BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Isolation of genomic DNA with NucleoSpin Blood XL-Maxi kit

Purpose: this protocol is used to isolate gDNA from a frozen cell pellet of up to 1e8 cells, as an alternative to QIAamp DNA Blood Maxi Kit (Qiagen Cat.# 51192).

Materials

- NucleoSpin Blood XL (Clontech Cat. # 740950)

- RNase A (Clontech, Cat. # 740505), lyophilized, dissolve to 20 mg/ml in PBS.

- Absolute EtOH

- Centrifuge with a swing-out buckets rotor capable of reaching 4,000–4,500 x g is required.

Getting started

- Set a water bath to 56°C.
- Preheat Elution Buffer BE to 70°C.
- Equilibrate cell pellets to room temperature (15–25°C).
- Prepare Buffer BQ2 and Proteinase K according to the kit insert.
- Determine the number of columns you will use per cell pellet (maximum 10E8 cells per column). If you have more than 1e8 cells, split the sample over several columns and combine the eluted DNA at the end.
- Label one 50 ml centrifuge tube for every column that will be used.

Step 1. Lyse blood sample

- Resuspend up to 1e8 cells in a final volume of 10 mL PBS.
- Pipette cell suspension, 500 µL Proteinase K and 20 µL RNase A into a 50 mL tube 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.
- Add 10 mL Buffer BQ1 to the samples and vortex the mixture vigorously for 10 s. *Vigorous mixing is important to obtain high yield and purity of DNA.*
- Incubate samples at 56°C for 15 min.
- Let the lysate cool down to room temperature before proceeding with addition of ethanol.

Step 2. Precipitate DNA

- Add 10 mL absolute ethanol to each sample and mix by inverting the tube 10 times.

  High local ethanol concentration must be avoided by immediate mixing after addition.

  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 lead to reduced yields.

Step 3. Bind DNA

- For each preparation, take one NucleoSpin Blood XL column placed in a provided collection tube and load 15 mL of lysate. Avoid drops on the rim of the column. Close the tubes and centrifuge 3 min at 3750 rpm. 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 XL Column in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.

- Load 15 mL of the remaining lysate to the respective NucleoSpin Blood XL column. Avoid drops on the rim of the column. Centrifuge 3 min at 3750 rpm. *Remove the collection tube with the column carefully from the rotor and avoid having the flow-through come in contact with the column outlet.*
- Discard the flow-through and place the column back into the collection tube.

Step 4. Wash silica membrane

- *1st wash:* add 7.5 mL Buffer BQ2 to the NucleoSpin Blood XL column. Centrifuge 2 min at 3750 rpm. It is not necessary to discard the flow-through after the first washing step.
- *2nd wash:* add 7.5 mL Buffer BQ2. Centrifuge 10 min at 3750 rpm. Remove the column carefully from the rotor to avoid having the flow-through get in contact with the column outlet.
  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

- Insert the column into a new 50 ml collection tube and apply 1000 µL of preheated (70°C) Buffer BE directly to the center of the silica membrane. Incubate at room temperature for 2 min. Centrifuge at 3750 rpm for 2 min.
- If higher yield is desired, reuse flow-through of Buffer BE directly onto the membrane of the NucleoSpin Blood XL column and close the cap. Incubate at 4°C for 1-2hrs (or overnight if desired). Centrifuge at 3750 rpm for 2 min.

Step 6. Dilute and store gDNA

- Measure the concentration of gDNA (for example using NanoDrop). If concentration is higher than 200 ng/ µL, dilute gDNA with Buffer BE. Store at 4°C for short term or -20°C for long term.
- Next, please see: “Protocol for gDNA sample submission for PCR and sequencing”