SALIVA VS BLOOD

Can saliva replace blood for DNA collection and analysis?

DNAgenotek®

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There should be no argument that most people would prefer non-invasive, fast and easy sample collection over painful, inconvenient and potentially hazardous blood draws. However, blood collection is generally considered the gold standard and processes for collection have been established in hospitals, clinics and labs worldwide.

So, is replacing blood with saliva a real possibility?

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 method … 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.[1]

We know blood collection will always be necessary in health care procedures, as blood contains some biomarkers, such as proteins and antibodies, which may not be present in saliva. In cases where the purpose of collection is strictly for genomic DNA analysis, it can be debated that saliva is the better way to collect DNA.

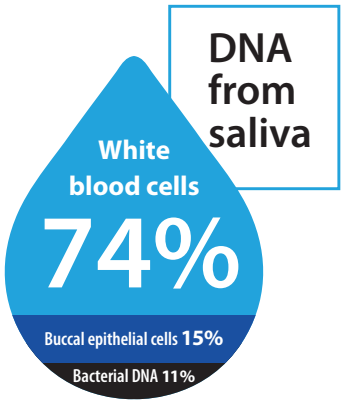
[1] Reynolds, J.D., et al. Comparison of High Density Genotyping Results from Saliva and Blood Samples on Affymetrix GeneChip® GenomeWide SNP 6.0 Arrays. Poster. Affymetrix Clinical Services Laboratory.

DNA quality from saliva samples versus blood samples

First and foremost, is saliva a viable option?

It is important to have high quality DNA within your sample type to get accurate results downstream. DNA from saliva must perform equivalently in downstream applications to DNA isolated from blood to merit a change of sample type. This means the quality and quantity of DNA extracted from saliva must meet the standards set for and achieved by blood. It may surprise you to know that there is some confusion surrounding the real source of genomic DNA in saliva; most people assume the sole source is buccal epithelial cells.

However, studies show that up to 74%[2] of the DNA in saliva comes from white blood cells, an excellent source of high quantity and high quality genomic DNA. Yielding virtually the same amount of DNA per volume and the same quality of DNA as blood, saliva can be considered equivalent to blood for genetic applications. The major issue with DNA from saliva arises when naturally occurring enzymes and bacteria in saliva degrade the DNA and compromise the sample quality.



Oragene® and ORAcollect® self-collection kits are designed with an integrated stabilization chemistry that maintains the integrity of saliva's high molecular weight DNA by inhibiting degradation and preventing bacterial growth. DNA collected with Oragene/ORAcollect is > 23 kb in fragment size; the amount of bacteria has minimal practical significance because the vast majority is of human origin.

When compared to other oral sampling methods, a 2 mL saliva sample collected with Oragene yields approximately 11% bacterial DNA, substantially lower than the bacterial DNA in mouthwash (66%) and cytobrushes (more than 88%).[3]

Many researchers continue to question the impact of bacterial content from saliva on sequencing. The literature clearly demonstrates that when performing sequencing, bacterial content has no impact on variant calling. Dr. Cory McLean of 23andMe® presented a poster in which he described whole genome sequencing (WGS) of 50 saliva samples. The DNA extracted from these archived Oragene/saliva samples was sequenced using Illumina technology, to a median depth of 44.9-fold coverage and covering 97.8%-98.2% of the genome.[4]

After identifying the variants in these samples, McLean compared the results to data from the same cohort previously determined using a genotyping array. He observed a 99.91%-99.97% concordance, indicating that Oragene/saliva samples provide consistent results across technology platforms.

A poster presented by the Broad Institute included this statement:

To date, we have sequenced over 1,585 (Oragene) saliva samples to 30× 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.[5]

[2] Thiede, C., et al. (2000). Buccal swabs but not mouthwash samples can be used to obtain pretransplant DNA fingerprints from recipients of allogeneic bone marrow transplant. *Bone Marrow Transplantation.* 25(5):575-577.

[3] James, C., Iwasiow, R.M., Birnboim, H.C. (2011). Human genomic DNA content of saliva samples collected with the Oragene® self-collection kit. DNA Genotek. PD-WP-011.

