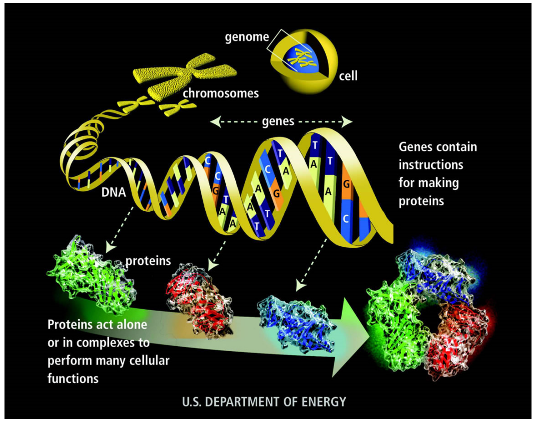
Synthetic life

WiSe 2017/18

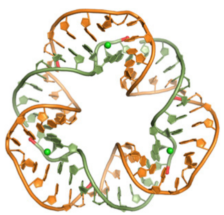
Zbigniew Pianowski

From DNA to proteins



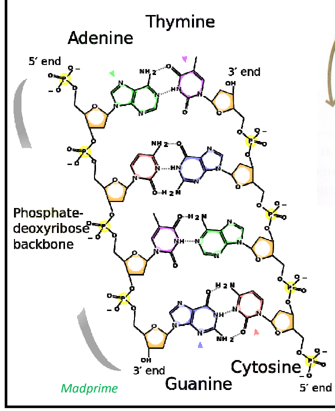
https://www.youtube.com/watch?v=gG7uCskUOrA

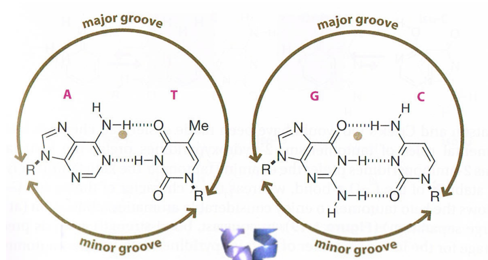
CHAPTER 1

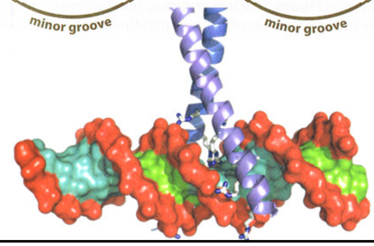


OLIGONUCLEOTIDES

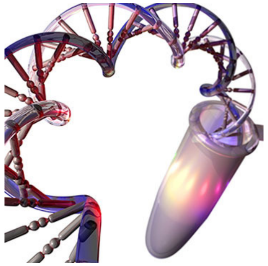
Canonical nucleobases and Watson-Crick pairing in DNA

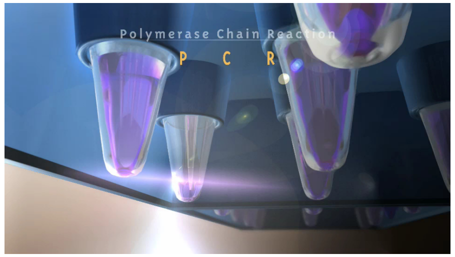






PCR – Polymerase Chain Reaction





National Library of Medicine,
National Institutes of Health

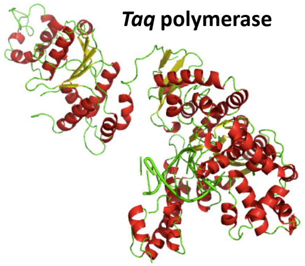
PCR – Polymerase Chain Reaction

Brian W. Schaller, Yellowstone Park

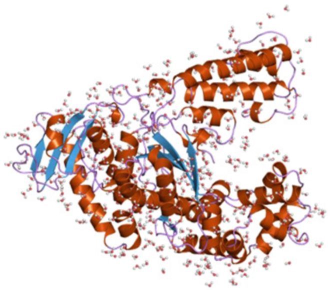
Thermus aquaticus is a thermophilic bacteria from hot springs in Yellowstone Park
70^0C – optimum, living range: 50-80^0C

It is a source of thermostable enzymes

PCR – Polymerase Chain Reaction



„Adenosine"

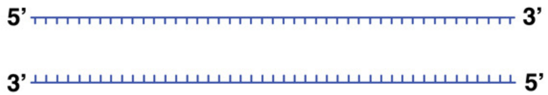


Taq polymerase withstands denaturing conditions (hot temperatures) detrimental for most enzymes. *Activity optimum: 75-80^0C, half-life at 95^0C > 2.5 h*

1990 – Kary Mullis optimized the PCR technique with *Taq* polymerase (1993 Nobel Prize)

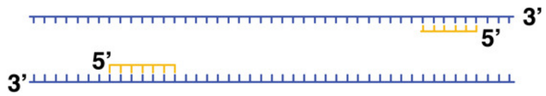
PCR – Polymerase Chain Reaction

We begin with a single molecule of DNA.

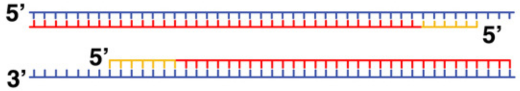


We can melt the DNA (break the hydrogen bonds holding the helix together) by heating it to 98 degrees.

PCR – Polymerase Chain Reaction

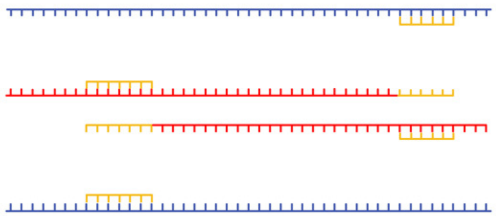


Two DNA primers (18-22 bp, T_m: 50-60^{0}C) are designed to anneal to a known sequence. The primers are separated in the sequence that we are targeting by a few hundred base pairs. Cooling the reaction from 98^{0}C to a more moderate temperature allows annealing to take place.



Now we have two primed templates. With dNTPs and DNA polymerase in the reaction mixture, new DNA is synthesized.

