AMPLIQON
PCR ENZYMES & REAGENTS

Genotyping using Q-Extract DNA Extraction PCR Kit

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 Kit from Ampliqon is the ideal, fast and convenient solution for genotyping. The performance of Q-Extract DNA Extraction PCR Kit has 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 using mammalian tissue e.g. mouse ear or tail*
- PCR-ready DNA in 8 minutes
- Minimal handling
- Reliable PCR results
- Red dye for direct gel loading and visualisation of pipetting
- Scalable set-up
- Automation-friendly
- Results obtained 1-2 hours after sample collection

*Other samples such as saliva, bacteria and various mammalian tissues can be used.

This kit combines the user-friendly DNA extraction of Q-Extract Solution with the convenience and excellent PCR performance of Taq DNA Polymerase 2x Master Mix RED. The non-toxic Q-Extract DNA Extraction Solution is designed for rapid and efficiently extraction of PCR-ready for geneotyping. 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.

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.

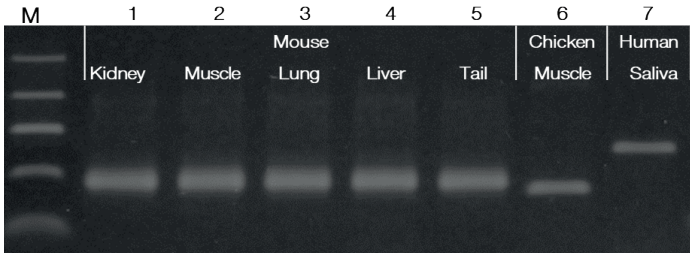


Figure 2. Amplification of DNA from various samples. Q-Extract DNA Extraction PCR Kit was used to extract and amplify genomic DNA from various mammalian tissues. M: DNA marker Iqon Low DNA Ladder. Lane 1-5: Different mouse tissues as depicted, GADPH (266 bp). Lane 6: Chicken muscle tissue, HRPT1 (245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

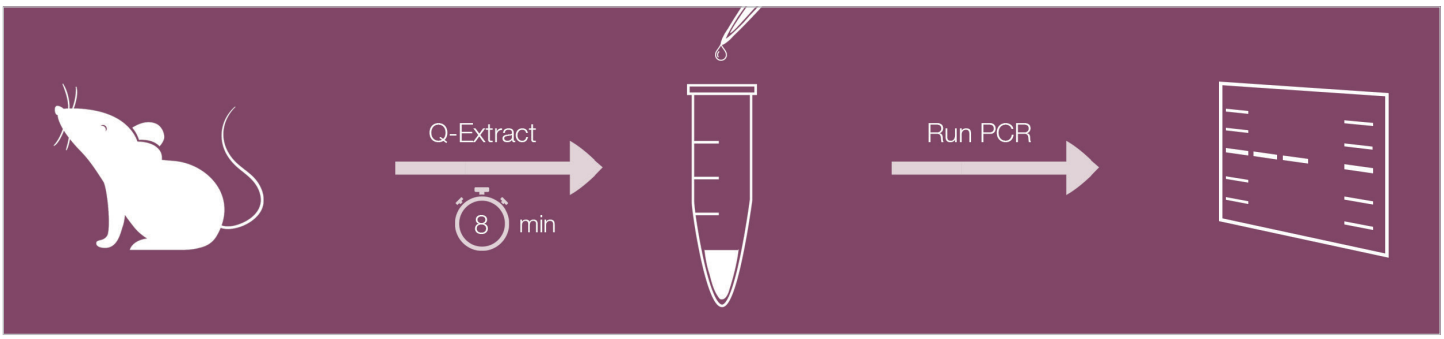


Figure 1. Illustration of the genotyping workflow, when using Q-Extract DNA Extraction PCR Kit. The PCR-ready DNA is extracted in 8 minutes, from mouse tail tissue or other mammalian tissues, using the Q-Extract DNA Extraction Solution. The extracted DNA is ready for PCR without further handling such as vortex, centrifugation or dilutions. The extracted DNA is amplified using Taq DNA Polymerase 2x Master Mix RED.

Q-Extract DNA Extraction PCR Kit

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).

