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AAV viral DNA and whole RNA recovery for AAV pool experiments in rhesus macaque

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ABSTRACT

This protocol outlines procedures to extract viral DNA and whole RNA from rhesus macaque tissue that had been treated with AAV *in vivo*

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protocols.io

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

Created: May 08, 2023

Last Modified: May 23,

2023

PROTOCOL integer ID:

81617

DNA/RNA extraction

3h 8m

(brain or liver) and $\underline{\mathsf{A}}^{1}$ mL TRIzol reagent

TRIzol Reagent Thermo Fisher Scientific Catalog #15596026 to bead homogenizer tubes.

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

2 Homogenize tissue in using the following settings:

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

■ Cycles: 2

Incubate for 00:05:00

5m

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

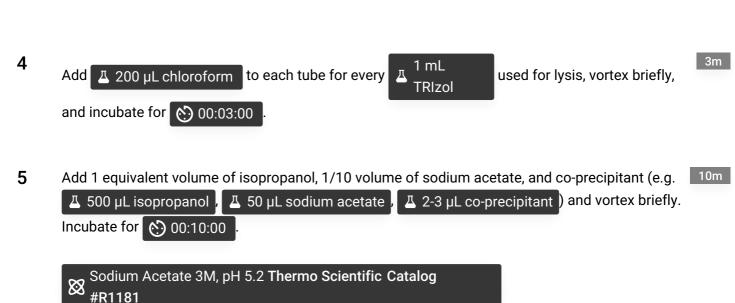
Equipment	
BEADBLASTER 24 MICROTUBE HOMOGENIZER, 115V	NAME
Tissue Homogenizer (24 position)	TYPE
Benchmark Scientific	BRAND
D2400	SKU
https://www.benchmarkscientific.com/product/d2400/	LINK

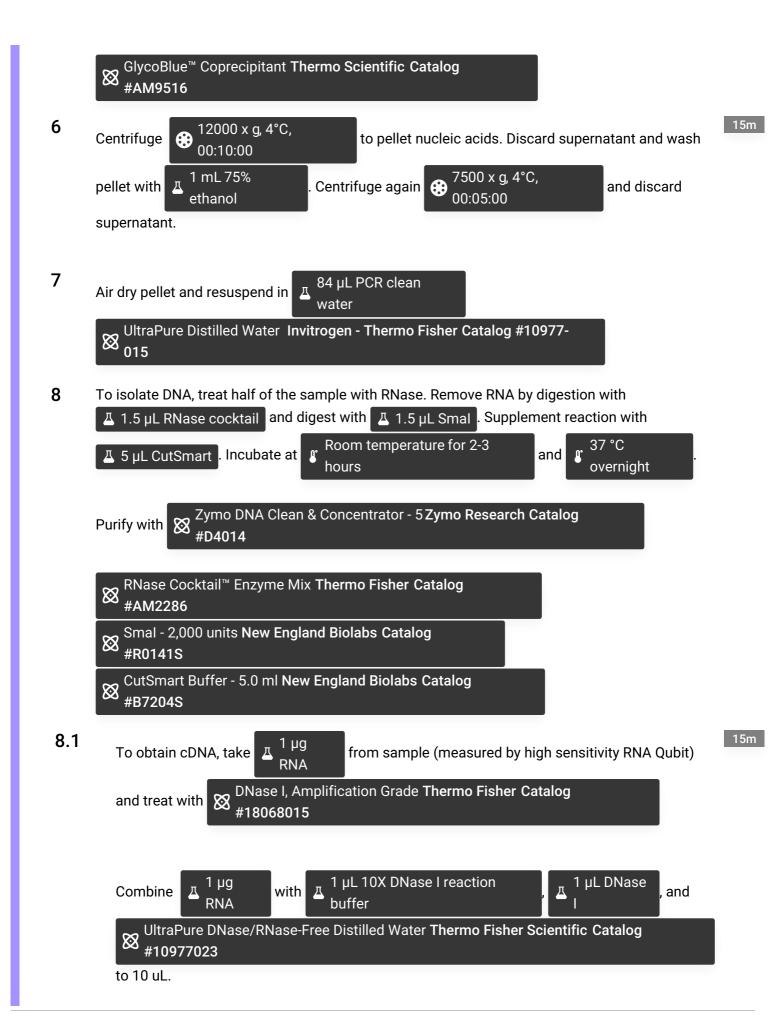
Note

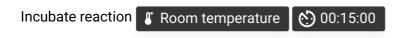
Samples can be stored at -20 °C for up to year in TRIzol.

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



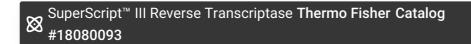








15m



9 Incubate \$\ 65 \cdot \cdot \cdot 00:05:00 \) and place on ice.

5m

- Prepare cDNA synthesis mix according to manufacturer's specifications. Add

 10 µL cDNA synthesis mix to each RNA primer mixture and mix by gently flicking the tubes.
- 11 Incubate as follows:

1h 55m

- \$ 50 °C **③** 00:50:00
- \$\circ\$ 25 °C \(\circ\$ 00:10:00
- \$\mathbb{8} 85 °C \\ \mathbb{\circ} 00:05:00
- 12 Store samples at -80 °C until ready to use.

PCR amplification

3h 8m

13 Use Zymo DNA purification of PCR product according to manufacturer's suggested protocol.

Zymo DNA Clean & Concentrator - 5 Zymo Research Catalog #D4014 14 Dilute PCR product 1:100 and use as template for an additional round of PCR amplification 50s around the variable region with primers containing Read1 and Read2 sequences by 10 cycles of for (*) 00:00:10 Q5 High-Fidelity DNA Polymerase - 500 units New England Biolabs Catalog using #M0491L and the following primers: Forward: 5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGcgatgttccagattacgcttgag -3' Reverse: 5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGattttgtaatccagaggttgattatcg - 3' 15 Use Zymo DNA purification of PCR product according to manufacturer's suggested protocol. Zymo DNA Clean & Concentrator - 5 Zymo Research Catalog #D4014 50s 16 Append Illumina flow cell adapters and unique indices by PCR amplification with NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) - 96 rxnsNew England **Biolabs Catalog #E7600S** by 10 cycles of \$\ 98 \circ for \(\frac{\chi}{2}\) 00:00:10 § 59 °C for **(:)** 00:00:30 (c) 00:00:10 using Q5 High-Fidelity DNA Polymerase - 500 units New England Biolabs Catalog #M0491L

17 Run PCR products on a freshly-prepared 2%

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

and gel purify amplified 200 bp PCR product.

gel