PacBio

Application note

High-molecular-weight DNA extraction from human blood and saliva using Nanobind kits for HiFi long-read sequencing

Blood and saliva are attractive sample types for large-scale genomic and clinical research studies, where ease of collection, storage, and processing are critical. Extracting high-quality DNA from these sample types is key for optimal sequencing performance, particularly for highly accurate long reads generated with PacBio® HiFi sequencing.

The comprehensiveness of HiFi long reads performs best with long molecules, requiring purified high-molecular-weight (HMW) DNA from robust extraction methods like PacBio Nanobind® technology. Nanobind extraction technology enables consistent recovery of high-quality HMW DNA from both blood and saliva, supporting high-performance sequencing on standard sample types.

This application note demonstrates that DNA extracted from whole blood and saliva samples using the Nanobind kits yields high-quality HiFi sequencing data. Paired blood-saliva samples from the same subjects were analyzed in parallel to show similar HiFi sequencing metrics and high concordance in variant calling performance. Together these results demonstrate the end-to-end performance of the PacBio portfolio in generating the high-quality genomic data needed to support population-scale clinical research.

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	Blood	Saliva
Collection method	Invasive and requires medical professionals	Non-invasive and self-collected
Sample storage / shipment conditions	Cold (-20°C)	Room temperature
DNA yield	200 µL: 1 to 12 µg 1 mL: 3 to 70 µg	~1 to ~45 µg
Sequencing output	104–122 Gb HiFi yield/Revio® SMRT® Cell	98–125 Gb HiFi yield/Revio SMRT® Cell
% human mapped read	99.9 % human mapped read	75 to 95 % human mapped read
Ideal project types	• Research hospitals • Large-scale projects where it is possible to collect blood and store it frozen • Projects with low tolerance to sample variability	• Population genetic studies (higher participation rates compared to blood) • Remote studies where no cold chain is available • Studies with subject aversion to blood sampling • Interest in salivary microbiota characterization

Table 1. Overview of DNA extraction from human blood and saliva samples with Nanobind kits.

Nanobind extraction workflow

PacBio Nanobind kits are optimized to extract HMW DNA molecules across sample types. Nanobind magnetic disk technology protects HMW DNA from shearing during the extraction process. Nanobind technology enables manual extraction from various sample types and high-throughput extraction from blood using Thermo Fisher and Hamilton systems. The Nanobind DNA extraction workflow follows four basic steps:

1. First, samples are lysed using a specific buffer optimized for each sample type.
2. DNA binds to Nanobind disks, which shield the DNA from damage during the extraction process.
3. DNA is washed three times with ethanol-based buffers, removing contaminants.
4. Finally, DNA is released from the Nanobind disk during elution without fragmentation, resulting in HMW DNA.

Figure 1. PacBio sample prep workflow with Nanobind kits.

The whole blood extraction workflow can be automated using the KingFisher Presto magnetic head (24 or 96 pins depending on sample volume) to handle the magnetic disk from one plate to another. PacBio has developed scripts for the Thermo Fisher KingFisher Apex and Hamilton NIMBUS Presto systems. KingFisher Apex is semi-automated, with manual plate filling and one user interaction during the run. NIMBUS Presto is a walkaway solution with automated plate filling. Both systems can extract up to 96 samples per run with a duration time of 2–2.5 hrs. Sequencing performance is consistent across manual and automatic extraction.

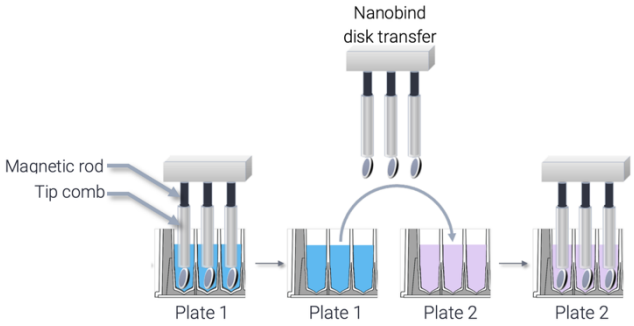

Figure 2. For automated Nanobind HT extraction kits, each plate contains a different buffer or sample solution. The Nanobind disk is transferred from one plate to another and mixed.

