SOP: Isolation of pAAV Production Plasmids Using ZymoPURE II Plasmid Maxiprep Kit

Approvals:

Preparer: Hetal Doshi Date: 11OCT2022
Reviewer: Dr. Maggie Bryans Date: 15OCT2022

1. Purpose:

1.1. To isolate pAAV plasmids: pAAV-GFP, pAAV-DJ and pHelper using ZymoPure II plasmid Maxiprep Kit

2. Scope and Applicability:

2.1. This SOP covers the isolation of pAAV plasmids (pAAV-GFP, pAAV-DJ and pHelper) used for helper-free triple transfection of HEK293 cells to produce AAV viral particles

3. Summary of Method:

3.1. Set up overnight mini culture from the glycerol stock of Stbl3 cells transformed with appropriate plasmid
3.2. Scale up the overnight culture to 150ml culture
3.3. Harvest cells and perform maxi prep.

4. References:

4.1. Stbl3 cells product information sheet https://tools.thermofisher.com/content/sfs/manuals/oneshot_stbl3_man.pdf
4.2. ZymoPURE II Plasmid maxiprep Kit insert https://files.zymoresearch.com/protocols/_d4202_d4203_zymopure_ii_plasmid_maxiprep.pdf

5. Definitions:

5.1. N/A

6. Precautions:

6.1. Use BL2 safety measures and discard waste in biohazard containers.
6.2. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick etc
6.3. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.

7. Responsibilities:

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials:

8.1. Glycerol stock of stbl3 cells transformed with pAAV-DJ vector, stbl3 cells transformed with pHelper vector, stbl3 cells transformed with pAAV-GFP vector generated from 4.5. AAV-DJ Helper Free Expression System by Cell Biolabs, Catalog # VPK-410-DJ
8.2. LB Broth Miller Catalog# BP1426-500, Fisher Bioreagents
8.3. Ampicillin stock 1000X stock (100mg/ml concentration)
8.4. Incubator shaker at 37°C and 225 rpm
8.5. Spectrophotometer, cuvette, cuvette rack
8.6. Serological pipettes 1ml, 5ml, 10ml, 25ml and 50ml, pipette aid

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8.7. Micropipettes and micropipette tips
8.8. ZymoPURE II Plasmid maxiprep kit
8.9. 50 ml conical tubes
8.10. Sorvall RC 5C Plus centrifuge chilled at 4°C
8.11. Nalgene Centrifuge Bottles with Sealing Cap, polypropylene copolymer polypropylene screw closure; silicone gasket; 250ml Catalog# 3141
8.12. Beaker 250ml
8.13. Eppendorf centrifuge
8.14. Eppendorf centrifuge

9. Procedure:

9.1. **Day 1: Set up overnight mini culture from glycerol stock**

9.1.1. Prepare a 15ml conical tube with 2ml LB/Amp in the BSC by adding 2 µl of 1000X stock of ampicillin to the 2ml of LB broth
9.1.2. Swab the working area on the lab bench with 70% Ethanol. Partially thaw the glycerol stock of bacteria with appropriate plasmid on ice. Using good aseptic technique pipette 20µl of glycerol stock to the 15 ml conical tube prepared in step 9.1.1.
9.1.3. Incubate the tube at 37°C shaking at 250 rpm overnight
9.1.4. Repeat step 9.1.1. through 9.1.3. for remaining plasmids to be isolated

9.2. **Day 2: Scale up to 150ml culture**

9.2.1. After ~ 12-18 hours take an aliquot of the culture, dilute ten-fold and measure using a standard spectrophotometer.
9.2.2. When OD_{600} for a tenfold diluted culture is 0.2-0.35, inoculate 1000ml Erlenmeyer flask containing 150ml LB/Amp broth with 1ml of overnight mini culture
9.2.3. Incubate the flask at 37°C shaking at 250 rpm for ~12-18 hours. (Note: Don't let the culture time exceed 18h)

