

Applications

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A versatile method for the automated isolation and qPCR analysis of Mouse tail genomic DNA

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Abstract

Isolation of genomic DNA from mouse tails with magnetic beads can be automated with the *epMotion* pipetting system from Eppendorf. The Invitex InviMag® Tissue DNA Kit and the Invitrogen ChargeSwitch® gDNA Micro Kit were used in this study. Combining the *epMotion* 5070 with the Eppendorf MixMate for efficient mixing of magnetic beads in 96 well deepwell plates, a semi-automated isolation protocol was established for small sample numbers. Alternatively, a fully automated isolation method was developed on the *epMotion* 5075 LH. We demonstrate here that the isolated gDNA is suitable for sensitive downstream applications such as qPCR. All qPCR assays were set-up with the same *epMotion* that was being used for the purification process. Using either the purified DNA or highly pure commercially available mouse genomic DNA from Blood (Promega) as templates in the PCR reactions, results from a GAPDH qPCR assay were compared.

Introduction

Standard methods for extracting DNA from mouse tails can be laborious and time consuming. The usage of phenol/chloroform is hazardous, the digestion steps with Proteinase K may require several hours [1]. High-throughput users, e.g. in genotyping projects, have increasingly turned to 96-well silica filterplate based methods to isolate genomic DNA from many samples with robotic workstations. Low to medium throughput users or users with varying sample numbers have little options to automate their procedures.

Here we describe a method employing a small, easy to operate benchtop automated pipetting system, the *epMotion*, to extract DNA from mouse tails in an automated or semi-automated fashion. The method is highly flexible and can be adapted for 1–72 samples per run. It can be used with different bead based kits as is being demonstrated by using two commercially available DNA Extraction Kits, the Invitex InviMag Tissue DNA Kit and the Invitrogen ChargeSwitch gDNA Micro Kit. For the subsequent set-up of the PCR analysis reactions the same high precision *epMotion* pipetting system was used. Automated set-up of qPCR reactions significantly reduces the operator variability and the likelihood of false negative or positive results due to pipetting errors.

Materials and Methods

Automated pipetting system:

Eppendorf *epMotion* 5075 LH with a gripper tool or Eppendorf *epMotion* 5070, 1-channel dispensing tool TS 1000-1, 1-channel dispensing tool TS 50-1, 8-channel dispensing tool TM1000-8, reservoir rack with 30 ml and 100 ml Tubes, 24-Thermorack for 1.5 ml Tubes, Promega Deep well Magnabot Magnetic Separation Device.

Equipment:

Eppendorf MixMate, Eppendorf Thermomixer comfort, Eppendorf BioPhotometer, Eppendorf Mastercycler *ep realplex*, Eppendorf Mastercycler *ep gradient*, Sartorius precision balance, DNA electrophoresis apparatus, UV Gel Documentation system.

Reagents and Samples:

Invitex InviMag Tissue DNA Kit, Invitrogen ChargeSwitch gDNA Micro Kit. Mouse tail samples (provided by the University Hospital Eppendorf and Lilly GmbH, Hamburg). Mouse genomic DNA (Promega). 5Prime RealMasterMix, GAPDH primers (TIB Molbiol, Berlin).

Consumables:

Eppendorf Plate Deepwell 96/1000µl, Eppendorf twin.tec PCR Plates 96 skirted, Eppendorf twin.tec PCR Plates 96 semi-skirted. Eppendorf 1.5 ml and 0.5 ml Tubes, Eppendorf UVette cuvettes. Eppendorf epTIPS motion Filter 1000 µl and 50 µl, Eppendorf epTIPS motion 1000 µl and 50 µl.

