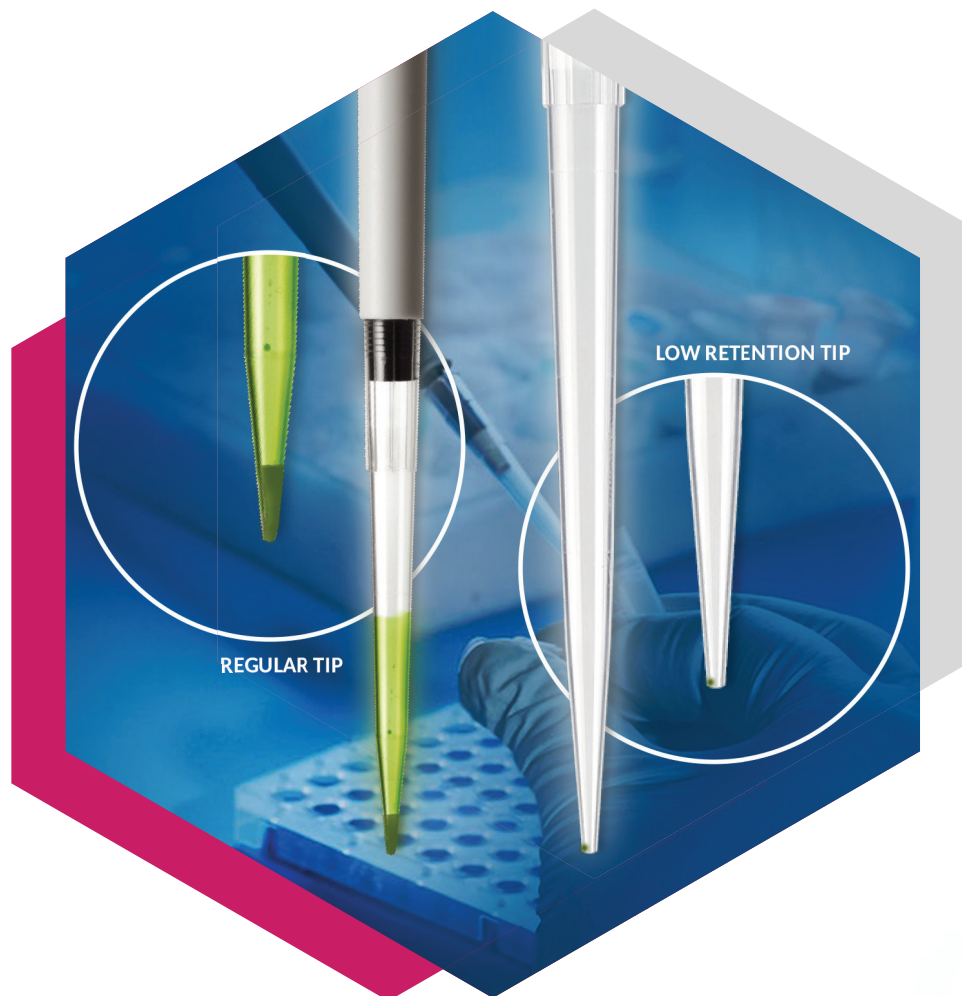




Evaluating Low Retention Tips for DNA Handling

Introduction:

In molecular biology workflows, accurate pipetting is crucial for ensuring the integrity and reproducibility of experimental results. At AHN to cater this need, pipette tips with low retention properties have been designed to minimize sample loss by preventing liquids from adhering to the tip surface. This is especially important for handling DNA samples, where maximizing recovery can directly impact applications such as PCR, sequencing, and cloning. This study aims to compare the performance of AHN low retention tips with other well-known brands.

Background

Pipetting accuracy is a key factor in DNA quantification and handling. Traditional pipette tips are made of polypropylene, a hydrophobic material that can sometimes retain small amounts of liquid. When working with precious or low-concentration DNA samples, even minimal retention can lead to inaccurate quantification, reduced reaction efficiency, and inconsistent results. This will affect the final results of gene expression assay, NGS, Epigenetics etc.

Low retention pipette tips feature surface modifications that aims to reduce the adhesion of liquids to the inner walls of the tips, thus minimizing sample loss. These tips are particularly beneficial when handling low-concentration or precious samples where any loss could compromise experimental results. Despite various claims by manufacturers, the effectiveness of low retention tips can vary significantly between brands, which necessitates a comparative study to validate their performance.

