AMPLIQON
PCR ENZYMES & REAGENTS

# Genotyping using Q-Extract DNA Extraction PCR Kits

Genotyping is a technology used for detection of minor genetic differences. Genotyping determines differences in genetic material by comparing one DNA sequence to another DNA sequence or a reference. Genotyping is used in research and diagnostic medicine.

Q-Extract DNA Extraction PCR Kits from Ampliqon are the ideal, fast and convenient solution for genotyping. The performance of Q-Extract DNA Extraction PCR Kits have been tested using different mammalian matrices and also against similar genotyping PCR kits from other suppliers. Furthermore, the performance and handling time of the included Q-Extract DNA Extraction Solution was compared to similar fast DNA extraction solutions from other suppliers.

### Features

- Genotyping of various matrices*
- PCR-ready DNA in 8 minutes
- Minimal handling
- Reliable PCR results
- Red or blue dye for direct gel loading and visualisa tion of pipetting
- Scalable set-up
- Automation-friendly
- Results obtained 1-2 hours after sample collection

*Mammalian tissues e.g. mouse ear or tail, plant leaves, bird feathers, fish fins, bacteria, saliva.

DNA is extracted in just 8 minutes from many different mammalian sample types e.g. mouse tails, ears and salvia. Depending on the sample size, the DNA extraction is performed in either PCR tubes or 1.5 ml tubes using a thermocycler or heating block, respectively. The one-reagent one-tube extraction set-up is easily scaled for e.g. automation on robotic platforms.

Q-Extract DNA Extraction PCR Kits combine the user-friendly DNA extraction of Q-Extract Solution with the convenience and excellent PCR performance of either Taq DNA Polymerase 2x Master Mix RED or TEMPase Hot Start DNA Polymerase 2x Master Mix BLUE.
The non-toxic Q-Extract DNA Extraction Solution is designed for rapid and efficiently extraction of PCR-ready for genotyping.

## Efficient amplification of DNA extracted from different types of mammalian tissues

Q-Extract DNA Extraction PCR Kit was used to extract and amplify genomic DNA from five different mouse tissues (kidney, muscle, lung, liver and tail), chicken muscle tissue and human saliva, (fig. 2). For DNA extraction, 0.5 -10 mg tissue or 20 µl saliva was added to 100 µl of Q-Extract DNA Extraction Solution. The extracted DNA was then amplified using the Taq DNA Polymerase 2x Master Mix RED and species specific primers. The results show that the Q-Extract DNA Extraction PCR Kit can be used for many different mammalian samples and also saliva.

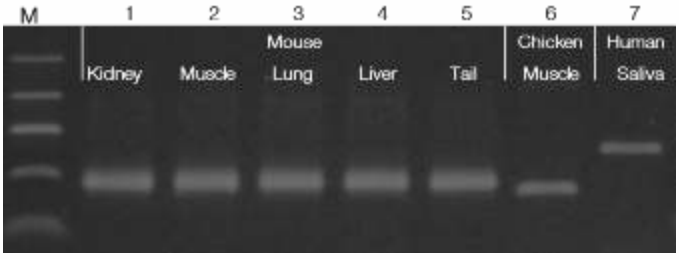



Figure 2. Amplification of DNA from various samples. Q-Extract DNA Extraction PCR Kit was used to extract and amplify genomic DNA from various mammalian tissues. M: DNA marker Iqon Low DNA Ladder. Lane 1-5: Different mouse tissues as depicted, GADPH (266 bp). Lane 6: Chicken muscle tissue, HRPT1 (245 bp) and Lane 7: Human saliva, DMD17 (415 bp).

