Apex DNA Extraction Solution combined with real-time PCR

Overview

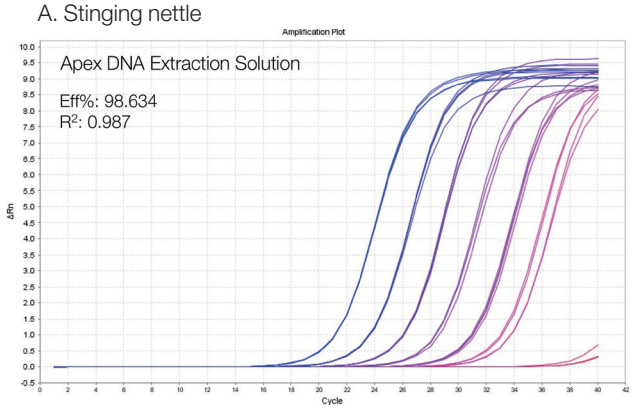
Apex DNA Extraction Solution is designed for rapid and efficient extraction of PCR-ready DNA from various sample types; mammalian tissues (such as mouse tail, and ear snips), plant leaves, fish fins, saliva and bacteria. The non-toxic Apex DNA Extraction Solution enables the extraction of PCR-ready DNA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

Here we describe how PCR-ready DNA extracted from stinging nettle (*Urtica dioica*) and ivy (*Hedera helix*) are used for successful real-time PCR using qPCR 2X GREEN Master Mix, High ROX.

DNA extracted using Apex DNA Extraction Solution is suitable for real-time PCR applications

Apex DNA Extraction Solution was used to extract the PCR-ready DNA from leaves of stinging nettle and ivy. To examine the abillity of the PCR-ready DNA extracts to be used for real-time PCR applications the extracts were amplified using primers targeting chloroplast DNA (trnL 72 bp). For this experiment 5-fold serial dilutions of the extracted DNA were prepared. 7 dilutions (0.2 µl/ reaction) all in dublicate were included in the experiment (figure 1).

A. Stinging nettle



B. Ivy

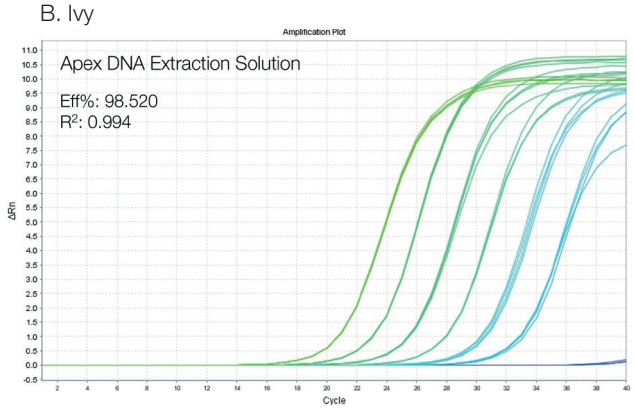


Figure 1. Data showing log amplification plots of 5-fold serial dilutions of stinging nettle and ivy all in dublicates. 7 dilutions (0.2 µl of each dilution/reaction) of each dublicate were included. A: Amplification plots of DNA extracted from stining nettle using Apex DNA Extraction Solution. B. Amplification plots of DNA extracted from ivy using Apex DNA Extraction Solution. Cycling was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) with the following protocol: 95 °C, 15 min; followed by 40 cycles of 95 °C, 15 sec; 60 °C, 60 sec (table 3).

Conclusion

The results from this experiment show that it is possible to utilize the DNA extracted using Apex DNA Extraction Solution for real-time PCR amplification with high efficeincy.

Table 1. Sample sizes of different matrices

Sample	Apex 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. Real-time PCR reaction mix

Component	Volume	Conc.
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 dilutions)	1 µl	0.2 µl/RXN
Total volume	25 µl	-

*qPCR 2X GREEN Master Mix, High ROX

Table 3. 2-step real-time PCR protocol

Step	Temp.	Time	Cycles
Initial heating	95 °C	15 min	1
Denaturation Annealing/Elongation	95 °C 60 °C	15 sec 60 sec	40
Final elongation	72 °C	4 min	1
End	4 °C	∞	1

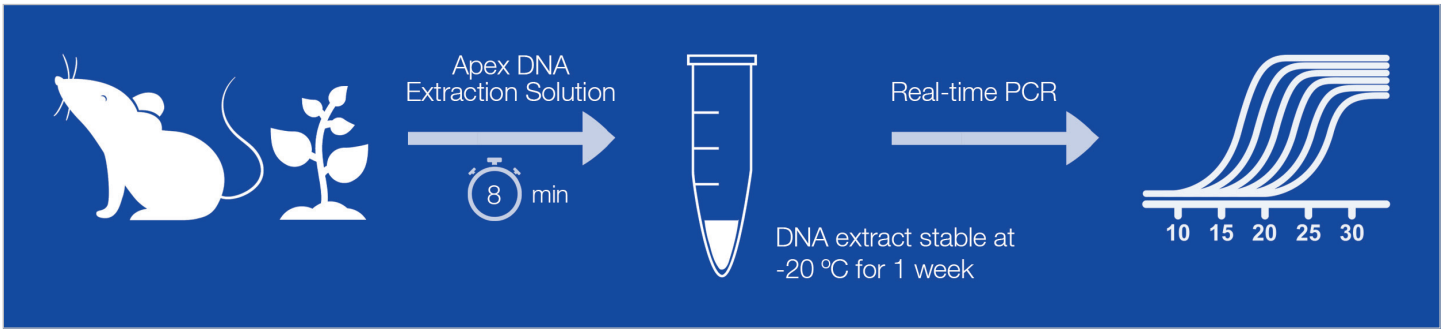
Apex DNA Extraction Solution Protocol

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 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 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.



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)