Ultra-Rapid Sequencing (PCR)

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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 ~52 minutes. The protocol outlines DNA extraction, PCR, library preparation for Oxford Nanopore Sequencing, and sequencer preparation and loading as executed for the 52 minute demonstration.

PROTOCOL CITATION

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https://protocols.io/view/ultra-rapid-sequencing-pcr-bs7bnhin

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Preparation

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

- **12.5 µl**
  Q5 Hot Start High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog #M0494L**
- **1.25 µl** forward primer
- **1.25 µl** reverse primer
- **9 µl** Nuclease-free Water **Contributed by users**

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

Epicentre QuickExtract™ DNA Extraction

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

3 Pre-heat thermocycler and pause for combined PCR and fragmentation protocol 11m 18s

Pre-heat two heat blocks (**65 °C** and **98 °C**)

DNA Extraction (in heat blocks)

65 °C for **00:06:00**

98 °C for **00:02:00**

PCR (in thermocycler)

98 °C for **00:00:30**

28 cycles of

98 °C for **00:00:05**

64 °C for **00:00:05**

72 °C for **00:00:08**

72 °C for **00:00:30**

hold at **20 °C**

Fragmentation (in thermocycler)

30 °C for **00:01:00**

80 °C for **00:01:00**

hold at **20 °C**

DNA Extraction

4 When acquired, place **20 µg** tumor tissue into **2 mL** DNA extraction tube

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

6 Place in pre-heated heat block at **65 °C** 8m

- briefly vortex after 3 minutes of incubation

7 Place in pre-heated heat block at **98 °C**

8 Remove tube, vortex briefly, spin down

9 Incubate on ice for **00:00:30** 30s

Target Amplification

10 Add **1 µl** extracted DNA to PCR reaction tube

11 Place in thermocycler and unpause combined protocol

11.1 During PCR, prepare ONT tagmentation mix by mixing the following in a **0.2 mL** PCR tube

- **2.5 µl**
  FRA (Fragmentation Mix) **Oxford Nanopore Technologies Catalog #SQK-RAD004**

11.2 During PCR, prepare ONT sequencing buffer

- **34 µl**
  SQB (Sequencing Buffer) **Oxford Nanopore Technologies Catalog #SQK-RAD004**
  SQB
- **25.5 µl**
  LB (Loading Beads) **Oxford Nanopore Technologies Catalog #SQK-RAD004**

4.5 µl nuclease free water **Contributed by users**

11.3 During PCR, prepare ONT flush buffer

- Add **30 µl**
  FLT (Flush Tether) **Oxford Nanopore Technologies Catalog #EXP-FLP002** into tube
  FB (Flush Buffer) **Oxford Nanopore Technologies Catalog #EXP-FLP002**

Mix and spin down

11.4 Prime flow cell with **800 µl** of mixed FB (Flush Buffer) **Oxford Nanopore Technologies Catalog #EXP-FLP002** and let incubate for **00:05:00** 5m

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

12 Let thermocycler run through the PCR protocol 26m

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 ONT rapid library preparation tagmentation. 10s

Library Preparation

14 Add **7.5 µl** PCR product to tagmentation mix tube, flick to mix, and spin down

15 Place in thermocycler and unpause combined protocol

16 Let thermocycler run through fragmentation protocol

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

18 Add **1 µl** ONT Rapid Adapter to tagmentation tube, flick to mix, and spin down

19 Incubate sequencing library for **00:05:00** at room temperature, flicking to mix occasionally and spinning down 5m

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

Sequencing

20 Add the entire **11 µl** library to the sequencing mix tube

21 Pipette entire **75 µl** sequencing mix into the SpotOn port, by slowly squeezing droplets onto the ramp/port

22 Unpause sequencing run in MinKNOW software