



TECHNISCHE  
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# DNA Sequencing

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- How can we gather information on the structure of DNA?
- What tools can we use?

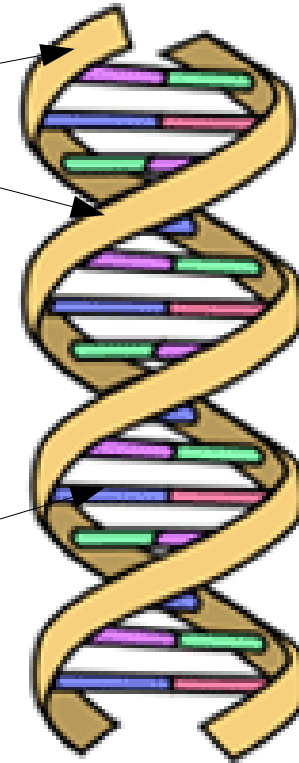
- DNA - structure and relevant properties
- Sequencing - extraction and preparation of the DNA
- Sequencing - acquisition of data
- Processing the Data

- Storage of genetical information
  - Manual for the production of proteins
- Present in every living cell

# DNA - structure

Backbones out of a chain of  
phosphate desoxyribose

— = Adenin  
— = Thymin  
— = Cytosin  
— = Guanin



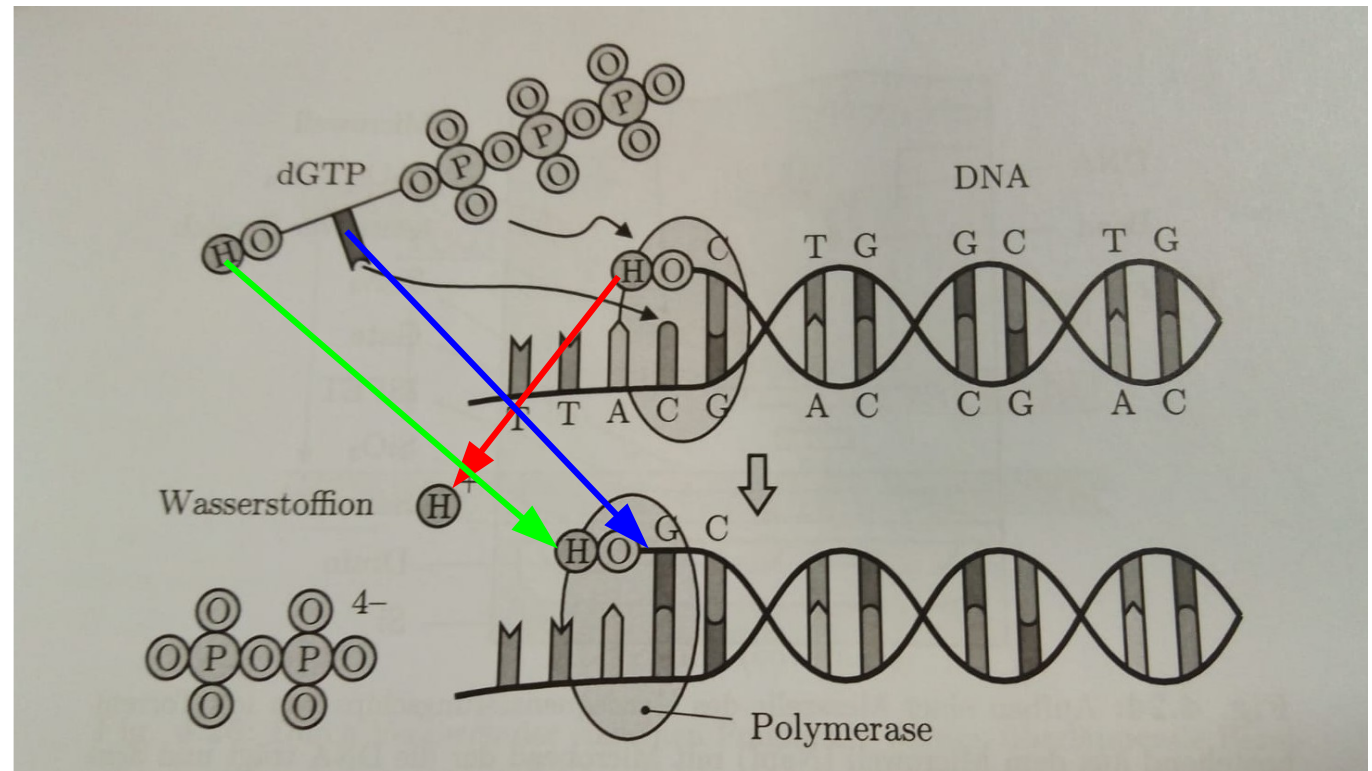
DNA

Backbones are connected  
through base pairs.  
Adenine is always paired with  
Thymine  
Cytosine is always paired with  
Guanine.

