VDL704.2 PHENOL/CHLOROFORM/ISOAMYL ALCOHOL EXTRACTION OF DNA

1. Purpose
   1.1. The purpose of this protocol is to purify DNA.
   1.2. This procedure is routinely performed in the Vector Development Laboratory (VDL) following Good Laboratory Practices (GLP).

2. Abbreviations and Definitions
   2.1. SOP  Standard Operating Procedure
   2.2. VDL  Vector Development Laboratory
   2.3. GLP  Good Laboratory Practice
   2.4. TE  Tris-EDTA

3. Equipment, Materials, and Reagents
   NOTE: All materials in contact with cells must be sterile, pyrogen-free and used according to the manufacturer’s directions unless stated otherwise. Equivalent materials and equipment may be used but all changes must be recorded.

3.1. Equipment
   3.1.1. Vortex
   3.1.2. Microcentrifuge

3.2. Materials
   3.2.1. Sterile pipet tips VWR
   3.2.2. 1.7 ml tubes Axygen

3.3. Reagents
   3.3.1. Phenol/Chloroform/Isoamyl Alcohol (25:24:1) Invitrogen
   3.3.2. 95% Ethanol (ice cold) VDL
   3.3.3. Glycogen Roche
   3.3.4. NaOAc Sigma
   3.3.5. 3M NaOAc VDL
   3.3.6. 70% Ethanol (ice cold) VDL
   3.3.7. 1X TE Amresco
   3.3.8. Sterile Water Baxter

3.4. Starting Materials
   3.4.1. Begin with 30 μL of purified viral vector.

3.5. Test Sample Identification
3.5.1. The bar code on the plasmid will be scanned and compared to the computer database to ensure the correct sample is being processed.
3.5.2. One copy of the barcode will be printed and applied to a copy of this SOP for the final record.

4. Procedure

4.1. Add 70 μL 1X TE buffer to the digested plasmid DNA.
4.2. Add 100 μL phenol:chloroform:isoamyl alcohol to the digested plasmid DNA.
4.3. Vortex thoroughly.
4.4. Spin the tube in a microcentrifuge at 14,000 rpm for 5 minutes to separate the phases.
4.5. Carefully transfer the top aqueous layer to a clean 1.7 mL tube. Discard the interface and lower phase into an organic waste container.
4.6. Add 400 μL ice-cold 95% EtOH, 50 μL 3 M NaOAc, and 1 μL glycogen to the retained aqueous layer.
4.7. Vortex thoroughly.
4.8. Spin the tube in a microcentrifuge at 14,000 rpm for 5 minutes.
4.9. A white DNA pellet should be visible on the bottom of the tube.
4.10. Carefully remove and discard the supernatant.
4.11. Wash the pellet with ice-cold 70% ethanol.
4.13. Spin in a microcentrifuge at 14,000 rpm for 5 minutes.
4.14. The DNA pellet should again be visible on the bottom of the tube.
4.15. Carefully aspirate off the supernatant.
4.16. Air dry the pellet for approximately 15 minutes at room temperature to evaporate residual ethanol.
4.17. When the pellet is dry, dissolve the DNA pellet in 10 μL sterile 1X TE buffer and store at -20°C until use.

5. Data Collection and Management

5.1. A final report will be produced from the raw data and inserted into the laboratory notebook and a copy will be stored in the VDL permanent files.
5.2. Deviations of the protocol will be recorded in the laboratory notebook and on the Lab Meeting Sheet.
6. Review and Revisions

Written by: ___________________________
Director, VDL

Reviewed by: ___________________________
Director, Vector Production

Reviewed by: ___________________________
Director, QA/QC

Date Issued: 1/30/2006 replaces VDL 704.0

Annual Review:

2011

Reviewed without changes  Changed and this version archived

Reviewed by: ___________________________

QA/QC by: ___________________________

Date Issued: 5/24/11 replaces VDL 704.1

2012

Reviewed without changes  Changed and this version archived

Reviewed by: ___________________________

QA/QC by: ___________________________

Date: ___________________________

2013

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Reviewed by: ___________________________

QA/QC by: ___________________________

Date: ___________________________