	Thermo Fisher KingFisher Apex	Hamilton NIMBUS Presto
Instrument design	Benchtop sample purification system	Robotic liquid handler with integrated KingFisher Presto sample purification system
Samples per run	24 to 96	24 to 96
Hands-on time	45 min	20 min
Total time	~2 hrs	~2.5 hrs
Max throughput per week	1920 samples	1920 samples

Table 2. Workflow overview of automated extraction with Thermo Fisher and Hamilton systems.

Figure 3. KingFisher Apex system (top) and NIMBUS Presto system (bottom).

Blood samples

Whole blood is a common and easily accessible source of DNA that, with proper handling, provides high-quality input for PacBio HiFi sequencing.

Best practices for handling human whole blood samples

Blood collection anticoagulant: HiFi sequencing performance is best for samples stored with potassium EDTA (K2 EDTA) as an anticoagulant. Samples stored in sodium heparin (NaHep) and citrate (NaCit) also perform well in limited testing.

Storage: Inappropriate storage temperature can cause DNA degradation. The amount of DNA extracted and HiFi sequencing performance (yield, read length) decline if samples are not stored in low temperatures following collection. Samples can be stored at 4°C for a maximum of 2 days or at -20°C for a longer period of time. No systematic differences have been observed in DNA QC or sequencing results between fresh and frozen blood samples. Blood samples must be refrigerated or frozen as quickly as possible after being drawn. Blood samples should be aliquoted to avoid repeated freeze-thaws.

White blood cell (WBC) counts: The amount of DNA extracted increases proportionally to WBC count (Figure 4). WBC counts above 4×10^6 cell/mL yield more than 3 µg of DNA.

Input requirements and Nanobind kits used

Starting blood volume:

- 200 µL for manual extraction using Nanobind CBB (PN 102-301-900) or PanDNA (PN 102-762-700) kits
- 200 µL for HT extraction using Nanobind HT CBB kit (PN 102-762-700) – up to 96 samples per run
- 1 mL using Nanobind HT 1 mL blood kit (PN 102-762-800) – up to 24 samples per run

Blood DNA extraction results

Extracted from 200 µL human whole blood will yield between 1 to 12 µg of DNA and 1 mL extraction will yield 3 to 70 µg. Purity ratio 260/280 is between 1.8 to 2.0 and 260/230 ratio can vary from 1.3–2.2 (Figure 5). Samples with UV purities within the expected range should sequence well. The mode size of DNA extracted from human whole blood measured with the Femto Pulse system (Agilent Technologies) is typically 100 kb+.

To demonstrate HT extraction performance, we used 96 samples of 200 µL whole blood each from 8 subjects (12 replicates). Subjects were selected to represent normal physiological range WBC counts. From extraction on the Hamilton NIMBUS Presto, we obtained consistent DNA recovery and purity), indicating high reproducibility across replicates.

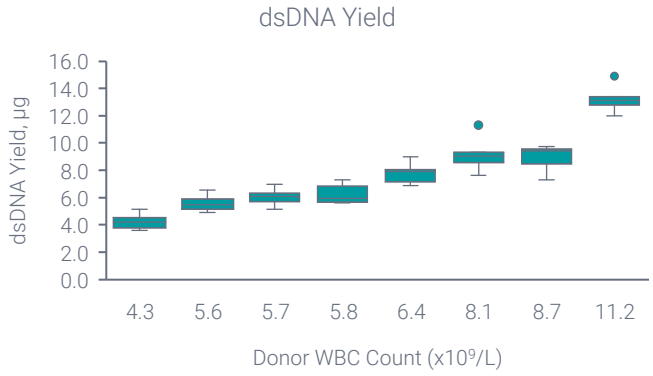

Figure 4. DNA extraction yield from 200 µL of whole blood versus WBC count using the Nanobind HT CBB kit on the Hamilton NIMBUS Presto system on 8 donors with 12 replicates.

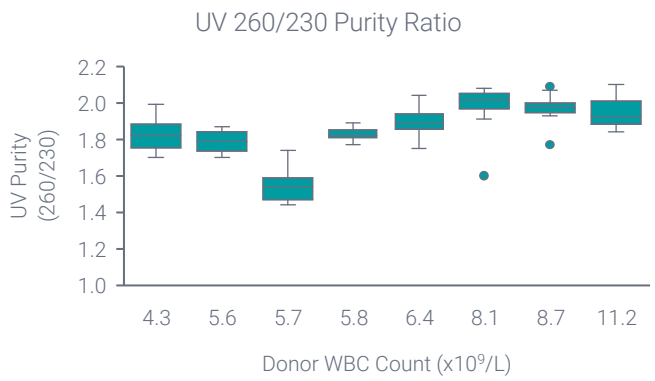

Figure 5. DNA 260/280 purity ratio from 200 µL of whole blood versus WBC count using the Nanobind HT CBB kit on KingFisher Apex system on 8 donors with 12 replicates.

