# Synthetic Biology Environmental Scan

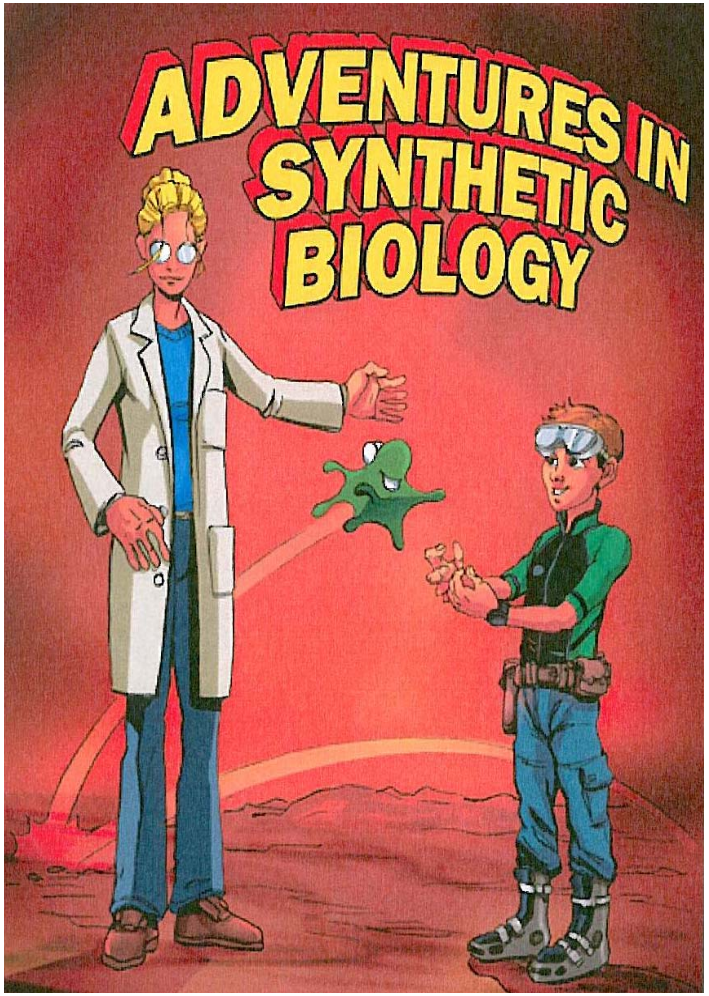
## Technologies & Markets

December 2006

Life Sciences SCOTLAND

iti Life Sciences real possibilities

# Synthetic biology - life is what we make it




Front cover, *Nature* Nov. 24th 2005

***A scientist discovers that which exists. An engineer creates that which never was.***

Theodore von Karman

**Science**: biology & systems biology

↕

**Engineering**: genetic engineering & synthetic biology

**Definitions:**

*Discipline orientated to the intentional design, modeling, construction, debugging and testing of artificial living systems*

Thomas Knight, MIT

*a) the design and construction of new biological parts, devices, and systems and*
*b) the re-design of existing, natural biological systems for useful purposes.*

http://syntheticbiology.org

# Introduction

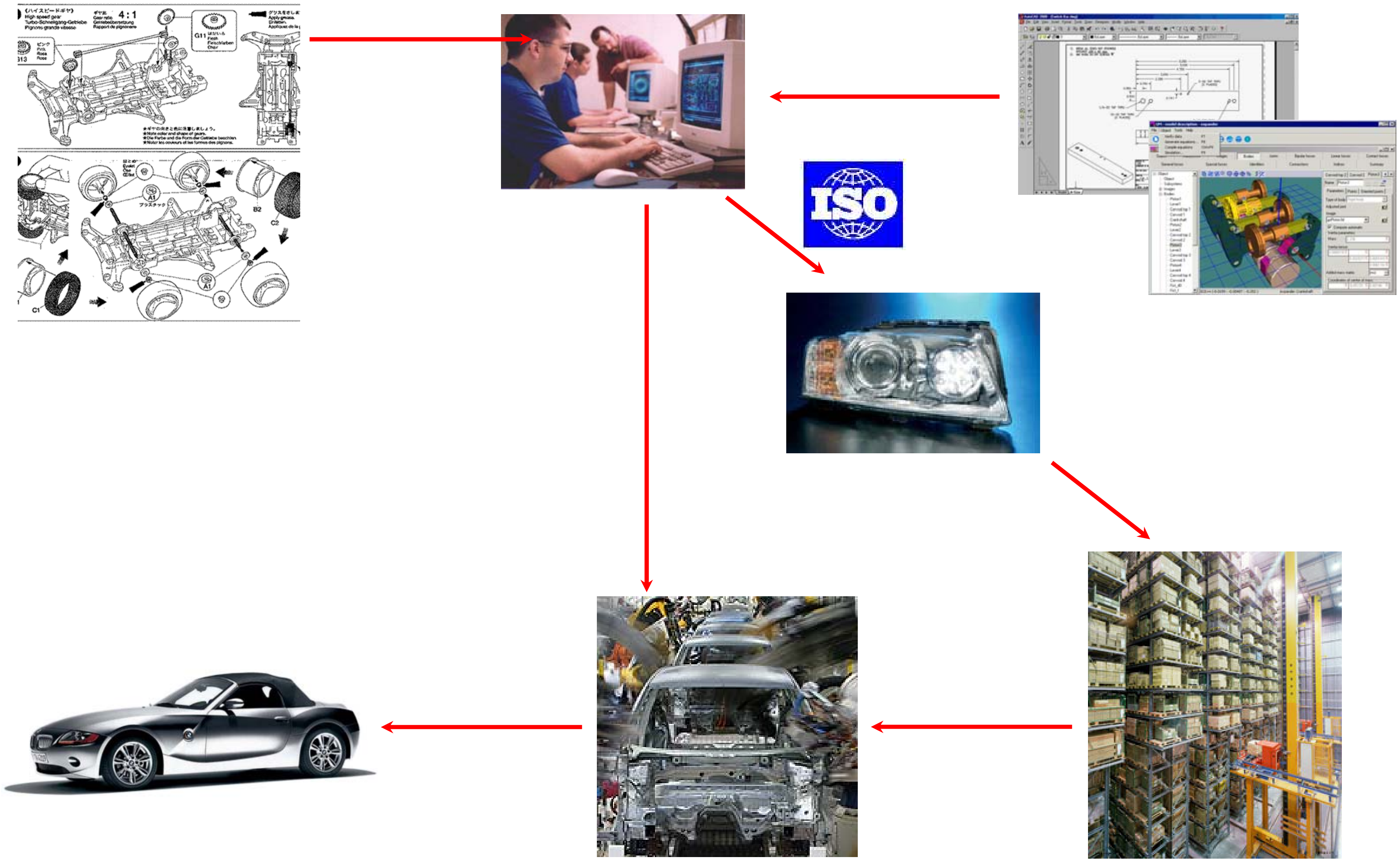
- Relatively young area – definitions emerging
- Very early days and ITI Life Sciences learning as we go
- Promises a lot – what's hype and what's not
- Several applications already emerging
- Technology may allow us to re-visit areas such as biofuels, bioremediation, cost-effective production of natural medicines….
  - Provide tools to circumvent technical impasses facing some of these areas?
- Risks / challenges/ considerations: safety, misuse, ethics, IP

