DNA Sequencing

ENCODE: ENCyclopedia Of DNA Elements

Objective:
To identify all functional elements in the human genome sequence

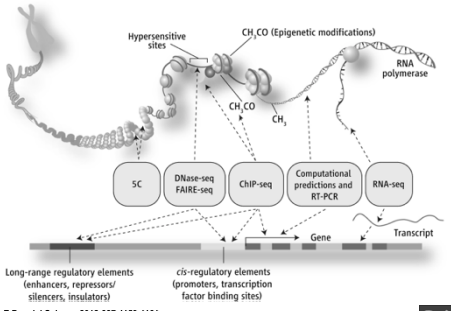
ENCODE By the Numbers

- **147** cell types studied
- **80%** functional portion of human genome
- **20,687** protein-coding genes
- **18,400** RNA genes
- **1640** data sets
- **30** papers published this week
- **442** researchers
- **$288 million** funding for pilot, technology, model organism, and current project

E Pennisi Science 2012;337:1159-1161

Science AAAS

Zooming in.A diagram of DNA in ever-greater detail shows how ENCODE's various tests (gray boxes) translate DNA's features into functional elements along a chromosome.



E Pennisi Science 2012;337:1159-1161

Science AAAS

DNA Sequencing

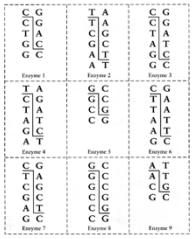
Restriction enzymes (1973; Boyer & Cohen) cleave the polynucleotide to smaller fragments.

These smaller fragments (100-200 base pairs) are sequenced.

The two strands are separated.

DNA Sequencing

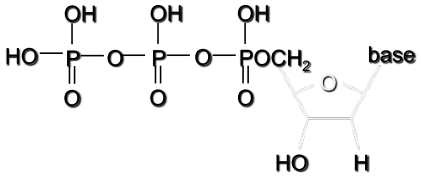
Restriction enzymes cleave the polynucleotide to smaller fragments in specific patterns.



DNA Sequencing

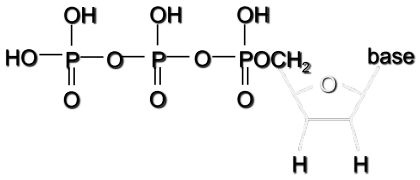
Single stranded DNA divided in four portions.

Each tube contains adenosine, thymidine, guanosine, and cytidine plus the triphosphates of their 2'-deoxy analogs.



DNA Sequencing

The first tube also contains the 2,'3'-dideoxy analog of adenosine triphosphate (ddATP); the second tube the 2,'3'-dideoxy analog of thymidine triphosphate (ddTTP), the third contains ddGTP, and the fourth ddCTP.



DNA Sequencing

Each tube also contains a "primer," a short section of the complementary DNA strand, labeled with radioactive phosphorus (^{32}P).

DNA synthesis takes place, producing a complementary strand of the DNA strand used as a template.

DNA synthesis stops when a dideoxynucleotide is incorporated into the growing chain.

DNA Sequencing

The contents of each tube are separated by electrophoresis and analyzed by autoradiography.

There are four lanes on the electrophoresis gel.

Each DNA fragment will be one nucleotide longer than the previous one.

DNA Profiling

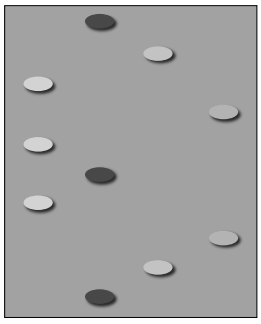
DNA sequencing involves determining the nucleotide sequence in DNA.

The nucleotide sequence in regions of DNA that code for proteins varies little from one individual to another, because the proteins are the same.

Most of the nucleotides in DNA are in "noncoding" regions and vary significantly among individuals.

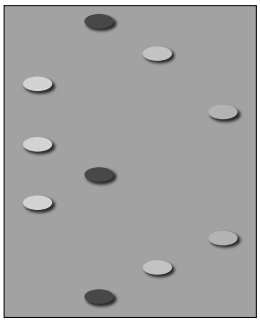
Enzymatic cleavage of DNA give a mixture of polynucleotides that can be separated by electrophoresis to give a "profile" characteristic of a single individual.

The bloody glove?