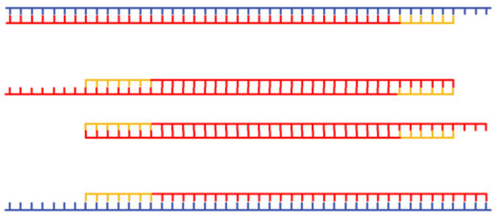
PCR – Polymerase Chain Reaction



The DNA is molten for another cycle.
Because there is a vast molar excess of primers, when we cool the mixture, we again anneal primers

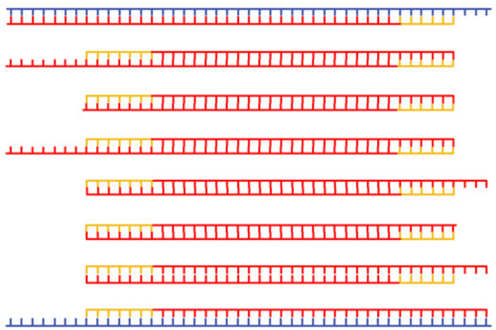
PCR – Polymerase Chain Reaction

New DNA is synthesized



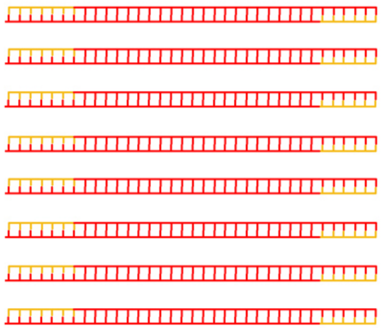
PCR – Polymerase Chain Reaction

In the next cycle, we begin to see DNA molecules whose ends are defined by the primers



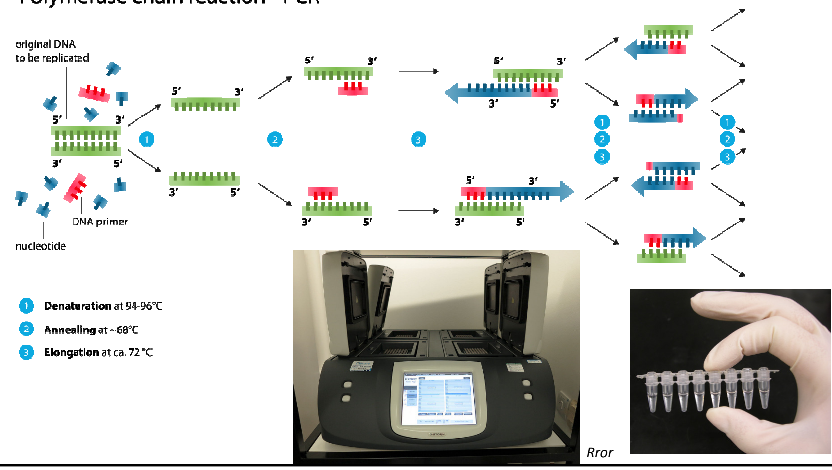
PCR – Polymerase Chain Reaction

After many cycles of melting, annealing, and replication, the overwhelming majority of DNA molecules in the mixture have ends defined by the primers

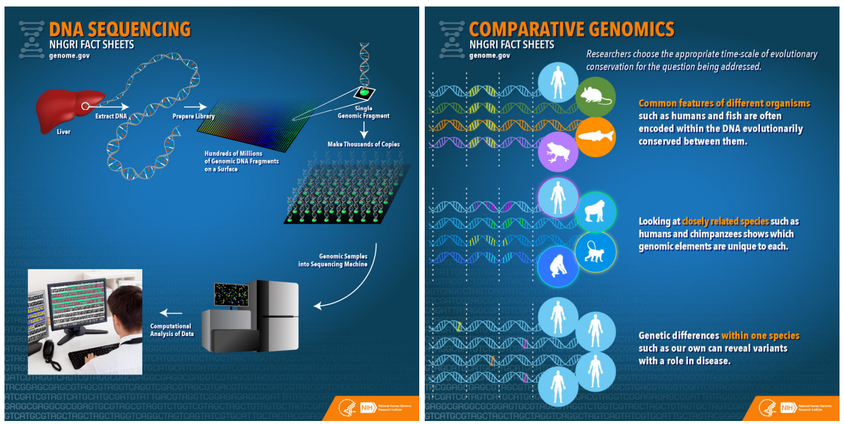


https://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-chain-reaction-PCR-3D-animation.html

Polymerase chain reaction - PCR

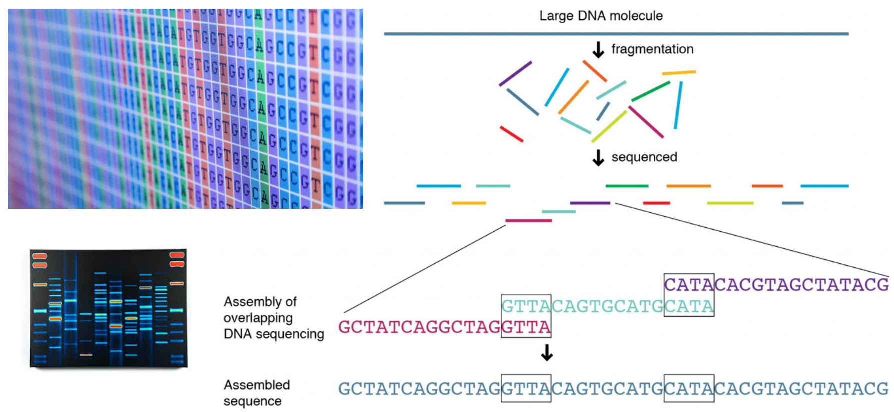


DNA sequencing



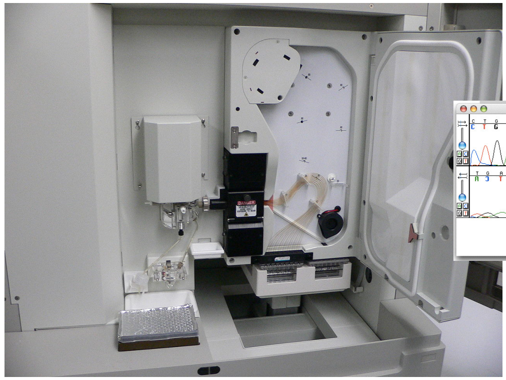
National Library of Medicine, National Institutes of Health

