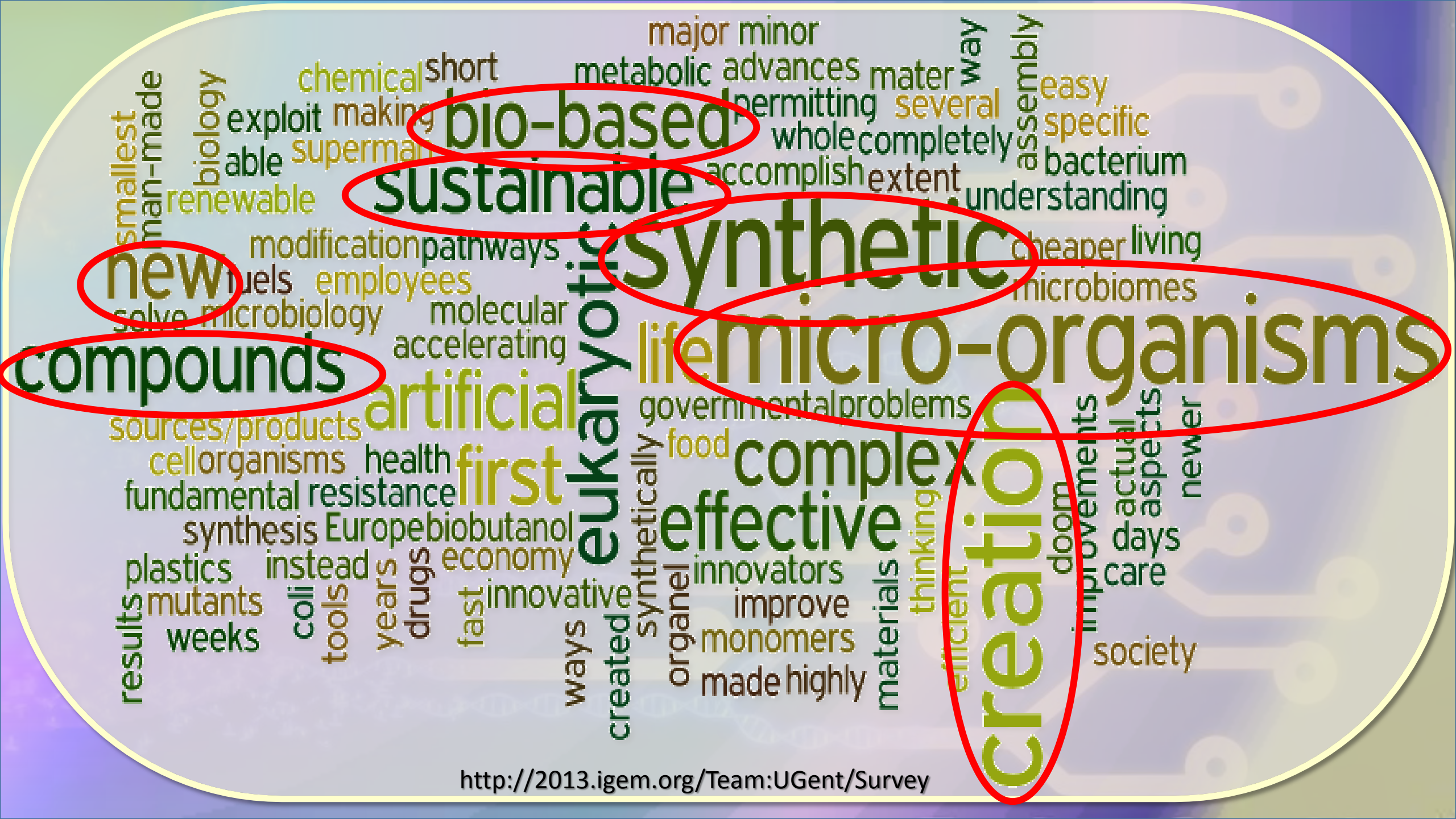
DECRYPTING SYNTHETIC BIOLOGY:
Engineering Microbes

AILEEN N. BAYOT-CUSTODIO, MSc

University Researcher II

National Institute of Molecular Biology and Biotechnology,
University of the Philippines Los Baños

November 26, 2015



http://2013.igem.org/Team:UGent/Survey

MANCHESTER 1824

The University of Manchester

March 1 – June 28, 2015

http://www.manchester.ac.uk/

Manchester Institute of Biotechnology

Discovery through innovation

http://www.mib.manchester.ac.uk/

SYNBIOCHEM

Manchester Synthetic Biology Research Centre
for Fine and Speciality Chemicals

ERIKO TAKANO

Professor of Synthetic Biology
SYNBIOCHEM Research Theme Director

Eriko's main field of interest is the synthetic biology of antibiotic production, including novel antibiotic discovery by post genomics, the systems biology of the metabolic switch from primary to secondary metabolism, and the regulation of antibiotic production through signaling molecules.

Current Research:

- Engineered micro-compartments for monoterpenoid production using synthetic biology
- Butyrolactone signaling circuits for synthetic biology
- Synthetic biology of polyketide biosynthesis
- Synthetic biology for antibiotic discovery and development

Outline:

- What is synthetic biology?
- Difference between SynBio and genetic engineering
- How SynBio was revolutionized: Craig Venter's story
- SynBio research areas
- Applications of SynBio
- How do you engineer microbes?
- SynBio Engineering Cycle: Focus on yeast producing artemisinin
- Insights about Synthetic Biology
- Paving the way for synthetic biology at BIOTECH-UPLB
- iGEM (International Genetically Engineered Machine) Competition

BIOTECH

What is Synthetic Biology?

- **Synthetic Biology** *is the application of science, technology and engineering to facilitate and accelerate the* ***design, manufacture and/or modification*** *of genetic materials in living organisms*[1]

The ***creation*** *of new biologically based* ***parts, devices and systems*** *and/or the* ***redesign*** *of* ***existing natural biological systems*** *for useful purposes. Applying* ***engineering principles*** *of iterative* ***Design/Build/Test*** *cycles and* ***plug-and-play***[2]

[1]Opinion on Synthetic Biology I: Definition. 2014, September. SCHER, SCENIHR, SCCS. European Commission

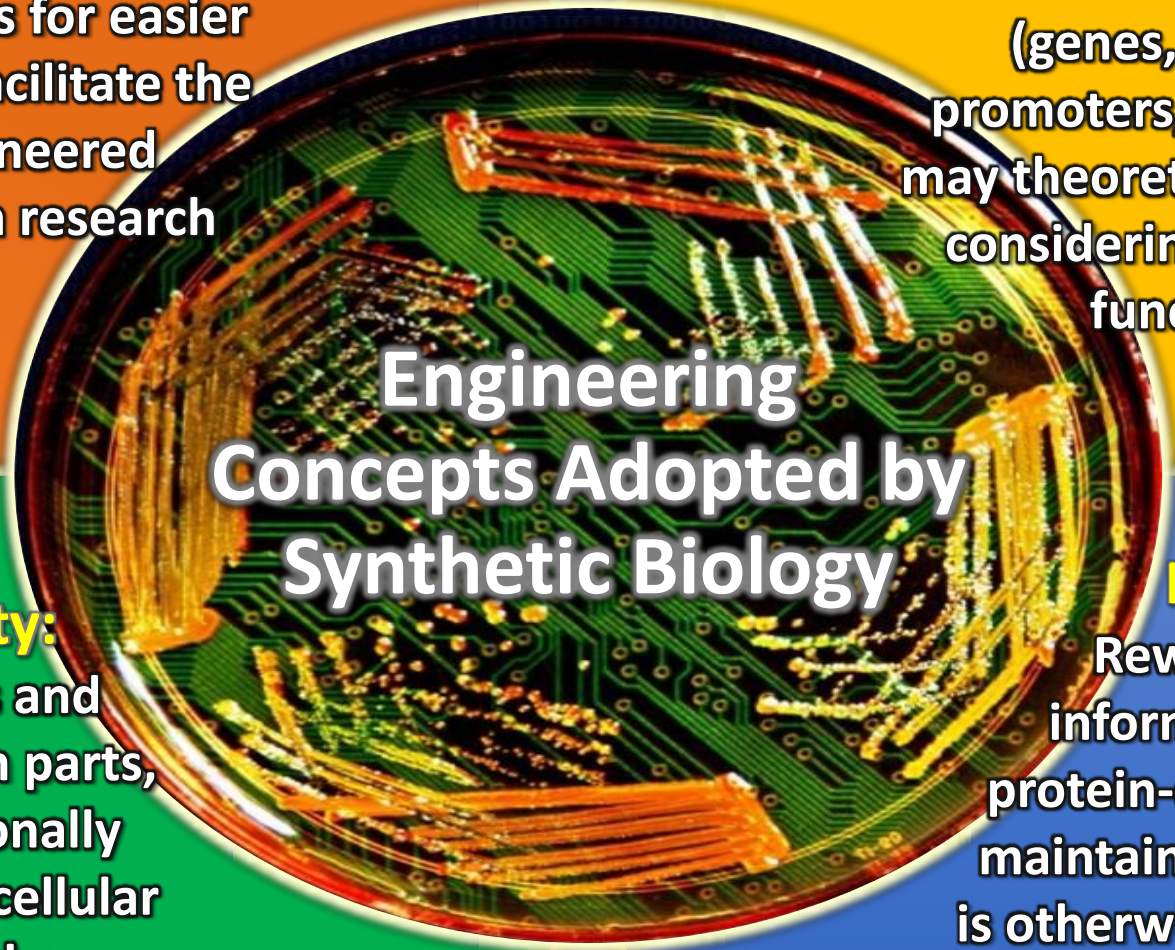
[2]Manchester Synthetic Biology Research Center for Fine and Specialty Chemicals, University of Manchester

How is Synthetic Biology Different from Genetic Engineering?

- Synthetic biology adopts classical engineering concepts such as **standardization**, **modularization**, **orthogonality**, and **refactoring** and attempts to apply these to the engineering of biological systems[1].
- These allow increasing **programmability** and **robustness**[2].
  - **Programmability**: ability to tightly control a biological system that has been engineered to generate a specified task; enables precise quantification of an output/ response, when a specified input is given
  - **Robustness**: stability of an engineered unit, with consistent performance that is independent of where it is implemented.

Opinion on Synthetic Biology I: Definition. 2014, September. SCHER, SCENIHR, SCCS. European Commission
Leonard et al., 2008. Trends in Biotechnol. 26:12. pp. 674-681

Engineering Concepts Adopted by Synthetic Biology



Standardization:

Aims for standardization of nucleotide sequences for easier engineering and to facilitate the exchange of engineered sequences between research groups.

Modularization:

Closely relates to hierarchical abstraction in which modules (genes, protein domains, promoters, and genetic circuits) may theoretically be used without considering internal molecular functional details.

Orthogonality:

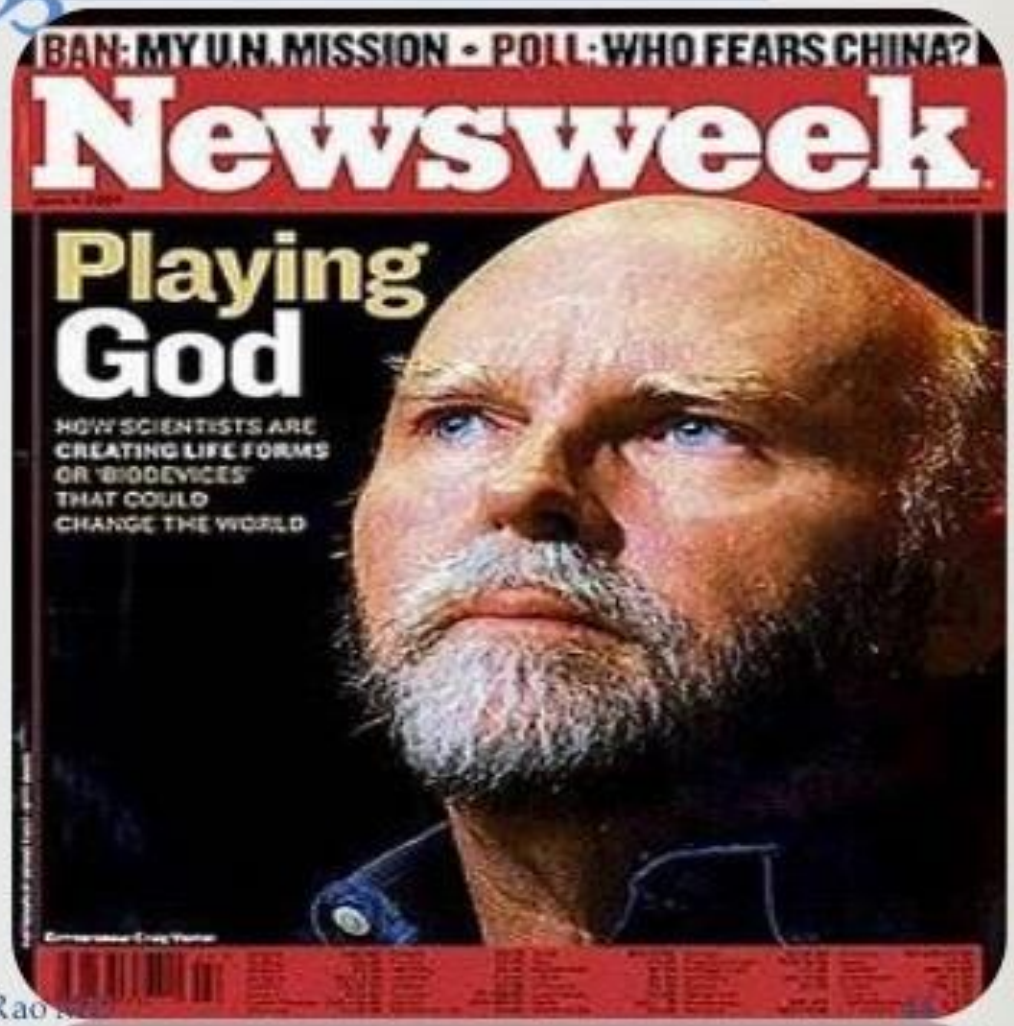
Employing parts and devices made from parts, which are functionally orthogonal to the cellular machinery of the engineered host organism.