Isolation of genomic DNA with magnetic beads:

By binding DNA to solid resin magnetic beads, DNA can be cleared from the lysate and can be washed from contaminants in subsequent washing steps. The lysis of the samples and the binding and elution of the DNA were performed in kit specific buffers. The reagent buffers are removed after the beads are fixed with a magnetic separator at the sides of the wells. During the washing steps and the elution, the beads are resuspended by mixing either with a Eppendorf MixMate or by automated tip mixing on the epMotion. After elution, the DNA is transferred to another tube.

gDNA isolation with epMotion 5070 or epMotion 5075LH:

The epMotion methods were programmed according to the user manuals of Invitex and Invitrogen. The epMotion 5070 was equipped with 1-channel dispensing tool TS 1000-1 or 8-channel dispensing tool TM 1000-8, deepwell plates, Magnabot magnetic separator, MTP 96 well plate, 30 ml and 100 ml reservoirs, 24-Thermorack and 1000 µl Tips (Fig. 1). The lysis step was performed in 1.5 ml tubes in the Eppendorf Thermomixer comfort for 2–4 h at 56 °C.

The buffers for the purification process were placed in 30 ml or 100 ml reservoir tubs. After lysis the samples were placed in a 24-Thermorack. The magnetic beads and the binding buffer were added to the 1.5 ml sample tubes and mixed by tip mixing. For washing steps and elution, the samples were transported to the deepwell plate on the magnetic separator.

In the epMotion 5070 method the sedimented beads in the deepwell plate were efficiently resuspended by mixing with the Eppendorf MixMate. Therefore, the plate was transferred manually to the MixMate aside of the epMotion 5070. After eluting the DNA from the beads, the elution buffer containing the DNA was transferred to a 96 well elution plate by the epMotion for further processing. With the epMotion 5075 LH, a completely automated isolation was performed. The resuspension of the beads was done by tip mixing during the buffer additions. For this purpose, the deepwell plate was transported to a free position on the worktable by the gripper. Both methods can be used with the Invitex InviMag Tissue DNA Kit. The Invitrogen ChargeSwitch gDNA Micro Kit requires resuspending the beads by tip mixing. Therefore, only the epMotion 5075LH method was used for this kit.

Characterizing and quantification of genomic DNA:

The samples were analyzed by gel electrophoresis on a 1% TAE agarose gel. The DNA fragments were separated with a voltage of 90 V and visualized with UV light. The photometric analysis of the purity and concentration of the isolated DNA was done with the Eppendorf BioPhotometer. DNA concentration calculated from the 260 nm absorption and the 260 nm/280 nm ratio were recorded.

A



B

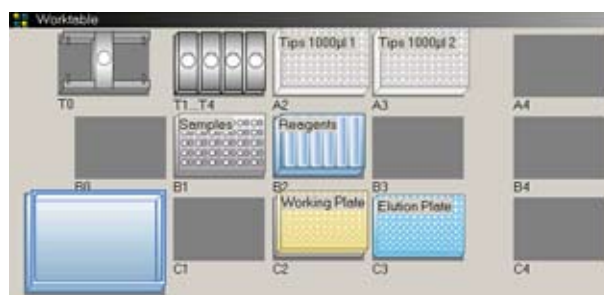


Fig. 1: Worktable for semi-automated gDNA isolation on the epMotion 5070 (A) and fully automated process on the epMotion 5075 LH (B).

Downstream applications:

In a qPCR reaction, the murine housekeeping gene GAPDH was amplified. This gene codes for the enzyme Glyceraldehyde 3-phosphate dehydrogenase that catalyzes the transformation of Glyceraldehyde 3-phosphate to 1,3-Bisphosphoglycerate in the glycolysis. The primer sequences are shown in Table 1.

Tab. 1: Primer sequences for GAPDH qPCR assay.

Forward Primer	5'-ATG ACA TCA AGA AGG TGG TG-3'
Reverse Primer	5'-CAT ACC AGG AAA TGA GCT TG-3'

Using these primers, a 177 bp GAPDH fragment was amplified. The 5Prime RealMasterMix SYBR ROX 2.5x was used in all experiments on the Eppendorf Mastercycler ep realplex. The PCR system was optimized for primer concentrations and optimum annealing temperatures (data not shown).

With the real-time PCR reactions different samples of approximately the same concentration and single samples in different concentrations (1:10 dilutions) were compared. DNA from different isolations was used as templates. Also, genomic mouse DNA isolated from blood (Promega) was used as a template and the results from mouse tail and blood DNA were compared with linear regression.

All real-time PCR reactions were set-up with the epMotion 5070, equipped with a TS 50 dispensing tool and two Eppendorf twin.tec PCR plates. In the first plate, the DNA dilution series for the standard curve was prepared automatically and in the second plate, the diluted DNA was combined with real-time PCR MasterMix and primers for cycling.

Restriction digestion assays were performed with the restriction enzyme *HindIII* (from *Haemophilus influenzae*). Restriction enzyme assays were incubated overnight at 37°C and the reaction was analyzed on a 1% TAE agarose gel.

Results and Discussions

Automatic gDNA Isolation:

Both methods, run on the epMotion 5070 and the MixMate or on the epMotion 5075 with tip-mixing, revealed comparable results when the Invitex InviMag Tissue DNA Kit was used. The methods took between 60 and 100 minutes, depending on the number of samples.

The results of the gel electrophoresis indicate homogeneous, non-sheared DNA with reasonable molecular weight (Fig. 2 A–B). For the Invitrogen ChargeSwitch gDNA Micro Kit the epMotion 5075 LH method with tip mixing delivered good results (Fig. 2C).

The yield obtained with the Invitex kit chemistry was significantly higher than that of the Invitrogen kit. With the semi-automated procedure around 5 µg DNA per mg tissue was obtained with the Invitex chemistry, whereas 2 µg DNA per mg tissue was obtained with the Invitrogen ChargeSwitch chemistry. Also, the 260/280 nm ratio of the isolated DNA was higher when prepared with the Invitex procedure. Typical values were within the range of 1.5–1.7, depending on the sample.

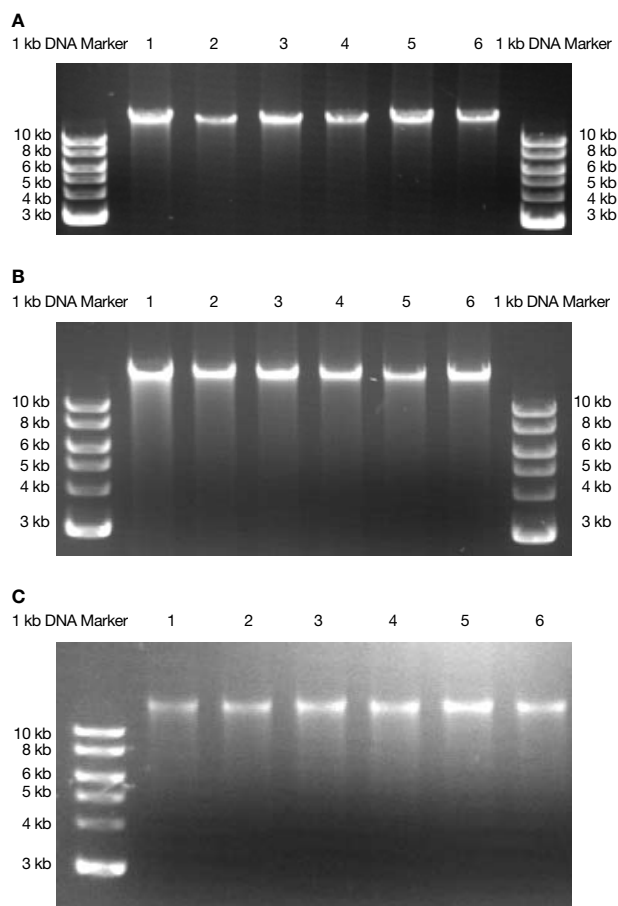


Fig. 2: Agarose gel analysis of isolated DNA:

A: Samples from the epMotion 5070/MixMate gDNA isolation procedure using the Invitex kit.

B: Samples from the epMotion 5075 LH gDNA isolation procedure using the Invitex kit.

C: Samples from the epMotion 5075 LH gDNA isolation procedure using the Invitrogen kit.

