DIFFINITY

DNA RapidExtract™ Kit

FAST • SIMPLE • EFFICIENT

Applications

Diffinity Genomics®

CHIRAL TECHNOLOGIES
DAICEL GROUP

IN COLLABORATION WITH
KANEKA

Introduction

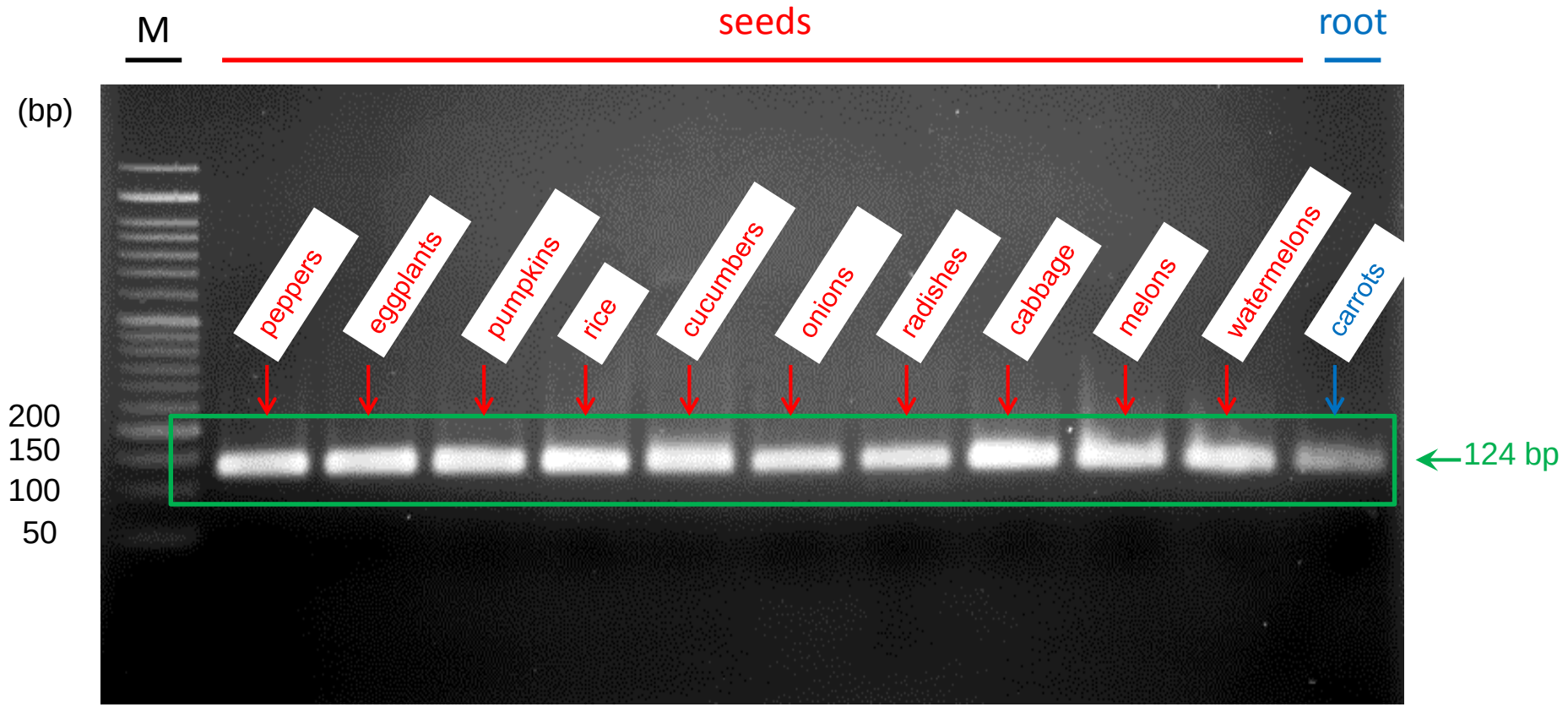
DNA can be extracted from any biological material such as plant or animal tissue, cells, virus particles, blood and environmental samples. DNA extraction is the starting point for numerous applications in fundamental research, diagnostics and agricultural genomics.

In collaboration with Kaneka, Chiral Technologies/Diffinity Genomics offers the Diffinity DNA RapidExtract™ Kit designed to lyse sample in a single incubation step in 10 minutes. One kit can be used for many sample types.

Here, data are presented on rapid DNA extractions yielding PCR-ready DNA.