Objective

The primary objective is to compare the DNA retention properties of AHN low retention tips with other leading brands. The study aims to quantify the amount of DNA retained in each type of tip and provide insights into the effectiveness of low retention tips from different manufacturers.

Methodology

- **Selection of Pipette tips**

The study involves well-known brands low retention tips.

All pipette tips used in this study are certified DNase/RNase-free.



- **DNA sample preparation**

- DNase-free molecular grade water was used to dilute the bacterial DNA to preferred dilutions.
- The samples were thoroughly mixed to maintain homogeneity.

- **Pipette Procedure**

- Fixed volume of 200 μ L of DNA solution was aspirated and dispensed 10 times using 200 μ L tips from each brand
- Following ten aspirations, the same tips were rinsed with 25 μ L of DNase-free water to capture any remaining DNA. 5 μ L of the rinse were then transferred to a new 0.5 mL tube to measure any DNA loss or leftover DNA.

- **Residual DNA Quantification**

The amount of DNA in the rinse buffer was measured using an Invitrogen™'s Qubit™ 1X dsDNA HS Assay Kit (Cat. # Q33230) protocol.

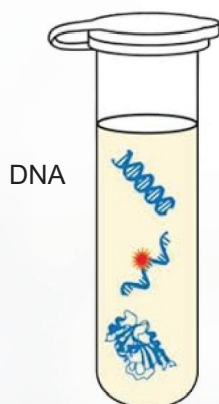
To each 0.5mL tube containing 5 μ L of rinse, 195 μ L of fluorescent buffer is added which labels the residual DNA. The solution was analyzed on Qubit 4 Fluorometer for residual fluorescent signal associated with retention of DNA solutions on the pipette tip. A reading of "Out of range" indicates a DNA concentration below 0.005 ng/ μ L.

Qubit fluorescence quantification technology



Figure 1 Qubit 4 Fluorometer

DNA dye Selectivity



Qubit™ fluorometers are designed to detect fluorescent dyes in Qubit™ assays, which are highly specific to target molecules in a given sample. For instance, the Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit (Cat. # Q33230) employs dyes that emits fluorescence only when bound to DNA, enabling highly sensitive detection, even at low concentrations. These dyes are absorbed by the DNA in the sample within minutes, and measurements can be completed in seconds. The Qubit™ fluorometer then interpolates the fluorescence readings against a pre-established standard curve.

Figure 2: Qubit™ 1X dsDNA HS assay kit - DNA dye selectivity



The fluorescent dyes in the assay bind selectively to DNA. Qubit™ fluorometers utilize advanced curve-fitting algorithms to generate a calibration curve based on standard samples with known DNA concentrations. The DNA concentration of an unknown sample is subsequently determined by comparing its relative fluorescence units (RFUs) to those of the standards.

