노벨 생리의학상 수상

Sir Richard J. Roberts 교수 초청강연

노벨상으로 가는길

The Path to the Nobel Prize

일 시 : 2017년 9월 8일 오전 11시

장 소 : 고려대학교 하나스퀘어 강당

Profile

Sir Richard J. Roberts

Dr. Richard J. Roberts is the Chief Scientific Officer at New England Biolabs, Beverly, Massachusetts. He received a Ph.D. in Organic Chemistry in 1968 from Sheffield University and then moved as a postdoctoral fellow to Harvard. From 1972 to 1992, he worked at Cold Spring Harbor Laboratory, eventually becoming Assistant Director for Research under Dr. J.D. Watson. He began work on the newly discovered Type II restriction enzymes in 1972 and these enzymes have been a major research theme. Studies of transcription in Adenovirus-2 led to the discovery of split genes and mRNA splicing in 1977, for which he received the Nobel Prize in Medicine in 1993. During the sequencing of the Adenovirus-2 genome computational tools became essential and his laboratory pioneered the application of computers in this area. DNA methyltransferases, as components of restriction-modification systems are also of active interest and the first crystal structures for the HhaI methyltransferase led to the discovery of base flipping. Bioinformatic studies of microbial genomes to find new restriction systems are a major research focus as is the elucidation of DNA methyltransferase recognition sequences using SMRT sequencing and a new approach to m5C containing recognition sequences.

The Path to the Nobel Prize

Richard J. Roberts

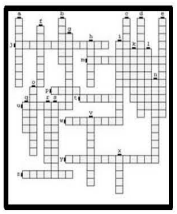
New England Biolabs

Korea University, September 8th , 2017

Born in 1943 in Derby, England

Junior School – 1950-1954

St. Stephen's School, Bath



Bath, England

At age 11, I wanted to become a detective.

My parents supported my curiosity and love of puzzles.

My father helped build a chemistry lab in the kitchen where I learned to love fireworks and explosives.

City of Bath Boy's School 1954-62

I increased my love for mathematics. I learned to play the violin and joined the school orchestra.

Being close to the limestone escarpment of the Mendips (Cheddar Gorge) I became interested in caving.

I also discovered jazz for which Bath became quite famous.

Sheffield University 1962 – 1965 - 1968

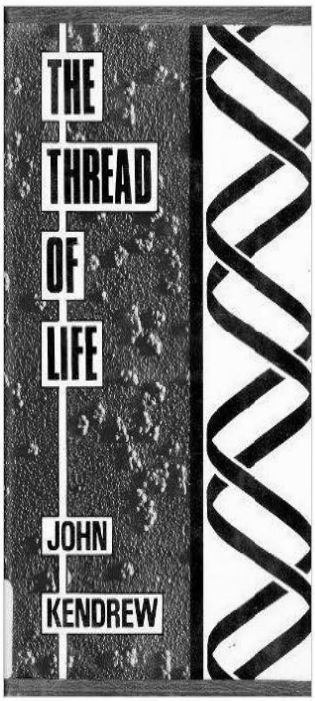
This interest in chemistry plus a fascination with games and puzzles led me to pursue a career in research.

It was a chance to be a detective and solve chemical puzzles in the world of science.

I got my B.Sc. In 1965 and stayed to do graduate work with Professor W.D. Ollis. He emphasized the use of problem-solving to make learning fun, rather than a chore.

Kazu Kurosawa

My "real" mentor
in Sheffield



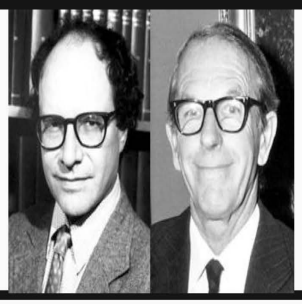
Post-Doc at Harvard 1969 - 1972

Molecular Biology

Sequenced
$tRNA^{Gly}$ in the
laboratory of Jack
Strominger

Nobel Prize in Chemistry, 1980

Walter Gilbert "For their contributions concerning the determination
Fred Sanger of base sequences in nucleic acids"



DNA SEQUENCING

In 1969 I visited Fred's lab to learn RNA sequencing

Cold Spring Harbor Laboratory 1972-1992

Cold Spring Harbor Laboratory

In 1972, after a 10-minute interview, James Watson offered me a position at Cold Spring Harbor Laboratory. He wanted me to sequence the DNA of the virus SV40.