# Classic engineering & synthetic biology

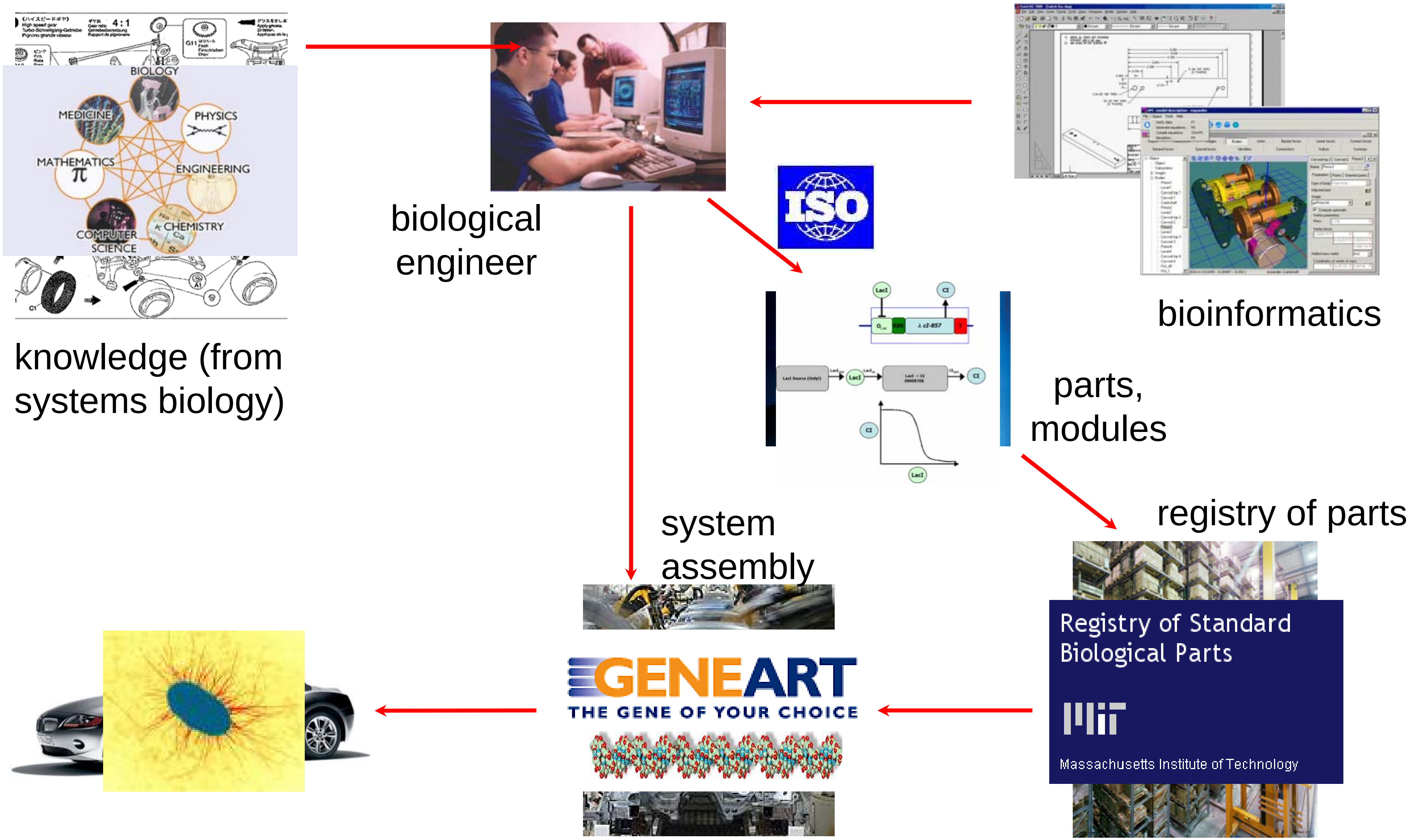
| Engineering | | Synthetic Biology | | Current Biotech |
|---|---|---|---|---|
| Solid knowledge base - mathematical models - *in silico* testing of new design variants | → | From molecular biology to systems biology – implementing a knowledge base | ≠ | Detailed knowledge of selected molecular mechanisms of selected model systems |
| Abstraction hierarchies | → | Modularity of "parts", orthogonal systems | ≠ | "Everything depends on everything" |
| Plug-and-play compatibility, standardization | → | Standardized cloning, parts design, measurement protocols | ≠ | Every DNA construct a piece of scientific art |
| Decoupling of design and fabrication | → | *De novo* DNA design and automatic assembly | ≠ | Design and fabrication are part of the same research and conducted by one and the same scientist |

