# Standardized Preanalytical Stabilization of Human Saliva Prevents Genomic DNA Degradation and Allows for Detection of Rare Tumor Mutations Using dPCR

[1]Authors: Franziska Kaiser[1]✉, Lisa Erkelenz[1], Julius Albers[2], Oezlem Karalay[2], Daniel Groelz[1]

[1]PreAnalytiX GmbH, Hombrechtikon, Switzerland; [2]QIAGEN GmbH, Hilden, Germany

✉Please contact Franziska.Kaiser@qiagen.com for further questions or go to www.preanalytix.com

## Introduction

Saliva is an easy-to-use specimen that offers the possibility to examine a wide range of analytes including but not limited to human genomic DNA (gDNA). In contrast to blood sampling, whole saliva collection is non-invasive and may even be performed at home without the involvement of medical personnel. Further, saliva collection is considered as a more convenient method of collection from difficult donors such as children. As long as nucleic acid stability is maintained, the sample can be shipped to the testing laboratory at ambient temperature to avoid logistical costs for maintaining the cooling chain. Several publications show that saliva samples harbor the potential to be used in early detection of various cancer entities such as head and neck cancer or non-small cell lung cancer. *EGFR* gene mutations for example can be detected in saliva of patients with non-small cell lung carcinoma (Kaczor-Urbanowicz et al., 2017; Wei et al., 2014). When working with saliva samples, standardized preanalytical workflows are of high importance in order to reduce errors and enable reproducible and reliable analytical test results. Therefore, experiments presented here were performed according to ISO 4307:2021-10 Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for saliva – Isolated human DNA.

## Materials and Methods

Saliva was collected from consented, apparently healthy adult donors into PAXgene Saliva Collectors and in 15 ml tubes without a stabilization reagent (n = 10). PAXgene Saliva stabilized samples, as well as unstabilized samples, were stored at room temperature (RT) or at 4°C. gDNA was extracted from 200 µl of the stabilized and unstabilized saliva samples directly after collection and after between 1 hour and 14 days storage using the QIAGEN QIAamp® DNA Mini Kit manually or automated on the QIAGEN QIAcube® Connect. The gDNA samples were analyzed for human DNA quantity and degradation status using the QIAGEN Investigator® Quantiplex® Pro RGQ Kit. The Investigator Quantiplex Pro Kit detects a smaller (91 bp) and a longer amplification product (353 bp) targeting the same locus. The longer target is more susceptible to DNA degradation, which allows for the assessment of the degradation status of the DNA in form of the degradation index. The degradation index is the quantification of the small fragment divided by the quantification of the long fragment. While an ideal degradation index of 1 indicates no degradation, values above 5 indicate DNA fragments of less than 300 bp. The DNA profile was determined with the Agilent 4200 TapeStation® System using Genomic DNA ScreenTape® analysis. Artificial DNA, including non-small cell lung cancer mutations *EGFR* exon19 mutation c.2235_2249del, *EGFR* L858R point mutation in exon 21 (c.2573T>G) or *EGFR* T790M point mutation in exon 20 (c.2369C>T), were spiked in stabilized saliva samples at calculated mutation rates of 0.25%, 0.5%, 1.0%, 1.5% and 2.0%. The QIAGEN QIAcuity® Digital PCR Platform was used to detect the respective mutations and determine the mutation rate using the QIAGEN dPCR LNA® Mutation Assays in 26k nanoplates.

## Results

### Onset of human gDNA degradation directly after saliva collection in unstabilized samples.

- Human saliva gDNA degrades quickly in unstabilized samples, whereas DNA integrity is maintained in PAXgene Saliva Collector stabilized samples
- Yield determined by quantification of a 91 bp product using the Investigator Quantiplex Pro assay decreased severely in unstabilized samples stored at RT within the first four days after saliva collection (**Figure 1A**)
- Increased degradation index in the same samples (**Figure 1B**)
- gDNA yield determined by qPCR remained more stable in unstabilized saliva samples stored at 4°C in comparison to unstabilized samples at RT, however, these samples also showed an increase in degradation as detected by the degradation index (**Figure 1B**)

