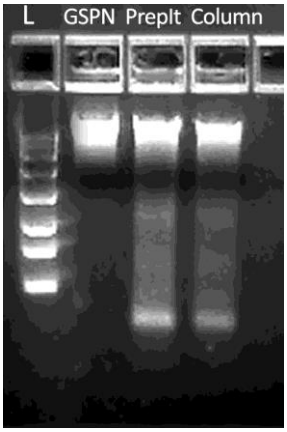
Comparison of DNA yields and purity from GSPN isolated saliva and Competitor isolated saliva with or without an additional RNase A step.

5 x 2ml saliva samples from 5 individuals were collected into GeneFix GFX-02 saliva collectors and stored at room temperature for several days. The tubes were vortexed to mix then 3 x 500ml aliquots from each tube were isolated through either GeneFix Saliva-Prep kit (GSPN-50), a competing kit (Kit O), or Kit O with an additional RNase step added. All samples were resuspended in 100ul TE buffer and analysed by Qubit dsdNA BR assay for DNA yield, Qubit RNA BR assay for RNA concentration and Nanodrop absorbance for A280/280 and a260/230 purity ratios.
In addition DNA from the GSPN and Kit O isolations were compared against a column based DNA isolation kit to assess DNA integrity and show presence of contaminating RNA. Samples were also tested for protein contamination using the Qubit Protein assay.

Results:

Sample		Qubit dsDNA ng/ul	Nanodrop/UV absorbance ng/ul	A260/280	A260/230
Saliva 1	GSPN	68.5	109.37	1.75	1.47
	Kit O	67.4	244.48	1.82	0.97
	RNase A + Kit O	65.1	148.66	1.61	0.67
Saliva 2	GSPN	58.6	69.11	1.77	2.20
	Kit O	55.9	135.02	1.88	1.20
	RNase A + Kit O	51.6	58.26	1.72	0.93
Saliva 3	GSPN	28.0	40.14	1.84	1.69
	Kit O	28.6	65.32	1.97	1.53
	RNase A + Kit O	28.3	37.47	1.73	1.07
Saliva 4	GSPN	76.3	133.29	1.77	1.80
	Kit O	64.4	236.25	1.83	1.25
	RNase A + Kit O	60.4	123.28	1.65	0.81
Saliva 5	GSPN	57.3	121.71	1.77	1.68
	Kit O	54.8	217.29	1.81	1.10
	RNase A + Kit O	55.2	116.96	1.62	0.71

These samples also contained protein



Conclusions:

1 GSPN tends to give marginally higher DNA yields than Kit O (with or without the additional RNAse A step), as measured by the Qubit Fluorometric assay.
2 Using Nanodrop concentrations for Kit O samples can result in substantial overestimation of the amount of DNA by as much as 4 times.
3 A260/280 values for GSPN and Kit O look comparable, however the high levels of RNA in the Kit O samples will artificially boost the A260/280 values from Kit O as pure RNA has an absorbance of 2.0 at 260nm, rather than 1.8 for pure DNA.
4 After RNase A treatment the A260/280 value for the Kit O samples is noticeably lower than the GSPN A260/280 ratios, and more accurately reflects the lower purity of the Kit O samples.
5 A260/230 values are significantly higher for GSPN samples than for Kit O samples.
6 Samples with a high DNA concentration can show protein contamination when isolated through Kit O. There was no protein detected in any of the GSPN samples.
7 The agarose gel demonstrates the high molecular weight intact DNA in all 3 samples, but significant RNA contamination present in the Kit O and column samples.