## 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<sup>™</sup> 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 <u>Size selection of</u> HMW DNA by semi-selective DNA precipitation protocol. Sequencing performance was assessed using the MinION.

## Materials

- 1 ml of human saliva
- Isohelix GeneFiX<sup>™</sup> 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<sup>™</sup> 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  $\mu l$  of RNase A.
- 3. Add 5 ml of Buffer G2 and 95  $\mu l$  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.
- Take 3 μg of extracted DNA and perform a<u>Size selection of HMW DNA by semi-selective DNA precipitation</u> 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