Refactoring:

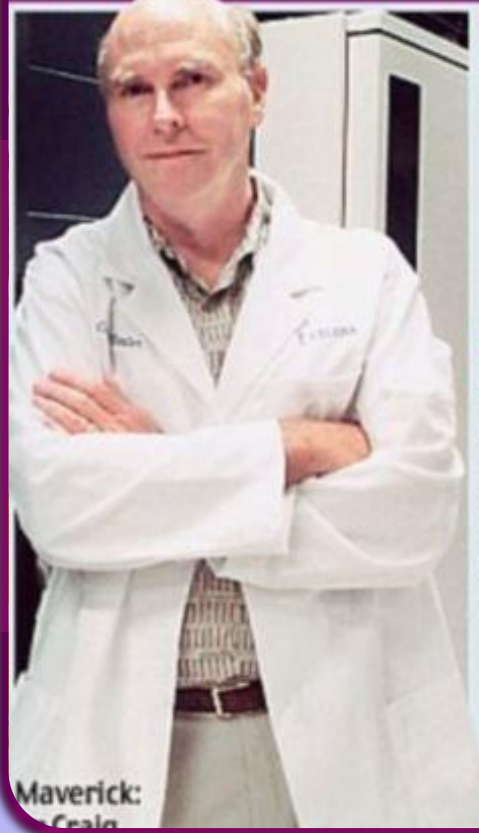
Rewriting of genetic information, so that the protein-coding information is maintained, but the sequence is otherwise randomized and all regulatory elements are replaced by specifically designed DNA parts

Craig venter creates revolution in Synthetic Biology

Craig Venter's team (and the associated paper in Science) that they have successfully synthesized the complete genome of the bacterium *Mycoplasma genitalium* is an important step towards achieving what is becoming known as "synthetic biology". By constructing complete DNA sequences from scratch, the door is being opened to transforming common laboratory chemicals into new living organisms; that are engineered with specific purposes in mind. And perhaps not surprisingly, this manipulation of DNA at the nan scale is increasingly being seen as part of the "nanotechnology revolution".



HOW TO MAKE ARTIFICIAL LIFE



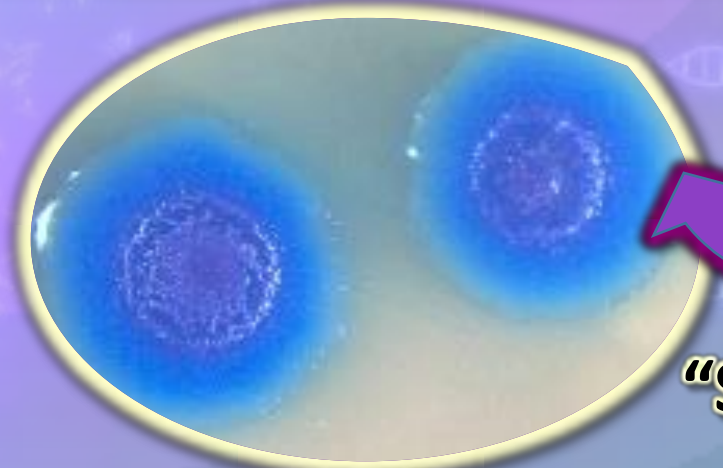
Maverick: Craig

1 Entire DNA of Mycoplasma mycoides, a bug that usually infects goats, is decoded.

Synthetic DNA code

2 Researchers buy fragments of DNA from a mail order catalogue. Each of the four bottles of chemicals contains a section of the code.

3 The fragments are put into yeast, which 'stitches' them together, gradually building a synthetic copy of the original DNA.



"Synthia"

Artificial DNA

Natural DNA

4 The artificial DNA is put into a recipient bacterium, which then grows and divides, creating two daughter cells, one with the artificial DNA and one with the natural DNA.

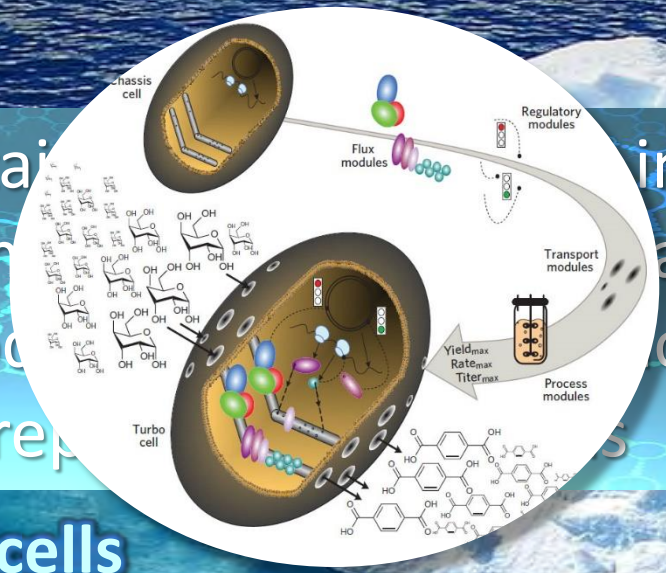
5 Antibiotics in the petri dish kill the bacterium with the natural DNA, leaving the one with the synthetic DNA to multiply.

6 Within just a few hours, all traces of the recipient bug are wiped out and bugs with artificial DNA thrive. New life has been created.

Graphic by John Lawson

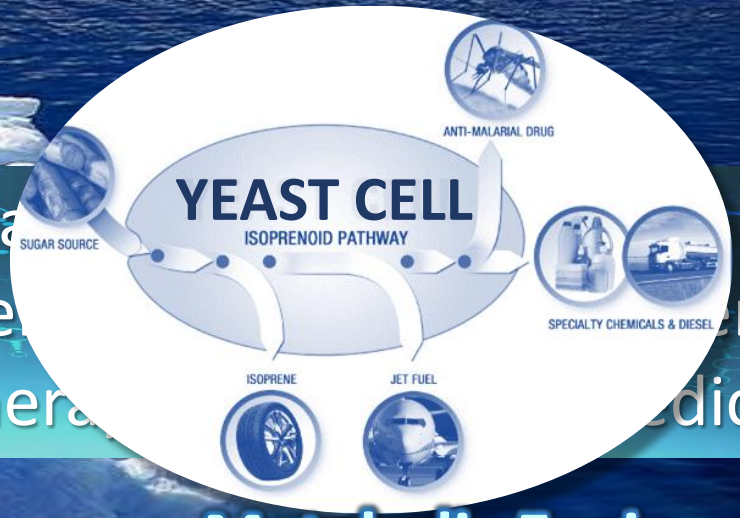
7 Possible uses are bugs capable of producing clea fuels and sucking carbon dioxide out of the atmosphere. Also microbe capable of mopping up oil slicks (above) or generating drugs, including the flu vaccine

Contai… synth… introd… self-rep… …into which …al could be …ovel living, …S



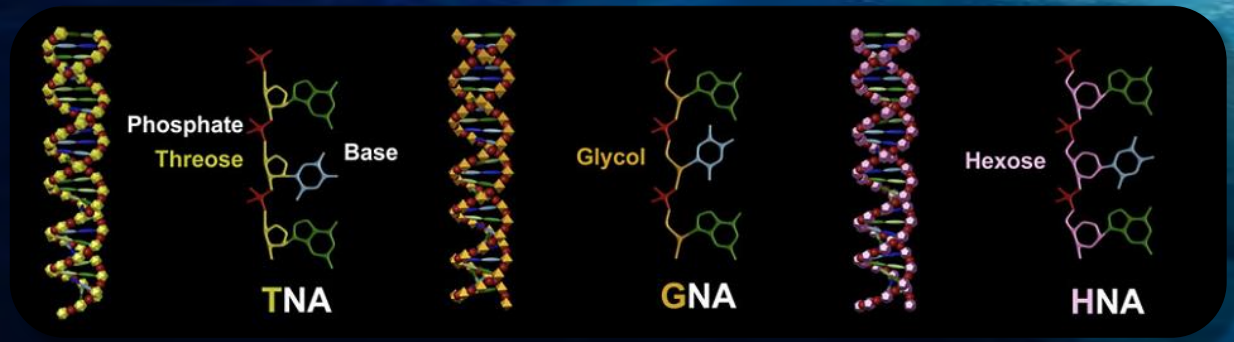
Protocells

Chemica… engineer… gene thera… …trait …ering, …edicine



Metabolic Engineering

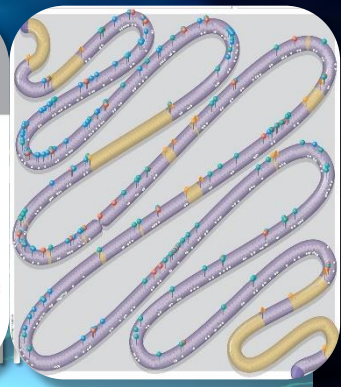
SYNTHETIC BIOLOGY RESEARCH AREAS



Orthogonal biosystems / xenobiology

TOTAL SYNTHESIS OF YEAST DESIGNER CHROMOSOME *SYNIII*

The chromosome is represented snake-like, with the positions of "designer changes" indicated by pins and white diamonds, and the deleted segments indicated in yellow, using the native chromosome sequence as a reference. The approximate position of nucleosomes (protein "packaging" for the DNA in the chromosome) are indicated by the small dots in the center of the chromosome. The positions of the changes are roughly to scale.



Synthetic genomics and DNA synthesis

Opinion on Synthetic Biology I: Definition. 2014, September. SCHER, SCENIHR, SCCS. European Commission

Applications of Synthetic Biology

Applications Health

- Synthetic tools for novel drug discovery and therapeutic approaches
- Genetically engineered organisms/viruses to fight diseases
- 'Synthetic' pathogens or components thereof for diagnosis and vaccine development
- Biosynthesis of pharmaceuticals

Applications Environment

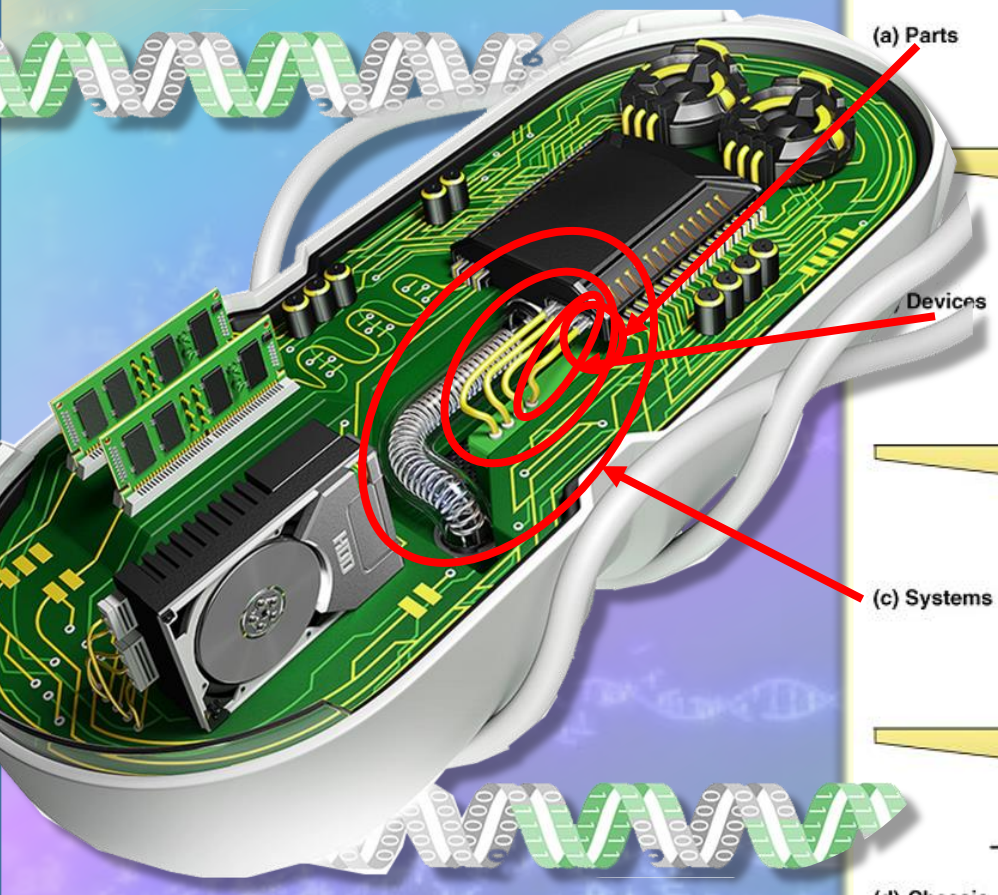
- *Environmental biosensors for monitoring pollution*
- *Removal of environmental pollution: bioremediation*
- *Production of environmentally-friendly chemicals from renewable sources*