Genome sequencing

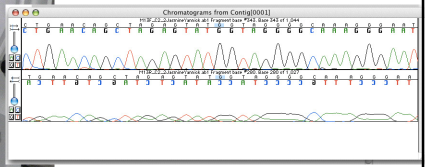


Genome sequencing

Electropherograms are commonly used to sequence portions of genomes



Mark Pellegrini

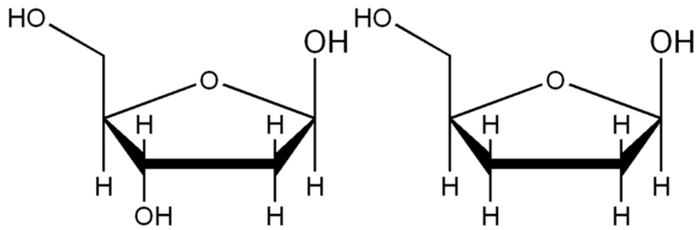


Tom David

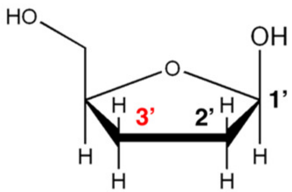
An ABI PRISM 3100 Genetic Analyzer. Such capillary sequencers automated the early efforts of sequencing genomes.

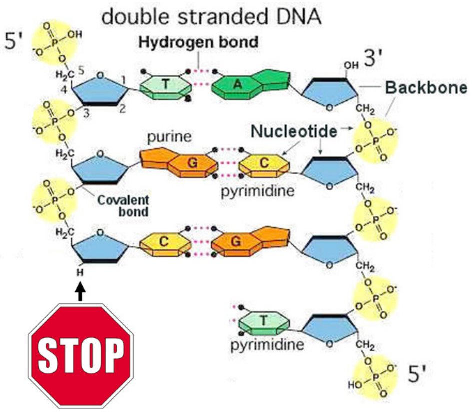
Sanger sequencing

What good is dideoxyribose?

