Nanoparticle-based nucleic acid extraction from eukaryotic sources

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Aim

To create efficient, flexible, and user-friendly nucleic acid extraction procedures for eukaryotic sources such as cultured cells, organoids, tissues, and body fluids.

Background

During the COVID-19 pandemic, a shortage of commercial kits for SARS-CoV-2 RNA extraction prompted the development of an extraction technology at NTNU. It utilizes a customized lysis buffer and silica-coated, superparamagnetic iron oxide nanoparticles, providing high specificity and enabling automated extraction. This technology laid the foundation for development of extraction procedures for other biological sources.

Results

Cell lines

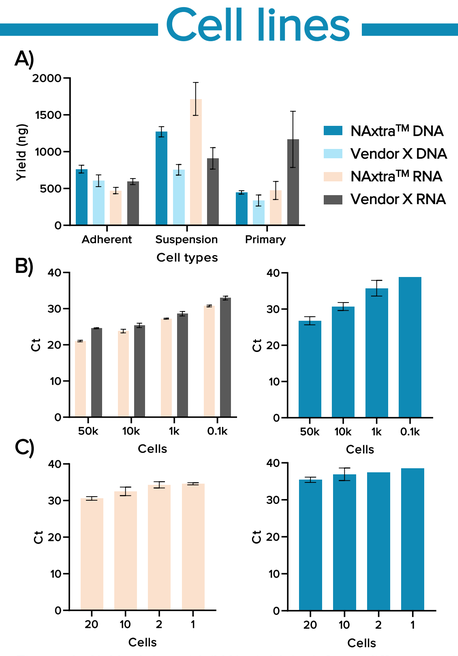

Figure 1. A) Nucleic acid (NA) yield (ng) from different cell types, comparing the NAxtra™ DNA/RNA extraction procedures to a commonly used kit by vendor X. Adherent = HAP1, suspension = JJN-3 and primary = fibroblasts, input = 100 000 cells, n = 3, error bars indicate ±1 SD. **B) (RT-)qPCR for NAs extracted using the NAxtra™ RNA/DNA extraction procedures, compared to a kit by vendor X for RNA extraction.** Cells = HAP1, n = 3, error bars indicate ±1 SD. **C) (RT-)qPCR for NAs extracted using a tailored low cell input procedure.** Cells = HAP1, n = 3, error bars indicate ±1 SD.

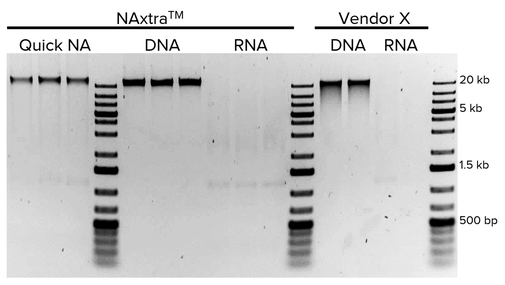

Figure 2. Nucleic acid (NA) extraction from 50 000 cells (HAP1), comparing NAxtra™ to a commonly used kit by vendor X. Automated extraction steps take ~15 minutes for the NAxtra™ Quick NA protocol and ~22 minutes for the DNA/RNA protocols.

Organoids

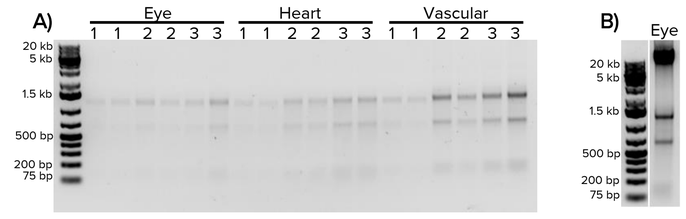

Figure 3. A) RNA extraction from 1-3 organoids of different types (eye, heart, vascular) using the NAxtra™ RNA protocol. RNA integrity (RIN) values ranged from 8.1-10. **B) NAxtra™ Quick NA extraction** from 11 eye organoids.