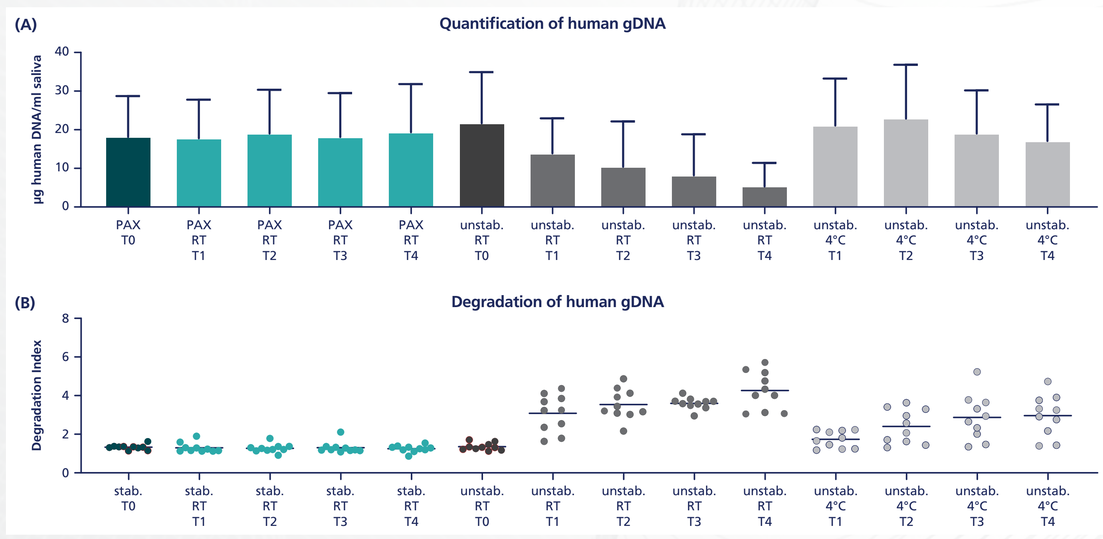


**Figure 1:** Saliva was collected from ten apparently healthy consented adult donors into PAXgene Saliva Collector for stabilization and into 15 ml tubes without stabilization reagent (n = 10). PAXgene saliva samples were kept at RT, unstabilized samples were kept both at RT and at 4°C for 4 days. Human gDNA extraction was performed directly after collection (T0) and on every following day (T1–T4) using the QIAGEN QIAamp DNA Mini Kit. Human gDNA was quantified (A) and the degradation index was determined in stabilized samples (green) and unstabilized samples (dark gray – RT; gray – 4°C) using Investigator Quantiplex Pro RGQ Kit.

- TapeStation Analysis revealed that after only 4 hours, the DNA profile in unstabilized samples was affected and further deteriorated over storage time whereas DNA profile was maintained in PAXgene Saliva samples (**Figure 2**)

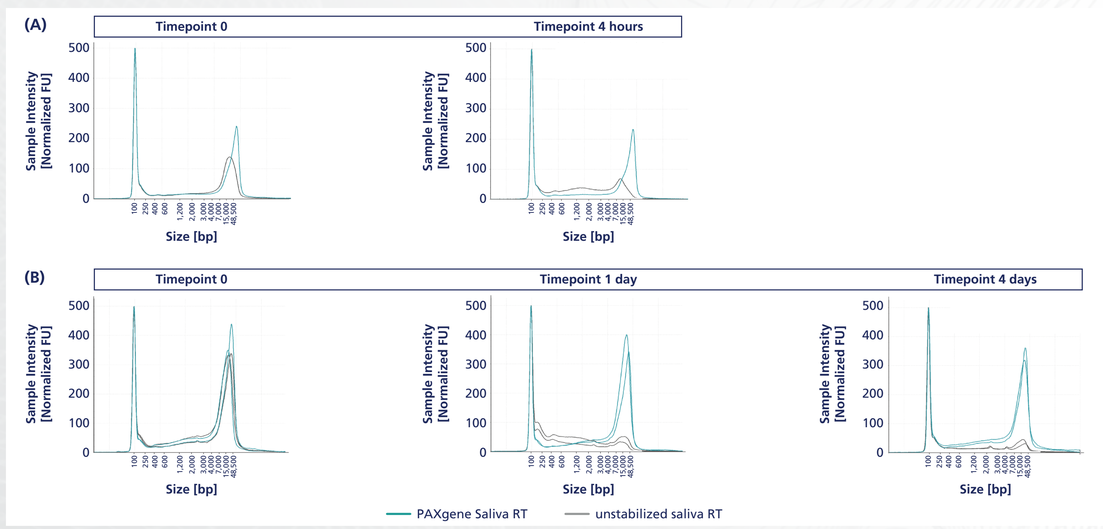


**Figure 2:** Representative profiles of human gDNA samples. Human gDNA profiles were determined with the Agilent 4200 TapeStation System using Genomic DNA ScreenTape analysis with gDNA from PAXgene Saliva Collector stabilized samples (green) and unstabilized saliva samples (gray). **A.** gDNA was purified at timepoint 0 directly after collection (left graph) and after storage for four hours (right graph). **B.** gDNA was purified at timepoint 0 (left) and after storage for one day (middle) and four days (right) at RT.