Heinemann & Panke, *Bioinformatics* (in press); Endy, *Nature* 438:449

# What is real engineering - example



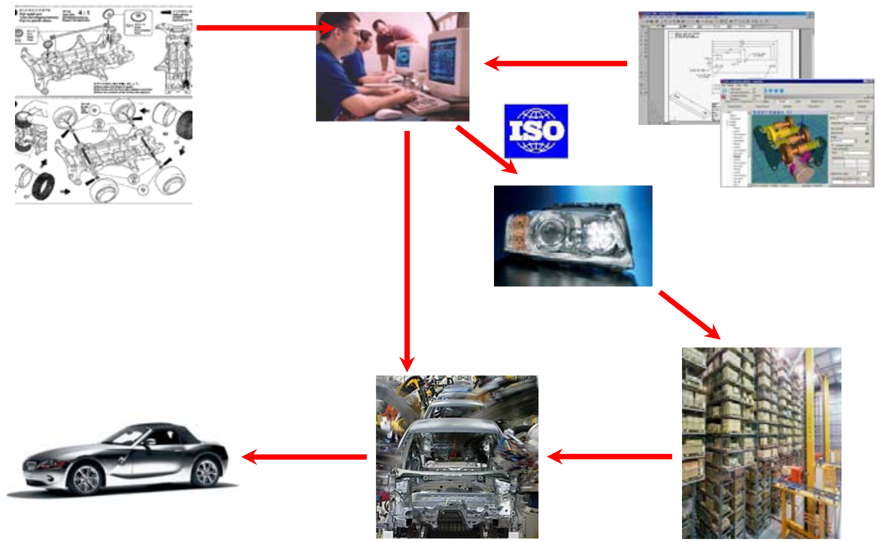
# Synthetic biology or real bioengineering



# Vision: system-level biological engineering

- Forward-**engineering design** (modeling, calculation) of ...

- ... robust, reliable **parts, modules** with defined, standardized interfaces allowing plug and play ...



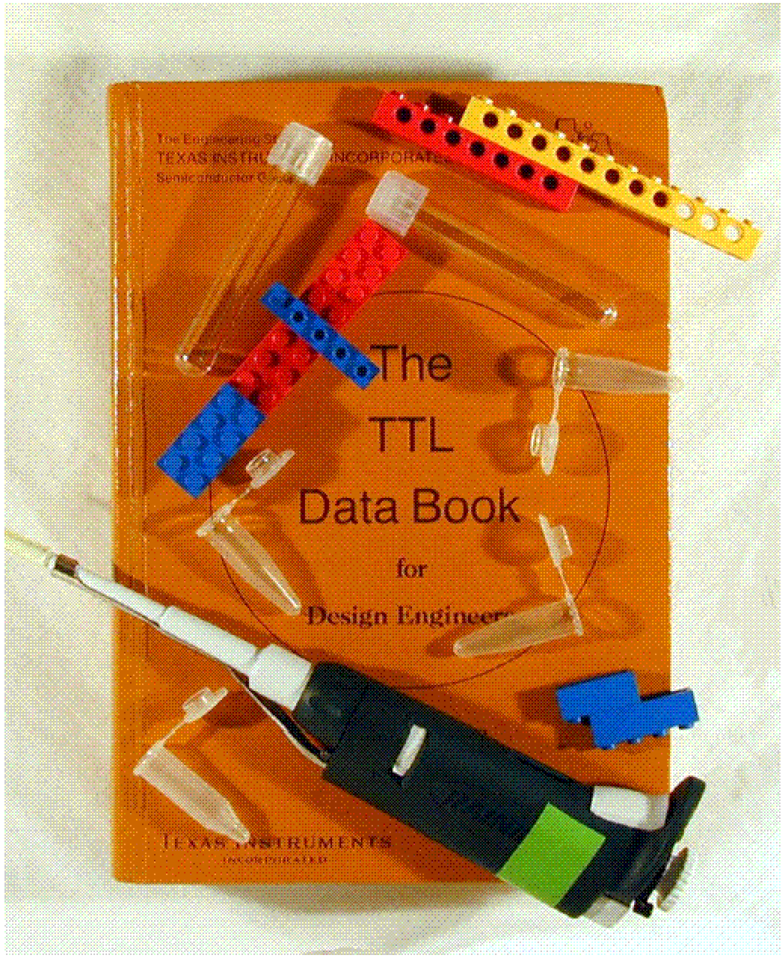

- ... and **combination** of parts and modules **into systems** (requiring system level design)

# Tools and enabling technologies

- Standardised cloning
- De novo DNA synthesis
- Chassis Engineering

# Standardised Cloning

# Standardised cloning





Courtesy of Tom Knight (MIT)

Electrical engineers very familiar with the transistor-transistor logic (TTL) data book of:

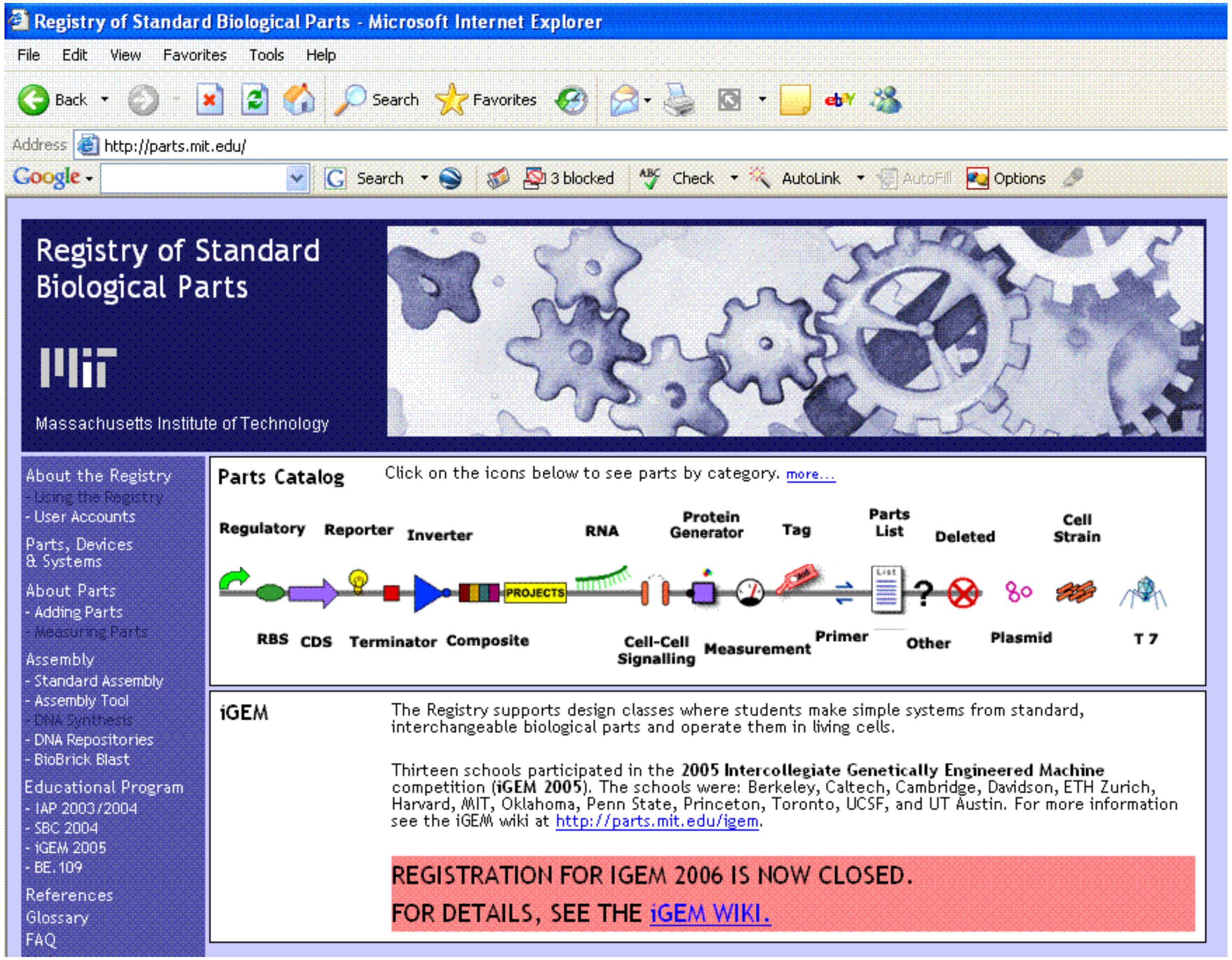
- well characterised,
- properly standardised
- plug and playable…

