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Protocol status: In development
We are still developing and optimizing this protocol

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Rapid Sequencing gDNA

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ABSTRACT

ONT Rapid sequencing kit use in a classroom setting.

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Funders Acknowledgement:
Biotechnology Program

Library Preparation

2m

1 DNA tagmentation

Thaw kit components at Room temperature , spin down briefly using a microfuge and mix by pipetting as indicated below:

- Lambda DNA (50 µg/ml): thaw at RT, briefly spin down, mix well by pipetting
- Fragmentation Mix (FRA): not frozen, briefly spin down, mix well by pipetting
- Rapid Adapter (RAP): not frozen, briefly spin down, mix well by pipetting
- Sequencing Buffer (SQB): thaw at RT, briefly spin down, mix well by pipetting*
- Loading Beads (LB): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use
- Sequencing Tether (SQT): thaw at RT, briefly spin down, mix well by pipetting

2 Prepare the DNA in Nuclease-free water

2.1 Transfer ~ 200 ng genomic DNA into a 1.5 ml Eppendorf DNA LoBind tube

2.2 Adjust the volume to 3.75 µL with Nuclease-free water

2.3 Mix by flicking the tube to avoid unwanted shearing

2.4 Spin down briefly in a microfuge

2.5 In a 0.2 ml thin-walled PCR tube, mix the following:

3.75 µL 200 ng template DNA

1.25 µL FRA

2.6 Mix gently by flicking the tube, and spin down.

2.7 Incubate the tube at 30 °C for 00:01:00 and then at 80 °C for 00:01:00 . 2m

Briefly put the tube on ice to cool it down.