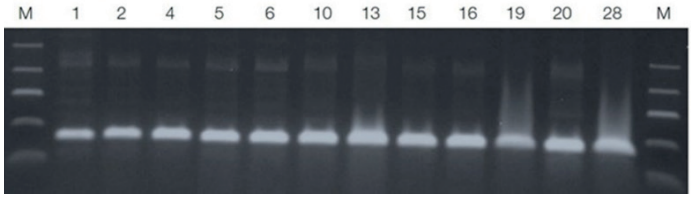
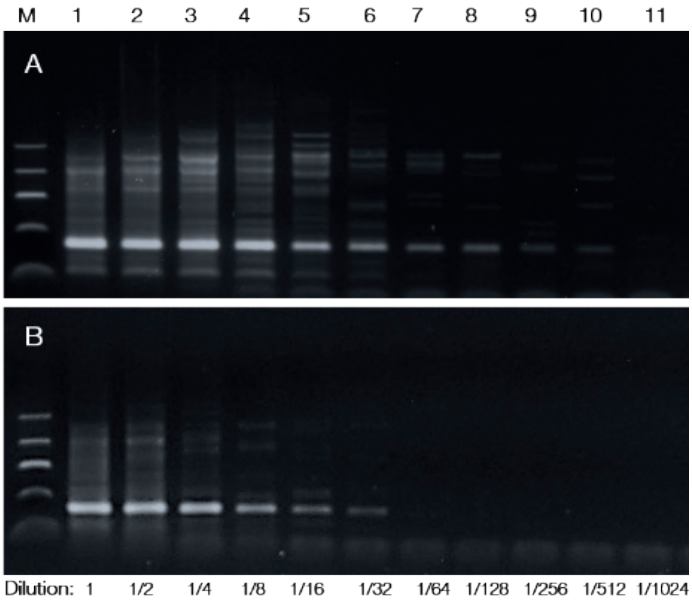


Figure 3. Flexibility of Q-Extract DNA Extraction protocol. M: DNA marker Iqon Low DNA Ladder. Lanes 1-28: Varying amounts (mg) of chicken muscle tissue, HRPT1 (245 bp) as specified on the top of each lane.



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	15 sec	
Elongation	72 °C	20 sec	
End	4 °C	∞	1

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).

Q-Extract PCR DNA Extract PCR Kit provides the user with 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, panel A or a fast 3-step PCR protocol, panel B, (fig. 4). Specific PCR results are obtained using both standard and fast 3-step protocol. We have recently shown that it is possible to save ~1 hour of PCR run time using fast 2-step-protocol and still get specific PCR amplicons of high yields. 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).

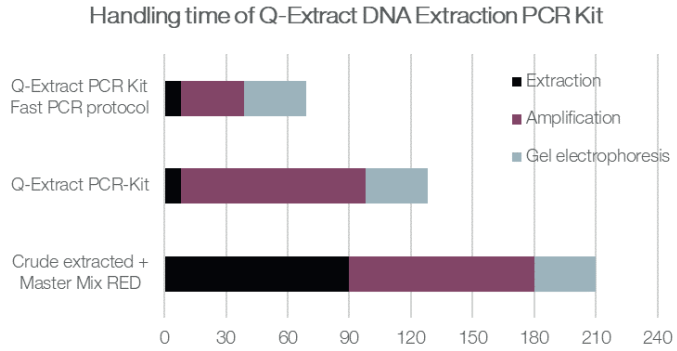


Figure 5. Total time from sample to result required for DNA extraction, amplification and gel electrophoresis when using Q-Extract DNA Extraction Kit with either fast 2-step PCR protocol* or standard PCR protocol. Furthermore, time to result of the standard crude extraction protocol** is also included.

*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.)

Q-Extract DNA Extraction PCR Kit

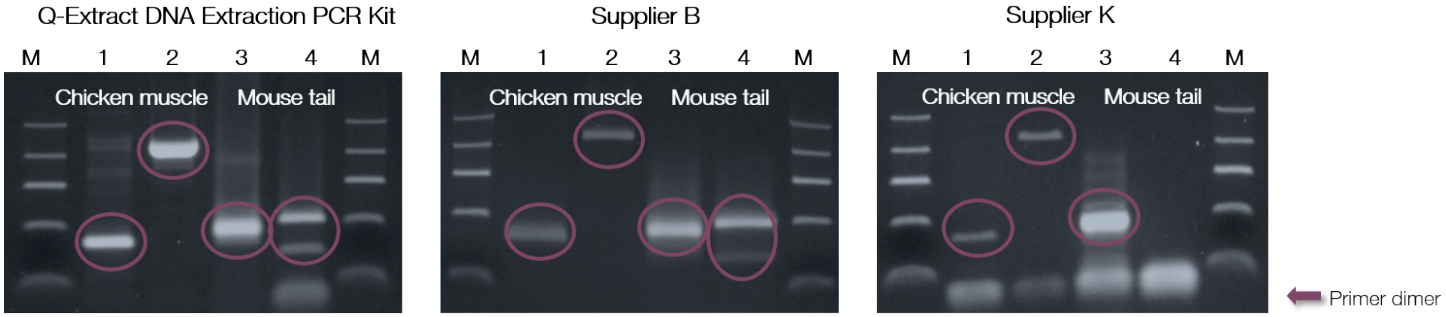


Figure 6. Performance of Q-Extract DNA Extraction PCR Kit was compared to similar genotyping PCR kits from supplier B and K. DNA was extracted from chicken muscle (lane 1-2) and mouse tail (lane 3-4) and amplified using DNA extraction and PCR reagents provided in the respective genotyping PCR Kits. M: 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. 8).