transistors

Can we write synthetic biology's TTL data book?

# Biobrick warehouse

Making good progress - standardized cloning: design of cloning strategies that can be repeated over and over again (automation) according to the same protocol and....




Warehouse for modular, DNA based parts that are combined to generate devices and systems

Parts:
Promoters
Ribosome binding sites
Coding sequences
(eg reporters)
Terminators
Degradation tags

Devices:
Toggle switches

# De novo Synthesis

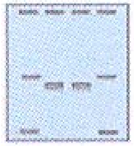
# *De novo* synthesis (typical vs optimised)

Design oligos

↓

Synthesize oligos

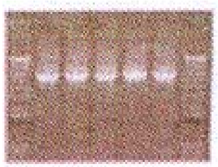
↓



Purify oligos and turn into ds inserts

↓

Amplify and purify

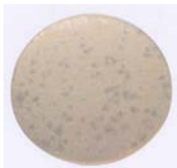


↓

Circularize

↓

Place in *E. coli* & pick



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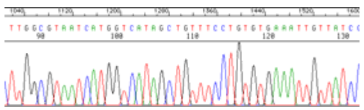
Sequence phages

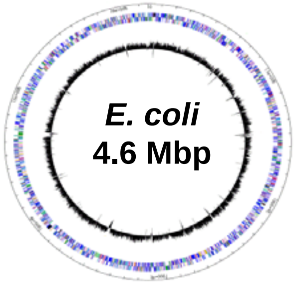


- The **Church**-lab has come up with a microchip-based low-error frequency and fast DNA-synthesis technology
- 1 error in 1400 base pairs (PAGE purification – 1 in 450bp)
- Able to synthesise all 21 genes that encode the proteins of the *E. coli* 30S ribosomal subunit (14.6kb) in 3 days
- Aim to drive cost of synthesis error-free oligos well below $1/bp

Tian *et al*., Nature 432: 1050 (2004)

# Chassis Engineering

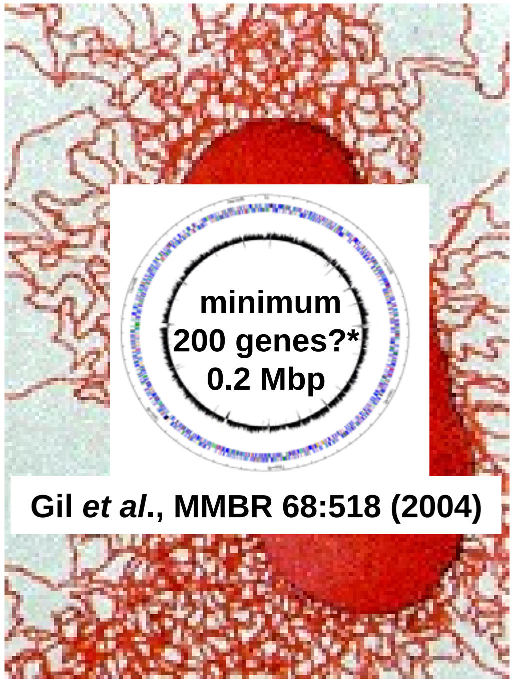
# Minimal genomes – reduced complexity + chassis to build upon



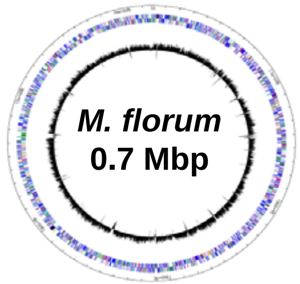

**Stripping down**

**Blattner-lab**
**Science 312: 1044 (2006)**

**MDS41-3**
**4.1 Mbp**




*Mesoplasma florum*
**Knight lab**




## Minimal genome as a chassis

## Reduced complexity – improved orthogonality

Rationally re-consitute more complex organisms
Rewrite genome: redefine codons, expand genetic code

