



Last modified: May 2018

Last reviewed: May 2018

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

Purpose: this protocol is used to isolate gDNA from frozen cell pellets.

Materials

- NucleoSpin Blood XL (Clontech Cat. # 740950)
- RNase A (Clontech Cat. # 740505) - Dissolve to 20 mg/ml in PBS, store at -20°C
- 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 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.
- Determine the number of columns needed per cell pellet. The maximum capacity of one column is 2e7 to 1e8 cells (read the Practical Considerations Appendix if the pellet contains more than 1e8 cells).
- Label one 50 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 50 mL conical (e.g. Falcon™ 50mL Conical Centrifuge Tubes) can be used.*

Step 1. Lyse Cell pellet

- Add 8.5 mL PBS and 500 μ L of Proteinase K to each conical. (*8.5mL PBS assumes the cell pellet volume is 1mL, bringing the final volume to 10mL. If splitting pellet over multiple columns, see the Practical Considerations Appendix for appropriate volumes*)
- Add cell pellet and mix well by vortexing. *If splitting pellet, split after vortexing, there should be 10mL per conical.*
- Add 20 μ L RNase A (20 mg/mL) to the 50mL 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.
- Add 10 mL Buffer BQ1 to the samples and vortex the mixture vigorously for 10 s. *Vigorous mixing is important for DNA yield and purity.*
- Incubate samples at 56°C for 15-45 minutes vortexing every 10 minutes until solution is clear.
- 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 result in reduced yields due to evaporation of the ethanol.*

Step 2. Precipitate DNA

- Add 10 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 after addition.*

Step 3. Bind DNA

- For each prep, take one NucleoSpin Blood XL column placed in the provided conical 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 contact 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 EB directly to the center of the silica membrane.
- For a) maximum yield elution (recommended): Incubate at 4°C overnight.
 b) rapid (reduced yield) elution: Incubate at 70°C for 5 minutes.
 (See Practical Considerations Appendix for description of elution conditions).
- Centrifuge at 3750 rpm for 2 min.

Step 6. Dilute and store gDNA

- Measure the concentration of gDNA (for example using NanoDrop or Qubit). If concentration is higher than 200 ng/µL, dilute gDNA with Buffer EB. *We recommend calculating the dilutions based on an elution volume of 900 uL because there is always some loss to the column.* Store at 4°C for short term or -20°C for long term.
- Next, please see: “gDNA sample submission protocol”

Practical Considerations Appendix

Splitting Pellets:

If you have more than 1×10^8 cells, the cell pellet will need to be split over multiple columns during the lysis steps, and the eluted DNA will be combined at the end. The pellet should be resuspended with 8.5 mL PBS and 500 μ L Proteinase K for every 1×10^8 cells, rounded up (ex: 1.8×10^8 would be resuspended in a total volume of 17 mL PBS and 1000 μ L Proteinase K; 2.4×10^8 would be resuspended in 25.5 mL PBS and 1500 μ L Proteinase K).

After vortexing to fully resuspend the pellet, the solution can be split into the total number of conicals needed. Each conical should contain a total of 10 mL of cell suspension. Lyse the cells by adding 10 mL of lysis buffer to each. Each conical can then be treated as a separate column for the rest of the protocol until elution and dilution.

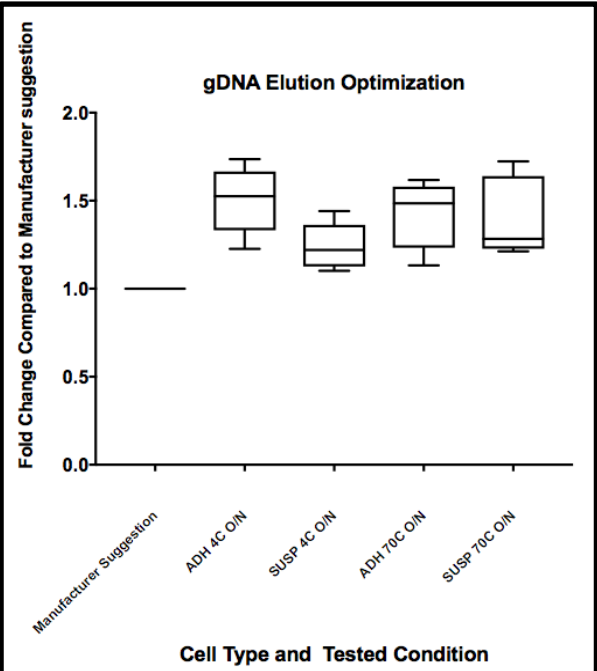
After the final DNA is eluted in 1000 μ L for each column, the columns corresponding to the same original cell pellet can be combined. The quantification can then be performed on this combined sample. When calculating the dilutions, be sure to use the appropriate starting volume; instead of calculating based on 900 μ L for a single column, use a total volume of 1800 μ L for two columns, or 2700 μ L for three columns.

RNase:

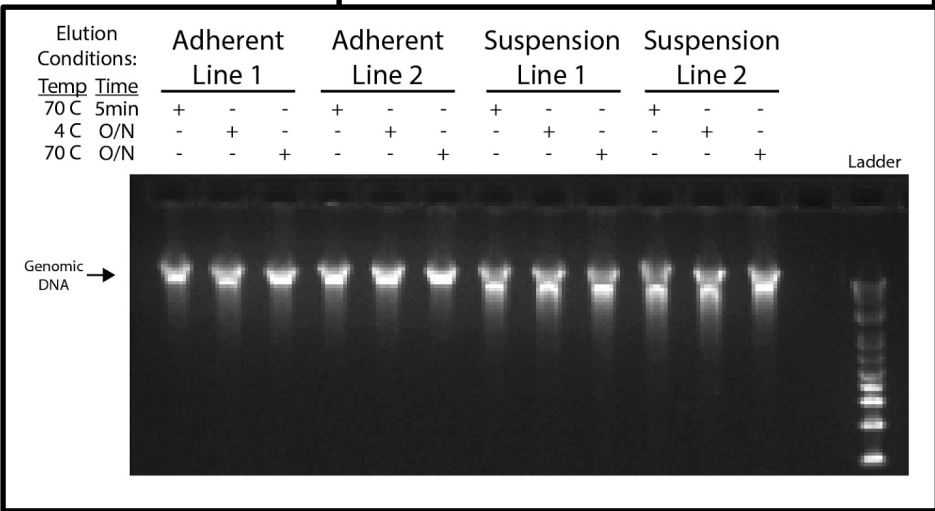
The original NucleoSpin Blood XL protocol does not call for the addition of RNase. However, the presence of RNA contamination in gDNA samples can skew quantification when using absorbance-based methods for determining sample concentrations. Furthermore, large amounts of RNA contamination can have a negative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

Increasing gDNA Yields:

We have found that increasing the duration of sample elution from the NucleoSpin columns can result in higher gDNA yields. More specifically, eluting samples from NucleoSpin columns overnight typically increases the amount of recovered gDNA by ~1.5X as compared to the manufacturer’s suggested 5 minute elution. These increased yields have been observed when isolating gDNA from both adherent and suspension cell lines. When eluting overnight the temperature of the incubation has no significant impact on the amount of gDNA recovered. Importantly, elution buffer should always be warmed to 70C before adding to columns (regardless of the overnight elution conditions).



We have assessed gDNA quality by agarose gel electrophoresis and find that overnight elution yields high quality gDNA. For example, we observe well formed gDNA bands with no obvious signs of fragmentation (streaking).



We have also run PCR amplification tests and find that overnight elution has no negative impact on the ability to PCR amplify from recovered gDNA.