[4] McLean, C., et al. (2012). Whole-genome sequencing of 50 LRRK2 G2019S carriers discordant for Parkinson's disease. Presented at the American Society for Human Genomics 2012.

[5] Dodge, S., et al. (2016). Sequencing Whole Genomes with DNA Derived from Saliva. Poster session presented at: 2016 Advances in Genome Biology and Technology Meeting (AGBT).

DNA purity, absorbance ratios

Absorbance at 230 nm is used to measure various contaminants, such as phenol and phenolic compounds, carbohydrates and other organics. While phenolics can be of concern, DNA Genotek® products do not contain these chemical compounds. Saliva samples contain a large amount of carbohydrates (from the heavily glycosylated protein mucin) and some protein, which is removed during extraction. In some cases, small amounts of carbohydrate are left behind and greatly inflate the absorbance reading because carbohydrates absorb very strongly at 230 nm, so even small quantities can lead to a poor absorbance ratio.

When extracting saliva samples with prepIT®•L2P, the median A_{260}/A_{280} ratio is between 1.6 and 1.9,[4] comparable to blood, which is on average 1.8. The presence of these carbohydrates does not affect downstream application and therefore A_{260}/A_{230} is not a useful method to assess the suitability for downstream use of DNA extracted from saliva samples.

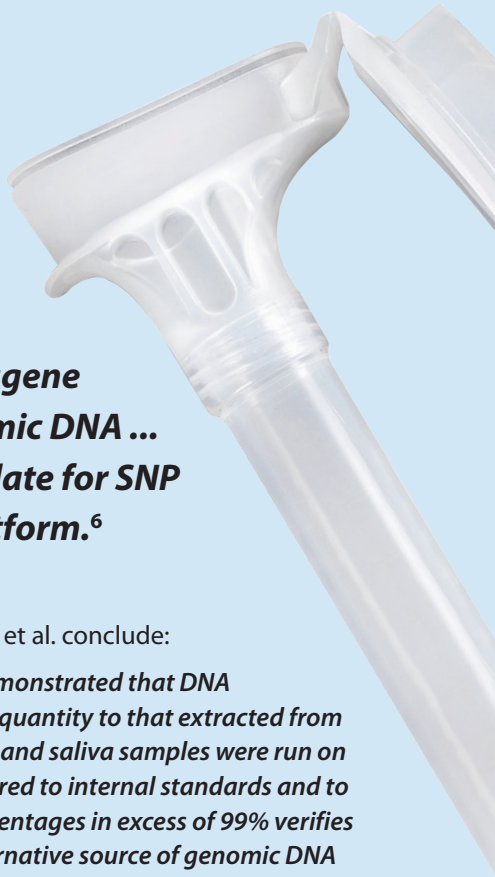
To accurately measure the purity of DNA extracted from saliva, the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}), rather than A_{260}/A_{230}, should be calculated. A_{260}/A_{280} is commonly used to assess DNA contamination of protein solutions, since proteins (in particular, the aromatic amino acids) absorb light at 280 nm. A ratio of about 1.8 is generally accepted as pure DNA. These ratios are typically indicative of a DNA sample that will perform well on downstream applications when all other quality control metrics are attained (e.g., high molecular weight on gel, acceptable concentrations by fluorescent-based quantification method).

Absorbance

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

Testimonials

on sample quality



Extensive research clearly 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?

Saliva's non-invasive collection method makes for a better alternative to blood samples and is comparable downstream.

In their study, Bahlo et al. state:

... saliva collected using the Oragene kit provides good quality genomic DNA ... comparable to blood as a template for SNP genotyping on the Illumina platform.[6]

In the previously mentioned poster, Reynolds et al. conclude:

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.[1]

In their 2012 study, Abraham et al. state: Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping.

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 Taqman assays or genome-wide chip arrays.[7]

6 Bahlo, M., et al. (2010). Saliva-derived DNA performs well in large-scale, high-density single-nucleotide polymorphism microarray studies. *Cancer Epidemiol Biomarkers Prev.* 19(3):794-798.