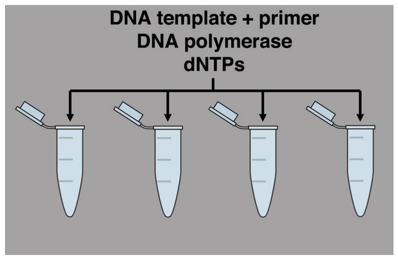


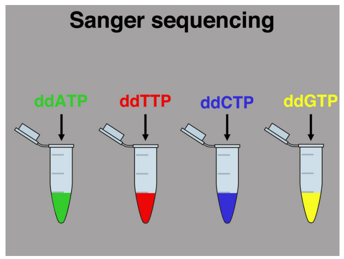
Sanger sequencing





Sanger sequencing

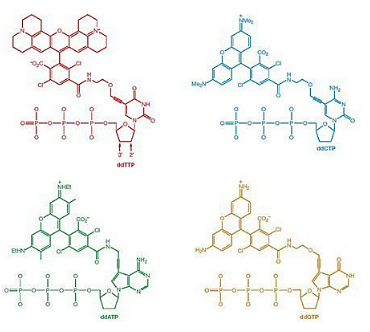


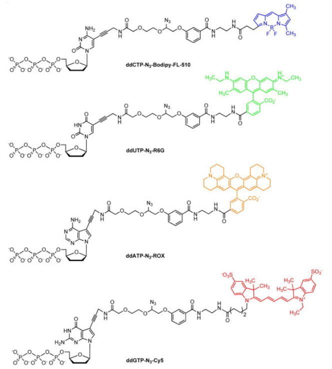


Sanger sequencing

Fluorescent dideoxynucleotides

standard | *chemically cleavable (reduction)*





J. Ju *et al. PNAS* **2005**, *102 (17)*, 5926-5931

Sanger sequencing

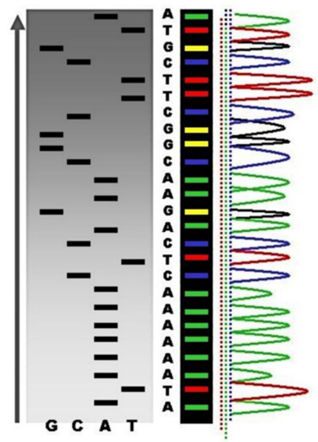
Sequencing Gel

TACGTA
ATGCATTAGGGCCTGGCTCTTT

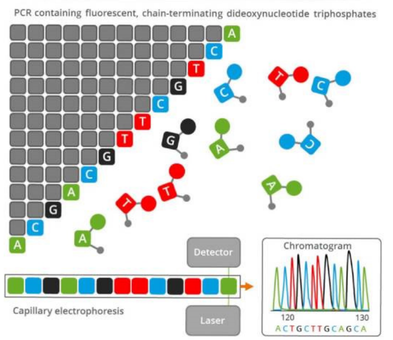
TACGTAA
ATGCATTAGGGCCTGGCTCTTT

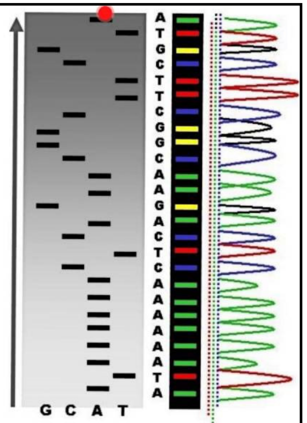
TACGTAATCCCGGA
ATGCATTAGGGCCTGGCTCTTT

TACGTAATCCCGGACCGA
ATGCATTAGGGCCTGGCTCTTT



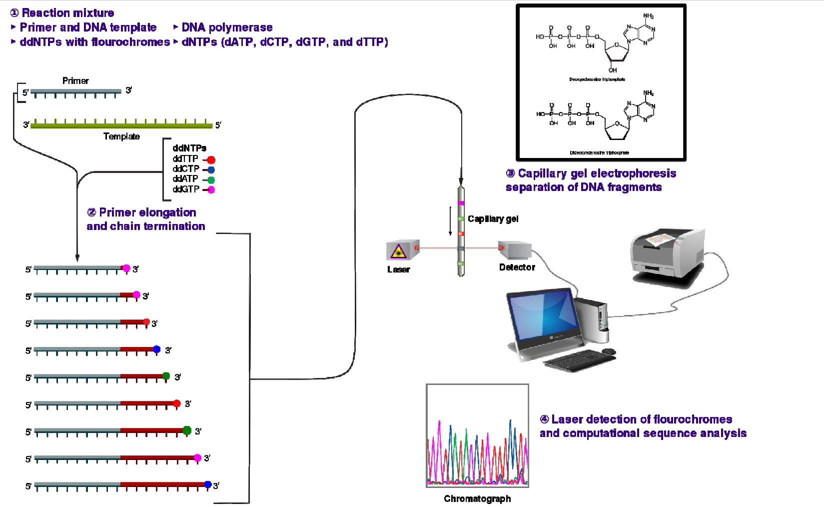
Sanger Sequencing



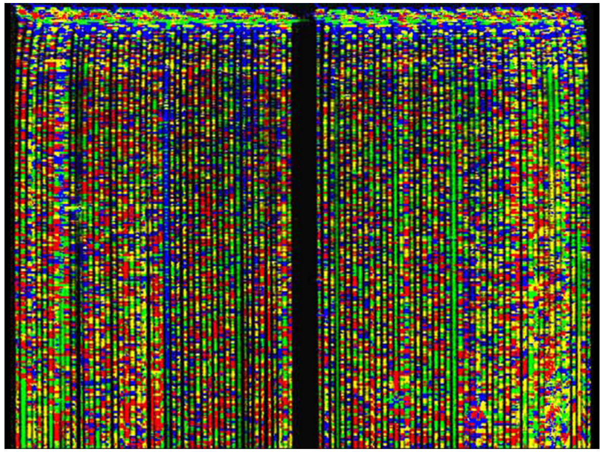


Sanger sequencing uses ddNTPs (dideoxynucleotide triphosphates) which do not have a free 3' OH mixed in with dNTPs
Whenever the DNA polymerase incorporates a ddNTP it won't be able to add any other nucleotides
Then gel electrophoresis is used to separate the DNAvv

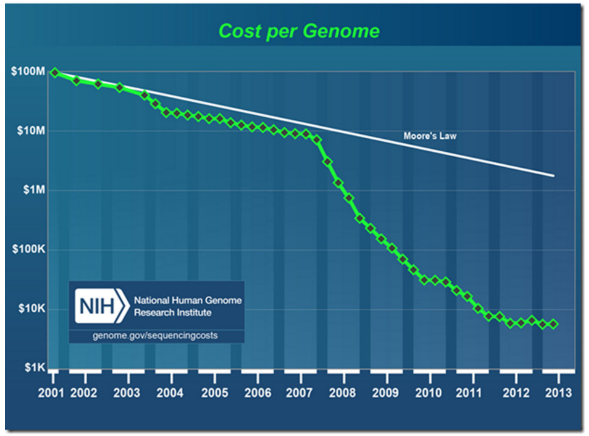
https://www.youtube.com/watch?v=ONGdehkB8jU (from 0:50)



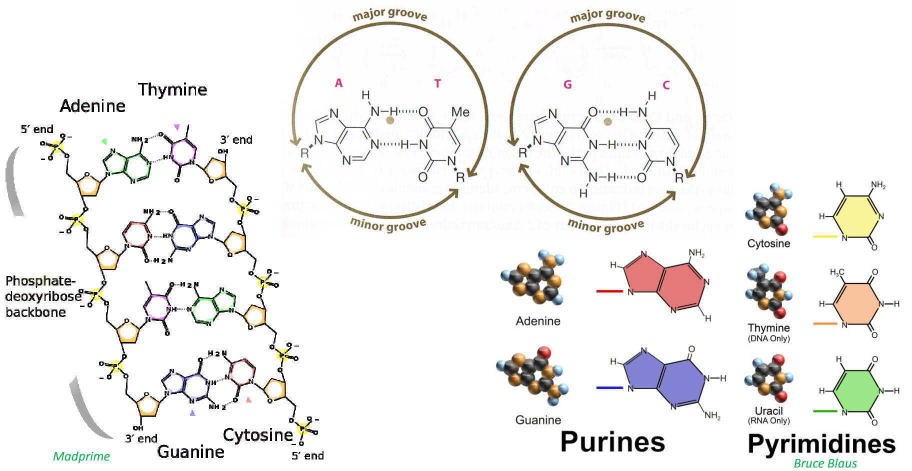
Sanger sequencing



Sanger sequencing



Canonical nucleobases and Watson-Crick pairing in DNA



Hoogsten base pairing of canonical DNA nucleobases

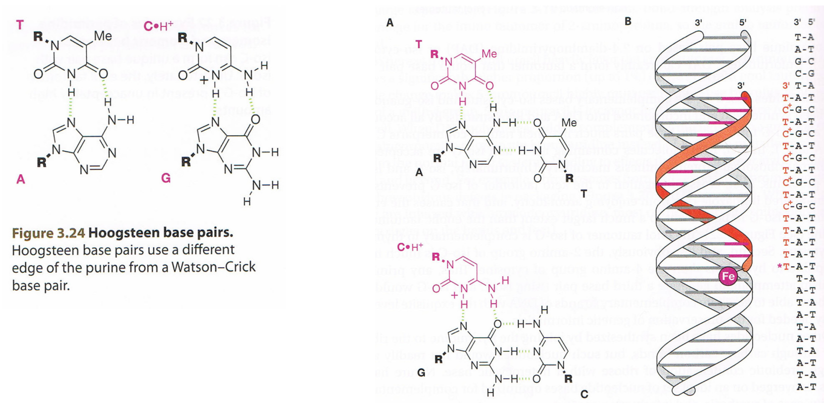
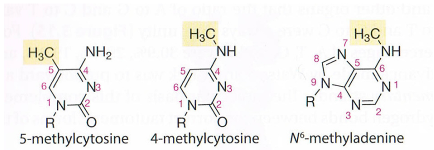
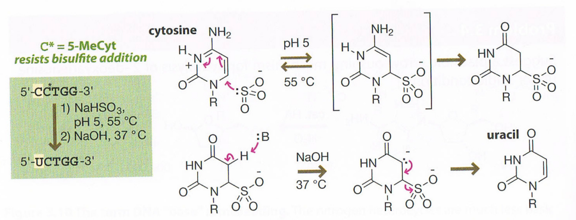


Figure 3.24 Hoogsteen base pairs. Hoogsteen base pairs use a different edge of the purine from a Watson–Crick base pair.

