sentetik biyoloji çağı

& *rekombinant DNA teknolojisi*

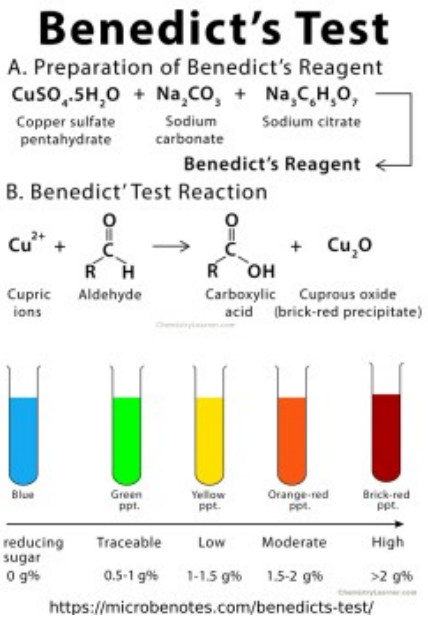
engin d

insanlık tarihi boyunca:
diyabet ve metabolik sendrom
tip 1 diyabet - 20'li yaşlarda ölüm demekti...

J Community Hosp Intern Med Perspect. 2012; 2(2): 10.3402/jchimp.v2i2.18701.
Published online 2012 Jul 16. doi: 10.3402/jchimp.v2i2.18701

Eber papirüsü

diabetes = sifon
sürekli idrar akışı...



$1

Frederick Banting (right) joined by Charles Best 1924

insülin "sifon"u geri döndürüyor

http://www.t1dliving.com/history-of-type-1-diabetes/

A chain	B chain	
Gly	Phe	1
Ile	Val	
Val	Asn	
Glu	Gln	
Gln	His	5
Cys	Leu	
Cys	Cys	
Ala	Gly	
Ser	Ser	
Val	His	10
Cys	Leu	
Ser	Val	
Leu	Glu	
Tyr	Ala	
Gln	Leu	15
Leu	Tyr	
Glu	Leu	
Asn	Val	
Tyr	Cys	
Cys	Gly	20
Asn	Glu	
	Arg	
	Gly	
	Phe	
	Phe	25
	Tyr	
	Thr	
	Pro	
	Lys	
	Ala	30

Frederick Sanger

https://en.wikipedia.org/wiki/Frederick_Sanger

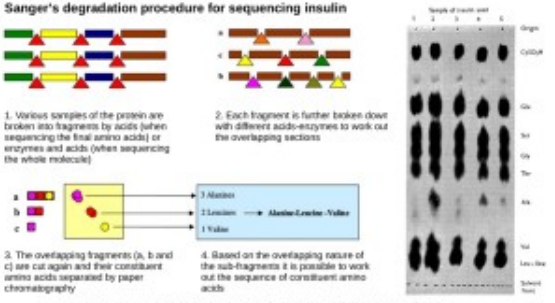
- Proteinler amorf yapılar mıdır?
- Hayır, amino asitlerin proteine özgü sırada yer alır...

Lilly

150 g pankreas / hayvan

20 mg insülin

Dünyanın ihtiyacını karşılamak için birkaç yüz milyon - milyar mertebesinde hayvanın pankreası... / yıl



https://www.whatisbiotechnology.org/index.php/exhibitions/sanger/insulin

'60 - '70ler: enzim saflaştır - aktivitesine bak yılla

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 12, pp. 2913–2917, December 1971

Specific Cleavage of Simian Virus 40 DNA by Restriction Endonuclease of Hemophilus Influenzae*

(gel electrophoresis/electron microscopy/DNA mapping/DNA fragments/tumor virus)

KATHLEEN DANNA AND DANIEL NATHANS

Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Communicated by Albert L. Lehninger, September 22, 1971

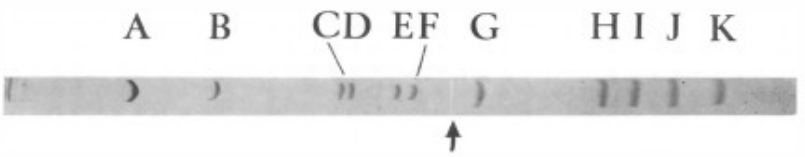


TABLE 1. *Molecular weights of SV40 DNA fragments produced by cleavage with* H. influenzae *restriction endonuclease*