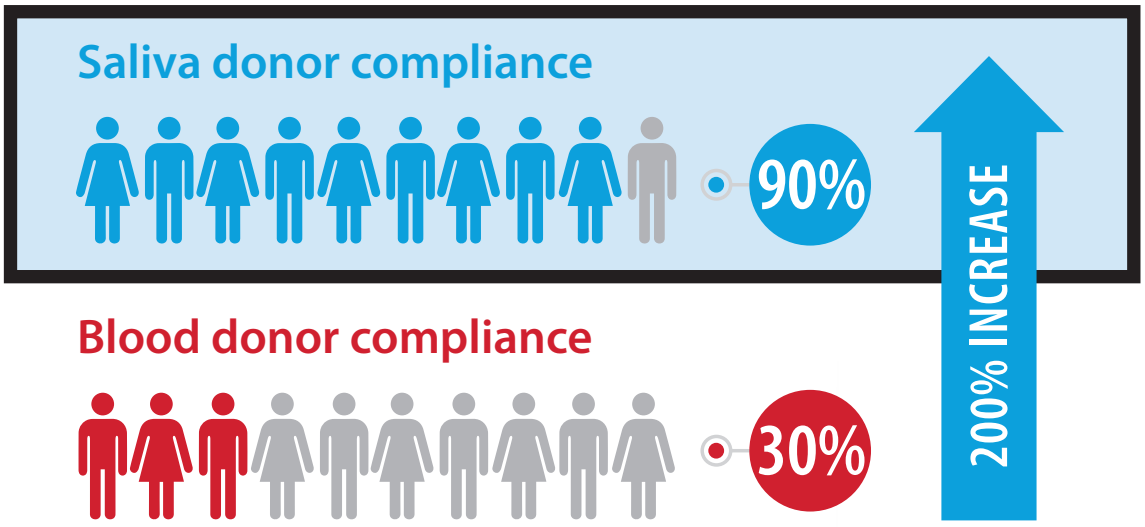
7 Abraham, J.E., et al. (2012). Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics. 5:19. doi: 10.1186/1755-8794-5-19.

Functionality and ease of use

Blood collection and donor compliance

Providing blood samples can be difficult and stressful for children, for distant donors and for other individuals who are difficult to collect from.

Donor recruitment is one of the most challenging aspects of a research project. Researchers require a sample type that fosters a high participation rate and ready access to donors to keep collection costs to a minimum.[8]



Blood collection is often viewed as the gold standard for high quality DNA; however, study results show that invasive venipuncture is the main reason for recruitment refusal. Additionally, blood collection requires access to clinical infrastructure. These two factors contribute to increased trial costs.[9,10] Saliva is a reliable sample type for high quality DNA and has proven advantages over blood (see table on next page). With saliva collection, researchers save time and effort in recruiting donors and are likely to spend less on donor incentives.

[8] https://www.dnagenotek.com/US/pdf/MK-006.pdf

[9] Hemmes, M., et al. (2010). PS1-11: Specimen collection within the Cancer Research Network: a critical appraisal. *Clin Med Res.* 8(3-4):191.

[10] Anthonappa, R.P., et al. (2013). Evaluation of long-term storage stability of saliva as a source of human DNA. *Clin Oral Invest.* 17:1719-1725.

Blood versus saliva (using Oragene) for DNA collection

Blood	Saliva (using Oragene)
Invasive	Non-invasive
Disliked by donors (impractical for children); low donor participation rate.	Easy to use (practical for children); high donor participation rate.
Requires a trained phlebotomist.	Does not require trained personnel.
Requires refrigeration and rapid processing (in less than a week).	Can be stored at room temperature.
Requires cold-chain transportation or in-clinic collection.	Can be collected at home and mailed back through regular post.
Expected compliance rate: 30%[11]	Expected compliance rate: 70-95%[11]

The better choice

Related content:

DNA saliva kits or traditional blood collection: which is more cost effective?

Saliva is easier.

Overcoming challenges in DNA sample collection.

[11] https://www.dnagenotek.com/US/saliva-is-easier.html

Saliva collection and donor compliance

In a clinical setting, care providers can order genetic tests for patients and **their close relatives to do from home for convenience and simplicity.**

DNA collection from saliva 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.

