Building Living Machines with Biobricks

The Promise of Synthetic Biology

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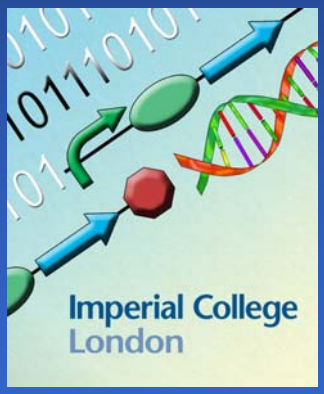


Complementary Material on Biological Engineering

Imperial College London

Department of Bioengineering

Division of Molecular Biosciences

Imperial College London

100 years of living science

100

Engineering Concepts

What is Engineering?

- **Definition**

Engineering is the application of science in the design, planning, construction, and maintenance of buildings, machines, and other manufactured things

- **Key Notions:**

Three key engineering notions have triggered innovation and accelerated development: **Abstraction**, **Standardisation** and **Characterisation**

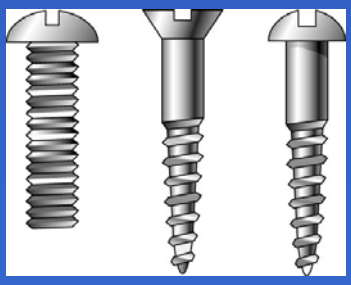
Parallel with other industrial revolutions			
Engineering Concepts	**Mechanical Industrial Revolution**	**Digital Industrial Revolution**	**Synthetic Biology Revolution**
Abstraction	Iron, Steel --> Nuts & Bolts --> Jet Engine --> Plane	Silicon --> Microprocessors --> Computers --> Computer Networks	DNA --> BioBricks Parts --> Devices --> Systems
Standardisation	Nuts, Bolts, Screws sizes, Material properties, ISO norms	Electronic interfaces (USB, Serial port, PCI), Communication protocols (HTTP, GSM), Programming languages (Java, C++, ANSI)	Biochemical interfaces, standardised host, standard genetic assembly
Characterisation	Mechanical component tolerance, stress and fatigue resistance	CPU Speed, Bandwidth, Quality of Service and Error Management	Performances in different conditions (hosts, media cultures, temperatures)

Engineering Concepts

Abstraction:

Definition: The process of generalization by reducing the information content of a concept or an observable phenomenon, typically in order to retain only information which is relevant for a particular purpose

Economic advantages: Different companies can specialise in specific issues



The humble screw: *Without standards, we could not fix things together…*

Standardisation:

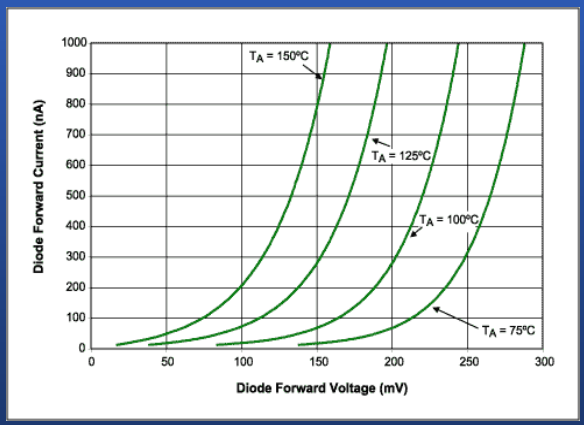
Definition: The process of establishing a standard (i.e a basis for comparison) among competing entities in a market

Economic Advantages: Standards promote innovation and competition. Their application is voluntary and does not block or restrict technological progress

Characterisation:

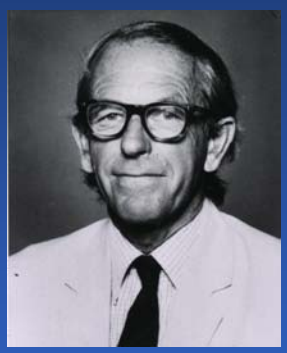
Definition: Quantification of behaviour and performances of a system with regards to specifications

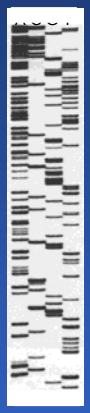
Economic Advantages: It is easier to build up trust in the market