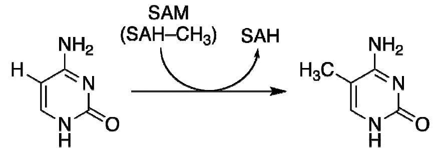
H. E. Moser, P. B. Dervan *Science* **1987**, *238*, 645-650

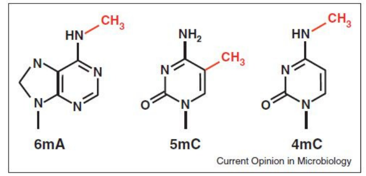
Modifications of nucleobase structures tolerated by polymerases



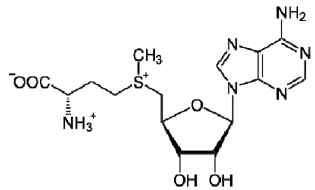


Modifications of nucleobases

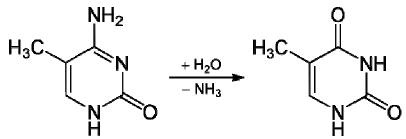




Chemical structures of common modified bases generated by DNA methyltransferases.

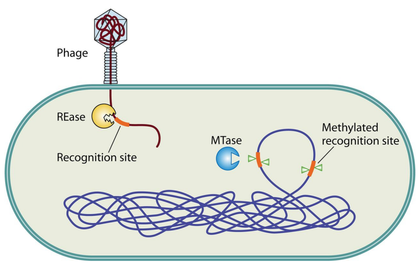


S-Adenosylmethionine (SAM)

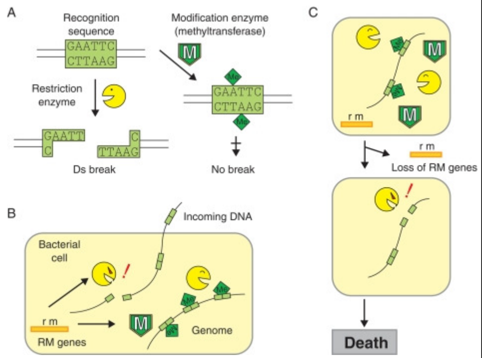


Restriction modification system

„Immune system" of bacteria and archaea against atacking viruses



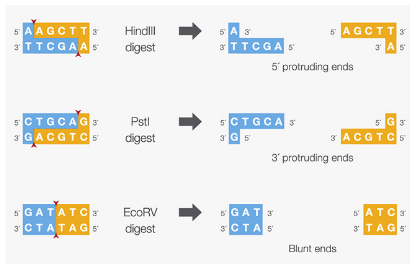
K. Vasu, V. Nagaraja
Microbiol. Mol. Biol. Rev. **2013**, *77(1)*, 53-72

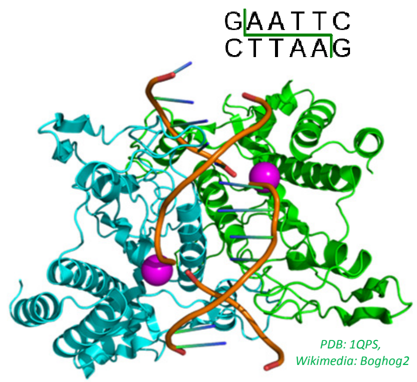


K. Ishikawa *et al. DOI: 10.1093/dnares/dsq027*

EcoI – a typical restriction enzyme

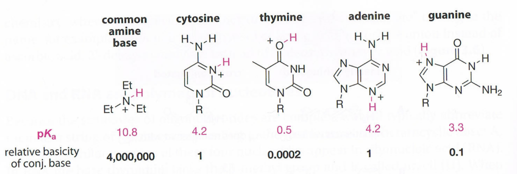
Products of restriction enzymes

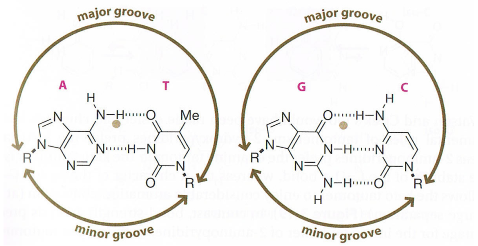




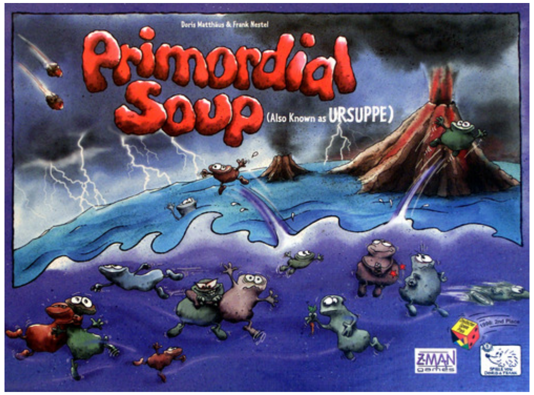
Structure of the homodimeric restriction enzyme EcoRI (cyan and green cartoon diagram) bound to double stranded DNA (brown tubes). Two catalytic magnesium ions (one from each monomer) are shown as magenta spheres and are adjacent to the cleaved sites in the DNA made by the enzyme (depicted as gaps in the DNA backbone).

Why are A, C, G and T the letters of genetic alphabet.





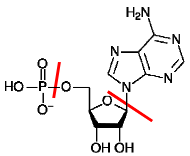
Prebiotic synthesis of nucleotides

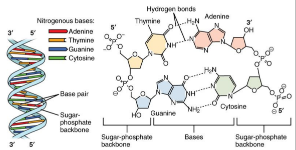


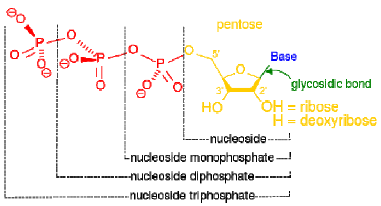
State of the art

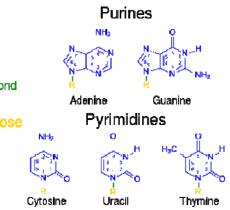
Nucleotides - components

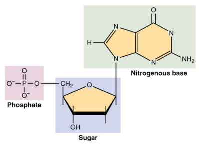
RNA – most likely evolutionarily older („RNA World") than DNA → prebiotic origin of ribose + A, C, G, and U nucleobases



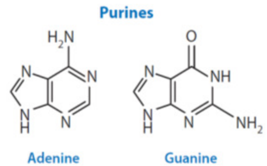


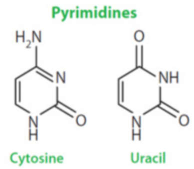




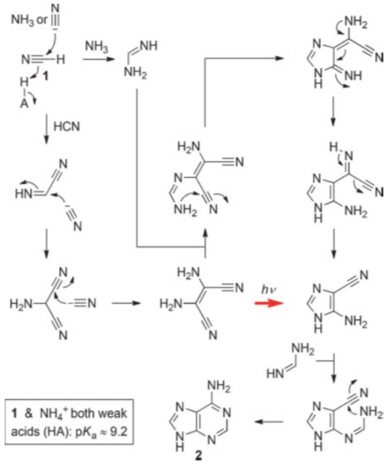


Prebiotic synthesis of nucleobases





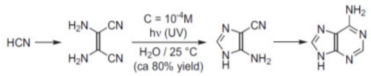
Prebiotic synthesis of adenine



1960 - Oró's synthesis of adenine **2** from hydrogen cyanide **1** and ammonia (general acid–base catalysis, presumed to operate in most steps, is only shown once).
Heating ammonium cyanide at 70⁰C for a few days
→ 0.5% adenine
Heating HCN with liquid ammonia in a sealed tube → 20% adenine

The photochemical shortcut discovered by Ferris and Orgel is shown by the red arrow.
Optimized yields – up to 20% for adenine, 3% for guanine

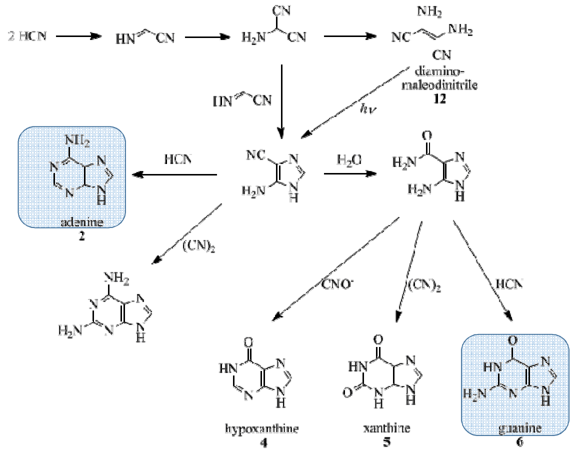
Eutectic freezing (-20⁰C) increases the yield of DAMN formation by concentrating HCN between pure ice crystals



J. Oro *Biochem. Biophys. Res. Commun.* **1960**, *2*, 407.

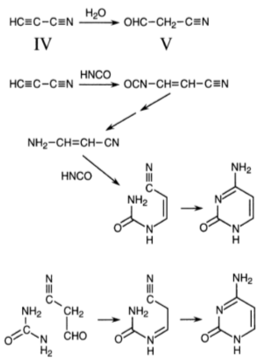
J. P. Ferris, L. E. Orgel, *J. Am. Chem. Soc.* **1966**, *88*, 1074

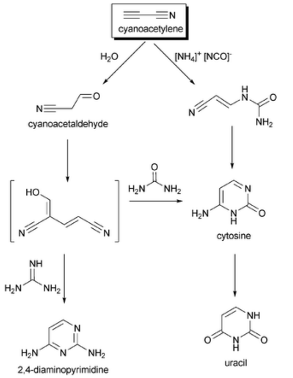
Prebiotic synthesis of purines



Prebiotic synthesis of pyrimidines

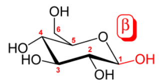
Cyanoacetylene is a major product of electric discharges in the mixture of nitrogen and methane

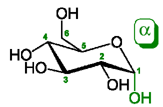


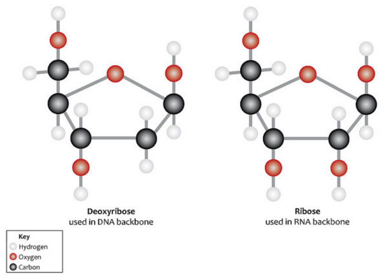


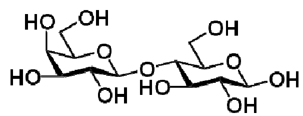
Cyanoacetylene incubated with saturated solution of urea yields up to 50% cytosine.
Other methods typically yield up to 5% cytosine.It is further converted to uracil by hydrolysis.

Carbohydrates

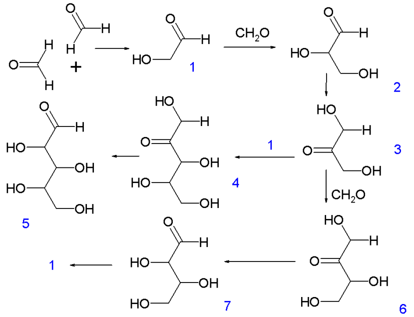








Formose reaction



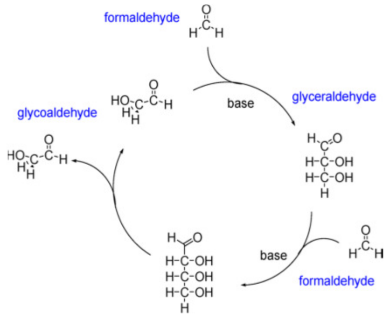
Alexander Butlerov (1828-1886)
St. Petersburg, Kazan, Russia

Ronald Breslow (1931-)
Columbia University, USA

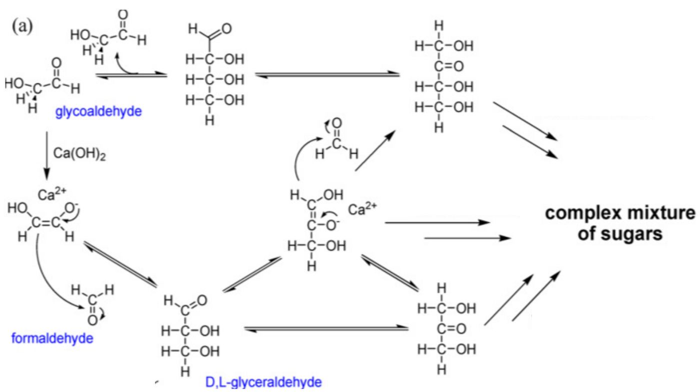
The reaction begins with two formaldehyde molecules condensing to make glycolaldehyde **1** which further reacts in an aldol reaction with another equivalent of formaldehyde to make glyceraldehyde **2**. An aldose-ketose isomerization of **2** forms dihydroxyacetone **3** which can react with **1** to form ribulose **4**, and through another isomerization ribose **5**. Molecule **3** also can react with formaldehyde to produce tetrulose **6** and then aldoltetrose **7**. Molecule **7** can split into **2** in a retro-aldol reaction.

Formose reaction as an autocatalytic process

Formose reaction starts in concentrated alkaline aqueous solutions of formaldehyde
alkali are typically calcium, magnesium or lead

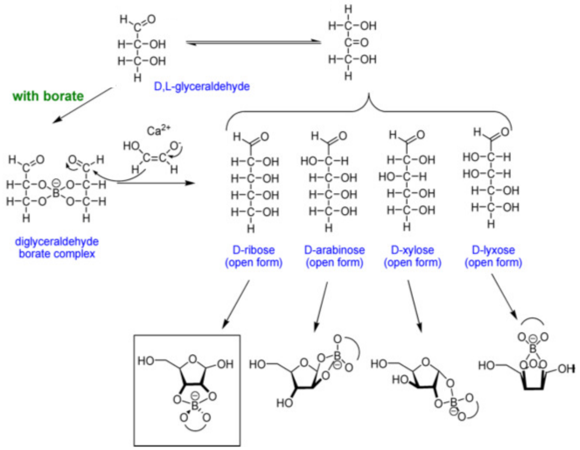


Formose reaction under standard basic catalysis – Ca(OH)$_2$



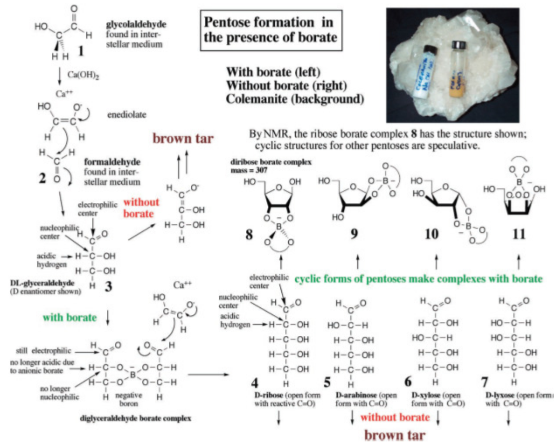
Escosura

Formose reaction in presence of borates



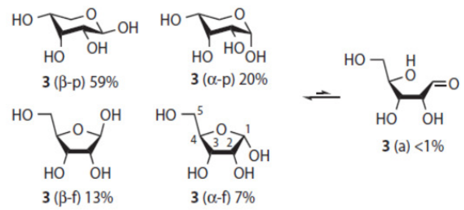
Escosura

Formose reaction in presence of borates



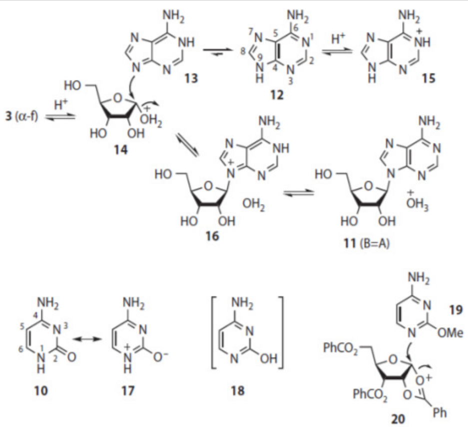
A. Ricardo, M. A. Carrigan, A. N. Olcott, S. A. Benner *Science* **2004**, *303*, 196

Prebiotic synthesis of nucleosides



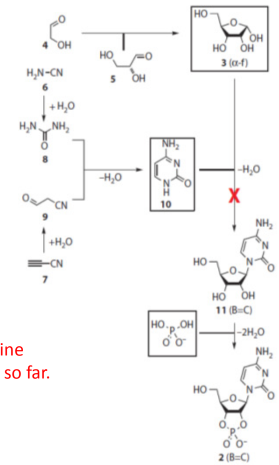
The difficulties of assembling beta-ribonucleosides by nucleobase ribosylation:

The many different forms of ribose **3** adopted in aqueous solution. The pyranose (p) and furanose (f) forms interconvert via the open-chain aldehyde (a), which is also in equilibrium with an open-chain aldehyde hydrate (not shown).



Adenine tautomerism and the ribosylation step necessary to make the adenosine **11** thought to be needed for RNA assembly. The low abundance of the reactive entities **13** and **14** is partly responsible for the low yield of **11**. The reason for the lower nucleophilicity of *N1* of the pyrimidines, and the conventional synthetic chemist's solution to the problems of ribosylation.

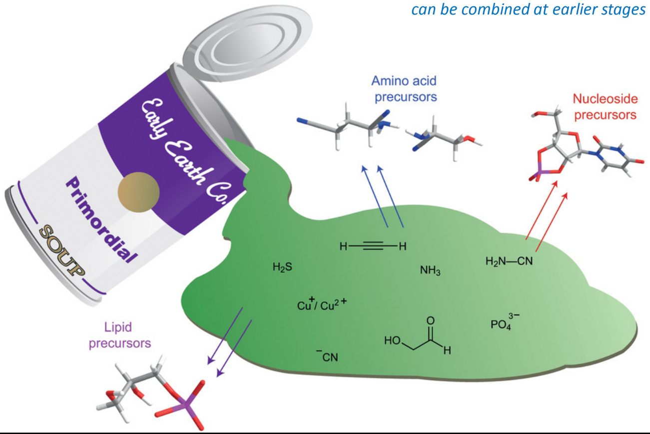
Prebiotic synthesis of pyrimidine nucleosides



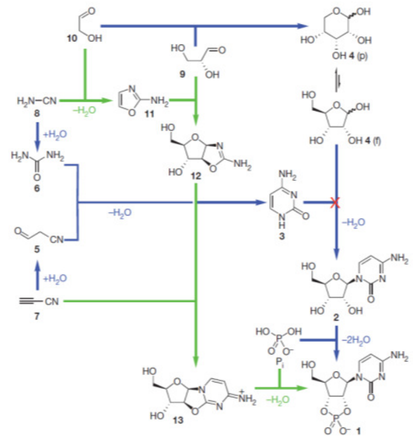
No direct synthesis of pyrimidine nucleosides from ribose reported so far.