Dry swabs can be used when collecting samples at home as an alternative to blood; however, without stabilization, dry swab samples are prone to bacterial growth and DNA degradation, rendering the sample unusable once it reaches the lab.[12,13] Dry procedures require the donor to insert a cytobrush, buccal swab or other collection device into the mouth and scrape tissue from the gum and cheek surfaces. These methods collect primarily buccal cells and a high proportion of bacteria that stick to the gum line. Conversely, DNA samples collected from saliva, where the donor spits into a collection device, are quite different, because they target whole saliva and offer lower bacterial contamination, higher yields and better quality than other oral sample collection methods.

Did you know?

Oragene's stabilization chemistry inactivates the SARS-CoV-2 virus, limiting transmission of COVID-19 during collection, processing and sample analysis.

Oragene saliva kits have demonstrated improved compliance rates and faster collection and extraction process turnaround time when compared to blood, rendering health care more efficient, as indicated in the testimonials of genetic researchers on the next page.

[12] Hansen, et al. (2007). Collection of blood, saliva and buccal cell samples in a pilot study on the Danish nurse cohort comparison of the response rate and quality of genomic DNA. *Cancer Epidemiol Biomarkers Prev.* 2072-2076.

[13] Galbete, C., et al. (2013). Lifestyle factors modify obesity risk linked to PPARG2 and FTO variants in an elderly population: a cross-sectional analysis in the SUN project. *Genes Nutr.* 8(1):61-67.

It should further be mentioned that Oragene/ORAcollect 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.

Related content: Reliability & stability of Oragene samples for WGS & exome sequencing

Testimonials

on collection and donor compliance

The authors of "New Saliva DNA Collection Method Compared to Buccal Cell Collection Techniques for Epidemiological Studies" state:

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.[14]

In their study, Viltrop et al. state:

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.[15]

Zhang et al. affirm: ***… 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 ...***[16]

[14] Rogers, N.L., et al. (2007). New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. *Am J Hum Biol.* 19:319-326.

[15] Viltrop, T., et al. (2009). Comparison of DNA extraction methods for multiplex polymerase chain reaction. *Anal Biochem.* 398(2):260-262.

[16] Zhang, L., et al. (2009). A rapid and reliable test for BRCA1 and BRCA2 founder mutation analysis in paraffin tissue using pyrosequencing. *J Molecular Diagn.* 11(3):176-181.

DNA stability and cost comparison of blood versus saliva

Blood collection may be perceived to be free for many institutions that have established blood collection labs/service centres; however, there are real costs to sample collection even within these environments. These costs include phlebotomists, lab technicians, medical supplies, shipping requirements and freezer storage.[17]

Oragene/ORAcollect products come in a variety of formats with differing yield and stability capabilities, with between 48% and 80% lower collection costs. Additional savings are introduced because these products enable at-home collection, use only standard shipping via regular mail at room temperature and eliminate refrigeration.

Related content: DNA saliva kits or traditional blood collection: which is more cost effective?

Transport cost and sample stability comparison

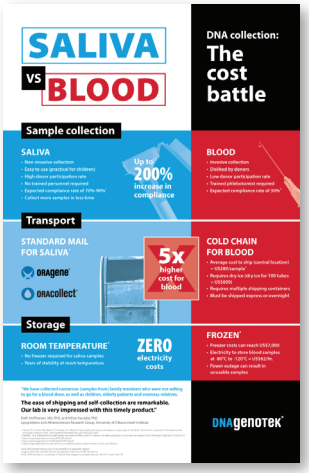
Sample stability during transportation is important and can become unpredictable with temperature fluctuations and delays. This is not a concern when using Oragene/ORAcollect devices, as samples are immediately stabilized at ambient temperature.

