Bi430/530
Theory of Recombinant DNA Techniques

Prerequisite: Molecular Biology (Bi 334)

First half:
DNA basics: isolation, detection, sequencing, gene cloning

Second half:
Manipulation of DNA and genomes
Stem cells and cloning
Transgenic animals and plants

FRIDAY May 1: Midterm exam
WEDNESDAY JUNE 10: Final exam

Syllabus

Bi 430 / 530

(CRN 60373 /60390)

Theory of Recombinant DNA Techniques

Syllabus, Spring 2021

Lecture/discussion:

M-W-F, 10:15 – 11:20. In light of the continuing (but hopefully waning) COVID-19 pandemic, our class meetings will be held remotely at the scheduled times, using Zoom. Class meetings will generally cover a set of lecture materials, posted as a powerpoint file ahead of class. All Zoom class meetings will be recorded, and can be accessed later if you need to miss a class for any reason.

Instructor:

Dr. Michael Bartlett --- SRTC Room 458 --- 503-725-3858 --- micb@pdx.edu

Office hours:

Mondays 2 – 3:00 PM, Thursdays 11AM – noon, or by appointment. All office hours will be held using Zoom.

Required reading:

All course readings will be posted online.

Syllabus--first half

The basics of DNA manipulation

Class Schedule

Week	Date	Topics	Quiz/ assign	Questions to address
1	M	The molecular revolution; DNA manipulation and biosafety		How are recombinant DNA risks defined and managed?
	W	Isolation of DNA and RNA		How is useful DNA and RNA isolated?
	F	Isolation of DNA and RNA II	Q1	
2	M	Visualization and detection of DNA, RNA, and protein		How are DNA, RNA and proteins detected and measured?
	W	Detection of *specific* DNA, RNA, and protein molecules	Q2	How can specific DNA, RNA and protein molecules be identified in a complex mixture?
	F	Enzymes for manipulation of nucleic acids	A1	How can DNA be modified in the test tube?
3	M	DNA amplification by PCR	Q3	Why is PCR such a versatile tool for nucleic acid studies?
	W	DNA sequencing	(A1 due)	What DNA sequences exist in nature, and what are they for?
	F	The human genome and its implications	Q4	How is the human genome accessed and used?

Syllabus--first half

The basics of DNA manipulation

4	M	Bioinformatics		How is biological sequence and functional information used?
	W	Bioinformatics II	Q5	
	F	Genome-scale measurements: microarrays, RNAseq, chromatin immunoprecipitation, proteomes	A2	How can all of the genes in a genome be studied at once?
5	M	Cloning genes I: plasmids and transformation	Q6	How is DNA moved into and between biological systems?
	W	Cloning genes II: special vectors and large DNA fragments	(A2 due)	
	F	**Midterm exam**		

“Molecular Cloning” (2012), Green and Sambrook (4th ed.)

Chapter 1: Isolation and Quantification of DNA
Chapter 2: Analysis of DNA
Chapter 3: Cloning and Transformation with Plasmid Vectors
Chapter 4: Gateway Recombinational Cloning
Chapter 5: Working with Bacterial Artificial Chromosomes and Other High-Capacity Vectors
Chapter 6: Extraction, Purification, and Analysis of RNA from Eukaryotic Cells
Chapter 7: Polymerase Chain Reaction
Chapter 8: Bioinformatics
Chapter 9: Quantification of DNA and RNA by Real-Time Polymerase Chain Reaction
Chapter 10: Nucleic Acid Platform Technologies
Chapter 11: DNA Sequencing
Chapter 12: Analysis of DNA Methylation in Mammalian Cells
Chapter 13: Preparation of Labeled DNA, RNA, and Oligonucleotide Probes
Chapter 14: Methods for In Vitro Mutagenesis
Chapter 15: Introducing Genes into Cultured Mammalian Cells
Chapter 16: Introducing Genes into Mammalian Cells: Viral Vectors
Chapter 17: Analysis of Gene Regulation Using Reporter Systems
Chapter 18: RNA Interference and Small RNA Analysis
Chapter 19: Expressing Cloned Genes for Protein Production, Purification, and Analysis
Chapter 20: Cross-Linking Technologies for Analysis of Chromatin Structure and Function
Chapter 21: Mapping of In Vivo RNA-Binding Sites by UV-Cross-Linking Immunoprecipitation (CLIP)
Chapter 22: Gateway-Compatible Yeast One-Hybrid and Two-Hybrid Assays

Readings from this manual will be posted

Syllabus--second half

Applications of rDNA

6	M	Cloning genes III: library construction and screening, recombination-based engineering, cloning in prokaryotes other than *E. coli*		How can a specific piece of DNA be identified and cloned?
	W	Protein expression I		How can cells be made to produce useful products?
	F	Protein expression II	Q7	
7	M	Mutagenesis, protein engineering, altering the genetic code		How can genes & organisms be altered for practical purposes?
	W	Applied mutagenesis: metabolic engineering, genome shuffling, synthetic genomes	Q8	
	F	Applied mutagenesis II	A3	
8	M	Cloning in *Saccharomyces cerevisiae*. Cloning in higher eukaryotic cells: cell culture, embryonic and induced pluripotent stem cells, organismal cloning	Q9	Why is yeast such a useful model system for eukaryotes? Why are stem cells so useful? How can an organism be cloned?
	W	Cloning in eukaryotic cells: transformation and viral transduction	(A3 due)	How is new DNA added to eukaryotic cells?
	F	Cloning in eukaryotic cells: selection strategies and genetic control	Q10	How are added genes controlled?