RESTRICTION ENZYMES

At CSHL I started investigating the enzyme Endonuclease R, which I heard about at Harvard during a lecture by Dan Nathans.

The enzyme cut DNA into specific pieces. I thought that if there were more of these enzymes, I could cut DNA into manageable-sized fragments and use them to sequence DNA. Soon my lab had a whole collection of restriction enzymes.

During the 70's and early 80's, about 75 out of the 100 known Type II enzymes were isolated in my laboratory at Cold Spring Harbor.

Dan Nathans
Werner Arber
Hamilton Smith

Nobel Prize in Physiology or Medicine 1978

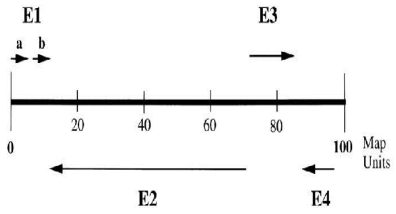
"For the discovery of restriction enzymes and their applications to problems of molecular genetics"

Cold Spring Harbor Laboratory

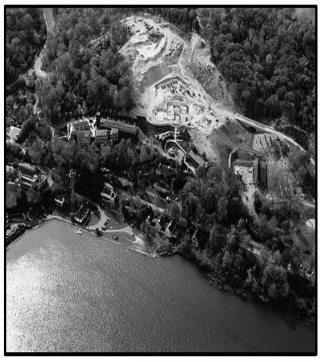
Some of these restriction enzymes were used to map adenovirus-2 DNA, a project in which Phil Sharp, in Joe Sambrook's lab, was also involved.

In 1974, my post-doc Richard Gelinas, started working with adenovirus-2 mRNA. We thought we could identify the DNA promoter regions by sequencing the 5' end of all the mRNAs and mapping them to the DNA. The promoter would be upstream of the 5' end of the mRNA.

Adenovirus-2 transcription in 1974



Do eukaryotic promoters look like prokaryotic promoters?



Cold Spring Harbor Laboratory 1972-1992

Norton Zinder

A very good friend who often rescued me from Jim Watson's wrath.

mRNA

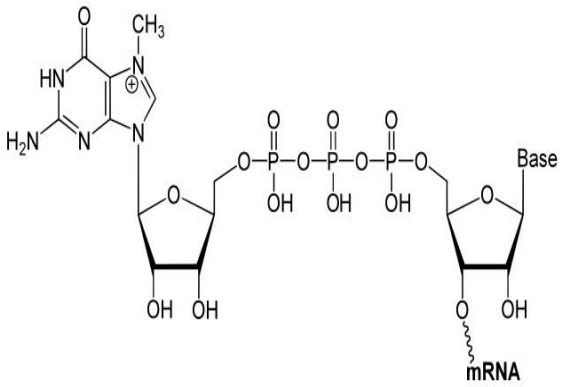
5′-pppN ——————————— AAAAAA$_{OH}$ 3′

↓ RNAse T1

5′-pppN——Gp ——Gp ——Gp ——Gp ——AAAAAA$_{OH}$

Look for all 5′-pppN———Gp

A key discovery – mRNA caps in Ad2 mRNA



S. Sommer, M. Salditt-Georgieff, S. Bachenheimer, J. E. Darnell, Y. Furuichi, M. Morgan and A.J. Shatkin
Nucleic Acids Research **3**: 750-765 (1976) .
Moss, B., and Koczot, F. J. Virol. 17: 385-392 (1976)

