

INTERNATIONAL-DMD (I-DMD) PROJECT

THE AMOUNT OF DNA TO BE SENT IS 15µg WHOSE MINIMUM CONCENTRATION MUST BE 37.5 ng / µl.

DNA EXTRACTION METHOD FROM WHOLE PERIPHERAL BLOOD SUGGESTED FOR NGS ANALYSIS OF DYSTROPHIN MUTATIONS

Forward

A standardized and cost-effective DNA extraction method is essential to assure reproducible results, whatever the DNA source. The DNA quality is particularly relevant for the next-generation sequencing (NGS) technologies. Indeed, non-standardized DNA qualification can negatively impact sequencing performances (especially if DNA was partially or complete degraded), resulting in a waste of samples, low confidence results and higher costs.

For these reasons, in our lab, we tested four different DNA extraction protocols (QIAamp DNA Blood Mini Kit from QIAGEN, illustra Nucleon BACC3 from GE Healthcare Life Sciences, gDNA Extraction Kit from Agilent and Phenol/chloroform method) using fresh blood, and blood stored at 4°C or -20°C.

Our finding highlight the efficiency of Nucleon extraction method for both fresh and stored blood (Fig. 1), in addition this is less expensive and time-consuming method and requires a small blood amount (1,5 ml), suggesting its benefit for NGS technologies.

Following the detailed method we have set up using the illustra Nucleon BACC3 kit.

Starting from 1,5 ml of blood, collected in EDTA Tubes:

- Add 4,5 ml of Reagent A (included in the kit)
- Rotary mix (or by inversion) for 5 minutes at room temperature
- Centrifuge at 1300 g for 5 minutes
- Discard the supernatant
- Add 340 µl of Reagent B (included in the kit) and resuspend the pellet
- Transfer the suspension to a clean 1.5 ml microcentrifuge tube.
- Add 100 µl of sodium perchlorate solution (included in the kit)
- Rotary mix (or by inversion) for 5 minutes at room temperature
- Incubate 5 min at 65°C
- Add 580 µl of cold chloroform
- Rotary mix (or by inversion) for 15 minutes at room temperature
- Centrifuge at 1300 g for 3 minutes
- Holding the tube vertically, transfer the upper phase to a clean 1.5 ml microcentrifuge tube
- Add 880 µl of cold absolute ethanol.
- Mix gently by inversion until the precipitate appears
- Pick up the precipitate with a Pasteur with a closed-end
- Disconnect by tapping and resuspend the precipitate into 100 µl of water (in a clean 1.5 ml microcentrifuge tube).
- Assess the DNA concentration (i.e using Nanodrop, Qubit)
- Evaluate the DNA integrity by agarose-gel.

DNA extraction was also performed on the QIA Symphony SP workstation using the QIA Symphony DNA kit. Extractions were performed according to the manufacturer's instructions (QIAGEN GmbH, Hilden, Germany). Quality and quantity of the extracted DNA was comparable with the DNA obtained by Illustra Nucleon BACC3 kit.

Fig.1. Different evaluations determining the Nucleon BACC3 as useful method for DNA extraction.

DNA EXTRACTION using
illustra Nucleon BACC3 (GE Healthcare Life Sciences)

FROM

FRESH BLOOD BLOOD STORED AT 4°C BLOOD STORED AT -20°C

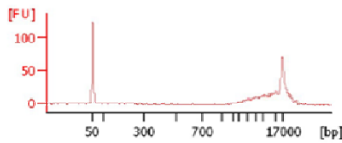
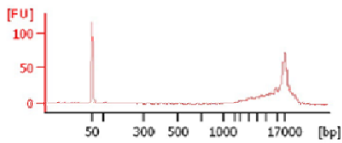
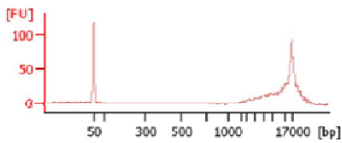
Nanodrop
quantification

ng/ul: 297.8
260/280: 1.82
260/230: 1.94

ng/ul: 295.0
260/280: 1.84
260/230: 1.88

ng/ul: 294.2
260/280: 1.74
260/230: 1.78

Bioanalyzer
quality
evaluation



DNA integrity
by agarose-
gel