Syllabus--second half

Applications of rDNA

9	M	Gene therapy and CRISPR-Cas9		How is gene therapy being done? How is Crispr-Cas9 being used
	W	CRISPR-Cas9 II	Q11	
	F	Nucleic acid vaccines	A4	How can the immune system be programmed to prevent infectious diseases?
10	M	*Memorial Day (no class)*		
	W	Transgenic animals	Q12	How and why are transgenic animals and plants made?
	F	Genetic manipulation of plants	(A4 due)	
Finals		**Final exam**, Wed. June 9, 10:15 – 12:05		

Recombinant DNA Techniques during a viral pandemic?

Grading:

Grading:	Bi 430	Bi 530*	
	40%	30%	Quizzes: lowest two quiz scores are dropped
	30%	20%	Homework: lowest homework score is dropped
	15%	15%	Midterm exam
	15%	15%	Final exam
	-----	20%	* In-class presentations, to be given during the second half of the class

Grading cut-offs will be as follows: 93% and up, A; 90 and up, A-; 88 and up, B+; 82 and up, B; 80 and up, B-; 77 and up, C+; 68 and up, C; 65 and up, C-; 62 and up, D+; 53 and up, D; 50 and up, D-; under 50, F.

There are no makeup exams. You must take both exams or you cannot earn a passing grade.

Academic dishonesty (cheating, plagiarism, etc.) will result in a zero for the assignment, and will be reported to student affairs, as described in the PSU Code of Conduct: https://www.pdx.edu/dos/psu-student-code-conduct

If you are a student with a documented disability and have registered with the Disability Resource Center, please contact me immediately to arrange academic accommodations.

Readings

Readings to be posted online

Information from: “Molecular Cloning, A Laboratory Manual” Sambrook and Russell (2012), Cold Spring Harbor Laboratory Press

Various papers (PDFs) through out the term

If you would like additional resources for specific topics, let me know

Introduction to DNA manipulation

1) The simplicity of a DNA-based information system makes direct, deliberate genetic manipulation possible

2) This represents an unprecedented power for human interaction with living systems

3) Benefits and costs of technology require continuous assessment

Guide to readings: Day 1

Discovery of the genetic code

- *Nirenberg 1967*. Essay: “Will Society Be Prepared?”, predicts and ponders the effects of DNA manipulation.
- *NIH Nirenberg Papers*. Some historical context for the discovery of the genetic code.

Basic lab safety and recombinant DNA

- *0.1 MC4 Safety*: short guide to lab safety from the Molecular Cloning manual.

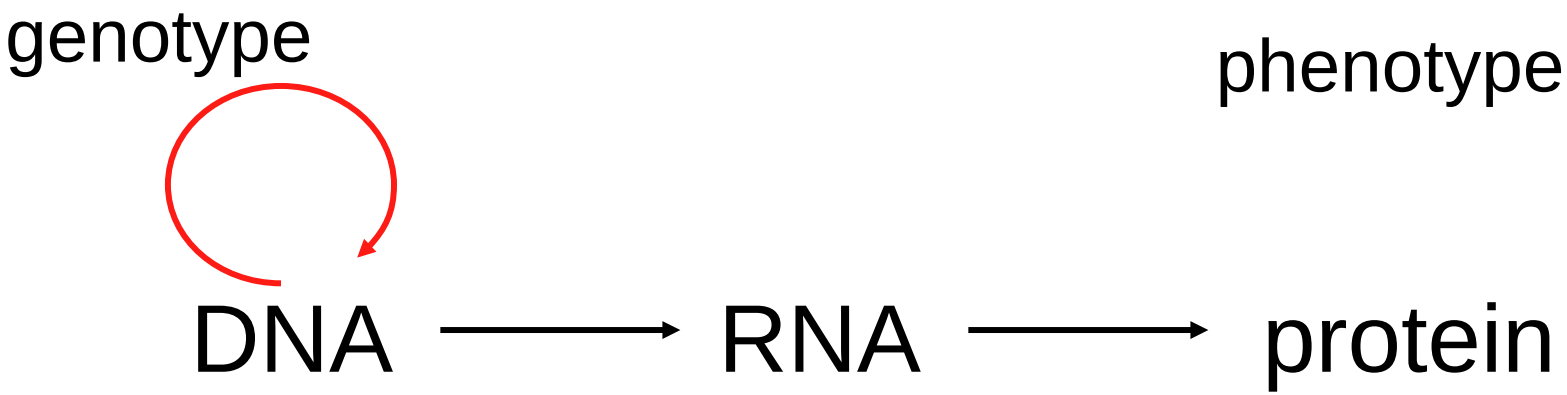
Genetic modification in the 21st century

- *Reboot the debate…* by Jennifer Kuzma. Both product and process are important for genetic engineering (2016).