The blood must be refrigerated immediately and, if transported to a remote lab, specially packaged, labeled, and kept refrigerated during the journey. The samples must be labeled as a hazardous material for shipment. Handling liquid blood samples is an expensive process, often adding millions of dollars to the total cost of a clinical trial. Shipping a small, dry-iced biosample via FedEx within the United States can be over $175 per shipment.[18]

Some blood-specific all-in-one insulated shipping container packages can cost around $77 for only 5 tubes of blood; for 500 tubes of blood, the cost would be over $7,700. Simple containers, which will require added insulation, can be as expensive or even more costly.[19] Dry ice for 100 blood samples can cost over $600.[9] And because blood needs to be extracted soon after collection, samples must be shipped express/overnight to the lab to maintain DNA stability. Costs to ship to a central location in the U.S. can range from $40 to $60 via Express UPS[20] or over $175 via standard FedEx.[18]

Samples collected with Oragene/ORAcollect devices have been validated for room temperature storage and are robust enough to endure temperature fluctuations that might be experienced during transport. Oragene/ORAcollect samples have been proven to withstand 3 multiple freeze-thaw cycles ranging from -20°C to +50°C — ensuring optimal sample stability during transportation.[21] Oragene/saliva samples can be stored at room temperature for years without DNA degradation and shipped via regular mail (full shipping details are found here).

All in all, shipping blood samples is 5x more costly than shipping saliva samples.



[17] https://research.utoronto.ca/human-blood-sample-collection-research-purposes

[18] https://www.neoteryx.com/microsampling-blog/clinical-trials-when-costs-add-up

[19] https://www.uline.ca/BL_2157/Insulated-Shipping-Kits

[20] http://williamlabs.com/wp-content/uploads/2018/03/packmaxq-whitepaper.pdf

[21] https://www.sciencedirect.com/science/article/abs/pii/S1570023216314453

Storage cost and sample stability comparison

Researchers often want to biobank collected DNA samples for future research, sometimes for months or years.

Unstabilized samples may undergo changes if there is any delay between collection and freezing, although even an immediate freezing process at the point of collection is no guarantee of sample safety. One risk involved with frozen samples is unexpected power failures.

Blood can be suitably stored for many years at around -80°C; blood samples stored at higher temperatures, such as -20°C, can negatively impact DNA yield.[22] Storing blood samples at these temperatures adds significant freezer costs. A progress report on energy assessment for laboratories for Sustainability at Caltech reported that the average individual kWh per week for lab-grade freezers was 209.22 kWh, generating an estimated electricity cost of $1,632 per year.[23]

Freezing samples also incurs significant costs for equipment and energy. Saliva samples collected in Oragene and ORAcollect can be stored at ambient temperature, thereby eliminating the expense and complexity associated with frozen samples.

The Office of Campus Sustainability for the University of Michigan reported that ultra-low temperature freezers have significant operating costs and can account for 5% or more of a laboratory's electricity use, costing $750 to $1,000 per year.[24] These costs can be even higher if you need to redraw the samples from the freezer.[25] Purchase of a lab-grade freezer can cost up to $7,000.[9]

Annual electricity costs for lab-grade freezer = up to $1,000

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 ...[26]

[22] https://www.thermofisher.com/blog/biobanking/long-term-storage-impacts-blood-dna-yield-but-not-integrity-or-methylation/

[23] https://studylib.net/doc/18516547/progress-report---sustainability-at-caltech

[24] http://sustainability.umich.edu/media/files/ULT%20Freezer%20Flyer%20-%20Nov%202018.pdf

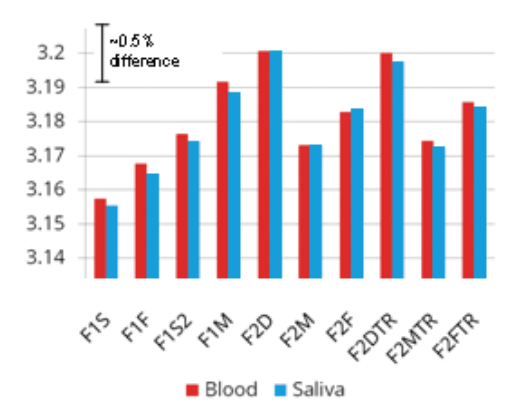
[25] NTNU HUNT and CONOR Project Price List. (2017). https://www.ntnu.edu/documents/140075/1268289603/Price+list NTNU+or+CONOR+assignmentsHUNT+biobank+2017.pdf/5f3fbb0a-a45a-4a74-a761-8f7364156e3b

[26] Daksis, J.I., Erikson, G.H. (2007). Heteropolymeric Triplex-Based Genomic Assay® to detect pathogens or single-nucleotide polymorphisms in human genomic samples. *PLoS ONE*. 2(3):e305

We now have the technical ability to get the wrong answers with unprecedented speed. If we put the wrong stuff into the front end of our analytical pipeline, we will not only lose the war on cancer, we'll pollute the scientific literature with incorrect data that will take us a long time to sort out. This is a crisis that requires disruptive innovation.

