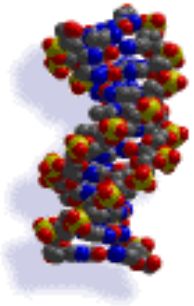
DNA Analysis - Basic Tools and Techniques



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Why are archaeologists interested in studying ancient DNA (aDNA)?

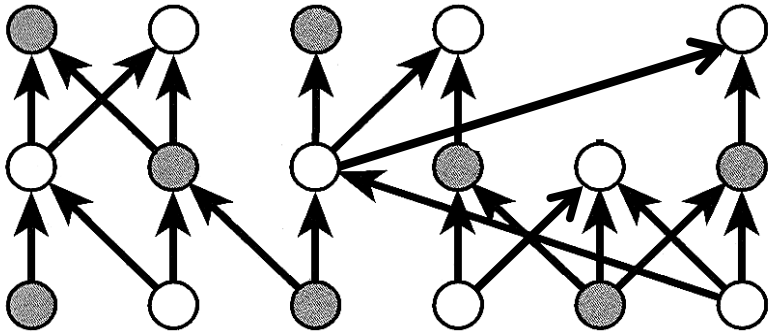
King Richard III of England (1452–1485)

Vindija cave (Croatia)

Oriental rat flea (*Xenopsylla cheopis*)

Why are DNA molecules so informative?

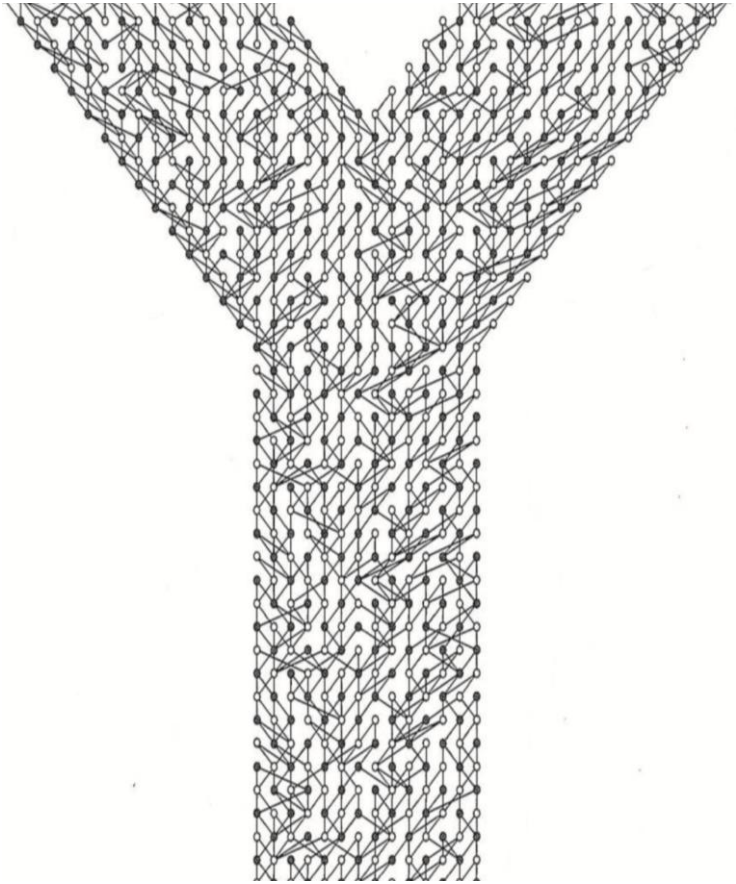
Deoxyribo**N**ucleic **A**cid (**DNA**) is a molecule that carries all instructions used in the growth, development, functioning and reproduction in all known living beings.



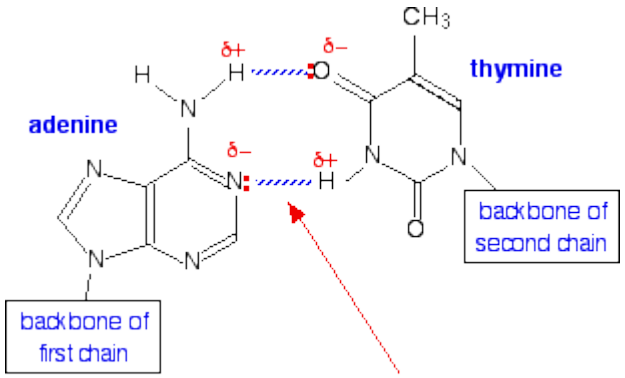
3th generation

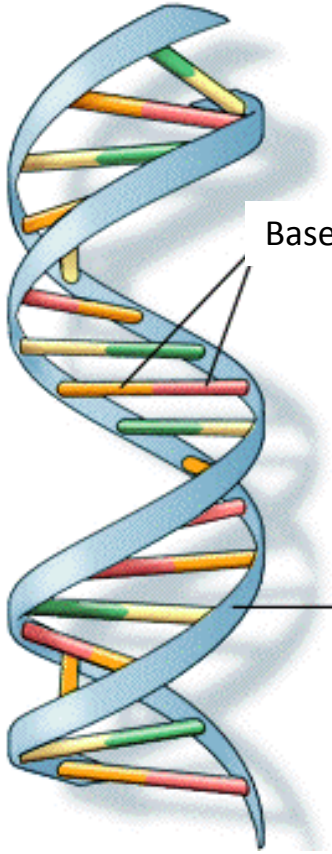
2nd generation

1st generation



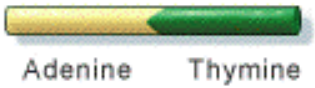
DNA is a time traveller!