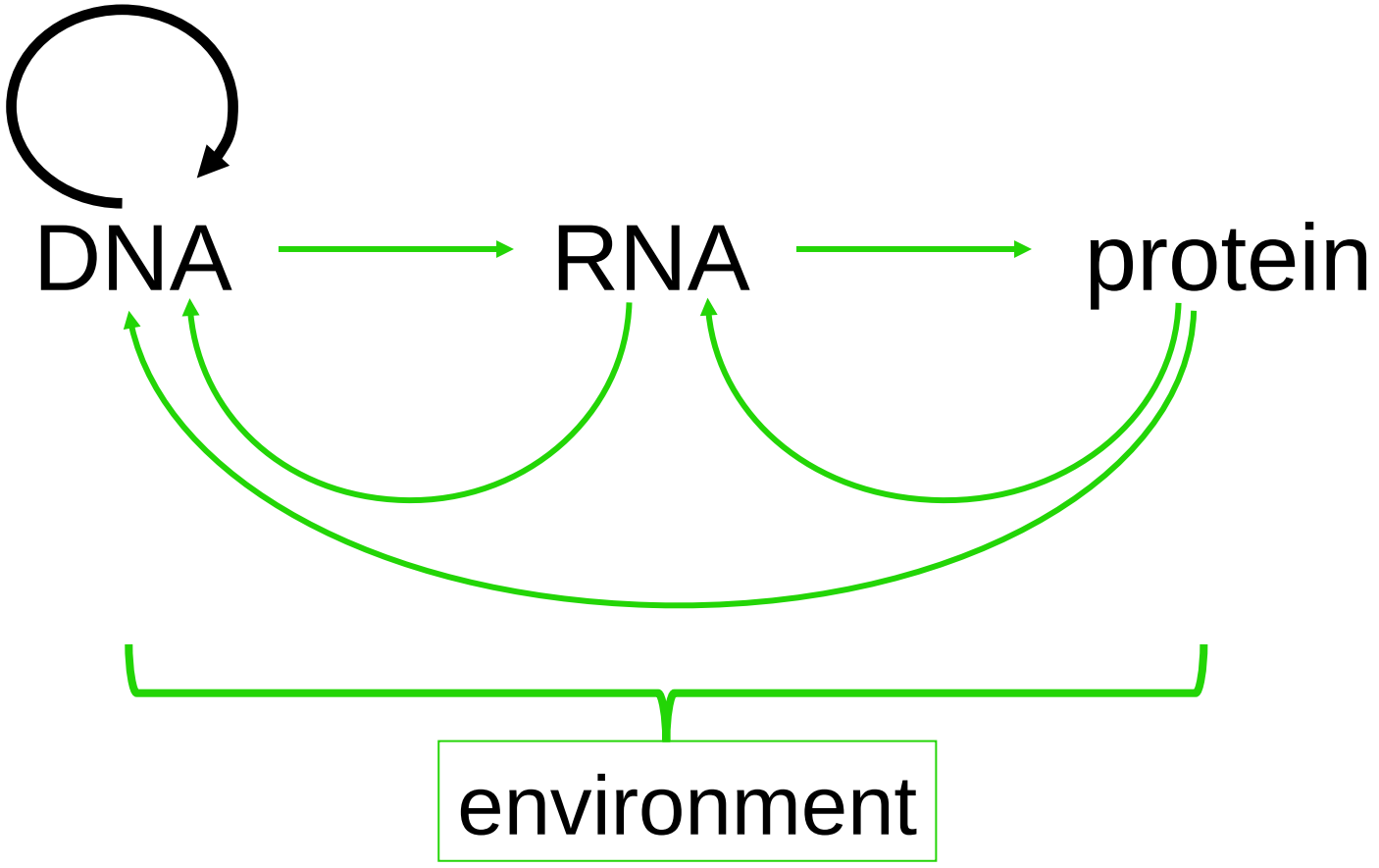
Video: Paul Berg discussing the Asilomar meeting and contemporary analysis of the risks of rDNA

- https://youtu.be/QSKe15I4vyM (part I)
- https://youtu.be/Eg2Sz_-l9UI (part II)

