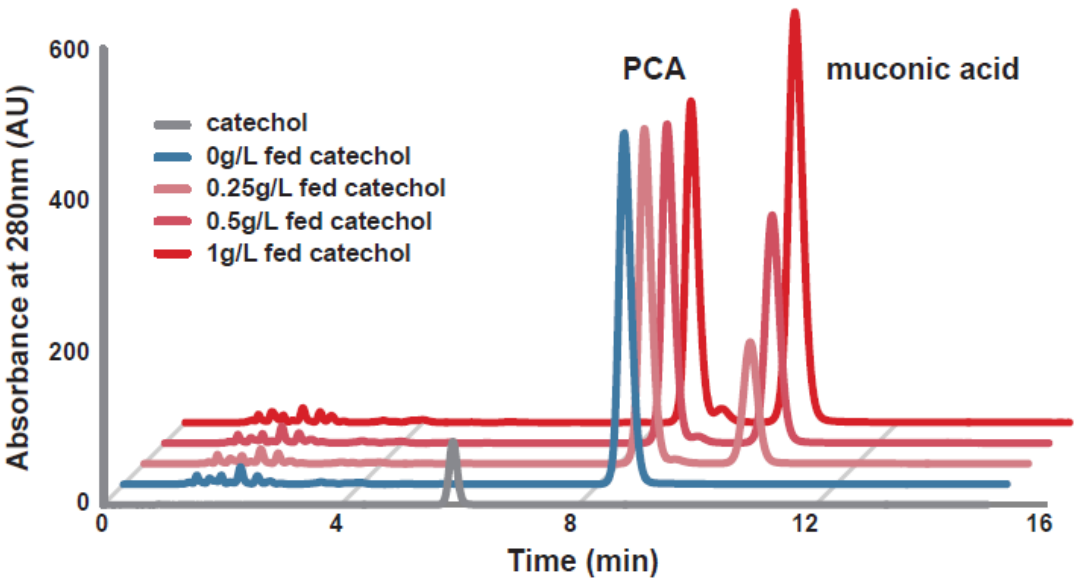
Amyris "Best of Breed" Organism Development

- Unique ability to engineer robust strains at industrial scale
- Proprietary processes to validate yeast production capability
- The only "big data" repository of yeast design data



Moving beyond terpenes





Cell Systems
Report

Efficient Multiplexed Integration of Synergistic Alleles and Metabolic Pathways in Yeasts via CRISPR-Cas

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11 genes
24 kb of DNA
a new pathway
in a single transformation!

AMYRIS

Summary

Purposeful
Our Mission
is to use synthetic biology
to solve big problems

Predictable
Our optimized terpene pathway
can produce thousands
of useful molecules

Profitable
Our technology
enables speed
to target molecule

thanks for listening

And thanks to all Amyrisians for making it real!