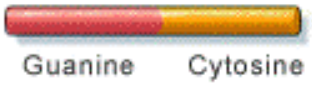


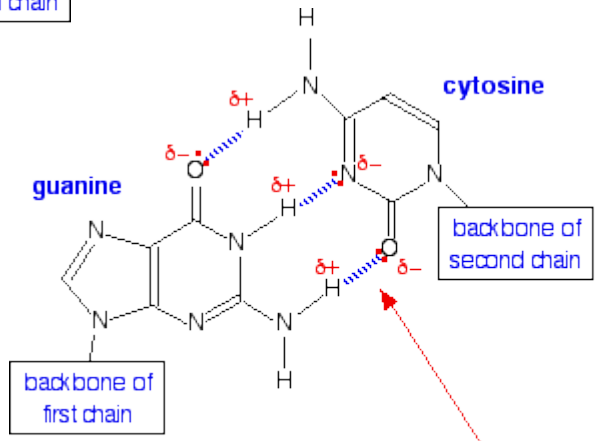


Base pairs

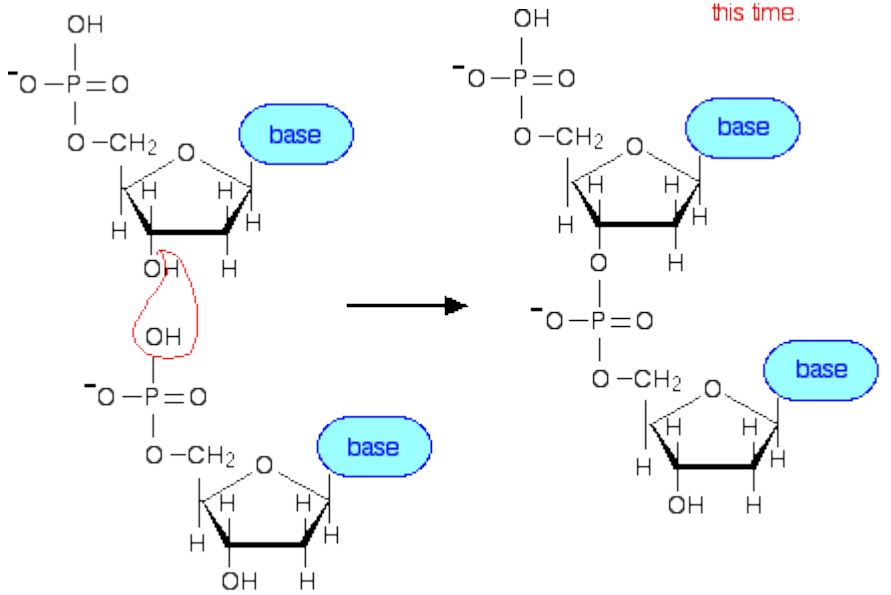
Sugar phosphate backbone

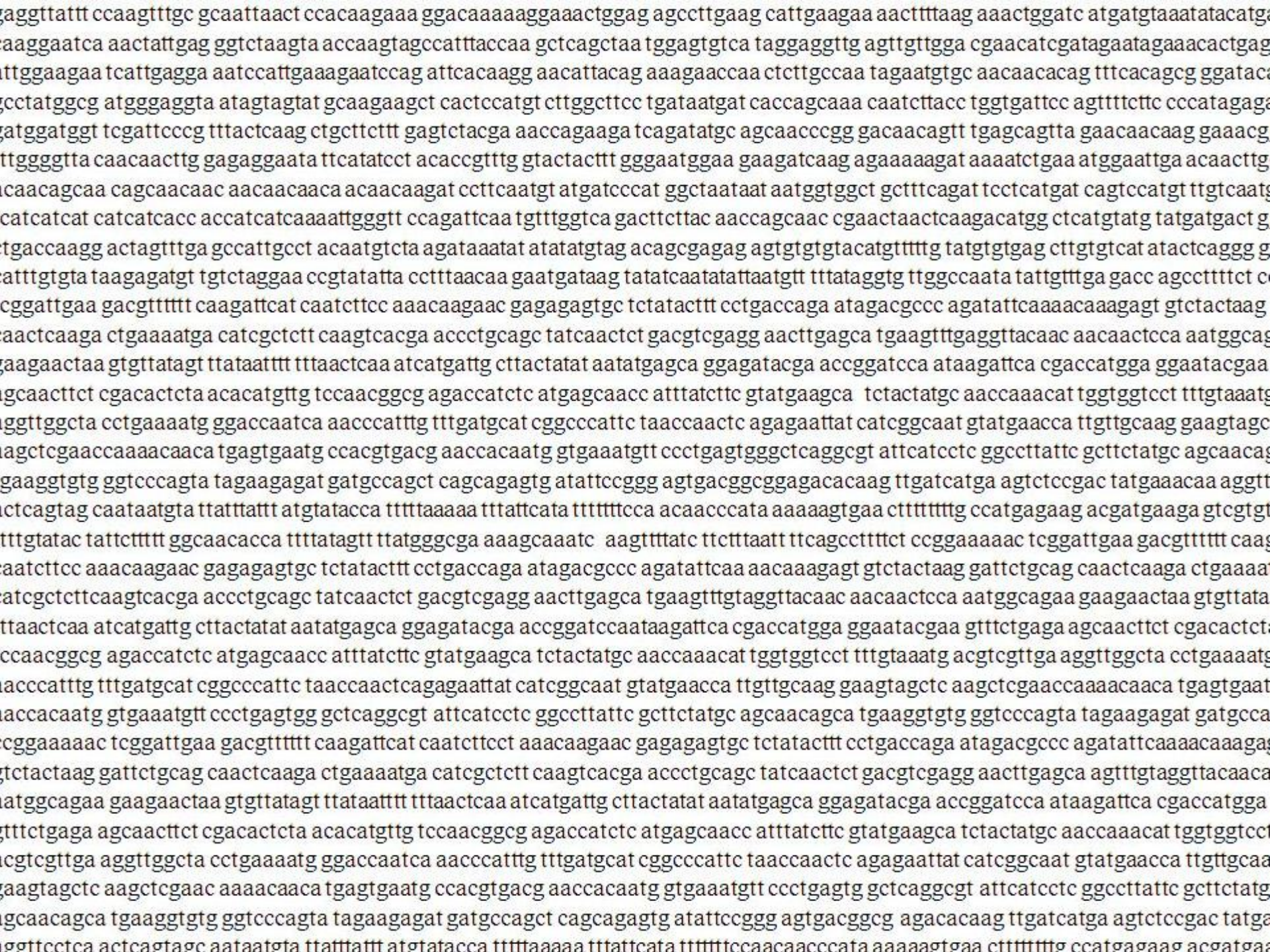




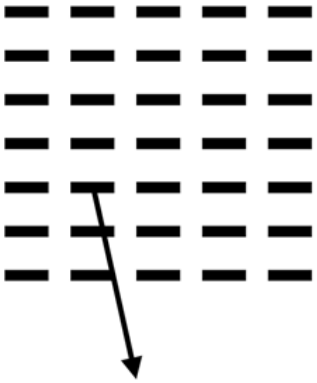


Notice **3** hydrogen bonds this time.





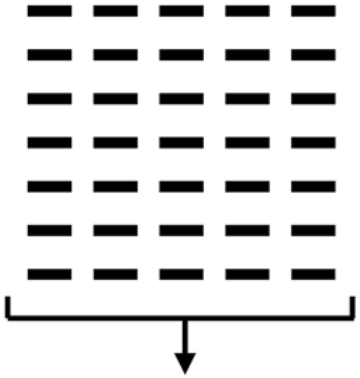
1st generation of DNA sequencers
(started 1987)



one fragment (ca 1000 bp long) per run

(human genome project /1990 – 2003/)

2nd and 3rd generations of DNA sequencers
(last ten years)



millions of fragments per run

MinION

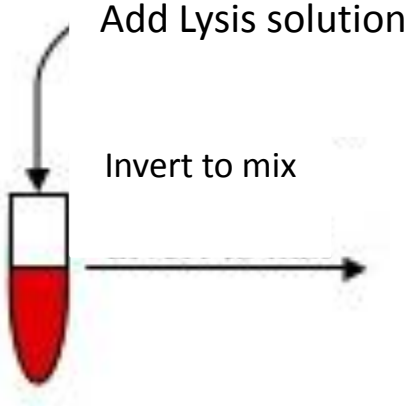
How to investigate DNA?

1. DNA isolation
2. PCR methods
3. Sequencing (1st, 2nd or 3th generation)
4. Bioinformatics

DNA isolation

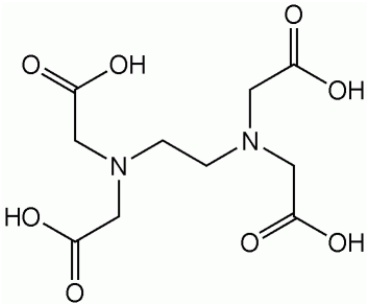
1) Cell lysis
2) Removing proteins and some other compounds
3) DNA purification

Archaeological sample need specific pretreatment because there is high possibility of **contamination** from microenvironment of the fossil, but also present day humans !!!

