Uppsala biobanking: gDNA purification with MACHEREY-NAGEL's NucleoSpin 96 Blood kits on the Hamilton Genomic Starlet

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Introduction

Uppsala Biobank is an infrastructure for medical research and centre of competence for biobank related matters.

Uppsala Biobank is a collaboration between Uppsala University and Uppsala County Council and represents the only biobank of these two principals. It was founded due to the benefits gained from having one central biobank. All sample collections containing biobank samples, sample collections for research or sample collections for medical care and treatment, under either of these two principals are included in Uppsala Biobank.

Uppsala Biobank also provides services such as collection of biobank samples, storage of samples as well as a sample management IT system. As the first biobank in Sweden, Uppsala Biobank has, in collaboration with Clinical Chemistry and Pharmacology at Akademiska University Hospital, developed innovative and unique logistics solutions in which the hospital infrastructure is used for efficient biobanking. This logistic solution enables effective collection of biobank samples via the hospital.

In 2010 the Uppsala Umeå Comprehensive Cancer Consortium (U-CAN) started with the purpose to become an international leader in terms of high-quality longitudinal biobanking of certain cancers. The collected sample material, along with detailed patient information, will primarily be used for biomarker studies to optimize diagnosis, therapy and follow-up in cancer diseases. Uppsala Biobank developed such an infrastructure for collecting, processing, and storage of blood or other liquid specimens biobank samples in a simple and automated manner for U-CAN (see page 2 for detailed description of the established workflow).

Since summer 2010, U-CAN has collected liquid sample from cancer patients suffering from colorectal -, prostate – or brain cancer and from leukaemia and lymphoma patients. From each patient an EDTA whole blood sample was collected in a collection tube, which is stored in -80°C, until DNA extraction is performed.

The collection for U-CAN also includes EDTA plasma, Citrate plasma and serum. These primary tubes were aliquoted into 2D barcoded Micronic 0.5 mL tubes, which were stored at low temperature in automated freezer. The total numbers of U-CAN donors at Uppsala Biobank are approximately 4500, and today (2015) about 15 other research projects are already using the Uppsala Biobank infrastructure.

The sample handling process (aliquotation, NA extraction) uses robotic equipment from Hamilton to facilitate and ensure uniform sample management.

Figure 1:
The Hamilton Genomic STARlet

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Method Description

For aliquotation of the EDTA blood sample a Microlab STAR liquid handling station from Hamilton was used.

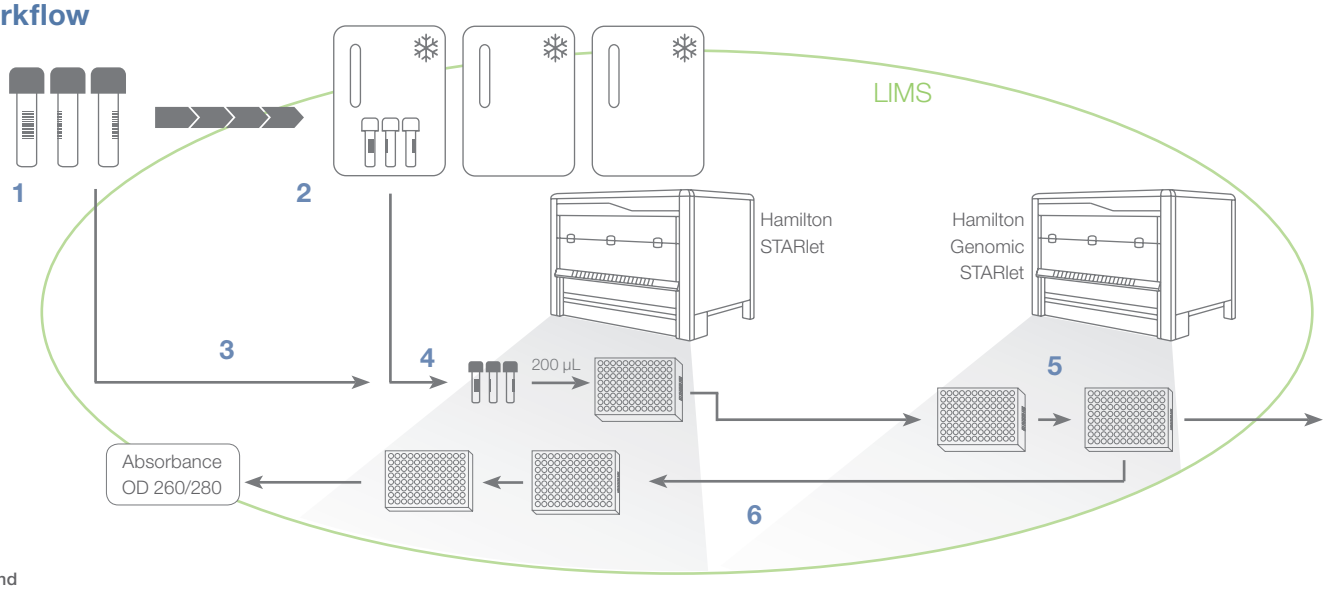
For the extraction of the genomic DNA out of the blood sample (fresh and frozen), the NucleoSpin Blood kit (PN 740665.4) from MACHEREY-NAGEL was used. The Extraction was performed fully automated on a Genomic STARlet standardised solution (4 Channel, manual load) from Hamilton using an integrated vacuum station. All Steps - from 200 µL aliquoted blood in a Micronic 96-1 rack, as a source until the eluate was obtained in the target storage plate, Micronic 96-1 rack, were carried out on this instrument without any user intervention.

