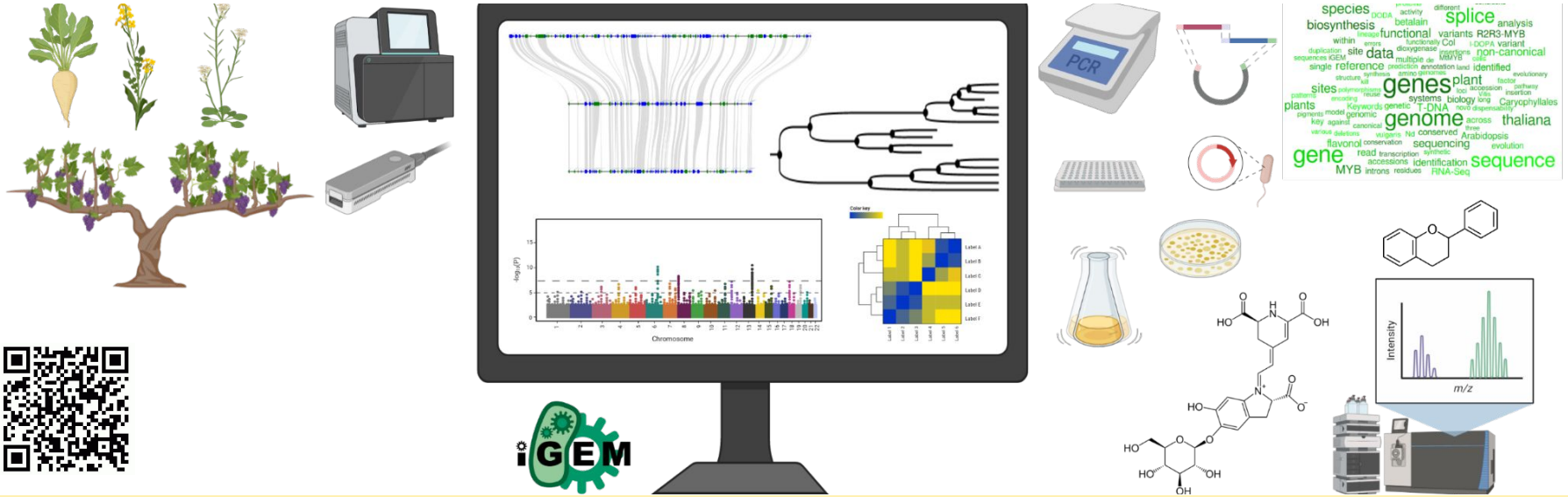
Technische Universität Braunschweig



Plant DNA extraction

Prof. Dr. Boas Pucker
(Plant Biotechnology and Bioinformatics)

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: **LMChemBSc12**
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

- Feedback, comments, or questions: b.pucker[a]tu-bs.de

Who discovered DNA and when?

Discovery of DNA

- Isolation of DNA by Friedrich Miescher in 1869
  - Working in the castle in Tübingen

Friedrich Miescher
(1844-1895)

