Optimized DNA extraction from low-mass samples for HiFi long-read sequencing

Deborah Moine, Sarah Kingan, Christine Lambert, Juniper Lake, Vicky Liu, Lin Wang, Jeff Zhou, Shreyasee Chakraborty, Davy Lee, Heather Ferrao, PacBio, 1305 O'Brien Drive, Menlo Park, CA 94025

Introduction

Non-invasive sample types such as saliva, buccal, and dried blood spots (DBS) are essential for scaling genomic research. However, conventional extraction workflows often yield fragmented, low-input DNA — limiting their suitability for HiFi long-read sequencing, which requires high-quality, high-molecular-weight DNA.

Here, we present:

Optimized workflows for saliva and buccal samples, enabling improved HMW DNA recovery and long-read sequencing performance.

An exploratory evaluation of DBS samples, assessing feasibility and defining future workflow development.

In this poster, "buccal" refers to saliva samples collected using ORAcollect™ devices.

Saliva collection Plan (n = 5 per group)

- **Group 1:** Adults (>18 years), A1 to A5
- **Group 2:** Children (4–10 years), C1 to C5
- **Group 3:** Toddlers (1–3 years), T1 to T5
- **Group 4:** Infants (3 months–1 year), I1 to I5
- **Group 5:** Newborns (0–3 months), N1 to N5

HMW DNA extraction

- **Saliva samples** were collected using Oragene™ or ORAcollect™ devices from DNA Genotek. HMW DNA was extracted from 500 µL saliva using a modified Nanobind CBB workflow. **Saliva and buccal yield from 1 to 7.5 µg of DNA**
- **DBS samples** were collected on blood cards, and DNA was isolated from 6–8 × 3 mm punches using Nanobind CBB or QIAGEN MagAttract modified workflows. **DBS yield: 0.2 to 0.5 µg of DNA**

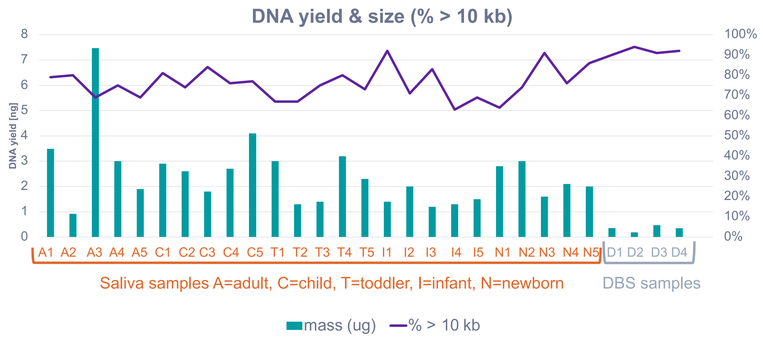

Figure 1. DNA yield from Saliva (1 to 7.5 µg) and DBS (184-476 ng) and DNA GQN at 10 kb measurement on Femto pulse system, from saliva (63% to 92% > 10 kb) and DBS (90% to 94% > 10 kb)

HiFi sequencing: WGS and PureTarget

	Whole Genome Sequencing	PureTarget Sequencing
Workflow	HiFi library preparation, native DNA sequencing of whole genome	Library preparation using the CRISPR-Cas9 system to target without PCR
Input material	500 ng of HMW DNA (GQN 10kb > 7)	1 µg of HMW DNA (GQN 30kb > 5)
Coverage	20X	≥100X on targeted region
Result	Comprehensive variant detection and methylation for whole genome	Comprehensive variant detection and methylation for targeted region
Throughput	1 to 2 samples per Revio SMRT Cell	Up to 96 samples per Revio SMRT Cell
Cost	~ 400 USD	~ 100 USD
TAT	2 days for 4 to 8 samples	3 days for 96 samples

WGS result

HiFi WGS libraries were prepared following standard PacBio procedures and sequenced using SPRQ chemistry on the Revio system.

Saliva samples were multiplex by donor age group to evaluate read length and % human DNA (typically ~75-95% human), unmapped reads were used to classify the salivary microbiome using sourmash tool (https://github.com/PacificBiosciences/pb-metagenomics-tools/tree/master/Taxonomic-Profiling-Sourmash). Subset of saliva samples were sequenced on one SMRT Cell.

