

Automated Purification of Genomic DNA from Tissue with the Macherey-Nagel NucleoMag[®] Tissue Kit on the epMotion[®] 5075

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Abstract

The purification of genomic DNA from tissue samples with the NucleoMag 96 Tissue Kit is designed for manual or automated small-scale preparation of highly pure genomic DNA from tissue, cells or bacteria pellets. Here we show the implementation of the NucleoMag Tissue Kit from

Macherey-Nagel on the epMotion 5075t/m. The combination allows a walk away purification in less than 120 minutes for untreated cells and less than 100 minutes for prelysed tissue and bacteria pellets.

Introduction

The procedure of the NucleoMag 96 Tissue kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.

Different samples, like tissue, cells or bacteria pellets are lysed with lysis buffer T 1 and Proteinase K. DNA is then bound to the magnetic beads. Contaminants are removed through a series of washing steps with different wash buffers.

Finally, remaining salts are removed with an additional 80 % ethanol wash step. The purified DNA is eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.

This application note describes the configuration and preparation of the epMotion 5075 to automate this kit.

Materials and Methods

Required Labware

Eppendorf epMotion 5075t or 5075m
 Dispensing Tool TM 1000-8
 Dispensing Tool TM 300-8
 Reservoir Rack
 Reservoirs 30 mL/ Reservoirs 100 mL
 Reservoir 400 mL

NucleoMag Sep (Magnetic separator)
 NucleoMag 96 Tissue Kit

Required Consumables:

epT.I.P.S.[®] Motion 1000 µL with filter
 epT.I.P.S. Motion 300 µL with filter
 Square-well Block as processing plate
 Microtiterplate to collect the eluates

Samples

Tissue, cells or bacteria pellets

Method

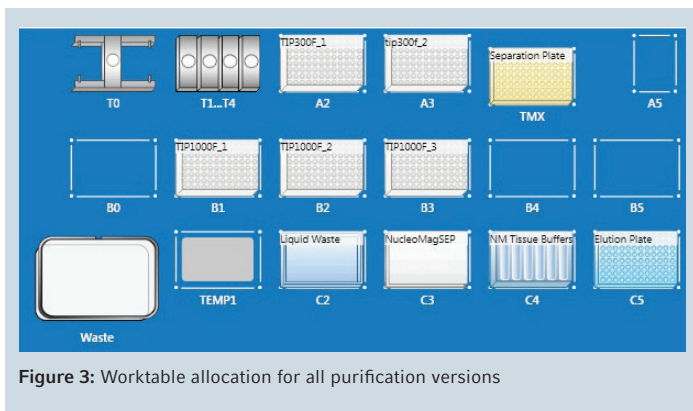
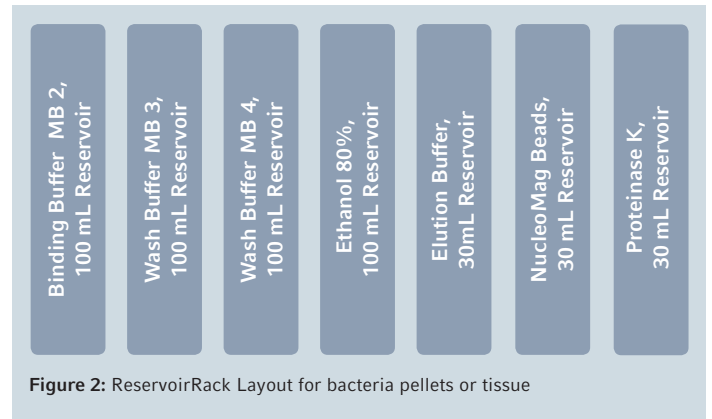
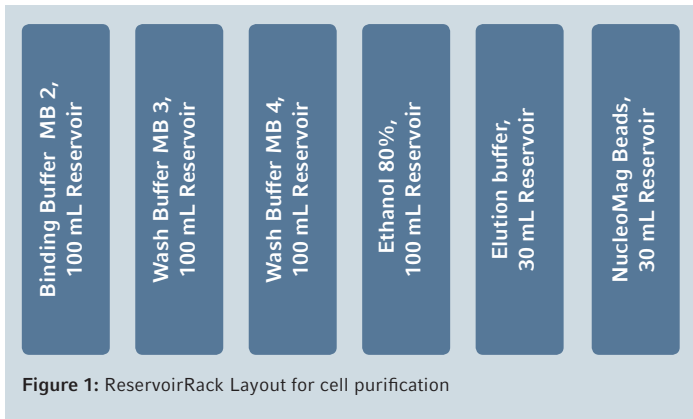
This protocol is developed to process up to 96 samples in parallel on the epMotion 5075m or 5075t workstation. This kit is suitable for up to 20 mg tissue, up to 10⁶ cells or bacteria pellets from up to 1 mL overnight culture. For tissue