mRNA

3′- ^{m7}G-5′-ppp-5′-N——————————— AAAAAA$_{OH}$ 3′

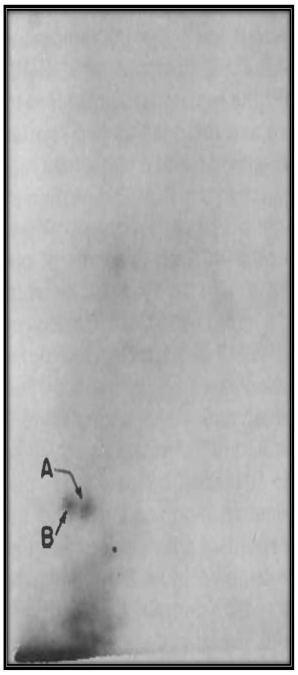
↓ RNAse T1

3′- ^{m7}G-5′-ppp-5′-N——Gp ——Gp ——Gp ——Gp ——AAAAAA$_{OH}$

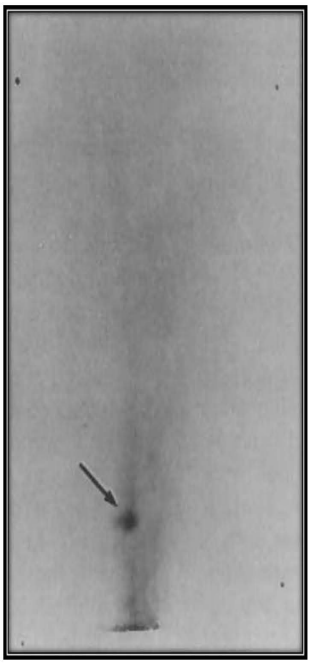
Look for all 3′– ^{m7}G-5′-ppp-5′-N———Gp

The 5' terminal T1 oligonucleotides of late Ad2 mRNA

(2-24 hours post-infection)



(15-24 hours post-infection)



Cell, Vol. 11, 533-544, July 1977, Copyright © 1977 by MIT

One Predominant 5′-Undecanucleotide in Adenovirus 2 Late Messenger RNAs

Richard E. Gelinas and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

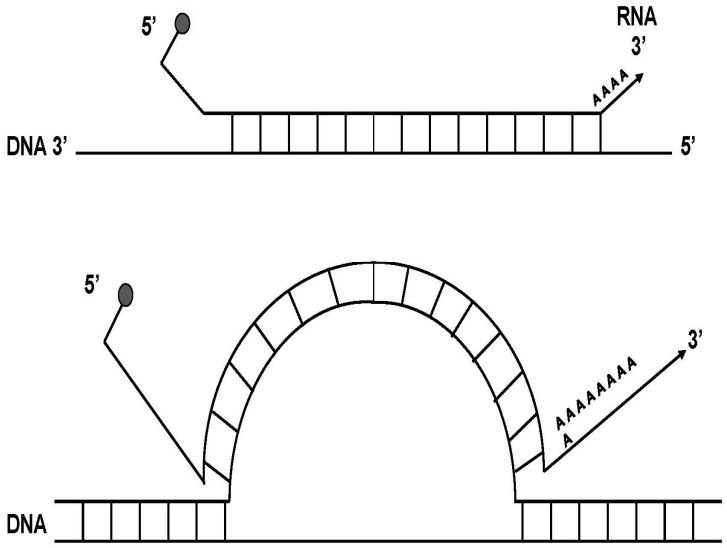
Oligonucleotides containing the 5′ termini of adenovirus 2 mRNA are selectively retained on columns of dihydroxyboryl cellulose. When total late adenovirus 2 mRNA was treated with RNAase T1, a single 5′ terminal oligonucleotide was isolated, although in several states of methylation. This oligonucleotide has the general structure $m^7G^{5'}ppp^{6'}$ÅmCmU($C_{11}U_{10}$)G. Since at least twelve individual species of mRNA must be present late after infection, this finding was unexpected and its significance is discussed.

