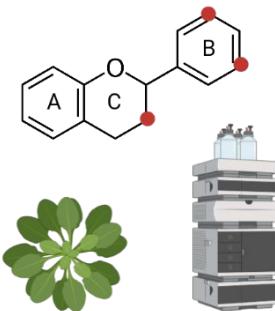
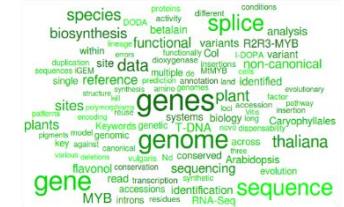
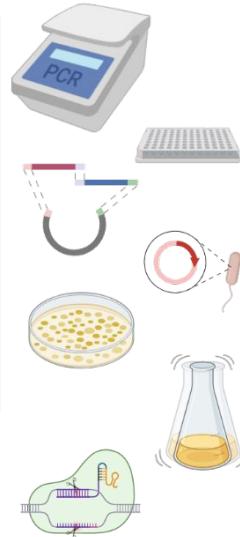
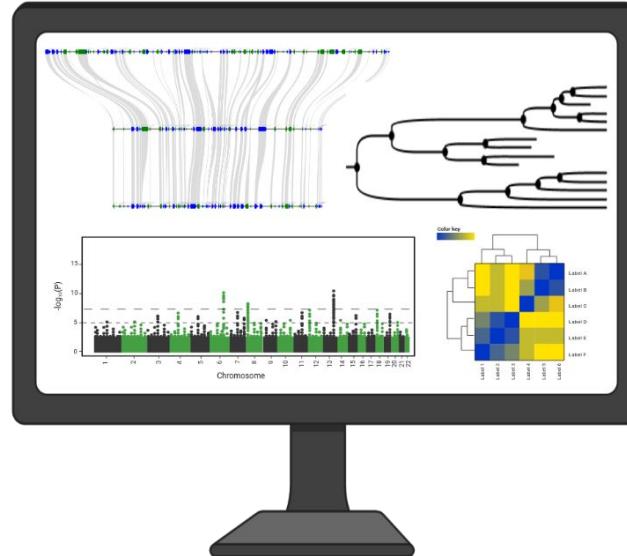


Plant Biotechnology and Bioinformatics



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

My figures and content can be re-used in accordance with CC BY 4.0, but this might not apply to all images/logos. Some figure were constructed using bioRender.com.

Importance of DNA and ‘genes’

- Genetic modifications enable substantially improved crops (e.g. gene editing via CRISPR/Cas)
- Genetically engineered plants are strictly regulated in the EU (cultivation is practically impossible)
- GMO classification is based on process not product in EU, but differently regulated in many other countries
- Slow change in society through science education (e.g. ÖkoProg, GeneSprout Initiative, RePlanet)