Tissues

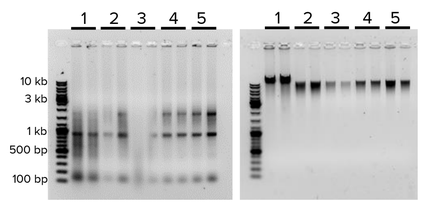

Figure 4. Optimalization of lysis buffer to PBS ratio during pre-treatment (bead-beating). NAxtra™ RNA (left) and DNA (right) extraction from mouse liver, preceded by bead-beating in 1 = PBS only, 2 = 1 part lysis buffer, 3 parts PBS, 3 = 1 part PBS, 1 part lysis buffer, 4 = 3 parts lysis buffer, 1 part PBS, 5 = lysis buffer only. The best results are achieved with bead-beating in only lysis buffer for RNA and only PBS for DNA.

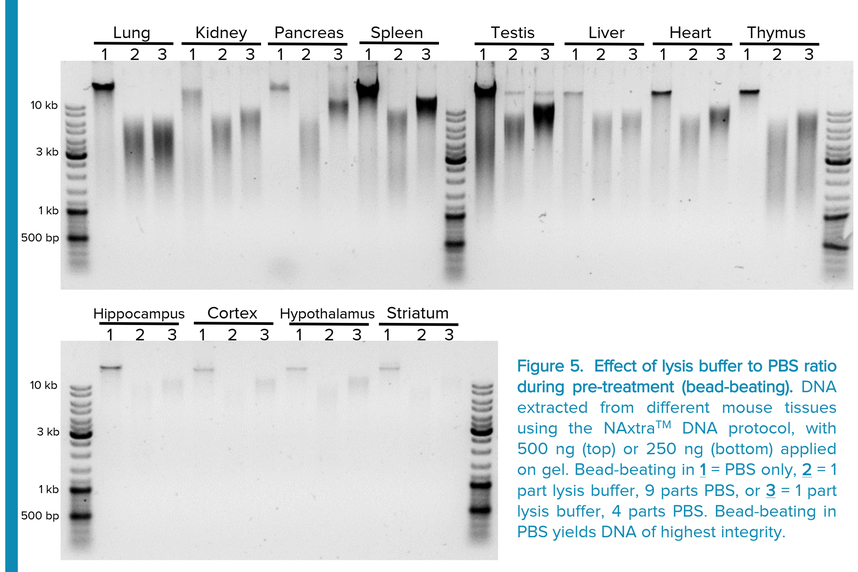

Figure 5. Effect of lysis buffer to PBS ratio during pre-treatment (bead-beating). DNA extracted from different mouse tissues using the NAxtra™ DNA protocol, with 500 ng (top) or 250 ng (bottom) applied on gel. Bead-beating in 1 = PBS only, 2 = 1 part lysis buffer, 9 parts PBS, or 3 = 1 part lysis buffer, 4 parts PBS. Bead-beating in PBS yields DNA of highest integrity.

Extraction procedures

Tailored protocols for nucleic acid extraction from cells, organoids, tissues and blood, including options for **pre-treatment** (for tissue and blood; Figure 4-6), **nuclease treatment,** or extraction from **low cell numbers** (down to one haploid cell; Figure 1.C) have been developed based on the NTNU extraction technology commercialized as NAxtra™ by Lybe Scientific AS. **Automated extraction** of 12 and 96 samples can be performed on robot systems KingFisher™ Duo Prime and Flex, respectively. Duration of the automated extraction steps ranges from 11-27 minutes depending on the input material and desired output.

