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Protocol: Isolation of genomic DNA with NucleoSpin Blood L – Midi Kit

Purpose: This protocol is used to extract gDNA from frozen cell pellets using the NucleoSpin Blood L Midi Kit. The manufacturer's protocol recommends this kit for cell pellets between 5e6 – 2e7 cells. It is strongly encouraged to first test this kit on not-infected cells to ensure this same range applies to the cells of interest. gDNA recovery from the column can be estimated using an average value of ~6.6 pg gDNA per cell.

Materials:

- NucleoSpin Blood L – Midi Kit (Clontech Cat. #740954.20)
- RNase A (Clontech, Cat. #740505), lyophilized, dissolve to 20 mg/ml in H2O; store at -20°C
- Absolute ethanol
- Centrifuge with a swing-out buckets rotor capable of reaching 3500 x g is required

Getting started:

- Set a water bath to 56°C.
- Preheat Elution Buffer BE on top of a heat block set to 70°C.
- Equilibrate cell pellets to room temperature (15–25°C).
- Prepare Buffer BQ2 by adding ethanol and prepare Proteinase K by resuspending in Proteinase Buffer according to the kit insert.
- Label one 15 mL centrifuge tube for every column that will be used. *These tubes do not have to be the specific collection tubes provided in the NucleoSpin kit. Any 15 mL conical (e.g. Falcon™ 15mL Conical Centrifuge Tubes) can be used.*

Step 1. Lyse cell pellet

1. Add 1 mL PBS and 150 uL of Proteinase K to each conical.
    - 1 mL PBS assumes the cell pellet volume is 1 mL, bringing the final volume to approximately 2 mL.
2. Add cell pellet and mix well by vortexing.
3. Add 4.1 uL RNase A (20 mg/mL) to the 15 mL conical and vortex.
    - If the cell pellet is still clumpy, incubate at 56°C for 5-20 min and vortex the tube several times to further break up the cells.
4. Add 2 mL of Buffer BQ1 and vortex the mixture vigorously for 10s.

- Vigorous mixing is important for DNA yield and purity.

5. Incubate samples at 56°C in the water bath for 15-45 minutes, vortexing every 10 minutes until solution is clear.
6. Let the lysate cool down to room temperature before proceeding with ethanol precipitation.
    - Make sure that the lysate has cooled down to room temperature (about 5 min) before loading it onto the columns. Loading of hot lysate may results in reduced yields due to evaporation of the ethanol.

Step 2. Precipitate DNA

7. Add 2 mL absolute ethanol to each sample and *immediately* mix by inverting the tube 10 times.
    - High local ethanol concentration must be avoided by immediate mixing.

Step 3. Bind DNA

8. For each prep, take one NucleoSpin Blood L Midi column placed in the provided conical and load 3 mL of lysate. Avoid drops on the rim of the column. Close the tubes and centrifuge 3 min at 3250 x g. Discard flow through.
    - The lysate will usually start to flow through the column even before centrifugation. This will not adversely affect DNA yield or purity.
    - Keep NucleoSpin Blood L Midi columns in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.
9. Load the remaining 3 mL of lysate to the respective NucleoSpin Blood L Midi columns. Avoid drops on the rim of the columns. Close the tubes and centrifuge 5 min at 3250 x g.
    - Remove the collection tubes with the columns carefully from the rotor and avoid having the flow-through come in contact with the column outlets.
10. Discard the flow-through and place the column back into the collection tube.

Step 4. Wash silica membrane

11. **1st wash:** add 2 mL of Buffer BQ2 to the NucleoSpin Blood L Midi columns. Centrifuge 2 min at 3250 x g. It is not necessary to discard the flow-through after the first washing step.
12. **2nd wash:** add 2 mL of Buffer BQ2. Centrifuge 10 min at 3250 x g. Remove the columns carefully from the rotor to avoid having the flow-through contact the column outlets. Discard flow through.
    - A longer centrifugation during this second washing step insures that residual ethanol from Buffer BQ2 is removed from the silica membrane of the column so no further drying step is necessary.

Step 5. Elute DNA

See the Practical Considerations Appendix for alternative elution conditions that may improve gDNA yields.

13. Insert the column into a new 15 mL collection tube and apply 200 uL of preheated (70°C) Elution Buffer BE directly to the center of the silica membrane.
15. Incubate overnight at 4°C, or at room temperature for 5 minutes.
16. Centrifuge at 3250 x g for 2 min to elute.

Step 6. Dilute and store gDNA

17. Measure the concentration of gDNA (for example, using NanoDrop or Qubit). If concentration is higher than 200 ng/uL, dilute gDNA with Elution Buffer BE.
    - We recommend calculating the dilutions based on a precisely measured elution final volume because there is always some loss due to the column.
18. Store at 4°C for short term, or -20°C for long term.
19. Next, please see: "**Guidelines for gDNA sample submission for PCR and sequencing**."

Practical Considerations Appendix

RNase:

The original NucleoSpin Blood L protocol does not call for the addition of RNAse. However, the presence of RNA contamination in gDNA samples can skew quantification when using absorbance-based methods for determining sample concentrations. Furthermore, large amounts of RNA contamination can have a negative impact on subsequent PCR steps. For these reasons we highly recommend using RNase when performing large scale gDNA isolations.

Elution Volume:

The Genomic DNA from Blood User Manual lists the following elution procedures for highest yield and/or concentration:

- High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90–100% of bound nucleic acid can be eluted
- High concentration: Perform one elution step with 60% of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution Maximal yield of bound nucleic acid is about 80%.
- High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–100% of bound nucleic acid is eluted in the standard elution volume at a high concentration.