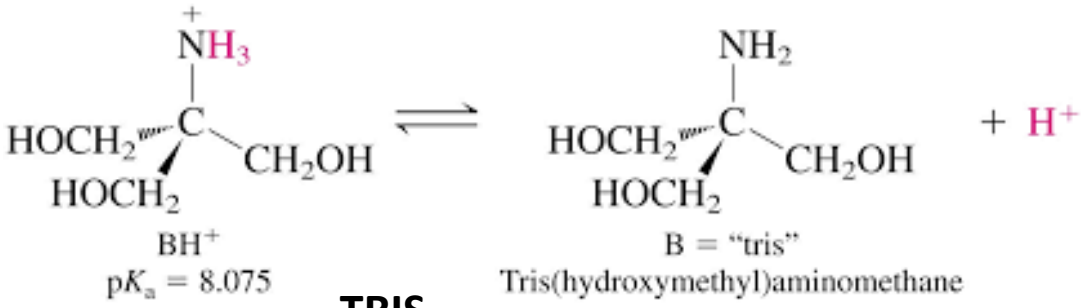


Incubate at 37 - 65 °C 5’ to 12h with occasional or continuous stiring

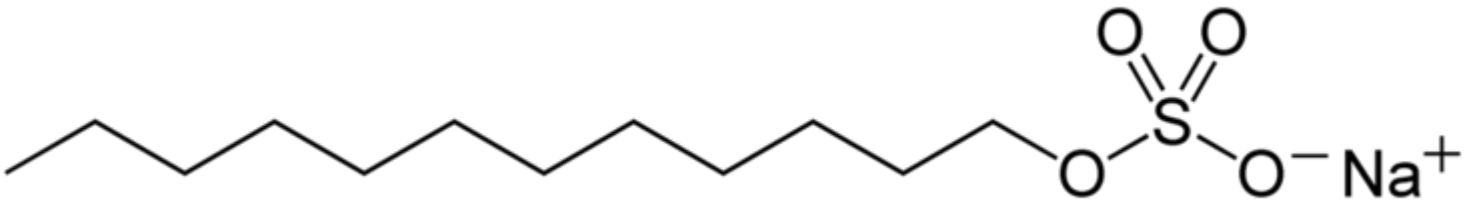
EDTA



General solution of lysis buffer contains: TRIS-HCl, EDTA, SDS or CTAB



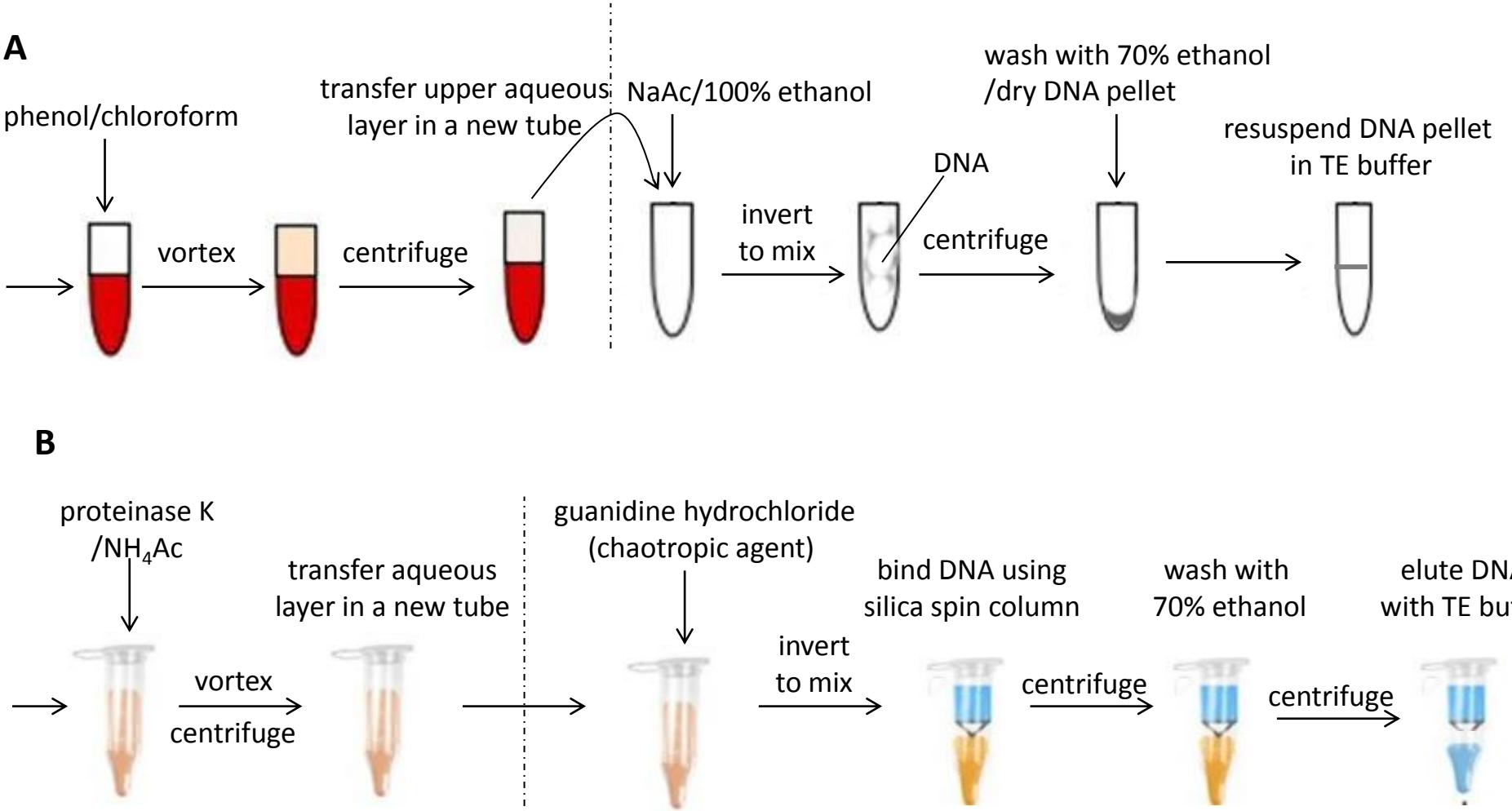
TRIS

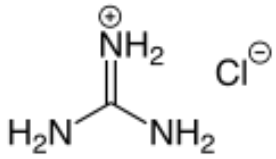


SDS

2) Removing proteins and some other compounds

3) DNA purification





Chaotropic agent - disrupt the hydrogen bonding network between water molecules

• DNA sample from two ~40,000-year-old Austrian cave bears was a mixture of bacterial, fungal, plant and other DNAs. Less than 6% of the recovered DNA was determined to be of cave bear (J. P. Noonan et al. 2005: Genomic sequencing of Pleistocene cave bears. Science 309, 597–599.)

The precautions usually applied in ancient DNA laboratory against DNA contamination:

1) complete separation of ancient laboratory and their rooms from other laboratories
2) direct delivery of all equipment and reagents to the laboratory
3) positive pressure generated with filtered air that excludes particles larger than 0.2 µm
4) UV irradiation and bleach treatment of all surfaces
5) bone surface was removed prior to extraction

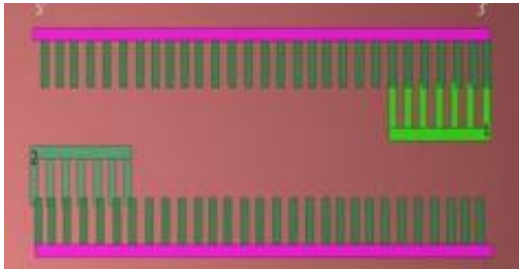
PCR methods

Polymerase
Chain
Reaction

= amplifying a specific DNA fragment *in vitro* up to billion copies
(Kary B. Mullis - Nobel Prize in Chemistry 1993)

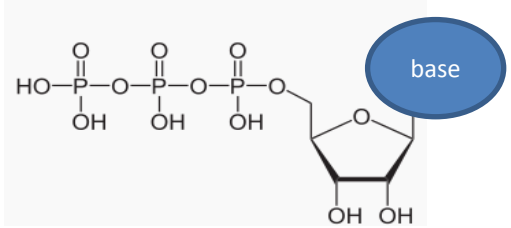
Thermal cycler

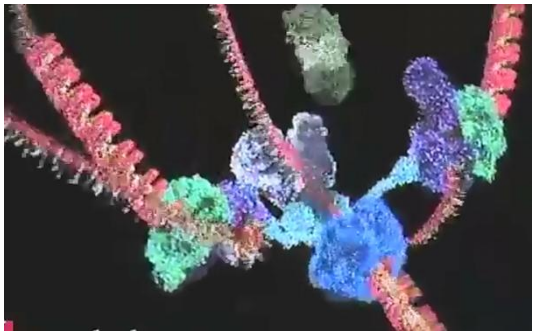
Thermal cycler device is able to specificaly increase and decrease temperature

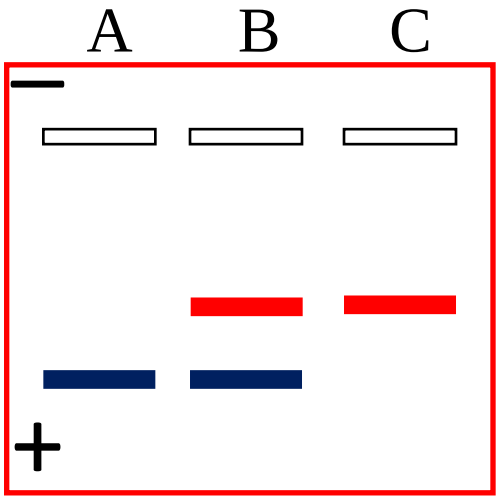


PCR ingredients:

1) DNA
2) PCR buffer with $MgCl_2$
3) Taq DNA polymerase
4) dNTP mix (A, C, G, T)
5) Primer 1
6) Primer 2