Product	Electron microscopy: % length ± 1 SD	Electron microscopy: Molecular weight (× 10^{-6})	Distribution of label: %	Distribution of label: Molecular weight (× 10^{-6})	s	Sedimentation analysis: Molecular weight $\left[\frac{S2}{S1} = \left(\frac{M2}{M1}\right)^{0.385}\right]$ (× 10^{-6})	Sedimentation analysis: Molecular weight $\left[\frac{S2}{S1} = \left(\frac{M2}{M1}\right)^{0.29}\right]$ (× 10^{-6})
A	21.8 ± 1.6	6.5	24	7.2	10.1	6.1	9.4
					9.8		
					9.7		
					9.4		
B	13.9 ± 1.4	4.2	18	5.4	9.2	4.6	7.5
					8.9		
					8.9		
C	10.6 ± 0.7*	3.2*	10.5*	3.2*			
D	10.6 ± 0.7*	3.2*	10.5*	3.2*	8.2	3.2	5.9
					8.2		
E	7.7 ± 1.4	2.3	7.5†	2.3†	7.6	2.4	4.7
F			7.5†	2.3†			
G			7	2.1	7.3	2.0	4.2
H			3.9	1.2	7.0	1.7	3.6
I			5.3	1.0‡			
J			4.1	0.87‡			
K			3.6	0.74‡			

* These values were obtained with a mixture of *C* and *D*. Percent distribution of label was divided by 2.
† These values were obtained with a mixture of *E* and *F*. Percent distribution of label was divided by 2.
‡ Molecular weights were estimated from mobilities of the products in a 5% polyacrylamide gel, with *A* through *H* as standards (see Fig. 5).

Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 2904–2909, October 1972

Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of *Escherichia coli*

(molecular hybrids/DNA joining/viral transformation/genetic transfer)

DAVID A. JACKSON*, ROBERT H. SYMONS†, AND PAUL BERG

Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305

Contributed by Paul Berg, July 31, 1972

ABSTRACT **We have developed methods for covalently joining duplex DNA molecules to one another and have used these techniques to construct circular dimers of SV40 DNA and to insert a DNA segment containing lambda phage genes and the galactose operon of *E. coli* into SV40 DNA. The method involves: (*a*) converting circular SV40 DNA to a linear form, (*b*) adding single-stranded homodeoxypolymeric extensions of defined composition and length to the 3′ ends of one of the DNA strands with the enzyme terminal deoxynucleotidyl transferase (*c*) adding complementary homodeoxypolymeric extensions to the other DNA strand, (*d*) annealing the two DNA molecules to form a circular duplex structure, and (*e*) filling the gaps and sealing nicks in this structure with *E. coli* DNA polymerase and DNA ligase to form a covalently closed-circular DNA molecule.**

MATERIALS AND METHODS

DNA. (*a*) Covalently closed-circular duplex SV40 DNA [SV40(I)] (labeled with [^{3}H]dT, 5 × 10^4 cpm/μg), free from SV40 linear or oligomeric molecules [but containing 3–5% of nicked double-stranded circles—SV40(II)] was purified from SV40-infected CV-1 cells (Jackson, D., & Berg, P., in preparation). (*b*) Closed-circular duplex λ*dvgal* DNA labeled with [^{3}H]dT (2.5 × 10^4 cpm/μg), was isolated from an *E. coli* strain containing this DNA as an autonomously replicating plasmid (see ref. 3) by equilibrium sedimentation in CsCl–ethidium bromide gradients (4) after lysis of the cells with detergent. A more detailed characterization of this DNA will be published later. Present information indicates that the

Yaşamın fiziksel ve kimyasal esasları

1912'ler için çok devrimsel...

1950 - Alexander Todd

H-phosphonate synthesis

Nucleotides Part XXXII.* Synthesis of a Dithymidine Dinucleotide Containing a 3′ : 5′-Internucleotidic Linkage.