Performance of Q-Extract DNA Extraction Solution compared to an equivalent DNA extraction solution

The performance of Q-Extract DNA Extraction Solution was compared to similar DNA extraction solution from supplier L, (fig. 7). DNA was extracted from 3 mg of chicken muscle tissue using the respective DNA extraction protocols. A four-fold serial dilution of the two DNA extracts was amplified using Taq DNA Polymerase 2x Master Mix RED. The obtained results indicate that the quality and yield of DNA extract using Q-Extract DNA Extraction Solution is slightly better than that of supplier L. The Q-Extract DNA Extraction Solution protocol provided reliable PCR-ready DNA in 9 minutes which is close to the 10 minutes provided by protocol from supplier L, (fig. 8).

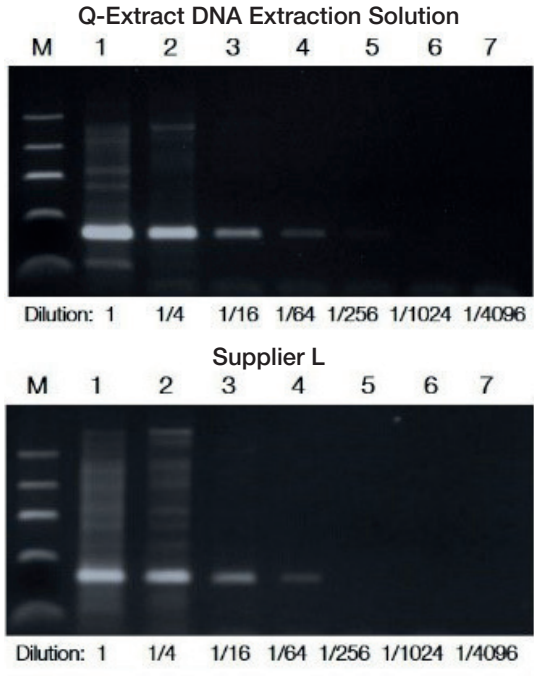


Figure 7. The performance of Q-Extract DNA Extraction Solution was compared to the DNA extraction solution from competitor L. A four-fold serial dilution of DNA extracts was amplified using Taq DNA Polymerase 2x Master Mix RED. M: DNA marker Iqon Low DNA Ladder. Lane 1-7: Chicken HRTP1 (245 bp).

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

Total handling time of the Q-Extract DNA Extraction Solution protocol was estimated in the laboratory and compared to three similar fast DNA extraction protocols performed according to the manuals from the respective manufacturers, L, K and B, (fig. 8). 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. 8.

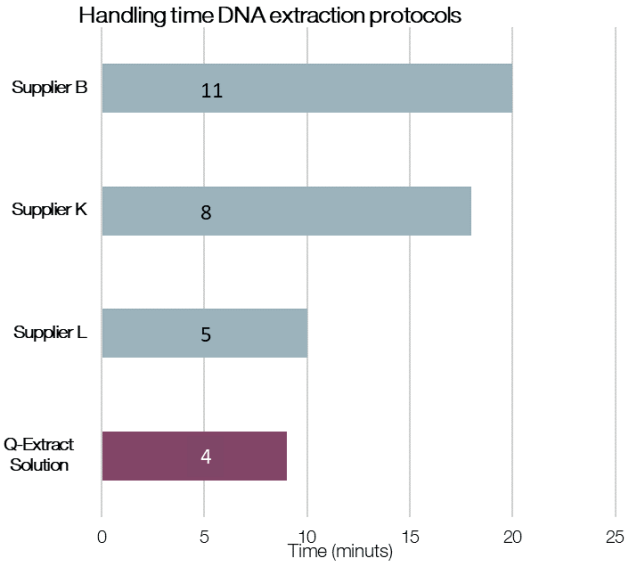


Figure 8. Total handling required for DNA extraction when using either Q-Extract DNA Extraction Solution or similar DNA extraction protocols from supplier L, K and B. Number of handling steps for each protocol is indicated.

Q-Extract DNA Extraction PCR Kit

Ordering information

Product	Reactions	Cat #
Q-Extract DNA Extraction Solution	100 500	A560001 A560004
Q-Extract DNA Extraction PCR Kit Incl. Taq DNA Polymerase 2x Master Mix RED	100 500	A570001 A570004
SAMPLES: Q-Extract DNA Extraction Solution Q-Extract DNA Extraction PCR Kit	 20 20	 A560099 A570099

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

More sizes and variants available at www.ampliqon.com

Q-Extract DNA Extraction Solution

Q-Extract DNA Extraction PCR Kit

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