Technical note

HIFI SEQUENCING PERFORMANCE OF SALIVA DNA SAMPLES COLLECTED WITH DNA GENOTEK ORAGENE DEVICES AND EXTRACTED USING NANOBIND KITS

Introduction

In recent years, saliva has emerged as an attractive DNA source for large-scale genomic and clinical research studies due to its ability to be non-invasively self-collected and stabilized at room temperature. Despite these advantages, apprehensions surrounding DNA quality and yield have delayed its use with long-read sequencing, where high-quality, high-molecular-weight (HMW) DNA is key for optimal yield and performance.

In this technical note we describe PacBio® HiFi sequencing results for saliva DNA samples collected and stabilized in DNA Genotek™ Oragene™ devices and extracted using the Nanobind® PanDNA or CBB kits.

The Nanobind kits contain reagents for cell lysis and gDNA extraction from saliva. Following lysis, the DNA binds to Nanobind disks protecting it from damage during extraction, resulting in HMW DNA. A Short Read Eliminator (SRE) size selection step helps remove DNA fragments below 10 kb as part of the library preparation workflow. The extraction workflow takes approximately 2 hours (25 min hands-on time). Salivary DNA stabilized in Oragene devices, extracted using the Nanobind kits and sequenced on a single SMRT® Cell on the Revio® system typically generates >20-fold HiFi coverage of the human genome, which is sufficient for comprehensive variant calling or ***de novo*** assembly.

Workflow recommendation and overview

PacBio saliva extraction protocol is compatible with Oragene•DISCOVER and Oragene•Dx devices which stabilize collected saliva at room temperature from 1 year (Dx) to 5 years (DISCOVER). 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.

Figure 1. *Oragene device and Nanobind kit*

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 (not from buccal epithelial cells). 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.

DNAgenotek™ PacBio

For optimal Nanobind DNA extraction, sufficient DNA mass of >2 µg of DNA in 500 µL saliva is required in the starting sample. To ensure adequate DNA mass, we recommend a preliminary quantification of DNA concentration directly from the stabilized saliva samples using the Qubit dsDNA BR Assay Kit prior to extraction. This pre-measurement step will give an approximative value of DNA content (result shown in figure 3). Insufficient DNA yield during extraction is often linked to improper saliva collection. Factors such as consuming food or drink within 30 minutes of collection, difficulty producing serous saliva, or providing an inadequate volume can significantly impact DNA recovery, making proper collection of saliva into Oragene devices essential.

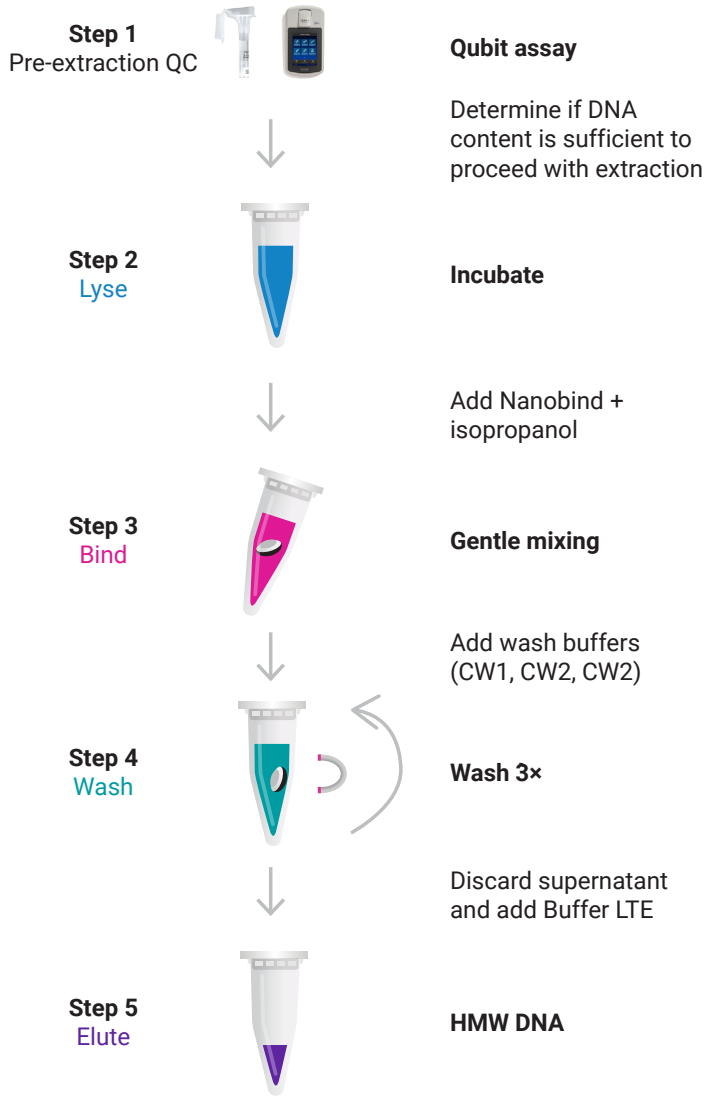

Figure 2. *Nanobind extraction workflow for a saliva sample collected in an Oragene device.*

DNA QC metrics and sequencing results

Saliva samples were collected from 30 donors, 90% of which had pre-analytical DNA mass >2 µg. HMW DNA was extracted from 27 samples with 93% yielding >500 ng. Following extraction, DNA was quantified using the Qubit dsDNA BR assay kit and characterized using the Femto Pulse system (Agilent Technologies). HiFi libraries were prepared for a subset of samples 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 1 summarizes the sequencing data for five representative samples. The samples yielded between 4.7 and 15.9 µg of HMW DNA. The HiFi sequencing yield was 119 to 133 Gb of HiFi data resulting in 27x to 40x coverage per genome, sufficient for comprehensive WGS variant detection. Between 75% to 95% of reads mapped to the human reference genome (GRCh38).