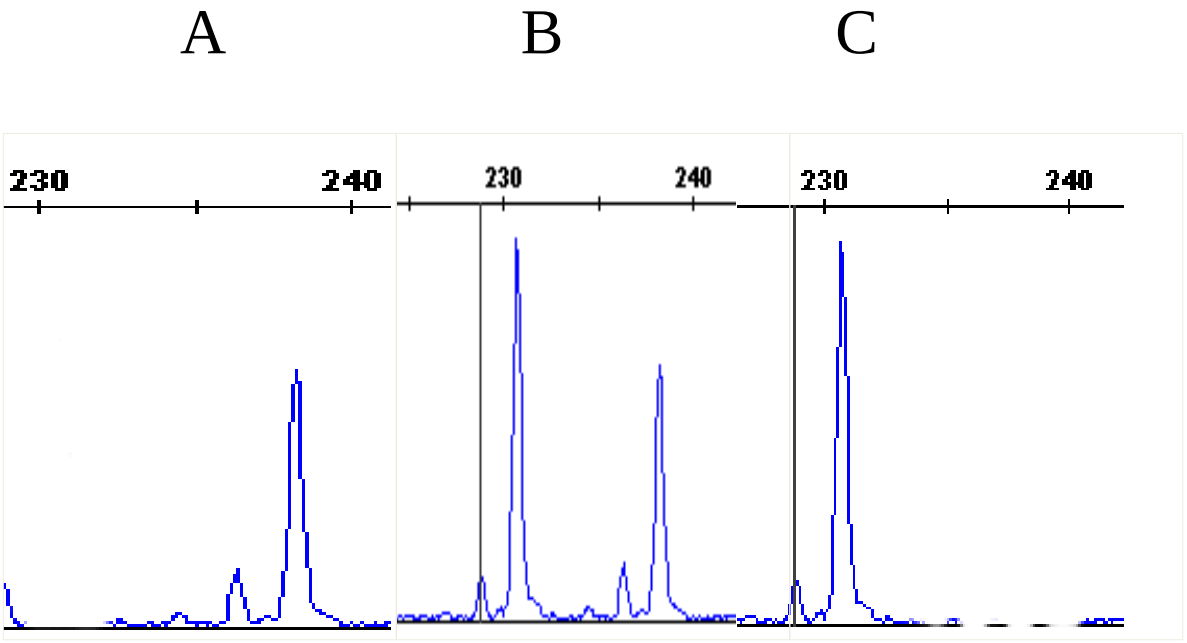


direct detection by electrophoresis (e.g. microsatellites)

→ PCR products

↓

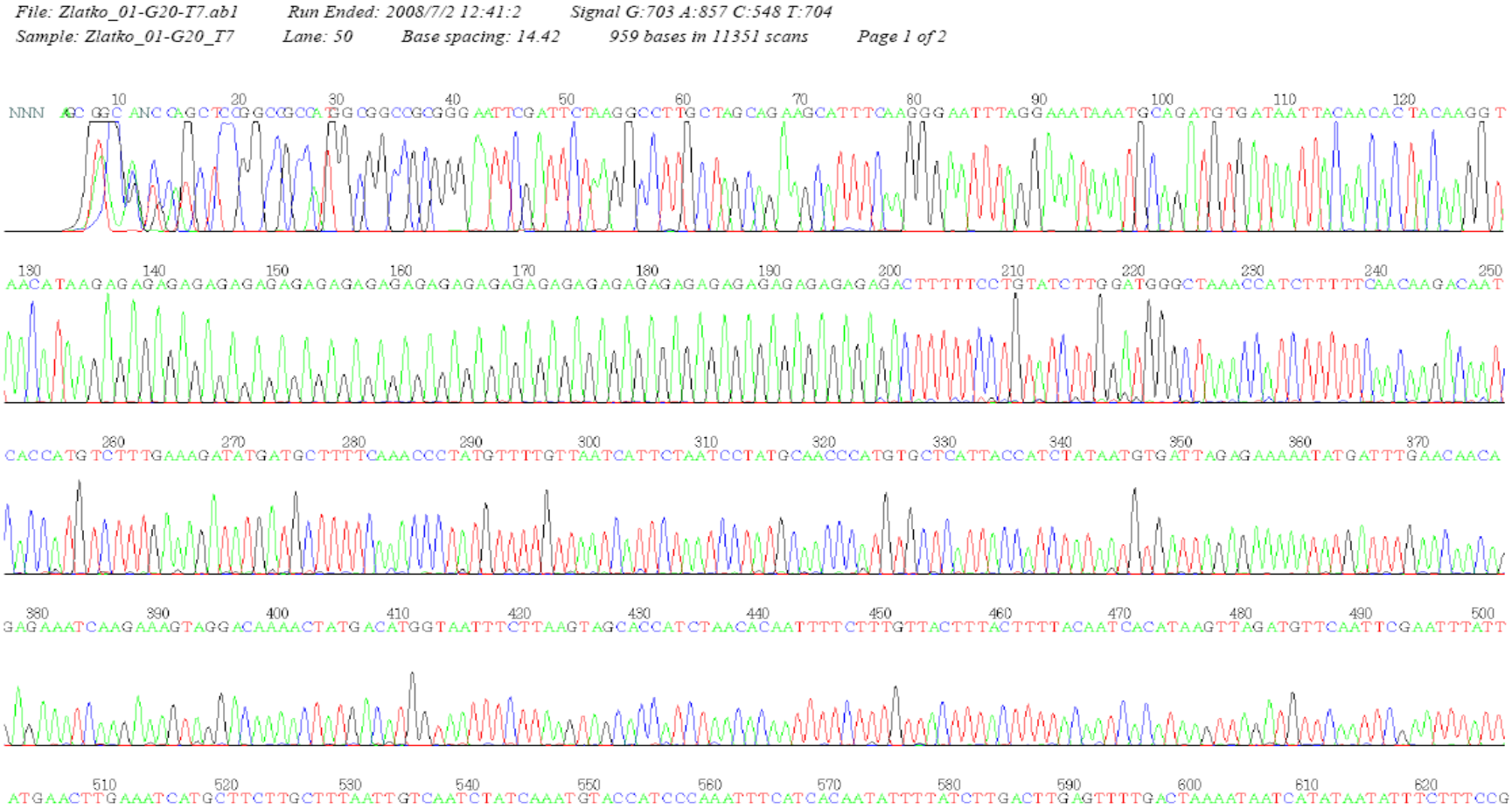
sequencing
(1st generation)



→ PCR products

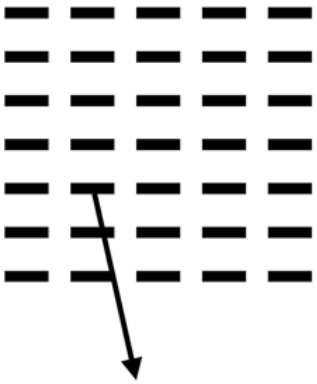
↓

sequencing
(1st generation)



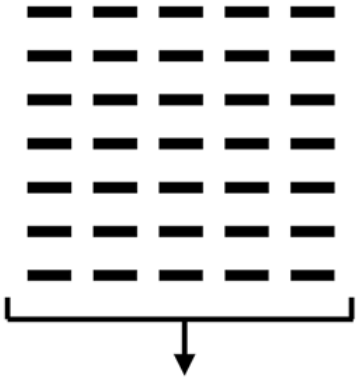
NGS sequencing

1st generation of DNA sequencers
(started 1987)



one fragment (ca 1000 bp long) per run

2nd and 3rd generations of DNA sequencers
(last ten years)



