

Why is DNA an important molecule in biology?

Newsweek, May 23, 2005



SAME DNA. SMALLER CHROMOSOMES.

THE ALL-NEW MIDSIZE H3. LIVING UP TO THE OFF-ROAD REPUTATION HUMMER MADE FAMOUS. COMING SOON. STARTING AT \$29,500. VEHICLE SHOWN \$30,195.*

"MSRP. TAX, TITLE, LICENSE, DEALER FEES AND OPTIONAL EQUIPMENT ARE EXTRA. 1.800.REAL.4WD ID GENERAL MOTORS CORPORATION: 2005



DNA still sells cars in the USA

Subaru: "Genetic superstar" Toyota: "Has a great set of genes



GENETIC INDIVIDUALITY:

Each of us is a genetically unique individual, and the genes determine who we are.



"...revealing what it is that makes you, you." -American television ad for ancestry.com 2015

The importance of DNA in biology

Major basis of heritable variation (genotype-phenotype)

Transmitted (can help reconstruct history)

Present in all living entities (DNA/RNA)

Stable molecule (ancient DNA – oldest = horse in permafrost = 500 000 years, forensic)

String of letters, can be easily analyzed with computers (compared to anatomical traits for taxonomy)

Genetic Individuality

Slide from S. Gilbert

Genes determine who you are, and they act the same in each person.

LIFE Magazine, First Days of Creation, 1990:

"The result of fertilization is a single nucleus that contains an entire biological blueprint for a new individual, genetic information governing everything from the length of the nose to the diseases that will be inherited."

Standupgirl.com (anti-Choice website):

"And even more amazingly, intelligence and personality—the way you look and feel were already in place in your genetic code. At the moment of conception you were essentially and uniquely you."



Disclaimer: DNA is not the cause of everything

Monozygotic twins are not identical

Cardiovascular disease associates better with lifestyle than with DNA sequence (Mozaffarian 2008)

Lung cancer associated with smoking habits

Drug metabolism is mostly due to the microbiome

Several genes associated with autism, depression, etc. were "lost" in larger studies

Distilbene: anti-miscarriage drug, increases cancer risks in daughters and malformations in grand-daughters

What the HGP Taught us with the first genome sequenced: Genes act differently and non-additively in different people

Cockayne syndrome: Mutation in the DNA repair enzyme ERCC6 at position 5q12.1. Homozygous recessive persons are characterized by growth failure, impaired neural development, premature aging, sensitivity to sunlight.

Usher Syndrome: Mutation in the retinal and cochlear basement membrane myosin MYO7A at 11q13. Homozygous recessive persons are characterized by congenital deafness and gradual loss of vision.

James Watson, presently 90 years old; not deaf, blind, nor stunted



Slide from S. Gilbert

Manipulating DNA

What can we do with DNA ?

What can we do with DNA ?

Extract, purify

Make more

Amplify Clone Synthetize

Examine

Quantify Examine length Stain, probe Sequence Examine 3D structure Measure physical properties of DNA molecules

Modify

Cut Ligate Recombine fragments Introduce foreign DNA Mutate

Extract DNA

Break cells, remove lipids and proteins,

precipitate DNA, remove liquid, resuspend in aquaeous solution



Be aware of contaminants!

Extract DNA

Break cells, remove lipids and proteins,

precipitate DNA, remove liquid, resuspend in aquaeous solution



Be aware of contaminants!

(DNA from mitochondria, viruses, bacteria, researcher, symbionts...)

Amplify DNA



Mix: Genomic DNA Probes (oligonucleotides) Nucleotides Taq polymerase Ions (MgCl2)

Cycles of Denaturation, Annealing, Elongation

PCR: Polymerase Chain Reaction

Amplifies DNA fragments of between 0.1 and 10 kb (up to 40 kb)

Amplify DNA

Polymerase chain reaction - PCR



- **Denaturation** at 94-96°C
- 2 Annealing at ~68°C

Elongation at ca. 72 °C

Cloning vs. PCR



Amplify DNA

DNA fragments 5 kb-15 kb: plasmids in bacteria ~10 kb: lambda phage-based vectors Up to 40 kb: fosmids in bacteria ~100-300 kb: bacterial artificial chromosomes (BAC)

First "synthetic" cell developed by scientists



Gibson et al. 2016

Cut DNA with restriction enzymes



Blunt ends, 3' protruding ends, 5' protruding ends

Cut DNA with restriction enzymes



Examine length of DNA



TAE (Tris-acetate-EDTA) buffer



Ligate DNA



Fragments have to be phosphorylated but only on one strand Dephosphorylate the vector to inhibit selfcircularization



Probe DNA: Fluorescent In Situ Hybridization





Probes for telomere sequences

Sanger sequencing

800 bp long Starts based on oligonucleotide (primer) ~4 euros per reaction

Dye terminator sequencing



120

McGovern 2015

130

GTGTGAGCTGTGATCGGT







Ding et al 2015



Illumina sequencing



For transcriptome: 2x 75 bp For whole genome: 2x 150 bp https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology/2-channel-sbs.html

Output of Illumina sequencing



Synthetize DNA



Gene Synthesis Service Options

Types	Gene Length	Price (No hidden charge promise) †	Starting Turnaround Time (Business Days) *	Starting Turnaround Time with Plasmid Prep Service (Business Days)
Standard Gene Synthesis ^{Guaranteed}	≤ 8 kb	View your discounted price online in as short as 1 minute	8	10
Fast Gene Synthesis ^{Guaranteed}	≤ 5 kb		7	9
Rush Gene Synthesis ^{Guaranteed}	≤4 kb		4US Manufacture	6 ^{US Manufacture}
GenPlus HT Gene Synthesis	≤ 3 kb		18	20
GenPlus Economy Gene Synthesis	≤ 8 kb		15	17
GenBrick [®] Gene Synthesis	> 8 kb		23	25

Introduce foreign DNA

most widespread transgenic crop in 2005-2015 = soybean resistant to glyphosate



Manipulating RNA

What can we do with RNA ?

What can we do with RNA ?

Extract, purify

Make more

 $RNA \rightarrow DNA \rightarrow RNA$ (reverse transcription, transcription)

Examine

Quantify Examine length Stain, probe Sequence Examine 3D structure Measure physical properties of RNA molecules

Modify

Mostly via DNA

Extract RNA



RT-qPCR

BlazeTaq[™] Two-Step RT-qPCR Kit



https://www.youtube.com/watch?v=iu4s3Hbc_bw

RNAseq

Starting with tissues/organs/single cells

Single-cell RNA-Seq (scRNA-Seq)







CRISPR





Can recognize and cut a specific DNA sequence (recognized by guide RNA) More versatile than restriction enzymes, Zn finger nucleases and transcription activator-like effector nucleases (TALENs).



Gene is disrupted

Gene has a new sequence

Creating mutants with CRISPR/Cas9

GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGCAGCGGATGCG	Wild type
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTGCAGCGGATGCG	
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTAGCGGATGCG	
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGCAGCGGATGCG	Deletion
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACAGCGGATGCG	
GAGTTCTACAGCGTGAACCACATGCGGATGCG	
AGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGACAGCGGATGCG	
TACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGGCTTTAAAGCGGATGCG	
CAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGCAAGGAAACTGCGGATGCG	Insertion

Agraulis vanillae



Ongoing clinical trials using CRISPR



Retina disease

Beta-thalassemia Sickle cell disease

Fundamental research is important

bacteria Thermus aquaticus



DNA and its observable effects

Phenotype = observable attributes of an individual

Genotype = inheritable genetic material = DNA or RNA

How do genotypes map onto phenotypes ?

