High molecular weight gDNA extraction from human saliva

19th August 2019

This protocol describes a method to extract high molecular weight genomic DNA from human saliva. The saliva was pooled from multiple individuals to account for variability among samples, and stored in an Isohelix GeneFiX™ Saliva DNA Collection Device. The extraction was carried out using the QIAGEN Blood and Cell Culture DNA Midi Kit followed by size selection with the size selection of HMW DNA by semi-selective DNA precipitation protocol. Sequencing performance was assessed using the MinION.

Materials

- 1 ml of human saliva
- Isohelix GeneFiX™ Saliva DNA Collection Device
- QIAGEN Blood and Cell Culture DNA Midi Kit
- QIAGEN RNase A
- Proteinase K
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water
- Isopropanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8)
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Refrigerated centrifuge with capacity for 15 ml Falcon tubes
- Vortex mixer
- Incubator or water bath with capacity for 37°C and 50°C

Method

Step 1

Collect 1 ml of saliva and store it at room temperature in an Isohelix GeneFiX™ Saliva DNA Collection Device until ready to extract the DNA.

Step 2

Transfer 1 ml of saliva to a 15 ml Falcon tube and add 19 µl of RNase A.

Step 3

Add 5 ml of Buffer G2 and 95 µl of Proteinase K to the tube, and mix by inverting the tube.

Step 4

Incubate the tube for 15 minutes at 37°C, and then for 1 hour at 50°C.

Step 5

Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.

Step 6

Pour the lysate through the column.

Step 7

Purify the lysate according to the standard protocol (steps 3–6, pages 50–52).

Step 8

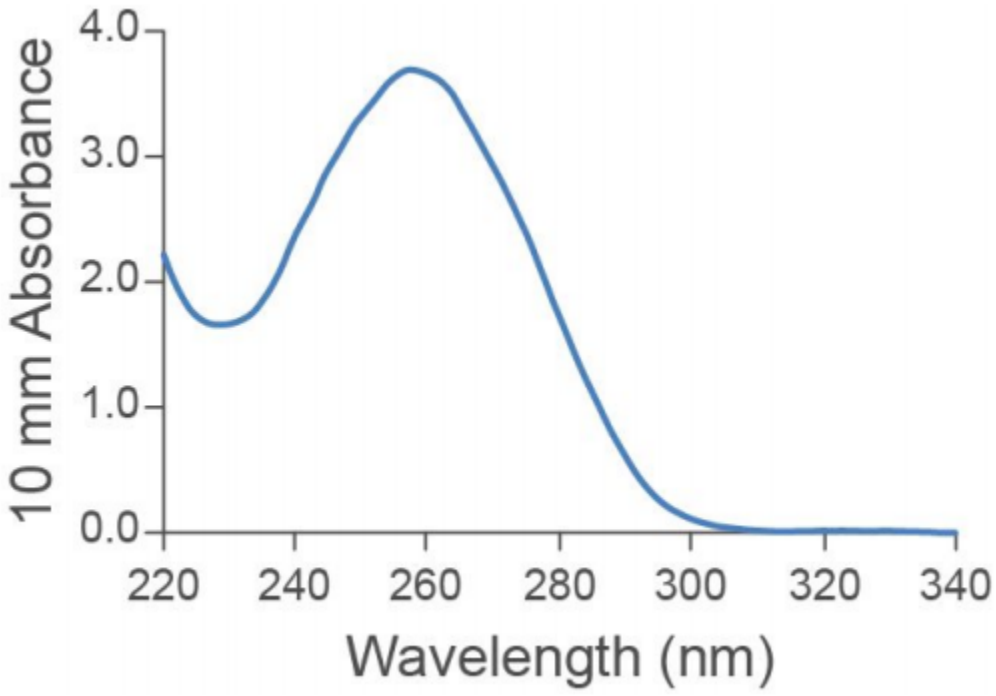
To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 150 µl TE buffer.

Step 9

Take 3 µg of extracted DNA and perform a size selection using the size selection of HMW DNA by semi-selective DNA precipitation the expected DNA recovery size selection is ~40-50%.

Results

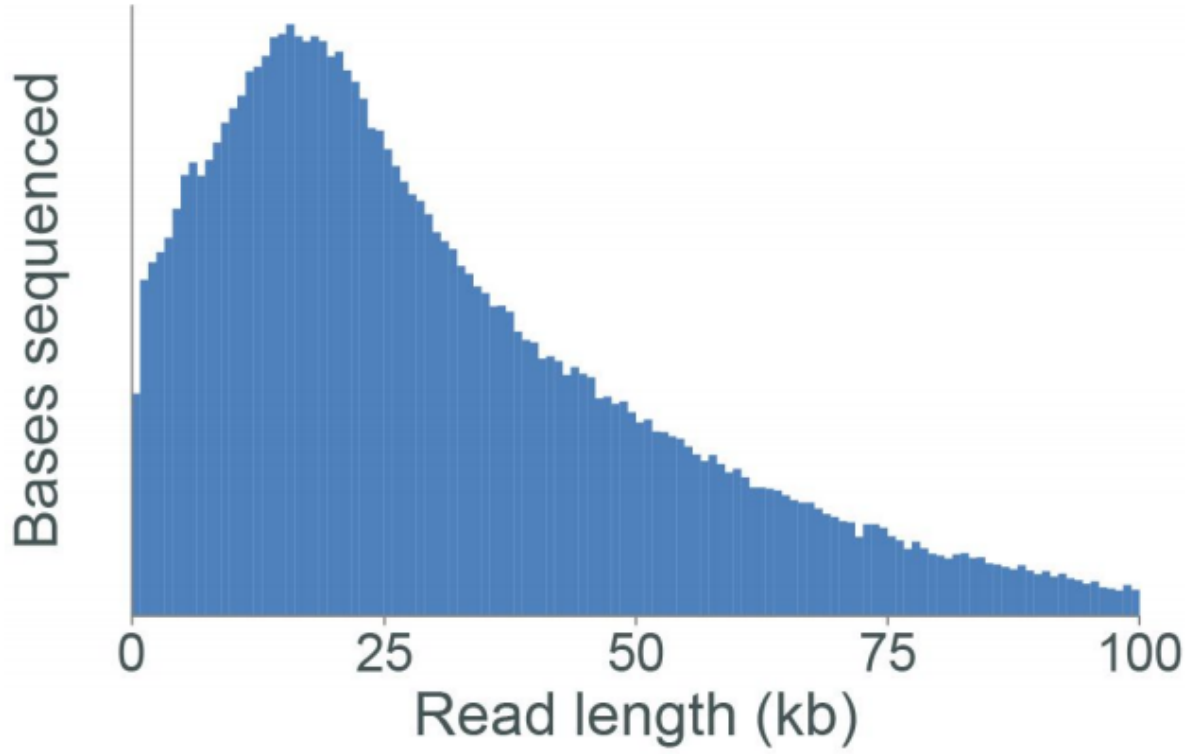
- **Yield:** 6-12 µg
- **OD 260/280:** 2.01
- **OD 260/230:** 2.61



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109):

- Typical output: ★★★ (8+ Gb in 48 h on FLO-MIN106D) for the Ligation Sequencing Kit, equivalent to the Lambda DNA supplied with the Control Expansion pack (EXP-CTL001).
- Read length profile:



Date	Change note
September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol.
October 2021	Removed reference to QIAGEN TissueRuptor

	II and probes from the materials list.