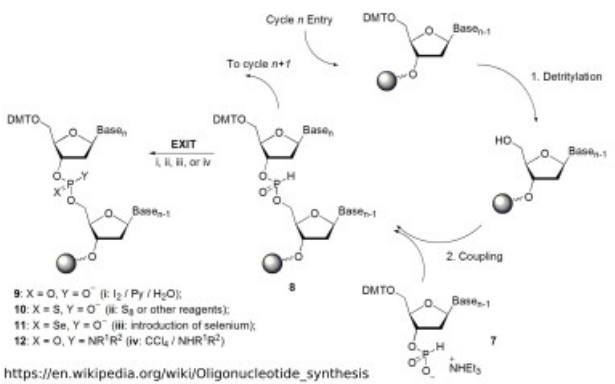
By A. M. Michelson and Sir Alexander R. Todd.

[Reprint Order No. 6258.]

A dithymidine dinucleotide (V) has been synthesised by condensing 3′-*O*-acetylthymidine with thymidine 3′-(benzyl phosphorochloridate) 5′-(dibenzyl phosphate) and subsequently removing the protecting groups. This represents the first preparation of a dinucleotide by chemical means and since the synthetic material behaves towards enzymes exactly as the dinucleotidic fragments obtained by degrading deoxyribonucleic acids the postulate of a 3′ : 5′-internucleotidic linkage in the latter is further confirmed. Thymidine-3′ thymidine-5′ phosphate has also been prepared and by-products isolated include a dinucleoside pyrophosphate and a dinucleotide pyrophosphate.

In previous papers of this series we have described the preparation of the 3′- and 5′-phosphates of the natural deoxyribonucleosides thymidine (I; R = R′ = H) (Michelson and Todd, *J.*, 1953, 951), deoxycytidine (Michelson and Todd, *J.*, 1954, 34), deoxyadenosine and deoxyguanosine (Hayes, Michelson, and Todd, *J.*, 1955, 808), and have identified the

* Part XXXI, *J.*, 1955, 2206.



https://en.wikipedia.org/wiki/Oligonucleotide_synthesis

1950 - Har Gobind Khorana

Phosphodiester synthesis

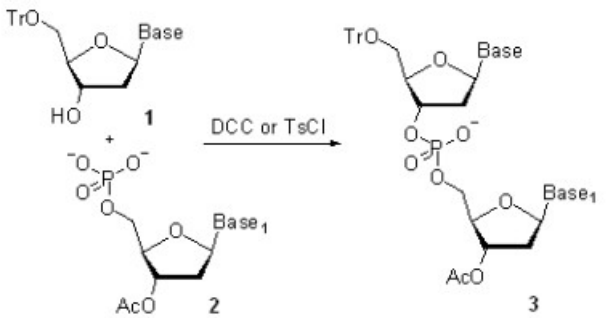
[Contribution from the Chemistry Division of the British Columbia Research Council]

Studies on Polynucleotides. I. A New and General Method for the Chemical Synthesis of the C_3'–C_3' Internucleotidic Linkage. Syntheses of Deoxyribo-dinucleotides[1]

By P. T. Gilham and H. G. Khorana

Received April 14, 1958

A new method has been developed for the specific synthesis of the naturally-occurring (C_3'–C_5') internucleotidic linkage; it involves reaction of a suitably protected deoxynucleotide with a second protected deoxy-nucleoside or -nucleotide in the presence of dicyclohexylcarbodiimide or *p*-toluenesulfonyl chloride. By this approach the three dinucleoside phosphates VIIa, VIIb and VIIc have been prepared in good yield. Procedures are described for the synthesis of deoxyribo-dinucleotides bearing 5′- or 3′-phosphoryl end-groups; these are illustrated by the synthesis of the two isomeric dithymidine dinucleotides (XII and XIV), and a mixed dinucleotide (XVI) containing the nucleosides, deoxyadenosine and thymidine. The results of enzymic and acidic degradative experiments are recorded and these provide additional characterization of the synthetic compounds. Some general observations on the scope and mechanism of this method of "phosphodiester" synthesis also are included.



1960 - R. Letsinger[22] and C. Reese

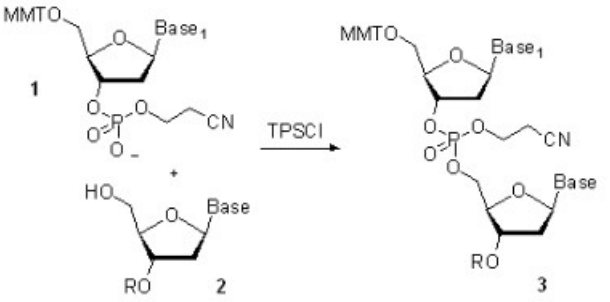
Phosphotriester synthesis

Synthesis of Oligothymidylates *via* Phosphotriester Intermediates

Robert L. Letsinger and Kelvin K. Ogilvie

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. Received December 23, 1968

Abstract: A procedure is described for the large-scale preparation of β-cyanoethyl ester derivatives of 5′-*O*-monomethoxytrityl TpT, TpTpT, and TpTpTpT. The essential feature is a double phosphorylation, the first step of which involves reaction of a terminal 3′-OH of a nucleoside with β-cyanoethyl phosphate and mesitylenesulfonyl chloride and the second step, condensation of the resulting phosphodiester with thymidine in the presence of triisopropylbenzenesulfonyl chloride. The products are separated by chromatography on silica gel with ethyl acetate and tetrahydrofuran. They may be converted in high yield to the corresponding demethoxytritylated derivatives and thence to TpT, TpTpT, and TpTpTpT, respectively, by successive treatment with aqueous acetic acid and ammonium hydroxide.



katı fazda sentez ????

https://en.wikipedia.org/wiki/Oligonucleotide_synthesis

1970-80 - Matteucci, M. D.
Caruthers, M. H.

Phosphite triester synthesis

Synthesis of Deoxyoligonucleotides on a Polymer Support[1]

M. D. Matteucci and M. H. Caruthers*

Contribution from the Department of Chemistry, University of Colorado, Boulder, Colorado 80309. *Received September 18, 1980*

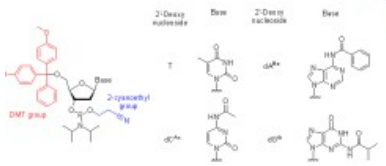
Abstract: The development of a new method for synthesizing deoxyoligonucleotides is described. The synthesis begins by derivatizing high-performance liquid chromatography grade silica gel to contain 5′-*O*-(dimethoxytrityl)deoxynucleosides linked through the 3′-hydroxyl to a carboxylic acid functional group on the support. This matrix is then packed into a column which is attached to a pump and a series of valves. The chemical steps for the addition of one nucleotide to the support are as follows: (1) detritylation using $ZnBr_2$ in nitromethane (30 min); (2) condensation of a 5′-*O*-(dimethoxytrityl)deoxynucleoside (3′-methoxytetrazoyl)phosphine with the support-bound nucleoside (60 min); (3) blocking unreacted, support-bound nucleoside hydroxyl groups with diethoxytriazolylphosphine (5 min); (4) oxidation of phosphites to phosphates with I_2 (5 min). Completed deoxyoligonucleotides are isolated by sequential treatment with thiophenol and ammonium hydroxide, purification by reverse-phase chromatography, and treatment with 80% acetic acid. The method is extremely fast (less than 2.5 h are needed for each nucleotide addition cycle), yields in excess of 95% per condensation are obtained, and isolation of the final product is a simple one-step column purification. The syntheses of d(C-G-T-C-A-C-A-A-T-T) and d(A-C-G-C-T-C-A-C-A-A-T-T) were carried out as a test of this method. Yields of support-bound deoxyoligonucleotides were 64% and 55%; the isolated yield of deoxydecanucleotide was 30%. Both synthetic products were homogeneous and biologically active by every criteria so far tested.