Plant samples | Extraction of PCR-ready DNA

DNA was extracted from plant seeds and tissues using Diffinity DNA RapidExtract Kit.
PCR amplification was followed by gel electrophoresis to verify the kit performance.



M: 50 bp DNA Ladder

Extraction and amplification protocols are on the next page.

Plant samples - DNA extraction and Amplification protocols

DNA extraction protocol

1. Cut samples into 5 mm pieces and place one of them in a PCR tube.
2. Add 100 µl of Solution A to mechanically disrupted tissue, mix thoroughly and incubate the PCR tube at 98 ℃ for 8 minutes on a heat block.
3. Cool the tube to room temperature.
4. Add 14 µl of Solution B and mix well by pipetting.

Primers

Housekeeping gene: tRNA leucine

>Forward
CGGACGAGAATAAAGATAGAGT
>Reverse
TTTTGGGGATAGAGGGACTTGA

PCR mixture

10 x Kaneka High-Speed DNA Polymerase Buffer	2.0 µl
2.0 mM dNTP	2.0 µl
Kaneka High-Speed DNA Polymerase	0.2 µl
10 µM primers	1.2 µl
DNA Extract	1.0 µl
Sterile distilled water	fill up to 20.0 µl

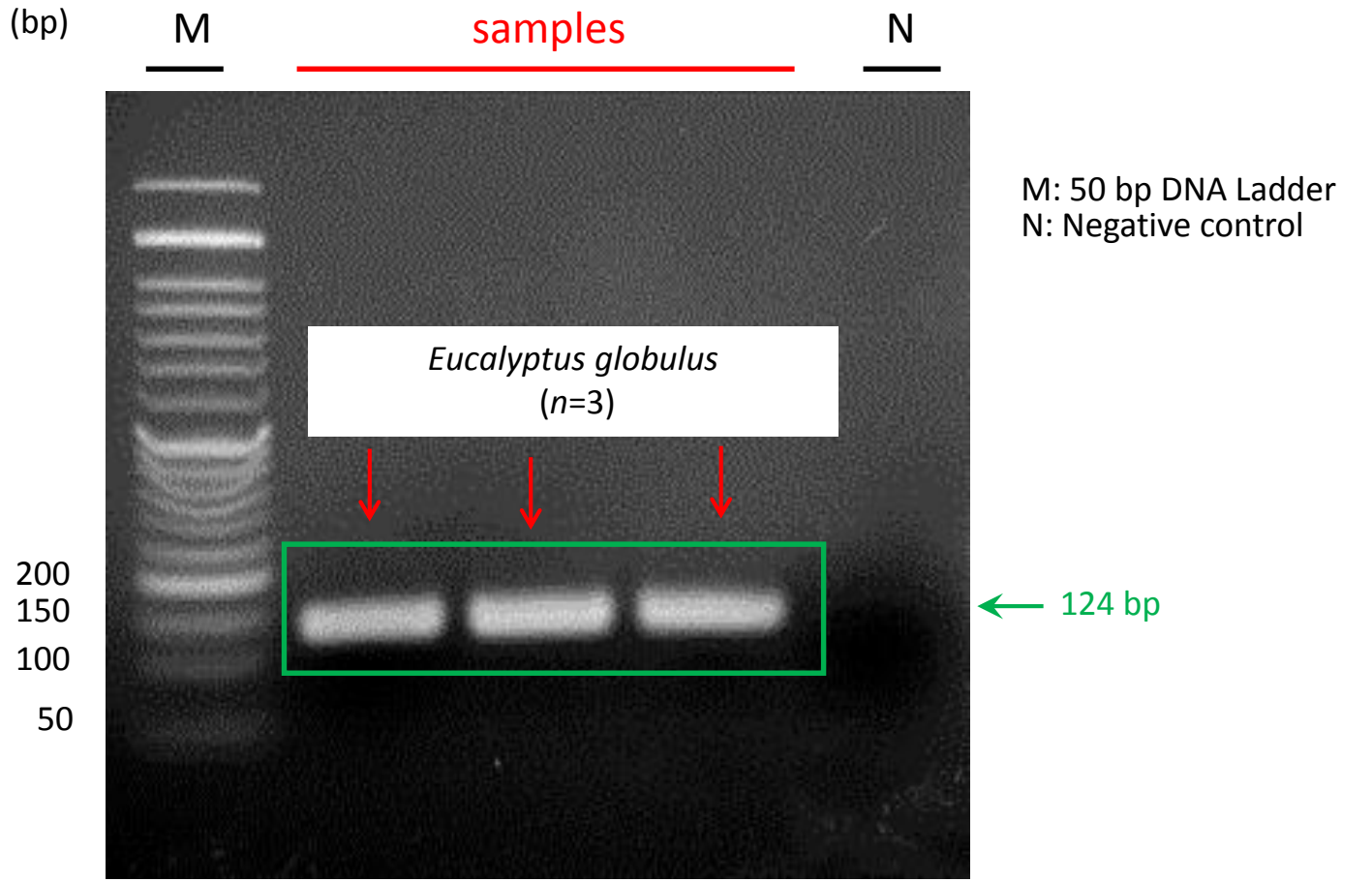
Thermal cycler
Biometra T3000
(Biometra GmbH, Germany)