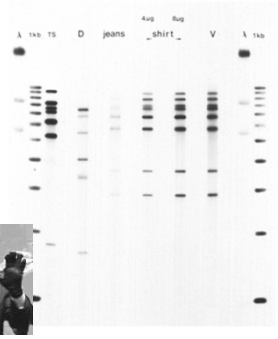
Sequence of fragment
T
TG
TGA
TGAC
TGACA
TGACAT
TGACATA
TGACATAC
TGACATACG
TGACATACGT

A bloody glove?



Sequence of fragment	Sequence of original DNA
T	A
TG	AC
TGA	ACT
TGAC	ACTG
TGACA	ACTGT
TGACAT	ACTGTA
TGACATA	ACTGTAT
TGACATAC	ACTGTATG
TGACATACG	ACTGTATGC
TGACATACGT	ACTGTATGCA

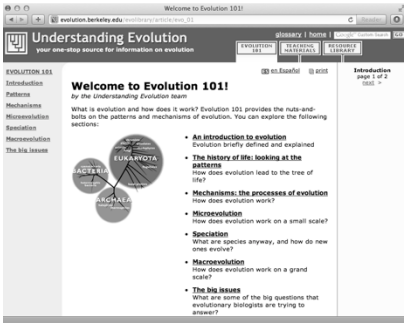
Genetic Fingerprinting



OJ Simpson and the bloody glove!

Forensics
Paternity
ID-military
Food
Wine
Anthropology
Evolution

http://evolution.berkeley.edu/evolibrary/article/evo_01



PCR: Polymerase Chain Reaction

PCR

When a sample of DNA is too small to be sequenced or profiled, the *polymerase chain reaction* (PCR) is used to make copies ("amplify") portions of it.

PCR amplifies DNA by repetitive cycles of the following steps.

1. Denaturation
2. Annealing ("priming")
3. Synthesis ("extension" or "elongation")

PCR

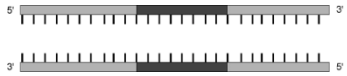
(*a*) Consider double-stranded DNA containing a polynucleotide sequence (the target region) that you wish to amplify.

Target region

(*b*) Heating the DNA to about 95°C causes the strands to separate. This is the denaturation step.

PCR

(*c*) Cooling the sample to ~60°C causes one primer oligonucleotide to bind to one strand and the other primer to the other strand. This is the annealing step.



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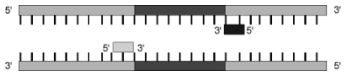
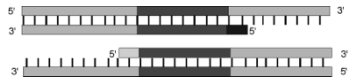


(*d*) In the presence of four DNA nucleotides and the enzyme DNA polymerase, the primer is extended in its 3' direction. This is the synthesis step and is carried out at 72°C.

PCR

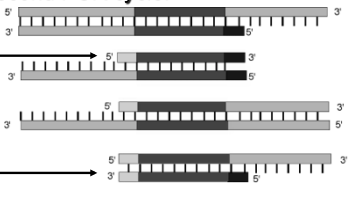
This completes one cycle of PCR.



(*e*) The next cycle begins with the denaturation of the two DNA molecules shown. Both are then primed as before.

PCR

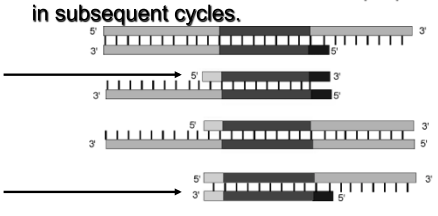
(*f*) Elongation of the primed fragments completes the second PCR cycle.



(*g*) Among the 8 DNAs formed in the second cycle are two having the structure shown.

PCR

The two contain only the target region and and are the ones that increase disproportionately in subsequent cycles.



(*g*) Among the 8 DNAs formed in the second cycle are two having the structure shown.