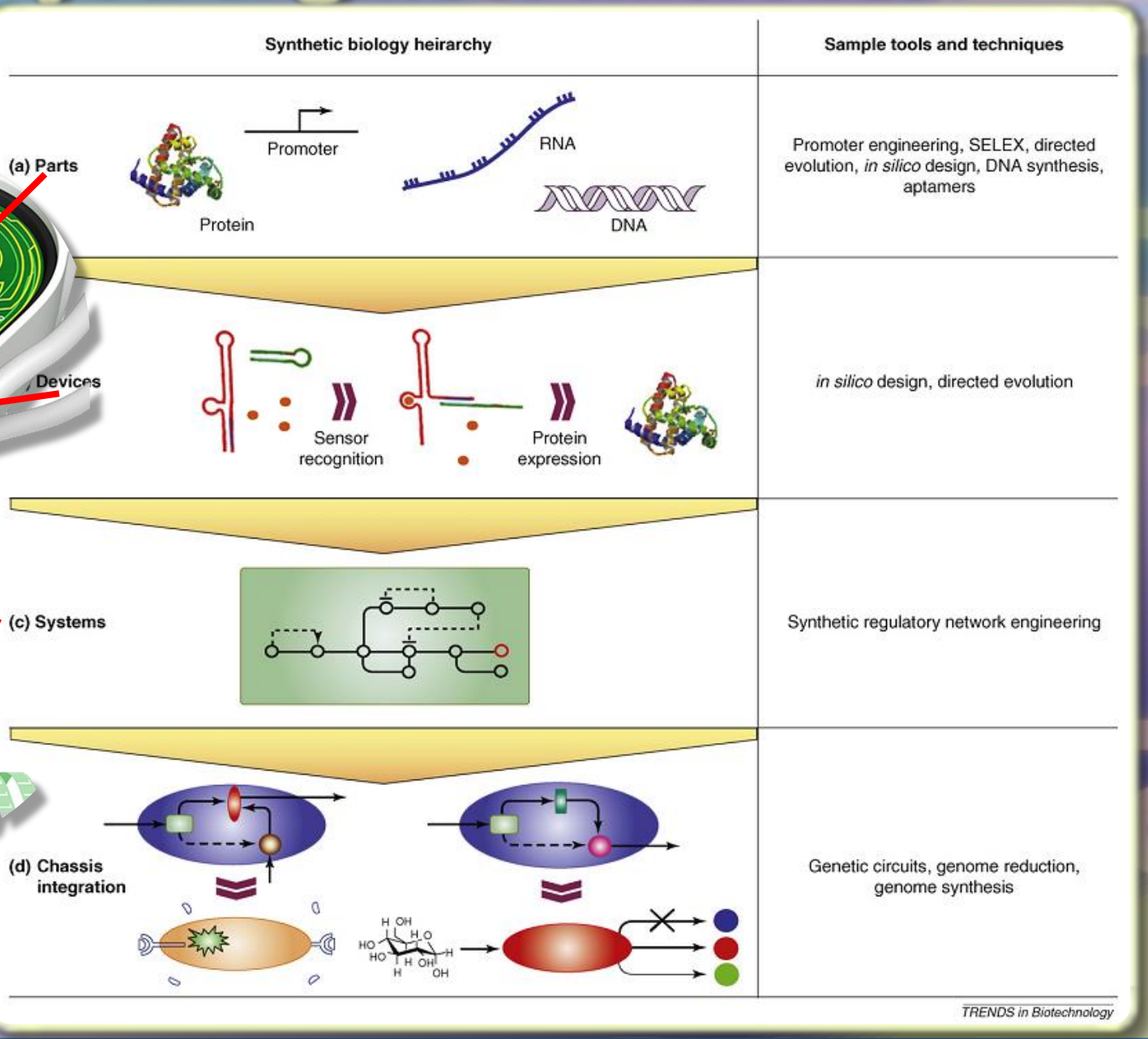
Applications Energy/Biofuels

GE/designed pathways to higher-chain alcohols, isoprenoids, biodiesel, alkanes (bacteria, yeast and other fungi)	Sugar → biodiesel, drop-in fuels
GE/designed pathways to isobutanol, biodiesel, alkanes (bacteria, yeast and other fungi)	Lignocellulose → biodiesel, drop-in fuels
GE/designed pathways to higher-chain alcohols, isoprenoids, secreted fatty acids and alkanes (mostly in cyanobacteria)	Light + H_2O + CO_2 → drop-ins by microalgae
GE enhancement of H_2 production (green algae, cyanobacteria)	Light + H_2O + CO_2 → hydrogen by microalgae

König, et al., 2013. Current Genomics. 14:11-24

How do you engineer microbes?





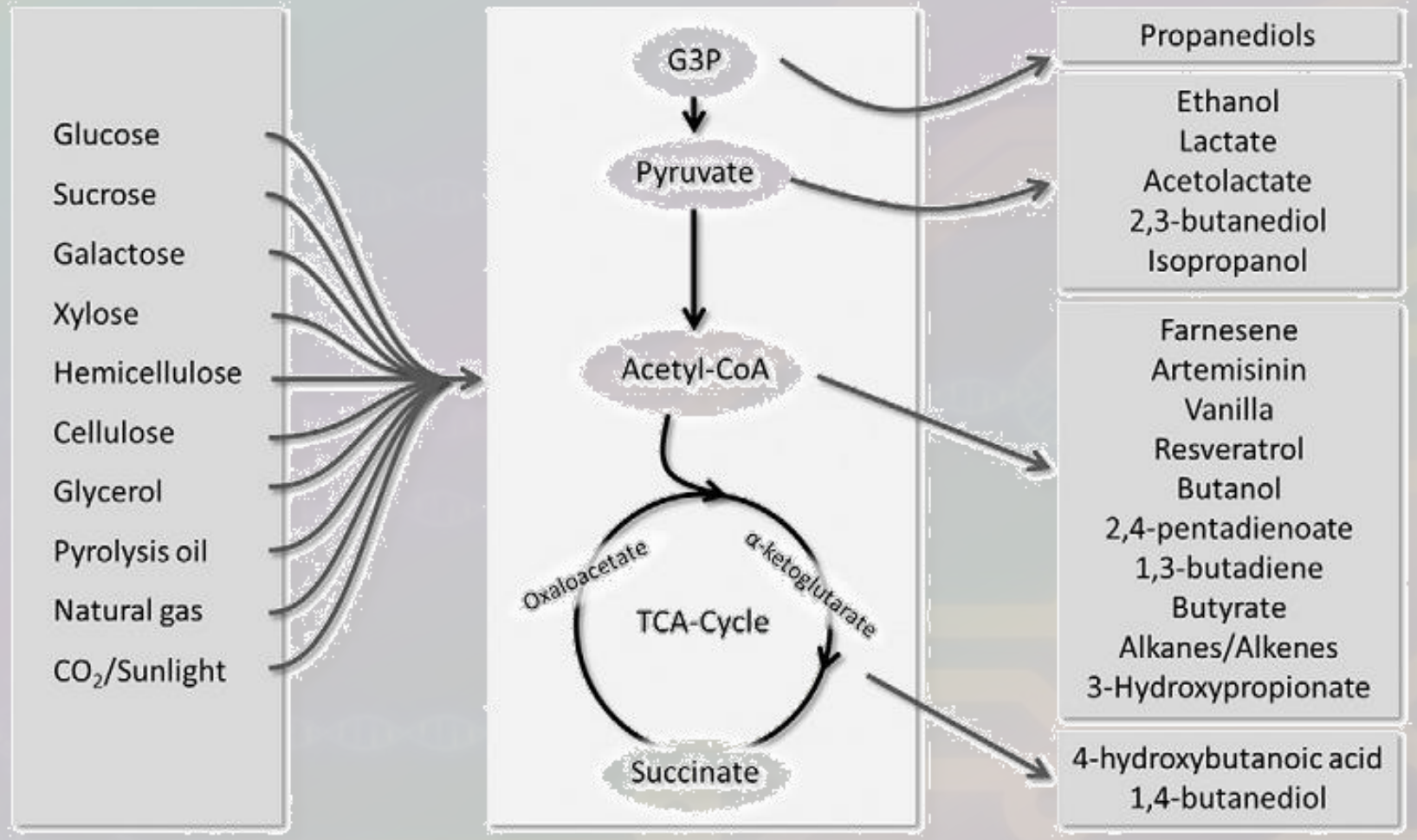
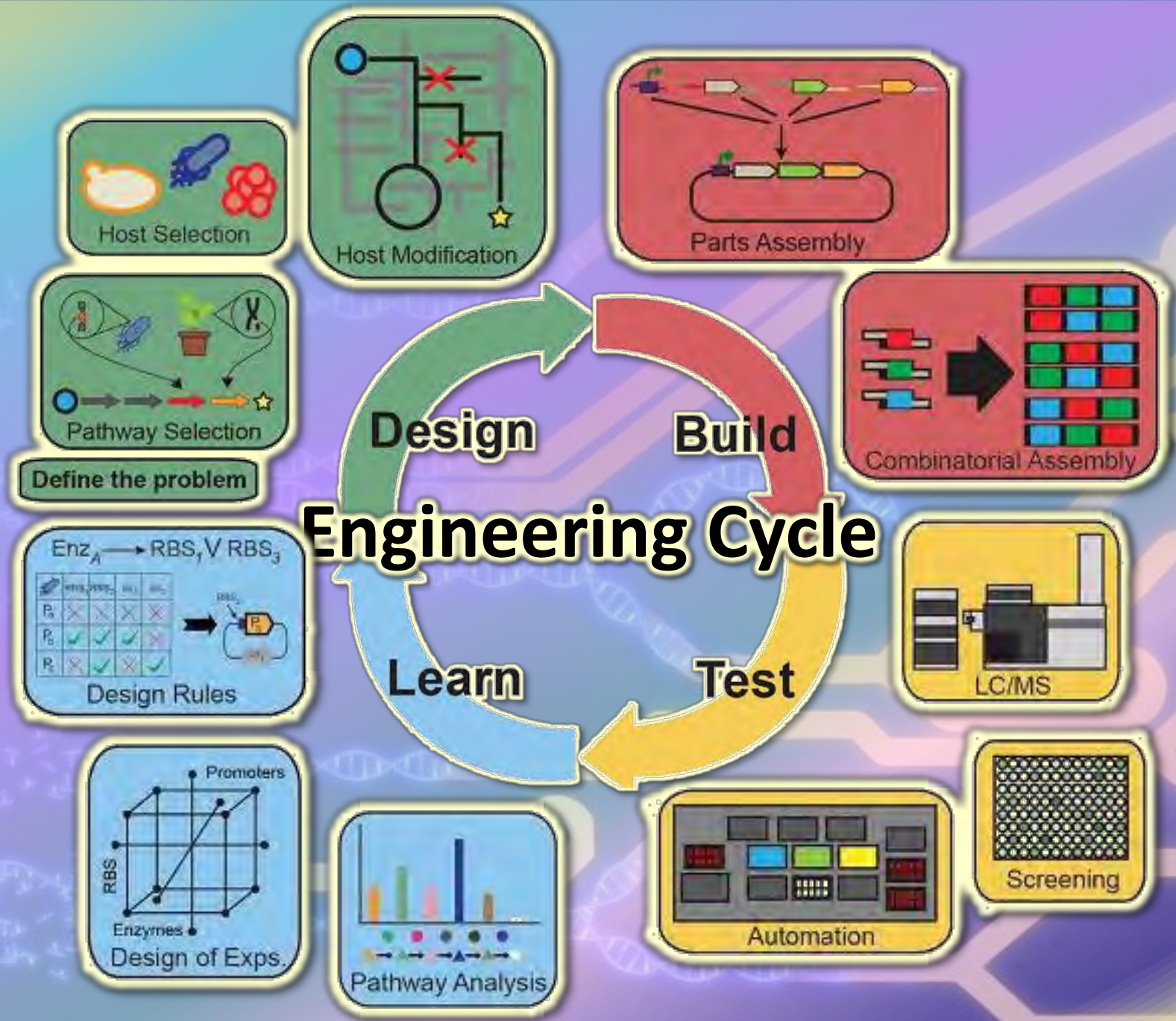


Fig. 3. The bow tie structure of metabolism with the main metabolic precursor metabolites residing in the center of the three super-pathways: catabolic reactions, central metabolism and anabolic reactions. Key precursor metabolites are: G3P (glyceraldehyde 3-phosphate), pyruvate, acetyl-CoA (acetyl-Coenzyme A), oxaloacetate, a-ketoglutarate, succinate, fatty acids, prenyl-pyrophosphates, and acyl-thioesters.

