
Low, Medium and High Volume Nucleic Acid Extraction

Genomic DNA extraction out of 1 - 10 ml blood with the chemagic Magnetic Separation Module I



Introduction

The human histocompatibility leukocyte antigens (HLA) are a group of highly polymorphic glycoproteins exerting defined biological functions: They harbor peptides derived from self or foreign protein antigens, bring them up to the cell surface and present them to immunocompetent T cells. Due to this, they play a central role in the physiology of the immune system and are the major barrier for solid or bone marrow transplantation. In order for the transplantation to be successful, the patient and the donor must be HLA matched. HLA allelic polymorphisms can be identified by a variety of techniques. More recently, molecular DNA methods have been employed to discriminate alleles of HLA genes. These techniques include direct sequencing (SBT) or SSO/SSP analysis of the HLA genes. Each of these techniques has its own intrinsic capability

for characterizing particular HLA loci, however, a combination of these techniques is generally required to completely defining the entire HLA haplotype. To search for the optimal HLA matching between donor and receiver, a high resolution molecular HLA typing with up to 50 to 100 PCR reactions is necessary.

One of the most important factors for reliable results is high yield and high purity of genomic DNA. To perform these reactions and in addition for prospective analysis, an amount of genomic nucleic acid is required, that exceeds often the yield of standard extraction protocols of 20 - 100 μ l whole blood.

Therefore a reliable and reproducible high-throughput purification of long size genomic DNA from a large volume of whole blood with a yield of up to 100 μ g DNA is of advantage.

Material and Methods

Instruments

Sample Preparation

Tecan Genesis Freedom 150/8 (Fig.1) equipped with 4 teflon coated standard steel tips and 4 disposable tip adapter. Carrier for primary sample tubes, for disposable tips, for reagents and for microplates or deep well plates are positioned on the worktable.

A barcodereader (PosID) is able to read the barcodes on the primary samples as well as on the mikroplates. Two hotels store the deep well plates. A robotic manipulator arm (RoMa) transports the deep well plates from the storage hotels to the pipetting positions on the worktable and under deck onto the carrier axis of the chemagic Magnetic Separation Module I.



Fig.2: chemagic Magnetic Separation Module I equipped with 96 metal rod head.

1: Electromagnet, 2: 96 metal rod head



Fig. 1: Tecan Freedom 150 Workstation with chemagic Magnetic Separation Module I attached. Robotic arm can load the carrier axis through the worktable.

Magnetic Separation

The chemagic Magnetic Separation Module I (Fig. 2) is positioned on the right hand side of the Tecan Freedom. The carrier axis leads under the Freedom worktable. All positions of the carrier axis can be loaded and deloaded from the robotic manipulator arm through the hole in the freedom worktable. The magnetic separation module is controlled from the Tecan Gemini Software by serial interface.

The chemagic Magnetic Separation Module I can be used with exchangeable heads which allow the processing in 96-well-plates (working volumes up to 2 ml) using a head with 96 magnetizable metal rods or in larger tubes (working volumes up to 50 ml) using a 12 rod head. This results in two different processes: Medium volume nucleic acid extraction out of 10 – 300 µl whole blood processed in deep well plates and high volume nucleic acid extraction out of 1 - 10 ml whole blood processed in 15 or 50 ml Falcon tubes.

Medium Volume Nucleic Acid Extraction

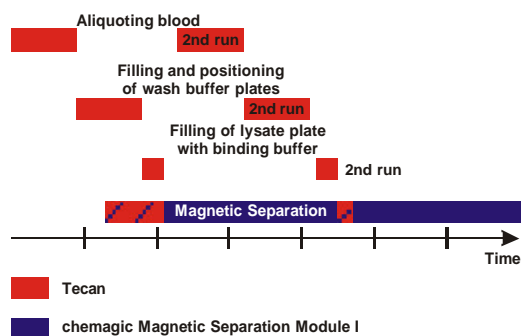
96 Extractions out of 100 µl whole blood

The primary samples are placed in the carriers on the worktable, the deep well plates have to be positioned in the hotels, reagents and disposables have to be on the deck and the chemagic Magnetic Separation Module I has to be equipped with the 96 rod head.

The barcode of the primary samples are read by the barcode reader (PosID). The robotic arm gets the deep well plates out of the hotel on the pipetting position. The Liquid Handling arm is preparing the following deep well plates:

- S 100 µl blood with 200 µl Lysis Buffer and 150 µl Magnetic Beads
- S 4x 500 µl Wash Buffer
- S 100 µl Elution Buffer

The robotic arm moves the deep well plates under deck onto the carrier axis of the separator and the Tecan software starts the chemagic protocol. The following diagram shows the chronological sequence.



The final Product, the nucleic acid stock solution can be quantified by measuring the OD and the quality is checked by agarose gel electrophoresis. Typical yields of this procedure are 3 - 4 µg of purified genomic DNA.

High Volume Nucleic Acid Extraction

12 Extractions out of 2 ml whole blood

The Tecan instrument has to be loaded with primary samples, reagents and disposables.

The magnetic separator has to be equipped with the 12 rod head.

The barcode of the primary samples are read by the barcode reader (PosID). The Liquid Handling arm is preparing the following 50 ml Falcon tubes:

- S 2 ml blood with 3 ml Lysis Buffer, 8 ml Binding Buffer and 240 µl Magnetic Beads
- S 5 ml Wash Buffers
- S 500 µl Elution Buffer

Typical yields of this procedure are 80 – 100 µg of purified genomic DNA.

The process for extraction of 12 samples needs about 70 minutes.

The system is able to run more several batches in one run without manual interruption.