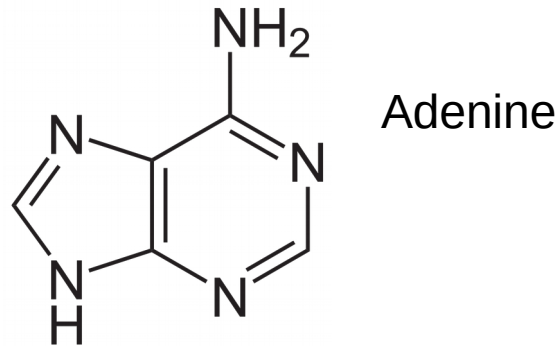
- Information is encoded by the sequence of Bases  
(A- Adenine, T- Thymine, C- Cytosine, G- Guanine)
- Every Base has a single possible Partner-Base to bond with
- Each of the two strands contains the entire information

# DNA – polymerase chain reaction

- Splitting the 2 Strands
- Completing each strand with the complementary base using enzymes (PCR)
- repeat



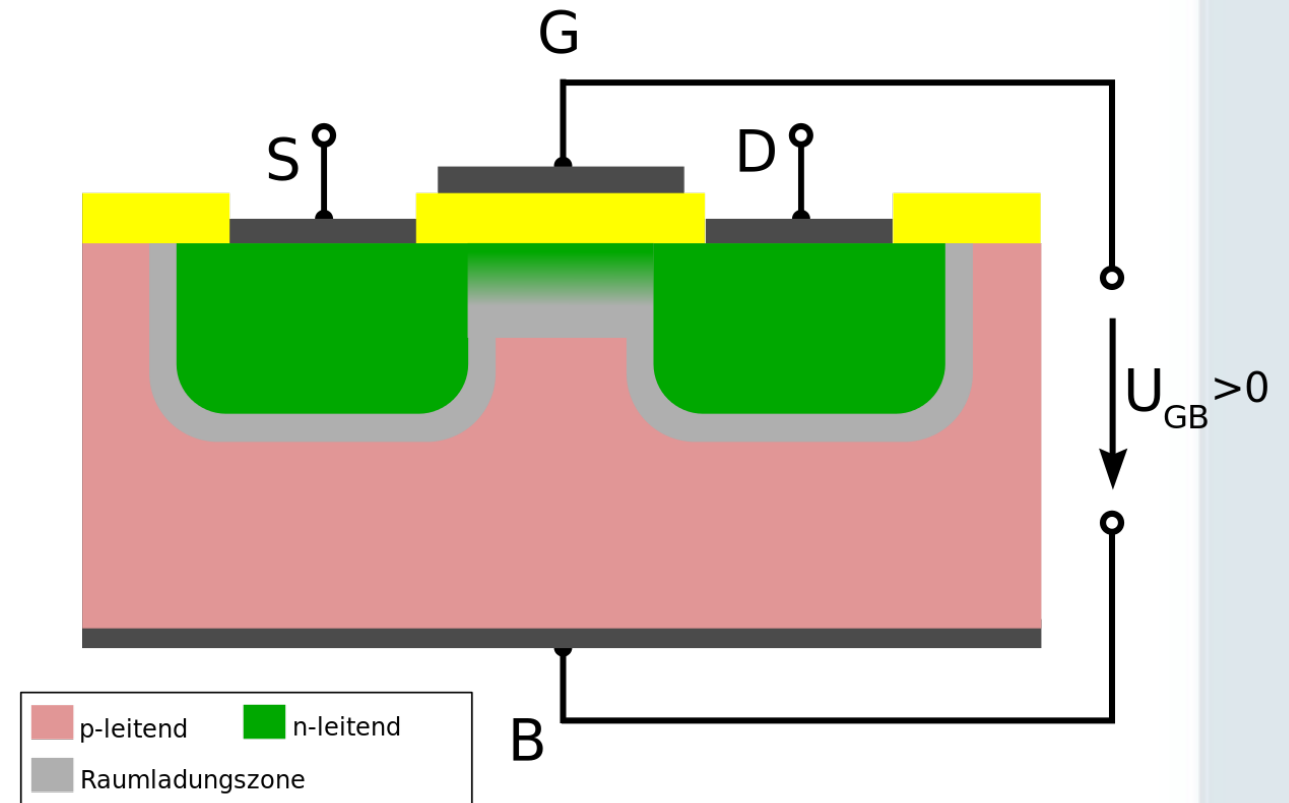
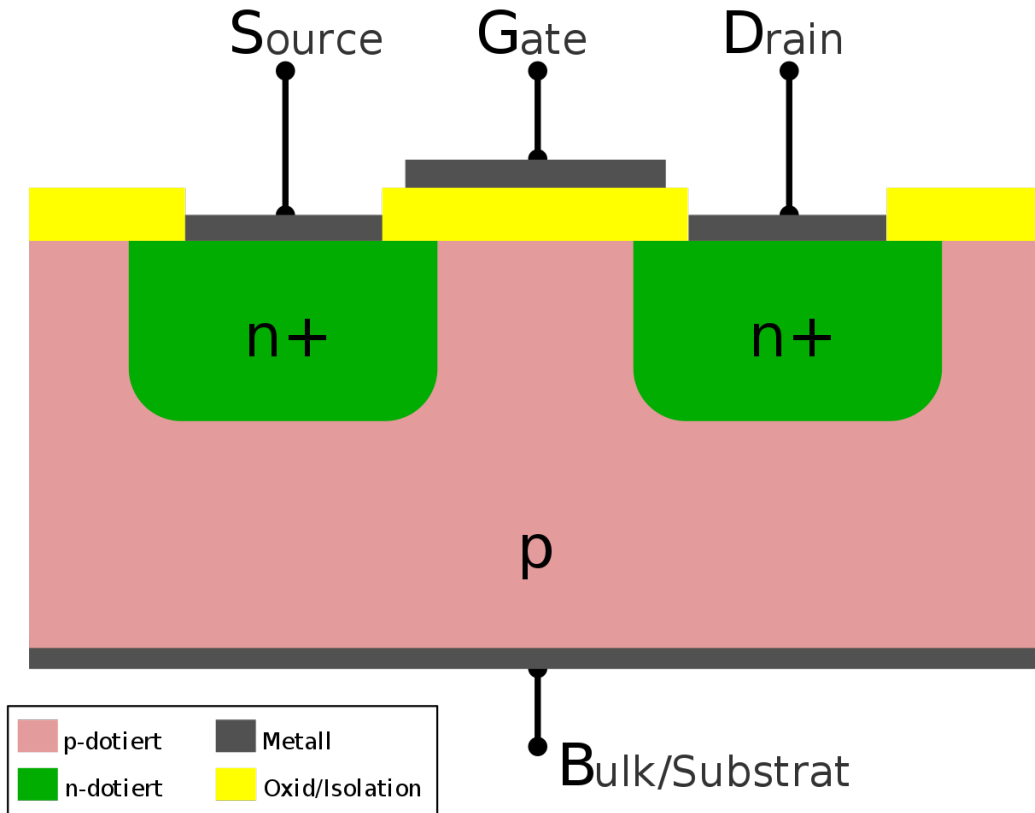
- What information are we looking for?
  - We are looking for the sequence of the bases, not the complete chemical structure (detection of „building blocks“, not single atoms)



