

Comparison of DNA from blood, Oragene®/saliva and buccal swab samples on the Illumina® BeadChip 610Quad assay

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Introduction

Non-invasive and easy-to-use DNA sample collection technologies can greatly improve the success of a research study by ensuring donor compliance and acceptance into a study. However, not all non-invasive collection kits offer the same analytical performance. The purpose of this study is to demonstrate the performance benefits of DNA from Oragene®/saliva samples over DNA collected from buccal swab samples. Similar collection benefits can be appreciated with both of these methods; however their respective performance is far from similar. Oragene [DNA Genotek] is a non-invasive sample collection method that yields large amounts of high quality DNA from a 2 mL saliva sample that is stable for several years at room temperature.

DNA from blood, Oragene/saliva and buccal swab samples were collected, purified and processed on the Illumina® BeadChip 610Quad assay. DNA from blood is used as the control sample in this study.

Methods and results

Samples from 16 donors were collected using 3 different methodologies. A – Venous blood collection using EDTA tubes, B – Saliva collection using Oragene-DNA (OG-500) from DNA Genotek, C – buccal sample using a cotton swab.

DNA from each sample was purified and processed on the Illumina BeadChip 610Quad. After purification, each sample was quantified using absorbance and the A_{260}/A_{280} ratios were determined on a Nanodrop instrument [see Figure 1]. The samples were then normalized to 100 ng/ μ L and re-quantified using PicoGreen® [see Figure 2]. The diluted samples were then processed on the Illumina BeadChip 610Quad as per manufacturer's instructions.

Quantification of samples by absorbance (A_{260})

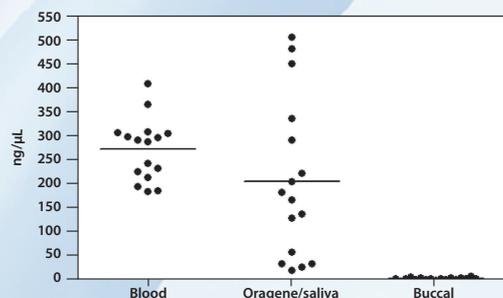


Figure 1: Total nucleic acid quantification of blood, saliva and buccal samples as measured by absorbance (A_{260}).

PicoGreen quantification of normalized samples

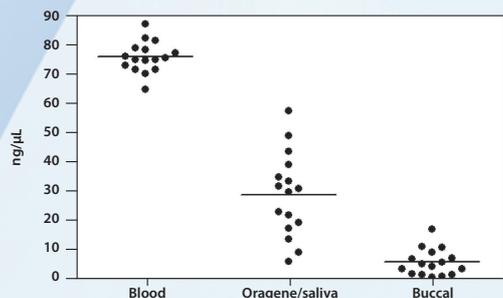


Figure 2: DNA concentration as measured using PicoGreen. Blood and saliva samples were diluted prior to quantification to what was expected to be 100 ng/ μ L based on absorbance (A_{260}) data. The amount of RNA measured by absorbance was not taken into consideration resulting in lower than expected DNA values as measured by PicoGreen.

Both blood and saliva had sufficient DNA with A_{260}/A_{280} ratios having averages greater than 1.7. In contrast, the buccal collection had much lower amounts of DNA and poor A_{260}/A_{280} ratios (Average = 1.2) [Figure 3]. It should be noted that after sample normalization the amount of DNA as quantified by PicoGreen was not the expected 100 ng/ μ L: for blood the average was 80 ng/ μ L and for saliva the average was 30 ng/ μ L. The lower than expected values, as measured by PicoGreen, are due to quantification of RNA fragments by absorbance. Considering that saliva contains considerably more RNA than blood this resulted in lower DNA quantification for saliva. This illustrates the importance of properly quantifying samples by PicoGreen.

A_{260}/A_{280} ratios



Figure 3: A_{260}/A_{280} ratios for blood, saliva and buccal samples.

Conclusion

When all samples were processed on the Illumina BeadChip 610Quad both blood and saliva had average call rates greater than 99.5%, with all samples having a call rate greater than 99%. The reproducibility between blood and saliva was calculated to be 99.99%. In contrast the average call rate for buccal samples was 97.2%, with some as low as 89.8% [see Figure 4].

Illumina 610Quad Bead Chip call rates

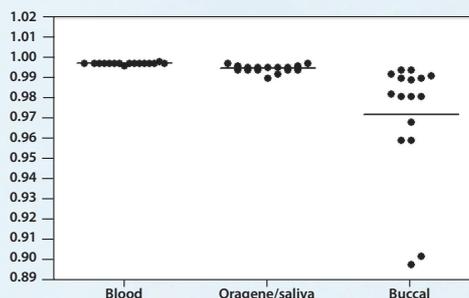


Figure 4: Illumina 610Quad Bead Chip call rates for blood, saliva and buccal samples. Blood and saliva had excellent call rates >0.99. Buccal samples had significantly lower call rates due to lower DNA quality (A_{260}/A_{280}) and lower DNA quantity (A_{260}).

Oragene is a proven non-invasive collection method that can be reliably used for microarray testing as demonstrated in this study.