All samples were eluted in 130 µl elution buffer. 10 µl of the samples were subjected to analysis on a 1% agarose gel. DNA markers in first and last lane (2a and 2b) and in first lane (2c): 1 kb DNA ladder (New England Biolabs).

qPCR and Restriction Enzyme Analysis:

For qPCR analysis, the DNA samples were normalized to approximately the same concentration. Usually, 2 ng of isolated DNA were subjected to qPCR analysis. When DNA samples obtained with different methods were analyzed in the same qPCR assay 2 ng and 0.2 ng DNA template was used as template to separate the amplification curves. The GAPDH fragment could successfully and specifically be amplified from the isolated DNA samples (Fig 5). The real-time PCR reactions with different samples in the same concentrations showed comparable Ct values. Ct values between 18.04 and 18.76 or between 23.25 and 23.93 were obtained when different DNA samples from the *epMotion 5070/MixMate* or *epMotion 5075LH* procedure with the Invitex kit were used as template depending on the concentration. Ct values between 21.20 and 21.97 were obtained when DNA from the *epMotion 5075 LH* with the Invitrogen Kit was used as template (Fig. 4). Since all samples were different and individually normalized the variations in the observed Ct values were expected.

In a quantitation experiment the DNA obtained from one individual sample with the Invitex process was compared to a commercially available, highly pure Mouse genomic DNA from blood, available from Promega. Both samples were diluted in 1:10 dilutions with the *epMotion 5070* and subjected to qPCR analysis. For the Invitex DNA, the calculated Ct value for 1 ng DNA was 19.11 (Fig. 6 A and C). The correlation coefficient for the assay (R^2) was 0.997 (Fig. 6 C). The DNA isolated from blood showed a calculated Ct value of 19.55 for 1 ng DNA. The correlation coefficient (R^2) was 0.999 (Fig. 6 B and D). These experiments were repeated with other purified samples and gave similar results (data not shown).

The restriction digestion analysis demonstrated that all analyzed samples could be digested (Fig. 3).

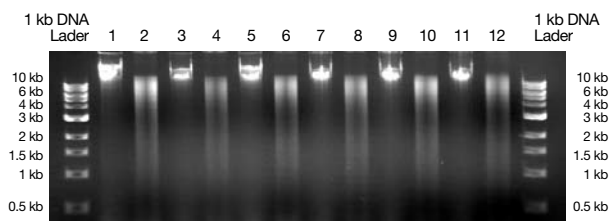


Fig. 3 Restriction digestion assay: Standard: 1 kb DNA Ladder from NEB. Samples from the *epMotion 5070/MixMate* process using the Invitex kit were digested with *HindIII* over night and analyzed together with non-digested controls on agarose gels. The samples were applied in restriction buffer to a 1% agarose gel.

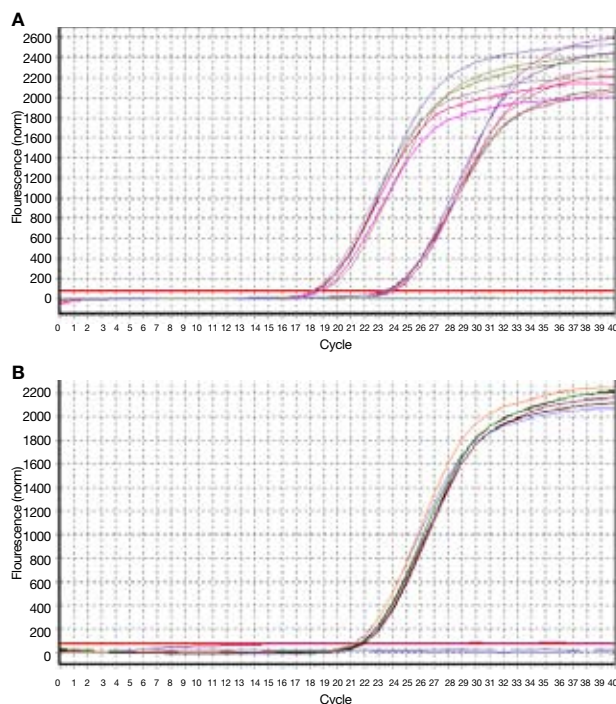


Fig. 4: GAPDH qPCR amplification with isolated genomic DNA: DNA from different mouse tail samples was isolated with the described automated methods and kits.