### Detection of cancer mutations down to a mutation rate of 0.25%

- Complete Sample to Insight Workflow when using the PAXgene Saliva Collector in combination with the QIAcuity for mutation detection analyses (**Figure 3**)

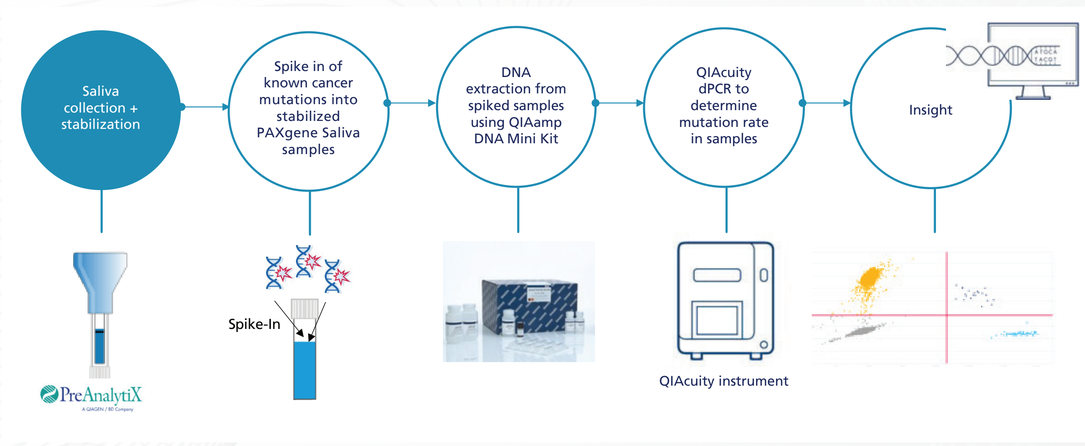


**Figure 3:** Sample to Insight Workflow when using PAXgene Saliva samples and the QIAcuity system to detect mutation rates in stabilized saliva samples.

- In stabilized samples, spiked-in cancer mutations could be reliably detected by dPCR using the QIAcuity down to mutation rates of 0.25% (**Figure 4**)
- With the dPCR LNA® Mutation Assay setup both mutant and wild-type alleles are detected simultaneously in a duplexing reaction enabling the calculation of absolute copies of wild type and mutant copies as well as the frequency of respective target mutations

