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Preparation of dsRNA viruses for next-generation sequencing

Microbial Genomics

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1 Works for me dx.doi.org/10.17504/protocols.io.ugnetve

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EXTERNAL LINK

https://doi.org/10.1099/mgen.0.000315

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Wilcox AH, Delwart E, Díaz-Muñoz SL, Next-generation sequencing of dsRNA is greatly improved by treatment with the inexpensive denaturing reagent DMSO. Microbial Genomics 5(11). doi: 10.1099/mgen.0.000315

MATERIALS

NAME	CATALOG #	VENDOR
NEBNext Second Strand Synthesis (dNTP-free) Reaction Buffer - 0.4 ml	B6117S	New England Biolabs
E.coli DNA Ligase - 1,000 units	M0205L	New England Biolabs
DNA Polymerase I (E.coli) - 2,500 units	M0209L	New England Biolabs
DMSO	D1435	Sigma Aldrich
Nuclease-free water	AM9932	Ambion
Qubit dsDNA HS Assay Kit	Q32851	Thermo Fisher Scientific
DNase I (e.g., NEB #M0303) and DNase I Cleanup Reagants or Kit for Removal of DNA Prior to Depletion	View	New England Biolabs
Millex-GP Syringe Filter Unit, 0.22 µm	SLGP033RS	Emd Millipore
Qubit RNA HS Assay Kit	Q32852	Thermo Fisher Scientific
RNase H	M0297S	New England Biolabs
RNeasy® Mini Kit	74104	Qiagen
SuperScript™ III First-Strand Synthesis System	18080051	Thermo Fisher Scientific
RNAse AT/1 Mix	EN0551	Thermo Scientific
RNeasy MinElute Cleanup Kit	74204	Qiagen
Nucleospin Gel and PCR cleanup	740609.250	Macharey Nagal

1 Viral lysates should be passed through a 0.22µm filter to remove host debris, then treated with nucleases to degrade extracapsular nucleic acids. We added 25µl DNAse I, 50µl RNAse A/T1 and 1X DNAse I Buffer to 1ml filtrate, and incubated for 1 hour 30 minutes at 37ºC.

2 RNA should be extracted using a commercially available kit. We used an RNeasy Mini Kit (Qiagen). Kits that require carrier RNA are not recommended because this will interfere with sequencing.

3 Quantify the RNA using Qubit. Note that low viral titres and/or small viral genomes may result in the amount of RNA being below the Qubit's limit of detection.

4 Add 50% DMSO to RNA sample, and incubate for 1 hour 30 minutes at 65ºC. Purify RNA using a column cleanup kit (we recommend Qiagen's RNeasy MinElute Cleanup Kit) or your preferred other method (e.g. ethanol precipitation, TRIzol purification).

5 First strand synthesis. Add 5µl purified RNA to 1µl random hexamers, 1µl dNTPs and 3µl nuclease-free water, and incubate for 5 minutes at 65ºC. Place on ice for 1 minute, then add 0.01M DTT, 5mM MgCl2, 1X reverse transcriptase buffer, 1µl RNAseOUT and 1µl Superscript III enzyme to each reaction, using nuclease-free water to bring reactions to a total volume of 20µl. Incubate in a thermal cycler for 10 minutes at 25ºC, 50 minutes at 50ºC and 5 minutes at 85ºC. Place reactions on ice.

6 Second strand synthesis. Add 1µl dNTPs, 0.5µl DNA ligase, 2µl DNA polymerase I, 0.5µl RNAse H, 1X second strand synthesis buffer, and use nuclease-free water to bring the total volume to 40µl. Incubate for 5 hours at 16ºC, and purify DNA using your preferred method (we used a Macharey-Nagal Nucleospin Gel and PCR Clean-up kit). Quantify DNA using Qubit.

7 Prepare DNA libraries for sequencing using your chosen platform and library preparation kit (we used Nextera XT).