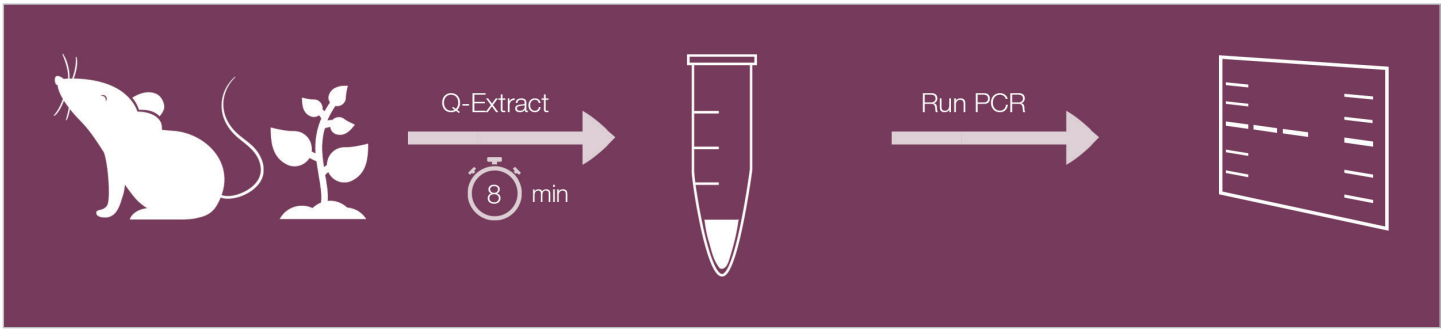
AMPLIQON
PCR ENZYMES & REAGENTS

Q-Extract DNA Extraction Solution for Genotyping of Plant DNA

Overview

The Q-Extract DNA Extraction Solution is designed for rapid and efficient extraction of PCR-ready DNA from various sample types; mammalian tissues (such as mouse tail, and ear snips), plant leaves, fish fins, saliva and bacteria. The non-toxic Q-extract DNA Extraction Solution enables the extraction of PCR-ready DNA in just 8 minutes.

Here we describe the use of Q-extract DNA Extraction Solution protocol for the extraction of PCR-ready plant DNA from stinging nettle (*Urtica dioica*) and ivy (*Hedera helix*) followed by end-point PCR using Taq DNA Polymerase 2x Master Mix RED (Ampliqon A/S).



Q-Extract Extraction Protocol

1. Add ~10 mg of plant leaves* to a tube containing 100 µl Q-Extract DNA Extraction Solution. Make sure that the leaves are completely covered by the Q-Extract DNA Extraction Solution.

2. Vortex the tube for 15 sec.

3. Transfer the tube to a heat block or a thermal cycler and incubate at

    1. 65 °C for 6 min
    2. 98 °C for 2 min
    3. 4 °C (or cool down on ice)

The DNA extract is now ready for PCR.

DNA extracts are stable at -20 °C for one week or long-term storage at -80 °C.

*Recommended sample sizes from various sample types are shown in table 1.

Table 1. Sample sizes of different matrices

Sample	Q-Extract DNA Extraction Solution 100 µl	500 µl
Tissue*	0.5 – 10 mg	10 – 50 mg
Plant**	2 – 10 mg	10 – 50 mg
E. coli	1 colony (Φ 0.5 - 2 mm)	1 colony (Φ 0.5 - 5 mm)
Saliva	10 – 20 µl	50 - 100 µl

* Examples of tested tissues include mouse tail snip, mouse organs and chicken breast.
**Examples of tested plant materials include leaves from stinging nettle and ivy.

Table 2. PCR reaction mix

Component	Volume	Conc.
Taq 2x Master Mix RED	12.5 µl	1x
Forward primer (10 µl)	0.5 µl	0.2 µM
Reverse primer (10 µl)	0.5 µl	0.2 µM
PCR Grade Water	6.5 µl	-
DNA extracts (5-fold diluted)*	1 µl	0.2 µl/RXN
Total volume	25 µl	-

* or 10 fold serial dilutions hereof, see figure 2.

