The Eureka Moments in Science

Tokyo, May 26, 2015

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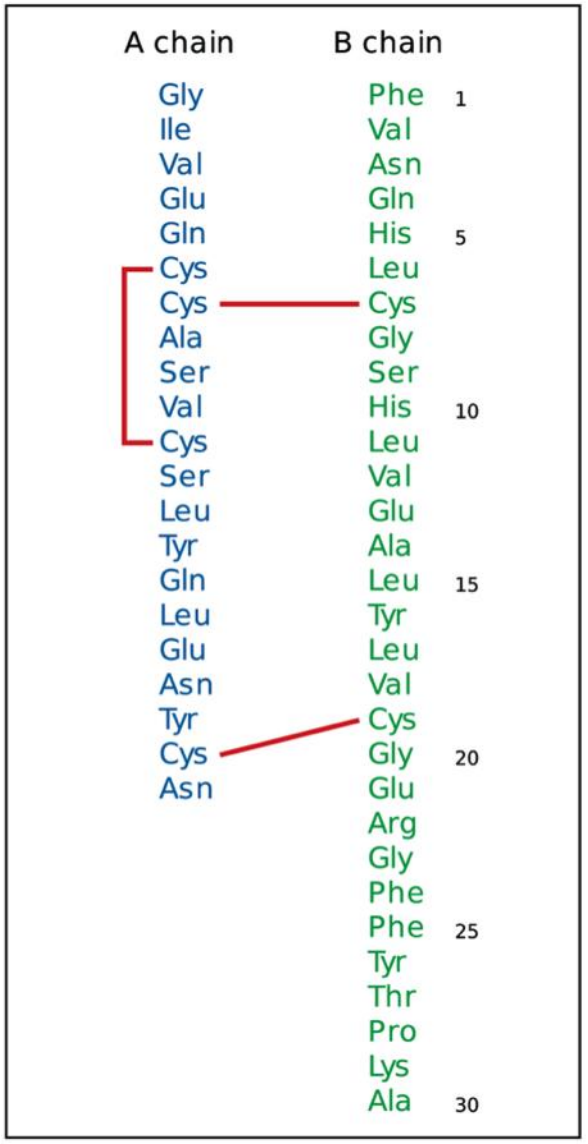
Nobel Prizes and Life Sciences

