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Isolation of DNA by the help of robotized detection on paramagnetic particles

Zdenek Janicek, Dalibor Huska, Libuse Trnkova, Ivo Provaznik, Jaromir Hubalek, Rene Kizek*

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Abstract

High-quality DNA isolation is the most important step in its study. This step is without questions enabled by paramagnetic particles (MPs). Paramagnetic particles bring to the traditional DNA isolation procedures many new possibilities, above all automation of the complete process. In this work, we were focused on optimization of DNA isolation by the help of paramagnetic particles with use of automated pipetting station.

Keywords: Paramagnetic particles, DNA isolation, Electrochemical detection,

Introduction

Automation enables especially significant reduction of contamination of sample and experimental mistakes caused by human factor. Proposition of this procedure is very important for both diagnostic and criminalistic purposes (Huska et al. 2008, Huska et al. 2009). Due to this fact, sample collection and subsequent isolation of analyte represent one of the crucial moments of supposed analysis. In consideration of increasing pressure on reduction of quantity of material available for analysis (hair, drop of blood serum or full blood, several cells of tissue), possibility of contamination of sample by personnel increases (Huska et al. 2009). Obtained false positive result subsequently can influence findings of investigation or proposal of therapeutic procedure. Possible change into process of nucleic acids isolation can bring paramagnetic microparticles (Palecek and Fojta 2007). As it is has been demonstrated, connection of isolation of nucleic acids by paramagnetic particles with subsequent electrochemical detection brings simple technological procedure (Palecek et al. 2002). Aim of this work consisted in testing of differently modified paramagnetic microparticles for DNA isolation.

Zdenek Janicek, Ivo Provaznik

Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Kolejni 4, CZ-612 00 Brno, Czech Republic

Dalibor Huska Rene Kizek*

Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

*Tel: +420 545 133 350, Fax: +420 545 212 044
E-mail: kizek@sci.muni.cz

Libuse Trnkova

Department of Chemistry, Masaryk University, Kotlarska 2, CZ-611 37 Brno, Czech Republic

Jaromir Hubalek

Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Udolni 53, CZ-602 00 Brno, Czech Republic

Materials and methods

Electrochemical DNA analysis was carried out by the help of apparatus AUTOLAB analyzer (EcoChemie, Netherlands) in connection with VA-Stand 663 (Metrohm, Switzerland). Square wave voltammetric (SWV) measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: potential step 5 mV, frequency 260 Hz. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s. Isolation of nucleic acids proceeded on paramagnetic particles chemagic viral DNA, food DNA, DNA plant, DNA blood, and DNA tissue, all from Chemagen (USA). For automated isolation, apparatus epMotion 5075 from company Eppendorf (Germany) was used. Fully automated isolation was carried out on automated pipetting system epMotion 5075 (Eppendorf, Germany). The position of B4 is a magnetic separator (Promega). The positions of C1 and C4 can be thermostated (Epthermoadapter PCR96). The pipetting provides a robotic arm with adapters (TS50, TS300, TS1000) and Gripper (TG-T). The samples are placed in the position B3 in adapter Ep0.5/1.5/2ml. Module Reservoir is located in the position B1, where washing solutions and waste are available. The device is controlled by the epMotion control panel. The tips are located in the A4 (ePtips 50), A3 (ePtips 300) and A2 (ePtips 1000) positions. PCR 96 plates are

used. The resulting volumes of collected samples ranged from 10 to 30 µl depending on the procedure.

Results and discussion

We created universal program by the help of software shipped with automatic pipetting station. We also optimized individual technical parameters and setting. Procedure was based on optimization, which was carried out at manual isolation. The most important parameters, which play crucial role in DNA isolation by MPs, are: i) pH of binding solution, in accordance with our results, the most suitable pH is 1,6; ii) ionic strength, which was in our experiments simulated by NaCl with final optimal concentration of 850 mM and iii) time of interaction between DNA and paramagnetic particles – for our experiments, the most suitable – optimal - interaction time is 15 min. In addition, we determined that with increasing intensity of shaking, increases also effectivity of DNA isolation in comparison with unshaken samples for more than 30%. Affectivity of DNA capturing by the help of epMotion was determined as 80%.

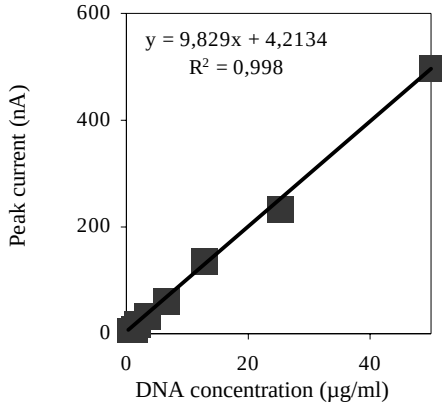

Figure 1: Dependence of DNA concentration on signal height. DNA was obtained by fully automated procedure.

Conclusion

Isolation of DNA can be carried out fully automatically, selectively and sensitively, with effectivity higher than 80 %, up to 60 min by the help of paramagnetic particles.

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