Cyanosulfidic chemistry

The aldol chemistry of sugars and cyanide chemistry of nucleobases can be combined at earlier stages than glycosylation.

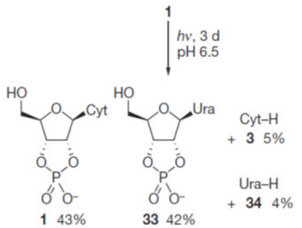


Cyanosulfidic chemistry



M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

Cyanosulfidic chemistry



Photochemistry of *beta*-ribocytidine-2',3'-cyclic phosphate **1**. Under conditions of irradiation that destroy most other pyrimidine nucleosides and nucleotides, **1** undergoes partial hydrolysis and slight nucleobase loss. Ura, N1-linked uracil; Cyt–H, cytosine; Ura–H, uracil.

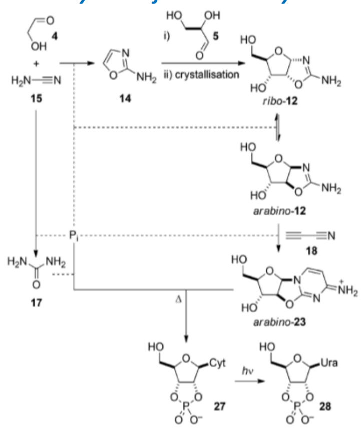
M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

J. D. Sutherland, *Angew. Chem. Int. Ed.* **2016**, *55*, 104-121.

B. H. Patel, C. Percivalle, D. J. Ritson, C. D. Duffy, J. D. Sutherland, *Nat. Chem.* **2015**, *7*, 301–307.

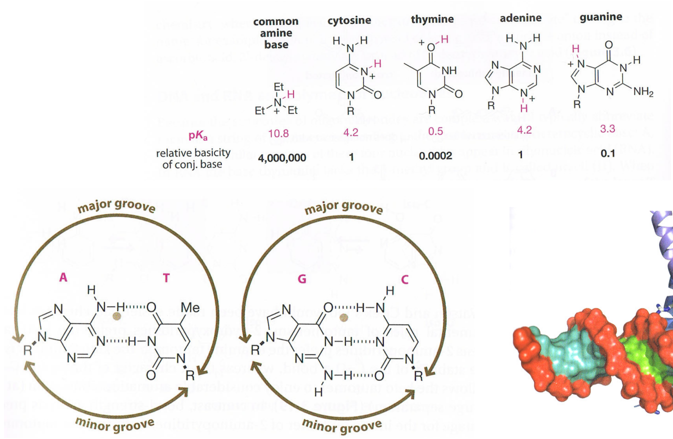
J. D. Sutherland, *et al. Nat. Chem.* **2013**, *5*, 383–389.

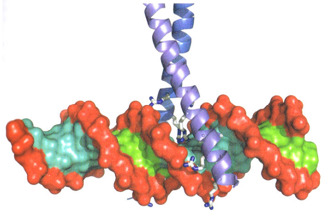
Cyanosulfidic chemistry

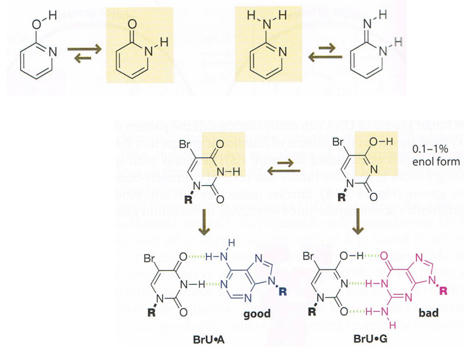


Potentially prebiotic synthesis of activated pyrimidine ribonucleotides. Catalysis, and reaction control through pH and chemical buffering, is indicated by dashed lines.

How else could it end up?







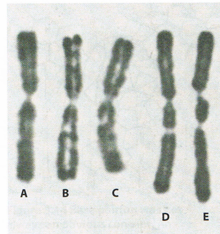
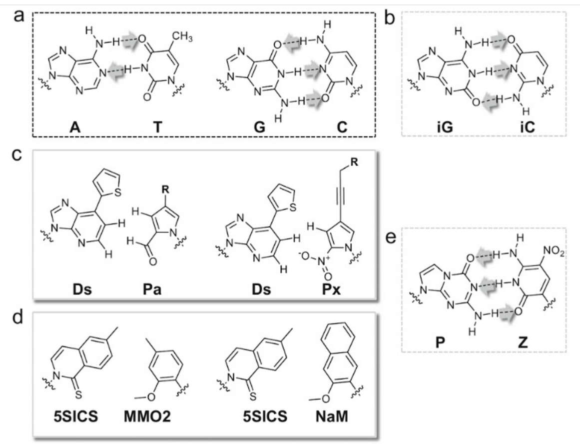
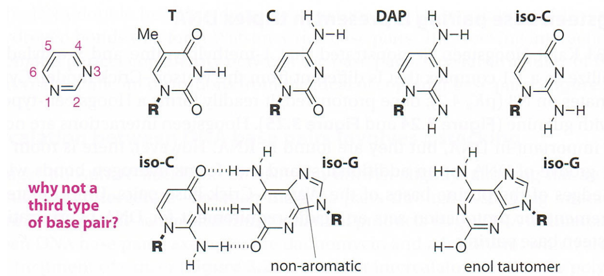


Figure 3.20 Chromosome 1 from hamster cells exposed to bromodeoxyuridine. (A) Normal chromosome. (B–E) Aberrant chromosomes. (From T.C. Hsu and C.E. Somers, *Proc. Natl. Acad. Sci. USA* 47: 396–403, 1961. With permission from the MD Anderson Cancer Center.)

Natural and non-natural base pairs that function in polymerase reactions



Alternative base pairs – synthetic biology



DAP – one tautomer forms a base pair with guanine

iso-C/iso-G
- specificity (the enol tautomer of iso-G, stabilized by aromatization, complementary to thymine)
- the 2-amino group of iso-C hydrolyses easily to uracil