

# DNA Extraction

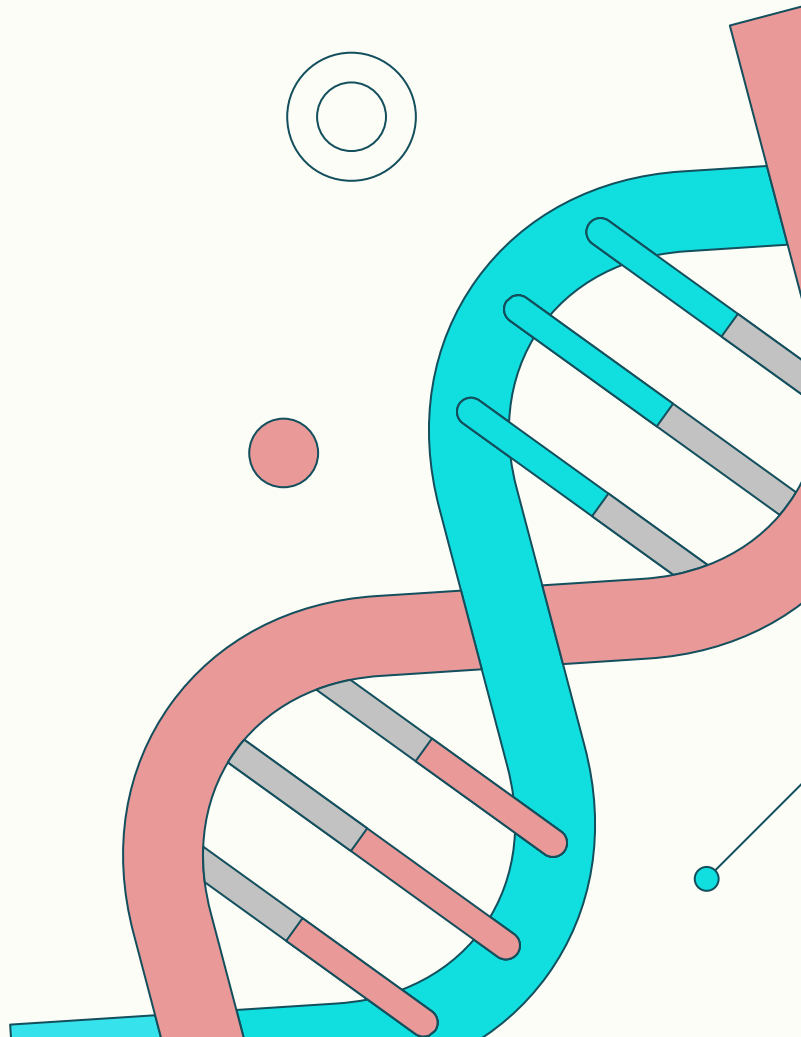
## from isolates or environmental samples

Miguel Simões, Emilie Maillard



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# Introduction



# Introduction

## Why extract DNA ?

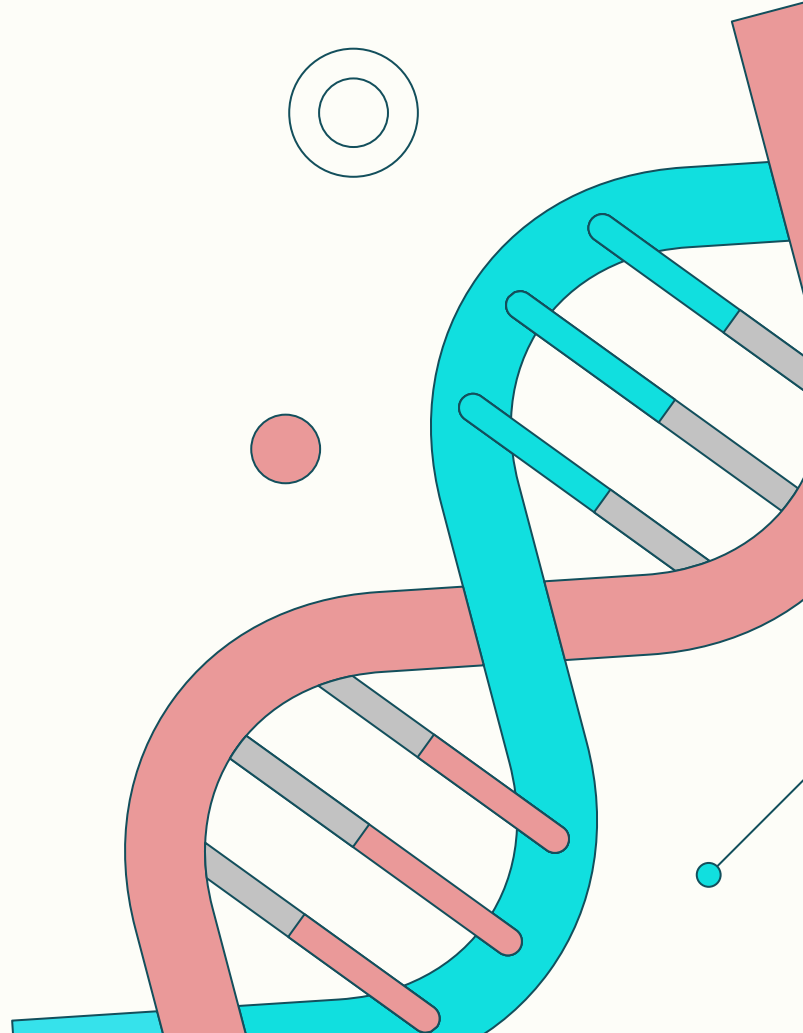
- Biodiversity monitoring
- Detection of rare / invasive species
- Obtaining genetic material to identify and study organisms
- Measuring diversity, detecting genes of interest [1]

Environmental samples	Isolates (pure culture)
→ Complex mixture of many organisms	→ Single organism
→ Presence of inhibitors	→ Clean biomass
→ Low biomass = low DNA yield	→ High DNA concentration



