Case Study: Creating a successful facility for large-scale extraction of DNA

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Introduction

Driven by the needs of several large population cohort studies, we have implemented a high-throughput facility for consistently high quality DNA purification from whole blood at KI Biobank. We have a routine throughput of up to 1000 samples per day using two parallel systems. Automation and modern extraction chemistry have given many benefits, and since starting the operation in May 2011 we have extracted DNA from over 100 000 individuals.

Our early experience clearly demonstrated the potential of the new systems in speed and cost. But we also experienced several difficult challenges. The modern genotyping platforms have also developed rapidly and we found that some of them are very sensitive to DNA quality parameters we didn't anticipate. We also experienced working environment problems due to the larger scale of operation.

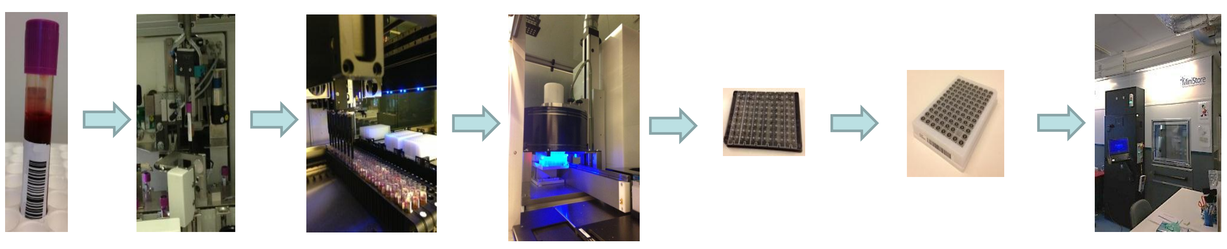
We have now devoted much effort to solving these problems, and have learnt many lessons.

Aim

Optimize our automated high-throughput workflow for DNA extraction for use our in-house genotyping analysis (SNP-analysis) pipeline that delivers in real-time results back to researchers/patients.

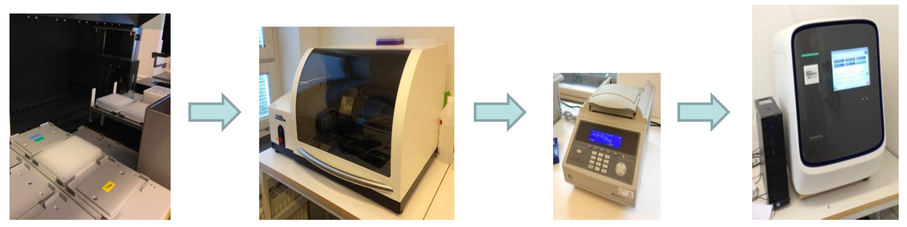
Workflow

Extraction



4 ml Blood sample from study donors | Automated lid removal | Aliquoting of 0.4 ml whole blood to extraction plate | Extraction | Measurement Yield: 10-15 µg | 150 µl DNA in 300 µl tubes | Storage at -20°C

Genotyping-analysis

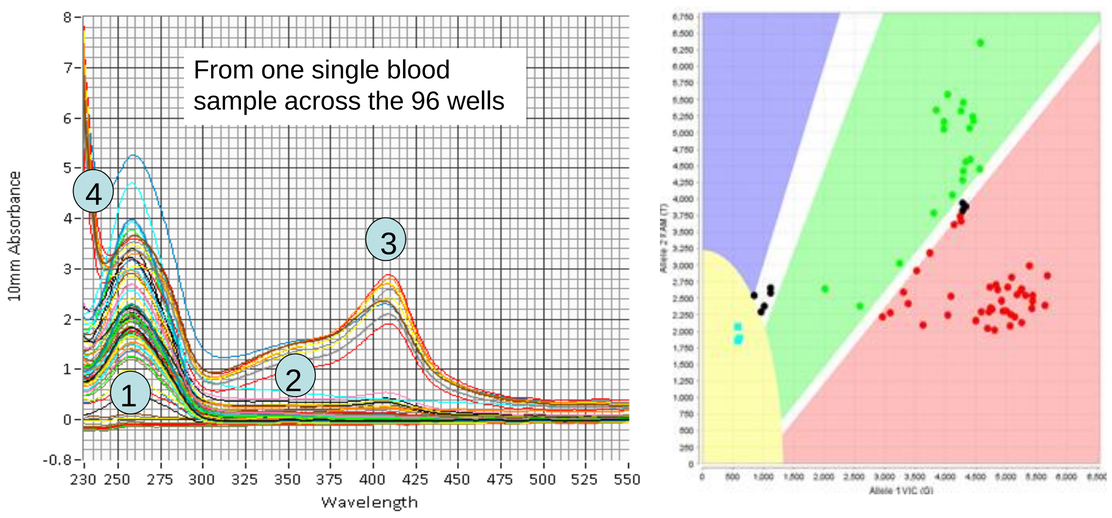


Pipetting from 96-well to 384 well | Pipetting from 384 well to array plate | PCR | SNP- Analysis

Issues

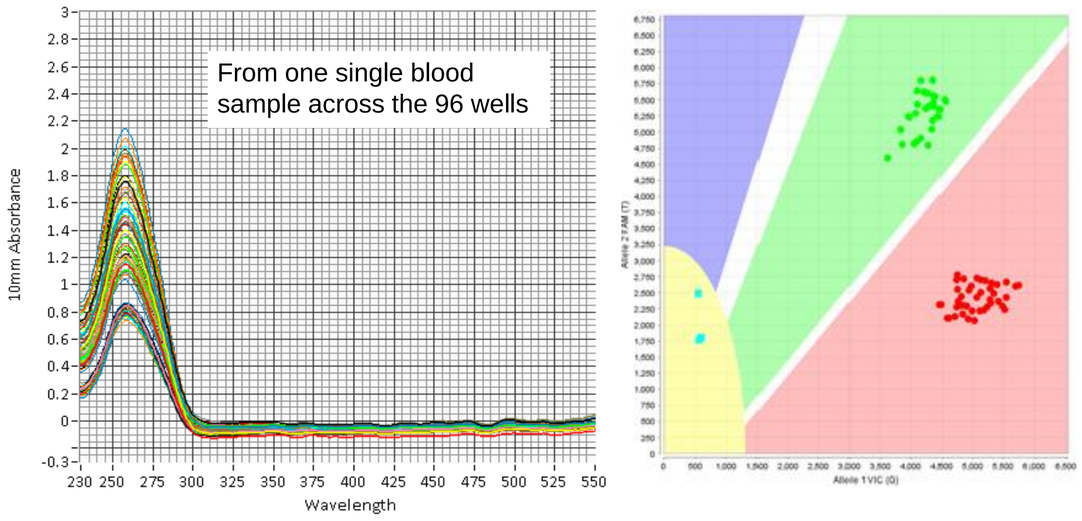
- [1] Low quality or no DNA extraction
- [2] Magnetic bead contamination
- [3] Hemoglobin contamination
- [4] Salt contamination
- [5] Environmental issues caused by large amounts of chemicals

Non optimized



Left: Full spectra UV-measurement; Right: Scattered non-clustered genotyping results

KI optimized



Left: Full spectra UV-measurement; Right: Scattered clustered genotyping results

Results and conclusion

After optimizing over 50 protocol steps, including developing non-foaming aspiration, dispense and mixing routines, improved magnetic separation and washing procedures (all of which are now included in the standard product), we saw a dramatic improvement in DNA-quality and genotyping results. The genotyping results using a 256 SNP array had call rates so poor before optimization, that no reliable data could be obtained. After the DNA extraction optimization the call rate increased (>99%) and this platform is now used on a daily routine with one of our most important translational healthcare research projects involving 200 000 participants.

The working environmental issues [5] that caused skin reactions was solved by mounting the instruments to the ventilation system.

Future objectives

We will start to implement new protocols such as extraction of DNA from larger volumes of blood and extracting RNA from blood.

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