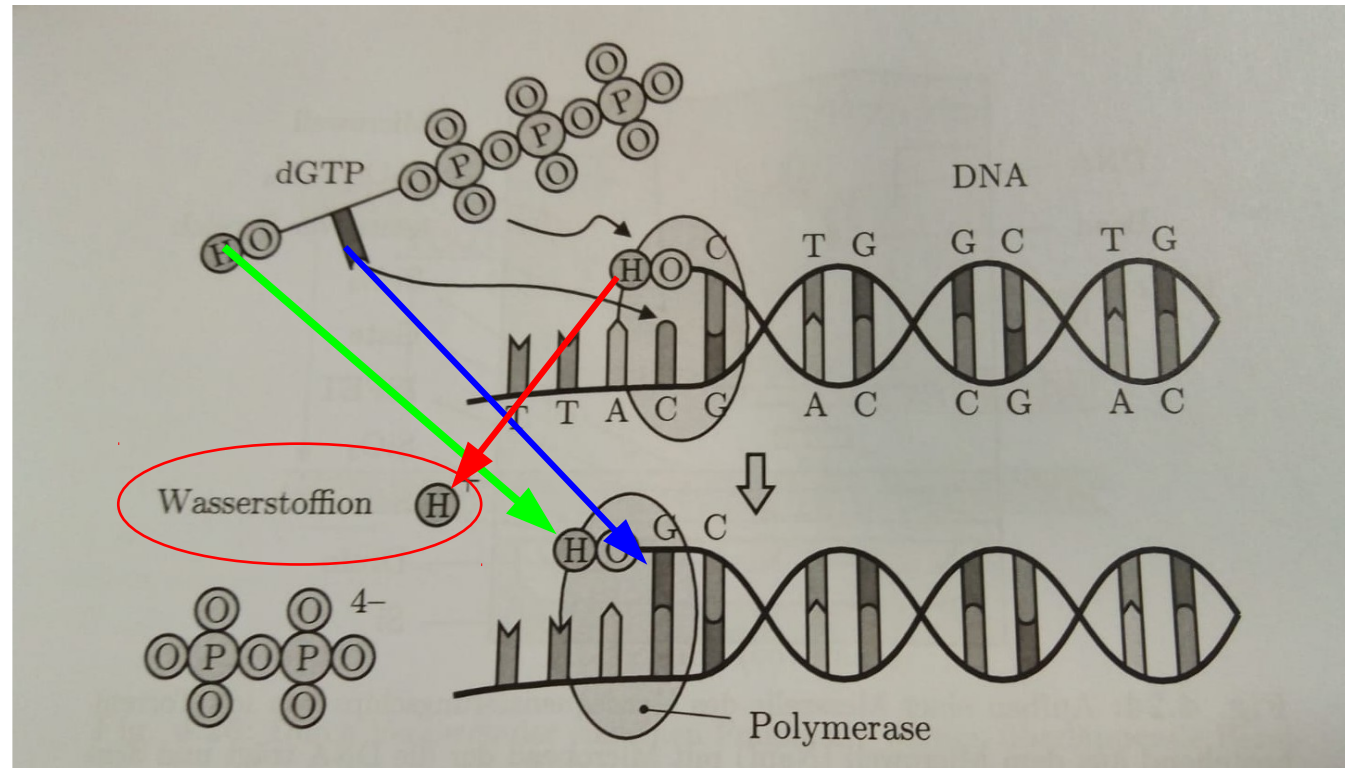
- How can we transform this information into a signal, that can be processed by a computer?
  - We need a setup, that reacts differently to each the 4 different bases AND can produce those signals very quickly (DNA consist of several Billion Base-Pairs)



- Field Effect transistor (FET)

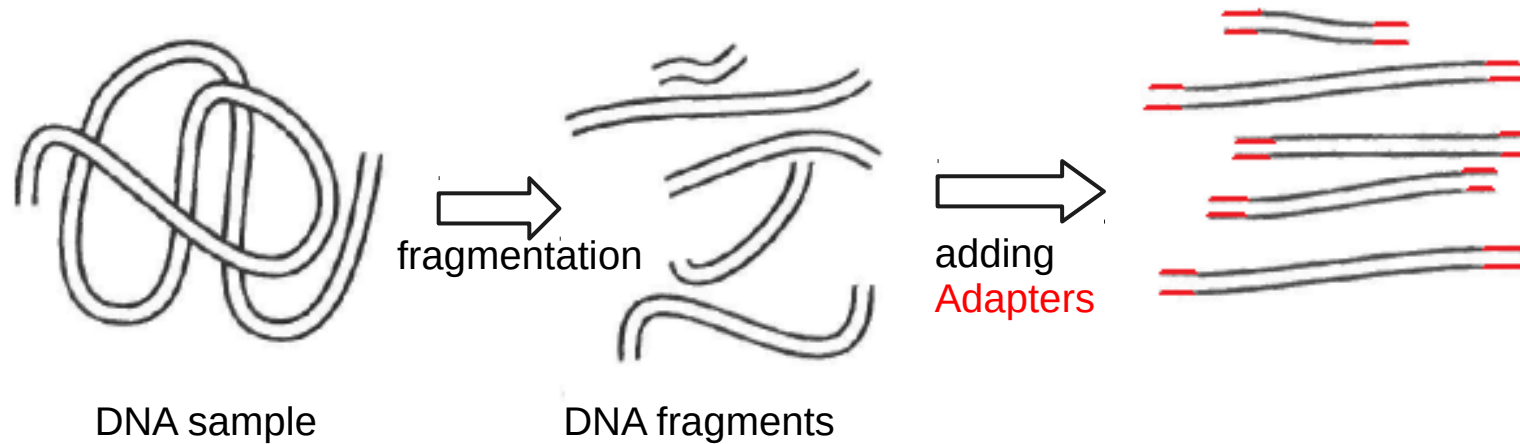


- Ion sensitive FET (sensitive to Hydrogen Ions)
  - Gate becomes conducting in the presence of Hydrogen Ions in the sample solution