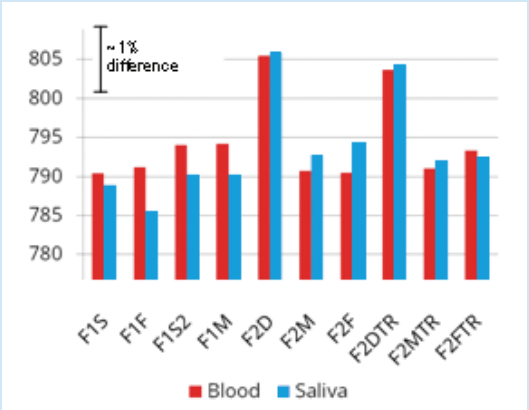
– Carolyn Compton, Biorepository Chief, National Cancer Institute, USA

Number of detected SNPs (millions) in each sample



Samples shown in both charts: family 1/2, member M/F/S/D. replicate TR.

Number of detected INDELs (millions) in each sample



Saliva and downstream applications

DNA sample quality is incredibly important when it comes to downstream applications. DNA from saliva collected with Oragene/ORAcollect is reliable for many downstream applications, such as:

- Single-nucleotide polymorphisms (SNPs)/copy number variation (CNV) microarrays
- Next generation sequencing (NGS)
- HLA typing
- Microsatellite analysis
- Whole genome amplification

Saliva DNA stabilized with Oragene/ORAcollect is comparable to blood for a variety of DNA analyses. Here are some examples of compatible downstream applications that are proven to be reliable with saliva:

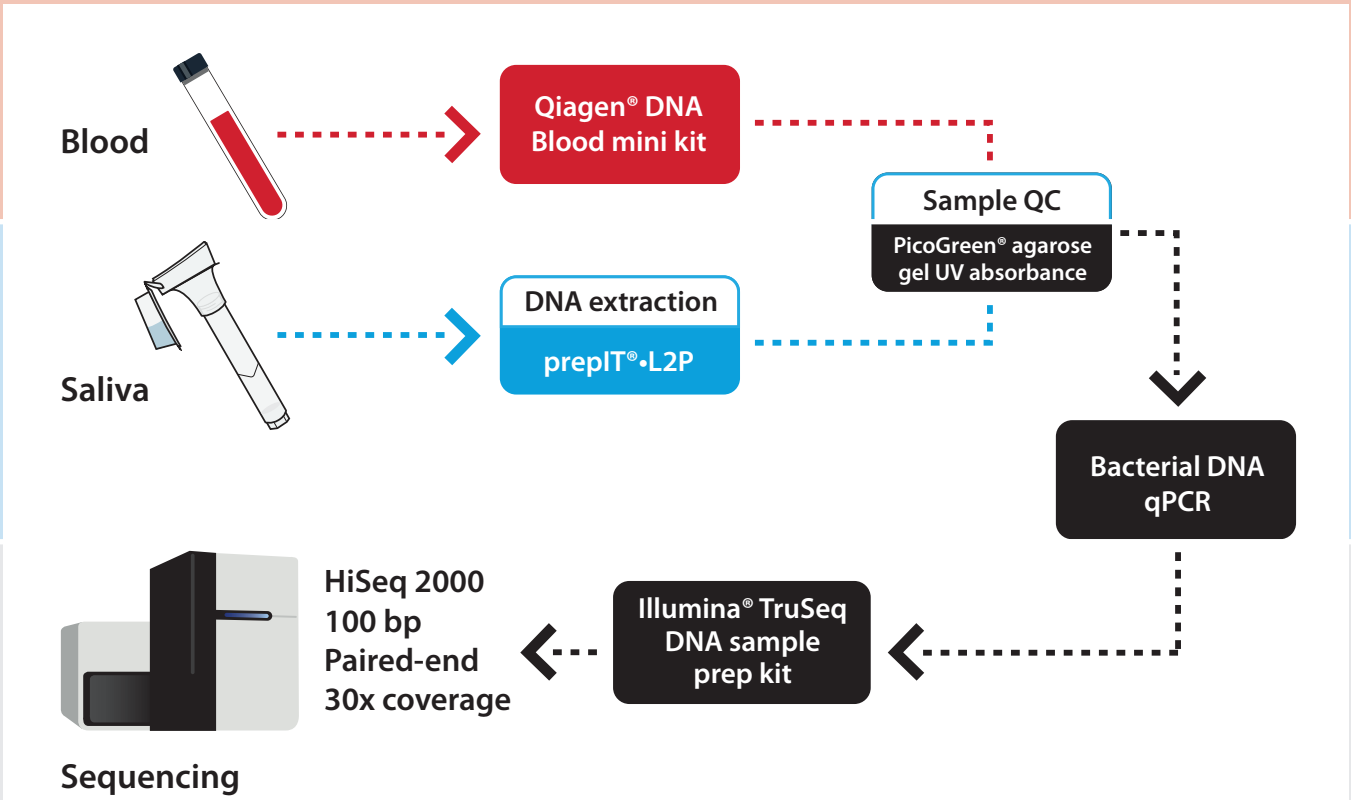
Whole genome sequencing (WGS)