Information flow in the cell

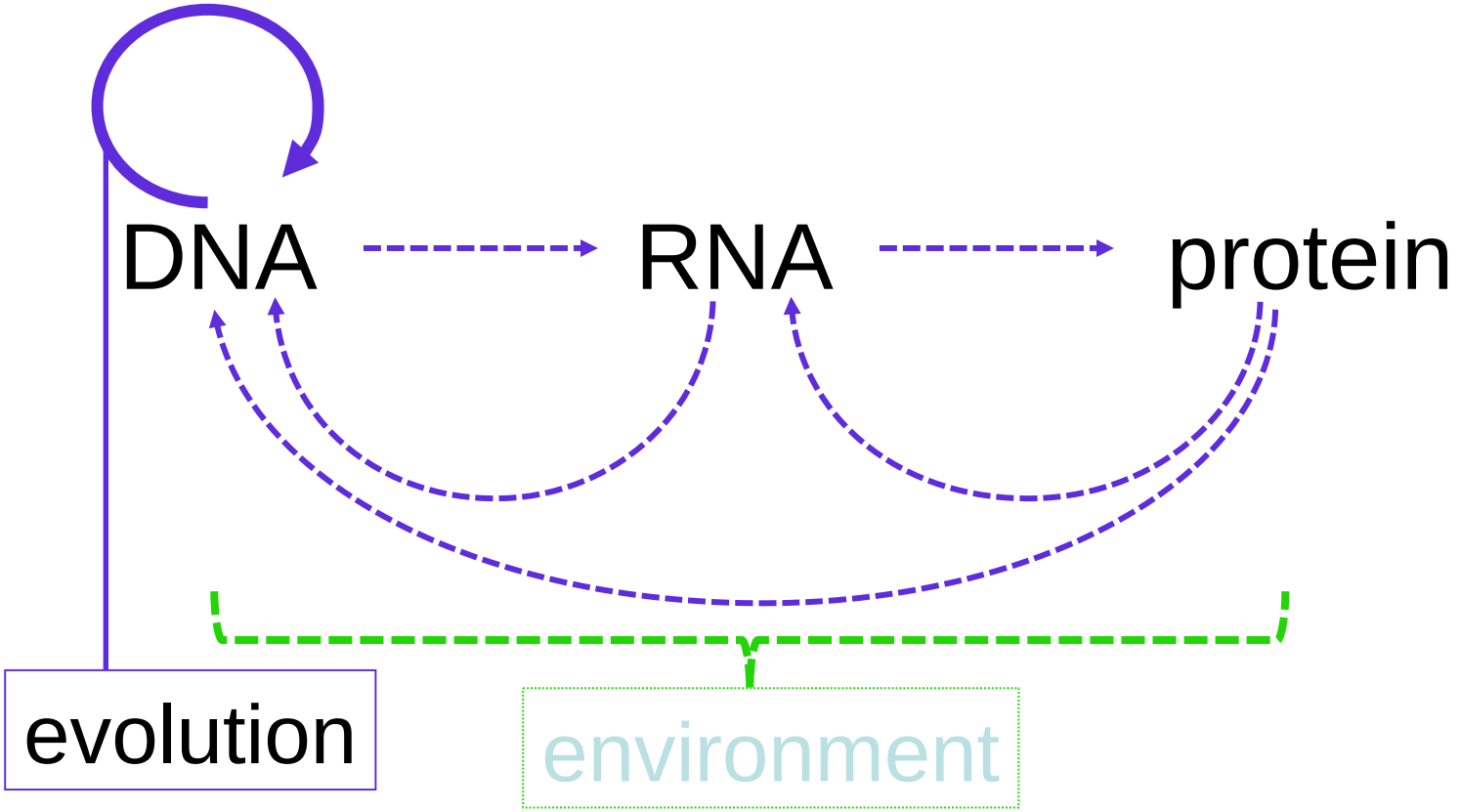


Organisms respond to their environment via information from sensory input, causing changes in gene expression



Dynamic but mostly <u>transient</u> modification of DNA program

Evolution: species respond to environment over long time frames via additions, deletions, rearrangements, and mutations in the DNA program



-- Heritable modifications of genetic program
-- Reflects adaptation to environment over long time scales

DNA structure

Rosalind Franklin and Maurice Wilkins:

X-Ray fiber diffraction pattern of pure B-form DNA (1953)

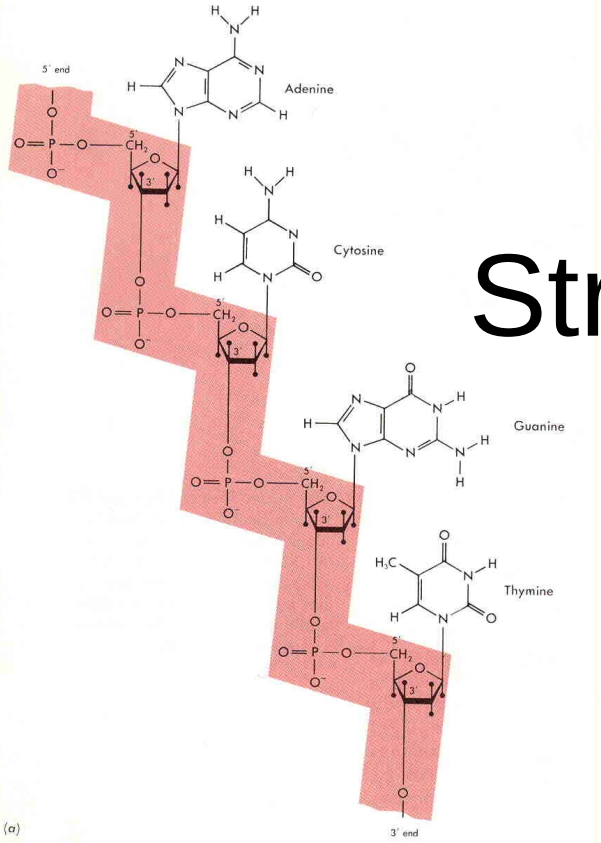
James Watson and Francis Crick:

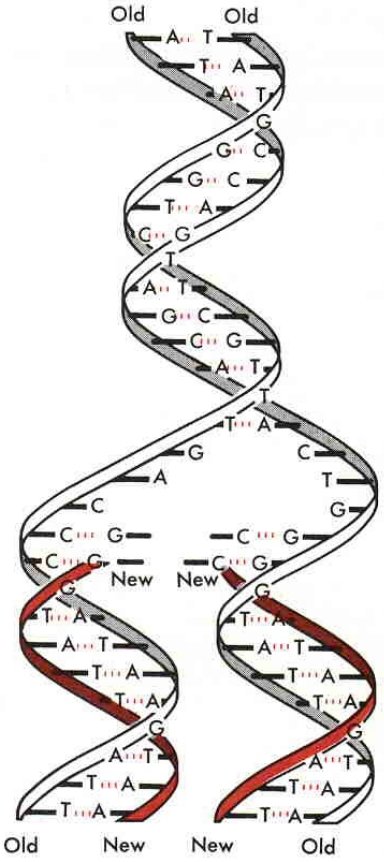
Proposed two antiparallel, helical strands forming a stable duplex with DNA bases on interior of the molecule, joined by hydrogen bonds (1953)

DNA had been known as the element of genetic transmission at least since 1947, when Avery showed that DNA could “transform” bacterial colony morphology

Why was the structure so important?

