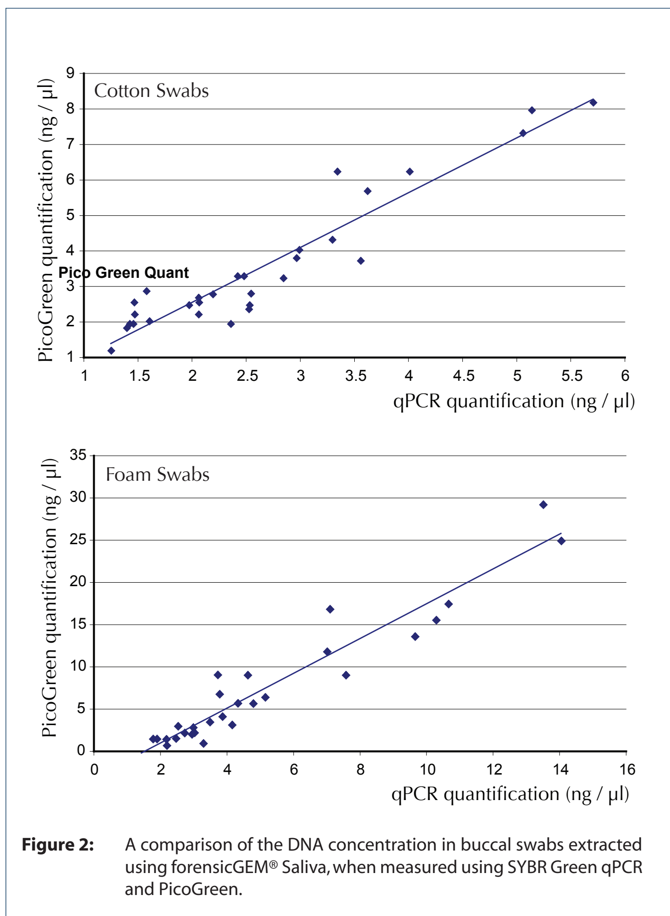

Figure 2: A comparison of the DNA concentration in buccal swabs extracted using forensicGEM® Saliva, when measured using SYBR Green qPCR and PicoGreen.

Discussion

The results demonstrate that rapid sample normalisation can be performed using intercalating dyes on ZyGEM DNA extracts if a sample of the DNA is taken before the 95°C heat. Alternatively, the optical density of the cell suspensions provide an excellent predictor of expected DNA yield.

As extraction efficiency is unlikely to change from one day to the next, a single calibration standard curve can be generated to correlate observed ODs or fluorescence with ng quantities.

It is notable that different swab types interfere with Pico Green to different extents. We have observed that some swabs release agents that fluoresce at the PicoGreen wavelengths (data not shown).

If PicoGreen is your preferred method for estimating human gDNA yield from buccal swabs, then we would advise different calibration curves for different swab types.

Omitting the heat-kill step from the ZyGEM workflow

The purpose of the 95°C step in ZyGEM extraction methods is to heat-inactivate the *forensic*GEM/*prep*GEM proteinase.

For many PCR applications this step can be dropped altogether. No detectable proteolytic degradation of the DNA polymerase occurs because of the e dilution factor of using only a small portion of the extract and the rapid ramping to 95°C as the first step of a PCR.

As a result, DNA can often be used immediately after the 75°C DNA extraction step. Figure 3 shows an example of a profile obtained from a buccal swab where the DNA was extracted using *forensic*GEM but the 95°C step was omitted from the method.

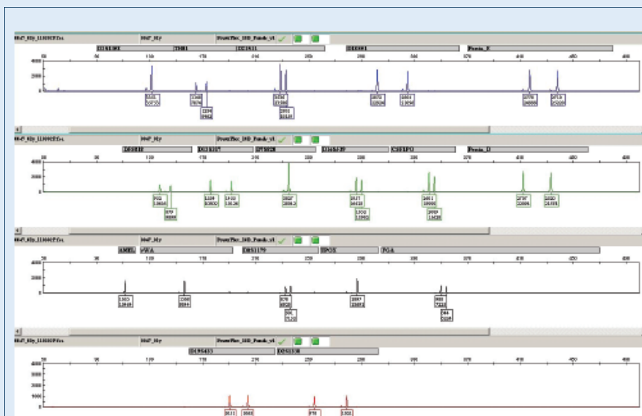

Figure 3: Promega PowerPlex 18 profile obtained from DNA extracted from a buccal swab using *forensic*GEM. No 95°C step was used.

If you feel that omitting the 95°C step would improve your workflow, we recommend empirically testing whether the change is possible with your own procedue.

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