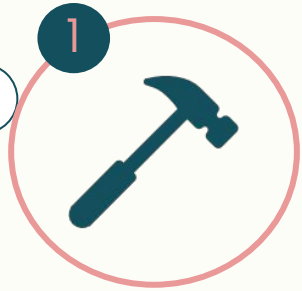
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# Scientific principle



# Scientific principle

## Overview



Cell lysis



Clearing of lysate



DNA binding



Washing



Elution

# ○ Scientific principle

## Cell lysis



**Objective:** Break down the cell wall and membrane to release the DNA [2]

Methods	Function	Advantage	Limit
<b>Mechanical (bead-beating)</b>	Break down wall/membrane	Effective against resistant cell	Can fragment DNA
<b>Chemical (SDS, CTAB)</b>	Solubilize membrane	Easy, works on many cells	Slow, may co-extract inhibitors
<b>Enzymatic (lysozyme, proteinase K)</b>	Dissolve membranes	Preserve DNA	Costly

# Scientific principle

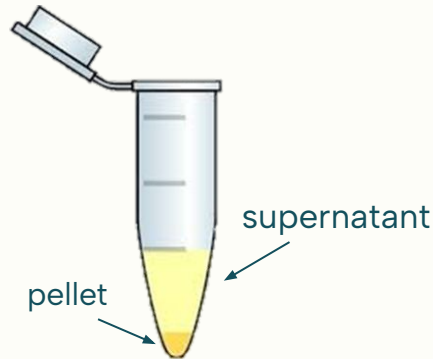
## Clearing of lysate



**Objective:** Remove cellular debris and protein from lysate [3]

→ Prevents column clogging and improves DNA purity

Centrifugation	Filtration	Precipitating agents
Pellet debris DNA stays in supernatant	Remove solid particles	Remove proteins & inhibitors

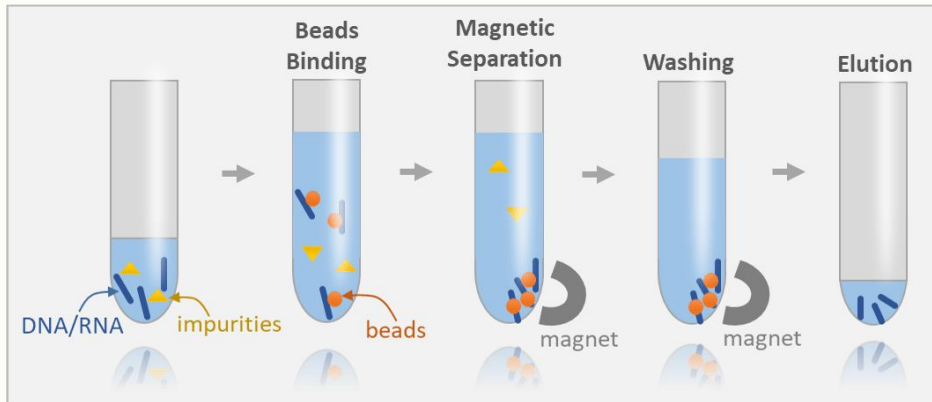


# Scientific principle

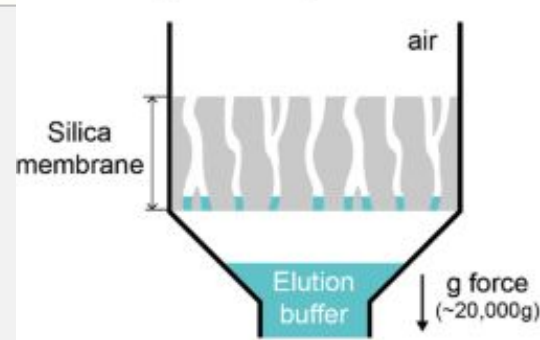
## DNA binding

**Objective:** Bind DNA to matrix while leaving contaminants in the solution

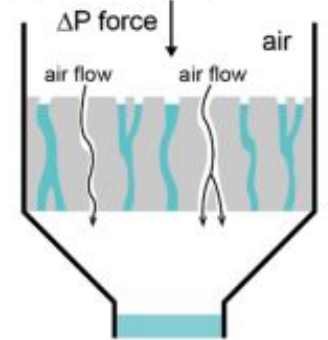
- In presence of chaotropic salts, DNA loses hydration and bind to silica or magnetic beads [4]



with magnetic bead



with centrifugation



with vacuum

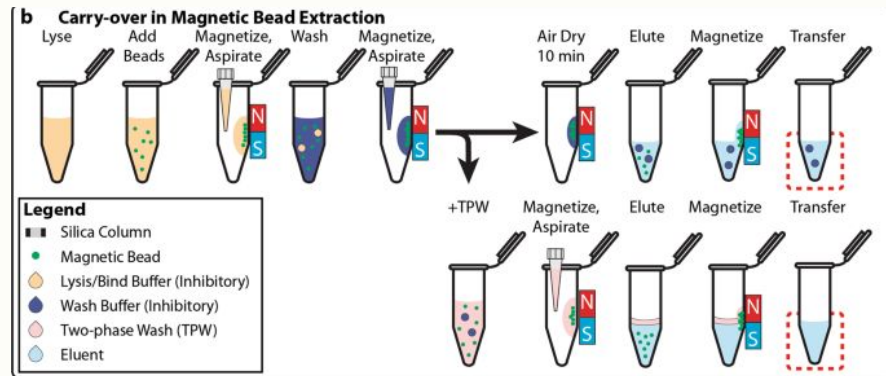
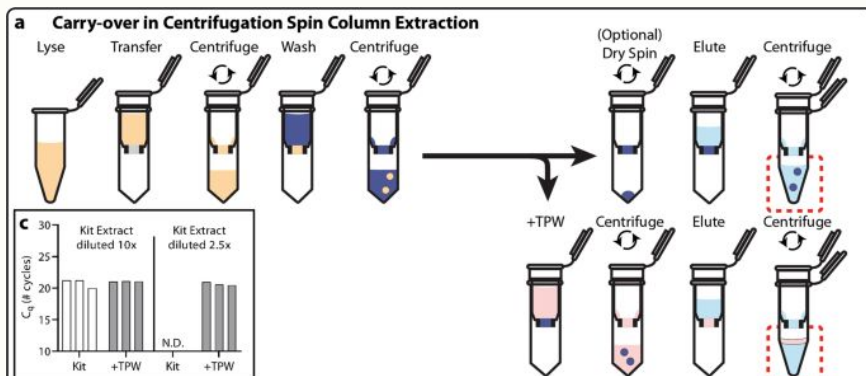
# Scientific principle