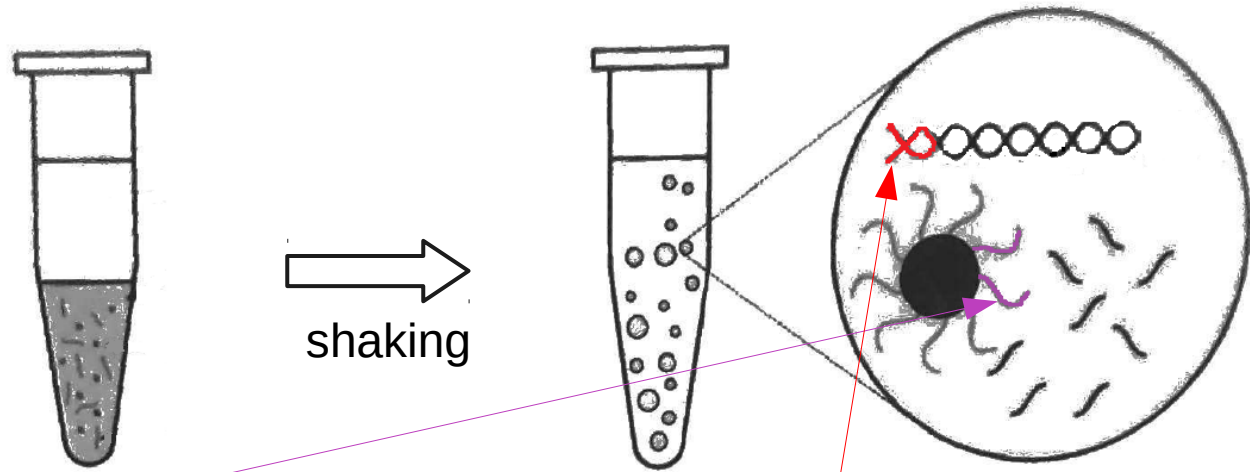
# Sample Preparation 1

- Splitting the DNA into many short fragments (using enzymes or with physical force)
- Adding Adapters to the ends of the fragments



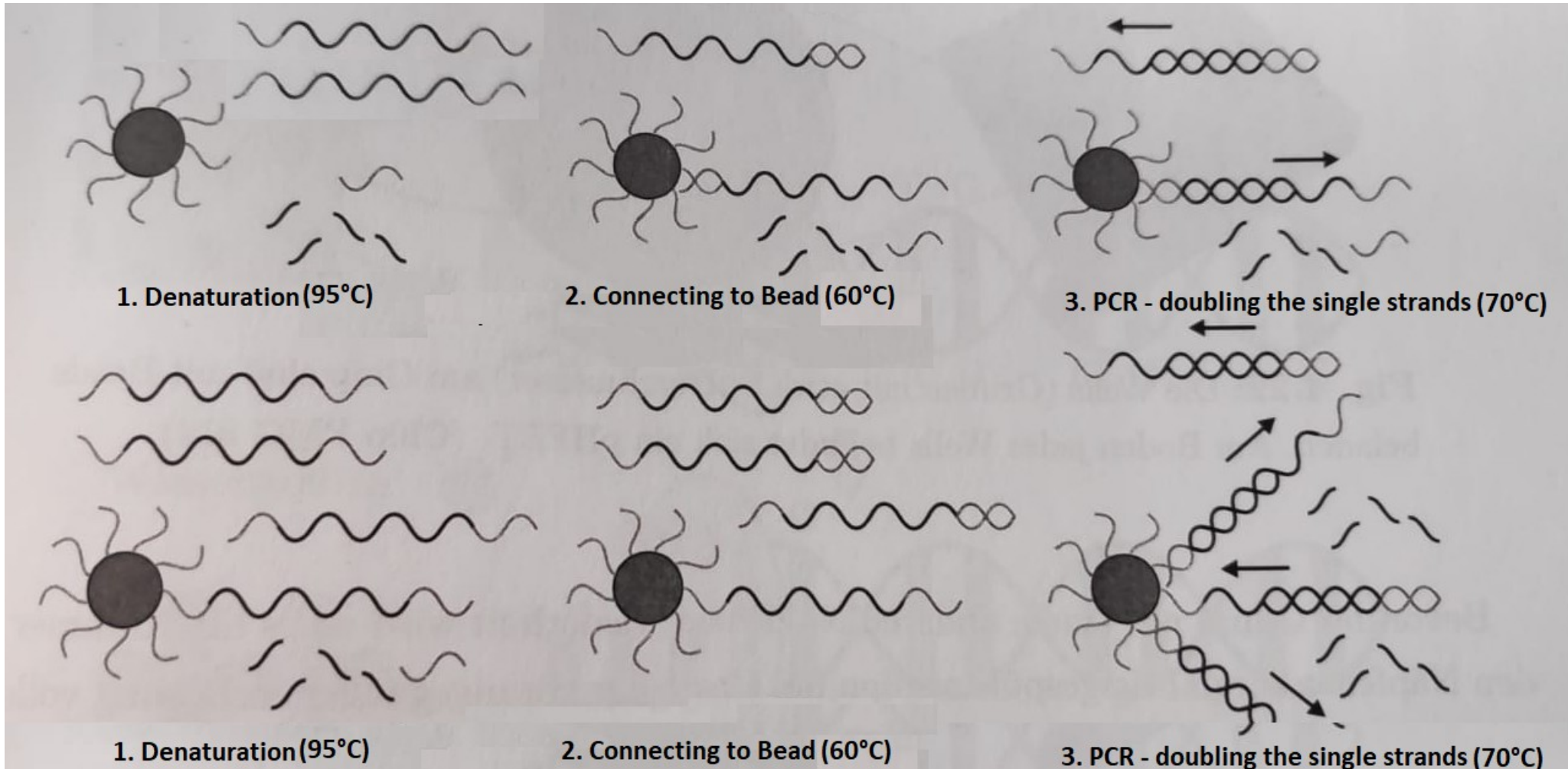
# Sample preparation 2- emPCR 1

- emPCR - „emulsion polymerase chain reaction“
- emulsion of aqueous solution in oil
- Aqueous Phase contains:
  - DNA fragments
  - Supply molecules for DNA replication
  - Polymerase (enzyme)
  - Beads

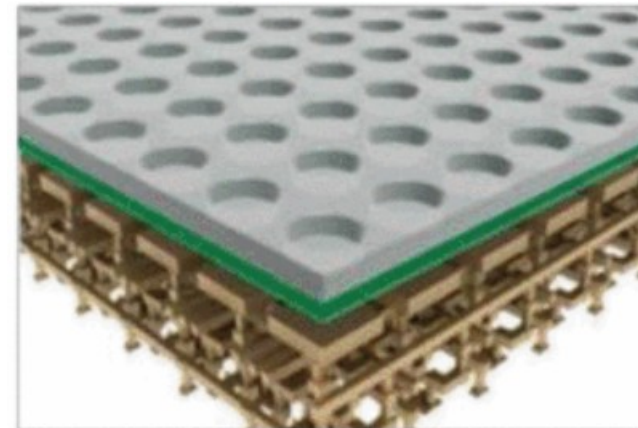
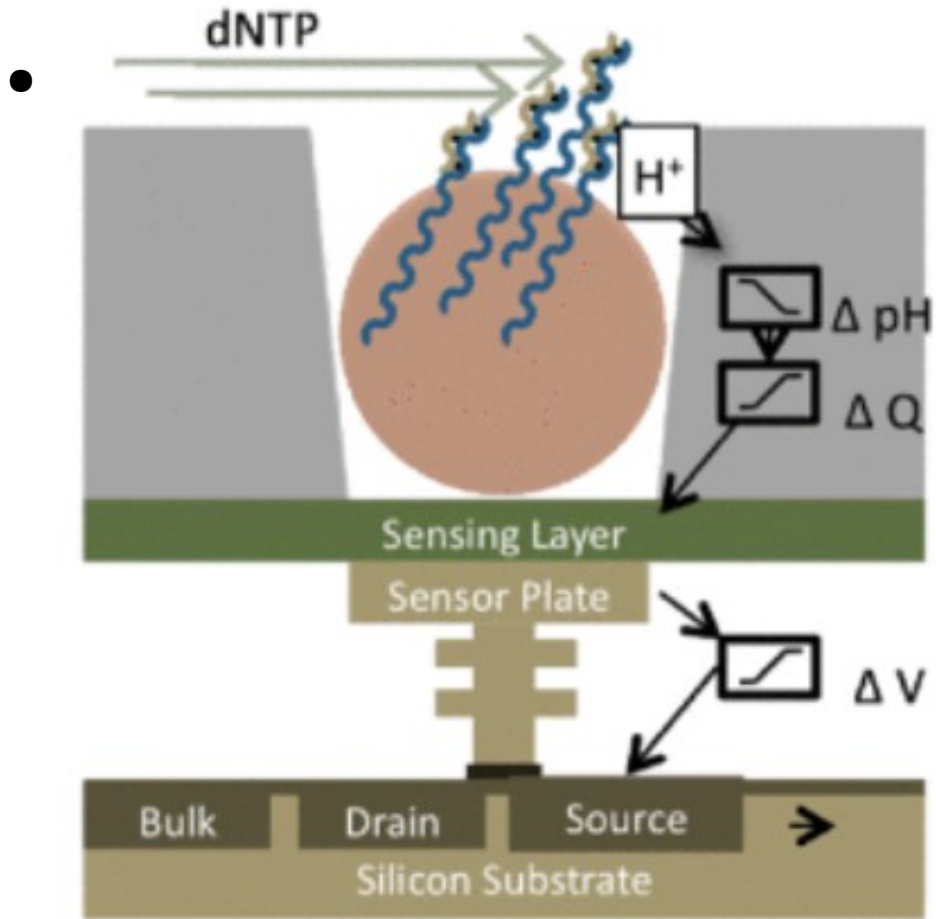


