Last modified: May 2019; overnight 70°C lysis
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Protocol: Isolation of genomic DNA with NucleoSpin Blood Kits

Purpose: This protocol is used to extract gDNA from frozen cell pellet(s) using the NucleoSpin Blood Kits. The manufacturer's protocol recommends different column sizes for cell pellets of various sizes. We strongly encourage users to first test the chosen kit on representative but non-valuable cells to ensure the protocol performs well for the cells of interest. gDNA recovery from the column can be estimated using an average value of ~6.6 pg gDNA per cell.

The possible kit sizes are:

- Mini Kit -- for pellets less than 5e6 cells
- L Midi Kit -- for pellets between 5e6 – 2e7 cells
- XL Maxi Kit -- for pellets of 2e7 – 1e8 cells

Note prior to starting: *If using the Mini kit, pellet your cells initially in 200 uL PBS to simplify the protocol.*

Materials:

- Machery Nagel NucleoSpin Blood Kit:
  - Mini (Clontech Cat. #740951)
  - L Midi (Clontech Cat. #740954.20)
  - XL Maxi (Clontech Cat. #740950)
- PBS
- RNase A (Clontech, Cat. #740505), lyophilized, dissolve to 20 mg/ml in H2O
- Absolute ethanol
- Extra 1.5 mL, 15 mL, or 50 mL centrifuge tubes (e.g. Falcon)
- A tabletop centrifuge:
  - For the Mini kit: a microcentrifuge capable of 11,000 x g
  - For the L Midi and XL Maxi: swing-out buckets rotor capable of 3,500 g
- Vortex
- Heat block and water bath

Getting started:

- Prepare the heat block, water bath, and reagents as described in the manufacturer's protocol.

- Equilibrate cell pellets to room temperature (15–25°C).
- For the Mini Kit: spin down cells and resuspend in 200 uL PBS, if not previously done.
- For the XL Maxi Kit: If your cell pellet contains more than the manufacturer's recommended amount, divide the pellet across multiple columns.

Instructions:

Use the manufacturer's protocol with the following modifications:

Step 1: Lysing cell pellets

- Incubate the cell pellet, PBS, Proteinase K and lysis buffer mixture at **70°C overnight** in the water bath.

We have determined that incubating the samples overnight with Proteinase K increases gDNA yields, improves gDNA quality, and increases the likelihood of PCR success.

Step 2. Precipitating DNA

- Remove the lysate from the water bath the following morning, allowing it to cool to room temperature before adding RNase A.

- Add RNase A (20 mg/mL) and incubate for 5 min at room temperature.
  - Mini: add 1 uL
  - L Midi: add 4.1 uL
  - XL Maxi: add 20 uL

The original NucleoSpin Blood Mini 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 gDNA isolations.

- When proceeding with the ethanol precipitation, be sure to immediately mix the sample after adding absolute ethanol to avoid high local ethanol concentration.

Step 3. Binding DNA

- Avoid drops on the rim of the column and keep 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.

Step 5. Eluting DNA

- Incubate the column with pre-warmed (to 70˚C) elution butter at room temperature for 5 min. An overnight incubation at 4˚C can lead to a modest increase in yield.
- Optional, recommended for precious samples: use a PCR inhibitor removal kit on eluted gDNA (Zymo Research, #D6030).

While not necessary, running the gDNA through a PCR inhibitor removal column can reduce the likelihood of PCR failure. There is no sample loss associated with the inhibitor removal columns.

Step 6. Diluting and storing gDNA

- Measure the concentration of gDNA (ex. NanoDrop or Qubit), and, if necessary, dilute to 200 ng/uL with elution buffer. Calculate the dilutions based on the actual measured volume of eluted DNA, as there is always some loss due to the column.

The NanoDrop and Qubit quantifications are often correlated, but the Qubit detects dsDNA, while NanoDrop will detect any nucleic acids. Thus, NanoDrop readings are often 2 – 3 fold higher than Qubit. We recommend the use of Qubit.

- Store at 4°C for short term or -20°C for long term.