the presence of a 7-methylguanosine residue linked via a 5′,5′-triphosphate bridge to the 5′ terminal residue of the mRNA chain. The unusual linkage of the 7-methylguanosine residue provided a way of isolating 5′ terminal oligonucleotides containing these structures. These oligonucleotides are unique in that they contain a 3′-phosphate at one terminus (for example, after RNAase T1 cleavage, they contain 3′-Gp) and a 2′,3′-*cis* diol from the 7-methylguanosine at the other terminus. Oligonucleotides containing a 2′,3′-*cis* diol have previously been isolated on affinity columns of dihydroxyboryl cellulose (DBAE-cellulose) (Weith, Wiebers and Gilham, 1970), and this method has been of value for the isolation of 3′ terminal oligonucleotides from ribosomal RNAs (Rosenberg, 1974) and capped structures generated by RNAase T2 cleavage of avian sarcoma virus mRNA (Furuichi et al., 1975b). We have extended their approach and used

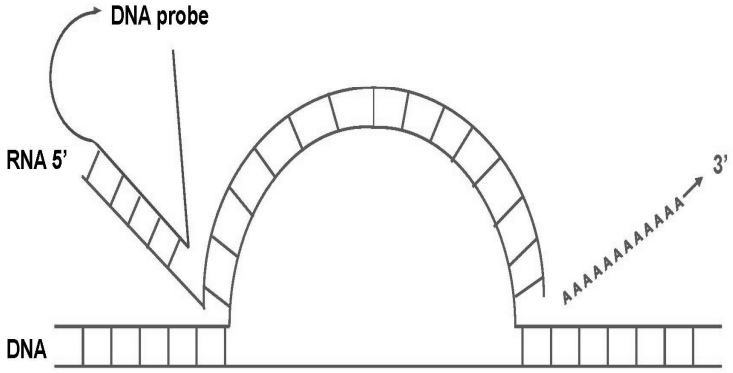
Unexpected behaviour of the capped oligonucleotide

1. Hybridization to individual DNA fragments across the Ad2 genome all gave the same cap
2. Pure mRNA for several well known proteins contained this same cap (D. Klessig)
3. When mRNA was hybridized to its main coding region and treated gently with RNAse the cap was lost
4. Ad2-SV40 hybrids showed the capped oligonucleotide was not coded adjacent to the main mRNA body (B.S. Zain)
5. Early RNAs have a different terminal oligonucleotide that is not lost by mild RNase treatment

An explanation and an experiment



THE 1977 R-LOOP EXPERIMENT



Cold Spring Harbor Laboratory

Through the course of these molecular biology experiments, we discovered that the genes in adenovirus must be split into at least two pieces. I then devised an electron microscope experiment that proved visually that this was true. Fortunately, Louise Chow and Tom Broker worked in a lab close to ours

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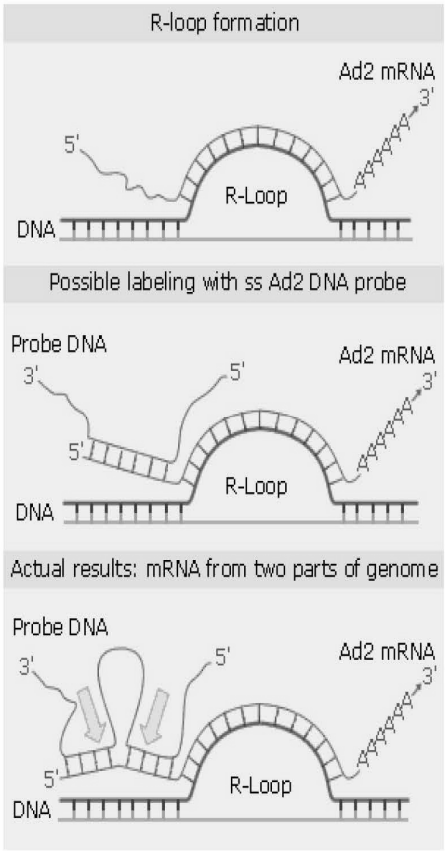