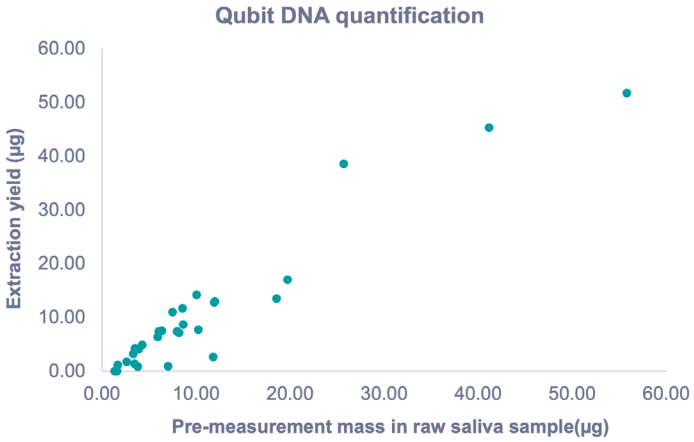

Figure 3. *Extraction yield versus Pre-measurement mass in raw sample. Pre-analytical measurement is predictive of DNA mass recovered.*

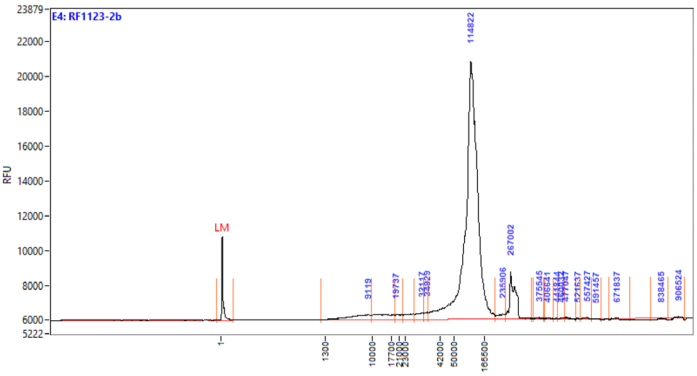

Figure 4. *Size distribution of HMW saliva DNA extracted with Nanobind kits and quantified on the Femto Pulse system.*

Sample	DNA yield (µg)	GQN_{10kb}	HiFi yield (Gb)	Mean HiFi read length (bp)	Median HiFi QV	Mean coverage	% Reads mapped
1	**10.0**	8.2	**135**	15551	Q35	**37×**	87%
2	**15.9**	7.9	**125**	13068	Q36	**32×**	83%
3	**5.9**	7.4	**119**	12069	Q35	**27×**	75%
4	**11.5**	8.6	**134**	14387	Q34	**40×**	95%
5	**5.7**	9.1	**135**	13892	Q35	**40×**	95%

Table 1. *Sequencing data for saliva samples prepared with Nanobind kits.*

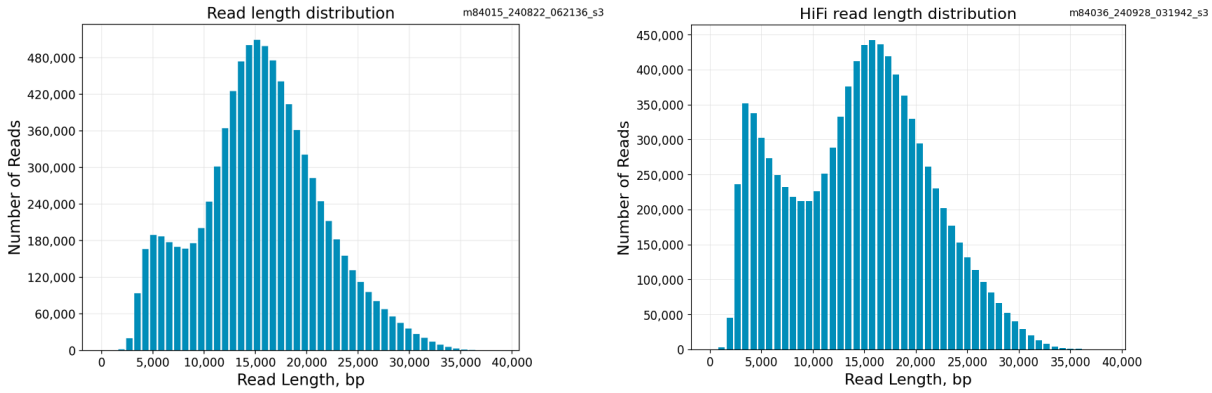

Figure 5. *HiFi read length distribution plot for saliva sample 1 (135 Gb HiFi) and sample 4 (134 Gb HiFi) sequenced on the Revio system. Both samples had human genome coverage > 37x*

Conclusion

This technical note demonstrates the HiFi sequencing performance of saliva samples collected and stabilized with Oragene devices and extracted using Nanobind kits. These results show that saliva can be a viable alternative to blood for obtaining high-quality long-read sequencing data. The non-invasive nature of saliva, the ability to self-collect samples using the Oragene devices, and their stability during storage and transportation at ambient temperature enables more efficient, cost-effective recruitment for large studies and facilitates the use of long-read sequencing in population genomics studies that leverage saliva samples.

REFERENCES

1. Procedure & checklist – Extracting HMW DNA from saliva collected in DNA Genotek Oragene devices using Nanobind kits
2. Procedure & checklist – Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0
3. Guide & overview – Nanobind PanDNA kit
4. Guide & overview – Nanobind CBB kit
5. Brochure – Revio system
6. Oragene™ Dx (OGD-600) datasheet
7. Oragene™ DISCOVER (OGR-600) datasheet

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