

Whole Genome Sequencing for Rapid Characterization of Rabies Virus Using Nanopore Technology

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jove.com/video/65414

Materials

Name	Company	Catalog Number	Comments
Brand name			
Software			
Sequencing software (<i>MinKnow</i>)	Oxford Nanopore Technologies	https://community.nanoporetech.com/downloads	
Bioinformatics tool kit (<i>Guppy</i>)	Oxford Nanopore Technologies	https://community.nanoporetech.com/docs/prepare/library_prep_protocols/Guppy-protocol/v/gpb_2003_v1_revao_14dec2018	
Equipment			
Thermal cycler (<i>miniPCR™ mini16 thermal cycler</i>)	Cambio	MP-QP-1016-01	
Homogenizer (<i>Precellys Evolution Touch Homogenizer</i>)	Bertin Instruments	EQ02520-300	
Cold Racks (0.2-0.5mL) (<i>PCR Mini-cooler with transparent lid</i>)	BRAND	781260	
Pipettor			
(<i>Pipetman L Fixed F1000L, 1000 uL</i>)	Gilson	SKU: FA10030	
(<i>Pipetman L Fixed F100L, 100 uL</i>)	Gilson	SKU: FA10024	
(<i>Pipetman L Fixed F10L, 10 uL</i>)	Gilson	SKU: FA10020	
(<i>Pipetman L Fixed F1L, 1 uL</i>)	Gilson	SKU: FA10025	
(<i>Pipetman L Fixed F20L, 20 uL</i>)	Gilson	SKU: FA10021	
(<i>Pipetman L Fixed F250L, 250 uL</i>)	Gilson	SKU: FA10026	
Fluorometer (<i>Qubit 4 Fluorometer</i>)	ThermoFisher scientific/Fisher scientific	Q33238	
Laptop			

(Any brand with ~2 GB of drive space, minimum of 512 GB storage space, MSI installer [GPU])			
Microcentrifuge (Refrigerated centrifuge)	ThermoFisher scientific/Fisher scientific	75004081	
Vortex mixer (Basic vortex mixer)	ThermoFisher scientific/Fisher scientific	88882011	
Magnetic rack (DynaMag -2 Magnet)	ThermoFisher scientific/Fisher scientific	12321D	
Sequencing device (MinION)	Oxford Nanopore Technologies	MinION Mk1B	
RNA Extraction			
RNA extraction kit (Qiagen RNeasy Mini Kit 250)	Qiagen	74106	
RNA stabilizing reagents (RNA later)	Invitrogen	AM7020	
(DNA/RNA Shield)	Zymo Research	R1100-50	
PCR			
Nuclease-free Water (Nuclease-free Water [not DEPC-treated])	ThermoFisher scientific/Fisher scientific	AM9937	
Master mix for first strand cDNA synthesis (LunaScript RT SuperMix Kit)	New England Biolabs	E3010S	
DNA amplification master mix (Q5® Hot Start High-Fidelity 2X Master Mix [NEB])	New England Biolabs	M0494L	
Primer (Scheme) (Custom DNA oligos)	Invitrogen		
SPRI Bead Clean-up			
SPRI beads (Aline Biosciences PCR Clean DX)	Cambio	AL-AC1003-50	
Ethanol, Pure Absolute, >99.8% (GC) [Riedel-De Haen]	Merck	818760	
Short Fragment buffer (SFB expansion pack)	Oxford Nanopore Technologies	EXP-SFB001	
DNA Quantification			
DNA quantification kit (Qubit® dsDNA HS Assay Kit)	ThermoFisher scientific/Fisher scientific	Q32854	
DNA quantification assay tubes (Qubit™ Assay Tubes)	ThermoFisher scientific/Fisher scientific	Q32856	
End Prep and barcoding (Qubit™ Assay Tubes)			
End Prep master mix (NEBNext Ultra End Repair/dA-Tailing Module)	New England Biolabs	E7546L	
Barcoding kit			
*Chemistry 9 (Native Barcoding Expansion 1-12)	Oxford Nanopore Technologies	*Chemistry 9 EXP-NBD104	
(Native Barcoding Expansion 13-24)		EXP-NBD114	
(Native Barcoding Expansion 96)		EXP-NBD196	
*Chemistry 14 (not compatible)	Oxford Nanopore Technologies	*Chemistry 14 (not compatible)	
(Native Barcoding Kit 24 V14)		SQK-NBD114.24	
(Native Barcoding Kit 96 V14)		SQK-NBD114.96	