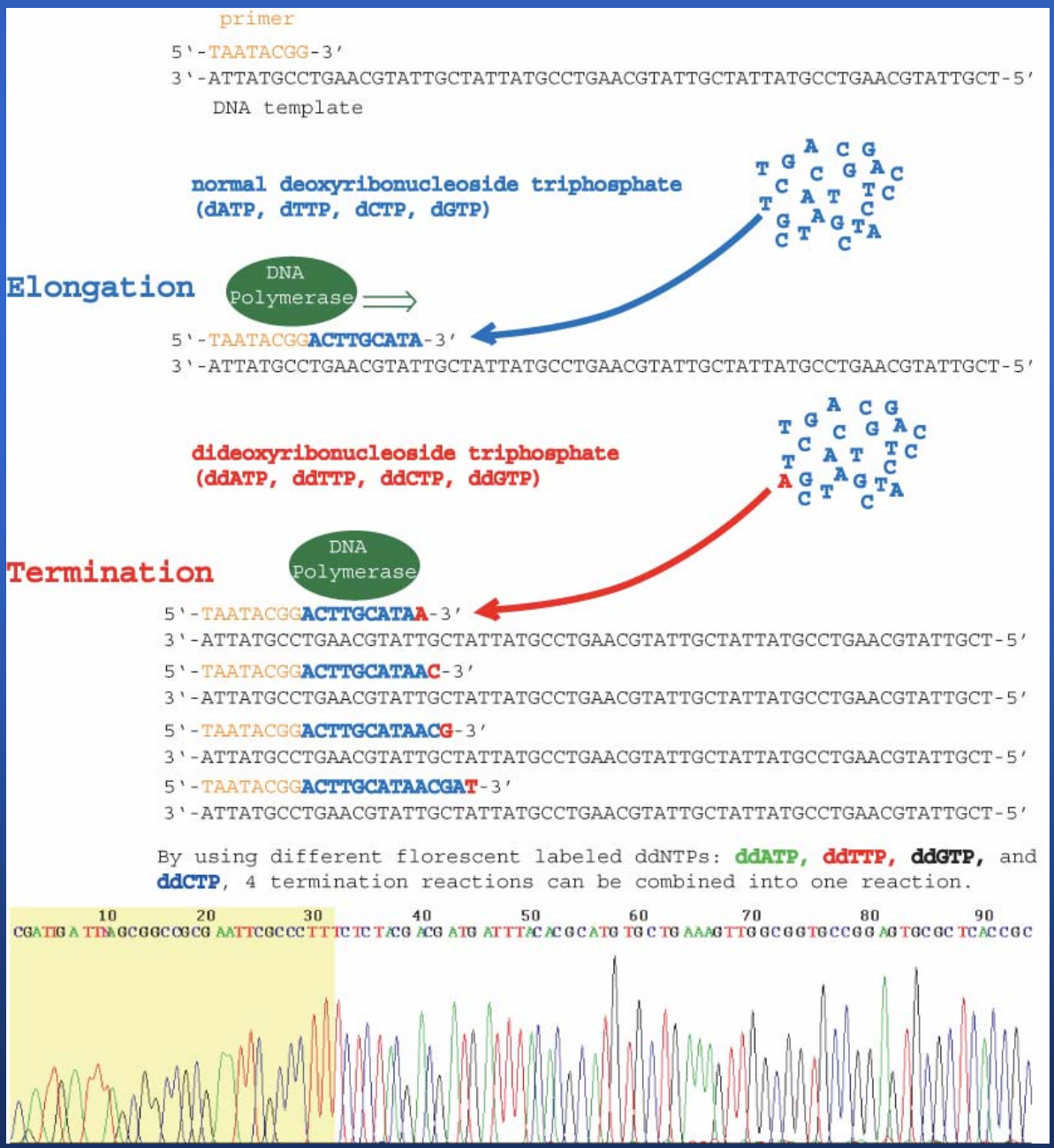
Characterisation of a Diode

DNA Sequencing



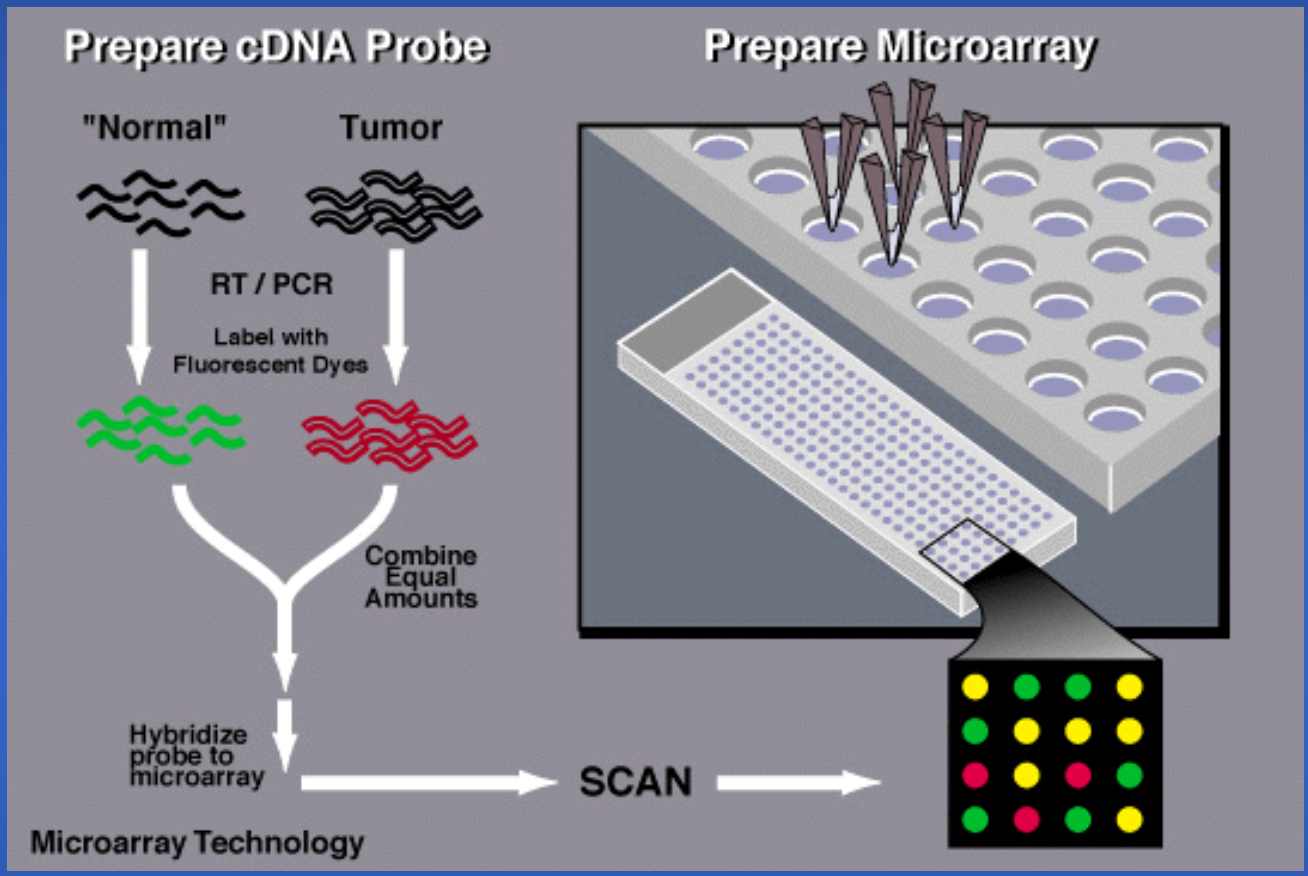


Frederick Sanger developed the DNA sequencing technique in the late 1970s. It was used to determine the order of bases within genes. Now it is used routinely to confirm DNA sequences (fidelity) in molecular biology research.



Oligonucleotide Microarray

The technique was developed to allow quick detection of a large number of expressed or activated genes (mRNA). Such transcriptional profiles are used to study which genes are turned on or off in abnormal disease states like cancer.



Up-regulated

Down-regulated

Normal

Polymerase Chain Reaction (PCR)

