

Resource Summary Report

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Viral Sequencing, from Gunk to Graph (One-Step four-primer PCR alternative)

DOI:10.17504/protocols.io.bd5ni85e

Type: Protocol

Proper Citation

David Eccles 2020. Viral Sequencing, from Gunk to Graph (One-Step four-primer PCR alternative). protocols.io dx.doi.org/10.17504/protocols.io.bd5ni85e

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bd5ni85e>

Authors: David Eccles

Group: Coronavirus Method Development Community

Summary: This is a fast "gunk to graph" protocol for analysing viral RNA from nasopharyngeal swabs. The approach involves swab lysis and inactivation at the point of sampling, uses a cellulose binding / wash protocol to reduce extraction cost, incorporates sample-specific barcodes during first-strand synthesis, nanopore rapid-attachment primers during PCR amplification, and nanopore sequencing with parallel RAMPART analysis for fast assembly and phylogenetics. Note: this is a one-step RT-PCR reaction protocol. For an alternative method that allows more multiplexing flexibility, see the protocol that this has been forked from: <https://dx.doi.org/10.17504/protocols.io.bd3yi8pw>

Affiliations: Malaghan Institute of Medical Research (NZ)

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