Erling Norrby

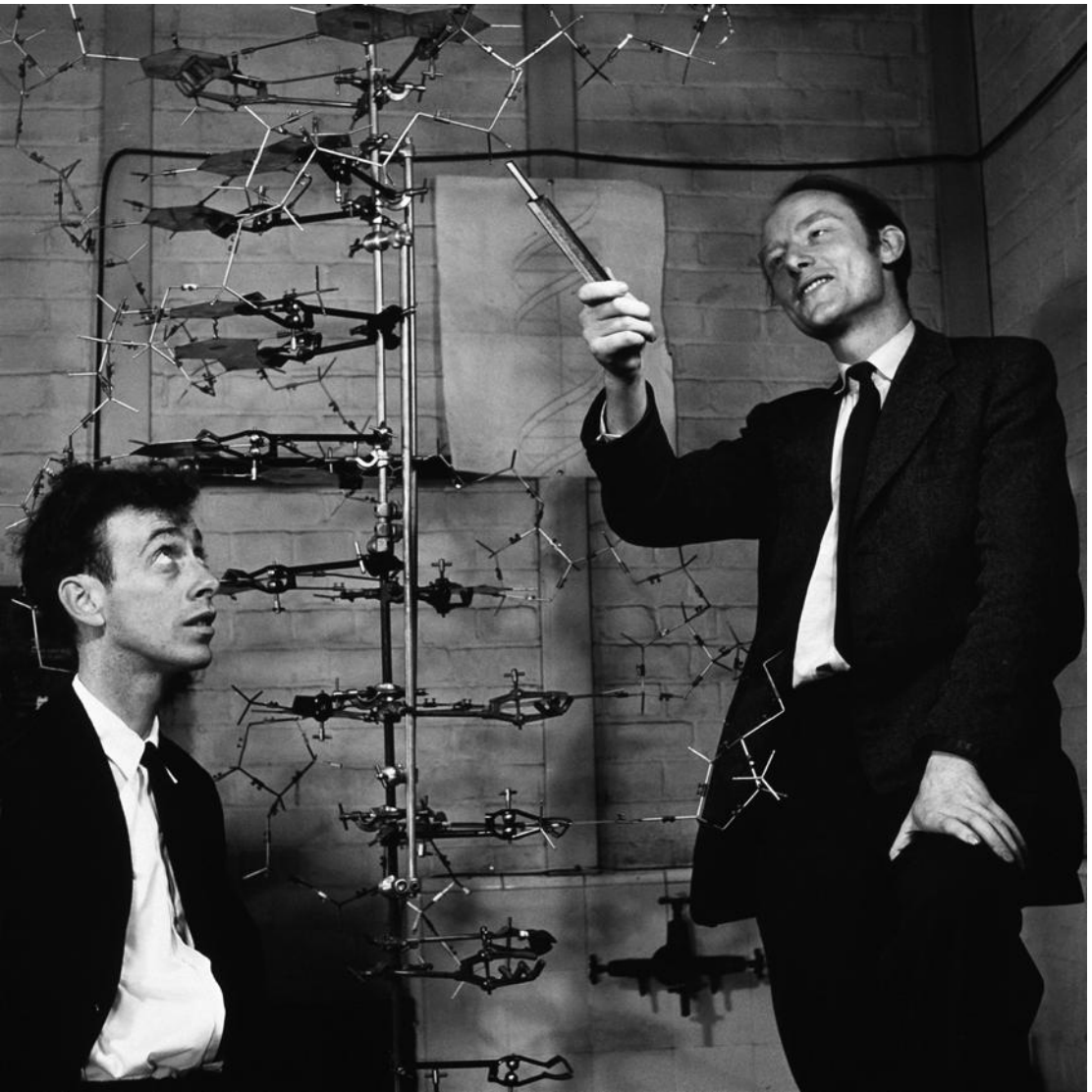
Nobel Prizes and Nature's Surprises

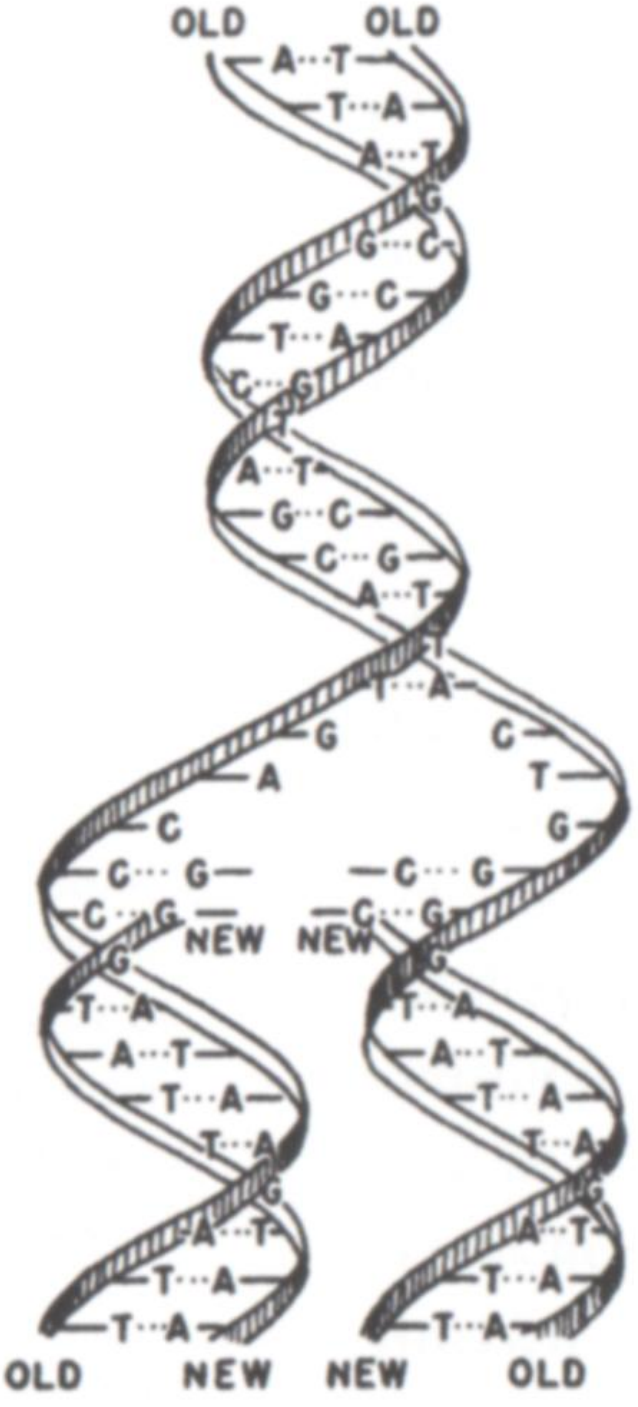
Erling Norrby

Frederick Sanger receiving the Nobel Prize in Chemistry 1958

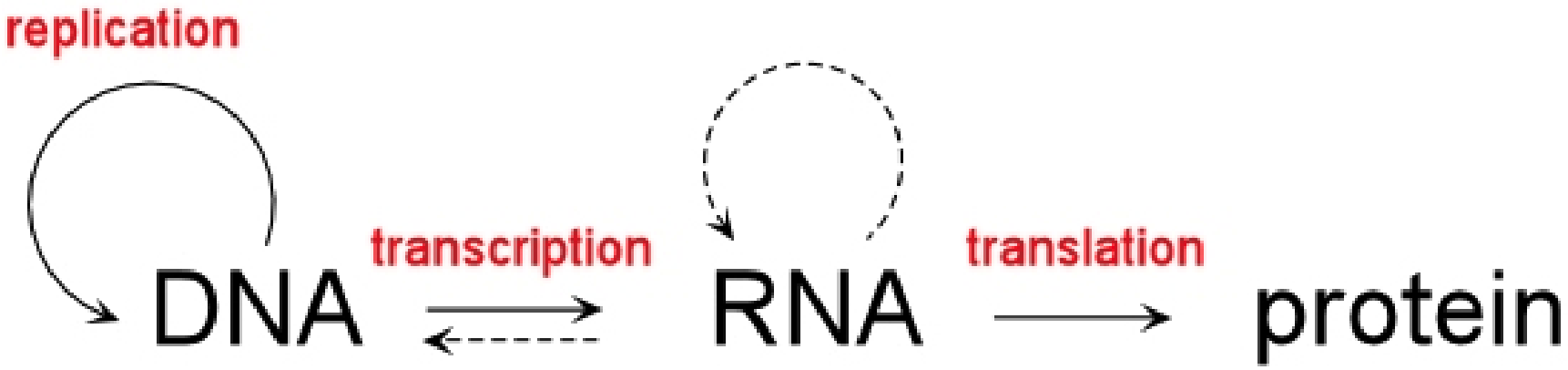


The structure of DNA
February 28, 1953

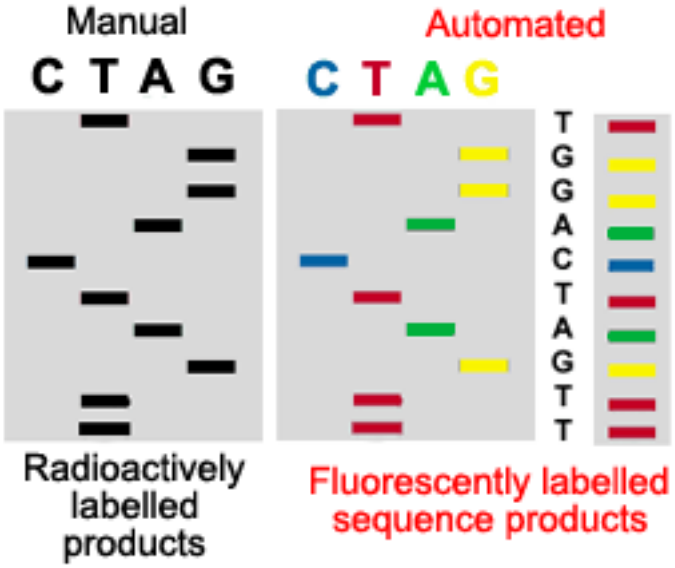