<https://www.jic.ac.uk/research-impact/purple-tomatoes/>
doi: 10.1038/nbt.1506



<https://givegenesachance.eu/>

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>

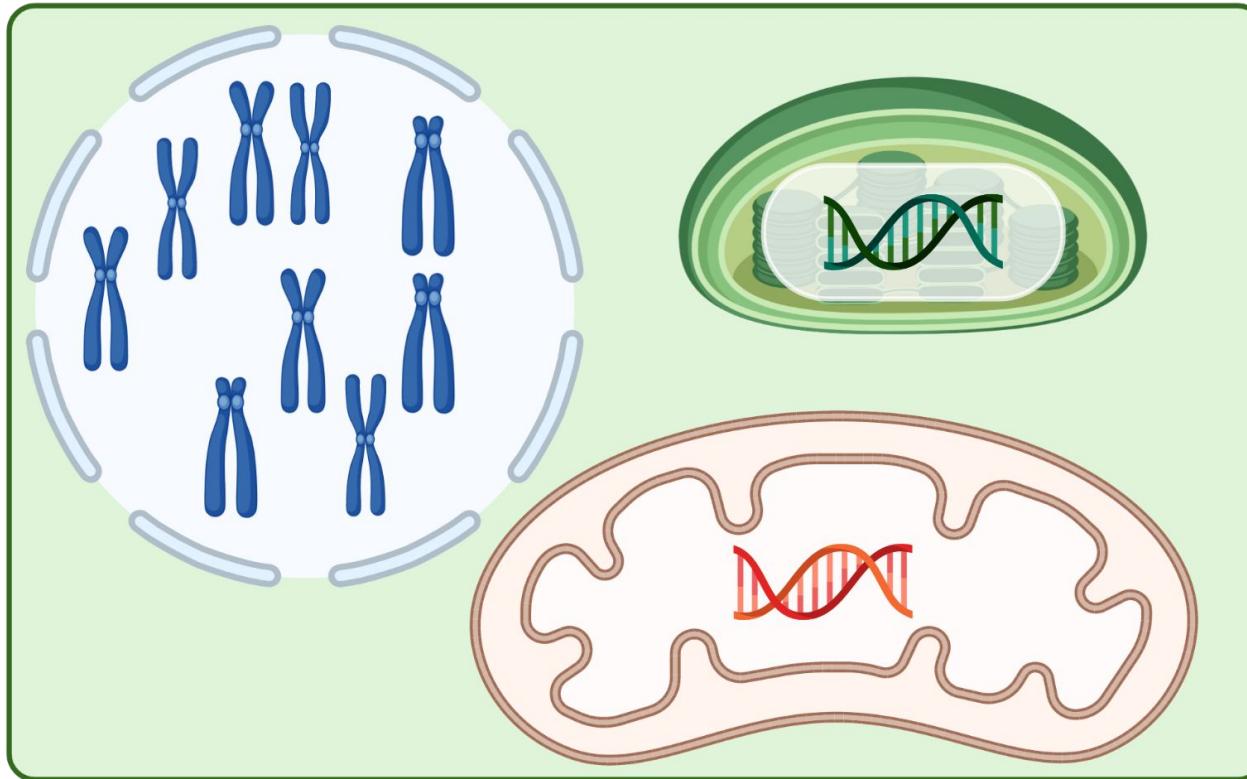


MOLECULAR STRUCTURE OF NUCLEIC ACIDS

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

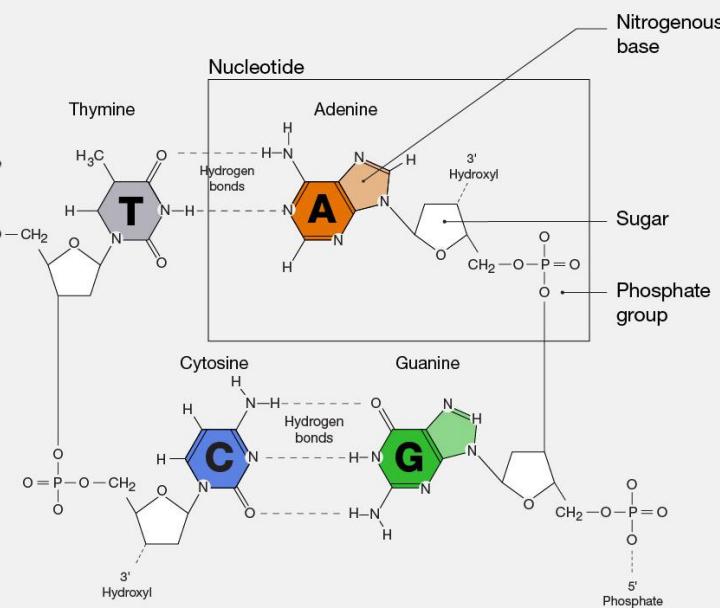
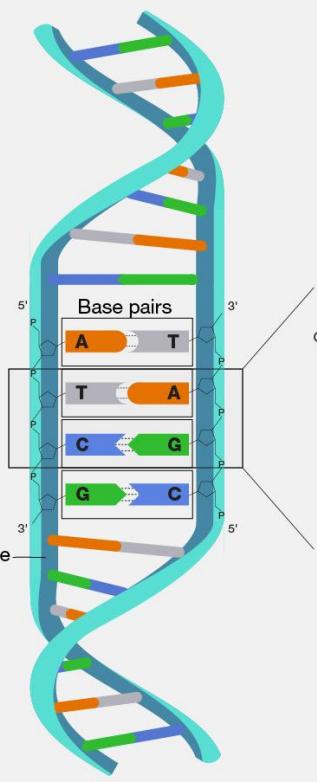
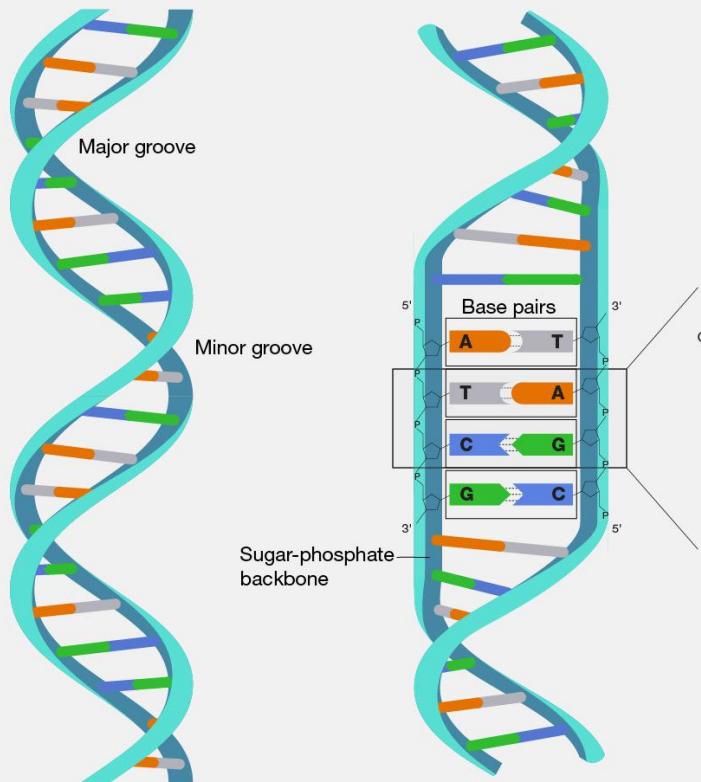


- Nucleome: DNA in nucleus (gDNA)
- Plastome: DNA in chloroplast and other plastids (ptDNA)
- Chondrome: DNA in mitochondria (mtDNA)