Structure of DNA





DNA structure suggested:

-- Mechanism for replication

-- Stabile information storage -- but accessing the information not difficult

The DNA structure provided a new template for hypotheses regarding biological phenomena

DNA is very easy to work with…

- Easy to isolate -- plasmids, genomic DNA, PCR, etc.
- Stable -- not as chemically reactive as RNA (even archaeologically stable!)
- Easy to propagate and move from cell to cell
- Easy to make specific constructs
- Easy to make specific mutations

- Very easy to sequence
- Predictable behavior (the genetic code)

- Sequence lends itself to analysis (genome projects)

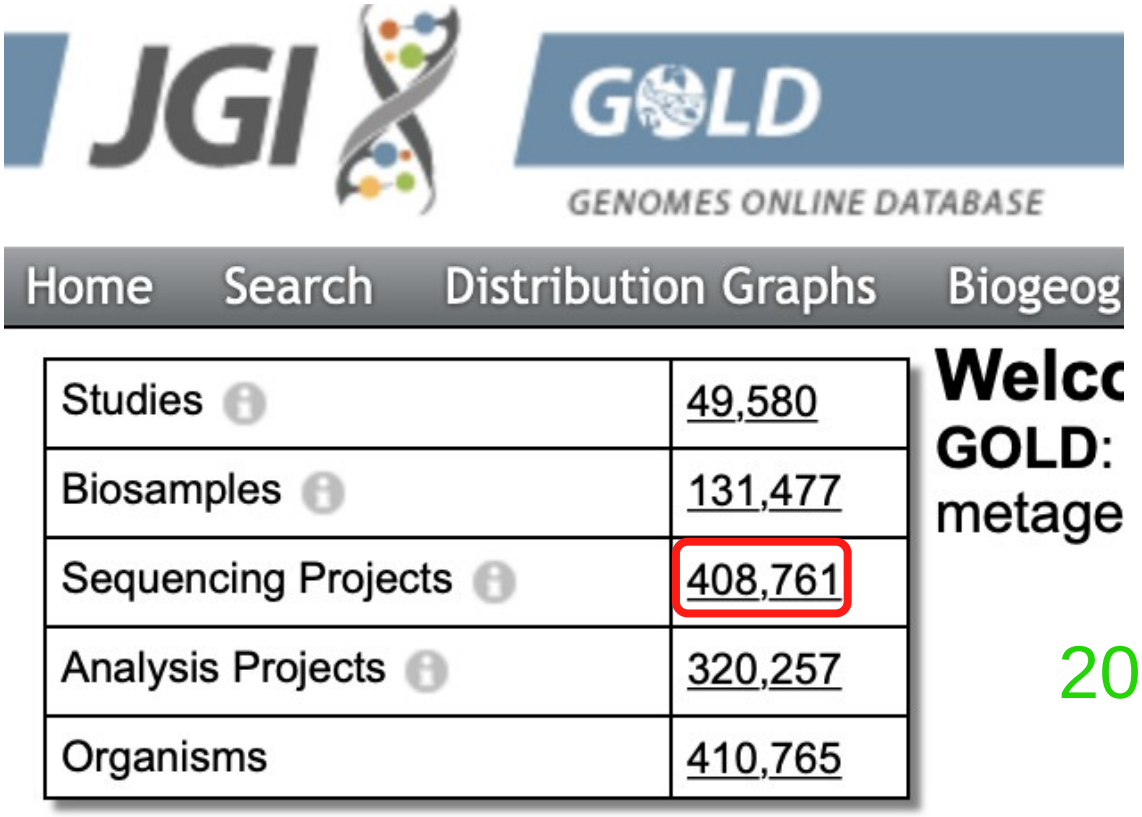
DNA is very easy to sequence: early completed genomes

Genome sequenced	Year	Genome size
Bacteriophage ϕX174	1977	5.38 kb
Plasmid pBR322	1979	4.3 kb
Bacteriophage λ	1982	48.5 kb
Epstein–Barr virus	1984	172 kb
Yeast chromosome III	1992	315 kb
Haemophilus influenzae	1995	1.8 Mb
Saccharomyces cerevisiae	1996	12 Mb
Ceanorhabditis elegans	1998	97 Mb
Drosophila melanogaster	2000	165 Mb
Homo sapiens	2000	3000 Mb
Arabidopsis thaliana	2000	125 Mb