The same blood samples were extracted in parallel on KingFisher Apex and NUMBUS Presto system from 1 mL whole blood (Figure 6). Yield varied from 5.5 to 65 µg and correlated with WBC count.

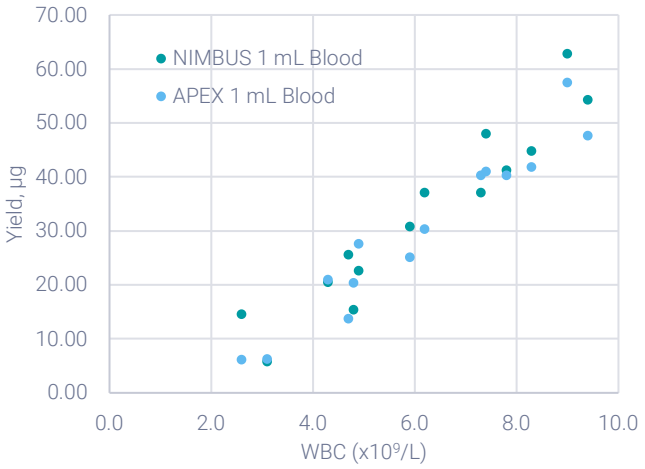

Figure 6. DNA extraction yield from 1 mL of whole blood versus WBC count using the Nanobind HT 1 mL blood kit on Apex and NUMBUS Presto systems.

Saliva samples

Saliva is an attractive sample type because it is non-invasive, can be self-collected, and is stable at room temperature. Despite these advantages, variation in DNA quality and yield have prevented widespread use with long-read sequencing, where high-quality DNA is key for optimal performance.

The optimal sequencing results presented here from DNA samples collected and stabilized in DNA Genotek™ Oragene™ devices (Figure 7) and extracted using the Nanobind CBB or PanDNA kits demonstrate saliva as a viable sample type for HiFi long-read sequencing.

Figure 7. Oragene saliva collection device.

Best practices for saliva samples

Oragene saliva collection device: PacBio saliva extraction protocol is compatible with Oragene•DISCOVER and Oragene•Dx devices which stabilize collected saliva at room temperature. The stabilization solution in the Oragene device lyses cells in saliva and stabilizes the DNA by preventing chemical and enzymatic DNA degradation and inhibiting bacterial growth. DNA is stabilized in solution, allowing the most straightforward HMW DNA isolation.

Sample collection guidelines: Improper saliva sample collection may lead to low DNA yields. Ensure the samples are collected according to the manufacturer's instructions for use that are included with the Oragene saliva collection devices (see instructional video here). In particular, subjects should:

- Avoid eating, drinking, smoking, chewing gum, or brushing their teeth for 30 minutes prior to collecting a saliva sample.
- Ensure the proper volume of saliva is collected as indicated by the 'Fill To' line on the Oragene device.

Shipping and storage conditions: Saliva samples collected in Oragene devices are stable at room temperature (RT) from 1 year (Dx) to 5 years (DISCOVER). Samples can be shipped at RT by standard post mail.

Extraction yield: Saliva samples are inherently complex, containing a proportion of DNA from microbes. DNA from stabilized saliva samples is generally ~75–95% human, whereas DNA from buccal samples can be as low as 10% human. The majority of DNA isolated from saliva comes from white blood cells in contrast to

epithelial cells from buccal samples. Due to individual biological variation, saliva gDNA yields are more variable than yields from blood, with ~1–45 µg of HMW DNA typically obtained from a 500 µL aliquot of stabilized saliva sample. Saliva collected and stabilized in the Oragene tube buffer must contain >2 µg of DNA in 500 µL for efficient Nanobind extraction. DNA content can be checked prior to extraction with the Qubit dsDNA BR (broad-range) assay.