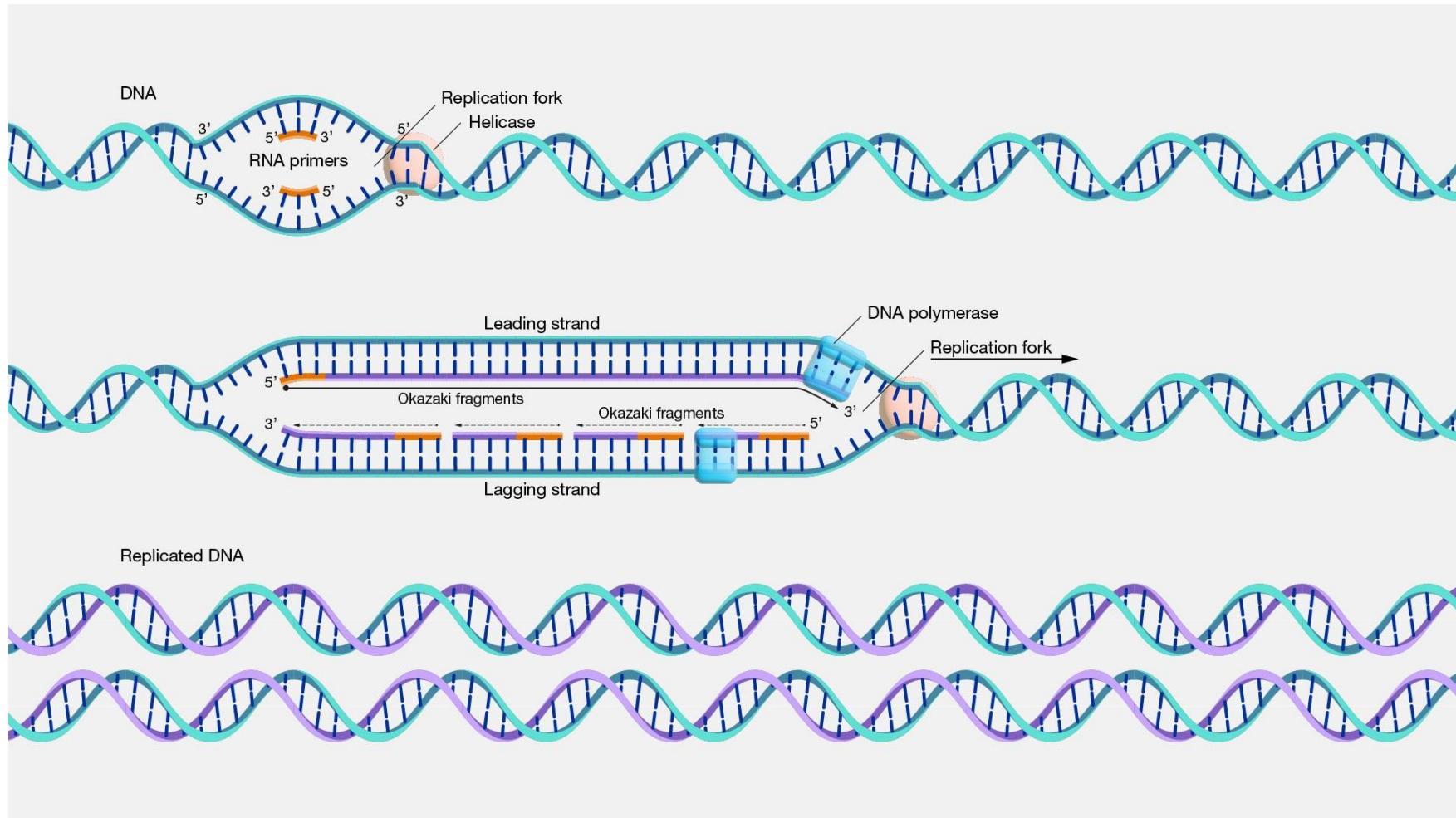


Biochemical DNA properties

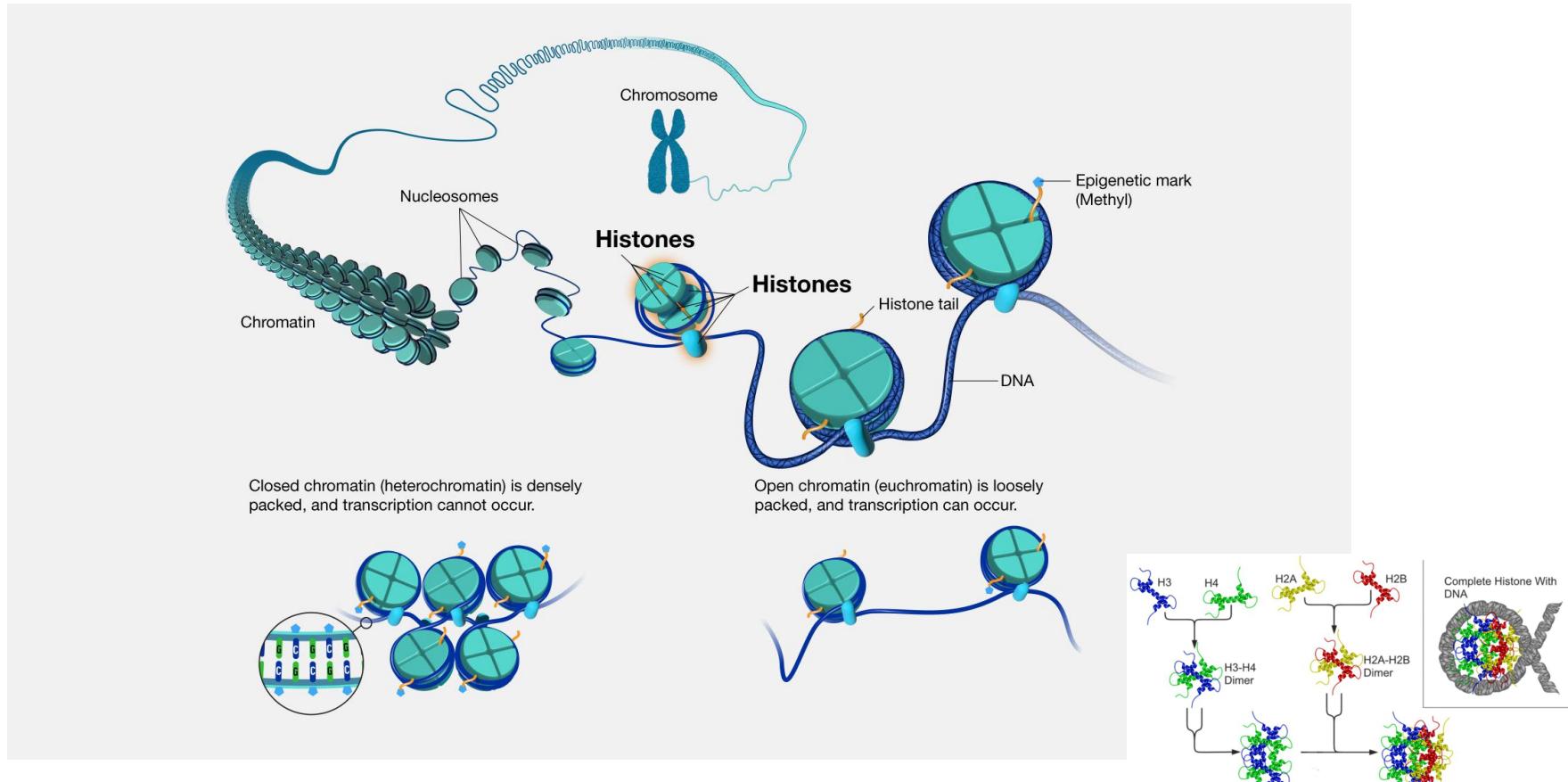
Deoxyribonucleic acid (DNA)



DNA replication



DNA in the nucleus



What are challenges during DNA extraction from plants?