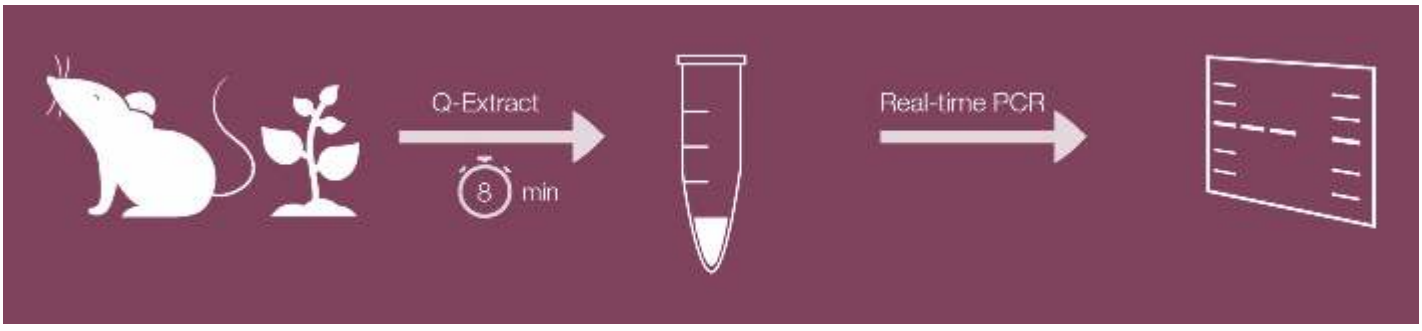


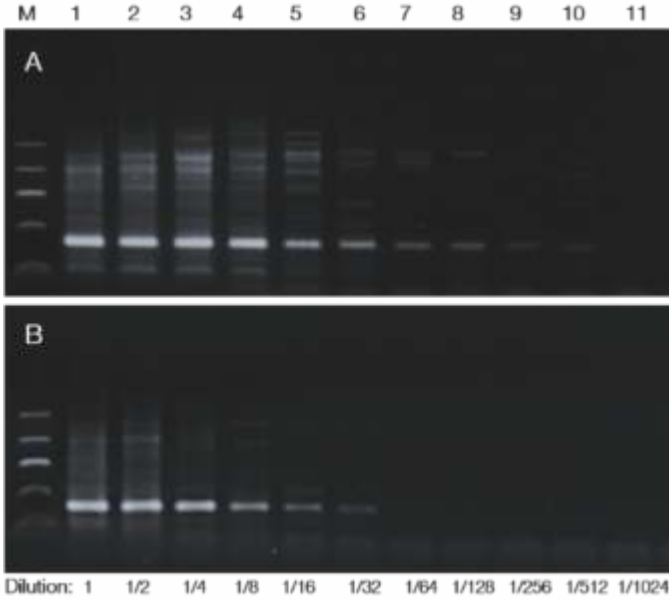

Figure 1. Illustration of the genotyping workflow, when using Q-Extract DNA Extraction PCR Kit. The PCR-ready DNA is extracted in 8 minutes (6 min at 65 °C + 2 min at 98 °C), using the Q-Extract DNA Extraction Solution. The extracted DNA is ready for PCR without further handling such as vortex, centrifugation or dilutions. The extracted DNA is amplified using either Taq DNA Polymerase 2x Master Mix RED or TEMPase Hot Start DNA Polymerase 2x Master Mix Blue.

# Q-Extract DNA Extraction PCR Kits

## Flexible sample handling

The flexibility of sample handling was tested by adding varying amounts (mg) of chicken muscle tissues to 100 µl of Q-Extract DNA Extraction Solution. The extracted DNA was subsequently amplified using Taq DNA Polymerase 2x Master Mix RED. The results show that the Q-Extract DNA Extraction protocol provides the user with a high degree of flexibility over a wide range of applied sample amounts, (fig. 3).


Figure 3. Flexibility of Q-Extract DNA Extraction protocol. M: DNA marker Iqon Low DNA Ladder. Lanes 1-28: Varying amounts (mg) of chicken muscle tissue, HRPT1 (245 bp) as specified on the top of each lane.