## Washing



**Objective:** Remove all contaminants while keeping the DNA fix to the matrix

- Wash buffers (ethanol-based) removes salts, protein and inhibitors [5]
- Multiple washes improve DNA purity



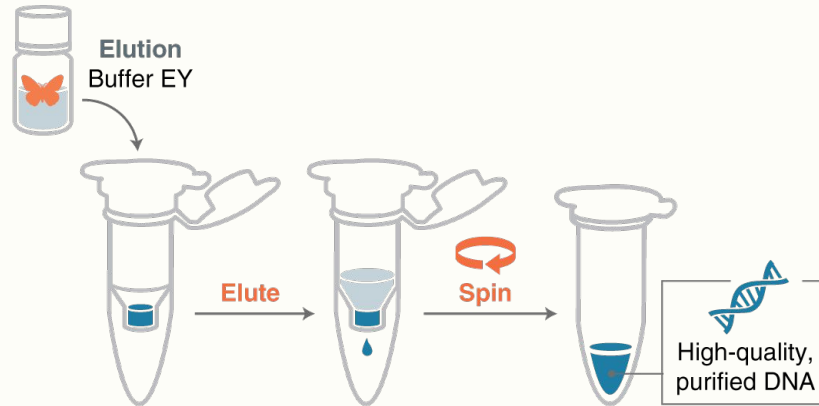
# Scientific principle

## Elution



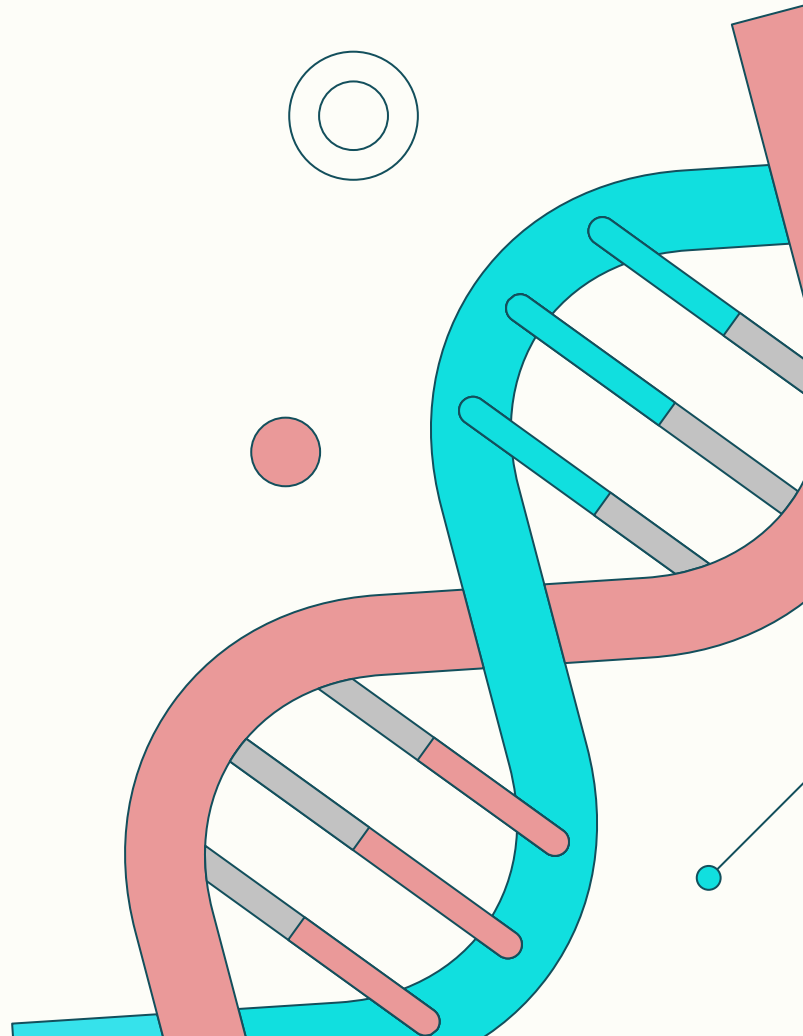
**Objective:** Release DNA from the matrix and collected it in a clean buffer

- Elution buffer (low salt or water) breaks interaction between matrix and DNA [6]