Katı fada sentez ve
OTOMASYON

Synthesis by the phosphoramidite method

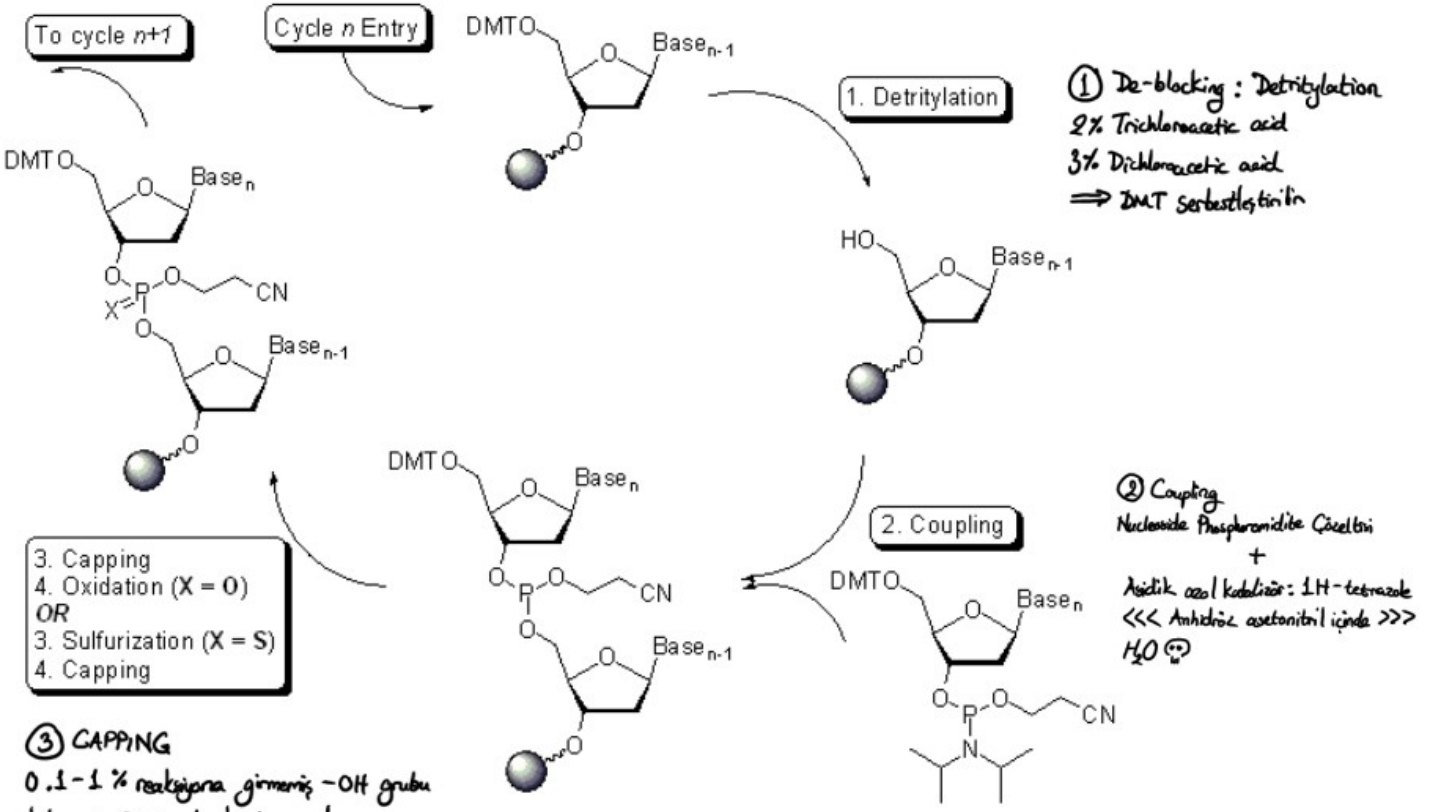
doğal olarak bulunan nükleozid-3' ya da 5'-fosfatlar yüksek verim ile nükleik asit sentezlemek için yeterli kadar reaktif değiller !

YAN TEPKİMELER!



3'-O-(N,N-diisopropyl phosphoramidite) türevleri → internükleozidik bağların kurulması çok daha özgül ve hızlı ...

