From primary blood to DNA – fully automated DNA extraction

The Department of Human Genetics at the Radboud University Nijmegen Medical Center provides care for patients and families with hereditary diseases, and performs ground-breaking research into the relationship between genes and diseases. The main areas of interest for the Center's DNA laboratory are hereditary cancers, kidney disease, hereditary deafness and blindness, mental retardation and neuromuscular disease, including mitochondrial disorders.

The laboratory's research activities require a reliable, highly reproducible and standardized method for DNA extraction from full blood samples. We are using a Tecan Freedom EVO® 150 Workstation, combined with a Chemagic Magnetic Separation Module I from Chemagen, to automate our DNA extraction method using magnetic beads.

Figures 1 and 2 show the experimental set-up for the DNA extraction, which includes a Tecan Freedom EVO 150 Workstation with PosID™ barcode reader, and a liquid handling (LiHa) arm with eight tips in mixed configuration. A robotic manipulator (RoMa) arm with a centric gripper transports buckets with BD Falcon™ tubes through an opening in the benchtop (under the deck) onto the carrier axis of the Chemagic Magnetic Separation Module I (Chemagen), which is adjacent to the Tecan Workstation. There is also a dispenser for larger volumes on the Workstation.

The extraction is performed in 50 ml Falcon™ tubes, which are organized in a 4 x 3 (=12) configuration in the buckets, in order to fit into the Chemagic Magnetic Separation Module I (see Figure 3). The module consists of an electromagnet and a metal 12-rod head. The rods are dipped into the Falcon™ tubes, which contain the sample and a magnetic bead suspension, so that when the electromagnet is switched on, the rods are magnetized and the beads are separated.

DNA extraction from full blood without aliquoting

No aliquoting or sample distribution is necessary for this application, so the original primary blood samples are used. Each sample's barcode is read from the primary tube at the beginning of the process and passed through the whole process to the final 2 ml Eppendorf vials containing the extracted DNA. All of the barcodes and the sample positions are stored and documented within the LIMS.

The LiHa arm is used to prepare the tubes, which contain approximately 7 ml blood with lysis buffer, M-PVA Magnetic Beads, washing buffer and elution buffer. The RoMa arm moves the buckets with the samples under deck onto the carrier axis of the separator and the Tecan software starts the Chemagic protocol for the magnetic separation. Centrifugation steps do not need to be carried out with this protocol and sample preparation using Chemagen's Chemagic kits can be completed very quickly.

The high magnetite content of the magnetic beads makes extractions from any sample volume very easy and, consequently, the low levels of non-specific binding ensure that highly pure nucleic acids are isolated from crude samples and critical sample materials. The isolated DNA can then be used directly in a variety of downstream applications.

The final product, the nucleic acid stock solution, is quantified by measuring the OD 280 / 260 with a Tecan GENios™ reader, and normalized in order to get a standard working concentration. The OD 280 value represents the DNA quality and the OD 260 represents the DNA concentration; typical yields from this procedure are 30 µg DNA/ml sample.

Downstream processing

The nucleic acid stock solution is suitable for use in a number of downstream processes, including Southern blotting, PCR, multiplex ligation-dependent probe amplification (MLPA), sequencing and conformation sensitive capillary electrophoresis.

Performance, throughput and validation

With the current set-up, the average throughput is 100-150 samples per week and 5,000 samples per year. With each two hour extraction run, 12 samples can be extracted as a batch and the maximum throughput per day is four runs with a total of 48 samples.

The instrument set-up and the method have been thoroughly validated by comparison with manual methods and the results have been published. Quality assessment studies have been undertaken by the European Molecular Diagnostics Network in 1-2 cycles per year with results scoring at the end of the study, and this is comparable with the German „Ringversuche" method.

Frans Hol, PhD, Clinical Molecular Geneticist, Radboud University Medical Center Nijmegen, Dept. Human Genetics – 120, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands

Figure 1: *Fully automated DNA extraction set-up: Tecan Freedom EVO 150 Workstation and Chemagic Magnetic Separation Module I (Chemagen).*

Tecan Freedom EVO 150 Workstation [1] with PosID barcode reader [2], LiHa arm with 8 tips in mixed configuration [3] and RoMa arm with centric gripper [4]. Buckets are transported through an opening in the benchtop under deck onto the carrier axis [5] of the Chemagic Magnetic Separation Module I (Chemagen) [6].

Figure 2: *Fully automated DNA extraction set-up in detail.*

PosID barcode reader [1], LiHa arm [2], RoMa arm with centric gripper [3], heat incubator [4] and buckets [5] with samples.

The Radboud University Medical Center Nijmegen uses Chemagen magnetic beads with a Freedom EVO Workstation to extract DNA from blood samples

Figure 3: *Chemagic Magnetic Separation Module I (Chemagen). Electromagnet [1] and metal 12-rod head [2].*

Automating DNA extraction procedures has several advantages:

- *The entire process is fully automated, from the primary blood sample to the final DNA as a nucleic acid stock solution, ready for further experiments*
- *It is much faster (two hours) compared with the manual method, which needs an overnight incubation*
- *It results in a much better and more reproducible quality of DNA*
- *The DNA has a lower viscosity and so can be pipetted much more easily in downstream processes*
- *The set-up allows excellent documentation of the process and results, and complete sample identification and tracking*
- *Combining Tecan instruments with Chemagen kits and the Magnetic Separation Module has been well established by Tecan, and both instruments can be controlled using one software package*
- *The system has been running very reliably, for over a year, and this is particularly important since aliquots of original blood samples are not stored, meaning that if a sample is lost then it would be necessary to go back to the patient for a second blood sample*