Can saliva replace blood for DNA collection and analysis?

Posted on DNA Genotek's blog, The Genetic Link

"Blood has proven a very consistent and reliable source of genetic material for many avenues of testing and research, but it can also be a time consuming, expensive and invasive collection ... Finding a comparable source of genetic material, such as saliva, that is more cost effective, more stable and less invasive would be extremely beneficial to the scientific community."

— a statement made by Affymetrix in the poster, entitled Comparison of high density genotyping results from saliva and blood samples on Affymetrix GeneChip® GenomeWide SNP 6.0 arrays.

Many things need to be considered in this debate. First and foremost, is saliva a viable option?

**Quality**

Saliva DNA must perform equivalently in downstream applications to that of blood to merit a change of sample type. This means the quality and quantity of DNA extracted from saliva must meet the standards set and achieved by blood. It might surprise you to know that much confusion surrounds the real source of genomic DNA in saliva. Surprisingly, most people assume the source of DNA in saliva is strictly buccal epithelial cells. However, studies show that up to 74% of the DNA in saliva comes from white blood cells which are an excellent source of large amounts of high quality genomic DNA. Yielding virtually the same amount of DNA per volume and the same DNA quality as blood, saliva can be considered equivalent to blood for genetic applications. However, the major issue with DNA from saliva arises when naturally degrading enzymes and bacteria within the sample attack DNA integrity and decrease quality very quickly.

Oragene® self-collection kits† are designed to maintain the integrity of DNA in saliva as they contain reagents to preserve the high molecular weight DNA by inhibiting degradation and preventing bacterial contamination. The majority of DNA obtained with Oragene is >23 kb in fragment size and the amount of bacteria has minimal practical significance as the vast majority is of human origin (average only 11.8% bacteria).³

When compared to other oral sampling methods, such as buccal swabs or mouthwash, a 2 mL saliva sample collected with Oragene yields approximately 11% bacterial DNA, substantially lower than mouthwash at 66% and cytobrushes at over 88% bacterial DNA.³

There should be no argument that most people would prefer a non-invasive, fast and easy sample collection over a painful, inconvenient, and hazardous blood draw. However, blood collection is often considered the golden standard for DNA quality and it is an established practice across hospitals, clinics, and labs worldwide. So, is replacing blood with saliva a real possibility?

We know blood collection will always be a necessary practice in healthcare as blood contains some biomarkers that may not be present in saliva. Proteins, antibodies, and metabolites are some examples of such biomarkers. However, when the purpose of collection is strictly for genomic DNA analysis, saliva makes a lot of sense.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER

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Absorbance at 230 is used to measure various contaminants such as phenol and phenolic compounds, carbohydrates and other organics. Saliva samples contain a large amount of carbohydrates (from the heavily-glycosylated protein mucin). While protein is removed during extraction, small amounts of this carbohydrate is left behind. Carbohydrates absorb very strongly at 230 nm so even small quantities of carbohydrate can greatly inflate the 230 reading leading to a poor ratio. The presence of these carbohydrates does not affect downstream application and therefore A\textsubscript{260}/A\textsubscript{230} is not a useful method to assess the suitability for downstream use of DNA extracted from saliva samples. Phenolics can be of concern; however, these are not within Oragene and prepIT•L2P reagents, so this is not an issue for Oragene/saliva samples.

To accurately measure the purity of DNA extracted from saliva, A\textsubscript{260}/A\textsubscript{280} should be calculated. The ratio of absorbance at 260 nm vs 280 nm is commonly used to assess DNA contamination of protein solutions, since proteins (in particular, the aromatic amino acids) absorb light at 280 nm. When extracting with prepIT•L2P the median A\textsubscript{260}/A\textsubscript{280} ratio is between 1.6–1.9. These ratios are typically indicative of a DNA sample that will perform well on your downstream application given that all your other QC metrics pass (high molecular weight on gel, acceptable concentrations by fluorescent based quantification method).

Multiple studies confirm DNA extracted from Oragene/saliva samples result in DNA of the highest integrity, performing equivalently to blood for the most demanding applications including microarrays and sequencing (targeted and whole genome).

A study conducted in 2010 by Bahlo, M. et al., stated “… saliva collected using the Oragene kit provides good quality genomic DNA … comparable to blood as a template for SNP genotyping on the Illumina platform.”

Affymetrix concludes in the previously mentioned poster, “Concentration and purity QC metrics have demonstrated that DNA extracted from saliva is of similar quality and quantity to that extracted from the paired blood sample … The paired blood and saliva samples were run on the GWS6.0 arrays, analyzed and then compared to internal standards and to each other. Call rates and reproducibility percentages in excess of 99% verifies that saliva can be used successfully as an alternative source of genomic DNA for use in high density genotyping.”

In further agreement, another study, entitled Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping by Abraham, J.E. et al., stated “DNA quality, as assessed by genotype call rates and genotype concordance between matched pairs of DNA was high (>$97\%$) for each measure in both blood and saliva-derived DNA. … We conclude that DNA from saliva and blood samples is comparable when genotyping using either Taqman or genome-wide chip arrays.”

A question that many researchers continue to ask is: what impact does bacterial content from saliva have on sequencing? The literature clearly demonstrates that when performing sequencing, the bacterial content has no impact on variant calling. Dr. Cory McLean of 23andMe presented a poster in which he described WGS of 50 saliva samples. The DNA extracted from these archived Oragene/saliva samples were sequenced using Illumina technology, to a median depth of 44.9 fold coverage and covered 97.8 – 98.2\% of the genome.