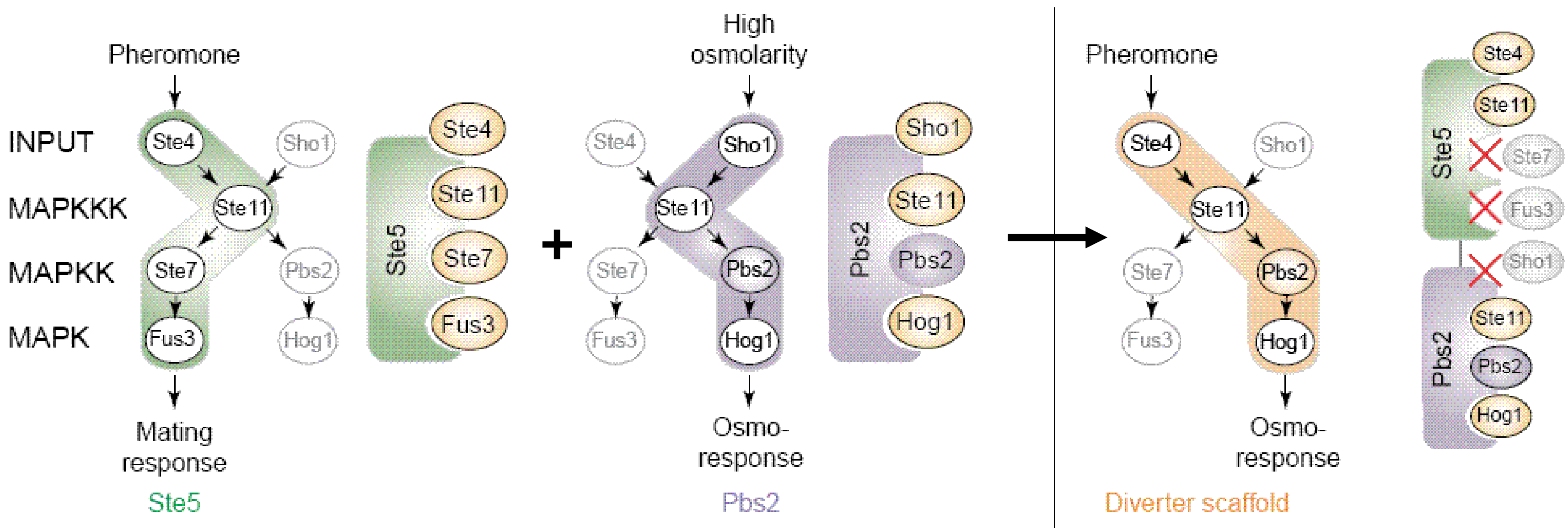
***200 genes is theoretically sufficient to sustain life under the most favourable laboratory conditions**

# Examples and applications

- Modular protein parts
- Engineering of systems

# Modular protein parts – example

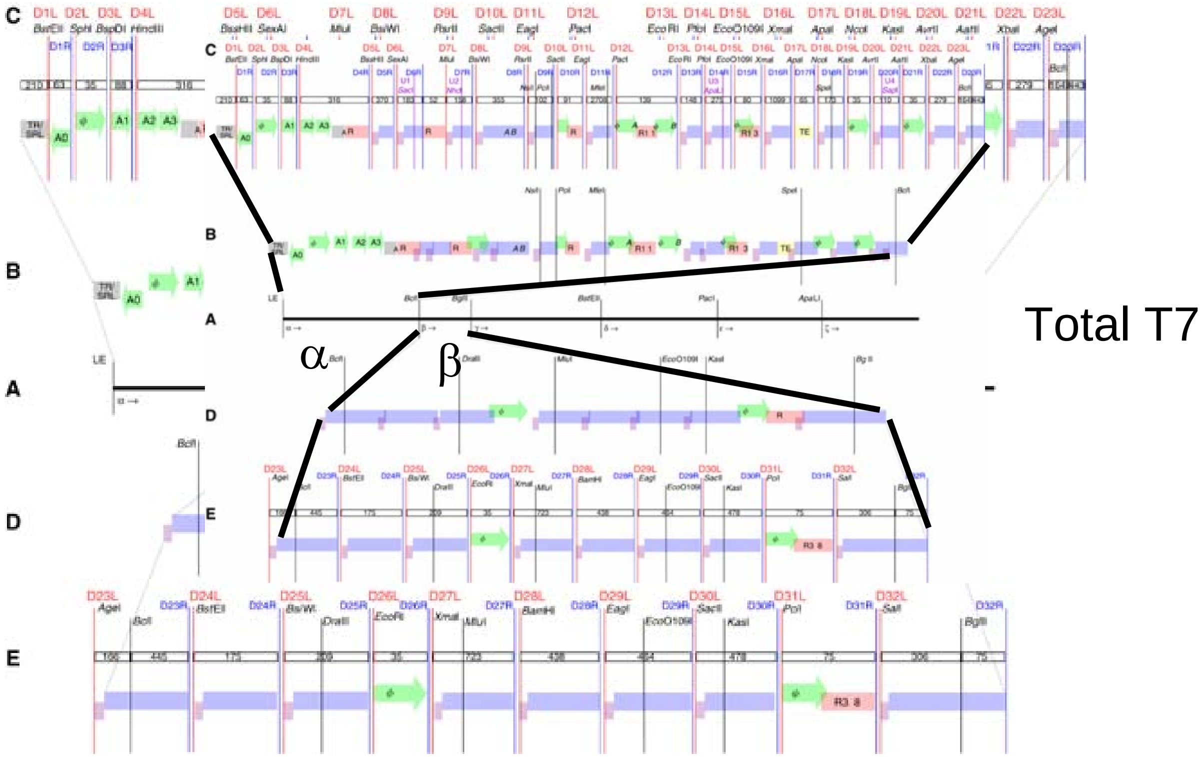
- MAPK signalling cascades – kinases in cascade are attached to a protein scaffold
- Cells bearing a hybrid constructed scaffold generate an osmolarity response to a mating signal




**WA Lim lab, eg , Science 299 (2003): 1061**

# Engineering of systems – example 1.

Is it possible to adapt biological systems to our ideas of engineering standards? i.e. Easier to study and manipulate - re-engineering 25% of phage T7 genome, eliminating “double use” sequences and introducing restriction sites