DNA extraction challenges

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

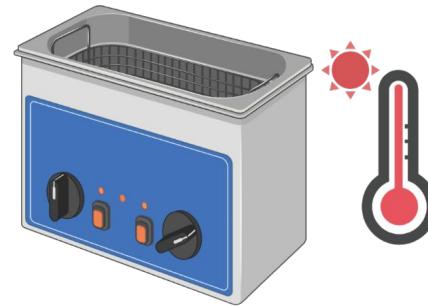
Breaking the cell wall



Mortar &
pestle



Ribolyzer



Ultrasonic
& heat

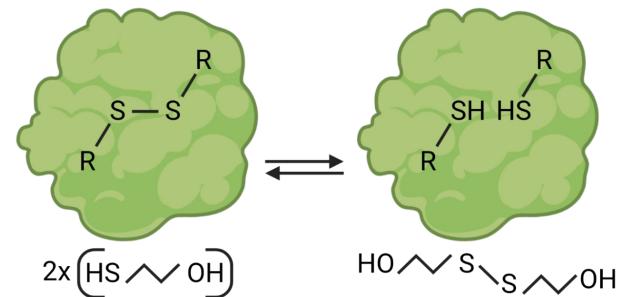
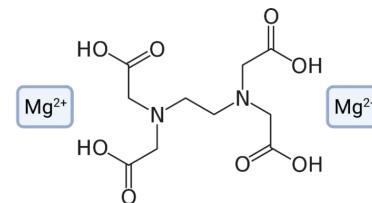


Plant cell composition - potential contaminants

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
- β -mercaptoethanol
- Proteases