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# DNA quality



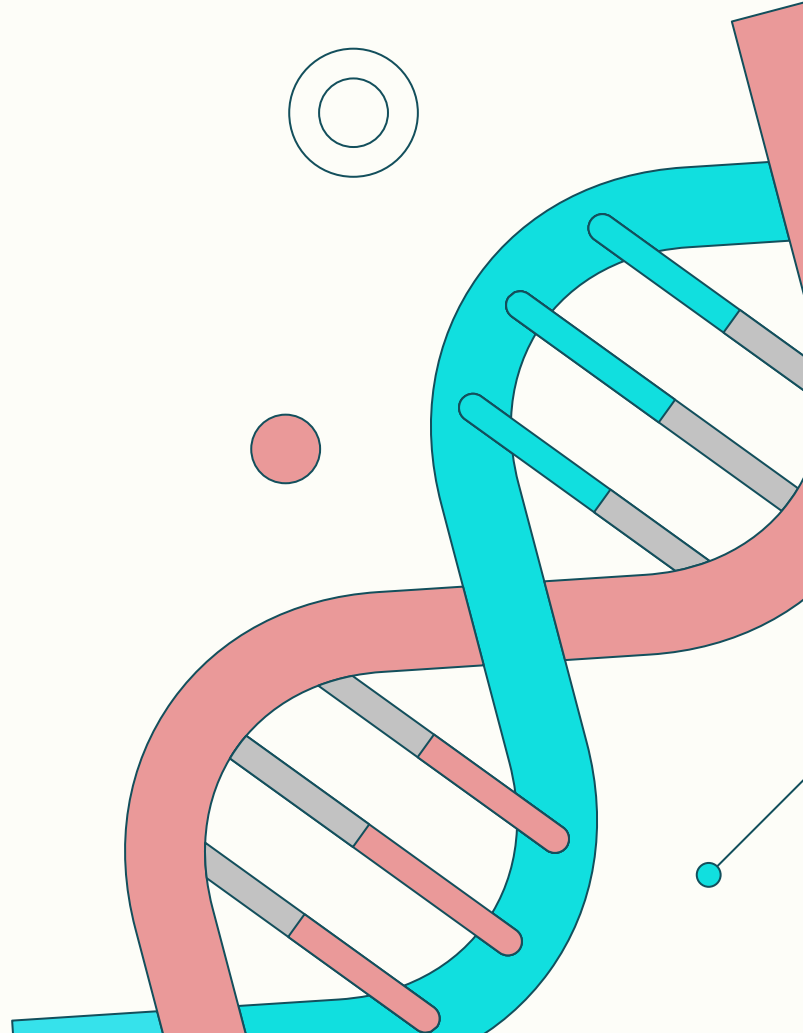
# Assessing DNA quality

Features	Method	Measurement
<b>Concentration</b>	Qubit fluorometer	dsDNA measurement
<b>Purity</b>	NanoDrop	Ratio 260/280 $\approx$ 1.8 Ratio 260/230 $\approx$ 2.0-2.2
<b>Integrity</b>	Agarose gel	Check for fragmentation
<b>Absence of inhibitors</b>	PCR test	



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# Applications in research






## DNA extraction from **Isolates**:

- Medical diagnostics & research:  
Vaccine development
- Forensics:  
DNA profiling for criminal investigation
- Biotechnology:  
Gene cloning
- Agriculture:  
Genetic modification

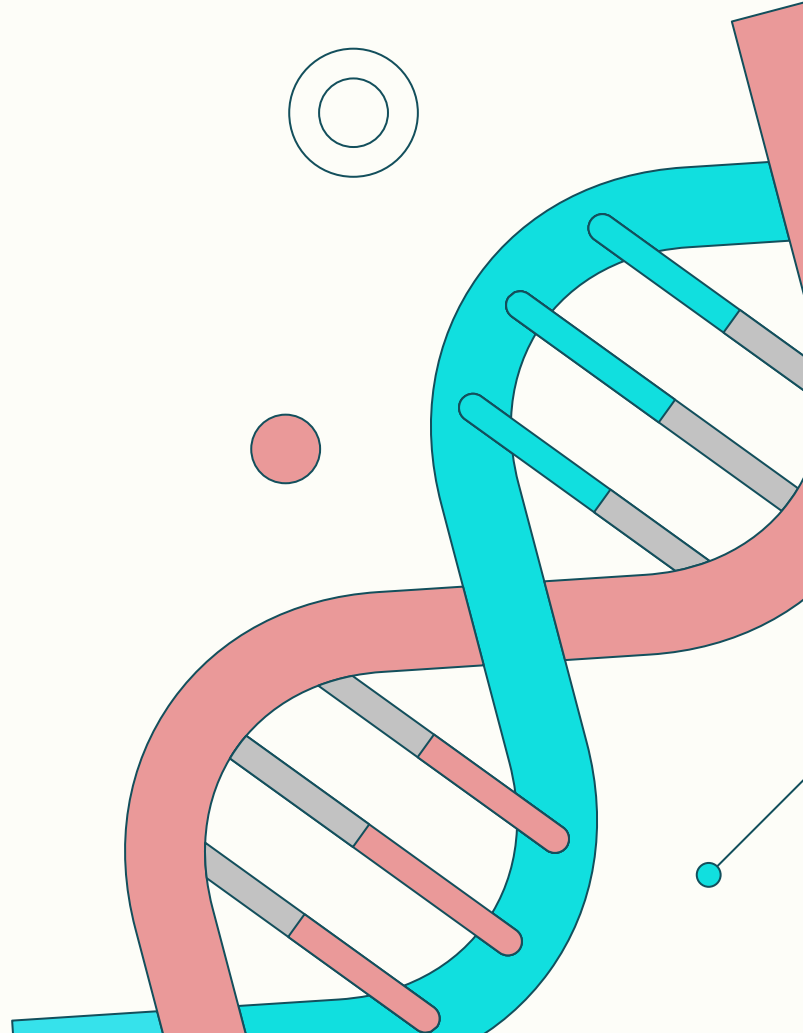


## DNA extraction from **Environment**:

- Microbial ecology:  
Studying unculturable microbes
  - Paleontology:  
Analyzing ancient DNA
  - Biodiversity monitoring:  
Species detection
  - Bioremediation:  
Genetic pollution degradation
- 

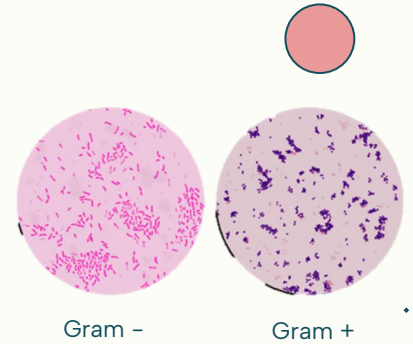
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# Limitations