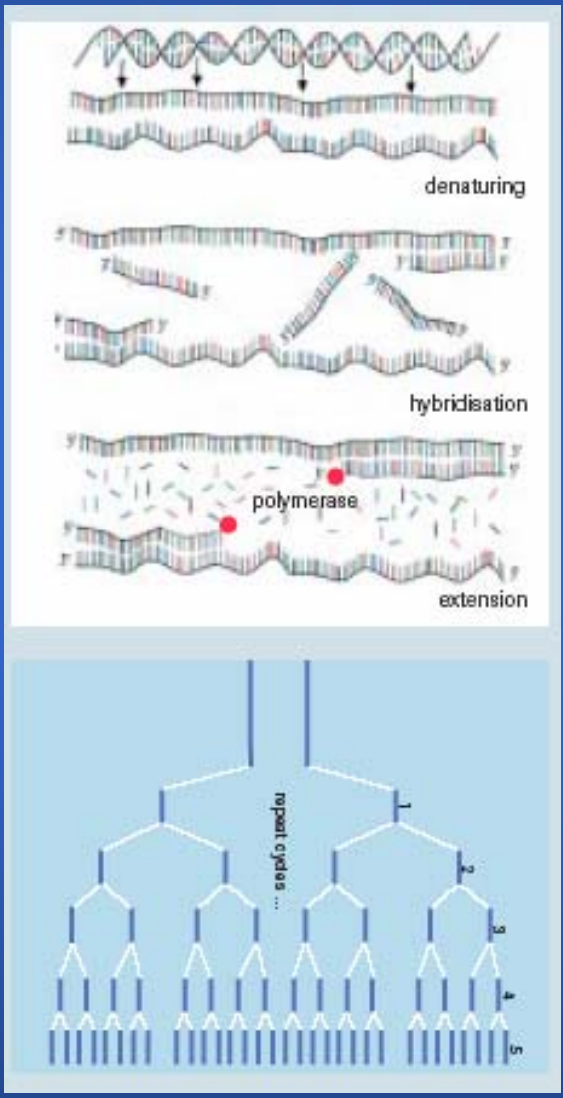
The purpose of PCR is to make a large number of copies of a gene of interest. This allows researchers the ability to amplify any DNA sequence from any source.

There are 3 steps in a PCR repeated for 30-40 cycles.

• Denaturation at 95˚C: the double stranded DNA melts open to single stranded DNA.

• Annealing/hybridisation: The primers anneal to the DNA.

• Extension 68˚C or 72˚C: The polymerase copies the template adding the dNTPs from 5’ to 3’.

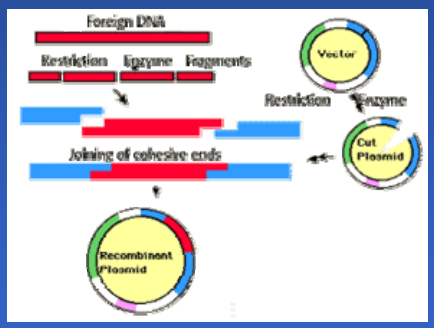


(Workflow from Roche)

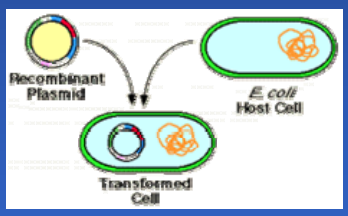
Recombinant DNA

Recombinant DNA is a form of artificial DNA which is engineered through the combination or insertion of one or more DNA strands, thereby combining DNA sequences which would not normally occur together.

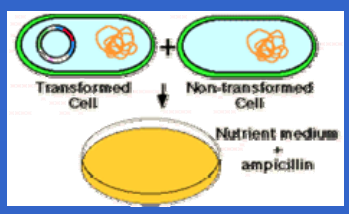
1. Creating recombinant DNA



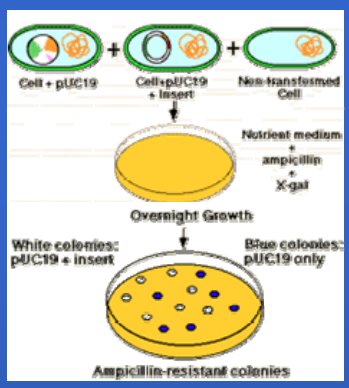
2. Transformation/incorporating the recombinant plasmid into bacteria



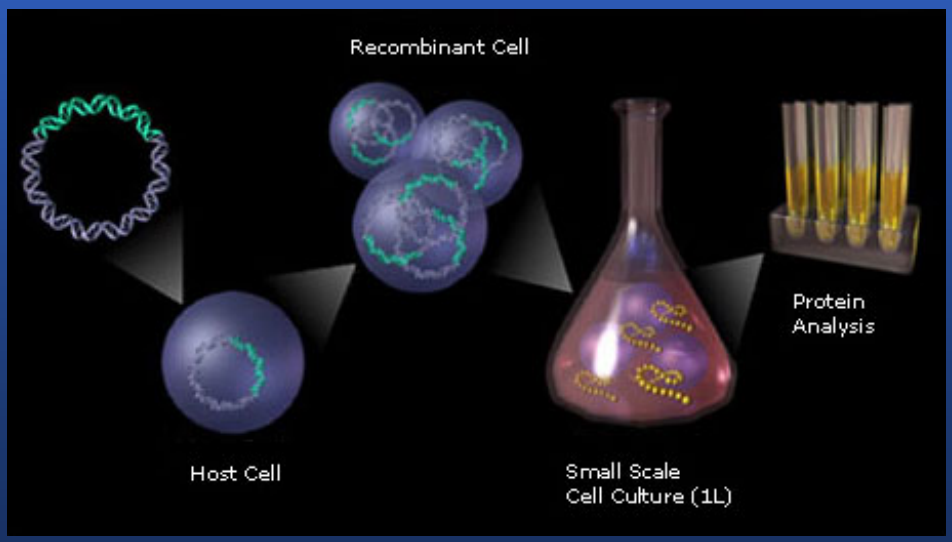
3. Selecting bacteria resistant to antibiotic