Input requirements and Nanobind kits used

- Initial 50°C incubation needs to be performed once per sample. Saliva samples must be stored at RT. Do not refrigerate.
- Saliva DNA extraction is performed from 500 µL of stabilized sample using Nanobind CBB (PN 102-301-900) or PanDNA (PN 102-762-700) kits.

Saliva DNA extraction results

DNA extracted from 500 µL of stabilized saliva will typically yield between ~1 to ~45 µg depending on the subject. Improper saliva sample collection may lead to low DNA yields. Purity ratio 260/280 is between 1.4 to 2.0. and 260/230 ratio can vary from 1.0–1.7. Samples with UV purities within the expected range should sequence well. UV purities outside of these ranges may indicate abnormalities in the extraction process. The mode size of extracted human saliva DNA measured on the Femto Pulse system (Agilent Technologies) is typically 80 kb+ with 70% or more of the DNA molecules ≥10 kb. This corresponds to a genome quality number (GQN) of 7.0 or higher at 10 kb.

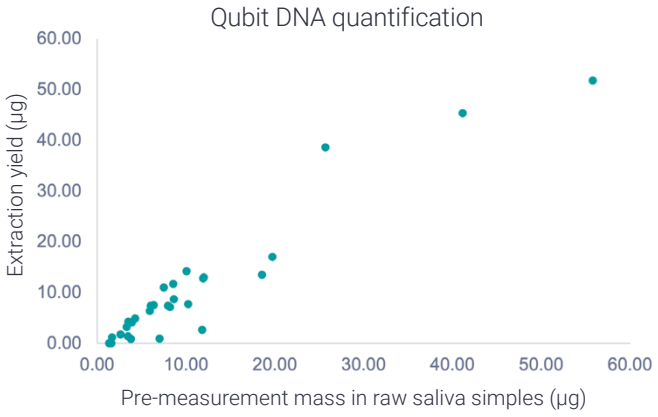

Figure 8. Extraction yield versus pre-measurement mass in raw samples. Pre-analytical measurement is predictive of DNA mass recovered.

Saliva samples were collected from 30 subjects, 90% of which had pre-analytical DNA mass >2 µg. HMW DNA was extracted from 27 samples with 93% yielding >500 ng (Figure 8). Following extraction, DNA was quantified using the Qubit dsDNA BR assay kit and characterized using the Femto Pulse system (Agilent Technologies) (Figure 9).

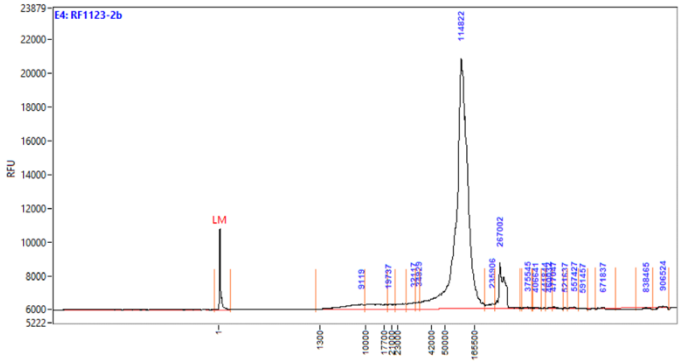

Figure 9. Size distribution of HMW saliva DNA extracted with Nanobind kits and quantified on the Femto Pulse system.

Blood and saliva whole genome sequencing (WGS) performance

HiFi WGS library prep for sequencing on the Revio system with SPRQ™ chemistry requires a minimum of 500 ng of HMW DNA, with 70% or more of the DNA ≥10 kb. All extracted blood and saliva samples matched this requirement. Results presented here are for samples sequenced from manual extraction, but consistent results can be obtained with HT automated extraction.

We sequenced 5 paired whole blood (200 µL) and saliva samples collected from 5 subjects and extracted using Nanobind kits. HiFi WGS libraries were prepared using the SMRTbell® prep kit 3.0 and sequenced on the Revio system using SPRQ chemistry. Each sample was sequenced on a single Revio SMRT Cell. Table 3 summarizes DNA QC and sequencing data for the 10 samples. Samples yielded between 1.88 and 13.6 µg of HMW DNA. The HiFi sequencing yield was 98 to 135 Gb of HiFi data resulting in 30x to 39x coverage per genome, sufficient for comprehensive WGS variant detection. For saliva samples, between 93% to 98% of reads mapped to the human reference genome (GRCh38).