DNA yield and purity were measured using the Trinean Drop-Sense 96 device, integrated into the Microlab STAR liquid handling station. 3 µL DNA was transferred from each tube into the Drop-Sense 96 plate by using the Microlab STAR liquid handling station.

For SNP detection the samples were submitted to the Uppsala SNP detection facility and processed according to their published procedure (TaqMan SNP Genotyping Assays from ThermoFisher; www.thermofisher.com https://tools.thermofisher.com/content/sfs/manuals/TaqMan_SNP_Genotyping_Assays_man.pdf). The SNP with ID rs1126960-A/C located on the IRX3 gene was used.

For integrity analysis, a 0.8% agarose gel was used. 2 µL of each DNA was mixed with 2 µL loading dye and subjected for gel analysis at 70 V for 4 h. As marker 4 µL “GeneRuler 1 kb DNA” ladder from Thermo Fisher was used.

Workflow



Legend

1. *Blood samples, collected using the Uppsala university hospital integrated biobank infrastructure, or from clinical trials and/or clinical research studies in Sweden and around the world, arrive at Uppsala Biobank and they are registered into LIMS* and stored in -80°C freezers.*
2. *When a sample withdrawal request is filed to Uppsala Biobank, a sample pick list is generated. The samples on the list are picked from the –80°C freezers and placed in the Hamilton STARlet to generate a 96-well plate with 200 µL aliquots.*
3. *Blood samples can alternatively enter directly the processing queue: In less than 4 hours, including pre and post pipetting steps, DNA from 96 200 µL blood samples is extracted (step 4-6). DNA can be extracted from fresh samples or from samples that have been frozen for years.*
4. *The whole blood samples are aliquoted into a 96 deep well plate by the Hamilton STARlet instrument. Also, back-up blood samples are made and stored in the -80°C freezers.*
5. *The 96 deep well plate is transferred to a Hamilton Genomic STARlet instrument for DNA extraction according to a protocol for the MACHEREY-NAGEL NucleoSpin 96 blood kit. Out of 200 µL whole blood, approximately 5 µg DNA in ~ 100 µL volume, is extracted. The DNA amount is dependent on the white blood cell content. The DNA eluate is transferred into 2D labelled tubes in a 1D labelled rack (storage rack marked in green).*
6. *The storage rack is transferred back to the Hamilton STARlet instrument. A few microliter of each DNA preparation is analyzed for DNA quality and quantity measurements, using absorbance technique. The result file from the DNA extraction process and measurements are combined in the LIMS system, and the donor information is updated.*

**LIMS: The Laboratory Information Management System (LIMS) used at Uppsala Biobank is a commercially available system custom made to perfectly match the sample collection and sample handling processes within the Uppsala Biobank. The LIMS system receives information from different databases, robotics systems and registrations and keeps record of donor ID, sample types, sample history and sample storage to maintain complete traceability of the samples.*

Results

A subset of approximately 1000 U-CAN samples have been used for automated DNA extraction, using a Hamilton Microlab STARlet for aliquotation and a Genomic STARlet from Hamilton for NA extraction running MACHEREY-NAGEL NA Extraction kits (see Workflow).

For Uppsala Biobank the sample quality is defined by yield, purity, integrity (degree of fragmentation) and SNP detection as a functional qualification criterion.

Purity

The purity of the DNA was analysed in a subset of 800 of the extracted sample. In >95% of the analysed sample (761 of 800) an OD 260/280 value of >1.7 was measured. Across each of the analysed 96 well plates a remarkable uniformity of the results was obtained (see figures 2 and 3: plate 3 & 10 as example). Except some outliers (in this case 1 or 3 drop outs and 4 or 3 values below 1.7 for plate 3 and 10, respectively), the other values are all between 1.7 and 2.0.

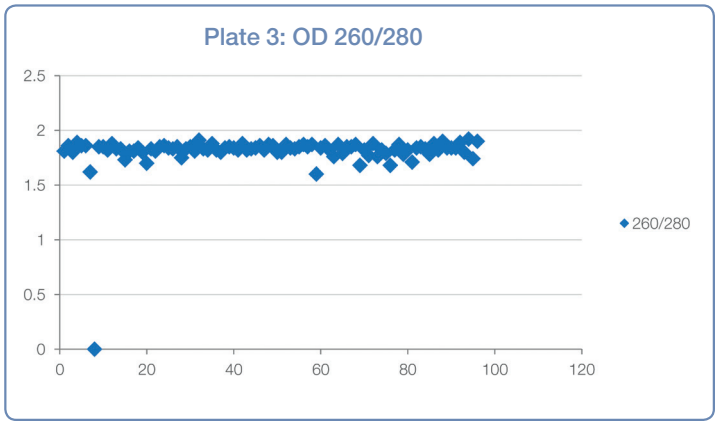


Figure 2: DNA purity, OD 260/280 of plate 3

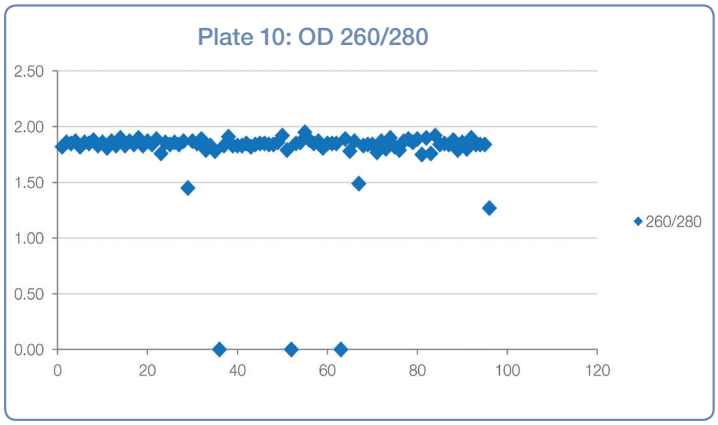


Figure 3: DNA purity, OD 260/280 of plate 10

Yield

Due to the fact that the blood sample came from different individuals, the measured concentration was observed to be within a bandwidth of 20-100 ng/µL in a 96 well plate reflecting most probably the difference of white blood cell content in the 96 individual patient samples of one extraction run (see figures 4 and 5: plate 7 & 8 as an example). Only a few outliers could be identified with concentrations above 100 ng or below 20 ng/µL. Zero values are coming from drop outs.

On average over all analysed sample the concentration is 52 ng/µL (excluding the drop outs) or on average 6.24 µg DNA per sample could be extracted.

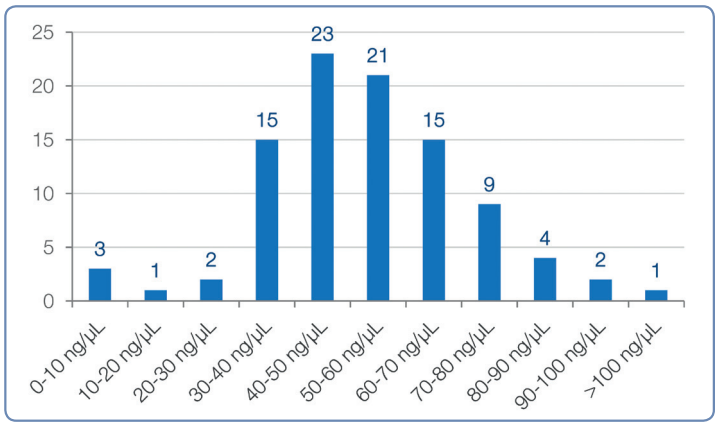


Figure 4: Concentration measurement of samples from plate 7

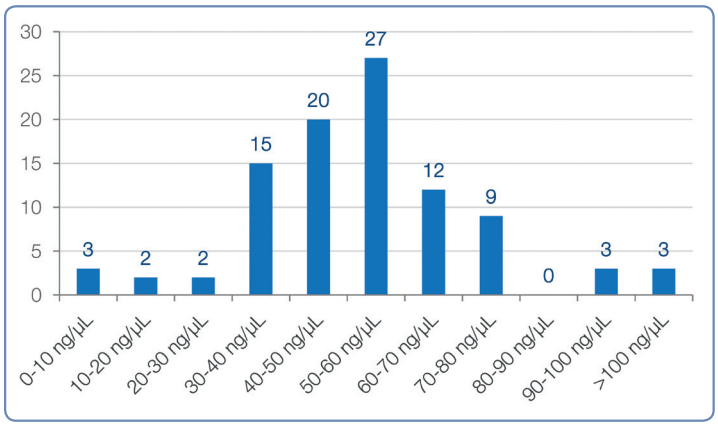


Figure 5: Concentration measurement of samples from plate 8

Quality: SNP & Fragmentation

SNP detection of the samples was successfully performed (see figures 6 and 7: SNP data from plate 2 & 3) indicating that the DNA is of high quality. DNA from one plate was subjected to gel electrophoresis to analyse the integrity of the obtained genomic DNA. The extracted DNA is of high molecular weight (see figure 8, only one quarter of the samples of one run are shown), implying that the extraction process by using MACHEREY-NAGEL NucleoSpin 96 Blood extraction kits and vacuum is a very gentle procedure.