A) Invitex Kit samples: Approximately 2 ng DNA was used as template from different samples obtained with the *epMotion 5070/MixMate* procedure (left curves). Approximately 0.2 ng DNA was used as template from samples obtained with the *epMotion 5075LH* (right curves). Different template DNA amounts were used to separate the amplification curves in the plot.

B) Approximately 2 ng of extracted DNA from different mouse tails was used for the Invitrogen Kit samples. All samples could successfully be amplified by qPCR.

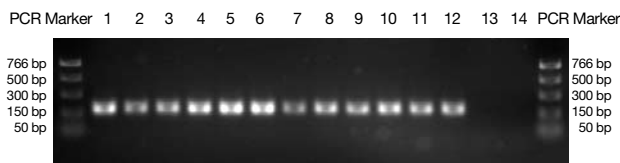


Fig. 5 Agarose gel analysis of the GAPDH qPCR products.

Standard: PCR Marker from NEB. 12 samples purified with the Invitex kit were subjected to GAPDH qPCR analysis (see also Fig 4A). 5 μ l of the amplification reactions were analyzed on a 2% agarose gel. Only the specific 177 bp fragment was detected. Negative controls (lane 13, 14) show no amplification products.

Conclusion

The results demonstrate that genomic DNA can successfully be purified from mouse tails with the *epMotion* 5070 and the Eppendorf MixMate or the *epMotion* 5075 LH. The procedure is highly flexible and can be tailored to different throughput, automation and budget needs. Both *epMotion* methods yield comparable results. With the Eppendorf MixMate, magnetic beads can efficiently be resuspended in Eppendorf Plates Deepwell. Tip mixing within the *epMotion* 5075 LH process was equally efficient in resuspending the beads throughout the purification process, as was also demonstrated before [3].

The protocols can easily be adapted to different kit chemistries with the help of the *epMotion* software. The Invitex InviMag Tissue DNA Kit proved to be most versatile in our hands as it worked equally well with both

variants of the method and furthermore gave best yields. The obtained DNA was in the range of 15–20 μg when samples amounts of 3–5 mg were used as starting material.

To check for compatibility with common downstream applications, restriction digestion and quantitative realtime-PCR were performed with the isolated DNA. With all samples, the GAPDH 177 bp PCR product was successfully amplified with good efficiency. In a dilution series, the DNA isolated with the Invitex chemistry was compared with commercially available highly pure genomic DNA from blood. No Ct shift was observed and the two standard curves were nearly identical. These results suggest that the purified DNA obtained with the automated methods were of equal quality and contained no PCR inhibitors.