purification 225 µL cleared lysate (external lysis step at 56°C for 1-3 hours or overnight, followed by centrifugation), for cell purification 200 µL suspension in T1 lysis buffer (without Proteinase K, which will automatically be added in the first epMotion step) is prefilled into each well of the separation plate. All subsequent steps are automated and will be carried out in this plate. This includes dispensing of buffers and beads, removal of the supernatants as well as transport and mixing steps. After the lysis step magnetic beads and binding buffer are added. During mixing and incubation step the DNA is bound to the magnetic beads. Magnetic beads are separated with the NucleoMag SEP and the supernatant is removed. Unspecifically bound contaminants are removed through several washing steps with wash buffers MB3 and MB4. Remaining salts are removed with an additional 80 % ethanol wash step, which replaces the MB5 buffer. After the last washing step residual ethanol is removed in a drying step of 15 minutes at 70°C on the integrated TMX module of the epMotion. Finally, the eluate is transferred to a dedicated elution plate. Both versions of the purification (for tissue or for cells and bacterias) are available as preprogrammed methods.

A purification process with 96 tissue samples with re-use tips for the wash steps requires 240x 1000µL tips and 104x 300 µL tips.

For the method the following positions of the worktable are occupied:

| Position | Labware | Comment |
|----------|----------------------------------|---------|
| A2 | 300 µL filtertips | |
| A3 | 300 µL filtertips | |
| TMX | Separation Plate (Lysed samples) | |
| B1 | 1000 µL filtertips | |
| B2 | 1000 µL filtertips | |
| B3 | 1000 µL filtertips | |
| C2 | Liquid Waste (400 mL reservoir) | |
| C3 | NucleoMag_Sep | |
| C4 | Reagent reservoirs | |
| C5 | Elution Plate | |