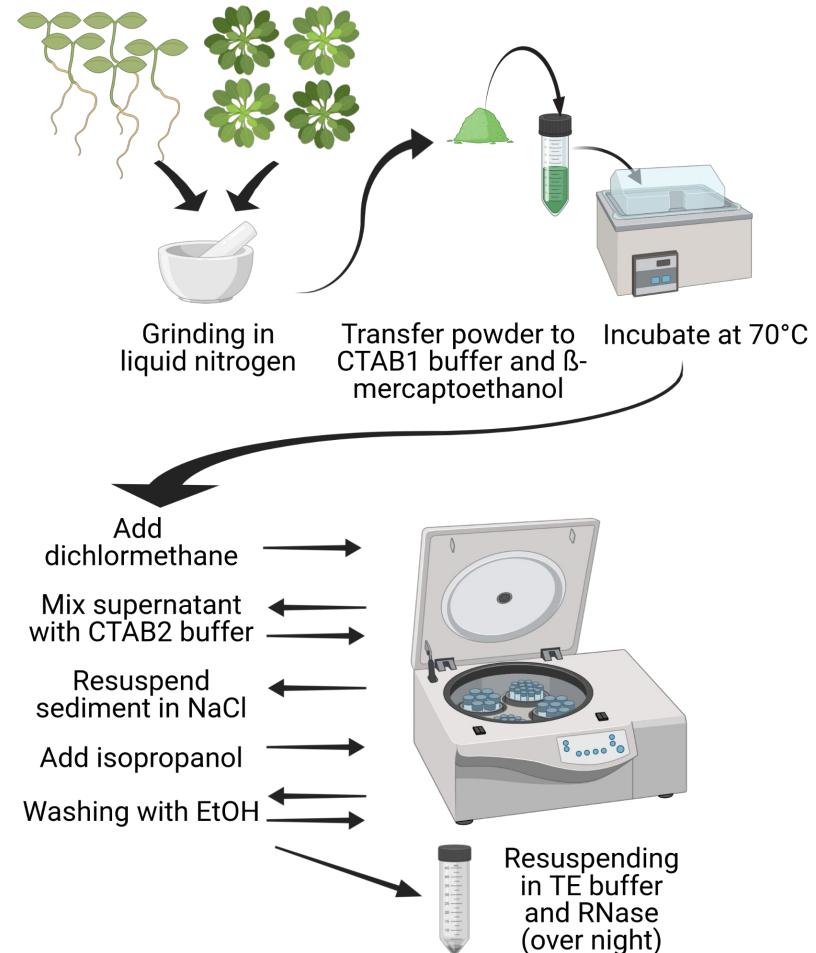
DNA extraction methods

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



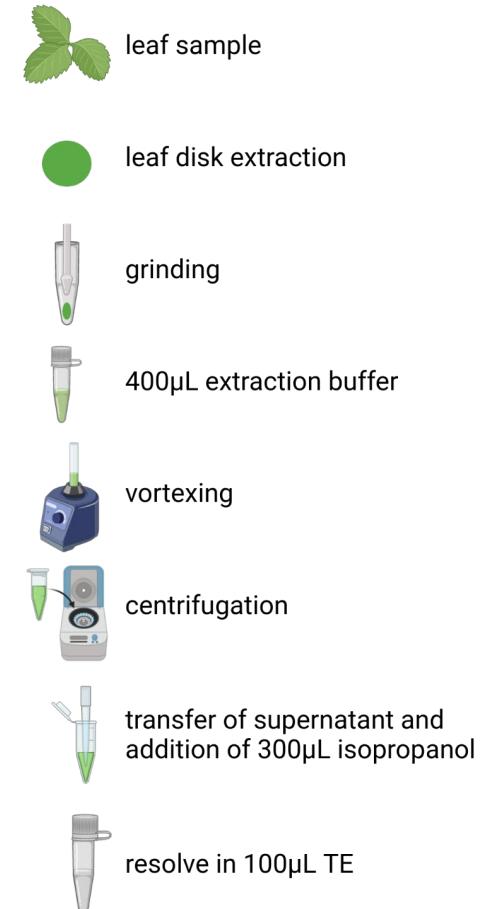
CTAB: Cetyltrimethylammonium bromide

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



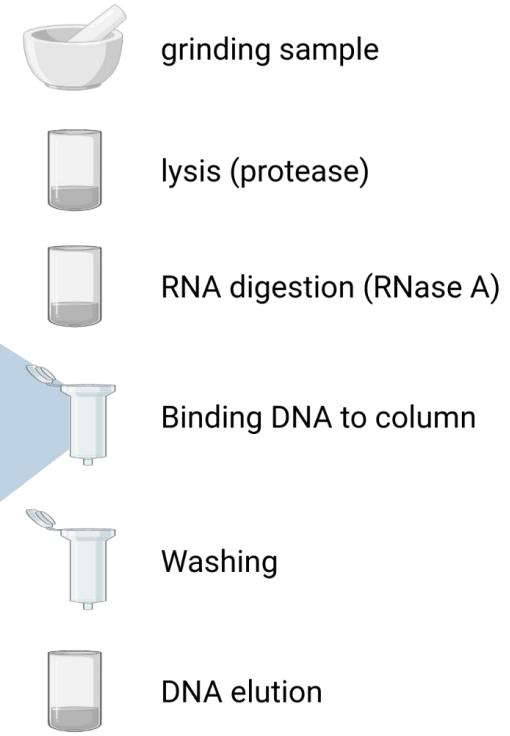
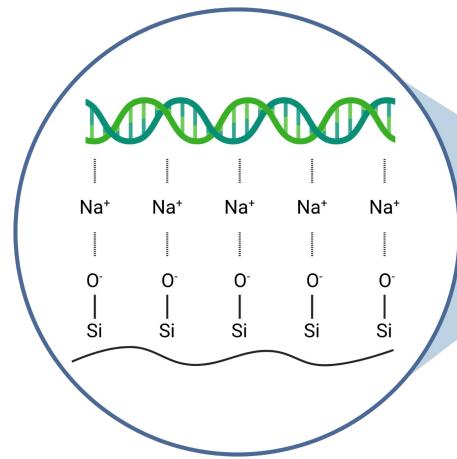
Edwards

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



Kit-based DNA extraction

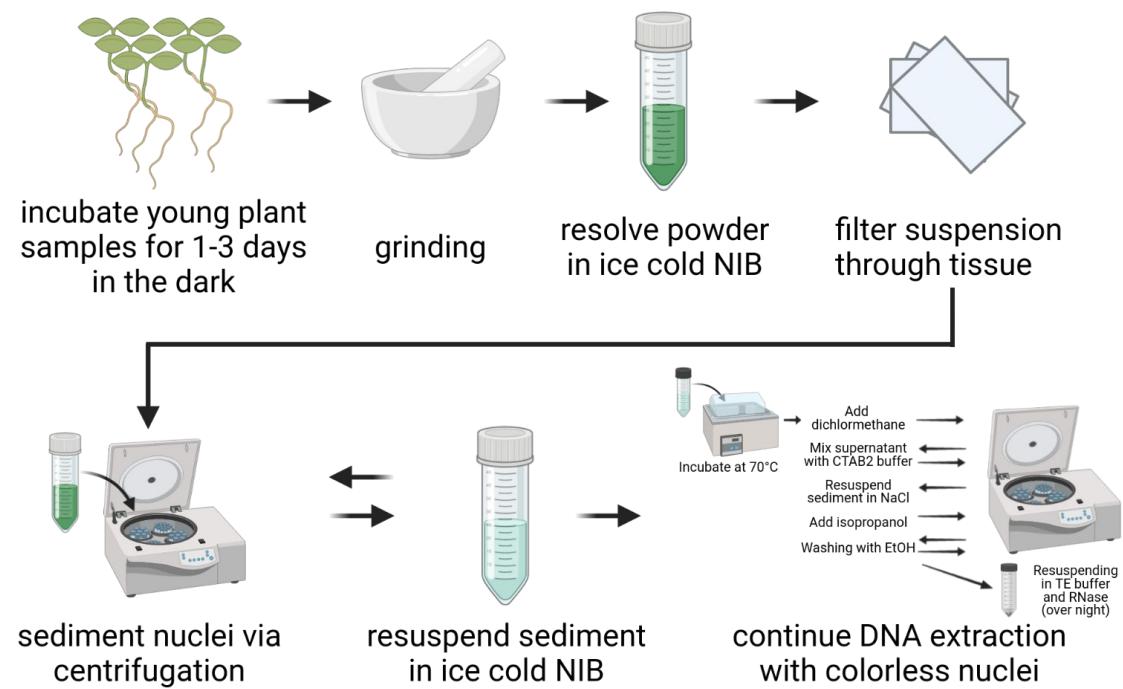
- High DNA purity
- Fragment length and total amount limited
- Modest time commitment
- High material costs



Guanidinium chloride is chaotropic agent that disrupts shell of hydration around DNA => binding to silica