Standard 3-step PCR protocol:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial | 95 °C | 5 min | 1 |
| Denaturation | 95 °C | 30 sec | 35 |
| Annealing | 55 °C | 30 sec | |
| Elongation | 72 °C | 30 sec | |
| End | 4 °C | ∞ | 1 |

Fast 3-step PCR protocol:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial | 95 °C | 3 min | 1 |
| Denaturation | 95 °C | 15 sec | 35 |
| Annealing | 55 °C | 1 5 | |
| sec Elongation | 72 °C | 2 0 | |
| sec | | | |

Figure 4. The performance of Q-Extract DNA Extraction PCR Kit when using either the standard 3-step PCR protocol, panel A, or the Fast 3-step PCR protocol, panel B. Q-Extract DNA Extraction PCR Kit was used to extract and amplify genomic DNA from mouse tail tissue. M: DNA marker Iqon Low DNA Ladder. Lane 1-11 Amplification of a two-fold serial dilution of the extracted mouse genomic DNA, mouse GADPH (266 bp).

## Fast genotyping results

The Q-Extract DNA Extraction PCR Kit was used to extract and amplify 2-fold dilutions of DNA from mouse tail tissue. The amplification was performed using either a standard 3-step PCR protocol or a fast 3-step PCR protocol, panel A or panel B, respectively, (fig. 4). Specific PCR results are obtained using both the standard (130 min) as well as the fast 3-step protocol (70 min). When using Q-Extract DNA Extraction PCR Kit, results (from sample to result) are obtained in 70 to 210 minutes, depending on the combination of applied DNA extraction method as well as applied PCR protocol, (fig. 5).

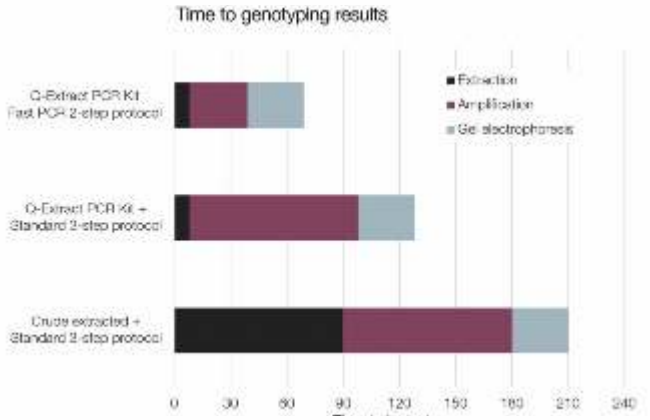



Figure 5. Total time (in minutes) from sample to result required for DNA extraction, amplification and gel electrophoresis when using Q-Extract DNA Extraction Kit with either fast PCR protocol*
or standard PCR protocol. Furthermore, time to result when using the crude extraction protocol** is also included.

*Fast 2-step PCR protocol for Taq DNA Polymerase 2x Master Mix: https://ampliqon.com/ en/pcr-technology/application-notes/ **(Truett GE et al. 2000. Biotechniques 29 (1): 52-54.)

## Storage of DNA extracts

DNA extracts can be stored at -20 °C for one week. Long term storage at -80 °C.