D. Jullesson et al. 2015. Biotechnology Advances 33. 1395–140



Petzold, et al. 2015. Frontiers in Bioengineering and Biotechnology. doi: 10.3389/fbioe.2015.00135

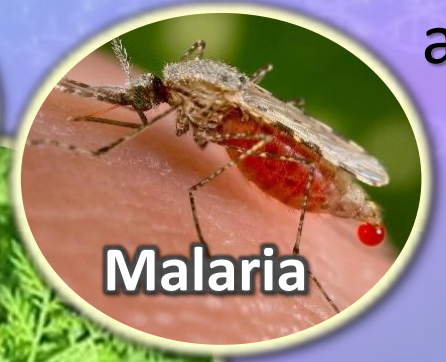
Design

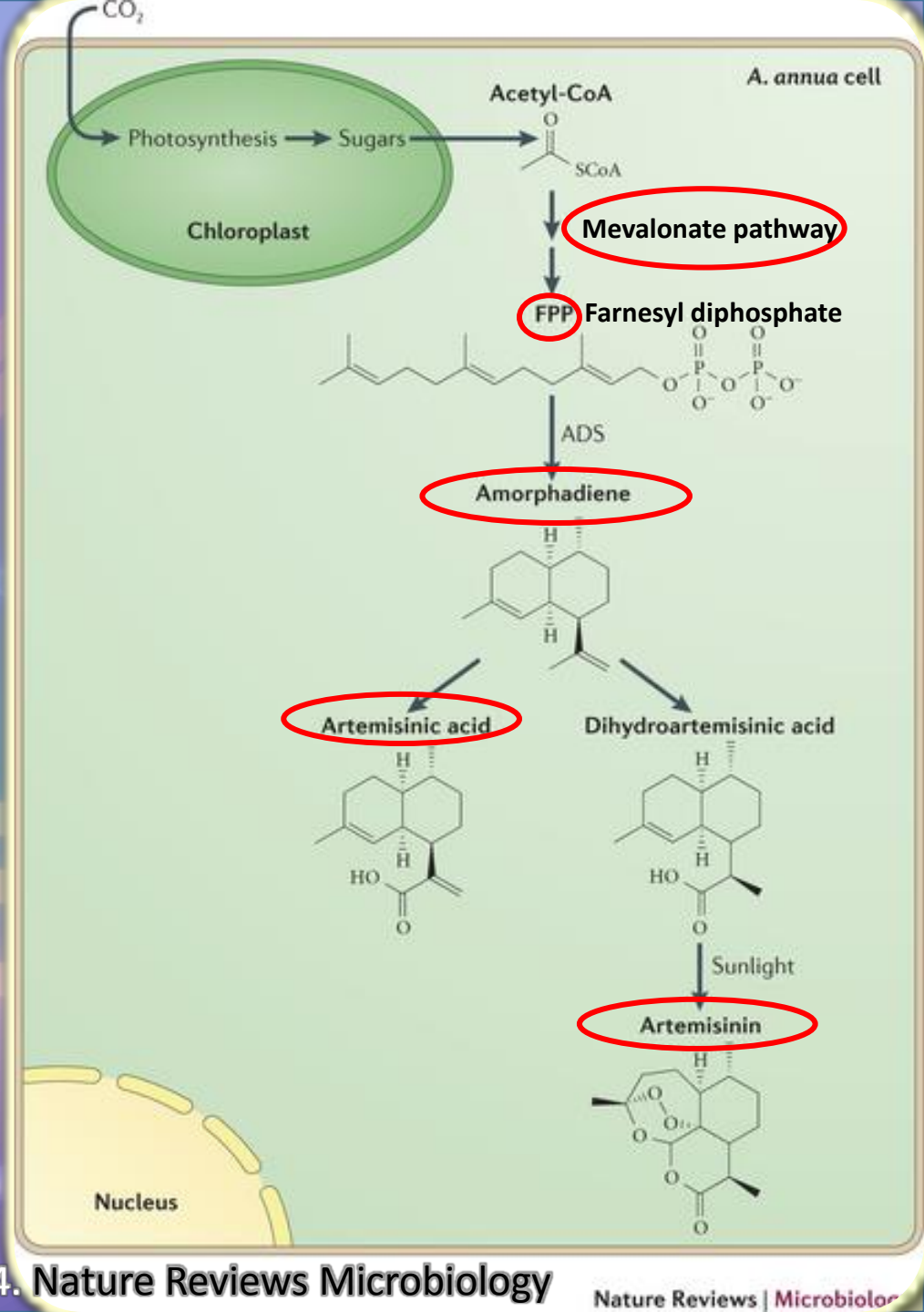
Define the problem

Define the Problem: Metabolic Engineering of Microorganisms for Artemisinin Production

Goal:

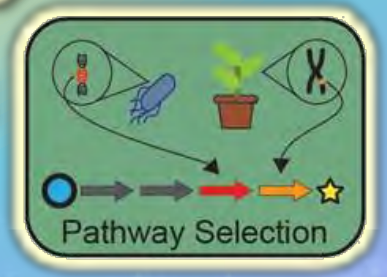
To produce affordable artemisinin using biological methods.