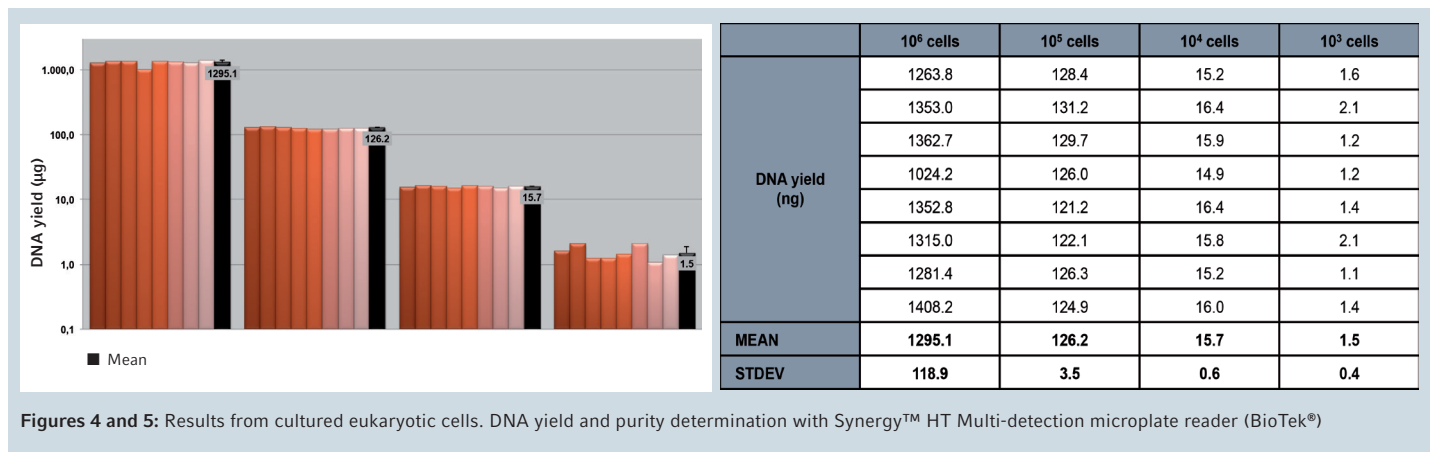
Results and Discussion

Purification results from tissue: Genomic DNA resulting from the aforementioned method was analyzed by gel electrophoresis of 10 μ L eluate or PCR product via 1% TAE agarose gel; Yield and purity were determined by UV spectroscopy. Furthermore a qPCR with SensiFast[®] Probe

Lo-Rox Kit (Bioline[®]) on an Applied Biosystems[®] 7500 was used to check for the absence of PCR inhibitors.

Purification of cells

HeLa cells in the range of 10^6 down to 10^3 were tested.



Figures 4 and 5: Results from cultured eukaryotic cells. DNA yield and purity determination with Synergy[™] HT Multi-detection microplate reader (BioTek[®])