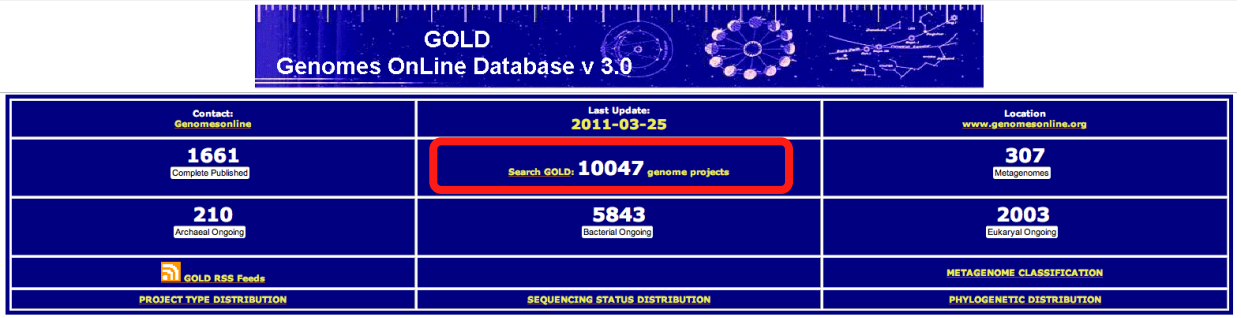
Etc….

Genomes OnLine Database (GOLD)

https://gold.jgi.doe.gov/



2021

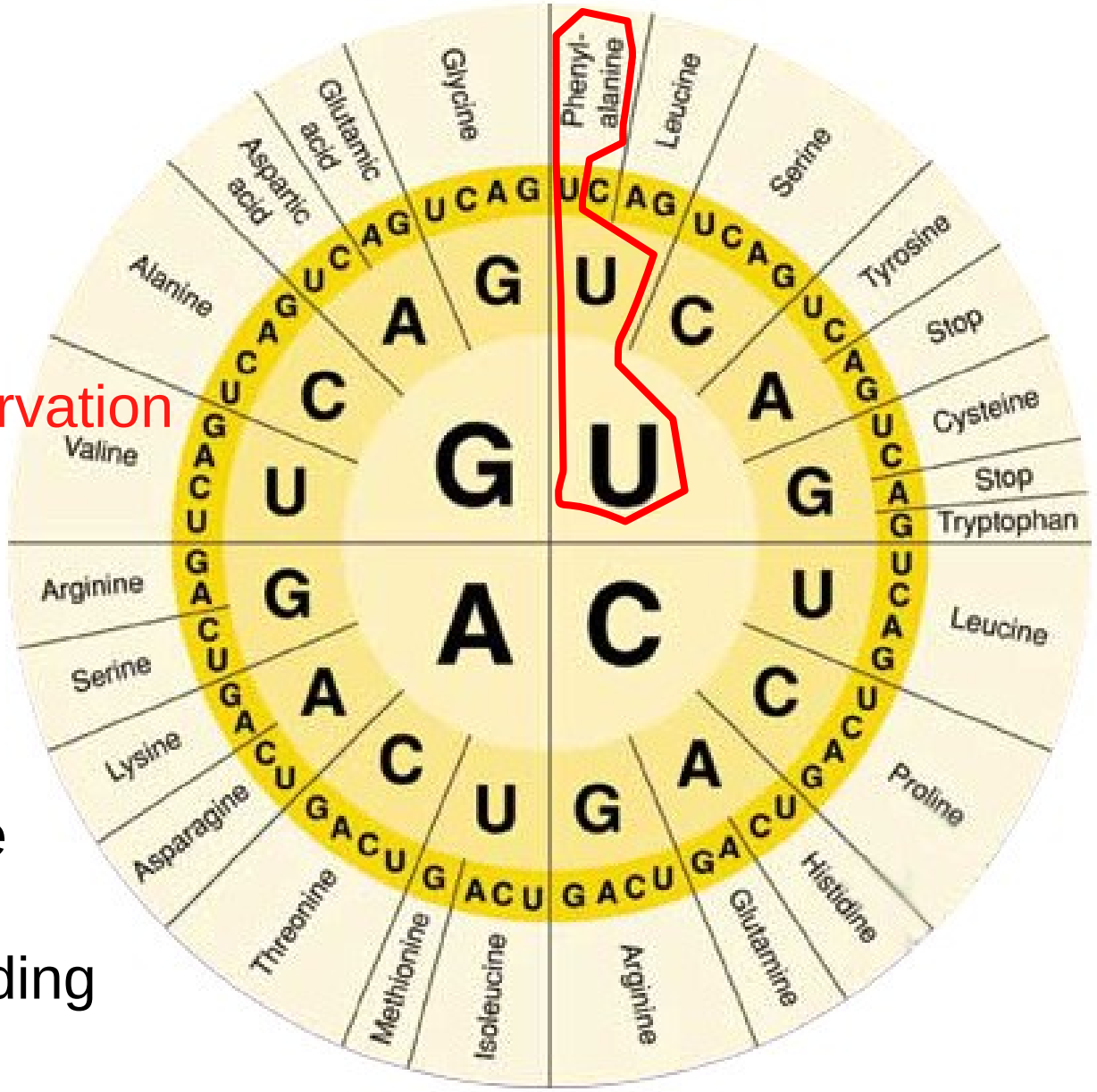


2011

The behavior of DNA (genes) is predictable

Gene sequence conservation often indicates functional similarity

Non-protein coding information sequences can also sometimes be detected by homology (for example, DNA binding protein binding sites)



the genetic code

The genetic code and the roots of biotechnology

1961
Marshall Nirenberg and Heinrich J. Matthaei:
polyU mRNA encodes poly-phenylalanine

1966
Nirenberg and colleagues had deciphered the 61 codons (and 3 nonsense codons) for the 20 common amino acids

1968
Nobel prize for Nirenberg, Holley, and Khorana

1966
George and Muriel Beadle (geneticist & author) write:

“ The deciphering of the DNA code has revealed our possession of a language much older than hieroglyphics, a language as old as life itself, a language that is the most living language of all--even if its letters are invisible and its words are buried deep in the cells of our bodies.”