Paddon and Keasling. 2014. Nature Reviews Microbiology

Design



Glucose

G3P

Pyruvate

Acetyl-CoA

DXF Pathway

DXP → MEP → CDP-ME → CDP-MEP → MEcPP → HMBPP

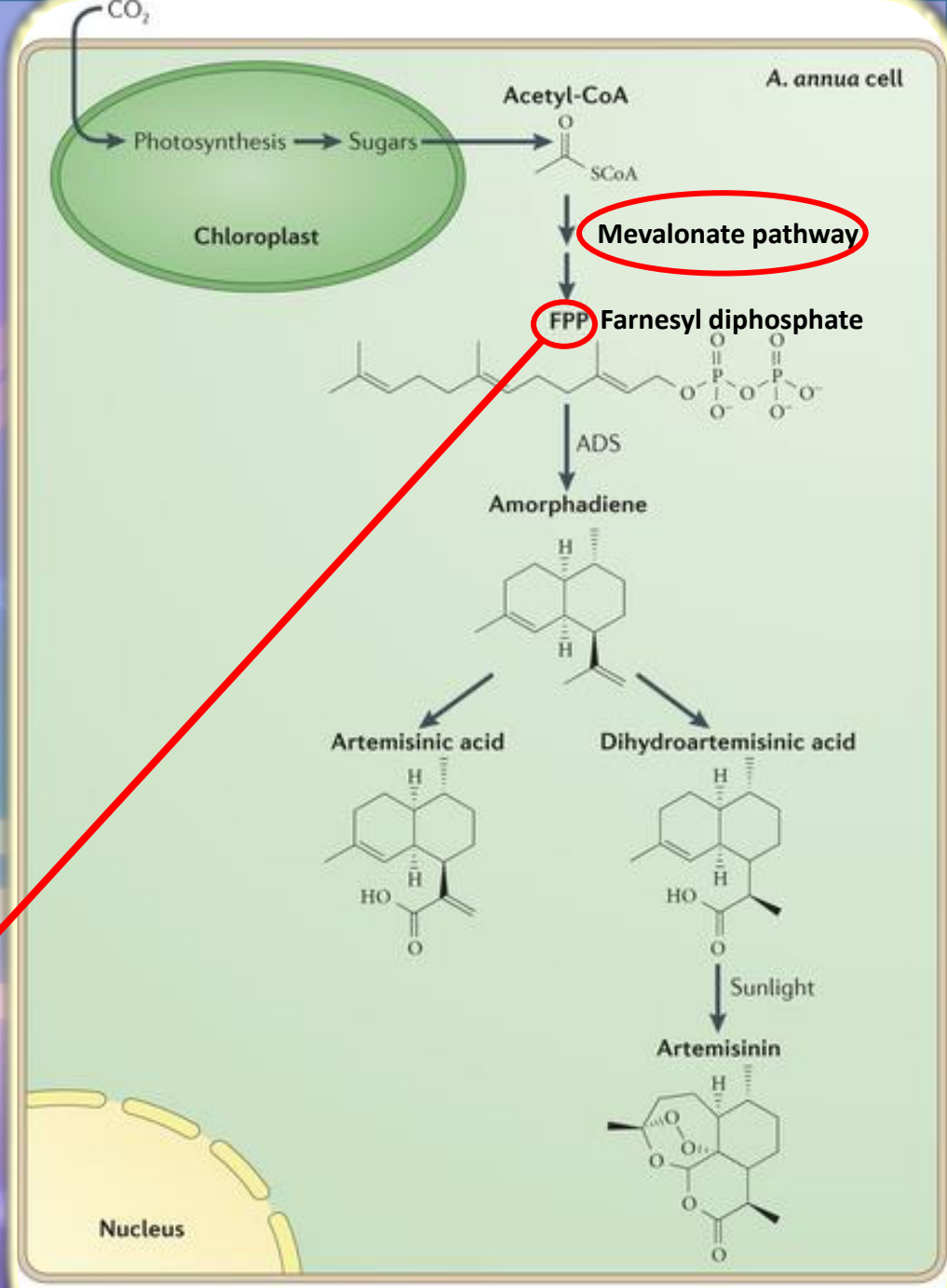
√

Mevalonate Pathway

AA-CoA → HMG-CoA → MEV → MEVP → MEVPP → IPP ↔ DMAPP

FPP

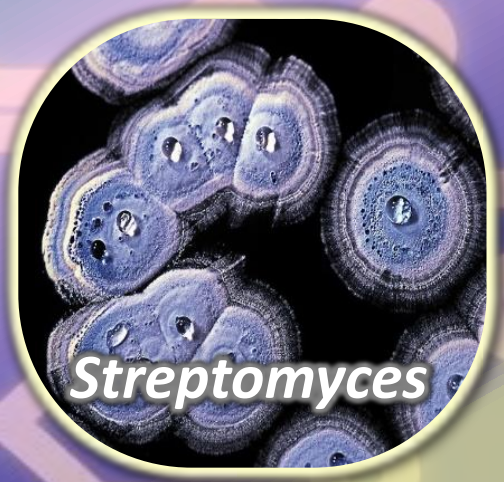
C_{15} Isoprenoids



Design

Host Selection

√

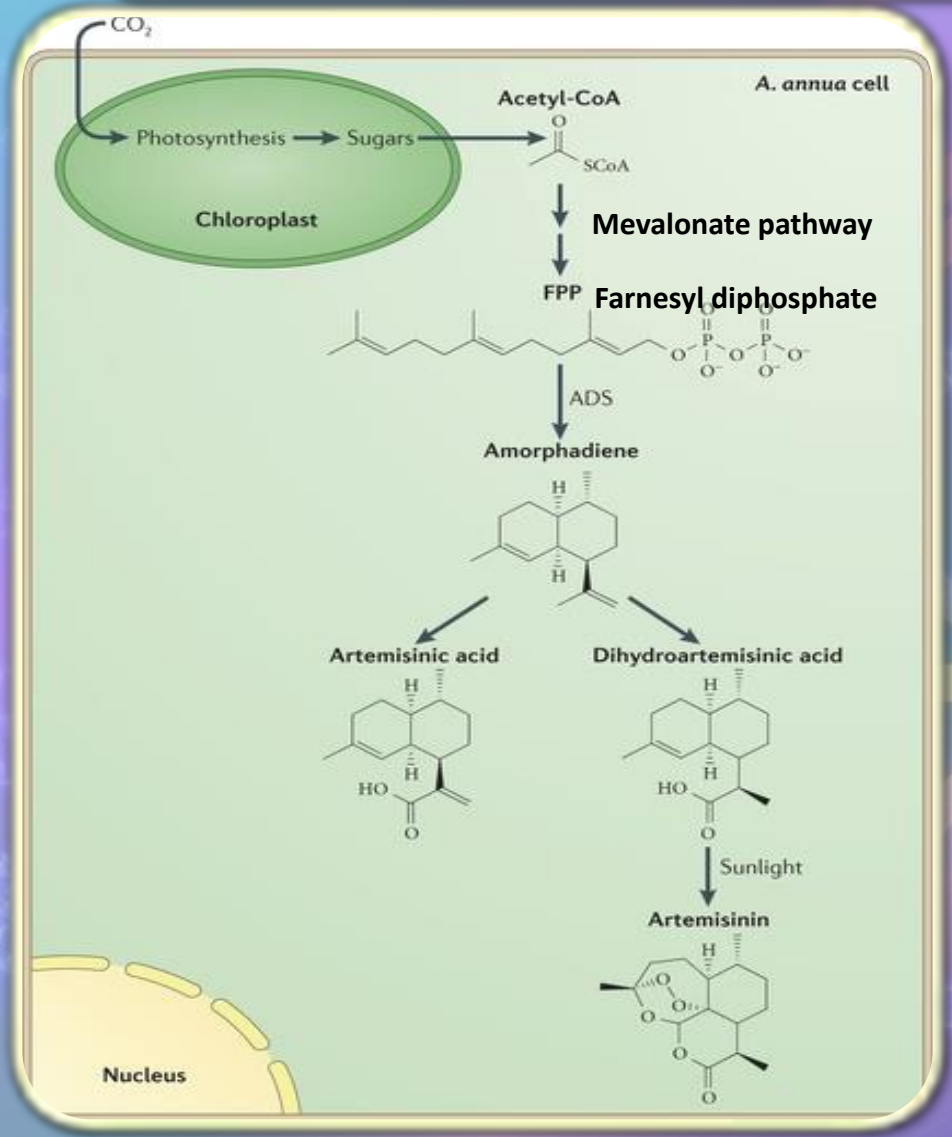


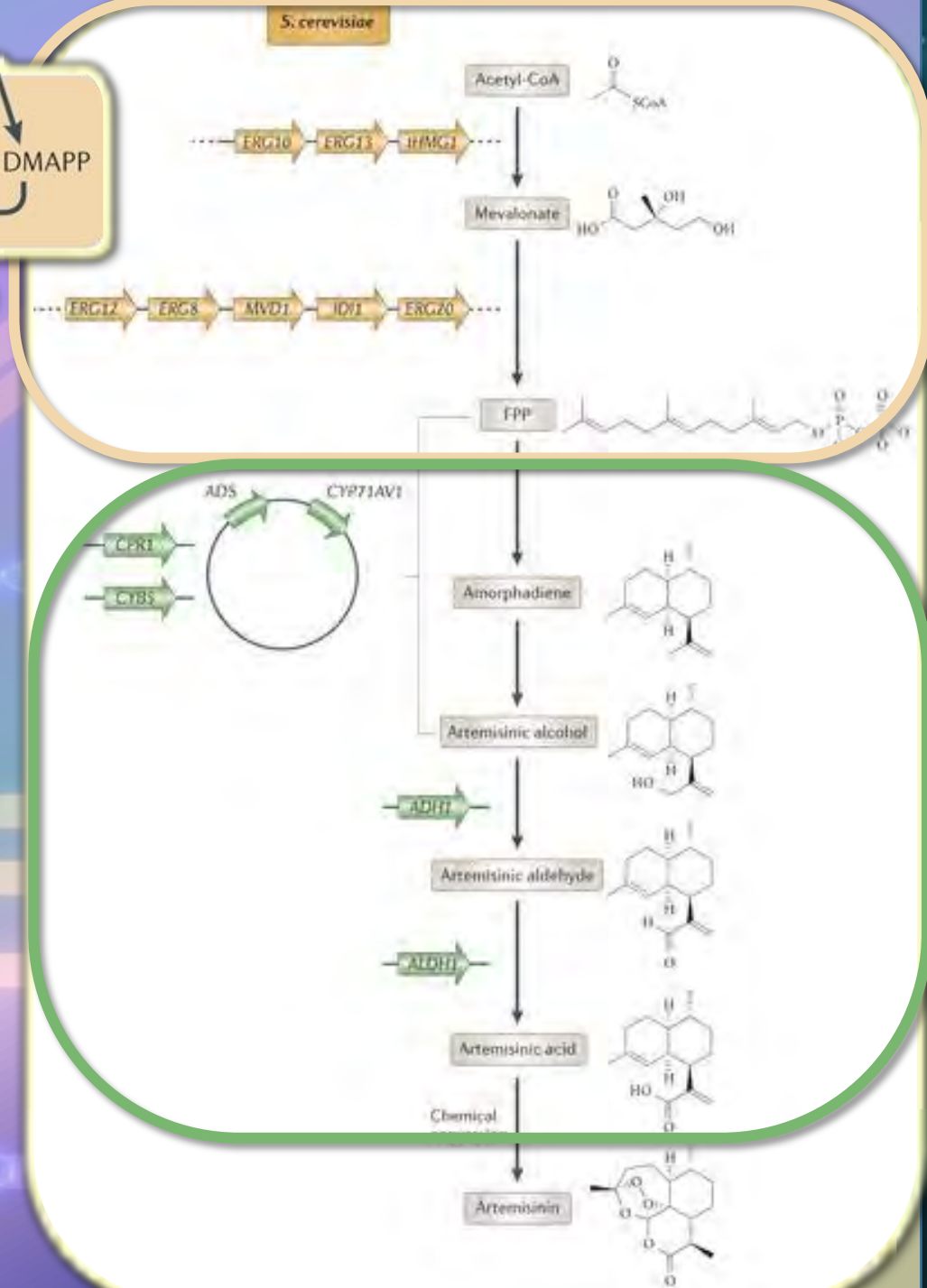
Design

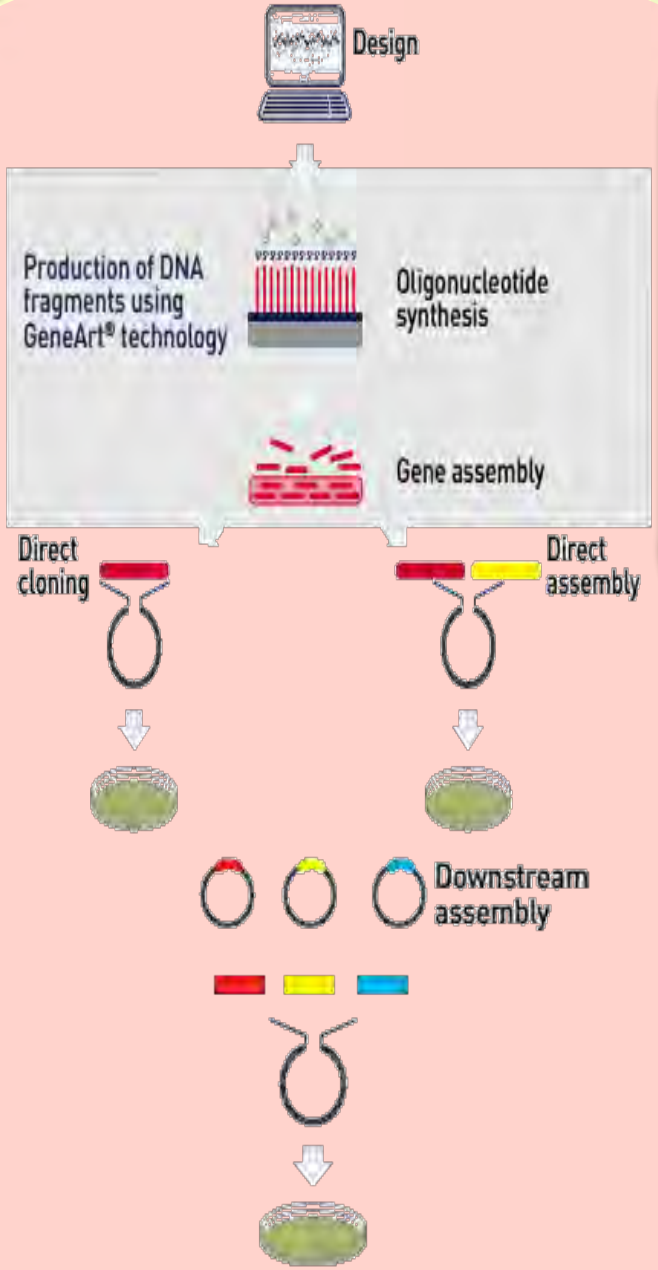
Mevalonate Pathway

AA-CoA → HMG-CoA → MEV → MEVP → MEVPP → IPP ↔ DMAPP

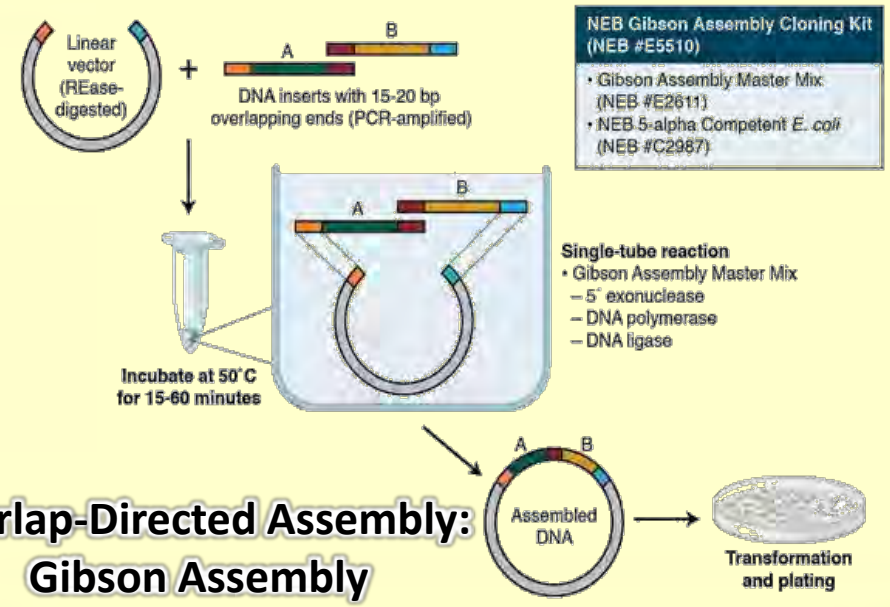
IPP → FPP



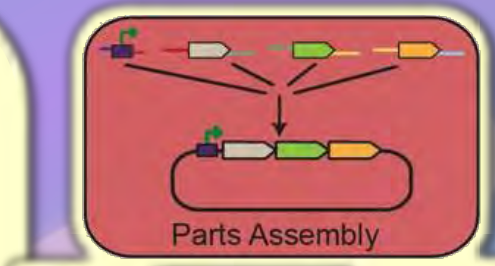




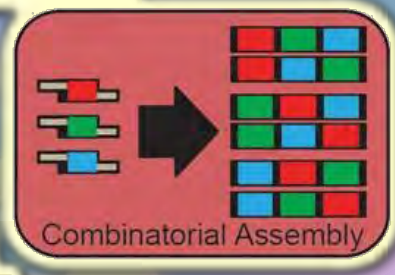
DNA Synthesis and Assembly to produce a synthetic gene

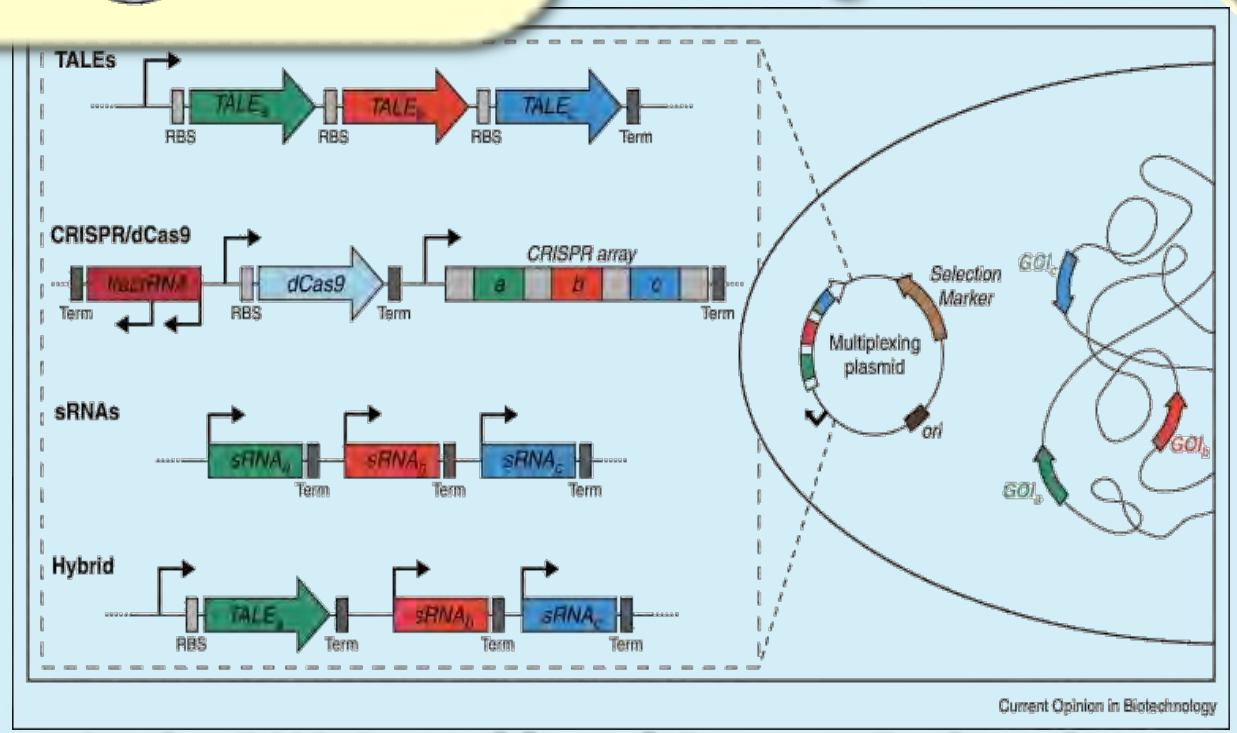


Overlap-Directed Assembly: Gibson Assembly



Build



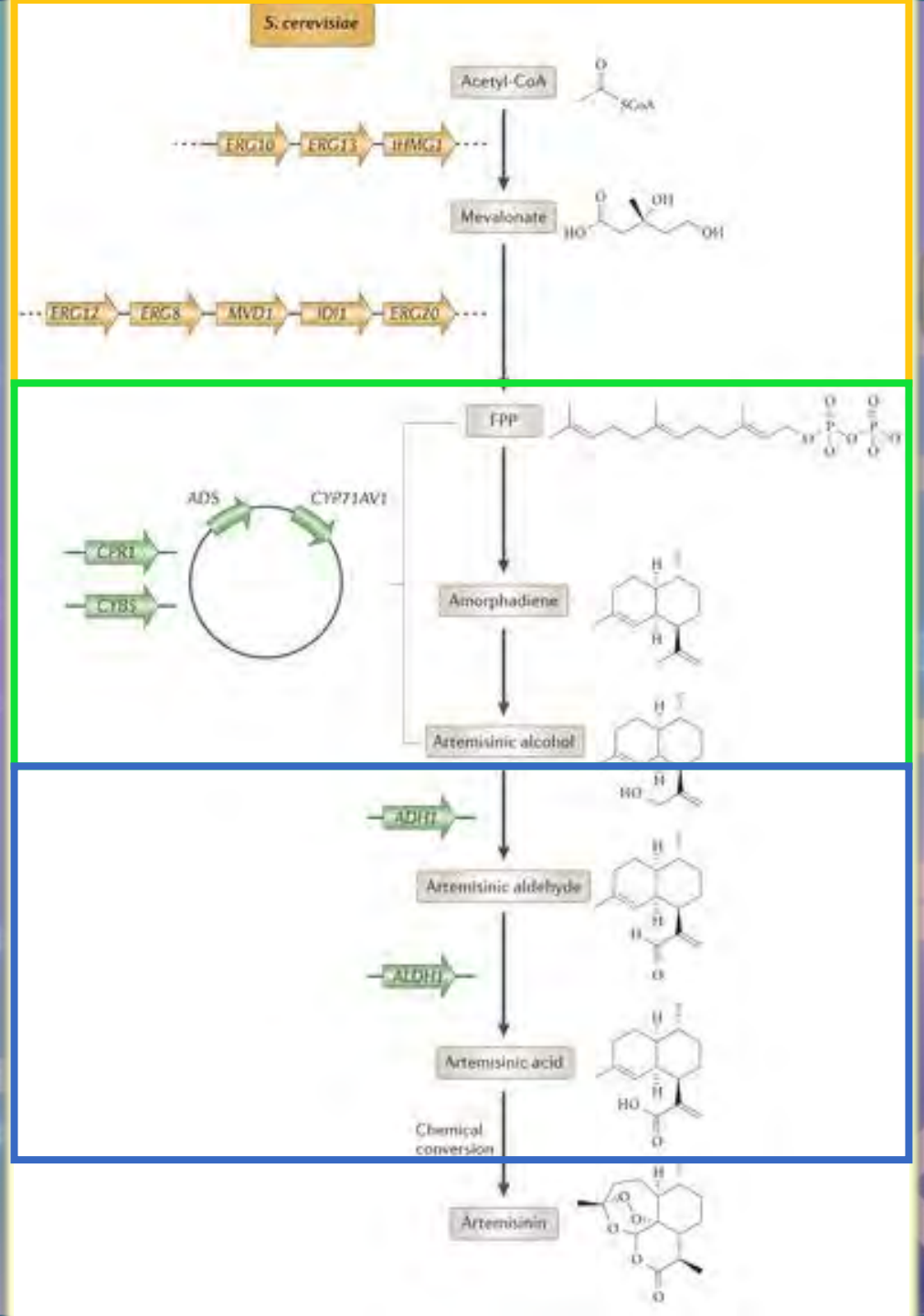


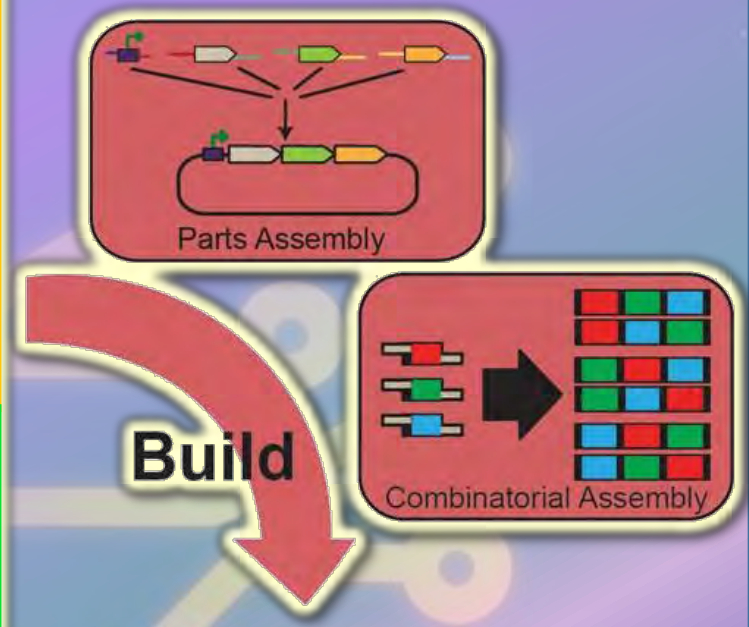
***In vivo* DNA Assembly and Genome Engineering**