Oragene/saliva samples yield human genomes comparable to blood samples with high quality results and low error rates. A study conducted by Seven Bridges and DNA Genotek compared blood and saliva samples in whole genome sequencing using Illumina's HiSeq 2000 100bp Paired-end 30x coverage.[27]

The team found:

- No significant difference in the total number of variants in single-nucleotide polymorphisms (SNPs) and INDELs (insertions and deletions) called from blood and saliva.
- Concordance differences in saliva/blood pairs are eliminated when blood data are down sampled to a coverage equal to saliva.
- Bacterial reads do not accumulate enough to affect mutation calling.

[27] https://www.dnagenotek.com/ROW/pdf/MK-00426.pdf



In the same study, the team investigated the source of unaligned reads in both the blood and saliva sample data.

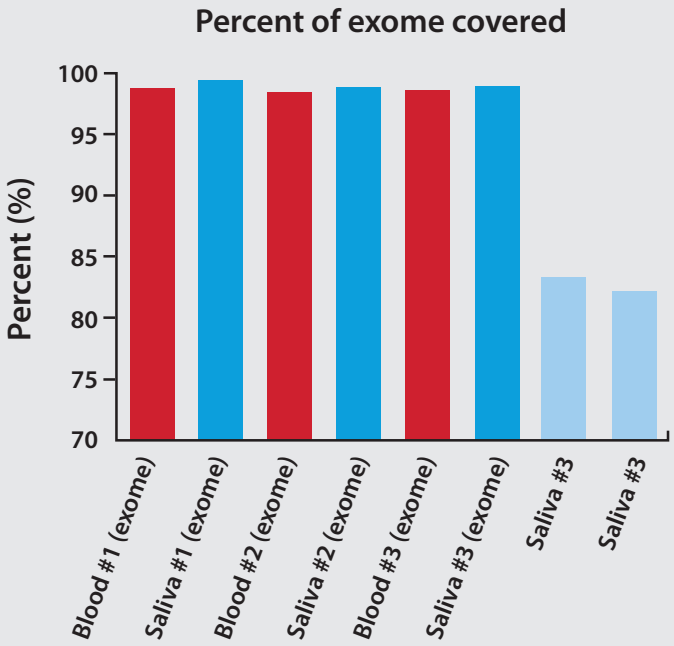
> ***We show that many of the reads failing to map to the human reference either align directly to species contained in the human microbiome database or bear similarities to other known bacterial and viral species. Overall, our analysis shows that there is no significant difference in variants detected between saliva and blood when samples are sequenced to the same coverage.***[28]

[28] https://www.dnagenotek.com/ROW/pdf/MK-00373.pdf

Exome sequencing

Saliva is compatible with Illumina Genome Analyzer II for whole exome sequencing (WES). A study was conducted using Oragene/saliva samples and blood to evaluate if saliva was a reliable source of DNA for next generation sequencing.