Cold Spring Harbor – 1972-1992

1977 discovery of RNA splicing

Cell, Vol. 12, 1-8, September 1977, Copyright © 1977 by MIT

An Amazing Sequence Arrangement at the 5′ Ends of Adenovirus 2 Messenger RNA

Louise T. Chow, Richard E. Gelinas, Thomas R. Broker and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

The 5′ terminal sequences of several adenovirus 2 (Ad2) mRNAs, isolated late in infection, are complementary to sequences within the Ad2 genome which are remote from the DNA from which the main coding sequence of each mRNA is transcribed. This has been observed by forming RNA displacement loops (R loops) between Ad2 DNA and unfractionated polysomal RNA from infected

teristic of that of the host genome (Lewin, 1975a, 1975b). For example, long polyadenylated transcripts appear in the nucleus, but only a small percentage of this nuclear RNA appears as polyadenylated mRNA on cytoplasmic polysomes (Philipson et al., 1971). These mRNAs are "capped" at their 5′ ends (Moss and Koczot, 1976; Sommer et al., 1976). Gelinas and Roberts (1977) found that most Ad2 mRNAs isolated at late times during infection contain the same "capped" 11 nucleotide sequence at their 5′ ends. This sequence was sensitive to ribonuclease cleavage in mRNA:DNA hybrids (Gelinas and Roberts, 1977; Klessig, 1977) and led to the suggestion that this 5′ terminal sequence might not be coded immediately adjacent to the main body of the mRNA.

Cold Spring Harbor Laboratory – 1978-1992

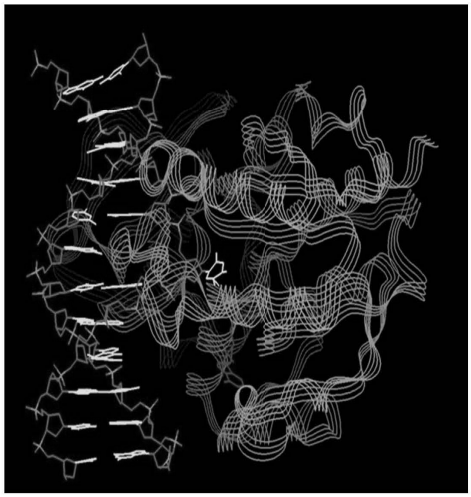
1. Sequenced some splice junctions
2. Sequenced Adenovirus-2 DNA
3. Pioneered sequence assembly programs
4. Began developing BIOinformatics

Nobel Prize in Physiology or Medicine 1993

"The Nobel Assembly at the Karolinska Institute in Stockholm, Sweden, has awarded the Nobel Prize in Physiology or Medicine for 1993 jointly to Richard J. Roberts and Phillip A. Sharp for their discovery of split genes."

Cold Spring Harbor/NEB – 1991-1993

With Xiaodong Cheng - discovery of base flipping in M.HhaI



1974 - New England Biolabs

1984 – North Beverly, MA

In 1992 I moved to New England Biolabs as a Research Director. I am now the Chief Scientific Officer.

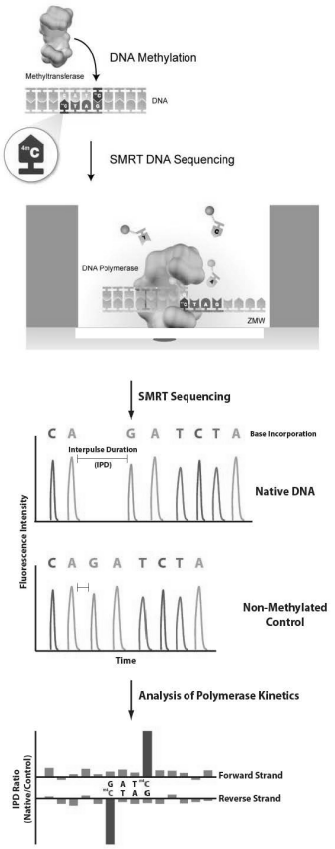
NEW ENGLAND BioLabs Inc.