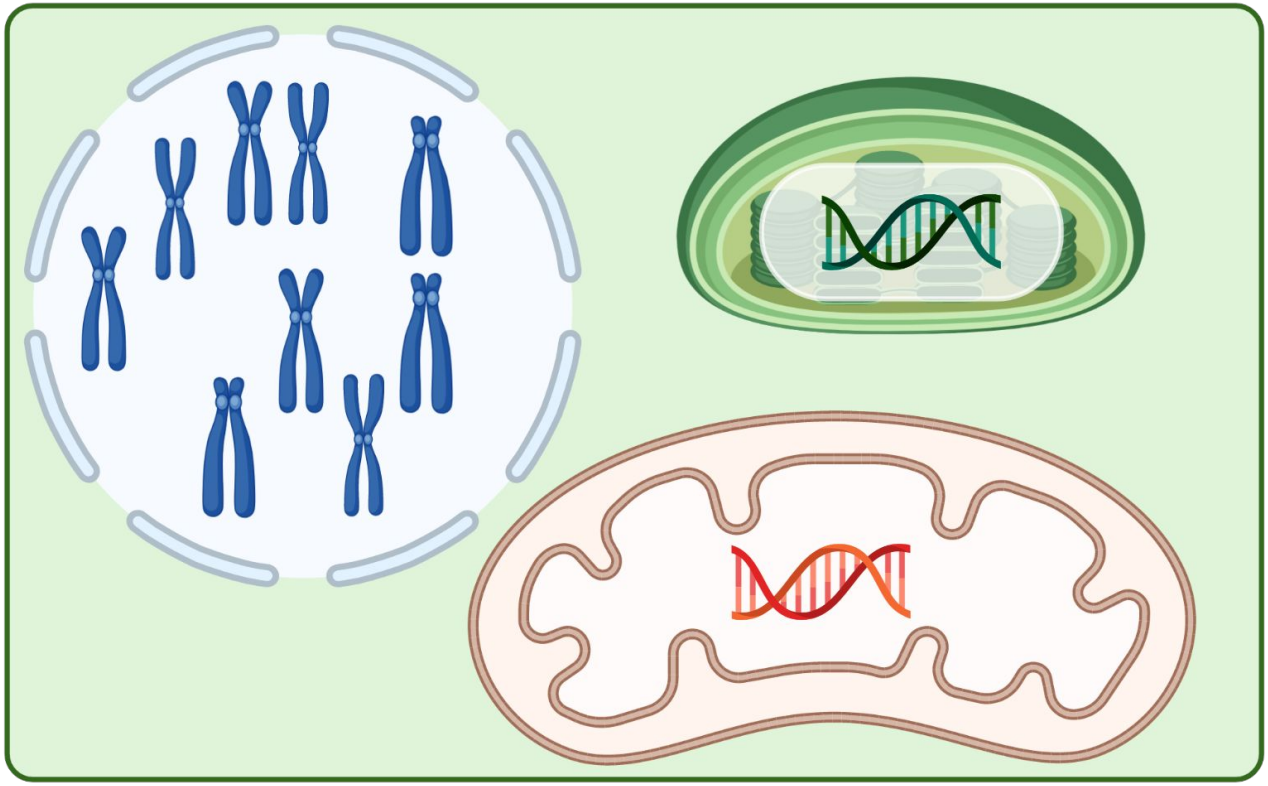
- WATSON, J., CRICK, F. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature* **171,** 737–738 (1953). https://doi.org/10.1038/171737a0

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

Plant DNA



- Nucleome: DNA in nucleus (gDNA)
- Plastome: DNA in chloroplast and other plastids (ptDNA)
- Chondrome: DNA in mitochondria (mtDNA)

What are challenges during DNA extraction?

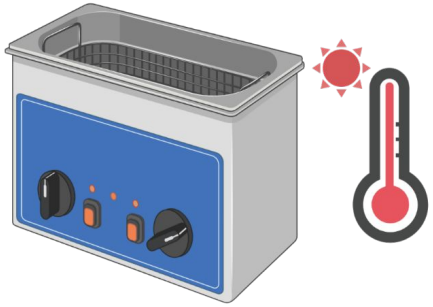
DNA extraction challenges

- Cell wall
- Contaminations (proteins, specialized metabolites)
- Other nucleic acids (RNAs)
- DNases
- DNA fragmentation

Breaking the cell wall

Mortar &
pestle

Ribolyzer



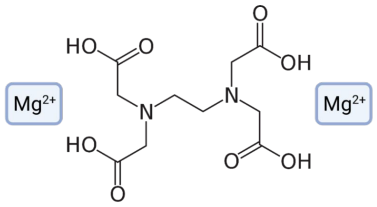
Ultrasonic
& heat

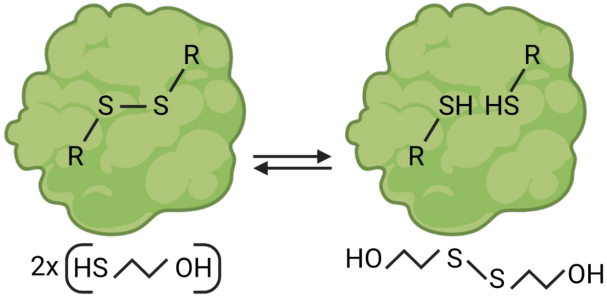
Plant cell composition - potential contaminants

macromolecule	Percentage of total dry weight	Number of molecules per cell
protein	55	3,000,000
RNA	20	-
DNA	3	-
lipid	9	20,000,000

How to inactivate DNases?