Table 3. 2-step real-time PCR protocol

Step	Temp.	Time	Cycles
Initial heating	95 °C	5 min	1
Denaturation Annealing Elongation	95 °C 60 °C 72 °C	30 sec 30 sec 30 sec	30
Final elongation	72 °C	4 min	1
End	4 °C	∞	1

End-point PCR of plant DNA extracted using Q-Extract DNA Extraction PCR Kit

The Q-Extract DNA Extraction PCR Kit consists of Q-Extract DNA extraction solution and Taq DNA Polymerase 2x Master Mix RED 1.5 mM $MgCl_2$. Q-Extract DNA Extraction Solution was used to extract the PCR-ready DNA from leaves of stinging nettle and ivy. DNA samples were extracted in dublicates (figure 1).

The extracted DNA samples were amplified using Taq DNA Polymerase 2x Master Mix RED 1.5 mM $MgCl_2$ (Ampliqon) using primers targeting genomic plant DNA (ITS 335 bp) and chloroplast DNA (trnL 380 bp). For this study 0.2 µl/reaction of the DNA samples extracted using either Q-Extract DNA Extract Solution or a PCR buffer without lysing agents (no treatment) were used to set up the PCR reactions. All amplifications were performed in dublicates. Figure 2 shows amplifications results of DNA extracted from stinging nettle. Figure 3 shows amplifications results of DNA extracted from ivy.

All DNA extracts using Q-Extract DNA Extraction Solution were amplified with high specificity and high yields for both genomic plant DNA (ITS 335 bp) and chloroplast DNA (trnL 380 bp). No difference in amplification yields were observed for chloroplast DNA (trnL 380 bp) between DNA samples extracted using Q-Extract DNA Extraction Solution or DNA samples extracted using a PCR buffer without lysing agents (No treatment).

On the contrary, a huge difference in amplification yields were observed for genomic plant DNA (ITS 335 bp), between DNA samples extracted using Q-Extract DNA Extraction Solution or DNA samples extracted using a PCR buffer without lysing agents (No treatment). DNA samples extracted using Q-Extract DNA Extraction Solution showed a much higher yield for the amplification of genomic plant DNA (ITS 335 bp) than for DNA samples extracted using a PCR buffer without lysing agents (No treatment). This difference can be explained by the sensitivity towards heat treatment of the chloroplast versus the plant nucleus as well as the higher numbers of chloroplast DNA compared to genomic DNA. This clearly indicates that the lysing agents, within Q-Extract DNA Extraction is required to lyse the plant nucleus effectively.

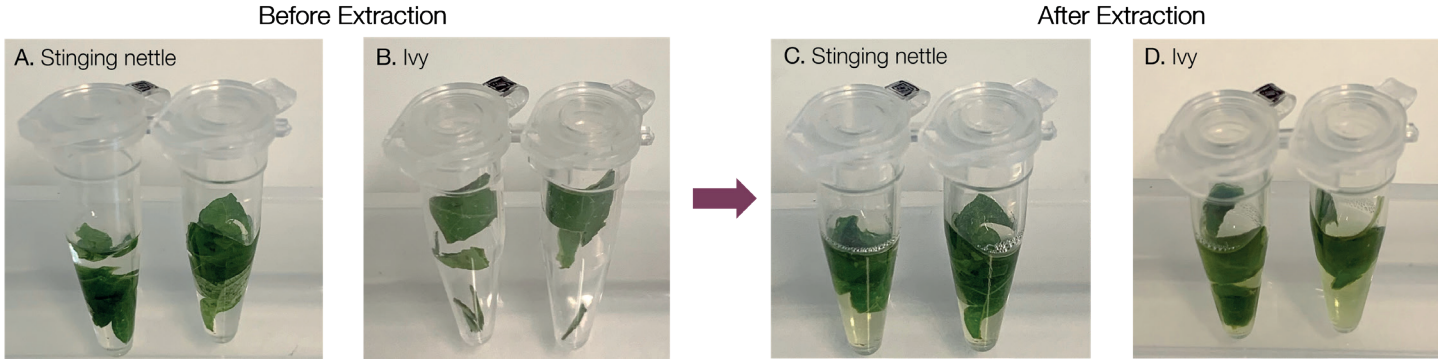


Figure 1. DNA extracts of stinging nettle and ivy. PCR tubes in duplicates with 100 µl Q-Extract DNA Extraction Solution + 10 mg leaves from either stinging nettles or ivy. A. stinging nettle and B. Ivy before extraction with Q-Extract DNA Extraction. C. Stinging nettle and D. ivy after extraction with Q-Extract DNA Extraction.