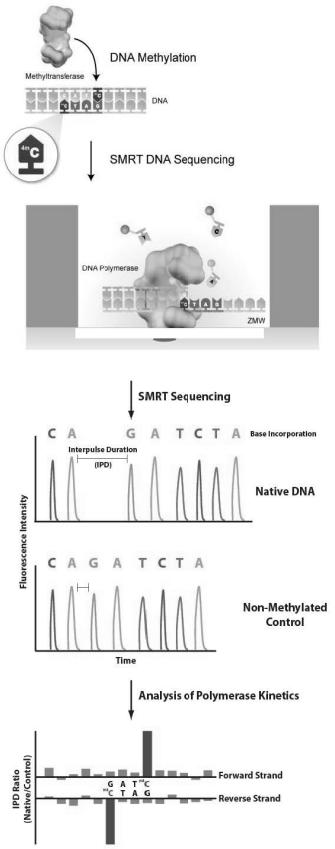
2005 – Ipswich, MA

New England Biolabs – 1992-present

Cloning, BIOinformatics and genomics

1. Cloning restriction enzyme genes for commercial production
2. Exploring the limits of bioinformatics
3. Genome sequencing to find restriction enzyme genes


DNA Methylation
Methyltransferase
DNA
SMRT DNA Sequencing
DNA Polymerase
ZMW
SMRT Sequencing
C A G A T C T A
Base Incorporation
Interpulse Duration
(IPD)
Native DNA
Fluorescence Intensity
C A G A T C T A
Non-Methylated
Control
Time
Analysis of Polymerase Kinetics
IPD Ratio
(Native/Control)
Forward Strand
Reverse Strand


DNA Methylation
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Time
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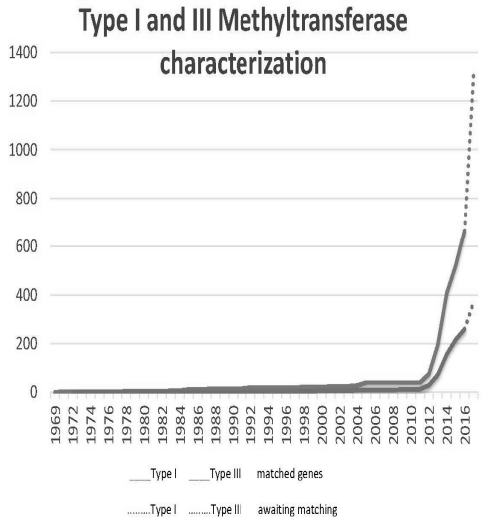
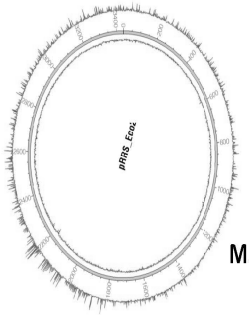
Forward Strand
Reverse Strand

