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We use this protocol and it's working

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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*

DNA/RNA extraction

3h 8m

1 Add 100 mg tissue sample (brain or liver) and 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: 5m

- Speed: 5.0 m/s
- Time: 30 seconds
- Pause: 1 minute
- Cycles: 2

Incubate for 00:05:00 .

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 ºC for up to year in TRIzol.

3 Centrifuge the homogenizer tubes containing the TRIzol solution and homogenized tissue using the following parameters: 12000 x g, 4°C, 00:05:00. Transfer the supernatant to a new tube (microcentrifuge tube or similar). 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

4 Add 200 µL chloroform to each tube for every 1 mL TRIzol used for lysis, vortex briefly, and incubate for 00:03:00. 3m

5 Add 1 equivalent volume of isopropanol, 1/10 volume of sodium acetate, and co-precipitant (e.g. 500 µL isopropanol, 50 µL sodium acetate, 2-3 µL co-precipitant) and vortex briefly. Incubate for 00:10:00. 10m

Sodium Acetate 3M, pH 5.2 **Thermo Scientific Catalog #R1181**

GlycoBlue™ Coprecipitant **Thermo Scientific Catalog #AM9516**

6 Centrifuge 12000 x g, 4°C, 00:10:00 to pellet nucleic acids. Discard supernatant and wash pellet with 1 mL 75% ethanol. Centrifuge again 7500 x g, 4°C, 00:05:00 and discard supernatant. 15m

7 Air dry pellet and resuspend in 84 µL PCR clean water

UltraPure Distilled Water **Invitrogen - Thermo Fisher Catalog #10977-015**

8 To isolate DNA, treat half of the sample with RNase. Remove RNA by digestion with 1.5 µL RNase cocktail and digest with 1.5 µL SmaI. Supplement reaction with 5 µL CutSmart. Incubate at Room temperature for 2-3 hours and 37 °C overnight.

Purify with Zymo DNA Clean & Concentrator - 5 **Zymo Research Catalog #D4014**

RNase Cocktail™ Enzyme Mix **Thermo Fisher Catalog #AM2286**

SmaI - 2,000 units **New England Biolabs Catalog #R0141S**

CutSmart Buffer - 5.0 ml **New England Biolabs Catalog #B7204S**

8.1 To obtain cDNA, take 1 µg RNA from sample (measured by high sensitivity RNA Qubit) and treat with DNase I, Amplification Grade **Thermo Fisher Catalog #18068015** 15m

Combine 1 µg RNA with 1 µL 10X DNase I reaction buffer, 1 µL DNase I, and UltraPure DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #10977023** to 10 uL.

Incubate reaction Room temperature 00:15:00

8.2 Inactivate DNase I by adding 1 µL 25 mM EDTA. Heat 65 °C 00:15:00 15m

8.3 To convert DNase I treated RNA to cDNA, take 1-5 µL sample and combine with 1 µL oligo(dT), 1 µL dNTP and fill to 10 µL using UltraPure water.

SuperScript™ III Reverse Transcriptase **Thermo Fisher Catalog #18080093**

9 Incubate 65 °C 00:05:00 and place on ice. 5m

10 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 00:50:00
- 25 °C 00:10:00
- 50 °C 00:50:00
- 85 °C 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 around the variable region with primers containing Read1 and Read2 sequences by 10 cycles of 98 °C for 00:00:10, 60 °C for 00:00:30, and 72 °C for 00:00:10 using Q5 High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0491L** 50s

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**

16 Append Illumina flow cell adapters and unique indices by PCR amplification with 50s

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

by 10 cycles of 98 °C for 00:00:10, 59 °C for 00:00:30, and 72 °C for 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** gel

and gel purify amplified 200 bp PCR product.