Figure 2. The extracted DNA from stinging nettle was amplified using Taq DNA Polymerase 2x Master Mix RED 1.5 mM $MgCl_2$ (Ampliqon) and two primer sets targeting: A. Genomic plant DNA (ITS 335 bp) or B. Chloro- plast DNA (trnL 380 bp). The positive controls are 1 ng/reaction of DNA purified from stinging nettle using DNeasy Plant Mini Kit (Qiagen). Also included are duplicates of samples extracted in buffer without lysing agents, but using the Q-Extraction protocol (No treatment). All DNA extracts are amplified in duplicates. DNA marker is Iqon PCR Ladder (Ampliqon).

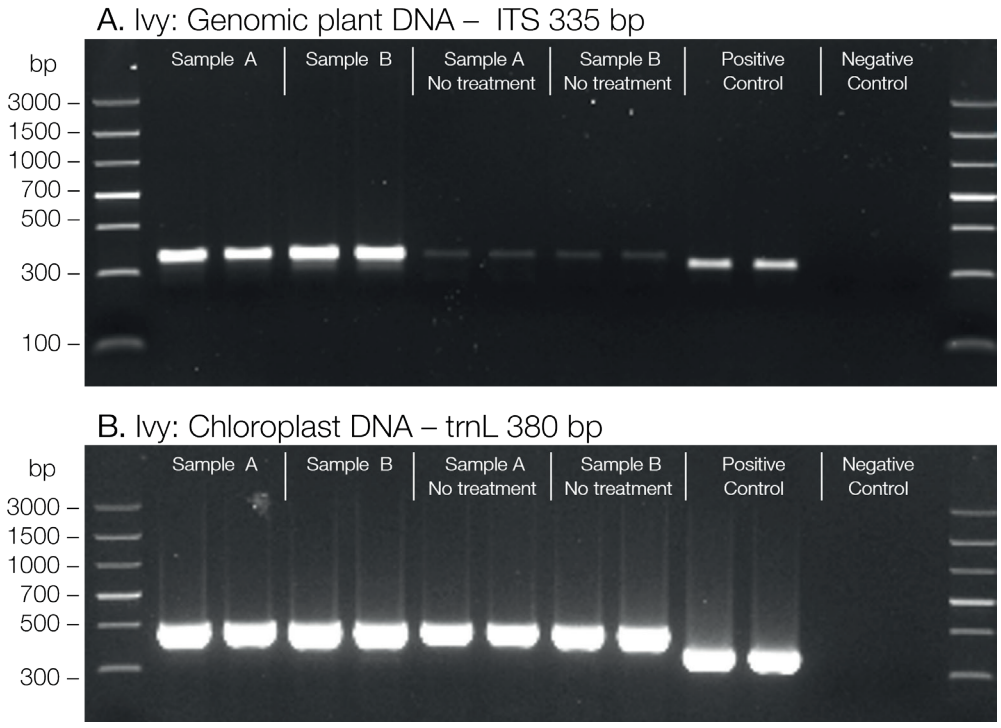


Figure 3. The extracted DNA from ivy was amplified using Taq DNA Polymerase 2x Master Mix RED 1.5 mM $MgCl_2$ (Ampliqon) and two primer sets targeting: A. Genomic plant DNA (ITS 335 bp) or B. Chloroplast DNA (trnL 380 bp). The positive controls are 1 ng/reaction of DNA purified from stinging nettle using DNeasy Plant Mini Kit (Qiagen). Also included are duplicates of samples extracted in buffer without lysing agents, but using the Q-Extraction protocol (No treatment). All DNA extracts are amplified in duplicates. DNA marker is Iqon PCR Ladder (Ampliqon).

Conclusion

PCR-ready DNA is easily extracted from plant leaves using Q-Extract DNA Extraction Solution with the fast 8 minutes protocol also applied for extraction of PCR-ready DNA from mammalian tissues, saliva and bacteria. Q-Extract DNA Extraction Solution is ideal for the extraction of PCR-ready DNA from plant material such as leaves, especially when analyzing plant genomic DNA.

Ordering information

Product	RXN*	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
Q-Extract DNA Extraction Hot Start PCR Kit incl. TEMPase Hot Start DNA Polymerase 2x Master Mix A BLUE	100 500	A574401 A574404
SAMPLES: Q-Extract DNA Extraction Solution Q-Extract DNA Extraction PCR Kit Q-Extract DNA Extraction Hot Start PCR Kit	 20 20 20	 A560099 A570099 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 Solution

Q-Extract DNA Extraction PCR Kit

Q-Extract DNA Extraction Hot Start PCR Kit

PCR ENZYMES MADE IN DENMARK

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