Genes for the overexpression of mevanolate pathway were integrated into the yeast genome

Transformation of plasmids containing genes for conversion of amorphadiene to artemisinic alcohol

Genes for conversion to artemisinic acid where further integrated into the yeast chromosome





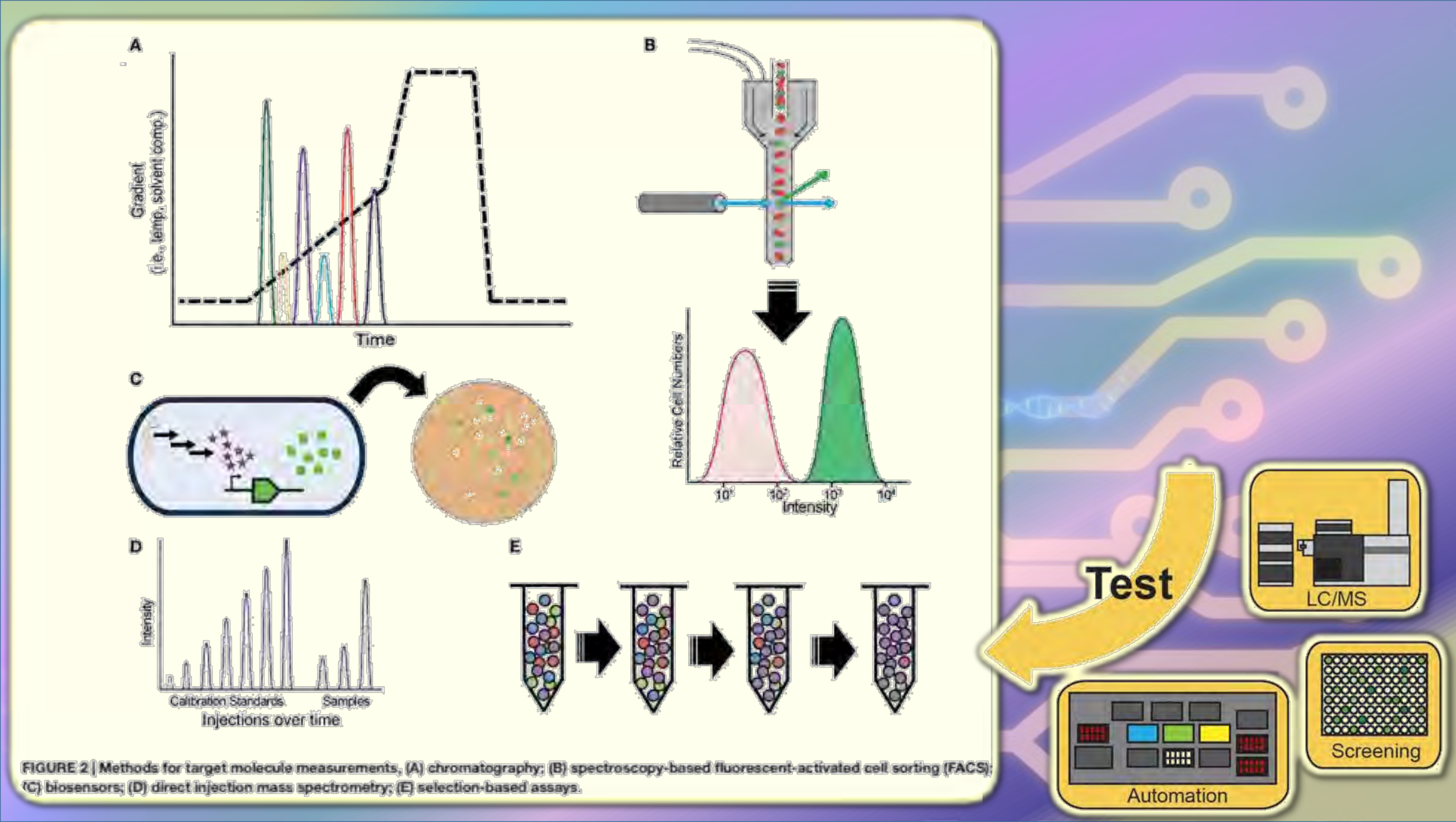
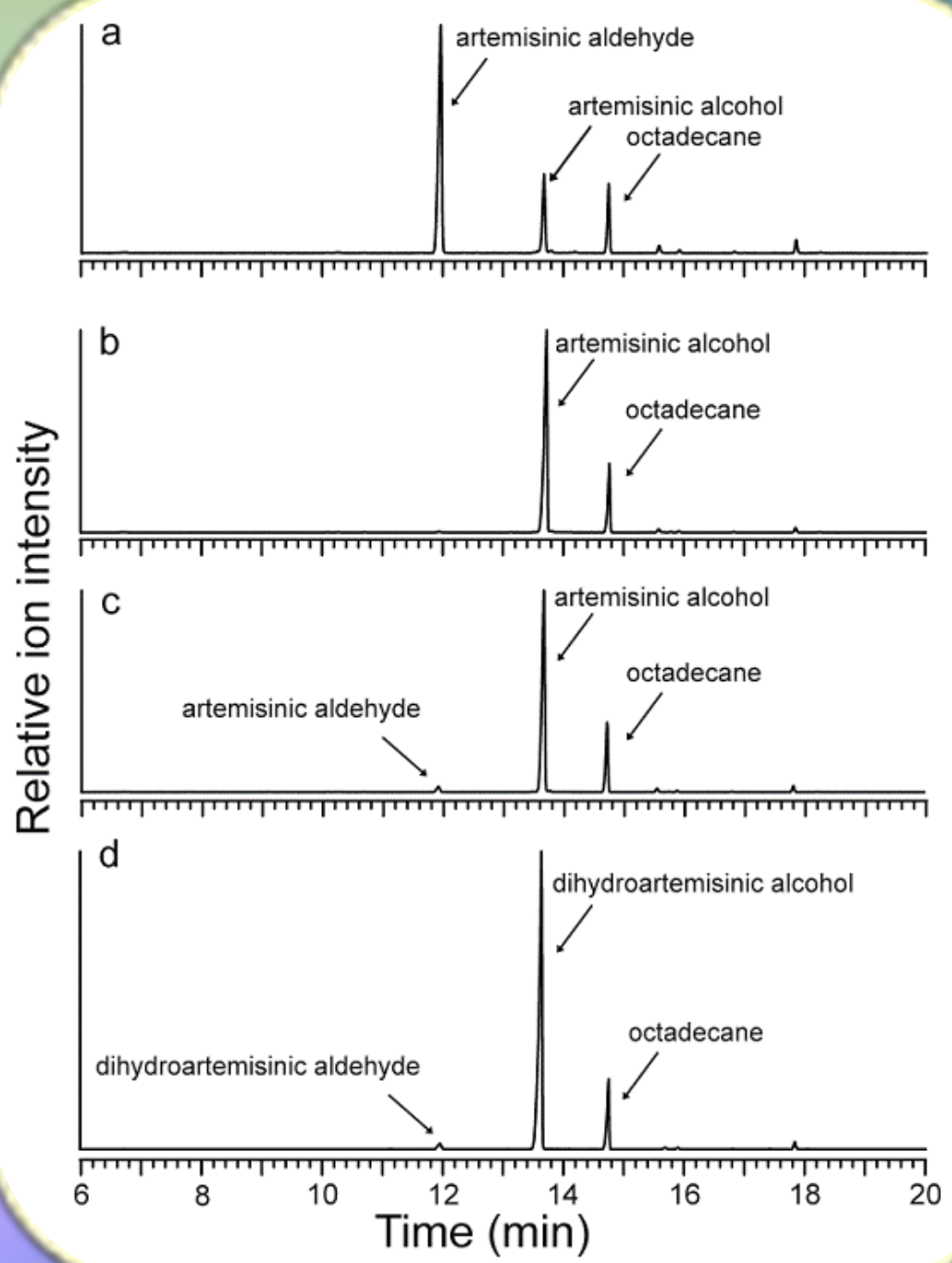
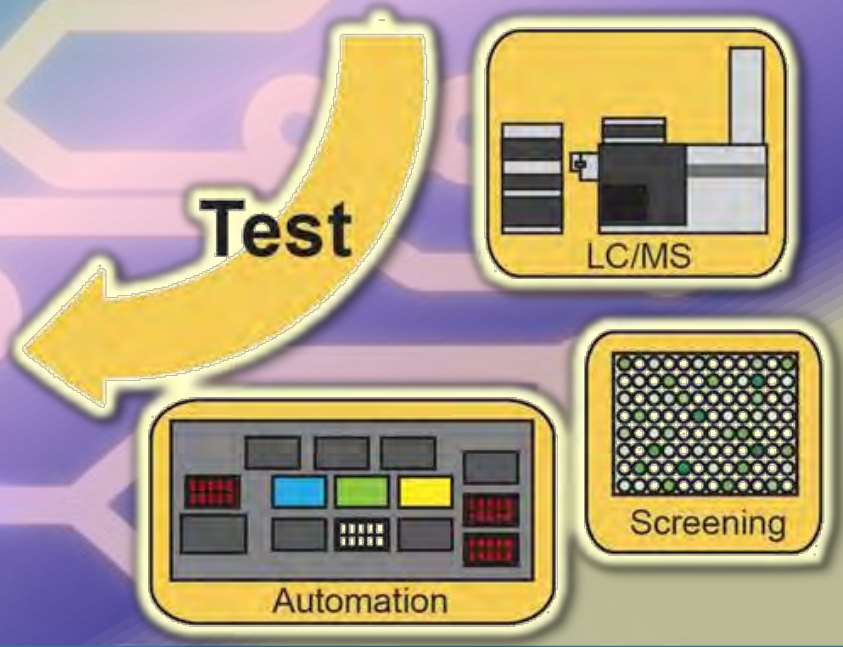


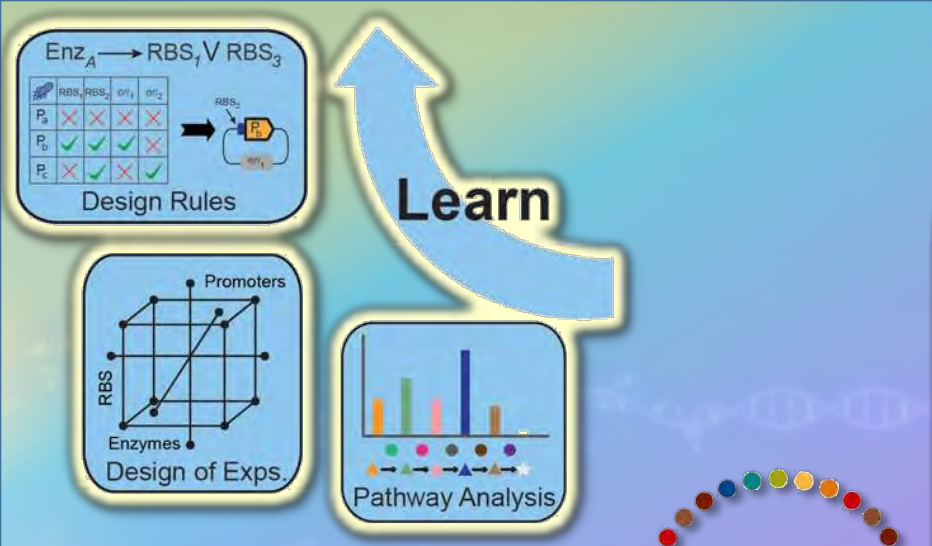
FIGURE 2 | Methods for target molecule measurements, (A) chromatography; (B) spectroscopy-based fluorescent-activated cell sorting (FACS); (C) biosensors; (D) direct injection mass spectrometry; (E) selection-based assays.