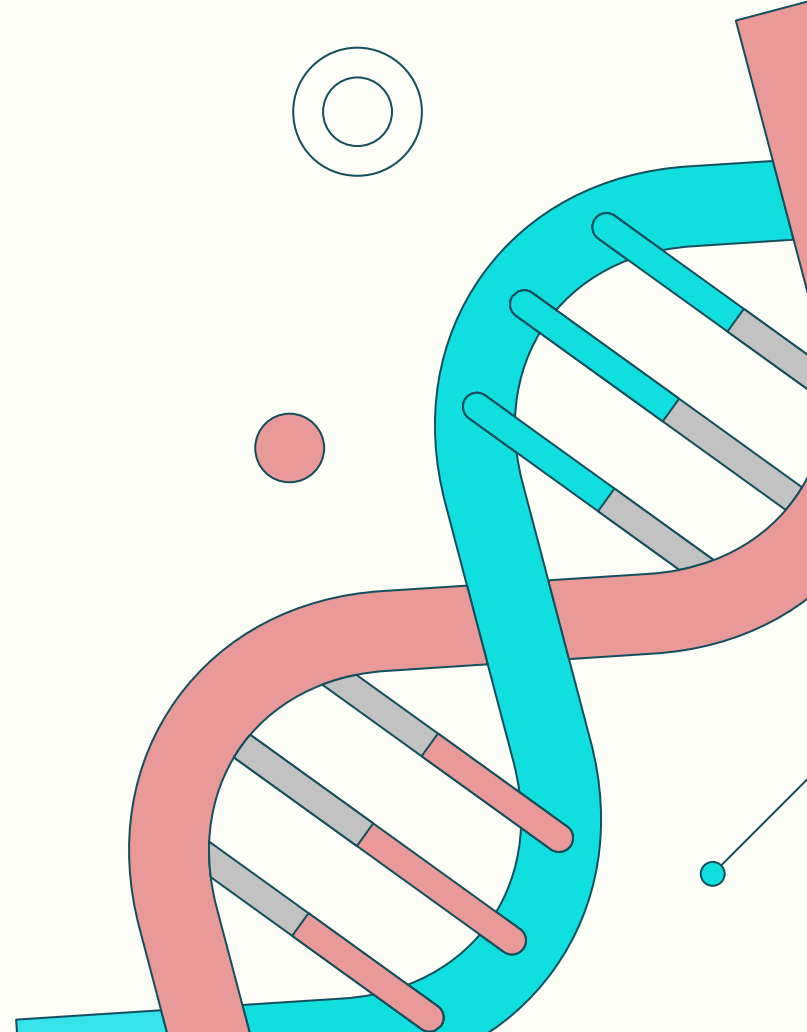
# Limitations

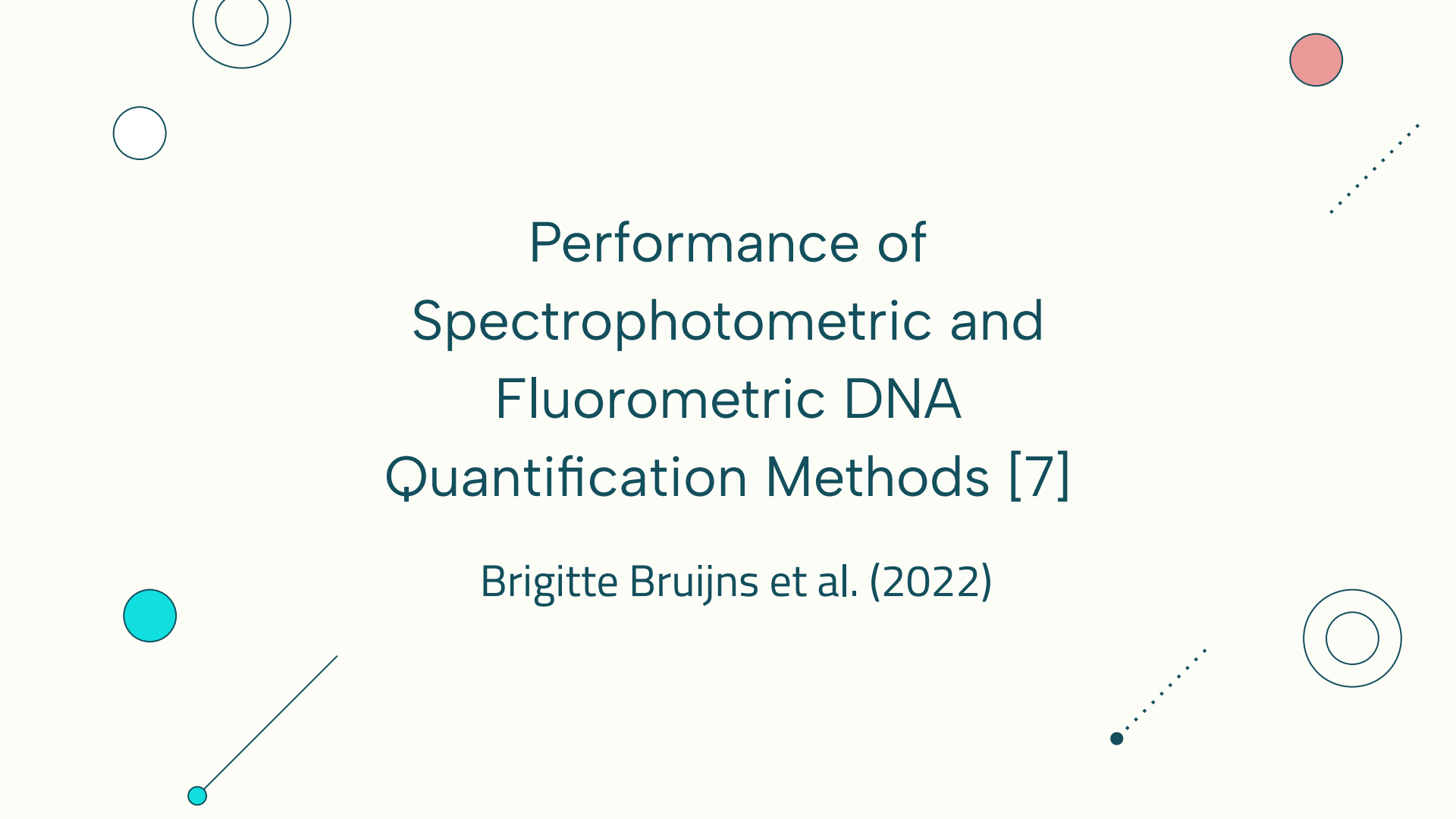
- **Lysis bias:** resistant microbe may not lyse
- **Co-extraction of inhibitors (humic acids, polyphenol)**
- **Extracellular DNA:** may distort community profile
- **DNA fragmentation:** mechanical lysis can break DNA
- **Low quantity in certain environments**
- **Variability between kits and protocols:** kit-dependent results