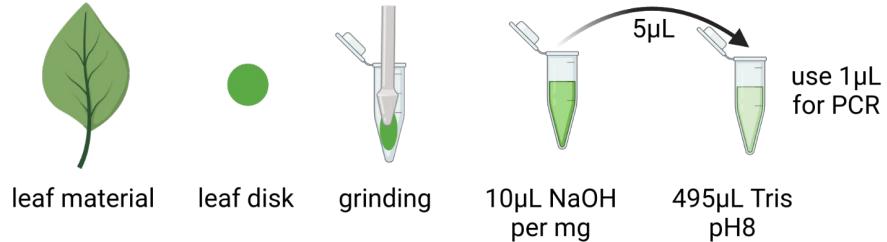
Nucleus isolation

- 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

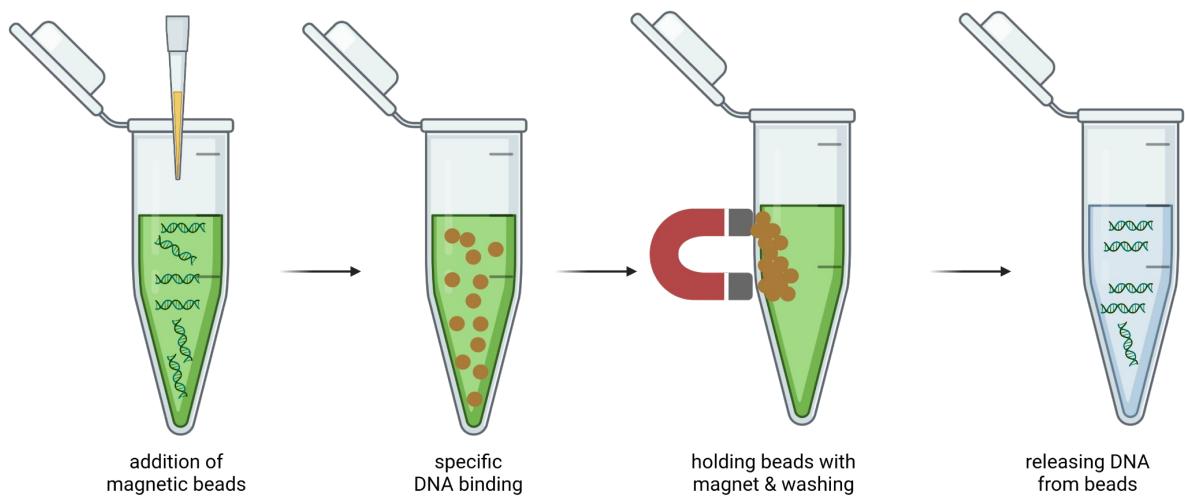


Wang et al., 1993: 10.1093/nar/21.17.4153



Magnetic beads

- High DNA quality
 - 20-30 nm in size
 - silica coated
 - compose of Fe_3O_4
- Modest time commitment
- High material costs

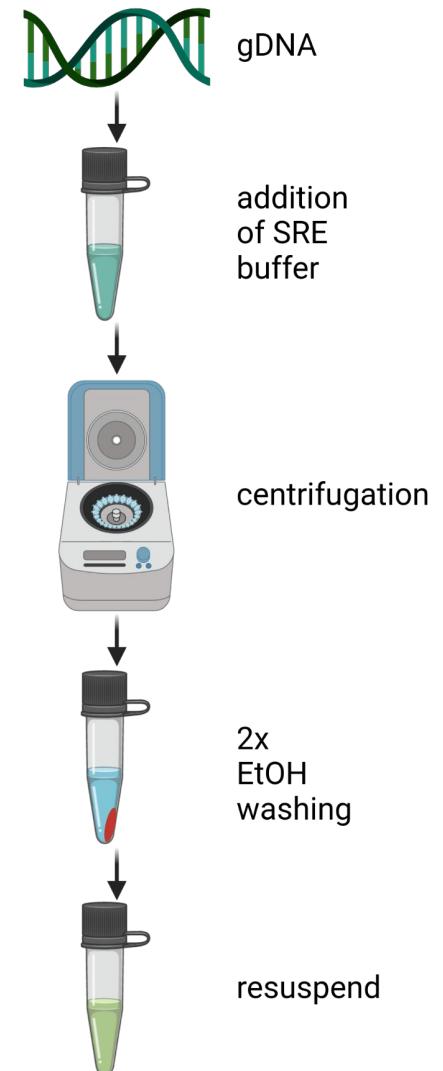


DNA precipitation

- 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

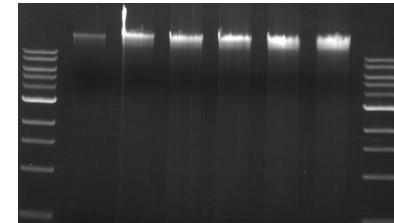
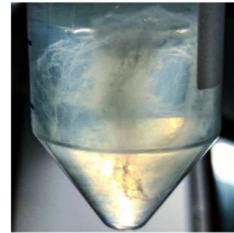


How to assess the DNA quality?



DNA quality check

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



Optical check

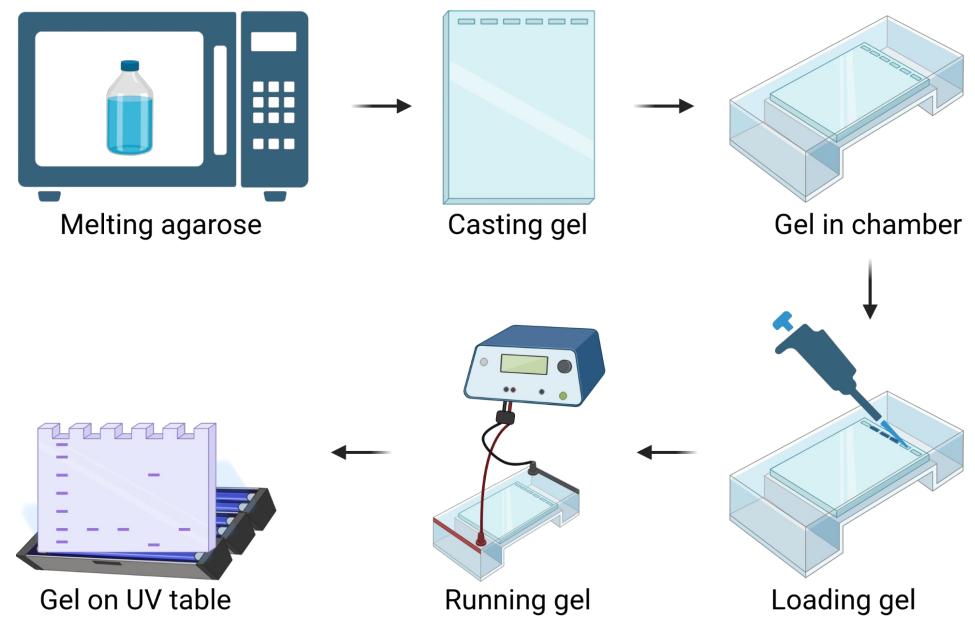
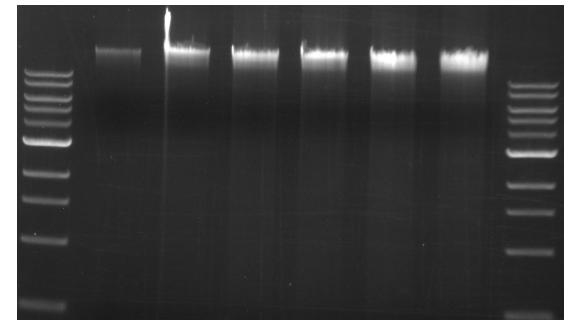
- DNA can be seen in precipitation steps
- Presence of sediment can be checked after centrifugation steps
- White color is often caused by contaminants
- Perfectly clean DNA is almost not visible



