

VERSION 2

MAY 23, 2023

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♠ Library tissue handling, viral DNA extraction, and NGS sample preparation V.2

Forked from a private protocol

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ABSTRACT

This protocol describes the procedure to isolate viral DNA from AAV-transfected tissue and prepare it for next-generation sequencing.

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Protocol status: Working We use this protocol and it's working

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2023

PROTOCOL integer ID:

82290

Marmoset tissue extraction (library selections)

- 1 Raise and house marmosets in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees.
 - For adult marmosets, ensure that there is no detectable neutralizing antibodies for AAV9. This can be conducted by The Penn Vector Core at the University of Pennsylvania (https://gtp.med.upenn.edu/intranethome/core-facilities-internal/immunology-core)
- 2 Inject marmosets with desired dose of library (e.g. 2 x 10¹² vector genomes of library) intravenously (e.g. via the femoral vein).
- **3** At four weeks post-injection, euthanize marmosets and perfuse with 1X phosphate buffered saline.
- 4 Flash freeze tissue (e.g. using 2-methylbutane chilled with dry ice). Separate the brain into coronal blocks and flash freeze the blocks. Store tissue at -80 °C until ready for processing.

DNA Extraction

5

100 mg tissue Add sample

(brain, liver, or spinal cord) and 🎩

1 mL TRIzol reagent

Use prefilled tubes with 1.5 mm Zirconium beads or 2.8 mm stainless steel beads.

Equipment	
Prefilled 2.0ml tubes, Zirconium Beads, 1.5mm Triple-Pure - High Impact, 50pk	NAME
Homogenizer tubes (1.5 mm Zirconium beads)	TYPE
Benchmark Scientific	BRAND
D1032-15	SKU
https://www.benchmarkscientific.com/product/d1032-group/	LINK

Equipment	
Prefilled 2.0ml tubes, Stainless Steel, 2.8mm Acid Washed, 50pk	NAME
Homogenizer tubes (2.8 stainless steel)	TYPE
Benchmark Scientific	BRAND
D1033-28	SKU
https://www.benchmarkscientific.com/product/d1032-group/	LINK

6 Homogenize tissue in using the following settings:

> ■ Speed: 5.0 m/s ■ Time: 30 seconds ■ Pause: 1 minute

Cycles: 2



Equipment	
BEADBUG 6, SIX POSITION HOMOGENIZER, 115V	NAME
Tissue homogenizer (6 position)	TYPE
Benchmark Scientific	BRAND
D1036	SKU
https://www.benchmarkscientific.com/product/d1036/	LINK

Equipment	
BEADBLASTER 24 MICROTUBE HOMOGENIZER, 115V	NAME
Tissue Homogenizer (24 position)	TYPE
Benchmark Scientific	BRAND
D2400	SKU
https://www.benchmarkscientific.com/product/d2400/	LINK

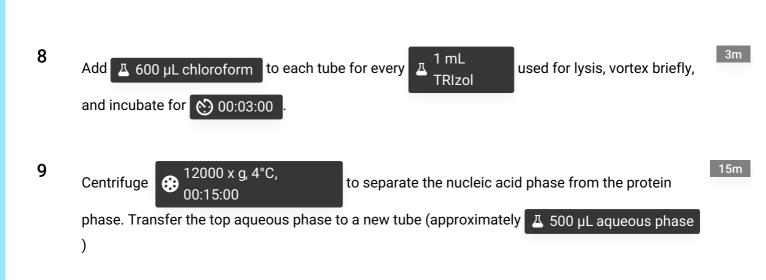
Note

Samples can be stored at -20 °C for up to year in TRIzol.

5m

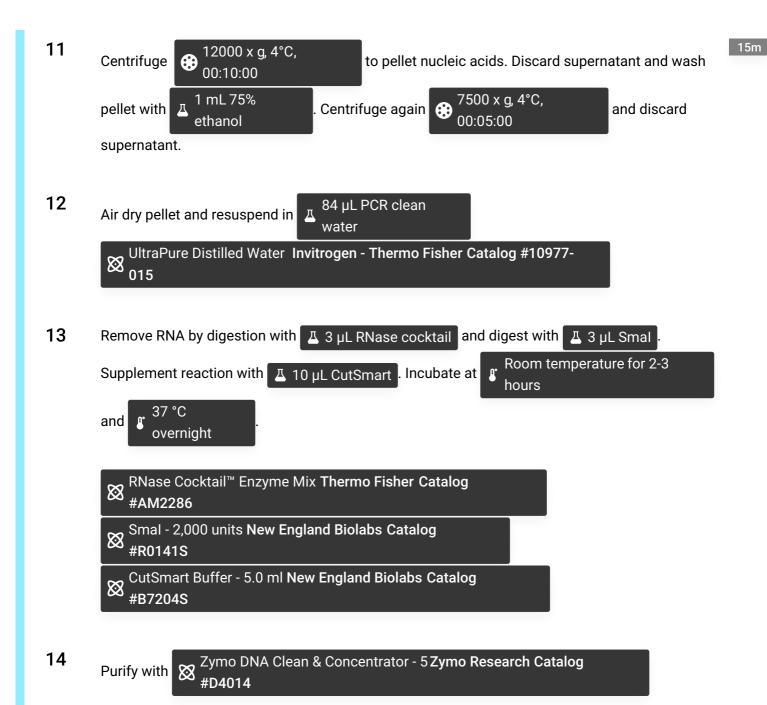
Equipment	
Centrifuge 5425/5425 R - Microcentrifuge	NAME
Refrigerated centrifuge	TYPE
Eppendorf	BRAND
2231000909	SKU
https://www.eppendorf.com/us-en/eShop- Products/Centrifugation/Microcentrifuges/Centrifuge-5425-5425R-p-PF-934144	LINK