- Heat
- EDTA
- ß-mercaptoethanol
- Proteases



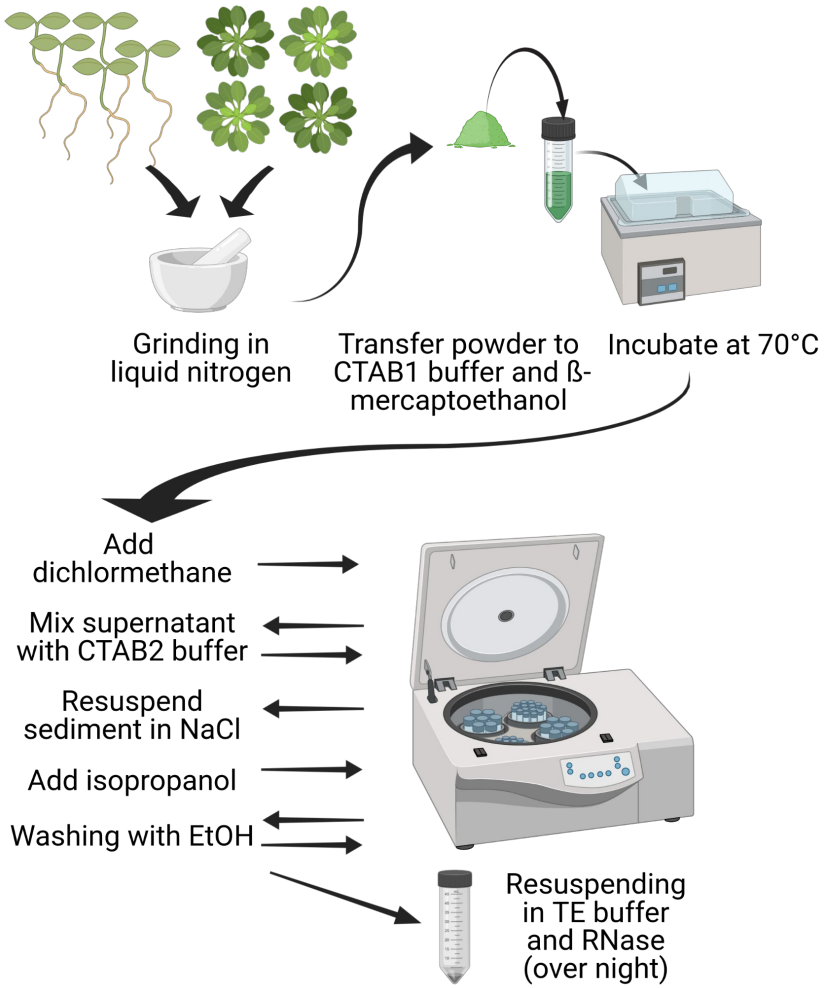


DNA extraction methods

- CTAB
- Edwards
- Kit-based
- Nucleus isolation
- Leaf lysis methods
- Magnetic beads

CTAB: Cetyltrimethylammonium bromide

- High DNA quality (suitable for sequencing)
- Substantial time commitment
- Low material costs



Edwards

- Good DNA quality (sufficient for PCR)
- Modest time commitment
- Low material costs


leaf sample


leaf disk extraction


grinding


400µL extraction buffer


vortexing


centrifugation


transfer of supernatant and addition of 300µL isopropanol


resolve in 100µL TE

Edwards et al., 1991: 10.1093/nar/19.6.1349

Kit-based DNA extraction

- High DNA purity
- Fragment length and total amount limited
- Modest time commitment
- High material costs


grinding sample


lysis (protease)


RNA digestion (RNase A)