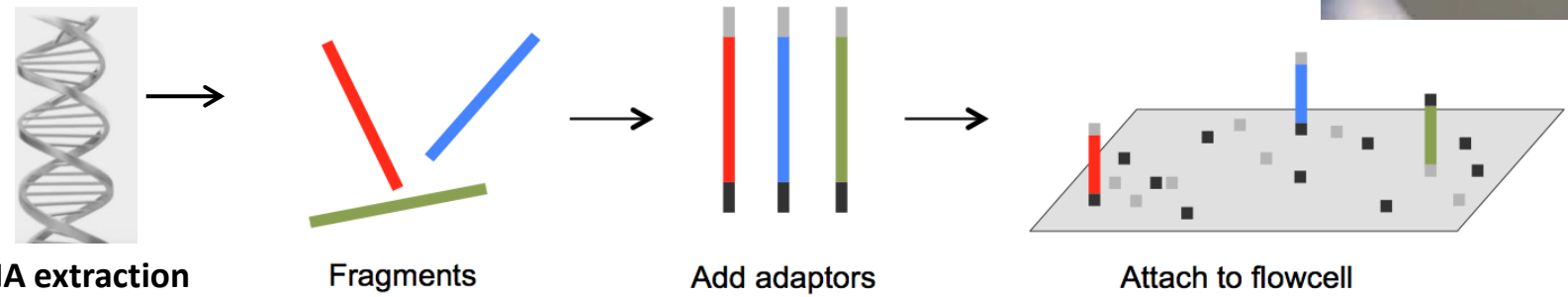
millions of fragments per run

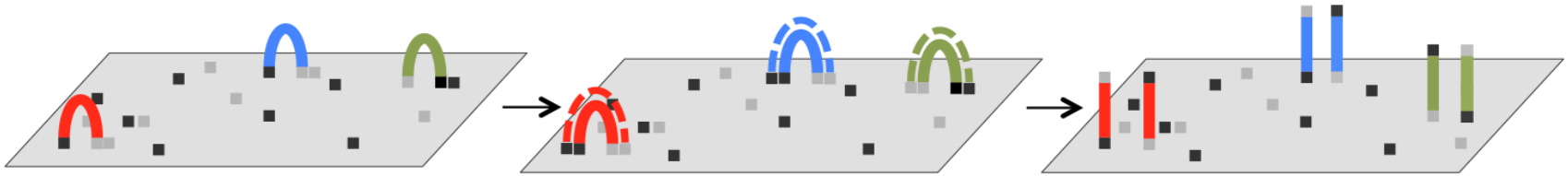
NGS = **N**ext **G**eneration **S**equencing