4. Insertional mutagenesis identifies plasmids with DNA inserts



Taken from http://www.biology.arizona.edu

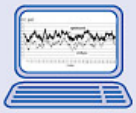


Taken from http://www.gene.com

DNA Synthesis

DNA synthesis is the process of synthesizing any DNA sequence in a test tube. This allows biologists to create DNA of different lengths, from individual genes to whole genomes.

Bioinformatics



- Design fragments and oligos
- Align every oligo with each other and check for potential false hybridisations

Oligosynthesis

- Chemical synthesis of short DNA oligonucleotides

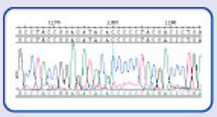
Gene Assembly

- Self-organising molecules: smart designed short DNA oligonucleotides

Cloning

- Cloning of the synthesized gene

Sequencing



- Sequencing of the synthesized gene
- Reading of every nucleotide ensures 100% correct end product

Final Documentation

- Final documentation and data transfer to the customer

Taken from www.geneart.com

GENEART
THE GENE OF YOUR CHOICE

An Open Source Approach to Biology

Why Open source ?

- **To Foster Innovation**
  - Simple things given away for free
  - Complex systems built more easily
  - Complex systems (where a profit can be made) can then be patented

Linux : *the most famous open source project.*

- **To Improve the Quality of the Parts**
  - Everyone can check the parts and the experimental data
  - More eyes, fewer errors go unnoticed

Collaboration in Synthetic Biology

- **Biobricks Foundation**
  - Repository of Biobricks at MIT
  - Website at parts.mit.edu

Parts.mit.edu: *The Biobrick Registry*

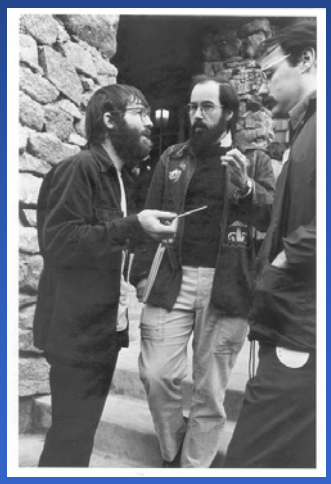
- **OpenWetWare**
  - Online Community
  - Research Material, Information…
  - Educational Material
  - Use of Wiki Software

OpenWetWare: *the Synthetic Biology Online Community*

Open Discussions on Biology

Ethics and BioSafety

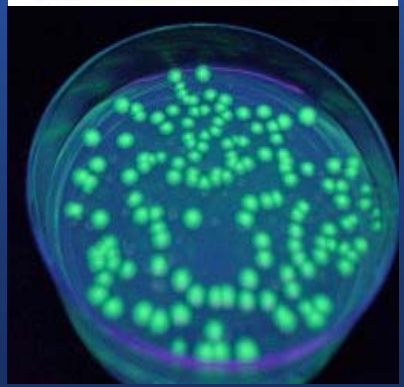
- **Recombinant DNA (1973):** the powerful technique led scientists to
  - Have a Moratorium until 1975
  - Develop Protocols of Self-Governance (Asilomar Conference)
- **A Generation Later**: New questions raised by DNA Synthesis



The Asilomar Conference (February 1975)
The conference was organized by Paul Berg to discuss the potential biohazards and regulation of biotechnology.

Intellectual Property

- **Goal:** Creation of a legal framework where innovation thrives
- **How:** Striking a trade off between protection for the inventor and open-ness so experimentation can take place



An example of Patent in Biotech: the use of GFP

The fluorescent protein GFP originates in jellyfish. Its use was patented in the early 90s. However, the GFP Biobrick is in the Registry allowing researchers to use it for their projects.