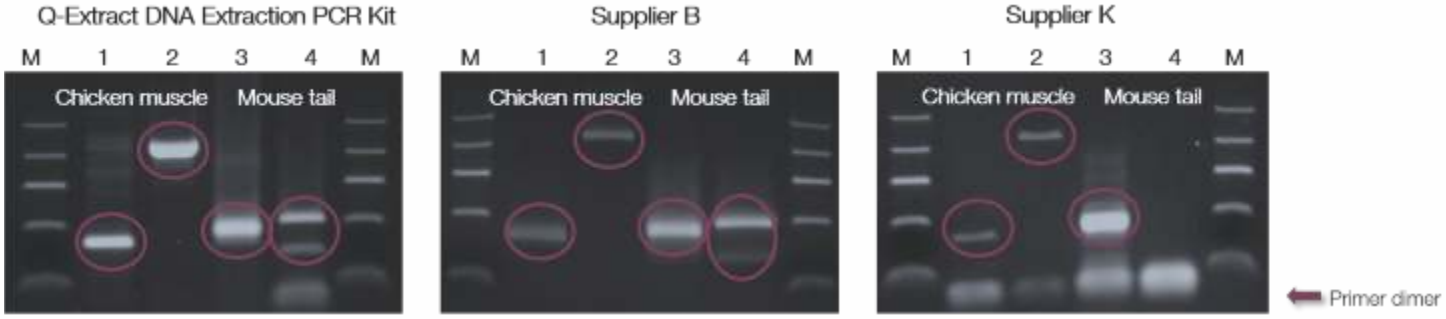



Figure 6. Performance of Q-Extract DNA Extraction PCR Kit was compared to similar genotyping PCR kits from supplier B and K. DNA was extracted from chicken muscle (lane 1-2) and mouse tail (lane 3-4) and amplified using DNA extraction and PCR reagents provided in the respective genotyping PCR Kits. M: DNA marker Iqon Low DNA Ladder. Lane 1: Chicken HRTP1 (245 bp), Lane 2: Chicken GADPH (775 bp), Lane 3: Mouse GADPH (265 bp) and Lane 4: Mouse B-actin (318 bp). Correct/expected amplicons are encircled.

## Performance of Q-Extract DNA Extraction PCR Kit compared to two equivalent PCR kits

DNA was extracted from chicken muscle or mouse tail. Both DNA extracts were tested with two different primer sets, respectively, (fig. 6). Each extraction and amplification were conducted according to the supplier manuals and using the DNA extraction reagents and PCR master mixes provided in the respective kits. The result shows that the Q-Extract DNA Extraction PCR Kit performs equally well or better than supplier B and L on the four DNA targets tested. The applied extraction protocol from supplier B and K are very similar and less user-friendly than the Q-Extract DNA Extraction protocol, (fig. 7).

## Q-Extract DNA Extraction Solution provides the easiest and fastest DNA extraction protocol

Total handling time of the Q-Extract DNA Extraction Solution pro- tocol was estimated in the laboratory and compared to three si- milar fast DNA extraction protocols performed according to the manuals from the respective manufacturers, L, K and B, (fig. 7).
The total handling time for extraction of DNA using Q-Extract DNA Extraction Solution was faster than the handling time for all three suppliers, but very close to supplier L. Furthermore, Q-Extract DNA Extraction Solution only requires 4 handling steps, which is lower than all the three competitors. Numbers of handling steps are indicated on the bars of the respective competitor in fig. 7.

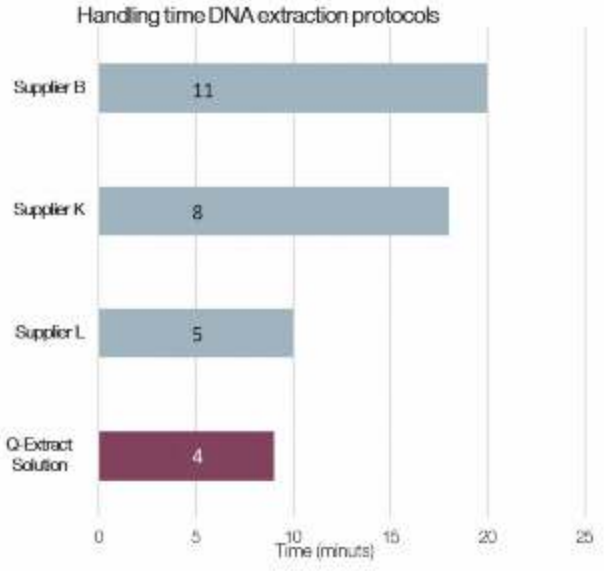



Figure 7. Total handling required for DNA extraction when using either Q-Extract DNA Extraction Solution or similar DNA extraction protocols from supplier L, K and B. Number of handling steps for each protocol is indicated.