KORUYUCU GRUPLAR
DMT : 4,4'-dimethoxytrityl
exocyclic amino grupları özgül olmayan reaksiyonlardan korumak gerek



① De-blocking : Detritylation
2% Trichloroacetic acid
3% Dichloroacetic acid
⟹ DMT serbestleştirilir

② Coupling
Nucleoside Phosphoramidite Çözeltisi
+
Asidik azol katalizör : 1H-tetrazole
<<< Anhidröz asetonitril içinde >>>
H_2O 💀

③ CAPPING
0.1-1 % reaksiyona girmemiş -OH grubu kalıyor → n-1 shortmers !
acetic anhydride + 1-methylimidazole

④ OXIDATION
Yeni oluşturulmuş trikoordine fosfit triester bağı doğal ve stabil değil
pyridine / lutidine / collidine

Coupling efficiency (%) Overall yield of oligonu

Department of Biochemistry Technische Hochschule Darmstadt Embo practical Course

Automated Chemical and Enzymic Gene Synthesis

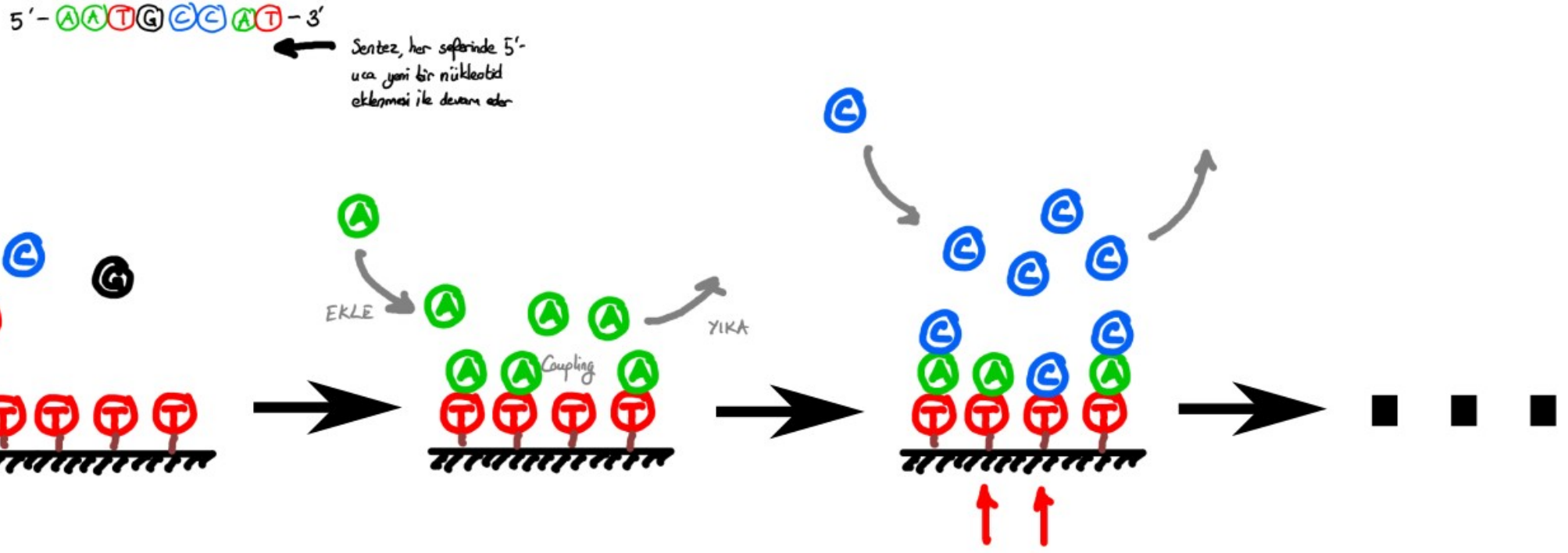
March 21st to April 3rd, 1982

The teaching staff will include: J. H. van Boom, Leiden. M. H. Caruthers, Colorado. H. J. Fritz, Köln. M. J. Gait, Cambridge, U. K. H. G. Gassen, W. Hillen, Darmstadt. K. Itakura, City of Hope, L. A. H. Kössel, Freiburg. H. Köster, Hamburg. K. E. Norris, Bagsvaerd. F. Ohtsuka, Osaka. H. Schott, Tübingen. H. Seliger, Ulm. O. C. Uhlenbeck, Urbana. E.-L. Winnacker, München and others.