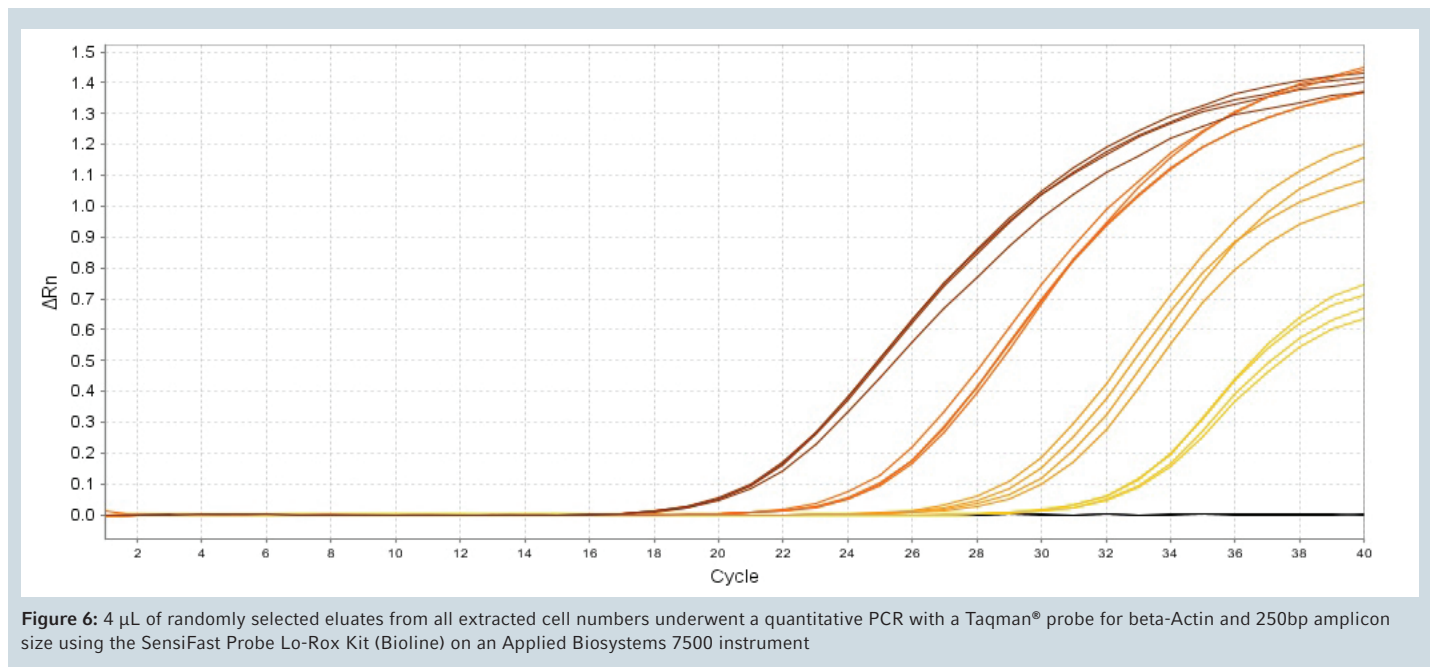
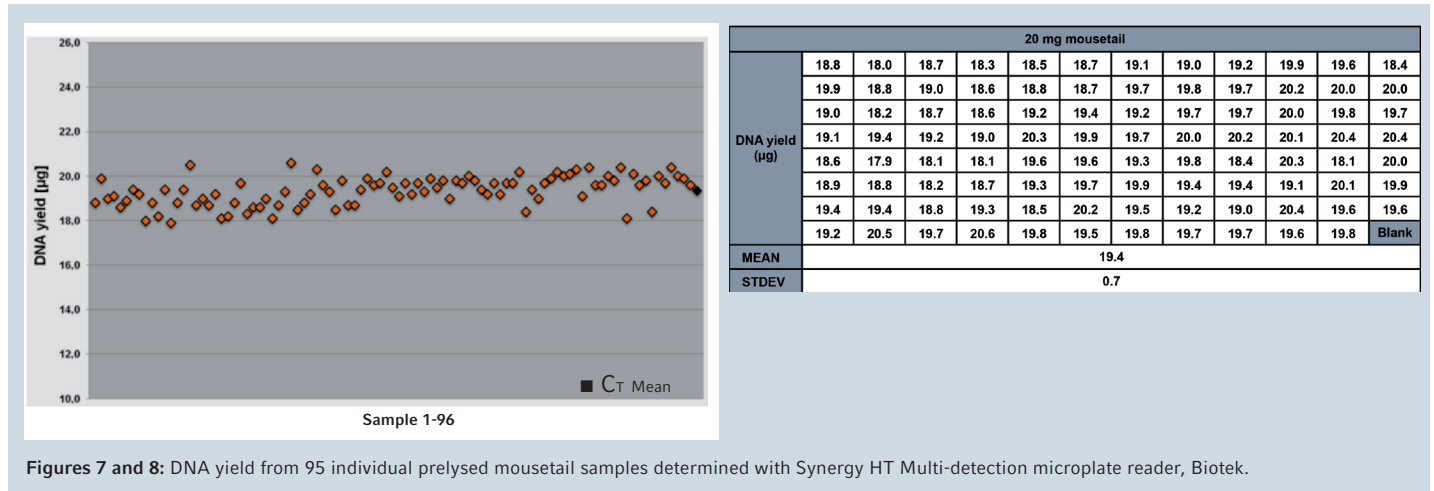


Figure 6: 4 μ L of randomly selected eluates from all extracted cell numbers underwent a quantitative PCR with a Taqman[®] probe for beta-Actin and 250bp amplicon size using the SensiFast Probe Lo-Rox Kit (Bioline) on an Applied Biosystems 7500 instrument

Mousetails

DNA isolation from 20 mg mousetails



Figures 7 and 8: DNA yield from 95 individual prelysed mousetail samples determined with Synergy HT Multi-detection microplate reader, Biotek.