(photo credit: Hanna Schilbert)

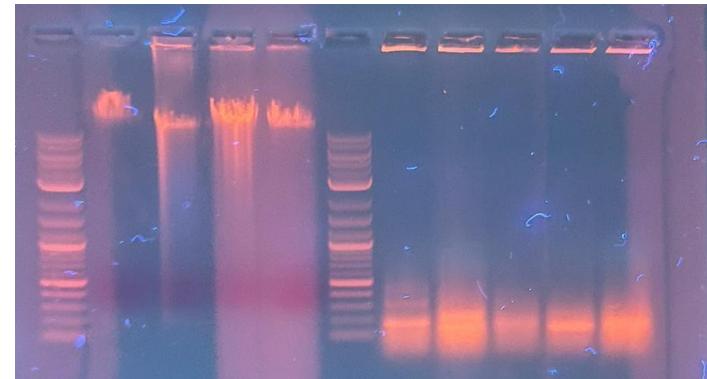
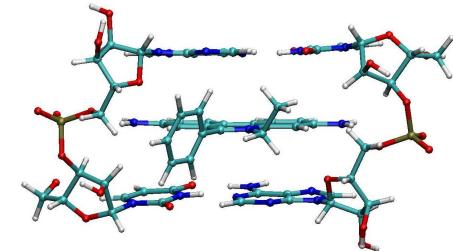
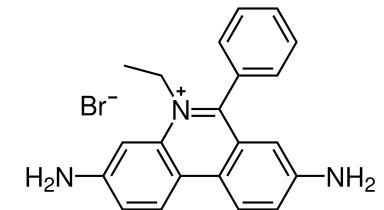
Agarose gel

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



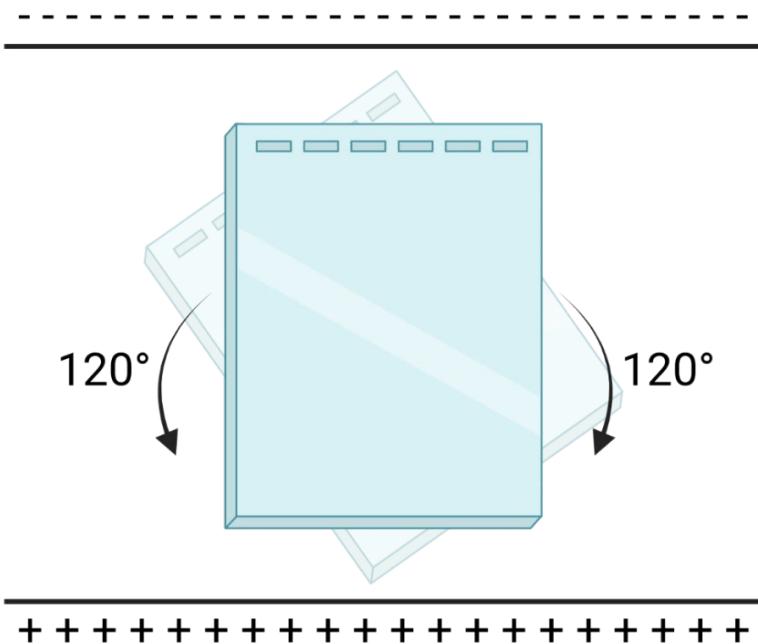
Ethidium bromide and alternatives

- Mutagenic substance due to intercalation
- Impact on humans/mammals not clear
- Disposal of EtBr remains controversial subject
- Alternatives: SYBR dyes, GelRed



(photo credit: Benjamin Harder)

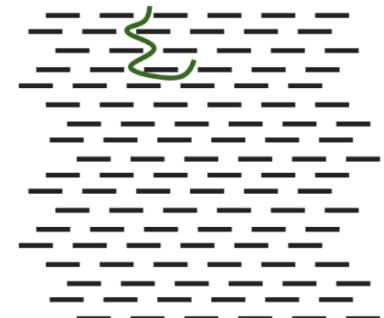
Pulse Field Gel Electrophoresis (PFGE)



