Ultra-Rapid Sequencing (LAMP)

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ABSTRACT

This protocol accompanies the paper "Ultra-Rapid Somatic Variant Detection via Real-Time Threshold Sequencing." This protocol was followed to initiate a sequencing run that resulted in a somatic variant call from known tumor tissue in less than 30 minutes. The protocol outlines DNA extraction, LAMP, library preparation for Oxford Nanopore Sequencing, and sequencer preparation and loading.

PROTOCOL CITATION

Jack Wadden 2021. Ultra-Rapid Sequencing (LAMP). **protocols.io** https://protocols.io/view/ultra-rapid-sequencing-lamp-btvmnn46

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Preparation

1 Prepare the LAMP reaction tube by mixing the following components in a **0.2 mL** PCR tube

WarmStart LAMP Kit (DNA and RNA) - 500 rxns **New England Biolabs Catalog #E1700L**

- **12.5 µl**
- **2.5 µl** **10 Micromolar (µM)** LAMP primer mix
- **9 µl** Nuclease-free Water **Contributed by users**

2 Prepare the DNA extraction tube by mixing the following components in a **0.2 mL** PCR tube

Epicentre QuickExtract™ DNA Extraction

- **100 µl** of Solution **Epicentre Catalog #QE09050**

3 Prepare ONT sequencing buffer

SQB (Sequencing Buffer) **Oxford Nanopore**

- **34 µl** **Technologies Catalog #SQK-RAD004** SQB

LB (Loading Beads) **Oxford Nanopore**

- **25.5 µl** **Technologies Catalog #SQK-RAD004**
- **4.5 µl** nuclease free water **Contributed by users**

4 Prepare ONT fragmentation mix

FRA (Fragmentation Mix) **Oxford Nanopore**

- **2.5 µl** **Technologies Catalog #SQK-RAD004**
- **5.6 µl** nuclease free water **Contributed by users**

5 Prepare ONT loading buffer

FLT (Flush Tether) **Oxford Nanopore**

- Add **30 µl** **Technologies Catalog #EXP-FLP002** into tube of

FB (Flush Buffer) **Oxford Nanopore**

Technologies Catalog #EXP-FLP002

- Mix and spin down

6 Prepare flow cell with **800 µl** of loading buffer and let incubate for **00:05:00** 5m

7 Start sequencing run in MinKNOW software and pause run immediately after flow cell QC check

8 Pre-heat thermocycler initiate, then pause, combined Ultra-Rapid Program 19m

DNA Extraction

65 °C for **00:01:00**

98 °C for **00:02:00**

hold at **20 °C**

LAMP

65 °C for **00:14:00**

Fragmentation

30 °C for **00:01:00**

80 °C for **00:01:00**

hold at **20 °C**

DNA Extraction

9 When acquired, place **20 µg** tumor tissue into DNA extraction tube

10 Vortex on high for **00:00:30** 30s

11 Place in pre-heated thermocycler and unpause combined program

12 Let thermocycler run through the DNA extraction protocol

13 Either let thermocycler draw DNA down to room temperature or quench in ice for **00:00:10** . Pause thermocycler program to pre-heat for LAMP. Briefly vortex on high and spin down. 10s

Target Amplification

14 Add **1 µl** extracted DNA to LAMP reaction tube, being careful to avoid leftover tissue or other debris in tube.

15 Place in thermocycler and unpause combined protocol

16 Let thermocycler run through the LAMP protocol 26m

17 Either let thermocycler draw DNA down to room temperature or quench in ice water for **00:00:10** . Pause thermocycler program to pre-heat for ONT rapid library preparation tagmentation. 10s

Library Preparation

18 Add **1.9 µl** LAMP product to tagmentation mix, flick to mix, and spin down

19 Place in thermocycler and unpause combined protocol

20 Let thermocycler run through the tagmentation protocol

21 Either let thermocycler draw DNA down to room temperature or quench in ice water for **00:00:10** 10s

22 RAP (Rapid Adapter) **Oxford Nanopore**

Add **1 µl** **Technologies Catalog #SQK-RAD004** to tagmentation tube, flick to mix, and spin down

23 Incubate sequencing library for **00:02:00** at room temperature, flicking to mix occasionally and spinning down 2m

23.1 During the final 1m of incubation, re-prime the ONT flow-cell with SpotOn port open, using **200 µl** FB (Flush Buffer) **Oxford Nanopore** of **Technologies Catalog #EXP-FLP002** mix

Sequencing

24 Add the entire 11ul library to the sequencing mix tube

25 Pipette entire **75 µl** sequencing mix into the MinION Spot On port, by slowly squeezing droplets onto the ramp/port.

26 Unpause sequencing run in MinKNOW software