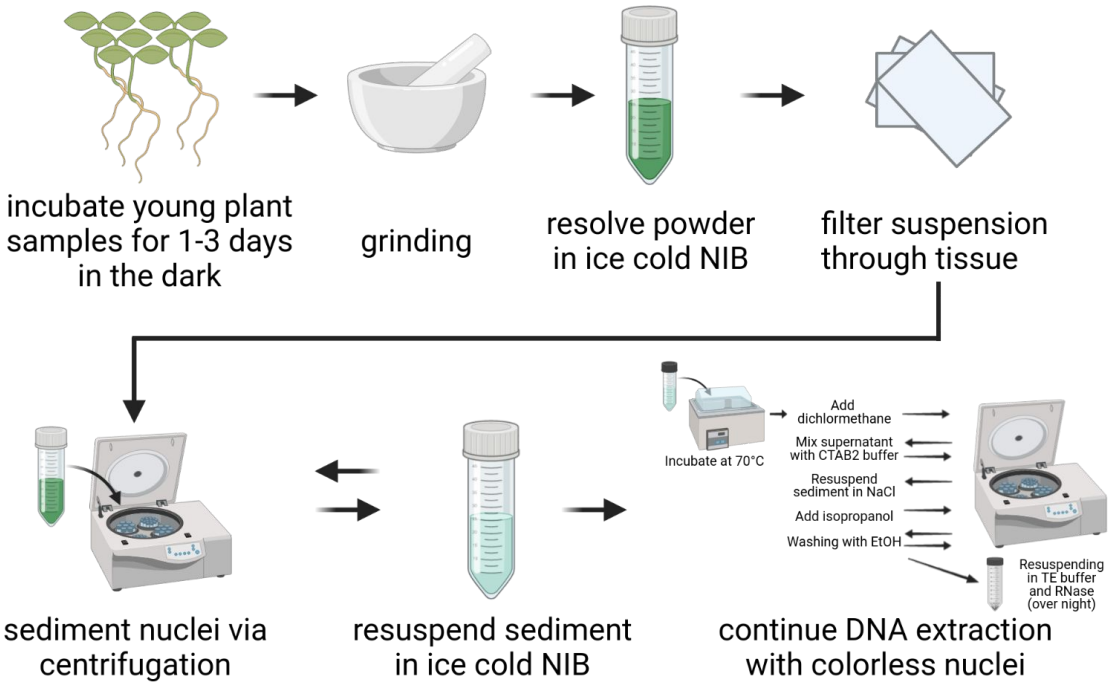

Binding DNA to column


Washing


DNA elution

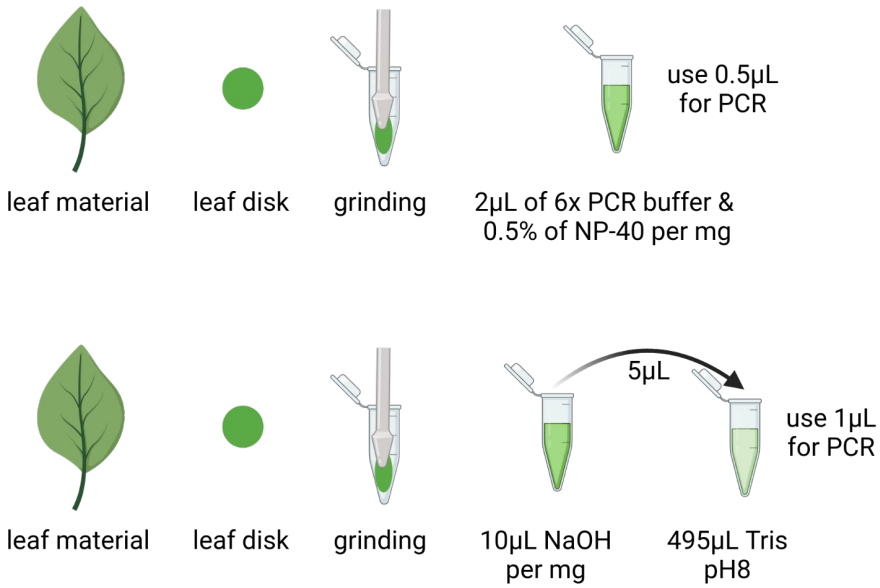
Nucleus isolation

- Excellent DNA quality
- Very high time commitment
- Modest material costs



Leaf lysis methods

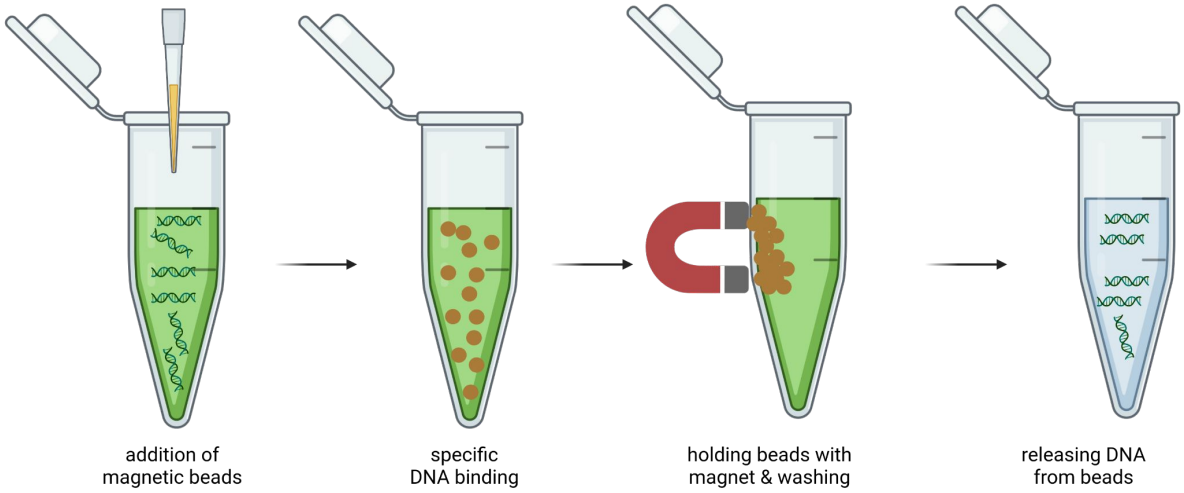
- Low DNA quality/purity; only suitable for immediate use in PCR
- Extremely fast
- Low costs



Wang et al., 1993: 10.1093/nar/21.17.4153

Magnetic beads

- High DNA quality
- Modest time commitment
- High material costs

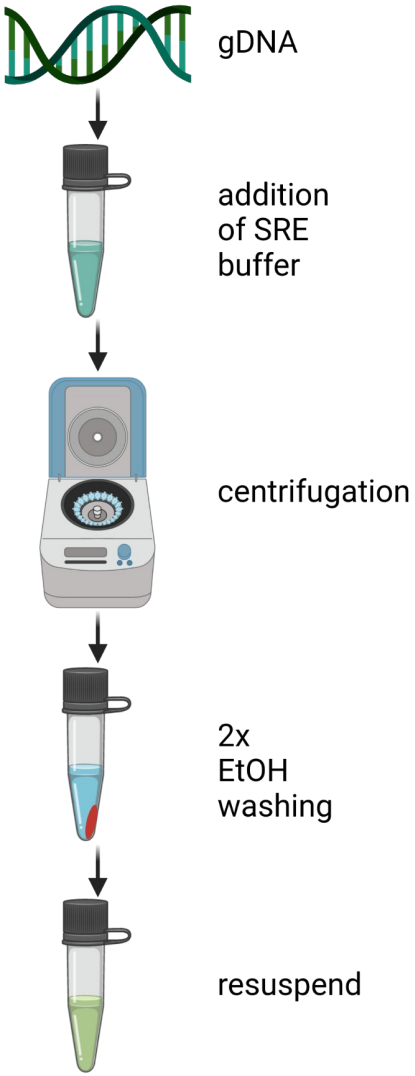


DNA precipitation

- Cation necessary to neutralize negative charge of DNA
- Low temperature facilitates flocculation of nucleic acids
- DNA concentration needs to be high enough
- DNA precipitation requires:
  - 35% isopropanol + 0.5M salt
  - 75% ethanol + 0.5M salt

Short Read Eliminator (SRE) kit

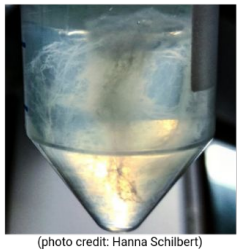
- Proprietary salt mixture allows DNA precipitation
- Removal of DNA fragments <10kb
- Depletion of DNA fragments <20kb



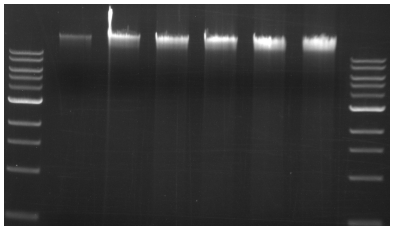
How to assess the DNA quality?