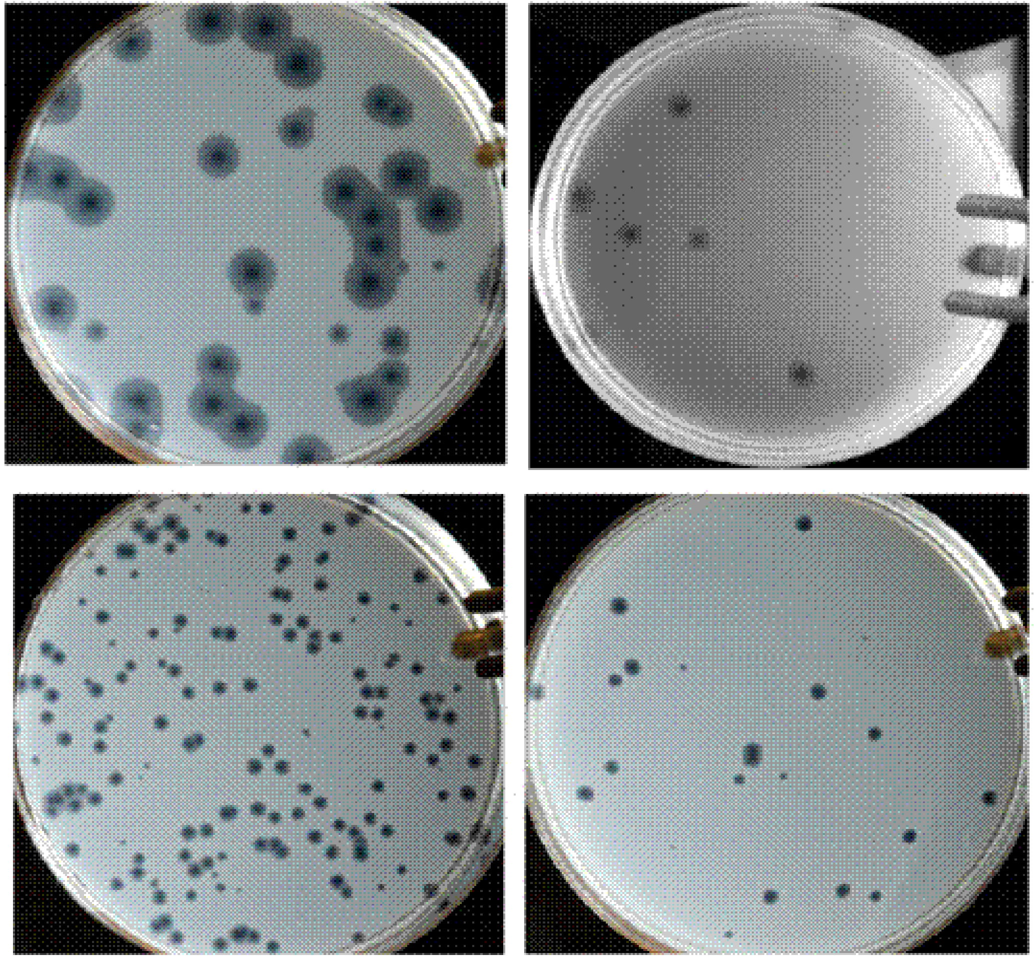



**Same information after re-engineering. Note de novo synthesis of DNA**

**Endy lab, Mol. Syst. Biol. 2005**

# Engineering of systems – example 1.

Re-engineering 25% of T7 genome and still get functional phage particles



wild type T7

α-wt

α-β-wt

wt-β-wt

# Engineering of systems – example 2.

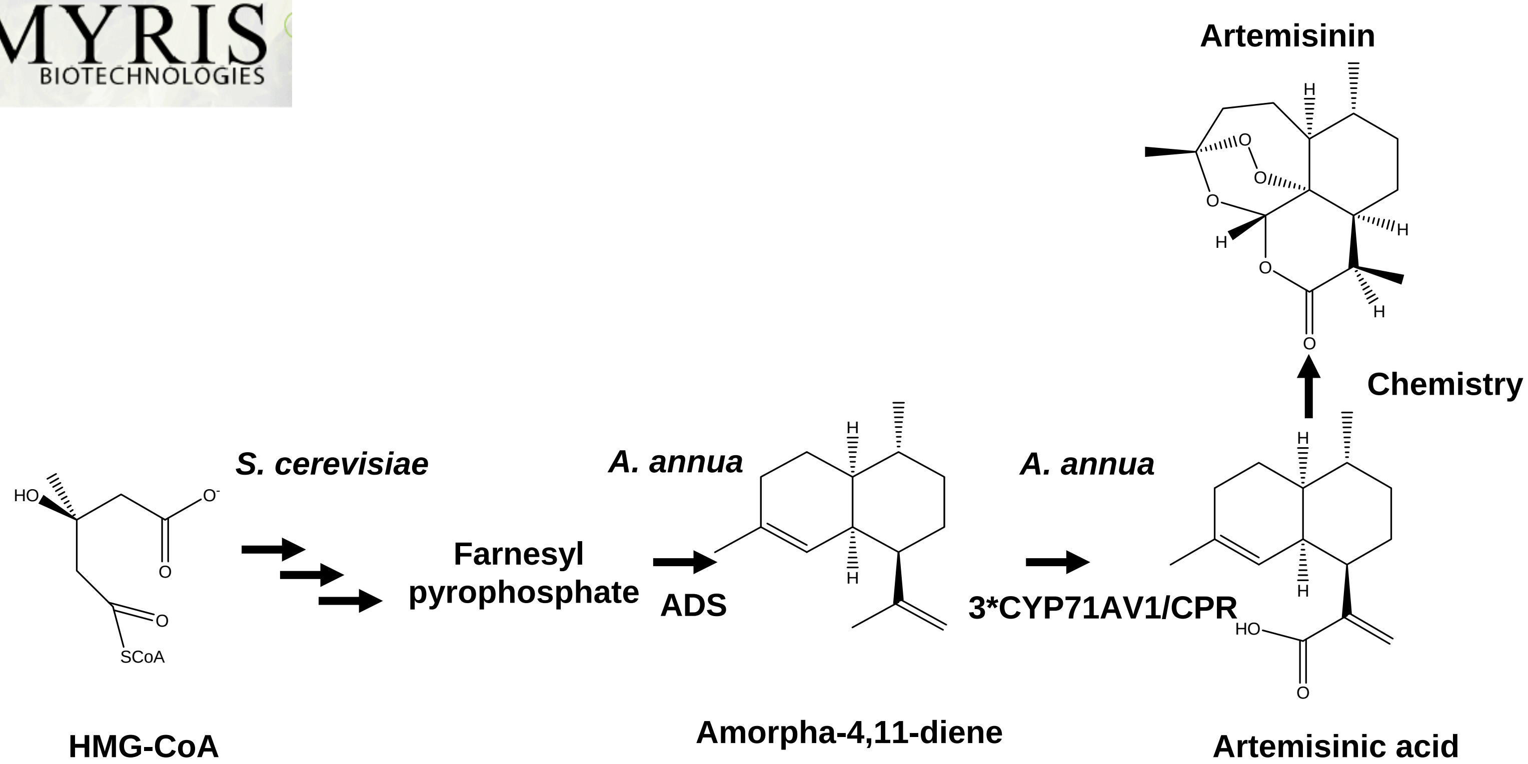
- **Artificial pathway building** - synthetic biology should enable organisms to perform complex multi-step syntheses of natural products by assembling “cassettes” of animal or plant genes that code for all of the enzymes in a synthetic pathway.

