Application note: XME-1

Isolation of high purity, high quality DNA from buccal swabs using the Isohelix Xtreme DNA Kit (Cat. No: XME-5/50)

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, is becoming ever more important. Isohelix buccal swabs have been specifically designed for enhanced yields, and together with Isohelix Dri-Capsules provide a means of obtaining stabilised DNA samples through off-site sampling or self-sampling programs. The new Isohelix Xtreme DNA Kit now offers the ability to isolate very high purity, intact DNA from such samples, suitable for use in all downstream applications and for archival purposes.

Here we describe the isolation of typical buccal swab samples and analysis of the purified DNA to show yield, purity and performance in the PCR environment of the intact genomic DNA isolated using the Isohelix Xtreme DNA Kit.

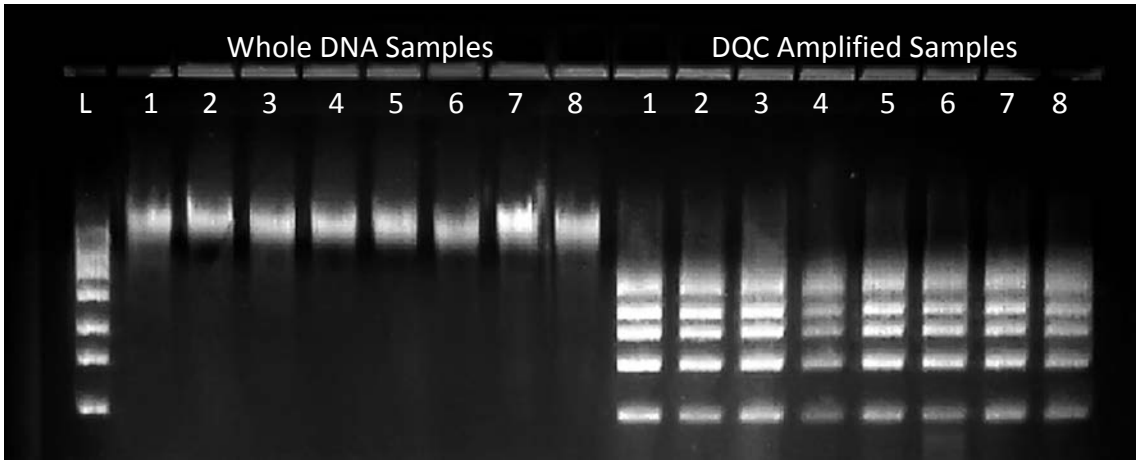
Buccal swabs were taken by several adult volunteers using Isohelix SK1 buccal swabs, according to the manufacturer's instructions. Each swab was taken by swabbing for periods of 30 seconds -1 minute, and the swab heads snapped off into the 5ml tubes provided with the swabs. The DNA was isolated using the Xtreme DNA Kit according to the instructions provided. Samples were eluted with 2 volumes of elution buffer pre-heated to 70^0C to give a final volume of 200µl for each DNA sample.
Yield and DNA concentration was calculated using Qubit dsDNA BR assays, 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 a range of Isohelix buccal swabs isolated with Xtreme DNA kit:

	Qubit dsDNA BR Results		Nanodrop Ratios	
Sample	Sample concentration µg/ml	Total dsDNA yield in µg	A260/280	A260/230
1	12.2	2.44	1.83	2.65
2	13.0	2.60	1.86	2.91
3	12.2	2.44	1.89	2.21
4	11.7	2.34	1.86	3.32
5	11.9	2.38	1.94	3.56
6	11.3	2.26	1.94	3.51
7	16.9	3.38	1.81	2.72
8	14.8	2.96	1.86	2.60

In this experiment, yields from a single adult swab range from 2.26µg to 3.38µg (mean 2.60µg) as measured in the Qubit dsDNA assay. This assay is specific for double-stranded DNA and gives a more accurate measure of DNA concentration than those obtained from the Nanodrop which tends to overestimate DNA concentration as UV absorbance data is unable to distinguish between dsDNA and other nucleotides present in the sample such as RNA and ssDNA.
A260/280 ratios for all samples are > 1.8 and A260/230 ratios are > 2 in all samples indicating very high DNA purity.

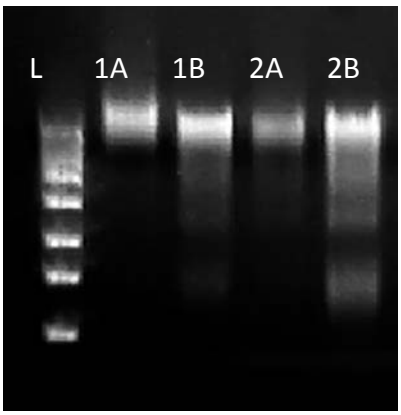
These samples were further analysed by running both whole DNA and DQC amplified samples on a 2.2% Agarose Flash Gel to check DNA integrity and quality.



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.

In a separate experiment paired sets of Isohelix buccal swabs were isolated with either Xtreme DNA or another leading column based DNA purification kit. Absorbance ratios were measured and whole DNA run on an agarose gel to compare purity of the DNA from the Xtreme DNA Kit against the alternative kit.

Sample	A260/280	A260/230
1A Xtreme DNA Kit	1.81	1.83
1B Alternative Column Kit	1.86	0.81
2A Xtreme DNA Kit	1.80	1.92
2B Alternative Column Kit	1.79	0.79



Although the A260/280 ratios are similar for both kits, the A260/230 ratios for the samples isolated using the alternative column based kit are both <1 suggesting the presence of impurities in these samples. Agarose gel comparison of whole DNA from both kits also indicates the presence of degraded or sheared DNA in the samples from the alternative column based kit as shown by the smeared appearance of the DNA on the gel.

These results together with the absorbance ratios, whole DNA and PCR results shown above demonstrate that good yields of very pure, intact, high quality genomic DNA can be isolated from buccal swabs (in this case Isohelix SK1 buccal swabs) using the Isohelix Xtreme DNA Kit.