## Performance of Q-Extract DNA Extraction Hot Start PCR Kit

The performance of Q-Extract DNA Extraction Hot Start PCR Kit was compared to that of Q-Extract DNA Extraction PCR Kit. The comparison shows that the Q-Extract DNA Extraction Hot Start PCR Kit provides high PCR yield and more specific amplification especially on the mouse β-aktin target (absence of primer dimers).

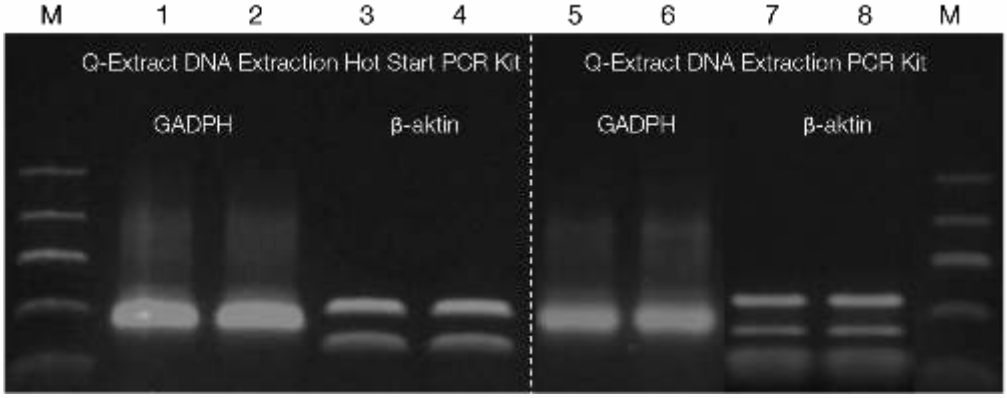



Figure 8. The performance of Q-Extract DNA Extraction Hot Start PCR Kit (lanes 1-4) was compared to the performance of Q-Extract DNA Extraction PCR Kit (lanes 5-8). DNA extracts were extracted in dublicates from mouse liver tissue. Lanes 1,2:5 and 6: Mouse GADPH (265 bp) and lanes 3,4,7 and 8: Mouse β-aktin (318 bp).

# Q-Extract DNA Extraction PCR Kits

## Applications

- Fast extraction of PCR-ready DNA
- Genotyping of mammalian tissues, plant leaves and fish fins
- Genotyping using either end-point PCR or real-time PCR

Plant leaves

Fish fins

Combined with real-time PCR

## Ordering

| Product | RXN* | Cat # |
|---|---|---|
| Q-Extract DNA Extraction Solution | 100<br>500 | A560001<br>A560004 |
| Q-Extract DNA Extraction PCR Kit Incl. Taq DNA Polymerase | 100<br>500 | A570001<br>A570004 |
| Q-Extract DNA Extraction Hot Start PCR Kit incl. TEMPase Hot Start DNA Polymerase 2x Master Mix A BLUE | 100<br>500 | A574401<br>A574404 |
| SAMPLES:<br>Q-Extract DNA Extraction Solution<br>Q-Extract DNA Extraction PCR Kit<br>Q-Extract DNA Extraction Hot Start PCR Kit | <br>20<br>20<br>20 | <br>A560099<br>A570099<br>A574499 |

* 1 reaction = 100 µl Q-Extract DNA Extraction Solution + 12.5 µl Taq DNA Polymerase 2x Master Mix RED (final PCR reaction 25 µl)

Q-Extract DNA Extraction

Q-Extract DNA Extraction PCR

Q-Extract DNA Extraction

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PCR ENZYMES MADE IN DENMARK

For more information or ordering please contact your local distributer AL-Labortechnik & Diagnostik GmbH

Email:office@al-labor.at

Phone: +43 (0) 7472 234 230



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