

Saliva DNA isolation from GeneFix™ Saliva Collectors using GeneFix™ Saliva DNA Midi Kit Cat. No: GMS-12

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 the 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 the complete 2ml saliva sample collected and stabilised within GeneFix™ Saliva Collectors, using the GeneFix™ Saliva DNA Midi Kit, and analysis of the purified DNA to show yield, purity and performance in the PCR environment of the intact genomic DNA.

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 Midi Kit uses silica membrane spin column technology and is designed to process the whole stabilised saliva sample (total volume 4ml) in one straightforward procedure. After a lysis step and addition of ethanol, the sample is run through silica membrane midi columns and eluted with 400µl elution buffer pre-heated to 70°C, according to the manufacturer's instructions.

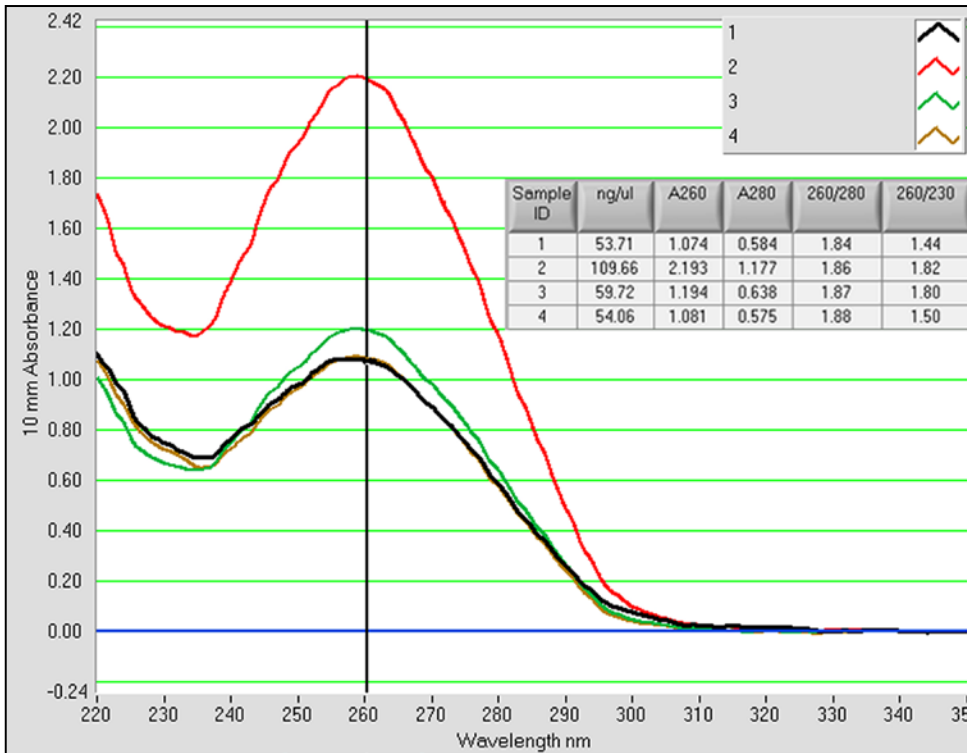
DNA yield and concentration was calculated using Qubit dsDNA BR assay. Absorbance ratios, both A260/280 and A260/230 were measured on a Nanodrop to assess purity, the quality of the DNA was checked by running whole DNA samples on an agarose FlashGel and the performance of the isolated DNA in the PCR 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 2ml saliva isolated with the GeneFix™ Saliva DNA Midi kit:

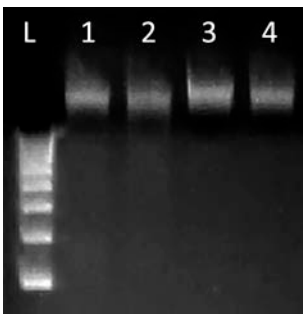
Sample	Qubit dsDNA BR Results		Nanodrop Results			
	Sample concentration µg/ml	Total dsDNA yield in µg	A260/280	A260/230	Sample concentration µg/ml	DNA yield in µg
1	26.1	10.44	1.84	1.44	53.71	21.48
2	76.8	30.72	1.86	1.82	109.66	43.86
3	48.1	19.24	1.87	1.80	59.72	23.89
4	43.2	17.28	1.88	1.50	54.06	21.62

In this experiment, yields from a 2ml adult saliva sample range from 10.4µg to 30.72µg (mean 19.4µg) as measured in the Qubit dsDNA assay, or from 21.5µg to 43.9µg (mean 27.7µg) by nanodrop. A260/280 ratios for all samples are > 1.8 and A260/230 ratios are >1.4 in all samples indicating very high DNA purity.

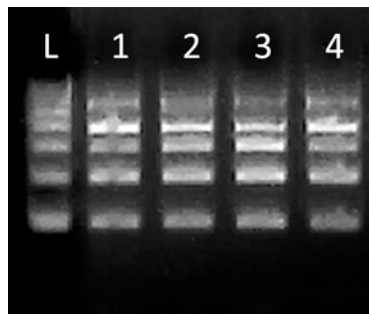


These samples were further analysed by running both whole DNA (10ng per lane by Qubit dsDNA BR assay) and DQC amplified samples (20ng DNA per PCR reaction) on a 2.2% Agarose Flash Gel to check DNA integrity and quality.

Whole DNA
10ng/lane



DQC Amplified DNA
20ng DNA per PCR reaction



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 and the whole DNA and PCR results shown above demonstrate that high 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.