Ligation mastermix (<i>Blunt/TA Ligase Master Mix</i>)	New England Biolabs	M0367S	
Adapter Ligation			
Adapter ligation master mix (<i>NEBNext Quick Ligation Module</i>)	New England Biolabs	E6056S	
(<i>NEBNext Ultra II Ligation Module</i>)	New England Biolabs	E7595S	
(<i>Blunt/TA Ligase Master Mix</i>)	New England Biolabs	M0367S	
Adapter mix			
* <i>Chemistry 9</i>	Oxford Nanopore Technologies	* <i>Chemistry 9</i> EXP-AMII001	
(<i>Adapter Mix II [AMII]</i>)			
* <i>Chemistry 14</i>	Oxford Nanopore Technologies	* <i>Chemistry 14</i> EXP-NBA114	
(<i>Native adapter [NA]</i>)			
Sequencing			
Flowcell priming kit			
* <i>Chemistry 9</i>	Oxford Nanopore Technologies	* <i>Chemistry 9</i> EXP-FLP002	
(<i>Flush Buffer [FB]</i>)			
(<i>Flush Tether [FT]</i>)			
* <i>Chemistry 14</i>	Oxford Nanopore Technologies	* <i>Chemistry 14</i> EXP-FLP004	
(<i>Flow Cell Flush [FCF]</i>)			
(<i>Flow Cell Tether [FCT]</i>)			
Ligation Sequencing Kit			
* <i>Chemistry 9</i>	Oxford Nanopore Technologies	* <i>Chemistry 9</i> SQK-LSK109	
Adapter Mix (<i>Adapter Mix [AMX]</i>)			
Ligation Buffer (<i>Ligation buffer [LNB]</i>)			
Short Fragment Buffer (<i>Short Fragment buffer [SFB]</i>)			
Sequencing Buffer (<i>Sequencing Buffer [SQB]</i>)			
Elution Buffer (<i>Elution buffer [EB]</i>)			
Loading Beads (<i>Loading Beads [LB]</i>)			
Sequencing Tether (<i>Sequencing Tether [SQT]</i>)			
* <i>Chemistry 14</i>	Oxford Nanopore Technologies	* <i>Chemistry 14</i> SQK-LSK114	
Adapter Mix (<i>Ligation Adapter [LA]</i>)			
Ligation Buffer (<i>Ligation buffer [LNB]</i>)			
Short Fragment Buffer (<i>Short Fragment buffer [SFB]</i>)			
Sequencing Buffer (<i>Sequencing Buffer [SB]</i>)			
Elution Buffer (<i>Elution buffer [EB]</i>)			
Loading Beads			

(Loading Beads [LIB])			
Sequencing Tether (Flow Cell Tether [FCT])			
Library solution (Library solution [LIS])			
Flush buffer (Flow Cell Flush [FCF])			
Flow Cell			
*Chemistry 9	Oxford Nanopore Technologies	*Chemistry 9 FLO-MIN106D	
(Flow Cell [R9.4.1])			
*Chemistry 14	Oxford Nanopore Technologies	*Chemistry 14 FLO-MIN114	
(Flow Cell [R10.4.1])			
Flow Cell wash			
Flowcell wash kit (Flow cell wash kit)	Oxford Nanopore Technologies	EXP-WSH004	
Consummables			
Surface decontaminant			
(DNA Away Surface Decontaminant, Squeeze Bottle [Molecular Bio])	ThermoFisher scientific/Fisher scientific	7010PK	
(RNase Away Surface Decontaminant, Bottle [Molecular Bio])	ThermoFisher scientific/Fisher scientific	7002PK	
PCR 8-Tube Strip 0.2ml, individual cap (PCR 8-Tube Strip 0.2ml, with Individual attached Flat Caps, Sterile, DNase/RNase, Pyrogen Free, Natural [Greiner])	Greiner	608281	
PCR Tube 0.2ml (PCR Tube 0.2ml, Natural [Domed Cap] Bagged in 500s, Non-Sterile [Greiner])	Greiner	671201	
1000µL Filter Tips (500) (Stacked 1000µL Filter Tips [500])	ThermoFisher scientific/Fisher scientific	11977724	
100µL Filter Tips (1000)	ThermoFisher scientific/Fisher scientific	11947724	
10µL Filter Tips (1000) (Stacked 100µL Filter Tips [1000])	ThermoFisher scientific/Fisher scientific	11907724	
Reinforced tubes tubes (2ml) with screw caps and o-rings (Fisherbrand™ Bulk tubes)	ThermoFisher scientific/Fisher scientific	15545809	
Microcentrifuge tube (1.5ml) (1.5 ml Eppendorf Tubes [500])	Eppendorf	1229888	
DNA LoBind Tubes (1.5ml) (DNA LoBind Tubes)	ThermoFisher scientific/Fisher scientific	10051232	
Cryobabies labels			
Gloves (S/M/L)			
Paper towel			