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# Scientific article





# Performance of Spectrophotometric and Fluorometric DNA Quantification Methods [7]

Brigitte Bruijns et al. (2022)

# Introduction

**Why?** Quantification important when little DNA available (forensics, clinical setting)

## Expectations?

Spectrophotometry measurements >  
Fluorometry measurements

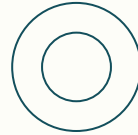
**Goal?** Comparing different quantification methods and analyst's influence



<https://clpmag.com/diagnostic-technologies/single-dna-test-can-screen-over-50-genetic-diseases/>



# Methods/Materials



7 different **DNA samples** containing 10ngDNA/ $\mu$ L:

Qubit, AccuGreen, AccuClear, TaqMan, Salmon, Herring, Jurkat, MilliQ.

3 different **analysts** (in triplo measurements)

**Spectrophotometric DNA quantification:** Nanodrop

**Fluorometry DNA quantification:** AccuGreen high Sensitivity Kit

AccuClear Ultra High Sensitivity Kit

Qubit dsDNA HS Assay Kit

**Statistical analysis:** one-way ANOVA ( $\alpha = 0.05$ )




Factors: "Analyst", "Method", "Analyst x Method"



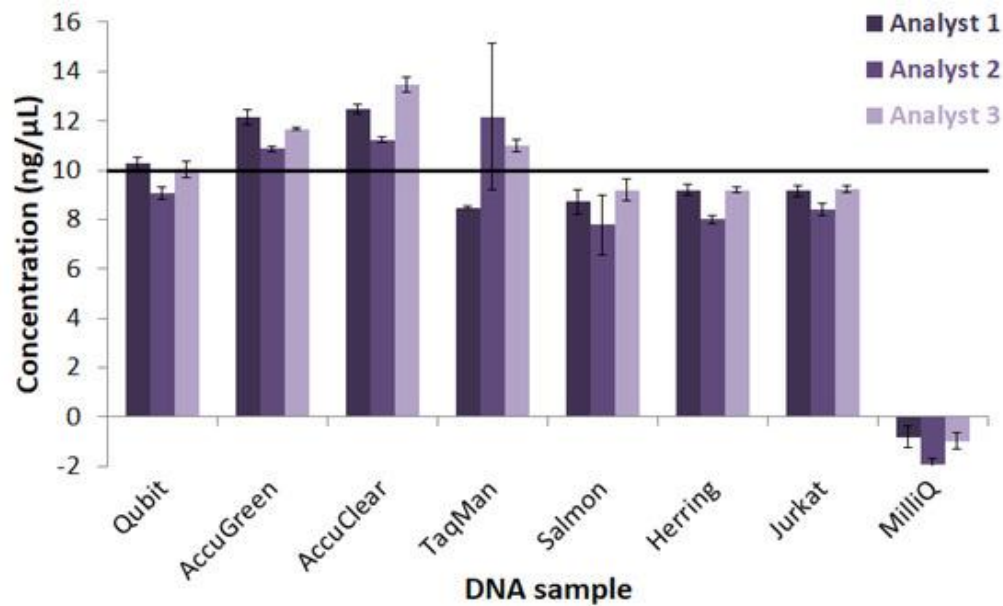


# Results: Spectrophotometry

<i>Sample\Ratio</i>	<b>260/280 nm</b>	<b>260/230 nm</b>
<b>Fish Samples</b>	1.7 - 2	>1.5
<b>Remaining Samples</b>	>2	<0.5