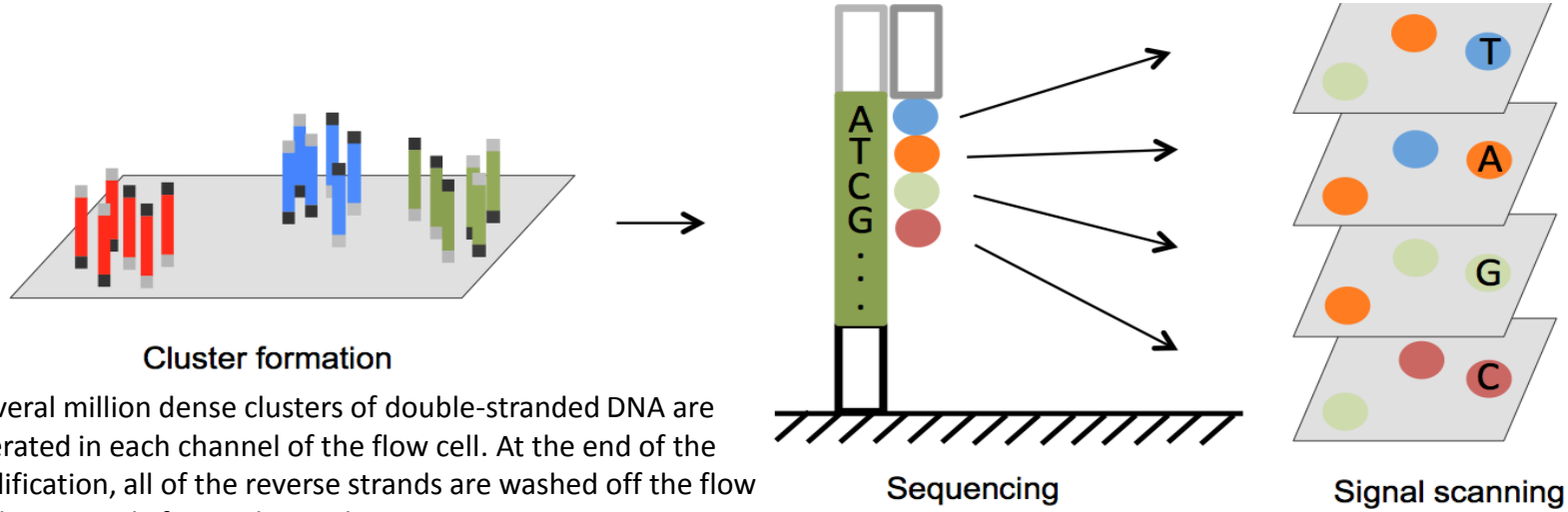
1) Sequencig by synthesis

2) Nanopore sequencing

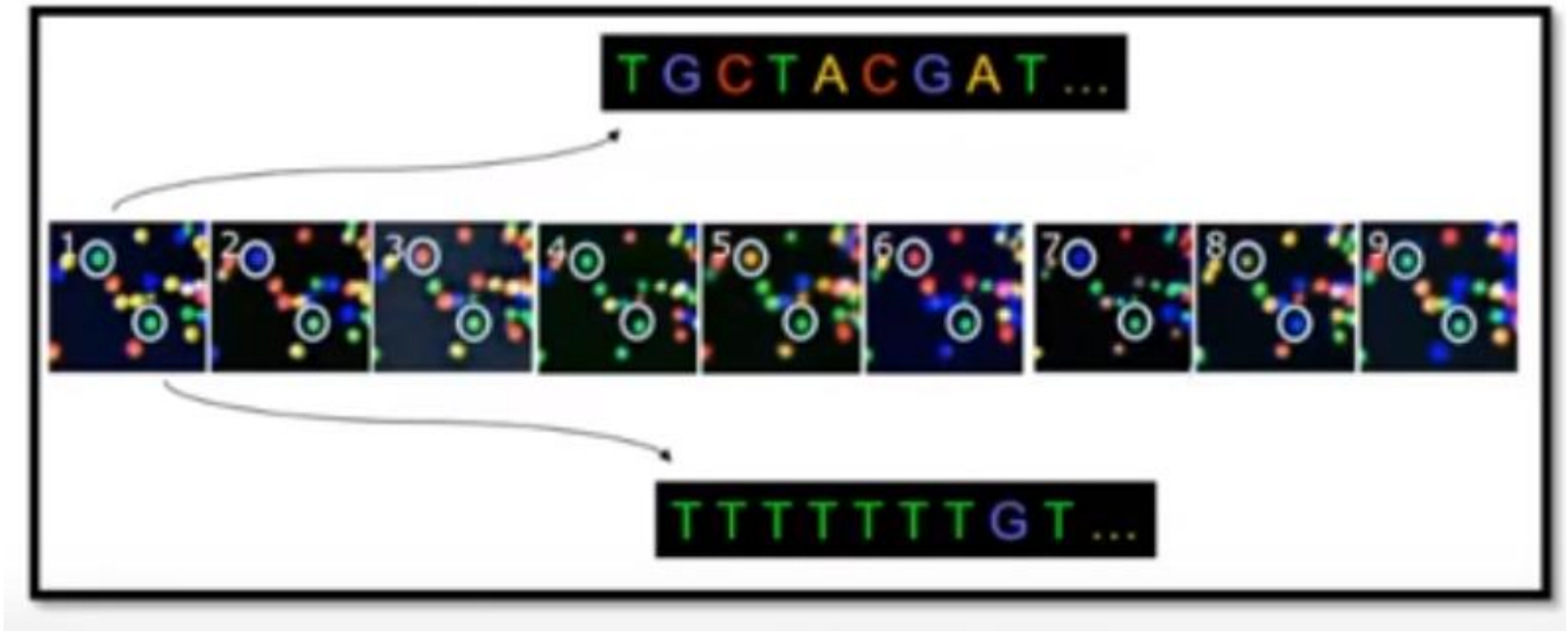
Sequencing by synthesis



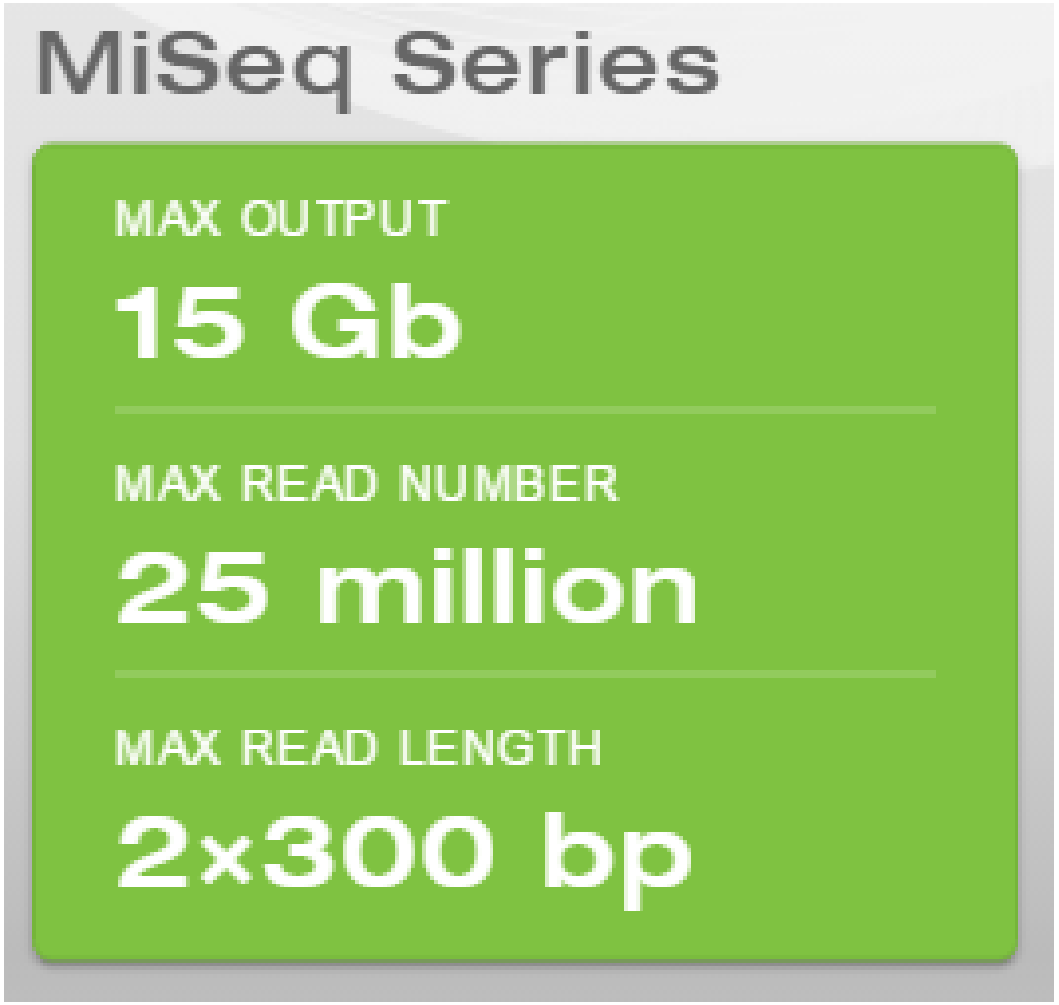




* Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell. At the end of the amplification, all of the reverse strands are washed off the flow cell, leaving only forward strands.

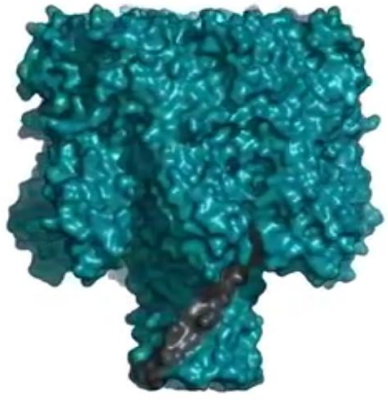

TGCTACGATC...
1
2
3
4
5
6
7
8
9
TTTTTTTGT...