- Jay Keasling’s Amyris Biotechnologies has engineered a new metabolic pathway in *E. coli* (and *S. cerevisiae*) that allows the rapid and inexpensive production of a precursor to the **malaria drug artemisinin**
  - Very effective antimalarial which is active against all known strains of malaria
  - But artemisinin is relatively scarce (extracted from dry leaves of the sweet wormwood tree) - too costly for developing countries

- Genes coding for in essence reactions along the pathway are selected from multiple organisms. The **optimized DNA is then *de novo* synthesized**
  - Current genetic engineering problem - multiple codons coding for the same amino acid and different organisms make different choices of which codon to use, so when the gene of one organism is put into another, expression levels may be affected.
  - De novo route means codon-optimized DNA (i.e. can only make one correct protein product) can be synthesised

# Engineering of systems – example 2.

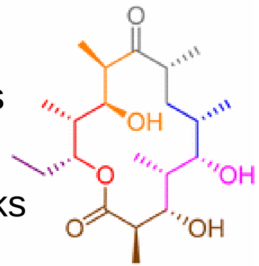
AMYRIS BIOTECHNOLOGIES




Very short turnaround times from strain generation to strain generation - very different situation to **genetic engineering**

# Also opportunity for generation of....

- **High-value pharmaceuticals (natural &/or complex) – lower cost production**
  - **Arixtra -** anticoagulant that has a highly complex chemical structure (contains 5 sugar units) and is difficult to manufacture on a large scale at low cost
  - Isoprenoids – members of class include Artemisinin and Taxol.
  - **Polyketides** – complex natural products produced by microorganisms. Numerous drugs spanning many therapeutic areas: antibiotics (erythromycin), immunosuppressants (rapamycin) and anticancers (doxorubicin). Polyketides are synthesised in blocks of modules. The DNA responsible for each block can be synthesised de novo. These blocks of DNA can then be combined in many different ways to create novel molecules

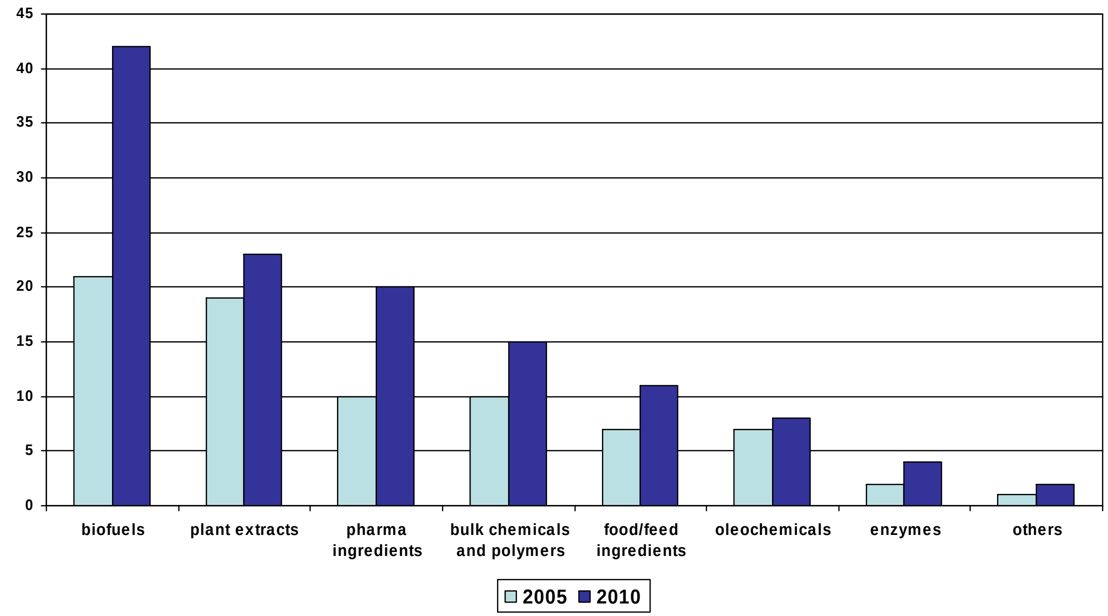



6dEB

- **Biofuels**
  - Synthetic Genomics plans to develop a proof of concept in either of two bio-energy applications - **hydrogen or ethanol**.
- **Specialty chemicals, feedstocks and intermediates**
  - BASF's vitamin B12: reduced complex 8-step chemical synthesis to new 1-step fermentation process. Reduces overall costs and overall environmental impact
  - Most organic chemicals derived from petroleum. Nature produces ~200 billion tonnes of plant biomass per annum by photosynthesis. Many chemicals normally got from fossil resources can be got from biomass (depends on economics)
- **Others: polymers, nutraceuticals**
  - Replacing polymers from oil with polymers made from sugars (DuPont's Sorona) or corn (Cargill Dow's NatureWorks)

# White biotech / industrial biotech

**Impact of industrial biotechnology (in billion EUR)**




2005: 77 billion EUR IB related sales in chemicals (7% sales of the chemical industry)
2010: 125 billion EUR IB related sales in chemicals (10% sales of the chemical industry)

McKinsey - 2006

- McKinsey & Co. predicts that by 2010 industrial biotechnology will account for 10% of all chemical industry sales (equates to €125 billion).
- Much of the projected growth in adoption of industrial biotechnology is attributable to biofuels (ethanol and biodiesel – see ITI LS' Biofuels foresighting).

# Leading players

Several synthetic biology start-ups have attracted venture funding:

synthetic genomics

- $30m from VC Draper Fisher Jurvetson and two other investors

CODON DEVICES

- $13m from Flagship Ventures, Alloy Ventures, Kleiner Perkins Caufield & Byers (KPCB) and Vinod Khosla
  - Founded by synthetic biology pioneers including George Church, Drew Endy and Jay Keasling.
  - Created a stretch of DNA more than 35,000 letters long (approx. ten genes present). Competing with Blue Heron Biotechnology, DNA 2.0 and Geneart.

