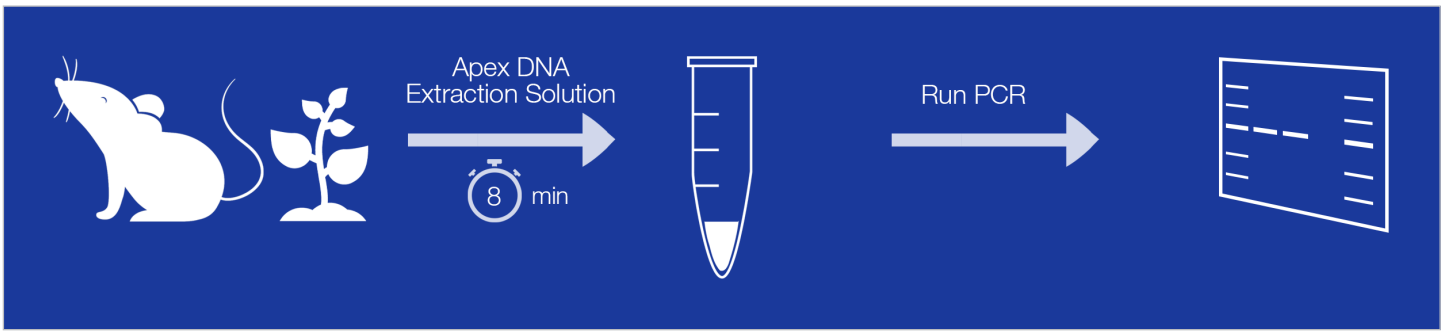
Apex DNA Extraction Solution for Genotyping of Plant DNA

Overview

The Apex 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 Apex DNA Extraction Solution enables the extraction of PCR-ready DNA in just 8 minutes.

Here we describe the use of Apex 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 2.0X Taq RED Master Mix (Apex).



Apex DNA Extraction Solution Protocol

1. Add ~10 mg of plant leaves* to a tube containing 100 µl Apex DNA Extraction Solution.
   Make sure that the leaves are completely covered by the Apex 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 20 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	Apex DNA Extraction Solution 100 µl	Apex DNA Extraction Solution 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.
2X Taq RED Master Mix	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 Extract-Amp RED PCR Kit

The Extract-Amp RED PCR Kit consists of Apex DNA Extraction Solution and 2.0X Taq RED Master Mix Kit 1.5mM $MgCl_2$ (Apex). Apex 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 2.0X Taq RED Master Mix Kit 1.5mM $MgCl_2$ 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 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 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 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 Apex DNA Extraction Solution or DNA samples extracted using a PCR buffer without lysing agents (No treatment). DNA samples extracted using Apex 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 DNA Extraction is required to lyse the plant nucleus effectively.

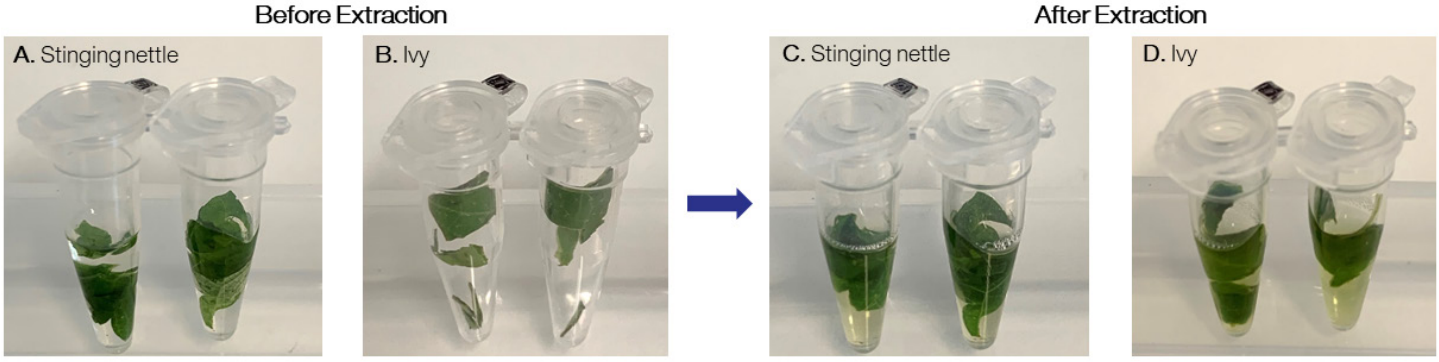


Figure 1. DNA extracts of stinging nettle and ivy. PCR tubes in duplicates with 100 µl DNA Extraction Solution + 10 mg leaves from either stinging nettles or ivy. A. stinging nettle and B. Ivy before extraction with DNA Extraction Solution. C. Stinging nettle and D. ivy after extraction with DNA Extraction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