PCR program
94℃, 1 min
94℃, 15 sec
60℃, 15 sec } x35
72℃, 15 sec

Electrophoresis and staining DNA
After 2% agarose gel electrophoresis, DNA fragments are visualized by staining with ethidium bromide.

Eucalyptus leaves | Extraction of PCR-ready DNA

DNA was extracted from eucalyptus leaves using Diffinity DNA RapidExtract Kit. PCR amplification was followed by gel electrophoresis to verify the kit performance.



Extraction and amplification protocols are on the next page.

Eucalyptus leaves – DNA extraction and Amplification protocols

DNA extraction protocol

1. Cut eucalyptus leaves into 5 mm pieces and place one of them in a PCR tube.
2. Add 100 µl of Solution A to mechanically disrupted tissue, mix thoroughly and incubate the PCR tube at 98 ℃ for 8 minutes on a heat block.
3. Cool the tube to room temperature.
4. Add 14 µl of Solution B and mix well by pipetting.
5. Dilute the mixture solution 10-fold with distilled water*.

*Impurities originated from eucalyptus leaves may decrease the DNA amplification. It can be improved by diluting the extract solution with distilled water.

Primers

Housekeeping gene: tRNA leucine

>Forward
CGGACGAGAATAAAGATAGAGT
> Reverse
TTTTGGGGATAGAGGGACTTGA

PCR mixture

10 x Kaneka High-Speed DNA Polymerase Buffer	2.0 µl
2.0 mM dNTP	2.0 µl
Kaneka High-Speed DNA Polymerase	0.2 µl
10 µM primers	1.2 µl
DNA Extract (10-fold diluted)	1.0 µl
Sterile distilled water	fill up to 20.0 µl

Thermal cycler
Biometra T3000
(Biometra GmbH, Germany)

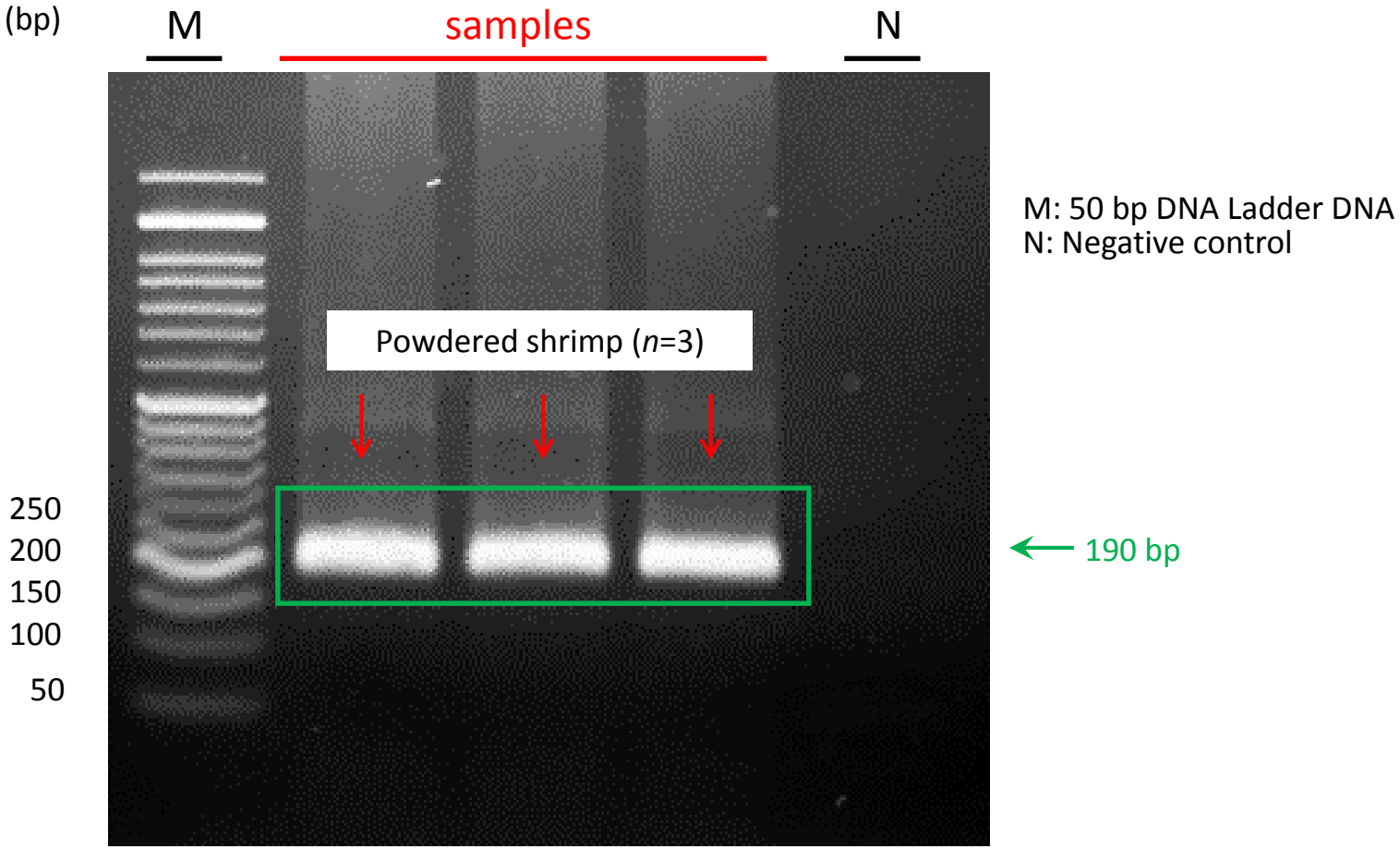
PCR program
94℃, 1 min
94℃, 15 sec
60℃, 15 sec
72℃, 15 sec
} x40