Central dogma of molecular biology



Sanger receiving the Nobel Prize in Chemistry in 1980



Reading the books of life

1. Human genomes. Predisposition to disease development, drug metabolism, forensic matters, our history.
2. Determination of genetic relatedness. The trees of life (not viruses). Linnaeus empirical classification of plants overhauled.
3. Identification of previously unknown forms of life and viruses. The dominance and ubiquitousness of life in the invisible world.
4. Microbial genomes. Diagnosis, antibiotic sensitivity, vaccine development, metagenomics – the beneficial effects of microbes.

CRISP-R

A tool available since 2013 for gene editing – adding, disrupting or changing the sequence of specific genes – and for gene regulation throughout the Three of Life

The three large conundrums

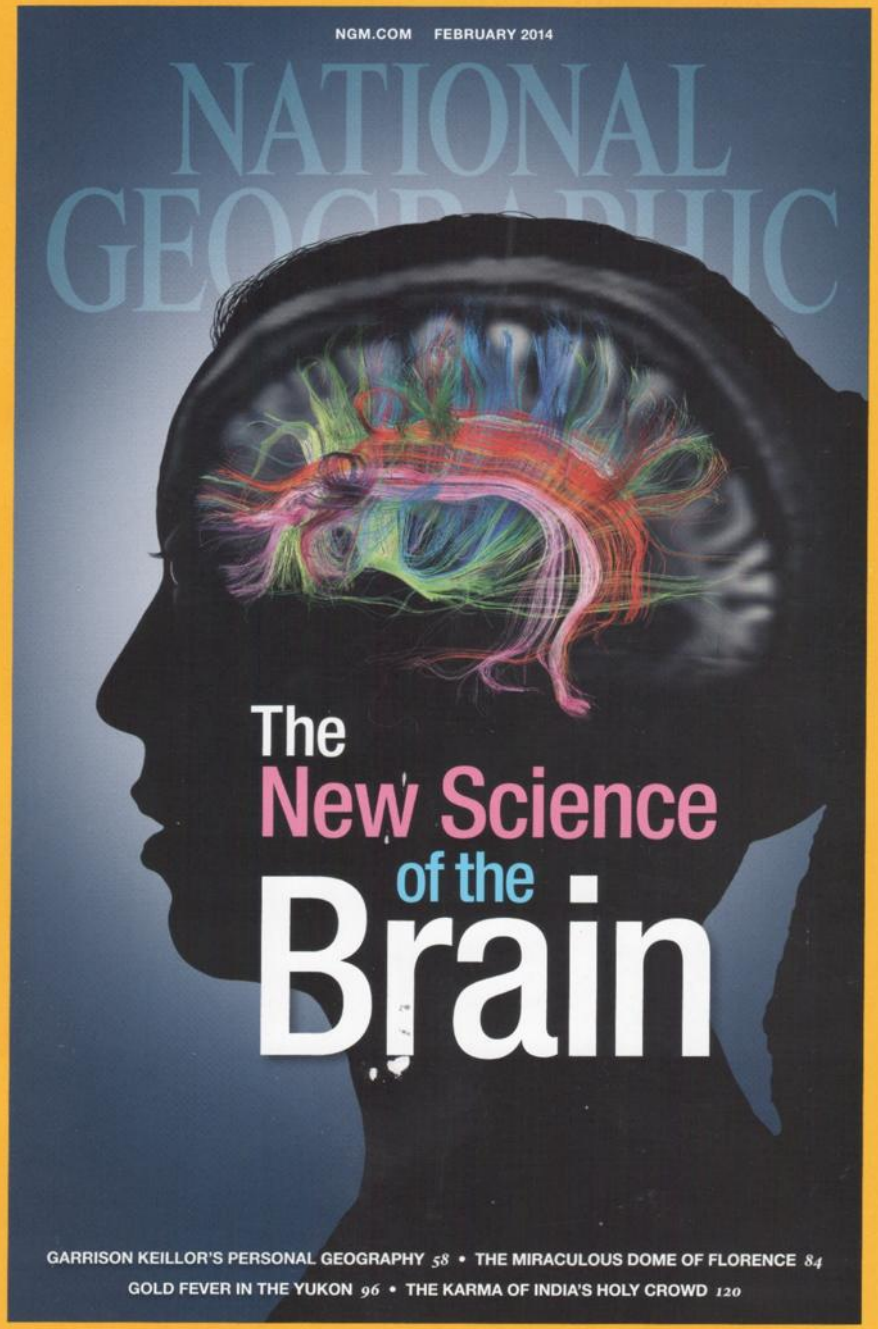
1. The origin of the Universe.
2. The origin of Life.
3. The origin of (Self-)Consciousness.