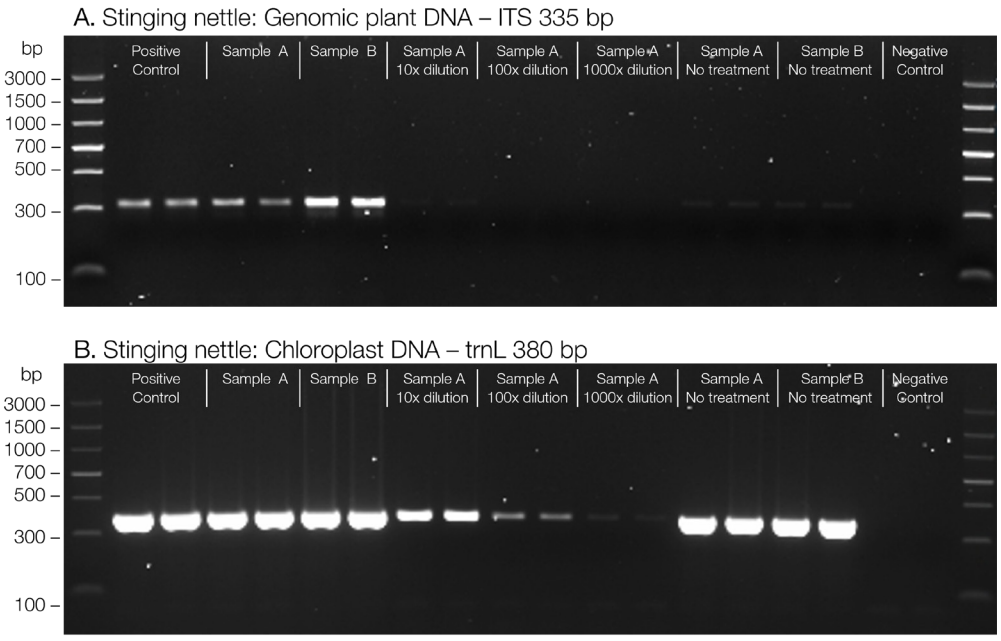


Figure 2. The extracted DNA from stinging nettle was amplified using 2.0X Taq RED Master Mix Kit 1.5mM $MgCl_2$ (Apex) and two primer sets targeting: A. Genomic plant DNA (ITS 335 bp) or B. Chloroplast DNA (trnL 380 bp). The positive controls are 1 ng/reaction of DNA purified from stinging nettle using DNeasy Plant Mini Kit (Qiagen). Also included are duplicates of samples extracted in buffer without lysing agents, but using the Apex DNA Extraction Solution protocol (No treatment). All DNA extracts are amplified in duplicates. DNA marker is ECON PCR Ladder (Apex).

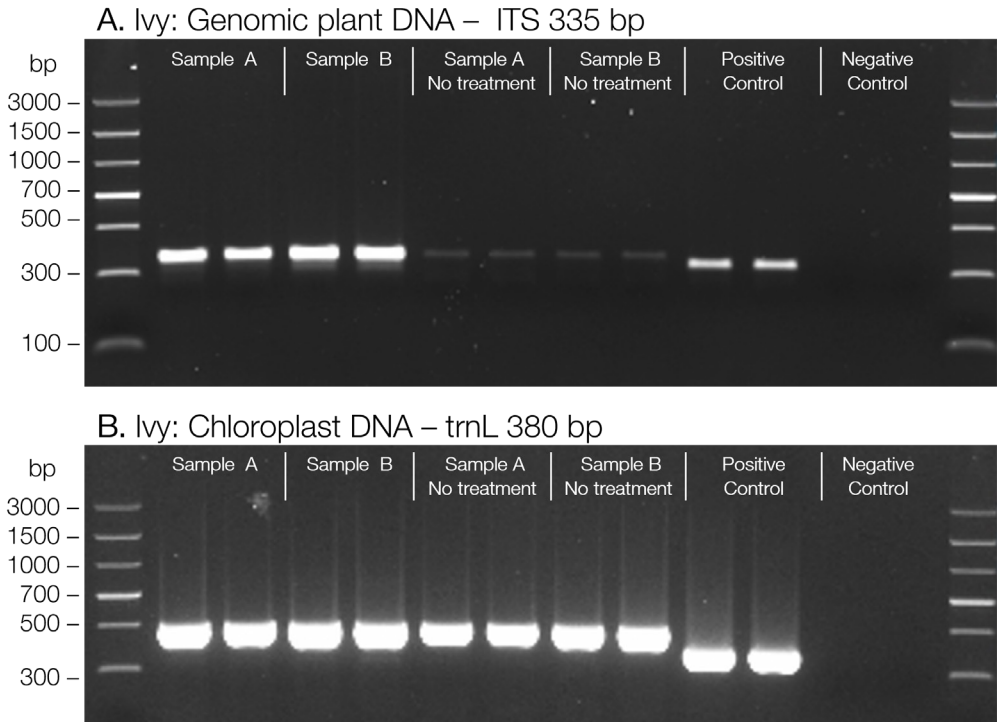


Figure 3. The extracted DNA from ivy was amplified using 2.0X Taq RED Master Mix Kit 1.5mM $MgCl_2$ (Apex) and two primer sets targeting: A. Genomic plant DNA (ITS 335 bp) or B. Chloroplast DNA (trnL 380 bp). The positive controls are 1 ng/reaction of DNA purified from stinging nettle using DNeasy Plant Mini Kit (Qiagen). Also included are duplicates of samples extracted in buffer without lysing agents, but using the Apex DNA Extraction Solution protocol (No treatment). All DNA extracts are amplified in duplicates. DNA marker is ECON PCR Ladder (Apex).

Conclusion

PCR-ready DNA is easily extracted from plant leaves using Apex DNA Extraction Solution with the fast 8 minutes protocol also applied for extraction of PCR-ready DNA from mammalian tissues, saliva and bacteria. Apex 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 #
Apex DNA Extraction Solution	100 500	42-503 42-503B
Extract-Amp RED PCR Kit incl. DNA Extraction Solution and 2.0X Taq RED Master Mix	100 500	42-502 42-502B

* 1 reaction = 100 µl DNA Extraction Solution + 12.5 µl 2.0X Taq RED Master Mix (final PCR reaction 25 µl)