Monitor the production of artemisinin, its intermediates and related genes to determine the levels of expression

- By LC MS
- By microarray and proteomics
- By transcriptomics and qPCR





UNITED AGAINST MALARIA

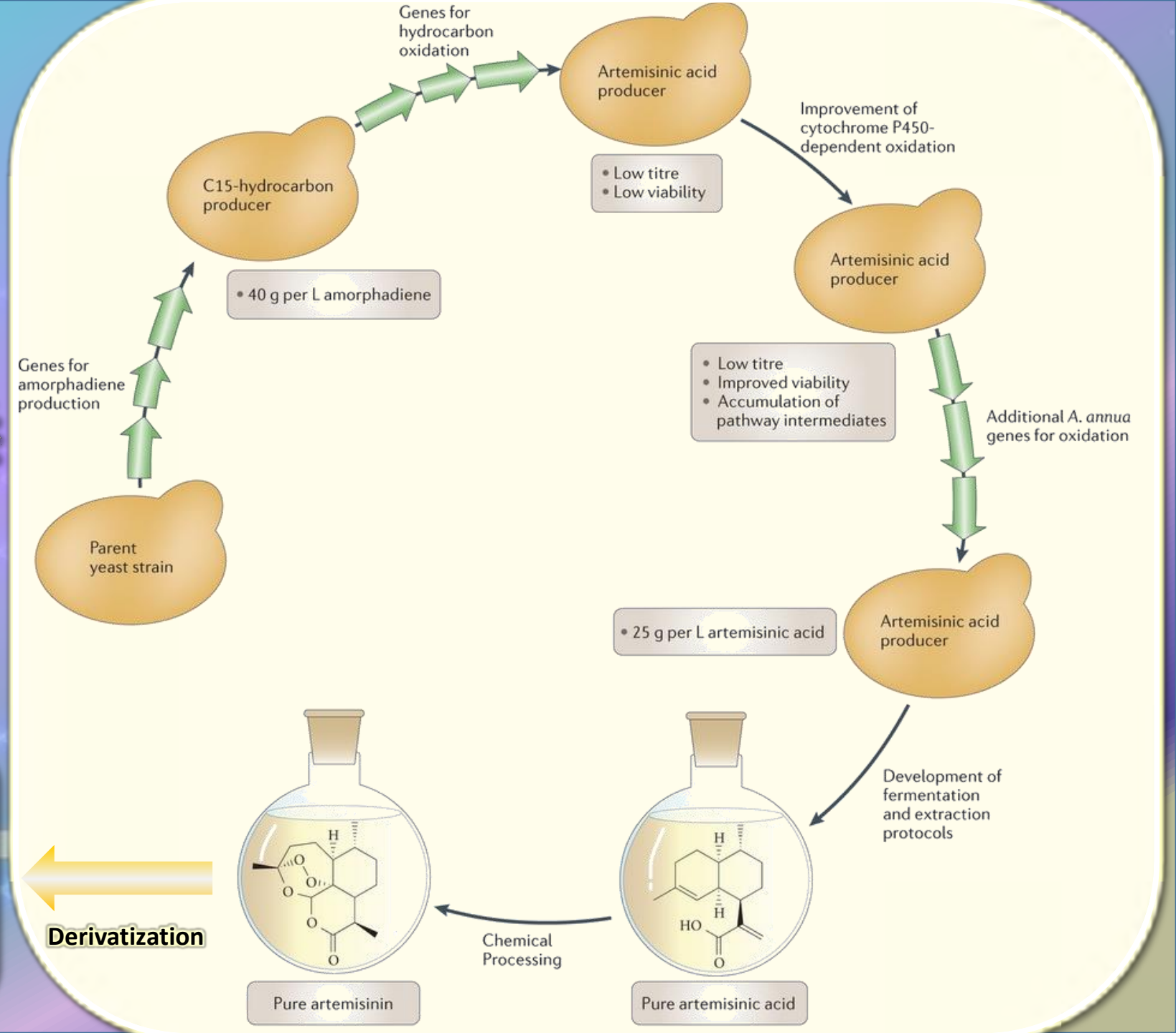
ACT therapies

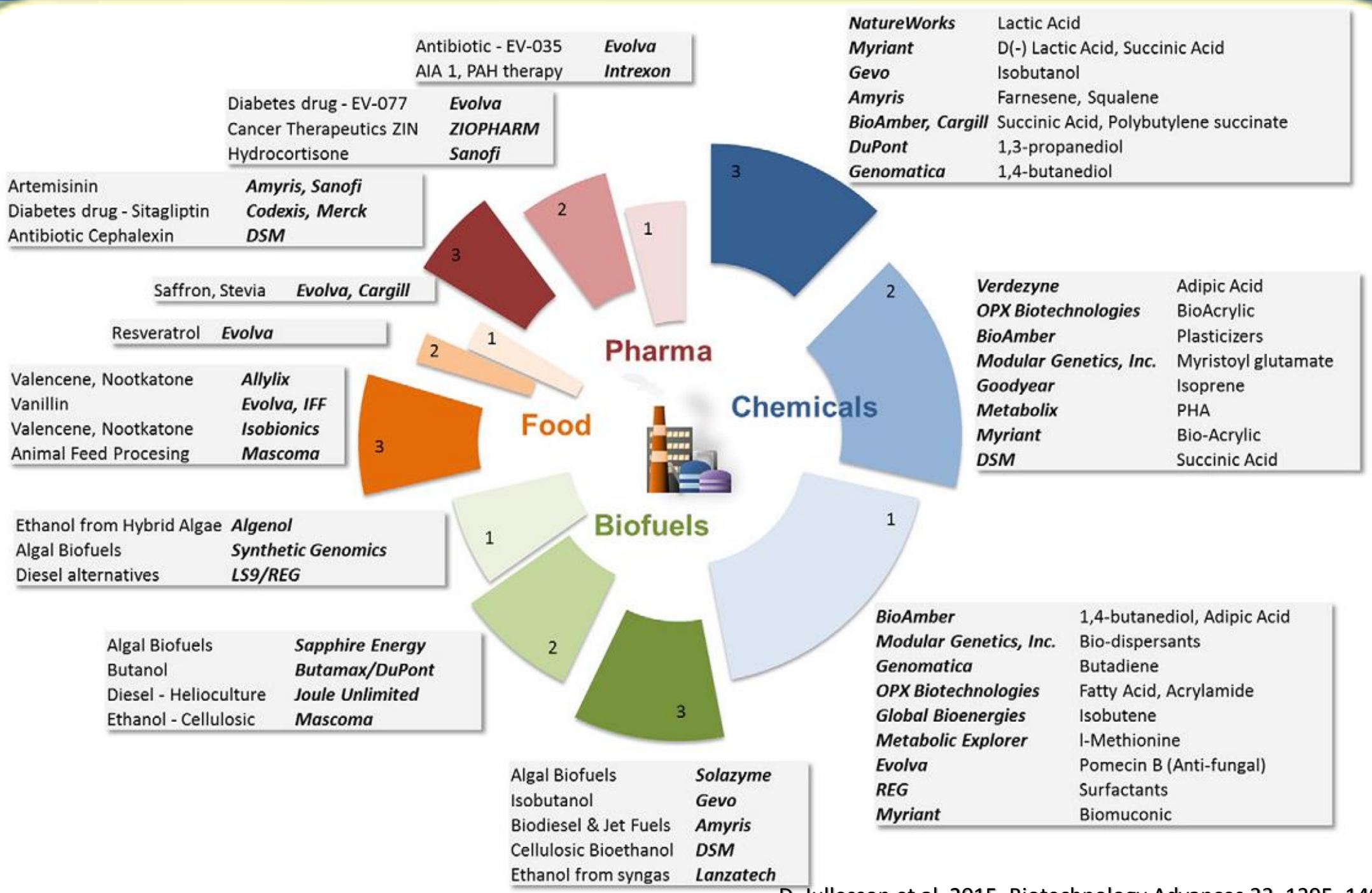
Artemisinin-based combination therapies (ACT) manufacturing

- **Artesunate**
- **Artemether**
- **Dihydroartemisinin**

Artemisinin derivatives

Derivatization





D. Jullesson et al. 2015. Biotechnology Advances 33. 1395–140

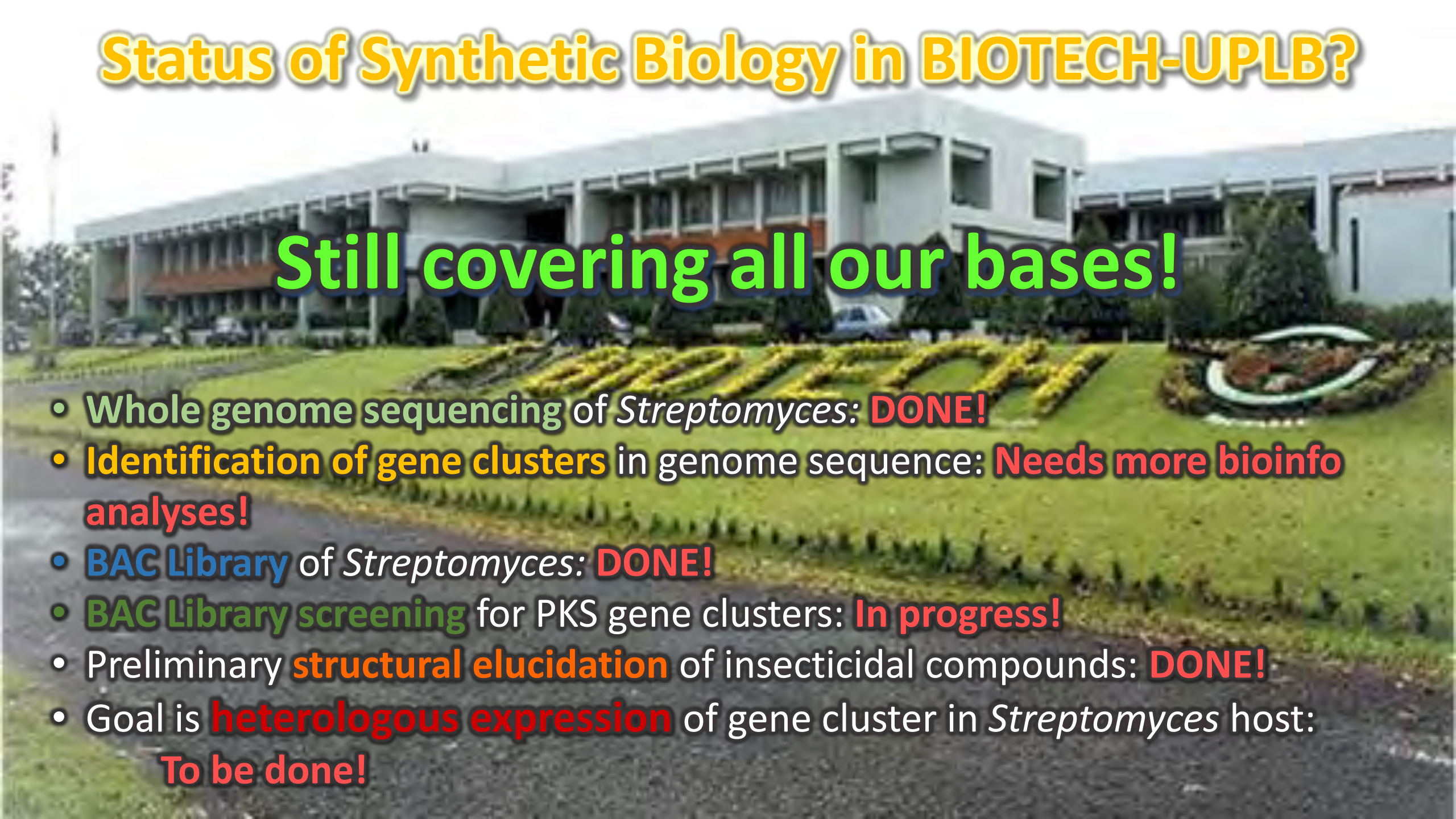
Insights about Synthetic Biology

- **SynBio is a lot of work!**
  - Need to have a strategic, well thought out plan!
  - Need to know your compounds
  - Need to know the biosynthetic pathway of your donor organism
  - Need to know the pathway of your host organism
  - Need to know your gene sequences
  - Need structural elucidation!
  - Expect **A LOT** of troubleshooting!
- You need a **knowledgeable mentor and team**!
  - Chemist, Bioengineer, Bioinformatician, Microbiologist
- Your **product needs to make a difference**!
- **You need funding**! Lots of it!
- **Bioethics and biosafety** are included in proposals

