ABSTRACT

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

- **GlycoBlue™ Coprecipitant**
  - Thermo Scientific Catalog #AM9516
  - Step 5

- **CutSmart Buffer - 5.0 ml**
  - New England Biolabs Catalog #B7204S
  - Step 8

- **SuperScript® III Reverse Transcriptase**
  - Thermo Fisher Catalog #18080093
  - Step 5

- **SmaI - 2,000 units**
  - New England Biolabs Catalog #R0141S
  - Step 8.3

- **UltraPure DNase/RNase-Free Distilled Water**
  - Thermo Fisher Scientific Catalog #10977023
  - Step 8

- **Q5 High-Fidelity DNA Polymerase - 500 units**
  - New England Biolabs Catalog #M0491L
  - Step 8.1

- **Sodium Acetate 3M, pH 5.2**
  - Thermo Scientific Catalog #R1181
  - In 2 steps

- **Zymo DNA Clean & Concentrator - 5**
  - Zymo Research Catalog #D4014
  - In 3 steps

- **DNase I, Amplification Grade**
  - Thermo Fisher Catalog #18068015
  - Step 8.1

- **UltraPure™ Low Melting Point Agarose**
  - Thermo Fisher Scientific Catalog #16520050
  - Step 8

- **TRIzol Reagent**
  - Thermo Fisher Scientific Catalog #15596026
  - Step 1

- **UltraPure Distilled Water**
  - Invitrogen - Thermo Fisher Catalog #10977-015
  - Step 7

- **RNase Cocktail&trade; Enzyme Mix**
  - Thermo Fisher Catalog #AM2286
  - Step 8

- **NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) - 96 rxns**
  - New England Biolabs Catalog #E7600S
  - Step 16

**DNA/RNA extraction**

1. Add 200 mg tissue sample (brain or liver) and 1 mL TRizol reagent

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.protocols.io | https://dx.doi.org/10.17504/protocols.io.3byl4jo68lo5/v1
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.

<table>
<thead>
<tr>
<th>Equipment</th>
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<tr>
<td><strong>Prefilled 2.0ml tubes, Zirconium Beads, 1.5mm Triple-Pure - High Impact, 50pk</strong></td>
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<tr>
<td>Homogenizer tubes (1.5 mm Zirconium beads)</td>
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<td>Benchmark Scientific</td>
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<tr>
<td><strong>Prefilled 2.0ml tubes, Stainless Steel, 2.8mm Acid Washed, 50pk</strong></td>
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<tr>
<td>Homogenizer tubes (2.8 stainless steel)</td>
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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.
Samples can be stored at -20 °C 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).
4. Add 200 µL chloroform to each tube for every 1 mL TRizol used for lysis, vortex briefly, and incubate for 00:03:00.

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.

Sodium Acetate 3M, pH 5.2 Thermo Scientific Catalog #R1181  
https://dx.doi.org/10.17504/protocols.io.3byl4jo68lo5/v1  
Oct 23 2023
GlycoBlue™ Coprecipitant
Scientific Catalog #AM9516

6 Centrifuge 12,000 x g, 4°C, 00:10:00 to pellet nucleic acids. Discard supernatant and wash pellet with 1 mL 75% ethanol. Centrifuge again 7,500 x g, 4°C, 00:05:00 and discard supernatant.

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

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
Research Catalog #D4014

RNase Cocktail™ Enzyme Mix
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
Fisher Catalog #18068015

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

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.

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.

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:
- 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.

13 Use Zymo DNA purification of PCR product according to manufacturer’s suggested protocol.
Zymo DNA Clean & Concentrator - 5 Zymo
Research Catalog #D4014
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
- $98 \, ^\circ \mathrm{C}$ for 00:00:10
- $60 \, ^\circ \mathrm{C}$ for 00:00:30
- and $72 \, ^\circ \mathrm{C}$ for 00:00:10
using Q5 High-Fidelity DNA Polymerase - 500 units
New England Biolabs Catalog #M0491L
and the following primers:
- Forward: 5'- TCGTCGGCGAGTCGATGTGATAAGACAGcgatgttccagattacgcttgag -3'
- Reverse: 5'- GTCTCGTGGGTCTAGAGATTGTGATGATAAGACAGattttgtaatccagaggttgattatcg - 3'

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

Append Illumina flow cell adapters and unique indices by PCR amplification with
- NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) - 96 rxns
New England Biolabs Catalog #E7600S
by 10 cycles of
- $98 \, ^\circ \mathrm{C}$ for 00:00:10
- $59 \, ^\circ \mathrm{C}$ for 00:00:30
- and $72 \, ^\circ \mathrm{C}$ for 00:00:10
using Q5 High-Fidelity DNA Polymerase - 500 units
New England Biolabs Catalog #M0491L

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.