9.3. **Day 3: Harvest and Maxi prep**

9.3.1. After ~ 12-18 hours take an aliquot of the culture, dilute ten-fold and measure using a standard spectrophotometer.
9.3.2. When OD_{600} for a tenfold diluted culture is of 0.2-0.35, harvest the culture by transferring all the culture using a clean sterile 50ml serological pipette to an autoclaved Nalgene centrifuge 250ml bottle
9.3.3. Centrifuge bacterial culture at 4000 x g for 10 minutes at 4°C in Sorvall RC 5C Plus centrifuge to pellet cells. Discard supernatant in a beaker labelled waste
9.3.4. Prewarm the ZymoPURE Elution Buffer at 50°C by incubating in a water bath
9.3.5. Add 14ml of ZymoPURE P1 (Red) to the bacterial cell pellet and resuspend completely by vortexing and pipetting with a serological pipette
9.3.6. Add 14ml of ZymoPURE P2 (Blue) and immediately mix by gently inverting the bottle 6 times. (**Note: Do not vortex**) Incubate the tube at room temperature for 3 minutes. Cells are completely lysed when the solution appears clear, purple, and viscous.
9.3.7. Add 14ml of ZymoPURE P3 (Yellow) and mix gently by thoroughly inverting the bottle (**Note: Do not vortex**) Invert the bottle for additional 5 times after the sample turns

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completely yellow. The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form

9.3.8. Ensure the plug is attached to the Luer Lock at the bottom of the ZymoPURE Syringe Filter-X. Place the syringe filter upright in a tube rack and load the lysate in the Zymo PURE syringe filter-X with 25ml serological pipette. Wait 5-8 minutes for the precipitate to float to the top.

9.3.9. Remove the luer lock plug from the bottom of the syringe and place it into clean 50 ml conical tube. Place the plunger in the syringe and push the solution through the ZymoPURE Syringe filter-X in one continuous until approximately 35 ml of cleared lysate is recovered. Save the cleared lysate

9.3.10. Add 14ml of ZymoPURE Binding Buffer to the cleared lysate from step 9.3.9 and mix thoroughly by inverting the tube 8 times

9.3.11. Remove the 50ml Reservoir from the top of the Zymo-Spin V-PX Column assembly. Ensure the connection between the 15ml Reservoir-X and Zymo-Spin VP-X Column is finger tight and place the assembly into a new 50ml conical tube.

9.3.12. Add 10ml of the mixture from Step 9.3.11 into the 15 ml Reservoir-X and Zymo-Spin VP-X Column Assembly, and centrifuge at 500 X g for 2 minutes in Eppendorf 5804 R centrifuge. Discard the flow through and repeat the step until the entire mixture has passed through the column.

9.3.13. Add 10ml of ZymoPURE Wash 1 to the Zymo-spin V-RX Column Assembly and centrifuge the column at 500 x g for 2 minutes in Eppendorf 5804 R centrifuge. Discard the flow through.

9.3.14. Confirm 88ml of 95% ethanol is added to the 23ml ZymoPURE Wash 2 (concentrate) if not already added add the ethanol and label appropriately

9.3.15. Add 10ml of ZymoPURE Wash 2 to the Zymo-spin V-RX Column Assembly and centrifuge at 500 x g for 2 minutes in Eppendorf 5804 R centrifuge. Discard flow through and repeat this wash step

9.3.16. Remove and discard the 15ml Reservoir-X from Zymo-Spin V-PX Column. Place the Zymo-spin V-PX Column in a collection tube and centrifuge at 16,000g for 1 minute in Eppendorf 52424 R centrifuge, to remove any residual was buffer

9.3.17. Transfer the Zymo-Spin V-PX Column into a clean new 1.5ml tube and add 400µl of prewarmed ZymoPURE Elution buffer directly to the column matrix. Incubate at room temperature for 10 minutes. Centrifuge at 16,000 x g for 1 minute in Eppendorf 52424 R centrifuge

9.3.18. Store the eluted plasmid DNA at – 20 °C.

9.3.19. Analyze the quantity and quality of plasmids by nanodrop and agarose gel electrophoresis.

10. Attachments/Figures

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11. History:

Revision Number	Effective Date	Preparer	Description of Change
0	15OCT2022	Hetal Doshi	Initial release