DBS samples SRE step was skipped, as they yielded < 500 ng of DNA and DNA repair step was extended to 30 min at 52C

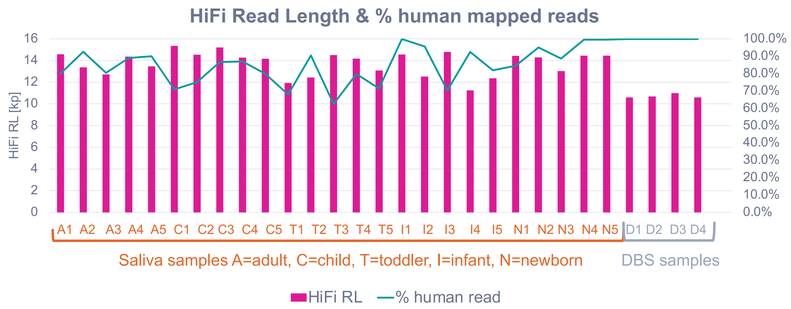

Figure 2. HiFi read length for saliva (11.2 to 14.8 kp) and DBS (~11 kp) and percentage of human mapped reads (70% to 99% for saliva and 99.9% for DBS).

Group	**newborn**
Sample	N5
Age of the participant	1 month
Method of collection	OCD-100A
Reads	8.44 M
Length	**14,289 bp**
Yield	**120.6 Gb**
Human mapped reads	99%
Mean Coverage	**38.4 X**

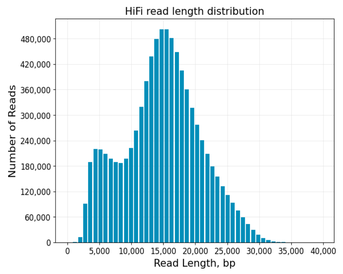

Figure 3. HiFi sequencing metrics for one SMRT Cell and HiFi Read Length distribution plot

Figure 4. Phylum-level composition of N-S5 is predominantly Bacillota (82%), with Actinomycetota (12%), Pseudomonadota (5%), and Other taxa (1%)

PureTarget result saliva and buccal

To assess cost-effective targeted approaches using non-invasive samples, saliva and buccal DNA were directly compared using the PureTarget carrier panel (12 genes) on the Revio system (SPRQ chemistry).

Nine-plex saliva and eight-plex buccal libraries were prepared using PureTarget 24 manual protocol with 2 µg per sample, followed by target enrichment analysis on SMRT Link to obtain sample target coverage.

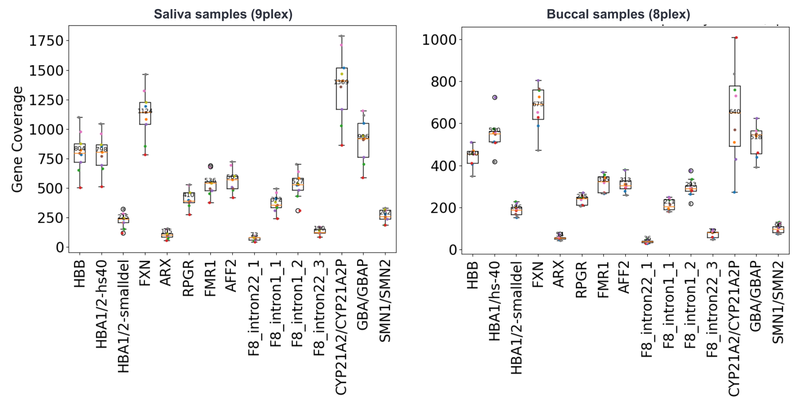

Fig 5. Coverage in PureTarget carrier panel across 9 saliva and 8 buccal samples

Disease	Gene	Mean coverage saliva	Mean coverage buccal
Fragile X syndrome, FRAXE type	AFF2	~583	~313
Early-infantile epileptic encephalopathy (EIEE1) and Partington syndrome (PRTS)	ARX	~105	~54
Congenital adrenal hyperplasia (CAH)	CYP21A2/CYP21A2P	~1369	~640
Hemophilia A	F8	~73 to ~527	~36 to ~283
Fragile-X disease (FXS)	FMR1	~536	~315
Friedreich ataxia (FRDA)	FXN	~1124	~675
Gaucher disease	GBA/GBAP1	~906	~518
Alpha thalassemia (AT)	HBA1/2	~798 / ~225	~550 / ~186
Sickle Cell Anemia and Beta thalassemia	HBB	~804	~440
X-linked retinitis pigmentosa	RPGR	~410	~235
Spinal muscular atrophy	SMN1/2	~262	~98
Classical-like Ehlers-Danlos syndrome	TNXB	~1369	~640

Fig 6. Saliva provided increased coverage across targets, but buccal samples also delivered reliable performance, indicating that both non-invasive sample types are compatible with PureTarget workflows.

Conclusion

- Optimized workflows enable high-quality HMW DNA recovery from saliva and buccal samples, supporting robust HiFi long-read WGS and PureTarget targeted sequencing.
- Exploratory DBS results support continued expansion into low-input applications.

Acknowledgements

The authors would like to thank DNA Genotek team who helped collecting samples used to generate data for the poster.

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