- Data Analysis**

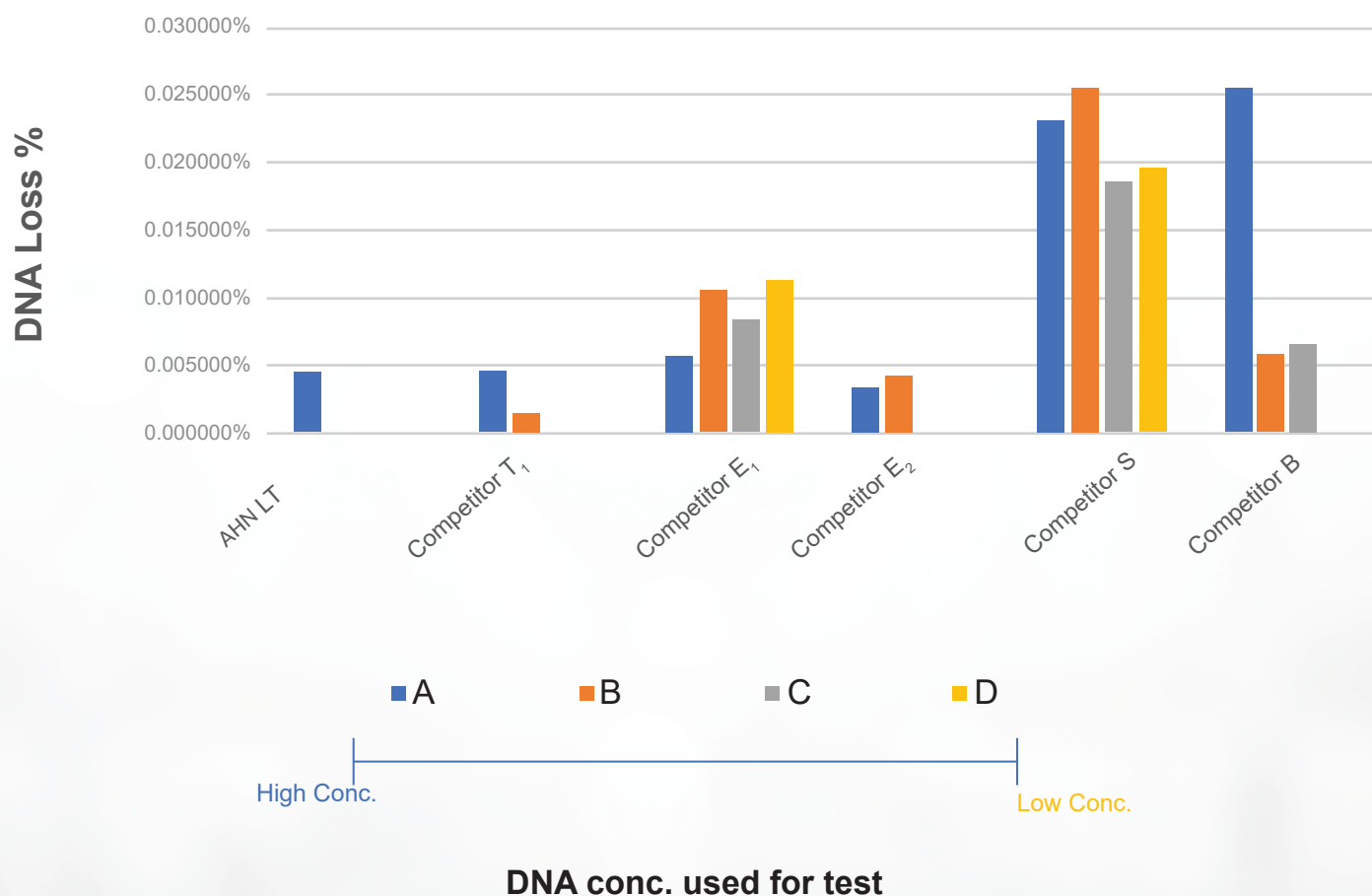
DNA retention was calculated as the percentage of the original DNA amount that remained in the tip after pipetting. This was determined using the formula:

$$\text{Percentage of DNA retained} = \frac{(\text{Amount of DNA detected in the rinse buffer}) \times 100}{\text{Original amount of DNA}}$$

Results and Discussion

- Comparison of DNA retention**

Low retention property of various brand tips- DNA Loss



Tip Brands	DNA loss in %			
	A ng/μL	B ng/μL	C ng/μL	D ng/μL
AHN LT	0.004375 %	0	0	0
Competitor T ₁	0.004575 %	0.00144 %	0	0
Competitor E ₁	0.0058 %	0.01055 %	0.00835%	0.0112 %
Competitor E ₂	0.0033 %	0.00412 %	0	0
Competitor S	0.0232 %	0.0257 %	0.018625 %	0.0197 %
Competitor B	0.0255 %	0.0059 %	0.006575 %	0

• Factors Influencing Results

Material composition: Differences in the polypropylene quality and the additives used during the manufacturing may have impacted tips performance.

Surface Treatment Variation: The effectiveness of low retention properties depended on the quality and type of surface treatment applied by each manufacturer.

Conclusion

The study demonstrated significant differences in DNA retention among the pipette tips tested. The results showed that AHN Low Retention tips demonstrates the lowest DNA loss across all concentrations, with a negligible loss of 0.0043% at High concentration and no detectable loss at lower concentration, indicating highly effective low retention properties.

Competitor E₁ and other branded tips showed substantially higher DNA loss, underscoring the benefits of low retention technology for minimizing DNA loss.

These findings suggest that low retention tips, particularly those from AHN provide superior performance for DNA, RNA and protein handling, making them valuable for applications requiring high accuracy and minimal sample loss.



Recommendations

Laboratories working with DNA, RNA, protein and other molecular/biological samples, particularly at low concentrations, should consider using low retention tips to enhance accuracy and minimize sample loss.