DNA quality check

- Optical inspection
- Agarose gel
- NanoDrop
- Qubit
- qPCR (next lecture)



(photo credit: Hanna Schilbert)



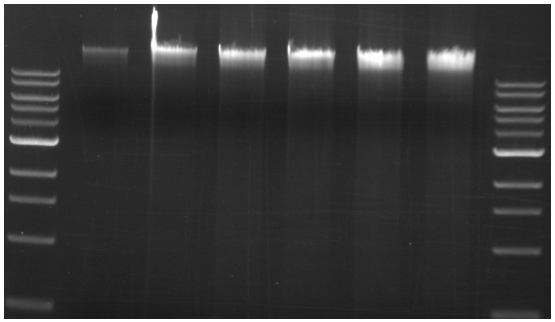
Optical check

- DNA can be seen in precipitation steps
- Presence of sediment can be checked after centrifugation steps
- White color is often caused by contaminants
- Perfectly clean DNA is almost not visible


(photo credit: Hanna Schilbert)

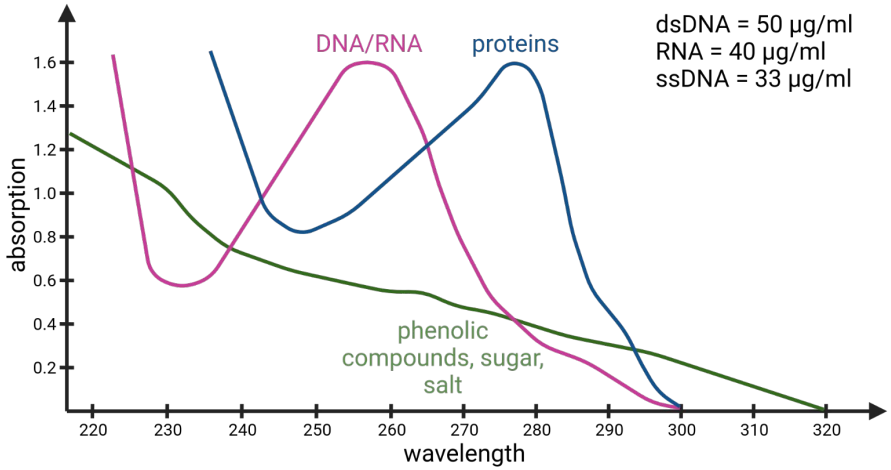
Agarose gel

- Agarose is a special polysaccharide
- DNA moves through the gel from minus (cathode) to plus (anode)
- DNA is separated by size



NanoDrop

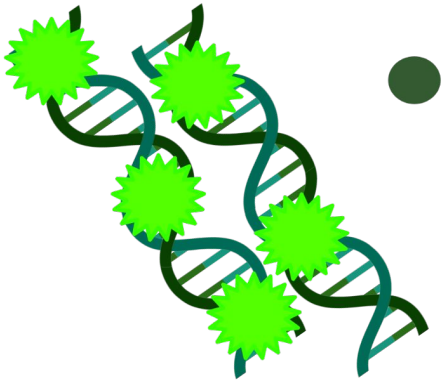
- Photometric analysis of nucleic acids
- Nucleic acid absorbance peak at 260nm
- Protein peak absorbance at 280nm (aromatic amino acids)
- OD_{260}/OD_{230} = small fragment/phenolic compound contamination
- OD_{260}/OD_{280} = protein contamination

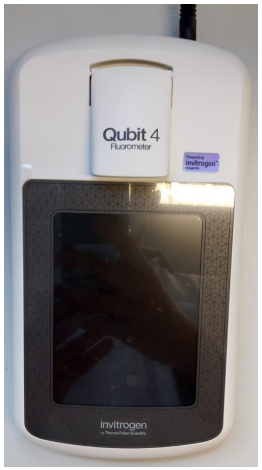


dsDNA = 50 µg/ml
RNA = 40 µg/ml
ssDNA = 33 µg/ml

Qubit

- Fluorescence-based (PicoGreen) measurement of nucleic acid concentration
- dsDNA HS Assay: 10pg/µL - 100ng/µL
- dsDNA BR Assay: 50pg/µL - 200ng/µL





How to store DNA?

DNA storage

- EDTA in buffer prevents degradation by DNases
- Store at 4°C for up to a week
- Store at -20°C for long term
- High molecular weight DNA should not be frozen to avoid breaks

Time for questions!

Questions

1. Who discovered DNA?
2. What is a plant genome?
3. What are challenges during the DNA extraction?
4. Which different DNA extraction methods exist?
5. What are the important steps of a DNA extraction method?
6. How can the DNA quality be checked?
7. What is the concept of a DNA quality check method?