

Saliva DNA isolation from GeneFix™ Saliva Collectors using GeneFix™ Saliva DNA Mini Kit Cat. No: GSS-50

With the use of advanced DNA analysis applications becoming routine in many labs, the need for high purity, high quality, intact genomic DNA samples from non-invasive sampling methods such as buccal swabbing and saliva collection, is becoming ever more important. In addition to the well established range of buccal swab kits and related DNA stabilisation and isolation solutions, Isohelix have recently launched a new GeneFix™ Saliva Collection device for the collection, stabilisation and transport of 2ml saliva samples, fully supported by the introduction of 3 new GeneFix™ Saliva DNA isolation kits. Both the GeneFix™ Saliva Collector and the 3 GeneFix™ Saliva DNA kits have been specifically designed for enhanced yields of highly purified intact genomic DNA, together with ease of handling and opportunities for high throughput. The new Isohelix GeneFix™ Saliva Collectors and Kits now offer the ability to isolate high yields of very high purity, intact DNA from saliva samples, suitable for use in all downstream applications and for archival purposes.

Here we describe isolation of DNA from saliva samples collected and stabilised with GeneFix™ Saliva Collectors, using the GeneFix™ Saliva DNA Mini Kit, and analysis of the purified DNA to show yield, purity and performance in the PCR environment of the intact genomic DNA. The GeneFix™ collectors collect a 2ml saliva sample in to 2ml stabilisation buffer giving a total volume of 4ml, the GeneFix™ mini kit will process 0.5ml -2ml volumes of stabilised saliva. If you require to process the whole 4ml in one go, the GeneFix™ Midi Kit should be used instead.

Saliva samples from several adult volunteers were collected using new Isohelix GeneFix™ Saliva Collectors. Each GeneFix™ collector is pre-filled with 2ml stabilisation buffer, into which the volunteer delivers a 2ml saliva sample. Once mixed with the stabilisation buffer, the saliva sample is stable at room temperature for at least 12 months prior to DNA isolation. The GeneFix™ Saliva DNA Mini Kit uses silica membrane spin column technology and is designed to process up to 2ml stabilised saliva sample, from the 4ml total volume, in one straightforward procedure. After a lysis step and addition of ethanol, the sample is run through silica membrane mini columns and eluted with 100µl elution buffer pre-heated to 70°C, according to the manufacturer’s instructions.

DNA yield and concentration was calculated using Qubit dsDNA BR assay. Absorbance ratios, both A260/280 and A260/230 were measured on a Nanodrop to assess purity, the quality of the DNA was checked by running whole DNA samples on an agarose FlashGel and the performance of the isolated DNA in the PCR environment was assessed by using the DNA in the Isohelix DQC Quality Check Kit, a multiplex PCR kit designed to show the integrity of isolated human genomic DNA.

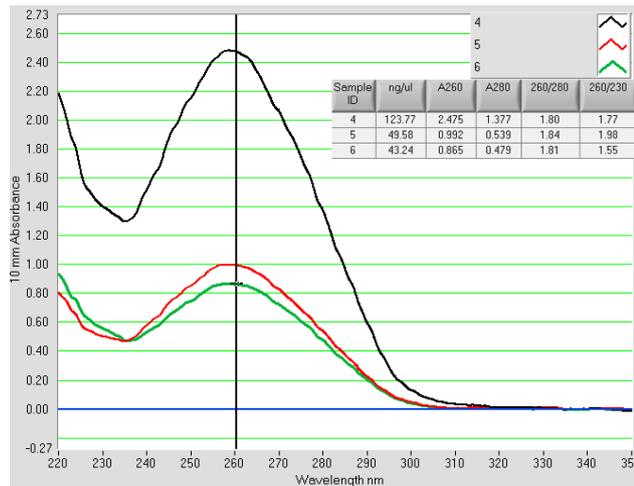
Results:

DNA concentration, yield and purity from saliva samples isolated with the GeneFix™ Saliva DNA Mini kit

Quant-iT dsDNA BR			
Sample	Concentration	Total µg	µg/ml of whole saliva
1	73.4 ug/mL	7.34	7.34
2	37.7 ug/mL	3.77	7.54
3	24.3 ug/mL	2.43	4.86
4	93.8 ug/mL	9.38	9.38
5	38.4 ug/mL	3.84	7.68
6	31.2 ug/mL	3.12	6.24
7	59.8 ug/mL	5.98	5.98
8	26.2 ug/mL	2.62	5.24
9	29.8 ug/mL	2.98	5.96

2ml stabilised saliva sample 
 1ml stabilised saliva sample 

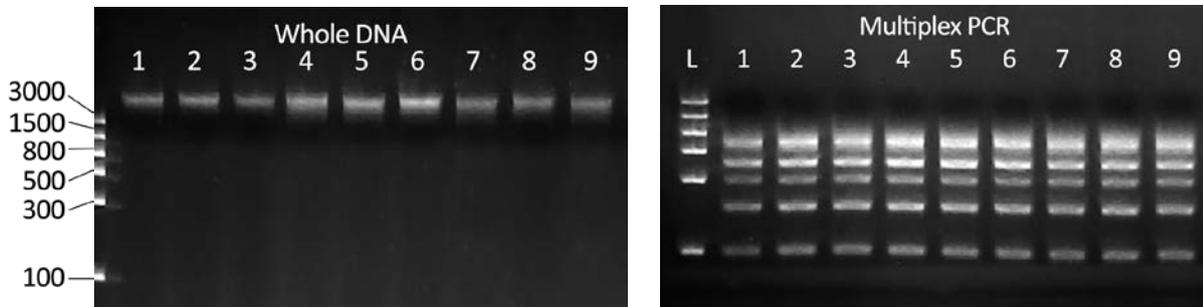
Nanodrop data			
Sample	ng/μl	A260/280	A260/230
1	108.84	1.80	1.51
2	51.52	1.80	1.74
3	37.44	1.82	1.49
4	123.77	1.80	1.77
5	49.58	1.84	1.98
6	43.24	1.81	1.55
7	174.12	1.84	1.45
8	67.12	1.89	1.88
9	63.08	1.89	1.81



Typical nanodrop scan for GSS isolated saliva DNA sample

In this experiment, yields of DNA per ml of saliva range from 4.86μg to 9.38μg (mean 6.69μg) as measured in the Qubit dsDNA assay, with mean concentrations of 75.67ng/μl (99.91ng/μl by nanodrop) for the 2ml samples and 31.27ng/μl (52.0ng/μl by nanodrop) for the 1ml samples. A260/280 ratios for all samples are > 1.8 and A260/230 ratios are >1.4 in all samples indicating very high DNA purity.

These samples were further analysed by running both whole DNA (10ng per lane by Qubit dsDNA BR assay) and DQC amplified samples (10ng DNA per PCR reaction) on a 2.2% Agarose FlashGel to check DNA integrity and quality.



The whole DNA samples all show high molecular weight intact genomic DNA with no evidence of fragmentation or shearing. The expected result from the DQC amplified samples is a pattern of 6 bands of 100bp, 200bp, 300bp, 400bp, 500bp and 600bp in size. The 500bp band is derived from an internal Lambda DNA control, the remaining 5 bands are amplified from different areas of the human genome and the presence of all 6 bands indicates both a successful multiplex PCR amplification and the presence of intact human genomic DNA.

The Qubit results together with the nanodrop absorbance ratios, the whole DNA gel and the multiplex PCR results shown above demonstrate that good yields of very pure, intact, high quality genomic DNA are isolated from saliva samples collected and stabilised in GeneFix™ Saliva Collectors using the GeneFix™ Saliva DNA Midi Kit.