DNA path through gel

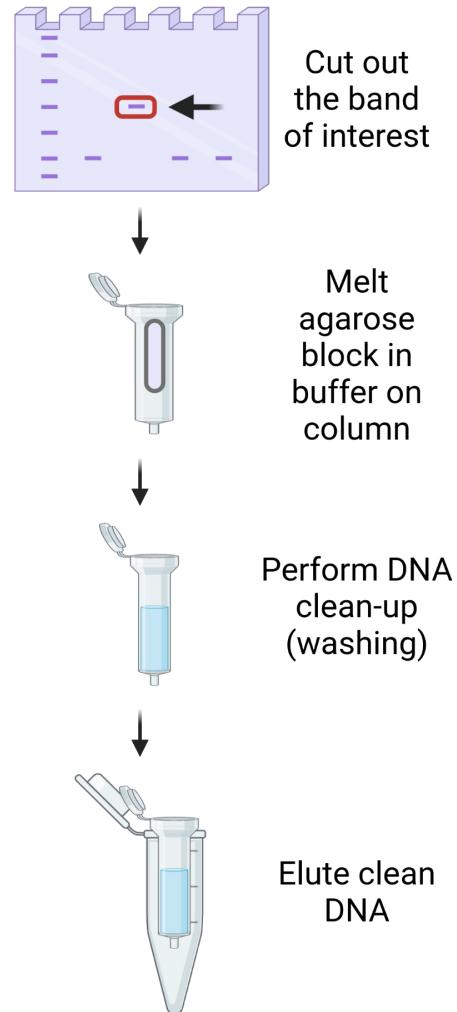


Concept of PFGE

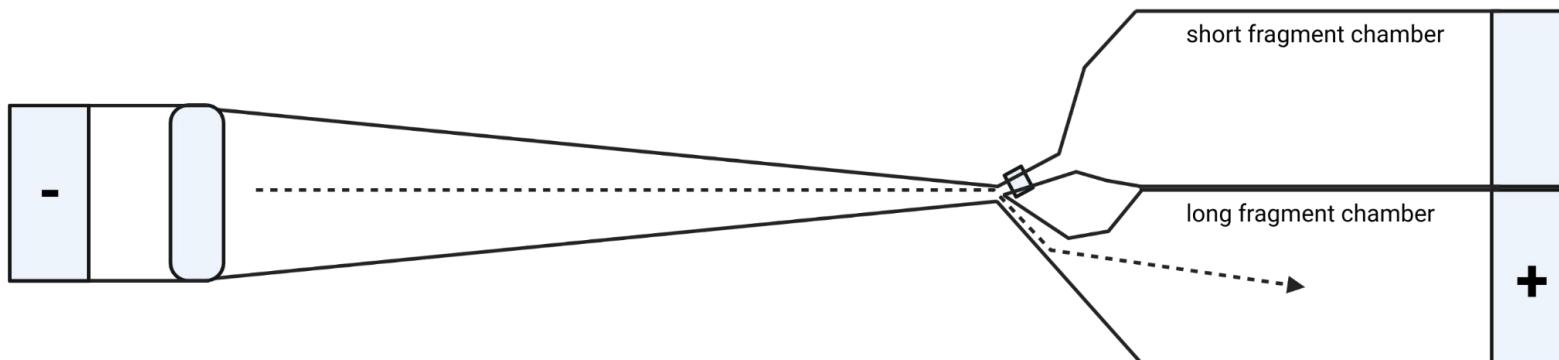
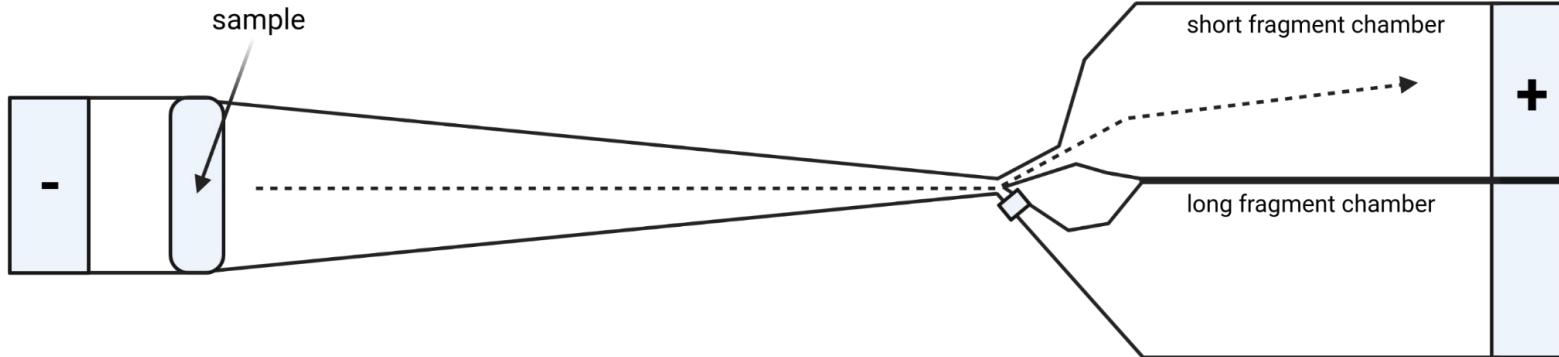


DNA extraction from gel

- Identification of correct band in gel
- Cut out the band within an agarose block
- Melt agarose block to release DNA
- Purify DNA on silica column

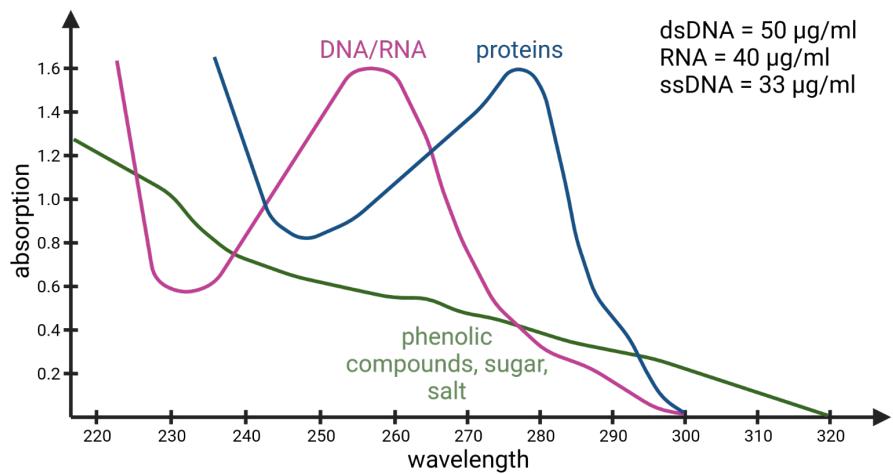


Blue Pippin



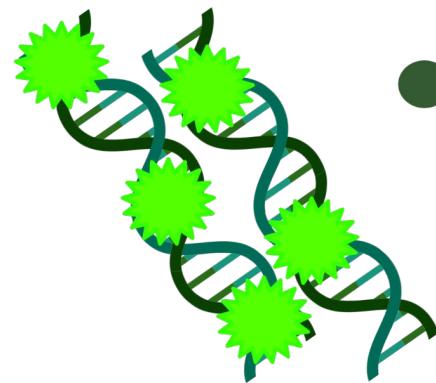
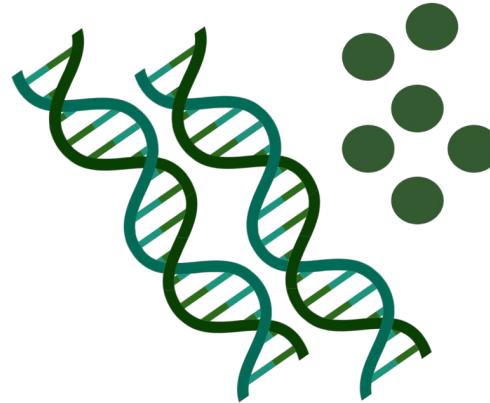
NanoDrop

- 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



Qubit

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



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?