

New Saliva-Prep2 Isolates High Purity Genomic DNA using GeneFix™ Collectors

The requirement for high purity, high quality intact genomic DNA samples from Non-Invasive sampling methods such as buccal and saliva collection, is becoming ever more important. Isohelix manufactures a broad range of DNA/RNA collection and isolation products, including the GeneFix™ device, which quickly collects 1ml or 2ml saliva into a stabilization buffer giving 5 years of DNA preservation. Further to this the DNA isolation is crucial in maintaining the yields and quality of the DNA sample, especially when using sensitive downstream analysis methods.

The new Saliva-Prep2 kit (GSPN) produces high purity DNA yet maintains a simple, straightforward protocol that uses widely available and low-cost benchtop equipment. The Saliva-Prep2 kit (GSPN) is widely and easily adaptable to varying volumes of saliva/buffer mix.

Here we describe the isolation of 1ml of stabilized saliva collected from 10 adult volunteers using GFX-02 collection devices (total sample volume 2ml saliva + 2ml stabilization buffer) using the Saliva-Prep2 kit, and analysis of the yield, purity and qPCR performance of the isolated DNA.

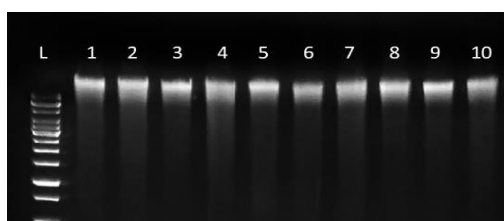
Results

DNA Concentration, Yield and Purity from 1ml stabilized saliva (0.5ml raw saliva) Isolated with the GeneFix™ Saliva-Prep2 (GSPN) DNA Kit:

Sample	Qubit dsDNA BR Results			Nanodrop Results	
	Sample Conc ng/μl	dsDNA yield in μg	Expected μg DNA from 2ml Sample	A260/280	A260/230
1	67.3	6.73	26.92	1.82	1.69
2	55.6	5.56	22.24	1.86	1.67
3	80.7	8.07	32.28	1.81	1.71
4	137.0	13.70	54.80	1.83	1.85
5	42.9	4.29	17.16	1.80	2.04
6	52.9	5.29	21.16	1.82	1.82
7	99.3	9.93	39.72	1.87	1.98
8	65.9	6.59	26.36	1.78	1.77
9	56.9	5.69	22.76	1.86	1.85
10	81.3	8.13	32.52	1.81	1.62
Mean	74.0	7.40	29.59	1.83	1.80

DNA yields are ideal for all downstream applications, including SNP and NGS. Nanodrop absorbance ratios show high purity supporting the agarose gel results of little or no RNA contamination

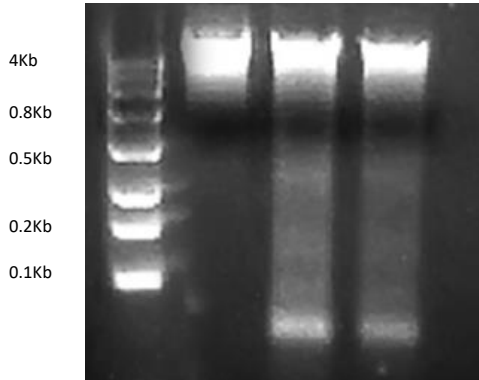
Whole DNA on 2.2% Agarose FlashGel against 1Kb Markers



- All samples show high molecular weight bands of intact DNA, sized significantly higher than the top marker of 10kb.
- There is no evidence of degradation or shearing.
- No RNA contamination is visible.

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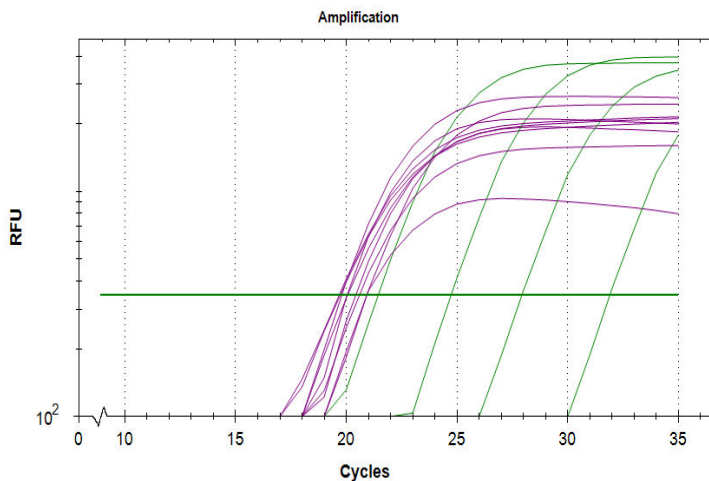
Whole DNA from Saliva-Prep2 (GSPN) using 1.2% Agarose FlashGel against 4Kb Markers:



Saliva-Prep2 PPTation Column

- The smears and bright band of very small material indicates RNA contamination present in the alternative precipitation-based kit and a silica column spin kit.
- The Saliva-Prep 2 sample shows no RNA contamination on a gel, and even by Qubit RNA assay the level is base line, and not further reduced by adding an RNase step.

Real-time PCR Data on GSPN isolated Human DNA from Saliva samples:



- Saliva samples (Purple) run on qPCR assay alongside DNA standards ranging from 5,800 starting copies with dilutions down to 5.8 copies.
- DNA isolated via GSPN is pure, high quality, ideal for sensitive downstream applications such as qPCR.
- The data also confirms that there are no PCR inhibitors

Conclusions:

- DNA Isolated using the Saliva-Prep2 kit (GSPN) shows consistently high yields and purity as supported by Qubit and Nanodrop data, and are suitable for all downstream applications, confirmed from qPCR results against human genomic standards.
- Gel documentation shows clean, intact genomic DNA without RNA contamination.
- The protocol takes up to 2 hours 15 minutes, and can be reduced by 30 minutes if desired.
- No special equipment is required for the GSPN kit, and it can also be used for manual and efficient high throughput DNA isolation using DeepWell Plates, multichannel pipettes and benchtop equipment.
- Saliva-Prep2 (GSPN) is supplied as a complete kit and requires no additional solvents or other chemicals.