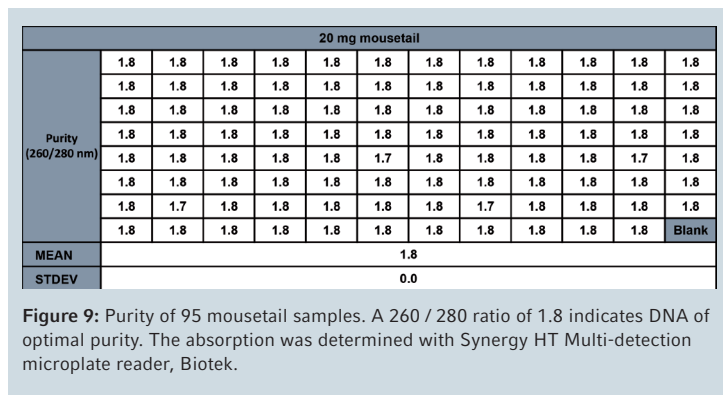


Figure 9: Purity of 95 mousetail samples. A 260 / 280 ratio of 1.8 indicates DNA of optimal purity. The absorption was determined with Synergy HT Multi-detection microplate reader, Biotek.

Other tissues

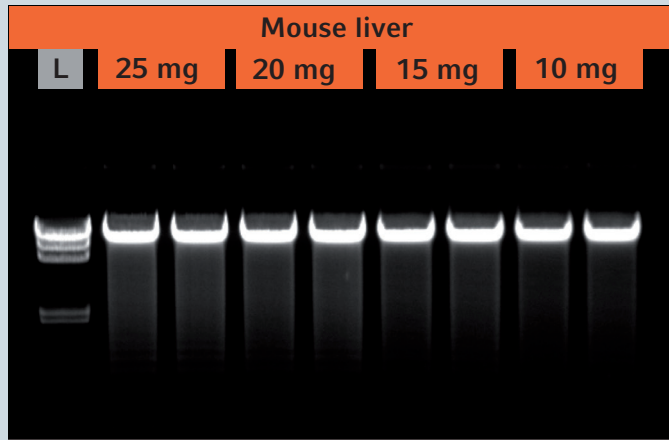


Figure 10: Purified gDNA from 4 different input amounts of mouse liver samples. 10 μ L eluate respectively per lane of an agarose gel (1 %)

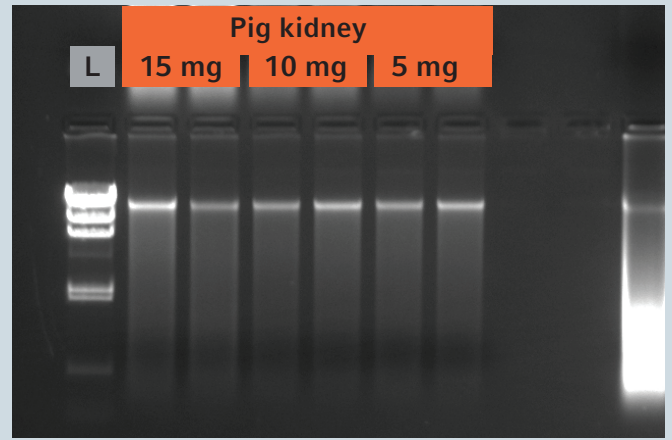


Figure 13: Results from pig kidney.