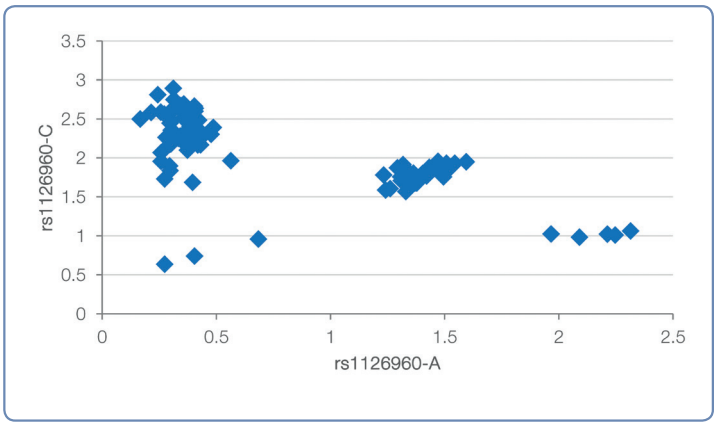


Figure 6: SNP data from plate 2

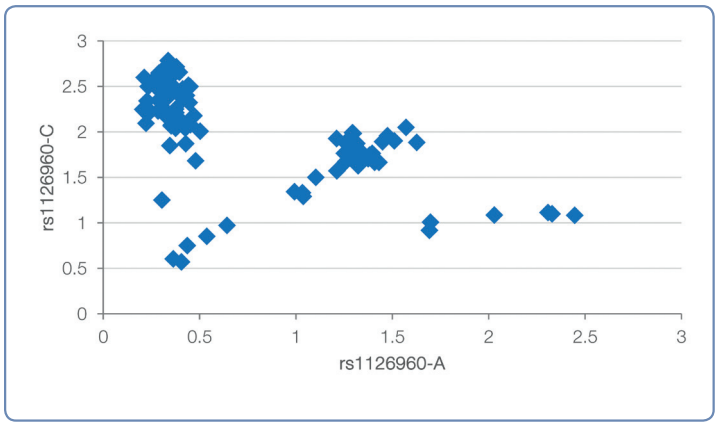


Figure 7: SNP data from plate 3

The outcome of the trial shows that the established workflow using Hamilton liquid handling instruments is performing very well: on average the expected amount of genomic DNA (at least 5 µg) could be obtained from 200 µL blood, fresh or frozen, and the purity measured by OD 260/280, was in the range between 1.7-2.0 for the majority (>95%) of samples.
SNP detection could be performed with high scoring data indicating functional usability of the extracted genomic DNA and there was no fragmentation of the genomic DNA and the DNA was of high molecular weight as analysed by agarose gel-electrophoresis.

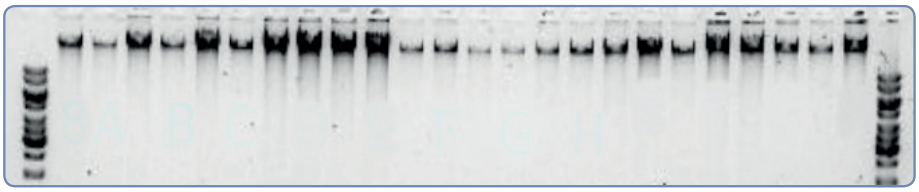


Figure 8. Extracted DNA
(only one quarter of the samples of one run are shown)

Summary

Uppsala Biobank is a well-established infrastructure for sample collection, sample handling and sample tracking for samples to be used for current and future medical research. The number of research projects and sample types that utilizes the Uppsala Biobank infrastructure are constantly increasing, showing that this initiative creating a hospital integrated biobank service has been very successful.

In the near future, Uppsala Biobank expects to handle more than 1000 donors per month. Approximately one third of these donors are donating whole blood for DNA extraction purposes. An increasing number of customers are expected to request direct extraction of DNA. Other expected future extraction requests of biobank samples are RNA from stabilized blood, free circulating DNA in blood, DNA from saliva, and DNA and RNA from tissue, either in formalin fixed paraffin embedded, or frozen in OTC. With the current installed automation and extraction instruments from Hamilton the Uppsala Biobank is well prepared to handle the expected future throughput and tasks.

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