After identifying the variants in these samples Dr. McLean compared the results to data from the same cohort previously determined using a genotyping array and observed a 99.91 – 99.97\% concordance, indicating that Oragene/saliva samples provide consistent results across different technology platforms. In addition, a poster recently presented by the Broad Institute stated:

“To date, we have sequenced over 1,585 (Oragene) saliva samples to 30\times coverage using the HiSeqX (Illumina)… Given this experience, we are confident sequencing patient samples from (Oragene) saliva can be cost effective and produce high quality results for research and clinical studies.”

Extensive research clearly exists and validates saliva as an equivalent alternative to blood for genomic DNA, but why would institutions currently using blood samples change their procedures to incorporate saliva? What benefits exist to outweigh the status quo?

**Functionality/ease of use**

When collecting a sample from a distant relative, a child or someone with a psychiatric disorder, obtaining a blood sample can be difficult and stressful on the patient. Saliva collection for DNA
improves patient care and donor compliance by providing a simple, painless alternative and removes the inconvenience, anxiety and cost of going to a clinic for a blood draw. But not all saliva kits are created equal. There are 3 methods for collecting oral DNA samples – dry, wet and non-invasive procedures. Dry procedures require the donor to insert a cytobrush, buccal swab or other collection device into the mouth where tissue is scraped from the gum and cheek surfaces. These methods collect primarily buccal cells and a high proportion of bacteria which stick to the gumline.

However, DNA samples collected from saliva where the donor spits into a collection device are quite different and offer higher yields and DNA quality than other oral DNA sample collection methods. One study, titled New Saliva DNA Collection Method Compared to Buccal Cell Collection Techniques for Epidemiological Studies, states:

“Whole-saliva collection provided an average DNA yield that was significantly greater than all other [oral] methods... Median yield [of Oragene/saliva]... was approximately three times the median yield of the oral rinse, and more than 12 times the median yields for the buccal swab and brush methods.”

Oragene saliva kits have been shown to improve compliance rates and speed up collection and extraction processes when compared to blood, rendering healthcare more efficient.

Abraham, J.E. et al. states “… advantages of saliva … include lower overall cost, lower infection risk, increased patient convenience, acceptability, compliance, and uptake.”

Bahlo, M. et al. reports in their study “The Oragene kit … presents minimal inconvenience to the participant, resulting in high response rates. Further, we have shown that saliva samples can be sent in the mail to a central collection point, thereby reducing transportation costs and the risk of duplication.”

Another work by Viltrop T. et al. states “Saliva collection is a painless procedure with no risk of disease transmission and no requirements for specialized medical personnel. Also, saliva collection allows wider population sampling as it is possible to collect DNA samples by mail.”

Zhang, L. et al. also affirms “… saliva collection (Oragene•DNA self-collection kit) … is especially attractive for maximizing the participation rate … [and] clinical situations in which patients and/or their relatives are not available for on-site whole blood collection. We have also adopted this test to provide rapid turnaround (1 week) results.”

And in their 2009 breast cancer study, Ambroson, C.B. et al. transitioned from blood to saliva collection using Oragene kits to reduce costs and to facilitate participation.

It should further be mentioned, Oragene/saliva samples are compatible with high-throughput DNA processing, enabling seamless integration into existing automated lab extraction procedures. With convenience established for the donor, clinician, and lab, our focus now turns to cost.

Cost

The price associated with blood collection may be perceived to be free for many institutions that have established blood collection labs/service centers; however, there are real costs to sample collection even within these environments. Phlebotomists, lab technicians, medical supplies, and shipping requirements (dry ice, containers, and overnight delivery) add to an estimated $40 per sample, not including freezer storage.

DNA from saliva, collected with Oragene, in comparison, comes in a variety of formats with differing yield and stability capabilities which cost between 48% – 80% less than blood. More savings are introduced as Oragene products enable at-home collection, standard shipping via regular mail at room temperature and zero refrigeration.

Daksis, J.I. et al. states, “The acquisition of high quality DNA for molecular assay from oral samples offers clear advantages in cost, handling, storing and shipping over acquisition of samples from blood. … It therefore opens the way for convenient point of care testing…”

Basham, R.J. et al. claims “…saliva [Oragene•DNA]… allows cost-efficient storage and shipping. In contrast, whole blood must either be extracted within a few days, or stored at −70°C until extracted.”

Abraham J.E et al. continues “… commercial extraction of DNA from saliva is cheaper than from blood.”
And another study by Nishiya, D.M. et al. reports “Obtaining blood biospecimens presents logistical and financial challenges. As a result, saliva biospecimen collection is becoming more frequent because of the ease of collection and lower cost.”

That being said, many clinicians and labs benefit from their institution’s internal procurement established for blood collection and are therefore exposed to minimal, if any, costs. But when working with children, psychiatric patients, or remote donors a blood sample is less convenient and more time consuming when a practical alternative exists.

I close with Abraham, J.E et al. “… [Oragene•DNA kits] are more expensive than blood sampling kits however this cost has to be counter-balanced by the increased inconvenience to the patient and the cost of trained staff required to obtain blood samples.”

Conclusion

In conclusion, can saliva replace blood for DNA collection and analysis? Yes, and in fact it has started to already.

Oragene is currently used as an alternative when blood is not a viable option in 250 hospitals worldwide and adopted by more than 6,000 researchers in over 100 countries. Saliva is increasingly becoming an equally important standard practice in healthcare and research for DNA based sample collection, especially for children, patients with mental health disabilities, and those who cannot efficiently access a blood clinic.

See more articles on DNA and RNA from saliva on The Genetic Link at http://blog.dnagenotek.com

References

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