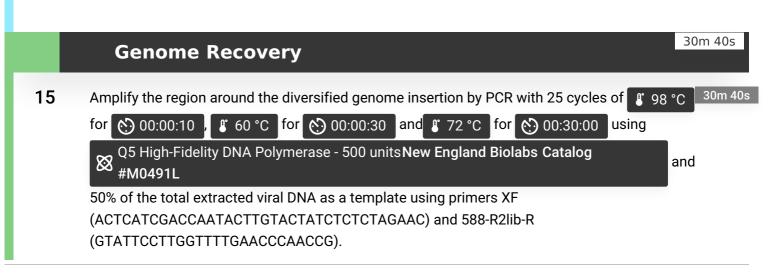
Equipment	
DNA LoBind® Tubes	NAME
Microcentrifuge tubes	TYPE
Eppendorf	BRAND
022431021	SKU
https://www.eppendorf.com/us-en/eShop-Products/Laboratory- Consumables/Tubes/DNA-LoBind-Tubes-p-PF-56252	LINK



Equipment	
Centrifuge 5425/5425 R - Microcentrifuge	NAME
Refrigerated centrifuge	TYPE
Eppendorf	BRAND
2231000909	SKU
https://www.eppendorf.com/us-en/eShop- Products/Centrifugation/Microcentrifuges/Centrifuge-5425-5425R-p-PF-934144	LINK

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Eppendorf	BRAND
022431021	SKU
https://www.eppendorf.com/us-en/eShop-Products/Laboratory- Consumables/Tubes/DNA-LoBind-Tubes-p-PF-56252	LINK





Index addition

1m 40s

Dilute PCR product 1:100 and use as template for an additional round of PCR amplification around the variable region with primers containing Read1 and Read2 sequences by 10 cycles of \$\ 98 \circ \text{for } \colon 00:00:10 \, \ \ 59 \circ \text{for } \colon 00:00:10 \,

50s

Q5 High-Fidelity DNA Polymerase - 500 units New England Biolabs Catalog

using #M0491L

and primers 588i-lib-PCR1-6bpUID-F (CACTCATCGACCAATACTTGTACTATCTCTCT) and 588i-lib-PCR1-R (GTATTCCTTGGTTTTGAACCCAACCG).

17 Purify with

Zymo DNA Clean & Concentrator - 5 Zymo Research Catalog #D4014

18 Append Illumina flow cell adapters and unique indices by PCR amplification with

50s

gel.

NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) - 96 rxns**New England**Biolabs Catalog #E7600S

by 10 cycles of \$\mathbb{E}\$ 98 °C for \$\infty\$ 00:00:10 , \$\mathbb{E}\$ 59 °C for \$\infty\$ 00:00:30 , and \$\mathbb{E}\$ 72 °C for \$\infty\$

00:00:10 using

Q5 High-Fidelity DNA Polymerase - 500 units New England Biolabs Catalog

Clean up and validation

19 Run PCR products on a freshly-prepared 2%

UltraPure™ Low Melting Point Agarose **Thermo Fisher Scientific Catalog** #16520050

- Verify the expected size of the band and extract from the gel.
- 21 If desired, verify the nucleotide diversity at the randomized insertion site by Sanger sequencing.

Note

If additional material is needed for Sanger sequencing, perform an additional PCR amplification using 15-20 cycles of \$\mathbb{E}\$ 98 °C for \$\mathbb{O}\$ 00:00:10 , \$\mathbb{E}\$ 60 °C for \$\mathbb{O}\$ 00:00:30 and \$\mathbb{E}\$ 72 °C for \$\mathbb{O}\$ 00:00:10 with primers NGS-QC-F (AATGATACGGCGACCACCGAG) and NGS-QC-R (CAAGCAGAAGACGGCATACGA).