

## GeneFix GFX: Stabilisation of RNA samples for up to 120 Hours (5 Days)

### Methods:

Three 2ml saliva samples were collected from separate donors (N, R, & A) into GeneFix GFX-02 saliva collectors, following the kit collection instructions. Samples were then incubated at room temperature (23°C ± 5°C). To test the stability of RNA in the kits, 300µl aliquots of each sample were extracted using a column RNA isolation kit at the following time points after incubation: 0 Hours (Fresh), 24 Hours, 48 Hours, & 120 Hours.

Following extraction, samples were analysed for sample yield, purity, & integrity using Qubit RNA assay, Nanodrop assay, & 1% agarose gel electrophoresis. In addition, SYBR Green rt-qPCR reactions were run using 10ng of each sample targeting the human ACTB gene, to assess suitability of collected samples for downstream applications.

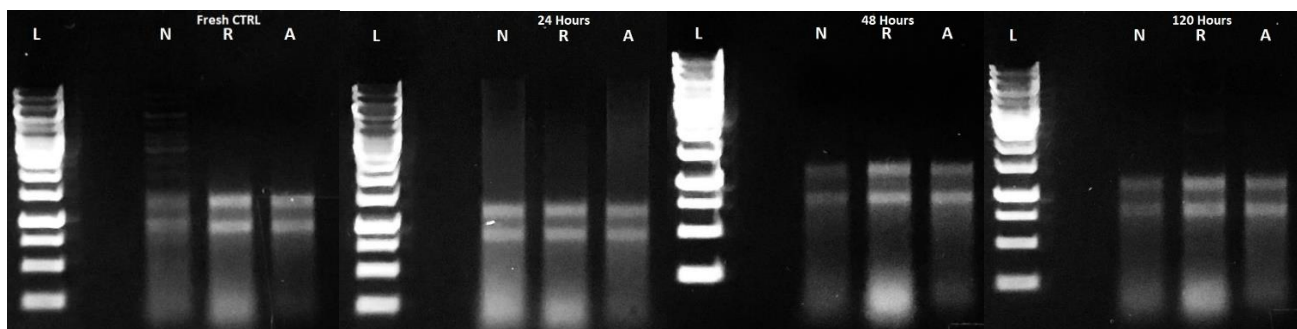
### Results:

Fresh Ctrl	Sample N	Sample R	Sample A	Mean
Qubit Yield (ng/ul)	33.4	109.0	53.9	<b>65.4</b>
A260/280	2.15	2.11	2.11	<b>2.1</b>
A260/230	2.02	2.06	1.29	<b>1.8</b>

24 Hours	Sample N	Sample R	Sample A	Mean
Qubit Yield (ng/ul)	34.9	82.7	51.6	<b>56.4</b>
A260/280	1.98	2.02	1.96	<b>2.0</b>
A260/230	1.50	2.16	1.69	<b>1.8</b>

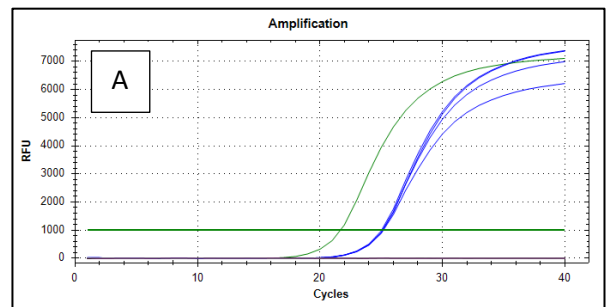
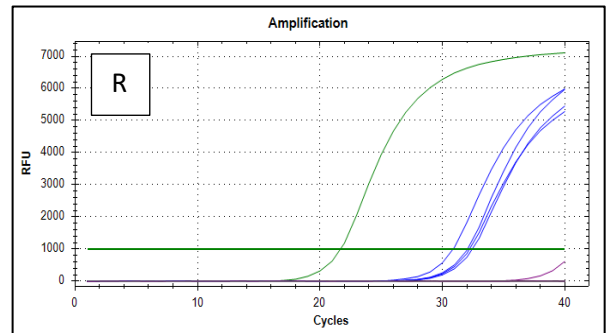
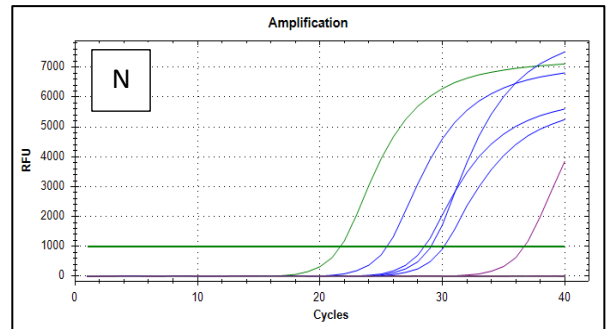
48 Hours	Sample N	Sample R	Sample A	Mean
Qubit Yield (ng/ul)	29.1	70.0	41.5	<b>46.9</b>
A260/280	1.97	2.02	1.97	<b>2.0</b>
A260/230	1.48	2.03	1.72	<b>1.7</b>

120 Hours	Sample N	Sample R	Sample A	Mean
Qubit Yield (ng/ul)	24.7	101.0	53.1	<b>59.6</b>
A260/280	2.00	2.05	2.01	<b>2.0</b>
A260/230	1.56	1.67	1.43	<b>1.6</b>



- Qubit RNA, Nanodrop, & gel analysis show that saliva total RNA samples stabilised using GeneFix collectors maintain yield, purity, and sample integrity stored at room temperature up to 120 hours following collection.

ACTB rt-qPCR	+RT Cq	-RT Cq	$\Delta$ Cq	% DNA Contamination
10ng RNA Std	18.35	28.39	-10.04	<b>0.15</b>
Fresh N	25.45	N/A	N/A	<b>0</b>
24h N	28.50	36.66	-8.16	<b>0.51</b>
48h N	29.06	N/A	N/A	<b>0</b>
120h N	30.13	N/A	N/A	<b>0</b>
Fresh R	30.85	N/A	N/A	<b>0</b>
24h R	32.42	N/A	N/A	<b>0</b>
48h R	32.04	N/A	N/A	<b>0</b>
120h R	32.20	N/A	N/A	<b>0</b>
Fresh A	25.17	N/A	N/A	<b>0</b>
24h A	25.13	N/A	N/A	<b>0</b>
48h A	25.10	N/A	N/A	<b>0</b>
120h A	25.03	N/A	N/A	<b>0</b>



- ACTB rt-qPCR of collected GFX RNA samples reported consistent amplification of the target gene.
- Signal from each donor sample was consistent, and did not degrade over the 120 hour incubation period.

### Conclusions:

- RNA samples collected from saliva and stored in GeneFix DNA/RNA collectors are stable for up to 120 hours following collection.
- Stabilised samples produce reliable, repeatable results during downstream rt-qPCR analysis.