

Introduction

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

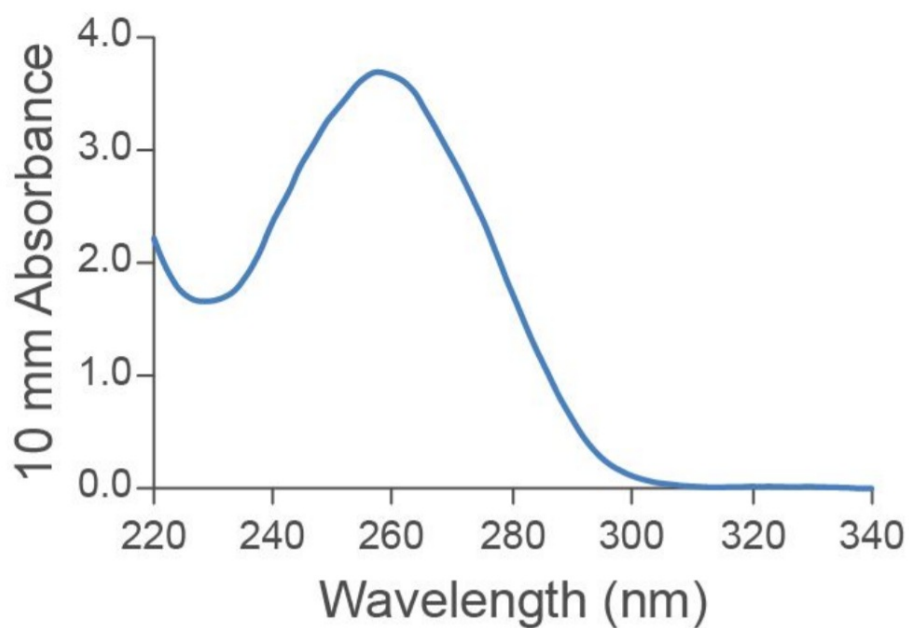
- 1 ml of human saliva
- [Isohelix GeneFiX™ Saliva DNA Collection Device](#)
- [QIAGEN Blood and Cell Culture DNA Midi Kit](#)
- [Qubit dsDNA BR Assay Kit \(ThermoFisher Scientific\)](#)
- [QIAGEN RNase A](#)
- [Proteinase K](#)
- Isopropanol
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Vortex mixer
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Vortex mixer
- Refrigerated centrifuge and rotor for 15 ml tubes
- Incubator or water bath with capacity for 37°C and 50°C

Method

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.
2. Transfer 1 ml of saliva to a 15 ml Falcon tube and add 19 µl of RNase A.
3. Add 5 ml of Buffer G2 and 95 µl of Proteinase K to the tube, and mix by inverting the tube.
4. Incubate the tube for 15 minutes at 37°C, and then for 1 hour at 50°C.
5. Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.
6. Pour the lysate through the column.
7. Purify the lysate according to the [standard protocol](#) (steps 3-6, pages 50-52).
8. To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 150 µl TE buffer.
9. Take 3 µg of extracted DNA and perform a [Size selection of HMW DNA by semi-selective DNA precipitation](#) The expected DNA recovery after size selection is ~40-50%.

Results

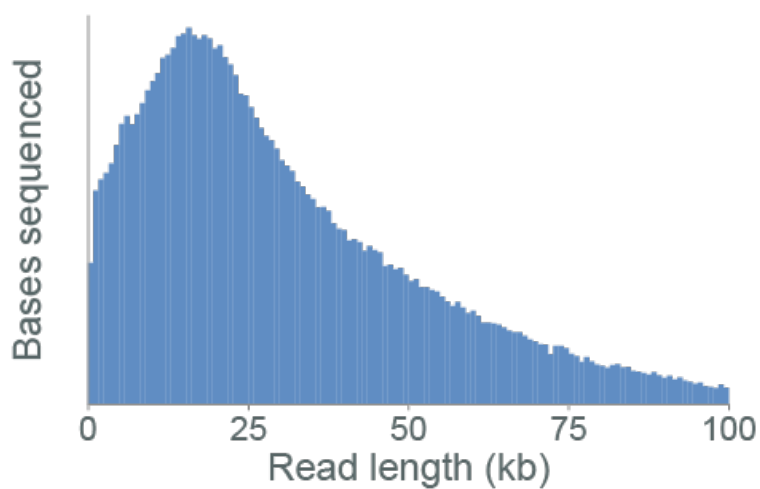
- **Yield:** 6-12 µg
- **OD 260-280:** 2.01
- **OD 260/230:** 2.61



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol