

Salivalyse: A Rapid Method for Direct-PCR using GeneFix Saliva Samples

Traditional PCR & qPCR workflows for the analysis of DNA samples often require lengthy extraction and purification processes to ensure that samples are free of contaminants in order to ensure that they will run correctly downstream. In recent years, the need for a quick, efficient method of DNA preparation for PCR has become apparent. For this, Isohelix has developed a new method for the rapid preparation of GeneFix Saliva samples for use in PCR assays, providing quick, reliable results in a fraction of the time. The following describes a method of rapidly preparing saliva samples for qPCR using Salivalyse reagent.

Methods & Materials

Ten saliva samples were collected from adult donors using GeneFix GFX-01 collectors. The collected samples were then stored for five days at ambient temperature ($23^{\circ}\text{C} \pm 5^{\circ}\text{C}$) prior to processing using the Salivalyse SEK-50 kit, using 200 μl sample aliquots and following the instructions in the kit protocol. The prepared samples were then run on downstream SYBR qPCR using ACTB and GAPDH primer targets. Prior to this, samples were also run on a 1.0% agarose gel to analyse sample integrity.

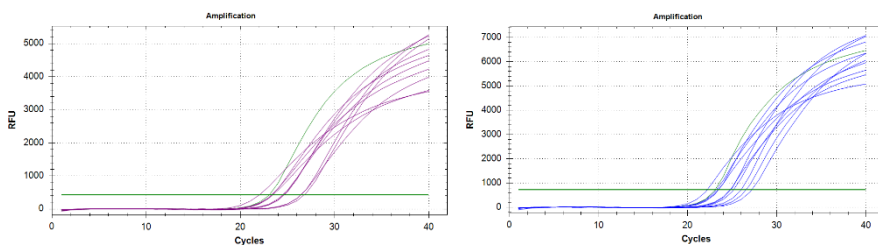
In addition, a further four GFX-01 saliva samples were collected to evaluate sample stability following Salivalyse treatment. Three aliquots per donor were processed using Salivalyse, with one of each aliquot stored at ambient temperature (AT), $+4^{\circ}\text{C}$, and -20°C respectively for five days. After this incubation samples were run on qPCR for analysis.

Results:

Salivalyse-prepared samples amplify successfully across multiple donors and gene targets:

Sample ID	1	2	3	4	5	6	7	8	9	10	Mean
Cq (ACTB)	24.67	22.05	26.78	23.46	23.38	26.43	24.50	24.59	26.43	23.33	24.56
Cq (GAPDH)	25.13	22.17	25.99	23.38	23.43	27.17	24.71	24.79	26.51	23.19	24.65

Table 1: qPCR run data of ten samples processed using Salivalyse reagent, with accompanying amplification curves shown below:



- All samples prepared using Salivalyse and run on qPCR showed consistent, reliable amplification of both gene targets tested (Table 1).
- Agarose gel analysis of the Salivalyse processed samples showed high quality, high molecular weight gDNA present for all collected samples.

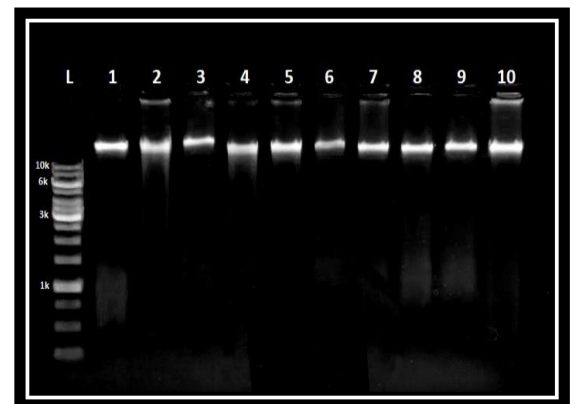


Figure 1: 1.0% agarose gel of the ten samples processed using Salivalyse reagent.

Samples prepared using Salivalyse remain stable for up to 5 days at Ambient Temperature:

Sample ID	ACTB Cq	GAPDH Cq
A 5 Days AT	23.68	23.56
B 5 Days AT	22.73	22.87
N 5 Days AT	23.47	23.35
T 5 Days AT	25.07	25.07
RT Mean	23.74	23.71
A 5 Days 4°C	23.59	23.62
B 5 Days 4°C	22.85	23.02
N 5 Days 4°C	23.16	23.42
T 5 Days 4°C	24.86	24.93
4°C Mean	23.62	23.75
A 5 Days -20°C	23.90	23.67
B 5 Days -20°C	22.79	22.97
N 5 Days -20°C	23.49	23.51
T 5 Days -20°C	25.81	25.33
-20°C Mean	24.00	23.87

Table 2: qPCR data of processed samples stored at different temperatures for five days prior to running on PCR. Each letter corresponds to a different donor.

- All samples tested amplified successfully and consistently. Samples stored at ambient temperature for five days prior to the PCR assay did not significantly differ in Cq values to those stored at either +4°C or -20°C for the same period, indicating that the sample quality was not affected.
- In addition, the mean Cq values of these samples were not significantly higher than the ten samples analysed immediately after Salivalyse preparation (Table 1), showing that samples remain stable after processing.

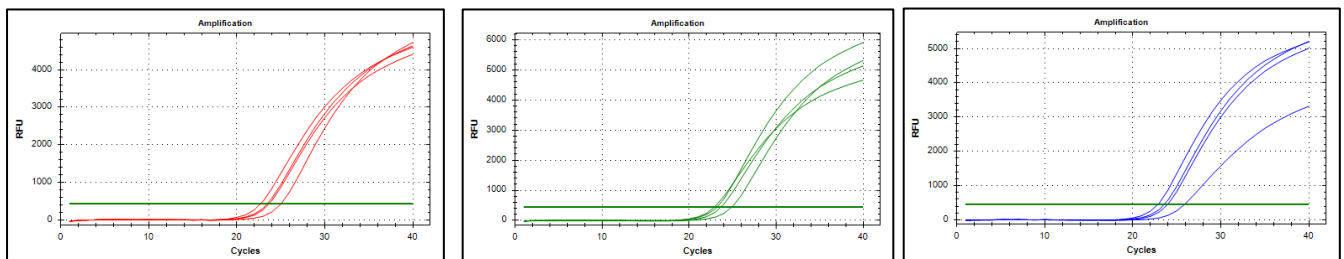


Figure 2: Amplification curves of the samples detailed in (Table 2). From left to right: Ambient temperature samples, refrigerated samples (+4°C), and frozen samples (-20°C).

Conclusions:

- GeneFix Salivalyse allows for rapid preparation of PCR-Ready DNA from saliva, with samples ready for use within 20 minutes following Proteinase K treatment.
- Salivalyse provides reliable amplification of DNA from GeneFix samples, generating reproducible results.
- Designed and optimised for use in both conventional PCR & qPCR.
- Only small volumes of sample needed for processing, precious samples can be retained & conserved for other downstream work.
- Salivalyse-treated samples remain stable at room temperature for up to 5 days, allowing for flexibility in workflows.