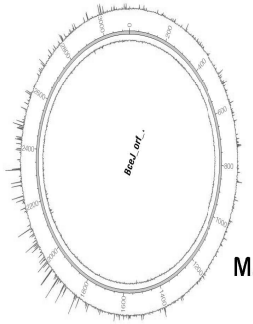


Single-strand plasmid methylation



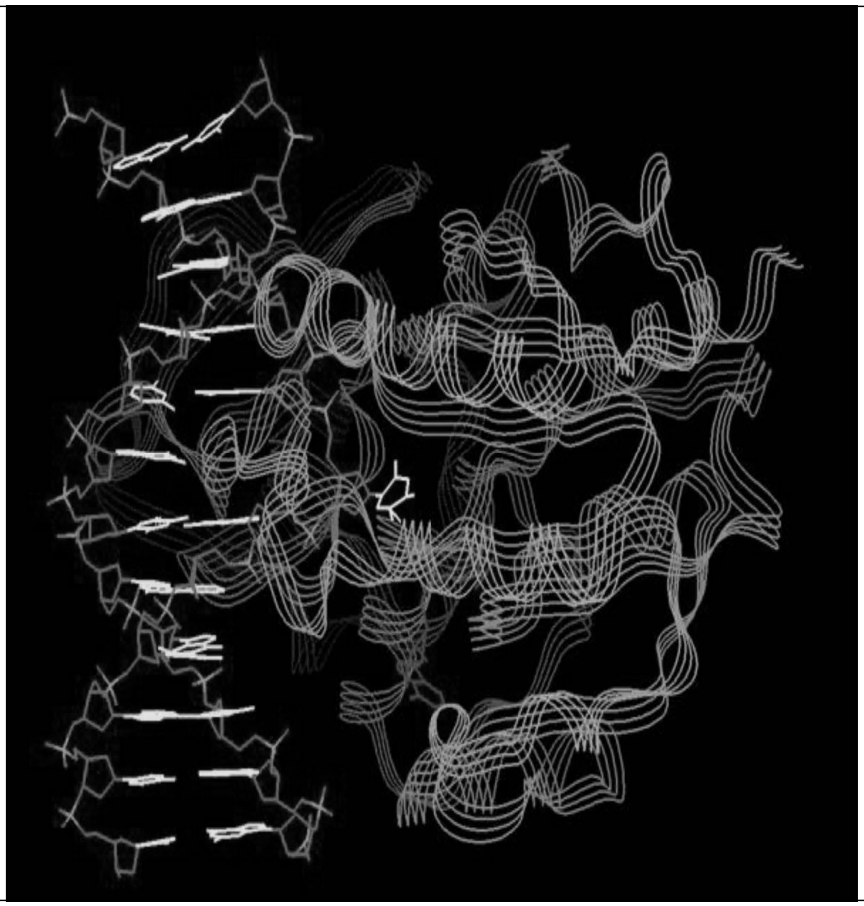
M.EcoGIX

Escherichia coli TY-2482

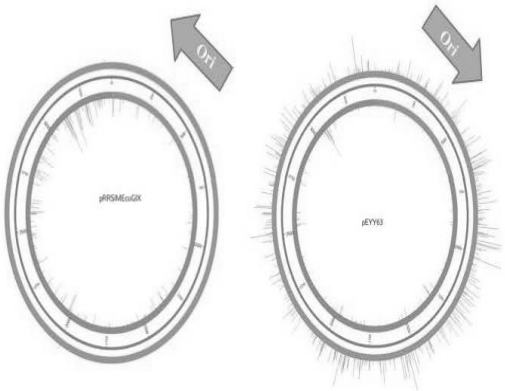


M.BceJIII

Burkholderia cenocepacia J2315



M.EcoGIX: Inverting Origin Switches
Modified Strand



What has been learned so far?

1. Many solitary MTases are found in bacteria and archaea. Could they be involved in epigenetic phenomena?

2. Much more variability in recognition sequences than expected.

3. Type II MTases specificity matches that of the REase

4. Many new non-specific MTases discovered on prophages

Advantages of PacBio sequencing

1. Methylase recognition sequences are easily determined

2. Functional annotation of methylase genes becomes straightforward

3. Closing bacterial genomes is greatly simplified

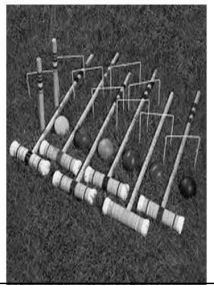
4. Accuracy of sequencing is enhanced

Puzzles and Games

I have always been fascinated by puzzles and games.

One of my favorites is croquet, which combines the skill of snooker with the strategy of chess.