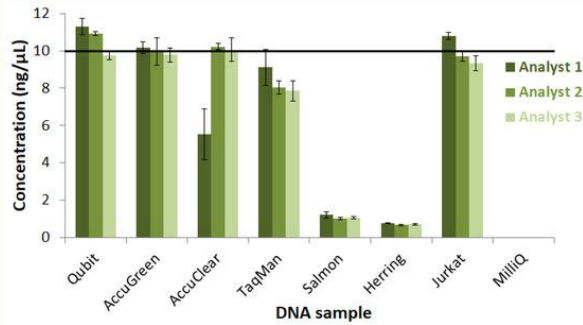


# Results: Spectrophotometry

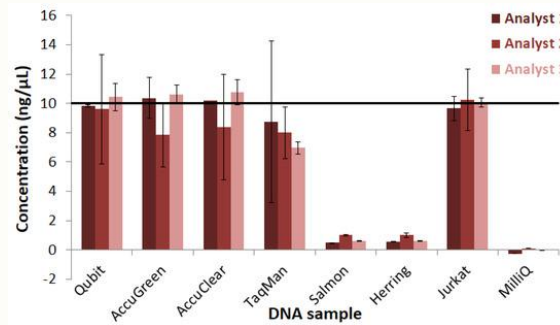


# Results: Fluorometry

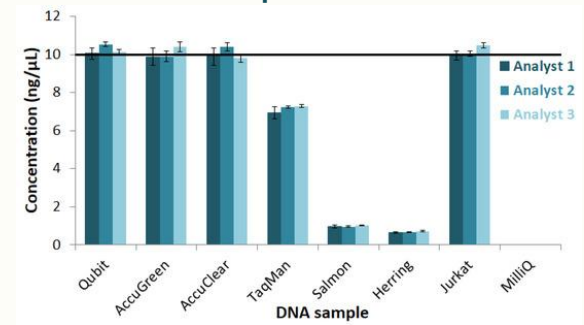
## AccuGreen



## AccuClear



## Qubit

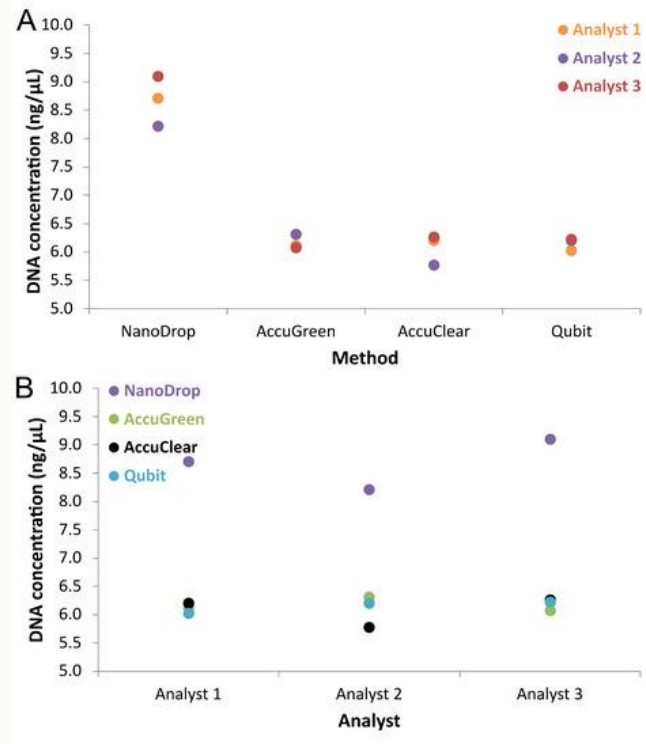


# Statistical Analysis

Analyst: Non-significant difference

Analyst x Method: Non-significant difference

Method: Significant difference



# Conclusion/Discussion

Spectrophotometry: + Simpler (no calibration), faster.  
- non-specificity, measures everything that absorbs 260nm, and no distinction between (dsDNA, ssDNA, RNA)

Fluorometry: + All fluorometry-analyzed DNA samples correct.  
+ Exact dsDNA measurements  
- more preparation, create standard curve (Qubits standard curve may be imprecise, AccuClear better)  
- Slow (except for AccuClear, can do 96 samples at a time)  
- No purity measurements.

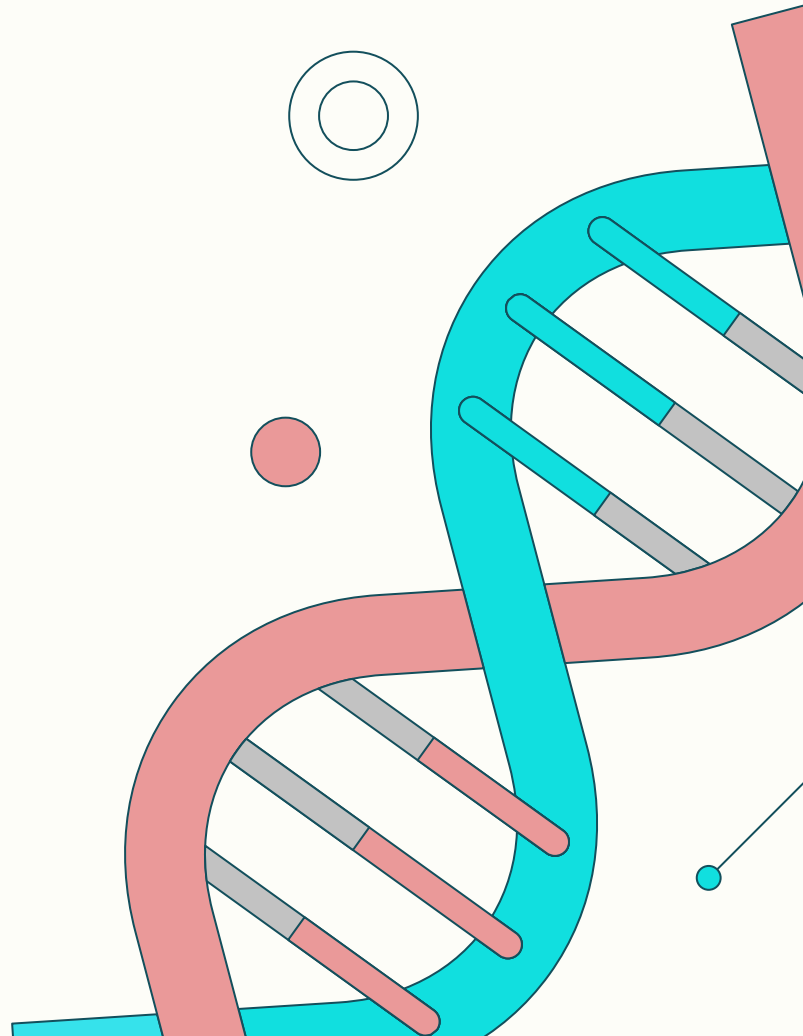
Fish DNA: only 10% of DNA is dsDNA (same as tested in other paper)

Measurements: Large standard variations, so in triplo better

Conclusion: For purity and a dsDNA concentration measurement use both methods!