Management of information in biology

1. Immunology – a clonal selection phenomenon.
2. The olfactory sense – a restricted number of receptors and a combinatorial system.
3. The brain memory functions – an instruction (combinatorial?) system

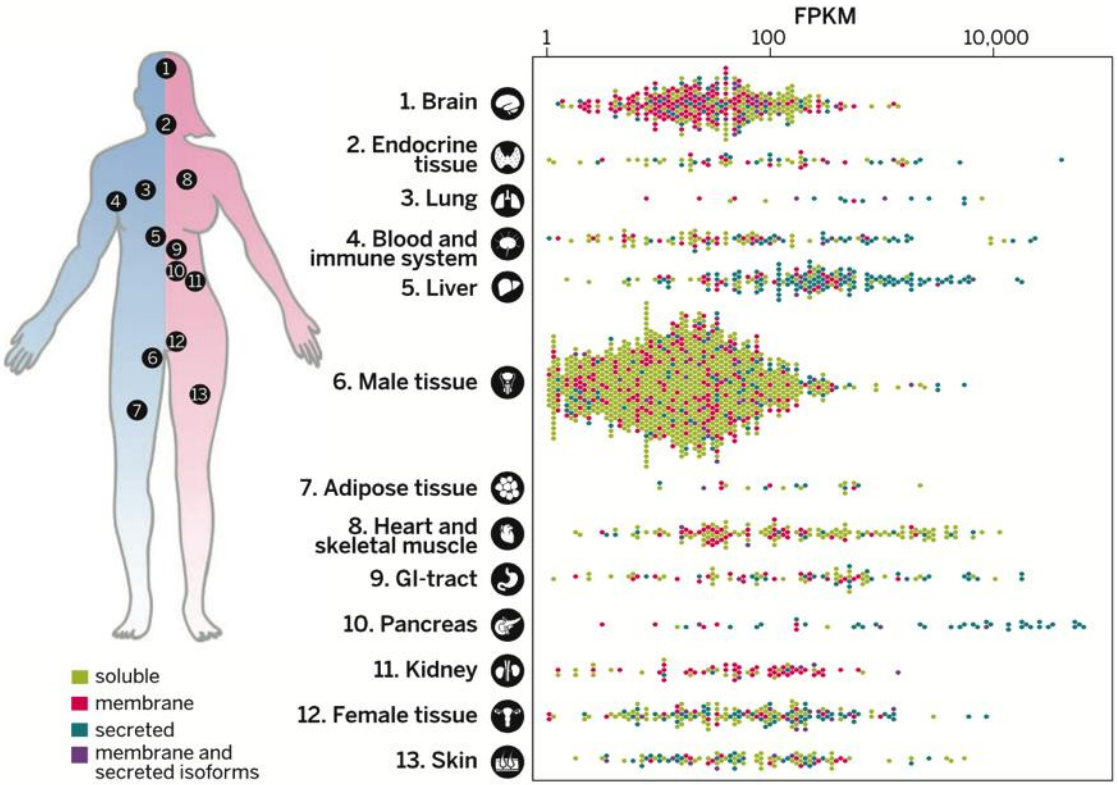
NGM.COM FEBRUARY 2014

NATIONAL GEOGRAPHIC



The New Science of the Brain

Mathias Uhlén et al.,
Science, 23 January2015 • vol 347 issue 6220



The human tissue–enriched proteins. All tissue-enriched proteins are shown for 13 representative tissues or groups of tissues, stratified according to their predicted subcellular localization. Enriched proteins are mainly intracellular in testis, mainly membrane bound in brain and kidney, and mainly secreted in pancreas and liver.

Synthetic Genomics DNA Assembly Tools

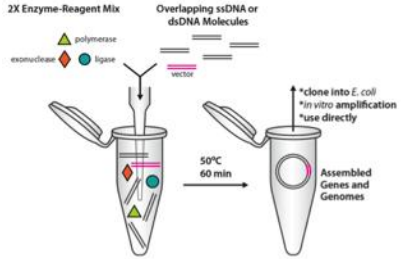
RESEARCH ARTICLE

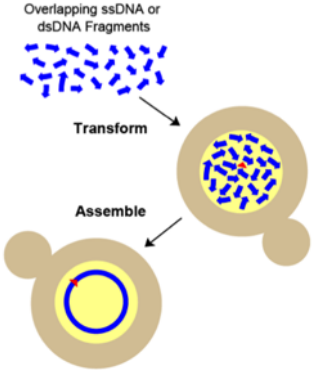
Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia A... Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stock... Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimi... Clyde A. Hutchison III, Hamilton O. Smith*

genome, we needed to establish convenient and reliable methods for the assembly and cloning of much larger synthetic DNA molecules.

Strategy for synthesis and assembly. The native 580,076-bp *M. genitalium* genome sequence (*Mycoplasma genitalium* G37 ATCC 33530 genomic sequence; accession no. L43967) (*3*) was partitioned into 101 cassettes of approxi-





PNAS

One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome

Daniel G. Gibson[a,1], Gwynedd A. Benders[b], Kevin C. Axelrod[a], Jayshree Zaveri[a], Mikkel A. Algire[a], Monzia Moodie[a], Michael G. Montague[a], J. Craig Venter[a], Hamilton O. Smith[b], and Clyde A. Hutchison III[b,1]

[a]The J. Craig Venter Institute, Synt... San Diego, CA 92121

Contributed by Clyde A. Hutchisor...

We previously reported asse... *Mycoplasma genitalium* JCVI-... *myces cerevisiae* by recombi... ments to produce a 592-kb cir... demonstrating assembly of t... lapping fragments in a single ... greatly simplifies the assembl... synthetic and natural fragme...