CODA

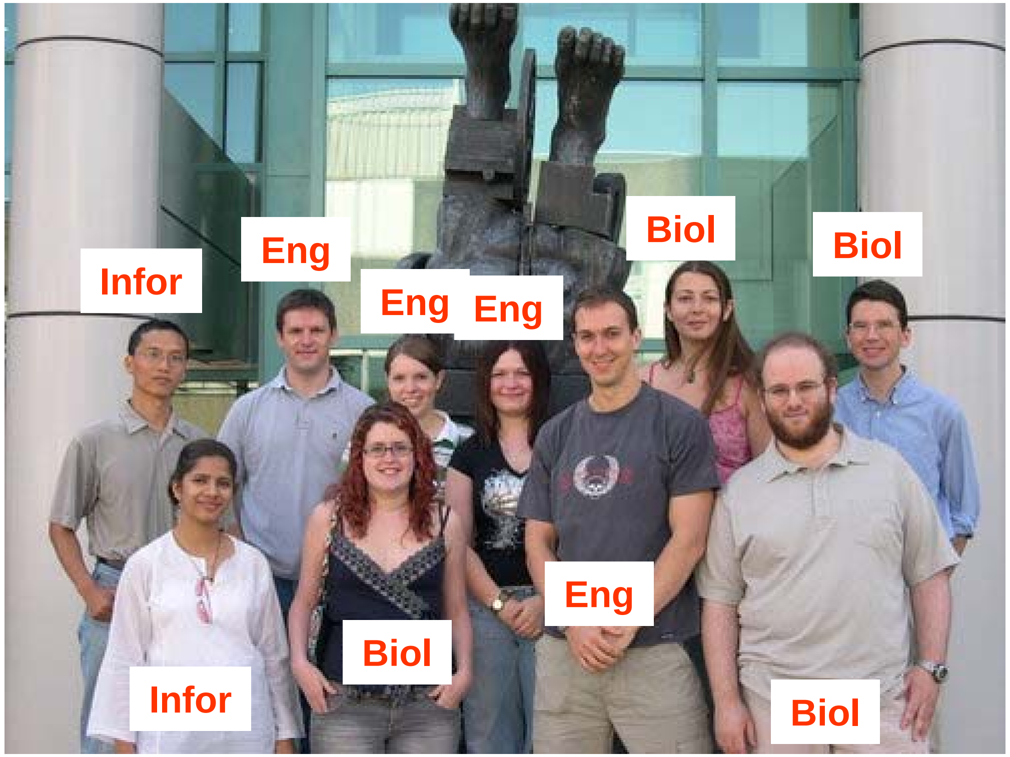
- $0.8m in Series A funding from the Tech Coast Angels.

AMYRIS BIOTECHNOLOGIES

- In October 2006, raised $20 million in a Series A funding from Khosla Ventures, KPCB, and Texas Pacific Group Ventures.
  - Follows a large grant from the Bill & Melinda Gates Foundation awarded to Amyris and OneWorld Health (first nonprofit pharma in US). Amyris has also started a second programme developing biofuels

# The International Genetically Engineered Machine competition

iGEM 2006




THE UNIVERSITY OF EDINBURGH
2006 iGEM

1st place: Best Poster
1st place: Best Real World Application
3rd place: Best Device

U of E team designed and modeled a biosensor that can detect the WHO guideline level of 10 ppb for arsenic in drinking water and emit a pH signal in response. A proof of concept Biobrick construct has shown a pH response to a concentration of arsenic of 5 ppb.

**37 teams (selection below) took part in iGEM 2006.**

# MIT's iGEM team set out to sweeten *E. coli*

- The students looked for genes that convert chemicals naturally made by bacteria into chemical precursors of aromatic compounds, as well as genes that convert the precursors to the aromatics themselves
  - e.g. isoamyl acetate is a component of the ripe-banana smell

- Plasmids were then generated with these plant genes under the control of bacterial promoters. They also eliminated the gene responsible for *E. coli*'s natural smell. The result was a new strain of *E. coli* that smells of bananas.

- One aim of iGEM is to stock shelves of the Registry of Standard Biological Parts.

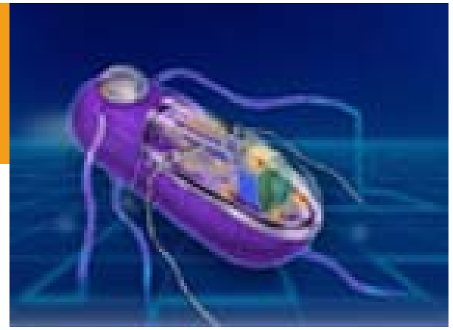
- While the projects are executed largely by undergraduate students (with faculty guidance), the designs represent some of the most complex biologically engineered machines to date.

# Ethics & risks (biosecurity and biosafety)

- Synthetic biology community keen to **regulate themselves** in order to ensure good practice and to address a range of concerns about their research
- Self-regulation largely focused on **biosecurity** (i.e. preventing new opportunities for bioterrorists) in an attempt to act proactively and prevent overly restrictive regulations being imposed
- However, concerns raised by groups such as Greenpeace and Genewatch UK (object to self-regulation) deal with the **biosafety** aspects, i.e. the uncertainties and unintentional consequences of synthetic-biology research, as opposed to its deliberate misuse.
- Biosafety is going to be a significant issue, especially in Europe. Compare SB with genetically modified crops – similar characteristics in terms of public perception of **novelty**, **uncertainty** and **controllability**
- **Ethics**
  - Laurie Zoloth's recent lecture on ethics & SB: http://syntheticbiology.org/Documents.html)
  - Markus Schmidt - Coordinator of the EU FP6 New and Emerging Science and Technology (NEST) "SYNBIOSAFE: Biosafety and ethical aspects of synthetic biology“. http://www.markusschmidt.eu/
- **IP -** sharing discoveries (e.g. parts) key but out of sync with biotech's modus operandi.

# Next steps…

- This is an ongoing process and this *Environmental Scan* is the precursor to a more in-depth foresighting report which will be released later in 2007
- Further analysis will seek to establish:
  - What is simply hype and what represents true opportunity
  - The level of maturity of key technologies
  - The dependency on systems biology
  - Expertise and appetite level within Scotland
  - The barriers – including safety/security, IP cost and competion
- The primary goal of the foresighting process is to identify specific opportunities (e.g. DNA synthesis) and establish the potential for generating IP
- To ensure that our foresighting is as comprehensive and rigorous as possible, and to determine if and where opportunities lie, we would very much welcome dialogue with our members



- To arrange a discussion, please contact
  - foresighting@itilifesciences.com