The programme will consist of practical work, lectures and seminars on solid-phase chemical synthesis of oligodeoxynucleotides, phosphotriester method as related to the phosphoroamidite procedure, automated oligonucleotide synthesis, oligonucleotides from DNA fragmentation, enzymic ligation of oligonucleotides, recombination of R.N.A., survey of oligonucleotide separation and analysis, ligation of synthetic genes (promotors) to plasmid vectors. A maximum of 15 students will be accepted. Applications should include a short curriculum vitae and a description of present research interests. The registration fee for the practical course is 200,- DM. Upon motivated request some fellowships for board and lodging will be granted.

The weekend March 27/28th will be kept free from practical work to allow the participation in a workshop entitled **Prospects of Automation in Gene Synthesis**
Informal application is sufficient for the workshop. The number of participants will be limited to 100. The registration fee is 60,- DM. **The closing date for applications is Januar 15th.** Those accepted will be notified not later than January 31st, 1982. Applications should be sent to Dr. H. G. Gassen, TH Darmstadt Institut für Organische Chemie und Biochemie D 61 Darmstadt, Petersenstraße 22 Phone: (06151)16 36 53 Telex: 419579

Coupling efficiency (%)	Overall yield of oligonucleotide (%)				
	20-mer	40-mer	60-mer	80-mer	100-mer
90	12	1.5	0.18	0.02	0.003
95	36	13	4.6	1.7	0.6
98	67	45	30	20	13
99	82	67	55	45	37
99.5	90	82	74	67	61



Psychic spies from China try to steal your mind's elation
And little girls from Sweden dream of silver screen quotation
And if you want these kind of dreams it's Californication
It's the edge of the world and all of Western civilization
The sun may rise in the East at least it's settled in a final location
It's understood that Hollywood sells Californication
Pay your surgeon very well to break the spell of aging
Celebrity skin, is this your chin, or is that war you're waging?
First born unicorn
Hardcore soft porn
Dream of