| | Mouse liver | | | | Mouse lung | | | |
|----------------------|-------------|-------|-------|-------|------------|-------|-------|-------|
| | 25 mg | 20 mg | 15 mg | 10 mg | 25 mg | 20 mg | 15 mg | 10 mg |
| DNA yield (μ g) | 29.2 | 25.9 | 20.5 | 16.8 | 14.1 | 12.3 | 9.5 | 7.1 |
| | 27.0 | 25.8 | 20.7 | 16.9 | 14.8 | 12.1 | 10.4 | 7.7 |
| | 28.1 | 25.7 | 20.9 | 16.9 | 14.6 | 12.9 | 10.0 | 7.9 |
| | 29.6 | 25.3 | 20.7 | 16.9 | 14.3 | 12.6 | 10.1 | 7.6 |
| | 29.4 | 24.3 | 20.0 | 16.5 | 15.1 | 12.0 | 10.0 | 7.8 |
| | 29.2 | 22.4 | 21.7 | 16.6 | 15.7 | 12.0 | 11.0 | 8.1 |
| | 28.9 | 24.9 | 19.3 | 17.8 | 14.8 | 11.3 | 10.9 | 7.0 |
| | 29.1 | 24.2 | 19.4 | 16.2 | 14.0 | 11.6 | 9.9 | 7.2 |
| MEAN | 28.8 | 24.8 | 20.4 | 16.8 | 14.7 | 12.1 | 10.2 | 7.6 |
| STDEV | 0.9 | 1.2 | 0.8 | 0.5 | 0.6 | 0.5 | 0.5 | 0.4 |

Figure 11: DNA yield from several amounts of mouse liver and mouse lung, respectively

| | Mouse liver | | | | Mouse lung | | | |
|---------------------|-------------|-------|-------|-------|------------|-------|-------|-------|
| | 25 mg | 20 mg | 15 mg | 10 mg | 25 mg | 20 mg | 15 mg | 10 mg |
| Purity (260/280 nm) | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 1.9 | 1.9 |
| | 1.8 | 1.8 | 1.7 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 |
| | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 1.9 | 1.9 |
| | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 | 2.0 |
| | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 1.9 | 2.0 | 2.0 |
| | 1.8 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 | 1.9 |
| | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 | 1.9 |
| | 1.8 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 | 1.9 |
| MEAN | 1.8 | 1.9 | 1.8 | 1.9 | 2.0 | 2.0 | 1.9 | 1.9 |
| STDEV | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 |

Figure 12: The 260 / 280 ratio of at least 1.8 indicated highly pure DNA extracted from different starting amounts of mouse liver and mouse lung, respectively.

Cross contamination

The cross contamination determination was done with mousetail samples in a checker board patten. No amplification for empty wells proves the absence of cross contamination.

| | | DNA yield [µg] of 20 mg mousetail | | | | | | | | | | | |
|---|------|-----------------------------------|------|------|------|------|------|------|------|------|------|------|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 20.2 | 0.0 | 20.8 | 0.0 | 20.2 | 0.0 | 20.3 | 0.0 | 21.7 | 0.0 | 22 | 0.0 | |
| B | 0.0 | 20.7 | 0.0 | 20.3 | 0.0 | 21.1 | 0.0 | 20.8 | 0.0 | 21.9 | 0.0 | 21.5 | |
| C | 19.1 | 0.0 | 19.6 | 0.0 | 20.7 | 0.0 | 21.5 | 0.0 | 21.7 | 0.0 | 21.1 | 0.0 | |
| D | 0.0 | 20 | 0.0 | 20.6 | 0.0 | 20.8 | 0.0 | 21.7 | 0.0 | 22.2 | 0.0 | 21.9 | |
| E | 18.3 | 0.0 | 19.6 | 0.0 | 20.3 | 0.0 | 21.8 | 0.0 | 21.7 | 0.0 | 22.1 | 0.0 | |
| F | 0.0 | 20.3 | 0.0 | 20.3 | 0.0 | 20.8 | 0.0 | 20.9 | 0.0 | 22.4 | 0.0 | 21.7 | |
| G | 20.2 | 0.0 | 20.1 | 0.0 | 20.5 | 0.0 | 21 | 0.0 | 21.7 | 0.0 | 21.8 | 0.0 | |
| H | 0.0 | 20.8 | 0.0 | 20.8 | 0.0 | 21.3 | 0.0 | 20.9 | 0.0 | 22.7 | 0.0 | 20.2 | |

Figure 14: No PCR amplification was observed with eluates from negative controls.

