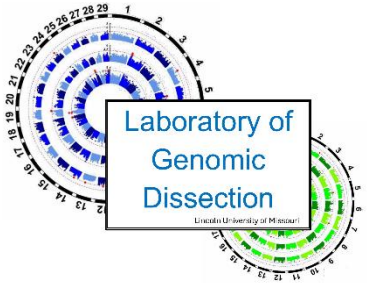

DNA extraction using the enzymatic salting-out protocol

Adapted from Radheshyam et al. (2013)

The salting-out method can be used to obtain higher yields of high-molecular-weight DNA. This method isolates high-quality samples for applications such as whole-genome sequencing. It is especially suitable for PCR amplification and short- and long-read sequencing. This protocol was designed based on the equipment available at this laboratory.

Basic principle

The enzymatic salting-out DNA extraction method combines the use of salt and enzymes to isolate DNA from cells.

Required reagents

1. 70% Ethanol
2. A Genomic DNA Clean & Concentrator Kit (e.g., kit from Zymo Research Corporation)
3. Absolute Ethanol
4. D-Sucrose powder
5. Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA) powder
6. MgCl flakes
7. NaCl powder
8. Pancreatic Ribonuclease A from Bovine
9. PCR water
10. Proteinase K solution
11. Sodium Dodecyl Sulfate (SDS) powder
12. TE pH 8.0 1x solution
13. Tris-HCl Buffer (1 M, pH 7.6) solution
14. Triton X-100

Required equipment and consumables

1. 15 mL conical bottom centrifuge tubes
2. 2 mL Eppendorf tubes
3. Heat block
4. Magnetic stirrer
5. Nalgene Oak Ridge high-speed polycarbonate centrifuge tubes 50 mL
6. Refrigerated centrifuge able to reach 10 K rpm
7. Scale and a scientific scale

Precautions

1. Label the solution with its components, concentration, date, and safety information. Include the name of the researcher who conducted all procedures.
2. Always confirm molecular weights in the bottle and adjust calculations if needed.
3. The particle size of the Sodium Dodecyl Sulfate (SDS) powder is very small and can be easily inhaled. Handle this reagent with care. SDS powder should be handled inside a ventilated hood while wearing a mask.

Working Solutions

Buffer A:

Procedure

1. **Dissolve Sucrose**:
    - In a beaker, add 109.5 g of sucrose to a small volume of distilled water (about 100-200 mL).
    - Stir until fully dissolved.
2. **Add Tris-HCl**:
    - Add 10 mL of 1 M Tris-HCl (pH 7.6) buffer to the sucrose solution.
3. **Make a 1 M $MgCl_2$ solution and add some to the mixture**:
    - Depending on what type of $MgCl_2$ form you have, weigh the following: 1) for Magnesium Chloride ($MgCl_2$, MW = 95.21 g/mol), weigh 95.21 g, or 2) for the hexahydrate form ($MgCl_2 \cdot 6H_2O$, MW = 203.30 g/mol), weigh 203.3 g.
    - Add 800 mL of distilled water to the powder in a beaker and stir until completely dissolved.
    - Measure the total volume of the solution and add distilled water up to 1 L. Transfer the solution to a final container.
    - Add 5 mL of this 1 M $MgCl_2$ solution to the mixture.
4. **Add Triton X-100**:
    - Add 1% of Triton X-100 based on the final volume.
    - For a 1% solution in 1 L, you would need 10 g of Triton X-100 (or 10 mL if it's the same density, but typically 10 g).

5. **Adjust the Volume**:
    - After adding all the components, bring the total volume of the solution up to 1 L with distilled water.
6. **Mix Well**:
    - Stir the solution gently to ensure all components are well mixed. Make sure everything is mixed thoroughly.

Buffer B

Procedure

1. Calculate Required Amounts

- **EDTA**:
    - The molecular weight of disodium EDTA is approximately 372.24 g/mol.
    - For a 25 mM solution in 1 liter:

      25 mM = 25 mmol/L × 372.24 g/mol = 9.306 g
- **NaCl**:
    - The molecular weight of NaCl is approximately 58.44 g/mol.
    - For a 75 mM solution in 1 liter:

      75 mM = 75 mmol/L × 58.44 g/mol = 4.383 g
- **Pancreatic Ribonuclease A**:
    - To achieve a concentration of 20 µg/mL in 1 L:

      20 µg/mL = 20 µg × 1,000 mL = 20,000 µg = 20 mg

2. Prepare the Solution

- **Dissolve EDTA:**
    - Weigh out 9.306 g of disodium EDTA
    - Dissolve in about 800 mL of distilled water
- **Adjust pH:**
    - Use a pH meter to check the pH of the solution

    - Adjust to pH 8.0 using either NaOH (to raise pH) or HCl (to lower pH)
- **Add NaCl:**
    - Weigh 4.383 g of NaCl.
    - Add it to the EDTA solution and stir until fully dissolved.
- **Add Ribonuclease A:**
    - Weigh out 20 mg of pancreatic Ribonuclease A and add it to the solution.
    - Stir gently to dissolve completely.
- **Adjust Volume:**
    - Transfer the solution to a 1 L volumetric flask.
    - Add distilled water to bring the final volume up to 1 L.
- **Mix Well:**
    - Ensure the solution is mixed thoroughly.

DNA extraction procedure

1. Use liquid blood as input. Add 3 mL of blood from each sample to a labeled Nalgene high-speed polycarbonate tube. Add 17 mL of **Buffer A**. Keep the solution on ice for 10-15 min.
2. Centrifuge your sample at 10,000 rpm for 10 min at 4°C. Discard the supernatant by tilting each tube. Be mindful to keep the centrifugation pellet within the tube.
3. Resuspend the centrifugation pellet in 1.8 mL of **Buffer B** by vortexing at low speed.
4. Label a new set of 15 mL tubes and transfer the suspension using a sterile transfer pipette. Include any debris.
5. Add 150 µl of 10% SDS and 20 µl Proteinase K (20 mg/mL) to the solution.
6. Incubate the suspension at 56°C (low-speed rocker or orbiter shaker in water bath; if not possible, vortex gently every hour). Incubation time is determined by tissue type. For blood, incubate for 3 hours.
7. Add saturated NaCl (700 µL) and mix the solution vigorously for 15 min.
8. Label two sets of 2 mL tubes. Divide the solution from each 15 mL tube into two matching, labeled 2 mL tubes. If you can perform centrifugation of 15 mL tubes, you do not need to split the solution.
9. Spin the mixture at 10,000 rpm at 4°C for 10 min.

10. Label a set of 15 mL tubes and transfer the supernatant from each of the matching 2 mL tubes (from the same sample). Do not transfer any debris and discard the pellet. DNA is precipitated by mixing the supernatant with two volumes of ice-cold Absolute Ethanol. Gradually add the Absolute Ethanol to the walls of the tube and invert the 15 mL tube till the DNA threads are visible (white threads). Adding Absolute Ethanol suddenly to the solution can increase the risk of precipitating salts and causing DNA precipitation to be missed.
11. Label another set of 2 mL tubes.
12. Collect the extracted DNA threads using 1,000 mL cut tips and transfer them to the new labeled 2 mL tubes (step 11). Try to decrease the amount of solution taken while capturing all the DNA threads.
13. Wash the collected DNA threads three times with ice-cold 70% ethanol. Add 500 µL of 70% ethanol and mix the solution gently with the same tip several times.
14. Eliminate residual ethanol with the same tip.
15. Air-dry the threads for 10 minutes in a heat block (40°C), keeping the lid open. Do not let the DNA threads dry completely; otherwise, DNA renaturation may become more difficult.
16. Use 100 µL TE 1X buffer to renature the DNA threads.
17. Incubate the DNA samples in a heat block (40°C for 30 minutes), keeping the tube open to eliminate residual ethanol.
18. Perform an additional DNA clean-up using a Genomic DNA Clean & Concentrator Kit (e.g., Zymo Research Corporation and follow its instructions.)
19. Measure the DNA concentration for each sample and dilute as needed with PCR water. Measure the quality parameter using a Qubit (ideal) or a Nanodrop. A260/280 values should be around 1.8, and A260/230 ≥ 2.0.

Applications

1. Extracted genomic DNA can be used for next-generation sequencing and animal genotyping.
2. Extracted DNA has several downstream applications in various techniques such as polymerase chain reaction (PCR) and DNA cloning.

Bibliography

Radheshyam, M., Brijesh, K., & Shyam, S. (2013). Evaluation of Salt-Out Method for the Isolation of Dna From Whole Blood : a Pathological Approach. *International Journal of Life Sciences Biotechnology and Pharma Research*, *2*, 7. http://www.ijlbpr.com/currentissue.php