- Beads are covered in **Adapter** molecules, that match the **Adapter** molecules on the DNA fragments
- The emulsion is prepared, so that every droplet contains one bead, and at most one DNA fragment

# Sample preparation- emPCR 2



# Measurement setup



Chip 3-D Cross Section

*Development of the Ion Torrent CMOS chip for DNA sequencing, James Bustillo et al., 2013*

# Measurement Process

## Adding Solution supplying a single type of base fragment

- If the supplied base matches the single strand, it gets incorporated by the polymerase
- This reaction produces Hydrogen Ions
- Hydrogen Ions are detected by the Ion sensitive Field Effect transistor
- The signal is registered and sent to the CPU

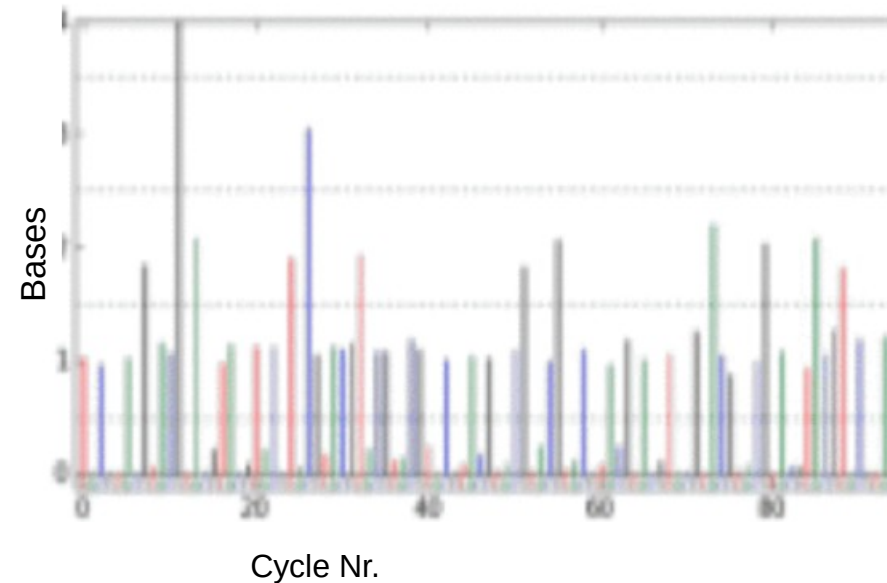
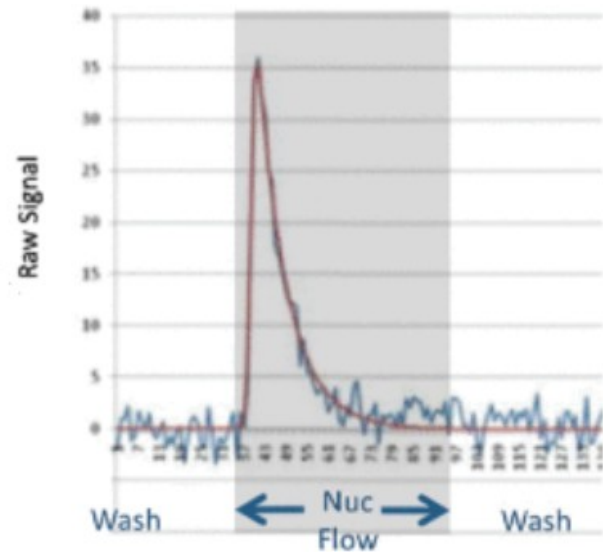
Washing solution is used, to wash away the remaining base