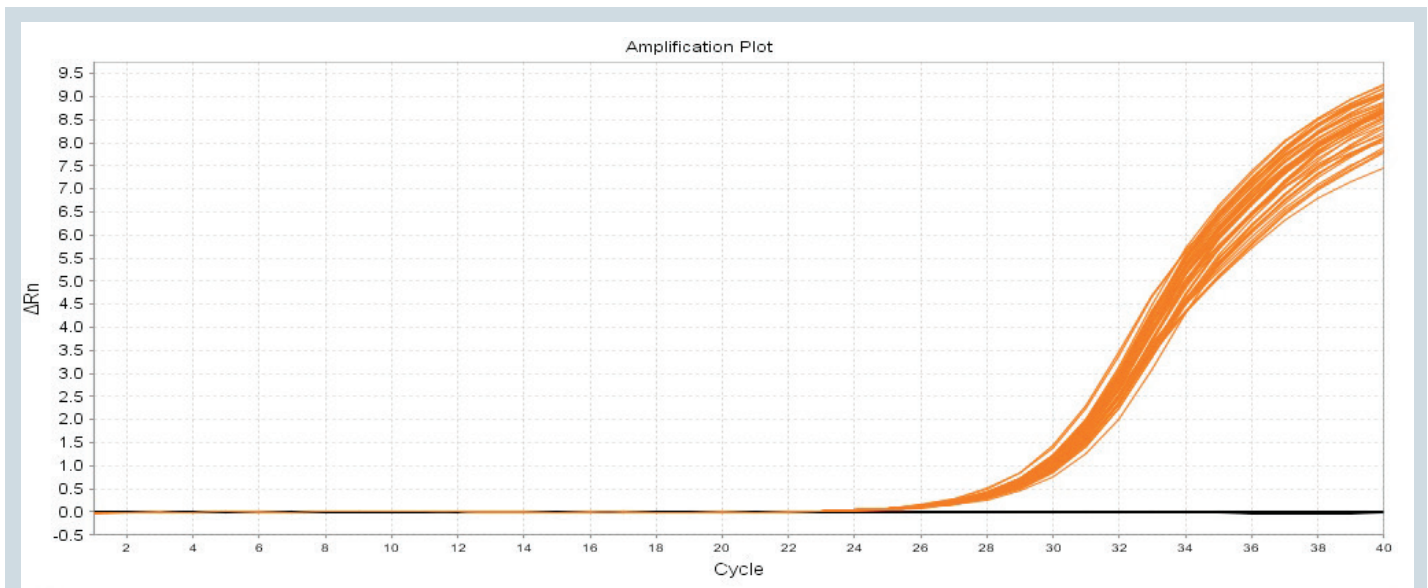


Figure 15: Amplification curves of the cross-contamination experiment

Conclusion

The purified DNA is suitable for a full range of downstream methods. The results from the electrophoresis analysis, qPCR as well as photometric measurements show the performance of the described procedure. The run time

for the automated extraction of 96 samples is between 100 and 120 minutes depending on the sample material. The use of ePT.I.P.S. in SafeRacks along with the re-use tips function, has a direct impact on cost.

Ordering information

| Description | Order no. international |
|---|-------------------------|
| epMotion® 5075t | 5075 000.302 |
| epMotion® 5075m | 5075 000.305 |
| ReservoirRack | 5075 754.002 |
| TM 1000-8 Dispensing tool | 5280 000.258 |
| TM 300-8 Dispensing tool | 5280 000.231 |
| epT.I.P.S.® Motion 1000 µL SafeRack with filter | 0030 014.650 |
| epT.I.P.S.® Motion 300 µL with filter | 0030 014.456 |
| Reservoir 30 mL | 0030 126.505 |
| Reservoir 100 mL | 0030 126.513 |
| Reservoir 400 mL | 5075 751.364 |
| Macherey-Nagel | |
| NucleoMag® 96 Tissue | REF 744400 |
| NucleoMag® SEP | REF 744900 |
| Square well block | REF 740481 |

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