My problem-solving nature is accompanied by a dry sense of humor as evidenced by my appearance as Dr. December in the 1997 Calendar "The Stud Muffins of Science", and my regular trips to the IgNobel Awards (the "opposite" of the Nobel Prizes) at Harvard University.



Honours and Humanitarian Work

I was awarded an Honorary Degree (Doctor of Science) by the University of Bath in 1994.

A refurbished science department at Beechen Cliff School (previously the City of Bath Boys' School) was also named after me.

In 2005, a large expansion to the Chemistry Department at the University of Sheffield was named after me.

I was knighted in Queen Elizabeth's 2008 Birthday Honours List.

I have helped to organize the Nobel Laureates for several good causes including gaining the release of some Bulgarian nurses from jail in Libya.

Join the Nobel Laureates Pro-GMO campaign

110 Nobel Laureates have written an open letter to Greenpeace and every UN Ambassador urging an acknowledgment that GMO technology is basically safe and should be supported for the sake of the Developing World, who desperately need better yielding crops with added nutritional value.

http://supportprecisionagriculture.org/

The Importance of Luck!

1. Billiards
2. Kazu Kurosawa
3. Harvard not Wisconsin
4. Looking for eukaryotic promoters in Adenovirus-2
5. Taking an early plane
6. Studying bacterial RM systems

Acknowledgments

Richard Gelinas
Louise Chow
Tom Broker

Sayeeda Zain (Ad2-SV40 hybrids)
Dan Klessig (fiber mRNA)

Many lab members, colleagues collaborators and friends over 50 years

Karen Otto (Slides)

Laureates Letter Supporting Precision Agriculture (GMOs)

To the Leaders of Greenpeace, the United Nations and Governments around the world

The United Nations Food & Agriculture Program has noted that global production of food, feed and fiber will need approximately to double by 2050 to meet the demands of a growing global population. Organizations opposed to modern plant breeding, with Greenpeace at their lead, have repeatedly denied these facts and opposed biotechnological innovations in agriculture. They have misrepresented their risks, benefits, and impacts, and supported the criminal destruction of approved field trials and research projects.

We urge Greenpeace and its supporters to re-examine the experience of farmers and consumers worldwide with crops and foods improved through biotechnology, recognize the findings of authoritative scientific bodies and regulatory agencies, and abandon their campaign against “GMOs” in general and Golden Rice in particular.

Scientific and regulatory agencies around the world have repeatedly and consistently found crops and foods improved through biotechnology to be as safe as, if not safer than those derived from any other method of roduction. There has never been a single confirmed case of a negative health outcome for humans or animals from their consumption. Their
environmental impacts have been shown repeatedly to be less damaging to the environment, and a boon to global biodiversity.

Greenpeace has spearheaded opposition to Golden Rice, which has the potential to reduce or eliminate much of the death and disease caused by a vitamin A deficiency (VAD), which has the greatest impact on the poorest people in Africa and Southeast Asia.

The World Health Organization estimates that 250 million people, suffer from VAD, including 40 percent of the children under five in the developing world.

Based on UNICEF statistics, a total of one to two million preventable deaths occur annually as a result of VAD, because it compromises the immune system, putting babies and children at great risk. VAD itself is the leading cause of childhood blindness globally affecting 250,000 – 500,000 children each year. Half die within 12 months of losing their eyesight.

WE CALL UPON GREENPEACE to cease and desist in its campaign against Golden Rice specifically, and crops and foods improved through biotechnology in general;

WE CALL UPON GOVERNMENTS OF THE WORLD to reject Greenpeace's campaign against Golden Rice specifically, and crops and foods improved through biotechnology in general; and to do everything in their power to oppose Greenpeace's actions and accelerate the access of farmers to all the tools of modern biology, especially seeds improved through biotechnology. Opposition based on emotion and dogma contradicted by data must be stopped.

How many poor people in the world must die before we consider this a "crime against humanity"?

Sincerely,