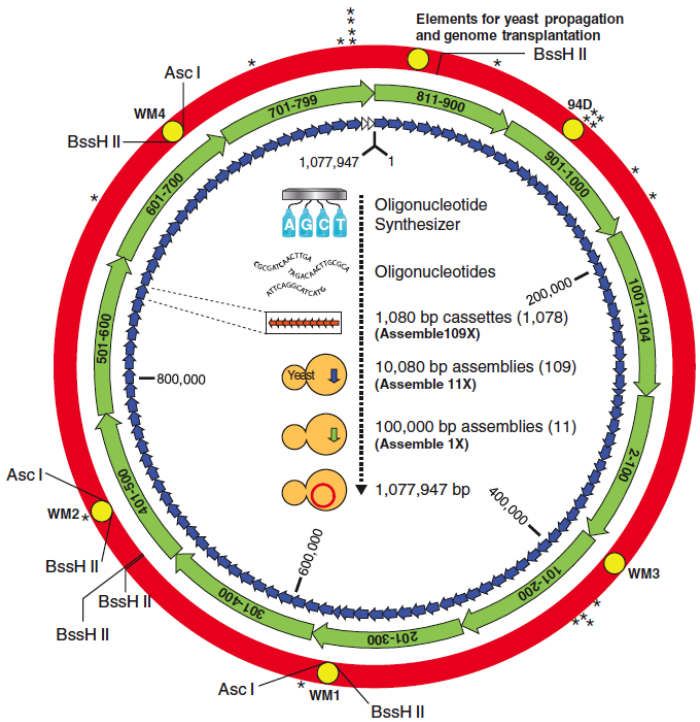
Enzymatic assembly of DNA molecules up to several hundred kilobases

Daniel G Gibson[1], Lei Young[1], Ray-Yuan Chuang[1], J Craig Venter[1,2], Clyde A Hutchison III[2] & Hamilton O Smith[2]

overlapping DNA molecules and then incubated at 50 °C for as few as 15 min (Online Methods). This approach dramatically simplifies the construction of large DNA molecules from constituent parts.

Exonucleases that recess double-stranded DNA from 5′ ends will not compete with polymerase activity. Thus, all enzymes required for DNA assembly can be simultaneously active in a single isothermal reaction. Furthermore, circular products can be enriched as they are not processed by any of the three enzymes in the reaction. We optimized a 50 °C isothermal assembly system using the activities of the 5′ T5 exonuclease (Epicentre), Phusion

We describe an isothermal, single-reac... ing multiple overlapping DNA ... d action of a 5′ exonuclease, a ... (A ligase. First we recessed DN... tranded DNA overhangs that sp... alently joined them. This asse... lessly construct synthetic and ... s and entire genomes, and cou... ring tool.



Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides

Daniel G. Gibson*

The J. Craig Venter Institute, Synthetic Biology Group, 9704 Medical Center Drive, Rockville, MD 20850, USA

Received January 1, 2009; Re...

RESEARCH ARTICLE

ABSTRACT

Here it is demonstrate... *myces cerevisiae* can... least 38 overlapping... otides and a linear do... transformation event... overlap by as few as... as 200 nucleotides in... scheme for assembl... oligonucleotides could... synthetic DNA molecul...

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] ... Hamilton O. Smith,[2] J. Craig ...

We report the design, synthe... JCVI-syn1.0 genome starting... into a *M. capricolum* recipie... synthetic chromosome. The ... including "watermark" sequ... mutations acquired during t... and are capable of continuo...

crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the

Chemical synthesis of the mouse mitochondrial genome

Daniel G Gibson[1], Hamilton O Smith[2], Clyde A Hutchison III[2], J Craig Venter[1,2] & Chuck Merryman[1]

We describe a one-step, isothermal assembly method for synthesizing DNA molecules from overlapping oligonucleotides. The method cycles between *in vitro* recombination and amplification until the desired length is reached. As a demonstration of its simplicity and robustness, we synthesized the entire 16.3-kilobase mouse mitochondrial genome from 600 overlapping 60-mers.

Chemical synthesis of long DNA sequences that encode various

and estimate that one individual could reconstruct the entire 16.3-kb molecule in just 5 d (**Supplementary Fig. 1**).