Results

The results from more than 1500 donor samples showed very high consistency of DNA in yield ($76 \mu\text{g} \pm 9 \mu\text{g}$) and purity (1.8 ± 0.09). The purified DNA was used for routine typing of HLA-A, B, C, DRB1 and DQB1 alleles by PCR-SSP. Typical Results are shown in Figure 3.

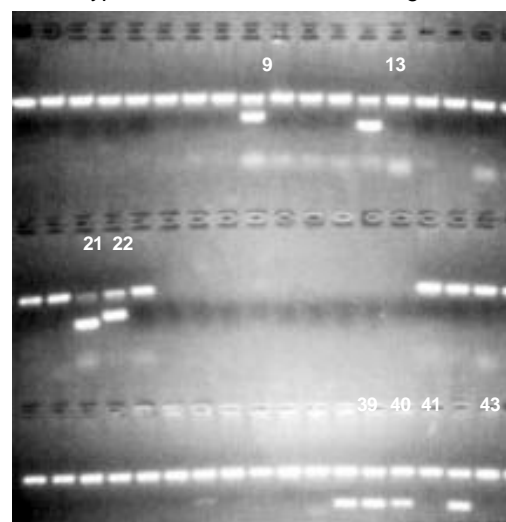


Figure 3: PCR-SSP analyses of HLA-DRB1 and HLA-DQB1 alleles. Genomic DNA was amplified with 45 different allele-specific primer pairs targeted at genes DRB1 through to DRB5 and DQB1. Allele-specific PCR products appear as a intensive bands below the 439 bp control amplicon (human growth hormone gene) in lanes 9, 13, 21,22, 39, 40, 41 and 43. (Data kindly provided Dr. S. Ferencik, Institute of Immunology, Director: Prof. Dr. med. H. Grosse-Wilde, University Hospital of Essen, Germany)

Conclusion

Classical methods of DNA and RNA isolation are based on column or precipitation methods. These techniques require centrifugation or vacuum steps and often have lengthy processing times as well as volume limitations, complicating their integration into automated high throughput processes. Additionally, the automated processing of these methods may cause cross contamination of the purified nucleic acids making this method useless for many diagnostic purposes.

Sample preparation using chemagen's chemagic Kits in combination with a fully automated robotic Tecan workstation are carried out quickly. No centrifugation steps are employed. Due to the high magnetite content of the particles, isolations from both, very small (e.g. 1 µl) and very large (e.g. 20 ml) sample volumes, are easily accomplished.

The isolated products can be used directly in a variety of downstream applications. chemagen has developed a novel module which eliminates automation obstacles. In this system, magnetic separation is achieved through the use of an electromagnet and magnetizable and rotatable metal rods, which are immersed into the magnetic bead suspension. With this apparatus, separated particles can be easily resuspended in subsequent buffers by switching off the electromagnet and rotating the rods at approximately 2,000 rpm. The normally difficult resuspension is in this case quick and thorough, resulting in products with both high yields and purities.

Credits

This application note has been made possible by:

Dr. Stephan Jacobs,
chemagen Biopolymer-Technologie AG
Arnold-Sommerfeld-Ring 2
D-52499 Baesweiler
Germany

Tecan Asia (Pte) Ltd., 80, Marine Parade, #13-04, Singapore 449269, Singapore, T +65 644 41 886, F +65 644 41 836
Tecan Sales Austria GmbH, Untersbergstrasse 1a, A-5082 Grödig / Salzburg, Austria, T +43 62 46 89 33, F +43 62 46 72 770
Tecan Sales International GmbH, Untersbergstrasse 1a, A-5082 Grödig / Salzburg, Austria, T +43 62 46 89 33, F +43 62 46 72 770
Tecan Benelux B.V.B.A., Vaartdijk 55, B-2800 Mechelen, Belgium, T +32 15 42 13 19, F +32 15 42 16 12
Tecan Benelux B.V.B.A., Industrieweg 30, NL-4283 GZ Giessen, Netherlands, T +31 18 34 48 17 4, F +31 18 34 48 06 7
Tecan Deutschland GmbH, Theodor-Storm-Straße 17, D-74564 Crailsheim, Germany, T +49 79 51 94 170, F +49 79 51 50 38
Tecan France S.A., Parc d'Activités de Pissaloup, Bâtiment Hermes II, Rue Edouard Branly, F-78190 Trappes, France, T +33 1 30 68 81 50, F +33 1 30 68 98 13
Tecan Italia S.r.l., Via F.lli Cervi, Palazzo Bernini, Centro Direzionale Milano 2, I-20090 Segrate (Mi), Italy, T +39 02 215 21 28, F +39 02 215 97 441
Tecan Japan Co. Ltd., Meiji Seimei Fuchu Building 10F, 1-40 Miyamachi, Fuchu City, Tokyo, Japan, T +81 42 334 88 55, F +81 42 334 04 01
Tecan Nordic AB, Box 208, SE-431 23 Mölndal, Sweden, T +46 31 75 44 000, F +46 31 75 44 010
Tecan Portugal, Quinta da Fonte - Edifício Pedro I, 2780-730 Paço d'Arcos, Portugal, T +351 21 000 82 16, F +351 21 000 16 75
Tecan Sales Switzerland AG, Seestrasse 103, CH-8708 Männedorf, Switzerland, T +41 1 922 89 22, F +41 1 922 89 23
Tecan Spain, Sabino de Arana, 32, E-08028 Barcelona, Spain, T +34 93 490 01 74, F +34 93 411 24 07
Tecan UK, Theale Court, 11-13 High Street, Theale, UK-Reading RG7 5AH, United Kingdom, T +44 11 89 300 300, F +44 11 89 305 671
Tecan US, P.O. Box 13953, Research Triangle Park, NC 27709, USA, T +1 919 361 5200, F +1 919 361 5201