The public reaction to the deciphering of the genetic code

Wow

"…just as big a breakthrough in biology as [Newton's discovery of gravitation in the seventeenth century] was in physics." --John Pfeiffer, journalist, 1961

Optimism

"No stronger proof of the universality of all life has been developed since Charles Darwin's 'The Origin of Species' demonstrated that all life is descended from one beginning. In the far future, the hope is that the hereditary lineup will be so well known that science may deal with the aberrations of DNA arrangements that produce cancer, aging, and other weaknesses of the flesh." Chicago Sun-Times, 1962

Caution

…knowledge gained from the genetic code “might well lead in the foreseeable future to a means of directing mutations and changing genes at will.” 1961, A. G. Steinberg of Case Western Reserve University

…knowledge of the genetic code could “lead to methods of tampering with life, of creating new diseases, of controlling minds, of influencing heredity, even perhaps in certain desired directions.”1961, Arne Wilhelm Kaurin Tiselius, 1948 Nobel Laureate in Chemistry

1967

"Will Society Be Prepared?" Marshall Nirenberg, editorial in *Science* (see letter online)

same language, with minor variations. Simple genetic messages now can be synthesized chemically. Genetic surgery, applied to microorganisms, is a reality. Genes can be prepared from one strain of bacteria and inserted into another, which is then changed genetically. Such changes are inheritable. Thus far, it has not been possible to program mammalian

What may be expected in the future? Short but meaningful genetic messages will be synthesized chemically. Since the instructions will be written in the language which cells understand, the messages will be used to program cells. Cells will carry out the instructions, and the program may even be inherited. I don't know how long it will take before it will be possible to program cells with chemically synthesized messages. Certainly the experimental obstacles are formidable. However, I have little doubt that the obstacles eventually will be overcome. The only question is when. My guess is that cells will be programmed with synthetic messages within 25 years. If efforts along those lines were intensified, bacteria might be programmed within 5 years.

Nirenberg, 1967

"When man becomes capable of instructing his own cells, he must refrain from doing so until he has sufficient wisdom to use this knowledge for the benefit of mankind....

[D]ecisions concerning the application of this knowledge must ultimately be made by society, and only an informed society can make such decisions wisely."

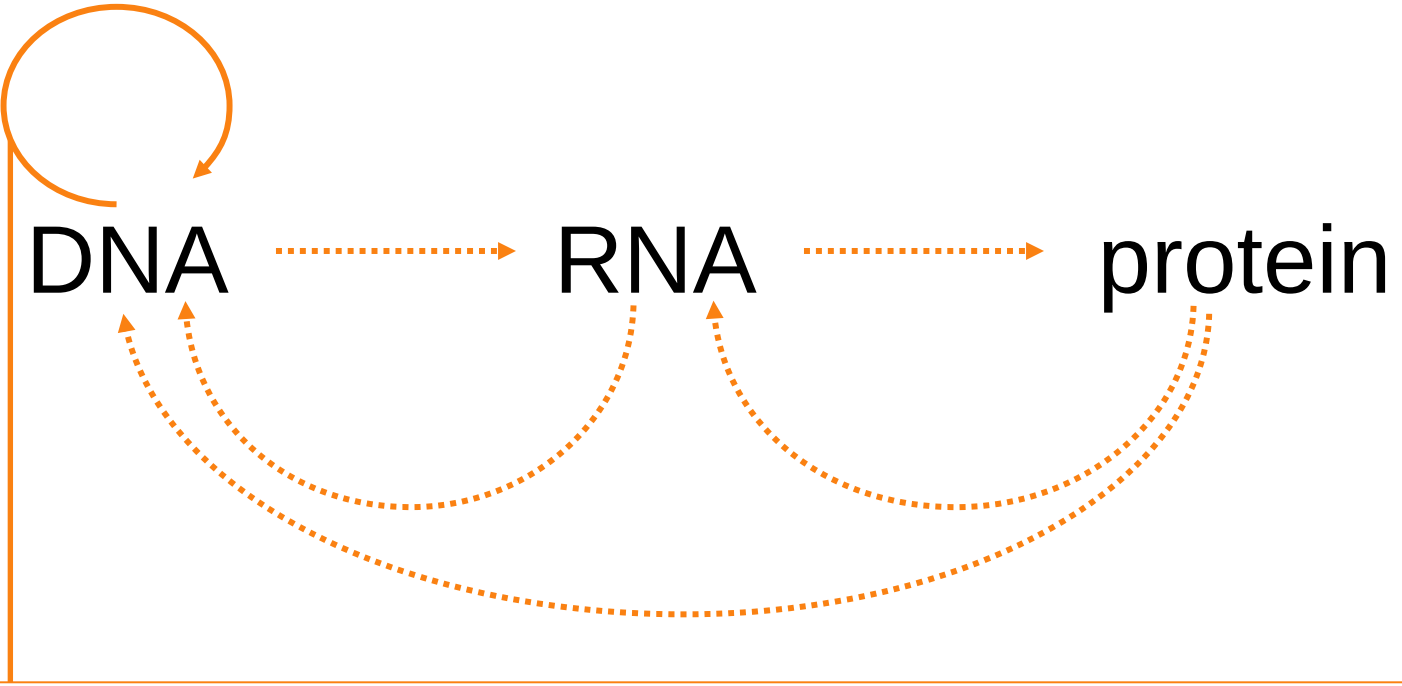
Response from Joshua Lederberg, 1967 (see letter):