PCR

Cycle	Total DNAs	Contain only target
0 (start)	1	0
1	2	0
2	4	0
3	8	2
4	16	8
5	32	22
10	1,024	1,004
20	1,048,566	1,048,526
30	1,073,741,824	1,073,741,764

Recombinant Methods

Recombinant DNA : GMOs

Restriction enzymes (1973), plasmids, promoters, recombinant DNA (rDNA) -> New Organisms -> Genentech et. al. (1976)

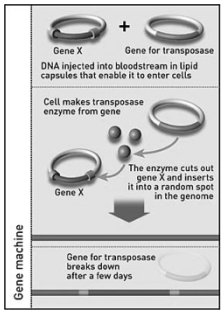
ABSTRACT

The Cohen-Boyer licensing program, by any variety of metrics, was widely successful. Recombinant DNA (rDNA) products provided a new technology platform for a range of industries, resulting in over US$35 billion in sales for an estimated 2,442 new products. Over the duration of the life of the patents (they expired in December 1997), the technology was licensed to 468 companies, many of them fledgling biotech companies who used the licenses to establish their legitimacy. Over the 25 years of the licensing program, Stanford and the University of California system accrued US$255 million in licensing revenues (to the end of 2001), much of which was subsequently invested in research and research infrastructure. In many ways, Stanford's management of the Cohen-Boyer patents has become the gold standard for university technology licensing. Stanford made pragmatic decisions and was flexible, adapting its licensing strategies as circumstances changed.

http://www.nytimes.com/1999/12/07/business/robert-a-swanson-52-co-founder-of-genentech.html

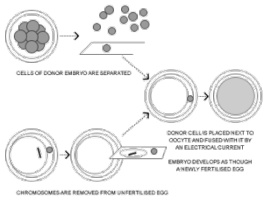
Transgenic Crops

Monsanto Syngenta Luis?



CLONING

Hello Dolly, and
Lassie, and Tabby



DNA Mutations

Understanding Evolution : Personal response question

Mutation is a random process.

a. agree
b. disagree

Ribozyme structure comes from Scott, W.G., Finch, J.T., Klug, A. (1995) The crystal structure of an all-RNA hammerhead ribozyme: a proposed mechanism for RNA catalytic cleavage. Cell 81: 991-1002

DNA Mutations

Substitution

CTGGAG
CTGGGG

Insertion

CTGGAG
CTGGTGGAG

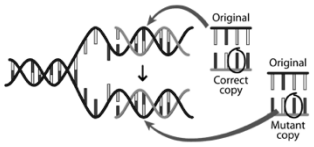
Deletion

CTGGAG
CTAG

Frameshift

The fat cat sat
hef atc ats at

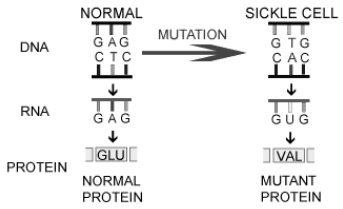
DNA Mutations



Enzymatic corrections are common
But, they are not always done

DNA Mutations

Sickle Cell Anemia



RNA

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Science AAAS

Published by AAAS

COMPARING DIFFERENT KINDS OF SMALL RNAs

	siRNA	microRNA	piRNA
Length	21–24 nucleotides	20–25 nucleotides	21–31 nucleotides
Organization	Double-stranded	Single-stranded	Single-stranded
Requires Dicer for maturation?	Yes	Yes	No
Found in	Animals, plants, fungi, protists	Animals, plants, protists	Only animals
Function	Controlling gene expression, blocking transposons	Controlling gene expression	Blocking transposons

M Leslie Science 2013;339:25-27

Published by AAAS

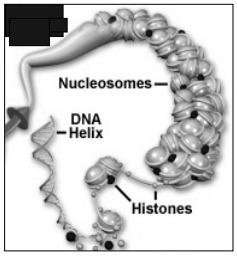
Epigenetics

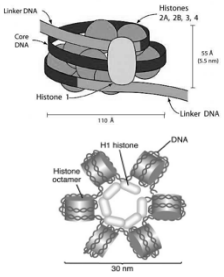
Epigenetics

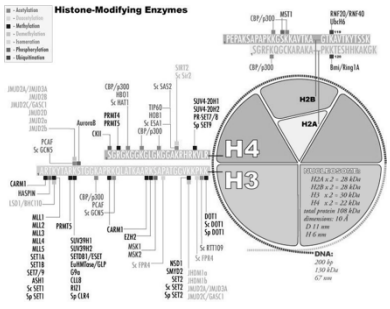
- Chemical reactions switch parts of the genome off and on at strategic times and locations.
- Epigenetics is the study of these reactions and the factors that influence them.
- View video: http://learn.genetics.utah.edu/content/epigenetics/intro/

http://learn.genetics.utah.edu/content/epigenetics/control/

Nucleosomes & Histones







Neoplasia is characterized by "methylation imbalance"