Electrophoresis and staining DNA

After 2% agarose gel electrophoresis, DNA fragments are visualized by staining with ethidium bromide.

Powdered shrimp | Extraction of PCR-ready DNA

DNA was extracted from powdered shrimp using Diffinity DNA RapidExtract Kit. PCR amplification was followed by gel electrophoresis to verify the kit performance.



Extraction and amplification protocols are on the next page.

Powdered shrimp – DNA extraction and Amplification protocols

DNA extraction protocol

1. Add 100 µl of Solution A to 20 mg powdered shrimp placed in a PCR tube.
2. Mix the sample well by pipetting and incubate the PCR tube at 98 ℃ for 8 minutes on a heat block.
3. Cool the tube to room temperature.
4. Add 14 µl of Solution B and mix well by pipetting.

Primers — 16S ribosomal RNA gene

> Forward
TTATATAAAGTCTRGCCTGCC
>Reverse1
GTCCCTCTAGAACATTTAAGCCTTTTC
> Reverse2
GTCCCTTTATACTATTTAAGCCTTTTC
> Reverse3
GTCCCCCCAAATTATTTAAGCCTTTTC

Mixed in a ratio of 1:1:1

Thermal cycler
Biometra T3000
(Biometra GmbH, Germany)

<PCR program>
94℃, 1 min
94℃, 15 sec
60℃, 15 sec
72℃, 15 sec
} x35

PCR mixture

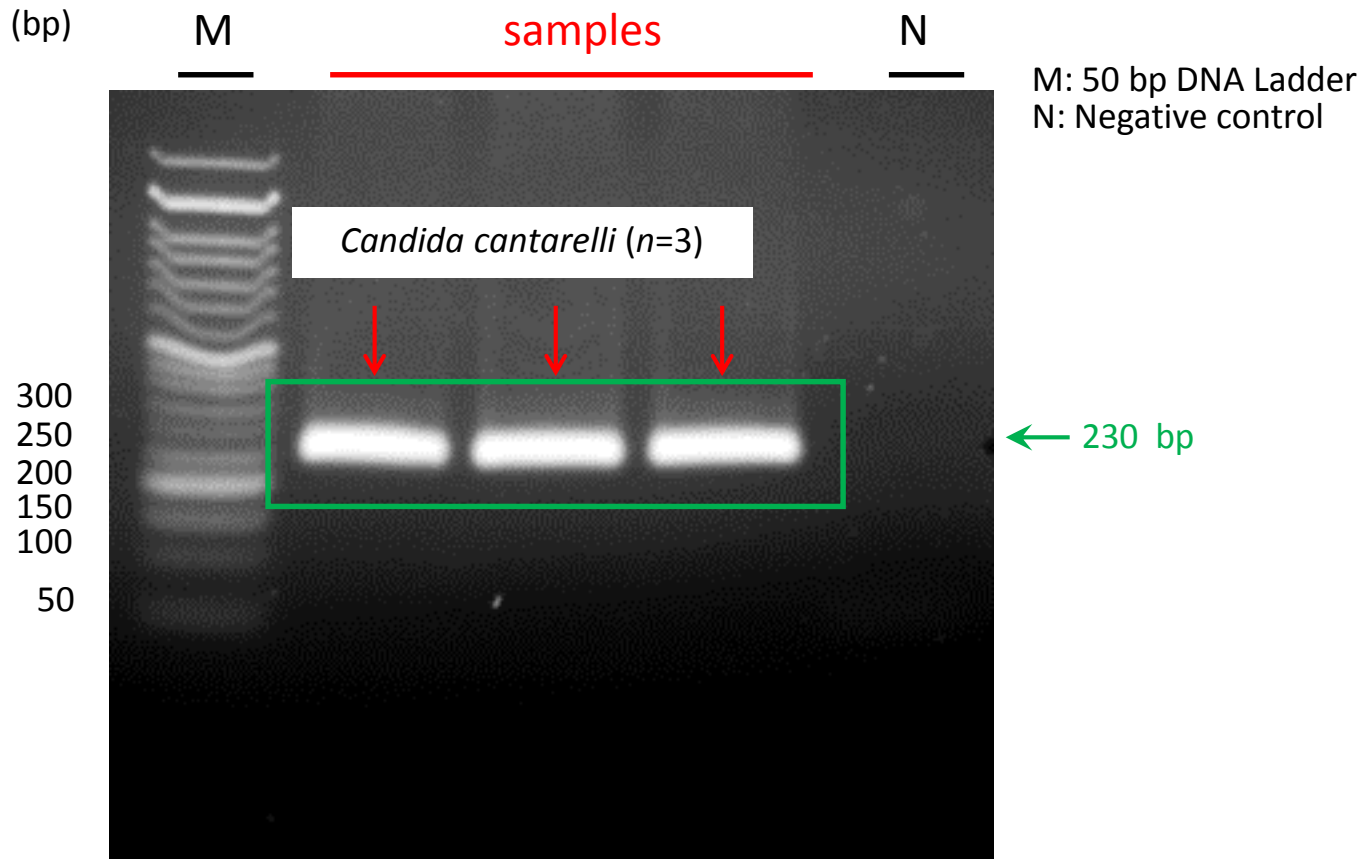
10 x Kaneka High-Speed DNA Polymerase Buffer	2.0 µl
2.0 mM dNTPs	2.0 µl
Kaneka High-Speed DNA Polymerase	0.2 µl
10 µM primers	1.2 µl
DNA Extract	1.0 µl
Sterile distilled water	fill up to 20.0 µl