Donor	Sample type	DNA yield (µg)	HiFi yield (Gb)	HiFi mean length (kb)	Median HiFi QV	Human mean coverage	% Human read mapped
Donor A	Blood	4.1	122	13.8	Q37	39	99.95
	Saliva	8.8	101	13.9	Q35	30	92.69
Donor B	Blood	2.0	113	14.4	Q36	36	99.95
	Saliva	6.2	98	14.2	Q34	30	96.25
Donor C	Blood	1.9	104	13.7	Q37	33	99.94
	Saliva	13.6	126	15.4	Q34	39	96.23
Donor D	Blood	4.6	119	14.8	Q35	38	99.95
	Saliva	9.5	125	14.8	Q35	39	98.2
Donor E	Blood	3.1	114	13.7	Q37	36	99.95
	Saliva	8	126	13.7	Q35	39	98.08

Table 3. Sequencing data for paired blood and saliva samples extracted with Nanobind kits.

Paired saliva and blood samples were assessed for genotype concordance of high-quality (GQ≥20) variants across GRCh38 without masking. Each sample was analyzed as a singleton using v3a1 (code is available at the PacBio GitHub page). We observed high variant calling concordance between paired blood and saliva samples from the same subject as shown in Figure 10.

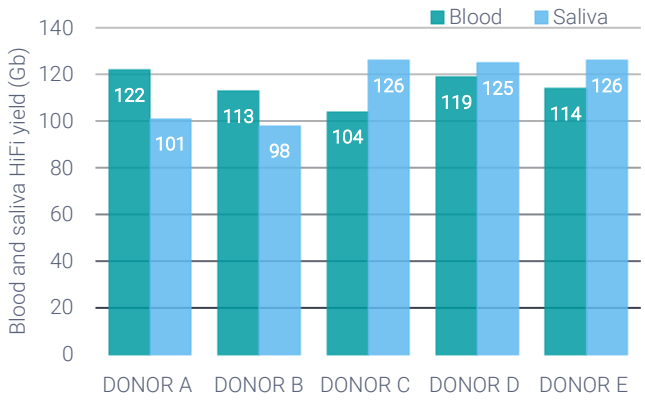

Figure 10. Paired saliva and blood samples show similar HiFi yield within subjects.

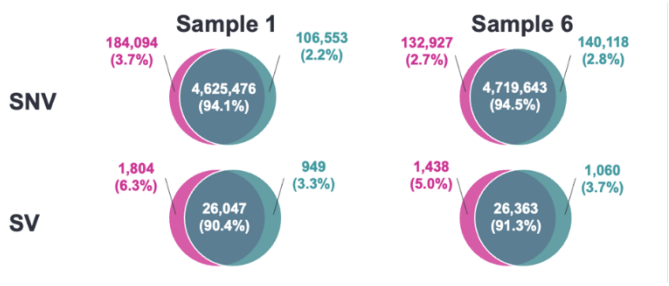

Figure 11. Results for two representative samples #1 and #6 from the same donor. For comparison, a technical replicate of blood from the same subject had concordance of 94.1% for SNVs and 91.5% for SVs, while blood samples from two different donors had concordance of 21.7% for SNVs and 44.6% for SVs (not shown).

Conclusion

Sample preparation is a critical factor that impacts sequencing yield, read length, and ultimately performance of variant calling and genome assembly for WGS projects. These results demonstrate Nanobind extraction technology as a reliable method for achieving optimal HiFi sequencing performance from whole blood and saliva samples.

Blood is already a well-established and reliable sample type, yielding high quality DNA. This is ideal for projects that require especially low failure rates, as in pediatric research. However, saliva is gaining traction as a non-invasive, convenient, and cost-effective sample alternative with comparable HiFi performance. This sample type is particularly promising for standard genetic testing, population-scale screening, and when convenience, accessibility, and non-invasive collection are priorities.

References

Guides & overviews – Nanobind HT kits | Nanobind CBB kit | Nanobind PanDNA kit

Protocol overviews – Nanobind HT kits | Nanobind CBB kit | Nanobind PanDNA kit

PacBio extraction automation scripts

Technical note – HiFi sequencing performance of saliva DNA samples collected with DNA Genotek Oragene devices and extracted using Nanobind kits

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