Exome enriched saliva and blood samples identified a similar mean number of variants: 28,738 from saliva and 28,067 for blood. 98.5% coverage of the exon regions were targeted by the SureSelect All Exon kit for both exome enriched saliva and blood samples.[29]



Single-nucleotide polymorphism (SNP) genotyping

Another study was conducted to investigate the use of genomic DNA extracted from saliva collected with Oragene•DNA self-collection kits for SNP and CNV analysis on Illumina BeadChip technologies. The performance compared paired blood and saliva samples to demonstrate the intra-donor reproducibility of the results.[30]

Donor	# CNVs in saliva		# Common CNVs in saliva replicates	# CNVs in blood	# Common CNVs in saliva and blood
	Replicate 1	Replicate 2			
1	26	24	23	13	13
2	16	21	15	9	7
3	17	18	15	10	9
4	18	21	16	8	7

Saliva intra-donor CNV reproducibility and saliva/blood CNV concordance on the Illumina Human1M-Duo.

- Greater than 90% reproducibility between saliva replicates.
- Greater than 80% reproducibility between paired blood and saliva.
- Saliva collected using the Oragene•DNA self-collection kit provides genomic DNA of sufficient quality for genotyping on the Illumina Human610-Quad and both genotyping and CNV analysis on the Human1M-Duo BeadChip arrays.
- Both saliva and blood samples performed better on the Human1M-Duo.
- DNA from saliva does not vary over time, as demonstrated through intra-donor genotyping concordance and CNV reproducibility of samples taken from the same donor on different days.
- DNA from saliva generates highly concordant data compared with DNA from blood for the same donor, as demonstrated by the genotyping concordance and CNV reproducibility.

[29] https://www.dnagenotek.com/ROW/pdf/MK-00014.pdf

[30] https://www.dnagenotek.com/ROW/pdf/MK-008.pdf

HLA typing

Saliva is compatible with Illumina® HiSeq™ 2000 for HLA typing using NGS. Blood and saliva samples were sequenced in an internal study for HLA typing to evaluate the performance of DNA from Oragene/saliva samples compared to DNA from blood samples collected from the same individuals.[31]

Sequencing metrics from Illumina HiSeq 2000

illumina®

Donor	1		2		3		4	
Sample Type	**Saliva**	**Blood**	**Saliva**	**Blood**	**Saliva**	**Blood**	**Saliva**	**Blood**
Yield (Mb)	3,989	3,810	3,886	3,830	4,005	3,594	3,246	3,550
% > = Q30 bases	86.9	87.4	87.0	87.2	87.5	86.9	87.3	87.4
Mean quality score	34.4	34.5	34.4	34.5	34.6	34.4	34.5	34.6
Mean coverage	133.61	148.96	164.07	189.54	159.85	164.75	108.12	130.21

- Prepared saliva and blood libraries were of equivalent quality (see table above).
- Samples were successfully barcoded and multiplexed in a single sequencing run.
- Saliva and blood had similar mean quality scores of approximately 34.5.
- Mean coverage for both saliva and blood exceeded 100.
- HLA call concordance between saliva and blood was 100%.
- HLA calls were 100% concordant with previously reported results for these donors using current HLA-typing methodologies.

This study illustrates that DNA from Oragene/saliva samples is a dependable alternative to blood for HLA typing, including Next Generation Sequencing applications. In agreement with previous exome and whole genome sequencing studies we demonstrated that Oragene/saliva samples are a reliable source of DNA for Next Generation Sequencing applications.[31]

[31] https://www.dnagenotek.com/ROW/pdf/MK-00111.pdf

SO ...

CAN SALIVA REPLACE BLOOD for DNA collection and analysis?

Absolutely.

In fact, saliva has begun to replace blood already; it's been adopted by more than **6,000** researchers in over **100** countries and in hundreds of hospitals worldwide.

Saliva is increasingly becoming a standard practice in health care and research for DNA-based sample collection, especially when collection is required for children, for donors who are difficult to collect from and for anyone who cannot easily access a blood clinic.

DNAgenotek®

www.dnagenotek.com