Electrophoresis and staining DNA

After 2% agarose gel electrophoresis, DNA fragments are visualized by staining with ethidium bromide.

Candida colonies | Extraction of PCR-ready DNA

DNA was extracted from candida colonies using Diffinity DNA RapidExtract Kit. PCR amplification was followed by gel electrophoresis to verify the kit performance.



Extraction and amplification protocols are on the next page.

Candida colonies – DNA extraction and Ampliciation protocols

DNA extraction protocol

1. Pick up candida colonies in solid media with inoculation loops. Add 100 µl of Solution A to the picked colonies placed in a PCR tube.
2. Mix the sample well by pipetting and incubate the PCR tube at 98 ℃ for 8 minutes on a heat block.
3. Cool the tube to room temperature.
4. Add 14 µl of Solution B and mix well.

Primers — 18S ribosomal RNA gene

>Forward
GTAACAAGGTYTCCGT
> Reverse
CGTTCTTCATCGATG

PCR mixture

10 x TaKaRa Taq HS Buffer	2.0 µl
2.5 mM dNTPs	1.6 µl
TaKaRa Taq HS DNA Polymerase	0.1 µl
10 µM primers	1.2 µl
DNA Extract	1.0 µl
Sterile distilled water	fill up to 20.0 µl

Thermal cycler
Biometra T3000
(Biometra GmbH, Germany)

PCR Program
94℃, 1 min
94℃, 15 sec
50℃, 15 sec } x35
72℃, 30 sec

Electrophoresis and staining DNA
After 2% agarose gel electrophoresis, DNA fragments are visualized by staining with ethidium bromide.