

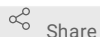


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Ultra-Rapid Sequencing (PCR)

Jack Wadden¹¹University of Michigan - Ann Arbor

1 Works for me



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Jack Wadden
University of Michigan - Ann Arbor

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

Jack Wadden 2021. Ultra-Rapid Sequencing (PCR). [protocols.io](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**
- briefly vortex after 3 minutes of incubation

8m













- 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
of Technologies Catalog #EXP-FLP002
- Mix and spin down
- 11.4 Prime flow cell with  800 µl of mixed 5m
-  FB (Flush Buffer) Oxford Nanopore
- Technologies Catalog #EXP-FLP002 and let
- incubate for  00:05:00
- 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 10s
program to pre-heat for ONT rapid library preparation tagmentation.

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** of **Technologies Catalog #EXP-FLP002** 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