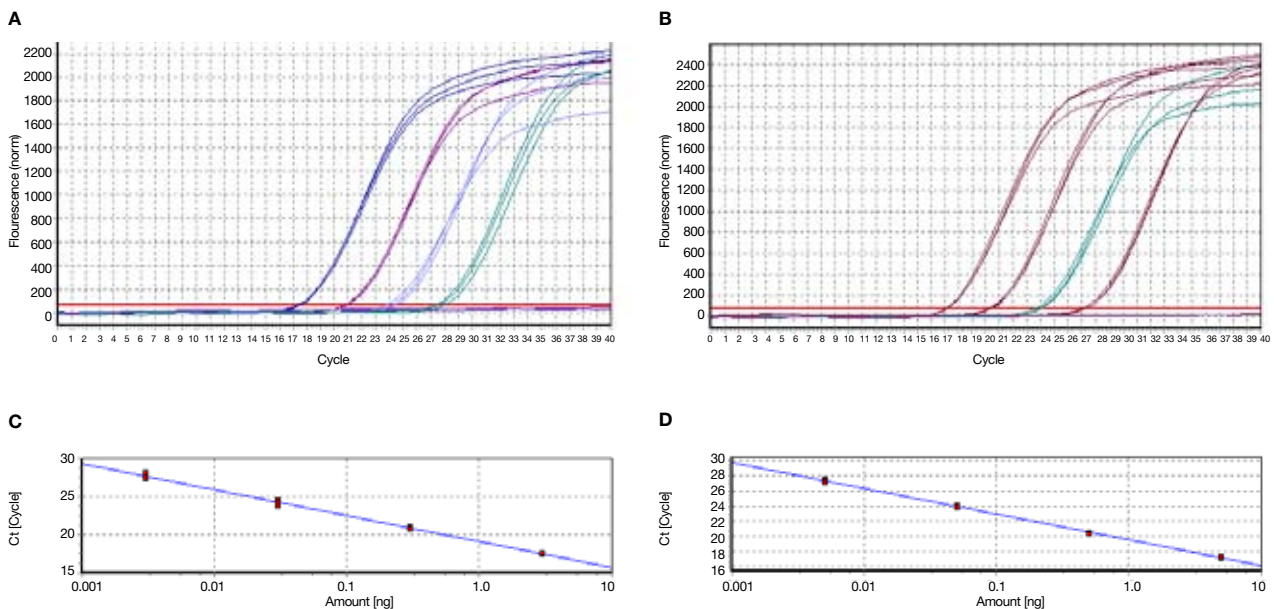


Fig. 6 Dilution series of isolated gDNA (Invitex procedure) and Promega gDNA from Blood:

- A)** 1:10 dilutions of mouse tail gDNA samples
- B)** 1:10 dilutions of Promega gDNA
- C)** linear regression of Ct values from Figure 6 A.
- D)** linear regression of Ct values from Figure 6 B.

Please note that both assays generate similar quantitations. The calculated y-intercept equals the Ct of 1 ng DNA template. Since it is 19.11 for the standard curve generated with the purified sample and 19.55 for the standard curve obtained with the Promega gDNA sample, no Ct shift for the purified DNA from mouse tails is observed.

All qPCR reactions were automatically set-up with the *epMotion* 5070 to standardize the set-up process. As suggested by Bustin [2], the automated set-up eliminates pipetting errors and standardizes the set-up process making results more reproducible.

In summary, the *epMotion* system is both flexible and precise enough for applications that utilize higher volumes, such as the purification of genomic DNA from mouse tails, and lower volumes, such as the set-up of qPCR reactions.

References

- [1] Sambrook J., Fritsch E., Maniatis T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.
- [2] Bustin SA. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J. Endocrinology. 2002;29:23-39.
- [3] Lindner S., Wehrhahn D. Reproducible and easy automated Purification of Plant genomic DNA, Eppendorf Application Note 124, January 2006.

Ordering information

Product	International order no.	North American order no.
epMotion® 5075 LH 230 V	5075 000.008	n/a
epMotion® 5075 LH 120 V	n/a	960020006
epMotion® 5070 LH 230 V	5070 000.018	n/a
epMotion® 5070 LH 120 V	n/a	960000005
MixMate	5353 000.014	022674200
Mastercycler ep <i>realplex</i> ⁴ S 230 V	6302 000.601	n/a
Mastercycler ep <i>realplex</i> ⁴ S 120 V	n/a	950020318
Dispensing tool TS 1000	5280 000.053	960001036
Dispensing tool TM 1000-8	5280 000.258	960001061
Dispensing tool TM 300-8	5280 000.231	960001052
Gripper	5282 000.018	960002270
Holder for gripper	5075 759.004	960002211
Reservoir Rack	5075 754.002	960002148
Reservoirs 100 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
Reservoirs 30 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.505	960051009
epTIPS Motion 1000 µl Filter	0030 003.993	960050100
epTIPS Motion 300 µl Filter	0030 003.977	960050061
epTIPS Motion 50 µl Filter	0030 003.950	960050029
twin.tec PCR Plate 96, skirted, green	0030 128.664	951020443

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