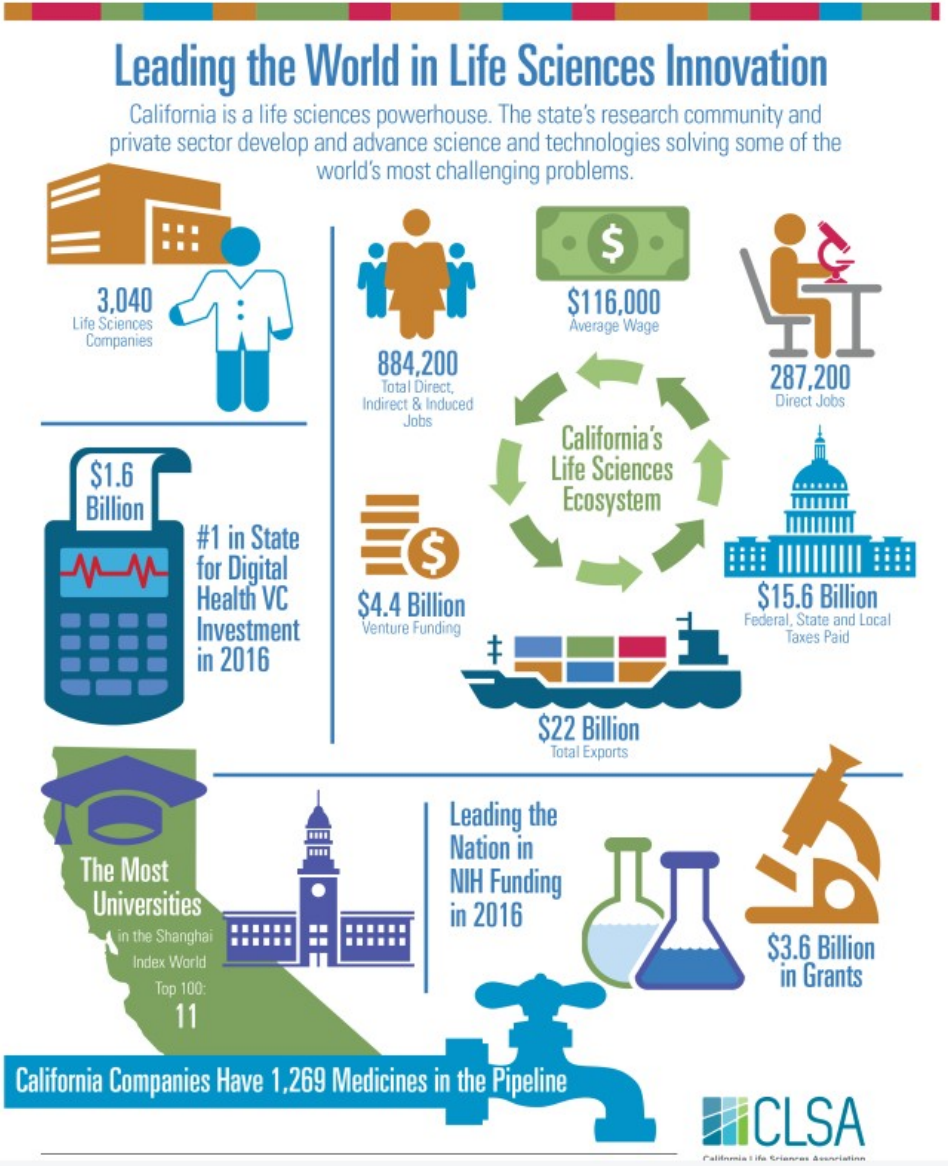
Californication

er 1973

ılly Functional Bacterial Plasmids *In Vitro*

nsformation/endonuclease/antibiotic resistance)

Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†



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Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

Communicated by Norman Davidson, July 18, 1973

ABSTRACT The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins.

*Eco*RI-generated fragments have been inserted into appropriately-treated *E. coli* by transformation (7) and have been shown to form biologically functional replicons that possess genetic properties and nucleotide base sequences of both parent DNA species.

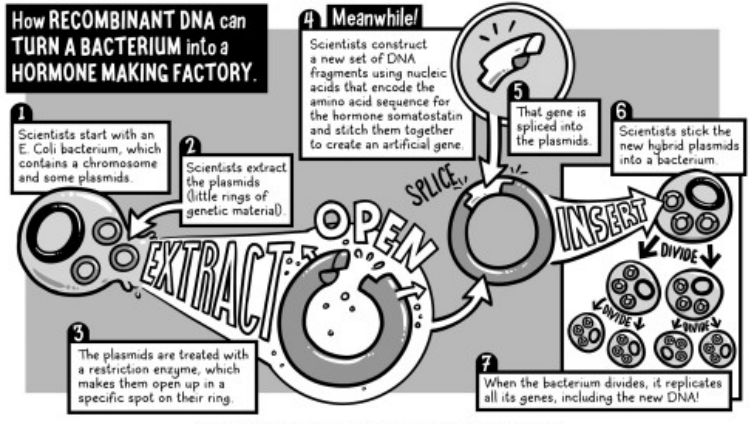
MATERIALS AND METHODS

E. coli strain W1485 containing the RSF1010 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R

Plasmid pSC101

EcoRI kesimi

Kurbağa ribozomal DNA'sı



https://www.gene.com/stories/proof-of-concept

> Science. 1977 Dec 9;198(4321):1056-63. doi: 10.1126/science.412251.

Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin

K Itakura, T Hirose, R Crea, A D Riggs, H L Heyneker, F Bolivar, H W Boyer

PMID: 412251 DOI: 10.1126/science.412251

Abstract

A gene for somatostatin, a mammalian peptide (14 amino acid residues) hormone, was synthesized by chemical methods. This gene was fused to the Escherichia coli beta-galactosidase gene on the plasmid pBR322. Transformation of E. coli with the chimeric plasmid DNA led to the synthesis of a polypeptide including the sequence of amino acids corresponding to somatostatin. In vitro, active somatostatin was specifically cleaved from the large chimeric protein by treatment with cyanogen bromide. This represents the first synthesis of a functional polypeptide product from a gene of chemically synthesized origin.