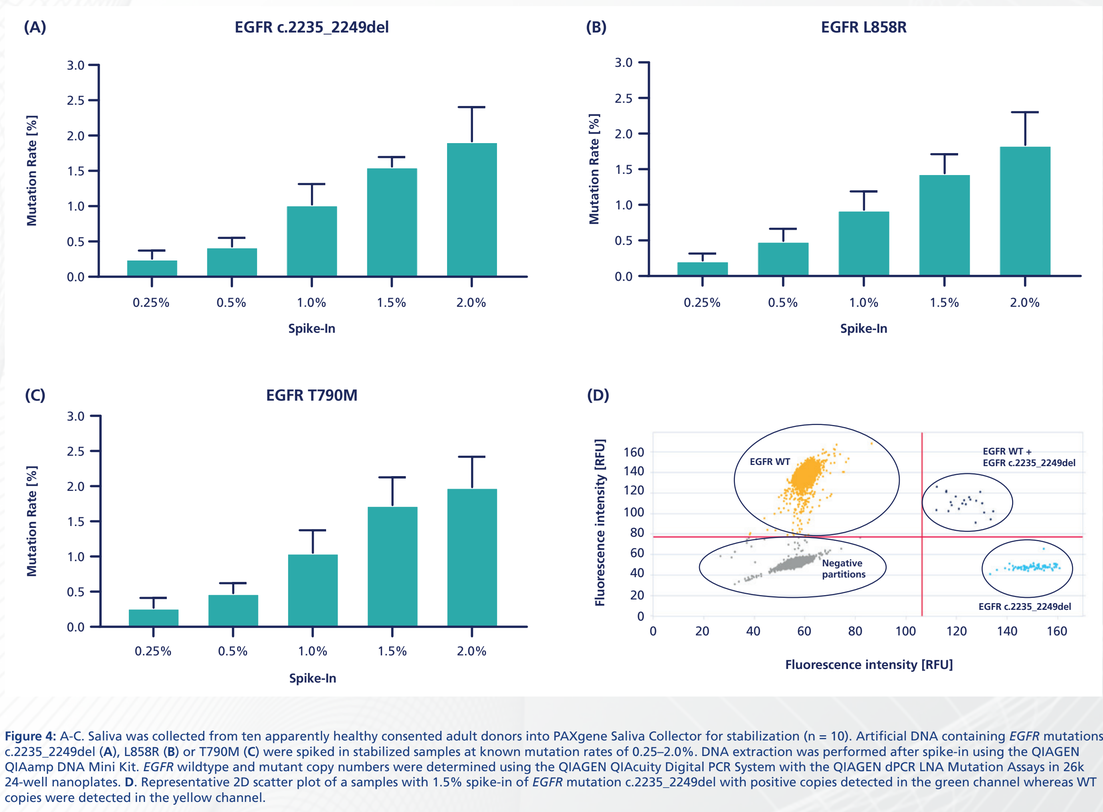


**Figure 4:** A-C. Saliva was collected from ten apparently healthy consented adult donors into PAXgene Saliva Collector for stabilization (n = 10). Artificial DNA containing *EGFR* mutations c.2235_2249del (**A**), L858R (**B**) or T790M (**C**) were spiked in stabilized samples at known mutation rates of 0.25–2.0%. DNA extraction was performed after spike-in using the QIAGEN QIAamp DNA Mini Kit. *EGFR* wildtype and mutant copy numbers were determined using the QIAGEN QIAcuity Digital PCR System with the QIAGEN dPCR LNA Mutation Assays in 26k 24-well nanoplates. **D.** Representative 2D scatter plot of a samples with 1.5% spike-in of *EGFR* mutation c.2235_2249del with positive copies detected in the green channel whereas WT copies were detected in the yellow channel.

## Conclusion

- Storage of saliva samples requires stabilization directly after collection as gDNA undergoes severe degradation when left unstabilized
- Genomic DNA from human saliva is stabilized when collected with the PAXgene Saliva Collector. The pre-filled stabilization solution in the PAXgene Saliva Collector preserves human gDNA levels by protecting DNA from degradation.
- In unstabilized samples, gDNA degradation starts immediately after collection and is constantly increasing.
- After one day only, qPCR results are no longer reliable in unstabilized samples as the severe DNA degradation affects the amplification of the qPCR target.
- Stabilized human saliva samples can be tested to detect cancer mutations using Digital PCR down to a mutation rate of 0.25%. While these proof of principle studies were performed with *EGFR* mutations, this detection method can easily be transferred onto other known mutations causing cancer entities such as oral or head and neck squamous cell carcinomas (Cui et al., 2021).

### References:

Cui Y, Kim HS, Cho ES, Han D, Park JA, Park JY, Nam W, Kim HJ, Cha IH, Cha YH. Longitudinal detection of somatic mutations in saliva and plasma for the surveillance of oral squamous cell carcinomas. PLoS One. 2021 Sep 3;16(9):e0256979. doi: 10.1371/journal.pone.0256979. PMID: 34478472; PMCID: PMC8415592.

Kaczor-Urbanowicz KE, Martín Carreras-Presas C, Kaczor T, Tu M, Wei F, Garcia-Godoy F, Wong DT. Emerging technologies for salivaomics in cancer detection. J Cell Mol Med. 2017 Apr;21(4):640-647. doi: 10.1111/jcmm.13007. Epub 2016 Nov 13. PMID: 27862926; PMCID: PMC5345659.

Wei F, Lin CC, Joon A, Feng Z, Troche G, Lira ME, Chia D, Mao M, Ho CL, Su WC, Wong DT. Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer. Am J Respir Crit Care Med. 2014 Nov 15;190(10):1117-26. doi: 10.1164/rccm.201406-1003OC. PMID: 25317990; PMCID: PMC5447327.

### Disclaimer:

The PAXgene Saliva Collector is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.



Better samples. More to explore.