MANCHESTER 1824
The University of Manchester

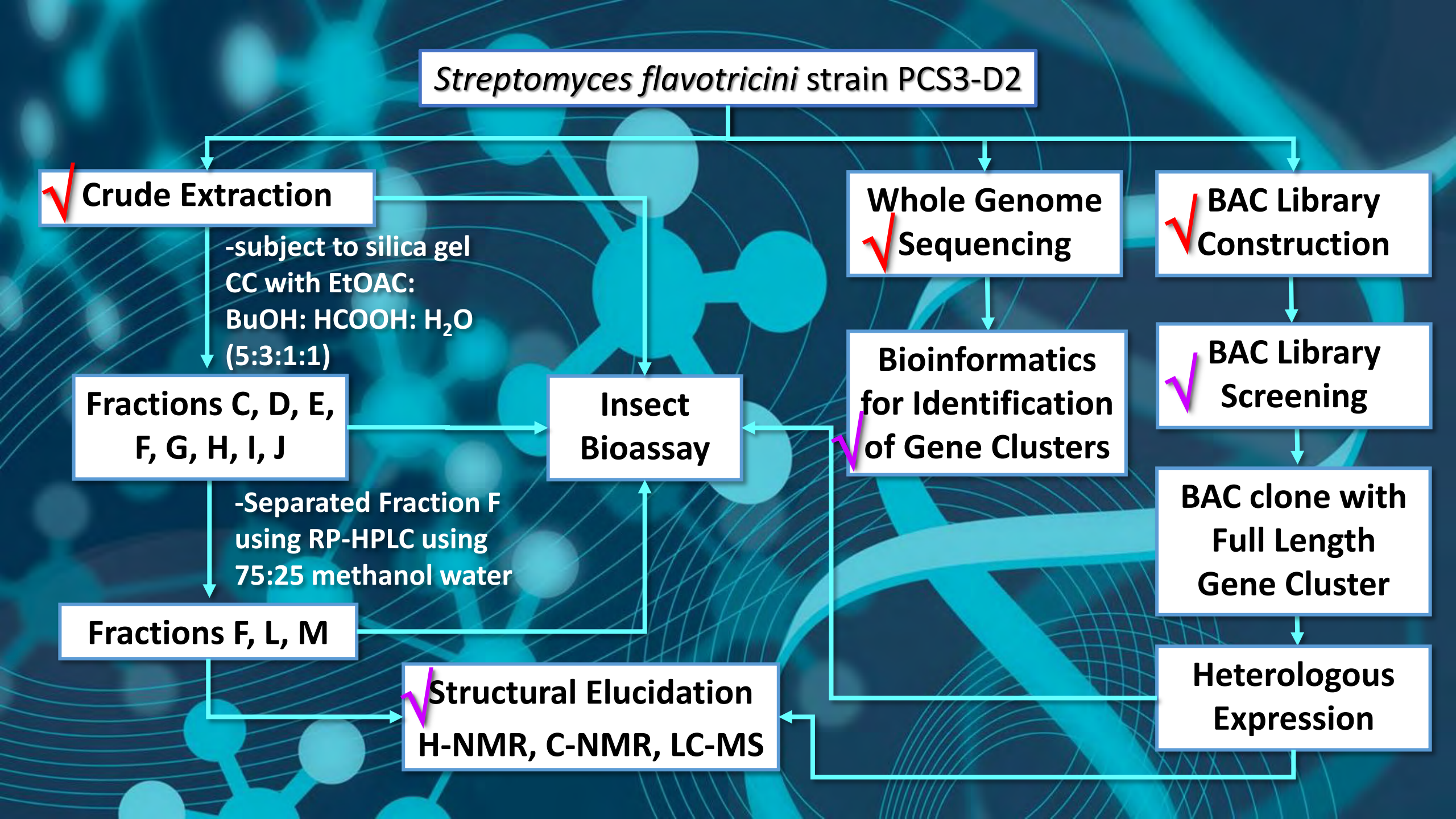
Manchester Institute of Biotechnology
Discovery through innovation

Status of Synthetic Biology in BIOTECH-UPLB?



Still covering all our bases!

- **Whole genome sequencing** of *Streptomyces:* **DONE!**
- **Identification of gene clusters** in genome sequence: **Needs more bioinfo analyses!**
- **BAC Library** of *Streptomyces:* **DONE!**
- **BAC Library screening** for PKS gene clusters: **In progress!**
- Preliminary **structural elucidation** of insecticidal compounds: **DONE!**
- Goal is **heterologous expression** of gene cluster in *Streptomyces* host: **To be done!**


Streptomyces flavotricini strain PCS3-D2
√ Crude Extraction
-subject to silica gel CC with EtOAC: BuOH: HCOOH: H_2O (5:3:1:1)
Fractions C, D, E, F, G, H, I, J
-Separated Fraction F using RP-HPLC using 75:25 methanol water
Fractions F, L, M
Insect Bioassay
√ Structural Elucidation H-NMR, C-NMR, LC-MS
Whole Genome √ Sequencing
Bioinformatics √ for Identification of Gene Clusters
√ BAC Library Construction
√ BAC Library Screening
BAC clone with Full Length Gene Cluster
Heterologous Expression

Synthetic Biology

based on standard parts

About

The International Genetically Engineered Machine (iGEM) Foundation is an independent, non-profit organization dedicated to education and competition, the advancement of synthetic biology, and the development of an open community and collaboration.

iGEM runs three main programs: the **iGEM Competition** - an international competition for students interested in the field of synthetic biology; the **Labs Program** - a program for academic labs to use the same resources as the competition teams; and the **Registry of Standard Biological Parts** - a growing collection of genetic parts use for building biological devices and systems.

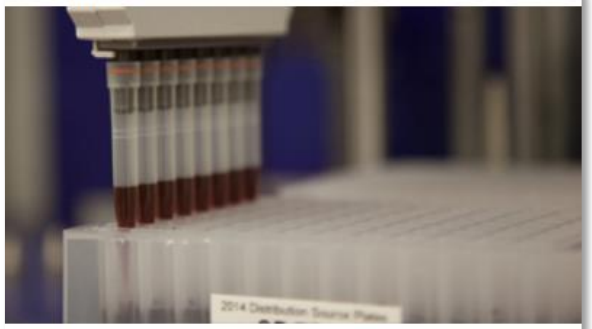
Competition

iGEM runs the premiere student competition in synthetic biology. Student teams are given a kit of biological parts and work over the summer to build and test biological systems in living cells, ranging from bacteria to mammalian cells.

Labs Program

Academic labs can also be part of the iGEM community. They can subscribe to the Labs Program to conduct their own research using synthetic biology\ and have access to the same resources as the iGEM competition teams.

Registry



iGEM runs the Registry of Standard Biological Parts. The Registry has over 20,000 standard biological parts, with open access to all participating groups.

Synthetic Biology

based on standard parts

Competition

The iGEM competition is an annual, world wide, synthetic biology event aimed at undergraduate university students, as well as high school and graduate students. Multidisciplinary teams work all summer long to build genetically engineered systems using **standard biological parts** called Biobricks. iGEM teams work inside and outside the lab, creating sophisticated projects that strive to create a positive contribution to their communities and the world.

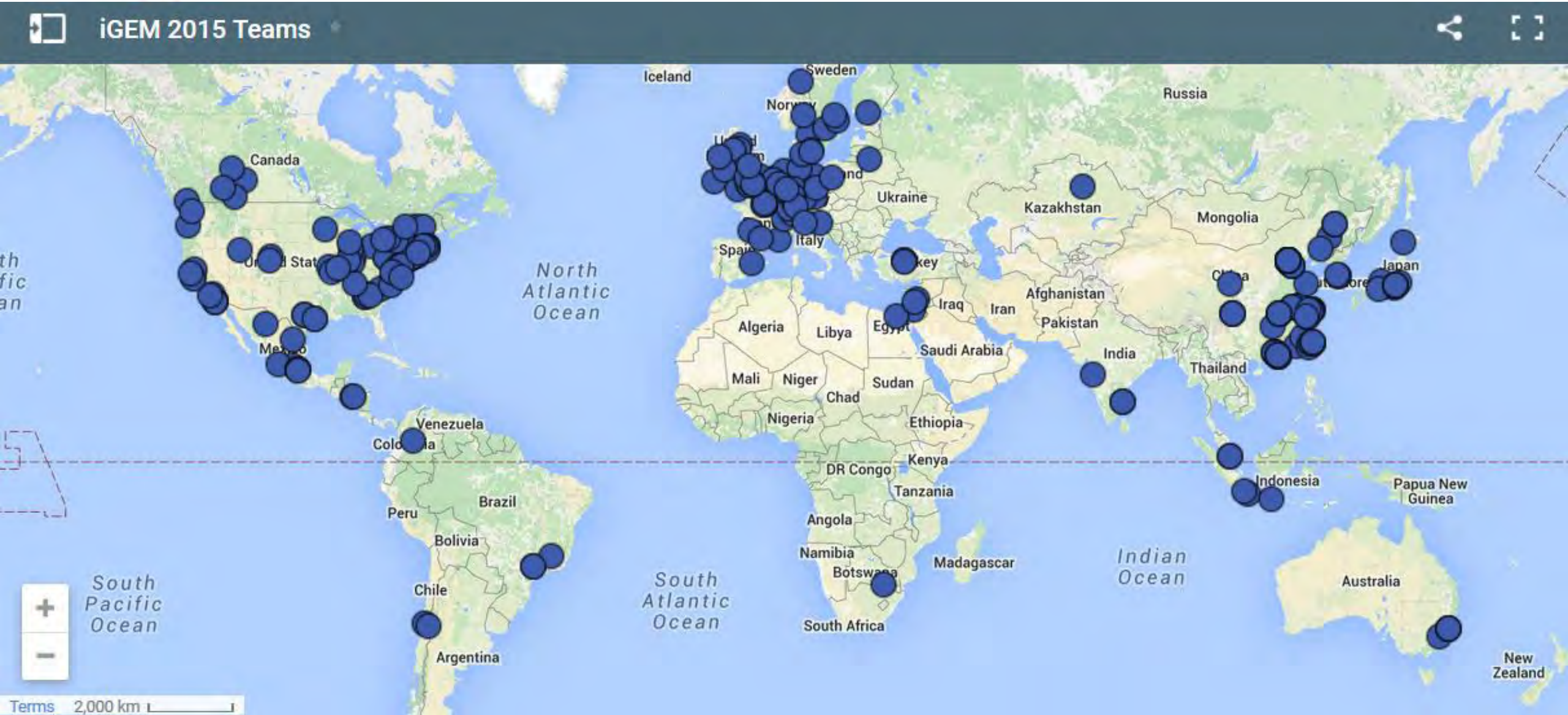
- Energy
- Environment
- Food and Nutrition
- Foundational Advance
- Health and Medicine
- Information Processing
- Manufacturing
- New Application
- Art and Design
- Community Labs
- Hardware
- High School
- Measurement
- Policy and Practices
- Software

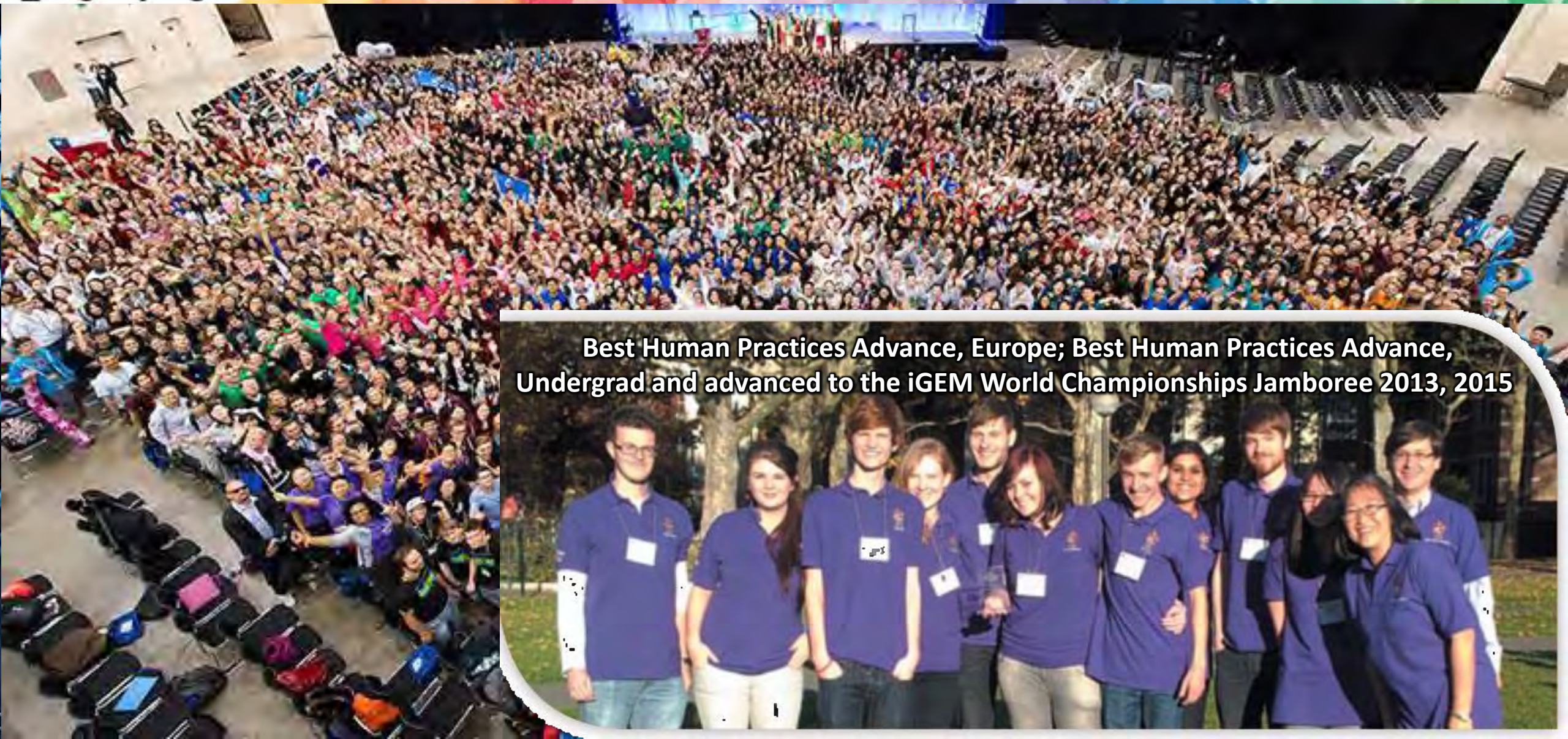
Registration and Other Fees:

- Team registration: A $4000 USD team registration fee is required for each team.
- Giant Jamboree attendance fees: attendance fees for the Giant Jamboree in Boston are $695 per attendee.

Synthetic Biology

based on standard parts





Best Human Practices Advance, Europe; Best Human Practices Advance, Undergrad and advanced to the iGEM World Championships Jamboree 2013, 2015

Acknowledgments

UPLB

BIOTECH

PCIEERD-DOST

The University of Manchester

Manchester Institute of Biotechnology

Discovery through innovation

BCDA

Bases Conversion and Development Authority


CHEERS!!!
UNIVERSITY OF MANCHESTER
MANCHESTER
1824
The University of Manchester