(paraphrased)

-- We need to be particularly careful with manipulation of the germ cell lines (heritable changes).

-- Considerations governing control of our biology are equally important to considerations governing control of our cultural institutions (given that culture is mutable and heritable)

Human activity: transient modifications of environment, permanent modifications of DNA program



Human intervention: genetics (indirect), rDNA (direct)

The first recombinant DNA molecules: 1972

- Paul Berg and co-workers at Stanford Univ.
- SV40 (Simian virus 40) had the genes from the E. coli galactose operon inserted

In 1974, a voluntary moratorium was declared on recombinant DNA research

- "…new technology created extraordinary novel avenues for genetics and could ultimately provide exceptional opportunities for medicine, agriculture and industry….

  …concerns that unfettered pursuit of this research might engender unforeseen and damaging consequences for human health and the Earth's ecosystems."

- 1975: The Asilomar Conference on Recombinant DNA

http://nobelprize.org/nobel_prizes/chemistry/articles/berg/index.html

1975: The Asilomar Conference on Recombinant DNA

- In attendance: internationally prominent scientists, government officials, doctors, lawyers, members of the press

- Conclusion:

  "…recombinant DNA research should proceed, but under strict guidelines."

- The moratorium was lifted, and "… guidelines were subsequently promulgated by the National Institutes of Health and by comparable bodies in other countries."

http://nobelprize.org/nobel_prizes/chemistry/articles/berg/index.html

The Asilomar principles:

1) containment should be an essential consideration in the experimental design
2) the effectiveness of the containment should match the estimated risk as closely as possible.

Additional suggestions:

Use biological barriers to limit the spread of recombinant DNA

- Fastidious bacterial hosts (able to grow only with specific nutrients) that are unable to survive in natural environments
- nontransmissible and equally fastidious vectors (plasmids, bacteriophages, or other viruses) that are able to grow in only specified hosts

The Asilomar principles:

Safety factors

- physical containment, exemplified by the use of hoods or where applicable, limited access or negative pressure laboratories
- strict adherence to good microbiological practices, which would limit the escape of organisms from the experimental situation
- education and training of all personnel involved in the experiments would be essential to effective containment measures.

Regulation of biotechnology: US National Institutes of Health (NIH) Guidelines

- stipulations of biosafety and containment measures for recombinant DNA research

- delineations of critical ethical principles and safety reporting requirements for human gene transfer research

See http://oba.od.nih.gov/rdna/rdna.html

“Reboot the debate” (essay by Jennifer Kuzma, posted)

Previous U.S. regulations of genetically modified organisms have focused mainly on the *product*, with little concern given to the *process* used to obtain the organism (following the Coordinated Framework for Reg. of Biotech [CFRB] of 1986)

Some processes are imprecise and introduce certain kinds of uncertainty, while others are much more precise

Some products are clearly innocuous, while others are potentially (or may be actually) dangerous

Both process and product need to be considered in debate on genetic modification

The question of ‘synthetic biology’

- Synthetic biology: biological systems that are
  - Programmable
  - Self-referential*
  - Modular

- The complexity of biological systems being created will likely lead to unexpected behaviors

- Rationale for this kind of work needs to be clearly stated. What is the utility of synthetic biological entities? How can public trust be retained?

* refers to biological entities or systems that are composed of elements that can interact, respond to changes, self-modify, replicate or expand.

Synthetic biology: four areas of ecological risk assessment

1)Specific physiology of the organism. Does it produce toxins, for example?

2)How will synthetic organism affect its environment? Will it affect biodiversity, for example?

3)Could the synthetic organism evolve quickly, adapting to new environments?

4)Can the synthetic organism transfer its genes to other organisms?

What can we do with recombinant DNA technology?

- begin to learn how cells, tissues, organisms, communities work, interact, respond to the environment (gain scientific knowledge)
- improve human health
- industrial production of useful enzymes, metabolic products
- improve industrial process
- raise agricultural productivity
- investigate problems of geneology, paternity, anthropology, archaeology
- investigate criminal cases
- etc….

Recombinant DNA technology in medicine

- Understand molecular mechanisms of disease
- Predict and diagnose of disease
- Animal models for human diseases
- Therapies
  - nucleic acids: gene therapy
  - production of pharmacologically active proteins
  - small biomolecule synthesis and testing
- Antimicrobial strategies
  - Vaccines
  - Antibiotic development and production

Summary:

1) The simplicity and predictability of a DNA-based information system makes genetic manipulation possible

2) This represents an unprecedented level of interaction with living systems

3) Benefits versus costs of recombinant DNA technology require continuous assessment