Blood

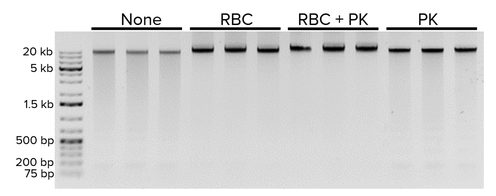

Figure 6. Effect of different pre-treatments (None, RBC = red blood cell lysis buffer, PK = proteinase K) on automated nucleic acid (NA) extraction using the NAxtra™ Quick NA protocol for blood (100 µl) collected in EDTA tubes.

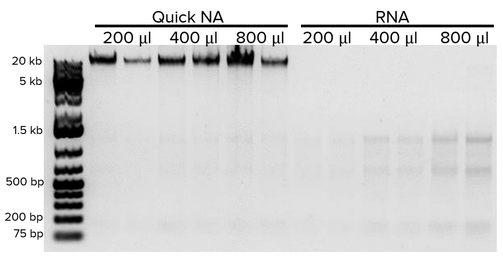

Figure 7. Automated nucleic acid (NA) extraction from blood collected in PAXgene blood RNA tubes, using the NAxtra™ Quick NA protocol or RNA protocol. Pre-treatment with proteinase K. Input volume of blood-mixture ranging from 200-800 µl.

Method

Synthesis

The process of synthesizing iron oxide nanoparticles, illustrated in Figure 8, can be varied to produce particles with different sizes, coatings, and magnetic properties.

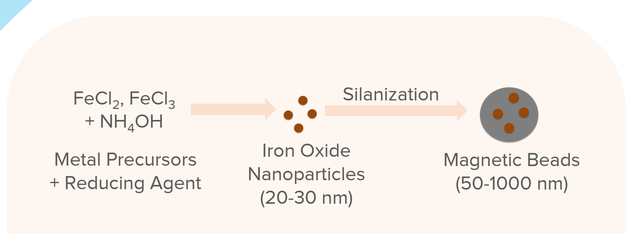

Figure 8. General synthesis of iron oxide nanoparticles from metal precursors by coprecipitation, followed by coating of particles with silica.

Extraction

Lysis of sample in a customized lysis buffer.

Addition of silica-coated, superparamagnetic iron oxide nanoparticles for binding of nucleic acids. Particle-bound nucleic acids are extracted using a magnet.

Removal of impurities by several washing steps.

Elution of pure nucleic acids in nuclease free water.

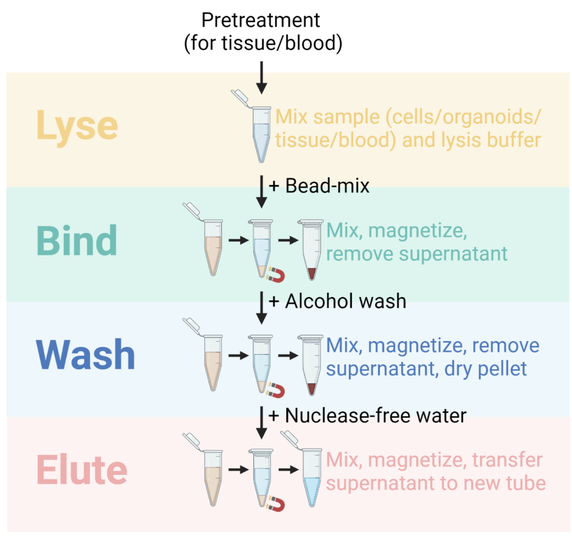

Figure 9. General principles of the nanoparticle-based nucleic acid extraction technology developed at NTNU (NAxtra™). Created with BioRender.com.

Summary

NAxtra™ is a **fast, flexible and cost-efficient nucleic acid extraction method** for **cells, organoids, tissues and blood.** The resulting nucleic acids have **high integrity** and are **suitable for downstream applications** such as (RT-)qPCR and next-generation sequencing.

Future perspective

Cheaper and more efficient molecular methods help accelerate advancements in molecular research. We are currently developing procedures for extraction of circulating, cell-free nucleic acids, and implementing NAxtra™ for single cell transcriptomics and epigenomics.