Automated genomic DNA purification from mouse ear using QuickPick™ gDNA reagents and MagRo™ 8-M robotic workstation

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ABSTRACT

The QuickPick™ gDNA purification reagents together with the PickPen® magnetic tools (PickPen® 1-M, PickPen® 8-M and MagRo™ 8-M) provide a fast and simple means of purifying genomic DNA both manually and automatically. The purified genomic DNA is of high quality, suitable for all commonly used downstream applications. The QuickPick™ SML gDNA kit is a universal kit intended for use with a variety of samples. A customized purification of genomic DNA from mouse ear with MagRo™ 8-M robotic workstation is described.

INTRODUCTION

Tissues are difficult materials to purify DNA from; the hardness of the tissue needs stronger homogenization and lysis methods. However, by optimizing the lysis time, temperature and mixing, the sample preparation leads to a well homogenized sample.

For some downstream applications the crude tissue lysate can be directly used as the template. Frequently, several different analyses are needed from the same sample the and thereby the purification of DNA is indispensable for long term storage. The QuickPick™ gDNA reagents have proven to give excellent results when purifying DNA from mouse ear combined with customer's laboratory-made lysis and elution buffers.

PRINCIPLE OF THE QUICKPICK™ SML gDNA KIT

The DNA in the sample is released from cells using a Lysis buffer and Proteinase K solution. The released DNA is bound specifically to the magnetic particles in the presence of Binding Buffer. PickPen® magnetic tool is used to capture the magnetic particles with bound DNA and to carry out subsequent washes to remove contaminants. Finally, DNA is eluted from the particles using Elution Buffer, and DNA is ready for use in downstream applications.

MATERIALS & METHODS

Genomic DNA was purified using QuickPick™ gDNA reagents in combination with MagRo™ 8-M robotic workstation. The kit insert protocol was followed with minor exceptions:

1. Lysis buffer was prepared in the laboratory.
2. Proteinase K from *Tritirachium album* was purchased from Roche Diagnostics (Germany).
3. Elution volume was reduced to 60 µl to obtain sufficient DNA concentrations for downstream applications.
4. Elution buffer was prepared in the laboratory.

Mouse ear clips (3-5 mg) were placed into the wells of a 1 ml deep well plate. The sample plate and the other consumables needed were placed on MagRo™ 8-M deck.

The automated protocol for 96 samples starts with adding Proteinase K solution and laboratory-made lysis buffer into the samples by MagRo™ 8-M. The sample, Proteinase K solution and lysis buffer are incubated on the PlateShaker on MagRo™ 8-M for 15 minutes.

After the lysis step MagRo™ 8-M adds Magnetic Particles and Binding Buffer mix into the lysed samples. During the incubation of the suspension on a shaker for the DNA binding MagRo™ 8-M dispenses Wash Buffers and Elution Buffer into the empty plates.

After the incubation MagRo™ 8-M collects the Magnetic Particles and performs washing steps and elutes DNA into elution buffer. After the elution Magnetic Particles are collected and discarded.

The eluates containing the DNA are ready for downstream applications.

The MagRo™ 8-M protocol was carried out in 3.5 hours including all reagent dispensing, lysis and purification steps with PickPen® tool.

Allele-specific PCR reactions were carried out for detecting mouse *sprouty* gene using PCR Amplitaq Gold-kit from Roche Diagnostics (Germany). The reactions were performed using 2 µl of purified eluate as a template with the total reaction volume of 25 µl. The primers (T_M of 64°C) were specific for *sprouty* gene.

RESULTS

The genomic DNA was purified from ear samples of 48 mice with QuickPick™ gDNA reagents and MagRo™ 8-M robotic workstation. The genomic DNA was used as the template in PCR for detection of an allele specific *sprouty* gene. Based on the PCR results 24 mice were identified to carry the *sprouty* gene by showing clear DNA bands with a size of 1000 bp (Fig. 1). These results demonstrate that the purified genomic DNA is consistently of high quality and is suitable for sensitive down-steam analysis such as PCR.

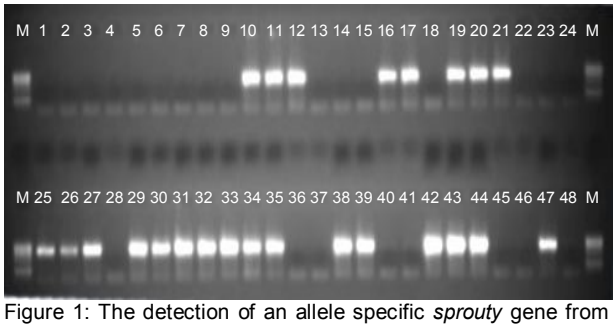

Figure 1: The detection of an allele specific *sprouty* gene from 48 mice samples by using PCR. The genomic DNA was purified with QuickPick™ gDNA reagents and MagRo™ 8-M robotic workstation. M: molecular marker, lanes 1-48: PCR products.

CONCLUSIONS

Fully automated, medium-throughput DNA purification from mouse ear was successfully performed. The quality of DNA is suitable for commonly used downstream applications such as genotyping with allele specific PCR. Automation does not damage the DNA; the resulting products were of the expected size. No inhibition of the reactions was detected due to high quality DNA without contamination.

Short lysis time and automated purification allows the customer to purify and analyze 96 samples in one day. The time from the beginning of placing the samples and other consumables on MagRo™ 8-M to purified DNA is hands-off time with no user intervention needed. This allows the customer to make preparations for the following analyzing process or other laboratory work.

All purification processes in MagRo™ 8-M are customized and optimized for the user. The processes can also be split into their own processes so that the customer can dispense e.g. Wash and Elution Buffers beforehand and purify the samples in MagRo™ 8-M or manually with PickPen® 8-M. By having these processes the customer saves lots of time during and between the sample purifications when MagRo™ 8-M can be used for reagent dispensing.

The customized processes have different variables to choose from e.g. number of samples, buffer volumes, incubation times and temperatures. These variables increase the flexibility of MagRo™ 8-M which, however, don't make it difficult to use. The customized processes are ready-to-run and one process may have several variables which to choose thus giving more diversity to customer comparing to other commercial robots.

The applicability of MagRo™ 8-M and the Bio-Nobile™ solution for DNA purification allows customers to save money by using their own reagents. All the QuickPick™ gDNA reagents can be bought separately and used as a whole kit or combined with laboratory-made reagents. The plastic ware usage is also decreased to minimum. For example the processes use only the needed amount of plates and only one tip for each reagent.

MagRo™ 8-M robotic workstation can be used for different magnetic particle based applications such as DNA, RNA and protein purifications as well as immunoprecipitation. These applications may have pre- and post-dispensing of samples and liquids combined with purification in one process. MagRo™ 8-M can also be used as a liquid handler e.g. for PCR reagent and sample dispensing.