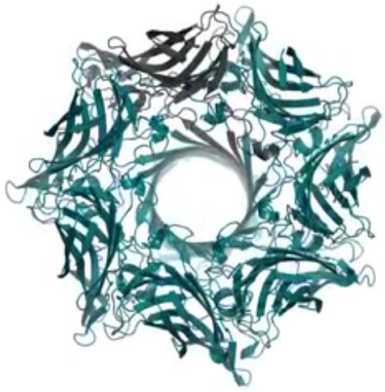
Illumina NGS sequencers (sequencing by synthesis)

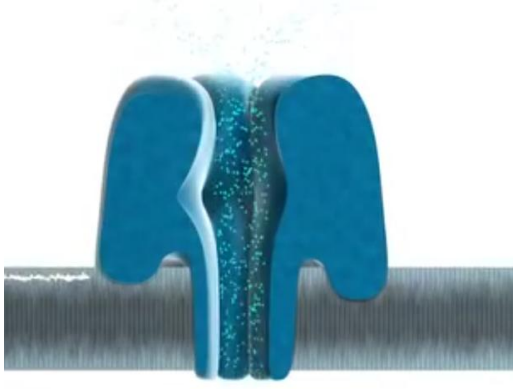


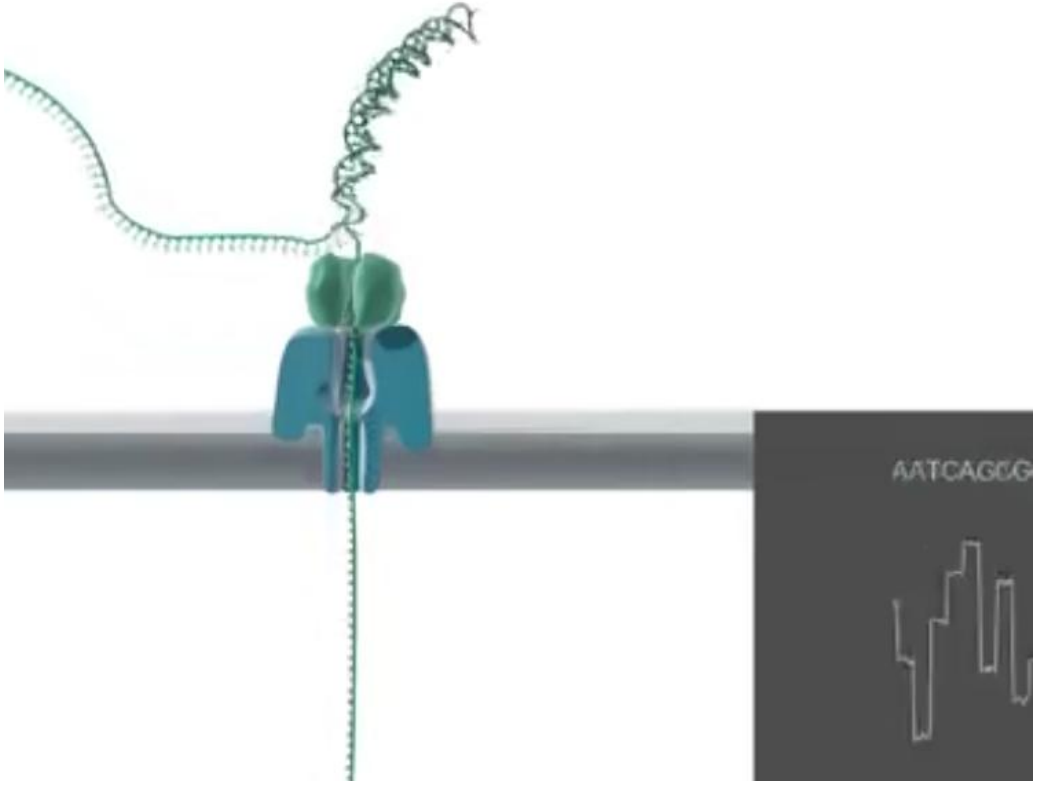

reference
NGS data

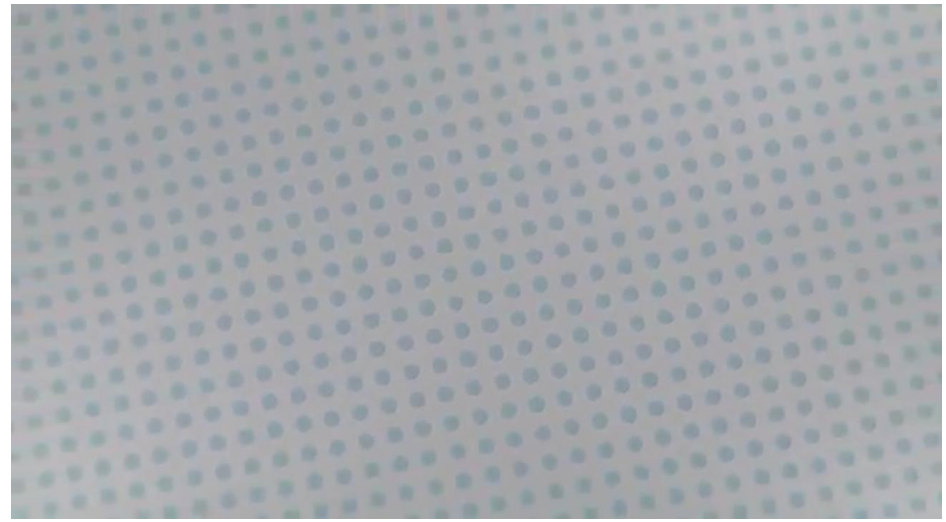
Nanopore Sequencing

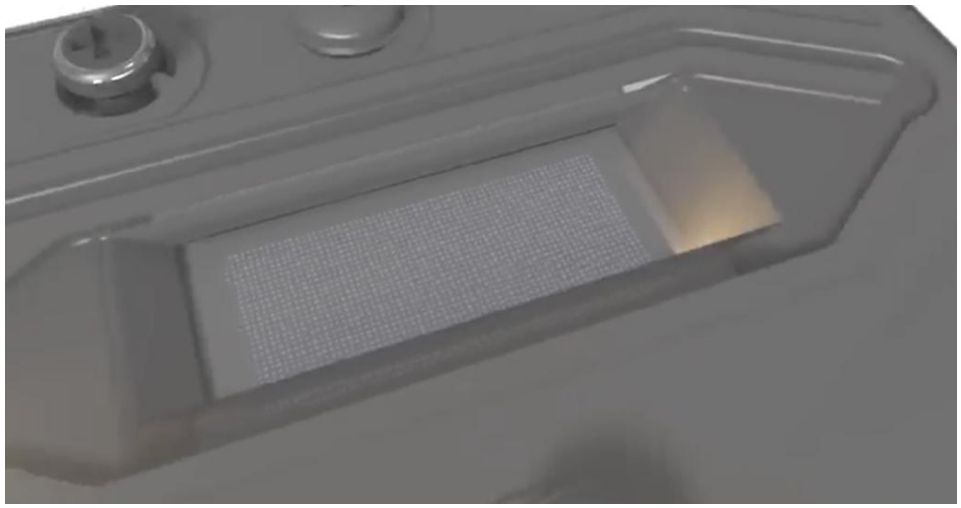












- yield : 21 Gb, read number 2.2 millions, longest read: 200Kb, run time 48h,, weight 87g