We recently described a one-step, isothermal *in vitro* recombination system capable of joining overlapping double-stranded DNA molecules up to hundreds of kilobases long[7]. The assembly reaction mixture in this system contains three separate enzymes (T5 exonuclease, Phusion polymerase and *Taq* ligase) that work in harmony to join multiple DNA fragments. In a typical reaction the assembly is accomplished in as few as 15 min. This method is robust and amenable to automation. For these reasons, we adapted it for assembly beginning at the oligo level. We optimized several parameters including the number of oligos used in a single reaction, their length, the amount of overlap, orientation, oligo concentration in the reaction, reaction temperature and reaction time (**Supplementary Tables 1–9** and **Supplementary Note 1**) to maximize efficiency of oligonucleotide assembly into a linearized pUC19 cloning vector.

We synthesized the entire 16,299-bp mouse mitochondrial genome from 600 overlapping 60-mer oligonucleotides. The overall assembly strategy encompassed four subassembly steps (**Fig. 1,**

Goldstein & Brown,
”A Century of Cholesterol and Coronaries: From Plaques to Genes to Statins,”
***Cell*, 161, March 26, 2015**

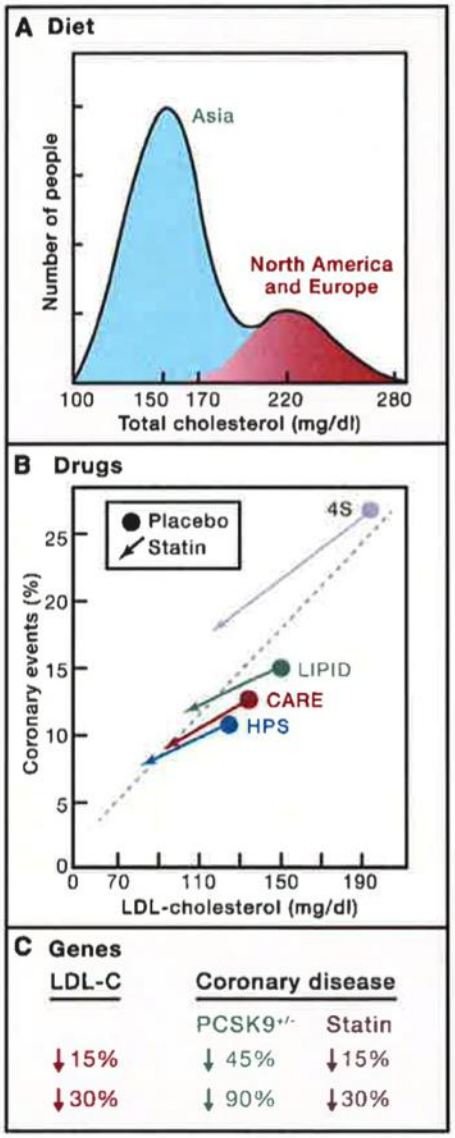


Figure 5. Diagram Illustrating the Effects of Diet, Drugs, and Genes on Plasma LDL and Coronary Disease

Conditions furthering Eureka moments in science

1. Choose an important problem.
2. Be heterodogmatic and bold.
3. Learn from nature. In biology, unravel the tinkering of evolution.
4. Be well informed about advancing technologies and means of information storage, processing and retrieval.
5. Stimulate the problem solving brain by combining adherence to the selected problem (obsessionalism) and by dialectic interactions with other members of the research group.
6. Have some luck and be aware of the role of serendipity.

Arne Tiselius, The Nobel Prize Ceremony in 1947

“When a new thought is born, or when one of the deep secrets of Nature yields to the searching scientist – in this very act of creation – there is a pure and primitive happiness deeper than anything of this kind which can ever be granted a human being to experience.”

“In any case we do not believe that to you even the highest awards and the most whole-hearted recognition can be more than a faint reflection of the deep satisfaction you must have experienced in your work.”