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# Conclusion





# Conclusion

## Why is DNA extraction important ?

- Forensics
- Medical Research
- Paleontology

## How does it work?

Lyse → Clear → Bind → Wash → Elute

## Limitations?

Difficulties in lysing, extracellular DNA, DNA fragmentation





# Conclusion (Paper Takeaways)

In triplo measurements needed

Use both quantification methods for more complete results

Incoherence: 260/280 nm ratio states fish samples are pure dsDNA  
Fluorometry/Spectrophotometry states fish samples are not pure





# References

- [1] Promega. (n.d.). *DNA Purification: DNA Extraction Methods*. Retrieved December 11, 2025. Available at: <https://ch.promega.com/resources/guides/nucleic-acid-analysis/dna-purification/>
- [2] Rieder, J., Jemmi, E., Hunter, M. E., & Adrian-Kalchhauser, I. (2024). *A Guide to Environmental DNA Extractions for Non-Molecular Trained Biologists, Ecologists, and Conservation Scientists*. *Environmental DNA*, 6, e70002. Available at: <https://boris-portal.unibe.ch/server/api/core/bitstreams/b915e30d-701a-4033-ba89-877d05895a7b/content>
- [3] Robe, P., Nalin, R., Capellano, C., Vogel, T. M., & Simonet, P. (2003). Extraction of DNA from soil. *European Journal of Soil Biology*, 39(4), 183–190. Available at: <https://www.sciencedirect.com/science/article/pii/S1164556303000335>
- [4] Bairoliya, A., Smith, J., & Kumar, R. (2022). *Extracellular DNA in environmental samples: Occurrence, extraction, quantification, and impact on microbial biodiversity assessment*. *Environmental Microbiology Reports*, 14(6), 910–926. Available at: <https://www.sciencedirect.com/science/article/pii/S0304389423000250>
- [5] Jue, E., Witters, D., & Ismagilov, R. F. (2020). *Two-phase wash to solve the ubiquitous contaminant-carryover problem in commercial nucleic-acid extraction kits*. *Scientific Reports*, 10, 1940. Available at: <https://www.nature.com/articles/s41598-020-58586-3>
- [6] Gibson, J. (2025, March 10). *Essential Tips for PCR & DNA Cleanup: Maximize Your DNA Purity and Yield*. NEBinspired Blog. New England Biolabs. Available at: <https://www.neb.com/en/nebinspired-blog/essential-tips-for-pcr-and-dna-cleanup-maximize-your-dna-purity-and-yield?srsId=AfmBOoo2VICV0cZqvfVvZIO6TKa4cITVn7H7zbtM8UGLV4uJNxcSmp8w>
- [7] Bruijns, B., Hoekema, T., Oomens, L., Tiggelaar, R., & Gardeniers, H. (2022). *Performance of spectrophotometric and fluorometric DNA quantification methods*. *Analytica*, 3(3), 371–384. Available at: <https://www.mdpi.com/2673-4532/3/3/25>



# Appendix

**Table 1.** DNA concentrations (in ng/ $\mu$ L) of the samples as measured by the three analysts with all four quantification methods.

Sample	Spectrophotometric						Fluorometric					
	Nanodrop			AccuGreen			AccuClear			Qubit		
	1	2	3	1	2	3	1	2	3	1	2	3
Qubit (Q)	10.3 $\pm$ 0.3	9.1 $\pm$ 0.2	10.0 $\pm$ 0.3	11.3 $\pm$ 0.4	10.9 $\pm$ 0.1	9.8 $\pm$ 0.2	9.8 $\pm$ 0.1	9.6 $\pm$ 3.7	10.4 $\pm$ 1.0	10.0 $\pm$ 0.3	10.5 $\pm$ 0.1	10.1 $\pm$ 0.1
AccuGreen (AG)	12.1 $\pm$ 0.3	10.9 $\pm$ 0.1	11.7 $\pm$ 0.1	10.2 $\pm$ 0.3 <sup>a</sup>	10.0 $\pm$ 0.7	9.8 $\pm$ 0.4	10.4 $\pm$ 0.4	7.9 $\pm$ 2.2	10.6 $\pm$ 0.6	9.9 $\pm$ 0.5	9.9 $\pm$ 0.3	10.4 $\pm$ 0.3
AccuClear (AC)	12.5 $\pm$ 0.2	11.2 $\pm$ 0.1	13.5 $\pm$ 0.3	5.5 $\pm$ 1.4	10.2 $\pm$ 0.2	10.1 $\pm$ 0.6	10.2 <sup>b</sup>	8.4 $\pm$ 3.6	10.8 $\pm$ 0.8	9.9 $\pm$ 0.4	10.4 $\pm$ 0.2	9.8 $\pm$ 0.6
TaqMan (TM)	8.5 $\pm$ 0.1	12.2 $\pm$ 3.0	11.0 $\pm$ 0.3	9.1 $\pm$ 0.9	8.0 $\pm$ 0.3	7.9 $\pm$ 0.6	8.7 $\pm$ 5.6	8.0 $\pm$ 1.8	7.0 $\pm$ 0.4	6.9 $\pm$ 0.3	7.3 $\pm$ 0.3	7.2 $\pm$ 0.1
Salmon (S)	8.7 $\pm$ 0.5	7.8 $\pm$ 1.2	9.2 $\pm$ 0.5	1.2 $\pm$ 0.2	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.5 $\pm$ 0.0	1.0 $\pm$ 0.1	0.6 $\pm$ 0.0	1.0 $\pm$ 0.1	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
Herring (H)	9.2 $\pm$ 0.2	8.0 $\pm$ 0.2	9.2 $\pm$ 0.1	0.8 $\pm$ 0.0	0.7 $\pm$ 0.0	0.7 $\pm$ 0.0	0.6 $\pm$ 0.0	1.0 $\pm$ 0.2	0.6 $\pm$ 0.0	0.6 $\pm$ 0.0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.0
Jurkat (J)	9.2 $\pm$ 0.2	8.4 $\pm$ 0.3	9.2 $\pm$ 0.2	10.8 $\pm$ 0.2	9.7 $\pm$ 0.3	9.3 $\pm$ 0.4	9.7 $\pm$ 0.8	10.2 $\pm$ 2.1	10.1 $\pm$ 0.3	9.9 $\pm$ 0.2	10.0 $\pm$ 0.1	10.5 $\pm$ 0.3
MilliQ (M)	-0.8 $\pm$ 0.4	-1.9 $\pm$ 0.3	-1.0 $\pm$ 0.3	$\leq$ 0.05	$\leq$ 0.05	$\leq$ 0.05	-0.3 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	$\leq$ 0.05	$\leq$ 0.05	$\leq$ 0.05

<sup>a</sup> n = 2. <sup>b</sup> n = 1.

Table 1: Exact measurements of scientific paper