The next Base is supplied

Cycle is repeated until the entire strand is completed

# Output signal

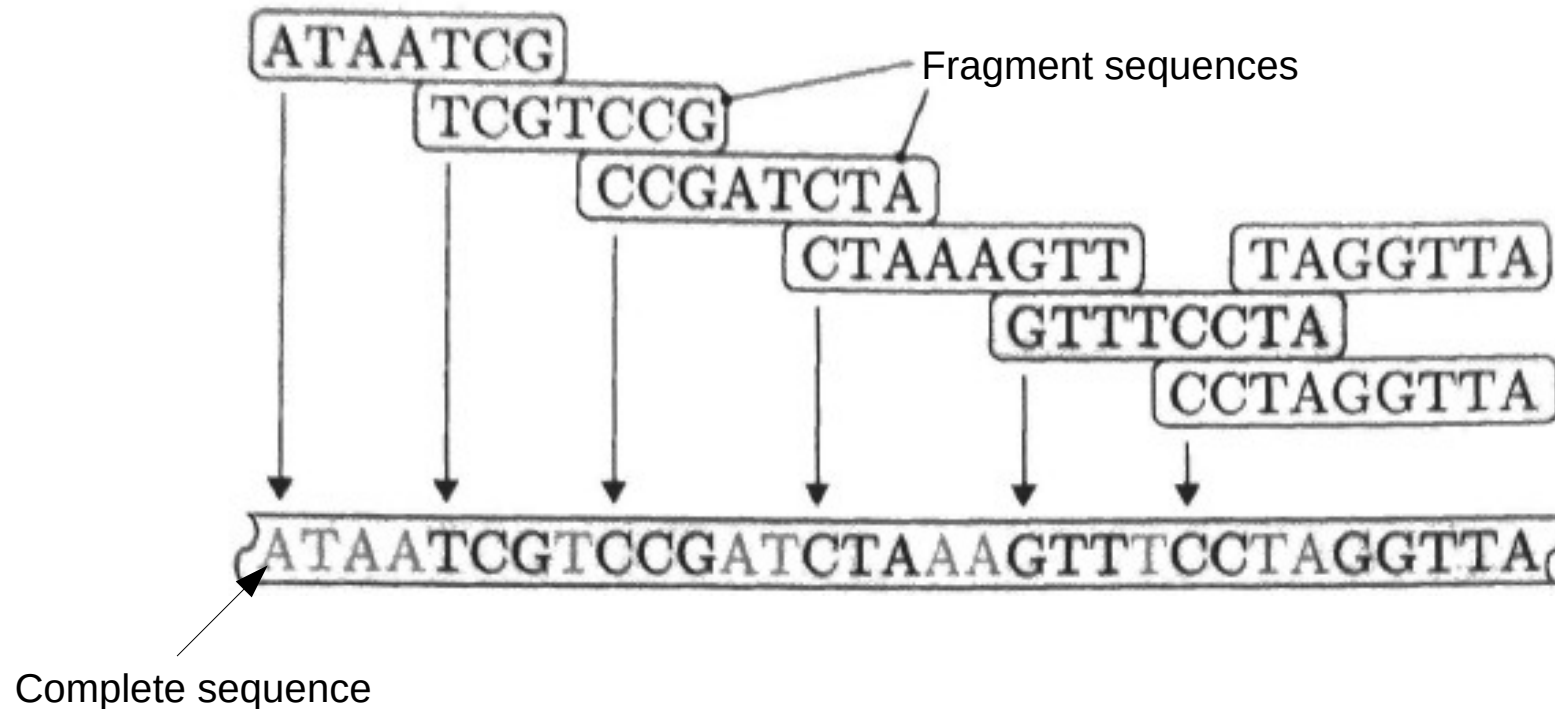
- A sequence containing a repetition of several bases results in more hydrogen produced, and therefore higher peaks.





# Processing the Data

- The sequences of the single fragments can be used to reconstruct the entire Data sequence



# Thank you for your attention !

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