Aberration Types

SNP Insertion (CNV) Deletion Indel Inversion Translocation Complex change (Epigenetic change)

Estimation of mutation rates

Mutation accumulation lines, sequencing family trio, across a phylogeny

Coding versus cis-regulatory

Coding Cis-regulatory Gene loss Gene amplification (Gene rearrangement)

Levels of dominance



Can be quantified as deviation from midpoint between parents

Different kinds of phenotypes

Morphology

Color

Size and shape

Presence/



absence Aristote, Historia animalium, book I, 2, 300BC Position Physiology

Behavior

Genotype & Phenotype = what engenders = what is apparent

DNA/RNA • Regulation of gene expression

- Biochemical reactions
- Subcellular architecture
- Assembly of cells
- Organism morphology and behavior

distinction appeared at the origin of life: etc.



Francis Crick Central Dogma A reductionist view of the GP relationship



Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

Crick 1958 Crick 1970

The genotype-phenotype-fitness map



Salazar-Cuidad & Martin-Riera 2013



Hallgrimsson et al. 2014



Plasticity: one genotype \rightarrow **several phenotypes**

Daphnia

with helmet

Nemoria arizonaria caterillars

spring: caterpillars feed on catkins

summer: caterpillars feed on leaves

Water crowfoot plant

leaves growing above water

leaves growing below water

Commodore butterly: Michael Wild, CC-BY-SA-3.0 (winter), Svdmolen, CC-BY-SA-3.0 (summer)

helmet

Daphnia: Agrawal et al (1999)

Nemoria arizonaria caterillars: Sadava *et al* (2014)

Water crowfoot plant: J R Crellin, CC BY-NC-ND 3.0

Desert locusts

solitary

gregarious

Commodore butterfly

winter

summer

A Lalouette (genitalia) JN Lorenzi (SARS-CoV-2) R Vijendravarma (organ size)

Flora Borne PhD (3 years) Manon Monier M2+PhD (3 years)

Isabelle Nuez Technician

Co-supervision: F. Graner & I

A Lalouette (genitalia) JN Lorenzi (SARS-CoV-2) R Vijendravarma (organ size)

Flora Borne PhD (3 years)

Manon Monier M2+PhD (3 years)

Isabelle Nuez Technician

Co-supervision: F. Graner & I

Bioadhesives

Natural polymer that can act as an adhesive: binds two items together and resists their separation

Mussel glue

water resistant

25 proteins

3,4-dihydroxyphenylalanine (DOPA)

Fly Glue

Stick to a wide variety of substrates

How adhesive is the glue?

What makes it adhesive?

Glue thickness varies from 0 to 20 µm

P{w[+m]=Sgs3-GFP}* animal

Borne et al. 2020 JEB

Experimental set up

Adhesion force does not vary between substrates except for teflon

* teflon different from every other substrates (ANOVA followed by Tukey tests, p < 0.05)

Borne et al. 2020 JEB

Low, Medium and Strong Adhesion Species

Monier, unpublished

How adhesive is the glue?

D. melanogaster glue is universal. moth eggs < Drosophila glue < mussel, barnacle < superglue 10-100 kPa 100-300 kPa 300-1000 kPa 10 MPa

~15 000 times the pupa weight Like very strong adhesive tapes

Borne et al. 2020 JEB

What makes it adhesive?

How adhesive is the glue?

D. melanogaster glue is universal.moth eggs < Drosophila glue < mussel, barnacle < superglue</td>10-100 kPa100-300 kPa300-1000 kPa10 MPa

~15 000 times the pupa weight Like very strong adhesive tapes

Borne et al. 2020 JEB

What makes it adhesive?

8 major proteins Ongoing RNAi and CRISPR

unpublished

simulans salivary glands rom L3 wandering larva

100 um

Nuclei volume (um 3)

1649.000

Cell volume (um 3)

293807.000

2315.000

15348.000

3D reconstruction on Imaris

Confocal microscope acquisition